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Short communication

Chiral 6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles: Novel antitumor DNA monoalkylating agents

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ABSTRACT

New chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles were synthesized and screened for their *in vitro* activity as anti-cancer agents in three human tumor cell lines, colorectal adenocarcinoma, melanoma and breast adenocarcinoma. (*R*)-6-Hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole and the corresponding benzylcarbamate showed selectivity for breast cancer cell lines with IC_{50} values of 2.4 μM and 2.2 μM, respectively. The latter also showed significant activity against colorectal adenocarcinoma cancer cell lines (IC_{50} = 8.7 μM). In contrast, the 7-hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole gave moderate anti-cancer activity. The performance against breast cancer cell lines (IC_{50} = 1.0 μM) of a potential bisalkylating agent, a (3*R*)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole, wasn't significantly different from the one observed for the monoalkylating derivatives indicating that the main mechanism of action may in fact be the monoalkylation process.

1. Introduction

DNA is the molecular target for many of the drugs that are used in cancer therapeutics. In fact, targeting the DNA of tumor cells with small molecules has been one of the most effective clinical strategies since the development of the nitrogen mustard, mechlorethamine. However, the first DNA interacting agents showed significant toxicity, which led to the search for new less toxic compounds and capable of targeting tumor DNA more specifically. Of particular interest are the minor groove binders, a group of DNA interactive agents which bind to specific regions of the genome and show significant *in vitro* and *in vivo* toxicity towards cancer cells [1–4].

Alkylating agents are minor groove binding agents that induce permanent DNA damage and often exhibit potent antitumor activity. A range of alkylating agents is known including monoalkylating (reacts only one DNA strand) and bifunctional alkylating drugs. The latter were found to crosslink the two complementary strands of DNA, which usually results in more potent and efficacious agents. Nevertheless, finding anti-cancer agents that target DNA selectively with the appropriate level of toxicity is still an important research area. The lethality of the alkylating agents results from the cell recognized of the DNA lesion.

Compounds that contain two reactive centers capable of binding covalently to DNA include thioimidazoles **1** [5,6], bis(hydroxymethyl) pyrrole derivatives **2** [7,8] and **3** [9], dihydropyrrolizine biscarbamates **4** [9–14] and **5** [14]. Studies on the mechanism of action of bifunctional electrophilic pyrroles and pyrrolizines indicate that these compounds act as DNA damaging agents via a S_N1 type reaction [10,11]. In a similar manner, it has been shown that bis(hydroxymethyl)-8*H*-3a-azacyclopenta[*a*]indene-1-yl **6a,b** and bis(hydroxymethyl)-8*H*-3a-azacyclopenta[*a*]indene-1-yl biscarbamates **6c,d** act as antitumor bifunctional DNA alkylating agents [17]. On the other hand, 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-c]thiazole biscarbamates **7**–**9** show good antileukemic activity (Fig. 1) [9,15,16].

We have been interested in the synthesis of chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives [18,19] and in the use of 2,2-dioxo-

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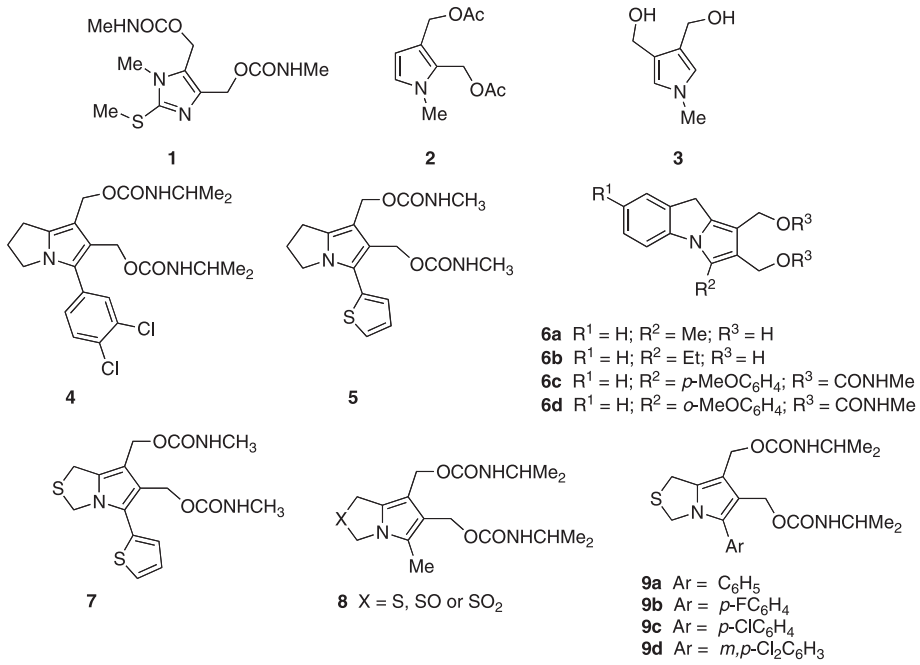


