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Original article

Synthesis and in vitro antitumour evaluation of benzothiazole-2-carbonitrile derivatives

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Abstract – Novel benzothiazole derivatives have been synthesised via the corresponding imino-1,2,3-dithiazoles. The cytotoxicity of some of these polyheterocyclic compounds was studied. Our results show that 2-cyano derivatives exhibit a medium in vitro antitumour activity.

imino-1,2,3-dithiazoles / antitumour activity / benzothiazoles / benzodioxines

1. Introduction

The benzothiazole ring is present in various marine or terrestrial natural compounds which have useful biological activities [1–4]. Because we are interested in heterocyclic systems with potential pharmacological value, we decided to synthesise new benzothiazole derivatives which are related to synthetic thiazoles which have shown antitumour activity [5]. Novel dioxinobenzothiazoles were also prepared with the aim to enhance the antiproliferative activity.

In this paper, we describe the biological evaluation of 4,7-dimethoxybenzothiazoles and dioxinobenzothiazoles prepared via *N*-arylimino-1,2,3-dithiazoles which have proved to be highly versatile intermediates in heterocyclic chemistry [6–8].

2. Chemistry

2.1. 4,7-Dimethoxybenzothiazoles

4,5-Dichloro-1,2,3-dithiazolium chloride **1** is a pale greenish yellow solid, insoluble in organic solvents. It is completely stable in a dry inert atmosphere but reacts slowly with moisture to form 4-chloro-1,2,3-dithiazol-5-one. This compound is readily prepared from chloroacetonitrile and disulfur dichloride [9], reacts rapidly with 2,5-dimethoxy-aniline, in dichloromethane at room temperature, to give the stable *N*-arylimino-1,2,3-dithiazoles **2** in high yield (84%). Pyrolysis of these imines gave 2-cyanobenzothiazoles **3** by cyclisation of the *ortho* carbon onto sulfur with liberation of the other sulfur atom and hydrogen chloride [10] (*figure 1*).

Removal (hydrolysis and decarboxylation) of the cyano group in the thiazole ring was performed by vigorous heating of compound **3a** in concentrated hydrochloric acid. The decyanated benzothiazole **4** was isolated in good yield (70%) (*figure 2*). Using standard conditions for the transformation of cyano groups into carboxylic acid or amido groups [11], the starting thiazole **3a** was treated with aqueous sodium hydroxide to give the acid **5**, or with sulfuric acid to provide the amide **6**, in quite good yields. The substituted amide **7** was prepared by treatment of the acid **5** 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 (*figure 2*).

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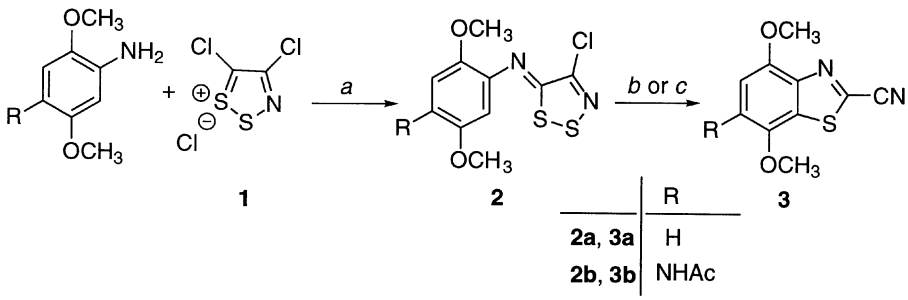

Figure 1. Reactions and conditions: a) pyridine, dichloromethane, –15 °C, 3 h (**2a**, 40%) or room temperature, 3 h (**2b**, 84%); b) toluene, sealed tube, reflux, 4 h (**3a**, 70%); c) $PyHBr_3$, pyridine, reflux, 1.5 h (**3b**, 72%).

Decyanation of compound **3b**, using the conditions described above, afforded the amino decyanated benzothiazole **8**, in which the amino group was easily reprotected, to give **9**, by treatment with acetic anhydride in the presence of pyridine. The quinone **10** was obtained by oxidation of **3b** with cerium ammonium nitrate (*figure 3*).

Starting from the commercially available isothiocyanate **11**, the 2-alkoxyderivative **13** was synthesised by a Jacobson process via the intermediate thiocarbamate **12** (*figure 4*). In this case, an electron releasing group is present in the 2-position of the benzothiazole ring.

