European Journal of Medicinal Chemistry 46 (2011) 1861–1873

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and cytotoxic activity of benzo[*a*]acronycine and benzo[*b*]acronycine substituted on the A ring

Thomas Gaslonde [a,*], Fabiola Covello [a], Laura Velazquez-Alonso [a], Stéphane Léonce [b], Alain Pierré [b], Bruno Pfeiffer [b], Sylvie Michel [a], François Tillequin [a]

[a] *Laboratoire de Pharmacognosie, Université Paris Descartes, UMR/CNRS 8638, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006 Paris, France*
[b] *Institut de recherche Servier, Division recherche cancérologie, 125 chemin de ronde, 78290 Croissy sur Seine, France*

ARTICLE INFO

Article history:
Received 11 January 2011
Received in revised form
16 February 2011
Accepted 18 February 2011
Available online 26 February 2011

Keywords:
Acronycine
Benzo[*a*]acronycine
Benzo[*b*]acronycine
Cytotoxicity

ABSTRACT

The impact of substitutions at position 10 in the A ring of the cytotoxic benzo[*a*]acronycine and benzo[*b*]acronycine series has been explored. 10-Bromobenzo[*a*] and 10-bromobenzo[*b*]acronycine were prepared in 12% and 15% yield respectively from commercially available chemicals. Their 1,2-dihydro-1,2-dihydroxy diesters were synthesized. The different derivatives were tested against two cell lines KB-3-1 and L1210. Their cytotoxic activities were found in the same range of magnitude as their non-substituted counterparts. These structure–activity relationships permitted to conclude that the introduction of a substituent at position 10 maintains the activity in both the benzo[*a*] and [*b*]acronycine series and open the way to further pharmacomodulations.

1. Introduction

Acronycine (**1**), a natural pyranoacridone first isolated from *Acronycia baueri* Schott (Rutaceae) [1] was shown to exhibit antitumor properties against a large panel of tumor cell lines [2,3] (Chart 1). Nevertheless, its moderate potency and its low solubility in aqueous solvents hampered subsequent clinical trials, which only gave poor results [4]. Consequently, several efforts were performed toward the design of more potent derivatives in acronycine series. Substitution of the aromatic A ring at position 11 gave rise to bioactive compounds. Indeed, 11-methoxyacronycine (**2**), 10,11-dimethoxyacronycine (**3**) and 11-aminoacronycine (**4**) were found to inhibit the proliferation of human HL60 promyelocytic leukemic cell line or murine leukemic L1210 cell line, indicating that substitution on A ring is not detrimental to the activity [5–7]. The first significant improvements in terms of potency were obtained with compounds substituted on the 1,2 double bond of the pyran ring, such as diesters of *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine that exhibited marked antitumor properties when compared with acronycine [8,9]. Subsequently, structural analogs including an additional aromatic ring fused onto the acronycine skeleton were developed, in the three isomeric benzo [*a*], benzo[*b*] and benzo[*c*]acronycine (**5**, **6**, and **7**, respectively) series [10–12]. When tested against L1210 cell proliferation, both benzo[*a*]acronycine (**5**) and benzo[*b*]acronycine (**6**) were found some 5-fold more cytotoxic than acronycine (**1**). On the opposite, benzo[*c*]acronycine (**7**) displayed a cytotoxicity within the same order of magnitude as acronycine [13]. *cis*-1,2-Diesters in the first two series displayed a better antitumor activity *in vivo* at a 32-fold smaller dose than acronycine itself [10,11]. The most interesting of them, (±)-*cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (**8**) was selected for phase I clinical trials under the code S23906-1 [14]. Its mechanism of action implies elimination of the acetoxy group at position 1 to generate a carbocation that is responsible for the alkylation of NH_2 groups of DNA guanine units in the minor groove [15,16]. The covalent binding to DNA of S23906-1 (**8**) was shown to induce a marked destabilization of the double helix, with the formation of single-stranded DNA [15,17,18], resulting in an increase of the cyclin E level in cells which were arrested in the S-phase and subsequently underwent apoptosis [18,19].

In this context, the positive role played by the substitution on A ring of acronycine stimulated us to explore the impact of substitutions in the A ring of the most cytotoxic benzo[*a*]acronycine and benzo[*b*]acronycine series. In order to avoid a steric hindrance at carbon 1, unfavorable to biological activity, as shown by the weak activity of benzo[*c*]acronycine, position 10 was chosen to insert the substituent. We report herein the synthesis and the biological evaluation of 10-bromobenzo[*a*] and benzo[*b*]acronycine. The

* Corresponding author. Tel.: +33 1 53 73 98 05; fax: +33 1 40 46 96 58.
E-mail address: thomas.gaslonde@parisdescartes.fr (T. Gaslonde).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.02.050

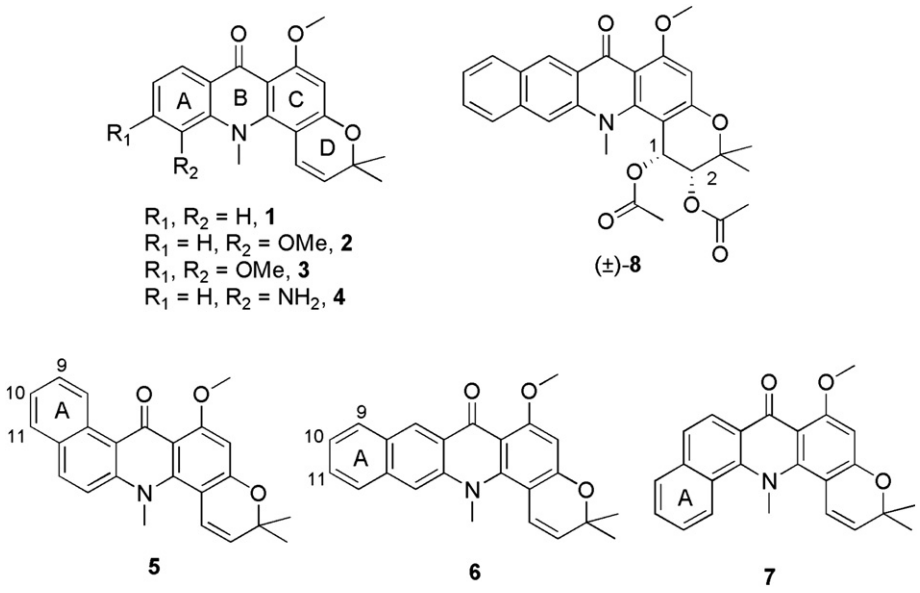


Chart 1. Structures of acronycine derivatives (**1**–**4**), benzo[*a*]acronycine (**5**), benzo[*b*]acronycine (**6**), benzo[*c*]acronycine (**7**) and (±)-*cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (S23906-1) (**8**).

corresponding 1,2-dihydro-1,2-dihydroxy diesters were also prepared in both series, in order to compare their activity with their non-substituted counterparts.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 10-bromobenzo[a]acronycine (9)

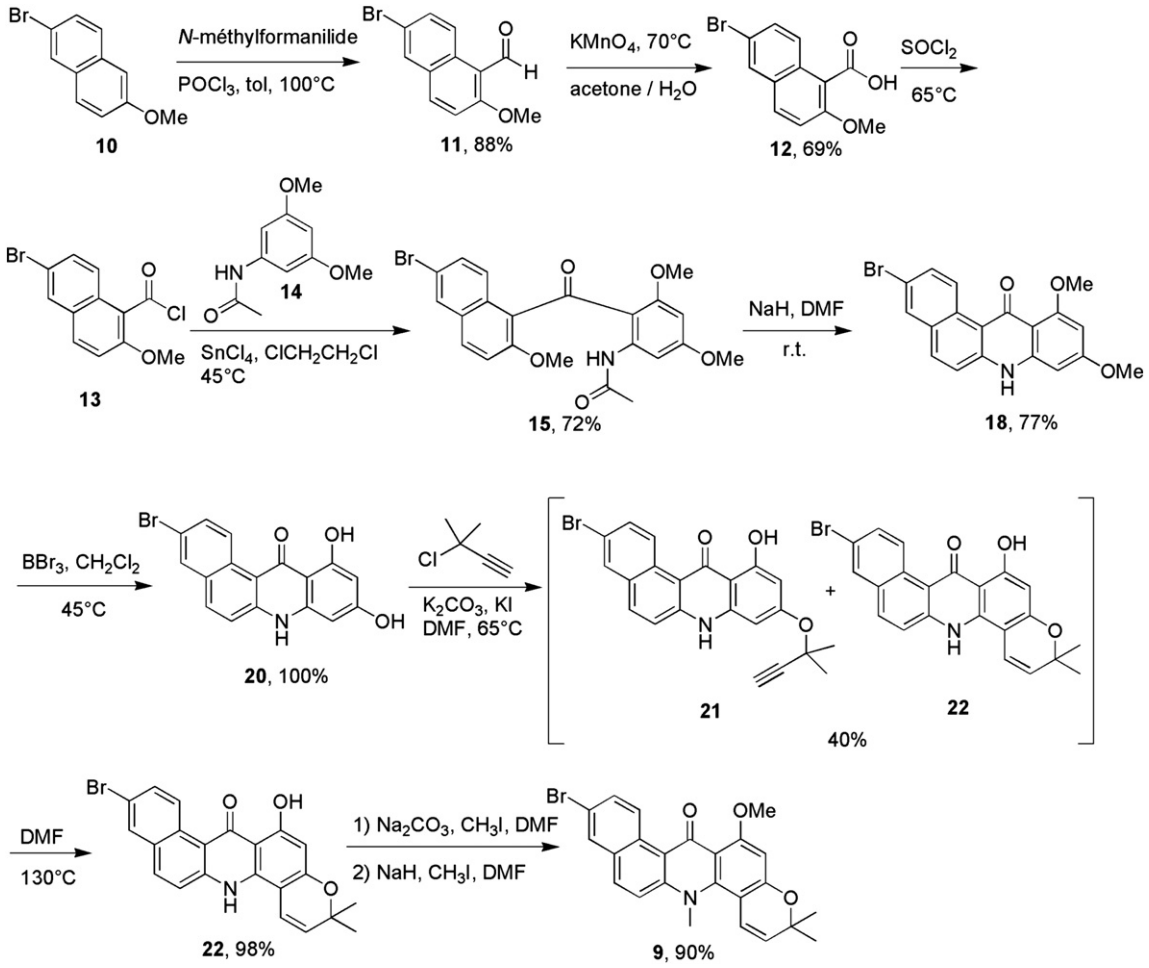
Synthesis of 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**9**) was envisioned through the cyclization in alkaline medium of an intermediate diarylketone, prepared by Friedel–Crafts acylation of an aniline precursor with a suitable naphthalenecarboxylic acid [9,20,21] (Scheme 1 and Chart 2).

Optimization of the conditions described by Nguyên-Hóan [22] for the formylation of the commercially available 2-bromo-6-methoxynaphthalene (**10**) by *N*-methylformanilide and phosphorus oxychloride, gave 6-bromo-2-methoxy-1-naphthaldehyde (**11**) in a better 88% yield. Oxidation to carboxylic acid **12** was performed by reaction with potassium permanganate in acetone. Subsequent treatment with thionyl chloride afforded the corresponding acyl chloride **13**. Friedel–Crafts acylation of 3,5-dimethoxyacetanilide (**14**) with 6-bromo-2-methoxy-1-naphthoyl chloride (**13**) using stannic chloride as catalyst gave the desired diarylketone **15**, accompanied by smaller amounts of compound **16** (Chart 2), resulting from a trans-amidification reaction of diarylketone **15**, and 2-bromo-6-methoxynaphthalen (**10**) formed by decarboxylation of **13**. It should be noted that the choice of the Lewis acid catalyst had a dramatic influence on the course of the reaction. For instance, the use of aluminium chloride gave a mixture of the desired diarylketone **15** and its regioisomer **17** (51% and 14% respectively). Cyclization of diarylketone **15** to the corresponding benzoacridone was achieved by the use of sodium hydride in dimethylformamide. Thus, 3-bromo-9,11-dimethoxybenzo[*a*]acridin-12(7*H*)-one (**18**) was obtained in 77% yield, accompanied by smaller amounts of diarylethylene **19** isolated in 14% yield. The acridone **18** was deprotected upon treatment with boron tribromide in dichloromethane to give 3-bromo-9,11-dihydroxybenzo[*a*]acridin-12(7*H*)-one (**20**) in quantitative yield. Regioselective O-alkylation of compound **20** at position 3 by 3-chloro-3-methylbut-1-yne was successfully performed in the presence of potassium carbonate and potassium iodide [23], to give the expected inseparable mixture of propargylic ether **21** and 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**22**), in a 70/30 ratio (NMR) in 40% overall yield, accompanied by smaller amounts of the rearranged C-alkylation products 3-bromo-12-hydroxy-9,9-dimethyl-8-methylidene-10-(1,1-dimethylpropyn-1-oxy)-8,9-dihydro-13*H*-benzo[*a*]pyrrolo[1,2,3-*fg*]acridin-13-one (**23**) and 3-bromo-10,12-dihydroxy-9,9-dimethyl-8-methylidene-8,9-dihydro-13*H*-benzo[*a*]pyrrolo[1,2,3-*fg*]acridin-13-one (**24**), isolated in 4% and 1% yield, respectively [6]. The crude mixture of **21** and **22** was heated at 130 °C in DMF for 3 h, in order to achieve the thermal rearrangement of the propargylic ether. N- and O-Methylation reactions of acridone **22** by methyl iodide in DMF were performed in a one pot process involving successive additions of a weak (sodium carbonate) and a strong (sodium hydride) base in the reaction medium, affording the desired bromobenzo[*a*]acronycine (**9**) in 90% yield.

2.1.2. Synthesis of 10-bromobenzo[b]acronycine (25)

The same successful synthetic strategy used for the preparation of the angular 10-bromobenzo[*a*]acronycine (**9**) was followed for the synthesis of 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**25**) (Scheme 2 and Chart 3).

