chemistry proceedings

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Proceeding Paper

Synthesis, Characterization and Preliminary Antibacterial Evaluation against *Staphylococcus aureus* of a New 2,4,5-Tri(hetero)arylimidazole Derivative Based on Azaindole Heterocycle †

Nuna L. P. Ramos [1], Rui Oliveira [2], Susana P. G. Costa [1] and Maria Manuela M. Raposo [1,*]

[1] Centre of Chemistry, Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal; nuna.ramos15@gmail.com (N.L.P.R.); spc@quimica.uminho.pt (S.P.G.C.)
[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal; ruipso@bio.uminho.pt
* Correspondence: mfox@quimica.uminho.pt
† Presented at 25th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2021; Available online: https://ecsoc-25.sciforum.net.

Abstract: Imidazole derivatives are known for their varied biological applications, such as antibacterial, antifungal, antioxidant, antiviral, antiparasitic and anticancer compounds, among others. Therefore, numerous imidazole derivatives have been synthesized and developed in recent years as potential drugs in the treatment of several diseases. In this communication, we report the synthesis of a new imidazole derivative, substituted at positions 2, 4 and 5 with heterocyclic groups, using a simple synthetic methodology and an easy purification procedure. The new compound was characterized by the usual spectroscopic techniques (NMR, UV-Vis absorption and emission). The evaluation of the novel imidazole derivative as a potential antibiotic drug was carried out against the Gram-positive bacterium *Staphylococcus aureus*, using the disk test diffusion method. The results showed a dose–response effect against the bacterium under study, revealing that the rational design of this imidazole derivative is quite promising to improve the antibacterial activity of imidazole derivatives.

Keywords: imidazole; synthesis; antibacterial activity; *Staphylococcus aureus*

Citation: Ramos, N.L.P.; Oliveira, R.; Costa, S.P.G.; Raposo, M.M.M. Synthesis, Characterization and Preliminary Antibacterial Evaluation against *Staphylococcus aureus* of a New 2,4,5-Tri(hetero)arylimidazole Derivative Based on Azaindole Heterocycle. *Chem. Proc.* **2022**, *8*, 104. https://doi.org/10.3390/ecsoc-25-11781

Academic Editor: Julio A. Seijas

Published: 14 November 2021

1. Introduction

The imidazole ring shows various interesting properties and is one of the most prominent structures in numerous natural products, such as histidine, histamine and vitamin B_{12} and is a component of DNA bases. Due to the characteristics of this heterocyclic ring and the binding properties to several analytes and biological structures [1–5], this core is also combined with several synthetic drugs with therapeutic use in medicine, such as omeprazole, eprosartan and metronidazole [6–8].

Over the past years, chemists have synthetized a diverse range of new imidazole derivatives as possible chemotherapeutic agents for the treatment of several diseases, such as antibacterial, antifungal, anti-inflammatory, antiviral, antiparasitic and anticancer [8–11]. This fact can be explained by taking into account that imidazole derivatives are polar and ionizable and can, therefore, be used to optimize the solubility and bioavailability parameters of existing molecules. On the other hand, the structure of the imidazole ring allows binding to numerous enzymes and receptors at the biological level through various types of hydrogen bonds and π-π stacking interactions, leading to a wide range of biological activities [7,12–14].

One of the major problems that humanity faces is the increase of multidrug-resistant bacteria, which is why the constant discovery of new potential drugs is important to combat

these microorganisms. The most reported example in the literature is the resistance of *Staphylococcus aureus*. Soon after the widespread use of penicillin, resistance to penicillin rapidly appeared in the clinic. Years later, a new antibiotic, methicillin, was developed [15], which effectively controlled infections caused by penicillin-resistant *S. aureus*. However, two years after the use of methicillin, the first strain of methicillin-resistant *S. aureus* (MRSA) was isolated [13,15].

In addition to the varied antibacterial activities [16–18], imidazoles also display interesting photophysical properties, namely tunable absorption and fluorescence by the careful choice and placement of electron donors and/or acceptor substituents at the imidazole core for the development of fluorescent bioimaging probes [13,16,19].

In this communication, we report the synthesis and the spectroscopic characterization of a new 2,4,5-tri(hetero)arylimidazole derivative based on azaindole heterocycle with potential antibiotic activity against *S. aureus* bacteria.

