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European Journal of Medicinal Chemistry 43 (2008) 2677–2687
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Structure–activity relationships in the acronycine and benzo[*b*]acronycine series: Role of the pyran ring

Quyên Do [a], Huong Doan Thi Mai [a], Thomas Gaslonde [a], Bruno Pfeiffer [b], Stéphane Léonce [b], Alain Pierré [b], Sylvie Michel [a], François Tillequin [a,*], Hanh Dufat [a]

[a] *Laboratoire de Pharmacognosie de l'Université Paris Descartes, U.M.R./C.N.R.S. n° 8638, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France*
[b] *Institut de Recherches Servier, Division Recherche Cancérologie, 125 Chemin de Ronde, 78290 Croissy sur Seine, France*

Received 20 July 2007; received in revised form 18 January 2008; accepted 24 January 2008
Available online 3 February 2008

Abstract

In order to explore the structure–activity relationships in the acronycine series, simplified analogues of *cis*-1,2-diacetoxy-1,2-dihydroacronycine and *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (S23906-1, under clinical trials) lacking the fused pyran ring, but possessing an acetoxymethyl leaving group at position 4 were prepared. These new analogues only displayed marginal antiproliferative activity compared to the parent compounds. The presence of the angularly fused dimethylpyran ring appears as an indispensable structural requirement to observe significant cytotoxic activity in this series.

Keywords: Acridone; Benzo[*b*]acridone; Acronycine; Cytotoxicity

1. Introduction

The pyranoacridone alkaloid acronycine (**1**), originally isolated from *Acronychia baueri* Schott (Rutaceae) [1–3], has shown antitumor properties against a large panel of murine solid tumor models [4,5]. However, its moderate potency and poor solubility in water severely hampered the subsequent clinical trials, which were rapidly discontinued due to modest therapeutic effects and dose-limiting gastrointestinal toxicity after oral administration [6]. Consequently, the development of structural analogues with increased potency and/or better solubility in biocompatible solvents was highly desirable (Chart 1).

Our efforts toward the obtainment of more potent derivatives were guided by a hypothesis of bioactivation of the 1,2-double bond of acronycine into the corresponding epoxide in vivo [7]. Significant improvements in terms of solubility and potency were obtained with derivatives modified in the pyran ring, which had a similar reactivity toward nucleophilic agents as acronycine epoxide, but an improved chemical stability. Such compounds are exemplified by diesters of *cis*-1,2-dihydroxy-1,2-dihydroacronycine (i.e., diacetate **2**) [8] and *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine ((±)-*cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one) [9]. Representatives of the latter series are considered as valuable drug candidates [10]. For instance, diacetate **3**, developed under the code S23906-1, is currently under phase I clinical trials. Their mechanism of action implies alkylation of the 2-amino group of DNA guanine residues by the carbocation resulting from the elimination of the ester leaving group at position 1 of the drug [11–14], inducing a marked destabilization of the double helix, with the formation of single-stranded DNA [15], which finally leads to cell apoptosis [16].

In the course of the exploration of the structure–activity relationships [17,18], the presence of a methoxy electron-donating group at C-6, which facilitates the formation and stabilization of a carbocation at the benzylic position 1, was

* Corresponding author. Tel.: +33 1 53 73 98 10; fax: +33 1 40 46 96 58.
E-mail address: francois.tillequin@univ-paris5.fr (F. Tillequin).

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doi:10.1016/j.ejmech.2008.01.033

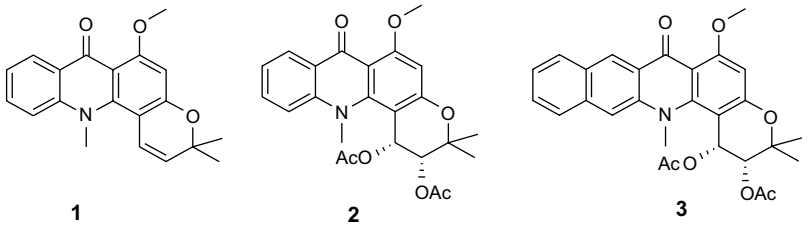

Chart 1.

shown to have a marked influence on the activity and appeared as an important structural feature to observe potent cytotoxic effects in both acronycine and benzo[*b*]acronycine series [8,12]. The surprising activity of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine monoesters at position 2 could be rationalized on the basis of spontaneous transesterification into the isomeric *cis*-monoesters at position 1 [12,14]. In contrast, Michael acceptors in the benzo[*b*]acronycine series, possessing a 1,2-pyran double bond substituted at position 2 by an acyl group, were devoid of significant cytotoxic activity, most probably in correlation with the high delocalization of the electrons in the large polyaromatic chromophore [19].

The purpose of this work is to determine whether the presence of the angularly fused pyran ring present in acronycine and S23906 is important or not to observe biological activity. We describe here the synthesis and antiproliferative activities of simplified compounds in the acridone and benzo[*b*]acridone series, lacking the fused pyran ring, but possessing an acyloxymethyl leaving group at position 4, hence potentially able to undergo additions onto intracellular nucleophilic targets. Esters of 4-hydroxymethyl-acridones and -benzo[*b*]acridones bearing alkoxy substituents at position 3 or positions 1 and 3, which mimic the methoxy group and the pyran ring oxygen of acronycine derivatives, respectively, have been envisaged.

2. Synthesis and biological activity of 1,3-dialkoxy-4-hydroxymethylacridone and benzo[*b*]acridone esters

2.1. Chemistry

The access to 4-acetoxymethyl-1,3-dimethoxy-10-methylacridone (**4**), a simplified analogue of *cis*-1,2-dihydroxy-1,2-dihydroacronycine diacetate (**2**), was envisioned from the readily available 1,3-dimethoxy-10-methylacridone-4-carbaldehyde (**5**). Indeed, this latter compound can be conveniently prepared through Ullmann condensation of 2-chlorobenzoic acid and 3,5-dimethoxyaniline, followed by simultaneous cyclization and formylation of the intermediate carboxylic diarylamine under Vilsmeier–Haack conditions, and final *N*-methylation [20]. Sodium borohydride reduction of **5** smoothly afforded 4-hydromethyl-1,3-dimethoxy-10-methylacridone (**6**) in almost quantitative yield. Acetylation of **6** with acetic anhydride in pyridine gave **4**, which was purified by column chromatography over neutral alumina, using a mixture of cyclohexane and acetone as solvent. In contrast, all attempts to purify **4** over silica gel column, using dichloromethane–methanol mixture as solvent, only resulted in the isolation of 1,3-dimethoxy-4-methoxymethyl-10-methylacridone (**7**), giving evidence for the high reactivity of **4** toward nucleophilic agents under mild acidic conditions (Chart 2).

A similar approach was first considered for the synthesis of a 1,3-dialkoxy-4-hydroxymethylbenzo[*b*]acridone ester analogue of S23906-1 (**3**). Condensation of 3-bromo-2-naphthoic acid (**8**) with 3,5-dimethoxyaniline (**9**) under Ullmann conditions gave 3-(3,5-dimethoxyphenyl)-amino-2-naphthoic acid (**10**). However, Vilsmeier–Haack formylation of **10**, by treatment with phosphoryl chloride in *N*,*N*-dimethylformamide, led to 1,3-dimethoxybenzo[*b*]acridone-6-carbaldehyde (**11**), accompanied by smaller amounts of 1,3-dimethoxybenzo[*b*]acridone (**12**), which could not be further formylated at position 4. Consequently, obtainment of a conveniently substituted benzoacridone basic core bearing a carbaldehyde group at position 4 was envisaged through periodate oxidation of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine (**13**), since this

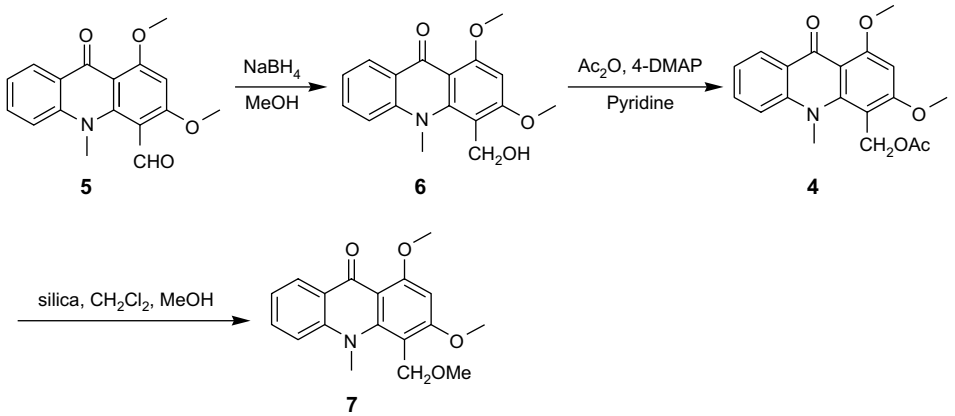

Chart 2.

method had previously given satisfactory results for pyran ring opening in the acronycine series [21,22]. Thus, treatment of **13** with sodium periodate in acetone afforded the dialdehyde **14** in almost quantitative yield. Reduction of the carbaldehyde groups of **14** with sodium borohydride gave the diol **15** in 68% yield. Finally, **15** was acetylated to the desired ester **16** upon treatment with excess acetic anhydride in pyridine. Due to reactivity similar to that of **4** in the presence of silica, purification of **16** was conducted by column chromatography over neutral alumina, using dichloromethane as eluent (Charts 3 and 4).

