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Synthesis and Cytotoxic Evaluation of Novel Thiazolocarbazoles. Part II[1]

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Novel thiazolocarbazole derivatives have been synthesized *via* the corresponding imino-1,2,3-dithiazoles. In vitro antitumor activity of these polyheterocyclic compounds was studied.

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 and a number of structure–activity studies have already been made to determine the essential structural requirements associated with its biological activity.[2–4] In connection with recent works on the interest aroused by thiazoloacridines (**I**)[5–7], thiazoloquinolines (**II**)[8] and substituted benzothiazoles (**III**)[9,10], we recently described the synthesis of new derivatives in which the thiazole ring was fused with various heterocyclic structures (**IV**, **V**)[11,12]. As a continuation of this work, we recently decided to prepare novel linear tetracyclic thiazolocarbazoles (**VI**, **VII**) derivatives by fusing the carbazole and the thiazole rings[1,13] (Fig. 1). The resulting structures were inspired by natural marine alkaloids (*e.g.* dercitine and kuanoniamines[2]) or by the much studied terrestrial ellipticine[14–17] and showed a modest antitumor activity. Among all the compounds prepared, the two most promising structures were the 9-ethyl-9*H*-1-thia-3,9-diazacyclopenta[*b*]fluorene-2-carbonitrile **VI** ($R_1 = CN$, $R_2 = C_2H_5$) and the 4,10-dimethyl-9*H*-1-thia-3,9-diaza-cyclopenta[*b*]fluorene-2-carbonitrile **VII** ($R_1 = CN$) which showed an intermediate cytotoxic activity with no real effect on the cell cycle.

To ascertain the real impact of the lead compounds and with the aim of enhancing the biological activity of such products, wide modifications of the carbonitrile function in position 2 of the thiazole ring were studied. Inspired by previous work on ellipticine and congeners,[14–17] substitution in position 6 of the thiazolocarbazole skeleton by a hydroxy or methoxy group was also investigated with the aim of studying the influence of these modifications on the cytotoxic activity of such derivatives. In this paper we describe the synthetic route and the biological evaluation of these novel substituted polyheterocyclic compounds.

MATERIALS AND METHODS

Chemistry

Melting points were determined using a Köfler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer Paragon 1000PC

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Abbreviations: broad singulet (b.s.); 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI); 2-[4-(2-hydroxethyl)-piperazin-1-yl]ethanesulfonic acid (HEPES); 1-hydroxybenzotriazole (HOBt); phosphate buffered solution (PBS); ribonuclease (RNase).

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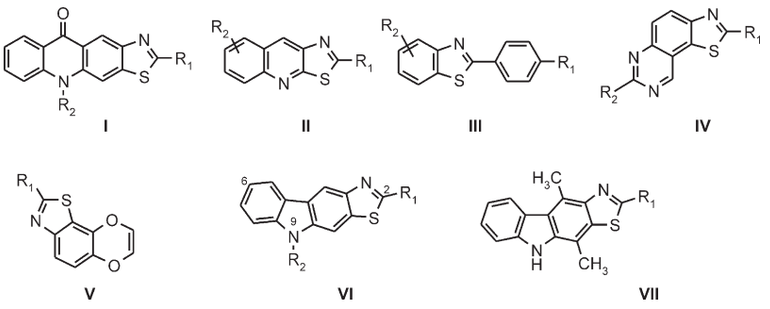


FIGURE 1 Structures (I–VII).

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 parts 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 with bp 40–60°C. Other solvents were used without purification. Thin-layer chromatography was performed on Merck Kieselgel 60 F254 aluminium backed plates. Compounds **3**, **4**, **10** and **12** were prepared according to procedures described by Lamazzi *et al.*[1] and Chabane *et al.*[13] Compound **1** is commercially available.

Synthesis of 2-substituted-6-ethyl-6H-[1,3]thiazolo[4,5]carbazoles

6-Ethyl-6H-[1,3]thiazolo[4,5-c]carbazole **5**

Compound **3** (1.0 g, 3.61 mmol) was refluxed for 3 h in 2 mL of HBr 48% and 8 mL of acetic acid. The solvent was removed under reduced pressure. The residue was poured into water (10 mL), neutralized with a satured solution of sodium hydrogenocarbonate and then extracted with ethyl acetate (2 × 20 mL). The extracts were dried over magnesium sulfate, and the solvent was removed under vacuum. The residue was purified by column chromatography with dichloromethane as eluent. Yield: 95%, white needles, mp 116°C (Found M^+, 252.0717, $C_{15}H_{12}N_2S$ requires 252.0721); ν_{max} (KBr)/cm^{-1} 3045, 2965, 1588, 1477, 1233, 747; δ_H(400 MHz, DMSO d_6) 1.34 (t, 3H, *J* 7.1 Hz, CH_3), 4.58 (q, 2H, CH_2N), 7.36 (t, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.55 (t, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.76 (d, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.87 (d, 1H, *J* 8.8 Hz, $H_{ar.}$), 8.05 (d, 1H, *J* 7.5 Hz, $H_{ar.}$), 8.19 (d, 1H, *J* 8.8 Hz, $H_{ar.}$), 9.36 (s, 1H, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 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).

