

SYNTHESIS AND ANTITUMORAL EVALUATION OF 12-SUBSTITUTED 6,7-DIHYDROBENZO[4,5]CYCLOHEPT[1,2-*b*]INDOLE DERIVATIVES

Benoît Joseph^a, Virginie Chapellier^a, Jean-Yves Mérour^{a*}, and Stéphane Léonce^b

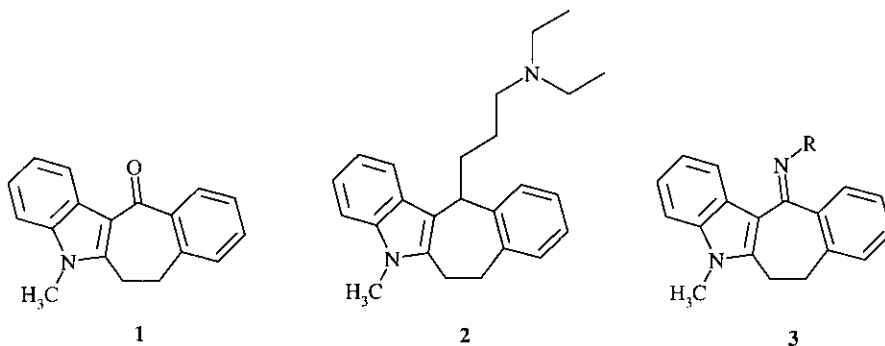
^a *Institut de Chimie Organique et Analytique UPRES-A CNRS 6005, Université d'Orléans, BP 6759, 45067 Orléans Cedex 2, France*

^b *Institut de Recherche Servier, 11 rue des Moulineaux 92150 Suresnes, France*

Abstract- The synthesis of 12-substituted 6,7-dihydrobenzo[4,5]cyclohept[1,2-*b*]indole derivatives (**2-3**) are described. The antiproliferative activity of some of them were evaluated.

Despite great effort, the development of curative antitumor drugs (cancer is a major disease at a worldwide level, accounting for over 7 millions death per year) has been only partially successful. In the ongoing search for new effective chemotherapeutic agents, a wide variety of new drugs have been prepared from taxanes, vinca alkaloids, camptothecin, duocarmycins and podophyllotoxins.¹ These drugs are classified into: alkylating agents, nucleic acids intercalating agents, topoisomerase inhibitors, spindle poisons.² One of the most important classes of anticancer drug is that of the DNA-binding agents, which can bind either in the major or minor grooves, or can intercalate in between base pairs of double-stranded DNA.

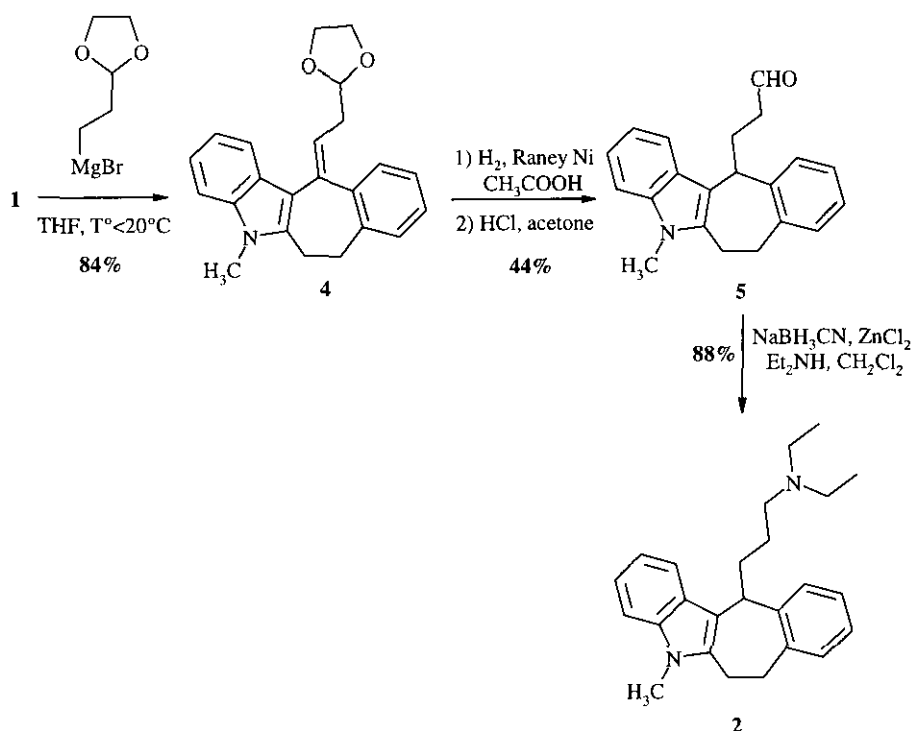
In a recent paper, we have described an effective route for the preparation of a new tetracyclic indolic compound (**1**) and its X-Ray crystallographic analysis.³



