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Original article

Synthesis and biological evaluation of modified acridines: the effect of *N*- and *O*- substituent in the nitrogenated ring on antitumor activity

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Abstract

A series of new acridines has been prepared by cyclodehydration of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)anthranilic acid in acidic media following classical procedures. All these compounds have in common a dioxygenated ring fused to the acridine. The tetracyclic system possesses a linear or angular structure formed by intramolecular cyclisation. The last ring and the substituent of the system modify, in an interesting way, the antitumor activity of acridines. Several of the studied compounds displayed significant cytotoxic activity (inhibition of L1210 and HT-29 cell proliferation). The most cytotoxic compound **13a**, shows more activity than *m*-AMSA in inhibiting L1210 and HT-29 cell proliferation and this compound has been selected as a development candidate for further evaluation. The activity results also indicate that the new 11-*O*-substituted compounds are of considerable interest with high levels of cytotoxic activity. The angular or non-linear dioxinoacridine **10** was equiactive with the linear structure **7**. Pentacyclic analogues (**14** and **15**) were more cytotoxic than the tetracyclic compounds (up to twofold).

Keywords: Acridines; Antitumor agents; Cytotoxicity; Polycyclic systems

1. Introduction

Acridine derivatives are one of the more studied chemotherapeutic compounds, widely used as antimalarial [1], antiprotozoal [2], antibacterial [3] and antitumor agents [4].

Antitumor cytotoxic agents with DNA-intercalative properties have been studied. These compounds are characterized by the presence of a planar chromophore, generally a tri- or tetracyclic ring system and one or two flexible substituent groups. The acridine derivatives known as DNA-intercalators, such as *nitracrine* **1** [5], *m*-AMSA **2** [6], DACA **3** [7] and others DNA-intercalative agents as *proflavine* **4** [8] and *ellipticine* **5** [9] exhibit cytotoxic activity and some of them have been found to be clinically useful (Fig. 1). Amsacrine **2** is the best-known compound of 9-anilinoacridines series. It was one of the first DNA-intercalating agents to be considered as a topoisomerase II inhibitor. The significant clinical use of several of these compounds is limited by problems such as side effects, drug resistance and poor bioavailability, which have encouraged further modifications to these compounds. At present, almost all the reported antitumor agents in the acridine series have been derived from pattern compounds and they have incorporated changes in the substituents or heterocyclic system modifications.

The intercalation process is the strongest type of reversible binding to the double helical DNA in compounds with suffi-

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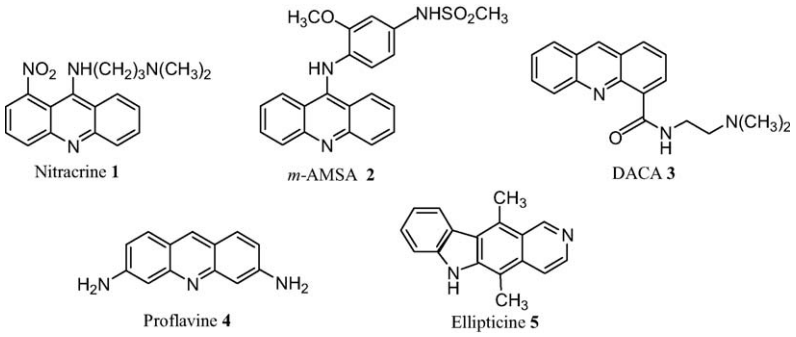


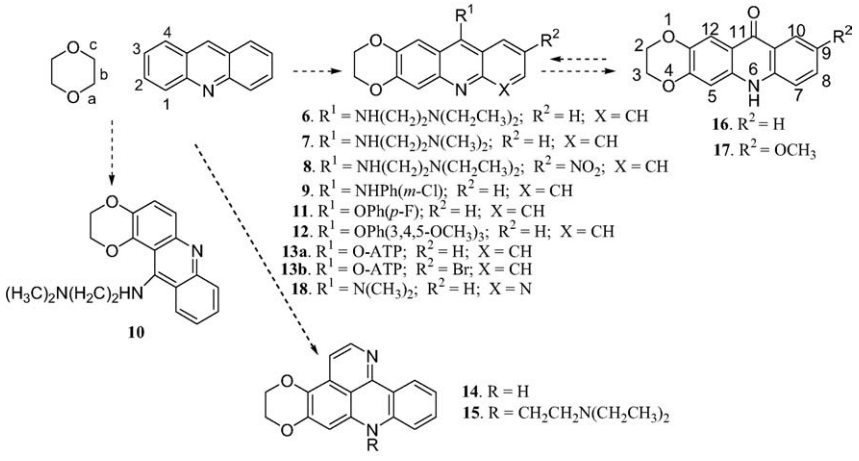
Fig. 1. General formula of known anticancer agents.

ciently large coplanar aromatic chromophore. The compounds showed in Fig. 1 are complex molecules, not obviously related in any way except in the possession of coplanar chromophores that favor the intercalation; for this reason they are designated as DNA intercalators. Moreover, the cytotoxicity of most of the clinically useful DNA-intercalating agents involves the inhibition of the enzyme DNA-topoisomerase I or II. Several detailed SAR studies of acridine-based DNA-intercalating agents suggest that the mode of binding is important and the chromophore will locate to give maximum overlap with the DNA base pairs. The intercalative binding appears to be a necessary but not sufficient condition for the antiproliferative activity [10].

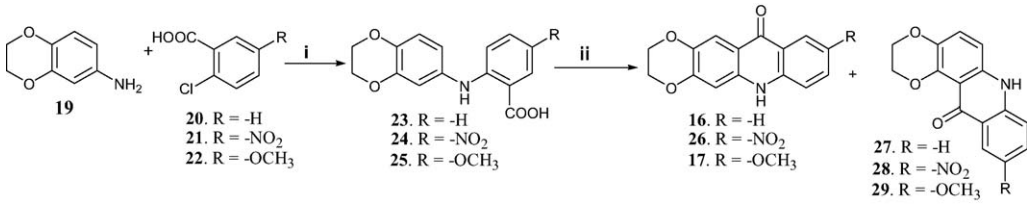
It is consequently of great interest to find new types of polycyclic compounds possessing potent antitumor activities and reduced side effects. Recently, Antonini et al. [11] have prepared several bis functionalized acridine-4-carboxamides with two basic side chains as important antitumor agents. Herein we report the synthesis and biological evaluation of a series of novel acridines with a 1,4-dioxine group fused at 2,3- or 4,5-positions. There is only few previous reports of this ring system in the literature [4d–g] and we give the first account here of their biological properties.

Other modifications at the C-11 of the acridine nucleus and at the D ring have been made (Scheme 1). Thus, several acridine derivatives have been prepared, which vary in the atom linked to 11- position, in the size and electronic character of the lateral chain and in the kind of the 9-substituent (compounds **6**–**17**). The introduction of *N*-substituent are the most known but the *O*-substituents as ATP at the 11-position of the acridine derivatives is unknown up to date, and this is the first time that ATP was enclosed in antitumor agents (compounds **13a** and **13b**). It is well known that all cells need Adenosine triphosphate (ATP) to survive. Recently, the effects of drug binding on the ATP hydrolysis were studied [12]. Several modifications affecting ATP binding or hydrolysis have been made and used to study the mechanism of ATP-dependent anticancer drug transport. Now, the research is still ongoing and the results are not available. For our part, we think that the study of compounds containing ATP framework can be assist to understand the mechanism of ATP anticancer effects.

Also, the substitution of the benzene (D ring) by a pyridine nucleus has been considered (compound **18**). Both of them are bioisostere groups. Our aim was to verify the effect on the cytotoxic activity and the cell cycle selectivity of the additional dioxygenated ring of compounds compared to tricyclic derivatives and if the substitution of the C-11, believed essential for antitumor activity, could afford similar or enhanced biological properties. In vitro cytotoxicity data against leukemic cell lines and selectivity for the cellular cycle are described. The struc-



Scheme 1.



Scheme 2. Conditions: **i**, K_2CO_3/Cu/xylene **ii**, TFA/CH_2Cl_2 or H_2SO_4 or PPA.

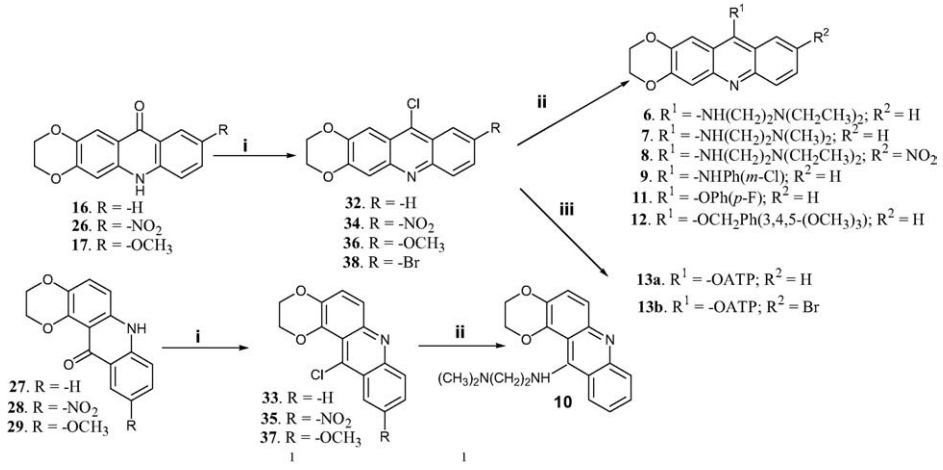
ture-activity relationships should provide chemical leads towards the design of novel anticancer compounds.

