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Structure–Activity Study of L-Amino Acid-Based N-Type Calcium Channel Blockers

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Abstract—Synthesis and structure–activity relationship (SAR) study of L-amino acid-based N-type calcium channel blockers are described. The compounds synthesized were evaluated for inhibitory activity against both N-type and L-type calcium channels focusing on selectivity to reduce cardiovascular side effects due to blocking of L-type calcium channels. In the course of screening of our compound library, *N*-(*t*-butoxycarbonyl)-L-aspartic acid derivative **1a** was identified as an initial lead compound for a new series of N-type calcium channel blockers, which inhibited calcium influx into IMR-32 human neuroblastoma cells with an IC_{50} of 3.4 μM. Compound **1a** also exhibited blockade of N-type calcium channel current in electrophysiological experiment using IMR-32 cells (34% inhibition at 10 μM, $n=3$). As a consequence of conversion of amino acid residue of **1a**, compound **12a**, that include *N*-(*t*-butoxycarbonyl)-L-cysteine, was found to be a potent N-type calcium channel blocker with an IC_{50} of 0.61 μM. Thus, L-cysteine was selected as a potential structural motif for further modification. Optimization of C- and N-terminals of L-cysteine using *S*-cyclohexylmethyl-L-cysteine as a central scaffold led to potent and selective N-type calcium channel blocker **21f**, which showed improved inhibitory potency (IC_{50} 0.12 μM) and 12-fold selectivity for N-type calcium channels over L-type channels.

Introduction

Voltage-dependent calcium channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization and regulate numerous intracellular functions including contraction, secretion, neurotransmitter release and gene expression.[1] Based on pharmacological and electrophysiological properties, these calcium channels are classified into several subtypes as L, N, P, Q, R or T-types. Among these channels, the N, P, Q, and R-type channels have all been shown to play key roles in neurotransmitter release.[2]

N-Type calcium channels are located at presynaptic terminals throughout neurons and directly mediate spinal transmission of pain signals from the peripheral to the central nervous system. ω-Conotoxin MVIIA, a 25-amino acid peptide, is a selective blocker of N-type calcium channels which shows analgesic activity when administered intrathecally.[3] Based on clinical observations reported for ω-Conotoxin MVIIA, it has been suggested that selective and orally active N-type calcium channel blockers could be useful for the treatment of pain. Therefore, synthetic efforts have focused on small-molecule, non-peptide N-type calcium channel blockers for analgesia or neuroprotection. A number of small-molecule N-type calcium channel blockers have been reported.[4] Examples of reported calcium channel blockers are flunarizine,[5] cilnidipine,[6] NS-649,[7] SB-206284,[8] PD176078,[4b] and E2050.[9] Some of these calcium channel blockers have been shown to be active in animal pain models.[5] However, most of these compounds block also the other calcium channel subtypes including L-type channels as well as sodium and potassium channels. Inhibition of cardiac L-type calcium channels causes hypotensive side effects, although blocking of neuronal L-type calcium channels shows neuroprotective effects.[10]

Here, we describe the synthesis and structure–activity relationships (SAR) of a novel series of L-amino

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acid-based N-type calcium channel blockers focusing on selectivity versus L-type calcium channels. The SAR study was examined based on inhibitory potency of calcium influx into IMR-32 human neuroblastoma cells,[11] as well as blockade of N-type calcium channel current in electrophysiological study. In order to estimate the selectivity versus L-type calcium channels, we also evaluated blocking activity of calcium influx into AtT-20 mouse pituitary tumor-derived cells.[12]

Chemistry

The synthesis of aspartic acid ester **1** was achieved by sequential esterification as shown in Scheme 1. Half ester **3** was obtained by esterification of Boc-L-Asp-OBzl with the appropriate alcohol in the presence of EDC and DMAP followed by deprotection of benzyl ester. Reaction of **3** with 4-methoxybenzylalcohol gave Boc-L-Asp diester **1a–e**. Compound **2**, an enantiomer of **1a,** was synthesized from Boc-D-Asp-OBzl by the same method as described for compound **1a**.

L-Serine ester **4** was prepared from Boc-L-Ser by reaction with 4-methoxybenzyl chloride and then with acid chloride as shown in Scheme 1. The L-serine derivatives **6-8** was obtained from commercially available Boc-L-Ser (OBzl) by catalytic hydrogeneration of the benzene ring using rhodium on alumina and subsequent condensation of carboxylic acid **5** with the appropriate alcohol or amine.

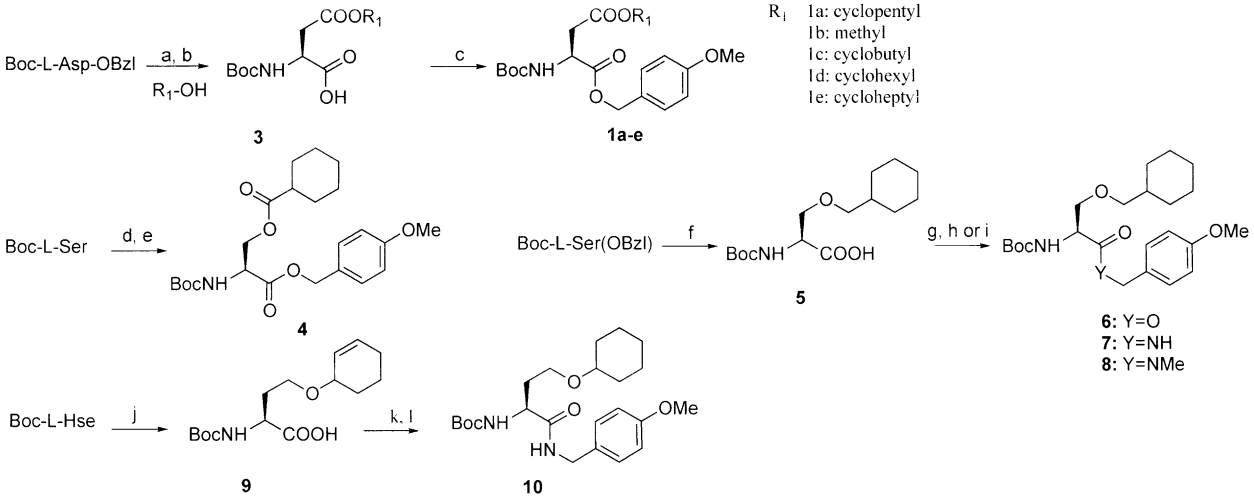
A similar method was applied for the ether formation of L-homo-serine (L-Hse) ether **9** by treatment with cyclohexene-1-bromide and Boc-L-Hse. Subsequent catalytic reduction of compound **9** and condensation with 4-methoxybenzylamine provided the Boc-L-Hse ether **10**.

Scheme 2 outlines the synthesis of the L-cysteine-based N-type calcium channel blockers. Treatment of L-cysteine with cyclohexylmethylbromide in the presence of aqueous sodium hydroxide in ethanol afforded *S*-cyclohexylmethyl-L-cysteine. Subsequent reaction of *S*-cyclohexylmethyl-L-cysteine with di-*tert*-butyl dicarbonate furnished *N*-Boc-protected compound **11** from L-cysteine by one-pot procedure. Reaction of carboxylic acid **11** with appropriately substituted benzylamines in the presence of EDC and HOBt and subsequent deprotection produced intermediates **13a–f** for N-terminal modification. **13a** was converted to the compounds **14–20**, which have various acyl group at the N-terminal by the reaction with corresponding carboxylic acid. Reaction of **13a–f** with *N*-Boc-(*R*)-thiazolidine-4-carboxylic acid in the presence of EDC and HOBt afforded compounds **21a–f**. Compounds (**21–26**) shown in Table 3 were synthesized from **21f** by deprotection and subsequent condensation or reductive amination.

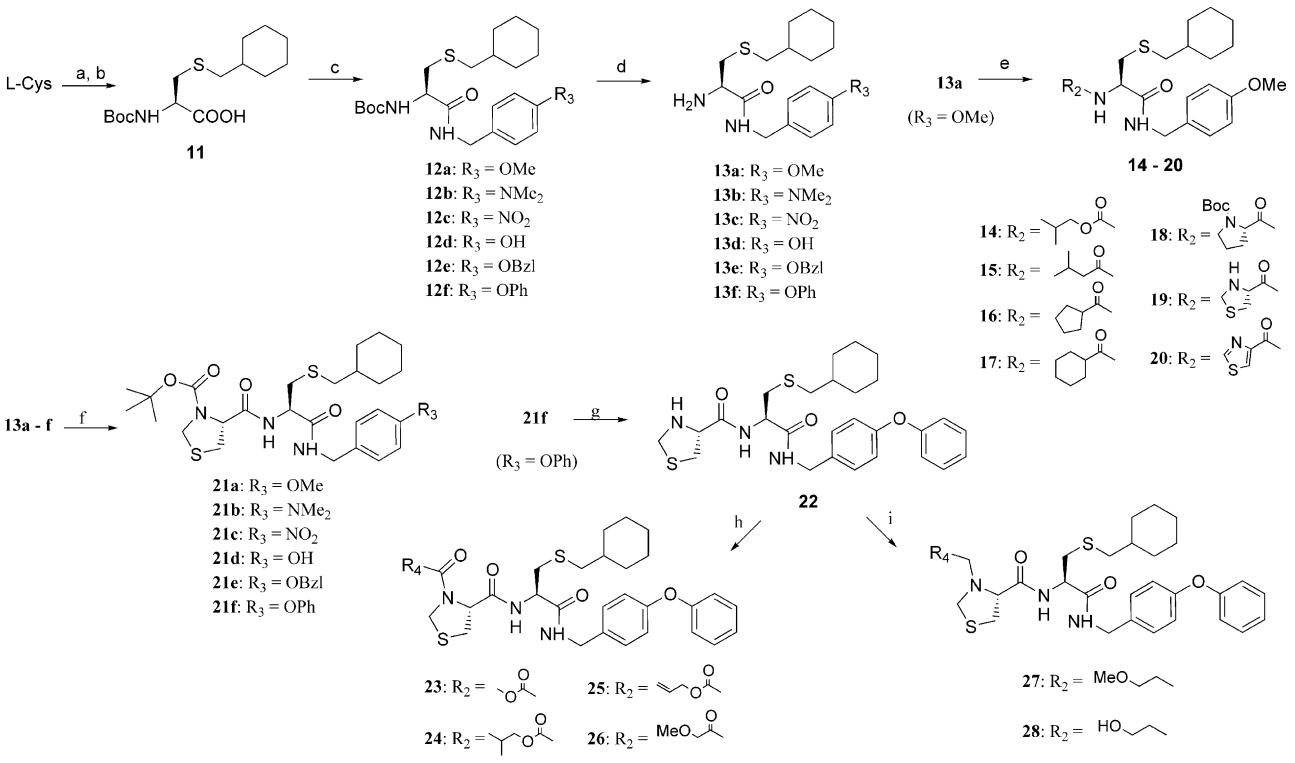
Results and Discussion

In the course of screening of our compound library, *N*-(*t*-butoxycarbonyl)-L-aspartic acid derivative **1a** was identified as an initial lead compound.[13,14] The compound **1a** had moderate N-type calcium channel blocking activity (IC_{50} of 3.4 μM; IMR-32 assay[11]) and selectivity over L-type calcium channels (IC_{50} of 15 μM; in AtT-20 assay,[12] selectivity ratio L/N = 4.4). Compound **2**, the enantiomer of **1a** derived from D-Asp, showed slightly higher activity in IMR-32 assay (IC_{50} = 2.0 μM) than **1a**. Furthermore, inhibitory activity for L-type calcium channels in AtT-20 assay was increased by 5-fold (IC_{50} = 2.9 μM, L/N = 1.5). Due to lack of selectivity of the D-Asp derivative **2**, all of the following compounds were synthesized in optically active forms, which were derived from the corresponding L-amino acids.