6-Ethyl-6H-[1,3]thiazolo[4,5-c]carbazole-2-carboxamide **6**

A stirred solution of carbonitrile 3 (1.0 g, 3.6 mmol) in 10 mL of ethanol was treated with 10% aqueous potassium hydroxyde (4.0 mL, 3.9 mmol). After 6 h at reflux and evaporation under reduced pressure of ethanol, the reaction mixture was extracted with ethyl acetate, and washed with water. The combined extracts were dried over magnesium sulfate. Evaporation of the solvent led to the crude amide. Recrystallization in ethanol yielded a white solid (0.95 g, 90%), mp = 260°C (Found M^+, 295.0772, $C_{16}H_{13}N_3OS$ requires 295.0779); ν_{max} (KBr)/cm^{-1} 3420, 2976, 1670, 1590, 1501, 1238, 752; δ_H(400 MHz, DMSO $d_6 + D_2O$) 1.35 (t, 3H, *J* 7.1 Hz, CH_3), 4.58 (q, 2H, *J* 7.1 Hz, CH_2), 7.39 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.56 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.78 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.95 (d, 1H, *J* 9.0 Hz, $H_{ar.}$), 8.09 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.19 (d, 1H, *J* 9.0 Hz, $H_{ar.}$); m/z 295 (M^+, 100%), 280 ($M^+ - CH_3$, 71).

(6-Ethyl-6H-[1,3]thiazolo[4,5-c]carbazol-2-yl)-carboxylic Acid **7**

A stirred solution of carbonitrile 3 (0.5 g, 1.8 mmol) in 10 mL of ethanol was treated with 10% aqueous potassium hydroxyde (18.0, 10.1 mmol). After 12 h at room temperature and evaporation under reduced pressure of ethanol, the aqueous phase was acidified with HCl 2N. The combined extracts were dried over magnesium sulfate. Evaporation of the solvent yielded the crude acid as a yellow solid (0.4 g, 99%). This compound was used directly in the next step without further purification; mp = 138°C (Found M^+, 296.0621, $C_{16}H_{12}N_2O_2S$ requires 296.0619); ν_{max} (KBr)/cm^{-1} 3364, 2470, 1698, 1590, 1484, 1242, 743; δ_H(400 MHz, DMSO $d_6 + D_2O$) 1.35 (t, 3H, *J* 7.1 Hz, CH_3), 4.60 (q, 2H, *J* 7.1 Hz, CH_2), 7.40 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.58 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.81 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.00 (d, 1H, *J* 9.0 Hz, $H_{ar.}$), 8.10 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.27 (d, 1H, *J* 9.0 Hz, $H_{ar.}$); m/z 296 (M^+, 2%), 252 ($M^+ - CO_2$, 89), 237 ($M^+ - CO_2$, CH_3, 100).

N-[2-(*N*,*N*-dimethylamino)ethyl]-6-ethyl-6H-[1,3]-thiazolo[4,5-c]carbazole-2-carboxamide **8**

To a stirred solution of carboxylic acid **7** (0.5 g, 1.68 mmol) in dry DMF (5 mL) were added at 0°C 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.36 g, 1.85 mmol), hydroxybenzotriazole (0.25 g, 1.85 mmol), and *N*,*N*-dimethylethylenediamine (1.85 mL, 16.8 mmol). The solution was stirred at room temperature for 18 h. After evaporation of the solvent, the residue was poured into water (50 mL) and extracted with ethyl acetate (2 × 50 mL). The extracts were dried over magnesium sulfate, and the solvent was removed under vacuum. The residue was purified by column chromatography with dichloromethane/methanol (90/10) as eluent. Yield: 56% (0.35 g), white powder, mp = 151°C (Found M^+, 366.1506, $C_{20}H_{22}N_4OS$ requires 366.1514); ν_{max} (KBr)/cm^{-1} 3417, 3057, 2968, 1668, 1521, 1243, 726; δ_H(400 MHz, $CDCl_3 + D_2O$) 1.46 (t, 3H, *J* 7.1 Hz, CH_3), 2.34 (s, 6H, 2 × CH_3), 2.61 (t, 2H, *J* 6.1 Hz, $CH_2N(CH_3)_2$), 3.64 (t, 2H, *J* 6.1 Hz, $CONHCH_2$), 4.43 (q, 2H, *J* 7.1 Hz, CH_2N), 7.37 (t, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.45–7.56 (m, 2H, $H_{ar.}$), 7.58 (d, 1H, *J* 9.1 Hz, $H_{ar.}$), 8.05–8.12 (m, 2H, $H_{ar.}$); m/z 366 (M^+, 3%), 295 ($M^+ - 71$, 9), 58 ([$N^+(CH_3)_2 = CH_2$], 100).

Synthesis of Imidazolines

A stirred mixture of cyanothiazolocarbazole **3**, **10** or **12** (1 mmol) and ethylenediamine (5 mmol) in anhydrous ethanol (5 mL) under argon was heated under reflux for 2 h. The solvent was removed in vacuo and water (5 mL) was added to the crude residue. The precipitated solid was collected and treated with an excess of ethereal HCl. The solid was collected and washed several times with anhydrous ether to give the white imidazoline dihydrochloride.

2-(4,5-Dihydro-1H-imidazol-2-yl)-6-ethyl-6H-1-thia-3,6-diaza-cyclopenta[c]fluorene **9**

Yield: 98%, white needles, mp = 218°C (Found M^+, 320.1083, $C_{18}H_{16}N_4S$ requires 320.1095); ν_{max} (KBr)/cm^{-1} 3408–2976, 1612, 1578, 1478, 1245, 1138, 752; δ_H(400 MHz, DMSO d_6) 1.35 (t, 3H, *J* 7.2 Hz, CH_3), 3.51 (t, 2H, *J* 9.1 Hz, CH_2), 3.92 (t, 3H, *J* 9.1 Hz, CH_2), 4.59 (q, 2H, *J* 7.2 Hz, CH_2), 7.32–7.47 (m, 2H, NH, $H_{ar.}$), 7.57 (td, 1H, *J* 7.7 Hz, *J* 1.1 Hz, $H_{ar.}$), 7.78 (d, 1H, *J* 8.3 Hz, $H_{ar.}$), 7.92 (d, 1H, *J* 8.9 Hz, $H_{ar.}$), 8.07 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.17 (d, 1H, *J* 8.9 Hz, $H_{ar.}$); m/z 320 ($M + H^+$, 100%).

