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Synthesis of novel 2,3-dihydro-1,4-dioxino[2,3-*g*]quinoline derivatives as potential antitumor agents

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Abstract—New dioxinoquinolines (**1**–**8**) have been synthesized and their antiproliferative properties have been tested against several cell lines. The treatment of the 6-acetamido-2,3-dihydro-1,4-benzodioxine (**10**) with phosphorous oxychloride in the presence of DMF leads to a mixture of linear and angular tricyclic compounds. The key intermediates were modified and cyclized giving the corresponding dioxinoquinolines. In general, these compounds have a moderate citotoxycity.

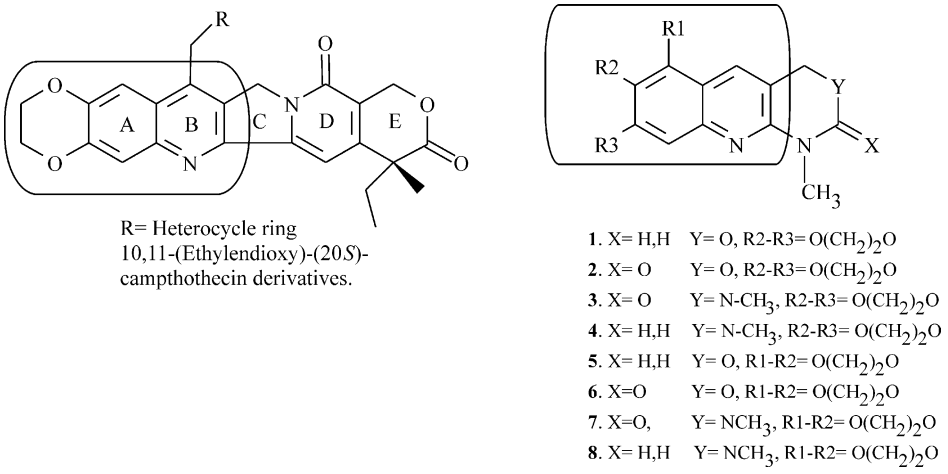

Figure 1. Analogue compounds of camptothecin.

1. Introduction

Several polycyclic analogues of natural or semi-synthetic antitumor agents are well known, and have attracted considerable interest because of their significant anticancer activity, due to the inhibition of topoisomerase I or topoisomerase II, or to intercalation on DNA.[1–5] Structure–activity studies on antitumor compounds related to 1,4-benzodioxine derivatives show that fused 1,4-dioxine systems retained significant activity.[6]

Given their great biological properties, compounds containing the quinoline system have been the subject of many synthetic studies. In a general program, we synthesize antitumor agents related to natural products (camptothecin, ellipticine and podophyllotoxin, among others). Our aim is to develop series of synthetic molecules which are more accessible and more amenable to optimisation through analogue synthesis. This paper describes the preparation of quinolines fused with a 1,4-dioxane group at positions 5–6 or 6–7 of the quinoline ring (Fig. 1). The new linear or angular tetracyclic compounds can be considered simplified camptothecin analogues having certain modifications in the lactone ring, and suppression of *C*, and *D* rings. These struc-

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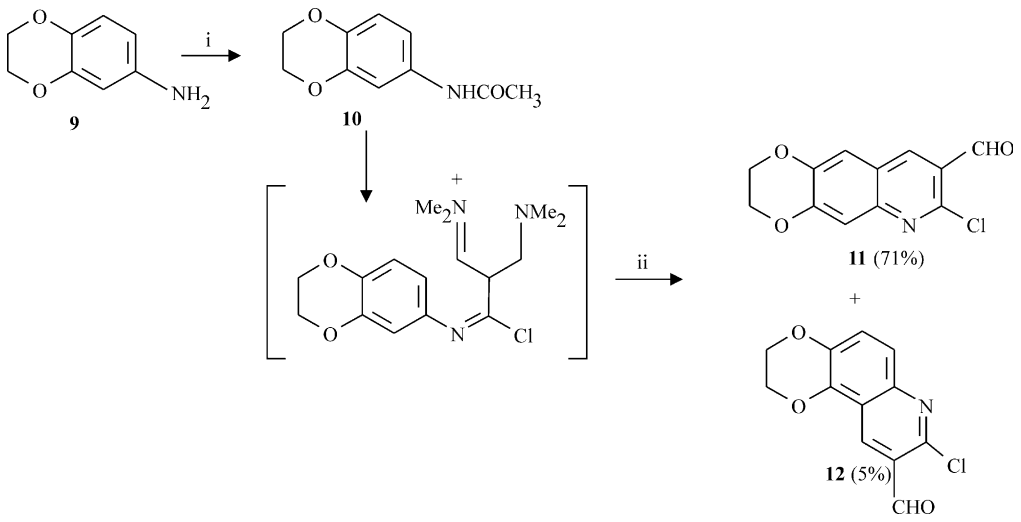


Scheme 1. Reagents and conditions: i $(CH_3CO)_2O$, 140 °C; ii $POCl_3$/DMF, 90 °C.

tures were designed by pharmacomodulation of camptothecin in order to simplify the molecule and produce an amelioration of its properties, thus the results may provide key data about the critical structure necessary for the activity of related agents. These compounds are weakly basic and possess, or not, an electrophilic centre on the ring *D*. Camptothecin inhibits topoisomerase I by stabilizing a covalent enzyme–DNA complex,[7] but does not affect isolated enzyme or isolated DNA, suggesting that a drug binding site is formed when the complex topisomerase I-DNA is formed.[8] Several studies on camptothecin derivatives show that the lactone ring, as an electrophilic group, is the critical region for the antitumor activity.[9] The clinical application of camptothecin in the treatment of cancer has been limited by severe toxic side effects during the administration of the drug, and by solubility problems.[10]

