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# Synthesis of *N*-Benzyl- and *N*-Phenyl-2-amino-4,5-dihydrothiazoles and Thioureas and Evaluation as Modulators of the Isoforms of Nitric Oxide Synthase

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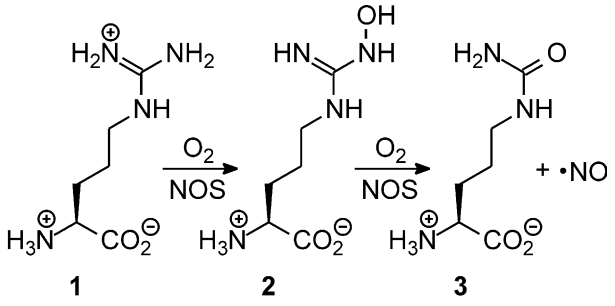
**Abstract**—Inhibition of the isoforms of nitric oxide synthase (NOS) has important applications in therapy of several diseases, including cancer. Using 1400W [*N*-(3-aminomethylbenzyl)acetamidine], thiocitrulline and $N^{\delta}$-(4,5-dihydrothiazol-2-yl)ornithine as lead compounds, series of *N*-benzyl- and *N*-phenyl-2-amino-4,5-dihydrothiazoles and thioureas were designed as inhibitors of NOS. Ring-substituted benzyl and phenyl isothiocyanates were synthesised by condensation of the corresponding amines with thiophosgene and addition of ammonia gave the corresponding thioureas in high yields. The substituted 2-amino-4,5-dihydrothiazoles were approached by two routes. Treatment of simple benzylamines with 2-methylthio-4,5-dihydrothiazole at 180 °C afforded the corresponding 2-benzylamino-4,5-dihydrothiazoles. For less nucleophilic amines and those carrying more thermally labile substituents, the 4,5-dihydrothiazoles were approached by acid-catalysed cyclisation of *N*-(2-hydroxyethyl)thioureas. This cyclisation was shown to proceed by an $S_N2$-like process. Modest inhibitory activity was shown by most of the thioureas and 4,5-dihydrothiazoles, with *N*-(3-aminomethylphenyl)thiourea ($IC_{50} = 13\,\mu M$ vs rat neuronal NOS and $IC_{50} = 23\,\mu M$ vs rat inducible NOS) and 2-(3-aminomethylphenylamino)-4,5-dihydrothiazole ($IC_{50} = 13\,\mu M$ vs rat neuronal NOS and $IC_{50} = 19\,\mu M$ vs human inducible NOS) being the most potent. Several thioureas and 4,5-dihydrothiazoles were found to stimulate the activity of human inducible NOS in a time-dependent manner.


## Introduction

Nitric oxide (•NO) is the smallest known messenger molecule in biological systems. It is synthesised from L-arginine **1** by the various isoforms of nitric oxide synthase (NOS), yielding L-citrulline **3** as a co-product. The process comprises two separate mono-oxygenation steps with $N^{G}$-hydroxyarginine **2** as an intermediate (Scheme 1). Both steps require molecular oxygen ($O_2$) and NADPH. There are two main groups of isoforms of NOS, a constitutive $Ca^{2+}$/ calmodulin-dependent type (cNOS) and an inducible $Ca^{2+}$/ calmodulin-independent form (iNOS). cNOS can be further sub-divided into endothelial and neuronal forms (eNOS and nNOS, respectively). Underactivity and overactivity of each of these isoforms can be associated with disease states.

Excessive NO production by eNOS within blood vessel walls is thought to be the basis for conditions such as septic- and cytokine-induced circulatory shock. In these conditions, the sGc–cGMP pathway is excessively activated, which leads to high levels of NO and so contributes to profound vasodilatation and hypotension.[1]




**Scheme 1.** Conversion of L-arginine **1** to L-citrulline **3** and nitric oxide, via L-$N^{G}$-hydroxyarginine **2**, mediated by nitric oxide synthases.

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However, if too little NO is produced, this can lead to conditions such as high blood pressure, angina and impotence. Recently, it has been shown that a reduction in the activity and synthesis of NO within the endothelium may contribute to the initiation and progression of atherosclerosis.[2] Release of NO from the pelvic nerve neurons located in the human corpus cavernosum is known to cause penile erection. NOS inhibitors have been shown to prevent this action while nitric oxide sources mimic the effect.[3] An overexpression of nNOS in circulating neutrophils has been found in patients with Parkinson's disease[4] and nNOS activity is thought to be linked to migraine headaches; these are believed to result from abnormal activity in large cerebral blood vessels and high levels of nNOS occur in the vasodilator nerves that supply the large cerebral blood vessels.[5] NO production by iNOS is essential for the defence mechanism of an organism; however, NO produced by iNOS has been related to several pathological conditions, including cancer,[6] arthritis[7] and diabetes.[8] Thus selective and potent inhibition of the isoforms of NOS is an important goal in medicinal chemistry.

The structures of several reported inhibitors of NOS are shown in Figure 1. Many of the early inhibitors, for example L-NMMA **4**,[9] are close analogues of the substrate L-arginine **1**. Isosteric replacement of the terminal guanidine of **1** with an acetamidine gives the non-selective L-NIO **5a**.[10] The homologue L-NIL **5b**,[11] however, shows some selectivity for inhibition of iNOS. This selectivity (iNOS vs eNOS) increases when the carboxylate of **5b** is replaced by the diol motif in **6**[12] but iNOS vs nNOS selectivity is poor. In each of these inhibitors, a binding motif can be recognised in which the guanidine/amidine ligates to the haem iron at the active site of the enzyme, while additional binding contacts recognise the amine, the carboxylate and, possibly, the N–H near the haem ligand. In $N^G$-nitroarginine **7**[13] and its esters and dipeptides,[14] which are also NOS inhibitors, the ligand is a nitroguanidine; imidazoles have also been used as ligands for haem-Fe in our non-isoform-selective inhibitor **8**[15] and related compounds.[16] Substitution on the imidazole of **8** and replacement with less electron-rich heterocycles led to weaker inhibition,[15] which is consistent with their weaker potential ligation to iron. More recent highly iNOS-selective inhibitors[17–22] contain cyclic amidines (e.g. **9**[22]) to bind to the iron but lack the amino-acid motif. Most interesting are the *N*-(3-aminomethylbenzyl)acetamidine 1400W **10** (a highly selective inhibitor of rat iNOS)[23] and the lower homologue **11** (a selective inhibitor of nNOS);[24] this pair of compounds illustrates exquisitely the possibility of switching isoform selectivity radically through subtle changes in the spatial relationship between the haem-Fe-binding group and remote functionalities. Sulfur-containing groups have also been used as the ligands for iron. The simplest such compound, thiocitrulline **12** is relatively unselective,[25] as is our potent inhibitor **13**[26] and *N*-aryl-*S*-alkylisothioureas such as **14**.[27] The tetrahydrobiopterin binding site has also received some attention in the development of isoform-selective inhibitors.[28,29] 7-Nitroindazole **15**, which is reported to be competitive with both the substrate **1** and with tetrahydrobiopterin, is claimed[30] to be moderately selective for inhibition of the nNOS isoform.

In the present study, we sought to explore the inhibitory activity of hybrids between the highly selective **10** and **11** and our potent dihydrothiazole **13**. The structures of the designed target compounds are shown in Figure 2. In particular, we sought to explore whether the isoform selectivity shown by **10** and **11** could be translated into analogous compounds carrying different haem-ligating head groups. We report here the synthesis of these series of compounds and their evaluation as inhibitors of nNOS and iNOS. Although Collins et al.[24] note particularly the

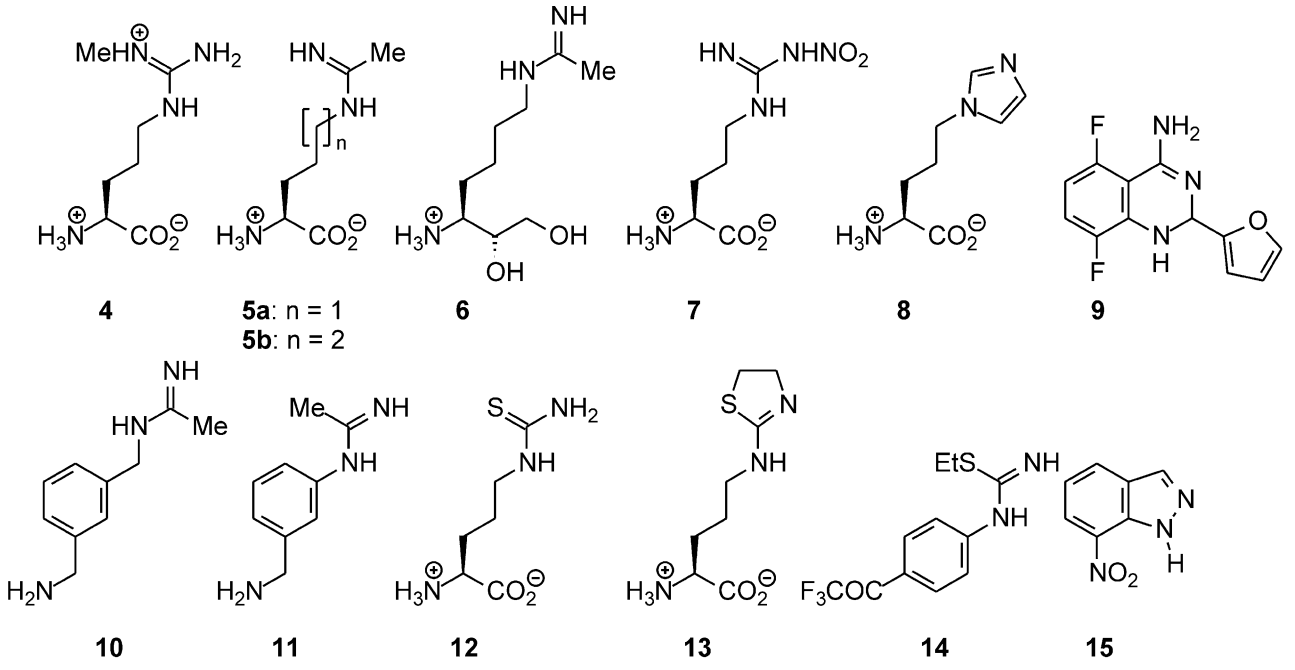



**Figure 1.** Structures of known inhibitors of the isoforms of NOS.

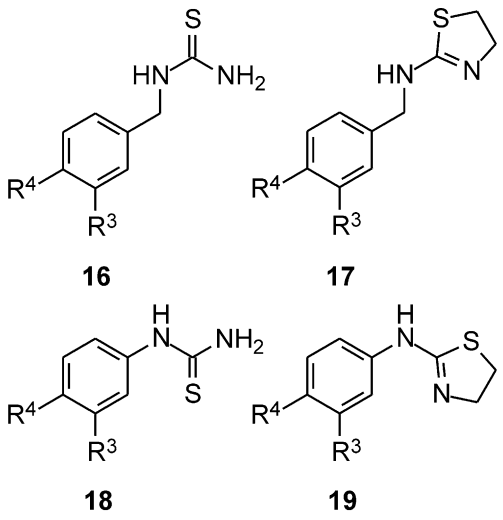



**Figure 2.** Structures of target *N*-benzyl and *N*-phenyl thioureas and 2-amino-4,5-dihydrothiazoles.

importance of the 3-aminomethyl group for binding of **10** and **11** to NOS isoforms, we used a range of different substituents on the benzene ring to test whether this substitution is still optimal when the ligand for the haem iron is sulfur, rather than nitrogen.

## Chemical Synthesis

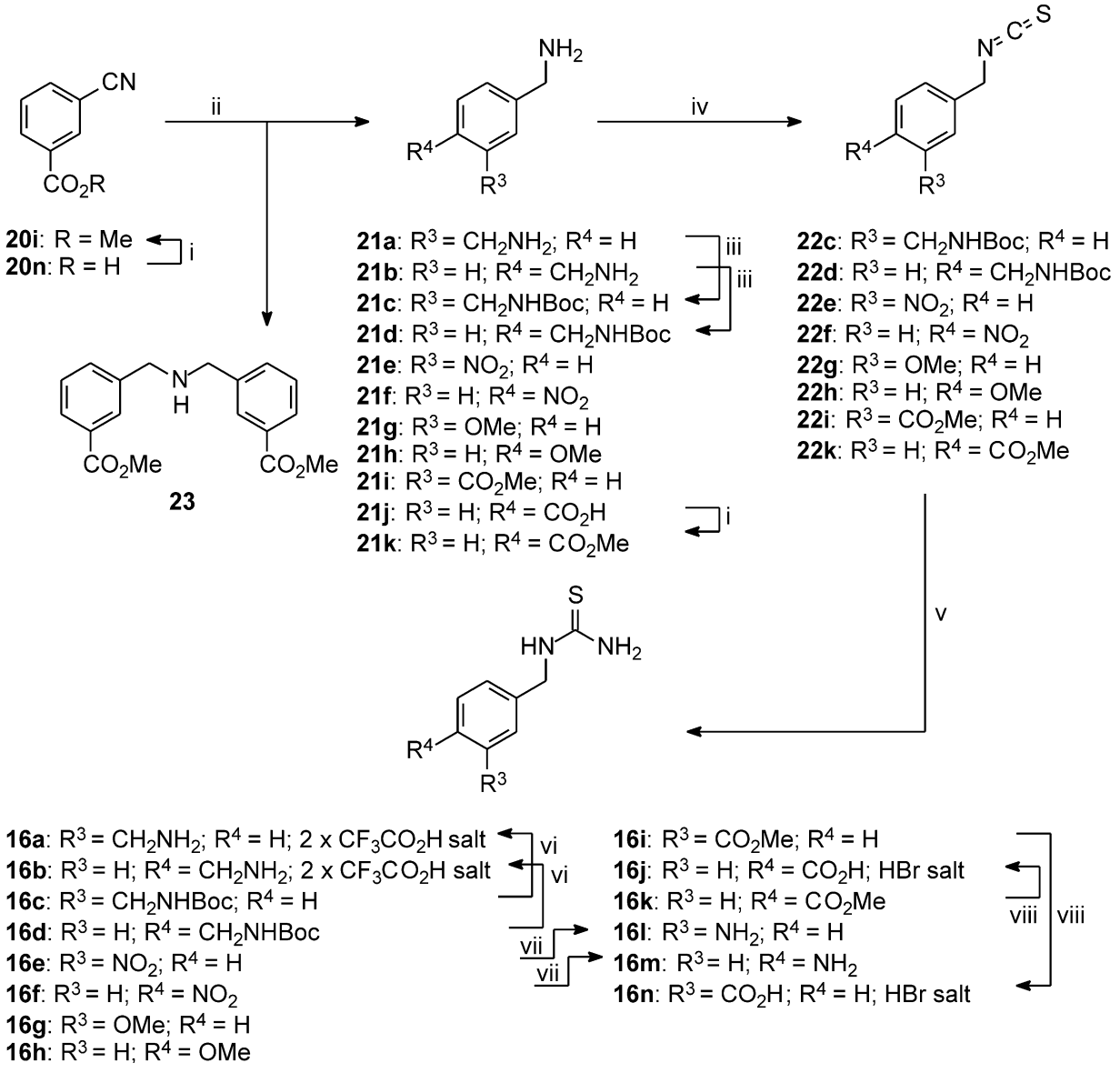
The synthesis of the first series of target compounds, the *N*-benzylthioureas is shown in Scheme 2. The primary strategy was to add ammonia to the electrophilic isothiocyanate unit in the benzylisothiocyanates **22**. A common route was developed to prepare these from the corresponding benzylamines **21**. However, certain additional substituents on the benzyl group required prior protection. 1,3-Bis(aminomethyl)benzene **21a** was treated carefully with a sub-stoichiometric amount of di-*tert*-butyl dicarbonate to give, after a simple aqueous workup to remove excess **21a**, a high yield of the crystalline mono-Boc derivative **21c**. Similar treatment of the analogous *para* diamine **21b** furnished the corresponding mono-Boc derivative **21d**. To protect the carboxylic acid in the 4-aminomethylbenzoic acid **21j**, the methyl ester **21k** was formed in the usual way by reaction with acidic methanol. However, the corresponding 3-aminomethylbenzoic acid is not commercially available and the corresponding methyl ester **21i** had to be synthesised by an alternative route. Using a nitrile as a synthon for the aminomethyl unit, 3-cyanobenzoic acid **20n** was converted to its methyl ester **20i**; the relatively modest yield of 42% may have been due to competing Pinner reaction of the nitrile with the acidic methanol. Hydrogenation of **20i** gave two products, the required simple reduction product **21i** and the secondary amine **23**. The latter is formed by transimination of the intermediate iminomethylbenzene with the primary amine **21i** and subsequent hydrogenation of the new imine. The 3-nitro-, 4-nitro- 3-methoxy- and 4-methoxybenzylamines **21e–h**, respectively, are commercially available; thus the set of (protected/substituted)benzylamines **21** was now in place. Using our previously developed method,[26] the benzylamines **21** were treated with thiophosgene in the presence of calcium carbonate as an insoluble mild base in a mixed organic/aqueous solvent system. The corresponding isothiocyanates **22c–i,k** were obtained in yields ranging from 37 to 83%. Simple treatment of **22c–i,k** with ammonia gave the *N*-benzylthioureas **16c–i,k** in good to excellent yields. In some cases, deprotection or further modification of the substituent was necessary. Acidolysis removed the Boc protecting groups from **16c,d** to give the analogous *N*-(aminomethyl)benzylthioureas **16a,b** as their bis(trifluoroacetate) salts. Selective reduction of the nitro groups of **16e,f** with tin(II) chloride provided the aminobenzylthioureas **16l,m**, the lower homologues of **16a,b**. Hydrolysis of the protecting esters in **16i,k** with hydrobromic acid afforded the required carboxylic acids **16n,j**, respectively.

