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Synthesis and biological evaluation of *N*-mercaptoacylcysteine derivatives as leukotriene A_4 hydrolase inhibitors

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ABSTRACT

We studied synthetic modifications of *N*-mercaptoacylamino acid derivatives to develop a new class of leukotriene A_4 (LTA_4) hydrolase inhibitors. *S*-(4-Dimethylamino)benzyl-L-cysteine derivative **2a** (SA6541) showed inhibitory activity against LTA_4 hydrolase (IC_{50}, 270 nM) and selectivity over other metallopeptidases except angiotensin-converting enzyme (ACE, IC_{50}, 520 nM). Modification at the *para*-substituent of the phenyl ring of compound **2a** improved LTA_4 hydrolase inhibitory activity as well as selectivity over ACE. Finally, we obtained *S*-(4-cyclohexyl)benzy-L-cysteine derivatives **11l** and **16i** as potent and selective LTA_4 hydrolase inhibitors.

Leukotriene A_4 (LTA_4) hydrolase (EC 3.3.2.6) is a bifunctional zinc-containing metalloenzyme.[1] One of its functions is a highly substrate-specific epoxide hydrolase activity, which involves converting an unstable epoxide fatty acid derivative LTA_4 into a diol leukotriene B_4 (LTB_4), a potent proinflammatory mediator.[2] This catalytic reaction is the final and rate-determining step in LTB_4 biosynthesis. Therefore, inhibition of LTA_4 hydrolase would be a suitable approach for treatment of a variety of inflammatory diseases.[3] Another function is its intrinsic arginyl aminopeptidase activity. The biological role of which has not been elucidated thus far.[1]

Previously, we reported that 4-arylalkylthio-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline derivatives had inhibitory activities against LTA_4 hydrolase. Compounds **1a** and **1b**, in particular, exhibited more potent and selective inhibitory activities against the enzyme than its lead compound, captopril (Fig. 1).[4] This result prompted us to obtain more selective LTA_4 hydrolase inhibitors. On the basis of a previous study[4] and structural similarities to the zinc-containing metalloenzyme, we had screened other ACE inhibitors, *N*-mercaptoacylamino acid derivatives,[5] for inhibitory activities against LTA_4 hydrolase.[4,6,7a] Among the derivatives, we found that *S*-(4-dimethylamino)benzyl-L-cysteine derivative **2a** (SA6541, Fig. 1) possessed the desired inhibitory activity[7a] (Table 1).

Compound **2a** exhibited good anti-inflammatory effects after oral administration in murine.[7] Although the compound showed selectivity over other metallopeptidases, it still retained the ACE[8] inhibitory activity (Table 1). Therefore, we synthesized a new series of *N*-mercaptoacylamino acid derivatives to develop more potent and more selective LTA_4 hydrolase inhibitors than compound **2a**.

Compound **2a** and all the compounds listed in Table 2–5 were synthesized as below. Synthesis of **2a** was conducted as shown in Scheme 1.[7a] 4-Dimethylaminobenzyl alcohol **3** was treated with

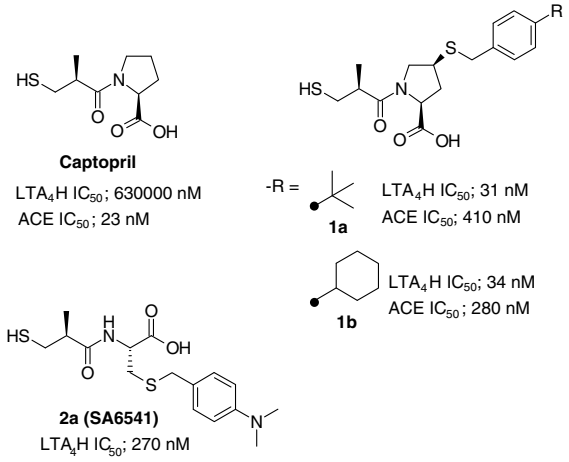

Figure 1. Structures of 4-arylalkylthio-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline derivatives and compound **2a** (SA6541).

