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Novel and orally bioavailable inducible nitric oxide synthase inhibitors: synthesis and evaluation of optically active 4,5-dialkyl-2-iminoselenazolidine derivatives

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Abstract—We have previously reported that (4*R*,5*R*)-5-ethyl-2-imino-4-methylthiazolidine (**3**) strongly inhibits inducible nitric oxide synthase (iNOS). In a successive search for strong and selective iNOS inhibitors, we, herein, describe the synthesis of the selenium analogue of **3** (**4**: ES-2133) and its related optically active compounds and examine their in vitro and in vivo inhibitory activity against iNOS. In addition, an alternative synthetic method to the selected compound **4** and its pharmacokinetic profile is also reported.

Nitric oxide (NO), an important bioregulator and ubiquitous biomessenger existing in a wide variety of organisms, is produced by oxidation of L-arginine catalyzed by nitric oxide synthase (NOS). Over the past decades, there has been extensive scientific interest in the fundamental biochemistry and physiological roles of NO.[1–3] Based on its endogenous regulation, NOS has been structurally classified as constitutive NOS (cNOS) and inducible NOS (iNOS). cNOS has been subdivided into endothelial NOS (eNOS) found in the vascular endothelium and known to be implicated in vascular tone and platelets aggregation, and neuronal NOS (nNOS) found in the brain and known to regulate neuronal transmission and cerebral blood flow.[4,5] On the other hand, iNOS is mainly expressed in macrophages, 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 ileitis, and colitis.[6–8] Different approaches have been taken to inhibit excessive production of NO, and many compounds including amino acids and non amino acids derivatives[9–12] have been synthesized and investigated in connection with a number of inflammatory diseases in animal models and in clinical trials.[10,13–17]

In the course of our search for selective iNOS inhibitors,[18,19] we have previously reported the structure–activity relationships (SARs) of a series of 4,5-dialkyl-2-iminothiazolidine derivatives as iNOS inhibitors and shown that (4*R*,5*R*)-5-ethyl-2-imino-4-methylthiazolidine (**3**: ES-1537, Fig. 1) strongly inhibits iNOS.[20] Very recently, we reported a novel synthetic method of the *cis*- and *trans*-4,5-diethyl-2-iminoselenazolidine **1** via aziridine as intermediates and demonstrated that the inhibitory activity of **1** against iNOS is in the same range as that of the thiazolidine analogue **2**.[21]

On the other hand, selenium-containing compounds such as Ebselen have been studied in the field of

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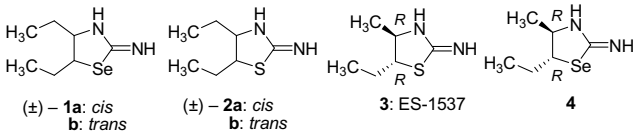


Figure 1.

pharmacology.[22,23] Therefore, the biological role of compounds containing selenium is of intense current interest for medicinal chemists. Herein, we describe the synthesis of the selenium analogue of **3**, that is, (4*R*,5*R*)-5-ethyl-2-imino-4-methylselenazolidine (**4**) and its related optically active selenazolidine derivatives, and evaluate their in vitro and in vivo inhibitory activity against iNOS. In addition, an alternative synthetic method and pharmacokinetic profile of selected compound **4** are also reported.

According to a previously reported procedure,[21] reaction of the *O*-methanesulfonyl β-amino alcohol hydrochloride **7**,[20] synthesized from the commercially available amino alcohol **5** in seven steps, with potassium selenocyanate afforded a mixture of **4** and **8** (Scheme 1). The mixture was then reacted with methyl chloroglyoxylate and triethylamine to afford a mixture of **9** and **10**, which were immediately separated by column chromatography to give the *N*-oxalyl derivatives **9** and **10**. Hydrolysis of **9** and **10** with potassium carbonate in methanol afforded **4** and **8**, respectively.

^{1}H NMR chemical shifts of the methyl group directly connected to the selenazolidine ring of **4** and **8** are 1.25 and 1.56 ppm, respectively. By comparing ^{1}H NMR chemical shift of the methyl group in the thiazolidine analogue of **4** with that of the methyl group in the thiazolidine analogue of **8**, we deduced that compound **4** is the selenium analogue of **3**.

In order to confirm the structure of **4**, we carried out an X-ray analysis of this compound. According to the ORTEP view illustrated in Figure 2, it is clear that compound **4** possesses a *transoid* configuration. In addition, Flack parameter[24] analysis together with experimental data revealed that compound **4** has a 4*R* and 5*R* configuration.