2.2. Pharmacology

Compounds **6**, **4**, **7**, **15**, and **16** were evaluated *in vitro* for their cytotoxicity against two tumor cell lines, a murine leukemia cell line (L1210) and a human epidermoid carcinoma cell line (KB-3-1), in comparison with *cis*-1,2-diacetoxy-1,2-dihydroacronycine (**2**) and *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (**3**). The results (IC_{50}) are reported in Table 1.

2.3. Results and discussion

As expected, diacetate **16**, with an acyloxymethyl leaving group at position 4, displayed significant cytotoxic activity against both cell lines, even if 10- to 50-fold less potent than its pentacyclic pyran counterpart **3**. Similarly, the fact that compounds **6**, **7**, and **15**, whose structures do not include an ester leaving group at the benzylic position, were found almost inactive appears to be in full agreement with the mechanism of action previously established for *cis*-1,2-dihydroxy-1,2-dihydroacronycine and *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine esters and diesters [12–14]. In contrast, the lack of activity of 4-acetoxymethyl-1,3-dimethoxy-10-methylacridin-9(10*H*)-one (**4**) is more surprising, but can be most probably rationalized on the basis of high chemical instability of this compound which readily reacts with nucleophiles, as shown by its facile total conversion into **7** in the presence of methanol in slightly acidic medium. In this context, it seems reasonable to hypothesize that **4** should be destroyed in the culture medium, cell membrane or cytoplasm before it can reach its nuclear target. This latter assumption prompted us to synthesize and evaluate 3-alkoxy-4-hydroxymethyl-acridone and -benzo[*b*]acridone esters, lacking the methoxy electron-donating group at C-1, known to facilitate the formation and stabilization of a carbocation at the benzylic position [12].

3. Synthesis and biological activity of 3-alkoxy-4-hydroxymethylacridone and benzo[*b*]acridone esters

3.1. Chemistry

In a first attempt, access to 4-hydroxymethyl-3-methoxy-10-methylacridone (**17**) was envisioned through reduction of a carbaldehyde obtained by formylation of 3-methoxyacridone (**18**) [23]. Condensation of 2-chlorobenzoic acid (**19**) with 3-methoxyaniline (**20**) gave 2-(3-methoxyphenyl)-aminobenzoic acid (**21**) [23]. Treatment of **21** under Vilsmeier–Haack conditions only resulted in cyclization into 3-methoxyacridone (**18**) in a moderate 10% yield, without simultaneous formylation reaction, and all further attempts to formylate **18** at position 4 remained unsuccessful. Therefore, a second approach, whose key step was the oxidation of the methyl group of a 4-methylacridone, was considered. The starting material, 3-methoxy-2-methylaniline (**22**), was prepared from commercially available 2-methyl-3-nitrophenol, which was converted to 2-methoxy-6-nitrotoluene (**23**) in 94% yield by treatment with methyl iodide in *N*,*N*-dimethylformamide in the presence of sodium hydride. Reduction of **23** by zinc wool in acetic acid afforded **22** in 92% yield. Ullmann condensation of **22** with 2-chlorobenzoic acid (**19**) gave 2-(3-methoxy-2-methylphenyl)-aminobenzoic acid (**24**) [24,25] in an acceptable 52% yield. Cyclization of **24** by trifluoroacetic anhydride in dichloromethane [26,27] gave 3-methoxy-4-methylacridone (**25**) in 85% yield, which was methylated to 3-methoxy-4,10-dimethylacridone (**26**) in 72% yield by the use of methyl iodide and sodium hydride in tetrahydrofuran. Conversion of **26** into the desired 4-hydroxymethyl-3-methoxy-10-methylacridone (**17**) was ensured in a one-pot process involving benzylic bromination by *N*-bromosuccinimide followed by hydrolysis of the bromine atom. Under those conditions, compound **17** was isolated

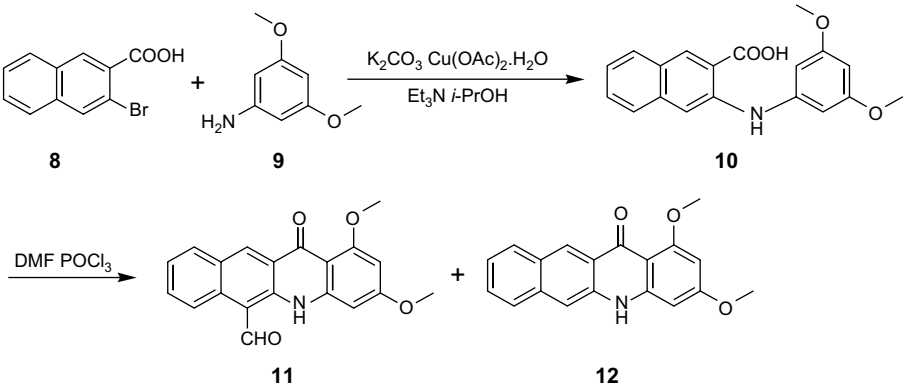

Chart 3.

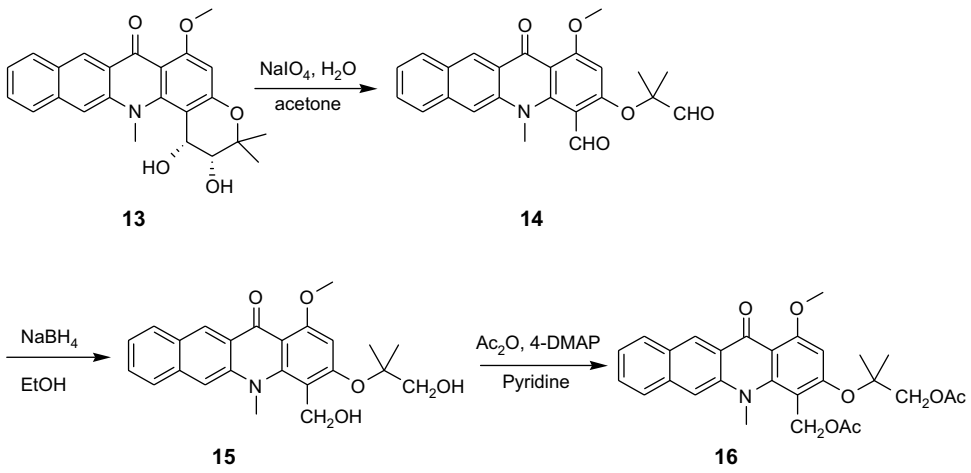

Chart 4.

in 25% yield after column chromatography over silica gel, accompanied by 2-bromo-3-methoxy-4,10-dimethylacridone (**27**) obtained in 3% yield. Finally, acetylation of **17** with acetic anhydride in anhydrous pyridine gave 4-acetoxymethyl-3-methoxy-10-methylacridone (**28**) in 65% yield (Charts 5 and 6).

