Synthesis and *in Vitro* Cytotoxic Evaluation of *N*-Substituted Benzo[5,6]cyclohepta[*b*]indoles

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A new series of *N*-substituted benzo[5,6]cyclohepta[*b*]indole derivatives were synthesised and evaluated for *in vitro* cytotoxic activities against L1210 murine leukemia and HT29 cell lines. Among them, several compounds showed potent antitumor activity and blocked cell cycle progression of L1210 cells in G_2+M phase.

Key words synthesis; indole; seven-membered ring; cytotoxicity; cell cycle progression analysis

The DNA molecule is an important target of many antitumor agents.[1)] Conformational changes in the double helix is the result of the binding of drugs to DNA involving modification in the mechanism of DNA replication and consequently cell killing. Different derivatives such as ellipticine or related structures,[2)] DACA[3)] and TAS-103[4)] (Fig. 1) are highly toxic to tumour cells through their capacity to intercalate into DNA and to poison DNA topoisomerases.

On the basis of these facts, we became interested in the synthesis of related ellipticine skeleton with a seven-membered ring to evaluate their antitumoral potential. First, we reported the synthesis of tetracyclic derivative **1**,[5)] which exhibited potent cytotoxic activity *in vitro* on L1210 murine leukemia cells. Unfortunately, a non specific L1210 cell cycle effect and a toxicity at 50 μM were also observed for the latter. From these pharmacological results, we investigated the preparation and the reactivity of 5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one **2** or 5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-6-one **3** (Fig. 2), with a view to designing potent anticancer agents.[6)]

In the present study, *N*-substituted benzo[5,6]cyclohepta-[*b*]indole derivatives A (Fig. 2) were synthesised and evaluated. Three structural modifications were investigated to improve the antitumour activity: 1) length of the amino linker chain, 2) nature of the amino substituents, and 3) nature of the indole substituent in position-5.

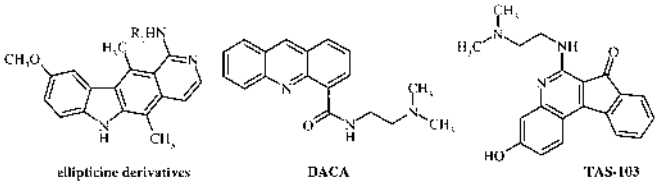

Fig. 1

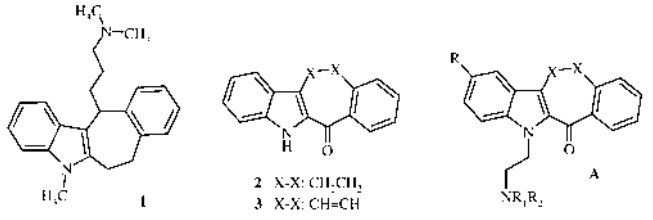

Fig. 2

Chemistry Following the procedure used for the preparation of **2**,[6)] we first prepared the fused 5-methoxyindole ring system **9** starting from either 5-methoxy-1-phenylsulfonylindole-3-carboxaldehyde **4a**[7)] or 5-methoxy-1-*tert*-butoxycarbonylindole-3-carboxaldehyde **4b** (Chart 1). A Wittig reaction of **4** with the ylide generated from **5**[8)] gave an *E*/*Z* mixture of alkenes **6** in 60—65% yield. Compounds **6** were hydrogenated over 10% Pd/C catalyst to afford **7** (96% yield). Saponification of ester **7a** gave the corresponding acid **8a** in 65% yield. The same conditions applied to **7b** afforded the *N*-deprotected acid **8b** (84% yield). Intramolecular acidic cyclisation[9)] of acid **8b** afforded tetracyclic compound **9** in 65% yield.

With **8a**, the cyclisation led to **9** and the unexpected 5-methoxy-6-phenylsulfonyl-5,6,11,12-tetrahydrobenzo[5,6]-cyclohepta[*b*]indol-6-one **10** in 20—30% yield (Chart 2). The structures of **9** and **10** were confirmed by one and two dimensional (1D, 2D) NMR, (NOESY) and (HETCOR) data (Fig. 3). No cyclisation on position-4 of the indole ring was observed.

All derivatives **11**—**15** and **19**, **20** were synthesised by reaction of an alkyl halide (chloride or bromide) with an indolic anion, which was conveniently prepared from **2**, **3**, **9**, **18** in the presence of sodium hydride or KOH in *N*,*N*-dimethylformamide (DMF). First, we obtained 5,6,11,12-tetrahydrobenzo derivatives **11** and **12** in 65—73% yield from **2** with 2-(dimethylamino)ethyl chloride or with 3-(dimethylamino)propyl chloride; then, the 5,6-dihydrobenzo derivatives **13** and **14** were similarly obtained from **3** in 60—70% yield. *N*-Alkylation of **3** with an excess of 1,3-dibromopropane afforded **15** which was treated by 2-aminoethanol to lead to the derivative **16** in 46% yield (Chart 3).

A number of reports have discussed the synthesis of dimeric compounds designed as potential bis-intercalating agents.[10)] Treatment of **3** with an appropriate amount of 1,6-dibromohexane gave the dimeric form **17** in 60% yield (Chart 4). Unfortunately, the latter has not been tested due to its low solubility in solvents.

N-Alkylation of **9** was performed to give a mixture of 5-methoxy-5,6-dihydrobenzo and 5-methoxy-5,6,11,12-tetrahydrobenzo derivatives not separable by column chromatography. To solve this problem, only 5-methoxy-5,6-dihydrobenzo derivatives were prepared as a pure form. The unsaturated ketone **18** was obtained in 90% yield by treatment of **9** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

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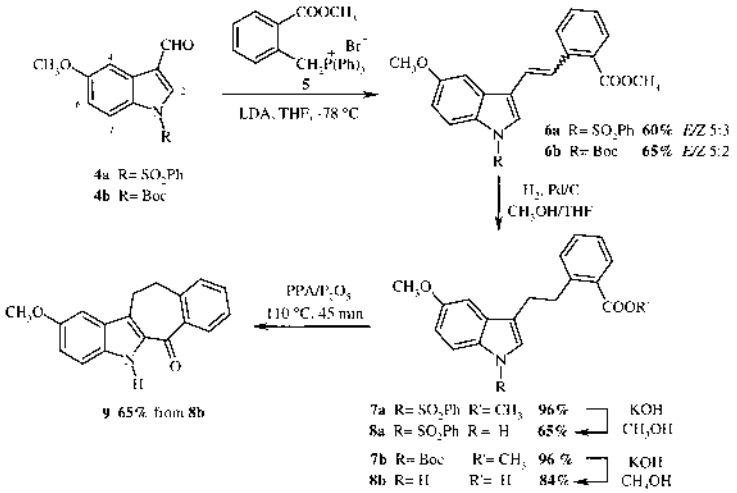