Accordingly, the acyl chloride **26** was first obtained from the commercially available 3-hydroxy-2-naphthoic acid in four steps, following the procedure of Murphy et al. [24]. Friedel–Crafts reaction of 3,5-dimethoxyacetanilide (**14**) with compound **26** afforded the desired diarylketone **27** in a good 81% yield, accompanied by 9% of the trans-amidified diarylketone **28**. Surprisingly, in alkaline medium, the diarylketone **27** did not cyclize to the acridone **29**, but gave the 4-aryl-2-quinolinone **30** in 50% yield, accompanied by the deprotected diarylketone **31** isolated in 10% yield. Compound **30** can be considered as arising from an intramolecular aldol condensation involving the acetyl group [20,21]. Therefore, the diarylketone **27** was first deprotected by acid hydrolysis of the amide group and the resulting aminodiarylketone **31** cyclized, upon treatment with sodium hydride, to the acridone **29** in a good 74% overall yield from **27**. It should be noted that, under similar alkaline conditions, compound **28**, without hydrogen



Scheme 1. Synthesis of 10-bromobenzo[*a*]acronycine (**9**).

atom at α-position of the carbonyl amide group, also gave the acridone **29**, although in a smaller 30% yield. Conversion of the dimethoxyacridone **29** to the corresponding dihydroxyacridone **32** could only be successfully conducted when a solution of hydrogen bromide in acetic acid was used as deprotecting agent. Treatment of **32** with 3-chloro-3-methylbut-1-yne gave as previously an inseparable mixture of the desired propargylic ether **33** and cyclized compound **34**, accompanied by 9-bromo-5-hydroxy-1,1-dimethyl-2-methyliden-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (**35**) isolated in 4% yield. After heating in dimethylformamide at 130 °C for 3.5 h, the mixture of **33** and **34** gave pure 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*] acridin-7-one (**34**) in 38% overall yield from **32**. Finally, methylation at *N*-14 and *O*-6 was achieved using the similar one pot method previously used for the preparation of **9** from **22**. Under these conditions, the desired linear bromobenzo[*b*]acronycine **25** was obtained in 82% yield, together with small amount of its phenolic counterpart **36**, isolated in 12% yield.

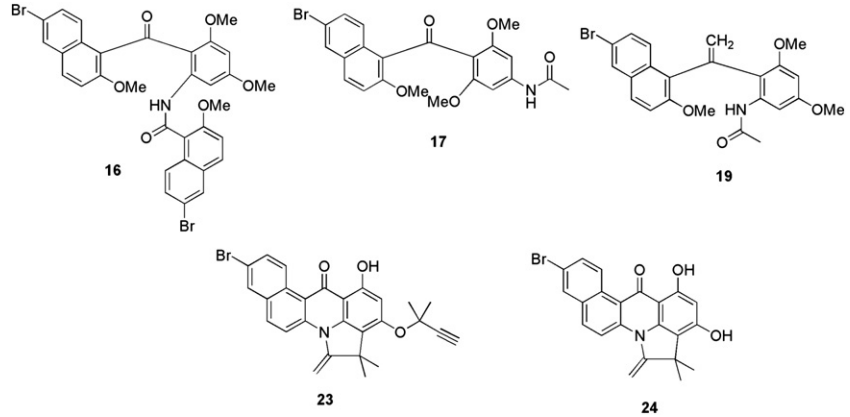
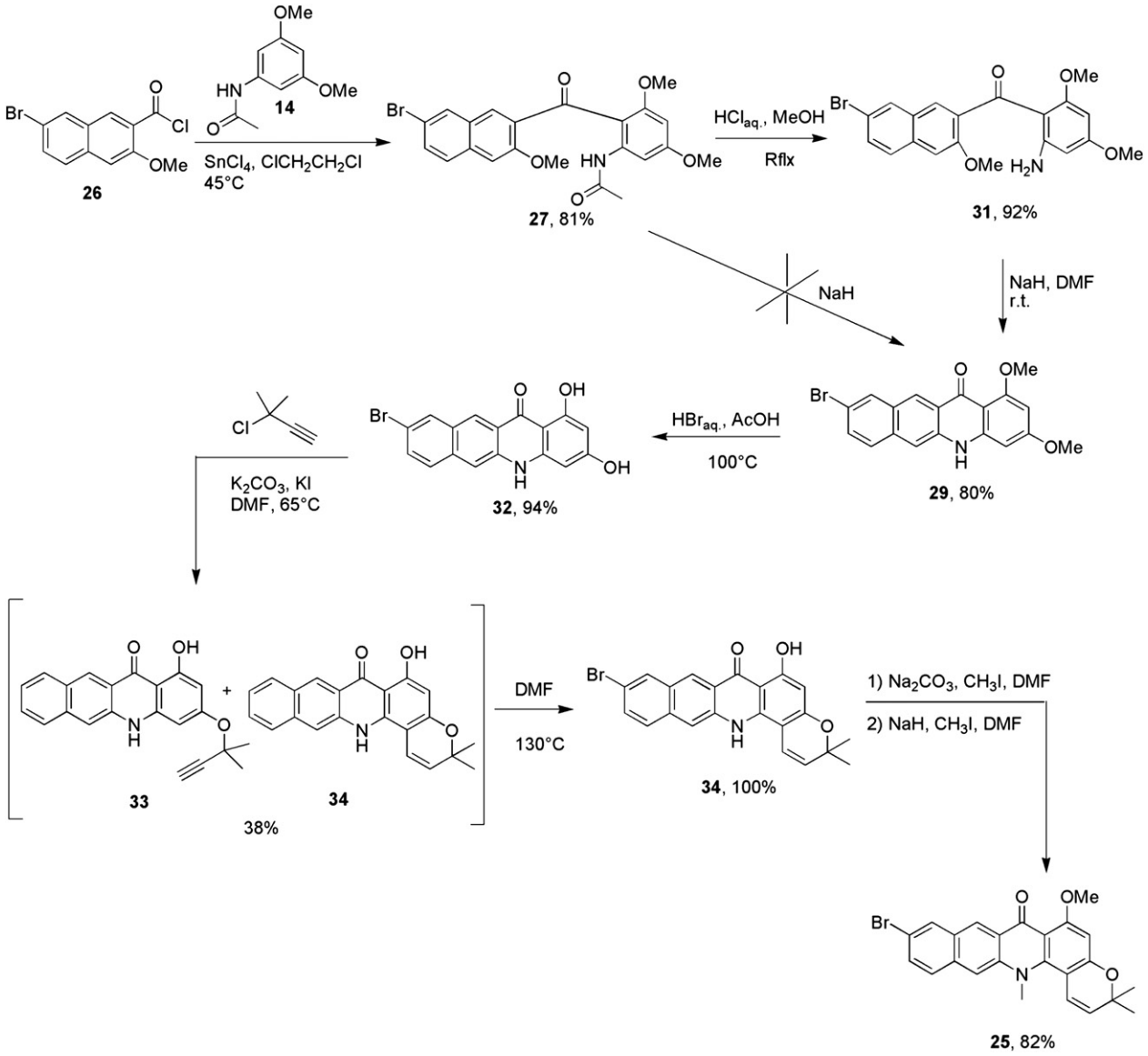


Chart 2. Structures of secondary products **16**, **17**, **19**, **23** and **24** obtained from synthesis described in Scheme 1.



Scheme 2. Synthesis of 10-bromobenzo[*b*]acronycine (**25**).

2.1.3. Synthesis of 1,2-diesters in 10-bromobenzo[a]acronycine and 10-bromobenzo[b]acronycine series

The (±)-*cis*-diols **37** and **38** were conveniently obtained in 64% and 97% yield, respectively, by catalytic osmium tetroxide oxidation of **9** and **25**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidative agent [8] (Schemes 3 and 4). Acetylation of the diols **37** and **38** was ensured by use of excess acetic anhydride to give the corresponding diacetates **39** and **40** in 93 and 98% yield, respectively. Finally, treatment of the angular and the linear diols **37** and **38** with *N*,*N*′-carbonyldiimidazole in 2-butanone afforded the cyclic carbonates **41** and **42**.

2.2. Pharmacology

The biological profile of the newly synthesized molecules in both benzo[*a*] and benzo[*b*]acronycine series was determined by their capacity to inhibit proliferation of murine leukemia cell line (L1210) and human epidermoid carcinoma cell line (KB-3-1). The results are reported in Tables 1 and 2 . As a general rule, compounds belonging to both bromobenzo[*a*] and bromobenzo[*b*]acronycine series exhibited similar biological profiles. Indeed, diacetates and cyclic carbonates **39**–**42** displayed submicromolar IC_{50}, in the range of 0.011–0.6 μM, and were found more active against KB-3-1 solid tumor cell line than leukemic L1210 cell line. Also, compounds **9** and **25** with the 1,2 double bond and the corresponding diols **37** and **38** were less active. These results are in full agreement with the mechanism of action previously established for S-23096-1 in the benzo[*b*]acronycine series. When compared to their previously reported unsubstituted analogs, the cytotoxic activity of the 10-bromo compounds lie within the same range of potency, excepted for compound **9**, which is *ca.* 5-fold more potent against both cell lines than its unsubstituted counterpart **5** (Chart 4).

3. Conclusion

Novel benzoacronycine analogs have been synthesized in the 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*] pyrano[3,2-*h*]acridin-7-one and 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one series. The different derivatives were tested against two cell lines KB-3-1 and

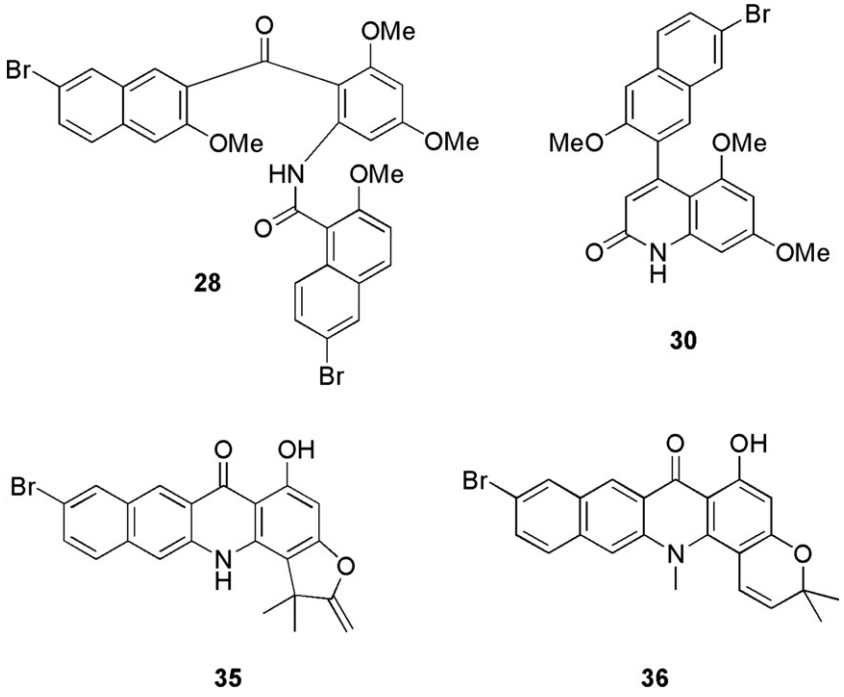


Chart 3. Structures of secondary products **28**, **30**, **35** and **36** obtained from synthesis described in Scheme 2.

L1210. Their cytotoxic potencies were found within the same range of magnitude as their unsubstituted counterparts. Especially, the activity of (±)-*cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine **40** is similar to that of compound **8** that recently underwent phase I clinical trials. These results permit to conclude that the introduction of a substituent at position 10 in the A aromatic ring is not detrimental to the activity in both the benzo[*a*] and [*b*]acronycine series and open the way to further pharmacomodulations.

4. Experimental section

4.1. Chemistry

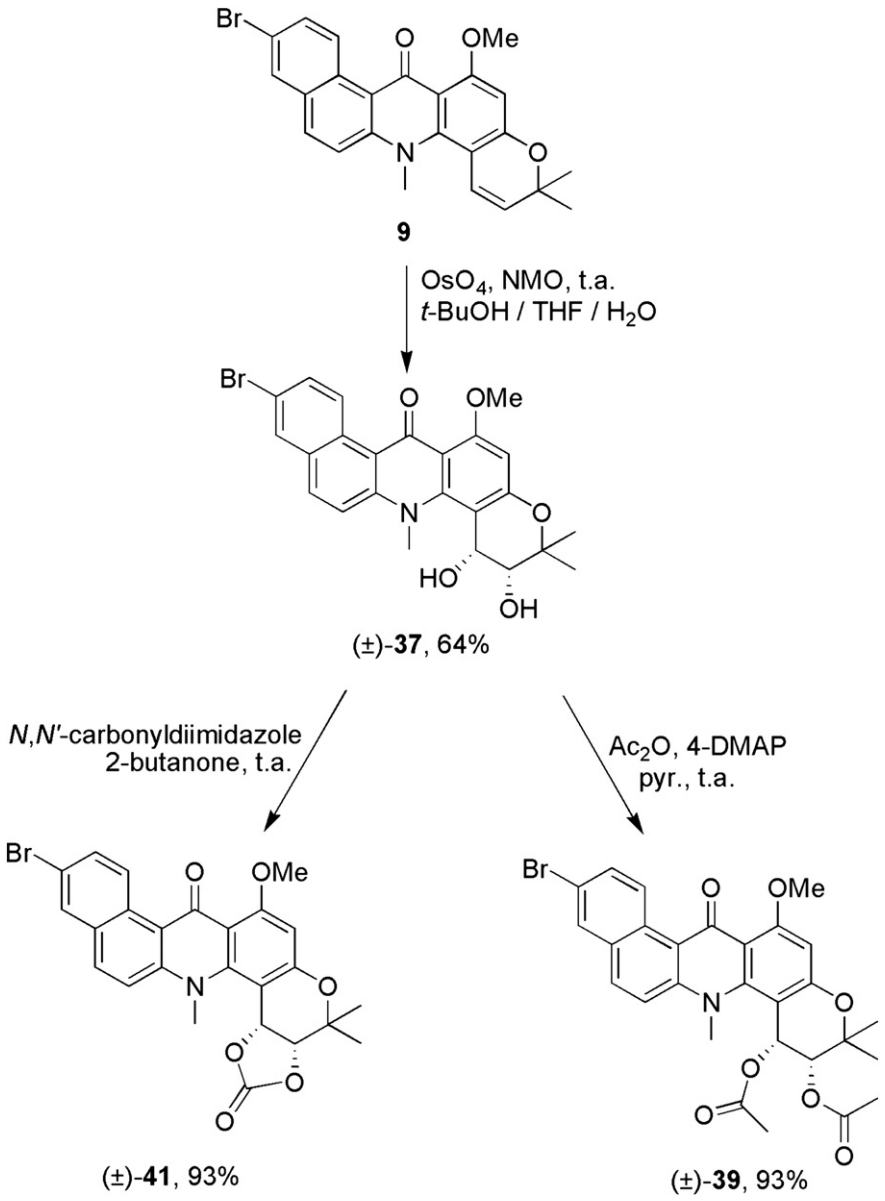
The melting points were determined on a Leica VM apparatus and are not corrected. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets on a Nicolet-510 FT-IR instrument. UV spectra were recorded in spectroscopic grade MeOH on a Jenway 6705 spectrometer. ^1H NMR (δ [ppm], J [Hz]) and ^{13}C NMR spectra were recorded at 400 and 75 MHz, using Bruker Avance 400 and Bruker Avance 300 spectrometers, respectively. When necessary, the signals were unambiguously assigned by 2D NMR techniques: $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^{13}C-^1H$ HMQC, and $^{13}C-^1H$ HMBC. These experiments were performed using standard Bruker micro-programs. Mass spectra were recorded with a ZQ 2000 Waters spectrometer using electron spray ionization (MS (ESI^+ or ESI^-)). Flash column chromatographies were conducted using silica gel 60 SDS (20–45 μm or 35–70 μm) with an overpressure of 300 mbar. Thin layer chromatographies were performed using Merck TLC Silica gel 60 F_{254}.

