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Synthesis and Biological Properties of New Benz[*h*]isoquinoline Derivatives

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In order to determine the importance of the pyrrolic nitrogen atom in a series of suitably substituted γ-carbolines derivatives for their cytotoxic and antitumor properties, a series of structurally related benz[*h*]isoquinolines were synthesized. When compared to the "parent" γ-carbolines, these new compounds showed greatly decreased effects on topoisomerases I and II. Whereas the 8-hydroxylated derivatives retained a significant cytotoxicity, all the new compounds were devoid of antitumor effect in the P388 murine ip-ip system.

Keywords antitumor activity; topoisomerase; benz[*h*]isoquinoline; γ-carboline analogue

γ-Carboline derivatives **1a, b** have recently been shown to have cytotoxic and antitumor activities.[1,2] They were also recognised as topoisomerase II poisons.[3] Study of the structure–activity relationship revealed the key role of the dialkylaminoalkylamino side-chain and the 4-methyl group for these activities.[1] However, the substitution of the pyrrolic nitrogen atom (NH or NCH_3) appears to be of minor importance. This observation, along with the growing interest in the related potent antitumor compound **2**,[2,4-6] which belongs to the benzo[*e*]pyrido[4,3-*b*]indole series, led us to focus on the benz[*h*]isoquinoline ring system, in which the pyrrole nucleus of the γ-carbolines **1** is replaced by a benzene ring.

In this paper, we report the synthesis of the analogues **3a—d** and their biological studies, *i.e.*, cytotoxicity, antitumor activity and stimulation of the DNA topoisomerase I and II cleavable complexes.

Chemistry

To prepare these structural analogues, we used Eloy and Deryckere's strategy for the preparation of isoquinolines,[7] depicted in Chart 1. Starting from compound **5**, Reformatsky reaction followed by dehydration (step i) led to the ester **5**. Saponification of this compound (ii) gave the acid **6** from which the azide **7** was obtained by using the conventional method (iii). This azide was subjected to thermal transformation. In constrast to related examples, only it decomposition was observed. Thus, at this stage we resorted to the little known thermal cyclization of vinyl urethanes.[8] The urethane **8** was obtained by heating the azide **7** in ethanol (iv) and then its thermal cyclization into benz[*h*]isoquinolone **9** was achieved in boiling diphenyl ether in the presence of a catalytic amount of tributylamine (v). This reaction is actually one of the few known examples of thermal cyclization of a urethane.[8,9] The last three steps, chlorination (vi), substitution (vii) and demethylation (viii) to give the phenolic analogues **3c, d** were performed under the usual conditions.

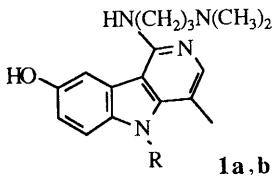
Biological Results

The IC_{50} values of compounds **3a—d** tested against L1210 and P388 cultured cells are reported in Table I, which includes the results obtained with compounds **3b** and **3d** in the *in vivo* P388 leukemia model.

The results in the topoisomerase I and II cleavable complexes formation assay shown in Table II indicate that compounds **3a—d** are inactive towards topoisomerase I and that compounds **3a** and **3b** are inactive towards topoisomerase II (MIC > 10 μM). On the other hand, compounds **3c** and **3d** display a minimal topoisomerase II poisoning activity with MIC between 3 and 10 μM. In comparison, the "parent" compounds **1a** and **2** are highly active towards both topoisomerase I (MIC < 0.1 μM) and topoisomerase II (MIC < 0.5 μM).

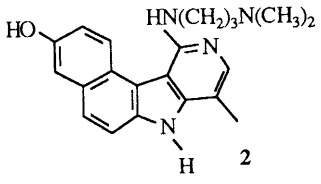
Discussion and Conclusion

From a biological point of view, it has already been reported that the antitumor γ-carbolines (**1a, 1b**) and