A similar approach was used for the preparation of 4-acetoxymethyl-3-methoxy-5-methylbenzo[*b*]acridin-12(5*H*)-one (**29**). Ullmann condensation of 3-methoxy-2-methylaniline (**22**) with 3-bromo-2-naphthoic acid (**8**) gave 3-(3methoxy-2-methylphenyl)-amino-2-naphthoic acid (**30**) in 40% yield. Cyclization of **30** with trifluoroacetic anhydride in dichloromethane gave 3-methoxy-4-methylbenzo[*b*]acridin-12(5*H*)-one (**31**) in 47% yield. Methylation of **31** upon treatment with methyl iodide and sodium hydride in tetrahydrofuran afforded 3-methoxy-4,5-dimethylbenzo[*b*]acridin-12(5*H*)-one (**32**) in 94% yield. Benzylic bromination with *N*-bromosuccinimide followed by hydrolysis gave 4-hydroxymethyl-3-methoxy-5-methylbenzo[*b*]acridin-12(5*H*)-one (**33**) in 13% overall yield. Acetylation of **33** afforded the desired acetate **29** in 65% yield (Chart 7).

Table 1
Cytotoxic Activity of Compounds **6**, **4**, **7**, **15**, **16**, **17**, **28**, **33**, and **29** in comparison with *cis*-1,2-diacetoxy-1,2-dihydroacronycine (**2**) and *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (S23906-1) (**3**)

Compound	Cytotoxicity IC_{50} (μM)	
	L1210	KB-3-1
6	23.9	34.1
4	39.9	17.7
7	51.7	36.2
15	1.3	26.2
16	3	4.9
17	13.9	n.d.
28	24	n.d.
33	34.6	22.5
29	18.5	7.9
2	3.4	n.d.
3	0.79	0.095

n.d., Not determined.

3.2. Pharmacology

When evaluated *in vitro* against L1210 and KB-3-1 cell lines under conditions similar to those previously described for their 1-methoxy counterparts, compounds **17**, **28**, **33**, and **29** were found almost devoid of significant cytotoxic activity, as shown in Table 1.

4. Conclusion

In conclusion, the simplified compounds lacking the fused pyran ring, prepared on the models of *cis*-1,2-dihydroxy-1,2-dihydroacronycine and *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine esters and diesters only displayed marginal antiproliferative activity compared to the parent compounds **2** and **3**. In terms of structure–activity relationships, the presence of the angularly fused dimethylpyran ring appears as an indispensable structural requirement to observe significant cytotoxic activity. This 2,2-dimethylpyran motif fused onto an aromatic or heteroaromatic basic core is frequently encountered in natural products, in which it arises from the condensation of a phenol with an active isoprene unit. It appears as a ''privileged structure'' present in numerous bioactive compounds [28,29], including several cytotoxic, antitumor, or antineoplastic agents exemplified by the isoflavonoids amorphispironone and tephrosin [30], the quinolone 4,8,8-trimethyl-1,8-dihydro-2*H*-pyrano[2,3-*h*]quinolin-2-one [31], the phloroglucinol derived calanone [32], and the quinone β-lapachone [33].

5. Experimental section

5.1. Chemistry

Melting points were determined on a hot stage Reichert microscope and are not corrected. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets or sodium chloride films on a Nicolet 510 FT-IR instrument. UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on

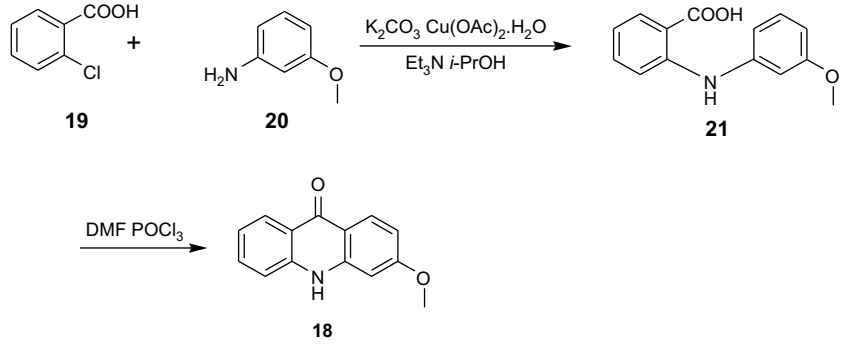

Chart 5.

a Beckman DU 640 spectrophotometer. ^{1}H NMR (δ [ppm], J [Hz]) were recorded at 400 MHz, using a Bruker Avance 400 spectrometer. ^{13}C NMR spectra were recorded at 75 MHz or 100 MHz, using Bruker AC-300 or Avance 400 spectrometers. When necessary, the signals were unambiguously assigned by 2D NMR techniques: ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HMQC, and ^{13}C–^{1}H HMBC. These experiments were performed using standard Bruker microprograms. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electrospray ionization (ES-MS; $V_c = 30$ V), or with a Nermag R-10-10C spectrometer using desorption-chemical ionization (DCI-MS; reagent gas: NH_3). Flash column chromatographies were performed using silica gel SDS (Chromatogel 60, 35–70 μm) with an overpressure of 300 mbar.

*5.1.1. 4-Hydroxymethyl-1,3-dimethoxy-10-methylacridin-9(10H)-one (**6**)*

Sodium borohydride (80 mg, 2.2 mmol) was added at 0 °C to a solution of **5** (130 mg, 0.44 mmol) in MeOH (5 mL) and the reaction mixture was stirred at 25 °C for 30 min. After addition of water (15 mL), the mixture was extracted with CH_2Cl_2 (5 × 50 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.5:0.5 to 95:5) gave **6** (122 mg, 93%) as a pale yellow amorphous solid. UV λ nm (MeOH) (log ε) 215 (3.88), 251 (4.12), 274 (4.83), 297 (4.44), 387 (4.26); IR (KBr) ν, cm^{-1} 3370, 3047, 2934, 1649, 1620, 1594, 1578, 1555, 1507, 1459, 1391, 1320, 1239, 1207, 1133, 1097, 1055, 1029, 942, 805, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (1H, dd, $J = 8$, 1.5 Hz, H_8), 7.65 (1H, td, $J = 8$, 1,5 Hz, H_6), 7.38 (1H, dd, $J = 8$, 1,5 Hz, H_5), 7.23 (1H, td, $J = 8$, 1,5 Hz, H_7), 6.36 (1H, s, H_2), 4.76 (2H, s, CH_2–OH), 4.04 (3H, s, C_1–OCH_3), 4.02 (6H, s, C_3–OCH_3 et NCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177,2 (C_9), 164.0 (C_3), 162.9 (C_1), 149.6 (C_{4a}), 145.0 (C_{10a}), 132.8 (C_6), 127.0 (C_8), 125.0 (C_{8a}), 121.6 (C_7), 116.1 (C_5), 111.0 (C_{9a}), 108.2 (C_4), 89.2 (C_2), 58.4 (C_4–CH_2OH), 56.4 (C_1–OCH_3), 56.0 (C_3–OCH_3), 44.8 (NCH_3); ES-MS *m/z* 300 $[MH]^+$, 322 $[MNa]^+$. Anal. Calcd. for $C_{17}H_{17}NO_4$: C 68.21; H 5.72; N 4.68%. Found: C 68.14; H 5.78; N 4.72%.

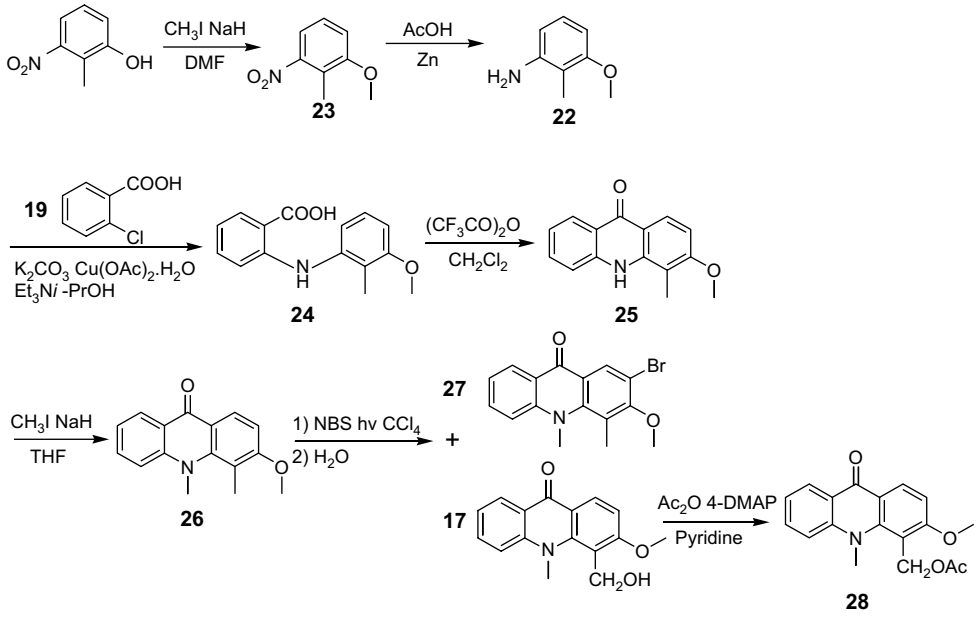

Chart 6.

