Synthesis, Cytotoxic Activity, and Mechanism of Action of Furo[2,3-*c*]acridin-6-one and Benzo[*b*]furo[3,2-*h*]acridin-6-one Analogues of Psorospermin and Acronycine

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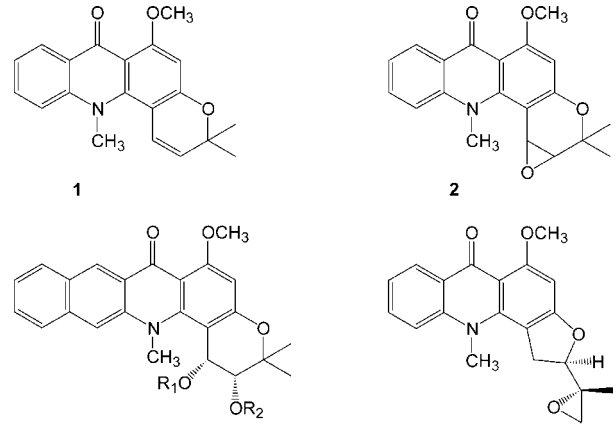
Compounds possessing the epoxyfuran system present in the natural cytotoxic dihydrofuroxanthone psorospermin (**4**) fused onto the acridone or benzo[*b*]acridone chromophores present in the antitumor acronycine (**1**) and S23906-1 (**3**) were prepared. The basic furoacridone and benzofuroacridone cores bearing an isopropenyl substituent at a convenient position were synthesized by condensation of 1,3-dihydroxyacridone (**7**) or 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**9**) with (*E*)-1,4-dibromo-2-methylbut-2-ene. In both series, the (2*R**,1′*S**) epoxides, with the same relative configuration as psorospermin, were the most active compounds, exhibiting cytotoxic properties with IC_{50} values in the 10−100 nM range. As in the acronycine and psorospermin series, the new compounds act through alkylation of the DNA guanine units. However, a strong difference was noted in the DNA alkylation site between the benzopyranoacridone S23906-1, which alkylates DNA guanine units at position N-2 in the minor groove, and the new 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one derived epoxide **21**, which alkylates DNA guanine units at position N-7 in the major groove.

Introduction

The natural pyranoacridone acronycine (**1**, Chart 1), first isolated from *Acronychia baueri* Schott (Rutaceae) in 1948,[1,2] was subsequently shown to exhibit a broad spectrum of activity against numerous experimental tumors models, including sarcoma, myeloma, carcinoma, and melanoma.[2,3] However, the moderate potency and very low solubility in aqueous solvents of this alkaloid severely hampered the subsequent clinical trials, which gave poor results.[4]

Following the isolation of the unstable acronycine epoxide (**2**) from several New-Caledonian *Sarcomelicope* species, efforts toward the design of more potent derivatives were guided by a hypothesis of bioactivation of the 1,2-double bond of acronycine into the corresponding oxirane in vivo.[2,5] Significant improvements in terms of potency were obtained with derivatives modified in the pyran ring, which had a similar reactivity toward nucleophilic agents as acronycine epoxide but an improved chemical stability. Such compounds are exemplified by diesters of *cis*-and *trans*-1,2-dihydroxy-1,2-dihydroacronycine, which exhibited marked antitumor properties, with a broadened spectrum and increased potency when compared to acronycine.[6] Further on, structural analogues in the related benzo[*a*]acronycine and benzo[*b*]acronycine series, including an additional aromatic ring fused onto the natural alkaloid skeleton, were developed, and esters and diesters of 1,2-dihydro-1,2-diols in these latter series proved even more potent.[7−9] For instance, diacetate **3** (S23906-1), which recently underwent phase I clinical trials, demonstrated comparable and/or better activity than paclitaxel, vinorelbine, and irinotecan when evaluated against aggressive orthotopic models of human ovarian, lung, and colon cancers, respectively.[10] The mechanism of its action was shown to imply alkylation of the 2-amino group of DNA guanine residues in the minor groove, by the carbocation resulting from the elimination of the ester leaving group at position 1 of the drug.[8,11]

Chart 1. Acronycine (**1**), Acronycine Epoxide (**2**), (±)-*cis*-1,2-Diacetoxy-1,2-dihydrobenzo[*b*]acronycine S23906-1 (**3**), (±)-*cis*-1,2-Carbonyloxy-1,2-dihydrobenzo[*b*]acronycine S23905-1 (**27**), and Psorospermin (**4**)



Psorospermin (**4**) is a natural dihydrofuroxanthone, which was isolated by Kupchan in 1980 from the roots of *Psorospermum febrifugum* Sprach. (Guttiferae), based on its significant activity in vivo against P388 mouse leukemia.[12] The stereochemistry and absolute configuration of this compound were established later by the group of Cassady.[13] Psorospermin, as well as some of its derivatives such as O^5-methylpsorospermin, exhibits significant cytotoxicity against a variety of tumor cell lines. Its mechanism of action implies interaction with DNA, but in contrast with acronycine derivatives, it has been shown to

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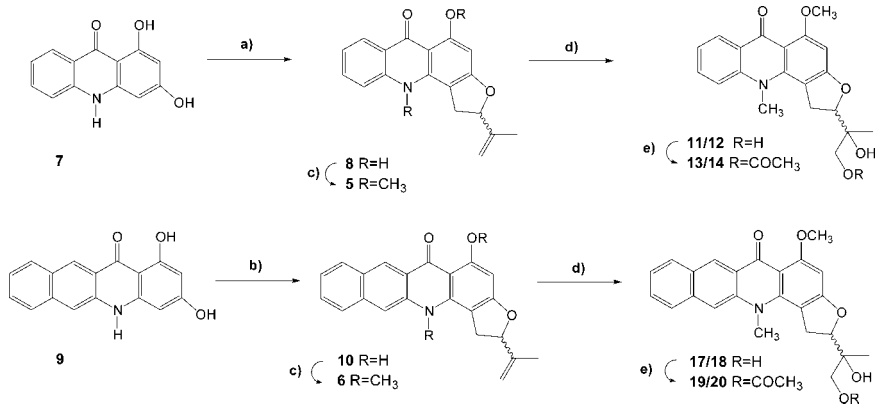
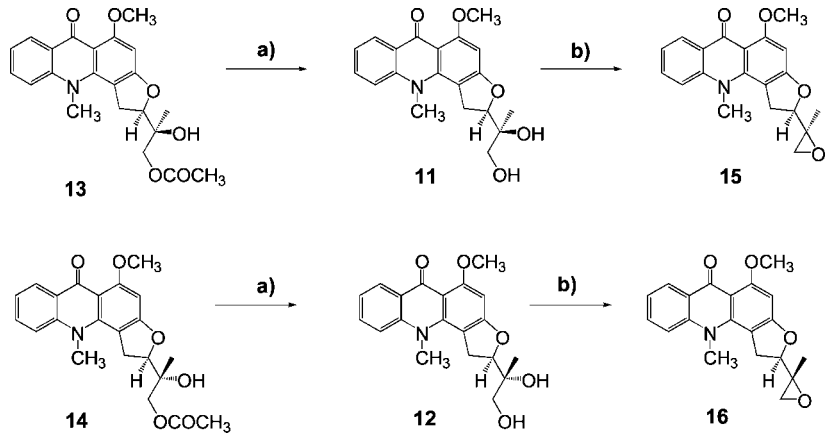
Scheme 1. Synthesis of Diastereomeric Mixture of Monoacetates in the 11*H*-Furo[2,3-*c*]acridin-6-one and 13*H*-Benzo[*b*]furo[3,2-*h*]-acridin-6-one Series[a]



[a] (a) Dibromoisoprene, K_2CO_3, acetone, room temp; (b) dibromoisoprene, NaOH, EtOH, 40 °C; (c) NaH, Me_2SO_4, DMF, room temp; (d) OsO_4, NMO, *t*-BuOH/THF/H_2O 10:3:1, room temp; (e) Ac_2O, pyridine, 0 °C.

Scheme 2. Synthesis of Epoxides (**15**, **16**) in the 11*H*-Furo[2,3-*c*]acridin-6-one Series[a]



[a] (a) K_2CO_3, MeOH; (b) (i) MsCl, pyridine, (ii) K_2CO_3, 18-crown-6, acetone.

covalently bind, through its exocyclic oxirane group, to the N-7 position of the guanine units in the major groove.[14]

In a continuation of our studies on the structure−activity relationships in the acronycine[15] and psorospermin[16] series, we describe here the synthesis and the biological properties of (±)-2-isopropenyl-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**5**), (±)-2-isopropenyl-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**6**), the corresponding epoxides, and related dihydrodiol esters and diesters.

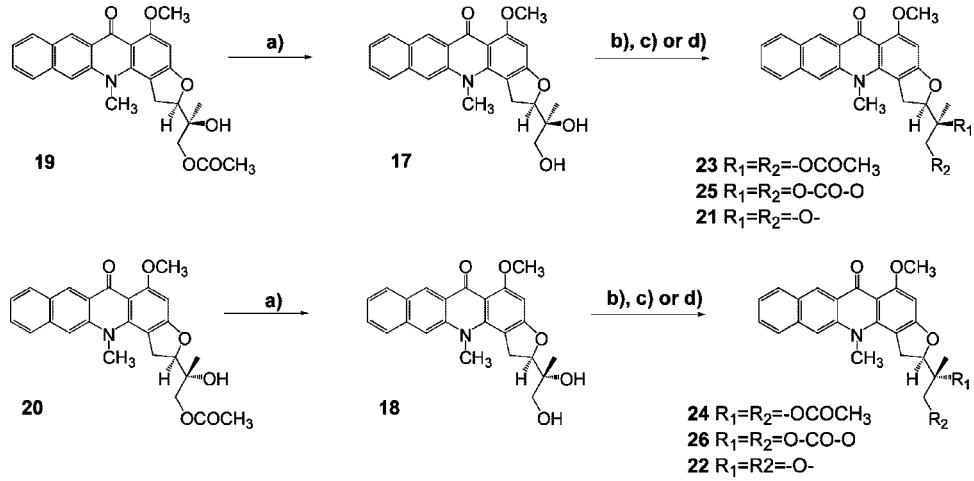
Chemistry

Nickl described in 1958 the first synthesis of a 2-isopropenyl-2,3-dihydrofuran fused onto an aromatic ring, by treatment of a phenol precursor with (*E*)-1,4-dibromobut-2-ene ("dibromoisoprene") in alkaline medium.[17] Although described as giving moderate yields, this method was anticipated to permit construction of the desired basic 1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one and 1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one cores bearing an isopropenyl substituent at the required 2-position in a single step. Also, it had previously given satisfactory results in the synthesis of the related natural alkaloid rutacridone.[18] Consequently, the syntheses of **5** and **6** were envisioned according to Scheme 1.

