Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 Vol. 17 (6), pp. 397–401

Synthesis and Cytotoxic Evaluation of Novel Thiazolocarbazoles

CHRISTELLE LAMAZZI[a,b], HADJILA CHABANE[a,b], VALERIE THIERY[b], ALAIN PIERRE[c], STEPHANE LEONCE[c], BRUNO PFEIFFER[d], PIERRE RENARD[d], GÉRALD GUILLAUMET[a] and THIERRY BESSON[b,*]

[a]*Institut de Chimie Organique et Analytique, UMR-CNRS 6005, Université d'Orléans, rue de Chartres, BP 6759, F-45067 Orléans cedex 2, France;* [b]*Laboratoire de Génie Protéique et Cellulaire, EA3169, Groupe de Chimie Organique, UFR Sciences Fondamentales et Sciences pour l'Ingénieur, Bâtiment Marie Curie, Université de la Rochelle, F-17042 La Rochelle cedex 1, France;* [c]*Institut de Recherche Servier, Division de Cancérologie Expérimentale, 11 Rue des Moulineaux, 92150 Suresnes, France;* [d]*A.D.I.R., 1 Rue Carle Hébert, 92415 Courbevoie cedex, France*

(Received 20 March 2002)

Novel thiazolocarbazole derivatives 4 and 5 have been synthesized *via* the corresponding imino-1,2,3-dithiazoles 3. *In vitro* antitumor activity of these polyheterocyclic compounds was studied and the results show that 2-cyano derivatives exhibit a medium *in vitro* antiproliferative effect.

Keywords: Imino-1,2,3-dithiazoles; Appel salt; Carbazoles; Thiazoles; Cytotoxic activity

INTRODUCTION

The thiazole ring is present in various marine or terrestrial natural compounds which possess useful biological activities.[1–3] As we are interested in original heterocyclic systems with pharmacological potential, we decided to prepare new tetracyclic thiazolocarbazole derivatives by fusing the carbazole and the thiazole rings. The resulting structures are related to recently described thiazoloacridines (**I**),[4–6] thiazoloquinolines (**II**)[7] and substituted benzothiazoles (**III**)[8,9] which exhibit interesting antitumor activity (Figure 1).

Fusion of the thiazole ring onto the carbazole skeleton suggested the use of imino-1,2,3-dithiazoles which proved to be highly versatile intermediates in heterocyclic synthesis.[10–13] In this paper we describe the synthetic route to these new polyheterocyclic compounds (**IV**).

MATERIALS AND METHODS

Chemistry

Instrumentation

Mps were determined using a Kofler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer Paragon 1000PC instrument. ^{1}H and ^{13}C-NMR were recorded on a JEOL JNM LA400 (400 MHz) spectrometer (Centre Commun d'Analyses, Université de la Rochelle); chemical shifts (δ) are reported in part per million (ppm) downfield from tetramethylsilane (TMS) which was used as internal standard. Coupling constants J are given in Hz. Mass spectra were recorded on a Varian MAT311 spectrometer in the Centre Régional de Mesures Physiques de l'Ouest (CRMPO), Université de Rennes. Chromatography was carried out on silica gel 60 at medium pressure. Light petroleum refers to the fraction b.p. 40–60°C. Other solvents were used without purification. Thin-layer chromatography was performed on Merck Kieselgel 60 F254 aluminium backed plates.

Synthesis

Synthesis of Imino-1,2,3-dithiazoles **3**; General Procedure

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride **1** (1 mmol) was added to

*Corresponding author. Tel.: +33-5-46-45-82-76. Fax: +33-5-46-45-82-47/65. E-mail: tbesson@univ-lr.fr

Abbreviations: HEPES, 2-[4-(2-hydroxethyl)-piperazin-1-yl]ethanesulfonic acid; PBS, phosphate buffered solution; RNase, ribonuclease

ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1475636021000005703

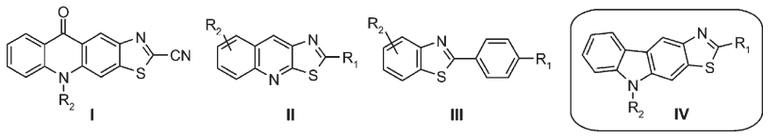


FIGURE 1 Structures of compounds (**I**), (**II**) and (**III**).

a stirred solution of the starting amine **2** (1 mmol) in dichloromethane (10 mL). After 1 h, pyridine (2 mmol) was added and the red mixture stirred for 30 min. The solvent was removed in vacuo and the crude residue purified by column chromatography (light petroleum/ethyl acetate) to afford the required compounds **3** (see Table I).