Fig. 1. Chemical structures of some compounds that contain two reactive centers capable of binding covalently to DNA.

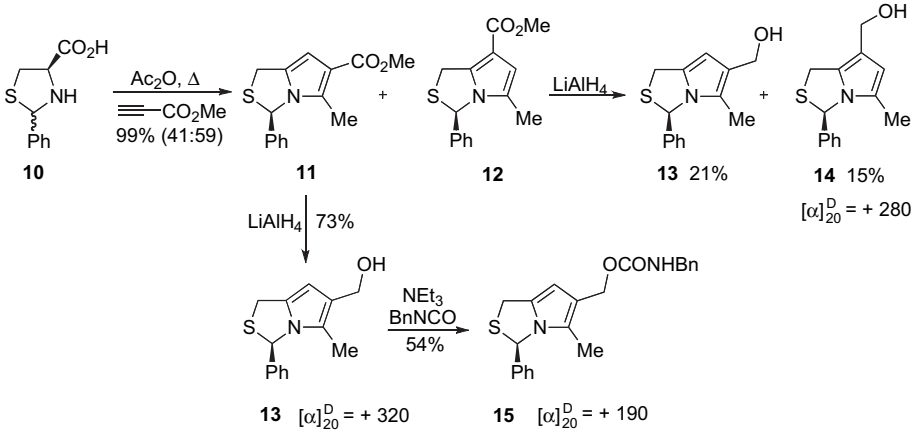
1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles and 2,2-dioxo-1*H*,3*H*-pyrrazolo[1,5-*c*]thiazoles, as precursors of new reactive intermediates (aza- and diaza-fulvenium methides), which are very versatile building blocks for the synthesis of heterocyclic compounds [20–23]. In this context, we decided to carry out the biological evaluation of new 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles as anti-cancer agents. In particular, we wonder what will be the behaviour of monoalkylating 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives, since the established idea that bisalkylating agents are required to ensure efficient activities led the study of the monofunctional derivatives to be neglected.

2. Results and discussion

2.1. Chemistry

Chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **11** and **12** were synthesized in 99% yield as a regioisomeric mixture (41:59) following a known synthetic procedure [18] (Scheme 1). The reaction proceeds via 1,3-dipolar cycloaddition of methyl propiolate with the bicyclic münchnone, generated *in situ* from thiazolidine **10**, followed by loss of carbon dioxide. The mixture of products cannot be separated by flash chromatography. However, 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **11** was isolated in pure form by selective crystallization from diethyl ether/hexane. The structural assignment of compound **11** was established by X-ray crystallography (Fig. 2). The experimental data allowed us to conclude that we were in the presence of the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole regioisomer bearing the carboxylate group at C-6 with *R* configuration assigned to the chiral centre C-3.

The reduction of (*R*)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6-carboxylate **11** was carried out with lithium aluminium hydride affording the corresponding alcohol **13** in 73% yield, which was converted into (*R*)-6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole benzylcarbamate **15** in 54% yield by reacting with benzyl isocyanate (Scheme 1). The regioisomeric alcohols 6-hydroxymethyl-pyrrolo[1,2-*c*]thiazole **13** and 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **14** were obtained by the reduction of the mixture of compounds **11** and **12** with lithium aluminium hydride and these derivatives could



Scheme 1. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **11**-**15**.

