Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 313–316

4,5-Disubstituted-1,3-oxazolidin-2-imine derivatives: a new class of orally bioavailable nitric oxide synthase inhibitor

Shigeo Ueda,[a,*] Hideo Terauchi,[b] Akihiro Yano,[c] Motoharu Ido,[c] Masashi Matsumoto[c] and Motoji Kawasaki[a]

[a]*Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd, Enoki-cho 33-94, Suita, Osaka 564-0053, Japan*
[b]*Pharmaceutical Research & Technology Center, Dainippon Pharmaceutical Co., Ltd, Ebie 1-5-51, Fukushima, Osaka 553-0001, Japan*
[c]*Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd, Enoki-cho 33-94, Suita, Osaka 564-0053, Japan*

Received 29 September 2003; revised 7 November 2003; accepted 10 November 2003

Abstract—In our search for a novel class of inducible nitric oxide synthase (iNOS) inhibitors, 1,3-oxazolidin-2-imine was found to weakly inhibit iNOS. Further modifications of this compound resulted in a remarkable increase in both the in vivo and in vitro inhibitory activity and selectivity for iNOS.

1. Introduction

There has been extensive scientific interest in the fundamental biochemistry and diverse physiological roles of nitric oxide (NO) since this simple gaseous radical molecule was first shown to have actions identical to those proposed for the endothelium-derived relaxing factor.[1,2] NO is produced by oxidation of L-arginine catalyzed by nitric oxide synthase (NOS) which utilizes molecular oxygen and cofactors.[3,4] NOS has been divided into two major sub-enzymes, that is, a constitutive NOS (cNOS), which is found in the vascular endothelium and in the brain, and an inducible NOS (iNOS), which is present in activated macrophages. Although the major function of iNOS is thought to serve in host defense mechanism, several studies have indicated that iNOS is also implicated in NO excessive production which causes inflammatory diseases such as hypotension,[5] rheumatoid arthritis[6] and colitis.[7] On the other hand, cNOS has been further subdivided into endothelial NOS (eNOS), which is implicated in blood pressure regulation,[8] and neuronal NOS (nNOS), which regulates neuronal transmission.[9] Indeed, structural analogues of L-arginine such as N^G-nitro-L-arginine (L-NNA), N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) have been synthesized and investigated in connection with a number of inflammation diseases in animal models as well as clinical trials. However, due to lack of selectivity for iNOS, no compound has been approved for the treatment of these conditions. Therefore potent and selective iNOS inhibitors that do not interfere with cNOS physiological functions have long been needed.

To date, non-L-arginine type iNOS selective inhibitors such as *S*-ethylisothiourea,[10] 2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine,[10] 2-iminopiperidines,[11] 2-aminopyridine[12] and 1,2-dihydro-4-quinazolinamine[13] have been reported. Among these inhibitors, (1*S*,5*S*,6*R*,7*R*)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]-heptane hydrochloride (ONO-1714)[14] and *S*-[2-[(1-iminoethyl)amino]ethyl]-L-homocysteine (GW274150)[15] have been reported to potently and selectively inhibit iNOS and are currently undergoing evaluation in clinical Phase II and I trials, respectively. To synthesize compounds which show more selectivity for iNOS than ONO-1714 dose has been obvious interest to us, because, to our knowledge, ONO-1714 is the most proceeding compound as iNOS inhibitor in clinical trial and because the more selectivity would lead to the less side effect.

Keywords: Inducible nitric oxide synthase inhibitor; 1,3-Oxazolidin-2-imine; Selectivity.
* Corresponding author. Tel.: +81-6-6337-5906; fax: +81-6-6338-7656; e-mail: shigeo-ueda@dainippon-pharm.co.jp

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.010

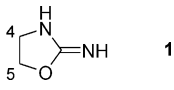


Figure 1. Structure of the lead 1,3-oxazolidin-2-imine (**1**).

In our search for a novel class of iNOS inhibitors by means of random screening, we have found that the 1,3-oxazolidin-2-imine **1** (Fig. 1) weakly inhibits iNOS with an IC_{50} value of 7.9 μM. Further modification of this compound resulted in the discovery of a new series of 1,3-oxazolidin-2-imine derivatives with improved inhibitory activity and selectivity for iNOS. Here, we report the synthesis of these new compounds and their structure–activity relationships (SARs).