2.2. Dioxinobenzothiazoles [12]

The chemistry of the salt **1** described above also allows a rapid access to the dioxinobenzothiazoles **15–18**, from the starting 6-amino-2,3-dihydro-1,4-benzodioxin (*figure 5*). The bromination (NBS, CCl_4, AIBN)–debromination (NaI, acetone) sequence previously described in several syntheses of benzodioxins [13], lead to the expected products **19** and **20** (*figure 5*), whilst heating of the brominated intermediate **21** in pyridine, in the presence of one equivalent of copper iodide (CuI), allowed an alternative access to the linear compound **15** (the use of the standard method afforded the angular brominated derivative **22**) (*figure 6*).

3. Pharmacology

Fifteen compounds were evaluated in vitro for their antiproliferative activity using the murine L1210 leukaemia cell line [14]. The results expressed as IC_{50}

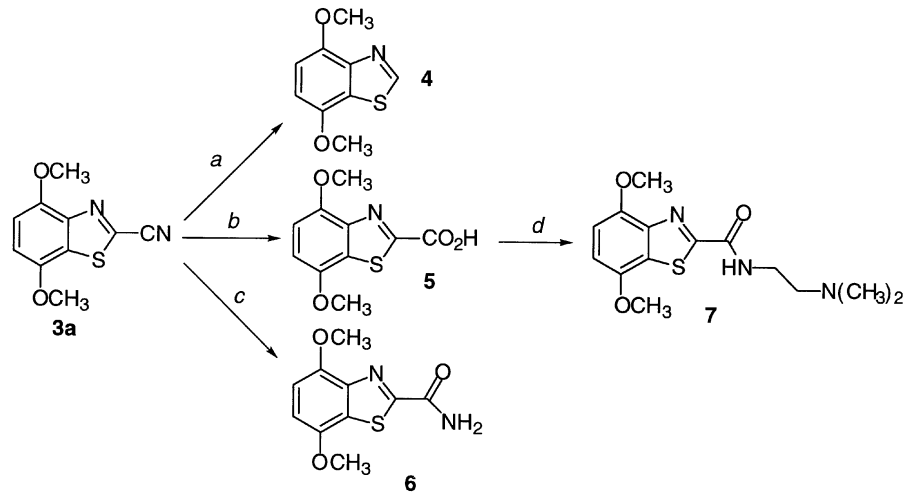

Figure 2. Reactions and conditions: a) conc. HCl, reflux, 8 h, 70%; b) NaOH 10%, 80 °C, 3.5 h, 64%; c) conc. H_2SO_4, room temperature, 2 h, 72%; d) *N*,*N*-dimethylethylenediamine, EDCI, HOBT, DMF, 0 °C, 4 days, 43%.

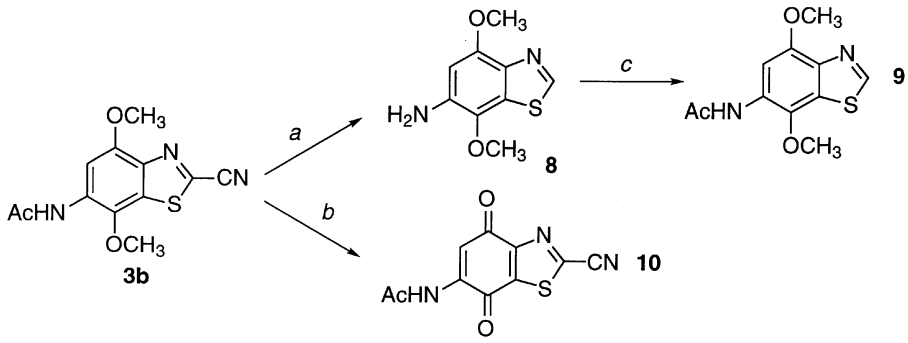

Figure 3. Reactions and conditions: a) conc. HCl, reflux, 1.5 h, 83%; b) CAN, CH_3CN, H_2O, room temperature, 15 min, 56%; c) Ac_2O, pyridine, room temperature, 12 h, 56%.

(concentration reducing the cell proliferation by 50%) are reported in *table I*. Cell cycle perturbations induced by the most active compounds (IC_{50} < 20 μM) were also investigated.

