Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3679–3680

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Time-Dependence and Preliminary SAR Studies in Inhibition of Nitric Oxide Synthase Isoforms by Homologues of Thiocitrulline

Claire L. M. Goodyer,[a] Edwin C. Chinje,[b] Mohammed Jaffar,[b] Ian J. Stratford[b] and Michael D. Threadgill[a,*]

[a]*Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[b]*School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

Received 20 May 2003; revised 1 August 2003; accepted 11 August 2003

Abstract—Treatment of N^{α}-Cbz-N^{ε}-(2-hydroxyethylaminothiocarbonyl)-L-lysine *N*-(2-hydroxyethyl)amide with boiling hydrochloric acid gave N^{ε}-(4,5-dihydrothiazol-2-yl)-L-lysine. This was a weak and non-isoform selective inhibitor of NOS, whereas N^{ε}-aminothiocarbonyl-L-lysine and its methyl ester were potent, with $IC_{50} = 13$ and 18 μM, respectively, against human iNOS and $IC_{50} = 3$ and 8 μM, respectively, against rat nNOS. Time dependence was observed for inhibition of nNOS by the ester.

Nitric oxide (NO·) is biosynthesised from L-arginine in two steps catalysed by nitric oxide synthases (NOSs). There are three isoforms, endothelial NOS (eNOS) and neuronal NOS (nNOS) (constitutive Ca^{2+}-dependent forms) and an inducible form (iNOS). Selective inhibition of each of these has potential applications in therapy several diseases, including cancer.[1–3] Early NOS inhibitors included the arginine analogues N^{ω}-monomethyl-L-arginine (L-NMMA), N^{δ}-(iminoethyl)-L-ornithine (L-NIO), N^{ω}-nitro-L-arginine (L-NNA) and its methyl ester (L-NAME). These bind to the guanidinium region of the arginine-binding site[4] and are competitive reversible inhibitors, which show potency but little isoform selectivity.[5] We reported[3] that N^{δ}-(4,5-dihydrothiazol-2-yl)-L-ornithine **2** was a potent inhibitor of rat iNOS and rat nNOS. This is a close analogue of the known inhibitor L-thiocitrulline **1**, which is also relatively non-isoform-selective (Fig. 1).[1]

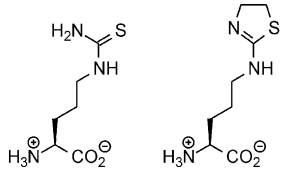


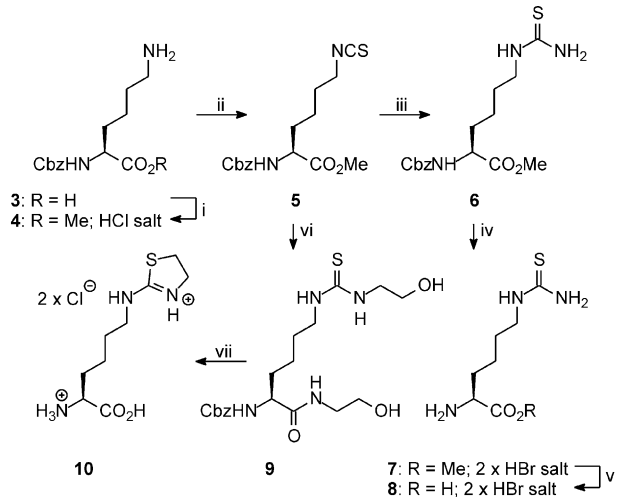
Figure 1. Structures of NOS inhibitors L-thiocitrulline 1 and N^{δ}-(4,5-dihydrothiazol-2-yl)-L-lysine **2**.