Chart 1

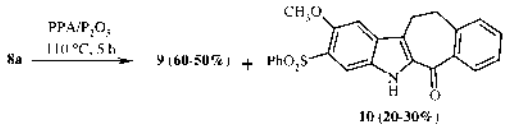

Chart 2

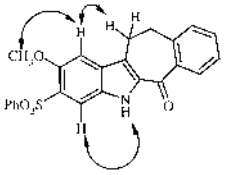

Fig. 3. Correlations in the NOESY Spectrum of **10**

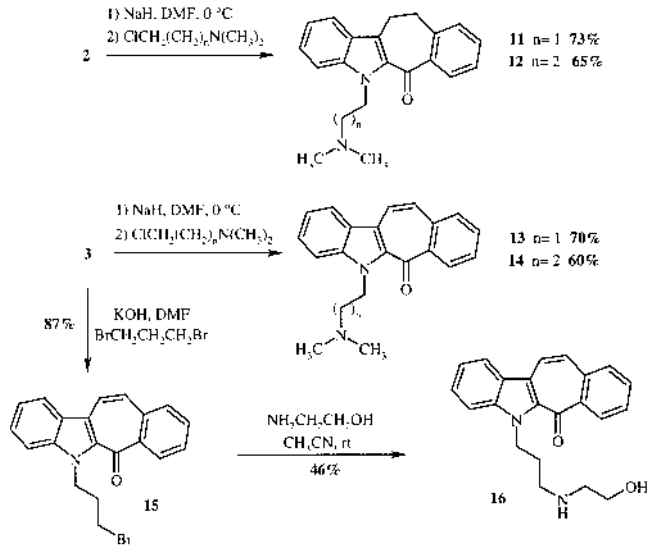

Chart 3

in dioxane. Final alkylation led to **19** and **20** in fair yields (Chart 5).

Pharmacology The cytotoxic properties of only five compounds **11**, **13**, **14**, **16** and **19** were assessed by a cell growth inhibition assay using the murine L1210 leukemia and HT29 (colon cancer) cell lines.[11] The results are expressed as IC_{50} (concentration reducing the cell proliferation by 50%). Similarly, to examine the effect of the new derivatives on cell cycle progression, flow cytometric analysis was performed. All pharmacological results are reported in Table 1.

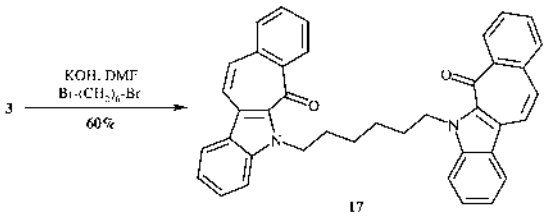

Chart 4

Results and Discussion

The 5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one derivative **11** was first evaluated. The calculated IC_{50} value with the murine L1210 leukemia cell line was 16.8 μM. The unsaturated compound **13** is significantly more active (IC_{50}=3.4 μM) than its saturated counterpart **11**. Next, we investigated the effect of the amino alkyl chain on the cytotoxic profile. Insertion of one methylene in the alkyl chain as in **14** or modification of the terminal amino part as **16** slightly improves the cytotoxicity (**14**: IC_{50}=2.9 μM, **16**: IC_{50}=1.9 μM), although it's inferior to that measured with the tumour-active drug adriamycine. Similarly, compound **14** shows the same overall activity against HT29 cells (IC_{50}=2.4 μM). In the 5-methoxy series, compound **19** has the same potency against L1210 or HT29 cell lines as **14**.

Compounds **13**, **14**, **16**, and **19** induce an accumulation of L1210 cells in the G_2+M phase of the cell cycle. Among them, compound **13** causes a massive G_2+M arrest at a concentration of 2.5—5 μM.

These preliminary biological results show that *N*-substituted 5,6-dihydrobenzo-[5,6]cyclohepta[*b*]indol-6-one derivatives are an interesting new class of cytotoxic compounds worthy of further development.

Experimental

Chemistry Melting points were determined using a Büchi capillary instrument and are uncorrected. The infrared (IR) spectra of compounds were

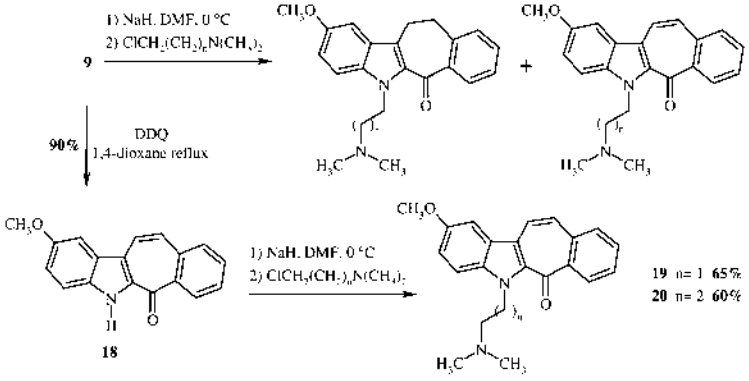

Chart 5

Table 1. Inhibition of L1210 Cell Proliferation and L1210 Cell Cycle Progression by Compounds **11**, **13**, **14**, **16** and **19**

Compound	IC_{50} (μM) L1210	HT29	% of L1210 cells[a] in the G_2+M phase (μM)
Adriamycine	0.024	0.066	+++[c] (0.1 μM)
11	16.8	NT[b]	+ (50 μM)
13	3.4	NT	+++ (2.5—5 μM)
14	2.9	3.1	++ (5 μM)
16	1.9	NT[b]	++ (5 μM)
19	2.9	2.4	+ (10 μM)

a) 24% of untreated control cells were in the G_2+M phase of the cell cycle. *b*) Not tested. *c*) +30—50%, ++ 50—70%, +++ >70%.

recorded on a Perkin Elmer FT-IR paragon 1000 spectrometer. NMR spectra were recorded at 300 °K in $CDCl_3$ on a Bruker Avance DPX 250. Chemical shifts are expressed in parts per million relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Mass spectra were recorded on Perkin-Elmer SCIEX API 300 using ionspray methodology. The elemental compositions of the compounds agreed to within 0.4% of the calculated value. Thin-layer chromatography (TLC) was run on precoated silica gel plates (Merck $60F_{254}$) and the spots visualised using an ultraviolet lamp. Flash chromatography was carried out on a column using Merck Silica gel 60 (40—63 μm) as the stationary phase. Tetrahydrofuran (THF) was distilled under nitrogen from sodium and benzophenone before use. All reactions requiring anhydrous conditions were conducted in flame-dried apparatus.

