N-Phenylamidines as Selective Inhibitors of Human Neuronal Nitric Oxide Synthase: Structure−Activity Studies and Demonstration of in Vivo Activity

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Selective inhibition of the neuronal isoform of nitric oxide synthase (NOS) compared to the endothelial and inducible isoforms may be required for treatment of neurological disorders caused by excessive production of nitric oxide. Recently, we described *N*-(3-(aminomethyl)-benzyl)acetamidine (**13**) as a slow, tight-binding inhibitor, highly selective for human inducible nitric oxide synthase (iNOS). Removal of a single methylene bridge between the amidine nitrogen and phenyl ring to give *N*-(3-(aminomethyl)phenyl)acetamidine (**14**) dramatically altered the selectivity to give a neuronal selective nitric oxide synthase (nNOS) inhibitor. Part of this large shift in selectivity was due to **14** being a rapidly reversible inhibitor of iNOS in contrast to the essentially irreversible inhibition of iNOS observed with **13**. Structure−activity studies revealed that a basic amine functionality tethered to an aromatic ring and a sterically compact amidine are key pharmacophores for this class of NOS inhibitors. Maximal nNOS inhibition potency was achieved with *N*-(3-(aminomethyl)phenyl)-2-furanylamidine (**77**) (K_{i-nNOS} = 0.006 μM; K_{i-eNOS} = 0.35 μM; K_{i-iNOS} = 0.16 μM). Finally, α-fluoro-*N*-(3-(aminomethyl)-phenyl)acetamidine (**74**) (K_{i-nNOS} = 0.011 μM; K_{i-eNOS} = 1.1 μM; K_{i-iNOS} = 0.48 μM) had excellent brain penetration and inhibited nNOS in a rat brain slice assay as well as in the rat brain (cerebellum) in vivo. Thus, *N*-phenylamidines should be useful in validating the role of nNOS in neurological disorders.

Introduction

Nitric oxide (NO) is believed to be involved in a number of physiological processes including inflammation, regulation of blood pressure, transcription, apoptosis, mitochondrial electron transport, platelet adhesion, opioid tolerance, neurotransmission, and defense mechanisms.[1−5] The synthesis of NO is catalyzed by the inducible, endothelial, and neuronal isoforms of nitric oxide synthase (NOS).[6−9] Each of the isoforms converts L-arginine to L-citrulline and nitric oxide utilizing NADPH and O_2 as substrates, as well as flavin-adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin, and heme as cofactors. Although similar in their catalytic mechanism, the NOS isoforms are distinguished by their regulation. The neuronal and endothelial isoforms are present constitutively and are regulated by their dependence on Ca^{2+}/calmodulin concentration, while the inducible isoform does not depend on Ca^{2+} concentration since it already has tightly bound calmodulin. Furthermore, comparison of the primary sequence of the three known human NOS genes reveals an isoform sequence homology of approximately 50% which indicates that there may be isoform structural variability in the active site.

When highly regulated, the production of NO is beneficial to the host organism. It is, however, believed that a number of disease states arise from excessive or inappropriate production of NO including cerebral ischemia, septic shock, and arthritis. Since the three isoforms are found in separate tissues and are involved in unique physiological processes, it is critical that selective inhibition of one isoform over another be achieved to avoid complications resulting from inhibition of normal physiological NO production by all three isoforms.[10,11] Thus, the primary goal of our research is to obtain selective inhibitors of the neuronal and inducible isoforms of nitric oxide synthase over the endothelial isoform.

The first reported NOS inhibitors[12] have been limited to analogues of L-arginine including N^G-methyl-L-arginine (**1**, L-NMMA),[13−16] N^G-nitro-L-arginine (**2**, L-NNA),[14,16,17] N^G-nitro-L-arginine methyl ester (**3**, L-NAME),[15] N^G-amino-L-arginine (**4,** L-NAA),[14,16] and N^δ-(iminoethyl)-L-ornithine (**5**, L-NIO)[15] (Chart 1). The L-arginine analogues have minimal selectivity; therefore compounds with greater selectivity are needed to dissect the pharmacological effects derived from each isoform. Griffith[18,19] and Furfine[20] have reported that L-thiocitrulline (**6**) and *S*-alkyl-L-thiocitrullines (**7**) are potent NOS inhibitors with selectivity for the neuronal isoform. A number of non-amino acid NOS inhibitors that exhibit variable isoform selectivity have appeared in the literature including bisisothioureas (**8**),[21] 7-nitroindazole (**9**),[22] aminoguanidine (**10**),[23] 2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine (**11a**), 2-iminoazaheterocycles (**11b**),[24] and *N*-phenylisothioureas (**12**)[25] (Chart 2).

