Synthesis and Evaluation of New 6-Amino-Substituted Benzo[c]phenanthridine Derivatives

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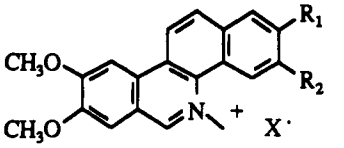
Different 7,8,9,10-tetrahydrobenzo[c]phenanthridin-6(5*H*)-ones (**10a–e**) were prepared by using a one-pot procedure which includes the preparation of various 6- and 7-alkoxy-1-naphthylisocyanates from 1-naphthylamines and triphosgene, followed by addition of 1-*N*-morpholino-1-cyclohexenes, and cyclization of the resulting amides upon heating in the presence of hydrogen chloride. Subsequent aromatization, chlorination, and substitution with (dimethylamino)alkylamines, followed by a demethylation or a selective desisopropylation, allowed us to synthesize the derivatives **6a–i** and **7a–h** bearing a [(dimethylamino)alkyl]amino side chain at their 6-position. These compounds, as the other analogs **5a–b**, were devised to further study the structure–activity relationships in the benzo[c]phenanthridine family of antitumor alkaloids led by fagaronine (**1a**) and nitidine (**1b**). Topoisomerases I and II cleavable complex assay and evaluation of the cytotoxicity and antitumor properties were performed. In vitro cytotoxicity (L1210 and Calc 18) shows a relationship between the cytotoxicity of these compounds and their topoisomerase poisoning properties. However, all these compounds were devoid of significant antitumor effect on the P388 murine leukemia system.

Many alkaloids of the benzo[c]phenanthridine family display antitumor properties.[1] The most active so far reported[2] are the benzo[c]phenantridinium salts fagaronine (**1a**) and nitidine (**1b**). The latter has been the subject of a NCI investigation in 1976, but a narrow antitumor spectrum,[3, 4] a certain toxicity,[5, 6] and an instability[7-9] were then reported. Fagaronine was also shown to inhibit many reverse transcriptases,[10] and more recently fagaronine as well as nitidine were shown to inhibit HIV-1 and -2 reverse transcriptases.[11] Moreover since fagaronine was proven to poison topoisomerase I,[12] this could provide a mechanism of action for the antileukemia activity of this class of compound. Further synthetic benzo[c]phenantridinium[13, 14] and analogs derived from this ring system have been prepared.[6,8,15-23] Despite an apparent limited success in the field of biological applications, recent patents, one disclosing the use of benzo[c]phenanthridine alkaloids in association with adriamycine for the treatment of multiple drug-resistant tumors,[24] another claiming the antitumor properties of the new 5-ethylated analogs of the benzo[c]phenantridinium derivatives,[25] may prove to be of interest in the future.

From the structure–activity relationships point of view, two main remarks can be drawn: (i) for all the compounds so far synthesized and studied, the iminium charge on the benzo[c]phenanthridine ring seem to be necessary for their biological action,[4,26,27] and (ii) the reactivity of the iminium toward nucleophilic attack[28] has been put forward to explain the antileukemia activity of these series.[29] However, such a transformation leads to unstable products,[7,28] which could be responsible for the acute toxicity reported for nitidine **1b**.[5] Indeed, the presence of this iminium could even account for the low antitumor activity.[8,9] Substitution pattern[9] and the nature of substituents on the benzo[c]phenanthridinium system appear to be critical for these series to display antitumor properties. For example, *O*-methylfagaronine **1c**, isofagaronine **1d**, and the 2,3:8,9-bis(methylenedioxy) derivative (avicine) are less active than their parent compounds **1a** and **1b**.[13,14]



1a R_1 = OH, R_2 = OCH_3 : Fagaronine

1b R_1, R_2 = OCH_2O : Nitidine

1c R_1 = OCH_3, R_2 = OCH_3

1d R_1 = OCH_3, R_2 = OH : isofagaronine

Moreover, for the analogs derived from various related ring systems which have been synthesized,[6,8,15,17-22] data published are generally insufficient to infer an eventual effect of substitution pattern on their possible antitumor activity.

In our laboratory, various derivatives of ellipticine have been synthesized[30] with the aim to increase antitumor properties. For example when compared to the antitumor drug elliptinium **2**, the analogs **3a-b** and the less related compound **4** have been found highly active in various experimental tumor models and are still undergoing clinical trials[31-33] as new anticancer agents.

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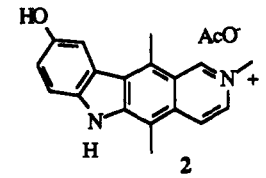

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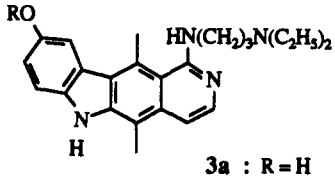
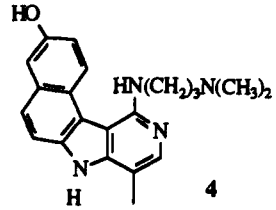


3a : R = H
3b : R = Me



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With respect to compound **2**, the main change in the structure of the ellipticine analogs **3a–b** is the replacement of the iminium chromophore by a [(dialkylamino)alkyl]-amino side chain placed next to the formerly quaternary nitrogen. Since this change has proven to increase the scope of antitumor properties in vivo in the above mentioned series,[34] we decided to try to perform the same change starting from the antitumor benzo[c]phenanthridine alkaloids as a model. Interestingly in the course of this previous work a correlation between antitumor properties and topoisomerases poisoning was proposed.[34]

After the synthesis of nitidine and *O*-methylfagaronine analogs **5a–b** bearing such [(dimethylamino)alkyl]amino side chain on their 6 position,[35] we undertook the preparation of hydroxylated analogs **6f–i** and **7f–h** in order to study the role of the phenolic function present in fagaronine's structure. In this paper, we report the synthesis of the benzo[c]phenanthridine derivatives **6a–i** and **7a–h**. The results of the biological studies of all the above-mentioned analogs are also given. In view of the results obtained with the ellipiticine analogs **3a–b** and compound **4** and since fagaronine is a poison of topoisomerase I,[12] a biochemical evaluation was also undertaken.

