Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 Vol. 17 (6), pp. 369–374

Cytotoxic Bis-3,4-dihydro-β-carbolines and Bis-β-carbolines

WEI-QUN JIANG[a], CATHERINE CHARLET-FAGNÈRE[a], JANOS SAPI[a], JEAN-YVES LARONZE[a,*], PIERRE RENARD[b], BRUNO PFEIFFER[b] and STÉPHANE LÉONCE[c]

[a]*UMR 6013 "Isolement, Structure, Transformations et Synthèses de Produits Naturels", IFR 53 "Biomolécules", Faculté de Pharmacie, 51 rue Cognacq Jay, F-51096 Reims Cédex, France;* [b]*ADIR, Groupe Servier, 1 rue Carle Hébert, F-92415 Courbevoie Cédex, France;* [c]*Institut de Recherche Servier, 11 rue des Moulineaux, F-92150 Suresnes, France*

(Received 20 March 2002)

Ten bis-β-carboline 1, 2 and bis-3,4-dihydro-β-carboline 3, 4 derivatives, linked between carbons 1 and 1′ by a polymethylene spacer, were synthesized from bis-tryptamine amides 9, 10. Some of them display a micromolar IC_{50} towards L-1210 cells.

Keywords: Bis-β-carboline; Bis-3,4-dihydro-β-carboline; Cytotoxic activity

INTRODUCTION

In continuation of our work toward potential anti-cancer molecules,[1] we intended to apply our indole chemistry to the synthesis of bis-β-carbolines linked between C(1) and C(1′) by a polymethylene spacer, following the successful approach that had led to bis-naphthamides,[2] bis-imidazoacridones, bis-anthrapyrazoles,[3,4] bis-pyridocarbazoles,[5] pyrrolobenzodiazepines (PBD),[6–8] and bis-acridinecarboxamides.[9] Some of these derivatives are reported to be mixed topoisomerase I and II inhibitors.[10] The nature of their interaction with DNA is not fully understood, though polyintercalation was sometimes suggested[11] as a possible mechanism. Recently, several bis-β-carboline-3-carboxamides have proved to be intercalating agents, one of them having micromolar-range activity on two cancer cell lines.[12]

This approach could seem reminiscent of Porthoghese's "message-address" concept.[13] It has been recently exemplified in the fields of acetylcholinesterase inhibitors,[14–16] antimalarials,[17] and vitamin D.[18]

It should be noted that unlike what Porthoghese's hypothesis states, monomers do not need to have a significant biological activity, as it was recently observed for bis-catechol inhibitors of HIV-1 integrase.[19]

This paper deals with the preparation of some bis-β-carbolines **1**, **2** and bis-3,4-dihydro-β-carbolines **3**, **4** and their cytoxicity activities towards experimental L-1210 cells.

MATERIALS AND METHODS

Chemistry

Melting points, determined on a Reichert hot plate apparatus, are uncorrected. IR spectra were recorded on a BOMEM FTIR apparatus with COSMIC interferometer. UV spectra (in MeOH) were recorded on a Unicam 8700 spectrometer. ^{1}H- and ^{13}C-NMR spectra, using TMS as internal standard, were measured on a Bruker AC 300 apparatus at 300 and 75 MHz, respectively. MS spectra were obtained on a VG Autospec (Fisons) spectrometer.

Acylation of Tryptamine or 5-methoxytryptamine by Dichlorides 7 (Y = Cl)

General procedure starting from tryptamine: To a solution of tryptamine **5** (13.2 mmol) and triethylamine (13.2 mmol) in CH_2Cl_2 was added dropwise dichloride **7** (6.6 mmol). The reaction mixture was stirred at room temperature under nitrogen for 3–4 h, then poured into ice-water (100 ml), the white

*Corresponding author. Fax: +33-0-326918025. E-mail: jy.laronze@univ-reims.fr

ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1475630210000056631

precipitate was filtered off, washed with water and CH_2Cl_2 and dried to afford diamide **9**.

General procedure starting from 5-methoxytryptamine: To a cold solution of 5-methoxytryptamine chlorhydrate **6** (1.28 mmol) in water (3–4 ml) and CH_2Cl_2 (25 ml) was added dropwise (30 min) an aqueous (10 ml) solution of NaOH (6.6 mmol) and dichloride **7** (0.77 mmol). Stirring was maintained for 4–5 h, then the reaction mixture was diluted with CH_2Cl_2. The separated organic layer was washed with water (2 × 30 ml), brine (30 ml), dried and evaporated to dryness affording diamide **10**.