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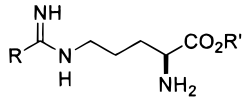
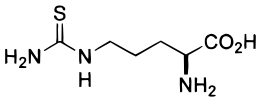
Chart 1. Amino-Acid-Based NOS Inhibitors



1	R = NHMe	R' = H	L-NMMA
2	R = $NHNO_2$	R' = H	L-NNA
3	R = $NHNO_2$	R' = Me	L-NAME
4	R = $NHNH_2$	R' = H	L-NAA
5	R = Me	R' = H	L-NIO



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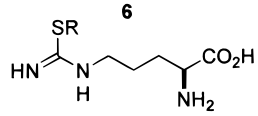
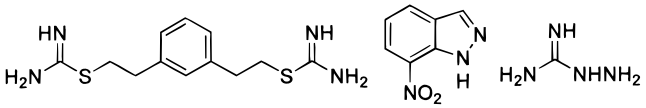
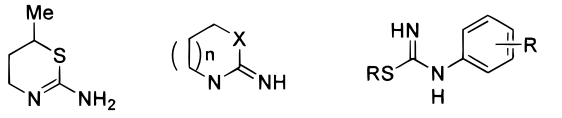


7 R = Et, Me

Chart 2. Non-Amino-Acid-Based NOS Inhibitors

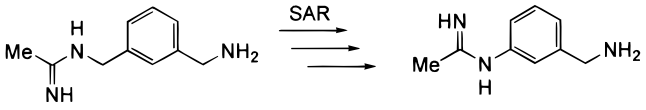


8 **9** **10**



11a **11b** (X = S, O, NH) **12**

Chart 3. Structures of Inducible and Neuronal Selective NOS Inhibitors



13 iNOS selective **14** nNOS selective

During our course of investigations directed toward the discovery of selective NOS inhibitors, *N*-(3-(aminomethyl)benzyl)acetamidine (**13**) was identified as a selective inhibitor of iNOS over eNOS and nNOS (Chart 3).[26] Inhibition of both constitutive isozymes by **13** was rapidly reversible in contrast to the essentially irreversible inhibition of iNOS. The binding constant for the initial reversible complex between **13** and iNOS was 2.0 μM, which was the K_i value for **13** against nNOS. Therefore, much of the selectivity for iNOS could be attributed to interactions that developed slowly. Further studies on **13** revealed that removal of a single methylene unit from **13** to give a substituted *N*-(3-(aminomethyl)phenyl)acetamidine (**14**) provided an inhibitor that exhibited selectivity for nNOS over iNOS and eNOS. This dramatic reversal of isoform selectivity initiated our investigations into the structure−activity relationship of this novel class of non-amino-acid-based NOS inhibitor. A series of structurally modified, substituted *N*-phenylamidines were targeted for evaluation. Herein, we report the synthesis and evaluation of novel, substituted *N*-phenylamidines as inhibitors of human neuronal nitric oxide synthase both in vitro and in vivo.[27]

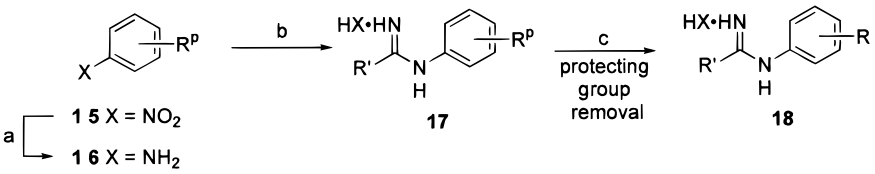
Chemistry

The majority of the targeted *N*-phenylamidines were synthesized as illustrated in Scheme 1. Many of the aniline precursors were commercially available. The key transformation of this sequence involved the use of functionalized thioimidates to convert substituted anilines to *N*-phenyl-substituted acetamidines. The exploitation of thioimidates as general reagents for the synthesis of substituted acetamidines has been limited due to the strong unpleasant odor associated with the liberated thiol. The facile ability of thioimidates to react with nonnucleophilic amines such as substituted anilines, however, suggested these reagents would be superior to conventional *O*-alkyl imidates. Furthermore, a wide array of substituted thioimidates are readily accessible from carboxamides via conversion to the corresponding thioamides with Lawesson's reagent followed by sulfur alkylation with a reactive halide as depicted in Scheme 2. Our initial target syntheses utilized *S*-benzyl thioimidates resulting in the generation of benzylmercaptan as an obnoxious reaction byproduct. The use of 2-naphthylmethyl bromide as a preferred alkylating agent for the conversion of thioamides to thioimidates was developed to advantageously provide reagents that efficiently react with amines to afford acetamidines and a nonodorous thiol byproduct easily removed in a simplistic reaction process.[28]