2. Chemistry

Scheme 2 shows the synthetic pathway employed in the preparation of compounds (**16** and **17**, **26**–**29**). The *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)anthranilic acid **23** was prepared by direct nucleophilic displacement (Ullmann conditions) [13] of the 2-chlorobenzoic acid **20** with the 2,3-dihydro-1,4-benzodioxin-6-amine. In this reaction, the substitution of the halogen atom of the ortho-halogenobenzoic acid by aniline is promoted by copper catalyst. The ring closure of this acid derivative was carried out by different pathways. The standard synthesis of 11-substituted compounds from the diarylamine (**23**–**25**) using $POCl_3$ lead directly the 11-chloroderivatives contaminated with side products which were difficult to remove, resulting in modest yields of pure compounds. When the acid **23** was refluxed in $POCl_3$ the cyclisation proceeded fast, affording the chloroacridines **32** and **33** [4f], corresponding to the ring closure in the two possible positions (C7 or C5, respectively) (Scheme 3). However, when compound **23** was treated with TFA in CH_2Cl_2 the compound of cyclisation in position C7 was regioselectively obtained (tetracyclic ketone **16**), which refluxed in phosphorus oxychloride gave the chloroacridine **32** (Scheme 3). Another way of classical cyclisation consisted in refluxing the carboxylic acid **(23–25)** in sulfuric acid under air for 4 h or using polyphosphoric acid at 110 °C [14].

In order to study the role of the substituents (electron-withdrawing versus electron-donating) in the 9 position of the acridine nucleus, we have synthesized different derivatives with a nitro, bromo or methoxy group. Our first approach to the synthesis of the nitro substituted acridine **8** was to prepare the corresponding acridinone **26** from the 4-nitrodiphenylamine carboxylic acid **24**, obtained by Ullmann reaction between **19** and **21**. In this step, the carboxylic acid was cyclised using TFA to form a mixture of the two isomers "linear" and "non linear" acridones in 22% **(26)** and 64% **(28)** yield, respectively. The main step of the synthesis of this series of compounds was the nucleophilic attack of the corresponding chloroacridines by alkylamine, diarylamine or alcohol. The conversion of **26** in the chloroderivative **34** by refluxing with $POCl_3$ following by alkylation of the corresponding *N,N*-diethylaminoethylamine let us to obtain compound **8**. However, the direct nitration of the corresponding acridones would be the most convenient way to introduce a nitro function in the acridine nucleus. Thus, the nitro derivative **26** was obtained in 68% yield when **16** were treated with a mixture of nitric acid and acetic acid at 0 °C. Under these conditions only one isomer was obtained and the nitro function is located at the para position of the amino group.

Ullmann condensation of 2-chloro-5-methoxy benzoic acid with 2,3-dihydro-1,4-benzodioxin-6-amine afforded the diphenylamine carboxylic acid **25**. The intramolecular cyclisation of **25** with phosphorus oxychloride gave the two isomeric "linear" and "non-linear" compounds **36** and **37**. Attempts to obtain the corresponding *N*-substituted acridine by direct alkylation of **36** and **37** were not successful and only the corresponding acridones **17** and **29** together with degradation compounds were obtained.



Scheme 3. Conditions**: i**, $POCl_3$ **ii,** a) R^1H/K_2CO_3/DMF b) R^1H/NaH/THF or DMF **iii**, Adenosin triphosphate disodium salt, Aliquat®, H_2O:CH_3CN.

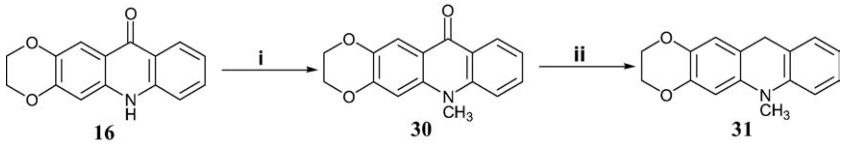
Compound **38** which contains a bromine atom at the C-9 position was obtained from the chloro derivative **32** by treatment with *N*-bromosuccinimide in methanol following the standard procedure [15].

The conversion of the chloroderivative intermediates in the corresponding *N*- and *O*- substituted acridines was achieved by applying classical procedures, following different methods of alkylation. The acridines (**6–8** and **10**) possessing a diamino chain in the C ring were prepared by nucleophilic substitution from the corresponding chloroacridines **32–34** with the primary alkylamine in dry dimethyl formamide (DMF) using K_2CO_3 as a base [16]. These were again isolated and purified as free bases. For the preparation of the aniline **9** and the ether **12**, the chloroacridine **32** was coupled with the appropriate aniline or alcohol, respectively, using NaH as a base in DMF. Being the most hindered substituent (3,4,5-trimethoxybenzyloxy) the most difficult to couple at the C-11 position (compound **12**). For the preparation of the diaryl ether **11**, K_2CO_3 was used as a base or the phenoxy salt was previously formed by treatment with Na in anhydrous methanol. This last method was preferable and provides **11** in satisfactory yield. For the preparation of **13a**, which have an ATP (adenosine triphosphate) group at the C-11 position, another synthetic method was selected. The preparation of this compound requires very carefully controlled conditions and preferably an inert atmosphere. The utilization of ammonium salts as phase transfer catalysts for the introduction of an ATP group at the C-11 position of acridine derivatives has not previously been reported. In this case, the treatment of chloroderivative **32** with a twofold excess of the adenosine triphosphate disodium salt with respect to the acridine and a catalyst amount of Aliquat® in H_2O/CH_3CN (1:1) under ultrasonic irradiation led to the formation of **13a**. We have found that ultrasonic irradiation affords a significant amelioration in the preparation of these compounds versus the classical ways. The same procedure was applied to the double halogenated acridine **38** and the corresponding ATP-*O*-acridine **13b** was obtained in a low yield. These compounds containing ATP unit were relatively stables. The spectrum of ^{1}H-NMR of **13a** it confirms this stability on having turned out to be unaltered after three months. After one year the compound stored at 4 °C was hydrolysed in a 55% to the corresponding acridinone (checked by NMR-spectra).

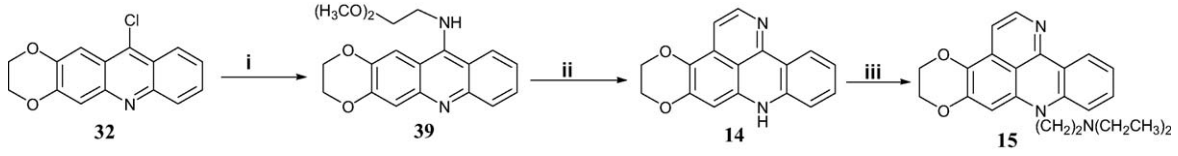
N-methylation of the acridinone **16** by treatment with excess of methyl iodide in THF in presence of NaH afforded the *N*-methylacridinone **30** which was reduced to **31** using $LiAlH_4$ in THF in 94% yield (Scheme 4).

Condensation of **32** with dimethoxyethylamine gave the stable acetal **39**, which was regioselectively cyclized by reflux treatment with PTSA (*p*-toluenesulfonic acid monohydrate) in toluene to the pentacyclic system **14** [16]. Subsequent treatment of **14** with the diethylaminoethyl chloride in DMF provided **15** (Scheme 5). Pyrido[4,3,2-*kl*]acridine, tetracyclic analogues related to the pentacyclic nucleus **14** were isolated from marine organisms [17].

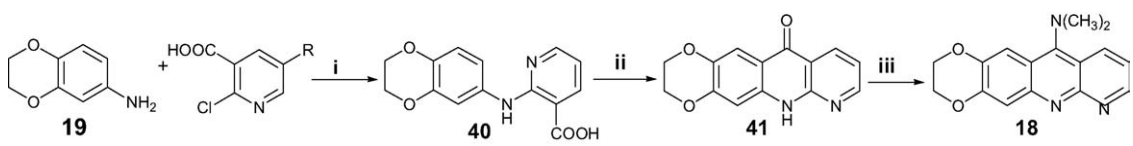
The formation of the nicotinic acid **40** was carried out by nucleophilic substitution of the 2-chloropyridine-3-carboxilic acid using NaH in DMF in 60% yield. Further, the cyclisation in the presence of TFA gave only the "linear" isomer **41** in 72% yield. The cyclisation employing PPA or H_2SO_4 was unsuccessful. For the preparation of the required 11-substituted acridine, an alternative route via the corresponding acridone **41** appeared to be an attractive way for the direct amination. The one pot reaction of **41** and dimethylamine hydrochloride in hexamethylphosphoric triamide (HMPT) at 220 °C (20 h) gave the 11-dimethylaminoacridine **18** in 40% yields without passing through the chloroacridine derivative [18].



Scheme 4. Conditions: **i**, NaH/CH_3I/THF **ii**, $LiAlH_4/BH_3$/THF.



Scheme 5. Conditions: **i**, $(CH_3O)_2CHCH_2NH_2$/pyridine **ii**, PTSA/toluene **iii**, RCl/K_2CO_3/DMF.



Scheme 6. Conditions: **i**, NaH/DMF **ii**, TFA/CH_2Cl_2 **iii**, $(CH_3)_2NH$/HMPT.

Scheme 6 depicts the synthesis of **18**, which has a pyridine ring instead of the benzene ring in the acridine system. Both of them in medicinal chemistry are considered bioisostere groups.

3. Results and discussion

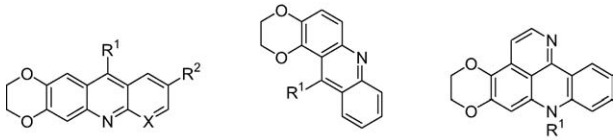
Biological data for the compounds studied are presented in Table 1 as IC_{50} values.

In vitro cytotoxic potency of these derivatives toward the L1210 (leukemia) [18] and HT-29 (human colon carcinoma) [19] cellular lines is described and compared to that of reference drugs, also are reported in Table 1 their effect on the L1210 cell cycle. The structure-activity relationships in this series were examinate by three types of structural changes: a) Insertion of a substituent in the C-11 or C-12 position, b) the introduction of a different substituent at the D ring of the acridine system, and c) the linear or angular structure of the heterocyclic system.

Some of the new compounds possess an interesting cytotoxic activity against L1210 and HT-29 cellular lines. The IC_{50} for every compound tested against each cell line are in the micromolar range with the exception of **16–18** which are the least effective in the series. The unsubstituted compound **6** is more active than the nitro derivative **8**. The introduction of a brome atom at C-9 position is detrimental for the activity. When an aminoalkylamino group was appended at the C-11 position, the antitumor activity increased and reached its maximum value with a dimethylaminoethylamino group, but the replacement of dimethylamino (**7**) by diethylamino (**6**) does not result in any loss of potency. It is particularly interesting to note that the ATP group afforded the most potent derivative (compound **13a**). This compound was 104- and 490-fold more potent than compound **6** against L1210 and HT-29 cell lines, respectively, and the only difference is the presence of the ATP framework instead of the dialkylaminoethylamino group at the C-11. However, the substituted analogue **13b** shows a decrease potency (**13b** was 157 fold less active than the acridine derivative **13a** against L1210 cells and 442 fold less active than **13a** against HT-29 cells).