2-(4,5-Dihydro-1H-imidazol-2-yl)-6-methyl-6H-1-thia-3,6-diaza-cyclopenta[c]fluorene **11**

Yield: 98%, white needles, (Found $[M + H]^+$, 307.1016, $C_{17}H_{15}N_4S$ requires 307.1017); ν_{max} (KBr)/cm^{-1} 3450, 2950, 1598, 1480, 1265, 1154, 1030, 748; δ_H(400 MHz, DMSO d_6) 4.02 (s, 2H, CH_2), 4.08 (s, 2H, CH_2), 4.24 (s, 3H, CH_3), 7.43 (t, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.61 (t, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.79 (d, 1H, *J* 8.3 Hz, $H_{ar.}$), 7.92 (d, 1H, *J* 8.3 Hz, $H_{ar.}$), 7.99 (d, 1H, *J* 9.2 Hz, $H_{ar.}$), 8.23 (d, 1H, *J* 9.2 Hz, $H_{ar.}$), 8.53 (bs, 1H, N), 11.46 (s, 2H, NH); δ_C(100 MHz, DMSO d_6) 29.78, 36.44, 44.93, 110.64, 111.95, 113.23, 120.00, 120.10, 120.64, 121.73, 126.31, 129.12, 139.41, 140.41, 144.23, 146.52, 157.88; m/z 307 ($M + H^+$, 100%).

2-(4,5-Dihydro-1H-imidazol-2-yl)-5H-4,10-dimethyl[1,3]thiazolo[5,4-b]carbazole **13**

Yield: 98%, yellow needles, mp > 260°C (Found M^+, 320.1084, $C_{18}H_{16}N_4S$ requires 320.1095); ν_{max} (KBr)/cm^{-1} 3446, 1596, 1576, 1259, 1082, 1031, 743; δ_H(400 MHz, DMSO $d_6 + D_2O$) 2.69 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 3.72–3.66 (m, 4H, 2 × CH_2), 7.22 (t, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.45 (t, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.55 (d, 1H, *J* 8.0 Hz, $H_{ar.}$), 8.24 (d, 1H, *J* 8.0 Hz, $H_{ar.}$); m/z 320 (M^+, 100%).

Synthesis of Methoxycarbazole Derivatives

2-(9-Ethyl-9H-carbazol-3-yl)-isoindole-1,3-dione **14**

A stirred mixture of 3-amino-9-ethyl-9*H*-carbazole 1 (5.0 g, 23.77 mmol) in 100 mL of chloroform and phthalic anhydride (5.3 g, 35.8 mmol) was heated to reflux for 6 h. The reaction mixture was then cooled, washed with water, and dried over magnesium sulfate. Evaporation of the solvent yielded the crude product as yellow needles (7.1 g, 88%), mp = 212°C (Found M^+, 340.1205, $C_{22}H_{16}N_2O_2$ requires 340.1211); ν_{max} (KBr)/cm^{-1} 3458, 3063, 2967, 2933, 2872, 1714, 1717, 1601, 1487, 1470, 1380, 1235, 1106, 714; δ_H(400 MHz, $CDCl_3$) 1.32 (t, 3H, *J* 7.1 Hz, CH_3), 4.46 (q, 2H, *J* 7.1 Hz, CH_2), 7.22 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.47–7.51(m, 2H, $H_{ar.}$), 7.62–7.76 (m, 2H, $H_{ar.}$), 7.89 −7.93 (m, 2H, $H_{ar.}$), 7.96–8.00 (m, 2H, $H_{ar.}$), 8.13 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.20 (d, 1H, *J* 1.7 Hz, $H_{ar.}$); δ_C(100 MHz, $CDCl_3$) 13.81, 37.68, 108.66, 108.82, 119.16, 119.36, 120.76, 122.64, 122.66, 123.29, 123.61, 124.35, 126.15, 131.95, 134.23, 139.35, 140.45, 168.07; m/z 340 (M^+, 100%), 325 ($M^+ - CH_3$, 86).

2-(6-Acetyl-9-ethyl-9H-carbazol-3-yl)-isoindole-1.3-dione **15**

The acetyl chloride (3.9 g, 29.37 mmol) then the anhydrous aluminium chloride (1.25 mL, 17.6 mmol) were added at 0°C under an argon atmosphere, to a solution of carbazole **14** (4.0 g, 11.76 mmol) in 160 mL of dichloromethane. The cooling bath was removed and the stirred mixture was heated at 30°C for 3 h. After cooling the residue was hydrolyzed with water, HCl 2N and extracted with dichloromethane. The organic layers were washed with a saturated solution of sodium hydrogenocarbonate then dried over magnesium sulfate and evaporated *in vacuo*. The product was washed with ethanol to yield the title compound as yellow needles (4.4 g, 98%), mp = 232°C(Found M^+, 382.1312, $C_{24}H_{18}N_2O_3$

requires 382.1317); ν_{max} (KBr)/cm^{-1} 3567, 3452, 3070, 2983, 1773, 1717, 1667, 1595, 1493, 1249, 1113, 797, 712; δ_H(400 MHz, DMSO d_6) 1.34 (t, 3H, *J* 7.1 Hz, CH_3), 2.66 (s, 3H, CH_3), 4.51 (q, 2H, *J* 7.1 Hz, CH_2), 7.55 (dd, 1H, *J* 2.0 Hz, *J* 8.7 Hz, $H_{ar.}$), 7.73 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.80 (d, 1H, *J* 8.7 Hz, $H_{ar.}$), 7.91 −7.95 (m, 2H, $H_{ar.}$), 7.97–8.01 (m, 2H, $H_{ar.}$), 8.10 (dd, 1H, *J* 1.5 Hz, *J* 8.8 Hz, $H_{ar.}$), 8.36 (d, 1H, *J* 2.0 Hz, $H_{ar.}$), 8.86 (d, 1H, *J* 1.5 Hz, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 13.81, 26.57, 37.98, 108.38, 109.41, 119.47, 122.28, 122.34, 123.59, 123.67, 123.81, 125.07, 126.72, 129.07, 131.83, 134.34, 139.93, 143.10, 167.87, 197.46; m/z 382 (M^+, 100%), 367 ($M^+ - CH_3$, 75).