Scheme 1 shows the syntheses of the target lysine derivatives. CbzLysOMe **4** was converted to isothiocyanate **5** with thiophosgene. Addition of ammonia then gave the thiourea **6** in high yield. Debenzylation (HBr/AcOH) removed the Cbz to give compound **7**.[6] The ester was hydrolysed with aqueous hydrobromic acid, yielding ε-aminothiocarbonyl-L-lysine **8**.[7] This sequence gave both **8** and its ester **7** for evaluation, by analogy with L-NNA and L-NAME. Reaction of **5** with 2-aminoethanol in boiling acetone not only formed the required *N*′-(2-hydroxyethyl)thiourea but also displaced the ester OMe group, giving **9**. Boiling with hydrochloric acid not only effected cyclisation to the 4,5-dihydrothiazole but also hydrolysed the α-amide and Cbz protection, giving N^{ε}-(4,5-dihydrothiazol-2-yl)-L-lysine **10**.[8]

Compounds **7**, **8** and **10** were evaluated for inhibition of rat brain nNOS and of human iNOS (hiNOS).[9] L-NMMA (an amino-acid inhibitor) and 7-nitroindazole (7-NI; a non-amino acid inhibitor)[10] were used for comparison. The assay used the conversion of L-[U-^{14}C]-arginine to L-[U-^{14}C]-citrulline.[11] Compounds were initially evaluated at 100 μM. Incubation of the inhibitors with the enzyme for various periods before addition of L-[U-^{14}C]-Arg to start the reaction revealed that the inhibition of nNOS by **7** increased markedly

*Corresponding author. Tel.: +44-1225-386840; Fax: +44-1225-386114; e-mail: m.d.threadgill@bath.ac.uk

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.018



Scheme 1. Synthesis of lysine-derived thioureas **7**, **8** and 4,5-dihydrothiazole **10**. Reagents and conditions: (i) MeOH, $SOCl_2$, 4 days, 65%; (ii) $CSCl_2$, $CaCO_3$, H_2O, $CHCl_3$, 16 h, 52%; (iii) NH_3, CH_2Cl_2, 4 h, 59%; (iv) HBr, HOAc, 15 h, 100%; (v) aq HBr (48%), 16 h, 100%; (vi) $H_2N(CH_2)_2OH$, acetone, reflux, 4 h, 53%; (viii) aq HCl (6 M), reflux, 36 h, 76%.

Table 1. Time-dependence of inhibition of NOS isoform activity by known inhibitors L-NMMA, 7-nitroindazole (7NI) and by **7**, **8**

Compd	Isoform	Percentage inhibition[a] of NOS activity by compounds (100 μM) with pre-incubation (t min)[b]				
		$t=0$	$t=5$	$t=10$	$t=15$	$t=20$
L-NMMA	nNOS	94±1	99±2	96±1	98±1	97±2
7-NI	nNOS	59±1		74±3	75±1	74±1
	hiNOS	77±2	61±5	68±2	58±2	56±2
7	nNOS[c]	14±1	83±11	90±2	86±1	85±1
	hiNOS[d]	77±5	79±1	67±1	73±2	70±3
8	nNOS[c]	97±4	97±3	98±5	97±1	97±1
	hiNOS[d]	97±2	97±1	97±3	97±2	97±3

[a]Values are means of three experiments ± standard deviation.
[b]Pre-incubation of the inhibitor with the enzyme preparation, prior to initiating NOS activity by addition of L-[^{14}C]-arginine.
[c]Rat brain nNOS.
[d]Recombinant human iNOS.

Table 2. IC_{50} values for inhibition of NOS isoforms (μM)[a]

Compd	iNOS[b]	hiNOS[c]	nNOS[d]
1		<5	17
2	8.1[e]		1.3
7		13	3
8		18	8
10		>100	>100

[a]IC_{50} values were measured using a 15-min pre-incubation of the inhibitor with the enzyme.
[b]Rat iNOS.
[c]Recombinant human iNOS.
[d]Rat brain nNOS.
[e]Data taken from ref 2. IC_{50} measured without pre-incubation.

with increasing pre-incubation time (Table 1) but this effect was not shown for hiNOS. No time-dependence was seen for inhibition of either isoform by the amino-acids L-NMMA or **8**. Owing to the potential for time-dependence, IC_{50} values were determined for **7**, **8** and **10** against both isoforms with 15-min pre-incubation (Table 2).