The in vitro data of the acridinones **16** and **17** depicted a lower activity for these compounds compared with the corresponding acridines due to they do not have a nucleofil group to DNA binding. Also, the corresponding aminated compound **18** with a low antitumor activity suggests that the basic group on the acridine system are significant for the enhancement of the antitumor activity (**18** have very low pKa, due to steric deconjugation). The decrease of activity of **16-18** is more notable against HT-29 that against L1210. The introduction of a phenoxy group in the C-11 enhances a decrease of the antitumor activity but the compounds were active. Compound **11** possesses a remarkable antitumor activity but is lower than that displayed by **7**. The substituents of the phenoxy group are important for the antitumor activity and the *p*-fluoro group is considered the best substituent for the antitumoral activity while the 3,4,5-trimethoxyphenyl group showed a reduced activity (**11** was 4 fold more active than **12**). Compound **9** possessing substituted aniline showed less activity than the alkylamines **6** and **7**. Substitution pattern in the C-9 position of the target

Table 1
Cytotoxicity of compounds **6–18**



Compound	Structure	R^1	R^2	X	IC_{50} (μM) L1210	IC_{50} (μM) HT-29	Cellular cycle
6	A	a	H	CH	4.8 ± 0.08	5.9 ± 0.03	S/G_2M (76%) 25 μM
7	A	b	H	CH	4.2 ± 0.05	4.0 ± 0.04	G_2M (85%) 10 μM
8	A	a	NO_2	CH	7.6 ± 0.03	4.7 ± 0.02	N.S. 50 μM
9	A	c	H	CH	65.2 ± 0.07	37.2 ± 1.02	N.T.
10	B	b	-	-	4.0 ± 0.05	3.8 ± 0.05	G_2M (88%) 5 μM
11	A	d	H	CH	11.5 ± 0.06	16.2 ± 0.06	N.S.
12	A	e	H	CH	43.1 ± 0.05	64.5 ± 0.04	N.T.
13-a	A	f	H	CH	0.046 ± 0.04	0.012 ± 0.03	G_1 (89%) 0.5 μM
13-b	A	f	Br	CH	7.2 ± 0.05	5.3 ± 0.04	N.S. 2 μM (apoptosis)
14	C	H	H	CH	0.4 ± 0.06	0.8 ± 0.03	N.S. 2 μM
15	C	a	H	CH	0.9 ± 0.02	0.5 ± 0.02	N.S. 5 μM (apoptosis)
16	A	=O	H	–	> 10 (83%)	N.S.	N.S.
17	A	=O	OCH_3	–	> 10 (91%)	N.S.	N.S.
18	A	g	H	N	> 10 (82%)	N.S.	N.S.
31	A	–	–	CH	> 10 (67%)	N.S.	N.S.
9-Aminoacridine					45.0 ± 0.03	37 ± 0.05	G_2M (68%)
m-AMSA					0.05 ± 0.05	0.08 ± 0.09	G_2S (62%)

a) 2-(Diethyl)ethylendiamino. b) 2-(dimethyl)ethylendiamino. c) *m*-chloroanilino. d) *p*-fluorophenoxyde. e) (3,4,5-trimethoxy)benzyloxy. f) ATP. g) dimethylamine. N.T. non tested. N.S. non significant.

compound was found to be of relevant interest. For these compounds with electron-withdrawing or electron-donating substituents their activity were also shown in Table 1.

A brome atom leads to the less active compound but with apoptosis effect (the activity decreased 157-fold, comparing compounds **13a** and **13b**, but **13b** showed problems of instability) and a nitro group decreased 1.5 times the activity (see compounds **6** and **8**). The cytotoxicity was decreased for the acridinones (**16** and **17**) and reduced acridines (**18**). Compounds **13a**, **14** and **15** represent a novel structural type of antitumor agents. Removal of the alkylaminoalkyl group of **15** increases the cytotoxic activity on L1210 cell line, but in contrast, decreases the activity against human colon carcinoma (HT-29). It is worth noting that while **13a** is a potent cytotoxic compound the analogue cyclic **18** was poorly effective in all biological tests. The much greater relative cytotoxicity of the acridine series may be due to their lipophilic/hydrophilic character, which is known to contribute in vitro antitumor activity.

In order to explore further the role of the linear or angular structure in conferring antitumoral activity the compounds **10**, **14** and **15** were evaluated. The angular derivative **10** showed similar activity to the linear analogue **7**, while the pentacyclic compound **14** was 10 fold times more potent than **7**. Finally, the *N*-substituted pentacyclic compound **15** showed less cytotoxic activity than the unsubstituted compound **14**. The reason for this is not clear; the possible correlation between hydrophilic character and in vitro cytotoxic activity is not linear.

Some of these compounds could block the cell cycle progression of L1210 cells in the G_2/M phase. Thus the angular compound **10** was more specific on disturb cell cycle at G_2/M phase (> 85% at 5 μM). The linear acridine analogue **7** showed also high selectivity for the G_2/M phase of the cell cycle (induced the accumulation of > 85% of cells at 10 μM). More interesting was the cellular selectivity showed by the acridine **13a**. This compound perturbs the cell cycle by accumulating cells in the G_1 phase (more of 85% in a 0.5 μM). In contrast, the cytotoxicity of classical acridines induced a marked accumulation of cells in the G_2 phase. Perturbation of cell cycle control in the G_1 phase is a critical fact for the tumor progression. Therefore, compounds with the ability to arrest cells in the G_1 phase can be considered as a new class of efficient compounds against tumors [20,21].

In conclusion, we have designed and synthesized novel acridine analogues. In particular, the introduction of ATP substructure enhanced the cytotoxic activity, and **13a** represents the most active compound of this series. The nature of the substituent in position C-11 is very important for cytotoxicity as can be seen from the in vitro activity of the dialkylaminoalkylamino group of **6** and **7** and the arylamino group of **9**. Concerning the chromophore, there are pronounced differences in activity between class A and C, in the case compounds **14** and **15** (class C) are more effective than **6** and **7** (class A). **13a** is the most cytotoxic derivative and possesses an ATP substituent, this group is able to form hydrogen bonds with other polar groups. On the other hand the triphosphate side substituent with strong electrostatic repulsions between the negatively charged drug and nucleic acid functions difficult the DNA intercalation. The SAR studies may, however, lead to the development of new more potent compounds.

Interestingly, the fact that **13a** induced a perturbation of the cell cycle G_1 different from that of classical acridines G_2 suggests that they act, at the molecular level, with a different mechanism of action. Moreover UV-visible studies of **13a** show that the cytotoxic activity is not by DNA intercalation.

Taking into account the cytotoxicity data observed above, further modifications are underway to increase potency of the studied acridines.

4. Experimental section

4.1. Chemistry

Melting points were obtained on a MFB-595010M Gallenkamp apparatus in open capillary tubes and are uncorrected. IR spectra were obtained using a FTIR Perkin-Elmer 1600 Infrared Spectrophotometer. Only noteworthy IR absorptions are listed (cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini-200 (200 and 50.3 MHz, respectively) or Varian Gemini-300 (300 and 75.5 MHz) Instrument using $CDCl_3$ as solvent with tetramethylsilane as internal standard or $(CD_3)_2CO$. Other ^{1}H-NMR spectra and heterocorrelation ^{1}H-^{13}C (HMQC and HMBC) experiments were recorded on a Varian VXR-500 (500 MHz). Chemical shifts are in parts per million (ppm). Standard abbreviations are used. Mass spectra were recorded on a Hewlett-Packard 5988-A using chemical ionization (NH_3 or CH_4) or electronic impact. Column chromatography was performed with silica gel (E. Merck, 70-230 mesh). Reactions were monitored by TLC using 0.25 mm silica gel F-254 (E. Merck). Microanalysis was determined on a Carlo Erba–1106 analyzer. Compounds were analyzed for C, H, and N, and analytical results obtained for these elements were within ±0.4% of the calculated values for the formula shown. All reagents were of commercially quality or were purified before use. Organic solvents were of analytical grade or were purified by standard procedures. Commercial products were obtained from Sigma-Aldrich. The organic extracts were dried over anhydrous Na_2SO_4. ^{1}H-NMR and ^{13}C-NMR data of the compounds **16**, **23**, **30**, **32** and **33** were checked with the results reported before [4e,22].

4.1.1. General procedure to obtain N- and O- substituted compounds

Method A. A suspension of the corresponding chloroacridine (1 mmol), the appropriate amine (2.5 mmol) and anhydrous K_2CO_3 (2 mmol) in dry DMF (5 ml) was stirred at 80 °C for 3 h under an inert atmosphere. The cooled mixture was extracted with ether (3 × 15 ml). The organic layers were dried, filtered and concentrated under *vacuum*. The crude product was subjected to column chromatography eluting with a hexane/ethyl acetate 1:1 mixture as eluent.

Method B. A suspension formed by NaH (60%, 3 mmol), and the corresponding aniline or alcohol (2 mmol) in 15 ml of

anhydrous DMF (for the amine) or THF (for the alcohol), was stirred at room temperature under argon atmosphere. After 40 min, the chloro-derivative **32** was drop-wise added (1 mmol) and stirred at 100 °C for 24 h. The cooled mixture was hydrolyzed with water, the solvent removed and extracted with ether (3 × 15 ml). The combined organic layers were dried, filtered and concentrated. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate 1:1 as eluent to give the corresponding *N*- or *O*- substituted compound.

Method C. A suspension of anhydrous K_2CO_3 (6 mmol) and the corresponding phenol (3 mmol) in dry DMF was stirred at room temperature under an inert atmosphere for 30 min. Then, the suitable chloroacridine was added (1 mmol) and stirred at 100 °C for 6 h. The cooled mixture was extracted with ether (3 × 15 ml) and the combined organic layers were dried, filtered and concentrated under vacuum. The residue was chromatographed on a silica gel column using hexane/ethyl acetate 1/1 to afford the *O*-substituted compound.

