Synthesis and Antitumour Evaluation of Benzopsoralen Analogues

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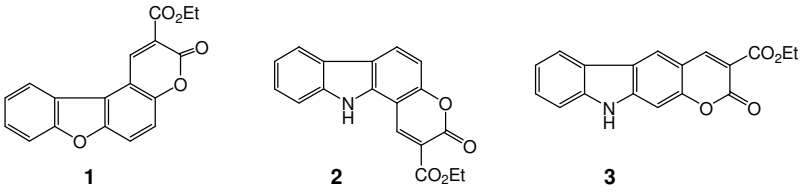
Abstract: The synthesis of five new analogues of benzopsoralens, derived from xanthen-9-one and carbazole is described. The preparation of the hydroxylated precursors, their formylation and the formation of the pyranone ring, by the reaction between *ortho*-formylated heterocycles and diethyl malonate in EtOH, will be discussed. The inhibitory effect on the growth of human tumour cell lines (MCF-7, SF-268 and NCI-460) of the final products was evaluated and is discussed in terms of the 3D-geometry and electronic density distribution. The compounds showing significant biological activity in this study were those having angular structures.

1. Introduction.- Psoralens are natural products derived from coumarin. Some of their derivatives are photosensitising drugs and are used for treatment of various skin diseases (*e.g.* cutaneous malignant melanoma) [1], blood decontamination, and some AIDS related infections [2]. In a previous publication, our research group prepared psoralen analogues (*e.g.* **1**) based on dibenzofuran [3] and studied their properties. Some of them showed sensitization of singlet oxygen with high quantum efficiency [4] and good ability to inhibit the *in vitro* growth of three human tumour cell lines representing different tumour types, MCF-7 (breast cancer), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) [5].

In view of the antitumour properties referred above for compounds of type **1**, it was decided to evaluate the effects on the biological properties of modifications on the furan ring. The first modification consisted on the replacement of the oxygen atom by NH giving **2** and **3**, isosters of oxopyranodibenzofuran (**1**). The replacement of the furan ring by 1,4-pyranone gave xanthone derivatives, **4**, **5** and **6**, close analogues of xanthonolignoids studied previously by members of our group [6].



The five tetracyclic compounds which were synthesized were tested for their growth inhibitory activity against three human tumor cell lines.

2. Results and discussion.- *2.1. Chemistry.* In order to build the pyranone ring by the same method that was used before [3], *ortho*-hydroxyaldehydes compounds, were needed as starting materials.

The hydroxylated xanthen-9-ones **7** were prepared by known procedures [7] and were then formylated, to give products **8**, by different methods.

The first attempt to formylate the hydroxylated xanthones **7** used the *Duff* reaction [7, 8]. By this method, hydroxyaldehydes **8b**, **8c**, **8d**, and **8a** were obtained in 56, 32, 17, and 13% yields, respectively (*Scheme 1*). The *Reimer-Tiemann* formylation [9] gave compound **8a** in an even lower yield (6%). In the ^{1}H-NMR spectra of all the hydroxyaldehydes **8**, a low field signal was observed corresponding to the H-bonded OH, and the lowest signal of all (δ = 13.54 ppm) corresponded to compound **8a**.

INSERT SCHEME 1

The pyranone ring was built from *ortho*-formylhydroxyxanthen-9-ones **8b-8c**, by reflux with diethyl malonate in EtOH in the presence of AcOH and pyridine [10] (*Scheme 2*) and compounds **4** and **5** were obtained in 50 and 55% yields, respectively. In the ^{1}H-NMR spectra, the signals of the hydroxyl and aldehyde groups were replaced by a low field singlet, corresponding to H-1 of the pyranone ring. It is interesting to point out that the low field singlet for compound **4** appears at δ 10.57 and for compound **5** at 9.21 ppm. As a consequence of the low yields of the formylation of hydroxyxanthenones **7a** and **7d**, the preparation of the corresponding oxopyrano-xanthenones, was not accomplished.

INSERT SCHEME 2

For the synthesis of pyranoxanthen-9-one **6**, another approach was used. 3-Methoxyxanthen-9-one was reduced [11] to the 3-methoxyxanthene (77%, $LiAlH_4$) (*Scheme 3*).

Vilsmeier formylation [12] ($POCl_3$ and *N*-methylformanilide) gave the aldehyde **9a** in 31% yield, which was demethylated quantitatively, using BBr_3 [13], to give the aldehyde **9b** (*Scheme 3*).

The usual method was applied for the construction of the pyranone ring, reacting **9b** with diethyl malonate, and xanthene **10** was obtained in high yield (73%). Oxidation of **10** by chromium (VI) oxide [7a] gave **6** in 83% yield (*Scheme 3*).

INSERT SCHEME 3

Aldehydes **11b** and **11c** were obtained from commercial 2-hydroxycarbazole. The *Reimer-Tiemann* formylation gave **11b** in 20% yield [14] (*Scheme 4*), whereas **11c** was prepared in 32% by subsequent metalation and treatment with DMF [16] from the carbamate **11a**, which was obtained in good yield (*c.f.* [15]).

INSERT SCHEME 4

The *ortho*-formylhydroxycarbazoles **11b** and **11c** were then converted to the oxopyranocarbazoles **2** and **3**, in 17 and 27% yield, respectively, following the usual method [10].

The structures of the final products were confirmed by mass spectrometry, IR and NMR spectroscopy.

2.2. *Biological activity* **-** *Effect of compounds on the growth of human tumour cell lines*

The ability of oxopyranoxanthenones **4**, **5** and **6** and the oxopyranocarbazoles **2** and **3** to inhibit the *in vitro* growth of MCF-7, NCI-H460 and SF-268 cell lines was evaluated and the results, given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}), are summarized in Table 1.

