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N-Substituted 5-amino-4-cyanopyrazoles: synthesis and reactivity studies

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Masters Dissertation
Master Degree in Medicinal Chemistry

Performed under the supervision of:
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Doctor Elina Margarida Ribeiro Marinho

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All the best for you!

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

N-Substituted 5-amino-4-cyanopyrazoles: synthesis and reactivity studies

Abstract

A literature search indicates that the presence of the pyrazole nucleus in different structures allowed to prepare materials that were applied in technological areas, in medicine, pharmacology or agriculture. Pyrazoles are important building blocks in synthetic and medicinal chemistry because they can be incorporated into a variety of aromatic/heteroaromatic structures and, depending on the substitution pattern, can present a wide range of biological activities, including anticancer activity.

In this work, 5-amino-4-cyanopyrazoles were synthetized by the reaction of 2-ethoxymethylene-malononitrile and substituted hydrazines, using experimental methods previously developed and optimized in the research group. The *o*-aminonitrile unit present in these pyrazoles allowed them to be used as starting materials in the preparation of an imidate derivative that was subsequently used in the synthesis of dimeric structures, by reaction with triethylorthoformate and acid catalysis. The use of acid also induced cleavage of the imidate function, regenerating the starting pyrazole ring, and this contributed to reduce the yield of the dimeric structures that were prepared.

5-Amino-4-cyanopyrazoles were also used as precursors in the synthesis of pyrazolo[3,4-*d*]pyrimidines through the reaction with *N,N*-dimethylacetamide dimethyl acetal, *N,N*-dimethylformamide diethyl acetal or triethylorthoformate, followed by the addition of aromatic, heteroaromatic or alkyl amines, in the presence of acid. An optimization study of the experimental conditions for the synthesis of these compounds and also to isolate the product, was also performed. This approach allowed to prepare 59 pyrazolo[3,4-*d*]pyrimidines by reaction with aromatic amines, 4 with heteroaromatic amines and 3 with alkyl amines. The presence of acetic acid resulted in partial acetylation of the amines, reducing the amount of free amine available to generate the pyrazolo[3,4-*d*]pyrimidine, which contributed to reduce the yield of the product.

A selection of these pyrazolo[3,4-*d*]pyrimidine derivatives was tested for their anticancer activity using the cell line Hs578t of triple negative breast cancer, at ICVS (University of Minho). One of the tested structures demonstrated promising anticancer activity, with an IC_{50} of 4.95 μM. Future work should focus on the synthesis of analogous pyrazolo[3,4-*d*]pyrimidines, aiming to complete the SAR study of this family of compounds.

Keywords: 5-amino-4-cyanopyrazoles, anticancer activity, triple-negative breast cancer, *N,N*-dimethylacetamide dimethyl acetal, *N,N*-dimethylformamide diethyl acetal, pyrazolo[3,4-*d*]pyrimidines, triethylorthoformate

5-Amino-4-cianopirazoles *N*-substituídos: estudos de síntese e reatividade

Resumo

Os trabalhos reportados na literatura evidenciam que a presença do núcleo de pirazole em diferentes estruturas permitiu preparar materiais que encontraram aplicações em áreas tecnológicas, em medicina, farmacologia ou agricultura. Os pirazoles são blocos importantes em química sintética e medicinal, porque podem ser incorporados em diversas estruturas aromáticas/heteroaromáticas e, dependendo do padrão de substituição, podem apresentar uma ampla gama de atividades biológicas, incluindo atividade anticancerígena.

Neste trabalho foram sintetizados 5-amino-4-cianopirazoles pela reação do 2-etoximetileno-malononitrilo e hidrazinas substituídas, utilizando métodos anteriormente desenvolvidos e otimizados no grupo de investigação. A unidade de *o*-aminonitrilo presente nestes pirazoles permitiu que fossem usados como reagentes de partida para a síntese do imidato e, na presença de trietilortoformiato e catálise ácida, a formação de estruturas diméricas. A utilização de ácido provoca a clivagem da função imidato regenerando o pirazole de partida, o que reduz o rendimento de formação das estruturas diméricas.

5-Amino-4-cianopirazoles também funcionaram como precursores para a síntese de pirazolo[3,4-*d*]pirimidinas através da reação com *N,N*-dimetilacetamida dimetil acetal, *N,N*-dimetilformamida dietil acetal ou trietilortoformiato, seguida da adição de aminas aromáticas, heteroaromáticas e alquílicas, na presença de ácido. Foi necessário realizar um estudo de otimização das condições experimentais para a síntese destes compostos e também para o isolamento do produto. Sintetizaram-se 59 pirazolo[3,4-*d*]pirimidinas com aminas aromáticas, 4 com heteroaromáticas e 3 com alquílicas. A presença de ácido acético resulta também na acetilação das aminas, reduzindo a quantidade de amina livre disponível para gerar a pirazolo[3,4-*d*]pirimidina, o que contribui para diminuir o rendimento do produto.

Uma seleção destes derivados de pirazolo[3,4-*d*]pirimidinas foi testada quanto à sua atividade anticancerígena usando a linha celular Hs578t do cancro da mama triplo negativo, pelo ICVS (Universidade do Minho). Verificou-se que uma das estruturas testadas apresentou uma atividade anticancerígena promissora, com um valor de IC_{50} de 4.95 μM. Trabalhos futuros irão incidir na síntese de pirazolo[3,4-*d*]pirimidinas análogas, de forma a completar o estudo SAR nesta família de compostos.

Palavras-chave: 5-amino-4-cianopirazoles, atividade anticancerígena, cancro da mama triplo negativo, *N,N*-dimetilacetamida dimetil acetal, *N,N*-dimetilformamida dietil acetal, pirazolo[3,4-*d*]pirimidinas, trietilortoformiato

Index

List of Figures

List of Tables

List of Schemes

List of abbreviations and terms

Abbreviations	
δ	Chemical shift (expressed in ppm units)
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
^{13}C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
^{15}N NMR	Nitrogen-15 nuclear magnetic resonance spectroscopy
aq.	Aqueous
brs	Broad singlet (in the ^{1}H NMR spectra analysis)
Comp.	Compound
d	Doublet (in the ^{1}H NMR spectra)
DCM	Dichloromethane
dd	Doublet of doublets (in the ^{1}H NMR spectra)
DMADEA	*N,N*-dimethylacetamide dimethyl acetal
DMFDEA	*N,N*-dimethylformamide diethyl acetal
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
dt	Doublet of triplets (in the ^{1}H NMR spectra analysis)
Eq.	Equivalent
EtOH	Ethanol
ESI-MS	Electrospray ionization mass spectrometry
FTIR-ATR	Fourier Transform Infrared Spectroscopy - Attenuated Total Reflection
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Single Quantum Coherence
i	Intense (in the IR spectra)
IC_{50}	Half-maximal inhibitory concentration (the concentration at which a drug is able to inhibit a particular biological process by 50%)
ICVS	Life and Health Sciences Research Institute
IR	Infrared spectroscopy
J	Coupling constant (expressed in Hz)
l	Large (in the IR spectra)
m	Medium (in the IR spectra)
m	Multiplet (in the ^{1}H NMR spectra)
MeOH	Methanol
m.p.	Melting point

MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium assay
MW	Microwave irradiation
m/z	Mass-to-charge ratio (mass spectroscopy)
NEt_3	Triethylamine
ppm	Parts per million
q	Quartet (in the ^1H NMR spectra)
qd	Quartet of doublets (in the ^1H NMR spectra)
R_f	Retention factor
r.t.	Room temperature
s	Singlet (in the ^1H NMR spectra)
SAR	Structure-activity relationship
t	Triplet (in the ^1H NMR spectra)
td	Triplet of doublets (in the ^1H NMR spectra)
TEOF	Triethylorthoformate
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography
tt	Triplet of triplets (in the ^1H NMR spectra)
UV	Ultraviolet light
w	Weak (in the IR spectra)

Chapter 1 – Introduction

1.1. Pyrazoles and pharmacological importance

Pyrazoles are heterocycles of the azole family that consist of a 5-membered ring with two nitrogen atoms in adjacent positions. The simplest molecule has the molecular formula $C_3H_4N_2$ (Figure **1.1**). The pyrazole scaffold contains three nucleophilic (N_1, N_2, C_4) and two electrophilic (C_3, C_5) positions. They constitute a class of compounds particularly useful in organic synthesis, given their versatile chemistry.[1–4]

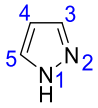


Figure 1.1: Chemical structure of pyrazole.

The presence of the pyrazole nucleus in different structures led to diverse applications in different areas such as technology[1], agrochemicals[1,5–8], medicine[1,9] and pharmacology[2,3,5,6,8,9]. They are important targets in medicinal chemistry because, depending on the substitution pattern, they can present a wide range of biological activities such as antidepressant[1,9], antituberculosis[1], antibacterial[1,3,6], antifungal[1,3,6], leishmanicides[3], antiviral[1,3,6,9], antichagas[3], anti-inflammatory[3,5,6,10], anti-psychotic[1], antihyhypertensive[6], antihyperglycemic[3] or anticancer[1,3,6,9,10] agents. A number of drugs have been developed through functionalization of pyrazoles and these include celecoxib (anti-inflammatory)[1,3], CDPPB (anti-psychotic)[1], lonazolac (anti-inflammatory)[1,3], pyrazofurin (antitumoral, antiviral)[3], mepirizole (anti-inflammatory)[1], formycin (antitumoral, antiviral)[3], fluviol B (antimicrobial)[3], nostacine A (cytotoxic)[3] (Figure **1.2**).

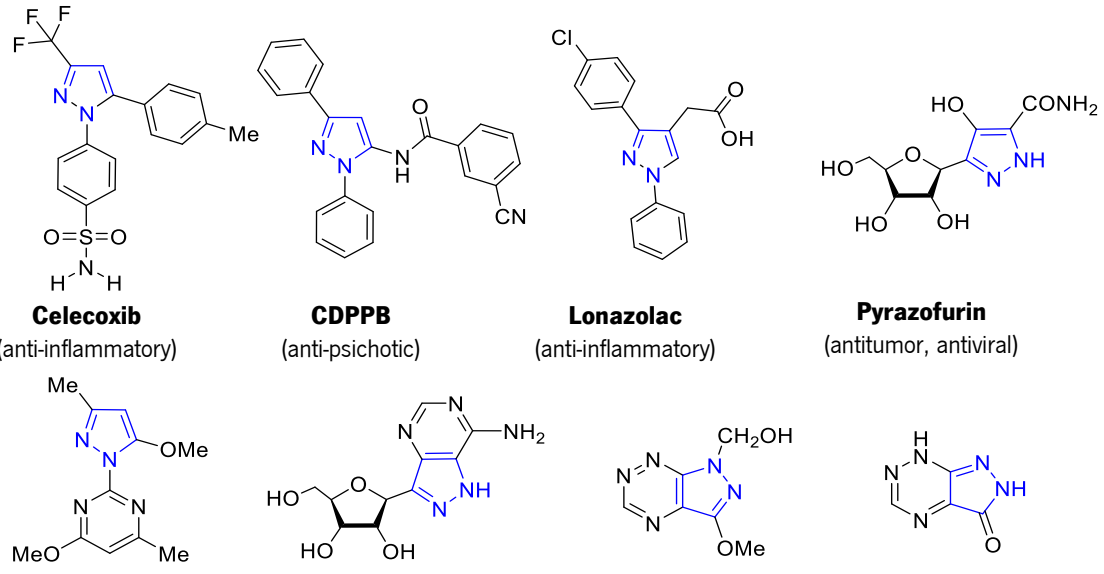
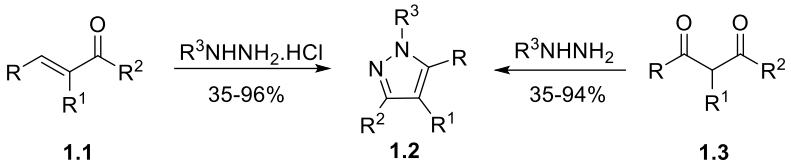


Figure 1.2: Examples of drugs containing the pyrazole unit currently on the market.

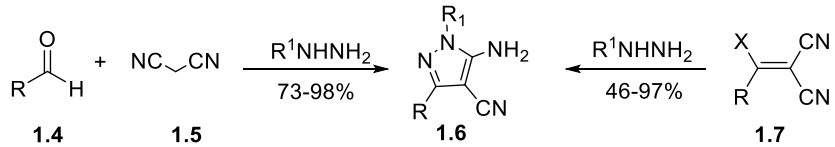
1.2. Synthesis of pyrazoles

Over the years, a wide variety of synthetic methods have been reported to prepare the pyrazole ring **1.2**.[1,3] The most common involve the reaction of an α,β-unsaturated carbonyl compound **1.1**[11] or a 1,3-dicarbonyl compounds **1.3** with substituted hydrazines (Scheme **1.1**). Several experimental conditions were used, for example conventional heating in water[11], microwave irradiation at 130°C in the presence of potassium carbonate[12], I_2-mediated oxidative C-N bond formation[13], ionic liquid [BMIM][BF_4][14] or ethanol[15,16] as solvent, and as catalysts *p*-toluene sulfonic acid (*p*-TSA)[17], silica-supported sulfuric acid ($H_2SO_4.SiO_2$)[18] or Fe_3O_4@CeO_2MnPs[10], $Sc(OTf)_3$[19] and copper nitrate ($Cu(NO_3)_2.3H_2O$).[20] Nanomaterials have also been used as nanoorganocatalysts.[21,22]



Scheme 1.1: Synthesis of pyrazole derivatives **1.2** from α,β-unsaturated carbonyl compounds **1.1** or 1,3-dicarbonyl compounds **1.3** and substituted hydrazines.

The presence of the *o*-aminonitrile motif is particularly important as it can be used to generate a variety of fused heterocyclic derivatives. 5-Amino-1*H*-pyrazole-4-carbonitriles **1.6** have been synthesized by efficient, eco-friendly, economical and fast processes (Scheme **1.2**).[23,24] These methods involve the reaction of substituted hydrazines with aromatic aldehydes **1.4** and malononitrile **1.5**, performed under different conditions such as water containing an ionic liquid, 1-butyl-3-methylimidazolium hydroxide ([Bmim]OH)[24], ethanol and water as solvents[25] or using aqueous glycerol-K_2CO_3 as catalyst.[23] Other common methods reported in the literature involve the reaction of substituted hydrazines with malononitrile derivatives **1.7** in the presence of $Ru^{II}(bpy)_3Cl_2{\cdot}6H_2O$ irradiated with a blue LED[26], HCl[27], NaOH[27,28] or in ethanol.[7,8,29]



Scheme 1.2: Different routes for the synthesis of 5-amino-4-cyanopyrazoles **1.6**.

1.3. Synthesis of pyrazolo[3,4-*d*]pyrimidines

The synthesis of pyrazolo[3,4-*d*]pyrimidines and their biological properties have been intensively studied as can be documented in several reviews.[4,30–35] The 5-amino-4-cyanopyrazole ring can be used as a starting material for the synthesis of pyrazolo[3,4-*d*]pyrimidines. These fused heterocycles (Figure **1.3**) have drawn considerable attention due to their structural similarity with the purine scaffold.[4,36,37] Pyrazolo[3,4-*d*]pyrimidine derivatives have a considerable pharmacological importance as anti-inflammatory[36–38], antimicrobial[36–39], antiviral[36–39], anticancer[36–38], antileukemic[36], tuberculostatic[36,38] agents, among others.

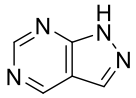
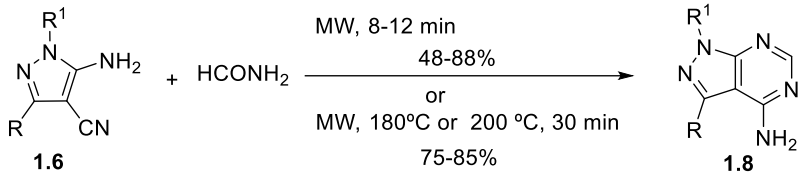


Figure 1.3: Chemical structure of pyrazolo[3,4-*d*]pyrimidine.

Some synthetic methods reported in the literature for the preparation of pyrazolo[3,4-*d*]pyrimidines use microwave (MW) irradiation and conventional heating and some of these examples will be described below. Microwave synthesis can be considered a convenient alternative when compared with traditional methods, as it is a simple/mild and environmentally friendly procedure that originates good yields under reduced reaction times.[40–42] Heravi M. *et al.*[40] reported the reaction of 5-amino-1*H*-pyrazole-4-carbonitrile **1.6** and formamide (Scheme **1.3**), using solid acids such as silica-supported H_2SO_4, tungstophosphoric acid ($H_3PW_{12}O_{40}$), molybdophosphoric acid ($H_3PMo_{12}O_{40}$), or silica-supported $H_3PW_{12}O_{40}$/SiO, under microwave irradiation (1000 W for 8-12 minutes). The 4-aminopyrazolo[3,4-*d*]pyrimidines **1.8** were isolated in 48-88% yield after 8-12 minutes at 1000 W (Scheme **1.3**).[40] Later, Todorovic N. *et al.*[41] reported the synthesis of pyrazolo[3-4*d*]pyrimidines **1.8** from pyrazole **1.6** and formamide but without catalysis. The product was isolated in good yield after 30 minutes at 180°C or 200°C, under MW irradiation.[41]

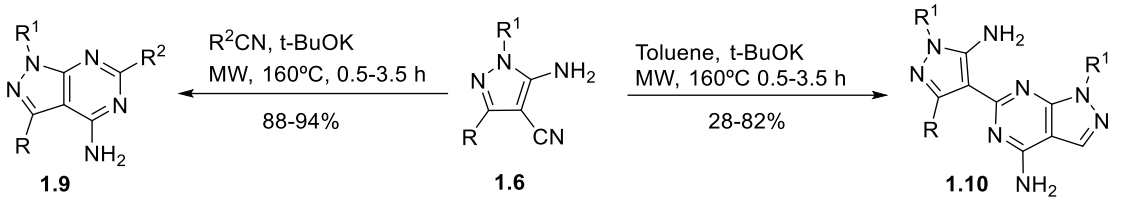


R=H
R^1=H, CMe_3, Me, C_6H_5, 2,4-NO_2-C_6H_3

Scheme 1.3: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.8** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

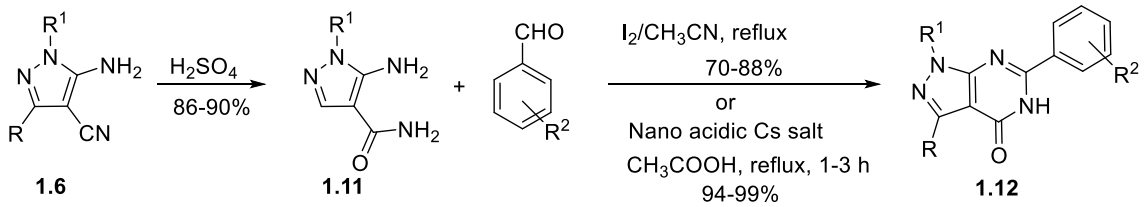
Smith C. *et al.*[42] reported the reaction of **1.6** with various aryl nitriles in the presence of potassium tert-butoxide (t-BuOK) under MW conditions at 160°C (Scheme **1.4**). Pyrazolo[3,4-*d*]pyrimidines **1.9** were obtained after 0.5 to 3.5 hours in excellent yield (88-94%). Using the same reaction conditions, but

replacing the nitrile by toluene, used as solvent, led to the structurally more complex dimeric pyrazolo[3,4-*d*]pyrimidines **1.10** isolated in 28-82% yield.[42]



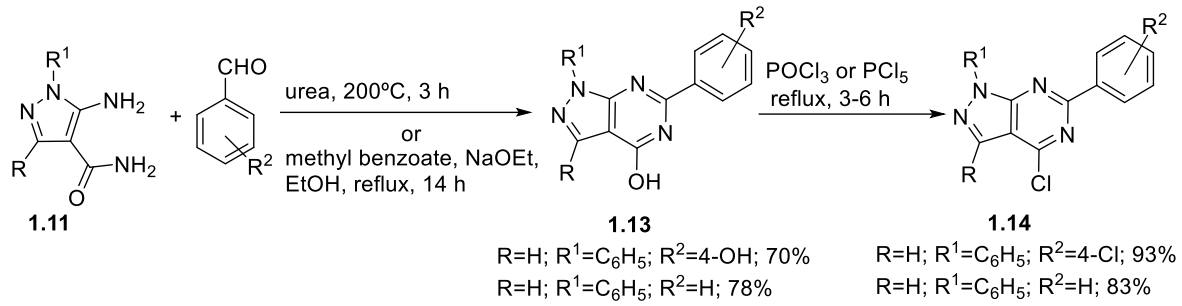
Scheme 1.4: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.9-1.10** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

Another method for the synthesis of pyrazolo[3,4-*d*]pyrimidines uses the reaction of pyrazole **1.6** with concentrated sulfuric acid at room temperature, which led to the 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolcarboxamide **1.11** (Scheme **1.5**).[43,44] The cyclocondensation of **1.11** with aromatic aldehydes occurred under reflux of acetonitrile and in the presence of iodine. New pyrazolo[3,4-*d*]pyrimidines **1.12** were obtained in good yield (70-88%).[43] Bamoharram F. *et al.*[45] prepared new pyrazolo[3,4-*d*]pyrimidines from **1.11**, using Preyssler nanoparticles ($Cs_{12}H_2[NaP_5W_{30}O_{110}]$) as a heterogeneous acid catalyst. After 1-3 hours under reflux of acetic acid, pyrazolo[3,4-*d*]pyrimidinones **1.12** were obtained in excellent yields (94-99%).[45]



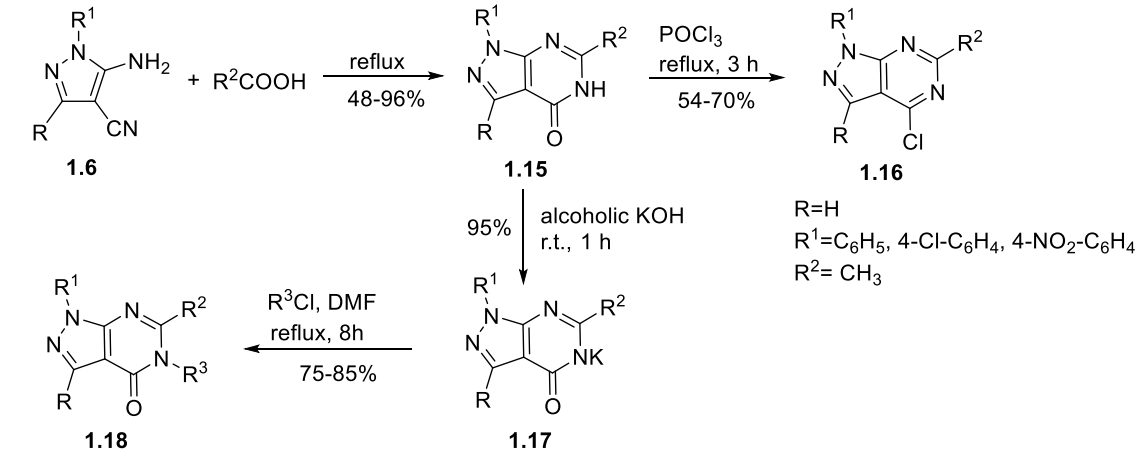
Scheme 1.5: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.12** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

Das J. *et al.*[44] prepared new pyrazolo[3,4-*d*]pyrimidinones **1.13** isolated in 70% yield from cyclization of **1.11** that occurred in the presence of urea at 200°C after 3 hours (Scheme **1.6**). When **1.13** was treated with phosphorous pentachloride or phosphorous oxychloride, the final product **1.14** was isolated in 93% yield.[44] More recently, Gaber A. *et al.*[46] reacted intermediate **1.11** with methyl benzoate and sodium ethoxide under reflux in ethanol (14 hours), leading to pyrazolo[3,4-*d*]pyrimidinone **1.13** with 78% yield. In the next step, **1.13** was reacted with phosphorous oxychloride under reflux (6 hours), obtaining pyrazolo[3,4-*d*]pyrimidines **1.14** in good yield (83%).[46]



Scheme 1.6: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.13-1.14** from intermediate **1.11**.

Another method for the synthesis of pyrazolo[3,4-*d*]pyrimidines used the reaction of pyrazole **1.6** with formic acid or acetic acid under reflux for condensation and intramolecular cyclization, generating pyrazolopyrimidinone **1.15** (48-96% yield), after 7-14 hours (Scheme **1.7**).[38,47,48] In the next step, **1.15** was reacted with phosphoryl trichloride and after 3 hours under reflux, pyrazolo[3,4-*d*]pyrimidines **1.16** were obtained in yields between 54 and 70%.[47] Sherbiny F. *et al.*[48] performed the reaction of **1.15** with alcoholic potassium hydroxide at room temperature, forming the potassium salt **1.17** (95%), after 1 hour. The synthesis proceeded with the reaction of intermediate **1.17** with different alkyl chlorides in DMF, yielding the corresponding pyrazolo[3,4-*d*]pyridimines **1.18** (75-85%).[48]

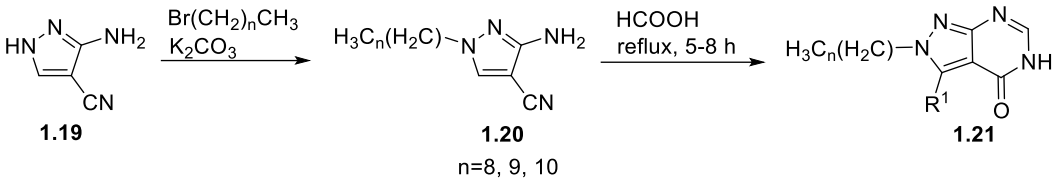


Scheme 1.7: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.15-1.18** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

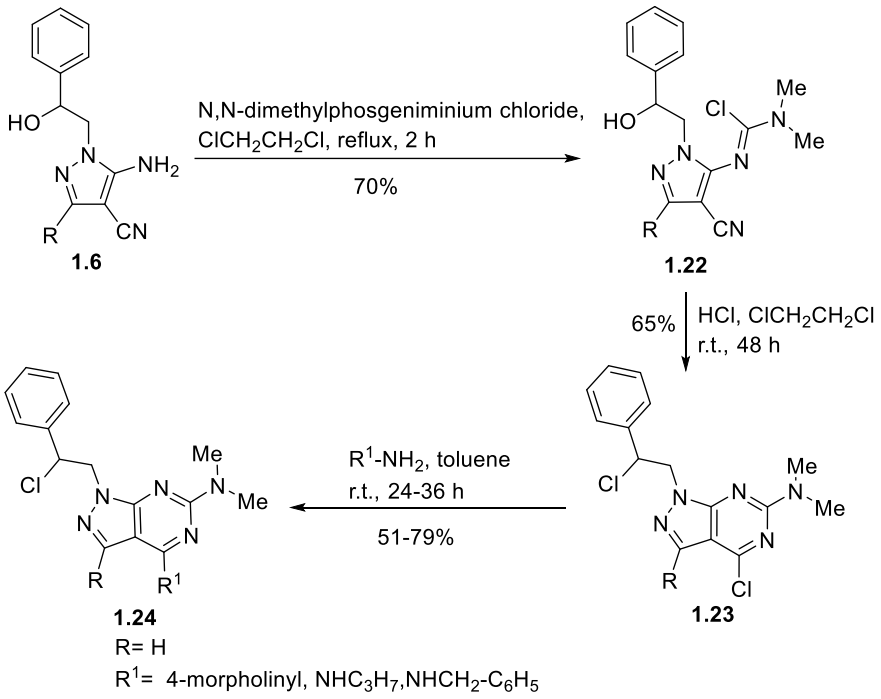
La Motta C. *et al.*[49] described the synthesis of pyrazolo[3,4-*d*]pyrimidinone **1.21** in a two-steps process initiated by alkylation of the commercially available 3-amino-4-pyrazolecarbonitrile **1.19** with the appropriate alkyl bromide in the presence of K_2CO_3 leading to *N*-alkylpyrazoles **1.20** (Scheme **1.8**). Cyclization of **1.20** with formic acid under reflux originated the pyrazolo[3,4-*d*]pyrimidin-4-ones **1.21**.[49]

Schenone S. *et al.*[50] reported the synthesis of pyrazolo[3,4-*d*]pyrimidines **1.24** in a two-steps reaction in which compound **1.6** was reacted with *N,N*-dimethylphosgeniminium chloride in dichloroethane under

reflux leading to the corresponding dimethylcarbamimidic chloride derivative **1.22** (Scheme **1.9**). This compound cyclized in the presence of hydrochloric acid forming the intermediate **1.23** (R=H) in 65% yield, after 48 hours. In the next step, **1.23** was reacted with amines in toluene to generate pyrazolopyrimidine **1.24** (34-90%).[50]

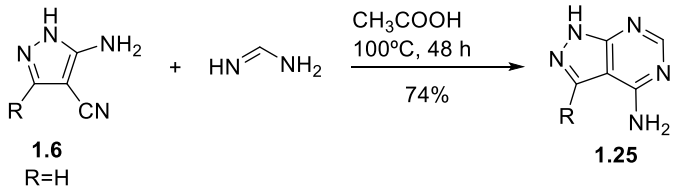


Scheme 1.8: Synthesis of pyrazolo[3,4-*d*]pyrimidin-4-ones **1.21** from 3-amino-4-pyrazolecarbonitrile **1.19**.



Scheme 1.9: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.24** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

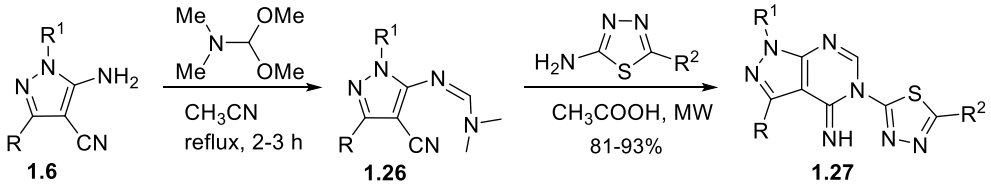
Arava V. *et al.*[51] performed a simple reaction of pyrazole **1.6** with formamidine in acetic acid at 100°C. After 48 hours, pyrazolo[3,4-*d*]pyrimidine **1.25** was isolated in 74% yield (Scheme **1.10**).[51]



Scheme 1.10: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.25** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

Song J. *et al.*[37] reported the synthesis of pyrazolopyrimidines from substituted aminopyrazoles **1.6** and *N,N*-dimethylformamide dimethyl acetal, leading to amidines **1.26** (Scheme **1.11**). The amidine

undergoes condensation with 2-amino-5-substituted-1,3,4-thiadiazoles under MW irradiation, generating pyrazolopyrimidines **1.27** in good yield (81-93%).[37]

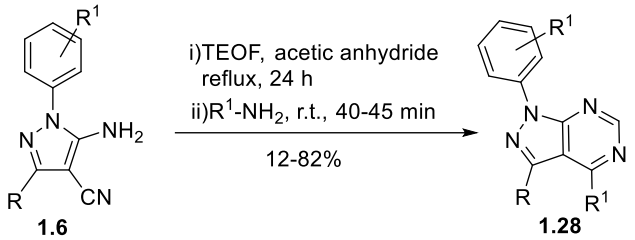


1.6 **1.26** **1.27**

R= H
R^1= H, Ph
R^2= CF_3, 2-FPh, 3-FPh, 4-CF_3Ph

Scheme 1.11: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.27** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

Gupta S. *et al.*[7] performed the reaction of **1.6** with triethylorthoformate and acetic anhydride under reflux, originating the imidate that was then cyclized with primary amines to generate pyrazolo[3,4-*d*]pyrimidines **1.28** (12-82%) (Scheme **1.12**).[7]



1.6 **1.28**

R= H
R^1= 4-Me, 4-OMe, 4-CO_2H, 4-CN, 4-NO_2, 4-CF_3, 4-Cl

Scheme 1.12: Synthesis of pyrazolo[3,4-*d*]pyrimidines **1.28** from 5-amino-1*H*-pyrazole-4-carbonitrile **1.6**.

1.4. Objectives

The objectives of this work were to synthesize 5-amino-4-cyanopyrazoles as starting reagents for the synthesis of dimeric structures and pyrazolo[3,4-*d*]pyrimidines. Dimeric structures had already been previously synthesized in the research group but in a very low yield, so the aim was also to improve the yield of these compounds.

In a recent MSc Master Dissertation, Figueiredo synthesized adenine derivatives from 5-amino-4-cyanoimidazoles. These compounds showed to be promising drug candidates in the Hs578t cell line of triple negative breast cancer because they demonstrated a high affinity for tumor cells (MCF-7) and low toxicity in normal cells (MCF-10).[52] The objective of this work was to synthesize structures similar to the previous ones, replacing the imidazole ring by a pyrazole ring, forming pyrazolo[3,4-*d*]pyrimidine derivatives, and testing their biological activity in the same cell lines. This would allow us to understand the importance of replacing an imidazole by a pyrazole ring in the purine nucleous, on the anticancer activity of the compounds.

Chapter 2 - Results and discussion

2.1. Synthesis of 5-amino-4-cyanopyrazoles

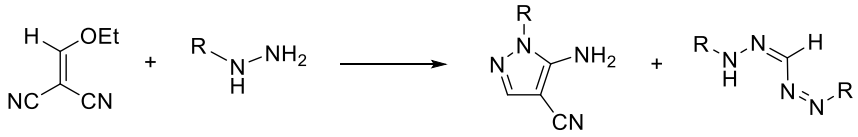
This section reports the synthesis of 5-amino-4-cyanopyrazoles to subsequently study their reactivity. These compounds are not commercially available, and the synthetic method used was previously developed in the research group.[53]

2.1.1. Synthesis and mechanistic discussion

- **Reaction of 2-(ethoxymethylene)malononitrile with aromatic hydrazines**

The reaction of 2-(ethoxymethylene)malononitrile **2.1** with 1 equivalent of substituted hydrazines **2.2a-k** was performed in EtOH except, in one case, where no was used solvent. Addition of triethylamine (NEt_3) allowed to neutralize the salt when the hydrazine used was in the form of the hydrochloride salt. The temperatures used ranged from room temperature to 110°C. Table **2.1** summarizes the experimental conditions that were performed in order to optimize the synthesis of 5-amino-4-cyanopyrazoles **2.3**.

Table 2.1: Experimental conditions for the reaction of 2-(ethoxymethylene)malononitrile **2.1** with aromatic hydrazines **2.2a-k**



2.1 | **2.2a.** R=C_6H_5; **2.2b.** R=2-F-C_6H_4; **2.2c.** R=3-F-C_6H_4; **2.2d.** R=4-F-C_6H_4; **2.2e.** R=4-OMe-C_6H_4; **2.2f.** R=4-CO_2H-C_6H_4; **2.2g.** R=4-Me-C_6H_4; **2.2h.** R=4-Cl-C_6H_4; **2.2i.** R=4-Br-C_6H_4; **2.2j.** R=4-NO_2-C_6H_4; **2.2k.** R=2,5-F-C_6H_3 | **2.3a.** R=C_6H_5; **2.3b.** R=2-F-C_6H_4; **2.3c.** R=3-F-C_6H_4; **2.3d.** R=4-F-C_6H_4; **2.3e.** R=4-OMe-C_6H_4; **2.3f.** R=4-CO_2H-C_6H_4; **2.3g.** R=4-Me-C_6H_4; **2.3h.** R=4-Cl-C_6H_4; **2.3i.** R=4-Br-C_6H_4; **2.3j.** R=4-NO_2-C_6H_4; **2.3k.** R=2,5-F-C_6H_3 | **2.4a, f, k**

Entry	Reagents 1.	Reagents 2.	Experimental Conditions	Product (yield)
1	1.28 mmol	**2.2a.** 1 eq.	EtOH (2 mL), r.t., 35 min	**2.3a.** 12%
2	1.28 mmol	**2.2a.** 1 eq.	EtOH (2 mL), 40°C, 70 min	**2.3a.** 10%
3	1.25 mmol	**2.2a.** 1 eq.	Neat, 40°C, 30 min	Complex mixture containing **2.3a** [a)]
4	2.53 mmol	**2.2a.** 1 eq.	EtOH (2 mL), 80°C, 1 h 45 min	F_1= **2.3a.** 22% F_2= **2.4a.** 12%
5	1.23 mmol	**2.2a.** 1 eq.	EtOH (1 mL), 80°C, 10 h 45 min	**2.3a.** 36%
6	1.20 mmol	**2.2a.** 1 eq.	EtOH (2 mL), 110°C, 1.5 h	**2.3a.** 45%

7	1.22 mmol	**2.2a.** 1 eq.	EtOH (2.5 mL), 110°C, 3 h	**2.3a.** 56%
8	1.24 mmol	**2.2a.** 1 eq.	EtOH (2 mL), 110°C, 6 h	**2.3a.** 63%
9	0.70 mmol	**2.2b.HCl.** 1 eq.	EtOH (1 mL), r.t., 49.5 h	Complex mixture containing **2.3b** [a)]
10	0.70 mmol	**2.2b.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), r.t., 49.5 h	**2.3b.** 82%
11	0.50 mmol	**2.2b.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), 50°C, 30.5 h	Complex mixture containing **2.3b** [a)]
12	1.23 mmol	**2.2c.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 24 h	**2.3c.** 56%
13	1.64 mmol	**2.2c.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 40°C, 24 h	**2.3c.** 62%
14	0.83 mmol	**2.2c.HCl.** 1 eq.	EtOH (3 mL), NEt_3 (1 eq.), 60°C, 48 h	**2.3c.** 49%
15	1.28 mmol	**2.2c.HCl.** 1 eq.	EtOH (5 mL), NEt_3 (1 eq.), 80°C, 27 h	**2.3c.** 39%
16	0.82 mmol	**2.2d.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 48 h	**2.3d.** 51%
17	0.49 mmol	**2.2d.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 60°C, 46 h	**2.3d.** 58%
18	0.29 mmol	**2.2e.HCl.** 1 eq.	EtOH (0.8 mL), NEt_3 (1 eq.), r.t., 26 h	**2.3e.** 13%
19	1.20 mmol	**2.2f.** 1 eq.	EtOH (2 mL), r.t., 24 h	F_1**= 2.3f.** 70% F_2**= 2.3f** + **2.4f** (1.1:1) [a)]
20	1.20 mmol	**2.2f.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 24 h	**2.3f** + **2.2f** (2.7:1) [a)]
21	0.82 mmol	**2.2f.** 1 eq.	EtOH (3 mL), 60°C, 28.5 h	**2.3f.** 32%
22	1.26 mmol	**2.2f.** 1 eq.	EtOH (5.5 mL), 80°C, 19 h	**2.3f.** 54%
23	0.82 mmol	**2.2g.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 24 h	**2.3g.** 66%
24	0.83 mmol	**2.2g.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 60°C, 24 h	**2.3g.** 75%
25	0.83 mmol	**2.2h.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 24 h	**2.3h.** 42%
26	0.84 mmol	**2.2h.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 60°C, 24 h	**2.3h.** 79%
27	0.65 mmol	**2.2i.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), r.t., 48 h	**2.3i.** 69%
28	0.37 mmol	**2.2i.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), 60°C, 48 h	**2.3i.** 55%
29	0.33 mmol	**2.2j.** 1 eq.	EtOH (3 mL), 40°C, 63 h	**2.3j.** 59%
30	0.33 mmol	**2.2j.** 1 eq.	EtOH (4.5 mL), 80°C, 26 h	**2.3j.** 25%
31	1.23 mmol	**2.2k.** 1 eq.	EtOH (2 mL), r.t., 18 h	**2.3k.** 77%
32	0.82 mmol	**2.2k.** 1 eq.	EtOH (3 mL), 60°C, 47 h	**2.3k.** 61%
33	0.87 mmol	**2.2k.** 1 eq.	EtOH (4.5 mL), 80°C, 28 h	**2.3k.** 71%
34	1.25 mmol	**2.2k.** 1 eq.	EtOH (5 mL), 80°C, 54 h	**2.3k** + **2.4k** (7.1:1) [a)]

[a)] By ^{1}H NMR.

The reaction of compound **2.1** with phenylhydrazine **2.2a**, in ethanol, at room temperature (35 minutes) or at 40°C (70 minutes) led to pyrazole **2.3a** (entries 1-2) in poor yield, confirmed by ^{1}H NMR. When the reaction was performed without solvent at 40°C for 30 minutes, the resulting oil, was identified as a complex mixture containing traces of product **2.3a** (entry 3). A signal around δ_H 6.60 ppm, assigned to the NH_2 protons and δ_H 7.77 ppm to the C-H, confirmed the presence of this product. The isolation of the pure yellow crystalline compound was possible when the reaction mixture was heated at 80°C (entries 4 and 5). The product **2.3a** was isolated in 22% after 1 hour and 45 minutes, when the starting material

was no longer identified by TLC (entry 4). The mother liquor was concentrated in the rotary evaporator, and the orange solid precipitated, was filtered and identified as the product **2.4a** by ^{1}H NMR, was isolated in 12% yield. A signal around δ_H 8.69 ppm, assigned to the C-H protons and δ_H 11.42 ppm to the NH, confirmed the presence of formazan **2.4a** (Figure **2.1**). By electrospray ionization mass spectrometry (ESI-MS), the value of m/z obtained for compound **2.4a** (M=224.27g/mol) was 223.14 (M-1). Increasing the reaction time to 10 hours and 45 minutes resulted in an increase in the isolated yield of **2.3a** to 36% (entry 5). The mother liquor contained a complex mixture (4 spots on TLC) and was therefore discarded. The use of a higher temperature (110°C) for 1.5, 3 or 6 hours led also to **2.3a** (entries 6-8). The reaction time of 6 hours led to the best isolated yield of this product (63%).

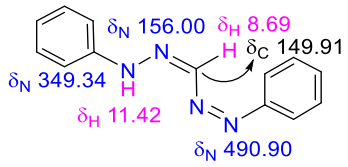


Figure 2.1: Characterization data (^{1}H, ^{13}C, ^{15}N and ESI-MS) of formazan **2.4a**.

The reaction of compound **2.1** with 2-fluorophenylhydrazine hydrochloride **2.2b** in ethanol, at room temperature (49.5 hours) led to a complex mixture containing **2.3b**, by ^{1}H NMR (entry 9). As the hydrazine is in the hydrochloride form, the reaction was repeated adding 1 equivalent of triethylamine and pyrazole **2.3b** was obtained in 82% yield (entry 10). This result proved that triethylamine is necessary in order to neutralize the hydrazine to subsequently participate in the pyrazole synthesis. Increasing the temperature to 50°C led to a complex mixture containing **2.3b** as confirmed by ^{1}H NMR of the oil (entry 11).

3-Fluorophenylhydrazine hydrochloride **2.2c** was reacted with **2.1** and 1 equivalent of triethylamine, in ethanol, at room temperature for 24 hours (entry 12). The beige solid **2.3c**, was identified as a pure product in 56% yield. The mother liquor contained a complex mixture (4 spots on TLC) and was therefore discarded. Increasing the temperature to 40°C, increased the yield to 62% (entry 13). The reaction was repeated at 60°C (48 hours, entry 14) and 80°C (27 hours, entry 15). In both cases, the yields obtained were lower which indicates that heating should be avoided in this reaction.

4-Fluorophenylhydrazine hydrochloride **2.2d** was reacted with **2.1** and triethylamine (1 eq.), in ethanol, at room temperature (entry 16). After 48 hours, TLC confirmed the absence of starting material and the product **2.3d** was isolated in 51% yield. Increasing the temperature to 60°C, increased the yield to 58% (entry 17).

The pure product **2.3e** was isolated in only 13% yield, when compound **2.1** was combined with 4-methoxyphenylhydrazine **2.2e** and triethylamine (1 eq.), in ethanol, at room temperature for 26 hours (entry 18). The very low yield is probably due to the fact that we started with a very small amount of hydrazine **2.2e** (49.8 mg) and, upon filtration, the solid was almost all retained on the filter paper.

4-Hydrazinylbenzoic acid **2.2f** was reacted with **2.1** in ethanol at room temperature for 24 hours, when the starting material was no longer identified by TLC (entry 19). The pure product **2.3f** was isolated as an orange solid in good yield (70%). A second crop of solid precipitated, after partial removal of the solvent in rotatory evaporator, and was filtered leading a mixture of **2.3f** and **2.4f** (1.1:1), by ^{1}H NMR. To try to avoid the formation of product **2.4f**, the reaction was repeated and NEt_3 was added (entry 20). It was expected that NEt_3 would increase the rate of cyclization of product **2.3f**, preventing the formation of **2.4f**. However, a mixture of **2.3f** and **2.2f** was isolated in a molar ratio of 2.7:1, indicating that NEt_3 decreased the reaction rate. The reaction was repeated at 60°C (28.5 hours, entry 21) and 80°C (20 hours, entry 22). In both cases, the yields obtained were lower which indicates that heating is not improving the reaction rate.

The reaction of compound **2.1** with 4-tolylhydrazine hydrochloride **2.2g** and triethylamine (1 eq.), in ethanol, at room temperature (24 hours) led to pyrazole **2.3g** isolated in 66% yield (entry 23). Increasing the temperature to 60°C (24 hours), improved the yield to 75% (entry 24).

The reaction of compound **2.1** with (4-chlorophenyl)hydrazine hydrochloride **2.2h**, in ethanol, at room temperature led to pyrazole **2.3h** (entry 25) in 42% yield, after 24 hours. The mother liquor contained a complex mixture (4 spots on TLC) and was therefore discarded. Increasing the temperature to 60°C (entry 26), resulted in an increase of the yield to 79%.

The pure product **2.3i** was obtained in 69% yield, when compound **2.1** was combined with (4-bromophenyl)hydrazine hydrochloride **2.2i** and triethylamine (1 eq.), in ethanol, at room temperature for 48 hours (entry 27). Increasing the temperature to 60°C (entry 28), decreased the yield to 55%.

The reaction of compound **2.1** with (4-nitrophenyl)hydrazine **2.2j**, in ethanol, at 40°C led to pyrazole **2.3j** (entry 29) in 59% yield, after 63 hours. The mother liquor contained a complex mixture (4 spots on TLC) and was therefore discarded. Increasing the temperature to 80°C, resulted in a decrease in the isolated yield of this product after 26 hours (entry 30).

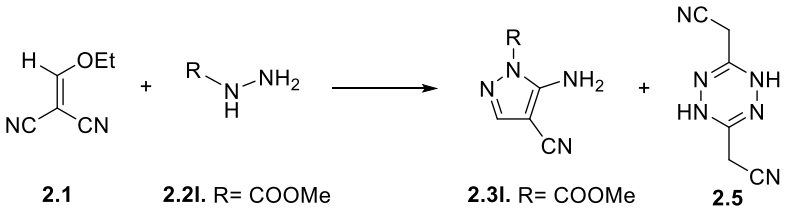
2,5-Difluorophenylhydrazine **2.2k** was combined with compound **2.1** in ethanol at room temperature. After 18 hours TLC showed the absence of starting material and the pure product **2.3k** was isolated as a yellow solid in 77% yield (entry 31). The temperature was increased to 60°C (47 hours,

entry 32) and 80°C (28 hours, entry 33) but the yields were lower. The reaction time was increased to 56 hours at 80°C and products **2.3k** and **2.4k** were isolated in a ratio of 7.1:1 (entry 34).

- **Reaction of 2-(ethoxymethylene)malononitrile with alkyl and acyl hydrazines**

The reaction of 2-(ethoxymethylene)malononitrile **2.1** with 1 or 2 molar equivalents of substituted hydrazines **2.2l-q** was performed mainly in EtOH and, in a few cases, in the absence of solvent or with different solvents (CH_3CN, aqueous $NaHCO_3$, DMSO, MeOH). Once again, triethylamine was used to neutralize reaction mixture when the hydrazine was in the form of the hydrochloride salt. The temperatures used ranged from -10°C to 110°C. Table **2.2** summarizes the experimental conditions used to prepare the 5-amino-4-cyanopyrazoles.

Table 2.2: Experimental conditions for the reaction of 2-(ethoxymethylene)malononitrile **2.1** with different hydrazines **2.2l-q**



2.1

2.2l. R= COOMe
2.2m. R=COOEt
2.2n. R= CH_2COOEt
2.2o. R=$COCH_2CN$
2.2p. R=H
2.2q. R=COMe

2.3l. R= COOMe
2.3m. R=COOEt
2.3n. R= CH_2COOEt

2.5

Entry	Reagents 2.1.	Reagents 2.2.	Experimental Conditions	Product (yield)
1	0.88 mmol	**2.2l.** 1 eq.	EtOH (1.5 mL), 25°C, 4 days	F_1= **2.3l.** 10% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
2	0.98 mmol	**2.2l.** 1 eq.	EtOH (2 mL), 40°C, 4.5 h	F_1= **2.3l.** 13% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
3	0.82 mmol	**2.2l.** 1 eq.	EtOH (2.5 mL), 60°C, 28 h	F_1= **2.3l.** 34% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
4	1.19 mmol	**2.2l.** 1 eq.	EtOH (2 mL), 80°C, 12 h	F_1= **2.3l.** 31% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
5	0.96 mmol	**2.2l.** 2 eq.	EtOH (2 mL), 80°C, 40 h	F_1= **2.3l.** 20% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
6	1.16 mmol	**2.2l.** 1 eq.	EtOH (2 mL), 80°C, 48 h	F_1= **2.3l.** 28% F_2= Complex mixture containing **2.3l** and malononitrile [a)]
7	1.19 mmol	**2.2l.** 1 eq.	EtOH (4 mL), 80°C, 48 h	F_1= **2.3l**. 17%

				F_2= Complex mixture containing **2.3l** and malononitrile [a)]
8	1.22 mmol	**2.2l.** 1 eq.	EtOH (8 mL), 80°C, 48 h	F_1= **2.3l.** 13% F_2= Complex mixture containing **2.3l** [a)]
9	1.26 mmol	**2.2l.** 1 eq.	EtOH (2 mL), 100°C, 45 h	Complex mixture [a)]
10	0.85 mmol	**2.2m.** 1 eq.	EtOH (1 mL), r.t., 48 h	**2.3m.** 51%
11	0.82 mmol	**2.2m.** 1 eq.	EtOH (1 mL), 60°C, 48 h	**2.3m.** 27%
12	0.85 mmol	**2.2n.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 3 days	**2.3n.** 26%
13	0.85 mmol	**2.2n.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), r.t., 10.5 days	**2.3n.** 51%
14	1.27 mmol	**2.2n.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 40°C, 53 h	**2.3n.** 22%
15	1.19 mmol	**2.2n.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 65°C, 19 h	**2.3n.** 25%
16	1.17 mmol	**2.2n.HCl.** 1 eq.	EtOH (5 mL), 65°C, 40 h	Mixture of **2.2n** + ammonium salt (1.4:1) [a)]
17	0.87 mmol	**2.2n.HCl.** 1 eq.	CH_3CN (5 mL), NEt_3 (1 eq.), 65°C, 3 days	Complex mixture containing **2.3n** [a)]
18	0.89 mmol	**2.2n.HCl.** 1 eq.	$NaHCO_3$ aq. (0.05 M), (2 mL), 65°C, 64 h	Complex mixture containing **2.3n** [a)]
19	0.86 mmol	**2.2n.HCl.** 1 eq.	EtOH (5 mL), CH_3COOH (1 eq.), 65°C, 64 h	Complex mixture containing **2.3n** [a)]
20	1.23 mmol	**2.2n.HCl.** 1 eq.	EtOH (2 mL), NEt_3 (1 eq.), 100°C, 48 h	Complex mixture containing **2.3n** [a)]
21	0.54 mmol	**2.2n.HCl.** 1 eq.	EtOH (4 mL), NEt_3 (1 eq.), 110°C, 9 h	F_1= **2.3n.** 27% F_2= Complex mixture containing **2.3n** [a)]
22	0.87 mmol	**2.2o.** 1 eq.	EtOH (1 mL), r.t., 48 h	F_1= **2.5.** 25% F_2= Complex mixture containing **2.3o** [a)]
23	0.82 mmol	**2.2o.** 1 eq.	EtOH (1 mL), r.t., 24 h	F_1= **2.5.** 7% F_2= Complex mixture containing **2.3o** [a)]
24	0.82 mmol	**2.2o.** 1 eq.	EtOH (1 mL), -10°C, 48 h	Complex mixture [a)]
25	0.81 mmol	**2.2o.** 1 eq.	EtOH (1 mL), -10°C, 6.5 h	Complex mixture [a)]
26	0.84 mmol	**2.2o.** 1 eq.	MeOH (1 mL), -10°C, 6.5 h	Complex mixture [a)]
27	0.82 mmol	**2.2o.** 1 eq.	EtOH (1 mL), 60°C, 48 h	Complex mixture [a)]
28	0.84 mmol	**2.2p.HCl.** 1 eq.	i) CH_3CN (2 mL), NEt_3 (1 eq.), r.t., 50 min ii) 80°C, 70 min	Complex mixture [a)]
29	1.23 mmol	**2.2p.HCl.** 1 eq.	i) EtOH (2 mL), NEt_3 (1 eq.), r.t., 30 min ii) 110°C, 45 h	Complex mixture [a)]
30	0.80 mmol	**2.2p.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), 0°C, 24 h	Complex mixture containing EtOH + NEt_3 + malononitrile [a)]
31	0.80 mmol	**2.2p.HCl.** 1 eq.	EtOH (1 mL), 0°C, 24 h	Complex mixture [a)]
32	0.84 mmol	**2.2p.HCl.** 1 eq.	EtOH (1 mL), NEt_3 (1 eq.), -10°C, 48 h	Complex mixture containing NEt_3 + ammonium salt + malononitrile [a)]
33	0.82 mmol	**2.2p.HCl.** 1 eq.	i) Neat, 150°C, 7 h ii) NEt_3 (1 eq.), r.t., 2.5 h	Complex mixture [a)]
34	0.82 mmol	**2.2p.HCl.** 1 eq.	i) DMSO (200 µL), 150°C, 5 h ii) NEt_3 (1 eq.), r.t., 2.5 h	Complex mixture [a)]
35	0.80 mmol	**2.2p.** 1 eq.	EtOH (0.5 mL), r.t., 48h	Complex mixture [a)]

36	0.80 mmol	**2.2p.** 1 eq.	EtOH (40 mL), r.t., 24 h	Complex mixture containing ammonium salt + malononitrile [a)]
37	0.80 mmol	**2.2p.** 1 eq.	EtOH (1 mL), 0°C, 24 h	**2.1** + ammonium salt (5.3:1) [a)]
38	0.86 mmol	**2.2p.** 1 eq.	EtOH (1 mL), -10°C, 48 h	Complex mixture containing ammonium salt + malononitrile [a)]
39	0.88 mmol	**2.2p.** 1 eq.	EtOH (0.5 mL), 80°C, 3 h	Complex mixture [a)]
40	0.90 mmol	**2.2p.** 1 eq.	EtOH (1.5 mL), 80°C, 16.5 h	Complex mixture [a)]
41	0.86 mmol	**2.2p.** 1 eq.	CH_3CN (1 mL), 80°C, 25 h	**2.1** + ammonium salt (4.3:1) [a)]
42	0.82 mmol	**2.2p.** 1 eq.	Neat, 150°C, 7.5 h	Complex mixture [a)]
43	0.83 mmol	**2.2p.** 1 eq.	DMSO (100 µL), 150°C, 9 h	Complex mixture [a)]
44	0.82 mmol	**2.2q.** 1 eq.	EtOH (1 mL), 10°C, 47 h	Complex mixture [a)]
45	0.83 mmol	**2.2q.** 1 eq.	EtOH (1 mL), r.t., 47 h	Complex mixture [a)]
46	0.84 mmol	**2.2q.** 1 eq.	EtOH (1 mL), 60°C, 20 h	Complex mixture [a)]
47	0.90 mmol	**2.2q.** 1 eq.	EtOH (1 mL), 110°C, 23 h	Complex mixture [a)]
48	0.78 mmol	**2.2q.** 1 eq.	DMSO (100 µL), 150°C, 6 h	Complex mixture [a)]

[a)] By ^{1}H NMR.

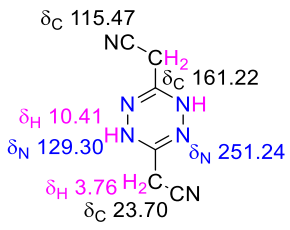
Methyl hydrazinecarboxylate **2.2l** was combined with **2.1** in ethanol at 25°C for 4 days. The product **2.3l** was isolated in the first crop in 10% yield (entry 1). The reaction was repeated at 40°C for 4.5 hours and product **2.3l** was isolated in 13% yield (entry 2). At 60°C (28 hours, entry 3) and 80°C (12 hours, entry 4) product **2.3l** was isolated in 34% and 31% yield, respectively. To try to accelerate the reaction, the amount of hydrazine was increased to 2 equivalents (entry 5). After 40 hours at 80°C, the yield was 20%. To try to understand if the concentration would influence the rate of reaction, the experiment was repeated using 2, 4 and 8 mL (entries 6-8) of ethanol, at 80°C. The decrease in concentration led to a decrease in yield, 28%, 17% and 13%, respectively. Increasing the temperature to 100°C (45 hours) led to a complex mixture (entry 9). As the yields of the isolated product, in these reactions, were very low, the mother liquors were concentrated in the rotary evaporator and the solid that precipitated was filtered and identified by ^{1}H NMR as a complex mixture containing **2.3l** and malononitrile (entries 1-9). The formation of the malononitrile probably results from cleavage of the starting material **2.1**.

The reaction of compound **2.1** with ethyl hydrazinecarboxylate **2.2m**, in ethanol, at room temperature led to a pyrazole **2.3m** (entry 10) in 51% yield, after 48 hours. Increasing the temperature to 60°C, led to a decrease in yield to 27% (entry 11).

The reaction between **2.1**, ethyl hydrazinoacetate hydrochloride **2.2n** and triethylamine (1 eq.) was carried out in ethanol at room temperature. After 3 days, TLC showed the absence of starting material and a beige product **2.3n** was isolated in 31% yield (entry 12). The yield increased to 51% when the reaction was allowed to proceed for 10.5 days (entry 13). Increasing the temperature to 40°C (53 hours,

entry 14) or 65°C (19 hours, entry 15), resulted in a decreased yield. The reaction was repeated at 65°C without NEt_3 and only a **2.2n** and ammonium salt were isolated in a 1.4:1 ratio (entry 16). The use of NEt_3 allowed to isolate the product, suggesting that the reaction requires the presence of the non-protonated hydrazine. Using acetonitrile as solvent at 65°C for 3 days (when the starting material was no longer identified by TLC), a complex mixture containing traces of **2.3n** was isolated (entry 17). A signal around δ_H 6.71 ppm, assigned to the NH_2 protons, a signal at δ_H 7.55 ppm for the C-H proton, and δ_H 4.81 ppm, assigned to the CH_2 protons, confirmed the presence of this product. In an attempt to replace NEt_3, by a more ecological solution, the reaction was repeated at 65°C in the presence of aqueous $NaHCO_3$ 0.05 M (64 hours, entry 18) or acetic acid (64 hours, entry 19). In both cases, complex mixture containing traces of **2.3n** was also formed. Increasing the temperature (100°C), led to a complex mixture after 48 hours (entry 20), but **2.3n** could be detected in the reaction mixture, by ^{1}H NMR. At 110°C for 9 hours, the product **2.3n** was isolated in a first crop in 27% yield (entry 21). A second crop of solid precipitated, after concentration in the rotary evaporator, was filtered, leading to a complex mixture containing **2.3n**.

The reaction between **2.1** and 2-cyanoacetohydrazide **2.2o** (1 eq.) at room temperature for ‘48 hours (entry 22) and 24 hours (entry 23) originated product **2.5** in 25% and 7% yield, respectively. In the ^{1}H NMR, a signal around δ_H 10.42 ppm, integrating for 1H, was assigned to the N-H proton and at δ_H 3.76 ppm, integrating for 2H, assigned to the CH_2, confirmed the presence of compound **2.5** (Figure **2.2**). In the ^{13}C NMR, a signal around δ_C 115.47 ppm, assigned to the CN group and δ_C 161.22 ppm for the C=N. The existence of a CH_2 at δ_C 23.70 ppm was confirmed, by DEPT. The mother liquor was a complex mixture where the pyrazole **2.3o**, could be identified. In a new experiment, the temperature was lowered to -10°C for 48 hours, and a complex mixture was again obtained, where no signs of the desired pyrazole were seen (entry 24). The reaction was repeated for only 6.5 hours in ethanol (entry 25) and methanol (entry 26) and a complex mixture was obtained in both cases. The temperature was raised to 60°C for 48 hours and a complex mixture was obtained (entry 27).



2.5

Figure 2.2: Characterization data (^{1}H, ^{13}C, ^{15}N) for compound **2.5**.

Hydrazine hydrochloride **2.2p** reacted with 2-(ethoxymethylene)malononitrile **2.1** and 1 equivalent of NEt_3, used to neutralize the hydrazine. The reaction was performed in acetonitrile initially at room temperature and then at 80°C (entry 28). TLC showed the absence of starting material, and the resulting oil proved to be a complex mixture. It was not possible to identify the desired product, probably due to the high insolubility of hydrazine **2.2p** under these conditions. The same result was obtained when ethanol was used as solvent, first at room temperature (30 minutes) and then at 110°C (45 hours) (entry 29). The temperature was lowered to 0°C, and the reaction mixture was stirred for 24 hours. The resulting oil showed to be a complex mixture containing ethanol, NEt_3 and malononitrile (entry 30). The reaction was repeated under the same conditions but without NEt_3, and a complex mixture was also obtained (entry 31). The temperature was further lowered to -10°C and the resulting oil show again to be a complex mixture (entry 32). Increasing the temperature to 150°C, without solvent (7 hours, entry 33) or in DMSO (5 hours, entry 34) followed by addition of NEt_3 and stirring for 2.5 hours at room temperature, led to an oil that proved to be a complex mixture.

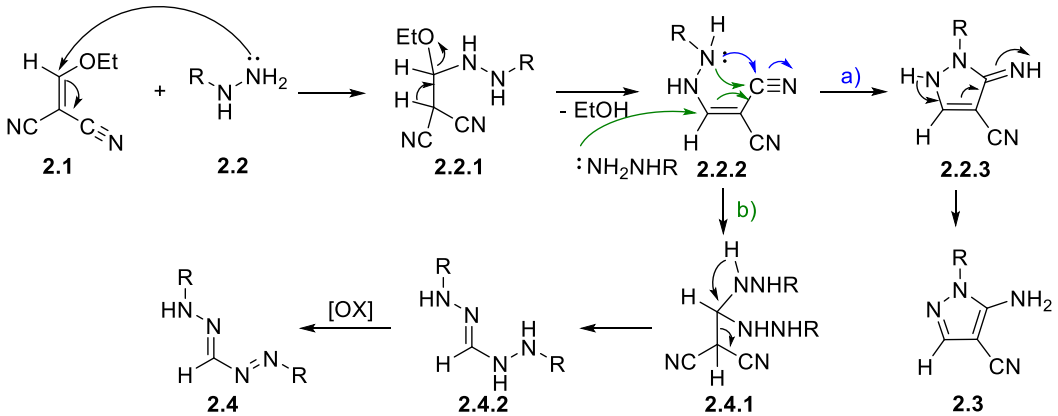
Hydrazine monohydrate **2.2p** was also reacted with 2-(ethoxymethylene)malononitrile **2.1** in ethanol at room temperature. After 48 hours, TLC showed the absence of starting material and the resulting oil was again a complex mixture (entry 35). Dilution of the reaction mixture was highly increased (40 mL of ethanol) and the spectrum of the solid isolated after 24 hours at room temperature showed a mixture of starting material **2.1** (δ_H 8.38 ppm, CH), ammonium salt and malononitrile (δ_H 4.45 ppm, CH_2) (entry 36). Malononitrile probably arises from cleavage of the 2-(ethoxymethylene)malononitrile **2.1**. This is probably why the reaction cannot proceed. In a new experiment, the temperature was lowered to 0°C (24 hours) and the ^{1}H NMR spectrum of the oil showed a mixture of **2.1** and ammonium salt in a 5.3:1 molar ratio (entry 37). Lowering the temperature to -10°C (entry 38) led to a complex mixture containing both starting materials, ammonium salt and malononitrile. Bussenius J. *et al.*[54] synthesized the desired pyrazole at 80°C for 3 hours. The successful experimental procedure was reproduced, and the resulting oil proved to be a complex mixture where traces of malononitrile were also present (entry 39). Increasing the reaction time to 16.5 hours (entry 40) led to similar results. When acetonitrile was used as solvent, under the same conditions, the reaction did not take place as only the starting material **2.1** and ammonium salt (4.3:1) were identified (entry 41). The reaction was repeated at 150°C without solvent (7.5 hours, entry 42) or in DMSO (9 hours, entry 43). In both cases, the resulting oil proved to be a complex mixture. No further attempts were made in order to prepare this product.

Acetohydrazide **2.2q** was reacted with **2.1** in ethanol (1 mL) at 10°C. After 47 hours, TLC showed the absence of starting material but the resulting solid showed to be a complex mixture, by ^{1}H NMR (entry

44). The reaction was repeated at room temperature and after 47 hours the solid obtained was also identified as a complex mixture (entry 45). Increasing the temperature to 60°C (20 hours, entry 46) and 110°C (23 hours, entry 47), led again to a complex mixture. In both cases, in the ^{1}H NMR spectrum, the CH_3 signal of the **2.2q** acetyl group (δ_H 1.8 ppm) was not seen. This could mean that, increasing the temperature, was leading to cleavage of the acetyl group and the reaction was not proceeding through the intended synthetic route. At 150°C in DMSO, a black solid was isolated which was complex mixture, by ^{1}H NMR (entry 48). Again, no further attempts were made in order to prepare this product.

To try to understand formation of the ammonium salt, 2-(ethoxymethylene)malononitrile **2.1** was solubilized in ethanol (2 mL) and the mixture was stirred for 48 hours and 7 days. After 48 hours, reagent **2.1** was isolated in 43% yield. A complex mixture was identified in the mother liquor, where traces of **2.1** and a large amount of ammonium salt were seen. After 7 days, reagent **2.1** was isolated in 16% yield. The mother liquor was a complex mixture with an enormous amount of ammonium salt and malononitrile, by ^{1}H NMR. Malononitrile probably arises from cleavage of the starting material **2.1**.