An initial structure–activity study was started with replacement of the alkyl group on the side chain ester using the L-Asp skeleton as a structural motif (see Table 1: compounds **1a–1e**). Methyl ester (**1b**: R_1 = methyl) showed extremely weak activity for N-type calcium

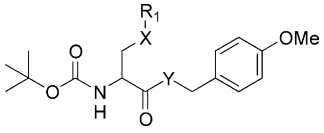


Scheme 1. Reagents: (a) R_1-OH, EDC, DMAP, CH_2Cl_2; (b) 5% Pd/C, H_2, AcOEt; (c) 4-methoxybenzylalcohol, EDC, DMAP, CH_2Cl_2; (d) 4-methoxybenzyl chloride. DMF, K_2CO_3, NaI; (e) cyclohexanecarbonyl chloride, Et_3N, CH_2Cl_2; (f) Rh–Al_2O_3, H_2, *i*-PrOH; (g) 4-methoxybenzyl alcohol, EDC, HOBt, DMF; (h) 4-methoxybenzylamine, EDC, HOBt, DMF; (i) 4-methoxybenzylamine, EDC, HOBt, DMF; (j) cyclohexene-3-bromide, NaH; (k) H_2, Pd/C, EtOH; (l) 4-methoxybenzylamine, EDC, DMAP.



Scheme 2. Reagents: (a) cyclohexylmethyl bromide, 2 N NaOH, EtOH; (b) Boc_2O, 2 N NaOH, EtOH; (c) 4-substituted benzylamine, EDC, HOBt, dichloromethane; (d) 4 N HCl/dioxane; (e) carboxylic acid, EDC, HOBt, dichloromethane; (f) *N*-Boc-(*R*)-thiazolidine-4-carboxylic acid, EDC, HOBt, dichloromethane; (g) 4 N HCl/dioxane; (h) carboxylic acid, EDC, HOBt, dichloromethane; (i) aldehyde, $NaBH_3CN$, AcOH, EtOH.

Table 1. In vitro inhibition of calcium influx in IMR-32 and AtT-20 assays



Compd		X	R_1	Y	IC_{50} (μM)[a]		Selectivity ratio (L/N)
					N-type (IMR-32)	L-type (AtT-20)	
1a	L-Asp	CO–O	Cyclopentyl	O	3.4	15	4.4
2	D-Asp	CO–O	Cyclopentyl	O	2.0	2.9	1.5
1b	L-Asp	CO–O	Methyl	O	26	14	0.5
1c	L-Asp	CO–O	Cyclobutyl	O	15	28	1.9
1d	L-Asp	CO–O	Cyclohexyl	O	2.3	16	7.0
1e	L-Asp	CO–O	Cycloheptyl	O	2.6	12	4.6
4	L-Ser	O–CO	Cyclohexyl	O	1.6	10	6.3
6	L-Ser	O–CH_2	Cyclohexyl	O	1.2	8.4	7.0
7	L-Ser	O–CH_2	Cyclohexyl	NH	0.95	3.8	4.0
8	L-Ser	O–CH_2	Cyclohexyl	NMe	1.8	1.4	0.8
10	L-Hse	CH_2–O	Cyclohexyl	NH	4.0	13	3.3
12a	L-Cys	S–CH_2	Cyclohexyl	NH	0.61	1.7	2.8

[a]Values represent means of multiple determinations performed in duplicate.

channels (IC_{50} = 26 μM) and low selectivity (L/N = 0.5). In the course of modification of ring size on the side chain ester (**1a** and **1c–1e**), compound **1c** (R_1 = cyclobutyl) showed 4-fold decreased N-type inhibitory activity and selectivity. In contrast, compounds **1d** (R_1 = cyclohexyl) and **1e** (R = cycloheptyl) showed similar potency to the initial lead compound **1a**. With respect to the selectivity for N-type calcium channels, cyclohexyl ester was better than cyclopentyl ester. Thus, the compound **1d**, which has cyclohexyl ester function at the side chain and 4-methoxy benzyl ester group at the α-carbonyl, was selected for further SAR study.

Replacement of the amino acid skeleton was next investigated. L-Ser analogue, the reverse type ester of **1a**, showed increased N-type blocking activity in IMR-32 assay (**4**, $IC_{50} = 1.6\,\mu M$). Similar results were observed for the L-Ser ether (**6**, $IC_{50} = 1.2\,\mu M$), which had 3-fold improved N-type calcium channel blocking potency than the lead compound **1a**.

The ester group was converted to the amide group since the ester function seems to be unstable against metabolic hydrolysis. Conversion to amide was achieved without losing potency in IMR-32 assay (**7**, $IC_{50} = 0.95\,\mu M$) or selectivity (L/N = 4.0). The *N*-methyl analogue **8** (Y = NMe) was also synthesized for the same purpose, however, this compound was not selective (L/N = 0.8).

In terms of the oxygen position on the side chain, interesting SAR was observed by comparison between L-Ser ether **7** and L-Hse ether **10.** The N-type calcium channel inhibitory activity of **10** was decreased 4-fold by this minor modification. This observation was similar to the comparison between L-Asp ester **1a** and L-Ser ester **4**. These results also showed that the position of oxygen on the side chain has a significant influence on the inhibitory activity. Thus, we decided to replace L-serine by L-cysteine residue in order to modify side chain hetero atom. L-Cysteine analogue was among the most potent analogues in this series (**12a**, $IC_{50} = 0.61\,\mu M$). The inhibitory activity for N-type calcium channels of **12a** was also evaluated by electrophysiological experiments. This compound blocked N-type calcium channel current in IMR-32 cells (66% at 10 μM, $n = 3$). Activities of selected compounds in electrophysiological experiments were summarized in Table 4.

The SAR of L-cysteine-based N-type calcium channel blockers was next investigated for N-terminal substituent R_2 (Table 2). While replacement of the *tert*-butoxycarbonyl group by an *iso*-butoxycarbonyl group resulted in slight loss of activity (**14**, IC_{50} 0.72 μM), removal of the *tert*-butoxycarbonyl group resulted in 10-fold loss of inhibitory activity for N-type calcium channels in IMR-32 assay (**13a**, IC_{50} 5.8 μM). This observation suggested that the lipophilic substituents enhance potency; however, substitution of the carbamate moiety with an acyl group showed decreased potency by 2-fold (**15**, IC_{50} 1.2 μM) compared to compound **12a**. Similarly, substitution with cycloalkyl group (**16** and **17**) also reduced N-type blocking activity.

Screening of *N*-acyl group indicated that substitution by *N*-*tert*-butoxycarbonyl-thiazolidine-4-carbonyl increased N-type inhibitory potency (**21a**, IC_{50} 0.39 μM). Interestingly, compound **18**, which has an L-proline residue instead of a thiazolidine-4-carbonyl group on the N-terminal, showed lower potency (IC_{50} 0.88 μM) than **21a**. Comparison of **21a** and **18** showed that the sulfur atom led to a further enhancement of potency. Further modification of the sulfur-containing ring structure was considered, but compound **19** (IC_{50} 2.7 μM), which does not have a *t*-butoxycarbonyl group on the thiazolidine ring, and compound **20**, which has thiazole-4-carbonyl moiety, showed very weak inhibitory activity for N-type calcium channels.

The inhibitory activity of compound **21a** for N-type calcium channels was also confirmed by electrophysiological study using IMR-32 cells (Table 4, 48% inhibition at 10 μM, $n = 3$).[10] Therefore, the following SAR study was performed with compound **21a**, which has an *N*-t-butoxycarbonyl-thiazolidine-4-carbonyl function at its N-terminal.

Replacement of substituents R_3 on the benzene ring did not significantly affect on the potency for compounds **21b** ($R_3 = NMe_2$, IC_{50} 0.61 μM) and **21c** ($R_3 = NO_2$; IC_{50} 0.60 μM). Although both of these compounds showed weaker activity than compound **21a**, there was no difference in the N-type inhibitory activity between compounds **21b** and **21c**. These results suggested that there was no influence of the electronic effect of the substituent on the benzene ring. On the other hand, compound **21d** with a free phenol moiety showed 5-fold lower potency ($R_3 = OH$; IC_{50} 1.8 μM). Compound **21e**, which had benzyloxy moiety, was synthesized to estimate steric effects.

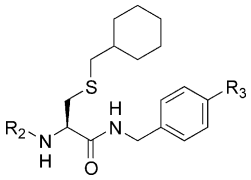
Compound **21e** showed slightly lower activity than compound **21a**.

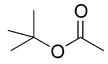
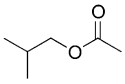
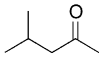
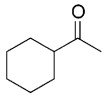
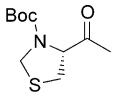
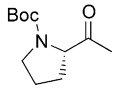
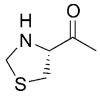
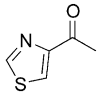
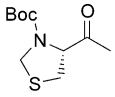
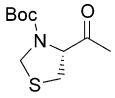
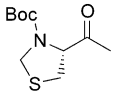
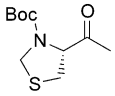
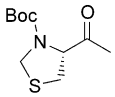
As a consequence of modification of substituents on the benzene ring, compound **21f,** which had a phenoxy residue, was found to be the most potent N-type blocker among this series of compounds. Compound **21f** blocked N-type calcium channels with an IC_{50} of 0.14 μM and showed good selectivity over L-type calcium channels (selectivity ratio IC_{50} L-type/N-type = 12).

