*Journal of Enzyme Inhibition and Medicinal Chemistry*, December 2004 Vol. 19 (6), pp. 567–575





# Synthesis and Cytotoxic Activity of Thiazolofluorenone Derivatives

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

[a]*Groupe de Chimie Organique, Laboratoire de Biotechnologies et de Chimie Bio-organique, FRE 2766, Université de La Rochelle, Avenue Michel Crépeau, 17042 La Rochelle, France;* [b]*Institut de Chimie, Organique et Analytique, UMR-CNRS 6005, Université d' Orléans, rue de Chartres, BP 6759, F-45067, Orléans cedex 2, France;* [c]*Division de Cancérologie Expérimentale, Institut de Recherche Servier, 125 chemin de Ronde, 78290, Croissy sur Seine, France;* [d]*Laboratoires Servier, 1 Rue Carle Hébert, 92415, Courbevoie cedex, France*

*(Received 4 December 2003; In final form 11 February 2004)*

**The synthesis and biological evaluation of some novel thiazolofluorenones, thiazolofluorenes and thiazoloanthraquinones, substituted with amino side-chains are described. These polyheterocyclic compounds have been synthesized *via* the corresponding imino-1,2,3-dithiazoles. Their cytotoxic activity and their eventual selective effect on a phase of the cell cycle were evaluated *in vitro*, using the murine lymphocytic L1210 leukaemia cell line.**

*Keywords*: Imino-1,2,3-dithiazoles; Fluorenones; Thiazoles; Cytotoxic activity

*Abbreviations*: 2-[4-(2-hydroxethyl)-piperazin-1-yl]ethanesulfonic acid (HEPES); Phosphate buffered solution (PBS); Ribonuclease (RNase)

## INTRODUCTION

The occurrence of the thiazole ring in various natural and synthetic products has generated interest in many groups on account of its useful biological properties.[1–5] Thus, in our laboratory we launched a research program dealing with the preparation and pharmacological evaluation of some novel thiazolo derivatives. We recently reported the regiocontrolled synthesis of substituted thiazoloheterocycles (**I**–**III**)[6–8] mainly related to marine or terrestrial alkaloids (e.g. dercitine, kuanoniamine and ellipticine). Among all the compounds prepared, the novel linear tetracyclic 4.10-dimethyl-9*H*-1-thia-3.9-diaza-cyclopenta[*b*]fluorene-2-carbonitrile **III** showed an intermediate cytotoxic activity with no real effect on the cell cycle (Figure 1).

Our interest in biologically active compounds as potential antitumor candidates, focussed our studies on the synthesis of a series of derivatives in which the thiazole ring was fused with fluorenone (**IV**–**VI**), fluorene (**VII**) and anthraquinone (**VIII**). The resulting structures are related to recently described thiazoloacridines (**IX**), thiazoloquinolines (**X**) and substituted benzothiazoles (**I**–**III**, **XI**) which exhibit interesting antitumor activity[3–11] (Figure 2). In this paper we describe the synthetic route and the biological evaluation to these novel substituted polyheterocyclic compounds (**IV**–**VIII**). These original skeletons (**IV**–**VIII**) which have not been previously described are structurally close to carbazole with a carbonyl or methylene group replacing the nitrogen atom of carbazole. In the hope to study the importance of the lead compounds and with the aim to enhance the cytotoxic activity of such products, wide modifications by an amino side-group of key synthetic precursors having a carbonitrile function in position 2 of the thiazole ring were studied.

## 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

*Corresponding author. Tel.: +33-546-458-276. Fax: +33-546-458-247. E-mail: vthiery@univ-lr.fr

ISSN 1475-6366 print/ISSN 1475-6374 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/14756360400004607

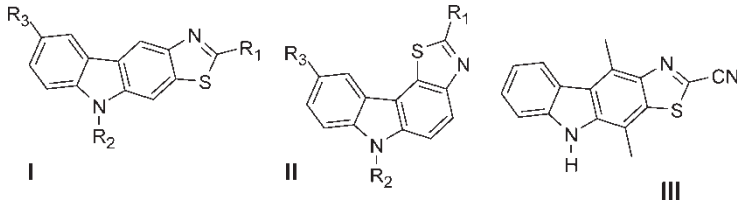


FIGURE 1 Structures (**I**–**III**).



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 parts 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.

### *Synthesis of Iminodithiazoles*

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride (1.15 mmol) was added to a stirred solution of amine (0.95 mmol) in dichloromethane (10 mL). After 15 min, pyridine (2.3 mmol) was added and the mixture stirred for 2 h. The solvent was removed *in vacuo* and the crude residue purified by column chromatography (dichloromethane 100%) to afford the required compound.

#### 2-(4-Chloro-[1,2,3]Dithiazol-5-Ylideneamino)-Fluoren-9-One **2**

This compound was prepared from commercially available 2-aminofluorenone. Yield: 98%, yellow needles, mp = 210°C. Found $M^+$, 329.9695. $C_{15}H_7N_2OS_2Cl$ requires 329.9688; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3048, 3017, 1713, 1607, 1452, 1127, 861, 731; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.36 (t, 1H, *J* 8.3 Hz, $H_{ar.}$), 7.41-7.43 (m, 2H, $H_{ar.}$), 7.60–7.63 (m, 2H, $H_{ar.}$), 7.80 (d, 1H, *J* 8.7 Hz, $H_{ar.}$), 7.90 (d, 1H, *J* 8.7 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 115.27, 120.31, 121.66, 124.59, 126.51, 129.11, 134.38, 135.01, 135.87, 142.33, 144.09, 148.04, 151.61, 188.14, 192.90; *m/z* 330 ($M^+$, 100%).

#### 3-(4-Chloro-[1,2,3]Dithiazol-5-Ylideneamino)-Fluoren-9-One **3**

This compound was prepared from commercially available 3-aminofluorenone. Yield: 84%, yellow needles, mp = 224°C. Found $M^+$, 329.9686. $C_{15}H_7N_2OS_2Cl$ requires 329.9688; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3050, 3002, 1701, 1575, 1191, 866, 736; $\delta_H$ (400 MHz, $CDCl_3$) 7.07 (dd, 1H, *J* 1.7 Hz, *J* 8.0 Hz, $H_{ar.}$), 7.30–7.36 (m, 2H, $H_{ar.}$), 7.51–7.52 (m, 2H, $H_{ar.}$), 7.68 (dd, 1H, *J* 0,8 Hz, *J* 7.8 Hz, $H_{ar.}$), 7.76 (d, 1H, *J* 7.8 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, $CDCl_3$) 112.21, 118.62, 120,49, 124.32, 126.34, 129.65, 131.90, 134.68, 134.73, 143.34, 146.84, 147.81, 157.19, 160,21, 192.50; *m/z* 330 ($M^+$, 100%).