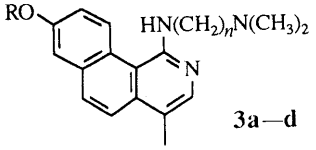


1a, b

1a : R = CH_3
1b : R = H

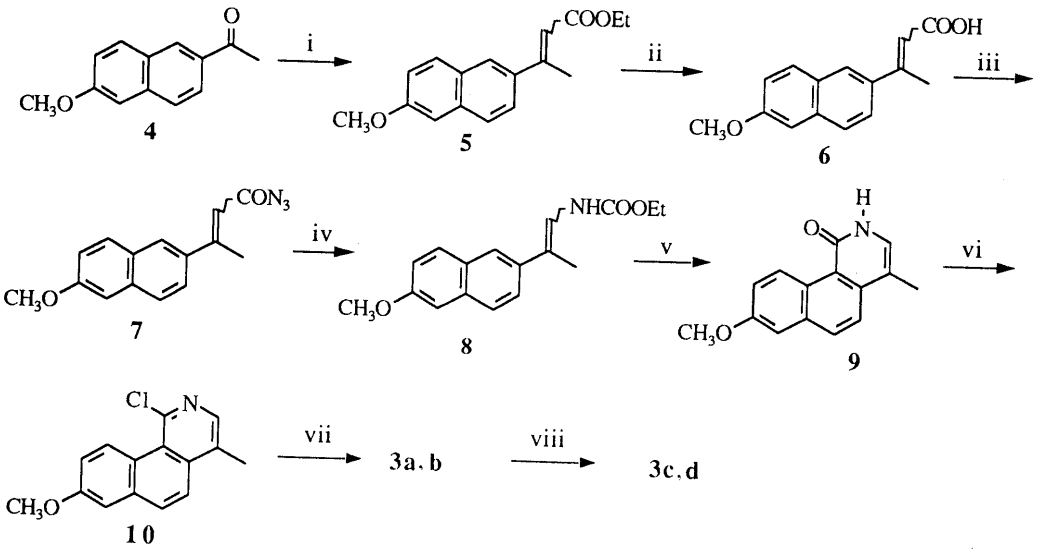


2



3a—d

3a : n = 2, R = CH_3
3b : n = 3, R = CH_3
3c : n = 2, R = H
3d : n = 3, R = H



i : 1) $BrZnCH_2CO_2Et$, Δ. 2) H^+, Δ. ii : NaOH, H_2O-EtOH. iii : 1) $ClCO_2Et$, NEt_3. 2) NaN_3. iv : EtOH, Δ. v : Ph_2O, NBu_3, Δ. vi : $POCl_3$, Δ. vii : Amines, Δ. viii : HBr 47%, Δ

Chart 1

TABLE I. *In Vitro* and *In Vivo* Evaluation of Compounds **3a—d**

	3a	**3c**	**3b**	**3d**
R	CH_3	H	CH_3	H
IC_{50} (μM/l) L1210	10	4	2.4	0.3
IC_{50} (μM/l) P388	3.5	0.3	2.8	0.15
T/C (dose in mg/kg) (ip/ip)	ND	ND	111 (10) 97 (15) 97 (25)	100 (10) 115 (15)

TABLE II. Results of DNA Topoisomerase I and II Assays

Compound	Topoisomerase I MIC (μM)	Topoisomerase II MIC (μM)
1a	<0.1	0.5
2	<0.1	0.3
3a	>10	>10
3b	>10	>10
3c	>10	10
3d	>10	3—10

benzo[*e*]pyrido[4,3-*b*]indole (**2**) are nearly equally active in the topoisomerase I and II cleavable complexes stimulation assays.[2,10,11] The results presented in this paper show that compounds **3a—d** essentially lack the topoisomerase I poisoning properties of the "parent" compounds, whereas the analogues **3c** and **3d** retain some topoisomerase II poisoning properties. These results could explain the lower cytotoxicity and the lack of significant antitumor activity observed.

It is known that a phenolic function is essential for the topoisomerase II poisoning activity of the γ-carbolines **1a**, **b**, the benzopyridoindole **2** and the ellipticine series.[2,3,11,12] This also seems to be true in the present case.

It is interesting to note that while replacement of the pyrrole nucleus by a benzene ring does not completely abolish the topoisomerase II poisoning activity, the topoisomerase I poisoning properties are completely lost. This could indicate that the pyrrole nucleus is only essential for topoisomerase I poisoning. Since a similar loss was also observed when the angular benzene ring of the benzo[*e*]pyrido[4,3-*b*]indole (**2**) was displaced to the benzo[*g*]position,[11] the geometry change of the OH group relative to the whole molecule which results from a pyrrole to a benzene ring replacement is probably an important factor.

In conclusion, the synthesis of the benz[*h*]isoquinoline analogues **3a—d** and their biochemical and biological evaluations indicate that replacement of the pyrrole nucleus of the γ-carbolines **1a**, **b** by a benzene ring is not an interesting approach to find new compounds with improved cytotoxic and antitumor properties.

