Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1975–1978

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Substituted 2-Aminopyridines as Inhibitors of Nitric Oxide Synthases

William K. Hagmann,[a,*] Charles G. Caldwell,[a] Ping Chen,[a] Philippe L. Durette,[a] Craig K. Esser,[a] Thomas J. Lanza,[a] Ihor E. Kopka,[a] Ravi Guthikonda,[a] Shrenik K. Shah,[a] Malcolm MacCoss,[a] Renee M. Chabin,[b] Daniel Fletcher,[d] Stephan K. Grant,[b] Barbara G. Green,[b] John L. Humes,[c] Theresa M. Kelly,[b] Sylvie Luell,[d] Roger Meurer,[d] Vernon Moore,[d] Stephen G. Pacholok,[c] Tony Pavia,[b] Hollis R. Williams[c] and Kenny K. Wong[b]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories Rahway, NJ 07065, USA*
[b]*Department of Biochemistry, Merck Research Laboratories Rahway, NJ 07065, USA*
[c]*Department of Inflammation Research, Merck Research Laboratories Rahway, NJ 07065, USA*
[d]*Department of Pharmacology Merck Research Laboratories Rahway, NJ 07065, USA*

Received 22 May 2000; accepted 28 June 2000

Abstract—A series of substituted 2-aminopyridines was prepared and evaluated as inhibitors of human nitric oxide synthases (NOS). 4,6-Disubstitution enhanced both potency and specificity for the inducible NOS with the most potent compound having an IC_{50} of 28 nM.

In recent years, nitric oxide (NO) has emerged as one of the most interesting, and seemingly ubiquitous, mediators of normal and pathophysiological processes.[1–3] In mammalian cells, NO is produced by the oxidation of L-Arginine by nitric oxide synthase (NOS). There are three isoforms of NOS: the constitutively expressed neuronal NOS (n-NOS) and endothelial cell NOS (e-NOS) and the inducible NOS (i-NOS). n-NOS is believed to have a role in the production of NO as a neurotransmitter. e-NOS is found primarily in vascular endothelium where it regulates blood pressure and vascular tone. i-NOS expression is induced in activated macrophage and other cell types by numerous inflammatory stimuli including endotoxin (LPS) and cytokines (e.g. IL-1) and has a role in host defense and possibly chronic inflammatory conditions. Transgenic mice that have the NOS genes knocked-out confirm many of the biological roles of the respective NOS isoforms.[4–8]

The induction of i-NOS by inflammatory stimuli and the prolonged production of copious amounts of NO by some activated inflammatory cells strongly suggest a role for i-NOS in both host defense and tissue destruction associated with acute and chronic inflammation. As such, i-NOS may have a role in a variety of diseases including septic shock, arthritis, and inflammatory bowel disease. The objective of our program was to identify potent and selective inhibitors of i-NOS with appropriate pharmacological properties. Several groups have identified 2-aminopyridines as NOS inhibitors. A detailed report of the in vitro and in vivo properties of 2-amino-4-methylpyridine **3** has appeared.[9,10] The structure–activity relationship for a variety of substituted 2-aminopyridines is described herein.

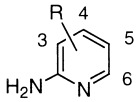
Synthesis of 2-Aminopyridines

The synthesis of 2-aminopyridine derivatives has been extensively reviewed.[11–13] Several of the compounds in Tables 1 and 2 are also commercially available. Among the methods employed to prepare some of the compounds described, the Chitchibabin reaction, Hofmann, Curtius, Lossen and Neber-type rearrangements, and halogen replacements were employed.[14] The synthesis of 2-aminopyridine from pyridine-*N*-oxides has also been described (Scheme 1).[15] Pyridine-*N*-oxides were treated with 4-chloro-2,2-dimethyl-1,3(2*H*)-benzoxazine to give 3-(2-pyridyl)-1,3-benzoxazinones. Subsequent treatment with strong acid afforded the 2-aminopyridine.

*Corresponding author. Tel.: +1-732-594-7249; fax: +1-732-594-5966; e-mail: william_hagmann@merck.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00389-9

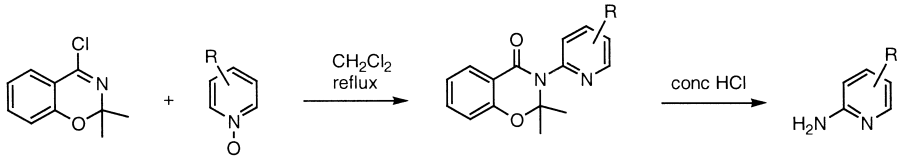
Table 1. Inhibition of nitric oxide synthases by substituted 2-aminopyridines[a]