2-Amino-4,5-dihydrothiazoles can be prepared by at least three independent routes. Firstly, the dihydrothiazole can be introduced as a single unit. Stokker et al.[31] used this approach in synthesising the corresponding 2-benzylamino-4,5-dihydrothiazole by treatment of 5-*t*-butyl-2-hydroxy-3-iodobenzylamine with 2-methylthio-4,5-dihydrothiazole **26** in boiling ethanol. Other workers[32] have used similar conditions for reactions of primary amines with this electrophilic dihydrothiazole synthon. However, Hirashima et al.[33] noted that the reaction of **26** with substituted 2-phenylethylamines gave only low yields of 2-amino-4,5-dihydrothiazoles, even under forcing conditions in boiling pentan-1-ol. In our laboratory, 3-methoxybenzylamine **21g** and 4-methoxybenzylamine **21h** failed to react with **26** in boiling ethanol and the condensation required strongly forcing conditions, heating the amines **21g,h** with **26** in the absence of solvent at 180 °C for 4 h, to achieve even moderate yields of the 2-(methoxybenzylamino)-4,5-dihydrothiazoles **17g,h**, respectively. Under these conditions, substituents more sensitive than methoxy would not be expected to survive; indeed, treatment of the mono-Boc-protected diamine **21c** with neat **26** at this temperature led only to unidentifiable degradation products. Secondly, double alkylation of *N*-substituted thioureas with 1,2-dibromoethane gives 2-alkylamino-4,5-dihydrothiazoles but the yields achievable by this method are usually poor to modest.[26] Thirdly, Caujolle et al. reported[34] introduction of the $CH_2CH_2$ unit in two steps, reaction of the isothiocyanate with 2-aminoethanol to give the *N*-(2-hydroxyethyl)thiourea and acid-catalysed cyclisation. Hence, as shown in Scheme 3, the benzylisothiocyanates **22c–k** were treated with 2-aminoethanol in boiling acetone to give the corresponding *N*-benzylthioureas **24c–k** in satisfactory yields. In the cases of isothiocyanates carrying strong electron-withdrawing groups on the benzene ring, low yields of the 3-(benzylaminothiocarbonyl)-2,2-dimethyltetrahydro-1,3-oxazoles **25e,f,k** were also formed. These products of formal condensation with the solvent are unlikely to have arisen from reaction of the hydroxyethylthioureas with acetone, since this process should not be sensitive to the substitution on the benzene ring in the manner observed. It is more likely that 2,2-dimethyltetrahydro-1,3-oxazole is

formed by reversible condensation of 2-aminoethanol with acetone; this heterocycle is a sterically hindered nucleophile at nitrogen (owing to the adjacent *gem*-dimethyl) and reacts only with the more electrophilic isothiocyanates.

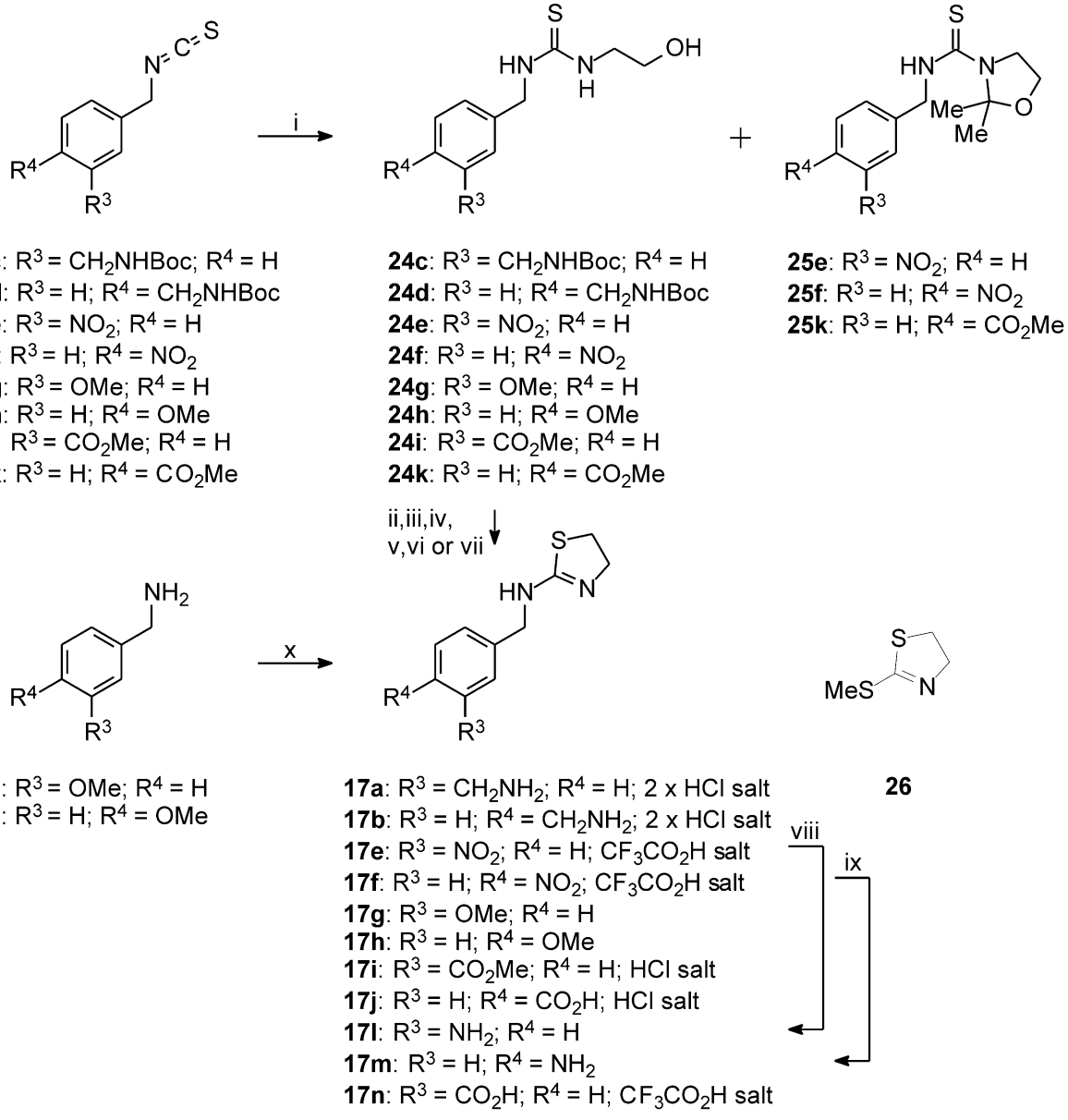
Treatment of the isomeric *N*-(2-hydroxyethyl)thioureas **24c**,**d** with boiling hydrochloric acid for prolonged periods efficiently closed the dihydrothiazole rings and simultaneously removed the Boc protection, giving **17a**,**b** in satisfactory yields as their dihydrochloride salts. Similar treatment of the *para*-substituted ester **24k** again closed the dihydrothiazole ring and hydrolysed the ester protection to afford the target carboxylic acid **17j**. However, application of this method to the *meta* isomer **24i** gave incomplete ester hydrolysis; the product mixture was re-esterified to give **17i** for purification before acid-catalysed hydrolytic deprotection with aqueous trifluoroacetic acid to give the 2-(3-carboxybenzylamino)-4,5-dihydrothiazole **17n**. Since these cyclisation conditions are relatively forcing and cleavage of sensitive substituents is a risk, a milder cyclisation was developed. *N*-(2-Hydroxyethyl)-*N*′-(3-methoxybenzyl)thiourea **24g** was treated with neat trifluoroacetic acid at ambient temperature for 2 h; direct $^1$H NMR analysis showed that cyclisation to **17g** was complete. Cyclisation of the nitrobenzyl analogues **24e**,**f** to **17e**,**f** with trifluoroacetic acid required longer reaction time. Finally, the nitrobenzylaminodihydrothiazoles were reduced to the corresponding aminobenzyl compounds **17l**,**m** with tin(II) chloride under neutral conditions.

The preparations of the lower homologues, the *N*-phenylthioureas **18** and the 2-phenylamino-4,5-dihydrothiazoles **19**, followed sequences similar to those for the benzyl compounds above, although a smaller range of substituents was investigated (Scheme 4). The

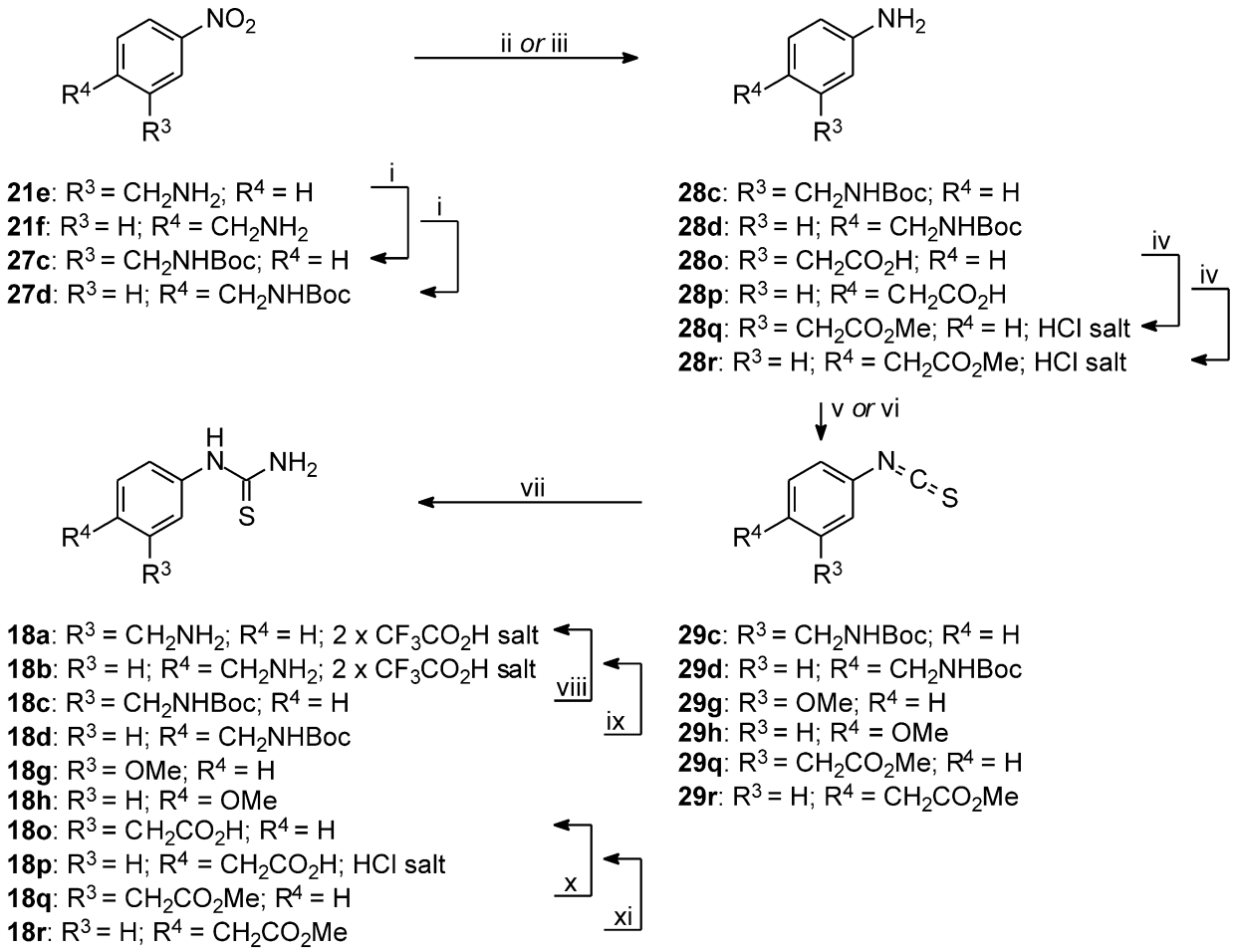



**Scheme 2.** Synthesis of *N*-benzylthioureas 16. *Reagents and conditions*: (i) MeOH, $SOCl_2$, 4 d; (ii) MeOH, $H_2$, Pd/C, 16 h; (iii) $Boc_2O$ (0.3 equiv), $Et_3N$, $CH_2Cl_2$, 16 h; (iv) $CSCl_2$, $CaCO_3$, $H_2O$, $CHCl_3$, 16 h; (v) $NH_3$, $CH_2Cl_2$, 3.5 h, 0 °C; (vi) $CF_3CO_2H$, 5 min; (vii) $SnCl_2$, EtOH, reflux, 1 h; (viii) aq. HBr (50%), 16 h.

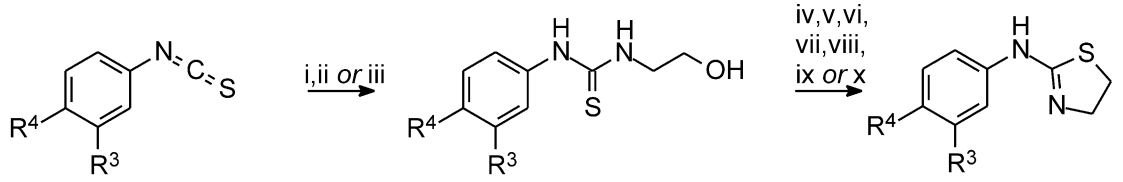
commercially available methoxyphenylisothiocyanates **29g**,**h** reacted with ammonia to give the corresponding thioureas **18g**,**h**. Protection of the aliphatic amine nitrogens in the nitrobenzylamines **21e**,**f** with Boc (giving **27c**,**d**) was followed by reduction of the nitro group with tin(II) chloride (under neutral conditions to avoid deprotection) afforded the Boc-amino-anilines **28c**,**d**. The methyl esters **28q**,**r** were formed by treatment of the aminophenylacetic acids **28o**,**p** with methanol and thionyl chloride. As in the benzylamine series, amines **28c**,**d**,**q**,**r** were converted efficiently to the isothiocyanates **29c**,**d**,**q**,**r** with thiophosgene. Again, these reacted with ammonia to furnish the thioureas **18c**,**d**,**q**,**r**. Acidolytic deprotection removed the Boc groups, giving the aminomethylphenylthioureas **18a**,**b** as their trifluoroacetate salts, whereas hydrolysis with aqueous acid yielded the carboxymethylthioureas **18o**,**p**. As shown in Scheme 5, the same set of phenylisothiocyanates **29c**,**d**,**g**,**h**,**q**,**r** was used to prepare the *N*-(2-hydroxyethyl)thioureas **30c**,**d**,**g**,**h**, **q**,**r**; in contrast to the benzyl series, there was no evidence of formation of the acetone adducts (the 2,2-dimethyltetrahydrooxazoles). The conditions for the cyclisations were selected according to the type of simultaneous deprotection also required. Cyclisation/deprotection of **30c** was effected by both the boiling hydrochloric acid and the trifluoroacetic acid methods, giving **19aA** and **19aB**, respectively. The *para*-substituted isomer **19b** was prepared by the trifluoroacetic acid method only, as were the two 2-(methoxyphenylamino)-4,5-dihydrothiazoles **19g**,**h**. Boiling hydrochloric acid ring-closed and deprotected the esters **30q**,**r**, giving the 2-(carboxymethylphenylamino)-4,5-dihydrothiazoles **19o**,**p**, respectively, in good yield.




