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Synthesis, cytotoxic activity, and DNA binding properties of antitumor *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine cinnamoyl esters

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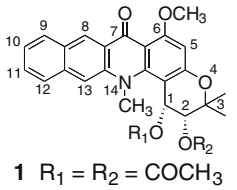
ABSTRACT

Monocinnamoyl esters at position 2 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 and their acetyl derivatives at position 1 were prepared as stabilized analogues of the anticancer alkylating agent S23906-1. Monocinnamoyl esters at position 2 were slower DNA alkylators than the reference 2-monoacetate. Mixed esters bearing an acetyl ester group at position 1 and a cinnamoyl ester group at position 2 alkylated DNA slower than S23906-1. A strong correlation was observed between cytotoxicity and DNA alkylation kinetics, with slower alkylators displaying more potent antiproliferative activities. The most cytotoxic compounds proved to be significantly active in vivo against murine C-38 adenocarcinoma implanted in mice, but less potent than S23906-1.

1. Introduction

There is an urgent need for the discovery and development of new anticancer agents. Much effort is directed toward the identification of cytostatic agents targeting the cell cycle pathway, angiogenesis or cell differentiation, but conventional cytotoxic agents interfering with DNA remain actively searched as well.[1] Indeed, the current development of small molecules that bind directly to DNA, such as ecteinascidin 743 (ET-743, Trabectedin),[2] which induces stabilization of the DNA double helix after alkylation of the 2-amino groups of guanine residues, clearly indicate the resurgence of interest in DNA alkylating agents. The mechanism of action of the acronycine derivative (±)-*cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**1**),[3] which recently underwent phase I clinical trials under the code S23906-1, also 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.[4–6] However, in strong contrast with ecteinascidin 743, DNA alkylation by S23906-1, induces a marked destabilization of the double helix, with the formation of single-stranded DNA,[7] which finally leads to cell apoptosis.[8]

Surprisingly, monoesters at position 2 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, exemplified by acetate **2**, which is the major metabolite of **1** in vivo, also exhibit both potent cytotoxic activities in vitro and marked antitumor activities in vivo, despite the lack of leaving group at the benzylic position 1.[5,6] These latter results can be rationalized on the basis of a spontaneous transesterification process, leading to the more reactive but unstable isomeric *cis*-monoesters at position 1 in aqueous media.



1 $R_1 = R_2 = COCH_3$
2 $R_1 = H$ $R_2 = COCH_3$

The ultimate metabolite of both S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**) is the diol

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(±)-*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 (**3**), which is devoid of any ester leaving group and consequently biologically inactive.

In this context, stabilized esters presenting a better stability toward hydrolytic processes appear as possible new drug candidates, provided they are still able to undergo additions onto intracellular nuclophilic targets. Recent results obtained in the field of prodrugs of anticancer agents underline the stabilizing effect toward hydrolysis of the 'acrylic' double bond conjugated with the aromatic ring in various cinnamoic esters.[9] In a continuation of our studies on the structure activity relationships in the benzoacronycine series,[10–16] we describe here the synthesis and biological activities of monocinnamoyl esters at position 2 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, and their acetyl derivatives at position 1. Several types of substituents on the cinnamoic aromatic ring have been envisaged, including halogens (Cl or Br), electron withdrawing groups (NO_2 or CF_3) and electron donating groups (OCH_3).

2. Results

2.1. Chemistry

Treatment 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 (**3**)[3] with 1 equiv of the appropriate cinnamoyl chloride in dry pyridine afforded the corresponding monoesters at the less hindered 2-position (**4**–**16**) with an excellent regioselectivity, as previously observed for their benzoic conterparts.[5,17] Further treatment of monoesters **4**–**16** with excess acetic anhydride gave the desired mixed esters **17**–**29** in almost quantitative yield.

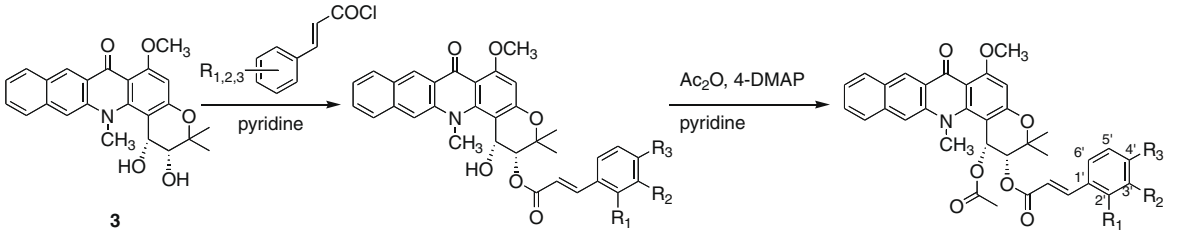
2.2. Cytotoxic activity

Monocinnamoyl esters **4**–**16** and the corresponding mixed esters **17**–**29** 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 S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**). The results (IC_{50}) are reported in Tables 1 and 2.

Table 1
Cytotoxic activity of monocinnamoyl esters **4**–**16** against murine leukemia cell line L1210 and human epidermoid carcinoma cell line KB-3-1, in comparison with S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**)

Compound	Cytotoxicity L1210 IC_{50} (μM)	Cytotoxicity KB-3-1 IC_{50} (μM)
1	0.79	0.10
2	0.62	0.16
4	0.12	0.03
5	0.04	0.008
6	0.05	0.02
7	0.06	0.02
8	0.035	0.006
9	0.035	0.006
10	0.05	0.006
11	0.08	0.02
12	0.09	0.02
13	0.10	0.015
14	0.24	0.03
15	0.13	0.03
16	1.1	0.08

As expected, the cinnamoyl esters **4**–**16** as well as their acetyl counterparts **17**–**29** displayed significant cytotoxic properties, most of them with submicromolar IC_{50} against both cell lines. With a few exceptions (compounds **16**, **17**, **25**, and **28**), all of them were found more potent than the reference compounds in the series, S23906-1 (**1**) and **2**. As a general rule, monocinnamoyl esters **4**–**16** were found more potent against both cell lines than the corresponding acetates **17**–**29**. The IC_{50} values of the esters unsubstituted on the cinnamic aromatic ring (**4** and **17**) were within the same range of magnitude as the parent compounds. Substitution by electron withdrawing groups (NO_2 or CF_3) or electron donating groups (OCH_3) did not result in a significant increase of the antiproliferative activity against KB-3-1 cell line, except in the case of the acetylated trifluoromethylcinnamate **26**. Excellent results in terms of cytotoxicity against both cell lines were obtained with compounds bearing halo substituents at position 4 on the cinnamic aromatic ring. Indeed, the most active monocinnamate derivative, (±)-*cis*-



Monocinnamoyl esters

4 R = H $R_1 = R_2 = R_3 = H$
5 R = H $R_1 = R_2 = H$ $R_3 = Cl$
6 R = H $R_2 = R_3 = H$ $R_1 = Cl$
7 R = H $R_1 = R_3 = H$ $R_2 = Cl$
8 R = H $R_1 = H$ $R_2 = R_3 = Cl$
9 R = H $R_2 = H$ $R_1 = R_3 = Cl$
10 R = H $R_1 = R_2 = H$ $R_3 = Br$
11 R = H $R_1 = R_3 = H$ $R_2 = Br$
12 R = H $R_1 = R_2 = H$ $R_3 = CF_3$
13 R = H $R_1 = R_3 = H$ $R_2 = CF_3$
14 R = H $R_1 = R_2 = H$ $R_3 = NO_2$
15 R = H $R_1 = R_2 = H$ $R_3 = OCH_3$
16 R = H $R_1 = H$ $R_2 = R_3 = OCH_3$

Mixed acetyl-cinnamoyl esters

17 R = $COCH_3$ $R_1 = R_2 = R_3 = H$
18 R = $COCH_3$ $R_1 = R_2 = H$ $R_3 = Cl$
19 R = $COCH_3$ $R_2 = R_3 = H$ $R_1 = Cl$
20 R = $COCH_3$ $R_1 = R_3 = H$ $R_2 = Cl$
21 R = $COCH_3$ $R_1 = H$ $R_2 = R_3 = Cl$
22 R = $COCH_3$ $R_2 = H$ $R_1 = R_3 = Cl$
23 R = $COCH_3$ $R_1 = R_2 = H$ $R_3 = Br$
24 R = $COCH_3$ $R_1 = R_3 = H$ $R_2 = Br$
25 R = $COCH_3$ $R_1 = R_2 = H$ $R_3 = CF_3$
26 R = $COCH_3$ $R_1 = R_3 = H$ $R_2 = CF_3$
27 R = $COCH_3$ $R_1 = R_2 = H$ $R_3 = NO_2$
28 R = $COCH_3$ $R_1 = R_2 = H$ $R_3 = OCH_3$
29 R = $COCH_3$ $R_1 = H$ $R_2 = R_3 = OCH_3$

Table 2
Cytotoxic activity of mixed esters **17**–**29** against murine leukemia cell line L1210 and human epidermoid carcinoma cell line KB-3-1, in comparison with S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**)

Compound	Cytotoxicity L1210 IC_{50} (μM)	Cytotoxicity KB-3-1 IC_{50} (μM)
1	0.79	0.10
2	0.62	0.16
17	1.1	0.15
18	0.5	0.07
19	0.6	0.09
20	Insoluble	Insoluble
21	0.4	0.05
22	0.6	0.09
23	0.7	0.08
24	0.6	0.12
25	1.5	0.3
26	0.7	0.06
27	0.5	0.14
28	1.2	0.24
29	0.9	0.15

2-(3,4-dichlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**8**) was about 20–30-fold more potent than S-23906-1 (**1**) and its monoacetyl counterpart **2**. Similarly, (±)-*cis*-1-acetoxy-(3,4-dichlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**21**) was the most interesting compound in the mixed ester series, but was only two-fold more potent than the reference compound S-23906-1 (**1**).

2.3. DNA alkylation

The compounds were also evaluated for their ability to form covalent complexes with DNA using gel shift assay. S23906-1 (**1**) and its mono-acetylated derivative **2** were previously analysed for DNA alkylation[4] and revealed in kinetics reaction that half of the DNA was alkylated ($t_{1/2}$) after 30 and 5 min, respectively (Tables 3 and 4).