2. Chemistry

New potential anticancer agents (**1**–**8**) were synthesised from aniline **9** by a straightforward high-yield process (Scheme 1). Acylation of the 6-amino-2,3-dihydro-1,4-benzodioxine with acetic anhydride in the presence of triethylamine afforded the corresponding acetamide **10**. The key intermediates **11** and **12** were obtained from **10** by bis-formylation at the side chain under Vilsmeier conditions.[11–15]

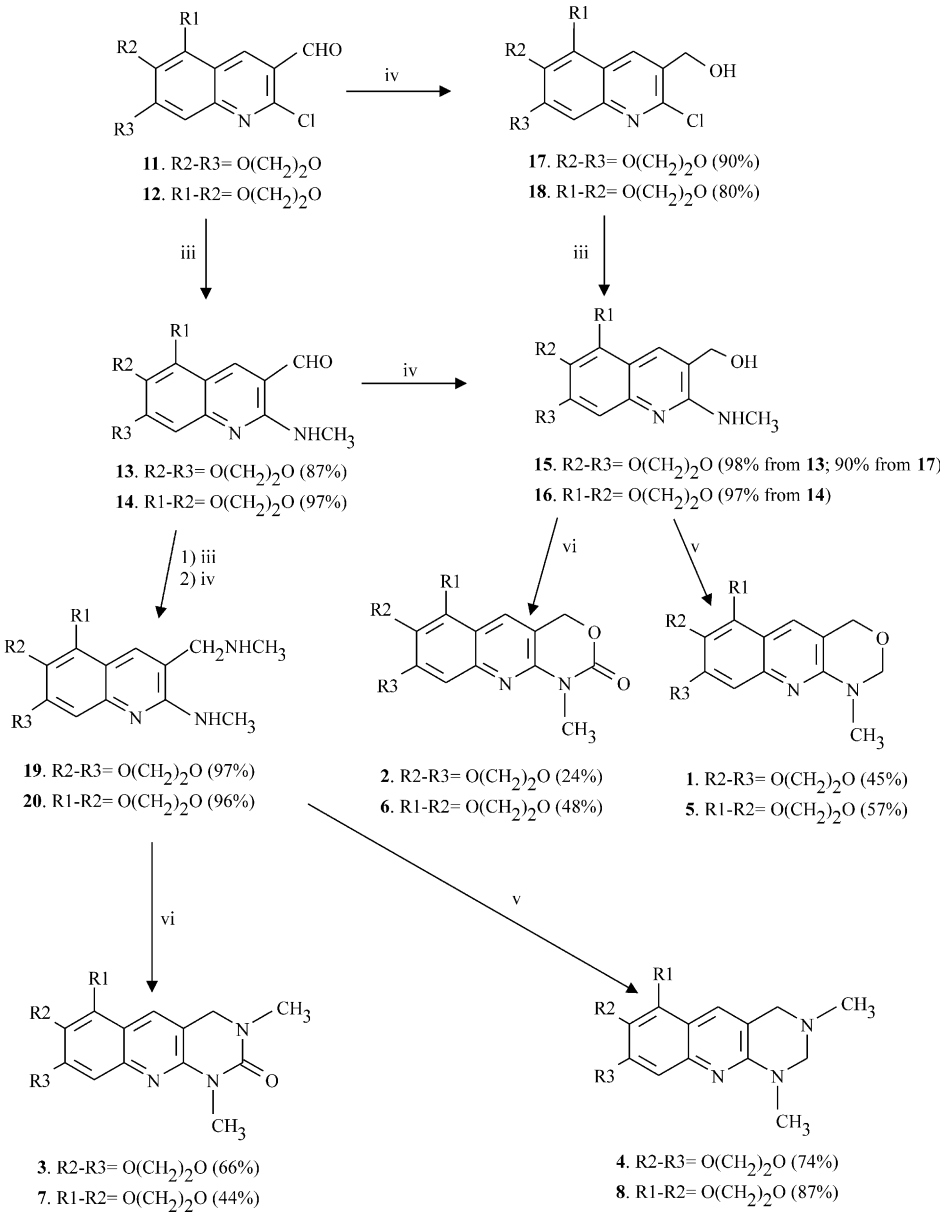
The dialdehyde obtained by heating the acetamide **10** with phosphorous oxychloride in DMF at 80–90 °C, was quickly cyclized in situ to give **11** in good yield (71%). In this case, two cyclization pathways were possible; linear (**11**) and angular (**12**) tricyclic compounds were obtained. The best conditions of the Vilsmeier reaction were achieved in an one-pot procedure and provided compound **11** in 71% yield, whereas the phenanthrene analogue **12** was obtained in only 5%, which was attributed to steric effects. The structures of **11,** and **12** were confirmed by ^1H and ^{13}C NMR. The structural assignment of **12** was confirmed by the appearance of two aromatic double doublets ($J = 9.2$ and $J = 0.5$ Hz) at 7.38 and 7.49 ppm (attributed to H-5 and H-6). In contrast, the H-9, and H-10 of compound **11** appeared as two doublets ($J = 0.5$ Hz).

To improve the yields and scope of the synthesis of these tricyclic compounds, we tested a variety of conditions for the $POCl_3$–DMF reactions and found that regioselectivity is governed by temperature and addition of the $POCl_3$ reagent. A temperature higher than 90 °C favoured the formation of the angular isomer **12**. In our experience, the linear isomer **11** is most favoured when the $POCl_3$ was added slowly and the temperature did not exceed 90 °C.

Transformation of **11** and **12** to the aminoalcohols **15** or **16**, respectively, was attempted in two ways: reduction of the aldehyde group followed by 2-chloro substitution, or firstly substitution followed by reduction of the aminocarbaldehyde intermediate; the last strategy is preferable. Substitution of the 2-chloro group by methylamine gave 87% (compound **13**) and 97% yield (compound **14**), whereas the same substitution of the corresponding chloro-alcohols gave the desired compounds after long reaction times (14 days). This result is understandable; since the aldehyde group is more activating than the hydroxy-methyl group to nucleophilic substitution of the adjacent chlorine, moreover alcohol **15** has solubility troubles which decreased the yield of reaction.