The mechanism proposed for the reaction leading to 5-amino-4-cyanopyrazoles **2.3** and compound **2.4** from 2-(ethoxymethylene)malononitrile **2.1** and hydrazines **2.2** is presented in Scheme **2.1**. Nucleophilic attack of the amino group of hydrazine **2.2** to the activated carbon of **2.1**, leads to intermediate **2.2.1**, which eliminates ethanol and generates intermediate **2.2.2**. Following path a), intramolecular cyclization by nucleophilic attack of the pair of electrons on the amine to the carbon of the cyano group, forming intermediate **2.2.3**. Tautomeric equilibrium generates pyrazoles **2.3**.



Scheme 2.1: Proposed mechanism for the synthesis of 5-amino-4-cyanopyrazoles **2.3** and compound **2.4**.

By path b), nucleophilic attack by the electron pair of a second unit of hydrazine **2.2** to **2.2.2**, leads to **2.4.1**. Elimination of HCN generates **2.4.2** which undergoes an oxidation process to form compound **2.4**. This path probably occurs when the mother liquor is concentrated in the rotary evaporator, induced by the temperature of the water bath.

2.1.2. Analytical and spectroscopic characterization

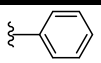
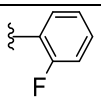
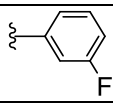
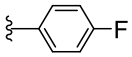
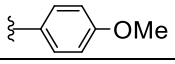
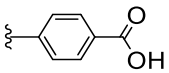
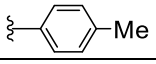
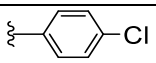
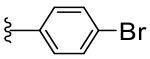
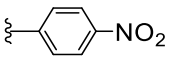
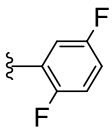
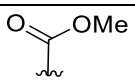
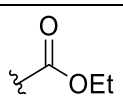
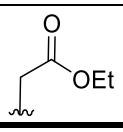
The structures of the synthesized compounds were assigned on the basis of IR, ^{1}H, ^{13}C and ^{15}N NMR spectroscopic data. The physical and analytical data are presented below.

- **Physical and analytical data**

Table **2.3** presents the melting point range and the best isolated yield for each compound **2.3a-n**. Compounds herein presented will be later submitted to elemental analysis.

Table 2.3: Physical and analytical data for pyrazoles **2.3a-n**

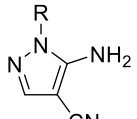
2.3

Comp.	R	Yield (%)	m.p. (°C)	Chemical Formula	Calculated values (%)		
					C	H	N
2.3a		63	147-149	$C_{10}H_8N_4$	65.14	4.39	30.42
2.3b		82	125-127	$C_{10}H_7FN_4$	59.40	3.50	27.72
2.3c		62	165-168	$C_{10}H_7FN_4$	59.40	3.50	27.72
2.3d		58	162-164	$C_{10}H_7FN_4$	59.40	3.49	27.71
2.3e		13	133-135	$C_{11}H_{10}N_4O$	61.66	4.71	26.16
2.3f		70	281-283	$C_{11}H_8N_4O_2$	57.89	3.54	24.56
2.3g		75	136-138	$C_{11}H_{10}N_4$	66.65	5.09	28.26
2.3h		79	160-162	$C_{10}H_7ClN_4$	54.93	3.23	25.63
2.3i		69	151-153	$C_{10}H_7BrN_4$	45.65	2.68	21.30
2.3j		59	153-155	$C_{10}H_7N_5O_2$	52.40	3.08	30.57
2.3k		77	178-180	$C_{10}H_6F_2N_4$	54.55	2.75	25.45
2.3l		34	193-195	$C_6H_6N_4O_2$	43.37	3.65	33.73
2.3m		51	129-131	$C_7H_8N_4O_2$	46.67	4.48	31.10
2.3n		51	196-198	$C_8H_{10}N_4O_2$	49.48	5.20	28.86

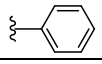
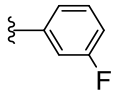
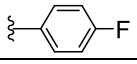
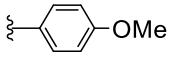
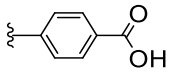
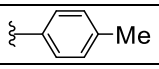
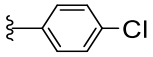
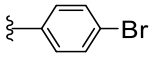
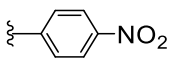
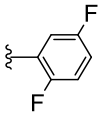
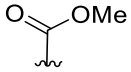
- **Infrared Spectroscopy**

Pyrazoles **2.3a-n** show an intense band in the 2212-2243 cm^{-1} range attributed to the stretching vibration of the CN group. The weak to medium intensity bands in the 3465-3053 cm^{-1} range correspond to the stretching vibrations of the N-H and C_{sp2}-H bonds. The intense band at 1714, 1749, 1749 and 1738 cm^{-1} was assigned to the stretching vibration of the carbonyl group of compounds **2.3f** and **2.3l-n**, respectively. The stretching vibrations of the C=C and C=N bonds as well as the bending vibration of the N-H bond are represented by several bands of weak to strong intensity between the 1504 and 1693 cm^{-1} (Table **2.4**).

Table 2.4: IR spectroscopic data (FTIR-ATR) for the pyrazoles **2.3a-n**



2.3

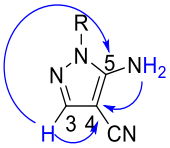
Comp.	R	4000-3000	CN	CO	1700-1500
2.3a		3319m, 3223m, 3053w	2223i	---	1640i, 1599m, 1591m, 1561m, 1536i
2.3b		3321l, 3197l	2224i	---	1645m, 1567i, 1538i, 1504i
2.3c		3454m, 3306l, 3216l	2227i	---	1640i, 1612m, 1598m, 1563m, 1537i
2.3d		3457m, 3300m, 3213w	2243i	---	1639i, 1562i, 1538i, 1514i
2.3e		3446i, 3301i, 3158m, 3064m	2212i	---	1639i, 1616w, 1591m, 1567m, 1533i, 1517i
2.3f		3424l, 3296l	2225i	1714i	1693w, 1644i, 1611m, 1567m, 1539i, 1515w
2.3g		3314l, 3178l	2216i	---	1660m, 1614w, 1590w, 1539i, 1513m
2.3h		3456m, 3297m, 3182l	2243i	---	1635i, 1600w, 1586w, 1559m, 1533i
2.3i		3455m, 3297m, 3183m	2242i	---	1637i, 1593w, 1583w, 1560m, 1534i,
2.3j		3361l, 3259l	2232m	---	1651m, 1596i, 1533m, 1505i
2.3k		3462m, 3330m, 3238l	2235i	---	1650i, 1632w, 1568m, 1538i, 1507i
2.3l		3452m, 3276l, 3211l, 3175l, 3137l	2220i	1749i	1635i, 1561m, 1529w
2.3m		3448m, 3281w, 3125l	2216i	1749i	1636i, 1559i, 1528w
2.3n		3465l, 3339l, 3152l	2213i	1738i	1667m, 1585m, 1538m

- **^{1}H-NMR Spectroscopy**

Table **2.5** summarizes the ^{1}H NMR signals assigned to pyrazoles **2.3a-n**. The signal of the C-H protons of the pyrazole unit appears between δ_H 7.54-7.89 ppm and the amine protons as a broad singlet between δ_H 6.52-7.72 ppm. The OH group of compound **2.3f** appears as a broad signal with a chemical shift of 12.57 ppm. The singlet at δ_H 4.81 ppm corresponds to the CH_2 of compound **2.3m**. In Figure **2.3**, the ^{1}H NMR spectrum of pyrazole **2.3a**, with some key signals assigned, was used to illustrate the spectrum of pyrazoles **2.3**.

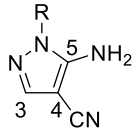
- **^{13}C-NMR Spectroscopy**

Table **2.6** summarizes the ^{13}C NMR signals assigned to pyrazoles **2.3a-n**. In the ^{13}C NMR spectra of **2.3a-n**, carbon C-3 is usually visible around δ_C 140.79-144.03 ppm. In the HMBC correlation spectrum it is possible to see the interaction of H-3 with C-5 and C-4. The presence of the CN group was confirmed by the signal at δ_C 113.77-115.15 ppm. The carbonyl group of compounds **2.3f**, **2.3l-n** appear at δ_C 166.65, 150.77, 150.36 and 167.47 ppm, respectively. Figure **2.4** shows the ^{13}C NMR spectrum of **2.3a** with key signals assigned.

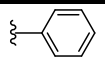
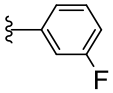
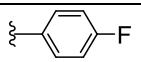
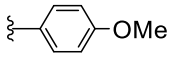
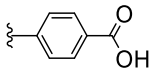
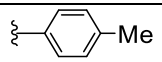
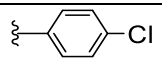
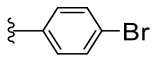
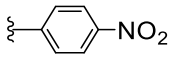
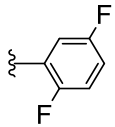
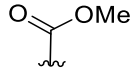


2.3

Table 2.5: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) of the pyrazole **2.3a-n**



2.3

Comp.	R	NH_2	C-H	R
2.3a		6.66 (brs, 2H)	7.78 (s, 1H)	7.52 (td, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.4 Hz), 7.50 (d, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz), 7.41 (td, 1H, $H_{4'}$, J2.0 Hz, 8.4 Hz)
2.3b		6.73 (brs, 2H)	7.78 (s, 1H)	7.54-7.57 (m, 1H, $H_{4'}$), 7.49 (td, 1H, $H_{6'}$, J1.2 Hz, 8.4 Hz), 7.43 (td, 1H, $H_{3'}$, J1.2 Hz, 8.4 Hz), 7.35 (td, 1H, $H_{5'}$, J1.2 Hz, 8.4 Hz)
2.3c		6.82 (brs, 2H)	7.80 (s, 1H)	7.56 (dd, 1H, $H_{5'}$, J2.4 Hz, 8.8 Hz), 7.35-7.38 (m, 1H, $H_{6'}$), 7.35 (s, 1H, $H_{2'}$), 7.30 (td, 1H, $H_{4'}$, J2.4 Hz, 8.8 Hz)
2.3d		6.68 (brs, 2H)	7.76 (s, 1H)	7.52 (dd, 2H, $H_{2'}$ + $H_{6'}$, J5.2 Hz, 8.8 Hz), 7.35 (t, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.3e		6.52 (brs, 2H)	7.72 (s, 1H)	7.37 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.8 Hz), 7.05 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.8 Hz), 3.79 (s, 3H, OCH_3)
2.3f		6.86 (brs, 2H)	7.83 (s, 1H)	12.57 (brs, 1H, OH), 8.06 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.4 Hz, 8.8 Hz), 7.65 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.4 Hz, 8.8 Hz)
2.3g		6.59 (brs 2H)	7.74 (s, 1H)	7.35 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.4 Hz), 7.31 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 2.35 (s, 3H, CH_3)
2.3h		6.75 (brs, 2H)	7.79 (s, 1H)	7.57 (dt, 2H, $H_{3'}$ + $H_{5'}$, J2.4 Hz, 8.8 Hz), 7.51 (dt, 2H, $H_{2'}$ + $H_{6'}$, J2.4 Hz, 8.8 Hz)
2.3i		6.76 (brs, 2H)	7.79 (s, 1H)	7.70 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz), 7.45 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz)
2.3j		7.04 (brs, 2H)	7.89 (s, 1H)	8.35 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.8 Hz), 7.83 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.8 Hz)
2.3k		6.86 (brs, 2H)	7.80 (s, 1H)	7.41-7.53 (m, 3H, $H_{3'}$, $H_{4'}$, $H_{6'}$)
2.3l		7.72 (brs, 2H)	7.82 (s, 1H)	3.93 (s, 3H, OCH_3)

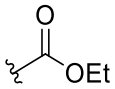
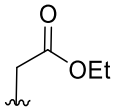
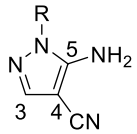
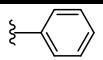
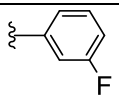
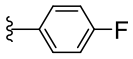
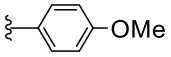
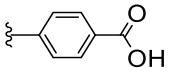
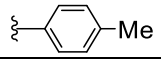
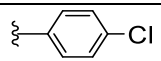
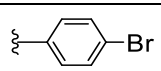
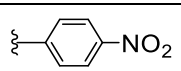
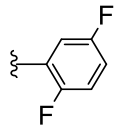
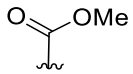
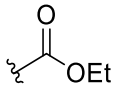
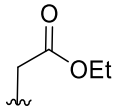
2.3m		7.71 (brs, 2H)	7.80 (s, 1H)	4.39 (q, 2H, J7.2 Hz, OCH_2), 1.31 (t, 3H, J7.2 Hz, CH_3)
2.3n		6.69 (brs, 2H)	7.54 (s, 1H)	4.81 (s, 2H, CH_2), 4.12 (q, 2H, J7.2 Hz, OCH_2), 1.19 (t, 3H, J7.2 Hz, CH_3)

Table 2.6: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for pyrazoles **2.3a-n**



2.3

Comp.	R	C_3	C_4	C_5	CN	R
2.3a		141.70	73.40	151.22	114.79	137.47 ($C_{1'}$), 129.48 ($C_{3'}$ + $C_{5'}$), 127.90 ($C_{4'}$), 124.15 ($C_{2'}$ + $C_{6'}$)
2.3b		142.19	72.05	152.67	114.79	156.80 (d, $C_{2'}$, J249.70 Hz), 131.44 (d, $C_{4'}$, J7.80 Hz), 129.46 (s, $C_{6'}$), 125.26 (d, $C_{5'}$, J3.60 Hz), 124.66 (d, $C_{1'}$, J12.40 Hz), 116.99 (d, $C_{3'}$, J19.20 Hz)
2.3c		142.10	73.62	151.48	114.63	162.10 (d, $C_{3'}$, J244.00 Hz), 138.83 (d, $C_{1'}$, J10.00 Hz), 131.21 (d, $C_{5'}$, J9.00 Hz), 120.17 (d, $C_{6'}$, J3.00 Hz), 114.74 (d, $C_{4'}$, J21.00 Hz), 111.50 (d, $C_{2'}$, J24.00 Hz)
2.3d		141.68	73.19	151.44	114.76	161.22 ($C_{4'}$, J244.00 Hz), 133.77 ($C_{1'}$, J3.00 Hz), 126.88 ($C_{2'}$ + $C_{6'}$, J9.00 Hz), 116.40 ($C_{3'}$ + $C_{5'}$, J23.00 Hz)
2.3e		141.23	72.93	151.24	114.92	158.82 ($C_{4'}$), 130.24 ($C_{1'}$), 126.18 ($C_{2'}$ + $C_{6'}$), 114.59 ($C_{3'}$ + $C_{5'}$), 55.48 (OCH_3)
2.3f		142.40	73.94	151.57	114.61	166.65 (CO), 141.03 ($C_{1'}$), 130.63 ($C_{3'}$ + $C_{5'}$), 129.75 ($C_{4'}$), 123.54 ($C_{2'}$ + $C_{6'}$)
2.3g		141.47	73.25	151.15	114.85	137.48 ($C_{4'}$), 134.99 ($C_{1'}$), 129.88 ($C_{3'}$ + $C_{5'}$), 124.14 ($C_{2'}$ + $C_{6'}$), 20.63 (CH_3)
2.3h		141.99	73.48	151.43	114.66	136.30 ($C_{1'}$), 132.23 ($C_{4'}$), 129.44 ($C_{3'}$ + $C_{5'}$), 126.03 ($C_{2'}$ + $C_{6'}$)
2.3i		142.02	73.52	151.39	114.63	136.76 ($C_{1'}$), 120.63 ($C_{4'}$), 132.36 ($C_{3'}$ + $C_{5'}$), 126.25 ($C_{2'}$ + $C_{6'}$)

2.3j		143.05	74.37	151.97	114.38	145.73 ($C_{4'}$), 142.80 ($C_{1'}$), 124.97 ($C_{3'}$ + $C_{5'}$), 124.19 ($C_{2'}$ + $C_{6'}$)
2.3k		142.50	72.08	152.82	114.68	157.78 (dd, $C_{5'}$, J 3.00 Hz, 242.00 Hz), 153.52 (dd, $C_{2'}$, J 3.00 Hz, 246.00 Hz), 125.37 (d, $C_{1'}$, J 14.00 Hz), 118.28 (dd, $C_{3'}$, J 9.50 Hz, 31.00 Hz), 118.06 (dd, $C_{4'}$, J 8.10 Hz, 32.00 Hz), 116.46 (d, $C_{6'}$, J 26.00 Hz)
2.3l		144.03	72.24	154.82	113.77	150.77 (CO), 54.71 (OCH_3)
2.3m		143.89	72.27	154.89	113.77	150.36 (CO), 64.22 (OCH_2), 13.91 (CH_3)
2.3n		140.79	72.11	152.62	115.15	167.47 (CO), 61.17 (OCH_2), 48.75 (CH_2), 14.05 (CH_3)

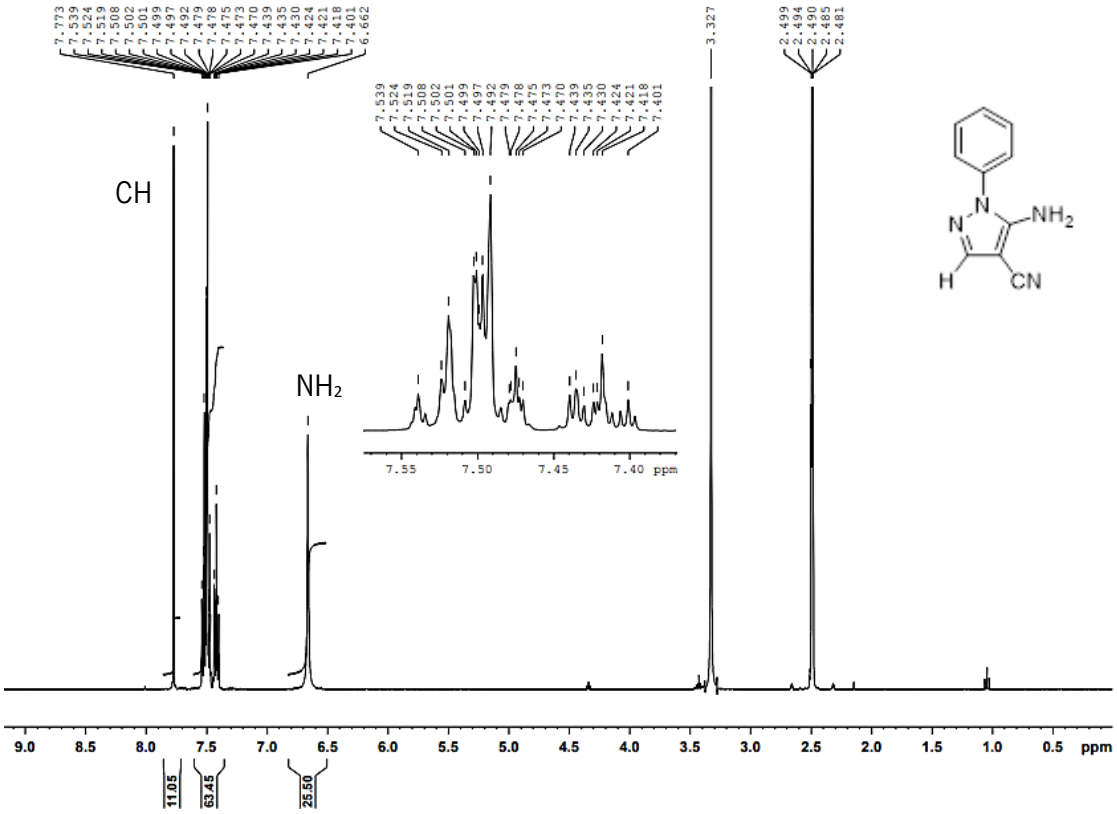


Figure 2.3: ^{1}H NMR spectrum for compound **2.3a** in DMSO-d_6 solution (^{1}H: 400 MHz).

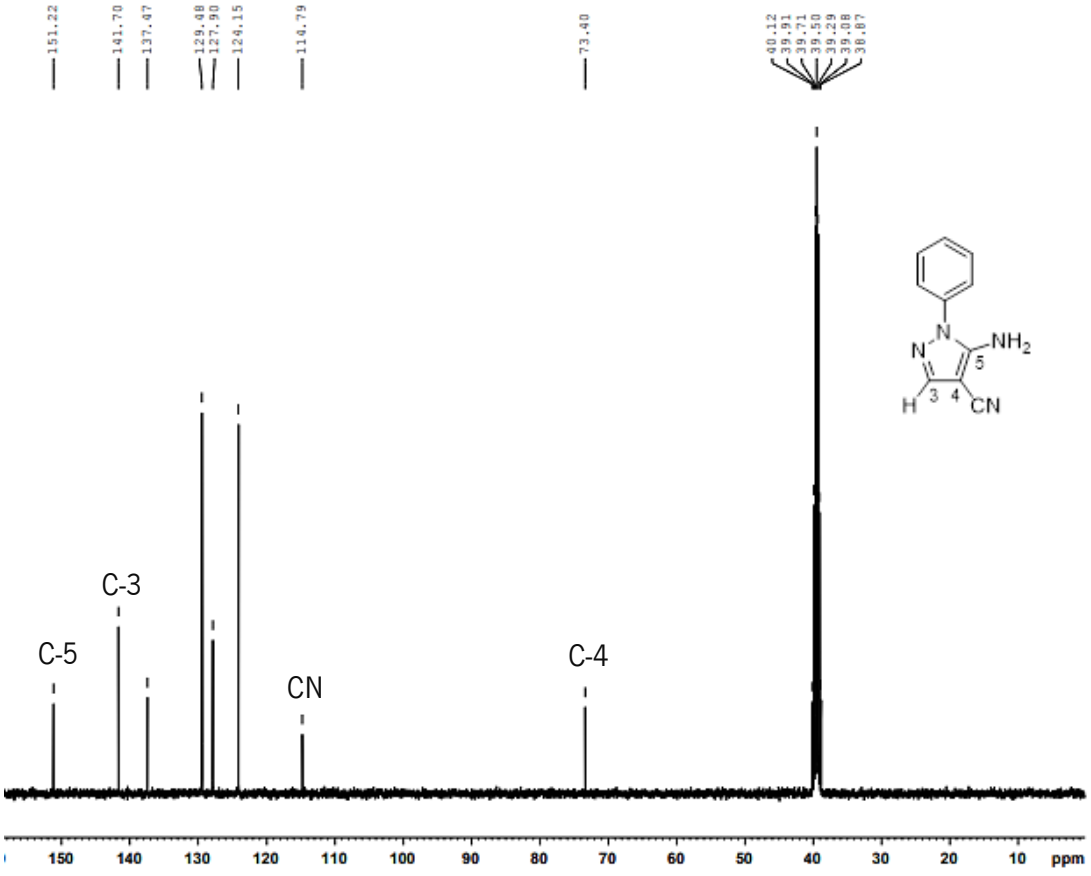
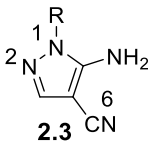


Figure 2.4: ^{13}C NMR spectrum for compound **2.3a** in DMSO-d_6 solution (^{13}C: 100 MHz).

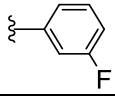
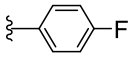
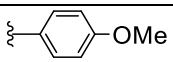
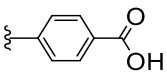
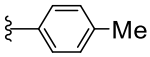
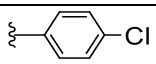
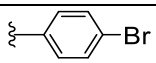
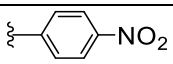
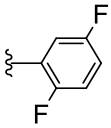
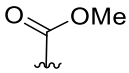
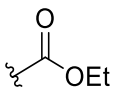
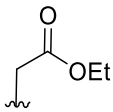
- **^{15}N-NMR Spectroscopy**

In the ^{15}N HMBC correlation spectra, nitrogen atoms N-1 and N-2 were identified around δ_N 177.88-291.72 ppm. The values of the nitrogen atom NH_2 were identified around δ_N 54.80-70.08 ppm. The values of the nitrogen chemical shifts for pyrazoles are summarized in Table **2.7**. Figure **2.5** shows the ^{15}N NMR correlation spectrum of **2.3a** with key signals assigned.

Table 2.7: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) of the pyrazoles **2.3a-n**



2.3

Comp.	R	NH_2	N_1	N_2	N_6	R
2.3a		55.78	193.56	290.18	[a]	–
2.3b		57.18	180.81	291.72	[a]	–
2.3c		56.83	191.59	289.32	[a]	–
2.3d		55.90	191.15	290.31	[a]	–
2.3e		55.32	192.20	291.10	[a]	–
2.3f		57.26	192.66	288.84	[a]	–
2.3g		55.35	193.08	290.31	[a]	–
2.3h		56.25	191.13	289.62	[a]	–
2.3i		56.76	191.21	289.33	[a]	–
2.3j		58.33	191.62	288.35	[a]	369.35 (NO_2)
2.3k		57.81	179.41	290.77	[a]	–
2.3l		70.08	194.60	280.95	[a]	–
2.3m		70.00	194.58	281.01	[a]	–
2.3n		54.80	177.88	289.40	[a]	–

[a] N_6 was never visible in the ^{15}N spectrum.

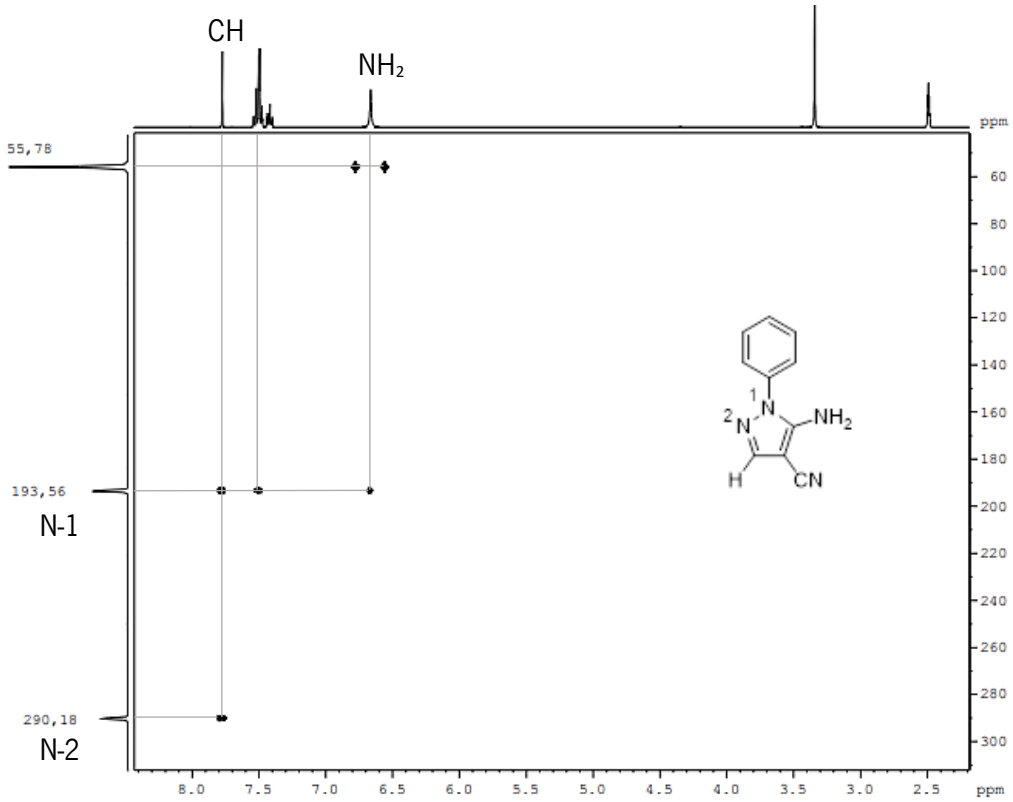
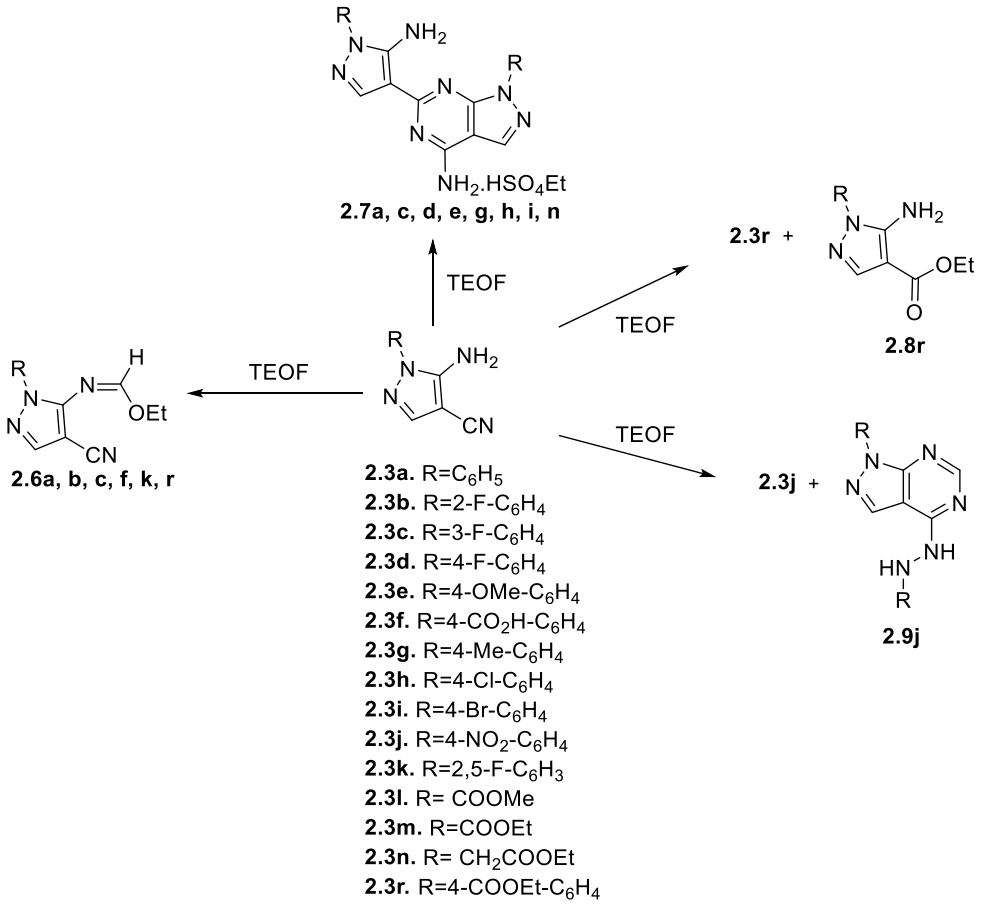


Figure 2.5: ^{15}N HMBC spectrum for compound **2.3a** in DMSO-d_6 solution (^{15}N: 40 MHz).

2.2. Reaction of 5-amino-4-cyanopyrazoles with triethylorthoformate

The *o*-aminonitrile motif in the aromatic ring has been used to prepare fused pyrimidine systems by a simple one-pot reaction involving triethylorthoformate (TEOF).[55] In this work, the substituted 5-amino-4-cyanopyrazoles **2.3** synthesized were used to study their reactivity with TEOF (Scheme **2.2**). Dimeric structures have been previously synthesized in our research group but in a low yield (15-25%).[53] The objective of this work was to optimize the conditions for the formation of dimeric structures and to isolate them in a better yield. An alternative method to their synthesis was also studied involving the initial formation of the imidate function.



Scheme 2.2: Reaction of 5-amino-4-cyanopyrazoles **2.3** with TEOF.

2.2.1. Synthesis of imidate

The reaction of 5-amino-4-cyanopyrazoles **2.3** with TEOF was performed in the absence of solvent and using 3 or 6 molar equivalents of TEOF. Table **2.8** summarizes the experimental conditions used to prepare the compounds **2.6**.

The reaction of compound **2.3a-c**, **2.3k** with 3 equivalents of TEOF, at 150°C (6-16 hours) led to the corresponding compounds **2.6a-c** and **2.6k** in 42-78% yield (entries 1-3, 8).

Pyrazole **2.3f** was reacted with 3 equivalents of TEOF at 150°C for 3 hours, and **2.6f** was isolated in 54% yield (entry 4). When the reaction proceeded for 6 hours, a mixture of **2.6f** and **2.6r**, in a 1:1 molar ratio, was obtained (entry 5). When the reaction proceeded for 15 hours at 150°C, only product **2.6r** was isolated in 46% yield (entry 6). The reaction was repeated with 6 equivalents of TEOF for 3 hours and product **2.6r** was isolated in 47% yield (entry 7). These results indicate that a longer reaction time and/or large amount of TEOF lead to esterification of the carboxyl group.

Table 2.8: Experimental conditions for the reaction of 5-amino-4-cyanopyrazoles **2.3** with TEOF

Entry	Reagents		Experimental conditions	Product (yield)
	2.3.	TEOF		
1	**2.3a.** 0.27 mmol	3 eq.	150°C, 16 h	**2.6a.** 62%
2	**2.3b.** 0.14 mmol	3 eq.	150°C, 6 h	**2.6b.** 78%
3	**2.3c.** 0.26 mmol	3 eq.	150°C, 8 h	**2.6c.** 42%
4	**2.3f.** 0.23 mmol	3 eq.	150°C, 3 h	**2.6f.** 54%
5	**2.3f.** 0.35 mmol	3 eq.	150°C, 6 h	**2.6f** + **2.6r** (1:1) [a)]
6	**2.3f.** 0.35 mmol	3 eq.	150°C, 15 h	**2.6r.** 46%
7	**2.3f.** 0.23 mmol	6 eq.	150°C, 3 h	**2.6r.** 47%
8	**2.3k.** 0.23 mmol	3 eq.	150°C, 6 h	**2.6k.** 63%
9	**2.3l.** 0.34 mmol	3 eq.	150°C, 6 h	Complex mixture [a)]

[a)] By ^{1}H NMR.

The reaction of compound **2.3l** with 3 equivalents of TEOF, at 150°C for 6 hours, led to be a complex mixture (entry 9).

These reactions could not be followed by TLC, so different reaction times were experimented for each pyrazole derivative and only the time that gave the pure product in the highest yield was reported.

2.2.2. Synthesis of dimeric pyrazole derivatives

In our research group, the *o*-aminonitrile motif in the aromatic ring has been used to prepare fused pyrimidine systems by a simple one-pot reaction involving TEOF (method A). The reaction of pyrazoles **2.3** with TEOF was performed in ethanol in the presence of acid (H_2SO_4, TFA, CH_3COOH and HNO_3) (Table **2.9**). The temperatures used, ranged from 50°C to 110°C.

Table 2.9: Experimental conditions for the reaction of pyrazoles **2.3** with TEOF and acid catalysis (method A)

Entry	Reagents		Experimental conditions	Product (yield)
	2.3.	TEOF		
1	**2.3a.** 1.25 mmol	3 eq.	EtOH (9 mL), H_2SO_4 (0.5 eq.), 110°C, 21 h	Complex mixture containing **2.3a** and traces of **2.7a** [a)]
2	**2.3a.** 0.29 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 68 h	F_1= **2.7a.** 31% F_2= Complex mixture [a)]
3	**2.3b.** 0.25 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 16 days	Complex mixture containing **2.7b**, **2.3b** and ammonium salt [a)]
4	**2.3c.** 0.28 mmol	3 eq.	EtOH (24.5 mL), H_2SO_4 (0.5 eq.), 110°C, 54 h	Complex mixture containing **2.7c** and ammonium salt [a)]
5	**2.3c.** 0.24 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 68 h	Complex mixture containing **2.3c** [a)]
6	**2.3c.** 0.27 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 50°C, 17 days	F_1= **2.7c.** 4%

				F_2= Complex mixture containing **2.3c** and ammonium salt [a]
7	**2.3c.** 0.27 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 50°C, 29 days	F_1= **2.7c.** 24% F_2= Complex mixture containing **2.3c** and ammonium salt [a]
8	**2.3c.** 0.20 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (1 eq.), 50°C, 6.5 days	Complex mixture containing **2.7c**, **2.3c** and ammonium salt [a]
9	**2.3d.** 0.18 mmol	3 eq.	EtOH (1.5 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	F_1=**2.7d.** 43% F_2= Complex mixture containing **2.3d** and ammonium salt [a]
10	**2.3e.** 0.24 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 22 h	F_1= **2.7e.** 48% F_2= Complex mixture containing **2.3e** and ammonium salt [a]
11	**2.3f.** 0.23 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	**2.3r.** 69%
12	**2.3f.** 0.24 mmol	3 eq.	EtOH (1 mL), TFA (0.5 eq.), 50°C, 3 days	**2.3r + 2.6r** (1.7:1) [a]
13	**2.3f.** 0.24 mmol	3 eq.	EtOH (1 mL), CH_3COOH (0.5 eq.), 50°C, 3 days	**2.3f.** 24%
14	**2.3f.** 0.24 mmol	3 eq.	EtOH (1 mL), HNO_3 (0.5 eq.), 50°C, 3 days	**2.3f.** 35%
15	**2.3f.** 0.23 mmol	3 eq.	EtOH (11 mL), HNO_3 (0.5 eq.), 110°C, 31 h	**2.3f.** 49%
16	**2.3f.** 0.22 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 100°C, 48 h	**2.3r** + **2.8r** (1:1.6) + ammonium salt [a]
17	**2.3g.** 0.23 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	F_1= **2.7g.** 14% F_2= Complex mixture containing **2.3g** and ammonium salt [a]
18	**2.3h.** 0.23 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	F_1= **2.7h.** 47% F_2= Complex mixture containing **2.3h** and ammonium salt [a]
19	**2.3i.** 0.18 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	F_1= **2.7i.** 22% F_2= Complex mixture containing **2.3i** and ammonium salt [a]
20	**2.3j.** 0.13 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 6 days	**2.3j** + **2.9j** (1:1.8) [a]
21	**2.3j.** 0.13 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 8 days	Complex mixture containing **2.9j** [a]
22	**2.3k.** 0.23 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 68 h	Complex mixture containing traces of **2.3k** and **2.7k** [a]
23	**2.3k.** 0.26 mmol	3 eq.	i) EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 12 days ii) 80°C, 56 h	Complex mixture containing ammonium salt [a]
24	**2.3l.** 0.31 mmol	3 eq.	EtOH (2 mL), H_2SO_4 (0.5 eq.), 50°C, 4 days	**2.3l.** 66%
25	**2.3l.** 0.33 mmol	3 eq.	EtOH (2 mL), H_2SO_4 (0.5 eq.), 50°C, 10.5 days	Complex mixture [a]
26	**2.3m.** 0.28 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 3 days	Complex mixture [a]
27	**2.3n.** 0.26 mmol	3 eq.	EtOH (1 mL), H_2SO_4 (0.5 eq.), 50°C, 4 days	F_1= **2.7n.** 21% F_2= Complex mixture [a]
28	**2.3r.** 0.20 mmol	3 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 5.5 days	**2.3r.** 46%

[a] By ^1H NMR.

The reaction of **2.3a** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 110°C for 21 hours, led to a complex mixture containing **2.3a** and traces of **2.7a** (entry 1, Table **2.9**). Decreasing the temperature to 50°C, led to a beige solid after 68 hours identified as the dimeric structure **2.7a**, isolated in 31% yield (entry 2). The mother liquor was a complex mixture containing **2.3a** and ammonium salt.

Pyrazole **2.3b** was reacted with 3 equivalents of TEOF and 0.5 equivalents of sulfuric acid at 50°C, in ethanol. After 16 days, an oil was obtained which proved to be a complex mixture containing **2.7b**, **2.3b** and ammonium salt (entry 3). The same result was obtained when the reaction was repeated for less time (10 days).

The reaction of **2.3c** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 110°C for 54 hours, led to a complex mixture containing **2.7c** and ammonium salt (entry 4). The use of a higher temperature led to variability in the volume of solvent used throughout the reaction, leading to solvent replenishment over time, making a total of 24.5 mL of ethanol used. Decreasing the temperature to 50°C and performing the reaction for 68 hours led to a complex mixture containing **2.3c**, by ^{1}H NMR (entry 5). Increasing the reaction time to 17 days, led to the dimeric structure **2.7c**, isolated in 4% yield (entry 6). The mother liquor was a complex mixture containing **2.7c** and a large amount of ammonium salt. Increasing the reaction time to 29 days, increased to 24% the reaction yield (entry 7). Once again, the mother liquor remained a complex mixture containing **2.7c** and a large amount of ammonium salt, by ^{1}H NMR. To try to reduce the reaction time, the amount of H_2SO_4 was increased to 1 equivalent (entry 8). After 6.5 days, a solid was isolated which proved to be a complex mixture containing **2.7c**, **2.3c** and ammonium salt.

The reaction of **2.3d** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 50°C for 72 hours, led to the pure product **2.7d** in 43% yield (entry 9). The mother liquor was a complex mixture containing **2.3d** and ammonium salt.

Pyrazole **2.3e** was reacted with 3 equivalents of TEOF and 0.5 equivalents of sulfuric acid at 50°C, in ethanol. After 22 hours, the solid that precipitated from the reaction mixture was the pure product **2.7e**, isolated in 48% yield (entry 10). The mother liquor was a complex mixture containing **2.3e** and ammonium salt.

The reaction of **2.3f** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 50°C for 72 hours, induced the esterification of the carboxyl group, and the product **2.3r** was isolated in 69% yield (entry 11). In an attempt to avoid esterification, the reaction was repeated under the same conditions using different acids such as TFA, CH_3COOH and HNO_3. From the TFA catalyzed reaction an orange solid was isolated which was a mixture of the products **2.3r** + **2.6r** in a 1.7:1 molar ratio (entry 12). With CH_3COOH and HNO_3, no reaction took place and only the reagent **2.3f** was isolated in 24% and 35% yield, respectively (entries 13-14). Using HNO_3 as catalyst, the temperature was increased to 110°C and, after 31 hours, the reagent **2.3f** was isolated in 49% yield (entry 15). The use of a higher temperature led to variability in the volume of solvent used throughout the reaction, leading to solvent replenishment

over time, making a total of 11 mL of ethanol used. In a last attempt to isolate the corresponding dimeric structure, the reaction was carried out with sulfuric acid catalysis at 100°C (entry 16). After 48 hours, a mixture of **2.3r** and **2.8r** in a 1:1.6 molar ratio was isolated and identified by ^{1}H NMR. The elevated temperature and the use of a strong acid, H_2SO_4, led to hydrolysis of the cyano group, forming compound **2.8r**.

Pyrazole **2.3g** was reacted with 3 equivalents of TEOF and 0.5 equivalents of sulfuric acid at 50°C, in ethanol (entry 17). After 72 hours, the product **2.7g** was isolated in 14% yield. The mother liquor was a complex mixture containing **2.3g** and ammonium salt.

Pyrazole **2.3h** was reacted with 3 equivalents of TEOF and 0.5 equivalents of sulfuric acid at 50°C, in ethanol (entry 18). After 72 hours, the product **2.7h** was isolated in 47% yield. The mother liquor was a complex mixture containing **2.3h** and ammonium salt.

The reaction of **2.3i** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 50°C for 72 hours, led to a pure product **2.7i** in 22% yield (entry 19). The mother liquor was a complex mixture containing **2.3i** and ammonium salt.

Pyrazole **2.3j** was reacted with 3 equivalents of TEOF and 0.5 equivalents of H_2SO_4 at 50°C. After 6 days, TLC showed the absence of starting material, and a mixture of **2.3j** and **2.9j** in 1:1.8 molar ratio (by ^{1}H NMR) was obtained (entry 20). The reaction was repeated for a total of 8 days and a complex mixture containing **2.9j** was isolated (entry 21). A possible explanation for the formation of the product **2.9j** was the presence of (4-nitrophenyl)hydrazine **2.2j** in the reaction mixture. However, by ^{1}H NMR, pyrazole **2.3j** was pure without any signals of the presence of **2.2j**. As the nitro group is electron withdrawing and the reaction is performed in the presence of acid, the opening of the pyrazole ring may have occurred, generating (4-nitrophenyl)hydrazine **2.2j**. Thus, **2.2j** becomes available in the reaction medium, leading to the formation of **2.9j**.

Pyrazole **2.3k** was reacted with 3 equivalents of TEOF and 0.5 equivalents of H_2SO_4 at 50°C (entry 22). After 68 hours, a complex reaction mixture was formed, containing traces of **2.3k** and **2.7k**, by ^{1}H NMR. The reaction was repeated for 12 days at 50°C and followed by ^{1}H NMR (entry 23). At the end of this time, the reaction mixture still had a large amount of starting material **2.3k** and ammonium salt and a small amount of **2.7k**. The temperature was raised to 80°C for 56 hours, but the solid isolated was identified as a complex mixture containing ammonium salt.

Pyrazole **2.3l** was reacted with 3 equivalents of TEOF and 0.5 equivalents of H_2SO_4 at 50°C for 4 days (entry 24) and 10.5 days (entry 25). After 4 days, the starting material **2.3l** was recovered in 66% yield. In the mother liquor, a complex mixture containing ammonium salt was identified. After 10.5 days,

the ^{1}H NMR showed a complex mixture where the signals of the reagent could not be identified.

Pyrazole **2.3m** was reacted with 3 equivalents of TEOF and 0.5 equivalents of sulfuric acid at 50°C, in ethanol (entry 26). After 3 days, the solid that precipitated from the reaction mixture was isolated and shown to be a complex mixture.

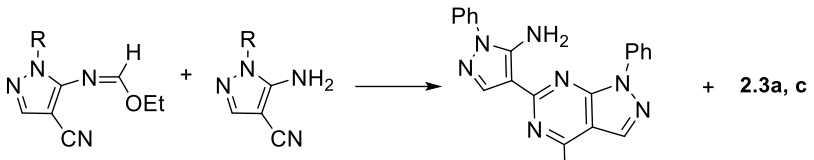
The reaction of **2.3n** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 50°C for 4 days, led to **2.7n** in 21% yield (entry 27). The mother liquor proved to be a complex mixture containing **2.7n** and a large amount of ammonium salt, by ^{1}H NMR.

The reaction of **2.3r** with 3 equivalents of TEOF and sulfuric acid catalysis (0.5 eq.) at 50°C for 5.5 days, led to the recovery if the starting material **2.3r** in 46% yield (entry 28). In the mother liquor, a complex mixture containing ammonium salt was identified.

The presence of sulfuric acid catalysis causes hydrolysis of the cyano group, releasing the ammonium salt. This competitive process is challenging, because as time passes, more pyrazole **2.3** is hydrolyzed and less pyrazole is available to react through the desired synthetic pathway. Therefore, the yields of the products obtained are very low. Increasing the temperature was also not possibly because it increases the rate of acid-catalyzed hydrolysis. For these reasons, it was decided to abandon this synthetic method.

In order to be able to generate dimeric structures **2.7** in higher yields, an attempt was made to react pyrazole **2.3** with imidate **2.6** under acid catalysis (method B, Table **2.10**).

Table 2.10: Experimental conditions for the reaction of pyrazoles **2.3** with imidate **2.6** and acid catalysis (method B)



Entry	Reagents		Experimental conditions	Product (yield)
	2.6.	2.3.		
1	**2.6a.** 0.23 mmol	**2.3a.** 1 eq.	CH_3COOH (0.4 mL), 118°C, 5h	**2.6a** + **2.3a** (1:1) [a]
2	**2.6a.** 0.19 mmol	**2.3a.** 1 eq.	EtOH (0.5 mL), H_2SO_4 (1 eq.), 50°C, 65 h	**2.7a** + **2.3a** (1:1.2) [a]
3	**2.6c.** 0.15 mmol	**2.3c.** 1 eq.	EtOH (0.5 mL), CH_3COOH (1 eq.), 50°C, 3 days	**2.6c** + **2.3c** (1.1:1) [a]
4	**2.6c.** 0.19 mmol	**2.3c.** 1 eq.	EtOH (0.5 mL), H_2SO_4 (0.5 eq.), 50°C, 28 h	**2.3c.** 90% [a]

[a] By ^{1}H NMR.

Imidate **2.6a** was reacted with 1 equivalent of pyrazole **2.3a** in CH_3COOH at 118°C for 5 hours (entry 1). The resulting solid showed to be a mixture of starting material **2.6a** and **2.3a** in a 1:1 molar ratio. The reaction was repeated in ethanol and H_2SO_4 at 50°C (entry 2). After 65 hours, a mixture of **2.7a** and **2.3a** in a 1:1.2 molar ratio was isolated. The imidate function is very sensitive to nucleophilic attack in the presence of acid. For this reason, it was theorized that, in the presence of sulfuric acid, the imidate function in **2.6** was cleaving, to generate the pyrazole. This is why mostly pyrazole **2.3a** was isolated.

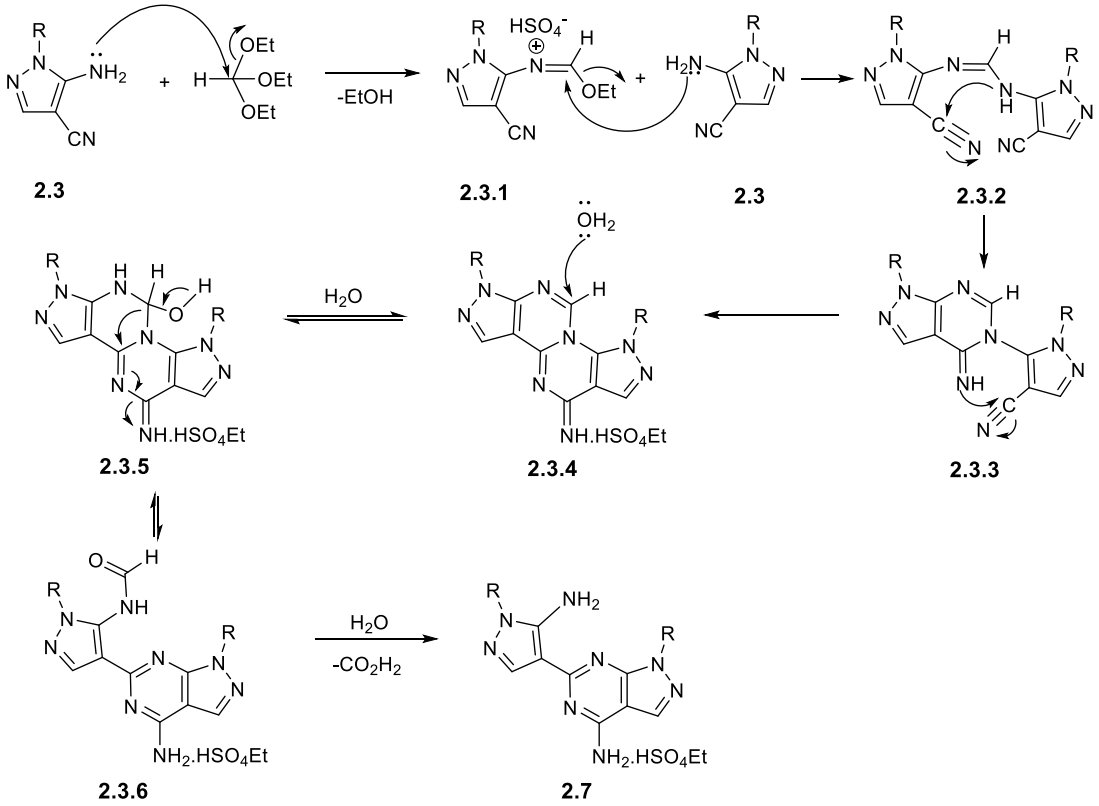
To avoid this process, the reaction was repeated under the same experimental conditions, but using CH_3COOH, as catalyst. After 3 days, the solid that precipitated from the reaction mixture was isolated and identified as a mixture of **2.6c** and **2.3c** in a 1.1:1 molar ratio (entry 3). In a separate experiment, the amount of H_2SO_4 was reduced to 0.5 equivalents (entry 4). After 28 hours only compound **2.3c** was isolated in 90% yield. Thus, sulfuric acid was found to be too strong for these reactions, cleaving the imidate function and regenerating the pyrazole.

In both methods, TLC showed 4 or 5 spots, one of them identified as the starting pyrazole. For this reason, it was not possible to follow the reactions by TLC, because the pyrazole spot was always present, conveying the idea that the reaction was not yet completed. No further attempts were made to perform these reactions due to this fact, together with the difficulty to isolate the pure product, as cleavage of the imidate function could not be avoided in the presence of acid.

The proposed mechanism for the formation of **2.7** is shown in Scheme **2.3**, inspired by the previous studies on the reaction of anthranilonitrile with TEOF.[55] It begins with nucleophilic attack of amine **2.3** to the central carbon of triethylorthoformate, generating an unstable tetrahedral intermediate that evolves to **2.3.1** by elimination of two ethanol molecules. A cascade reaction is initiated by the acid-catalyzed nucleophilic attack of a second molecule of **2.3**, forming the symmetrical amidine **2.3.2**. After two consecutive intramolecular cyclization reactions (**2.3.2** and **2.3.3**), structure **2.3.4** is formed, previously isolated as a salt in analogous structures.[55] The formation of the final compound **2.7** may result from hydrolysis and ring opening of the pyrimidine nucleus in compound **2.3.4**, as illustrated in the scheme.

The proposed mechanism for the formation of compound **2.8r** is shown in Scheme **2.4**. In acid medium, the nitrogen atom of the cyano group of **2.3r** may be protonated, giving rise to intermediate **2.3.7**. Nucleophilic attack by ethanol to the carbon of the cyano group leads to **2.3.8**, that may be protonated in acidic medium (**2.3.9**) accelerating its hydrolysis. This intermediate reacts with the water

present in the medium, forming **2.3.10**, in equilibrium with **2.3.11**, which ultimately loses ammonia and leads to the ester formation in compound **2.8r**.



Scheme 2.3: Proposed mechanism for the formation of pyrazolo[3,4-*d*]pyrimidine **2.7**.

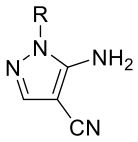
Scheme 2.4: Proposed mechanism for the formation of compound **2.8r**.

2.2.3. Analytical and spectroscopic characterization

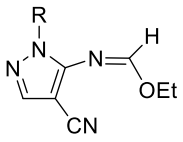
- **Physical and analytical data**

Table **2.11** presents the melting point range and the isolated yields for all the compounds **2.3r**, **2.6** and **2.7**. Compounds herein presented will be later submitted to elemental analysis.

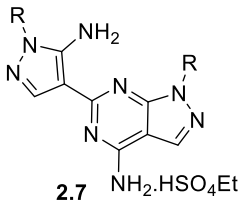
Table 2.11: Physical and analytical data of compounds **2.3r**, **2.6** and **2.7**



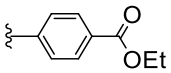
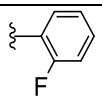
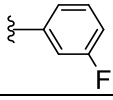
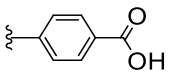
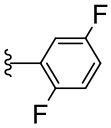
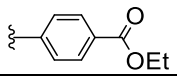
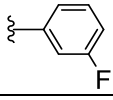
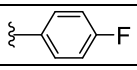
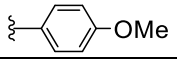
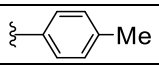
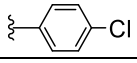
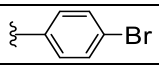
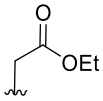
2.3r



2.6



2.7

Comp.	R	Yield (%)	m.p. (°C)	Chemical Formula	Calculated values (%)		
					C	H	N
2.3r		69	198-200	$C_{13}H_{12}N_4O_2$	60.92	4.73	21.87
2.6a		62	[a]	$C_{13}H_{12}N_4$	69.61	5.40	24.98
2.6b		78	60-62	$C_{14}H_{13}FN_4O$	61.76	4.81	20.58
2.6c		42	69-71	$C_{13}H_{11}FN_4O$	60.45	4.30	21.70
2.6f		54	156-158	$C_{14}H_{12}N_4O_2$	62.68	4.52	20.88
2.6k		63	43-45	$C_{13}H_{10}F_2N_4O$	56.52	3.66	20.28
2.6r		47	51-53	$C_{16}H_{16}N_4O_3$	61.52	5.17	17.94
2.7a		31	244-246	$C_{20}H_{16}N_8.H_2SO_4Et$	53.43	4.48	22.66
2.7c		24	225-227	$C_{20}H_{14}F_2N_8.H_2SO_4Et$	49.81	3.80	21.12
2.7d		43	215-217	$C_{20}H_{14}F_2N_8.H_2SO_4Et$	49.81	3.80	21.12
2.7e		48	240-242	$C_{22}H_{20}N_8O_2.H_2SO_4Et$	51.98	4.73	20.21
2.7g		14	212-214	$C_{22}H_{20}N_8.H_2SO_4Et$	55.16	5.02	21.44
2.7h		47	209-211	$C_{20}H_{14}Cl_2N_8.H_2SO_4Et$	46.90	3.58	19.89
2.7i		22	219-221	$C_{20}H_{14}Br_2N_8.H_2SO_4Et$	40.51	3.09	17.18
2.7n		21	170-172	$C_{14}H_{20}N_8.H_2SO_4Et$	42.02	5.09	21.78

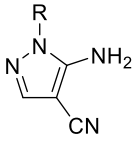
[a] Compound was isolated as an oil.

- **Infrared Spectroscopy**

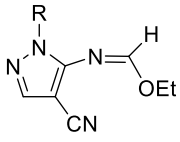
Pyrazoles **2.6** and **2.3r** show an intense band in the 2222-2235 cm^{-1} range attributed to the stretching vibration of the CN group. The weak to medium intensity bands in the 2931-3527 cm^{-1} range correspond to the stretching vibrations of the N-H and C_{sp2}-H bonds. The stretching vibration of the carbonyl groups of compounds **2.3r**, **2.6f** and **2.6r** leads to an intense band in the 1682-1779 cm^{-1} range.

As expected, for compounds **2.7** the absence of the band corresponding to the stretching vibration of the cyano group was observed. The stretching vibrations of the C=C and C=N bonds as well as the bending vibration of the N-H bond generate several bands of weak to strong intensity between the 1509 and 1688 cm^{-1}. The stretching vibration of the carbonyl groups of compound **2.7n** show a medium band at 1753 and 1716 cm^{-1} (Table **2.12**).

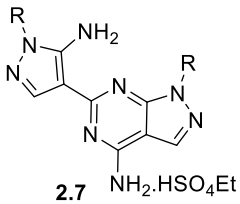
Table 2.12: IR spectroscopic data (FTIR-ATR) of compounds **2.3r**, **2.6** and **2.7**



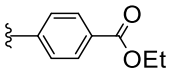
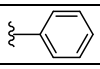
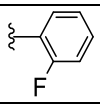
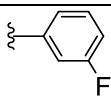
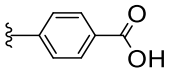
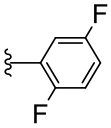
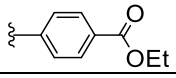
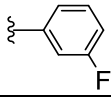
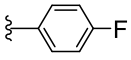
2.3r

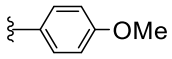
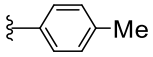
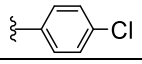
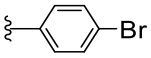
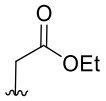


2.6



2.7

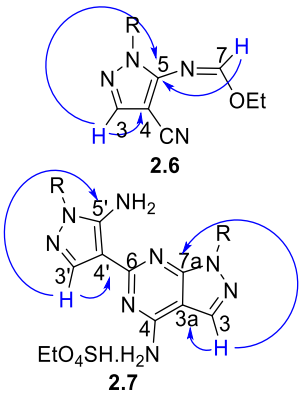
Comp.	R	4000-2700	CN	CO	1700-1500
2.3r		3462w, 3330m, 3238w	2235i	1779i	1650i, 1568m, 1538i, 1507m
2.6a		2931w	2222i	–	1593m, 1549m
2.6b		2985w	2226i	–	1626i, 1539m, 1510i
2.6c		3139l, 2989l	2225i	–	1623i, 1611i, 1543m
2.6e		3527l, 3114l, 2982l	2226i	1682i	1622i, 1603i, 1541m, 1513m
2.6k		3116m, 3074w, 3004w, 2952w	2222i	–	1623i,1537m, 1516i
2.6r		3083l, 2985m, 2960l	2225i	1705i	1623i, 1604i, 1541i, 1512m
2.7a		3333l, 3115l	–	–	1672i, 1620m, 1596m, 1538i, 1509w
2.7c		3379w, 3297w, 3124m	–	–	1673i, 1615m, 1591i, 1539m, 1511w
2.7d		3363l, 3116w	–	–	1672i, 1626w, 1594i, 1540m, 1510i

2.7e		3458l, 3334l	—	—	1684i, 1627i, 1601i, 1561w, 1521i
2.7g		3410w, 3320l, 2981w	—	—	1672i, 1632m, 1598i, 1538w, 1516i
2.7h		3313l, 3074l	—	—	1669i, 1633m, 1599i, 1538i
2.7i		3367w, 3311w, 3116m	—	—	1672i, 1635m, 1602i, 1591w, 1538i, 1514m
2.7n		3436m, 3337m, 3066l	—	1753m, 1716m	1688i, 1633w, 1602m, 1549i, 1528m

- **^{1}H-NMR Spectroscopy**

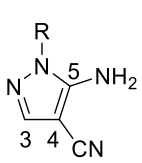
Table **2.13** and Table **2.14** summarize the ^{1}H NMR signals assigned to compounds **2.3r**, **2.6**, **2.7**, **2.8r** and **2.9j**. For compounds **2.6** the signal for H-3 and H-7 appears as a singlet between δ_H 8.16-8.22 ppm and δ_H 8.53-8.57 ppm, respectively. For compounds **2.7** the signal for the C-H proton of the pyrazole unit (H-3') appears between δ_H 7.95-8.20 ppm and for the fused pyrazole ring (H-3) at δ_H 8.32-8.51 ppm. The amine protons lead to a broad singlet between δ_H 8.14-9.68 ppm. For compound **2.9j** the signal for H-3 and H-6 appears as a singlet at δ_H 8.82 ppm and δ_H 8.99 ppm, respectively. In Figure **2.6** and Figure **2.7**, an example of the ^{1}H NMR spectrum of compounds **2.6c** and **2.7n** is shown, with some key signals assigned.

- **^{13}C-NMR Spectroscopy**

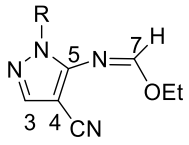


For compounds **2.6** the two signals around δ_C 141.98-143.08 ppm and δ_C 162.30-162.72 ppm were assigned to carbons C-3 and C-7, respectively. The HMBC correlation spectra confirms the suggested structure, where proton H-7 correlates with C-5 and with the ethyl group and also proton H-3 correlates with C-4 and C-5. In some cases, it is possible to see the correlation between this proton and the first carbon atom of the R groups, four bonds away. Additionally, the presence of the CN group for compounds **2.3r** and **2.6** was confirmed by the signal at δ_C 113.73-114.54 ppm and the signal assigned to the carbonyl carbon is visible in the region at δ_C 164.98-166.53 ppm. Table **2.15** summarizes the ^{13}C NMR signals assigned to compounds **2.3r**, **2.6** and **2.8r**. Figure **2.8** shows the ^{13}C NMR spectrum of **2.6c** with key signals assigned. In the ^{13}C NMR spectra of dimeric structures **2.7** (Table **2.16**) carbons C-3 and C-3' are usually visible around δ_C 135.13-139.85 ppm. In the HMBC correlation spectrum it is possible to see the interaction of H-3 or H3' with C-7a and C-3a or C-5'and C-4', respectively. Figure **2.9** shows the ^{13}C NMR spectrum of **2.7n** with key signals assigned.