Table 3
Kinetics of DNA alkylation by halogenated monocinnamoyl esters **4**, **5**, **8**, and **10**, in comparison with S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**)

Compound	DNA alkylation $t_{1/2}$ (min)
1	30
2	5
4	20
5	60
8	60
10	60

Table 4
Kinetics of DNA alkylation by halogenated mixed esters **17**, **18**, **21**, and **23**, in comparison with S23906-1 (**1**) and (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**)

Compound	DNA alkylation $t_{1/2}$ (min)
1	30
2	5
17	90
18	360
21	>1440
23	360

Kinetics of DNA alkylation reactions were determined in both series for the most active halogeno derivatives **5**, **8**, and **10** on the one hand, and **18**, **21**, and **23** on, the other hand, in comparison with unsubstituted compounds **4** and **17**, and reference compounds **1** and **2**. The results are summarized on Tables 3 and 4 and a representative experiment is presented in Figure 1. As a general rule, monocinnamoyl esters alkylate DNA faster than the corresponding mixed esters. In both series, a strong correlation between antiproliferative activity and DNA alkylation kinetics can be observed. Indeed, the most cytotoxic compounds, monocinnamate **8** and mixed ester **21**, appear as the slowest DNA alkylators in their respective series.

2.4. Antitumor in vivo evaluation

The most cytotoxic compounds in both series, **8** and **21**, were selected for an in vivo evaluation, comparatively with compound **1** (S23906-1), on the murine C38 colon adenocarcinoma model implanted sc. Although less active and less potent than compound **1**, compound **8** administered twice by iv route at the optimal dose of 2 mg/kg proved to be significantly active, inhibiting tumor growth by 90% and with one out of seven mice tumor-free on day 90 (Fig. 2). Compound **8** was toxic when administered at 4 mg/kg since 6 mice out of seven died after the treatment.

In the same experiment, compound **21** administered at the optimal dose of 4 mg/kg induced 84% of tumor growth inhibition without tumor-free mice on day 90 (Fig. 3). Compound **1** (S 23906-1), administered at its optimal dose of 4 mg/kg induced 99% of tumor growth inhibition with five mice out of seven tumor-free on day 90.

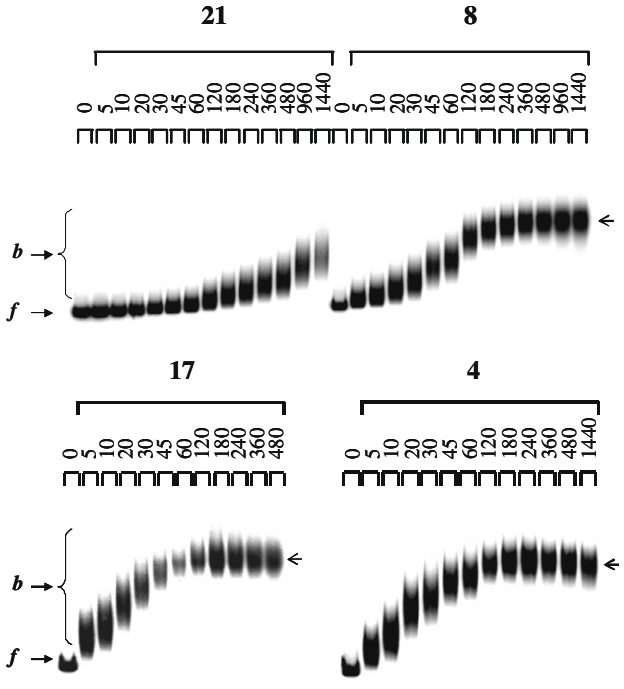


Figure 1. Kinetics for DNA alkylation measured using electromobility shift assay—the radiolabelled 117 bp DNA fragment was incubated with 50 μM of the indicated compound at room temperature for the time specified on the top of the lanes (min). '***b***' and '***f***' correspond to bound or free DNA, respectively. Open arrow refers to the reached plateau for the alkylation of the DNA by compound **8**. The $t_{1/2}$ referred in Tables 3 and 4 corresponds to the incubation time requires to obtain one half of the migration retardation obtained at the plateau. When the plateau is far to be reached as for compound **21** the $t_{1/2}$ is referred as > of the maximum of experiment time (24 h, 1440 min).

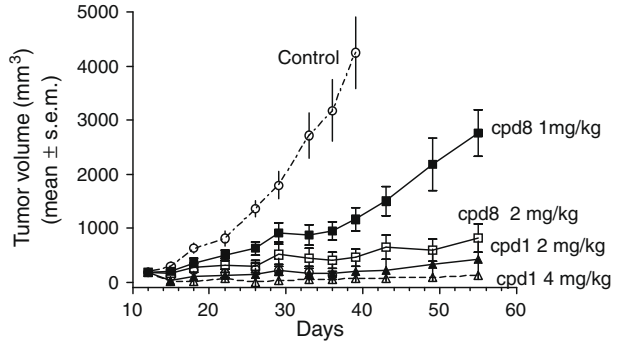


Figure 2. In vivo evaluation of compound **8** (S72419-1) administered by the iv route, in comparison with S23906-1 (**1**), on an established C38 colon adenocarcinoma (sc implantation) in mice.

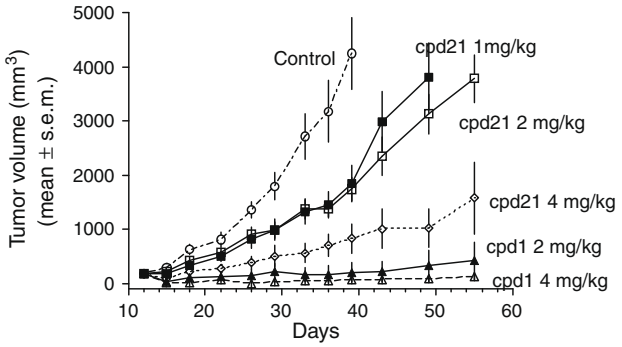


Figure 3. In vivo evaluation of compound **21** (S72422-1) administered by the iv route, in comparison with S23906-1 (**1**), on an established C38 colon adenocarcinoma (sc implantation) in mice.

3. Discussion and conclusion

Introduction of a cinnamoyl ester group at position 2 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 resulted, as expected, in the obtainment of slower DNA alkylators than the reference monoacetyl ester **2**. Similarly, mixed esters bearing an acetyl ester group at position 1 and a cinnamoyl ester group at position 2 alkylated DNA slower than S23906-1 (**1**). A strong correlation between cytotoxicity in vitro and DNA alkylation kinetics was observed. Indeed, slower alkylators displayed more potent antiproliferative activities in both series. When tested in vivo against the murine C-38 adenocarcinoma model implanted in mice, the most cytotoxic compounds, **8** and **21**, proved to be significantly active, but less potent than the reference compound S23906-1 (**1**). The lack of direct correlation between cytotoxic properties in vitro and antitumor activity in vivo has been previously observed in the benzoacronycine series.[3] It should be most probably related to the fact that several other factors (i.e., distribution, metabolism, and general toxicity) modulate the biological activity in vivo.

4. Experimental

4.1. General

Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electrospray ionization (ESI-MS; Vc = 30 V). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets or sodium chloride films on a Perkin–Elmer 257 instrument. ^1H NMR (δ [ppm], J [Hz]) spectra and ^{13}C NMR spectra were run at 400 MHz and 100 MHz, respectively, using a Bruker AVANCE-400 spectrometer. 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 microprograms. Flash column chromatographies were conducted using silica gel 60 Merck (35–70 μm) with an overpressure of 300 mbar. Microanalyses were in agreement with calculated values ±0.4%. The starting (±)-*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 (**3**) was prepared essentially as previously described.[3]

4.2. General procedure for the synthesis of monocinnamoyl esters 4–16

Thionyl chloride (7.30 mL, 100 mmol), was added dropwise to a suspension of the appropriate cinnamoic acid (10 mmol) in anhydrous CH_2Cl_2 (40 mL). The reaction mixture was stirred under reflux for 3 h, filtered, and the solvent and excess thionyl chloride were removed under reduced pressure to give the crude cinnamoyl chloride as a whitish solid, which was immediately used without further purification in the following step. The crude cinnamoyl chloride (1.15 mmol) was added 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 (419 mg, 1.03 mmol) in dry pyridine (10 mL). The reaction mixture was stirred at room temperature for 48 h and then evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) afforded the corresponding (±)-*cis*-2-cinnamoyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one **4**–**16** as a yellow amorphous solid in 23–42% yield.

4.3. (±)-*cis*-2-Cinnamoyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (4)

Yield 34%, UV λ nm (log ε) (MeOH) 236 (3.23), 283 (3.74), 330 (2.63), 340 (2.83), 436 (2.53); IR (KBr) ν 3415, 2982, 2932, 1707, 1637, 1610, 1585, 1469, 1467, 1395, 1205, 1167, 1148, 1086 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.86 (1H, s, H-8), 8.00 (1H, dd, J = 8, 1.5, H-9), 7.83 (1H, dd, J = 8, 1.5, H-12), 7.67 (1H, d, J = 16, OCOCH=C*H*), 7.65 (1H, s, H-13), 7.51 (1H, td, J = 8, 1.5, H-11), 7.44–7.42 (2H, m, H-2′, H-6′), 7.40 (1H, td, J = 8, 1.5, H-10), 7.34–7.30 (3H, m, H3′, H4′, H5′), 6.42 (1H, d, J = 16, OCOC*H*=CH), 6.29 (1H, s, H-5), 5.55 (1H, d, J = 5.5, H-2), 5.42 (1H, m → d, J = 5.5 upon D_2O addn., H-1), 4.02 (3H, s, OCH_3), 3.96 (3H, s, NCH_3), 2.13 (1H, br s, D_2O exch., OH-1), 1.59 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.8 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 149.8 (C-14a), 142.1 (OCOCH=*C*H), 142.1 (C-13a), 135.8 (C-1′), 135.3 (C-12a), 130.6 (C-4′), 129.5 (C-9), 128.9 (2C, C-3′, C-5′), 128.6 (C-8a), 128.2 (2C, C-2′, C-6′), 128.1 (C-11), 127.8 (C-8), 126.8 (C-12), 125.5 (C-7a), 124.4 (C-10), 116.8 (OCO*C*H=CH), 112.0 (C-13), 111.1 (C-6a), 102.0 (C-14b), 93.8 (C-5), 76.7 (C-3), 72.1 (C-2), 64.1 (C-1), 56.3 (OCH_3), 42.4 (NCH_3), 25.5 (CH_3-C-3), 22.6 (CH_3-C-3); ESI-MS m/z 536 $[MH]^+$. Anal. Calcd for ($C_{33}H_{29}NO_6$): C, 74.00; H, 5.46; N, 2.62. Found: C, 73.82; H, 5.48; N, 2.57.