3-(1,3-Dioxo-isoindol-2-yl)-6-acetate-9-ethyl-9H-carbazole **16**

To a solution of acetylcarbazole **15** (4.5 g, 11.7 mmol) in anhydrous dichloromethane (30 mL) was added *meta*-chloroperbenzoic acid (5.3 g, 30.4 mmol). The reaction mixture was cooled in an ice bath and trifluoroacetic acid (0.9 mL, 11.6 mmol) was added dropwise over 10 min. The reaction mixture was then stirred at room temperature for 3 h. The mixture was allowed to cool and the chlorobenzoic acid was filtered off. The extract was washed with brine and a saturated aqueous solution of hydrogenocarbonate. The organic phases were dried over magnesium sulfate. The solvent was removed under vacuum. The residue was washed with ethanol to yield red needles (4.5 g, 97%). This compound **16** was used directly in the next step without further purification; mp = 222°C (Found M^+, 398.1263, $C_{24}H_{18}N_2O_4$ requires 398.1266); ν_{max} (KBr)/cm^{-1} 3466, 3064, 2978, 2937, 2880, 1750, 1725, 1491, 1457, 1383, 1227; δ_H(400 MHz, DMSO d_6) 1.32 (t, 3H, *J* 7.1 Hz, CH_3), 2.30 (s, 3H, CH_3), 4.48 (q, 2H, *J* 7.1 Hz, CH_2), 7.24 (dd, 1H, *J* 2.1 Hz, *J* 9.0 Hz, $H_{ar.}$), 7.49 (dd, 1H, *J* 2.8 Hz, *J* 8.7 Hz, $H_{ar.}$), 7.67 (d,1H, *J* 9.0 Hz, $H_{ar.}$), 7.74 (d, 1H, *J* 9.8 Hz, $H_{ar.}$), 7.91–7.94 (m, 2H, $H_{ar.}$), 7.97–8.00 (m, 2H, $H_{ar.}$), 8.18 (d, 1H, *J* 2.8 Hz $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 13.85, 26.50, 37.91, 108.38, 109.46, 119.47, 122.28, 122.34, 123.59, 123.67, 123.81, 125.07, 126.72, 129.07, 131.83, 134.34, 139.93, 143.12, 167.87, 197.46; m/z 398 (M^+, 100%), 367 ($M^+ - CH_3$, 75).

2-(9-Ethyl-6-hydroxy-9H-carbazol-3-yl)-isoindole-1,3-dione **17**

To a solution of ester **16** (4.5 g, 11.2 mmol) in 50 mL of ethanol was added 0.5 mL of sodium ethoxide, prepared by addition of sodium (0.46 g, 20 mmol) to 200 mL of anhydrous EtOH. The resulting solution was stirred at room temperature for 6 h, then acidified at pH 6 with resin Dowex 50X8-400 and filtered off. The filtrate was evaporated to dryness. The resulting material was purified by column chromatography with dichloromethane/petroleum ether (50/50) as eluent to afford the required product (2.7 g, 68%) as orange needles, mp = 244°C; ν_{max} (KBr)/cm^{-1} 3366, 3288, 3154, 2960, 2175, 1666, 1606, 1579, 1474, 1372, 1249, 1215, 932, 838, 782; δ_H(400 MHz, DMSO d_6) 1.30 (t, 3H, *J* 7.0 Hz, CH_3), 4.41 (q, 2H, *J* 7.0 Hz, CH_2), 6.99 (dd, 1H, *J* 2.2 Hz, *J* 8.7 Hz, $H_{ar.}$), 7.39–7.47 (m, 3H, $H_{ar.}$), 7.63 (d, 1H, *J* 8.8 Hz, $H_{ar.}$), 7.90–7.92 (m, 2H, $H_{ar.}$), 7.96–7.99 (m, 2H, $H_{ar.}$), 8.06 (d, 1H, *J* 2.2 Hz, $H_{ar.}$), 9.09 (s, 1H, OH); δ_C(100 MHz, DMSO d_6) 13.83, 37.85, 109.02, 109.09, 113.33, 119.52, 119.97, 122.76, 122.88, 123.63, 124.78, 131.92, 138.18, 139.90, 143.76, 167.95, 170,25; m/z 356 (M^+, 100%).

