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Microwave-assisted Synthesis of Novel Thiazolocarbazoles and Evaluation as Potential Anticancer Agents. Part III

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Novel 6-substituted thiazolocarbazole derivatives have been synthesized under microwave irradiation *via* the corresponding imino-1,2,3-dithiazoles. *In vitro* antitumor potential of these polyheterocyclic compounds was evaluated. Among all the tested thiazolocarbazoles, compound 10 is the most effective in inhibiting cell growth.

Keywords: Microwave chemistry; Imino-1,2,3-dithiazoles; Appel salt; Carbazoles; Thiazoles; Cytotoxic and antiproliferative activities

INTRODUCTION

Because a large number of natural products and target drug compounds contain an aromatic heterocyclic core, synthetics routes towards these molecules are usually quite challenging. Microwave assisted chemistry is only a 20 years old technology, and a recent understanding of this technology shows that it is quite selective for molecules that need to receive energy and when the usual synthetic methods require forcing conditions or prolonged reaction times. For all these reasons, the various possibilities offered by this technology are particularly attractive for multi-step synthesis of aromatic heterocycles required in drug discovery strategies, in which fast high yielding protocols and purification or avoidance facility are highly desirable.[3,4]

The main activity of our research group consists in performing microwave-assisted synthesis of heterocyclic structures exhibiting pharmaceutical value. Our target molecules s are inspired by natural marine or terrestrial alkaloids for which interesting biological activities have been detected. Thus, we recently described the synthesis of new thiazole compounds[1,2] derived from natural alkaloids extracted from marine organisms (*e.g.* dercitine and kuanoniamines) or terrestrial plants (*e.g.* ellipticine) (Figure 1).[5,6,7] In these studies, our strategy consisted in combining the thiazole ring with various heterocyclic structures in the hope of detecting interesting cytotoxicity profiles. 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 **I** and the 4,10-dimethyl-9*H*-1-thia-3,9-diazacyclopenta[*b*]fluorene-2-carbonitrile **II** that showed a significant cytotoxic activity without real effect on the cell cycle. These results prompted us to investigate the effect of pharmacomodulation at the level of the nitrile function (R_2) and the homocycle (R_1) of the tetracycle **IV**, starting from the lead compound **II**.

Inspired by previous works on ellipticine and congeners,[8] we decided to explore the influence of substituting the 6-position (R_1) of the thiazolocarbazole skeleton, by halogen (*e.g.* bromine or chlorine) or by a hydroxyl group. In this paper, we re-investigate the synthesis of the parent compound **III** under microwave irradiation and report the benefits associated with the microwave methodology for the synthesis of various analogues **IV**. The cytotoxic and antiproliferative effects of these new substituted polyheterocyclic compounds are also described and discussed.

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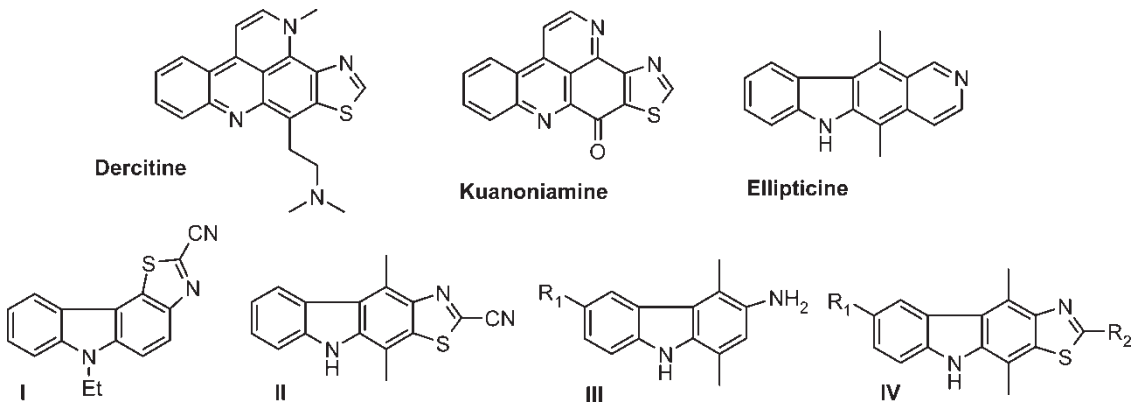


FIGURE 1 Structures (I–IV).

