Bioorganic & Medicinal Chemistry Letters 18 (2008) 4529–4532

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of *N*-mercaptoacylproline and *N*-mercaptoacylthiazolidine-4-carboxylic acid derivatives as leukotriene A_4 hydrolase inhibitors

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ARTICLE INFO

Article history:
Received 2 May 2008
Revised 9 July 2008
Accepted 11 July 2008
Available online 15 July 2008

Keywords:
LTA_4 hydrolase
LTA_4 hydrolase inhibitor
Epoxide hydrolase
N-mercaptoacyl-L-proline
N-mercaptoacylthiazolidine-4-carboxylic acid

ABSTRACT

We studied the synthetic modification of structurally similar *N*-mercaptoacyl-L-proline and (4*R*)-*N*-mercaptoacylthiazolidine-4-carboxylic acid to obtain potent leukotriene A_4 (LTA_4) hydrolase inhibitors. An *N*-mercaptoacyl group, (2*S*)-3-mercapto-2-methylpropionyl group, was effective for both scaffolds. Additional introduction of a large substituent such as 4-isopropylbenzylthio (**3f**), 4-*tert*-butylbenzylthio (**3l**) or 4-cyclohexylbenzylthio group (**3m**) with (*S*)-configuration at the C_4 position of proline yielded much more potent LTA_4 hydrolase inhibitors (IC_{50}; 52, 31, and 34 nM, respectively) than captopril (IC_{50}; 630,000 nM).

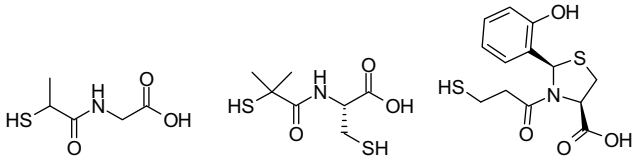
Labile epoxide leukotriene A_4 (LTA_4) is transformed into leukotriene B_4 (LTB_4) by LTA_4 hydrolase in the 5-lipooxygenase (5-LO) pathway of arachidonic acid metabolism. LTB_4 is a lipid mediator that induces chemokinetic, chemotactic and aggregation responses in polymorphonuclear leukocytes,[1,2] and it promotes adhesion of these cells to endothelial cell monolayers.[3] LTB_4 is produced in various cells, such as neutrophils, monocytes, macrophages, keratinocytes, lymphocytes and mast cells.[4] It plays important pathological roles in inflammatory diseases, such as rheumatoid arthritis,[5] psoriasis[6] and bowel disease.[7] Recent studies suggest that LTB_4 is involved in vascular inflammation and arteriosclerosis.[8] Prevention of either LTB_4 biosynthesis by inhibiting the 5-LO pathway, or LTB_4 binding to receptors, would be a suitable approach for designing anti-inflammatory drugs.

LTA_4 hydrolase (EC 3.3.2.6) locates in the cytosol[9] and nucleus.[10] Its membrane-bound form[11] has also been reported. This enzyme is a bifunctional zinc metalloenzyme,[12] which is a member of the MA clan of metallopeptidases such as angiotensin-converting enzyme (ACE) and thermolysin.[13] One of the functions is highly substrate-specific epoxide hydrolase activity. This catalytic reaction is rate-determining, and is the final step in LTB_4 biosynthesis. The other function is intrinsic arginyl aminopeptidase activity, which acts on various substrates.[14]

Several LTA_4 hydrolase inhibitors have been reported.[15] The general aminopeptidase inhibitor, bestatin, inhibits the epoxy hydrolase activity (IC_{50}; 4 μM).[16] The ACE inhibitor captopril also detracts the activity (IC_{50}; 14 μM).[17]

Since captopril retained a weak inhibitory activity against LTA_4 hydrolase, we examined other thiol compounds including SA446, which is a potent ACE inhibitor containing (4*R*)-thiazolidine-4-carboxylic acid (Fig. 1).[18–23] However, these compounds did not show any inhibitory activity.[24]

We examined the effects of the *N*-mercaptoacyl part of *N*-mercaptoacyl-L-proline **1**[25] and (4*R*)-*N*-mercaptoacylthiazolidine-4-carboxylic acid **2**[23] (Table 1). Among the derivatives, only (2*S*)-3-mercapto-2-methylpropionyl group (**1e** [captopril] and **2e**)



tiopronin (Thiola®) **bucillamine (Rimatil®)** **SA446**

Figure 1. Structures of tiopronin, bucillamine, and SA446.