2-(9-Ethyl-6-methoxy-9H-carbazol-3-yl)-isoindole-1,3-dione **18**

To a solution of compound **17** (1.0 g, 2.8 mmol) in tetrahydrofuran (30 mL) was carefully added sodium hydride (60% dispersion in mineral oil, 0.224 g, 5.6 mmol). After being stirred for 30 min, the reaction mixture was cooled in an ice bath and methyl iodide (0.35 mL, 5.6 mmol) was added dropwise. After 3 h at room temperature, the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane, washed with water and dried over magnesium sulfate. The solvent was removed *in vacuo* to yield a dark oil which was purified using column chromatography (eluent: petroleum ether/ethyl acetate: 60/40). Yield: 45%; yellow needles, mp = 170°C (Found M^+, 370.1312, $C_{23}H_{18}N_2O_3$ requires 370.1317); ν_{max} (KBr)/cm^{-1} 3472, 2976, 2948, 2888, 2824, 1782, 1724, 1710, 1606, 1480, 1378, 1210, 1078, 717; δ_H(400 MHz, DMSO d_6) 1.31 (t, 3H, *J* 7.0 Hz, CH_3), 3.83 (s, 3H, OCH_3), 4.45 (q, 2H, *J* 7.0 Hz, CH_2), 7.12 (dd, 1H, *J* 2.5 Hz, *J* 8.8 Hz, $H_{ar.}$), 7.44 (dd, 1H, *J* 2.0 Hz, *J* 8.8 Hz, $H_{ar.}$), 7.56 (d, 1H, *J* 8.7 Hz, $H_{ar.}$), 7.67 (dd, 1H, *J* 8.7, $H_{ar.}$), 7.72 (d, 1H, *J* 2.5 Hz, $H_{ar.}$) 7.90–7.99 (m, 4H, $H_{ar.}$), 8.17 (d, 1H, *J* 2.0 Hz, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 13.83, 37.85, 109.02, 109.09, 113.33, 119.52, 119.97, 122.76, 122.88, 123.63, 124.78, 131.92, 138.18, 139.90, 143.76, 167.95, 170,25; m/z 370 (M^+, 100%), 355 ($M^+ - CH_3$, 58).

6-Methoxy-3-amino-9-ethyl-9H-carbazole **19**

A stirred mixture of compound **18** (0.3 g, 0.81 mmol) and hydrazine (0.23 mL, 7.9 mmol) in dry ethanol (30 mL) was heated to reflux for 2 h, then cooled and filtered through a bed of Celite. The solvent was removed *in vacuo*. The residue was dissolved in dichloromethane, washed with a satured solution of sodium hydrogenocarbonate, and dried over magnesium sulfate. The solvent was removed *in vacuo* to yield the crude title compound as a brown solid (0.14 g, 70 %), mp > 250°C (Found M^+, 240.1257, $C_{15}H_{16}N_2O$ requires 240.12626); ν_{max} (KBr)/cm^{-1} 3459, 3057, 2982, 2946, 2894, 1766, 1715, 1601, 1486, 1386, 1113, 871, 799, 721; δ_H(400 MHz, $CDCl_3$) 1.36 (t, 3H, *J* 7.3 Hz, CH_3), 3.81(s, 3H, CH_3), 4.26 (q, 2H, *J* 7.3 Hz, CH_2), 6.92 (dd, 1H, *J* 1.5 Hz, *J* 8.8 Hz, $H_{ar.}$), 7.07 (dd, 1H, *J* 2.4 Hz, *J* 8.8 Hz, $H_{ar.}$), 7.19 (d, 1H, *J* 8.8 Hz, $H_{ar.}$), 7.24 (d, 1H, *J* 8.3 Hz, $H_{ar.}$),

7.40 (d, 1H, *J* 1.5 Hz, $H_{ar.}$), 7.47 (d, 1H, *J* 2.4 Hz, $H_{ar.}$); δ_C(100 MHz, $CDCl_3$) 13.84, 37.60, 56.15, 103.35, 107.00, 109.06, 109.12, 114.83, 116.12, 122.54, 123.32, 135.56, 136.82, 153.19; m/z 240 (M^+, 100%).

4-Chloro-5-(9-ethyl-6-methoxycarbazolylimino)-5H-1,2,3-dithiazole **20**

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride[19] (0.24 g, 1.14 mmol) was added to a stirred solution of amine **19** (0.23 g, 0.95 mmol) in dichloromethane (10 mL). After 15 min, pyridine (0.16 mL, 1.9 mmol) was added and the mixture stirred for 2 h. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (light petroleum/ethyl acetate: 9/1) to afford the required compound (0.34 g, 93%) as yellow needles, mp = 146°C(Found M^+, 375.0281, $C_{17}H_{14}N_3OClS_2$ requires 375.0266); ν_{max} (KBr)/cm^{-1} 2979, 2936, 2888, 2830, 1599, 1483, 1442, 1327, 1209, 1133, 866, 845, 763, 693; δ_H(400 MHz, DMSO d_6) 1.30 (t, 3H, *J* 7.0 Hz, CH_3), 3.83 (s, 3H, CH_3), 4.42 (q, 2H, *J* 7.0 Hz, CH_2), 7.11 (dd, 1H, *J* 2.3 Hz, *J* 9.0 Hz, $H_{ar.}$), 7.40 (dd, 1H, *J* 1.8 Hz, 8.9, $H_{ar.}$), 7.54 (d, 1H, *J* 8.7 Hz, $H_{ar.}$), 7.68 (d, 1H, *J* 8.7 Hz, $H_{ar.}$), 7.7 (d, 1H, *J* 2.3 Hz, $H_{ar.}$), 8.13 (d, 1H, *J* 1.8 Hz, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 13.87, 37.25, 55.68, 103.61, 110,03, 110,26, 112.10, 115.45, 118.73, 122.45, 122.57, 135.12, 138.43, 141.33, 147.38, 153.42, 155.63; m/z 375 (M^+, 100%).