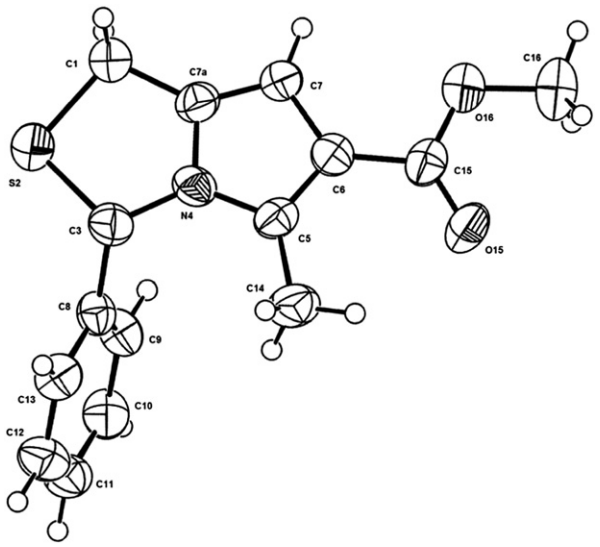


Fig. 2. X-Ray structure of methyl (3*R*)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6-carboxylate (**11**).

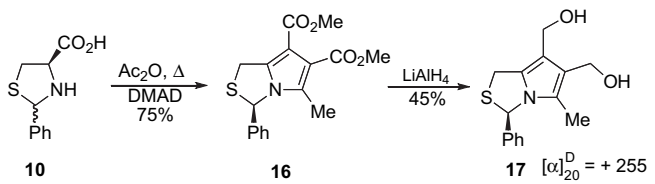
be separated by flash chromatography. Reaction of alcohol **14** with benzyl isocyanate afforded the corresponding 7-hydroxymethyl-(*R*)-pyrrolo[1,2-*c*]thiazole benzylcarbamate. The formation of this carbamate was confirmed by the proton NMR spectrum of the crude product. However, attempts to purify the compound led only to degradation products. Thus, due to the lack of stability we decided not to proceed with the biological evaluation studies of this compound.

The synthesis of chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **16** was also carried out following a procedure described in the literature [18]. Heterocycle **16** underwent reduction with lithium aluminium hydride to afford chiral *(3R)*-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) in 45% yield (Scheme 2).

2.2. Anti-cancer activity

In vitro studies of the anti-cancer activity of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **11**, **13**, **14**, **15** and **17** have been carried out against melanoma (A375), colorectal adenocarcinoma (WiDR) and breast (MCF7) cancer human cell lines. Cells were incubated during 24 h, 48 h, 72 h and 96 h with a DMSO solution of the selected compounds, washed and then cell proliferation was evaluated by MTT test. Control experiments were carried out performing the incubation with only DMSO solution for the same times. The comparison of the activity of the compounds can be made by analyzing the corresponding IC_{50} values calculated from the dose-response curves (Table 1).

The 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6-carboxylate **11** did not show considerable anti-cancer activity against the three cell lines tested, with IC_{50} values (72 h incubation time) of 56.2 μM, 44.6 μM and 48.8 μM for A375, WiDr and MCF7 cells, respectively. These were the expected results since compound **11** bearing an ester group at C-6 cannot act as an alkylating agent. On the other hand, the study of the biological activity of 6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **13** led to very interesting results. IC_{50} values of 62.9 μM for A375 melanoma cell lines, 15.4 μM for WiDR colorectal adenocarcinoma cell lines and 2.4 μM for MCF7 breast cancer cell lines were obtained for compound **13**. Although showing moderate activity against melanoma cell lines, 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **13** is more active against breast cancer cell lines and a concentration of just 2.4 μM allows the inhibition of cell proliferation by 50%. Similar results were obtained for 6-hydroxymethyl-(*R*)-pyrrolo[1,2-*c*]thiazole benzylcarbamate **15** with IC_{50} of 2.2 μM for MCF7 breast cancer cell line. Thus, selectivity for breast cancer cell lines was observed for both 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **13** and **15**. It is worth noticing that compound **15** also showed significant activity against colorectal adenocarcinoma cell lines (IC_{50} = 8.7 μM).