#### 4-Chloro-([1,2,3]Dithiazol-5-Ylidenamino)-Anthraquinone **4**

This compound was prepared from commercially available 2-aminoanthraquinone. Yield: 91%, red needles, mp = 250°C. Found $M^+$, 357.9636. $C_{16}H_7N_2O_2S_2Cl$ requires 357.9637; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3253, 2949, 2867, 1608, 1515, 1449, 1336, 1300, 1107, 977, 829; $\delta_H$ (400 MHz, $CDCl_3$) 7.51 (dd, 1H, *J* 2.3 Hz, 8.3 Hz, $H_{ar.}$), 7.78–7.83 (m, 2H, $H_{ar.}$), 8.10 (d, 1H, *J* 2.3 Hz, $H_{ar.}$), 7.30–7.34 (m, 2H, $H_{ar.}$), 8.42 (d, 1H, *J* 8.3 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, $CDCl_3$) 102.55, 117.01,

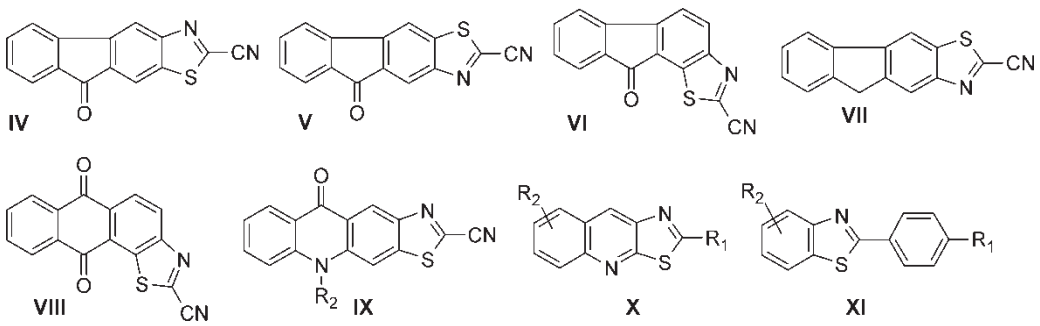


FIGURE 2 Structures (**IV**–**XI**).

125.78, 127.25, 127.33, 127.34, 129.88, 131.18, 133.56, 134.13, 134.15, 134.39, 135.55, 156.16, 182.10, 182.61; *m/z* 358 ($M^+$, 100%).

(4-Chloro-[1,2,3]Dithiazol-5-Ylidene)-(9H-Fluoren-2-Yl)-Amine **5**

This compound was prepared from commercially available 2-aminofluorene. Yield: 86%, red needles, mp = 175°C. Found $M^+$, 315.9905. $C_{15}H_9N_2S_2Cl$ requires 315.9895; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3043, 2919, 1554, 1395, 1145, 852, 735; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.96 (s, 1H, $CH_2$), 7.26 (dd, 1H, *J* 7.8 Hz, *J* 1.4 Hz, $H_{ar.}$), 7.31 (t, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.38 (t, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.44 (s, 1H, $H_{ar.}$), 7.58 (d, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.90 (d, 1H, *J* 7.8 Hz, $H_{ar.}$), 8.00 (d, 1H, *J* 7.8 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 36.55, 116.49, 118.48, 119.97, 121.23, 125.15, 126.82, 126.87, 139.35, 140,53, 143.18, 144.85, 147.07, 149.52, 158.62; *m/z* 316 ($M^+$, 100%).

(3-Bromo-9H-Fluoren-2-Yl)-(4-Chloro-[1,2,3]Dithiazol-5-Ylidene)Amine **7**

This compound was prepared from 2-amino-3-bromofluorenone **6**. Yield: 76%, yellow needles, mp = 175°C. Found $M^+$, 393.8999. $C_{15}H_6BrClN_2S_2$ requires 393.9000; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3060,1693, 1632, 1609, 1535, 1485, 1397; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.26 (dd, 1H, *J* 2.0 Hz, *J* 8.0 Hz, $H_{ar.}$), 7.41 (td, 1H, *J* 2.0 Hz, *J* 8.0 Hz, $H_{ar.}$), 7.51 (td, 1H, *J* 1.7 Hz, *J* 7.3 Hz, $H_{ar.}$), 7.55 (d, 1H, *J* 1.7 Hz, $H_{ar.}$), 7.70 (d, 1H, *J* 8.1 Hz, $H_{ar.}$), 8.14 (dd, 1H, *J* 1.7 Hz, *J* 8.1 Hz, $H_{ar.}$), 8.23 (d, 1H, *J* 8.3 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 36.86, 114.48, 114.77, 120,00, 125.04, 125.13, 127.14, 127.42, 140.04, 141.58, 143.40, 144.01, 147.79, 148.26, 160,25; *m/z* 394 ($M^+$, 100%).

### *Bromination*

3-Bromo-9*H*-Fluorene-2-Ylamine **6**

Under an inert atmosphere, to a solution of aminofluorene (1.1 g, 5.6 mmol) in acetic acid (50 mL) was added dropwise bromine (5.6 mmol, 0.23 mL). After 1.5 h under stirring at room temperature, acetic acid was removed *in vacuo*. The crude residue was basified with NaOH 30% (30 mL). The mixture was dissolved in ethyl acetate and washed with sodium thiosulfate solution (20 mL) and then purified by column chromatography (eluent: dichloromethane 100%). Yield: 25%, white needles, mp = 140°C (Lit. 137–139°C). Found $M^+$, 259.0031. $C_{13}H_{10}BrN$ requires 258.9996; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3425, 3355, 3041, 2925, 1615, 1415, 862, 760; $\delta_H$ (400 MHz, $CDCl_3$) 3.77 (s, 1H, $CH_2$), 6.95 (s, 1H, $H_{ar.}$),7.21 (t, 1H, *J* 7.5–Hz, $H_{ar.}$) 7.32 (t, 1H, *J* 7.5–Hz, $H_{ar.}$), 7.46 (d, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.59 (d, 1H, *J* 7.5 Hz, $H_{ar.}$), 7.80 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, $CDCl_3$) 36.53, 108.22, 112.22, 118.81, 123.84, 124.83, 125.70, 126.81, 134.22, 141.06, 142.32, 142.79, 144.13; *m/z* 259 ($M^+$, 100%).

### *Synthesis of Thiazolocarbonitriles*

Under an inert atmosphere, a mixture of dithiazole (0.51 mmol) and pyridinium tribromide (0.53 mmol) in pyridine (8 mL) was heated at reflux for 4.5 h. After cooling, pyridine was removed *in vacuo* and the crude residue purified by column chromatography (eluent: dichloromethane 100%).