Finally, we examined the cyclization of the secondary amines to tetracyclic systems. Acylation of aminoalcohols **15**–**16** with dimethyl carbonate and butyl lithium afforded the carbamate **2** in low yield (24%), and the carbamate **6** (48%) respectively, while cyclization using formaldehyde[16] gave amino-ethers **1** in 45%, and **5** in 57% yield, respectively (Scheme 2). The analogous compounds **4** and **8** were obtained from the corresponding diamines **19**–**20**, using the same formaldehyde methodology.

The low yields obtained in the preparation of compounds **1, 2, 5,** and **6**, is possible due to competing reaction of the nitrogen atom of the pyridine ring.



Scheme 2. Reagents and conditions: iii CH_3NH_2 (40%)/MeOH, 90 °C. iv. $NaBH_4$/MeOH. v. HCHO/APTS/toluene reflux. vi. NaOMe/$(CH_3O)_2CO$, 100 °C.

The oxopyrimidoquinolines **3** and **7** were synthesized in acceptable yields by reacting the corresponding diaminocompounds **19** or **20** with dimethyl carbonate in the presence of butyl lithium.

The purity of the products was checked by TLC, IR, ^1H NMR, ^{13}C NMR. All new compounds gave satisfactory analysis and spectral data.

Further studies on the chemical behaviour and on the biological activities of these derivatives are in progress.

Table 1. In vitro anticancer activity of the compounds **1**–**8** (expressed as mean log GI_{50})

Cell line	Compounds							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Leukemia (RPMI-8226)	−5.65	−4.00	−4.00	−4.00	−4.21	−6.02	−4.00	−4.48
Non small cell lung cancer (EKVX)	−4.41	−5.87	−4.25	−4.56	−4.00	−5.21	−4.12	−4.00
Cancer colon (KM12)	−4.36	−4.23	−4.12	−4.67	−4.00	−5.43	−4.05	−4.45
CNS cancer (SF-268)	−4.87	−4.57	−5.23	−4.00	−4.00	−4.65	−4.00	−4.00
Renal cancer (A498)	−4.00	−4.73	−4.00	−4.00	−4.00	−4.32	−4.00	−5.43
Breast cancer (HS-578T)	−7.95	−5.78	−4.32	−5.22	−5.55	−4.67	−4.56	−4.00

3. Pharmacological results

These compounds were tested in vitro by the National Cancer Institute, and were evaluated against 60 human tumor cell lines derived from eight cancer types (leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer and renal cancer).[17,18] Representative results are summarized in Table 1. Results are presented as log GI_{50}, where GI_{50} represents the molar concentration required to inhibit cell growth by 50%.

In general, most of the compounds have only moderate activity (all compounds showed 50% growth inhibition at 10^{-4} M) (Table 1). With a few exceptions, all compounds have similar activity across the cell lines; there is little effect of linear and angular systems, or in the nature of the 4th ring. Moreover, compound **1**, the most active, showed selectivity to breast cancer (HS578T) ($GI_{50} = 1.10^{-8}$ M), and leukemia. Compound **2** has selectivity against non-small cell lung cancer ($GI_{50} = 10^{-6}$), and breast cancer lines.

In conclusion, our results demonstrate that the tetracyclic compounds incorporating a 1,4-benzodioxine group show reduced antitumor activity compared to camptothecin. Studies aimed at increasing the antitumor activity of other tetracyclic systems are currently in progress.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a Varian Gemini-300 or 500-spectrometer using tetramethylsilane as internal standard and $CDCl_3$ as solvent, chemical shifts are given in ppm and coupling constants are expressed in Hz. ^{13}C NMR spectra were recorded on a Varian Gemini 75.5 or 50.3 MHz spectrometer. Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a FTIR Perkin Elmer 1600 spectrophotometer. Mass spectra were recorded on a Hewlett-Packard spectrometer 5988-A (70 eV). Chromatography was carried out on SiO_2 (silica gel 60, SDS, 60–200 μm). Microanalyses were determined on a Carlo Erba 1106 Analyser at Serveis Cientifico-Tècnics, Universitat de Barcelona and all the new compounds have given C, H, N analyses within ±0.4% of the theoretical values. All reagents were of commercial quality or purified before use and the organic solvents were of analytical grade or purified by standard procedures.

4.1.1. 1 - Methyl - 1,2,8,9 - tetrahydro[1,4]dioxino[2,3-*g*]-[1,3]oxazino[4,5-*b*]quinoline (1). A suspension of the aminoalcohol **15** (190 mg, 0.77 mmol), a catalytic amount of PTSA, enough amount of molecular sieves 4 Å and paraformaldehyde (212 mg, 2.35 mmol) in anhydrous toluene (25 mL) was stirred at 110 °C for 24 h. Then, the cooled mixture was filtered and the filtrate was washed with a saturated solution of $NaHCO_3$. The resulting organic layer was dried, filtered and concentrated affording the desired product which was purified by crystallization from hexane/EtOAc. The oxazinoquinoline **1** was obtained as a white solid (90 mg, 45% yield): Mp: 140–142 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1070 (C-*O*-C, st.); 1230 (ArC-*O*-C, st.). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.16 (s, 3H, CH_3N); 4.29 (cs, 4H, $\mathbf{OCH_2CH_2O}$); 4.87 (s, 2H, H-2); 4.90 (d, $J = 1.2$ Hz, 2H, H-4); 6.95 (s, 1H, H-6); 7.18 (s, 1H, H-11); 7.26 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 32.4 (CH_3, CH_3N); 64.2 and 64.5 (CH_2, $\mathbf{OCH_2CH_2O}$); 68.6 (CH_2, C4); 81.3 (CH_2, C2); 111.7 (CH, C11); 111.9 (CH, C6), 116.4 (C, C5a); 118.5 (C, C4a); 129.1 (CH, C5); 140.7 (C, C6a); 143.4 (C, C11a); 146.2 (C, C10a); 152.1 (C, C12a). MS (EI) (*m*/*z*) 57 (26%); 201 (40%); 228 (45%, $C_{13}H_{12}N_2O_2^+$); 244 (1%, $C_{13}H_{12}N_2O_3^+$); 258 (100%, $C_{14}H_{14}N_2O_3^+$). Anal. calcd for ($C_{14}H_{14}N_2O_3$): C, 65.11%; H%, 5.46%; N, 10.85%. Found: C, 64.89%; H%, 5.67%; N, 10.75%.