Scheme 2. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **16** and **17**.

Surprisingly, 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **14** gave moderate anti-cancer activity against the three cell lines tested. Considering that this derivate is a potential alkylating agent, differing from 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **13** by only the position of the hydroxymethyl group, it would be expected to show similar activity.

The results of the cell proliferation using different concentrations of 6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole benzylcarbamate (**15**) against MCF7 breast cancer cell lines are presented in Fig. 3.

Some conclusions regarding structure-activity relationships can be redrawn based on the biological evaluation of these 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **11**, **13**, **15** and **14**. The low anti-cancer activity observed for compound **11** in contrast with the high performance shown by the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **13** and **15** allows to conclude that the mechanism of action which leads to cell proliferation inhibition must involve DNA damage via mono-alkylation since the presence of a hydroxymethyl group or the corresponding benzylcarbamate is required. The mechanism of the monoalkylation can be rationalized considering that the electrophilic reactivity of the hydroxymethyl group or the corresponding carbamate is enhanced by participation of the ring nitrogen, similar to the mechanism of action of mitomycin and pyrrolizidine alkaloid pyrrole metabolites [1–4] (Scheme 3). On the other hand, the position of hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole substituent is crucial. In fact, derivative **13** with the hydroxymethyl at C-6 is active whereas the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **14** with this group at C-7 showed low activity. This observation raises the question of whether the main mechanism of action of some of the compounds **1**–**9** previously reported as bifunctional alkylating agents [5–16] is in fact the bisalkylation or the monoalkylation process.

In order to get further insight into structure-activity relationships, *in vitro* studies of the anti-cancer activity of (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) was carried out. This compound showed moderate activity against melanoma cell lines, with IC_{50} value of 27.2 μM (72 h incubation time). However, 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **17** was more active against colorectal adenocarcinoma cell lines (IC_{50} = 7.1 μM) and showed very high performance against breast cancer cell lines with a concentration of just 1.0 μM allowing the inhibition of cell proliferation by 50%. The results of the cell proliferation using different concentrations of (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) against MCF7 breast cancer cell lines are presented in Fig. 4.

It is worth noticing that 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **17** only differs from 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **13** because it has an additional hydroxymethyl group. Compound **17** is chiral with *R*

Table 1
IC_{50} values of compounds **11**, **13**, **14**, **15** and **17**.

Compound	IC50 (μM) [a]											
	A375				WiDR				MCF7			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
11	73.5	65.1	56.2	40.0	54.2	57.1	44.6	40.9	128.4	55.5	48.8	30.6
13	102.8	69.8	62.9	36.8	55.5	17.1	15.4	11.0	26.4	4.3	2.4	1.9
15	37.4	32.4	42.1	27.6	30.9	8.7	8.7	14.8	8.8	4.4	2.2	1.9
14	128.0	106.0	62.1	45.3	143.6	95.3	63.5	65.1	110.2	63.9	29.9	19.9
17	97.3	36.1	27.2	16.9	60.0	9.1	7.1	3.2	12.2	1.9	1.0	0.6

[a] Concentration needed to inhibit cell proliferation by 50% as determined from dose-response curves by exponential decay fitting ($r^2 > 0.9$).

configuration having a phenyl group at C-3 and is a potential bisalkylating agent since it bears two hydroxymethyl groups. Despite these structural features the activity of compound **17** against breast cancer cell lines is not significantly different from the one observed for the monoalkylating 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivative **13**. This result indicates that the main mechanism of action of the studied 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles may in fact be the monoalkylation process.