4. Results and discussion

The 2-cyano-4,7-dimethoxybenzothiazole derivatives **3a** and **3b** were first evaluated and found practically

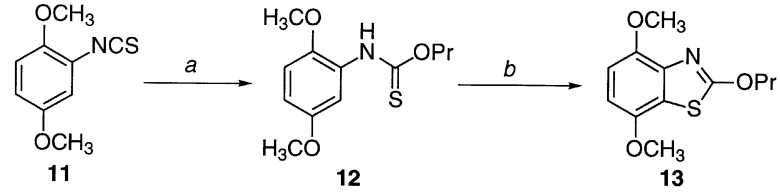

Figure 4. Reactions and conditions: a) PrOH, NaH, 83%; b) K_3FeCN_6, NaOH, 28%.

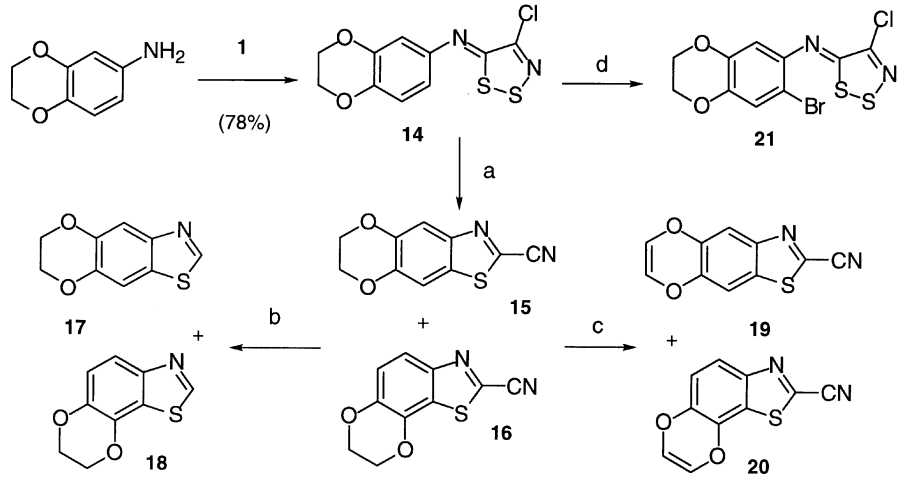

Figure 5. Reagents and conditions: a) $PyHBr_3$ (1 eq.), DMF/pyridine, reflux, 90 min, 58% (29% for **15** and 29% for **16**); b): HCl, reflux, 2.5 h, 80% (**17**) and 65% (**18**); c) i) NBS/AIBN, CCl_4, hν, reflux, 10 h; ii) NaI, acetone, reflux, 1.5 h, 69% (**19**) and 27% (**20**); d) Br_2 (1 eq.), CH_3COOH, room temperature, 2 h, 98%.

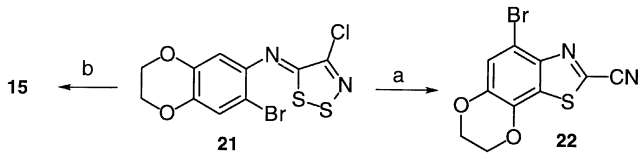

Figure 6. Reagents and conditions: a) $PyHBr_3$ (1 eq.), DMF/pyridine, reflux, 2 h, 25%; b) CuI, pyridine, reflux, 90 min, 55%.

equipotent on cell proliferation with IC_{50}'s of, respectively, 20.6 μM and 25.2 μM. Also, both were found to be able to almost totally block the cells in the G_2 + M phase of the cell cycle. Suppression of the 2-cyano substituent (compounds **4** and **9**) or its replacement by a 2-carboxy (compound **5**), a 2-aminocarbonyl (compound **6**), a 2-[2-(*N*,*N*-dimethylamino)ethylaminocarbonyl] (compound **7**) or a 2-propoxy (compound **13**) substituent led to inactive derivatives. Compound **10** which is a quinone derivative was found significantly more active that its 4,7-dimethoxy counterpart **3b** with an IC_{50} of 5μM versus 25.2 μM. This compound was unfortunately devoid of any specific effect on the cell cycle.

All the 2-cyano-dioxinobenzothiazoles have also shown a relatively interesting antiproliferative activity. As for the 4,7-dimethoxybenzothiazoles, removal of the cyano substituent present in the 2-position of the dioxinobenzothiazole ring (compound **17**) involved the lost of any activity (IC_{50} > 100 μM). The unsaturated compounds **19** and **20** were found significantly more active than their saturated conterparts **15** and **18**, with IC_{50}'s of, respectively, 18.2 μM and 31.7 μM, confirming the results already published on dioxinocoumarins [15, 16]. The dioxinobenzothiazole **19** was found able to block L1210 cells in the G_2 + M phase of the cell cycle.