Thiazolocarbazoles **4** And **5**; Thermolysis General Procedure

N-(4-Chloro-5*H*-1,2,3-dithiazol-5-yliden)arylamines **3** were heated under argon at 200°C for 1 or 2 min. The product was isolated by column chromatography (light petroleum/ethyl acetate) to afford the expected products **4** and **5** (see Table I).

6H-1-Thia-3,6-diazacyclopenta[c]fluorene-2-carbonitrile **4a**: brown needles, mp = 230°C (Found M^+, 249.0361. $C_{14}H_7N_3S$ requires 249.0361); ν_{max} (KBr)/cm^{-1} 3346 (NH), 2920, 2230 (CN), 1586, 1475, 1328, 731; δ_H(400 MHz, $CDCl_3$ + D_2O) 7.45 (td, 1H, *J* 7.5 and 1.1 Hz, H_{ar}), 7.56 (td, 1H, *J* 7.5 and 1.1 Hz, H_{ar}), 7.61 (d, 1H, *J* 8.2 Hz, H_{ar}), 7.76 (d, 1H, *J* 8.9 Hz, H_{ar}), 8.06 (d, 1H, *J* 8.2 Hz, H_{ar}), 8.25 (d, 1H, *J* 8.9 Hz, H_{ar}); m/z 249 (M^+, 100%), 196 (M^+-53, 6), 170 (M^+-79, 2), 153 (M^+-96, 6).

6-Methyl-6H-1-thia-3,6-diazacyclopenta[c]fluorene-2-carbonitrile **4b**: yellow needles, mp = 238°C (Found M^+, 263.0532. $C_{15}H_9N_3S$ requires 263.0517); ν_{max} (KBr)/cm^{-1} 3067, 2945, 2221 (CN), 1586, 1478, 1259, 1134, 914, 801, 727; δ_H(400 MHz, $CDCl_3$) 4.03 (s, 3H, CH_3), 7.45 (t, 1H, *J* 7.8 Hz, H_{ar}), 7.57–7.64 (m, 2H, H_{ar}), 7.74 (d, 1H, *J* 9.0 Hz, H_{ar}), 8.07 (d, 1H, *J* 7.8 Hz, H_{ar}), 8.28 (d, 1H, *J* 9.0 Hz, H_{ar}); δ_C(100 MHz, $CDCl_3$) 29.8, 109.6, 110.6, 113.7, 114.4, 120.7, 121.1, 122.1, 126.4, 127.0, 131.7, 139.9, 145.7, 146.1; m/z 263 (M^+, 100%), 248 (M^+-CH_3, 9).

6-Ethyl-6H-1-thia-3,6-diazacyclopenta[c]fluorene-2-carbonitrile **4c**: pale yellow needles, mp = 154–156°C (Found M^+, 277.0675. $C_{16}H_{11}N_3S$ requires 277.0673); ν_{max} (KBr)/cm^{-1} 3080, 2983, 2226 (CN), 1585, 1477, 1338, 1247, 1145, 801, 745; δ_H(400 MHz, DMSO-d_6) 1.36 (t, 3H, *J* 7.1 Hz, CH_3), 4.55 (q, 2H, *J* 7.1 Hz, CH_2), 7.38 (t, 1H, *J* 7.7 Hz, H_{ar}), 7.58 (td, 1H, *J* 7.7 and 1.1 Hz, H_{ar}), 7.76 (d, 1H, *J* 8.3 Hz, H_{ar}), 7.95 (d, 1H, *J* 8.3 Hz, H_{ar}), 7.97 (d, 1H, *J* 9.1 Hz, H_{ar}), 8.22 (d, 1H, *J* 9.1 Hz, H_{ar}); δ_C(100 MHz, DMSO-d_6) 13.9, 37.7, 110.3, 111.6, 113.4, 113.7, 120.3, 120.4, 120.5, 121.7, 126.2, 128.3, 130.9, 138.6, 139.4, 146.3; m/z 277 (M^+, 65%), 262 (M^+-CH_3, 100). (Anal. Calcd. for $C_{16}H_{11}N_3S$: C, 69.29; H, 3.99; N, 15.15. Found: C, 69.15; H 4.24; N 15.14%).