Chemistry

The key step is based on the preparation[36,37] of 7,8,9,-10-tetrahydrobenzo[c]phenanthridin-6(5*H*)-ones from 1-naphthylisocyanates and cyclohexanone enamines, which is probably one of the simplest ways to obtain the benzo-[c]phenanthridine ring system.[21,25,38–42] Thus, starting from the readily available hydrochloride salts of 1-naphthylamines[43] **8a–e**, we were able to prepare the corresponding isocyanates by the action of triphosgene in boiling *o*-dichlorobenzene. Direct treatment of the hot isocyanate solution with the enamines **9a–b** gave the intermediate amides, which, without isolation and upon further heating of the still hydrogen chloride-saturated medium, underwent a cyclization to give the hardly soluble compounds **10a–e** in a 20–50% yield (see Scheme I).

5a $R_1 + R_2 = OCH_2O$
5b $R_1, R_2 = OCH_3$

6a-i: n = 2 7a-h: n = 3

	a	b	c	d	e	f	g	h	i
R1	H	OMe	H	OMe	OiPr	OH	H	OH	OH
R2	H	H	OMe	H	H	H	OH	H	H
R3	H	OMe	H	H	OMe	H	H	OH	OMe

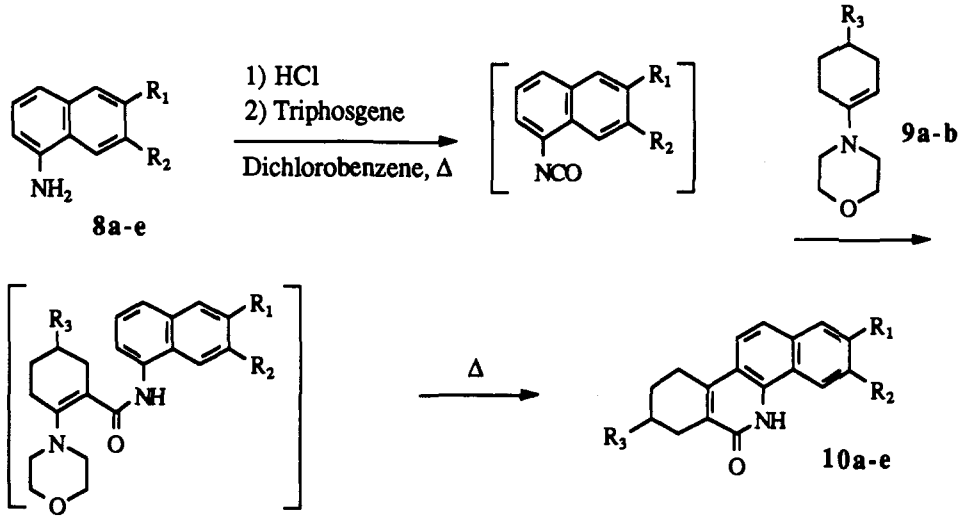
Note : this table is usable for all the relevant schemes

The organic layer left after the usual workup still contained some uncyclized amide. Upon treatment with cold sulfuric acid for 1 week, it yielded some more compounds **10a–d**, thus raising the yield up to 30–70%. A difference between these two cyclization methods arose when starting from 6-isopropoxy-1-aminonaphthalene (**8e**). The heating of the medium led to 42% of **10e**, whereas the sulfuric acid treatment of the remnant amide gave, instead of and as expected,[44] 22% of the deprotected 2-hydroxy-8-methoxy derivative **10i**. The synthesis of compounds **6a–i**, **7a–h**, was achieved in four straightforward steps (Scheme II), and selective cleavage of the isopropyl ether **6e** into **6i** was achieved with boiling hydrochloric acid.

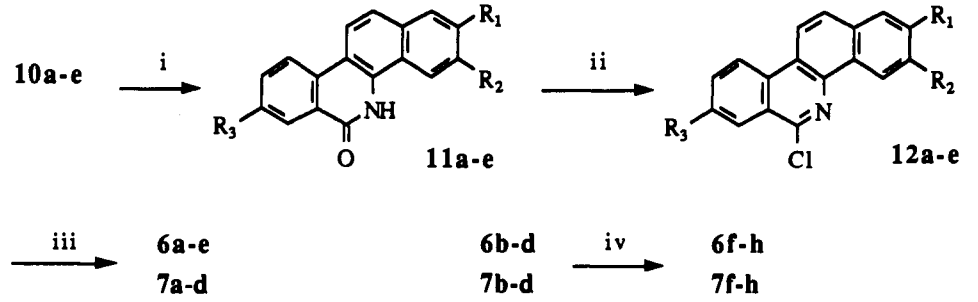
Biological Results

Cytotoxicity and Antitumor Properties. Compounds **5a–b**, **6a–i**, and **7a–h** were tested on cultured murine lymphoblastic leukemia cells (L1210) and human mammary adenocarcinoma cells (Calc 18), using the well

Scheme I



Scheme II[a]



[a](i) 10% Pd/C, diphenyl oxide, reflux; (ii) $POCl_3$, reflux; (iii) $Me_2N(CH_2)_2NH_2$, reflux; (iv) HBr, 47%, reflux.

Table I. Cytotoxicity toward L1210 and Calc 18

compd	IC50 (μM) L1210	IC50 (μM) Calc 18
6a	>90	nd
7a	5.2	nd
6b	1.7	1.5
7b	2.5	3.0
6c	2.5	nd
7c	2.6	nd
6d	3.0	3.0
7d	3.4	2.5
6f	0.5	1
7f	2.6	3.0
6g	0.5	3.0
7g	3.4	2.5
6h	0.5	1.0
7h	4.5	>10
6i	nd	1.5
5a	0.3	0.6
5b	>5	3.0

Table II. Cytotoxicity toward P388 and P388 CPTO.3

compd	P388 IC50 (μM)	P388 IC90 (μM)	P388 CPTO.3 IC50 (μM)	P388 CPTO.3 IC90 (μM)
1a	2.0	6	>10	>10
5a	0.9	3.5	0.15	3.5
camptothecin	0.015	0.07	0.8	6.5

described conditions. The cells were counted after 24 h of incubation (L1210) or after 96 h of incubation (Calc 18) in the presence of the given concentration of the drugs (see Table I). Compound **5a**, which is the most cytotoxic against L1210 and Calc 18 cells, was also tested on a murine leukemia P388 CPT0.3 cell line which has acquired a resistance to Camptothecin,[45] a known topoisomerase I poison. In contrast with fagaronine (**1a**) or camptothecin, compound **5a** was found cytotoxic on both sensitive (P388) and resistant (P388 CPT0.3) cell lines (see Table II). The most cytotoxic compounds were also studied in vivo on the P388 leukemia murine model, and all the results are reported in Table III.