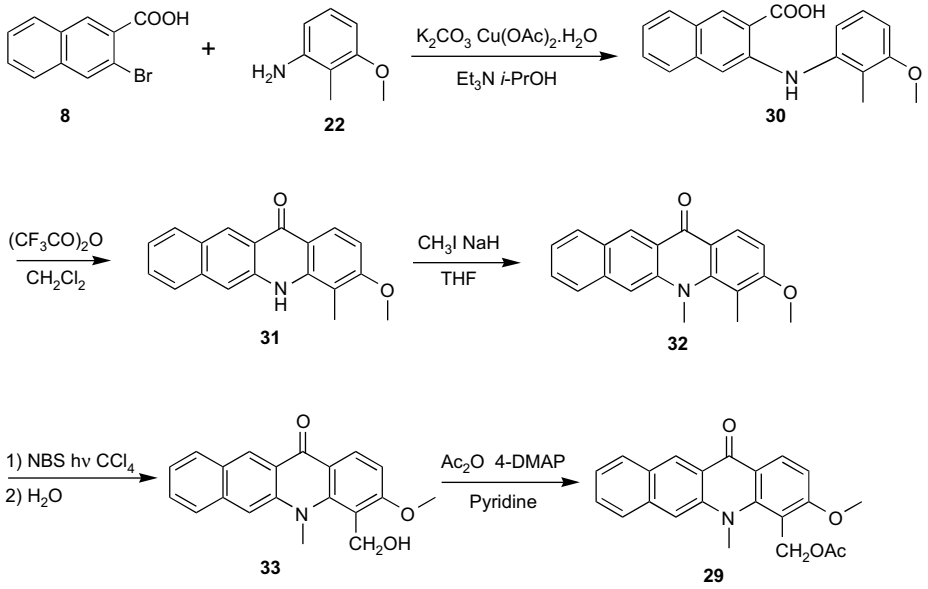

Chart 7.

*5.1.2. 4-Acetoxymethyl-1,3-dimethoxy-10-methylacridin-9(10H)-one (**4**)*

Acetic anhydride (92 μL, 0.1 mmol) was added to an ice-cooled solution of **6** (30 mg, 0.1 mmol) and 4-dimethylaminopyridine (2 mg) in dry pyridine (1.5 mL). After stirring at 25 °C for 3 h, the reaction mixture was poured on cold water (10 mL). The precipitate was filtered, washed with water (2 × 5 mL), and dried in vacuum over P_2O_5. Column chromatography over neutral alumina (solvent: cyclohexane, then cyclohexane/Me_2CO, 99:1 to 95:5) gave **4** (21 mg, 61%) as a whitish amorphous solid. UV λ nm (MeOH) (log ε) 215 (3.79), 251 (4.11), 274 (4.82), 297 (4.42), 386 (4.00); IR (KBr) ν, cm^{-1} 2997, 2925, 2845, 1727, 1638, 1630, 1602, 1587, 1578, 1496, 1468, 1440, 1392, 1313, 1237, 1210, 1143, 1091, 1058, 1013, 958, 767; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, dd, $J = 8$, 1.5 Hz, H_8), 7.62 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.32 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7.22 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.38 (1H, s, H_2), 5.22 (2H, s, CH_2OAc), 4.05 (3H, s, OCH_3), 3.99 (3H, s, NCH_3), 3.86 (3H, s, OCH_3), 2.17 (3H, s, $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7 (C_9), 171.3 ($OCOCH_3$), 164.8 (C_3), 163.6 (C_1), 150.8 (C_{4a}), 145.2 (C_{10a}), 132.8 (C_6), 127.0 (C_8), 125.3 (C_{8a}), 121.8 (C_7), 116.2 (C_5), 111.0 (C_{9a}), 102.9 (C_4), 89.4 (C_2), 60.2 (CH_2OAc), 56.4 (C_1–OCH_3), 56.1 (C_3–OCH_3), 44.3 (NCH_3), 21.2 ($OCOCH_3$); ES-MS *m/z* 364 $[MNa]^+$. Anal. Calcd. for $C_{19}H_{19}NO_5$: C 66.85; H 5.61; N 4.10%. Found: C 68.84; H 5.66; N 4.07%.

*5.1.3. 1,3-Dimethoxy-4-methoxymethyl-10-methylacridin-9(10H)-one (**7**)*

Preparation of **7** followed the procedure described for the preparation of **4** from **6**, but the final column chromatography was conducted over silica gel (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99.8:0.2 to 98:2). Compound **7** (18 mg, 56%) was obtained as a pale yellow amorphous solid. UV λ nm (MeOH) (log ε) 215 (3.47), 251 (3.76), 273 (4.49), 296 (4.07), 385 (3.89); IR (KBr) ν, cm^{-1} 3014, 2922, 1632, 1596, 1579, 1498, 1460, 1392, 1312, 1214, 1137, 1128, 1092, 1051, 941, 762; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, dd, $J = 8$, 1.5 Hz, H_8), 7.60 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.38 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7.22 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.36 (1H, s, H_2), 4.46 (2H, s, CH_2OCH_3), 4.03 (3H, s, C_1–OCH_3), 4.01 (3H, s, NCH_3), 4.00 (3H, s, C_3–OCH_3), 3.53 (3H, s, CH_2OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0 (C_9), 164.6 (C_3), 163.0 (C_1), 150.6 (C_{4a}), 145.2 (C_{10a}), 132.8 (C_6), 127.1 (C_8), 125.2 (C_{8a}), 121.6 (C_7), 116.2 (C_5), 110.9 (C_{9a}), 105.7 (C_4), 89.4 (C_2), 67.2 (CH_2OCH_3), 58.8 (CH_2OCH_3), 56.4 (C_1–OCH_3), 56.2 (C_3–OCH_3), 43.6 (NCH_3); ES-MS *m/z* 314 $[MH]^+$, 336 $[MNa]^+$. Anal. Calcd. for $C_{18}H_{19}NO_4$: C 68.99; H 6.11; N 4.47%. Found: C 69.06; H 6.08; N 4.41%.

*5.1.4. 3-(3,5-Dimethoxyphenyl)-amino-2-naphthoic acid (**10**)*

A mixture of **8** (3.2 g, 13.1 mmol), **9** (2.0 g, 13.1 mmol), potassium acetate (2.65 g), cupric acetate monohydrate (0.08 g), and triethylamine (1.5 mL) in 2-propanol (50 mL) was heated under reflux for 48 h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between CH_2Cl_2 and 1 N aqueous HCl. The aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 90:10) gave **10** (2.5 g, 58%) as a pale yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (1H, br s, NH), 8.73 (1H, s, H_1), 7.79 (1H, d, $J = 8$ Hz, H_8), 7.67 (1H, s, H_4), 7.58 (1H, d, $J = 8$ Hz, H_5), 7.47 (1H, t, $J = 8$ Hz, H_6), 7.28 (1H, t, $J = 8$ Hz, H_7), 6.55 (2H, d, $J = 2.5$ Hz, $H_{2'}$, $H_{6'}$), 6.27 (1H, t, $J = 2.5$ Hz, $H_{4'}$), 3.84 (6H, s, 2 × OCH_3); ^{13}C NMR

(75 MHz, $CDCl_3$) δ 172.8 (COOH), 143.2 (C_3), 142.9 (2C, $C_{3'}$, $C_{5'}$), 137.6 (C_{4a}), 135.3 (2C, C_1, C_{4a}), 129.5 (C_8), 129.3 (C_6), 126.4 ($C_{1'}$), 126.0 (C_5), 124.2 (C_{8a}), 123.5 (C_7), 109.8 (C_4), 99.9 (2C, $C_{2'}$, $C_{6'}$), 95.6 ($C_{4'}$), 55.4 (2C, $2 \times OCH_3$); ES-MS *m/z* 324 $[MH]^+$, 346 $[MNa]^+$. Anal. Calcd. for $C_{19}H_{17}NO_4$: C 70.58; H 5.30; N 4.33%. Found: C 70.54; H 5.28; N 4.32%.