5. Conclusion

In conclusion, we have described the synthesis of novel benzothiazoles and dioxinobenzothiazoles, among which the 2-cyano derivatives exhibit interesting in vitro antitumour activity. Presence of unsaturation in the dioxin moiety, in combination with the cyano group in the 2-position of the thiazole ring, did not really involve any specific effect on the cell cycle. Our results suggest that introduction of the thiazolo-2-carbonitrile ring into more extended and more complexe heterocyclic moieties could open the door to promising applications.

6. Experimental protocols

6.1. Chemistry

Melting points were determined using a Kofler banc and are uncorrected. IR spectra were recorded on a Perkin-Elmer Paragon 1000PC instrument. ^{1}H- and ^{13}C-NMR were recorded on a JEOL JNM LA400 (400 Mhz) spectrometer (Centre Commun d'Analyse, Université de La Rochelle); chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Mass spectra were recorded on a Varian MAT311 in the Centre de Mesure Physiques de L'Ouest (C.R.M.P.O.), Université de Rennes. Chromatography was carried out on silica gel 60

Table I. Characteristics and pharmacological activity of synthesised compounds.

Compound	M.p. (°C)	Formula	IC_{50} (μM)	% of L1210 cells in the G_2 + M phase[a] (μM)
3a	174	$C_{10}H_8N_2O_2S$	20.6	64% (50)
3b	173	$C_{12}H_{11}N_3O_3S$	25.2	77% (100)
4	108	$C_9H_9NO_2S$	> 100	n.e.[b]
5	> 230	$C_{10}H_9NO_4S$	> 100	n.e.
6	240	$C_{10}H_{10}N_2O_3S$	> 100	n.e.
7	144	$C_{14}H_{19}N_3O_3S$	> 100	n.e.
9	154	$C_{11}H_{12}N_2O_3S$	> 100	n.e.
10	242	$C_{10}H_5N_3O_3S$	5	non specific
13	73	$C_{12}H_{15}NO_3S$	> 100	n.e.
15	178	$C_{10}H_6N_2O_2S$	58.4	n.e.[b]
16	156	$C_{10}H_6N_2O_2S$	42.3	n.e.
17	142	$C_9H_7NO_2S$	> 100	n.e.
19	176	$C_{10}H_4N_2O_2S$	18.2	41% (50)
20	150	$C_{10}H_4N_2O_2S$	31.7	n.e.
22	250	$C_{10}H_5BrN_2O_2S$	80.8	n. e.

[a]24% of untreated control cells were in the G_2 + M phase of the cell cycle. [b]n.e.: not evaluated (for IC_{50} > 30 μM).

at medium pressure and the sample mixtures were applied to the column preadsorbed onto silica. Light petroleum refers to the fraction b.p. 40–60 °C. Further solvents were used without purification. Thin-layer chromatography was performed on Merck Kieselgel 60 F_{254} aluminium backed plates.

Spectral data for compounds **14**–**22** are consistent with assigned structures as previously described in ref. [12].

6.1.1. 2,5-Dimethoxy-N-(4-chloro-5H-dithiazol-5-ylidene)aniline **2a**

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride (15.42 g, 73.7 mmol) and pyridine (10.8 mL, 134 mmol) were added to a stirred solution of 2,5-dimethoxyaniline (10.3 g, 67 mmol) in dichloromethane (150 mL) cooled at –15 °C. After 2 h, the mixture was filtered and the solvent removed in vacuo. The crude residue was purified by column chromatography (eluent: light petroleum/ethyl acetate: 9/1) to afford compound **2a** as an orange oil (7.8 g, 40%); IR (film): ν 2 938 and 2 833 (CH_3), 1 586 (C=N), 1 498, 1 464, 1 278, 1 223, 1 195, 1 165, 1 044 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.78 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.69 (d, 1H, *J* = 2.9 Hz, 6-H), 6.75 (dd, 1H, *J* = 2.9 Hz and *J′* = 8.8 Hz, 4-H), 6.93 (d, 1H, *J′* = 8.8 Hz, 3-H); ^{13}C-NMR ($CDCl_3$): δ 56.65, 57.21, 106.11, 112.91, 114.43, 141.71, 144.68, 148.48, 154.97, 161.02; MS (EI): *m/z* 288 (M^+), 188, 148; HRMS: calc. for $C_{10}H_9ClN_2O_2S_2$: 287.9794, found: 287.9801.