2. Materials and Methods

Commercial reagents were supplied by Sigma-Aldrich (St. Louis, MO, USA), Acros, Fluka, Panreac, Liofilchem and were used as received. Thin layer chromatography (TLC) was performed on silica gel 60 plates with fluorescence indicator F254 (Macherey-Nagel, Düren, Germany). The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance III device at 400 MHz and 100.6 MHz, respectively, using the solvent peak as an internal reference. The assignment of the ^{1}H and ^{13}C signals was performed using two-dimensional heteronuclear correlation techniques, using as solvent DMSO-d_6 with a 99.9% deuteration degree, containing 0.1% v/v tetramethylsilane from Sigma-Aldrich. The UV-visible absorption spectra were made using a Shimadzu UV/2501PC spectrophotometer, and the fluorescence emission spectra using a FluoroMax-4 (HORIBA) spectrofluorimeter using 9,10-diphenylanthracene as fluorescence standard.

For the study of antibacterial activity, the bacterial strain of the bacteria *S. aureus* ATCC 6538 was used. Compound **3** was applied in sterilized Whatman No. 1 filter paper, 6 mm diameter disks.

2.1. Synthesis and Spectroscopic Characterization of Imidazole Derivative 3

The 7-azaindole-3-carboxaldehyde **1** (1 mmol), furil **2** (1 mmol), NH_4OAc (20 mmol) and I_2 (5 mol %) were dissolved in ethanol (5 mL), followed by stirring and heating at reflux for 2 h. The reaction was monitored by TLC, using a mixture of dichloromethane/methanol (9:1) as eluent. Then, the reaction mixture was diluted with water (15 mL), having a small amount of $Na_2S_2O_3$, and was cooled in an ice bath. The resulting crude product, which precipitated, was purified by recrystallization from ethanol to give the pure imidazole derivative **3** as a brown solid in 16% yield, m.p. = [238–240] °C (Figure 1).

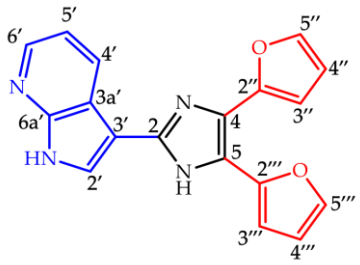


Figure 1. Structure of imidazole derivative **3**.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.62–6.63 (m, 2H, H4″ and H4‴), 6.87 (d, J = 3.2 Hz, 2H, H3″ and H3‴), 7.20–7.23 (q ap, J = 4.8 and 8.0 Hz, 1H, H5′), 7.76 (d, J = 0.8 Hz, 2H, H5″ and H5‴), 8.19 (d, J = 2.8 Hz, 1H, H2′), 8.29 (dd, J= 1.2 and 4.4 Hz, 1H, H6′), 8.69 (dd, J = 1.6 and 8.0 Hz, 1H, H4′), 11.96 (s, 1H, H_{imid}) ppm. The indole NH was not visible in these conditions.

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 104.94 (C3′), 107.34 (C3″ and C3‴), 111.83 (C4″ and C4‴), 116.63 (C5′), 117.44 (C3a′), 124.90 (C2′), 129.78 (C4′), 142.10 (C2″ + C2‴ + C5″ + C5‴), 143.71 (C2 and C6′), 148.58 (C6a′) ppm. The signals for carbons 4 and 5 were not visible, as already reported in the literature for similar systems [19].

UV/Vis (acetonitrile, nm): λ_{max} (log ε) = 321 (4.3); λ_{em} (Φ_F) = 455 (0.14).

2.2. Antibacterial Activity of Imidazole Derivative **3**

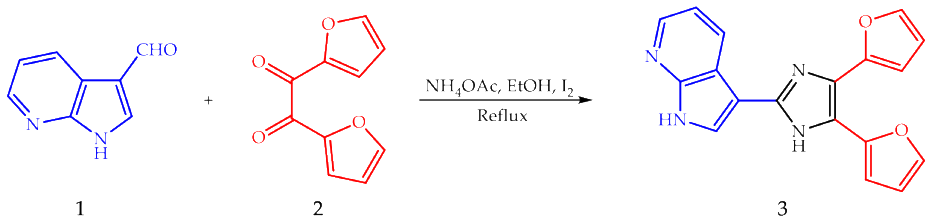
The inoculum suspension was prepared by selecting two morphologically similar colonies of *S. aureus* from overnight growth at 37 °C on solid Luria-Bertani agar medium (LB agar; 1% *w*/*v* tryptone, 0.5% *w*/*v* yeast extract, 1% *w*/*v* NaCl, 2% *w*/*v* agar) with a sterile loop. These colonies were suspended in LB liquid medium (the same composition as LB agar but without agar) and were incubated for 4–6 h at 37 °C with 200 rpm. The turbidity of the culture was visually adjusted to 0.5 of the McFarland scale with sterilized deionized water.