4.4. (±)-*cis*-2-(4-Chlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (5)

Yield 40%, UV λ nm (log ε) (MeOH) 226 (3.37), 234 (3.35), 284 (3.78), 341 (2.87), 382 (2.06), 437 (2.55), 496 (1.34); IR (KBr) 3425, 2974, 2928, 1718, 1640, 1613, 1589, 1492, 1393, 1202, 1151, 1088, 1033, 1009, 819 ν cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ

8.82 (1H, s, H-8), 7.98 (1H, dd, *J* = 8, 1.5, H-9), 7.78 (1H, dd, *J* = 8, 1.5, H-12), 7.64 (1H, d, *J* = 16, OCOCH=C*H*), 7.61 (1H, s, H-13), 7.49 (1H, td, *J* = 8, 1.5, H-11), 7.39 (1H, td, *J* = 8, 1.5, H-10), 7.35 (2H, d, *J* = 8.5, H-2′, H-6′), 7.27 (2H, d, *J* = 8.5, H-3′, H-5′), 6.43 (1H, d, *J* = 16, OCOC*H*=CH), 6.25 (1H, s, H-5), 5.55 (1H, d, *J* = 5, H-2), 5.42 (1H, m → d, *J* = 5 upon D_2O addn., H-1), 3.99 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 2.35 (1H, br s, D_2O exch., OH-1), 1.58 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.6 (*C*O-O-C-2), 162.6 (C-6), 159.4 (C-4a), 149.2 (C-14a), 145.1 (OCOCH=*C*H), 142.0 (C-13a), 136.6 (C-4′), 135.7 (C-12a), 132.5 (C-1′), 129.5 (C-9), 129.4 (2C, C-2′, C-6′), 129.2 (2C, C-3′, C-5′), 128.6 (C-8a), 128.2 (C-11), 127.9 (C-8), 126.8 (C-12), 125.5 (C-7a); 124.4 (C-10), 117.5 (OCO*C*H=CH), 111.9 (C-13), 111.7 (C-6a), 101.9 (C-14b), 93.8 (C-5), 76.8 (C-3), 72.2 (C-2), 64.1 (C-1), 56.1 (OCH_3), 42.3 (NCH_3), 25.4 (*C*H_3-C-3), 22,6 (*C*H_3-C-3); ESI-MS *m/z* 570 and 572 $[MH]^+$, 592 and 594 $[MNa]^+$. Anal. Calcd for ($C_{33}H_{28}ClNO_6$): C, 69.53; H, 4.95; N, 2.46. Found: C, 69.72; H, 5.02; N, 2.47.

4.5. (±)-*cis*-2-(2-Chlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (6)

Yield 40%, UV λ nm ($\log\varepsilon$) (MeOH) 206 (3.54), 223 (3.42), 229 (3.41), 284 (3.87), 325 (2.93); IR (KBr) ν 3343, 3056, 2982, 2932, 2846, 1720, 1642, 1615, 1588, 1495, 1390, 1153, 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.81 (1H, s, H-8), 8.09 (1H, d, *J* = 16, OCOCH=C*H*), 7.97 (1H, dd, *J* = 8, 1.5, H-9), 7.76 (1H, dd, *J* = 8, 1.5, H-12), 7.59 (1H, s, H-13), 7.49 (1H, dd, *J* = 7.5, 1.5, H-6′), 7.48 (1H, td, *J* = 8, 1.5, H-11), 7.38 (1H, td, *J* = 8, 1.5, H-10), 7.34 (1H, dd, *J* = 7.5, 1.5, H-3′), 7.24 (1H, td, *J* = 7.5, 1.5, H-5′), 7.15 (1H, td, *J* = 7.5, 1.5, H-4′), 6.48 (1H, d, *J* = 16, OCOC*H*=CH), 6.21 (1H, s, H-5), 5.54 (1H, d, *J* = 5.5, H-2), 5.42 (1H, m → d, *J* = 5.5 upon D_2O addn., H-1), 3.95 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 2.55 (1H, br s, D_2O exch., OH-1), 1.58 (3H, s, CH_3-C-3), 1.50 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.5 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 149.7 (C-14a), 142.3 (OCOCH=*C*H), 142.0 (C-13a), 135.8 (C-2′), 135.2 (C-12a), 132.3 (C-1′), 131.4 (C-5′), 130.2 (C-3′), 129.6 (C-9), 128.6 (C-8a), 128.3 (C-11), 127.9 (C-8), 127.7 (C-6′), 127.1 (C-4′), 126.9 (C-12), 125.6 (C-7a), 124.4 (C-10), 119.6 (OCO*C*H=CH), 111.9 (C-13), 110.9 (C-6a), 101.9 (C-14b), 93.8 (C-5), 76.8 (C-3), 72.4 (C-2), 64.2 (C-1), 56.2 (OCH_3), 42.4 (NCH_3), 25.5 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS *m/z* 570 and 572 $[MH]^+$, 592 and 594 $[MNa]^+$, 608 and 610 $[MK]^+$. Anal. Calcd for ($C_{33}H_{28}ClNO_6$): C, 74.00; H, 5.46; N, 2.62. Found: C, 74.11; H, 5.51; N, 2.54.

4.6. (±)-*cis*-2-(3-Chlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (7)

Yield 31%, UV λ nm ($\log\varepsilon$) (MeOH) 219 (3.49), 283 (3.85), 328 (2.94), 340 (2.95), 437 (2.62); IR (KBr) ν 3378, 2920, 2846, 1712, 1642, 1588, 1203, 1149, 1083 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.82 (1H, s, H-8), 7.98 (1H, dd, *J* = 8, 1.5, H-9), 7.78 (1H, dd, *J* = 8, 1.5, H-12), 7.62 (1H, d, *J* = 16, OCOCH=C*H*), 7.60 (1H, s, H-13), 7.49 (1H, td, *J* = 8, 1.5, H-11), 7.41–7.37 (2H, m, H-2′, H-10), 7.30–7.22 (3H, m, H4′, H5′, H6′), 6.46 (1H, d, *J* = 16, OCOC*H*=CH), 6.25 (1H, s, H-5), 5.55 (1H, d, *J* = 5.5, H-2), 5.42 (1H, m → d, *J* = 5.5 upon D_2O addn., H-1), 3.99 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 2.35 (1H, br s, D_2O exch., OH-1), 1.59 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.8 (*C*O-O-C-2), 162.6 (C-6), 159.4 (C-4a), 149.8 (C-14a), 144.9 (OCOCH=*C*H), 142.0 (C-13a), 135.9 (C-1′), 135.7 (C-12a), 134.9 (C-3′), 130.5, 130.2 (C-4′, C-5′), 129.6 (C-9), 128.6 (C-8a), 128.3 (C-11), 128.0 (C-2′), 127.9 (C-8), 126.8 (C-12), 126.4 (C-6′), 125.5 (C-7a), 124.5 (C-10), 118.6 (OCO*C*H=CH), 111.9 (C-13), 110.9 (C-6a), 101.9 (C-14b), 93.8 (C-5), 76.8 (C-3), 72.4 (C-2), 64.1 (C-1), 56.2 (OCH_3), 42.4 (NCH_3), 25.5 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS *m/z* 570 and 572 $[MH]^+$, 592 and 594 $[MNa]^+$. Anal. Calcd for ($C_{33}H_{28}ClNO_6$): C, 69.53; H, 4.95; N, 2.46. Found: C, 69.52; H, 4.91; N, 2.41.

4.7. (±)-*cis*-2-(3,4-Dichlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (8)

Yield 31%, UV λ nm ($\log\varepsilon$) (MeOH) 283 (4.09), 338 (3.23), 436 (2.89), 437 (2.90); IR (KBr) ν 3351, 2982, 2928, 1724, 1642, 1615, 1588, 1491, 1394, 1200, 1149, 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, s, H-8), 7.97 (1H, dd, *J* = 8, 1.5, H-9), 7.74 (1H, dd, *J* = 8, 1.5, H-12), 7.61 (1H, d, *J* = 16, OCOCH=C*H*), 7.56 (1H, s, H-13), 7.50 (1H, d, *J* = 2, H-2′), 7.47 (1H, td, *J* = 8, 1.5, H-11), 7.38 (1H, td, *J* = 8, 1.5, H-10), 7.36 (1H, d, *J* = 8.5, H-5′), 7.23 (1H, dd, *J* = 8.5, 2, H-6′), 6.48 (1H, d, *J* = 16, OCOC*H*=CH), 6.22 (1H, s, H-5), 5.56 (1H, d, *J* = 5.5, H-2), 5.43 (1H, m → d, *J* = 5.5 upon D_2O addn., H-1), 3.97 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 2.56 (1H, br s, D_2O exch., OH-1), 1.58 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2 (C-7), 166.6 (*C*O-O-C-2), 162.5 (C-6), 159.5 (C-4a), 149.8 (C-14a), 143.6 (OCOCH=*C*H), 141.9 (C-13a), 135.7 (C-12a), 134.5 (C-4′), 134.3 (C-1′), 133.2 (C-3′), 130.9 (C-5′), 129.9 (C-2′), 129.5 (C-9), 128.5 (C-8a), 128.3 (C-11), 127.9 (C-8), 127.1 (C-6′), 126.8 (C-12), 125.3 (C-7a), 124.4 (C-10), 119.3 (OCO*C*H=CH), 111.9 (C-13), 110.8 (C-6a), 102.0 (C-14b), 93.7 (C-5), 76.8 (C-3), 72.5 (C-2), 64.1 (C-1), 56.0 (OCH_3), 42.4 (NCH_3), 25.6 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS *m/z* 604, 606 et 608 $[MH]^+$. Anal. Calcd for ($C_{33}H_{27}Cl_2NO_6$): C, 65.57; H, 4.50; N, 2.32. Found: C, 65.43; H, 4.64; N, 2.27.

