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Original article

Chiral 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles with anti-breast cancer properties

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ABSTRACT

The synthesis and biological evaluation of 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles as anticancer agents against MCF7 breast cancer cell lines is reported. The design of the new compounds has been guided considering (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*] thiazole as the lead compound due to its good performance against MCF7 breast cancer cell lines ($IC_{50} = 1.0$ μM). The structural changes included the removal of the phenyl group at C-3, the replacement of this group by a 3,4,5-trimethoxyphenyl group, the removal of the methyl group at C-5 from the lead scaffold and the replacement of this group by a phenyl substituent. Overall, these studies showed that the combined presence of a phenyl group at C-3 and a methyl group at C-5 in the 1*H*,3*H*-pyrrolo[1,2-*c*] thiazole ring system is essential to ensure high cytotoxicty against MCF7 breast cancer cell lines. To probe whether the absolute configuration of the lead compound might affect the anticancer activity, its enantiomer was prepared and the activity against MCF7 cells was evaluated. (3*S*)-6,7-Bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole proved to be the most active compound so far studied, with IC_{50} value of 0.5 μM.

1. Introduction

The major challenge in cancer chemotherapy is the selective inactivation of DNA in cancer cells. 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 compounds that are less toxic 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 mono-alkylating (reacting with only one DNA strand) and bifunctional alkylating drugs. The latter were found to crosslink the two complementary strands of DNA, which is expected to inhibit DNA replication and subsequently cell proliferation and usually results in more potent and efficacious agents.

Mitomycin C (**1**) is a naturally occurring antitumor alkylating agent which finds use in clinical practice as a useful chemotherapeutic agent. The mitomycin C metabolite **2** is the active specie having two electrophilic centers (C-1 and C-10) which enables DNA interstrand cross-linking reactions. On the other hand, several pyrrolizine alkaloids (e.g. **3**), one of the most abundant class of biologically active natural products, also undergo metabolic activation to give dehydropyrrolizines bearing two reactive functionalities (e.g. **4**, C-7 and C-9) which allow DNA cross-linking. The structural similarities between the activated pyrrolizine alkaloids and mitomycin C, both having a pyrrole-containing substructure, and the demonstration that they target preferentially the same sequences of the DNA led to the search of new pyrrole-derived bifunctional alkylating agents. In fact, Hopkins et al. have shown that bis(hydroxymethyl) pyrroles **5** and **6** and the antitumor 1*H*-pyrrolizine **7** also show selectivity for the same site of DNA interstrand cross-linking. Studies on the mechanism of action of bifunctional electrophilic pyrroles

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indicate that these compounds act as DNA damaging agents via a S_N1 type reaction and that the electrophilic reactivity is enhanced by participation of the ring nitrogen [5–11] (Fig. 1).

Pyrrole-derived alkylating agents that contain two reactive centers capable of binding covalently to DNA and showing antitumor activity include also dihydropyrrolizine **8** [12], 8*H*-3a-azacyclopenta[*a*]indene-1-yl derivatives **9** [13], 1*H*,3*H*-pyrrolo[1,2-c]thiazoles **10**–**11**, 2,3-dihydropyrrolo[2,1-*b*]thiazoles **12** [9,14,15], 4*H*-benzo[*d*]pyrrolo[2,1-*b*]thiazoles **13** [16], dihydropyrrolo[2,1-*a*]isoquinolines **14**, dihydropyrrolo[1,2-*a*]isoquinolines **15** and 5,10-dihydropyrrolo[1,2-b]isoquinolines **16** [17–19] (Fig. 2).

We have recently describe the synthesis of chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **17**–**20** and their *in vitro* activity as anticancer agents in three human tumor cell lines, colorectal adenocarcinoma, melanoma and breast adenocarcinoma [20]. The selection of monoalkylating pyrrole-derived compounds **17**–**19** and bisalkylating 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **20** aimed to determine whether bisalkylating agents are in fact required to ensure efficient activities. (*R*)-6-Hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) and the corresponding benzylcarbamate **18** showed selectivity for MCF7 breast cancer cell lines with IC_{50} values of 2.4 μM and 2.2 μM, respectively (Table 1). The latter also showed significant activity against colorectal adenocarcinoma cancer cell lines ($IC_{50} = 8.7$ μM). Surprisingly, 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **19** gave moderate anticancer activity against the three cell lines tested. Considering that this compound is a monoalkylating agent, differing from 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **17** by only the position of the hydroxymethyl group, it would be expected to show similar activity. The low activity shown by the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **19** gave insight into structure-activity relationships. Furthermore, the bisalkylating (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**20**), although showing very high performance against MCF7 breast cancer cell lines (IC_{50} = 1.0 μM) was not significantly different from that observed for the monoalkylanting 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives indicating that the main mechanism of action may in fact be the monoalkylation process.

The selectivity of the studied compounds **17**–**20** to MCF7 cell lines led us to focus our attention on the design of new 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles aiming to find more efficient alkylating agents with anti-breast cancer properties. Herein, the synthesis and the biological evaluation of new alkylating 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles as anticancer agents against MCF7 breast cancer cell lines is reported.

2. Results and discussion

2.1. Chemistry

Pyrrolo[1,2-*c*]thiazoles **24** and **25** were prepared as outlined in Scheme 1, following a known general synthetic procedure [20–22]. The 1,3-dipolar cycloaddition of the bicyclic münchnone generated in situ from thiazolidine **21a** with methyl propiolate was followed by loss of carbon dioxide giving the regioisomeric pyrrolo[1,2-*c*]thiazoles **22** and **23** as a 41:59 mixture. Reduction of this mixture with lithium aluminum hydride afforded the corresponding alcohols **24** and **25**, which were separated by flash chromatography. The structural assignment of 6-hydroxymethyl- and 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles, **24** and **24**, was supported by two-dimensional HSQC, HMBC and NOESY spectra (400 MHz). In the NOESY spectrum of compound **24** methyl protons show connectivity with H-3 and H-9 protons whereas in the NOESY spectrum of compound **25** connectivities were observed between methyl protons and protons H-6 and H-3.

The same synthetic strategy was applied for the preparation of 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **27** in good overall yield (Scheme 1). It should be noted that 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **27b** is obtained as single enantiomer with *R* configuration.

The synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives unsubstituted at C-5 requires the initial synthesis of *N*-formylthiazolidines **28** which was carried out by reacting thiazolidines **21c** or **21d** with formic acid and acetic anhydride [23,24]. Treatment of *N*-formylthiazolidines **28** with triethylamine and tosyl chloride generated the corresponding dipole which was trapped with dimethyl acetylene dicarboxylate to give 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **29** [25]. The reduction of these heterocycles with lithium aluminum hydride afforded the target alcohols **30** (Scheme 2).

The stereochemistry of the (3*R*)-3-(pyridin-3-yl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **29b** was established by single-crystal X-ray crystallography (Fig. 3). Using the small anomalous dispersion of Mo Kα X-ray radiation by the sulfur atom, a refinement of the Flack parameter in the structural model (1439 Friedel pairs, $R\ (I > 2\sigma) = 0.0448$ for 3267 reflections and 201 parameters, Flack parameter: 0.11(8)) allowed us to unambiguously assign the *R* configuration to the chiral center C-3.