5-Methoxy-1-*tert*-butoxycarbonylindole-3-carboxaldehyde (4b) A solution of 5-methoxyindole-3-carboxaldehyde[7] (1.0 g, 5.7 mmol), di-*tert*-butyldicarbonate (1.50 g, 6.9 mmol) and 4-dimethylaminopyridine (100 mg, 0.82 mmol) in anhydrous acetonitrile (100 ml) was stirred at 40 °C for 12 h. After cooling, the solvent was evaporated *in vacuo*. The residue was partitioned between CH_2Cl_2 (50 ml) and 10% aqueous HCl (50 ml), then the aqueous phase was separated and extracted with CH_2Cl_2 (2×30 ml). The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give **4b** (1.54 g, 98%). mp 134—135 °C (EtOAc). IR (KBr) cm^{-1}: 1734, 1676 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.70 (s, 9H, CH_3), 3.89 (3H, s, CH_3), 7.01 (1H, dd, J=2.5, 9.1 Hz, H_6), 7.76 (1H, d, J=2.5 Hz, H_4), 8.01 (1H, d, J=9.1 Hz, H_7), 8.19 (1H, s, H_2), 10.06 (1H, s CHO). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 28.0 (3 CH_3), 55.7 (CH_3), 85.5 (C), 103.7 (CH), 115.5 (CH), 115.9 (CH), 121.3 (C), 126.9 (C), 130.4 (C), 136.7 (CH), 148.1 (C), 157.2 (CO), 185.8 (CO). Ionspray-MS *m*/*z*: 276 (M^++1). *Anal.* Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.77; H, 6.09; N, 4.98.

2-{(*E*/*Z*)-2-[5-Methoxy-1-(phenylsulfonyl)-1*H*-3-indolyl]-1-ethenyl}-benzoic Acid Methyl Ester (6a) To a suspension of (2-carbomethoxybenzyl)triphenyl phosphonium bromide **5**[8] (9.36 g, 19 mmol) in anhydrous THF (120 ml), 2 M lithium diisopropylamide (LDA) in heptane (9.6 ml, 19 mmol) was added dropwise at −78 °C. After 30 min, a solution of **4a** (2.0 g, 6.34 mmol) in THF (20 ml) was added dropwise with vigorous stirring at −78 °C. The mixture was stirred at −78 °C for 1 h, then for 1.5 h at room temperature and THF was evaporated *in vacuo*. The residue was partitioned between EtOAc (30 ml) and 10% aqueous HCl (30 ml), the aqueous phase was separated and extracted with EtOAc (2×30 ml). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The crude oil was purified by column chromatography (eluent petroleum ether/CH_2Cl_2 1 : 1) to afford **6a** as two isomers in 60% yield (1.7 g, *E*/*Z* ratio 5:3, *E* isomer was first eluted).

E-Isomer: mp 139—140 °C (MeOH). IR (KBr) cm^{-1}: 1714 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 3.91 (6H, s, CH_3), 6.99 (1H, dd, J=2.6, 9.1 Hz, H_6), 7.08 (1H, d, J=16.6 Hz, =CH), 7.28—7.53 (6H, m, H_4+H_{Ar}), 7.68 (1H, s, H_2), 7.74 (1H, br d, J=8.0 Hz, H_{Ar}), 7.86—7.97 (4H, m, H_7+H_{Ar}), 8.15 (1H, d, J=16.6 Hz, =CH). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 52.0 (CH_3), 55.6 (CH_3) 103.3 (CH), 114.2 (CH), 114.5 (CH), 121.2 (C), 121.8 (CH), 125.3 (CH), 126.2 (CH), 126.6 (2 CH), 127.1 (CH), 128.1 (C), 128.2 (CH), 129.2 (2 CH), 129.9 (C), 130.2 (C), 130.7 (CH), 132.1 (CH), 133.7 (CH), 137.8 (C), 139.1 (C), 156.8 (C), 167.6 (CO). IS-MS *m*/*z*: 448 (M^++1). *Anal.* Calcd for $C_{25}H_{21}NO_5S$: C, 67.10; H, 4.73; N, 3.13. Found: C, 66.89; H, 4.91; N, 2.99.

Z-Isomer: mp 152—154 °C (MeOH). IR (KBr) cm^{-1}: 1714 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 3.67 (3H, s, CH_3), 3.86 (3H, s, CH_3), 6.61 (1H, d, J=12.0 Hz, =CH), 6.71 (1H, d, J=2.2 Hz, H_4), 6.86 (1H, dd, J=2.2, 9.5 Hz, H_6), 7.01 (1H, s, H_2), 7.14—7.53 (7H, m, =CH+H_{Ar}), 7.68 (2H, d, J=7.0 Hz, H_{Ar}), 7.80 (1H, d, J=9.0 Hz, H_7), 8.01 (1H, dd, J=1.2, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 52.0 (CH_3), 55.4 (CH_3), 102.1 (CH), 114.2 (CH), 114.4 (CH), 118.9 (CH), 119.1 (C), 125.1 (CH), 126.7 (2 CH), 127.4 (CH), 129.0 (C), 129.1 (2CH), 129.3 (CH), 130.5 (C), 130.6 (C), 131.0 (CH), 132 (CH), 132.1 (CH), 133.6 (CH), 138.0 (C), 139.6 (C), 156.4 (C), 167.4 (CO). IS-MS *m*/*z*: 448 (M^++1). *Anal.* Calcd for $C_{25}H_{21}NO_5S$: C, 67.10; H, 4.73; N, 3.13. Found: C, 67.02; H, 4.62; N, 3.00.

2-{(*E*/*Z*)-2-[5-Methoxy-1-(*tert*-butoxycarbonyl)-1*H*-3-indolyl]-1-ethenyl}benzoic Acid Methyl Ester (6b) With the same methodology but using **4b** as starting material, **6b** was isolated after column chromatography (eluent petroleum ether/CH_2Cl_2 1 : 1) in 65% yield as a yellow oil (*E*/*Z* ratio 5 : 2, *E* isomer was first eluted).