On the basis of the literature of antitumor drugs,^{1,2} we considered the preparation of new simple derivatives of **1** to evaluate their antitumor activities. The present work reports the preparation of **2-3** from **1** by functionalisation of the keto group by substituents found in different antitumor agents.^{4,5}

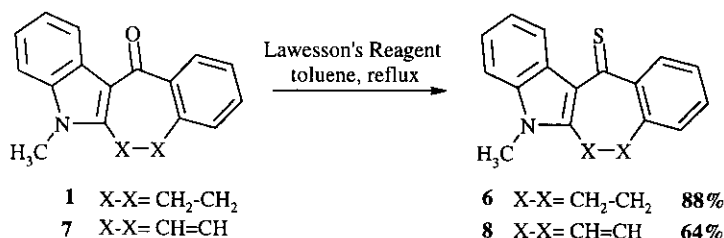
After reactivity studies of the ketonic carbonyl group which have shown the relative inertness of the keto group towards Wittig reagents or amino derivatives, we planned to obtain derivative (**2**) *via* a condensation of an appropriate Grignard reagent and derivatives (**3**) *via* a nucleophilic substitution of a thioalkyl intermediate easily available.

The first synthetic method is illustrated in Scheme 1. Treatment of **1** with an excess (12 eq.) of freshly prepared acetal Grignard reagent⁶ in THF gave **4** in 84% yield. Use of less than 12 equivalents of Grignard reagent afforded **4** with lower yields due to the presence of residual starting material. Reduction of the double bond was only effective under pressure (400 psi) in acetic acid in the presence of Raney Ni for 30 h. After mild acid hydrolysis of the crude mixture, the desired aldehyde (**5**) was isolated in 44% yield. The moderate yield is explained by the formation of the corresponding alcohol during the first step of the reaction through deprotection of the starting material (traces of water in the medium) and reduction of the free aldehyde generated. Finally, reductive amination of aldehyde was performed with ZnCl₂ and NaBH₃CN in the presence of diethylamine to afford the aminoalkyl product (**2**) in 88% yield.⁷