Next, as depicted in Scheme 2, a series of 2-iminoselenazolidine derivatives (**4a**–**c** and **8a**–**c**) were synthesized from the corresponding *O*-methanesulfonyl β-amino alcohol hydrochlorides **7a**–**c** by similar synthetic procedure to that of **4** and **8**. The chemical structure of **4a** and **8a** were determined by comparing their ^{1}H NMR signals with those of their sulfur analogues,[20] and the chemical structures of **4b**,**c**, and **8b**,**c** were determined by comparing their ^{1}H NMR signals with those of their enantiomers (**4b** = **4a**, **8b** = **8a**, **4c** = **4**, **8c** = **8**).

The inhibitory activity of the synthesized compounds (**4**, **4a**–**c**, **8**, and **8a**–**c**) against iNOS and nNOS was evaluated according to previously reported methods,[25,26] and their selectivity for iNOS was determined from IC_{50}nNOS/IC_{50}iNOS ratio.

As shown in Table 1, compounds **4** and **8c** strongly inhibited iNOS with IC_{50} values of 9.3 and 9.4 nM, respectively, indicating that strong inhibition of iNOS requires 4*R*,5*R*-configuration for substituents on the

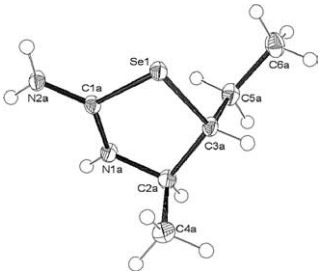
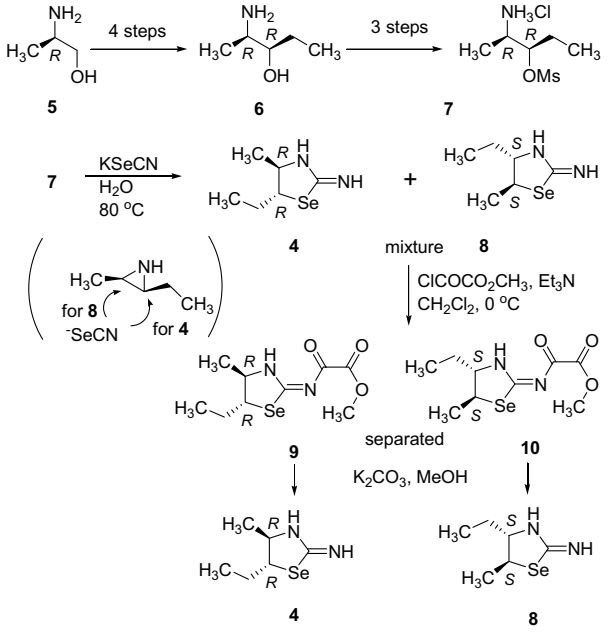
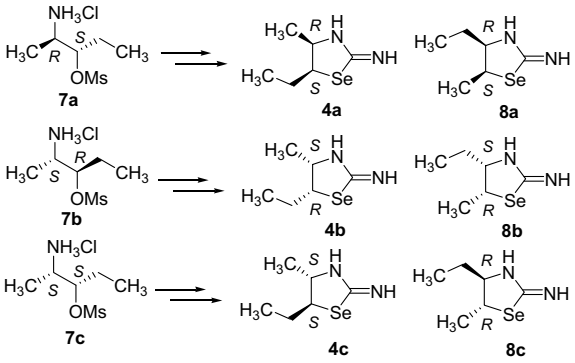


Figure 2. ORTEP view of X-ray structure of (4*R*,5*R*)-5-ethyl-2-imino-4-methylselenazolidine (**4**).

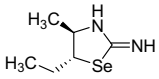
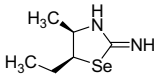
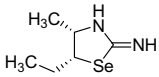
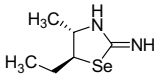
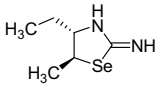
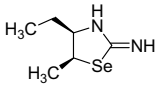
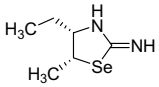
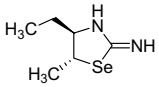
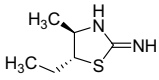


Scheme 1. Synthetic approach to compound **4** via aziridine intermediate.



Scheme 2. Synthesis of other selenazolidine derivatives (**4a**–**c** and **8a**–**c**).