4.1.1. 6-Bromo-2-methoxy-1-naphthaldehyde (*11*)

N-Methylformanilide (160 mL, 1.3 mol) and phosphorus oxychloride (185 mL, 2.0 mol) were added to a solution of 2-bromo-6-methoxynaphthalen (**10**) (51.4 g, 0.22 mol) in anhydrous toluene (260 mL). The solution was heated at 100 °C for 5 h. The cooled reaction mixture was cautiously added to a solution of sodium acetate (250 g) in water (820 mL). After evaporation of the solvents under reduce pressure the residue is solubilized in ethyl acetate (1 L) and water (1 L), and extracted with ethyl acetate (6 × 1 L). The combined organic layers were washed with water (500 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give dark oil which crystallized upon cooling. The filtered solid was recrystallized in dichloromethane/cyclohexane to give 23.5 g of 6-bromo-2-methoxy-1-naphthaldehyde (**11**). Additionally, the combined filtrates were evaporated under reduce pressure and furnished after column chromatography on silica gel (C_6H_{12}/AcOEt: 9/1: V/V) 27 g more of desired solid **11**. Compound **11** was obtained as white needles (88%), m.p. 103–104 °C (CH_2Cl_2/C_6H_{12}). ^1H NMR ($CDCl_3$) δ: 4.07 (s, 3H, OMe); 7.34 (d, 1H, J = 9 Hz, H-3), 7.67 (dd, 1H, J = 9 and 2 Hz, H-7), 7.94 (d, 1H, J = 2 Hz, H-5), 7.98 (d, 1H, J = 9 Hz, H-4), 9.19 (d, 1H, J = 9 Hz, H-8), 10.87 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$) δ: 56.6 (OMe), 89.7 (C-1), 113.7 (C-3), 116.7 (C-4a), 118.5 (C-6), 126.9 (C-8), 129.8 (C-8a), 130.0 (C-8), 132.9 (C-7), 136.3 (C-4), 163.9 (C-2), 191.6 (CHO). IR (KBr) cm^{-1}: 3099, 2986, 2947, 2881, 2842, 2804, 1666, 1584, 1498, 1456, 1394, 1343, 1266, 1250, 1172, 1153, 1075, 1048, 881, 820, 807. UV λ_{max} (MeOH) nm (log ϵ): 226 (4.56), 247 (4.54), 313 (3.83), 362 (3.71). MS (ESI^+) m/z: 265 and 267 $[M + Na]^+$.

4.1.2. 6-Bromo-2-methoxy-1-naphthaoic acid (*12*)

A solution of 6-bromo-2-methoxy-1-naphthaldehyde (**11**) (20.0 g, 75 mmol) in anhydrous acetone (400 mL) was heated at 70 °C. After addition of a solution of potassium permanganate (32 g) in water (1 L) for 2 h, the reaction was stirred and heated further 2 h. An aqueous solution of potassium hydroxide was added to the reaction mixture to obtain pH = 10. After filtration, the solution was washed with ethyl acetate (200 mL) and acidified with hydrochloric acid (10%) to pH = 2. The precipitate was filtered, washed with water (3 × 100 mL) and dried in a vacuum over P_2O_5 to give 6-bromo-2-methoxy-1-naphthaoic acid (**12**) as a white amorphous solid (14.53 g, 69%). ^1H NMR ($CDCl_3$) δ: 4.12 (s, 3H, OMe), 7.37 (d, 1H, J = 9 Hz, H-3), 7.65 (dd, 1H, J = 9 and 2 Hz, H-7),

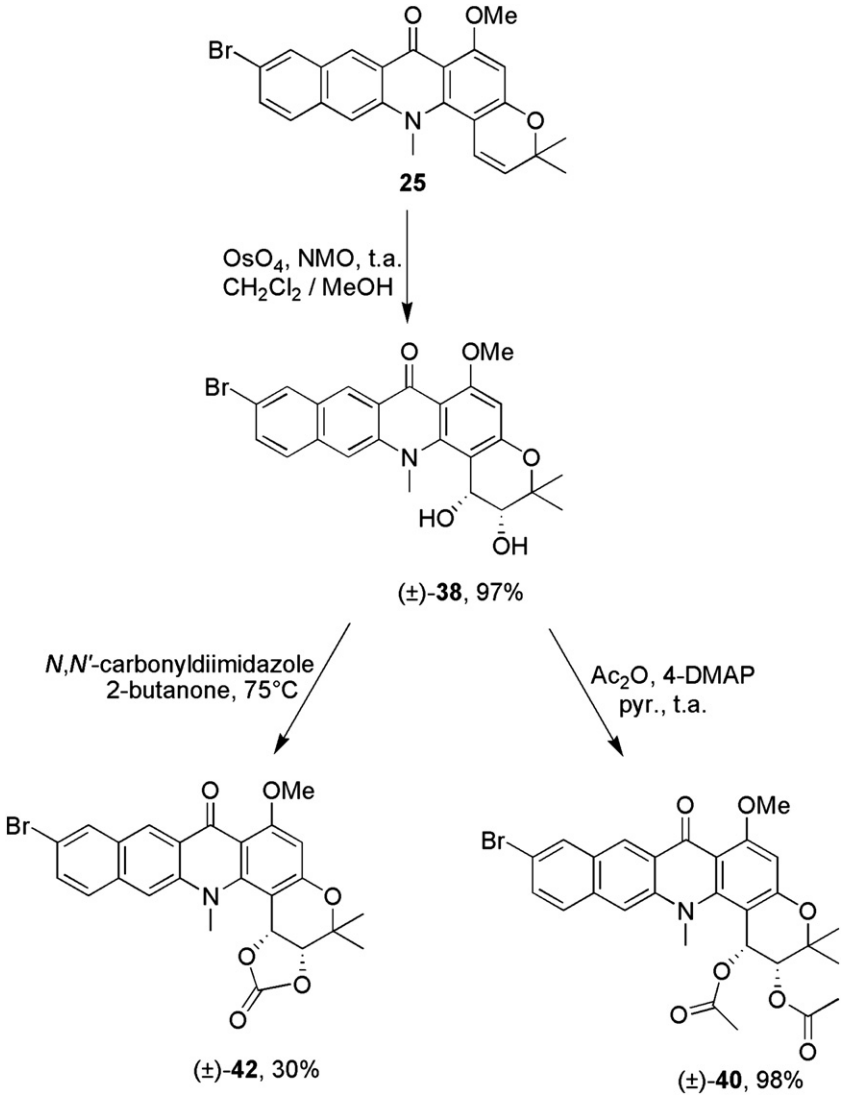


Scheme 3. Synthesis of 1,2-diesters in 10-bromobenzo[*a*]acronycine series.

7.92 (d, 1H, $J = 9$ Hz, H4), 7.98 (d, 1H, $J = 2$ Hz, H-5), 8.53 (d, 1H, $J = 9$ Hz, H-8). ^{13}C NMR ($CDCl_3$) δ: 57.3 (OMe), 113.9 (C-3), 114.6 (C-1), 118.5 (C-6), 126.8 (C-8), 130.1 (C-5 and C-4a), 130.4 (C-8a), 131.6 (C-7), 132.8 (C-4), 156.2 (C-2), 168.8 (COOH). IR (KBr) cm^{-1}: 2982, 2943, 2889, 1700, 1669, 1588, 1499, 1433, 1343, 1293, 1254, 1149, 1083, 1017, 916, 873, 818. UV λ_{max} (MeOH) nm (log ϵ): 235 (4.82), 272 (3.70), 282 (3.78), 293 (3.65), 331 (3.39), 343 (3.42). MS (ESI^-) *m*/*z*: 279 and 281 $[M - H]^-$.

*4.1.3. (6-Bromo-2-methoxynapht-1-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**15**) and (6-bromo-2-methoxynapht-1-yl)-[2-(6-bromo-2-methoxy-1-naphthamido)-4,6-dimethoxyphenyl] ketone (**16**)*

Thionyl chloride (12.0 mL) was added to 6-bromo-2-methoxy-1-naphthaoic acid (**12**) (2.10 g, 7.47 mmol). The reaction mixture was stirred at 65 °C for 3 h and evaporated under reduced pressure to give crude 6-bromo-2-methoxy-1-naphthaoyl chloride (**13**) which was immediately used in the following step without further purification. Stannic chloride (1.0 mL, 8.86 mmol) was added to a solution of the crude 6-bromo-2-methoxy-1-naphthaoyl chloride (**13**) in anhydrous dichloroethane (10 mL). The reaction mixture was heated to 45 °C for 30 min. Then, a solution of 3,5-dimethoxyacetanilide (**14**) (0.96 g, 4.92 mmol) in anhydrous dichloroethane (25 mL) was added, and the mixture was heated at 45 °C for 20 h. The reaction mixture was added to cold water (300 mL) and extracted with dichloromethane (3 × 150 mL). The combined organic layers were washed with an aqueous solution of potassium hydroxide (10%) to remove excess of 6-bromo-2-methoxy-1-naphthaoic acid (**12**), dried over $MgSO_4$ and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 95/5: V/V) gave successively 2-bromo-6-methoxynaphthalene (210 mg), (6-bromo-2-methoxynapht-1-yl)-[2-(6-bromo-2-methoxy-1-naphthamido)-4,6-dimethoxyphenyl]ketone (**16**) (47 mg, 1%) as a white amorphous solid, (6-bromo-2-methoxynapht-1-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**15**) (1.67 g, 72%) as a yellowish amorphous solid and 3,5-dimethoxyacetanilide (**14**) (124 mg). (6-*Bromo*-2-*methoxynapht*-1-*yl*)-[2-(6-*bromo*-2-*methoxy*-1-*naphthamido*)-4,6-*dimethoxyphenyl*]*ketone* (**16**). ^{1}H NMR ($CDCl_3$) δ: 3.09 (s, 3H, OMe-6′),



Scheme 4. Synthesis of 1,2-diesters in 10-bromobenzo[*b*]acronycine series.

3.81 (s, 3H, OMe-2), 3.95 (s, 3H, OMe-2″), 3.99 (s, 3H, OMe-4′), 6.07 (d, 1H, $J = 2$ Hz, H-5′), 7.27 (d, 1H, $J = 9$ Hz, H-3), 7.32 (d, 1H, $J = 9$ Hz, H-3″), 7.41 (dd, H, $J = 9$ and 1.5 Hz, H-7), 7.43 (d, 1H, $J = 9$ Hz, H-8), 7.55 (dd, 1H, $J = 9$ and 2 Hz, H-7″), 7.72 (d, 1H, $J = 9$ Hz, H-4), 7.80 (d, 1H, $J = 9$ Hz, H-4″), 7.93 (d, 1H, $J = 1.5$ Hz, H-5), 7.95 (d, 1H, $J = 2$ Hz, H-5″), 7.99 (d, 1H, $J = 9$ Hz, H-8″), 8.46 (d, 1H, $J = 2$ Hz, H-6′), 12.42 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 55.7 (OMe-6′), 55.9 (OMe-4′), 56.6 (OMe-2″), 57.0 (OMe-2), 94.9 (C-5′), 97.5 (C-3′), 110.2 (C-2′), 114.2 (C-3″), 114.6 (C-3), 117.5 (C-6), 117.9 (C-6″), 121.0 (C-4a″), 125.6 (C-8), 126.2 (C-8″), 128.9 (C-4a), 129.0 (C-4), 129.3 (C-8a), 129.9 (C-1), 130.0 (C-1″ and C-8a″), 130.07 (C-5), 130.14 (C-5″), 130.2 (C-7), 130.9 (C-4 and C-7″), 144.1 (C-1′), 152.9 (C-2), 154.3 (C-2″), 163.1 (C-6′), 165.9 (C-4′), 166.6 (CONH), 197.3 (ArCOAr′). IR (KBr) cm^{-1}: 3350, 3124, 3062, 3003, 2937, 1606, 1587, 1520, 1497, 1446, 1271, 1252, 1143, 1077, 898. UV λ_{max} (MeOH) nm (log ϵ): 235 (5.08), 283 (4.21), 294 (4.22). MS (ESI$^+$) *m/z*: 700, 702 and 704 [M + Na]$^+$. (6-*Bromo*-2-*methoxynapht*-1-*yl*)-(2-*acetamido*-4,6-*dimethoxyphenyl*)*ketone* (**15**). ^{1}H NMR ($CDCl_3$) δ: 2.29 (s, 3H, COMe), 3.05 (s, 3H, OMe-6′), 3.82 (s, 3H, OMe-2), 3.89 (s, 3H, OMe-4′), 5.98 (d, 1H, $J = 2.5$ Hz, H-5′), 7.29 (d, 1H, $J = 9$ Hz, H-3), 7.42 (m, 2H, H-7 and H-8), 7.74 (d, 1H, $J = 9$ Hz, H-4), 7.94 (s, 1H, H-5), 8.15 (d, 1H, $J = 2.5$ Hz, H-3′), 12.24 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 25.9 (COMe), 55.7 (OMe-4′, OMe-6′), 57.0 (OMe-2), 94.4 (C-5′), 97.0 (C-3′), 109.6 (C-1′), 114.6 (C-3), 117.5 (C-6), 125.8 (C-8), 128.8 (C-4), 129.2 (C-8a), 129.4 (C-4a), 130.0 (C-1, C-5), 130.2 (C-7), 144.7 (C-2′), 152.8 (C-2), 163.3 (C-6′), 166.0 (C-4′), 170.1 (CONH), 197.5 (ArCOAr). IR (KBr) cm^{-1}: 3203, 3126, 3004, 2959, 2935, 2834, 1697, 1611, 1584, 1518, 1497, 1444, 1273, 1247, 1199, 1160,

Table 1
Cytotoxicity of bromo compounds **22**, **23**, **24**, **9**, **37**, **39**, **41** in comparison with non-substituted compounds **5**, **44**, **43** in benzo[*a*]acronycine series.