Reaction of 1,3-dihydroxyacridone (**7**)[19] with (*E*)-1,4-dibromo-2-methylbut-2-ene[20] at 40 °C, in the presence of potassium carbonate in acetone, gave (±)-2-isopropenyl-5-hydroxy-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**8**) in a moderate 23% yield. Simultaneous N- and O-methylation of **8** was ensured by the use of sodium hydride as base and dimethyl sulfate as alkylating agent, in dimethylformamide, to give (±)-2-isopropenyl-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**5**) in almost quantitative yield. The same two-step sequence, using 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**9**) as starting material, successively afforded (±)-2-isopropenyl-5-hydroxy-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**10**) and (±)-2-isopropenyl-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**6**). Phase sensitive NOESY and HMQC experiments performed on the dimethyl derivatives **5** and **6** permitted us to ascribe unambiguously angular structures to compounds **5**, **6**, **8**, and **10**.[21]

Attempts to directly epoxidize the isopropenyl group of **5** or **6**, using an oxidizing reagent such as *m*-chloroperbenzoic acid or hydrogen peroxide, proved unsuccessful. Consequently, a multistep approach, involving oxirane formation through the alkaline cyclization of the monomesylate of a vicinal diol, was envisaged, since this method had previously given good results in the course of the synthesis of psorospermin analogues in the quinobenzoxazine series (Scheme 2).[22] Catalytic osmium tetroxide oxidation of **5**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent,[9,23] led to a diasteroisomeric mixture of racemic (2*R**,1′*S**) (**11**) and (2*R**,1′*R**) (**12**) diols,

Scheme 3. Synthesis of Esters (**19**−**26**) and Epoxides (**21**, **22**) in the 13*H*-Benzo[*b*]furo[3,2-*h*]acridin-6-one Series[a]



[a] (a) K_2CO_3, MeOH; (b) Ac_2O, pyridine, 4-DMAP; (c) *N*,*N*′-carbonyldiimidazole, 2-butanone; (d) (i) MsCl, pyridine, (ii) K_2CO_3, 18-crown-6, acetone.

which proved difficult to separate, even on a small scale for analytical purposes. In contrast, the corresponding (2*R**,1′*S**) (**13**) and (2*R**,1′*R**) (**14**) monoacetates on the primary alcohol, prepared by treatment of the diastereoisomeric mixture of diols with acetic anhydride in anhydrous pyridine under argon, could be easily separated by OPLC on a preparative scale. After separation, each of them conveniently regenerated the corresponding diol in quantitative yield upon treatment with potassium carbonate. Mesylation of the primary alcohol of diols **11** and **12** with methanesulfonyl chloride in pyridine afforded the corresponding mesylates, which were immediately submitted without purification to alkaline treatment with potassium carbonate in the presence of 18-crown-6 ether in acetone to afford the desired (2*R**,1′*S**) and (2*R**,1′*R**) epoxides **15** and **16**, respectively.

When applied to (±)-2-isopropenyl-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**6**), the same reaction sequence permitted preparation of (2*R**,1′*S**) and (2*R**,1′*R**) diols **17** and **18**, (2*R**,1′*S**) and (2*R**,1′*R**) monoacetates **19** and **20**, and (2*R**,1′*S**) and (2*R**,1′*R**) epoxides **21** and **22** (Scheme 3). In this latter series, which was anticipated to exhibit better biological activities, (2*R**,1′*S**) and (2*R**,1′*R**) diacetates **23** and **24** and (2*R**,1′*S**) and (2*R**,1′*R**) cyclic carbonates **25** and **26** were also prepared from diols **17** and **18**, by treatment with acetic anhydride in the presence of 4-dimethylaminopyridine and with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux, respectively.

The relative (2*R**,1′*S**) configurations of epoxides **15** and **21**, on the one hand, and (2*R**,1′*R**) of epoxides **16** and **22**, on the other hand, were deduced from a thorough ^{1}H NMR study, in comparison with data previously established for epimeric pairs in the related epoxyrotenone,[24] epoxytubaic acid,[13b] and psorospermin[13a,b] series. Indeed, the chemical shift difference between the two signals of the geminal methylene protons of the epoxide group is systematically smaller in the case of a (2*R*,1′*S*) configuration ($\Delta(\delta H_a - \delta H_b) \approx 0.09-0.12$ ppm) than in the case of a (2*R*,1′*R*) configuration ($\Delta(\delta H_a - \delta H_b) \approx 0.24-0.29$ ppm). Thus, the relative configuration (2*R**,1′*S**) was attributed to compounds of higher R_f, **15** ($\Delta(\delta H_a - \delta H_b) = 0.10$ ppm) and **21** ($\Delta(\delta H_a - \delta H_b) = 0.09$ ppm), whereas the relative configuration (2*R**,1′*R**) was attributed to compounds of lower R_f, **16** ($\Delta(\delta H_a - \delta H_b) = 0.26$ ppm) and **22** ($\Delta(\delta H_a - \delta H_b) = 0.27$ ppm). The relative configurations of the various diols (**11**, **12**, **17**, **18**), esters (**13**, **14**, **19**, **20**), and diesters (**23**-**26**) were further deduced from those of the corresponding epoxides.

Table 1. Cytotoxicity of 11*H*-Furo[2,3-*c*]acridin-6-one Derivatives **5**, **8**, and **11**−**16**, in Comparison with Acronycine (**1**) and S23906-1 (**3**)

compd	cytotoxicity (IC_{50}, μM)[a] L1210 cells	cytotoxicity (IC_{50}, μM)[a] KB-3-1 cells	cell cycle perturbation (μM), L1210[b]
1 (acronycine)	23	3.7	G2 M ++ 50 μM
3 (S23906-1)	0.6	0.1	S +++ 5 μM
5	4.7	5.9	S + 25 μM
8	2.8	5.2	S + 20 μM
11	25	34	nt
12	28	46	nt
13	16	24	nt
14	22	48	nt
15	1.5	1.3	MS ++ 10 μM
16	0.03	0.06	MS ++ 0.1 μM

[a] Inhibition of cell proliferation measured by the MTT assay (mean of at least three values obtained in separate experiments). [b] L1210 cells arrested in the indicated phase after a 21 h exposure to the given concentration. nt: not tested.

Results and Discussion

All the new 1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one and 1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one derivatives were first 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). The results (IC_{50}) are reported in Tables 1 and 2.

In the 11*H*-furo[2,3-*c*]acridin-6-one series, both (±)-2-isopropenyl-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**5**) and (±)-2-isopropenyl-5-hydroxy-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**8**) exhibited cytotoxic properties, with IC_{50} values between 2 and 6 μM against the two cell lines. The corresponding diols and monoacetates (**11**−**14**) only displayed marginal activities or were found inactive. As expected, the epoxides **15** and **16** were the most active compounds in the series. However, a dramatic difference of potency was observed between the two diastereoisomers. Indeed, the (2*R**,1′*S**) epoxide (**15**) exhibited cytotoxic properties with micromolar IC_{50} against both L1210 and KB-3-1 cells, whereas the (2*R**,1′*R**) isomer (**16**) was found to be nearly 100-fold more potent in inhibiting cell proliferation. Interestingly, a recent study on O^5-methylpsorospermin has also shown that the (±)-

Table 2. Cytotoxicity of 13*H*-Benzo[*b*]furo[3,2-*h*]acridin-6-one Derivatives **6**, **10**, and **17**−**26**, in Comparison with Acronycine (**1**) and S23906-1 (**3**)

compd	cytotoxicity (IC_{50}, μM)[a] L1210 cells	KB-3-1 cells	cell cycle perturbation (μM), L1210[b]
1 (acronycine)	23	3.7	G2 M ++ 50 μM
3 (S23906-1)	0.7	0.1	S +++ 5 μM
6	4.1	6.1	G2 M + 10 μM
10	10	6.6	nt
17	2.4	8.2	nt
18	4.5	5.7	nt
19	0.8	6.7	G2 M ++ 5 μM
20	4.2	5.5	G2 M ++ 20 μM
21	0.04	0.08	S +++ 0.5 μM
22	0.06	0.04	MS +++ 0.1 μM
23	2.3	6.9	G2 M ++ 20 μM
24	3.9	8.2	nt
25	2.1	7.9	nt
26	25	8.4	nt

[a] Inhibition of cell proliferation measured by the MTT assay (mean of at least three values obtained in separate experiments). [b] L1210 cells arrested in the indicated phase after a 21 h exposure to the given concentration. nt: not tested.

(2*R**,1′*R**) diasteroisomeric pair, containing the naturally occurring (2*R*,1′*R*) epoxyfuroxanthone, was much more active than the isomeric (±)-(2*S**,1′*R**) pair when tested against a large range of tumor cells.[25]

In the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series, the cytotoxic activities displayed by the diols **17** and **18**, the esters **19** and **20**, and the diesters **23**−**26** were found to be within the same order of magnitude as those displayed by (±)-2-isopropenyl-5-hydroxy-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**10**) and (±)-2-isopropenyl-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (**6**). As in the 11*H*-furo[2,3-*c*]acridin-6-one series, the epoxides **21** and **22** were the most active compounds, and the (2*R**,1′*S**) isomer was also the most potent against both cell lines. Nevertheless, the difference between the cytotoxic activities of the (2*R**,1′*R**) and (2*R**,1′*S**) diastereoisomers was much less important than that observed in the 11*H*-furo[2,3-*c*]acridin-6-one series. Both compounds exhibited IC_{50} in the 10−100 nM range. The potency of compounds **21** and **22** is noteworthy, being more potent than the reference compound S23906-1 (**3**).

The perturbation of the cell cycle induced by the new derivatives was studied on the L1210 cell line.