6-(2-Dimethylaminoethyl)-6H-1-thia-3,6-diazacyclopenta[c]fluorene-2-carbonitrile **4d**: brown oil (Found M^+, 320.1083. $C_{18}H_{16}N_4S$ requires 320.1095); ν_{max} (oil)/cm^{-1} 2909, 2772, 2225 (CN), 1586, 1463, 1334, 1120, 746; δ_H(400 MHz, $CDCl_3$) 2.36 (s, 6H, CH_3), 2.75 (t, 2H, *J* 7.5 Hz, CH_2), 4.53 (t, 2H, *J* 7.5 Hz, NCH_2), 7.35–7.45 (m, 2H, H_{ar}), 7.55–7.62 (m, 2H, H_{ar}), 7.70 (d, 1H, *J* 9.0 Hz, H_{ar}), 7.99 (d, 1H, *J* 7.9 Hz, H_{ar}), 8.22 (d, 1H, *J* 9.0 Hz, H_{ar}); m/z 320 (M^+, 2%), 262 (10), 249 (9), 58 (100).

Ethyl 3-(6H-2-cyano-1-thia-3,6-diazacyclopenta[c]-fluoren-6-yl)-propionate **4e**: pale yellow needles, mp = 96–98°C (Found M^+, 349.0884. $C_{19}H_{15}N_3O_2S$ requires 349.0885); ν_{max} (KBr)/cm^{-1} 3056, 2982, 2226 (CN), 1732 (CO), 1585, 1476, 1331, 1185, 1060, 802, 745; δ_H(400 MHz, $CDCl_3$) 0.98 (t, 3H, *J* 7.1 Hz, CH_3), 2.88 (t, 2H, *J* 7.6 Hz, CH_2COOEt), 3.90 (q, 2H, *J* 7.1 Hz, $COOCH_2$), 4.82 (t, 2H, *J* 7.6 Hz, NCH_2), 7.42 (t, 1H, *J* 7.7 Hz, H_{ar}), 7.60 (t, 1H, *J* 7.7 Hz, H_{ar}), 7.83 (d, 1H, *J* 8.3 Hz, H_{ar}), 8.01 (d, 1H, *J* 8.3 Hz, H_{ar}), 8.07 (d, 1H, *J* 9.1 Hz, H_{ar}), 8.29 (d, 1H, *J* 9.1 Hz, H_{ar}); δ_C(100 MHz, $CDCl_3$) 14.0, 33.7, 39.3, 61.1, 109.7, 110.8, 113.6, 114.7, 120.9, 121.0, 121.4, 122.2, 126.5, 128.9,

TABLE I Synthesis of the imines **3** and thermolysis results

Starting material	R	Yield of **3** (%)	Yield of **4** (%)	Yield of **5** (%)
2a	H	50	5	N.D.[a]
2b	Me	75	30	N.D.
2c	Et	70	40	10
2d	$CH_2CH_2N(CH_3)_2$	34	4	N.D.
2e	CH_2CH_2COOEt	70	40	10
2f	$(CH_2)_2COOCH_2Ph$	65	45	N.D.
2g	$(CH_2)_2CONHCH_2Ph$	50	25	N.D.

[a]N.D.: not detected

131.9, 139.0, 139.7, 147.1, 170.9; m/z 349 (M^+, 44%), 275 (3), 262 (100). (Anal. Calcd. for $C_{19}H_{15}N_3O_2S$: C, 65.31; H, 4.32; N, 12.02. Found: C, 65.22; H 4.35; N 11.84%).