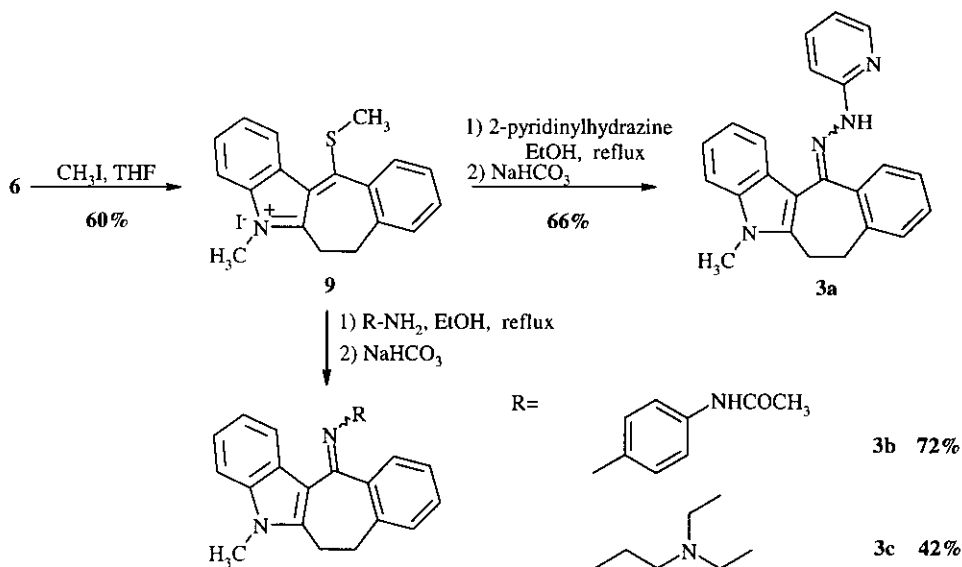
Scheme 1

The general synthetic route to 12-*N*-substituted derivatives (**3**) is outlined in Schemes 2 and 3. Thionation of **1** with Lawesson's reagent in hot anhydrous toluene afforded the desired new thione (**6**) in 88% yield.⁸ The ¹³C NMR spectrum showed a signal for C=S at δ 224.9 ppm (for **1**, C=O was observed at δ 188.1 ppm).



Scheme 2

Similarly in our laboratory, the same procedure was applied to the unsaturated derivative (**7**)³ to afford **8** in 64% yield. Alkylation of **6** with iodomethane in THF gave the red crystalline iodide salt (**9**) in 60% yield. In our hands, attempts to obtain the iodide salt from the thione (**8**) were unsuccessful. Reaction of **9** with 2-pyridinylhydrazine in refluxing ethanol gave hydrazone (**3a**) in 66% yield. By NMR analysis, only one isomer for **3a** was observed. Two primary amines *N*-(4-aminophenyl)acetamide, *N,N*-diethylethylenediamine) were also used to afford the imines (**3b-c**) in moderate to fair yields (42-72%). For information, attempts to reduce **3b-c** to their corresponding amines with sodium borohydride or lithium aluminium hydride failed.



Scheme 3

The antiproliferative activity of compounds (**2**) and (**3a**) were assessed using the murine L1210 leukemia and Lewis lung carcinoma cell lines.⁹

Table: Growth-inhibitory activity of **2** and **3a**

Compound	IC ₅₀ L1210 (μM)	IC ₅₀ LLC (μM)
adriamycine	0.024	0.019
2	4.7	11.6
3a	> 50	NT ^a

^a Not tested

The biological results were compared with the reference compound adriamycine (Table). Modest *in vitro* cytotoxicity was observed for **2**. Hydrazone (**3a**) was found inactive.

This study represents an effective route to prepare derivatives (**2-3**) through a fonctionnalisation of the keto group. Unfortunately, the cytotoxicity of **2** (or **3a**) was significantly less compared to adriamycine.

EXPERIMENTAL

Melting points were determined using a Büchi SMP-20 melting point apparatus and are uncorrected. The IR spectra of compounds were recorded on a Perkin Elmer FTIR paragon 1000 spectrophotometer. NMR spectra were recorded at 300°K in CDCl₃ or DMSO-d₆ on a Bruker Avance DPX 250. Chemical shifts are expressed in parts per million and referenced to TMS. MS spectra were recorded on Perkin-Elmer SCIEX API 300 using ionspray methodology. Thin layer chromatography was performed on precoated plate of silica gel 60F₂₅₄ (Merck) and the spots visualised using an ultraviolet lamp. Flash chromatography was conducted Merck silica gel 60 (0.040 mm-0.063 mm) as the stationary phase. All air- and moisture sensitive reactions were conducted under a prepurified argon atmosphere. Anhydrous solvents or reagents were transferred *via* syringe.