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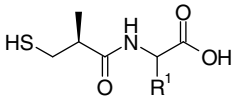
Table 1
Inhibitory activities of compound **2a** against other metallopeptidases

Enzyme	Compound **2a**
	IC_{50} or % inhibition at 100 μM
LTA_4 hydrolase	0.27 μM
Angiotensin-converting enzyme	0.52 μM
Aminopeptidase M	42%
Endopeptidase 24.11	16%
Endothelin-1 converting enzyme	8%
Type I collagenase	3%
Type III collagenase	No inhibition

Table 2
R^1 modifications of *N*-[(2*S*)-3-mercapto-2-methylpropionyl]amino acid



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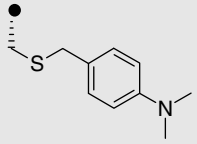
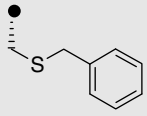
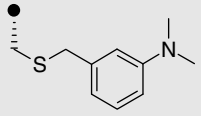
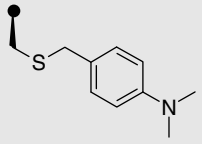
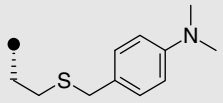
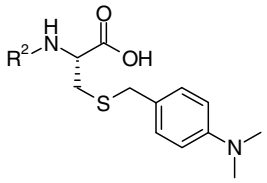
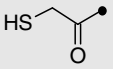
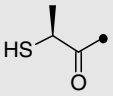
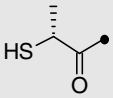
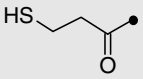
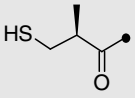
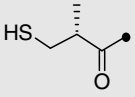
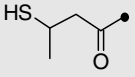
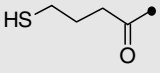
Compound	$-R^1$	IC_{50} (nM)
		LTA_4 hydrolase
2a (SA6541)		270
2b		>10,000
2c		>10,000
2d		>10,000
2e		>10,000

hydrobromic acid, followed by the reaction of resultant bromide **4** with *N*-Boc-L-cysteine to give *N*-Boc-*S*-(4-dimethylamino)benzyl-L-cysteine **5**. After removing the *N*-Boc group of compound **5**, coupling reactions with 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate[4] gave *N*-[(2*S*)-3-benzoylthio-2-methylpropionyl]-*S*-(4-dimethylamino)benzyl-L-cysteine **6**. Ammonolysis of compound **6** gave *S*-(4-dimethylamino)benzy-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-cysteine **2a**.

Compounds **2b–e** (Table 2), **7a–g** (Table 3), and **11q** (Table 4) were prepared in a similar way as compound **2a**. Compound **2b** was obtained via coupling reaction of *S*-benzyl-L-cysteine with 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate to give *N*-[(2*S*)-3-benzoylthio-2-methylpropionyl]-*S*-benzyl-L-cysteine followed by deprotection with aqueous ammonia yielding the desired compound. Synthesis of compounds **2c** and **11q** was conducted with 3-dimethylaminobenzyl alcohol and 4-diethylaminobenzyl alcohol as starting materials, respectively. Compounds **2d** and **2e** were

Table 3
R^2 modifications of *S*-(4-dimethylamino)benzyl-L-cysteine



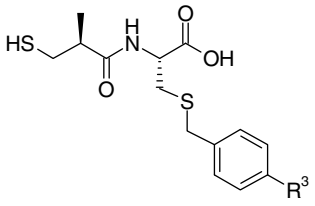
Compound	R^2-	IC_{50} (nM)
		LTA_4 hydrolase
7a		470
7b		>10,000
7c		>10,000
7d		>10,000
2a (SA6541)		270
7e		1500
7f		7400
7g		>10,000

prepared from *N*-Boc-D-cysteine and *N*-Boc-L-homocysteine, respectively. Introduction of the R^2 moieties of compounds **7a–g** was accomplished by using corresponding active esters derived from *S*-benzoylthioalkanoic acids[9] instead of 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate in Scheme 1.

Syntheses of compounds **11a–p** and **11r–u** in Table 4 were treated as shown in Scheme 2. Appropriate 4-substituted benzyl chlorides and bromides (**8a–p** and **8r–u**) were combined with L-cysteine in the presence of aqueous sodium hydroxide to yield *S*-(4-substituted)benzyl-L-cysteine derivatives **9a–p** and **9r–u**.[10] The resultant compounds were then coupled with 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate to yield *N*-[(2*S*)-3-benzoylthio-2-methylpropionyl]-*S*-(4-substituted)benzyl-L-cysteine derivatives (**10a–p** and **10r–u**). Reaction of compounds **10a–p** and **10r–u** with aqueous ammonia gave compounds **11a–p** and **11r–u**. Compound **12** (Table 5) was synthesized in a similar way by using L-penicillamine instead of L-cysteine.

