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Design, synthesis, and biological evaluation of new thiazolo[5,4-*d*]pyrimidine derivatives as potent antiproliferative agents†

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A series of thiazolo[5,4-*d*]pyrimidine derivatives were synthesized and evaluated for their antiproliferative activities against several human cancer cell lines. Structure–activity relationship studies were carried out, showing that most of the target compounds had good inhibition against the tested cell lines. Among them, compound **7i** exhibited potent inhibition against human gastric cancer cells MGC-803 and HGC-27 with IC_{50} values of 4.64 and 5.07 μM, respectively and around 12-fold selectivity between MGC-803 and GES-1, indicating a relatively low toxicity to normal cells. The potency and low toxicity of compound **7i** make the thiazolo[5,4-*d*]pyrimidine an attractive scaffold for designing new derivatives selectively targeting MGC-803 cells.

Scaffold diversity has been highly pursued in the construction of small-molecule libraries, highlighting the importance of molecular scaffolds in the identification of new drugs. Of particular interest are the heterocyclic scaffolds,[1–4] which are prevalent in drugs and natural products. Modifications in the biologically privileged scaffolds have been recognized as an important strategy to search new bioactive compounds for the treatment of diseases. Compounds bearing the bicyclic thiazolo[5,4-*d*]pyrimidine scaffold have been proved to exhibit diverse biological activities such as anti-tumor,[5–9] antimicrobial,[10] anti-inflammatory,[11] antinociceptive[12] and human cytomegalovirus inhibitory.[13] For example, compound **A** as a TRPV1 (vanilloid receptor 1) antagonist is orally active and exerts reversal of carrageenan-induced thermal hyperalgesia models in rats (Fig. 1).[14] Compound **B** is a highly potent and selective hA_3AR (human adenosine A_3 receptor) antagonist with a hA_3AR K_i value reaching 18 nM.[15] Moreover, 7-thia-8-oxoguanosine **C** is a guanosine analogue showing immunostimulatory activity both *in vivo* and *in vitro*,[16,17] in addition to a broad-spectrum antiviral activity.[18–20] Also, thiazolidinone **D** was reported to be active against HEPG2 (human liver carcinoma cell line) targeting thymidylate synthase.[21] Therefore, modifications based on this scaffold may be a viable strategy for designing new antiproliferative agents. Our previous work has demonstrated that the thiazolo[5,4-*d*]pyrimidine scaffold could be used for designing apoptosis-inducing agents.[22] Following our previous work, herein we report the synthesis and the antiproliferative activity against three human cancer cell lines of a series of thiazolo[5,4-*d*]pyrimidine derivatives. We also explored the selectivity of such compounds to MGC-803 cells over GES-1 cells.

The general synthetic route was illustrated in Scheme 1. The intermediate derivatives **5a–b** were synthesized following the previously reported procedure.[23] Then the isothiocyanate analogs reacted with **5a–b** under alkaline conditions (cesium carbonate) in acetonitrile to give the key active intermediates **6a–f**.[24] The target compounds **7a–j** were readily obtained by refluxing indicated amines with **6** and triethylamine in isopropanol.

All the target compounds were screened for their antiproliferative activities against human gastric cancer cell lines (MGC-803 and HGC-27) and a human lung cancer cell line (H1650) by the MTT assay (the cells were purchased from the Cell Bank, Shanghai Institutes for Biological Sciences, Shanghai, China), and 5-fluorouracil (5-FU) was employed as the reference drug.[25] The antiproliferative results are summarized in Table 1. Generally, most of the synthesized compounds presented moderate to good inhibition toward the tested cancer cell lines, and in particular, were more sensitive to the MGC-803 cell line than the other two cell lines with IC_{50} values of less than 10 μM. For MGC-803 cells, compounds **7a**, **7c**, and **7i** showed the best inhibitory effect with IC_{50} values of around 5 μM, comparable to 5-FU. For HGC-27 and H1650 cells, this series of compounds displayed a decreased inhibitory activity with IC_{50} values of more than 10