MATERIALS AND METHODS

Chemistry

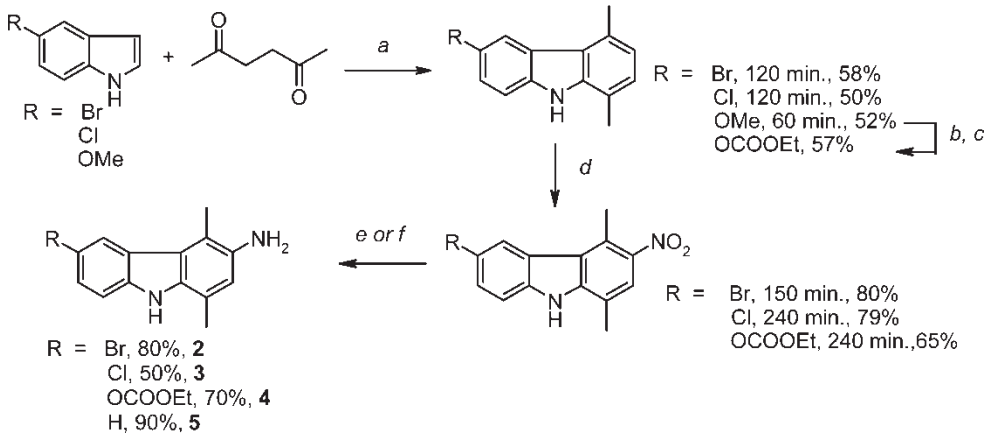
Commercial reagents were used as received without additional purification. Melting points were determined using a Köfler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Paragon 1000PC instrument. ^{1}H and ^{13}C-NMR were recorded on a JEOL NMR LA400 (400 MHz) 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. The mass spectra (HRMS) were recorded on a Varian MAT311 spectrometer in the Centre Régional de Mesures Physiques de l'Ouest (CRMPO), Université de Rennes. Column chromatography was performed by using Merck silica gel (70–230 mesh) at medium pressure. Light petroleum refers to the fraction boiling point 40–60°C. Other solvents were used without purification. Analytical thin layer chromatography (tlc) was performed on Merck Kieselgel 60 F254 aluminium backed plates.

Focused microwave irradiations were carried out with CEM *Discover*™ focused microwave reactor (300 W, 2450 MHz, monomode system), which has *in situ* magnetic variable speed rotation, irradiation monitored by a PC computer, infrared measurement and continuous feedback temperature control. Experiments may be performed at atmospheric pressure or under pressure in pressure-rated reaction tubes with continuous pressure measurement.

Spectral data for compounds **2**, **3**, **4**, **5**, **9**, **13** are consistent with assigned structures as previously described by Chabane[3,4,8] and Lancelot.[9,10] (Scheme 1)

General Procedure for the Preparation of N-(4-Chloro-5H-1,2,3-dithiazol-5-ylidene) Derivatives (6–9)

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride **1** (3.5 mmol) was added to a solution of amine **2–5** (3.3 mmol) in dichloromethane (20 mL) and pyridine (7.0 mmol). The mixture was stirred at room temperature for 2 h and then washed twice with water (20 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by



SCHEME 1 Synthetic route to 6-substituted-1,4-dimethylcarbazoles. Reaction conditions and yields: (a) PTSA, ethanol, reflux, 79°C, 40 W, μw; (b) HBr 48%, 100°C, 40 W, 90 min, μw, 85%; (c) ethyl chloroformate, triethylamine, μw, 95%; (d) HNO_3, AcOH, rt; (e) for R = Br, Cl: ammonium formate, Pd-C, ethanol, 79°C, 40 W, μw, **4** (70%), **5** (90%); (f) $SnCl_2.2H_2O$, ethanol, 79°C, oil bath, **2** (80%), **3** (50%).

column chromatography with dichloromethane as the eluent, afforded the title compounds as stable solids.