E-Isomer: IR (film) cm^{-1}: 1721, 1675 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.72 (9H, s, CH_3), 3.95 (3H, s, CH_3), 3.99 (3H, s, CH_3), 7.03 (1H, dd, J=2.5, 9.1 Hz, H_6), 7.16 (1H, d, J=16.4 Hz, =CH), 7.30 (1H, t, J=8.0 Hz, H_{Ar}), 7.51 (1H, t, J=8.0 Hz, H_{Ar}), 7.61 (1H, d, J=2.5 Hz, H_4), 7.75 (1H, s, H_2), 7.77 (1H, d, J=8.2 Hz, H_{Ar}), 7.97 (1H, dd, J=1.0, 7.7 Hz, H_{Ar}), 8.13 (1H, br d, J=9.1 Hz, H_7), 8.20 (1H, d, J=16.4 Hz, =CH). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.8 (3 CH_3), 51.8 (CH_3), 55.4 (CH_3), 83.5 (C), 102.9 (CH), 113.3 (CH), 115.8 (CH), 118.9 (Cq), 122.6 (CH), 125.1 (CH), 125.9 (CH), 126.6 (CH), 126.9 (CH), 127.8 (C), 129.2 (C), 130.5 (C), 130.5 (CH), 131.9 (CH), 139.1 (C), 149.2 (C), 156.1 (CO), 167.5 (CO). IS-MS *m*/*z*: 408 (M^++1). *Anal.* Calcd for $C_{24}H_{25}NO_5$: C, 70.75; H, 6.18; N, 3.44. Found: C, 70.93; H, 6.30; N, 3.25.

Z-Isomer: IR (film) cm^{-1}: 1721, 1675 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.60 (9H, s, CH_3), 3.69 (3H, s, CH_3), 3.91 (3H, s, CH_3), 6.72—6.77 (2H, m, =CH+H_4), 6.90 (1H, dd, J=2.5, 8.5 Hz, H_6), 7.14—7.20 (2H, m, H_{Ar}), 7.27—7.41 (3H, m, =CH+H_{Ar}), 7.95—8.07 (2H, m, H_7+H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.6 (3 CH_3), 52.2 (CH_3), 55.3 (CH_3), 83.2 (C), 103.1 (CH), 113.3 (CH), 115.7 (CH), 118.5 (C), 123.5 (CH), 125.1 (CH), 125.7 (CH), 126.7 (CH), 126.9 (CH), 127.6 (C), 129.4 (C), 130.5 (C), 130.5 (CH), 133.0 (CH), 139.3 (C), 149.9 (C), 156.2 (CO), 168.5 (CO). IS-MS *m*/*z*: 408 (M^++1). *Anal.* Calcd for $C_{24}H_{25}NO_5$: C, 70.75; H, 6.18; N, 3.44. Found: C, 70.97; H, 6.25; N, 3.37.

2-{2-[5-Methoxy-1-phenylsulfonyl-1*H*-3-indolyl]ethyl}benzoic Acid

Methyl Ester (7a) A mixture of **6a** (1.42 g, 3.18 mmol) and 10% Pd/C (150 mg) in MeOH/THF (30 ml, 3/1 v/v) was shaken in a Parr apparatus under 45 psi of hydrogen at room temperature for 2 h. The catalyst was removed by filtration, and solvents evaporated. The crude residue was purified by column chromatography (eluent petroleum ether/CH_2Cl_2 1 : 1) to give **7a** (1.37 g, 96%) as a white solid. mp 121—122 °C (MeOH). IR (KBr) cm^{-1}: 1724 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.92 (2H, t, J=7.5 Hz, CH_2), 3.29 (2H, t, J=7.5 Hz, CH_2), 3.84 (6H, s, CH_3), 6.91 (1H, dd, J=2.5, 9.0 Hz, H_6), 7.08 (1H, d, J=2.5 Hz, H_4), 7.12 (1H, dd, J=1.2, 8.0 Hz, H_{Ar}), 7.25—7.54 (6H, m, H_2+H_{Ar}), 7.77—7.92 (4H, m, H_7+H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.1 (CH_2), 34.2 (CH_2), 51.8 (CH_3), 55.6 (CH_3), 102.2 (CH), 113.6 (CH), 114.5 (CH), 123.1 (C), 123.6 (CH), 126.2 (CH), 126.6 (2 CH), 129.0 (2 CH), 129.3 (C), 129.9 (C), 130.7 (CH), 131.2 (CH), 132.0 (CH), 132.1 (C), 133.5 (CH), 138.2 (C), 143.2 (C), 156.3 (CO), 167.7 (CO). IS-MS *m/z*: 450 (M^++1). *Anal.* Calcd for $C_{25}H_{23}NO_5S$: C, 66.80; H, 5.16; N, 3.12. Found: C, 66.56; H, 4.98; N, 3.01.

2-{2-[5-Methoxy-1-(*tert*-butoxycarbonyl)-1*H*-3-indolyl]ethyl}benzoic Acid Methyl Ester (7b) Using the same procedure and the same eluent for the final purification, **7b** was prepared from **6b** in 96% yield as an oil. IR (film) cm^{-1}: 1724, 1674 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.72 (9H, s, CH_3), 3.02 (2H, t, J=8.0 Hz, CH_2), 3.41 (2H, t, J=8.0 Hz, CH_2), 3.93 (3H, s, CH_3), 3.95 (3H, s, CH_3), 7.00 (1H, dd, J=2.5, 9.1 Hz, H_6), 7.26 (1H, d, J=2.5 Hz, H_4), 7.29—7.35 (2H, m, H_2+H_{Ar}), 7.44—7.51 (2H, m, H_{Ar}), 7.97 (1H, dd, J=1.4, 8.0 Hz, H_{Ar}), 8.09 (1H, br d, J=9.1 Hz, H_7). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.3 (CH_2), 28.0 ($3CH_3$), 34.4 (CH_2), 51.7 (CH_3), 55.4 (CH_3), 82.8 (C), 101.8 (CH), 112.7 (CH), 115.6 (CH), 120.5 (C), 122.9 (CH), 123.1 (C), 125.9 (CH), 129.2 (C), 130.5 (CH), 131.1 (CH), 131.4 (C), 131.9 (CH), 143.5 (C), 149.6 (C), 155.6 (CO), 167.6 (CO). IS-MS *m/z*: 410 (M^++1). *Anal.* Calcd for $C_{24}H_{27}NO_5$: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.12; H, 6.77; N, 3.40.