Among the compounds tested the angular pyranocarbazole **2** was found to have the best inhibitory activity against all the three cell lines, while pyranocarbazole **3,** a compound with a related structure but with a different orientation of the pyranone ring, was found to be ineffective as growth inhibitor even when tested at concentrations of 150 μM.

The pyranoxanthenone **4** exhibited only a growth inhibitory effect against MCF-7 cells being ineffective against SF-268 and NCI-H460 cell lines. This result is in agreement with that observed for xanthonolignoids [6, 17] where the equivalent angular xanthonolignoid revealed some selectivity to MCF-7 cells [6]. This different cell line response could reflect a possible tumour type-specific sensitivity of this compound for the breast cancer cell line. Compound **5** with the same functional groups, but a different orientation, showed a three fold decrease in its inhibitory activity against MCF-7 cell, but without the selectivity detected with compound **4**.

Although the number of the compounds tested is limited, some structural features important to the growth inhibitory effect can be inferred. Angular compounds showed in general higher activity (*c.f.* compound **2** in the three cell lines and compound **4** on MCF-7). It has been previously observed that angular compound **1** [5] was active on these cell lines (Table 1).

INSERT TABLE 1

2.3. Analysis of the molecular electrostatic potential superimposed onto total electron density (MEP/TED) and 3D-geometry

All compounds possess close molecular volumes (Table 2). However, as observed in a previous study [5], the growth inhibitory activity presented by some of these compounds is due to the 3D-geometry and electronic density distribution.

INSERT TABLE 2

A simple inspection of the MEP/TED figures and structure of these compounds, Table 3, suggests that the compounds **4**, **5** and **2** are expected to present antitumour activity, since they show similarities in 3D-geometry and charge distribution when compared with those observed for the compound ethyl 3-oxo-3*H*-benzofuro[3,2-*f*]chromene-2-carboxylate (compound **1**) [5]. However, it is compound **2** that shows the greatest ability to inhibit the *in vitro* growth of MCF-7, NCI-H460 and SF-268 cell lines (Table 2), which is comparable to that observed before for compound **1** [5]. The charge distribution over the atoms of these molecules is surprisingly similar (Figure 1).

INSERT FIG. 1

The oxopyranoxanthenones, **4** and **5,** present similar conformation, but they show specific variations in the charge distribution at some critical points. It was observed that compound **5** had only a slight inhibitory effect, while **4** showed a moderate growth inhibitory effect only against the MCF-7 cell line. This reflects the strict dependence between tumour type-specific sensitivity and a combination of 3D-geometry and a specific charge distribution (Figure 2).

INSERT FIG. 2

These two compounds display a negative charge density localized in the internal part of the molecule, due to a carbonyl group (compound **4**), and an oxygen atom from the xanthenone ring (compound **5**). The local electronic effect caused by the carbonyl group in **4** seems to be responsible for its specificity to MCF-7 cell line. Despite this, the behaviour presented by compounds **4** and **5** suggests that a negative charge localized in this region is able to lower the tumour growth inhibitory activity. This is in agreement with the observations reported in a previous work, where the inhibitory activity of some benzopsoralens (including compound **1**, [5]) was studied. A fifth interaction point must be introduced in the previously proposed model, involving the internal part of the condensed ring system of the bioactive compound and the bioreceptor. This point must be of major importance in the association bioactive compound/ bioreceptor, inducing the tumour growth inhibitory activity if the electronic density is positive, as it is the case for compounds **1** and **2**.

3. Conclusions

The synthesis of five new analogues of benzopsoralens, derived from xanthen-9-one and carbazole was described.

Some of the compounds synthesized (**4**, **5**, and **2**) showed an interesting growth inhibitory effect against the MCF-7, SF-268 and NCI-H460 cell lines. All the compounds showing some biological activity (**4**, **5** and **2**) have angular structures revealing the importance of the global molecular topology as previously observed for xanthonolignoids.

Specific 3D-geometry and charge distribution are responsible for the biological activity displayed by some of these compounds. The behaviour presented by

compound **2**, very similar to that presented by compound **1**, and by compounds **4** and **5** suggests that a fifth interaction point must be included in the previously proposed model [5], involving the internal part of the condensed ring of the bioactive compound and the bioreceptor(s). This seems to play a major role in the interaction between the bioactive compound and the bioreceptor(s).

Experimental Part

1. Chemistry

General: Light petroleum refers to solvent boiling in the range 40-60º. Column chromatography (CC) was performed on, silica gel 60 (70-230 mesh, *Merck*). Melting points were determined on a *Gallenkamp* apparatus and are uncorrected. UV spectra were recorded in EtOH on a *SHIMADZU UV-2501 PC* and data are presented in λ_{max} (nm), (log ε [mol^{-1} dm^3 cm^{-1}]). IR were recorded on a *Diffus-IR Bomem MB-Series FTIR* spectrometer in cm^{-1}. NMR spectra were obtained on a *Varian Unity Plus at 300 MHz* (^1H) and 75.4 (^{13}C) and the assignments were based on irradiation and 2D-NMR techniques (HMQC and HMBC), respectively. The solvent was $CDCl_3$ (if not stated otherwise) and δ is in ppm, relative to internal $SiMe_4$. Elemental analyses were carried out with a *LECO CHNS-932*. EIMS and HRMS spectra were carried out with an *AutoSpecE spectrometer*, in m/z (rel. %).

General method of Duff *formylation of hydroxyxanthen-9-ones:* To a soln. of 2-hydroxyxanthen-9-one (212 mg, 1.00 mmol) in AcOH (99.8%, 15 ml), hexamethylenetetramine (HMTA) (475 mg, 3.39 mmol) was added and the mixture was refluxed for 4 h. After cooling a 10% soln. of HCl (30 ml) was added and the mixture was refluxed for 20 min. The mixture was then poured onto ice

(100 g) and extracted with Et_2O (3 x 60 ml). The org. extracts were combined, dried ($MgSO_4$) and the solvent partly removed. The reaction was followed by TLC (Et_2O/petrol, 1:1).