4.1.2. 8 - Methyl - 2,3,8,9 - tetrahydro[1,4]dioxino[2,3-*f*]-[1,3]oxazino[4,5-*b*]quinoline (5). The compound **5** was prepared following the same procedure described above for **1**, starting from the aminoalcohol **16** (70 mg, 0.286 mmol). This methodology afforded **5** (42 mg, 0.162 mmol) as a white solid in 57% yield. Mp: 138–140 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1066 (C-*O*-C, st.); 1242 (ArC-*O*-C, st.). ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.17 (s, 3H, CH_3N); 4.33 (cs, 4H, $\mathbf{OCH_2CH_2O}$); 4.89 (s, 2H, H-9); 4.96 (s, 2H, H-11); 7.09 (d, $J = 9.2$ Hz, 1H, H-5); 7.24 (d, $J = 9.2$ Hz, 1H, H-6); 7.71 (s, 1H, H-12). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 32.4 (CH_3, CH_3N); 64.2 and 64.6 (CH_2, $\mathbf{OCH_2CH_2O}$); 68.7 (CH_2, C4); 81.3 (CH_2, C2); 114.6 (C, C12a); 117.4 (C, C11a), 118.9 (CH, C6); 120.4 (C, C4a); 123.7 (CH, C12); 136.5 (C, C4a); 136.6 (C, C12b); 142.8 (C, C6a); 151.8 (C, C7a). Anal. calcd for ($C_{14}H_{14}N_2O_3$): C, 65.11%; H%, 5.46%; N, 10.85%. Found: C, 64.96%; H%, 5.81%; N, 10.61%.

4.1.3. 1-Methyl-2-oxo-1,2,8,9-tetrahydro[1,4]dioxino[2,3-*g*][1,3]oxazino[4,5-*b*] quinoline (2). A suspension of the aminoalcohol **15** (63 mg, 0.255 mmol) and sodium methoxide (35 mg, 0.64 mmol) in 5 mL of dimethyl carbonate was heated at 100 °C for 24 h. Then, the solvent was removed under reduced pressure. The solid obtained was dissolved in CH_2Cl_2 and washed several times with water. The washed organic layer was dried, filtered and concentrated affording a crude mixture which was purified by silica gel column chromatography. When a mixture of hexane/EtOAc, 70/30 was used as eluent, a white solid was obtained (17 mg, 24% yield) which was identified as the oxazinoquinoline **2**: Mp: 232–233 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1064 (C-*O*-C); 1244 (ArC-*O*-C); 1730 (CO, st.). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.51 (s, 3H, CH_3N); 4.29 (cs, 4H, $\mathbf{OCH_2CH_2O}$); 5.19 (d, $J = 1.0$ Hz, 2H, $\mathbf{CH_2}$OCO); 7.07 (s, 1H, H-6); 7.31 (s, 1H, H-11); 7.58 (d, $J = 0.5$ Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 30.2 (CH_3, CH_3N); 64.3 and 64.4 (CH_2, $\mathbf{OCH_2CH_2O}$); 66.1 (CH_2, $\mathbf{CH_2}$OCO); 112.0 (CH, C6); 113.4 (CH, C11); 113.8 (C, C4a); 120.6 (C, C5a); 130.4 (CH, C5); 142.9 (C, C11a); 143.3 (C, C6a); 147.3

(C, C10a); 147.8 (C, C12a); 153.3 (C; C2). MS (EI) (*m*/*z*) 63 (60%), 83 (100%), 85 (65%), 201 (38%), 227 (33%, $C_{13}H_{12}N_2O_2^+$), 272 (34%, $C_{14}H_{12}N_2O_4$). Anal. calcd for ($C_{14}H_{12}N_2O_4$): C, 61.76%; H, 4.44%; N, 10.29%. Found: C, 61.55%; H, 4.68%; N, 10.09%.

4.1.4. 8-Methyl-9-oxo-2,3,8,9-tetrahydro[1,4]dioxino[2,3-*f*][1,3]oxazino[4,5-*b*] quinoline (6). Following the same procedure described above, and starting from the aminoalcohol **16** (85 mg, 0.348 mmol) the compound **6** (45 mg, 0.4 mmol) was obtained as a white solid in 48% yield. Mp: 214–215 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1095 (C-*O*-C); 1288 (ArC-*O*-C); 1698 (CO, st.). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.59 (s, 3H, CH_3N); 4.40 (cs, 4H, O**CH_2CH_2**O); 5.32 (d, $J=1.1$ Hz, 2H, **CH_2**OCO); 7.27 (d, $J=9$ Hz, 1H, H-5); 7.46 (dd, $J=9.0$, $J=0.8$ Hz, 1H, H-6); 8.09 (d, $J=0.8$ Hz, 1H, H-12). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 30.0 (CH_3, CH_3N); 64.5 and 64.6 (CH_2, O**CH_2CH_2**O); 66.2 (CH_2, **CH_2**OCO); 114.8 (C, C11a); 117.0 (C, C12a); 120.8 (C, C6); 122.0 (CH, C5); 125.2 (CH, C12); 136.5 (C, C12b); 138.8 (C, C4a); 142.3 (C, C6a); 147.5 (C, C7a); 152.3 (C; C9). Anal. calcd for ($C_{14}H_{12}N_2O_4$): C, 61.76%; H, 4.44%; N, 10.29%. Found: C, 61.60%; H, 4.68%; N, 9.98%.