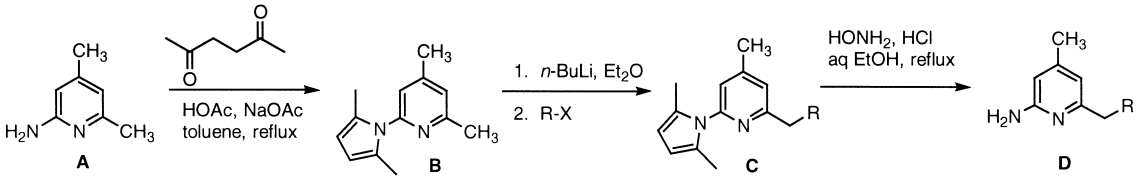
e-NOS

Compound	R	i-NOS IC_{50} (μM)	e-NOS IC_{50} (μM)	Selectivity e-NOS/i-NOS	n-NOS IC_{50} (μM)	Selectivity n-NOS/i-NOS
1	H	1.9	2.8	1.5	4.8	2.5
2	3-CH_3	0.94	1.20	1.3	ND	ND
3	4-CH_3	0.17	0.072	0.4	0.075	0.4
4	5-CH_3	0.6	3.1	5.2	ND	ND
5	6-CH_3	2.0	0.82	0.4	ND	ND
6	3,4-$(CH_3)_2$	0.076	0.15	2.0	ND	ND
7	3,5-$(CH_3)_2$	14.6	3.6	0.2	2.0	0.1
8	4,5-$(CH_3)_2$	0.81	0.6	0.7	0.34	0.4
9	4,6-$(CH_3)_2$	0.11	0.045	0.4	ND	ND
10	5,6-$(CH_3)_2$	7.2	2.8	0.4	2.2	0.3
11	4-C_2H_5	0.23	0.23	1.0	ND	ND
12	4-n-C_3H_7	>50	ND	ND	ND	ND
13	4-Cl	>50	ND	ND	ND	ND
14	4-CF_3	13.2	72.4	5.5	ND	ND
15	3-C_2H_5, 4-CH_3	2.7	3.3	1.2	ND	ND
16	3-*n*-C_3H_7, 4-CH_3	34.5	ND	ND	ND	ND
17	3-NH_2, 4-CH_3	0.059	0.081	1.4	ND	ND
18	5-C_2H_5, 4-CH_3,	1.3	3.4	2.6	0.61	0.5
19	4-CH_3, 6-C_2H_5	0.33	0.049	0.15	42% @ 0.1	0.3
20	4-CH_3, 6-*n*-C_3H_7	0.11	1.0	9.1	0.09	0.8
21	4-CH_3, 6-*i*-C_3H_7	0.11	0.2	1.8	1.2	10.9
22	4-CH_3, 6-*n*-C_4H_9	0.046	40% @ 0.1	>2.7	59% @ 0.1	<2.2
23	4-CH_3, 6-*i*-C_4H_9	0.028	0.15	5.4	0.10	3.6
24	4-CH_3, 6-*i*-C_5H_{11}	0.076	1.9	25	0.51	6.7
25	4-CH_3, 6-$(CH_2)_3$Ph	12.7	17.2	1.4	3.3	0.3
N^g-Methyl-L-arginine		10	8.7	0.9	2.7	0.3
N-Iminoethyl-L-lysine		1.4	7.9	5.6	18.4	13.1
Aminoguanidine		101	500	5.0	118	1.1

[a]ND = not determined. NOS activity was determined by comparing conversion of ^{3}H-(L)-arginine to ^{3}H-(LL)-citrulline in the presence of compound with control. The assay mixture (pH 7.5) containing 1 μM ^{3}H-(L)-arginine (2 μCi), cofactors and inhibitor or aq DMSO (control) was incubated for 30 min at room temperature. The reaction was quenched by adding a slurry of Dowex 50W-X8 resin which removed unreacted substrate. The concentration of ^{3}H-(L)-citrulline in the supernatant was determined on a scintillation counter. For each inhibitor, the percent inhibition was determined (2×) at 10 different concentrations and an IC_{50} calculated using SIGMAPLOT.



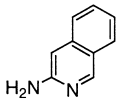
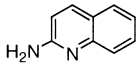
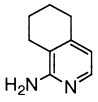
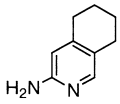
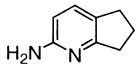
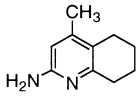
Scheme 1.



Scheme 2.

