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Bioorganic & Medicinal Chemistry 12 (2004) 4101–4116
Bioorganic & Medicinal Chemistry

4,5-Dialkylsubstituted 2-imino-1,3-thiazolidine derivatives as potent inducible nitric oxide synthase inhibitors

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Received 28 April 2004; revised 22 May 2004; accepted 22 May 2004
Available online 17 June 2004

Abstract—In the course of our search for selective iNOS inhibitors, we have previously reported that 2-imino-1,3-oxazolidine derivatives (**1**) and 2-aminothiazole derivatives (**2**) are selective iNOS inhibitors. In order to find more potent iNOS inhibitors, we focused our efforts on the synthesis and evaluation of the inhibitory activity against iNOS and selectivity for iNOS both in vitro and in vivo of a series of 2-imino-1,3-thiazolidine derivatives (**3**), which are analogues of **1** and **2**. Our results show that among the compounds synthesized (4*R*,5*R*)-5-ethyl-2-imino-4-methyl-1,3-thiazolidine [(4*R*,5*R*)-**14a**: ES-1537] exhibited potent inhibitory activity and selectivity for iNOS. In addition, ES-1537 had good pharmacokinetic profile in rats with BA value of 80%. It is therefore expected that ES-1537 may be therapeutically useful for the treatment of diseases related to excess production of NO.

1. Introduction

Nitric oxide (NO) is an important bioregulator and ubiquitous biomessenger existing in a wide variety of organisms. Over the past decades, there has been extensive scientific interest in the fundamental biochemistry and physiological roles of NO.[1,2] NO is produced by oxidation of L-arginine catalyzed by nitric oxide synthase (NOS), which utilizes NADPH, molecular oxygen, and cofactors such as tetrahydrobiopterin, flavin mononucleotide, flavin adenine dinucleotide, and heme-contained porphyrin.[3]

Based on its endogenous regulation, NOS has been structurally classified as constitutive NOS (cNOS), which requires Ca^{2+}/calmmodulin for its activation, and inducible NOS (iNOS), which is independent of Ca^{2+}/calmmodulin for its activation. Furthermore, cNOS has been subdivided into endothelial NOS (eNOS) found in the vascular endothelium, and neuronal NOS (nNOS) found in the brain. eNOS is known to be implicated in vascular tone and platelet aggregation, while nNOS has been shown to regulate neuronal transmission and cerebral blood flow.[4,5] iNOS, on the other hand, is mainly expressed in macrophages activated by inflammatory cytokines or lipopolysaccharide (LPS) stimuli, and its major function is thought to serve in host defense mechanism. However, it has also been reported that iNOS is implicated in the uncontrolled production of NO that causes inflammatory diseases such as shock condition, inflammatory arthritis, chronic ileitus, and colitis.[6–8]

The first reported NOS inhibitors have been limited to L-arginine analogues such as N^G-nitro-L-arginine,[9] N^G-monomethyl-L-arginine (L-NMMA),[10] N^G-nitro-L-arginine methyl ester.[11] Some of these compounds have been investigated in clinical trials, however, their lack of selectivity for iNOS hampered further investigation. Therefore, compounds with selective inhibition against iNOS over its other isoforms are needed to avoid complications resulting from inhibition of NO normal

Keywords: 2-Imino-1,3-thiazolidine; Inducible nitric oxide synthase inhibitor; Orally bioavailable.
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doi:10.1016/j.bmc.2004.05.031

physiological production and to dissect the natural pharmacological effects derived from iNOS isoforms.

Indeed, many kinds of non-L-arginine type NOS inhibitors such as N^{δ}-iminomethyl-L-ornithine,[12] *S*-alkyl-L-thiocitrulline,[13] 7-nitroindazole,[14] *N*-phenylisothioureas,[15] 2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine,[16] (1*S*,4*S*,6*R*,7*R*)-7-chloro-3-imino-5-methyl-2-azabicyclo-[4.1.0]heptane hydrochloride,[17] and 2-aminopyridine[18] have been reported to exhibit potent inhibition with variable selectivity for iNOS and its isoforms. In addition, compounds such as flavonoids,[19] coumarins,[20] and diterpenes[21] isolated from natural product have been reported to inhibit iNOS. These inhibitors have been investigated in connection with a number of inflammatory diseases in animal models and clinical trials.

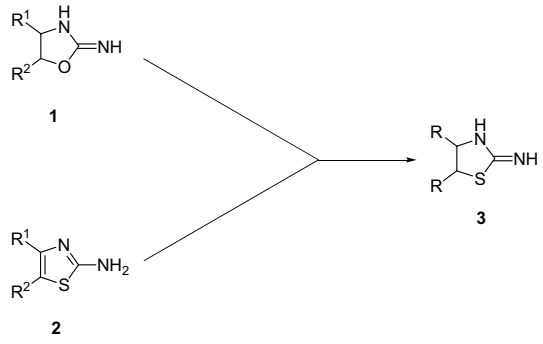
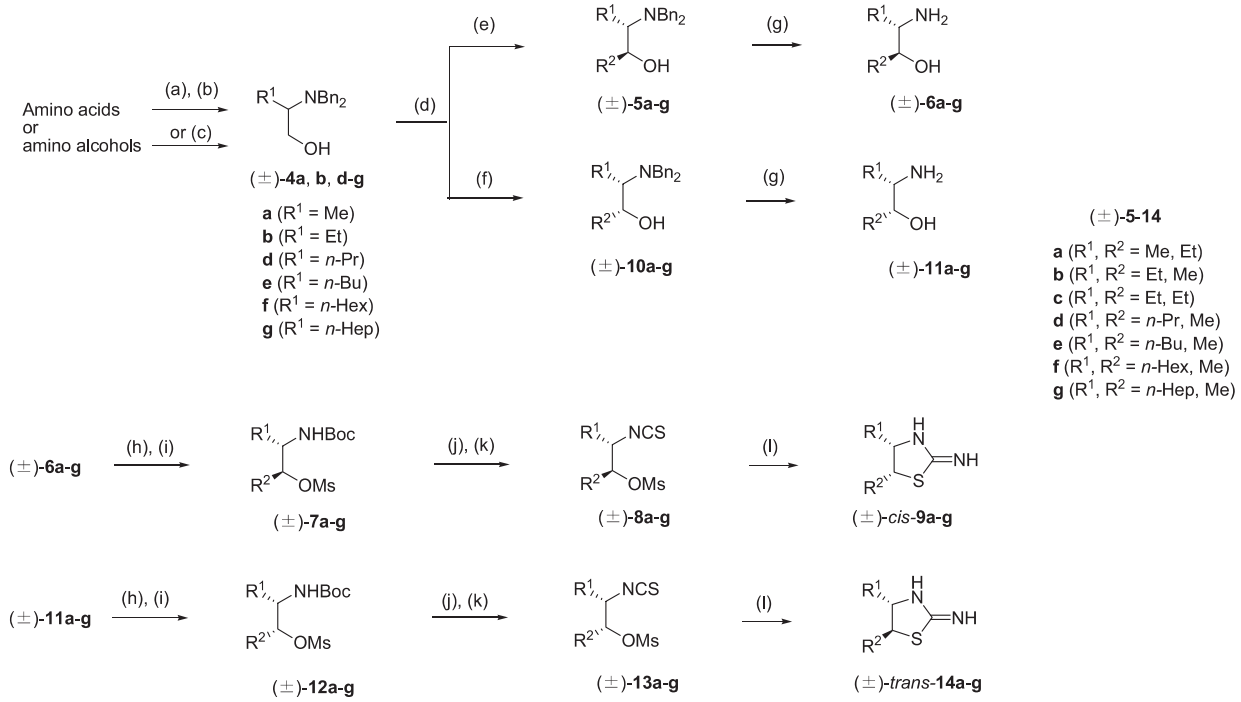


Chart 1. Molecular design of the 2-imino-1,3-thiazolidine derivatives **3**.

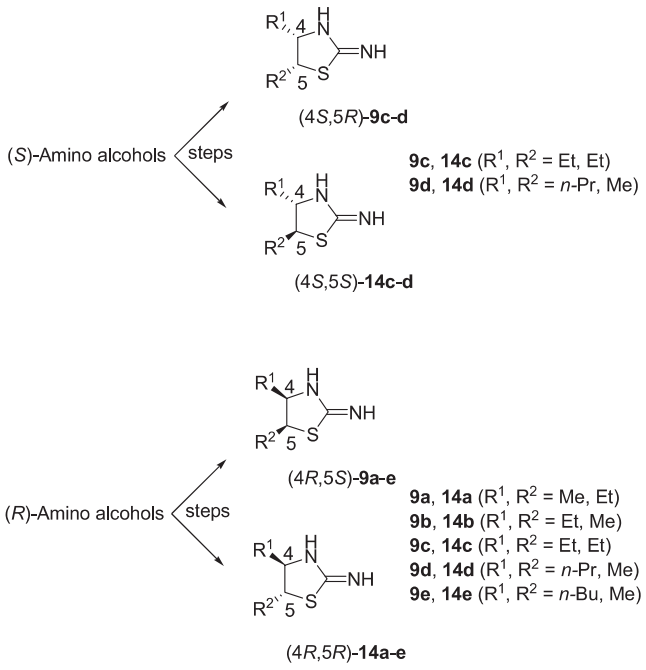
In the course of our search for selective iNOS inhibitors, we have previously reported that 2-imino-1,3-oxazolidine derivatives (**1**)[22] and 2-aminothiazole derivatives (**2**)[23] (Chart 1) are selective iNOS inhibitors. Since the inhibitory activity of **1** against iNOS is higher than that of **2** but both **1** and **2** have same selectivity for iNOS, we focused our efforts in this field on evaluating the inhibitory activity of a series of analogues of **1** and **2** that is 2-imino-1,3-thiazolidine derivatives against iNOS. In this paper, we report the synthesis, structure–activity relationships (SARs) and evaluation of the inhibitory activity and selectivity for iNOS both in vitro and in vivo of a series of novel 4,5-disubstituted 2-imino-1,3-thiazolidine derivatives (**3**) (Chart 1).

2. Chemistry

The synthesis of 4,5-disubstituted 2-imino-1,3-thiazolidine derivatives (±)-*cis*-**9** is outlined in Scheme 1. *N*,*N*-dibenzylamino alcohols (±)-**4** were obtained from commercially available amino acids or amino alcohols by reported procedure with some modifications.[24] Swern oxidation of (±)-**4**, resulting in intermediate aldehyde, followed by diastereoselective alkylation with alkylzinc reagent under chelation control[25] gave secondary alcohols (±)-**5**. Hydrogenation of (±)-**5** using Pearlman's catalyst gave free amines (±)-**6**. Sequential protection of the amine and hydroxy groups of the resulting (±)-**6** using Boc_2O and MsCl gave mesylates (±)-**7**. Conversion of (±)-**7** to isothiocyanates (±)-**8** was achieved by removal of Boc group of (±)-**7** using hydrogen chloride in EtOH and the following reaction with thiophosgen in



Scheme 1. Synthesis of racemic 4,5-disubstituted 2-imino-1,3-thiazolidine derivatives as iNOS inhibitors. Reagents and conditions: (a) BnBr, K_2CO_3, H_2O, 100 °C; (b) $LiAlH_4$, THF, rt; (c) BnBr, K_2CO_3, CH_3CN, 60 °C; (d) Swern oxi.; (e) $(R^2)_2Zn$, toluene, 0 °C; (f) R^2MgBr, THF, 0 °C; (g) H_2, 20% $Pd(OH)_2$–C, MeOH, rt; (h) Boc_2O, CH_2Cl_2, rt; (i) MsCl, Et_3N, CH_2Cl_2, 0 °C; (j) 30% HCl/EtOH, rt; (k) $CSCl_2$, $CaCO_3$, $CHCl_3$, H_2O, 0 °C; (l) 28% NH_3 aq, dioxane, 60 °C.



Scheme 2. Synthesis of the optically active 4,5-disubstituted 2-imino-1,3-thiazolidine derivatives as iNOS inhibitors. The reagents and conditions of synthesis for these derivatives are the same as those for the racemic derivatives.

$CHCl_3$–H_2O system. (±)-**8** was subjected to aqueous ammonia in dioxane to give (±)-*cis*-**9**. On the other hand, the synthesis of (±)-*trans*-**14** from (±)-**4** was conducted in a similar manner to that for the preparation of (±)-*cis*-**9** except for the use of Grignard's reagent[24,25] (condition f) instead of dialkylzinc (condition e), which was subjected to intermediate aldehyde.

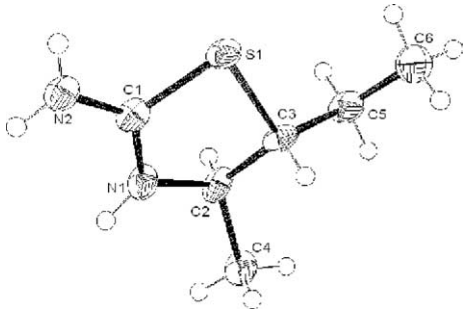


Figure 1. ORTEP view of X-ray structure of (4*R*,5*R*)-**14a**.