9-Methoxy-6-ethyl-6H-[1,3]thiazolo[4,5-c]carbazole-2-carbonitrile **21**

N-(4-Chloro-5*H*-1,2,3-dithiazol-5-yliden)amine **20** (0.1 g, 0.27 mmol) was heated under argon at 200°C for 5 min in a graphite bath. The products were isolated by column chromatography (dichloromethane 100%). The angular isomer **21** was obtained in 60% and the linear counterpart **22** in 10%. yellow needles, mp = 216°C (Found M^+, 307.0791, $C_{17}H_{13}N_3OS$ requires 307.0779); ν_{max} (KBr)/cm^{-1} 2993, 2943, 2221, 2165, 1590, 1484, 1336, 1037, 825, 774; δ_H(400 MHz, DMSO d_6) 1.33 (t, 3H, *J* 7.0 Hz, CH_3), 3.92 (s, 3H, OCH_3), 4.58 (q, 2H, *J* 7.0 Hz, CH_2), 7.25 (dd, 1H, *J* 2.2 Hz, 9.1 Hz, $H_{ar.}$), 7.45 (d, 1H, *J* 2.2 Hz, $H_{ar.}$), 7.76 (d, 1H, *J* 8.9 Hz, $H_{ar.}$), 8.04 (d, 1H, *J* 9.1 Hz, $H_{ar.}$), 8.27 (d, 1H, *J* 8.9 Hz, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 14.17, 37.83, 56.79, 102.98, 111.52, 111.94, 113.03, 113.93, 115.53, 120,76, 121.62, 128.55, 130,60, 134.26, 138.90, 145.98, 154.46; m/z 307 (M^+, 100%).

8-Methoxy-5-ethyl-5H-[1,3]thiazolo[5,4-b]carbazole-2-carbonitrile **22**

Yield 10%, yellow needles, (Found M^+, 307.0785, $C_{17}H_{13}N_3OS$ requires 307.0779); ν_{max} (KBr)/cm^{-1} 2993, 2943, 2221, 2165, 1590, 1484, 1336, 1037, 825, 774; δ_H(400 MHz, DMSO d_6) 1.44 (t, 3H, *J* 7.0 Hz, CH_3), 3.95 (s, 3H, OCH_3), 4.36 (q, 2H, *J* 7.0 Hz, CH_2), 7.20 (dd, 1H, *J* 2.5 Hz, 8.8 Hz, $H_{ar.}$), 7.36 (d, 1H, *J* 8.8 Hz, $H_{ar.}$), 7.68 (d, 1H, *J* 2.5 Hz, $H_{ar.}$), 7.77 (s, 1H, $H_{ar.}$), 8.80 (s, 1H, $H_{ar.}$); δ_C(100 MHz, DMSO d_6) 13.49, 38.11, 56.19, 98.92, 104.31, 109.59, 113.67, 116.40, 116.59, 122,78, 125.64, 132.02, 134.03, 136.38, 141.53, 145.72, 154.28; m/z 307 (M^+, 100%).

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.[18] Cells were exposed to graded concentrations of the compounds for 48 h and results expressed in IC_{50} values (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls).

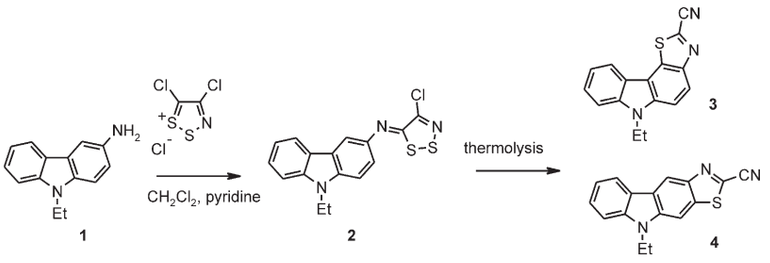
For the cell cycle analysis, L1210 cells ($2,5 \times 10^5$ cells/mL) were incubated for 21 h with various concentrations of the compounds, then fixed by 70% ethanol (v/v), washed and incubated in PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide for 30 min at 20°C. For each sample, 1×10^4 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_2 phase of the cell cycle, and were obtained in at least three independent experiments.

RESULTS AND DISCUSSION

Chemistry

As we showed in our study of the chemistry of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel salt)[19] and its derivatives, fusion of the thiazole ring onto the carbazole skeleton suggested the use of 5-(*N*-arylimino)-4-chloro-5*H*-1,2,3-dithiazoles which have proved to be highly versatile intermediates in heterocyclic synthesis.[20–24]

As previously described by our research group, synthesis of the thiazolocarbazoles **3**, **4** and **10** was performed in two steps from *N*-alkyl-3-aminocarbazole.[1] Using a standard method applied to the preparation of *N*-arylimino-1,2,3-dithiazoles, the starting aminocarbazoles were condensed with 4,5-dichloro-1,2,3-dithiazolium chloride in dichloromethane at room temperature, followed by addition of pyridine, to give the desired imino-1,2,3-dithiazolocarbazoles in good yields (Scheme 1). The thermolysis procedure consisted in heating the neat imines under argon at 200–250°C (metal bath) for 1 or 2 minutes. Whatever conditions were used, the major product obtained in reasonable yields, was



SCHEME 1 Synthetic route to thiazolocarbazoles **3** and **4**.

the angular isomer **3** (a low yield of its linear conterpart was detected for the *N*-ethyl derivative).