Benzyl 3-(6H-2-Cyano-1-thia-3,6-diazacyclopenta[c]-fluoren-6-yl)-propionate **4f**: yellow needles, mp = 165°C (Found M^+, 411.1042. $C_{24}H_{17}N_3O_2S$ requires 411.1041); ν_{max} (KBr)/cm^{-1} 3052, 2941, 2226 (CN), 1720 (CO), 1584, 1461, 1325, 1203, 751; δ_H(400 MHz, DMSO-d_6) 2.97 (t, 2H, *J* 6.6 Hz, CH_2COOBn), 4.84 (t, 2H, *J* 6.6 Hz, NCH_2), 4.92 (s, 2H, CH_2Bn), 7.10–7.12 (m, 2H, H_{ar}), 7.23–7.23 (m, 3H, H_{ar}), 7.42(t, 1H, *J* 7.7 Hz, H_{ar}), 7.59 (td, 1H, *J* 7.7 and 1.0 Hz, H_{ar}), 7.83 (d, 1H, *J* 8.4 Hz, H_{ar}), 8.0 (d, 1H, *J* 8.4 Hz, H_{ar}), 8.05 (d, 1H, *J* 9.1 Hz, H_{ar}), 8.25 (d, 1H, *J* 9.1 Hz, H_{ar}); δ_C(100 MHz, DMSO-d_6) 33.3, 39.1, 65.7, 110.8, 112.2, 113.5, 113.8, 120.4, 120.8, 121.7, 126.4, 127.8, 127.9, 128.1, 128.2, 131.2, 135.5, 138.8, 139.4, 146.3, 170.8; m/z 411 (M^+, 60%), 350 (16), 320 (19), 262 (100).

N-Benzyl-3-(6H-2-cyano-1-thia-3,6-diazacyclopenta [c]fluoren-6-yl)-propionamide **4g**: pale brown, mp = 154–156°C (Found M^+, 410.1202. $C_{24}H_{18}N_4OS$ requires 410.1201); ν_{max} (KBr)/cm^{-1} 3271 (NH), 3082, 2223 (CN), 1641 (CO), 1549, 1439, 1331, 1228, 1121, 1027, 744; δ_H(400 MHz, $CDCl_3$ + D_2O) 2.76 (t, 2H, *J* 6.2 Hz, CH_2CONH), 4.21 (s, 2H, CH_2Bn), 4.83 (t, 2H, *J* 6.2 Hz, NCH_2), 6.83 (d, 2H, J 7.0 Hz, H_{ar}), 7.05–7.18 (m, 3H, H_{ar}), 7.43 (t, 1H, *J* 7.9 Hz, H_{ar}), 7.55–7.65 (m, 2H, H_{ar}), 7.78 (d, 1H, *J* 9.0 Hz, H_{ar}), 7.99 (d, 1H, *J* 7.9 Hz, H_{ar}), 8.17(d, 1H, *J* 9.0 Hz, H_{ar}); δ_C(100 MHz, $CDCl_3$) 36.0, 39.9, 43.7, 109.9, 111.0, 113.6, 114.6, 120.9, 121.0, 121.3, 122.2, 126.5, 127.5, 128.5, 128.7, 131.7, 137.2, 139.0, 139.7, 147.1, 169.6; m/z 410 (M^+, 93%), 262 (100), 249 (10), 148 (29).

9-Ethyl-9H-1-thia-3,9-diazacyclopenta[b]fluorene-2-carbonitrile **5c**: pale yellow needles, mp = 183–185°C (Found M^+, 277.0675. $C_{16}H_{11}N_3S$ requires 277.0673); ν_{max} (KBr)/cm^{-1} 3080, 2924, 2219 (CN), 1598, 1476, 1350, 1287, 1237, 1120, 1071, 745; δ_H(400 MHz, DMSO-d_6) 1.36 (t, 3H, *J* 7.2 Hz, CH_3), 4.45 (q, 2H, *J* 7.2 Hz, CH_2), 7.28 (t, 1H, *J* 7.4 Hz, H_{ar}), 7.56 (d, 1H, *J* 7.6 Hz, H_{ar}), 7.63 (d, 1H, *J* 8.2 Hz, H_{ar}), 8.33 (d, 1H, *J* 7.7 Hz, H_{ar}), 8.35 (s, 1H, H_{ar}), 8.99 (s, 1H, H_{ar}); δ_C(100 MHz, DMSO-d_6) 13.1, 37.4, 110.7, 109.4, 113.8, 115.9, 119.6, 121.3, 121.7, 125.1, 127.6, 132.1, 133.8, 140.7, 141.3, 147.5; m/z 277 (M^+, 71%), 262 (M^+-CH_3, 100). (Anal. Calcd. for $C_{16}H_{11}N_3S$: C, 69.29; H, 3.99; N, 15.15. Found: C, 69.04; H 4.24; N 15.07).