4.8. (±)-*cis*-2-(2,4-Dichlorocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (9)

Yield 30%, UV λ nm ($\log\varepsilon$) (MeOH) 203 (3.61), 206 (3.61), 227 (3.49), 283 (3.90), 331 (2.93), 341 (2.95); IR (KBr) ν 3394, 2982, 2920, 2846, 1716, 1638, 1584, 1495, 1394, 1153, 1091, 815 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, s, H-8), 8.01 (1H, d, *J* = 16, OCOCH=C*H*), 7.95 (1H, dd, *J* = 8, 1.5, H-9), 7.70 (1H, dd, *J* = 8, 1.5, H-12), 7.53 (1H, s, H-13), 7.44 (1H, td, *J* = 8, 1.5, H-11), 7.40 (1H, d, *J* = 8.5, H-6′), 7.36 (1H, td, *J* = 8, 1.5, H-10), 7.36 (1H, d, *J* = 2, H-3′), 7.11 (1H, dd, *J* = 8.5, 2, H-5′), 6.44 (1H, d, *J* = 16, OCOC*H*=CH), 6.17 (1H, s, H-5), 5.56 (1H, d, *J* = 5.5, H-2), 5.43 (1H, m → d, *J* = 5.5 upon D_2O addn., H-1), 3.92 (3H, s, NCH_3), 3.91 (3H, s, OCH_3), 2.80 (1H, br s, D_2O exch., OH-1), 1.59 (3H, s, CH_3-C-3), 1.51 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3 (C-7), 166.5 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 149.8 (C-14a), 141.9 (C-13a), 140.9 (OCOCH=*C*H), 136.7 (C-4′), 135.7 (2C, C-12a, C-2′), 130.9 (C-1′), 130.0 (C-3′), 129.5 (C-9), 128.5 (C-8a), 128.4 (C-6′), 128.3 (C-11), 127.9 (C-8), 127.6 (C-5′), 126.8 (C-12), 125.5 (C-7a), 124.4 (C-10), 120.2 (OCO*C*H=CH), 111.9 (C-13), 110.8 (C-6a), 101.9 (C-14b), 93.6 (C-5), 76.8 (C-3), 72.6 (C-2), 64.2 (C-1), 56.1 (OCH_3), 42.4 (NCH_3), 25.6 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS *m/z* 604, 606, and 608 $[MH]^+$, 626, 628, and 630 $[MNa]^+$. Anal. Calcd for ($C_{33}H_{27}Cl_2NO_6$): C, 65.57; H, 4.50; N, 2.32. Found: C, 65.71; H, 4.53; N, 2.39.

4.9. (±)-*cis*-2-(4-Bromocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (10)

Yield 32%, UV λ nm ($\log\varepsilon$) (MeOH) 284 (3.84), 340 (2.87), 438 (2.51); IR (KBr) ν 3335, 2986, 2928, 2846, 1712, 1642, 1615,

1583, 1495, 1394, 1269, 1087, 815, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.79 (1H, s, H-8), 7.97 (1H, dd, J = 8, 1.5, H-9), 7.75 (1H, dd, J = 8, 1.5, H-12), 7.64 (1H, d, J = 16, OCOCH=C*H*), 7.57 (1H, s, H-13), 7.47 (1H, td, J = 8, 1.5, H-11), 7.44 (2H, d, J = 8.5, H3′, H5′), 7.37 (1H, td, J = 8, 1.5, H-10), 7.27 (2H, d, J = 8.5, H-2′, H-6′), 6.48 (1H, d, J = 16, OCOC*H*=CH), 6.22 (1H, s, H-5), 5.55 (1H, d, J = 5, H-2), 5.42 (1H, m → d, J = 5 upon D_2O addn., H-1), 3.98 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 2.55 (1H, br s, D_2O exch., OH-1), 1.53 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.8 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 149.8 (C-14a), 145.2 (OCOCH=*C*H), 142,0 (C-13a), 135.8 (C-12a), 133.1 (C-1′), 132.3 (2C, C-3′, C-5′), 129.6 (2C, C-2′, C-6′), 129.6 (C-9), 128.6 (C-8a), 128.3 (C-11), 127.9 (C-8), 126.8 (C-12), 125.5 (C-7a), 125.0 (C-4′), 124.5 (C-10), 117.8 (OCO*C*H=CH), 112.0 (C-13), 111.0 (C-6a), 102.0 (C-14b), 93.8 (C-5), 76.8 (C-3), 72.3 (C-2), 64.1 (C-1), 56.2 (OCH_3), 42.4 (NCH_3), 25.5 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS m/z 614 and 616 $[MH]^+$, 636 and 638 $[MNa]^+$. Anal. Calcd for ($C_{33}H_{28}BrNO_6$): C, 64.50; H, 4.59; N, 2.28. Found: C, 64.38; H, 4.69; N, 2.34.

4.10. (±)-*cis*-2-(3-Bromocinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (11)

Yield 30%, IR (KBr) ν 3354, 2977, 2912, 2847, 1716, 1645, 1612, 1584, 1490, 1458, 1271, 1207, 1153, 1088, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, s, H-8), 7.97 (1H, dd, J = 8, 1.5, H-9), 7.76 (1H, dd, J = 8, 1.5, H-12), 7.62 (1H, d, J = 16, OCOCH=C*H*), 7.58 (1H, s, H-13), 7.56 (1H, t, J = 1.5, H-2′), 7.48 (1H, td, J = 8, 1.5, H-11), 7.43 (1H, dt, J = 8, 1.5, H-4′), 7.38 (1H, td, J = 8, 1.5, H-10), 7.32 (1H, dt, J = 8, 1.5, H-6′); 7.16 (1H, t, J = 8, H-5′), 6.48 (1H, d, J = 16, OCOC*H*=CH), 6.21 (1H, s, H-5), 5.56 (1H, d, J = 5, H-2), 5.42 (1H, m → d, J = 5 upon D_2O addn., H-1), 3.95 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 3.58 (1H, br s, D_2O exch., OH-1), 1.59 (3H, s, CH_3-C-3), 1.50 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 166.6 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 149.8 (C-14a), 144.8 (OCOCH=*C*H), 142.0 (C-13a), 136.2 (C-1′), 135.8 (C-12a), 133.4 (C-4′), 130.9 (C-2′), 130.4 (C-5′), 129.6 (C-9), 128.6 (C-8a), 128.3 (C-11), 127.9 (C-8), 126.9 (2C, C-12, C-6′), 125.5 (C-7a), 124.5 (C-10), 123.1 (C-3′), 118.6 (OCO*C*H=CH), 111.9 (C-13), 111.0 (C-6a), 101.9 (C-14b), 93.8 (C-5), 76.8 (C-3), 72.4 (C-2), 64.1 (C-1), 56.2 (OCH_3), 42.4 (NCH_3), 25.5 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS m/z 614 and 616 $[MH]^+$, 636 and 638 $[MNa]^+$. Anal. Calcd for ($C_{33}H_{28}BrNO_6$): C, 64.50; H, 4.59; N, 2.28. Found: C, 64.59; H, 4.53; N, 2.22.

4.11. (±)-*cis*-2-(4-Trifluoromethylcinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (12)

Yield 24%, IR (KBr) ν 3327, 2950, 2922, 2848, 1721, 1648, 1613, 1588, 1498, 1459, 1393, 1320, 1281, 1166, 1009, 974, 834, 737, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.82 (1H, s, H-8), 7.99 (1H, dd, J = 8, 1.5, H-9), 7.77 (1H, dd, J = 8, 1.5, H-12), 7.73 (1H, d, J = 16, OCOCH=C*H*), 7.68 (1H, s, H-13), 7.61–7.52 (4H, m, H-2′, H-6′, H-3′, H-5′), 7.49 (1H, td, J = 8, 1.5, H-11), 7.39 (1H, td, J = 8, 1.5, H-10), 6.56 (1H, d, J = 16, OCOC*H*=CH), 6.26 (1H, s, H-5), 5.59 (1H, d, J = 5.5, H-2), 5.45 (1H, m → d, J = 5.5 upon D_2O addn., H-1), 4.00 (3H, s, OCH_3), 3.96 (3H, s, NCH_3), 2.42 (1H, br s, D_2O exch., OH-1), 1.58 (3H, s, CH_3-C-3); 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C-7), 167.1 (*C*O-O-C-2), 162.3 (C-6), 159.6 (C-4a), 149.9 (C-14a), 144.2 (OCOCH=*C*H), 141.5 (C-13a), 137.9 (C-12a), 135.4 (C-1′), 131.8 (q, J = 35, C-4′), 129.2 (C-8), 128.3 (3C, C-11, C-2′, C-6′), 127.8 (C-8a), 126.7 (C-9), 125.8 (2C, C-3′, C-5′), 124.4 (C-7a), 124.2 (C-12), 122.4 (C-10), 120.5 (OCO*C*H=CH), 120.1 (q, J = 270, CF_3), 111.6 (C-13), 110.3 (C-6a), 102.5 (C-14b), 93.3 (C-5), 76.9 (C-3), 72.6 (C-2), 64.1 (C-1), 55.6 (OCH_3), 42.2 (NCH_3), 25.6 (*C*H_3-C-3), 22.5 (*C*H_3-C-3); ESI-MS m/z 604 $[MH]^+$. Anal. Calcd for ($C_{34}H_{28}F_3NO_6$).: C, 67.66; H, 4.68; N, 2.32. Found: C, 67.50; H, 4.65; N, 2.37.