Topoisomerases Inhibition. The ability of the compounds to induce the formation of topoisomerase I or II cleavable complex is shown in Table IV. The compounds can be classified into three groups : (i) compound **5a**, **6b,h**, **7h**, **6i** which are able to stimulate both topoisomerase I and II cleavable complex formation; (ii) compounds **6d,f,g** which are able to stimulate only topoisomerase II cleavable complex formation; and (iii) compounds **5b**, **7b,d,f,g** which are inactive in the topoisomerase I and II assays. The properties of **1a** (fagaronine) appear unique compared to the series since this compound is active against topoisomerase I in the cleavable complex assay but not against topoisomerase II.[12]

Each compound was examined with five concentrations at 0.1, 0.3, 1.0, 3.0, and 10 μM on the DNA cleavage reaction

Table III. Studies on the *in Vivo* P388 Leukaemia Tests

compd	dose (mg/kg)	T/C
6f	5	93
	10	138
	20	118
	40	101
6g	5	92
	10	83
	20	102
7g	5	91
	10	90
	20	73
6h	5	119
	10	111
	20	111
5a	5	99
	10	103
	20	92
1a[2]	100	265

Table IV. Inhibition of DNA Topoisomerase I and II Activities

compd	MIC (μM) topoisomerase I	MIC (μM) topoisomerase II
1a	0.1	>10
5a	0.3	1
5b	>10	>10
6b	1	10
7b	>10	>10
6d	>10	10
7d	>10	>10
6f	>10	1
7f	>10	>10
6g	>10	10
7g	>10	>10
6h	1	3
7h	3	10
6i	1	10
camptothecin	0.01	>100
etoposide	>100	0.1

using topoisomerase I or topoisomerase II except camptothecin and etoposide which had been examined at 0.001, 0.01, 0.1, 1, 10, and 100 μM. MIC corresponds to the minimal concentration (μM) of agent able to stimulate the DNA cleavage reaction.

Discussion and Conclusion

The use of the condensation of various naphthylisocyanates with cyclohexanone enamines allowed us to prepare some 7,8,9,10-tetrahydrobenzo[*c*]phenanthridin-6(5*H*)-ones. From these intermediates, 16 new benzo[*c*]phenanthridines derivatives bearing a [(dimethylamino)alkyl]-amino side chain on their 6-position were easily obtained.

The salified 6-[[(dimethylamino)alkyl]amino]benzo[c]-phenanthridines derivatives turned out to keep a water solubility as the benzo[c]phenanthridinium alkaloids. This chromophore alteration had been devised to avoid the formation of water-insoluble compounds arising from nucleophilic attack of the iminium function.[9,28] since it could probably be the cause of the loss of antitumor properties observed for aqueous solution of alkaloids on standing.[7,8]

Since fagaronine has recently been shown to stimulate the cleavable complex of topoisomerase I,[12] the same biological experiments were conducted on these new derivatives. The nitidine analog **5a** and compounds **6bh**, **7h**, and **6i** actually turned out to stimulate both topoisomerase I and II cleavable complexes formation, with compound **5a** displaying the highest activity. In addition compounds **6d,f,g** were found to stimulate only the formation of topoisomerase II cleavable complex (see Table IV).

Concerning topoisomerase I, a methoxy or hydroxy function on R_3 seems to be an important feature which enhances the activity. On the other hand, compound **5a** bearing a methylenedioxy group or compound **6f** bearing a phenolic function on R_1 display the highest topoisomerase II poisoning properties. Interestingly, another important parameter seems to be the size of the dibasic side chain which greatly influences the stimulation of the topoisomerase cleavable complex formation of the series. Thus, replacement of the [(dimethylamino)ethyl]amino side chain by a [(dimethylamino)propyl]amino considerably decreases (**7h**) or abolishes (compounds **5b**, **7b,d,g,f**) the topoisomerase I and II poisoning properties.

The cytotoxicities of these compounds might be related to the topoisomerase poisoning. Indeed, the most cytotoxic compounds (**5a**, **6h**) are presenting the highest score on the topoisomerase I and II assays. Furthermore, the inactive derivatives **7g**, **7f**, **7d**, **5b**, and **7b** are presenting a 3–9-fold decrease of cytotoxicities. Nitidine (**2a**) has previously been found to be 10-fold more cytotoxic than fagaronine (**1a**) on KB cells, presumably because of the presence of the methylenedioxy group.[6,26] Our results are in agreement with this trend (compare **5a** and **5b**). Additional data, especially topoisomerases poisoning of nitidine chloride (which was unavailable), would surely provide a better understanding of role of the methylenedioxy group. Another experiment showed that, contrary to Fagaronine,[12] compound **5a** kept its cytotoxicity toward the P388 CPT0.3 cell line which has acquired a resistance toward Camptothecin.[45] This cross-resistance pattern, along with the results of Table IV, emphasize the differences between compound **5a** and fagaronine regarding their mechanisms of cytotoxicity. A similar lack of cross resistance was found with the same cell line with agents which also stimulate formation of both topoisomerase I and II cleavable complexes (Intoplicine or Saintopine, J-F Riou, unpublished results). This phenomenom can be explained by the level of topoisomerase II poisoning which is important enough to obtain similar IC_{50} with the camptothecin-sensitive and -resistant cell lines.

