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Heterocyclic Analogues of L-Citrulline as Inhibitors of the Isoforms of Nitric Oxide Synthase (NOS) and Identification of N^{δ}-(4,5-Dihydrothiazol-2-yl)ornithine as a Potent Inhibitor

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Abstract—L-Thiocitrulline is a known potent inhibitor of several isoforms of nitric oxide synthase (NOS). To explore the structure–activity relationships (SARs) for this molecule in more depth than has previously been reported, three analogues substituted at the sulphur of the isothioureas have been synthesised. In two of these, the *S*-substituent was 'tied back' sterically by cyclisation to the nitrogen remote from the amino-acid unit. N^{δ}-(4,5-Dihydrothiazol-2-yl)ornithine was identified as an inhibitor of rat inducible and constitutive isoforms of NOS and of a constitutive NOS derived from a human tumour xenograft. Analogous N^{δ}-(thiazol-2-yl)ornithines were less active, whereas the corresponding N^{δ}-(oxazol-2-yl)ornithine and N^{δ}-(pyrimidin-2-yl)ornithine failed completely to inhibit NOS. A new efficient preparation of the critical synthetic intermediate, N^{α}-Boc-thiocitrulline *t*-butyl ester, has been developed. Further exploration of the SAR with 2-amino-5-(heterocyclylthio)pentanoic acids (synthesised from 2-(Boc-amino)-5-bromopentanoic acid *t*-butyl ester), with *N*-(4-aminobutyl)thiourea and with 2-(4-aminobutylamino)-4,5-dihydrothiazole enabled refinement of our previous model for binding of the substrate, L-arginine, and the inhibitors to NOS.

Introduction

Nitric oxide (•NO) is the smallest known messenger molecule in biological systems and inter alia is responsible for maintaining cardiovascular homeostasis.[1] It is biosynthesised from L-arginine **1** by the various isoforms of nitric oxide synthase (NOS), yielding L-citrulline **3** as a co-product. As shown in Scheme 1, the process comprises two separate mono-oxygenation steps and involves N^{G}-hydroxyarginine **2** as an intermediate. Both steps require molecular oxygen (O_2) and NADPH. There are two main groups of isoforms of NOS, the constitutive Ca^{2+}/calmodulin-dependent types (cNOS) and an inducible Ca^{2+}/calmodulin-independent form (iNOS). The cNOS types can be further divided[2] into the neuronal form (nNOS) and the endothelial form (eNOS). Several known inhibitors of the isoforms of the enzyme are analogues of the substrate **1** or of the co-product **3** (Scheme 1); they include N^{G}-monomethyl-L-arginine (NMMA, **4**),[3] N^{G}-nitro-L-arginine (NOARG, **5a**)[3,4] and its methyl ester (NAME, **5b**), N^{δ}-iminomethyl-L-ornithine (NIO, **6**) and L-thiocitrulline **7**. The K_i values reported for these inhibitors are comparable with the K_m for the substrate **1**.[5]

NOS inhibitors that have particular tissue or isozyme specificities open up a variety of therapeutic possibilities.[6] For example, *N*-(3-(aminomethyl)benzyl)acetamidine is a highly selective inhibitor of murine iNOS.[7,8] Recently, NOS inhibitors have been used selectively to modulate tumour blood flow, oxygenation and redox status.[9–11] In our previous paper,[12] we reported our identification of *S*-2-amino-5-(imidazol-1-yl)pentanoic acid **8** as an inhibitor of two rat and one human isoforms of NOS, and our approaches to designing prodrugs which would be bioreduced selectively to inhibitors in hypoxic tumour tissue. In the

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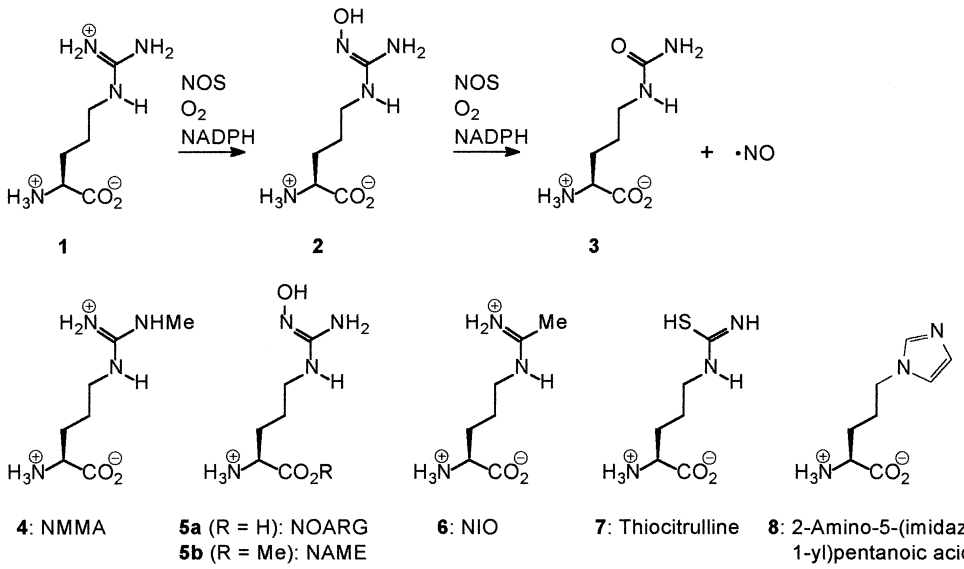


Scheme 1. Steps in the NOS-catalysed oxidation of L-arginine **1** to L-citrulline **3**, via N^G-hydroxyarginine **3** and structures of known inhibitors **4**–**8** of NOS.

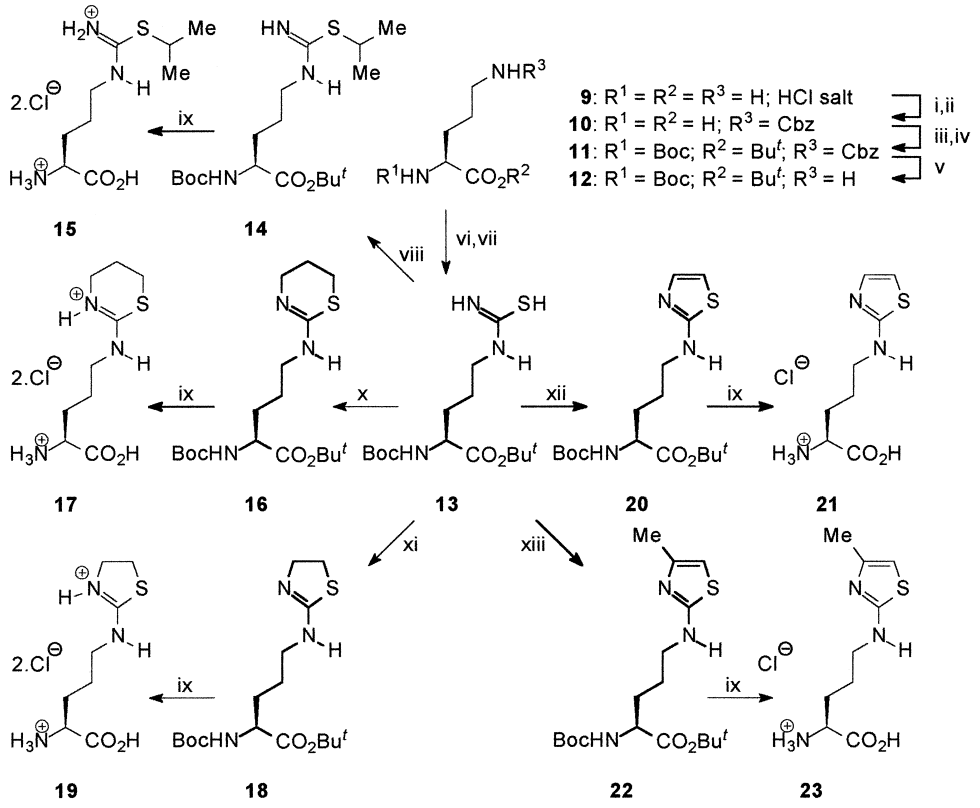
present paper, we report our findings on the development of new, more potent inhibitors, the contribution of these findings to the establishment of models for binding of amino-acid inhibitors to NOS, and our identification of a new strong inhibitor of the isoforms of the enzyme.