Similarly, the optically active compounds (4*S*,5*R*)-**9c**,**d**, (4*S*,5*S*)-**14c**,**d**, (4*R*,5*S*)-**9a**–**e**, and (4*R*,5*R*)-**14a**–**e** were synthesized from the corresponding optically active amino alcohols (Scheme 2). The absolute configuration of each optically active 2-imino-1,3-thiazolidine derivative was confirmed by X–ray crystallographic analysis of (4*R*,5*R*)-**14a** as a representative example (Fig. 1). This analysis supports the structure depicted in Tables 2 and 3.

3. Results and discussion

The inhibitory activity against iNOS and nNOS of the synthesized compounds that is (±)-*cis*-**9a**–**g**, (±)-*trans*-**14a**–**g**, (4*S*,5*R*)-**9c**,**d**, (4*S*,5*S*)-**14c**,**d**, (4*R*,5*S*)-**9a**–**e**, and (4*R*,5*R*)-**14a**–**e** was evaluated according to a previously reported procedure[26,27] with some modifications, and selectivity for iNOS of synthesized compounds was defined as the ratio of IC_{50} value of nNOS to iNOS.

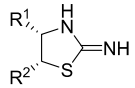
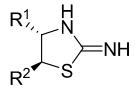
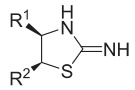
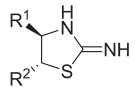
Our initial investigation of the 2-imino-1,3-thiazolidine derivatives focused on introduction of aliphatic

Table 1. Inhibitory activity of disubstituted 2-imino-1,3-thiazolidine derivatives

Structure	Compound	R^1	R^2	Inhibitory activity[a] (IC_{50})		Selectivity[b]
				iNOS (μM)[c]	nNOS (μM)[d]	nNOS/iNOS
(±)-*cis*	**9a**	Me	Et	0.060	0.98	16
	9b	Et	Me	0.053	0.38	7.2
	9c	Et	Et	0.034	0.72	21
	9d	*n*-Pr	Me	0.0085	0.11	12
	9e	*n*-Bu	Me	0.022	0.20	9.1
	9f	*n*-Hex[e]	Me	0.14	0.89	6.4
	9g	*n*-Hep[f]	Me	0.34	0.60	1.8
(±)-*trans*	**14a**	Me	Et	0.012	0.29	24
	14b	Et	Me	0.011	0.038	3.5
	14c	Et	Et	0.014	0.53	38
	14d	*n*-Pr	Me	0.015	0.16	11
	14e	*n*-Bu	Me	0.031	0.12	3.9
	14f	*n*-Hex[e]	Me	0.13	0.12	0.9
	14g	*n*-Hep[f]	Me	0.28	0.045	0.16
	1a[22]			0.041	0.92	22
	2a[23]			4.1	5.5	1.3
	L-NMMA			19	4.3	0.23

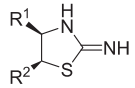
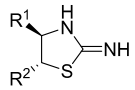
[a] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight concentrations.
[b] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.
[c] iNOS activity were evaluated by the procedure described in the Experimental section.
[d] nNOS activity were evaluated by the procedure described in the Experimental section.
[e] Hexyl.
[f] Heptyl.

Table 2. Inhibitory activity of optically active isomers of **9c**, **9d**, **14c**, and **14d**

Structure	Entry	Compound	R^1	R^2	Inhibitory activity[a] (IC_{50})		Selectivity[b]
					iNOS (μM)[c]	nNOS (μM)[d]	nNOS/iNOS
	1	(4*S*,5*R*)-**9c**	Et	Et	0.40	1.9	4.8
	2	(4*S*,5*R*)-**9d**	*n*-Pr	Me	0.032	0.25	7.8
	3	(4*S*,5*S*)-**14c**	Et	Et	0.16	0.92	5.8
	4	(4*S*,5*S*)-**14d**	*n*-Pr	Me	0.0069	0.043	6.2
	5	(4*R*,5*S*)-**9c**	Et	Et	0.020	0.39	20
	6	(4*R*,5*S*)-**9d**	*n*-Pr	Me	0.0057	0.092	16
	7	(4*R*,5*R*)-**14c**	Et	Et	0.011	0.50	45
	8	(4*R*,5*R*)-**14d**	*n*-Pr	Me	0.014	0.27	19

[a] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight concentrations.
[b] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.
[c] iNOS activity were evaluated by the procedure described in the Experimental section.
[d] nNOS activity were evaluated by the procedure described in the Experimental section.

Table 3. Inhibitory activity of (4*R*)-configurated 2-imino-1,3-thiazolidine derivatives

Structure	Compound	R^1	R^2	Inhibitory activity[a] (IC_{50})		Selectivity[b]
				iNOS (μM)[c]	nNOS (μM)[d]	nNOS/iNOS
	(4*R*,5*S*)-**9a**	Me	Et	0.040	0.96	24
	(4*R*,5*S*)-**9b**	Et	Me	0.050	0.91	18
	(4*R*,5*S*)-**9e**	*n*-Bu	Me	0.015	0.27	18
	(4*R*,5*R*)-**14a**	Me	Et	0.0066	0.38	58
	(4*R*,5*R*)-**14b**	Et	Me	0.0094	0.12	13
	(4*R*,5*R*)-**14e**	*n*-Bu	Me	0.038	0.61	16

[a] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight concentrations.
[b] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.
[c] iNOS activity were evaluated by the procedure described in the Experimental section.
[d] nNOS activity were evaluated by the procedure described in the Experimental section.

substituents into the 4- and 5-position of the thiazolidine ring affording the (±)-*cis*-**9a**–**g** or (±)-*trans*-**14a**–**g** derivatives.

As shown in Table 1, when a propyl and a methyl groups were inserted into the 4-position and the 5-position, respectively, of the thiazolidine ring, the inhibitory activity against iNOS of the resulting compound (±)-*cis*-**9d** was greatly improved compared to that of the thiazole analogue **2a** (R^1, R^2 = *n*-Pr, Me) [(±)-*cis*-**9d**: $IC_{50} = 0.0085\ \mu M$, 480-fold that of **2a**]. From this finding, it is suggested that the strong inhibitory activity of (±)-*cis*-**9d** against iNOS is caused by a hydrogen bond between the amidine moiety of (±)-*cis*-**9d** and the carboxylic acid moiety of Glu371 in iNOS oxygenase domain.[28] This suggestion is supported by previous reports showing that compounds with an amidine moiety strongly inhibit iNOS.[16,17] In addition, it has been reported that interaction between the sulfur atom of NOS inhibitors and the heme iron in NOS oxygenase domain increases the inhibitory activity against NOS.[29] Thus, it is assumed that the strong inhibitory activity against iNOS of (±)-*cis*-**9d** is due to the interaction between sulfur and heme iron. This assumption is in accordance with our result showing that the inhibitory activity against iNOS of (±)-*cis*-**9d** is five times stronger than that of the oxazolidine analogue (±)-*cis*-**1a** (R^1, R^2 = *n*-Pr, Me).

When alkyl groups such as methyl, ethyl, or butyl were introduced into the 4-position of the thiazolidine ring, the inhibitory activity against iNOS of the resulting compounds ranged from 0.011 to 0.060 μM [(±)-*cis*-**9a**–**c**,**e**, (±)-*trans*-**9a**–**e**; Table 1]. These results indicate that small size substituents at the 4-position of the thiazolidine ring are well tolerated in iNOS inhibition. Additional increase in the size of the substituent at the 4-position, that is a hexyl or heptyl group reduced the inhibitory activity against iNOS [(±)-*cis*-**9f**,**g**, (±)-*trans*-**14f**,**g**; Table 1].

Next, the selectivity for iNOS of the synthesized compounds is shown in Table 1. Interestingly, compounds with an ethyl group at the 5-position of the thiazolidine ring showed moderate selectivity for iNOS [(±)-*cis*-**9a**,**c**, (±)-*trans*-**14a**,**c**], especially, (±)-*trans*-**14c** exhibited good selectivity for iNOS (nNOS/iNOS = 38) among all the

racemic derivatives synthesized. These results are in agreement with those of our previous report describing the oxazolidine derivatives.[22] On the other hand, an increase in the size of the substituent at the 4-position of the thiazolidine ring attenuated the selectivity for iNOS, especially (±)-*trans*-**14g** contrastively showed nNOS selectivity (nNOS/iNOS = 0.16).

From the aforementioned findings, we selected compounds with the best inhibitory activity against iNOS and the best selectivity for iNOS among the racemic derivatives [(±)-*cis*-**9d** and(±)-*trans*-**14c**], and further evaluated the inhibitory activity against iNOS and selectivity for iNOS of their optically active isomers.

As shown in Table 2, among the four isomers with 4-propyl-5-methyl substitution, the inhibitory activity against iNOS of compounds with *S* configuration at the 5-position was higher than that of compounds with *R* configuration (entry 6 vs entry 8 and entry 4 vs entry 2). In the case of 4,5-diethyl substitution, the inhibitory activity against iNOS of compounds with *R* configuration at the 4-position was higher than that of compounds with *S* configuration (entry 5 vs entry 3 and entry 7 vs entry 1).

On the other hand, among the four isomers with 4-propyl-5-methyl substitution, the selectivity for iNOS of compounds with *R* configuration at the 4-position was better than that of compounds with *S* configuration (entry 6 vs entry 4 and entry 8 vs entry 2). In the case of 4,5-diethyl substitution, a similar result to that above was obtained (entry 5 vs entry 3 and entry 7 vs entry 1).

As the trend of good selectivity for iNOS was limited to compounds with *R* configuration at the 4-position (entries 5–8 vs entries 1–4), we evaluated the inhibitory activity and selectivity for iNOS of other racemic derivatives with *R* configuration at the 4-position that is (±)-*cis*-**9a**,**b**,**e** and (±)-*trans*-**14a**,**b**,**e** depicted in Table 1.

As shown in Table 3, (4*R*,5*R*)-**14a** (ES-1537) showed strong inhibitory activity against iNOS and good selectivity for iNOS ($IC_{50} = 0.0066\,\mu M$, nNOS/iNOS = 58). Interestingly, among compounds depicted in Table 2, the configuration at the 5-position of compounds showing strong inhibitory activity against iNOS is '*S*' (entries 4 and 6), however, the configuration at the 5-position of (4*R*,5*R*)-**14a** is not '*S*' but '*R*' (Table 3). Therefore, the inhibitory activity against iNOS and selectivity for iNOS of (4*R*,5*R*)-**14a** is greatly affected with *R* configuration rather than *S* configuration at the 5-position as well as at the 4-position.

Other *R*-configurated derivatives showed strong inhibitory activity against iNOS in the nanomol and/or subnanomol order and moderate selectivity for iNOS.

The strong inhibitory activity against iNOS of (4*R*,5*R*)-**14a** was supported by a docking study (Fig. 2). As expected, the amidine moiety of (4*R*,5*R*)-**14a** tightly coordinated to the carboxylic acid group of Glu371, and the ethyl group of (4*R*,5*R*)-**14a** was placed in the hydrophobic cavity above the porphyrin ring of iNOS oxygenase domain. In addition, interaction between the sulfur atom of the compound and the iron atom of heme may be expected.[30]

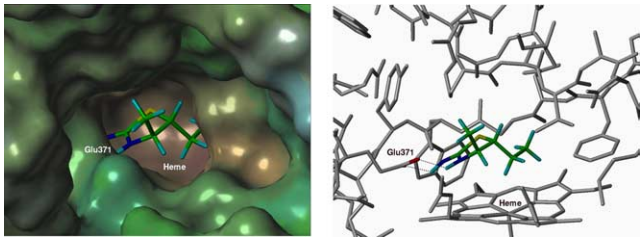


Figure 2. Left: Docking model of compound (4*R*,5*R*)-**14a** in complex with iNOS in a surface representation. The compound resides in the small pocket above the heme. Right: Expected binding mode of (4*R*,5*R*)-**14a** (green carbon atoms, yellow sulfur atom) in complex with iNOS oxygenase domain (gray). Interaction of (4*R*,5*R*)-**14a** with iNOS is mainly due to a hydrogen bond between the amidine moiety of the inhibitor (light blue hydrogen atoms connected to blue nitrogen atoms) and the carboxylic acid group (red oxygen atoms, residue of Glu371). In addition, interaction of the sulfur atom of (4*R*,5*R*)-**14a** with the iron atom of heme may be expected.