12-[(E)-2-(1,3-Dioxolan-2-yl)ethylidene]-5-methyl-5,6,7,12-tetrahydrobenzo[4,5]cyclohepta[b]indole (4). To a suspension of magnesium turnings (300 mg, 12.3 mmol) in THF (2 mL) and dibromoethane (10 drops) was added carefully 2-(2-bromoethyl)-1,3-dioxolane (1.5 mL, 12.7 mmol) in THF (13 mL) under vigorous stirring, the temperature of the reaction was kept below 20°C. After the addition, the resulting mixture was stirred for 1 h. Then, a solution of **1** (200 mg, 0.8 mmol) in THF (3 mL) was added and the mixture was stirred at rt for 1 h. THF was removed *in vacuo*. The residue was partitioned between 10% aqueous HCl and methylene chloride and separated. The organic phases were combined, dried (MgSO₄) and evaporated. The crude compound was purified by column chromatography (eluent 3:7 petroleum ether/methylene chloride) to give 220 mg (84%) of **4** as a white compound: mp 208-209 °C (petroleum ether-methylene chloride); IR (KBr) ν 1142 (C-O) cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ : 2.64-3.62 (m, 6H); 3.55 (s, 3H, CH₃); 3.87-4.00 (m, 4H); 5.01 (t, 1H, *J* = 4.6 Hz, CH-O); 6.18 (t, 1H, *J* = 7.6 Hz, CH=); 7.11-7.31 (m, 7H, H_{Ar}); 7.82-7.85 (m, 1H, H_{Ar}); ¹³C NMR (62.90 MHz, CDCl₃) δ : 27.4 (CH₂), 29.1

(NCH₃), 31.3 (CH₂), 34.3 (CH₂), 65.0 (2, CH₂O), 104.3 (CHO₂), 108.4, 113.3 (q), 119.2, 119.4, 121.0, 122.2, 125.9 (q), 126.0, 127.0, 128.2, 128.3, 134.8 (q), 136.5 (q), 136.7 (q), 138.6 (q), 142.0 (q); MS: *m/z* 346 (M⁺+1); *Anal.* Calcd for C₂₃H₂₃NO₂: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.15; H, 6.53; N, 3.88.

3-(5-Methyl-5,6,7,12-tetrahydrobenzo[4,5]cyclohepta[b]indol-12-yl)propanal (5). A mixture of **4** (250 mg, 0.72 mmol) and Raney Ni washed with AcOH (6 g) in AcOH/CH₂Cl₂ (10 mL) was shaken in a Parr apparatus under 400 psi of hydrogen at rt for 30 h. The catalyst was filtered through celite and the solvent of the filtrate was removed *in vacuo*. The residue was partitioned between ethyl acetate and water was separated, then washed with saturated aqueous NaHCO₃. The combined organic phases were dried (MgSO₄) and evaporated. The crude compound was dissolved in acetone (10 mL) and 10% aqueous HCl (2 mL). The mixture was stirred for 2 h, then acetone was removed *in vacuo*. The suspension was basified by addition of saturated aqueous NaHCO₃ and extracted with EtOAc. The organic phases were combined, dried (MgSO₄) and evaporated. The crude residue was purified by column chromatography (eluent 3:7 petroleum ether/methylene chloride) to give 96 mg (44%) of **5** as an amorphous compound; IR (film) ν 2827, 2728 (CHO), 1714 (C=O) cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ : 2.25-2.51 (m, 4H, CH₂); 2.84 (td, 1H, *J* = 4.0, 16.0 Hz, CH₂); 3.00 (dt, 1H, *J* = 4.0, 16.0 Hz, CH₂); 3.20 (dt, 1H, *J* = 4.0, 16.0 Hz, CH₂); 3.58 (s, 3H, CH₃); 3.58-3.66 (m, 1H, CH₂); 4.24 (dd, 1H, *J* = 4.6 Hz, CH); 7.12-7.25 (m, 7H, H_{Ar}); 7.63 (m, 1H, H_{Ar}); 9.70 (s, 1H, CHO); ¹³C NMR (62.90 MHz, CDCl₃) δ : 26.6 (CH₂), 29.2 (NCH₃), 30.3 (CH₂), 31.7 (CH₂), 42.8 (CH), 43.0 (CH₂), 108.4, 112.0 (q), 117.5, 118.9, 120.9, 126.5, 126.9, 127.7 (q), 129.9, 130.3, 135.3 (q), 136.1 (q), 139.5 (q), 142.7 (q), 202.3 (CHO); MS: *m/z* 304 (M⁺+1); *Anal.* Calcd for C₂₁H₂₁NO: C, 83.13; H, 6.98; N, 4.62. Found: C, 82.80; H, 6.80; N, 4.70.