A procedure for the selective alkylation of 2-amino-4,6-dimethylpyridine is outlined in Scheme 2.[16–18] 2-Amino-4,6-dimethylpyridine A was treated with acetonylacetone with the removal of water to form pyridylpyrrole B. Reaction of B with one equiv of *n*-butyllithium in diethylether formed the anion on the 6-methyl group which was subsequently allowed to react with an electrophile R-X to form C. However, if LDA is used as the base in THF solvent, alkylation occurs primarily on the 4-methyl group. The pyrrole protecting group was removed by reaction with hydroxylamine hydrochloride in refluxing aqueous ethanol to form the 6-substituted methyl product D. This method allowed the preparation of **19**–**25**. Compounds were evaluated as inhibitors of the three human NOS isoforms (Tables 1 and 2).

Table 2. Inhibition of nitric oxide synthases by fused-ring aminopyridines[a]

Compound	Structure	i-NOS IC_{50} (μM)	e-NOS IC_{50} (μM)	Selectivity e-NOS/i-NOS	n-NOS IC_{50} (μM)	Selectivity n-NOS/i-NOS
32		4.6	1.4	0.3	6.6	1.4
33		>50	ND	ND	ND	ND
34		1.7	ND	ND	ND	ND
35		29	24	0.8	11	0.4
36		4.1	16.6	4.0	10.6	2.6
37		>50	>50	ND	>50	ND
38		4.0	19.8	5.0	2.4	0.6

[a]ND = not determined. See footnote to Table 1 for assay conditions.

Discussion

The introduction of a single methyl group to the 4-position of 2-aminopyridine, as in **3**, significantly enhanced the inhibition of all three NOS isoforms, in particular the constitutive enzymes (Table 1). The other single positional isomers were less impressive. Interestingly, 2-amino-4-methylpyridine (**3**) is available by prescription in some parts of the world as an analgesic and for the treatment of hypotension; possibly associated with its inhibition of the constitutive NOS's. Introduction of a second methyl group suggested that the 6-position may enhance selectivity and/or potency with respect to **3**. Substitution at the 5-position looked to be deleterious in this series. As seen with **3** versus **11**–**14**, at least for i-NOS, the 4-methyl group was optimal. The 4-Cl (**13**) and 4-CF_3 (**14**) groups, which would be expected to be nearly isosteric with methyl, lost nearly all the activity and may suggest that the pK_a of the aminopyridine system plays a role in inhibition.

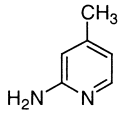
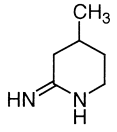
Despite the initial results obtained with a 4,6-dimethyl substitution (**9**), larger alkyl groups at the 6-position along with the 4-CH_3 group offered the most potent and selective compounds in this series (**19–24**). As the size of the alkyl group increased from methyl to isopentyl, potency for e-NOS decreased while i-NOS potency remained relatively constant, thus increasing i-NOS selectivity. The 6-*iso*-butyl (**23**) and 6-*iso*-pentyl (**24**) analogues were the most potent and selective inhibitors of i-NOS that were obtained in this series. The larger phenylpropyl (**25**) was much less potent.

Finally, a series of fused bicyclic analogues were prepared and evaluated as inhibitors of NOS (Table 2). 3,4-Dimethyl substitution (**6**) was much more potency enhancing than were its fused unsaturated (**32**) and saturated (**35**) analogues. This was also true for 4,5-disubstitution (**8** versus **33** and **36**). However, 2-aminoquinoline (**34**) was more potent than the 5,6-dimethylpyridyl (**10**) analogue as well as the saturated derivatives (**37** and **38**).

Similar patterns of potency enhancement for the saturated 2-iminopiperidine analogues have been reported.[19] 4-Methyl and 4,6-dimethyl substitution enhanced potency for all three isoforms of NOS. An in vivo comparison of aminopyridine **3** and iminopiperidine **39** revealed certain shortcomings of the 2-aminopyridine series (Table 3). Pyridine **3** was more potent in raising mean arterial pressure (MAP) in rats, a result of its greater potency versus e-NOS. This hypertensive effect is consistent with the clinical use of **3** to treat hypotension.[20] On the other hand, **3** was less potent than **39** in a model of LPS-induced nitrate production in mice which reflects either its lesser potency versus i-NOS or poorer oral bioavailability.

In summary, we have explored the SAR of a series of substituted 2-aminopyridines and obtained significant increases in selectivity for inhibition of i-NOS. 4-Alkyl and 4,6-dialkyl substituents provided the most potent and selective inhibitors of the inducible NOS isoform. However, the 2-aminopyridine analogues

Table 3. In vivo comparison of aminopyridine **3** and iminopiperidine **39**

	 **3**	 **39**
i-NOS: IC_{50} (μM)	0.17	0.016
e-NOS: IC_{50} (μM)	0.072	0.22
LPS[a]: ED_{50} (mg/kg po)	25	0.15
BP[b]: HD_{40} (mg/kg iv)	0.11	1.8

[a]LPS = LPS-induced NO_x increase in mouse plasma: i-NOS mediated increase in NO products (ED_{50} = effective dose for a 50% reduction w/ respect to control).
[b]BP = Blood pressure elevation in anesthetized rats: ec-NOS mediated decrease in mean aterial blood pressure (HD_{40} = dose to increase MAP by 40 mm versus control).

are not as potent or selective (in vitro or in vivo) as their saturated counterparts, the 2-iminopiperidines.