Experimental

Chemistry Melting points are uncorrected, ^{1}H-NMR spectra were recorded on a Bruker AC-200 MHz spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts (δ) are reported in part per million relative to Me_4Si as an internal standard, and coupling constants (*J*) are given in hertz (Hz). Signal identification was often done with the help of NOE effects. Elemental analyses were performed by the Service Central de Micronalyses (CNRS-ICSN, Gif-sur-Yvette, France) on the isolated products, unless otherwise stated. Pure commecially available solvents were used, without further treatment.

Ethyl 3-[2-(6-Methoxynaphthyl)]but-2-enoate (5) Activated zinc powder (36 g, 0.549 mol) in dry toluene (20 ml) was heated at boiling temperature. Then a solution of 6-methoxy-2-acetylnaphthalene (**4**) (100 g, 0.5 mol) and ethyl bromoacetate (55.6 ml, 0.5 mol) in dry toluene (0.5 l) was added dropwise in order to maintain a gentle reflux without exterior heating, but taking the usual precautions at the beginning of addition, since this reaction is exothermic and starts quite suddenly. At the end of this addition, reflux was resumed by heating for 1 h. After cooling, the reaction mixture was poured into 20% sulfuric acid (0.9 l), and extracted with methylene chloride. The organic solution was washed with 5% sulfuric acid, and water, before being dried over magnesium

sulfate. The oily residue obtained after evaporation to dryness was dissolved in acetic acid (500 ml), then hydrochloric acid (2.3 ml) was added and the solution was boiled for 3 h. After the usual work-up, the crude ester was saponified without further purification. A sample was purified by chromatography over alumina (neutral 6% water) with heptane–ethyl acetate mixture (1 : 1, v/v), mp 79 °C. ^{1}H-NMR δ: 1.35 (t, 3H, J=7.2, $OCH_2\underline{CH_3}$), 2.69 (d, 3H, J=1.2, CH_3), 3.84 (s, 3H, OCH_3), 4.25 (q, 2H, J=7.2, $O\underline{CH_2}CH_3$), 6.29 (q, 1H, J=1.2, H2), 7.13 (d, 1H, J=2.5, H4′), 7.17 (dd, 1H, J=2.5, 8.7, H6′), 7.69 (dd, 1H, J=1.9, 8.7, H2′), 7.70—7.78 (m, 2H, H3′, H7′), 7.90 (d, 1H, J=1.9, H8′). *Anal.* Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.8; H, 6.6.

3-[2-(6-Methoxynaphthyl)]but-2-enoic Acid (6) The above-described ester and sodium hydroxide (50 g) were dissolved in 75% methanol (800 ml) and heated at reflux for 1 h. The resulting solution was acidified with concentrated hydrochloric acid and the finely divided precipitate was collected by filtration. After drying, it was washed with diethyl ether yielding 33 g of the acid **6** (28% from **4**), mp 203 °C. ^{1}H-NMR δ: 2.71 (d, 3H, J=1.2, CH_3), 3.94 (s, 3H, OCH_3), 6.32 (d, 1H, J=1.2, H2), 7.15—7.21 (m, 2H, H4′, H6′), 7.59 (dd, 1H, J=1.8, 8.7, H2′), 7.73—7.80 (m, 2H, H3′, H7′), 7.92 (d, 1H, J=1.2, H8′). *Anal.* Calcd for $C_{15}H_{14}O_3$: C, 74.5; H, 5.8. Found: C, 74.36; H, 5.82.

Ethyl-*N*-1-[2-(6-Methoxynaphethyl)prop-1-enyl]carbamate (8) A suspension of the acid **6** (12.5 g, 0.054 mol) in triethylamine (8.8 ml, 0.06 mol) and acetone (140 ml) was cooled to 0 °C. Then ethyl chloroformate (6.75 ml, 0.0705 mol) in acetone (80 ml) was added dropwise. The suspension was stirred at room temperature for 1 h, then cooled to 0 °C and sodium azide (5.2 g, 0.08 mol) in water (12.5 ml) was added dropwise. This solution was stirred overnight before pouring it into a mixture of crushed ice (500 g) and water (1.5 l). The suspension was extracted with methylene chloride, and the organic layer was washed with water and evaporated to dryness without heating. Ethanol (200 ml) was immediately added to the resulting oily azide **7**, before heating at reflux until nitrogen evolution ceased. The mixture was evaporated to dryness and the residue obtained was recrystallized from cyclohexane over Norit. An analytical sample in solution in methylene chloride was purified by flash chromatography over alumina and, after concentration, recrystallized from aqueous ethanol, mp 93 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.28 (t, 3H, J=7.0, $CH_2\underline{CH_3}$), 2.11 (d, 3H, J=1.1, CH_3), 3.89 (s, 3H, OCH_3), 4.18 (q, 2H, J=7.0, $\underline{CH_2}CH_3$), 6.97 (d, 1H, J=10.2, H1), 7.15 (dd, 1H, J=2.5, 8.8, H7′), 7.30 (d, 1H, J=2.5, H5′), 7.57 (dd, 1H, J=1.6, 8.8, H3′), 7.75—7.85 (m, 3H, H1′, H4′, H8′), 9.27 (d, 1H, J=10.2, NH). *Anal.* Calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.81; O, 16.82. Found: C, 71.3; H, 6.9; N, 5.1; O, 16.5. Nota: This vinyl urethane decomposes in DMSO-d_6 over a 24 h period.