*5.1.5. 1,3-Dimethoxybenzo[b]acridin-12(5H)-one-6-carbaldehyde (**11**) and 1,3-dimethoxybenzo[b]acridin-12(5H)-one (**12**)*

The diarylamine **10** (650 mg, 20 mmol) was slowly added (40 min at 25 °C) to a well-stirred mixture of dry DMF (2 mL) and $POCl_3$ (1 mL), and stirring was continued for 2 h. The dark viscous liquid was diluted with water (10 mL), and 2 M NaOH aqueous solution (20 mL) was added. The solution was boiled for 0.5 h and filtered. The precipitate was washed with water (2×10 mL), and dried in vacuum over P_2O_5. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 95:5) successively gave **11** (393 mg, 59%) and **12** (85 mg, 14%) as yellow amorphous solids.

*5.1.5.1. 1,3-Dimethoxybenzo[b]acridin-12(5H)-one-6-carbaldehyde (**11**).* ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (1H, s, CHO), 9.20 (1H, s, H_{11}), 8.77 (1H, d, $J = 8$ Hz, H_7), 8.10 (1H, d, $J = 8$ Hz, H_{10}), 7.90 (1H, t, $J = 8$ Hz, H_8), 7.74 (1H, t, $J = 8$ Hz, H_9), 7.07 (1H, d, $J = 1.5$ Hz, H_4), 6.61 (1H, d, $J = 1.5$ Hz, H_2), 4.12 (3H, s, OCH_3), 4.05 (3H, s, OCH_3); ES-MS *m/z* 334 $[MH]^+$. Anal. Calcd. for $C_{20}H_{15}NO_4$: C 72.06; H 4.54; N 4.20%. Found: C 72.14; H 4.58; N 4.12%.

5.1.5.2. . 1,3-Dimethoxybenzo[*b*]acridin-12(5*H*)-one (**12**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (1H, s, H_{11}), 7.94 (1H, d, $J = 8$ Hz, H_{10}), 7.70 (1H, d, $J = 8$ Hz, H_7), 7.45 (1H, s, H_6), 7.43 (1H, t, $J = 8$ Hz, H_8), 7.30 (1H, t, $J = 8$ Hz, H_9), 6.15 (1H, d, $J = 1.5$ Hz, H_4), 6.02 (1H, d, $J = 1.5$ Hz, H_2), 3.93 (3H, s, OCH_3), 3.81 (3H, s, OCH_3); ES-MS *m/z* 306 $[MH]^+$, 328 $[MNa]^+$. Anal. Calcd. for $C_{19}H_{15}NO_3$: C 74.74; H 4.95; N 4.59%. Found: C 74.83; H 5.01; N 4.52%.

*5.1.6. 1-Methoxy-5-methyl-3-(2-oxo-1,1-dimethylethoxy)benzo[b]acridin-12-oxo-5,12-dihyro-4-carbaldehyde (**14**)*

A solution of sodium periodate (526 mg, 3.14 mmol) in water (40 mL) was added dropwise to a solution of (±)-*cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**13**) (500 mg, 1.23 mmol) in acetone (40 mL). The reaction mixture was stirred at 25 °C for 4 days, filtered, and evaporated under reduced pressure. After addition of water (100 mL), the residue was extracted with EtOAc (4×100 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/Me_2CO, 99:1 to 80:20) gave **14** (470 mg, 94%) as a pale yellow amorphous solid. UV λ nm (MeOH) (log ε) 251 (4.44), 275 (4.73), 313 (4.82), 430 (4.01); IR (KBr) ν, cm^{-1} 3055, 2975, 2912, 2845, 1730, 1644, 1621, 1596, 1555, 1486, 1460, 1387, 1321, 1291, 1235, 1251, 1139, 1117, 1088, 1043, 1007, 877, 815; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (1H, s, C_4–CHO), 9.86 (1H, s, C_3–O–$C(CH_3)_2CHO$), 8.92 (1H, s, H_{11}), 8.03 (1H, d, $J = 8$ Hz, H_{10}), 7.91 (1H, d, $J = 8$ Hz, H_7), 7.81 (1H, s, H_6), 7.57 (1H, t, $J = 8$ Hz, H_8), 7.45 (1H, t, $J = 8$ Hz, H_9), 5.97 (1H, s, H_2), 3.98 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 1.66 (6H, s, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.1 (C_3–O–$C(CH_3)_2CHO$), 185.7 (C_4–*C*HO), 177.3 (C_{12}), 167.6 (C_3), 165.6 (C_1), 149.8 (C_{4a}), 140.4 (C_{5a}), 135.9 (C_{6a}), 129.6 (C_{10}), 129.2 (C_{10a}), 128.6 (C_8), 128.2 (C_{11}), 127.2 (C_7), 125.2 (C_9), 124.9 (C_{11a}), 113.7 (C_6), 109.5 (C_{12a}), 91.8 (C_2), 85.3 (C_4), 77.1 ($C(CH_3)_2$), 55.7 (OCH_3), 45.3 (NCH_3), 21.9 ($C(CH_3)_2$); DCI-MS *m/z* 404 $[MH]^+$. Anal. Calcd. for $C_{24}H_{21}NO_5$: C 71.45; H 5.25; N 3.47%. Found: C 71.51; H 5.29; N 3.43%.

*5.1.7. 4-Hydroxymethyl-5-methyl-3-(1,1-dimethyl-2-hydroxyethoxy)-1-methoxybenzo[b]acridin-12(5H)-one (**15**)*

Sodium borohydride (43 mg, 1.14 mmol) was added to a solution of **14** (80 mg, 0.19 mmol) in EtOH (5 mL) and the reaction mixture was stirred at 25 °C for 24 h. After addition of water (20 mL), the mixture was extracted with CH_2Cl_2 (5×50 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Silica gel column chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/EtOH, 99:1 to 70:30) gave **15** (55 mg, 68%) as a pale yellow amorphous solid. UV λ nm (MeOH) (log ε) 251 (3.95), 280 (4.82), 289 (4.77), 342 (4.13), 440 (3.74); IR (KBr) ν, cm^{-1} 3342, 2962, 2848, 2356, 1638, 1611, 1581, 1463, 1436, 1358, 1327, 1243, 1201, 1131, 1089, 1023, 920, 869, 803; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (1H, s, H_{11}), 8.08 (1H, d, $J = 8$ Hz, H_{10}), 7.98 (1H, d, $J = 8$ Hz, H_7), 7.93 (1H, s, H_6), 7.58 (1H, t, $J = 8$ Hz, H_8), 7.42 (1H, t, $J = 8$ Hz, H_9), 6.51 (1H, s, H_2), 5.00 (1H, t, $J = 5$ Hz, D_2O exch., C_4–CH_2–O*H*), 4.86 (1H, s, D_2O exch., $C(CH_3)_2$–CH_2O*H*), 4.67 (2H, d, $J = 5$ Hz, C_4–CH_2–OH), 4.06 (3H, s, OCH_3), 3.96 (2H, s, $C(CH_3)_2$–CH_2–OH), 3.92 (3H, s, NCH_3), 1.28 (6H, s, $C(CH_3)_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.7 (C_{12}), 165.1 (C_3), 163.1 (C_1), 150.6 (C_{4a}), 143.0 (C_{5a}), 136.5 (C_{6a}), 130.2 (C_{10}), 129.1 (C_8), 128.7 (C_{10a}), 127.9 (C_{11}), 127.4 (C_7), 125.9 (C_{11a}), 125.4 (C_9), 113.5 (C_6), 110.2 (C_{12a}), 92.1 (C_2), 78.1 (2C, C_4, $C(CH_3)_2$), 70.0 ($C(CH_3)_2CH_2$–OH), 57.1 (C_4–CH_2OH), 56.9 (OCH_3), 45.4 (NCH_3), 27,6 ($C(CH_3)_2$); ES-MS *m/z* 407 $[M]^+$. Anal. Calcd. for $C_{24}H_{25}NO_5$: C 70.74; H 6.18; N 3.44%. Found: C 70.81; H 6.21; N 3.42%.