Chiral 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **32a** was obtained as described in literature [26]. The synthesis of (3*R*)-5-(4-fluorophenyl)-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate (**32b**) was achieved using the same methodology. The process involves the selective synthesis of *N*-acyl-2-

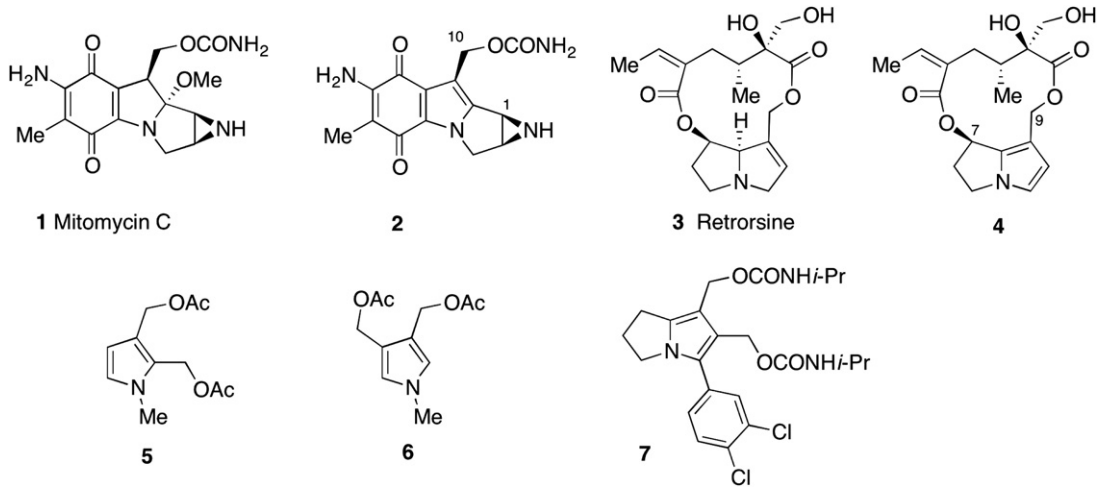


Fig. 1. Chemical structures of some DNA bifunctional alkylating agents.

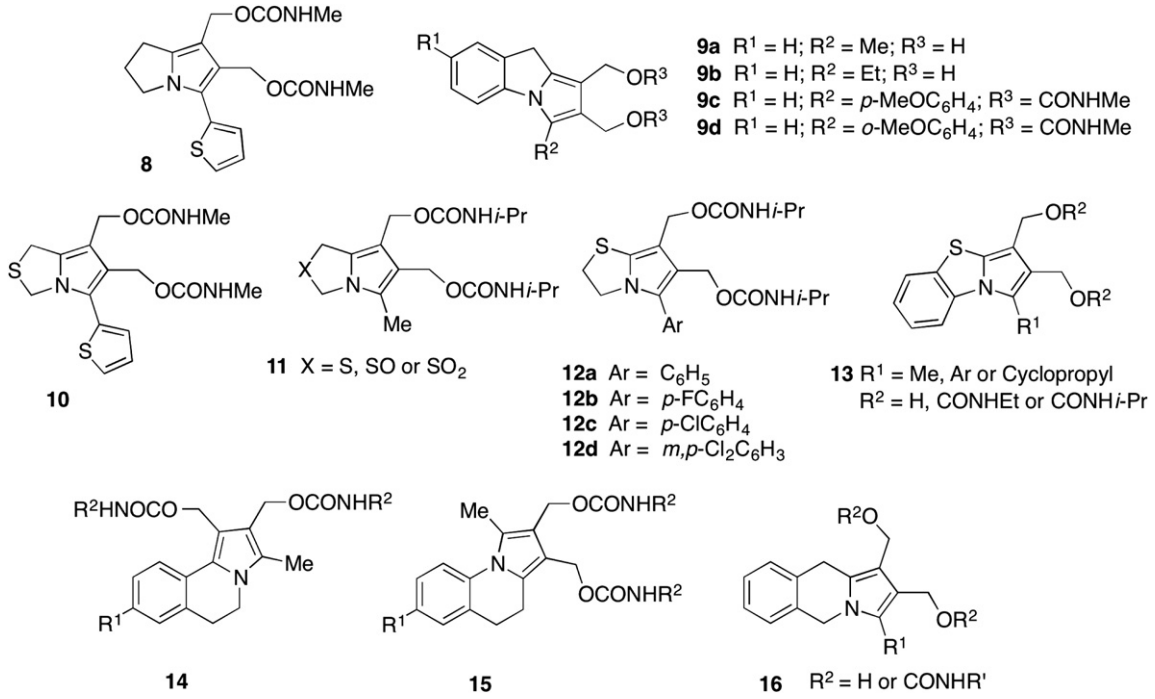
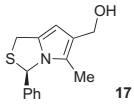
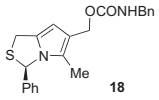
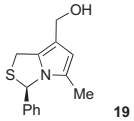
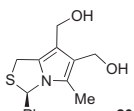


Fig. 2. Chemical structures of some compounds that contain two reactive centers capable of binding covalently to DNA.

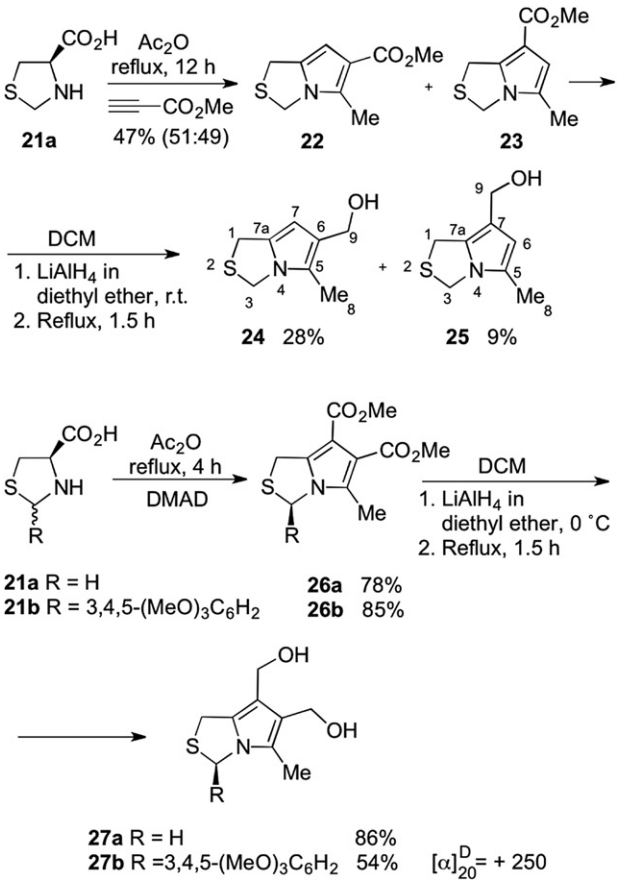
phenyl-1,3-thiazolidine-4-carboxylic acids as pure stereoisomers with (2*R*,4*R*) configuration by treating the triethylamine salt of thiazolidine **21c** with the appropriate acid chloride. The reaction of (2*R*,4*R*)-*N*-acyl-2-phenyl-1,3-thiazolidine-4-carboxylic acids **31** with dimethyl acetylene dicarboxylate in refluxing acetic anhydride gave chiral (3*R*)-3-aryl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **32** in

Table 1
Cytotoxicity of compounds **17**-**20** against MCF7 breast cancer cell lines [20].[a]

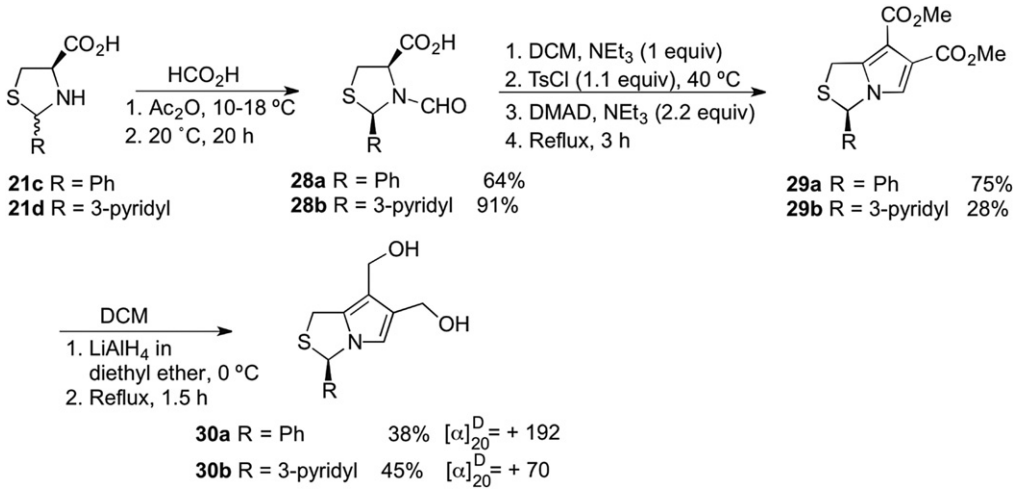
Compound	IC_{50} (μM)[b]			
	MCF7			
	24 h	48 h	72 h	96 h
 **17**	26.4	4.3	2.4	1.9
 **18**	8.8	4.4	2.2	1.9
 **19**	110.2	63.9	29.9	19.9
 **20**	12.2	1.9	1.0	0.6

[a] 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.