8-Methoxy-4-methylbenz[*h*]isoquinolin-1(2H)-one (9) A solution of the above-mentioned crude vinyl urethane (3 g) and tributylamine (1 g) in diphenyl ether (50 ml) was heated at reflux for 72 h. The cooled solution was poured into an excess of heptane, and the resulting precipitate was filtered and recrystallized in xylene thus yielding 1.1 g (43% from **6**) of compound **9**, mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.22 (s (br), 1H, H3), 7.34 (dd, 1H, J=2.8, 9.4, H9), 7.49 (d, 1H, J=2.8, H7), 7.75 (d, 1H, J=8.8, H5), 8.16 (d, 1H, J=8.8, H6), 10.15 (d, 1H, J=9.4, H10), 11.4 (s, 1H, NH). *Anal.* Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85%. Found: C, 75.2; H, 5.4; N, 5.8.

1-Chloro-8-methoxy-4-methylbenz[*h*]isoquinoline (10) Benz[*h*]isoquinolinone **9** (3.24 g, 1.2 mmol) in $POCl_3$ (25 ml, 0.25 mol) was heated at reflux for 1 h. The cooled mixture was slowly poured into an excess of crushed ice and the solution was basified with concentrated aqueous ammonia. The resulting precipitate was collected by filtration, washed with water, dried and recrystallized from cyclohexane to yield 3.2 g (92%) of compound **10**, mp 135 °C. ^{1}H-NMR δ: 2.64 (s, 3H, CH_3), 4.03 (s, 3H, OCH_3), 7.26 (d, 1H, J=2.8, H7), 7.33 (dd, 1H, J=2.8, 9.4, H9), 7.77 (d, 1H, J=9.2, H5), 7.89 (d, 1H, J=9.2, H6), 8.22 (s, 1H, H3), 9.77 (d, 1H, J=9.4, H10). *Anal.* Calcd for $C_{15}H_{11}NClO$: C, 70.18; H, 4.32; N, 5.46; Cl, 13.81. Found: C, 70.4; H, 4.4; N, 5.5; Cl, 13.9.

1-{[2-(Dimethylamino)ethyl]amino}-8-methoxy-4-methylbenz[*h*]isoquinoline (3a) The chlorinated compound **10** (1.5 g, 5.8 mmol) was dissolved in 2-dimethylaminoethylamine (5 ml) and heated at reflux under argon for 12 h. The crude mixture was evaporated to dryness, the residue was dissolved in 1 N hydrochloric acid and the aqueous layer was washed with methylene chloride. Upon basification of this aqueous layer, the crude base precipitated. It was extracted with methylene chloride, and the organic layer was washed with water, dried (Na_2CO_3) and evaporated to dryness. The crude crystals obtained were recrystallized from hexane, yielding 1.37 g (76%) of compound **3a**, mp 104 °C. ^{1}H-NMR δ: 2.30 (s, 6H, NMe_2), 2.49 (d, 3H, J=0.8, CH_3), 2.74 (m, 2H, $CH_2$2′), 3.64 (m, 2H, $CH_2$1′), 3.97 (s, 3H, OCH_3), 6.10 (s(br), 1H, NH), 7.26 (dd, 1H, J=2.8, 8.8, H9), 7.29 (d, 1H, J=2.8, H7), 7.74 (d, 1H, J=9.1, H5), 7.83 (d, 1H, J=9.1, H6), 7.98 (d, 1H, J=0.8, H3), 9.12 (d, 1H, J=8.8, H10). *Anal.* Calcd for $C_{19}H_{23}N_3O$: C, 73.75; H, 7.49; N, 13.58. Found: C, 73.5; H, 7.4; N, 13.5.