1,6-Hexanedioic acid bis-(tryptamin-N_b-yl)-amide (**9a**). Yield: 69%; m.p.: 188°C; IR (KBr) ν 3399, 3256, 2944, 1653, 1630, 1560 cm^{-1}; $UV_{\lambda_{max}}$ 290, 283, 274, 221, 206 nm; ^1H NMR (DMSO-d_6) δ 10.83 (s, 2H, NH_{ind}), 7.91 (brs, 2H, NH), 7.02–7.56 (m, 10H, ArH), 3.35 (m, 4H, $-CH_2NH-$), 2.86 (t, J = 7.4, 4H, $-CH_2-$;), 2.10 (m, 4H, $-CH_2CO-$), 1.54 (m, 4H, $-CH_2-$); ^{13}C NMR (DMSO-d_6) δ 172.1 (CO), 136.5 (C_{7a}), 127.5 (C_{3a}), 122.8 (C_2), 121.1 (C_6), 118.5 (C_4, C_5), 112.1 (C_3), 111.6 (C_7), 39.2 ($-CH_2NH-$), 35.6 ($-CH_2CO-$), 25.5 ($-CH_2CH_2NH-$), 25.3 ($-CH_2CH_2CO-$); MS (EI^+) *m/z* 431 ($M^+ + 1$, 2), 430 (M^+, 4), 288 (6), 271 (5), 143 (90), 130 (100); HREIMS: calc. for $C_{26}H_{30}N_4O_2$ 430.2369, found 430.2365.

1,8-Octanedioic acid bis-(tryptamin-N_b-yl)-amide (**9b**). Yield: 90%; m.p.: 177–178°C; IR (KBr) ν 3397, 3262, 2940, 1655, 1630, 1560 cm^{-1}; $UV_{\lambda_{max}}$ 291, 283, 222, 204 nm; ^1H NMR (DMSO-d_6) δ 10.80 (s, 2H, NH_{ind}), 7.86 (brs, 2H, NH), 6.97–7.54 (m, 10H, ArH), 3.35 (m, 4H, $-CH_2NH-$), 2.87 (t, J = 7.4, 4H, $-CH_2CH_2NH-$), 2.10 (t, J = 7.7, 4H, $-CH_2CO-$), 1.55 (m, 4H, CH_2), 1.26 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ 172.2 (CO), 136.4 (C_{7a}), 127.4 (C_{3a}), 122.7 (C_2), 121.0 (C_6), 118.4 (C_5), 118.3 (C_4), 112.1 (C_3), 111.5 (C_7), 39.6 ($-CH_2NH-$), 35.7 ($-CH_2CO-$), 28.7 ($-CH_2CH_2NH-$), 25.4 (2 × CH_2); MS(EI^+) *m/z* 458 (M^+, 14), 316 (16), 299 (11), 143 (100); HREIMS: calc. for $C_{28}H_{34}N_4O_2$ 458.2682, found 458.2672.

1,12-Dodecanedioic acid bis-(tryptamin-N_b-yl)-amide (**9c**) Yield: 82%; amorphous. IR (KBr) ν 3398, 3267, 2916, 1655, 1632, 1570 cm^{-1}; $UV_{\lambda_{max}}$ 290, 283, 274, 223, 207 nm; ^1H NMR (DMSO-d_6) δ 10.83 (s, 2H, NH_{ind}), 7.93 (brs, 2H, NH), 7.08–7.57 (m, 10H, ArH), 3.35 (m, 4H, $-CH_2NH-$), 2.85 (t, J = 7.5, 4H, $-CH_2CH_2NH-$), 2.08 (m, 4H, $-CH_2CO-$), 1.52 (m, 4H, $-CH_2-$), 1.22 (m, 8H, $-CH_2-$); ^{13}C NMR (DMSO-d_6) δ 172.3 (CO), 136.5 (C_{7a}), 127.5 (C_{3a}), 122.8 (C_2), 121.1 (C_6), 118.4 (C_4, C_5), 112.2 (C_3), 111.6 (C_7), 39.5 ($-CH_2NH-$), 35.8 ($-CH_2CO-$), 29.0 (3 × CH_2), 25.6 (2 × CH_2); MS (EI^+) *m/z* 514 (M^+, 5), 372 (31), 355 (10), 229 (21), 212 (13), 143 (100); HREIMS: calc. for $C_{32}H_{42}N_4O_2$ 514.3308, found 514.3307;

1,6-Hexanedioic acid bis-(5-methoxytryptamin-N_b-yl)-amide (**10a**) Yield: 96%; amorphous; IR (KBr) ν 3408, 3290, 2933, 1651, 1634, 1530 cm^{-1}; $UV_{\lambda_{max}}$ 309, 297, 278, 222 nm; ^1H NMR ($CDCl_3$) δ 8.27 (s, 2H, NH_{ind}), 6.84–7.24 (m, 8H, ArH), 5.74 (brs, 2H, NH), 3.83 (s, 6H, OCH_3), 3.57 (m, 4H, $-CH_2NH-$), 2.92 (t, J = 7.3, 4H, $-CH_2CH_2NH-$), 2.05 (m, 4H, $-CH_2CO-$), 1.52 (m, 4H, $-CH_2-$); ^{13}C NMR ($CDCl_3$) δ 172.9 (CO), 154.0 (C_5), 131.6 (C_{7a}), 127.7 (C_{3a}), 123.0 (C_2), 112.5 (C_3), 112.2 (C_7), 112.0 (C_4), 100.6 (C_6), 56.0 (OCH_3), 39.5 ($-CH_2NH-$), 36.2 ($-CH_2CO-$), 25.0 ($-CH_2CH_2NH-$), 25.2 ($-CH_2CH_2CO-$); MS (EI^+) *m/z* 490 (M^+, 17), 318 (21), 301 (16), 173 (100); HREIMS: calc. for $C_{28}H_{34}N_4O_4$ 490.2580, found 490.2584.

