Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1023–1026

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

3,4-Dihydro-1-isoquinolinamines: A Novel Class of Nitric Oxide Synthase Inhibitors with a Range of Isoform Selectivity and Potency

Haydn Beaton,[a] Peter Hamley,[a,*] David J. Nicholls,[b] Alan C. Tinker[a,*] and Alan V. Wallace[b]

[a]*Department of Medicinal Chemistry, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, LE11 5RH, UK*
[b]*Department of Bioscience, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, LE11 5RH, UK*

Received 19 December 2000; revised 5 February 2001; accepted 16 February 2001

Abstract—3-Phenyl-3,4-dihydro-1-isoquinolinamine is a weak inhibitor of iNOS and nNOS. Structural variation of **5a** results in inhibitors with a range of potency and selectivity for the NOS enzymes, including a potent and very selective iNOS inhibitor **5j**.

The nitric oxide synthases (NOS) are a family of haem-containing oxygenases which convert the natural amino-acid L-arginine into nitric oxide.[1] Three enzyme isoforms have been characterised. Two of these are constitutive and calcium dependent: neuronal NOS (nNOS or NOS-I) found in nervous tissue and endothelial NOS (eNOS or NOS-III) found in the vascular endothelium. eNOS has a pivotal role in maintaining vascular homeostasis. The third enzyme isoform, inducible NOS (iNOS or NOS-II) is synthesised de novo in response to various pathological challenges. It is not calcium-dependent and produces nitric oxide at much higher, cytotoxic concentrations. Overexpression of iNOS has been implicated in a number of inflammatory disorders,[2] in particular rheumatoid arthritis and septic shock. Overstimulation of nNOS (by excessive calcium concentrations) is thought to contribute to neurological diseases, for example, stroke.[3]

Many inhibitors of NOS have been described in the literature. The best known are analogues of the substrate L-arginine such as *N*-monomethyl arginine (L-NMMA, **1**), *N*-nitroarginine (L-NA, **2a**) and its methyl ester (L-NAME, **2b**) and *N*-iminoethyllysine (L-NIL, **3**).[4] However, their utility is limited by low potency and undesirable cardiovascular side effects arising from inhibition of eNOS. Aminoguanidine (AA, **4**)[5] is a frequently used inhibitor that is not particularly selective. More structurally diverse inhibitors including guanidine derivatives,[6] isothioureas[7] and amidines[8] have also been described but so far there have been few reports of selective and potent iNOS inhibitors.[8b]

We have discovered that the 3,4-dihydro-1-isoquinolinamine derivative **5a** is an inhibitor of the human iNOS and nNOS isoforms of modest potency (IC_{50} 9 and 12 μM, respectively) but some selectivity against eNOS. The aromatised analogue of **5**, 3-phenyl-1-isoquinolinamine, has no significant inhibitory activity against any of the isoforms. This paper describes the effects of structural variation in this novel class of compounds which produces inhibitors showing a wide range of potency and isoform selectivity, including compound **5j**, which is one of the most selective iNOS inhibitors reported to date.

Chemistry

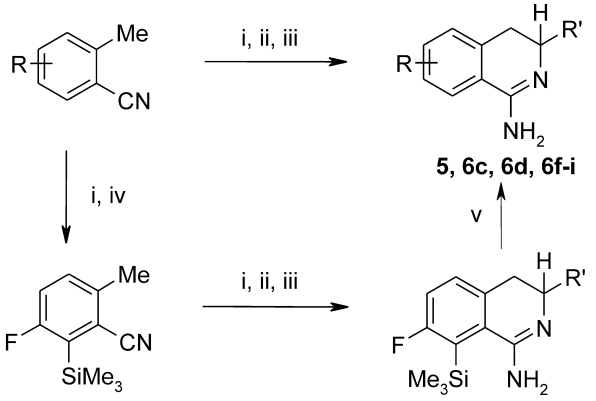
Most compounds were made by a new approach that we have recently described[9] based on the cycloaddition reaction between *o*-tolunitrile derivatives and silyl-aldimines (Scheme 1). This affords amino dihydro-isoquinolines directly in moderate to good yields. In the case of 5-fluoro-2-methylbenzonitrile, deprotonation occurred preferentially at the 6-position. This problem was overcome by addition of one equivalent of trimethylsilyl