3. Conclusions

Herein, chiral 6-hydroxymethyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives as new antitumor DNA monoalkylating agents with selectivity for MCF7 breast cancer cell lines are reported (IC_{50}~2 μM). The low activity shown by the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivative bearing the hydroxymethyl group at C-7 gave insight into structure-activity relationships. Furthermore, the potential bisalkylating agent (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole, although showing very high performance against breast cancer cell lines (IC_{50} = 1.0 μM), it wasn't significantly different from the one observed for the monoalkylating 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives indicating that the main mechanism of action may in fact be the monoalkylation process.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were recorded on an instrument operating at 400 MHz. ^{13}C NMR spectra were recorded on an instrument operating at 75.5 MHz or at 100 MHz. The solvent is deuterochloroform except where indicated otherwise; chemical shifts are expressed in parts per million related to internal TMS, and coupling constants (*J*) are in hertz. Microanalyses were performed using an EA 1108-HNS-O Fisons instrument. Mass spectra were recorded on an HP GC 6890/MSD5973 instrument under electron impact (EI) at 70 eV. HRMS spectra were obtained on a VG Autospect M spectrometer (TOF MS EI^+ or ESI). Optical rotations were measured on an Optical Activity AA-5 electrical polarimeter. M.p.s were recorded on a Reichert hot stage and are uncorrected. Flash column chromatography was performed with silica gel 60 as the stationary phase. Dimethyl (3*R*)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **16** was prepared as described in the literature [18].

4.1.1. *Methyl (3R)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole-6-carboxylate (**11**) and methyl (3R)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole-7-carboxylate (**12**)*

2-Phenylthiazolidine-4-carboxylic acid (**10**) (3.80 g, 18.18 mmol), methyl propiolate (2.5 mL, 21.27 mmol) and Ac_2O (60 mL) were heated at 110 °C overnight. The reaction was cooled to room temperature and was diluted with CH_2Cl_2 (150 mL). The organic phase was washed with saturated aqueous solution of $NaHCO_3$ and with water, dried (Na_2SO_4) and evaporated off. The crude product was purified by flash chromatography [hexane–ethyl acetate (2:1)] giving a mixture of **11** and **12** (41:59) in 99% yield (4.90 g, 17.95 mmol). Compound **11** could be separated by selective crystallization with diethyl ether-hexane. *Methyl (3R)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole-6-carboxylate* (**11**): pale yellow solid, mp 87–89 °C; IR (KBr): 1695, 1227, 1170, 1070 cm^{-1}; ^{1}H NMR δ: 2.17 (3H, s), 3.78 (3H, s), 4.02 (1H, d, J = 13.2 Hz), 4.27 (1H, d, J = 13.2 Hz), 6.25 (1H, s), 6.32 (1H, s) 7.00–7.02 (2H, m, ArH), 7.30–7.34 (3H, m, ArH); ^{13}C NMR δ: 11.8, 28.2, 50.8, 64.0, 101.7, 116.6, 125.4, 128.6, 129.1, 132.0, 133.1, 141.1, 165.8; MS (EI): *m/z* 273 $[M]^+$ (100), 242 (18), 152 (99), 121 (58); Anal. Calcd for $C_{15}H_{15}NO_2S$: C, 65.9; H, 5.5; N, 5.1; S, 11.7. Found: C, 66.3; H, 5.7; N, 5.1; S, 11.5. *Methyl (3R)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole-7-carboxylate* (**12**): ^{1}H NMR δ: 2.22 (3H, s), 3.81 (3H, s), 4.34 (1H, d, J = 14.8 Hz), 4.51 (1H, dd, J = 0.8 and 14.8 Hz), 6.27 (1H, d, J = 0.8 Hz), 6.36 (1H, s), 7.26–7.38 (5H, m,