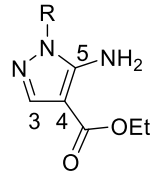
Table 2.13: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for compounds **2.3r**, **2.6** and **2.8r**



2.3r



2.6



2.8r

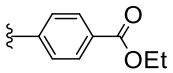
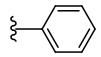
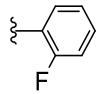
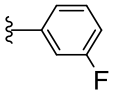
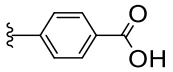
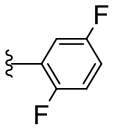
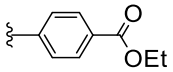
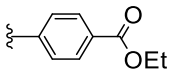
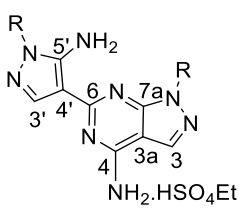
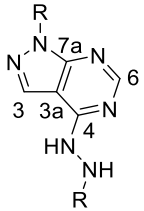
Comp.	R	NH_2	C-H	R	OEt
2.3r		6.88 (brs, 2H)	7.84 (s, 1H, H_3)	8.07 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz), 7.68 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 4.33 (q, 2H, J7.2 Hz, OCH_2), 1.33 (t, 3H, J7.2 Hz, CH_3)	—
2.6a		—	8.53 (s, 1H, H_7) 8.16 (s, 1H, H_3)	7.62 (dt, 2H, $H_{2'}$ + $H_{6'}$, J1.6 Hz, 8.8 Hz), 7.50 (tt, 2H, $H_{3'}$ + $H_{5'}$, J1.6 Hz, 8.8 Hz), 7.41 (tt, 1H, $H_{4'}$, J1.6 Hz, 8.8 Hz)	4.29 (q, 2H, J7.2 Hz, OCH_2), 1.29 (t, 3H, J7.2 Hz, CH_3)
2.6b		—	8.56 (s, 1H, H_7) 8.20 (s, 1H, H_3)	7.54-7.59 (m, 1H, $H_{4'}$ + $H_{6'}$), 7.46 (td, 1H, $H_{3'}$, J1.2 Hz, 8.4 Hz), 7.37 (td, 1H, $H_{5'}$, J1.2 Hz, 8.4 Hz)	4.15 (q, 2H, J7.2 Hz, OCH_2), 1.20 (t, 3H, J7.2 Hz, CH_3)
2.6c		—	8.53 (s, 1H, H_7) 8.19 (s, 1H, H_3)	7.51-7.59 (m, 3H, $H_{2'}$ + $H_{5'}$ + $H_{6'}$), 7.28 (td, 1H, $H_{4'}$, J1.2 Hz, 8.8 Hz)	4.32 (q, 2H, J7.2 Hz, OCH_2), 1.29 (t, 3H, J7.2 Hz, CH_3)
2.6f		—	8.56 (s, 1H, H_7) 8.22 (s, 1H, H_3)	12.93 (brs, 1H, OH), 8.06 (dd, 2H, $H_{3'}$ + $H_{5'}$, J1.6 Hz, 7.2 Hz), 7.80 (dd, 2H, $H_{2'}$ + $H_{6'}$, J1.6 Hz, 7.2 Hz)	4.33 (q, 2H, J7.2 Hz, OCH_2), 1.30 (t, 3H, J7.2 Hz, CH_3)
2.6k		—	8.56 (s, 1H, H_7) 8.22 (s, 1H, H_3)	7.44-7.58 (m, 3H, $H_{3'}$ + $H_{4'}$ + $H_{6'}$)	4.18 (q, 2H, J7.2 Hz, OCH_2), 1.20 (t, 3H, J7.2 Hz, CH_3)
2.6r		—	8.57 (s, 1H, H_7) 8.22 (s, 1H, H_3)	8.08 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.6 Hz), 7.85 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.6 Hz), 4.36 (q, 2H, J7.2 Hz, OCH_2), 1.33 (t, 3H, J7.2 Hz, CH_3)	4.31 (q, 2H, J7.2 Hz, OCH_2), 1.29 (t, 3H, J7.2 Hz, CH_3)
2.8r		6.52 (brs, 2H)	7.76 (s, 1H, H_3)	8.08 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz), 7.72 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 4.33 (q, 2H, J7.2 Hz, OCH_2), 1.33 (t, 3H, J7.2 Hz, CH_3)	4.21 (q, 2H, J7.2 Hz, OCH_2), 1.26 (t, 3H, J7.2 Hz, CH_3)

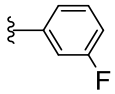
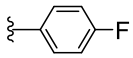
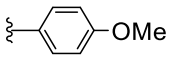
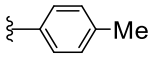
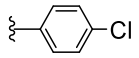
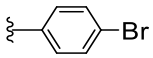
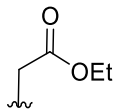
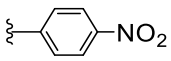
Table 2.14: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for compounds **2.7** and **2.9j**



2.7



2.9j

Comp.	R	N-H [a]	C-H	R
2.7a		9.66 (brs, 1H) 8.33 (brs, 1H)	8.51 (s, 1H, H_3) 8.20 (s, 1H, $H_{3'}$)	7.91 (d, 2H, $H_{o''}$ + $H_{o'''}$, J7.6 Hz), 7.53-7.59 (m, 6H, H_o + $H_{o'}$ + H_m + $H_{m'}$ + $H_{m''}$ + $H_{m'''}$), 7.46 (t, 2H, H_p + $H_{p'}$, J7.6 Hz)
2.7c		8.99 (brs, 1H) 8.16 (brs, 1H)	8.46 (s, 1H, H_3) 8.19 (s, 1H, $H_{3'}$)	7.98 (dd, 1H, $H_{o'}$, J2.4 Hz, 8.4 Hz), 7.91 (dt, 1H, H_o, J2.4 Hz, 8.4 Hz), 7.62 (t, 1H, $H_{m'}$, J8.4 Hz), 7.58 (t, 1H, $H_{m'''}$, J8.4 Hz), 7.49 (dt, 1H, $H_{o'''}$, J2.4 Hz, 8.4 Hz), 7.45 (t, 1H, $H_{o''}$, J8.4 Hz), 7.29 (dd, 1H, $H_{p'}$, J2.4 Hz, 8.4 Hz), 7.23 (dd, 1H, H_p, J2.4 Hz, 8.4 Hz)
2.7d		9.08 (brs, 1H) 8.24 (brs, 1H)	8.42 (s, 1H, H_3) 8.15 (s, 1H, $H_{3'}$)	8.05 (dd, 2H, H_o + $H_{o'}$, J4.8 Hz, 8.8 Hz), 7.62 (dd, 2H, $H_{o''}$ + $H_{o'''}$, J4.8 Hz, 8.8 Hz), 7.41 (t, 2H, $H_{m''}$ + $H_{m'''}$, J8.8 Hz), 7.39 (t, 2H, H_m + $H_{m'}$, J8.8 Hz)
2.7e		9.67 (brs, 1H) 8.34 (brs, 1H)	8.41 (s, 1H, H_3) 8.11 (s, 1H, $H_{3'}$)	7.86 (d, 2H, $H_{o''}$ + $H_{o'''}$, J8.8 Hz), 7.47 (d, 2H, H_o + $H_{o'}$, J8.8 Hz), 7.11 (d, 2H, $H_{m''}$ + $H_{m'''}$, J8.8 Hz), 7.09 (d, 2H, H_m + $H_{m'}$, J8.8 Hz), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3)
2.7g		9.37 (brs, 1H) 8.27 (brs, 1H)	8.45 (s, 1H, H_3) 8.16 (s, 1H, $H_{3'}$)	7.83 (d, 2H, H_o + $H_{o'}$, J8.4 Hz), 7.45 (d, 2H, $H_{o''}$ + $H_{o'''}$, J8.4 Hz), 7.38 (d, 2H, $H_{m''}$ + $H_{m'''}$, J8.4 Hz), 7.35 (d, 2H, H_m + $H_{m'}$, J8.4 Hz), 2.38 (s, 3H, CH_3), 2.37 (s, 3H, CH_3)
2.7h		9.18 (brs, 1H) 8.33 (brs, 1H)	8.44 (s, 1H, H_3) 8.19 (s, 1H, $H_{3'}$)	8.04 (d, 2H, $H_{o''}$ + $H_{o'''}$, J8.8 Hz), 7.63 (s, 2H, H_o + $H_{o'}$), 7.62 (s, 2H, $H_{m''}$ + $H_{m'''}$), 7.61 (s, 2H, H_m + $H_{m'}$)
2.7i		9.00 (brs, 1H) 8.34 (brs, 1H)	8.42 (s, 1H, H_3) 8.18 (s, 1H, $H_{3'}$)	8.04 (d, 2H, $H_{o''}$ + $H_{o'''}$, J8.8 Hz), 7.76 (dd, 2H, $H_{m''}$ + $H_{m'''}$, J3.2 Hz, 8.8 Hz), 7.73 (dd, 2H, H_m + $H_{m'}$, J3.2 Hz, 8.8 Hz), 7.56 (dt, 2H, H_o + $H_{o'}$, J3.2 Hz, 8.8 Hz)
2.7n		9.68 (brs, 1H) 8.14 (brs, 1H)	8.32 (s, 1H, H_3) 7.95 (s, 1H, $H_{3'}$)	5.41 (s, 2H, CH_2), 4.93 (s, 2H, CH_2), 4.15 (q, 4H, J7.2 Hz, OCH_2), 1.20 (t, 6H, J7.2 Hz, CH_3)
2.9j		10.90 (brs, 1H) 10.16 (s, 1H)	8.99 (s, 1H, H_6) 8.82 (s, 1H, H_3)	8.51 (dt, 2H, H_m + $H_{m'}$, J2.0 Hz, 9.2 Hz), 8.39 (dt, 2H, H_o + $H_{o'}$, J2.0 Hz, 9.2 Hz), 8.19 (dt, 2H, $H_{m''}$ + $H_{m'''}$, J2.0 Hz, 9.2 Hz), 7.06 (dt, 2H, $H_{o''}$ + $H_{o'''}$, J2.0 Hz, 9.2 Hz)

[a] The protonated amine linked to C_4 in compound **2.7** cannot be seen in the ^{1}H NMR spectrum as the signal is probably incorporated in the peak for water, always broad and slightly shifted to tower field.

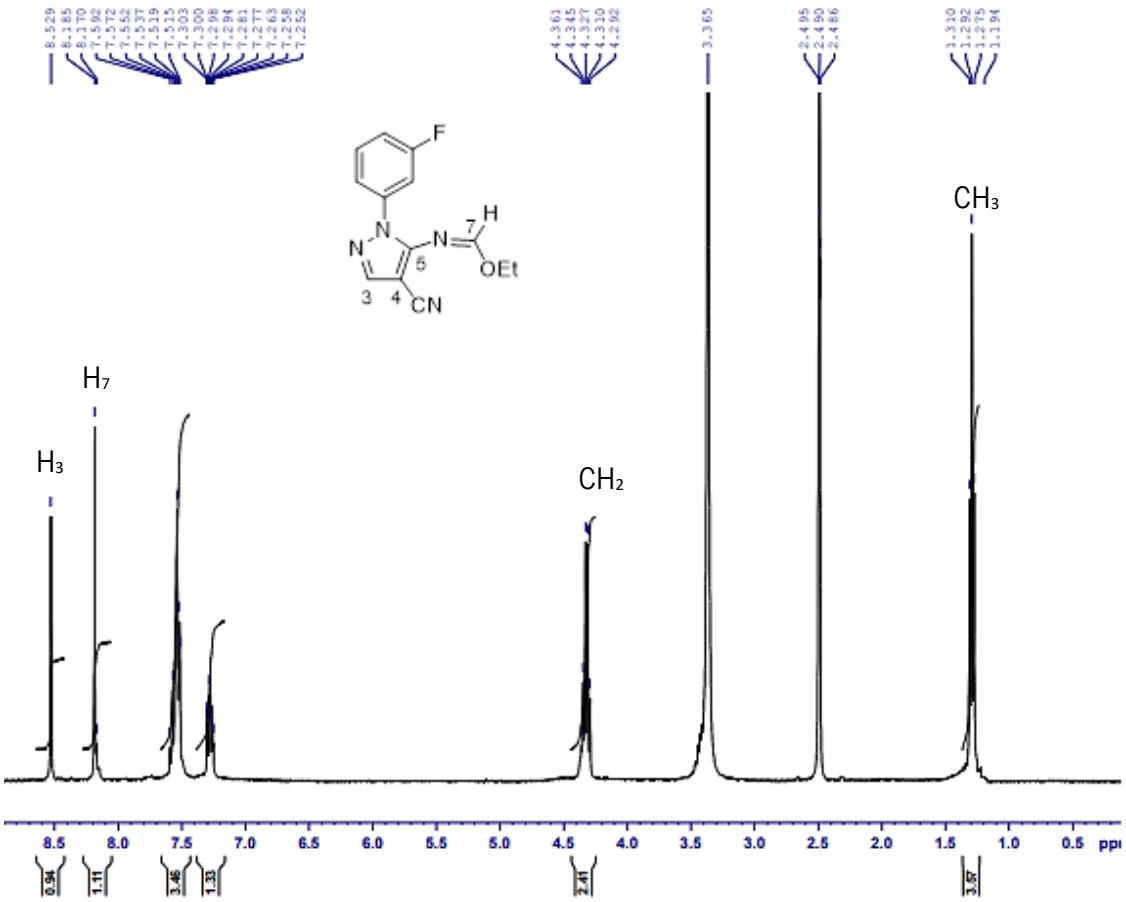


Figure 2.6: ^{1}H NMR spectrum for compound **2.6c** in DMSO-d_6 solution (^{1}H: 400 MHz).

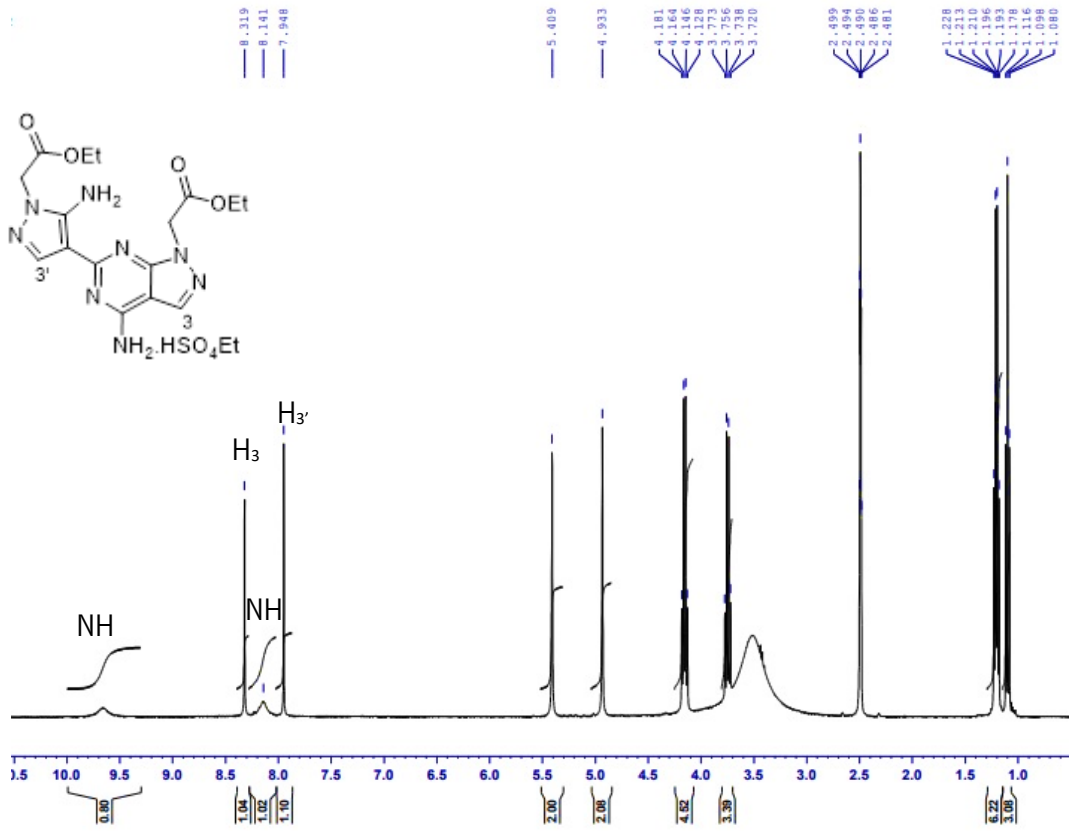
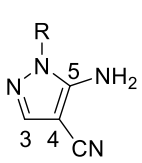
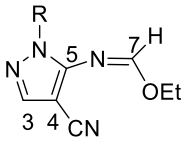


Figure 2.7: ^{1}H NMR spectrum for compound **2.7n** in DMSO-d_6 solution (^{1}H: 400 MHz).

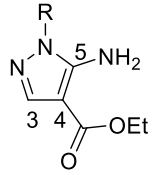
Table 2.15: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for compounds **2.3r**, **2.6** and **2.8r**



2.3r



2.6



2.8r

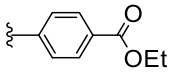
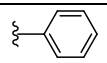
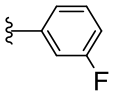
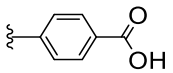
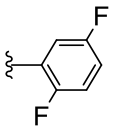
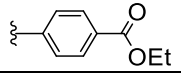
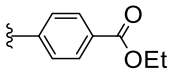
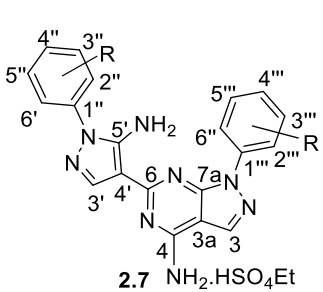
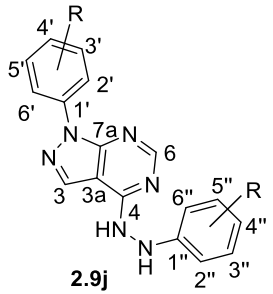
Comp.	R	C_3	C_4	C_5	C_7	CN	R	OEt
2.3r		142.47	73.97	151.58	—	114.54	165.05 (CO), 141.31 (C_1), 130.44 ($C_{3'}$ + $C_{5'}$), 128.58 ($C_{4'}$), 123.59 ($C_{2'}$ + $C_{6'}$), 60.99 (OCH_2), 14.15 (CH_3)	—
2.6a		141.98	81.77	150.44	162.30	114.04	137.63 ($C_{1'}$), 128.98 ($C_{3'}$ + $C_{5'}$), 127.94 ($C_{4'}$), 123.78 ($C_{2'}$ + $C_{6'}$)	63.99 (OCH_2), 14.91 (CH_3)
2.6b		142.76	80.97	151.81	162.50	113.84	155.81 (d, $C_{2'}$, J 250.00 Hz), 131.62 (d, $C_{4'}$, J 8.00 Hz), 128.93 (s, $C_{6'}$), 125.06 (d, $C_{5'}$, J 3.00 Hz), 124.86 (d, $C_{1'}$, J 12.00 Hz), 116.59 (d, $C_{3'}$, J 20.00 Hz)	63.93 (OCH_2), 13.54 (CH_3)
2.6c		142.35	82.13	150.84	162.66	113.92	161.79 (d, $C_{3'}$, J 243.00 Hz), 138.94 (d, $C_{1'}$, J 11.00 Hz), 130.89 (d, $C_{5'}$, J 8.00 Hz), 119.65 (d, $C_{6'}$, J 2.00 Hz), 114.79 (d, $C_{4'}$, J 20.00 Hz), 110.95 (d, $C_{2'}$, J 26.00 Hz)	64.21 (OCH_2), 13.82 (CH_3)
2.6f		142.56	82.29	150.95	162.62	113.91	166.53 (CO), 140.88 ($C_{1'}$), 130.20 ($C_{3'}$ + $C_{5'}$), 129.93 ($C_{4'}$), 123.24 ($C_{2'}$ + $C_{6'}$)	64.23 (OCH_2), 13.81 (CH_3)
2.6k		143.08	81.13	151.99	162.72	113.73	157.52 (dd, $C_{5'}$, J 2.40 Hz, 242.00 Hz), 152.35 (dd, $C_{2'}$, J 2.60 Hz, 248.00 Hz), 125.51 (d, $C_{1'}$, J 26.00 Hz), 118.28 (dd, $C_{3'}$, J 8.00 Hz, 30.00 Hz), 118.06 (dd, $C_{4'}$, J 9.00 Hz, 30.00 Hz), 115.94 (d, $C_{6'}$, J 27.00 Hz)	64.08 (OCH_2), 13.59 (CH_3)
2.6r		142.66	82.40	151.04	162.68	113.90	164.98 (CO), 141.23 ($C_{1'}$), 130.10 ($C_{3'}$ + $C_{5'}$), 128.77 ($C_{4'}$), 123.31 ($C_{2'}$ + $C_{6'}$), 61.04 (OCH_2), 14.14 (CH_3)	64.29 (OCH_2), 13.84 (CH_3)
2.8r		140.97	95.12	150.14	—	—	165.06 and 163.45 (CO), 141.71 (C_1), 130.45 ($C_{3'}$ + $C_{5'}$), 128.21 ($C_{4'}$), 122.99 ($C_{2'}$ + $C_{6'}$), 60.95 and 59.12 (OCH_2), 14.45 and 14.16 (CH_3)	59.12 (OCH_2), 14.45 (CH_3)

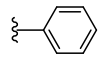
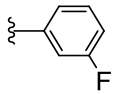
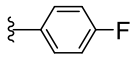
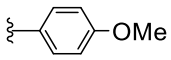
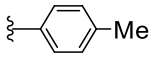
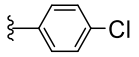
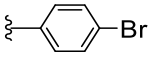
Table 2.16: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for structures **2.7** and **2.9j**

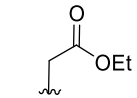
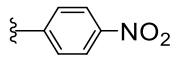


2.7



2.9j

Comp.	R	$C_{3'}$	$C_{4'}$	$C_{5'}$	C_3	C_{3a}	C_{7a}	C_4	C_6	R
2.7a		138.83	96.44	148.76	136.16	97.78	151.48	152.11	153.29	137.68 ($C_{1'''}$), 137.52 ($C_{1''}$), 129.58 ($C_{3''}$ + $C_{5''}$ or $C_{3'''}$ + $C_{5'''}$), 129.50 ($C_{3''}$ + $C_{5''}$ or $C_{3'''}$ + $C_{5'''}$), 127.85 ($C_{4''}$), 127.54 ($C_{4'''}$), 123.70 ($C_{2''}$ + $C_{6''}$), 121.99 ($C_{2'''}$ + $C_{6'''}$)
2.7c		139.68	98.98	148.64	135.89	98.35	152.89	155.06	155.52	162.24 (d, $C_{3''}$, J 242.00 Hz), 162.21 (d, $C_{3'''}$, J 243.00 Hz), 139.74 (d, $C_{1''}$, J 11.00 Hz), 139.36 (d, $C_{1'''}$, J 10.00 Hz), 131.30 (d, $C_{5''}$, J 8.00 Hz), 131.21 (d, $C_{5'''}$, J 8.00 Hz), 119.44 (d, $C_{6''}$, J 3.00 Hz), 117.03 (d, $C_{6'}$, J 3.00 Hz), 114.24 (d, $C_{4''}$, J 21.00 Hz), 113.46 (d, $C_{4'''}$, J 21.00 Hz), 110.71 (d, $C_{2'''}$, J 25.00 Hz), 108.30 (d, $C_{2''}$, J 26.00 Hz)
2.7d		139.36	[a]	148.63	135.54	97.94	[a]	152.36	154.84	161.01 (d, $C_{4'}$, J 244.00 Hz), 160.45 (d, $C_{4''}$, J 243.00 Hz), 134.21 (d, $C_{1''}$, J 2.00 Hz), 134.19 (d, $C_{1'''}$, J 2.00 Hz), 126.17 (d, $C_{2'''}$ + $C_{6''}$, J 8.00 Hz), 123.74 (d, $C_{2''}$ + $C_{6'}$, J 8.00 Hz), 116.32 (d, $C_{3''}$ + $C_{5''}$, J 22.00 Hz), 116.22 (d, $C_{3'''}$ + $C_{5'''}$, J 23.00 Hz)
2.7e		138.65	93.20	148.49	135.13	97.63	151.78	152.89	154.46	158.61 ($C_{4''}$), 158.07 ($C_{4'''}$), 131.13 ($C_{1'''}$), 130.59 ($C_{1''}$), 125.62 ($C_{2''}$ + $C_{6'}$), 123.36 ($C_{2'''}$ + $C_{6''}$), 114.60 ($C_{3''}$ + $C_{5''}$), 114.47 ($C_{3'''}$ + $C_{5'''}$), 55.49 and 55.45 (OCH_3)
2.7g		138.83	[a]	148.55	135.60	97.78	[a]	151.71	153.65	137.26 ($C_{4''}$), 136.76 ($C_{4'''}$), 135.53 ($C_{1''}$), 135.21 ($C_{1'''}$), 129.83 ($C_{3''}$ + $C_{5''}$), 129.84 ($C_{3'''}$ + $C_{5'''}$), 123.64 ($C_{2'''}$ + $C_{6''}$), 121.79 ($C_{2''}$ + $C_{6'}$), 20.66 and 20.63 (CH_3)
2.7h		139.74	98.15	148.72	135.97	98.13	151.49	152.45	154.63	136.70 ($C_{1''}$), 136.99 ($C_{1'''}$), 131.93 ($C_{4''}$), 131.19 ($C_{4'''}$), 129.54 ($C_{3''}$ + $C_{5''}$), 129.45 ($C_{3'''}$ + $C_{5'''}$), 125.41 ($C_{2''}$ + $C_{6'}$), 123.21 ($C_{2'''}$ + $C_{6''}$)
2.7i		139.85	[a]	148.56	135.78	98.19	[a]	152.65	155.07	137.53 ($C_{1''}$), 137.19 ($C_{1'''}$), 132.37 ($C_{3''}$ + $C_{5''}$), 132.27 ($C_{3'''}$ + $C_{5'''}$), 125.53 ($C_{2'''}$ + $C_{6''}$), 123.22 ($C_{2''}$ + $C_{6'}$), 120.10 ($C_{4''}$), 119.19 ($C_{4'''}$)

2.7n		137.65	94.71	150.16	135.32	96.14	151.93	150.95	152.84	167.56 and 167.67 (CO), 61.39, 61.34 and 61.20 (OCH_2), 48.65 and 48.59 (CH_2), 15.11, 14.07 and 14.04 (CH_3)
2.9j		–	–	–	137.85	103.06	149.33	152.87	152.00	146.15 ($C_{4'}$), 141.89 ($C_{1'}$), 125.38 ($C_{3'}$ + $C_{5'}$), 122.30 ($C_{2'}$ + $C_{6'}$), 151.97 ($C_{1''}$), 141.31 ($C_{4''}$), 125.70 ($C_{3''}$ + $C_{5''}$), 113.16 ($C_{2''}$ + $C_{6''}$)

[a] Not visible.

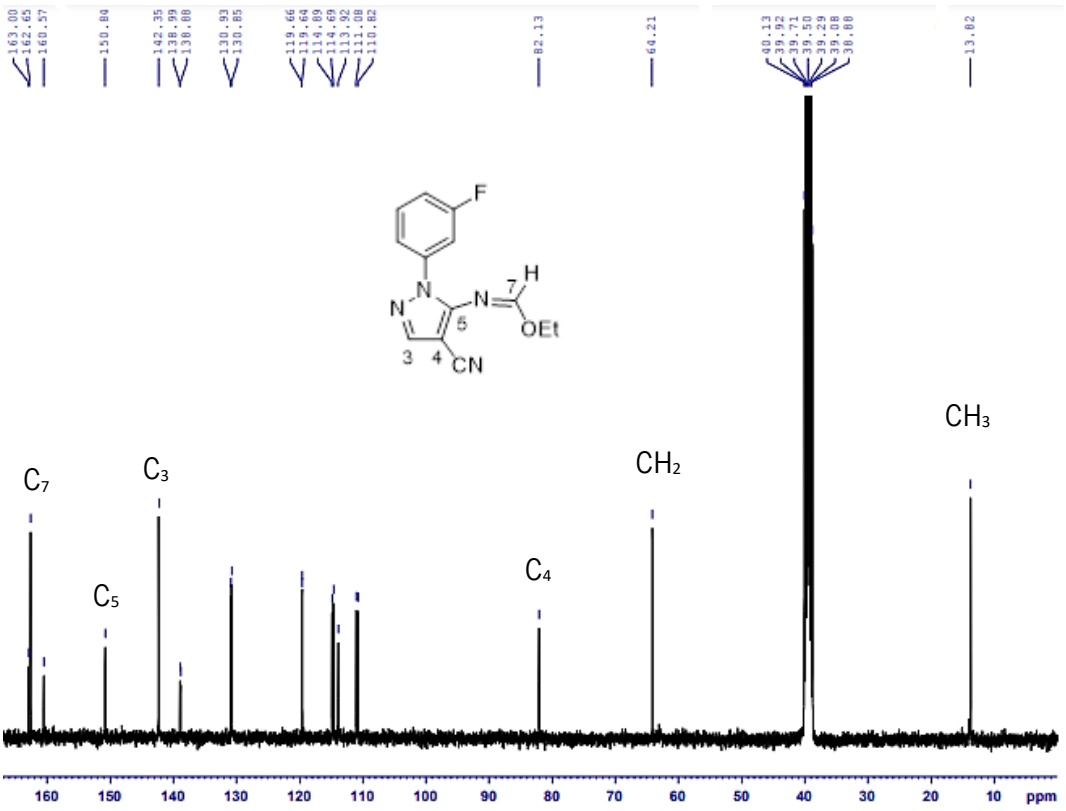


Figure 2.8: ^{13}C NMR spectrum for compound **2.6c** in DMSO-d_6 solution (^{13}C: 100 MHz).

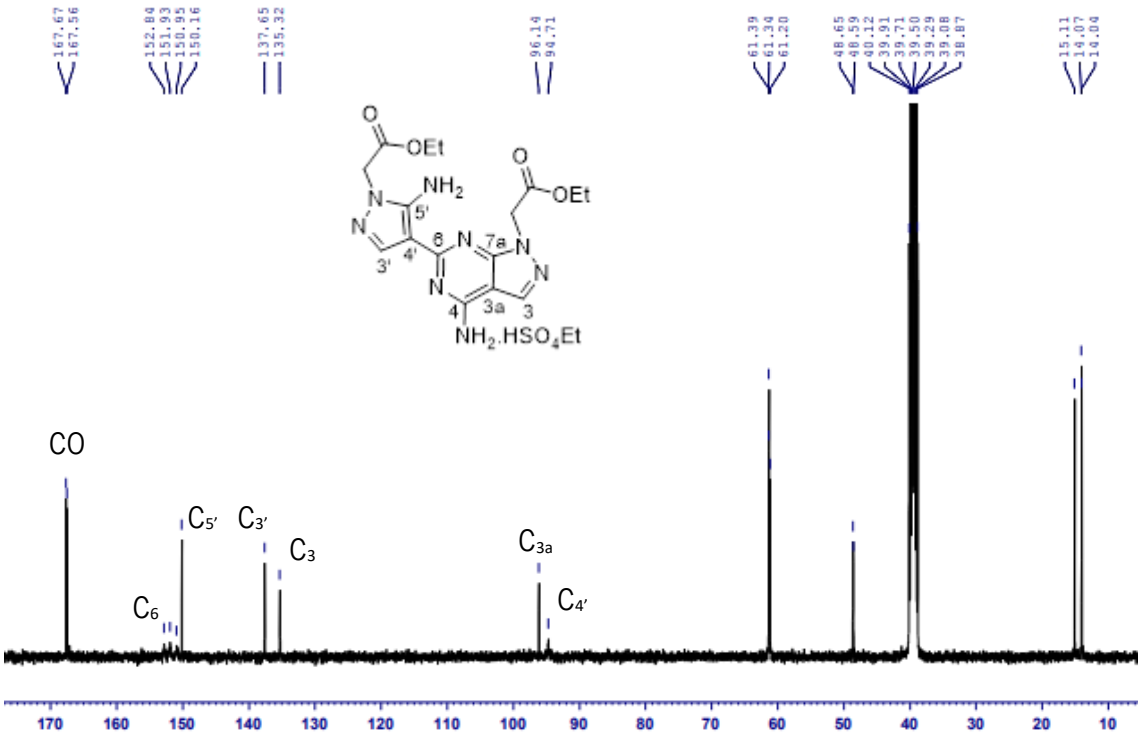
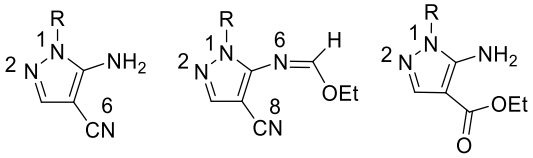


Figure 2.9: ^{13}C NMR spectrum for compound **2.7n** in DMSO-d_6 solution (^{13}C: 100 MHz).

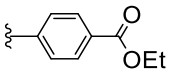
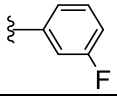
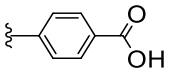
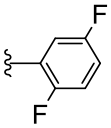
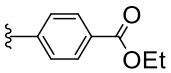
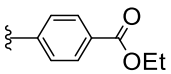
- **^{15}N-NMR Spectroscopy**

In the ^{15}N HMBC correlation spectra, nitrogen atoms N-1 and N-2 of pyrazoles **2.6** were identified around δ_N 197.15-209.71 ppm and δ_N 288.81-302.49 ppm, respectively. The chemical shift values for the nitrogen atom N-6 were identified around δ_N 219.43-220.91 ppm. For dimeric structure **2.7**, nitrogen atoms N-1, N-1', N-2, N-2' and N-6 were identified around δ_N 177.69-312.76 ppm. For compound **2.9j**, nitrogen atoms N-5 and N-7 were identified at δ_N 168.67 and δ_N 229.71 ppm, respectively. The values of the nitrogen chemical shifts for all the compounds are summarized in Table **2.17** and Table **2.18**. Figure **2.10** and Figure **2.11** shows the ^{15}N NMR spectrum of **2.6c** and **2.7n**, with key signals assigned.

Table 2.17: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) for compounds **2.3r**, **2.6** and **2.8r**

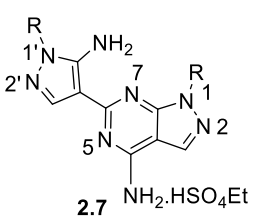


2.3r **2.6** **2.8r**

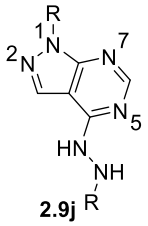
Comp.	R	NH_2	N_1	N_2	N_6	N_8
2.3r		[a]	192.39	288.81	[b]	–
2.6a		–	209.71	299.13	220.91	[b]
2.6b		–	198.70	302.49	219.44	[b]
2.6c		–	207.27	298.60	219.43	[b]
2.6f		–	208.19	298.56	220.04	[b]
2.6k		----	197.15	302.02	[a]	[b]
2.6r		–	207.78	298.11	[a]	[b]
2.8r		[a]	193.11	290.10	–	–

[a] Not visible; [b] N_6 or N_8 was never visible in the ^{15}N correlation spectrum.

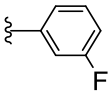
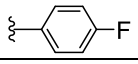
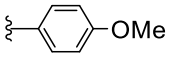
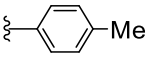
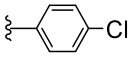
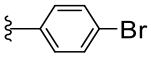
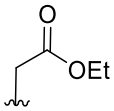
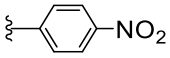
Table 2.18: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) for compounds **2.7** and **2.9j**



2.7



2.9j

Comp.	R	N-H	$N_{1'}$	$N_{2'}$	N_1	N_2	N_5	N_7	R
2.7a		a)	194.09	288.14	200.90	308.37	a)	a)	–
2.7c		a)	193.07	286.36	197.00	304.40	a)	a)	–
2.7d		a)	192.12	287.39	197.28	305.78	a)	a)	–
2.7e		56.44	193.11	288.80	200.41	309.31	a)	a)	–
2.7g		a)	194.06	287.83	199.95	307.41	a)	a)	–
2.7h		a)	192.32	286.73	197.48	305.09	a)	a)	–
2.7i		a)	192.60	286.40	196.82	304.20	a)	a)	–
2.7n		a)	177.69	288.30	186.28	312.76	a)	a)	–
2.9j		104.71	–	–	203.40	312.75	168.67	229.71	368.79 369.96

a) Not visible.

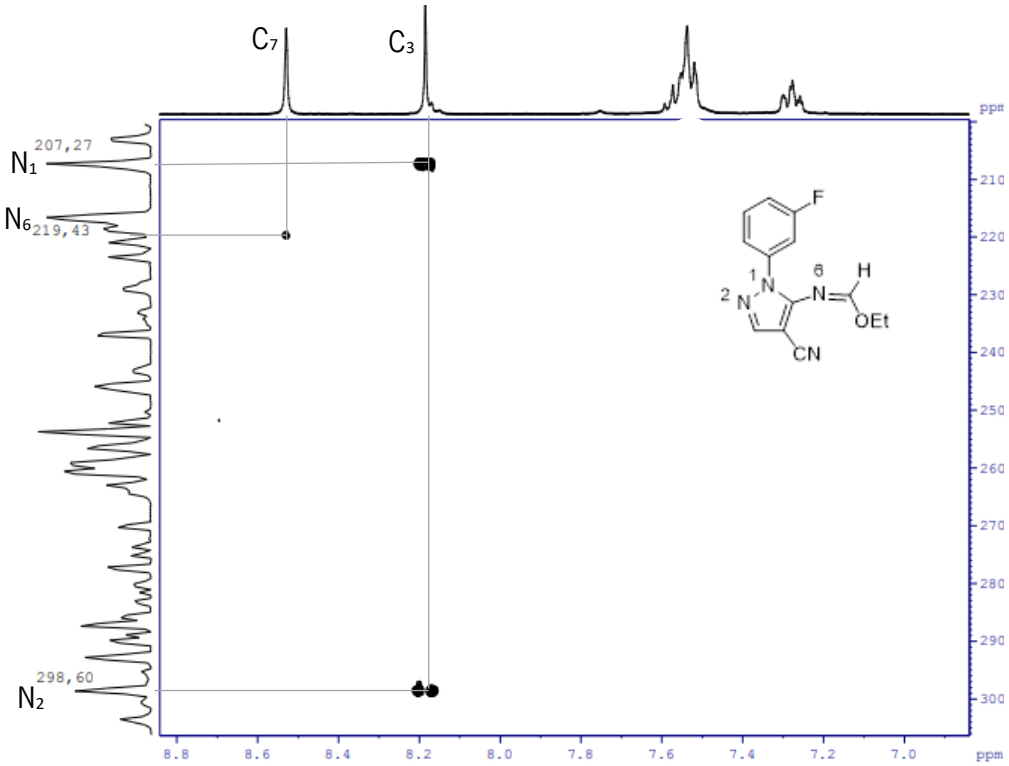


Figure 2.10: ^{15}N HMBC spectrum for compound **2.6c** in DMSO-d_6 solution (^{15}N: 40 MHz).

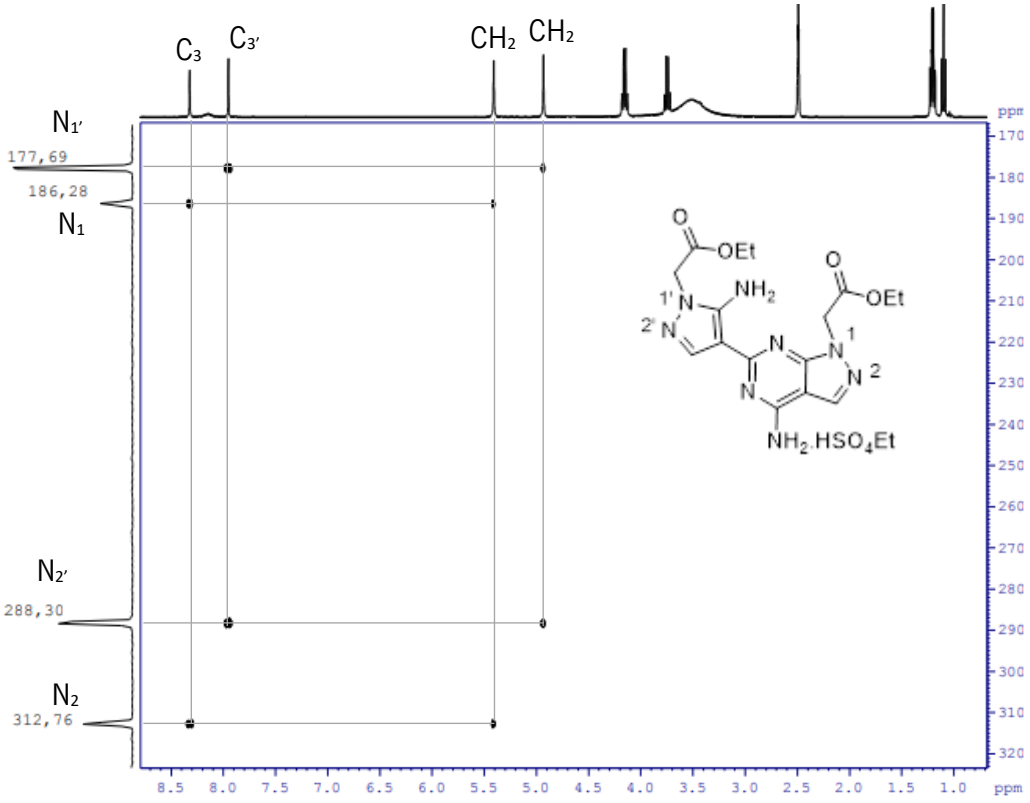


Figure 2.11: ^{15}N HMBC spectrum for compound **2.7n** in DMSO-d_6 solution (^{15}N: 40 MHz).

2.3. Reaction of 5-amino-4-cyanopyrazoles with DMFDEA and DMADEA

The *N*-substituted 5-amino-4-cyanopyrazoles were also reacted with *N*,*N*-dimethylformamide diethyl acetal (DMFDEA) and *N*,*N*-dimethylacetamide dimethyl acetal (DMADEA). This reaction was performed in order to prepare the corresponding amidines and to further react these compounds with aromatic, heteroaromatic and alkyl amines for the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives. The methodology used is based in the successful approach to generate purine derivatives reported in the literature by our research group.[56]

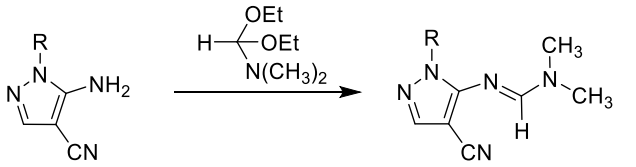
2.3.1. Synthesis of amidines

- **Reaction with DMFDEA**

The reaction of pyrazoles **2.3a-n** with DMFDEA was performed in the absence of solvent or in DCM or DCM/EtOH and using 1.5-5 molar equivalents of DMFDEA. These reactions were followed by ^{1}H NMR and the starting reagent was only absent after 24 and 48 hours for compounds **2.10a-m** and **2.10n**,

respectively. It was not possible to follow the reaction by TLC because the spot of compound **2.10** had the same retention factor (R_f) as that of the starting material **2.3** in all the solvent mixtures used as eluent (n-hexane:ethyl acetate (1:1), ethyl acetate:petroleum ether (3:2 or 3:1), DCM:EtOH (9:1)). Table **2.19** summarizes the experimental conditions to prepare **2.10**.

Table 2.19: Experimental conditions for the reaction of pyrazoles **2.3a-n** with DMFDEA



2.3a. R=C_6H_5
2.3b. R=2-F-C_6H_4
2.3c. R=3-F-C_6H_4
2.3d. R=4-F-C_6H_4
2.3f. R=4-CO_2H-C_6H_4
2.3g. R=4-Me-C_6H_4
2.3h. R=4-Cl-C_6H_4
2.3i. R=4-Br-C_6H_4
2.3j. R=4-NO_2-C_6H_4
2.3k. R=2,5-F-C_6H_3
2.3l. R= COOMe
2.3m. R=COOEt
2.3n. R= CH_2COOEt

2.10a. R=C_6H_5
2.10b. R=2-F-C_6H_4
2.10c. R=3-F-C_6H_4
2.10d. R=4-F-C_6H_4
2.10f. R=4-CO_2H-C_6H_4
2.10g. R=4-Me-C_6H_4
2.10h. R=4-Cl-C_6H_4
2.10i. R=4-Br-C_6H_4
2.10j. R=4-NO_2-C_6H_4
2.10k. R=2,5-F-C_6H_3
2.10l. R= H
2.10m. R=COOEt
2.10n. R= CH_2COOEt
2.10r. R=4-COOEt-C_6H_4

Entry	Reagents		Experimental conditions	Product (yield)
	2.3.	DMFDEA		
1	**2.3a.** 0.43 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10a.** 97% [a]
2	**2.3b.** 0.22 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10b.** 82% [a]
3	**2.3c.** 0.24 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10c.** 95% [a]
4	**2.3d.** 0.25 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10d.** 92% [a]
5	**2.3f.** 0.23 mmol	3 eq.	i) DCM (5.5 mL), r.t., 56.5 h ii) 50°C, 31 h	**2.3f.** 96%
6	**2.3f.** 0.22 mmol	4 eq.	EtOH (100 µl), DCM (0.5 mL), r.t., 24 h	**2.10f.** 96% [a]
7	**2.3f.** 0.22 mmol	5 eq.	Neat, reflux, 2 h	**2.10r.** 80% [a]
8	**2.3g.** 0.27 mmol	1.5 eq.	DCM (1.5 mL), r.t., 24 h	**2.10g.** 90% [a]
9	**2.3h.** 0.23 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10h.** 98% [a]
10	**2.3i.** 0.14 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10i.** 97% [a]
11	**2.3j.** 0.07 mmol	1.5 eq.	DCM (1 mL), r.t., 24 h	**2.10j.** 70% [a]
12	**2.3k.** 0.23 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.10k.** 88% [a]
13	**2.3l.** 0.26 mmol	2 eq.	DCM (0.5 mL), r.t., 24 h	**2.10l.** 35%
14	**2.3m.** 0.23 mmol	2 eq.	DCM (0.5 mL), r.t., 24 h	**2.10m.** 85% [a]
15	**2.3n.** 0.26 mmol	2 eq.	DCM (1.5 mL), r.t., 48 h	**2.10n.** 73% [a]

[a] Isolated as an oil.

The reaction of compounds **2.3a-d**, **2.3g-k** with 1.5 equivalents of DMFDEA and DCM (0.5-1.5 mL), at room temperature (24 hours) led to a respectively compounds **2.10a-d**, **2.10g-k** in 82-98% yield (entries 1-4, 8-12).

Pyrazole **2.3f** reacted with 3 equivalents of DMFDEA in DCM (5.5 mL) at room temperature (entry 5). After 56.5 hours, in the ^{1}H NMR spectrum, signals from the starting reagents were still visible, so it was decided to increase the temperature to 50°C (31 hours). The starting pyrazole **2.3f** was isolated in 96% yield. The reaction was repeated with 100 µL of EtOH to help solubilize the pyrazole and 4 equivalents of DMFDEA (entry 6). After 24 hours product **2.10f** was isolated in 96% yield. The reaction was repeated with 5 equivalents of DMFDEA, without solvent under reflux (2 hours) and the esterified compound **2.10r** was obtained with 80% yield (entry 7).

Pyrazole **2.3l** reacted with 2 equivalents of DMFDEA in DCM (5.5 mL) at room temperature for 24 hours (entry 13). These conditions caused the cleavage of the COOMe group, leaving a proton in that position. Compound **2.10l** was obtained in 35% yield. The mother liquor was an oil which showed to be a complex mixture containing the product **2.10l**, by ^{1}H NMR.

The reaction of compounds **2.3m-n** with 2 equivalents of DMFDEA and DCM (0.5-1.5 mL), at room temperature (24 and 48 hours), led to compounds **2.10m-n** in 85% and 73% yield, respectively (entries 14-15).

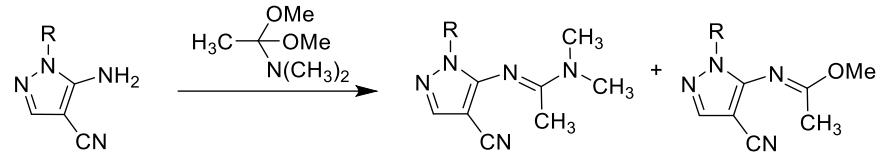
Compounds **2.10** were obtained as an oil after a flash chromatography to remove the excess of DMFDEA used. These oils were used for the next step in the formation of adenine derivatives (Section 2.4).

- **Reaction with DMADEA**

The preparation of pyrazolo[3,4-*d*]pyrimidines where the proton in the 6-position was replaced by a methyl group was considered an important addition to the arsenal of compounds available for biological screening. The success in the use of DMFDEA to incorporate the proton led us to explore the reaction of the analogous DMADEA with 5-amino-4-cyanopyrazoles that would ultimately allow to incorporate the methyl group in the desired position.

The reaction of pyrazoles **2.3a-n** with DMADEA was performed in DCM or DCM and EtOH, using 1.5-4 molar equivalents of DMADEA. These reactions were followed by ^{1}H NMR as the R_f of a spot of the product overlapped with that of the reagent in TLC (n-hexane:ethyl acetate (1:1), ethyl acetate:petroleum ether (3:2 or 3:1), DCM:EtOH (9:1)). Table **2.20** summarizes the experimental conditions to prepare compounds **2.11** and **2.12**.

Table 2.20: Experimental conditions for the reaction of pyrazoles **2.3** with DMADEA



2.3a. R=C_6H_5
2.3c. R=3-F-C_6H_4
2.3d. R=4-F-C_6H_4
2.3f. R=4-CO_2H-C_6H_4
2.3g. R=4-Me-C_6H_4
2.3h. R=4-Cl-C_6H_4
2.3i. R=4-Br-C_6H_4
2.3n. R= CH_2COOEt

2.11a. R=C_6H_5
2.11c. R=3-F-C_6H_4
2.11d. R=4-F-C_6H_4
2.11f. R=4-CO_2H-C_6H_4
2.11g. R=4-Me-C_6H_4
2.11h. R=4-Cl-C_6H_4
2.11i. R=4-Br-C_6H_4
2.11n. R= CH_2COOEt

2.12a. R=C_6H_5
2.12c. R=3-F-C_6H_4
2.12d. R=4-F-C_6H_4
2.12f. R=4-CO_2H-C_6H_4
2.12g. R=4-Me-C_6H_4
2.12h. R=4-Cl-C_6H_4
2.12i. R=4-Br-C_6H_4
2.12n. R= CH_2COOEt

Entry	Reagents		Experimental conditions	Product (yield)
	2.3.	DMADEA		
1	**2.3a.** 0.43 mmol	1.5 eq.	DCM (0.5 mL), r.t., 25 h	**2.11a** + **2.12a** (1.1:1) [a,b]
2	**2.3a.** 0.45 mmol	1.5 eq.	DCM (0.5 mL), 50°C, 25 h	**2.11a** + **2.12a** (1:1.1) [a,b]
3	**2.3c.** 0.26 mmol	1.5 eq.	DCM (0.5 mL), r.t., 25 h	**2.11c** + **2.12c** (1:1.5) [a,b]
4	**2.3d.** 0.19 mmol	1.5 eq.	DCM (0.5 mL), r.t., 48 h	**2.11d** + **2.12d** (1:1.2) [a,b]
5	**2.3f.** 0.23 mmol	4 eq.	EtOH (100 µL), DCM (0.5 mL), r.t., 48 h	**2.11f** + **2.12f** (1:1) [a,b]
6	**2.3g.** 0.25 mmol	2.5 eq.	DCM (1.5 mL), r.t., 48 h	**2.11g** + **2.12g** (1:1.1) [a,b]
7	**2.3h.** 0.23 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.11h** + **2.12h** (1.5:1) [a,b]
8	**2.3i.** 0.19 mmol	1.5 eq.	DCM (0.5 mL), r.t., 24 h	**2.11i** + **2.12i** (1.5:1) [a,b]
9	**2.3n.** 0.27 mmol	2 eq.	DCM (1.5 mL), r.t., 24 h	**2.11n** + **2.12n** (1:1.1) [a,b]

[a] Isolated as an oil; [b] By ^{1}H NMR.

Pyrazole **2.3a** reacted with 1.5 equivalents of DMADEA in DCM at room temperature and 50°C (entries 1-2). In both cases, after 25 hours, the reaction product was a mixture of **2.11a** and **2.12a** in a molar ratio of 1.1:1 and 1:1.1, respectively, by ^{1}H NMR.

The reaction of compounds **2.3c-d**, **2.3h-i** with DMADEA (1.5 eq.) in DCM led to mixtures of **2.11c** + **2.12c** (1:1.5, entry 3), **2.11d** + **2.12d** (1:1.2, entry 4), **2.11h** + **2.12h** (1.5:1, entry 7) and **2.11i** and **2.12i** (1.5:1, entry 8), after 24 or 48 hours at room temperature.

Pyrazole **2.3f** reacted at room temperature with 4 equivalents of DMADEA in DCM and EtOH to improve the solubility of the pyrazole (entry 5). After 48 hours, the resulting oil was a mixture of **2.11f** and **2.12f** in a molar ratio of 1:1, by ^{1}H NMR.

The reaction of compound **2.3g** with 2.5 equivalents of DMADEA in DCM, at room temperature (48 hours) led to a mixture of **2.11g** and **2.12g** in molar ratio of 1:1.1 (entry 6).

The reaction of compound **2.3n** with 2 equivalents of DMADEA and DCM, at room temperature (24 hours) led to a mixture of **2.11n** and **2.12n** in molar ratio of 1:1.1 (entry 9).

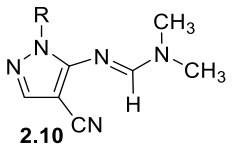
Compounds **2.11** and **2.12** were isolated as an oil and no attempts were made to separate them. These mixtures were directly used for the next step in the formation of adenine derivatives (Section 2.4).

2.3.2. Analytical and spectroscopic characterization

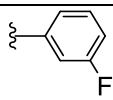
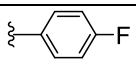
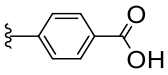
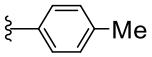
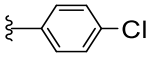
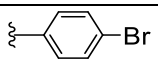
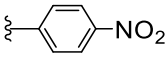
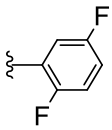
- **Physical and analytical data**

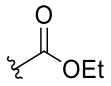
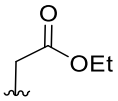
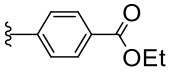
Table **2.21** presents the melting point range for **2.10l** and the isolated yields for all and new compounds **2.10a-n** and **2.10r**. Compounds herein presented will be later submitted to elemental analysis.

Table 2.21: Physical and analytical data of compounds **2.10a-n** and **2.10r**



2.10

Comp.	R	Yield (%)	m.p. (°C)	Chemical Formula	Calculated values (%)		
					C	H	N
2.10a		97	[a]	$C_{13}H_{13}N_5$	65.25	5.48	29.27
2.10b		82	[a]	$C_{13}H_{12}FN_5$	60.69	4.70	27.22
2.10c		95	[a]	$C_{13}H_{12}FN_5$	60.69	4.70	27.22
2.10d		92	[a]	$C_{13}H_{12}FN_5$	60.69	4.70	27.22
2.10f		96	[a]	$C_{14}H_{13}N_5O_2$	59.36	4.63	24.72
2.10g		90	[a]	$C_{14}H_{15}N_5$	66.38	5.97	27.65
2.10h		98	[a]	$C_{13}H_{12}ClN_5$	57.04	4.42	25.59
2.10i		97	[a]	$C_{13}H_{12}BrN_5$	49.07	3.80	22.01
2.10j		70	[a]	$C_{13}H_{12}N_6O_2$	54.93	4.25	29.56
2.10k		88	[a]	$C_{13}H_{11}F_2N_5$	56.73	4.03	25.44

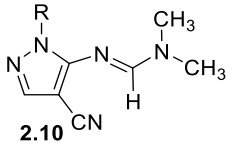
2.10l	H	35	212-214	$C_7H_9N_5$	51.52	5.56	42.92
2.10m		85	[a]	$C_{10}H_{13}N_5O_2$	51.06	5.57	29.77
2.10n		73	[a]	$C_{11}H_{15}N_5O_2$	53.00	6.07	28.10
2.10r		80	[a]	$C_{16}H_{17}N_5O_2$	61.72	5.50	22.49

[a] Compound isolated as an oil.

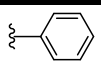
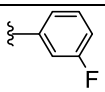
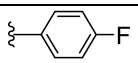
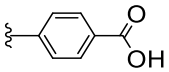
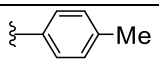
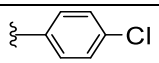
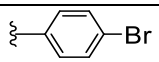
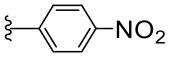
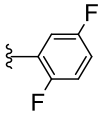
- **Infrared Spectroscopy**

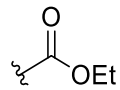
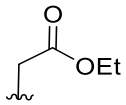
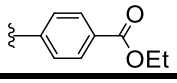
Compounds **2.10a-n** and **2.10r** show an intense band in the 2207-2256 cm^{-1} range attributed to the stretching vibration of the CN group (Table **2.22**). The weak to medium intensity bands in the 2818-3527 cm^{-1} range correspond to the stretching vibrations of the N-H and C_{sp2}-H bonds. The stretching vibration of the carbonyl groups of compounds **2.10f**, **2.10m-n** and **2.10r** show an intense band in the 1682-1750 cm^{-1} range.

Table 2.22: IR spectroscopic data (FTIR-ATR) for compounds **2.10a-n** and **2.10r**



2.10

Comp.	R	4000-2700	CN	CO	1800-1500
2.10a		2922w	2214i	—	1625i, 1595i, 1564m, 1532i, 1507i
2.10b		2985w	2256i	—	1626i, 1539i, 1510i
2.10c		2926w	2211i	—	1626i, 1599i, 1528m, 1513i
2.10d		2982w	2211i	—	1633i, 1532m, 1512i
2.10f		3527w, 3114w, 2982w	2226i	1682i	1622i, 1603i, 1541i, 1513i
2.10g		3112w, 2922w	2207i	—	1622i, 1584sh, 1563w, 1506i
2.10h		3121w, 2924w	2211i	—	1627i, 1597m, 1532i
2.10i		3184w, 3124w, 2923w	2212i	—	1627i, 1592m, 1531i, 1506w
2.10j		3281w, 3106m, 2925w	2214i	—	1627i, 1592m, 1568w, 1939m, 1505i
2.10k		3088w, 2927w, 2818w	2208i	—	1634i, 1609i, 1517m, 1506m

2.10l	H	3112m	2221i	—	1644i, 1595i, 1515m, 1506i
2.10m		3170w, 2856w	2208i	1699i	1631i, 1574i, 1512m
2.10n		3106w, 2927m	2212i	1750i	1628i, 1541i, 1514m
2.10r		2922w	2214i	1705i	1626i, 1605i, 1533m, 1505i

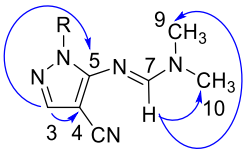
- **^{1}H-NMR Spectroscopy**

Table **2.23** summarizes the ^{1}H NMR signals assigned to compounds **2.10a-n** and **2.10r**. The signal for H-3 and H-7 appears as a singlet between δ_H 7.70-8.08 ppm and δ_H 8.08-8.34 ppm, respectively. The CH_3 signal appears between δ_H 2.79 ppm and δ_H 3.14 ppm. For compounds **2.10c-d**, **2.10i-j** and **2.10r**, the H-9 signal appears as a doublet with a coupling constant J 0.4 Hz. In Figure **2.12**, an example of the ^{1}H NMR spectrum of compound **2.10h** is shown, with some key signals assigned.

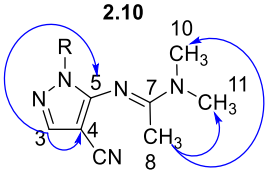
The signal for H-3 and H-8 of compounds **2.11** and **2.12** appears as a singlet between δ_H 7.77-8.20 ppm and δ_H 2.02-2.10 ppm, respectively. For compounds **2.11**, the signal for H-10 and H-11 appears between δ_H 2.99 ppm and δ_H 3.15 ppm. For compounds **2.12**, the signal for H-9 appears as a singlet between δ_H 3.75 ppm and δ_H 3.80 ppm. Table **2.24** summarizes the ^{1}H NMR signals assigned to compounds **2.11** and **2.12**.

- **^{13}C-NMR Spectroscopy**

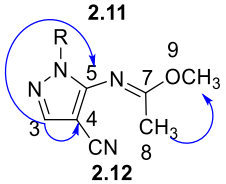
In the ^{13}C NMR spectrum of amidines **2.10** (Table **2.25**) carbons C-3 and C-7 are usually visible around δ_C 140.99-143.21 ppm and δ_C 156.10-156.93 ppm, respectively. The carbons of the CH_3 group appear between δ_C 33.86 ppm and δ_C 40.62 ppm. In the HMBC correlation spectrum it is possible to see the interaction of H-3 or H-7 with C-4, C-5 and CH_3, respectively. Figure **2.13** shows the ^{13}C NMR spectrum of **2.10h** with key signals assigned.



2.10



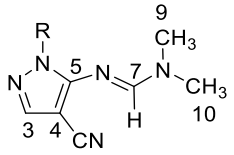
2.11



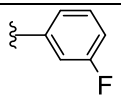
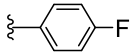
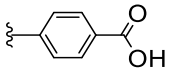
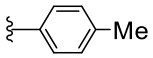
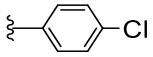
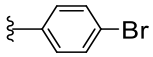
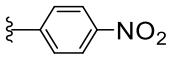
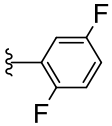
2.12

For compounds **2.11** and **2.12** (Table **2.26**) carbons C-3 and C-8 are usually visible around δ_C 141.43-142.29 ppm and δ_C 16.30-17.84 ppm, respectively. The carbons of the CH_3 groups for compounds **2.11a** and **2.11c** appear between δ_C 37.46 ppm and δ_C 38.55 ppm. For the compounds **2.12a** and **2.12c**, carbons C-9 are visible around δ_C 54.79 ppm and δ_C 54.89 ppm, respectively. In the HMBC correlation spectrum it is possible to see the interaction of H-3 or H-8 with C-4, C-5 and CH_3, respectively.