The most potent epoxide in the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one **21** was also evaluated for its ability to form covalent complexes with DNA using gel shift assay. As shown in Figure 1A,B, in vitro interaction of **21** with a radiolabeled DNA fragment reveals only a weak gel shift for the highest concentrations and longer incubation time. This very weak retardation contrasts with that obtained previously using S23906-1 and derivatives.[8,11a,b] Since the large gel shift obtained using S23906-1 was previously attributed to its peculiar mode of action destabilizing the double-stranded DNA helix,[26] we evaluated the ability of compound **21** to stabilize or destabilize the DNA helix using melting temperature studies (Figure 1C). In contrast to the negative ΔT_m obtained using S23906-1, compound **21** induces a positive ΔT_m at equivalent concentrations. This result suggests that compound **21** classically stabilizes the DNA helix upon binding.

The epoxide group is often associated with alkylation of the DNA at guanines on the major group through the covalent bonding to the N-7 position of guanine. Sometimes, this position spontaneously leads to a depurinate guanine nucleotide, leading

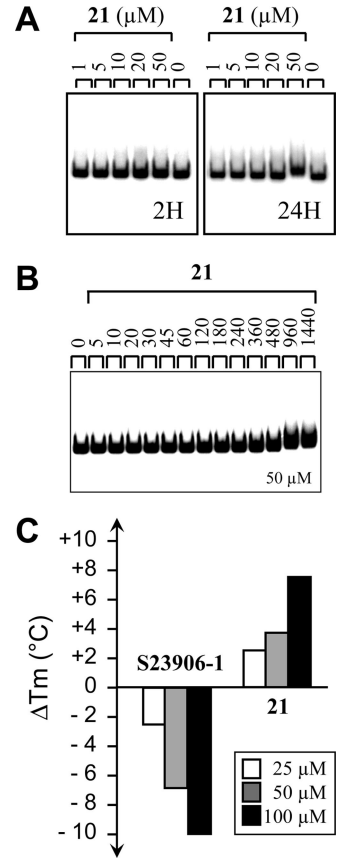


Figure 1. Gel shift assays for alkylation of a 117-bp radiolabeled DNA fragment using (A) increasing concentrations of compound **21** as specified on the top of the lanes after 2 or 24 h incubation times or (B) a fixed concentration of 50 μM of compound **21** incubated with the DNA from 5 min to 24 h as specified on the top of the lanes (min). (C) Variation of the melting temperature of double-stranded XH-24 bp oligonucleotides incubated alone or with compound **21** or **3** (S23906-1) prior to ethanol precipitation of the alkylated DNA and subsequent melting temperature measurement as described in the Experimental Section. Positive ΔT_m values correspond to stabilization of the DNA helix by the compound, whereas negative ΔT_m values correspond to destabilization of the double strand of DNA helix.

to an AP site being labile under heating step classically using for sequencing gel analyses.[27] As presented in Figure 2, compound **21** induces a DNA cleavage at guanine positions as indicated by the migration profile that is similar to that obtained in the "G-track" lane generated using dimethyl sulfate and piperidine treatment as a classical Maxam and Gilbert sequencing lane for guanines. To confirm that alkylation occurs at guanine positions, compound **21** was incubated with oligonucleotides containing GC, AT, or IC base pairs as single types of base pairs in DNA oligonucleotides. Figure 3 indicated the covalent bonding of compound **21** using its intrinsic fluorescence measured in samples in which the free alkylating compound was from free and alkylated oligonucleotide by ethanol precipitation. The bonding occurs using GC-containing oligonucleotides and, to a weaker extend, to AT and IC base pair DNAs. Further insight into the base recognition was established by incubating compound **21** with a hairpin oligonucleotide containing either guanines or 7-deazaguanines as targets (Figure 4). Strong alkylation of DNA containing guanines (blue line) but not 7-deazaguanines (red lines) indicated the importance of the presence of a nitrogen atom on guanines for the bonding reaction, strongly suggesting that the N-7 position of guanines in the major groove is the target for compound **21**. Finally, the

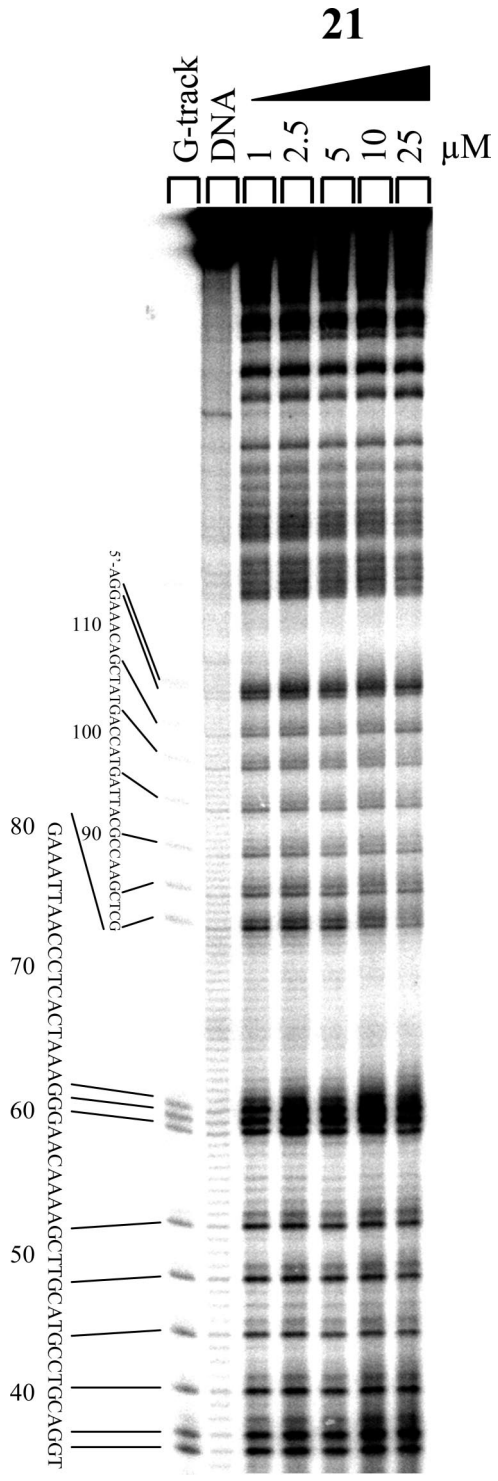


Figure 2. Identification of the alkylation sites of compound **21** using denaturing polyacrylamide gels. Increasing amounts (1−25 μM) of compound **21** were incubated for 24 h in 1 mM sodium cacodylate buffer with the 265-bp radiolabeled DNA fragment prior to heating for 5 min at 90 °C in the formamide containing loading buffer and separation under electrophoresis in denaturing conditions. G-track lane is used as a marker for the DNA sequence specified on the left of the figure.

ability of compound **21** to alkylate DNA within the cells was evaluated from comparison to previously described S23906-1 and its highly potent derivative S23905-1 (**27**) using their fluorescence properties after genomic DNA extraction of treated KB-3-1 cells. Such experiments indicated that compound **21** has a potent DNA alkylating drug within the cell (Figure 5).

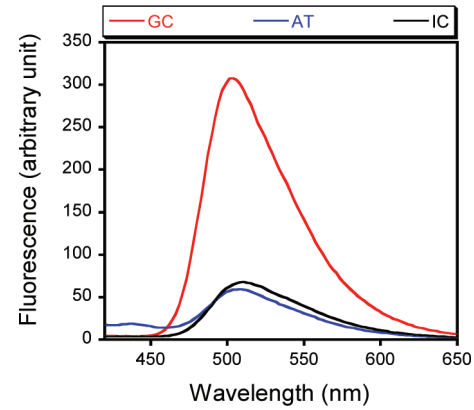


Figure 3. Alkylation of DNA at GC bp. Compound **21** (5 μM) was incubated with the 30-mer oligonucleotides containing A·T (blue line), G·C (red line), or I·C (black line) base pairs only. After 24 h of incubation at room temperature, the DNA samples were precipitated with cold ethanol and the fluorescence of compound **21** covalently linked to the various DNA was scanned from 420 to 650 nm using an excitation wavelength of 354 nm.

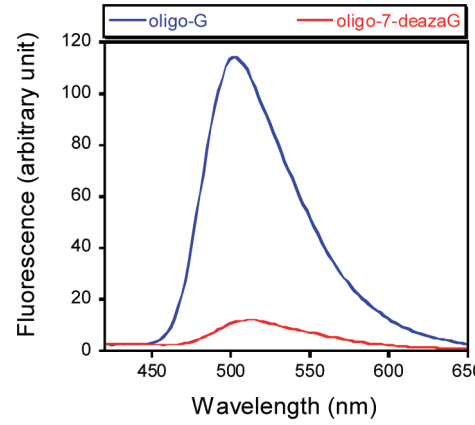


Figure 4. Alkylation of DNA at N-7 position of guanines. Compound **21** (5 μM) was incubated with the short hairpin oligonucleotides containing guanines (in blue) or 7-deazaguanines (in red) for 24 h at room temperature. After removal of the free compound using ethanol precipitation of the oligonucleotides, the fluorescence of compound **21** covalently linked to the oligonucleotides was scanned from 420 to 650 nm using an excitation wavelength of 354 nm.

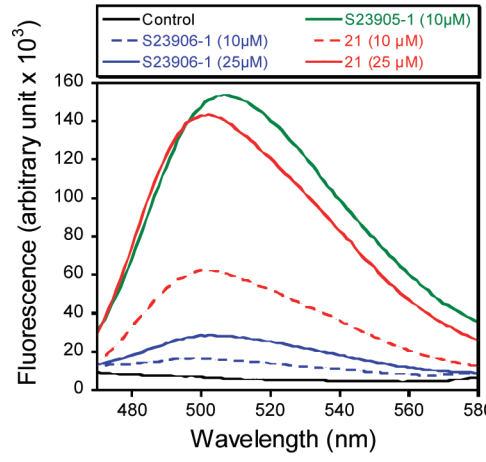


Figure 5. Formation of covalent DNA adducts in cells. KB-3-1 cells were treated or not (black lane) for 24 h with 10 (dashed lanes) or 25 μM (plain lanes) compound **21** (red lanes) or **3** (S23906-1) (blue lanes) or with 10 μM **27** (S23905-1) (green lane) used as controls. Genomic DNA was quantified and analyzed for fluorescence emission using a 300 nm excitation wavelength. The fluorescence spectra were normalized regarding the DNA quantification.