9-Oxo-9*H*-[3.1]Thiazolo-Cyclopenta[B]Fluorene-2-Carbonitrile **8**

This compound was prepared from precursor **2**. Yield: 51%, yellow needles, mp > 250°C. Found $M^+$, 262.0205. $C_{15}H_6N_2OS$ requires 262.0200; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3073, 3005, 2924, 2233, 1706, 1616, 1105, 740; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.48 (t, 1H, *J* 7.7 Hz, $H_{ar.}$), 7.69–7.73 (m, 2H, $H_{ar.}$), 7.88 (d, 1H, *J* 7.7 Hz, $H_{ar.}$), 8.36 (s, 1H, $H_{ar.}$), 8.67 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 113.19, 115.35, 120.17, 122.03, 124.46, 130.53, 134.29, 134.69, 136.14, 137.99, 142.11, 142.32, 143.14, 152.02, 191.10; *m/z* 262 ($M^+$, 100%).

10-Oxo-10*H*-[1,3]-Thiazolocyclopenta[A]-Fluorene-2-Carbonitrile **9**

This compound was prepared from precursor **2**. Yield: 18%, yellow needles, mp > 250°C. Found $M^+$, 262.0202. $C_{15}H_6N_2OS$ requires 262.0200; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3072, 2960, 2920, 2231, 1711, 16023, 751; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.43 (t, 1H, *J* 7.4 Hz, $H_{ar.}$), 7.64 7.68 (m, 2H, $H_{ar.}$), 7.95 (d, 1H, *J* 7.4 Hz, $H_{ar.}$), 8.19 (d, 1H, *J* 8.3 Hz, $H_{ar.}$), 7.53 (d, 1H, *J* 8.3 Hz, $H_{ar.}$); *m/z* 262 ($M^+$, 100%).

9-Oxo-9*H*-[1,3]Thiazolo-Cyclopenta[B]Fluorene-2-Carbonitrile **10**

This compound was prepared from precursor **3**. Yield: 52%, yellow needles, mp > 250°C Found $M^+$, 262.0201. $C_{15}H_6N_2OS$ requires 262.0200; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3389, 2986, 2966, 2932, 2870, 2223, 1757, 1603, 1476, 1288, 780; $\delta_H$ (400 MHz, $CDCl_3$) 7.41 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.62 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.71–7.77 (m, 2H, $H_{ar.}$), 8.25–8.26 (m, 2H, $H_{ar.}$); *m/z* 262 ($M^+$, 100%).

6,11-Dioxo-6,11-Dihydro-Anthra[2,1-D]Thiazole-2-Carbonitrile **11**

Compound **4** (2.5 mmol) was irradiated in the presence of graphite (0.5 g, 10 wt % to the reactants). The irradiation was programmed to obtain a constant temperature (150°C) with a maximal power output of 150 W. After cooling, the mixture was dissolved in dichloromethane and purified by column chromatography (silica gel). Elution with light petroleum-dichloromethane 30/70 gave the required benzothiazole.

Yield: 56%, yellow needles, mp > 250°C. Found $M^+$, 290.0147. $C_{16}H_6N_2O_2S$ requires 290.0150; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3082, 2970, 2878, 2237, 2008, 1670, 1547, 1332, 1296, 713; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.98 8.01 (m, 2H, $H_{ar.}$), 8.25–8.30 (m, 2H, $H_{ar.}$),

8.46 (d, 1H, *J* 8.5 Hz, $H_{ar.}$), 8.73 (d, 1H, *J* 8.5 Hz, $H_{ar.}$); *m/z* 290 ($M^+$, 100%).

9*H*-[3,1]-Thiazolocyclopenta[B]Fluorene-2-Carbonitrile **12**

Under an inert atmosphere, a suspension of compound **7** (0.5 mmol), copper iodide (0.5 mmol) in pyridine (8 mL) was irradiated during 15 min. The irradiation was programmed to obtain a constant temperature (105°C) with a maximal power output of 150 W. After cooling, the mixture was dissolved in dichloromethane, washed with sodium thiosulfate solution (20 mL) and purified by column chromatography (eluent: dichloromethane). Yield: 80%, yellow needles, mp = 170°C. Found $M^+$, 248.0405. $C_{15}H_8N_2S$ requires 248.0408; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3057, 2916, 2228, 1471, 1404, 1138, 862; $\delta_H$ (400 MHz, $CDCl_3$) 4.10 (s, 1H, $CH_2$), 7.40–7.47 (m, 2H, $H_{ar.}$), 7.62 (d, 1H, *J* 6.9 Hz, $H_{ar.}$), 7.88 (d, 1H, *J* 6.9 Hz, $H_{ar.}$), 8.31 (s, 1H, $H_{ar.}$), 8.33 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, $CDCl_3$) 36.54, 11.97, 113.29, 120.79, 121.29, 125.49, 127.37, 128.61, 134.93, 135.22, 139.55, 143.56, 144.00, 144.13, 151.62; *m/z* 248 ($M^+$, 100%).

### *Synthesis of Imidazolines*

A stirred mixture of cyanothiazolo derivatives (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 a white imidazoline dihydrochloride.

2-(4,5-Dihydro-1*H*-Imidazol-2-Yl)-[3,1]-Thiazolocyclopenta[B]Fluoren-9-One **13**

This compound was prepared from precursor **8**. Yield: 98%, white needles, mp > 250°C. Found $MH^+$, 306.0704. $C_{17}H_{12}N_3OS$ requires 306.0701; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3451, 2938, 2865, 1630, 1519, 1418, 748; $\delta_H$ (400 MHz, DMSO-$d_6$) 4.09 (bs, 4H, $CH_2$), 7.48 (t, 1H, *J* 7.4 Hz, $H_{ar.}$), 7.71–7.74 (m, 2H, $H_{ar.}$), 8.29 (s, 1H, $H_{ar.}$), 8.36 (s, 1H, NH), 8.76 (s, 1H, $H_{ar.}$), 11.50 (s, 1H, NH); $\delta_C$ (100 MHz, DMSO-$d_6$) 36.49, 45.04, 115.80, 119.86, 122.01, 124.48, 130.54, 134.30, 134.73, 136.18, 141.93, 142.98, 143.20, 150,98, 152.17, 157.59; *m/z* 306 ($MH^+$, 100%).