1,8-Octanedioic acid bis-(5-methoxytryptamin-N_b-yl)-amide (**10b**) Yield: 97%; amorphous; IR (KBr) ν 3394, 3296, 2934, 1643,1531 cm^{-1}; $UV_{\lambda_{max}}$ 310, 298, 278, 223 nm; ^1H NMR ($CDCl_3$) δ 8.56 (s, 2H, NH_{ind}), 6.86–7.25 (m, 8H, ArH), 5.60 (brs, 2H, NH), 3.85 (s, 6H, OCH_3), 3.61 (m, 4H, $-CH_2NH-$), 2.96 (t, J = 7.2, 4H, $-CH_2CH_2NH-$), 2.06 (m, 4H, $-CH_2CO-$), 1.52 (m, 4H, $-CH_2-$),1.18 (m, 4H, $-CH_2-$); ^{13}C NMR ($CDCl_3$) δ 172.9 (CO), 153.9 (C_5), 131.7 (C_{7a}), 127.6 (C_{3a}), 123.1 (C_2), 112.1 (C_4), 112.0 (C_3), 111.9 (C_7), 100.5 (C_6), 55.9 (OCH_3), 39.5 ($-CH_2NH-$), 36.4 ($-CH_2CO-$), 28.5 ($-CH_2CH_2CO-$), 25.4 ($-CH_2CH_2NH-$), 25.1 (CH_2); MS (EI^+) *m/z* 518 (M^+, 16), 346 (17), 227 (6), 214 (17), 173 (100); HREIMS: calc. for $C_{30}H_{38}N_4O_4$ 518.2893, found 518.2912.

1,12-Dodecanedioic acid bis-(5-methoxytryptamin-N_b-yl)-amide (**10c**) Yield: 96%; amorphous; IR (KBr) ν 3387, 3291, 2926, 1651, 1485 cm^{-1}; $UV_{\lambda_{max}}$ 310, 297, 279, 221 nm; ^1H NMR ($CDCl_3$) δ 8.55 (s, 2H, NH_{ind}), 6.86–7.27 (m, 8H, ArH), 5.80 (brs, 2H, NH), 3.84 (s, 6H, OCH_3), 3.57 (m, 4H, $-CH_2NH-$), 2.93 (t, J = 7.5, 4H, $-CH_2CH_2NH-$), 2.11 (m, 4H, $-CH_2CO-$), 1.56 (m, 4H, $-CH_2-$),1.22 (m, 8H, $-CH_2-$); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO), 153.5 (C_5), 131.6 (C_{7a}), 127.4 (C_{3a}), 123.0 (C_2), 112.0 (C_4), 111.8 (C_3), 111.7 (C_7), 100.2 (C_6), 55.7 (OCH_3), 39.6 ($-CH_2NH-$), 36.4 ($-CH_2CO-$), 29.0 ($-CH_2CH_2CO-$), 28.9 (CH_2), 28.8 (CH_2), 25.5 (CH_2), 25.0 ($-CH_2CH_2NH-$); MS (EI^+) *m/z* 574 (M^+, 3), 402 (4), 386 (1), 173 (100); HREIMS: calc. for $C_{34}H_{46}N_4O_4$ 574.3519, found 574.3509.

Bischler–Napieralsky Cyclization of Diamides 9, 10

General procedure: A mixture of diamide **9** or **10** (2.5 mmol) and freshly distilled $POCl_3$ (10 ml) was refluxed under nitrogen for 10 min, then cooled and stirring was maintained at room temperature for 24 h. After evaporation of $POCl_3$ the residue was treated with ice-water (100 ml), CH_2Cl_2 (200 ml) was added, the mixture was adjusted to pH 14 with NaOH (10%), and stirred for 3 h. After separation the organic layer was washed with water (2 × 50 ml) and saturated NaCl solution (2 × 50 ml), dried ($MgSO_4$), filtered and finally evaporated to dryness to afford dihydro-β-carbolines **3** or **4**.

1-[1-(3,4-Dihydro-β-carbolin-1-yl)-but-4-yl]-3,4-dihydro-β-carboline (**3a**). Yield: 81%; m.p.: 188°C (dec.); IR (KBr) ν 3420, 3067, 2942, 1641 cm^{-1}; $UV_{\lambda_{max}}$ 349, 325, 318, 240, 227, 204 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 7.15–7.56 (m, 8H, ArH), 3.80 (t, J = 7.7, 4H, $-CH_2N=$), 2.82 (t, J = 7.7, 4H, $-CH_2CH_2N=$), 2.71 (m, 4H, $CH_{2_{chain}}$), 1.73 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 163.1 (C1), 137.4 (C_{8a}), 128.3 (C_{9a}), 124.9 (C_{4b}), 124.4 (C_7), 119.8 (C_5), 119.7 (C_6), 116.7 (C_{4a}), 112.2(C_8), 47.3 (C_3), 34.1 ($C_{1'}$), 26.2 ($C_{2'}$)*, 19.1 (C_4)*; (*indicates interchangeable signals in the whole paper) MS (EI^+) *m/z* 394 (M^+, 9), 364 (11), 350 (44), 210 (100), 197 (41), 182 (17); HREIMS: calc. for $C_{26}H_{26}N_4$ 394.2157, found 394.2152.