None of the compounds shows useful isoform selectivity. The potency of **1** is retained in the higher homologue **7** but insertion of an additional CH_2 into the linker between the amino-acid and the dihydrothiazole decreased potency markedly (**10** vs **2**). 1400 W (*N*-3-aminomethylbenzyl)acetamidine) is a slow-binding inhibitor of rat iNOS, taking 10 min of pre-incubation with the enzyme to exert full inhibition.[12] However, slow binding to nNOS has not been reported; homothiocitrulline ester **7** is the first compound for which this has been observed. The ^{1}H NMR spectrum of a solution of **7** in D_2O was unchanged after 40 min; thus, ester hydrolysis is highly unlikely under the conditions of the enzyme inhibition assay. Such slow binding may reflect the need for **7** to adjust its conformation during the binding process; the origin of this effect will be the subject of future study.

Acknowledgements

We thank AICR for financial support.

References and Notes

1. Marletta, M. A. *J. Med. Chem.* **1994**, *37*, 1899.
2. Schmidt, H. H. H. W.; Walter, U. *Cell* **1994**, *78*, 919.
3. Ulhaq, S.; Chinje, E. C.; Naylor, M. A.; Jaffar, M.; Stratford, I. J.; Threadgill, M. D. *Bioorg. Med. Chem.* **1999**, *7*, 1787.
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. Ogden, J. E.; Moore, P. K. *Trends Biotech.* **1995**, *13*, 70.
6. Data for **7**: IR ν_{max} 1240, 1742, 3030 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.43–1.90 (6H, m, β,γ,δ-H_6), 3.67 (3H, s, CH_3), 3.29 (2H, t, $J=7.8$ Hz, Lys ε-H_2), 4.01 (1H, m, α-H), 7.52 (1H, br NH), 8.34 (1H, s, NH); MS m/z 220.1119 (M+H) ($C_8H_{18}N_3O_2S$ requires 220.1120).
7. Data for **8**: IR ν_{max} 1194, 1739, 3429 cm^{-1}; NMR ($CDCl_3$) δ_H 1.5–2.0 (6H, m, β,γ,δ-H_6), 2.96 (2H, t, $J=7.8$ Hz, ε-H_2), 4.01 (1H, t, $J=6.6$ Hz, α-H); NMR (CD_3OD) δ_C 22.3, 27.4, 30.1, 43.9, 52.7, 128.8, 170.4; MS m/z 206.0956 (M+H) ($C_7H_{16}N_3O_2S$ requires 206.0963).
8. Data for **10**: IR ν_{max} 1651, 1750, 3413 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.44 (6H, m, β,γ,δ-H_6), 2.80 (2H, m, ε-H_2), 3.35 (2H, m, thiazole 5-H_2), 3.49 (1H, m, α-H), 3.58 (2H, m, thiazole 4-H_2), 8.10 (3H, br, N^+H_3) 8.50 (2H, br, 2×NH); NMR ($(CD_3)_2SO$) δ_C 21.6, 29.5, 30.9, 38.6, 44.8, 51.8, 57.6, 169.6, 171.0; MS m/z 232.1115 (M+H) ($C_9H_{18}N_3O_2S$ requires 232.1120).
9. Goodyer, C. L. M.; Chinje, E. C.; Jaffar, M.; Stratford, I. J.; Threadgill, M. D. *Bioorg. Med. Chem.* **2003**, *11*, 4189.
10. Wolff, D. J.; Gribbin, B. J. *Arch. Biochem. Biophys.* **1994**, *311*, 300.
11. Ulhaq, S.; Chinje, E. C.; Naylor, M. A.; Jaffar, M.; Stratford, I. J.; Threadgill, M. D. *Bioorg. Med. Chem.* **1998**, *6*, 2139.
12. Garvey, E. P.; Oplinger, J. A.; Furfine, E. S.; Kiff, R. J.; Laszlo, F.; Whittle, B. J. R.; Knowles, R. G. *J. Biol. Chem.* **1997**, *272*, 4959.