Table 2.23: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for compounds **2.10a-n** and **2.10r**



2.10

Comp.	R	C-H	CH_3	R
2.10a		7.97 (s, 1H, H_3) 8.24 (s, 1H, H_7)	2.95 (s, 3H, H_9) 3.09 (s, 3H, H_{10})	7.74 (dd, 2H, $H_{2'}$ + $H_{6'}$, J1.2 Hz, 8.4 Hz), 7.47 (t, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 7.33 (tt, 1H, $H_{4'}$, J1.2 Hz, 8.4 Hz)
2.10b		7.99 (s, 1H, H_3) 8.24 (s, 1H, H_7)	2.79 (s, 3H, H_9) 3.06 (s, 3H, H_{10})	7.52 (tt, 1H, $H_{4'}$, J1.6 Hz, 6.8 Hz), 7.49 (dd, 1H, $H_{6'}$, J1.6 Hz, 7.6 Hz), 7.39 (td, 1H, $H_{3'}$, J1.2 Hz, 8.4 Hz), 7.32 (td, 1H, $H_{5'}$, J1.2 Hz, 8.4 Hz)
2.10c		8.00 (s, 1H, H_3) 8.27 (s, 1H, H_7)	2.99 (d, 3H, H_9, J0.4 Hz) 3.12 (s, 3H, H_{10})	7.70 (d, 1H, $H_{6'}$, J8.4 Hz), 7.67 (d, 1H, $H_{2'}$, J8.4 Hz), 7.48-7.52 (m, 1H, $H_{5'}$), 7.15-7.21 (m, 1H, $H_{4'}$, J 1.2 Hz, 2.0 Hz, 8.4 Hz)
2.10d		7.97 (s, 1H, H_3) 8.24 (s, 1H, H_7)	2.95 (d, 3H, H_9, J0.4 Hz) 3.10 (s, 3H, H_{10})	7.77 (dd, 2H, $H_{2'}$ + $H_{6'}$, J5.2 Hz, 8.8 Hz)7.31 (t, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.10f		8.02 (s, 1H, H_3) 8.28 (s, 1H, H_7)	2.99 (s, 3H, H_9) 3.11 (s, 3H, H_{10})	8.00 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.8 Hz), 7.88 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.8 Hz)
2.10g		7.94 (s, 1H, H_3) 8.22 (s, 1H, H_7)	2.94 (s, 3H, H_9) 3.09 (s, 3H, H_{10})	7.59 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.4 Hz), 7.26 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 2.32 (s, 3H, CH_3)
2.10h		7.99 (s, 1H, H_3) 8.26 (s, 1H, H_7)	2.97 (s, 3H, H_9) 3.10 (s, 3H, H_{10})	7.80 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.4 Hz, 8.8 Hz), 7.53 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.4 Hz, 8.8 Hz)
2.10i		7.99 (s, 1H, H_3) 8.26 (s, 1H, H_7)	2.97 (d, 3H, H_9, J0.4 Hz) 3.11 (s, 3H, H_{10})	7.74 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.65 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.10j		8.08 (s, 1H, H_3) 8.34 (s, 1H, H_7)	3.03 (d, 3H, H_9, J0.4 Hz) 3.14 (s, 3H, H_{10})	8.31 (d, 2H, $H_{3'}$ + $H_{5'}$, J7.2 Hz), 8.17 (d, 2H, $H_{2'}$ + $H_{6'}$, J7.2 Hz)
2.10k		8.01 (s, 1H, H_3) 8.26 (s, 1H, H_7)	2.82 (s, 3H, H_9) 3.07 (s, 3H, H_{10})	7.36-7.50 (m, 3H, $H_{3'}$, $H_{4'}$, $H_{6'}$)
2.10l	H	7.70 (brs, 1H, H_3)	2.94 (s, 3H, H_9)	12.75 (s, 1H, NH)

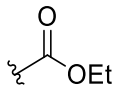
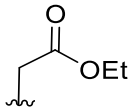
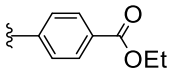
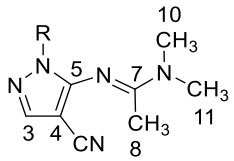
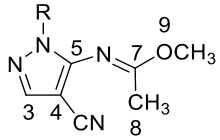
		8.09 (s, 1H, H_7)	3.04 (s, 3H, H_{10})	
2.10m		7.94 (s, 1H, H_3) 8.08 (s, 1H, H_7)	2.94 (s, 3H, H_9) 3.04 (s, 3H, H_{10})	4.01 (q, 2H, J7.2 Hz, OCH_2), 1.15 (t, 3H, J7.2 Hz, CH_3)
2.10n		7.75 (s, 1H, H_3) 8.22 (s, 1H, H_7)	2.95 (s, 3H, H_9) 3.08 (s, 3H, H_{10})	4.82 (s, 2H, CH_2), 4.12 (q, 2H, J7.2 Hz, OCH_2), 1.17 (t, 3H, J7.2 Hz, CH_3)
2.10r		8.04 (s, 1H, H_3) 8.30 (s, 1H, H_7)	3.00 (d, 3H, H_9, J0.4 Hz) 3.13 (s, 3H, H_{10})	8.05 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz), 8.00 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 4.31 (q, 2H, J7.2 Hz, OCH_2), 1.32 (t, 3H, J7.2 Hz, CH_3)

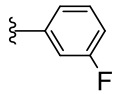
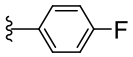
Table 2.24: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for compounds **2.11** and **2.12**



2.11



2.12

Comp.	R	C-H	CH_3	R
2.11a		7.99 (s, 1H, H_3)	2.05 (s, 3H, H_8) 3.00 (s, 3H, H_{10}) 3.05 (s, 3H, H_{11})	7.57 (dd, 2H, $H_{2'}$ + $H_{6'}$, J1.6 Hz, 8.0 Hz), 7.49 (dd, 2H, $H_{3'}$ + $H_{5'}$, J1.6 Hz, 8.0 Hz), 7.32 (tt, 1H, $H_{4'}$, J1.6 Hz, 8.0 Hz)
2.12a		8.17 (s, 1H, H_3)	2.07 (s, 3H, H_8) 3.76 (s, 3H, H_9)	7.64 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.0 Hz), 7.52 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.0 Hz), 7.41 (tt, 1H, $H_{4'}$, J2.0 Hz, 8.0 Hz)
2.11c		8.03 (s, 1H, H_3)	2.08 (s, 3H, H_8) 3.04 (s, 3H, H_{10}) 3.10 (s, 3H, H_{11})	7.58 (d, 1H, $H_{6'}$, J8.4 Hz), 7.56 (d, 1H, $H_{2'}$, J8.4 Hz), 7.47-7.51 (m, 1H, $H_{5'}$), 7.17 (td, 1H, $H_{4'}$, J2.4 Hz, 8.4 Hz)
2.12c		8.20 (s, 1H, H_3)	2.10 (s, 3H, H_8) 3.80 (s, 3H, H_9)	7.50-7.55 (m, 1H, $H_{2'}$ + $H_{5'}$), 7.47 (d, 1H, $H_{6'}$, J7.2 Hz), 7.28 (td, 1H, $H_{4'}$, J2.4 Hz, 8.4 Hz)
2.11d		7.98 (s, 1H, H_3)	2.06 (s, 3H, H_8) 3.03 (s, 3H, H_{10}) 3.08 (s, 3H, H_{11})	7.62 (dd 2H, $H_{2'}$ + $H_{6'}$, J5.2 Hz, 8.8 Hz), 7.29 (t, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.12d		8.16 (s, 1H, H_3)	2.08 (s, 3H, H_8) 3.76 (s, 3H, H_9)	7.67 (dd, 2H, $H_{2'}$ + $H_{6'}$, J5.2 Hz, 8.8 Hz), 7.35 (t, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)

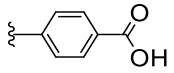
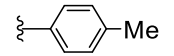
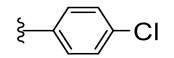
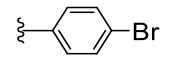
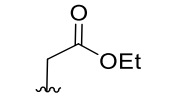
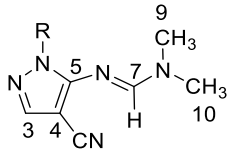
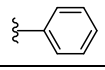
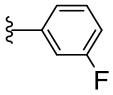
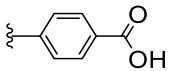
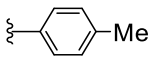
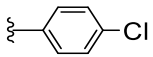
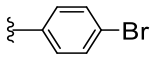
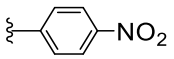
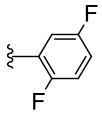
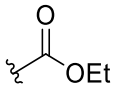
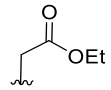
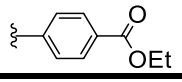
2.11f		8.16 (s, 1H, H_3)	2.04 (s, 3H, H_8) 3.04 (s, 3H, H_{10}) 3.10 (s, 3H, H_{11})	7.92 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 7.52 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.4 Hz)
2.12f		8.17 (s, 1H, H_3)	2.06 (s, 3H, H_8) 3.78 (s, 3H, H_9)	7.97 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.8 Hz), 7.61 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.8 Hz)
2.11g		7.95 (s, 1H, H_3)	2.03 (s, 3H, H_8) 2.99 (s, 3H, H_{10}) 3.06 (s, 3H, H_{11})	7.44 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.4 Hz), 7.23 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 2.32 (s, 3H, CH_3)
2.12g		8.13 (s, 1H, H_3)	2.05 (s, 3H, H_8) 3.75 (s, 3H, H_9)	7.49 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.4 Hz), 7.30 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.4 Hz), 2.34 (s, 3H, CH_3)
2.11h		8.01 (s, 1H, H_3)	2.07 (s, 3H, H_8) 3.02 (s, 3H, H_{10}) 3.09 (s, 3H, H_{11})	7.63 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.52 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.12h		8.19 (s, 1H, H_3)	2.09 (s, 3H, H_8) 3.78 (s, 3H, H_9)	7.70 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.58 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.11i		8.00 (s, 1H, H_3)	2.07 (s, 3H, H_8) 3.02 (s, 3H, H_{10}) 3.09 (s, 3H, H_{11})	7.69 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.56 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.12i		8.18 (s, 1H, H_3)	2.09 (s, 3H, H_8) 3.78 (s, 3H, H_9)	7.71 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.65 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)
2.11n		7.77 (s, 1H, H_3)	2.02 (s, 3H, H_8) 3.08 (s, 3H, H_{10}) 3.15 (s, 3H, H_{11})	4.74 (s, 2H, CH_2), 4.10 (q, 2H, J7.2 Hz, OCH_2), 1.15 (t, 3H, J7.2 Hz, CH_3)
2.12n		7.95 (s, 1H, H_3)	2.04 (s, 3H, H_8) 3.80 (s, 3H, H_9)	4.90 (s, 2H, CH_2), 4.12 (q, 2H, J7.2 Hz, OCH_2), 1.18 (t, 3H, J7.2 Hz, CH_3)

Table 2.25: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for compounds **2.10a-n** and **2.10r**



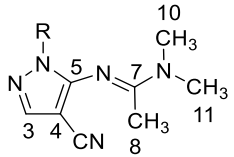
2.10

Comp.	R	$C_3 + C_7$	C_4	C_5	CH_3	CN	R
2.10a		141.85 (C_3) 156.63 (C_7)	78.25	154.82	34.15 (C_9) 40.12 (C_{10})	115.67	138.53 ($C_{1'}$), 128.67 ($C_{3'}$ + $C_{5'}$), 126.90 ($C_{4'}$), 123.22 ($C_{2'}$ + $C_{6'}$)
2.10b		142.54 (C_3) 156.53 (C_7)	76.99	155.87	33.86 (C_9) 40.12 (C_{10})	115.57	156.18 (d, $C_{2'}$, J 250.00 Hz), 130.82 (d, $C_{4'}$, J 8.00 Hz), 129.06 (s, $C_{6'}$), 125.73 (d, $C_{1'}$, J 12.00 Hz), 124.70 (d, $C_{5'}$, J 4.00 Hz), 116.40 (d, $C_{3'}$, J 20.00 Hz)
2.10c		142.24 (C_3) 156.75 (C_7)	78.51	155.20	34.24 (C_9) 40.22 (C_{10})	115.50	161.70 (d, $C_{3'}$, J 242.00 Hz), 139.95 (d, $C_{1'}$, J 10.00 Hz), 130.49 (d, $C_{5'}$, J 9.00 Hz), 118.72 (d, $C_{6'}$, J 3.00 Hz), 113.47 (d, $C_{4'}$, J 21.00 Hz), 109.93 (d, $C_{2'}$, J 26.00 Hz)
2.10d		141.90 (C_3) 156.70 (C_7)	78.12	154.75	34.18 (C_9) 40.16 (C_{10})	115.63	160.47 (d, $C_{4'}$, J 243.00 Hz), 134.91 (d, $C_{1'}$, J 3.00 Hz), 125.37 (d, $C_{2'}$ + $C_{6'}$, J 9.00 Hz), 115.49 (d, $C_{3'}$ + $C_{5'}$, J 23.00 Hz)
2.10f		142.35 (C_3) 156.70 (C_7)	78.50	155.25	34.28 (C_9) 40.21 (C_{10})	115.56	167.28 (CO), 141.21 ($C_{1'}$), 129.78 ($C_{3'}$ + $C_{5'}$), 130.99 ($C_{4'}$), 122.38 ($C_{2'}$ + $C_{6'}$)
2.10g		141.64 (C_3) 156.70 (C_7)	78.10	154.59	34.11 (C_9) 40.08 (C_{10})	115.73	136.34 ($C_{4'}$), 136.14 ($C_{1'}$), 129.07 ($C_{3'}$ + $C_{5'}$), 123.18 ($C_{2'}$ + $C_{6'}$), 20.54 (CH_3)
2.10h		142.20 (C_3) 156.76 (C_7)	78.34	154.97	34.28 (C_9) 40.23 (C_{10})	115.58	137.38 ($C_{1'}$), 131.13 ($C_{4'}$), 128.73 ($C_{3'}$ + $C_{5'}$), 124.73 ($C_{2'}$ + $C_{6'}$)
2.10i		142.20 (C_3) 156.71 (C_7)	78.35	154.94	34.26 (C_9) 40.20 (C_{10})	115.54	137.78 ($C_{1'}$), 131.63 ($C_{3'}$ + $C_{5'}$), 124.98 ($C_{2'}$ + $C_{6'}$), 119.47 ($C_{4'}$)
2.10j		143.21 (C_3) 156.93 (C_7)	78.92	155.99	34.54 (C_9) 40.42 (C_{10})	115.37	144.96 ($C_{4'}$), 143.63 ($C_{1'}$), 124.50 ($C_{3'}$ + $C_{5'}$), 122.90 ($C_{2'}$ + $C_{6'}$)
2.10k		142.87 (C_3) 156.60 (C_7)	77.02	155.96	33.92 (C_9) 40.12 (C_{10})	115.42	157.40 (dd, $C_{5'}$, J 2.00 Hz, 240.00 Hz), 152.65 (dd, $C_{2'}$, J 2.00 Hz, 246.00 Hz), 126.41 (d, $C_{1'}$, J 25.00 Hz), 117.73 (dd, $C_{3'}$, J 9.40 Hz, 22.8 Hz), 117.33 (dd, $C_{4'}$, J 8.10 Hz, 23.80 Hz), 115.89 (d, $C_{6'}$, J 26.00 Hz)
2.10l	H	141.81 (C_3) 156.45 (C_7)	77.69	[a]	33.90 (C_9) 40.18 (C_{10})	115.81	–

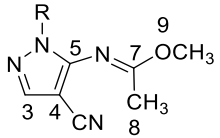
2.10m		141.87 (C_3) 156.58 (C_7)	[a]	156.58	33.92 (C_9) 40.13 (C_{10})	115.81	162.35 (CO), 54.90 (OCH_2), 14.69 (CH_3)
2.10n		140.99 (C_3) 156.10 (C_7)	75.77	154.51	33.91 (C_9) 40.14 (C_{10})	115.84	167.54 (CO), 61.02 (OCH_2), 48.66 (CH_2), 14.00 (CH_3)
2.10r		142.60 (C_3) 156.74 (C_7)	78.61	155.46	34.34 (C_9) 40.62 (C_{10})	115.48	165.07 (CO), 142.24 ($C_{1'}$), 129.82 ($C_{3'}$ + $C_{5'}$), 127.60 ($C_{4'}$), 122.52 ($C_{2'}$ + $C_{6'}$), 60.84 (OCH_2), 14.13 (CH_3)

[a] Not visible.

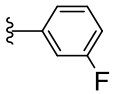
Table 2.26: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for compounds **2.11a**, **2.11c**, **2.12a** and **2.12c**



2.11



2.12

Comp.	R	C_3	C_4	C_5	C_7	CH_3	R
2.11a		141.43	81.28	154.29	161.40	16.30 (C_8) 37.46 (C_{10}) 38.44 (C_{11})	138.51 ($C_{1'}$), 128.75 ($C_{3'}$ + $C_{5'}$), 126.91 ($C_{4'}$), 123.49 ($C_{2'}$ + $C_{6'}$)
2.12a		141.94	82.67	150.51	168.40	17.69 (C_8) 54.79 (C_9)	137.90 ($C_{1'}$), 129.11 ($C_{3'}$ + $C_{5'}$), 127.96 ($C_{4'}$), 122.90 ($C_{2'}$ + $C_{6'}$)
2.11c		141.84	81.47	154.59	161.68	16.42 (C_8) 37.54 (C_{10}) 38.55 (C_{11})	161.74 ($C_{3'}$, J 241.00 Hz), 139.93 ($C_{1'}$, J 10.00 Hz), 130.58 ($C_{5'}$, J 10.00 Hz), 118.48 ($C_{6'}$, J 3.00 Hz), 113.52 ($C_{4'}$, J 21.00 Hz), 109.68 ($C_{2'}$, J 26.00 Hz)
2.12c		142.29	83.03	150.79	168.77	17.84 (C_8) 54.89 (C_9)	161.04 ($C_{3'}$, J 243.00 Hz), 138.93 ($C_{1'}$, J 11.00 Hz), 130.96 ($C_{5'}$, J 9.00 Hz), 119.36 ($C_{6'}$, J 3.00 Hz), 114.78 ($C_{4'}$, J 21.00 Hz), 110.69 ($C_{2'}$, J 25.00 Hz)

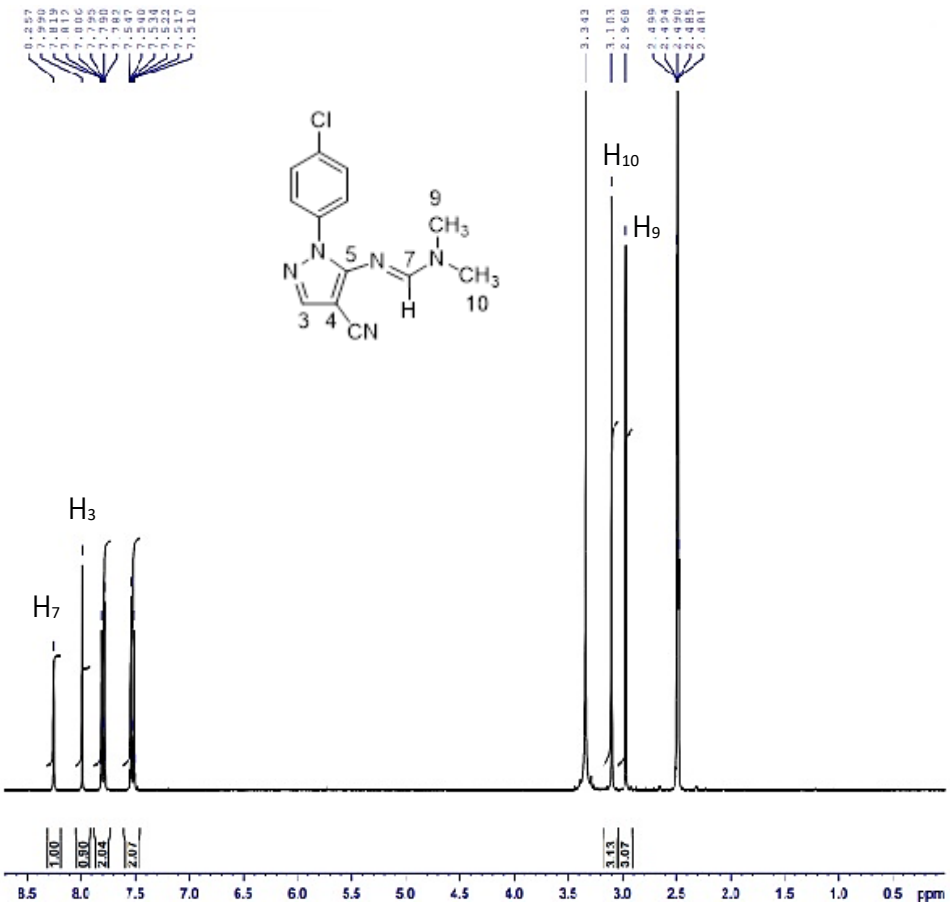


Figure 2.12: ^{1}H NMR spectrum for compound **2.10h** in DMSO-d_6 solution (^{1}H: 400 MHz).

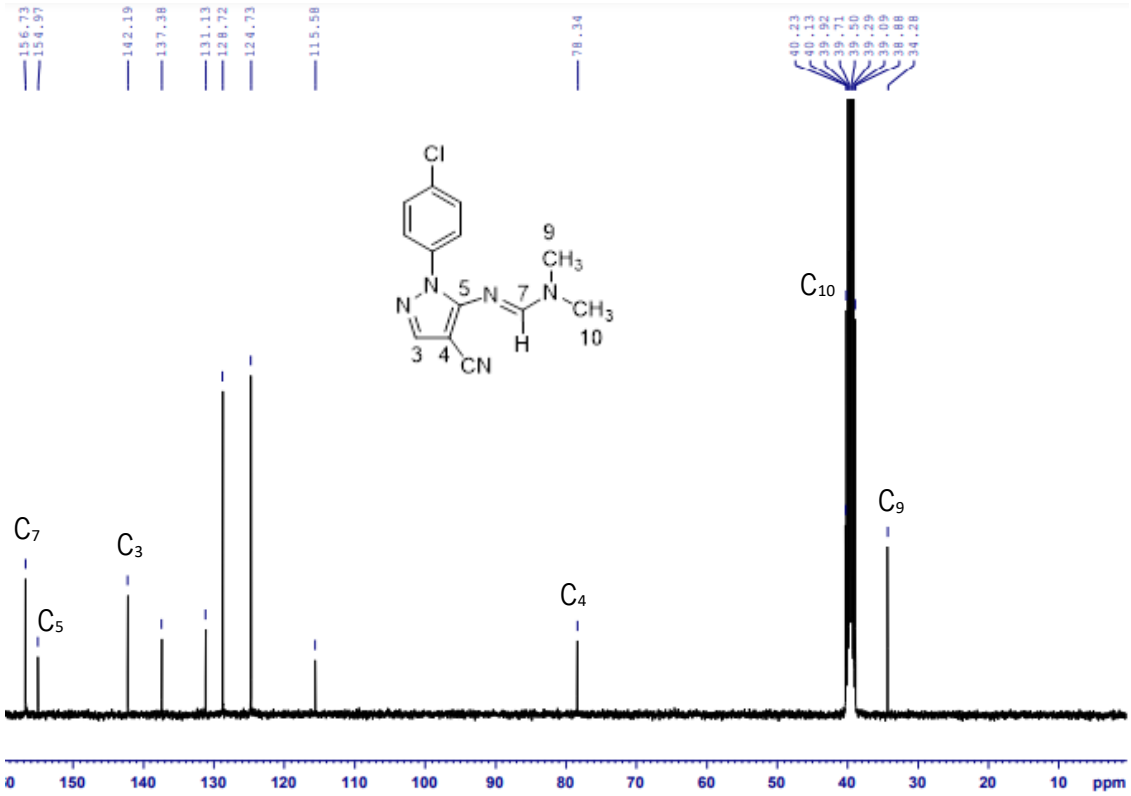


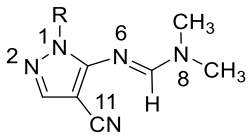
Figure 2.13: ^{13}C NMR spectrum for compound **2.10h** in DMSO-d_6 solution (^{13}C: 100 MHz).

- **^{15}N-NMR Spectroscopy**

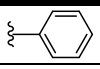
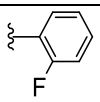
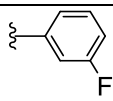
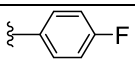
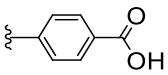
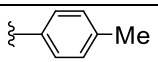
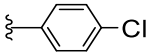
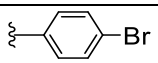
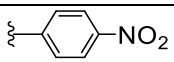
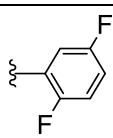
In the ^{15}N HMBC correlation spectra, nitrogen atoms N-1 and N-2 of amidines **2.10** were identified around δ_N 192.60-205.98 ppm and δ_N 291.76-304.20 ppm, respectively. The chemical shift values of the nitrogen atom N-8 were identified around δ_N 95.97-104.11 ppm. The values of all the nitrogen chemical shifts are summarized in Table **2.27**. Figure **2.14** shows the ^{15}N NMR correlation spectrum of **2.10h**, with key signals assigned.

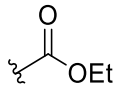
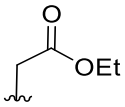
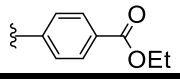
The nitrogen atoms N-1 and N-2 of compounds **2.11** and **2.12** were identified around δ_N 204.30-208.30 ppm and δ_N 292.91-298.63 ppm, respectively. The chemical shifts values of the nitrogen atom N-6 were identified around δ_N 199.50-218.30 ppm. For compounds **2.11a** and **2.11c**, nitrogen atom N-9 was identified at δ_N 94.94 ppm and δ_N 98.79 ppm, respectively. The values of the nitrogen chemical shifts compounds are summarized in Table **2.28**.

Table 2.27: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) for compounds **2.10a-n** and **2.10r**



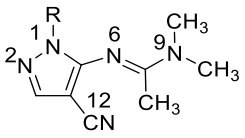
2.10

Comp.	R	N_1	N_2	N_6	N_8	N_{11}	R
2.10a		205.98	293.46	192.63	101.30	a)	—
2.10b		195.55	297.14	191.14	100.77	a)	—
2.10c		203.67	292.41	191.91	101.84	a)	—
2.10d		204.00	293.35	191.60	100.79	a)	—
2.10f		204.85	292.48	192.11	101.71	a)	—
2.10g		205.95	293.72	192.79	99.84	a)	—
2.10h		203.68	304.20	191.65	101.44	a)	—
2.10i		203.76	292.30	191.79	101.53	a)	—
2.10j		202.90	291.90	191.21	104.11	a)	369.40
2.10k		194.08	296.44	190.22	101.79	a)	—
2.10l	H	b)	b)	b)	b)	a)	—

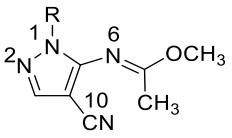
2.10m		[b]	[b]	[b]	95.97	[a]	—
2.10n		192.60	294.75	190.12	100.29	[a]	—
2.10r		204.28	291.76	191.74	102.29	[a]	—

[a] N_{11} was never visible in the ^{15}N spectrum; [b] Not visible.

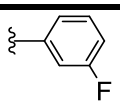
Table 2.28: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) for compounds **2.11a**, **2.11c**, **2.12a** and **2.12c**



2.11



2.12

Comp.	R	N_1	N_2	N_6	N_9	N_{10} + N_{12}
2.11a		206.70	294.21	199.90	94.94	[a]
2.12a		208.30	298.63	218.30	—	[a]
2.11c		204.30	292.91	199.50	98.79	[a]
2.12c		206.30	298.03	217.40	—	[a]

[a] N_{10} and N_{12} was never visible in the ^{15}N spectrum.

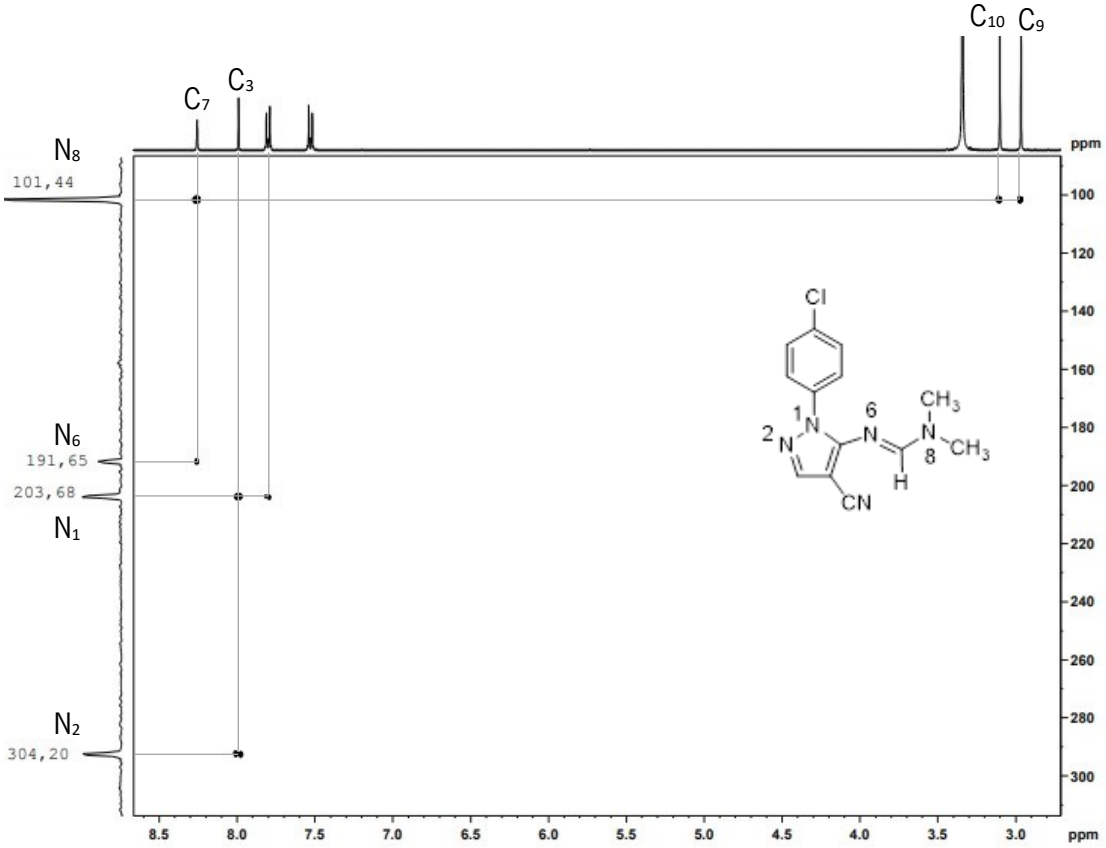


Figure 2.14: ^{15}N HMBC spectrum for compound **2.10h** in DMSO-d_6 solution (^{15}N: 40 MHz).

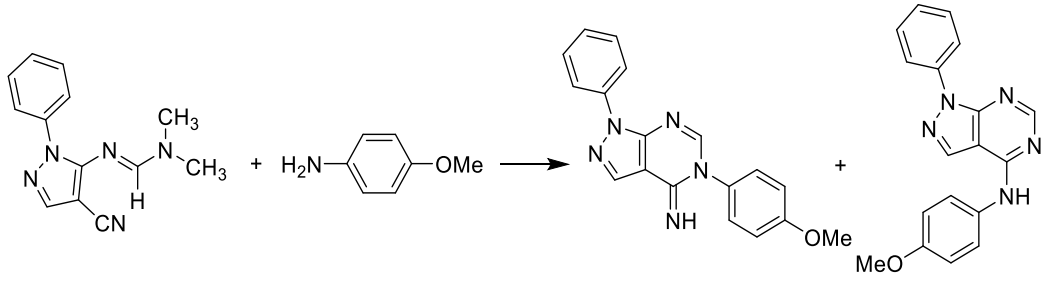
2.4. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives

Adenine derivatives were previously synthesized in our research group, from the reaction of 5-amino-4-cyanoimidazoles with TEOF or DMFDEA followed by addition of a primary aromatic amine.[56] The aim of this work is to synthesize the analogous pyrazolo[3,4-*d*]pyrimidine derivatives starting from an appropriate substituted pyrazole. The access to imidates **2.6** and amidines **2.10**, **2.11** and **2.12** allowed to perform the reaction with aromatic, alkyl and heteroaromatic primary amines for the synthesis of the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives.

2.4.1. Reactions with aromatic amines

The initial studies were performed using the reaction of compound **2.10a** with aromatic amine **2.13f** to optimize the experimental conditions that would allow to isolate the pure pyrazolo[3,4-*d*]pyrimidine in the highest yield (Table **2.29**). Several experimental conditions were tested to prepare these compounds, varying the equivalents of amine, the acid and the temperature.

Table 2.29: Optimization of experimental conditions to generate pyrazolo[3,4-*d*]pyrimidines



2.10a **2.13f** **2.14f** **2.15f**

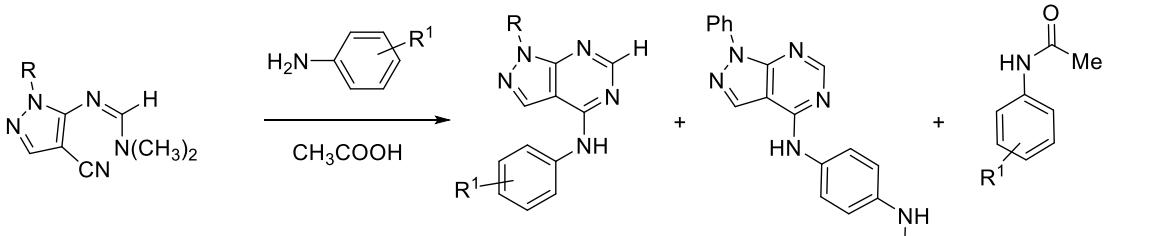
Entry	Reagents		Reaction conditions	Product (yield)
	2.10a.	**2.13f.**		
1	0.29 mmol	1 eq.	TFA (500 µL), reflux, 2 h	**2.14f.** 22%
2	0.54 mmol	1 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15f.** 49%
3	0.54 mmol	1 eq.	CH_3COOH (500 µL), 118°C, 2 h	**2.15f.** 62%
4	0.42 mmol	1.6 eq.	i) **2.13f** (1 eq.), CH_3COOH (500 µL), reflux, 1 h ii) **2.13f** (0.6 eq.), CH_3COOH (500 µL), reflux, 1 h	**2.15f.** 61%
5	0.24 mmol	2 eq.	i) **2.13f** (1 eq.), CH_3COOH (500 µL), reflux, 1 h ii) **2.13f** (1 eq.), CH_3COOH (500 µL), reflux, 1 h	**2.15f.** 53%
6	0.30 mmol	2 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15f.** 75%

The experimental conditions initially used involved the combination of compound **2.10a** with 1 equivalent of 4-methoxyaniline **2.13f** in TFA (entry 1). After 2 hours under reflux, TLC showed the absence of starting material and compound **2.14f** was isolated in 22% yield. The mother liquor was a

dark oil which showed to be a complex mixture, by ^{1}H NMR. The use of acetic acid (entry 2) under the same conditions used in entry 1, led to the formation of **2.15f**, isolated in 49% yield. In the mother liquor, a large amount of **2.10a** remained and the presence of the amine **2.13f** was not detected, by ^{1}H NMR. The yield of product **2.15f** increased to 62% (entry 3), when the reaction mixture was stirred in a closed vessel at 118°C for 2 hours. The mother liquor continued to show the presence of **2.10a** and the absence of **2.13f**. The reaction was repeated with 1.6 equivalents of the aromatic amine and 1 mL of CH_3COOH (entry 4). Initially, 1 equivalent of **2.13f** was added to 500 µL of acid and after 1 hour under reflux another 0.6 equivalents of **2.13f** was added, together with more acid (500 µL). After 2 hours of reflux, product **2.15f** was isolated in 61% yield. In the mother liquor, compound **2.10a** continued to be identified. The reaction was repeated, increasing the amount of amine **2.13f** to 2 equivalents (entry 5). The reaction started with 1 equivalent of **2.13f** and after 1 hour another equivalent of **2.13f** was added. Pure compound **2.15f** was obtained, but the yield decreased to 53%. In the mother liquor, a complex mixture remained where the signals for the acetylated amine were identified. The use of 2 equivalents of amine (entry 6) added as a single portion led to the isolation of product **2.15f** in 75% yield. This experimental condition was considered to be the best for the formation of pyrazolo[3,4-*d*]pyrimidine derivatives.

Using the previously optimized method (method A), the remaining pyrazolo[3,4-*d*]pyrimidine derivatives were synthesized (Table **2.30**). The reactions were followed by TLC, and the product was isolated when the amine was no longer present.

Table 2.30: Experimental conditions for the reaction of compounds **2.10** with different aromatic amines **2.13a-m**



2.10a. R=C_6H_5
2.10b. R=2-F-C_6H_4
2.10c. R=3-F-C_6H_4
2.10d. R=4-F-C_6H_4
2.10e. R=4-OMe-C_6H_4
2.10f. R=4-CO_2H-C_6H_4
2.10g. R=4-Me-C_6H_4
2.10h. R=4-Cl-C_6H_4
2.10i. R=4-Br-C_6H_4
2.10j. R=4-NO_2-C_6H_4
2.10k. R=2,5-F-C_6H_3
2.10m. R=COOEt
2.10n. R=CH_2COOEt
2.10r. R=4-COOEt-C_6H_4

2.13a. R^1=H
2.13b. R^1=2-OH
2.13c. R^1=3-OH
2.13d. R^1=4-OH
2.13e R^1=3-OMe
2.13f. R^1=4-OMe
2.13g. R^1=3-Me
2.13h. R^1=3-Br
2.13i. R^1=4-Br
2.13j. R^1=4-Cl
2.13k. R^1=4-CN
2.13l. R^1=4-NH_2
2.13m. R^1=4-Cl-2-NO_2

2.15a-bo

2.16a

2.17d. R^1=4-OH
2.17f. R^1=4-OMe
2.17h. R^1=3-Br
2.17i. R^1=4-Br
2.17l. R^1=4-NH_2

2.15*

2.15a. R=C_6H_5; R^1=H
2.15b. R=C_6H_5; R^1=2-OH
2.15c. R=C_6H_5; R^1=3-OH
2.15d. R=C_6H_5; R^1=4-OH
2.15e. R=C_6H_5; R^1=3-OMe
2.15f. R=C_6H_5; R^1=4-OMe
2.15g. R=C_6H_5; R^1=3-Me
2.15h. R=C_6H_5; R^1=3-Br
2.15i. R=C_6H_5; R^1=4-Br
2.15j. R=C_6H_5; R^1=4-Cl
2.15k. R=C_6H_5; R^1=4-CN
2.15l. R=C_6H_5; R^1=4-NH_2
2.15m. R=2-F-C_6H_4; R^1=3-OMe
2.15n. R=2-F-C_6H_4; R^1=4-OMe
2.15o. R=2-F-C_6H_4; R^1=3-Me
2.15p. R=3-F-C_6H_4; R^1=H
2.15q. R=3-F-C_6H_4; R^1=3-OH
2.15r. R=3-F-C_6H_4; R^1=4-OH
2.15s. R=3-F-C_6H_4; R^1=3-OMe
2.15t. R=3-F-C_6H_4; R^1=4-OMe
2.15u. R=3-F-C_6H_4; R^1=3-Me
2.15v. R=3-F-C_6H_4; R^1=3-Br
2.15w. R=3-F-C_6H_4; R^1=4-Br
2.15x. R=3-F-C_6H_4; R^1=4-Cl
2.15y. R=4-F-C_6H_4; R^1=H
2.15z. R=4-F-C_6H_4; R^1=4-OH
2.15aa. R=4-F-C_6H_4; R^1=4-OMe
2.15ab. R=4-F-C_6H_4; R^1=3-Me
2.15ac. R=4-F-C_6H_4; R^1=3-Br
2.15ad. R=4-CO_2H-C_6H_4; R^1=H
2.15ae. R=4-CO_2H-C_6H_4; R^1=3-OH
2.15af. R=4-CO_2H-C_6H_4; R^1=4-OH
2.15ag. R=4-CO_2H-C_6H_4; R^1=3-OMe
2.15ah. R=4-CO_2H-C_6H_4; R^1=4-OMe
2.15ai. R=4-CO_2H-C_6H_4; R^1=3-Me
2.15aj. R=4-CO_2H-C_6H_4; R^1=3-Br
2.15ak. R=4-CO_2H-C_6H_4; R^1=4-Br
2.15al. R=4-CO_2H-C_6H_4; R^1=4-Cl
2.15am. R=4-CO_2H-C_6H_4; R^1=4-CN
2.15an. R=4-Me-C_6H_4; R^1=H
2.15ao. R=4-Me-C_6H_4; R^1=4-OH
2.15ap. R=4-Me-C_6H_4; R^1=4-OMe
2.15aq. R=4-Me-C_6H_4; R^1=3-Me
2.15ar. R=4-Me-C_6H_4; R^1=4-Cl
2.15as. R=4-Cl-C_6H_4; R^1=H
2.15at. R=4-Cl-C_6H_4; R^1=4-OH
2.15au. R=4-Cl-C_6H_4; R^1=3-OMe
2.15av. R=4-Cl-C_6H_4; R^1=4-OMe
2.15aw. R=4-Cl-C_6H_4; R^1=3-Me
2.15ax. R=4-Cl-C_6H_4; R^1=4-Br
2.15ay. R=4-Cl-C_6H_4; $R^1$4-Cl
2.15az. R=4-Cl-C_6H_4; R^1=4-CN
2.15ba. R=4-Br-C_6H_4; R^1=H
2.15bb. R=4-Br-C_6H_4; R^1=4-OH
2.15bc. R=4-Br-C_6H_4; R^1=4-OMe
2.15bd. R=4-Br-C_6H_4; R^1=4-Br
2.15be. R=4-NO_2-C_6H_4; R^1=4-OMe
2.15bf. R=2,5-F-C_6H_3; R^1=**H**
2.15bg. R=2,5-F-C_6H_3; R^1=4-OH
2.15bh. R=2,5-F-C_6H_3; R^1=3-OMe
2.15bi. R=2,5-F-C_6H_3; R^1=4-OMe
2.15bj. R=2,5-F-C_6H_3; R^1=3-Br
2.15bk. R=2,5-F-C_6H_3; R^1=4-Cl
2.15bl. R=H; R^1=4-Br
2.15bm. R=CH_2COOEt; R^1=4-OMe
2.15bn. R=CH_2COOEt; R^1=4-Cl
2.15bo. R=COOEt-C_6H_4; R^1=4-OMe

Entry	Reagents		Experimental conditions	Product (yield)
	2.10.	**2.13.**		
1	**2.10a.** 0.21 mmol	**2.13a.** 2 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15a.** 55%
2	**2.10a.** 0.17 mmol	**2.13b.** 2 eq.	CH_3COOH (500 µL), 118°C, 7 h	Complex mixture containing **2.15b** [a)]
3	**2.10a.** 0.25 mmol	**2.13b.** 2 eq.	CH_3COOH (500 µL), 118°C, 15.5 h	Complex mixture containing **2.15b** [a)]
4	**2.10a.** 0.25 mmol	**2.13c.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	Complex mixture containing **2.15c** [a)]
5	**2.10a.** 0.22 mmol	**2.13d.** 2 eq.	CH_3COOH (500 µL), 118°C, 5 h	**2.15d.** 70%
6	**2.10a.** 0.27 mmol	**2.13e.** 2 eq.	CH_3COOH (500 µL), 118°C, 14 h	**2.15e.** 68%
7	**2.10a.** 0.30 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15f.** 75%
8	**2.10a.** 0.25 mmol	**2.13g.** 2 eq.	CH_3COOH (500 µL), 118°C, 14 h	**2.15g.** 60%
9	**2.10a.** 0.27 mmol	**2.13h.** 2 eq.	CH_3COOH (500 µL), 118°C, 7 h	**2.15h.** 52%
10	**2.10a.** 0.27 mmol	**2.13i.** 2 eq.	CH_3COOH (500 µL), 118°C, 10 h	**2.15i.** 67%
11	**2.10a.** 0.27 mmol	**2.13j.** 2 eq.	CH_3COOH (500 µL), 118°C, 10 h	**2.15j.** 79%
12	**2.10a.** 0.21mmol	**2.13k.** 2 eq.	CH_3COOH (500 µL), 118°C, 6 h	**2.15k.** 45%
13	**2.10a.** 0.25 mmol	**2.13l.** 2 eq.	CH_3COOH (500 µL), 118°C, 13.5 h	Complex mixture [a)]
14	**2.10a.** 0.26 mmol	**2.13l.** 2 eq.	CH_3COOH (500 µL), 118°C, 4 h	**2.16a** + **2.17l** (1.1:1) [a)]
15	**2.10a.** 0.25 mmol	**2.13l.** 2 eq.	CH_3COOH (500 µL), 60°C, 5.5 h	**2.10a** + **2.13l** (3.2:1) [a)]
16	**2.10a.** 0.24 mmol	**2.13l.** 2 eq.	i) CH_3COOH (400 µL), 60°C, 24 h ii) 80°C, 3 days	**2.15l.** 6%
17	**2.10a.** 0.27 mmol	**2.13m.** 2 eq.	CH_3COOH (400 µL), 118°C, 5.5 h	**2.10a** + **2.13m** (1:1) [a)]
18	**2.10b.** 0.21 mmol	**2.13e.** 2 eq.	CH_3COOH (500 µL), 118°C, 6 h	Complex mixture containing **2.15m** [a)]

19	**2.10b.** 0.20 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15n.** 4%
20	**2.10b.** 0.16 mmol	**2.13g.** 2 eq.	CH_3COOH (500 µL), 118°C, 6 h	Complex mixture containing **2.15o** [a)]
21	**2.10c.** 0.39 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15p.** 32%
22	**2.10c.** 0.20 mmol	**2.13c.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	Complex mixture containing **2.15q** [a)]
23	**2.10c.** 0.20 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15r.** 53%
24	**2.10c.** 0.24 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), reflux, 2.5 h	**2.15s.** 48%
25	**2.10c.** 0.24 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15t.** 54%
26	**2.10c.** 0.20 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15u.** 48%
27	**2.10c.** 0.13 mmol	**2.13h.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15v.** 22%
28	**2.10c.** 0.23 mmol	**2.13i.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15w** + **2.17i** (1:3.4) [a)]
29	**2.10c.** 0.13 mmol	**2.13j.** 2 eq.	CH_3COOH (500 µL), 118°C, 14 h	**2.15x.** 52%
30	**2.10d.** 0.23 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15y.** 42%
31	**2.10d.** 0.23 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15z.** 50%
32	**2.10d.** 0.18 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15aa.** 52%
33	**2.10d.** 0.20 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 4.5 h	**2.15ab.** 47%
34	**2.10d.** 0.21 mmol	**2.13h.** 2 eq.	CH_3COOH (400 µL), 118°C, 4.5 h	**2.15ac + 2.17h** (1:1) [a)]
35	**2.10f.** 0.20 mmol	**2.13a.** 2 eq.	CH_3COOH (500 µL), 118°C, 7 h	**2.15ad.** 79%
36	**2.10f.** 0.20 mmol	**2.13c.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15ae.** 67%
37	**2.10f.** 0.20 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15af.** 76%
38	**2.10f.** 0.22 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15ag.** 62%
39	**2.10f.** 0.20 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), 118°C, 7 h	**2.15ah.** 73%
40	**2.10f.** 0.19 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15ai.** 62%
41	**2.10f.** 0.20 mmol	**2.13h.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15aj.** 30%
42	**2.10f.** 0.22 mmol	**2.13i.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15ak.** 39%
43	**2.10f.** 0.22 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15al.** 57%
44	**2.10f.** 0.20 mmol	**2.13k.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15am** + **2.10f** (2.5:1) [a)]
45	**2.10f.** 0.20 mmol	**2.13l.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	Complex mixture [a)]
46	**2.10g.** 0.23 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15an.** 48%
47	**2.10g.** 0.24 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15ao.** 46%
48	**2.10g.** 0.24 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	Complex mixture [a)]
49	**2.10g.** 0.19 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 5.5 h	**2.15ap.** 53%
50	**2.10g.** 0.24 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15aq.** 59%
51	**2.10g.** 0.23 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15ar.** 48%
52	**2.10h.** 0.20 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15as.** 48%
53	**2.10h.** 0.20 mmol	**2.13c.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	Complex mixture [a)]
54	**2.10h.** 0.21 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15at.** 53%
55	**2.10h.** 0.17 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15au.** 53%
56	**2.10h.** 0.24 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 4.5 h	**2.15av.** 62%

57	**2.10h.** 0.20 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15aw.** 46%
58	**2.10h.** 0.22 mmol	**2.13h.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	Complex mixture [a]
59	**2.10h.** 0.21 mmol	**2.13i.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15ax.** 57%
60	**2.10h.** 0.19 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15ay.** 68%
61	**2.10h.** 0.23 mmol	**2.13k.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15az** + **2.10h** (3.3:1) [a]
62	**2.10i.** 0.13 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15ba.** 52%
63	**2.10i.** 0.13 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15bb**. 66%
64	**2.10i.** 0.13 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.15bc.** 51%
65	**2.10i.** 0.10 mmol	**2.13i.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15bd** + **2.17i** (1:1) [a]
66	**2.10j.** 0.05 mmol	**2.13f.** 2 eq.	CH_3COOH (300 µL), 118°C, 4 h	**2.15be.** 11%
67	**2.10k.** 0.15 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15bf.** 11%
68	**2.10k.** 0.19 mmol	**2.13d.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15bg.** 57%
69	**2.10k.** 0.21 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), 118°C, 7.5 h	**2.15bh.** 40%
70	**2.10k.** 0.20 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), reflux, 2 h	**2.15bi.** 55%
71	**2.10k.** 0.19 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	Complex mixture [a]
72	**2.10k.** 0.19 mmol	**2.13h.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	Complex mixture containing **2.15bj** and **2.17h** [a]
73	**2.10k.** 0.25 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.15bk.** 38%
74	**2.10m.** 0.20 mmol	**2.13i.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15bl** + **2.17i** (1:3.2) [a]
75	**2.10m.** 0.20 mmol	**2.13i.** 1.2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.15bl.** 62%
76	**2.10n.** 0.24 mmol	**2.13a.**1.2eq.	CH_3COOH (400 µL), 118°C, 5.5 h	Complex mixture [a]
77	**2.10n.** 0.26 mmol	**2.13f.** 1 eq.	CH_3COOH (500 µL), reflux, 1.5 h	**2.15bm.** 41%
78	**2.10n.** 0.24 mmol	**2.13j.** 1 eq.	CH_3COOH (500 µL), 118°C, 5.5 h	**2.15bn.** 46%
79	**2.10r.** 0.15 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), reflux, 2 h	F_1= **2.15bo.** 67% F_2= **2.17f.** 33%
80	**2.10r.** 0.20 mmol	**2.13j.** 2 eq.	CH_3COOH (500 µL), 118°C, 6 h	Complex mixture [a]

[a] By ^1H NMR.

Compound **2.10a** was reacted with amines **2.13a**, **2.13d-k** originating the respective pyrazolo[3,4-*d*]pyrimidine derivatives **2.15a**, **2.15d-k** in a yield ranging from 45% to 79% (entries 1, 5-12). The reaction between compounds **2.10a** and **2.13b** at 118°C, for 7 hours (entry 2) or 15.5 hours (entry 3) originated a small amount of a complex mixture containing the desired product **2.15b**, by ^1H NMR. Compound **2.10a** was reacted with *p*-phenylenediamine **2.13l** for 13.5 hours leading also to a complex mixture (entry 13). Decreasing the reaction time to 4 hours, led to a mixture of compounds **2.16a** and **2.17l** in a 1.1:1 molar ration, by ^1H NMR (entry 14). The formation of product **2.16a** resulted from the reaction of both amino groups of *p*-phenylenediamine with the amidine function of two pyrazole units **2.10a**. This product was identified by ^1H, ^{13}C and ^{15}N NMR (Figure **2.15**). In the ^1H and ^{13}C NMR spectrum of compound **2.16a**, most of the protons/carbon atoms give rise to two signals that were

assigned to each of the heteroaromatic ring structures. It was not possible to separate these signals and associate them to the corresponding pyrazolopyrimidine, so they will be grouped and identified for each of these protons/carbons for only one of the rings. The spectrum was initially registered at 20°C but some of the signals were broad and difficult to assign. Running the spectrum at 80°C led to a better resolution and the data reported corresponds to the chemical shift values at this temperature.

In the ^{1}H NMR, the broad signal centered at δ_H 9.94 ppm, integrating for (1+1)H, was assigned to the N-H proton of the central aromatic ring. The signal for H-3 and H-6, each integrating for (1+1)H, appears as a singlet at δ_H 8.35/8.44 ppm and δ_H 8.47/8.52 ppm, respectively. In the ^{13}C NMR spectrum, the signals at δ_C 133.22/133.25 ppm and δ_C 155.63 ppm were assigned to carbons C-3 and C-6, respectively. In the aromatic ring, only two signals were identified for the C-H carbon, in ^{13}C NMR spectrum (δ_C 121.96/122.12 ppm). The two remaining carbons of this ring show a single signal at δ_C 134.64 ppm. For the two phenyl group, duplicate chemical shift values were obtained for carbons at position 2'' + 6'' (δ_C 120.53/120.56 ppm), 3'' + 5'' (δ_C 128.61/128.63 ppm) and 4'' (δ_C 125.82/125.86 ppm), and a single value for carbon at position 1 (δ_C 138.54 ppm).

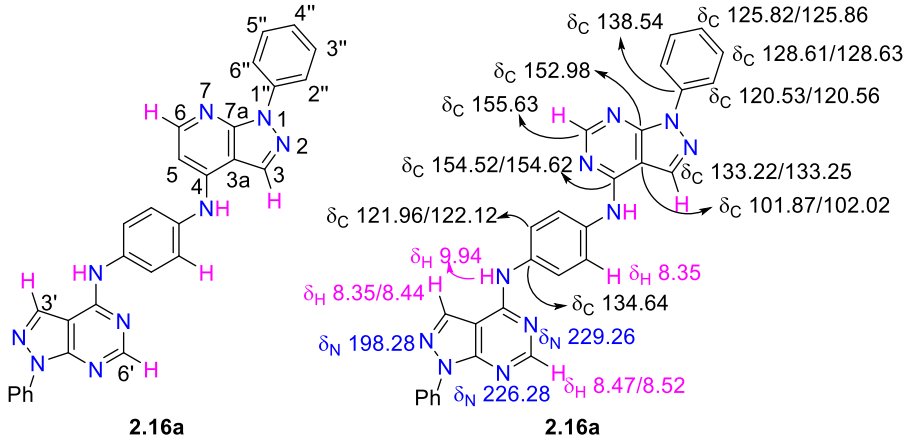


Figure 2.15: Carbon numbering used for chemical shift assignment (left) and characterization data (^{1}H, ^{13}C, ^{15}N) for compound **2.16a** (right).

Compound **2.17l** was formed by a side reaction when the 4-aminoaniline **2.13l** reacted with acetic acid present in the reaction mixture. The reaction was repeated at 60°C for 5.5 hours (entry 15). A mixture of compounds **2.10a** and **2.13l** was isolated in a 3.2:1 molar ratio, by ^{1}H NMR. When the mixture was maintained at 60°C and the reaction time was increased to 24 hours, followed by 3 days at 80°C, the product **2.15l** was isolated in 6% yield. In the mother liquor, a mixture of the starting materials was identified, by ^{1}H NMR. It is possible that the yield of this reaction could be improved if it was carried out for a longer time period.

Compound **2.10a** was reacted with **2.13m** and a 1:1 mixture of the starting materials was isolated

after 5.5 hours (entry 17). The reaction most likely needs more time to allow for the formation of the desired product. However, after 5.5 hours the reaction mixture started darkening and the products were isolated, even though the TLC showed the presence of the starting material.

The reaction of compound **2.10b** with 3-methoxyaniline **2.13e** and *m*-toluidine **2.13g** originated a complex mixture containing **2.15m** and **2.15o**, respectively, by ^{1}H NMR (entries 18 and 20). The combination of **2.10b** with 4-methoxyaniline **2.13f** led to compound **2.15n** isolated in a low yield (4%, entry 19) probably due to the high solubility of this product in the reaction mixture. In the mother liquor, a complex mixture containing **2.15n** was identified, by ^{1}H NMR. In the reactions with compound **2.10b**, the desired product is formed, but it is very difficult to isolate. In the first two cases (entries 18 and 20), it was not possible to separate the product by using different solvent mixtures. The presence of the fluorine atom in the 2 position of the aromatic ring may be responsible for a stereochemical impediment. For this reason, reactions with this substituent were abandoned.

Compound **2.10c** was reacted with amines **2.13a**, **2.13d-h** and **2.13j** originating the respective pyrazolo[3,4-*d*]pyrimidine derivatives **2.15p**, **2.15r-v** and **2.15x**, isolated in 22% to 54% yield (entries 21, 23-27 and 29). These compounds were very soluble in all the organic solvents that were used (alcohols, acetone, ethyl acetate, diethyl ether, dioxane, tetrahydrofuran, petroleum ether). The reaction between **2.10c** and 3-aminophenol **2.13c** originated a complex mixture containing **2.15q** (entry 22). It was not possible to isolate product **2.15q** because it was highly soluble in all the organic solvents referred above. A mixture of **2.15w** and **2.17i** in a 1:3.4 molar ratio was obtained when compound **2.10c** was reacted with 4-bromoaniline **2.13i** (entry 28).

The reaction between compound **2.10d** and **2.13a**, **2.13d** and **2.13f-g** originated the respective derivatives **2.15y-ab** in 42% to 52% yield (entries 30-33). Once again it was verified that compounds with fluoro-substituted aromatic rings are very soluble, which justifies their moderate isolated yield. The mother liquor was a complex mixture containing **2.15y-ab**, by ^{1}H NMR. When compound **2.10d** reacted with 3-bromoaniline **2.13h**, a mixture of compounds **2.15ac** and **2.17h** in a 1:1 molar ratio (by ^{1}H NMR) was isolated (entry 34).

Compound **2.10f** was reacted with amines **2.13a** and **2.13c-j**, leading to the corresponding pyrazolo[3,4-*d*]pyrimidines derivatives **2.15ad-al** in 30% to 79% yield (entries 35-43). The reaction of **2.10f** with **2.13k** originated a mixture of compounds **2.15am** and **2.10f** in a 2.5:1 molar ratio, by ^{1}H NMR (entry 44). In the mother liquor, the signals for **2.10f** were identified and the amine signals were not observed, indicating that probably the reaction required more amine in order to consume the remaining starting material **2.10f**. A complex mixture was obtained when compound **2.10f** was reacted

with *p*-phenylenediamine **2.13l** (entry 45) and this reaction was not investigated further.

The derivatives **2.15an-ao** and **2.15ap-ar** (48-59% yield) were obtained when **2.10g** was reacted with **2.13a**, **2.13d**, **2.13f-g** and **2.13j** (entries 46-47 and 49-51). The reaction of compound **2.10g** with **2.13e** originated a complex mixture (entry 48) and was abandoned.

Compound **2.10h** was reacted with **2.13a**, **2.13d-g** and **2.13i-j** originating compounds **2.15as, 2.15at-aw** and **2.15ax-ay**, respectively, in 46-68% yield (entries 52, 54-57 and 59-60). Complex mixtures were obtained when compound **2.10h** was reacted with amines **2.13c** or **2.13h** (entries 53 and 58). When compound **2.10h** was reacted with 4-aminobenzonitrile **2.13k**, a mixture of **2.15az** and **2.10h** was formed in a 3.3:1 molar ration, by ^{1}H NMR (entry 61). In the mother liquor, only the starting material **2.10h** was identified indicating that more amine would be necessary in order to consume the remaining pyrazole.

The reaction between compound **2.10i** and aniline **2.13a**, 4-aminophenol **2.13d** and 4-methoxyaniline **2.13f**, generated the respective compounds **2.15ba-bc** in 51% to 66% yield (entries 62-64). A mixture of **2.15bd** and **2.17i** in a 1:1 molar ratio was isolated when **2.10i** was reacted with **2.13i** (entry 65).

Compound **2.10j** was reacted with 4-methoxyaniline **2.13f** leading to derivative **2.15be** in 11% yield (entry 66). The very low isolated yield is due to the fact that we started with a very small amount of compound **2.10j** (14 mg) and, upon filtration, the solid was almost all retained on the filter paper.

The reaction between compound **2.10k** and **2.13a**, **2.13d-f** and **2.13j** originated the pure derivatives **2.15bf-bi** and **2.15bk** in 11-57% yield (entries 67-70 and 73). A complex mixture was obtained when **2.10k** was reacted with 3-methylaniline **2.13g** (entry 71). The reaction of **2.10k** and **2.13h** originated a complex mixture where signals for **2.15bj** and **2.17h** were identified, by ^{1}H NMR (entry 72). Due to the solubility of product **2.15bg** it was not possible to separate it from the product mixture.

Compound **2.10m** was reacted with amine **2.13l** leading to a mixture of **2.15bl** and **2.17l** in a 1:3.2 molar ratio, by ^{1}H NMR (entry 74). Since the amount of **2.17l** (amino-acetylate) was too high compared with **2.15bl**, the quantity of amine was reduced to 1.2 equivalents (entry 75). The pure compound **2.15bl** was obtained in 62% yield.

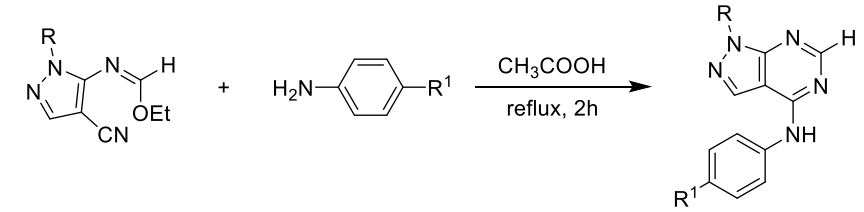
A complex mixture was obtained when compound **2.10n** was reacted with 1.2 equivalents of aniline **2.13a** (entry 76). Compound **2.10n** was reacted with 1 molar equivalent of 4-methoxyaniline **2.13f** and 4-chloroaniline **2.13j** originating derivatives **2.15bm-bn** in 41% and 46% yield, respectively (entries 77-78).

The pyrazolo[3,4-*d*]pyrimidine **2.15bo** was obtained in 67% yield from the reaction of **2.10r** with 2 equivalents of 4-methoxyaniline **2.13f** under reflux (entry 79). In a second crop the pure compound **2.17f** was isolated in 33% yield. The reaction of **2.10r** and 4-chloroaniline **2.13j** led to a complex mixture (entry 80).

In some cases, the ^{1}H NMR spectrum showed the presence of a secondary product, identified as the corresponding acetylated amine **2.17**. The contaminated solid was washed with cold ethanol to remove the acetylated amine but the pyrazolo[3,4-*d*]pyrimidine derivative **2.15** was also partially solubilized, reducing the isolated yield of the pure product.

Another method for the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives uses the reaction of imidate **2.6** (method B) with primary aromatic amines **2.13**, under reflux in acetic acid. Table **2.31** summarizes the experimental conditions that were used to prepare compounds **2.15**.

Table 2.31: Experimental conditions for the reaction of imidates **2.6** with primary aromatic amines **2.13**



2.6a. R=C_6H_5
2.6b. R=2-F-C_6H_4
2.6f. R=4-COOH-C_6H_4
2.6r. R=4-COOEt-C_6H_4

2.13a. R^1=H
2.13f. R^1=4-OMe

2.15f. R=C_6H_5, R^1=4-OMe
2.15n. R=2-F-C_6H_4, R^1=4-OMe
2.15ad. R=4-COOH-C_6H_4, R^1=H
2.15ah. R=4-COOH-C_6H_4, R^1=4-OMe
2.15bo. R=4-COOEt-C_6H_4, R^1=4-OMe

Entry	Reagents		Experimental conditions	Product (yield)
	2.6.	**2.13.**		
1	**2.6a.** 0.43 mmol	**2.13f.** 1 eq.	CH_3COOH (500 µL), reflux, 1.5 h	**2.15f.** 54%
2	**2.6b.** 0.15 mmol	**2.13f.** 1 eq.	CH_3COOH (500 µL), reflux, 2 h	Mixture containing **2.15n** [a]
3	**2.6f.** 0.11 mmol	**2.13a.** 1 eq.	CH_3COOH (500 µL), reflux, 45 min	**2.15ad.** 83%
4	**2.6f.** 0.08 mmol	**2.13f.** 1 eq.	CH_3COOH (500 µL), reflux, 45 min	**2.15ah.** 56%
5	**2.6r.** 0.09 mmol	**2.13f.** 1 eq.	CH_3COOH (500 µL), reflux, 2.5 h	**2.15bo.** 46%

[a] By ^{1}H NMR.

The reaction of compounds **2.6a**, **2.6f**, and **2.6r** with aromatic amines **2.13a** or **2.13f** under reflux of acetic acid for 45 minutes to 2.5 hours, successfully generated the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **2.15** (entries 1, 3-5). The products were collected in 46-83% yield.

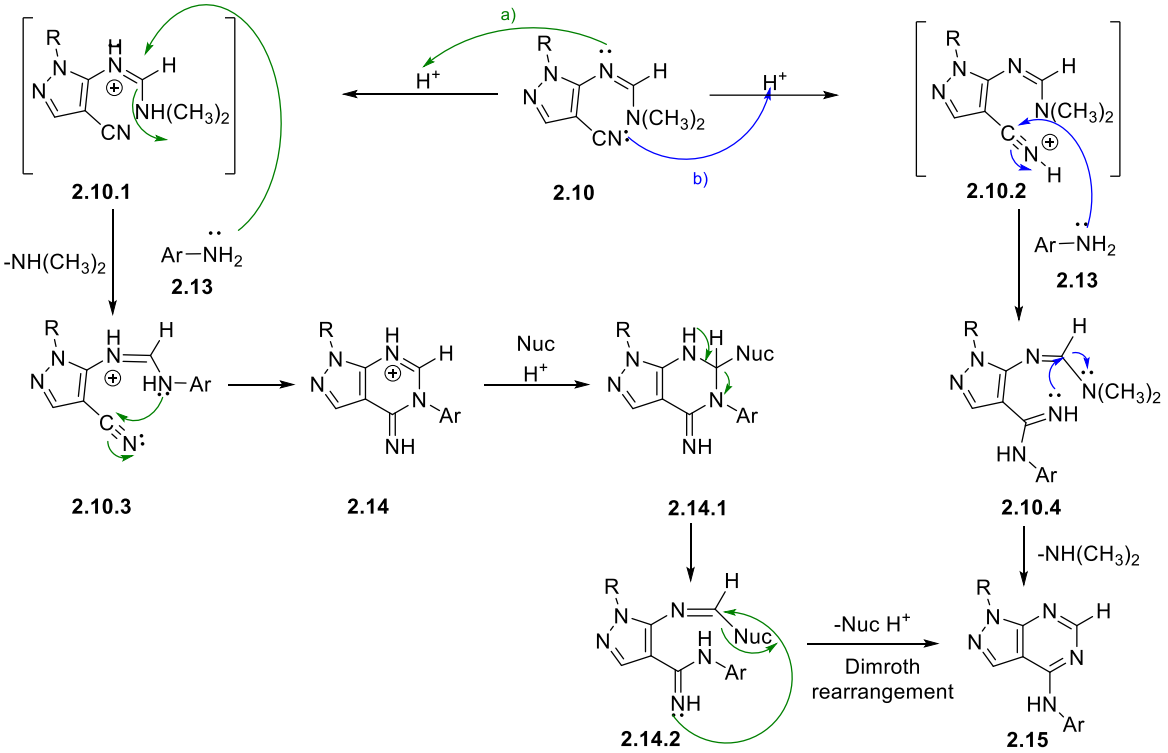
The reaction of compound **2.6b** with 4-methoxyaniline **2.10f** (1 eq.) under reflux in acetic acid for 2 hours, led to a mixture containing product **2.15n**, as confirmed by ^{1}H NMR (entry 2). The product

could not be selectivity separated from the reaction mixture using the combination of different solvents.

Comparing the yields and experimental conditions of methods A (from amidine) and B (from imidate), we can conclude that they are both very similar. Method A was selected to prepare most of the pyrazolo[3,4-*d*]pyrimidine derivatives, as the amidine **2.10** was prepared at room temperature, while the imidate **2.6** required heating at 150°C.

The mechanism proposed for the formation of pyrazolo[3,4-*d*]pyrimidine derivatives **2.15** from the reaction of amidine **2.10** with aromatic amines **2.13** is presented in Scheme **2.5**. In acid medium, we can consider that compound **2.10** has two possible protonation sites: either the amidine function, leading to an intermediate **2.10.1** that evolves through pathway a) or the nitrile, leading to an unstable nitrilium salt **2.10.2** that rapidly evolves through pathway b). Following pathway a), nucleophilic attack by the pair of electrons of the amine group of **2.13** to intermediate **2.10.1**, would giving rise to **2.10.3**, which undergoes intramolecular cyclization, generating compound **2.14**. In acid media, a nucleophilic species (possibly the solvent) can attack the pyrimidine ring, generating the intermediate **2.14.1**. Ring opening leading to **2.14.2**, followed by ring closure to generate the more stable aromatic structure leads to the pyrazolo[3,4-*d*]pyrimidine **2.15** (Dimroth rearrangement).

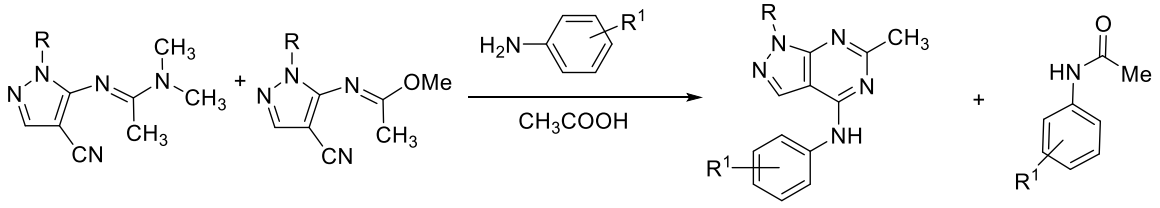
Following pathway b), nucleophilic attack by the pair of electrons of the amine group of **2.13** occurs on the positively charged carbon atom of the nitrilium salt in **2.10.2**, leading to **2.10.4**. Pyrazolo[3,4-*d*]pyrimidine **2.15** can be formed from **2.10.4** by intramolecular cyclization.



Scheme 2.5: Proposed mechanism for the formation of pyrazolo[3,4-*d*]pyrimidine derivatives **2.15**.

The mixture of amidines **2.11** and **2.12** was also used to react with aromatic amines to synthesize pyrazolo[3,4-*d*]pyrimidine derivatives **2.18**. Reactions occurred at 118°C for 4.5-5 hours and CH_3COOH (400-500 µL) was used as solvent. Table **2.32** summarizes the experimental conditions that were used.

Table 2.32: Experimental conditions for the reaction of **2.11** and **2.12** with aromatic amines **2.13**



2.11a. R=C_6H_5
2.11c. R=3-F-C_6H_4
2.11d. R=4-F-C_6H_4
2.11f. R=4-CO_2H-C_6H_4
2.11g. R=4-Me-C_6H_4
2.11h. R=4-Cl-C_6H_4
2.11i. R=4-Br-C_6H_4
2.11n. R= CH_2COOEt

2.12a. R=C_6H_5
2.12c. R=3-F-C_6H_4
2.12d. R=4-F-C_6H_4
2.12f. R=4-CO_2H-C_6H_4
2.12g. R=4-Me-C_6H_4
2.12h. R=4-Cl-C_6H_4
2.12i. R=4-Br-C_6H_4
2.12n. R= CH_2COOEt

2.13a. R^1=H
2.13e R^1=3-OMe
2.13f. R^1=4-OMe
2.13g. R^1=3-Me
2.13j. R^1=4-Cl

2.18a. R=C_6H_5, R^1=4-OMe
2.18b. R=3-F-C_6H_4, R^1=4-OMe
2.18c. R=3-F-C_6H_4, R^1=4-Cl
2.18d. R=4-F-C_6H_4, R^1=3-Me
2.18e. R=4-Me-C_6H_4, R^1=H
2.18f. R=4-Cl-C_6H_4, R^1=3-Me
2.18g. R=4-Br-C_6H_4, R^1=4-OMe
2.18h. R= CH_2COOEt, R^1=H

2.17a. R^1=H
2.17g. R^1=3-Me
2.17j. R^1=4-Cl

Entry	Reagents		Experimental conditions	Product (yield)
	2.11. + **2.12.**	**2.13.**		
1	**a.** 0.45 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 4.5 h	**2.18a.** 14%
2	**c.** 0.24 mmol	**2.13f.** 2 eq.	CH_3COOH (500 µL), 118°C, 5 h	**2.18b.** 17%

3	**c.** 0.24 mmol	**2.13j.** 2 eq.	CH_3COOH (500 µL), 118°C, 5 h	**2.18c.** 17%
4	**d.** 0.17 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.18d.** 27%
5	**f.** 0.21 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	Complex mixture [a)]
6	**g.** 0.22 mmol	**2.13a.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.18e** + **2.17a** (4.3:1) [a)]
7	**g.** 0.23 mmol	**2.13e.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	Complex mixture [a)]
8	**h.** 0.21 mmol	**2.13g.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.18f** + **2.17g** (5.5:1) [a)]
9	**i.** 0.17 mmol	**2.13f.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.18g.** 34%
10	**n.** 0.21 mmol	**2.13a.** 1 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.18h** + **2.17a** (5.7:1) [a)]
11	**n.** 0.25 mmol	**2.13j.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.17j.** 56%

[a)] By ^1H NMR.