Table 1. Inhibitory activity of 2-iminoselenazolidines against iNOS and nNOS[a]

Compound (stereo)	Structure	Inhibitory activity[b]		Selectivity[e]
		iNOS IC_{50}(nM)[c]	nNOS IC_{50} (nM)[d]	iNOS/nNOS
4 (4*R*,5*R*)		9.3	790	85
4a (4*R*,5*S*)		66	1300	20
4b (4*S*,5*R*)		340	6900	20
4c (4*S*,5*S*)		34	350	10
8 (4*S*,5*S*)		21	130	6.2
8a (4*R*,5*S*)		21	280	13
8b (4*S*,5*R*)		80	740	9.3
8c (4*R*,5*R*)		9.4	92	9.8
3[20] (4*R*,5*R*)		6.6	380	58
L-NMMA		19,000	4300	0.23

[a] All compounds were isolated as fumarates and were analytically pure.
[b] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight different concentrations.
[c] Inhibitory activity against iNOS and nNOS was evaluated according to previously reported procedures.[25,26]
[d] Inhibitory activity against iNOS and nNOS was evaluated according to previously reported procedures.[25,26]
[e] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.

selenazolidine ring. This finding is in agreement with that in our previous study with the thiazolidine derivatives.[20] Among the oxazolidines,[18] thiazolidines,[20] and selenazolidines we synthesized so far, compound **4** shows the best selectivity for iNOS (IC_{50}nNOS/IC_{50}iNOS = 85).[27]

Inversion of the *R*-configuration at the 4-position of **4** to the *S*-configuration reduced the inhibitory activity against iNOS and nNOS and the selectivity for iNOS (**4b**). On the other hand, when substituents at the 4- and 5-positions of **4** were inverted (compound **8c**), the inhibitory activity against iNOS stayed the same as that of **4**, while the inhibitory activity against nNOS increased as compared to that of **4**.

The order of inhibitory activity against iNOS of the series of **4** was (4*R*,5*R*) > (4*S*,5*S*) > (4*R*,5*S*) > (4*S*,5*R*), and that of the series of **8** was (4*R*,5*R*) > (4*S*,5*S*) = (4*R*,5*S*) > (4*S*,5*R*). These findings indicate that the *transoid* configuration is preferable for strong iNOS inhibition.

Interestingly, the inhibitory activity against nNOS of compounds **8** and **8a–c** was stronger than that of compounds **4** and **4a–c** (**8c** vs **4**, **8** vs **4c**, **8b** vs **4b**, and **8a** vs **4a**). These results indicate that strong inhibitory activity against nNOS requires 4-ethyl and 5-methyl substituent on the selenazolidine ring.

Next, in order to determine the kinetics of inhibition of the series of 2-iminoselenazolidine derivatives against iNOS, Lineweaver–Burk double-reciprocal plot analysis of the kinetics of iNOS inhibition in the presence of different concentrations of **4** (ES-2133) was performed.

As shown in Figure 3, double-reciprocal plots of the kinetics of iNOS inhibition in the presence of different concentrations of **4** resulted in lines intersecting at the same point on the *y*-axis. This result indicates that **4** competitively inhibits L-arginine binding to iNOS with a K_i value of 6.0 nM (K_i was determined by plotting the gradient of each double-reciprocal plot against concentration of **4**).

Next, the in vivo inhibitory activity of the selected compound **4** against iNOS was determined by evaluating its effects on plasma nitrite/nitrate levels in LPS–treated mice. As shown in Table 2, compound **4**, given orally,

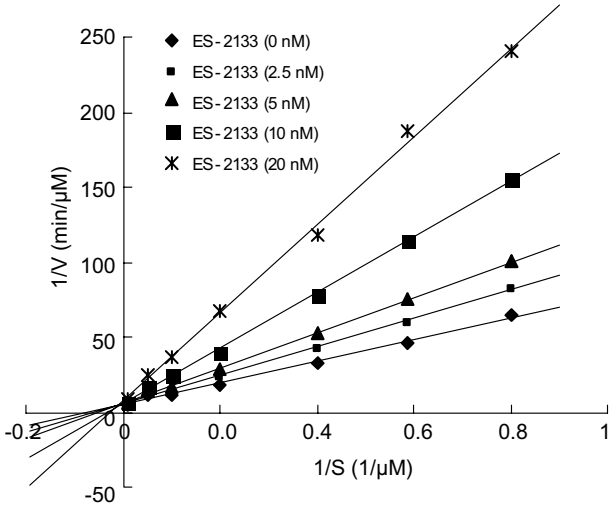


Figure 3. Reciprocal plot of the kinetics of inhibition of iNOS binding to L-arginine in the presence of different concentrations of **4**. The inhibitory activity against iNOS was determined by measuring the conversion of L-[H^3]-arginine to L-[H^3]-citrulline according to a reported procedure.[18] Each point is the average of duplicate determinations.