4.2. General procedure for the preparation of 2-oxopyrimidoquinolines (3 and 7)

A solution of the corresponding amine (1 mmol) in dry THF (6 mL) was cooled to 0 °C under argon atmosphere. Then a solution of butyllithium (1.2 mmol) in hexane (1.6 M) was dropwise added. The mixture was stirred at the same temperature for 30 min, and then at room temperature for another 2 h. After a solution of dimethyl carbonate (1.2 mmol) was added, the resulting mixture was stirred at room temperature for 12 h. A solution of saturated NH_4Cl was added, and the reaction mixture was extracted with ether (3×20 mL), dried over Na_2SO_4, filtered off, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with hexane/ethyl acetate or by crystallisation.

4.2.1. 1,3-Dimethyl-2-oxo-1,2,3,4,8,9-hexahydro-[1,4]dioxino[2,3-*g*]pyrimido[4,5-*b*] quinoline (3). Starting from the amine (97 mg, 0.374 mmol) in THF (6 mL), and following the general procedure described above the title compound was obtained as a white solid (70 mg, 0.245 mmol) in a 66% yield. Mp: 220–222 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1062 (C-*O*-C, st); 1247 (ArC-*O*-C, st); 1662 (CO, st). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.07 (s, 3H, CH_3-N); 3.53 (s, 3H, CH_3N); 4.35 (cs, 4H, CH_2–); 4.43 (d, $J=1$ Hz, 2H, C4-H_2); 7.07 (s, 1H, H-6); 7.33 (s, 1H, H-11); 7.55 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 29.0 (CH_3); 35.5 (CH_3); 48.9 (CH_2, C-4); 64.3 and 64.4 (CH_2, O**CH_2CH_2**O), 111.6 (CH, C-6); 113.0 (CH, C-11); 114.1 (C, C4a); 120.3 (C, C5a); 130.9 (CH, C5); 142.6 (C, C11a); 142.8 (C, C6a); 146.8 (C, C10a); 149.2 (C, C12a); 155.2 (C-2). Anal. calcd for ($C_{15}H_{15}N_3O_3$): C, 63.15%; H, 5.30%; N, 14.73%. Found: C, 62.79%; H, 5.57%; N, 14.58%.

4.2.2. 4.4.2. 8,10 - Dimethyl - 9 - oxo - 2,3,8,9,10,11-hexahydro-[1,4]dioxino[2,3-*f*]pyrimido[4,5-*b*] quinoline (7). Starting from the amine (76 mg, 0.293 mmol), and following the general procedure described before the title compound was obtained as a white solid (37 mg, 0.129 mmol) in a 44% yield. Mp: 232–234 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1064 (C-*O*-C, st); 1250 (ArC-*O*-C, st); 1670 (CO, st). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.07 (s, 3H, CH_3-N); 3.53 (s, 3H, CH_3N); 4.37 (cs, 4H, CH_2-); 4.46 (d, $J=1.3$ Hz, 2H, C11-H_2); 7.20 (d, $J=9.1$ Hz, 1H, H-5); 7.39 (dd, $J=9.1$ Hz, $J=0.8$ Hz, 1H, H-6); 7.96 (d, $J=0.8$ Hz, 1H, H-12). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 28.9 (CH_3); 35.5 (CH_3); 49.1 (CH_2, C-4); 64.3, and 64.6 (CH_2, O**CH_2CH_2**O), 115.1 (C, C-11a); 116.6 (C, C-12a); 120.5 (CH, C6); 121.3 (CH, C5); 125.6 (CH, C12); 136.2 (C, C12b); 138.1 (C, C4a); 142.2 (C, C6a); 148.9 (C, C7a); 155.1 (C, C9). Anal. calcd for ($C_{15}H_{15}N_3O_3$): C, 63.15%; H, 5.30%; N, 14.73%. Found: C, 62.82%; H, 5.64%; N, 14.51%.

4.3. General procedure for obtaining pyrimidoquinolines (4 and 8)

To a solution of the corresponding amine (1 mmol) in dry toluene (50 mL), a catalytic amount of the PTSA, molecular sieves 4 Å (200 mg), and paraformaldehyde (3 mmol) were added. The mixture was stirred at 110 °C for 24 h. After cooling, the mixture was made basic with saturated solution of $NaHCO_3$, and extracted with ether (2×30 mL). The organic layers were dried, the solvent was removed under vacuo to give the desired pyrimidoquinoline. The crude product was recrystallized from hexane/ethyl acetate mixtures.

4.3.1. 1,3 - Dimethyl - 1,2,3,4,8,9 - hexahydro - [1,4]dioxino[2,3-*g*]pyrimido[4,5-*b*]quinoline (4). Starting from the amine (111 mg, 0.428 mmol), and following the general procedure described before, the title compound was obtained as a white solid (85.9 mg, 0.316 mmol) in a 74% yield. Mp: 139–141 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1060 (C-*O*-C, st); 1242 (ArC-*O*-C, st). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.48 (s, 3H, CH_3-N); 3.20 (s, 3H, CH_3N); 3.90 (cs, 2H, CH_2–); 4.19 (s, 2H, C4-H_2); 4.29 (cs, 4H, CH_2); 6.94 (s, 1H, H-6); 7.25 (s, 1H, H-11); 7.30 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 34.1 (CH_3); 41.0 (CH_3); 55.5 (CH_2, C-4); 64.3 and 64.5 (CH_2, O**CH_2CH_2**O), 72.2 (CH_2); 111.2 (CH, C-11); 111.7 (CH, C-6); 115.7 (C, C5a); 118.5 (C, C4a); 131.7 (CH, C5); 140.4 (C, C10a); 146.1 (C, C6a); 146.1 (C, C10a); 152.2 (C-12a). Anal. calcd for ($C_{15}H_{17}N_3O_2.2H_2O$): C, 58.62%; H, 6.89%; N, 13.67%. Found: C, 58.87%; H, 6.57%; N, 13.74%.