Finally, the structure–activity relationship of modification of the substituents on the nitrogen atom of thiazolidine was investigated (Table 3). In contrast to the results with modification of the N-terminal acyl group R_1 (Table 2), no significant influence of substituents was observed on the N-type inhibitory activity. However, it should be noted that most of these compounds showed increased inhibitory activity and selectivity for N-type calcium channels compared to the compounds shown in Table 2. These results indicated that the phenoxy moiety played a crucial role in inhibitory potency and selectivity. Among the compounds shown in Table 3, compounds **23** (R_4 = methoxycarbonyl; IC_{50} 0.12 μM) and **26** (R_4 = 2-methoxyacetyl; IC_{50} 0.12 μM) were equipotent to compound **21f**.

In conclusion, the SAR study of a series of L-cysteine-based compounds led to the discovery of novel neuronal N-type calcium channel blockers. Compound **21f**, which had phenoxy functionarity on the C-terminal benzene ring, was a potent N-type calcium channel blocker and was 12-fold more selective for N-type over L-type calcium channels and could be a potential lead compound for further modification.

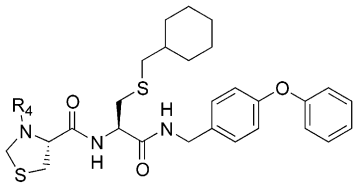
Table 2. Modification of the N-terminal acyl group R_2 and C-terminal R_3 of L-cysteine; in vitro inhibition of calcium influx in IMR-32 and AtT-20 assays

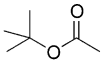
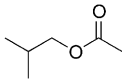
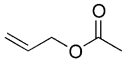
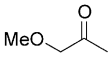
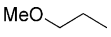


Compd	R_2	R_3	IC_{50} (μM)[a]		Selectivity ratio (L/N)
			N-type (IMR-32)	L-type (AtT-20)	
12a		OMe	0.61	1.7	2.8
13a	H	OMe	5.8	—	—
14		OMe	0.72	1.5	2.1
15		OMe	1.2	2.7	2.3
16		OMe	0.92	2.3	2.5
17		OMe	1.3	1.9	1.5
21a		OMe	0.39	0.68	1.7
18		OMe	0.88	1.9	2.2
19		OMe	2.7	3.5	1.3
20		OMe	1.9	2.7	1.4
21b		NMe_2	0.61	0.95	1.6
21c		NO_2	0.60	0.50	0.8
21d		OH	1.8	2.8	1.6
21e		Obzl	0.50	1.5	3.0
21f		OPh	0.14	1.7	12

[a]Values represent means of multiple determinations performed in duplicate.

Table 3. Modification of substituents R_4 on the nitrogen atom of the thiazolidine ring; in vitro inhibition of calcium influx in IMR-32 and AtT-20 assays



Compd	R_4	IC_{50} (μM)[a]		Selectivity ratio (L/N)
		N-type (IMR-32)	L-type (AtT-20)	
21f		0.14	1.7	12
22	H	0.29	2.0	6.9
23		0.12	0.87	7.3
24		0.36	1.2	3.3
25		0.33	0.44	1.3
26		0.12	0.93	7.8
27		0.20	0.60	3.0
28		0.35	1.1	3.1
ω-Conotoxin MVIIA		0.0062	n.t.	
Nifedipine		n.t.	0.034	

[a]Values represent means of multiple determinations performed in duplicate. n.t., not tested.

Table 4. Activities of selected compounds in electrophysiological experiments

Compd	Electrophysiology (IMR-32, 10 μM, $n=3$) inhibition %
1a	34
6	38
12a	66
21a	48
21f	25 (3 μM)[a]

[a]Compound **21f** was insoluble in assay medium at 10 μM concentration.

Experimental

Chemistry

Melting points were determined in Yanaco micro melting point apparatus and are uncorrected. Analytical samples were homogeneous on TLC and afforded spectroscopic results consistent with assigned structures. ^{1}H NMR spectra were obtained using a Varian GEMINI-200, VXR-200s, VXR-500, or MERCURY300 spectrometer. Mass spectra were obtained on a PerSeptive Voyager Elite spectrometer, HITACHI M1200H or JEOL JMS-DX303HF spectrometer. IR spectra were measured on a Perkin–Elmer FT-IR 1760X or JASCO FT/IR-430 spectrometer. Elemental analyses for carbon, hydrogen, nitrogen, sulfur were carried out on a Perkin-Elmer PE2400 SeriesII CHNS/O analyzer and are within ±0.4% of theory for the formulas given. Optical rotations were measured by JASCO DIP-1000 polarimeter. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm) or Fuji Silysia FL60D]. Thin-layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F254). Compounds evaluated for calcium channel inhibition were further checked optical purity by chiral

HPLC analysis performed by using DAICEL Chiralcel OD or Chiralpack AD column eluting with *n*-hexane/2-propanol (5–40%) (with or without 0.1% of diethylamine), detection at 225 nm. All the compounds analyzed showed sufficient optical purity (>95% ee).

General procedure for preparation of aspartic acid derivatives 1a–1e

***N*-(*t*-Butoxycarbonyl)-ω-cyclopentyloxy-L-aspartic acid (3a).** To a solution of 3.2 g (10 mmol) of *N*-(*t*-Butoxycarbonyl)-aspartic acid α-benzylester, 1.7 g (20 mmol) of cyclopentylalcohol, and 127 mg (1.0 mmol) of DMAP in 20 mL of dichloromethane was added 3.8 g (20 mmol) of EDC–HCl at room temperature. The resulting mixture was stirred for 2 h and 1 N HCl was added. The products were extracted with dichloromethane, and the extracts were washed with brine, dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 9/1) to give quantitative amount (4.2 g, 10 mmol) of *N*-(*t*-butoxycarbonyl)-ω-cyclopentyloxy-L-aspartic acid α-benzylester. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (5H, s), 5.50 (1H, d, J = 8.3 Hz), 5.25–5.11 (3H, m), 4.64–4.56 (1H, m), 2.97 (1H, dd, J = 5.1, 16.9 Hz), 2.77 (1H, dd, J = 4.8, 16.9 Hz), 1.88–1.54 (8H, m), 1.43 (9H, s).

A solution of 4.2 g (10 mmol) of *N*-(*t*-Butoxycarbonyl)-ω-cyclopentyloxy-L-aspartic acid α-benzylester in 25 mL of ethyl acetate was stirred in the presence of a catalytic amount (500 mg) of 5%Pd on carbon under hydrogen atmosphere for 13 h. The catalyst was removed by filtration and the residue was concentrated in vacuo to give 3.0 g (9.9 mmol, 99%) of **3a**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.54 (1H, d, J = 8.1 Hz), 5.24–5.17 (1H, m), 4.63–4.55 (1H, m), 2.97 (1H, dd, J = 4.8, 17.1 Hz), 2.79 (1H, dd, J = 4.8, 17.0 Hz), 1.87–1.55 (8H, m), 1.45 (9H, s).

***N*-(*t*-Butoxycarbonyl)-ω-cyclopentyloxy-L-aspartic acid α-4-methoxybenzylester (1a).** To a solution of 76 mg (0.25 mmol) of **3a**, 39 mg (0.28 mmol) of 4-methoxybenzylalcohol, and 5.0 mg (0.04 mmol) of DMAP in 1.0 mL of dichloromethane was added 97 mg (0.51 mmol) of EDC. The mixture was stirred for 2 h at room temperature and 1 N hydrochloric acid was added and the product was extracted with dichloromethane. The extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 9/1) to give 81 mg (0.19 mmol, 76%) of **1a**. mp: 61.2–62.1 °C; $[\alpha]_D^{25}$ −16.0° (*c* 1.15, MeOH); IR (KBr) ν 3382, 2970, 1724, 1615, 1588, 1517, 1368, 1344, 1250, 1166, 1033, 980, 826, 517, 417 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.27 (2H, d, J = 8.6 Hz), 6.88 (2H, d, J = 8.6 Hz), 5.48 (1H, d, J = 8.8 Hz), 5.18–5.04 (3H, m), 4.60–4.51 (1H, m), 3.81 (3H, s), 2.96 (1H, dd, J = 4.8, 17.0 Hz), 2.76 (1H, dd, J = 4.8, 16.8 Hz), 1.83–1.54 (8H, m), 1.43 (9H, s); MS (FAB) m/z 422 $(M+H)^+$. Anal. calcd for $C_{22}H_{31}NO_7$: C, 62.69%; H, 7.41%; N, 3.32%. Found: C, 62.74%; H, 7.42%; N, 3.31%.

***N*-(*t*-Butoxycarbonyl)-ω-cyclopentyloxy-D-aspartic acid α-4-methoxybenzylester (2).** All analytical data of the compound **2** were identical to that of the compound **1a** except for optical rotation. $[\alpha]_D^{25}$ +15.1° (*c* 0.92, MeOH). Anal. calcd for $C_{22}H_{31}NO_7$: C, 62.69%; H, 7.41%; N, 3.32%. Found: C, 62.62%; H, 7.44%; N, 3.29%.

***N*-(*t*-Butoxycarbonyl)-ω-methyl-L-aspartic acid α-4-methoxybenzylester (1b).** IR (KBr) ν 3411, 2980, 1725, 1709 cm^{-1}; MS (MALDI) m/z 406 $(M+K)^+$, 390 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31–7.24 (2H, m), 6.92–6.84 (2H, m), 5.48 (1H, d, J = 6.8 Hz), 5.16 (1H, d, J = 11.8 Hz), 5.08 (1H, d, J = 11.8 Hz), 4.63–4.54 (1H, m), 3.81 (3H, s), 3.62 (3H, s), 3.00 (1H, dd, J = 17.2, 4.8 Hz), 2.80 (1H, dd, J = 17.2, 4.8 Hz), 1.43 (9H, s); HRMS (MALDI-TOF) calcd for $C_{18}H_{25}NO_7Na$: m/z 390.1529 $(M+Na)^+$. Found: m/z 390.1552 $(M+Na)^+$.