(6-Bromo-1,4-Dimethyl-9*H*-Carbazol-3yl)-(4-Chloro-[1,2,3]Dithiazol-5-ylidene)-Amine (**6**)

This compound was prepared from precursor **2**. Yield: 80%, red solid mp = 190°C (Found M^+, 422.926, $C_{16}H_{11}BrClN_3S_2$ requires 422.927); υ_{max} (KBr)/cm^{-1} 3360, 1606, 1444, 1278, 1160, 851, 768; δ_H (400 MHz, DMSO d_6) 2.56 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 7.18 (s, 1H, H_{ar}), 7.53 (d, 1H, *J* 2.4 Hz, H_{ar}), 8.26 (s, 1H, H_{ar}), 11.50 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 14.13, 16.72, 113.07, 115.48, 118.80, 120.63, 122.53, 124.41, 125.17, 127.64, 137.84, 139.10, 140.83, 147.20, 156.74.

(6-Chloro-1,4-Dimethyl-9*H*-Carbazol-3yl)-(4-Chloro-[1,2,3]Dithiazol-5-ylidene)-Amine (**7**)

This compound was prepared from precursor **3**. Yield: 79%, red solid mp = 170°C (Found M^+, 378.9771, $C_{16}H_{11}Cl_2N_3S_2$ requires 378.9766); δ_H (400 MHz, DMSO d_6) 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.18 (s, 1H, H_{ar}), 7.43 (d, 1H, *J* 8.8 Hz, H_{ar}), 7.56 (d, 1H, *J* 8.8 Hz, H_{ar}), 8.13 (s, 1H, H_{ar}), 11.49 (s, 1H, NH).

Ethyl[6-(4-Chloro-[1,2,3]Dithiazol-5-ylidene-amino)-5,8-Dimethyl-9*H*-Carbazol-3-yl] Carbonate (**8**)

This compound was prepared from precursor **4**. Yield: 76%, orange solid, mp = 170°C; υ_{max} (KBr)/cm^{-1} 3354, 2988, 2361, 1758, 1518, 1497, 1369, 1256, 1010, 851; δ_H (400 MHz, DMSO d_6) 1.31 (t 3H, *J* 7.2 Hz, CH_3), 2.55 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.26(q, 2H, *J* 7.2 Hz, CH_2), 7.13 (s, 1H, H_{ar}), 7.26 (dd, 1H, *J* 2.4 Hz, *J* 8.8 Hz, H_{ar}), 7.54 (d, 1H, *J* 8.8 Hz, H_{ar}), 7.96 (d, 1H, *J* 2.4 Hz, H_{ar}), 11.47 (s, 1H, NH); δ_C (100 MHz, DMSO d_6)14.25, 14.38, 17.01, 64.48, 111.67, 114.77, 115.50, 119.09, 119.26, 121.52, 122.52, 123.50, 138.37, 138.44, 140.94, 143.91, 147.25, 154.17, 157.15.

General Procedure for the Preparation of 2-Cyanocarbazoles

A solution of *N*-arylimino-1,2,3-dithiazoles **6–9** (0.71 mmol) in *N*-methylpyrrolidin-2-one (10 mL) was irradiated under microwave. The irradiation was programmed to maintain a constant temperature (200°C) with a maximal power output of 75 W. After cooling, the reaction mixture was extracted with ethyl acetate (2 × 15 mL). The organic layer was washed with water, then dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography on silica gel using petroleum-dichloromethane (20/80) as eluent.

6-Bromo-4,10-Dimethyl-9*H*-1-Thia-3,9-Diazacyclopenta[*B*]Fluorene-2-Carbonitrile (**10**)

This compound was prepared from precursor **6**. Yield: 52% yellow needles mp > 260°C (Found M^+, 356.9765, $C_{16}H_{10}BrN_3S$ requires 356.9758); υ_{max} (KBr)/cm^{-1} 3395, 2919, 2222, 1858, 1603, 1449, 1287, 1129, 800; δ_H (400 MHz, DMSO-d_6) 2.74 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 7.55 (d, 1H, *J* 8.6 Hz, H_{ar}), 7.65 (dd, 1H, *J* 1.2 Hz, *J* 8.6 Hz, H_{ar}), 8.37 (d, 1H, *J* 1.2 Hz, H_{ar}), 11.78 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 15.29, 16.72, 109.22, 111.36, 112.97, 113.83, 124.66, 125.02, 127.11, 128.06, 129.05, 130.37, 134.20, 139.81, 140.06, 144.65.