2. Results and discussion

Starting from the 1,3-oxazolidin-2-imine (**1**), we first found that the 5-bromomethyl-1,3-oxazolidin-2-imine (**2**) potently inhibits iNOS with an IC_{50} value 16-fold that of **1** (IC_{50} = 0.50 μM versus 7.9 μM, Table 1). This finding led us to suggest that introduction of an appropriately-sized hydrophobic alkyl group at the 4- or 5-position of **1** would increase the inhibitory activity and selectivity for iNOS (Chart 1).

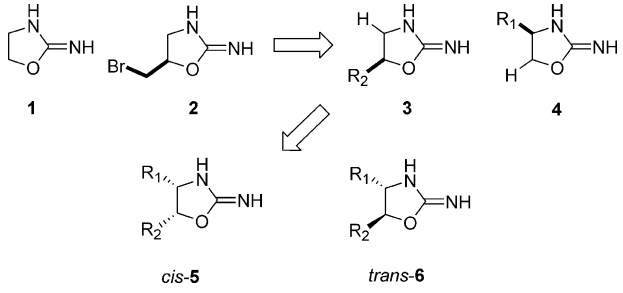
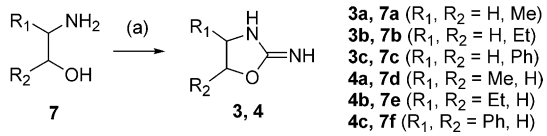
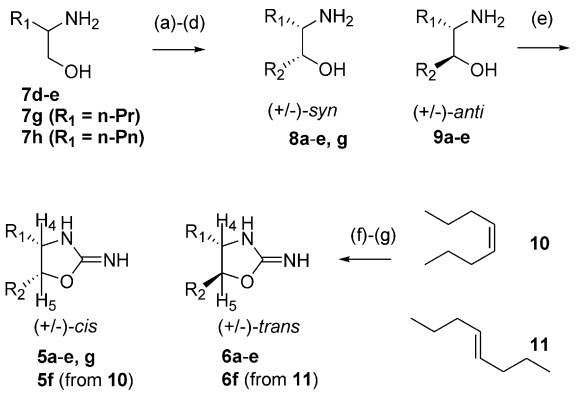


Chart 1. Design of the 1,3-oxazolidin-2-imine derivatives.



Scheme 1. Synthesis of monosubstituted 1,3-oxazolidin-2-imine derivatives as iNOS inhibitor: (a) BrCN, EtOH, rt (35–80%).

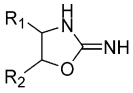


Scheme 2. Synthesis of disubstituted 1,3-oxazolidin-2-imine derivatives as iNOS inhibitor: (a) BnBr, K_2CO_3, CH_3CN, 60 °C (>95%); (b) Swern oxi. (87–95%); (c) R_2MgBr, THF, −30 °C (65–75%) or $(R_2)_2Zn$, toluene, 0 °C (33–67%); (d) H_2, $Pd(OH)_2$, MeOH (70–85%); (e) BrCN, EtOH, rt (46–78%); (f) NBS, NH_2CN, CH_2Cl_2, 0 °C; (g) concd H_2SO_4, acetone, rt (35–40% for two steps).

First, the monosubstituted 1,3-oxazolidin-2-imine derivatives **3a–c** and **4a–c** were synthesized by the route depicted in Scheme 1. In addition, the disubstituted derivatives **5a–g** and **6a–f** were prepared by the reaction sequences shown in Scheme 2,[16–19] and their stereochemical configurations were confirmed based on the nuclear Overhauser effects between 4-H (position 4) and 5-H (position 5) using ^1H NMR analysis.

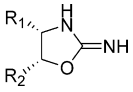
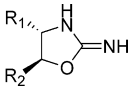
Next, the inhibitory activity of the synthesized compounds (**3a–c**, **4a–c**, **5a–g** and **6a–f**) against iNOS and nNOS was evaluated according to previously reported

Table 1. Inhibitory activity of monosubstituted 1,3-oxazolidin-2-imine derivatives (**1–4**)[a]

Structure	Compound	R_1	R_2	Inhibitory activity[b]		Selectivity nNOS/iNOS[d]
				iNOS IC_{50} (μM)[c]	nNOS IC_{50} (μM)[c]	
 (±)	**1**	H	H	7.9	34	4.3
	2	H	$BrCH_2$	0.50	0.40	0.8
	3a[f]	H	Me	0.074	1.4	19
	3b	H	Et	0.28	1.8	6.4
	3c	H	Ph	n.s. @ 100[e]	n.s. @ 100[e]	n.d.[g]
	4a[f]	Me	H	1.5	9.8	6.5
	4b[f]	Et	H	1.6	2.8	1.8
	4c	Ph	H	28% @ 100[e]	18% @ 100[e]	n.d.[g]
	L-NMMA			19	4.3	0.23
	L-NNA			80	0.5	0.0063
	ONO-1714			0.003	0.015	5