Unfortunately, all the analogs prepared were devoid of significant antitumor activity against the *in vivo* P388 murine model. This points out that, for these compounds, either topoisomerase poisoning is insufficient to confer the *in vivo* activity and has to be improved or an additional event (another mechanism of action, problems in intracellular penetration, ...) has to be considered.

In conclusion, the synthetic approaches developed here to prepare new functionnalized benzo[c]phenanthridine derivatives associated with their biochemical and biological evaluation is providing interesting clues to further explore the antitumor potential of this class of compound.

Experimental Section

Biological Studies. Topoisomerase Inhibition. Topoisomerase I and II were prepared from calf thymus as already described.[46] Topoisomerase I or II inhibitions were evaluated using the DNA cleavage assay which was carried out according to the procedure described previously.[47] Each compound was evaluated for its minimal inhibitory concentration (MIC), corresponding to the lowest concentration (μM) able to produce a detectable stimulation of the DNA cleavage reaction.

Growth Inhibition Assay. Camptothecin was obtained from the Sigma Chemical Co. (St. Louis, MO). Stock solutions at 1 mg/mL in dimethyl sulfoxide were stored at −20 °C, and further dilutions in water were made just before use.

Calc 18 human mammary adenocarcinoma cells, P388 murine leukemia cells, and P388 CPT0.3 cells resistant to camptothecin[45] were incubated at 37 °C for 96 h in the presence of various concentrations of drug and evaluated for viability by neutral red staining according to published procedure.[48] The concentration of drugs giving 50% of growth inhibition (IC50) were determined.

L1210 (ATCC-CCL 219) cells were cultivated in Dulbecco's MEM supplemented with 10% fetal calf serum at 37 °C in water-jacketed CO_2 incubators (5% CO_2). Cells were seeded at 10^5 cells/mL in 1-mL microwell plates. After 24 h (usually (3–4) × 10^5 cells/mL), tested compounds were added in duplicate at various concentrations and incubated for 24 h. Cells were counted with a Coulter-Counter ZM (Coultronics Inc.). The dose inhibiting the growth by 50% (IC50) was extrapolated from regression curves obtained with experimental points without significant toxicity.

***In Vivo* Assay.** Female BDF_1 mice were inoculated IP with 10^6 P.388 leukemia cells (J_0) (10 controls and 6 animals in each test group), and test compounds were injected intraperitonealy as 0.2-mL solutions (PBS) at days 1, 3, and 7 after leukemia inoculation.

T/C are expressed as the ratio of the mean survival time of treated animals on the mean survival time of controls multiplied by 100.

Chemistry. ^{1}H NMR spectra were recorded on a Bruker AC-200 MHz spectrometer. Shifts are given according to the TMS signal. In each series, some attribution could only be done with the help of 2D NMR ^{1}H spectra. Mass spectra were performed by the Département de Chimie Organique, Spectrométrie de Masse (Université Paris-Sud, Batiment 410, Orsay, France), with a direct introduction of the salts and using the gazeous ammonia ionisation technique. Elemental analyses were performed by the service central de microanalyses (CNRS ICSN, Gif-sur-Yvette, France) and were within ±0.4% of theoretical values for the mentioned empirical formula. Dry solvents used were of a commercially available quality.

1-*N*-Morpholino-4-methoxy-1-cyclohexene (9b). In a Dean–Stark apparatus 4-methoxycyclohexanone[49] (10 g, 0.077 mol), morpholine (7.3 mL, 0.084 mol), and *p*-toluenesulfonic acid (0.7 g, 0.05 mol) were heated at refluxing temperature in toluene (300 mL) for 16 h. After removal of the solvent, the oil obtained was then distilled (bp(0.15 mm) = 153–160 °C), yielding 9.8 g of the enamine **9b** which was used in the next step without further purification and could also be stored at 4 °C for some months without decomposition.

Compounds 10a–e: General Procedure. Under a well-ventilated hood, 1-naphthylamine **8a–e** (0.06 mol) was dissolved in dry dichlorobenzene (200 mL). Hydrogen chloride was bubbled through the solution until complete precipitation of the naphthylamine hydrochloride. Then triphosgene (Aldrich, 5.59 g, 0.0202 mol, 1.01 equiv) was added. The mixture was quickly heated to boiling temperature under vigorous stirring. Heating was maintained until obtention of a clear solution of the isocyanate (10 min), and the solution was left to cool for another 10-min period. The required enamine (**9a–b**, 0.066 mol, 1.1 equiv) was then quickly added, the hot mixture was stirred for 10 min and boiling was resumed 15 min more. The solution was left to

Table V. Experimental Data for Tetrahydrobenzo[c]phenanthridinones 10, Benzo[c]phenanthridinones 11, and 6-Chlorobenzo[c]phenanthridines 12