**Scheme 3.** Synthesis of *N*-benzyl-*N*′-(2-hydroxyethyl)thioureas **24** and 2-(benzylamino)-4,5-dihydrothiazoles **17**. *Reagents and conditions*: (i) $H_2N(CH_2)_2OH$, acetone, reflux 4 h; (ii) aq HCl (6 M), reflux, 36 h (**17a**,**b**,**j**); (iii) $CF_3CO_2H$, 16 h (**17e**); iv, $CF_3CO_2H$, reflux, 15 h (**17f**); (v) $CF_3CO_2H$, 2 h (**17g**); (vi) aq HCl (6 M), reflux, 40 h, then MeOH, $SOCl_2$, 4 d (**17i**); (vii) aq $CF_3CO_2H$ (50%), reflux, 16 h (**17n**); (viii) $SnCl_2$, EtOH, reflux, 1 h; (ix) $SnCl_2$, EtOH reflux 1.5 h; (x) **26**, 180 °C, 4 h.




**Scheme 4.** Synthesis of *N*-phenylthioureas **18**. *Reagents and conditions*: (i) $Boc_2O$, $Et_3N$, $CH_2Cl_2$, 16 h; (ii) $SnCl_2$, EtOH, reflux, 30 min (**28c**); (iii) $SnCl_2$, EtOH, reflux, 1 h (**28d**); (iv) MeOH, $SOCl_2$, 4 d; (v) $CSCl_2$, $CaCO_3$, $H_2O$, $CHCl_3$, 2 h (**29c**); (vi) $CSCl_2$, $CaCO_3$, $H_2O$, $CHCl_3$, 16 h (**29d,q,r**); (vii) $NH_3$, $CH_2Cl_2$, 3.5 h; (viii) $CF_3CO_2H$, 5 min; (ix) $CF_3CO_2H$, 2 h; (x) aq HCl (1 M), 9 d; (xi) aq HCl (6 M), 16 h.

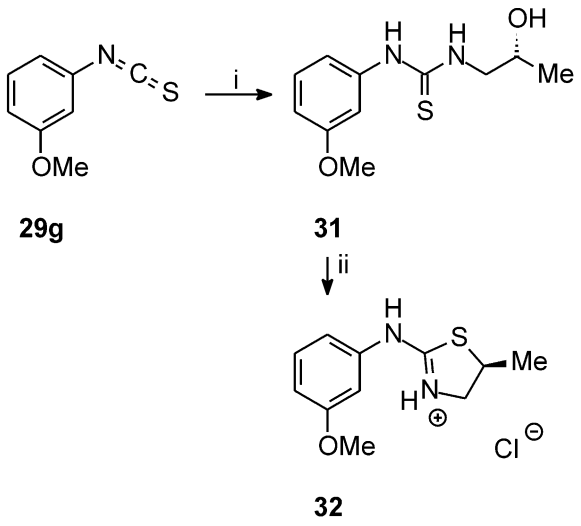



**29c**: $R^3 = CH_2NHBoc$; $R^4 = H$
**29d**: $R^3 = H$; $R^4 = CH_2NHBoc$
**29g**: $R^3 = OMe$; $R^4 = H$
**29h**: $R^3 = H$; $R^4 = OMe$
**29q**: $R^3 = CH_2CO_2Me$; $R^4 = H$
**29r**: $R^3 = H$; $R^4 = CH_2CO_2Me$

**30c**: $R^3 = CH_2NHBoc$; $R^4 = H$
**20d**: $R^3 = H$; $R^4 = CH_2NHBoc$
**30g**: $R^3 = OMe$; $R^4 = H$
**30h**: $R^3 = H$; $R^4 = OMe$
**30q**: $R^3 = CH_2CO_2Me$; $R^4 = H$
**30r**: $R^3 = H$; $R^4 = CH_2CO_2Me$

**19aA**: $R^3 = CH_2NH_2$; $R^4 = H$; 2 x HCl salt
**19aB**: $R^3 = CH_2NH_2$; $R^4 = H$; 2 x $CF_3CO_2H$ salt
**19b**: $R^3 = H$; $R^4 = CH_2NH_2$; 2 x $CF_3CO_2H$ salt
**19g**: $R^3 = OMe$; $R^4 = H$
**19h**: $R^3 = H$; $R^4 = OMe$; $CF_3CO_2H$ salt
**19o**: $R^3 = CH_2CO_2H$; $R^4 = H$; HCl salt
**19p**: $R^3 = H$; $R^4 = CH_2CO_2H$; HCl salt

**Scheme 5.** Synthesis of *N*-phenyl-*N*′-(2-hydroxyethyl)thioureas **30** and 2-(phenylamino)-4,5-dihydrothiazoles **19**. *Reagents and conditions*: (i) $H_2N(CH_2)_2OH$, acetone, reflux, 2 h (**30c,g**); (ii) $H_2N(CH_2)_2OH$, acetone, reflux, 4 h (**30d,q,r**); (iii) $H_2N(CH_2)_2OH$, acetone, reflux, 1.5 h (**30h**); (iv) aq HCl (6 M), reflux, 43 h (**19aA**); (v) $CF_3CO_2H$, 5 min (**19aB**); (vi) $CF_3CO_2H$, 2 h (**19b**); (vii) $CF_3CO_2H$, 3 h (**19g**); (viii) $CF_3CO_2H$, reflux, 15 h (**19h**); (ix) aq HCl (6 M), reflux, 36 h (**19o**); (x) aq HCl (6 M), 16 h.




**Scheme 6.** Stereochemical outcome of acid-catalysed cyclisation to form the 4,5-dihydrothiazole. *Reagents and conditions*: (i) *R*-1-aminopropan-2-ol, acetone, reflux, 2 h; (ii) aq HCl (6 M), reflux, 24 h.

To investigate briefly the mechanism of the acid-catalysed ring-closure of the 2-hydroxyethylthioureas, the homochiral *R*-N-(2-hydroxypropyl)thiourea **31** was synthesised by treatment of 3-methoxyphenylisothiocyanate **29g** with *R*-1-aminopropan-2-ol (Scheme 6). Cyclisation with boiling hydrochloric acid gave the corresponding 2-(3-methoxyphenylamino)-5-methyl-4,5-dihydrothiazole **32**, with specific rotation $[\alpha]_D^{20} = -32.4°$. Since the racemate has not been formed, the cyclisation proceeds, at least in part, by an $S_N2$-like process.

## Biological Evaluation

The *N*-benzylthioureas **16**, the 2-benzylamino-4,5-dihydrothiazoles **17**, the *N*-phenylthioureas **18**, the 2-phenylamino-4,5-dihydrothiazoles **19** and selected *N*-(2-hydroxyethyl)thioureas **24** and **30** were evaluated for their inhibition of the activities of the isoforms of NOS, generally according to the method published previously.[15] The known inhibitors L-NMMA **4**, 1400W **10**, thiocitrulline **12** and 7-nitroindazole **15** were also subjected to the test system, for comparison. Inhibition of the activity of nNOS was measured using an enzyme preparation from rat brain (in which the large majority of the NOS activity is nNOS), whereas most of the assays of activity against iNOS were performed using a preparation of recombinant human iNOS overexpressed in an HT1080 cell line.[35] Selected compounds were also evaluated for inhibition of iNOS as a crude preparation from the lungs of rats previously treated with lipopolysaccharide (LPS). The assays were based on the conversion of [$^{14}$C]-arginine to [$^{14}$C]-citrulline. As a screen for inhibitory activity, all compounds were tested at 100 μM concentration against human iNOS and rat nNOS. Assays were performed in two modes, simultaneous addition of the test compound to the enzyme preparation and of [$^{14}$C]-arginine (to start the enzymic reaction) and pre-incubation of the test compound with the enzyme preparation for 10 min before initiation of the enzymic reaction by addition of [$^{14}$C]-arginine. This pre-incubation has been reported to be optimum for the inhibitory activity of 1400W **11**, which is a slow-binding selective inhibitor of iNOS;[23] this pre-incubation was investigated since many of the test compounds can be considered to be analogues of **11** and may also be slow-binding. We have also noted[35] that an amino-acid-type inhibitor, $N^{\varepsilon}$-homothiocitrulline methyl ester requires at least 5 min pre-incubation with rat nNOS to exert its full inhibitory potency. $IC_{50}$ values were determined (with 15 min pre-incubation) for compounds **18a**,**b** and **19a**, which showed consistent activity in the general screen. Compounds **16a**,**h**, **17a**, **18a** and **19a** were also evaluated at 100 μM for their inhibition of rat iNOS, without pre-incubation.

The results of the biological evaluation of the test compounds are shown in Table 1. Pre-incubation of the compounds with the rat nNOS preparation has little or no effect on the inhibition of this isoform by most of the compounds. Many of the compounds (**16b**,**g**,**h**,**j**,**l**–**n**, **17g**,**h**, **j**,**l**–**n**, **18g**,**h**,**o**,**p**, **19g**,**h**,**o**,**p**, **24g**,**h** and **30g**,**h**) are inactive or have only weak activity against this isoform. Significant activity was shown by all the new compounds carrying the aminomethyl group on the benzene ring, consistent with the view of Collins et al.[24] that this group is optimal in the amidine series of inhibitors, for example **10** and **11**. Thus inhibitory activity was shown by **16a** and by **17a**, the *N*-benzylthiourea and the 2-benzylamino-4,5-dihydrothiazole most closely related to 1400W **11** with an aminomethyl group located *meta* on the benzene ring. The corresponding *para*-substituted analogues **16b** and **17b**, respectively, were less potent. The *N*-(aminomethylphenyl)thioureas **18a**,**b** and the 2-(aminomethylphenylamino)-4,5-dihydrothiazoles **19a**,**b** were also active. Again, the *meta* substituted isomers were more potent than the corresponding *para* compounds, in that **18a** had $IC_{50} = 13$ μM, whereas **18b** had $IC_{50} = 41$ μM, when assayed with 15-min pre-incubation. Interestingly, 7-nitroindazole **15**, which is claimed to be selective for nNOS inhibition,[30] showed $IC_{50} = 40$ μM in this system, making compounds **18a** and **19a** more potent than this lead compound in our nNOS system and similar in potency to L-NMMA **4**, 1400W **10** and thiocitrulline **12**.

In contrast with the results for nNOS, pre-incubation of the test compounds with the human iNOS enzyme preparation had a profound effect on the inhibition. The inhibition caused by **16g**,**h**, **17a**,**g**,**h**, **18g** and **19g** appeared to decrease to a greater or lesser extent with pre-incubation, although these compounds had only moderate potency. In contrast, the inhibition of human iNOS by the most potent compound, **19a**, increased with pre-incubation, suggesting that slow binding may be involved. This effect was also observed with the weaker inhibitor **19b**. The most potent inhibitor of human iNOS was the *meta*-aminomethylphenylamino-4,5-dihydrothiazole **19a**, with $IC_{50} = 19$ μM. Interestingly, the corresponding thiourea **18a** was much less potent, with $IC_{50} = 260$ μM. Again, **19a**, was more potent in this system than 7-nitroindazole **15** but less potent than the other 'standard' inhibitors **4**, **10** and **12**.

Most striking, however, was the strong *stimulation* of the activity of human iNOS by **16j**,**l**,**m**,**n**, **17j**,**m**, **18o**, **19p** and **24g**. In each case, pre-incubation abolished this stimulation and, in some cases, led to weak inhibition. Compounds **17l**,**n** and **24h** were weaker stimulators (with no pre-incubation) but switched to become significant inhibitors when pre-incubated with the enzyme preparation for 10 min before initiation of the enzyme-catalysed generation of nitric oxide. We have previously noted[15] the phenomenon of stimulation of rat iNOS activity by *S*-2-amino-5-(3-nitrotriazol-1-yl)pentanoic acid and by *S*-2-amino-5-(3-aminotriazol-1-yl)pentanoic acid, although the effects were much weaker. A structure–activity tendency is evident for this stimulatory effect. The most effective stimulators in the benzyl series **16**, **17** carry either amines or carboxylic acids attached directly to the benzene ring. In the phenyl series, only **18o** and **19p** are stimulatory; these carry $CH_2CO_2H$ as substituents on the benzene ring, giving the same distance between the carboxylate and the sulfur as in their stimulatory isomers **16j** and **16n**, respectively. The molecular origin of this stimulatory effect remains unclear, although it is consistent with a model in which there are two binding sites for these compounds, the substrate (arginine)-binding site at the catalytic centre and a (possibly remote) allosteric site. To rationalise the data in terms of this model, the binding of the compounds to the arginine site would be inhibitory (and competitive with arginine) and binding to the allosteric site would be stimulatory, possibly through inducing a conformational change in the enzyme protein. To fit the observed dependence on pre-incubation, binding to the allosteric site would be fast (leading to stimulation of nitric oxide synthesis without pre-incubation), whereas inhibitory binding to the substrate-binding site would be slow. Then the overall effect observed after pre-incubation would be the sum of the stimulatory and inhibitory effects (leading to apparent diminution of stimulation or switch to apparent inhibition).

**Table 1.** Inhibition of human iNOS, rat iNOS and rat nNOS by the thioureas **16**,**18**, the *N*-(2-hydroxyethyl)thioureas **24**, **30**, the 4,5-dihydrothiazoles **17**, **19** and, for comparison, by the known inhibitors L-NMMA **4**, 1400W **10**, thiocitrulline **12** and 7-nitroindazole **15**

| Compd | % inhibition (human iNOS)[a] | | | % inhibition (rat iNOS)[a] | % inhibition (rat nNOS)[a,b] | | |
|---|---|---|---|---|---|---|---|
| | No pre-incubation[b] | 10 min pre-incubation[b] | $IC_{50}$ (15 min pre-incubation)[b] (μM) | No pre-incubation[b] | No pre-incubation[b] | 10 min pre-incubation[b] | $IC_{50}$ (15 min pre-incubation)[b] (μM) |
| **4** | | | <4 | | 94±1 | 96±1 | 9 |
| **10** | 79±1 | 82±1 | <4 | | | | 12 |
| **12** | | | <5 | | | | 17 |
| **15** | 77±2 | 68±2 | 24 | | 59±1 | 74±3 | 40 |
| **16a** | 34±1 | 44±2 | | 23±2 | 39±5 | 47±1 | |
| **16b** | 29±5 | 32±4 | | | 11±6 | 20±2 | |
| **16g** | 35±2 | 7±3 | | | −3±1 | 6±4 | |
| **16h** | 26±6 | 2±5 | | 5±3 | 8±1 | 14±1 | |
| **16j** | −56±12 | 3±1 | | | −0.4±0.4 | 6±1 | |
| **16l** | −58±1 | 9±1 | | | −1±6 | 9±6 | |
| **16m** | −55±11 | 12±1 | | | −0.3±1 | 12±2 | |
| **16n** | −46±5 | 7±2 | | | 14±1 | −0.4±2 | |
| **17a** | 48±1 | 26±3 | | 5±3 | 55±6 | 59±1 | |
| **17b** | −30±2 | 51±2 | | | 33±3 | 65±2 | |
| **17g** | 34±4 | 8±4 | | | 8±1 | 14±1 | |
| **17h** | 26±6 | 3±13 | | | 10±2 | 13±1 | |
| **17j** | −45±1 | 5±1 | | | 0.4±4 | −11±1 | |
| **17l** | −36±1 | 12±2 | | | 5±2 | 17±5 | |
| **17m** | −47±10 | 19±2 | | | 8±2 | 12±1 | |
| **17n** | −39±1 | 34±3 | | | 5±3 | 13±3 | |
| **18a** | 35±4 | 40±5 | 260 | 98±1 ($IC_{50}$ = 23 μM) | 98±3 ($IC_{50}$ = 10 μM) | 67±1 | 13 |
| **18b** | 57±1 | 52±1 | 89 | | 48±4 | 44±1 | 41 |
| **18g** | 17±6 | 2±1 | | | −4±1 | 5±1 | |
| **18h** | 26±7 | 28±1 | | | −1±3 | 4±2 | |
| **18o** | −65±5 | −3±14 | | | 9±6 | 5±2 | |
| **18p** | 15±2 | 13±3 | | | −7±8 | 15±6 | |
| **19a** | 62±2 | 86±1 | 19 | 34±1 ($IC_{50}$ = 190 μM) | 66±1 ($IC_{50}$ = 21 μM) | 89±1 | 13 |
| **19b** | 16±4 | 40±1 | | | 56±1 | 56±1 | |
| **19g** | 26±2 | −1±12 | | | 7±1 | 20±2 | |
| **19h** | 1±1 | 7±3 | | | −2±1 | 17±1 | |
| **19o** | −1±4 | −8±14 | | | 4±2 | 9±3 | |
| **19p** | −58±4 | 9±2 | | | 9±2 | 14±5 | |
| **24g** | −58±7 | 8±1 | | | −1±4 | 5±1 | |
| **24h** | −25±1 | 44±5 | | | −1±1 | 5±1 | |
| **30g** | −10±1 | −1±1 | | | 0±0.6 | 1±1 | |
| **30h** | 6±3 | 6±2 | | | 2±1 | 2±3 | |

[a]Concentration of test compound 100 μM.
[b]Pre-incubation refers to the time between addition of the test compound and addition of [$^{14}$C]-arginine to initiate the enzymic reaction.