*Corresponding author. Tel.: +44-509-644541; fax: +44-509-645507; e-mail: peter.hamley@astrazeneca.com; alan.tinker@astrazeneca.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00119-6

chloride to the reaction mixture to quench the unwanted anion, then adding a second equivalent of base to generate the required lithiated species. The silyl group is removed quantitatively after cyclisation by fluoride treatment.

A few simple 3,4-dihydroisoquinolinamines (i.e., substituted by H or methyl at position 3) were prepared by reaction of a phenethyl halide derivative with ethyl thiocyanate and a Lewis acid[10] to give a cyclic thiomidate which in turn was reacted with ammonia to give isoquinolinamines **6b** and **6e**. The benzazepine homologue **6j** could be prepared similarly from 3-bromo-1-phenylpropane (Scheme 2).



Scheme 1. (i) LDA, DMPU, THF, −78 °C; (ii) R′CH=NSiMe$_3$ (prepared from R′CHO and LiHMDS, THF, 0 °C); (iii) HCl; (iv) Me$_3$SiCl; (v) TBAF, THF.

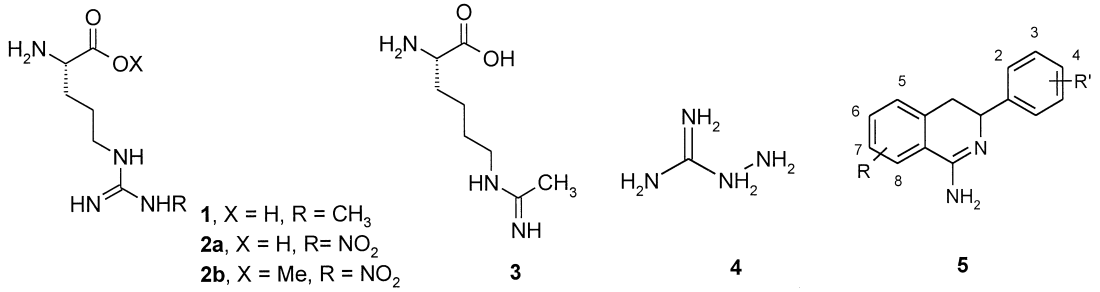
Biology

Compounds were assayed for their ability to inhibit the conversion of [3H]-L-Arg to [3H]-L-citrulline catalysed by: (i) iNOS from human DLD-1 cells; (ii) nNOS from rat cerebellum; and (iii) eNOS frum HUVEC cells using minor modifications[11] of methods already described.[12,13]

Results and Discussion

The effects of substitution of the 3-phenyl compound **5a** were first investigated. Surprisingly, substitution at any of the unsubstituted aryl positions on either the isoquinoline ring, the phenyl substituent, or the amidine group by even a single methyl group led to loss of activity against iNOS relative to the parent **5a** (data not shown). This suggests that **5a** is close to the maximum size acceptable for binding to the enzyme active site for this series: indeed only fluorine was tolerated in some positions (Table 1). Whilst fluorine substitution *meta* or *para* in the phenyl substituent or at positions 5 or 6 in the isoquinoline ring can be made with little or no effect on either the potency or selectivity profile of the compounds, dramatic effects occur on substitution at the isoquinoline 7 or 8 position. While the former is almost inactive, the latter results in a 34-fold increase in iNOS activity without significantly affecting the activity against the other isoforms (cf. compound **5j** vs **5d**). Thus, compound **5j** has good potency combined with 100-fold selectivity for iNOS against nNOS and about 1000-fold

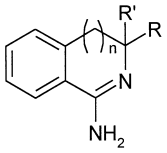
Table 1. Inhibition of NOS isoforms by 3-phenylisoquinolinamines