6-Chloro-4,10-Dimethyl-9*H*-1-Thia-3,9-Diazacyclopenta[*B*]Fluorene-2-Carbonitrile (**11**)

This compound was prepared from precursor **7**. Yield: 61% yellow needles, mp > 260°C (Found M^+, 311.0293, $C_{16}H_{10}ClN_3S$ requires 311.0284); υ_{max} (KBr)/cm^{-1} 2936, 2862, 2228, 1728, 1610, 1462, 1292, 806; δ_H (400 MHz, DMSO-d_6) 2.70 (s, 3H, CH_3), 3.13 (s, 3H, CH_3), 7.52 (d, 1H, *J* 8.8 Hz, H_{ar}), 7.65 (d, 1H, *J* 8.8 Hz, H_{ar}), 8.19 (s, 1H, H_{ar}), 11.72 (s, 1H, NH).

Ethyl[2-Cyano-4,5-Dimethyl-9*H*-1-Thia-3,9-Diazacyclopenta[*B*]Fluoren-6-yl]Carbonate (**12**)

This compound was prepared from precursor **8**. Yield: 61% yellow needles, mp 256°C (Found M^+, 365.0839, $C_{19}H_{15}N_3O_3S$ requires 365.0834); υ_{max} (KBr)/cm^{-1} 3388, 2980, 2222, 1740, 1474, 1278, 1122, 998, 810, 506; δ_H (400 MHz, DMSO-d_6)1.33 (t, 3H, *J* 7.1 Hz, CH_3), 2.74 (s, 3H, CH_3), 3.17 (s, 3H, CH_3), 4.28 (q, 2H, *J* 7.1 Hz, CH_2), 7.38 (d, 1H, *J* 8.8 Hz, H_{ar}), 7.59 (d, 1H, *J* 8.8 Hz, H_{ar}), 8.11 (s, 1H, H_{ar}), 11.75 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 16.48, 19.87, 110.38, 112.61, 117.58, 119.43, 120.22, 123.91, 125.03, 126.38, 126.95, 129.80, 138.39, 139.39.

General Procedure for the Preparation of Imidazolines (14–16)

Under an inert atmosphere, a stirred mixture of carbonitrile **10**, **12**, **13** (1.1 mmol) and ethylenediamine (10 mmol) in anhydrous ethanol (5 mL) was heated under reflux for 2 h in a microwave oven (78°C, 40 W). The solvent was removed in vacuo and water (5 mL) was added to the crude residue. The precipitated solid was collected and washed with light petroleum.

6-Bromo-2-(4,5-Dihydro-1*H*-Imidazol-2-yl)-4,10-Dimethyl-9*H*-1-Thia-3,9-diazacyclopenta[*B*] Fluorene (**14**)

This compound was prepared from precursor **10**. Yield: 79%, light yellow solid, mp > 260°C (Found M^+, 398.0207, $C_{18}H_{15}BrN_4S$ requires 398.0200); υ_{max} (KBr)/cm^{-1} 3269, 2916, 1603, 1522, 1444, 1294, 482; δ_H (400 MHz, DMSO-d_6) 2.71 (s, 3H, CH_3), 3.20 (s, 3H, CH_3), 3.50 (bs, 2H, CH_2), 3.90 (bs, 2H, CH_2), 7.24 (s, 1H, NH), 7.52 (d, 1H, *J* 8.4 Hz, H_{ar}), 7.59 (dd, 1H, *J* 2.0 Hz, *J* 8.4. Hz, H_{ar}), 8.34 (d, 1H, *J* 2.0 Hz, H_{ar}), 11.61 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 15.30, 16.27, 44.88, 55.10, 109.08, 110.73, 112.66, 120.41, 124.50, 125.09, 125.34, 128.06, 134.38, 138.39, 139.69, 145.79, 154.60, 159.62.