*4.1.1.1. 11-[2-(Diethyl)ethylendiamine]-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**6**)*. Compound **6** (0.12 g; 52% yield) was obtained as a colourless oil following the general procedure (method **A**) starting from compound **32** (0.18 g, 0.66 mmol) and 2-(diethylamino)ethylamine (0.19 g, 1.65 mmol). IR (KBr) υ cm^{-1}: 3500 (N–H), 1485 (C=C), 1297 (Ar–O), 1065 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.12 (t, $J = 7$ Hz, 6H, CH_3), 2.65 (t, $J = 7$ Hz, 4H, CH_2N), 2.69 (t, $J = 7$ Hz, 2H, CH_2N), 3.82 (t, $J = 7$ Hz, 2H, CH_2N), 4.39 (m, 4H, CH_2O), 7.23 (m, 2H, C8-H and C9-H), 7.58 and 7.59 (s, 2H, C5-H and C12-H), 8.01 (d, $J = 7$ Hz, C10-H), 8.21 (d, $J = 7$ Hz, C7-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 46.1 (CH_2, CH_2N), 46.5 (CH_2, CH_2N), 52.5 (CH_2, CH_2N), 64.4 and 64.6 (CH_2, CH_2O), 107.2 (CH, C-5), 112.5 (CH, C-12), 114.1 (C, C-10a), 122.0 and 122.7 (CH, C-7, C-9), 124.2 (C, C-11a), 128.9 and 129.1 (CH, C-8, C-10), 141.9 and 142.0 (C, C-4a, C-12a), 149.1 and 149.9 (C, C-9, C-5a, C-6a), 151.8 (C, C-11). MS (EI) (*m*/*z*, %): 351 (M, 5), 293 (M-58, 2), 252 (M-99, 23), 86 (100). Anal. Calcd for $C_{21}H_{25}N_3O_2$; C, 71.77; H, 7.17; N, 11.96. Found: C, 72.01; H, 6.98; N, 11.67.

*4.1.1.2. 11-[2-(Dimethyl)ethylendiamine]-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**7**)*. Starting from acridine **32** (0.08 g, 0.25 mmol) and *N*,*N*-dimethylethylendiamine (0.05 g, 0.625 mmol) was obtained compound **7** as an oil (0.05 g, 68%), following the general procedure (method **A**). IR (NaCl) υ cm^{-1}: 3395 (N–H), 1461(C=C), 1287 (Ar–O), 1121 (C–O). ^{1}H-NMR (CD_3OD, 200 MHz) δ: 3.08 (s, 6H, CH_3N), 3.40 (bs, 2H, CH_2N), 3.56 (bs, 2H, CH_2N), 4.62 (m, 4H, CH_2O), 7.39 (s, 1H, C5-H), 7.62 (bs, 2H, C9-H and C12-H), 7.77 (d, $J = 8$ Hz, 1H, C7-H), 7.97 (bs, 1H, C8-H), 8.46 (d, $J = 8$ Hz, 1H, C10-H). ^{13}C-NMR (CD_3OD, 50.3 MHz) δ: 35.5 (CH_3, CH_3N), 43.9 (CH_2, CH_2N), 55.2 (CH_2, CH_2N), 64.9 (CH_2, CH_2O), 66.9 (CH_2, CH_2O), 111.7 (CH, C-5), 112.1 (C, C-10a), 119.3 (CH, C-12), 122.0 (C, C-11a), 124.6 and 125.2 (CH, C-7, C-9), 128.9 (CH, C-8), 136.4 (CH, C-10), 138.1 and 138.5 (C, C-4a, C-12a), 148.1 and 149.2 (C, C-5a, C-6a), 150.9 (C, C-11). Anal. Calcd for $C_{19}H_{21}N_3O_2$; C, 70.57; H, 6.55; N, 12.99. Found: C, 70.87; H, 6.79; N, 12.83.

*4.1.1.3. 9-Nitro-11-[2-(diethyl)ethylendiamine]-2,3-dihydro-1,4-dioxino[2,3-b] acridine (**8**)*. Starting from the chloroacridine **34** (0.08 g, 0.25 mmol), *N*,*N*-diethylethylendiamine (0.073 g, 0.625 mmol) and following the general procedure (method **A**), compound **8** (0.065 g, 68% yield) was obtained as a colourless oil. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.11 (t, $J = 7$ Hz, 6H, CH_3CH_2), 2.72 (m, 6H, CH_2N), 3.81 (bs, 2H, CH_2N), 4.46 (m, 4H, CH_2O), 6.63 (bs, 1H, NH), 7.32 (t, $J = 7.4$ Hz, 1H, C7-H), 7.61 (t, $J = 7.4$ Hz, 1H, C8-H), 7.71 (s, 1H, C5-H), 7.98 and 8.06 (m, 2H, C10-H and C12-H). ^{13}C -NMR ($CDCl_3$, 50.3 MHz) δ: 11.7 (CH_3, CH_3CH_2), 45.9 and 46.1 (CH_2, CH_2N), 52.1 (CH_2, CH_2N), 64.0 and 65.3 (CH_2, CH_2O), 108.9 (CH, C-5), 112.1 (C, C-10a), 116.2 (C, C-11a), 122.2 (CH, C-10), 122.9 (CH, C-12), 129.7 (CH, C-7), 130.0 (CH, C-8), 139.2 and 139.8 (C, C-4a, C-12a), 140.2 (C, C-9), 151.2 (C, C-6a), 152.0 (C, C-5a), 161.1 (C, C-11). MS (EI) (*m*/*z*, %) 396 (M, 1), 293 (1), 267 (2), 86 (100). Anal. Calcd for $C_{21}H_{24}N_4O_4$; C, 63.62; H, 6.10; N, 14.13. Found: C, 63.45; H, 6.34; N, 13.98.

*4.1.1.4. 11-(m-Chlorophenylamino)-2,3-dihydro-1,4-dioxino [2,3-b]acridine (**9**)*. Starting from 3-chloroaniline (0.14 g, 1.1 mmol), the acridine **32** (0.15 g, 0.55 mmol) and operating *via* the general procedure (procedure **B**), compound **9** was obtained as a colourless oil (0.065 g, 45%). IR (KBr) υ cm^{-1}: 3388 (N-H), 1596 (Ar–H), 1127 (Ar–O), 1075 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.25 (m, 4H, CH_2O), 6.86 (m, 1H, Ar), 7.12 (m, 1H, Ar), 7.28 (m, 7H, Ar), 7.62 (m, 1H, Ar), 7.93 (bs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 64.3 and 64.4 (CH_2, CH_2O), 109.9 (CH, C-5), 113.8 (CH, C-2′), 117.2 (C, C-10a), 117.3 (CH, C-12), 121.2 (C, C-11a), 127.1 and 127.4 (CH, C-7, C-9), 128.8, 130.2, 130.3 and 131.5 (CH, C-8, C-4′, C-5′, C-6′), 131.9 (CH, C-10), 135.2 (C, C-3′), 140.2 (C, C-6a), 143.3 and 143.4 (C, C-4a, C-12a), 143.5 (C, C-1′), 151.9 (C, C-5a), 161.9 (C, C-11). Anal. Calcd for $C_{21}H_{15}ClN_2O_2$; C, 69.52; H, 4.17; N, 7.72. Found: C, 69.67; H, 4.36; N, 7.37.

*4.1.1.5. 12[(2-Dimethyl)ethylendiamino]-2,3-dihydro-1,4-dioxino[3,4-b]acridine (**10**)*. Starting from compound **33** (0.05 g, 0.18 mmol), *N,N*-dimethylethylendiamine (0.04 g, 0.45 mmol) and following the general procedure **A** it was obtained compound **10** (0.04 g, 70% yield) as a yellow solid. m.p. (hexane/ethyl acetate): 103–104 °C. ^{1}H-NMR (CD_3OD, 200 MHz) δ: 2.27 (s, 6H, CH_3N), 2.47 (t, $J = 8$ Hz, 2H, CH_2N), 3.74 (t, $J = 8$ Hz, 2H, CH_2-N), 7.23 (m, 2H, C10-H, C11-H), 7.33 (d, $J = 12$ Hz, 1H, C5-H), 7.50 (t, $J = 8$ Hz, 1H, C9-H), 6.75 (d, $J = 12$ Hz, 1H, C6-H), 8.15 (d, $J = 8$ Hz, 1H, C8-H). ^{13}C-NMR (CD_3OD, 50.3 MHz) δ: 46.9 (CH_3, CH_3N), 51.7 (CH_2, CH_2N), 61.5 (CH_2, CH_2NAr), 66.4 (CH_2, CH_2O), 67.4 (CH_2, CH_2O), 102.3 (C, C-12a), 118.2 (C, C-11a), 120.5 (CH, C-5), 122.0 (CH, C-9), 124.8 (CH, C-6), 127,0 (CH, C-10), 127.1 (CH, C-11), 131.4 (CH, C-8), 137.7 (C, C-4a and C-

12b), 139.2 (C, C-6a), 144.8 (C, C-7a), 146.8 (C, C-12). Anal. Calcd for $C_{19}H_{21}N_3O_2$; C, 70.57; H, 6.55; N, 12.99. Found: C, 70.23; H, 6.88; N, 12.67.