The reaction of compounds **2.11a** + **2.12a** and **2.11c-d** + **2.12c-d** with aromatic amines **2.13f**, **2.13g** or **2.13j** at 118°C for 4.5 hours to 5 hours, successfully generated the corresponding pyrazolo[3,4-*d*]pyrimidines derivatives **2.18a-d** (entries 1-4).The products were collected in 14-27% yield. The poor isolated yield may be due to an incomplete reaction as all the experiments we performed for 5 hours. The procedure requires improvement and increasing the reaction time is a viable possibility to be tested.

The complex mixture was obtained when compounds **2.11f** + **2.12f** and **2.11g** + **2.12g** reacted with amines 3-methoxyaniline **2.13e** and 4-chloroaniline **2.13j**, by ^1H NMR (entries 5 and 7).

The reaction of compounds **2.11g** + **2.12g** and **2.11h** + **2.12h** with 2 equivalents of aniline **2.13a**, led to a mixture of compounds **2.18e** + **2.17a** and **2.18f** + **2.7g** in a 4.3:1 and 5.5:1 molar ratio, respectively, by ^1H NMR (entries 6 and 8).

Compound **2.18g** was obtained in 34% yield, from the reaction of **2.11i** and **2.12i** with 2 equivalents of 4-methoxyaniline **2.13f** (entry 9).

The reaction of compounds **2.11n** + **2.12n** with aniline **2.13a** (1 eq.) generated a mixture of compounds **2.18h** and **2.17a** in a 5.7:1 molar ratio, by ^1H NMR (entry 10). When compounds **2.11n** + **2.12n** were reacted with 2 equivalents of 4-chloroaniline **2.13j** only acetylated amine **2.17j** was isolated in 56% yield (entry 11). The mother liquor still contained a large amount of acetylated amine **2.17j** and a small amount of the desired product. In this case, acetylation of the amine seems to be the preferential pathway, considerably decreasing the amount of free amine to participate in the formation of the desired pyrazolo[3,4-*d*]pyrimidine derivative **2.18**.

2.4.2. Reaction with other amines

Amidines **2.10** were also combined with heteroaromatic, cyclic and alkyl amines for the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives and the experimental conditions were summarized in Table **2.33**.

The product **2.20** was isolated in 34% of yield when compound **2.10a** was combined with 3-aminopyrazole **2.19** at 118°C for 6 hours (entry 1).

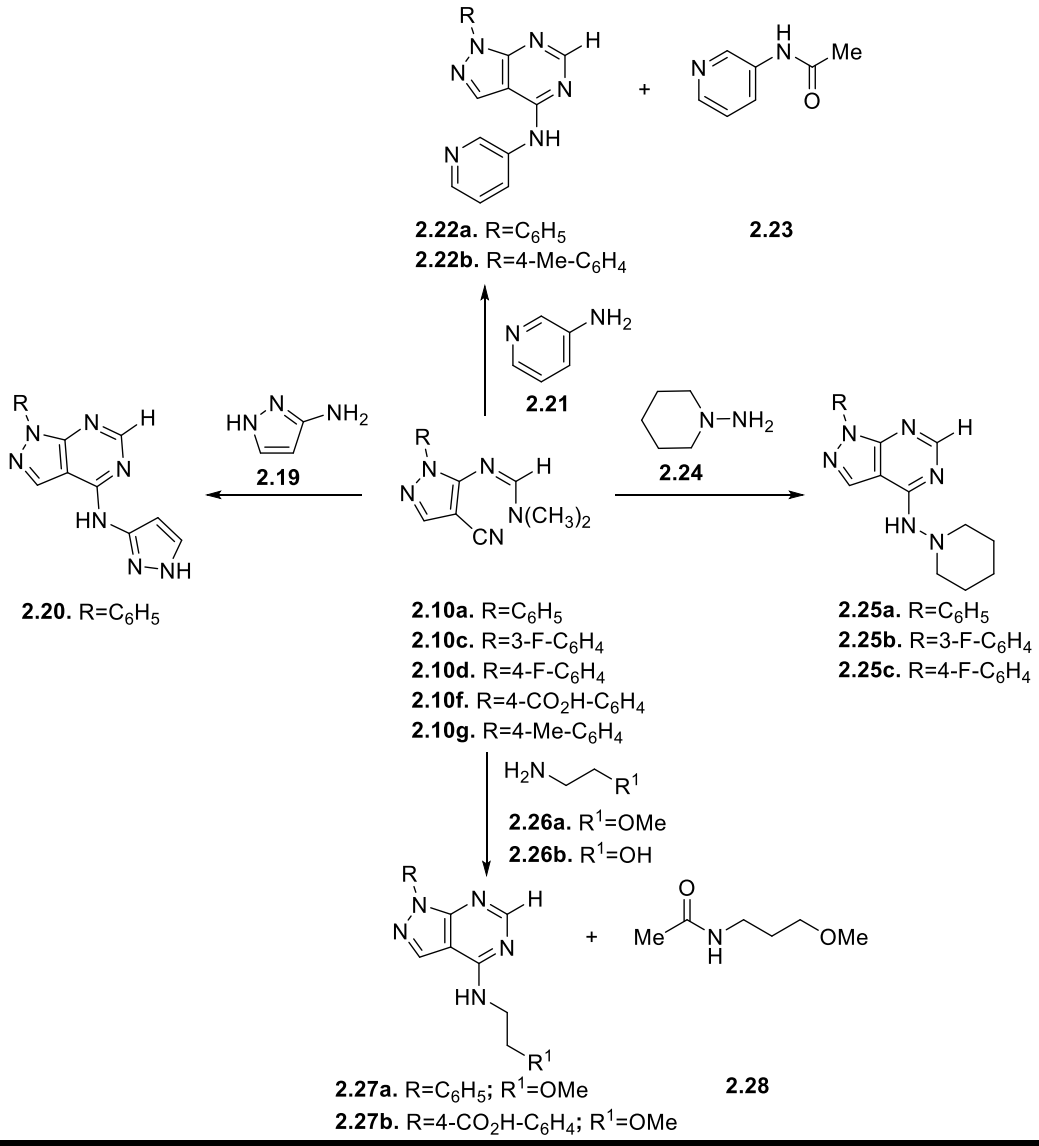
The reaction of compound **2.10a** with 3-aminopyridine **2.22** originated a mixture of compounds **2.23a** and **2.23** in a 1:1 molar ratio, by ^{1}H NMR (entry 2). The pure product **2.23b** was isolated in 54% yield, from the reaction of amidine **2.10g** with 3-aminopyridine 22 (entry 3).

Compounds **2.10a** and **2.10c** were reacted with 1-aminopiperidine **2.24** originating derivatives **2.25a-b** in 38% and 25% yield, respectively (entries 4-5). When amidine **2.10d** was reacted with the same amine **2.24,** a complex mixture containing **2.25c** was obtained (entry 6). The product could not be selectively separated from the reaction mixture using the combination of different solvents.

The reaction of compounds **2.10a** with 2-methoxyethylamine **2.26a** led to the isolation of a mixture of compounds **2.27a** and **2.28** in a 2.5:1 molar ratio, by ^{1}H NMR (entry 7). The pure product **2.27b** was isolated in 28% yield from the reaction of amidine **2.10f** with 2-methoxyethylamine **2.26a** (entry 9).

A complex mixture was obtained when compounds **2.10a** and **2.10f** were reacted with amine **2.26b** (entries 8 and 10).

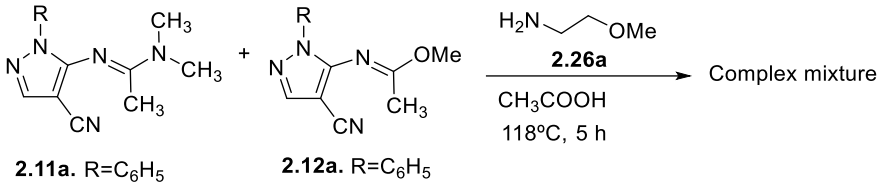
Table 2.33: Experimental conditions for the reaction of **2.10** with amines **2.19**, **2.21**, **2.24** and **2.26**



Entry	Reagents		Experimental conditions	Product (yield)
	2.10.	Amines		
1	**2.10a.** 0.23 mmol	**2.19.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.20.** 34%
2	**2.10a.** 0.22 mmol	**2.21.** 2 eq.	CH_3COOH (400 µL), 118°C, 6 h	**2.22a** + **2.23** (1:1) [a]
3	**2.10g.** 0.24 mmol	**2.21.** 2 eq.	CH_3COOH (400 µL), 118°C, 5.5 h	**2.22b.** 54%
4	**2.10a.** 0.23 mmol	**2.24.** 2 eq.	CH_3COOH (400 µL), 118°C, 6.5 h	**2.25a.** 38%
5	**2.10c.** 0.23 mmol	**2.24.** 2 eq.	CH_3COOH (400 µL), 118°C, 5 h	**2.25b.** 25%
6	**2.10d.** 0.23 mmol	**2.24.** 2 eq.	CH_3COOH (400 µL), 118°C, 6.5 h	Complex mixture containing **2.25c** [a]
7	**2.10a.** 0.25 mmol	**2.26a.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	**2.27a + 2.28** (2.5:1) [a]
8	**2.10a.** 0.23 mmol	**2.26b.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	Complex mixture [a]
9	**2.10f.** 0.18 mmol	**2.26a.** 2 eq.	CH_3COOH (400 µL), 118°C, 6.5 h	**2.27b.** 28%
10	**2.10f.** 0.19 mmol	**2.26b.** 2 eq.	CH_3COOH (400 µL), 118°C, 4 h	Complex mixture [a]

[a] By ^1H NMR.

The mixture of substituted pyrazoles **2.11a** and **2.12a** was reacted with 2-methoxyethylamine **2.26a** (2 eq.) in CH_3COOH (400 μL) at 118°C (Scheme **2.6**). After 5 hours, an oil was isolated and identified as a complex mixture, by ^1H NMR.



2.11a. R=C_6H_5 **2.12a.** R=C_6H_5

Scheme 2.6: Reaction of substituted pyrazoles **2.11a** and **2.12a** with 2-methoxyethylamine **2.26a**.

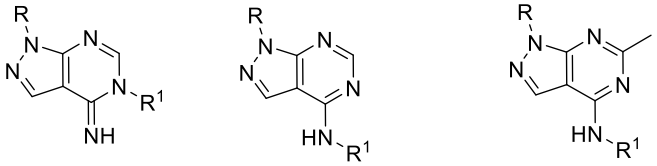
The reaction of amidine **2.10**, **2.11** and **2.12** with these amines originated the desired products although in low yield and some complex mixtures. Further studies will be required in order to optimize the reaction, varying, for example, the amount of amine, the acid used, the temperature and the reaction time.

2.4.3. Analytical and spectroscopic characterization

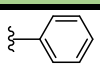
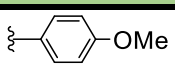
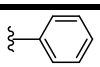
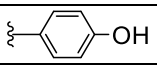
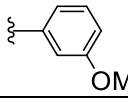
- **Physical and analytical data**

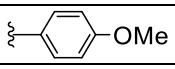
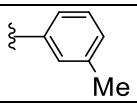
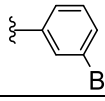
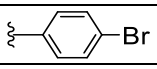
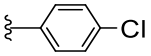
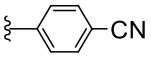
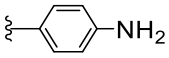
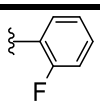
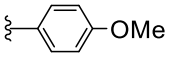
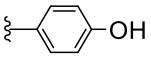
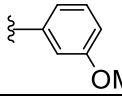
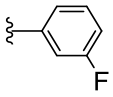
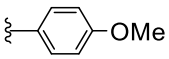
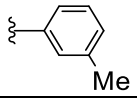
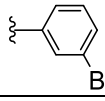
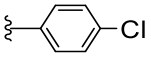
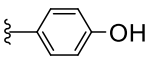
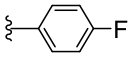
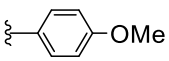
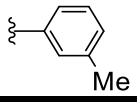
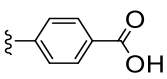
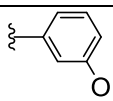
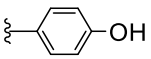
Table **2.34** presents the melting point range and the isolated yield for all pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27**. Compounds herein presented will be later submitted to elemental analysis.

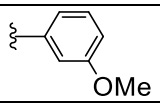
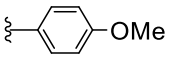
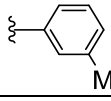
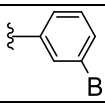
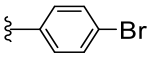
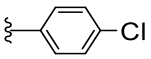
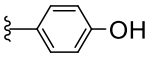
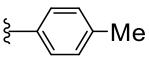
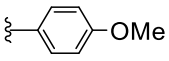
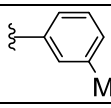
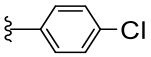
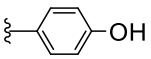
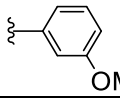
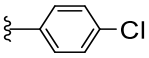
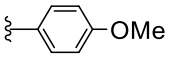
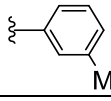
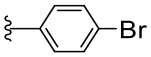
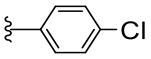
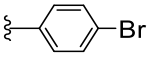
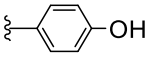
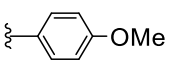
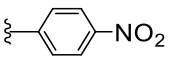
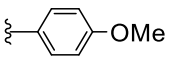
Table 2.34: Physical and analytical data for pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27**

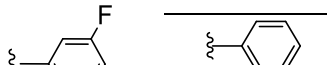
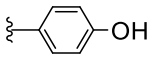
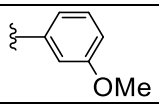
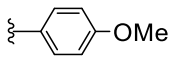
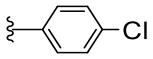
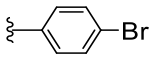
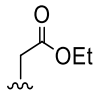
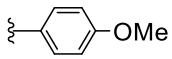
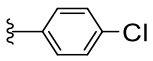
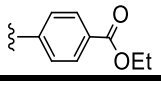
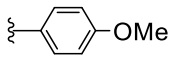
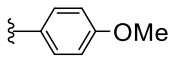
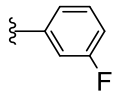
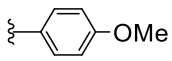
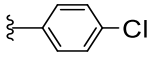
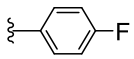
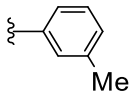
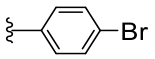
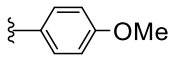
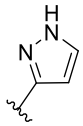
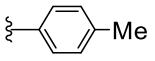
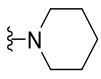
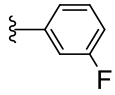
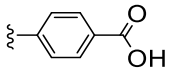
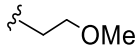


2.14f **2.15**, **2.20**, **2.22**, **2.25**, **2.27** **2.18**

Comp.	R	R^1	Yield (%)	m.p. (°C)	Chemical Formula	Calculated values (%)		
						C	H	N
2.14f			22	[a]	$C_{18}H_{15}N_5O$	68.13	4.76	22.07
2.15a			55	195-197	$C_{17}H_{13}N_5$	71.06	4.56	24.37
2.15d			70	257-259	$C_{17}H_{13}N_5O$	67.32	4.32	23.09
2.15e			68	199-201	$C_{18}H_{15}N_5O$	68.13	4.76	22.07

2.15f			75	213-215	$C_{18}H_{15}N_5O$	68.13	4.76	22.07
2.15g			60	196-198	$C_{18}H_{15}N_5$	71.74	5.02	23.24
2.15h			52	191-193	$C_{17}H_{12}BrN_5$	55.75	3.30	19.12
2.15i			67	212-214	$C_{17}H_{12}BrN_5$	55.75	3.30	19.12
2.15j			79	184-186	$C_{17}H_{12}ClN_5$	63.46	3.76	21.77
2.15k			45	274-276	$C_{18}H_{12}N_6$	69.22	3.87	26.91
2.15l			6	[a]	$C_{17}H_{14}N_6$	67.54	4.67	27.80
2.15n			4	158-160	$C_{18}H_{14}FN_5O$	64.47	5.67	20.88
2.15p			32	192-194	$C_{17}H_{12}FN_5$	66.88	3.96	22.94
2.15r			53	258-260	$C_{17}H_{12}FN_5O$	63.55	3.76	21.80
2.15s			48	189-191	$C_{18}H_{14}FN_5O$	64.47	5.67	20.88
2.15t			54	191-193	$C_{18}H_{14}FN_5O$	64.47	5.67	20.88
2.15u			48	213-215	$C_{18}H_{14}FN_5$	67.70	4.42	21.93
2.15v			22	211-213	$C_{17}H_{11}BrFN_5$	53.14	2.89	18.23
2.15x			52	195-197	$C_{17}H_{11}ClFN_5$	60.10	3.26	20.61
2.15y			42	170-172	$C_{17}H_{12}FN_5$	66.88	3.96	22.94
2.15z			50	257-259	$C_{17}H_{12}FN_5O$	63.55	3.76	21.80
2.15aa			52	166-168	$C_{18}H_{14}FN_5O$	64.47	5.67	20.88
2.15ab			47	198-200	$C_{18}H_{14}FN_5$	67.70	4.42	21.93
2.15ad			79	264-266	$C_{18}H_{13}N_5O_2$	65.25	3.95	21.14
2.15ae			67	313-315	$C_{18}H_{13}N_5O_3$	62.24	3.77	20.16
2.15af			76	295-297	$C_{18}H_{13}N_5O_3$	62.24	3.77	20.16

2.15ag			62	315-317	$C_{19}H_{15}N_5O_3$	63.15	4.18	19.38
2.15ah			73	293-295	$C_{19}H_{15}N_5O_3$	63.15	4.18	19.38
2.15ai			62	320-322	$C_{19}H_{15}N_5O_2$	66.08	4.38	20.28
2.15aj			30	317-319	$C_{18}H_{12}BrN_5O_2$	52.70	2.95	17.07
2.15ak			39	230-232	$C_{18}H_{12}BrN_5O_2$	52.70	2.95	17.07
2.15al			57	333-335	$C_{18}H_{12}ClN_5O_2$	59.11	3.31	19.15
2.15an			48	180-182	$C_{18}H_{15}N_5$	71.74	5.02	23.24
2.15ao			46	>360	$C_{18}H_{15}N_5O$	68.13	4.76	22.07
2.15ap			53	196-198	$C_{20}H_{19}N_5O$	69.55	5.54	20.28
2.15aq			59	183-185	$C_{19}H_{17}N_5$	72.36	5.43	22.21
2.15ar			48	221-223	$C_{18}H_{14}ClN_5$	64.38	4.20	20.86
2.15as			48	210-212	$C_{17}H_{12}ClN_5$	63.46	3.76	21.77
2.15at			53	238-240	$C_{17}H_{12}ClN_5O$	60.45	3.58	20.73
2.15au			53	201-203	$C_{18}H_{14}ClN_5O$	61.46	4.01	19.91
2.15av			62	183-185	$C_{18}H_{14}ClN_5O$	61.46	4.01	19.91
2.15aw			46	215-217	$C_{18}H_{14}ClN_5$	64.38	4.20	20.86
2.15ax			57	>360	$C_{17}H_{11}BrClN_5$	50.96	2.77	17.48
2.15ay			68	223-225	$C_{17}H_{11}Cl_2N_5$	57.32	3.11	19.66
2.15ba			52	230-232	$C_{17}H_{12}BrN_5$	55.75	3.30	19.12
2.15bb			66	232-234	$C_{17}H_{12}BrN_5O$	53.42	3.16	18.32
2.15bc			51	217-219	$C_{18}H_{14}BrN_5O$	54.56	3.56	17.67
2.15be			11	[a]	$C_{18}H_{14}N_6O_3$	59.67	3.89	23.19

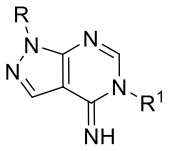
2.15bf			11	[a]	$C_{17}H_{11}F_2N_5$	63.16	3.43	21.66
2.15bg			57	266-268	$C_{17}H_{11}F_2N_5O$	60.18	3.27	20.64
2.15bh			40	165-167	$C_{18}H_{13}F_2N_5O$	61.19	3.71	19.82
2.15bi			55	185-187	$C_{18}H_{13}F_2N_5O$	61.19	3.71	19.82
2.15bk			38	258-260	$C_{17}H_{10}ClF_2N_5$	57.08	2.82	19.58
2.15bl	H		62	283-285	$C_{11}H_8BrN_5$	45.54	2.78	24.14
2.15bm			41	132-134	$C_{16}H_{17}N_5O_3$	58.71	5.23	21.39
2.15bn			46	195-197	$C_{15}H_{14}ClN_5O_2$	54.31	4.25	21.11
2.15bo			67	218-220	$C_{21}H_{19}N_5O_3$	64.77	4.92	17.98
2.18a			14	198-200	$C_{19}H_{17}N_5O$	68.87	5.17	21.13
2.18b			17	212-214	$C_{19}H_{16}FN_5O$	65.32	4.62	20.05
2.18c			17	188-190	$C_{18}H_{13}ClFN_5$	61.11	3.70	19.80
2.18d			27	234-236	$C_{19}H_{16}FN_5$	68.46	4.84	21.01
2.18g			34	228-230	$C_{19}H_{16}BrN_5O$	55.62	3.93	17.07
2.20			34	218-220	$C_{14}H_{11}N_7$	60.64	4.00	35.36
2.22b			54	258-260	$C_{17}H_{14}N_6$	67.54	4.67	27.80
2.25a			38	196-198	$C_{16}H_{18}N_6$	65.29	6.16	28.55
2.25b			25	203-205	$C_{16}H_{17}FN_6$	61.53	5.49	26.91
2.27b			28	264-266	$C_{15}H_{15}N_5O_3$	57.50	4.83	22.35

[a] The isolated amount was not enough for m.p. measurement.

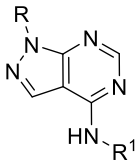
- **Infrared Spectroscopy**

Table **2.35** shows the data of the IR spectra of pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27**. The weak to medium intensity bands in the 2700-3500 cm^{-1} range correspond to the stretching vibrations of the N-H and C_{sp2}-H bonds. Compound **2.15k** showed an intense band at 2221 cm^{-1} attributed to the stretching vibration of the CN group. The stretching vibration of the carbonyl group of the compounds led to an intense band between 1669 and 1751 cm^{-1}.

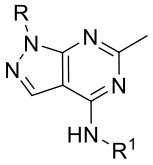
Table 2.35: IR spectroscopic data (FTIR-ATR) of the pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27**



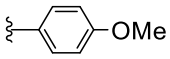
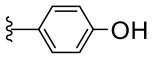
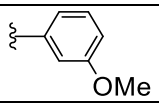
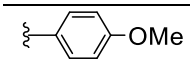
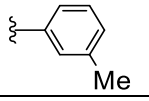
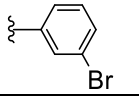
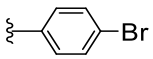
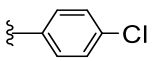
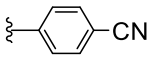
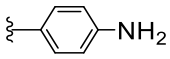
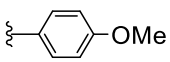
2.14f

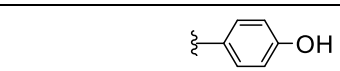
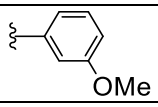
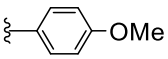
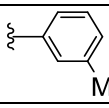
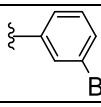
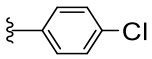
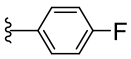
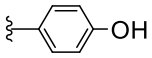
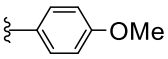
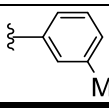
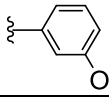
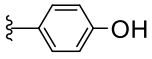
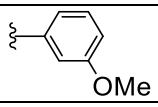
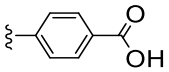
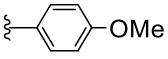
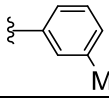
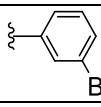
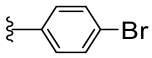
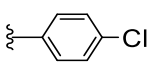
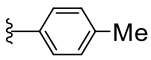
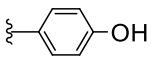


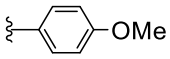
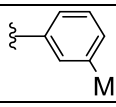
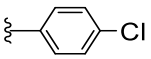
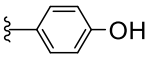
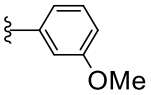
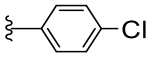
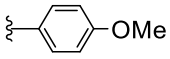
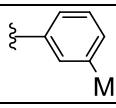
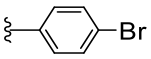
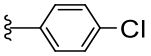
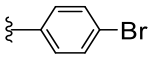
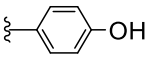
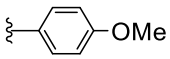
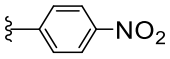
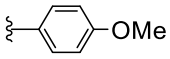
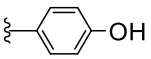
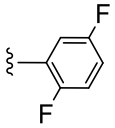
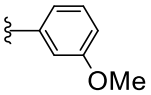
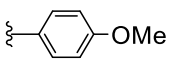
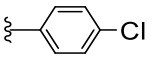
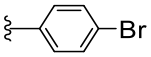
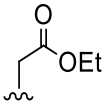
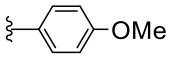
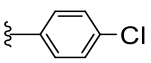
2.15, **2.20**, **2.22**, **2.25**, **2.27**

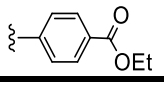
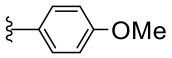
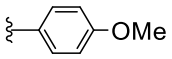
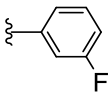
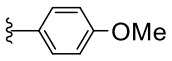
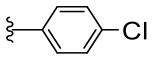
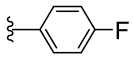
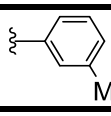
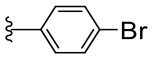
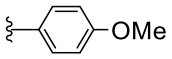
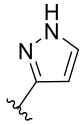
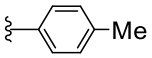
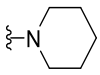
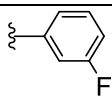
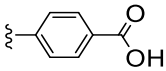
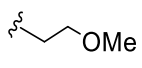


2.18

Comp.	R	R^1	4000-2700	CN	CO	1700-1500
2.14f			[a]			
2.15a			3196l, 3057l, 2870w	–	–	1604m, 1579i, 1537w, 1503i
2.15d			3268l, 3060w, 2872l, 2801w	–	–	1587i, 1505i
2.15e			3336m, 3105w, 2958l	–	–	1627m, 1608m, 1584i, 1567i, 1534m
2.15f			3239w, 3123w, 2997l	–	–	1615m, 1580i, 1502i
2.15g			3213w, 2857l	–	–	1606m, 1590m, 1575i, 1501i
2.15h			3341m, 3102w	–	–	1622i, 1592m, 1575m, 1563i, 1526m
2.15i			3339m	–	–	1622i, 1599m, 1577m, 1561i, 1526m, 1501i
2.15j			3308w, 3216w, 3125l	–	–	1624i, 1599m, 1563i, 1528m, 1501i
2.15k			3330m, 3217w, 3126w	2221i	–	1626i, 1609m, 1598m, 1564i, 1525m, 1505i
2.15l			[a]			
2.15n			3285m, 3130w, 3065w	–	–	1628i, 1580m, 1568i, 1533m, 1505i
2.15p			3278l, 2902l	–	–	1605m, 1569i, 1539m

2.15r			3181w, 2877l, 2806w	—	—	1590i, 1511m
2.15s			3167w, 3005l, 2920l	—	—	1575i, 1539sh
2.15t			3125m, 2999m, 2838w	—	—	1615m, 1580i, 1502i
2.15u			3229w, 3015w	—	—	1608m, 1594i, 1574i
2.15v			3303l	—	—	1597m, 1568i, 1540w
2.15x			3201l, 3062w, 2872l	—	—	1600i, 1582i, 1574m, 1534m
2.15y			3243l, 2841l	—	—	1616m, 1577i, 1542sh, 1506i
2.15z			3242l, 2877l	—	—	1621sh, 1599i, 1514i
2.15aa			3240w, 3074w, 3005l	—	—	1645w, 1616m, 1576i, 1506i
2.15ab			3401l, 3095w, 2820l	—	—	1609m, 1595m, 1569i, 1506i
2.15ad			2800-3500 broad fringed band, 2959l	—	1672m	1604i, 1585i, 1568i, 1534m
2.15ae			3177l	—	1673m	1593i, 1518m
2.15af			2800-3400 broad fridged band, 2770l	—	1673m	1673sh, 1601i, 1513i
2.15ag			2800-3300 broad fridged band, 2951l	—	1669m	1588i, 1574i, 1514i
2.15ah			2800-3500 broad fridged band, 2961l	—	1674m	1594i, 1575i, 1507i
2.15ai			3205l, 2928w	—	1675m	1597i, 1575i, 1515i
2.15aj			3199w, 3062w, 2928l	—	1680m	1602m, 1568i, 1539w, 1515i
2.15ak			2700-3300 broad fridged band, 2951l	—	1674m	1600i, 1575i, 1558m, 1514i
2.15al			2700-3350 broad fridged band, 2968l	—	1675m	1603i, 1580i, 1563i
2.15an			3311m, 3222w, 3127w	—	—	1627i, 1611m, 1580i, 1559i, 1515m
2.15ao			3198w, 3030w	—	—	1607m, 1514i

2.15ap			3223l, 3000w, 2838w	—	—	1591i, 1509i
2.15aq			3310m, 3127w	—	—	1630i, 1603m, 1586i, 1560i, 1539m
2.15ar			3303w, 3125w	—	—	1634i, 1612m, 1576i, 1562i, 1533m, 1516i
2.15as			3292l, 3056w, 2800l	—	—	1606m, 1575i, 1558m, 1539w
2.15at			3393l, 3022l, 2873w	—	—	1655w, 1601i, 1589i, 1539w, 1511m
2.15au			3367m, 3097w, 2961w	—	—	1627m, 1585i, 1563i, 1532m
2.15av			3213l, 2907l, 2836w	—	–	1615m, 1575i, 1539m
2.15aw			3237w, 2834l	—	—	1611m, 1569i, 1540m
2.15ax			3224l, 2947w	—	—	1602m, 1567i
2.15ay			3290w, 3118w	—	—	1660m, 1603i, 1532i
2.15ba			3206l, 3051w, 2803l	—	—	1606m, 1583i, 1575i, 1538m
2.15bb			3240l, 2935w	—	—	1599i, 1585m, 1575m
2.15bc			3183w, 2836l	—	—	1614m, 1576i, 1540m, 1511i
2.15be			[a]			
2.15bf			[a]			
2.15bg			3134l, 2939w	—	—	1611sh, 1582i, 1563i, 1539m, 1511i
2.15bh			3247l, 3080w, 2959l	—	—	1694i, 1576m, 1548w, 1521i
2.15bi			3286w, 3131w, 3073l, 2836w	—	—	1626i, 1575i, 1533m, 1512i, 1507i
2.15bk			3421m, 3122w	—	—	1625i, 1585i, 1576sh, 1517i
2.15bl	H		3205l, 3164w, 2931l, 2880w	—	—	1597m, 1575i, 1540m
2.15bm			3311l, 2936l	—	1751i	1610m, 1576m, 1511i
2.15bn			3365m, 2985m	—	1735i	1623i, 1574i, 1538m

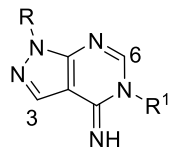
2.15bo			3373m, 2935w	—	1751i	1611m, 1576i, 1549m, 1511i
2.18a			3186l, 2908w	—	—	1576i, 1502i
2.18b			3196w, 2910l	—	—	1600m, 1581i, 1539m, 1511m
2.18c			3290l, 3188w, 3115w	—	—	1660m, 1599i, 1548i
2.18d			3298l, 3187l, 2910l	—	—	1667m, 1610w, 1576i, 1507i
2.18g			3172l, 3003l	—	—	1579i, 1538m,1510i
2.20			3271w, 3160l, 2969w, 2877w	—	—	1659i, 1593i, 1558m,
2.22b			3291l, 3192w, 3123w, 3014w	—	—	1683m, 1632i, 1576m, 1558i, 1533m, 1516i
2.25a			3401l, 3215l, 3062w, 2813w	—	—	1612i, 1588i, 1545w, 1502i
2.25b			3401l, 3215w, 3091l	—	—	1611i, 1599i, 1549i, 1507i
2.27b			3243w, 2946w	—	1680i	1600i, 1514m

[a)] The isolated amount was not enough to make the IR spectrum.

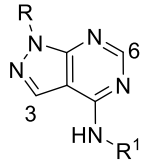
- **^{1}H-NMR Spectroscopy**

Table **2.36** summarizes the ^{1}H NMR signals assigned to pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.20**, **2.22**, **2.25** and **2.27**. For these compounds, the signal for H-3 and H-6 appears as a singlet between δ_H 8.03-8.71 ppm and δ_H 8.32-8.72 ppm, respectively. The amine protons appear as a singlet or a broad singlet between δ_H 8.57-11.10 ppm. The ^{1}H NMR spectrum of compound **2.15al** is presented in Figure **2.16**, with some key signals assigned. For pyrazolo[3,4-*d*]pyrimidine derivatives **2.18**, the signal for H-3 appears as a singlet between δ_H 8.10-8.56 ppm (Table **2.37**). The CH_3 protons also appear as singlets between δ_H 2.48-2.59 ppm. The amine protons appear as a broad singlet between δ_H 9.71-10.23 ppm.

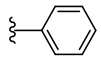
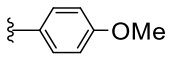
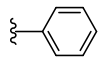
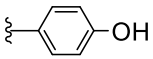
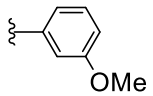
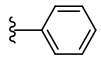
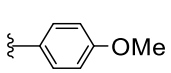
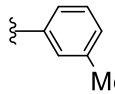
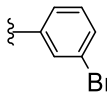
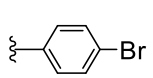
Table 2.36: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.20**, **2.22**, **2.25** and **2.27**

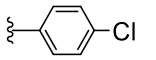
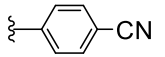
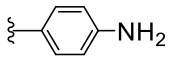
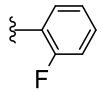
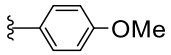
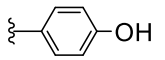
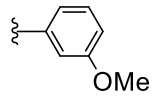
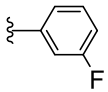
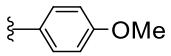
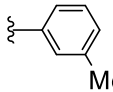
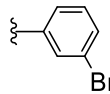
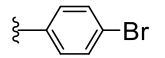


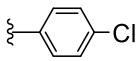
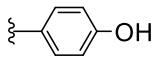
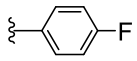
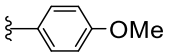
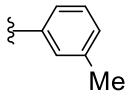
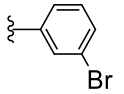
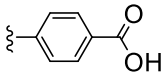
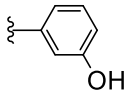
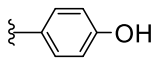
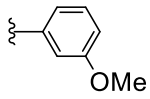
2.14f

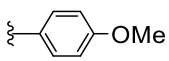
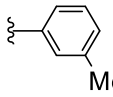
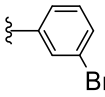
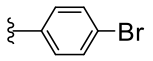
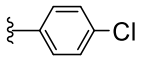
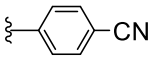
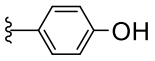
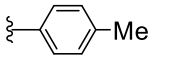
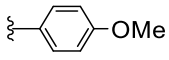
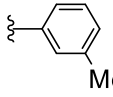
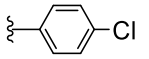


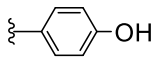
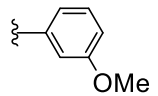
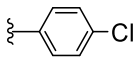
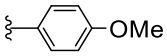
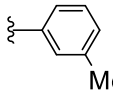
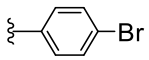
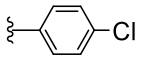
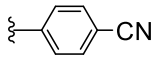
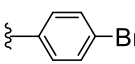
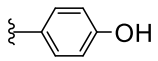
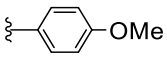
2.15, **2.20**, **2.22**, **2.25**, **2.27**

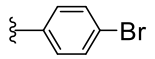
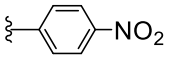
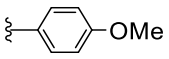
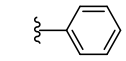
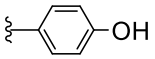
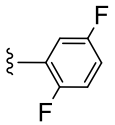
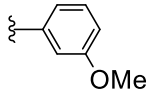
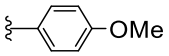
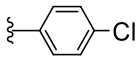
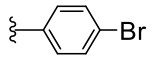
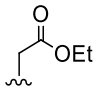
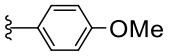
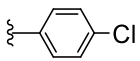
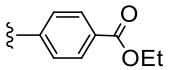
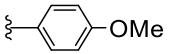
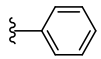
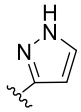
Comp.	R	R^1	C-H	NH	R	R^1
2.14f			8.18 (s, 1H, H_3) 8.32 (s, 1H, H_6)	11.10 (s, 1H)	8.02 (dd, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 7.6 Hz), 7.56 (td, 2H, $H_{3'}$ + $H_{5'}$, *J* 1.2 Hz, 7.6 Hz), 7.40 (td, 1H, $H_{4'}$, *J* 1.2 Hz, 7.6 Hz)	7.58 (d, 2H, $H_{2''}$ + $H_{6''}$, *J* 8.8 Hz), 6.95 (dd, 2H, $H_{3''}$ + $H_{5''}$, *J* 8.8 Hz), 3.70 (s, 3H, OCH_3)
2.15a [a)]			8.44 (s, 1H, H_3) 8.51 (s, 1H, H_6)	9.98 (s, 1H)	8.20 (dt, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 7.6 Hz), 7.55 (td, 2H, $H_{3'}$ + $H_{5'}$, *J* 1.2 Hz, 7.6 Hz), 7.37 (td, 1H, $H_{4'}$, *J* 1.2 Hz, 7.6 Hz)	7.82 (dd, 2H, $H_{2''}$ + $H_{6''}$, *J* 1.2 Hz, 7.6 Hz), 7.41 (td, 2H, $H_{3''}$ + $H_{5''}$, *J* 1.2 Hz, 7.6 Hz), 7.16 (d, 1H, $H_{4''}$, *J* 7.6 Hz)
2.15d			8.38 (brs, 1H, H_3) 8.42 (s, 1H, H_6)	9.99 (s, 1H)	8.18 (d, 2H, $H_{2'}$ + $H_{6'}$, *J* 8.0 Hz), 7.55 (d, 2H, $H_{3'}$ + $H_{5'}$, *J* 8.0 Hz), 7.34 (tt, 1H, $H_{4'}$, *J* 1.2 Hz, 8.0 Hz)	9.39 (brs, 1H, OH), 7.52 (brs, 2H, $H_{2''}$ + $H_{6''}$), 6.81 (d, 2H, $H_{3''}$ + $H_{5''}$, *J* 8.8 Hz)
2.15e [a)]			8.46 (s, 1H, H_3) 8.52 (s, 1H, H_6)	9.94 (brs, 1H)	8.19 (dt, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 8.8 Hz), 7.57 (dt, 2H, $H_{3'}$ + $H_{5'}$, *J* 1.2 Hz, 8.8 Hz), 7.37 (tt, 1H, $H_{4'}$, *J* 1.2 Hz, 8.8 Hz)	7.53 (s, 1H, $H_{2''}$), 7.42 (dd, 1H, $H_{6''}$, *J* 1.6 Hz, 8.4 Hz), 7.31 (t, 1H, $H_{5''}$, *J* 8.4 Hz), 6.73 (ddd, 1H, $H_{4''}$, *J* 1.6 Hz, 2.4 Hz, 8.4 Hz), 3.81 (s, 3H, OCH_3)
2.15f [a)]			8.26 (brs, 1H, H_3) 8.44 (s, 1H, H_6)	9.85 (brs, 1H)	8.19 (dt, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 7.2 Hz), 7.54 (tt, 2H, $H_{3'}$ + $H_{5'}$, *J* 1.2 Hz, 7.2 Hz), 7.34 (tt, 1H, $H_{4'}$, *J* 1.2 Hz, 7.2 Hz)	7.65 (dd, 2H, $H_{2''}$ + $H_{6''}$, *J* 2.0 Hz, 8.8 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, *J* 2.0 Hz, 8.8 Hz), 3.79 (s, 3H, OCH_3)
2.15g [a)]			8.38 (s, 1H, H_3) 8.48 (s, 1H, H_6)	9.89 (brs, 1H)	8.16 (dt, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 7.2 Hz), 7.54 (tt, 2H, $H_{3'}$ + $H_{5'}$, *J* 1.2 Hz, 7.2 Hz), 7.34 (tt, 1H, $H_{4'}$, *J* 1.2 Hz, 7.2 Hz)	7.62 (s, 1H, $H_{2''}$), 7.60 (s, 1H, $H_{6''}$), 7.28 (t, 1H, $H_{5''}$, *J* 7.2 Hz), 6.98 (d, 1H, $H_{4''}$, *J* 7.2 Hz), 2.34 (s, 1H, CH_3)
2.15h [a)]			8.54 (s, 1H, H_3) 8.57 (s, 1H, H_6)	10.11 (brs, 1H)	8.19 (d, 2H, $H_{2'}$ + $H_{6'}$, *J* 7.2 Hz), 7.56 (td, 2H, $H_{3'}$ + $H_{5'}$, *J* 2.0 Hz, 7.2 Hz), 7.37 (dd, 1H, $H_{4'}$, *J* 2.0 Hz, 7.2 Hz)	8.21 (brs, 1H, $H_{2''}$), 7.84 (dd, 1H, $H_{6''}$, *J* 1.2 Hz, 8.0 Hz), 7.34 (t, 1H, $H_{5''}$, *J* 8.0 Hz), 7.30 (dt, 1H, $H_{4''}$, *J* 1.2 Hz, 8.0 Hz)
2.15i [a)]			8.52 (s, 1H, H_3) 8.53 (s, 1H, H_6)	10.08 (brs, 1H)	8.19 (dt, 2H, $H_{2'}$ + $H_{6'}$, *J* 1.2 Hz, 7.6 Hz), 7.53 (t, 2H, $H_{3'}$ + $H_{5'}$, *J* 7.6 Hz), 7.36 (tt, 1H, $H_{4'}$, *J* 1.2 Hz, 7.6 Hz)	7.84 (dt, 2H, $H_{2''}$ + $H_{6''}$, *J* 2.0 Hz, 8.8 Hz), 7.56 (dt, 2H, $H_{3''}$ + $H_{5''}$, *J* 2.0 Hz, 8.8 Hz)

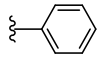
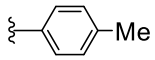
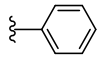
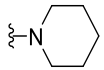
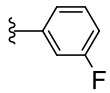
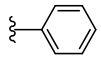
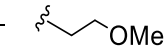
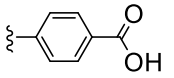
2.15j [a)]			8.51 (s, 1H, H_3) 8.53 (s, 1H, H_6)	10.09 (brs, 1H)	8.19 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 1.2 Hz, 7.2 Hz), 7.56 (tt, 2H, $H_{3'}$ + $H_{5'}$, J 1.2 Hz, 8.8 Hz), 7.36 (tt, 1H, $H_{4'}$, J 1.2 Hz, 7.2 Hz)	7.89 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 7.43 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)
2.15k [a)]			8.59 (s, 1H, H_3) 8.60 (s, 1H, H_6)	10.36 (brs, 1H)	8.16 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 7.2 Hz), 7.55 (tt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz), 7.37 (tt, 1H, $H_{4'}$, J 2.0 Hz, 7.2 Hz)	8.11 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 7.2 Hz), 7.79 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 7.2 Hz)
2.15l			8.51 (s, 1H, H_3) 8.60 (s, 1H, H_6)	10.37 (brs, 1H)	8.13 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.0 Hz), 7.59 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 8.0 Hz), 7.21 (td, 1H, $H_{4'}$, J 2.4 Hz, 8.8 Hz)	NH_2 (not visible in the spectrum), 7.86 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 7.64 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz)
2.15n [a)]			8.24 (brs, 1H, H_3) 8.34 (s, 1H, H_6)	9.86 (brs, 1H)	7.64 (dd, 1H, $H_{6'}$, J 1.2 Hz, 8.4 Hz), 7.56 (m, 1H, $H_{4'}$), 7.45 (td, 1H, $H_{3'}$, J 1.2 Hz, 8.4 Hz), 7.39 (m, 1H, $H_{5'}$)	7.66 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.8 Hz), 3.80 (s, 3H, OCH_3)
2.15p [a)]			8.46 (s, 1H, H_3) 8.53 (s, 1H, H_6)	10.03 (s, 1H)	8.10-8.16 (m, 1H, $H_{6'}$), 8.10 (t, 1H, $H_{2'}$, J 2.0 Hz), 7.59 (q, 1H, $H_{5'}$, J 2.0 Hz), 7.15-7.19 (m, 1H, $H_{4'}$)	7.81 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 1.2 Hz, 6.8 Hz), 7.41 (tt, 2H, $H_{3''}$ + $H_{5''}$, J 1.2 Hz, 6.8 Hz), 7.12-7.16 (m, 1H, $H_{4''}$)
2.15r [a)]			8.12 (brs, 1H, H_3) 8.44 (s, 1H, H_6)	9.79 (brs, 1H)	8.08-8.12 (m, 2H, $H_{2'}$ + $H_{6'}$), 7.56 (q, 1H, $H_{5'}$, J 2.4 Hz), 7.13 (tdd, 1H, $H_{4'}$, J 0.8 Hz, 2.4 Hz, 8.4 Hz)	9.15 (brs, 1H, OH), 7.47 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.83 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)
2.15s			8.55 (brs, 1H, H_3) 8.56 (s, 1H, H_6)	10.20 (brs, 1H)	8.12-8.15 (m, 1H, $H_{6'}$), 8.11 (s, 1H, $H_{2'}$), 7.58 (q, 1H, $H_{5'}$, J 2.4 Hz), 7.16-7.20 (m, 1H, $H_{4'}$)	7.53 (s, 1H, $H_{2''}$), 7.42 (dd, 1H, $H_{6''}$, J 1.2 Hz, 8.4 Hz), 7.30 (t, 1H, $H_{5''}$, J 8.4 Hz), 6.71 (ddd, 1H, $H_{4''}$, J 1.2 Hz, 2.4 Hz, 8.4 Hz), 3.78 (s, 3H, OCH_3)
2.15t [a)]			8.28 (brs, 1H, H_3) 8.47 (s, 1H, H_6)	9.90 (brs, 1H)	8.08-8.14 (m, 2H, $H_{2'}$ + $H_{6'}$), 7.58 (q, 1H, $H_{5'}$, J 1.2 Hz), 7.14 (tdd, 1H, $H_{4'}$, J 1.2 Hz, 2.4 Hz, 8.4 Hz)	7.65 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.99 (d, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz), 3.80 (s, 3H, OCH_3)
2.15u			8.52 (brs, 1H, H_3) 8.54 (s, 1H, H_6)	10.16 (s, 1H)	8.13 (d, 1H, $H_{6'}$, J 8.8 Hz), 8.10 (s, 1H, $H_{2'}$), 7.58 (tdd, 1H, $H_{5'}$, J 2.4 Hz, 8.8 Hz), 7.16 (tdd, 1H, $H_{4'}$, J 1.2 Hz, 2.4 Hz, 8.8 Hz)	7.64 (s, 1H, $H_{2''}$), 7.62 (s, 1H, $H_{6''}$), 7.28 (td, 1H, $H_{5''}$, J 2.0 Hz, 7.6 Hz), 6.96 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.33 (s, 1H, CH_3)
2.15v			8.61 (s, 1H, H_3) 8.63 (s, 1H, H_6)	10.09 (brs, 1H)	8.11-8.14 (m, 2H, $H_{2'}$ + $H_{6'}$), 7.61 (q, 1H, $H_{5'}$, J 2.4 Hz), 7.18-7.21 (m, 1H, $H_{4'}$)	8.27 (brs, 1H, $H_{2''}$), 7.81 (d, 1H, $H_{6''}$, J 8.0 Hz), 7.37 (t, 1H, $H_{5''}$, J 8.0 Hz), 7.31 (ddd, 1H, $H_{4''}$, J 0.8 Hz, 2.0 Hz, 8.0 Hz)
2.15w			8.60 (s, 1H, H_3) 8.60 (s, 1H, H_6)	10.04 (s, 1H)	8.12-8.15 (m, 2H, $H_{2'}$ + $H_{6'}$), 7.58 (q, 1H, $H_{5'}$, J 2.4 Hz), 7.19-7.21 (m, 1H, $H_{4'}$)	7.86 (d, 2H, $H_{2''}$ + $H_{6''}$, J 9.2 Hz), 7.54 (d, 2H, $H_{3''}$ + $H_{5''}$, J 9.2 Hz)

2.15x [a)]			8.53 (s, 1H, H_3) 8.56 (s, 1H, H_6)	10.13 (brs, 1H)	8.10-8.16 (m, 2H, $H_{2'}$ + $H_{6'}$), 7.56-7.62 (m, 1H, $H_{5'}$), 7.15-7.17 (m, 1H, $H_{4'}$)	7.88 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 7.44 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)
2.15y [a)]			8.42 (s, 1H, H_3) 8.50 (s, 1H, H_6)	10.00 (brs, 1H)	8.21 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 5.2 Hz, 8.8 Hz), 7.37 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	7.81 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 1.2 Hz, 7.2 Hz), 7.41 (dd, 2H, $H_{3''}$ + $H_{5''}$, J 1.2 Hz, 7.2 Hz), 7.14 (tt, 1H, $H_{4''}$, J 1.2 Hz, 7.2 Hz)
2.15z [a)]			8.11 (brs, 1H, H_3) 8.40 (s, 1H, H_6)	9.79 (brs, 1H)	8.20 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 4.8 Hz, 8.8 Hz), 7.35 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	OH (not visible in the spectrum), 7.47 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 6.83 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)
2.15aa [a)]			8.24 (brs, 1H, H_3) 8.43 (s, 1H, H_6)	9.87 (brs, 1H)	8.20 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 5.2 Hz, 8.8 Hz), 7.36 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	7.64 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 6.8 Hz), 6.99 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 6.8 Hz), 3.79 (s, 3H, OCH_3)
2.15ab			8.50 (brs, 1H, H_3) 8.52 (s, 1H, H_6)	10.15 (s, 1H)	8.21 (dd, 2H, $H_{2'}$ + $H_{6'}$ J 4.8 Hz, 8.8 Hz), 7.40 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	7.66 (s, 1H, $H_{2''}$), 7.65 (s, 1H, $H_{6''}$), 7.28 (t, 1H, $H_{5''}$, J 7.2 Hz), 6.96 (d, 1H, $H_{4''}$, J 7.2 Hz), 2.34 (s, 1H, CH_3)
2.15ac			8.58 (s, 1H, H_3) 8.60 (s, 1H, H_6)	10.34 (brs, 1H)	8.22 (dd, 2H, $H_{2'}$ + $H_{6'}$ J 5.2 Hz, 8.8 Hz), 7.42 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	8.28 (s, 1H, $H_{2''}$), 7.82 (d, 1H, $H_{6''}$, J 8.0 Hz), 7.36 (t, 1H, $H_{5''}$, J 8.0 Hz), 7.24 (s, 1H, $H_{4''}$)
2.15ad [a)]			8.50 (s, 1H, H_3) 8.55 (s, 1H, H_6)	10.06 (brs, 1H)	OH (not visible in the spectrum), 8.42 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 7.2 Hz), 8.12 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz)	7.82 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 1.2 Hz, 8.4 Hz), 7.41 (tt, 2H, $H_{3''}$ + $H_{5''}$, J 1.2 Hz, 8.4 Hz), 7.17 (tt, 1H, $H_{4''}$, J 1.2 Hz, 8.4 Hz)
2.15ae [a)]			8.50 (s, 1H, H_3) 8.56 (s, 1H, H_6)	9.85 (brs, 1H)	12.64 (brs, 1H, OH), 8.42 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 7.2 Hz), 8.13 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz)	OH (not visible in the spectrum), 7.38 (s, 1H, $H_{2''}$), 7.22 (d, 1H, $H_{6''}$, J 8.0 Hz), 7.19 (d, 1H, $H_{5''}$, J 8.0 Hz), 6.61 (ddd, 1H, $H_{4''}$, J 0.8 Hz, 2.4 Hz, 8.0 Hz)
2.15af			8.58 (brs, 1H, H_3) 8.46 (s 1H, H_6)	10.05 (s, 1H)	12.73 (brs, 1H, OH), 8.40 (d, 2H, $H_{2'}$ + $H_{6'}$, J 7.2 Hz), 8.10 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz)	9.44 (brs, 1H, OH), 7.51 (brs, 2H, $H_{2''}$ + $H_{6''}$), 6.81 (d, 2H, $H_{3''}$ + $H_{5''}$, J 7.2 Hz)
2.15ag			8.58 (s, 1H, H_3) 8.59 (brs, 1H, H_6)	10.24 (brs, 1H)	OH (not visible in the spectrum), 8.42 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 7.2 Hz), 8.11 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 7.2 Hz)	7.54 (s, 1H, $H_{2''}$), 7.43 (dd, 1H, $H_{6''}$, J 1.2 Hz, 8.0 Hz), 7.31 (t, 1H, $H_{5''}$, J 8.0 Hz), 6.72 (ddd, 1H, $H_{4''}$, J 0.8 Hz, 2.4 Hz, 8.0 Hz), 3.78 (s, 3H, OCH_3)

2.15ah [a)]			8.31 (brs, 1H, H_3) 8.48 (s, 1H, H_6)	9.92 (brs, 1H)	OH (not visible in the spectrum), 8.40 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 7.2 Hz), 8.11 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz)	7.65 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 6.8 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 6.8 Hz), 3.79 (s, 3H, OCH_3)
2.15ai			8.57 (brs, 1H, H_3) 8.59 (brs, 1H, H_6)	10.20 (s, 1H)	OH (not visible in the spectrum), 8.41 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 8.8 Hz), 8.11 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 8.8 Hz)	7.66 (s, 1H, $H_{2''}$), 7.64 (s, 1H, $H_{6''}$), 7.29 (td, 1H, $H_{5''}$, J 2.0 Hz, 7.6 Hz), 6.96 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.34 (s, 1H, CH_3)
2.15aj [a)]			8.59 (s, 1H, H_3) 8.60 (s, 1H, H_6)	10.13 (brs, 1H)	OH (not visible in the spectrum), 8.42 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 8.12 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	8.20 (brs, 1H, $H_{2''}$), 7.83 (ddd, 1H, $H_{6''}$, J 1.2 Hz, 2.4 Hz, 8.0 Hz), 7.35 (t, 1H, $H_{5''}$, J 8.0 Hz), 7.30 (ddd, 1H, $H_{4''}$, J 1.2 Hz, 2.4 Hz, 8.0 Hz)
2.15ak			8.57 (s, 1H, H_3) 8.59 (brs, 1H, H_6)	10.32 (s, 1H)	12.92 (brs, 1H, OH), 8.40 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 6.8 Hz), 8.10 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 6.8 Hz)	7.84 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 6.8 Hz), 7.55 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 6.8 Hz)
2.15al			8.58 (s, 1H, H_3) 8.60 (brs, 1H, H_6)	10.33 (s, 1H)	12.91 (brs, 1H, OH), 8.40 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 8.11 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	7.89 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 8.8 Hz), 7.44 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.8 Hz)
2.15am			8.71 (s, 1H, H_3) 8.72 (s, 1H, H_6)	10.65 (s, 1H)	OH (not visible in the spectrum), 8.42 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz), 8.13 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	8.15 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz), 7.87 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz)
2.15an			8.50 (brs, 1H, H_3) 8.51 (s, 1H, H_6)	10.20 (brs, 1H)	8.05 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.4 Hz), 7.36 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.4 Hz), 2.36 (s, 3H, CH_3)	7.85 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 8.4 Hz), 7.40 (tt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.4 Hz), 7.14 (tt, 1H, $H_{4''}$, J 2.0 Hz, 8.4 Hz)
2.15ao [a)]			8.09 (brs, 1H, H_3) 8.39 (s, 1H, H_6)	9.00-10.00 (brs, 2H, NH + OH)	8.03 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz), 7.33 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz), 2.37 (s, 3H, CH_3)	9.00-10.00 (brs, 2H, NH + OH), 7.47 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.82 (d, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz)
2.15ap [a)]			8.23 (brs, 1H, H_3) 8.42 (s, 1H, H_6)	9.82 (brs, 1H)	8.04 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.4 Hz), 7.34 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.4 Hz), 2.37 (s, 3H, CH_3)	7.65 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 9.2 Hz), 6.99 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 9.2 Hz), 3.79 (s, 3H, OCH_3)
2.15aq			8.48 (brs, 1H, H_3) 8.51 (s, 1H, H_6)	10.12 (s, 1H)	8.05 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.4 Hz), 7.35 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.4 Hz), 2.36 (s, 3H, CH_3)	7.66 (s, 1H, $H_{2''}$), 7.65 (s, 1H, $H_{6''}$), 7.28 (td, 1H, $H_{5''}$, J 2.0 Hz, 7.6 Hz), 6.95 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.34 (s, 1H, CH_3)
2.15ar [a)]			8.48 (s, 1H, H_3) 8.52 (s, 1H, H_6)	10.07 (brs, 1H)	8.05 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 8.8 Hz), 7.35 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 8.8 Hz), 2.38 (s, 3H, CH_3)	7.89 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 7.43 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)

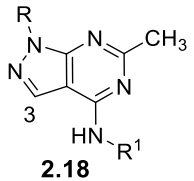
2.15as [a)]			8.44 (s, 1H, H_3) 8.51 (s, 1H, H_6)	10.02 (brs, 1H)	8.26 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 6.8 Hz), 7.59 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 6.8 Hz)	7.80 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 8.4 Hz), 7.41 (tt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.4 Hz), 7.16 (tt, 1H, $H_{4''}$, J 2.0 Hz, 8.4 Hz)
2.15at [a)]			8.14 (brs, 1H, H_3) 8.42 (s, 1H, H_6)	9.80 (brs, 1H)	8.25 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 6.8 Hz), 7.58 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 6.8 Hz)	9.15 (brs, 1H, OH), 7.47 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 6.82 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 8.8 Hz)
2.15au [a)]			8.47 (s, 1H, H_3) 8.53 (s, 1H, H_6)	9.98 (brs, 1H)	8.26 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 7.59 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	7.51 (s, 1H, $H_{2''}$), 7.40 (ddd, 1H, $H_{6''}$, J 0.8 Hz, 2.4 Hz, 8.0 Hz), 7.30 (t, 1H, $H_{5''}$, J 8.0 Hz), 6.74 (ddd, 1H, $H_{4''}$, J 0.8 Hz, 2.4 Hz, 8.0 Hz), 3.80 (s, 3H, OCH_3)
2.15av [a)]			8.27 (brs, 1H, H_3) 8.45 (s, 1H, H_6)	9.88 (brs, 1H)	8.26 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 6.8 Hz), 7.58 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 6.8 Hz)	7.64 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 6.8 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 6.8 Hz), 3.79 (s, 3H, OCH_3)
2.15aw			8.50 (brs, 1H, H_3) 8.52 (s, 1H, H_6)	10.14 (s, 1H)	8.25 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 7.2 Hz), 7.60 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz)	7.64 (s, 1H, $H_{6''}$), 7.64 (s, 1H, $H_{2''}$), 7.28 (t, 1H, $H_{5''}$, J 7.6 Hz), 6.95 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.33 (s, 3H, CH_3)
2.15ax			8.56 (s, 1H, H_3) 8.57 (brs, 1H, H_6)	10.33 (brs, 1H)	8.26 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 7.62 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	7.85 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 7.57 (d, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz)
2.15ay [a)]			8.52 (s, 1H, H_3) 8.54 (s, 1H, H_6)	10.11 (s, 1H)	8.26 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 6.8 Hz), 7.60 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 6.8 Hz)	7.87 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 6.8 Hz), 7.43 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.4 Hz, 6.8 Hz)
2.15az			8.54 (s, 1H, H_3) 8.67 (s, 1H, H_6)	10.62 (s, 1H)	8.26 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 6.8 Hz), 7.58 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 6.8 Hz)	8.15 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz), 7.86 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz)
2.15ba [a)]			8.45 (s, 2H, H_3 + H_6)	10.02 (brs, 1H)	8.27 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz), 7.65 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	7.81 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 1.2 Hz, 6.8 Hz), 7.41 (t, 2H, $H_{3''}$ + $H_{5''}$, J 6.8 Hz), 7.16 (tt, 1H, $H_{4''}$, J 1.2 Hz, 6.8 Hz)
2.15bb [a)]			8.14 (brs, 1H, H_3) 8.42 (s, 1H, H_6)	9.76 (brs, 1H)	8.20 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 9.2 Hz), 7.72 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 9.2 Hz)	9.34 (brs, 1H, OH), 7.47 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.83 (d, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz)
2.15bc [a)]			8.27 (brs, 1H, H_3) 8.45 (s, 1H, H_6)	9.88 (brs, 1H)	8.20 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.4 Hz, 8.8 Hz), 7.72 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 2.4 Hz, 8.8 Hz)	7.64 (dd, 2H, $H_{2''}$ + $H_{6''}$, J 2.4 Hz, 8.8 Hz), 7.00 (dd, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.8 Hz), 3.80 (s, 3H, OCH_3)

2.15ad			8.53 (s, 1H, H_3) 8.55 (s, 1H, H_6)	10.12 (brs, 1H)	8.21 (d, 2H, $H_{2'}$ + $H_{6'}$, J8.8 Hz), 7.74 (d, 2H, $H_{3'}$ + $H_{5'}$, J8.8 Hz)	7.83 (d, 2H, $H_{2''}$ + $H_{6''}$, J8.8 Hz), 7.56 (d, 2H, $H_{3''}$ + $H_{5''}$, J8.8 Hz)
2.15be			8.50 (brs, 1H, H_3) 8.55 (s, 1H, H_6)	10.23 (brs, 1H)	8.62 (d, 2H, $H_{3'}$ + $H_{5'}$, J9.2 Hz), 8.45 (d, 2H, $H_{2'}$ + $H_{6'}$, J9.2 Hz)	7.67 (brs, 2H, $H_{2''}$ + $H_{6''}$), 7.00 (d, 2H, $H_{3''}$ + $H_{5''}$, J8.8 Hz), 3.78 (s, 3H, OCH_3)
2.15bf			8.45 (s, 1H, H_3) 8.54 (brs, 1H, H_6)	10.29 (brs, 1H)	7.47-7.66 (m, 1H, $H_{3'}$ + $H_{4'}$ + $H_{6'}$)	7.83 (d, 2H, $H_{2''}$ + $H_{6''}$, J7.6 Hz), 7.41 (t, 2H, $H_{3''}$ + $H_{5''}$, J7.6 Hz), 7.15 (t, 1H, $H_{4''}$, J7.6 Hz)
2.15bg [a]			8.12 (brs, 1H,H_3) 8.33 (s, 1H, H_6)	9.78 (brs, 1H)	7.51-7.59 (m, 1H, $H_{6'}$), 7.48-7.51 (m, 1H, $H_{3'}$), 7.36-7.42 (m, 1H, $H_{4'}$)	9.14 (brs, 1H, OH), 7.47 (d, 2H, $H_{2''}$ + $H_{6''}$, J8.8 Hz), 6.82 (d, 2H, $H_{3''}$ + $H_{5''}$, J8.8 Hz)
2.15bh			8.55 (brs, 1H, H_3) 8.47 (s, 1H, H_6)	10.24 (brs, 1H)	7.62-7.68 (m, 1H, $H_{6'}$), 7.56-7.60 (m, 1H, $H_{3'}$), 7.45-7.49 (m, 1H, $H_{4'}$)	7.54 (s, 1H, $H_{2''}$), 7.43 (dd, 1H, $H_{6''}$, J2.4 Hz, 8.4 Hz), 7.31 (t, 1H, $H_{5''}$, J8.4 Hz), 6.73 (ddd, 1H, $H_{4''}$, J0.8 Hz, 2.4 Hz, 8.4 Hz), 3.78 (s, 3H, OCH_3)
2.15bi [a]			8.26 (brs, 1H, H_3) 8.37 (s, 1H, H_6)	9.90 (brs, 1H)	7.37-7.60 (m, 3H, $H_{3'}$ + $H_{4'}$ + $H_{6'}$)	7.64 (dt, 2H, $H_{2''}$ + $H_{6''}$, J2.0 Hz, 8.8 Hz), 7.00 (dt, 2H, $H_{3''}$ + $H_{5''}$, J2.0 Hz, 8.8 Hz), 3.80 (s, 3H, OCH_3)
2.15bk			8.48 (s, 1H, H_3) 8.58 (brs, 1H, H_6)	10.37 (brs, 1H)	7.63-7.67 (m, 1H, $H_{6'}$), 7.55-7.61 (m, 1H, $H_{3'}$), 7.47-7.49 (m, 1H, $H_{4'}$)	7.89 (d, 2H, $H_{2''}$ + $H_{6''}$, J8.8 Hz), 7.45 (d, 2H, $H_{3''}$ + $H_{5''}$, J8.8 Hz)
2.15bl	H		8.29 (brs, 1H, H_3) 8.41 (s, 1H, H_6)	10.09 (s, 1H)	13.67 (brs, 1H)	7.86 (dd, 2H, $H_{2''}$ + $H_{6''}$, J2.0 Hz, 8.8 Hz), 7.55 (dd, 2H, $H_{3''}$ + $H_{5''}$, J2.0 Hz, 8.8 Hz)
2.15bm [a]			8.03 (brs, 1H, H_3) 8.35 (s, 1H, H_6)	9.75 (brs, 1H)	5.17 (s, 2H, CH_2), 4.17 (q, 2H, OCH_2, J7.2 Hz), 1.21 (t, 3H, CH_3, J7.2 Hz)	7.64 (dd, 2H, $H_{2''}$ + $H_{6''}$, J2.4 Hz, 8.8 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, J2.4 Hz, 6.8 Hz), 3.80 (s, 3H, OCH_3)
2.15bn			8.33 (brs, 1H, H_3) 8.44 (s, 1H, H_6)	10.24 (brs, 1H)	5.24 (s, 2H, CH_2), 4.14 (q, 2H, OCH_2, J7.2 Hz), 1.18 (t, 3H, CH_3, J7.2 Hz)	7.89 (dd, 2H, $H_{2''}$ + $H_{6''}$, J2.0 Hz, 7.2 Hz), 7.44 (dd, 2H, $H_{3''}$ + $H_{5''}$, J2.0 Hz, 7.2 Hz)
2.15bo [a]			8.31 (brs, 1H, H_3) 8.48 (s, 1H, H_6)	9.93 (brs, 1H)	8.42 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 8.8 Hz), 8.12 (dd, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 8.8 Hz), 4.35 (q, 2H, OCH_2), 1.35 (t, 3H, CH_3)	7.64 (dd, 2H, $H_{2''}$ + $H_{6''}$, J2.0 Hz, 7.2 Hz), 6.99 (dd, 2H, $H_{3''}$ + $H_{5''}$, J2.0 Hz, 7.2 Hz), 3.79 (s, 3H, OCH_3)
2.20			8.37 (brs, 1H, H_3) 8.47 (s, 1H, H_6)	10.43 (s, 1H)	8.17 (dd, 2H, $H_{2'}$ + $H_{6'}$, J2.0 Hz, 7.6 Hz), 7.53 (tt, 2H, $H_{3'}$ + $H_{5'}$, J2.0 Hz, 7.6 Hz), 7.33 (tt, 1H, $H_{4'}$, J2.0 Hz, 7.6 Hz)	9.93 (brs, 1H, NH), 7.64 (s, 1H, $H_{4''}$), 6.69 (s, 1H, $H_{5''}$)

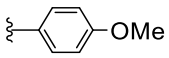
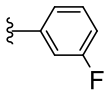
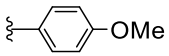
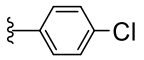
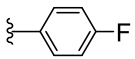
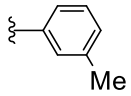
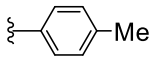
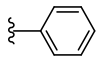
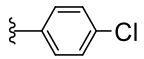
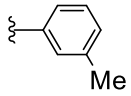
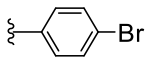
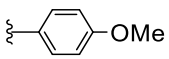
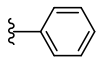
2.22a			8.57 (s, 1H, H_3) 8.59 (s, 1H, H_6)	10.42 (sl, 1H)	8.19 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.4 Hz), 7.57 (t, 2H, $H_{3'}$ + $H_{5'}$, J 8.4 Hz), 7.37 (t, 1H, $H_{4'}$, J 8.4 Hz)	9.00 (s, 2H, $H_{2''}$), 8.32-8.35 (m, 2H, $H_{4''}$ + $H_{6''}$), 7.44 (qd, 2H, $H_{5''}$, J 1.6 Hz, 4.8 Hz)
2.22b			8.55 (s, 1H, H_3) 8.56 (s, 1H, H_6)	10.39 (s, 1H)	8.06 (d, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.4 Hz), 7.36 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.4 Hz), 2.37 (s, 3H, CH_3)	9.00 (s, 2H, $H_{2''}$), 8.32-8.35 (m, 2H, $H_{4''}$ + $H_{6''}$), 7.44 (qd, 2H, $H_{5''}$, J 1.6 Hz, 4.8 Hz)
2.25a			8.27 (s, 1H, H_3) 8.50 (s, 1H, H_6)	9.44 (s, 1H)	8.18 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 7.54 (tt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz), 7.33 (tt, 1H, $H_{4'}$, J 2.0 Hz, 8.8 Hz)	3.00-3.10 (m, 2H, $H_{2''}$ + $H_{6''}$) and 2.56-2.62 (m, 2H, $H_{2''}$ + $H_{6''}$), 1.60-1.72 (m, 5H, $H_{3''}$ + $H_{5''}$ + $H_{4'}$), 1.10-1.20 (m, 1H, $H_{4''}$)
2.25b			8.31 (s, 1H, H_3) 8.52 (s, 1H, H_6)	9.51 (s, 1H)	8.13-8.15 (m, 1H, $H_{6'}$), 8.10-8.12 (m, 1H, H_2), 7.56-7.61 (m, 1H, $H_{5'}$), 7.14-7.19 (m, 1H, $H_{4'}$)	3.00-3.11 (m, 2H, $H_{2''}$ + $H_{6''}$) and 2.57-2.66 (m, 2H, $H_{2''}$ + $H_{6''}$), 1.60-1.73 (m, 5H, $H_{3''}$ + $H_{5''}$ + $H_{4'}$), 1.10-1.20 (m, 1H, $H_{4''}$)
2.27a			8.36 (s, 1H, H_3) 8.43 (s, 1H, H_6)	8.57 (t, 1H, J 5.6 Hz)	8.17 (d, 2H, $H_{2'}$ + $H_{6'}$, J 7.2 Hz), 7.53 (tt, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 7.2 Hz), 7.33 (tt, 1H, $H_{4'}$, J 2.0 Hz, 7.2 Hz)	3.50-3.65 (m, 4H, CH_2,), 3.29 (s, 3H, OCH_3)
2.27b			8.40 (s, 1H, H_3) 8.47 (s, 1H, H_6)	8.62 (t, 1H, J 5.6 Hz)	12.94 (brs, 1H, OH), 8.39 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 8.09 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	3.69 (q, 2H, CH_2, J 5.6 Hz), 3.55 (t, 2H, CH_2, J 5.6 Hz), 3.28 (s, 3H, OCH_3)

[a] This spectrum was obtained at 80°C.