***N*-(*t*-Butoxycarbonyl)-ω-cyclobutyloxy-L-aspartic acid α-4-methoxybenzylester (1c).** Mp: 47.1–48.5 °C; $[\alpha]_D^{25}$ −22.0° (*c* 1.29, MeOH); IR (neat) ν 3375, 2980, 1730 cm^{-1}; MS (MALDI) m/z 446 $(M+K)^+$, 430 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31–7.24 (2H, m), 6.92–6.85 (2H, m), 5.48 (1H, d, J = 9.0 Hz), 5.15 (1H, d, J = 11.8 Hz), 5.07 (1H, d, J = 11.8 Hz), 5.01–4.86 (1H, m), 4.62–4.52 (1H, m), 3.81 (3H, s), 2.96 (1H, dd, J = 17.2, 5.2 Hz), 2.76 (1H, dd, J = 17.2, 4.8 Hz), 2.37–2.22 (2H, m), 2.12–1.43 (13H, m). Anal. calcd for $C_{21}H_{29}NO_7$: C, 61.90%; H, 7.17%; N, 3.44%. Found: C, 61.65%; H, 7.23%; N, 3.39%.

***N*-(*t*-Butoxycarbonyl)-ω-cyclopentyloxy-L-aspartic acid α-4-methoxybenzylester (1d).** Mp: 57.6–58.8 °C; $[\alpha]_D^{25}$ +7.45° (*c* 1.05, $CHCl_3$); IR (KBr) ν 3433 (NH), 2942, 1748, 1713 (C=O), 1513 (amide), 1366, 1342, 1247, 1176, 1028, 821 cm^{-1}; MS (FAB) m/z 436 $(M+H)^+$, 380, 214, 180, 165, 121; ^{1}H NMR (200 MHz, $CDCl_3$) 7.27 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 5.52–5.42 (1H, m), 5.15 (1H, d, J = 12.0 Hz), 5.07 (1H, d, J = 12.0 Hz), 4.75–4.64 (1H, m), 4.62–4.50 (1H, m), 3.81 (3H, s), 2.98 (1H, dd, J = 4.4, 16.8 Hz), 2.76 (1H, dd, J = 5.0, 16.8 Hz), 1.90–1.52 (6H, m), 1.43 (9H, s), 1.40–1.22 (4H, m); HRMS (MALDI-TOF) calcd for $C_{23}H_{33}NO_7Na$: m/z 458.2155 $(M+Na)^+$. Found: m/z 458.2184 $(M+Na)^+$.

***N*-(*t*-Butoxycarbonyl)-ω-cycloheptyloxy-L-aspartic acid α-4-methoxybenzylester (1e).** Mp: 46.3–47.3 °C; $[\alpha]_D^{25}$ −23.0° (MeOH, *c* 1.02); IR (KBr) ν 3390, 2928, 2858, 1730, 1703, 1618, 1516, 1458, 1421, 1391, 1368, 1343, 1296, 1251, 1207, 1174, 1111, 1075, 1026, 973, 912, 876, 815, 781, 759, 555, 489 cm^{-1}; MS (MALDI) m/z 488 $(M+K)^+$, 472 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.27 (2H, d, J = 8.4 Hz), 6.88 (2H, d, J = 8.4 Hz), 5.48 (1H, d, J = 8.4 Hz), 5.15 (1H, d, J = 12.2 Hz), 5.07 (1H, d, J = 12.2 Hz), 4.94–4.82 (1H, m), 4.61–4.52 (1H, m), 3.81 (3H, s), 2.95 (1H, dd, J = 4.4, 16.8 Hz), 2.76 (1H, dd, J = 4.8, 16.8 Hz), 1.90–1.75 (2H, m), 1.68–1.34 (19H, m). Anal. calcd for $C_{24}H_{35}NO_7$: C, 64.12%; H, 7.85%; N, 3.12%. Found: C, 64.18%; H, 7.94%; N, 3.12%.

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexyl-L-serine-4-methoxybenzylester (4).** To a solution of 82 mg (0.4 mmol) of *N*-(*t*-butoxycarbonyl)-L-serine in 0.5 mL of dimethylformamide was added 60 μL (0.41 mmol) of 4-methoxybenzyl chloride, 55 mg (0.40 mmol) of potassium carbonate, and 60 mg (0.4 mmol) of sodium iodide. The resulting mixture was stirred for overnight and poured into water. The product was extracted with ethyl acetate, and the organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 2/1) to give 97 mg (0.30 mmol, 75%) of *N*-(*t*-butoxycarbonyl) - L - serine - 4 - methoxybenzylester. ^{1}H NMR (200 MHz, $CDCl_3$) 7.29 (2H, d, $J=9.0$ Hz), 6.88 (2H, d, $J=9.0$ Hz), 5.53–5.38 (1H, m), 5.15 (2H, s), 4.45–4.30 (1H, m), 4.00–3.80 (2H, m), 3.80 (3H, s), 2.30 (1H, broad), 1.44 (9H, m). HRMS (MALDI-TOF) calcd for $C_{16}H_{23}NO_6Na$: *m*/*z* 348.142305 $(M+Na)^+$. Found: *m*/*z* 348.1457

To a solution of 35 mg (0.11 mmol) of *N*-(*t*-butoxycarbonyl)-L-serine-4-methoxybenzylester in 1 mL of dichloromethane was added 23 μL (0.17 mmol) of triethylamine and 17 μL (0.13 mmol) of cyclohexanecarbonyl chloride at 0 °C. After stirring overnight, the mixture was poured into water. The product was extracted with dichloromethane, and the organic layers were washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 5/1) to give 44 mg (0.10 mmol, 94%) of **4**. Mp: 64.4–66.0 °C; $[\alpha]_D^{25}$ −28.3° (*c* 1.26, MeOH); IR (neat) ν 3369, 2934, 2857, 1739, 1719, 1614, 1587, 1516.,454, 1368, 1344, 1304, 1249, 1163, 1133, 1034, 824 cm^{-1}; MS (FAB) *m*/*z* 436 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33–7.24 (2H, m), 6.91–6.83 (2H, m), 5.28 (1H, d, $J=8.1$ Hz), 5.14 (1H, d, $J=12.3$ Hz), 5.09 (1H, d, $J=12.3$ Hz), 4.64–4.52 (1H, m), 4.45 (1H, dd, $J=1.2$, 3.7 Hz), 4.26 (1H, dd, $J=11.2$, 3.4 Hz), 3.81 (3H, s), 2.27–2.10 (1H, m), 1.89–1.53 (5H, m), 1.53–1.05 (5H, m), 1.44 (9H, s). Anal. calcd for $C_{23}H_{33}NO_7$: C, 63.43%; H, 7.64%; N, 3.22%. Found: C, 63.56%; H, 7.71%; N, 3.21%.

General procedure for preparation of L-serine *O*-ether derivatives (6–8)

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexylmethyl-L-serine (5).** A mixture of *N*-(*t*-butoxycarbonyl)-*O*-benzyl-L-serine (3.0 g, 10 mmol), Rh–Al_2O_3 (10% w/w, 295 mg), and *i*-propylalcohol (60 mL) was stirred under hydrogen atmosphere at room temperature overnight. The resulting mixture was filtered to remove catalyst. The filtrate was concentrated under reduced pressure to give 2.9 g (9.8 mmol, 97%) of the crude product. Recrystallization from *n*-hexane gave 2.6 g (8.6 mmol, 86%) of **5** as colorless crystals. $[\alpha]_D^{25}$ +19.2° (*c* 1.4, $CHCl_3$); MS (FAB) 324 $(M+Na)^+$; IR (neat) ν 3446, 3331, 2978, 2925, 2853, 2668, 1714, 1505, 1451, 1393, 1367, 1295, 1248, 1208, 1166, 1124, 1060, 1027 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.35 (d, $J=8.0$ Hz, 1H), 4.46–4.36 (m, 1H), 3.90–3.80 (m, 1H), 3.83 (dd, $J=9.0$, 5.0 Hz, 1H), 3.27 (d, $J=7.0$ Hz, 2H), 1.90–1.38 (m, 15H), 1.33–1.07 (m, 3H), 1.03–0.80 (m, 2H); HRMS (MALDI-TOF) calcd for $C_{15}H_{27}NO_5Na$: *m*/*z* 324.178691 $(M+Na)^+$. Found: *m*/*z* 324.1807.

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexylmethyl-L-serine 4-methoxybenzylester (6).** To a solution of **5** (40.9 mg, 0.14 mmol) in 1.0 mL of dichloromethane was added 4-methoxybenzylalcohol (17 μL, 0.14 mmol), EDC (40 mg, 0.21 mmol), and DMAP (1.6 mg, 0.01 mmol) and stirred for 3 h at room temperature. The resulting mixture was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 8/1) to give 36 mg (0.09 mmol, 63%) of **6** as a colorless oil. $[\alpha]_D^{25}$ −25.45° (*c* 0.62, MeOH); IR (neat) ν 3446, 2926, 1717 cm^{-1}; MS (MALDI) *m*/*z* 444 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.29 (2H, d, $J=8.4$ Hz), 6.88 (2H, d, $J=8.4$ Hz), 5.36 (1H, br. d, $J=8.8$ Hz), 5.18 (1H, d, $J=12.0$ Hz), 5.06 (1H, d, $J=12.0$ Hz), 4.48–4.34 (1H, m), 3.81–3.76 (4H, m), 3.60 (1H, dd, $J=9.8$, 3.2 Hz), 3.19 (1H, dd, $J=9.4$, 6.6 Hz), 3.09 (1H, dd, $J=9.4$, 6.4 Hz), 1.74–1.13 (19H, m); HRMS (MALDI-TOF) calcd for $C_{23}H_{35}NO_6Na$: *m*/*z* 444.2362 $(M+Na)^+$. Found: *m*/*z* 444.2364 $(M+Na)^+$.

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexylmethyl-L-serine 4-methoxybenzylamide (7).** Mp: 86.2–88.0 °C; $[\alpha]_D^{25}$ −9.05° (*c* 1.18, MeOH) IR (KBr) ν 3337, 2922, 2853, 1685, 1661, 1530, 1514, 1461, 1368, 1304, 1248, 173, 1131, 1040, 633 cm^{-1}; MS (MALDI) *m*/*z* 459 $(M+K)^+$, 443 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19 (2H, d, $J=8.2$ Hz), 6.85 (2H, d, $J=8.2$ Hz), 6.80–6.66 (1H, br), 5.52–5.26 (1H, br), 4.44 (1H, dd, $J=5.2$, 15.0 Hz), 4.36 (1H, dd, $J=5.8$, 15.0 Hz), 4.30–4.15 (1H, br), 3.89–3.79 (4H, m), 3.44 (1H, dd, $J=7.0$, 9.2 Hz), 3.27 (1H, dd, $J=6.1$, 9.3 Hz), 3.20 (1H, dd, $J=6.1$, 9.3 Hz), 1.94 (5H, m), 1.44 (9H, s), 1.34–1.04 (4H, m), 0.96–0.74 (2H, m). Anal. calcd for $C_{23}H_{36}N_2O_5$: C, 65.69% ; H, 8.63%; N, 6.66%. Found: C, 65.68% ; H, 8.67%; N, 6.65%.