Chemical Synthesis

S-2-Amino-5-(imidazol-1-yl)pentanoic acid **8**[12] and thiocitrulline **7** are inhibitors of NOS that may be considered to bind to the substrate (arginine) binding site through the α-amino acid zwitterion moiety, presenting the imidazole 3-*N* and the sulphur of the (iso)thiourea as strong ligands for the haem iron. The approach adopted in the present study was to investigate the effects on NOS inhibitory activity of incorporating the (iso)thiourea unit of **7** into aromatic and partly saturated heterocycles and to test the requirement for the CH_2NH group, which may form a hydrogen bond in the active site of the enzymes and thus contribute to binding. The common synthetic intermediate for the target azathiaheterocycles **17**, **19**, **21** and **23** and for the *S*-alkylisothiourea **15** was the protected thiocitrulline **13** (Scheme 2). Ornithine **9** was protected at the δ-amine with Cbz, selectivity being achieved by temporary complexation of the carboxylate and the α-amine with Cu^{2+}, in a modification of the method employed by us[13] and by Yajima et al.[14] for the selective ε-acylation of lysine; Clarke and Waight[15] have prepared Orn(Cbz)OH by this method. Boc and *t*-butyl ester protection were then introduced at the exposed amine and carboxylic acid of Orn(Cbz)OH **10**, giving BocOrn(Cbz)OBu^t **11**. Hydrogenolysis exposed the ε-amine in **12**. The thiourea unit in **13** was introduced in very high yield in two steps, formation of the isothiocyanate with thiophosgene and reaction with ammonia; the intermediate was not isolated. An alternative sequence, reaction with benzoyl isothiocyanate and hydrolysis of the benzoyl group from the intermediate *N*-benzoylthiourea, gave poor yields (< 25%) of **13**.

The simple *S*-alkyl thiocitrulline derivative **15** was prepared efficiently by alkylation of the thiourea **13** at sulphur with 2-iodopropane under basic conditions, followed by acidolytic cleavage of the Boc and Bu^t ester protecting groups of the intermediate **14**. The first example in which the isothiourea is incorporated into a heterocycle is the dihydrothiazine **17**. Synthesis of this target molecule was achieved by alkylation of **13** at sulphur with 1,3-dibromopropane, with cyclisation being completed in one pot through intramolecular alkylation at the less sterically hindered nitrogen. Deprotection of the intermediate **16** gave **17** in 17% overall yield from **13**. The five-membered ring analogue **19** was prepared, via **18**, in slightly better yield (23%), using 1,2-dibromoethane as the bifunctional electrophile. For comparison of biological activity of aromatic with non-aromatic heterocycles, the thiazole-amino-acids **21** and **23** were prepared. Hantzsch condensations of the protected thiocitrulline **13** with chloroacetaldehyde and with chloroacetone under the standard neutral conditions gave the thiazole **20** and the 4-methylthiazole **22**, respectively, in satisfactory yields. The usual acidolytic deprotection then provided the target thiazolyl amino acids **21** and **23**.

Each of the target amino acids **15**, **17**, **19**, **21** and **23** shown in Scheme 2 contains the sulphur atom and both of the nitrogen atoms of the thiourea present in the lead inhibitor thiocitrulline **7**. To test the requirement for these particular atoms for NOS inhibitory activity, amino acids analogous to the active (dihydro)thiazoles **19** and **21** and to the dihydrothiazine **17** were synthesised, in which one or more of these atoms have been replaced by an alternative heteroatom. 2-Substituted 4,5-dihydrooxazoles are usually prepared by cyclisation of *N*-(2-hydroxyethyl)amides with thionyl chloride or similar dehydrating agents[16] but 2-alkylamino-4,5-dihydrooxazoles are reported relatively infrequently in the

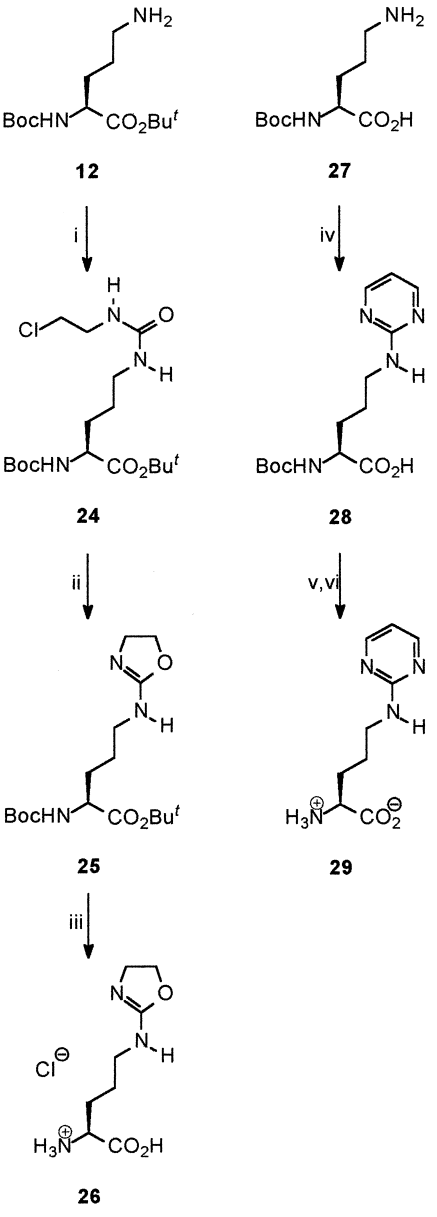


Scheme 2. Synthesis of *S*-isopropylthiocitrulline **15**, the potent inhibitor **19** and related compounds from the critical intermediate N^{α}-Boc-thiocitrulline *t*-butyl ester **13**. Reagents: (i) $CuCO_3$, BnOCOCl, Na_2CO_3; (ii) EDTA Na_2^+ salt; (iii) $Me_2C{=}CH_2$, concd H_2SO_4, 1,4-dioxane; (iv) Boc_2O, Et_3N, H_2O; (v) H_2, Pd/C, EtOH; (vi) $CSCl_2$, $CaCO_3$, $CHCl_3$; (vii) NH_3, MeOH; (viii) Me_2CHI, Et_3N, MeCN; (ix) HCl, CH_2Cl_2; (x) $Br(CH_2)_3Br$, KOBu^t, THF; (xi) $Br(CH_2)_2Br$, K_2CO_3, THF, Δ; (xii) $ClCH_2Cl$, THF; (xiii) $ClCH_2COMe$, THF.

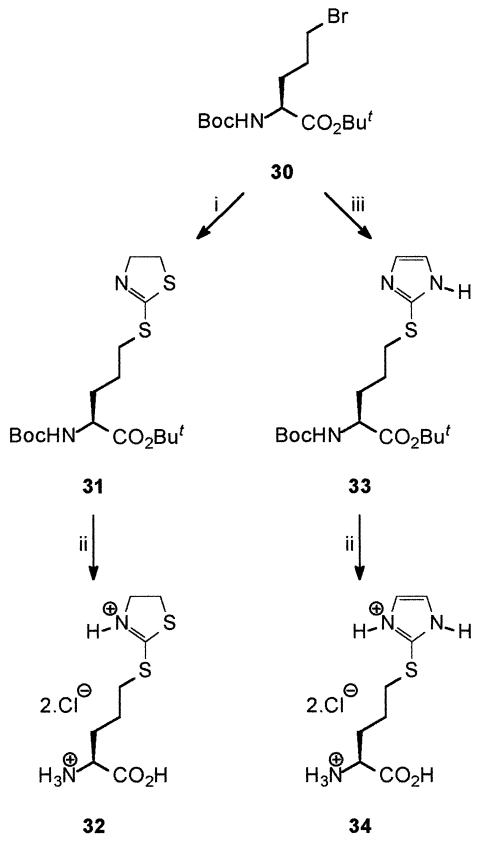
literature.[17,18] As shown in Scheme 3, BocOrnOBu^t **12** was carbamoylated efficiently by 2-chloroethyl isocyanate to give the urea **24**. Cyclisation was effected by the method developed by Wong et al.[19] using potassium fluoride on alumina as the base, to give the 4,5-dihydrooxazole **24** in high yield, as shown in Scheme 3. Acidolytic deprotection gave the dihydrooxazolyl amino acid **26**. The analogous pyrimidine **29** (Scheme 3) was synthesised from BocOrnOH **27**,[20] protection of the acid function being unnecessary in this case during the S_NAr reaction with 2-chloropyrimidine. Removal of the Boc group of **28** was followed by neutralisation during chromatography to afford **29**.