6.1.2. 4-Acetamido-2,5-dimethoxy-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene) aniline **2b**

Under an inert atmosphere, 4,5-dichloro-1,2,3-dithiazolium chloride (220 mg, 1.04 mmol) was added to a stirred solution of 4-acetamido-2,5-dimethoxyaniline (200 mg, 0.95 mmol) in dichloromethane (10 mL). After 1 h, pyridine (110 mg, 1.39 mmol) was added and the mixture stirred for 15 min. The solvent was removed in vacuo and the crude residue purified by column chromatography (eluent: dichloromethane/ethyl acetate: 4/1) to afford compound **2b** (275 mg, 84%) as an orange powder; m.p. 155 °C; IR (KBr): ν 3 506 (NH), 1 671 (C=O), 1 483, 1 402, 1 214, 1 041, 862 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.22 (s, 3H, $NHCOCH_3$), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.72 (s, 1H, 6-H), 7.80 (s, 1H, NH), 8.28 (s, 1H, 3-H); ^{13}C-NMR ($CDCl_3$): δ 25.01, 56.20, 56.24, 102.98, 104.85, 126.62, 133.91, 141.64, 143.81, 147.80, 158.88, 168.24; MS (EI): *m/z* 345 (M^+), 252 (M–CNSCl), 195; HRMS: calc. for $C_{12}H_{12}ClN_3O_3S_2$: 345.0009, found: 345.0016.

6.1.3. 4,7-Dimethoxybenzothiazole-2-carbonitrile **3a**

In a closed system, a solution of **2a** (871 mg, 3.02 mmol) in toluene (5 mL) was heated in an oil bath at 200–210 °C for 8 h. After cooling, the toluene was removed in vacuo. The crude residue was purified by column chromatography (eluent: dichloromethane/light petroleum: 6/4) and recrystallised from ethanol to afford **3a** (471 mg, 70%) as yellow needles; m.p. 174 °C; IR (KBr): ν 2 229 (nitrile), 1 595 (C=N), 1 501, 1 454, 1 280, 1 140, 1 094, 1 046, 969 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.98 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.93 (s, 2H, 5-H and 6-H); ^{13}C-NMR ($CDCl_3$): δ 56.36, 56.49, 108.10, 108.44, 112.97, 126.32, 135.55, 143.75, 147.59, 148.91; MS (EI): *m/z* 220 (M^+), 205 (M^+–CH_3), 191; HRMS: calc. for $C_{10}H_8N_2O_2S$: 220.0306, found: 220.0303.

6.1.4. 6-Acetamido-4,7-dimethoxybenzothiazole-2-carbonitrile **3b**

Under an inert atmosphere, a mixture of **2b** (1.60 g, 4.63 mmol) and pyridinium perbromide (1.63 g, 5.10 mmol) in pyridine (20 mL) was heated at reflux for 2.5 h. After cooling, pyridine was removed in vacuo and the crude residue purified by column chromatography (eluent: dichloromethane/ethyl acetate: 95/5). Recrystallisation from ethanol afforded compound **3b** (927 mg, 72%) as yellow needles; m.p. 173 °C; IR (KBr): ν 3 390 (NH), 2 226 (nitrile), 1 683 (C=O), 1 600 (C=N), 1 523, 1 448, 1 420, 1 240, 1 132, 1 050 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.30 (s, 3H, $NHCOCH_3$), 3.96 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 7.86 (bs, 1H, NH), 8.34 (s, 1H, 5-H); ^{13}C-NMR ($CDCl_3$): δ 25.20, 56.68, 59.88, 101.65, 112.78, 128.05, 132.37, 132.79, 134.56, 139.33, 151.25, 168.62; MS (EI): *m/z* 277 (M^+), 220 (M^+–$CNOCH_3$); HRMS: calc. for $C_{12}H_{11}N_3O_3S$: 277.0521, found: 277.0524.

6.1.5. 4,7-Dimethoxybenzothiazole **4**

A suspension of compound **3a** in concentrated hydrochloric acid (20 mL) was heated at reflux for 8 h. The mixture was cooled at 0 °C, neutralised to pH 8 using saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. The combined extracts were dried over $MgSO_4$ and the solvent removed in vacuo. After recrystallisation from hexane, compound **4** (177 mg, 70%) was obtained as colourless needles; m.p. 108 °C; IR (KBr): ν 3 074 ($CH_{unsat.}$), 2 959 (CH_3), 1 594 (C=N), 1 500, 1 456, 1 439, 1 345, 1 263, 1 184, 1 150 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.97 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.78 (d, 1H, *J* = 8.6 Hz, $H_{arom.}$), 6.86 (d, 1H, *J* = 8.6 Hz, $H_{arom.}$), 8.91 (s, 1H, 2-H); ^{13}C-NMR ($CDCl_3$): δ 55.29, 55.63, 104.81, 106.39, 123.95, 144.11,

147.59, 152.49, 152.53; MS (EI): *m/z* 195 (M^+), 180 (M^+–CH_3), 166; HRMS: calc. for $C_9H_9NO_2S$: 195.0354, found: 195.0361.