1-[1-(3,4-Dihydro-β-carbolin-1-yl)-hex-6-yl]-3,4-dihydro-β-carboline (**3b**). Yield: 92%; m.p.: 146°C; IR (KBr) ν 3135, 3063, 2932, 1601, 1547 cm^{-1}; $UV_{\lambda_{max}}$ 349, 318, 240, 228, 207 nm; ^1H NMR ($CDCl_3$) δ 10.35 (brs, 2H, NH_{ind}), 7.12–7.57 (m, 8H, ArH), 3.85 (t, J = 7.8, 4H, $-CH_2N=$), 2.85 (t, J = 7.7, 4H, $-CH_2CH_2N=$), 2.68 (t, J = 7.6, 4H, $CH_{2_{chain}}$), 1.69 (m, 4H, $CH_{2_{chain}}$), 1.37 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$) δ 162.2 (C_1), 137.2 (C_{8a}), 129.0 (C_{9a}), 125.4 (C_{4b}), 124.2 (C_7), 120.0 (C_6), 119.9 (C_5), 116.7 (C_{4a}), 112.2 (C_8), 47.8 (C_3), 34.7 ($C_{1'}$), 28.0 ($C_{2'}$), 26.0 ($C_{3'}$)*, 19.5 (C_4)*; MS (EI^+) *m/z* 422 (M^+, 100), 278 (24), 237 (37), 195 (71), 155 (56); HREIMS: calc. for $C_{28}H_{30}N_4$ 422.2470, found 422.2461.

1-[1-(3,4-Dihydro-β-carbolin-1-yl)-dec-10-yl]-3,4-dihydro-β-carboline (**3c**). Yield: 35%; m.p.: 134°C; IR (KBr) ν 3360, 3235, 2930, 1609, 1551 cm^{-1}; $UV_{\lambda_{max}}$ 349, 318, 242, 227, 207 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 10.1 (brs, 2H, NH_{ind}), 7.22–7.67 (m, 8H, ArH), 3.98 (t, J = 7.7, 4H, $-CH_2N=$), 3.27 (t, J = 7.7, 4H, $-CH_2CH_2N=$), 3.14 (t, J = 7.5, 4H, $CH_{2_{chain}}$), 1.83 (m, 4H, $CH_{2_{chain}}$),1.48 (m, 4H, $CH_{2_{chain}}$), 1.33 (m, 8H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 169.9 (C_1), 141.5 (C_{8a}), 128.9 (C_{9a}), 124.8 (C_7), 124.3 (C_{4b}), 123.8 (C_{4a}), 121.5 (C_6), 121.0 (C_5), 113.1 (C_8), 41.7 (C_3), 32.1 ($C_{1'}$), 28.4 ($C_{2'}$), 28.3 ($C_{3'}$), 28.2 ($C_{4'}$), 27.3 ($C_{5'}$), 18.3 (C_4); MS (EI^+) *m/z* 478 (M^+, 63), 197 (53), 184 (100); HREIMS: calc. for $C_{32}H_{38}N_4$ 478.3096, found 478.3095.

1-[1-(6-Methoxy-3,4-dihydro-β-carbolin-1-yl)-but-4-yl]-6-methoxy-3,4-dihydro-β-carboline (**4a**). Yield: 73%; m.p.: 212°C; IR (KBr) ν 3186, 2940, 1605, 1547 cm^{-1}; $UV_{\lambda_{max}}$ 372, 328, 315, 205 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 6.93–7.37 (m, 6H, ArH), 3.83 (s, 6H, OCH_3), 3.78 (t, J = 7.5, 4H, $-CH_2N=$), 2.85 (t, J = 7.5, 4H, $-CH_2CH_2N=$), 2.66 (m, 4H, $CH_{2_{chain}}$), 1.83 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 164.0 (C_1), 154.4 (C_6), 133.7 (C_{8a}), 128.9 (C_{9a}), 125.3 (C_{4b}), 117.2 (C_{4a}), 116.7 (C_7), 113.7 (C_8), 55.9 (OCH_3), 47.0 (C_3), 26.7 ($C_{1'}$), 19.5 ($C_{2'}$)*, 19.4 (C_4)*; MS (EI^+) *m/z* 454 (M^+, 8), 410 17), 240 (100), 227 (36); HREIMS: calc. for $C_{28}H_{30}N_4O_2$ 454.2369, found 454.2368.

1-[1-(6-Methoxy-3,4-dihydro-β-carbolin-1-yl)-hex-6-yl]-6-methoxy-3,4-dihydro-β-carboline (**4b**). Yield: 73.3%; m.p.: 207°C; IR (KBr) ν 2930, 1605, 1547 cm^{-1}; $UV_{\lambda_{max}}$ 362, 328, 232, 212 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 6.91–7.40 (m, 6H, ArH), 3.84 (s, 6H, OCH_3), 3.79 (t, J = 7.6, 4H, $-CH_2N=$), 2.90 (t, J = 7.6, 4H, $-CH_2CH_2N=$), 1.68 (m, 4H, $CH_{2_{chain}}$), 1.44 (m, 4H, $CH_{2_{chain}}$), 1.26 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 165.2 (C_1), 154.3 (C_6), 134.2 (C_{8a}), 127.8 (C_{9a}), 124.8 (C_{4b}), 118.1 (C_{4a}), 117.5 (C_7), 113.7 (C_8), 99.9 (C_5), 55.5 (OCH_3), 45.5 (C_3), 29.5($C_{1'}$), 27.7 ($C_{2'}$), 26.8 ($C_{3'}$)*, 19.2 (C_4)*; MS (EI^+) *m/z* 482 (M^+, 37), 310 (11), 293 (8), 281 (12), 267 (28), 253 (11), 241 (23), 227 (57); HREIMS: calc. for $C_{30}H_{34}N_4O_2$ 482.2682, found 482.2685.