	R	R′	iNOS IC_{50} (μM)	nNOS IC_{50} (μM)	eNOS IC_{50} (μM)
5a	H	H	9	12	n.s.@100[a]
5b	H	2-F	33	13	44
5c	H	3-F	9	10	n.s.@100[a]
5d	H	4-F	5.5	9	40
5e	H	4-Cl	46%@100[a]	30	86
5f	8-F	H	0.55	2	80
5g	5-F	4-F	4.7	5	44
5h	6-F	4-F	9	20	100
5i	7-F	4-F	43%@100[a]	44	55
5j	8-F	4-F	0.16	16	n.s.@100[a]
5k	8-Cl	H	6	25%@100[a]	n.s.@100[a]
5l	8-Cl	4-F	5	100	39%@100[a]
	Standards :				
1	L-NMMA		0.3	0.1	0.3
2b	L-NAME		14	0.15	2.7
3	L-NIL		0.13	1.6	2.4
4	AG		4.5	6	72

[a]Percent inhibition at test concentration n.s. = no significant effect (<25% inhibition).

Table 2. Inhibition of NOS isoforms by alkyl and heteroaryl substituted isoquinolinamines



	R	R′	n	iNOS IC_{50} (μM)	nNOS IC_{50} (μM)	eNOS IC_{50} (μM)
6a	H	H	1	32	0.4	2
6b	CH_3	H	1	18	3	4
6c	C≡CH	H	1	2.7	0.4	0.9
6d	*c*-Pr	H	1	1.3	1.5	3.6
6e	CH_3	CH_3	1	n.s@100[a]	10	n.s.@100[a]
6f	2-Thienyl	H	1	2.7	2	8
6g	2-Furanyl	H	1	1.2	0.9	5
6h	2-Thiazolyl	H	1	4.2	8	19
6i	2-Imidazolyl	H	1	47%@100[a]	n.s.@100[a]	n.s.@100[a]
6j	H	H	2	n.s.@100[a]	2	12

[a]See footnote to Table 1.

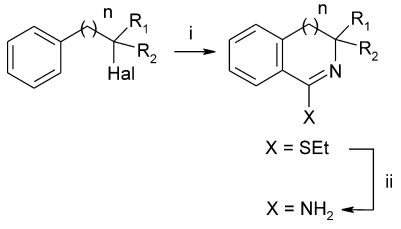
selectivity against eNOS. The suspicion that compound **5a** represented a close to maximum size fit for the active site of NOS led us to examine the preparation of smaller analogues (Table 2).

The 3-unsubstituted compound **6a** has a reversed selectivity compared to that of **5** resulting from a reduction in iNOS potency combined with an increase in activity against nNOS and eNOS. Small alkyl substituents or five-membered heterocycles at position 3 give compounds with little selectivity. Disubstitution at the 3-position as in **6e** and increase in amidine ring size to seven (**6j**) reduces potency.

Some of the more potent and selective compounds were examined for their ability to inhibit nitric oxide synthesis in intact DLD-1 cells (Table 3). Although the absolute value of the IC_{50} in these experiments is much higher than in the enzyme assays (largely due to the much higher arginine concentration of ~1 mM in the cells) the compounds show a similar rank order of potency. Several show potency in the cell comparable or superior to that of the standard inhibitors such as L-NMMA **1**, L-NAME **2b** or aminoguanidine **4**.

Table 3. Inhibitory activity of selected compounds in intact DLD-1 cells

Compound	IC_{50} (mM)	Compound	IC_{50} (mM)
1 (L-NMMA)	170	**5j**	83
2b (L-NAME)	~300	**5l**	350
4 (AG)	1100	**6d**	300
5	470	**6h**	880
5f	130		



Scheme 2. (i) EtSCN, $SnCl_4$; (ii) R_3NH_2.

In conclusion, we have described a series of dihydroisoquinolines which led to the discovery of a highly selective iNOS inhibitor. The levels of selectivity achieved demonstrate that good isoform selectivity is indeed an achievable goal and points the way to compounds which could not only more closely define the role of inducible nitric oxide synthase, but also offer a novel therapy for inflammatory diseases.