Examination of the activity of the most potent inhibitors of the activities of the isoforms of NOS reveals moderate selectivity. Interestingly, the claimed[30] nNOS-selective inhibitor **15** showed no selectivity for rat NOS over human iNOS, although this may have been a species effect. Whereas 2-(3-aminomethylphenylamino)-4,5-dihydrothiazole **19a** was the most potent inhibitor of human iNOS ($IC_{50}=19\,\mu M$ after 15 min pre-incubation), it was similarly active against rat nNOS ($IC_{50}=13\,\mu M$ after 15 min pre-incubation); interestingly, it was much less potent against rat iNOS ($IC_{50}=190\,\mu M$ with no pre-incubation) than against rat nNOS ($IC_{50}=21\,\mu M$ with no pre-incubation). A different species effect was seen with the corresponding *N*-(3-aminomethylphenyl)thiourea **18a**. Here, little selectivity was seen between inhibition of rat iNOS ($IC_{50}=23\,\mu M$ with no pre-incubation) and inhibition of rat nNOS ($IC_{50}=10\,\mu M$ with no pre-incubation) but the compound was 20-fold selective for inhibition of rat nNOS ($IC_{50}=13\,\mu M$ with 15-min pre-incubation) over inhibition of human iNOS ($IC_{50}=260\,\mu M$ with 15-min pre-incubation). The *para* isomer **18b**, however, showed only 2-fold selectivity in the same (rat nNOS vs human iNOS) comparison.

## Conclusions

In this paper, we have described our design of novel inhibitors of the isoforms of NOS, replacing the amidines in the highly isoform-selective inhibitors 1400W **11** and its lower homologue **12** with thiourea and 4,5-dihydrothiazole units for ligation to the haem iron; this replacement was based on the potent activity of thiocitrulline **13** and of the analogous dihydrothiazole **14**. The effect of varying the nature and position of the substituents on the aryl ring were also examined. The thioureas were synthesised in good yields by reaction of the corresponding isothiocyanates with ammonia, whereas the 2-(substituted amino)-4,5-dihydrothiazoles were prepared by two routes, reaction of benzylamines with 2-methylthio-4,5-dihydrothiazole under forcing conditions or acid-catalysed cyclisation of *N*-(2-hydroxyethyl)thioureas.

The most potent inhibitory activity was seen where the substituent was aminomethyl, the same substituent as in **11** and **12**, although the position (*meta* or *para*) of this substituent was of limited importance. The isoform-selectivity for inhibition of iNOS and nNOS, where observed, was generally limited. However, **18a** showed useful selectivity for inhibition of rat nNOS over human iNOS. Most compounds showed modest inhibitory potency but the activities of **18a** and **19a** were comparable to those of the widely used experimental inhibitor thiocitrulline **13**,[15] of **8**[15] and of our previous ω-isothiourea ornithine-based inhibitors, $N^{\delta}$-(imino(isopropylthio))methyl)ornithine,[26] $N^{\delta}$-(4,5-dihydro-1,3-thiazin-2-yl)ornithine[26] and $N^{\delta}$-(4,5-dihydro-1,3-thiazol-2-yl)ornithine **14**.[26] Most striking, however, is the stimulation of the activity of human iNOS by the *N*-(aminobenzyl) and *N*-(carboxybenzyl) thioureas and 2-(aminobenzylamino) and 2-(carboxybenzyl) 4,5-dihydrothiazoles. The mechanistic origin of this stimulation, which may have experimental or therapeutic applications, will be the subject of intense further study.

## Experimental

NMR data were recorded on either JEOL/Varian GX 270 or EX 400 spectrometers, using solutions in $CDCl_3$, unless otherwise stated. IR spectra were recorded on samples as KBr discs, unless otherwise stated. Mass Spectra were recorded using a VG Analytical Mass Spectrometer in the FAB positive ion mode, unless otherwise stated. Solutions in organic solvents were dried with $MgSO_4$ and solvents were evaporated under reduced pressure. Experiments were conducted at ambient temperature, unless otherwise stated. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block.

***N*-(3-(Aminomethyl)phenylmethyl)thiourea bis(trifluoroacetate) salt (16a)**. Compound **16c** (100 mg, 300 μmol) was stirred in $CF_3CO_2H$ (3 mL) for 5 min. Evaporation gave **16a** (140 mg, 99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1172, 1780, 3200 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 4.00 (2H, m, $CH_2N^+H_3$), 4.65 (2H, m, $CH_2NH$), 7.15 (3H, br, $N^+H_3$), 7.32 (4H, m, Ar-$H_4$), 8.10 (4H, br, $NH+N^+H_3$); MS *m*/*z* 196.0905 (M + H) ($C_9H_{14}N_3S$ requires 196.0908), 179 ($M-NH_3$), 162 ($M-2\times NH_3$).

***N*-(4-(Aminomethyl)phenylmethyl)thiourea bis(trifluoroacetate) salt (16b)**. Compound **16d** was treated with $CF_3CO_2H$, as for the synthesis of **16a** (reaction time 2 h), to give **16b** (99%) as white crystals: mp 197–199 °C; IR $\nu_{max}$ 1188, 3293 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.99 (4H, s, $2\times CH_2$), 4.64 (3H, br, $NH_3$), 7.29 (1H, br, NH), 7.30 (2H, d, $J=8.0$ Hz, Ar 3,5-$H_2$), 7.37 (2H, d, $J=8.0$ Hz, Ar 2,6-$H_2$), 8.16 (3H, br, $N^+H_3$); MS *m*/*z* 196.0908 (M + H) ($C_9H_{14}N_3S$ requires 196.0920), 179 ($M-NH_3$).

**1,1-Dimethylethyl *N*-(3-(thioureidomethyl)phenylmethyl)carbamate (16c)**. $NH_3$ was passed through **22c** (400 mg, 1.5 mmol) in $CH_2Cl_2$ (40 mL) for 30 min. The mixture was stirred for 3 h at 0 °C. Evaporation and chromatography (EtOAc/hexane 4:1) gave **16c** (300 mg, 72%) as white crystals: mp 70–72 °C; IR $\nu_{max}$ 1164, 1608, 3308 $cm^{-1}$; NMR $\delta_H$ 1.40 (9H, s, $Bu^t$), 4.18 (2H, br, $CH_2$), 4.62 (2H, br, $CH_2$), 5.30 (1H, br, NH), 6.01 (2H, br, $NH_2$), 7.21 (5H, m, Ar-$H_4$ + NH); MS *m*/*z* 296.1426 (M + H) ($C_{14}H_{22}N_3O_2S$ requires 296.1433), 240 ($M-Me_2C{=}CH_2$); Found C, 56.50; H, 7.11; $C_{14}H_{21}N_3O_2S$ requires C, 56.50; H, 7.09%.

**1,1-Dimethylethyl *N*-(4-(thioureidomethyl)phenylmethyl)carbamate (16d)**. Compound **22d** was treated with $NH_3$, as for the synthesis of **16c** (chromatographic eluant EtOAc), to give **16d** (71%) as pale yellow crystals: mp 104–106 °C; IR $\nu_{max}$ 1171, 1686, 3355 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.38 (9H, s, $Bu^t$), 4.08 (2H, d, $J=6.0$ Hz, $CH_2$), 4.57 (2H, br, $CH_2$), 7.20 (6H, m, Ar-$H_4$ + $NH_2$), 7.37 (1H, br, NH), 7.98 (1H, br, NH); MS *m*/*z* 591

(2 M + H), 296.1423 (M + H) ($C_{14}H_{22}N_3O_3S$ requires 296.1421), 249 (M−$Me_2C{=}CH_2$); Found C, 56.6: H, 7.09; N, 13.90; $C_{14}H_{21}N_3O_2S$ requires C, 56.72; H, 7.16; N, 14.23%.

***N*-(3-Nitrophenylmethyl)thiourea (16e)**. Compound **22e** was treated with $NH_3$, as for the synthesis of **16d**, to give **16e** (68%) as yellow crystals: mp 143–145 °C; IR $\nu_{max}$ 1159, 1347, 1529, 3292 $cm^{-1}$; NMR $\delta_H$ 4.77 (2H, s, $CH_2$), 7.20 (2H, br, $NH_2$), 7.64 (1H, dd, $J$ = 8.2, 7.8 Hz, Ar 5-H), 7.74 (1H, d, $J$ = 7.8 Hz, Ar 4-H), 8.10 (1H, s, Ar 2-H), 8.11 (1H, d, $J$ = 8.2 Hz, Ar 6-H); MS $m/z$ 212.0498 (M + H) ($C_8H_{10}N_3O_2S$ requires 212.0494), 196 (M−$NH_2$).

***N*-(4-Nitrophenylmethyl)thiourea (16f)**. Compound **22f** was treated with $NH_3$, as for the synthesis of **16d**, to give **16f** (69%) as a colourless oil: (lit.[36] mp 113.5–115 °C); IR (film) $\nu_{max}$ 1159, 1344, 1563, 3213 $cm^{-1}$; NMR $\delta_H$ 4.82 (2H, br, $CH_2$), 5.82 (2H, br, $NH_2$), 7.26 (1H, s, NH), 7.50 (2H, d, $J$ = 8.6 Hz, Ar 2,6-$H_2$), 8.21 (2H, d, $J$ = 8.6 Hz, Ar 3,5-$H_2$); MS $m/z$ 422 (2 M + H), 212.0490 (M + H) ($C_8H_{10}N_3O_2S$ requires 212.0494), 196 (M−$NH_2$).

***N*-(3-Methoxyphenylmethyl)thiourea (16g)**. Compound **22g** was treated with $NH_3$, as for the synthesis of **16d**, to give **16g** (99%) as a colourless oil: (lit.[37] mp 102 °C); IR (film) $\nu_{max}$ 1046, 2835, 3273 $cm^{-1}$; NMR $\delta_H$ 3.80 (3H, s, Me), 4.20 (1H, br, NH), 4.77 (2H, m, $CH_2$), 5.83 (2H, s, $NH_2$), 6.86 (3H, m, Ar 2,4,6-$H_3$), 7.27 (1H, m, Ar 5-H); MS $m/z$ 197.0751 (M + H) ($C_9H_{13}N_2OS$ requires 197.0749), 121 (M−$NHCSNH_2$).

***N*-(4-Methoxyphenylmethyl)thiourea (16h)**. Compound **22h** was treated with $NH_3$, as for the synthesis of **16c** [chromatographic eluant EtOAc/hexane (1:1)], to give **16h** (99%) as white crystals: mp 124–126 °C (lit.[38] mp 135 °C); IR $\nu_{max}$ 1177, 2800, 3165 $cm^{-1}$; NMR $\delta_H$ 3.87 (3H, s, Me), 4.38 (2H, br, $CH_2$), 5.75 (2H, br, $NH_2$), 6.88 (1H, br, NH), 6.90 (2H, d, $J$ = 8.4 Hz, Ar 3,5-$H_2$), 7.23 (2H, d, $J$ = 8.4 Hz, Ar 2,6-$H_2$); MS $m/z$ 393 (2 M + H), 197.0749 (M + H) ($C_9H_{13}N_2OS$ requires 197.0751); Found C, 54.80: H, 6.13; N, 14.14; $C_9H_{12}N_2OS$ requires C, 54.30; H, 6.11; N, 14.10%.

**Methyl 3-(thioureidomethyl)benzoate (16i)**. Compound **22i** was treated with $NH_3$, as for the synthesis of **16d**, to give **16i** (99%) as pale yellow crystals: mp 123–125 °C; IR $\nu_{max}$ 1202, 1710, 3418 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.89 (3H, s, Me), 4.88 (2H, s, $CH_2$), 7.44 (1H, t, $J$ = 7.8 Hz, Ar 5-H), 7.56 (1H, d, $J$ = 7.8 Hz, Ar 4-H), 7.90 (1H, d, $J$ = 7.8 Hz, Ar 6-H), 8.03 (1H, s, Ar 2-H); MS $m/z$ 449 (2 M + H), 225.0708 (M + H) ($C_{10}H_{13}N_2O_2S$ requires 225.0698), 211 (M−Me).

**4-(Thioureidomethyl)benzoic acid hydrobromide (16j)**. Compound **16k** was treated with HBr, as for the synthesis of **16n**, to give **16j** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1176, 1705, 3382 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.88 (2H, s, $CH_2$), 7.41 (2H, d, $J$ = 8.3 Hz, Ar 3,5-$H_2$), 7.97 (2H, d, $J$ = 8.3 Hz, Ar 2,6-$H_2$); MS $m/z$ 211.0541 (M + H) ($C_9H_{11}N_2O_2S$ requires 211.0547).

**Methyl 4-(thioureidomethyl)benzoate (16k)**. Compound **22k** was treated with $NH_3$, as for the synthesis of **16h**, to give **16k** (99%) as white crystals: mp 131–133 °C; IR $\nu_{max}$ 1179, 1711, 3409 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.82 (3H, s, Me), 4.70 (2H, s, $CH_2$), 7.18 (2H, br, $NH_2$), 7.38 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.90 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 8.06 (1H, br NH); MS $m/z$ 225.0690 (M + H) ($C_{10}H_{13}N_2O_2S$ requires 225.0698).

***N*-(3-Aminophenylmethyl)thiourea (16l)**. Compound **16e** (90 mg, 0.4 mmol) was boiled under reflux with $SnCl_2{\cdot}2H_2O$ (200 mg, 1.2 mmol) in EtOH (5 mL) for 1 h. The solution was cooled to 0 °C, basified with 5% aq $NaHCO_3$, extracted with EtOAc and washed with saturated brine. Drying, evaporation and chromatography (EtOAc/hexane 5:1) gave **16l** (40 mg, 55%) as pale buff crystals: mp 141–143 °C; IR $\nu_{max}$ 1166, 3289 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.34 (2H, s, $CH_2$), 4.25 (1H, br, NH), 4.58 (2H, br, $NH_2$), 6.62 (3H, m, Ar 2,4,6-$H_3$), 6.68 (1H, br, NH), 7.05 (1H, t, $J$ = 7.8 Hz, Ar 5-H); MS $m/z$ 182.0757 (M + H) ($C_8H_{12}N_3S$ requires 182.0752).

***N*-(4-Aminophenylmethyl)thiourea (16m)**. Compound **16f** was treated with $SnCl_2$, as for the synthesis of **16l** (reaction time 3 h; chromatographic eluant EtOAc/AcOH/ hexane (10:1:1), to give **16m** (95%) as pale orange crystals: mp >270 °C; IR $\nu_{max}$ 1179, 3422 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 4.54 (2H, s, $CH_2$), 6.69 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.06 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$); MS $m/z$ 182.0746 (M + H) ($C_8H_{12}N_3S$ requires 182.0752), 164 (M−$NH_3$).

**3-(Thioureidomethyl)benzoic acid hydrobromide (16n)**. Compound **16i** (80 mg, 360 μmol) was stirred in aq HBr (50%, 5 mL) for 16 h. Evaporation gave **16n** (70 mg, 98%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1704, 3298 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.89 (2H, s, $CH_2$), 7.45 (1H, t, $J$ = 7.8 Hz, Ar 5-H), 7.57 (1H, d, $J$ = 7.8 Hz, Ar 4-H), 7.91 (1H, d, $J$ = 7.8 Hz, Ar 6-H), 8.08 (1H, s, Ar 2-H); MS $m/z$ 225.0708 (M + H) ($C_{10}H_{13}N_2O_2S$ requires 225.0698).

**2-(3-(Aminomethyl)phenylmethylamino)-4,5-dihydrothiazole dihydrochloride (17a)**. Compound **24c** (83 mg, 240 μmol) was boiled under reflux for 36 h in aq HCl (6 M, 4 mL). Evaporation gave **17a** (59 mg, 47%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1632, 3429 $cm^{-1}$; NMR $\delta_H$ 3.63 (2H, t, $J$ = 7.4 Hz, thiazole 5-$H_2$), 3.99 (2H, t, $J$ = 7.4 Hz, thiazole 4-$H_2$), 4.06 (2H, brq, $J$ = 5.9 Hz, $CH_2N^+H_3$), 4.77 (2H, d, $J$ = 6.2 Hz, $ArCH_2N$thiazole) 7.50 (4H, m, Ar-$H_4$), 8.70 (3H, br, $N^+H_3$), 10.89 (2H, s, 2 × NH); MS $m/z$ 222.1067 (M + H) ($C_{11}H_{16}N_3S$ requires 222.1065).