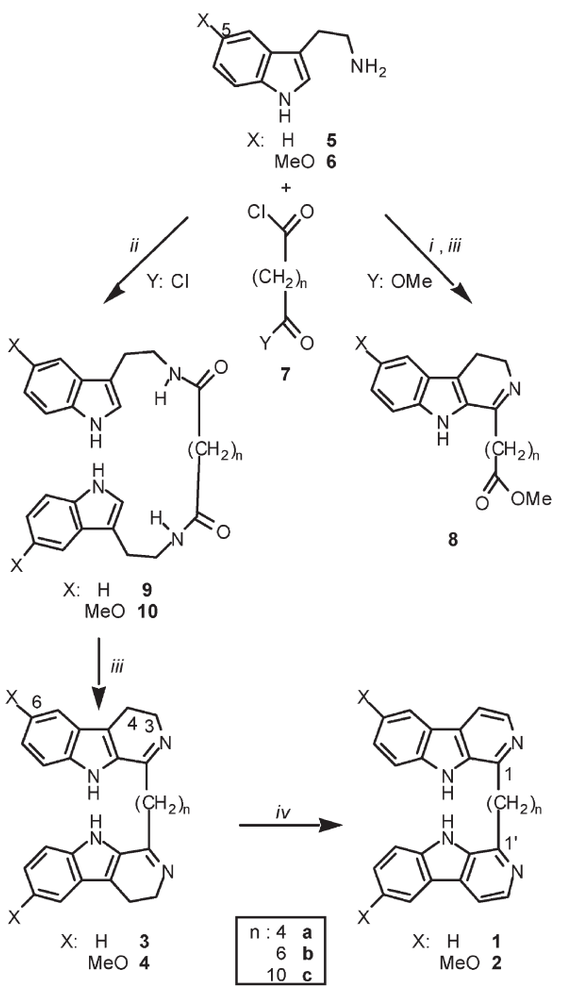
1-[1-(6-Methoxy-3,4-dihydro-β-carbolin-1-yl)-dec-10-yl]-6-methoxy-3,4-dihydro-β-carboline (**4c**) Yield: 28%; m.p.: 124°C; IR (KBr) ν 3403, 3288, 2926, 1651, 1537 cm^{-1}; $UV_{\lambda_{max}}$ 392, 328, 234, 212 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 6.97–7.33 (m, 6H, ArH), 3.88 (t, J = 7.6, 4H, $-CH_2N=$), 3.86 (s, 6H, OCH_3), 2.83 (t, J = 7.6, 4H, $-CH_2CH_2N=$), 2.65 (t, J = 7.5, 4H, $CH_{2_{chain}}$), 1.63 (m, 4H, $CH_{2_{chain}}$), 1.34 (m, 8H, $CH_{2_{chain}}$), 1.26 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 163.5 (C_1), 153.9 (C_6)C6), 132.7 (C_{8a}), 128.7 (C_{9a}), 124.9 (C_{4b}), 116.5 (C_{4a}), 115.8 (C_7), 113.0 (C_8), 100.1 (C_5), 55.5 (OCH_3), 46.8 (C_3), 29.4($C_{1'}$), 28.9 ($C_{2'}$), 28.9 ($C_{3'}$), 27.2 ($C_{4'}$), 27.1 ($C_{5'}$), 19.1 (C_4); MS (EI^+) *m/z* 538 (M^+, 37), 344 (38), 227 (36), 214 (100); HREIMS: calc. for $C_{34}H_{42}N_4O_2$ 538.3308, found 538.3313.

Preparation of 1,1′-bis-β-carbolines 1, 2

General procedure for aromatization of **3**, **4**. A mixture of 3,4-dihydro-β-carboline **3** or **4** (0.3 mmol) and 10% Pd catalyst on charcoal (100 mg) in *p*-cymene (10 ml) was refluxed under nitrogen for 6–8 h. At the end of the reaction the catalyst was filtered off on celite, washed with CH_2Cl_2–MeOH (5 × 10 ml), and the extracts were evaporated to dryness under reduced pressure. The residue was purified by preparative thin layer chromatography (eluant: CH_2Cl_2:MeOH:NH_4OH 9:1:0.2) to afford the corresponding bis-β-carbolines **1**, **2**.