Table 4. Effects of selected compounds on plasma nitrite/nitrate levels in LPS-treated mice[a]

Compound	Inhibitory activity $ID_{50} \pm SD$ (mg/kg, po)
(4*R*,5*S*)-**9d**	0.35 ± 0.02
(4*R*,5*S*)-**9e**	0.38 ± 0.04
(4*R*,5*R*)-**14a**	0.13 ± 0.03
(4*R*,5*R*)-**14c**	0.15 ± 0.03
(4*S*,5*S*)-**14d**	0.31 ± 0.03

[a] The effects of each compound on plasma nitrite/nitrate levels were evaluated according to previously reported methods[17,31] with some modifications (see Experimental section).

Next, the inhibitory activity of selected compounds against iNOS in vivo was determined by evaluating their effects on plasma nitrite/nitrate levels in LPS-treated mice. As shown in Table 4, (4*R*,5*R*)-**14a**, given orally, strongly inhibited the increase in plasma nitrite/nitrate levels in mice (ID_{50} value of 0.13 mg/kg). Other selected compounds also inhibited the increase in plasma nitrite/nitrate levels (range of ID_{50} is 0.15–0.38 mg/kg). Finally, in order to investigate the pharmacokinetic profile of (4*R*,5*R*)-**14a**, we evaluated the bioavailability of this compound in rats.

As shown in Table 5, intravenous administration of (4*R*,5*R*)-**14a** at a dose of 0.3 mg/kg had a mean integrated area under plasma concentration (AUC) of 110 (ng·h/mL), while its oral administration at the same dose exhibited an AUC of 88 (ng·h/mL). Thus oral bioavailability of (4*R*,5*R*)-**14a** was 80%. Considering the strong inhibitory activity and high selectivity of (4*R*,5*R*)-**14a** for iNOS, as well as its pharmacokinetic profile, it is suggested that this compound might be therapeutically useful for the treatment of diseases related to excess production of NO.

Table 5. Rat pharmacokinetic data of (4*R*,5*R*)-**14a**

Route (dosage)	Parameters	Mean ± SD[a]
iv (0.3 mg/kg)	AUC (0–3) (ng·h/mL)[b]	110 ± 10
	$t_{1/2}$ (h)[c]	0.75 ± 0.10
	CL (L/h/kg)[d]	2.8 ± 0.3
	Vss (L/kg)[e]	3.0 ± 0.1
po (0.3 mg/kg)	C_{max} (ng/mL)[f]	50 ± 10
	t_{max} (h)[g]	0.75 ± 0.3
	AUC (0–3) (ng·h/mL)[b]	88 ± 19
	$t_{1/2}$ (h)[c]	0.82 ± 0.19
	BA (%)[h]	80 ± 18

[a] Each value represents the mean ± standard deviation (SD) of three animals in this study (see Experimental section).
[b] Integrated area under plasma concentration versus time curve from 0 to 3 h.
[c] Pharmacokinetic half-life.
[d] Plasma clearance.
[e] Steady-state volume of distribution.
[f] Maximum plasma concentration of unchanged compound in plasma.
[g] Time of maximum concentration.
[h] Oral bioavailability.

4. Conclusions

In this paper, we described the synthesis, SARs, and evaluation of the inhibitory activity and selectivity for NOS both in vitro and in vivo of a series of 2-imino-1,3-thiazolidine derivatives. Our results show that: (1) Introduction of small size alkyl groups into the 2-iminothiazolidine frameworks led to strong inhibition against iNOS at the nanomol order. (2) An increase in the size of the alkyl group at the 4-position led to a better selectivity for nNOS. (3) Among the synthesized compounds with appropriately sized substituents, compounds with *R* configuration at the 4-position had better selectivity for iNOS than those with *S* configuration. (4) Introduction of differently sized alkyl groups into the 2-iminothiazolidine led to the discovery of (4*R*,5*R*)-5-ethyl-2-imino-4-methyl-1,3-thiazolidine [(4*R*,5*R*)-**14a**: ES-1537], which showed strong inhibitory activity against iNOS and good selectivity for iNOS (IC_{50} value of 0.0066 μM, nNOS/iNOS ratio of 58). In vivo study revealed that ES-1537, given orally, strongly inhibited the increase in plasma nitrite/nitrate levels in LPS-treated mice with ID_{50} value of 0.13 mg/kg. In addition, ES-1537 had a good pharmacokinetic profile with 80% bioavailability. It is therefore suggested that ES-1537 might be therapeutically useful for treatment of diseases related to excess production of NO.

5. Experimental

5.1. Synthesis

In general, melting points were obtained on a Yanagimito micro-melting point apparatus MP-J3 and are uncollected. ^{1}H NMR spectra were recorded at 300 MHz on a JEOL spectrometer using deuterated solvent with tetramethylsilane as an internal standard and chemical shift values are indicated in parts per million. MS spectrum were recorded on a Shimadz QP-8000. Column chromatography was carried out on Kieselgel 60 (Merck; 70–230 mesh) or Chromatorex®-NH (Fuji Silysia Chemical; 100–200 mesh).

5.2. Synthesis of (±)-*N*,*N*-dibenzyl-2-aminopropan-1-ol [(±)-4a]

To a solution of 2-aminopropan-1-ol (50.0 g, 666 mmol) in acetonitrile (1.33 L) was added K_2CO_3 (138 g, 99.8 mmol) and benzylbromide (228 g, 1.33 mol). After stirring at 60 °C for 8 h, the reaction mixture was cooled to room temperature, and the resulting precipitates were filtered off. The mother liquor was concentrated under reduced pressure to give crude material that was partitioned into chloroform and water. The organic phase was dried over anhydrous magnesium sulfate, and evaporated in vacuo to give oily product (157 g). This compound was used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 6.8$ Hz), 2.98 (1H, m), 3.12 (1H, br), 3.35 (1H, t, $J = 7.8$ Hz), 3.36 (2H, d, $J = 13.4$ Hz), 3.46 (1H, t, $J = 10.5$ Hz), 3.82 (2H, d, $J = 13.2$ Hz), 7.21–7.38 (10H, m). MS m/z 256 $[M+1]^+$.

The following *N*,*N*-dibenzylamino-alcohol derivatives were synthesized according to a synthetic method similar to that of (±)-**4a**.

5.2.1. (±)-*N*,*N*-Dibenzyl-2-aminobutan-1-ol [(±)-4b]. ^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 7.4$ Hz), 1.24 (1H, m), 1.78 (1H, td, $J = 7.7$, 4.0 Hz), 2.71 (1H, m), 3.40 (1H, t, $J = 10.4$ Hz), 3.42 (2H, d, $J = 13.2$ Hz), 3.52 (1H, dd, $J = 10.4$, 5.0 Hz), 3.82 (2H, d, $J = 13.2$ Hz), 7.21–7.34 (10H, m). MS m/z 270 $[M+1]^+$.

5.2.2. (±)-*N*,*N*-Dibenzyl-2-aminopentan-1-ol [(±)-4d]. ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, t, $J = 7.1$ Hz), 1.13–1.43 (3H, m), 1.68 (1H, m), 2.79 (1H, m), 3.18 (1H, br), 3.40 (1H, t, $J = 10.4$ Hz), 3.40 (2H, d, $J = 13.2$ Hz), 3.50 (1H, dd, $J = 10.6$, 5.1 Hz), 3.81 (2H, d, $J = 13.2$ Hz), 7.21–7.36 (10H, m). MS m/z 284 $[M+1]^+$.

5.2.3. (±)-*N*,*N*-Dibenzyl-2-aminohexan-1-ol [(±)-4e]. ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 7.0$ Hz), 1.13–1.37 (5H, m), 1.73 (1H, m), 2.78 (1H, m), 3.21 (1H, br), 3.40 (1H, t, $J = 10.3$ Hz), 3.41 (2H, d, $J = 13.4$ Hz), 3.50 (1H, dd, $J = 10.6$, 5.1 Hz), 3.82 (2H, d, $J = 13.4$ Hz), 7.21–7.33 (10H, m). MS m/z 298 $[M+1]^+$.

5.3. Synthesis of (±)-*N*,*N*-dibenzyl-2-aminoctan-1-ol [(±)-4f]

To an aqueous solution of 10% K_2CO_3 (200 mL) was added 2-aminoctanoic acid (5.00 g, 31.4 mmol) and BnBr (21.5 g, 126 mmol). After stirring at 80 °C over night, the reaction mixture was cooled to room temperature, and extracted with chloroform (50 mL × 2). The organic phase was dried over magnesium sulfate and evaporated in vacuo to give a crude oil (16.7 g). To a

suspension of $LiAlH_4$ (2.38 g, 62.9 mmol) in THF (150 mL) was added the crude oil in THF (25 mL) at 0 °C and the reaction mixture was allowed to warm to room temperature after which it was stirred over night. The reaction mixture was quenched by H_2O (2.4 mL), 15% aqueous sodium hydroxy solution (2.4 mL), and H_2O (7.2 mL) successively. The resulting white precipitate was filtered off, and the filtrate was concentrated to give a crude product. Purification by column chromatography on silica gel using AcOEt/hexane (1/3) as an eluent gave the title compound (8.89 g, 87%). ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J = 6.7$ Hz), 1.15–1.40 (9H, m), 1.69 (1H, m), 2.77 (1H, m), 3.19 (1H, br), 3.40 (1H, t, $J = 10.3$ Hz), 3.40 (2H, d, $J = 13.2$ Hz), 3.49 (1H, dd, $J = 10.5$, 5.0 Hz), 3.82 (2H, d, $J = 13.2$ Hz), 7.21–7.37 (10H, m). MS m/z 326 $[M+1]^+$. Anal. Calcd for $C_{22}H_{31}NO$: C, 81.18; H, 9.60; N, 4.30. Found: C, 81.08; H, 9.54; N, 4.35.

5.3.1. (±)-*N*,*N*-Dibenzyl-2-aminononan-1-ol [(±)-4g]. This compound was synthesized according to a synthetic method similar to that of (±)-**4f**. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J = 6.8$ Hz), 1.15–1.40 (11H, m), 1.72 (1H, m), 2.78 (1H, m), 3.18 (1H, s), 3.40 (1H, t, $J = 10.3$ Hz), 3.40 (2H, d, $J = 13.2$ Hz), 3.50 (1H, dd, $J = 10.5$, 5.0 Hz), 3.82 (2H, d, $J = 13.4$ Hz), 7.21–7.37 (10H, m). MS m/z 340 $[M+1]^+$. Anal. Calcd for $C_{23}H_{33}NO \cdot 0.25H_2O$: C, 80.30; H, 9.82; N, 4.07. Found: C, 80.24; H, 9.66; N, 4.17.

5.4. Synthesis of (±)-*N*,*N*-dibenzyl-4-aminopentan-3-ol [(±)-5a] and (±)-*N*,*N*-dibenzyl-4-aminopentan-3-ol [(±)-10a]

To a stirred solution of oxalylchloride (14.0 mL, 160 mmol) in CH_2Cl_2 (333 mL) was added dimethylsulfoxide (23.0 mL, 320 mmol) in CH_2Cl_2 (66.5 mL) over 10 min at −60 °C. The stirred reaction mixture was then treated within 10 min with crude (±)-**4a** (34.0 g) in CH_2Cl_2 (133 mL), and stirred for an additional 30 min at the same temperature. Subsequently, triethylamine (93.0 mL, 666 mmol) was added to the reaction mixture with vigorous stirring under −45 °C. The resulting milky mixture was washed successively with water (600 mL × 3) and brine, and then dried over magnesium sulfate. Filtration followed by evaporation in vacuo gave a crude aldehyde (33.7 g) that was used for the next step without further purification. To a stirred solution of the crude aldehyde (17.2 g) in toluene (136 mL) was added 1 M Et_2Zn in hexane (136 mL, 136 mmol) at 0 °C. The reaction mixture was then stirred over night at the same temperature, and quenched by satd NH_4Cl (13.6 mL) with stirring for an additional 1 h. The resulting white precipitate was filtered off, and the filtrate was concentrated in vacuo to give a crude oil. Purification by column chromatography on silica gel using AcOEt/*n*-hexane (1/10) as eluent gave (±)-**5a** (11.9 g, 62%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, t, $J = 7.3$ Hz), 1.00 (3H, t, $J = 6.6$ Hz), 1.14 (1H, m), 1.53 (1H, m), 2.55 (1H, dq, $J = 9.4$, 6.5 Hz), 3.30 (2H, d, $J = 13.4$ Hz), 3.41 (1H, td, $J = 8.9$, 2.7 Hz), 3.83 (2H, d, $J = 13.4$ Hz), 4.47 (1H, br), 7.20–7.34 (10H, m). MS m/z 284 $[M+1]^+$. Anal. Calcd for $C_{19}H_{25}NO$: C, 80.52; H, 8.89; N, 4.94. Found: C, 80.61; H, 8.91; N, 5.01. In the case of the synthesis of (±)-**10a**, crude aldehyde (15.8 g) was treated with 3 M ethylmagnesiumbromide followed by similar work up [AcOEt/*n*-hexane (1/8)] to afford (±)-**10a** (13.2 g, 75%). ^{1}H NMR ($CDCl_3$) δ: 0.87 (3H, t, $J = 7.4$ Hz), 1.11 (3H, d, $J = 6.8$ Hz), 1.29 (1H, m), 1.58 (1H, br), 1.75 (1H, m), 2.72 (1H, qu, $J = 6.6$ Hz), 3.47 (2H, d, $J = 13.7$ Hz), 3.54 (1H, m), 3.76 (2H, d, $J = 13.9$ Hz), 7.19–7.36 (10H, m). MS m/z 284 $[M+1]^+$. Anal. Calcd for $C_{19}H_{25}NO$: C, 80.52; H, 8.89; N, 4.94. Found: C, 80.26; H, 8.91; N, 5.04.