Target compounds **32** and **34** (Scheme 4) lack the potential hydrogen-bond donor N–H at the position corresponding to the $CH_2N\boldsymbol{H}$ of arginine, the substrate of NOS. Thus synthesis and evaluation of these compounds will test the contribution of this hydrogen bond to the binding of the more potent inhibitors and may contribute to understanding the mode of binding of the natural substrate. The synthetic sequence to both targets was different to that used for the other heterocyclic amino acids above, in that the (protected) amino acid unit was introduced as an electrophile, the 5-bromopentanoate ester **30**.[12,21,22] Treatment of **30** with thiazole-2-thiolate and with the anion derived from imidazole-2-thione/2-mercaptoimidazole lead to the formation of the *S*-alkylated products **31** and **33** in moderate and excellent yields, respectively. Acidolytic deprotection removed the Boc and Bu^t ester groups to afford the targets **32** and **34** as their dihydrochloride salts.

Scheme 5 shows the synthetic approaches to the target compounds **39** and **41**, which represent the lead inhibitors thiocitrulline **7** and the 4,5-dihydrothiazolyl amino acid **19**, respectively, but which lack the carboxylic acid moiety. These were designed to test the requirement for electrostatic or hydrogen-bonding interactions between the α-carboxylate of the inhibitors and the enzyme. Such compounds, if potent in their NOS-inhibitory activity, may be easier to formulate as drugs and may penetrate cell membranes more readily than their zwitterionic parents. Reaction of the mono-Boc protected putrescine **35**[23] with carbon disulphide and methylation without isolation of the intermediate dithiocarbamate salt gave the dithiocarbamate ester **36**. The thiourea **38** was formed in good yield by displacement of methanethiolate by ammonia. Cyclisation to the 4,5-dihydrothiazole **40** with 1,2-dibromoethane was effected under reaction conditions analogous to those used for the protected dihydrothiazolyl amino-acid **18**. Removal of the Boc protection from the aminobutyl chains of **36**, **38** and **40** under the usual conditions furnished the target aminobutyl compounds **37**, **39** and **41**, respectively, as their hydrochloride salts.



Scheme 3. Synthesis of N^{δ}-(4,5-dihydrooxazol-2-yl)ornithine **26** and N^{δ}-(pyrimidin-2-yl)ornithine **29**. Reagents: (i) $Cl(CH_2)_2NCO$, THF; (ii) KF/alumina, MeCN; (iii) HCl, CH_2Cl_2; (iv) 2-chloropyrimidine, Et_3N, MeOH, Δ; (v) HCl, H_2O, EtOAc; (vi) NH_3, H_2O, MeOH.



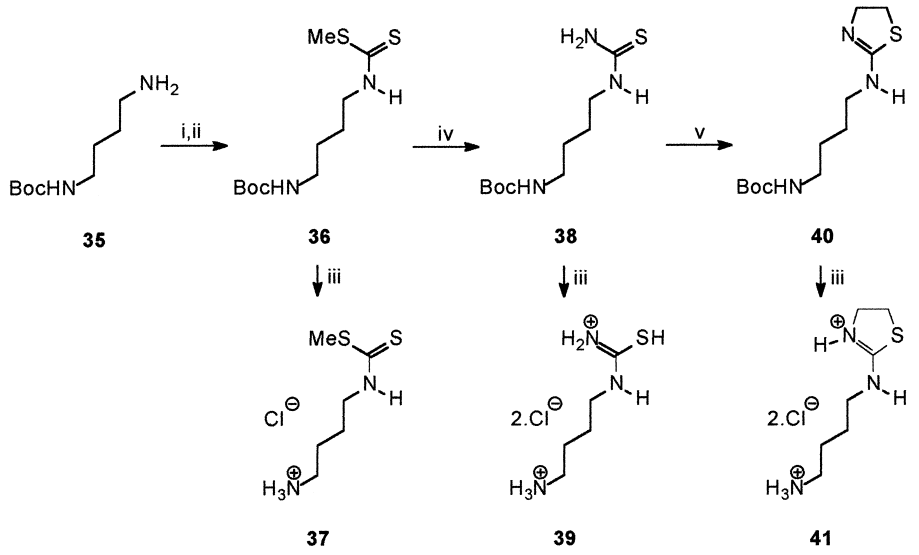
Scheme 4. Synthesis of sulphide-linked analogues **32** and **34**. Reagents: (i) 4,5-dihydrothiazole-2-thiol, $NaHCO_3$, MeOH; (ii) HCl, CH_2Cl_2; (iii) imidazole-2-thiol, $NaHCO_3$, MeOH.

Evaluation as Inhibitors of NOS

All the candidate inhibitors synthesised as described above were evaluated for their inhibitory activity against iNOS and nNOS derived from rat lung and from rat brain, respectively. As described in the Experimental section, the assay was based on the conversion of [^{14}C]-**1** to [^{14}C]-**3**.

As an initial screen, most compounds were tested at 1.0 mM against the two isoforms of the rat enzyme; the results are shown in Table 1. The initial screen was conducted at 2.0 mM for **29** and at 100 μM for **37**, **39** and **41**. IC_{50} values were also obtained for selected compounds. Taking thiocitrulline **7** as the lead compound, the effect of introducing a bulky alkyl substituent at sulphur was tested with the *S*-isopropyl isothiourea **15**. This compound retained significant activity but was ca. tenfold less potent than **7**. In the corresponding dihydrothiazine **17**, the conformation of the *S*-substituent is constrained by linkage back to the nitrogen of the isothiourea; the inhibitory activity is maintained, relative to **15**. However, imposition of further conformational constraint by incorporating the isothiourea into the 5-membered dihydrothiazole ring of **19** led to a threefold increase in potency, as judged by IC_{50} against the two rat enzymes (against rat nNOS: **17**: $IC_{50} = 15\,\mu M$; **19**: $IC_{50} = 4.3\,\mu M$). However, when these (constrained) isothioureas were examined for their inhibitory activity using the cNOS of human origin, the dihydrothiazole **19** appeared to be slightly more potent than the lead compound thiocitrulline **7** (**7**: $IC_{50} = 2.0\,\mu M$; **19**: $IC_{50} = 1.3\,\mu M$). As found for the NOS of rodent origin, **15** and **17** were ca. 10-fold less potent than **7** against the human enzyme. Interestingly, the isoform selectivity of **19** is different to that of **7**; whereas **7** is threefold more active against rat iNOS than against rat nNOS, **19** is twofold more active against rat nNOS than against rat iNOS.

With the potent activity of **19** having been established, the structure–activity relationship around the five-membered ring was explored. Conversion of the ring to the aromatic thiazole **21** reduced activity markedly, with $IC_{50} = 200\,\mu M$ against the rat iNOS, and introduction of



Scheme 5. Synthesis of methyl *N*-(4-aminobutyl)dithiocarbamate **37**, *N*-(4-aminobutyl)thiourea **39** and 2-(4-aminobutylamino)-4,5-dihydrothiazole **41**. Reagents: (i) CS_2, Et_3N, THF; (ii) MeI; (iii) HCl, CH_2Cl_2; (iv) NH_3, MeOH; (v) $Br(CH_2)_2Br$, K_2CO_3, THF, Δ.