6.1.6. 4,7-Dimethoxybenzothiaole-2-carboxylic acid **5**

A suspension of compound **3a** (204 mg, 0.93 mmol) in 10% aqueous sodium hydroxide (10 mL) was heated at 80 °C for 3.5 h. After cooling to room temperature, the mixture was poured onto iced water and acidified to pH 1 using 10% aqueous hydrochloric acid. The yellow precipitate that separated was filtered under vacuum and dried. The product was purified by column chromatography (eluent: hexane/ethyl acetate/methanol: 1/1/0.5 then methanol). Recrystallisation from water afforded compound **5** (141 mg, 64%) as yellow needles; m.p. 230 °C (dec); IR (KBr): ν 3 367 (broad: OH), 2 838 (CH_3), 1 632 (C=O), 1 499, 1 383, 1 263, 1 189, 1 097, 1 044, 974 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.90 (s, 2H, $H_{arom.}$); ^{13}C-NMR (DMSO-d_6): δ 55.92, 56.11, 106.04, 107.53, 126.56, 144.48, 147.54, 148.19, 162.07, 169.85; MS (EI): *m/z* 209 (M–CH_2O), 195 (M–CO_2), 180 (M–CO_2, CH_3), 166; HRMS could not be measured due to the absence of the molecular pic.

6.1.7. 4,7-Dimethoxybenzothiazole-2-carboxamide **6**

A solution of compound **3a** (703 mg, 3.19 mmol) in concentrated sulfuric acid (5 mL) was stirred at room temperature for 2 h. After cooling at 0 °C, the mixture was basified using 10% aqueous sodium hydroxide and then extracted with ethyl acetate. The combined extracts were dried over $MgSO_4$. Removal of the solvent in vacuo followed by a recrystallisation from ethanol gives compound **6** (548 mg, 72%) as amber needles; m.p. 240 °C; IR (KBr): ν 3 406 (NH), 1 682 (C=O), 1 600 (C=N), 1 558, 1 505, 1 118, 799 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.97 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 5.73 (bs, 1H, NH), 6.84 (d, 1H, *J* = 8.6 Hz, $H_{arom.}$), 6.89 (d, 1H, *J* = 8.6 Hz, $H_{arom.}$), 7.41 (bs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 55.98, 56.15, 107.50, 108.33, 126.58, 143.89, 147.44, 148.33, 161.17, 163.60; MS (EI): *m/z* 238 (M^+), 223 (M^+–CH_3), 209, 192, 180; HRMS: calc. for $C_{10}H_{10}N_2O_3S$: 238.0412, found: 238.0415.

6.1.8. 4,7-Dimethoxybenzothiazole-2-[2-(N,N-dimethylamino)ethyl]carboxamide **7**

Under an inert atmosphere, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (1.22 g, 6.47 mmol), 1-hydroxybenzotriazole (840 mg, 6.47 mmol) and *N*,*N*-dimethylethylenediamine (0.7 mL, 6.47 mmol) were added to a stirred solution of compound **5** (703 mg, 2.94 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred for 4 days, allowing the temperature to increase slowly. Water was then added with cooling and the crude material extracted with ethyl acetate. The combined extracts were washed with water (three times) and dried over $MgSO_4$. After removal of the solvent in vacuo, the product was purified by column chromatography (eluent: dichloromethane/methanol: 95/5) and recrystallised from hexane/ethanol to afford compound **7** (393 mg, 43%) as pale yellow needles; m.p. 144 °C; IR (KBr): ν 3 168 (NH), 2 950 (CH_3), 2 824 (CH_2), 1 662 (C=O), 1 540, 1 276, 1 188, 1 143, 1 090, 1 045 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.24 (s, 6H, $N(CH_3)_2$), 2.50 (t, 2H, *J* = 6.25 Hz, CH_2–N), 3.54 (q, 2H, *J* = 6.25 Hz, NH–CH_2), 3.91 (s, 3H, OCH_3), 3.99 (s, 3H OCH_3), 6.77 (d, 1H, *J* = 8.7 Hz, $H_{arom.}$), 6.82 (d, 1H, *J* = 8.7 Hz, $H_{arom.}$), 7.73 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$): δ 37.47, 45.29 (×2), 56.05, 56.34, 57.86, 106.40, 107.07, 128.11, 144.29, 148.29, 148.39, 159.89, 163.77; MS (EI): *m/z* 309 (M^+), 58 ($(CH_3)_2N{=}CH_2^+$); HRMS: calc. for $C_{14}H_{19}N_3O_3S$: 309.1147, found: 309.1155.