compd	yield[d]	mp, °C	formula	anal.	^{1}H NMR[a]
10a	73	>260[b]	$C_{17}H_{15}NO$	CHN	1.82 and 1.79 (m, 4 H, CH_2-8, CH_2-9), 2.62 (m, 2 H, CH_2-10), 3.01 (m, 2 H, CH_2-7), 7.52–7.60 (m, 2 H, H-2, H-3), 7.65 (m, 2 H, H-11, H-12), 7.73 (m, 1 H, H-1), 8.13 (m, 1 H, H-4)
10b	54	>260	$C_{19}H_{19}NO_3$	CHN	2.19 (m, 2 H, CH_2-10), 2.99 (m, 2 H, CH_2-9), 3.27 (m, 2 H, CH_2-7), 3.56–3.81 (2s, 6 H, OCH_3), 4.05 (s, br, 1 H, H-8), 7.32 (m, 2 H, H-1, H-3), 7.75 (m, 2 H, H-11, H-12), 8.29 (d, 1 H, J = 9.0, H-4)
10c	31	>260	$C_{18}H_{17}NO_2$	CHN	1.87 (m, 4 H, CH_2-8, CH_2-9), 2.84 (m, 2 H, CH_2-10), 3.15 (m, 2 H, CH_2-7), 3.97 (s, 3 H, OCH_3), 7.32 (d, 1 H, J = 8.8, H-2), 7.66–7.99 (m, 4 H, H-1, H-4, H-11, H-12)
10d	58	>260	$C_{18}H_{17}NO_2$	CHNO	1.88 (m, 4 H, CH_2-8, CH_2-9), 2.69 (m, 2 H, CH_2-10), 3.12 (s, 2 H, CH_2-7), 3.91 (s, 3 H, OCH_3), 7.26 (m, 2 H, H-1, H-3), 7.74 (m, 2 H, H-11, H-12), 8.19 (d, 1 H, J = 9.7, H-4)
10e	42	>260	$C_{21}H_{23}NO_3$	CHNO	1.27 (d, 6 H, J = 6.2, CH_3-iPr), 2.12 (m, 2 H, CH_2-9), 2.90–3.25 (m, 4 H, CH_2-7, CH_2-10), 3.45 (s, 3 H, OCH_3), 4.00 (m, 1 H, H-8), 4.68 (sept, 1 H, J = 6.2, HiPr), 7.26–7.30 (m, 2 H, H-1, H-3), 7.67 (m, 2 H, H-11, H-12), 8.22 (d, J = 9.8, 1 H, H-4)
10i	22	>260	$C_{18}H_{19}NO_4 \cdot H_2O$	CHNO	1.82–2.00 (m, 2 H, CH_2-9), 2.39 (m, 1 H, H-7), 2.75–2.95 (m, 3 H, H-7, CH_2-10), 3.91 (s, 3 H, OCH_3), 3.64 (m, 1 H, H-8), 7.09 (dd, 1 H, J = 2.5, 9.0, H-3), 7.16 (d, 1 H, J = 2.5, H-1), 7.43 (d, 1 H, J = 8.9 H-12), 7.63 (d, 1 H, J = 8.9, H-11), 8.69 (d, 1 H, J = 9.0, H-4), 9.93 (s br, 1 H, NH), 11.81 (s br, 1 H, OH)
11a	77	>260[c]	$C_{17}H_{11}NO$	CHN	7.24–7.72 (m, 8H, Ar), 8.00 (d, 1 H, J = 8.3, H-1), 8.07 (d, 1 H, J = 8.1, H-10)
11b	89	>260	$C_{19}H_{15}NO_3$	CHN	3.80 et 3.82 (2s, 6 H, OCH_3), 6.93 (m, 2 H, H-1, H-3), 7.36 (m, 3 H, H-7, H-9, H-12), 7.61 (d, 1 H, J = 9.4, H-11), 7.75 (d, 1 H, J = 8.6, H-10), 7.95 (d, 1 H, J = 8.2, H-4)
11c	44	>260	$C_{18}H_{13}NO_2$	CHN	3.85 (s, 3 H, OCH_3), 6.93 (d, 1 H, J = 8.6, H-2), 7.48 (m, 4 H, H-1, H-12, H-8, H-9), 7.68 (m, 2 H, H-10, H-11), 8.06 (m, 2 H, H-4, H-7)
11d	84	>260	$C_{18}H_{13}NO_2$	CHNO	3.77 (s, 3 H, OCH_3), 6.88 (m, 2 H, H-1, H-3), 7.46 (m, 3 H, H-8, H-9, H-10), 7.74 (m, 2 H, H-11, H-12), 8.00, 8.04, (2s, 2 H, H-7, H-4)
11e	88	>260	$C_{21}H_{19}NO_3$	CHNO	1.27 (d, 6 H, J = 6.1, CH_3-iPr), 3.69 (2, 3 H, OCH_3), 4.58 (m, 1 H, HiPr), 6.90–7.76 (m, 8H, Ar)
12a	77	152	$C_{17}H_{10}NOCl$	CHNCl	7.54–7.78 (m, 3 H, H-2, H-3, H-9), 7.85–8.01 (m, 2 H, H-1, H-8), 7.98 (d, 1 H, J = 9.0, H-12), 8.43 (d, 1 H, J = 9.0, H-11), 8.51 (dd, 1 H, J = 1.1, 8.3, H-10), 8.61 (dd, 1 H, J = 0.5, 8.4, H-7), 9.27 (ddd, 1 H, J = 0.5, 1.5, 6.5, H-4)
12b	73	177	$C_{19}H_{14}NO_3Cl$	CHNCl	3.98–4.02 (2s, 6 H, OCH_3), 7.26 (m, 1 H, H-1), 7.38 (dd, 1 H, J = 2.5, 9.1, H-3), 7.51 (dd, 1 H, J = 2.6, 9.1, H-9), 7.77 (d, 1 H, J = 2.6, H-7), 7.89 (d, 1 H, J = 9.0, H-12), 8.36 (d, 1 H, J = 9.0, H-11), 8.51 (d, 1 H, J = 9.1, H-10), 9.14 (d, 1 H, J = 9.1, H-4)
12c	63	157	$C_{18}H_{12}NO_2Cl$	CHNCl	4.11 (s, 4 H, OCH_3), 7.33 (dd, 1 H, J = 2.6, 8.8, H-2), 7.78 (ddd, J = 1.1, 7.1, 8.2, H-8), 7.87 (d, 1 H, J = 9.0, H-12), 7.93 (ddd, 1 H, J = 1.2, 7.1, 8.3, H-9), 7.97 (d, 1 H, J = 8.8, H-1), 8.36 (d, 1 H, J = 9.0, H-11), 8.55 (dd, 1 H, J = 1.1, 8.3, H-10), 8.64 (d, 1 H, J = 2.5, H-4), 8.68 (d, 1 H, J = 8.2, H-7)
12d	91	191	$C_{18}H_{12}NO_2Cl$	CHNOCl	3.98 (s, 3 H, OCH_3), 7.27 (d, 1 H, J = 2.6, H-1), 7.36 (dd, 1 H, J = 2.6, 9.2, H-3), 7.73 (m, 2 H, H-8, H-9), 7.92 (d, 1 H, J = 9.0, H-12), 8.44 (d, 1 H, J = 9.0, H-11), 8.52 (d, 1 H, J = 8.5, H-10), 8.62 (d, 1 H, J = 8.3, H-7), 9.18 (d, 1 H, J = 9.2, H-4)
12e	89	131	$C_{21}H_{19}NO_3Cl$	CHNOCl	1.44 (d, 6 H, J = 6.0, CH_3-iPr), 4.02 (s, 3 H, OCH_3), 4.77 (sept, 1 H, J = 6.0, H-iPr), 7.27 (d, 1 H, J = 2.4, H-1), 7.34 (dd, 1 H, J = 2.4, 9.1, H-3), 7.52 (dd, 1 H, J = 2.7, 9.1, H-9), 7.79 (d, 1 H, J = 2.7, H-7), 7.87 (d, 1 H, J = 9.0, H-12), 8.36 (d, 1 H, J = 9, H-11), 8.52 (d, 1 H, J = 9.1, H-10), d, 1 H, J = 9.1, H-4)