4.12. (±)-*cis*-2-(3-Trifluoromethylcinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (13)

Yield 23%, IR (KBr) ν 3336, 2982, 2926, 2849, 1713, 1641, 1591, 1488, 1396, 1333, 1270, 1027, 1164, 1144, 1121, 1084, 816, 740, 687, 657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.70 (1H, s, H-8), 7.94 (1H, dd, J = 8, 1.5, H-9), 7.77 (1H, d, J = 16, OCOCH=C*H*), 7.66 (1H, dd, J = 8, 1.5, H-12), 7.64 (1H, m, H-2′), 7.56–7.52 (2H, m, H-4′, H-6′), 7.50 (1H, s, H-13), 7.43–7.33 (3H, m, H-11, H-5′, H-10), 6.64 (1H, d, J = 16, OCOC*H*=CH), 6.15 (1H, s, H-5), 5.59 (1H, d, J = 5.5, H-2), 5.44 (1H, m → d, J = 5.5 upon D_2O addn., H-1), 3.93 (3H, s, NCH_3), 3.89 (3H, s, OCH_3), 3.52 (1H, br s, D_2O exch., OH-1), 1.60 (3H, s, CH_3-C-3), 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2 (C-7), 166.7 (*C*O-O-C-2), 162.5 (C-6), 159.5 (C-4a), 149.8 (C-14a), 144.6 (OCOCH=*C*H), 141.9 (C-13a), 135.7 (C-12a), 135.1 (C-1′), 131.6 (q, J = 32, C-3′), 131.2 (C-6′), 129.5 (2C, C-9, C-5′), 128.5 (C-8a), 128.2 (C-11), 127.9 (C-8), 126.9 (2C, C-12, C-4′), 125.2 (C-7a), 124.8 (C-2′), 124.4 (C-10), 119.4 (OCO*C*H=CH), 119.2 (q, J = 270, CF_3), 111.9 (C-13), 111.8 (C-6a), 102.0 (C-14b), 93.6 (C-5), 76.8 (C-3), 72.5 (C-2), 64.2 (C-1), 56.0 (OCH_3), 42.4 (NCH_3), 25.6 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS m/z 604 $[MH]^+$. Anal. Calcd for ($C_{34}H_{28}F_3NO_6$): C, 67.66; H, 4.68; N, 2.32. Found: C, 67.72; H, 4.74; N, 2.28.

4.13. (±)-*cis*-1-Hydroxy-6-methoxy-3,3,14-trimethyl-2-(4-nitrocinnamoyloxy)-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (14)

Yield 37%, UV λ nm ($\log\varepsilon$) (MeOH) 233 (3.35), 287 (3.74), 379 (2.17), 418 (2.51), 425 (2.53); IR (KBr) ν 3348, 2982, 2932, 1718, 1640, 1617, 1589, 1570, 1518, 1492, 1396, 1343, 1024, 1165, 1151, 1087, 1029 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.72 (1H, s, H-8), 8.13 (2H, d, J = 8.5, H-3′, H-5′), 7.94 (1H, dd, J = 8, 1.5, H-9), 7.77 (1H, d, J = 16, OCOCH=C*H*), 7,69 (1H, dd, J = 8, 1.5, H-12), 7.55 (2H, d, J = 8.5, H-2′, H-6′), 7.51 (1H, s, H-13), 7.43 (1H, td, J = 8, 1.5, H-11), 7.38 (1H, td, J = 8, 1.5, H-10), 6.68 (1H, d, J = 16, OCOC*H*=CH), 6.21 (1H, s, H-5), 5.59 (1H, d, J = 5, H-2), 5,44 (1H, m → d, J = 5 upon D_2O addn., H-1), 3.95 (3H, s, OCH_3), 3.93 (3H, s, NCH_3), 2.35 (1H, br s, D_2O exch., OH-1), 1.60 (3H, s, CH_3-C-3), 1.51 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9 (C-7), 167.7 (*C*O-O-C-2), 162.6 (C-6), 159.3 (C-4a), 149.2 (C-14a), 147.3 (C-4′), 143.1 (OCOCH=*C*H), 142.9 (C-13a), 135.5 (C-12a), 132.4 (C-1′), 129.5 (C-9), 128.8 (C-8a), 128.7 (2C, C-2′, C-6′), 128.2 (C-11), 127.9 (C-8), 126.9 (C-12), 126.7 (C-7a), 124.4 (C-10), 124.1 (2C, C-3′, C-5′), 117.1 (OCO*C*H=CH), 111.3 (C-13), 104.6 (C-6a), 101.9 (C-14b), 93.6 (C-5), 76.6 (C-3), 72.2 (C-2), 64.1 (C-1), 56.1 (OCH_3), 42.3 (NCH_3), 25.4 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS m/z 581 $[MH]^+$, 603 $[MNa]^+$, 619 $[MK]^+$. Anal. Calcd for ($C_{33}H_{28}N_2O_8$): C, 68.27; H, 4.86; N, 4.83. Found: C, 68.11; H, 4.91; N, 4.91.

4.14. (±)-*cis*-1-Hydroxy-6-methoxy-2-(4-methoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (15)

Yield 42%, UV λ nm ($\log\varepsilon$) (MeOH) 232 (3.46), 286 (3.88), 379 (2.08), 437 (2.66); IR (KBr) ν 3351, 2928, 1712, 1638, 1592, 1506, 1394, 1153, 1079 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.85 (1H, s, H-8), 7.99 (1H, dd, J = 8, 1.5, H-9), 7.82 (1H, dd, J = 8, 1.5, H-12), 7.64 (1H, s, H-13), 7.62 (1H, d, J = 16, OCOCH=C*H*), 7.51 (1H, td, J = 8, 1.5, H-11), 7.39 (1H, td, J = 8, 1.5, H-10), 7.37 (2H, d, J = 8.5, H-2′, H-6′), 6.82 (2H, d, J = 8.5, H-3′, H-5′), 6.28 (1H, d, J = 16,

OCOC*H*=CH), 6.26 (1H, s, H-5), 5.54 (1H, d, *J* = 5, H-2), 5.42 (1H, m → d, *J* = 5 upon D_2O addn., H-1), 4.00 (3H, s, CH_3O-C-6), 3.95 (3H, s, NCH_3), 3.94 (3H, s, CH_3O-C-4′), 2.18 (1H, br s, D_2O exch., OH-1), 1.58 (3H, s, CH_3-C-3), 1.49 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 167.3 (*C*O-O-C-2), 162.6 (C-6), 161.4 (C-4′), 159.5 (C-4a), 149.8 (C-14a), 146.5 (OCOCH=*C*H), 142.1 (C-13a), 135.8 (C-12a), 130.1 (2C, C-2′, C-6′), 129.6 (C-9), 128.6 (C-8a), 128.3 (C-11), 127.9 (C-8), 126.9 (C-12), 126.8 (C-1′), 125.8 (C-7a), 124.5 (C-10), 114.4 (2C, C3′, C5′), 114.2 (OCO*C*H=CH), 112.0 (C-13), 111.1 (C-6a), 102.1 (C-14b), 93.9 (C-5), 76.8 (C-3), 71.9 (C-2), 64.1 (C-1), 56.3 (*C*H_3O-C-6), 55.4 (*C*H_3O-C-4′), 42.4 (NCH_3), 25.5 (*C*H_3-C-3), 22.7 (*C*H_3-C-3); ESI-MS *m/z* 566 $[MH]^+$. Anal. Calcd for ($C_{34}H_{31}NO_7$): C, 72.20; H, 5.52; N, 2.48. Found: C, 72.09; H, 5.39; N, 2.57.

4.15. (±)-*cis*-1-Hydroxy-6-methoxy-2-(3,4-dimethoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (16)

Yield 25%, IR (KBr) *ν* 3356, 2937, 1716, 1700, 1643, 1616, 1538, 1513, 1494, 1459, 1394, 1335, 1262, 1203, 1149, 1132, 1089, 1027, 814, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, s, H-8), 7.98 (1H, dd, *J* = 8, 1.5, H-9), 7.74 (1H, dd, *J* = 8, 1.5, H-12), 7.66 (1H, d, *J* = 16, OCOCH=C*H*), 7.57 (1H, s, H-13), 7.45 (1H, td, *J* = 8, 1.5, H-11), 7.36 (1H, td, *J* = 8, 1.5, H-10), 6.97 (1H, dd, *J* = 8.5, 1.5, H-6′), 6.87 (1H, d, *J* = 1.5, H-2′), 6.74 (1H, d, *J* = 8.5, H-5′), 6.38 (1H, d, *J* = 16, OCOC*H*=CH), 6.11 (1H, s, H-5), 5.57 (1H, d, *J* = 5.5, H-2), 5.43 (1H, m → d, *J* = 5.5 upon D_2O addn., H-1), 3.97 (3H, s, NCH_3), 3.85 (6H, s, 2 × OCH_3), 3.66 (3H, s, OCH_3), 2.82 (1H, br s, D_2O exch., OH-1), 1.59 (3H, s, CH_3-C-3), 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2 (C-7), 167.3 (*C*O-O-C-2), 162.6 (C-6), 159.5 (C-4a), 151.4 (C-4′), 149.9 (C-14a), 149.2 (C-3′), 146.5 (OCOCH=*C*H), 141.9 (C-13a), 135.8 (C-12a), 129.5 (C-9), 128.5 (C-8a), 128.2 (C-11), 127.9 (C-8), 127.2 (C-1′), 126.8 (C-12), 125.3 (C-7a), 124.4 (C-10), 123.2 (C-6′), 114.7 (OCO*C*H=CH), 111.8 (C-13), 110.9 (C-5′), 110.8 (C-6a), 109.4 (C-2′), 102.2 (C-14b), 93.7 (C-5), 76.8 (C-3), 71.9 (C-2), 64.2 (C-1), 56.0, 55.9, 55.7 (3 × OCH_3), 42.3 (NCH_3), 25.5 (*C*H_3-C-3), 22.6 (*C*H_3-C-3); ESI-MS *m/z* 596 $[MH]^+$. Anal. Calcd for ($C_{35}H_{33}NO_8$): C, 70.58; H, 5.58; N, 2.35. Found: C, 70.50; H, 5.42; N, 2.41.