2-(4,5-Dihydro-1*H*-Imidazol-2-Yl)-[1,3]-Thiazolocyclopenta[A]Fluoren-10-One **14**

This compound was prepared from precursor **9**. Yield: 98%, white needles, mp > 250°C. Found $MH^+$, 306.0623. $C_{17}H_{12}N_3OS$ requires 306.0622; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3451, 2938, 2865, 1630, 1519, 1418, 748; $\delta_H$ (400 MHz, $D_2O$) 3.78-3.84 (m, 4H, $CH_2$), 7.13-7.24 (m, 4H, $H_{ar.}$), 7.42 (t, 1H, *J* 7.2 Hz, $H_{ar.}$), 7.52 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, $D_2O$) 45.54, 116.84, 120.07, 122.71, 125.06, 130.92, 133.54, 134.37, 137.52, 137.54, 142.36, 143.64, 152.63, 156.35, 157.53, 194.00; *m/z* 306 ($MH^+$, 100%).

2-(4,5-Dihydro-1*H*-Imidazol-2-Yl)-[1,3]-Thiazolo-Cyclopenta[B]Fluoren-9-One **15**

This compound was prepared from precursor **10**. Yield: 98%, white needles, mp > 250°C. Found $M^+$, 305.1623. $C_{17}H_{11}N_3OS$ requires 305.1622; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3400–3200, 2865, 1630, 1519; $\delta_H$ (400 MHz, $D_2O$) 3.76–3.82 (m, 4H, $CH_2$), 7.13–7.24 (m, 4H, 4 × $H_{ar.}$), 7.37 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 8.52 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, $D_2O$) 45.54, 116.84, 120.07, 122.71, 125.06, 130.92, 133.54, 134.37, 137.52, 137.54, 142.36, 143.64, 152.53, 156.35, 157.53; 194.00; *m/z* 305 ($M^+$, 100%).

2-(4,5-Dihydro-1*H*-Imidazolyl)Anthra[2,1-D]Thiazole-6,11-Dione **16**

This compound was prepared from precursor **11**. Yield: 98%, yellow needles, mp > 250°C. Found $MH^+$, 334.1623. $C_{18}H_{11}N_3O_2S$ requires 334.1622; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3660-3400, 1664, 1596, 1328, 1297, 961, 716; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.52 (t, 2H, *J* 10.0 Hz, $CH_2$), 3.95 (t, 2H, *J* 9.9 Hz, $CH_2$), 7.54 (s, 1H, NH), 7.96–7.97 (m, 2H, $H_{ar.}$), 8.22–8.28 (m, 2H, $H_{ar.}$), 8.38 (d, 1H, *J* 8.4 Hz, $H_{ar.}$), 8.55 (d, 1H, *J* 8.5 Hz, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 125.46, 126.88, 127.00, 127.02, 127.72, 128.32, 129.13, 131.33, 132.62, 132.97, 134.68, 134.93, 157.32, 181.89, 182.11; *m/z* 334 ($MH^+$, 100%).

2-(4,5-Dihydro-1*H*-Imidazol-2-Yl)-9*H*-[3,1]-Thiazolocyclopenta[B]Fluorene **17**

This compound was prepared from precursor **12**. Yield: 80%, white needles, mp > 250°C. Found $M^+$, 291.0831. $C_{17}H_{13}N_3S$ requires 291.0830; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3367, 2941, 2855, 1601, 1516, 1306; $\delta_H$ (400 MHz, DMSO-$d_6$) 3.48 (t, 2H, *J* 9.9 Hz, $CH_2$), 3.88 (t, 2H, *J* 10.0 Hz, $CH_2$), 4.08 (s, 1H, $CH_2$), 7.35–7.44 (m, 3H, 2 × $H_{ar.}$, NH), 7.62 (d, 1H, *J* 7.3 $H_{ar.}$), 7.98 (d, 1H, *J* 7.3 Hz, $H_{ar.}$), 8.23 (s, 1H, $H_{ar.}$), 8.65 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 36.17, 44.97,55.13, 113.24, 119.85, 120,57, 125.36, 127.07, 127.64, 134.36, 139.90, 140.56, 142.36, 143.72, 152.20, 159.20, 159.47; *m/z* 291 ($M^+$, 100%).

### *Synthesis of 2-substituted Thiazolofluorenone Derivatives*

*N*-(2-Dimethylamino-Ethyl)-9-Oxo-9*H*-[3,1]-Thiazoloycyclopenta[B]Fluorene-2-Carboxamidine **18**

A stirred mixture of carbonitrile **8** (1 mmol) and *N,N*-dimethylethylenediamine (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 a white imidazoline dihydrochloride. Yield: 85%, white needles, mp > 250°C Found $MH^+$, 351.1281. $C_{19}H_{19}N_4OS$ requires 351.1280; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3451, 3210, 3145, 2887, 2656, 2475, 1712, 1693, 1615, 1469, 747; $\delta_H$ (400 MHz, $D_2O$) 2.55 (s, 6H, 2 × $CH_3$), 3.50 (t, 2H, *J* 6.3 Hz, $CH_2$), 3.86 (t, 2H, *J* 6.3 Hz, $CH_2$), 7.30 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.46–7.56 (m, 3H, $H_{ar.}$), 8.00–8.02 (m, 2H, $H_{ar.}$); $\delta_C$ (100 MHz, $D_2O$) 44.11, 55.10, 115.78, 121.60, 122.98, 125.67, 131.49, 134.98, 135.32, 137.59, 142.97, 144.19, 152.74, 155.88, 194.72; *m/z* 351 ($MH^+$, 100%).

### 2-(1*H*-Benzimidazol-2-Yl)-[3,1]-Thiazolocyclopenta[B]Fluoren-9-One **19**

A stirred mixture of carbonitrile **8** (0.3 mmol) and 2-aminoaniline (9.2 mmol) in anhydrous ethanol (5–mL) under argon was heated under reflux for 24 h. The solvent was removed *in vacuo* and water (5 mL) was added to the crude residue. The solid was collected and washed several times with anhydrous ether and then recrystallized with ethanol. Yield: 45%, orange needles, mp = 222°C. Found $M^+$, 353.0628. $C_{21}H_{11}N_3OS$ requires 353.0622; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3392, 3339, 3068, 2966, 2850, 1713, 1643, 1426, 1310, 745; $\delta_H$ (400 MHz, DMSO-$d_6$) 7.30–7.32 (m, 3H, 2 $H_{ar.}$, NH), 7.42 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.64–7.68 (m, 4H, $H_{ar.}$), 7.87 (d, 1H, *J* 7.3 Hz, $H_{ar.}$), 8.19 (s, 1H, $H_{ar.}$), 8.55 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 112.24, 114.99, 118.40, 119.71, 121.38, 122.73, 124.01, 124.55, 129.66, 133.33, 134.54, 135.57, 140.50, 142.00, 143.54, 153.64, 159.97, 191.59; *m/z* 353 ($M^+$, 100%).