References and Notes

1. (a) For reviews see: Kerwin, J. F., Jr.; Lancaster, J. R.; Feldman, P. L., Jr. *J. Med. Chem.* **1995**, *38*, 4343. (b) Boughton-Smith, N. K.; Tinker, A. C. *IDrugs* **1998**, *1*, 321.
2. (a) Connor, J. R.; Manning, P. T.; Settle, S. L.; Moore, W. M.; Jerome, G. M.; Webber, R. K.; Tjoeng, F. S.; Currie, M. G. *Eur. J. Pharmacol.* **1995**, *273*, 15. (b) Salvemini, D.; Wang, Z.-Q.; Wyatt, P. S.; Bourdon, D. M.; Marino, M. H.; Manning, P. T.; Currie, M. G. *Br. J. Pharmacol.* **1996**, *118*, 829.
3. Eliasson, M. J. L.; Huang, Z.; Ferrante, R. J.; Sasamata, M.; Molliver, M. E.; Snyder, S. H.; Moskowitz, M. A. *J. Neurosci.* **1999**, *19*, 5910.
4. Moore, W. M.; Webber, R. K.; Jerome, G. M.; Tjoeng, F. S.; Misko, T. P.; Currie, M. G. *J. Med. Chem.* **1994**, *37*, 3886.
5. (a) Wolff, D. J.; Lubeskie, A. *Arch. Biochem. Biophys.* **1995**, *316*, 290. (b) Misko, T. P.; Moore, W. M.; Kasten, T. P.; Nickols, G. A.; Corbett, J. A.; Tilton, R. G.; McDaniel, M. L.; Williamson, J. R.; Currie, M. G. *Eur. J. Pharmacol.* **1993**, *233*, 119.
6. (a) Hamley, P.; Tinker, A. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1573. (b) Lee, Y.; Marletta, M. A.; Martasek, P.; Roman, L. J.; Masters, B. S. S.; Silverman, R. B. *Bioorg. Med. Chem.* **1999**, *7*, 1097.

7. Shearer, B.; Lee, S.; Oplinger, J. A.; Frick, L. W.; Garvey, E. P.; Furfine, E. S. *J. Med. Chem.* **1997**, *40*, 1901.
8. (a) Webber, R. K.; Metz, S.; Moore, W. M.; Connor, J. R.; Currie, M. G.; Fok, K. F.; Hagen, T. J.; Hansen, D. W., Jr.; 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. (b) Hagen, T. J.; Bergmanis, A. A.; Kramer, S. W.; Fok, K. F.; Schmelzer, A. E.; Pitzele, B. S.; Swenton, L.; Jerome, G. M.; Kornmeier, C. M.; Moore, W. M.; Branson, L. F.; Connor, J. R.; Manning, P. T.; Currie, M. G.; Hallinan, E. A. *J. Med. Chem.* **1998**, *41*, 3675.
9. Beaton, H.; Hamley, P.; Tinker, A. C. *Tetrahedron Lett.* **1998**, *39*, 1227.
10. (a) Gittos, M. W.; Robinson, M. R.; Verge, J. P.; Davies, R. V.; Iddon, B.; Suschitzky, H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 33. (b) Gomez-Parra, V.; Gracian, D.; Madronero, R. *An. Quim.* **1974**, *70*, 980.
11. Macdonald, J.; Gentile, R. J.; Murray, R. J. Int. Patent Appl. WO 94/21621, 1994.
12. (a) Bredt, D. S.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 682. (b) Forstermann, U.; Schmidt, H. H. H. W.; Kohlhaas, K. L.; Murad, F. *Eur. J. Parmacol.* **1992**, *225*, 161.
13. The initial arginine concentration used (3 μM) was chosen as being equal to or lower than measured values of K_m (arginine), which varied from 3 to 15 μM depending on isoform. Assays were performed at 22 °C for 10 min (Type I and III) and 1 h (Type II). Results quoted are a mean of at least two determinations.