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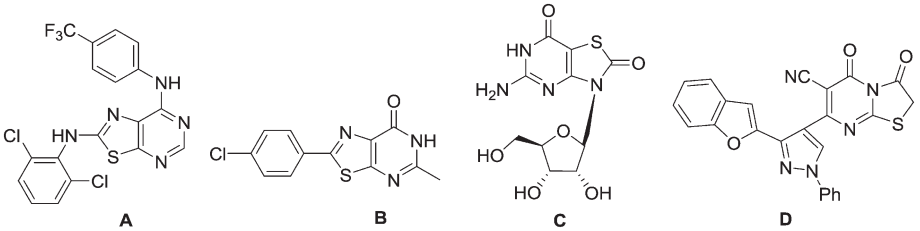
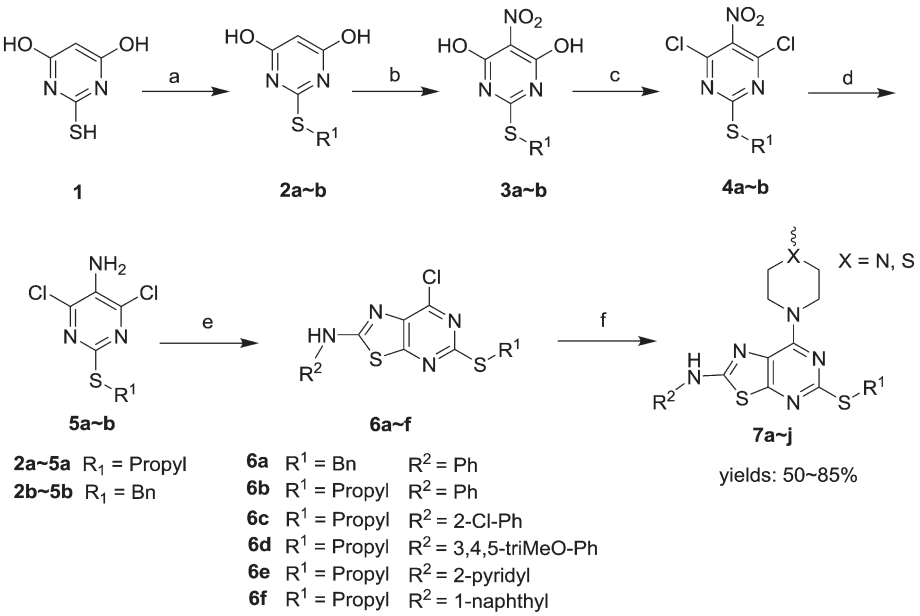


Fig. 1 Pharmaceutically important thiazolo-pyrimidine derivatives.



Scheme 1 Reagent and conditions: (a) alkyl bromide, TEA, methanol, reflux, 2 h; (b) fuming nitric acid, AcOH, 25–45 °C, 1 h; (c) $POCl_3$, DMA, reflux, 2 h; (d) Fe, AcOH, methanol, rt ~ reflux; (e) R^2SCN, Cs_2CO_3, acetonitrile, rt, overnight; (f) TEA, isopropanol, reflux, 6 h.

μM. Only compound **7i** showed a slightly better antiproliferative activity than 5-FU. For H1650 cells, a similar trend was observed; compounds **7b** and **7e** were slightly more potent than 5-FU with IC_{50} values of 8.49 and 5.79 μM, respectively. In general, this series of compounds did not show remarkable structure–activity relationships, highlighting the importance of the thiazolo[5,4-*d*]pyrimidine scaffold in the activity observed. The prioritized one is the compound **7i**, which potently inhibited the growth of MGC-803 and HGC27 cells and will be subjected to further mechanistic studies.

The potent inhibition of compounds **7a** and **7i** against MGC-803 cells prompted us to explore the selectivity between MGC-803 cells and GES-1 cells. As shown in Table 2, these two compounds showed good selectivity with SI values of >12.5 and 12.0, respectively, indicating low toxicity (IC_{50} > 55 μM against GES-1 cells). The potency and low toxicity of compounds **7a** and **7i** make the thiazolo[5,4-*d*]pyrimidine an attractive scaffold for designing new derivatives selectively targeting MGC-803 cells.