Table 1. Inhibition of the isoforms of NOS by know inhibitors and by heterocyclic analogues of L-citrulline **3**

Compound	% Inhibition of rat iNOS (initial screen)	% Inhibition of rat nNOS (initial screen)	IC_{50} (-M) versus rat iNOS	IC_{50} (-M) versus rat nNOS	IC_{50} (-M) versus H647 cNOS
5a (NOARG)				3.0 ± 0.2	
5b (NAME)				6.0 ± 0.3	
6 (NIO)				2.3 ± 0.1	
7 (triocitrulline)			1.7 ± 0.1	4.6 ± 0.2	1.9 ± 0.2
8	99 ± 2 (1.0 mM)	100 ± 2 (1.0 mM)	31.6 ± 1.5	18.5 ± 1.3	13.0 ± 0.6
15	96 ± 5 (1.0 mM)	97 ± 3 (1.0 mM)	14.5 ± 0.7		23.5 ± 0.9
17	84 ± 2 (1.0 mM)	86 ± 2 (1.0 mM)	24.5 ± 1.8	13.0 ± 0.5	27.5 ± 1.7
19	100 ± 7 (1.0 mM)	93 ± 1 (1.0 mM)	8.1 ± 2.2	4.3 ± 0.1	1.3 ± 0.1
21	81 ± 4 (1.0 mM)	71 ± 2 (1.0 mM)	199 ± 33		
23	26 ± 4 (1.0 mM)	14 ± 10 (1.0 mM)			
26	18 ± 6 (1.0 mM)	12 ± 3 (1.0 mM)			
29	3 ± 2 (2.0 mM)	8 ± 3 (2.0 mM)			
32	6 ± 6 (1.0 mM)	−2 ± 2 (1.0 mM)			
34	15 ± 1 (1.0 mM)	5 ± 2 (1.0 mM)			
37	5 ± 6 (100 μM)	4 ± 3 (100 μM)			
39	25 ± 5 (100 μM)	13 ± 4 (100 μM)			
41	64 ± 2 (100 μM)	49 ± 1 (100 μM)		100 ± 10	

a methyl substituent on the thiazole virtually abolished activity in **23**. Replacement of the soft ligand sulphur in **19** with oxygen, a potentially harder ligand, in the dihydrooxazole **26** also abolished inhibitory activity. The pyrimidine **29** was also inactive, even at 2.0 mM.

In compounds **32** and **34**, the heterocycle is linked to the amino-acid unit through a lipophilic, non-hydrogen-bonding sulphur, rather than through the potential hydrogen-bond donor N−H so far examined. The thiazolyl sulphide **32** is the strict analogue of the 4,5-dihydrothiazolylamino compound **19**; however, although **19** was a potent inhibitor of the rat enzymes (IC_{50} = 8.1 μM versus rat iNOS and IC_{50} = 4.3 μM versus rat nNOS), the *S*-linked analogue **32** did not inhibit either rat isoform at the standard initial test concentration, 1.0 mM. The corresponding imidazole **34** was also inactive.

Whereas evaluation of the sulphides **32** and **34** tested the requirement for a hydrogen-bonding N−H link between the heterocycle and the amino acid, the importance of the α-carboxylate to binding and inhibition was examined using **37**, **39** and **41**. These aminobutylamino compounds were evaluated at a standard test concentration 100 μM. As expected, the dithiocarbamate **37** was inactive but **39**, the 'decarboxylated analogue' of thiocitrulline **7** showed weak activity, being >100-fold less active than the lead compound. Interestingly, removal of the carboxylate had a lesser deleterious effect on the inhibitory activity in the 4,5-dihydrothiazole series, in that **41** was some 20-fold less active than **19** against the rat isoforms of NOS, as judged by IC_{50} values.

In the present study, the compounds synthesised and evaluated have a wide range of inhibitory potencies

against the isoforms of NOS. Some structure–activity concepts can be gleaned; these can be rationalised in terms of a model for the binding of these agents to the substrate binding site of the enzymes, as shown in Figure 1. In the simpler model we developed in our previous study[12] using 2-amino-5-azolylpentanoic acids, it was evident that ligation of the heterocyclic nitrogen to the haem iron atom was important for binding. We also speculated that there may be binding sites for the anionic α-carboxylate and cationic α-ammonium groups of the amino acid unit, located at appropriate distances from the haem to allow presentation of the heterocycle to the iron. The findings of the present study allow us to present a refined model for the binding of the sulphur-containing inhibitors (Fig. 1). The known inhibitor, thiocitrulline **7**, could bind at the substrate binding site as shown, with electrostatic and/or hydrogen-bonding recognition of the α-ammonium and α-carboxylate functions and ligation of the soft sulphur centre to the haem iron. In addition, it may be speculated that there is a further hydrogen-bond from the isothiourea N−H to residues in the NOS protein, as shown in Figure 1. The most effective inhibitor amongst the 2-amino-5-azolylpentanoic acids, the imidazole **8**, fits this model but lacks the possibility of this hydrogen bond. The *S*-isopropyl group in **15** also fits the model but it is evident that this addition of an alkyl group to the sulphur of thiocitrulline reduces affinity either by sterically obstructing approach of the ligand to the iron atom or by preventing it being negatively charged. Methylation of thiocitrulline at sulphur has been reported[24] to reduce inhibitory activity by ca. 10-fold, which is consistent with our findings. However, when the steric restriction of the approach of the sulphur to the iron is lessened by 'tying back' the *S*-substituent to the nitrogen in the 4,5-dihydrothiazole **19**, the inhibitory activity is restored to the potency of thiocitrulline **7**. Thus we propose that **19** binds as shown in Figure 1 and that the diminution of activity upon alkylation at sulphur in thiocitrulline is a steric effect, rather than prevention of formation of a thiolate anion.

In compound **32**, a sulphide links the dihydrothiazole and the amino-acid units. This link is not capable of being a hydrogen-bond donor and provides a direct test for the putative hydrogen-bond shown for the binding of the substrate L-arginine and the inhibitors **7** and **19**. The inactivity of **32** attests to the importance of this attractive interaction in this sulphur-ligand series. Feldman et al.[25] observed a similar requirement in that L-arginine **1** is the substrate for the isoforms of NOS but the analogue L-indospicine (in which the guanidine is replaced by $CH_2C(=N+H_2)NH_2$) does not bind. In contrast, this hydrogen bond appears to be unnecessary in the strong binding[12] of the imidazole **8** and of 2-amino-5-(5-methyl-2-nitrophenylthio)pentanoic acid.[26] Finally, removal of the anionic α-carboxylate diminishes but does not abolish inhibitory activity. Thus comparison of the interactions of **39** with those of **7** and of the aminobutylaminodihydrothiazole **41** with the potent thiazole-amino acid **19** (Fig. 1) suggest that the binding of this carboxylate anion is not one of the major determinants in recognition and binding of the substrate and the inhibitors by the isoforms of NOS. This observation provides a potential lead for the development of new inhibitors with uncharged side-chains, based on the 1-substituted imidazole ligand **8** and the 2-(substituted amino)-4,5-dihydrothiazole ligand **19**. This refined model is consistent with those previously proposed[26–28] for binding of **1**, of isothiourea NOS inhibitors and of

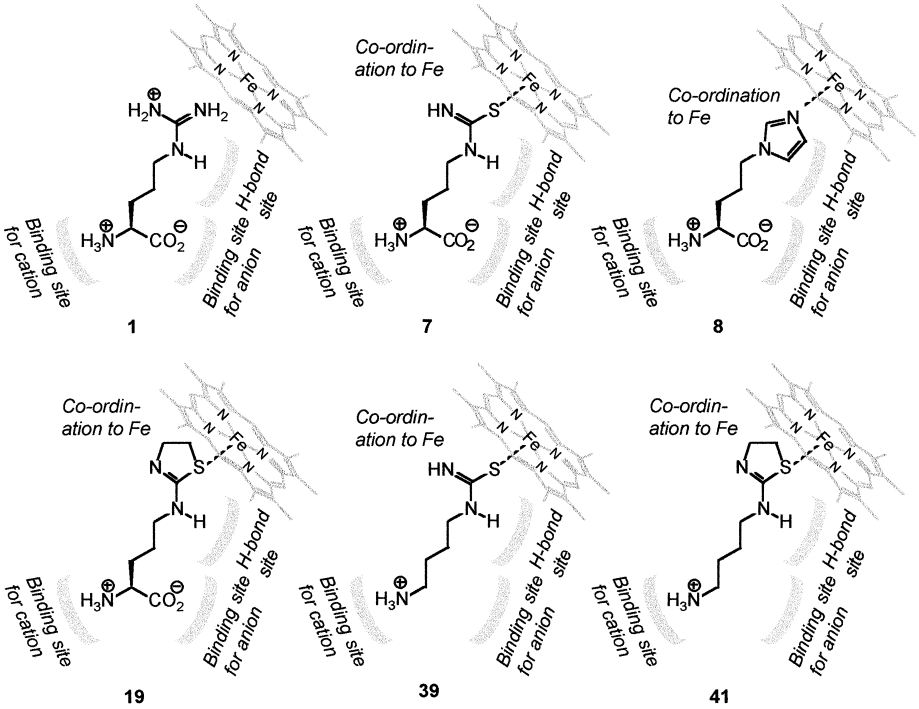


Figure 1. Models for binding of the substrate **1**, the inhibitors thiocitrulline **7**, 2-amino-5-(imidazol-1-yl)pentanoic acid **8**, N^{δ}-(4,5-dihydrothiazol-2-yl)ornithine **19** and the 'decarboxylated' analogues **39** and **41** to the substrate binding site of NOS.

some known amino-acid NOS inhibitors to the substrate binding site.