2-{2-[5-Methoxy-1-(phenylsulfonyl)-1*H*-3-indolyl]ethyl}benzoic Acid (8a) A solution of ester **7a** (500 mg, 1.1 mmol) in MeOH 95% (20 ml) and potassium hydroxide (997 mg, 17.6 mmol) was stirred at reflux for 60 h. The solvent was removed *in vacuo*, water (20 ml) was added to the residue and the pH was adjusted to 1 by careful addition of 10% aqueous HCl. After extraction with CH_2Cl_2 (2×20 ml), the organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was separated by column chromatography (eluent CH_2Cl_2) to give **8a** (315 mg, 65%) as crystals. mp 127—129 °C (MeOH). IR (KBr) cm^{-1}: 3300—2400 (OH), 1700 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$+D_2O) δ: 2.96 (2H, t, J=7.5 Hz, CH_2), 3.37 (2H, t, J=7.5 Hz, CH_2), 3.79 (3H, s, CH_3), 6.89 (1H, dd, J=2.5, 9.0 Hz, H_6), 6.95 (1H, d, J=2.5 Hz, H_4), 7.12 (1H, d, J=8.0 Hz, H_{Ar}), 7.25-7.51 (6H, m, H_2+H_{Ar}), 7.79 (2H, d, J=8.0 Hz, H_{Ar}), 7.87 (1H, d, J=9.0 Hz, H_7), 8.06 (1H, dd, J=1.0, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.0 (CH_2), 34.4 (CH_2), 55.6 (CH_3), 102.1 (CH), 113.4 (CH), 114.6 (CH), 123.0 (C), 123.8 (CH), 126.4 (CH), 126.6 (2 CH), 128.0 (C), 129.1 (2CH), 129.9 (C), 131.5 (CH), 131.8 (CH) 132.1 (C), 132.9 (CH), 133.5 (CH), 138.2 (C), 144.2 (C), 156.3 (C), 172.2 (CO). IS-MS *m/z*: 436 (M^++1). *Anal.* Calcd for $C_{24}H_{21}NO_5S$: C, 66.19; H, 4.86; N, 3.22. Found: C, 65.93; H, 4.97; N, 3.36.

2-[2-(5-Methoxy-1*H*-3-indolyl)ethyl]benzoic Acid (8b) Following the same methodology, compound **8b** was obtained from **7b** in 84% yield as crystals. mp 143—145 °C (MeOH). IR (KBr) cm^{-1}: 3403 (NH), 3300—2400 (OH), 1708 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$+D_2O) δ: 3.05 (2H, t, J=8.0 Hz, CH_2), 3.43 (2H, t, J=8.0 Hz, CH_2), 3.86 (3H, s, CH_3), 6.85 (1H, dd, J=2.5, 9.0 Hz, H_6), 6.89 (1H, d, J=3.5 Hz, H_2), 7.11 (1H, d, J=2.5 Hz, H_4), 7.22 (1H, d, J=9.0 Hz, H_7), 7.31 (2H, t, J=8.0 Hz, H_{Ar}), 7.47 (1H, t, J=8.0 Hz, H_{Ar}), 7.71 (1H, br s, NH), 8.06 (1H, br d, J=8.1 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 27.5 (CH_2), 35.1 (CH_2), 55.8 (CH_3), 100.8 (CH), 111.6 (CH), 112.0 (CH), 115.8 (C), 122.2 (CH), 125.8 (CH), 127.8 (C), 129.6 (C), 130.4 (C), 131.1 (CH), 131.4 (C), 131.8 (C), 144.0 (C), 153.7 (C), 168.1 (CO). IS-MS *m/z*: 296 (M^++1). *Anal.* Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.53; H, 5.91; N, 4.63.

2-Methoxy-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one (9) Finely powdered **8b** (300 mg, 1.02 mmol) was added to polyphosphoric acid (PPA, 1.7 g) and phosphorus pentoxide (233 mg) with stirring at 90 °C. After the addition was complete, the mixture was stirred at 90 °C for 1 h, then cooled, ice was added, and the mixture was neutralised with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (2×15 ml). The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The crude residue was purified by column chromatography (eluent CH_2Cl_2) to afford **9** (184 mg, 65%) as crystals. mp 171—173 °C (EtOAc–petroleum ether). IR (KBr) cm^{-1}: 3314 (NH), 1613 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 3.15—3.21 (2H, m, CH_2), 3.26—3.30 (2H, m, CH_2), 3.88 (3H, s, CH_3), 7.00—7.07 (2H, m, H_{Ar}), 7.31—7.52 (4H, m, H_{Ar}), 8.13 (1H, dd, J=1.6, 8.2 Hz, H_{Ar}), 9.33 (1H, br s, NH). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 24.6 (CH_2), 36.2 (CH_2), 55.9 (CH_3), 101.2 (CH), 113.3 (CH), 118.8 (CH), 125.7 (C), 127.1 (CH), 127.5 (C), 130.3 (2CH), 132.5 (C), 132.7 (CH), 133.9 (C), 137.5 (C), 141.2 (C), 154.5 (C), 184.3 (CO). IS-MS *m/z*: 278 (M^++1). *Anal.* Calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 78.24; H, 5.41; N, 4.87.

2-Methoxy-3-phenylsulfonyl-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one (10) Following the same methodology, compound **8a** afforded **9** and **10**, respectively, in 50—60% and 20—30% yields. Compound **10**: mp 231—233 °C (MeOH); IR (KBr) cm^{-1}: 3310 (NH), 1611 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 3.06—3.23 (4H, m, CH_2), 3.69 (3H, s, CH_3), 7.32 (1H, s, H_{Ar}), 7.40—7.46 (2H, m, H_{Ar}), 7.52—7.66 (4H, m, H_{Ar}), 7.89 (2H, d, J=7.2 Hz, H_{Ar}), 7.95 (1H, d, J=7.5 Hz, H_{Ar}); 8.24 (1H, s, H_{Ar}), 12.03 (1H, br s, NH). ^{13}C-NMR (62.90 MHz, DMSO-d_6) δ: 23.8 (CH_2), 34.9 (CH_2), 56.2 (CH_3), 103.4 (CH), 114.6 (CH), 123.9 (C), 127.0 (CH), 127.8 (2 CH), 128.3 (C), 128.9 (2 CH), 129.7 (CH), 130.4 (CH), 130.5 (C), 130.7 (C), 133.0 (CH), 133.2 (CH), 136.2 (C), 137.0 (C), 141.1 (C), 141.3 (C), 150.2 (C), 184.0 (CO). IS-MS *m/z*: 418 (M^++1). *Anal.* Calcd for $C_{24}H_{19}NO_4S$: C, 69.05; H, 4.59; N, 3.36. Found: C, 68.79; H, 4.40 N, 3.19.