Indeed, the measured fluorescence was much higher than the reference compound S23906-1 but not has much as the highly potent cyclic carbonate derivative S23905-1 (**27**). Interestingly, genomic DNA alkylation level correlates with the cytotoxicity level of each compound, in agreement with the role of alkylation in the cytotoxic function of those alkylating agents.

Conclusion

In conclusion, the presence of an epoxy substitution on the furan ring appears as an important structural requirement to observe a marked cytotoxic activity against L1210 and KB-3-1 cell lines, in both 11*H*-furo[2,3-*c*]acridin-6-one series and 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series.

As previously observed in the isomeric benzo[*b*]acronycine series, the cytotoxic activity appeared to be strongly correlated with the ability of the compounds to give covalent adducts with DNA. However, a strong difference could be noted in the DNA alkylation site between the two series. Indeed, *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 esters and diesters alkylate DNA guanine units at position N-2 in the minor groove, whereas the new epoxides in the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series alkylate DNA guanine units at position N-7 in the major groove. From a structure−activity viewpoint, the ester pyran or epoxy furan pharmacophore appears to play a crucial role at the site of guanine DNA allkylation. New epoxides in the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series alkylate DNA at the same guanine N-7 position as natural xanthones belonging to the psorospermin series.

Experimental Section

Chemistry. Melting points were determined on a hot stage Reichert microscope and are uncorrected. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electrospray ionization (ESI-MS; $V_c = 30$ V) or with a Nermag R-10-10C spectrometer using desorption−chemical ionization (DCI-MS; reagent gas, NH_3). 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 were run at 400 MHz and ^{13}C NMR spectra at 100 MHz, using a Bruker AVANCE-400 spectrometer. When necessary, the structures of the novel compounds were ensured and the signals 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. Column chromatographies were carried out with silica gel 20−45 μm. Flash column chromatographies were conducted using silica gel 60 Merck (35−70 μm) with an overpressure of 300 mbar. Optimum phase layer chromatography (OPLC) was performed on a BIONISIS-OPLC 50 apparatus. In analytical mode, 5 cm × 20 cm BIONISIS-HTSorb F254 BSLA013 silica plates were used (granulometry, 11 μM; porosity, 6 nm; thickness, 0.2 mm). In preparative mode, 20 cm × 20 cm BIONISIS-HTSorb F254 BSLPG001 silica plates were used (granulometry, 11 mM; porosity, 6 nm; thickness, 0.5 mm) with an external pressure of 50 bar and an eluent flow of 1000 μL/min. Microanalyses were in agreement with calculated values ± 0.4%.

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.[28] 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 that reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

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.

DNA Restriction Fragments. The 117- and 265-bp DNA fragments were obtained from the pBS plasmid digestion using EcoRI and PvuII restriction enzymes in their respective digestion buffers and were then labeled at the EcoRI site using α-[^{32}P]dATP (GE Healthcare) and AMV reverse transcriptase (Ozyme). The radiolabeled DNAs were then purified by electrophoresis on a nondenaturing 10% (w/v) polyacrylamide gel with the desired 3′-end-labeled product being cut out of the gel and eluted overnight in 500 mM ammonium acetate, 10 mM magnesium acetate.

Gel Shift Studies. A typical cross-linking reaction consisted of incubating 50 μM drug with the 117-bp radiolabeled DNA in 1 mM Na cacodylate, pH 7.0 (Tris buffer must be avoided due to the presence of reactive amine functions) and incubating in the dark at room temperature during the period specified in the legend. After the desired incubation time, 5 μL of a 50% glycerol containing tracking dye solutions was added to each DNA sample, and they were then resolved by electrophoresis under nondenaturing conditions in 6% polyacrylamide gels for about 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 then analyzed on a phosphorimager (Molecular Dynamics 445SI).

Identification of the Alkylation Site by Thermal Cleavage of the DNA Using Denaturing Polyacrylamide Gels. The 265-bp radiolabeled DNA fragments was incubated with compound **21** at the desired concentration for 24 h at 37 °C in 10 μL of binding buffer (1 mM Na cacodylate, pH 7.0) prior to adding 5 μL of an 80% formamide solution containing tracking dyes. The water was removed by drying under vacuum for 10 min, and the DNA samples were heated at 90 °C for 4 min and chilled in ice for 4 min prior to electrophoresis on polyacrylamide gels under denaturating conditions (8% acrylamide containing 8 M urea) for about 90 min at 65 W at room temperature in 1× TBE buffer (89 mM Tris base, 89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3). After electrophoresis, gels were dried and analyzed as described above. Quantification of the cleavage peaks was performed using the ImageQuant software relative to the guanine track obtained from classical Maxam and Gilbert procedure.

Spectrometric and in Vitro Fluorescence Studies of Alkylated Oligonucleotides. Thermal denaturation studies were conducted using double stranded XH-24bp oligonucleotide as previously described (David-Cordonnier et al., 2005). For fluorescence studies using various oligonucleotide sequences, compound **21** (5 μM) was incubated with double strand AT (5′-TATATATAAAAATATATATTTAAATATATA and complementary strand), GC (5′-CGCGCGCGGGGGCGCGCGCCCGGG CGCGCG and complementary strand), or IC (5′-CICICICII-IIICICICICCCIIICICICI and complementary strand) oligonucleotides or hairpin oligonucleotides (stem underlined) containing guanines (5′-CTATGACTCTGTCATAG) or 7-deazaguanines (5′-CTAT7dGACTCT7dGTCATA7dG) (0.4 μM) in binding buffer containing 1 mM sodium cacodylate, pH 7.0. After a 24 h incubation time at room temperature, the various DNAs were ethanol-precipitated to remove the free compound. The fluorescence of the compound covalently linked to the various DNA was measured from 420 to 650 nm using an excitation wavelength of 354 nm.

Detection of −DNA Adducts in KB-3-1 Cells. KB31 cells from epidermoid carcinoma were grown as described above. KB-3-1 cells were harvested by trypsinization and plated (1.5×10^6 in 10 cm diameter dishes) 24 h before treatment with the test drugs at the

desired concentrations for a further 24 h of incubation. Cells were then collected by centrifugation (1300 rpm, 5 min), washed twice with 10 mL of PBS buffer, and resuspended in 2 mL of PBS containing 5 mM $MgCl_2$. Sequential addition of SDS (200 μL of a 10% solution) and proteinase K (80 μL at 10 mg/ml) followed each time by mild agitation for 5 min was performed before further addition of 200 μL of 0.1 M EDTA, pH 7.5, and incubation for 4 h at 37 °C. Final addition of 80 μL of 5 M NaCl was performed before DNA extraction using 3 mL of phenol/chloroform/isoamylic alcohol (25:24:1) and centrifugation at 4000 rpm for 10 min, and two subsequent extractions with 3 mL of chloroform/isoamylic alcohol (24:1) prior to DNA precipitation with cold ethanol. The absorption of the DNA dissolved in 200 μL of H_2O was measured at 260 nm to estimate the quantity of collected DNA. The fluorescence of either the free compounds or their respective adducts linked to genomic DNA was measured using a SPEX Fluorolog spectrofluorimeter with an excitation wavelength of 300 nm and an emission range from 450 to 580 nm.

2-Isopropenyl-5-hydroxy-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (8). (*E*)-1,4-Dibromo-2-methylbut-2-ene[20] (0.75 g, 3.3 mmol) was added dropwise to a solution of 1,3-dihydroxyacridone (**7**)[19] (1 g, 2.2 mmol) in dry acetone (50 mL) in the presence of dry potassium carbonate (3 g). The mixture was stirred for 3 h at 40 °C. The reaction mixture was filtered, and solvent was removed under reduced pressure. Purification by flash chromatography (solvent, cyclohexane/ethyl acetate 90:10, then 85:15) afforded **8** (300 mg, 23%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60 (s, 3H, CH_3-3′), 3.06 (dd, J = 15, 8.5 Hz, 1H, H-1a), 3.52 (dd, J = 15, 8.5 Hz, 1H, H-1b), 4.95 (d, J = 2 Hz, 1H, H-2′a), 5.10 (d, J = 2 Hz, 1H, H-2′b), 5.45 (t, J = 8.5 Hz, 1H, H-2), 6.14 (s, 1H, H-4), 7.25 (td, J = 8, 1 Hz, 1H, H-8), 7.65 (dd, J = 8, 1 Hz, 1H, H-10), 7.75 (td, J = 8, 1.5 Hz, 1H, H-9), 8.16 (dd, J = 8, 1 Hz, 1H, H-7), 11.20 (s, 1H, NH), 14.60 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.1 (CH_3-3′), 37.4 (C-1), 86.2 (C-2), 88.9 (C-4), 102.0 (C-5a), 112.8 (C-2′), 114.35 (C-11b), 114.3 (C-10), 121.4 (C-8), 125.0 (C-6a), 127.3 (C-7), 132.4 (C-9), 140.5 (C-1′), 142.9 (C-10a), 143.0 (C-11a), 165.3 (C-3a + C-5), 182.0 (C-6); DCI-MS m/z 294 [MH^+]; IR (KBr) ν cm^{-1} 3295, 2918, 2849, 1652, 1646, 1602, 1558, 1259, 1170, 753; UV λ nm (MeOH) 244, 260, 268, 297, 327, 392. Anal. ($C_{18}H_{15}NO_3$) C, H, N.