Ethyl 3-(6H-2-Cyano-1-thia-3,9-diazacyclopenta[b]-fluoren-9-yl)-propionate **5e**: pale yellow needles, mp = 128–130°C (Found M^+, 349.0877. $C_{19}H_{15}N_3O_2S$ requires 349.0885); ν_{max} (KBr)/cm^{-1}, 2985, 2225 (CN), 1729 (CO), 1600, 1537, 1480, 1185; δ_H(400 MHz, $CDCl_3$) 1.00 (t, 3H, *J* 7.1 Hz, CH_3), 2.86 (t, 2H, *J* 6.8 Hz, CH_2COOEt), 3.93 (q, 2H, *J* 7.1 Hz, $COOCH_2$), 4.60 (t, 2H, *J* 6.8 Hz, NCH_2), 7.26 (t, 1H, *J* 7.7 Hz, H_{ar}), 7.60 (td, 1H, *J* 7.7 and 1.1 Hz, H_{ar}), 7.61 (d, 1H, *J* 8.2 Hz, H_{ar}), 8.26 (d, 1H, *J* 8.2 Hz, H_{ar}), 8.29 (s, 1H, H_{ar}), 8.89 (s, 1H, H_{ar}); δ_C(100 MHz, $CDCl_3$) 13.7, 32.6, 38.7, 60.1, 101.0, 109.6, 113.7, 115.6, 119.7, 121.2, 121.6, 124.9, 127.4, 132.2, 133.6, 140.5, 141.2, 145.3, 170.8; m/z 277 (M^+, 71%), 262 (M^+-CH_3, 100). (Anal. Calcd. for $C_{19}H_{15}N_3O_2S$: C, 65.31; H, 4.32; N, 12.02. Found: C, 65.21; H 4.05; N 11.88).

Decyanation Procedure

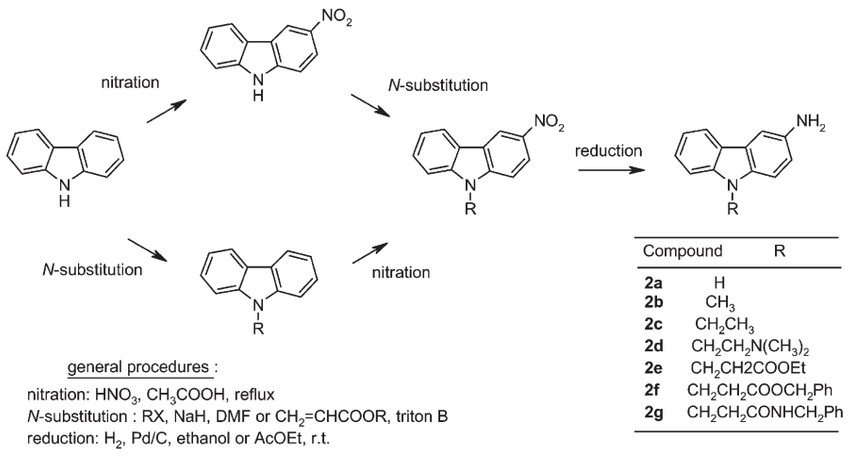
9-Ethyl-9H-1-thia-3,9-diazacyclopenta[c]fluorene **6c**: a suspension of benzothiazole **5c** (1 g, 3.61 mmol) in concentrated hydrochloric acid (20 ml) was heated at reflux for 3 h. The mixture was allowed to cool to room temperature and neutralised (pH8). The mixture was washed with dichloromethane and the combined extracts were dried. Recrystallisation of the crude product from hexane gave the expected product 6c. White needles, mp = 115–117°C (Found M^+, 252.0717. $C_{15}H_{12}N_2S$ requires 252.0721); ν_{max} (KBr)/cm^{-1}, 2965, 1588, 1477, 1233, 1153, 1091, 836; δ_H(400 MHz, DMSO-d_6) 1.34 (q, 3H, *J* 7.1 Hz, CH_3), 4.58 (t, 2H, *J* 7.1 Hz, CH_2), 7.36 (t, 1H, *J* 7.5 Hz, H_{ar}), 7.55 (t, 1H, *J* 7.7 Hz, H_{ar}), 7.76 (d, 1H, *J* 8.3 Hz, H_{ar}), 7.87 (d, 1H, *J* 8.8 Hz, H_{ar}), 8.05 (d, 1H, *J* 7.7 Hz, H_{ar}), 8.19 (d, 1H, *J* 8.8 Hz, H_{ar}), 9.36 (s, 1H, H_{ar}); δ_C(100 MHz, $CDCl_3$) 14.0, 38.0, 108.2, 109.0, 115.3, 119.6, 120.8, 121.0, 121.7, 125.5, 126.6, 137.5, 139.6, 148.0, 149.8; m/z 252 (M^+, 69%), 237 (M^+-CH_3, 100). (Anal. Calcd. for $C_{15}H_{12}N_2S$: C, 71.39; H, 4.79; N, 11.10. Found: C, 71.36; H 5.08; N 11.09%).