[a] Solvent used: CF_3COOD for compounds 10a–e and 11a–e; DMSO-d_6 for compound 10i; $CDCl_3$ for compounds 12a–e. [b] Lit.[37] mp 340 °C. [c] Lit.[50] mp 329 °C. [d] In percent.

crystallize, and the precipitate was filtered and washed with CH_2Cl_2. The filtrate was collected and kept apart. The precipitate was then successively washed thoroughly with H_2O, EtOH, and CH_2Cl_2 and dried to yield 20–50% of analytically pure 7,8,9,-10-tetrahydrobenzo[c]phenanthridin-6(5H)-ones 10a–e. The filtrate mentioned above was evaporated to dryness and the residue treated with stirring with cold 70% sulfuric acid (150 g) for 1 week.[36] After dilution in 1 L of water, the same purification procedure as above was applied, followed by a recrystallization in dichlorobenzene. Additional 10–20% of analytically pure compounds 10a–d were thus obtained. In the case of the 6-isopropoxy-1-naphthylamine 10e, the precipitate obtained through the sulfuric acid treatment was washed with water and boiling methanol, thus yielding 22% of the hydroxy compound 12i as a monohydrate (see Table V).

Compounds 11a–e: General Procedure. Compounds 10a–e (0.025 mol) were heated for 24 h in refluxing diphenyl ether (50 mL) in the presence of 0.5 g of 10% palladium over charcoal. The mixture was diluted in an excess of n-heptane and filtered. The precipitate was taken up repeatedly in boiling dichlorobenzene and filtered. For the compounds 13a and 13b, the precipitate was extracted with the same solvent but using a continuous extraction apparatus during a week. The compounds crystallized on cooling(see Table V).

Compounds 12a–e: General Procedure. Compounds 11a–e (0.022 mol) were heated in phosphorous oxychloride (40 mL) during 2 h. The mixture was cautiously poured on an excess of crushed ice and basified with concentrated ammonia. The precipitate was filtered, washed with water, dried, and recrystallized from n-heptane(see Table V).

Compounds 6a–e and 7a–d: General Procedure. Under an inert atmosphere, compounds 12a–e (5 mmol) in the required [(dimethylamino)alkyl]amine (0.2 mol) were heated at reflux for 3 h. The mixture was then evaporated to dryness and dissolved in CH_2Cl_2. The organic layer was washed with 5% aqueous NaOH and H_2O and then dried over K_2CO_3 before evaporation to dryness. In the case of compound 14e, a chromatography over alumina washing first with CH_2Cl_2 and then eluting with a mixture of CH_2Cl_2–(EtOH/NH_3 1 N), 9–1 v/v, was necessary to obtain the expected product 12e as a solid, which gave an homogeneous spot on TLC. All the free bases were amorphous and characterized under their crystalline bismesylate salts form prepared from acetone which were used for the biological studies (see Table VI).

Compounds 6f–h and 7f–h: General Procedure. The free bases 6b–d or 7b–d (0.8 mmol) were boiled for 5 h in 47% hydrobromic acid (30 mL or 50 mL in the case of 6b and 7b). The solution was evaporated to dryness in vacuo, and the solid residue obtained was triturated in methanol, filtered, washed with methanol, and dried (see Table VI).

6-{[2′-(Dimethylamino)ethyl]amino}-2-hydroxy-8-methoxybenzo[c] phenanthridine (6i). Compound 6e (0.11 g, 0.27 mmol) was boiled for 3 h in 33% hydrochloric acid (50 mL), and the solution was evaporated to dryness in vacuo. The residue obtained was triturated in dry methanol, filtered, and washed with methanol, yielding 0.02 g of a solid. The NMR spectrum of this solid showed the presence of some amount (less than 20%) of dihydroxy compound 6h along the monohydroxy derivative 6i. From the filtered solution the pure monohydroxy compound 6i precipitated on standing overnight. This gave, after drying, 0.06 g (46%) of 6i: mp >260 °C dec; ^{1}H NMR δ (DMSO-d_6) 2.88, 2.91 (2s, 6 H, NMe_2), 3.55 (m, 2 H, CH_2-2′), 3.97 (s, 3 H, OCH_3), 4.15 (m, 2 H, CH_2-1′), 7.13–7.18 (m, 2 H, H-1, H-3), 7.46 (dd, 1 H, J = 2.2, 9.0, H-9), 7.54 (d, 1 H, J = 8.9, H-12), 7.96 (d, 1 H, J =