2-Isopropenyl-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo-[2,3-*c*]acridin-6-one (5). A solution of **8** (400 mg, 1.36 mmol) in dry DMF (50 mL) was added slowly, under nitrogen atmosphere, to an ice-cooled suspension of NaH (165 mg of 80% oil dispersion, 5.46 mmol) in dry DMF (5 mL), and the mixture was stirred under argon for 15 min at 0 °C. After dropwise addition of dimethyl sulfate (0.52 mL, 5.46 mmol), the reaction mixture was stirred at room temperature for 1 h, then poured carefully onto ice−water and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with water (3 × 150 mL), dried over $NaSO_4$, filtered, and evaporated under reduced pressure to afford pure **5** (445 mg, 97%) as a yellowish amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H, CH_3 3′), 3.42 (dd, J = 15, 8.5 Hz, 1H, H-1a), 3.80 (dd, J = 15, 8.5 Hz, 1H, H-1b), 3.88 (s, 3H, N-CH_3), 3.97 (s, 3H, O-CH_3), 4.98 (d, J = 1 Hz, 1H, H-2′a), 5.13 (d, J = 1 Hz, 1H, H-2′b), 5.25 (t, J = 8.5 Hz, 1H, H-2), 6.33 (s, 1H, H-4), 7.22 (td, J = 8, 1.5 Hz, 1H, H-8), 7.30 (dd, J = 8, 1.5 Hz, 1H, H-10), 7.60 (td, J = 8, 1.5 Hz, 1H, H-9), 8.39 (dd, J = 8, 1.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (CH_3-3′), 37.4 (C-1), 39.2 (N-CH_3), 56.3 (O-CH_3), 86.2 (C-2), 88.9 (C-4), 102.0 (C-5a), 112.8 (C-2′), 114.35 (C-11b), 114.3 (C-10), 121.4 (C-8), 125.0 (C-6a), 127.3 (C-7), 132.4 (C-9), 140.5 (C-1′), 142.9 (C-10a), 143.0 (C-11a), 161.3 (C-3a + C-5), 182.0 (C-6); DCI-MS m/z 322 [MH^+]; IR (KBr) ν cm^{-1} 2970, 2935, 2850, 1632, 1599, 1585, 1498, 1398, 1199, 1093, 756; UV λ nm (MeOH) 210, 227, 250, 261, 269, 296, 325, 384. Anal. ($C_{20}H_{19}NO_3$) C, H, N.

(±)-(2*R,1′*S**)- and (2*R**,1′*R**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (13 and 14).** Compound **5** (350 mg, 1.09 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol, 1.4 mL) and *N*-methylmorpholine *N*-oxide dihydrate (300 mg, 2.18 mmol) in *t*-BuOH/THF/H_2O (10:3:1, 30 mL). The reaction mixture was stirred at room temperature for 4 days. After addition of saturated aqueous $NaHSO_3$, the mixture was stirred for 1 h and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent CH_2Cl_2/MeOH 98:2) gave 345 mg of a diastereomeric mixture of diols **11** and **12** (0.98 mmol, 90%), which was solubilized in dry pyridine (20 mL). The solution was cooled (ice−water bath), and acetic anhydride (0.18 mL, 1.96 mmol) was added. The mixture was stirred for 3 h and evaporated. The residue was extracted with CH_2Cl_2 (200 mL) and the solution washed with water (2 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent CH_2Cl_2/MeOH 98:2) afforded a diastereomeric mixture of monoacetates **13** and **14** (300 mg, 77%). Separation of the diasteroisomers was carried out by optimum phase layer chromatography (solvent CH_2Cl_2/MeOH 99:1), which gave successively **13** (80 mg, 26%) and **14** (90 mg, 30%) as amorphous white solids.

(±)-(2*R,1′*S**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (13).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 3H, CH_3-3′), 2.14 (s, 3H, $COCH_3$), 2.54 (br s, 1H, OH), 3.68 (m, 2H, CH_2-1), 3.90 (s, 3H, O-CH_3), 3.94 (s, 3H, N-CH_3), 4.21 (s, 2H, CH_2-2′), 4.83 (t, J = 8.5 Hz, 1H, H-2), 6.28 (s, 1H, H-4), 7.23 (td, J = 8, 1.5 Hz, 1H, H-8), 7.29 (dd, J = 8, 1.5 Hz, 1H, H-10), 7.60 (td, J = 8, 1.5 Hz, 1H, H-9), 8.37 (dd, J = 8, 1.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5 (C-3′), 20.9 ($COCH_3$), 32.8 (C-1), 39.4 (N-CH_3), 56.4 (O-CH_3), 68.7 (C-2′), 72.9 (C-1′), 85.0 (C-2), 88.9 (C-4), 102.5 (C-11b), 110.0 (C-5a), 114.5 (C-10), 121.6 (C-8), 125.4 (C-6a), 127.4 (C-7), 132.7 (C-9), 142.2 (C-11a), 143.5 (C-10a), 163.6 (C-5), 165.0 (C-3a), 171.3 ($COCH_3$), 177.6 (C-6); DCI-MS m/z 398 [MH^+]; IR (KBr) ν cm^{-1} 3335, 2932, 2854, 1735, 1627, 1600, 1499, 1398, 1250, 1091, 1025, 757; UV λ nm (MeOH) 213, 225, 250, 263, 269, 295, 323, 385. Anal. ($C_{22}H_{23}NO_6$) C, H, N.

(±)-(2*R,1′*R**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (14).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 3H, CH_3 3′), 2.14 (s, 3H, $COCH_3$), 2.36 (br s, 1H, OH), 3.58 (dd, J = 16, 9 Hz, 1H, H-1a), 3.71 (dd, J = 16, 9 Hz, 1H, H-1b), 3.90 (s, 3H, O-CH_3), 3.93, (s, 3H, N-CH_3), 4.16 (d, J = 11.5 Hz, 1H, H-2′a), 4.35 (d, J = 11.5 Hz, 1H, H-2′b), 4.81 (t, J = 9 Hz, 1H, H-2), 6.26 (s, 1H, H-4), 7.22 (td, J = 8, 1.5 Hz, 1H, H-8), 7.28 (dd, J = 8, 1.5 Hz, 1H, H-10), 7.60 (td, J = 8, 1.5 Hz, 1H, H-9), 8.37 (d, J = 8, 1.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4 (CH_3-3′). 21.0 ($COCH_3$), 32.7 (C-1), 39.4 (N-CH_3), 56.3 (O-CH_3), 68.5 (C-2′), 72.6 (C-1′), 86.3 (C-2), 88.9 (C-4), 102.5 (C-11b), 110.0 (C-5a), 114.5 (C-10), 121.6 (C-8), 125.2 (C-6a), 127.4 (C-7), 132.7 (C-9), 143.4 (C-10a), 145.2 (C-11a), 163.5 (C-5), 164.9 (C-3a), 171.1 ($COCH_3$), 177.6 (C-6); DCI-MS m/z 398 [MH^+]; IR KBr ν cm^{-1} 3330, 2928, 2845, 1720, 1631, 1588, 1506, 1398, 1250, 1196, 753; UV λ nm (MeOH) 213, 225, 250, 263, 269, 296, 323, 385. Anal. ($C_{22}H_{23}NO_6$) C, H, N.

(±)-(2*R,1′*S**)-2-(1,2-Dihydroxy-1-methylethyl)-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (11).** Potassium carbonate (300 mg) was added to a solution of **13** (80 mg, 0.2 mmol) in CH_2Cl_2/MeOH (4:1, 30 mL). The mixture was stirred for 1 h, filtered, and evaporated under reduced pressure. The residue was partitioned between CH_2Cl_2 (30 mL) and 0.05 N aqueous HCl (30 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give **11** (75 mg, 98%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$/CH_3OD, 1:1) δ 0.98 (s, 3H, CH_3 −3′), 3.35 (d, J = 12 Hz, 1H, H-2′a), 3.40−3.60 (m, 3H, CH_2-1 + H-2′b), 3.73 (s, 3H, O-CH_3), 3.78 (s, 3H, N-CH_3), 4.72 (t, J = 8.5 Hz, 1H, H-2), 6.14 (s, 1H, H-4), 7.05 (td, J = 8, 1 Hz, 1H, H-8), 7.22 (dd, J = 8, 1 Hz, 1H, H-10), 7.47 (td, J = 8, 1 Hz, 1H, H-9), 8.12 (dd, J = 8, 1 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$/CH_3OD, 1:1) δ 17.8 (CH_3-3′), 32.2 (C-1), 38.8 (N-CH_3), 55.4 (O-CH_3), 66.6 (C-2′), 72.9 (C-1′), 84.7 (C-2), 88.6 (C-4), 102.7 (C-11b), 109.0 (C-5a), 114.4 (C-10), 121.2 (C-8), 124.2 (C-6a), 126.4 (C-7), 132.7 (C-9), 143.1 (C-10a), 144.8 (C-11a),

162.9 (C-5), 165.5 (C-3a), 178.0 (C-6); DCI-MS *m/z* 356 [MH^+]; IR (KBr) ν cm^{-1} 3405, 3275, 2916, 1620, 1592, 1557, 1510, 1196, 1095, 749; UV λ nm (MeOH) 213, 225, 248, 262, 270, 295, 325, 386. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

(±)-(2*R,1′*R**)-2-(1,2-Dihydroxy-1-methylethyl)-5-methoxy-11-methyl-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (12).** The procedure described for the preparation of **11** from **13** applied to **14** (65 mg, 0.163 mmol) gave **12** (55 mg 95%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 3H, CH_3-3′), 2.32 (s, br, 1H, OH), 2.67 (s, br, 1H, OH), 3.55−3.70 (m, 2H, H-1a + H-2′a), 3.75 (dd, J = 15, 8.5 Hz, 1H, H-1b), 3.88 (m, 1H, H-2′b), 3.91 (s, 3H, O-CH_3), 3.94 (s, 3H, N-CH_3), 4.85 (t, J = 8.5 Hz, 1H, H-2), 6.28 (s, 1H, H-4), 7.23 (td, J = 8, 1 Hz, 1H, H-8), 7.29 (dd, J = 8, 1 Hz, 1H, H-10), 7.60 (td, J = 8, 1 Hz, 1H, H-9), 8.38 (dd, J = 8, 1 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1 (C-3′). 33.1 (C-1), 39.3 (N-CH_3), 56.0 (O-CH_3), 68.2 (C-2′), 73.5 (C-1′), 88.1 (C-2), 88.7 (C-4), 102.5 (C-11b), 109.6 (C-5a), 114.5 (C-10), 121.6 (C-8), 124.8 (C-6a), 127.2 (C-7), 132.8 (C-9), 143.3 (C-10a), 144.9 (C-11a), 163.2 (C-5), 165.1 (C-3a), 177.5 (C-6); DCI-MS *m/z* 356 [MH^+]; IR (KBr) ν cm^{-1} 3365, 2920, 2845, 1619, 1588, 1499, 1401, 1196, 1087, 1017, 757; UV λ nm (MeOH) 213, 226, 250, 263, 269, 296, 323, 385. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