### 2-[1-(2-Diethylamino-Ethyl)-4.5-Dihydro-1*H*-Imidazole]-[3,1]-Thiazolocyclo Penta[B] Fluoren-9-One **20**

A stirred mixture of carbonitrile **8** (0.2 mmol) and *N,N*-diethyldiethylenetriamine (5.7 mmol) in anhydrous ethanol (5 mL) under argon was heated under reflux for 18 h. The solvent was removed *in vacuo.* The crude residue was washed with a solution NaOH (5 mL) and extracted with dichloromethane (5 mL). The product was isolated by column chromatography (dichloromethane 100%). Yield: 39%, yellow needles, mp > 220°C. Found $M^+$, 404.1674. $C_{23}H_{24}N_4OS$ requires 404.1670; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 2965, 2871, 2796, 1706, 1613, 1573, 1106, 765; $\delta_H$ (400 MHz, $CDCl_3$) 1.04 (bs, 6H, 2 × $CH_3$), 2.57–2.63 (m, 4H, 2 × $CH_2$), 2.71–2.74 (m, 2H, $CH_2$), 3.71 (t, 2H, *J* 10.3 Hz, $CH_2$), 3.95–4.01 (m, 4H, 2 × $CH_2$), 7.35 (t, 1H, *J* 7.8 $H_{ar.}$), 7.54 (t, 1H, *J* 7.8 $H_{ar.}$), 7.61 (d, 1H, *J* 7.8 Hz, $H_{ar.}$), 7.73 (d, 1H, *J* 7.8 Hz, $H_{ar.}$), 8.03 (s, 1H, $H_{ar.}$), 8.24 (s, 1H, $H_{ar.}$); $\delta_C$ (100 MHz, DMSO-$d_6$) 11.60, 45.38, 47.25, 50.90, 52.40, 53.29, 113.29, 120.26, 120.74, 124.68, 129.56, 133.75, 135.09, 135.50, 141.43, 142.22, 144.04, 153.90, 158.30, 161.41, 192.44; *m/z* 404 ($M^+$, 100%).

### 9-Oxo-9*H*-[3,1]-Thiazolocyclopenta[B]Fluorene-2-Carboximidic Acid Ethyl Ester **21**

A stirred mixture of carbonitrile **8** (0.5 mmol) and 2.5 N NaOH (0.5 mmol) in anhydrous ethanol (5 mL) under argon was stirred at room temperature for 2 h. The solvent was removed *in vacuo.* The crude residue was extracted with ethyl acetate (5 mL). The product was isolated by column chromatography (dichloromethane 100%). Yield: 87%, yellow needles, mp = 230°C. Found $M^+$, 308.0618. $C_{17}H_{12}N_2O_2S$ requires 308.0619; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3566, 3368, 3030, 1722, 1610, 1519, 1488; $\delta_H$ (400 MHz, DMSO-$d_6$) 1.49 (t, 3H, *J* 7.3 Hz, $CH_3$), 4.49 (q, 2H, *J* 7.3 Hz, $CH_2$), 7.35 (t, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.54 (t, 1H, *J* 7.3, $H_{ar.}$), 7.61 (d, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.74 (d, 1H, *J* 7.3 Hz, $H_{ar.}$), 7.98 (s, 1H, $H_{ar.}$), 8.33 (s, 1H, $H_{ar.}$), 8.94 (s, 1H, H); $\delta_C$ (100 MHz, DMSO-$d_6$) 14.13, 63.21, 113.66, 120.53, 120.78, 124.73, 129.68, 134.12, 135.10, 135.46, 141.43, 142.71, 143.84, 153.16, 159.50, 160.36, 192.11; *m/z* 308 ($M^+$, 100%).

### 9-Oxo-9*H*-[3,1]Thiazolocyclopenta[B]Fluorene-2-Carboximidic Acid 2-Dimethylamino-Ethyl Ester **22**

A stirred mixture of carbonitrile **8** (0.5 mmol), 2.5 N NaOH (0.5 mmol) and dimethylaminoethanol (0.5 mmol) in dry THF (5 mL) under argon was stirred at room temperature for 2 h. The solvent was removed *in vacuo.* The crude residue was extracted with ethyl acetate (5 mL). The product was isolated after recrystallization in ethanol.

Yield: 67%, yellow needles, mp = 220°C. Found $MH^+$, 352.1078. $C_{19}H_{17}N_3O_2S$ requires 352.1069; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3392, 3339, 3068, 2966, 2850, 1713, 1643, 1426, 1310, 745; $\delta_H$ (400 MHz, DMSO-$d_6$) 2.25 (s, 6H, 2 × $CH_3$), 2.68 (t, 2H, *J* 5.4 Hz, $CH_2$), 4.40–4.45 (m, 2H, $CH_2$), 7.43 (t, 1H, *J* 7.8 $H_{ar.}$), 7.66–7.69 (m, 2H, $H_{ar.}$), 7.87 (d, 1H, *J* 7.8 Hz, $H_{ar.}$), 8.22 (s, 1H, $H_{ar.}$), 8.51 (s, 1H, $H_{ar.}$), 9.41 (s, 1H, NH); $\delta_C$ (100 MHz, DMSO-$d_6$) 45.92, 57.57, 65.45, 113.67, 120.64, 120.82, 124.78, 129.75, 134.20, 135.14, 135.51, 141.54, 142.73, 143.87, 153.24, 192.14; *m/z* 352 ($MH^+$, 100%).

### N-Hydroxy-9-oxo-9*H*-[3.1]thiazolo-cyclopenta[b]fluorene-2-carboxamidine **23**

A stirred mixture of carbonitrile **8** (0.5 mmol), hydroxylamine hydrochloride (0.52 mmol) and trihydrate sodium acetate (0.52 mmol) in anhydrous ethanol (5 mL) under argon was heated under reflux for 4 h. The solvent was removed *in vacuo* and water (5 mL) was added to the crude residue. The precipitated solid was collected and recrystallized in ethanol. Yield: 50%, yellow needles, mp > 250°C. Found $M^+$, 295.0424. $C_{15}H_9N_3O_2S$ requires 295.0415; $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3487, 3456, 3386, 3292, 3050, 2902, 1709, 1613, 1417, 963; $\delta_H$ (400 MHz, DMSO-$d_6$) 6.17 (s, 2H, NH), 7.40 (t, 1H, *J* 7.9 Hz, $H_{ar.}$), 7.64–7.67 (m, 2H, $H_{ar.}$), 7.83 (d, 1H, *J* 7.9 Hz, $H_{ar.}$), 8.06 (s, 1H, $H_{ar.}$), 8.49 (s, 1H, $H_{ar.}$), 10,59 (s, 1H, OH); $\delta_C$ (100 MHz,

DMSO-$d_6$) 114.98, 118.21, 121.45, 124.15, 129.69, 132.82, 134.50, 135.68, 140.48, 141.42, 143.68, 146.93, 152.75, 163.42, 191.91; *m/z* 295 ($M^+$, 100%).