Variation in Position-3 of the N*-ethyl-thiazolocarbazole 5*

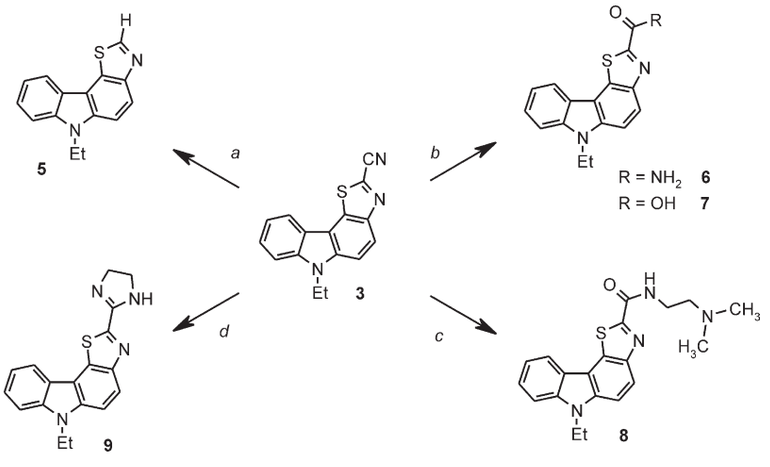
Removal (hydrolysis and decarboxylation) of the cyano group on the thiazole ring was performed by vigorous heating of compound **5** in 48% hydrobromic acid. The de-cyanated thiazolocarbazole was isolated in good yield (95%). Using standard conditions for the transformation of cyano groups into amido groups or carboxylic acids, the starting thiazole **5** was treated with aqueous potassium hydroxide to give the carboxamide **6** (90%) or, after a longer reaction time, to provide quantitatively the carboxylic acid **7** (Scheme 2). The substituted amide **8** was prepared by treatment of the acid with *N*,*N*-dimethylethylenediamine in the presence of 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt), a method commonly used for the coupling of amino acids. The imidazoline **9** was readily obtained by treatment of the carbonitrile **5** with ethylenediamine in refluxing ethanol in the conventional manner.

Following the same strategy, the 9-methyl-9*H*-1-thia-3,9-diazacyclopenta[*b*]fluorene-2-carbonitrile[13] **10** was treated by the appropriate diamine to give the corresponding imidazoline **11** in very good yield (98%) (Scheme 3).

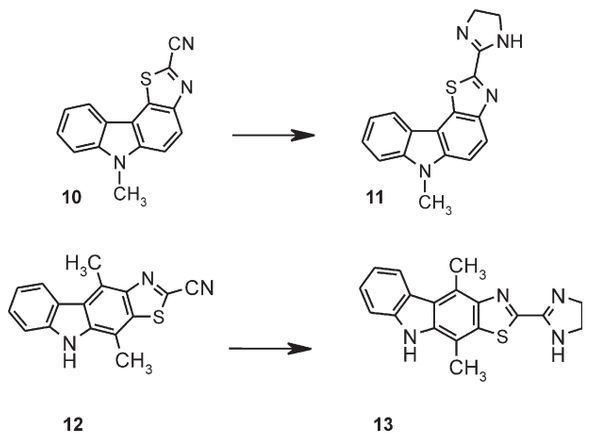
In connection with our work on the utility of 4,5-dichloro-1,2,3-dithiazolium chloride in the preparation of novel thiazolo heterocyclic systems, we recently described access to a dimethylthiazolocarbazole ring **VII** which is structurally very close to the natural alkaloid ellipticine or pyridocarbazole congeners. The synthesis of this little known thiazolocarbazole ring was performed in five steps via the intermediate 3-amino-1,4-dimethylcarbazole which was prepared from the starting 5-bromoindole.[13] The cyano derivative **12** obtained was also transformed into the corresponding imidazoline **13** (Scheme 3). For all the compounds prepared, we expected that the imidazoline ring may lead to cationic molecules with a better water solubility which would have an important impact on their biological properties (*e.g.* for DNA binding ability).

Synthesis of 6-Methoxy thiazolo[4,5-c]carbazoles

A first approach, which consists in formation of the thiazole moiety before introduction of



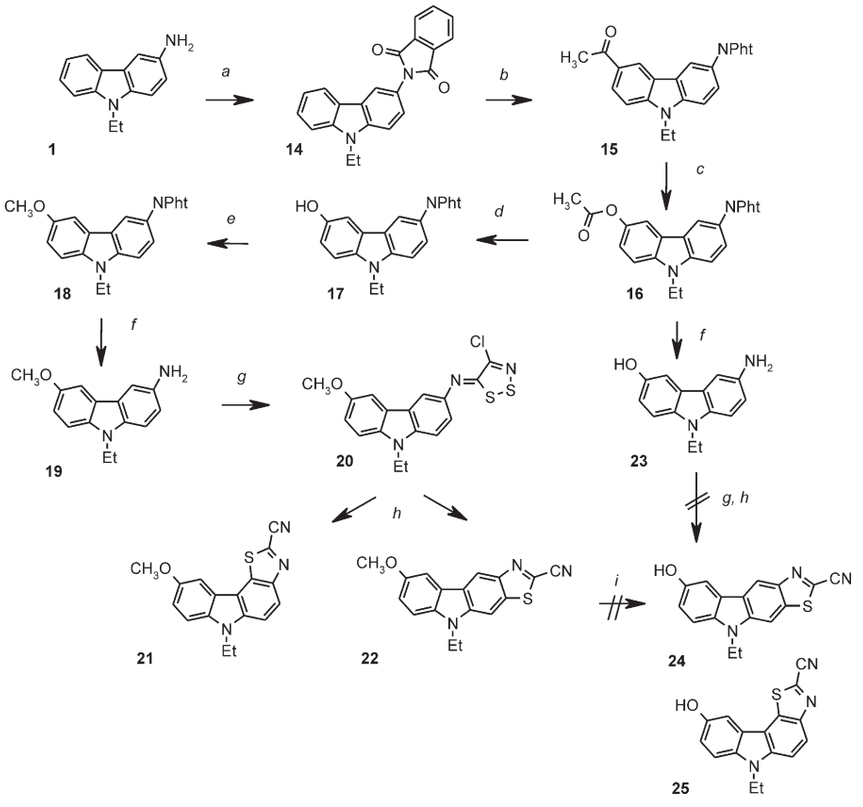
SCHEME 2 Synthetic modulations of compound **3**. Reaction conditions and yields: (a) HBr 48%, reflux, 3h, 95%; (b) KOH 10%, reflux, 6h, 90%; (c) i-KOH 10%, reflux, 12h, 99% of **7**; ii-EDCL, HOBt, DMF, $(CH_3)_2NCH_2CH_2NH_2$, 56%; (d) $H_2NCH_2CH_2NH_2$, EtOH, reflux 2h, 95%.