The following (±)-*N*,*N*-dibenzyl-amino alcohol derivatives were synthesized according to a synthetic method similar to that of (±)-**5a** and (±)-**10a**.

5.4.1. (±)-*N*,*N*-Dibenzyl-3-aminopentan-2-ol [(±)-5b]. Yield 33%. ^{1}H NMR ($CDCl_3$) δ: 1.09 (3H, t, $J = 7.5$ Hz), 1.10 (3H, d, $J = 6.0$ Hz), 1.37 (1H, m), 2.28 (1H, dt, $J = 9.2$, 5.7 Hz), 3.45 (2H, d, $J = 13.4$ Hz), 3.61 (1H, qd, $J = 9.2$, 6.1 Hz), 3.86 (2H, d, $J = 13.2$ Hz), 4.34 (1H, br), 7.21–7.33 (10H, m). MS m/z 284 $[M+1]^+$. Anal. Calcd for $C_{19}H_{25}NO$: C, 80.52; H, 8.89; N, 4.94. Found: C, 80.47; H, 8.87; N, 5.05.

5.4.2. (±)-*N*,*N*-Dibenzyl-4-aminohexan-3-ol [(±)-5c]. Yield 70%. ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 7.2$ Hz), 1.07 (3H, t, $J = 7.5$ Hz), 1.11 (1H, m), 1.31 (1H, m), 1.57 (1H, m), 1.79 (1H, m), 2.37 (1H, td, $J = 9.3$, 5.5 Hz), 3.40 (1H, td, $J = 8.7$, 2.6 Hz), 3.46 (2H, d, $J = 13.2$ Hz), 3.87 (2H, d, $J = 13.2$ Hz), 4.19 (1H, s), 7.20–7.33 (10H, m). MS m/z 298 $[M+1]^+$. Anal. Calcd for $C_{20}H_{27}NO$: C, 80.76; H, 9.15; N, 4.71. Found: C, 80.73; H, 9.16; N, 4.81.

5.4.3. (±)-*N*,*N*-Dibenzyl-3-aminohexan-2-ol [(±)-5d]. Yield 44%. ^{1}H NMR ($CDCl_3$) δ: 0.97 (3H, t, $J = 7.1$ Hz), 1.09 (3H, d, $J = 6.0$ Hz), 1.27 (1H, m), 1.41–1.54 (2H, m), 1.69 (1H, m), 2.33 (1H, td, $J = 9.1$, 5.4 Hz), 3.44 (2H, d, $J = 13.4$ Hz), 3.60 (1H, qd, $J = 9.1$, 6.0 Hz), 3.85 (2H, d, $J = 13.4$ Hz), 4.33 (1H, s), 7.21–7.33 (10H, m). MS m/z 298 $[M+1]^+$. Anal. Calcd for $C_{20}H_{27}NO$: C, 80.76; H, 9.15; N, 4.71. Found: C, 80.52; H, 9.11; N, 4.88.

5.4.4. (±)-*N*,*N*-Dibenzyl-3-aminoheptan-2-ol [(±)-5e]. Yield 50%. ^{1}H NMR ($CDCl_3$) δ: 0.96 (3H, t, $J = 7.1$ Hz), 1.09 (3H, d, $J = 6.0$ Hz), 1.23–1.46 (5H, m), 1.71 (1H, m), 2.32 (1H, td, $J = 9.3$, 5.4 Hz), 3.45 (2H, d, $J = 13.4$ Hz), 3.60 (1H, m), 3.85 (2H, d, $J = 13.4$ Hz), 4.34 (1H, br), 7.20–7.33 (10H, m). MS m/z 312 $[M+1]^+$. Anal. Calcd for $C_{21}H_{29}NO$: C, 80.98; H, 9.38; N, 4.50. Found: C, 80.97; H, 9.09; N, 4.66.

5.4.5. (±)-*N*,*N*-Dibenzyl-3-aminononan-2-ol [(±)-5f]. Yield 40%. ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t,

$J = 6.6$ Hz), 1.09 (3H, d, $J = 6.0$ Hz), 1.21–1.44 (10H, m), 1.72 (1H, m), 2.32 (1H, dt, $J = 9.3$, 5.4 Hz), 3.44 (2H, d, $J = 13.3$ Hz), 3.60 (1H, m), 3.85 (2H, d, $J = 13.3$ Hz), 4.34 (1H, br), 7.21–7.33 (10H, m). MS m/z 340 $[M+1]^+$. Anal. Calcd for $C_{23}H_{33}NO$: C, 81.73; H, 9.80; N, 4.13. Found: C, 81.58; H, 9.90; N, 4.37.

5.4.6. (±)-*N*,*N*-Dibenzyl-3-aminodecan-2-ol [(±)-5g]. Yield 40%. ^1H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 6.7$ Hz), 1.09 (3H, d, $J = 6.0$ Hz), 1.21–1.44 (11H, m), 1.72 (1H, m), 2.32 (1H, dt, $J = 9.3$, 5.4 Hz), 3.44 (2H, d, $J = 13.4$ Hz), 3.60 (1H, m), 3.85 (2H, d, $J = 13.2$ Hz), 4.34 (1H, s), 7.21–7.33 (10H, m). MS m/z 354 $[M+1]^+$. Anal. Calcd for $C_{24}H_{35}NO{\cdot}0.1H_2O$: C, 81.12; H, 9.98; N, 3.94. Found: C, 81.22; H, 10.04; N, 4.08.

5.4.7. (±)-*N*,*N*-Dibenzyl-3-aminopentan-2-ol [(±)-10b]. Yield 77%. ^1H NMR ($CDCl_3$) δ: 1.00 (3H, t, $J = 7.5$ Hz), 1.19 (3H, d, $J = 6.6$ Hz), 1.50 (1H, m), 1.77 (1H, m), 2.58 (1H, td, $J = 7.0$, 4.7 Hz), 3.64 (2H, d, $J = 13.5$ Hz), 3.74 (2H, d, $J = 13.7$ Hz), 3.90 (1H, m). MS m/z 284 $[M+1]^+$. Anal. Calcd for $C_{19}H_{25}NO{\cdot}0.4H_2O$: C, 78.52; H, 8.95; N, 4.82. Found: C, 78.70; H, 8.76; N, 4.92.

5.4.8. (±)-*N*,*N*-Dibenzyl-4-aminohexan-3-ol [(±)-10c]. Yield 68%. ^1H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J = 7.3$ Hz), 0.99 (3H, d, $J = 7.3$ Hz), 1.27–1.84 (4H, m), 2.58 (1H, td, $J = 7.0$, 4.1 Hz), 3.60 (1H, m), 3.68 (4H, s), 7.20–7.39 (10H, m). MS m/z 298 $[M+1]^+$. Anal. Calcd for $C_{20}H_{27}NO{\cdot}0.1H_2O$: C, 80.28; H, 9.16; N, 4.68. Found: C, 80.33; H, 9.21; N, 4.95.

5.4.9. (±)-*N*,*N*-Dibenzyl-3-aminohexan-2-ol [(±)-10d]. Yield 65%. ^1H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 6.9$ Hz), 1.18 (3H, d, $J = 6.6$ Hz), 1.35–1.72 (4H, m), 2.30 (1H, br), 2.65 (1H, td, $J = 6.3$, 4.8 Hz), 3.62 (2H, d, $J = 13.6$ Hz), 3.73 (2H, d, $J = 13.7$ Hz), 3.89 (1H, m), 7.20–7.35 (10H, m). MS m/z 298 $[M+1]^+$. Anal. Calcd for $C_{20}H_{27}NO$: C, 80.76; H, 9.15; N, 4.71. Found: C, 81.01; H, 8.91; N, 5.07.

5.4.10. (±)-*N*,*N*-Dibenzyl-3-aminoheptan-2-ol [(±)-10e]. Yield 73%. ^1H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 7.1$ Hz), 1.17 (3H, d, $J = 6.6$ Hz), 1.23–1.50 (6H, m), 2.33 (1H, br), 2.64 (1H, m), 3.62 (2H, $J = 13.5$ Hz), 3.74 (2H, $J = 13.5$ Hz), 3.89 (1H, m), 7.20–7.34 (10H, m). MS m/z 312 $[M+1]^+$. Anal. Calcd for $C_{21}H_{29}NO$: C, 80.98; H, 9.38; N, 4.50. Found: C, 80.87; H, 9.29; N, 4.58.

5.4.11. (±)-*N*,*N*-Dibenzyl-3-aminononan-2-ol [(±)-10f]. Yield 78%. ^1H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J = 6.7$ Hz), 1.18 (3H, d, $J = 6.6$ Hz), 1.25–1.48 (9H, m), 1.70 (1H, m), 2.33 (1H, m), 2.64 (1H, m), 3.62 (2H, d, $J = 13.7$ Hz), 3.73 (2H, d, $J = 13.6$ Hz), 3.90 (1H, m), 7.21–7.33 (10H, m). MS m/z 340 $[M+1]^+$. Anal. Calcd for $C_{23}H_{33}NO{\cdot}0.2H_2O$: C, 80.51; H, 9.81; N, 4.08. Found: C, 80.76; H, 9.80; N, 4.23.

5.4.12. (±)-*N*,*N*-Dibenzyl-3-aminodecan-2-ol [(±)-10g]. Yield 71%. ^1H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J = 6.8$ Hz), 1.18 (3H, d, $J = 6.6$ Hz), 1.20–1.48 (11H, m), 1.70 (1H, m), 2.32 (1H, br), 2.64 (1H, m), 3.62 (2H, d, $J = 13.7$ Hz), 3.73 (2H, d, $J = 13.5$ Hz), 3.89 (1H, m), 7.20–7.34 (10H, m). MS m/z 354 $[M+1]^+$. Anal. Calcd for $C_{24}H_{35}NO$: C, 81.53; H, 9.98; N, 3.96. Found: C, 81.28; H, 9.86; N, 4.10.

5.5. Synthesis of (±)-2-aminopentan-3-ol [(±)-6a]

To a solution of (±)-**5a** (5.89 g, 20.8 mmol) in MeOH (400 mL) was added 20% $Pd(OH)_2$–C (1.47 g) in one portion under argon. The mixture was stirred under 1 atm of hydrogen over night, and the catalyst was removed by filtration through Celite and washed with MeOH. The combined filtrate was evaporated under reduced pressure to afford the title compound (±)-**6a** (2.00 g, 93%). ^1H NMR ($CDCl_3$) δ: 0.99 (3H, t, $J = 7.4$ Hz), 1.10 (3H, d, $J = 6.4$ Hz), 1.37 (1H, m), 1.55 (1H, m), 1.90–2.10 (2H, br), 2.75 (1H, qu, $J = 6.4$ Hz), 3.12 (1H, dq, $J = 3.7$, 3.4 Hz). MS m/z 104 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 104–105 °C. Anal. Calcd for $C_5H_{13}NO{\cdot}C_2H_2O_4$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.63; H, 7.82; N, 7.32.

The following amino alcohol derivatives were synthesized according to a synthetic method similar to that of (±)-**6a**.

5.5.1. (±)-3-Aminopentan-2-ol [(±)-6b]. Yield 67%. Mp 28–30 °C (hexane). ^1H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 7.4$ Hz), 1.19 (3H, d, $J = 6.0$ Hz), 1.26 (1H, m), 1.62 (1H, m), 2.15–2.35 (3H, br), 2.42 (1H, m), 3.46 (1H, qu, $J = 6.3$ Hz). MS m/z 104 $[M+1]^+$. Anal. Calcd for $C_{21}H_{29}NO{\cdot}0.1H_2O$: C, 57.21; H, 12.68; N, 13.34. Found: C, 57.15; H, 12.50; N, 13.15.