1-Hydroxy-9-oxoxanthene-2-carbaldehyde **(8a)**. Yellow crystals (13%). M.p.: 149-152º (EtOH). IR (Nujol): 1679 (C=O), 1645 (C=O), 1614, 1573, 1465, 1283, 1255, 1063, 828, 769, 711, 639. ^{1}H-NMR: 13.54 (*s*, OH); 10.62 (*s*, CHO); 8.34 (*dd*, *J* = 7.8, 1.5, H-C(8)); 8.24 (*d*, *J* = 8.7, H-C(3)); 7.86 (*ddd*, *J* = 8.4, 7.5, 2.1, H-C(6)); 7.62 (*br d*, *J* = 8.4, H-C(5)); 7.51 (*ddd*, *J* = 8.0, 7.2, 1.2, H-C(7)); 6.91 (*d*, *J* = 9.0, H-C(4)). EI-MS: 241 (13, $[M+1]^+$), 240 (78, M^+), 239 (100), 212 (16), 183 (6), 155 (8), 127 (9), 92 (6). HR-EI-MS: 240.0415 (M^+, $C_{14}H_8O_4$; calc. 240.0423).

2-Hydroxy-9-oxoxanthene-1-carbaldehyde **(8b)**. Product **8b** precipitated as yellow crystals (135 mg, 56%). M.p.: 164-166º (EtOH) (lit. [8b] 163º (petrol)). IR (Nujol): 1640 (strong, C=O), 1465, 1330, 1304, 1233, 1211, 1152, 1057, 880, 846, 817, 752. ^{1}H-NMR: 13.02 (*s*, OH); 11.59 (*s*, CHO); 8.34 (*dd*, *J* = 7.8, 1.5, H-C(8)); 7.78 (*ddd*, *J* = 7.8, 7.5, 1.5, H-C(6)); 7.75 (*d*, *J* = 9.6, H-C(4) or H-C(3)); 7.53 (*br d*, *J* = 7.8, H-C(5)); 7.48-7.40 (*m*, 2H, H-C(7), and H-C(3) or H-C(4)).

For other hydroxyxanthenones the reflux duration was variable and estimated by TLC (5 h, 2 h and 7 h, for 1, 3 and 4-hydroxyxanthen-9-one, resp.). It was necessary to use column chromatography or preparative chromatography to obtain 1-hydroxy-9-oxoxanthene-2-carbaldehyde and 4-hydroxy-9-oxoxanthene-3-carbaldehyde. All the aldehydes were obtained as yellow crystals.

3-Hydroxy-9-oxoxanthene-4-carbaldehyde **(8c)**. Yellow crystals (32%). M.p.: 212-215º (EtOH) (lit. [18]: 223º (EtOH)). IR (Nujol): 1650 (strong, C=O), 1615,

1463, 1414, 1344, 1316, 1215, 1168, 1068, 819, 760, 735, 680. ^{1}H-NMR: 12.66 (*s*, OH); 10.74 (*s*, CHO); 8.48 (*d*, *J* = 9.0, H-C(1)); 8.36 (*dd*, *J* = 7.8, 1.8, H-C(8)); 7.78 (*ddd*, *J* = 8.6, 7.5, 1.5, H-C(6)); 7.56 (*br d*, *J* = 8.4, H-C(5)); 7.47 (*ddd*, *J* = 7.5, 6.6, 1.0, H-C(7)); 6.99 (*d*, *J* = 9.0, H-C(1)). MS-EI: 241 (16, $[M+1]^+$), 240 (100, M^+), 239 (97), 222 (4), 194 (8), 155 (6), 127 (7), 92 (4), 69 (7). HR-EI-MS: 240.0423 (M^+, $C_{14}H_8O_4$; calc. 240.0423).

4-Hydroxy-3-oxoxanthene-3-carbaldehyde **(8d)**. Yellow crystals (17%). M.p.: 166-169º. IR (Nujol): 1667 (strong, C=O), 1632 (C=O), 1612, 1463, 1345, 1293, 1234, 1216, 1297, 1019, 982, 904, 829, 806, 759, 743, 689. ^{1}H-NMR: 11.70 (*s*, OH); 10.10 (*s*, CHO); 8.36 (*dd*, *J* = 7.8, 1.8, H-C(8)); 7.96 (*d*, *J* = 8.1, H-C(2) or H-C(1)); 7.82 (*ddd*, *J* = 8.7, 6.9, 1.5, H-C(6)); 7.68 (*br d*, *J* = 8.7, H-C(5)), 7.58 (*d*, *J* = 8.4, H-C(1) or H-C(2)); 7.46 (*ddd*, *J* = 8.1, 6.9, 1.0, 1H, H-C(7)). MS-EI: 241 (116, $[M+1]^+$), 240 (100, M^+), 239 (54), 222 (4), 194 (17), 183 (6), 155 (6), 127 (6), 120 (6), 92 (3), 69 (3), 63 (3). HR-EI-MS: 240.0420 (M^+, $C_{14}H_8O_4$; calc. 240.0423).

General method of formation of the pyranone ring: To a soln. of 2-hydroxy-9-oxoxanthene-1-carbaldehyde **8b** (38 mg, 0.16 mmol) in EtOH (6 ml) diethyl malonate (35 mg, 0.22 mmol, previously dissolved in 1 ml EtOH), one drop of AcOH and one drop of piperidine were added and the mixture was refluxed for 3.5 h. A precipitate formed on cooling and was filtered off and washed with cold EtOH.