Conclusion

In this paper, we have described our exploration of the structure–activity relationships for inhibition of the isoforms of NOS, using thiocitrulline **7** as a known lead compound. Synthesis and evaluation of series of N^{δ}-(heterocyclyl)ornithines, 2-amino-5-(heterocyclylthio)-pentanoic acids and analogues lacking the carboxylate have led to identification of N^{δ}-(4,5-dihydrothiazol-2-yl)ornithine **19** as a potent new inhibitor of the isoforms of NOS with potency similar to those of the known inhibitors NOARG **5a**, NAME **5b**, NIO **6** and thiocitrulline **7**. Our enzyme-inhibition data have led us to reinforce and refine our previous model[12] for binding of inhibitors to the L-arginine substrate binding site of the enzymes (Fig. 1). In particular, we have shown that the α-carboxylate anion in the new lead **19** is desirable but not essential for inhibitory activity. The results of our further exploration of the structure–activity relationships in this region of 2-substituted 4,5-dihydrothiazoles (derived from **19**) and 1-substituted imidazoles (derived from **8**) will be reported separately, as will our use of **19** as a 'warhead' delivered by tissue-selective prodrugs.

Experimental

General methods

NMR spectra were recorded on samples in $CDCl_3$, unless otherwise stated. IR spectra were recorded on KBr discs, unless otherwise stated. Mass spectra were obtained by electron-impact (EI), chemical-ionisation (CI) or fast atom bombardment (FAB) techniques in the positive ion mode, unless otherwise stated. The stationary phase for chromatography was silica gel. Melting points are uncorrected. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. All chiral amino acids are of L configuration, unless otherwise stated. The brine was saturated. N^{δ}-Cbz-ornithine **10** was prepared essentially by the method of Clarke and Waight,[15] as modified by us[13] for the synthesis of N^{ε}-Cbz-lysine.

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-(phenylmethoxycarbonyl)-L-ornithine 1,1-dimethylethyl ester (11). Orn(Cbz)OH **10**[15] (54.0 g, 202 mmol) was stirred vigorously with 2-methylpropene (160 mL) and H_2SO_4 (15 mL) in 1,4-dioxan (200 mL) at 0°C for 6 h. The solution was added during 20 min to Et_3N (120 mL) and water (200 mL) at 10°C. Di-*t*-butyl dicarbonate (44.0 g, 202 mmol) was added. The mixture was stirred for 5 h. The evaporation residue, in EtOAc, was washed (aq $KHSO_4$, water, brine) and was dried. Evaporation and chromatography (hexane:ethyl acetate, 2:1) gave **11** (28.0 g, 82%) as a colourless oil, (lit.[29], oil); ^{1}H NMR δ 1.43 (9H, s, Bu^t), 1.46 (9H, s, Bu^t), 1.83–2.60 (4H, m, β, γ-H_4), 3.20 (2H, m, δ-H_2), 4.18 (1H, m, α-H), 4.85 (1H, br, NH), 5.02–5.20 (3H, m, $PhCH_2$ + NH), 7.35 (5H, s, Ph-H_5); ^{13}C NMR δ 27.78, 28.31, 28.49, 30.32, 53.36, 66.44, 79.77, 82.17, 128.42, 128.57, 128.89, 135.83, 155.39, 171.32, 172.67; MS (FAB) *m*/*z* 423 (M + H).

N^{α}-(1,1-Dimethylethoxycarbonyl)ornithine 1,1-dimethylethyl ester (12). Compound **11** (20.3 g, 48 mmol), in EtOH (120 mL), was stirred with H_2 in the presence of Pd/C (10%, 700 mg) for 48 h. Filtration (Celite®), evaporation and chromatography (CH_2Cl_2:MeOH, 19:1) gave **12** (15.3 g, 77%) as an oil, (lit.[29] oil); ^{1}H NMR δ 1.43 (9H, s, Bu^t), 1.46 (9H, s, Bu^t), 1.88–2.32 (4H, m, β,γ-H_2), 4.18 (1H, br, α-H), 4.38 (2H, t, J = 7 Hz, δ-H_2), 5.13 (2H, br, NH_2).

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-aminothiocarbonylornithine 1,1-dimethylethyl ester (13). Thiophosgene (2.07 g, 18 mmol) was stirred vigorously with **12** (2.0 g, 6.6 mmol) and $CaCO_3$ (740 mg, 6.8 mmol) in $CHCl_3$ (45 mL) and water (5 mL) for 16 h. The suspension was filtered. The aqueous phase of the filtrate was separated and was extracted thrice with $CHCl_3$. The combined organic filtrate and extracts were dried and the solvent was evaporated. The residue was taken up in MeOH (30 mL) and cooled to −4°C. NH_3 was passed through the solution for 20 min and the mixture was stirred for 3 h at 0°C. Evaporation and chromatography (EtOAc: hexane, 4:1) gave **13** (1.68 g, 89%) as a white solid: mp 45–47°C (lit.[30] oil); ^{1}H NMR δ 1.44 (9H, s, Bu^t), 1.47 (9H, s, Bu^t), 1.68–1.90 (4H, m, β, γ-H_4), 3.51 (2H, m, δ-H_2), 4.13 (1H, m, α-H), 5.28 (1H, d, J = 7 Hz, α-NH), 6.21 (1H, br, NH), 6.60 (1H, br, NH); ^{13}C NMR δ 26.34, 27.94, 28.30, 32.33, 49.34, 51.53, 77.47, 80.29, 155.45, 166.82, 171.54.

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-(imino(1-methylethylthio)methyl)ornithine 1,1-dimethylethyl ester (14). Compound **13** (500 mg, 1.4 mmol) was stirred with 2-iodopropane (367 mg, 2.2 mmol) and Et_3N (450 mg, 4.4 mmol) in dry MeCN (5 mL) at 50°C for 16 h. Evaporation and chromatography (EtOAc:Me_2CO, 1:1) gave **14** (378 mg, 68%) as a colourless oil. ^{1}H NMR δ 1.25 (6H, d, J = 7 Hz, 2×Me), 1.84 (18H, s, 2×Bu^t), 1.89–2.20 (4H, m, β,γ-H_4), 3.49 (2H, t, J = 7 Hz, δ-H_2), 3.60 (1H, m, C*H*Me_2), 4.23 (1H, *t*, J = 6 Hz, α-H), 5.26 (1H, m, NH); MS (FAB) *m*/*z* 390.2425 (M + H) ($C_{18}H_{36}N_3O_4S$ requires 390.2427).

N^{δ}-(Imino(1-methylethylthio)methyl)ornithine dihydrochloride (15). Compound **14** (250 mg, 640 mmol) was treated with HCl, as for the synthesis of **21** except that the solvent was CH_2Cl_2 and the material was recrystallised (EtOH:CH_2Cl_2), to give **15** (62%) as a highly hygroscopic white solid: ^{1}H NMR δ (D_2O) 1.38 (6H, d, J = 7 Hz, 2 (Me), 1.73–2.05 (4H, m, β,γ-H_4), 3.44 (2H, t, J = 7 Hz, δ-H_2), 3.79 (1H, septet, J = 7 Hz, $CHMe_2$), 4.03 (1H, t, J = 6 Hz, α-H); MS (FAB) *m*/*z* 235.1351 (M + H) ($C_9H_{21}N_3O_2S$ requires 235.1356).