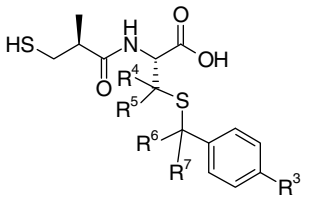
Scheme 3 represents the synthesis of compounds **16a–i** (Table 5). Corresponding alcohols **13a–i** together with L-cysteine were treated with hydrochloric acid at 55–65 °C followed by *N*-Boc protection for isolation and purification by flash chromatography, yielding *N*-Boc-*S*-substituted-L-cysteine derivatives **14a–i**. After removing the *N*-Boc groups of **14a–i**, coupling reactions with 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate gave

Table 4
R^3 modifications of *S*-benzyl-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-cysteine



Compound	R^3	IC_{50} (nM)	
		LTA_4 hydrolase	ACE
2b	H	>10,000	34
11a	F	>10,000	100
11b	Cl	1700	250
11c	Br	610	280
11d	I	15	140
11e	CH_3	7200	240
11f	CF_3	140	340
11g	C_2H_5	280	21
11h	*n*-Pr	72	210
11i	*i*-Pr	200	300
11j	*t*-Bu	24	130
11k	Ph	600	280
11l	*c*-Hex	79	4000
11m	OCH_3	2400	100
11n	OCF_3	400	260
11o	OC_2H_5	640	340
11p	OPh	1700	370
2a (SA6541)	$N(CH_3)_2$	270	520
11q	$N(C_2H_5)_2$	900	4600
11r	CN	530	300
11s	NO_2	4900	320
11t	SCH_3	46	300
11u	SO_2CH_3	160	300

Table 5
R^3, R^4, R^5, R^6, and R^7 modifications of *S*-benzyl-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-cysteine

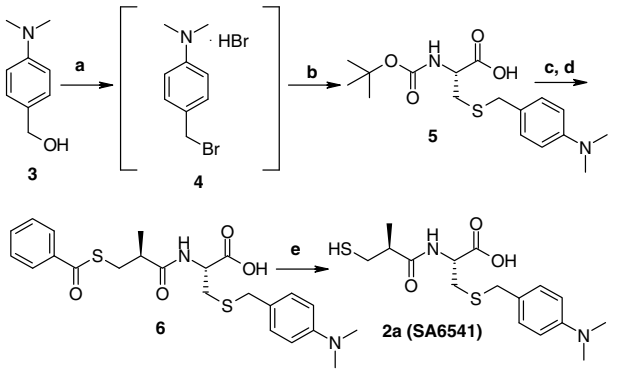


Compound	R^3	R^4	R^5	R^6	R^7	IC_{50} (nM)	
						LTA_4 hydrolase	ACE
11i	*i*-Pr	H	H	H	H	200	300
12	*i*-Pr	CH_3	CH_3	H	H	>10,000	>10,000
16a	*i*-Pr	H	H	H	CH_3	55	200
16b	*i*-Pr	H	H	H	C_2H_5	67	340
16c	*i*-Pr	H	H	H	*n*-Pr	180	420
16d	*i*-Pr	H	H	H	*i*-Pr	520	430
16e	*i*-Pr	H	H	H	*n*-Bu	510	3600
16f	*i*-Pr	H	H	H	Ph	91	1700
16g	*i*-Pr	H	H	CH_3	CH_3	79	260
11l	*c*-Hex	H	H	H	H	79	4000
16h	*c*-Hex	H	H	H	Ph	210	>10,000
16i	*c*-Hex	H	H	CH_3	CH_3	55	3000