5.5.2. (±)-4-Aminohexan-3-ol [(±)-6c]. Yield 84%. Mp 43–44 °C (hexane). ^1H NMR ($CDCl_3$) δ: 0.99 (3H, t, $J = 7.4$ Hz), 1.10 (3H, d, $J = 6.4$ Hz), 1.39 (1H, m), 1.55 (1H, m), 1.85 (3H, br), 2.75 (1H, qu, $J = 6.4$ Hz), 3.11 (1H, m). MS m/z 118 $[M+1]^+$. Anal. Calcd for $C_6H_{15}NO$: C, 61.49; H, 12.90; N, 11.95. Found: C, 61.25; H, 12.65; N, 11.71.

5.5.3. (±)-3-Aminohexan-2-ol [(±)-6d]. Yield 95%. ^1H NMR ($CDCl_3$) δ: 0.95 (3H, t, $J = 6.8$ Hz), 1.22 (3H, d, $J = 6.0$ Hz), 1.31–1.56 (4H, m), 2.67 (1H, td, $J = 7.9$, 3.0 Hz), 3.55 (1H, m), 3.35–3.70 (3H, br). MS m/z 118 $[M+1]^+$. Anal. Calcd for $C_6H_{15}NO$: C, 61.49; H, 12.90; N, 11.95. Found: C, 61.38; H, 12.42; N, 11.76.

5.5.4. (±)-3-Aminoheptan-2-ol [(±)-6e]. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 7.0$ Hz), 1.68 (3H, d, $J = 6.2$ Hz), 1.24–1.61 (6H, m), 2.03 (3H, br), 2.44 (1H, m), 3.41 (1H, qu, $J = 6.3$ Hz). MS m/z 132 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 100–102 °C. MS m/z 132 $[M+1]^+$. Anal. Calcd for $C_7H_{17}NO{\cdot}0.75C_2H_2O_4$: C, 51.37; H, 9.38; N, 7.05. Found: C, 51.69; H, 9.52; N, 7.19.

5.5.5. (±)-3-Aminononan-2-ol [(±)-6f]. Yield 98%. ^{1}H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J = 6.6$ Hz), 1.17 (3H, d, $J = 6.1$ Hz), 1.10–1.59 (10H, m), 2.42 (1H, m), 3.39 (1H, qu, $J = 6.3$ Hz). MS m/z 160 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 124–125 °C. MS m/z 160 $[M+1]^+$. Anal. Calcd for $C_9H_{21}NO{\cdot}C_2H_2O_4$: C, 52.29; H, 9.30; N, 5.62. Found: C, 52.83; H, 9.25; N, 5.68.

5.5.6. (±)-3-Aminodecan-2-ol [(±)-6g]. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J = 6.8$ Hz), 1.18 (3H, d, $J = 6.2$ Hz), 1.23–1.38 (12H, m), 2.11 (3H, br), 2.46 (1H, m), 3.42 (1H, qu, $J = 6.4$ Hz). MS m/z 174 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 124–126 °C. MS m/z 174 $[M+1]^+$. Anal. Calcd for $C_{10}H_{23}NO{\cdot}C_2H_2O_4$: C, 54.73; H, 9.57; N, 5.32. Found: C, 54.71; H, 9.52; N, 5.31.

5.5.7. (±)-2-Aminopentan-3-ol [(±)-11a]. Yield 75%. ^{1}H NMR ($CDCl_3$) δ: 0.99 (3H, t, $J = 7.4$ Hz), 1.02 (3H, d, $J = 6.6$ Hz), 1.43 (1H, qu, $J = 7.2$ Hz), 1.72 (3H, br), 3.00 (1H, qd, $J = 6.5$, 3.5 Hz), 3.37 (1H, td, $J = 6.5$, 3.5 Hz). MS m/z 104 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 124–126 °C. MS m/z 104 $[M+1]^+$. Anal. Calcd for $C_5H_{13}NO{\cdot}C_2H_2O_4$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.52; H, 8.02; N, 6.99.

5.5.8. (±)-3-Aminopentan-2-ol [(±)-11b]. Yield 76%. ^{1}H NMR ($CDCl_3$) δ: 0.97 (3H, t, $J = 7.4$ Hz), 1.09 (3H, d, $J = 6.4$ Hz), 1.19 (1H, m), 1.46 (1H, m), 1.74 (3H, br), 2.67 (1H, qu, $J = 4.3$ Hz), 3.71 (1H, qd, $J = 6.4$, 4.0 Hz). MS m/z 104 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/E_2O solution to give the oxalate as a white solid, mp 108–110 °C. MS m/z 104 $[M+1]^+$. Anal. Calcd for $C_5H_{13}NO{\cdot}C_2H_2O_4$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.35; H, 7.84; N, 7.29.

5.5.9. (±)-4-Aminohexan-3-ol [(±)-11c]. Yield 97%. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 7.4$ Hz), 1.00 (3H, d, $J = 7.4$ Hz), 1.24–1.65 (4H, m), 2.55–2.78 (3H, br), 2.77 (1H, m), 3.49 (1H, qu, $J = 4.2$ Hz). MS m/z 118 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as an amorphous. Anal. Calcd for $C_6H_{15}NO{\cdot}0.65H_2O$: C, 43.89; H, 8.43; N, 6.40. Found: C, 43.53; H, 8.38; N, 6.79.

5.5.10. (±)-3-Aminohexan-2-ol [(±)-11d]. Yield 87%. Mp 74–76 °C (Et_2O/hexane). ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H, t, $J = 7.0$ Hz), 1.09 (3H, d, $J = 6.0$ Hz), 1.16–1.50 (4H, m), 1.58–1.80 (3H, br), 2.77 (1H, qu, $J = 4.1$ Hz), 3.69 (1H, m). MS m/z 118 $[M+1]^+$. Anal. Calcd for $C_6H_{15}NO{\cdot}0.25H_2O$: C, 59.22; H, 12.84; N, 11.51. Found: C, 59.62; H, 12.62; N, 11.70.

5.5.11. (±)-3-Aminoheptan-2-ol [(±)-11e]. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 6.8$ Hz), 1.09 (3H, d, $J = 6.4$ Hz), 1.18–1.44 (6H, m), 1.85 (3H, br), 2.77 (1H, m), 3.71 (1H, m). MS m/z 132 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 98–100 °C. MS m/z 132 $[M+1]^+$. Anal. Calcd for $C_7H_{17}NO{\cdot}C_2H_2O_4$: C, 48.86; H, 8.66; N, 6.33. Found: C, 48.87; H, 8.58; N, 6.41.

5.5.12. (±)-3-Aminononan-2-ol [(±)-11f]. Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.08 (3H, d, $J = 6.2$ Hz), 1.16–1.42 (8H, m), 1.72 (3H, br), 2.75 (1H, m), 3.69 (1H, m). MS m/z 160 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 124–126 °C. MS m/z 160 $[M+1]^+$. Anal. Calcd for $C_9H_{21}NO{\cdot}C_2H_2O_4$: C, 53.00; H, 9.30; N, 5.62. Found: C, 53.01; H, 9.28; N, 5.79.

5.5.13. (±)-3-Aminondecan-2-ol [(±)-11g]. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.10 (3H, d, $J = 6.4$ Hz), 1.20–1.50 (12H, m), 1.91 (3H, br), 2.78 (1H, m), 3.73 (1H, m). MS m/z 174 $[M+1]^+$. The title compound was treated with oxalic acid in EtOH/*n*-hexane solution to give the oxalate as a white solid, mp 110–112 °C. MS m/z 174 $[M+1]^+$. Anal. Calcd for $C_9H_{21}NO{\cdot}0.85C_2H_2O_4{\cdot}0.25$EtOH: C, 54.39; H, 9.86; N, 5.66. Found: C, 54.41; H, 9.56; N, 5.45.

5.6. Synthesis of (±)-*N*-*tert*-butoxycarbonyl-*O*-methanesulfonyl-2-aminopentan-3-ol [(±)-7a]

To a solution of (±)-**6a** (10.3 g, 100 mmol) in CH_2Cl_2 (250 mL) was added Boc_2O (21.8 g, 100 mmol) at room temperature. The reaction mixture was stirred for 2 h at the same temperature, and concentrated under reduced pressure to give crude carbamate (20.5 g) that was used for the next step without further purification. To a solution of the carbamate (20.5 g) and Et_3N (20.9 mL, 150 mmol) in CH_2Cl_2 (150 mL) was added methanesulfonyl chloride (14.3 g, 125 mmol) dropwise. After stirring for 2 h, the reaction mixture was washed with water, dried over magnesium sulfate. Filtration, evaporation in vacuo, and purification by column chromatography (AcOEt/hexane = 1/2) gave the title compound (±)-**7a** (25.3 g, 90%) as a white solid. The mesylate can

be recrystallized readily from petroleum ether: mp 49–51 °C. ^{1}H NMR ($CDCl_3$) δ: 1.02 (3H, t, $J = 7.4$ Hz), 1.22 (3H, d, $J = 7.0$ Hz), 1.45 (9H, s), 1.77 (1H, qu, $J = 7.2$ Hz), 3.06 (3H, s), 3.98 (1H, br), 4.55 (1H, td, $J = 6.7$, 3.5 Hz), 4.60 (1H, br). MS m/z 282 $[M+1]^+$. Anal. Calcd for $C_{11}H_{23}NO_5S$: C, 46.96; H, 8.24; N, 4.98; S, 11.40. Found: C, 46.96; H, 8.28; N, 5.07; S, 11.44.

The following mesylate derivatives were synthesized according to a synthetic method similar to that of (±)-**7a**.

5.6.1. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminopentan-2-ol [(±)-7b]. Yield 92%. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 7.4$ Hz), 1.43 (3H, d, $J = 6.4$ Hz), 1.43–1.72 (2H, m), 1.45 (9H, s), 3.02 (3H, s), 3.61 (1H, m), 4.51 (1H, br d, $J = 9.2$ Hz), 4.83 (2H, qd, $J = 8.6$, 2.7 Hz). MS m/z 282 $[M+1]^+$. Anal. Calcd for $C_{11}H_{23}NO_5S \cdot 0.25H_2O$: C, 46.96; H, 8.24; N, 4.98; S, 11.40. Found: C, 46.36; H, 8.12; N, 5.03; S, 11.15.

5.6.2. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-4-aminohexan-3-ol [(±)-7c]. Yield 79%. Mp 43–45 °C (hexane). ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, d, $J = 7.4$ Hz), 1.01 (3H, d, $J = 7.5$ Hz), 1.40 (9H, s), 1.48–1.62 (2H, m), 1.79 (2H, qu, $J = 7.4$ Hz), 3.08 (s, 3H), 3.73 (1H, m), 4.52 (1H, br d, $J = 9.5$ Hz), 4.63 (1H, td, $J = 6.8$, 2.7 Hz). MS m/z 296 $[M+1]^+$. Anal. Calcd for $C_{12}H_{25}NO_5S$: C, 48.79; H, 8.53; N, 4.74; S, 10.85. Found: C, 8.54; H, 8.54; N, 4.93; S, 10.90.

5.6.3. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminohexan-2-ol [(±)-7d]. Yield 73%. ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, t, $J = 6.9$ Hz), 1.27–1.64 (8H, m), 1.53 (9H, s), 3.09 (3H, s), 3.70 (1H, br), 4.50 (1H, m), 4.80 (1H, m). MS m/z 296 $[M+1]^+$. Anal. Calcd for $C_{12}H_{25}NO_5S$: C, 48.79; H, 8.53; N, 4.74; S, 10.85. Found: C, 48.77; H, 8.88; N, 5.01; S, 11.03.

5.6.4. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminoheptan-2-ol [(±)-7e]. Yield 89%. Mp 57–58 °C (hexane).^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 7.0$ Hz), 1.24–1.60 (6H, m), 1.42 (3H, d, $J = 6.4$ Hz), 1.44 (9H, s), 2.99 (3H, s), 3.68 (1H, m), 4.49 (1H, d, $J = 9.5$ Hz), 4.79 (1H, m). MS m/z 310 $[M+1]^+$. Anal. Calcd for $C_{13}H_{27}NO_5S$: C, 50.46; H, 8.80; N, 4.53; S, 10.36. Found: C, 50.43; H, 8.75; N, 4.59; S, 10.36.

5.6.5. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminononan-2-ol [(±)-7f]. Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.21–1.50 (10H, m), 1.42 (3H, d, $J = 6.4$ Hz), 1.45 (9H, s), 3.00 (3H, s), 3.68 (1H, m), 4.49 (1H, d, $J = 9.7$ Hz), 4.80 (1H, dq, $J = 6.4$, 2.7 Hz). MS m/z 338 $[M+1]^+$. Anal. Calcd for $C_{15}H_{31}NO_5S$: C, 53.38; H, 9.26; N, 4.15; S, 9.50. Found: C, 53.15; H, 9.22; N, 4.19; S, 9.59.