6.1.9. 6-Amino-4,7-dimethoxybenzothiazole **8**

A solution of compound **3b** (93 mg, 0.34 mmol) in concentrated hydrochloric acid (7 mL) was heated at reflux for 1.5 h. After cooling at room temperature, the mixture was basified to pH 8 using saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. The combined extracts were washed with water and brine, and dried over $MgSO_4$. The solvent was removed in vacuo and the crude residue was purified by column chromatography (eluent: light petroleum/ethyl acetate: 1/1) to afford compound **8** (59 mg, 83%) as a brown powder; m.p. 153 °C; IR (KBr): ν 3 440/3 310 (NH_2), 1 614 (C=N), 1 500, 1 463, 1 398, 1 237, 1 043, 987 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.86 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.40 (s, 1H, 5-H), 8.60 (s, 1H, 2-H); ^{13}C-NMR ($CDCl_3$): δ 56.09, 58.75, 98.02, 128.61, 133.24, 137.35, 137.48, 148.11, 150.38; MS (EI): *m/z* 210 (M^+), 195 (M^+–CH_3), 154 (M^+–C_3H_4O).

6.1.10. 6-Acetamido-4,7-dimethoxybenzothiazole **9**

Acetic anhydride (2.2 mL, 23.33 mmol) was added to a stirred solution of **8** (265 mg, 1.26 mmol) in pyridine (15 mL). The mixture was strirred overnight at room temperature. Then, water was added, the product was extracted with ethyl acetate, purified by column chromatography (eluent: ethyl acetate/light petroleum: 7/3) and recrystallised from dichloromethane/light petroleum to afford compound **9** (179 mg, 56%) as pale yellow needles; m.p. 154 °C; IR (KBr): ν 3 296 (NH), 2 963, 1 668 (C=O), 1 606 (C=N), 1 532, 1 463, 1 386, 1 254, 1 222, 1 042 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.25 (s, 3H, $NHCOCH_3$), 3.93 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3),

7.78 (bs, 1H, NH), 8.18 (s, 1H, 5-H), 8.79 (s, 1H, 2-H); ^{13}C-NMR ($CDCl_3$): δ 25.09, 56.35, 59.74, 100.80, 126.26, 128.94, 135.58, 140.60, 149.89, 150.81, 168.42; MS (EI): *m/z* 252 (M^+), 237 (M^+–CH_3), 195; HRMS: calc. for $C_{11}H_{12}N_2O_3S$: 252.0569, found: 252.0567.

6.1.11. 6-Acetamido-2-cyano-4,7-dioxobenzothiazole **10**

A solution of cerium ammonium nitrate (2.59 g, 4.72 mmol) in water (10 mL) was added to a stirred solution of **3b** (524 mg, 1.89 mmol) in acetonitrile (20 mL) over 10 min. After 15 min the mixture was extracted with ethyl acetate, the combined extracts were dried over $MgSO_4$ and the solvents removed in vacuo. The crude residue was purified by column chromatography (eluent: dichloromethane/ethyl acetate: 95/5) to afford compound **10** (261 mg, 56%) as orange needles; m.p. 242 °C; IR (KBr): ν 3 266 (NH), 3 108 ($CH_{unsat.}$), 2 238 (nitrile), 1 708 (C=O), 1 522, 1 427, 1 331, 1 194, 1 141, 1 014 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.32 (s, 3H, $NHCOCH_3$), 7.94 (s, 1H, 5-H), 8.13 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$): δ 25.04, 111.15, 115.47, 138.29, 140.03, 142.88, 153.75, 169.02, 175.34, 178.42; MS (EI): *m/z* 247 (M^+), 205; HRMS: calc. for $C_{10}H_5N_3O_3S$: 247.0052, found: 247.0046.