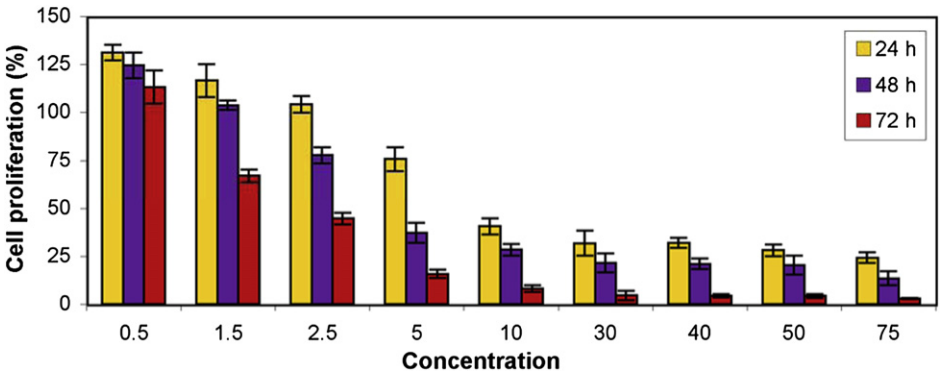
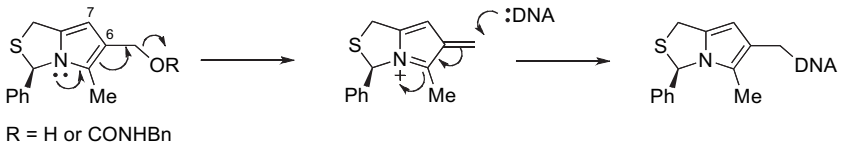


Fig. 3. Values of cell proliferation of *(3R)*-6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole benzylcarbamate (**15**) against MCF7 breast cancer cell lines.



Scheme 3. The proposed mechanism of DNA monoalkylation by 6-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles.

ArH); ^{13}C NMR δ: 12.1, 30.4, 51.0, 64.9, 106.9, 112.2, 125.7, 128.7, 129.1, 140.3, 140.9, 165.1; MS (EI): *m/z* 273 $[M]^+$ (100), 258 (88), 240 (23), 152 (87), 121 (58).

*4.1.2. General procedure for compounds **13**, **14** and **17***

A solution of the appropriate 5-methyl-1*H*,3*H*-pyrrolo[1,2-*c*] thiazole-carboxylate **11** and/or **12** (17.40 mmol) in dry dichloromethane (170 mL) was added dropwise to a suspension of lithium aluminium hydride (1.1 eq., 0.76 g, 19.14 mmol) in anhydrous diethyl ether (120 mL) at 0 °C. The solution was refluxed for 1.5 h after the addition was completed and then cooled on an ice bath. The excess of hydride was carefully decomposed by addition of ethyl acetate followed by slow addition of water (0.8 mL), NaOH 15% (0.8 mL) and water (2.4 mL). The mixture was filtered through celite and the inorganic residue was washed with several portions of hot dichloromethane. The filtrate was dried (Na_2SO_4) and the solvent evaporated off. The crude product was purified by flash chromatography [hexane–ethyl acetate] or recrystallisation.

For the synthesis of compound **17** 2.2 equivalents of lithium aluminium hydride (1.52 g, 38.28 mmol) were used. In this case the excess of hydride was eliminated by the addition of ethyl acetate followed by slow addition of water (1.6 mL), NaOH 15% (1.6 mL) and water (4.8 mL).

*4.1.2.1. (3R)-6-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**13**).* The starting material was compound **11**. Yield: 73%, pale orange solid, mp 53–55 °C (from ethyl ether-petroleum ether); IR (KBr): 3415, 1457, 1340, 989 cm^{-1}; ^{1}H NMR δ: 1.46 (1H, bs), 1.86 (3H, s), 4.04 (1H, d, J = 12.8 Hz), 4.29 (1H, d, J = 12.8 Hz), 4.44 (2H, s), 5.92 (1H, s), 6.22 (1H, s) 7.03–7.04 (2H, m, ArH), 7.25–7.33 (3H, m, ArH); ^{13}C NMR δ: 9.9, 28.7, 58.1, 64.2, 100.1, 122.9, 124.9, 126.6, 128.4, 129.0, 133.0, 141.8; MS (EI): *m/z* 245 $[M]^+$ (100), 227 (13), 124 (74), 106 (19); HRMS (EI): *m/z* 245.0883 ($C_{14}H_{15}NOS$ $[M]^+$, 245.0874). $[\alpha]_{20}^{D} = +320$ (*c* 1, CH_2Cl_2).