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



Scheme 1. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **22**-**27**.



Scheme 2. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **29** and **30**.

good yield. Reduction of heterocycles **32** with lithium aluminum hydride afforded the corresponding alcohols **33** (Scheme 3).

Chiral 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **36** with *S* configuration was prepared as outlined in Scheme 4. It was demonstrated that the reaction conditions described for the selective synthesis of (2*S*,4*R*)-*N*-aroyl-2-phenyl-1,3-thiazolidine-4-carboxylic acids [26] can also be applied to the preparation of *N*-acetyl derivatives. Thus, the reaction of thiazolidine **21c** with acetyl chloride in dry pyridine allows the exclusive formation of (2*S*,4*R*)-*N*-acetyl-2-phenyl-1,3-thiazolidine-4-carboxylic acid (**34**) in 99% yield. 1,3-Thiazolidine **34** reacts with dimethyl acetylene dicarboxylate in refluxing acetic anhydride to afford (3*S*)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **35** which was converted into chiral 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **36** upon reaction with lithium aluminum hydride.

6,7-Bis(hydroxymethyl)-1*H*,3*H*-pyrazolo[1,5-*c*]thiazole (**38**) was prepared by reduction of 1*H*,3*H*-pyrazolo[1,5-*c*]thiazole **37** [27] with lithium aluminum hydride (Scheme 5).

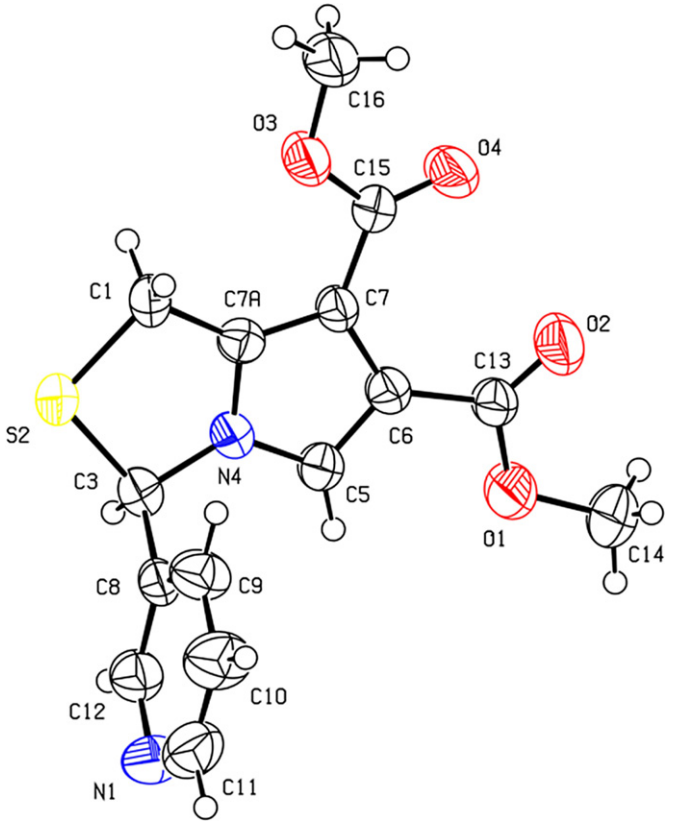
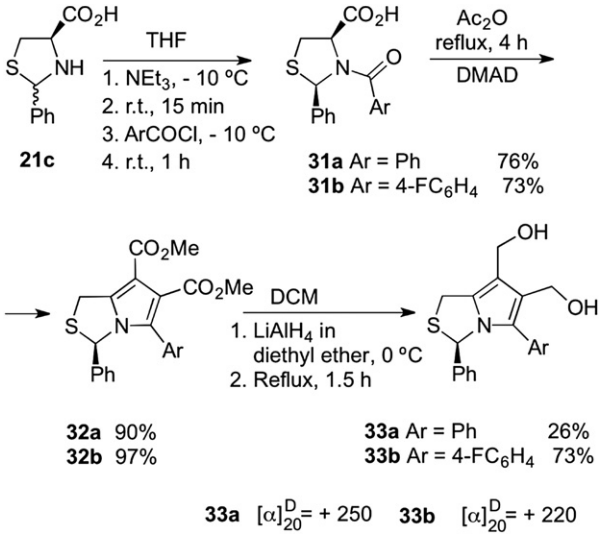


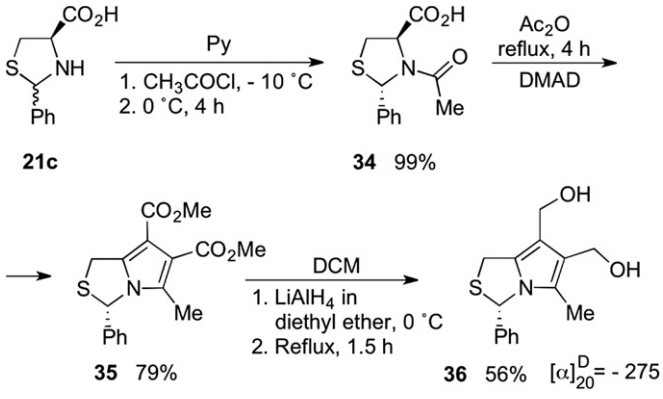
Fig. 3. X-ray structure of dimethyl (3*R*)-3-(pyridin-3-yl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate **29b**.

2.2. Anticancer activity

In vitro studies of the anticancer activity of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **24**, **25**, **27**, **30**, **33** and **36** have been carried out against MCF7 breast cancer 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 was made by the analysis of the corresponding IC_{50} values calculated from the dose response curves (Table 3).



Scheme 3. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **32** and **33**.



Scheme 4. Synthesis of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **35** and **36**.

The design of new compounds has been guided considering (3*R*)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **19** as the lead compound and making structural changes aiming not only to obtain derivatives with higher anti-breast cancer activity but to gather data regarding structure-activity relationships (Fig. 4). This type of information is extremely valuable for planning further structural modifications.

The possibility of preparing benzylcarbamate derivatives was ruled out based on the cytotoxicity shown by compounds **17**, **18** and **19** against non-tumoral fibroblast HFF1 cells (Table 2). In fact, (*R*)-6-hydroxymethyl-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**17**) required a concentration of 154.0 μM for the inhibition of cell proliferation by 50% whereas in the case of the corresponding benzylcarbamate **18** a concentration of only 48.2 μM was needed. Thus, it was observed that 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **17** show significant selectivity for cancer cell lines in contrast with the benzylcarbamate derivative. Some degree of selectivity was also observed for 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **19** since it showed an IC_{50} value of 103.5 μM (96 h incubation time) against non tumoral fibroblast HFF1 cell line but an IC_{50} value of 19.9 μM against MCF7 breast cancer cell line.