6.1.12. 4,7-Dimethoxy-2-propyloxybenzothiazole **13**

A mixture of **12** (698 mg, 2.73 mmol) and 30% aqueous sodium hydroxide (2.9 mL, 21.84 mmol) in ethanol (3 mL) was added dropwise to a solution of K_3FeCN_6 (3.6 g, 10.62 mmol) in water (5 mL) heated at 85 °C. After 1.5 h, the mixture was allowed to cool to room temperature and extracted with dichloromethane. The combined extracts were dried over $MgSO_4$ and the solvents removed in vacuo. The crude residue was purified by column chromatography (eluent: light petroleum/ethyl acetate: 7/1). Recrystallisation from hexane afforded compound **13** (191 mg, 28%) as colourless needles; m.p. 73 °C; IR (KBr): ν 2 964 and 2 833 (CH_2/CH_3), 1 534, 1 500, 1 467, 1 382, 1 340, 1 262, 1 097, 1 051 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.05 (t, 3H, J = 7 Hz, CH_3), 1.86 (sextuplet, 2H, J = 7 Hz, CH_2), 3.89 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.57 (t, 2H, J = 7 Hz, CH_2), 6.63 (d, 1H, J = 8.5 Hz, $H_{arom.}$), 6.77 (d, 1H, J = 8.5 Hz, $H_{arom.}$); ^{13}C-NMR ($CDCl_3$): δ 10.29, 22.20, 55.93, 56.35, 73.68, 103.90, 107.25, 121.18, 139.71, 146.32, 147.86, 173.04; MS (EI): *m/z* 253 (M^+), 211, 196; HRMS: calc. for $C_{12}H_{15}NO_3S$: 253.0773, found: 253.0774.

6.2. Antiproliferative activity

L1210 cells (murine leukaemia) provided by the NCI, Frederik, USA were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% foetal 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 ref. [14]. Cells were exposed to graded concentrations of the compounds for 48 h and results 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 (2.5×10^5 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 an ATC3000 flow cytometer (Brucker, France) using an argon laser (Spectra-Physics) emitting 400 mW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter.

Data are displayed as linear histograms and results are expressed as the percentage of cells found is the G_2 + M phase of the cell cycle.

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References

[1] Gunawardana G.P., Kohmoto S., Gunasekara S.P., McConnel O.J., Koehn F.E., J. Am. Chem. Soc. 110 (1988) 4856–4858.

[2] Gunawardana G.P., Kohmoto S., Burres N.S., Tetrahedron Lett. 30 (1989) 4359–4362.

[3] Gunawardana G.P., Koehn F.E., Lee A.Y., Clardy J., He H.Y., Faulkner J.D., J. Org. Chem. 57 (1992) 1523–1526.

[4] Carroll A.R., Scheuer P.J., J. Org. Chem. 55 (1990) 4426–4431.

[5] Shi D.F., Bradshaw T.D., Wrigley S., McCall C.J., Lelieveld P., Fichtner I., Stevens M.F.G., J. Med. Chem. 39 (1996) 3375–3384.

[6] Rees C.W., J. Heterocycl. Chem. 29 (1992) 639–651.

[7] Besson T., Emayan K., Rees C.W., J. Chem. Soc. Perkin Trans. 1 (1995) 2097–2102.

[8] Besson T., Guillaumet G., Lamazzi C., Rees C.W., Synlett. (1997) 704–706.

[9] Appel R., Janssen H., Siray M., Knoch F., Chem. Ber. 118 (1985) 1632–1643.

[10] English R.F., Rakitin O.A., Rees C.W., Vlasova O.G., J. Chem. Soc. Perkin Trans. 1 (1997) 201–205.

[11] March J., in: Advanced Organic Chemistry, 4th edition, Wiley-Interscience Publication, 1992, p. 887.

[12] Besson T., Guillard J., Tetrahedron 55 (1999) 5139–5144. In this paper the multistep synthesis of such compounds was transposed to a focused microwave oven.

[13] Besson T., Ruiz N., Coudert G., Guillaumet G., Tetrahedron 51 (1995) 3197–3204.

[14] Léonce S., Pérez V., Casabianca-Pignède M.R., Anstett M., Bisagni E., Atassi, G., Invest. New Drugs 14 (1996) 169–180.

[15] Csik G., Besson T., Coudert G., Guillaumet G., Nocentini S., J. Photochem. Photobiol. B: Biol. 19 (1993) 119–124.

[16] Csik G., Ronto G., Nocentini S., Averbeck S., Averbeck D., Besson T., Coudert G., Guillaumet, G., J. Photochem. Photobiol. B: Biol. 24 (1994) 129–139.