4.16. General procedure for the synthesis of mixed esters 17–29

Acetic anhydride (0.15 mL, 1.57 mmol) was added to an iced-cooled solution (0 °C) of the appropriate (±)-*cis*-2-cinnamoyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one **4**–**16** (0.15 mmol) and 4-dimethylaminopyridine (0.01 g) in dry pyridine (5 mL). After stirring at 25 °C for 5 h, the reaction mixture was poured on cold water (20 mL). The precipitate was filtered, washed with water (3 × 15 mL), and dried *in vacuum* over P_2O_5, to afford the corresponding (±)-*cis*-1-acetoxy-2-cinnamoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one **17**–**29** as a yellow amorphous solid in 70–100% yield.

4.17. (±)-*cis*-1-Acetoxy-2-cinnamoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (17)

Yield 71%, UV λ nm (logε) (MeOH) 222 (3.40), 236 (3.35), 286 (3.82), 337 (2.93), 431 (2.57); IR (KBr) *ν* 2974, 2935, 1735, 1638, 1618, 1577, 1429, 1328, 1205, 1155, 1086, 1029 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = 8, 1.5, H-12), 7.62 (1H, d, *J* = 16, OCOCH=C*H*), 7.57–7.52 (2H, m, H-11, H-13), 7.46–7.44 (2H, m, H2′, H6′), 7.41 (1H, td, *J* = 8, 1.5, H-10), 7.36–7.32 (3H, m, H-3′, H-4′, H-5′), 6.62 (1H, d, *J* = 5, H-1), 6.37 (1H, d, *J* = 16, OCOC*H*=CH), 6.35 (1H, s, H-5), 5.67 (1H, d, *J* = 5, H-2), 4.05 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 1.95 (3H, s, OCOC**H**$_3$), 1.64 (3H, s, CH_3-C-3), 1.48 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5 (C-7), 171.1 (*C*O-O-C-1), 166.5 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 146.5 (OCOCH=*C*H), 142.5 (C-13a), 136.0 (C-12a), 135.3 (C-1′), 130.7 (C-4′), 129.8 (C-9), 128.9 (C-3′, C-5′), 128.8 (C-8a), 128.4 (3C, C11, C2′, C6′), 128.2 (C-8), 126.8 (C-12), 126.0 (C-7a), 124.6 (C-10), 116.9 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.9 (C-14b), 94.4 (C-5), 76.7 (C-3), 69.2 (C-2), 66.1 (C-1), 56.5 (OCH_3), 43.0 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 20.4 (OCO*C*H_3); ESI-MS *m/z* 578 $[MH]^+$. Anal. Calcd for ($C_{35}H_{31}NO_7$): C, 72.78; H, 5.41; N, 2.42. Found: C, 72.90; H, 5.37; N, 2.32.

4.18. (±)-*cis*-1-Acetoxy-2-(4-chlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (18)

Yield 98%, UV λ nm (logε) (MeOH) 226 (3.44), 286 (3.74), 375 (2.02), 383 (2.06), 431 (2.46); IR (KBr) 2982, 2928, 1735, 1720, 1648, 1591, 1491, 1400, 1226, 1206, 1153, 1086, 1033, 1009, 819 *ν* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = 8, 1.5, H-12), 7.55 (1H, d, *J* = 16, OCOCH=C*H*), 7.53 (1H, td, *J* = 8, 1.5, H-11), 7.52 (1H, s, H-13), 7.41 (1H, td, *J* = 8, 1.5, H-10), 7.38 (2H, d, *J* = 8.5, H-2′, H-6′), 7.30 (2H, d, *J* = 8.5, H-3′, H-5′), 6.60 (1H, d, *J* = 5, H-1), 6.35 (1H, s, H-5), 6.34 (1H, d, *J* = 16, OCOC*H*=CH), 5.66 (1H, d, *J* = 5, H-2), 4.05 (3H, s, OCH**3**), 3.72 (3H, s, NCH**3**), 1.94 (3H, s, OCOCH**3**), 1.63 (3H, s, CH_3-C-3), 1.51 (3H, s CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.3 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 144.9 (OCOCH=*C*H), 142.4 (C-13a), 136.7 (C-4′), 135.9 (C-12a), 132.5 (C-1′), 129.8 (C-9), 129.5 (C-2′, C6′), 129.3 (C-3′, C-5′), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 126.8 (C-12), 125.9 (C-7a), 124.7 (C-10), 117.5 (OCO*C*H=CH), 111.6 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.3 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 612 and 614 $[MH]^+$, 634 and 636 $[MNa]^+$. Anal. Calcd for ($C_{35}H_{30}ClNO_7$): C, 68.68; H, 4.94; N, 2.29. Found: C, 68.57; H, 5.01; N, 2.17.

4.19. (±)-*cis*-1-Acetoxy-2-(2-chlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (19)

Yield 94%, UV λ nm (logε) (MeOH) 224 (3.31), 230 (3.31), 286 (3.79), 338 (2.87), 434 (2.49); IR (KBr) *ν* 3056, 2978, 2939, 1747, 1720, 1646, 1588, 1495, 1398, 1157, 1087, 1029, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.04 (1H, d, *J* = 16, OCOCH=C*H*), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = 8, 1.5, H-12), 7.55 (1H, dd, *J* = 7.5, 1.5, H-3′), 7.53 (1H, td, *J* = 8, 1.5, H-11), 7.52 (1H, s, H-13), 7.41 (1H, td, *J* = 8, 1.5, H-10), 7,36 (1H, dd, *J* = 7.5, 1 Hz, H-6′), 7.26 (1H, td, *J* = 7.5, 1.5, H-5′), 7.21 (1H, td, *J* = 7.5, 1, H-4′), 6.60 (1H, d, *J* = 5, H-1), 6.36 (1H, d, *J* = 16, OCOC*H*=CH), 6.35 (1H, s, H-5), 5.66 (1H, d, *J* = 5, H-2), 4.03 (3H, s, OCH**3**), 3.72 (3H, s, NCH**3**), 1.96 (3H, s, OCOCH**3**), 1.64 (3H, s, CH_3-C-3), 1,53 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178,4 (C-7), 171.2 (*C*O-O-C-1), 165.9 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 142.4 (C-13a), 142.1 (OCOCH=*C*H), 135.9 (C-12a), 135.2 (C-2′), 132.2 (C-1′), 131.5 (C-5′), 130.3 (C-6′), 129.7 (C-9), 128.8 (C-8a), 128,4 (C-11), 128.2 (C-8), 127.7 (C-3′), 127.1 (C-4′), 126.8 (C-12), 126.0 (C-7a), 124.6 (C-10), 119.3 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.5 (C-2), 66.2 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.2 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 612 and 614 $[MH]^+$. Anal. Calcd for ($C_{35}H_{30}ClNO_7$): C, 68.68; H, 4.94; N, 2.29. Found: C, 68.74; H, 5.03; N, 2.27.

4.20. (±)-*cis*-1-Acetoxy-2-(3-chlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (20)

Yield 99%, UV λ nm (log ε) (MeOH) 206 (3.60), 220 (3.53), 226 (3.51), 286 (3.88), 327 (2.99); IR (KBr) ν 3056, 2982, 2016, 2846, 1724, 1642, 1592, 1401, 1157, 1087, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.55 (1H, d, J = 16, OCOCH=C*H*), 7.54 (1H, td, J = 8, 1.5, H-11), 7.52 (1H, s, H-13), 7.45 (1H, t, J = 1.5, H-2′), 7.41 (1H, td, J = 8, 1.5, H-10), 7.34–7.25 (3H, m, H-4′, H-6′, H-5′), 6.60 (1H, d, J = 5, H-1), 6.37 (1H, d, J = 16, OCOC*H*=CH), 6.35 (1H, s, H-5), 5.66 (1H, d, J = 5, H-2), 4.05 (3H, s, OCH_3), 3.72 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.63 (3H, s, CH_3-C-3), 1.51 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.1 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 144.8 (OCOCH=*C*H), 142.4 (C-13a), 135.9 (C-12a), 135.8 (C-1′), 135.0 (C-3′), 130.6 (C-5′), 130.2 (C-4′), 129.8 (C-9), 128.8 (C-8a), 128.4 (C-8), 128.2 (C-11), 128.0 (C-2′), 126.8 (C-12), 126.6 (C-6′), 126.0 (C-7a), 124.7 (C-10), 118.4 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.4 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS m/z 612 and 614 $[MH]^+$. Anal. Calcd for ($C_{35}H_{30}ClNO_7$): C, 68.68; H, 4.94; N, 2.29. Found: C, 68.79; H, 4.89; N, 2.33.

4.21. (±)-*cis*-1-Acetoxy-(3,4-dichlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (21)

Yield 84%, UV λ nm (log ε) (MeOH) 230 (3.61), 286 (3.97), 337 (3.10), 433 (2.75); IR (KBr) ν 3056, 2978, 2935, 2854, 1747, 1646, 1576, 1398, 1157, 1087, 1033, 815, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.56–7.48 (4H, m, H-11, H-2′, H-13, OCOCH=C*H*), 7.43–7.39 (2H, m, H-10, H-5′), 7.27 (1H, dd, J = 8.5, 2, H-6′), 6.60 (1H, d, J = 5, H-1), 6.35 (1H, d, J = 16, OCOC*H*=CH), 6.34 (1H, s, H-5), 5.66 (1H, d, J = 5, H-2), 4.05 (3H, s, OCH_3), 3.71 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.63 (3H, s, CH_3-C-3), 1.51 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 165.9 (*C*O-O-C-2), 163.1 (C-6), 160.3 (C-4a), 150.4 (C-14a), 143.6 (OCOCH=*C*H), 142.4 (C-13a), 135.9 (C-12a), 134.7 (C-1′), 134.0 (C-3′), 133.4 (C-4′), 130.9 (C-5′), 129.9 (C-2′), 129.8 (C-9), 128.8 (C-8a), 128.4 (C-8), 128.2 (C-11), 127.3 (C-6′), 126.8 (C-12), 126.0 (C-7a), 124.7 (C-10), 118.8 (OCO*C*H=CH), 111.6 (C-13), 111.5 (C-6a), 97.7 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.5 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3) ; 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21,2 (OCO*C*H_3); ESI-MS m/z 646, 648, and 650 $[MH]^+$. Anal. Calcd for ($C_{35}H_{29}Cl_2NO_7$): C, 65.02; H, 4.52; N, 2.17. Found: C, 64.90; H, 4.42; N, 2.09.