***N,N*-Diethyl-*N*-[3-5-methyl-5,6,7,12-tetrahydrobenzo[4,5]cyclohepta[b]indol-12-yl]propylamine (2).** Aldehyde (**5**) (150 mg, 0.50 mmol), diethylamine (0.1 mL, 1.00 mmol), NaBH₃CN (38 mg, 0.60 mmol) and ZnCl₂ (35 mg, 0.25 mmol) in methanol (10 mL) were stirred at 50 °C overnight. After cooling and evaporation of solvent, The residue was partitioned between 10% aqueous HCl and methylene chloride and extracted twice. The organic phases were combined, dried (MgSO₄) and evaporated. The crude compound was purified by column chromatography (85:15 methylene chloride/methanol) to give 160 mg (88%) of **2** as a crystalline compound: mp 186-187 °C (petroleum ether-ethyl acetate); ¹H NMR (250 MHz, CDCl₃) δ : 1.22 (t, 6H, *J* = 7.3 Hz, CH₃); 1.70-2.10 (m, 4H, CH₂); 2.75-3.03 (m, 8H, CH₂); 3.18 (dt, 1H, *J* = 16.8, 3.5 Hz, CH₂); 3.50-3.60 (m, 1H, CH₂); 3.57 (s, 3H, CH₃); 4.20 (t, 1H, *J* = 8.5 Hz, CH); 7.12-7.25 (m, 7H, H_{Ar}); 7.55 (d, 1H, *J* = 7.5 Hz, H_{Ar}); ¹³C NMR (62.90 MHz, CDCl₃) δ : 8.6 (2, CH₃), 22.5 (CH₂), 26.6 (CH₂), 29.2 (NCH₃), 31.7 (CH₂), 35.0 (CH), 43.4 (CH₂), 46.6 (2, CH₂), 51.3 (CH₂), 108.6, 111.8 (q), 117.2, 119.1, 121.1, 126.7, 127.1, 127.3 (q), 129.7, 130.5, 135.4 (q), 136.1 (q), 139.4

(q), 142.7 (q); MS: m/z 361 ($M^+ + 1$); *Anal.* Calcd for $C_{25}H_{32}N_2$: C, 83.28; H, 8.95; N, 7.77. Found: C, 82.98; H, 9.03; N, 7.56.