[a] All compounds were analytically pure.
[b] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight concentrations.
[c] iNOS and nNOS activity were evaluated by previously reported procedure.[20,21]
[d] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.
[e] Percent inhibition at test concentration; n.s., no significant effect (<15% inhibition).
[f] Salted with fumaric acid.
[g] n.d., not determined.

Table 2. Inhibitory activity of disubstituted 1,3-oxazolidin-2-imine derivatives (**5**–**6**)[a]

Structure	Compound	R_1	R_2	Inhibitory activity[b]		Selectivity
				iNOS IC_{50} (μM)[c]	nNOS IC_{50} (μM)[c]	nNOS/iNOS[d]
 (+/−)-*cis*	**5a**[e]	Me	Et	0.16	0.85	5.3
	5b[e]	Et	Me	0.12	0.68	5.7
	5c[f]	Et	Et	0.13	1.2	9.2
	5d	*n*-Pr	Me	0.041	0.92	22
	5e[e]	*n*-Pn[g]	Me	0.23	3.6	16
	5f[e]	*n*-Pr	*n*-Pr	6.8	57	8.4
	5g	Me	Me	0.15	1.6	11
 (+/−)-*trans*	**6a**	Me	Et	0.12	1.7	14
	6b[e]	Et	Me	0.17	2.3	14
	6c	Et	Et	0.056	2.5	45
	6d	*n*-Pr	Me	0.12	0.57	4.8
	6e[e]	*n*-Pn[g]	Me	0.50	0.79	1.6
	6f[e]	*n*-Pr	*n*-Pr	2.5	73	29

[a] All compounds were analytically pure.
[b] IC_{50} values for iNOS and nNOS were determined by testing each compound at eight concentrations.
[c] iNOS and nNOS activity were evaluated by previously reported procedure.[20,21]
[d] Selectivity was defined as the ratio of IC_{50} value of nNOS to iNOS.
[e] Salted with fumaric acid.
[f] Salted with HBr.
[g] Pentyl.

methods,[20,21] and their selectivities for iNOS were determined from nNOS/iNOS ratio.

As shown in Table 1, when a methyl group was inserted into the 5-position of **1**, the inhibitory activity and selectivity for iNOS of the resulting compound **3a** were significantly improved (**3a**: $IC_{50} = 0.074$ μM, 107-fold that of **1**, nNOS/iNOS = 19, 4.4-fold that of **1**). In contrast, introduction of a methyl group into the 4-position of **1** produced only a slight improvement in both the inhibitory activity and selectivity for iNOS (**4a**: $IC_{50} = 1.5$ μM, nNOS/iNOS = 6.5). Compounds **3b** and **4b** with an ethyl group at the 4- or 5-position of **1** respectively, had moderate inhibitory activity for iNOS. However, a bulky phenyl moiety appended at the 4- or 5-position of **1** resulted in a complete loss of inhibitory activity against both iNOS and nNOS (**3c** and **4c**). These results indicate that the size of substituents at the 4- and 5-position of **1** is very important for iNOS inhibitory activity.

In further modifications of compound **1**, the effect of a substitution at both the 4- and 5-positions on the inhibitory activity and selectivity for iNOS were investigated.

As shown in Table 2, compounds **5d**[22] and **6c**[23] showed strong inhibitory activity against iNOS [**5d**: $IC_{50} = 0.041$ μM (193-fold that of **1**), **6c**: $IC_{50} = 0.056$ μM (141-fold that of **1**)]. As iNOS oxygenase domain has been reported to have a hydrophobic, small pocket,[24] it is suggested that interaction of **5d** or **6c** with this pocket is responsible for the strong inhibitory activity of these two compounds against iNOS.

Interestingly, **5c** and **6d** with contrastive configurations to those of **6c** and **5d**, respectively, showed much lower inhibitory activity and selectivity for iNOS. And as expected, increasing the steric bulk at both the 4- and 5-positions of **1** resulted in a dramatic decrease in both the inhibitory activity and selectivity for iNOS (**5f** and **6f**), while other disubstituted compounds generally displayed moderate inhibition of iNOS.