N^{δ}-(4,5-Dihydro-1,3-thiazin-2-yl)-N^{α}-(1,1-dimethylethoxycarbonyl)ornithine 1,1-dimethylethyl ester (16). Compound **13** (440 mg, 1.3 mmol) was stirred with $Br(CH_2)_3Br$ (572 mg, 2.8 mmol) and KOBu^t (140 mg, 1.3 mmol) in THF (3 mL) at 35°C for 16 h. Evaporation and

chromatography (EtOAc→EtOAc:EtOH, 2:1) gave **16** (125 mg, 25%) as a colourless oil: IR (film) ν 3300, 1710, 1650, 1605 cm^{-1}; ^1H NMR δ 1.44 (9H, s, Bu^t), 1.47 (9H, s, Bu^t), 1.70–1.95 (4H, m, β,γ-H_4), 2.20–2.40 (2H, m, thiazine 5-H_2), 3.15–3.25 (2H, m, δ-H_2), 3.60–4.00 (4H, m, thiazine 4,6-H_4), 4.22 (1H, m, α-H), 5.42 (1H, d, J = 7.9 Hz, α-NH), 9.05 (1H, br, δ-NH); ^{13}C NMR δ 26.81, 28.04, 28.99, 30.56, 31.77, 40.80, 43.29, 49.52, 53.66, 82.17, 82.33, 156.02, 164.15, 171.58; MS (EI) m/z 387 (M), 277 (M−Boc).

N^{δ}-(4,5-Dihydro-1,3-thiazin-2-yl)ornithine hydrochloride (17). A solution of **16** was treated with HCl, as for the synthesis of **21**, to give **17** (67%) as a highly hygroscopic white solid: ^1H NMR ($(CD_3)_2SO$) δ 1.62–1.98 (4H, m, β,γ-H_4), 2.02 (2H, quintet, J = 6 Hz, thiazine 5-H_2), 3.18 (2H, t, J = 6 Hz, δ-H_2), 3.40–3.60 (4H, m, thiazine 4,6-H_4), 3.89 (1H, m, α-H), 8.4 (3H, br, N + H_3), 8.6 (1H, br, NH), 9.2 (1H, br, NH); ^{13}C NMR ($(CD_3)_2SO$) δ 21.70, 22.34, 26.68, 49.08, 51.67, 51.93, 62.08, 163.67, 170.80; MS (FAB) m/z 232.1137 (M + H) ($C_9H_{18}N_3O_2S$ requires 232.1120).

N^{δ}-(4,5-Dihydrothiazol-2-yl)-N^{α}-(1,1-dimethylethoxycarbonyl)ornithine 1,1-dimethylethyl ester (18). Compound **13** (500 mg, 1.4 mmol) was heated under reflux with $Br(CH_2)_2Br$ (541 mg, 2.9 mmol) and K_2CO_3 (199 mg, 1.4 mmol) in THF (10 mL) for 16 h. Filtration, evaporation and chromatography (EtOAc:hexane, 1:1→EtOAc:CH_2Cl_2:Et_3N, 10:4:1) gave **18** (200 mg, 35%) as a colourless oil: ^1H NMR δ 1.46 (9H, s, Bu^t), 1.48 (9H, s, Bu^t), 1.65–1.93 (4H, m, β,γ-H_4), 3.14 (2H, t, J = 7 Hz, thiazole 5-H_2), 3.35 (2H, m, δ-H_2), 3.61 (2H, t, J = 7 Hz, thiazole 4-H_2), 4.17 (1H, m, α-H), 5.38 (1H, d, J = 8 Hz, α-NH), 5.79 (1H, br, δ-NH); MS (FAB) m/z 372.1962 (M + H) ($C_{17}H_{30}N_3O_4S$ requires 372.1957).

N^{δ}-(4,5-Dihydrothiazol-2-yl)ornithine dihydrochloride (19). Compound **18** was treated with HCl, as for the synthesis of **21**, to give **19** (67%) as a highly hygroscopic white solid: ^1H NMR (D_2O) δ 1.67–1.86 (4H, m, β,γ-H_4), 3.53–3.65 (2H, m, δ-H_2), 3.24 (2H, t, J = 7 Hz, thiazoline 4-H_2), 3.93–4.07 (1H, m, 2-H), 4.24 (2H, t, J = 7 Hz, thiazoline 5-H_2); ^{13}C NMR (D_2O) δ 26.72, 28.91, 30.32, 31.69, 48.21, 58.34, 167.65, 172.36; MS (FAB) m/z 218.0979 (M + H) ($C_8H_{16}N_3O_2S$ requires 218.0963).

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-(thiazol-2-yl)ornithine 1,1-dimethylethyl ester (20). Compound **13** (350 mg, 1.0 mmol) was stirred with chloroacetaldehyde (100 mg, 1.3 mmol) in THF (5 mL) for 2 h. Evaporation and chromatography (EtOAc:hexane, 1:1) gave **20** (230 mg, 61%) as a white solid: mp 69–71°C; ^1H NMR δ 1.44 (9H, s, Bu^t), 1.45 (9H, s, Bu^t), 1.71–1.91 (4H, m, β,γ-H_4), 3.36 (2H, m, δ-H_2), 4.25 (1H, m, α-H), 5.18 (1H, d, J = 7 Hz, α-NH), 5.72–5.83 (1H, br, δ-NH), 6.48 (1H, d, J = 3.7 Hz, thiazole 4-H), 7.11 (1H, d, J = 3.7 Hz, thiazole 5-H); ^{13}C NMR δ 24.04, 28.00, 28.68, 31.60, 45.47, 53.51, 79.81, 82.15, 106.38, 139.10, 155.47, 170.32, 171.67; MS (FAB) m/z 743 (2M + H), 372.1966 (M + H) ($C_{17}H_{30}N_3O_4S$ requires 372.1957).

N^{δ}-(Thiazol-2-yl)ornithine hydrochloride (21). A solution of **20** (300 mg, 810 μmol) in THF (5 mL) was saturated with HCl at 0°C and was stirred at 20°C for 30 min. The precipitate was collected by filtration under N_2, washed (THF) and dried to give **21** (130 mg, 65%) as a white solid: mp 213–215°C; ^1H NMR (DMSO-d_6) δ 1.87–2.10 (4H, m, β,γ-H_4), 3.49 (2H, t, J = 7.0 Hz, δ-H_2), 4.10 (1H, brt, J = 6 Hz, α-H), 6.90 (1H, d, J = 3 Hz, thiazole 4-H), 7.23 (1H, d, J = 3 Hz, thiazole 3-H); MS (FAB) m/z 216.0805 (M + H) ($C_8H_{14}N_3O_2S$ requires 216.0808).

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-(4-methylthiazol-2-yl)ornithine 1,1-dimethylethyl ester (22). Compound **13** (350 mg, 1.0 mmol) was stirred with chloroacetone (187 mg, 2.0 mmol) in THF (6 mL) for 1 h. Evaporation and chromatography (CH_2Cl_2:hexane, 1:1) gave **22** (230 mg, 61%) as an oil: ^1H NMR δ 1.44 (9H, s, Bu^t), 1.46 (9H, s, Bu^t), 1.75–1.95 (4H, m, β,γ-H_4), 2.20 (3H, s, thiazole-Me), 3.28 (2H, t, J = 6 Hz, δ-H_2), 4.15–4.23 (1H, m, α-H), 5.10 (1H, d, J = 7 Hz, α-NH), 5.19–5.26 (1H, br, δ-NH), 6.02 (1H, s, thiazole 5-H); ^{13}C NMR δ 16.65, 24.78, 28.33, 28.53, 31.91, 46.03, 53.47, 79.81, 82.19, 99.87, 144.32, 155.49, 169.91, 171.56; MS (FAB) m/z 386.2112 (M + H) ($C_{18}H_{32}N_3O_4S$ requires 386.2114).

N^{δ}-(4-Methylthiazol-2-yl)ornithine hydrochloride (23). Compound **22** was treated with HCl, as for the synthesis of **21**, to give **23** (65%) as a white solid: mp 250°C; ^1H NMR (D_2O) δ 1.43–1.82 (4H, m, β,γ-H_4), 2.01 (3H, s, thiazole-Me), 3.32 (2H, t, J = 7 Hz, 5-H_2), 3.65 (1H, t, J = 6 Hz, α-H), 6.07 (1H, s, thiazole 5-H); ^{13}C NMR (D_2O) δ 17.81, 24.78, 29.94, 46.21, 56.46, 102.83, 139.20, 162.30, 172.10; MS (FAB) m/z 230.0962 (M + H) ($C_9H_{16}N_3O_2S$ requires 230.0965).