5-Methyl-6,7-dihydrobenzo[4,5]cyclohepta[b]indole-12-thione (6). A mixture of **1** (886 mg, 3.39 mmol) and Lawesson's reagent (2.06 g, 5.09 mmol) in anhydrous toluene (80 mL) was refluxed for 3 h. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (eluent: 7:3 methylene chloride/petroleum ether) to give 826 mg (88%) of **6** as an amorphous compound; IR (film) ν 1071 (C=S) cm^{-1} ; 1H NMR (250 MHz, $CDCl_3$) δ : 2.92-2.97 (m, 2H, CH_2); 3.11-3.16 (m, 2H, CH_2); 3.50 (s, 3H, CH_3); 7.12-7.33 (m, 6H, H_{Ar}); 7.84 (dd, 1H, $J = 8.0$ Hz, H_{Ar}); 8.93 (d, 1H, $J = 7.1$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ : 28.9 (CH_2), 29.8 (NCH_3), 32.1 (CH_2), 109.0, 122.9, 123.8 (2 C), 126.8, 127.1, 127.9 (q), 129.6, 130.3 (2 C), 132.9 (q), 137.8 (q), 145.7 (q), 150.9 (q), 224.9 (C=S); MS: m/z 278 ($M^+ + 1$); *Anal.* Calcd for $C_{18}H_{15}NS$: C, 77.94; H, 5.45; N, 5.05. Found: C, 78.25; H, 5.60; N, 5.17.

5-Methyl-5,12-dihydrobenzo[4,5]cyclohepta[b]indole-12-thione (8). Following the same procedure used for **6** but substituting **1** by **7**, purification of the residue by silica gel chromatography (eluent: 7:3 methylene chloride/petroleum ether) to give **8** (64%) as a crystalline compound: mp 135-136 °C (methanol); IR (film) ν 1069 (C=S) cm^{-1} ; 1H NMR (250 MHz, $CDCl_3$) δ : 3.76 (s, 3H, CH_3); 7.09 (d, 1H, $J = 11.8$ Hz, $CH=$); 7.26 (d, 1H, $J = 11.8$ Hz, $CH=$); 7.30-7.43 (m, 3H, H_{Ar}); 7.49-7.57 (m, 3H, H_{Ar}); 8.82-8.86 (m, 1H, H_{Ar}); 9.11-9.15 (dd, 1H, $J = 1.3, 7.0$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, $CDCl_3$) δ : 29.9 (NCH_3), 109.3, 117.6, 123.0, 123.5, 126.0, 127.7 (q), 129.7, 129.8, 129.9 (q), 130.7, 133.1 (2 C), 135.3, 137.5 (q), 139.1 (q), 147.4 (q), 215.4 (C=S); MS: m/z 276 ($M^+ + 1$); *Anal.* Calcd for $C_{18}H_{13}NS$: C, 78.51; H, 4.76; N, 5.09. Found: C, 78.32; H, 4.58; N, 5.20.

5-Methyl-12-methylsulfanyl-6,7-dihydrobenzo[4,5]cyclohepta[b]indolium iodide (9). To a solution of thioketone (**6**) (340 mg, 1.23 mmol) in THF (20 mL) was added dropwise a solution of methyl iodide (1 mL, 16.0 mmol). The mixture was stirred at rt for 24 h. The red precipitates were collected, washed with ether to provide 309 mg (60%) of **9** as red crystals: mp 170-180 °C (washing with THF); 1H NMR (250 MHz, $CDCl_3$) δ : 2.67 (s, 3H, CH_3); 3.35-3.40 (m, 2H, CH_2); 3.63-3.74 (m, 2H, CH_2); 7.50-7.60 (m, 7H, H_{Ar}); 8.39-8.41 (m, 1H, H_{Ar}). The compound (**9**) was used in the next step without further characterisation.

5-Methyl-5,6,7,12-dihydrobenzo[4,5]cyclohepta[b]indol-12-one-12-(2-pyridyl)hydrazone (3a). 2-Pyridinylhydrazine (222 mg, 2.16 mmol) was added to a solution of **9** (300 mg, 0.72 mmol) in ethanol (30 mL) and the mixture was heated to reflux for 2 h. Ethanol was removed *in vacuo*, 20 mL of methylene chloride and saturated aqueous $NaHCO_3$ were added. The product was extracted twice. The organic phase was dried ($MgSO_4$), then evaporated. The crude compound was washed by methanol and filtered to afford 167 mg (66%) of **3a** as white solid; mp 267-268 °C (dichloromethane-methanol); IR (KBr) ν 3325 (NH),