*4.1.1.6. 11-(p-Fluorophenoxy-2,3-dihydro-1,4-dioxino[2,3-b] acridine (**11**)*. Compound **11** (0.045 g, 64%) was obtained by the general procedure (method **C**) from as the *p*-fluorophenol (0.065 g, 0.6 mmol) and the acridine **32** (0.05 g, 0.19 mmol). IR (KBr) υ cm^{-1}: 1501 (Ar–H), 1189 (Ar–O), 1068 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.39 (m, 4H, CH_2O), 6.79 (m, 2H, C2′-H and C6′-H), 7.40 (m, 2H, Ar), 7.67 (m, 2H, Ar), 7.99 (d, J = 8.2 Hz, 1H, C10-H), 8.21 (d, J = 8.2 Hz, 1H, C7-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 64.3 and 64.4 (CH_2, CH_2O), 105.5 (CH, C-5), 112.8 (CH, C-7), 116.3 (C, C-10a), 116.5 (CH, d, J = 8 Hz, C-2′ and C-6′), 117.0 (CH, C-10), 119.3 (CH, C-12), 122.1 (CH, C-9), 125.1 (CH, C-8), 129.4 (CH, d, J = 37 Hz, C-3′, C-5′), 144.8 (C, C-11a), 148.1, 148.9, and 149.5 (C, C-1′, C-11, C-4a, C-12a), 153.3 and 155.2 (C, C-5a, C-6a), 158.6 (C, d, J = 265 Hz, C-4′). MS (m/z, %): 347 (M, 100), 252 (M-95, 14), 196 (3), 152 (6). Anal. ($C_{21}H_{14}FNO_3$) C, H, N. Anal.Calcd for $C_{21}H_{14}FNO_3$; C, 72.62; H, 4.06; N, 4.03. Found: C, 72.96; H, 4.34; N, 3.89.

*4.1.1.7. 11-(3,4,5-Trimethoxybenzyloxy)-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**12**)*. Compound **12** (colorless oil, 0.16 g, 60% yield) was obtained using the general procedure (method B) starting from 3,4,5-trimethoxybenzylalcohol (0.25 g, 1.24 mmol) and the chloroderivative **32** (0.17 g, 0.62 mmol). IR (KBr) υ cm^{-1} (2 HCl): 3505 (N–H), 1204 (Ar–O), 1136 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.85 (m, 9H, CH_3O), 4.25 (m, 4H, CH_2O), 4.60 (s, CH_2O), 6.62 (s, 2H, C2′-H and C6′-H), 7.33 (m, 5H, Ar), 8.22 (bs, 1H Ar). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 55.6 (CH_3, CH_3O), 60.4 (CH_3, CH_3O), 63.7 (CH_2, CH_2O), 63.8 (CH_2, CH_2O), 64.7 (CH_2, CH_2O), 103.4 (CH, C-2′, C-6′), 112.2 (CH, C-5), 116.2 (C, C-10a), 116.6 (CH, C-12), 120.6 (CH, C-7), 124.2 (C, C-11a), 125.8 (CH, C-9), 132.6 (CH, C-8), 137.0 (CH, C-10), 140.0 and 141.2 (C, C-4a, C-12a), 144.2 (C, C-5a, C-6a), 149.8 (C, C-11), 151,9 (C, C-1′), 152.8 (C, C-3′, C-4′, C-5′). MS (EI) (*m*/*z*, %) 433 (M, 1), 368 (1), 252 (100), 253 (17), 197 ($C_{10}H_{13}O_4^+$, 59). Anal.Calcd for $C_{25}H_{23}NO_6$; C, 69.27; H, 5.35; N, 3.23. Found: C, 69.01; H, 5.76; N, 3.56.

*4.1.1.8. 11-(Adenosin-5′-triphosphate)-2,3-dihydro-1,4-dioxino [2,3-b]acridine (**13a**)*. A solution of chloroacridine **32** (120 mg, 0.44 mmol) and KI (103.5 mg, 0.62 mmol) in CH_3CN 10 ml was stirred under argon atmosphere at room temperature for 60 minutes. After adenosin triphosphate disodium salt (408 mg, 0.74 mmol) and Aliquat® (0.3 ml) in CH_3CN:H_2O 1:1 (20 ml) were added and the mixture was introduced in an ultrasonic irradiation bath for 24 h. Finally the CH_3CN was removed and the mixture was extracted with with CH_2Cl_2. The resulting organic layers were dried, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (metanol /NH_4OH) affording 150 mg (46% yield) of the compound **13a**. IR (KBr) υ cm^{-1}: 3490 (OH), 2924 (NH), 1729 (C=N), 1260 (Ar–O), 1087 (C–O). ^{1}H-NMR (CD_3OD, 200 MHz) δ: 1.99 (bs, 5H, OH), 3.04 (s, 1H, CH-O), 3.24 (m, 2H, CH_2O), 3.39 (s, 1H, CH-O), 3.45 (m, 1H, CH-O), 4.23 (m, 4H, CH_2-O), 7.11 (s, 1H, C5-H), 7.18 (m, 2H, C9-H and C10-H), 7.41 (m, 1H, C7-H), 7.48 (m, 1H, C8-H), 7.87 (s, 1H, C12-H), 8.38 and 8.39 (m, 2H, C2-H and C6-H), 9.86 (bs, 2H, NH_2). ^{13}C-NMR (CD_3OD, 50.3 MHz) δ: 48.1 (CH, CH-O), 61.5 (CH_2, CH_2O), 62.3 (CH_2, CH_2O), 63.1 (CH_2, CH_2O), 102.1 (CH, C-5), 109.8 (CH, C-9), 112.2 (CH, C-12), 116.1 (CH, C-7), 120.3 (CH, C-8), 123.8 (CH, C-10), 126.1 (CH, C-2′), 132.3 (CH, C-6′), 134.8 (C, C-3a'), 136.1 (C, C-10a), 137.4 (C, C-11a), 138.1 (C, C-6a), 139.8 (C, C-12a), 140.3 (C, C-5a), 141.1 (C, C-4a), 142.7 (C, C-11), 149.4 (C, C-7a), 178.1 and 178.9 (C, C-4′). Anal.Calcd for $C_{25}H_{25}P_3N_6O_{15}$; C, 40.45; H, 3.39; N, 11.32. Found: C, 40.41; H, 3.53; N, 11.11.

*4.1.1.9. 11-(Adenosin-5′-triphosphate)-9-bromo-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**13b**)*. Was prepared from the chloroacridine **38** (130 mg, 0.37 mmol) as described above for **13a**. The residue was purified by silica gel column chromatography (metanol/NH_4OH) affording 75 mg (24% yield) of the compound **13b** as an oil. IR (KBr) υ cm^{-1}: 3510 (OH, N–H), 2938 (NH), 1220 (Ar–O), 1082 (C–O). ^{1}H-NMR (CD_3OD, 200 MHz) δ: 2.01 (bs, 5H, OH), 3.12 (s, 1H, CH–O 3.29 (m, 2H, CH_2O), 3.45 (s, 1H, CH–O), 4.30 (m, 4H, CH_2–O), 7.08 (s, 1H, C5-H), 7.28 (m, 1H, Ar), 7.50 (m, 1H, C7-H), 7.56 (m, 1H, C8-H), 7.80 (s, 1H, C12-H), 8.30 (m, 2H, Ar), 8.98 (bs, 2H, NH_2). This compound **13b** was unstable.

*4.1.1.10. 6,9-Dihydro-5H-dioxino[2,3-i]pyrido[4,3,2-kl]acridine (**14**)*. A mixture of the acetal **39** (125 mg, 0.37 mmol) and a catalytic amount of PTSA in dry toluene (20 ml) was stirred in a 130 °C oil bath for 1 h. Then was allowed to room temperature and the mixture was poured drop-wise into an aqueous solution of ammonium hydroxide cooled to 0 °C and extracted with ethyl acetate (3 × 20 ml). The organic layers were washed with water, dried on sodium sulphate and the solvent was concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate 100% as eluent to give the compound **14** as an orange solid (25 mg, 24% yield). m.p. (hexane/ethyl acetate) 349–350 °C. ^{1}H-NMR (CD_3OD, 200 MHz) δ: 4.34 (s, 4H, CH_2); 6.33 (s, 1H, C8-H); 7.01 (m, 2H, Ar); 7.16 (d, J = 6 Hz, 1H, C13-H); 7.38 (m, 1H, C9-H); 7.95 (d, J = 6 Hz, 1H, C10-H); 8.23 (d, J = 8 Hz, 1H, C3-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 62.3 (CH_2-O), 93.7 (CH, C-8), 106.3 (CH, C-2), 112.5 (CH, C-10), 118.1 (CH, C-13), 123.6 (C, C-13a), 124.1 (C, Ar), 129.2 (CH, C-11 and C-12), 129.6 (CH, C-3), 136.4 (C, C-3a), 143.2 (CH, C-2), 144.3 (C, C-7a, C-3b), 148.1 (C, C-8a and C-9a), 149.2 (C, C-13b). Anal. Calcd for $C_{17}H_{12}N_2O_2$; C, 73.90; H, 4.38; N, 10.14. Found: C, 73.21; H, 4.65; N, 9.89.

*4.1.1.11. 6-(N,N-diethylaminoethyl)-6,9-dihydro-5H-dioxino [2,3-i]pyrido[4,3,2-kl]acridine (**15**)*. Prepared from the compound **14** (100 mg, 0.35 mmol) and 2-diethylaminoethyl chlor-

ide hydrochloride (200 mg, 1.05 mmol) as described above in general procedure A, the compound **15** was obtained as a dark oil (98 mg, 75% yield). ^{1}H-NMR (CD_3OD, 200 MHz) δ: 1.09 (t, $J = 7$ Hz, 6H, CH_3), 2.65 (q, $J = 7$ Hz, 6H, CH_2-N), 3.78 (t, $J = 7$ Hz, 2H, CH_2), 4.32 (s, 4H, CH_2), 6.20 (s, 1H, C8-H), 7.02 (m, 3H, Ar), 7.39 (d, $J = 8.2$ Hz, 1H, C10-H), 7.97 (d, $J = 6$ Hz, 1H, C13-H), 8.23 (d, $J = 8.2$ Hz, 1H, C2-H). ^{13}C-NMR (CD_3OD, 50.3 MHz) δ: 9.3 (CH_3), 42.9 (CH_2), 45.0 (CH_2), 45.9 (CH_2), 61.7 (CH_2-O), 62.6 (CH_2-O), 93.0 (CH, C-8), 106.9 (CH, C-12), 111.2 (CH, C-13), 117.9 (CH, C-11), 122.6 (CH, C-10), 123.1 (C, Ar), 124.5 (C, C-13a), 128.8 (CH, C-3), 138.9 (C, C-3a), 144.6 (C, C-7a, C-3b), 141.8 (CH, C-2), 147.2 (C, C-8a, C-9a), 148.4 (C, C-13b). Anal. Calcd for $C_{23}H_{25}N_3O_2$; C, 73.57; H, 6.71; N, 11.19. Found: C, 73.87; H, 6.98; N, 10.87.