Table VI. Experimental Data for the 6-Aminated Benzo[c]phenanthridines 6 and 7

compd	yield[a]	mp, °C	formula	anal.	CI[b]	^{1}H NMR (DMSO-d_6)
6a	50	160	$C_{23}H_{29}N_3O_8S_2 \cdot 0.7$ $MeSO_3H \cdot 0.8H_2O$	CHNS		2.41 (s, 6 H, CH_3SO_3H), 2.91, 2.93 (2s, 6 H, NMe_2), 3.60 (m, 2 H, CH_2-2'), 4.14 (m, 2 H, CH_2-1'), 7.59–7.99 (m, 6 H, H-12, H-1, H-3, H-2, H-8, H-9), 8.41 (d, 1 H, J = 8.3, H-7), 8.55 (d, 1 H, J = 9.1, H-11), 8.76 (d, 1 H, J = 8.0, H-10), 9.11 (d, 1 H, J = 9.6, H-4)
6b	76	>260	$C_{25}H_{33}N_3O_9S_2 \cdot 2H_2O$	CHNS		2.36 (s, 6 H, CH_3SO_3H), 2.91, 2.93 (2s, 6 H, NMe_2), 3.57 (m, 1 H, CH_2-2'), 3.91–3.96 (2s, 6 H, OCH_3), 4.12 (m, 2 H, CH_2-1'), 7.25 (dd, 1 H, J = 2.6, 9.0, H-3), 7.39 (d, 1 H, J = 2.6, H-1), 7.50 (dd, 1 H, J = 2.5, 9.1, H-9), 7.67 (d, 1 H, J = 9.0, H-12), 7.81 (d, 1 H, J = 2.5, H-7), 8.44 (d, 1 H, J = 9.0, H-11), 8.64 (d, 1 H, J = 9.1, H-10), 8.96 (d, 1 H, J = 9.1, H-4)
6c	81	182	$C_{24}H_{31}N_3O_7S_2 \cdot 0.7$ $MeSO_3H \cdot 0.2H_2O$	CHNOS	346	2.32 (s, 6 H, CH_3SO_3H), 2.91, 2.93 (2s, 6 H, NMe_2), 3.56 (m, 2 H, CH_2-2'), 3.98 (s, 3 H, OCH_3), 4.05 (m, 2 H, CH_2-1'), 7.27 (dd, 1 H, J = 2.7, 8.8, H-2), 7.71 (d, 1 H, J = 8.7, H-12), 7.77 (m, 1 H, H-8), 7.88 (m, 1 H, H-9), 7.90 (d, 1 H, J = 8.8, H-1), 8.36–8.46 (m, 2 H, H-10, H-11), 8.47 (d, 1 H, J = 2.7, H-4), 8.74 (d, 1 H, J = 8.0, H-7)
6d	90	210	$C_{24}H_{31}N_3O_7S_2$	CHNS		2.37 (s, 6 H, CH_3SO_3H), 2.90, 2.92 (2s, 6 H, NMe_2), 3.57 (m, 2 H, CH_2-2'), 3.91 (s, 3 H, OCH_3), 4.11 (m, 2 H, CH_2-1'), 7.26 (dd, 1 H, J = 2.6, 9.1, H-3), 7.41 (d, 1 H, J = 2.6, H-1), 7.65–7.72 (m, 2 H, H-9, H-12), 7.86 (m, 1 H, H-8), 8.37 (d, 1 H, J = 8.1, H-7), 8.50 (d, 1 H, J = 9.1, H-11), 8.71 (d, 1 H, J = 8.2, H-10), 8.99 (d, 1 H, J = 9.1, H-4)
6e	30	202	$C_{27}H_{37}N_3O_8S_2 \cdot 2H_2O$	CHNOS		1.35 (d, 6 H, J = 6.0, CH_3-iPr), 2.35 (s, 6 H, CH_3SO_3H), 2.91, 2.93 (2, 6 H, NMe_2), 3.57 (m, 1 H, CH_2-2'), 3.97 (s, 3 H, OCH_3), 4.11 (m, 2 H, CH_2-1'), 4.81 (sept, 1 H, J = 6.0, H-iPr), 7.21 (dd, 1 H, J = 2.4, 9.0, H-3), 7.38 (d, 1 H, J = 2.4, H-1), 7.50 (dd, 1 H, J = 2.3, 9.1, H-9), 7.65 (d, 1 H, J = 8.9, H-12), 7.80 (d, 1 H, J = 2.3, H-7), 8.42 (d, 1 H, J = 8.9, H-11), 8.63 (d, 1 H, J = 9.1, H-10), 8.95 (d, 1 H, J = 9.0, H-4)
7a	58	217	$C_{24}H_{31}N_3O_6S$	CHNS		2.18 (m, 2 H, CH_2-2'), 2.43 (s, 6 H, CH_3SO_3H), 2.80–2.83 (2s, 6 H, NMe_2), 3.29 (m, 2 H, CH_2-3'), 3.93 (m, 2 H, CH_2-1'), 7.62–7.98 (m, 6 H, H-12, H-1, H-3, H-2, H-8, H-9), 8.01 (d, 1 H, J = 6.7, H-7), 8.55 (d, 1 H, J = 9.0, H-11), 8.77 (d, 1 H, J = 8.2, H-10), 9.05 (dd, 1 H, J = 2.1, 7.1, H-4)
7b	89	>260	$C_{26}H_{25}N_3O_9S_2 \cdot 2H_2O$	CHNS		2.18 (m, 2 H, CH_2-2'), 2.36 (s, 6 H, CH_3SO_3H), 2.78, 2.81 (2s, 6 H, NMe_2), 3.26 (m, 2 H, CH_2-3'), 3.87 (m, 2 H, CH_2-1'), 3.91, 3.96 (2s, 6 H, OCH_3), 7.26 (dd, 1 H, J = 2.6, 9.1, H-3), 7.39 (d, 1 H, J = 2.6, H-1), 7.51 (dd, 1 H, J = 2.3, 9.1, H-9), 7.68 (d, 1 H, J = 9.0, H-12), 7.86 (d, 1 H, J = 2.3, H-7), 8.43 (d, 1 H, J = 9.0, H-11), 8.63 (d, 1 H, J = 9.1, H-10), 8.93 (d, 1 H, J = 9.1, H-4)