We initially investigated if the presence of the phenyl group at C-3 is a requirement for the observed biological activity (Table 3). Thus, anticancer activity of C-3 unsubstituted 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **27a** against MCF7 cell lines was studied. It was observed that this structural change resulted in near 22-fold decrease in activity ($IC_{50} = 22.6$ μM for 72 h incubation time). The anticancer activity of C-3 unsubstituted 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole derivatives **24** and **25**, bearing only one hydroxymethyl group at C-6 and C-7, respectively, was also evaluated. Compound **24** required a concentration of 191.5 μM for the inhibition of cell proliferation by 50% whereas the corresponding 3-phenyl substituted derivative **17** showed an IC_{50} value of 2.4 μM (72 h incubation time). Similarly, 7-hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **25** showed significantly lower activity than the 3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **19** with an IC_{50} value of 40.9 μM. The replacement of the phenyl group of the

Scheme 5. Synthesis of 1*H*,3*H*-pyrazolo[1,5-*c*]thiazole derivative **38**.

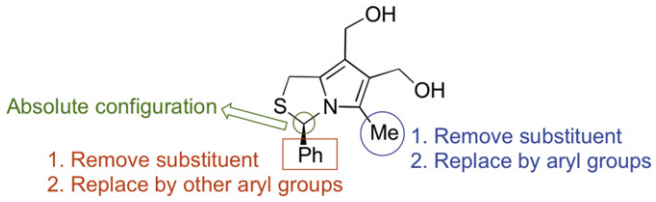


Fig. 4. Chemical modifications on 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **20**.

lead compound **20** by another aryl group was carried out. The new 3-(3,4,5-trimethoxyphenyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **27b** showed low activity against MCF7 breast cancer cell lines. By comparing the results of **27a**, **24**, **25** and **20** against MCF-7 cells, we concluded that the phenyl group at C-3 is critical for the anticancer activity.

We then turned our attention to the removal of the methyl group from the lead scaffold **20** and the replacement of this group by aryl substituent (Table 3). Thus, 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **30a** was prepared and its cytotoxicity against MCF7 cells evaluated. The moderated activity observed for this heterocycle (19.4 μM for 48 h incubation time) when compared with 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **20** did not justify a complete study. The C-5 unsubstituted 3-pyridyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **30b** also showed low activity ($IC_{50} = 61.7$ μM for 72 h incubation time). The evaluation of the anticancer activity of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **33** resulting from the replacement of methyl substituent of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **20** by phenyl and *p*-fluorophenyl groups was also carried out. In both cases a significant decrease of the activity against MCF7 cells was observed (Table 3). Nevertheless, these results have demonstrated that the methyl group at C-5 plays a role in the anticancer activity of 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **20**.

Overall, these studies showed that the combined presence of phenyl group at C-3 and a methyl group at C-5 in the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole ring system is important to the cytotoxic activity against MCF7 breast cancer cell line.

The 6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrazolo[1,5-*c*]thiazole (**38**) did not show anticancer activity against MCF7 breast cancer cell line.

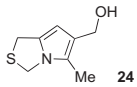
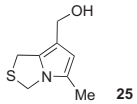
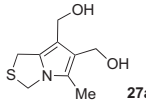
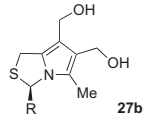
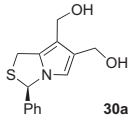
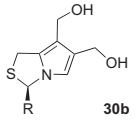
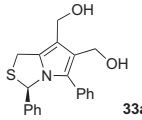
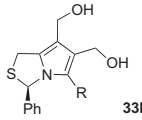
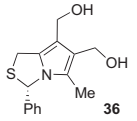
To probe whether the absolute configuration of the lead compound **20**, a chiral compound with *R* configuration, might affect the anticancer activity, its enantiomer was prepared and the activity against MCF7 cells was evaluated (Table 3 and Fig. 5). (3*S*)-6,7-Bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**36**) proved to be the most active compound so far studied, with IC_{50} value of 0.5 μM (72 h incubation time).

Table 2
Cytotoxicity of compounds **17**, **18** and **19** against non tumoral fibroblast HFF1 cell lines.[a]

Compound	IC_{50} (μM)[b,c]
17	154.0
18	48.2
19	103.5

[a] Cells were incubated during 96 h with a DMSO solution of the selected compounds, washed and then cell proliferation was evaluated by MTT test.
[b] Concentration needed to inhibit cell proliferation by 50% as determined from dose–response curves by exponential decay fitting ($r^2 > 0.9$).
[c] Calculated for $n = 3$.

Table 3
Cytotoxicity of compounds **24**, **25**, **27**, **30**, **33** and **36** against MCF7 breast cancer cell lines.[a]

Compound	IC_{50} (μM)[b]			
	MCF7			
	24 h	48 h	72 h	96 h
 **24**	186.3	155.6	191.5	82.3
 **25**	75.9	54.7	40.9	44.6
 **27a**	37.8	38.1	22.6	21.5
 **27b** R = 3,4,5-$(MeO)_3C_6H_2$	>200	127.1	54.5	59.9
 **30a**	70.8[c]	19.4[c]	ND	ND
 **30b** R = 3-pyridyl	>200	128.1	61.7	30.6
 **33a**	92.6	61.8	52.1	70.2
 **33b** R = 4-FC_6H_4	56.7	46.3	44.6	26.4
 **36**	1.75	0.54	0.54	0.30

ND = Not determined.
[a] 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.
[b] Concentration needed to inhibit cell proliferation by 50% as determined from dose–response curves by exponential decay fitting ($r^2 > 0.9$).
[c] Calculated for $n = 2$.

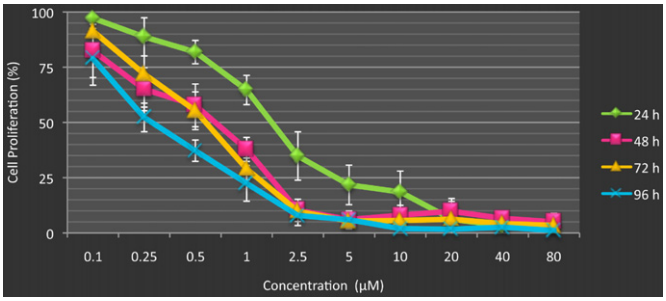


Fig. 5. Values of cell proliferation of (3*S*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**36**).

3. Conclusions

Herein, the design, synthesis and biological evaluation of a series of new hydroxymethyl-1*H*,3*H*-pyrrolo[1,2-c]thiazoles as anticancer agents against MCF7 breast cancer cell lines is reported. Several structural changes on (3*R*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**20**), selected as the lead compound due to its good performance against MCF7 breast cancer cell lines (IC_{50} = 1.0 μM), have been carried out. The study allowed us to redraw some conclusions regarding structure-activity relationships. Thus, the results of the *in vitro* activity have demonstrated that the presence of a phenyl group at C-3 and a methyl group at C-5 in the 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole ring system is crucial. Furthermore, the enantiomer of derivative **20**, (3*S*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**36**), proved to be the most active compound so far studied, with IC_{50} value of 0.5 μM.

4. Experimental

4.1. Chemistry

4.1.1. General methods

^{1}H NMR spectra were recorded on an instrument operating at 400 MHz. ^{13}C NMR spectra were recorded on an instrument operating at 100 MHz. Chemical shifts are expressed in parts per million related to internal TMS, and coupling constants (*J*) are in hertz. IR spectra were recorded on a Nicolet 6700 FTIR spectrometer. Mass spectra were recorded under electron impact (EI) or electrospray ionization (ESI). HRMS spectra were obtained on a VG Autospect M spectrometer (TOF MS EI+ or ESI). Microanalyses were performed using an EA1108-HNS-O Fisons instrument. Melting points were determined in open glass capillary with an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity AA-5 electrical polarimeter. Flash column chromatography was performed with silica gel 60 as the stationary phase. TLC analyses were carried out on Merck Silica gel 60 F_{254} plates. Crystallographic data for compound **29b** was collected on a Bruker APEX CCD diffractometer.