(±)-(2*R,1′*S**)-5-Methoxy-11-methyl-2-(2-methyloxiran-2-yl)-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (15).** Mesyl chloride (0.013 mL, 0.16 mmol) was added to an ice-cooled solution of diol **11** (60 mg, 0.16 mmol) in dry pyridine (5 mL). The reaction mixture was stirred for 1 h and evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 (2 × 50 mL) and washed with water (50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give the crude mesylate, which was solubilized in anhydrous acetone (10 mL), in presence of potassium carbonate (300 mg) and 18-crown-6 (20 mg). The mixture was stirred for 24 h at 20 °C, filtered, and evaporated. Flash chromatography (solvent CH_2Cl_2, then CH_2Cl_2/MeOH 99:1) gave epoxide **15** (34 mg, 62.5%) as white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 3H, CH_3-3′), 2.77 (d, J = 4.5 Hz, 1H, H-2′a), 2.87 (d, J = 4.5 Hz, 1H, H-2′b), 3.55 (dd, J = 14.5, 8 Hz, 1H, H-1a), 3.70 (dd, J = 14.5, 9 Hz, 1H, H-1b), 3.89 (s, 3H, N-CH_3), 3.96 (s, 3H, O-CH_3), 4.71 (dd, J = 9, 8 Hz, 1H, H-2), 6.32 (s, 1H, H-4), 7.23 (td, J = 8, 1.5 Hz, 1H, H-8), 7.30 (dd, J = 8, 1.5 Hz, 1H, H-10), 7.61 (td, J = 8, 1.5 Hz, 1H, H-9), 8.38 (dd, J = 8, 1.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6 (CH_3-3). 34.0 (C-1), 39.4 (N-CH_3), 52.8 (C-2′), 56.4 (O-CH_3), 56.8 (C-1′), 85.0 (C-2), 88.9 (C-4), 101.9 (C-11b), 110.0 (C-5a), 114.5 (C-10), 121.6 (C-8), 125.3 (C-6a), 127.4 (C-7), 132.7 (C-9), 143.4 (C-10a), 145.2 (C-11a), 163.7 (C-5), 164.9 (C-3a), 177.6 (C-6); DCI-MS *m/z* 338[$MH]^+$, 360 [$MNa]^+$; IR (KBr) ν cm^{-1}: 2963, 2933, 2846, 1631, 1592, 1495, 1405, 1192, 1091, 1025, 757; UV λ nm (MeOH) 212, 225, 250, 262, 269, 295, 322, 384. Anal. ($C_{20}H_{19}NO_4$) C, H, N.

(±)-(2*R,1′*R**)-5-Methoxy-11-methyl-2-(2-methyloxiran-2-yl)-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (16).** The procedure described for the preparation of **15** from **11** applied to **12** (50 mg, 0.14 mmol) gave **16** (10 mg, 21%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 3H, CH_3-3′), 2.73 (d, J = 4.5 Hz, 1H, H-2′a), 2.99 (d, J = 4.5 Hz, 1H, H-2′b), 3.53 (dd, J = 14.5, 8 Hz, 1H, H-1a), 3.75 (dd, J = 14.5, 9 Hz, 1H, H-1b), 3.88 (s, 3H, N-CH_3), 3.96 (s, 3H, O-CH_3), 4.77 (dd, J = 9, 8 Hz, 1H, H-2), 6.31 (s, 1H, H-4), 7.22 (td, J = 8, 1.5 Hz, 1H, H-8), 7.29 (dd, J = 8, 1.5 Hz, 1H, H-10), 7.61 (td, J = 8, 1.5 Hz, 1H, H-9), 8.38 (dd, J = 8, 1.5 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.2 (CH_3-3′), 34.5 (C-1), 39.4 (N-CH_3), 51.1 (C-2′), 56.4 (O-CH_3), 57.7 (C-1′), 84.8 (C-2), 88.9 (C-4), 101.6 (C-11b), 110.0 (C-5a), 114.5 (C-10), 121.6 (C-8), 125.3 (C-6a), 127.4 (C-7), 132.7 (C-9), 143.4 (C-10a), 145.2 (C-11a), 163.7 (C-5), 165.2 (C-3a), 177.7 (C-6); DCI-MS *m/z* 338[$MH]^+$, 360 [$MNa]^+$; IR (KBr) ν cm^{-1} 2966, 2920, 2854, 1631, 1584, 1495, 1401, 1200, 1087, 1025, 757; UV λ nm (MeOH) 212, 225, 250, 263, 269, 295, 322, 380. Anal. ($C_{20}H_{19}NO_4$) C, H, N.

2-Isopropenyl-5-hydroxy-1,2-dihydro-13*H*-benzo[b]furo[3,2-*h*]-acridin-9-one (10). A solution of 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**9**)[7a] (2 g, 7.2 mmol) in 10 mL of dry DMF was added to a suspension of NaH (432 mg of 80% oil dispersion, 14.4 mmol) in dry DMF (15 mL) at 0 °C under argon. After 15 min of stirring, (*E*)-1,4-dibromo-2-methylbut-2-ene[20] (2.45 g, 10.8 mmol) was added dropwise at 0 °C. The mixture was stirred for 20 h at 20 °C, diluted with ice−water (50 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chomatography (solvent cyclohexane/EtOAc 9:1 to 8:2) gave compound **10** (460 mg, 20%) as an orange amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (s, 3H, CH_3-3′), 3.07 (dd, J = 14.5, 7.5 Hz, 1H, H-1a), 3.48 (dd, J = 14.5, 9.5 Hz, 1H, H-1b), 4.96 (d, J = 1 Hz, 1H, H-2′a), 5.11 (d, J = 1 Hz, 1H, H-2′b), 5.47 (dd, J = 9.5, 7.5 Hz, 1H, H-2), 6.12 (s, 1H, H-4), 7.44 (td, J = 8, 1.5 Hz, 1H, H-9), 7.60 (td, J = 8, 1.5 Hz, 1H, H-10), 7.98 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.02 (s, 1H, H-12), 8.14 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.88 (s, 1H, H-7), 11.13 (s, 1H, NH), 14.47 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.9 (CH_3) 32.5 (C-1), 88.0 (C-2), 90.7 (C-4), 100.2 (C-5a), 104.0 (C-13b), 112.9 (C-12), 113.2 (C-2′), 120.9 (C-6a), 125.5 (C-9), 127.6 (C-11 + C-7), 127.0 (C-7a), 129.8 (C-10), 130.6 (C-8), 136.9 (C-11a), 138.6 (C-12a), 140.3 (C-13a), 144.5 (C-1′), 166.1 (C-5 + C-3a), 182.3 (C-6); DCI-MS *m/z* 344 [$MH]^+$; IR (KBr) ν cm^{-1} 3324, 2925, 2846, 1648, 1595, 1508, 1449, 1347, 1264, 1159, 1109; UV λ nm (MeOH) 228, 265, 282, 327, 355. Anal. ($C_{22}H_{17}NO_3$) C, H, N.

2-Isopropenyl-5-methoxy-1,2-dihydro-13-methyl-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (6). Methylation of compound **10** (350 mg, 1 mmol), under conditions essentially similar to those described for the preparation of **8** from **5** afforded **6** (360 mg, 97%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H, CH_3-3′), 3.48 (dd, J = 14.5, 8 Hz, 1H, H-1a), 3.83 (dd, J = 14.5, 9.5 Hz, 1H, H-1b), 3.96 (s, 3H, N-CH_3), 4.00 (s, 3H, O-CH_3), 4.99 (d, J = 1 Hz, 1H, H-2′b), 5.15 (d, J = 1 Hz, 1H, H-2′a), 5.3 (dd, J = 9.5, 8 Hz, 1H, H-2), 6.33 (s, 1H, H-4), 7.40 (td, J = 8, 1.5 Hz, 1H, H-9), 7.51 (td, J = 8, 1.5 Hz, 1H, H-10), 7.59 (s, 1H, H-12), 7.85 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.01 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.93 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.2 (CH_3-3′), 37.3 (C-1), 39.7 (N-CH_3), 56.3 (O-CH_3), 86.4 (C-2), 88.5 (C-4), 103.0 (C-5a), 104.5 (C-13b), 110.0 (C-12), 112.9 (C-2′), 124.2 (C-9), 126.0 (C-6a), 126.6 (C-11), 128.2 (C-7a), 128.2 (C-10 + C-7), 129.6 (C-8), 134.9 (C-11a), 135.7 (C-12a), 141.0 (C-13a), 143.5 (C-1′), 165.0 (C-5 + C-3a), 182.5 (C-6); DCI-MS *m/z* 372 [$MH]^+$; IR (KBr) ν cm^{-1} 2925, 2850, 1641, 1617, 1586, 1497, 1459, 1397, 1085; UV λ nm (MeOH) 231, 268, 279, 287, 322, 346. Anal. ($C_{24}H_{21}NO_3$) C, H, N.

(±)-(2*R,1′*S**)- and (2*R**,1′*R**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (19 and 20).** Catalytic osmic oxidation of compound **6** (250 mg, 0.67mmol), under conditions similar to those described for **5**, using osmium tetroxide solution (2.5% in t-BuOH, 0.07 mmol 770 μL) and *N*-methylmorpholine *N*-oxide (190 mg) in a mixture of *t*-BuOH/THF/H_2O (10:3:1, 25 mL), gave, after the usual workup, a diastereomeric mixture of diols **19** and **20** (240 mg, 88%). Acetylation with acetic anhydride (1.2 mmol, 0.11 mL) in dry pyridine (20 mL) followed by flash chromatography (solvent CH_2Cl_2/MeOH 98:2) afforded a diastereomeric mixture of monoacetates (250 mg, 93%). Preparative thin layer chromatography performed on 50 mg of mixture (solvent CH_2Cl_2/MeOH 98:2) gave **19** (20 mg, 40%) and **20** (20 mg, 40%) as amorphous yellow solids.