1-[1-(β-Carbolin-1-yl)-but-4-yl]-β-carboline (**1a**) Yield: 42%; m.p.: 182°C; IR (KBr) ν 3256, 3207, 3155, 3067, 2941, 1628 cm^{-1}; $UV_{\lambda_{max}}$ 352, 287, 250, 233 nm; ^1H NMR (DMSO-d_6) δ 11.66 (brs, 2H, NH), 8.25 (d, J = 5.8, 2H, H-3), 8.20 (d, J = 8.1, 2H, H-5), 7.95 (d, J = 5.8, 2H, H-4), 7.62 (d, J = 8.1, 2H, H-8), 7.55 (t, J = 8.1, 2H, H-7), 7.25 (t, J = 8.1, 2H, H-6), 3.22 (m, 4H, $CH_{2_{chain}}$), 1.98 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR (DMSO-d_6) δ 145.9 (C_1), 140.6 (C_{8a}), 137.5 (C_3), 134.2 (C_{9a}), 128.0 (C_7), 127.4 (C_{4a}), 121.8 (C_5), 121.2 (C_{4b}), 119.3 (C_6), 112.8 (C_4), 112.1 (C_8), 33.4 ($C_{1'}$), 28.3 ($C_{2'}$); MS (EI^+) *m/z* 390 (M^+, 54), 208 (97), 195 (100); HREIMS: calc. for $C_{26}H_{22}N_4$ 390.1844, found 390.1829.

1-[1-(β-Carbolin-1-yl)-hex-6-yl]-β-carboline (**1b**) Yield: 43%; m.p.: 228°C; IR (KBr) ν 3198, 3144, 2933,



Conditions: *i* :1 eq. **5**; *ii* : 2 eq. **5** or **6**, Et_3N or NaOH; *iii* : $POCl_3$; *iv* : Pd-C, *p*-cymene, reflux.

SCHEME 1 Synthesis of bis-3,4-dihydro-β-carbolines and bis-β-carbolines.

1626, 1566 cm^{-1}; $UV_{\lambda_{max}}$ 351, 336, 289, 251, 240, 234 nm; ^1H NMR (DMSO-d_6) δ 11.36 (brs, 2H, NH), 8.23 (d, J = 5.1, 2H, H-3), 8.03 (d, J = 8.1, 2H, H-5), 7.81 (d, J = 5.1, 2H, H-4), 7.58 (d, J = 8.1, 2H, H-8), 7.42 (t, J = 8.1, 2H, H-7), 7.18 (t, J = 8.1, 2H, H-6), 3.14 (m, 4H, $CH_{2_{chain}}$), 1.76 (m, 4H, $CH_{2_{chain}}$), 1.25 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR (DMSO-d_6) δ 145.5 (C_1), 141.6 (C_{8a}), 135.6 (C_3), 134.6 (C_{9a}), 129.5 (C_{4a}), 128.7 (C_7), 121.7 (C_5), 121.3 (C_{4b}), 120.0 (C_6), 113.2 (C_4), 112.3 (C_8), 32.6($C_{1'}$), 28.0 ($C_{2'}$), 27.8 ($C_{3'}$); MS (EI^+) *m/z* 418 (M^+, 31), 237 (58), 209 (27), 195 (77); HREIMS: calc. for $C_{28}H_{26}N_4$ 418.2157, found 418.2100.

1-[1-(β-Carbolin-1-yl)-dec-10-yl]-β-carboline (**1c**) Yield: 25%; m.p.: 218°C; IR (KBr) *ν* 3398, 2924, 1626, 1500 cm^{-1}; $UV_{\lambda_{max}}$ 350, 337, 288, 249, 234 nm; ^1H NMR ($CDCl_3$ + CD_3OD) δ 10.58 (brs, 2H, NH), 8.25 (d, J = 5.4, 2H, H-3), 8.07 (d, J = 8.1, 2H, H-5), 7.79 (d, J = 5.4, 2H, H-4), 7.54 (d, J = 8.1, 2H, H-8), 7.49 (t, J = 8.1, 2H, H-7), 7.23 (t, J = 8.1, 2H, H-6), 3.12 (m, 4H, $CH_{2_{chain}}$), 1.80 (m, 4H, $CH_{2_{chain}}$), 1.32 (m, 4H, $CH_{2_{chain}}$), 1.16 (m, 4H, $CH_{2_{chain}}$), 1.08 (m, 4H, $CH_{2_{chain}}$); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 146.0 (C_1), 140.7 (C_{8a}), 137.0 (C_3), 134.4 (C_{9a}), 128.6 (C_{4a}), 128.1 (C_7), 121.4 (C_5), 121.4 (C_{4b}), 119.5 (C_6), 112.8 (C_4), 111.7 (C_8), 33.8 ($C_{1'}$), 29.3 ($C_{2'}$), 29.0 ($C_{3'}$), 28.9 ($C_{4'}$), 28.7 ($C_{5'}$); MS (EI^+) *m/z* 474 (M^+, 14), 293 (34), 195 (38), 182 (100); HREIMS: calc. for $C_{32}H_{34}N_4$ 474.2783, found 474.2793.