1-{[3-(Dimethylamino)propyl]amino}-8-methoxy-4-methylbenz[*h*]isoquinoline (3b) Starting from dimethylaminopropylamine and using the procedure described above, 1.51 g (85%) of compound **3b** was obtained, mp 112 °C. ^{1}H-NMR δ: 1.94 (m, 2H, $CH_2$2′), 2.22 (s, 6H, NMe_2), 2.49 (d, 3H, J=0.8, CH_3), 2.48 (m, 2H, $CH_2$3′), 3.64 (m, 2H, $CH_2$1′), 3.97 (s, 3H, OCH_3), 7.05 (s(1), 1H, NH), 7.27 (dd, 1H, J=2.8, 8.8, H9), 7.29 (d, 1H, J=2.8, H7), 7.74 (d, 1H, J=9.1, H5), 7.83 (d, 1H, J=9.1, H6), 8.00 (d, 1H, J=0.8, H3), 9.10 (d, 1H, J=8.8, H10). *Anal.* Calcd for $C_{20}H_{25}N_3O$: C, 74.27; H, 7.79; N, 12.99. Found: C, 74.3; H, 7.7; N, 12.9.

8-Hydroxy-1-{[2-(dimethylamino)ethyl]amino}-4-methylbenz[*h*]isoquinoline (3c) The base **3a** (1.6 mmol) was dissolved in 47% hydrobromic acid (20 ml) and heated at reflux for 3 h. The mixture was evaporated to dryness, and the residue was taken up in methanol. This mixture was filtered, and the solid was washed with methanol and dried to yield 97% of the phenolic compound **3c**, mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.94 (s, 6H, NMe_2), 3.57 (m, 2H, $CH_2$2′), 4.05 (m, 2H, $CH_2$1′), 7.39 (dd, 1H, J=2.5, 9.2, H9), 7.45 (d, 1H, J=2.5, H7), 7.78 (s, 1H, H3), 7.85 (d, 1H, J=8.9, H5), 8.25 (d, 1H, J=8.9, H6), 8.86 (d, 1 H, J=9.2, H10). *Anal.* Calcd for $C_{18}H_{22}Br_2N_3O$, 0.1 HBr, 0.25 H_2O: C, 46.12; H, 4.86; N, 9.00; Br, 35.80. Found: C, 46.1; H, 5.0; N, 9.0; Br, 35.8.

8-Hydroxy-1-{[3-(dimethylamino)propyl]amino}-4-methylbenz[*h*]-isoquinoline (3d) Using the same procedure as above, but starting from compound **3b**, gave a 98% yield of compound **3d**, mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.19 (m, 2H, $CH_2$2′), 2.48 (s, 3H, CH_3), 2.83 (s, 6H, NMe_2), 3.28 (m, 2H, $CH_2$3′), 3.70 (m, 2H, $CH_2$1′), 7.39 (dd, 1H, J=2.5, 9.2, H9), 7.45 (d, 1H, J=2.5, H7), 7.71 (s, 1H, H3), 7.83 (d, 1H, J=8.9, H5), 8.24 (d, 1H, J=8.9, H6), 8.75 (d, 1H, J=9.2, H10). *Anal.* Calcd for $C_{19}H_{24}Br_2N_3O$: C, 48.53; H, 5.14; N, 8.94; Br, 33.93. Found: C, 48.4; H, 5.3; N, 9.0; Br, 33.8.

Biology *In Vitro* Antiproliferative Effects: L1210 and P388 cytotoxicity determination: Compounds **3a—d** were teated on cultured murine lymphoblastic leukemia cells (L1210), under the usual conditions. The cells were counted after 48 h of incubation with the drugs. In the case of P388, the cells were incubated for 96 h in the presence of various concentrations of drug. Viability was evaluated by using of the neutral red staining method.[11] The concentration of the drug inhibiting growth by 50% (IC50) was then determined.

In Vivo Antitumor Effects on P388 Leukemia: Female BDF_1 mice were inoculated intraperitoneally with 10^6 P388 leukemia cells (J_0) (10 controls and 6 animals in each group) and test compounds were injected intraperitoneally as a 0.2 ml solution (PBS) at days 1,3 and 7 after leukemia inoculation. Values of T/C are expressed as the ratio of the mean survival time of treated animals to the mean survival time of controls multiplied by 100.

Preparation of Topoisomerases and DNA Cleavage Reaction: Topoisomerases I and II were prepared from calf thymus as already described.[13] Topoisomerase I or II poisoning was evaluated using the the DNA cleavage assays described previously.[14] Each compound was examined at five concentrations (0.1, 0.3, 1, 3 and 10 μM). The minimum inhibitory concentration (MIC), corresponds to the lowest concentration able to produce a detectable stimulation of the DNA cleavage reaction.

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