A protecting group strategy was necessary for the synthesis of targets incorporating amine or carboxylic acid functionalities according to Scheme 1. Acid-labile protecting groups proved to be the most compatible with the amidine functionality with the exception of compound **35**. In this case, the benzyloxycarbonyl (CBZ) group was used to protect the primary amine and was efficiently removed by catalytic hydrogenation.

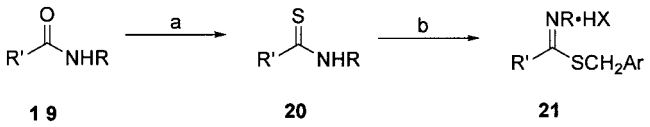
Compounds **36**−**42** and **44** were synthesized as outlined in Scheme 3. Treatment of 3-nitrobenzyl bromide with the appropriate amine and subsequent *N*-BOC protection in the required cases was followed by reduction of the nitro group to provide the requisite substituted aniline intermediates **24**. Conversion to the targeted amidines was achieved by reaction with thioimidates **21a**−**i** and removal of protecting groups when appropriate. Isothiourea **45** was prepared by alkylation of thiourea with 3-nitrobenzyl bromide followed by reduction of the nitro group and treatment with thioimidate **21b**. Similarily, alkylation of *m*-nitrophenacyl bromide (**26**) with thiourea followed by hydrogenation of the nitro group and treatment with thioimidate **21b** provided aminothiazole **66** (Scheme 4). Pyrazole **67** was prepared from 3-nitroacetophenone (**28**) in six steps as illustrated in Scheme 5. Pyrazole formation via an intermediate dimethyleneamide followed by protection as the *tert*-butoxycarbonylcarbamate provided the corresponding nitroaromatic **29**. Hydrogenolysis, treatment with thioimidate **21b**, and deprotection afforded **67**. Amine **35** was prepared from commercially available 3-nitrophenethyl alcohol (**30**) as depicted in Scheme 6. A one-pot reduction−protection sequence followed by alcohol to bromide interconversion provided **31**. Displacement with sodium azide, reduction to the primary amine, *N*-CBZ protection, and subsequent *N*-BOC deprotection provided the requisite aniline **32**. Treatment

Scheme 1[a]



[a] Reagents: (a) H_2, 10% Pd/C; (b) R′C(=NH·HX)SCH_2Ar (**21**), EtOH, rt; (c) HX/AcOH and/or 1,4-dioxane, rt.

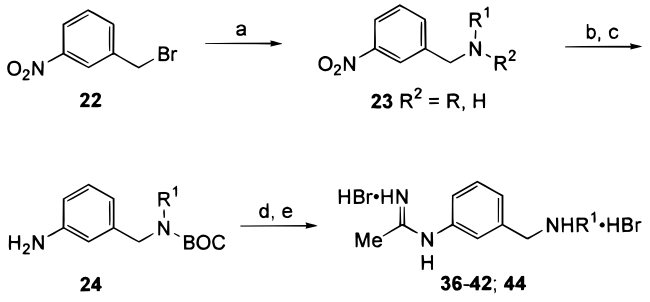
Scheme 2[a]



	R'	Ar
21a (R = H)	Me	phenyl
21b (R = H)	Me	2-napthyl
21c (R = Me)	Me	phenyl
21d (R = H)	FCH_2	phenyl
21e (R = H)	BOCNHCH$_2$	phenyl
21f (R = H)	$MeSCH_2$	2-napthyl
21g (R = H)	2-pyridyl	2-phenyl
21h (R = H)	2-thienyl	2-napthyl
21i (R = H)	2-furanyl	2-phenyl

[a] Reagents: (a) Lawesson's reagent; (b) $ArCH_2X$ (Ar = phenyl or 2-naphthyl, X = Cl, Br), $CHCl_3$, Δ.