Ethyl 3,12-dioxopyran[3,2-a]xanthene-2-carboxylate **(4)**. Compound **4** was obtained from 2-hydroxy-9-oxoxanthene-1-carbaldehyde **8b** as a yellow solid (27 mg, 50%. M.p.: 220-222º ($CHCl_3$/petrol); UV (log ε, EtOH): 395 (3.94), 377

(3.99), 298 (4.22). IR (Nujol): 1769 (strong, C=O), 1641 (C=O), 1611, 1594, 1562, 1466, 1327, 1249, 1227, 1207, 1119, 1035, 968, 900, 834, 799, 774, 725, 684. ^{1}H-NMR: 10.57 (*s*, H-C(1)); 8.34 (*dd*, *J* = 8.4, 1.7, H-C(11)); 7.84 (*d*, *J* = 9.0, H-C(5) or H-C(6)); 7.82 (*ddd*, *J* = 8.4, 7.0, 1.7, H-C(9)); 7.73 (*d*, *J* = 9.0, H-C(6) or H-C(5)); 7.58 (*br d*, *J* = 8.4, H-C(8)); 7.49 (*ddd*, *J* = 7.6, 7.0, 1.2, H-C(10)); 4.48 (*q*, *J* = 7.5, Et), 1.47 (*t*, *J* = 7.2, Et). ^{13}C-NMR: 177.76, 162.76, 155.83, 155.04, 153.92, 152.26, 146.11 (CH), 135.39 (CH), 126.52 (CH), 124.94 (CH), 124.90 (CH), 123.87 (CH), 122.19, 120.15, 117.76 (CH), 115.89, 115.72, 62.05 (CH_2), 14.24 (CH_3). MS-EI: 337 (21), 336 (100, M^+), 308 (9), 291 (66), 264 (81), 236 (32), 223 (3), 212 (5), 207 (8), 179 (12), 150 (9). HR-EI-MS: 336.0635 (M^+, $C_{19}H_{12}O_6$; calc. 336.0634). Anal. Calc. for $C_{19}H_{12}O_6$.1/4(H_2O): C 66.96, H 3.70; found: C 67.01, H 3.71.

Ethyl 3,7-dioxopyran[2,3-c]xanthene-2-carboxylate **(5)**. Compound **5** was obtained from 3-hydroxy-9-oxoxanthene-4-carbaldehyde **8c** as yellow crystals (26 mg, 55%). M.p.: 196-197º (EtOH). UV (log ε, EtOH): 350 (4.04), 328 (4.13), 307 (4.08), 276 (4.38). IR (Nujol): 1771 (strong, C=O), 1660 (C=O), 1624, 1463, 1262, 1232, 1083, 1019, 965, 793, 773, 722, 630. ^{1}H-NMR: 9.21 (*s*, H-C(1)); 8.59 (*d*, *J* = 9.0, H-C(6)); 8.39 (*dd*, *J* = 7.8, 1.5, H-C(8)); 7.84 (*ddd*, *J* = 8.7, 7.2, 1.5, H-C(10)); 7.67 (*br d*, *J* = 8.4, H-C(11)); 7.51 (*ddd*, *J* = 7.7, 7.2, 1.2, H-C(9)); 7.36 (*d*, *J* = 9.0, H-C(5)); 4.50 (*q*, *J* = 6.9, Et); 1.48 (*t*, *J* = 7.2, Et). ^{13}C-NMR: 175.25 (C(7)), 162.94 (CO_2Et), 159.41 (C(4a)), 155.65 (C(11a)), 155.52 (C(3)), 153.31 (C(12a)), 142.22 (C(1)), 135.37 (C(10)), 132.74 (C(6)), 126.97 (C(8)), 125.34 (C(9)), 121.88 (C(7a)), 118.06 (C(12b) ou C(2)), 117.95 (C(11)), 117.93 (C(12b) or C(2)), 113.29 (C(5)), 108.083 (C(6a)), 62.43 (CH_2), 14.26 (CH_3). MS-EI): 337 (18, [*M*+1] $^{+}$),

336 (83, M^+), 308 (4), 291 (85), 264 (100), 236 (28), 207 (24), 179 (8), 150 (8), 87 (6), 75 (4). HR-EI-MS: 336.0639 (M^+, $C_{19}H_{12}O_6$; calc. 336.0634).

*Synthesis of pyranoxanthen-9-one (**6**) via xanthene*

3-Methoxyxanthene. The 3-methoxyxanthen-9-one was reduced with $LiAlH_4$ in dry Et_2O giving an oil which was purified by column chromatography (TLC control: Et_2O/petrol, 3:7). The product was obtained as colourless crystals (Et_2O/petrol, 5:95) (77%). M.p.: 78-82º (CH_2Cl_2/petrol) (lit. [19]: 79-80º (hexane)). IR (KBr): 3059, 2996, 2840, 1604, 1577, 1486, 1444, 1314, 1237, 1150, 1093, 963, 836, 760. ^{1}H-RMN (acetone-d_6): 7.28-7.19 (*m*, H-C(8) and H-C(6)), 7.15 (*br d*, *J* = 8.3, H-C(1)), 7.09-7.01 (*m*, H-C(5) and H-C(7)), 6.67 (*dd*, *J* = 8.3, 2.5, H-C(2)), 6.62 (*d*, *J* = 2.5, H-C(4)), 4.00 (*s*, C*H*$_2$), 3.81 (*s*, OMe).

3-Methoxyxanthene-2-carbaldehyde **(9a).** *Vilsmeier* formylation of 3-methoxyxanthene with *N*-methylformanilide and $POCl_3$ in CH_2Cl_2 (9 h reflux) gave an oil which was purified by column chromatography. The starting 3-methoxyxanthene was partly recovered (20 mg, 31%) and the title compound was obtained as light yellow crystals (22 mg, 31%). M.p.: 156-158º (lit. [12a]: 154º). IR (KBr): 1671 (C=O), 1628, 1609, 1572, 1505, 1468, 1445, 1426, 1405, 1330, 1307, 1290, 1235, 1185, 1113, 1015, 963, 894, 830, 773, 753. ^{1}H-NMR: 10.34 (*s*, CHO), 7.77 (*s*, H-C(1)), 7.25-7.18 (*m*, H-C(6) and H-C(8)), 7.11-7.03 (*m*, H-C(7) and H-C(5)), 6.64 (*s*, H-C(4)), 4.01 (*s*, C*H*$_2$), 3.93 (*s*, OMe).