5-[3-(Dimethylamino)ethyl]-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one (11) To a solution of **2** (200 mg, 0.81 mmol) in anhydrous DMF (10 ml) was added portionwise sodium hydride (30 mg, 1.50 mmol, 60% oil dispersion) at 0 °C. The mixture was stirred for 15 min at 0 °C, then 2-(dimethylamino)ethyl chloride (0.18 ml, 1.6 mmol) diluted in anhydrous DMF (5 ml) was added and the solution was stirred at 90 °C overnight. After cooling, the solvent was removed *in vacuo* and the residue was partitioned between water and CH_2Cl_2 and extracted. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluent CH_2Cl_2/MeOH 98 : 2) to give **11** (188 mg, 73%) as yellow crystals. mp 96—97 °C (CH_2Cl_2–petroleum ether). IR (KBr) cm^{-1}: 1621 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.41 (6H, s, CH_3), 2.71 (2H, t, J=7.2 Hz, CH_2), 3.18—3.27 (4H, m, CH_2), 4.75 (2H, t, J=7.2 Hz, CH_2), 7.15 (1H, t, J=8.0 Hz, H_{Ar}), 7.26—7.48 (5H, m, H_{Ar}), 7.66 (1H, d, J=8.0 Hz, H_{Ar}), 7.92 (1H, dd, J=1.6, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 25.5 (CH_2), 35.2 (CH_2), 44.0 (CH_2), 46.0 (2 CH_3), 59.2 (CH_2), 110.5 (CH), 120.4 (CH), 121.1 (CH), 126.2 (C), 126.8 (CH), 127.0 (CH), 127.4 (C), 129.1 (CH), 129.9 (CH), 131.8 (CH), 132.4 (C), 139.3 (C), 139.7 (C), 140.6 (C), 187.4 (CO). IS-MS *m/z*: 319 (M^++1). *Anal.* Calcd for $C_{21}H_{22}N_2O$: C, 79.21; H, 6.96; N, 8.80. Found: C, 78.89; H, 7.07; N, 8.97.

5-[3-(Dimethylamino)propyl]-5,6,11,12-tetrahydrobenzo[5,6]cyclohepta[*b*]indol-6-one (12) Following the same methodology, compound **12** was obtained from **2** with 2-(dimethylamino)propyl chloride in 65% yield as an oil. IR (film) cm^{-1}: 1605 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 1.99—2.10 (2H, m, CH_2), 2.31 (6H, s, CH_3), 2.45 (2H, t, J=7.0 Hz, CH_2), 3.17—3.27 (4H, m, CH_2), 4.66 (2H, t, J=7.0 Hz, CH_2), 7.12 (1H, t, J=8.0 Hz, H_{Ar}), 7.23—7.47 (5H, m, H_{Ar}), 7.65 (1H, br d, J=8.0 Hz, H_{Ar}), 7.89 (1H, dd, J=1.5, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 24.5 (CH_2), 28.5 (CH_2), 35.0 (CH_2), 43.7 (CH_2), 45.2 ($2CH_3$), 56.8 (CH_2), 110.5 (CH), 120.1 (CH), 120.9 (CH), 125.9 (C), 126.6 (CH), 126.9 (CH), 127.4 (C), 128.9 (CH), 129.8 (CH), 131.6 (CH), 132.2 (C), 139.2 (C), 139.5 (C), 140.5 (C), 187.4 (CO). IS-MS *m/z*: 333 (M^++1). *Anal.* Calcd for $C_{22}H_{24}N_2O$: C, 79.48; H, 7.28; N, 8.43. Found: C, 79.75; H, 7.35; N, 8.32.

5-[3-(Dimethylamino)ethyl]-5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-6-one (13) Following the same methodology, compound **13** was obtained from **3** with 2-(dimethylamino)ethyl chloride in 70% yield. mp 88—89 °C (CH_2Cl_2–petroleum ether). IR (KBr) cm^{-1}: 1635 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.40 (6H, s, CH_3), 2.81 (2H, t, J=7.2 Hz, CH_2), 4.96 (2H, t, J=7.2 Hz, CH_2), 7.26 (1H, d, J=12.0 Hz, =CH), 7.34 (1H, t, J=8.0 Hz, H_{Ar}), 7.51—7.77 (6H, m, =CH+H_{Ar}), 8.07 (1H, d, J=8.0 Hz, H_{Ar}), 8.76 (1H, dd, J=1.6, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 44.1 (CH_2), 46.0 ($2CH_3$), 59.3 (CH_2), 110.6 (CH), 120.9 (CH), 121.3 (CH), 121.5 (CH), 122.0 (C), 125.1 (C), 127.3 (CH), 128.3 (CH), 129.2 (CH), 130.9 (CH), 131.5 (CH), 133.0 (CH), 136.1 (C), 136.2 (C), 137.6 (C), 139.8 (C), 181.3 (CO). IS-MS *m/z*: 317 (M^++1). *Anal.* Calcd for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.87; H, 6.18; N, 8.72.

5-[3-(Dimethylamino)propyl]-5,6-dihydrobenzo[5,6]cyclohepta-[*b*]indol-6-one (14) Following the same methodology, compound **14** was obtained from **3** with 2-(dimethylamino)propyl chloride in 60% yield. mp 102—103 °C (CH_2Cl_2–MeOH). IR (KBr) cm^{-1}: 1605 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.27—2.39 (2H, m, CH_2), 2.49 (6H, s, CH_3), 2.78 (2H, t, J=7.0 Hz, CH_2), 4.91 (2H, t, J=7.0 Hz, CH_2), 7.30 (1H, d, J=11.5 Hz, =CH), 7.35 (1H, t, J=8.0 Hz, H_{Ar}), 7.53—7.79 (6H, m, =CH+H_{Ar}), 8.08 (1H, d, J=8.0 Hz, H_{Ar}), 7.89 (1H, d, J=8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 28.5 (CH_2), 43.9 (CH_2), 45.2 (2 CH_3), 56.7 (CH_2), 110.8 (CH),

121.2 (CH), 121.5 (2CH), 122.1 (C), 124.9 (C), 127.2 (CH), 128.2 (CH), 129.0 (CH), 130.8 (CH), 131.4 (CH), 132.8 (CH), 135.9 (C), 136.0 (C), 137.5 (C), 139.7 (C), 181.3 (CO). IS-MS *m/z*: 331 ($M^+ + 1$). *Anal.* Calcd for $C_{22}H_{22}N_2O$: C, 79.97; H, 6.71; N, 8.48. Found: C, 80.33; H, 6.66; N, 8.57.