**2-(4-(Aminomethyl)phenylmethylamino)-4,5-dihydrothiazole dihydrochloride (17b)**. Compound **24d** (100 mg, 0.29 mmol) was boiled under reflux in aq HCl (6 M, 6 mL) for 36 h. Evaporation and recrystallisation ($Pr^iOH$) gave **17b** (40 mg, 47%) as pale yellow crystals: mp 198–200 °C; IR $\nu_{max}$ 1654, 3425 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.64 (2H, t, $J$ = 7.4 Hz, thiazole 5-$H_2$), 4.04 (2H, m, thiazole 4-$H_2$), 4.14 (2H, m, $CH_2$NHthiazole), 4.61 (2H, s, $CH_2N^+H_3$), 7.44 (2H, m, Ar 3,5-$H_2$),

7.53 (2H, m, Ar 2,6-$H_2$); MS *m/z* 443 (2 M + H), 222.1061 (M + H) ($C_{11}H_{16}N_3S$ requires 222.1065), 205 (M − $NH_3$).

**2-(3-Nitrophenylmethylamino)-4,5-dihydrothiazole trifluoroacetate salt (17e)**. Compound **24e** (120 mg, 470 μmol) was stirred for 16 h with $CF_3CO_2H$ (5 mL). Evaporation gave **17e** (170 mg, 99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1352, 1532, 1679, 3170 $cm^{-1}$; NMR $\delta_H$ 3.55 (2H, t, *J* = 7.8 Hz, thiazole 5-$H_2$), 4.05 (2H, t, *J* = 7.8 Hz, thiazole 4-$H_2$), 4.59 (2H, d, *J* = 4.7 Hz, $ArCH_2$), 7.61 (1H, dd, *J* = 8.2, 7.8 Hz, Ar 5-H), 7.69 (1H, d, *J* = 7.8 Hz, Ar 6-H), 8.17 (1H, s, Ar 2-H), 8.21 (1H, d, *J* = 8.2 Hz, Ar 4-H), 11.94 (1H, br, NH), 12.32 (1H, br NH); MS *m/z* 475 (2 M + H), 238.0640 (M + H) ($C_{10}H_{12}N_3O_2S$ requires 238.0650).

**2-(4-Nitrophenylmethylamino)-4,5-dihydrothiazole trifluoroacetate salt (17f)**. Compound **24f** (200 mg, 780 μmol) was boiled under reflux in $CF_3CO_2H$ (5 mL) for 15 h. Evaporation gave **17f** (240 mg, 99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1347, 1524, 1678, 3173 $cm^{-1}$; NMR $\delta_H$ 3.54 (2H, t, *J* = 7.8 Hz, thiazole 5-$H_2$), 4.04 (2H, t, *J* = 7.8 Hz, thiazole 4-$H_2$), 4.59 (2H, d, *J* = 5.1 Hz, $CH_2Ar$), 7.49 (2H, d, *J* = 8.6 Hz, Ar 2,6-$H_2$), 8.23 (2H, d, *J* = 8.6 Hz, Ar 3,5-$H_2$), MS *m/z* 238.0639 (M + H) ($C_{10}H_{12}N_3O_2S$ requires 238.0650), 222 (M − $NH_2$).

**2-(3-Methoxyphenylmethylamino)-4,5-dihydrothiazole (17g)**. Method A. Compound **24g** (290 mg, 1.2 mmol) was stirred in $CF_3CO_2H$ (5 mL) for 2 h. Evaporation and chromatography (EtOAc/MeOH 5:1) gave **17g** (200 mg, 75%) as white crystals: mp 94–96 °C; IR (film) $\nu_{max}$ 1681, 3200 $cm^{-1}$; NMR $\delta_H$ 3.46 (2H, t, *J* = 7.4 Hz, thiazole 5-$H_2$), 3.82 (3H, s, Me), 3.95 (2H, t, *J* = 7.8 Hz, thiazole 4-$H_2$), 4.43 (2H, d, *J* = 5.6 Hz, $CH_2Ar$), 6.87 (3H, m, Ar 2,4,6-$H_3$), 7.28 (1H, m, Ar 5-H), 12.25 (1H, s, NH), 12.36 (1H, s, NH); NMR $\delta_C$ 31.1, 48.7, 51.3, 55.2, 112.9, 113.9, 119.6, 130.0, 136.5, 160.0, 174.6; MS *m/z* 223.0890 (M + H) ($C_{11}H_{15}N_2OS$ requires 223.0905).

**2-(3-Methoxyphenylmethyl)-4,5-dihydrothiazole (17g)**. Method B. 3-Methoxybenzylamine **21g** was heated with 2-methylthio-4,5-dihydrothiazole **26** (320 mg, 2.4 mmol) at 180 °C for 4 h. Evaporation and chromatography (EtOAc/ MeOH 5:1) gave **17g** (163 mg, 30%) with properties as above.

**2-(4-Methoxyphenylmethylamino)-4,5-dihydrothiazole (17h)**. 4-Methoxybenzylamine **21h** was treated with **26**, as for the synthesis of **17g** (Method B) [chromatographic eluant $CH_2Cl_2$/MeOH (10:1)] to give **17h** (30%) as white crystals: mp 77–79 °C (lit.[33] mp 84–85 °C); NMR $\delta_H$ 3.38 (2H, t, *J* = 7.4 Hz, thiazole 5-$H_2$), 3.80 (3H, s, Me), 4.04 (2H, t, *J* = 7.4 Hz, thiazole 4-$H_2$), 4.40 (2H, s, $ArCH_2$), 6.87 (2H, d, *J* = 8.8 Hz, Ar 3,5-$H_2$), 7.25 (2H, d, *J* = 8.8 Hz, Ar 2,6-$H_2$); IR $\nu_{max}$ 1610, 2780, 3207 $cm^{-1}$; MS *m/z* 223.0912 (M + H) ($C_{11}H_{15}N_2OS$ requires 223.0905); Found C, 58.25: H, 6.44; N, 12.35: $C_{11}H_{14}N_2OS$. 0.25$H_2O$ requires C, 58.29; H, 6.40; N, 12.36%.

**Methyl 3-((4,5-dihydrothiazol-2-ylamino)methyl)benzoate hydrochloride (17i)**. Compound **24i** (70 mg, 260 μmol) was boiled under reflux for 40 h in aq HCl (6 M, 5 mL). The evaporation residue was stirred with MeOH (70 mL) and $SOCl_2$ (0.5 mL) for 4 d. Evaporation gave **17i** (50 mg, 67%) as a pale buff gum: IR (film) $\nu_{max}$ 1640, 1718, 3398 $cm^{-1}$; NMR $\delta_H$ 3.51 (2H, br, $CH_2$), 3.92 (3H, s, Me), 4.02 (2H, br, $CH_2$), 4.54 (2H, s, $CH_2Ar$), 7.47 (1H, t, *J* = 7.4 Hz, Ar-$H_5$), 7.57 (1H, d, *J* = 7.4 Hz, Ar-$H_4$), 7.95 (1H, s, Ar-$H_2$), 7.99 (1H, d, *J* = 7.4 Hz, Ar-$H_4$), 10.40 (1H, br, NH), 10.93 (1H, br, NH): MS *m/z* 251.0859 (M + H) ($C_{12}H_{14}N_2O_2S$ requires 251.0854).

**4-(4,5-Dihydrothiazol-2-ylaminomethyl)benzoic acid hydrochloride (17j)**. Compound **24k** was treated with aq HCl, as for the synthesis of **17a**, to give **17j** (99%) as pale yellow crystals: mp 138–140 °C; IR (film) $\nu_{max}$ 1656, 1697, 3430 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.57 (2H, t, *J* = 7.4 Hz, thiazole 5-$H_2$), 3.92 (2H, t, *J* = 7.4 Hz, thiazole 4-$H_2$), 4.73 (2H, s, $CH_2Ar$), 7.46 (2H, d, *J* = 8.2 Hz, Ar 3,5-$H_2$), 7.95 (2H, d, *J* = 8.2 Hz, Ar 2,6-$H_2$), 10.66 (1H, br, NH), 13.05 (1H, br, NH); MS *m/z* 237.0688 (M + H) ($C_{11}H_{13}N_2O_2S$ requires 237.0698).

**2-(3-Aminophenylmethylamino)-4,5-dihydrothiazole (17l)**. Compound **17e** was treated with $SnCl_2$, as for the synthesis of **16l** (chromatography omitted), to give **17l** (150 mg, 99%) as a colourless oil: IR (film) $\nu_{max}$ 1618; 3391 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.30 (2H, m, thiazole 5-$H_2$), 3.90 (2H, m, thiazole 4-$H_2$), 4.30 (2H, br, $CH_2Ar$), 6.62 (2H, m, Ar 4,6-$H_2$), 6.67 (1H, s, Ar 2-H), 7.04 (1H, t, *J* = 7.4 Hz, Ar 5-H); MS *m/z* 208.0905 (M + H) ($C_{10}H_{14}N_3S$ requires 208.0908), 196 (M − aminodihydrothiazole).

**2-(4-Aminophenylmethylamino)-4,5-dihydrothiazole (17m)**. Compound **17f** was treated with $SnCl_2$, as for the synthesis of **17l** (reaction time 1.5 h), to give **17m** (38%) as a colourless oil: IR (film) $\nu_{max}$ 1609, 3324 $cm^{-1}$; NMR $\delta_H$ 3.33 (2H, t, *J* = 7.4 Hz, thiazole 5-$H_2$), 3.64 (2H, br, $NH_2$), 4.02 (2H, t, *J* = 7.4 Hz, thiazole 4-$H_2$), 6.64 (2H, d, *J* = 8.6 Hz, Ar 2,6-$H_2$), 7.11 (2H, d, *J* = 8.6 Hz, Ar 3,5-$H_2$); NMR 35.7, 49.2), 60.4, 115.4, 129.0, 129.3, 146.0, 161.9; MS *m/z* 208.0911 (M + H) ($C_{10}H_{14}N_3S$ requires 208.0908).

**3-(4,5-Dihydrothiazol-2-ylaminomethyl)benzoic acid trifluoroacetate salt (17n)**. Compound **17i** (70 mg, 280 μmol) was stirred under reflux in aq $CF_3CO_2H$ (50%, 5 mL) for 16 h. Evaporation gave **17n** (70 mg, 99%) as a colourless hygroscopic gum: IR $\nu_{max}$ 1645, 1696, 3433 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.65 (2H, t, *J* = 7.8 Hz, thiazole 5-$H_2$), 4.04 (2H, t, *J* = 7.8 Hz, thiazole 4-$H_2$), 4.60 (2H, m, $CH_2Ar$), 7.54 (3H, m, Ar-$H_3$), 8.02 (1H, m, Ar-H); MS *m/z* 237.0698 (M + H) ($C_{11}H_{13}N_2O_2S$ requires 237.0698).

***N*-(3-Aminomethylphenyl)thiourea bis(trifluoroacetate) salt (18a)**. Compound **18c** was treated with $CF_3CO_2H$, as for the synthesis of **16a**, to give **18a** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1170, 3407 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 4.02 (2H, brq,

$J=5.9$ Hz, $CH_2$), 7.09 (3H, br, $N^+H_3$), 7.20 (1H, m, Ar 4-H), 7.40 (2H, m, Ar 5,6-$H_2$), 7.50 (1H, s, Ar 2-H), 8.16 (3H, br, $N^+H_3$), 9.87 (1H, s, NH); NMR ($(CD_3)_2SO$) $\delta_C$ 42.3, 123.4, 123.5, 124.8, 129.1, 134.5, 139.5, 181.1; MS $m/z$ 182.0768 (M+H) ($C_8H_{12}N_3S$ requires 182.0752), 164 (M−$NH_3$).

***N*-(4-Aminomethylphenyl)thiourea bis(trifluoroacetate) salt (18b)**. Compound **18d** was treated with $CF_3CO_2H$, as for the synthesis of **16a** (reaction time 2 h), to give **18b** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1173, 3369 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.96 (2H, q, $J=5.6$ Hz, $CH_2$), 7.38 (2H, d, $J=8.2$ Hz, Ar 2,6-$H_2$), 7.51 (2H, d, $J=8.2$ Hz, Ar 3,5-$H_2$), 7.51 (3H, br, $N^+H_3$), 8.11 (3H, br, $N^+H_3$), 9.83 (1H, s, NH); MS $m/z$ 182.0748 (M+H) ($C_8H_{12}N_3S$ requires 182.0752), 164 (M−$NH_3$).

**1,1-Dimethylethyl *N*-(3-thioureidophenylmethyl)carbamate (18c)**. Compound **29c** was treated with $NH_3$, as for the synthesis of **16c**, to give **18c** (38%) as pale yellow crystals: mp 198–200 °C; IR $\nu_{max}$ 1173, 3291 $cm^{-1}$, NMR $\delta_H$ 1.37 (9H, s, Bu$^t$), 4.01 (2H, d, $J=7.4$ Hz, $CH_2$), 6.96 (1H, d, $J=7.4$ Hz, NH), 7.23 (1H, d, $J=7.8$ Hz, Ar-H), 7.16–7.29 (5H, m, Ar-$H_{3+}NH_2$), 9.78 (1H, s, NH); MS $m/z$ 282.1295 (M+H) ($C_{13}H_{20}N_3O_2S$ requires 282.1276), 226 (M−$Me_2C{=}CH_2$).

**1,1-Dimethylethyl *N*-(4-thioureidophenylmethyl)carbamate (18d)**. Compound **29d** was treated with $NH_3$, as for the synthesis of **16c**, to give **18d** (66%) as white crystals: mp 89–91 °C; IR $\nu_{max}$ 1187, 1690, 3298 $cm^{-1}$; NMR $\delta_H$ 1.46 (9H, s, Bu$^t$), 4.30 (2H, d, $J=6.0$ Hz, $CH_2$), 5.03 (1H, br, NH), 6.25 (2H, br, $NH_2$), 7.19 (2H, d, $J=8.2$ Hz, Ar 3,5-$H_2$), 7.33 (2H, d, $J=8.2$ Hz, Ar 2,6-$H_2$), 8.39 (1H, br, NH); MS $m/z$ 282.1276 (M+H) ($C_{13}H_{20}N_3O_2S$ requires 282.1276), 226 (M−$Me_2C{=}CH_2$); Found C, 54.40: H, 6.73; N, 14.20: $C_{13}H_{19}N_3O_2S$ 0.5$H_2O$ requires C, 53.87; H, 6.78; N, 14.49%.

***N*-(3-Methoxyphenyl)thiourea (18g)**. 3-Methoxyphenylisothiocyanate **29g** was treated with $NH_3$, as for the synthesis of **16c** (chromatography omitted), to give **18g** (99%) as white crystals: mp 160–162 °C (lit.[39] mp 160 °C); IR $\nu_{max}$ 1166, 3149 $cm^{-1}$; NMR $\delta_H$ 3.73 (3H, s, Me), 6.67 (1H, d, $J=7.6$ Hz, Ar 4-H), 6.90 (1H, d, $J=7.6$ Hz, Ar 6-H), 7.10 (1H, s, Ar 2-H), 7.23 (1H, t, $J=7.6$ Hz, Ar 5-H), 7.53 (2H, br, $NH_2$) 9.62 (1H, s, NH); NMR ($(CD_3)_2SO$) $\delta_C$ 55.1, 108.6, 110.0, 115.0, 129.6, 140.3, 159.3, 180.9; MS $m/z$ 365 (2M+H), 183.0598 (M+H) ($C_8H_{11}N_2OS$ requires 183.0592); Found C, 52.50: H, 5.53; N, 15.40; $C_8H_{10}N_2OS$ requires C, 52.72; H, 5.53; N, 15.37%.

***N*-(4-Methoxyphenyl)thiourea (18h)**. 4-Methoxyphenylisothiocyanate **29h** was treated with $NH_3$, as for the synthesis of **18g**, to give **18h** (99%) as white crystals: mp 198–200 °C (lit.[40] mp 210 °C); IR $\nu_{max}$ 1171, 2838, 3154 $cm^{-1}$; NMR $\delta_H$ 3.71 (3H, s, Me), 6.89 (2H, d, $J=8.8$ Hz, Ar 3,5-$H_2$), 7.21 (2H, d, $J=8.8$ Hz, Ar 2,6-$H_2$), 7.23 (2H, br, $NH_2$), 9.47 (1H, s, NH); MS $m/z$ 183.0592 (M+H) ($C_8H_{10}N_2OS$ requires 183.0592).

**3-Thioureidophenylacetic acid hydrochloride (18o)**. Compound **18q** (50 mg, 220 μmol) was stirred in aq HCl (1 M, 3 mL) for 9 d. Evaporation gave **18o** (40 mg, 86%) as white crystals: mp 159–161 °C (lit.[41] mp 174–176 °C); IR $\nu_{max}$ 1157, 1730, 2500, 3337 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.67 (2H, s, $CH_2$), 7.15 (1H, d, $J=7.4$ Hz, Ar 4-H), 7.23 (1H, d, $J=8.6$ Hz, Ar 6-H), 7.25 (1H, s, Ar 2-H), 7.35 (1H, dd, $J=8.6$, 7.4 Hz, Ar 5-H); MS $m/z$ 211.0541 (M+H) ($C_9H_{11}N_2O_2S$ requires 211.0531).