References and Notes

1. Moncada, S.; Palmer, R. M.; Higgs, E. *Pharmacol. Rev.* **1991**, *43*, 109.
2. Nathan, C.; Xie, Q.-W. *Cell* **1994**, *78*, 915.
3. Kerwin, J. F.; Lancaster, J. R.; Feldman, P. L. *J. Med. Chem.* **1995**, *38*, 4343.
4. Huang, Z. H.; Huang, P. L.; Panahian, N.; Dalkara, T.; Fishman, M. C.; Moskowitz, M. A. *Science* **1994**, *265*, 1883.
5. MacMicking, J. D.; Nathan, C.; Hom, G.; Chartrain, N.; Trumbauer, M.; Stevens, K.; Xie, Q.-W.; Sokol, K.; Fletcher, D. S.; Hutchinson, N.; Chen, H.; Mudgett, J. S. *Cell* **1995**, *81*, 641.
6. Wei, X.-Q.; Charles, I. G.; Smith, A.; Ure, J.; Feng, G.-j.; Huang, F.-P.; Xu, D.; Muller, W.; Moncada, S.; Liew, F. Y. *Nature* **1995**, *375*, 408.
7. Huang, P. L.; Huang, Z.; Mashimo, H.; Bloch, K. D.; Moskowitz, M. A.; Bevan, J. A.; Fishman, M. C. *Nature* **1995**, *377*, 239.
8. Nelson, R. J.; Demas, G. E.; Huang, P. L.; Fishman, M. C.; Dawson, V. L.; Dawson, T. M.; Snyder, S. H. *Nature* **1995**, *378*, 383.
9. Faraci, W. S.; Nagel, A. A.; Verdries, K. A.; Vincent, L. A.; Xu, H.; Nichols, L. E.; Labasi, J. M.; Salter, E. D.; Pettipher, E. R. *Br. J. Pharmacol.* **1996**, *119*, 1101.
10. Pettipher, E. R.; Hibbs, T. A.; Smith, M. A.; Griffiths, R. J. *Inflamm. Res.* **1997**, *46*, S135.
11. Leffler, M. T. In *Organic Reactions*; Adams, R., Ed.; J. Wiley and Sons: NY, 1942; Vol. 1, pp 91–104.
12. Tomcufcik, A. S.; Starker, L. N. In *The Chemistry of Heterocyclic Compounds, Pyridine and Its Derivatives, Part 3*; Klingsberg, E., Ed.; Interscience: NY, 1962; pp 1–177.
13. Scriven, E. F. V. In *Comprehensive Heterocyclic Chemistry*; Vol. 2, Part 2A, Boulton, A. J., McKillop, A., Eds.; Pergamon: Oxford, 1984; pp 165–314.
14. The experimental details for the preparation of each individual compound have been reported: Esser, C.; Hagmann, W.; Hoffman, W.; Shah, S.; Wong, K.; Chabin, R.; Guthikonda, R.; MacCoss, M.; Caldwell, C.; Durette, P. US Patent 5,972,975, 1999; *Chem. Abstr.* **1997**, *125*, 142569.
15. Wachi, K.; Terada, A. *Chem. Pharm. Bull.* **1980**, *28*, 465.
16. Kaiser, E. M.; Bartling, G. J.; Thomas, W. R.; Nichols, S. B.; Nash, D. R. *J. Org. Chem.* **1973**, *38*, 71.
17. Levine, R.; Dimmig, D. A.; Kadunce, W. M. *J. Org. Chem.* **1974**, *39*, 3834.
18. Bruekelman, S. P.; Leach, S. E.; Meakins, G. D.; Tirel, M. D. *J. Chem. Soc. Perkin Trans. 1,* **1984**, 2801.
19. Webber, R. K.; Metz, S.; Moore, W. M.; Connor, J. R.; Currie, M. G.; Fok, K. F.; Hagen, T. J.; Hansen, D. W.; Jerome, G. M.; Manning, P. T.; Pitzele, B. S.; Toth, M. V.; Trivedi, M.; Zupec, M. E.; Tjoeng, F. S. *J. Med. Chem.* **1998**, *41*, 96.
20. Schoepke, H. G.; Shideman, F. E. *J. Pharmacol. Exper. Ther.* **1961**, *133*, 171.