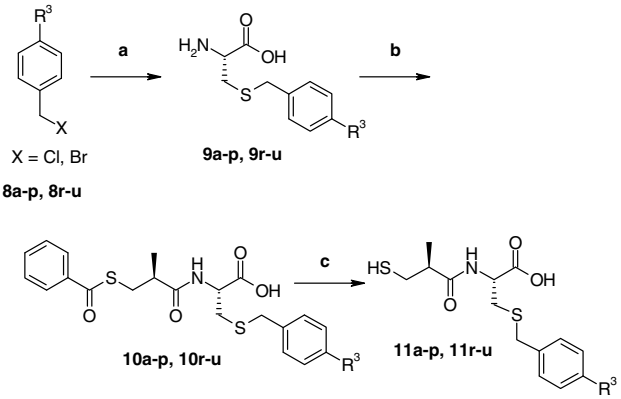
N-[(2*S*)-3-benzoylthio-2-methylpropionyl]-*S*-(α-substituted-4-substituted)benzyl-L-cysteine derivatives **15a–i**. A reaction of compounds **15a–i** with aqueous ammonia resulted in compounds **16a–i**.

Table 2 shows the effect of R^1 moiety of *N*-[(2*S*)-3-mercapto-2-methylpropionyl]amino acid derivatives. *S*-(4-dimethylamino)-benzyl-L-cysteine derivative **2a** (SA6541) exhibited LTA_4 hydrolase inhibitory activity. However, a regioisomer (**2c**), the epimer (**2d**) of compound **2a** or the desdimethylamino analog (**2b**) did not show the inhibitory activity. Neither did the derivative of L-homocysteine (**2e**) (IC_{50} > 10,000 nM).

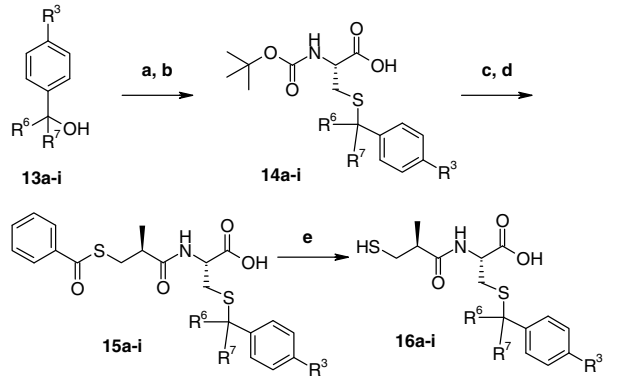
We then studied alterations in the *N*-mercaptoacyl moiety of the compound **2a** (Table 3). Introduction of the mercaptoacetyl group (**7a**) slightly reduced the inhibitory activity. Neither the 2-mercaptopropionyl group (**7b** and **7c**) nor the 3-mercaptopropionyl group



Scheme 1. Reagents and conditions: (a) 47% HBr aq 120 °C, 2.5 h; (b) Boc-L-Cys-OH, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 2.5 h; (c) 4 M HCl in dioxane, rt, 1 h; (d) 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate, triethylamine, DMF, CH_2Cl_2, rt, overnight; (e) 28% NH_3 aq, rt, 1 h.



Scheme 2. Reagents and conditions: (a) L-Cysteine HCl·H_2O, 2 M NaOH aq, EtOH, $CHCl_3$, rt, overnight; (b) 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate, triethylamine, DMF, rt, overnight; (c) 28% NH_3 aq, rt, 1 h.



Scheme 3. Reagents and conditions: (a) L-Cysteine HCl·H_2O, 2 M HCl aq, dioxane, 55–65 °C, overnight; (b) $(Boc)_2O$, triethylamine, THF, rt, 4 h; (c) 4 M HCl in dioxane, rt, 1 h; (d) 4-nitrophenyl (2*S*)-3-benzoylthio-2-methylpropionate, triethylamine, DMF, rt; (e) 28% NH_3 aq, rt, 1 h.

(**7d**) were efficacious for raising LTA_4 hydrolase inhibitory activity (IC_{50} > 10,000 nM). Compound **7e**, the epimer of **2a**, decreased the activity. The 3-mercaptobutyryl group (**7f**) markedly reduced LTA_4 hydrolase inhibitory activity, and the 4-mercaptobutyryl group (**7g**) decreased it even further (IC_{50} > 10,000 nM). These results suggest that the steric requirements of the enzyme surrounding the acyl moiety is highly stringent—much like those of *N*-mercaptoacyl-L-proline and (4*R*)-*N*-mercaptoacylthiazolidine-4-carboxylic acid derivatives which we previously reported.[4]