Table 2. Inhibitory effect of compound **4** on plasma nitrite/nitrate levels in LPS-treated mice

Compound	Inhibitory activity
	ID_{50}[a] mean ± SD[b] (mg/kg, po)
4	0.30 ± 0.03

[a] The effects of compound **4** on plasma nitrite/nitrate levels were evaluated according to a previously reported method[20] with some modifications. Compound **4** was orally administered three hours after LPS (1 mg/kg, iv) injection into C57BL/6 mice. Six hours after treatment with LPS, blood samples were collected from the abdominal aorta under nembutal anesthesia. Plasma was obtained by centrifugation, and the concentration of accumulated nitrite/nitrate was measured.

[b] Value represents the mean ± standard deviation (SD) of five experiments.

strongly inhibited LPS-induced increase in plasma nitrite/nitrate levels in mice with ID_{50} value of 0.30 mg/kg.

Next, we evaluated the bioavailability of **4** in rats. As shown in Table 3, intravenous administration of **4** at a dose of 0.3 mg/kg had a mean integrated area under plasma concentration (AUC) of 117 (ng·h/mL), while its oral administration at the same dose exhibited an AUC of 85 (ng·h/mL). Thus, the oral bioavailability of **4** in rats was 73%. When compound **4** was given orally to normal mice at 30 mg/kg/day for 2 weeks, no toxicity in, for instance, hematological test was observed. Considering the strong inhibitory activity and high selectivity of **4** 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.[28]

Finally, we investigated an alternative synthetic approach to **4** without resolution of the derivative of **4** in a sequential synthesis as shown in Scheme 1. According

Table 3. Pharmacokinetics data of **4** in rats

Route (dose)	Parameters	Mean ± SD[a]
iv (0.3 mg/kg)	AUC (0–8) (ng·h/mL)[b]	117 ± 15
	$t_{1/2}$ (h)[c]	0.95 ± 0.18
	CL (L/h/kg)[d]	3.3 ± 0.4
	V_{ss} (L/kg)[e]	2.6 ± 0.3
po (0.3 mg/kg)	C_{max}(ng/mL)[f]	45 ± 10
	t_{max} (h)[g]	0.5 ± 0.0
	AUC (0–8) (ng·h/mL)[b]	85 ± 9
	$t_{1/2}$ (h)[c]	1.2 ± 0.3
	BA (%)[h]	73 ± 8

[a] Each value represents the mean ± standard deviation (SD) of three animals (SD rat, female, 8 weeks old).

[b] Integrated area under plasma concentration versus time curve from 0 to 8 h.

[c] Pharmacokinetic half life.

[d] Plasma clearance.

[e] Steady-state volume of distribution.

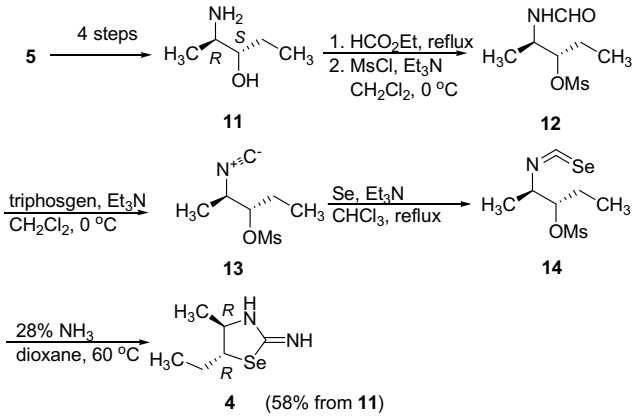
[f] Maximum plasma concentration of unchanged compound.

[g] Time of maximum concentration.

[h] Oral bioavailability.

to the literature,[29] *N*-formyl derivatives can be reacted with triphosgene, triethylamine, and selenium in one pot reaction to give the corresponding isoselenocyanate derivatives. Thus we used this synthetic method for the preparation of **4**. Sequential reaction of the corresponding amino alcohol **5** via **11**,[20] gave the requisite *N*-formyl derivative **12**, which was subjected to one pot reaction condition to yield not **14** as expected but a complex mixture. Thus, the intermediate isonitrile **13**, produced by the reaction of **12** with triphosgene and triethylamine, was isolated as a crude, which was immediately treated with elemental selenium to furnish isoselenocyanate **14** as a brown crude oil. The oil **14** was finally treated with 28% ammonia aqueous solution in dioxane to give **4** (Scheme 3).[30,31] The physical data of compound **4** synthesized by this method was identical to that of **4** synthesized by the method depicted in Scheme 1.