Among disubstituted compounds with an ethyl or *n*-propyl moiety at the 5-position, the *trans*-**6a**, **6c** and **6f** were more selective than the *cis* analogues **5a**, **5c** and **5f**. In particular, compound **6c** showed the best selectivity for iNOS (nNOS/iNOS = 45).[25] Thus the *trans* orientation was seen as favorable in terms of selectivity.

Finally, inhibitory activity of compounds **5d** and **6c** for iNOS in vivo was investigated by evaluating the effects of these two compounds on plasma nitrite/nitrate levels in lipopolysaccharide (LPS)-treated mice. As shown in

Table 3. Effects of selected compounds (±)-**5d** and (±)-**6c** on plasma nitrite/nitrate levels in LPS-treated mice[a]

Compound	Inhibitory activity	
	iNOS, IC_{50} (μM)[b]	% inhibition ± SD[c] (mg/kg, po)[d]
(±)-**5d**	0.041	71 ± 8.6 (3)
(±)-**6c**	0.056	75 ± 10 (10)

[a] The effects of each compound on plasma nitrite/nitrate levels were evaluated according to a previously reported method[14] with some modifications. Compounds were orally administered three hours after LPS (1 mg/kg, iv) injection into C57BL/6 mice. Six hours after LPS treatment, blood samples were collected under nembutal anesthesia from the abdominal aorta. Plasma was obtained by centrifugation, and the concentration of accumulated nitrite/nitrate was measured.
[b] See footnote c of Table 2.
[c] Each value represents the mean ± standard deviation (SD) of five animals.
[d] Dosage.

Table 3, both **5d** and **6c**, given orally, strongly inhibited the increase in plasma nitrite/nitrate levels in mice (**5d**: 71% at 3 mg/kg, **6c**: 75% at 10 mg/kg).

3. Conclusion

The series of 1,3-oxazolidin-2-imine derivatives described here represents a new class of iNOS inhibitor. The SARs of these novel inhibitors revealed that introduction of small substituents into the 1,3-oxazolidine ring enhances both the inhibitory activity and selectivity for iNOS. In addition, oral administration of selected 1,3-oxazolidin-2-imine derivatives attenuates the rise of plasma nitrite/nitrate levels induced by LPS in mice. These findings would aid in the design of more potent and selective iNOS inhibitors, because 1,3-oxazolidin-2-imine scaffold is easily synthesized from the corresponding amino alcohols and has diversity to introduce substituent at any position on its ring as well as to exchange oxygen atom by other one. Further investigation of the 1,3-oxazolidin-2-imine derivatives as well as other relative compounds is now in progress.

Acknowledgements

We gratefully acknowledged Dr. Nobuhide Watanabe and Dr. Katsumi Chiba for the thorough and helpful comments on this letter.