1592 (C=N) cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) δ : 2.86 (td, 1H, $J = 4.0, 16.0$ Hz, CH_2); 3.01 (dt, 1H, $J = 4.0, 16.0$ Hz, CH_2); 3.14 (dt, 1H, $J = 4.0, 16.0$ Hz, CH_2); 3.39 (td, 1H, $J = 4.0, 16.0$ Hz, CH_2); 3.60 (s, 3H, CH_3); 6.70-6.75 (m, 1H, H_{pyr}); 7.21-7.41 (m, 6H, H_{Ar}); 7.50-7.66 (m, 3H, H_{Ar} , H_{pyr}); 8.07-8.09 (m, 1H, H_{pyr}); 8.32-8.37 (m, 1H, H_{Ar}); 8.57 (s, 1H, NH); ^{13}C NMR (62.90 MHz, CDCl_3) δ : 27.4 (CH_2), 29.4 (NCH_3), 31.9 (CH_2), 107.3, 108.6, 111.3 (q), 115.2, 120.5, 120.7, 121.9, 125.4 (q), 127.0, 127.1, 129.3, 129.7, 134.5 (q), 136.8 (q), 138.2, 138.6 (q), 138.8 (q), 141.7 (q), 147.6 (q), 157.1 (q); MS: m/z 353 ($\text{M}^+ + 1$); Anal. Calcd for $\text{C}_{23}\text{H}_{20}\text{N}_4$: C, 78.38; H, 5.72; N, 15.90. Found: C, 78.12; H, 5.53; N, 15.93.

***N*-{4-[5-Methyl-5,6,7,12-tetrahydrobenzo[4,5]cyclohepta[*b*]indol-12-ylidene)amino]phenyl}acetamide (3b).** Following the same procedure used for 3a but substituting 2-pyridinylhydrazine by 4-acetamidoaniline, purification of the residue by silica gel chromatography (eluent: 95:5 methylene chloride/methanol) yielded 3b (72%) as a crystalline compound: mp 274-275 °C (methanol); IR (film) ν 3396 (NH), 1694 (C=O) cm^{-1} ; ^1H NMR (250 MHz, DMSO-d_6) δ : 1.99 (s, 3H, COCH_3); 3.20-3.32 (m, 4H, CH_2); 3.66 (s, 3H, NCH_3); 6.66-6.69 (m, 3H, H_{Ar}); 6.92 (t, 1H, $J = 8.0$ Hz, H_{Ar}); 7.13-7.19 (m, 2H, H_{Ar}); 7.36-7.39 (m, 4H, H_{Ar}); 7.44-7.48 (m, 1H, H_{Ar}); 8.41-8.45 (m, 1H, H_{Ar}); 9.75 (s, 1H, NH); ^{13}C NMR (62.90 MHz, DMSO-d_6) δ : 23.9 (CH_3), 27.2 (CH_2), 29.4 (NCH_3), 31.4 (CH_2), 109.2, 112.7 (q), 119.3 (2 C), 120.4, 121.2, 121.5 (2 C), 121.8, 125.5, 125.8, 128.1, 128.3, 128.7, 134.4 (q), 136.6 (q), 138.6 (q), 142.9 (q), 146.7 (q), 162.6 (q), 167.8 (C=O); MS: m/z 394 ($\text{M}^+ + 1$); Anal. Calcd for $\text{C}_{26}\text{H}_{23}\text{N}_3\text{O}$: C, 79.36; H, 5.89; N, 10.68. Found: C, 79.01; H, 6.03; N, 10.55