4.22. (±)-*cis*-1-Acetoxy-(2,4-dichlorocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (22)

Yield 100%, UV λ nm (log ε) (MeOH) 233 (3.54), 286 (3.99), 337 (3.05), 434 (2.66); IR (KBr) ν 2924, 2850, 1728, 1638, 1623, 1588, 1398, 1207, 1149, 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.95 (1H, d, J = 16, OCOCH=C*H*), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.56–7.48 (3H, m, H-11, H-13, H-6′), 7.43–7.39 (2H, m, H-10, H-3′), 7.20 (1H, dd, J = 8, 1.5, H-5′), 6.60 (1H, d, J = 5, H-1), 6.34 (1H, s, H-5), 6.33 (1H, d, J = 16, OCOC*H*=CH), 5.66 (1H, d, J = 5, H-2), 4.03 (3H, s, OCH_3), 3.72 (3H, s, NCH_3), 1.95 (3H, s, $OCOCH_3$), 1.64 (3H, s, CH_3-C-3), 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.2 (*C*O-O-C-1), 165.8 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 142.4 (C-13a), 140.9 (OCOCH=*C*H), 136.9 (C-4′), 136.0 (C-12a), 135.8 (C-2′), 130.8 (C-1′), 130.1 (C-3′), 129.7 (C-9), 128.8 (C-8a), 128.5 (C-6′), 128.4 (C-11), 128.2 (C-8), 127.6 (C-5′), 126.8 (C-12), 125.9 (C-7a), 124.7 (C-10), 119.8 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.7 (C-14b), 94.4 (C-5), 76.8 (C-3), 69.6 (C-2), 66.2 (C-1), 56.4 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS m/z 646, 648, and 650 $[MH]^+$. Anal. Calcd for ($C_{35}H_{29}Cl_2NO_7$): C, 65.02; H, 4.52; N, 2.17. Found: C, 65.13; H, 4.57; N, 2.19.

4.23. (±)-*cis*-1-Acetoxy-2-(4-bromocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (23)

Yield 96%, UV λ nm (log ε) (MeOH) 205 (3.61), 286 (3.87), 335 (2.94), 434 (2.61); IR (KBr) ν 3048, 2978, 2932, 2850, 1743, 1646, 1588, 1405, 1087, 1005, 916, 819, 730, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.90 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.54 (1H, d, J = 16, OCOCH=C*H*), 7.53 (1H, td, J = 8, 1.5, H-11), 7.52 (1H, s, H-13), 7.46 (2H, d, J = 8.5, H-3′, H-5′), 7.41 (1H, td, J = 8, 1.5, H-10), 7.30 (2H, d, J = 8.5, H-2′, H-6′), 6.60 (1H, d, J = 5, H-1), 6.36 (1H, d, J = 16, OCOC*H*=CH), 6.35 (1H, s, H-5), 5.67 (1H, d, J = 5, H-2), 4.02 (3H, s, OCH_3), 3.72 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.63 (3H, s, CH_3-C-3), 1.51 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.2 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 145.0 (OCOCH=*C*H), 142.4 (C-13a), 136.0 (C-12a), 132.9 (C1′), 132.2 (C3′, C5′), 129.8 (C-9), 129.7 (C-2′, C-6′), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 126.8 (C-12), 125,9 (C-7a), 125.1 (C-4′), 124.7 (C-10), 117.6 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.3 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS m/z 656 and 658 $[MH]^+$. Anal. Calcd for ($C_{35}H_{30}BrNO_7$): C, 64.03; H, 4.61; N, 2.13. Found: C, 63.92; H, 4.62; N, 2.05.

4.24. (±)-*cis*-1-Acetoxy-2-(3-bromocinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (24)

Yield 90%, IR (KBr) ν 2916, 2846, 1731, 1651, 1621, 1586, 1569, 1494, 1464, 1399, 1227, 1205, 1153, 1083, 1028, 747 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.61–7.52 (4H, m, H-2′, H-11, H-13, OCOCH=C*H*), 7.47 (1H, dt, J = 8, 1.5, H-4′), 7.41 (1H, td, J = 8, 1.5, H-10), 7.37 (1H, dt, J = 8, 1.5, H-6′), 7.22 (1H, t, J = 8, H-5′), 6.61 (1H, d, J = 5, H-1), 6.37 (1H, d, J = 16, OCOC*H*=CH), 6.36 (1H, s, H-5), 5.66 (1H, d, J = 5, H-2), 4.05 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.64 (3H, s, CH_3-C-3), 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.1 (*C*O-O-C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 144.7 (OCOCH=*C*H), 142.4 (C-13a), 136.1 (C-1′), 136.0 (C-12a), 133.5 (C-4′), 131.0 (C-2′), 130.5 (C-5′), 129.8 (C-9), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 127.0 (C-6′), 126.8 (C-12), 126.0 (C-7a), 124.7 (C-10), 123.1 (C-3′), 118.5 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.4 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS m/z 656 and 658 $[MH]^+$. Anal. Calcd for ($C_{35}H_{30}BrNO_7$): C, 64.03; H, 4.61; N, 2.13. Found: C, 64.20; H, 4.53; N, 2.21.

4.25. (±)-*cis*-1-Acetoxy-2-(4-trifluoromethylcinnamoyloxy)-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (25)

Yield 88%, IR (KBr) ν 2981, 2930, 2849, 1723, 1642, 1615, 1592, 1490, 1460, 1392, 1372, 1352, 1281, 1206, 1159, 1122, 1027, 1014, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, s, H-8), 8.01 (1H, dd, J = 8, 1.5, H-9), 7.84 (1H, dd, J = 8, 1.5, H-12), 7.66–7.53 (7H, m,

H-2′, H-6′, H-3′, H-5′, H-11, H-13, OCOCH=C*H*), 7.41 (1H, td, *J* = 8, 1.5, H-10), 6.61 (1H, d, *J* = 5, H-1), 6.45 (1H, d, *J* = 16, OCOC*H*=CH), 6.36 (1H, s, H-5), 5.68 (1H, d, *J* = 5, H-2), 4.05 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.65 (3H, s, CH_3-C-3), 1.53 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3 (C-7), 171.0 (*C*O-O-C-1), 165.9 (*C*O-O-C-2), 163.0 (C-6), 160.3 (C-4a), 150.3 (C-14a), 144.4 (OCOCH=*C*H), 142.3 (C-13a), 137.3 (C-12a), 135.9 (C-1′), 131.8 (q, *J* = 35, C-4′), 129.7 (C-8), 128.7 (C-11), 128.4 (2C, C-2′, C-6′), 128.3 (C-8a), 128.1 (C-9), 126.7 (C-12), 125.8 (2C, C-3′, C-5′), 125.5 (C-7a), 124.6, (C-10), 119.4 (OCO*C*H=CH), 118.4 (q, *J* = 270, CF_3), 111.6 (C-13), 111.3 (C-6a), 97.6 (C-14b), 94.3 (C-5), 77.2 (C-3), 69.4 (C-2), 66.0 (C-1), 55.4 (OCH_3), 42.9 (NCH_3), 24.8 (*C*H_3-C-3), 23.0 (*C*H_3-C-3), 21.1 (OCO*C*H_3); ESI-MS *m/z* 646 $[MH]^+$. Anal. Calcd for ($C_{36}H_{30}F_3NO_7$): C, 66.97; H, 4.68; N, 2.17. Found: C, 67.08; H, 4.53; N, 2.24.

4.26. (±)-*cis*-1-Acetoxy-2-(3-Trifluoromethylcinnamoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (26)

Yield 98%, IR (KBr) *v* 2982, 2926, 2835, 1729, 1646, 1621, 1589, 1573, 1494, 1462, 1400, 1373, 1332, 1270, 1227, 1205, 1159, 1127, 1089, 1033, 914, 868, 806, 746 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = 8, 1.5, H-12), 7.70–7.59 (4H, m, H-2′, H-4′, H-6′, OCOCH=C*H*), 7.54 (1H, td, *J* = 8, 1.5, H-11), 7.53 (1H, s, H-13), 7.45 (1H, t, *J* = 8, H-5′), 7.41 (1H, td, *J* = 8, 1.5, H-10), 6.62 (1H, d, *J* = 5, H-1), 6.44 (1H, d, *J* = 16, OCOC*H*=CH), 6.36 (1H, s, H-5), 5.67 (1H, d, *J* = 5, H-2), 4.05 (3H, s, OCH_3), 3.72 (3H, s, NCH_3), 1.95 (3H, s, $OCOCH_3$), 1.64 (3H, s, CH_3–C-3), 1.52 (3H, s, CH_3–C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.0 (*C*O–O–C-2), 163.1 (C-6), 160.4 (C-4a), 150.4 (C-14a), 144.5 (OCOCH=*C*H), 142.4 (C-13a), 136.0 (C-12a), 134.8 (C-1′), 131.7 (q, *J* = 30, C-3′), 131.4 (C-6′), 129.8 (C-5′), 129.5 (C-9), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 127.1 (C-4′), 126.8 (C-12), 126.0 (C-7a), 124.8 (C-2′), 124.7 (C-10), 119.2 (q, *J* = 270, CF_3), 118.9 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.8 (C-14b), 94.4 (C-5), 76.6 (C-3), 69.5 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3–C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 646 $[MH]^+$. Anal. Calcd for ($C_{36}H_{30}F_3NO_7$): C, 66.97; H, 4.68; N, 2.17. Found: C, 66.88; H, 4.74; N, 2.20.