N^{δ}-(2-Chloroethylaminocarbonyl)-N^{α}-(1,1-dimethylethoxycarbonyl)ornithine 1,1-dimethylethyl ester (24). 2-Chloroethylisocyanate (211 mg, 2.0 mmol) was added during 5 min to **12** (500 mg, 1.7 mmol) in THF (5 mL) at 0°C. The mixture was stirred at 20°C for 16 h. Evaporation and chromatography (EtOAc) gave **24** (530 mg, 78%) as a white solid: mp 103–105°C; ^1H NMR δ 1.44 (9 H, s, Bu^t), 1.46 (9 H, s, Bu^t), 1.62–1.96 (4H, m, β,γ-H_4), 3.21 (2H, t, J = 6.4 Hz, NCH_2), 3.33 (2H, t, J = 6.4 Hz, NCH_2), 3.61 (2H, t, J = 6.4 Hz, CH_2Cl), 4.15 (1H, m, α-H), 4.90 (1H, br, NH), 4.98 (1H, br, NH), 5.18 (1H, d, J = 7 Hz, α-NH); ^{13}C NMR δ 25.82, 28.00, 28.35, 30.60, 39.87, 42.12, 44.88, 53.62, 79.96, 82.19, 155.76, 158.30, 171.82; MS (FAB) m/z 791/789/787 (2M + H), 396/394 (M + H), 358 (M−HCl), 340/338 (M−$Me_2C{=}CH_2$), 296/294 (M−Boc).

N^{δ}-(4,5-Dihydrooxazol-2-yl)-N^{α}-(1,1-dimethylethoxycarbonyl)ornithine 1,1-dimethylethyl ester (25). Compound **24** (516 mg, 3.6 mmol) was heated under reflux with KF on alumina (40%, 516 mg, 3.6 mmol) in MeCN (10 mL) for 16 h. The mixture was cooled and filtered (Celite®). Evaporation and chromatography (EtOAc:hexane:Et_3N, 20:40:3) gave **25** (390 mg, 83%) as a colourless oil: ^1H NMR δ 1.45 (9H, s, Bu^t), 1.46 (9H, s, Bu^t), 1.55–1.90 (4H, m, β,γ-H_4), 3.20 (2H, m, δ-H_2), 3.75 (2H, t, J = 7 Hz, oxazole 4-H_2), 4.17 (1H, m, α-H),

4.25 (2H, t, $J=7.1$ Hz, oxazole 5-H_2), 4.43 (1H, m, δ-NH), 5.32 (1H, d, $J=7.9$ Hz, α-NH); ^{13}C NMR δ 25.68, 28.32, 28.90, 30.34, 42.63, 52.45, 53.69, 67.85, 79.57, 81.84, 155.50, 161.47, 171.85; MS (FAB) *m/z* 358 (M + H).

N^{δ}-(4,5-Dihydrooxazol-2-yl)ornithine hydrochloride (26). Compound **25** was treated with HCl, as for the synthesis of **21** except that the solvent was CH_2Cl_2, to give **26** (67%) as a highly hygroscopic white solid: ^{1}H NMR (D_2O) δ 1.62–1.98 (4H, m, β,γ-H_4), 3.58 (2H, m, 5-H_2), 3.75 (2H, t, $J=7$ Hz, oxazole 4-H_2), 4.00 (1H, m, α-H), 4.23 (2H, t, $J=7$ Hz, oxazole 5-H_2); ^{13}C NMR (D_2O) 24.98, 27.69, 29.25, 34.15, 54.62, 66.23, 158.23, 175.85; MS (FAB) *m/z* 202.1185 (M + H) ($C_8H_{16}N_3O_3$ requires 202.1193).

N^{α}-(1,1-Dimethylethoxycarbonyl)-N^{δ}-(pyrimidin-2-yl)-ornithine (28). BocOrnOH **27**[20] (2.00 g, 8.6 mmol) was heated under reflux with 2-chloropyrimidine (500 mg, 4.3 mmol) and Et_3N (430 mg, 4.2 mmol) in MeOH (60 mL) for 3 days. Evaporation and chromatography (acetone:MeOH, 1:1) gave **28** (347 mg, 26%) as a white solid: mp 164–166°C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.35 (9H, s, Bu^t), 1.87 (4H, m, 3,4-H_4), 3.35 (2H, m, 5-H_2), 4.35 (1H, m, 2-H), 7.03 (1H, t, $J=7.9$ Hz, pyrimidine 5-H), 8.30 (2 H, d, $J=7.9$ Hz, pyrimidine 4,6-H_2); MS (FAB) *m/z* 312 (M + H).

N^{δ}-(Pyrimidin-2-yl)ornithine (29). Compound **28** (710 mg, 2.2 mmol) was stirred with hydrochloric acid (4 M, 15 mL) and EtOAc (25 mL) for 4 h. Evaporation and chromatography (MeOH:35% aq NH_3, 49:1) gave **29** (300 mg, 63%) as a white hygroscopic solid: mp 195–197°C; ^{1}H NMR (D_2O) δ 1.75–2.00 (4H, m, 3,4-H_4), 3.04 (2H, t, $J=7.1$ Hz, 5-H_2), 4.27 (1H, m, 2-H), 6.77 (1H, t, $J=5.0$ Hz, pyrimidine 5-H), 8.32 (2H, d, $J=5.0$ Hz, pyrimidine 4,6-H_2); MS (FAB) *m/z* 211.1183 (M + H) ($C_9H_{15}N_4O_2$ requires 211.1195).

1,1-Dimethylethyl *S*-5-(4,5-dihydrothiazol-2-ylthio)-2-(1,1-dimethylethoxycarbonylamino)pentanoate (31). 4,5-Dihydrothiazole-2-thiol (500 mg, 4.3 mmol) was stirred with $NaHCO_3$ (354 mg, 4.3 mmol) in MeOH (10 mL) for 30 min. The solvent was evaporated. The residual Na salt was suspended in dry EtOH (5 mL) and was cooled to 0°C. Compound **30**[12] (400 mg, 1.3 mmol) was added and the mixture was stirred at 20°C for 6 h. Water (10 mL) was added and the mixture was extracted with EtOAc (3×10 mL). Washing (water, brine), drying, evaporation and chromatography (EtOAc:hexane, 1:2) gave **31** (230 mg, 44%) as a colourless oil: ^{1}H NMR δ 1.44 (9H, s, Bu^t), 1.46 (9H, s, Bu^t), 1.65–1.96 (4H, m, 3,4-H_4), 3.11 (1H, dt, $J=11$, 7 Hz, 5-H), 3.14 (1H, dt, $J=11$, 7 Hz, 5-H), 3.40 (2H, t, $J=8.0$ Hz, thiazole 5-H_2), 4.20 (1H, m, 2-H), 4.22 (2H, t, $J=8.0$ Hz, thiazole 4-H_2), 5.31 (1H, d, $J=8$ Hz, α-NH); *m/z* (FAB) 390 (M + H).

***S*-2-Amino-5-(4,5-dihydrothiazol-2-ylthio)pentanoic acid dihydrochloride (32).** Compound **31** was treated with HCl, as for the synthesis of **21**, to give **32** (63%) as a white solid: mp 160–162°C; Found: C, 28.70; H, 5.30; N, 8.13. $C_8H_{14}N_2O_2S_2.2HCl.2H_2O$ requires C, 28.75; H, 5.73; N, 8.38%; ^{1}H NMR ($(CD_3)_2SO$) δ 1.70–1.91 (4H, m, 3,4-H_4), 3.13–3.20 (2H, m, 5-H_2), 3.17 (2H, t, $J=7$ Hz, thiazole 4-H_2), 3.95 (1H, m, 2-H), 4.16 (2H, t, $J=7$ Hz, thiazole 5-H_2), 8.42–8.60 (3H, br, N + H_3). ^{13}C NMR ($(CD_3)_2SO$) δ 25.02, 28.98, 31.86, 31.97, 51.50, 62.10, 168.78, 170.83; MS (FAB) *m/z* 235 (M + H).