Thiazolidine-4-carboxylic acids **21a** [28] and **21c** [21], 3-formyl-2-phenylthiazolidine-4-carboxylic acid **28a** [25], (2*R*,4*R*)-3-phenyl-2-phenylthiazolidine-4-carboxylic acid **31a** [26], 1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles **26a** [22], **29a** [25] and **32a** [26] and 1*H*,3*H*-pyrazolo[1,5-*c*]thiazole **37** [27] were prepared as described in the literature.

4.1.2. General procedure for the synthesis of thiazolidine-4-carboxylic acids

A solution of the appropriate aldehyde (40 mmol) in ethanol (30 mL) was added to a solution of L-cysteine (4.8 g, 40 mmol) in

water (30 mL). After stirring overnight at room temperature the product was filtered and washed with diethyl ether.

*4.1.2.1. 2-(3,4,5-Trimethoxyphenyl)thiazolidine-4-carboxylic acid (**21b**).* Yield: 99%, white solid, mp 150–152 °C (lit. [29] 146–148 °C). The ^{1}H NMR spectrum obtained at 40 °C showed the presence of the two diastereoisomers (2*R*,4*R*) and (2*S*,4*R*) (ratio 50:50). ^{1}H NMR (400 MHz, CD_3OD) δ 3.25–3.37 and 3.44–3.50 (2 × m, 2H), 3.76 and 3.77 (2 × s, 3H), 3.85 (s, 6H), 4.04–4.07 and 4.35–4.38 (2 × m, 1H), 5.52 and 5.69 (2 × s, 1H), 6.87 and 6.90 (2 × s, 2H, ArH).

*4.1.2.2. 2-(Pyridin-3-yl)thiazolidine-4-carboxylic acid (**21d**).* Yield: 98%, white solid, mp 137–139 °C (lit. [30] 136–137 °C). The ^{1}H NMR spectrum showed the presence of the two diastereoisomers (2*R*,4*R*) and (2*S*,4*R*) (ratio 41:59). IR (KBr) 1725, 1317, 1249, 1188, 1158, 1050 cm^{-1}. Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23–3.35 (m, 1H), 3.42–3.55 (m, 1H), 4.05–4.11 (m, 1H), 5.64 (s, 1H), 7.45–7.52 (m, 1H, ArH), 8.03–8.14 (m, 1H, ArH), 8.48–8.55 (m, 1H, ArH), 8.70–8.74 (m, 1H, ArH); Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23–3.35 (m, 1H), 3.42–3.55 (m, 1H), 4.27–4.31 (m, 1H), 5.84 (s, 1H), 7.45–7.52 (m, 1H, ArH), 8.03–8.14 (m, 1H, ArH), 8.48–8.55 (m, 1H, ArH), 8.70–8.74 (m, 1H, ArH). Anal. Calcd for $C_9H_{10}N_2O_2S$: C, 51.41; H, 4.79; N, 13.32. Found: C, 51.16; H, 5.07; N, 13.15.

*4.1.3. (2R,4R)-3-formyl-2-(pyridin-3-yl)thiazolidine-4-carboxylic acid (**28b**)*

The compound was prepared according to a procedure described in the literature [23]. Yield: 91%, white solid, mp 195–197 °C (from ethanol) (lit. [23] 214 °C). Anal. Calcd for $C_{10}H_{10}N_2O_3S$: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.79; H, 4.62; N, 11.44; S, 13.54.

*4.1.4. (2R,4R)-3-(4-fluorobenzoyl)-2-phenylthiazolidine-4-carboxylic acid (**31b**)*

The compound was prepared according to a procedure described in the literature for the synthesis of (2*R*,4*R*)-3-aroyl-2-phenylthiazolidine-4-carboxylic acids [26]. Yield: 73%, white solid, mp 170–172 °C (from ethyl acetate). IR (KBr) 1714, 1605, 1425, 1296, 1231 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 50 °C) δ 3.40 (dd, J = 6.8 and 12.3 Hz, 1H), 3.53 (dd, J = 7.2 and 12.3 Hz, 1H), 5.20 (dd, J = 7.2 and 6.8 Hz, 1H), 6.14 (s, 1H, C*H*Ph), 6.96 (d, J = 8.5 Hz, 2H, ArH), 7.27–7.38 (m, 7H, ArH). MS (EI) *m*/*z* 332 (MH^+, 100%), 196 (15). HRMS (ESI-TOF) *m*/*z* 332.0763 (MH^+, $C_{17}H_{15}FNO_3S$ requires 332.0751). $[\alpha]_{20}^{D}$ = +90 (*c* 1, MeOH).

*4.1.5. (2S,4R)-3-Acetyl-2-phenylthiazolidine-4-carboxylic acid (**34**)*

The compound was prepared according to a procedure described in the literature for the synthesis of (2*S*,4*R*)-3-aroyl-2-phenylthiazolidine-4-carboxylic acids [26]. Yield: 99%, white solid, mp 179–181 °C (from ethyl acetate) (lit. [31] 189–190 °C) HRMS (ESI-TOF) *m*/*z* 252.0683 (MH^+, $C_{12}H_{14}NO_3S$ requires 252.0689).

4.1.6. General procedure for the synthesis of 1H,3H-pyrrolo[1,2-c] thiazole carboxylates

A solution of the appropriate thiazolidine-4-carboxylic acid (18 mmol), dipolarophile (1.5 equiv., 27 mmol) and Ac_2O (60 mL) was heated at 110 °C during the time indicated in each case. 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].

*4.1.6.1. Methyl 5-methyl-1H,3H-pyrrolo[1,2-c]thiazole-6-carboxylate (**22**) and methyl 5-methyl-1H,3H-pyrrolo[1,2-c]thiazole-7-carboxylate (**23**).* The starting material was **21a** and dipolarophile was methyl propiolate. Reaction time: 12 h. Yield: 47%, compounds **22** and **23** were obtained as a mixture (51:49). Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (s, 3H), 3.77 (s, 3H), 4.29 (s, 2H), 4.91 (s, 2H), 6.33 (s, 1H). MS (EI) *m*/*z* 197 (M^+, 100%), 182 (34), 166 (21), 138 (19), 120 (50). Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 3.78 (s, 3H), 4.03 (s, 2H), 4.88 (s, 2H), 6.23 (s, 1H). MS (EI) *m*/*z* 197 (M^+, 84%), 182 (100), 166 (16), 138 (18), 120 (36).

*4.1.6.2. Dimethyl (3R)-3-(3,4,5-trimethoxyphenyl)-5-methyl-1H,3H-pyrrolo[1,2-c]thiazole-6,7-dicarboxylate (**26b**).* The starting material was **21b** and dipolarophile was dimethyl acetylene dicarboxylate. Reaction time: 4 h. Yield: 85%, white solid, mp 125–127 °C (from diethyl ether). IR (KBr) 1692, 1591, 1543, 1448, 1420, 1235, 1130 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 3H), 3.80 (s, 6H), 3.84 (s, 9H), 4.32 (d, J = 14.8 Hz, 1H), 4.47 (d, J = 14.8 Hz, 1H), 6.23 (s, 1H, C*H*Ar), 6.27 (s, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5, 30.1, 51.4, 51.6, 56.3, 60.9, 65.4, 102.7, 106.9, 117.4, 130.9, 135.4, 138.5, 140.5, 153.9, 164.0, 165.2. MS (EI) *m*/*z* 421 (M^+, 33%), 389 (12), 212 (100), 197 (63), 153 (14). HRMS (EI-TOF) *m*/*z* 421.1196 (M^+, $C_{20}H_{23}NO_7S$ requires 421.1195). $[\alpha]_{20}^{D}$ = +175 (*c* 1, CH_2Cl_2).