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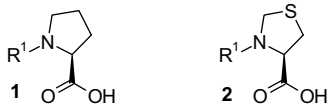
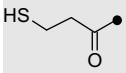
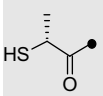
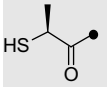
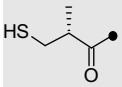
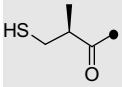
doi:10.1016/j.bmcl.2008.07.043

Table 1
LTA_4 hydrolase inhibitory activities of *N*-mercaptoacyl-L-proline and (4*R*)-*N*-mercaptoacylthiazolidine-4-carboxylic acid derivatives



R^1–	Compound **1**[a]	LTA_4 hydrolase % inhibition at 1 mM	Compound **2**[b]	LTA_4 hydrolase % inhibition at 1 mM
	**1a**	NI	**2a**	NI
	**1b**	ND	**2b**	NI
	**1c**	NI	**2c**	NI
	**1d**	5	**2d**	18
	**1e (captopril)**	59[c]	**2e**	58

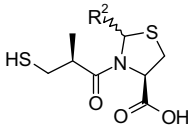
[a] Ref. 25.
[b] Ref. 23.
[c] IC_{50}: 630 μM, NI, no inhibition, ND, no data.

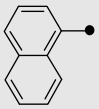
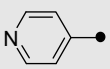
exhibited similar inhibitory potency against LTA_4 hydrolase, suggesting a strict steric requirement around the mercaptoacyl group. This result also suggests equivalency of the scaffolds of compounds **1** and **2** for the inhibition of LTA_4 hydrolase.

Table 2 shows the effect of R^2 on the (4*R*)-thiazolidine-4-carboxylic acid derivatives having the (2*S*)-3-mercapto-2-methylpropionyl group. Only compound **2g** that has a large substituent (R^2 = 1-naphtyl) showed an equipotent inhibitory activity with compound **2e**. Because the modification of R^1 or R^2 gave no more potent compound, we introduced R^3 to captopril (**1e**) (Table 3). *N*-Mercaptoacyl-4-substituted-L-proline derivatives have been reported to be ACE inhibitors;[26] the inhibition of LTA_4 hydrolase has not been observed.

Compound **3a** (R^3 = benzylthio) increased the inhibitory activity against LTA_4 hydrolase (175-fold) over captopril (**1e**, R^3 = H), as

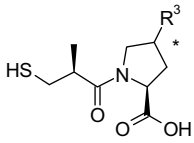
Table 2
LTA_4 hydrolase inhibitory activities of (4*R*)-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]thiazolidine-4-carboxylic acid derivatives[a]

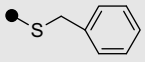
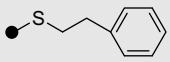
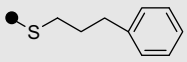
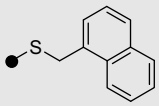
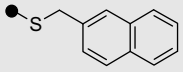
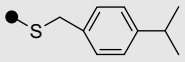
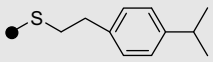
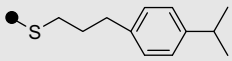
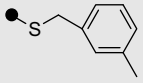
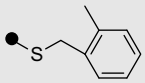
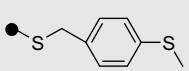
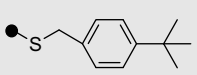
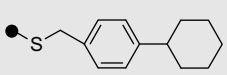
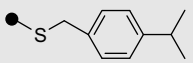


Compound	R^2–	LTA_4 hydrolase % inhibition at 1 mM
2e	H-	58
2f		NI
2g		58
2h		25
2i		36
2j		14

[a] Ref. 23, NI, no inhibition. Absolute configuration of R^2 is not determined.

Table 3
LTA_4 hydrolase and ACE inhibitory activities of *N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline derivatives



Compound	–R^3	Configuration[a]	IC_{50} (nM)	
			LTA_4 hydrolase	ACE
1e	–H	—	630,000	23[b]
3a		(*S*)	3600	2.4
3b		(*S*)	5100	0.18
3c		(*S*)	1100	0.029
3d		(*S*)	9200	3.2
3e		(*S*)	1100	4.2
3f		(*S*)	52	8.4
3g		(*S*)	790	19
3h		(*S*)	2700	15
3i		(*S*)	>10,000	0.9
3j		(*S*)	840	2.3
3k		(*S*)	120	4.1
3l		(*S*)	31	410
3m		(*S*)	34	280
4		(*R*)	290	3.3