Compounds	**22**	**23**	**24**	**9**	**5**	**37**	**39**	**44**	**41**	**43**
IC_{50} L1210, μM	3.4	51.1	7.3	0.49	2.5	7.6	0.4	0.73	0.08	0.059
IC_{50} KB-3-1, μM	1.7	37.1	2.5	1.3	8.6	5.5	0.040	0.14	0.015	0.015

Table 2
Cytotoxicity of bromo compounds **36**, **25**, **38**, **40**, **42** in comparison with non-substituted compounds **6**, **8**, **45** in benzo[*b*]acronycine series.

Compounds	**36**	**25**	**6**	**38**	**40**	**8**	**42**	**45**
IC_{50} L1210, μM	0.645	2.2	14.9	0.225	0.6	0.79	0.034	0.016
IC_{50} KB-3-1, μM	14.3	9.9	3.4	36.3	0.115	0.095	0.011	n.t.

n.t.: not tested.

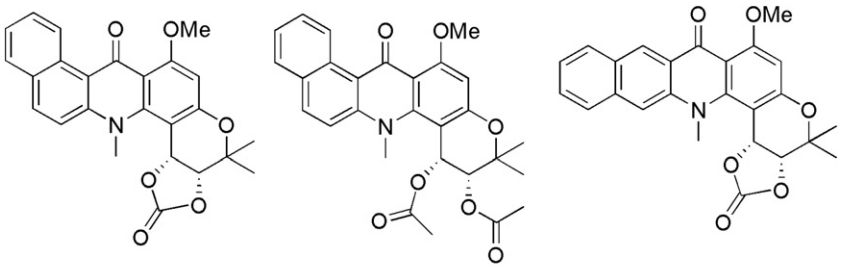


Chart 4. Reference 1,2-diesters in the benzo[*a*] and benzo[*b*]acronycine series.

1144, 1109, 1086, 904, 803. UV λ_{max} (MeOH) nm (log ϵ): 236 (4.91), 307 (4.24). MS (ESI$^+$) *m*/*z*: 480 and 482 $[M + Na]^+$, 496 and 498 $[M + K]^+$.

4.1.4. (6-Bromo-2-methoxynapht-1-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**15**) and (6-bromo-2-methoxynapht-1-yl)-(4-acetamido-2,6-dimethoxyphenyl)ketone (**17**)

Thionyl chloride (6.0 mL) was added to 6-bromo-2-methoxy-1-naphthaoic acid (**12**) (1.00 g, 3.56 mmol). The reaction mixture was stirred at 65 °C for 3 h and evaporated under reduced pressure to give crude 6-bromo-2-methoxy-1-naphthaoyl chloride (**13**) which was immediately used in the following step without further purification. Aluminium trichloride (0.87 g, 6.52 mmol) was added to a solution of the crude 6-bromo-2-methoxy-1-naphthaoyl chloride (**13**) in anhydrous dichloroethane (3 mL). The reaction mixture was heated to −30 °C for 30 min. Then, a solution of 3,5-dimethoxyacetanilide (**14**) (0.56 g, 2.87 mmol) in anhydrous dichloroethane (6 mL) was added, and the mixture was heated at −30 °C for 6 days. A cold solution of hydrochloric acid (400 mL, 15%) was added to the reaction mixture. After extraction with dichloromethane (3 × 50 mL), the combined organic layers were washed with an aqueous solution of potassium hydroxide (10%) to remove excess of 6-bromo-2-methoxy-1-naphthaoic acid (**12**), dried over $MgSO_4$ and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 95/5: V/V) gave successively (6-bromo-2-methoxynapht-1-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**15**) (670 mg, 51%) as a yellowish amorphous solid and (6-bromo-2-methoxynapht-1-yl)-(4-acetamido-2,6-dimethoxyphenyl)ketone (**17**) (180 mg, 14%) as a white amorphous solid. (6-*Bromo*-2-*methoxynapht*-1-*yl*)-(4-*acetamido*-2,6-*dimethoxyphenyl*)*ketone* (**17**). ^{1}H NMR (DMSO-d_6) δ: 2.04 (s, 3H, COMe), 3.47 (s, 6H, OMe6′ and OMe2′), 3.64 (s, 3H, OMe2), 6.95 (s, 2H, H-3′ and H-5′), 7.45 (d, 1H, J = 9 Hz, H-3), 7.58 (dd, 1H, J = 9 and 2 Hz, H-7), 7.74 (d, 1H, J = 9Hz, H-8), 7.98 (d, 1H, J = 9 Hz, H-4), 8.16 (d, 1H, J = 2 Hz, H-5), 10.09 (s, 1H, NH). ^{13}C NMR (DMSO-*d*6) δ: 24.8 (CO*Me*), 56.2 (OMe2′ and OMe6′), 57.4 (OMe2), 95.65 (C3′ and C5′), 116.3 (C3), 116.6 (C1′), 117.1 (C6), 126.8 (C4a), 127.2 (C8), 130.1 (C1), 130.2 (C8a), 130.3 (C5), 130.4 (C7), 131.4 (C4), 143.1 (C4′), 155.8 (C2), 158.9 (C2′ and C6′), 169.3 (CONH), 195.0 (ArCOAr). IR (KBr) cm^{-1}: 3308, 3001, 2966, 2935, 2838, 1681, 1658, 1602, 1533, 1499, 1453, 1405, 1273, 1250, 1231, 1130, 897. UV λ_{max} (MeOH) nm (log ϵ): 233 (4.76), 297 (4.10). MS (ESI$^+$) *m*/*z*: 480 and 482 $[M + Na]^+$, 496 and 498 $[M + K]^+$.

4.1.5. 3-Bromo-9,11-dimethoxybenzo[a]acridin-12(7H)-one (**18**) and 1-(6-bromo-2-methoxynapht-1-yl)-1-(2-acetamido-4,6-dimethoxyphenyl)ethylene (**19**)

A solution of (6-bromo-2-methoxynapht-1-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**15**) (15.00 g, 32.8 mmol) in anhydrous *N*,*N*-dimethylformamide (240 mL) was added to a suspension of sodium hydride (3.93 g, 98.2 mmol) in anhydrous *N*,*N*-dimethylformamide (100 mL). The reaction mixture was stirred at room temperature for 2 h and poured on cold water (3 L). The precipitate was filtered and dried in a vacuum over P_2O_5. The solid was washed with dichloromethane (3 × 70 mL) and filtered to give 3-bromo-9,11-dimethoxybenzo[*a*]acridin-12(7*H*)-one (**18**) (9.72 g, 77%) as an amorphous white solid. Then, the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 95/5: V/V) gave 1-(6-bromo-2-methoxynapht-1-yl)-1-(2-acetamido-4,6-dimethoxyphenyl)ethylene (**19**) (2.09 g, 14%) as white prisms, m.p. 175–176 °C (CH_2Cl_2). 3-*Bromo*-9,11-*dimethoxybenzo*[*a*]*acridin*-12(7*H*)-*one* (**18**). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 3.69 (s, 3H, OMe-11), 3.97 (s, 3H, OMe-9), 6.11 (m, 2H, H-8 and H-10), 7.14 (d, 1H, J = 9 Hz, H-6), 7.62 (d, 1H, J = 9 Hz, H-5), 7.71 (dd, 1H, J = 9 and 1.5 Hz, H-2), 7.84 (d, 1H, J = 1.5 Hz, H-4), 10.14 (d, 1H, J = 9 Hz, H-1). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 55.0 (OMe-11), 55.7 (OMe-9), 89.8 (C-10), 93.8 (C-8), 109.3 (C-11a), 114.0 (C-12a), 118.1 (C-6), 118.2 (C-3), 128.5 (C-1), 130.0 (C-4), 130.4 (C-4a), 130.7 (C-12b), 131.2 (C-2), 133.1 (C-5), 140.5 (C-7a), 142.6 (C-6a), 161.7 (C-9), 163.0 (C-11), 179.2 (C-12). IR (KBr) cm^{-1}: 3435, 3290, 3185, 3132, 3097, 3029, 2962, 1637, 1606, 1581, 1534, 1452, 1398, 1206, 1162, 1129, 1077, 831. UV λ_{max} (MeOH) nm (log ϵ): 203 (4.48), 218 (4.33), 245 (4.40), 253 (4.37), 291 (4.81), 319 (4.00), 357 (3.48), 373 (3.66), 392 (3.65). MS (ESI$^+$) *m*/*z*: 384 and 386 $[M + H]^+$, 406 and 408 $[M + Na]^+$. 1-(6-*Bromo*-2-*methoxynapht*-1-*yl*)-1-(2-*acetamido*-4,6-*dimethoxyphenyl*)*ethylene* (**19**). ^{1}H NMR ($CDCl_3$) δ: 2.17 (s, 3H, COMe), 3.32 (s, 3H, OMe-6′), 3.74 (s, 3H, OMe-2), 3.83 (s, 3H, OMe-4′), 5.61 (d, 1H, J = 2 Hz, CH_2), 5.72 (d, 1H, J = 2 Hz, CH_2), 6.13 (d, 1H, J = 2 Hz, H-5′), 7.20 (d, 1H, J = 9 Hz, H-3), 7.53 (dd, 1H, J = 9 and 2 Hz, H-7), 7.61 (d, 1H, J = 2 Hz, H-3′), 7.67 (d, 1H, J = 9 Hz, H-4), 7.93 (d, 1H, J = 2 Hz, H-5), 8.30 (d, 1H, J = 9 Hz, H-8), 8.33 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 24.7 (CO*Me*), 55.0 (OMe-6′), 55.4 (OMe-4′), 56.6 (OMe-2), 94.9 (C-5′), 98.1 (C-3′), 114.3 (C-3), 114.5 (C-1′), 117.5 (C-6), 125.2 (CH_2), 126.7 (C-1), 128.0 (C-4), 128.6 (C-8), 128.9 (C-7), 129.5 (C-5), 130.6 (C-4a), 131.8 (C-8a), 137.2 (C-2′), 138.3 (*C*=CH_2), 153.3 (C-2), 158.2 (C-6′), 159.9 (C-4′), 168.3 (CONH). IR (KBr) cm^{-1}: 3404, 3070, 2993, 2962, 2941, 2836, 1689, 1585, 1529, 1529, 1493, 1447, 1427, 1334, 1252, 1143, 1108, 1073, 821, 800. UV λ_{max} (MeOH) nm (log ϵ): 237 (4.85), 286 (4.00), 341 (3.48). MS (ESI$^+$) *m*/*z*: 478 and 480 $[M + Na]^+$, 494 and 496 $[M + K]^+$.

4.1.6. 3-Bromo-9,11-dihydroxybenzo[a]acridin-12(7H)-one (**20**)

Boron tribromide (19.7 mL, 208 mmol) was added to a solution of 3-bromo-9,11-dimethoxybenzo[*a*]acridin-12(7*H*)-one (**18**) (10.0 g, 26.0 mmol) in anhydrous dichloromethane (530 mL). The reaction mixture was stirred at 45 °C for 27 h, and then, added on icy water (1.2 L). The solid was filtered, washed with water (3 × 150 mL), washed with dichloromethane (3 × 50 mL) and dried in a vacuum over P_2O_5 to give 3-bromo-9,11-dihydroxybenzo[*a*]acridin-12(7*H*)-one (**20**) (9.29 g, 100%) as a yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ: 6.09 (d, 1H, J = 1.5 Hz, H-8), 6.39 (d, 1H, J = 1.5 Hz, H-10), 7.61 (d, 1H, J = 9 Hz, H-6), 7.82 (dd, 1H, J = 9 and 1.5 Hz, H-2), 8.15

(d, 1H, $J = 9$ Hz, H-5), 8.25 (d, 1H, $J = 1.5$ Hz, H-4), 9.95 (d, 1H, $J = 9$ Hz, H-1), 10.51 (s, 1H, OH-9), 10.51 (s, 1H, NH), 14.78 (s, 1H, OH-11). ^{13}C NMR (DMSO-d_6) δ: 91.4 (C-8), 97.5 (C-10), 106.0 (C-11a), 110.6 (C-12a), 118.4 (C-3), 119.8 (C-6), 128.6 (C-1), 130.3 (C-4a), 131.0 (C-4), 131.1 (C-12b), 131.8 (C-2), 135.0 (C-5), 142.0 (C-7a), 142.7 (C-6a), 164.0 and 164.1 (C-9 and C-11), 182.3 (C-12). IR (KBr) cm^{-1}: 3358, 3132, 3055, 3013, 1647, 1586, 1499, 1411, 1180, 1165, 1134, 826. UV λ_{max} (MeOH) nm (log ϵ): 202 (4.42), 223 (4.32), 238 (4.25), 256 (4.35), 294 (4.71), 325 (3.92), 389 (3.50). MS (ESI$^+$) m/z: 354 and 356 $[M + H]^+$.