5.6.6. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminodecan-2-ol [(±)-7g]. Yield 96%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.25–1.50 (12H, m), 1.42 (3H, d, $J = 6.4$ Hz), 1.45 (9H, s), 3.03 (3H, s), 3.68 (1H, m), 4.49 (1H, d, $J = 9.7$ Hz), 4.80 (1H, m). MS m/z 352 $[M+1]^+$. Anal. Calcd for $C_{16}H_{33}NO_5S$: C, 54.67; H, 9.46; N, 3.98; S, 9.12. Found: C, 54.61; H, 9.40; N, 4.06; S, 9.32.

5.6.7. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-2-aminopentan-3-ol [(±)-12a]. Yield 88%. Mp 62–64 °C (Et_2O/hexane). ^{1}H NMR ($CDCl_3$) δ: 1.02 (3H, t, $J = 7.4$ Hz), 1.14 (3H, d, $J = 6.8$ Hz), 1.45 (9H, s), 1.58–1.85 (2H, m), 3.05 (3H, m), 3.89 (1H, m), 4.69 (1H, m), 4.86 (1H, br). MS m/z 282 $[M+1]^+$. Anal. Calcd for $C_{11}H_{23}NO_5S \cdot 0.1Et_2O$: C, 47.41; H, 8.38; N, 4.85; S, 11.10. Found: C, 47.73; H, 8.36; N, 5.15; S, 10.80.

5.6.8. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminopentan-2-ol [(±)-12b]. Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 7.3$ Hz), 1.36 (1H, m), 1.40 (3H, d, $J = 6.6$ Hz), 1.49 (9H, m), 1.71 (1H, m), 3.05 (3H, s), 3.65 (1H, m), 4.62 (1H, br d, $J = 7.9$ Hz), 4.86 (1H, m). MS m/z 282 $[M+1]^+$. Anal. Calcd for $C_{11}H_{23}NO_5S$: C, 46.96; H, 8.24; N, 4.98, S; 11.40. Found: C, 47.05; H, 8.54; N, 5.03, S; 11.44.

5.6.9. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-4-aminohexan-3-ol [(±)-12c]. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.91–1.05 (6H, m), 1.24–1.81 (4H, m), 1.45 (9H, s), 3.03 (3H, s), 3.68 (1H, br), 4.71 (2H, br). MS m/z 296 $[M+1]^+$. Anal. Calcd for $C_{12}H_{25}NO_5S$: C, 48.79; H, 8.53; N, 4.74; S, 10.85. Found: C, 49.01; H, 8.72; N, 4.82; S, 10.47.

5.6.10. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminohexan-2-ol [(±)-12d]. Yield 80%. Mp 75–77 °C (hexane). ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H, t, $J = 7.0$ Hz), 1.24–1.62 (4H, m), 1.39 (3H, d, $J = 6.6$ Hz), 1.44 (9H, s), 3.03 (3H, s), 3.69 (1H, m), 4.60 (1H, m), 4.88 (1H, m). MS m/z 296 $[M+1]^+$. Anal. Calcd for $C_{12}H_{25}NO_5S$: C, 48.79; H, 8.53; N, 4.74; S, 10.85. Found: C, 48.73; H, 8.48; N, 4.90; S, 10.89.

5.6.11. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminoheptan-2-ol [(±)-12e]. Yield 93%. ^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 7.0$ Hz), 1.25–1.50 (6H, m), 1.40 (3H, d, $J = 6.4$ Hz), 1.45 (9H, s), 3.03 (3H, s), 3.69 (1H, br), 4.59 (1H, m), 4.89 (1H, m). MS m/z 310 $[M+1]^+$. Anal. Calcd for $C_{13}H_{27}NO_5S$: C, 50.46; H, 8.80; N, 4.53; S, 10.36. Found: C, 49.56; H, 8.72; N, 4.82; S, 10.42.

5.6.12. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminononan-2-ol [(±)-12f]. Yield 74%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.23–1.48 (10H, m), 1.39 (3H, d, $J = 6.6$ Hz), 1.45 (9H, s), 3.02 (3H, s), 3.68 (1H, br),

4.59 (1H, d, $J=9.0$ Hz), 4.88 (1H, m). MS m/z 338 $[M+1]^+$. Anal. Calcd for $C_{15}H_{31}NO_5S$: C, 53.38; H, 9.26; N, 4.15; S, 9.50. Found: C, 53.38; H, 9.22; N, 4.27; S, 9.49.

5.6.13. (±)-*N*-*tert*-Butoxycarbonyl-*O*-methanesulfonyl-3-aminodecan-2-ol [(±)-12g]. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J=6.7$ Hz), 1.20–1.62 (12H, m), 1.39 (3H, d, $J=6.6$ Hz), 1.45 (9H, s), 3.02 (3H, s), 3.68 (1H, br), 4.58 (1H, d, $J=9.2$ Hz), 4.88 (1H, m). MS m/z 352 $[M+1]^+$. Anal. Calcd for $C_{16}H_{33}NO_5S$: C, 54.67; H, 9.46; N, 3.98; S, 9.12. Found: C, 55.14; H, 9.45; N, 4.04; S, 8.79.

5.7. Synthesis of (±)-(1-ethyl-2-isothiocyanato)propyl methanesulfonate [(±)-8a]

To a solution of (±)-**7a** (5.94 g, 21.1 mmol) in EtOH (30 mL) was added 30% HCl/EtOH (30 mL) and the mixture was stirred for 1 h. The reaction mixture was then concentrated in vacuo to give a hydrochloride (4.72 g) that was used for the next step without further purification. To a stirred two phase solution of the hydrochloride (4.72 g) in H_2O (47 mL) and $CHCl_3$ (47 mL) was added $CSCl_2$ (3.69 g, 32.0 mmol) and $CaCO_3$ (6.41 g, 64.0 mmol) at 0 °C. After stirring at the same temperature for 2 h, the organic phase was separated and dried over magnesium sulfate. Filtration, evaporation, and purification by column chromatography (AcOEt/*n*-hexane = 1/3) gave the title compound (±)-**8a** (2.73 g, 58%). ^{1}H NMR ($CDCl_3$) δ: 1.04 (3H, t, $J=7.5$ Hz), 1.46 (3H, d, $J=6.6$ Hz), 1.77–1.90 (2H, m), 3.10 (3H, s), 4.02 (1H, m), 4.55 (1H, m). MS m/z 224 $[M+1]^+$. Anal. Calcd for $C_7H_{13}NO_3S_2$: C, 37.65; H, 5.87; N, 6.29; S, 28.72. Found: C, 37.79; H, 5.80; N, 6.29; S, 28.65.

The following isothiocyanate derivatives were synthesized according to a synthetic method similar to that of (±)-**8a**.

5.7.1. (±)-(2-Isothiocyanato-1-methyl)butyl methanesulfonate [(±)-8b]. Yield 83%. ^{1}H NMR ($CDCl_3$) δ: 1.04 (3H, t, $J=7.4$ Hz), 1.46 (3H, d, $J=6.8$ Hz), 1.79–1.90 (2H, m), 3.17 (3H, s), 4.02 (1H, m), 4.55 (1H, q, $J=5.7$ Hz). MS m/z 224 $[M+1]^+$. Anal. Calcd for $C_7H_{13}NO_3S_2$: C, 37.65; H, 5.87; N, 6.27; S, 28.72. Found: C, 37.85; H, 5.92; N, 6.33; S, 28.33.

5.7.2. (±)-(1-Ethyl-2-isothiocyanato)butyl methanesulfonate [(±)-8c]. Yield 76%. ^{1}H NMR ($CDCl_3$) δ: 1.03 (3H, t, $J=7.4$ Hz), 1.11 (3H, t, $J=7.3$ Hz), 1.74–1.92 (2H, m), 3.11 (3H, s), 3.77 (1H, ddd, $J=7.9$, 5.7, 4.0 Hz), 4.61 (1H, td, $J=6.5$, 4.0 Hz). MS m/z 238 $[M+1]^+$. Anal. Calcd for $C_8H_{15}NO_3S_2$: C, 40.48; H, 6.37; N, 5.90; S, 27.02. Found: C, 40.85; H, 6.44; N, 5.95; S, 27.21.

5.7.3. (±)-(2-Isothiocyanato-1-methyl)pentyl methanesulfonate [(±)-8d]. Yield 77%. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J=7.1$ Hz), 1.50 (3H, d, $J=6.4$ Hz), 1.40–1.78 (4H, m), 3.11 (3H, s), 3.75 (1H, m), 4.76 (1H, m). MS m/z 238 $[M+1]^+$. Anal. Calcd for $C_8H_{15}NO_3S_2$: C, 40.48; H, 6.37; N, 5.90; S, 27.02. Found: C, 40.11; H, 6.00; N, 6.23; S, 26.89.

5.7.4. (±)-(2-Isothiocyanato-1-methyl)hexyl methanesulfonate [(±)-8e]. Yield 52%. ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H, t, $J=7.0$ Hz), 1.27–1.76 (6H, m), 1.50 (3H, d, $J=6.4$ Hz), 3.08 (3H, s), 3.74 (1H, qu, $J=4.6$ Hz), 4.76 (1H, m). MS m/z 252 $[M+1]^+$. Anal. Calcd for $C_9H_{17}NO_3S_2$: C, 43.00; H, 6.82; N, 5.57; S, 25.51. Found: C, 43.15; H, 6.83; N, 5.65; S, 25.28.

5.7.5. (±)-(2-Isothiocyanato-1-methyl)octyl methanesulfonate [(±)-8f]. Yield 88%. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J=6.7$ Hz), 1.23–1.76 (10H, m), 1.50 (3H, d, $J=6.4$ Hz), 3.08 (3H, s), 3.74 (1H, qu, $J=4.6$ Hz), 4.76 (1H, m). MS m/z 280 $[M+1]^+$. Anal. Calcd for $C_{11}H_{21}NO_3S_2$: C, 47.28; H, 7.58; N, 5.01; S, 22.95. Found: C, 47.66; H, 7.63; N, 5.03; S, 22.66.

5.7.6. (±)-(2-Isothiocyanato-1-methyl)nonyl methanesulfonate [(±)-8g]. Yield 87%. ^{1}H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J=6.8$ Hz), 1.25–1.40 (11H, m), 1.50 (3H, d, $J=6.4$ Hz), 1.69 (1H, m), 3.10 (3H, s), 3.74 (1H, qu, $J=4.6$ Hz), 4.76 (1H, m). MS m/z 294 $[M+1]^+$. Anal. Calcd for $C_{12}H_{23}NO_3S_2$: C, 49.12; H, 7.90; N, 4.77; S, 21.85. Found: C, 49.23; H, 8.01; N, 4.84; S, 21.98.

5.7.7. (±)-(1-Ethyl-2-isothiocyanato)propyl methanesulfonate [(±)-13a]. Yield 65%. ^{1}H NMR ($CDCl_3$) δ: 1.05 (3H, t, $J=7.4$ Hz), 1.41 (3H, d, $J=6.8$ Hz), 1.67–1.91 (2H, m), 3.12 (3H, s), 4.14 (1H, m), 4.60 (1H, qu, $J=4.1$ Hz). MS m/z 224 $[M+1]^+$. Anal. Calcd for $C_7H_{13}NO_3S_2$: C, 37.65; H, 5.87; N, 6.27; S, 28.72. Found: C, 37.65; H, 5.90; N, 6.34; S, 28.87.

5.7.8. (±)-(2-Isothiocyanato-1-methyl)butyl methanesulfonate [(±)-13b]. Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 1.10 (3H, t, $J=7.4$ Hz), 1.47 (3H, d, $J=6.4$ Hz), 1.61–1.73 (2H, m), 3.09 (3H, s), 3.89 (1H, m), 4.81 (1H, qd, $J=6.4$, 4.1 Hz). MS m/z 224 $[M+1]^+$. Anal. Calcd for $C_7H_{13}NO_3S$: C, 37.65; H, 5.87; N, 6.27; S, 28.72. Found: C, 37.79; H, 5.86; N, 6.47; S, 28.71.

5.7.9. (±)-(2-Isothiocyanato-1-ethyl)butyl methanesulfonate [(±)-13c]. Yield 81%. ^{1}H NMR ($CDCl_3$) δ: 1.01–1.13 (6H, m), 1.61–1.89 (4H, m), 3.11 (3H, s), 3.98 (1H, qu, $J=4.4$ Hz), 4.63 (1H, qu, $J=4.1$ Hz). MS m/z 238 $[M+1]^+$. Anal. Calcd for $C_8H_{15}NO_3S_2$: C, 40.48; H, 6.37; N, 5.90; S, 27.02. Found: C, 40.79; H, 6.41; N, 6.00; S, 26.52.

5.7.10. (±)-(2-Isothiocyanato-1-methyl)pentyl methanesulfonate [(±)-13d]. Yield 83%. ^{1}H NMR ($CDCl_3$) δ:

0.98 (3H, t, $J = 7.1$ Hz), 1.33–1.67 (4H, m), 1.46 (3H, d, $J = 6.4$ Hz), 3.09 (3H, s), 3.98 (1H, qu, $J = 4.3$ Hz), 4.79 (1H, qd, $J = 6.4$, 3.8 Hz). MS m/z 238 $[M+1]^+$. Anal. Calcd for $C_8H_{15}NO_3S_2$: C, 40.48; H, 6.37; N, 5.90; S, 27.02. Found: C, 40.66; H, 6.29; N, 5.94; S, 27.43.