***N,N*-Diethyl-*N*-(5-methyl-5,6,7,12-tetrahydrobenzo[4,5]cyclohepta[*b*]indol-12-ylidene)-1,2-ethanediamine (3c).** Following the same procedure used for 3a but substituting 2-pyridinylhydrazine by *N,N*-diethylethylenediamine, purification of the residue by silica gel chromatography (eluent: 95:5 methylene chloride/methanol) yielded 3c (42%) as a crystalline compound: mp 211-212 °C (methanol); IR (film) ν 1615 (C=N) cm^{-1} ; ^1H NMR (250 MHz, DMSO-d_6) δ : 1.13 (t, 6H, $J = 7.1$ Hz, CH_3); 2.77-3.41 (m, 10H, CH_2); 3.63 (s, 3H, NCH_3); 4.05-4.20 (m, 2H, CH_2); 7.06-7.51 (m, 7H, H_{Ar}); 8.09 (d, 1H, $J = 7.1$ Hz, H_{Ar}); ^{13}C NMR (62.90 MHz, DMSO-d_6) δ : 9.4 (2, CH_3), 27.0 (CH_2), 29.3 (NCH_3), 31.0 (CH_2), 46.5 (CH_2), 46.8 (2, CH_2), 52.5 (CH_2), 109.1, 113.1 (q), 119.8, 120.5, 121.4, 125.4 (q), 125.5 (q), 125.9, 126.7, 128.9, 129.1, 136.3 (q), 137.4 (q), 137.6 (q), 140.7 (q); MS: m/z 360 ($\text{M}^+ + 1$); Anal. Calcd for $\text{C}_{24}\text{H}_{29}\text{N}_3$: C, 80.18; H, 8.13; N, 11.69. Found: C, 80.37; H, 8.01; N, 11.54.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Mr. Arnaud Mulnet.

REFERENCES

1. R.P. Hertzberg and R.K. Johnson, *Annu. Rep. Med. Chem.*, 1993, **28**, 167.
2. J.R. Prous, *The Year's Drug News*, Prous Science Publishers, Barcelona, 1994, section 14, pp. 404-471.
3. B. Joseph, O. Cornec, J.-Y. Mérour, X. Solans, and M. Font-Bardia, *J. Heterocycl. Chem.*, 1997, **34**, 525.
4. a) J.A. Spicer, S.A. Gamage, G.J. Atwell, G.J. Finlay, B.C. Baguley, and W.A. Denny, *J. Med. Chem.*, 1997, **40**, 1919; b) C. Alvarez-Ibarra, R. Fernandez-Granda, M.L. Quiroga, A. Carbonell, F. Cardenas, and E. Giralt, *J. Med. Chem.*, 1997, **40**, 668; c) A. Varvaserou, A. Tsotinis, Th. Siatra-Papastaikoudi, A. Papadaki-Valikari, D.E. Thurston, T.C. Jenkins, and L.R. Kelland, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 865.
5. G.-H. Kuo, M.A. Eissenstat, M.P. Wentland, R.G. Robinson, K.M. Klingbeil, D.W. Danz, and S.A. Coughlin, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 399.
6. A. Greiner, *Tetrahedron Lett.*, 1989, **30**, 3547.
7. S. Kim, C.H. Oh, J.S. Ko, K.H. Ahn, and Y.J. Kim, *J. Org. Chem.*, 1985, **50**, 1927.
8. a) M.P. Cava, and M.I. Levinson, *Tetrahedron*, 1985, **41**, 5061; b) A. Lévai, *J. Chem. Res. (S)*, 1992, 163.
9. The two murine L1210 leukemia and Lewis lung carcinoma (LLC) cell lines were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by microculture tetrazolium assay.¹⁰ Briefly, L1210 and LLC cells were exposed to graded concentrations of drug for 48 and 96 h, respectively (4 doubling times). Results are expressed as IC₅₀, the concentration which reduce by 50% the optical density of treated cells with respect to the density of untreated cells.
10. S. Leonce, V. Perez, M.R. Casabianca-Pignede, M. Anstett, E. Bisagni, A. Pierre, and G. Atassi, *Investigational New Drugs*, 1996, **14**, 169.

Received, 26th March, 1998