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexylmethyl-L-serine 4-methoxybenzylmethylamide (8).** Colorless oil; IR (neat) ν 3306, 2925, 2853, 1713, 1645, 1515 cm^{-1}; MS (MALDI) *m*/*z* 473 $(M+K)^+$, 457 $(M+Na)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.21–7.13 (2H, m), 6.89–6.80 (2H, m), 5.47–5.38 (1H, m), 5.00–4.70 (2H, m), 4.45 (0.3H, d, $J=16.6$ Hz), 4.30 (0.7H, d, $J=14.4$ Hz), 3.79 (3H, s), 3.66–3.47 (2H, m), 3.22–3.14 (2H, m), 3.01 (2.1H, s), 2.89 (0.9H, s), 1.74–0.74 (20H, m); HRMS (MALDI-TOF) calcd for $C_{24}H_{38}N_2O_5Na$: *m*/*z* 457.2678 $(M+Na)^+$. Found: *m*/*z* 457.2683 $(M+Na)^+$.

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexene-3-yl-L-homo-serine (9).** To a suspension of NaH (676 mg, 17.6 mmol) in dimethyformamide (3 mL) was added dropwise a solution of *N*-(*t*-butoxycarbonyl)-L-homo-serine (1.9 g, 8.8 mmol) in dimethylformamide (7 mL) at 0 °C and the mixture was stirred for 20 min. To this solution was added Bu_4NI (33 mg, 0.088 mmol) and the resulting mixture was stirred for 30 min, and 3-bromocyclohexene was added. After stirring for 2 h, sodium iodide (1.3 g, 8.8 mmol) was added and stirred at room temperature overnight. The mixture was poured into water and the

product was extracted with ethyl acetate. The organic layers were washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate/acetic acid, 5/5/0.1) to give 379 mg (1.2 mmol, 14%) of **9** as a pale brown oil. MS (APCI) *m/z* 298 $(M-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) 5.95–5.65 (3H, m), 4.37–4.24 (1H, m), 3.96–3.84 (1H, m), 3.80–3.62 (2H, m), 2.26–1.93 (4H, m), 1.92–1.50 (4H, m), 1.47 (9H, s).

***N*-(*t*-Butoxycarbonyl)-*O*-cyclohexyl-L-homo-serine 4-methoxybenzylamide (10).** A mixture of **9** (200 mg, 0.67 mmol), Pd/C (10%, 20 mg), and methanol was stirred under hydrogen atmosphere for 1 h. The reaction mixture was filtered to remove catalyst and the filtrate was concentrate under reduced pressure to give 177 mg (0.59 mmol, 88%) of *N*-(*t*-butoxycarbonyl)-*O*-cyclohexyl-L-homo-serine as a colorless oil. MS (APCI) *m/z* 300 $(M-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) 5.95–5.65 (1H, m), 4.37–4.22 (1H, m), 4.22–3.82 (1H, broad), 3.80–3.54 (2H, m), 3.44–3.26 (1H, m), 2.20–2.00 (2H, m), 1.95–1.60 (4H, m), 1.60–1.10 (6H, m), 1.46 (9H, s).

A mixture of *N*-(*t*-butoxycarbonyl)-*O*-cyclohexyl-L-homo-serine (150 mg, 0.50 mmol), 4-methoxybenzylamine (65 μL, 0.50 mmol), DMAP (6 mg), EDC (143 mg, 0.75 mmol), and dichloromethane (1 mL) was stirred for 2 h at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 3/1 to 2/1) to give 68 mg (0.16 mmol, 32%) of **10** as a colorless solid. mp: 55.1–57.7 °C; $[\alpha]_D^{25}$ −27.6° (*c* 0.93, MeOH); IR (film) ν 3319, 2933, 2858, 1706, 1661, 1614, 1514, 1454, 1392, 1367, 1249, 1175, 1103, 1035, 912, 824, 734, 647 cm^{-1}; MS (APCI) *m/z* 421 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.25–7.15 (2H, m), 7.00–6.80 (3H, m), 6.03–5.88 (1H, m), 4.52–4.15 (3H, m), 3.80 (3H, s), 3.71–3.42 (2H, m), 3.26–3.08 (1H, m), 2.10–1.95 (2H, m), 1.85–1.05 (10H, m), 1.42 (9H, s). Anal. calcd for $C_{23}H_{36}N_2O_5$ $1/4H_2O$: C, 64.99%; H, 8.66%; N, 6.59%. Found: C, 64.72%; H, 8.55%; N, 6.62%.

General procedure for preparation of L-cysteine derivatives

***N*-(*t*-Butoxycarbonyl) *S*-cyclohexylmethyl-L-cysteine (11).** To a solution of L-cysteine (242 g, 2.0 mol), EtOH (2 L), and 2 N NaOH (2 L) was added cyclohexylmethylbromide (308 mL, 2.21 mol) and tetrabutylammonium iodide (22.0 g, 60 mmol) and resulting mixture was stirred for 3 days. To the resulting reaction mixture was added Boc_2O (505 mL, 2.20 mol). After stirring for additional 1 day, ethanol was evaporated and the residue was acidified by adding 1 N HCl (1.3 L) at 0 °C. The mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give 621 g (1.96 mol, 98%) of *N*-Boc-*S*-cyclohexylmethyl-cysteine (**11**) as colorless solids. MS (MALDI): *m/z* 356 $(M+K)^+$, 340 $(M+Na)^+$; IR (KBr): υ 3151, 2925, 1741, 1691, 1522, 1160 cm^{-1}; ^1H NMR (200 MHz, CD_3OD): δ 4.29–4.25 (1H, m), 2.95 (1H, dd, *J* = 13.8 Hz, 4.8 Hz), 2.80 (1H, dd, *J* = 13.8 Hz, 7.8 Hz), 2.45 (2H, d, *J* = 6.9 Hz), 1.87–1.83 (2H, m), 1.76–1.60 (3H, m), 1.53–1.37 (10H, m), 1.34–1.09 (3H, m), 1.01–0.88 (2H, m); HRMS (MALDI-TOF) calcd for $C_{15}H_{27}NO_4SNa$: *m/z* 340.15585 $(M+Na)^+$. Found.: *m/z* 340.1543.

***N*-(*t*-Butoxycarbonyl) *S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (12a).** To a solution of *N*-Boc-*S*-cyclohexylmethyl-cysteine (**11**) (435 g, 1.37 mol) in DMF (1.0 L) was added HOBt (210 g, 1.37 mol), 4-methoxybenzylamine (179 mL, 1.37 mol), EDC (314 g, 1.64 mol, 1.2 equiv). After stirring for 2 h, the reaction mixture was poured onto ice and EtOH (500 mL) was added. Precipitation formed was collected by filtration and washed with water (500 mL), saturated aqueous $NaHCO_3$ (3 × 500 mL), and then water (500 mL). The resulting solid was recrystallized from ethanol EtOH (1.45 L) to give 486 g of *N*-Boc-*S*-cyclohexylmethyl-cysteine 4-methoxybenzylamide (**12a**) (1.10 mol, 81%). Mp: 98.1–101.5° C; $[\alpha]_D^{23.4}$: +8.78° (*c* 1.10, $CHCl_3$); MS (APCI): *m/z* 437 (M + H) +, 381, 337; IR (KBr): υ 3341, 2918, 2849, 1683, 1658, 1613, 1515, 1446, 1367, 1312, 1244, 1171, 1109, 1037, 1020, 864 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.25–7.18 (2H, m), 6.89–6.84 (2H, m), 6.68–6.50 (1H, m), 5.36 (1H, d, *J* = 7.0 Hz), 4.39 (2H, d, *J* = 5.6 Hz), 4.28–4.18 (1H, m), 3.80 (3H, s), 2.98 (1H, dd, *J* = 14.0, 5.8 Hz), 2.82 (1H, dd, *J* = 14.0, 7.0 Hz), 2.46 (1H, dd, *J* = 12.8, 7.0 Hz), 2.39 (1H, dd, *J* = 12.8, 6.6 Hz), 1.83–0.82 (20H, m). Anal. calcd for $C_{23}H_{36}N_2O_4S$: C, 63.27%; H, 8.31%; N, 6.42%; S, 7.34%. Found: C, 63.12%; H, 8.40%; N, 6.38%; S, 7.51%.