1,1-Dimethylethyl *S*-2-(1,1-dimethylethoxycarbonylamino)-5-(imidazol-2-ylthio)pentanoate (33). Imidazole-2-thiol (492 mg, 4.9 mmol) was stirred with $NaHCO_3$ (412 mg, 4.9 mmol) in MeOH (10 mL) for 30 min. The solvent was evaporated. The residual Na salt was suspended in dry EtOH (5 mL) and was cooled to 0°C. Compound **30**[12] (750 mg, 2.1 mmol) was added and the mixture was stirred at 20°C for 6 h. Water (10 mL) was added and the mixture was extracted with EtOAc (3×10 mL). Washing (water, brine), drying, evaporation and chromatography (EtOAc) gave **33** (700 mg, 89%) as a colourless oil: ^{1}H NMR δ 1.44 (9H, s, Bu^t), 1.45 (9H, s, Bu^t), 1.61–2.00 (4H, m, 3,4-H_4), 3.09 (2H, m, 5-H_2), 4.15 (1H, m, 2-H), 5.25 (1H, br, NH), 7.20 (2H, s, imidazole 4,5-H_2); MS (FAB) *m/z* 372 (M + H).

***S*-2-Amino-5-(imidazol-2-ylthio)pentanoic acid dihydrochloride (34).** Compound **33** was treated with HCl, as for the synthesis of **21**, to give **34** (63%) as a highly hygroscopic white solid: ^{1}H NMR (D_2O) δ 1.40–1.58 (2H, m, 4-H_2), 1.72–1.88 (2H, m, 3-H_2), 2.83 (2H, t, $J=7$ Hz, 5-H_2), 3.55 (1H, t, $J=6$ Hz, 2-H), 7.01 (2H, s, imidazole 4,5-H_2); ^{13}C NMR (D_2O) δ 25.87, 29.84, 34.70, 55.18, 124.81, 140.15, 175.30; MS (FAB) *m/z* 216.0820 (M + H) ($C_8H_{14}N_3O_2S$ requires 216.0867).

Methyl *N*-(4-(1,1-dimethylethoxycarbonylamino)butyl)-dithiocarbamate (36). CS_2 (1.37 g, 18 mmol) was added to **35**[23] (1.7 g, 9 mmol) in THF (10 mL) at 0°C, followed by Et_3N (900 mg, 9 mmol). The mixture was stirred at 0°C for 2 h. MeI (1.3 g, 9 mmol) was added and the reaction was stirred at 0°C for 10 min and at 20°C for 16 h. Filtration, evaporation and chromatography (CH_2Cl_2:EtOAc, 1:1) gave **36** (2.0 g, 80%) as colourless oil: Found: C, 47.70; H, 7.93; N, 9.95. $C_{11}H_{22}N_2O_2S_2$ requires C, 47.45; H, 7.96; N, 10.05%; ^{1}H NMR δ 1.94 (9H, s, Bu^t), 1.52–176 (4H, m, 2,3-H_4), 2.62 (3H, s, SMe), 3.15 (2H, m, 1-H_2), 3.55 (2H, m, 4-H_2), 4.96 (1H, br, NH), 8.15 (1H, br, NH); ^{13}C NMR δ 25.56, 27.31, 28.12, 39.67, 46.59, 78.95, 132.45, 156.06; MS (CI) *m/z* 279 (M + H).

Methyl *N*-(4-(aminobutyl)dithiocarbamate hydrochloride (37). Compound **36** was treated with HCl, as for the synthesis of **21**, to give **37** (71%) as a white solid: mp 119–121°C; ^{1}H NMR (D_2O) δ 1.62–1.80 (4H, m, 2,3-H_4), 2.58 (3H, s, SMe), 3.04 (2H, t, $J=6$ Hz, 4-H_2), 3.78 (2H, t, $J=6$ Hz, 1-H_2); MS (FAB) *m/z* 179.0696 (M + H) ($C_6H_{15}N_2S_2$ requires 179.0677).

1,1-Dimethylethoxycarbonyl *N*-(4-(thioureido)butyl)-carbamate (38). NH_3 was passed through **36** (780 mg, 2.8 mmol) in MeOH (10 mL) at 0°C for 10 min. The solution was stirred at 0°C for 2 h and at 20°C for 16 h. Evaporation and chromatography (CH_2Cl_2:EtOAc:Et_2O, 1:2:2) gave **38** (350 mg, 51%) as a white solid: mp

157–159°C; ^{1}H NMR δ 1.43 (9H, s, Bu^t), 1.60–1.71 (4H, m, 2,3-H_4), 3.20 (2H, m, 4-H_2), 3.52 (2H, m, 1-H_2), 4.93 (1H, br, NH), 6.51 (1H, br, SH), 7.07 (1H, br, NH); ^{13}C NMR δ 27.81, 28.39, 29.63, 43.35, 45.05, 79.71, 128.77, 156.77; MS (CI) *m*/*z* 248 (M + H).

***N*-(4-Aminobutyl)thiourea dihydrochloride (39).** Compound **38** was treated with HCl, as for the synthesis of **21**, to give **39** (47%) as a highly hygroscopic white solid: ^{1}H NMR ($(CD_3)_2SO$) δ 1.49 (2H, quintet, J = 6.6 Hz, 2-H_2), 1.72 (2H, quintet, J = 6.5 Hz, 3-H_2), 2.78 (2H, m, 1-H_2), 3.36 (2H, t, J = 6.5 Hz, 4-H_2), 4.18 (1H, br, NH), 8.00 (1H, br, NH), 8.07 (3H, s, N + H_3), 9.50 (1H, br) and 10.05 (1H, br) (NH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 29.06, 29.81, 45.85, 45.97, 176.37; MS (FAB) *m*/*z* 148.0904 (M + H) ($C_5H_{14}N_3S$ requires 148.0908).

4,5-Dihydro-2-(4-(1,1-dimethylethoxycarbonylamino)butylamino)thiazole (40). $Br(CH_2)_2Br$ (1.28 g, 6.8 mmol) was boiled under reflux with **38** (500 mg, 2.0 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in THF (10 mL) for 16 h. Filtration, evaporation and chromatography (EtOAc: hexane, 1:1→EtOAc:CH_2Cl_2:Et_3N, 10:4:1) gave **40** (200 mg, 40%) as a colourless oil: ^{1}H NMR δ 1.50 (9H, s, Bu^t), 1.55–1.90 (4H, m, butyl 2,3-H_2), 3.15 (1H, t, J = 7 Hz, butyl 1-H_2), 3.37 (2H, m, butyl 4-H_2), 3.50 (2H, t, J = 7 Hz, thiazole 5-H_2), 3.99 (2H, t, J = 7 Hz, thiazole 4-H_2), 4.72 (1H, br, NH); ^{13}C NMR δ 27.50, 28.40, 32.30, 41.13, 62.20, 79.19, 156.26, 158.74; MS (CI) *m*/*z* 280 (M + H).

2-(4-Aminobutylamino)-4,5-dihydrothiazole dihydrochloride (41). Compound **40** was treated with HCl, as for the synthesis of **21**, to give **41** as a white solid: mp 162–164°C; ^{1}H NMR ($(CD_3)_2SO$) δ 1.55–1.68 (4H, m, butyl 2,3-H_4), 2.79 (2H, m, butyl 4-H_2), 3.30 (2H, t, J = 6.5 Hz, butyl 1-H_2), 3.56 (2H, t, J = 7 Hz, thiazole 5-H_2), 3.89 (2H, t, J = 7 Hz, thiazole 4-H_2), 8.12 (3H, br, N + H_3), 10.40 (1H, br, NH); ^{13}C NMR ($(CD_3)_2SO$) δ 24.00, 24.76, 30.63, 38.78, 44.31, 48.64, 169.23; MS (FAB) *m*/*z* 159.0943 (M + H) ($C_7H_{15}N_2S$ requires 159.0955).

NOS inhibition studies

Measurements of the inhibitory activity of the test compounds against rat iNOS and rat nNOS and against cNOS derived from H647 human cells were made as described previously.[12] The results are shown in Table 1 as the mean of triplicate experiments ± standard deviation.

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