**4-Thioureidophenylacetic acid hydrochloride (18p)**. Compound **18r** (50 mg, 220 μmol) was stirred for 16 h in aq HCl (6 M, 5 mL). Evaporation gave **18p** (60 mg, 99%) as white crystals: mp 198–200 °C (lit.[40] mp 200–203 °C); IR $\nu_{max}$ 1181, 1699, 3313 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.65 (2H, s, $CH_2$), 7.26–7.32 (4H, m, Ar-$H_4$); MS $m/z$ 211.0541 (M+H) ($C_9H_{11}N_2O_2S$ requires 211.0531).

**Methyl 3-thioureidophenylacetate (18q)**. Compound **29q** was treated with $NH_3$, as for the synthesis of **16h**, to give **18q** (99%) as pale yellow crystals: mp 131–133 °C; IR $\nu_{max}$ 1160, 1730, 3409 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.61 (3H, s, Me), 3.65 (2H, s, $CH_2$), 7.00 (1H, d, $J=7.4$ Hz, Ar 4-H), 7.23 (1H, dd, $J=8.6$, 7.4 Hz, Ar 5-H), 7.25 (1H, s, Ar 2-H), 7.33 (1H, d, $J=8.6$ Hz, Ar 6-H), 7.35 (1H, br, NH), 9.71 (1H, s, NH); NMR ($(CD_3)_2SO$)$_C$ 40.1, 51.8, 121.6, 123.8, 125.4, 128.7, 134.9, 139.2, 171.5, 181.1; MS $m/z$ 225.0694 (M+H) ($C_{10}H_{13}N_2O_2S$) requires 225.0698).

**Methyl 4-thioureidophenylacetate (18r)**. Compound **29r** was treated with $NH_3$, as for the synthesis of **16d**, to give **18r** (93%) as a white powder: mp 121–123 °C; IR $\nu_{max}$ 1718, 3168, 3341 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.59 (3H, s, Me), 3.62 (2H, s, $CH_2$), 7.18 (2H, d, $J=8.2$ Hz, Ar 3,5-$H_2$), 7.31 (2H, d, $J=8.2$ Hz, Ar 2,6-$H_2$), 7.43 (2H, br, $NH_2$), 9.65 (1H, s, NH); MS $m/z$ 225.0687 (M+H) ($C_{10}H_{13}N_2O_2S$ requires 225.0698).

**2-(3-(Aminomethyl)phenylamino)-4,5-dihydrothiazole dihydrochloride (19aA)**. Compound **30c** was treated with HCl, as for the synthesis of **17a** (reaction time 43 h), to give **19aA** (99%) as pale buff crystals: mp 208–210 °C; IR $\nu_{max}$ 1640, 3432 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.65 (2H, t, $J=7.7$ Hz, thiazole 5-$H_2$), 4.01 (2H, t, $J=7.7$ Hz, thiazole 4-$H_2$), 4.11 (2H, m, $ArCH_2$), 7.39 (1H, d, $J=7.5$ Hz, Ar 4-H), 7.56 (2H, m, Ar 5,6-$H_2$), 7.62 (1H, s, Ar 2-H), 8.66 (3H, s, $N^+H_3$), 10.5 (1H, br, NH), 12.7 (1H, br, NH); MS $m/z$ 208.0918 (M+H) ($C_{10}H_{14}N_3S$ requires 208.0908); Found C, 37.91: H, 6.06; N, 13.29; $C_{10}H_{13}N_3S$ 2$H_2O$ 2HCl requires C, 38.50; H, 5.67; N, 13.00%.

**2-(3-(Aminomethylphenylamino)-4,5-dihydrothiazole bis(trifluoroacetate) salt (19aB)**. Compound **30c** was treated with $CF_3CO_2H$, as for the synthesis of **16a**, to give **19aB** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1674, 3398 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.62 (2H, t, $J=7.7$ Hz, thiazole 5-$H_2$), 4.0 (4H, m, thiazole 4-$H_2$ + Ar$CH_2$), 7.30 (4H, m, Ar-$H_4$), 7.86 (1H, br, NH), 8.18 (3H, m, $N^+H_3$), 9.73 (1H, br, NH); MS $m/z$ 208.0915 (M+H) ($C_{10}H_{14}N_3S$ requires 208.0908), 191 (M−$NH_3$).

**2 - (4 - (Aminomethyl)phenylamino) - 4,5 - dihydrothiazole bis(trifluoroacetate) salt (19b)**. Compound **30d** was treated with $CF_3CO_2H$, as for the synthesis of **16a** (reaction time 2 h), to give **19b** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1643, 3400 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.60 (2H, t, $J$ = 7.4 Hz, thiazole 5-$H_2$), 4.00 (2H, t, $J$ = 7.4 Hz, thiazole 4-$H_2$), 4.03 (2H, q, $J$ = 6.0 Hz $CH_2N^+H_3$), 4.59 (1H, br, NH), 7.37 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 7.54 (2H, d, $J$ = 8.2 Hz, Ar 5,6-$H_2$), 8.26 (3H, br, $NH_3$); MS $m/z$ 208.0914 (M + H) ($C_{10}H_{14}N_3S$ requires 208.0908), 191 (M − $NH_3$).

**2-(3-Methoxyphenylamino)-4,5-dihydrothiazole (19g)**. Compound **30g** (200 mg, 890 μmol) was stirred in $CF_3CO_2H$ (5 mL) for 3 h. Evaporation and chromatography (EtOAc) gave **19g** (130 mg, 70%) as pale buff crystals: mp 80–82 °C; IR $\nu_{max}$ 1674, 2850, 3238 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 4.17 (3H, s, Me), 3.92 (2H, t, $J$ = 7.6 Hz, thiazole 5-$H_2$), 4.35 (2H, t, $J$ = 7.6 Hz, thiazole 4-$H_2$) 7.24 (1H, d, $J$ = 8.2 Hz, Ar 4-H), 7.34 (1H, d, $J$ = 8.2 Hz, Ar 6-H), 7.41 (1H, s, NH), 7.73 (1H, t, $J$ = 8.2 Hz, Ar 5-H), 7.74 (1H, s, Ar 2-H); MS $m/z$ 209.0749 (M + H) ($C_{10}H_{13}N_2OS$ requires 209.0743).

**2-(4-Methoxyphenylamino)-4,5-dihydrothiazole trifluoroacetate salt (19h)**. Compound **30h** (200 mg, 880 μmol) was boiled under reflux in $CF_3CO_2H$ (5 mL) for 15 h. Evaporation gave **19h** (220 mg, 99%) as white crystals: mp 101–103 °C: IR $\nu_{max}$ 1674, 2750, 3409 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 2.29 (3H, s, Me), 3.60 (2H, m, $CH_2$) 3.75 (2H, m, $CH_2$), 7.02 (2H, d, $J$ = 6.9 Hz, Ar 3,5-$H_2$), 7.25 (2H, d, $J$ = 6.9 Hz, Ar 2,6-$H_2$), 7.97 (1H, br, NH); MS $m/z$ 209.0758 (M + H) ($C_{10}H_{13}N_2OS$ requires 209.0749).

**3-(4,5-Dihydrothiazol-2-ylamino)phenylacetic acid hydrochloride (19o)**. Compound **30q** was treated with aq HCl, as for the synthesis of **17a**, to give **19o** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1633, 1714, 3450 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.55 (2H, t, $J$ = 7.6 Hz, thiazole 5-$H_2$), 3.93 (2H, t, $J$ = 7.6 Hz, thiazole 4-$H_2$), 5.75 (2H, s, $CH_2Ar$), 7.25 (3H, m, Ar-$H_3$), 7.44 (2H, m, Ar-H + NH); NMR ($(CD_3)_2SO$) $\delta_C$ 31.0, 40.5, 48.7, 122.0, 124.3, 129.1, 129.8, 135.8 136.8, 171.2, 173.6; MS $m/z$ 237.0698 (M + H) ($C_{11}H_{13}N_2O_2S$ requires 237.0698).

**4-(4,5-Dihydrothiazol-2-ylamino)phenylacetic acid hydrochloride (19p)**. Compound **30r** was treated with aq HCl, as for the synthesis of **18p**, to give **19p** (99%) as a colourless hygroscopic gum: IR (film) $\nu_{max}$ 1633, 1736, 3423 $cm^{-1}$: NMR ($CD_3OD$) $\delta_H$ 3.67 (4H, s, thiazole 5-$CH_2$, $ArCH_2$), 4.04 (2H, s, thiazole 4-$CH_2$), 7.30 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.43 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$); MS $m/z$ 237.0697 (M + H) ($C_{11}H_{13}N_2O_2S$ requires 237.0698).

**Methyl 3-cyanobenzoate (20i)**. 3-Cyanobenzoic acid **20n** was treated with MeOH and $SOCl_2$, as for the synthesis of **28q**, to give **20i** (42%) as white crystals: mp 38–40 °C (lit.[42] mp 64–65 °C); IR $\nu_{max}$ 1720, 2228 $cm^{-1}$; NMR $\delta_H$ 3.96 (3H, s, Me), 7.95 (1H, t, $J$ = 7.8 Hz, Ar 5-H), 7.84 (1H, d, $J$ = 7.8 Hz, Ar 4-H), 8.27 (1H, d, $J$ = 7.8 Hz, Ar 6-H), 8.34 (1H, s, Ar 2-H); MS $m/z$ 162 (M + H), 147 (M − Me).

**1,1-Dimethylethyl *N*-(3-(aminomethyl)phenylmethyl)carbamate (21c)**. Di(*t*-butyl) dicarbonate (1.0 g, 4.9 mmol) was added slowly to 1,3-bis(aminomethyl)benzene **21a** (2.0 g, 15 mmol) and $Et_3N$ (2.9 g, 29 mmol) in $CH_2Cl_2$ (15 mL) at 0 °C and the mixture was stirred for 16 h. The evaporation residue, in $CH_2Cl_2$, was washed with aq $NaHCO_3$ and dried. Evaporation gave **21c** (900 mg, 78%) as white crystals: mp 61–64 °C (lit.[43] oil); NMR $\delta_H$ 1.51 (9H, s, $Bu^t$), 1.67 (2H, s, $NH_2$), 3.90 (2H, d, $J$ = 4.3 Hz, $CH_2NH_2$), 4.34 (2H, s, $CH_2$NHBoc), 5.10 (1H, br, NH), 7.25 (4H, m, Ar-$H_4$); MS $m/z$ 237 (M + H), 181 (M − $Me_2C{=}CH_2$), 164 (M − $Bu^tO$).

**1,1-Dimethylethyl *N*-(4-(aminomethyl)phenylmethyl)carbamate (21d)**. 1,4-Bis(aminomethyl)benzene **21b** was treated with $Boc_2O$, as for the synthesis of **21c**, to give **21d** (930 mg, 80%) as a colourless oil: (lit.[44] solid) NMR $\delta_H$ 1.46 (9H, s, $Bu^t$), 1.52 (2H, br, $NH_2$), 3.85 (2H, s, $CH_2NH_2$), 4.29 (2H, d, $J$ = 6.0 Hz, $CH_2$NHBoc), 5.89 (1H, br, NH), 7.24–7.28 (4H, m, Ar-$H_4$); MS $m/z$ 237 (M + H), 181 (M − $Me_2C{=}CH_2$), 164 (M − $Bu^tO$).

**Methyl 3-(aminomethyl)benzoate (21i)** and di(3-methoxycarbonylphenylmethyl)amine (**23**). Compound **20i** (900 mg, 5.6 mmol) in MeOH (30 mL) was treated with Pd/C (10%) and $H_2$ for 16 h. Filtration (Celite©), evaporation and chromatography (EtOAc) gave **23** (150 mg, 9%) as a colourless oil: (lit.[45] oil); IR (film) $\nu_{max}$ 1721, 3336 $cm^{-1}$; NMR $\delta_H$ 3.84 (4H, s, 2 × $CH_2$), 3.91 (6H, s, 2 × Me), 7.40 (2H, t, $J$ = 7.8 Hz, 2 × Ar 5-H), 7.52 (2H, d, $J$ = 7.8 Hz, 2 × Ar 4-H), 7.93 (2H, d, $J$ = 7.8 Hz, 2 × Ar 6-H), 8.02 (2H, s, 2 × Ar 2-H); MS $m/z$ 314 (M + H). Further elution gave **21i** (330 mg, 36%) as white crystals: mp 37–39 °C (lit.[45] oil); IR (film) $\nu_{max}$ 1719, 3453 $cm^{-1}$; NMR $\delta_H$ 3.92 (3H, s, Me), 3.95 (2H, s, $CH_2$), 7.40 (1H, t, $J$ = 7.8 Hz, Ar 5-H), 7.53 (1H, d, $J$ = 7.8 Hz, Ar 4-H), 7.92 (1H, d, $J$ = 7.8 Hz, Ar 6-H), 8.01 (1H, s, Ar 2-H); MS $m/z$ 166 (M + H), 121 (M − $NH_2$).

**Methyl 4-(aminomethyl)benzoate hydrochloride (21k)**. 4-Aminomethylbenzoic acid **21j** was treated with MeOH and $SOCl_2$, as for the synthesis of **28q**, to give **21k** (2.2 g, 99%) as white crystals: mp 153–155 °C (lit.[46] mp 235–238 °C); NMR ($(CD_3)_2SO$) $\delta_H$ 3.84 (3H, s, Me), 4.08 (2H, s, $CH_2$), 7.64 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.96 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 8.62 (3H, br, $NH_3$); MS $m/z$ 166 (M + H), 150 (M − $NH_2$).

**1,1-Dimethylethyl *N*-(3-(isothiocyanatomethyl)phenylmethyl)carbamate (22c)**. Compound **21c** (900 mg, 3.9 mmol), $CaCO_3$ (400 mg, 4.0 mmol), water (3 mL), thiophosgene (900 mg, 7.8 mmol) and $CHCl_3$ (25 mL) were stirred vigorously for 16 h. The mixture was extracted with $CHCl_3$. Drying, evaporation and chromatography (EtOAc/hexane 1:3) gave **22c** (400 mg, 37%) as a colourless oil (lit.[44] mp 43 °C); IR (film) $\nu_{max}$ 1670, 2060, 3353 $cm^{-1}$; NMR $\delta_H$ 1.46 (9H, s, $Bu^t$), 4.33 (2H, d, $J$ = 5.6 Hz, $CH_2$NHBoc), 4.70 (2H, s, $CH_2NCS$), 4.91 (1H, br, NH), 7.22–7.27 (3H, m, Ar 2,4,6-$H_3$), 7.34 (1H, t, $J$ = 7.8 Hz, Ar 6-H); MS $m/z$ 279.1163 (M + H) ($C_{14}H_{19}N_2O_2S$ requires 279.1167), 223 (M − $Me_2C{=}CH_2$), 179 (M − Boc), 164 (M − BocNH).

**1,1-Dimethylethyl *N*-(4-isothiocyanatomethyl)phenylmethyl)carbamate (22d)**. Compound **21d** was treated with thiophosgene, as for the synthesis of **22c**, to give **22d** (860 mg, 81%) as pale yellow crystals: mp 80–82 °C (lit.[44] mp 74 °C); IR $\nu_{max}$ 1682, 2091, 3358 $cm^{-1}$; NMR $\delta_H$ 1.46 (9H, s, Bu$^t$), 4.33 (2H, m, C$H_2$NHBoc), 4.70 (2H, s, $CH_2$), 4.88 (1H, br, NH), 7.24–7.35 (4H, m, Ar-$H_4$); MS *m*/*z* 279 (M + H), 164 (M−Bu$^t$O).

**3-Nitrophenylmethylisothiocyanate (22e)**. 3-Nitrobenzylamine **21e** was treated with thiophosgene, as for the synthesis of **22c** (chromatographic eluant EtOAc), to give **22e** (61%) as yellow crystals: mp 65–67 °C (lit.[47] mp 65–67 °C); IR $\nu_{max}$ 1347, 1526, 2135 $cm^{-1}$; NMR $\delta_H$ 4.82 (2H, s, $CH_2$), 7.62 (1H, t, *J* = 7.8 Hz, Ar 5-H), 7.69 (1H, d, *J* = 7.8, Hz, Ar 4-H), 8.20 (1H, s, Ar 2-H), 8.21 (1H, d, *J* = 7.8 Hz, Ar 6-H); MS *m*/*z* 193 (M−H).