3-Hydroxyxanthene-2-carbaldehyde **(9b)**. The 3-methoxyxanthene-2-carbaldehyde **9a** was demethylated with BBr_3 in dry CH_2Cl_2 at low temperature (dry ice/acetone) to give compound **9b** as a light yellow solid (67 mg, 100%). M.p.: 172-174º (lit. [12a] 159º). IR (Nujol): 1664 (strong, C=O), 1619, 1593,

1570, 1293, 1245, 1186, 1148, 968, 880, 869, 759, 726. ^{1}H-NMR: 11.15 (*s*, O*H*), 9.78 (*s*, C*H*O), 7.37 (*br s*, H-C(1)), 7.28-7.16 (*m*, H-C(6) and H-C(8)), 7.12-7.06 (*m*, H-C(5) and H-C(7)), 6.64 (*s*, H-C(4)), 4.04 (*s*, CH_2).

Ethyl 2-oxopyran[3,2-b]xanthene-3-carboxylate **(10)**. This compound was obtained by the general method already described (4.2), as beige thin needles (78 mg, 73%) M.p.: 240-242º (EtOH), IR (Nujol): 1769 (C=O), 1752 (C=O), 1632, 1615, 1564, 1456, 1303, 1295, 1275, 1233, 1195, 1136, 1033, 991, 907, 847, 797, 750, 731, 666. ^{1}H-NMR: 8.49 (*s*, H-C(4)), 7.43 (s, H-C(5)), 7.30-7.20 and 7.15-7.08 (*m*, H-C(7), H-C(8), H-C(9) and H-C(10)), 7.03 (*s*, H-C(12)), 4.42 (*q*, *J* = 6.9, Et), 4.13 (*s*, CH_2), 1.42 (*t*, *J* = 7.2, Et). ^{13}C-NMR: 163.53, 157.13, 157.09, 155.61, 150.89, 148.56 (CH), 129.98 (CH), 129.20 (CH), 128.48 (CH), 124.48 (CH), 119.53, 118.93, 116.95 (CH), 115.70, 114.02, 104.42 (CH), 62.07, 27.23, 14.53. MS-EI: 323 (20, [*M*+1] $^{+}$), 322 (100, M^{+}), 321 (73), 293 (13), 277 (23), 250 (17), 221 (17), 193 (8), 163 (6), 138 (8). HR-EI-MS: 322.0845 (M^{+}, $C_{19}H_{14}O_5$; calc. 322.0841).

Ethyl 2,6-dioxopyran[3,2-b]xanthene-3-carboxylate **(6)**. To a soln. of the pyranoxanthene **10** (32 mg, 0.10 mmol) in pyridine (5 ml) powdered chromium (VI) oxide (40 mg, 0.40 mmol) was added and the mixture was left stirring at room temperature for 2.5 h. The mixture became dark brown and a solid precipitated. The mixture was poured over HCl 2 M (30 ml) and was extracted with $CHCl_3$ (3x 30 ml) and EtOAc (4 x 30 ml). The extracts were combined, dried and the solvent was evaporated. The resulting solid was crystallized from a mixture of $CHCl_3$ and MeOH. The chromatographic control (TLC) was done using $CHCl_3$/MeOH, (99:1). The title compound was obtained as a white powder (28

mg, 83%). M.p.: 305-307º ($CHCl_3$/CH_3OH), decomposes above 300º. UV (log ε, EtOH): 360 (3.80), 267 (4.46). IR (Nujol): 1768 (C=O), 1652 (C=O), 1623, 1607, 1561, 1465, 1352, 1222, 1139, 1027, 973, 846, 795, 762, 692. ^{1}H-NMR ($CDCl_3$ + CD_3OD): 8.64 (*s*, H-C(4) or H-C(5)), 8.62 (*s*, H-C(4) or H-C(5)), 8.29 (*dd*, *J* = 8.1, 1.8, H-C(7)), 7.77 (*ddd*, *J* = 8.4, 7.2, 1.8, H-C(9)), 7.50 (*br d*, *J* = 8.4, H-C(10)), 7.42 (*ddd*, *J* = 7.9, 6.9, 1.2, H-C(8)), (*s*, H-C(12)), 4.38 (*q*, *J* = 6.9, Et), 1.38 (*t*, *J* = 7.2, Et). ^{13}C-NMR, δ ($CDCl_3$ + CD_3OD): 148.23 (CH), 135.80 (CH), 130.08 (CH), 126.68, 124.97 (CH), 118.02 (CH), 105.07 (CH), 62.13 (CH_2), 13.95 (CH_3). The signals described correspond to carbon atoms either than quaternary, due to the low solubility of the compound. MS-EI: 337 (21, $[M+1]^+$), 336 (98, M^+), 308 (4), 291 (100), 264 (93), 236 (24), 207 (18), 179 (8), 150 (6), 145 (5). HR-EI-MS: 336.0633 (M^+, $C_{19}H_{12}O_6$; calc. 336.0634). Anal. calc. for $C_{19}H_{12}O_6$: C 67.86, H 3.60; found: C 67.43, H 3.71.