***S*-Cyclohexylmethyl-L-cysteine 4-methoxybenzylamide hydrochloride (13a).** To a solution of *N*-Boc-*S*-cyclohexylmethyl-cysteine 4-methoxybenzylamide (**12a**) (486 g, 1.11 mol) in 1,4-dioxane (500 mL) was added HCl/dioxane (ca. 7 N, 800 mL) at room temperature. After stirring for 1 h, the reaction mixture was concentrated under reduced pressure to give quantitative amount (419 g) of *S*-cyclohexylmethyl-cysteine 4-methoxybenzylamide hydrochloride (**13a**) as a white amorphous solid. Mp: 82.4–84.0 °C; $[\alpha]_D^{23.4}$: +120.8° (*c* 1.03, $CHCl_3$); IR (KBr) ν 3338, 2924, 2851, 1666, 1561, 1514, 1248, 1178, 1038, 807 cm^{-1}; MS (MALDI) *m/z* 359 $(M+Na)^+$, 337 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.22 (m, 2H), 6.91–6.84 (m, 2H), 4.38 (d, *J* = 15 Hz, 1H), 4.33 (d, *J* = 15 Hz, 1H), 3.96 (dd, *J* = 8, 6 Hz, 1H), 3.77 (s, 3H), 3.01 (dd, *J* = 14, 6 Hz, 1H), 2.90 (dd, *J* = 14, 8 Hz, 1H), 2.44 (d, *J* = 7 Hz, 2H), 1.86–1.62 (m, 5H), 1.54–1.37 (m, 1H), 1.37–1.07 (m, 3H), 1.03–0.85 (m, 2H). Anal. calcd for $C_{18}H_{28}N_2O_2S$. HCl $0.5H_2O$: C, 56.60%; H, 7.92%; N, 7.33%; S, 8.39%. Found: C, 56.48%; H, 7.72%; N, 7.55%; S, 8.60%.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (21a).** To a solution of *S*-cyclohexylmethyl-cysteine 4-methoxybenzylamide hydrochloride **13a** (112 g,

0.30 mol) in DMF (400 mL) was added *N*-methylmorpholine (33 mL, 0.30 mol), HOBt (46 g, 0.30 mol), *N*-Boc-(*R*)-tiazolizine-4-carboxylic acid (191 g, 0.824 mol), and EDC (189 g, 0.989 mol, 1.2 equiv) at 0 °C. After stirring for 90 min, the reaction mixture was concentrated under reduced pressure and poured into water. The resulting mixture was cooled in ice-water bath. The precipitation formed was washed with saturated aqueous $NaHCO_3$ (500 mL × 3) and water (500 mL) and was collected by filtration. Recrystallization from ethanol (300 mL) gave 56 g (0.10 mol, 33.9%) **21a** as a white powder [mp: 114.1–115.7 °C; $[\alpha]_D^{22.5}$: −34.5° (*c* 0.93, $CHCl_3$)]. The mother liquor was concentrated under reduced pressure and purified by column chromatography on silica gel (*n*-dichloromethane/ethyl acetate, 19/1 to 4/1). Further purification by recrystallization from EtOAc (100 mL)/hexane (400 mL) gave 75.86 g (0.14 mol, 45.9%) of **21a**. Mp: 115.9–117.4 °C; $[\alpha]_D^{25.3}$: −37.3° (*c* 1.02, $CHCl_3$); IR (KBr) ν 3282, 2925, 1706, 1644, 1514 cm^{-1}; MS (MALDI) *m*/*z* 590 $(M+K)^+$, 574 $(M+Na)^+$; ^1H NMR (200 MHz, CD3OD) δ 7.22 (2H, d, *J* = 8.8 Hz), 6.85 (2H, d, *J* = 8.8 Hz), 4.66–4.45 (4H, m), 4.34 (1H, d, *J* = 14.6 Hz), 4.29 (1H, d, *J* = 14.6 Hz), 3.76 (3H, s), 3.35 (1H, dd, *J* = 7.2, 12.0 Hz), 3.12 (1H, dd, *J* = 4.6, 12.0 Hz), 2.98–2.73 (2H, br), 2.41 (2H, d, *J* = 7.0 Hz), 1.88–0.80 (20H, m). Anal. calcd for $C_{27}H_{41}N_4O_5S_2$: C, 58.78%; H, 7.49%; N, 7.62%; S, 11.62%. Found: C, 58.79%; H, 7.57%; N, 7.84%; S, 11.71%.

***N*-*iso*-Butoxycarbonyl-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (14).** Mp: 61.3–63.6 °C; $[\alpha]_D^{25}$ −19.3° (*c* 1.16, MeOH); IR (film) ν 3305, 2922, 2852, 1689, 1656, 1533, 1515, 1448, 1384, 1311, 1243, 1040, 808 cm^{-1}; MS (APCI) *m*/*z* 437 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.26–7.15 (2H, m), 6.92–6.80 (2H, m), 6.75–6.58 (1H, m), 5.58 (1H, d, *J* = 7.3 Hz), 4.40 (2H, d, *J* = 5.5 Hz), 4.27 (1H, td, *J* = 7.3, 5.5 Hz), 3.85 (2H, d, *J* = 6.6 Hz), 3.80 (3H, s), 2.99 (1H, dd, *J* = 13.9, 5.5 Hz), 2.82 (1H, dd, *J* = 13.9, 7.3 Hz), 2.44 (2H, d, *J* = 6.6 Hz), 2.02–1.55 (6H, m), 1.55–1.03 (4H, m), 1.03–0.77 (2H, m), 0.92 (6H, d, *J* = 7.0 Hz). Anal. calcd for $C_{23}H_{36}N_2O_4S$: C, 63.27%; H, 8.31%; N, 6.42%; S, 7.34%. Found: C, 63.09%; H, 8.31%; N, 6.42%; S, 7.63%.

***N*-(3-Methylbutanoyl)-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (15).** Mp: 106.1–108.7 °C; $[\alpha]_D^{25}$ −29.1° (*c* 1.06, MeOH); IR (film) ν 3283, 3092, 2925, 2852, 1635, 1515, 1449, 1384, 1301, 1251, 1176, 1111, 1036, 909, 810, 734 cm^{-1}; MS (APCI) *m*/*z* 421 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.26–7.16 (2H, m), 6.91–6.76 (3H, m), 6.43 (1H, d, *J* = 7.5 Hz), 4.50 (1H, td, *J* = 7.5, 5.5 Hz), 4.38 (2H, d, *J* = 5.5 Hz), 3.80 (3H, s), 2.95 (1H, dd, *J* = 13.6, 5.5 Hz), 2.76 (1H, dd, *J* = 13.6, 7.5 Hz), 2.47 (2H, d, *J* = 6.6 Hz), 2.20–1.95 (3H, m), 1.89–1.56 (6H, m), 1.56–1.05 (3H, m), 1.05–0.78 (2H, m), 0.94 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz). Anal. calcd for $C_{23}H_{36}N_2O_3S$: C, 65.68%; H, 8.63%; N, 6.66%; S, 7.62%. Found: C, 65.48%; H, 8.61%; N, 6.60%; S, 7.73%.

***N*-Cyclopentanecarbonyl-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (16).** Mp: 55.8–57.9 °C; $[\alpha]_D^{25}$ −14.7° (*c* 0.92, MeOH); IR (KBr) ν 3283, 2923, 2853, 1636, 1543, 1515 (APCI) *m*/*z* 433 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.24–7.05 (3H, m), 6.87–6.80 (2H, m), 6.59 (1H, d, *J* = 7.0 Hz), 4.62–4.52 (1H, m), 4.39 (1H, dd, *J* = 15.0, 5.8 Hz), 4.31 (1H, dd, *J* = 15.0, 5.4 Hz), 3.78 (3H, s), 2.93 (1H, dd, *J* = 13.4, 5.4 Hz), 2.79 (1H, dd, *J* = 13.4, 7.4 Hz), 2.66–2.50 (1H, m), 2.45 (2H, d, *J* = 7.0 Hz), 1.95–0.78 (19H, m). Anal. calcd for $C_{24}H_{36}N_2O_3S$: C, 66.63%; H, 8.39%; N, 6.48%; S, 7.41%. Found: C, 66.37%; H, 8.42%; N, 6.41%; S, 7.28%.

***N*-Cyclohexanecarbonyl-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (17).** Mp: 157.4–159.9 °C; $[\alpha]_D^{25}$ −8.9° (*c* 0.67, $CHCl_3$); IR (KBr) ν 3291, 2930, 2853, 1663, 1639, 1533, 1514, 1245 cm^{-1}; MS (APCI) *m*/*z* 447 $(M+H)^+$, 317, 169; ^1H NMR (200 MHz, $CDCl_3$) δ 7.24–7.16 (2H, m), 6.89–6.78 (3H, m), 6.46 (1H, d, *J* = 6.8 Hz), 4.52–4.42 (1H, m), 4.38 (2H, d, *J* = 5.8 Hz), 3.79 (3H, S), 2.95 (1H, dd, *J* = 13.6, 5.0 Hz), 2.74 (1H, dd, *J* = 13.6, 8.0 Hz), 2.47 (2H, d, *J* = 7.0 Hz), 2.20–2.05 (1H, m), 1.92–0.82 (21H, m). Anal. calcd for $C_{25}H_{38}N_2O_3S$: C, 67.23%; H, 8.58%; N, 6.27%; S, 7.18%. Found: C, 67.08%; H, 8.63%; N, 6.23%; S, 7.18%.

***N*-(*N*-(*t*-Butoxycarbonyl)-L-prolyl)-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (18).** Colorless oil; $[\alpha]_D^{25}$ −25.5° (*c* 0.92, $CHCl_3$); IR (neat) ν 3296, 2925, 1651, 1514, 1395, 1248 cm^{-1}; MS (MALDI) *m*/*z* 572 $(M+K)^+$, 556 $(M+Na)^+$; ^1H NMR (500 MHz, DMSO-d_6, 100 °C) δ 8.03–7.97 (1H, m), 7.57 (1H, d, *J* = 8.8 Hz), 7.18 (2H, d, *J* = 8.8 Hz), 6.85 (2H, d, *J* = 8.8 Hz), 4.45–4.40 (1H, m), 4.21 (2H, d, *J* = 7.5 Hz), 4.15–4.12 (1H, m), 3.73 (3H, s), 3.40–3.30 (2H, m), 2.85 (1H, dd, *J* = 15.0, 8.7 Hz), 2.76 (1H, dd, *J* = 15.0, 6.3 Hz), 2.42 (2H, d, *J* = 13.0 Hz), 2.11–2.03 (1H, m), 1.88–1.72 (4H, m), 1.69–1.58 (3H, m), 1.45–1.33 (2H, m), 1.26–1.10 (3H, m), 0.98–0.92 (2H, m); HRMS (MALDI-TOF) calcd for $C_{28}H_{43}N_3O_5S$ Na: *m*/*z* 556.2821 $(M+Na)^+$. Found: *m*/*z* 556.2806 $(M+Na)^+$.

***N*-((*R*)-Thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (19).** Mp: 127.1–129.0 °C; $[\alpha]_D^{25}$ −47.5° (*c* 1.01, $CHCl_3$); IR (KBr) ν 3270, 2919, 2849, 1638, 1548, 1515, 1447, 1303, 1254, 1175, 1038, 824, 692 cm^{-1}; MS (MALDI, Pos.) 474 $(M+Na)^+$, 452 $(M+H)^+$.; ^1H NMR (200 MHz, CD_3OD) δ 7.87 (1H, d, *J* = 7.4 Hz), 7.25–7.18 (2H, m), 6.90– 6.83 (2H, m), 6.78–6.70 (1H, m), 4.48–4.31 (3H, m), 4.30–4.21 (1H, m), 4.20–4.12 (1H, m), 4.10–4.00 (1H, m), 3.80 (3H, s), 3.41 (1H, dd, *J* = 11.0, 4.1 Hz), 3.10 (1H, dd, *J* = 11.0, 7.7 Hz), 2.92 (1H, dd, *J* = 13.7, 6.3 Hz), 2.83 (1H, dd, *J* = 13.7, 7.3 Hz), 2.50–2.38 (1H, b), 2.44 (2H, d, *J* = 6.9 Hz), 1.85–1.55 (5H, m), 1.50–1.35 (1H, m), 1.31–1.03 (3H, m), 0.98–0.84 (2H, m). Anal. calcd for $C_{22}H_{33}N_3O_3S_2$: C, 58.51%; H, 7.36%; N, 9.30%; S, 14.20%. Found: C, 58.16%; H, 7.34%; N, 9.18%; S, 14.22%.

