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Tricyclic thiazoles are a new class of angiogenesis inhibitors

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Abstract

Tricyclic thiazoleamine derivatives that were identified as hits in a screen against human umbilical vein endothelial cell proliferation were subjected to a structure-activity relationship study. Two structurally superimposable scaffolds—4*H*-thiochromeno[4,3-*d*]thiazol-2-amine and 5,6-dihydro-4*H*-benzo[6,7]cyclohepta[1,2-*d*]thiazol-2-amine derivatives—yielded low-micromolar inhibitors, and two among them **37** and **43** also exhibited antiangiogenic activity in an endothelial tube formation assay. Thus, **37** and **43** can serve as leads to develop a novel class of antiangiogenic agents.

Keywords

Tricyclic thiazoles; endothelial cells; angiogenesis

Angiogenesis plays an essential role in tumor growth and metastasis. Inhibitors of angiogenesis are emerging as a new class of anticancer drugs.^{1,2,3} In the clinic, it has been found that inclusion of an antiangiogenic drug like bevacizumab, sunitinib, or sorafenib in the combination chemotherapy produces significant survival benefits^{4,5} and hence, antiangiogenic drugs have become an integral part of front-line therapy in treating different types of cancers. Unfortunately, primary and acquired resistance to antiangiogenic therapy is becoming a real impediment and new agents with novel mechanisms of action are urgently needed to tackle this problem.⁶ Because proliferation of endothelial cells is an obligatory step for in vivo angiogenesis, direct growth inhibition of endothelial cells in culture has come to serve as a proxy for anti-angiogenesis screening. During a routine test of target compounds and intermediates synthesized in our laboratory, we discovered that four tricyclic thiazoles—**3**, **7**, **9**, and **11** (Table 1)—were moderately antiproliferative against human umbilical vein endothelial cells (HUVEC) in a [³H]-thymidine incorporation assay. In fact, these tricyclic thiazoles had been synthesized in the course of our development of methionine aminopeptidase (MetAP) inhibitors as antimycobacterial agents.⁷ It has been established earlier using fumagillin that inhibition of human MetAP2 leads to the growth

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Supplementary data: Supplementary data associated with this article which includes synthesis and analytical data for tricyclic thiazoles, protocols for cell proliferation assays, representative dose-response curves of HUVEC inhibition, and protocol for HUVEC tube formation assay can be found in the online version at <http://dx.doi.org/10.1016/j.bmcl.2013>.

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inhibition of HUVEC.^{8,9} However, tricyclic thiazoles **3**, **7**, **9**, and **11** did not inhibit (up to 20 μ M) either isoforms of human MetAPs (hMetAP1 and hMetAP2), suggesting that HUVEC inhibition proceeded through a different mechanism. Herein, we disclose a structure-activity relationship (SAR) study of this novel class of endothelial cell inhibitors.

The tricyclic thiazoles were synthesized as per our earlier procedure⁷ using a variation of Hantzsch thiazole synthesis. Two typical examples are shown in Scheme 1. Briefly condensation of 6-chlorothiochroman-4-one (eq. 1) or 1-benzosuberone (eq. 2) with thiourea in the presence of iodine at 100 °C generated the tricyclic thiazoleamines **36** and **32**, respectively, which upon neutralization served as starting materials for the subsequent steps. Thiazoleamine **37** (Scheme 1, eq. 1) was prepared by forming the Schiff base followed by reduction using sodium cyanoborohydride. Thiazoleamine **32** was treated with 2,6-difluorobenzoyl chloride in triethylamine containing dichloromethane to obtain the corresponding benzamide derivative **43** (Scheme 1, eq. 2). Thiazoleamine **45** (Table 2) was obtained by alkylating amine **30** with 6-azidohex-1-yl tosylate following the procedure of Salvatore et al.¹⁰ Benzamides **46** and **47** (Table 2) were synthesized by coupling thiazoleamine **30** and **32**, respectively, with 4-propynyloxybenzoic acid (see Supplementary data).