### *In Vitro* Cytotoxic Activity

L1210 cells (murine leukaemia), 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 previously.[12] Cells were exposed to graded concentrations of the compounds for 48 h and the results were expressed as $IC_{50}$ (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, $1 \times 10^4$ cells were analysed on a Epics XL/MCL flow cytometer (Beckman Coulter, France). Results are expressed as the percentage of cells in each phase of the cell cycle.

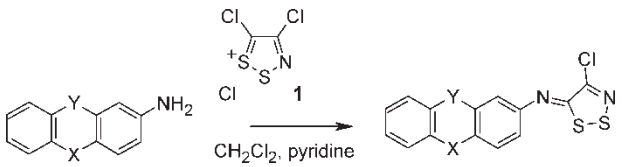
## RESULTS AND DISCUSSION

### Chemistry

As we previously described in our study of the chemistry of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel salt) and its derivatives, fusion of the thiazole ring onto the fluorenone, fluorene and anthraquinone skeletons 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.[13–17]

The synthesis of the thiazoloderivatives **8**–**12** was performed in two steps from the corresponding amino precursors **2**–**7**. Using a standard method applied for the preparation of *N*-arylimino-1,2,3-dithiazoles,[18,19] the starting amines 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 **2**-**7** in good yields (Scheme 1).

The best thermolysis procedure for the fluorenone derivatives **2** and **3** consisted in heating the imines under argon in pyridine at reflux in the presence of pyridinium tribromide. A short exploration of various alternatives has shown that heating at 200°C (neat) for 2 min, or at 140°C for 2 or 3 days in sealed tube in the presence of toluene, gave worse results. Exposing the same imines to microwave irradiation, neat in a glass vial with a screw cap lid, was also unsuccessful. The expected compounds **8**, **9**, **10** were then obtained in reasonable yields (51%, 18% and 52% respectively), the linear isomers **8** and **10** being mainly isolated besides a lower yield of their angular counterpart (Scheme 2).

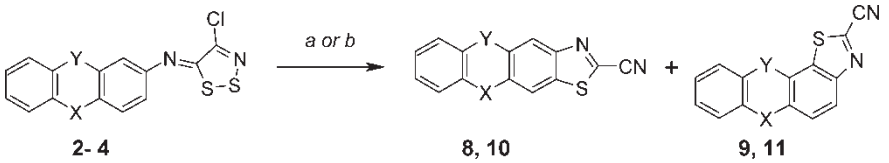



| Compound | X | Y | Yield % |
|---|---|---|---|
| **2** | – | C=O | 98 |
| **3** | C=O | – | 84 |
| **4** | C=O | C=O | 91 |
| **5** | – | $CH_2$ | 86 |

SCHEME 1 Synthetic routes to iminodithiazoles **2**–**5**.

Cyclisation of the 3-iminofluorene **5** was more difficult than for the other molecules and led to a complicated mixture of regioisomers difficult to separate even by column chromatography or recrystallization, accompanied by carbonaceous compounds. According to NMR data, the linear isomer **12** was the main product. In order to obtain regioselectively this linear isomer **12**, a mild procedure, which consisted of heating an *ortho* bromoimine **7** in the presence of cuprous iodide in pyridine at reflux, was applied and afforded it in good yield (Scheme 3).

Unfortunately, with the anthraquinone derivative **4**, whatever thermolysis conditions were used, no trace of the required thiazoloanthraquinone was detected besides the recovered starting imine. In connection with our recent work on the utility of microwaves in organic chemistry,[18,19] a solvent-free approach, including the use of graphite as support, allowed a rapid a safe heating of the starting imine. Graphite is one of the solids most efficiently heated by microwave and is also known for its ability to adsorb organic molecules. We recently showed that the strong thermal effect due to graphite/microwaves interaction can be efficiently use for the synthesis of various polyheterocyclic molecules for which traditional methods failed or are less attractive. Following a strategy which consisted in varying the ratio between the quantity of the reactant and the support (graphite), we showed that a short (2–5 min) microwave irradiation (150 W) of the starting imino-1,2,3-dithiazole **4** at 150°C in the presence of a small amount of graphite (10% by weight) surprisingly afforded the angular




| Starting material | X | Y | Compound | Yield % |
|---|---|---|---|---|
| **2** | - | C=O | **8** | 51 |
| **2** | - | C=O | **9** | 18 |
| **3** | C=O | - | **10** | 52 |
| **4** | C=O | C=O | **11** | 56 |
| **5** | - | $CH_2$ | - | - |

SCHEME 2 Synthetic routes to thiazolo derivatives **8**–**11**. Reaction conditions and yields: (a) **2** or **3** pyridinium tribromide, pyridine, reflux. (b) **4**, graphite, μW.

2-cyanobenzothiazole **11** (Scheme 1). No trace of the linear counterpart was detected.

It is known that the cyano group in position-2 of the benzothiazole ring is very reactive and that its transformation into acid, amide, amidine and imidate may be easily realised. According to this strategy, the condensation of 2-cyanobenzothiazoles with the commercially available ethylenediamine in various solvents (*e.g.* ethanol, THF) was studied to give the desired imidazolines **13**–**17** (Scheme 4).

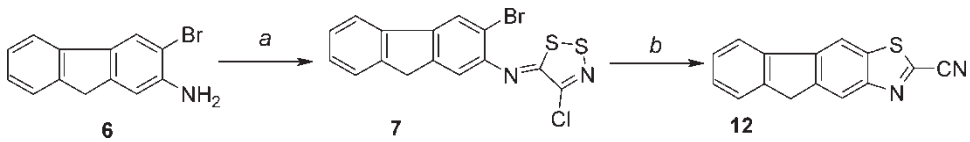
For all the prepared compounds, we expected that the basic side chain may give cationic molecules with a better water solubility and an important impact on their biological properties (*e.g.* for DNA binding ability). Our best candidate **8** which exhibits a good cytotoxicity and is easily prepared in good yield was modified into the amidines **18**, **23** by treatment of carbonitrile **8** with the appropriated amines. Amidates **21**, **22** were obtained in good yields from derivative **8** on reflux in alcohol in the presence of 1 equivalent of NaOH 2.5 N. Treatment of compound **8** with *N*,*N*-diethyldiethylenetriamine led to the *N*-alkylated imidazoline **20** in modest yield (39%). Diaminobenzene yielded 45% of the benzimidazole **19** (Scheme 5).[20,21]