The test solutions were prepared by serial dilutions of the imidazole derivative **3**, in DMSO, in order to prepare concentrations of 0.5, 1.5, and 3.0 µg/µL. Ampicillin (0.3 µg/µL) was used as a standard drug for antibacterial activity. For each Petri dish, previously prepared with LB agar medium, the top agar was prepared with 100 µL bacterial suspension and 5 mL of LB agar (with 1.5% of agar) at 50 °C. This mixture was poured on the plates (approximately 5 mL) and cooled down for 15 min. Sterilized disks were placed firmly onto the medium with the top agar. In each disk, 10 µL of the solutions or controls were applied and were allowed to diffuse at room temperature for 1–2 h before incubation at 37 °C for 18 h. These assays were performed in triplicate. After incubation, inhibition zones were measured at the point where no obvious growth of bacteria is detected by the unaided eye.

3. Results and Discussion

3.1. Synthesis and Spectroscopic Characterization of the Imidazole Derivative **3**

Imidazole derivative **3** was synthesized from 7-azaindole-3-carboxaldehyde (**1**) and furil (**2**), in the presence of ammonium acetate, using ethanol as solvent and iodine as the catalyst, for 2 h, giving the pure compound as a brown solid with 16% yield after recrystallization from ethanol (Scheme 1). Iodine as a catalyst in organic reactions acts as a mild Lewis acid and has the ability of bonding with the carbonyl oxygen, increasing the reactivity of the carbonyl compound [20].



1 2 3

Scheme 1. Synthesis of imidazole derivative **3**.

Pure imidazole derivative **3** was isolated in low yield (16%), probably due to losses during the recrystallization process, as well as the low reactivity of the heterocyclic aldehyde bearing a pyrrole electron-rich moiety which will enhance the electronic density on the carbonyl group, and, therefore, will induce a lower reactivity of this compound. After synthesis, imidazole derivative **3** was characterized by NMR spectroscopy (Figure 2).

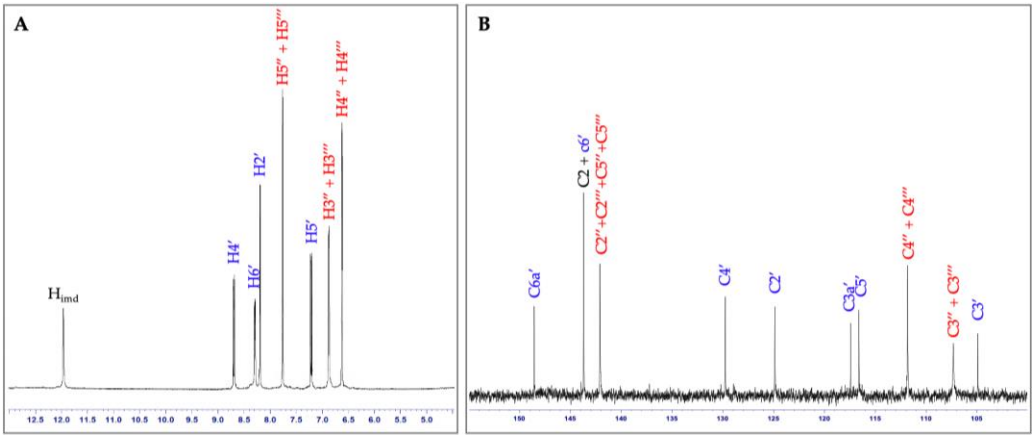


Figure 2. ^{1}H NMR (**A**) and ^{13}C NMR (**B**) spectra of imidazole derivative **3** (the colors correspond to the different moieties, as shown in Scheme 1).

Standard spectroscopic characterization of compound **3** was performed in acetonitrile (ACN) solutions with concentrations 1×10^{-5} and 1×10^{-6} mol/dm^3 (Figure 3). The relative quantum fluorescence yield of the compound was calculated using the fluorescence standard 9,10-diphenylanthracene in ethanol, whose absolute fluorescence quantum yield is 0.95 [21].

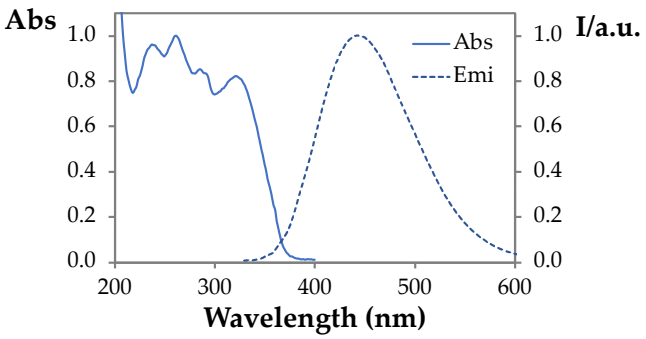


Figure 3. Normalized absorption and emission spectra of imidazole derivative **3**.