Synthesis of the pyranocarbazoles

Reimer-Tiemann *formylation.* 2-Hydroxycarbazole (2 g, 11 mmol), was treated with $CHCl_3$ and NaOH and gave, after column chromatography (Et_2O/petrol, 1:9), 2-hydroxycarbazole-1-carbaldehyde **11b** as yellow crystals, (0.46 g, 20%). M.p.: 219-220º (decomposes above 200º) (lit. [12c]: 232º (EtOH)). IR (Nujol): 3405 (N*H*), 1633 (C=O), 1598, 1318, 1278, 1206, 1170, 1115, 1085, 1032, 938, 784, 775, 757, 743, 722. ^{1}H-NMR (acetone-d_6): 11.16 (*br s*, O*H*), 10.69 (*s*, C*H*O), 10.33 (*br s*, N*H*), 8.27 (*d*, *J* = 8.1, H-C(4)), 8.06 (*br d*, *J* = 8.1, H-C(5)), 7.71 (*br d*, *J* = 8.1, H-C(8)), 7.39 (*ddd*, *J* = 7.9, 7.5, 1.5, H-C(7)), 7.25 (*ddd*, *J* = 7.9, 6.9, 1.0, H-C(6)), 6.85 (*d*, *J* = 8.4, H-C(3)).

Formylation via carbamate formation

i) Synthesis of the carbamate. A soln. of the 2-hydroxycarbazole (0.45 g, 5 mmol) in dry pyridine (2 ml) and *N,N*-diethylcarbamoyl chloride (0.745 g, 11 mmol) was heated for 4 h at 100°. The mixture was poured onto crushed ice (75 g) and extracted with Et_2O (3x 25 ml) and the org. extract was washed with 2 M NaOH (3x 25 ml) and H_2O (3x 25 ml) and dried ($MgSO_4$). After solvent evaporation the corresponding (*N,N*-diethylcarbamoyl)-carbazole was obtained as a brown oil. The carbamate was used without further purification.

2-(N,N-Diethylcarbamoyl)-carbazole **(11a)**. Light brown oil (0.634 g, 92%), which was used without purification. IR (film): 3408, 3307 (N*H*), 2975, 2934, 1700 (strong, C=O), 1633, 1611, 1461, 1422, 1380, 1326, 1271, 1230, 1166, 1119, 1002, 971, 865, 748, 727, 666. ^{1}H-NMR: 8.26 (*br s*, NH), 7.94 (*br d*, *J* = 7.8, H-C(5)), 7.89 (*d*, *J* = 8.4, H-C(4)), 7.40-7.33 (*m*, H-C(7) and H-C(8)), 7.24-7.18 (*m*, H-C(6)), 7.20 (*d*, *J* = 2.1, H-C(1)), 6.95 (*dd*, *J* = 8.4, 2.1, H-C(3)), 3.60-3.40 (*m*, Et), 1.40-1.20 (m, Et).

ii) Formylation of the carbamate. A soln. of *sec*-BuLi (6.75 ml of a 0.13 M soln. in cyclohexane, 1.3 mmol) was added under N_2 to a stirred mixture of TMEDA (0.783 g, 6.7 mmol) in dry THF at –78º. After 5 min. a soln. of the carbamate **11a** (0.634 g, 2.25 mmol) in dry THF (6 ml) was added. After 60 min. DMF (0.7 ml) was added, the mixture was left for 2 h while it reached room temperature and then it was left stirring for 48 h. During this period the solution changed from yellow to brown. A saturated soln. of ammonium chloride (75 ml) was added and the mixture was extracted with chloroform (3 x 20 ml). The combined org. extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure, giving a mixture of compounds as a brown oil. This mixture was

submitted to column chromatography (silica, ethyl EtOAc/light petroleum mixtures of increasing polarity). The main product isolated was the 2-hydroxycarbazole-3-carbaldehyde **11c** as an off-white solid (150 mg, 32%). M.p.: 232-233º (decomposes above 200º) (lit. [12c] 240º (EtOH)). IR (Nujol): 3371 (NH), 1704, 1644 (strong, C=O), 1326, 1250, 1205, 1167, 1015, 896, 871, 855, 822, 772, 721.^{1}H-NMR: 11.51 (*s*, OH), 10.77 (*br s*, N*H*), 10.04 (*s*, CHO), 8.51 (*s*, H-C(4)), 8.13 (*br d*, *J* = 7.5, H-C(5)), 7.54 (*br d*, *J* = 8.1, H-C(8)), 7.43 (*ddd*, *J* = 8.1, 7.2, 1.2, H-C(7)), 7.27 (*ddd*, *J* = 8.1, 7.2, 0.9, H-C(6)), 6.94 (*s*, H-C(1)).

iii) Formation of the pyranone ring

Ethyl 3-oxopyran[3,2-a]carbazole-2-carboxylate **(2)** was obtained, as described above, from 1-formyl-2-hydroxycarbazole **11b** (together with starting material, 17%) as a yellow solid after recrystallisation from $CHCl_3$ /cyclohexane (24 mg, 68%). M.p.: 211-212º (decomp.). UV (log ε, EtOH): 394 (4.39), 320 (4.40), 286 (4.40). IR (Nujol): 3341 (NH), 1746 (strong, C=O), 1614, 1345, 1330, 1257, 1217, 1193, 1117, 1089, 1034, 798, 776, 739, 666. ^{1}H-NMR (acetone-d_6): 11.49 (*br s*, N*H*), 9.25 (*br s*, H-(1)), 8.50 (*br d*, *J* = 8.7, H-C(6)), 8.18 (*br d*, *J* = 8.1, H-C(7)), 7.64 (*br d*, *J* = 8.1, H-C(10)), 7.48 (*ddd*, *J* = 8.1, 7.2, 1.2, H-C(9)), 7.32 (*ddd*, *J* = 7.4, 7.2, 0.9, H-C(8)), 7.22 (*br d*, *J* = 8.7, H-C(5)), 4.40 (*q*, *J* = 6.9, Et), 1.41 (*t*, *J* = 7.2, Et). ^{13}C-NMR (acetone-d_6): 164.00 (*C*O_2Et), 156.88 (C=O), 155.55 (C(4a)), 144.73 (C(1)), 140.97 (C(10a)), 137.88 (C(11a)), 127.53 (C(6)), 126.63 (C(9)), 123.51 (C(7a)), 121.22 (C(8)), 120.77 (C(7)), 120.21 (C(6a)), 116.57 (C(2)), 112.26 (C(10)), 108.27 (C(5)), 104.39 (C(11b)), 61.89 (CH_2),

14.56 (CH_3). MS-EI: 308 (20, $[M+1]^+$), 307 (100, M^+), 262 (19), 235 (33), 207 (11), 178 (17). HR-EI-MS: 307.0840 (M^+, $C_{18}H_{13}NO_4$; calc. 307.0845).