(±)-(2*R,1′*S**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (19).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 3H, CH_3-3′), 2.15 (s, 3H, $COCH_3$), 2.40 (br s, 1H, OH-1′), 3.65 (m, 2H, H-1a + H-1b), 3.98 (s, 6H, N-CH_3 + O-CH_3), 4.24 (s, 2H, H-2′a + H-2′b), 4.87 (t, J = 9.5 Hz, 1H, H-2), 6.29 (s, 1H, H-4), 7.30 (td, J = 8.0, 1.5 Hz, 1H, H-9), 7.53 (td, J = 8.0, 1.5 Hz, 1H, H-10), 7.59 (s, 1H, H-12), 7.85 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.01 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (C-3′), 26.9 ($CO\underline{C}H_3$), 32.5 (C-2′), 39.7 (N-CH_3), 56.4 (O-

CH_3), 68.4 (C-1), 72.6 (C-1′), 86.3 (C-2), 88.5 (C-4), 102.5 (C-5a + C-13b), 110.1 (C-12), 124.3 (C-9), 125.4 (C-11), 125.5 (C-6a), 126.7 (C-10 + C-7), 128.2 (C-7a), 129.6 (C-8), 135.8 (C-11a), 141.0 (C-12a), 147.9 (C-13a), 163.75 (C-3a), 165.4 (C-5), 178.4 (C$\underline{O}CH_3$), 182.1 (C-6); DCI-MS *m/z* 448 $[MH]^+$, 470 $[MNa]^+$; IR (KBr) ν cm^{-1} 3320, 2930, 1730, 1642, 1615, 1585, 1498, 1240, 1085; UV λ nm (MeOH) 232, 268, 280, 286, 323, 346. Anal. ($C_{26}H_{25}NO_6$) C, H, N.

(±)-(2*R,1′*R**)-2-(2-Acetoxy-1-hydroxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (20).** ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 3H, CH_3-3′), 2.15 (s, 3H, CO-CH_3), 2.37 (br s, 1H, OH-1′), 3.61 (dd, J = 14, 9 Hz, 1H, H-1a), 3.75 (dd, J = 14, 9 Hz, 1H, H-1b), 3.96 (s, 3H, N-CH_3), 3.97 (s, 3H, O-CH_3), 4.18 (d, J = 11 Hz, 1H, H-2′b), 4.38 (d, J = 11 Hz, 1H, H-2′a), 4.83 (t, J = 9 Hz 1H, H-2), 6.27 (s, 1H, H-4), 7.41 (td, J = 8.0, 1.5 Hz, 1H, H-9), 7.54 (td, J = 8.0, 1.5 Hz, 1H, H-10), 7.58 (s, 1H, H-12), 7.85 (dd, J = 8.0, 1.5 Hz, 1H, H-11), 8.02 (dd, J = 8.0, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3 (C-3′), 20.9 (CO$\underline{C}H_3$), 32.5 (C-1), 39.7 (N-CH_3), 56.35 (O-CH_3), 68.4 (C-2′), 72.6 (C-1′), 86.3 (C-2), 88.5 (C-4), 102.5 (C-5a + C-13b), 110.1 (C-12), 124.35 (C-9), 125.5 (C-6a), 126.7 (C-11), 128.2 (C-10 + C-7), 128.25 (C-7a), 129.6 (C-8), 135.7 (C-11a), 143.3 (C-12a), 145.8 (C-13a), 163.8 (C-3a), 165.4 (C-5), 178.5 (C$\underline{O}CH_3$), 182.1 (C-6); DCI-MS *m/z* 448 $[MH]^+$, 470 $[MNa]^+$; IR (KBr) ν cm^{-1} 3320, 2930, 1730, 1642, 1615, 1585, 1498, 1399, 1240, 1085; UV λ nm (MeOH) 232.5, 266.5, 279.5, 286, 323, 345.5. Anal. ($C_{26}H_{25}NO_6$) C, H, N.

(±)-(2*R,1′*S**)-2-(1,2-Dihydroxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (17).** The procedure described for the preparation of **11** from **13** applied to monoacetate **19** (89 mg, 0.20 mmol) gave **17** (79 mg 97%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$/MeOD 1:1) δ 1.03 (s, 3H, CH_3-3′), 3.38 (d, J = 11.0 Hz, 1H, H-2′a), 3.53 (d, J = 11.0 Hz, 1H, H-2′b), 3.56 (m, 2H, H-1a + H-1b), 3.78 (s, 3H, N-CH_3), 3.86 (s, 3H, O-CH_3), 4.77 (t, J = 9 Hz, 1H, H-2), 6.14 (s, 1H, H-4), 7.26 (td, J = 8, 1.5 Hz, 1H, H-9), 7.38 (td, J = 8, 1.5 Hz, 1H, H-10), 7.52 (s, 1H, H-12), 7.72 (dd, J = 8, 1.5 Hz, 1H, H-11), 7.85 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.70 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD 1:1) δ 20.0 (CH_3-3′), 32.5 (C-1), 39.0 (N-CH_3), 56.0 (O-CH_3), 67.5 (C-2′), 73.5 (C-1′), 86.5 (C-2), 89.8 (C-4), 104.1 (C-5a), 108.5 (C-13b), 111.5 (C-12), 124.7 (C-9), 125.5 (C-6a), 127.0 (C-11), 127.4 (C-7), 128.0 (C-7a), 128.5 (C-10), 129.5 (C-8), 136.0 (C-11a), 141.5 (C-12a), 145.5 (C-13a), 163.5 (C-3a), 166.5 (C-5), 177 (C-6); DCI-MS *m/z* 406 $[MH^+]$, 428 $[MNa^+]$; IR KBr ν cm^{-1} 3412, 2935, 1638, 1612, 1586, 1500, 1398, 1198, 1085, 1025; UV λ nm (MeOH): 230, 280, 324, 349.5. Anal. ($C_{24}H_{23}NO_5$) C, H, N.

(±)-(2*R,1′*R**)-2-(1,2-Dihydroxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (18).** The procedure described for the preparation of **11** from **13** applied to **20** (89 mg, 0.20 mmol) gave **18** (76 mg 94%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$/MeOD 1:1) δ 1.24 (s, 3H, CH_3-3′), 3.65 (m, 2H, H-1a + H-2′a), 3.77 (dd, J = 14, 9 Hz, 1H, H-1b), 3.88 (d, J = 11.0 Hz, 1H, H-2′b), 3.95 (s, 6H, N-CH_3 + O-CH_3), 4.85 (t, J = 9 Hz, 1H, H-2), 6.23 (s, 1H, H-4), 7.37 (td, J = 8, 1.5 Hz, 1H, H-9), 7.51 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (s, 1H, H-12), 7.83 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.05 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.90 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD 1:1) δ 20.1 (CH_3-3′), 32.9 (C-1), 39.6 (N-CH_3), 56.4 (O-CH_3), 68.4 (C-2′), 73.0 (C-1′), 88.2 (C-2), 88.4 (C-4), 102.7 (C-5a + C-13b), 110.1 (C-12), 124.3 (C-9), 125.4 (C-6a), 126.7 (C-11), 128.1 (C-10 + C-7), 128.2 (C-7a), 129.5 (C-8), 135.8 (C-11a), 141.0 (C-12a), 147.9 (C-13a), 163.7 (C-3a), 165.3 (C-5), 182.1 (C-6); DCI-MS *m/z* 406 $[MH^+]$, 428 $[MNa^+]$; IR KBr ν cm^{-1} 3394, 2928, 1637, 1612, 1586, 1498, 1399, 1085, 1024; UV λ nm (MeOH) 229.5, 280, 324, 348. Anal. ($C_{24}H_{23}NO_5$) C, H, N.

(±)-(2*R,1′*S**)-5-Methoxy-13-methyl-2-(2-methyloxiran-2-yl)-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]-acridin-6-one (21).** The same procedure as that described for the synthesis of **15** from **11**, applied to **17** (50 mg, 0.12 mmol), gave **21** (23 mg, 48%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 3H, CH_3-3′), 2.79 (d, J = 4.5 Hz, 1H, H-2′a), 2.88 (d, J = 4.5 Hz, 1H, H-2′b), 3.58 (dd, J = 14.5, 8 Hz, 1H, H-1a), 3.73 (dd, J = 14.5, 9.5 Hz, 1H, H-1b), 3.98 (s, 3H, N-CH_3), 3.99 (s, 3H, O-CH_3), 4.74 (dd, J = 9.5, 8 Hz, 1H, H-2), 6.29 (s, 1H, H-4), 7.41 (td, J = 8, 1.5 Hz, 1H, H-9), 7.54 (td, J = 8, 1.5 Hz, 1H, H-10), 7.60 (s, 1H, H-12), 7.86 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 (CH_3-3′), 33.0 (C-1), 39.0 (N-CH_3), 52.0 (C-2′), 56.4 (O-CH_3), 56.7 (C-1′), 84.0 (C-2), 87.5 (C-4), 102.0 (C-5a + C-13b), 110.1 (C-12), 124.3 (C-9), 125.4 (C-6a), 126.6 (C-11), 128.2 (C-7 + C-10), 128.4 (C-7a), 129.5 (C-8), 135.8 (C-11a), 141.0 (C-12a), 147.9 (C-13a), 163.9 (C-5 + C-3a), 181.0 (C-6); DCI-MS *m/z* 388$[MH]^+$; IR (KBr) ν cm^{-1} 2924, 1644, 1617, 1585, 1498, 1400, 1197, 1084, 1025; UV λ nm (MeOH) 231.5, 269, 280, 285, 324, 346. Anal. ($C_{24}H_{21}NO_4$) C, H, N.

(±)-(2*R,1′*R**)-5-Methoxy-13-methyl-2-(2-methyloxiran-2-yl)-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]-acridin-6-one (22).** The same procedure as that described for the synthesis of **15** from **11**, applied to **18** (30 mg, 0.074 mmol), gave **22** (10 mg, 34%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H, CH_3-3′), 2.74 (d, J = 5.0 Hz, 1H, H-2′a), 3.00 (d, J = 5.0 Hz, 1H, H-2′b), 3.59 (dd, J = 14.5, 9.5 Hz, 1H, H-1a), 3.79 (dd, J = 14.5, 8 Hz, 1H, H-1b), 3.98 (s, 3H, N-CH_3), 3.99 (s, 3H, O-CH_3), 4.80 (dd, J = 9.5, 8 Hz, 1H, H-2), 6.31 (s, 1H, H-4), 7.41 (td, J = 8, 1.5 Hz, 1H, H-9), 7.54 (td, J = 8, 1.5 Hz, 1H, H-10), 7.60 (s, 1H, H-12), 7.86 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.01 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.2 (CH_3-3′), 34.3 (C-1), 39.8 (N-CH_3), 51.1 (C-2′), 56.4 (O-CH_3), 56.5 (C-1′), 85.0 (C-2), 88.5 (C-4), 101.8 (C-5a + C-13b), 110.1 (C-12), 124.4 (C-9), 125.6 (C-6a), 126.8 (C-11), 128.2 (C-7 + C-10), 128.5 (C-7a), 129.6 (C-8), 135.8 (C-11a), 141.1 (C-12a), 145.9 (C-13a), 164.0 (C-3a), 165.8 (C-5), 178.5 (C-6); DCI-MS *m/z* 388$[MH]^+$; IR KBr ν cm^{-1} 2927, 1646, 1617, 1585, 1497, 1400, 1198, 1084; UV λ nm (MeOH) 232, 269, 280, 285, 324, 345. Anal. ($C_{24}H_{21}NO_4$) C, H, N.