A collection of 35 tricyclic thiazole derivatives (Table 1) comprising of thiazoleamines and their amides were synthesized and screened for their antiproliferative activities in HUVEC culture. Among a series of 4*H*,5*H*-naphto[1,2-*d*]thiazoleamines containing different patterns of methoxy substitutions on the A-ring (see eq. 1 in Scheme 1 for ring designation), all the parent primary amines **1**, **4**, **6**, **10**, and **12** failed to register an IC₅₀ below 10 μ M. Only a single furanyl substituted thiazoleamine **7** showed a moderate inhibition of HUVEC (4.5 μ M). We had acetamide, propanamide, and hexanamide derivatives in this series where both the hexanamides **3** and **9** inhibited HUVEC proliferation moderately (3.0 and 3.7 μ M, respectively), but none of the acetamides except **11** (4.2 μ M) exhibited HUVEC inhibition. Compounds **14** and **15**, comprising of an inversely fused tricyclic thiazole ring system, were ineffective. Next in our SAR effort, we produced and screened thiazoles embodying a contracted B-ring (**16–19**), a completely severed B-ring (**20–23**), and a totally eliminated A-ring (**24** and **25**). None of these compounds (**16–25**) except *N*-(6-methoxy-8*H*-indeno[1,2-*d*]thiazol-2-yl)acetamide (**17**, 4.4 μ M) exhibited HUVEC inhibition. We then introduced an oxygen atom in the B-ring to obtain chromenothiazole derivatives **26** through **29**, but they were all inactive. Finally, when we prepared and tested thiochromenothiazole derivatives **30** and **31**, both the parent thiazoleamine and its propanamide were found to inhibit HUVEC proliferation with an IC₅₀ around 2 μ M. Another variation we tried was to enlarge the B-ring to make seven-membered tricyclic thiazole derivatives **32** through **35** and in this set all of them exhibited IC₅₀ values below 10 μ M, except thiazoleamine **32**. Reading into the SAR data accumulated so far, we reasoned that the important feature contributing to the antiproliferative activity of the thiazoleamines against HUVEC is the tricyclic framework (chiefly based on the fact that compounds **20** through **25** were inactive). We also concluded that the amino group of tricyclic thiazoleamine is permissive to derivatization without significant loss in activity (IC₅₀'s of amine **30** and amides **3**, **9**, **31**, **33**, and **34** are below 4 μ M). Interestingly, further analysis revealed that the two structural scaffolds with good HUVEC inhibitory activity, namely thiochromenothiazole and benzocycloheptathiazole are perfectly superimposable (Figure 1) due to the larger atomic radius of sulfur in **31** spanning the space occupied by two methylene groups in the seven-membered ring counterpart **33**. Thus, we reckoned that further medicinal chemistry based upon thiochromenothiazole and benzocycloheptathiazole skeletons is likely to afford more potent inhibitors of HUVEC proliferation. We synthesized and evaluated 8-chloro substituted thiochromeno[1,2-*d*]thiazoleamine **36** which was found to be about as potent as the parent thiazoleamine **30** for

inhibiting the growth of HUVEC (Table 2). Interestingly, when thiazoleamine **36** was alkylated with 2-furanylmethyl group, the resulting secondary amine **37** became the most potent inhibitor of HUVEC in the entire collection of compounds. The potency of HUVEC inhibition of ethyl carbamate **38** was close to that of propanamide **31**. In the series of benzocycloheptathiazoles, imide **34** turned out to be more potent for HUVEC inhibition than the amide **33** (1.5 versus 3.7 μM). However, carbamate **39** was an inferior inhibitor. Remarkably, in the series of thiochromenothiazoles both amine (**30**, **36**, and **37**) and amide (**31** and **38**) derivatives were generally potent at inhibiting HUVEC proliferation, whereas in the case of benzocycloheptathiazoles—**32** through **34**, **39**, and **40**—strictly the amide **33** and imide **34** were good inhibitors, but the parent amines **32** and **40** were poor inhibitors. At this juncture it was deemed necessary to assess if the HUVEC inhibiting thiochromenothiazoles and benzocycloheptathiazoles were capable of exhibiting cell-type selectivity. We assessed the effects of these compounds on the proliferation of three cancer cell lines—Jurkat-T (T cell leukemia), BT474 (breast cancer), and HeLa (cervical cancer). Thiochromenothiazoles **30**, **31**, and **36** through **38** were in general only two-fold more selective to HUVEC. On the other hand, of the two benzocycloheptathiazole amides that inhibited HUVEC potently, **33** showed an impressive selectivity for HUVEC (>5 fold), and **34** was reasonably selective.

Upon a closer scrutiny of the cell inhibition data, we became curious with the observation that compounds **30**, **34**, **36**, and **37** manifested high inhibitory activities against Jurkat-T cells besides inhibiting HUVEC. A thorough literature search revealed that analogs of *N*-(4*H*,5*H*,6*H*-benzo[6,7]cyclohepta[2,1-*d*]thiazol-2-yl)2,6-difluorobenzamide and the corresponding inversely fused thiazole derivatives were claimed to be potential immunosuppressive agents.¹¹ Thus, we synthesized few more analogs consisting of 2,6-difluorobenzamide moiety—**41** and **42** derived from thiochromenothiazoles, and **43** and **44** derived from benzocycloheptathiazoles. Among these benzamides, **43** turned out to be a potent (1.5 μM) and highly selective inhibitor of HUVEC, while inhibition by **42** was moderate. Surprisingly, benzocycloheptathiazoleamide **44** was nearly ten times less potent than **43** and this can only be attributed to the presence of a fluoro group on the A-ring.