Ethyl 3-oxopyran[2,3-b]carbazole-2-carboxylate **(3)**. Compound **3** was obtained, as described above, from 3-formyl-2-hydroxycarbazole **11c**, together with starting material (27%), as a yellow solid (31 mg, 20%). M.p.: 335-336º (decomp.). UV (log ε, EtOH): 370 (4.49), 305 (4.89), 275 (5.08). IR (Nujol): 3271 (NH), 1765 (C=O), 1746 (C=O), 1636, 1596, 1569, 1276, 1241, 1200, 1044, 1016, 795, 725, 665. ^{1}H-NMR (DMSO-d_6): 11.91 (*br s*, N*H*), 8.88 (*s*, H-C(1)), 8.67 (*s*, H-C(11)), 8.13 (*br d*, *J* = 7.8, H-C(10)), 7.55 (*br d*, *J* = 7.8, H-C(7)), 7.46 (*br t*, *J* = 8.1, H-C(8)), 7.41 (*s*, H-C(5)), 7.27 (*br t*, *J* = 7.5, H-C(9)), 4.28 (*q*, *J* = 7.2, Et), (*t*, *J* = 6.9, Et). ^{13}C-NMR (DMSO-d_6): 163.11 (*C*O_2Et), 156.74 (*C*=O), 153.75 (C(4a)), 150.59 (C(1)), 144.04 (C(5a)), 141.01 (C(6a)), 126.89 (C(7)), 122.76 (C(10)), 121.91 (C(10a)), 121.16 (C(10b)), 120.60 (C(10)), 120.32 (C(9)), 112.08 (C(2)), 111.59 (C(7)), 110.73 (C(11a)), 96.83 (C(5)), 60.88 (*C*H_2), 14.20 (*C*H_3). MS-EI: 308 (20, $[M+1]^+$), 307 (100, M^+), 262 (45), 235 (41), 207 (25), 178 (25), 89 (12), 69 (51). HR-EI-MS: 307.0848 (M^+, $C_{18}H_{13}O_4N$; calc. 307.0845).

2. Tumour cell growth assay: The effects of compounds on the growth of tumour cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) for *in vitro* anticancer drug screening using the protein-binding dye sulforhodamine B (SRB) to assess growth inhibition [20, 21]. Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium supplemented with 5% heat-inactivated fetal bovine serum, 2 mM glutamine and 50 μg/mL of gentamicin at 37º in an humidified atmosphere

containing 5% CO_2. The optimal plating density of each cell line, that ensure exponential growth throughout all the experimental period, was the same as originally published [20] and was respectively $7.5x10^4$ cells/mL to NCI-H460, $1.5x10^5$ cells/mL to MCF-7 and SF-268. Cells were exposed for 48 h to five concentrations of compounds starting from a maximum concentration of 150 μM. Compounds, prepared in DMSO, were freshly diluted with cell culture medium immediately prior the assays. Final concentrations of DMSO (≤ 0.25%) did not interfere with the cell lines growth. For each test compound and for each cell line a dose-response curve was generated and the growth inhibition of 50% (GI50), corresponding to the concentration of compound that inhibits 50% of the net cell growth was determined as described [20]. Doxorubicin, used as a positive control, was tested in the same manner.

3. Theoretical calculations: The structure of the isolated molecules was initially optimized using the semi-empirical method PM3 [22]. Subsequentely, these structures were refined using the Density Functional Theory (DFT) [23]. The B3LYP hybrid functional and 6-31G* basis sets was used.

In the DFT optimizations, the Berny gradient was used. The requested convergence limit on RMS density matrix was 1×10^{-8} and the threshold values for the maximum force and the maximum displacement were 0.000450 and 0.001800 a.u. respectively.

The visualization of the structures was performed with GaussView 3 [24]. The molecular volumes were calculated using the facilities of Hyperchem 5.11 [25].

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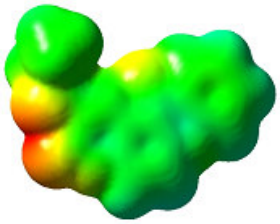
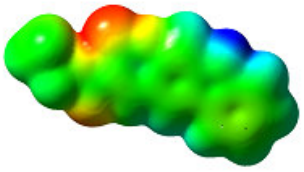
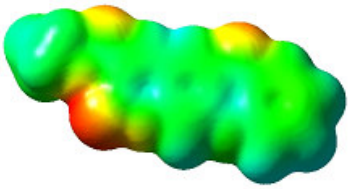
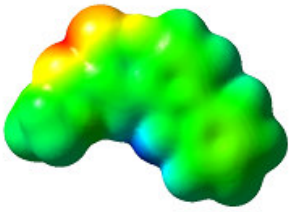
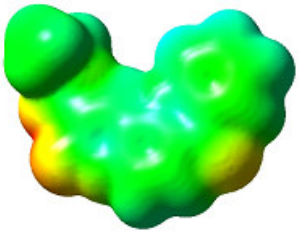
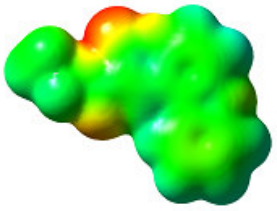
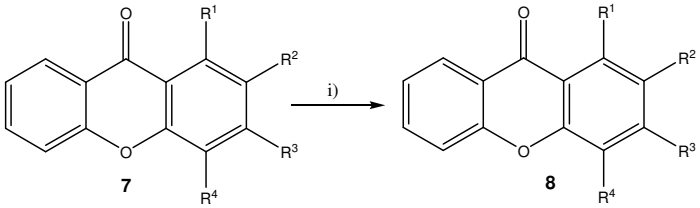
Table 1. Effect of compounds on the growth of human tumour cell lines