(±)-(2*R,1′*S**)-2-(1,2-Diacetoxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (23).** Acetic anhydride (5 mL) was added to an ice-cooled solution of diol **17** (40 mg, 0.098 mmol) in of dry pyridine (5 mL) containing 4-aminopyridine (10 mg). The reaction mixture was stirred for 4 h, and solvents were removed under reduced pressure. The residue was taken up in CH_2Cl_2 (100 mL) and the solution washed with water (50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent CH_2Cl_2/MeOH 99:1) afforded diacetate **23** (32 mg, 66%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 3H, C$\underline{H}_3$-3′), 2.08 (s, 3H, CO-C$\underline{H}_3$), 2.13 (s, 3H, CO-C$\underline{H}_3$), 3.78 (m, 2H, H-1a + H-1b), 3.99 (s, 6H, N-CH_3 + O-CH_3), 4.67 (d, J = 12.0 Hz, 1H, H-2′a), 4.73 (d, J = 12.0 Hz, 1H, H-2′b), 5.25 (t, J = 9.5 Hz, 1H, H-2), 6.29 (s, 1H, H-4), 7.39 (td, J = 8, 1.5 Hz, 1H, H-9), 7.54 (td, J = 8, 1.5 Hz, 1H, H-10), 7.60 (s, 1H, H-12), 7.86 (dd, J = 8, 1.5 Hz, 1H, H-11), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (CH_3-3′), 20.7 (CO$\underline{C}H_3$), 22.0 (CO$\underline{C}H_3$), 32.7 (C-1), 39.8 (N-CH_3), 56.4 (O-CH_3), 64.4 (C-2′), 82.58 (C-1′), 82.6 (C-2), 88.5 (C-4), 102.1 (C-5a + C-13b), 110.1 (C-12), 124.3 (C-9), 125.5 (C-6a), 126.7 (C-11), 128.2 (C-7 + C-10), 128.5 (C-7a), 129.6 (C-8), 135.7 (C-11a), 141.3 (C-12a), 145.2 (C-13a), 165.5 (C-3a + C-5), 171.0 (2 × C$\underline{O}CH_3$), 178.0 (C-6); DCI-MS *m/z* 490 $[MH]^+$, 512 $[MNa]^+$; IR (KBr) ν cm^{-1} 2925, 1742, 1648, 1618, 1585, 1496, 1400, 1240, 1085, 1025; UV λ nm (MeOH) 231, 269, 280, 324, 345. Anal. ($C_{28}H_{27}NO_7$) C, H, N.

(±)-(2*R,1′*R**)-2-(1,2-Diacetoxy-1-methylethyl)-5-methoxy-13-methyl-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one (24).** The procedure described for the preparation of **23** from **17** applied to **18** (40 mg, 0,098 mmol) gave **24** (28 mg, 58%) as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H, CH_3-3′), 2.00 (s, 3H, CO-CH_3), 2.06 (s, 3H, CO-CH_3), 3.68 (m, 2H, H-1a + H-1b), 3.98 (s, 3H, N-CH_3), 4.00 (s, 3H, O-CH_3), 4.57 (m, 2H, H-2′a + H-2′b), 5.24 (dd, J = 8.5, 9.5 Hz, 1H, H-2), 6.32 (s,

1H, H-4), 7.42 (td, $J = 8$, 1.5 Hz, 1H, H-9), 7.55 (td, $J = 8$, 1.5 Hz, 1H, H-10), 7.61 (s, 1H, H-12), 7.86 (dd, $J = 8$, 1.5 Hz, 1H, H-11), 8.02 (dd, $J = 8$, 1.5 Hz, 1H, H-8), 8.92 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.1 (CH_3-3′), 20.7 ($CO\underline{C}H_3$), 22.0 ($CO\underline{C}H_3$), 30.6 (C-1), 37.2 (N-CH_3), 56.4 (O-CH_3), 64.4 (C-2′), 82.6 (C-1′+C-2), 88.5 (C-4), 102.1 (C-5a + C-13b), 110.1 (C-12), 124.3 (C-9), 125.5 (C-6a), 126.7 (C-11), 128.2 (C-7 + C-10), 128.5 (C-7a), 129.6 (C-8), 135.7 (C-11a), 141.0 (C-12a), 145.2 (C-13a), 163.5 (C3-a + C-5), 170.0 (2 × $\underline{C}OCH_3$), 179.3 (C-6); DCI-MS m/z 490 $[MH]^+$, 512 $[MNa]^+$; IR KBr ν cm^{-1} 2924, 1742, 1647, 1618, 1585, 1498, 1400, 1240, 1085, 1025; UV λ nm (MeOH) 233, 268, 280, 324, 345 nm. Anal. ($C_{28}H_{27}NO_7$) C, H, N.

(±)-(2*R,1′*S**)-5-Methoxy-13-methyl-2-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]-acridin-6-one (25).** *N*,*N*′-Carbonyldiimidazole (60 mg, 0.37 mmol) was added to a solution of **17** (50 mg, 0.12 mmol) in 2-butanone (5 mL). The reaction mixture was stirred for 20 h at 20 °C. The solvent was removed by evaporation under reduced pressure. Flash chromatography (solvent CH_2Cl_2/MeOH 98:2) afforded **25** (34 mg, 64%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3-3′), 3.51 (dd, $J = 15$, 7.0 Hz, 1H, H-1a), 3.86 (dd, $J = 15$, 9.5 Hz, 1H, H-1b), 3.99 (s, 3H, N-CH_3), 4.00 (s, 3H, O-CH_3), 4.22 (d, $J = 9$ Hz, 1H, H-2′b), 4.56 (d, $J = 9$ Hz, 1H, H-2′a), 5.02 (dd, $J = 9.5$, 7 Hz, 1H, H-2), 6.31 (s, 1H, H-4), 7.42 (td, $J = 8$, 1.5 Hz, 1H, H-9), 7.55 (td, $J = 8$, 1.5 Hz, 1H, H-10), 7.61 (s, 1H, H-12), 7.87 (dd, $J = 8$, 1.5 Hz, 1H, H-11), 8.02 (dd, $J = 8$, 1.5 Hz, 1H, H-8), 8.91 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.1 (CH_3-3′), 32.5 (C-1), 39.0 (N-CH_3), 56.7 (O-CH_3), 70.3 (C-2′), 84.6 (C-1′), 85.8 (C-2), 89.1 (C-4), 103.1 (C-5a), 108.6 (C-13b), 111.5 (C-12), 124.9 (C-9), 125.4 (C-6a), 127.1 (C-11), 128.2 (C-7), 128.7 (C-10), 128.9 (C-7a), 129.6 (C-8), 135.9 (C-11a), 141.0 (C-12a), 145.6 (C-13a), 154.6 (CO carbonate), 163.6 (C-3a), 165.3 (C-5), 177.0 (C-6); DCI-MS m/z 432 $[MH]^+$, 454 $[MNa]^+$; IR (KBr) ν cm^{-1} 2927, 1801, 1642, 1617, 1586, 1498, 1400, 1198, 1085; UV λ nm (MeOH): 232, 269, 280, 286, 324, 346. Anal. ($C_{25}H_{21}NO_6$) C, H, N.

(±)-(2*R,1′*R**)-5-Methoxy-13-methyl-2-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-1,2-dihydro-13*H*-benzo[*b*]furo[3,2-*h*]-acridin-6-one (26).** The procedure described for the preparation of **25** from **17** applied to **18** (50 mg, 0.12 mmol) gave **26** (23 mg, 43%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 3H, CH_3-3′), 3.64 (dd, $J = 15$, 8.5 Hz, 1H, H-1a), 3.82 (s, 3H, O-CH_3), 3.90 (dd, $J = 15$, 9.5 Hz, 1H, H-1b), 3.96 (s, 3H, N-CH_3), 4.36 (d, $J = 9.0$ Hz, 1H, H-2′a), 4.64 (d, $J = 9.0$ Hz, 1H, H-2′b), 5.06 (dd, $J = 9.5$, 8.5 Hz, 1H, H-2), 6.44 (s, 1H, H-4), 7.42 (td, $J = 8$, 1.5 Hz, 1H, H-9), 7.58 (td, $J = 8$, 1.5 Hz, 1H, H-10), 7.92 (s, 1H, H-12), 8.00 (dd, $J = 8$, 1.5 Hz, 1H, H-11), 8.08 (dd, $J = 8$, 1.5 Hz, 1H, H-8), 8.66 (s, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.1 (CH_3-3′), 32.5 (C-1), 39.0 (N-CH_3), 56.6 (O-CH_3), 72.5 (C-2′), 83.0 (C-1′), 85.0 (C-2), 89.0 (C-4), 103.9 (C-5a), 108.3 (C-13b), 111.4 (C-12), 125.4 (C-9), 126.9 (C-6a), 127.4 (C-11), 128.1 (C-7), 128.7 (C-10 + C-7a), 129.5 (C-8), 136.4 (C-11a), 141.5 (C-12a), 145.5 (C-13a), 154.5 (CO carbonate), 163.2 (C-3a), 165.0 (C-5), 176.5 (C-6); DCI-MS m/z 432 $[MH]^+$; IR (KBr) ν cm^{-1} 2916, 1782, 1649, 1618, 1588, 1498, 1401, 1196, 1086; UV λ nm (MeOH) 231, 268, 279, 286, 322, 343. Anal. ($C_{25}H_{21}NO_6$) C, H, N.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **5**−**26**; elemental analysis data of compounds **5**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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