In Vitro Antitumor Activity

L1210 cells (murine leukemia), provided by the NCI (Frederick, USA), were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fœtal 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 the microculture tetrazolium assay as described in reference 16. Cells were exposed to graded concentrations of the compounds for 48 h and results are expressed as IC_{50} values (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls).

For the cell cycle 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 PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide for 30 min at 20°C. For each sample, 1×10^4 cells were analysed on a Epics XL/MCL flow cytometer (Beckman Coulter, France). Results are expressed as the percentage of cells found in the G_1 phase of the cell cycle, and were obtained from at least three independent experiments.



Compound	R
2a	H
2b	CH_3
2c	CH_2CH_3
2d	$CH_2CH_2N(CH_3)_2$
2e	$CH_2CH2COOEt$
2f	$CH_2CH_2COOCH_2Ph$
2g	$CH_2CH_2CONHCH_2Ph$

SCHEME 1 General method of synthesis of the starting amines **2**.

RESULTS AND DISCUSSION

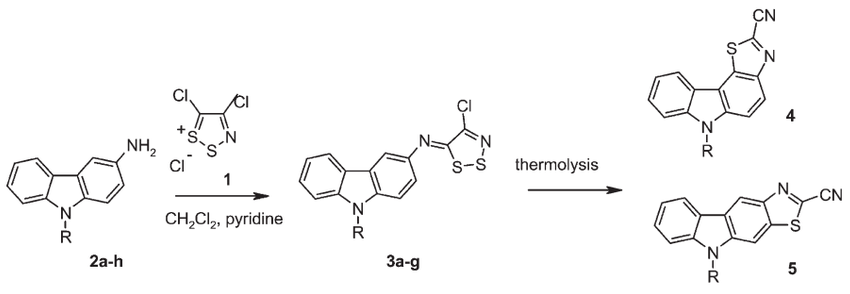
Chemistry

The thiazolocarbazole ring has been very rarely described in the literature and one of the most recent synthesis of such a ring was performed via the Fischer-indole synthesis,[14] a method which does not allow modulation of the substituents fixed at nitrogen. Studying the chemistry of 4,5-dichloro-1,2,3-dithiazolium chloride **1** (Appel salt) and its derivatives, we showed that 5-(*N*-arylimino)-4-chloro-5*H*-1,2,3-dithiazoles, which are stable crystalline solids, readily prepared in high yield from anilines and the salt **1**, cyclised on vigorous heating to give 2-cyanobenzothiazoles with sulfur and hydrogen chloride elimination.[10–13]

Synthesis of the novel thiazolocarbazoles **4** and **5** was performed in two steps from the starting 3-aminocarbazoles **2**; preliminary studies had shown that it was better to start from the *N*-substituted carbazoles.