7c	86	211	$C_{25}H_{33}N_3O_7S_2 \cdot 0.5H_2O$	CHNOS		2.25 (m, 2 H, CH_2-2'), 2.36 (s, 6 H, CH_3SO_3H), 2.77, 2.80 (2s, 6 H, NMe_2), 3.25 (m, 2 H, CH_2-3'), 3.87 (m, 2 H, CH_2-1'), 3.93 (s, 3 H, OCH_3), 7.29 (dd, 1 H, J = 2.6, 8.7, H-2), 7.70 (d, 1 H, J = 8.9, H-12), 7.71 (m, 1 H, H-8), 7.88 (m, 1 H, H-9), 7.90 (d, 1 H, J = 8.7, H-1), 8.37 (d, 1 H, J = 9.0, H-10), 8.45 (m, 1 H, H-11), 8.4 (d, 1 H, J = 2.6, H-4), 8.79 (d, 1 H, J = 8.9, H-7)
7d	78	248	$C_{25}H_{33}N_3O_7S_2 \cdot 2H_2O$	CHNS	360	2.23 (m, 2 H, CH_2-2'), 2.37 (s, 6 H, CH_3SO_3H), 2.78, 2.80 (2s, 6 H, NMe_2), 3.24 (m, 2 H, CH_2-3'), 3.85 (m, 2 H, CH_2-1'), 3.92 (s, 3 H, OCH_3), 7.27 (dd, 1 H, J = 2.5, 9.1, H-3), 7.42 (d, 1 H, J = 2.5, H-1), 7.64–7.72 (m, 2 H, H-12, H-9), 7.87 (m, 1 H, H-8), 8.42–8.51 (m, 2 H, H-7, H-11), 8.70 (d, 1 H, J = 8.9, H-10), 8.97 (d, 1 H, J = 9.1, H-4)
6f	49	>260	$C_{21}H_{23}N_3OBr_2 \cdot 0.5H_2O$	CHNBr		2.92, 2.95 (2s, 6 H, NMe_2), 3.63 (m, 2 H, CH_2-2'), 4.19 (m, 2 H, CH_2-1'), 7.23 (m, 2 H, H-1, H-3), 7.60–7.72 (m, 2 H, H-8, H-12), 7.50 (m, 1 H, H-9), 8.43 (d, 1 H, J = 9.2, H-7), 8.51 (d, 1 H, J = 8.5, H-11), 8.69 (d, 1 H, J = 8.2, H-10), 8.98 (d, 1 H, J = 9.7, H-4)
6g	51	>260	$C_{21}H_{23}N_3OBr_2 \cdot 0.5H_2O$	CHNBr	332	2.91, 2.96 (2s, 6 H, NMe_2), 3.62 (m, 2 H, CH_2-2'), 4.14 (m, 2 H, CH_2-1'), 7.16 (dd, 1 H, J = 2.5, 8.7, H-2), 7.66 (d, 1 H, J = 9.0, H-12), 7.71–7.91 (m, 3 H, H-1, H-8, H-9), 7.29 (d, 1 H, J = 9.0, H-11), 8.35 (d, 1 H, J = 2.5, H-4), 8.47 (d, 1 H, J = 7.1, H-10), 8.71 (d, 1 H, J = 8.2, H-7)
6h	59	>260	$C_{21}H_{23}N_3O_2Br_2 \cdot 1.5H_2O$	CHNBr	348	2.89, 2.94 (2s, 6 H, NMe_2), 3.57 (m, 2 H, CH_2-2'), 4.13 (m, 2 H, CH_2-1'), 7.14–7.19 (m, 2 H, H-1, H-3), 7.44 (dd, 1 H, J = 2.0, 9.0, H-9), 7.57 (d, 1 H, J = 8.9, H-12), d, 1 H, J = 2.0, H-7), 8.34 (d, 1 H, J = 8.9, H-11), 8.54 (d, 1 H, J = 9.0, H-10), 8.85 (d, 1 H, J = 9.8, H-4)
6f	81	>260	$C_{22}H_{25}N_3OBr_2 \cdot 1.1$ $H_2O0.1HBr$	CHNBr		2.28 (m, 2 H, CH_2-2'), 2.79, 2.82 (2s, 6 H, NMe_2), 3.28 (m, 2 H, CH_2-3'), 3.98 (m, 2 H, CH_2-1'), 7.28 (m, 2 H, H-1, H-3), 7.67–7.77 (m, 2 H, H-8, H-12), 7.95 (m, 1 H, H-9), 8.45 (d, 1 H, J = 9.1, H-7), 8.74 (m, 2 H, H-10, H-11), 8.82 (d, 1 H, J = 9.7, H-4)
7g	64	>260	$C_{22}H_{25}N_3OBr_2 \cdot 0.5H_2O$	CHNOBr	346	2.44 (m, 2 H, CH_2-2'), 2.77–2.82 (2s, 6 H, NMe_2), 3.31 (m, 2 H, CH_2-3'), 3.91 (m, 2 H, CH_2-1'), 7.21 (dd, 1 H, J = 2.3, 8.7, H-2), 7.72 (d, 1 H, J = 8.7, H-12), 7.77–7.96 (m, 3 H, H-1, H-8, H-9), 8.28–8.32 (m, 2 H, H-4, H-11), 8.58 (d, 1 H, J = 8.1, H-10), 8.73 (d, 1 H, J = 8.3, H-7)
7h	26	>260	$C_{22}H_{25}N_3O_2Br_2 \cdot 0.5H_2O$	CHN	362	2.22 (m, 2 H, CH_2-2'), 2.77, 2.80 (2s, 6 H, NMe_2), 3.25 (m, 2 H, CH_2-3'), 3.90 (m, 2 H, CH_2-1'), 7.22 (m, 2 H, H-1, H-3), 7.48 (dd, 1 H, J = 2.0, 9.0, H-9), 7.64 (d, 1 H, J = 8.9, H-12), 7.82 (d, J = 2.0, 1 H, H-7), 8.35 (d, 1 H, J = 8.9, H-11), 8.58 (d, 1 H, J = 9.0, H-10), 8.76 (d, 1 H, J = 9.7, H-4)

[a] In percent. [b] Chemical ionization (NH_3) m/z = MH^+ (100) (M: molecular mass of the base).

2.2, H-7), 8.36 (d, 1 H, J = 8.9, H-11), 8.60 (d, 1 H, J = 9.0, H-10), 8.92 (d, 1 H, J = 9.7, H-4). Anal. Calcd for $C_{22}H_{25}N_3O_2Cl_2 \cdot 0.3HCl \cdot 1.3H_2O$: C, 56.50; H, 5.80; N, 9.00; O, 11.30; Cl, 17.20. Found: C, 56.5; H, 6.0; N, 9.0; O, 11.3; Cl, 17.2.

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