In conclusion, we synthesized a series of 4,5-dialkyl-2-iminoselenazolidine derivatives by two synthetic methods, that is, through aziridine system and isoselenocyanate system. Among the synthesized oxa-, thia-, and selenazolidine derivatives, compound **4** showed strong



Scheme 3. Alternative synthetic approach to **4**.

inhibitory activity against iNOS and the best selectivity for iNOS. In vivo study, compound **4**, given orally, strongly inhibited LPS-induced increase in plasma nitrite/nitrate levels in mice with an ID_{50} value of 0.30 mg/kg. In addition, compound **4** had a good pharmacokinetic profile in rat with 73% bioavailability. It is, therefore, suggested that compound **4** might be therapeutically useful for treatment of diseases related to excess production of NO. Further studies using several animal models are now in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.01.013.

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27. Unpublished result: Selectivity of **4** for iNOS over eNOS was very high according to the evaluation by the reported procedure.[13]
28. Pharmacological and pharmacokinetic profile of compound **4** was similar to that of compound **3**.[20]
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30. Synthetic method and characteristic data are given for (4*R*,5*R*)-5-ethyl-2-imino-4-methylselenazolidine (**4**: ES-2133) as a representative (improved method): Compound **11** (0.22 g), synthesized from commercially available amino alcohol **5** for five steps according to our previous report,[20] was treated with excess of ethyl formate (1.6 mL) under reflux for 8 h and the reaction mixture was concentrated under reduced pressure to give *N*-formyl intermediate (0.15 g), which was subjected to methanesulfonylchloride (0.13 g) with triethylamine (0.16 mL) followed by usual work up to give *O*-mesylate **12** as a crude oil (0.21 g). Compound **12** (0.21 g) was reacted by triphosgene (0.15 g) and triethylamine (0.28 mL) in 5 mL of CH_2Cl_2 at 0 °C for 10 min under argon, and the reaction mixture was treated by excess amount of *n*-hexane under dry ice–acetone bath to precipitate white solid. The white solid was filtered off, and then the filtrate was concentrated to give crude isonitrile **13** (0.19 g), which was immediately subjected to selenium (0.90 g) and excess of triethylamine (0.45 mL) in 10 mL of $CHCl_3$ at reflux temperature under argon. Filtration and concentration of the reaction mixture gave crude isoselenocyanate **14** as crude brown oil (0.18 g). The crude **14** was treated by 5 mL of 28% ammonia aqueous solution and 5 mL of dioxane at 60 °C for 1 h, and the reaction mixture was concentrated to a half volume and extracted with $CHCl_3$ (10 mL × 3). The organic solution was dried with sodium sulfate, filtered off and concentrated to give crude material, which was purified by column chromatography on Chromatorex®-NH (Fuji Silysia Chemical) to afford selenazolidine **4** as a yellow oil (0.11 g, 58% for four

steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (3H, t, J = 7.2 Hz), 1.25 (3H, d, J = 6.4 Hz), 1.68–1.97 (2H, m), 3.85 (1H, qu, J = 4.9 Hz), 3.96 (1H, qu, J = 6.2 Hz), 4.63 (2H, br). APCI–MS m/z 192 $(M+1)^+$. This compound was treated with fumaric acid in ethanol/*n*-hexane solution to give its fumarate as a white solid; mp 145–148 °C. Anal. Calcd for $C_6H_{12}N_2Se{\cdot}0.5C_4H_4O_4$: C, 38.56; H, 5.66; N, 11.24; Se, 31.69. Found: C, 38.70; H, 5.63; N, 11.23; Se, 31.62. $[\alpha]_D$ +139 (c 0.84, MeOH). Characteristic data for other novel compounds (**4a**–**c**, **8**, and **8a**–**c**) were given in Supplementary data.

31. The crystal data of compound **4** are as follows: $C_6H_{12}N_2Se{\cdot}0.5C_4H_4O_4$; M_r = 249.17; monoclinic; $P2_1$; a = 6.4654(7) Å; b = 15.0711(17) Å; c = 11.8516(13) Å; β = 103.892(2); V = 1121.0(2) Å^3; Z = 4; D_c = 1.476 g/cm^3; F(0 0 0) = 504.00; μ(Mo K$_\alpha$) = 3.324 mm^{-1}; T = 100(2) K; R = 0.0223; S = 1.017. Flack's χ parameter (0.016(7)) indicated that the absolute configuration at the 4- and 5-positions of compound **4** is *R*,*R*. Full information on the crystal structure can be ordered from Cambridge Crystallographic Data Centre (CCDC), deposition number CCDC 257366.