*4.1.6.3. Dimethyl (3R)-5-(4-fluorophenyl)-3-phenyl-1H,3H-pyrrolo [1,2-c]thiazole-6,7-dicarboxylate (**32b**).* The starting material was **31b** and dipolarophile was dimethyl acetylene dicarboxylate. Reaction time: 4 h. Yield: 97%, white foam. IR (KBr) 1734, 1718, 1701, 1496, 1400, 1202, 1159, 1110 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.86 (s, 3H), 4.43 (d, J = 15.1 Hz, 1H), 4.57 (dd, J = 1.2 and 15.1 Hz, 1H), 6.26 (br s, 1H, CHPh), 6.74–6.75 (m, 2H, ArH), 6.83–6.87 (m, 2H, ArH), 6.98–7.01 (m, 2H, ArH), 7.12–7.17 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 51.8, 51.9, 65.6, 106.8, 115.0 (d, $^2J_{C-F}$ = 21.5 Hz), 119.8, 125.7, 128.7, 128.8, 130.8, 131.7 (d, $^3J_{C-F}$ = 8.4 Hz), 140.2, 141.4, 162.7 (d, $^1J_{C-F}$ = 247.5 Hz), 163.8, 165.0. MS (EI) *m*/*z* 411 (M^+, 49%), 379 (100), 290 (52), 258 (38), 228 (20), 121 (55). HRMS (EI-TOF) *m*/*z* 411.0945 (M^+, $C_{22}H_{18}FNO_4S$ requires 411.0941). $[\alpha]_{20}^{D}$ = +185 (*c* 1, CH_2Cl_2).

*4.1.6.4. Dimethyl (3S)-3-phenyl-5-methyl-1H,3H-pyrrolo[1,2-c]thiazole-6,7-dicarboxylate (**35**).* The starting material was **34** and dipolarophile was dimethyl acetylene dicarboxylate. Reaction time: 4 h. Yield: 79%, white solid, mp 160–162 °C (from ethyl acetate–hexane). IR (KBr) 1729, 1705, 1450, 1340, 1292, 1154, 1095 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.32 (d, J = 14.9 Hz, 1H), 4.48 (d, J = 14.9 Hz, 1H), 6.28 (s, 1H, CHPh), 7.05–7.07 (m, 2H, ArH), 7.33–7.35 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 30.0, 51.4, 51.6, 65.0, 106.8, 117.4, 125.6, 129.0, 129.3, 130.7, 140.1, 140.5, 164.0, 165.3. MS (EI) *m*/*z* 331 (M^+, 17%), 299 (100), 210 (21), 178 (98), 161 (20). HRMS (EI-TOF) *m*/*z* 331.0877 (M^+, $C_{17}H_{17}NO_4S$ requires 331.0878). $[\alpha]_{20}^{D}$ = −175 (*c* 1, CH_2Cl_2).

*4.1.7. Dimethyl (3R)-3-(pyridin-3-yl)-1H,3H-pyrrolo[1,2-c]thiazole-6,7-dicarboxylate (**29b**)*

Triethylamine (0.75 mL, 5.36 mmol) was added to a suspension of N-formylthiazolidine-4-carboxylic acid **28b** (1.16 g, 4.87 mmol) in dry dichloromethane (2.0 mL). This solution was added dropwise to a mixture of tosyl chloride (1.02 g, 5.36 mmol) in dichloromethane (2.0 mL) at 40 °C. Dimethyl acetylene dicarboxylate (0.74 mL, 5.45 mmol) was then added quickly followed by triethylamine (1.5 mL, 10.71 mmol). The reaction mixture was maintained refluxing for 3 h. After cooling to room temperature water was added and the organic layer was separated. The aqueous phase was extracted with dichloromethane and the organic extracts are combined, dried over anhydrous sodium sulfate and the solvent

evaporated off. Purification by flash chromatography [hexane-ethyl acetate (1:2), hexane-ethyl acetate (1:3), then hexane-ethyl acetate (1:4)] affords **29b**. Yield: 28%, orange solid, mp 98–100 °C (from diethyl ether). IR (KBr) 1703, 1682, 1517, 1248, 1220 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.78 (s, 3H), 3.87 (s, 3H), 4.41 (dd, J = 1.1 e 15.0 Hz, 1H), 4.52 (dd, J = 1.8 and 15.0 Hz, 1H), 6.40 (br s, 1H, CHAr), 6.89 (s, 1H), 7.36 (dd, J = 4.8 and 8.1 Hz, 1H, ArH), 7.60–7.64 (m, 1H, ArH), 8.59 (d, J = 2.1 Hz, 1H, ArH), 8.66 (dd, J = 1.6 and 4.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.8, 51.6, 51.6, 63.9, 108.0, 121.0, 121.1, 124.2, 133.7, 134.8, 142.6, 148.6, 151.2, 163.4, 163.6. MS (EI) m/z 318 (M^+, 16%), 286 (100), 228 (10), 200 (11), 164 (30), 123 (37). HRMS (EI-TOF) m/z 318.0678 (M^+, $C_{15}H_{14}N_2O_4S$ requires 318.0674). $[\alpha]_{20}^{D}$ = +40 (c 0.5, CH_2Cl_2).

4.1.8. 6-Hydroxymethyl-5-methyl-1H,3H-pyrrolo[1,2-c]thiazole (**24**) and 7-hydroxymethyl-5-methyl-1H,3H-pyrrolo[1,2-c]thiazole (**25**)

A solution containing a mixture of **22** and **23** (3.35 g, 17.01 mmol) in dry dichloromethane (200 mL) was added dropwise to a suspension of lithium aluminum hydride (1.1 equiv., 0.71 g, 18.71 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. Purification of the crude product by flash chromatography [hexane-ethyl acetate (2:1), then hexane-ethyl acetate (1:1)] gave, in order of elution, 7-hydroxymethyl-5-methyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **25** and 6-hydroxymethyl-5-methyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole **24**.

6-Hydroxymethyl-5-methyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**24**). Yield: 28%, brown solid, mp 73–75 °C (from diethyl ether). IR (KBr) 3285, 1421, 1358, 991 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.57 (br s, 1H), 2.22 (s, 3H), 4.04 (s, 2H), 4.45 (s, 2H), 4.86 (s, 2H), 5.84 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.0 (CH_3), 28.8 (C-1), 46.8 (C-3), 58.2 (CH_2), 100.1 (C-7), 122.5 (C-6), 124.3 (C-5), 131.9 (C-7a). MS (EI) m/z 169 (M^+, 100%), 151 (44), 136 (17), 106 (25). HRMS (EI-TOF) m/z 169.0558 (M^+, $C_8H_{11}NOS$ requires 169.0561).

7-Hydroxymethyl-5-methyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (**25**). Yield: 9%, yellowish oil. IR (film) 3399, 1426, 1391, 1258, 984 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.58 (br s, 1H), 2.19 (s, 3H), 4.06 (s, 2H), 4.43 (s, 2H), 4.85 (s, 2H), 5.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9 (CH_3), 28.1 (C-1), 46.8 (C-3), 58.2 (CH_2), 110.4 (C-6), 114.4 (C-7), 124.5 (C-5), 130.8 (C-7a). MS (EI) m/z 169 (M^+, 100%), 151 (28), 138 (31), 94 (21).

4.1.9. General procedure for the synthesis of 6,7-bis(hydroxymethyl)-1H,3H-pyrrolo[1,2-c]thiazoles and 6,7-bis(hydroxymethyl)-1H,3H-pyrazolo[1,5-c]thiazole

A solution containing the appropriate 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole or 1*H*,3*H*-pyrazolo[1,5-*c*]thiazole (1.66 mmol) in dry dichloromethane (16 mL) was added dropwise to a suspension of lithium aluminum hydride (2.2 equiv., 0.15 g, 3.65 mmol) in anhydrous diethyl ether (23 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 (0.2 mL), NaOH 15% (0.2 mL) and water (0.6 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 recrystallization.