Since the *S*-benzyl-L-cysteine derivative **2b** and the *S*-(3-dimethylamino)benzyl-L-cysteine derivative **2c** did not show inhibitory activities against LTA_4 hydrolase (Table 2), we made efforts only to introduce *para*-substituents (Table 4). Introduction of the fluorine atom (**11a**) did not appear to improve activity against LTA_4 hydrolase. An iodine atom (**11d**) created the most potent LTA_4 hydrolase inhibition in this series. Introduction of the methyl group (**11e**) contributed to only weak inhibitory activity against the enzyme and trifluoromethyl (**11f**), ethyl (**11g**), isopropyl (**11i**), and phenyl (**11k**) groups raised the activity moderately. Introduction of *n*-propyl (**11h**), *tert*-butyl (**11j**), and cyclohexyl (**11l**) groups improved the activities further. Notably, compound **11l** showed potent LTA_4 hydrolase inhibitory activity (IC_{50}; 79 nM) with a small inhibition against ACE (IC_{50}, 4000 nM). Introduction of alkoxy and phenoxy groups (**11m–p**), diethylamino (**11q**), cyano (**11r**), and nitro (**11s**) groups showed weak or moderate inhibitory activities against LTA_4 hydrolase. Adoption of the methylthio group made the compound (**11t**) potent; however, the methanesulfonyl group (**11u**) did not. Quantitative structure–activity relationship (QSAR) analysis by multi-regression analysis of compounds **11b–u** suggested a quadratic relation with mr (molar refractivity) of R^3. The optimum value for inhibition was mr_{opt} = 10.36, which corresponded to **11j** (R^3 = *t*-Bu).[11]

To examine the effects of substituents at β- and δ-positions on the *S*-benzyl-L-cysteine moiety, we modified these portions of compound **11i**. Though introduction of the *gem*-dimethyl group at the β-position (compound **12**, Table 5) resulted in loss of activity (IC_{50} > 10,000 nM), the same group at the δ-position improved the inhibitory activity against LTA_4 hydrolase (compound **16g**, Table 5). Comparison of conformational energies (ΔE)[12] among compounds **11i**, **12** and **16g** taking the active conformation (pose **a**), which will be discussed later, suggested that compound **12** had approximately 2–4 kcal/mol higher energy than the other two, a possible reason why compound **12** lost its inhibitory activity. Therefore, we focused on the modification at the benzyl (δ-methylene) position (R^6 and R^7, Table 5) of the *S*-benzyl-L-cysteine derivatives. Table 5 outlines these results. For those compounds with R^3 = *i*-Pr, compounds **16a** (R^6 = H and R^7 = CH_3), **16b** (R^6 = H and R^7 = C_2H_5), **16f** (R^6 = H and R^7 = Ph), and **16g** (R^6 and R^7 = CH_3) showed higher LTA_4 hydrolase inhibitory activities compared with that of compound **11i** (R^6 and R^7 = H). Among them, compound **16f** inhibited LTA_4 hydrolase with nineteen times more potency than ACE. LTA_4 hydrolase inhibitory activities of compounds **16d** (R^6 = H, R^7 = *i*-Pr) and **16e** (R^6 = H and R^7 = *n*-Bu) decreased a little. When R^3 was a cyclohexyl, ACE inhibitory activity of compound **16h** (R^6 = H and R^7 = Ph) markedly decreased, however, maintaining LTA_4 hydrolase inhibitory activity. A similar trend was also found for **11l** (R^6 and R^7 = H) and **16i** (R^6 and R^7 = CH_3) compounds.