Scheme 3[a]



[a] Reagents: (a) NHR^1R^2 or NH_2R^1, toluene, reflux; (b) BOC_2O, THF, satd $NaHCO_3$, rt; (c) H_2, 10% Pd/C; (d) **21b**, EtOH, rt; (e) HBr/AcOH, 1,4-dioxane, rt.

with thioimidate **21a** followed by protecting group removal provided **35**.

Compound **49** was prepared from commercially available 3-aminophenylacetic acid upon treatment with thioimidate **21a** in the presence of triethylamine followed by simultaneous recrystallization and esterification with methanol (Scheme 7). Urea **52** was readily synthesized upon treatment of 3-nitrobenzylamine with 4-morpholinecarbonyl chloride, reduction to the aniline, and amidine formation. Hydrazine **68** was prepared from 3-nitrophenylhydrazine following hydrazine protection, hydrogenolysis, amidine formation, and deprotection (Scheme 7). Carboxylate derivatives **50a** and **63** were prepared as in Scheme 8 from 3-nitrophenylbenzoic acid. Protection of the carboxylic acid as the *tert*-butyl ester followed by hydrogenation provided the required aniline. Acetamidine formation gave rise to **50a** directly, and subsequent ester removal provided **63**. Carboxylic acid **48** was prepared in a similar manner from 3-nitrophenylacetic acid via *tert*-butyl ester **50b**. Last, amide **51** was prepared in a similar fashion from 3-nitrophenylacetic acid following amide formation, hydrogenation, and amidine formation.

The majority of the synthesized compounds had satisfactory elemental analysis with the exception of compounds **35**, **43**, **46**, **49**, **50a**, **54**, **63**, **69**, **72**, and **75**. Although the mass spectral and ^{1}H NMR data for all compounds were consistent with the targeted structures, elemental analysis for these specific compounds indicated that an ammonium halide, derived from hydrolysis of the thioimidate reagent, was present in the synthesized samples. Despite our efforts to purify these compounds using crystallization or reverse-phase high-pressure liquid chromatography, homogeneity could not be reached, and these compounds were tested as is. There was no indication that the impurity had any effect on enzyme activity.

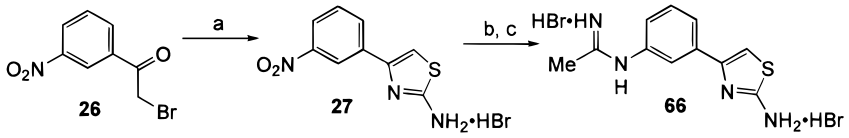
Results and Discussion

N-(3-(Aminomethyl)phenyl)acetamidine (**14**) was a potent and selective inhibitor of nNOS (Table 1). Inhibition was rapidly reversible against each of the three human NOS isoforms. Specifically, time courses in the presence of **14** showed no deviation from linearity when compared to untreated enzyme controls and were competitive with that of arginine.[29] Because of the distinct difference between **14** and the time-dependent benzylacetamidine derivative **13**, *N*-phenylamidine **14** was further studied. *N*-(3-(Aminomethyl)phenyl)acetamidine (**14**) was preincubated with human iNOS for up to 20 min in the absence of L-arginine and in the presence of cofactors. No differences in enzyme activity was observed compared to control when pretreated enzyme was highly diluted into standard assay conditions at 10 μM L-arginine.[29] Thus, the removal of the methylene bridge between the phenyl ring and the acetamidine nitrogen dramatically changed the kinetics of iNOS inhibition. For comparison, data presented here in the structure−activity development of **14** were taken at a single time point as previously described.[21]

In the initial structure−activity relationship studies of **14**, the optimal spatial preference of the free amino group in **14** was investigated. Shifting the aminomethyl functionality to the para position of the aromatic ring as in compound **33** resulted in a significant reduction in nNOS and eNOS inhibition but not iNOS inhibition. Removal of a methylene unit from **14** to give aniline **34** or incorporation of an additional methylene unit to give **35** resulted in a reduction in inhibition of all isoforms. The observed reduction in enzyme inhibition combined with reduced isoform selectivity suggested that alteration of the 3-aminomethyl substituent would have a profound effect on potency as well as isoform selectivity of NOS inhibition.