Analyzing the SAR of thiochromenothiazole and benzocycloheptathiazole derivatives thus far (Table 2), we concluded that the A-ring and the 2-amino group on the thiazole are the two apparent loci amenable for modifications to seek potency improvement and perhaps attachment of affinity probes. Thus, we prepared two thiochromenothiazoleamine derivatives **45** and **46**, and a benzocycloheptathiazoleamide **47**, all carrying probe attachable functionalities (via azide-alkyne Huisgen cycloaddition). Not only did these derivatives retain HUVEC inhibitory activity, but also, amide **46** which is a 2.9 μM inhibitor, may serve as a useful tool in the target identification studies.

In order to evaluate the effectiveness of these two classes of compounds in blocking angiogenesis, we performed in vitro tube formation assay¹² with a potent thiochromenothiazoleamine **37** and a selectively potent benzocycloheptathiazole derivative **43** (Figure 2). The HUVEC tube formation was inhibited by both **37** and **43** in a dose-dependent manner. An aggregate of tube length, size, and number of junctions was quantified (Figure 2). Thiazoleamine **37** inhibited HUVEC tube formation very potently ($\approx 35\%$ of control at 5 μM) whereas **43** showed a moderate inhibition ($\approx 75\%$ of control at 5 μM).

In conclusion, we identified tricyclic thiazole derivatives as inhibitors of HUVEC proliferation with distinct structures and new mechanisms. We prepared, in total, 47 analogs by systematically manipulating the three rings of the tricyclic thiazoles. Two tricyclic systems, thiochromenothiazoles and benzocycloheptathiazoles emerged as the most potent inhibitors. Two most potent HUVEC inhibitors **37** and **43** (1.1 and 1.5 μM respectively)

representing the two tricyclic scaffolds stated above, also inhibited endothelial tube formation. Also, benzocycloheptathiazole **43** is a very selective inhibitor of HUVEC and hence these compounds serve as potential leads for the development of promising antiangiogenic agents. We will pursue synthesis of more analogs related to these two lead structures to seek further improvement in potency and we plan to undertake a study to elucidate the mechanism of HUVEC inhibition by thiochromenothiazoles and benzocycloheptathiazoles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of **31** (green) and **33** (gray) were minimized (MM2) and were overlayed using Chem3D.

Control (DMSO)

37 (2 μ M) **37** (5 μ M) **37** (10 μ M) **43** (2 μ M) **43** (5 μ M) **43** (10 μ M) **Figure 2.**

Representative images of tubes formed on Matrigel after seeding with HUVEC and treating with DMSO, **37** or **43** (2, 5, and 10 μ M). Tubes were stained with Calcein-AM and observed under fluorescence microscope at 40 $\times$ magnification. Tube formation was quantified using AngioQuant v1.33 software (The MathWorks, Natick, MA). The experiments were performed in triplicate.

Scheme 1.

A typical synthesis of tricyclic thiazole derivatives. Conditions: *i.* thiourea, iodine, EtOH, 100 °C, 3 h, then aqueous NaHCO₃; *ii.* furfural/MgSO₄/MeOH and then NaBH₃CN; *iii.* 2,6-difluorobenzoyl chloride, Et₃N/DCM.

Table 1

Inhibition of HUVEC proliferation by thiazoles.

Entry	structure	Substituents (if not specified, R [#] = H)	HUVEC ^a (IC ₅₀ , μM)
1		R ² = H	>10
2		R ² = COMe	>10
3		R ² = CO(CH ₂) ₄ Me	3.0 ± 1.0
4		R ² = H, R ⁶ = OMe	>10
5		R ² = COMe, R ⁶ = OMe	>10
6		R ² = H, R ⁷ = OMe	>10
7		R ² = CH ₂ (2-furanyl), R ⁷ = OMe	4.5 ± 1.2
8		R ² = COMe, R ⁷ = OMe	>10
9		R ² = CO(CH ₂) ₄ Me, R ⁷ = OMe	3.7 ± 1.0
10		R ² = H, R ⁸ = OMe	>10
11		R ² = COMe, R ⁸ = OMe	4.2 ± 1.1
12		R ² = H, R ⁷ , R ⁸ = OMe	>10
13		R ² = COEt, R ⁷ , R ⁸ = OMe	>10
14		R ² = H	>10
15		R ² = COMe	>10
16		R ² = H, R ⁶ = OMe	>10
17		R ² = COMe, R ⁶ = OMe	4.4 ± 1.5
18		R ² = H, R ⁶ = F	>10
19		R ² = COEt, R ⁶ = F	>10
20		R ² = H, R ⁶ , R ⁷ = OMe	>10
21		R ² = COMe, R ⁶ , R ⁷ = OMe	>10
22		R ² = H, R ⁷ = Cl	>10
23		R ² = COMe, R ⁷ = Cl	>10
24		R ² = H	>10
25		R ² = COEt	>10
26		R ² = H, X = O	>10
27		R ² = COEt, X = O	>10
28		R ² = H, X = O, R ⁸ = F	>10
29		R ² = COEt, X = O, R ⁸ = F	>10
30		R ² = H, X = S	1.9 ± 0.3
31		R ² = COEt, X = S	2.0 ± 0.3
32		R ¹ , R ² = H, X = CH ₂	>10
33		R ¹ = H, R ² = COEt, X = CH ₂	3.7 ± 1.1
34		R ¹ , R ² = COEt, X = CH ₂	1.5 ± 0.3