Table 2.37: ^{1}H NMR spectroscopic data (400 MHz, DMSO-d_6) for pyrazolo[3,4-*d*]pyrimidine derivatives **2.18**



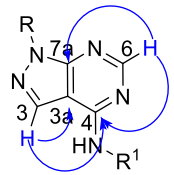
2.18

Comp.	R	R^1	C-H	CH_3	NH	R	R^1
2.18a [a]			8.13 (brs, 1H, H_3)	2.49 (s, 1H)	9.71 (brs, 1H)	8.20 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 1.2 Hz, 7.6 Hz), 7.53 (t, 2H, $H_{3'}$ + $H_{5'}$, J 7.6 Hz), 7.32 (t, 1H, $H_{4'}$, J 7.6 Hz)	7.67 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.99 (d, 2H, $H_{3''}$ + $H_{5''}$, J 8.8 Hz), 3.79 (s, 3H, OCH_3)
2.18b [a]			8.10 (brs, 1H, H_3)	2.55 (s, 1H)	9.85 (brs, 1H)	8.10-8.14 (m, 2H, $H_{6'}$ + $H_{2'}$), 7.54-7.60 (m, 1H, $H_{5'}$), 7.10-7.13 (m, 1H, $H_{4'}$)	7.67 (dt, 2H, $H_{2''}$ + $H_{6''}$, J 2.0 Hz, 6.8 Hz), 6.99 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 6.8 Hz), 3.80 (s, 3H, OCH_3)
2.18c			8.56 (brs, 1H, H_3)	2.59 (s, 1H)	10.23 (brs, 1H)	8.15 (d, 1H, $H_{6'}$, J 8.4 Hz), 8.12 (brs, 1H, $H_{2'}$), 7.56-7.62 (m, 1H, $H_{5'}$), 7.19 (tdd, 1H, $H_{4'}$, J 1.2 Hz, 2.4 Hz, 8.4 Hz)	7.92 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 7.45 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.8 Hz)
2.18d			8.37 (brs, 1H, H_3)	2.55 (s, 1H)	10.03 (brs, 1H)	8.19 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 4.8 Hz, 9.2 Hz), 7.39 (t, 2H, $H_{3'}$ + $H_{5'}$, J 9.2 Hz)	7.64 (s, 1H, $H_{6''}$), 7.62 (s, 1H, $H_{2''}$), 7.29 (t, 1H, $H_{5''}$, J 7.6 Hz), 6.94 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.33 (s, 3H, CH_3)
2.18e			8.39 (brs, 1H, H_3)	2.48 (s, 1H)	10.05 (brs, 1H)	8.04 (d, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz), 7.35 (d, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz), 2.35 (s, 3H, CH_3)	7.87 (d, 2H, $H_{2''}$ + $H_{6''}$, J 7.6 Hz), 7.40 (t, 2H, $H_{3''}$ + $H_{5''}$, J 7.6 Hz), 7.12 (t, 1H, $H_{4''}$, J 7.6 Hz)
2.18f			8.40 (brs, 1H, H_3)	2.48 (s, 1H)	10.04 (brs, 1H)	8.27 (dt, 2H, $H_{2'}$ + $H_{6'}$, J 8.8 Hz), 7.62 (dt, 2H, $H_{3'}$ + $H_{5'}$, J 8.8 Hz)	7.64 (s, 1H, $H_{6''}$), 7.63 (s, 1H, $H_{2''}$), 7.29 (t, 1H, $H_{5''}$, J 7.6 Hz), 6.95 (d, 1H, $H_{4''}$, J 7.6 Hz), 2.34 (s, 3H, CH_3)
2.18g [a]			8.12 (brs, 1H, H_3)	2.53 (s, 1H)	9.73 (brs, 1H)	8.19 (dd, 2H, $H_{2'}$ + $H_{6'}$, J 2.0 Hz, 8.8 Hz), 7.71 (dd, 2H, $H_{3'}$ + $H_{5'}$, J 2.0 Hz, 8.8 Hz)	7.64 (d, 2H, $H_{2''}$ + $H_{6''}$, J 8.8 Hz), 6.98 (dt, 2H, $H_{3''}$ + $H_{5''}$, J 2.0 Hz, 8.8 Hz), 3.79 (s, 3H, OCH_3)
2.18h			8.17 (brs, 1H, H_3)	2.48 (s, 1H)	9.98 (brs, 1H)	5.17 (s, 2H, CH_2), 4.14 (q, 2H, OCH_2, J 7.2 Hz), 1.19 (t, 3H, CH_3, J 7.2 Hz)	7.85 (d, 2H, $H_{2''}$ + $H_{6''}$, J 7.2 Hz), 7.39 (t, 2H, $H_{3''}$ + $H_{5''}$, J 7.2 Hz), 7.11 (t, 1H, $H_{4''}$, J 7.2 Hz)

[a] This spectrum was obtained at 80°C.

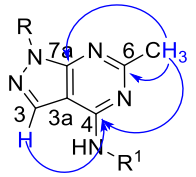
- **^{13}C-NMR Spectroscopy**

For pyrazolo[3,4-*d*]pyrimidine derivative **2.14f**, the two signals at δ_C 136.01 ppm and 148.82 ppm were assigned to carbons C-3 and C-6, respectively (Table **2.38**). The two signals around δ_C 132.03-136.87 ppm and δ_C 155.09-156.78 ppm were assigned to carbons C-3 and C-6, respectively, for pyrazolo[3,4-*d*]pyrimidine derivatives **2.15**, **2.20**, **2.22**, **2.25** and **2.27** (Table **2.38**). The HMBC correlation spectra confirm the suggested structure, where proton H-3 correlates with C-3a and C-4 and proton H-6 correlates with C-3a, C-4 and C-7a. Figure **2.17** shows the ^{13}C NMR spectrum of pyrazolo[3,4-*d*]pyrimidine **2.15al**, with key signals assigned.



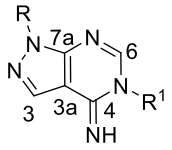
2.15, **2.20**, **2.22**, **2.25** and **2.27**

For pyrazolo[3,4-*d*]pyrimidine derivatives **2.18** the two signals around δ_C 133.11-134.15 ppm and δ_C 25.73-26.41 ppm were assigned to carbons C-3 and CH_3, respectively (Table **2.39**). The HMBC correlation spectra confirm the suggested structure, where proton H-3 correlates with C-4 and proton of CH_3 group correlates with C-4, C-6 and C-7a.

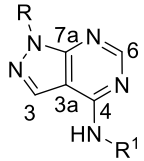


2.18

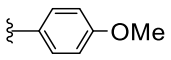
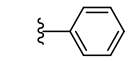
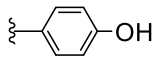
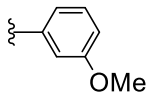
Table 2.38: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.20**, **2.22**, **2.25** and **2.27**

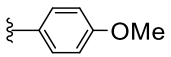
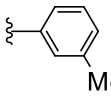
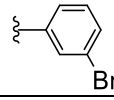
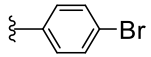
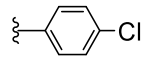
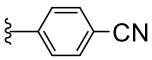
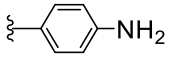
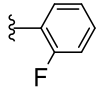
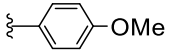
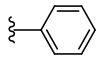
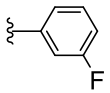
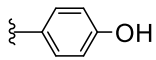
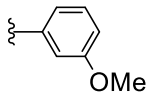


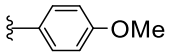
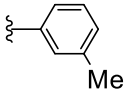
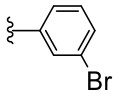
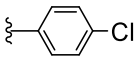
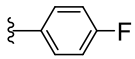
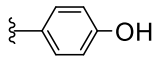
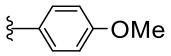
2.14f

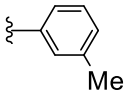
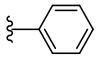
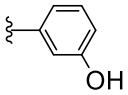
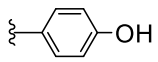
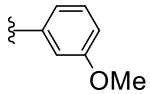
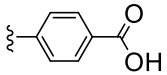
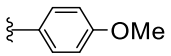
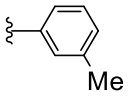
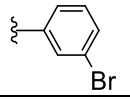
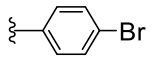
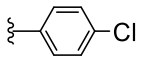
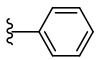
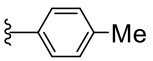
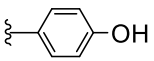
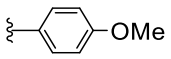


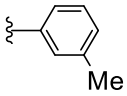
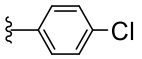
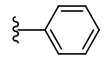
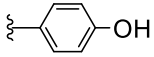
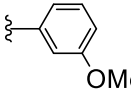
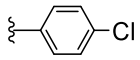
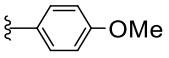
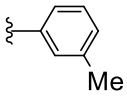
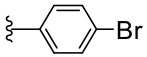
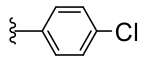
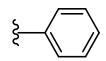
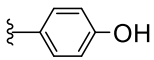
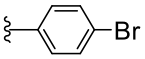
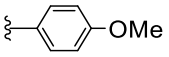
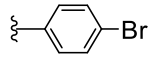
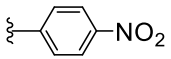
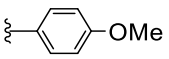
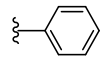
2.15, **2.20**, **2.22**, **2.25**, **2.27**

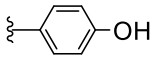
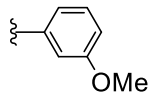
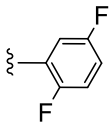
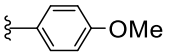
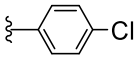
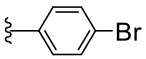
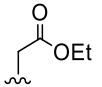
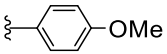
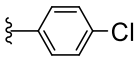
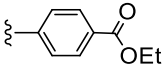
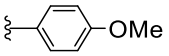
Comp.	R	R^1	C-H	C_{3a}	C_4	C_{7a}	R	R^1
2.14f			136.01 (C_3) 148.82 (C_6)	107.62	154.88	151.87	138.22 ($C_{1'}$), 129.23 ($C_{3'}$ + $C_{5'}$), 127.16 ($C_{4'}$), 121.78 ($C_{2'}$ + $C_{6'}$)	157.24 ($C_{4''}$), 129.45 ($C_{1''}$), 122.69 ($C_{2''}$ + $C_{6''}$), 114.11 ($C_{3''}$ + $C_{5''}$), 55.28 (OCH_3)
2.15a [a)]			133.25 (C_3) 155.59 (C_6)	102.07	154.56	152.98	138.56 ($C_{1'}$), 128.64 ($C_{3'}$ + $C_{5'}$), 125.88 ($C_{4'}$), 120.57 ($C_{2'}$ + $C_{6'}$)	138.51 ($C_{1''}$), 128.28 ($C_{3''}$ + $C_{5''}$), 123.51 ($C_{4''}$), 121.49 ($C_{2''}$ + $C_{6''}$)
2.15d			133.72 (C_3) 156.25 (C_6)	101.99	154.25	153.12	138.79 ($C_{1'}$), 129.16 ($C_{3'}$ + $C_{5'}$), 126.25 ($C_{4'}$), 120.74 ($C_{2'}$ + $C_{6'}$)	154.81 ($C_{4''}$), 129.93 ($C_{1''}$), 123.65 ($C_{2''}$ + $C_{6''}$), 115.31 ($C_{3''}$ + $C_{5''}$)
2.15e [a)]			133.21 (C_3) 155.53 (C_6)	102.13	154.47	152.94	138.48 ($C_{1'}$), 128.62 ($C_{3'}$ + $C_{5'}$), 125.88 ($C_{4'}$), 120.56 ($C_{2'}$ + $C_{6'}$)	159.41 ($C_{3''}$), 139.74 ($C_{1''}$), 128.99 ($C_{5''}$), 113.66 ($C_{6''}$), 109.11 ($C_{4''}$), 107.51 ($C_{2''}$), 54.85 (OCH_3)

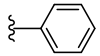
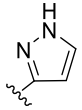
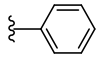
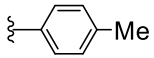
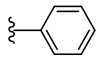
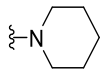
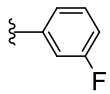
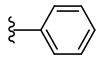
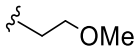
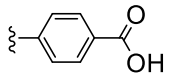
2.15f [a]			133.21 (C_3) 155.67 (C_6)	101.70	154.90	152.99	138.56 ($C_{1'}$), 128.60 ($C_{3'}$ + $C_{5'}$), 125.78 ($C_{4'}$), 120.51 ($C_{2'}$ + $C_{6'}$)	156.07 ($C_{4''}$), 131.37 ($C_{1''}$), 123.69 ($C_{2''}$ + $C_{6''}$), 113.85 ($C_{3''}$ + $C_{5''}$), 55.04 (OCH_3)
2.15g [a]			133.43 (C_3) 155.83 (C_6)	102.18	154.84	153.14	138.57 ($C_{1'}$), 128.86 ($C_{3'}$ + $C_{5'}$), 126.17 ($C_{4'}$), 120.85 ($C_{2'}$ + $C_{6'}$)	138.62 ($C_{1''}$), 137.91 ($C_{3''}$), 128.36 ($C_{5''}$), 124.60 ($C_{4''}$), 122.28 ($C_{2''}$), 118.99 ($C_{6''}$), 20.81 (CH_3)
2.15h [a]			133.14 (C_3) 155.47 (C_6)	102.33	154.11	152.88	138.41 ($C_{1'}$), 128.68 ($C_{3'}$ + $C_{5'}$), 126.00 ($C_{4'}$), 120.62 ($C_{2'}$ + $C_{6'}$)	140.36 ($C_{1''}$), 130.13 ($C_{5''}$), 125.70 ($C_{4''}$), 123.21 ($C_{2''}$), 121.21 ($C_{3''}$), 119.57 ($C_{6''}$)
2.15i [a]			133.16 (C_3) 155.46 (C_6)	102.25	154.14	152.89	138.43 ($C_{1'}$), 128.64 ($C_{3'}$ + $C_{5'}$), 125.94 ($C_{4'}$), 120.58 ($C_{2'}$ + $C_{6'}$)	138.06 ($C_{1''}$), 131.06 ($C_{3''}$ + $C_{5''}$), 122.92 ($C_{2''}$ + $C_{6''}$), 115.02 ($C_{4''}$)
2.15j [a]			133.16 (C_3) 155.97 (C_6)	102.21	154.19	152.89	138.44 ($C_{1'}$), 128.64 ($C_{3'}$ + $C_{5'}$), 125.94 ($C_{4'}$), 120.58 ($C_{2'}$ + $C_{6'}$)	137.60 ($C_{1''}$), 128.13 ($C_{3''}$ + $C_{5''}$), 127.13 ($C_{4''}$), 123.60 ($C_{2''}$ + $C_{6''}$)
2.15k [a]			133.28 (C_3) 155.44 (C_6)	102.83	153.97	153.04	138.40 ($C_{1'}$), 128.86 ($C_{3'}$ + $C_{5'}$), 126.32 ($C_{4'}$), 120.88 ($C_{2'}$ + $C_{6'}$)	143.26 ($C_{1''}$), 132.70 ($C_{3''}$ + $C_{5''}$), 120.61 ($C_{2''}$ + $C_{6''}$), 104.79 ($C_{4''}$)
2.15l [b]							—	
2.15n [a]			133.76 (C_3) 155.78 (C_6)	100.54	154.90	154.17	155.90 (d, $C_{2'}$, J 251.00 Hz), 130.14 (d, $C_{4'}$, J 7.00 Hz), 128.28 (s, $C_{6'}$), 125.13 (d, $C_{1'}$, J 12.00 Hz), 124.38 (d, $C_{5'}$, J 4.00 Hz), 116.22 (d, $C_{3'}$, J 20.00 Hz)	156.11 ($C_{4''}$), 131.38 ($C_{1''}$), 123.79 ($C_{2''}$ + $C_{6''}$), 113.88 ($C_{3''}$ + $C_{5''}$), 55.05 (OCH_3)
2.15p [a]			133.83 (C_3) 155.82 (C_6)	102.26	154.55	153.29	161.87 (d, $C_{3'}$, J 242.00 Hz), 139.94 (d, $C_{1'}$, J 11.00 Hz), 130.49 (d, $C_{5'}$, J 9.00 Hz), 115.90 (d, $C_{6'}$, J 3.00 Hz), 112.31 (d, $C_{4'}$, J 22.00 Hz), 107.21 (d, $C_{2'}$, J 28.00 Hz)	138.42 ($C_{1''}$), 128.29 ($C_{3''}$ + $C_{5''}$), 123.62 ($C_{4''}$), 121.53 ($C_{2''}$ + $C_{6''}$)
2.15r [a]			133.84 (C_3) 155.95 (C_6)	101.72	154.44	153.38	161.87 (d, $C_{3'}$, J 241.00 Hz), 140.03 (d, $C_{1'}$, J 11.00 Hz), 130.43 (d, $C_{5'}$, J 10.00 Hz), 115.84 (d, $C_{6'}$, J 3.00 Hz), 112.18 (d, $C_{4'}$, J 21.00 Hz), 107.15 (d, $C_{2'}$, J 27.00 Hz)	155.21 ($C_{4''}$), 129.56 ($C_{1''}$), 124.30 ($C_{2''}$ + $C_{6''}$), 115.12 ($C_{3''}$ + $C_{5''}$)
2.15s			134.26 (C_3) 156.25 (C_6)	102.73	154.51	153.35	162.15 (d, $C_{3'}$, J 242.00 Hz), 140.16 (d, $C_{1'}$, J 11.00 Hz), 131.05 (d, $C_{5'}$, J 9.00 Hz), 116.09 (d, $C_{6'}$, J 2.00 Hz), 112.79	159.54 ($C_{3''}$), 139.96 ($C_{1''}$), 129.51 ($C_{5''}$), 113.37 ($C_{6''}$), 109.06 ($C_{4''}$), 107.25 ($C_{2''}$), 55.09 (OCH_3)

							(d, $C_{4'}$, J 21.00 Hz), 107.39 (d, $C_{2'}$, J 27.00 Hz)	
2.15t [a]			133.80 (C_3) 155.89 (C_6)	101.88	154.88	153.31	161.85 (d, $C_{3'}$, J242.00 Hz), 139.99 (d, $C_{1'}$, J 11.00 Hz), 130.44 (d, $C_{5'}$, J 9.00 Hz), 115.82 (d, $C_{6'}$, J 3.00 Hz), 112.21 (d, $C_{4'}$, J 21.00 Hz), 107.14 (d, $C_{2'}$, J 27.00 Hz)	156.13 ($C_{4''}$), 131.22 ($C_{1''}$), 123.72 ($C_{2''}$ + $C_{6''}$), 113.86 ($C_{3''}$ + $C_{5''}$), 55.03 (OCH_3)
2.15u			134.30 (C_3) 156.35 (C_6)	102.62	154.61	153.39	162.16 (d, $C_{3'}$, J242.00 Hz), 140.18 (d, $C_{1'}$, J 11.00 Hz), 131.07 (d, $C_{5'}$, J 9.00 Hz), 116.10 (d, $C_{6'}$, J 3.00 Hz), 112.78 (d, $C_{4'}$, J 21.00 Hz), 107.39 (d, $C_{2'}$, J 27.00 Hz)	138.67 ($C_{1''}$), 138.01 ($C_{3''}$), 128.61 ($C_{5''}$), 124.61 ($C_{4''}$), 121.97 ($C_{2''}$), 118.71 ($C_{6''}$), 21.19 (CH_3)
2.15v			134.25 (C_3) 156.26 (C_6)	102.91	154.27	153.35	162.18 (d, $C_{3'}$, J242.00 Hz), 140.08 (d, $C_{1'}$, J 11.00 Hz), 131.16 (d, $C_{5'}$, J 9.00 Hz), 116.23 (d, $C_{6'}$, J 2.00 Hz), 112.98 (d, $C_{4'}$, J 21.00 Hz), 107.52 (d, $C_{2'}$, J 27.00 Hz)	140.87 ($C_{1''}$), 130.71 ($C_{5''}$), 125.88 ($C_{4''}$), 123.27 ($C_{2''}$), 121.24 ($C_{3''}$), 119.70 ($C_{6''}$)
2.15x [a]			133.77 (C_3) 155.75 (C_6)	102.42	154.24	153.25	161.89 (d, $C_{3'}$, J242.00 Hz), 139.88 (d, $C_{1'}$, J 10.00 Hz), 130.54 (d, $C_{5'}$, J 9.00 Hz), 115.97 (d, $C_{6'}$, J 3.00 Hz), 112.43 (d, $C_{4'}$, J 21.00 Hz), 107.28 (d, $C_{2'}$, J 27.00 Hz)	137.48 ($C_{1''}$), 128.18 ($C_{3''}$ + $C_{5''}$), 127.31 ($C_{4''}$), 122.71 ($C_{2''}$ + $C_{6''}$)
2.15y [a]			133.32 (C_3) 155.69 (C_6)	101.96	154.59	152.88	159.89 (d, $C_{4'}$, J242.00 Hz), 134.89 (d, $C_{1'}$, J 2.00 Hz), 122.59 (d, $C_{2'}$ + $C_{6'}$, J 8.00 Hz), 115.43 (d, $C_{3'}$ + $C_{5'}$, J 23.00 Hz)	138.52 ($C_{1''}$), 128.31 ($C_{3''}$ + $C_{5''}$), 123.60 ($C_{4''}$), 121.50 ($C_{2''}$ + $C_{6''}$)
2.15z [a]			133.31 (C_3) 155.81 (C_6)	101.42	154.40	152.94	159.80 (d, $C_{4'}$, J242.00 Hz), 134.97 (d, $C_{1'}$, J 3.00 Hz), 122.48 (d, $C_{2'}$ + $C_{6'}$, J 9.00 Hz), 115.36 (d, $C_{3'}$ + $C_{5'}$, J 22.00 Hz)	155.23 ($C_{4''}$), 129.66 ($C_{1''}$), 124.29 ($C_{2''}$ + $C_{6''}$), 115.11 ($C_{3''}$ + $C_{5''}$)
2.15aa [a]			133.29 (C_3) 155.78 (C_6)	101.59	154.98	152.90	159.84 (d, $C_{4'}$, J242.00 Hz), 134.95 (d, $C_{1'}$, J 3.00 Hz), 122.52 (d, $C_{2'}$ + $C_{6'}$, J 9.00 Hz), 115.39 (d, $C_{3'}$ + $C_{5'}$, J 23.00 Hz)	156.13 ($C_{4''}$), 131.33 ($C_{1''}$), 123.76 ($C_{2''}$ + $C_{6''}$), 113.89 ($C_{3''}$ + $C_{5''}$), 55.07 (OCH_3)

2.15ab			133.78 (C_3) 156.21 (C_6)	102.31	154.64	152.93	160.11 (d, $C_{4'}$, J242.00 Hz), 135.14 (d, $C_{1'}$, J 3.00 Hz), 122.79 (d, $C_{2'}$ + $C_{6'}$, J 9.00 Hz), 115.19 (d, $C_{3'}$ + $C_{5'}$, J 23.00 Hz)	138.75 ($C_{1''}$), 138.01 ($C_{3''}$), 128.62 ($C_{5''}$), 124.55 ($C_{4''}$), 121.95 ($C_{2''}$), 118.68 ($C_{6''}$), 21.19 (CH_3)
2.15ad a)			134.20 (C_3) 155.88 (C_6)	102.36	154.58	153.51	166.19 (CO), 141.82 ($C_{1'}$), 130.03 ($C_{3'}$ + $C_{5'}$), 128.00 ($C_{4'}$), 119.62 ($C_{2'}$ + $C_{6'}$)	138.42 ($C_{1''}$), 128.30 ($C_{3''}$ + $C_{5''}$), 123.64 ($C_{4''}$), 121.53 ($C_{2''}$ + $C_{6''}$)
2.15ae a)			134.27 (C_3) 155.86 (C_6)	102.38	154.60	153.53	166.26 (CO), 141.83 ($C_{1'}$), 130.02 ($C_{3'}$ + $C_{5'}$), 128.10 ($C_{4'}$), 119.61 ($C_{2'}$ + $C_{6'}$)	157.39 ($C_{3''}$), 139.42 ($C_{1''}$), 128.91 ($C_{5''}$), 112.37 ($C_{6''}$), 111.08 ($C_{4''}$), 108.90 ($C_{2''}$),
2.15af			134.68 (C_3) 156.52 (C_6)	102.37	154.52	153.71	166.80 (CO), 142.22 ($C_{1'}$), 130.56 ($C_{3'}$ + $C_{5'}$), 128.06 ($C_{4'}$), 119.74 ($C_{2'}$ + $C_{6'}$)	154.52 ($C_{4''}$), 129.83 ($C_{1''}$), 123.63 ($C_{2''}$ + $C_{6''}$), 115.34 ($C_{3''}$ + $C_{5''}$)
2.15ag			134.71 (C_3) 156.38 (C_6)	102.89	154.60	153.64	166.83 (CO), 142.13 ($C_{1'}$), 130.63 ($C_{3'}$ + $C_{5'}$), 128.29 ($C_{4'}$), 119.88 ($C_{2'}$ + $C_{6'}$)	159.60 ($C_{3''}$), 140.00 ($C_{1''}$), 129.60 ($C_{5''}$), 113.65 ($C_{6''}$), 109.15 ($C_{4''}$), 107.32 ($C_{2''}$), 55.16 (OCH_3)
2.15ah a)			134.20 (C_3) 155.99 (C_6)	102.22	154.93	153.57	166.23 (CO), 141.90 ($C_{1'}$), 130.03 ($C_{3'}$ + $C_{5'}$), 127.92 ($C_{4'}$), 119.60 ($C_{2'}$ + $C_{6'}$)	156.17 ($C_{4''}$), 131.24 ($C_{1''}$), 123.77 ($C_{2''}$ + $C_{6''}$), 113.91 ($C_{3''}$ + $C_{5''}$), 55.08 (OCH_3)
2.15ai			134.70 (C_3) 156.44 (C_6)	102.75	154.67	153.64	166.84 (CO), 142.06 ($C_{1'}$), 130.58 ($C_{3'}$ + $C_{5'}$), 128.46 ($C_{4'}$), 119.82 ($C_{2'}$ + $C_{6'}$)	138.69 ($C_{1''}$), 138.05 ($C_{3''}$), 128.65 ($C_{5''}$), 124.65 ($C_{4''}$), 122.01 ($C_{2''}$), 118.72 ($C_{6''}$), 21.21 (CH_3)
2.15aj a)			134.08 (C_3) 155.76 (C_6)	102.65	154.15	153.42	166.23 (CO), 141.69 ($C_{1'}$), 130.04 ($C_{3'}$ + $C_{5'}$), 128.26 ($C_{4'}$), 119.67 ($C_{2'}$ + $C_{6'}$)	140.25 ($C_{1''}$), 130.14 ($C_{5''}$), 125.82 ($C_{4''}$), 123.29 ($C_{2''}$), 121.04 ($C_{3''}$), 119.64 ($C_{6''}$)
2.15ak			134.59 (C_3) 156.22 (C_6)	102.94	154.23	153.50	166.73 (CO), 142.08 ($C_{1'}$), 130.57 ($C_{3'}$ + $C_{5'}$), 128.10 ($C_{4'}$), 119.78 ($C_{2'}$ + $C_{6'}$)	138.27 ($C_{1''}$), 131.51 ($C_{3''}$ + $C_{5''}$), 122.97 ($C_{2''}$ + $C_{6''}$), 115.32 ($C_{4''}$)
2.15al			134.61 (C_3) 156.25 (C_6)	102.90	154.28	153.56	166.72 (CO), 142.09 ($C_{1'}$), 130.57 ($C_{3'}$ + $C_{5'}$), 128.09 ($C_{4'}$), 119.80 ($C_{2'}$ + $C_{6'}$)	137.81 ($C_{1''}$), 128.62 ($C_{3''}$ + $C_{5''}$), 127.29 ($C_{4''}$), 122.66 ($C_{2''}$ + $C_{6''}$)
2.15an			133.43 (C_3) 156.02 (C_6)	102.35	154.59	152.84	136.39 ($C_{1'}$), 135.72 ($C_{4'}$), 129.58 ($C_{3'}$ + $C_{5'}$), 120.72 ($C_{2'}$ + $C_{6'}$), 20.56 (CH_3)	138.90 ($C_{1''}$), 128.76 ($C_{3''}$ + $C_{5''}$), 123.73 ($C_{4''}$), 121.43 ($C_{2''}$ + $C_{6''}$)
2.15ao a)			132.97 (C_3) 155.68 (C_6)	101.45	154.37	152.86	136.25 ($C_{1'}$), 135.28 ($C_{4'}$), 129.06 ($C_{3'}$ + $C_{5'}$), 120.58 ($C_{2'}$ + $C_{6'}$), 20.08 (CH_3)	155.24 ($C_{4''}$), 129.77 ($C_{1''}$), 124.30 ($C_{2''}$ + $C_{6''}$), 115.14 ($C_{3''}$ + $C_{5''}$)
2.15ap a)			132.88 (C_3) 155.57 (C_6)	101.58	154.87	152.77	136.20 ($C_{1'}$), 135.23 ($C_{4'}$), 129.00 ($C_{3'}$ + $C_{5'}$), 120.50 ($C_{2'}$ + $C_{6'}$), 20.02 (CH_3)	156.04 ($C_{4''}$), 131.41 ($C_{1''}$), 123.66 ($C_{2''}$ + $C_{6''}$), 113.84 ($C_{3''}$ + $C_{5''}$), 55.04 (OCH_3)

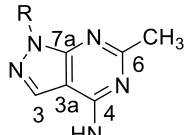
2.15aq			133.43 (C_3) 156.05 (C_6)	102.32	154.62	152.83	136.39 ($C_{1'}$), 135.70 ($C_{4'}$), 129.57 ($C_{3'}$ + $C_{5'}$), 120.76 ($C_{2'}$ + $C_{6'}$), 20.56 (CH_3)	138.83 ($C_{1''}$), 137.99 ($C_{3''}$), 128.61 ($C_{5''}$), 124.47 ($C_{4''}$), 121.90 ($C_{2''}$), 118.63 ($C_{6''}$), 21.26 (CH_3)
2.15ar a)			132.85 (C_3) 155.37 (C_6)	102.08	154.16	152.68	136.08 ($C_{1'}$), 135.40 ($C_{4'}$), 129.05 ($C_{3'}$ + $C_{5'}$), 120.57 ($C_{2'}$ + $C_{6'}$), 20.02 (CH_3)	137.64 ($C_{1''}$), 128.12 ($C_{3''}$ + $C_{5''}$), 127.09 ($C_{4''}$), 122.57 ($C_{2''}$ + $C_{6''}$)
2.15as a)			133.68 (C_3) 155.76 (C_6)	102.15	154.58	153.08	137.36 ($C_{1'}$), 130.12 ($C_{4'}$), 128.68 ($C_{3'}$ + $C_{5'}$), 121.86 ($C_{2'}$ + $C_{6'}$)	138.46 ($C_{1''}$), 128.30 ($C_{3''}$ + $C_{5''}$), 123.62 ($C_{4''}$), 121.54 ($C_{2''}$ + $C_{6''}$)
2.15at a)			133.68 (C_3) 155.88 (C_6)	101.60	154.42	153.15	137.42 ($C_{1'}$), 129.99 ($C_{4'}$), 128.68 ($C_{3'}$ + $C_{5'}$), 121.79 ($C_{2'}$ + $C_{6'}$)	155.22 ($C_{4''}$), 129.59 ($C_{1''}$), 124.27 ($C_{2''}$ + $C_{6''}$), 115.11 ($C_{3''}$ + $C_{5''}$)
2.15au a)			133.66 (C_3) 155.71 (C_6)	102.23	154.51	153.06	137.34 ($C_{1'}$), 130.14 ($C_{4'}$), 128.69 ($C_{3'}$ + $C_{5'}$), 121.87 ($C_{2'}$ + $C_{6'}$)	159.43 ($C_{3''}$), 139.67 ($C_{1''}$), 129.05 ($C_{5''}$), 113.72 ($C_{6''}$), 109.21 ($C_{4''}$), 107.57 ($C_{2''}$), 54.88 (OCH_3)
2.15av a)			133.63 (C_3) 155.81 (C_6)	101.76	154.90	153.09	137.39 ($C_{1'}$), 130.00 ($C_{4'}$), 128.61 ($C_{3'}$ + $C_{5'}$), 121.77 ($C_{2'}$ + $C_{6'}$)	156.12 ($C_{4''}$), 131.26 ($C_{1''}$), 123.72 ($C_{2''}$ + $C_{6''}$), 113.86 ($C_{3''}$ + $C_{5''}$), 55.04 (OCH_3)
2.15aw			134.12 (C_3) 156.25 (C_6)	102.51	154.62	153.14	137.54 ($C_{1'}$), 130.41 ($C_{4'}$), 129.16 ($C_{3'}$ + $C_{5'}$), 121.99 ($C_{2'}$ + $C_{6'}$)	138.71 ($C_{1''}$), 138.00 ($C_{3''}$), 128.61 ($C_{5''}$), 124.57 ($C_{4''}$), 121.99 ($C_{2''}$), 118.66 ($C_{6''}$), 21.20 (CH_3)
2.15ax			134.08 (C_3) 156.13 (C_6)	102.71	154.26	153.10	137.54 ($C_{1'}$), 130.41 ($C_{4'}$), 129.22 ($C_{3'}$ + $C_{5'}$), 122.08 ($C_{2'}$ + $C_{6'}$)	138.30 ($C_{1''}$), 131.54 ($C_{3''}$ + $C_{5''}$), 123.01 ($C_{2''}$ + $C_{6''}$), 115.32 ($C_{4''}$)
2.15ay a)			133.59 (C_3) 155.65 (C_6)	102.29	154.22	153.01	137.28 ($C_{1'}$), 130.20 ($C_{4'}$), 128.70 ($C_{3'}$ + $C_{5'}$), 121.89 ($C_{2'}$ + $C_{6'}$)	137.51 ($C_{1''}$), 128.16 ($C_{3''}$ + $C_{5''}$), 127.26 ($C_{4''}$), 122.68 ($C_{2''}$ + $C_{6''}$)
2.15ba a)			133.72 (C_3) 155.76 (C_6)	102.17	154.58	153.10	137.39 ($C_{1'}$), 131.62 ($C_{3'}$ + $C_{5'}$), 122.15 ($C_{2'}$ + $C_{6'}$), 118.23 ($C_{4'}$)	138.45 ($C_{1''}$), 128.36 ($C_{3'''}$ + $C_{5''}$), 123.62 ($C_{4''}$), 121.54 ($C_{2''}$ + $C_{6''}$)
2.15bb a)			133.72 (C_3) 155.88 (C_6)	101.63	154.43	153.17	137.86 ($C_{1'}$), 131.57 ($C_{3'}$ + $C_{5'}$), 122.07 ($C_{2'}$ + $C_{6'}$), 118.09 ($C_{4'}$)	155.21 ($C_{4''}$), 129.58 ($C_{1''}$), 124.27 ($C_{2''}$ + $C_{6''}$), 115.11 ($C_{3''}$ + $C_{5''}$)
2.15bc a)			133.69 (C_3) 155.84 (C_6)	101.80	154.92	153.13	137.83 ($C_{1'}$), 131.59 ($C_{3'}$ + $C_{5'}$), 122.09 ($C_{2'}$ + $C_{6'}$), 118.14 ($C_{4'}$)	156.14 ($C_{4''}$), 131.25 ($C_{1''}$), 123.75 ($C_{2''}$ + $C_{6''}$), 113.88 ($C_{3''}$ + $C_{5''}$), 55.06 (OCH_3)
2.15ad			133.63 (C_3) 155.63 (C_6)	102.36	154.16	153.02	137.71 ($C_{1'}$), 131.63 ($C_{3'}$ + $C_{5'}$), 122.15 ($C_{2'}$ + $C_{6'}$), 118.30 ($C_{4'}$)	137.96 ($C_{1''}$), 131.08 ($C_{3''}$ + $C_{5''}$), 122.98 ($C_{2''}$ + $C_{6''}$), 115.14 ($C_{4''}$)
2.15be b)			—					
2.15bf b)			—					

2.15bg a)			134.26 (C_3) 155.98 (C_6)	100.43	154.47	154.30	157.25 (dd, $C_{5'}$, J2.90 Hz, 241.00 Hz), 152.16 (dd, $C_{2'}$, J2.90 Hz, 247.00 Hz), 125.85 (dd, $C_{1'}$, J14.00 Hz, 25.00 Hz), 117.58 (dd, $C_{3'}$, J9.00 Hz, 32.00 Hz), 116.41 (dd, $C_{4'}$, J8.00 Hz, 32.00 Hz), 114.82 (d, $C_{6'}$, J26.00 Hz)	155.23 ($C_{4''}$), 129.61 ($C_{1''}$), 124.39 ($C_{2''}$ + $C_{6''}$), 115.13 ($C_{3''}$ + $C_{5''}$)
2.15bh			134.74 (C_3) 156.36 (C_6)	101.46	154.53	154.29	157.63 (dd, $C_{5'}$, J3.00 Hz, 244.00 Hz), 152.06 (dd, $C_{2'}$, J3.00 Hz, 247.00 Hz), 125.91 (dd, $C_{1'}$, J 14.00 Hz, 26 Hz), 118.18 (dd, $C_{3'}$, J9.00 Hz, 22.00 Hz), 117.23 (dd, $C_{4'}$, J8.00 Hz, 24.00 Hz), 115.47 (d, $C_{6'}$, J27.00 Hz)	159.99 ($C_{3''}$), 140.04 ($C_{1''}$), 129.60 ($C_{5''}$), 113.67 ($C_{6''}$), 109.14 ($C_{4''}$), 107.34 ($C_{2''}$), 55.14 (OCH_3)
2.15bi a)			134.23 (C_3) 155.93 (C_6)	100.61	154.89	154.24	157.25 (dd, $C_{5'}$, J3.00 Hz, 242.00 Hz), 152.15 (dd, $C_{2'}$, J3.00 Hz, 248.00 Hz), 125.81 (dd, $C_{1'}$, J14.00 Hz, 25.00 Hz), 117.59 (dd, $C_{3'}$, J9.00 Hz, 28.00 Hz), 116.43 (dd, $C_{4'}$, J8.00 Hz, 32.00 Hz), 114.81 (d, $C_{6'}$, J28.00 Hz)	156.15 ($C_{4''}$), 131.28 ($C_{1''}$), 123.80 ($C_{2''}$ + $C_{6''}$), 113.88 ($C_{3''}$ + $C_{5''}$), 55.03 (OCH_3)
2.15bk			134.71 (C_3) 156.30 (C_6)	101.52	156.30	154.27	157.65 (dd, $C_{5'}$, J3.00 Hz, 241.00 Hz), 152.46 (dd, $C_{2'}$, J3.00 Hz, 247.00 Hz), 125.87 (dd, $C_{1'}$, J14.00 Hz, 25.00 Hz), 118.20 (dd, $C_{3'}$, J9.00 Hz, 31.00 Hz), 117.26 (dd, $C_{4'}$, J8.00 Hz, 31.00 Hz), 115.44 (d, $C_{6'}$, J26.00 Hz)	137.51 ($C_{1''}$), 128.68 ($C_{3''}$ + $C_{5''}$), 127.35 ($C_{4''}$), 122.76 ($C_{2''}$ + $C_{6''}$)
2.15bl	H		132.36 (C_3) 155.09 (C_6)	100.71	154.68	153.98	–	138.78 ($C_{1''}$), 131.46 ($C_{3''}$ + $C_{5''}$), 122.72 ($C_{2''}$ + $C_{6''}$), 114.76 ($C_{4''}$)
2.15bm a)			132.03 (C_3) 155.16 (C_6)	100.21	154.74	153.54	167.08 (CO), 60.68 (OCH_2), 47.63 (CH_2), 13.49 (CH_3)	156.01 ($C_{4''}$), 131.47 ($C_{1''}$), 123.69 ($C_{2''}$ + $C_{6''}$), 113.84 ($C_{3''}$ + $C_{5''}$), 58.04 (OCH_3)
2.15bn			132.46 (C_3) 155.49 (C_6)	101.08	154.09	153.60	167.74 (CO), 61.29 (OCH_2), 48.01 (CH_2), 14.00 (CH_3)	138.06 ($C_{1''}$), 128.62 ($C_{3''}$ + $C_{5''}$), 127.10 ($C_{4''}$), 121.61 ($C_{2''}$ + $C_{6''}$)
2.15bo a)			134.36 (C_3) 156.07 (C_6)	102.08	154.97	153.66	164.90 (CO), 142.19 ($C_{1'}$), 129.92 ($C_{3'}$ + $C_{5'}$), 127.05 ($C_{4'}$), 119.70 ($C_{2'}$ + $C_{6'}$), 60.37 (OCH_2), 13.75 (CH_3)	156.22 ($C_{4''}$), 131.25 ($C_{1''}$), 123.81 ($C_{2''}$ + $C_{6''}$), 113.95 ($C_{3''}$ + $C_{5''}$), 55.12 (OCH_3)

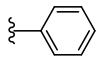
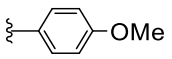
2.20			133.81 (C_3) 155.79 (C_6)	101.73	154.44	153.18	138.70 ($C_{1'}$), 129.37 ($C_{3'}$ + $C_{5'}$), 125.93 ($C_{4'}$), 120.71 ($C_{2'}$ + $C_{6'}$)	146.20 ($C_{1''}$), 128.72 ($C_{4''}$), 97.96 ($C_{5''}$)
2.22a			133.67 (C_3) 156.04 (C_6)	102.66	154.53	153.05	138.64 ($C_{1'}$), 129.25 ($C_{3'}$ + $C_{5'}$), 126.51 ($C_{4'}$), 120.88 ($C_{2'}$ + $C_{6'}$)	144.41 ($C_{4''}$), 142.57 ($C_{2''}$), 135.73 ($C_{1''}$), 128.26 ($C_{6''}$), 123.60 ($C_{5''}$)
2.22b			133.33 (C_3) 155.91 (C_6)	102.53	154.47	152.80	136.28 ($C_{1'}$), 135.74 ($C_{4'}$), 129.59 ($C_{3'}$ + $C_{5'}$), 120.81 ($C_{2'}$ + $C_{6'}$), 20.55 (CH_3)	144.34 ($C_{4''}$), 142.53 ($C_{2''}$), 135.83 ($C_{1''}$), 128.16 ($C_{6''}$), 123.55 ($C_{5''}$)
2.25a			136.29 (C_3) 156.05 (C_6)	99.79	157.90	153.97	138.92 ($C_{1'}$), 129.09 ($C_{3'}$ + $C_{5'}$), 126.06 ($C_{4'}$), 120.78 ($C_{2'}$ + $C_{6'}$)	55.93 ($C_{2''}$ + $C_{6''}$), 25.43 ($C_{3''}$ + $C_{5''}$), 22.93 ($C_{4''}$)
2.25b			136.87 (C_3) 156.28 (C_6)	99.97	157.87	154.35	162.14 ($C_{3'}$, *J* 241 Hz), 140.39 ($C_{1'}$, *J* 11 Hz), 131.01 ($C_{5'}$, *J* 9 Hz), 116.09 ($C_{6'}$, *J* 3 Hz), 112.52 ($C_{4'}$, *J* 21 Hz), 107.35 ($C_{3'}$, *J* 27 Hz)	55.89 ($C_{2''}$ + $C_{6''}$), 25.41 ($C_{3''}$ + $C_{5''}$), 22.92 ($C_{4''}$)
2.27a			133.80 (C_3) 156.45 (C_6)	101.84	156.60	152.84	138.93 ($C_{1'}$), 129.14 ($C_{3'}$ + $C_{5'}$), 126.12 ($C_{4'}$), 120.62 ($C_{2'}$ + $C_{6'}$)	70.36 (CH_2), 58.02 (OCH_3), 39.80 (CH_2)
2.27b			134.83 (C_3) 156.78 (C_6)	102.18	156.66	153.50	166.86 (CO), 142.41 ($C_{1'}$), 130.62 ($C_{3'}$ + $C_{5'}$), 127.91 ($C_{4'}$), 119.75 ($C_{2'}$ + $C_{6'}$)	70.36 (CH_2), 58.08 (OCH_3), 39.90 (CH_2)

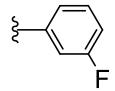
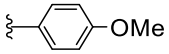
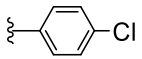
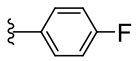
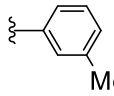
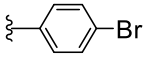
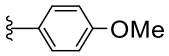
[a] This spectrum was obtained at 80°C; [b] The isolated amount was not enough to make the ^{13}C NMR spectrum.

Table 2.39: ^{13}C NMR spectroscopic data (100 MHz, DMSO-d_6) for pyrazolo[3,4-*d*]pyrimidines derivatives **2.18**



2.18

Comp.	R	R^1	C_3	CH_3	C_{3a}	C_4	C_6	C_{7a}	R	R^1
2.18a [a]			133.11	25.73	99.76	222.84	165.09	154.8	138.76 ($C_{1'}$), 128.56 ($C_{3'}$ + $C_{5'}$), 125.59 ($C_{4'}$), 120.48 ($C_{2'}$ + $C_{6'}$)	155.93 ($C_{4''}$), 131.68 ($C_{1''}$), 123.51 ($C_{2''}$ + $C_{6''}$), 113.86 ($C_{3''}$ + $C_{5''}$), 55.05 (OCH_3)

2.18b [a]			133.70	25.74	99.93	154.62	165.40	154.49	161.85 (d, $C_{3'}$, J 241.00 Hz), 140.21 (d, $C_{1'}$, J 11.00 Hz), 130.38 (d, $C_{5'}$, J 10.00 Hz), 115.76 (d,$C_{6'}$, J 3.00 Hz), 111.98 (d, $C_{4'}$, J 21.00 Hz), 107.05 (d, $C_{2'}$, J 27.00 Hz)	156.01 ($C_{4''}$), 131.53 ($C_{1''}$), 123.56 ($C_{2''}$ + $C_{6''}$), 113.87 ($C_{3''}$ + $C_{5''}$), 55.04 (OCH_3)
2.18c			134.15	26.38	100.88	154.52	165.81	154.00	162.18 (d, $C_{3'}$, J 242.00 Hz), 140.33 (d, $C_{1'}$, J 10.00 Hz), 131.11 (d, $C_{5'}$, J 10.00 Hz), 116.13 (d, $C_{6'}$, J 2.00 Hz), 112.73 (d, $C_{4'}$, J 21.00 Hz), 107.39 (d, $C_{2'}$, J 26.00 Hz)	138.11 ($C_{1''}$), 128.66 ($C_{3''}$ + $C_{5''}$), 127.10 ($C_{4''}$), 122.51 ($C_{2''}$ + $C_{6''}$)
2.18d			133.76	26.41	100.45	154.51	165.78	154.21	160.17 (d, $C_{4'}$, J 242.00 Hz), 135.40 (d, $C_{1'}$, J 3.00 Hz), 122.97 (d, $C_{2'}$ + $C_{6'}$, J 8.00 Hz), 116.09 (d, $C_{3'}$ + $C_{5'}$, J 23.00 Hz)	139.12 ($C_{1''}$), 138.16 ($C_{3''}$), 128.79 ($C_{5''}$), 124.53 ($C_{4''}$), 121.97 ($C_{2''}$), 118.63 ($C_{6''}$), 21.31 (CH_3)
2.18g [a]			133.67	25.80	99.92	154.76	165.48	154.39	138.09 ($C_{1'}$), 131.64 ($C_{3'}$ + $C_{5'}$), 122.22 ($C_{2'}$ + $C_{6'}$), 118.07 ($C_{4'}$)	156.12 ($C_{4''}$), 131.64 ($C_{1''}$), 123.70 ($C_{2''}$ + $C_{6''}$), 113.99 ($C_{3''}$ + $C_{5''}$), 55.05 (OCH_3)

[a] This spectrum was obtained at 80°C.

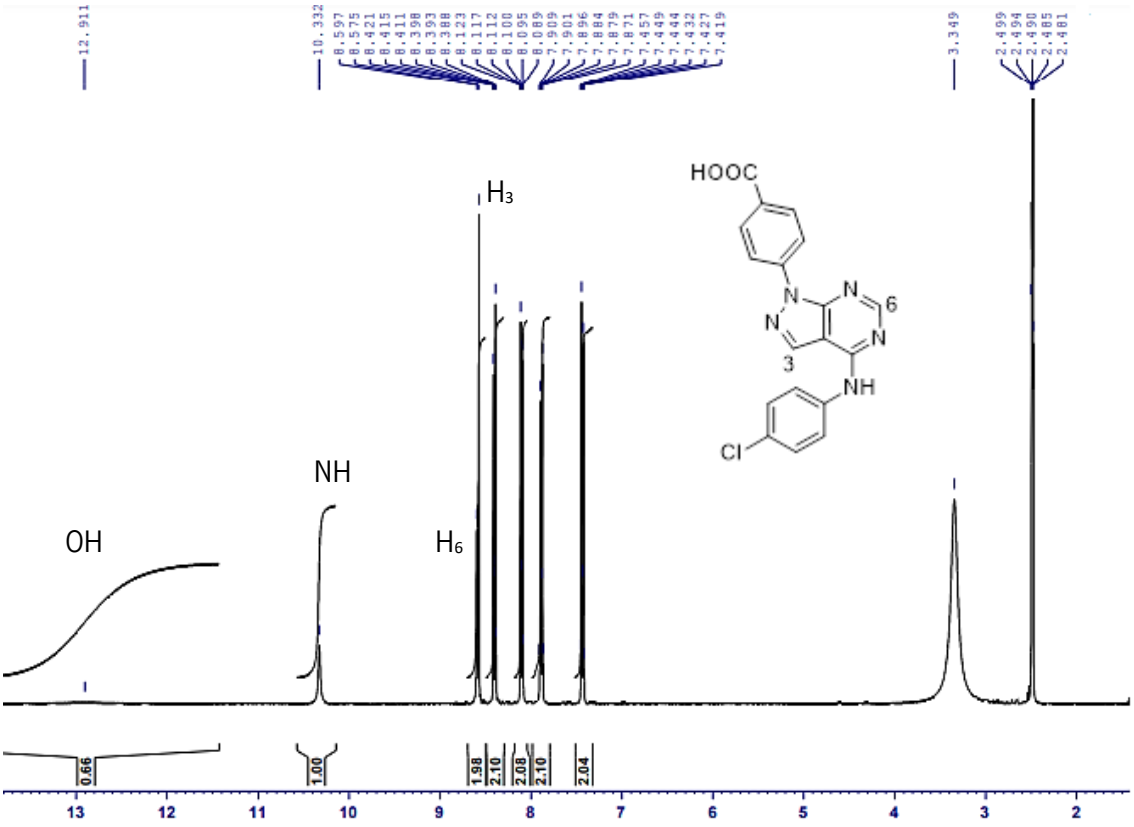


Figure 2.16: ^{1}H NMR spectrum for pyrazolo[3,4-*d*]pyrimidine derivative **2.15al** in DMSO-d_6 solution (^{1}H: 400 MHz).

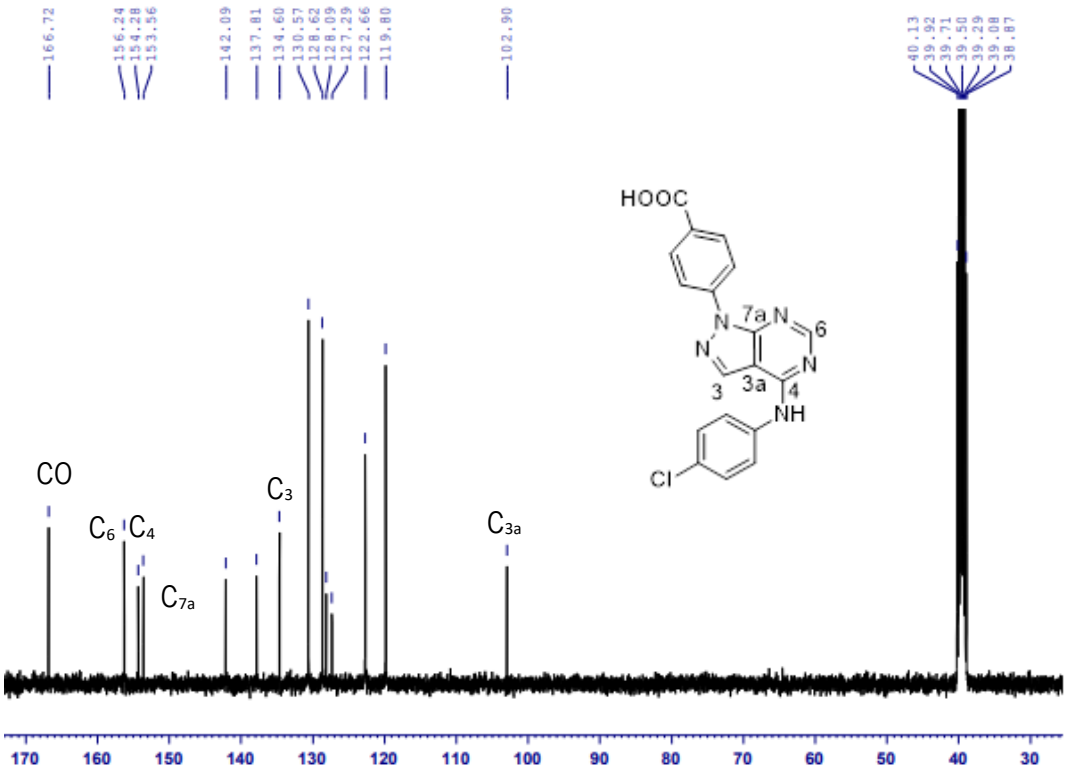
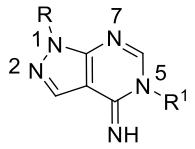


Figure 2.17: ^{13}C NMR spectrum for pyrazolo[3,4-*d*]pyrimidine derivative **2.15al** in DMSO-d_6 solution (^{13}C: 100 MHz).

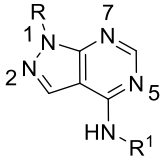
- **^{15}N-NMR Spectroscopy**

In the ^{15}N HMBC correlation spectra, nitrogen atoms N-1 and N-2 of pyrazolo[3,4-*d*]pyrimidines derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27** were identified around δ_N 182.33-203.42 ppm and δ_N 302.85-312.35 ppm, respectively. The values of the nitrogen atom N-5 and N-7 of compound **2.14f** were identified at δ_N 171.19 ppm and δ_N 212.89 ppm, respectively. These are significantly lower values than those identified for the remaining, fully aromatic compounds, where the values of the nitrogen atom N-5 and N-7 were identified around δ_N 222.84-232.72 ppm and δ_N 218.22-230.67 ppm, respectively. The values for the nitrogen atom N-H were identified around δ_N 94.30-113.55 ppm. The values of the nitrogen chemical shifts for all the compounds were summarized in Table **2.40**. Figure **2.18** shows the ^{15}N NMR correlation spectrum of pyrazolo[3,4-*d*]pyrimidine derivative **2.15al** with key signals assigned.

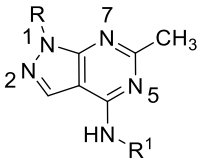
Table 2.40: ^{15}N NMR spectroscopic data (40 MHz, DMSO-d_6) for pyrazolo[3,4-*d*]pyrimidine derivatives **2.14f**, **2.15**, **2.18**, **2.20**, **2.22**, **2.25** and **2.27**



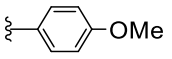
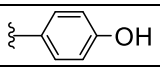
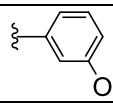
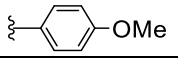
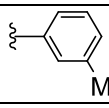
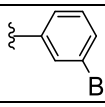
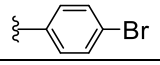
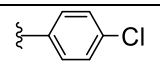
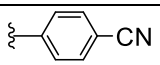
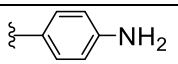
2.14f

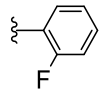
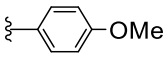
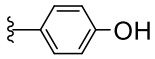
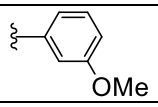
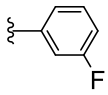
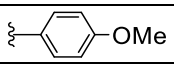
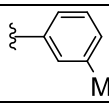
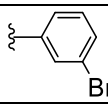
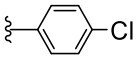
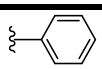
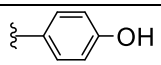
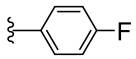
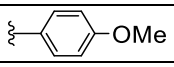
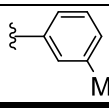
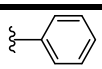
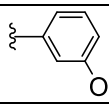
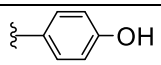
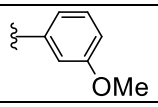
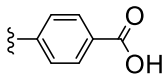
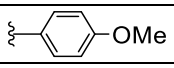
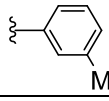
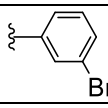
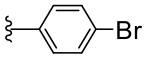
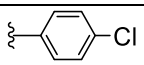
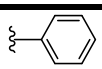
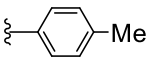
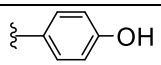
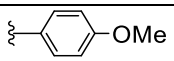


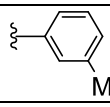
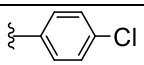
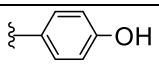
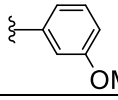
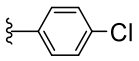
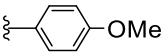
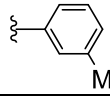
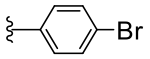
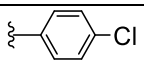
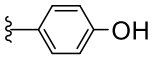
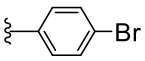
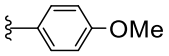
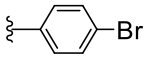
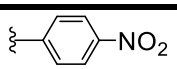
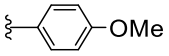
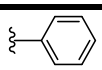
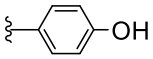
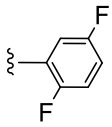
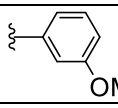
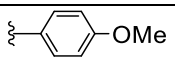
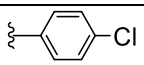
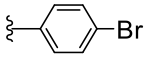
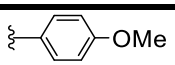
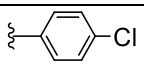
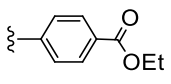
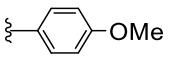
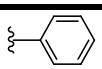
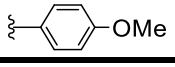
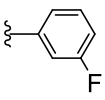
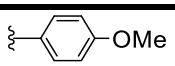
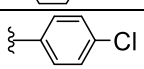
2.15, **2.20**, **2.22**, **2.25**, **2.27**

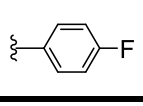
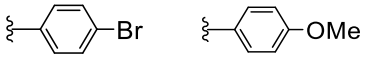
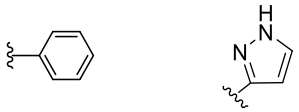
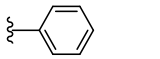
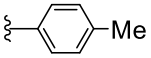
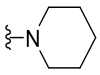
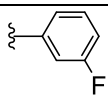
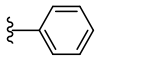
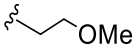
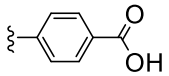


2.18

Comp.	R	R^1	N_1	N_2	N_5	N_7	NH	R^1
2.14f			203.42	305.16	171.19	212.89	[b]	—
2.15a [a]			198.24	306.93	231.03	226.80	[b]	—
2.15d			[b]	[b]	228.56	223.98	111.50	—
2.15e [a]			198.22	307.37	231.60	227.11	[b]	—
2.15f [a]			198.06	[b]	229.41	225.13	110.63	—
2.15g [a]			198.24	307.04	230.71	226.29	[b]	—
2.15h [a]			198.40	308.20	231.50	228.40	[b]	—
2.15i [a]			198.50	307.86	231.00	218.22	111.63	—
2.15j [a]			198.53	307.89	230.67	228.14	111.37	—
2.15k [a]			198.85	309.20	232.72	230.67	[b]	—
2.15l [c]					—			

2.15n [a]			[b]	[b]	229.25	224.00	110.56	—
2.15p [a]			196.40	305.81	231.70	226.20	[b]	—
2.15r [a]			[b]	[b]	230.71	223.75	[b]	—
2.15s			[b]	[b]	231.49	226.40	[b]	—
2.15t [a]			[b]	[b]	230.67	224.63	111.10	—
2.15u			196.40	[b]	230.90	225.80	113.55	—
2.15v			196.52	305.91	231.24	227.85	[b]	—
2.15x [a]			196.66	306.70	[b]	227.56	111.93	—
2.15y [a]			196.54	306.92	230.92	225.90	[b]	—
2.15z [a]			195.99	[b]	229.91	223.45	[b]	—
2.15aa [a]			196.50	[b]	229.82	224.31	[b]	—
2.15ab			196.41	[b]	230.23	225.50	113.30	—
2.15ad [a]			197.54	305.90	231.87	226.82	[b]	—
2.15ae [a]			197.46	305.45	232.46	226.36	[b]	—
2.15af			197.00	[b]	229.70	224.00	[b]	—
2.15ag			197.80	[b]	232.00	227.00	[b]	—
2.15ah [a]			197.40	[b]	230.70	224.90	[b]	—
2.15ai			197.51	[b]	231.22	226.30	[b]	—
2.15aj [a]			197.82	306.69	232.09	228.68	[b]	—
2.15ak			197.47	305.55	231.23	227.76	112.22	—
2.15al			197.40	305.41	231.00	227.70	112.30	—
2.15an			198.19	[b]	229.73	226.78	113.10	—
2.15ao [a]			198.01	[b]	229.72	224.27	[b]	—
2.15ap [a]			198.09	[b]	229.49	225.22	110.70	—

2.15aq			198.33	[b]	229.91	226.51	112.94	—
2.15ar [a]			198.49	308.27	230.85	227.90	[b]	—
2.15as [a]			196.30	306.00	231.21	226.26	[b]	—
2.15at [a]			196.02	[b]	230.32	223.47	[b]	—
2.15au [a]			198.46	306.03	231.73	226.42	[b]	—
2.15av [a]			196.15	[b]	230.35	224.49	[b]	—
2.15aw			196.47	[b]	230.72	225.84	113.48	—
2.15ax			196.34	305.93	230.64	227.10	[b]	—
2.15ay [a]			196.48	306.70	[b]	[b]	[b]	—
2.15ba [a]			196.35	305.88	231.40	226.30	[b]	—
2.15bb [a]			196.00	[b]	230.74	223.72	[b]	—
2.15bc [a]			196.05	[b]	230.21	224.32	111.12	—
2.15bd			196.60	306.44	[b]	[b]	[b]	—
2.15be [c]					—			
2.15bf [c]					—			
2.15bg [a]			[b]	[b]	230.12	222.87	[b]	—
2.15bh			[b]	[b]	230.76	225.33	[b]	—
2.15bi [a]			[b]	[b]	229.90	223.56	111.05	—
2.15bk			186.24	312.32	230.05	225.96	112.08	—
2.15bl	H		190.15	305.46	[b]	227.68	110.56	—
2.15bm [a]			182.33	311.46	227.76	222.28	[b]	—
2.15bn			182.91	312.35	228.25	225.08	111.49	—
2.15bo [a]			[b]	[b]	230.60	225.10	[b]	—
2.18a [a]			[b]	[b]	222.84	229.75	[b]	—
2.18b [a]			[b]	[b]	230.46	222.46	[b]	—
2.18c			[b]	[b]	231.15	224.89	111.56	—

2.18d			[b]	[b]	230.43	222.69	[b]	—
2.18g [a]			195.12	[b]	230.15	222.33	110.81	—
2.20			[b]	[b]	[b]	[b]	[b]	—
2.22a			198.46	307.52	230.18	228.23	[b]	317.58
2.22b			198.52	307.70	230.07	228.24	107.15	317.74
2.25a			196.60	304.27	224.10	220.40	[b]	—
2.25b			194.60	302.85	225.99	219.97	[b]	—
2.27a			197.97	303.96	[b]	[b]	94.30	—
2.27b			197.26	308.37	227.12	220.19	94.99	—

[a] This spectrum was obtained at 80°C; [b] Not visible in the spectrum; [c] The isolated amount was not enough to make the ^{15}N NMR spectrum.