4.1.9.1. 6,7-Bis(hydroxymethyl)-5-methyl-1H,3H-pyrrolo[1,2-c]thiazole (**27a**).

Yield: 86%, white foam. IR (film) 3359, 1430, 1404, 1374, 1032, 988 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.21 (s, 3H), 2.50 (br s, 2H), 4.06 (s, 2H), 4.50 (s, 4H), 4.86 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.9, 27.7, 46.8, 56.4, 56.6, 113.6, 122.6, 122.8, 130.5. MS (EI) m/z 199 (M^+, 100%), 181 (83), 164 (51), 162 (46), 152 (40), 108 (26). HRMS (EI-TOF) m/z 199.0665 (M^+, $C_9H_{13}NO_2S$ requires 199.0667).

4.1.9.2. (3R)-6,7-Bis(hydroxymethyl)-5-methyl-3-(3,4,5-trimethoxyphenyl)-1H,3H-pyrrolo[1,2-c]thiazole (**27b**).

Yield: 54%, with solid, mp 143–144 °C (from diethyl ether). IR (KBr) 3505, 3423, 1599, 1507, 1467, 1405, 1424, 1239, 1127, 968 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.71 (br s, 1H), 1.89 (s, 3H), 2.65 (br s, 1H), 3.79 (s, 6H), 3.83 (s, 3H), 4.09 (d, J = 12.8 Hz, 1H), 4.28 (d, J = 12.8 Hz, 1H), 4.49 (d, J = 12.3 Hz, 1H), 4.53 (d, J = 12.3 Hz, 1H), 4.58 (s, 2H), 6.16 (s, 1H), 6.27 (s, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.0, 27.7, 56.2, 56.4, 56.7, 60.9, 64.7, 102.8, 113.6, 123.3, 123.6, 131.6, 136.9, 138.1, 153.7. MS (EI) m/z 365 (M^+, 7%), 331 (18), 212 (100), 197 (47), 181 (14), 153 (16). HRMS (EI-TOF) m/z 365.1299 (M^+, $C_{18}H_{23}NO_5S$ requires 365.1297). $[\alpha]_{20}^{D}$ = +250 (c 0.5, CH_2Cl_2).

4.1.9.3. (3R)-6,7-Bis(hydroxymethyl)-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**30a**).

Yield: 38%, white solid, mp 108–109 °C (from diethyl ether). IR (KBr) 3406, 3365, 1521, 1456, 1340, 1159, 1024, 999 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.69 (br s, 1H), 2.83 (br s, 1H), 4.16 (d, J = 13.0 Hz, 1H), 4.22 (d, J = 13.0 Hz, 1H), 4.47 (d, J = 12.3 Hz, 1H), 4.52 (d, J = 12.3 Hz, 1H), 4.58 (s, 2H), 6.29 (s, 1H), 6.33 (s, 1H), 7.26–7.28 (m, 2H, ArH), 7.33–7.35 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 56.5, 57.8, 65.7, 113.9, 114.1, 127.1, 128.2, 128.9, 129.1, 133.9, 139.3. MS (EI) m/z 261 (M^+, 53%), 243 (36), 226 (24), 140 (31), 121 (100), 94 (18). HRMS (EI-TOF) m/z 261.0833 (M^+, $C_{14}H_{15}NO_2S$ requires 261.0824). $[\alpha]_{20}^{D}$ = +192 (c 0.5, CH_2Cl_2).

4.1.9.4. (3R)-6,7-Bis(hydroxymethyl)-3-(pyridin-3-yl)-1H,3H-pyrrolo[1,2-c]thiazole (**30a**).

Yield: 45%, colorless oil. IR (film) 3334, 1648, 1517, 1429, 1362, 1162, 996 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.36 (br s, 2H), 4.18 (d, J = 13.0 Hz, 1H), 4.25 (d, J = 13.0 Hz, 1H), 4.56 (s, 2H), 4.59 (s, 2H), 6.30 (s, 1H), 6.32 (s, 1H), 7.29–7.32 (m, 1H, ArH), 7.58 (d, J = 7.9 Hz, 1H, ArH), 8.48 (s, 1H, ArH), 8.57–8.58 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.39, 56.0, 57.2, 63.0, 113.5, 114.5, 124.1, 129.0, 133.8, 135.0, 136.0, 147.9, 149.9. MS (EI) m/z 262 (M^+, 41%), 244 (56), 227 (47), 122 (100), 108 (30), 94 (29). HRMS (EI-TOF) m/z 262.0772 (M^+, $C_{13}H_{14}N_2O_2S$ requires 262.0776). $[\alpha]_{20}^{D}$ = +70 (c 1, CH_2Cl_2).

4.1.9.5. (3R)-6,7-Bis(hydroxymethyl)-3,5-diphenyl-1H,3H-pyrrolo[1,2-c]thiazole (**33a**).

Yield: 26%, white foam. IR (KBr) 3390, 3132, 1398, 1385, 1005 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.54 (br s, 2H), 4.16 (d, J = 13.1 Hz, 1H), 4.36 (d, J = 13.1 Hz, 1H), 4.51 (s, 2H), 4.69 (s, 2H), 6.31 (s, 1H, C*H*Ph), 6.74–6.76 (m, 2H, ArH), 7.00-7.03 (m, 2H, ArH), 7.12–7.13 (m, 3H, ArH), 7.17–7.19 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.4, 56.7, 64.7, 114.2, 125.0, 125.3, 127.4, 127.9, 128.0, 128.4, 128.8, 129.6, 130.8, 133.3, 141.8. MS (ESI) m/z 360 ($[M + Na]^+$, 100%), 295 (3), 227 (9). HRMS (ESI-TOF) m/z 360.1032 ($[M + Na]^+$, $C_{20}H_{19}NO_2SNa$ requires 360.1029). $[\alpha]_{20}^{D}$ = +250 (c 1, CH_2Cl_2)

4.1.9.6. (3R)-6,7-Bis(hydroxymethyl)-(4-fluorophenyl)-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**33b**).

Yield: 73%, pale yellow foam. IR (film) 3151, 1496, 1454, 1423, 1398, 1339, 1223 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.59 (br s, 1H), 2.78 (br s, 1H), 4.15 (d, J = 13.1 Hz, 1H), 4.35 (d, J = 13.1 Hz, 1H), 4.45 (s, 2H), 4.68 (s, 2H), 6.25 (s, 1H, CHPh), 6.75–6.77 (m, 2H, ArH), 6.84–6.88 (m, 2H, ArH), 6.95–6.98 (m, 2H, ArH), 7.13–7.15 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.6,

56.7, 56.8, 64.9, 114.2, 115.1 (d, $^2J_{C-F}$ = 21.6 Hz), 125.4, 125.5, 127.0 (d, $^4J_{C-F}$ = 3.2 Hz), 127.7, 128.2, 128.6, 131.5 (d, $^3J_{C-F}$ = 8.1 Hz), 133.4, 141.8, 162.2 (d, $^1J_{C-F}$ = 247.8 Hz). MS (EI) *m/z* 355 (M^+, 9%), 337 (59), 321 (65), 200 (97), 186 (48), 121 (100). HRMS (EI-TOF) *m/z* 355.1035 (M^+, $C_{20}H_{18}NO_2SF$ requires 355.1042). $[\alpha]_{20}^{D}$ = +220 (*c* 1, CH_2Cl_2).