1-[1-(6-Methoxy-β-carbolin-1-yl)-dec-10-yl]-6-methoxy-β-carboline (**2c**) Yield: 22%; m.p.: 212°C; IR (KBr) *ν* 3133, 3063, 2928, 1601, 1566 cm^{-1}; $UV_{\lambda_{max}}$ 369, 296, 287, 257, 245, 232 nm; ^1H NMR ($CDCl_3$) δ 10.19 (brs, 2H, NH), 8.33 (d, J = 5.4, 2H, H-3), 7.81 (d, J = 5.4, 2H, H-4), 7.56 (d, J = 2.7, 2H, H-5), 7.43 (t, J = 8.1, 2H, H-8), 7.17 (dd, J = 8.1 and 2.7, 2H, H-7), 3.92 (s, 6H, OCH_3), 3.12 (m, 4H, $CH_{2_{chain}}$), 1.80 (m, 4H, $CH_{2_{chain}}$), 1.21 (m, 4H, $CH_{2_{chain}}$), 1.04 (m, 4H, $CH_{2_{chain}}$), 0.96 (m, 4H, $CH_{2_{chain}}$); MS (EI^+) *m/z* 534 (M^+, 28), 323 (48), 225 (41), 212 (100); HREIMS: calc. for $C_{34}H_{38}N_4O_2$ 534.3008, found 534.2995.

Biology

L-1210 cells (Murine Leukemia) provided by the NCI, Frederik, USA were cultivated in RPMI 1640 medium (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). Cytoxicity was measured by the micro-culture tetrazolium assay.[20] Cells were exposed to graded concentrations of the compounds for 48 h and results expressed as IC_{50} values (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls).

RESULTS AND DISCUSSION

Chemistry

We first investigated the stepwise approach **5** + **7** → **8** → **1**, based on two successive Bischler–Napieralsky (B–N) cyclizations (Scheme 1). This method turned out to be rather disappointing: for example, for n = 1, the yield of **8** from tryptamine did not exceed 24%. Similar difficulties have already been mentioned by Frost.[21]

Owing to the problems encountered, we finally turned back to the use of diamides **9**, **10** in a double B–N cyclization strategy which we had already used for the synthesis of large-ring bis-indole dilactams.[22] We observed that upon heating **9** (X = H) with $POCl_3$ for a short period (5–10 min, t.l.c. monitoring), dihydro-derivatives **3** (X = H) were obtained in yields varying from 69 to 97%. Aromatization of **3**

TABLE I Cytotoxicity against L-1210 cell line of bis β-carbolines (**1**, **2**) and their 3,4-dihydro analogs (**3**, **4**)

	X	n	IC_{50} (μM)*
1a	H	4	3.5
1b	H	6	3.5
1c	H	10	n.t.†
2c	OMe	10	19
3a	H	4	1.2
3b	H	6	1.1
3c	H	10	n.t.
4a	OMe	4	2.0
4b	OMe	6	5.3
4c	OMe	10	1.5

*Results are a mean of three experiments. †n.t.: Not tested.

(X = H) was performed with 10% Pd on charcoal in boiling *p*-cymene to give **1** (X = H). Isolated product yields tended to be generally low (25%–43%), due to tedious chromatographic purifications.

The synthesis of the respective methoxy-substituted derivatives (X = OMe) proved to be far more troublesome, as Bischler–Napieralsky ring closure dramatically depended on the quality of $POCl_3$, and the yields were generally lower (28–73%) than in the non-substituted series. Until now aromatization to **2c** has only been performed for $n = 10$, under the above mentioned conditions.

Biology

The *in vitro* cytotoxic potentials were evaluated by measurement of IC_{50} values of derivatives **1**, **2** and **3**, **4** toward leukemic cells (Table I). According to the tests, the presence of the 6-OMe group did not improve activity. More surprisingly, aromatic compounds **1**, **2** were in the same range of activities as **3**, **4**: this could be attributed to the fact that protonation of N(2) would be a more important factor than planarity of the molecule for DNA binding.

These preliminary results show that our β-carboline pseudo-dimers **1**, **2** or **3**, **4** are cytotoxic. Work is in progress in our laboratory to define the nature, the length and the attachment point of the linker to obtain better activities.

Acknowledgements

WQJ thanks the "Ministère des Affaires Etrangères" for a grant, and ADIR Company for financial support.

References

[1] Noé, E., Séraphin, D., Zhang, Q., Djaté, F., Hénin, J., Laronze, J.Y. and Lévy, J. (1996) "Synthesis of the new (cyclopenta[b]-pyrrolo[1,2-d])azepino[4,5-b]indole ring system", *Tetrahedron Letters* **37**, 5701–5704.

[2] For a review: "Bisnafide mesylate". *Drugs of the Future*, **21** (1996) 239–244.

[3] Cholody, W.M., Hernandez, L., Hassner, L., Scudiero, D.A., Djurickovic, D.B. and Michejda, C.J. (1995) "Bisimidazoacridones and related compounds: new antineoplastic agents with high selectivity against colon tumors", *Journal of Medicinal Chemistry* **38**, 3043–3052.

[4] Denny, W.A., Gamage, S.A., Spicer, J.A., Baguley, B.C. and Graeme, J. (1998) "Bis(acridinecarboxamide) and bis(phenazinecarboxamide) as antitumor agents. Patent Cooperation Treaty International Application WO 98 17,650", *Chemical Abstracts* **128**, 308499g.

[5] Okahana, H., Tsuzuki, S., Mori, J. and Furukawa, H. (1994) "Preparation of pyridocarbazoles as neoplasm inhibitors. Japan Patent 06 80,668 [94 80,668]", *Chemical Abstracts* **121**, 108767m.