2-(4,5-Dihydro-1*H*-Imidazol-2-yl)-4,10-Dimethyl-9*H*-1-Thia-3,9-Diaza Cyclopenta[*b*]Fluoren-6-ol **15**

This compound was prepared from precursor **12**. Yield: 44%, light yellow needles, mp = 255°C (Found M^+, 336.1026, $C_{18}H_{16}N_4OS$ requires 336.1044); υ_{max} (KBr)/cm^{-1} 3287, 2917, 1596, 1466, 1291; δ_H (400 MHz, DMSO-d_6) 2.67 (s, 3H, CH_3), 3.17 (s, 3H, CH_3), 3.51 (t, 2H, *J* 10.0 Hz, CH_2), 3.90 (t, 2H, *J* 10.0 Hz, CH_2), 6.96(dd, 1H, *J* 2.0 Hz, *J* 8.6 Hz, H_{ar}), 7.19 (s, 1H, NH), 7.37 (d, 1H, *J* 8.6 Hz, H_{ar}), 7.62 (d, 1H, *J* 2.0 Hz, H_{ar}), 11.07 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 15.31, 16.38, 44.96, 55.17, 107.83, 108.49, 111.22, 114.88, 121.49, 123.95, 124.84, 133.45, 134.95, 139.02, 145.23, 150.59, 153.75, 159.84.

2-(4,5-Dihydro-1*H*-Imidazol-2-yl)-4,10-Dimethyl-9*H*-1-Thia-3,9-Diazacyclopenta[*b*]Fluorene (**16**)

This compound was prepared from precursor **13**. Yield: 61%, light yellow solid, mp > 260°C (Found M^+, 320.1084, $C_{18}H_{16}N_4S$ requires 320.1095); υ_{max} (KBr)/cm^{-1} 3446, 2920, 1597, 1523, 1460, 1324, 1287, 1259, 1082, 1031, 985, 873, 743; δ_H (400 MHz, DMSO-d_6) 2.71 (s, 3H, CH_3), 3.21 (s, 3H, CH_3), 3.50 (bs, 2H, CH_2), 3.90 (bs, 2H, CH_2), 7.22 (t, 1H, *J* 7.6 Hz, H_{ar}), 7.45 (t, 1H, *J* 7.6 Hz, H_{ar}), 7.55 (d, 1H, *J* 7.6 Hz, H_{ar}), 8.24 (d, 1H, *J* 7.6 Hz, H_{ar}), 11.42 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 15.48, 16.45, 45.01, 55.20, 109.25, 110.85, 112.81, 120.50, 124.65, 125.17, 125.49, 128.23, 134.45, 138.49, 139.75, 145.83, 154.73, 159.73.

Pharmacology

Cell Culture

Three human breast carcinoma cell lines, MCF-7/6, MCF-7/AZ and MDA-MB-231 kindly provided by Dr. M. Mareel (Laboratoire de Cancérologie Expérimentale, Hôpital Universitaire, Ghent, Belgique) and one normal breast cell line, HTB-125 (LGC Promochem) were used in the present study. MCF-7/AZ and MCF-7/6 cells are variants of the human mammary carcinoma cell family MCF-7.[11] 7/AZ cells differ from 7/6 ones in their morphology and their differential invasiveness. MDA-MB-231 is classed both as a hormone-independent and as a highly invasive breast cancer cell line.[12]

All cell lines were cultured at 37°C in a 5% CO_2/95% air humidified atmosphere, in DMEM-HAM's F12 medium (1:1, v/v, Gibco), supplemented with 10% fetal calf serum (v/v, Dutscher) to which were added penicillin 100 U/mL and streptomycin 100 μg/mL.

In vitro drug sensitivity was performed by the CellTiter 96® Non-radioactive cell proliferation assay (Promega) which allows determination of the fraction of viable cells remaining after drug treatment.[13] The test compounds were dissolved in dimethyl sulphoxide (DMSO, Sigma-Aldrich) to give 1 mM stock solutions from which further dilutions in culture medium were made for a final concentration range of 10^{-5}–10^{-10} M.