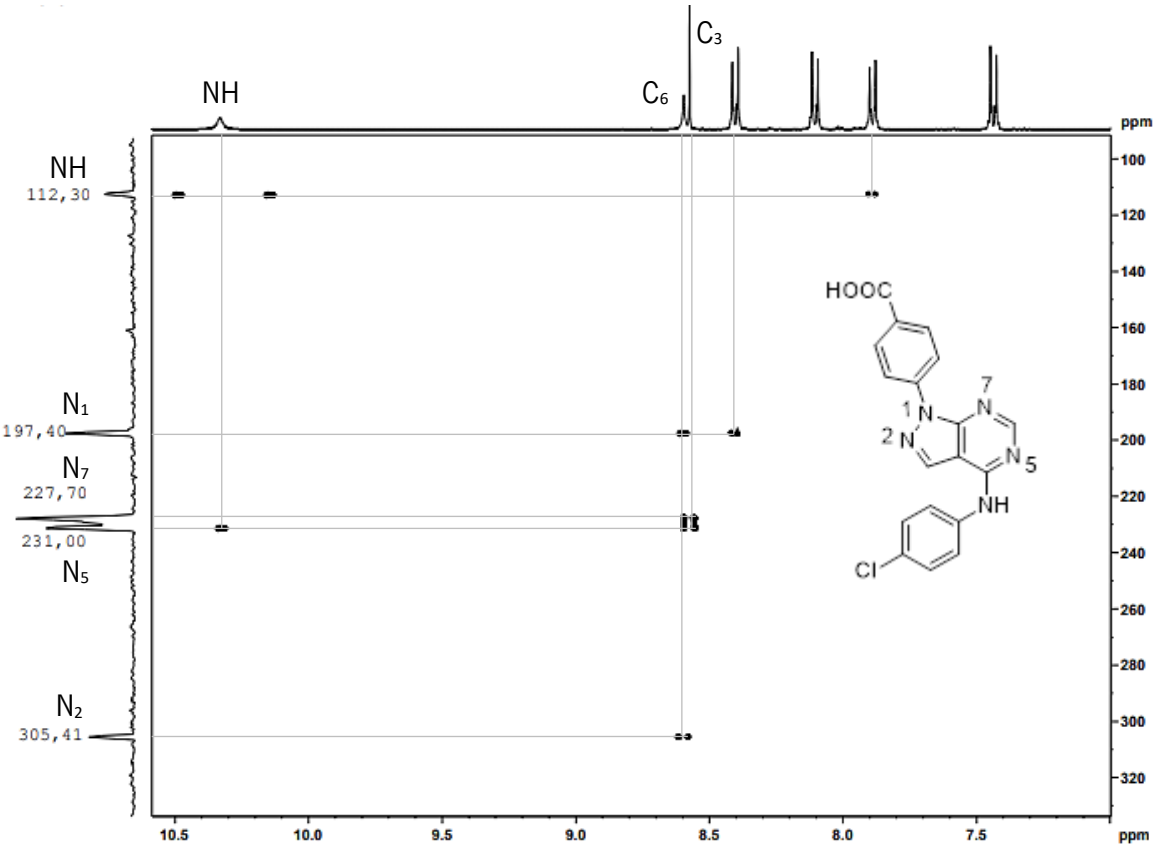


Figure 2.18: ^{15}N HMBC spectrum for pyrazolo[3,4-*d*]pyrimidine derivative **2.15al** in DMSO-d_6 solution (^{15}N: 40 MHz).

Chapter 3 – Biological tests

3.1. Viability screening of pyrazolo[3,4-*d*]pyrimidines

The biological tests were performed in the Life and Health Sciences Research Institute (ICVS) of the School of Medicine at the University of Minho. These assays were conducted by Mónica Cerqueira, Ana Silva and Doctor Marta Sílvia Costa, members of the Cancer Biomarkers and Therapeutics Research Team led by Professor Fátima Baltazar.

The triple-negative breast cancer cell line Hs578t was used to screen the *in vitro* cytotoxic activity of the synthesized pyrazolo[3,4-*d*]pyrimidines **2.15a**, **2.15d-2.15k**, **2.15r-2.15t**, **2.15z**, **2.15aa**, **2.15af**, **2.15ah**, **2.15ao, 2.15ap**, **2.15as, 2.15at**, **2.15bc**, **2.15bg**, **2.15bi**, **2.15bm**, **2.15bo**, **2.18a**, **2.18b**, **2.18g**, **2.20**, **2.25a** and **2.27b**, using paclitaxel as reference drug (Figure **3.1**). Cells were treated for 72 hours, with two different concentrations – 10 and 30 μM – of these compounds and evaluated for their viability, using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay. The concentrations were selected on the basis of previous results obtained by the research group.[57] The cell viability was determined after absorbance measurements at 490 nm and the percentage of cell survival was calculated. The results are shown in tables 3.1-3.5, where the pyrazolo[3,4-*d*]pyrimidine derivatives selected had only one substituent group R changed at a time, aiming to understand the effect of different groups in the biological activity.

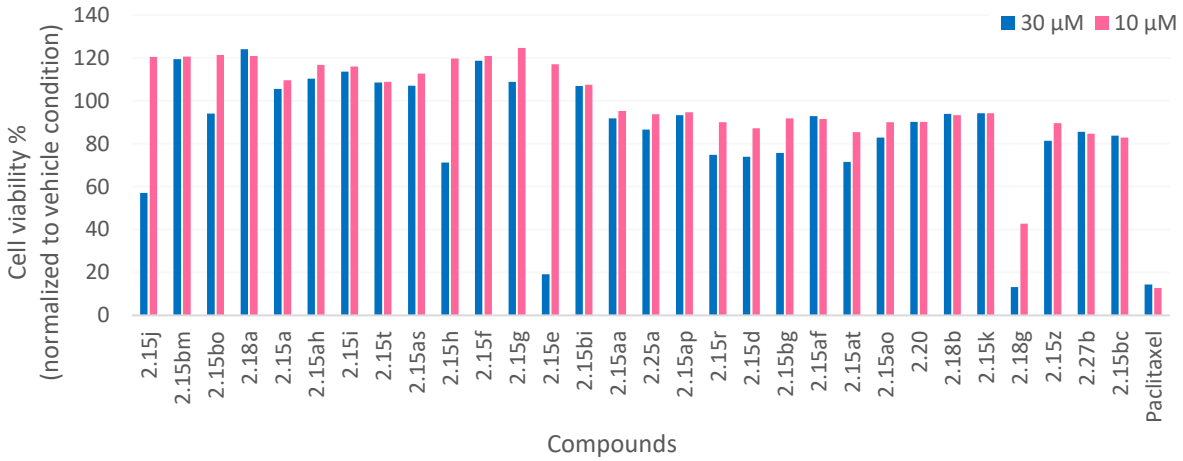
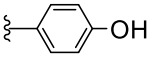
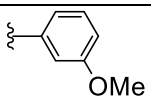
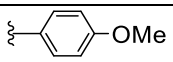
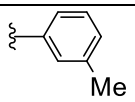
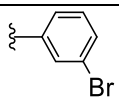
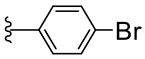
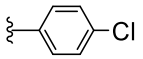
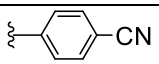
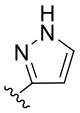
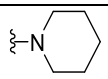


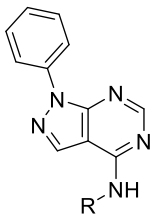
Figure 3.1: Cell viability for Hs578t cell line after 72 hours of treatment for the selected compounds and paclitaxel (10 μM and 30 μM).

In Table **3.1**, we combined the results of 11 compounds where the substituent R linked to the NH was changed, maintaining the phenyl ring linked to the pyrazole. All the compounds **2.15a**, **2.15d-2.15k**, **2.20** and **2.25a** tested did not demonstrate relevant biological activity. Comparing the effect of a substituent in the *para* position of the aromatic ring (compounds **2.15d**, **2.15f**, **2.15i-k**), indicated

that the presence of a chlorine atom enhanced the activity at 30 μM (57.1%) but at 10 μM, an equally poor performance was registered for all compounds. Comparing the effect of a substituent in the *meta* position of the aromatic ring (compounds **2.15e**, **2.15g**, **2.15i-h**), indicated that a methoxyl group performed far better than the remaining compounds at 30 μM (19.1%) but the activity was equally lost at 10 μM for the three derivatives. The presence of a simple phenyl ring (**2.15a**), an aromatic heterocycle (**2.20**) or an aliphatic heterocycle (**2.25a**) had no influence in the biological activity.

Table 3.1: Cell viability results for Hs578t cell line after 72 hours of treatment (10 μM and 30 μM)

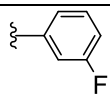
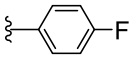
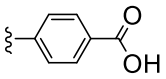
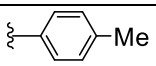
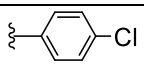
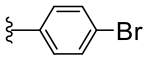
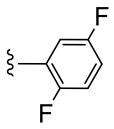
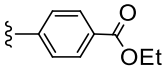
Comp.	R	Cell viability (%) [a]	
		10 μM	30 μM
2.15a		109.6	105.6
2.15d		87.2	73.95
2.15e		117.1	**19.1**
2.15f		120.9	118.6
2.15g		124.6	108.8
2.15h		119.7	71.3
2.15i		116.0	113.6
2.15j		120.5	57.1
2.15k		94.3	94.2
2.20		90.2	90.1
2.25a		93.8	86.5

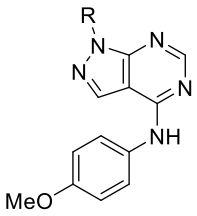


[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values.

Table **3.2** summarizes the cell viability data for compounds **2.15f**, **2.15t**, **2.15aa**, **2.15ah**, **2.15ap**, **2.15as**, **2.15bc**, **2.15bi**, **2.15bm**, **2.15bo**, where the 4-methoxyphenyl substituent was linked to the NH group and the substituent in the pyrazole nitrogen was varied. None of the compounds demonstrated significant biological activity, although we could consider that the presence of a bromine atom in the *para* position of the aromatic ring (**15bc**) slightly reduced the cell viability at both concentrations (10 and 30 μM).

Table 3.2: Cell viability results for Hs578t cell line after 72 hours of treatment (10 μM and 30 μM)

Comp.	R	Cell viability (%) [a]	
		10 μM	30 μM
2.15f		120.9	118.6
2.15t		108.8	108.5
2.15aa		95.3	91.8
2.15ah		116.8	110.4
2.15ap		94.6	93.4
2.15as		112.8	107.1
2.15bc		82.9	83.8
2.15bi		107.5	107.0
2.15bm		120.7	119.4
2.15bo		121.4	94.1

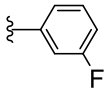
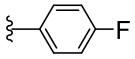
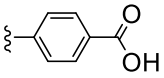
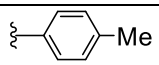
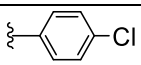
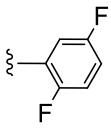


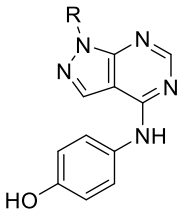
[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values.

Table **3.3** combines the viability results for compounds **2.15d**, **2.15r**, **2.15z**, **2.15af**, **2.15ao**, **2.15at** and **2.15bg**, with a 4-hydroxyphenyl substituent linked to the exocyclic NH and different aromatic substituents in the pyrazole nitrogen. Again, the chlorine atom in the 4-position of the phenyl ring (**2.15at**) has a slight effect on cell viability at 30 μM (71.5%), a result that is comparable to the one obtained for **2.15d** (R=Ph) and **2.15bg** (R=2,5-difluorophenyl). In general, the compounds exhibited no relevant activity.

Compound **2.27b**, with an alkyl substituent in the exocyclic NH and a 4-carboxyphenyl group in the pyrazole nitrogen, was also tested for its anticancer activity, but did not demonstrate significant biological activity (Table **3.4**).

Table 3.3: Cell viability results for Hs578t cell lines after 72 hours of treatment (10 μM and 30 μM)

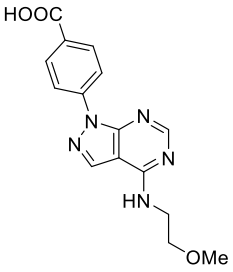
Comp.	R	Cell viability (%) [a]	
		10 μM	30 μM
2.15d		87.2	73.95
2.15r		107.5	107.0
2.15z		89.6	81.4
2.15af		91.5	92.9
2.15ao		90.1	82.9
2.15at		85.4	71.5
2.15bg		91.8	75.8



[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values.

Table 3.4: Cell viability result for Hs578t cell line after 72 hours of treatment (10 μM and 30 μM)

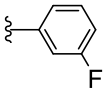
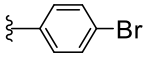
Comp.	Cell viability (%) [a]	
	10 μM	30 μM
2.27b	84.7	85.5

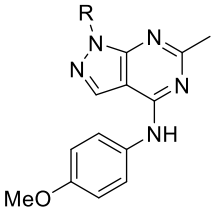


[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values.

Only three compounds were tested where the proton in the 6-position of the pyrazolo[3,4-*d*]pyrimidine ring was replaced by a methyl group (Table **3.5**). In this study, the 4-methoxyphenyl group was maintained at the NH in position 4 of the heterocyclic ring and only the substituent linked to the pyrazole nitrogen was changed. The compound with a bromine atom in the 4-position of the phenyl ring (**2.18g**) presented promising results for the anticancer activity at both concentrations tested (10 and 30 μM). Comparison with the biological activity of the analogous structure with a proton in the 6-position of the heterocycle (Table **3.2**, **2.15bc**) indicates that the presence of the methyl group is essential for the anticancer activity recorded.

Table 3.5: Cell viability results for Hs578t cell lines after 72 hours of treatment (10 μM and 30 μM)

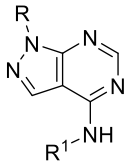
Comp.	R	Cell viability (%) [a]	
		10 μM	30 μM
2.18a		121.0	124.0
2.18b		93.3	94.0
2.18g		**42.8**	**13.2**



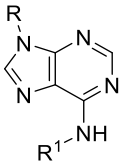
[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values.

Figueiredo *et al.* reported the synthesis of adenine derivatives (**2.29**), and tested them in the same cell line.[52,56] This allowed us to compare the effect of having a pyrazole ring (**2.15**) instead of an imidazole ring (**2.29**) in the fused heterocyclic structure, on the anticancer activity. Table **3.6** combines the cell viability data for both compounds' families, considering the same substitution pattern.

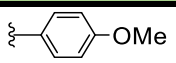
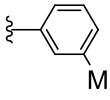
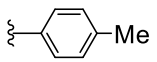
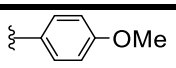
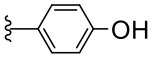
Table 3.6: Cell viability comparison of **2.15** and **2.29n** for Hs578t cell line after 72 hours of treatment (10 μM and 30 μM)



2.15



2.29

Comp.	R	R^1	Cell viability (%) [a]		Comp.	Cell viability (%) [a], [b]	
			10 μM	30 μM		10 μM	30 μM
2.15f			120.9	118.6	**2.29a**	90.0	30.6
2.15g			124.6	108.8	**2.29b**	102.3	46.7
2.15ap			94.6	93.4	**2.29c**	99.2	35.1
2.15ao			90.1	82.9	**2.29d**	106.5	74.2

[a] The results were obtained from at least three independent experiments, each one in triplicate and the data are presented as mean values. [b] Results reported by Figueiredo *et al.* in reference 56.

All the adenine derivatives **2.29a-d** displayed higher anticancer activity when compared to their analogs **2.15f** at a 30 μM concentration. At 10 μM, comparable poor results were registered. These results indicate that the nitrogen atom at position 7 (imidazole ring) appears to be important for biological activity, that is considerably reduced when the nitrogen atom is moved to position 2 (pyrazole ring). However, further studies need to be carried out in order to support and validate this conclusion and also

to potentiate the anticancer activity to the nM range.

In this work, pyrazolo[3,4-*d*]pyrimidine **2.18g** was the most promising compound, leading to the lowest cell viability values, and as such the half maximal inhibitory concentration (IC_{50}) was determined.

3.2. IC_{50} determination

The IC_{50} of the pyrazolo[3,4-*d*]pyrimidine derivative **2.18g** was determined by MTS assay using the Hs578t cancer cell line. Cells were treated with 7 different concentrations of compound **2.18g**, ranging from 0.1 to 40 μM (72 hours) or 5 to 60 μM (24 and 48 hours).

Compound **2.18g** exhibited an IC_{50} value of 4.95 μM (Table **3.7**) after 72 hours of incubation, having no anticancer effect at 24 and 48 hours timepoints.

Table 3.7: IC_{50} value for the **2.18g** in Hs578t cell line

Compound	IC_{50} (μM) in Hs578t cell line		
	24h	48h	72h
2.18g	>30	>30	4.95

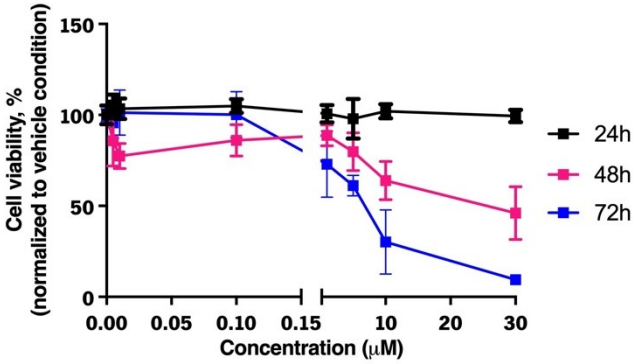
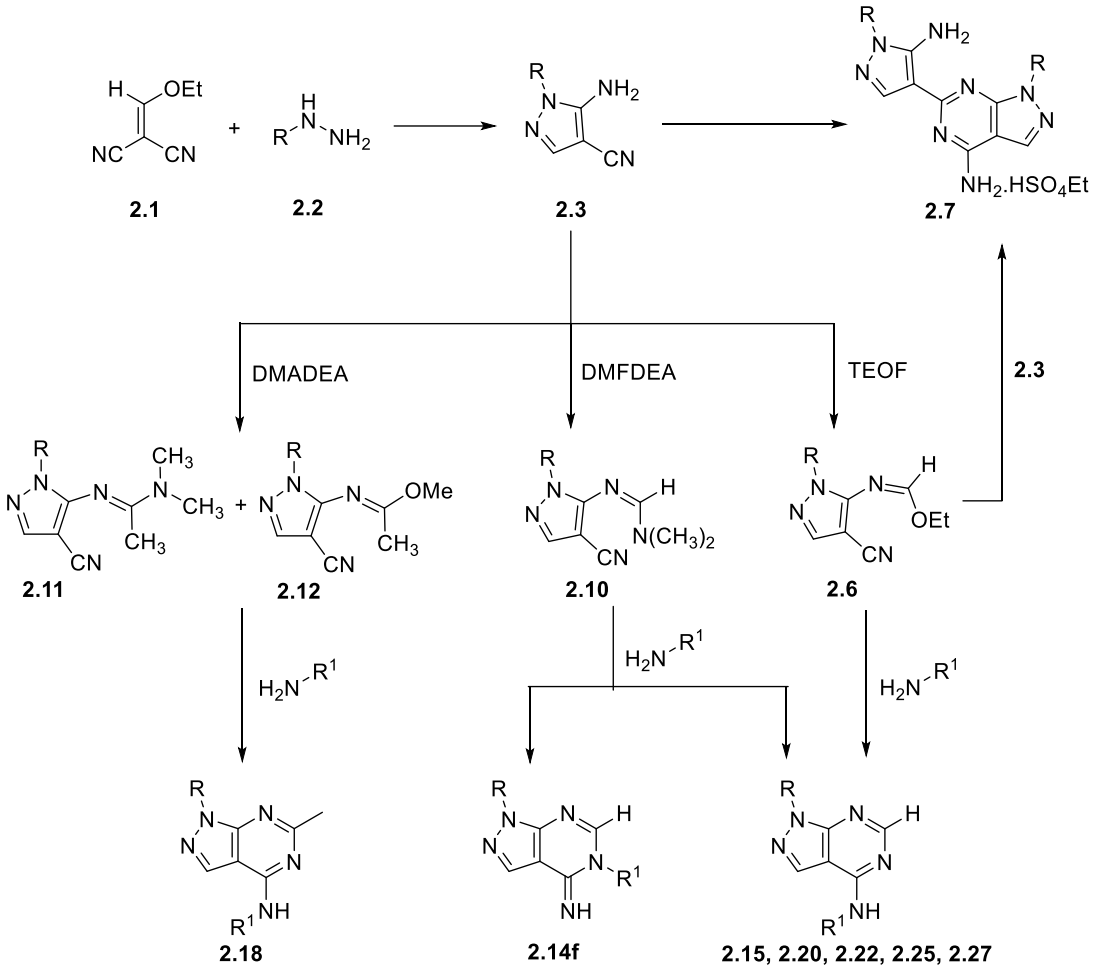


Figure 3.2: Effect of compound **2.18g** on Hs578t cell line viability after 24, 48 and 72 hours. The results were represented as the mean percentage with standard deviation.

Chapter 4 – Conclusions and future prospects

The objectives of this work were to synthesize 5-amino-4-cyanopyrazoles and use them as starting reagents for the synthesis of pyrazolo[3,4-*d*]pyrimidines (Scheme **4.1**).



Scheme 4.1: Studies of the reactivity of 5-amino-4-cyanopyrazoles **2.3**.

5-Amino-4-cyanopyrazole derivatives **2.3** were synthesized from the commercially available ethoxymethylene malononitrile **2.1** and aromatic hydrazines or alkyl hydrazines, using well established synthetic methods. Pyrazoles **2.3** were isolated as pure products in low to good yields (13-82%). Reactions with aromatic hydrazines occurred in a shorter period of time (6-63 hours) than reactions with alkyl hydrazines (28 hours - 10.5 days). Furthermore, the isolated yield of 5-amino-4-cyanopyrazoles was higher when the synthesis involved aromatic hydrazines. Future work should include a study of the reaction mechanism leading to the formation of the ammonium salt, in these reactions. It is possible that, by avoiding its formation, we could improve the isolated yield of the pyrazole derivatives, since this salt is always present in the mother liquor.

Studies on the reactivity of 5-amino-4-cyanopyrazoles **2.3** with TEOF were also carried out. Dimeric structures had been previously synthesized in the research group with a very low yield, so the objective was to improve the yield of these compounds. Imidates **2.6** were obtained in moderate to good yields (42-78%), when the reagents were combined and heated at 150°C, without catalysis. In the presence of sulfuric acid and heating at 50°C in ethanol, a dimeric structure **2.7** was isolated in low to moderate yields (14-48%). This is probably due to a competitive reaction where cleavage of the imidate function, regenerates the pyrazole ring, reducing the amount of imidate available to react with the amino group of another pyrazole **2.3**, ultimately leading to the desired structure. These fused pyrazolo[3,4-*d*]pyrimidines **2.7** will be tested for their biological activity, in particular as anticancer agents. This one-pot synthetic method did not allow to increase the isolated yield of these products. Thus, an alternative approach was tested, reacting imidate **2.6** with a new pyrazole unit **2.3**. In this case, only mixtures of starting reagents or starting reagents and dimeric structure **2.7** were isolated. This reaction was not investigated further, but in future work, it would be important to vary the experimental conditions, such as temperature, catalyst or reaction time.

Previous work in the research group allowed the synthesis of several adenine derivatives. Some of them proved to have a promising anticancer activity when tested on triple negative breast cancer cell lines. The aim of this work was also to replace the imidazole ring in the purine nucleous by a pyrazole ring, preparing several pyrazolo[3,4-*d*]pyrimidine derivatives **2.15**. The effect of altering the position of one nitrogen atom in the 5-membered ring on the anticancer activity would be accessed through the collaboration with partners at ICVS.

Two different methods were used to prepare pyrazolo[3,4-*d*]pyrimidine derivatives **2.15** by reacting different amines with imidate **2.6** or with amidines **2.10** and **2.11** + **2.12**. Amidines **2.10** were prepared from the reaction of pyrazoles **2.3** with DMFDEA. The reaction of **2.3** with DMADEA led to a mixture of amidines **2.11** with imidates **2.12**. The synthesis of amidines **2.10** was carried out at room temperature while the synthesis of the imidate required heating at 150°C. In addition, the yield of amidines **2.10** was excellent. For these reasons, most of the pyrazolo[3,4-*d*]pyrimidine derivatives **2.15** were prepared from amidines **2.10**. Pyrazolo[3,4-*d*]pyrimidines **2.15**, **2.18**, **2.20**, **2.22**, **2.25a-b** and **2.27b** were isolated in low to good yields (11-79%). In these reactions, acetylation of the amines by reaction with acetic acid was also occurring and is probably responsible for the low isolated yield of the products. Compound **2.14f** was obtained when studying the best conditions to prepare pyrazolo[3,4-*d*]pyrimidines. Further studies are required in order to optimize the reaction conditions (reaction time, temperature, acid and/or solvent) to improve the yield of these compounds.

Some of the synthesized pyrazolo[3,4-*d*]pyrimidines were tested for their anticancer activity on the Hs578t cell line. Compound **2.18g** (Figure **4.1**) was the only compound that demonstrated relevant anticancer activity, with a cell viability of 42.8% and 13.2% at 10 μM and 30 μM, respectively, and IC_{50} value of 4.95 μM. The methyl group at C-6 appears to have a key role in the activity of the compound. In addition, the presence of bromine at position 4 of the phenyl ring (**2.15bc**) also seems important. No relevant activity was registered for compound **2.15bc** where the methyl group was replaced by a proton and also for compound **2.18a** where the bromine substituent was also replayed by a proton in the aromatic ring. Future work should focus on the synthesis of pyrazolo[3,4-*d*]pyrimidines with the methyl group at C-6 and with the bromine atom at position 4 of an aromatic ring. Furthermore, the biological activity of the remaining compounds should also be tested.

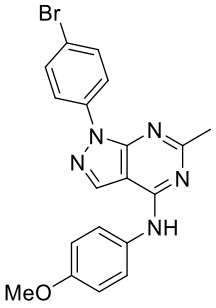


Figure 4.1: Structure of pyrazolo[3,4-*d*]pyrimidine **2.18g**.

Chapter 5 – Experimental procedures

5.1. Chemistry

5.1.1. Reagents and instrumentation

All reagents and solvents were purchased from commercial sources (Acros, Alfa-Aesar, Fluka, Merck and Sigma-Aldrich) and used without further purification. The reactions were followed by Thin Layer Chromatography (TLC) using aluminum plates coated with silica gel 60 and fluorescence indicator of 0.20 mm thickness (Macherey-Nagel, DC-Fertigfolien ALUGRAM Xtra SIL G/UV254). The eluent, varied according to the polarity of the compounds. In general, a mixture of ethyl acetate/petroleum ether (3:1 or 3:2), ethyl acetate/n-hexane (1:1), dichloromethane/ethyl acetate/acetic acid (8:4:0.5) and dichloromethane/ethanol (9:1) were used. The spots were visualized under ultraviolet light (UV - λ_{max} 254 nm) and in an iodine chamber. For flash chromatography silica gel MN Kieselgel 60 (230-400 mesh, particle size <0.063 mm) was used.

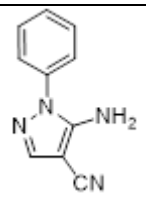
Some of the reactions were performed in a IKAMAG RCT basic, at different temperatures with magnetic stirring between 150-550 RPM. Other reactions were carried out in a Panasonic MIR-154 incubator, at -10°C and 0°C and with a magnetic stirring between 150-300 RPM. Solvent evaporation was performed on a Buchi RE-11 rotary evaporator, under reduced pressure and variable temperature. Petroleum ether refers to the boiling range 40-60°C.

The Infrared Fourier Transform Spectroscopy (FTIR) spectra were recorded on a Nicolet iS10 FT-IR spectrometer (Thermo-Fisher Scientific, Waltam, MA, USA) equipped with a diamond crystal cell for attenuated total reflection (ATR) operation. The spectra were acquired (32 scans per sample or background) in the range of 4000–500 cm^{-1} at a nominal resolution of 4 cm^{-1}. Melting points (°C) were determined in a Gallenkamp melting point apparatus. The NMR spectra were recorded at room temperature and in some cases at 80°C, on a Bruker Avance III 400 (^{1}H: 400 MHz, ^{13}C: 100.6 MHz, ^{15}N: 40.6 MHz), including the ^{1}H, ^{13}C and ^{1}H-^{15}N correlation spectra (HMQC and HMBC) and deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent. The chemical shifts, δ, were reported in ppm and the coupling constants, *J*, in hertz (Hz).

All compounds herein presented were identified on the basis of their analytical and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR with DEPT, HMBC and HMQC, ^{15}N NMR).

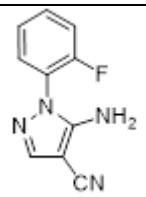
5.1.2. Synthesis addressed in section 2.1.

Synthesis of 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile 2.3a



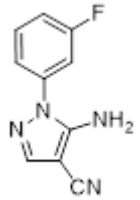
Phenylhydrazine **2.2a** (0.14 g; 122 µL; 1.24 mmol; 1 eq.) was added to an orange suspension of 2-(ethoxymethylene)malononitrile **2.1** (0.15 g; 1.24 mmol) in ethanol (2 mL) leading immediately to a brownish solution. The reaction mixture was stirred at 110°C for 6 hours, when TLC showed the absence of the starting material. The solution was concentrated in the rotary evaporator leading to a light brown solid. The resulting solid was cooled, filtered and washed with a mixture of water:ethanol (2:1) and identified as 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **2.3a** (0.14 g; 0.80 mmol; 63%).

Synthesis of 5-amino-1-(2-fluorophenyl)-1H-pyrazole-4-carbonitrile 2.3b



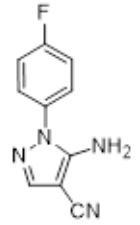
Triethylamine (0.07 g; 97 µL; 0.70 mmol; 1 eq.) was added to an orange suspension of 2-(ethoxymethylene)malononitrile **2.1** (0.08 g; 0.70 mmol) and 2-fluorophenylhydrazine hydrochloride **2.2b** (0.11 g; 0.70 mmol; 1 eq.) in ethanol (1 mL). The resulting brownish solution was stirred at room temperature for 49.5 hours. The solution was concentrated in the rotary evaporator leading to a light brown solid. The solid was filtered and washed with a 3:1 mixture of water and ethanol and was identified as 5-amino-1-(2-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3b** (0.12 g; 0.57 mmol; 82%).

Synthesis of 5-amino-1-(3-fluorophenyl)-1H-pyrazole-4-carbonitrile 2.3c



Triethylamine (0.17 g; 228 µL; 1.64 mmol; 1 eq.) was added to a beige suspension of 2-(ethoxymethylene)malononitrile **2.1** (0.20 g; 1.64 mmol) and 3-fluorophenylhydrazine hydrochloride **2.2c** (0.17 g; 1.64 mmol; 1 eq.) in ethanol (2 mL). The resulting dark solution was stirred at 40°C for 24 hours. The beige solid that precipitated on cooling in an ice bath was filtered and washed with a 3:1 mixture of water and ethanol. The product was identified as 5-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3c** (0.21 g; 1.02 mmol; 62%).

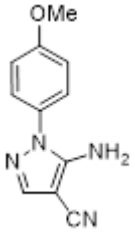
Synthesis of 5-amino-1-(4-fluorophenyl)-1H-pyrazole-4-carbonitrile 2.3d



Triethylamine (0.05 g; 68 µL; 0.49 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.06 g; 0.49 mmol) and (4-fluorophenyl)-hydrazine hydrochloride **2.2d** (0.80 g; 0.49 mmol; 1 eq.) in ethanol (1 mL). The brownish solution was stirred at 60°C. After 48 hours, TLC showed the absence of starting material and the orange solid was filtered, washed with a mixture of water ethanol (3:1) and identified as 5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3d** (0.06 g; 0.29 mmol; 58%).

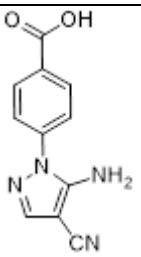
Synthesis of 5-amino-1-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile 2.3e

Triethylamine (0.03 g; 40 µL; 0.29 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.03 g; 0.29 mmol) and 4-methoxyphenylhydrazine hydrochloride **2.2e** (0.05 g; 0.29



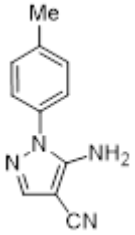
mmol; 1 eq.) in ethanol (0.8 mL). The brownish solution was stirred at room temperature. After 26 hours, TLC showed the absence of starting material and the beige solid was filtered, washed with diethyl ether and identified as 5-amino-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbonitrile **2.3e** (7.90 mg; 0.04 mmol; 13%).

Synthesis of 4-(5-amino-4-cyano-1H-pyrazol-1-yl)benzoic acid 2.3f



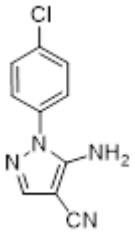
4-Hydrazinobenzoic acid **2.2f** (0.19 g; 1.23 mmol; 1 eq.) was added to an orange suspension of 2-(ethoxymethylene)malononitrile **2.1** (0.15 g; 1.23 mmol) in ethanol (2 mL). The reaction mixture was stirred at room temperature for 24 hours, when the TLC showed the absence of starting material. The resulting orange solid was filtered, washed with a mixture of water:ethanol (3:1) and identified as 4-(5-amino-4-cyano-1*H*-pyrazole-1-yl)benzoic acid **2.3f** (0.19 g; 0.84 mmol; 70%).

Synthesis of 5-amino-1-(4-tolyl)-1H-pyrazole-4-carbonitrile 2.3g



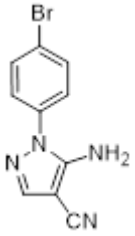
Triethylamine (0.08 g; 115 µL; 0.83 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.10 g; 0.83 mmol) and 4-tolylhydrazine hydrochloride **2.2g** (0.13 g; 0.83 mmol; 1 eq.) in ethanol (2 mL). The orange solution was stirred at 60°C. After 24 hours, TLC showed the absence of starting material and the orange solid was filtered, washed with a mixture of water:ethanol (3:1) and identified as 5-amino-1-(4-tolyl)-1*H*-pyrazole-4-carbonitrile **2.3g** (0.12 g; 0.62 mmol; 75%).

Synthesis of 5-amino-1-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile 2.3h



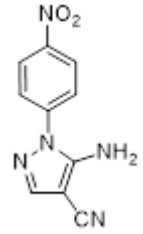
Triethylamine (0.08 g; 117 µL; 0.84 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.10 g; 0.84 mmol) and (4-chlorophenyl)hydrazine hydrochloride **2.2h** (0.15 g; 0.84 mmol; 1 eq.) in ethanol (2 mL). The brownish solution was stirred at 60°C. After 24 hours, TLC showed the absence of starting material and the orange solid that precipitated from the mixture was filtered and washed with a mixture of water:ethanol (3:1). The product was identified as 5-amino-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3h** (0.14 g; 0.66 mmol; 79%).

Synthesis of 5-amino-1-(4-bromophenyl)-1H-pyrazole-4-carbonitrile 2.3i



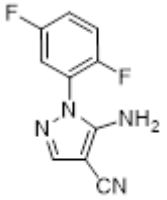
Triethylamine (0.07 g; 90 µL; 0.65 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.08 g; 0.65 mmol) and (4-bromophenyl)hydrazine hydrochloride **2.2i** (0.15 g; 0.65 mmol; 1 eq.) in ethanol (1 mL). The brownish solution was stirred at room temperature. After 48 hours, TLC showed the absence of starting material and the orange solid that precipitated from the mixture was filtered, washed with a mixture of water:ethanol (3:1) and identified as 5-amino-1-(4-bromophenyl)-1*H*-pyrazole-4-carbonitrile **2.3i** (0.12 g; 0.45 mmol; 69%).

Synthesis of 5-amino-1-(4-nitrophenyl)-1H-pyrazole-4-carbonitrile 2.3j



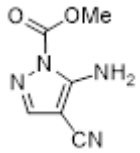
(4-Nitrophenyl)hydrazine **2.2j** (0.05 g; 0.33 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.04 g; 0.33 mmol) in ethanol (2 mL). The brownish suspension was stirred at 40°C for 63 hours and the evolution was followed by TLC. The deep orange solid that precipitated, was filtered, washed with a mixture of water:ethanol (3:1) and identified as 5-amino-1-(4-nitrophenyl)-1*H*-pyrazole-4-carbonitrile **2.3j** (0.04 g; 0.19 mmol; 59%).

Synthesis of 5-amino-1-(2,5-difluorophenyl)-1H-pyrazole-4-carbonitrile 2.3k



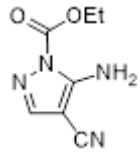
2,5-Difluorophenylhydrazine **2.2k** (0.18 g; 1.23 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.15 g; 1.23 mmol) in ethanol (2 mL). The brownish solution was stirred at room temperature. After 18 hours, TLC showed the absence of the starting material. The light brown solid that precipitated on cooling in an ice bath was filtered and washed with a 3:1 mixture of water and ethanol. The product was identified as 5-amino-1-(2,5-difluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3k** (0.21 g; 0.95 mmol; 77%).

Synthesis of methyl 5-amino-4-cyano-1H-pyrazole-1-carboxylate 2.3l



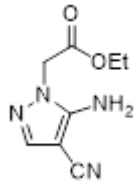
Methyl hydrazinecarboxylate **2.2l** (0.07 g; 0.82 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.10 g; 0.82 mmol) in ethanol (2.5 mL). The brownish solution was stirred at 60°C. After 28 hours TLC showed the absence of starting material. The beige solid that precipitated on cooling in an ice bath was filtered and washed with a 3:1 mixture of water and ethanol. The product was identified as methyl 5-amino-4-cyano-1*H*-pyrazole-1-carboxylate **2.3l** (0.05 g; 0.28 mmol; 34%).

Synthesis of ethyl 5-amino-4-cyano-1H-pyrazole-1-carboxylate 2.3m



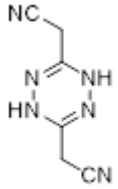
Ethyl hydrazinecarboxylate **2.2m** (0.09 g; 0.85 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.10 g; 0.85 mmol) in ethanol (1 mL). The light orange suspension was stirred at room temperature. After 48 hours, TLC showed the absence of the starting material. The beige solid that precipitated on cooling in an ice bath was filtered and washed with water. The product was identified as ethyl 5-amino-4-cyano-1*H*-pyrazole-1-carboxylate **2.3m** (0.08 g; 0.44 mmol; 51%).

Synthesis of ethyl 2-(5-amino-4-cyano-1H-pyrazole-1-yl)acetate 2.3n



Triethylamine (0.09 g; 118 µL; 0.84 mmol; 1 eq.) was added to a light brown suspension of 2-(ethoxymethylene)malononitrile **2.1** (0.10 g; 0.84 mmol) and ethyl hydrazinoacetate hydrochloride **2.2n** (0.13 g; 0.84 mmol; 1 eq.) in ethanol (2 mL). The resulting brownish solution was stirred at room temperature for 10.5 days. After 5 days at -20°C, the solution was concentrated in the rotary evaporator leading to a beige solid. The solid was filtered, washed with water and identified as of ethyl 2-(5-amino-4-cyano-1*H*-pyrazole-1-yl) acetate **2.3n** (0.08 g; 0.42 mmol; 51%).

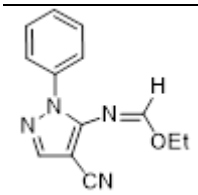
Synthesis of 2,2'-(1,4-dihydro-1,2,4,5-tetrazine-3,6-diyl)diacetonitrile 2.5



2-Cyanoacetohydrazide **2.2o** (0.09 g; 0.87 mmol; 1 eq.) was added to a solution of 2-(ethoxymethylene)malononitrile **2.1** (0.11 g; 0.87 mmol) in ethanol (1 mL). The light orange suspension was stirred at room temperature. After 48 hours, TLC showed the absence of the starting material. The orange solid that precipitated on cooling in an ice bath was filtered and washed with water. The product was identified as 2,2'-(1,4-dihydro-1,2,4,5-tetrazine-3,6-diyl)diacetonitrile **2.5** (0.04 g; 0.22 mmol; 25%).

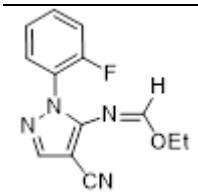
5.1.3. Synthesis addressed in section 2.2.

Synthesis of ethyl N-(4-cyano-1-phenyl-1H-pyrazol-5-yl)formimidate 2.6a



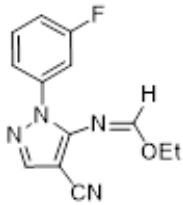
Triethylorthoformate (0.12 g; 136 µL; 0.82 mmol; 3 eq.) was added to 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **2.3a** (0.05 g; 0.27 mmol) and the brownish suspension was heated at 150°C for 16 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)formimidate **2.6a** (0.04 g; 0.17 mmol; 62%).

Synthesis of ethyl N-(4-cyano-1-(2-fluorophenyl)-1H-pyrazol-5-yl)formimidate 2.6b



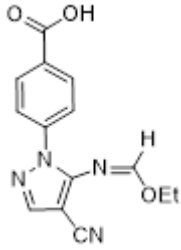
Triethylorthoformate (0.06 g; 68 µL; 0.41 mmol; 3 eq.) was added to 5-amino-1-(2-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3b** (0.03 g; 0.14 mmol) and the brownish suspension was heated at 150°C for 6 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl *N*-(4-cyano-1-(2-fluorophenyl)-1*H*-pyrazol-5-yl)formimidate **2.6b** (0.03 g; 0.11 mmol; 78%).

Synthesis of ethyl N-(4-cyano-1-(3-fluorophenyl)-1H-pyrazol-5-yl)formimidate 2.6c



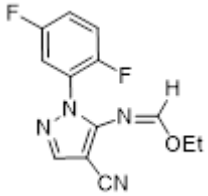
Triethylorthoformate (0.12 g; 131 µL; 0.78 mmol; 3 eq.) was added to 5-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3c** (0.05 g; 0.26 mmol) and the brownish suspension was heated at 150°C for 7 hours and 40 minutes. The resulting orange solid was filtered, washed with cold diethyl ether and identified as ethyl *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)formimidate **2.6c** (0.03 g; 0.11 mmol; 42%).

Synthesis of 4-(4-cyano-5-((ethoxymethylene)amino)-1H-pyrazol-1-yl)benzoic acid 2.6f



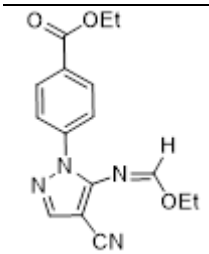
Triethylorthoformate (0.10 g; 115 µL; 0.69 mmol; 3 eq.) was added to 4-(5-amino-4-cyano-1*H*-pyrazole–1-yl)benzoic acid **2.3f** (0.05 g; 0.23 mmol) and the orange suspension was heated at 150°C for 3 hours. The resulting orange solid was filtered and washed with cold diethyl ether and identified as 4-(4-cyano-5-((ethoxy-methylene)amino)-1*H*- pyrazol-1-yl)benzoic acid **2.6f** (0.04 g; 0.12 mmol; 54%).

Synthesis of ethyl N-(4-cyano-1-(2,5-difluorophenyl)-1H-pyrazol-5-yl)formimidate 2.6k



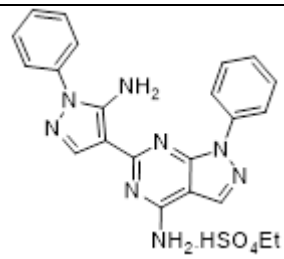
Triethylorthoformate (0.10 g; 114 µL; 0.68 mmol; 3 eq.) was added to 5-amino-1-(2,5-difluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3k** (0.05 g; 0.23 mmol) and the orange suspension was heated at 150°C for 6 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The light orange solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl *N*-(4-cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)formimidate **2.6k** (0.04 g; 0.15 mmol; 63%).

Synthesis of ethyl 4-(4-cyano-5-((ethoxymethylene)amino)-1H-pyrazol-1-yl)benzoate 2.6r



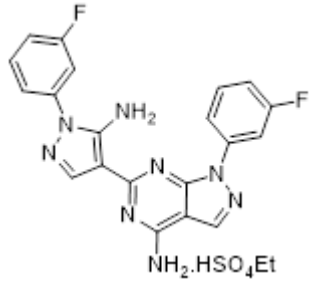
Triethylorthoformate (0.20 g; 230 µL; 1.38 mmol; 6 eq.) was added to 4-(5-amino-4-cyano-1*H*-pyrazol-1-yl)benzoic acid **2.3r** (0.05 g; 0.23 mmol) and the orange suspension was heated at 150°C for 3 hours. The resulting orange solid was filtered, washed with cold diethyl ether and identified as ethyl 4-(4-cyano-5-((ethoxy-methylene)amino)-1*H*-pyrazol-1-yl)benzoate **2.6r** (0.03 g; 0.11 mmol; 47%).

Synthesis of ethyl sulfate salt of 6-(5-amino-1-phenyl-1H-pyrazole-4-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7a



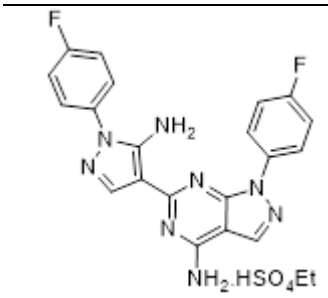
Triethylorthoformate (0.13 g; 143 µL; 0.86 mmol; 3 eq.) and H_2SO_4 (0.01 g; 8 µL; 0.14 mmol; 0.5 eq.) were added to a brownish solution of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **2.3a** (0.05 g; 0.29 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 50°C for 68 hours and the evolution was followed by TLC. The beige solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1-phenyl-1*H*-pyrazole-4-yl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimin-4-amine **2.7a** (0.04 g; 0.09 mmol; 31%).

Synthesis of ethyl sulfate salt of 6-(5-amino-1-(3-fluorophenyl)-1H-pyrazol-4-yl)-1-(3-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7c



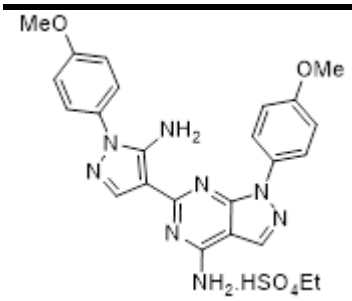
Triethylorthoformate (0.12 g; 136 µL; 0.81 mmol; 3 eq.) and H_2SO_4 (0.01 g; 7 µL; 0.14 mmol; 0.5 eq.) were added to a brownish solution of 5-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3c** (0.05 g; 0.27 mmol) in ethanol (1 mL). The reaction mixture was stirred at 50°C for 29 days and the evolution was followed by TLC. The beige solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1–(3-fluorophenyl)-1*H*-pyrazol-4-yl)-1-(3-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.7c** (0.03 g; 0.06 mmol; 24%).

Synthesis of ethyl sulfate salt of 6-(5-amino-1-(4-fluorophenyl)-1H-pyrazol-4-yl)-1-(4-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7d



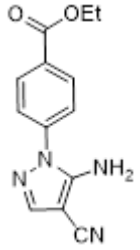
Triethylorthoformate (0.08 g; 89 μL; 0.54 mmol; 3 eq.) and H_2SO_4 (0.01 g; 5 μL; 0.09 mmol; 0.5 eq.) were added to a brownish solution of 5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3d** (0.04 g; 0.18 mmol) in ethanol (1.5 mL). The reaction mixture was stirred at 50°C for 72 hours and the evolution was followed by TLC. The beige solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4–yl)–1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.7d** (0.04 g; 0.08 mmol; 43%).

Synthesis of ethyl sulfate salt of 6-(5-amino-1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7e



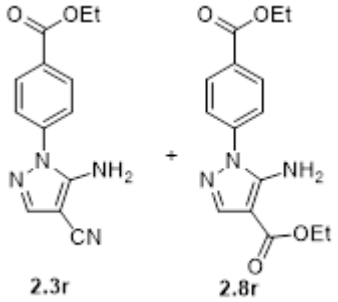
Triethylorthoformate (0.11 g; 122 μL; 0.73 mmol; 3 eq.) and H_2SO_4 (0.01 g; 6 μL; 0.12 mmol; 0.5 eq.) were added to a yellowish solution of 5-amino-1-(4-methoxyphenyl)-1*H*-pyrazole-4-carbonitrile **2.3e** (0.05 g; 0.24 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 50°C for 22 hours and the evolution was followed by TLC. The light pink solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-1-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.7e** (0.06 g; 0.12 mmol; 48%).

Synthesis of ethyl 4-(5-amino-4-cyano-1H-pyrazole-1-yl)benzoate 2.3r



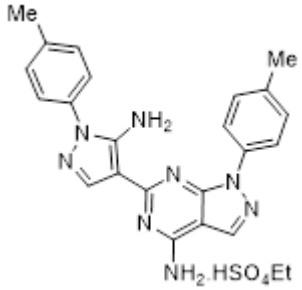
Triethylorthoformate (0.10 g; 114 μL; 0.69 mmol; 3 eq.) and H_2SO_4 (0.01 g; 6 μL; 0.11 mmol; 0.5 eq.) were added to an orange suspension of 4-(5-amino-4-cyano-1*H*-pyrazol-1-yl)benzoic acid **2.3e** (0.05 g; 0.23 mmol) in ethanol (1 mL). The reaction mixture was stirred at 50°C and the evolution was followed by TLC. After 3 days, the orange solid was filtered, washed with cold diethyl ether and identified as the ethyl-4-(5-amino-4-cyano-1*H*-pyrazole-1-yl)benzoate **2.3r** (0.04 g; 0.16 mmol; 69%).

Reaction of 4-(5-amino-4-cyano-1H-pyrazol-1-yl)benzoic acid 2.3f with TEOF



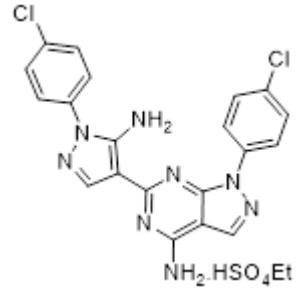
Triethylorthoformate (0.10 g; 111 μL; 0.66 mmol; 3 eq.) and H_2SO_4 (0.01 g; 6 μL; 0.11 mmol; 0.5 eq.) were added to an orange suspension of 4-(5-amino-4-cyano-1*H*-pyrazol-1-yl)benzoic acid **2.3f** (0.05 g; 0.22 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 100°C for 48 hours. The beige solid precipitate was filtered and washed with cold diethyl ether. The product was identified as a mixture of ethyl 4-(5-amino-4-cyano-1*H*-pyrazol-1-yl)benzoate **2.3r** and ethyl 5-amino-1-(4-(ethoxycarbonyl)phenyl)-1*H*-pyrazole-4-carboxylate **2.8r** (0.01 g) in a molar ration of 1:1.6, by ^{1}H NMR.

Synthesis of ethyl sulfate salt of 6-(5-amino-1-(4-tolyl)-1H-pyrazol-4-yl)-1-(4-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7g



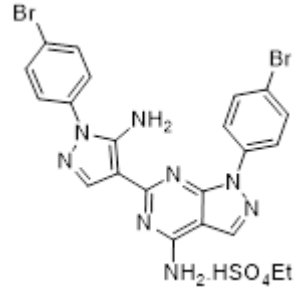
Triethylorthoformate (0.10 g; 116 µL; 0.70 mmol; 3 eq.) and H_2SO_4 (0.01 g; 6 µL; 0.12 mmol; 0.5 eq.) were added to a brownish solution of 5-amino-1-(4-tolyl)-1*H*-pyrazole-4-carbonitrile **2.3g** (0.05 g; 0.23 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 50°C for 72 hours and the evolution was followed by TLC. The beige solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1-(4-tolyl)-1*H*-pyrazol-4-yl)-1-(4-tolyl)-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine **2.7g** (0.02 g; 0.03 mmol; 14%).

Synthesis of ethyl sulfate salt of 6-(5-amino-1-(4-chlorophenyl)-1H-pyrazol-4-yl)-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7h



Triethylorthoformate (0.10 g; 114 µL; 0.68 mmol; 3 eq.) and H_2SO_4 (0.01 g; 6 µL; 0.11 mmol; 0.5 eq.) were added to a reddish solution of 5-amino-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3h** (0.05 g; 0.23 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 50°C for 72 hours and the evolution was followed by TLC. The orange solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.7h** (0.06 g; 0.11 mmol; 47%).

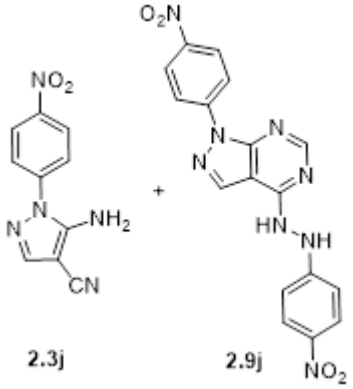
Synthesis of ethyl sulfate salt of 6-(5-amino-1-(4-bromophenyl)-1H-pyrazol-4-yl)-1-(4-bromophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.7i



Triethylorthoformate (0.08 g; 90 µL; 0.54 mmol; 3 eq.) and H_2SO_4 (0.01 g; 5 µL; 0.09 mmol; 0.5 eq.) were added to a yellowish solution of 5-amino-1-(4-bromophenyl)-1*H*-pyrazole-4-carbonitrile **2.3i** (0.05 g; 0.18 mmol) in ethanol (1 mL). The reaction mixture was stirred at 50°C for 72 hours and the evolution was followed by TLC. The beige solid precipitate was filtered, washed with cold diethyl ether and identified as the ethyl sulfate salt of 6-(5-amino–1-(4-bromophenyl)-1*H*-pyrazol-4-yl)-1-(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.7i** (0.03 g; 0.04 mmol; 22%).

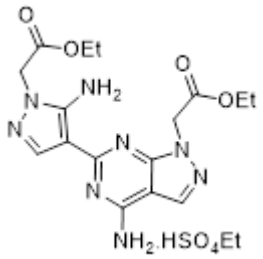
Reaction of 5-amino-1-(4-nitrophenyl)-1H-pyrazole-4-carbonitrile 2.3j with TEOF

Triethylorthoformate (0.06 g; 64 µL; 0.39 mmol; 3 eq.) and H_2SO_4 (0.01 g; 3 µL; 0.07 mmol; 0.5 eq.) were added to a light orange solution of 5-amino-1-(4-nitrophenyl)-1*H*-pyrazole-4-carbonitrile **2.3j** (0.03 g; 0.13 mmol) in ethanol (0.5 mL). The reaction mixture was stirred at 50°C for 6 days and the



evolution was followed by TLC. The orange solid precipitate was filtered and washed with cold ethanol. The product was identified as a mixture of the starting material **2.3j** and 1-(4-nitrophenyl)-4-(2-(4-nitrophenyl)hydrazineyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine **2.9j** (0.01 g) in a molar ration of 1:1.8, by ^{1}H NMR.

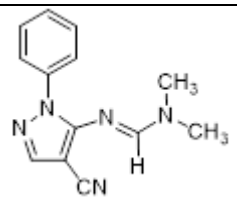
Synthesis of ethyl sulfate salt of ethyl 2-(5-amino-4-(4-amino-1-(2-ethoxy-2-oxoethyl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)-1H-pyrazole-1-yl)acetate 2.7n



Triethylorthoformate (0.12 g; 131 µL; 0.79 mmol; 3 eq.) and H_2SO_4 (0.01 g; 7 µL; 0.13 mmol; 0.5 eq.) were added to a brownish solution of ethyl 2-(5-amino-4-cyano-1*H*-pyrazole-1-yl)acetate **2.3n** (0.05 g; 0.26 mmol) in ethanol (1 mL). The reaction mixture was stirred at 50°C for 4 days and the evolution was followed by TLC. After 3 days in the freezer (-20°C), the precipitate obtained was filtered and washed with cold diethyl ether. The beige product was identified as the ethyl sulfate salt of ethyl 2-(5-amino-4-(4-amino-1-(2-ethoxy-2-oxoethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)-1*H*-pyrazol-1-yl) acetate **2.7n** (0.03 g; 0.05 mmol; 21%).

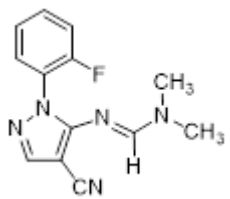
5.1.4. Synthesis addressed in section 2.3.

Synthesis of N'-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10a



DMFDEA (0.10 g; 112 µL; 0.65 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **2.3a** (0.08 g; 0.43 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.10 g; 0.42 mmol; 97%).

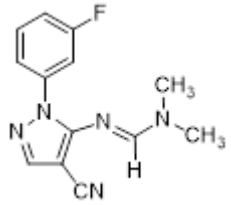
Synthesis of N'-(4-cyano-1-(2-fluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10b



DMFDEA (0.05 g; 58 µL; 0.34 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(2-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3b** (0.05 g; 0.22 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL)

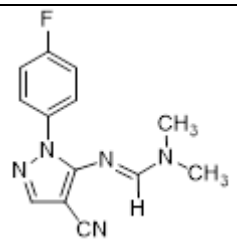
as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-(2-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylformimidamide **2.10b** (0.04 g; 0.18 mmol; 82%).

Synthesis of N'-(4-cyano-1-(3-fluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10c



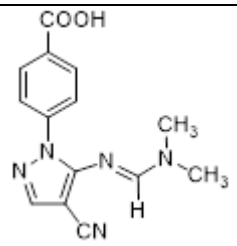
DMFDEA (0.05 g; 64 µL; 0.37 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3c** (0.05 g; 0.24 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylformimidamide **2.10c** (0.06 g; 0.23 mmol; 95%).

Synthesis of N'-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10d



DMFDEA (0.05 g; 64 µL; 0.37 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3d** (0.05 g; 0.25 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethyl-formimidamide **2.10d** (0.06 g; 0.23 mmol; 92%).

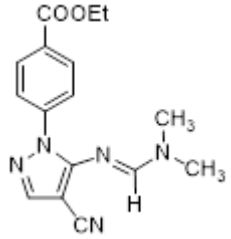
Synthesis of 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1H-pyrazol-1-yl)benzoic acid 2.10f



DMFDEA (0.13 g; 152 µL; 0.89 mmol; 4 eq.) was added to a light orange solution of4-(5-amino-4-cyano-1*H*-pyrazole-1-yl)benzoic acid **2.3f** (0.05 g; 0.22 mmol) in DCM (0.5 mL) and EtOH (100 µL). The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting light orange oil was identified as 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.06 g; 0.21 mmol; 96%).

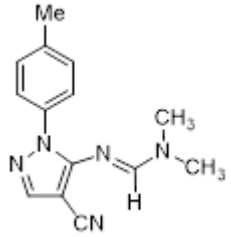
Synthesis of ethyl 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1H-pyrazol-1-yl)benzoate 2.10r

DMFDEA (0.16 g; 188 µL; 1.1 mmol; 5 eq.) was added to a light orange suspension of4-(5-amino-4-cyano-1*H*-pyrazole-1-yl)benzoic acid **2.3f** (0.05 g; 0.22 mmol). The reaction mixture was refluxed for



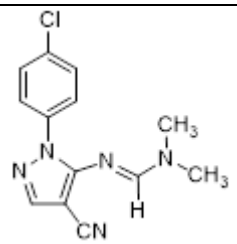
2 hours, leading to a homogeneous solution. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoate **2.10r** (0.05 g; 0.18 mmol; 80%).

Synthesis of N'-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10g



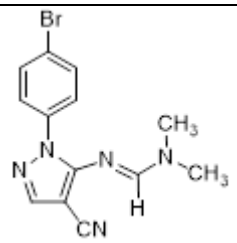
DMFDEA (0.06 g; 70 µL; 0.41 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(4-tolyl)-1*H*-pyrazole-4-carbonitrile **2.3g** (0.05 g; 0.27 mmol) in DCM (1.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-(p-tolyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylformimidamide **2.10g** (0.06 g; 0.24 mmol; 90%).

Synthesis of N'-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10h



DMFDEA (0.05 g; 59 µL; 0.35 mmol; 1.5 eq.) was added to a light orange suspension of 5-amino-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3h** (0.05 g; 0.23 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting light orange oil was identified as *N*-(1-(4-chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N,N*-dimethyl-formimidamide **2.10h** (0.06 g; 0.22 mmol; 98%).

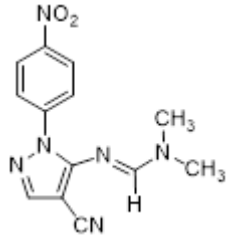
Synthesis of N'-(1-(4-bromophenyl)-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10i



DMFDEA (0.04 g; 51 µL; 0.30 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(4-bromophenyl)-1*H*-pyrazole-4-carbonitrile **2.3i** (0.05 g; 0.20 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting light orange oil was identified as *N*-(1-(4-bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N,N*-dimethylformimidamide **2.10i** (0.06 g; 0.19 mmol; 97%).

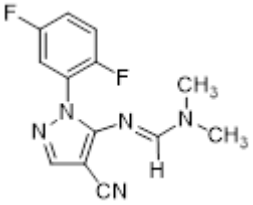
Synthesis of N'-(4-cyano-1-(4-nitrophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10j

DMFDEA (0.01 g; 17 µL; 0.10 mmol; 1.5 eq.) was added to a light orange suspension of 5-amino-1-



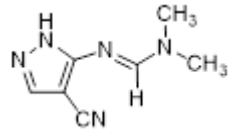
(4-nitrophenyl)-1*H*-pyrazole-4-carbonitrile **2.3j** (0.02 g; 0.07 mmol) in DCM (1.0 mL) leading immediately to a greenish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting light orange oil was identified as *N*-(4-cyano-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10j** (0.01 g; 0.05 mmol; 70%).

Synthesis of N'-(4-cyano-1-(2,5-difluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethyl-formimidamide 2.10k



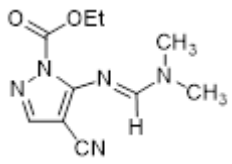
DMFDEA (0.05 g; 58 µL; 0.34 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(2,5-difluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3k** (0.05 g; 0.23 mmol) in DCM (0.5 mL) leading immediately to a reddish solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as *N*-(4-cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-formimidamide **2.10k** (0.06 g; 0.20 mmol; 88%).

Synthesis of N'-(4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10l



DMFDEA (0.08 g; 89 µL; 0.52 mmol; 2 eq.) was added to a beige suspension of methyl 5-amino-4-cyano-1*H*-pyrazole-1-carboxylate **2.3l** (0.04 g; 0.26 mmol) in DCM (0.5 mL) leading immediately to a light brown solution. The reaction mixture was stirred at room temperature for 24 hours. The solid precipitate was filtered and washed with water. The yellow solid was identified as *N*-(4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10l** (0.01 g; 0.09 mmol; 35%).