4.1.2. General procedure to obtain ketones

A suspension of the corresponding 2-(2,3-dihydro-1,4-benzodioxin-6-ylamino)-5-substituted benzoic acid (1 mmol) and trifluoroacetic acid (catalyst amount) in dry CH_2Cl_2 was stirred at reflux temperature for 24 h. The cooled mixture was basified (NaOH 1 N) and extracted with ether (3 × 25 ml). The combined organic layers were dried, filtered and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane 1:1).

*4.1.2.1. 2,3,6,11-Tetrahydro-1,4-dioxino[2,3-b]acridin-11-one (**16**)*. The acridone **16** was obtained as a white solid (0.32 g, 85% yield) following the general procedure to prepare acridones starting from the carboxylic acid **23** (0.4 g, 1.31 mmol). m.p. (ethyl acetate/hexane): 246–248 °C. IR (KBr) υ cm^{-1}: 3500 (NH), 1635 (C=O), 1299 (Ar–O), 1065 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.02 (bs, 1H, NH), 4.28 (s, 4H, CH_2O), 6.73 (m, 2H, C5-H, C8-H), 7.06 (d, $J = 8.4$ Hz, 1H, C7-H), 7.34 (m, 1H, C9-H), 8.00 (m, 2H, C10-H, C12-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 63.9 and 65.0 (CH_2, CH_2O), 102.7 (CH, C-5), 112.8 (CH, C-7), 114.5 (C, C-11a), 116.4 (CH, C-12), 120.7 (CH, C-9), 122.3 (C, C-10a), 126.5 (CH, C-10), 132.6 (CH, C-8), 140.1 and 141.3 (C, C-5a, C-12a), 145.1 (C, C-6a), 149.2 (C, C-4a), 178.0 (C, C=O). Anal. Calcd for $C_{15}H_{11}NO_3$; C, 71.14; H, 4.38; N, 5.53. Found: C, 71.59; H, 4.56; N, 5.67.

*4.1.2.2. 9-Methoxy-2,3,6,11-tetrahydro-1,4-dioxino[2,3-b]acridin-11-one (**17**)*. The acridone **17** (0.18 g, 64% yield) was prepared from the carboxylic acid **25** (0.3 g, 0.89 mmol) following the general procedure described above. Mp (ethyl acetate / hexane): > 275 °C. IR (KBr) υ cm^{-1}: 3500 (–OH), 1630 (C=O), 1298 (Ar–O), 1110 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.79 (s, 3H, CH_3O), 4.23 (m, 4H, CH_2–O), 6.74 (s, 1H, C5-H), 7.17 (m, 2H, C7-H, C8-H), 7.57 (m, 1H, C10-H), 7.71 (s, 1H, C12-H). Anal. Calcd for $C_{16}H_{13}NO_4$; C, 67.84; H, 4.63; N, 4.94. Found: C, 67.99; H, 4.85; N, 5.08.

*4.1.2.3. Dimethylamino-2,3-dihydro-1,4-dioxino[2,3-g]pyrido[2,3-b]quinoline (**18**)*. A suspension of tetracyclic ketone **41** (80 mg, 0.32 mmol) and dimethylamine hydrochloride (52 mg, 0.64 mmol) in HMPT (1 ml) was stirred at 220 °C for 20 hours. The cooled mixture was extracted with ether (3 × 15 ml) and the combined organic layers were washed several times with water in order to eliminate the HMPT, dried, filtered and concentrated. The desired compound **18** was obtained as yellow oil after purification by silica gel column chromatography using hexane / ethyl acetate 80:20 mixture as an eluent (39 mg, 39% yield). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 2.68 and 2.69 (s, 3H, CH_3N); 4.20 (m, 4H, CH_2O); 6.54 (m, 1H, C9-H); 6.71 (d, $J = 1.2$ Hz, 1H, C12-H); 6.97 (dd, $J_1 = 11$, $J_2 = 2.2$ Hz, C9-H), 7.20 (d, $J = 1.2$ Hz, 1H, C5-H); 8.25 (d, $J = 2.2$ Hz, 1H, C10-H); 8.48 (d, $J = 11.2$ Hz, C8-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 36.6 and 36.9 (CH_3, CH_3N); 64.38 and 64.40 (CH_2, CH_2O), 109.7 (CH, C-5), 113.5 (CH, C-12), 117.1 (CH, C-9), 130.4 (C, C-10a), 131.2 (C, C-11a), 140.3 (C, C-4a and C-12a), 141.4 (C, C-11), 143.3 (C, C-5a), 144.0 (C, C-6a), 159.3 (CH, C-10), 162.8 (CH, C-8). Anal. Calcd for $C_{16}H_{15}N_3O_2$; C, 68.31; H, 5.37; N, 14.94. Found: C, 68.78; H, 5.76; N, 14.65.

4.1.3. General procedure for the Ullmann reaction

A mixture of 6-amino-2,3-dihydro-1,4-benzodioxin **19** (2 mmol), the appropriate 5-substituted-2-chlorobenzoic acid (1 mmol), anhydrous K_2CO_3 (3 mmol) and Cu (catalyst amount) in xylene (40 ml) was stirred at reflux temperature for 4 hours. The cooled mixture was acidified with HCl 2N and the resulting product was extracted with ether (3 × 20 ml). The organic layers were dried, filtered and concentrated under reduced pressure. The crude product was subjected to column chromatography on silica gel eluting with ethyl acetate/methanol 90:10 mixture to give the corresponding benzoic acid derivatives.

*4.1.3.1. 2-(2,3-Dihydro-1,4-benzodioxin-6-yl)aminobenzoic acid (**23**)*. Starting from the 2-chlorobenzoic acid **(20)** (2 g, 12.7 mmol) and the amine **19** (5.4 g, 35.7 mmol) and following the general procedure of Ullmann reaction, compound **23** (2.5 g, 74% yield) was obtained as a white solid. Mp (ethyl acetate/hexane): 126–128 °C. IR (KBr) υ cm^{-1}: 3309 (–OH, –NH), 1690 (C=O), 1150 (C–O). ^{1}H-NMR ($CDCl_3$-CD_3OD, 200 MHz) δ: 4.20 (m, 4H, CH_2O), 6.80–7.48 (m, 5H, Ar), 8.02 (d, $J = 7.8$ Hz, 1H, C3-H), 8.12 (d, $J = 7.8$ Hz, 1H, C6-H). ^{13}C-NMR ($CDCl_3$–CD_3OD, 50.3 MHz) δ: 64.3 and 64.4 (CH_2, CH_2O), 116.2 (C, C-1), 117.7 (CH, C-5′), 126.7 (CH, C-3), 128.4 (CH, C-7′), 130.2 (CH, C-8′), 131.4 (CH, C-5), 133.5 (CH, C-6), 133.8 (CH, C-4), 140.5 and 140.8 (C, C-4′a, C-8′a), 141.7 (C, C-6′), 149.8 (C, C-2), 170.9 (C, COOH).

*4.1.3.2. 2-(2,3-Dihydro-1,4-benzodioxin-6-yl)amino-5-nitrobenzoic acid (**24**)*. Compound **24** was obtained as a yellow solid (3 g, 83% yield) from the 2-chloro-5-nitrobenzoic acid **(21)** (5.33 g, 26.45 mmol) and the aniline **19** (2.08 g, 13.7 mmol) and following the general procedure described above of Ullmann reaction. Mp (ethyl acetate / hexane): 135–136 °C. IR (KBr) υ cm^{-1}: 3500 (–OH), 1656 (C=O), 1064 (C–O). ^{1}H-NMR ($CDCl_3$–CD_3OD, 200 MHz) δ: 4.31 (m, 4H, CH_2O),

7.71 (m, 2H, C5′-H, C8′-H), 8.47 (m, 2H, C3-H, C7′-H), 8.94 (m, 2H, C4-H, C6-H), 9.60 (bs, 2H, OH, NH). ^{13}C-NMR ($CDCl_3$-CD_3OD, 50.3 MHz) δ: 64.3 and 64.4 (CH_2, CH_2O), 125.2 (CH, C-3), 126.9 (CH, C-6), 128.1 (CH, C-7′), 129.8 (CH, C-4), 131.1 (C, C-1), 133.4 (CH, C-5′); 135.8 (CH, C-8′); 141.2 (C, C-8′a); 142.1 (C, C-4′a); 144.2 (C, C-5); 146.7 (C, C-6′); 148.3 (C, C-2), 172.1 (C, COOH).

*4.1.3.3. 2-(2,3-Dihydro-1,4-benzodioxin-6-yl)amino-5-methoxybenzoic acid (**25**)*. Compound **25** (0.9 g, 56% yield) was obtained following the general procedure of Ullmann reaction from 2-chloro-5-methoxybenzoic acid **(22)** (1 g, 5.3 mmol) and aniline **19** (2.25 g, 14.9 mmol). Mp (ethyl acetate/hexane): 215–216 °C. IR (KBr) υ cm^{-1}: 3460 (–OH), 1682 (C=O), 1280 (Ar–O), 1054 (C–O). ^{1}H-NMR ($CDCl_3$–CD_3OD, 200 MHz) δ: 3.79 (s, 3H, CH_3O), 4.27 (s, 4H, CH_2O), 6.77 (m, 3H, C5′-H, C7′-H, C8′-H), 7.00 (m, 2H, C3-H, C4-H), 7.5 (d, J = 1.2 Hz, 1H, C6-H).