4.3.2. 8,10 - Dimethyl - 2,3,8,9,10,11 - hexahydro-[1,4]dioxino[2,3-*f*]pyrimido[4,5-*b*] quinoline (8). Starting from the amine (79 mg, 0.304 mmol), and following the general procedure described before, the title compound was obtained as an oil (72 mg, 0.265 mmol) in a 87% yield. This compound was unstable, and only the ^1H NMR was realized.

^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.39 (s, 3H, CH_3-N); 3.10 (s, 3H, CH_3N); 3.86 (s, 2H, CH_2–); 4.24 (cs,

4H, CH_2); 6.98 (d, $J=9.1$ Hz,1H, H-5); 7.13 (d, $J=9.1$ Hz, 1H, H-6); 7.64 (s, 1H, H-12).

4.3.3. *N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)acetamide (10). A solution of **9** (2.61 g, 17.26 mmol) in 50 mL of acetic anhydride was stirred at 140 °C for 8 h. The reaction mixture was cooled at 0 °C, basified with cold 5 N NaOH and extracted with CH_2Cl_2 (3×30 mL). Removal of the solvent, and purification by silica gel column chromatography with 4:6 EtOAc/hexane gave 2.9 g (15.01 mmol) of **10** (87%) as a white solid. Mp: 129–130 °C (hexane, ethyl acetate). IR (KBr), (cm^{-1}): 1067 (C-*O*-C, st); 1258 (ArC-*O*-C, st); 1664 (C=O, st); 3312 (NH, st). ^1H RMN ($CDCl_3$, 300 MHz) (ppm): 2.14 (s, 3H, **CH_3**-CO); 4.25 (m, 4H, O**CH_2CH_2**O); 6.79 (d, $J=8.5$ Hz, 1H, H-8); 6.86 (dd, $J_1=8.5$ Hz, $J_2=2.3$ Hz, 1H, H-7); 7.08 (bs, 1H, NH); 7.12 (d, $J=2.3$ Hz, 1H, H-5). ^{13}C-RMN ($CDCl_3$-CD_3OD, 50.3 MHz) (ppm): 22.9 (CH_3, **C**H_3-CO); 63.8 and 63.9 (CH_2, O**CH_2CH_2**O); 109.4 (CH, C5); 113.3 (CH, C7); 116.5 (CH, C8); 131.5 (C, C6); 139.8 (C, C8a); 142.8 (C, C4a); 169.5 (C, **C**O). Anal. calcd for ($C_{14}H_{12}N_2O_4$): C, 62.17%, H%, 5.74%, N, 7.25%. Found: C, 61.98%; H, 5.91%; N, 7.12%.

4.3.4. 7-Chloro-2,3-dihydro[1,4]dioxino[2,3-*g*]quinoline-8-carbaldehyde (11) and 8-Chloro-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline-9-carbaldehyde (12). A solution of phosphorus oxychloride (4 mL, 43.5 mmol) and anhydrous DMF (1.4 mL, 18.02 mmol) was stirred under argon atmosphere at 0 °C for 10 min. Then, the acetamide 10 (1.13 g, 5.84 mmol) was slowly added and the reaction mixture was stirred at 90 °C for 12 h. After, the cooled mixture was poured into ice (20 mL). The suspension obtained was filtered and the solid was dried in vacuo (P_2O_5) to give a yellow solid that was purified by silica gel column chromatography. Compound **12** was obtained as a yellow solid (66 mg, 5% yield) when a mixture of hexane/EtOAc, 80/20 was used as eluent. Compound **11**, white solid (1.04 g, 71% yield) was obtained using a mixture of hexane/EtOAc in the ratio 70/30.

4.3.5. Compound (11). Mp: 227–229 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1061 (C-*O*-C, st.); 1250 (ArC-*O*-C, st.); 1685 (CO, st.). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 4.41 (cs, 4H, O**CH_2CH_2**O); 7.34 (s, 1H, H-5); 7.48 (d, $J=0.5$ Hz, 1H, H-10); 8.54 (d, $J=0.5$ Hz, 1H, H-9), 10.48 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 64.1 and 64.6 (CH_2, O**CH_2CH_2**O); 113.6 (CH, C5); 113.8 (CH, C10); 122.5 (C, C9a); 124.7 (C, C8); 138.5 (CH, C9); 145.4 (C, C10a); 146.4 (C, C5a); 148.7 (C, C7); 150.5 (C, C4a); 189.2 (CH, CHO). MS (EI) (*m*/*z*) 83 (100%); 192 (60%, $C_9H_3ClNO_2^+$); 193 (56%, $C_9H_4ClNO_2^+$); 220 (20%, $C_{11}H_7ClNO_2^+$); 249 (80%, $C_{12}H_7ClNO_2^+$). Anal. calcd for ($C_{12}H_8ClNO_3$): C, 57.73%; H, 3.23%; N, 5.61%. Found: C, 57.51%; H, 3.42%; N, 5.72%.

4.3.6. Compound (12). Mp: 187–189 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1083 (C-*O*-C, st.); 1256 (ArC-*O*-C, st.); 1681 (CO, st.). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 4.36 (cs, 4H, CH_2O); 7.38 (d, $J_1=9.2$, $J_2=0.5$ Hz, 1H, H-5); 7.49 (dd, $J_1=9.2$ Hz, $J_2=0.5$ Hz, 1H, H-6), 8.88 (s, 1H, H-10); 10.45 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 64.3 and 64.6 (CH_2, O**CH_2CH_2**O); 119.0 (C, C10a); 121.0 (CH, C6); 125.2 (C, C9); 125.9 (CH, C5); 133.8 (CH, C10); 137.8 (C, C10b); 140.3 (C, C4a); 144.8 (C, C6a); 148.0 (C, C8); 189.0 (C, CHO). MS (EI) (*m*/*z*) 83 (100%), 192 (59%, $C_9H_3ClNO_2^+$); 193 (58%, $C_9H_4ClNO_2^+$); 220 (1%, $C_{11}H_7ClNO_2^+$); 249 (59%, $C_{12}H_8ClO_3^+$). Anal. calcd for ($C_{12}H_8ClNO_3$) C, 57.73%; H, 3.23%; N, 5.61%. Found: C, 57.45%; H, 3.51%; N, 5.74%.