Compound **3** exhibited a maximum wavelength of absorption at 321 nm, showing an intense absorption band. Regarding the emission properties, this imidazole showed a maximum emission wavelength at 455 nm, with a large Stokes' shift value of (134 nm), meaning it is possible to conclude that this compound has the potential to be used as a fluorescence probe in biological media since a large shift allows an improved separation of light from the matrix and the inherent light scattered by the sample [1,22]. Finally, the imidazole derivative showed a relative fluorescence quantum yield of 0.14.

3.2. Antibacterial Activity of Imidazole Derivative **3**

In vitro antibacterial studies of imidazole derivatives were performed against the Gram-positive bacterium *S. aureus* by disk test diffusion method (Figure 4). The compound inhibition halo values were measured in triplicate (Table 1), ampicillin was used as the standard antibiotic drug for the positive control, and DMSO was used as the negative control.

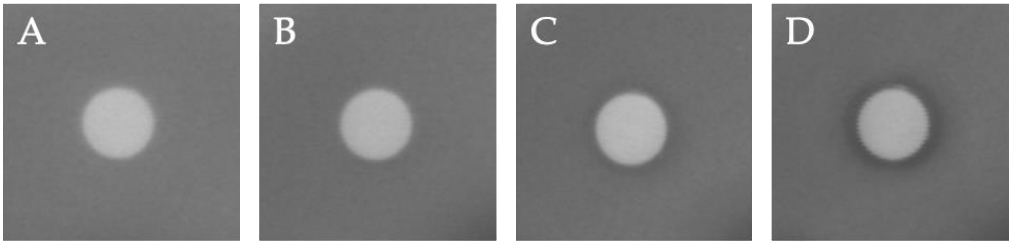


Figure 4. Disk test diffusion method of antibacterial activity of imidazole **3** against *S. aureus* ATCC 6538: (**A**) with 10 µL of DMSO, (**B**) 5 µg/disk, (**C**) 15 µg/disk and (**D**) 30 µg/disk.

Table 1. Inhibition halos and standard deviation of imidazole derivative **3** against *S. aureus* ATCC 6538 in the disk diffusion assay.

Compound	3 µg/disk	5 µg/disk	15 µg/disk	30 µg/disk
3	-	ND	ND	(8 ± 1)
Ampicillin	(23.0 ± 0.1)	-	-	-

ND: not detected; -: not determined.

Bearing in mind the results of the antibacterial activity, a clear inhibition zone is present around the test disk, whose diameter is larger when a higher concentration (30 µg/disk) is applied. However, in Figure 4C it is possible to visualize an inhibition of growth close to the disk markedly smaller than 30 µg/disk (Figure 4D), which was not measurable, thus suggesting that the antibacterial effect is dependent on the concentration that is applied to the disk. As expected, the broad-spectrum antibiotic ampicillin displayed considerably higher activity than imidazole **3**. Ampicillin is a semi-synthetic antibiotic, which is optimized for maximal activity and improved pharmacokinetic parameters. Based on previous work by this research group [23] and the rational design of the imidazole derivative **3**, it is possible to conclude that imidazole **3** is a new 2,4,5-tri(hetero)arylimidazole with the potential to be the basis for the development of novel drugs with antibiotic activity in medicinal chemistry.

4. Conclusions

Imidazole derivative **3** was synthesized using a simple synthetic methodology and an easy purification procedure and was characterized by the usual NMR and UV-Vis absorption and emission spectroscopies.

Compound **3** showed antibacterial activity against *S. aureus*, which can be considered a potential drug in the treatment of diseases caused by pathogenic bacteria.

Author Contributions: Conceptualization, M.M.M.R.; methodology, M.M.M.R., S.P.G.C. and R.O.; validation, N.L.P.R., M.M.M.R. and S.P.G.C.; formal analysis, N.L.P.R., M.M.M.R., S.P.G.C. and R.O.; investigation, N.L.P.R.; resources, M.M.M.R., S.P.G.C. and R.O.; writing—original draft preparation, N.L.P.R.; writing—review and editing, N.L.P.R., M.M.M.R., S.P.G.C. and R.O.; supervision, M.M.M.R., S.P.G.C. and R.O.; funding acquisition, M.M.M.R., S.P.G.C. and R.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Foundation for Science and Technology (FCT) through CQ/UM (UIDB/00686/2020) and CBMA/UM "Contrato-Programa" UIDB/04050/2020 funded by national funds. Thanks are also due to FCT for financial support to the Portuguese NMR Network (PTNMR, Bruker Avance III 400-Univ. Minho).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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