**4-Nitrophenylmethylisothiocyanate (22f)**. 4-Nitrobenzylamine **21f** was treated with thiophosgene, as for the synthesis of **22c** (chromatography omitted), to give **22f** (56%) as a colourless oil: (lit.[36] mp 37–38 °C); IR (film) $\nu_{max}$ 1347, 1531, 2070, 3079 $cm^{-1}$; NMR $\delta_H$ 4.88 (2H, s, $CH_2$), 7.52 (2H, d, *J* = 8.9 Hz, Ar 2,6-$H_2$), 8.25 (2H, d, *J* = 8.9 Hz, Ar 3,5-$H_2$); MS *m*/*z* 195 (M + H).

**3-Methoxyphenylmethylisothiocyanate (22g)**. 3-Methoxybenzylamine **21g** was treated with thiophosgene, as for the synthesis of **22c** (chromatographic eluant EtOAc/hexane (1:1)), to give **22g** (40%) as a colourless oil: (lit.[37] oil): IR (film) $\nu_{max}$ 2094 $cm^{-1}$; NMR $\delta_H$ 3.82 (3H, s, Me), 4.68 (2H, s, $CH_2$), 6.87 (3H, m, Ar 2,4,6-$H_3$), 7.30 (1H, t, *J* = 7.8 Hz, Ar 5-H); MS *m*/*z* 180.0460 (M + H) ($C_9H_{10}NOS$ requires 180.0483), 121 (M−NCS).

**4-Methoxyphenylmethylisothiocyanate (22h)**. 4-Methoxybenzylamine **18h** was treated with thiophosgene, as for the synthesis of **22g**, to give **22h** (80%) as a pale yellow oil: (lit.[48] oil); IR (film) $\nu_{max}$ 2087 $cm^{-1}$; NMR $\delta_H$ 3.81 (3H, s, Me), 4.63 (2H, s, $CH_2$), 6.93 (2H, d, *J* = 8.2 Hz, Ar 3,5-$H_2$), 7.22 (2H, d, *J* = 8.2 Hz, Ar 2,6-$H_2$); MS *m*/*z* 179 (M), 121 (M−NCS).

**Methyl 4-(isothiocyanatomethyl)benzoate (22k)**. Compound **21k** was treated with thiophosgene, as for the synthesis of **22f**, to give **22k** (83%) as a pale yellow oil: (lit.[49] oil); IR (film) $\nu_{max}$ 1715, 2089 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.88 (3H, s, Me), 5.05 (2H, s, $CH_2$), 7.49 (2H, d, *J* = 8.4 Hz, Ar 3,5-$H_2$), 7.98 (2H, d, *J* = 8.4 Hz, Ar 2,6-$H_2$); MS *m*/*z* 208 (M + H), 192 (M−Me).

**Methyl 3-(isothiocyanatomethyl)benzoate (22i)**. Compound **21i** was treated with thiophosgene, as for the synthesis of **22e**, to give **22i** (51%) as a colourless oil: (lit.[50] oil); IR (film) $\nu_{max}$ 1722, 2101 $cm^{-1}$; NMR $\delta_H$ 3.94 (3H, s, Me), 4.79 (2H, s, $CH_2$), 7.50 (1H, t, *J* = 7.8 Hz, Ar 5-H), 7.54 (1H, d, *J* = 7.8 Hz Ar 4-H), 8.0 (1H, d, *J* = 7.8 Hz, Ar 6-H), 8.03 (1H, s, Ar 2-H); MS *m*/*z* 208.0434 ($C_{10}H_{10}NO_2S$ requires 208.0432), 149 (M−NCS).

**1,1-Dimethylethyl *N*-(3-(*N*′-(2-hydroxyethyl)thioureidomethyl)phenylmethyl)carbamate (24c)**. Compound **22c** (210 mg, 1.0 mmol) in acetone (1 mL) was added dropwise during 30 min to 2-aminoethanol (60 mg, 1.0 mmol) in acetone (1 mL). The mixture was boiled under reflux for 4 h. Evaporation and chromatography (EtOAc/hexane 1:1) gave **24c** (90 mg, 30%) as a colourless oil: IR (film) $\nu_{max}$ 1170, 1642, 3413 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.45 (9H, s, Bu$^t$), 1.75 (1H, s, NHBoc), 3.75 (2H, m, C$H_2$C$H_2$O), 4.01 (2H, t, *J* = 6.2 Hz, $CH_2O$), 4.26 (2H, m, $ArCH_2$thiourea), 4.85 (2H, d, *J* = 4.7 Hz, $ArCH_2$-NBoc), 5.4 (1H, br, NH or OH), 7.16–7.33 (4H, m, Ar-$H_4$); MS *m*/*z* 340.1695 (M + H) ($C_{16}H_{26}N_3O_3S$ requires 340.1697), 284 (M−$Me_2C{=}CH_2$), 179, 164.

**1,1-Dimethylethyl *N*-(4-(N-(2-hydroxyethyl)thioureidomethyl)phenylmethyl)carbamate (24d)**. Compound **22d** was treated with 2-aminoethanol, as for the synthesis of **24c** (chromatography omitted), to give **24d** (41%) as an oil: NMR $\delta_H$ 1.44 (9H, s, Bu$^t$), 3.69 (4H, m, 2 × $CH_2$), 4.24 (2H, d, *J* = 5.4 Hz, $ArCH_2$), 4.67 (2H, s, $ArCH_2$), 5.04 (1H, s, OH), 6.88 (1H, br, NH), 7.18 (1H, br, NH), 7.20 (2H, d, *J* = 7.0 Hz, Ar 3,5-$H_2$), 7.26 (3H, m, Ar 2,6-$H_2$ + NH); IR (film) $\nu_{max}$ 1171, 1682, 3297 $cm^{-1}$; MS *m*/*z* 340.1695 (M + H) ($C_{16}H_{26}N_3O_3S$ requires 340.1684), 679 (2M + H), 284 (M−$Me_2C{=}CH_2$).

***N*-(2-Hydroxyethyl)-*N*′-(3-nitrophenylmethyl)thiourea (24e)** and 2,2-dimethyl-3-(***N*-(3-nitrophenylmethyl)aminothiocarbonyl)tetrahydrooxazole (25e)**. Compound **22e** was treated with 2-aminoethanol, as for the synthesis of **24c** (chromatographic eluant EtOAc), to give **25e** (50 mg, 19%) as a colourless oil: IR (film) $\nu_{max}$ 1144, 1346, 1534, 3413 $cm^{-1}$; NMR $\delta_H$ 1.80 (6H, s, 2 × Me), 3.82 (2H, br, oxazole 4-$H_2$), 4.05 (4H, t, *J* = 6.6 Hz, oxazole 5-$H_2$), 5.02 (2H, d, *J* = 5.4 Hz, C$H_2$Ar), 5.70 (1H, br, NH), 7.55 (1H, dd, *J* = 8.2, 7.8 Hz, Ar 5-H), 7.72 (1H, d, *J* = 7.8 Hz, Ar 4-H), 8.15 (1H, d, *J* = 8.2 Hz, Ar 6-H), 8.16 (1H, s, Ar 2-H); MS *m*/*z* 296.1066 (M + H) ($C_{13}H_{18}N_3O_3S$ requires 296.1069). Further elution gave **24e** (180 mg, 70%) as pale buff crystals: mp 79–81 °C; IR $\nu_{max}$ 1144, 1348, 1531, 3402 $cm^{-1}$; NMR $\delta_H$ 3.61 (2H, br, $NCH_2$), 3.82 (2H, t, *J* = 4.7 Hz, $OCH_2$), 4.89 (2H, d, *J* = 5.4 Hz, $ArCH_2$), 6.78 (1H, br, OH), 7.28 (1H, br, NH), 7.50 (1H, t, *J* = 7.4 Hz, Ar 5-H), 7.77 (1H, d, *J* = 7.4 Hz, Ar 4-H), 8.11 (1H, d, *J* = 7.4 Hz, Ar 6-H), 8.16 (1H, s, Ar 2-H); MS *m*/*z* 256.0753 (M + H) ($C_{10}H_{14}N_3O_3S$ requires 256.0756).

***N*-(2-Hydroxyethyl)-*N*′-(4-nitrophenylmethyl)thiourea (24f)** and 2,2-dimethyl-3-(N-(4-nitrophenylmethyl)aminothiocarbonyl)tetrahydrooxazole (**25f**). Compound **22f** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **25f** (13%) as a colourless oil: IR (film) $\nu_{max}$ 1143, 1346, 1541, 3380 $cm^{-1}$; NMR $\delta_H$ 1.79 (6H, s, 2 × Me), 3.82 (2H, m, $CH_2N$), 4.05 (2H, t, *J* = 6.2 Hz, $OCH_2$), 5.03 (2H, d, *J* = 5.4 Hz, $ArCH_2$), 5.67 (1H, br, NH), 7.49 (2H, d, *J* = 8.8 Hz, Ar 2,6-$H_2$) 8.17 (2H, d, *J* = 8.8 Hz, Ar 3,5-$H_2$); MS *m*/*z* 296.1063 (M + H) ($C_{13}H_{18}N_3O_3S$ requires 296.1069). Further elution gave **24f** (34%) as a colourless oil: IR (film) $\nu_{max}$ 1160, 1346, 1562, 3368 $cm^{-1}$; NMR $\delta_H$ 3.62 (2H, br, $CH_2Ar$), 3.82 (2H, t, *J* = 5.0 Hz, $NCH_2$), 4.90 (2H, d, *J* = 5.8 Hz, $OCH_2$), 6.54 (1H, br, OH), 7.10 (1H, br, NH), 7.26 (1H, br NH), 7.50 (2H, d, *J* = 8.9 Hz, Ar

2,6-$H_2$), 8.19 (2H, d, $J$ = 8.9 Hz, Ar 3,5-$H_2$); MS $m/z$ 256.0755 (M + H) ($C_8H_{10}N_3O_2S$ requires 256.0756).

***N*-(2-Hydroxyethyl)-*N'*-(3-methoxyphenylmethyl)thiourea (24g)**. Compound **22g** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **24g** (85%) as a colourless oil: NMR ($(CD_3)_2SO$) $\delta_H$ 3.36 (4H, m, 2 × $CH_2$), 3.67 (3H, s, Me), 4.55 (2H, m, $CH_2Ar$), 4.78 (1H, s, OH), 6.80 (1H, dd, $J$ = 7.4, 2.1 Hz, Ar 6-H), 6.86 (1H, dd, $J$ = 7.4, 2.1 Hz, Ar 4-H), 6.85 (1H, s, Ar 2-H), 7.23 (1H, dd, $J$ = 7.4 Hz, Ar 5-H), 7.52 (1H, br, NH), 7.92 (1H, br, NH); NMR ($(CD_3)_2SO$) $\delta_C$ 46.7, 48.5, 55.3, 112.9, 113.2, 119.7, 129.7, 159.6; MS $m/z$ 241.1009 (M + H) ($C_{11}H_{17}N_2O_2S$ requires 241.1011).

***N*-(2-Hydroxyethyl)-*N'*-(4-methoxyphenylmethyl)thiourea (24h)**. 3-Methoxyphenylisothiocyanate **22h** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e** (reaction time 2 h), to give **24h** (47%) as a colourless oil: IR (film) $\nu_{max}$ 1171, 3336 $cm^{-1}$; NMR $\delta_H$ 3.49 (3H, s, Me), 3.66 (2H, br, $CH_2N$), 3.81 (2H, d, $J$ = 4.8 Hz, $CH_2O$), 4.58 (2H, s, $ArCH_2$), 6.48 (1H, br, NH), 6.74 (1H, br, NH), 6.87 (2H, d, $J$ = 8.6 Hz, Ar 3,5-$H_2$), 7.25 (2H, d, $J$ = 8.6 Hz, Ar 2,6-$H_2$); MS $m/z$ 241.1011 (M + H) ($C_{11}H_{17}N_2O_2S$ requires 241.1018).

**Methyl 3-(*N'*-(2-hydroxyethyl)thioureidomethyl)benzoate (24i)**. Compound **22i** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e** (reaction time 2.5 h), to give **24i** (77%) as a colourless oil: IR (film) $\nu_{max}$ 1197, 1715, 3355 $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 3.60 (2H, m, $CH_2$), 3.66 (2H, m, $CH_2$), 3.89 (3H, s, Me), 4.80 (2H, s, $CH_2Ar$), 7.44 (1H, t, $J$ = 7.8 Hz, Ar-$H_5$), 7.56 (1H, d, $J$ = 7.8 Hz, Ar-$H_4$), 7.88 (1H, d, $J$ = 7.8 Hz, Ar-$H_4$), 7.96 (1H, s, Ar-$H_2$); MS $m/z$ 537 (2 M + H), 269.0955 (M + H) ($C_{12}H_{17}N_2O_3S$ requires 269.0960).

**Methyl 4-(*N'*-(2-hydroxyethyl)thioureidomethyl)benzoate (24k)** and 2,2-dimethyl-3-(***N*-(4-methoxycarbonylphenylmethyl)aminothiocarbonyl)tetrahydrooxazole (25k)**. Compound **22k** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **25k** (280 mg, 43%) as white crystals: mp 105–107 °C; IR $\nu_{max}$ 1199, 1701, 3359 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\nu\delta_H$ 1.69 (6H, s, $Me_2C$), 3.82 (3H, s, OMe), 3.64 (2H, t, $J$ = 6.3 Hz, oxazole 4-$CH_2$), 3.96 (2H, t, $J$ = 6.3 Hz, oxazole 5-$H_2$), 4.82 (2H, d, $J$ = 5.5 Hz, $ArCH_2$), 7.39 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.88 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 7.84 (1H, br, NH); MS $m/z$ 309.1263 (M + H) ($C_{15}H_{21}N_2O_3S$ requires 309.1273). Further elution gave **24k** (64%) as pale buff crystals: mp 75–77 °C; IR (film) $\nu_{max}$ 1194, 1714, 3351 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.47 (4H, m, 2 × $CH_2$), 3.82 (3H, s, Me), 4.74 (3H, m, $CH_2Ar$ + OH), 7.38 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$), 7.89 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 7.58 (1H, br, NH), 8.01 (1H, br, NH); MS $m/z$ 269.0961 (M + H) ($C_{12}H_{16}N_2O_3S$ requires 269.0960).

**1,1-Dimethylethyl *N*-(3-nitrophenylmethyl)carbamate (27c)**. Di-*tert*-butyl dicarbonate (1.7 g, 7.8 mmol) was added slowly to 3-nitrobenzylamine **21e** (1.0 g, 6.6 mmol) and $Et_3N$ (1.1 g, 11 mmol) in $CH_2Cl_2$ (25 mL) at 0 °C and the mixture was stirred for 16 h. The evaporation residue, in $CH_2Cl_2$, was washed with aq $NaHCO_3$ and dried. Evaporation and chromatography ($CH_2Cl_2$) gave **27c** (900 mg, 56%) as white crystals: mp 124–126 °C (lit.[51] mp 75–76 °C); NMR $\delta_H$ 1.39 (9H, s, $Bu^t$), 4.35 (2H, d, $J$ = 6.1 Hz, $CH_2$), 5.23 (1H, br, NH), 7.40 (1H, dd, $J$ = 8.9, 7.8 Hz, Ar 5-H), 7.54 (1H, d, $J$ = 7.8 Hz, Ar 6-H), 8.03 (1H, d, $J$ = 8.9 Hz, Ar 4-H), 8.04 (1H, s, Ar 2-H); MS $m/z$ 253.1184 (M + H) ($C_{12}H_{17}N_2O_4$ requires 253.1188), 197 (M − $Me_2C{=}CH_2$); Found C, 57.30: H, 6.35; N, 11.20; $C_{12}H_{15}N_2O_4$ requires C, 57.14; H, 6.35; N, 11.11%.

**1,1-Dimethylethyl *N*-(4-nitrophenylmethyl)carbamate (27d)**. 4-Nitrobenzylamine **21f** was treated with $Boc_2O$, as for the synthesis of **27c**, to give **27d** (91%) as white crystals: mp 111–114 °C (lit.[52] mp 109–110 °C); NMR $\delta_H$ 1.53 (9H, s, $Bu^t$), 4.42 (2H, d, $J$ = 5.9 Hz, $CH_2$), 5.05 (1H, br, NH), 7.44 (2H, d, $J$ = 8.6 Hz, Ar 2,6-$H_2$), 8.19 (2H, d, $J$ = 8.6 Hz, Ar 3,5-$H_2$); MS $m/z$ 505 (2 M + H), 406 (2 M + H − Boc), 275 (M + Na), 253 (M + H), 197 (M − $Me_2C{=}CH_2$), 180 ( M − $Bu^tO$).