4.3.7. 7-Methylamino-2,3-dihydro[1,4]dioxino[2,3-*g*]quinoline-8-carbaldehyde (13). To a solution of the aldehyde **11** (179 mg, 0.712 mmol) in methanol, a solution of 50 mL of methylamine (40% in water) solution was added and the resulting mixture was stirred at 90 °C for 24 h. Then, the methanol was removed and the suspension obtained was acidified with HCl 1N. The resulting mixture was stirred at room temperature for 12 h, then it was basified with a solution of NaOH 5N and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried, filtered and the solvent removed. The crude product obtained was purified by silica gel column chromatography yielding **13** (151 mg, 87% yield) as an orange solid. Mp: 150–152 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1063 (C-*O*-C, st.); 1247 (ArC-*O*-C, st.); 1664 (CO, st.). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.05 (d, $J=4.9$ Hz, 3H, CH_3-NH); 4.26 (cs, 4H, O**CH_2CH_2**O); 6.98 (s, 1H, H-10); 7.05 (s, 1H, H-5); 7.81 (bs, 1H, NH); 7.91 (s, 1H, H-9); 9.77 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 27.5 (CH_3, CH_3NH); 64.0 and 64.7 (CH_2, O**CH_2CH_2**O); 111.4 (CH, C5); 113.6 (CH, C10); 116.1 (C, C8); 117.1 (C, C9a); 141.0 (C, C10a); 146.8 (CH, C9); 147.8 (C, C5a); 150.4 (C, C4a); 154.8 (C, C7); 192.6 (CH, CHO). MS (EI) (*m*/*z*) 70 (99%); 188 (100%); 215 (26%, $C_{12}H_{11}N_2O_2^+$); 244 (97%, $C_{13}H_{12}N_2O_3$). Anal. calcd for ($C_{13}H_{12}N_2O_3$): C, 63.93%; H, 4.95%; N, 11.47%. Found: C, 63.63%; H, 5.31%; N, 11.76%.

4.3.8. 8-Methylamino-2,3-dihydro[1,4]dioxino[2,3-*f*]quinoline-9-carbaldehyde (14). Following the same procedure described above for **13**, and starting from the aldehyde **12** (0.73 mmol) the desired aminoaldehyde **14** (172 mg, 0.704 mmol) was obtained as a yellow solid in 95% yield. Mp: 158–159 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1057 (C-*O*-C, st.); 1179 (ArC-*O*-C, st.); 1665 (CO, st.). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.01 (d, $J=4.9$ Hz, 3H, CH_3-NH); 4.24 (cs, 4H, O**CH_2-CH_2**O); 7.07 (d, $J=9.1$ Hz, 1H, H-5); 7.13 (d, $J=9.1$ Hz, 1H, H-6); 7.73 (bs, 1H, NH); 8.26 (s, 1H, H-10); 9.78 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ(ppm): 27.3 (CH_3, CH_3NH); 64.0 and 64.7 (CH_2, O**CH_2CH_2**O); 113.6 (CH, C9); 116.3 (C, C10a); 118.8 (CH, C6); 125.3 (CH, C5); 136.0 (C, C10b); 137.4 (C, C4a); 141.4 (CH, C10); 146.4 (C, C6a); 154.2 (C, C8); 192.6 (CH, CHO). MS (EI) (*m*/*z*) 70 (99%); 188 (100%); 215 (26%, $C_{12}H_{11}N_2O_2^+$); 244 (97%, $C_{13}H_{12}N_2O_3$). Anal. calcd for ($C_{13}H_{12}N_2O_3$): C, 63.93%; H, 4.95%; N, 11.47%. Found: C, 63.50%; H, 5.05%; N, 11.34%.

4.4. Preparation of alcohols (15–18). General procedure

A solution of the corresponding aldehyde (1 mmol) in methanol (10 mL) was cooled at 0 °C and $NaBH_4$ (4.5

mmol) was added. The solution obtained was stirred at 0 °C for 10 min and then it was stirred at room temperature for 25 min more. After, the solvent was removed under reduced pressure and 20 mL of water were added. The suspension obtained was extracted several times with CH_2Cl_2 and the combined organic layers were dried, filtered and concentrated to yielding the desired aminoalcohol. The obtained alcohol was used without further purification or by silica gel column chromatography.

4.4.1. 7-Methylamino-8-hydroxymethyl-2,3-dihydro[1,4]-dioxino[2,3-*g*]quinoline (15). Method A. Compound **15** was obtained as a yellow solid (173 mg, 98% yield) starting from a solution of the aminoaldehyde **13** (174 mg, 0.712 mmol) and $NaBH_4$ (124 mg, 3.27 mmol) in methanol (20 mL) and following the general procedure, described above.