Except for the commercially available 3-amino-9-ethylcarbazole **2c**, the starting amines were prepared from carbazole. For **2a**, **2b**, **2d**, and **2e**, the general sequence of reactions (three steps) involves nitration of the carbazole skeleton, N^9-substitution and reduction of the nitro group to afford the corresponding amines (Scheme 1). Compounds **2f–h** were prepared in six steps from carbazole as previously described.[15]

Using a standard method used for the preparation of *N*-arylimino-1,2,3-dithiazoles,[10–13] the starting aminocarbazoles **2** were condensed with 4,5-dichloro-1,2,3,-dithiazolium chloride **1** in dichloromethane at room temperature, followed by addition of pyridine, to give the desired imino-1,2,3-dithiazolocarbazoles **3** in good yields (Scheme 2, Table I). The best thermolysis procedure consisted in heating the neat imines **3** under argon at 200–250°C (metal bath) for 1 or 2 minutes. A short exploration of various alternatives has shown that heating **3** for 2 or 3 days at 140°C (oil bath) in a sealed tube in the presence of toluene or exposing the imines to microwave irradiation, neat in glass vial with a screw cap lid, gave, in this case, less favourable results. Whatever conditions were used, the major product, obtained in reasonable yields, was the angular isomer **4** (Scheme 2). Only **3c** and **3e** afforded the linear counterpart **5c** and **5e** in a low yield (10%). Yields of compounds **3**, **4** and **5** are summarised in Table I.



SCHEME 2 Synthetic route to thiazolocarbazoles **4** and **5**.

TABLE II Cytotoxicity and antiproliferative activity results for compounds **4** and **5**

Compound	Formula	Cytotoxicity IC_{50} L1210 (μM)	% of L1210 cells in the cell cycle phases[a] G_1 (μM)
4b	$C_{15}H_9N_3S$	14.1	NE[b]
4c	$C_{16}H_{11}N_3S$	5.4	NS (25)[c]
4e	$C_{19}H_{15}N_3O_2S$	10.3	50–60 (10)
4f	$C_{24}H_{17}N_3O_2S$	7.7	60–80 (25)
4g	$C_{24}H_{18}N_4OS$	3.3	50–60 (10)
5c	$C_{16}H_{11}N_3S$	9.1	NS (25)
5e	$C_{19}H_{15}N_3O_2S$	19	NE

[a]% of untreated control cells in the phases of the cell cycle: 41% (G_1); 28% (S); 24% (G_2 + M); 1% (8N). [b]NE = not evaluated (for $IC_{50} > 10$ (μM). [c]NS = non specific, toxic at 25 μM.

Removal of the cyano group was carried out starting from **4c** by hydrolysis and decarboxylation in concentrate HCl, leading to **6c** in 95% yield.

In Vitro Antitumor Activity

In vitro antitumor activity of the compounds described have been assessed using the murine leukemia cell line. Selected data are listed in Table II for the most active compounds ($IC_{50} < 20$ μM). The low yields for compounds **4a** and **4d** did not allow a correct evaluation of their biological activity.

All the 2-cyanothiazolocarbazoles listed in Table II were found practically equipotent with respect to inhibition of cell proliferation; some of them were able to block partially the cells in the G_1 phase of the cell cycle.

Comparison of the data presented in Table II suggests that the angular derivatives **4** are more cytotoxic than their linear counterparts **5**. The nature of the substituents attached to the carbazole nitrogen does not seem to exert a significant influence on the cytotoxic activity. Evaluation of **6c**, the decyanated analog of **4c**, one of the most active of the 2-cyano derivatives, showed that such a pharmacomodulation induced suppression of the cytotoxic activity.

In conclusion, we have described the original synthesis of novel 2-cyanothiazolocarbazoles, which exhibit interesting in vitro cytotoxic activity. Unfortunately modifications of the substituent on the *N* atom of the carbazole ring, in combination with the cyano group in the 2-position of the thiazole, did not really induce any specific effect on the cell cycle. Our present results suggest that transformation of the cyano group into carboxylic acids, amides or amines (with appropriate substitution) could lead to promising results.

Acknowledgements

We thank *SERVIER* Laboratories and *the Comité de Charente-Maritime de la Ligue Nationale Contre le Cancer* for financial support.