5.7.11. (±)-(2-Isothiocyanato-1-methyl)hexyl methanesulfonate [(±)-13e]. Yield 69%. ^{1}H NMR ($CDCl_3$) δ: 0.94 (3H, t, $J = 7.2$ Hz), 1.33–1.66 (6H, m), 1.46 (3H, d, $J = 6.4$ Hz), 3.09 (3H, s), 3.96 (1H, m), 4.81 (1H, ddd, $J = 12.8$, 6.4, 3.7 Hz). MS m/z 252 $[M+1]^+$. Anal. Calcd for $C_9H_{17}NO_3S_2$: C, 43.00; H, 6.82; N, 5.57; S, 25.51. Found: C, 43.43; H, 6.95; N, 5.56; S, 24.68.

5.7.12. (±)-(2-Isothiocyanato-1-methyl)octyl methanesulfonate [(±)-13f]. Yield 78%. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, $J = 6.6$ Hz), 1.25–1.41 (8H, m), 1.46 (3H, d, $J = 6.4$ Hz), 1.55–1.64 (2H, m), 3.09 (3H, s), 3.96 (1H, m), 4.80 (1H, ddd, $J = 12.8$, 6.4, 3.7 Hz). MS m/z 280 $[M+1]^+$. Anal. Calcd for $C_{11}H_{21}NO_3S_2$: C, 47.28; H, 7.58; N, 5.01; S, 22.95. Found: C, 47.18; H, 7.54; N, 5.11; S, 23.11.

5.7.13. (±)-(2-Isothiocyanato-1-methyl)nonyl methanesulfonate [(±)-13g]. Yield 64%. ^{1}H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J = 6.7$ Hz), 1.20–1.66 (12H, m), 1.46 (3H, d, $J = 6.4$ Hz), 3.09 (3H, s), 3.97 (1H, m), 4.80 (1H, m). MS m/z 294 $[M+1]^+$. Anal. Calcd for $C_{12}H_{23}NO_3S_2$: C, 49.12; H, 7.90; N, 4.77; S, 21.85. Found: C, 49.55; H, 8.11; N, 4.96; S, 21.73.

5.8. Synthesis of (±)-*cis*-5-ethyl-2-imino-4-methyl-1,3-thiazolidine [(±)-cis-9a]

To a solution of (±)-**8a** (0.58 g, 2.6 mmol) in dioxane (5.2 mL) was added aqueous 28% NH_3 solution (5.2 mL). The reaction mixture was allowed to warm up to 60 °C with stirring for an additional 2 h. The reaction mixture was then concentrated to about half its volume under reduced pressure, and cooled in an ice bath. To this cooled mixture was added 10% sodium hydroxy solution (5.2 mL), and the mixture was extracted with $CHCl_3$ (5.2 mL × 2). The combined extracts were dried over sodium sulfate, concentrated in vacuo to give a crude oil. Purification by column chromatography using Chromatorex® and $CHCl_3$ as eluent gave the title compound (±)-*cis*-**9a** (0.21 g, 56%) as yellow oil: ^{1}H NMR ($CDCl_3$) δ: 0.97 (3H, t, $J = 7.2$ Hz), 1.23 (3H, d, $J = 6.8$ Hz), 1.56 (1H, m), 1.73 (1H, m), 3.80 (1H, m), 4.18 (1H, qu, $J = 6.7$ Hz). MS m/z 145 $[M+1]^+$. This compound was treated with fumaric acid in EtOH/Et_2O solution to give the fumarate as a white solid, mp 193–195 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}0.5C_4H_4O_4$: C, 47.50; H, 6.98; N, 13.85; S, 15.85. Found: C, 47.58; H, 6.93; N, 13.77; S, 15.64.

5.8.1. (±)-*cis*-4-Ethyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*cis*-9b]. Yield 64%. ^{1}H NMR ($CDCl_3$) δ: 1.05 (3H, t, $J = 7.4$ Hz), 1.24 (3H, d, $J = 6.8$ Hz), 1.59 (1H, m), 1.81 (1H, m), 3.76–3.90 (2H, m). MS m/z 145 $[M+1]^+$. This compound was treated with fumaric acid in EtOH solution to give the fumarate as a white solid, mp 139–141 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}1.5C_4H_4O_4$: C, 45.27; H, 5.70; N, 8.80; S, 10.07. Found: C, 45.40; H, 5.78; N, 8.78; S, 10.07.

5.8.2. (±)-*cis*-4,5-Diethyl-2-imino-1,3-thiazolidine [(±)-*cis*-9c]. Yield 84%. ^{1}H NMR ($CDCl_3$) δ: 0.93–1.07 (6H, m), 1.40–1.85 (4H, m), 3.30–3.60 (2H, br), 3.50 (1H, m), 3.83 (1H, m). MS m/z 159 $[M+1]^+$. This compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 159–160 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.5C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46.85; H, 6.00; N, 8.44; S, 9.50.

5.8.3. (±)-*cis*-2-Imino-5-methyl-4-propyl-1,3-thiazolidine [(±)-*cis*-9d]. Yield 58%. ^{1}H NMR ($CDCl_3$) δ: 0.97 (3H, t, $J = 7.1$ Hz), 1.23 (3H, d, $J = 6.9$ Hz), 1.41–1.79 (4H, m), 3.70–3.90 (2H, br), 3.78 (1H, m), 3.95 (1H, m). MS m/z 159 $[M+1]^+$. This compound was treated with fumaric acid in EtOH solution to give the fumarate as a white solid, mp 189–191 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.5C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46.91; H, 5.96; N, 8.41; S, 9.63.

5.8.4. (±)-*cis*-4-Butyl-2-imino-5-methyl-1,3-thiazolididine [(±)-*cis*-9e]. Yield 78%. ^{1}H NMR ($CDCl_3$) δ: 0.93 (3H, t, $J = 7.1$ Hz), 1.23 (3H, d, $J = 7.0$ Hz), 1.29–1.44 (3H, m), 1.46–1.60 (2H, m), 1.75 (1H, m), 3.78 (1H, qu, $J = 6.6$ Hz), 3.93 (1H, m), 4.68 (2H, br). MS m/z 173 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 138–141 °C. Anal. Calcd for $C_8H_{16}N_2S{\cdot}C_4H_4O_4$: C, 49.98; H, 6.99; N, 9.71; S, 11.12. Found: C, 49.91; H, 6.92; N, 9.67; S, 10.84.

5.8.5. (±)-*cis*-4-Hexyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*cis*-9f]. Yield 94%. ^{1}H NMR ($CDCl_3$) δ: 0.89 (3H, t, $J = 6.6$ Hz), 1.19–1.82 (10H, m), 1.23 (3H, d, $J = 6.4$ Hz), 3.78 (1H, qu, $J = 6.7$ Hz), 3.94 (1H, dt, $J = 8.5$, 5.8 Hz), 4.61 (2H, br). MS m/z 201 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 142–143 °C. Anal. Calcd for $C_{10}H_{19}N_2S{\cdot}1.5C_4H_4O_4{\cdot}0.25H_2O$: C, 50.85; H, 6.80; N, 7.41; S, 8.48. Found: C, 51.13; H, 6.98; N, 7.43; S, 8.49.

5.8.6. (±)-*cis*-4-Heptyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*cis*-9g]. Yield 98%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.6$ Hz), 1.20–1.40 (9H, m), 1.23 (3H, d, $J = 7.0$ Hz), 1.45–1.60 (2H, m), 1.75 (1H, m), 3.78 (1H, qu, $J = 6.6$ Hz), 3.93 (1H, dt, $J = 8.4$, 5.8 Hz), 4.58 (2H, br). MS m/z 215 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 135–136 °C. Anal. Calcd

for $C_{11}H_{22}N_2S{\cdot}C_4H_4O_4$: C, 54.52; H, 7.93; N, 8.48; S, 9.70. Found: C, 54.31; H, 7.93; N, 8.55; S, 9.50.

5.8.7. (±)-*trans*-5-Ethyl-2-imino-4-methyl-1,3-thiazolidine [(±)-*trans*-14a]. Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, $J = 7.3$ Hz), 1.24 (3H, d, $J = 6.4$ Hz), 1.56–1.82 (2H, m), 3.47 (1H, qu, $J = 4.8$ Hz), 3.95 (1H, dt, $J = 11.5$, 6.5 Hz). MS m/z 145 $[M+1]^+$. This compound was treated with fumaric acid in EtOH solution to give the fumarate as a white solid, mp 133–134 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}1.5C_4H_4O_4$: C, 45.27; H, 5.70; N, 8.80; S, 10.07. Found: C, 45.19; H, 5.71; N, 8.72; S, 9.70.

5.8.8. (±)-*trans*-4-Ethyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*trans*-14b]. Yield 60%. ^{1}H NMR ($CDCl_3$) δ: 1.00 (3H, t, $J = 7.4$ Hz), 1.40 (3H, d, $J = 6.4$ Hz), 1.47–1.60 (2H, m), 3.62–3.73 (2H, m). MS m/z 145 $[M+1]^+$. This compound was treated with fumaric acid in EtOH solution to give the fumarate as a white solid, mp 158–160 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}1.5C_4H_4O_4$: C, 45.27; H, 5.70; N, 8.80; S, 10.07. Found: C, 45.22; H, 5.77; N, 8.85; S, 10.13.

5.8.9. (±)-*trans*-4,5-Diethyl-2-imino-1,3-thiazolidine [(±)-*trans*-14c]. Yield 91%. ^{1}H NMR ($CDCl_3$) δ: 0.93–1.07 (6H, m), 1.50–1.78 (4H, m), 3.53 (1H, qu, $J = 4.6$ Hz), 3.82 (1H, m). MS m/z 159 $[M+1]^+$. This compound was treated with fumaric acid in EtOH/Et_2O solution to give its fumarate as a white solid, mp 159–160 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}C_4H_4O_4{\cdot}0.75H_2O$: C, 45.90; H, 6.83; N, 9.73; S, 11.14. Found: C, 46.22; H, 6.83; N, 9.36; S, 11.32.

5.8.10. (±)-*trans*-2-Imino-5-methyl-4-propyl-1,3-thiazolidine [(±)-*trans*-14d]. Yield 85%. ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, t, $J = 7.0$ Hz), 1.37–1.55 (4H, m), 1.41 (3H, d, $J = 6.6$ Hz), 3.64 (1H, qu, $J = 6.4$ Hz), 3.76 (1H, m). MS m/z 159 $[M+1]^+$. This compound was treated with fumaric acid in EtOH solution to give the fumarate as a white solid, mp 137–141 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.5C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 47.23; H, 6.24; N, 8.31; S, 9.34.

5.8.11. (±)-*trans*-4-Butyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*trans*-14e]. Yield 45%. ^{1}H NMR ($CDCl_3$) δ: 0.91 (3H, t, $J = 7.1$ Hz), 1.30–1.55 (6H, m), 1.40 (3H, d, $J = 6.8$ Hz), 3.61–3.79 (2H, m). MS m/z 173 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 124–126 °C. Anal. Calcd for $C_8H_{16}N_2S{\cdot}C_4H_4O_4$: C, 49.98; H, 6.99; N, 9.71; S, 11.12. Found: C, 50.06; H, 6.98; N, 9.69; S, 11.09.

5.8.12. (±)-*trans*-4-Hexyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*trans*-14f]. Yield 75%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.7$ Hz), 1.25–1.54 (10H, m), 1.39 (3H, d, $J = 6.6$ Hz), 3.60–3.78 (2H, m), 4.50 (2H, br). MS m/z 201 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 133–135 °C. Anal. Calcd for $C_{10}H_{19}N_2S{\cdot}1.5C_4H_4O_4$: C, 51.32; H, 7.00; N, 7.48; S, 8.56. Found: C, 51.57; H, 7.11; N, 7.60; S, 8.38.

5.8.13. (±)-*trans*-4-Heptyl-2-imino-5-methyl-1,3-thiazolidine [(±)-*trans*-14g]. Yield 95%. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, $J = 6.8$ Hz), 1.20–1.55 (12H, m), 1.39 (3H, d, $J = 6.6$ Hz), 3.60-3.77 (2H, m), 4.57 (2H, br). MS m/z 215 $[M+1]^+$. The title compound was treated with fumaric acid in EtOH/*n*-hexane solution to give the fumarate as a white solid, mp 132–135 °C. Anal. Calcd for $C_{11}H_{22}N_2S{\cdot}1.5C_4H_4O_4$: C, 52.56; H, 7.26; N, 7.21; S, 8.25. Found: C, 52.39; H, 7.25; N, 7.30; S, 7.98.