*5.1.8. 2-(4-acetoxymethyl-5-methyl-1-methoxy-12-oxo-5,12-dihydrobenzo[b]acridin-3-oxy)-2-methylpropyl acetate (**16**)*

An ice-cooled mixture of acetic anhydride (0.2 mL, 2.2 mmol) and dry pyridine (1.5 mL) was added to **15** (30 mg, 0.074 mmol) and 4-dimethylaminopyridine (2 mg). After stirring at room temperature for 24 h, the mixture was poured on cold water (20 mL). The precipitate was filtered, washed with water (2×5 mL), and dried in vacuum over P_2O_5. Column chromatography over neutral alumina (solvent: CH_2Cl_2, then

CH_2Cl_2/Me_2CO, 99:1 to 98:2) gave **16** (30 mg, 84%) as a yellow amorphous solid. UV λ nm (MeOH) (log ε) 251 (4.37), 280 (4.91), 289 (4.91), 339 (4.25), 435 (3.81); IR (KBr) ν, cm^{-1} 3054, 2913, 2846, 1738, 1649, 1620, 1584, 1495, 1463, 1440, 1402, 1325, 1327, 1248, 1204, 1146, 1088, 1066, 1018, 957; ^1H NMR (400 MHz, $CDCl_3$) δ 8.91 (1H, s, H_{11}), 8.01 (1H, d, $J = 8$ Hz, H_{10}), 7.85 (1H, d, $J = 8$ Hz, H_7), 7.63 (1H, s, H_6), 7.54 (1H, t, $J = 8$ Hz, H_8), 7.41 (1H, t, $J = 8$ Hz, H_9), 6.30 (1H, s, H_2), 5.25 (2H, s, C_4-CH_2-OAc), 4.30 (2H, s, $C(CH_3)_2-CH_2OAc$), 4.06 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 2.18 (3H, s, $C_4-CH_2OCOCH_3$), 2.04 (3H, s, $OC(CH_3)_2-CH_2OCOCH_3$), 1.60 (6H, s, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7 (C_{12}), 171.3 ($C(CH_3)_2CH_2OCOCH_3$), 170.7 ($C_4-CH_2-OCOCH_3$), 164.3 (C_3), 163.9 (C_1), 151.6 (C_{4a}), 142.4 (C_{5a}), 136.0 (C_{6a}), 129.7 (C_{10}), 128.7 (C_{10a}), 128.4 (C_8), 128.1 (C_{11}), 126.9 (C_7), 125.5 (C_{11a}), 124.6 (C_9), 112.3 (C_6), 110.4 (C_{12a}), 103.0 (C_4), 89.8 (C_2), 80.2 ($C_3-O-C(CH_3)_2$), 73.1 ($C(CH_3)_2CH_2-OCOCH_3$), 57.1 ($C_4-CH_2OCOCH_3$), 56.5 (OCH_3), 4.,9 (NCH_3), 23.8 ($C(CH_3)_2$), 22.4 ($C(CH_3)_2CH_2-OCOCH_3$), 21.2 ($C_4-CH_2OCOCH_3$); ES-MS *m/z* 492 $[MH]^+$, 514 $[MNa]^+$, 530 $[MK]^+$. Anal. Calcd. for $C_{28}H_{29}NO_7$: C 68.42; H 5.95; N 2.85%. Found: C 68.37; H 5.98; N 2.89%.

5.1.9. 2-(3-Methoxy-2-methylphenyl)-aminobenzoic acid (24)

A solution of **19** (1.33 g, 8.5 mmol), **22** (1.11 g, 8.1 mmol), potassium acetate (1.74 g), and cupric acetate monohydrate (0.05 g), and triethylamine (1 mL) in anhydrous 2-propanol (50 mL) was heated under reflux for 48 h. The reaction mixture was evaporated under reduced pressure and the residue was partitionned between CH_2Cl_2 (100 mL) and 1 N aqueous HCl (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 95:15) gave **24** (1.1 g, 52%) as whitish needles: m.p. 197–199 °C (recrystallized from CH_2Cl_2). IR (KBr) ν, cm^{-1} 2919, 1658, 1594, 1575, 1500, 1441, 1272, 1164, 776, 749, 730; ^1H NMR (400 MHz, $CDCl_3$) δ 9.15 (1H, s, NH), 8.02 (1H, d, $J = 8$ Hz, H_6), 7.30 (1H, t, $J = 8$ Hz, H_4), 7.19 (1H, t, $J = 8$ Hz, $H_{5'}$), 6.96 (1H, d, $J = 8$ Hz, $H_{4'}$), 6.81 (1H, d, $J = 8$ Hz, H_3), 6.75 (1H, d, $J = 8$ Hz, $H_{2'}$), 6.71 (1H, t, $J = 8$ Hz, H_5), 3.89 (3H, s, OCH_3), 2.15 (3H, s, $Ar-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2 (COOH), 158.8 ($C_{3'}$), 150.0 (C_2), 139.6 ($C_{1'}$), 135.3 (C_4), 132.5 (C_6), 126.6 ($C_{5'}$), 122.6 ($C_{2'}$), 117.9 ($C_{4'}$), 116.5 (C_5), 114.1 (C_3), 109.5 (C_1), 107.3 ($C_{6'}$), 55.8 (OCH_3), 10,6 ($Ar-CH_3$).; ES-MS *m/z* 258 $[MH]^+$, 280 $[MNa]^+$, 296 $[MK]^+$. Anal. Calcd. for $C_{15}H_{15}NO_3$: C 70.02; H 5.88; N 5.44%. Found: C 69.97; H 5.85; N 5.47%.

5.1.10. 3-Methoxy-4-methylacridin-9(10H)-one (25)

Trifluoroacetic anhydride (5.2 mL) was added to a solution of **24** (0.95 g, 3.7 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at room temperature for 48 h, evaporated under reduced pressure, and taken up by CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic phase was shaken for 15 min with 1 N NaOH (50 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **25** (0.75 g, 85%) as a pale yellow amorphous solid. IR (KBr) ν, cm^{-1} 2921, 2849, 1722, 1597, 1463, 1262, 1159; ^1H NMR (400 MHz, $CDCl_3$) δ 8.45 (1H, br s, NH), 8.39 (1H, dd, $J = 8$, 1.5 Hz, H_8), 8,34 (1H, d, $J = 9$ Hz, H_1), 7.61 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.40 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7,23 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.93 (1H, d, $J = 9$ Hz, H_2), 3.96 (3H, s, OCH_3), 2.36 (3H, s, $Ar-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6 (C_9), 160.8 (C_3), 147.0 (2C, C_{4a}, C_{10a}), 133.3 (C_6), 126.8 (C_8), 126.5 (C_1), 122.3 (C_{8a}), 121.5 (C_7), 120.7 (C_{9a}), 116.6 (C_5), 115.9 (C_4), 106.5 (C_2), 56.0 (OCH_3), 8,4 ($Ar-CH_3$); ES-MS *m/z* 240 $[MH]^+$; 262 $[MNa]^+$; 278 $[MK]^+$. Anal. Calcd. for $C_{15}H_{13}NO_2$: C 75.30; H 5.48; N 5.85%. Found: C 75.36; H 5.48; N 5.83%.

5.1.11. 3-Methoxy-4,10-dimethylacridin-9(10H)-one (26)

Sodium hydride (0.3 g of 50% oil dispersion, 12 mmol) was added to a solution of **25** (0.73 g, 3 mmol) in dry THF (20 mL). Methyl iodide (0.78 mL, 12.5 mmol) was added dropwise. The reaction mixture was stirred for 24 h at room temperature, diluted with water (20 mL), and extracted with CH_2Cl_2 (3×50 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2/hexane 9:1) afforded **26** (0.56 g, 72%) as a yellow amorphous solid. IR (KBr) ν, cm^{-1} 2921, 1632, 1592, 1557, 1462, 1445, 1408, 1283, 1263, 1237, 1101, 769, 687; ^1H NMR (400 MHz, $CDCl_3$) δ 8.41 (1H, dd, $J = 8$, 1.5 Hz, H_8), 8.37 (1H, d, $J = 9$ Hz, H_1), 7.68 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.44 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7.25 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.95 (1H, d, $J = 9$ Hz, H_2), 3.98 (3H, s, OCH_3), 3.85 (3H, s, NCH_3), 2.44 (3H, s, $Ar-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6 (C_9), 162.8 (C_3), 147.6 (C_{4a}), 147.1 (C_{10a}), 133.4 (C_6), 127.1 (C_8), 126.7 (C_1), 123.2 (C_{8a}), 121.4 (C_7), 119.8 (C_{9a}), 116.7 (C_5), 113.0 (C_4), 106.4 (C_2), 56.1 (OCH_3), 43.7 (NCH_3), 15.5 ($Ar-CH_3$); ES-MS *m/z* 254 $[MH]^+$; 276 $[MNa]^+$; 292 $[MK]^+$. Anal. Calcd. for $C_{16}H_{15}NO_2$: C 75.87; H 5.97; N 5.53%. Found: C 75.91; H 5.98; N 5.51%.