Structures of LTA_4 hydrolase analyzed by X-ray crystallography have been reported in which most ligands lie along the binding site of LTA_4 with a binding to catalytic Zn^{2+}.[13,14] Captopril, a weak LTA_4 hydrolase inhibitor, is also known to bind by its terminal S to Zn^{2+}. Previously, we described possible binding poses in the enzyme of mercaptoacylproline derivatives **1a**, **1b**, and captopril as a reference.[4] Their pyrrolidinyl and the mercaptoacyl parts were located over each other. In a similar way, docking poses within GOLD[15] of several potent compounds (**11d**, **11j**, **11l**, and **11t**) were examined (1H6S.pdb). Every compound bound to Arg563 by its carboxyl group, to Gly268 and Gly269 by its amide carbonyl O and to Zn^{2+} by its sulfhydryl S. Substituted benzyl portion elongated toward Phe340 and occupied a similar location within the pose **a** of compounds **1a** and **1b**[4] (Fig. 2). Another pose, whose substituted benzyl part heading Arg568 was comparable to pose **b** of compounds **1a** and **1b**,[4] was found for those except for compound **11l**. In *gem*-dimethyl analogs, while active compounds (**16g** and **16i**) docked in **a**- and **b**-like poses, inactive compound **12** did so only in **b**-like pose. This fact suggests that the binding of the active compounds occur in pose **a**. A docking study of compounds **2b** and **11g** into ACE (1UZF.pdb) gave analogous poses to **a** but not **b** in LTA_4 hydrolase. We also observed this pattern for the proline type inhibitor **1a** in its docking into ACE. Although estimation of binding free energies of compound **11j** calculated by MM/GBSA[16] did not give a clear preference between the two poses, five different runs indicated that pose **a** was more likely than **b**, and this tendency was also displayed by compound **1a**. Consequently, as a binding pose (active conformation) of compound **11j** as well as compound **1a**, pose **a** is plausible.

The groove of LTA_4 hydrolase around the cyclohexylbenzylthio group of compound **11l** in pose **a** was wide spread and comprised several amino acid residues (Asn291, Val322, Arg326, Glu348, Ser380, Glu384, and Lys565). However, the counterpart of ACE around compound **2b** consisting of residues (Thr282, Val379, Val380, Asp415, Asp453, Lys454, and Phe527) was narrow and limited. Particularly, Glu376 extends its side chain to obscure the space. Table 4 displays these results. LTA_4 hydrolase inhibitory activity increased with increasing bulkiness of R^3 from compound **2b** (R^3 = H) to compound **11j** (R^3 = *t*-Bu). Interaction between R^3 and the surrounding amino acid residues will be essential to express activity since compound **2b** had no activity. On the other hand, potent ACE inhibition was observed in compounds **2b** and **11g**, both with small R^3. The inhibitory activity appeared to gradually decrease with increasing bulkiness of R^3 and abruptly decreased in **11l** and **11q**. A large symmetrical substituent with respect to the bonding axis of R^3 in compounds **11l** and **11q** may not be able to circumvent the Glu376 side chain to effectively bind to ACE. For the non-active compound (**12**) with a *gem*-dimethyl group at β-position, Lys565 may hamper binding to LTA_4 hydrolase in addition to yielding an unfavorable conformational energy. When R^7 was large, the substituent would bump against His383 and Phe457 in ACE to reduce the inhibitory activity.

In conclusion, we studied synthetic modifications of the lead compound **2a** to develop potent and selective LTA_4 hydrolase inhibitors. Modification at the *para*-substituent of the phenyl ring of compound **2a** improved LTA_4 hydrolase inhibitory activity and made the iodo derivative **11d** the most potent (IC_{50}, 15 nM). Another modification at this position also improved selectivity for

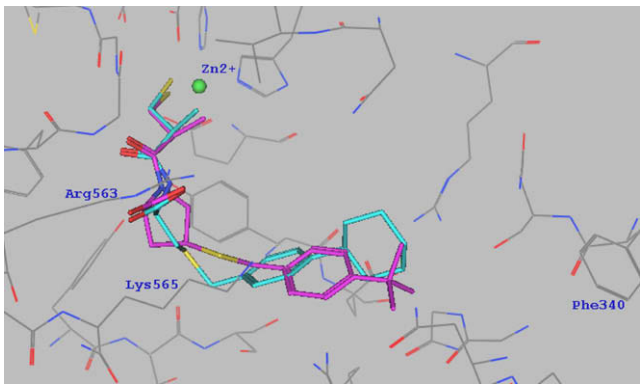


Figure 2. Plausible poses (pose **a**) of compounds **1a** (purple) and **11l** (cyan) docked into LTA_4 hydrolase.

LTA_4 hydrolase versus ACE. In particular, compounds **11l** and **16i** containing cyclohexyl group exhibited potent LTA_4 hydrolase inhibitory activities (IC_{50}, 79 and 55 nM, respectively) with a small inhibition of ACE (IC_{50}, 4000 and 3000 nM, respectively).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.042.

References and notes

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