5-(3-Bromopropyl)-5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-6-one (15) To a solution of **3** (200 mg, 0.81 mmol) and KOH (100 mg, 1.78 mmol) in anhydrous DMF (10 ml) was added a solution of 1,3-dibromopropane (0.51 ml, 2.56 mmol) diluted in anhydrous DMF (2 ml) at 0 °C. The mixture was stirred at room temperature overnight. After cooling, the solvent was removed *in vacuo*. The residue was partitioned between water (10 ml) and EtOAc (10 ml) and extracted. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*, and the crude residue was purified by column chromatography (eluent petroleum ether/CH_2Cl_2 2 : 8) to give **15** (130 mg, 87%) as yellow crystals. mp 150—152 °C (EtOAc–petroleum ether). IR (KBr) cm^{-1}: 1603 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.50—2.59 (2H, m, CH_2), 3.50 (2H, t, J=7.0 Hz, CH_2), 4.99 (2H, t, J=7.0 Hz, CH_2), 7.28 (1H, d, J=11.8 Hz, =CH), 7.36 (1H, t, J=8.0 Hz, H_{Ar}), 7.54—7.78 (6H, m, =CH+H_{Ar}), 8.10 (1H, d, J=8.0 Hz, H_{Ar}), 8.76 (1H, d, J=8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 31.2 (CH_2), 34.0 (CH_2), 44.6 (CH_2), 110.7 (CH), 121.0 (CH), 121.5 (2CH), 122.5 (C), 125.9 (C), 127.5 (CH), 128.4 (CH), 129.4 (CH), 130.9 (CH), 131.6 (CH), 133.2 (CH), 135.9 (C), 136.2 (C), 137.4 (C), 139.8 (C), 181.1 (CO); IS-MS *m/z*: 366 ($M^+ + 1$, ^{79}Br), 368 ($M^+ + 1$, ^{81}Br). *Anal.* Calcd for $C_{20}H_{16}BrNO$: C, 65.59; H, 4.40; N, 3.82. Found: C, 65.88; H, 4.23; N, 3.63.

5-{2-[(2-Hydroxyethyl)amino]ethyl}-5,6-dihydrobenzo[5,6]-cyclohepta[*b*]indol-6-one (16) A solution of **15** (190 mg, 0.52 mmol) and 2-aminoethanol (0.08 ml, 1.32 mmol) in acetonitrile (7 ml) was stirred at room temperature for 72 h. After evaporation, the crude residue was purified by column chromatography (eluent CH_2Cl_2/MeOH/NH_4OH 100 : 10 : 1) to give **16** (83 mg, 46%) as yellow crystals. mp 114—115 °C (CH_2Cl_2–MeOH). IR (KBr) cm^{-1}: 3014 (NH), 1606 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.07—2.18 (2H, m, CH_2), 2.73 (2H, t, J=7.0 Hz, CH_2), 2.77 (2H, t, J=5.1 Hz, CH_2), 2.82 (2H, br s, OH+NH), 3.66 (2H, t, J=5.1 Hz, CH_2), 4.88 (2H, t, J=7.0 Hz, CH_2), 7.23 (1H, d, J=11.6 Hz, =CH), 7.36 (1H, br t, J=8.0 Hz, H_{Ar}), 7.48—7.74 (6H, m, =CH+H_{Ar}), 8.04 (1H, d, J=8.0 Hz, H_{Ar}), 8.72 (1H, dd, J=1.7, 8.0 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 30.5 (CH_2), 43.7 (CH_2), 46.5 (CH_2), 51.2 (CH_2), 60.4 (CH_2), 110.7 (CH), 120.9 (CH), 121.4 (CH), 121.5 (CH), 122.5 (C), 125.0 (C), 127.5 (CH), 128.4 (CH), 129.3 (CH), 130.8 (CH), 131.6 (CH), 133.0 (CH), 136.0 (C), 136.1 (C), 137.2 (C), 139.7 (C), 181.2 (CO). IS-MS *m/z*: 347 ($M^+ + 1$). *Anal.* Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09. Found: C, 75.96; H, 6.28; N, 8.23.

5-[4-(6-Oxo-5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-5-yl)hexyl]-5,6-dihydrobenzo [5,6]cyclohepta[*b*]indol-6-one (17) To a solution of **3** (200 mg, 0.82 mmol) and KOH (50 mg, 0.89 mmol) in anhydrous DMF (10 ml) was added a solution of 1,6-dibromohexane (0.06 ml, 0.41 mmol) diluted in anhydrous DMF (1 ml) at 0 °C. The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*. The residue was partitioned between water (10 ml) and EtOAc (10 ml) and extracted. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluent petroleum ether/CH_2Cl_2 2 : 8) to give **17** (104 mg, 60%) as yellow crystals. mp 219—220 °C (MeOH). IR (KBr) cm^{-1}: 1617 (CO). ^{1}H-NMR (250 MHz, DMSO-d_6) δ: 1.48—1.58 (4H, m, CH_2), 1.85—2.00 (4H, m, CH_2), 4.87 (4H, t, J=7.0 Hz, CH_2), 7.28 (2H, d, J=10.5 Hz, =CH), 7.32—7.37 (2H, m, H_{Ar}), 7.51—7.55 (4H, m, H_{Ar}), 7.63 (2H, t, J=8.0 Hz, H_{Ar}), 7.69—7.79 (6H, m, =CH+H_{Ar}), 8.10 (2H, d, J=8.0 Hz, H_{Ar}), 8.76 (2H, d, J=8.0 Hz, H_{Ar}). The very low solubility of compound **17** did not allow us to perform ^{13}C-NMR. IS-MS *m/z*: 573 ($M^+ + 1$). *Anal.* Calcd for $C_{40}H_{32}N_2O_2$: C, 83.89; H, 5.63; N, 4.89. Found: C, 84.21; H, 6.78; N, 4.75.