4.1.7. *10-Bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7H-benzo[a]pyrano[3,2-h]acridin-7-one (**22**), 3-bromo-12-hydroxy-9,9-dimethyl-8-methylidene-10-(1,1-dimethylpropyn-1-oxy)-8,9-dihydro-13H-benzo[a]pyrrolo[1,2,3-fg]acridin-13-one (**23**) and 3-bromo-10,12-dihydroxy-9,9-dimethyl-8-methylidene-8,9-dihydro-13H-benzo[a]pyrrolo[1,2,3-fg]acridin-13-one (**24**)*

Anhydrous potassium carbonate (8.82 g, 42.1 mmol), anhydrous potassium iodide (7.00 g, 42.1 mmol) and 3-chloro-3-methylbut-1-yne (7.75 mL, 72.1 mmol) were added to a solution of 3-bromo-9,11-dihydroxybenzo[*a*]acridin-12(7*H*)-one (**20**) (5.00 g, 14.0 mmol) in anhydrous *N*,*N*-dimethylformamide (200 mL). The reaction mixture was stirred at 65 °C for 27 h, poured in water (2.5 L) and then, extracted with dichloromethane/methanol: 10/1: V/V (9 × 1 L). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/C_6H_{12}: 50/50 to 100/0: V/V) gave successively 3-bromo-12-hydroxy-9,9-dimethyl-8-methylidene-10-(1,1-dimethylpropyn-1-oxy)-8,9-dihydro-13*H*-benzo[*a*]pyrrolo[1,2,3-*fg*]acridin-13-one (**23**) (290 mg, 4%) as a orange-yellow amorphous solid, 3-bromo-10,12-dihydroxy-9,9-dimethyl-8-methylidene-8,9-dihydro-13*H*-benzo[*a*]pyrrolo[1,2,3-*fg*]acridin-13-one (**24**) (44.5 mg, 1%) as a orange-yellow amorphous solid, and a mixture of 3-bromo-11-hydroxy-9-(1,1-dimethylpropyn-1-oxy)-benzo[*a*]acridin-12(7*H*)-one (**21**) and 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**22**) (3.37 g). The mixture of compound **21** and **22** was dissolved in anhydrous *N*,*N*-dimethylformamide (200 mL). The solution was heated to 130 °C for 3.5 h, and poured on cold water (1 L). The solid was filtered, washed with water (3 × 50 mL) and dried in a vacuum over P_2O_5 to give 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**22**) (5.53 g, 40%) as a yellow amorphous solid. 3-*Bromo*-12-*hydroxy*-9,9-*dimethyl*-8-*methylidene*-10-(1,1-*dimethylpropyn*-1-*oxy*)-8,9-*dihydro*-13*H*-*benzo*[*a*]*pyrrolo*[1,2,3-*fg*]*acridin*-13-*one* (**23**). ^{1}H NMR ($CDCl_3$) δ: 1.61 (s, 6H, C-9Me$_2$), 1.81 (s, 6H, C-1″Me$_2$), 2.75 (s, 1H, H-3″), 5.01 (d, 1H, $J = 3.5$ Hz, H-1′), 5.59 (d, 1H, $J = 3.5$ Hz, H-1′), 7.15 (s, 1H, H-11), 8.00 (dd, 1H, $J = 9$ and 2 Hz, H-2), 7.93 (d, 1H, $J = 9$ Hz, H-5), 7.96 (d, 1H, $J = 2$ Hz, H-4), 8.24 (d, 1H, $J = 9$ Hz, H-6), 10.18 (d, 1H, $J = 9$ Hz, H-1), 12.89 (s, 1H, OH-12). ^{13}C NMR ($CDCl_3$) δ: 27.6 (C-9Me$_2$), 29.8 (C-1″Me$_2$), 46.0 (C-9), 72.4 (C-1″), 75.3 (C-3″), 85.1 (C-2″), 92.6 (C-1′), 99.2 (C-11), 104.6 (C-12a), 112.9 (C-9a), 115.3 (C-13a), 116.8 (C-6), 119.6 (C-3), 128.8 (C-1), 130.1 (C-4), 130.7 (C-3b), 131.0 (C-4a), 132.2 (C-2), 134.1 (C-5), 140.6 (C-9b), 142.4 (C-6a), 156.5 (C-10), 158.4 (C-8), 160.3 (C-12), 182.4 (CO). IR (KBr) cm^{-1}: 3267, 2992, 2958, 2854, 1667, 1626, 1498, 1385, 1339, 1138, 1087, 889, 830. UV λ_{max} (MeOH) nm (log ϵ): 207 (4.79), 261 (4.39), 307 (4.63), 420 (3.68). MS (ESI$^+$) m/z: 488 and 490 $[M + H]^+$, 510 and 512 $[M + Na]^+$. 3-*Bromo*-10,12-*dihydroxy*-9,9-*dimethyl*-8-*methylidene*-8,9-*dihydro*-13*H*-*benzo*[*a*]*pyrrolo*[1,2,3-*fg*]*acridin*-13-*one* (**24**). ^{1}H NMR (DMSO-d_6) δ: 1.74 (s, 6H, C-9Me$_2$), 4.56 (d, 1H, $J = 3$ Hz, H-1′), 4.71 (d, 1H, $J = 3$ Hz, H-1′), 6.43 (s, 1H, H-11), 7.87 (dd, 1H, $J = 9$ and 2 Hz, H-2), 8.24 (d, 1H, $J = 9$ Hz, H-5), 8.29 (d, 1H, $J = 2$ Hz, H-4), 8.35 (d, 1H, $J = 9$ Hz, H-6), 9.95 (d, 1H, $J = 9$ Hz, H-1), 10.51 (s, 1H, OH-10), 15.50 (s, 1H, OH-12). ^{13}C NMR (DMSO-d_6) δ: 28.1 (C-9Me$_2$), 43.8 (C-9), 84.2 (C-1′), 99.5 (C-11), 107.6 (C-12a), 107.8 (C-9a), 111.1 (C-13a), 118.9 (C-3), 120.4 (C-6), 128.9 (C-1), 129.7 (C-4a), 131.0 (C-4), 131.5 (C-13b), 132.0 (C-2), 135.3 (C-5), 136.3 (C-9b), 143.1 (C-6a), 161.2 (C-12), 165.1 (C-10), 172.7 (C-8), 183.2 (CO). IR (KBr) cm^{-1}: 3388, 2952, 2921, 2852, 1647, 1586, 1493, 1457, 1437, 1161, 1139, 1088, 829, 796. UV λ_{max} (MeOH) nm (log ϵ): 203 (4.59), 224 (4.38), 246 (4.29), 257 (4.32), 296 (4.67), 392 (3.50). MS (ESI$^+$) m/z: 422 and 424 $[M + H]^+$, 444 and 446 $[M + Na]^+$. 10-*Bromo*-6-*hydroxy*-3,3-*dimethyl*-3,14-*dihydro*-7*H*-*benzo*[*a*]*pyrano*[3,2-*h*]*acridin*-7-*one* (**22**). ^{1}H NMR (DMSO-d_6) δ: 1.45 (s, 6H, C-3Me$_2$), 5.75 (d, 1H, $J = 10$ Hz, H-2), 6.14 (s, 1H, H-5), 7.18 (d, 1H, $J = 10$ Hz, H-1), 7.83 (dd, 1H, $J = 9$ and 2 Hz, H-9), 7.98 (d, 1H, $J = 9$ Hz, H-13), 8.19 (d, 1H, $J = 9$ Hz, H-12), 8.25 (d, 1H, $J = 2$ Hz, H-11), 7.94 (d, 1H, $J = 9$ Hz, H-8), 11.49 (s, 1H, NH), 15.16 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 28.1 (C-3*Me*$_2$), 77.7 (C-3), 98.0 (C-5), 98.8 (C-14b), 106.7 (C-6a), 111.0 (C-7a), 116.6 (C-1), 118.7 (C-10), 120.1 (C-13), 126.5 (C-2), 128.7 (C-8), 129.9 (C-11a), 131.0 (C-11), 131.4 (C-7b), 132.0 (C-9), 135.2 (C-12), 136.5 (C-14a), 143.1 (C-13a), 159.0 (C-4a), 164.2 (C-6), 182.7 (CO). IR (KBr) cm^{-1}: 3379, 2967, 2921, 1642, 1583, 1546, 1488, 1375, 1174, 1145, 1124, 893, 833, 759. UV λ_{max} (MeOH) nm (log ϵ): 206 (4.67), 256 (4.38), 265 (4.42), 302 (4.87), 334 (4.05), 406 (3.60). MS (ESI$^+$) m/z: 422 and 424 $[M + H]^+$, 444 and 446 $[M + Na]^+$.

4.1.8. *10-Bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[a]pyrano[3,2-h]acridin-7-one (**9**)*

Anhydrous sodium carbonate (8.97 g, 84.6 mmol) and methyl iodide (10.5 mL, 170 mmol) were added to a solution of 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**22**) (7.14 g, 19.9 mmol) in anhydrous *N*,*N*-dimethylformamide (350 mL). The reaction mixture was heated to 45 °C for 24 h. Then, a suspension of sodium hydride (170 mmol) in anhydrous *N*,*N*-dimethylformamide (175 mL) and methyl iodide (5.3 mL, 85 mmol) were added, and the reaction mixture was heated at 40 °C for more 3 h. After addition of icy water (1 L), the solid was filtered, washed with water (3 × 200 mL) and dry in vacuum over P_2O_5. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.4/0.6: V/V) gave 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**9**) as an amorphous yellow solid (6.75 g, 90%). ^{1}H NMR ($CDCl_3$) δ: 1.53 (s, 6H, C-3Me$_2$), 3.78 (s, 3H, NMe), 4.01 (s, 3H, OMe), 5.49 (d, 1H, $J = 10$ Hz, H-2), 6.37 (s, 1H, H-5), 6.49 (d, 1H, $J = 10$ Hz, H-1), 7.37 (d, 1H, $J = 9$ Hz, H-13), 7.67 (dd, 1H, $J = 9$ and 2 Hz, H-9), 7.71 (d, 1H, $J = 9$ Hz, H-12), 8.83 (d, 1H, $J = 2$ Hz, H-11), 9.81 (d, 1H, $J = 9$ Hz, H-8). ^{13}C NMR ($CDCl_3$) δ: 27.0 (C-3*Me*$_2$), 45.0 (NMe), 56.5 (OMe), 76.4 (C-3), 95.3 (C-5), 103.1 (C-14b), 113.2 (C-6a), 117.4 (C-13), 118.6 (C-7a), 119.0 (C-10), 121.4 (C-1), 123.7 (C-2), 128.9 (C-8), 129.7 (C-11a), 129.8 (C-11), 130.7 (C-7b), 131.6 (C-9), 133.0 (C-12), 144.7 (C-13a and C-14a), 158.6 (C-4a), 162.1 (C-6), 179.0 (CO). IR (KBr) cm^{-1}: 2967, 2926, 2833, 1621, 1592, 1565, 1498, 1339, 1205, 1133, 1072, 1049, 876, 830, 722. UV λ_{max} (MeOH) nm (log ϵ): 210 (4.65), 254 (4.30), 302 (4.74), 402 (3.64). MS (ESI$^+$) m/z: 450 and 452 $[M + H]^+$, 472 and 474 $[M + Na]^+$.

4.1.9. *(7-Bromo-3-methoxynapht-2-yl)-[2-(7-bromo-3-methoxy-2-naphthamido)-4,6-dimethoxyphenyl]ketone (**28**) and (7-bromo-3-methoxynapht-2-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**27**)*

Thionyl chloride (18.0 mL) was added to 7-bromo-3-methoxy-2-naphthaoic acid (3.00 g, 10.7 mmol). The reaction mixture was stirred at 65 °C for 3 h and evaporated under reduced pressure to give crude 7-bromo-3-methoxy-2-naphthaoyl chloride (**26**) which was immediately used in the following step without further purification. Stannic chloride (2.1 mL, 18.3 mmol) was added to a solution of the crude 7-bromo-3-methoxy-2-naphthaoyl chloride (**26**) in anhydrous dichloroethane (15 mL). The reaction mixture was heated to 45 °C for 30 min. Then, a solution of 3,5-dimethoxyacetanilide (**14**) (1.20 g, 6.11 mmol) in anhydrous dichloroethane

(32 mL) was added, and the mixture was heated at 45 °C for 3 h. The reaction mixture was added to cold water (100 mL) and extracted with dichloromethane (3 × 150 mL). The combined organic layers were washed with an aqueous solution of potassium hydroxide (10%) to remove excess of 7-bromo-3-methoxy-2-naphthaoic acid, dried over $MgSO_4$ and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.7/0.3: V/V) gave successively (7-bromo-3-methoxynapht-2-yl)-[2-(7-bromo-3-methoxy-2-naphthamido)-4,6-dimethoxyphenyl]ketone (**28**) (211 mg, 9%) as a yellowish amorphous solid and (7-bromo-3-methoxynapht-2-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**27**) (2.26 g, 81%) as an amorphous white solid. (7-*Bromo*-3-*methoxynapht*-2-*yl*)-[2-(7-*bromo*-3-*methoxy*-2-*naphthamido*)-4,6-*dimethoxyphenyl*]*ketone* (**28**). ^{1}H NMR ($CDCl_3$) δ: 3.35 (s, 3H, OMe-6′), 3.81 (s, 3H, OMe-3), 3.96 (s, 3H, OMe-4′), 4.17 (s, 3H, OMe-3″), 6.18 (d, 1H, J = 2 Hz, H-5′), 7.08 (s, 1H, H-4), 7.20 (s, 1H, H-4″), 7.54 (dd, 1H, J = 9 and 2 Hz, H-6), 7.57 (dd, H, J = 9 and 2 Hz, H-6″), 7.60 (d, 1H, J = 9 Hz, H-5), 7.62 (d, 1H, J = 9 Hz, H-5″), 7.79 (s, 1H, H-1), 7.94 (d, 1H, J = 2 Hz, H-8), 8.03 (d, 1H, J = 2 Hz, H-8″), 8.24 (d, H, J = 2 Hz, H-3′), 8.62 (s, 1H, H-1″), 12.10 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 56.6 (OMe-6′), 55.8 (OMe-4′), 55.9 (OMe-3), 56.0 (OMe-3″), 95.0 (C-5′), 98.8 (C-3′), 105.5 (C-4), 106.5 (C-4″), 112.3 (C-1′), 117.6 (C-7), 118.0 (C-7″), 125.0 (C-4a″), 127.9 (C-5″), 128.0 (C-1), 128.2 (C-5), 129.3 (C-2 and C-2″), 130.6 (C-8), 130.8 (C-6), 131.0 (C-8″), 131.7 (C-6″), 132.9 (C-1″), 133.8 (C-8a), 134.5 (C-8a″), 135.7 (C-4a), 141.6 (C-2′), 155.2 (C-3″), 155.6 (C-3), 161.6 (C-6′), 164.3 (C-4′), 164.4 (CONH), 195.0 (ArCOAr). IR (KBr) cm^{-1}: 3282, 3003, 2920, 2850, 1669, 1609, 1575, 1526, 1447, 1305, 1260, 1208, 1157, 1059, 844, 726. UV λ_{max} (MeOH) nm (log ϵ): 201 (4.46), 234 (4.83). MS (ESI$^+$) m/z: 700, 702 and 704 $[M + Na]^+$, 716, 718 and 720 $[M + K]^+$. (7-*Bromo*-3-*methoxynapht*-2-*yl*)-(2-*acetamido*-4,6-*dimethoxyphenyl*)*ketone* (**27**). ^{1}H NMR ($CDCl_3$) δ: 2.23 (s, 1H, COMe), 3.28 (s, 3H, OMe-6′), 3.83 (s, 3H, OMe-3), 3.90 (s, 3H, OMe-4′), 6.07 (d, 1H, J = 2 Hz, H-5′), 7.08 (s, 1H, H-4), 7.53 (dd, 1H, J = 9 and 2 Hz, H-6), 7.60 (d, 1H, J = 9 Hz, H-5), 7.61 (s, 1H, H-1), 7.91 (d, 1H, J = 2 Hz, H-8), 8.03 (d, 1H, J = 2 Hz, H-3′), 11.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 25.8 (COMe), 56.8 (OMe-6′, OMe-4′ and OMe-3), 94.3 (C-5′), 97.0 (C-3′), 105.2 (C-4), 109.1 (C-1'), 117.6 (C-7), 126.8 (C-1), 128.2 (C-5), 129.4 (C-2), 130.4 (C-8), 130.7 (C-6), 133.5 (C-8a), 136.4 (C-4a), 143.6 (C-2′), 155.2 (C-3), 161.8 (C-6′), 164.3 (C-4′), 169.9 (CONH), 198.0 (ArCOAr). IR (KBr) cm^{-1}: 3289, 2920, 2850, 1609, 1582, 1446, 1303, 1252, 1206, 1159, 849. UV λ_{max} (MeOH) nm (log ϵ): 203 (4.33), 206 (4.37), 234 (4.80), 285 (4.12), 312 (4.18). MS (ESI$^+$) m/z: 480 and 482 $[M + Na]^+$.