*4.1.3.4. Nitro-2,3,6,11-tetrahydro-1,4-dioxino[2,3-b]acridin-11-one (**26**)*. A solution of acridone **16** (0.10 g, 0.29 mmol) in a mixture of acetic acid (5 ml) and nitric acid (0.05 ml) was cooled at 0 °C and stirred at room temperature overnight. The resulting mixture was basified with 2 N NaOH and the crude product was extracted with ether (3 × 10 ml). The organic layers were dried, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 90:10) to give **26** (0.08 g, 68%) as a solid. m.p. (ethyl acetate/hexane): 247–248 °C. IR (KBr) υ cm^{-1}: 1603 (C=O), 1505 (NO_2), 1136 (Ar–O), 1079 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.31 (s, 4H, CH_2O), 6.96 (d, J = 8.8 Hz, C7-H), 7.49 (bs, 1H, C5-H), 7.66 (s, 1H, C12-H), 8.31 (dd, J_1 = 8.8, J_2 = 3 Hz, 1H, C8-H), 8.85 (d, J = 3 Hz, 1H, C10-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 64.2 and 64.6 (CH_2, CH_2O), 110.6 (CH, C-5), 113.4 (C, C-10a), 114.2 (C, C-11a), 117.3 and 117.9 (CH, C-8, C-12), 127.3 (CH, C-10), 130.4 (CH, C-7), 140.3 (C, C-9), 143.7 (C, C-4a, C-12a), 145.3 and 146.4 (C, C-5a, C-6a), 179.0 (C, C=O). MS (*m*/*z*, %) 298 (M, 60), 272 (17), 226 (5), 201 (53), 167 (39), 135 (78), 65 (100). Anal. Calcd for $C_{15}H_{10}N_2O_5$; C, 60.41; H, 3.38; N, 9.39. Found: C, 60.65; H, 3.67; N, 9.12.

*4.1.3.5. Nitro-2,3,7,12-tetrahydro-1,4-dioxino[2,3-a]acridin-12-one (**28**)*. Following the general procedure indicated above from the 2-(2,3-dihydro-1,4-benzodioxin-6-amino)-5-nitrobenzoic acid (**24**) (0.5 g, 1.58 mmol). The residue was purified by silica gel column chromatography (hexane/ethyl acetate 90:10) to give **26** (0.1 g, 22% yield) and **28** (0.3 g, 64%) as a white solids. Analytical data of **28**: m.p. (ethyl acetate/hexane): 245–247 °C. IR (KBr) υ cm^{-1}: 3311 (NH), 1674 (C=O), 1579 (NO_2), 1128 (Ar-O), 1065 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.30 (m, 4H, CH_2O), 6.78 (m, 2 H, C5-H and C6-H), 6.94 (dd, J_1 = 9.4, J_2 = 8.4 Hz, 1H, C8-H), 8.06 (dd, J_1 = 9.4, J_2 = 2.8 Hz, 1H, C9-H), 8.90 (d, J_1 = 2.8, J_2 = 3 Hz, 1H, C11-H). MS (*m*/*z*, %) 298 (M, 55), 272 (35), 65 (100). Anal.Calcd for $C_{25}H_{10}N_2O_5$; C, 60.41; H, 3.38; N, 9.39. Found: C, 60.65; H, 3.76; N, 9.55.

*4.1.3.6. Methyl-2,3,6,11-tetrahydro-1,4-dioxino[2,3-b]acridin-11-one (**30**)*. Under an inert atmosphere, a suspension of NaH (60% in oil, 0.043 g, 1.78 mmol), the ketone **16** (0.15 g, 0.59 mmol) and CH_3I (0.11 ml, d 2.28, 1.78 mmol) in dry THF (15 ml) was stirred at 50 °C until the starting material was completely transformed (TLC elution with 7:3 hexane/ethyl acetate mixture). Then, the THF was removed in vacuum and the crude residue was extracted with CH_2Cl_2 (3 × 10 ml). The organic layers were dried, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 80:20) to give **30** (0.11 g, 69%) as a solid. m.p. (hexane/ethyl acetate): 229–230 °C. IR (KBr) υ cm^{-1}: 1594 (C=O), 1241 (Ar–O), 1065 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.71 (s, 3H, CH_3), 4.31 (m, 4H, CH_2O), 6.88 (s, 1H, C5-H), 7.21 (t, J = 8 Hz, 1H, C9-H), 7.40 (d, J = 8.8 Hz, 1H, C7-H), 7.62 (t, J = 8 Hz, 1H, C8-H), 7.91 (s, 1H, C12-H), 8.45 (d, J = 8 Hz, 1H, C10-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 33.7 (CH_3, CH_3), 64.0 and 65.1 (CH_2, CH_2O), 102.0 (CH, C-5), 114.2 (CH, C-7), 114.4 (CH, C-12), 117.5 (C, C-11a), 120.7 (CH, C-9), 121.2 (C, C-10a), 127.6 (CH, C-10), 133.2 (CH, C-8), 139.0 and 139.5 (C, C-4a and C-12a), 141.2 (C, C-6a), 149.5 (C, C-5a), 176.0 (C, C=O). Anal. Calcd for $C_{16}H_{13}NO_3$; C, 71.90; H, 4.90; N, 5.24. Found: C, 72.01; H, 5.23; N, 5.56

*4.1.3.7. Methyl-2,3,6,11-tetrahydro-1,4-dioxino[2,3-b]acridine (**31**)*. Under an inert atmosphere, a suspension of acridinone **30** (0.06 g, 0.23 mmol) and $LiAlH_4$ (0.034 g, 0.90 mmol) in dry THF (15 ml) was stirred at room temperature for 9 h. After, BH_3 was added (1.5 ml) and the reaction mixture was heated at 50 °C for 12 h. Then, the cooled mixture was extracted with ether (3 × 15 ml) and the combined organic layers were dried, filtered and concentrated in vacuum. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 95:5) to give **31** (0.05 g, 94%) as a colourless oil. IR (NaCl) υ cm^{-1}: 1502, 1240 (Ar–O–C), 1064 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.30 (s, 3H, CH_3N), 3.78 (s, 2H, CH_2), 4.22 (m, 4H, CH_2O), 6.41 (s, 1H, C5-H), 6.69 (s, 1H, C12-H), 6.87 (m, 2H, C7-H and C9-H), 7.16 (m, 2H, C8-H and C10-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 32.4 (CH_2, CH_2Ar), 33.1 (CH_3, CH_3N) 64.3 and 64.7 (CH_2, CH_2O), 101.2 (CH, C-5), 111.7 (CH, C-12), 115.2 (C, C-11a), 116.5 (CH, C-9), 119.9 (CH, C-8), 121.2 (C, C-10a), 126.7 (CH, C-10), 128.2 (CH, C-7), 138.1 and 139.0 (C, C-4a, C-12a), 141.3 and 141.7 (C, C-5a, C-6a). MS (*m*/*z*, %): 253 (M, 5), 252 (41), 237 (100), 153 (47). Anal. Calcd for $C_{16}H_{15}NO_2$; C, 75.87; H, 5.97; N, 5.53. Found: C, 75.98; H, 5.65; N, 5.88.

4.1.4. General procedure to prepare the chloroacridine derivatives

Method A. A solution containing the tetracyclic ketone (1 mmol) in $POCl_3$ (2 ml) was stirred at reflux temperature for 3 h. The cooled mixture was extracted with CH_2Cl_2

(3 × 20 ml) and the combined organic layers were washed with a saturated solution of $NaHCO_3$, dried, filtered and concentrated under reduced pressure. The resulting product was purified by silica gel column chromatography (hexane/ethyl acetate 50:50) giving the corresponding chlore derivative.

Method B. A suspension of the corresponding substituted benzoic acid and $POCl_3$ (4 ml) was stirred at reflux temperature for 4 h. Then, the cooled mixture was concentrated under vacuum, basified with NaOH 2N and extracted with CH_2Cl_2. The organic layers were dried, filtered and the solvent removed under reduced pressure. The purification of the crude product by silica gel column chromatography (hexane/ethyl acetate 1:1) afforded the two tetracyclic chlore-derivatives linear and angular isomers.

*4.1.4.1. 11-Chloro-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**32**) and 12-chloro-2,3-dihydro-1,4-dioxino [3,4-b]acridine (**33**)*. Compound **32** (0.45 g, 61% yield) and its isomer **33** (0.28 g, 38% yield) were obtained following the general procedure described above (method **B**) starting from the carboxylic acid **23** (0.73 mg, 0.27 mmol).

Compound **32** (0.15 g, 75% yield) can also be obtained following the method **A** from the ketone **16** (0.2 g, 0.74 mmol).

Compound **32**. m.p. (hexane): 115–117 °C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 4.45 (s, 4H, CH_2O), 7.55 (dt, $J_1 = 6$, $J_2 = 0.7$ Hz, 1H, C9-H), 7.63 (s, 1H, C12-H), 7.72 (dt, $J_1 = 6$, $J_2 = 0.7$ Hz, 1H, C8-H), 7.79 (s, 1H, C5-H), 8.12 (d, $J = 6$ Hz, 1H, C7-H), 8.33 (dd, $J_1 = 6$, $J_2 = 0.5$ Hz, 1H, C10-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 63.9 and 64.1 (CH_2, CH_2O), 123.3 (CH, C-5), 124.2 (CH, C-10), 124.6 (CH, C-9), 124.0 (C, C-12a), 126.8 (CH, C-7), 129.2 (C, C-10a), 129.5 (CH, C-8), 129.7 (CH, C-7), 135.9 (C, C-11), 137.0 (C, C-4a), 138.1 (C, C-5a), 146.0 (C, C-6a). Anal. Calcd for $C_{15}H_{10}ClNO_2$; C, 66.31; H, 3.71; N, 5.16. Found: C, 66.54; H, 3.86; N, 5.52.

Compound **33.** m.p. (hexane): 189–191 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.30 (m, 4H, CH_2O), 7.40 (d, $J = 9.6$ Hz, 1H, C5-H), 7.60 (m, 1H, C9-H), 7.70 (m, 1H, C10-H), 7.75 (d, $J = 9.6$ Hz, 1H, C6-H), 8.17 (d, $J = 8$ Hz, C8-H), 8.45 (d, $J = 8$ Hz, C11-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 64.0 and 64.1 (CH_2, CH_2O), 117.7 (C, C-11a), 121.6 (CH, C-5), 124.5 (CH, C-11), 125.1 (C, C-12a), 125.7 (CH, C-9), 127.1 (CH, C-10), 127.8 (CH, C-6), 130.7 (CH, C-8), 136.1 (C, C-12), 138.1 and 139.4 (C, C-6a, C-12b), 144.3 and 145.3 (C, C-4a, C-7a). Anal.Calcd for $C_{15}H_{10}ClNO_2$; C, 66.31; H, 3.71; N, 5.16. Found: C, 66.65; H, 3.85; N, 5.37.