SCHEME 3 Synthesis of imidazoline derivatives. Reaction conditions and yields: $H_2NCH_2CH_2NH_2$, EtOH, reflux 2h, **11** (98%), **13** (98%).

the substituent, was rapidly judged non-profitable in view of a second pathway in which the intermediate *N*-protected ester may be expected from the starting 3-amino-9-ethylcarbazole **1**. Substitution reactions on the carbazole ring have been well studied and have shown that a preliminary protection of the amino group allows a good orientation for the reaction.[25] Then, treatment of the starting amine **1** by phthalic anhydride in chloroform gave the carbazole **14** in a 88% yield and Friedel–Crafts acylation followed by a Baeyer–Villiger oxidation afforded the *N*-protected ester **16** in a very good yield (global yield: 95%) (Scheme 4). Saponification of the ester **16** with sodium ethanoate in ethanol gave the required 9-hydroxycarbazole **17** (68% yield) which on treatment with methyl iodide in the presence of sodium hydride led to the methoxy derivative **18** in modest yield (45%). Deprotection of the amino group of **18** was achieved in the presence of hydrazine in refluxing ethanol and the resulting amine **19** was condensed with Appel's salt. Following the procedure described previously[11,12], thermolysis of the resulting iminodithiazole **20** afforded the angular thiazolo[4,5-*c*]carbazole **21** (60%) accompanied by a small amount of its linear isomer **22** (10%).

Irrespective of the conditions were used, formation of the 6-hydroxy derivatives **24** was not possible from the methoxy-6-ethylthiazolo[4,5-*c*]carbazole-2-carbonitriles. Another route which consisted of forming the 6-hydroxythiazolcarbazole via the intermediate compound **23** gave unsatisfactory results.



SCHEME 4 Synthetic route to 6-methoxy-thiazolocarbazoles **21** and **22**. Reaction conditions and yields: (a) phtalic anhydride, $CHCl_3$, reflux, 18h, 88%; (b) acetyl chloride, $AlCl_3$, 30°C, 3h, 98%; (c) *m*CPBA, TFA, rt, 97%; (d) NaOEt, EtOH, rt, 6h, 68%; (e) NaH, CH_3I, rt, 45%; (f) NH_2NH_2, EtOH, reflux, 2h, 70%; (g) 4,5-dichloro-1,2,3-dithiazolium chloride, pyridine, rt, 93%; (h) 200°C, 5 min, 60% (**21**), 10% (**22**); (i) BBr_3, CH_2Cl_2, -50°C.

TABLE I Cytotoxicity and antiproliferative activity results for test compounds

Compound	Formula	Cytotoxicity IC_{50} L1210 (μM)	% of L1210 cells in the cell cycle phases[a] G_2 (μM)
3	$C_{16}H_{11}N_3S$	5.4	ns[c] (25)[d]
4	$C_{16}H_{11}N_3S$	9.1	ns[c] (25)[d]
5	$C_{15}H_{12}N_2S$	42.1	50–70 % G_2+M (100)
6	$C_{16}H_{13}N_3OS$	43.9	ne[b]
8	$C_{20}H_{22}N_4OS$	1.7	30–50 % G_2+M (5)
9	$C_{18}H_{15}N_4S$	4.1	43 % G_2+M (10)
10	$C_{15}H_9N_3S$	14.1	ne
11	$C_{17}H_{14}N_4S$	1.7	ns[c] (10)[e]
12	$C_{16}H_{11}N_3S$	3.8	ns[c] (10)[e]
13	$C_{18}H_{16}N_4S$	0.75	49 % G_2+M (2.5)
21	$C_{17}H_{13}N_3OS$	48.4	ne
22	$C_{17}H_{13}N_3OS$	30.3	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. [d] Toxic at 25 μM. [e] Toxic at 10 μM.

In Vitro Antitumor Activity

In vitro antitumor activity of the compounds described in this paper was assessed using the murine leukemia cell line. Selected data are listed in Table I for the most active compounds.

All the 2-cyanothiazolocarbazoles listed in Table I were found practically equipotent on cell proliferation inhibition. Some of them were able to block partially the cells in the G_2 phase of the cell cycle. In contrast with results obtained by other groups on the modification of ellipticine and congeners,[14–17] introduction of a methoxy group at position-6 of the thiazolo[4,5-*c*]carbazole ring did not really enhance the biological activity of such compounds. A lack of activity was obtained and the angular derivative **21** was not more cytotoxic than its linear counterpart **22**. Modifications at position-2 of the thiazole moiety was more promising. The ready transformation of the cyano group into an imidazoline ring seemed to induce an interesting enhancement of the cytotoxic activity of these compounds with a small specific effect on the cell cycle. This influence was particularly apparent for the 4,10-dimethyl-9*H*-1-thia-3,9-diazacyclopenta[*b*]fluorene derivative **13**.

In conclusion, we have described the synthesis of novel substituted thiazolo[4,5-*c*]carbazoles, which exhibit a modest in vitro cytotoxic activity. Among these results, modification of the substituent in the C-2 position of the thiazole moiety was particularly studied and showed an interesting change in biological activity by replacement of the cyano group by an imidazoline ring. Our present results suggest that transformation of the cyano group into various imidates (with appropriate substitution) could load to promising results.

Acknowledgements

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