2-Methoxy-5,6-dihydrobenzo[5,6]cyclohepta[b]indol-6-one (18) A solution of **9** (500 mg, 2.02 mmol) and DDQ (1.38 g, 6.08 mmol) in anhydrous dioxane (20 ml) was stirred under argon at reflux overnight. After cooling, the solution was diluted with CH_2Cl_2 (10 ml), then washed successively with 10% aqueous NaOH two times and water once. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The crude residue was purified by column chromatography (eluent CH_2Cl_2) to afford **18** (446 mg, 90%) as yellow crystals. mp >245 °C (EtOAc); IR (KBr) cm^{-1}: 3245 (NH), 1583 (CO). ^{1}H-NMR (250 MHz, DMSO-d_6) δ: 3.89 (3H, s, CH_3), 7.17 (1H, dd, J=2.2, 9.0 Hz, H_{Ar}), 7.46 (1H, d, J=11.6 Hz, =CH), 7.58 (1H, d, J=9.0 Hz, H_{Ar}), 7.71—7.90 (3H, m, H_{Ar}), 8.01 (1H, d, J=11.6 Hz, =CH); 8.04 (1H, d, J=8.2 Hz, H_{Ar}), 8.87 (1H, dd, J=1.2, 8.2 Hz, H_{Ar}), 12.37 (1H, br s, NH). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 55.6 (CH_3), 101.7 (CH), 113.8 (CH), 118.6 (CH), 119.8 (C), 123.0 (CH), 126.0 (2C), 128.2 (CH), 130.1 (CH), 132.2 (CH), 133.0 (C), 133.9 (C), 134.4 (CH), 136.8 (C), 137.3 (C), 154.7 (C), 177.1 (CO). IS-MS *m/z*: 276 ($M^+ + 1$). *Anal.* Calcd for $C_{18}H_{13}NO_2$: C, 78.53; H, 4.76; N, 5.09. Found: C, 78.15; H, 4.94; N, 4.96.

5-[3-(Dimethylamino)ethyl]-2-methoxy-5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-6-one (19) Following the methodology used for the preparation of **11**, compound **19** was obtained from **18** with 2-(dimethylamino)-ethyl chloride in 65% yield. mp 90—91 °C (petroleum ether); IR (KBr) cm^{-1}: 1619 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.36 (6H, s, CH_3), 2.75 (2H, t, J=7.5 Hz, CH_2), 3.87 (3H, s, CH_3), 4.81 (2H, t, J=7.5 Hz, CH_2), 7.06 (1H, d, J=11.5 Hz, =CH), 7.14 (1H, dd, J=2.2, 9.0 Hz, H_{Ar}), 7.30 (1H, d, J=2.2 Hz, H_{Ar}), 7.41 (1H, d, J=9.0 Hz, H_{Ar}), 7.47 (1H, d, J=11.5 Hz, =CH), 7.52—7.64 (3H, m, H_{Ar}), 8.71 (1H, dd, J=1.6, 7.9 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 44.3 (CH_2), 46.0 (2 CH_3), 56.0 (CH_2), 59.5 (CH_3), 101.3 (CH), 111.7 (CH), 118.6 (CH), 121.7 (2 CH), 125.4 (Cq), 128.2 (CH), 128.5 (CH), 130.9 (CH), 131.5 (CH), 132.9 (CH), 135.2 (C), 136.2 (C), 136.4 (C), 137.4 (C), 155.3 (C), 181.2 (CO). IS-MS *m/z*: 347 ($M^+ + 1$). *Anal.* Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.55; H, 6.45; N, 8.06.

5-[3-(Dimethylamino)propyl]-2-methoxy-5,6-dihydrobenzo[5,6]cyclohepta[*b*]indol-6-one (20) Following the methodology used for the preparation of **11**, compound **20** was obtained from **18** with 2-(dimethylamino)-propyl chloride in 60% yield as an oil. IR (film) cm^{-1}: 1619 (CO). ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 2.03—2.15 (2H, m, CH_2), 2.26 (6H, s, CH_3), 2.40 (2H, t, J=7.2 Hz, CH_2), 3.92 (3H, s, CH_3), 4.84 (2H, t, J=7.2 Hz, CH_2), 7.17 (1H, d, J=11.6 Hz, =CH), 7.17 (1H, dd, J=2.2, 9.0 Hz, H_{Ar}), 7.40 (1H, d, J=2.2 Hz, H_{Ar}), 7.52 (1H, d, J=9.0 Hz, H_{Ar}), 7.58—7.72 (4H, m, =CH+H_{Ar}), 8.73 (1H, dd, J=1.6, 7.8 Hz, H_{Ar}). ^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 28.8 (CH_2), 44.1 (CH_2), 45.3 (2CH_3), 55.9 (CH_2), 56.7 (CH_3), 101.0 (CH), 111.9 (CH), 118.5 (CH), 121.6 (2CH), 125.2 (C), 128.0 (CH), 128.3 (CH), 130.8 (CH), 131.4 (CH), 132.9 (CH), 135.2 (C), 136.1 (C), 136.2 (C), 137.3 (C), 155.2 (C), 181.1 (CO). IS-MS *m/z*: 361 ($M^+ + 1$). *Anal.* Calcd for $C_{23}H_{24}N_2O_2$: C, 76.64; H, 6.71; N, 7.77. Found: C, 76.37; H, 6.91; N, 7.89.

Antiproliferative Activity L1210 Murine leukemia cells provided by the NCI (Frederick, MD U.S.A.) were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fœtal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin and 10 mM HEPES buffer (pH=7.4). The cytotoxicity was measured by microculture tetrazolium assay as described in ref. 11. Cells were exposed for 48 h to graded concentrations in triplicate of the test drug compounds. Results are expressed as IC_{50} (mean, n=3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

Flow Cytometric Analysis L1210 cells (2.5×10^5 cells/ml) were incubated for 21 h with various concentrations of the compounds, then fixed by 70% ethanol (v/v), washed and incubated in phosphate buffer saline (PBS) containing 100 mg/ml RNAse and 24 mg/ml propidium iodide for 30 min at 20 °C. For each sample, 1×10^4 cells were analysed on an ATC3000 flow cytometer (Bruker) using an argon laser (Spectra-Physics) emitting 400 mW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter. Data are displayed as the percentage of cells found in the G_2+M phase of the cell cycle.

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