4.1.10. (7-Bromo-3-methoxynapht-2-yl)-(2-amino-4,6-dimethoxyphenyl)ketone (**31**) and 4-(7-bromo-3-methoxynapht-2-yl)-5,7-dimethoxy-1H-quinolin-2-one (**30**)

A solution of (7-bromo-3-methoxynapht-2-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**27**) (109 mg, 0.237 mmol) in anhydrous *N*,*N*-dimethylformamide (6 mL) was added to sodium hydride (1.63 mmol). The reaction mixture was stirred at room temperature for 2 days and poured on cold water (50 mL). The precipitate was filtered and dried in a vacuum over P_2O_5. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.2/0.8: V/V) gave (7-bromo-3-methoxynapht-2-yl)-(2-amino-4,6-dimethoxyphenyl)ketone (**31**) (10 mg, 10%) as an amorphous yellow solid and 4-(7-bromo-3-methoxynapht-2-yl)-5,7-dimethoxy-1*H*-quinolin-2-one (**30**) (50.7 mg, 50%) as an amorphous white solid. (7-*Bromo*-3-*methoxynapht*-2-*yl*)-(2-*amino*-4,6-*dimethoxyphenyl*)*ketone* (**31**). ^{1}H NMR ($CDCl_3$) δ: 3.22 (s, 3H, OMe-6′), 3.82 (s, 3H, OMe-4′), 3.86 (s, 3H, OMe-3), 5.63 (d, 1H, J = 2 Hz, H-5′), 5.79 (d, H, J = 2 Hz, H-3′), 6.50 (s, 2H, NH_2), 7.08 (s, 1H, H-4), 7.50 (dd, 1H, J = 9 and 2 Hz, H-6), 7.51 (s, 1H, H-1), 7.60 (d, 1H, J = 9 Hz, H-5), 7.98 (d, 1H, J = 2 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ: 55.3 (OMe-6′ and OMe-4′), 55.8 (OMe-3), 88.7 (C-5′), 91.9 (C-3′), 104.8 (C-4), 106.0 (C-1′), 117.2 (C-7), 125.1 (C-1), 128.2 (C-5), 129.7 (C-2), 129.8 (C-6), 130.1 (C-8), 132.9 (C-8a), 138.2 (C-4a), 154.2 (C-2′), 155.2 (C-3), 163.9 (C-6′), 165.2 (C-4′), 194.2 (ArCOAr). IR (KBr) cm^{-1}: 3456, 3316, 3052, 3001, 2959, 2924, 2846, 1615, 1584, 1460, 1274, 1252, 1208, 1165, 1141. UV λ_{max} (MeOH) nm (log ϵ): 233 (4.74), 297 (4.13), 353 (3.78). MS (ESI$^+$) m/z: 438 and 440 $[M + Na]^+$. 4-(7-*Bromo*-3-*methoxynapht*-2-*yl*)-5,7-*dimethoxy*-1*H*-*quinolin*-2-*one* (**30**). ^{1}H NMR ($CDCl_3$) δ: 3.33 (s, 3H, OMe-5), 3.80 (s, 3H, OMe-3′), 3.90 (s, 3H, OMe-7), 6.13 (d, 1H, J = 2 Hz, H-6), 6.38 (s, 1H, H-3), 6.54 (d, 1H, J = 2 Hz, H-8), 7.08 (s, 1H, H-4′), 7.54 (s, 1H, H-1′), 7.53 (dd, J = 9 and 2 Hz, H-6′), 7.65 (d, 1H, J = 9 Hz, H-5′), 7.93 (d, 1H, J = 2 Hz, H-8′), 12.40 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 55.4 (OMe-5), 55.6 (OMe-3′), 55.8 (OMe-7), 91.3 (C-8), 94.9 (C-6), 103.8 (C-4′), 106.2 (C-4a), 117.3 (C-7′), 118.9 (C-3), 126.1 (C-1′), 128.1 (C-5′), 129.6 (C-6′), 129.7 (C-8a′), 129.8 (C-8′), 132.8 (C-4a′), 134.9 (C-2′), 141.6 (C-8a), 149.2 (C-4), 156.3 (C-3′), 158.5 (C-5), 162.5 (C-7), 164.4 (C-2). IR (KBr) cm^{-1}: 3353, 3000, 2923, 2849, 1655, 1623, 1602, 1400, 1386, 1205, 978, 814. UV λ_{max} (MeOH) nm (log ϵ): 234 (4.86), 262 (4.43), 324 (4.08), 336 (4.03). MS (ESI$^+$) m/z: 440 and 442 $[M + H]^+$, 462 and 464 $[M + Na]^+$, 478 and 480 $[M + K]^+$.

4.1.11. (7-Bromo-3-methoxynapht-2-yl)-(2-amino-4,6-dimethoxyphenyl)ketone (**31**)

An aqueous solution of hydrochloric acid (20 mL, 35%) was added to a solution of (7-bromo-3-methoxynapht-2-yl)-(2-acetamido-4,6-dimethoxyphenyl)ketone (**27**) (2.26 g, 4.83 mmol) in methanol (30 mL). The reaction mixture was heated to reflux for 4 h. After dilution with cold water (100 mL), an aqueous solution of sodium hydroxide (20%) was added to obtain pH = 10. An extraction was performed with ethyl acetate (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give (7-bromo-3-methoxynapht-2-yl)-(2-amino-4,6-dimethoxyphenyl)ketone (**31**) (1.9 g, 92%).

4.1.12. 9-Bromo-1,3-dimethoxybenzo[b]acridin-12(5H)-one (**29**)

A solution of (7-bromo-3-methoxynapht-2-yl)-(2-amino-4,6-dimethoxyphenyl)ketone (**31**) (1.60 g, 3.84 mmol) in anhydrous *N*,*N*-dimethylformamide (40 mL) was added to a suspension of sodium hydride (19.2 mmol) in anhydrous *N*,*N*-dimethylformamide (20 mL). The reaction mixture was stirred at room temperature for 12 h. A hot filtration on Büchner funnel permitted to remove sodium salts. Then, after addition of cold water (300 mL), the precipitate was filtered, washed with water (3 × 50 mL), with methanol (50 mL) and, to finish, with ethyl acetate (2 × 50 mL). A yellow amorphous solid corresponding to 9-bromo-1,3-dimethoxybenzo[*b*]acridin-12(5*H*)-one (**29**) (1.18 g, 80%) was obtained and used in the following step without further purification. ^{1}H NMR (DMSO-d_6) δ: 3.84 (s, 3H, OMe), 3.88 (s, 3H, OMe), 6.24 (d, 1H, J = 2 Hz, H-4), 6.45 (d, J = 2 Hz, H-2), 7.61 (dd, 1H, J = 9 and 2 Hz, H-8), 7.76 (s, 1H, H-6), 7.92 (d, 1H, J = 9 Hz, H-7), 8.39 (d, 1H, J = 2 Hz, H-10), 8.72 (s, 1H, H-11), 11.36 (s, 1H, NH). IR (KBr) cm^{-1}: 3280, 3125, 2991, 2922, 1628, 1596, 1507, 1216, 1168, 1127. UV λ_{max} (MeOH) nm (log ϵ): 209 (4.24), 212 (4.24), 227 (4.25), 248 (4.21), 381 (4.75), 324 (4.05), 431 (3.70). MS (ESI$^+$) m/z: 384 and 386 $[M + H]^+$.

4.1.13. 9-Bromo-1,3-dihydroxybenzo[b]acridin-12(5H)-one (**32**)

An aqueous solution of bromhydric acid (20 mL, 48%) was added to a solution of 9-bromo-1,3-dimethoxybenzo[*b*]acridin-12(5*H*)-one (**29**) (400 mg, 1.04 mmol). The reaction mixture was heated to 110 °C for 48 h. An aqueous solution of sodium hydroxide (10%) was added to obtain pH = 4. The solid was filtered, washed with water (3 × 100 mL), dried in vacuum under P_2O_5, and to finish, was washed with dichloromethane (3 × 50 mL). 9-Bromo-1,3-dihydroxybenzo

[*b*]acridin-12(5*H*)-one (**32**) was obtained as a brown amorphous solid (350 mg, 94%). ^{1}H NMR (DMSO-d_6) δ: 5.97 (d, 1H, $J = 2$ Hz, H-2), 6.28 (d, $J = 2$ Hz, H-4), 7.66 (dd, 1H, $J = 9$ and 2 Hz, H-8), 7.85 (s, 1H, H-6), 7.96 (d, 1H, $J = 9$ Hz, H-7), 8.44 (d, 1H, $J = 2$ Hz, H-10), 8.85 (s, 1H, H-11), 10.71 (s, 1H, OH-3), 11.71 (s, 1H, NH), 13.97 (s, 1H, OH-1). ^{13}C NMR (DMSO-d_6) δ: 91.5 (C-4), 95.6 (C-2), 102.6 (C-12a), 112.2 (C-6), 117.4 (C-9), 121.0 (C-11a), 126.4 (C-11), 129.1 (C-10a), 129.3 (C-7), 131.5 (C-10), 132.0 (C-8), 134.7 (C-6a), 138.3 (C-5a), 144.9 (C-4a), 164.6 (C-1), 165.8 (C-3), 181.1 (C-12). IR (KBr) cm^{-1}: 3278, 3055, 2920, 1650, 1588, 1505, 1174, 1022, 1001, 817. UV λ_{max} (MeOH) nm (log ϵ): 207 (3.90), 224 (3.61), 284 (4.67), 355 (3.41), 369 (3.14), 453 (3.58). MS (ESI$^+$) *m*/*z*: 356 and 358 [M + H]$^+$.

4.1.14. 9-Bromo-5-hydroxy-1,1-dimethyl-2-methyliden-1,2-dihydrobenzo[b]furo[3,2-h]acridin-6(13H)-one (35) and 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (34)

Anhydrous potassium carbonate (643 mg, 4.66 mmol), anhydrous potassium iodide (774 mg, 4.66 mmol) and 3-chloro-3-methylbut-1-yne (523 μL, 4.66 mmol) were added to a solution of 9-bromo-1,3-dihydroxybenzo[*b*]acridin-12(5*H*)-one (**32**) (553 mg, 1.55 mmol) in anhydrous *N*,*N*-dimethylformamide (50 mL). The reaction mixture was stirred at 65 °C for 25 h, poured in water (150 mL) and then, extracted with dichloromethane/methanol: 10/1: V/V (4 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/C_6H_{12}: 50/50 to 100/0: V/V) gave successively 9-bromo-5-hydroxy-1,1-dimethyl-2-methyliden-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (**35**) (26 mg, 4%) as an orange amorphous solid, and a mixture of 9-bromo-1-hydroxy-3-(1,1-dimethylpropyn-1-oxy)benzo[*b*]acridin-12(5*H*)-one (**33**) and 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**34**). Without any further purification, the mixture of compound **33** and **34** was dissolved in anhydrous *N*,*N*-dimethylformamide (10 mL). The solution was heated to 130 °C for 3.5 h, and poured on cold water (80 mL). The solid was filtered, washed with water (3 × 20 mL) and dried in a vacuum over P_2O_5 to give 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**34**) (249 mg, 38%) as a red amorphous solid. 9-*Bromo*-5-*hydroxy*-1,1-*dimethyl*-2-*methyliden*-1,2-*dihydrobenzo*[*b*]*furo*[3,2-*h*]*acridin*-6(13*H*)-*one* (**35**). ^{1}H NMR (DMSO-d_6) δ: 1.71 (s, 6H, C-1Me$_2$), 4.56 (d, 1H, $J = 3$ Hz, CH_2a), 4.70 (d, 1H, $J = 3$ Hz, CH_2b), 6.31 (s, 1H, H-4), 7.70 (dd, 1H, $J = 2$ and 9 Hz, H-10), 7.94 (d, 1H, $J = 9$ Hz, H-11), 8.49 (d, 1H, $J = 2$ Hz, H-8), 8.53 (s, 1H, H-12), 8.91 (s, 1H, H-7), 10.15 (s, 1H, NH), 14.74 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 27.8 (C-1*Me*$_2$), 43.5 (C-1), 84.4 (CH_2), 90.9 (C-4), 104.3 (C-5a), 107.7 (C-13b), 114.2 (C-12), 118.1 (C-9), 120.8 (C-6a), 126.4 (C-7), 129.5 (C-7a), 129.7 (C-11), 131.7 (C-8), 132.2 (C-10), 134.8 (C-11a), 138.1 (C-12a), 138.9 (C-13a), 162.7 (C-3a), 165.8 (C-5), 172.6 (C-2), 182.4 (C═O). IR (KBr) cm^{-1}: 3400, 2960, 2921, 2865, 1645, 1594, 1507, 1497, 1470, 1283, 1162, 1141, 1077, 855, 824, 812. UV λ_{max} (MeOH) nm (log ϵ): 204 (4.48), 231 (4.29), 265 (4.54), 288 (4.81), 358 (4.05), 459 (3.59). MS (ESI$^+$) *m*/*z*: 422 and 424 [M + H]$^+$, 444 and 446 [M + Na]$^+$, 460 and 462 [M + K]$^+$. 10-*Bromo*-6-*hydroxy*-3,3-*dimethyl*-3,14-*dihydro*-7*H*-*benzo*[*b*]*pyrano*[3,2-*h*]*acridin*-7-*one* (**34**). ^{1}H NMR (DMSO-d_6) δ: 1.45 (s, 6H, C-3Me$_2$), 5.77 (d, 1H, $J = 10$ Hz, H-2), 6.05 (s, 1H, H-5), 7.13 (d, 1H, $J = 10$ Hz, H-1), 7.69 (dd, 1H, $J = 9$ and 2 Hz, H-11), 7.98 (d, 1H, $J = 9$ Hz, H-12), 8.21 (s, 1H, H-13), 8.48 (d, 1H, $J = 2$ Hz, H-9), 8.89 (s, 1H, H-8), 11.15 (s, 1H, NH), 14.42 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 28.1 (C-3*Me*$_2$), 77.9 (C-3), 96.3 (C-5), 98.5 (C-14b), 103.4 (C-6a), 113.6 (C-13), 116.6 (C-1), 117.9 (C-10), 120.8 (C-7a), 126.3 (C-8), 126.4 (C-2), 129.5 (C-8a), 129.6 (C-12), 131.6 (C-9), 132.1 (C-11), 134.7 (C-12a), 138.3 (C-13a), 139.2 (C-14a), 160.8 (C-4a), 164.8 (C-6), 181.9 (C═O). IR (KBr) cm^{-1}: 3388, 2960, 2929, 1647, 1587, 1552, 1498, 1345, 1278, 1166, 1126, 863, 820. UV λ_{max} (MeOH) nm (log ϵ): 214 (4.40), 253 (4.36), 276 (4.78), 300 (4.74), 315 (4.64), 372 (3.85), 468 (3.60). MS (ESI$^+$) *m*/*z*: 422 and 424 [M + H]$^+$, 444 and 446 [M + Na]$^+$.