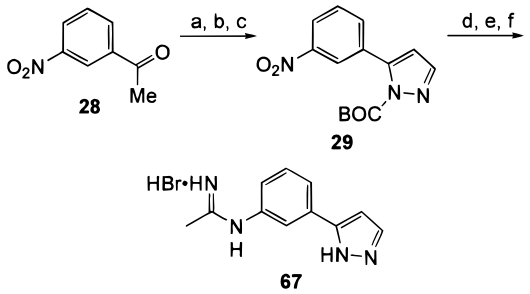
We next examined the effects of amine substitution at the 3-aminomethyl group. Simple monomethylation of **14** to give **36** had little effect on NOS inhibition. Incorporation of two methyl groups to give **37** decreased nNOS and iNOS inhibition approximately 4-fold while

Scheme 4[a]



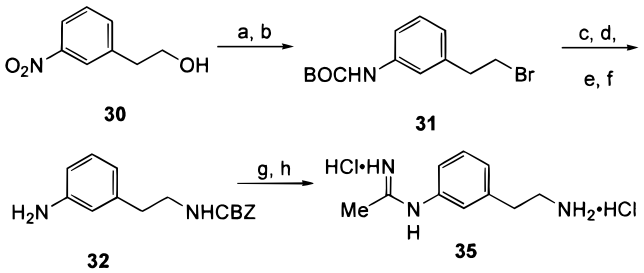
[a] Reagents: (a) thiourea, acetone, rt; (b) H_2, 10% Pd/C; (c) **21b**, EtOH, rt.

Scheme 5[a]



[a] Reagents: (a) $(MeO)_2CHNMe_2$, DMF, 120 °C; (b) H_2NNH_2, MeOH, H_2O, reflux; (c) BOC_2O, DMAP, CH_2Cl_2; (d) H_2, 10% Pd/C; (e) **21b**, EtOH, rt; (f) 48% HBr/AcOH, 1,4-dioxane, rt.

Scheme 6[a]



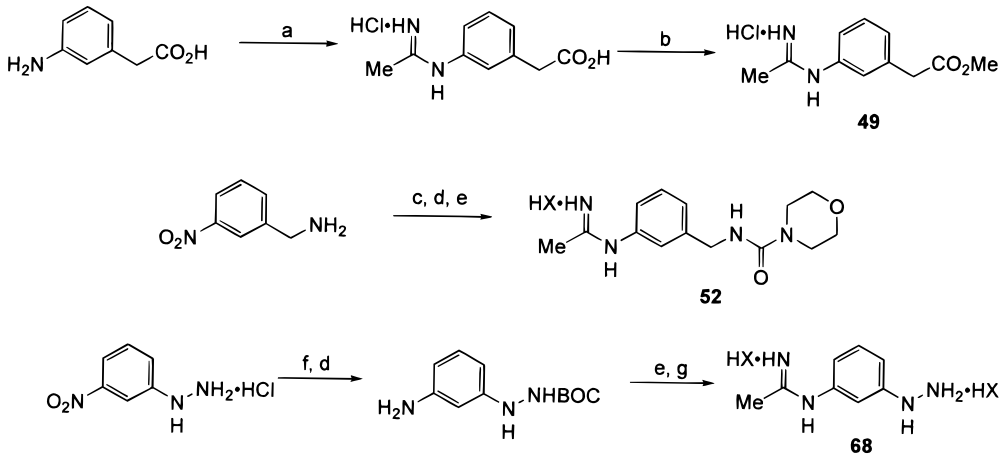
[a] Reagents: (a) H_2, 10% Pd/C, BOC_2O; (b) CBr_4, PPh_3; (c) NaN_3; (d) H_2, Pd/C; (e) CBZ-Cl; (f) TFA, CH_2Cl_2; (g) **21a**, EtOH, rt; (h) H_2, Pd/C, HCl.

having minimal influence on eNOS inhibition. In contrast, other monosubstitutions [2-pyridyl (**41**), *N*-hydroxyl (**42**), acetamidyl (**43**)] and disubstitutions [1,2,3,4-tetrahydroisoquinolinyl (**38**), morpholinyl (**40**)] generally resulted in equivalent or decreased inhibition on all isoforms of NOS. Interestingly, monosubstitution of the aminomethyl group with a 3-acetamidinobenzyl moiety (**44**) provided a potent and selective inhibitor of nNOS. These results suggested that a combination of sterics and electronics may be attenuating the potency of NOS inhibition. The fact that constraining the *N*-alkyl substituents of the aminomethyl group within a ring as in **38** results in a substantial reduction in nNOS inhibition relative to the nonconstrained analogues **37** and **44** indicates sterics as a component of NOS affinity. Moreover, a second effector of NOS affinity is revealed upon comparing inhibition by 2-aminopyridyl and hydroxylamine derivatives **41** and **42** to **14**. Both **41** and **42** are less potent NOS inhibitors than **14**, and this may be explained by the reduced basicity of the respective aminomethyl nitrogens, although subtle steric effects cannot be totally excluded.[32]