Method B. A suspension of the alcohol **17** (400 mg, 1.6 mmol) and a catalytic amount of KI in 35 mL of methylamine (40% in water) was stirred at 90 °C for 14 days, adding 5 mL of methylamine and a catalytic amount of KI every 12 h. Then, the cooled mixture was extracted with CH_2Cl_2 (3×40 mL) and the combined organic layers were dried, filtered and concentrated under reduced pressure yielding **15** as a yellow solid (354 mg, 90% yield). This compound was used without further purification. Mp: 189–190 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1070 (C-*O*-C, st.); 1239 (ArC-*O*-C, st.); 3376 (–OH, st.). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.07 (d, $J=3.5$ Hz, 3H, **CH_3**NH); 4.30 (cs, 4H, CH_2O); 4.56 (s, 2H, CH_2OH); 5.66 (bs, 1H, OH); 6.90 (s, 1H, H-10); 7.19 (s, 1H, H-5); 7.20 (s, 1H, H-9). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 28.1 (CH_3, CH_3NH); 64.0 (CH_2, CH_2OH); 64.3 and 64.6 (CH_2, O**CH_2CH_2**O); 111.2 (CH, C5); 112.3 (CH, C10); 118.1 (C, C9a); 120.3 (C, C8); 133.9 (CH, C9); 140.4 (C, C10a); 143.7 (C, C5a); 146.5 (C, C4a); 156.1 (C, C7). Anal. calcd for ($C_{13}H_{14}N_2O_3$): C, 63.40%; H, 5.73%; N, 11.38%. Found: C, 63.11%; H, 5.71%; N, 11.25%.

4.4.2. 8-Methylamino-9-hydroxymethyl-2,3-dihydro[1,4]-dioxino[2,3-*f*]quinoline (16). Following the general procedure of reduction of aldehydes described before, and starting from the aminoaldehyde **14** (150 mg, 0.614 mmol) the aminoalcohol **16** was obtained (147 mg, 0.596 mmol) as a yellow solid in 97% yield. Mp: 183–184 °C (methanol). IR (KBr) (cm^{-1}): 1088 (C-*O*-C, st.); 1242 (ArC-*O*-C, st.); 3380 (–OH, st.). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.04 (s, 3H, **CH_3**NH); 4.27 (cs, 4H, CH_2O); 4.61 (d, $J=0.5$ Hz, 2H, CH_2OH); 5.77 (bs, 1H, OH); 7.04 (d, $J=9.0$ Hz, 1H, H-5); 7.21 (d, $J=9.0$, 1H, H-6); 7.73 (d, $J=1.1$ Hz, 1H, H-9). ^{13}C NMR ($CDCl_3$ + CD3OD, 75.5 MHz) δ (ppm): 27.8 (CH_3, CH_3NH); 63.1 (CH_2, CH_2OH); 64.1 and 64.5 (CH_2, O**CH_2CH_2**O); 114.1 (C, C10a); 117.6 (CH, C6); 120.3 (CH, C5); 121.8 (C, C9); 128.1 (CH, C10); 136.4 (C, C10b); 136.7 (C, C4a); 142.7 (C. C6a); 155.8 (C, C8). Anal. calcd for ($C_{13}H_{14}N_2O_3$): C, 63.40%; H, 5.73%; N, 11.38%. Found: C, 63.32%; H, 5.61%; N, 5.49%.

4.4.3. 7-Chloro - 8 - hydroxymethyl - 2,3 - dihydro[1,4]dioxino[2,3-*g*]quinoline (17). Compound **17** was obtained as a white solid (362 mg, 90% yield) starting from a mixture of the aldehyde **11** (400 mg, 1.6 mmol) and $NaBH_4$ (60 mg, 1.6 mmol) in methanol (20 mL) and following the general procedure. Mp: 207–209 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1075, (C-*O*-C, st); 1235 (ArC-*O*-C, st); 3280 (–OH, st). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 4.36 (cs, 4H, CH_2O); 4.60 (dd, $J_1=5.7$ Hz, $J_2=1.0$ Hz, 2H, CH_2OH); 5.56 (t, $J=5.7$ Hz, 1H, OH); 7.31 (s, 1H, H-5); 7.47 (s, 1H, H-10); 8.22 (s, 1H, H-9). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 64.0 (CH_2, CH_2OH); 64.3 and 64.5 (CH_2, O**CH_2CH_2**O), 112.0 (CH, C10); 112.3 (CH, C5); 123.3 (C, C9a); 131.7 (C, C8); 134.7 (CH, C9); 142.6 (C, C5a); 144.8 (C, C10a); 146.8 (C, C7a); 147.4 (C, C4a). Anal. calcd for ($C_{12}H_{10}ClNO_3$): C, 57.27%; H, 4.01%; N, 5.57%. Found: C, 57.58%; H, 3.85%; N, 5.51%.

4.4.4. 8 - Chloro - 9 - hydroxymethyl - 2,3 - dihydro[1,4]-dioxino[2,3-*f*]quinoline (18). Following the general procedure for the preparation of alcohol described before, the compound **18** was obtained as a white solid (16 mg, 80% yield) starting from a mixture of the aldehyde **12** (205 mg, 0.82 mmol) and $NaBH_4$ (33 mg, 0.89 mmol) in methanol (12 mL) and following the general procedure. Mp: 173–174 °C (hexane, ethyl acetate). IR (KBr) (cm^{-1}): 1071, (C-*O*-C, st); 1243 (ArC-*O*-C, st); 3273 (–OH, st). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 4.42 (cs, 4H, CH_2O); 4.65 (dd, $J_1=5.5$ Hz, $J_2=1.0$ Hz, 2H, CH_2OH); 5.66 (t, $J=5.5$ Hz, 1H, OH); 7.37 (d, $J=9.0$ Hz, 1H, H-5); 7.45 (dd, $J=9.0$ Hz, $J=0.5$ Hz, 1H, H-6); 8.44 (d, $J=0.5$ Hz, 1H, H-10). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 60.1 (CH_2, CH_2OH); 64.4 and 64.7 (CH_2, O**CH_2CH_2**O), 119.4 (CH, C10a); 120.5 (CH, C6); 122.4 (CH, C5); 128.5 (CH, C10); 133.4 (C, C9); 136.1 (C, C10b); 139.9 (C, C4a); 141.8 (C, C6a); 146.4 (C, C8). Anal. calcd for ($C_{12}H_{10}ClNO_3$): C, 57.27%; H, 4.01%; N, 5.57%. Found: C, 57.12%; H, 4.34%; N, 5.89%.

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