Effects of Thiazolocarbazoles on Viability of Breast Cancer Cell Lines

Cells were preincubated in 96-well micro plates (2.2×10^5 cells per well) for 24 h at 37°C and 5% CO_2 to allow stabilization prior to addition of drugs. The individual cell lines were then treated with serial dilutions of thiazolocarbazoles ranging from 10^{-6} M to 10^{-10} M to determine dose-response curves of the cell lines for 24 h. A solution of MTT tetrazolium salt (15 μL) was then added to each well. The plates were incubated for a further 4 h to allow for MTT metabolism to formazan by the succinate-tetrazolium reductase system active only in viable cells. A solubilization/Stop solution (100 μL) was added to stop the MTT assay and the optical densities were red on a plate reader (VERSAmax, Molecular Devices) at 570 nm. The data were then analyzed to discern the % of toxicity according to the equation:

$$\%cytotoxicity = 100 - \left(\frac{DOtest}{DOcontrol} x100\right) \qquad (1)$$

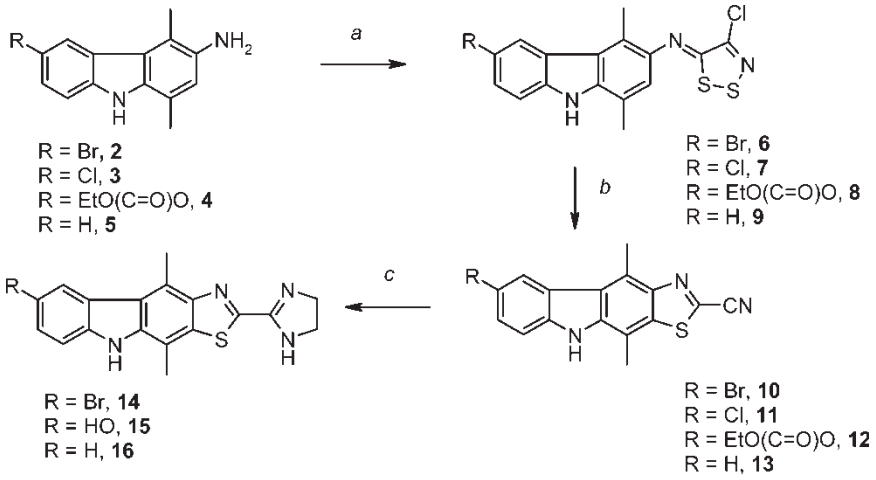
where DO test is the optical density at 570 nm recorded for the experimental sample; DO control is the optical density at 570 nm recorded in absence of drug.

The anti-proliferative effect of thiazolocarbazoles was tested with cells seeded at a density of 1×10^5 cells/well in 96-well culture plates. On day 0, 50 μL aliquots of medium containing graded concentrations (10^{-6}–10^{-10} M) of thiazolocarbazoles was added to each well of 96-well plates. After equilibration at 37°C in a humidified 5% CO_2 atmosphere, 50 μl of the cell suspension (5000 cells) were dispensed into all wells of the pre-equilibrated 96-well plate. After incubation at 37°C for 48 h in a humidified 5% CO_2 atmosphere, 15 μL of the MTT tetrazolium salt were added to each well. After 4 h of incubation, 100 μL of the solubilization/Stop solution were added to each well and culture plates were kept overnight in the incubator. The absorbance at 570 nm was recorded using an ELISA plate reader (VERSAmax, Molecular Devices). The data were then analyzed to discern the % of growth inhibition through a comparison of samples with cells not treated with drugs (control, 0% inhibition).

RESULTS AND DISCUSSION

Chemistry

The chemistry of *N*-arylimino-1,2,3 dithiazoles is one of the major interests of our research. Synthesis of the rare 6-substituted thiazolocarbazole ring



SCHEME 2 Synthetic route to 6-substituted thiazolocarbazoles. Reaction conditions and yields: (a) 4,5-dichloro-1,2,3-dithiazolium chloride, pyridine, rt, **6** (Br, 77%), **7** (Cl, 79%), **8** (OCOOEt, 76%), **9** (H, 60%); (b) NMP, 180°C, 80 W, μw, **10** (Br, 10 min, 85%), **11** (Cl, 30 min, 61%), **12** (OCOOEt, 20 min, 61%), **13** (H, 20 min, 66%); (c) $NH_2CH_2CH_2NH_2$, ethanol, 79°C, 40 W, μw, **14** (Br, 120 min, 79%), **15** (OH, 120 min, 88%), **16** (H, 80 min, 61%).