**1,1-Dimethylethyl *N*-(3-aminophenylmethyl)carbamate (28c)**. Compound **27c** was treated with $SnCl_2$, as for the synthesis of **28d** (reaction time 30 min), to give **28c** (31%) as a pale buff oil (lit.[53] oil); NMR $\delta_H$ 1.40 (9H, s, $Bu^t$), 3.59 (2H, br, $NH_2$), 4.15 (2H, d, $J$ = 5.7 Hz, $CH_2$), 4.70 (1H, br, NH), 6.55 (3H, m, Ar 2,4,6-$H_3$), 7.06 (1H t, $J$ = 7.7 Hz, Ar 5-H); MS $m/z$ 222 (M), 167 (M − $Me_2C{=}CH_2$), 121 (M − Boc), 106 (M − BocNH).

**1,1-Dimethylethyl *N*-(4-aminophenylmethyl)carbamate (28d)**. Compound **27d** was treated with $SnCl_2$, as for the synthesis of **16l** (chromatography omitted), to give **28d** (31%) as a colourless oil: (lit.[54] mp 75–76 °C); NMR $\delta_H$ 1.45 (9H s $Bu^t$), 3.8 (2H, br, $NH_2$), 4.18 (2H, d, $J$ = 5.1 Hz, $CH_2$), 4.73 (1H, br, NH), 6.64 (2H, d, $J$ = 8.2 Hz, Ar 2,6-$H_2$), 7.07 (2H, d, $J$ = 8.2 Hz, Ar 3,5-$H_2$); MS $m/z$ 445 (2 M + H), 222 (M + H), 165 (M − $Me_2C{=}CH_2$).

**Methyl 3-aminophenylacetate hydrochloride (28q)**. 3-Aminophenylacetic acid **28o** (2.0 g, 13.2 mmol) was stirred with MeOH (350 mL) and $SOCl_2$ (20 mL) for 4 d. Evaporation gave **28q** (2.6 g, 99%) as a colourless hygroscopic gum: (lit.[55] mp 167–170 °C); NMR ($(CD_3)_2SO$) $\delta_H$ 3.60 (2H, s, $CH_2$), 3.41 (3H, br, $N^+H_3$), 3.74 (3H, s, Me), 7.15 (3H, m, Ar-$H_3$), 7.41 (1H, m, Ar 5-H); MS $m/z$ 166 (M + H).

**Methyl 4-aminophenylacetate hydrochloride (28r)**. 4-Aminophenylacetic acid **28p** was treated with MeOH and $SOCl_2$, as for the synthesis of **28q**, to give **28r** (99%) as off-white crystals: mp 118–120 °C (lit.[56] mp 197–199 °C); NMR ($(CD_3)_2SO$) $\delta_H$ 3.47 (3H, br, $NH_3$), 3.59 (3H, s, Me), 3.68 (2H, s, $CH_2$), 7.19 (2H, d, $J$ = 8.6 Hz, Ar 3,5-$H_2$), 7.30 (2H, d, $J$ = 8.6 Hz, Ar 2,6-$H_2$); MS $m/z$ 166 (M + H), 121 (M − $CO_2H$).

**1,1-Dimethylethyl *N*-(3-isothiocyanatophenylmethyl)carbamate (29c)**. Compound **28c** was treated with thiophosgene, as for the synthesis of **22c** (reaction time 2 d),

to give **29c** (70%) as white crystals: mp 70–72 °C; IR $\nu_{max}$ 1675, 2100, 3180 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.39 (9H, s, $Bu^t$), 4.12 (2H, d, $J = 5.9$ Hz, $CH_2$), 7.23 (3H, m, Ar 2,4,6-$H_3$), 7.43 (1H, t, $J = 7.7$ Hz, Ar 5-H), 7.46 (1H, br, NH); MS $m/z$ 265.1012 (M + H) ($C_{13}H_{17}N_2O_2S$ requires 265.1011), 209 (M − $Me_2C{=}CH_2$); Found C, 58.50; H, 6.02; N, 10.40; $C_{13}H_{16}N_2O_2S$ requires C, 59.00; H, 6.06; N, 10.60%.

**1,1-Dimethylethyl *N*-(4-isothiocyanatophenylmethyl)carbamate (29d)**. Compound **28d** was treated with thiophosgene, as for the synthesis of **22c**, to give **29d** (26%) as a pale yellow powder: mp 113–115 °C; IR $\nu_{max}$ 1683, 2123, 3366 $cm^{-1}$; NMR $\delta_H$ 1.46 (9H, s, $Bu^t$), 4.29 (2H, d, $J = 5.6$ Hz, $CH_2$), 4.91 (1H, br, NH), 7.18 (2H, d, $J = 8.6$ Hz, Ar 2,6-$H_2$), 7.27 (2H, d, $J = 8.6$ Hz, Ar 3,5-$H_2$); MS $m/z$ 529 (2 M + H), 265.1006 (M + H) ($C_{13}H_{17}N_2O_2S$ requires 265.1011), 209 (M − $Me_2C{=}CH_2$); Found C, 58.10: H, 5.92; N, 10.30; $C_{13}H_{16}N_2O_2S$ 0.25$H_2O$ requires C, 58.08; H, 6.19; N, 10.42%.

**Methyl 3-isothiocyanatophenylacetate (29q)**. Compound **28q** was treated with thiophosgene, as for the synthesis of **22g**, to give **29q** (77%) as a pale yellow liquid: IR (film) $\nu_{max}$ 1738, 2119 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.61 (2H, s, $CH_2$), 3.71 (3H, s, Me), 7.26 (3H, m, Ar-$H_3$), 7.30 (1H, t, $J = 7.8$ Hz, Ar 5-H); MS $m/z$ 208.0432 (M + H) ($C_{10}H_{10}NO_2S$ requires 208.0432), 192 (M + H − Me), 148 (M + H − NCS).

**Methyl 4-isothiocyanatophenylacetate (29r)**. Compound **28r** was treated with thiophosgene, as for the synthesis of **22f**, to give **29r** (83%) as pale buff oil: (lit.[57] mp 168–170 °C); IR $\nu_{max}$ 1738, 2120 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.61 (3H, s, Me), 3.73 (2H, s, $CH_2$), 7.34 (2H, d, $J = 8.6$ Hz, Ar 2,6-$H_2$), 7.39 (2H, d, $J = 8.6$ Hz, Ar 3,5-$H_2$); MS $m/z$ 208 (M + H), 192 (M − Me).

**1,1-Dimethylethyl *N*-(3-(*N′*-(2-hydroxyethyl)thioureido)-phenylmethyl)carbamate (30c)**. Compound **28c** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e** (reaction time 2 h), to give **30c** (43%) as a colourless oil; IR $\nu_{max}$ (film) 1164, 1693, 3380 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.39 (9H, s, $Bu^t$), 3.50 (2H, br, C$H_2$NH), 3.52 (2H, m, $CH_2O$), 4.04 (2H, d, $J = 6.6$ Hz, C$H_2$NHBoc), 4.80 (1H, s, OH), 6.96 (1H, d, $J = 7.4$ Hz, Ar 4-H), 7.23 (2H, m, NH + Ar 2-H), 7.36 (2H, m, Ar 5,6-$H_2$), 7.66 (1H, br, NH), 9.60 (1H, br, NH); MS $m/z$ 651 (2 M + H), 326.1541 (M + H) ($C_{15}H_{24}N_3O_3S$ requires 326.1538), 270 (M − $Me_2C{=}CH_2$).

**1,1-Dimethylethyl *N*-4-(*N′*-(2-hydroxyethyl)thioureido-phenylmethyl)carbamate (30d)**. Compound **29d** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **30d** (45%) as a colourless oil; IR (film) $\nu_{max}$ 1166, 1689, 3323 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.39 (9H, s, $Bu^t$), 3.32 (2H, m, $CH_2$), 3.52 (2H, m, $CH_2$), 4.07 (2H, d, $J = 6.2$ Hz, C$H_2$NHBoc), 4.94 (1H, br, OH), 7.15 (2H, d, $J = 8.2$ Hz, Ar 3,5-$H_2$), 7.33 (2H, d, $J = 8.2$ Hz, Ar 2,6-$H_2$), 7.38 (1H, br, NH), 7.65 (1H, br, NH), 9.57 (1H, br, NH); MS $m/z$ 326.1552 (M + H) ($C_{15}H_{24}N_3O_3S$ requires 326.1538).

***N*-(2-Hydroxyethyl)-*N′*-(3-methoxyphenyl)thiourea (30g)**. 3-Methoxyphenylisothiocyanate **29g** was treated with 2-aminoethanol, as for the synthesis of **24d** (reaction time 2 h), to give **30g** (84%) as white crystals: mp 129–131 °C; IR $\nu_{max}$ 1149, 2900, 3186 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.52 (4H, m, 2 × $CH_2$), 3.70 (3H, s, Me), 4.80 (1H, br, OH), 6.65 (1H, d, $J = 8.2$ Hz, Ar 6-H), 6.90 (1H, d, $J = 7.4$ Hz, Ar 4-H), 7.20 (2H, m, Ar 2,5-$H_2$), 7.71 (1H, br, NH), 9.41 (1H, s, NH); MS $m/z$ 452 (2 M + H), 227.0846 (M + H) ($C_{10}H_{15}N_2O_2S$ requires 227.0854); Found C, 53.4: H, 6.28; N, 12.36; $C_{10}H_{14}N_2O_2S$ requires C, 53.08; H, 6.24; N, 12.38%.

***N*-(2-Hydroxyethyl)-*N′*-(4-methoxyphenyl)thiourea (30h)**. 4-Methoxyphenylisothiocyanate **29h** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e** (reaction time 1.5 h), to give **30h** (88%) as pale buff crystals: mp 147 °C (lit.[58] mp 146–147 °C); IR $\nu_{max}$ 1165, 2835, 3189, 3646 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.35 (3H, s, Me), 3.73 (4H, s, 2 × $CH_2$), 4.79 (1H, br, OH), 6.88 (2H, d, $J = 8.6$ Hz, Ar 3,5-$H_2$), 7.24 (2H, d, $J = 8.6$ Hz, Ar 2,6-$H_2$), 7.46 (1H, br, NH), 9.41 (1H, s, NH); MS $m/z$ 227.0850 (M + H) ($C_{10}H_{15}N_2O_2S$ requires 227.0854); Found C, 53.0; H, 6.17; N, 12.2; $C_{11}H_{14}N_2O_2S$ requires C, 53.08; H, 6.24; N, 12.38%.

**Methyl 3-(*N′*-(2-hydroxyethyl)thioureido)phenylacetate (30q)**. Compound **29q** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **30q** (64%) as a colourless oil: IR (film) $\nu_{max}$ 1061, 1732, 3293 $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 3.34 (2H, m, $CH_2N$), 3.52 (2H, br, $CH_2O$), 3.59 (3H, s, Me), 3.63 (2H, m, $CH_2Ar$), 4.70 (1H, s, OH), 6.97 (1H, d, $J = 7.4$ Hz, Ar 4-H) 7.24 (1H, dd, $J = 7.8$, 6.3 Hz, Ar 5-H), 7.28 (1H, s, Ar 2-H), 7.33 (1H, d, $J = 6.3$ Hz, Ar 6-H), 7.69 (1H, s, NH), 9.60 (1H, br, NH); MS $m/z$ 537 (2 M + H), 269.0953 (M + H) ($C_{12}H_{17}N_2O_3S$ requires 269.0960).

**Methyl 4-(*N′*-(2-hydroxyethyl)thioureido)phenylacetate (30r)**. Compound **29r** was treated with 2-aminoethanol, as for the synthesis of **24e** and **25e**, to give **30r** (27%) as pale yellow crystals: mp 53–55 °C; IR $\nu_{max}$ 1169, 1730, 3325, 3480 $cm^{-1}$; NMR $\delta_H$ 2.35 (1H, br, OH), 3.64 (2H, s, $CH_2$), 3.72 (3H, s, Me), 3.80 (4H, m, 2 × $CH_2$), 6.56 (1H, br, NH), 7.20 (2H, d, $J = 7.8$ Hz, Ar 3,5-$H_2$), 7.33 (2H, d, $J = 7.8$ Hz, Ar 2,6-$H_2$), 7.94 (1H, br, NH); MS $m/z$ 537 (2 M + H), 269.0933 (M + H) ($C_{12}H_{16}N_2O_3S$ requires 269.0960).

**(*R*)-*N*-(2-Hydroxypropyl)-*N′*-(3-methoxyphenyl)thiourea (31)**. Compound **29g** (500 mg, 3.0 mmol) in acetone (2.1 mL) was added dropwise during 30 min to (*R*)-1-aminopropan-2-ol (420 mg, 4.0 mmol) in acetone (2.1 mL). The mixture was boiled under reflux for 2 h. Evaporation and chromatography (EtOAc/hexane 1:1) gave **31** (270 mg, 38%) as a colourless oil: NMR $\delta_H$ 1.22 (3H, d, $J = 6.3$ Hz, Me), 3.46 (1H, m, C$H$NH), 3.81 (3H, s, OMe), 3.94 (1H, m, C$H$NH), 4.02 (1H, C$H$OH), 6.64 (1H, br, NH), 6.78 (3H, m, Ar 2,4,6-$H_3$), 7.33 (1H, t, $J = 8.2$ Hz, Ar 5-H), 7.76 (1H, br, NH); IR (film) $\nu_{max}$ 1180, 3369 $cm^{-1}$; $[\alpha]_D^{20} = -7.2°$ ($c$ 1.4 mg $mL^{-1}$, MeOH); MS $m/z$ 241.1007 ($C_{11}H_{17}N_2O_2S$ requires 241.1011).

**(*S*)-2-(3-Methoxyphenylamino)-5-methyl-4,5-dihydrothiazole hydrochloride (32).** Compound **31** (80 mg, 0.3 mmol) was boiled under reflux for 24 h in aq HCl (6 M, 4 mL). Evaporation gave **32** (70 mg, 99%) as a colourless hygroscopic gum; NMR $\delta_H$ 1.51 (3H, d, $J = 5.9$ Hz, thiazole-Me), 3.72 (1H, m, 4-H), 3.81 (3H, s, OMe), 4.11 (2H, m, 4-H + 5-H), 6.81 (1H, m, Ar 6-H), 6.88 (1H, d, $J = 8.2$ Hz, Ar 4-H), 7.30 (1H, s, Ar 2-H), 7.32 (1H, t, $J = 8.2$ Hz, Ar 5-H), 10.67 (1H, br, NH), 12.29 (1H, br, NH); IR (film) $\nu_{max}$ 1629, 3434 $cm^{-1}$; $[\alpha]_D^{20} = -32.4°$ (*c* 2.6 mg $mL^{-1}$, MeOH); MS *m*/*z* 223.08963 ($C_{11}H_{16}N_2OS$ requires 223.0905).

## NOS inhibition studies

Measurements of the inhibitory activity of the test compounds against rat nNOS and against rat iNOS were made essentially as described previously by us.[15]

For the studies with human iNOS, the enzyme was prepared as follows. An optimised mammalian expression vector (pEFIRES-P, courtesy of Dr. S. Hobbs, Cancer Research UK Centre for Cancer Therapeutics, ICR, London) was designed to express human iNOS cDNA (courtesy of Prof. Ian Charles, University of London). Expression was linked with the selectable marker gene (pac) at the level of mRNA and antibiotic selection (puromycin) directly enforced expression of the cDNA. This vector was been used to transfect the human fibrosarcoma cell line, HT1080 and a series of iNOS-expressing clones were produced. To avoid loss of viability through the cytotoxic consequences of excessive NO production, clones were grown in the presence of a non-toxic dose of **7** (100 μM). Routinely, clones were grown for 48 h in the absence of puromycin and **7** prior to extracting the iNOS enzyme. Cells were grown to near confluence and were harvested by trypsinisation. Cells were then washed twice in cold phosphate-buffered saline and homogenised in five volumes of ice-cold buffer containing HEPES (10 mM, pH 7.4), sucrose (320 mM), EDTA (100 μM), dithiothreitol (50 μM), leupeptin (10 μg $mL^{-1}$), soybean trypsin inhibitor (10 μg $mL^{-1}$) and aprotinin (2 μg $mL^{-1}$). The preparations were then sonicated using an MSE Soniprep 150 for 3 × 5 s at a nominal frequency of 23 KHz and oscillation amplitude between 5 and 10 μm. Samples were placed in ice between each sonication. These suspensions were allowed to stand in ice for a further 10 min, then centrifuged at 9000*g* for 15 min at 4 °C. The post-mitochondrial supernatant was treated with Dowex-50W [(200–400), 8% cross-linked, $Na^+$ form] to remove endogenous arginine. The supernatant was incubated with the resin for 5 min and centrifuged at 10,000 rpm for 5 min to pellet the resin. This process was repeated twice, after which the cytosol was treated as free of endogenous arginine and was used for assays of inhibition, using the usual protocol with and without pre-incubation of the test compounds with the preparation.

The results are shown in Table 1 as the mean of triplicate experiments ± SEM.

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