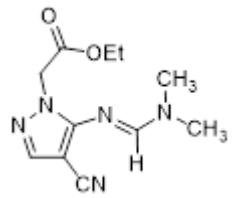
Synthesis of ethyl 4-cyano-5-(((dimethylamino)methylene)amino)-1H-pyrazole-1-carboxylate 2.10m



DMFDEA (0.07 g; 79 µL; 0.46 mmol; 2 eq.) was added to a yellowish suspension of ethyl 5-amino-4-cyano-1*H*-pyrazole-1-carboxylate **2.3m** (0.04 g; 0.23 mmol) in DCM (0.5 mL) leading immediately to a light brown solution. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl 4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazole-1-carboxylate **2.10m** (0.05 g; 0.20 mmol; 85%).

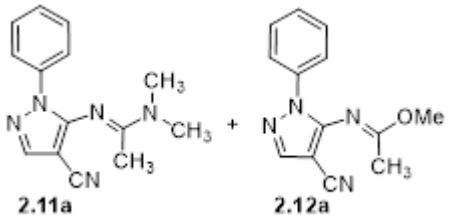
Synthesis of ethyl 2-(4-cyano-5-(((dimethylamino)methylene)amino)-1H-pyrazol-1-yl)acetate 2.10n

DMFDEA (0.06 g; 66 µL; 0.40 mmol; 2 eq.) was added to a beige suspension of ethyl 2-(5-amino-4-



cyano-1*H*-pyrazole-1-yl)acetate **2.3n** (0.05 g; 0.26 mmol) in DCM (1.5 mL) leading immediately to a light brown solution. The reaction mixture was stirred at room temperature for 48 hours. The reaction mixture was purified by dry flash chromatography, using acetonitrile (20 mL) as eluent. The brownish solution was concentrated in the rotatory evaporator. The resulting brownish oil was identified as ethyl 2-(4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)acetate **2.10n** (0.05 g; 0.19 mmol; 73%).

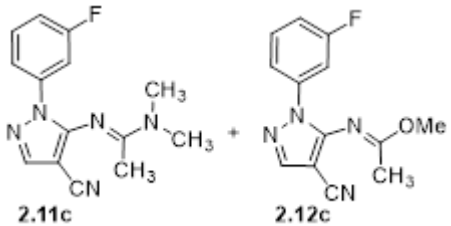
Reaction of 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile 2.3a with DMADEA



2.11a **2.12a**

DMADEA (0.09 g; 101 µL; 0.65 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **2.3a** (0.08 g; 0.43 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 25 hours. The resulting brownish oil was identified as a mixture of *N'*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylacetimidamide **2.11a** and methyl *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)acetimidate **2.12a** in a molar ratio of 1.1:1, by ^{1}H NMR.

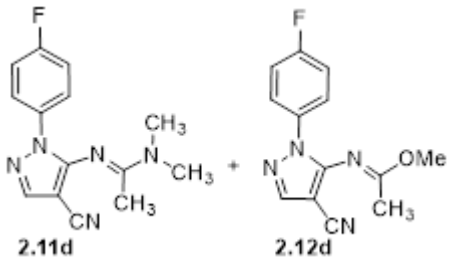
Reaction of 5-amino-1-(3-fluorophenyl)-1H-pyrazole-4-carbonitrile 2.3c with DMADEA



2.11c **2.12c**

DMADEA (0.05 g; 60 µL; 0.39 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3c** (0.05 g; 0.26 mmol) in DCM (0.5 mL) leading immediately to a reddish solution. The reaction mixture was stirred at room temperature for 25 hours. The resulting reddish oil was identified as a mixture of *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylacetimidamide **2.11c** and methyl *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)acetimidate **2.12c** in a molar ratio of 1:1.5, by ^{1}H NMR.

Reaction of 5-amino-1-(4-fluorophenyl)-1H-pyrazole-4-carbonitrile 2.3d with DMADEA

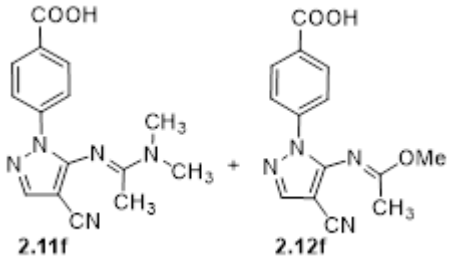


2.11d **2.12d**

DMADEA (0.04 g; 44 µL; 0.28 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1–(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3d** (0.04 g; 0.19 mmol) in DCM (0.5 mL) leading immediately to a light brown solution. The reaction mixture was stirred at room temperature for 48 hours. The resulting light brownish oil was identified as a mixture of *N*-(4-cyano–1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylacetimidamide **2.11d** and methyl *N*-(4-cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)acetimidate **2.12d** in a molar ratio of 1:1.2, by ^{1}H NMR.

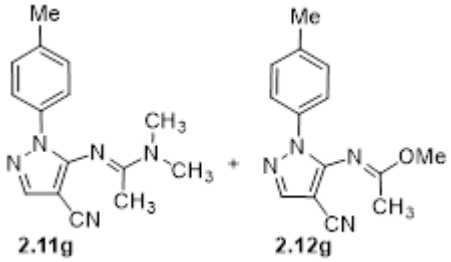
Reaction of 4-(5-amino-4-cyano-1H-pyrazol-1-yl)benzoic acid 2.3f with DMADEA

DMADEA (0.12 g; 145 µL; 0.94 mmol; 4 eq.) was added to a light orange solution of 4-(5-amino-4-



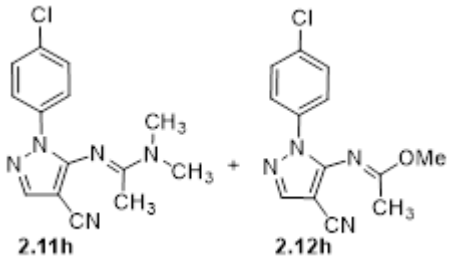
cyano-1*H*-pyrazole-1-yl)benzoic acid **2.3f** (0.05 g; 0.23 mmol) in DCM (0.5 mL) and EtOH (100 µL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 48 hours. The resulting brownish oil was identified as a mixture of 4-(4-cyano-5-((1-(dimethylamino)ethylidene)amino)-1*H*-pyrazol-1-yl) benzoic acid **2.11f** and 4-(4-cyano-5-((1-methoxyethylidene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.12f** in a molar ratio of 1:1, by ^{1}H NMR.

Reaction of 5-amino-1-(4-tolyl)-1H-pyrazole-4-carbonitrile 2.3g with DMADEA



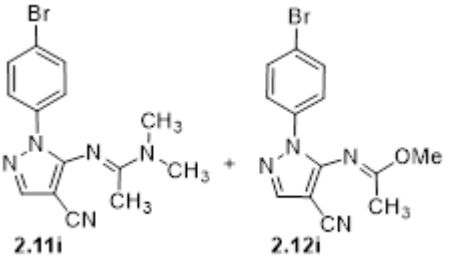
DMADEA (0.08 g; 97 µL; 0.63 mmol; 2.5 eq.) was added to a brownish suspension of 5-amino-1-(4-tolyl)-1*H*-pyrazole-4-carbonitrile **2.3g** (0.05 g; 0.25 mmol) in DCM (1.5 mL) leading immediately to a light brownish solution. The reaction mixture was stirred at room temperature for 48 hours. The resulting brownish oil was identified as a mixture of *N*-(4-cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylacetimidamide **2.11g** and methyl *N*-(4-cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)acetimidate **2.12g** in a molar ratio of 1:1.1, by ^{1}H NMR.

Reaction of 5-amino-1-(4-tolyl)-1H-pyrazole-4-carbonitrile 2.3h with DMADEA



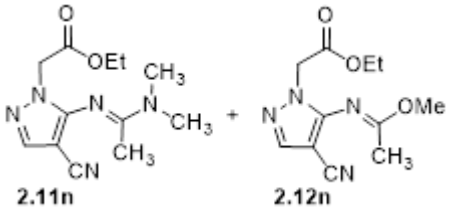
DMADEA (0.05 g; 54 µL; 0.35 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbonitrile **2.3h** (0.05 g; 0.23 mmol) in DCM (0.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The resulting brownish oil was identified as a mixture of *N*-(1-(4-chloro-phenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-acetimidamide **2.11h** and methyl *N*-(1-(4-chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)acetimidate **2.12h** in a molar ratio of 1.5:1, by ^{1}H NMR.

Reaction of 5-amino-1-(4-bromophenyl)-1H-pyrazole-4-carbonitrile 2.3i with DMADEA



DMADEA (0.04 g; 45 µL; 0.29 mmol; 1.5 eq.) was added to a brownish suspension of 5-amino-1-(4-bromophenyl)-1*H*-pyrazole-4-carbonitrile **2.3i** (0.05 g; 0.19 mmol) in DCM (0.5 mL) leading immediately to a reddish solution. The reaction mixture was stirred at room temperature for 24 hours. The resulting reddish oil was identified as a mixture of *N*-(1-(4-bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylacetimidamide **2.11i** and methyl *N*-(1-(4-bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)acetimidate **2.12i** in a molar ratio of 1.5:1, by ^{1}H NMR.

Reaction of ethyl 2-(5-amino-4-cyano-1H-pyrazole-1-yl)acetate 2.3n with DMADEA



DMADEA (0.07 g; 85 µL; 0.55 mmol; 2 eq.) was added to a beige suspension of ethyl 2-(5-amino-4-cyano-1*H*-pyrazole-1-yl)acetate **2.3n** (0.05 g; 0.27 mmol) in DCM (1.5 mL) leading immediately to a brownish solution. The reaction mixture was stirred at room temperature for 24 hours. The resulting brownish oil was identified as a mixture of ethyl 2-(4-cyano-5-((1-(dimethylamino)ethylidene)amino)-1*H*-pyrazol-1-yl)acetate **2.11n** and ethyl 2-(4-cyano-5-((1-methoxy-ethylidene)amino)-1*H*-pyrazol-1-yl)acetate **2.12n** in a molar ratio of 1:1.1, by ^{1}H NMR.

5.1.5. Synthesis addressed in section 2.4.

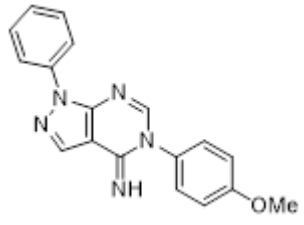
In the synthesis of **2.18**, a mixture of amidine **2.11** and imidate **2.12** was always used. As the molecular weight of the two structures is similar, the crude mixture was used in the reaction with amines, assuming that **2.11** + **2.12** had been formed in a quantitative yield. As such, only the total number of moles is referred in the experimental procedures.

General procedure for the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives

Method A: The amine **2.13**, **2.19**, **2.21**, **2.24** or **2.26** (1-2 eq.) was added to a solution of the amidine **2.10** or **2.11** + **2.12** in acetic acid and the mixture was refluxed or heated at 118°C. The reaction was controlled by TLC. The resulting solid was filtered and washed with cold ethanol.

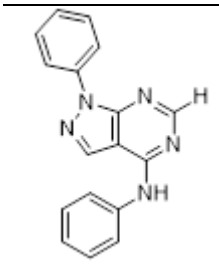
Method B: The amine **2.13** (1 eq.) was added to a solution of the imidate **2.6** in acetic acid and the mixture was refluxed. The reaction was controlled by TLC. The resulting solid was filtered and washed with cold ethanol.

Synthesis of 5-(4-methoxyphenyl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-imine 2.14f



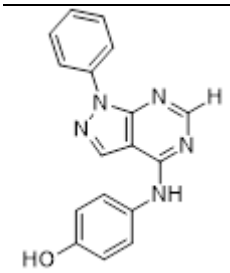
The 4-methoxyaniline **2.13f** (0.02 g; 0.21 mmol; 1 eq.) was added to a solution of the *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.21 mmol) in TFA (0.28 g; 188 µL; 2.5 mmol; 12 eq.) leading immediately to a brownish solution. The mixture was refluxed for 2 hours. The resulting light brown solid was filtered, washed with cold ethanol and identified as 5-(4-methoxyphenyl)-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-imine **2.14f** (0.01 g; 0.05 mmol; 22%).

Synthesis of 1-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15a



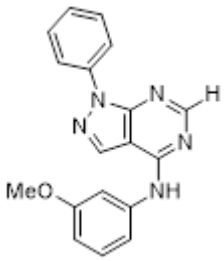
Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.21 mmol), acetic acid (500 µL; brown solution), aniline **2.13a** (0.04 g; 39 µL; 0.42 mmol; 2 eq.), reflux, 2 h. Product was isolated as a light brown solid and identified as 1-diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15a** (0.03 g; 0.12 mmol; 55%).

Synthesis of 4-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15d



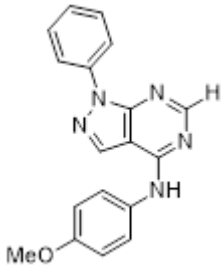
Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.22 mmol), acetic acid (500 µL; dark brown solution), 4-aminophenol **2.13d** (0.05 g; 0.44 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a grey solid and identified as 4-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15d** (0.05 g; 0.15 mmol; 70%).

Synthesis of (3-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15e



Prepared by the general method A. *N*-(C-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.27 mmol), acetic acid (500 µL; brown solution), 3-methoxyaniline **2.13e** (0.07 g; 60 µL; 0.54 mmol; 2 eq.), 118°C, 14 h. Product was isolated as a beige solid and identified as (3-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15e** (0.06 g; 0.18 mmol; 68%).

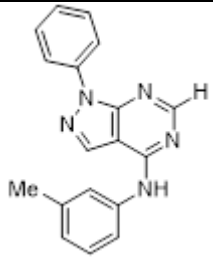
Synthesis of (4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15f



Method A: *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.07 g; 0.31 mmol), acetic acid (500 µL; brown solution), 4-methoxyaniline **2.13f** (0.08 g; 0.62 mmol; 2 eq.), reflux, 2 h. Product was isolated as a grey solid and identified as (4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15f** (0.07 g; 0.23 mmol; 75%).

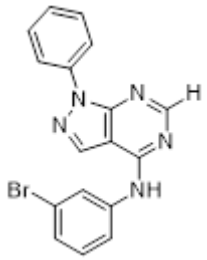
Method B: *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)formimidate **2.6a** (0.03 g; 0.14 mmol), acetic acid (500 µL; brown solution), 4-methoxyaniline **2.13f** (0.02 g; 0.14 mmol; 1 eq.), reflux, 1.5 h. Product was isolated as a grey solid and identified as (4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15f** (0.02 g; 0.07 mmol; 54%).

Synthesis of 1-phenyl-(m-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15g



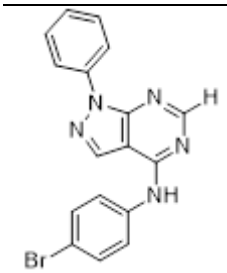
Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.25 mmol), acetic acid (500 µL; brown solution), m-toluidine **2.13g** (0.05 g; 54 µL; 0.50 mmol; 2 eq.), 118°C, 14 h. Product was isolated as a beige solid and identified as 1-phenyl(m-tolyl)-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine **2.15g** (0.05 g; 0.15 mmol; 60%).

Synthesis of (3-bromophenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15h



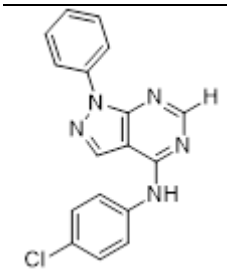
Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.27 mmol), acetic acid (500 µL; brown solution), 3-bromoaniline **2.13h** (0.09 g; 59 µL; 0.54 mmol; 2 eq.), 118°C, 7 h Product was isolated as a beige solid and identified as (3-bromophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15h** (0.05 g; 0.14 mmol; 52%).

Synthesis of (4-bromophenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15i



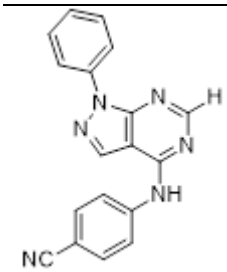
Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.27 mmol), acetic acid (500 µL; brown solution), 4-bromoaniline **2.13i** (0.09 g; 0.54 mmol; 2 eq.), 118°C, 10 h. Product was isolated as a beige solid and identified as (4-bromophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15i** (0.06 g; 0.18 mmol; 67%).

Synthesis of (4-chlorophenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15j



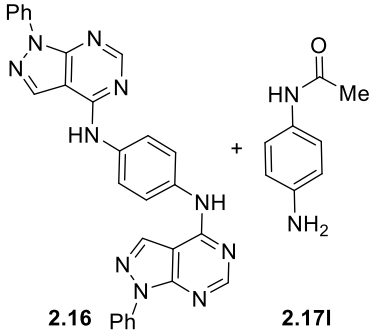
Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.27 mmol), acetic acid (500 µL; brown solution), 4-chloroaniline **2.13j** (0.07 g; 0.54 mmol; 2 eq.), 118°C, 10 h. Product was isolated as a beige solid and identified as (4-chlorophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15j** (0.06 g; 0.21 mmol; 79%).

Synthesis of 4-((1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)benzonitrile 2.15k



Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.21 mmol), acetic acid (500 µL; brown solution), 4-aminobenzonitrile **2.13k** (0.06 g; 0.42 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a grey solid and identified as 4-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino) **2.15k** (0.03 g; 0.01 mmol; 45%).

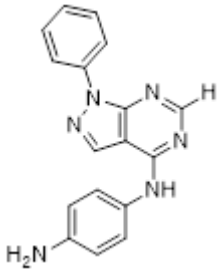
Reaction of (4-cyano-1-phenyl-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10a with* p-*phenylenediamine 2.13l



2.16 **2.17l**

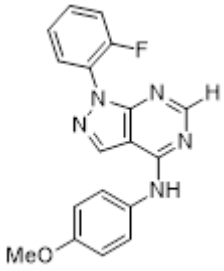
Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.24 mmol), acetic acid (500 µL; purple solution), *p*-phenylenediamine **2.13l** (0.07 g; 0.48 mmol; 2 eq.), 118°C, 4 h. A purple solid was isolated and was identified as a mixture of N^1,N^4-bis(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)benzene-1,4-diamine **2.16** and *N*-(4-aminophenyl)acetamide **2.17l** (0.03 g) in a molar ratio of 1.1:1, by ^{1}H NMR.

Synthesis of (1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)benzene-1,4-diamine 2.15l



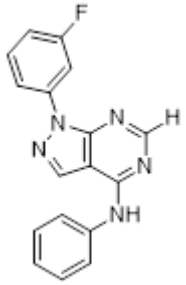
The *p*-phenylenediamine **2.13l** (0.06 g; 0.48 mmol; 2 eq.), was added to a solution of *N'*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.24 mmol) in acetic acid (500 µL) forming a purple solution. The reactional mixture was heated at 60°C for 24 h and 80°C for 3 days. The purple solid that precipitated on cooling in an ice bath was filtered and washed with cold ethyl ether. The product was identified as4-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino) **2.15l** (4.2 mg, 0.01 mmol; 6%).

Synthesis of 1-(2-fluorophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15n



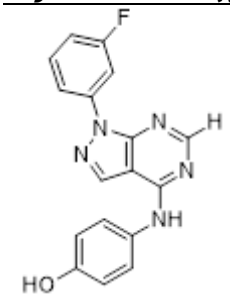
Prepared by the general method A. *N*-(4-Cyano-1-(2-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10b** (0.05 g; 0.20 mmol), acetic acid (500 µL; brown solution), 4-methoxyaniline **2.13f** (0.03 g; 0.40 mmol; 2 eq.), reflux, 2 h. Product was isolated as a brown solid and identified as 1-(2-fluorophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15n** (2.90 mg; 0.01 mmol; 4%).

Synthesis of 1-(3-fluorophenyl)-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15p



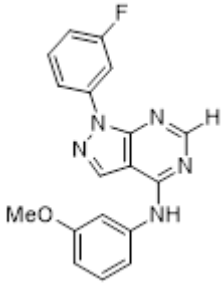
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.10 g; 0.40 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.04 g; 36 µL; 0.80 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as 1-(3-fluorophenyl)-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15p** (0.04 g; 0.01 mmol; 32%).

Synthesis of 4-((1-(3-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15r



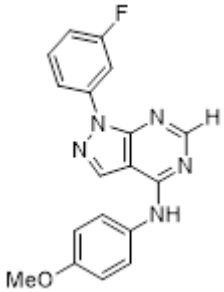
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d** (0.04g; 0.40 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a purple solid and identified as 4-((1-(3-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15r** (0.03 g; 0.11 mmol; 53%).

Synthesis of 1-(3-fluorophenyl)-N-(3-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15s



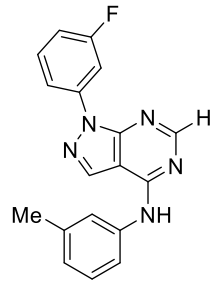
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.06 g; 0.24 mmol), acetic acid (400 µL; brown solution), 3-methoxyaniline **2.13e** (0.06 g; 54 µL; 0.48 mmol; 2 eq.), reflux, 2.5 h. Product was isolated as a light brown solid and identified as 1-(3-fluorophenyl)-*N*-(3-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15s** (0.04 g; 0.11 mmol; 48%).

Synthesis of 1-(3-fluorophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15t



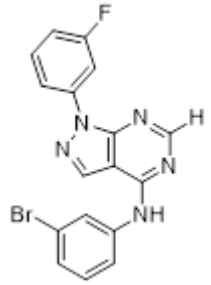
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.06 g; 0.24 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.06 g; 0.48 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a grey solid and identified as 1-(3-fluorophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15t** (0.04 g; 0.13 mmol; 54%).

Synthesis of 1-(3-fluorophenyl)-N-(m-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15u



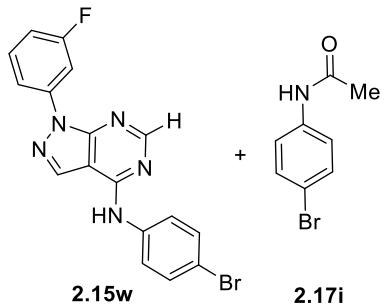
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.04 g; 43 µL; 0.40 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as 1-(3-fluorophenyl)-*N*-(*m*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15u** (0.03 g; 0.10 mmol; 48%).

Synthesis of N-(3-bromophenyl)-1-(3-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15v



Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.03 g; 0.13 mmol), acetic acid (400 µL; brown solution), 3-bromoaniline **13h** (0.05 g; 28 µL; 0.26 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a white solid and identified as *N*-(3-bromophenyl)-1-(3-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15v** (0.01 g; 0.03 mmol; 22%).

Reaction of N'-(4-cyano-1-(3-fluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10c with 4-bromoaniline 2.13i

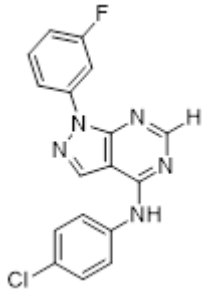


2.15w + **2.17i**

Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), 4-bromoaniline **2.13i** (0.08 g; 0.46 mmol; 2 eq.), 118°C, 5 h. A beige solid was isolated and was identified as a mixture of *N*-(4-bromophenyl)-1-(3-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15w** and (4-bromophenyl)-acetamide **2.17i** (0.02 g) in a molar ratio of 1:3.4, by ^{1}H NMR.

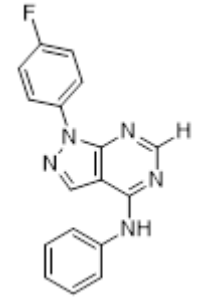
Synthesis of N-(4-chlorophenyl)-1-(3-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15x

Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10c** (0.03 g; 0.13 mmol), acetic acid (400 µL; yellow solution), 4-chloroaniline **2.13j**



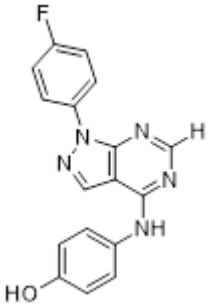
(0.04 g; 0.26 mmol; 2 eq.), 118°C, 14 h. Product was isolated as a grey solid and identified as *N*-(4-chlorophenyl)-1-(3-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15x** (0.02 g; 0.07 mmol; 52%).

Synthesis of 1-(4-fluorophenyl)-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15y



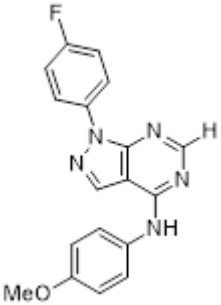
Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10d** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.04 g; 42 µL; 0.46 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a white solid and identified as 1-(4-fluorophenyl)-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15y** (0.03 g; 0.10 mmol; 42%).

Synthesis of 4-((1-(4-fluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15z



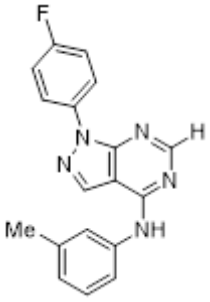
Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10d** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d** (0.03 g; 0.40 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as 4-((1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15z** (0.03 g; 0.10 mmol; 50%).

Synthesis of 1-(4-fluorophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15aa



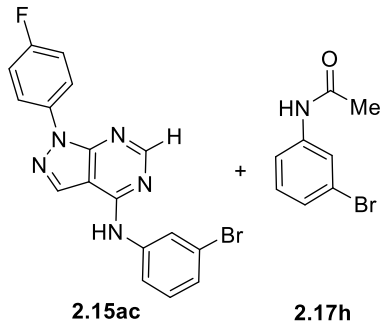
Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10d** (0.05 g; 0.18 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.04 g; 0.36 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a light pink solid and identified as 1-(4-fluorophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15aa** (0.03 g; 0.09 mmol; 52%).

Synthesis of 1-(4-fluorophenyl)-N-(m-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ab



Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-formimidamide **2.10d** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.04 g; 43 µL; 0.40 mmol; 2 eq.), 118°C, 4.5 h. Product was isolated as a beige solid and identifiedas 1-(4-fluorophenyl)-*N*-(m-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ab** (0.03 g; 0.09 mmol; 47%).

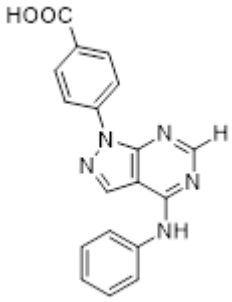
***Reaction of N'-(4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10d with* 3-bromoaniline *2.*13h**



2.15ac 2.17h

Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylformimidamide **2.10d** (0.05 g; 0.21 mmol), acetic acid (400 µL; brown solution), 3-bromoaniline **2.13h** (0.07 g; 46 µL; 0.42 mmol; 2 eq.), 118°C, 4.5 h. A brown oil was isolated which was identified as a mixture of *N*-(3-bromophenyl)-1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ac** and (3-bromophenyl)acetamide **2.17h** (0.02 g) in a molar ratio of 1:1, by ^{1}H NMR.

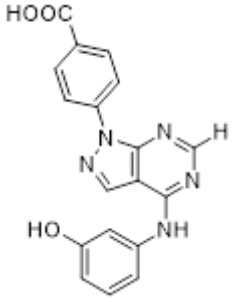
Synthesis of 4-(4-(phenylamino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ad



Method A: 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)-benzoic acid **2.10f** (0.06 g; 0.20 mmol), acetic acid (400 µL; orange solution), aniline **2.13a** (0.04 g; 36 µL; 0.40 mmol; 2 eq.), 118°C, 7 h. Product was isolated as a beige solid and identified as 4-(4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15ad** (0.05 g; 0.16 mmol; 79%).

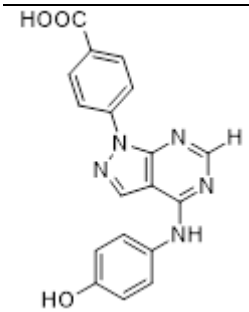
Method B: 4-(4-Cyano-5-((ethoxymethylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.6f** (0.03 g; 0.11 mmol), acetic acid (500 µL; orange solution), aniline **2.13a** (0.01 g; 11 µL; 0.11 mmol; 1 eq.), reflux, 45 min. Product was isolated as a beige solid and identified as 4-(4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15ad** (0.03 g; 0.09 mmol; 83%).

Synthesis of 4-(4-((3-hydroxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ae



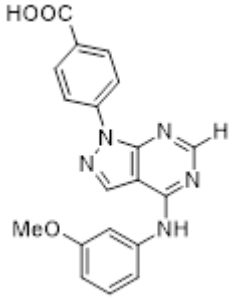
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.06 g; 0.20 mmol), acetic acid (400 µL; orange solution), 3-aminophenol **2.13c** (0.04 g; 0.40 mmol; 2 eq.), 118°C, 6 h. Product was isolated as an orange solid and identified as 4-(4-((3-hydroxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl) benzoic acid **2.15ae** (0.05 g; 0.13 mmol; 67%).

Synthesis of 4-(4-((4-hydroxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15af



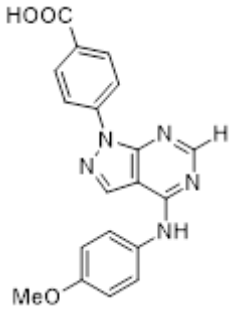
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.06 g; 0.20 mmol), acetic acid (400 µL; orange solution), 4-aminophenol **2.13d** (0.04 g; 0.40 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a purple solid and identified as 4-(4-((4-hydroxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15af** (0.05 g; 0.15 mmol; 76%).

Synthesis of 4-(4-((3-methoxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ag



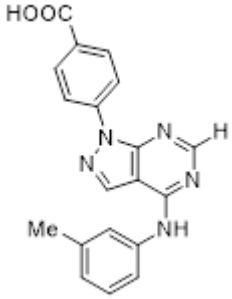
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1–yl)benzoic acid **2.10f** (0.06 g; 0.22 mmol), acetic acid (400 µL; orange solution), 3-methoxyaniline **2.13e** (0.05 g; 49 µL; 0.44 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as 4-(4-((3-methoxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-benzoic acid **2.15ag** (0.05 g; 0.14 mmol; 62%).

Synthesis of 4-(4-((4-methoxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ah



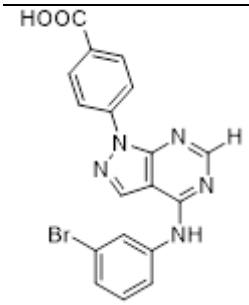
Method A: 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)-benzoic acid **2.10f** (0.06 g; 0.20 mmol), acetic acid (400 µL; orange solution), 4-methoxyaniline **2.13f** (0.05 g; 0.40 mmol; 2 eq.), 118°C, 7 h. Product was isolated as a beige solid and identified as 4-(4-((4-methoxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15ah** (0.05 g; 0.15 mmol; 73%). Method B: 4-(4-cyano-5-((ethoxymethylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.6f** (0.02 g; 0.08 mmol), acetic acid (500 µL; orange solution), 4-methoxyaniline **2.13f** (0.01 g; 0.08 mmol; 1 eq.), reflux, 45 min. Product was isolated as a beige solid and identified as 4-(4-((4-methoxy-phenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15ah** (0.02 g; 0.05 mmol; 56%).

Synthesis of 4-(4-(m-tolylamino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ai



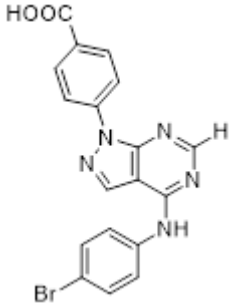
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-11-yl)-benzoic acid **2.10f** (0.05 g; 0.19 mmol), acetic acid (400 µL; orange solution), *m*-toluidine **2.13g**(0.04 g; 43 µL; 0.38 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a light brown solid and identified as 4-(4-(*m*-tolylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15ai** (0.04 g; 0.12 mmol; 62%).

Synthesis of 4-(4-((3-bromophenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15aj



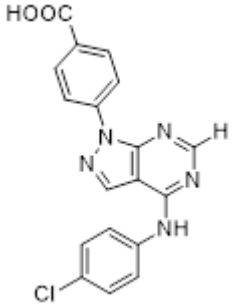
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)-amino)-1*H*-pyrazol-1–yl)benzoic acid **2.10f** (0.05 g; 0.20 mmol), acetic acid (400 µL; orange solution), 3-bromoaniline **2.13h** (0.08 g; 48 µL; 0.40 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as 4-(4-((3-bromophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15aj** (0.03 g; 0.06 mmol; 30%).

Synthesis of 4-(4-((4-bromophenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15ak



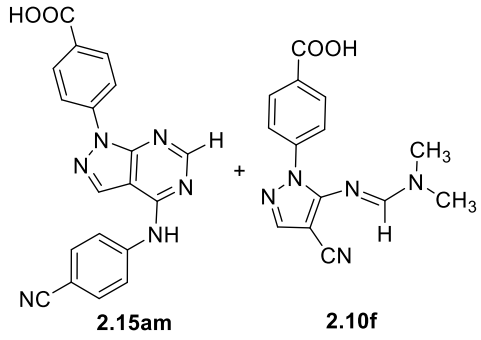
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)-amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.06 g; 0.22 mmol), acetic acid (400 µL; orange solution), 4-bromoaniline **13i** (0.08 g; 0.44 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as 4-(4-((4-bromophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-benzoic acid **2.15ak** (0.04 g; 0.09 mmol; 39%).

Synthesis of 4-(4-((4-bromophenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.15al



Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methy-lene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.06 g; 0.22 mmol), acetic acid (400 µL; orange solution), 4-chloroaniline **2.13j** (0.06 g; 0.44 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as 4-(4-((4-bromophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15al** (0.04 g; 0.13 mmol; 57%).

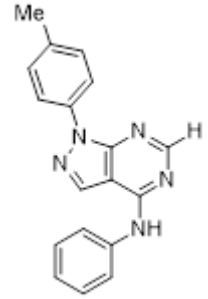
Reaction of 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1H-pyrazol-1-yl)benzoic acid 2.10f with 4-aminobenzonitrile 2.13k



2.15am **2.10f**

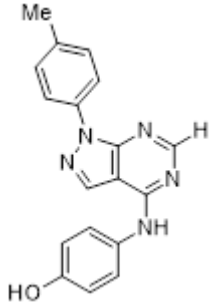
Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.05 g; 0.20 mmol), acetic acid (400 µL; orange solution), 4-aminobenzonitrile **2.13k** (0.05 g; 0.40 mmol; 2 eq.), 118°C, 5 h. An orange solid was isolated and identified as a mixture of 4-(4-((4-cyanophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoic acid **2.15am** and 4-(4-cyano-5-(((dimethylamino)methylene) amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.03 g) in a molar ratio of 2.5:1, by ^{1}H NMR.

Synthesis of N-phenyl-1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15an



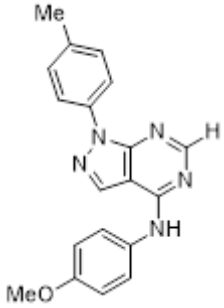
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.04 g; 42 µL; 0.46 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as N-phenyl-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15an** (0.03 g; 0.11 mmol; 48%).

Synthesis of 4-((1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15ao



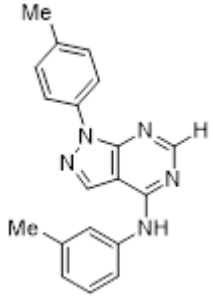
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.06 g; 0.24 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d** (0.05 g; 0.48 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a purple solid and identified as 4-((1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15ao** (0.03 g; 0.11 mmol; 46%).

Synthesis of N-(4-methoxyphenyl)-1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ap



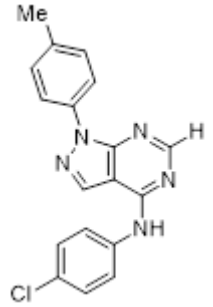
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.05 g; 0.19 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.05 g; 0.38 mmol; 2 eq.), 118°C, 5.5 h. Product was isolated as a light brown solid and identified as *N*-(4-methoxyphenyl)-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ap** (0.03 g; 0.10 mmol; 53%).

Synthesis of N-(m-tolyl)-1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15aq



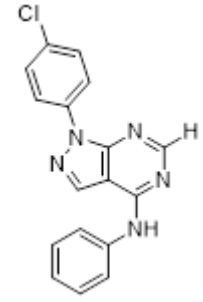
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.06 g; 0.24 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.05 g; 53 µL; 0.48 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a beige solid and identified as *N*-(*m*-tolyl)-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15aq** (0.04 g; 0.14 mmol; 59%).

Synthesis of N-(4-chlorophenyl)-1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ar



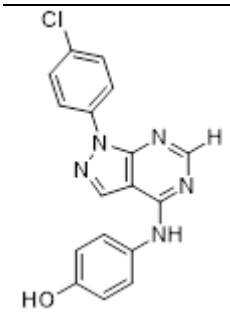
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), 4-chloroaniline **2.13j** (0.06 g; 0.46 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a beige solid and identified as *N*-(4-chlorophenyl)-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ar** (0.04 g; 0.11 mmol; 48%).

Synthesis of 1-(4-chlorophenyl)-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15as



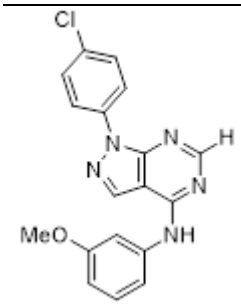
Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.04 g; 36 µL; 0.40 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as 1-(4-chlorophenyl)-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15as** (0.03 g; 0.10 mmol; 48%).

Synthesis of 4-((1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15at



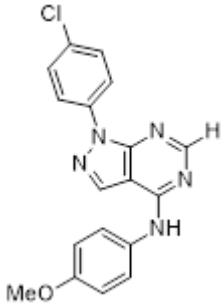
Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d** (0.05 g; 0.40 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a purple solid and identified as 4-((1-(4- chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15at** (0.04 g; 0.11 mmol; 53%).

Synthesis of 1-(4-chlorophenyl)-N-(3-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15au



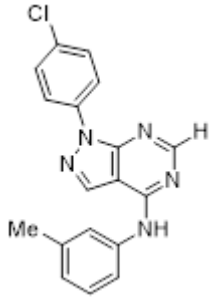
Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.17 mmol), acetic acid (400 µL; brown solution), 3-methoxyaniline **2.13e** (0.05 g; 45 µL; 0.34 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a light brown solid and identified as 1-(4-chlorophenyl)-*N*-(3-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15au** (0.03 g; 0.09 mmol; 53%).

Synthesis of 1-(4-chlorophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15av



Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.05 g; 0.40 mmol; 2 eq.), 118°C, 4.5 h. Product was isolated as a light brown solid and identified as 1-(4-chlorophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15av** (0.04 g; 0.12 mmol; 62%).

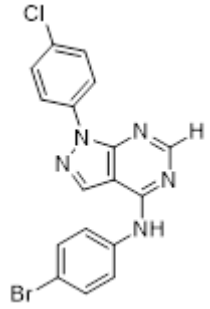
Synthesis of 1-(4-chlorophenyl)-N-(m-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15aw



Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.05 g; 47 µL; 0.40 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a light brown solid and identified as 1-(4-chlorophenyl)-*N*-(*m*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15aw** (0.03 g; 0.10 mmol; 46%).

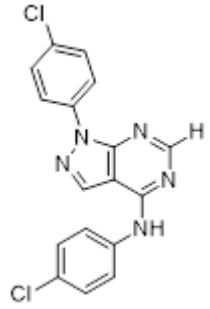
Synthesis of N-(4-bromophenyl)-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ax

Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.06 g; 0.21 mmol), acetic acid (400 µL; brown solution), 4-bromoaniline **2.13i**



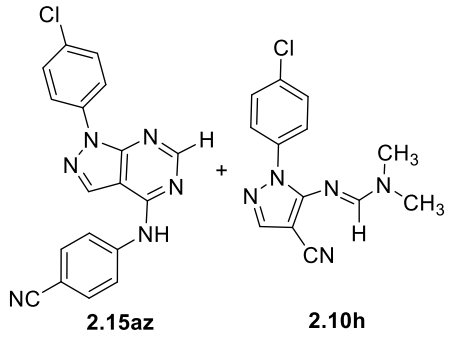
(0.07 g; 0.42 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a beige solid and identified as *N*-(4-bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ax** (0.05 g; 0.12 mmol; 57%).

Synthesis of N,1-bis(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ay



Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.05 g; 0.19 mmol), acetic acid (400 µL; brown solution), 4-chloro-aniline **2.13j** (0.05 g; 0.38 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a beige solid and identified as *N*,1-bis(4-chloro-phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ay** (0.05 g; 0.13 mmol; 68%).

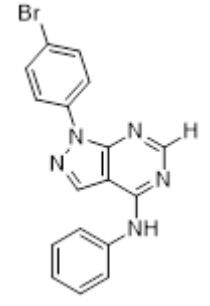
***Reaction of N'-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10h with* 4-aminobenzonitrile *2.*13k**



2.15az **2.10h**

Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.06 g; 0.23 mmol), acetic acid (400 µL; orange solution), 4-aminobenzonitrile **2.13k** (0.05 g; 0.46 mmol; 2 eq.), 118°C, 5 h. An orange solid was isolated which was identified as a mixture of 4-((1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4- yl)amino)benzonitrile **2.15az** and *N*-(1-(4-chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10h** (0.02 g) in a molar ratio of 3.3:1, by ^{1}H NMR.

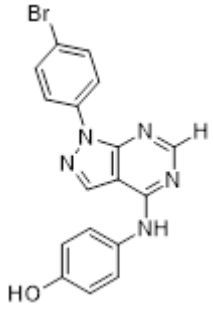
Synthesis of 1-(4-bromophenyl)-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15ba



Prepared by the general method A. *N*-(1-(4-Bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-formimidamide **2.10i** (0.04 g; 0.13 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.02 g; 24 µL; 0.26 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as 1-(4- bromophenyl)-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15ba** (0.02 g; 0.07 mmol; 52%).

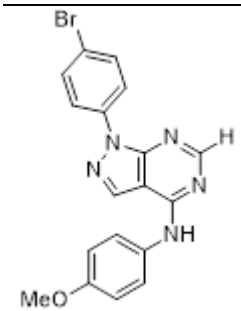
Synthesis of 4-((1-(4-bromophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15bb

Prepared by the general method A. *N*-(1-(4-Bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10i** (0.04 g; 0.13 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d**



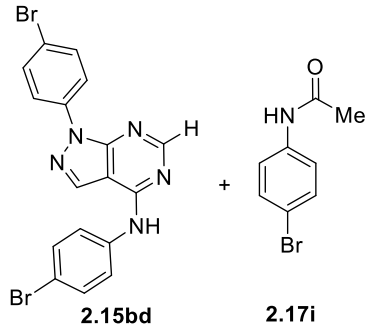
(0.03 g; 0.26 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a purple solid and identified as 4-((1-(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15bb** (0.03 g; 0.09 mmol; 66%).

Synthesis of 1-(4-bromophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bc



Prepared by the general method A. *N*-(1-(4-Bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10i** (0.04 g; 0.13 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.03 g; 0.26 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a brown solid and identified as 1-(4-bromophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bc** (0.03 g; 0.07 mmol; 51%).

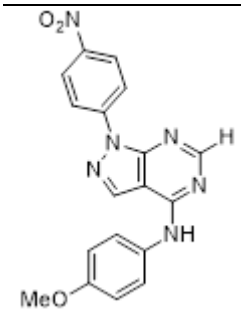
Reaction of N'-(1-(4-bromophenyl)-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10i with 4-bromoaniline 2.13i



2.15bd **2.17i**

Prepared by the general method A. *N*-(1-(4-Bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10i** (0.03 g; 0.10 mmol), acetic acid (400 µL; brown solution), 4-bromoaniline **2.13i** (0.03 g; 0.20 mmol; 2 eq.), 118°C, 4 h. A beige solid was isolated and was identified as a mixture of *N*,1-bis(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bd** and *N*-(4-bromophenyl)acetamide **2.17i** (0.02 g) in a molar ratio of 1:1, by ^{1}H NMR.

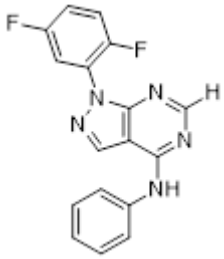
Synthesis of N-(4-methoxyphenyl)-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15be



Prepared by the general method A. *N*-(4-Cyano-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10j** (0.01 g; 0.05 mmol), acetic acid (300 µL; orange solution), 4-methoxyaniline **2.13f** (0.01 g; 0.10 mmol; 2 eq.), 118°C, 4 h. Product was isolated as an orange solid and identified as *N*-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15be** (2.0 mg; 0.006 mmol; 11%).

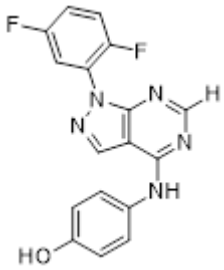
Synthesis of 1-(2,5-difluorophenyl)-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bf

Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10k** (0.04 g; 0.15 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.01



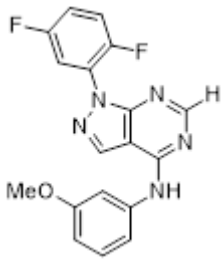
g; 13 µL; 0.30 mmol; 2 eq.), 118°C, 6 h. Product was isolated as an orange solid and identified as 1-(2,5-difluorophenyl)-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bf** (5.0 mg, 0.02 mmol; 11%).

Synthesis of 4-((1-(2,5-difluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenol 2.15bg



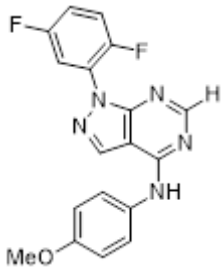
Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10k** (0.05 g; 0.19 mmol), acetic acid (400 µL; brown solution), 4-aminophenol **2.13d** (0.04 g; 0.38 mmol; 2 eq.), 118°C, 4 h. Product was isolated as a black solid and identified as 4-((1-(2,5-difluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino)phenol **2.15bg** (0.04 g; 0.11 mmol; 57%).

Synthesis of 1-(2,5-difluorophenyl)-N-(3-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bh



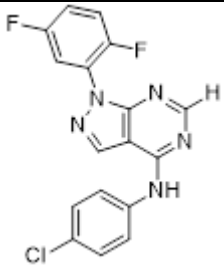
Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-formimidamide **2.10k** (0.05 g; 0.21 mmol), acetic acid (400 µL; brown solution), 3-methoxyaniline **2.13e** (0.05 g; 47 µL; 0.42 mmol; 2 eq.), 118°C, 7.5 h. Product was isolated as a grey solid and identified as 1-(2,5-difluorophenyl)-*N*-(3-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bh** (0.03 g; 0.08 mmol; 40%).

Synthesis of 1-(2,5-difluorophenyl)-N-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bi



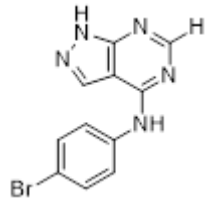
Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10k** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.05 g; 0.40 mmol; 2 eq.), reflux, 2 h. Product was isolated as a grey solid and identified as 1-(2,5-difluorophenyl)-*N*-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bi** (0.04 g; 0.11 mmol; 55%).

Synthesis of N-(4-chlorophenyl)-1-(2,5-difluorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bk



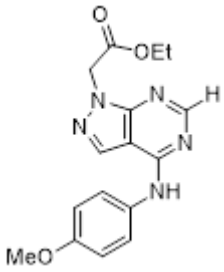
Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10k** (0.06 g; 0.25 mmol), acetic acid (400 µL; brown solution), 4-chloroaniline **2.13j** (0.06 g; 0.50 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a grey solid and identified as *N*-(4-chlorophenyl)-1-(2,5-difluorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bk** (0.03 g; 0.09 mmol; 38%).

Synthesis of N-(4-bromophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.15bl



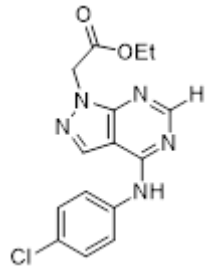
Prepared by the general method A. *N*-(4-Cyano-1-(2,5-difluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10k** (0.05 g; 0.20 mmol), acetic acid (400 µL; brown solution), 4-bromoaniline **2.13i** (0.04 g; 0.24 mmol; 1.2 eq.), 118°C, 4 h. Product was isolated as a beige solid and identified as *N*-(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.15bl** (0.04 g; 0.12 mmol; 62%).

Synthesis of ethyl 2-(4-((4-methoxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate 2.15bm



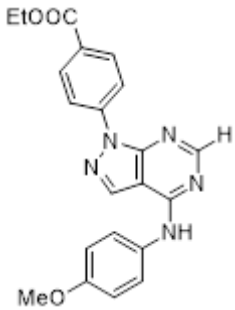
Prepared by the general method A. Ethyl 2-(4-cyano-5-(((dimethylamino)-methylene)amino)-1*H*-pyrazol-1-yl)acetate **2.10n** (0.06 g; 0.26 mmol), acetic acid (500 µL; brown solution), 4-methoxyaniline **2.13f** (0.03 g; 0.26 mmol; 1 eq.), reflux, 1.5 h. Product was isolated as a light brown solid and identified as ethyl 2-(4-((4-methoxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate **2.15bm** (0.04 g; 0.11 mmol; 41%).

Synthesis of ethyl 2-(4-((4-chlorophenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate 2.15bn



Prepared by the general method A. Ethyl 2-(4-cyano-5-(((dimethylamino)-methylene)amino)-1*H*-pyrazol-1-yl)acetate **2.10n** (0.06 g; 0.24 mmol), acetic acid (500 µL; brown solution), 4-chloroaniline **2.13j** (0.03 g; 0.29 mmol; 1.2 eq.), 118°C, 5.5 h. Product was isolated as a light brown solid and identified as ethyl 2-(4-((4-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate **2.15bn** (0.04 g; 0.11 mmol; 46%).

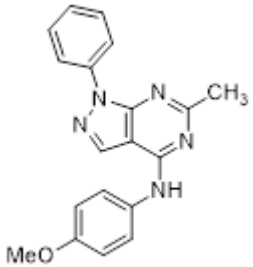
Synthesis of ethyl 4-(4-((4-methoxyphenyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoate 2.15bo



Method A: 4-(4-cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoate **2.10r** (0.05 g; 0.15 mmol), acetic acid (500 µL; orange solution), 4-methoxyaniline **2.13f** (0.04 g; 0.30 mmol; 2 eq.), reflux, 2 h. Product was isolated as a light brown solid and identified as ethyl 4-(4-((4-methoxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoate **2.15bo** (0.04 g; 0.10 mmol; 67%).

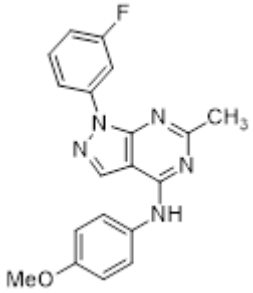
Method B: 4-(4-cyano-5-((ethoxymethylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.6f** (0.03 g; 0.09 mmol), acetic acid (500 µL; orange solution), 4-methoxyaniline **2.13f** (0.01 g; 0.09 mmol; 1 eq.), reflux, 2.5 h. Product was isolated as a light brown solid and identified as ethyl 4-(4-((4-methoxyphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)benzoate **2.15bo** (0.02 g; 0.04 mmol; 46%).

Synthesis of N-(4-methoxyphenyl)-6-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.18a



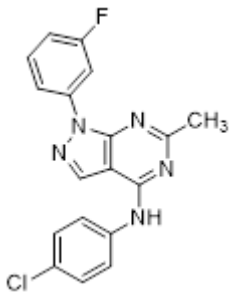
Prepared by the general method A. *N'*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11a** and methyl *N*-(4-cyano-1-phenyl-1*H*-pyrazol-5-yl)acetimidate **2.12a** (0.45 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.10 g; 0.90 mmol; 2 eq.), 118°C, 4.5 h. Product was isolated as a light pink solid and identified as *N*-(4-methoxyphenyl)- 6-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18a** (0.02 g; 0.06 mmol; 14%).

Synthesis of 1-(3-fluorophenyl)-N-(4-methoxyphenyl)-6-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.18b



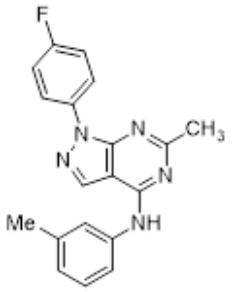
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11c** and methyl *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)acetimidate **2.12c** (0.24 mmol), acetic acid (500 µL; brown solution), 4-methoxyaniline **2.13f** (0.06 g; 0.48 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a white solid and identified as 1-(3-fluorophenyl)-*N*-(4-methoxyphenyl)-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18b** (0.01 g; 0.04 mmol; 17%).

Synthesis of N-(4-chlorophenyl)-1-(3-fluorophenyl)-6-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.18c



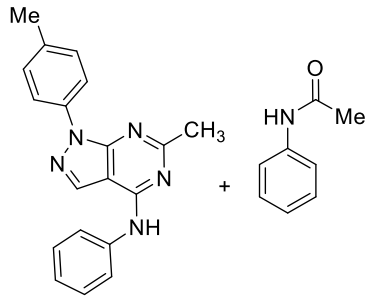
Prepared by the general method A. *N*-(4-Cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11c** and methyl *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)acetimidate **2.12c** (0.24 mmol), acetic acid (500 µL; brown solution), 4-chloroaniline **2.13j** (0.06 g; 0.48 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as *N*-(4-chloro-phenyl)-1-(3-fluorophenyl)-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18c** (0.01 g; 0.04 mmol; 17%).

Synthesis of 1-(4-fluorophenyl)-6-methyl-N-(m-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.18d



Prepared by the general method A. *N*-(4-Cyano-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11d** and methyl *N*-(4-cyano-1-(4-fluoro-phenyl)-1*H*-pyrazol-5-yl)acetimidate **2.12d** (0.17 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.04 g; 0.34 mmol; 36 µL; 2 eq.), 118°C, 5 h. Product was isolated as a light brown solid and identified as 1-(4-fluorophenyl)-6-methyl-*N*-(*m*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18d** (0.02 g; 0.05 mmol; 27%).

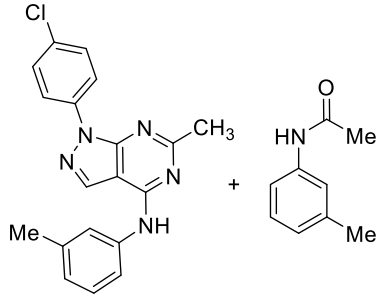
***Reaction of N'-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)-N,N-dimethylacetimidamide 2.11g and methyl N-(4-cyano-1-(p-tolyl)-1H-pyrazol-5-yl)acetimidate 2.12g with* aniline *2.*13a**



2.18e **2.17a**

Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11g** and methyl *N*-(4-cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)acetimidate **2.12g** (0.22 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.04 g; 40 µL; 0.44 mmol; 2 eq.), 118°C, 5 h. A beige solid was isolated and identified as a mixture of 6-methyl-*N*-phenyl-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18e** and *N*-phenylacetamide **2.17a** (0.02 g) in a molar ratio of 4.3:1, by ^{1}H NMR.

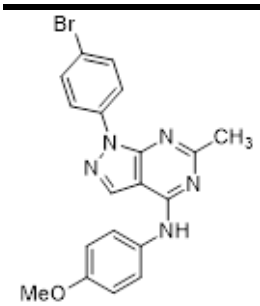
Reaction of N'-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylacetimidamide 2.11h and methyl N-(1-(4-chlorophenyl)-4-cyano-1H-pyrazol-5-yl)acetimidate 12h with m-toluidine 2.13g



2.18f **2.17g**

Prepared by the general method A. *N*-(1-(4-Chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11h** and methyl *N*-(1-(4-chlorophenyl)-4-cyano-1*H*-pyrazol-5-yl)acetimidate **2.12h** (0.21 mmol), acetic acid (400 µL; brown solution), *m*-toluidine **2.13g** (0.05 g; 45 µL; 0.42 mmol; 2 eq.), 118°C, 5 h. A grey solid was isolated and identified as a mixture of 1-(4-chlorophenyl)-6-methyl-*N*-(*m*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18f** and *N*-(*m*-tolyl)acetamide **2.17g** (0.02 g) in a molar ratio of 5.5:1, by ^{1}H NMR.

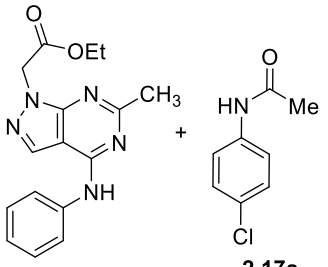
Synthesis of 1-(4-bromophenyl)-N-(4-methoxyphenyl)-6-methyl-1H-pyrazolo[3,4-d] pyrimidin-4-amine 2.18g



Prepared by the general method A. *N*-(1-(4-Bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)-*N,N*-dimethylacetimidamide **2.11i** and methyl *N*-(1-(4-bromophenyl)-4-cyano-1*H*-pyrazol-5-yl)acetimidate **2.12i** (0.17 mmol), acetic acid (400 µL; brown solution), 4-methoxyaniline **2.13f** (0.04 g; 0.34 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a light brown solid and identified as 1-(4-bromophenyl)-*N*-(4-methoxyphenyl)-6-methyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4-amine **2.18g** (0.02 g; 0.06 mmol; 34%).

Reaction of ethyl 2-(4-cyano-5-((1-(dimethylamino)ethylidene)amino)-1H-pyrazol-1-yl)acetate 2.11n and ethyl 2-(4-cyano-5-((1-methoxyethylidene)amino)-1H-pyrazol-1-yl)acetate 2.12n with aniline 2.13a

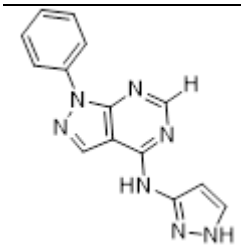
Prepared by the general method A. 2-(4-Cyano-5-((1-(dimethyl-amino)ethylidene)amino)-1*H*-pyrazol-1-yl)acetate **2.11n** and ethyl 2-(4-cyano-5-((1-methoxyethylidene)amino)-1*H*-pyrazol-1-yl)acetate **2.12n**



2.18h 2.17a

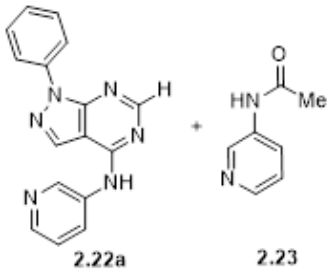
(0.25 mmol), acetic acid (400 µL; brown solution), aniline **2.13a** (0.02 g; 23 µL; 0.50 mmol; 2 eq.), 118°C, 5 h. A brown oil was identified as a mixture of ethyl 2-(6-methyl-4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)acetate **2.18h** and *N*-phenylacetamide **2.17a** (0.02 g) in a molar ratio of 5.7:1, by ^{1}H NMR.

Synthesis of 1-phenyl-N-(1H-pyrazol-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.20



Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.25 mmol), acetic acid (400 µL; brown solution), 3-aminopyrazole **2.19** (0.04 g; 0.50 mmol; 2 eq.), 118°C, 6 h. Product was isolated as a white solid and identified as 1-phenyl-*N*-(1*H*-pyrazol-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.20** (0.02 g; 0.09 mmol; 34%).

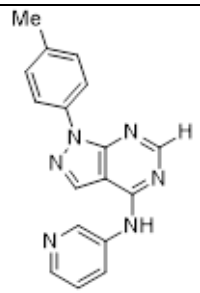
Reaction of N'-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10a with 3-aminopyridine 2.21



2.22a 2.23

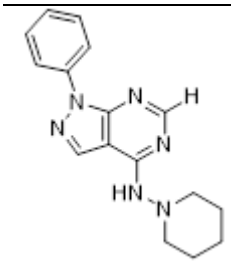
Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.22 mmol), acetic acid (400 µL; brown solution), 3-aminopyridine **2.21** (0.04 g; 0.44 mmol; 2 eq.), 118°C, 6 h. A light brown solid was identified as a mixture of 1-phenyl-*N*-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.22a** and *N*-(pyridin-3-yl)acetamide **2.23** (0.02 g) in a molar ratio of 1:1, by ^{1}H NMR.

Synthesis of N-(pyridin-3-yl)-1-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.22b



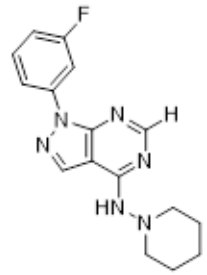
Prepared by the general method A. *N*-(4-Cyano-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10g** (0.06 g; 0.24 mmol), acetic acid (400 µL; brown solution), 3-aminopyridine **2.21** (0.05 g; 0.48 mmol; 2 eq.), 118°C, 5.5 h. Product was isolated as a beige solid and identified as *N*-(pyridin-3-yl)-1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.22b** (0.04 g; 0.13 mmol; 54%).

Synthesis of 1-phenyl-N-(piperidin-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.25a



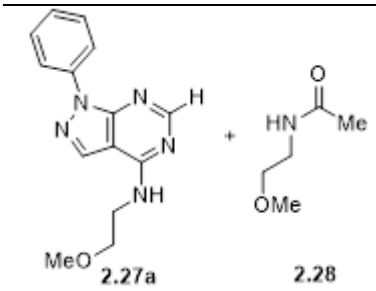
Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.05 g; 0.23 mmol), acetic acid (400 µL; brown solution), 1-aminopiperidine **2.24** (0.05 g; 49 µL; 0.45 mmol; 2 eq.), 118°C, 6.5 h. Product was isolated as a beige solid and identified as 1-phenyl-*N*-(piperidin-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.25a** (0.03 g; 0.09 mmol; 38%).

Synthesis of 1-(3-fluorophenyl)-N-(piperidin-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine 2.25b



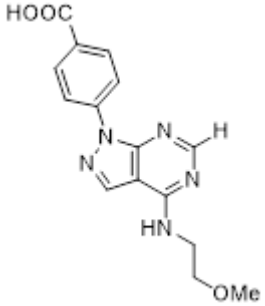
Prepared by the general method A. *N*-(4-cyano-1-(3-fluorophenyl)-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl-formimidamide **2.10c** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), 1-aminopyperidine **2.24** (0.05 g; 50 µL; 0.46 mmol; 2 eq.), 118°C, 5 h. Product was isolated as a beige solid and identified as 1-(3-fluorophenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.25b** (0.02 g; 0.06 mmol; 25%).

Reaction of N'-(4-cyano-1-phenyl-1H-pyrazol-5-yl)-N,N-dimethylformimidamide 2.10a with 2-methoxyethylamine 2.26a



Prepared by the general method A. *N*-(4-Cyano-1-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethylformimidamide **2.10a** (0.06 g; 0.23 mmol), acetic acid (400 µL; brown solution), 2-methoxyethylamine **2.26a** (0.03 g; 40 µL; 0.46 mmol; 2 eq.), 118°C, 4 h. A brown oil was identified as a mixture of *N*-(2 methoxyethyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2.27a** and *N*-(3-methoxypropyl)-acetamide **2.28** (0.02 g) in a molar ratio of 2.5:1, by ^{1}H NMR.

Synthesis of 4-(4-((2-methoxyethyl)amino)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)benzoic acid 2.27b



Prepared by the general method A. 4-(4-Cyano-5-(((dimethylamino)methylene)amino)-1*H*-pyrazol-1-yl)benzoic acid **2.10f** (0.05 g; 0.18 mmol), acetic acid (400 µL; brown solution), 2-methoxyethylamine **2.26a** (0.03 g; 34 µL; 0.36 mmol; 2 eq.), 118°C, 6.5 h. Product was isolated as a beige solid and identified as 4-(4-((2-methoxyethyl)amino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-1-yl)benzoic acid **2.27b** (0.02 g; 0.05 mmol; 28%).

5.2. Biology

5.2.1 Cell lines and culture conditions

A human basal breast cancer cell line, Hs578t (triple negative subtype) was used to evaluate the anticancer activity of the newly synthetized compounds . Hs578t cell line was obtained from American Type Culture Collection (ATCC, Virginia, USA). Hs578t cells were cultured in Dubelcco's Modified Eagle Medium, 4.5 g/L glucose (DMEM, Biochrom), supplemented with 10% heating activated Fetal Bovine Serum (FBS, Sigma-Aldrich) and 1% of antibiotic solution (Penicillin-Streptomycin, Gibco).

Cells were grown in a humidified incubator at 37 °C and 5% CO_2. Sub-culturing was performed using 80% confluence culture flasks. Then, the cells were reaped by washing the T75 flasks with phosphate-buffer saline (PBS 1x) and detached from the flasks using trypsin (Tryplex™ Express, Gibco) at 37 °C. After the confirmation that the cells were no longer adhered, 10% FBS medium was added to the flasks to inactivate trypsin. The culture medium was discarded, and cells resuspended in medium to determine the number of viable cells, Trypan blue exclusion method (Trypan Blue Solution, 4%, Gibco) and Neubauer chamber were utilized.

5.2.2 Cell viability assays - MTS assay

A first screen was performed to select the newly synthetized compounds with bioactivity against Hs578t cancer cells. Cells were plated into 96-well plates at a density of 3000 cells/well/100 μL and allowed to adhere overnight in complete medium. Then, cells were treated with selected compounds for 72 hours, with two different compound concentration (10 and 30 μM) in the respective culture medium. For this assay, 0.3% of DMSO and paclitaxel were used as reference drug. Cell Titer 96 Aqueous cell proliferation assay (MTS assay, Promega, Madison, WI, USA), was used to determine the effect of the studied compounds on cell viability. This colorimetric method was used to quantify viable cells, by measuring cell metabolic activity based on the MTS compound reduction (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) by NAD(P)H-dependent dehydrogenase to generate a colored formazan product that is soluble in cell culture medium. MTS solution was diluted in culture medium (1:10) and following 1 hour of incubation, the optical density was measured at 490 nm using the Varioskan Flash SkanIt (Thermo-Scientific) plate reader. The statistical analysis software, GraphPad Prism 9, was used to determine the compounds' activity from at least three independent experiments, each in triplicate. These values were obtained by application of a sigmoidal dose–response nonlinear regression, after logarithmic transformation.

5.2.3 IC_{50} determination

To determine the half-maximal IC_{50}, that means the concentration at which 50% of the cell growth is inhibited by drug treatment, the cells were plated into 96-well plates in triplicate at a density of $6x10^3$ cells per well, $5x10^3$ cells per well, or $3x10^3$ cells per well, for 24, 48 and 72 hours of treatment, respectively, and allowed to adhere overnight in 10% FBS culture medium. Cells were then treated with 7 different concentrations (from 0.05 to 30 μM) of compound **2.18g** or control (DMSO) in fresh medium.

After the incubation time, the compound cytotoxic effect was evaluated by the MTS assay (as explained before). The statistical analysis software, GraphPad Prism 9, was used to determine the compounds activity from at least three independent experiments, each in triplicate.

Chapter 6 – References

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