(9*H*-1-thia-3,9-diaza-cyclopenta[*b*]fluorene) was performed in six steps *via* the known 6-substituted-1,4-dimethyl-9*H*-carbazol-3-ylamines **2–5**, which were prepared from the starting commercially available 5-substituted indoles (Scheme 1). Our previous experience in the use of microwave in organic synthesis led us to check if there was any possibility to achieve better yields and cleaner reactions than with the purely thermal processes. Whatever the method, the microwave procedures were more rapid.

As previously described, the 6-bromo-1,4-dimethyl-9*H*-carbazole was prepared in 58% yield by treatment of the 5-bromoindole with hexane-2,4-dione in the presence of *p*-toluenesulphonic acid. Regioselective nitration in position 3 of the carbazole ring was achieved in good yield (80%) by treatment of 6-bromo-1,4-dimethyl-9*H*-carbazole with nitric acid in the presence of acetic anhydride. Using ammonium formate for catalytic transfer hydrogenation in ethanol, the reduction of the nitrocarbazole was accompanied by dehalogenation of the aromatic skeleton and led to the 3-amino-1,4-dimethylcarbazole **5** (90%).

In order to avoid dehalogenation, reduction of the 6-bromo-3-nitrocarbazole was performed with tin (II) chloride dihydrate, leading to the desired 6-bromo-3-amino-1,4-dimethylcarbazole **2** in good yield (80%). The analogue 6-chloro-3-amino-1,4-dimethylcarbazole **3** was prepared by the same synthetic pathway from 5-chloroindole.

N-arylimino-1,2,3-dithiazoles are highly versatile intermediates in heterocyclic synthesis. It is well known that reaction of 4,5-dichoro-1,2,3-dithiazolium chloride **1** with primary aromatic amines, in dichloromethane at room temperature, allows access to stable *N*-arylimino-4-chloro-5*H*-1,2,3-dithiazoles. Using a standard method applied to the preparation of *N*-arylimino-1,2,3-dithiazoles, the aminocarbazoles **2**, **3**, **4**, **5** 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 **6–9** in good yields (Scheme 2).

The best thermolysis procedure consisted in heating the imines **6–9** at 200°C in the presence of *N*-methylpyrrolidinone for 10 minutes, under microwave irradiation. The expected compounds **10–13** were then obtained in yields superior to 60% (Scheme 2). Cyanothiazolocarbazoles **10**, **12**, **13** were then treated with ethylenediamine in anhydrous ethanol to give the corresponding imidazolines **14**, **15**, **16** (Scheme 2). It must be noted that, for derivative **12**, the use of an excess of amine allows, the simultaneous aminolyse of the carbonate group and the transformation of the carbonitrile.

Pharmacology

Growth Inhibition and Cytotoxicity of Novel Synthesized Thiazolocarbazoles on Breast Cancer Cells

We have tested six synthetic thiazolocarbazoles for their activities in inhibiting the growth of three breast cancer cells lines (MCF-7/6, MCF-7/AZ and MDA-MB-231). We chose one hormone-independent cell line (MDA-MB231, invasive) known to be more aggressive and more resistant to drugs and two variants of the human mammary carcinoma cell family MCF-7 (MCF-7/6 invasive and MCF-7/AZ non-invasive). In order to determine whether the drop in cell number was due to thiazolocarbazole