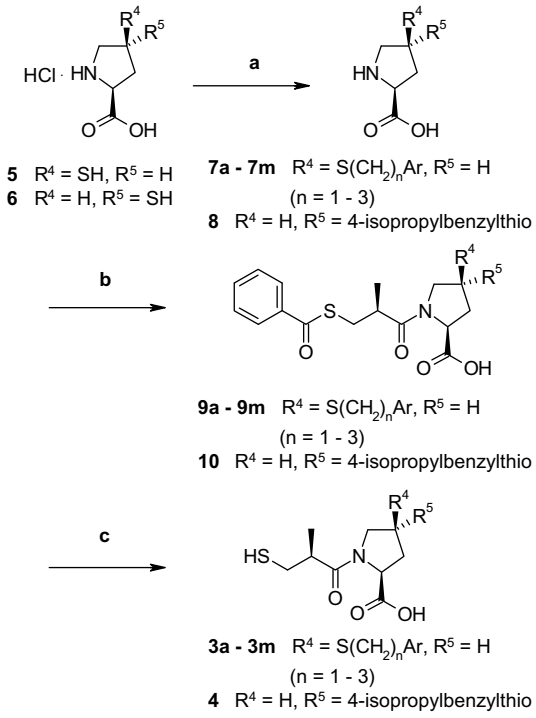
[a] Configuration of C_4 position of proline.
[b] Ref. 25.

well as with ACE[27] (10-fold) (Table 3). ACE inhibitory activities of compounds **3b** and **3c** increased remarkably with increasing bulkiness of R^3, yet LTA_4 hydrolase inhibition decreased (**3b**) or increased threefold (**3c**). Compound **3d** (R^3 = 1-naphtylmethylthio) was three times less potent than compound **3a**; compound **3e** (R^3 = 2-naphtylmethylthio) was three times more potent than compound **3a** against LTA_4 hydrolase, though these did not show significant change with ACE inhibitory activity.

The introduction of an isopropyl group (**3f**) to compound **3a** notably improved the inhibitory activity of LTA_4 hydrolase (12,000-fold) over captopril (**1e**). Conversions of the methylene moiety of the 4-isopropylbenzylthio group (**3f**) to ethylene (**3g**) or trimethylene (**3h**) reduced the inhibitory activities against LTA_4 hydrolase, but did not affect ACE. The 3-methylbenzylthio group (**3i**) lost the inhibitory activity completely. 2-Methylbenzylthio (**3j**) and 4-methythiobenzylthio groups (**3k**) exhibited moderate inhibitory activities against LTA_4 hydrolase. The replacement of 4-isopropyl of compound **3f** by *tert*-butyl (**3l**) or cyclohexyl group (**3m**) resulted in a potent LTA_4 hydrolase inhibitory activity similar to that of compound **3f**. The LTA_4 hydrolase inhibitory activities of these compounds were 20,000-fold more potent than that of captopril (**1e**). These substitutions were allowed to diminish the inhibitory ability against ACE.

The inversion of the stereochemistry of compound **3f** made compound **4** six times less potent against LTA_4 hydrolase.

N-Mercaptoacyl-4-arylalkylthio-L-proline derivatives **3a–m** and **4** were prepared as shown in Scheme 1. *cis*-4-Mercapto-L-proline **5**[28] was treated with the corresponding arylalkyl chlorides or bromides in the presence of aqueous sodium hydroxide to give compounds **7a–m**. The coupling reaction of compounds **7a–m** with 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate[29] yielded *cis*-4-arylalkylthio-*N*-[(2*S*)-3-benzoylthio-2-methylpropionyl]-L-proline derivatives **9a–m**. A reaction of compounds **9a–m** with aqueous ammonia gave *cis*-4-arylalkylthio-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline derivatives **3a–m**. Synthesis of *trans*-4-arylalkylthio-L-proline **4** was completed by a similar procedure using *trans*-4-mercapto-L-proline **6** as a starting material, which was prepared from *cis*-4-hydroxy-L-proline.



Scheme 1. Reagents and conditions: (a) corresponding arylalkyl chlorides or corresponding arylalkyl bromides, 2 M NaOH aq, EtOH, rt, overnight; (b) 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate, triethylamine, DMF, rt, overnight; (c) 28% NH_3 aq, rt, 1h.