*4.1.2.2. (3R)-6-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**13**) and (3R)-7-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**14**).* The starting material was a mixture of **11** and **12**. Purification by flash chromatography [hexane–ethyl acetate (2:1), then hexane–ethyl acetate (1:1)] yields in order of elution (3*R*)-7-hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**14**) and (3*R*)-6-hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**13**) in 15% and 21% yield, respectively. *(3R)-7-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole* **14**: white solid, mp 79–81 °C (from ethyl ether); IR (KBr): 3410, 1438, 1383, 1345, 1006 cm^{-1}; ^{1}H NMR δ: 1.46 (1H, bs), 1.83 (3H, s), 4.09 (1H, d, J = 12.8 Hz), 4.30 (1H, d, J = 12.8 Hz), 4.52 (2H, s), 5.98 (1H, s), 6.22 (1H, s), 7.05–7.07 (2H, m, ArH), 7.28–7.34 (3H, m, ArH); ^{13}C NMR δ: 12.1, 27.9, 58.3, 64.3, 111.3, 114.1, 125.1, 125.7, 128.4, 129.0, 131.8, 141.7; MS (EI): *m/z* 245 $[M]^+$ (100), 229 (16), 124 (79), 108 (17); HRMS (EI): *m/z* 245.0876 ($C_{14}H_{15}NOS$ $[M]^+$, 245.0874). $[\alpha]_{20}^{D} = +280$ (*c* 1, CH_2Cl_2). *(3R)-6-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**13**):* Compound **13** was identified by comparison with the specimen previously prepared (see above).

*4.1.2.3. (3R)-6,7-Bis(hydroxymethyl)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**17**).* The starting material was compound **16** [18]. Yield: 45%, white solid, mp 91–93 °C (from ethyl ether–ethyl acetate); IR (KBr): 3388, 1455, 1359, 1035, 1005 cm^{-1}; ^{1}H NMR δ: 1.83 (3H, s), 3.13 (1H, bs), 3.28 (1H, bs), 4.06 (1H, d, J = 12.8 Hz), 4.27 (1H, d, J = 12.8 Hz), 4.44 (1H, d, J = 12.0 Hz), 4.48 (1H, d, J = 12.0 Hz), 4.54 (2H, bs), 6.22 (1H, s) 7.03–7.05 (2H, m, ArH), 7.27–7.30 (3H, m, ArH); ^{13}C NMR δ: 9.9, 27.6, 56.2, 56.5, 64.3, 113.4, 123.0, 123.5, 125.7, 128.5, 129.0, 131.5, 141.6; MS (EI): *m/z* 275 $[M]^+$ (29), 257 (32), 239 (44), 162 (59), 121 (100), 77 (40); HRMS (EI): *m/z* 275.0991 ($C_{15}H_{17}NO_2S$ $[M]^+$, 275.0980). $[\alpha]_{20}^{D} = +255$ (*c* 1, CH_2Cl_2).

*4.1.3. (3R)-6-Hydroxymethyl-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole benzylcarbamate (**15**)*

Compound **15** was prepared according to a procedure described in the literature [15]. A solution of **13** (0.37 g; 1.51 mmol) and triethylamine (0.5 mL) in dry dichloromethane (18 mL) was cooled at 0 °C. Benzyl isocyanate (1.5 eq., 0.28 mL, 2.27 mmol) was added and the solution was refluxed for 2 h. The reaction mixture was concentrated to dryness in vacuo, the residue was dissolved in hot