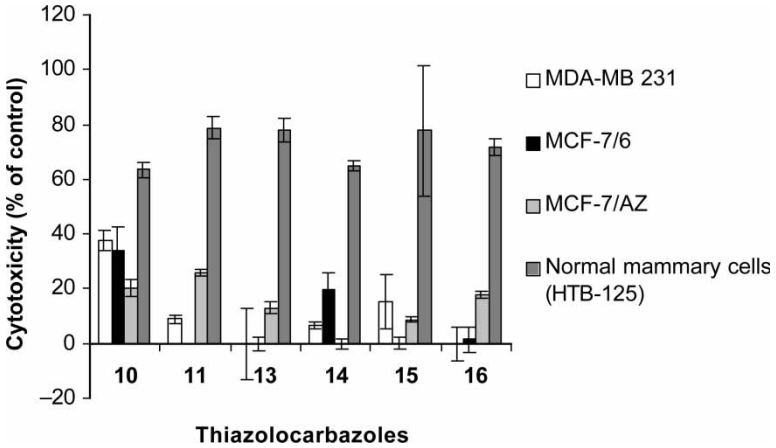


FIGURE 2 Toxicity of six thiazolocarbazoles on three breast cancer cell lines cultured for 24 h and treated with 1 μM of each compound. Comparison with one normal breast cell line.

toxicity, cytotoxic assays were performed on these same breast cancer cell lines and one normal breast cell line (HTB-125). As depicted in Figure 2, 24 h of thiazolocarbazole treatment at 1 μM revealed that these compounds possessed a low toxicity on the panel of three cancer cell lines. Less than 30% of the total cells were killed for all compounds at 1 μM, with the exception of **10**, which is able to reduce viable cell number by 38% in the MDA-MB-231 cell line. In the range of 10^{-7} and 10^{-10} M concentrations, no toxicity was recorded whatever the compounds and cell lines considered, suggesting that IC_{50} values were >1 μM. As shown in Figure 2, cytotoxic effects are very dependent on the cell lines investigated. Resistance was observed in the MCF-7/6 cell line for three compounds **11**, **15** and **13**, in the MCF-7/AZ for compound **14** and in the MDA-MB-231 for two compounds **16** and **13**. Only one compound, **10**, exhibited toxicity towards the panel of breast cancer cell lines tested. This analysis is in line with anti-proliferative results which revealed that cell growth was not affected by a drug dose lower than 1 μM. Compared with untreated exponentially growing cells, treatment with 1 μM of each compound resulted in a weak decrease in cell number after 48 h (Figure 3). Among all thiazolocarbazoles, **10** is the most effective in inhibiting cell growth with 26.5 and 53.8 percentage inhibition at 1 nM and 1 μM, respectively (Figure 3). Nevertheless, toxicity results suggest that the drop in cell number is probably due to the toxicity of **10**.

In this study, we have also tested the toxicity of the thiazolocarbazoles on one normal breast cell line (HTB-125). Results revealed the ability of all thiazolocarbazoles to kill normal breast cells, as cell viability is reduced by about 80%.

In conclusion, we have described the synthesis of novel 2-and 6-substituted dimethylthiazolocarbazoles among which the C-2 imidazoline derivatives exhibited interesting *in vitro* antitumor activity. Nevertheless, comparison with the unsubstituted skeleton in **13** and **16** suggests that introduction of an electron withdrawing group at C-6 does not enhance the activity and none of the test compounds appeared to be sufficiently selective.

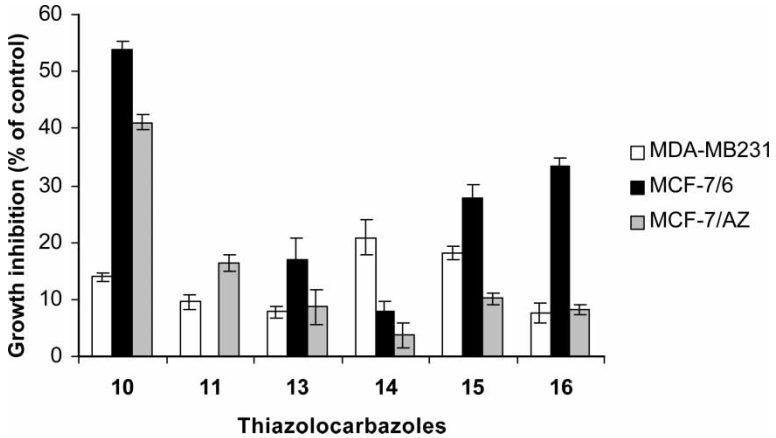


FIGURE 3 Effect of six thiazolocarbazoles on growth of breast cancer cells cultured for 48 h and treated with 1 μM of each compound. Results are expressed as % of growth inhibition (% relative to control). Each value was the mean of eight samples from one experiment.

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