5.1.12. 4-Hydroxymethyl-3-methoxy-10-methylacridin-9(10H)-one (17) and 2-bromo-3-methoxy-4,10-dimethylacridin-9(10H)-one (27)

N-Bromosuccinimide (197 mg, 1.11 mmol) was added to a solution of **26** (281 mg, 1.11 mmol) in CCl_4 (15 mL) the reaction mixture was irradiated (1000 W) for 15 min. After cooling, water (5 mL) was added and the resulting mixture was evaporated under reduced pressure. Column chromatography over silica gel (solvent: C_6H_6/EtOAc 7:3) gave **17** (75 mg, 25%) and **27** (10 mg, 3%) as bright yellow amorphous solids.

5.1.12.1. 4-Hydroxymethyl-3-methoxy-10-methylacridin-9(10H)-one (17).

IR (KBr) ν, cm^{-1} 2191, 2846, 2356, 1593, 1460, 1402, 1272, 1236, 1084, 817, 752; ^1H NMR (400 MHz,

$CDCl_3$) δ 8.49 (1H, d, $J = 9$ Hz, H_1), 8.43 (1H, dd, $J = 8$, 1.5 Hz, H_8), 7.72 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.48 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7.29 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.95 (1H, d, $J = 9$ Hz, H_2), 4,57 (2H, s, CH_2OH), 4.13 (3H, s, NCH_3), 4.03 (3H, s, OCH_3), 2.61 (1H, br s, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0 (C_9), 164.0 (C_3), 147.0 (C_{4a}), 146.0 (C_{10a}), 133.6 (C_6), 129.5 (C_1), 127.1 (C_8), 122.8 (C_{8a}), 121.6 (C_7), 119.6 (C_{9a}), 116.6 (C_5), 115.1 (C_4), 106.0 (C_2), 58.3 (CH_2OH), 56.3 (OCH_3), 43.7 (NCH_3); ES-MS *m/z* 270 $[MH]^+$. Anal. Calcd. for $C_{16}H_{15}NO_3$: C 71.36; H 5.61; N 5.20%. Found: C 71.42; H 5.67; N 5.13%.

*5.1.12.2. 2-Bromo-3-methoxy-4,10-dimethylacridin-9(10H)-one (**27**).* IR (KBr) ν, cm^{-1} 2950, 2927, 2852, 2356, 1732, 1629, 1609, 1436, 1398, 1265, 1090, 1051, 1009, 739, 704; ^1H NMR (400 MHz, $CDCl_3$) δ 8.56 (1H, s, H_1), 8,40 (1H, d, $J = 8$ Hz, H_8), 7.72 (1H, t, $J = 8$ Hz, H_6), 7.47 (1H, d, $J = 8$ Hz, H_5), 7.29 (1H, t, $J = 8$ Hz, H_7), 3.96 (3H, s, NCH_3), 3.87 (3H, s, OCH_3), 2.60 (3H, Ar–CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.5 (C_9), 160.4 (C_3), 146.8 (C_{4a}), 146.7 (C_{10a}), 133.9 (C_6), 129.5 (C_1), 127.2 (C_8), 123.1 (C_{8a}), 122.7 (C_{9a}), 121.9 (C_7), 121.4 (C_2), 116.7 (C_5), 111.7 (C_4), 56.5 (OCH_3), 42.4 (NCH_3), 15.4 (Ar–CH_3); ES-MS *m/z* 354/356 $[MNa]^+$, 370/372 $[MK]^+$. Anal. Calcd. for $C_{16}H_{14}BrNO_2$: C 57.85; H 4.25; N 4.22%. Found: C 57.91; H 4.19; N 4.26%.

*5.1.13. 4-Acetoxymethyl-3-methoxy-10-methylacridin-9(10H)-one (**28**)*

Acetylation of **17** (25 mg, 0.08 mmol) under conditions essentially similar to those described for the conversion of **6** to **4**, afforded **28** (18 mg, 65%) as a pale yellow amorphous solid. IR (KBr) ν, cm^{-1} 2919, 2846, 2356, 2333, 1727, 1608, 1581, 1452, 1405, 1270, 1097, 763; ^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (1H, d, $J = 9$ Hz, H_1), 8.42 (1H, dd, $J = 8$, 1.5 Hz, H_8), 7.72 (1H, td, $J = 8$, 1.5 Hz, H_6), 7.44 (1H, dd, $J = 8$, 1.5 Hz, H_5), 7.30 (1H, td, $J = 8$, 1.5 Hz, H_7), 6.97 (1H, d, $J = 9$ Hz, H_2), 4.56 (2H, s, CH_2OAc), 4.02 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 2.19 (3H, s, $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0 (C_9), 170,9 ($OCOCH_3$), 157.9 (C_3), 146,1 (2C, C_{4a}, C_{10a}), 133.9 (C_6), 1307 (C_1), 127.2 (C_8), 123.3 (C_{8a}), 122.2 (C_{9a}), 121.7 (C_7), 116.9 (C_5), 110.7 (C_4), 105.9 (C_2), 59.5 (CH_2–OAc), 56.7 (OCH_3), 43.2 (NCH_3), 21.3 ($OCOCH_3$); ES-MS *m/z* 334 $[MNa]^+$; 350 $[MK]^+$. Anal. Calcd. for $C_{18}H_{17}NO_4$: C 69.44; H 5.50; N 4.50%. Found: C 69.51; H 5.48; N 4.52%.

*5.1.14. 3-(3Methoxy-2-methylphenyl)-amino-2-naphthoic acid (**30**)*

Compound **30** was synthesized from **8** (5.56 g, 22.2 mmol) and **22** (3.04 g, 22.2 mmol) according to the procedure described for the preparation of **24** from **19** and **22**, using potassium acetate (4.7 g), cupric acetate monohydrate (0.14 g), and triethylamine (2.7 mL) in anhydrous 2-propanol (100 mL). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **30** (2.72 g, 40%) as a whitish amorphous solid. IR (KBr) ν, cm^{-1} 2918, 2844, 2560, 1660, 1578, 1524, 1433, 1292, 1216, 1148, 1112, 882, 758; ^1H NMR (400 MHz, $CDCl_3$) δ 8.84 (1H, br s, NH), 8.70 (1H, s, H_1), 7.76 (1H, d, $J = 8$ Hz, H_8), 7.50 (1H, d, $J = 8$ Hz, H_5), 7.40 (1H, t, $J = 8$ Hz, H_6), 7.23 (1H, t, $J = 8$ Hz, $H_{5'}$), 7.21 (1H, t, $J = 8$ Hz, H_7), 7.10 (1H, s, H_4), 7.09 (1H, d, $J = 8$ Hz, $H_{4'}$), 6.77 (1H, d, $J = 8$ Hz, $H_{6'}$), 3.91 (3H, s, OCH_3), 2.20 (3H, s, Ar–CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0 (COOH), 158.9 ($C_{3'}$), 144.8 (C_3), 140.3 ($C_{1'}$), 137.9 (C_{4a}), 135.2 (2C, C_1, C_{8a}), 129.4 (2C, C_8, C_6), 126.6 ($C_{5'}$), 125.9 (C_5), 122.9 (C_7), 121.9 ($C_{2'}$), 117.8 ($C_{4'}$), 113.5 (C_2), 108.1 (C_4), 106.8 ($C_{6'}$), 55.8 (OCH_3), 10.6 (Ar–CH_3); ES-MS *m/z* 308 $[MH]^+$, 330 $[MNa]^+$, 346 $[MK]^+$. Anal. Calcd. for $C_{19}H_{17}NO_3$: C 74.25; H 5.58; N 4.56%. Found: C 74.18; H 5.53; N 4.61%.