[6] Gregson, S.J., Howard, P.W., Hartley, J.A., Brooks, N.A., Adams, L.J., Jenkins, T.C., Kelland, L.R. and Thurston, D.E. (2001) "Design, synthesis, and evaluation of a novel pyrrolobenzodiazepine DNA-interactive agent with highly efficient cross-linking ability and potent cytotoxicity", *Journal of Medicinal Chemistry* **44**, 737–748.

[7] Gregson, S.J., Howard, P.W., Corcoran, K.E., Brooks, N.A., Jenkins, T.C., Kelland, L.R. and Thurston, D.E. (2001) "Synthesis of the first example of a C2–C3/C2′–C3′-endo unsaturated pyrrolo[2,1-c][1,4]benzodiazepine dimer", *Bioorganic and Medicinal Chemistry Letters* **11**, 2859–2862.

[8] Reddy, P.B.S., Damayanthi, Y. and Lown, W. (1999) "Design and efficient synthesis of novel DNA interstrand cross-linking agents: C-2 linked pyrrolo[2,1-*c*][1,4]benzodiazepine dimers", *Synlett*, 1112–1114.

[9] Antonini, I., Martelli, S. and Polucci, P. (1999) "Bis-acridinecarboxamides having antitumor activity. Patent Treaty Cooperation International Apply WO 99 06,372", *Chemical Abstracts* **130**, 168246m.

[10] Deady, L.W., Kaye, A.J., Finlay, G.J., Baguley, B.C. and Denny, W.A. (1997) "Synthesis and antitumor properties of N-[2-(dimethylamino)ethyl]carboxamide derivatives of fused tetracyclic quinolines and quinoxalines: a new class of putative topoisomerase inhibitors", *Journal of Medicinal Chemistry* **40**, 2042–2046.

[11] Lokey, R.S., Kwok, Y., Guelev, V., Pursell, C.J., Hurley, L.H. and Iverson, B.L. (1997) "A new class of polyintercalating molecules", *Journal of the American Chemical Society* **119**, 7202–7210.

[12] Xiao, S., Lin, W., Wang, C. and Yang, M. (2001) "Synthesis and biological evaluation of DNA targeting flexible side-chain substituted β-carboline derivatives", *Bioorganic and Medicinal Chemistry Letters* **11**, 437–441.

[13] Olmsted, S.L., Takemori, A.E. and Porthoghese, P.S. (1993) "A remarkable change of opioid receptor selectivity on the attachment of a peptidomimetic κ address element to the δ antagonist. Naltrindole: 5′-[(N2-alkylamidino)methyl]-naltrindole derivatives as a novel class of κ opioid receptor antagonists", *Journal of Medicinal Chemistry* **36**, 179–180.

[14] Hu, M.-K. and Lu, C.-F. (2000) "A facile synthesis of bis-tacrine isosters", *Tetrahedron Letters* **41**, 1815–1818.

[15] Carlier, P.R., Du, D.-M., Han, Y.-F., Liu, J., Perola, E., Williams, I.D. and Pang, Y.-P. (2000) "Dimerization of an inactive fragment of huperzine A produces a drug with twice the potency of the natural product", *Angewandte Chemie International Edition* **39**, 1775–1777.

[16] Guillou, C., Mary, A., Renko, D.Z., Gras, E. and Thal, C. (2000) "Potent acetylcholinesterase inhibitors: design, synthesis and structure–activity relationships of alkylene linked bis-galanthamine and galanthamine-galanthaminium salts", *Bioorganic and Medicinal Chemistry Letters* **10**, 637–639.

[17] Vennerstrom, J.L., Ager, Jr., A.L., Dorn, A., Andersen, S.L., Gerena, L., Ridley, R.G. and Milhous, W.K. (1998) "Bisquinolines 2. Antimalarial *N,N*-Bis(7-chloroquinolin-4-yl)heteroalkanediamines", *Journal of Medicinal Chemistry* **41**, 4360–4364.

[18] Sestelo, J.P., Mourino, A. and Sarandeses, L.A. (1999) "Design and synthesis of a 1,25-dihydroxyvitamin D_3 dimer as a potential chemical inducer of vitamin D receptor dimerization", *Organic Letters* **1**, 1005–1007.

[19] Zhao, H., Neamati, N., Mazumder, A., Sunder, S., Pommier, Y. and Burke, Jr., T.R. (1997) "Arylamide inhibitors of HIV-1 integrase", *Journal of Medicinal Chemistry* **40**, 1186–1194.
[20] Pierré, A., Kraus-Berthier, L., Atassi, G., Cros, S., Poupon, M.-F., Lavielle, G., Berlion, M. and Bizzari, J.-P. (1991) "Preclinical antitumor activity of a new vinca alkaloid derivative, S 12363", *Cancer Research* **51**, 2312–2318.
[21] Frost, J.R., Gaudillière, B.R.P. and Wick, A.E. (1985) "New evidence for the intermediacy of spiroindolenines in the Bischler–Napieralsky cyclization of N_β-acyltryptamines", *Chemical Communications*, 895–897.
[22] Charlet-Fagnère, C., Jiang, W.-Q. and Laronze, J.-Y. (1999) "Syntheses of large-ring bis-indolic dilactams", *Tetrahedron Letters* **40**, 1685–1688.