References and notes

1. Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524.
2. Pfeiffer, S.; Mayer, B.; Hemmens, B. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1714.
3. Marletta, M. A. *J. Biol. Chem.* **1993**, *268*, 12231.
4. Stuehr, D. J.; Griffith, O. W. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1992**, *65*, 287.
5. Thiemermann, C.; Vane, J. *Eur. J. Pharmacol.* **1990**, *182*, 591.
6. Grabowski, P. S.; Wright, P. K.; Van't Hof, R. J.; Helfrich, M. H.; Ohshima, H.; Ralston, S. H. *Br. J. Rheumatol.* **1997**, *36*, 651.
7. Seo, H. G.; Takata, I.; Nakamura, M.; Tatsumi, H.; Suzuki, K.; Fujii, J.; Taniguchi, N. *Arch. Biochem. Biophys.* **1995**, *324*, 41.
8. Rees, D. D.; Palmer, R. M. J.; Moncada, S. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3375.
9. Böhme, G. A.; Bon, C.; Lemaire, M.; Reibaud, M.; Piot, O.; Stutzmann, J. M.; Doble, A.; Blanchard, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9191.
10. Nakane, M.; Klinghofer, V.; Kuk, J.; Donnelly, J.; Budzik, G.; Pollock, J.; Basha, F.; Carter, G. *Mol. Pharmacol.* **1995**, *47*, 831.
11. Hansen, D. W.; Peterson, K. B.; Trivedi, M.; Webber, R. K.; Tjoeng, F. S.; Moore, W. M.; Jerome, G. M.; Kornmeier, C. M.; Manning, P. T.; Connor, J. R.; Misko, P. T.; Currie, M. G.; Pitzele, B. S. *J. Med. Chem.* **1998**, *41*, 1361.
12. Hagmann, W. K.; Galdwell, C. G.; Chen, P.; Durette, P. L.; Esser, C. K.; Lanza, T. J.; Kopka, I. E.; Guthikonda, R.; Shah, S. K.; MacCoss, M.; Chabin, R. M.; Fletcher, D.; Grant, S. K.; Green, B. G.; Humes, J. L.; Kelly, T. M.; Luell, S.; Meurer, R.; Moore, V.; Pacholok, S. G.; Pavia, T.; Williams, H. R.; Wong, K. K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1975.
13. Tinker, A. C.; Beaton, H. G.; Boughton-Smith, N.; Cook, T. R.; Cooper, S. L.; Fraser-Rae, L.; Hallam, K.; Hamley, P.; McInally, T.; Nicholls, D. J.; Pimm, A. D.; Wallace, A. V. *J. Med. Chem.* **2003**, *46*, 913.
14. Naka, M.; Nanbu, T.; Kobayashi, K.; Kamanaka, Y.; Komeno, M.; Yanase, R.; Fukutomi, T.; Fujimura, S.; Seo, H. G.; Fujiwara, N.; Ohuchida, S.; Suzuki, K.; Kondo, K.; Taniguchi, N. *Biochem. Biophys. Res. Commun.* **2000**, *270*, 663.
15. Young, R. J.; Beams, R. M.; Carter, K.; Clark, H. A. R.; Coe, D. M.; Chambers, C. L.; Davies, P. I.; Dawson, J.; Drysdale, M. J.; Franzman, K. W.; French, C.; Hodgson, S. T.; Hodson, H. F.; Kleanthous, S.; Rider, P.; Sanders, D.; Sawyer, D. A.; Scott, K. J.; Shearer, B. G.; Stocker, R.; Smith, S.; Tackley, M. C.; Knowles, R. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 597.
16. Reetz, M. T.; Drewes, M. W.; Schmitz, A. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1141.
17. Andrés, J. M.; Barrio, R.; Martínez, M. A.; Pedrosa, R.; Pérez-Encabo, A. *J. Org. Chem.* **1996**, *61*, 4210.
18. Ponsold, K.; Ihn, W. *Tetrahedron Lett.* **1970**, *11*, 1125.
19. Jung, S. H.; Kohn, H. *J. Am. Chem. Soc.* **1985**, *107*, 2931.
20. Misko, T. P.; Moore, W. M.; Kasten, T. P.; Nickols, G. A.; Corbette, J. A.; Tilton, R. G.; McDaniel, M. L.; Williamson, J. R.; Currie, M. G. *Eur. J. Pharmacol.* **1993**, *233*, 119.
21. Bredt, D. S.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 682.
22. Characteristic data are given for **5d**; mp 90–91 °C (hexane), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (3H, t, $J=7.0$ Hz), 1.24 (3H, d, $J=6.6$ Hz), 1.33–1.55 (4H, m), 3.86 (1H, m), 4.70 (1H, m). Anal. calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N, 19.70. Found: C, 58.74; H, 9.88; N, 19.86. APCI-MS m/z 143 $[M+1]^+$.
23. Characteristic data are given for **6c**; mp 69–70 °C (hexane), ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (3H, t, $J=7.5$ Hz), 0.97 (3H, t, $J=7.5$ Hz), 1.39–1.74 (4H, m), 3.47 (1H, dd, $J=6.3$, 12.5 Hz), 4.03 (1H, dd, $J=6.1$, 12.1 Hz). Anal. calcd for $C_7H_{14}N_2O{\cdot}0.1H_2O$: C, 58.39; H, 9.94; N, 19.45. Found: C, 58.09; H, 9.99; N, 19.30. APCI-MS m/z 143 $[M+1]^+$.
24. Crane, B. R.; Arvai, A. S.; Gachhui, R.; Wu, C.; Ghosh, D. K.; Getzoff, E. D.; Stuehr, D. J.; Tainer, J. A. *Science* **1997**, *278*, 425.
25. Unpublished results: Selectivity of both **5d** and **6c** for iNOS over eNOS was greater than that of ONO-1714 according to the evaluation by modified procedure.[26]
26. Wang, Y. X.; Poon, C. I.; Pang, C. C. Y. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 112.