***N*-(Thiazole-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-methoxybenzylamide (20).** Mp: 127.0–129.0 °C; $[\alpha]_D^{25}$ −11.2° (*c* 0.93, MeOH); IR (KBr) ν 3327, 2922, 1638,

1530, 1248 cm^{-1}; MS (MALDI) *m/z* 486 (M + K), 470 $(M+Na)^+$, 448 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 8.77 (1H, d, *J* = 1.8 Hz), 8.18 (1H, d, *J* = 7.8 Hz), 8.12 (1H, d, *J* = 1.8 Hz), 7.25–7.18 (2H, m), 6.88–6.81 (2H, m), 4.78–4.68 (1H, m), 4.51–4.33 (2H, m), 3.79 (3H, s), 3.16 (1H, dd, *J* = 14.0, 5.6 Hz), 2.92 (1H, dd, *J* = 14.0, 7.4 Hz), 2.57–2.41 (2H, m), 1.88–0.80 (11H, m). Anal. calcd for $C_{22}H_{29}N_3O_3S_2$: C, 59.03%; H, 6.53%; N, 9.39%; S, 14.33%. Found: C, 58.87%; H, 6.49%; N, 9.34%; S, 14.20%.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-dimethylaminobenzylamide (21b).** Amorphous solid; $[\alpha]_D^{25}$ −39.0° (*c* 1.02, $CHCl_3$); IR (film) ν 3293, 2925, 2851, 1646, 1523, 1448, 1367, 1255, 1163, 909, 804, 733 cm^{-1}; MS (APCI) *m/z* 587 $(M+Na)^+$, 565 $(M+H)^+$, 169, 134, 117; ^1H NMR (200 MHz, CD_3OD) δ 7.20–7.10 (2H, m), 6.77–6.67 (2H, m), 4.67–4.43 (4H, m), 4.29 (1H, d, *J* = 14.7 Hz), 4.26 (1H, d, *J* = 14.7 Hz), 3.41–3.30 (1H, m), 3.12 (1H, dd, *J* = 12.1, 4.8 Hz), 3.00–2.65 (8H, m), 2.41 (2H, d, *J* = 6.6 Hz), 1.88–0.80 (11H, m), 1.45 (9H, s). Anal. calcd for $C_{28}H_{44}N_4O_4S_2$: C, 59.54%; H, 7.85%; N, 9.92%; S, 11.35%. Found: C, 59.41%; H, 7.89%; N, 9.80%; S, 11.43%.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-nitrobenzylamide (21c).** Mp: 132.2–134.5 °C; $[\alpha]_D^{25}$ −27.8° (*c* 0.95, $CHCl_3$); IR (KBr) ν 3289, 3055, 2923, 2850, 1695, 1690, 1674, 1656, 1604, 1519, 1449, 1366, 1345, 1258, 1165, 1111, 857, 774, 733 cm^{-1}; MS (APCI) *m/z* 567 $(M+H)^+$, 511, 467; ^1H NMR (200 MHz, CD_3OD) δ 8.25–8.15 (2H, m), 7.61–7.51 (2H, m), 4.70–4.41 (6H, m), 3.45–3.32 (1H, m), 3.22–3.08 (1H, m), 3.04–2.72 (2H, m), 2.45 (2H, d, *J* = 6.6 Hz), 1.91–0.80 (11H, m), 1.45 (9H, s). Anal. calcd for $C_{26}H_{38}N_4O_6S_2$: C, 55.10%; H, 6.76%; N, 9.89%; S, 11.32%. Found: C, 55.18%; H, 6.72%; N, 9.83%; S, 11.41%.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-hydroxybenzylamide (21d).** Mp: 64.3–63.3 °C; $[\alpha]_D^{25}$ −45.0° (*c* 0.89, $CHCl_3$); IR (KBr) ν 3304, 2925, 2851, 1652, 1517, 1161 cm^{-1}; (MALDI) *m/z* 576 $(M+K)^+$, 560 $(M+Na)^+$; ^1H NMR (200 MHz, CD_3OD) δ 7.12 (2H, d, *J* = 8.4 Hz), 6.74–6.68 (2H, m), 4.65–4.44 (4H, m), 4.31 (1H, d, *J* = 14.8 Hz), 4.23 (1H, d, *J* = 14.8 Hz), 3.35 (1H, dd, *J* = 12.2, 7.4 Hz), 3.12 (1H, dd, *J* = 12.2, 4.8 Hz), 2.99–2.68 (2H, m), 2.41 (2H, d, *J* = 7.0 Hz), 1.88–0.81 (20H, m). Anal. calcd for $C_{26}H_{39}N_3O_5S_2$ $0.5H_2O$: C, 57.12%; H, 7.37%; N, 7.69%; S, 11.73%. Found: C, 57.34%; H, 7.21%; N, 7.71%; S, 11.93%.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-benzyloxybenzylamide (21e).** Amorphous solid; $[\alpha]_D^{25}$ −47.4° (*c* 0.82, $CHCl_3$); IR (KBr) ν 3293, 2925, 2851, 1703, 1649, 1511, 1381, 1367, 1241 cm^{-1}; MS (MALDI) *m/z* 666 $(M+K)^+$, 650 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44–7.18 (8H, m), 7.13 (1H, d, *J* = 7.8 Hz), 6.93–6.88 (2H, m), 5.04 (2H, s), 4.65–4.21 (6H, m), 3.32–3.12 (3H, m), 2.78 (1H, dd, *J* = 13.8, 6.3 Hz), 2.44–2.30 (2H, m), 1.80–0.78 (20H, m); HRMS (MALDI-TOF) calcd for $C_{33}H_{45}N_3O_5S_2Na$: *m/z* 650.2689 $(M+Na)^+$. Found: *m/z* 650.2697 $(M+Na)^+$.

***N*-(*N*-(*t*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (21f).** Amorphous solid; $[\alpha]_D^{25}$ −45.6° (*c* 0.61, $CHCl_3$); IR (KBr) ν 3293, 2925, 1704, 1649, 1507, 1489, 1367, 1239; MS (FAB) *m/z* 614 $(M+H)^+$, 558; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.23 (5H, m), 7.15–7.07 (2H, m), 7.01–6.91 (4H, m), 4.65–4.32 (6H, m), 3.33–3.13 (3H, m), 2.79 (1H, dd, *J* = 14.1, 6.3 Hz), 2.45–2.30 (2H, m), 1.83–0.78 (20H, m); HRMS (MALDI-TOF) calcd for $C_{32}H_{43}N_3O_5S_2Na$: *m/z* 636.2541 $(M+Na)^+$. Found: *m/z* 636.2553 $(M+Na)^+$.

***N*-((*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide hydrochloride (22).** Mp 75–77 °C; $[\alpha]_D^{25}$ −55.0 (*c* 1.10, MeOH); IR (KBr) ν 3239, 3039, 2923, 2850, 2706, 1656, 1589, 1547, 1507, 1489, 1447, 1356, 1238, 1166, 1105, 1070, 1015, 962, 908, 871, 846, 746, 691, 603, 499, 448, 421 cm^{-1}; MS (APCI) *m/z* 514 $(M+H)^+$; ^1H NMR (200 MHz, CD_3OD) δ 7.39–7.25 (m, 4H), 7.14–7.05 (m, 1H), 6.99–6.88 (m, 4H), 4.61–4.50 (m, 2H), 4.47–4.29 (m, 4H), 3.57 (dd, *J* = 12.0, 7.7 Hz, 1H), 3.23 (dd, *J* = 12.0, 7.2 Hz, 1H), 2.93 (dd, *J* = 13.6, 6.6 Hz, 1H), 2.80 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.53–2.36 (m, 2H), 1.90–0.80 (m, 11H). Anal. calcd for $C_{27}H_{35}N_3O_3S_2.0.5H_2O.HCl$: C, 57.99%; H, 6.67%; N, 7.65%; S, 11.47%. Found: C, 58.10%; H, 6.50%; N, 7.65%; S, 11.53%.

***N*-(*N*-Methoxycarbonyl-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (23).** Amorphous solid; $[\alpha]_D^{25}$ −52.0° (*c* 1.12, $CHCl_3$); IR (KBr) ν 3301, 3064, 2924, 2850, 1710, 1651, 1590, 1531, 1507, 1489, 1446, 1374, 1237, 1111, 1016, 962, 871, 767, 692, 499 cm^{-1}; MS (MALDI) *m/z* 610 $(M+K)^+$, 594 $(M+Na)^+$, 572 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–6.93 (m, 11H), 4.73–4.32 (m, 6H), 3.67 (s, 3H), 3.32 (dd, *J* = 12.0, 3.9 Hz, 1H), 3.28 (dd,*J* = 12.0, 6.9 Hz, 1H), 3.23–3.01 (br, 1H), 2.82 (dd, *J* = 13.8, 6.6 Hz, 1H), 2.48–2.34 (m, 2H), 1.82–1.52 (m, 5H), 1.49–1.04 (m, 4H), 0.96–0.81 (m, 2H). Anal. calcd for $C_{29}H_{37}N_3O_5S_2$: C, 60.92%; H, 6.52%; N, 7.35%; S, 11.22%. Found: C, 60.56%; H, 6.45%; N, 7.30%; S, 11.62%.