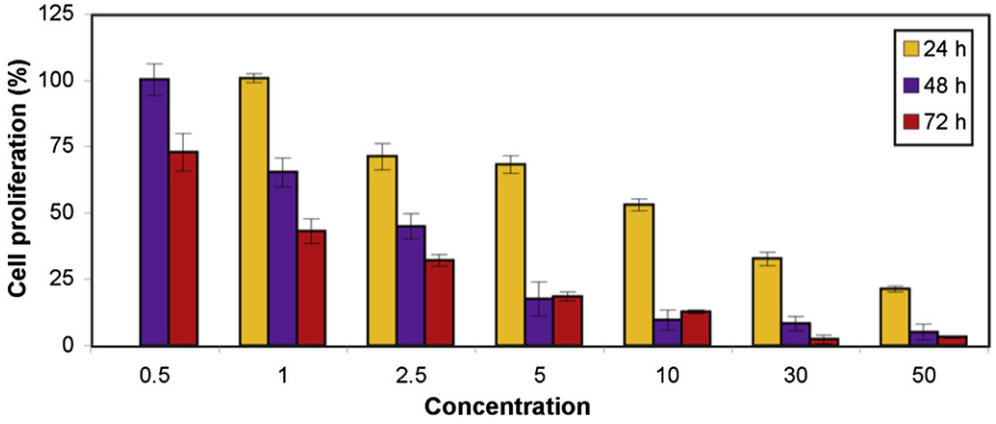


Fig. 4. Values of cell proliferation of *(3R)*-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) against MCF7 breast cancer cell lines.

ethyl acetate, treated with charcoal, filtered and concentrated to give a solid. The solid residue was washed with ether giving compound **15**. Yield 54% (0.31 g, 0.82 mmol), white solid, mp 95–97 °C; IR (KBr): 3331, 1698, 1536, 1255, 1138 cm^{-1}; ^1H NMR δ: 1.86 (3H, s), 4.04 (1H, d, J = 12.8 Hz), 4.28 (1H, d, J = 12.8 Hz), 4.36–4.37 (2H, m), 4.94 (2H, s), 5.94 (1H, s), 6.21 (1H, s) 7.03–7.05 (2H, m, ArH), 7.25–7.33 (8H, m, ArH); ^{13}C NMR δ: 10.0, 28.7, 45.1, 60.5, 64.3, 101.1, 120.0, 124.4, 125.7, 127.3, 127.4, 127.5, 128.4, 128.6, 129.0, 133.2, 138.6, 141.7, 156.8; HRMS (ESI): m/z 401.1298 ($C_{22}H_{22}N_2O_2S$ $[M + Na]^+$, 401.1294). $[\alpha]_{20}^{D} = +190$ (c 1, CH_2Cl_2).

4.2. Measurement of cell proliferation

The *in vitro* cytotoxic effect of the molecules was evaluated in human colorectal adenocarcinoma cell line WiDR, human melanoma cell line A375 and human breast adenocarcinoma cell line MCF7 purchased from American Type Culture Collection (ATCC). The cells lines were cultured with Dulbecco's Modified Eagle medium (Sigma–Aldrich, Inc; Sigma D-5648) supplemented with 10% heat-inactivated fetal bovine serum (Gibco Invitrogen Life Technologies; Gibco 2010-04), 1% Penicillin–Streptomycin (Gibco Invitrogen Life Technologies; 100 U/ml penicillin and 10 μg/mL streptomycin – Gibco 15140-122) and 100 μM sodium pyruvate (Gibco Invitrogen Life Technologies; Gibco 1360) at 37 °C in a humidified incubator with 95% air and 5% CO_2.

For each experiment, cells were plated in 24 multiwells (Corning Costar Corp), in a concentration of 50 000 cells/mL and kept in the incubator overnight, in order to allow the attachment of the cells. Cells were incubated during 24 h, 48 h, 72 h and 96 h with DMSO successive dilutions of the selected compounds.

The sensitivity of the cell lines to the compounds was analyzed using the MTT (3-4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, colorimetric assay (Sigma–Aldrich, Inc.; Sigma M2128) to measure cell proliferation. Cytotoxicity was expressed as the percentage of inhibition of cell proliferation correlated with control experiments where the incubation was carried out with only DMSO. This allows the calculation of the concentration that inhibits the culture cell proliferation in 50% (IC_{50}). Each experiment was performed in duplicate and repeated in three sets of tests.

In vitro studies of reference drugs have been carried out against melanoma (A375), colorectal adenocarcinoma (WiDR) and breast (MCF7) cancer human cell lines. For melanoma cancer human cell lines IC_{50} values of 62.23 and 6.49 μM were obtained for all-trans-retinoic acid and dacarbazine, respectively (72 h incubation time). In the case of colorectal adenocarcinoma cancer human cell lines 5-fluoracile showed an IC_{50} = 16.15 μM and oxaliplatin an IC_{50} = 1.83 μM. Epirubicin showed anti-cancer activity against breast cancer human cell lines with IC_{50} = 1.99 μM.

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