*5.1.15. 3-Methoxy-4-methylbenzo[b]acridin-12(5H)-one (**31**)*

Trifluoroacetic anhydride (5.6 mL) was added to a solution of **30** (1.24 g, 4.04 mmol) in CH_2Cl_2 (50 mL) and cyclization was further conducted as described for the preparation of **25** from **24**. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **31** (0.55 g, 47%) a yellow amorphous solid. IR (KBr) ν, cm^{-1} 2957, 2918, 2844, 2355, 1729, 1627, 1584, 1454, 1377, 1335, 1261, 1120, 1021, 796; ^1H NMR (400 MHz, $CDCl_3$) δ 9.06 (1H, s, H_{11}), 8.41 (1H, d, $J = 9$ Hz, H_1), 8.06 (1H, d, $J = 8$ Hz, H_{10}), 7.84 (1H, d, $J = 8$ Hz, H_7), 7.79 (1H, br s, NH), 7.73 (1H, s, H_6), 7.56 (1H, t, $J = 8$ Hz, H_8), 7.42 (1H, t, $J = 8$ Hz, H_9), 6.90 (1H, d, $J = 9$ Hz, H_2), 4.00 (3H, s, OCH_3), 2.40 (3H, s, Ar–CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.6 (C_{12}), 161.4 (C_3), 141.2 (C_{4a}), 137.7 (C_{5a}), 136.2 (C_{6a}), 129.9 (C_{10}), 128.6 (2C, C_{11a}, C_8), 128.5 (C_{11}), 127.2 (C_1), 126.2 (C_7), 124.2 (C_9), 121.5 (C_{10a}), 120.0 (C_4), 114.9 (C_{12a}), 111.2 (C_6), 105.4 (C_2), 56.0 (OCH_3), 14.1 (Ar–CH_3); ES-MS *m/z* 312 $[MNa]^+$, 328 $[MK]^+$. Anal. Calcd. for $C_{19}H_{15}NO_2$: C 78.87; H 5.23; N 4.84%. Found: C 78.79; H 5.28; N 4.77%.

*5.1.16. 3-Methoxy-4,5-dimethylbenzo[b]acridin-12(5H)-one (**32**)*

Methylation of **31** (0.55 g, 1.9 mmol) with excess methyl iodide (0.78 mL, 12.5 mmol) following the procedure described for the preparation of **26** from **25** afforded **32** (0.52 g, 94%) as yellow needles: m.p. 179–180 °C (recrystallized from CH_2Cl_2). IR (KBr) ν, cm^{-1} 2953, 2919, 2842, 1464, 1618, 1592, 1458, 1404, 1294, 1245, 1095, 1055, 776, 739; ^1H NMR (400 MHz, $CDCl_3$) δ 9.19 (1H, s, H_{11}), 8.37 (1H, d, $J = 9$ Hz, H_1), 8.01 (1H, d, $J = 8$ Hz, H_{10}), 7.86 (1H, d, $J = 8$ Hz, H_7), 7.72 (1H, s, H_6), 7.54 (1H, t, $J = 8$ Hz, H_8), 7.27 (1H, t, $J = 8$ Hz, H_9), 6.88 (1H, d, $J = 9$ Hz, H_2), 3.89 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 2,44 (3H, s, Ar–CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.6 (C_{12}), 163.4 (C_3), 148.3 (C_{4a}), 144.1 (C_{5a}), 136.3 (C_{6a}), 129.7 (C_{10}), 128.2 (2C, C_8, C_{11a}), 128.1 (C_{11}), 127.0 (C_1), 126.9 (C_7), 124.5 (C_9), 123.5 (C_{10a}), 119.0 (C_4), 113.0 (C_{12a}), 112.6 (C_6), 105.7 (C_2), 56.1 (OCH_3), 44,3 (NCH_3), 15.5 (Ar–CH_3); ES-MS *m/z* 304 $[MH]^+$, 326 $[MNa]^+$, 342 $[MK]^+$.

Anal. Calcd. for $C_{20}H_{17}NO_2$: C 79.19; H 5.65; N 4.62%. Found: C 79.26; H 5.68; N 4.61%.

5.1.17. 4-Hydroxymethyl-3-methoxy-5-methylbenzo[b]acridin-12(5H)-one (**33**)

N-Bromosuccinimide (0.17 g, 1.0 mmol) was added to a solution of **26** (0.30 g, 1.0 mmol) in CCl_4 (15 mL) the reaction mixture was irradiated (1000 W) for 15 min. After cooling, water (5 mL) was added and the resulting mixture was evaporated under reduced pressure. Column chromatography over silica gel (solvent: C_6H_6/EtOAc 7:3) gave **33** (0.08 g, 25%) as a bright yellow amorphous solid. IR (KBr) ν, cm^{-1} 2960, 2929, 2851, 2544, 1614, 1590, 1450, 1404, 1299, 1186, 1104, 820, 750, 641; ^1H NMR (400 MHz, $CDCl_3$) δ 8.89 (1H, s, H_{11}), 8.48 (1H, d, $J = 9$ Hz, H_1), 8.00 (1H, d, $J = 8$ Hz, H_{10}), 7.90 (1H, d, $J = 8$ Hz, H_7), 7.78 (1H, s, H_6), 7.54 (1H, t, $J = 8$ Hz, H_8), 7.42 (1H, t, $J = 8$ Hz, H_9), 6.93 (1H, d, $J = 9$ Hz, H_2), 4.51 (2H, s, CH_2OH), 4.20 (3H, s, OCH_3), 4.00 (3H, s, NCH_3), 2,15 (1H, br s large, D_2O exch., OH); ES-MS *m/z* 320 $[MH]^+$. Anal. Calcd. for $C_{20}H_{17}NO_3$: C 75.22; H 5.37; N 4.39%. Found: C 75.15; H 5.41; N 4.42%.

5.1.18. 4-Acetoxymethyl-3-methoxy-5-methylbenzo[b]acridin-12(5H)-one (**29**)

Compound **29**, synthesized from **33** (50 mg, 0.16 mmol) according to the procedure described for the preparation of **4** from **6**, was obtained as a yellow amorphous solid (36 mg, 65%). IR (KBr) ν, cm^{-1} 2955, 2919, 2849, 2366, 1730, 1641, 1591, 1502, 1462, 1412, 1266, 1094, 1011, 753; ^1H NMR (400 MHz, $CDCl_3$) δ 9.00 (1H, s, H_{11}), 8.54 (1H, d, $J = 9$ Hz, H_1), 8.05 (1H, d, $J = 8$ Hz, H_{10}), 7.91 (1H, d, $J = 8$ Hz, H_7), 7.80 (1H, s, H_6), 7.58 (1H, t, $J = 8$ Hz, H_8), 7.44 (1H, t, $J = 8$ Hz, H_9), 6.93 (1H, d, $J = 9$ Hz, H_2), 4.61 (2H, s, CH_2–OAc), 4.21 (3H, s, OCH_3), 4.02 (3H, s, NCH_3), 2.19 (3H, s, $OCOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2 (C_{12}), 170.8 ($OCOCH_3$), 164.9 (C_3), 148.7 (C_{4a}), 143.1 (C_{5a}), 136.3 (C_{6a}), 129.6 (C_{10}), 128.5 (C_8), 128.3 (C_{11a}), 128.2 (C_{11}), 126.9 (2C, C_1, C_7), 124.6 (C_9), 123.1 (C_{10a}), 118.6 (C_4), 112.6 (C_6), 112.3 (C_{12a}), 105.5 (C_2), 59.0 (CH_2–OAc), 56.5 (OCH_3), 42.8 (NCH_3), 21.5 ($OCOCH_3$); ES-MS *m/z* 362 $[MH]^+$. Anal. Calcd. for $C_{22}H_{19}NO_4$: C 73.12; H 5.30; N 3.88%. Found: C 73.10; H 5.33; N 3.81%.

5.2. Pharmacology

5.2.1. Cytotoxicity

L1210 and KB-3-1 cells were cultivated in RPMI 1640 or DMEM medium, respectively (Gibco), supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay (MTA) as described [34]. Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for four doubling times (48 h for L1210 cells and 96 h for KB-3-1 cells). Results are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

Acknowledgment

The authors thank Pr. M. Koch (Université Paris 5, France) for his stimulating interest in this work.

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