***N*-(*N*-(*iso*-Butoxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (24).** Mp: 76.2–77.8 °C; $[\alpha]_D^{25}$ −44.7° (*c* 0.91, $CHCl_3$); IR (KBr) ν 3454, 3298, 2960, 2923, 2849, 1719, 1653, 1535, 1489, 1415, 1358, 1242, 1110, 1022, 871, 764, 692, 584, 495 cm^{-1}; MS (MALDI) *m/z* 652 $(M+K)^+$, 636 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–6.92 (m, 11H), 4.73–4.29 (m, 6H), 3.84 (d, *J* = 6.6 Hz, 2H), 3.32 (dd, *J* = 12.3, 4.5 Hz, 1H), 3.29 (dd, *J* = 12.3, 6.6 Hz, 1H), 3.24–3.17 (br, 1H), 2.81 (dd, *J* = 13.5, 6.6 Hz, 1H), 2.44–2.32 (m, 2H), 1.99–1.54 (m, 6H), 1.49–1.04 (m, 4H), 0.95–0.86 (m, 8H). Anal. calcd for $C_{32}H_{43}N_3O_5S_2.0.5H_2O$: C, 61.71%; H, 7.12%; N, 6.75%; S, 10.30%. Found: C, 61.75%; H, 6.94%; N, 6.82%; S, 10.23%.

***N*-(*N*-Allyloxycarbonyl)-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (25).** Mp: 118.5–120.0 °C; $[\alpha]_D^{25}$ −51.5° (*c* 0.91, $CHCl_3$); IR (KBr) ν 3292, 3100, 2923, 2851, 1711, 1644, 1541, 1489, 1409, 1352, 1246, 1176, 1105, 967, 923, 873, 765, 691, 499 cm^{-1}; MS (MALDI)*m*/*z* 636 $(M+K)^+$, 620 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–6.93 (m, 11H), 5.93–5.80 (m, 1H), 5.32–5.21 (m, 2H), 4.74–4.28 (m, 8H), 3.32 (dd, *J* = 12.0, 3.9 Hz, 1H), 3.29 (dd, *J* = 12.0, 6.6 Hz, 1H), 3.22–3.04 (br, 1H), 2.81 (dd, *J* = 14.1, 6.6 Hz, 1H), 2.47–2.34 (m, 2H), 1.80–1.52 (m, 5H), 1.48–1.04 (m, 4H), 0.96–0.80 (m, 2H). Anal. calcd for $C_{31}H_{39}N_3O_5S_2$: C, 62.29%; H, 6.58%; N, 7.03%; S, 10.73%. Found: C, 62.18%; H, 6.48%; N, 6.99%; S, 11.12%.

***N*-(*N*-2-Methoxyacetyl-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (26).** Amorphous solid; $[\alpha]_D^{25}$ −52.4° (*c* 1.11, $CHCl_3$); IR (KBr) ν 3299, 3064, 2924, 2850, 2462, 1649, 1589, 1507, 1489, 1448, 1334, 1238, 1167, 1124, 1015, 962, 925, 871, 845, 749, 692, 573, 499, 439, 428 cm^{-1}; MS (APCI) *m*/*z* 586 $(M+H)^+$; ^1H NMR (500 MHz, DMSO-d_6) δ 8.11 (br, 1H), 7.89 (br, 1H), 7.38–7.34 (m, 2H), 7.29–7.27 (m, 2H), 7.13–7.09 (m, 1H), 6.99–6.93 (m, 4H), 4.89 (dd, *J* = 7.5, 4.0 Hz, 1H), 4.78 (d, *J* = 9.5 Hz, 1H), 4.48–4.42 (m, 2H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.12–4.01 (m, 2H), 3.30 (s, 3H), 3.34–3.27 (m, 1H), 3.17–3.07 (m, 1H), 2.89 (dd, *J* = 13.5, 6.3 Hz, 1H), 2.76 (dd, *J* = 13.5, 7.5 Hz, 1H), 2.43 (dd, *J* = 6.5, 1.5 Hz, 2H), 1.78–1.72 (m, 2H), 1.68–1.56 (m, 3H), 1.47–1.38 (m, 1H), 1.26–1.08 (m, 3H), 0.99–0.90 (m, 2H).

(500 MHz, DMSO-d_6, 100 °C) δ 8.16–8.04 (br, 1H), 7.97–7.85 (br, 1H), 7.38–7.34 (m, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.99–6.92 (m, 4H), 4.89 (dd, *J* = 7.0, 3.5 Hz, 1H), 4.79 (d, *J* = 9.0 Hz, 1H), 4.48–4.43 (m, 2H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.10 (d, *J* = 14.5 Hz, 1H), 4.04 (d, *J* = 14.5 Hz, 1H), 3.34–3.28 (m, 4H), 3.15–3.12 (m, 1H), 2.89 (dd, *J* = 13.0, 6.0 Hz, 1H), 2.76 (dd, *J* = 13.0, 7.0 Hz, 1H), 2.44 (dd, *J* = 13.0, 7.0 Hz, 1H), 2.43 (dd, *J* = 13.0, 6.0 Hz, 1H), 1.77–1.57 (m, 5H), 1.47–1.38 (m, 1H), 1.26–1.09 (m, 3H), 0.98–0.91 (m, 2H); HRMS (MALDI-TOF) calcd for $C_{30}H_{39}N_3O_5S_2Na$: *m*/*z* 608.2229 $(M+Na)^+$. Found: *m*/*z* 608.2204 $(M+Na)^+$.

***N*-(*N*-2-Methoxyethyl-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (27).** Colorless oil; IR (neat) ν 3332, 2925, 2851, 1643, 1574, 1487, 1385, 1363, 1193, 1073, 1015, 876, 865, 705, 558 cm^{-1}; MS (APCI) *m*/*z* 572 $(M+H)^+$; ^1H NMR (200 MHz, CD_3OD) δ 7.38–7.26 (m, 4H), 7.14–7.05 (m, 1H), 6.97–6.90 (m, 4H), 4.49 (dd, *J* = 7.6, 5.7 Hz, 1H), 4.37 (s, 2H), 4.20 (d, *J* = 10.0 Hz, 1H), 4.12–4.06 (m, 2H), 3.61–3.54 (m, 2H), 3.42–3.35 (m, 1H), 3.34 (s, 3H), 3.08 (dd, *J* = 11.0, 7.6 Hz, 1H), 2.96 (dd, *J* = 13.7, 5.7 Hz, 1H), 2.90–2.73 (m, 3H), 2.41 (d, *J* = 6.8 Hz, 2H), 1.90–0.80 (m, 11H); HRMS calcd for: HRMS (MALDI-TOF) calcd for $C_{30}H_{41}N_3O_4S_2Na$: *m*/*z* 594.2436 $(M+Na)^+$. Found: *m*/*z* 594.2409 $(M+Na)^+$.

***N*-(*N*-2-Hydroxyethyl-(*R*)-thiazolidine-4-carbonyl)-*S*-cyclohexylmethyl-L-cysteine 4-phenoxybenzylamide (28).** IR (KBr) ν 3300, 3066, 2923, 2850, 1650, 1590, 1507, 1489, 1447, 1307, 1238, 1167, 1132, 1056, 962, 871, 845, 748, 692, 498, 410 cm^{-1}; MS (APCI) *m*/*z* 558 $(M+H)^+$; ^1H NMR (200 MHz, CD_3OD) δ 7.33–7.24 (m, 4H), 7.13–7.04 (m, 1H), 6.97–6.89 (m, 4H), 4.50 (dd, *J* = 8.4, 5.3 Hz, 1H), 4.36 (s, 2H), 4.26 (d, *J* = 10.0 Hz, 1H), 4.13–4.05 (m, 2H), 3.80–3.60 (m, 2H), 3.43 (dd, *J* = 10.7, 2.8 Hz, 1H), 3.08 (dd, *J* = 10.7, 7.5 Hz, 1H), 2.99 (dd, *J* = 14.0, 5.3 Hz, 1H), 2.81 (dd, *J* = 14.0. 8.4 Hz, 1H), 2.82–2.58 (m, 2H), 2.40 (d, *J* = 7.0 Hz, 2H), 1.88–0.80 (m, 11H); HRMS (MALDI-TOF) calcd for $C_{29}H_{39}N_3O_4S_2$ Na: *m*/*z* 580.2280 $(M+Na)^+$. Found: *m*/*z* 580.2255 $(M+Na)^+$.

In vitro pharmacology

Cell culture. IMR-32 (human neuroblastoma cells) and AtT-20/D16v-F2 (mouse pituitary tumor-derived cells) were cultured at 37 °C under 5% CO_2–95% air in a humidified chamber.

IMR-32 cells. These were grown as already described[11] in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin. To achieve a differentiated phenotype, cells were incubated with DMEM containing 5% FBS, penicillin-streptomycin, 1 mM dibutyryl-cAMP and 2.5 μM 5-bromodeoxyuridine for 9–12 days.

AtT-20/D16v-F2 cells. AtT-20/D16v-F2, mouse pituitary tumor-derived cells, were grown in Dulbecco's Modified Eagle's medium containing 10% FBS, penicillin—streptomycin.[12]

$[Ca^{2+}]_i$ measurement. $[Ca^{2+}]_i$ was measured in cell suspension. IMR-32 or AtT-20/d16v-F2 cells were suspended in DMEM containing 10% Nu serum V culture supplement (Becton Dickenson), 10 mM HEPES. Cell suspension was incubated with 5 mM fura-2/AM for 30 min at 37 °C. Cells were resuspended in Krebs-Ringer HEPES solution [(in mM) NaCl 125, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2, D-glucose 6, KH_2PO_4 1.2 HEPES-NaOH 25 pH 7.4], and adjusted to 1.0×10^6 cells/mL (IMR-32), or 2.0×10^6 cells/mL (AtT20/D16v-F2). Fluorescence (λ_{Ex}: 340 nm and 380 nm, λ_{Em}: 500 nm) were detected by a fluorometer which was designed to measure fura-2 derived fluorescence (CAF-110 Japan Spectroscopic). Cell suspension was incubated with test compound and 10 μM nifedipine (IMR-32) or 3 μM ω-conotoxin MVIIC (AtT-20/D16v-F2) for 360 s before high-K^+ stimulus (IMR-32 cells: 80 mM K^+, AtT-20/D16v-F2 cells: 50 mM K^+). The increase in R340/380 (ΔR340/380) evoked by high-K^+ stimulus was used as an index of relative increase in $[Ca^{2+}]_i$ increase. Inhibition% was calculated by comparison of ΔR340/380 in each measurement with that in measurement in the absence of the test compounds. The values in Tables 1–3 represent the average of two sets of observations within an error range of 8.0% for both IMR-32 and AtT-20 assays.

Electrophysiological recording. The electrophysiological recordings were performed in the conventional whole-cell

configurations under voltage-clamp conditions using IMR-32 cells. Pipettes had a resistance of 3–6 MΩ. Membrane currents were measured using a patch clamp amplifier (Axopatch 2B Axon Instruments). The test compounds were applied using a rapid application method termed as a 'Y-tube method'.

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