X-ray crystallography of LTA_4 hydrolase with a bestatin molecule in the active site was reported, in which carbonyl O and the adjacent hydroxyl O of the ligand coordinated with the catalytic Zn ion.[30] Other small molecules, such as captopril, thioamine and amino hydroxamic acid derivatives, were also known to bind LTA_4 hydrolase by a similar ability to coordinate with Zn.[31] Several potent compounds (**3f**, **3l**, **3m**, and **4**) and captopril were subjected to a docking study to examine their binding mode in LTA_4 hydrolase (Fig. 2). The docking was performed within GOLD[32] with a distance constraint (1.5–3.5 Å) between the terminal S of the ligand and the catalytic Zn (tetrahedral) of LTA_4 hydrolase (1H6S.pdb). The obtained pose of captopril was similar to that of X-ray crystallography; coordination of the sulfhydryl S to the Zn ion, binding of the carboxyl O to Arg563, and the carbonyl O to Gly268 and Gly269.[31] The pyrrolidinyl and the mercaptopropionyl parts of the docked four compounds located over the captopril structure, and the substituted benzylthio group extended in a wide groove toward Phe340 (pose **a**) in the enzyme. Another pose (pose **b**) whose benzylthio group extended toward Arg568 was also found for the three compounds (**3f**, **3l**, and **4**). Torsional angle (C3–C4–*S*-C) classifying pose **a** and **b** of compound **3l** was 161° and 108°, respectively. The docking score ordinarily prioritizing binding poses was not effective between the two poses. Both the poses located deep inside the groove occupied different positions from the suggested binding position of LTA_4 in the enzyme.[30] The acyl part of compound **3l** was closely surrounded by several amino acid residues (Tyr267, Gly269, His295, Glu296, and Tyr383), suggesting limited tolerance of R^1 (Table 1). Substituent R^2 of compounds **2f–j** would be too close to the side chain of Tyr383 and be inappropriate to bind for them having particularly a twisted aromatic ring to the thiazolidine (Table 2). The wide cleft appeared to accept even larger R^3 than *tert*-butylbenzylthio group and be tolerable to sterically inversed compound **4** (Table 3). Analysis of ACE-captopril cocrystal revealed that the amide carbonyl oxygen of the ligand was hydrogen-bonded by two $N^{\varepsilon 2}$Hs of the imidazole parts of His353 and His513.[33] More flexible and efficient coordinating ability of these hydrogen-bonds than that of the two NHs (Gly268 and Gly269) of LTA_4 hydrolase main chain would have captopril more potent against ACE than LTA_4 hydrolase. Phenylalkylthio groups of compounds **3a–c** poses obtained by docking study with ACE (1UZF.pdb) located near hydrophobic area (Val379, Val380, Phe457, and Phe527), which appeared deficient in LTA_4 hydrolase. This suggests the preference for hydrophobicity

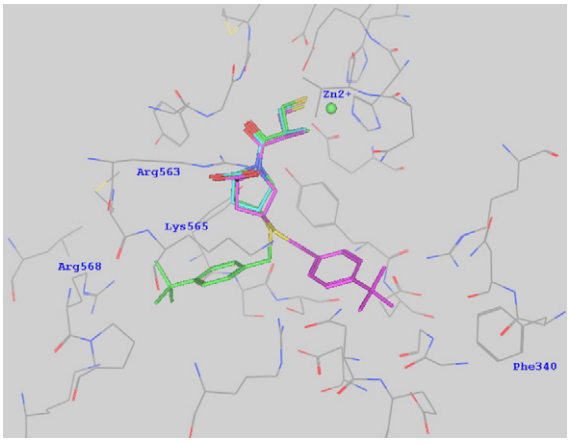


Figure 2. Possible poses of compound **3l** docked into LTA_4 hydrolase (pose **a**; purple, pose **b**; green). Captopril; blue

of R^3 of these compounds to ACE inhibitory activity and the difference between the two enzymes. Instead of these hydrophobic residues, the aminobutyl chain of Lys565 lying close to the phenylalkylthio groups may contribute to activity increase against LTA_4 hydrolase.

In conclusion, the inhibitory activities of *N*-mercaptoacyl-L-proline and (4*R*)-*N*-mercaptoacylthiazolidine-4-carboxylic acid derivatives against LTA_4 hydrolase were studied. The *N*-(2*S*)-3-mercapto-2-methylpropionyl group was necessary to bear the inhibitory activity for the L-proline and (4*R*)-thiazolidine-4-carboxylic acid derivatives. In addition to this chiral acyl group, the introduction of (*S*)-benzylthio group at the C_4 position of proline gave a potent LTA_4 hydrolase inhibitor, compound **3a** (IC_{50}; 3600 nM). Larger benzylthio groups such as 4-isopropylbenzylthio (**3f**), 4-*tert*-butylbenzylthio (**3l**) or 4-cyclohexylbenzylthio group (**3m**) yielded much more potent LTA_4 hydrolase inhibitors (IC_{50}; 52, 31, and 34 nM, respectively) than captopril (**1e**). In particular, compounds **3l** and **3m** decreased inhibitory ability against ACE.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.043.

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