*4.1.4.2. 9-Nitro-11-chloro-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**34**)*. Compound **34** (0.9 g, 57% yield) was prepared from the ketone **26** (0.15 g, 0.5 mmol) by following the general procedure described above (method **A**). IR (KBr) υ cm^{-1}: 1530 (NO_2), 1490 (C=C), 1240 (Ar–O), 1102 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.53 (m, 4H, CH_2O); 7.62 (t, $J = 7.8$ Hz, 1H, Ar); 7.78 (dd, $J_1 = 7.8$, $J_2 = 0.7$ Hz, 1H, Ar); 7.91 (s, 1H, C5-H); 8.15 (d, $J = 8.8$ Hz, 1H, C7-H); 8.83 (d, $J = 8.8$ Hz, C8-H). Anal.Calcd for $C_{15}H_9ClN_2O_4$; C, 56.89; H, 2.86; N, 8.85. Found: C, 57.15; H, 2.99; N, 8.96.

*4.1.4.3. 9-Methoxy-11-chloro-2,3-dihydro-1,4-dioxino[2,3-b] acridine (**36**) and 10-Methoxy-12-chloro-2,3-dihydro-1,4-dioxino [3,4-b]acridine (**37**)*. Compounds **36** (0.25 g, 50% yield) and **37** (0.104 g, 21% yield) were obtained by the general procedure described above (method **B**) from the carboxylic acid **25** (0.5 g, 1.66 mmol).

Compound **36**. m.p. (hexane/ethyl acetate): 235–237 °C. IR (NaCl) υ cm^{-1}: 1500 (C=C), 1226 (Ar–O), 1069 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.01 (s, 3H, CH_3O); 4.44 (s, 4H, CH_2O); 7.43 (m, 2H, C5-H, C8-H); 7.63 (s, 1H, C12-H); 7.74 (s, 1H, C10-H); 8.04 (d, $J = 9.2$ Hz, C7-H).

Compound **37**. m.p. (hexane/ethyl acetate): 173–175 °C. IR (NaCl) υ cm^{-1}: 1470 (C=C), 1265 (Ar–O), 1123 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.03 (s, 3H, CH_3O); 4.46 (m, 4H, CH_2O); 7.40 (m, 2H, C5-H, C9-H); 7.64 (d, $J = 3$ Hz, C11-H); 7.80 (d, $J = 12$ Hz, C6-H); 8.15 (d, $J = 12$ Hz, C8-H).

*4.1.4.4. 9-Bromo-11-chloro-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**38**)*. *N*-Bromosuccinimide (0.064 g, 0.36 mmol) was added to a cooled solution (0 °C) of compound **32** (0.1 g, 0.36 mmol) in acetonitrile (10 ml) and the mixture was stirred at room temperature for 3 h. Then, the solvent was removed and a solution of 5 N NaOH was added (10 ml). The suspension obtained was extracted with ether (3 × 10 ml), dried, filtered and concentrated. The bromo derivative **38** (0.05 g, 40% yield) was obtained as a yellow oil after purification of the crude of reaction by silica gel column chromatography (hexane/ethyl acetate 70:30). IR (KBr) υ cm^{-1}: 1520 (C=N), 1282 (Ar–O), 1105 (C–O), 875 (C–Br). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.47 (m, 2H, CH_2O); 4.59 (m, 2H, CH_2O); 7.62 (m, 1H, C8-H); 7.80 (m, 2H, C5-H, C12-H); 8.30 (m, 2H, C7-H, C10-H). Anal. Calcd for $C_{15}H_9BrClNO_2$; C, 51.89; H, 2.59; N, 4.00. Found: C, 52.12; H, 2.67; N, 4.34.

*4.1.4.5. 11-Aminoacetaldehyde dimethylacetal-2,3-dihydro-1,4-dioxino[2,3-b]acridine (**39**)*. A solution of the compound **32** (0.1 g, 0.37 mmol) and aminoacetaldehyde dimethylacetal (0.1 g, 0.92 mmol) in 2 ml of pyridine was stirred at 200 °C for 4 h into a modified Schlenk tube. After evaporation of the solvent the aminoacetal **39** was obtained (0.098 g, 78%) as a yellow solid, which was directly used in the next reaction without purification because of it instability. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.45 (s, 6H, CH_3), 4.17 (d, $J = 5$ Hz, 2H, CH_2N), 4.46 (m, 4H, CH_2–O), 4.91 (m, 1H, CH), 5.48 (s, 1H, NH), 7.18 (s, 1H, C5-H), 7.41 (m, 1H, Ar), 7.68 (m, 1H, Ar), 7.82 (m, 1H, Ar), 7.95 (s, 1H, C12-H), 8.41 (d, $J = 8.2$ Hz, 1H, C7-H). ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ: 38.2 (CH_2–N), 55.1 (CH_3), 64.0 and 60.5 (CH_2–O), 102.3 (CH, C-5), 104.5 (CH, C-12), 108.2 (CH, C-7), 112.5 (C, C-10a), 119.1 (CH, C-9), 121.8 (CH, C-8), 123.0 (C, C-11a), 138.2 (C, C-4a, C-12a), 142.8 and 143.1 (C, C-5a and C-6a), 151.1 (C, C-11). Anal. Calcd for $C_{19}H_{21}N_2O_4$; C, 67.05; H, 5.92; N, 8.23. Found: C, 67.33; H, 6.23; N, 8.45.

*4.1.4.6. 2-(2,3-Dihydro-1,4-benzodioxin-6-yl)aminonicotinic acid (**40**).* A suspension of 6-amino-2,3-dihydro-1,4-benzodioxin **19** (300 mg, 2 mmol), the 2-chloronicotinic acid (621 mg, 4 mmol) and NaH (60%, 105 mg, 4.3 mmol) in distilled DMF (3 ml) was stirred in an argon atmosphere at 90 °C for 24 h. The cooled mixture was neutralized with 2N HCl and extracted with ether (3 × 20 ml). The organic layers were dried, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 70:30) to give the corresponding nicotinic acid **40** as a white solid. m.p. (hexane/ethyl acetate): 130–131 °C. IR (KBr) υ cm^{-1}: 3400 (O–H and N–H), 1682 (C=O), 1290 (O–Ar), 1064 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.21 (m, 4H, CH_2O), 6.79 (m, 3H, C5-H, C5′-H, C8′-H), 7.18 (d, J = 2.6 Hz, 1H, C7′-H), 8.26 (bs, 2H, NH, OH), 8.54 (d, J = 7.4 Hz, C4-H), 8.79 (d, J = 7.6 Hz, C6-H). Anal.Calcd for $C_{14}H_{12}N_2O_4$; C, 61.76; H, 4.44; N, 10.29. Found: C, 62.10; H, 4.76; N, 10.45.

*4.1.4.7. 2,3,6,11-Tetrahydro-1,4-dioxino[2,3-g] pyrido[2,3-b] quinolin-11-ona (**41**).* A solution of nicotinic acid **40** (300 mg, 1.1 mmol) and a catalytic amount of trifluoroacetic acid in distilled CH_2Cl_2 was stirred at reflux temperature for 48 hours. The cooled mixture was basified with 2 N NaOH and extracted with CH_2Cl_2. The organic layers were dried, filtered and concentrated giving a crude product which was purified by silica gel column chromatography (hexane / ethyl acetate 80 / 20). Compound **41** was obtained as yellow oil (200 mg, 72% yield). IR (KBr) υ cm^{-1}: 1592 (C=O), 1267 (O–Ar), 1180 (C–O). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 4.21 (m, 4H, CH_2O), 6.52 (m, 1H, Ar), 6.78 (d, J = 8.4 Hz, 1H, Ar), 6.94 (dd, J_1 = 11, J_2 = 2.2 Hz, 1H, C9-H), 7.40 (bs, 1H, NH), 8.27 (s, 1H, C10-H), 8.43 (bs, 1H, C8-H). Anal. Calcd for $C_{14}H_{10}N_2O_3$; C, 66.14; H, 3.96; N, 11.02. Found: C, 66.45; H, 4.32; N, 11.45.

4.2. Biological experimental

4.2.1. Biological materials. cell culture and cytotoxicity [18]

L1210 leukemia and HT-29 cells were grown in nutrient medium RPMI 1640 or DNEM, respectively supplemented with 2 mM L-glutamine, 200 IU/ml penicillin, 50 μg/ml streptomycin, and 20% heat inactivated horse serum. They were incubated in a 5% CO_2 atmosphere at 37 °C. For the experiments the drugs were dissolved in dimethyl sulfoxide (0.5% final) and added to the cells in exponential phase of growth at an initial concentration of 0. 8×10^5 cells/ml. The cells were counted in quadruplicate after 48 h for L1210 cells and 96 h for HT-29 cells with Coultronics Coulter Counter and results were expressed as the drug concentration which inhibited cell growth by 50% as compared to the controls (IC_{50}). The IC_{50} values were calculated from regression lines obtained from the probit of the percent cell growth inhibition plotted as a function of the logarithm of the dose.

4.2.2. Inhibition of cellular proliferation and cell cycle effects [19]

L1210 leukemia cells were grown in nutrient medium RPMI 1640 supplemented with 2 mM L-glutamine, 200 IU/ml penicilium, 50 μg/ml streptomicyn, and 20% heat inactivated horse serum. They were incubated in a 5% CO_2 atmosphere at 37 °C for 21 h with several drug concentrations. Cells were then fixed by ethanol (70% v/v), then washed, and incubated with PBS containing 100 μM RNAse and 25 μM/ml propidium iodide for 30 min at room temperature. For each concentration, 10^4 cells were analyzed on an Epics XL flow cytomer (Modele Beckman Coulter, French). Results were expressed as a percentage of cells accumulated in each phase of the cell cycle and are indicated.

4.2.3. Interaction with DNA. UV-visible determinations [23]

Absorption spectra were recorded using a Kontron UVIKON 930 UV visible spectrophotometer. Measurements were made in different buffers at pH 7.0 at 20 °C. Spectra were recorded with a drug concentration of 50 μM, in the presence of 1 mM calf thymus DNA (purchased from Sigma Chemical) and 10 mM SDS, respectively.

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