4.27. (±)-*cis*-1-Acetoxy-6-methoxy-3,3,14-trimethyl-2-(4-nitrocinnamoyloxy)-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (27)

Yield 84%, UV λ nm (log ε) (MeOH) 233 (3.47), 287 (3.85), 431 (2.65); IR (KBr) *v* 3056, 2961, 2928, 2851, 1729, 1641, 1590, 1080, 1025, 740 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.19 (2H, d, *J* = 8.5, H-3′, H-5′), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = 8, 1.5, H-12), 7.63 (1H, d, *J* = 16, OCOCH=C*H*), 7.60 (2H, d, *J* = 8.5, H-2′, H-6′), 7.54 (1H, td, *J* = 8, 1.5, H-11), 7.52 (1H, s, H-13), 7.41 (1H, td, *J* = 8, 1.5, H-10), 6.61 (1H, d, *J* = 5, H-1), 6.49 (1H, d, *J* = 16, OCOC*H*=CH), 6.35 (1H, s, H-5), 5.67 (1H, d, *J* = 5, H-2), 4.05 (3H, s, OCH_3), 3.72 (3H, s, NCH_3), 1.95 (3H, s, $OCOCH_3$), 1.64 (3H, s, CH_3-C-3), 1.55 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 165.6 (*C*O-O-C-2), 163.1 (C-6), 160.3 (C-4a), 150.4 (C-14a), 148.7 (C-4′), 143.1 (OCOCH=*C*H), 142.4 (C-13a), 140.0 (C-1′), 135.9 (C-12a), 129.8 (C-9), 128.9 (2C, C-2′, C-6′), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 126.8 (C-12), 126.1 (C-7a), 124.7 (C-10), 124.2 (2C, C-3′, C-5′), 121.3 (OCO*C*H=CH), 111.6 (C-13), 111.4 (C-6a), 97.6 (C-14b), 94.3 (C-5), 76.5 (C-3), 69.7 (C-2), 66.1 (C-1), 56.5 (OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.0 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 623 $[MH]^+$. Anal. Calcd for ($C_{35}H_{30}N_2O_9$): C, 67.52; H, 4.86; N, 4.50. Found: C, 67.39; H, 4.71; N, 4.61.

4.28. (±)-*cis*-1-Acetoxy-6-methoxy-2-(4-methoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (28)

Yield 90%, UV λ nm (log ε) (MeOH) 230 (3.34), 286 (3.68), 308 (3.28), 373 (1.99), 418 (2.44), 425 (2.45); IR (KBr) *v* 3052, 2955, 2924, 2850, 1735, 1650, 1588, 1510, 1153, 1083, 1029, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, s, H-8), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.84 (1H, dd, *J* = *J* = 8, 1.5, H-12), 7.57 (1H, d, *J* = 16, OCOCH=C*H*), 7.54 (1H, td, *J* = 8, 1.5, H-11), 7.53 (1H, s, H-13), 7.41 (1H, td, *J* = 8, 1.5, H-10), 7.40 (2H, d, *J* = 8.5, H-2′, H-6′), 6.84 (2H, d, *J* = 8.5, H-3′, H-5′), 6.60 (1H, d, *J* = 5, H-1), 6.36 (1H, s, H-5), 6.23 (1H, d, *J* = 16, OCOC*H*=CH), 5.66 (1H, d, *J* = 5, H-2), 4.05 (3H, s, CH_3O-C-6), 3.81 (3H, s, NCH_3), 3.73 (3H, s, CH_3O-C-4′), 1.94 (3H, s, $OCOCH_3$), 1.63 (3H, s, CH_3-C-3), 1.59 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.8 (*C*O-O-C-2), 163.1 (C-6), 161.7 (C-4′), 160.5 (C-4a), 150.4 (C-14a), 146.1 (OCOCH=*C*H), 142.5 (C-13a), 135.9 (C-12a), 130.1 (2C, C-2′, C-6′), 129.7 (C-9), 128.8 (C-8a), 128.4 (C-11), 128.2 (C-8), 126.8 (2C, C-12, C-1′), 126.0 (C-7a), 124.6 (C-10), 114.4 (2C, C-3′, C-5′), 114.4 (OCO*C*H=CH), 111.7 (C-13), 111.4 (C-6a), 97.9 (C-14b), 94.4 (C-5), 76.8 (C-3), 68.9 (C-2), 66.2 (C-1), 56.5 (*C*H_3O-C-6), 55.5 (*C*H_3O-C-4′), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.1 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 608 $[MH]^+$; 630 $[MNa]^+$. Anal. Calcd for ($C_{36}H_{33}NO_8$): C, 71.16; H, 5.47; N, 2.31. Found: C, 71.01; H, 5.54; N, 2.34.

4.29. (±)-*cis*-1-Acetoxy-6-methoxy-2-(3,4-dimethoxycinnamoyloxy)-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (29)

Yield 98%, IR (KBr) *v* 2982, 2932, 2833, 1740, 1717, 1651, 1624, 1588, 1508, 1462, 1389, 1263, 1230, 1027, 1141, 1028, 905, 813, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, s, H-8), 8.01 (1H, dd, *J* = 8, 1.5, H-9), 7.90 (1H, dd, *J* = 8, 1.5, H-12), 7.58–7.53 (3H, m, H-11, H-13, OCOCH=C*H*), 7.41 (1H, td, *J* = 8, 1.5, H-10) ; 7.01 (1H, dd, *J* = 8.5, 1.5, H-6′), 6,98 (1H, d, *J* = 1.5, H-2′), 6.80 (1H, d, *J* = 8.5, H-5′), 6.61 (1H, d, *J* = 5, H-1), 6.36 (1H, s, H-5), 6.19 (1H, d, *J* = 16, OCOC*H*=CH), 5.68 (1H, d, *J* = 5, H-2), 4.05 (3H, s, CH_3O-C-6), 3.88 (6H, s, CH_3O-C-3′, CH_3O-C-4′), 3.73 (3H, s, NCH_3), 1.94 (3H, s, $OCOCH_3$), 1.64 (3H, s, CH_3-C-3), 1.52 (3H, s, CH_3-C-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4 (C-7), 171.1 (*C*O-O-C-1), 166.8 (*C*O-O-C-2), 163.1 (C-6), 160,4 (C-4a), 151.5 (C-4′), 149.3 (C-3′), 150.4 (C-14a), 146.4 (OCOCH=*C*H), 142.4 (C-13a), 135.9 (C-12a), 129.8 (C-9), 128.8 (C-8a), 128.4 (C-8), 128.2 (C-11), 127.0 (C-1′), 126.8 (C-12), 126.0 (C-7a), 124.7 (C-10), 123.4 (C-6′), 114.4 (OCO*C*H=CH), 111.6 (2C, C-13, C-6a), 111.0 (C-5′), 109.5 (C-2′), 97.9 (C-14b), 94.4 (C-5), 76.8 (C-3), 68.9 (C-2), 66.2 (C-1), 56.5, 56.1, 56.0 (3 × OCH_3), 42.9 (NCH_3), 24.9 (*C*H_3-C-3), 23.0 (*C*H_3-C-3), 21.2 (OCO*C*H_3); ESI-MS *m/z* 638 $[MH]^+$. Anal. Calcd for ($C_{37}H_{35}NO_9$): C, 69.69; H, 5.53; N, 2.20. Found: C, 69.82; H, 5.67; N, 2.29.

4.30. DNA alkylation

Kinetics for DNA alkylation were performed using a 117-bp DNA fragment obtained from *Eco*RI and *Pvu*II restriction enzymes digestion of the pBS plasmid which was then labeled at the *Eco*RI site using α-[^{32}P]-dATP (Amersham) and AMV reverse transcriptase (Ozyme) and purified as previously described (David-Cordonnier et al., 2002). The radiolabelled DNA fragment was then incubated with a fixed concentration of drug (50 μM) in 1 mM Na cacodylate, pH 7.0 in the dark and room temperature during 5, 10, 20, 30, 45 min, 1, 2, 3, 4, 6, 8, 16 and 24 h. At the desired incubation time, the reaction was stopped by adding 5 μL of a 50% glycerol containing tracking dyes solution and subsequent freezing of

the reaction samples at −80 °C. The DNA samples were then resolved by electrophoresis under non-denaturing conditions in 6% polyacrylamide gels for around 5 h at 300 V at room temperature in TBE buffer (89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3). Gels were transferred to Whatman 3MM paper, dried under vacuum at 80 °C, and finally analyzed on a phosphorimager (Molecular Dynamics 445SI). The $t_{1/2}$ corresponds to the incubation time requires to obtain one half of the maximum of alkylation retardation as a plateau (t_{max}). When the plateau is not reached the $t_{1/2}$ is referred as > of the half of the maximum of experiment time if the plateau is nearly reached or > of the maximum of experiment time (24 h) if the plateau is far to be reached (Fig. 1).

4.31. 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.[17] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 4 doubling times (48 h for L1210 cells and 96 h for KB-3-1 cells). Results are expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

4.32. Cell cycle analysis

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNAse and 50 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analyzed on an XLMCL flow cytometer (Beckman Coulter, France). Results are expressed as the% of cells in the S phase of the cell cycle.

4.33. Antitumor activity

The antitumor activity of the compounds **1, 8** and **21** was evaluated on the murine colon 38 adenocarcinoma implanted in B6D2F1 (C57B1/6 × DBA2) mice. The colon adenocarcinoma C38 (NCI, Frederick) was introduced by sc implantation of a tumor fragment into the dorsal flank. The compounds were solubilized in a preparation of cremophor ELP/ethanol (10% each in physiological saline) and administered by iv injection 12 and 22 days after the tumor graft.

Compounds were administered at their maximal tolerated dose (MTD) which was the highest non-toxic dose (4 mg/kg); MTD/2 (2 mg/kg) and MTD/4 (1 mg/kg). A dose is considered as toxic when it induces toxic deaths or when the weight loss is higher than 20%. Tumor were measured twice a wekk and tumor volume (Vt) were calculated using the following formula: length (mm) × width^2 (mm^2)/2. At the end of the experiment, on day 80, mice were palpated and tumor-free mice were recorded.

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