	GI_{50} (μM)		
Compounds	**MCF-7**	**SF-268**	**NCI-H460**
1	13.9±1.2	20.4±1.1	17.6±1.4 [**5**]
4	48.2 ± 3.4	≥ 150	≥ 150
5	138.8 ± 4.8	142.7 ± 5.9	145.7 ± 2.9
6	> 150	> 150	> 150
2	11.6 ± 0.5	35.9 ± 3.9	20.6 ± 1.5
3	> 150	> 150	> 150

Results are mean ± SEM of 1-6 independent experiments performed in duplicate. Doxorubicin was used as positive control: GI50 (MCF-7) = 42.8 ± 8.2 nM; GI50 (SF-268) = 93.0 ± 7.0 nM; GI50 (NCI-H460) = 94.0 ± 8.7 nM.

Table 2. Representation of the molecular electrostatic potential (MEP) superimposed onto total electron density (TED)

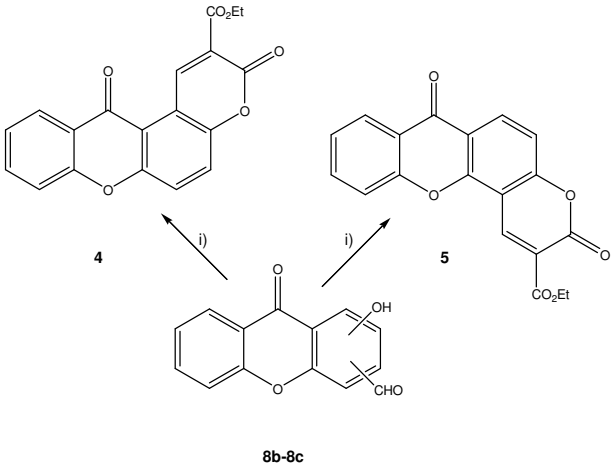
Compound	ED/PES	Molecular volume, Å^3	Compound	ED/PES	Molecular volume, Å^3
4		884.46	**3**		856.46
6		892.29	**2**		853.24
5		885.41	**1**		838.44



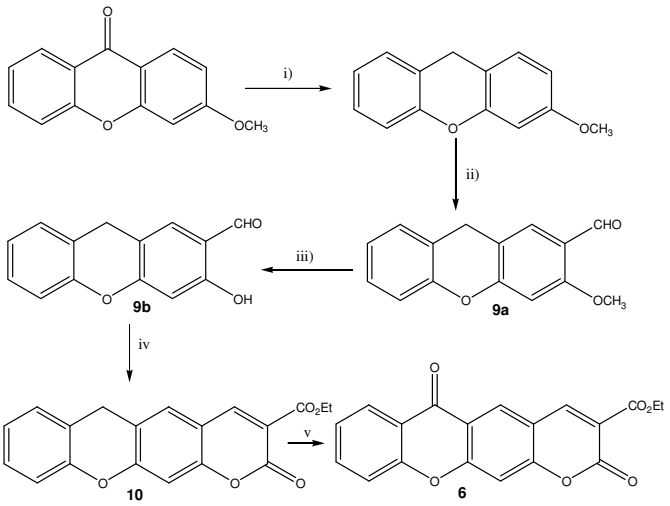
7a R^1=OH, R^2=H, R^3=R^4= H
7b R^1=H, R^2=OH R^3=R^4= H
7c R^1= R^2=H, R^3=OH, R^4=H
7d R^1= R^2=H, R^3=H, R^4= OH

8a R^1=OH, R^2=CHO, R^3=R^4= H
8b R^1=CHO, R^2=OH R^3=R^4= H
8c R^1= R^2=H, R^3=OH, R^4=CHO
8d R^1= R^2=H, R^3=CHO, R^4= OH

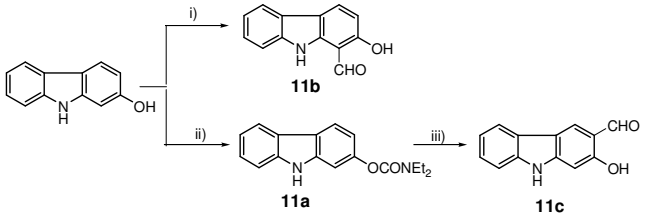
Scheme 1 Duff formylation of hydroxyxanthen-9-ones **7**. Reaction conditions: i) HMTA/ CH_3CO_2H/ reflux.



Scheme 2 Formation of the pyranone ring. i) $CH_2(CO_2Et)_2$, EtOH, piperidine, AcOH, reflux.



Scheme 3 Synthesis of the 2-formyl-3-hydroxyxanthene **9b** and pyranoxanthen-9-one **6**. Reaction conditions: i) 1. $LiAlH_4$, Et_2O, 1 h reflux. 2. NH_4Cl. ii) $POCl_3$, N-methyl formanilide, CH_2Cl_2, reflux, 9 h. iii) 1. BBr_3, CH_2Cl_2. 2. H_2O. iv) $CH_2(CO_2Et)_2$ /EtOH, 3 hrs. v) CrO_3/pyridine, 2.5 hrs.



Scheme 4 i) NaOH/$CHCl_3$. ii) $ClCONEt_2$/pyridine. iii) 1. *sec*-BuLi, TMEDA, THF, -78°C; 2. DMF, -78 ºC-25 ºC.

Figure 1 and 2 in separated files

Figure 1 Charge distribution for compounds **1** and **2**

Figure 2 Charge distribution for compounds **4** and **5**

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