*4.1.9.7. (3S)-6,7-Bis(hydroxymethyl)-5-methyl-3-phenyl-1H,3H-pyrrolo[1,2-c]thiazole (**36**).* Yield: 56%, white solid, mp 88–90 °C (from ethyl acetate–diethyl ether). IR (KBr) 3385, 1540, 1456, 1437, 1344, 1035, 1005 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.84 (s, 3H), 2.44 (br s, 2H), 4.07 (d, *J* = 12.8 Hz, 1H), 4.28 (d, *J* = 12.8 Hz, 1H), 4.47 (d, *J* = 12.3 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.57 (s, 2H), 6.22 (s, 1H), 7.04–7.06 (m, 2H, ArH), 7.26–7.33 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.0, 27.7, 56.4, 56.7, 64.4, 113.4, 123.0, 123.5, 125.7, 128.5, 129.0, 131.5, 141.6. MS (EI) *m/z* 275 (M^+, 42%), 257 (43), 241 (52), 226 (19), 162 (38), 154 (35), 136 (21), 121 (100). HRMS (EI-TOF) *m/z* 275.0979 (M^+, $C_{15}H_{17}NO_2S$ requires 275.0980). $[\alpha]_{20}^{D}$ = −275 (*c* 1, CH_2Cl_2).

*4.1.9.8. 6,7-Bis(hydroxymethyl)-1H,3H-pyrazolo[1,5-c]thiazole (**38**).* Yield: 34%, white solid, mp 111–112 °C (from diethyl ether). IR (KBr) 3323, 3167, 1564, 1510, 1439, 1385, 1024, 985 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.45 (br s, 2H), 4.08 (s, 2H), 4.56 (s, 2H), 4.70 (s, 2H), 5.14 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, DMSO-d_6) δ 26.1, 48.6, 53.6, 56.6, 113.3, 142.2, 155.3. MS (EI) *m/z* 186 (M^+, 7%), 168 (100), 122 (8), 110 (9). HRMS (EI-TOF) *m/z* 186.0467 (M^+, $C_7H_{10}N_2O_2S$ requires 186.0463)

*4.2. Crystall data for dimethyl (3R)-3-(pyridin-3-yl)-1H,3H-pyrrolo[1,2-c]thiazole-6,7-dicarboxylate (**29b**)*

$C_{15}H_{14}N_2O_4S$, *M* = 318.34, monoclinic, *Pc*, with unit cell, *a* = 8.0325(3) Å, *b* = 9.2745(4) Å, *c* = 10.5028(4) Å, α = 90.00°, β = 108.585(2)°, γ = 90.00°, *V* = 741.63(5) $Å^3$. It contains two molecules/unit cell. D_c = 1.426 g cm^{-3}, *Z* = 2, μ = 0.238 mm^{-1}. *R* [$I > 2\sigma$ (*I*)] = 0.0448 and *Rw* = 0.0967 for 3267 independent reflections.

4.3. Measurement of cell proliferation

The *in vitro* cytotoxic effect of the molecules was evaluated in human breast adenocarcinoma cells MCF7, HCC1954 and HCC1806 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.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.036.

References

[1] a) L.H. Hurley, Nat. Rev. Cancer 2 (2002) 188–200;
b) X. Cai, P.J. Gray Jr., D.D. Von Hoff, Cancer Treat. Rev. 35 (2009) 437–450.
[2] C. Oakman, S. Bessi, E. Zafarana, F. Galardi, L. Biganzoli, A. Di Leo, Brest Cancer Res. 11 (2009) 1–11.
[3] J. Hansson, R. Lewensohn, U. Ringborg, B. Nilsson, Cancer Res. 47 (1987) 2631–2637.
[4] B.D. Palmer, W.R. Wilson, S.M. Pullen, W.A. Denny, J. Med. Chem. 33 (1990) 112–121.
[5] S.R. Rajski, R.M. Williams, Chem. Rev. 98 (1998) 2723–2795.
[6] M.F. Weidner, S.T. Sigurdsson, P.B. Hopkins, Biochemistry 29 (1990) 9225–9233.
[7] J. Woo, S.Th. Sigurdsson, P.B. Hopkins, J. Am. Chem. Soc. 115 (1993) 3407–3415.
[8] W.K. Anderson, P.F. Corey, J. Med. Chem. 20 (1977) 812–818.
[9] W.K. Anderson, R.H. Mach, J. Med. Chem. 30 (1987) 2109–2115.
[10] W.K. Anderson, H.L. McPherson Jr., J. Med. Chem. 25 (1982) 84–86.
[11] W.K. Anderson, R.H. Mach, J. Heterocyclic Chem. 27 (1990) 1025–1030.
[12] W.K. Anderson, D.C. Dean, T. Endo, J. Med. Chem. 33 (1990) 1667–1675.
[13] R. Kakadiya, H. Dong, P.-C. Lee, N. Kapuriya, X. Zhang, T.-C. Chou, T.-C. Lee, K. Kapuriya, A. Shah, T.-L. Su, Bioorg. Med. Chem. 17 (2009) 5614–5626.
[14] D. Ladurée, J.-C. Lancelot, M. Robba, E. Chenu, G. Mathé, J. Med. Chem. 32 (1989) 456–461.
[15] I. Lalezari, E.L. Schwartz, J. Med. Chem. 31 (1988) 1427–1429.
[16] R. Chaniyara, S. Tala, C.-W. Chen, P.-C. Lee, R. Kakadiya, H. Dong, B. Marvania, C.-H. Chen, T.-C. Chou, T.-C. Lee, A. Shah, T.-L. Su, Eur. J. Med. Chem. 53 (2012) 28–40.
[17] W.K. Anderson, H.L. McPherson, J.S. New, A.C. Rick, J. Med. Chem. 27 (1984) 1321–1325.
[18] W.K. Anderson, A.R. Heider, N. Raju, J.A. Yucht, J. Med. Chem. 31 (1988) 2097–2102.
[19] R. Chaniyara, N. Kapuriya, H. Dong, P.-C. Lee, S. Suman, B. Marvania, T.-C. Chou, T.-C. Lee, R. Kakadiya, A. Shah, T.-L. Su, Bioorg. Med. Chem. 19 (2011) 275–286.
[20] M.I.L. Soares, A.F. Brito, M. Laranjo, A.M. Abrantes, M.F. Botelho, J.A. Paixão, A. Matos Beja, M. Ramos Silva, T.M.V.D. Pinho e Melo, Eur. J. Med. Chem. 45 (2010) 4676–4681.
[21] T.M.V.D. Pinho e Melo, M.I.L. Soares, D.M. Barbosa, A.M. d'A.R. Gonsalves, A.M. Beja, J.A. Paixão, M.R. Silva, Tetrahedron 56 (2000) 3419–3424.
[22] J.M. Kane, J. Org. Chem. 45 (1980) 5396–5397.
[23] J.-L. Fabre, D. Farge, C. James, D. Lavé, US Patent 4,529,728.
[24] Experimental procedure described for the synthesis of 7-cyano-3-(3-pyridyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole in H.G. Rajoharison, US Patent 5,124,459.
[25] T.M.V.D. Pinho e Melo, M.I.L. Soares, A.M. d'A.R. Gonsalves, J.A. Paixão, A.M. Beja, M.R. Silva, J. Org. Chem. 70 (2005) 6629–6638.
[26] T.M.V.D. Pinho e Melo, C.S.B. Gomes, A.M. d'A.R. Gonsalves, J.A. Paixão, A.M. Beja, M.R. Silva, L. Alte da Veiga, Tetrahedron 58 (2002) 5093–5102.
[27] O.B. Sutcliffe, R.C. Storr, T.L. Gilchrist, P. Rafferty, Tetrahedron 56 (2000) 10011–10021.
[28] A. Padwa, G.E. Fryxell, J.R. Gasdaska, M.K. Venkatramanan, G.S.K. Wong, J. Org. Chem. 54 (1989) 644–653.
[29] A.N. Skvortsov, V.M. Uvarov, D.A. De Vekki, E.P. Studentsov, N.K. Skvortsov, Russ. J. Gen. Chem. 80 (2010) 2007–2021.
[30] A. Banashek, M.I. Shchukina, J. Gen. Chem. USSR 31 (1961) 1374–1377.
[31] L. Szilágyi, Z. Györgydeák, J. Am. Chem. Soc. 101 (1979) 427–432.