References

[1] Molinski, T.F. (1993) "Marine pyridoacridine alkaloids: structure and biological chemistry", *Chemical Reviews* **93**, 1825–1838.
[2] Gunawardana, G.P., Kohmoto, S., Gunasekara, S.P., McConnel, O.J. and Koehn, F.E. (1988) "Dercitin, a new biologically active acridine alkaloid from a deep water sponge marine sponge, *Dercitus* sp.", *Journal of American Chemical Society* **110**, 4856–4858.
[3] Gunawardana, G.P., Kohmoto, S. and Burres, N.S. (1989) "New cytotoxic acridine alkaloids from two deep water marine sponges of the family Pachastrellidae", *Tetrahedron Letters* **30**, 4359–4362.
[4] Robin, M., Faure, R., Périchaud, A. and Galy, J.P. (2000) "Synthesis of new thiazolo[5,4-*a*]acridine derivatives", *Heterocycles* **53**, 387–395.
[5] Hanoun, J.P., Faure, R., Galy, J.P. and Elguero, J. (1996) "Azido/tetrazole equilibrium in the thiazolacridinone series", *Journal of Heterocyclic Chemistry* **33**, 747–750.
[6] Barbe, J., Boyer, G., Carignano, I., Elguero, J., Galy, J.P., Morel, S. and Oughedani, R. (1991) "Thiazolo[5,4-*a*]acridines", *Tetrahedron Letters* **32**, 6709–6710.
[7] Alvarez-Ibarra, C., Frenandez-Granda, R., Quiroga, M.L., Carbonell, A., Cardenas, F. and Giralt, E. (1997) "Synthesis and antitumor evaluation of new thiazolo[5,4-*b*]quinoline derivatives", *Journal of Medicinal Chemistry* **40**, 668–676.
[8] Wells, G., Bradshaw, T.D., Diana, P., Seaton, A., Shi, D.-F., Westwell, A.D. and Stevens, M.F.G. (2000) "Antitumor benzothiazoles. Part 10: the synthesis and antitumor activity of benzothiazole substituted quinol derivatives", *Bioorganic & Medicinal Chemistry Letters* **10**, 513–515.
[9] Bradshaw, T.D., Wrigley, S., Shi, D.-F., Schultz, R.J., Paull, K.D. and Stevens, M.F.G. (1998) "2-(4-Aminophenyl)benzothiazoles: novel agents with selective profiles of *in vitro* antitumor activity", *British Journal of Cancer* **77**, 745–752.
[10] Bénéteau, V., Besson, T., Guillard, J., Leonce, S. and Pfeiffer, B. (1999) "Synthesis and in vitro antitumour evaluation of benzothiazole-2-carbonitrile derivatives", *European Journal of Medicinal Chemistry* **34**, 1053–1060.
[11] Besson, T., Dozias, M.J., Guillard, J. and Rees, C.W. (1998) "New route to 2-cyanobenzothiazoles via *N*-arylimino-1,2,3-dithiazoles", *Journal of the Chemical Society, Perkin Transactions* **1**, 3925–3926.
[12] Bénéteau, V., Besson, T. and Rees, C.W. (1997) "Rapid synthesis of 2-cyanobenzothiazoles from *N*-aryliminodithiazoles under microwave irradiation", *Synthetic Communications* **27**, 2275–2280.
[13] Besson, T. and Rees, C.W. (1995) "Some chemistry of 4,5-dichloro-1,2,3-dithiazolium chloride and its derivatives", *Journal of the Chemical Society, Perkin Transactions* **I**, 1659–1662.
[14] Martarello, L., Joseph, D. and Kirsch, G. (1995) "Preparation of thiazolocarbazoles via the Fischer indole synthesis", *Journal of the Chemical Society, Perkin Transactions* **I**, 2941–2944.
[15] Besson, T., Joseph, B., Moreau, P., Viaud, M.C., Coudert, G. and Guillaumet, G. (1992) "Synthesis and fluorescent properties of new heterobifunctional fluorescent probes", *Heterocycles* **34**, 273–291.
[16] Léonce, S., Pérez, V., Casabianca-Pignède, M.R., Anstett, M., Bisagni, E. and Atassi, G. (1996) "In vitro cytotoxicity of S16020-2, a new olivacine derivative", *Investigational New Drugs* **14**, 169–180.