Entry	structure	Substituents (if not specified, R [#] = H)	HUVEC ^a (IC ₅₀ , μM)
35		R ¹ , R ² = H, X = O, R ⁸ -R ⁹ = OCH ₂ O	5.1 ± 1.1

^aIC₅₀ values are averages of three independent experiments and the ± standard deviation is given.

Table 2

Two classes of thiazoles: Antiproliferative activities against four cell lines (IC₅₀, μ M).

Entry	Structure	Substituents (if not specified, R [#] = H)	HUVEC ^a	Jurkat-T ^a	BT474 ^a	HeLa ^a	Selectivity index ^b
30		H	1.9 $\pm$ 0.3	0.9 $\pm$ 0.1	8.2 $\pm$ 1.4	6.8 $\pm$ 1.1	0.5/4.3/3.6
31		COEt	2.0 $\pm$ 0.3	4.9 $\pm$ 0.5	5.9 $\pm$ 1.1	8.0 $\pm$ 1.1	2.5/3.0/4.0
41		COPh(2,6-difluoro)	12.0 $\pm$ 2.2	11.8 $\pm$ 1.1	7.2 $\pm$ 1.3	13.1 $\pm$ 2.1	–
45		(CH ₂) ₆ N ₃	5.9 $\pm$ 1.1	ND	ND	ND	–
46		COPh(4-OCH ₂ C $\equiv$ CH)	2.9 $\pm$ 0.8	4.6 $\pm$ 0.9	0.3 $\pm$ 0.2	5.0 $\pm$ 1.1	1.6/0. 1/1.7
36		H	1.9 $\pm$ 0.4	1.6 $\pm$ 0.7	3.8 $\pm$ 1.1	11.9 $\pm$ 1.0	0.8/2.0/6.3
37		CH ₂ (2-furanyl)	1.1 $\pm$ 0.3	2.5 $\pm$ 0.6	2.3 $\pm$ 0.8	10.3 $\pm$ 1.1	2.3/2.1/9.4
38		CO ₂ Et	2.8 $\pm$ 0.4	6.3 $\pm$ 1.0	6.2 $\pm$ 1.1	6.5 $\pm$ 1.6	2.3/2.2/2.3
42		COPh(2,6-difluoro)	5.2 $\pm$ 0.4	17.3 $\pm$ 1.1	3.6 $\pm$ 1.1	>20	3.3/0.7/>4
32		R ¹ , R ² = H	19.1 $\pm$ 1.2	>20	>20	>20	–
33		R ² = COEt	3.7 $\pm$ 1.1	>20	>20	>20	>5/>5/>5
34		R ¹ , R ² = COEt	1.5 $\pm$ 0.3	2.8 $\pm$ 0.4	>20	5.9 $\pm$ 1.1	1.9/>13/4
39		R ² = CO ₂ Et	9.2 $\pm$ 1.2	>20	>20	>20	–
43		R ² = COPh(2,6-difluoro)	1.5 $\pm$ 0.4	>20	>20	15.2 $\pm$ 2.2	>13/>13/10
47		R ² = COPh(4-OCH ₂ C $\equiv$ CH)	7.3 $\pm$ 1.1	ND	ND	ND	–
40		H	14.8 $\pm$ 1.2	>20	>20	16.2 $\pm$ 2.3	–
44		COPh(2,6-difluoro)	13.5 $\pm$ 2.1	>20	>20	>20	–

^aIC₅₀ values are averages of three independent experiments and the $\pm$ standard deviation is given.^bSelectivity index: IC₅₀ values of Jurkat-T/BT474/HeLa compared to that of HUVEC. ND: Not determined.