4.1.15. 10-Bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (35) and 10-bromo-6-hydroxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (36)

Anhydrous sodium carbonate (1.67 g, 10.0 mmol) and methyl iodide (495 μL, 10.0 mmol) were added to a solution of 10-bromo-6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**34**) (423 mg, 1.00 mmol) in anhydrous *N*,*N*-dimethylformamide (22 mL). The reaction mixture was heated to 50 °C for 27 h. Then, a suspension of sodium hydride (10 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) and methyl iodide (495 μL, 10.0 mmol) were added, and the reaction mixture was heated at 40 °C for more 3 h. After addition of water (100 mL) and extraction with dichloromethane (3 × 100 mL), the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.7/0.3: V/V) gave 10-bromo-6-hydroxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**36**) (51 mg, 12%) as a red amorphous solid and 10-bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**35**) (371 mg, 82%) as a yellow amorphous solid. 10-*Bromo*-6-*methoxy*-3,3,14-*trimethyl*-3,14-*dihydro*-7*H*-*benzo*[*b*]*pyrano*[3,2-*h*]*acridin*-7-*one* (**35**). ^{1}H NMR ($CDCl_3$) δ: 1.56 (s, 6H, C-3Me$_2$), 3.90 (s, 3H, NMe), 4.01 (s, 3H, OMe), 5.55 (d, 1H, $J = 10$ Hz, H-2), 6.31 (s, 1H, H-5), 6.57 (d, 1H, $J = 10$ Hz, H-1), 7.57 (dd, 1H, $J = 9$ and 2 Hz, H-11), 7.62 (s, 1H, H-13), 7.72 (d, 1H, $J = 9$ Hz, H-12), 8.16 (d, 1H, $J = 2$ Hz, H-9), 8.81 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ: 27.0 (C-3*Me*$_2$), 44.8 (NMe), 56.5 (OMe), 76.6 (C-3), 94.2 (C-5), 103.2 (C-14b), 109.7 (C-6a), 112.1 (C-13), 118.1 (C-10), 121.9 (C-1), 123.3 (C-2), 126.2 (C-7a), 127.3 (C-8), 128.5 (C-12), 129.5 (C-8a), 131.3 (C-9), 131.6 (C-11), 134.1 (C-12a), 142.3 (C-13a), 147.4 (C-14a), 160.1 (C-4a), 163.3 (C-6), 177.8 (C═O). IR (KBr) cm^{-1}: 3058, 3006, 2969, 2953, 2921, 1645, 1633, 1614, 1583, 1559, 1489, 1458, 1197, 1139, 1120, 1088, 854, 805, 768. UV λ_{max} (MeOH) nm (log ϵ): 211 (4.54), 245 (4.36), 279 (4.73), 300 (4.72), 310 (4.73), 366 (3.87), 450 (3.72). MS (ESI$^+$) *m*/*z*: 472 and 474 [M + Na]$^+$, 488 and 490 [M + K]$^+$. 10-*Bromo*-6-*hydroxy*-3,3,14-*trimethyl*-3,14-*dihydro*-7*H*-*benzo*[*b*]*pyrano*[3,2-*h*]*acridin*-7-*one* (**36**). ^{1}H NMR ($CDCl_3$) δ: 1.57 (s, 6H, C-3Me$_2$), 4.00 (s, 3H, NMe), 5.58 (d, 1H, $J = 10$ Hz, H-2), 6.28 (s, 1H, H-5), 6.60 (d, 1H, $J = 10$ Hz, H-1), 7.65 (dd, 1H, $J = 9$ and 2 Hz, H-11), 7.74 (s, 1H, H-13), 7.80 (d, 1H, $J = 9$ Hz, H-12), 8.19 (d, 1H, $J = 2$ Hz, H-9), 8.89 (s, 1H, H-8), 14.48 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 27.1 (C-3*Me*$_2$), 44.2 (NMe), 76.8 (C-3), 97.8 (C-5), 101.3 (C-14b), 112.5 (C-13), 118.7 (C-10), 121.6 (C-1), 122.8 (C-7a), 123.4 (C-2), 126.7 (C-8), 128.7 (C-12), 129.3 (C-8a), 131.2 (C-9), 132.3 (C-11), 134.7 (C-12a), 142.1 (C-13a), 145.3 (C-14a), 162.5 (C-4a), 165.7 (C-6), 181.8 (C-7). IR (KBr) cm^{-1}: 3425, 3057, 2975, 1629, 1590, 1578, 1467, 1388, 1375, 1329, 1274, 1173, 1127, 866, 819. UV λ_{max} (MeOH) nm (log ϵ): 211 (4.40), 253 (4.24), 279 (4.60), 312 (4.67), 377 (3.84), 468 (3.57). MS (ESI$^+$) *m*/*z*: 436 and 438 [M + H]$^+$, 458 and 460 [M + Na]$^+$, 472 and 474 [M + K]$^+$.

4.1.16. (±)-cis-10-Bromo-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[a]pyrano[3,2-h]acridin-7-one (37)

10-Bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**9**) (100 mg, 222 μmol) was added to a solution of osmium tetroxide (2.5% m/V in *t*-BuOH) (240 μL) and *N*-methylmorpholine *N*-oxide hydrate (380 mg, 2.80 mmol) in *t*-BuOH/THF/H_2O: 10/3/1: V/V/V (3 mL). The reaction mixture was stirred at room temperature for 3.25 h. After addition of saturated

aqueous $NaHSO_3$ (5 mL), the reaction was stirred for 1 h and then extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 97/3: V/V) gave (±)-*cis*-10-bromo-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**37**) (69.2 mg, 64%) as a yellowish amorphous solid. ^{1}H NMR (DMSO-d_6) δ: 1.38 (s, 3H, C-3Me$_2$A), 1.42 (s, 3H, C-3Me$_2$B), 3.66 (t, 1H, J = 5 Hz, H-2), 3.83 (s, 3H, OMe), 3.93 (s, 3H, NMe), 4.63 (d, 1H, J = 9 Hz, OH-1), 5.05 (d, 1H, J = 5 Hz, OH-2), 5.07 (dd, 1H, J = 9 and 5 Hz, H-1), 6.27 (s, 1H, H-5), 7.72 (dd, 1H, J = 9 and 2 Hz, H-9), 7.81 (d, 1H, J = 9 Hz, H-13), 8.12 (d, 1H, J = 9 Hz, H-12), 8.22 (d, 1H, J = 2 Hz, H-11), 9.12 (d, 1H, J = 9 Hz, H-8). ^{13}C NMR (DMSO-d_6) δ: 22.8 (C-3*Me$_2$A*), 25.5 (C-3*Me$_2$B*), 42.5 (NMe), 56.4 (OMe), 64.2 (C-1), 70.6 (C-2), 78.1 (C-3), 95.5 (C-5), 103.9 (C-14b), 114.0 (C-6a), 117.7 (C-7a), 118.0 (C-10), 119.3 (C-13), 128.3 (C-8), 129.5 (C-11a), 130.4 (C-11), 130.7 (C-7b), 131.2 (C-9), 133.0 (C-12), 145.0 (C-13a), 147.3 (C-14a), 158.8 (C-4a), 160.5 (C-6), 177.8 (CO). IR (KBr) cm^{-1}: 3445, 3378, 2972, 2931, 2849, 1627, 1596, 1509, 1386, 1211, 1147, 1072, 1052, 889, 838, 815. UV λ_{max} (MeOH) nm (log ϵ): 205 (4.68), 254 (4.48), 298 (4.76), 383 (3.75), 400 (3.76). MS (ESI$^+$) m/z: 484 and 486 [M + H]$^+$, 506 and 508 [M + Na]$^+$.

4.1.17. (±)-cis-10-Bromo-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (**38**)

10-Bromo-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**25**) (100 mg, 222 μmol) was added to a solution of osmium tetroxide (2.5% m/V in *t*-BuOH) (250 μL) and *N*-methylmorpholine *N*-oxide hydrate (90 mg, 0.667 mmol) in CH_2Cl_2/MeOH: 4/1: V/V (8 mL). The reaction mixture was stirred at room temperature for 4.25 h. After addition of saturated aqueous $NaHSO_3$ (5 mL), the reaction was stirred for 1 h and then extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 98/2: V/V) gave (±)-*cis*-10-bromo-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 (**38**) (104.6 mg, 97%) as a yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ: 1.39 (s, 3H, C-3MeA), 1.43 (s, 3H, C-3MeB), 3.68 (t, 1H, J = 5 Hz, H-2), 3.82 (s, 3H, OMe), 3.89 (s, 3H, NMe), 4.64 (d, 1H, J = 10 Hz, OH-1), 5.08 (dd, 1H, J = 10 and 5 Hz, H-1), 5.10 (d, 1H, J = 5 Hz, OH-2), 6.20 (s, 1H, H-5), 7.66 (dd, 1H, J = 9 and 2 Hz, H-11), 7.95 (s, 1H, H-13), 8.00 (d, 1H, J = 9 Hz, H-12), 8.41 (d, 1H, J = 2 Hz, H-9), 8.64 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6) δ: 22.8 (C-3*MeB*), 25.6 (C-3*MeA*), 42.3 (NMe), 56.3 (OMe), 64.5 (C-1), 70.6 (C-2), 78.3 (C-3), 94.1 (C-5), 104.1 (C-14b), 110.3 (C-6a), 112.8 (C-13), 117.5 (C-10), 126.2 (C-8), 126.3 (C-7a), 129.1 (C-8a), 129.7 (C-12), 131.3 (C-9 and C-11), 134.3 (C-12a), 142.5 (C-13a), 150.3 (C-14a), 160.3 (C-4a), 161.7 (C-6), 176.8 (C=O). IR (KBr) cm^{-1}: 3361, 2956, 2925, 2851, 1633, 1606, 1590, 1501, 1458, 1388, 1205, 1143, 1092, 816. UV λ_{max} (MeOH) nm (log ϵ): 208 (4.45), 240 (4.26), 286 (4.82), 335 (3.95), 446 (3.67). MS (ESI$^+$) m/z: 506 and 508 [M + Na]$^+$, 522 and 524 [M + K]$^+$.

4.1.18. (±)-cis-1,2-Diacetoxy-10-bromo-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[a]pyrano[3,2-h]acridin-7-one (**39**)

Acetic anhydride (284 μL, 3.02 mmol) and 4-dimethylaminopyridine (1 mg) were added to a solution of (±)-*cis*-10-bromo-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**37**) (29.2 mg, 60.3 μmol) in anhydrous pyridine (2.0 mL). The reaction mixture was stirred for 24 h at room temperature. After addition of cold water (5 mL), the precipitate was filtered, washed with water (2 × 5 mL) and dried in a vacuum over P_2O_5 to give (±)-*cis*-1,2-diacetoxy-10-bromo-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**39**) (32 mg, 93%) as a yellowish amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.48 (s, 3H, C-3Me$_2$B), 1.58 (s, 3H, C-3Me$_2$A), 1.98 (s, 3H, MeCOC-1), 2.03 (s, 3H, MeCOC-2), 3.71 (s, 3H, NMe), 4.05 (s, 3H, OMe), 5.46 (d, 1H, J = 5 Hz, H-2), 6.39 (s, 1H, H-5), 6.54 (d, 1H, J = 5 Hz, H-1), 7.42 (d, 1H, J = 9 Hz, H-13), 7.72 (dd, 1H, J = 9 and 2 Hz, H-9), 7.91 (d, 1H, J = 9 Hz, H-12), 7.97 (d, 1H, J = 2 Hz, H-11), 9.72 (d, 1H, J = 9 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ: 20.8 (*Me*COC-2), 21.0 (*Me*COC-1), 23.4 (C-3*Me$_2$A*), 24.5 (C-3*Me$_2$B*), 42.9 (NMe), 56.5 (OMe), 65.6 (C-1), 69.6 (C-2), 76.4 (C-3), 95.9 (C-5), 97.8 (C-14b), 115.0 (C-6a), 117.2 (C-13), 119.2 (C-10), 119.4 (C-7a), 128.7 (C-8), 129.6 (C-11a), 129.9 (C-11), 130.8 (C-7b), 131.9 (C-9), 133.3 (C-12), 145.2 (C-13a), 147.5 (C-14a), 159.2 (C-4a), 162.1 (C-6), 170.6 (Me*C*OC-2), 171.1 (Me*C*OC-1), 179.0 (CO). IR (KBr) cm^{-1}: 2978, 2931, 1750, 1622, 1596, 1504, 1231, 1211, 1154, 1070, 908, 887, 831. UV λ_{max} (MeOH) nm (log ϵ): 206 (4.65), 252 (4.45), 296 (4.72), 379 (3.73), 396 (3.75). MS (ESI$^+$) m/z: 568 and 570 [M + H]$^+$.