### *In Vitro* Cytotoxic Activity

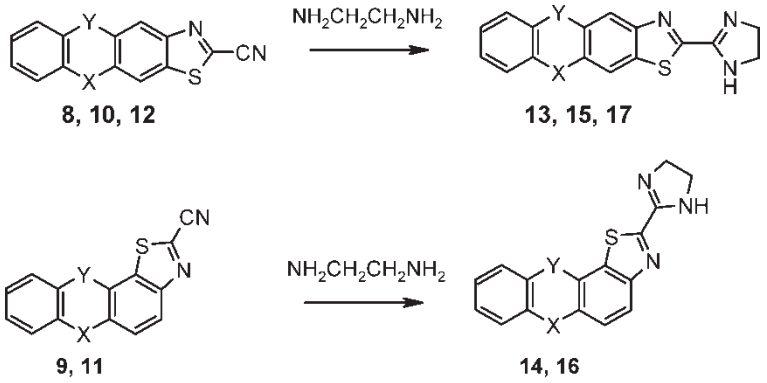
*In vitro* cytotoxic activity of sixteen compounds described in this paper was assessed using the murine L1210 leukaemia cell line. The results expressed as the $IC_{50}$ value (concentration reducing cell proliferation by 50%) are given in Tables I–III. Cell cycle perturbations induced by the most active compounds ($IC_{50} < 10\,\mu M$) were also investigated.

The 2-cyanothiazolocarbazoles **8**–**12** reported in Table I were first evaluated. Linear thiazolofluorenones **8** and **10**, with an inversed thiazole ring exhibited a similar activity. Compounds **8** and **10** were found practically equipotent on cell proliferation inhibition with $IC_{50}$'s of 6.8 μM and 8.7 μM, respectively. Also, both were found to partially block the cells in the $G_1$ phase of the cell cycle. With $IC_{50}$ of 21.4 μM, the angular derivative **9** appeared less active than the linear isomer **8**. All oxo derivatives **8**, **9**, **11** were more cytotoxic than the fluorene **12** with $IC_{50} > 10\,\mu M$. It must be noted that the cyano anthraquinone possessing two carbonyl functions **11** with $IC_{50}$ of 3.4 μM was found to block partially the cells in the $G_2$ phase of the cell cycle.

The nature of the substituents fixed at the C-2 position of the thiazole ring seems to exert a significant influence on the cytotoxic activity. Comparison of the data presented in Tables II and III showed that the replacement of the 2-cyano substituent by an imidazoline group led to more active derivatives. In all cases, compounds **13**–**17** with $IC_{50} < 4\,\mu M$ were more cytotoxic than their cyano counterparts **8**–**12**. Except for the fluorene derivative **17** which was devoid of any specific effect

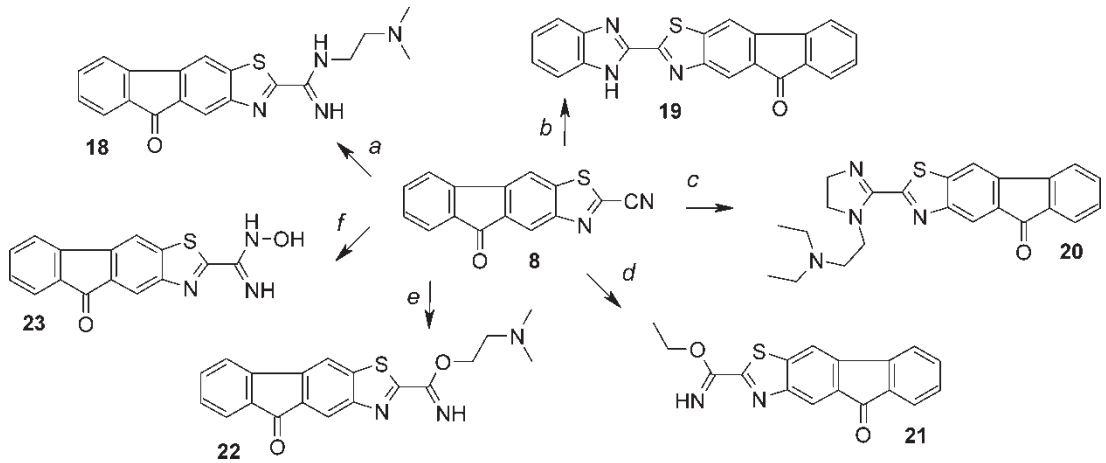



SCHEME 3 Synthesis of thiazolofluorene **12**. Reaction conditions and yields: (a) 4,5-dichloro-1,2,3-dithiazolium chloride **1**, pyridine, r.t., 76%. (b) CuI, pyridine, 105°C, 15 min., μW, 52%.




| Starting material | X | Y | Compound | Yield % |
|---|---|---|---|---|
| **8** | - | C=O | **13** | 98 |
| **9** | - | C=O | **14** | 98 |
| **10** | C=O | - | **15** | 98 |
| **11** | C=O | C=O | **16** | 98 |
| **12** | - | $CH_2$ | **17** | 80 |

SCHEME 4 Synthesis of imidazolines **13**–**17**.




SCHEME 5 Variation in position-2 of thiazolofluorenone **8**. Reaction conditions and yields: (a) *N*,*N*-dimethylethylenediamine, ethanol, reflux, 2 h, 85%. (b) 2-aminoaniline, ethanol, reflux, 24 h, 45%. (c) *N*,*N*-diethyldiethylenetriamine, ethanol, reflux, 18 h, 39%. (d) NaOH 2.5 N, ethanol, r.t., 2 h, 87%. (e) NaOH 2.5, *N*-dimethylaminoethanol, THF, r.t., 2 h, 67%. (f) hydroxylamine hydrochloride, AcONa, ethanol, reflux, 4 h, 50%.

TABLE I Cytotoxicity of carbonitriles **8**–**12**

| Compound | Formula | Cytotoxicity $IC_{50}$ L1210 (μM) | % of L1210 cells in G1/S/G2 + M phases[a] (μM) |
|---|---|---|---|
| **8** | $C_{15}H_6N_2OS$ | 6.8 | 56/26/18 (50) |
| **9** | $C_{15}H_6N_2OS$ | 21.4 | ne[b] |
| **10** | $C_{15}H_6N_2OS$ | 8.7 | 56/23/21 (50) |
| **11** | $C_{16}H_6N_2O_2S$ | 3.4 | 35/26/39 (5) |
| **12** | $C_{15}H_8N_2S$ | >10 | ne[b] |

[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).

TABLE II Cytotoxicity of imidazolines **13**–**17**

| Compound | Formula | Cytotoxicity $IC_{50}$ L1210 (μM) | % of L1210 cells in G1/S/G2 + M phases[a] (μM) |
|---|---|---|---|
| **13** | $C_{17}H_{12}N_3OS$ | 1.7 | 12/10/78 (10) |
| **14** | $C_{17}H_{11}N_3OS$ | 3.6 | 27/22/51 (5–20) |
| **15** | $C_{17}H_{12}N_3OS$ | 1.9 | 8/14/50/28[c] (10) |
| **16** | $C_{18}H_{11}N_3O_2S$ | 2.5 | 19/2/59 (20) |
| **17** | $C_{17}H_{13}N_3S$ | 2.7 | ns[b] |

[a] % of untreated control cells in the phases of the cell cycle: 41% ($G_1$); 28% (S); 24% ($G_2$ + M); 1% (8N). [b] ns = non specific, toxic at 25 μM. [c] 28% 8 N.