In light of the acceptable cytotoxicity and low toxicity of compounds **7a** and **7i**, their molecular properties were calculated online using the free molecular calculation services provided by Molsoft (http://molsoft.com/mprop). As shown in Table 3, compounds **7a** and **7i** exhibited acceptable molecular properties with a MolLogP value slightly higher than the desirable value.

In conclusion, a series of thiazolo-pyrimidine derivatives were prepared and evaluated for their antiproliferative activity toward several human cancer cell lines. Most of the target compounds showed moderate to good inhibition against the tested cell lines. Among them, compounds **7a** and **7i** exhibited potent inhibition against MGC-803 cells with IC_{50} values of 5.13 and 4.64 μM, respectively and showed at least 12-fold selectivity between MGC-803 and GES-1, indicating their low toxicity. These results indicated that the thiazolo[5,4-*d*]pyrimidine scaffold may serve as a template for designing new anticancer agents.

Conflict of interest

The authors declare no competing interests.

Acknowledgements

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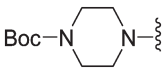
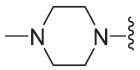
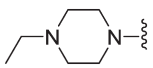
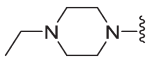
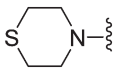
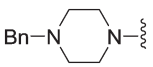
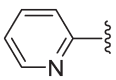
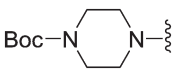
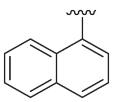
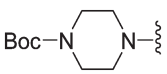
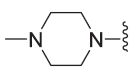
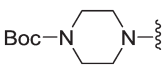
Table 1 Antiproliferative activities of thiazolo-pyrimidine derivatives against three cancer cell lines

Compound	R^1	R^2	R^3N	IC_{50}[a] (μM) MGC-803	HGC27	H1650
7a	Propyl	Ph		5.13 ± 0.71	16.17 ± 1.20	13.36 ± 1.12
7b	Propyl	Ph		9.84 ± 0.99	12.26 ± 1.50	8.49 ± 0.92
7c	Propyl	Ph		4.81 ± 0.68	18.90 ± 1.27	21.73 ± 1.65
7d	Propyl	3,4,5-triMeO-Ph		14.50 ± 0.65	27.56 ± 0.87	36.93 ± 3.06
7e	Propyl	2-Cl-Ph		6.07 ± 0.78	10.30 ± 1.01	5.79 ± 0.86
7f	Propyl	2-Cl-Ph		7.78 ± 0.89	10.62 ± 1.02	12.95 ± 2.16
7g	Propyl			8.63 ± 0.96	10.23 ± 0.79	13.13 ± 2.01
7h	Propyl			30.80 ± 1.48	13.50 ± 1.13	18.73 ± 2.66
7i	Bn-	Ph		4.64 ± 0.66	5.07 ± 0.70	13.83 ± 1.14
7j	Bn-	Ph		9.91 ± 0.99	>64	15.73 ± 0.36
5-FU	—	—	—	6.56 ± 0.31	8.22 ± 0.98	12.52 ± 1.53

[a] Inhibitory activity was assayed by exposure for 72 h to the substrate and expressed as the concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

Table 2 Selectivity of compounds **7a** and **7i** between MGC-803 and GES-1

Compound	IC_{50}[a] (μM) MGC-803	GES-1	SI[b]
7a	5.13 ± 0.71	>64	>12.5
7i	4.64 ± 0.66	55.84 ± 0.99	12.0

[a] Inhibitory activity was assayed by exposure for 72 h to the substance and expressed as the concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments. [b] The selectivity index (SI) was calculated based on the IC_{50} (GES-1) and IC_{50} (MGC803) data.

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Table 3 Molecular properties of **7a** and **7i**[a]

Compound	MW	HBA	HBD	Mol log *P*	MolPSA (A^2)	MV (A^3)
Desirable value	**<500**	**<10**	**<5**	**<5**	**<140**	—
7a	486.19	7	1	5.82	62.61	476.71
7i	448.15	6	1	5.47	43.92	411.88

[a] MW: molecular weight; HBA: number of hydrogen bond acceptors; HBD: number of hydrogen bond donors; Mol log *P*: log *P* value predicted by Molsoft; MolPSA: topological polar surface area; MV: molecular volume.

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