4.1.19. (±)-cis-1,2-Diacetoxy-10-bromo-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (**40**)

The procedure described for the preparation of **39** from **37**, applied to (±)-*cis*-10-bromo-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 (**38**) (35 mg, 72 μmol) gave (±)-*cis*-1,2-diacetoxy-10-bromo-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**40**) (40 mg, 98%) as a yellow amorphous product. ^{1}H NMR ($CDCl_3$) δ: 1.50 (s, 3H, C-3MeB), 1.59 (s, 3H, C-3MeA), 1.99 (s, 3H, MeCOC-1), 2.05 (s, 3H, MeCOC-2), 3.72 (s, 3H, NMe), 4.03 (s, 3H, OMe), 5.50 (d, 1H, J = 5 Hz, H-2), 6.33 (s, 1H, H-5), 6.58 (d, 1H, J = 5 Hz, H-1), 7.52 (s, 1H, H-13), 7.61 (dd, 1H, J = 9 and 2 Hz, H-11), 7.74 (d, 1H, J = 9 Hz, H-12), 8.19 (d, 1H, J = 2 Hz, H-9), 8.79 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ: 20.8 (*Me*COC-2), 21.2 (*Me*COC-1), 23.6 (C-3*MeA*), 24.6 (C-3*MeB*), 43.1 (NMe), 56.5 (OMe), 65.8 (C-1), 69.5 (C-2), 76.6 (C-3), 94.8 (C-5), 98.1 (C-14b), 111.4 (C-6a), 111.9 (C-13), 118.3 (C-10), 126.6 (C-7a), 127.2 (C-8), 128.5 (C-12), 129.7 (C-8a), 131.4 (C-9), 131.7 (C-11), 134.2 (C-12a), 142.8 (C-13a), 150.3 (C-14a), 160.5 (C-4a), 163.0 (C-6), 170.6 (Me*C*OC-2), 171.1 (Me*C*OC-1), 178.1 (C=O). IR (KBr) cm^{-1}: 3054, 2975, 2921, 2848, 1746, 1643, 1614, 1590, 1493, 1396, 1236, 1197, 1155, 1084, 1026, 913, 703. UV λ_{max} (MeOH) nm (log ϵ): 208 (4.48), 245 (4.26), 288 (4.80), 334 (3.97), 440 (3.68). MS (ESI$^+$) m/z: 568 and 570 [M + H]$^+$, 590 and 592 [M + Na]$^+$.

4.1.20. (±)-cis-10-Bromo-1,2-di-O-carbonyl-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[a]pyrano[3,2-h]acridin-7-one (**41**)

N,*N*′-Carbonydiimidazole (54 mg, 333 μmol) was added to a solution of (±)-*cis*-10-bromo-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**37**) (29.5 mg, 60.9 μmol) in 2-butanone (2 mL). The reaction mixture was stirred at room temperature for 5 h and water (5 mL) was added. An extraction with dichloromethane (3 × 10 mL) was performed and the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.4/0.6: V/V) gave (±)-*cis*-10-bromo-1,2-di-*O*-carbonyl-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**41**) (29 mg, 93%) as a yellowish amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 3H, C-3Me$_2$B), 1.63 (s, 3H, C-3Me$_2$B), 3.96 (s, 3H, NMe), 4.03 (s, 3H, OMe), 4.82 (d, 1H, J = 8 Hz, H-2), 6.27 (d, 1H, J = 8 Hz, H-1), 6.38 (s, 1H, H-5), 7.53 (d, 1H, J = 9 Hz, H-13), 7.72 (dd, 1H, J = 9 and 2 Hz, H-9), 7.92 (d, 1H, J = 9 Hz, H-12), 7.98 (d, 1H, J = 2 Hz, H-11), 9.62 (d, 1H, J = 9 Hz,

H-8). ^{13}C NMR ($CDCl_3$) δ: 22.1 (C-3*Me*$_2$*B*), 24.4 (C-3*Me*$_2$*A*), 43.9 (NMe), 56.6 (OMe), 70.9 (C-1), 74.1 (C-3), 78.8 (C-2), 96.4 (C-5), 97.2 (C-14b), 115.1 (C-6a), 117.7 (C-13), 119.5 (C-10), 119.9 (C-7a), 128.5 (C-8), 129.1 (C-11a), 130.0 (C-11), 130.9 (C-7b), 132.0 (C-9), 133.2 (C-12), 145.4 (C-13a), 147.3 (C-14a), 153.6 (OCOO), 158.4 (C-4a), 162.8 (C-6), 179.2 (CO). IR (KBr) cm^{-1}: 3023, 2980, 2937, 2851, 1812, 1625, 1594, 1501, 1396, 1174, 1143, 1050, 828. UV λ_{max} (MeOH) nm (log ϵ): 207 (4.66), 249 (4.45), 294 (4.73), 377 (3.73), 393 (3.77). MS (ESI$^+$) *m*/*z*: 510 and 512 [M + H]$^+$, 532 and 534 [M + Na]$^+$.

4.1.21. (±)-cis-10-Bromo-1,2-di-O-carbonyl-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (**42**)

N,*N*′-Carbonydiimidazole (54 mg, 333 μmol) was added to a solution of (±)-*cis*-10-bromo-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 (**38**) (30.0 mg, 62.0 μmol) in 2-butanone (2 mL). The reaction mixture was heated for 22 h at 75 °C, and water (5 mL) was added. An extraction with dichloromethane (3 × 10 mL) was performed and the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 99.3/0.7: V/V) gave (±)-*cis*-10-bromo-1,2-di-*O*-carbonyl-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 (**42**) as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 1.43 (s, 3H, C-3MeA), 1.60 (s, 3H, C-3MeB), 3.94 (s, 3H, NMe), 3.95 (s, 3H, OMe), 4.85 (d, 1H, *J* = 10 Hz, H-2), 6.28 (s, 1H, H-5), 6.33 (d, 1H, *J* = 10 Hz, H-1), 7.57 (dd, 1H, *J* = 9 and 2 Hz, H-11), 7.65 (s, 1H, H-13), 7.73 (d, 1H, *J* = 9 Hz, H-12), 8.13 (d, 1H, *J* = 2 Hz, H-9), 8.68 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 21.8 (C-3*MeA*), 24.5 (C-3*MeB*), 44.3 (NMe), 56.5 (OMe), 71.1 (C-1), 74.4 (C-3), 79.0 (C-2), 95.5 (C-5), 97.6 (C-14b), 111.5 (C-6a), 113.2 (C-13), 118.6 (C-10), 126.6 (C-7a), 126.9 (C-8), 128.7 (C-12), 129.8 (C-8a), 131.2 (C-9), 132.0 (C-11), 134.3 (C-12a), 142.5 (C-13a), 150.2 (C-14a), 153.8 (OCOO), 160.1 (C-4a), 163.8 (C-6), 178.8 (C=O). IR (KBr) cm^{-1}: 2984, 2953, 2921, 2848, 1793, 1641, 1618, 1587, 1497, 1396, 1203, 1178, 1097, 1088, 1026. UV λ_{max} (MeOH) nm (log ϵ): 209 (4.46), 291 (4.73), 437 (3.61). MS (ESI$^+$) *m*/*z*: 510 and 512 [M + H]$^+$, 532 and 534 [M + Na]$^+$, 548 and 550 [M + K]$^+$.

4.2. Pharmacology

Cell culture and 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 [25,26]. 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.

Appendix. Supporting information

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.02.050.

References

[1] G.K. Hughes, F.N. Lahey, J.R. Price, L.J. Webb, Alkaloids of the australian rutaceae, Nature 162 (1948) 223–224.
[2] G.H. Svoboda, Alkaloids of *Acronychia baueri*. II. Extraction of the alkaloids and studies of structure-activity relations, Llyodia 29 (1966) 206–224.
[3] G.H. Svoboda, G.A. Poore, P.J. Simpson, G.B. Boder, Alkaloids of *Acronychia baueri* Schott I. Isolation of the alkaloids and a study of the antitumor and other biological properties of acronycine, J. Pharm. Sci. 55 (1966) 758–768.
[4] J.H. Scarffe, A.R. Beaumont, D. Crowther, Phase I-II evaluation of acronycine in patients with multiple myeloma, Canc. Treat. Rep. 67 (1983) 93–94.
[5] T.-L. Su, K.A. Watanabe, Anticancer Acridone alkaloids. in: Atta-ur- Rahman, F.Z. Basha (Eds.), Studies in Natural Products Chemistry, Vol. 13. Elsevier, Amsterdam, 1993, pp. 347–382.
[6] T.-C. Chou, C.-C. Tzeng, T.-S. Wu, K.A. Watanabe, T.-L. Su, Inhibition of cell growth and macromolecule biosynthesis of human promyelocytic leukemic cells by acridone alkaloids, Phytother. Res. 3 (1989) 237–242.
[7] A. Elomri, S. Michel, M. Koch, E. Seguin, F. Tillequin, A. Pierré, Gh. Atassi, Synthesis and cytotoxic activity of 11-nitro and 11-amino derivatives of acronycine and 6-demethoxyacronycine, Chem. Pharm. Bull. 47 (1999) 1604–1606.
[8] A. Elomri, S. Mitaku, S. Michel, A.-L. Skaltsounis, F. Tillequin, M. Koch, A. Pierré, N. Guilbaud, S. Léonce, L. Kraus-Berthier, Y. Rolland, G. Atassi, Synthesis and cytotoxic and antitumor activity of esters in the 1,2-dihydroxy-1,2-dihydroacronycine series, J. Med. Chem. 39 (1996) 4762–4766.
[9] P. Magiatis, S. Mitaku, A.-L. Skaltsounis, F. Tillequin, M. Koch, A. Pierré, Gh. Atassi, Synthesis and biological activity of esters in the *trans*-1,2-dihydroxy-1,2-dihydroacronycine series, J. Nat. Prod. 61 (1998) 198–201.
[10] T.M. Nguyen, C. Sittisombut, S. Boutefnouchet, M.-C. Lallemand, S. Michel, M. Koch, F. Tillequin, R. Mazinghien, A. Lansiaux, M.-H. David-Cordonnier, B. Pfeiffer, L. Kraus-Berthier, S. Léonce, A. Pierré, Synthesis, antitumor activity, and mechanism of action of benzo[*a*]pyrano[3,2-*h*]acridin-7-one analogues of acronycine, J. Med. Chem. 49 (2006) 3383–3394.
[11] N. Costes, H. Le Deit, S. Michel, F. Tillequin, M. Koch, B. Pfeiffer, P. Renard, S. Léonce, N. Guilbaud, L. Kraus-Berthier, A. Pierré, G. Atassi, Synthesis and cytotoxic and antitumor activity of benzo[*b*]pyrano[3,2-*h*]acridin-7-one analogues of acronycine, J. Med. Chem. 43 (2000) 2395–2402.
[12] J.-B. Bongui, A. Elomri, D. Cahard, F. Tillequin, B. Pfeiffer, A. Pierré, E. Seguin, Synthesis and cytotoxic activity of acronycine analogues in the benzo[*c*]pyrano[3,2-*h*]acridin-7-one and naphtho[1,2-*b*][1,7] and [1,10]-phenanthrolin-7(14*H*)-one series, Chem. Pharm. Bull. 53 (2005) 1540–1546.
[13] Q.C. Nguyen, T.T. Nguyen, R. Yougnia, T. Gaslonde, H. Dufat, S. Michel, F. Tillequin, Acronycine derivatives: a promising series of anticancer agents, Anti-Cancer Agents Med. Chem. 9 (2009) 804–815.
[14] H.D.T. Mai, T. Gaslonde, S. Michel, F. Tillequin, M. Koch, J.-B. Bongui, A. Elomri, E. Seguin, B. Pfeiffer, P. Renard, M.-H. David-Cordonnier, W. Laine, C. Bailly, L. Kraus-Berthier, S. Léonce, J.A. Hickman, A. Pierré, Structure–activity relationships and mechanism of action of antitumor benzo[*b*]pyrano[3,2-*h*]acridin-7-one acronycine analogues, J. Med. Chem. 46 (2003) 3072–3082.
[15] M.-H. David-Cordonnier, W. Laine, A. Lansiaux, M. Kouach, G. Briand, A. Pierré, J.A. Hickman, C. Bailly, Alkylation of Guanine in DNA by S23906-1, a novel potent antitumor compound derived from the plant alkaloid acronycine, Biochemistry 41 (2002) 9911–9920.
[16] M.-H. David-Cordonnier, W. Laine, A. Lansiaux, F. Rosu, P. Colson, E. de Pauw, S. Michel, F. Tillequin, M. Koch, J. Hickman, A. Pierré, C. Bailly, Covalent binding of antitumor benzoacronycines to double-stranded DNA induces helix opening and the formation of single-stranded DNA: unique consequences of a novel DNA-bonding mechanism, Mol. Cancer Ther. 4 (2005) 71–80.
[17] M.-H. David-Cordonnier, W. Laine, T. Gaslonde, S. Michel, F. Tillequin, M. Koch, S. Léonce, A. Pierré, C. Bailly, Design of novel antitumor DNA alkylating agents: the benzacronycine series, Curr. Med. Chem.: Anti-Cancer Agents 4 (2004) 83–92.
[18] H.T. Nguyen, M.-C. Lallemand, S. Boutefnouchet, S. Michel, F. Tillequin, Antitumor *Psorospermum* xanthones and *Sarcomelicope* acridones: privileged structures implied in DNA alkylation, J. Nat. Prod. 72 (2009) 527–539.
[19] S. Léonce, V. Perez, S. Lambel, D. Peyroulan, F. Tillequin, S. Michel, M. Koch, B. Pfeiffer, Gh. Atassi, J.A. Hickman, A. Pierré, Induction of cyclin E and inhibition of DNA synthesis by the novel acronycine derivative S23906-1 precede the irreversible arrest of the tumor cells in S phase leading to apoptosis, Mol. Pharmacol. 60 (2001) 1383–1391.
[20] J. Adams, P. Gupta, J.R. Lewis, Rutaceous constituents 6. A biomimetic synthesis of acronycine, Chem. Ind. (London) (1976) 109–110.
[21] J.H. Adams, P.M. Brown, P. Gupta, M.S. Khan, J.R. Lewis, Rutaceous constituents. 13. A biomimetic synthesis of acronycine, Tetrahedron 37 (1981) 209–217.
[22] Nguyên Hóan, Dérivés du bromo-6-méthoxy-2-naphtaldéhyde-1 d'intérêt biologique, Bull. Soc. Chim. Fr. (1953) 309–314.
[23] A. Elomri, S. Michel, F. Tillequin, M. Koch, A novel synthesis of 6-demethoxyacronycine, Heterocycles 34 (1992) 799–806.
[24] R.A. Murphy, H.F. Kung, M.-P. Kung, J. Billings, Synthesis and characterization of iodobenzamide analogues: potential D-2 dopamine receptor imaging agents, J. Med. Chem. 33 (1990) 171–178.
[25] S. Léonce, A. Pierré, M. Anstett, V. Perez, A. Genton, J.P. Bizzari, Gh. Atassi, Effects of a new triazinoaminopiperidine derivative on adriamycin accumulation and retention in cells displaying P-glycoprotein-mediated multidrug resistance, Bio-Chem. Pharmacol. 44 (1992) 1707–1715.
[26] A. Pierré, T.A. Dunn, L. Kraus-Berthier, S. Léonce, D. Saint-Dizier, G. Regnier, A. Dhainaut, M. Berlion, J.P. Bizarri, Gh. Atassi, In vitro and in vivo circumvention of multidrug resistance by Servier 9788, a novel triazinoaminopiperidine derivative, Invest. New Drugs 10 (1992) 137–148.