TABLE III Cytotoxicity of fluorenone derivatives **18–23**

| Compound | Formula | Cytotoxicity $IC_{50}$ L1210 (μM) | % of L1210 cells in G1/S/G2 + M phases[a] (μM) |
|---|---|---|---|
| **13** | $C_{17}H_{12}N_3OS$ | 1.7 | 12/10/78 (10) |
| **18** | $C_{19}H_{19}N_4OS$ | 2.1 | ns[c] |
| **19** | $C_{21}H_{11}N_3OS$ | 2 | ns[c] |
| **20** | $C_{23}H_{24}N_4OS$ | 2.6 | 12/19/69 (10) |
| **21** | $C_{17}H_{12}N_2O_2S$ | >10 | ne[b] |
| **22** | $C_{19}H_{17}N_3O_2S$ | >10 | ne[b] |
| **23** | $C_{15}H_9N_3O_2S$ | 1.3 | ns[c] |

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

on the cell phase, all imidazoline derivatives **13–16** were able to block the cells in the $G_2$ phase of the cell cycle (Table II). Evaluation of the angular fluorenone **14** showed again a lower activity than for their linear isomers **13**, **15** which were equipotent on inhibition of cell proliferation with $IC_{50}$'s of 1.7 μM and 1.9 μM, respectively. However, **13** induced a massive accumulation of L1210 cells in the G2M phase, and **15** was able to induce polyploïdy.

The amidines **18**, **19**, and **23** with $IC_{50}$'s of 2.1 μM, 2 μM and 1.3 μM, respectively, exhibited a similar activity to that of **13** but were unfortunately devoid of any specificity on the cell phase when evaluated. Imidates **21**, **22** were found relatively less cytotoxic than compound **13** with $IC_{50}$'s > 10 μM. Whatever the nature of the amino basic side chain on C-2, the imidazoline or *N*-alkylated imidazoline **13**, **20** derivatives remained the best cytotoxic candidates. Introduction of a cationic side chain on the imidazoline (compound **20**) did not enhance the activity compared to the imidazoline **13**. Both exhibited a similar $IC_{50}$'s of 1.7 μM and 2.6 μM respectively and showed an equal specific effect by blocking, at 10 μM, 78% and 69% of cells in the $G_2$ + M phases, respectively (Table III).

In conclusion, we have described the synthesis of novel thiazolofluorenones, which exhibit interesting *in vitro* cytotoxic activity. Introduction of an imidazoline group on position-2 of the thiazolo ring enhanced the cytotoxic activity of these compounds with a specific effect on the cell cycle G2 phase. Our present results suggest that linear thiazolo fluorenone open the door 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] Wells, G., Bradshaw, T.D., Diana, P., Seaton, A., Shi, D.-F., Westwell, A.D. and Stevens, M.F.G. (2000) *Bioorg. Med. Chem. Lett.* **10**, 513–515.
[2] Bradshaw, T.D., Wrigley, S., Shi, D.-F., Schultz, R.J., Paull, K.D. and Stevens, M.F.G. (1998) *Br. J. Cancer* **77**, 745–752.
[3] Molinski, T.F. (1993) *Chem. Rev.* **93**, 1825–1838.
[4] Gunawardana, G.P., Kohmoto, S., Gunasekara, S.P., McConnel, O.J. and Koehn, F.E. (1988) *J. Am. Chem. Soc.* **110**, 4856–4858.
[5] Gunawardana, G.P., Kohmoto, S. and Burres, N.S. (1989) *Tetrahedron Lett.* **30**, 4359–4362.
[6] Chabane, H., Lamazzi, C., Thiéry, V., Guillaumet, G. and Besson, T. (2002) *Tetrahedron Lett.* **43**, 2483–2486.
[7] Lamazzi, C., Chabane, H., Thiéry, V., Pierre, A., Léonce, S., Pfeiffer, B., Renard, P., Guillaumet, G. and Besson, T. (2002) *J. Enz. Inhib. Med. Chem.* **17**, 397–401.
[8] Chabane, H., Lamazzi, C., Thiéry, V., Pierre, A., Léonce, S., Pfeiffer, B., Renard, P., Guillaumet, G. and Besson, T. (2003) *J. Enz. Inhib. Med. Chem.* **18**, 167–174.
[9] Robin, M., Faure, R., Périchaud, A. and Galy, J.P. (2000) *Heterocycles* **53**, 387–395.
[10] Hanoun, J.P., Faure, R., Galy, J.P. and Elguero, J. (1996) *J. Heterocycl. Chem.* **33**, 747–750.
[11] Barbe, J., Boyer, G., Carignano, I., Elguero, J., Galy, J.P., Morel, S. and Oughedani, R. (1991) *Tetrahedron Lett.* **32**, 6709–6710.
[12] Léonce, S., Pérez, V., Casabianca-Pignède, M.R., Anstett, M., Bisagni, E. and Atassi, G. (1996) *Investig. New Drugs* **14**, 169–180.
[13] Appel, R., Janssen, H., Siray, M. and Knoch, F. (1985) *Chem. Ber.* **118**, 1632–1643.
[14] Besson, T., Rees, C.W., Roe, D. and Thiéry, V. (2000) *J. Chem. Soc., Perkin Trans.* **1**, 555–561.
[15] Besson, T., Rees, C.W. and Thiéry, V. (1999) *Synthesis*, 1345–1348.
[16] Besson, T., Guillaumet, G., Lamazzi, C., Rees, C.W. and Thiéry, V. (1998) *J. Chem. Soc., Perkin Trans.* **1**, 4057–4059.
[17] Bénéteau, V., Besson, T. and Rees, C.W. (1997) *Synth. Commun.* **27**, 2275–2280.
[18] Frère, S., Thiéry, V. and Besson, T. (2003) *Synth. Commun.* **33**, 3789–3798.
[19] Frère, S., Thiéry, V., Bailly, C. and Besson, T. (2003) *Tetrahedron* **59**, 773–779.
[20] Bénéteau, V., Besson, T., Guillard, J., Léonce, S. and Pfeiffer, B. (1999) *Eur. J. Med. Chem.* **34**, 1053–1060.
[21] Besson, T., Dozias, M.J., Guillard, J. and Rees, C.W. (1998) *J. Chem. Soc., Perkin Trans.* **1**, 3925–3926.