The following optically active 2-imino-1,3-thiazolidine derivatives were synthesized according to a synthetic method similar to that of racemic compounds.

5.8.14. (4*R*,5*S*)-5-Ethyl-2-imino-4-methyl-1,3-thiazolidine·0.5fumarate [(4*R*,5*S*)-9a]. Mp 191–193 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}0.5C_4H_4O_4$: C, 47.50; H, 6.98; N, 13.85; S, 15.85. Found: C, 47.58; H, 6.93; N, 13.77; S, 15.64. $[\alpha]_D$ −90.7 (*c* 0.50, MeOH).

5.8.15. (4*R*,5*S*)-4-Ethyl-2-imino-5-methyl-1,3-thiazolidine·0.5fumarate [(4*R*,5*S*)-9b]. Mp 181–186 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}0.5C_4H_4O_4$: C, 47.50; H, 6.98; N, 13.85; S, 15.85. Found: C, 47.23; H, 6.90; N, 13.55; S, 15.44. $[\alpha]_D$ −95.2 (*c* 0.29, MeOH).

5.8.16. (4*S*,5*R*)-4,5-Diethyl-2-imino-1,3-thiazolidine·1.5fumarate [(4*S*,5*R*)-9c]. Mp 153–155 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.5C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 47.08; H, 6.09; N, 8.54; S, 9.58. $[\alpha]_D$ +44 (*c* 0.2, MeOH).

5.8.17. (4*R*,5*S*)-4,5-Diethyl-2-imino-1,3-thiazolidine·0.5fumarate [(4*R*,5*S*)-9c]. Mp 185–187 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}0.5C_4H_4O_4$: C, 49.98; H, 7.46; N, 12.95; S, 14.82. Found: C, 49.83; H, 7.47; N, 12.89; S, 14.60. $[\alpha]_D$ −58.5 (*c* 0.41, MeOH).

5.8.18. (4*S*,5*R*)-2-Imino-5-methyl-4-propyl-1,3-thiazolidine·1.25fumarate [(4*S*,5*R*)-9d]. Mp 153–155 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.25C_4H_4O_4$: C, 47.51; H, 6.31; N, 9.23; S, 10.57. Found: C, 47.51; H, 6.28; N, 9.37; S, 10.67. $[\alpha]_D$ +2.4 (*c* 0.41, MeOH).

5.8.19. (4*R*,5*S*)-2-Imino-5-methyl-4-propyl-1,3-thiazolidine·1.1fumarate [(4*R*,5*S*)-9d]. Mp 146–149 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.1C_4H_4O_4$: C, 47.89; H, 6.49; N, 9.80; S, 11.21. Found: C, 48.04; H, 6.64; N, 9.96; S, 11.16. $[\alpha]_D$ −2.5 (*c* 0.16, MeOH).

5.8.20. (4*R*,5*S*)-4-Butyl-2-imino-5-methyl-1,3-thiazolidine·fumarate [(4*R*,5*S*)-9e]. Mp 111–115 °C. Anal. Calcd for $C_8H_{16}N_2S{\cdot}C_4H_4O_4{\cdot}0.35EtOH$: C, 50.10; H, 7.32; N, 9.20; S, 10.53. Found: C, 49.96; H, 7.00; N, 9.55; S, 10.19. $[\alpha]_D$ +4.0 (*c* 0.45, MeOH).

5.8.21. (4*R*,5*R*)-5-Ethyl-2-imino-4-methyl-1,3-thiazolidine·0.5fumarate [(4*R*,5*R*)-14a]. Mp 158–164 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}0.5C_4H_4O_4$: C, 47.50; H, 6.98; N, 13.85; S, 15.85. Found: C, 47.26; H, 6.95; N, 13.64; S, 15.70. $[\alpha]_D$ + 138.9 (*c* 1.0, MeOH).

5.8.22. (4*R*,5*R*)-4-Ethyl-2-imino-5-methyl-1,3-thiazolidine·1.5fumarate [(4*R*,5*R*)-14b]. Mp 158–160 °C. Anal. Calcd for $C_6H_{12}N_2S{\cdot}1.5C_4H_4O_4$: C, 45.27; H, 5.70; N, 8.80; S, 10.07. Found: C, 45.22; H, 5.77; N, 8.85; S, 10.13. $[\alpha]_D$ +62.2 (*c* 0.20, MeOH).

5.8.23. (4*S*,5*S*)-4,5-Diethyl-2-imino-1,3-thiazolidine·1.5fumarate [(4*S*,5*S*)-14c]. Mp 141–144 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.5C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 47.73; H, 6.32; N, 8.43; S, 8.67. $[\alpha]_D$ −61.8 (*c* 0.34, MeOH).

5.8.24. (4*R*,5*R*)-4,5-Diethyl-2-imino-1,3-thiazolidine·1.5fumarate [(4*R*,5*R*)-14c]. Mp 147–149 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}C_4H_4O_4$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 47.03; H, 6.42; N, 8.53; S, 9.33. $[\alpha]_D$ +58 (*c* 0.2, MeOH).

5.8.25. (4*S*,5*S*)-2-Imino-5-methyl-4-propyl-1,3-thiazolidine·1.25fumarate [(4*S*,5*S*)-14d]. Mp 130–136 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.25C_4H_4O_4$: C, 47.51; H, 6.31; N, 9.23; S, 10.57. Found: C, 47.81; H, 6.45; N, 9.41; S, 10.33. $[\alpha]_D$ −56.6 (*c* 0.68, MeOH).

5.8.26. (4*R*,5*R*)-2-Imino-5-methyl-4-propyl-1,3-thiazolidine·1.1fumarate [(4*R*,5*R*)-14d]. Mp 124–129 °C. Anal. Calcd for $C_7H_{14}N_2S{\cdot}1.1C_4H_4O_4$: C, 47.89; H, 6.49; N, 9.80; S, 11.21. Found: C, 48.00; H, 6.61; N, 10.13; S, 10.96. $[\alpha]_D$ +53.1 (*c* 0.41, MeOH).

5.8.27. (4*R*,5*R*)-4-Butyl-2-imino-5-methyl-1,3-thiazolidine [(4*R*,5*R*)-14e]. Mp 120–124 °C. Anal. Calcd for $C_8H_{16}N_2S{\cdot}C_4H_4O_4{\cdot}0.25H_2O$: C, 49.21; H, 7.06; N, 9.57; S, 10.95. Found: C, 49.56; H, 6.93; N, 9.50; S, 10.66. $[\alpha]_D$ +49.3 (*c* 0.56, MeOH).

5.9. X–ray crystallographic analysis of (4*R*,5*R*)-14a

Colorless prismatic crystals of (4*R*,5*R*)-**14a** having approximate dimension of $0.30\times0.20\times0.05$ mm were obtained from ethanol solution by slow evaporation at room temperature. All measurements were made on a Bruker AXS APEX CCD area detector with graphite monochromated MoKα radiation. The structure of (4*R*,5*R*)-**14a** was solved by direct methods and refined by full-matrix least-squares methods with anisotropic temperature factors for nonhydrogen atoms. Hydrogen atoms were placed at their idealized positions and included in structure factor calculation with fixed isotropic thermal parameters. The crystal data are as follows: $C_6H_{12}N_2S{\cdot}0.5C_4H_4O_4$; $M_r = 202.27$; monoclinic; $P2_1$; $a = 15.4581(18)$ Å; $b = 8.4743(10)$ Å; $c = 17.581(2)$ Å; $\beta = 110.602(2)°$; $V = 2155.8(4)$ Å^3; $Z = 8$; $D_c = 1.246$ g/cm^3; $F(0\,0\,0) = 864.00$; μ (MoKα) = 0.273 mm^{-1}; $T = 100(2)$ K; $R = 0.070$; $S = 1.18$. Flack's χ parameter (−0.08(9)) indicated that the absolute structure of (4*R*,5*R*)-**14a** was *R*,*R* configuration.[32]

5.10. Docking study

Docking study between (4*R*,5*R*)-**14a** and crystal structure of iNOS (PDB code: 1N2N) was performed using the docking software FlexX (Tripos, Inc., St. Louis MO, USA).[33] The docking model was energy-minimized by using the molecular modeling software InsightII/Discover95.0 (Accerlys, Inc., San Diego, CA, USA) with cvff force field.[34]

5.11. In vitro biological assay

5.11.1. Preparation of partially purified iNOS enzyme.[26] Mouse RAW 264.7 macrophages cell culture were grown in Dulbecco's modified eagles medium (DMEM) supplemented by 10% fetal bovine serum under 5% CO_2 atmosphere at 37 °C. To this medium, lipopolysaccharide (LPS) and interferon-γ were added to the final concentration of 0.2 μg/mL and 100 unit/mL, respectively. After that the grown cell culture were collected from this medium, and to this cell culture was added Tris and dithiothreitol (DTT) to the final concentration of 50 mM and 100 μM, respectively, at pH 7.5. And then the culture was centrifuged at 100,000*g* for 30 min. And to the obtained supernatant was added Dowex HCR-W2 at 4 °C and stirred for 30 min. at the same temperature. The obtained supernatant was used as crude enzyme. NOS activity was measured by monitoring the conversion level of L-citrulline from L-arginine. To 70 mL of the obtained crude enzyme was added, 20 μL of Tris (pH 7.5) including 1 mM of NADPH, 10 μL of compounds, 20 μL of 10 μCi/mL L-[H^3]-arginine (0.5 μM) and 80 μL of Tris (pH 7.5) and incubated at 37 °C for 30 min. To this was added 200 μL of 0.1 M (pH 5.0) including 2 mM EDTA and 2 mM EGTA. To the resulting was added Dowex 50W-8X and stirred for 5 min. L-[H^3]-citrulline in the supernatant was monitored by scintillation counting.

5.11.2. Preparation of partially purified nNOS enzyme.[27] Std female Wistar rat (150–170 g) cerebella were homogenized after adding 50 mM of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.1) including 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 12.5 mM 2-mercaptoethanol, and 0.5 mM

EDTA, and then, centrifuged at 100,000*g* for 30 min. To the obtained supernatant was added Dowex HCR-W2 and the resulting was stirred for 30 min at 4 °C. The final supernatant was used as crude enzyme. NOS activity was measured by monitoring the conversion level of L-citrulline from L-arginine. To 100 μL of the obtained crude enzyme was added 60 μL of 50 mM Tris (pH 7.5) including 1 mM NADPH, 2 mM $CaCl_2$, and 300 nM calmodulin, 10 μL of compounds, 20 μL of 10 μCi/mL L-[H^3]-arginine (0.5 μM), and 10 μL of Tris (pH 7.5). The resulting mixture was incubated at 37 °C for 5 min and followed by adding 200 μL of 0.1 M sodium acetate buffer (pH 5.0) including 2 mM EDTA and 2 mM EGTA. To the resulting was added Dowex 50W-8X and stirred for 30 min. L-[H^3]-citrulline in the supernatant was monitored by scintillation counting.

5.12. Measurement (ID_{50} value) of NOx levels in plasma of LPS-treated mice

As following reported procedures,[17,31] male C57BL/6 mice (7 weeks old) purchased from Shizuoka Laboratory Animal Center (Hamamatu, Japan) were intraperioneally treated with 2.5 mg/kg LPS. Test-compounds (0.1–30 mg/kg) were administered orally 3 h after LPS treatment, and blood samples were taken from anesthetized mice by cardiac puncture 6 h after injection of LPS. The concentration of nitrite/nitrate in plasma was measured by the use of a colorimetric assay kit (Dojindo Lab., Japan). ID_{50} values were determined graphically by a linear regression model.

5.13. Measurement of pharmacokinetic data

(4*R*,5*R*)-**14a** was administered intravenously or orally at a dose of 0.3 mg/kg to fasting Wistar rats (200–250 g). Plasma samples were collected at 0.083, 0.25, 0.5, 1, 2, and 3 h after (4*R*,5*R*)-**14a** intravenous administration and 0.5, 1, 2, and 3 h after its oral administration. (4*R*,5*R*)-**14a** concentration in the plasma was measured using LC/MS (Agilent 1100 series LC/MSD system, Agilent Technologies) with a mobile phase: 5% Acetonitrile in 0.1% formic acid, HPLC Column: CAPCELL PACK C_{18}, Shiseido, and ionization mode: ESI. The pharmacokinetic parameter of (4*R*,5*R*)-**14a** were calculated from plasma concentration–time curves using moment method.

Acknowledgements

We gratefully acknowledged Dr. Nobuhide Watanabe and Dr. Katsumi Chiba for their helpful comments. Thanks are also due to members of the Department of Physico-Chemical Analysis for their elemental analyses and spectral measurements, and members of the Pharmacokinetics & Physico-Chemical Property Research Laboratories for their pharmacokinetics evaluation.

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