A series of compounds were targeted that replaced the amino group of the aminomethyl substituent. Replacement of the amino group with a hydroxyl group (**46**) resulted in a 15-fold reduction in nNOS inhibition and greater than a 25-fold reduction in iNOS inhibition relative to **14**. Incorporation of an α-methyl group (**47**) to the benzylic carbon of **46** significantly diminished nNOS inhibition as compared to **46** providing further evidence of steric factors influencing nNOS inhibition. The decreased nNOS inhibition and selectivity observed with hydroxyl derivatives **46** and **47** are also consistent with nitrogen basicity being a key component for tight nNOS binding. Although **14** and **46** both possess a hydrogen bond donor for potential interaction with the active site, **46** is a 15-fold less potent nNOS inhibitor. One possible explanation for this reduced nNOS inhibition may be the loss of a key charge−charge interaction between the enzyme and inhibitor. The potent inhibitors that possess a basic aminomethyl functionality are most likely charged at physiological pH, and the resulting ammonium ion may be interacting with the enzyme via a key charge−charge interaction. With the exception of isothiourea (**45**), other replacements of the amino group including carboxylic acid (**48**), methyl ester (**49**), *tert*-butyl ester (**50a**), amide (**51**), or urea (**52**) resulted in low or undetectable NOS inhibition providing additional support for the involvement of a charge−charge interaction.

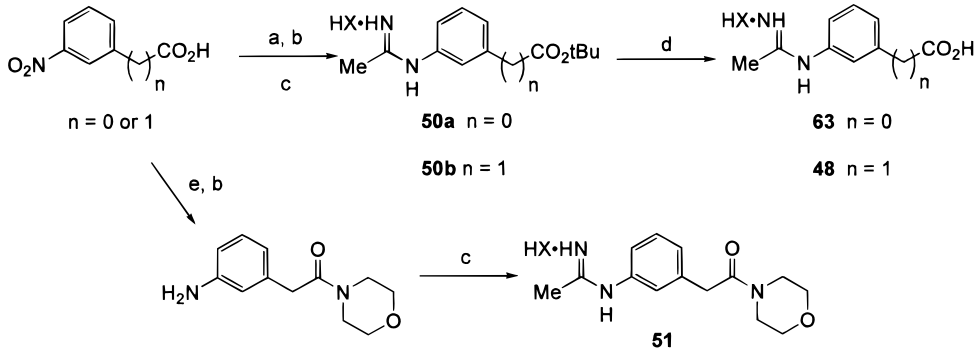
Given the observed effects from amine substitution and amine replacement, a thorough evaluation of aminomethyl replacements was next pursued. Replacement of the aminomethyl functionality with hydrogen (**53**) and electron-withdrawing groups such as fluoro (**54**), bromo (**55**), nitro (**56**), and cyano (**57**) resulted in a decreased ability to inhibit all isoforms of NOS. Similarily, replacement with electron-donating substituents such as amino (**34**), dimethylamino (**58**), methylthio (**59**), methoxy (**60**), and hydroxyl (**61**) as well as replacement with an acetyl group (**62**), carboxylic acid (**63**), carboxylate ester (**50**), amide (**64**), and sulfonamide (**65**) resulted in substantial loss of NOS inhibition. The limited analogues derived from aminomethyl replacements that inhibited NOS all possessed a basic amino group including pyrazole **67**, hydrazine **68**, benzamidine **69**, and amidine **70**. Of this subset, **69** was the most potent which may be rationalized by the presence of a basic nitrogen as well as hydrogen bond donor and charge−charge interaction capabilities that are maintained for interaction with the enzyme. The fact that **69** was a 14-fold weaker nNOS inhibitor than **14** may reflect an unoptimal spatial orientation of the benzamidine in **69** relative to the aminomethyl group in **14**. It is interesting to compare the inhibition potencies of amidine **70** to the corresponding methylene homologue **43** since these two compounds reiterate the importance of functional group spatial orientation. Simply adding one methylene unit (**70** → **43**) between the amine and the aromatic ring resulted in a 4−8-fold increase in NOS inhibition. The observed activity of the above compounds clearly establishes the aminomethyl group as a key pharmacophore for potent NOS inhibition with this class of inhibitor.

Scheme 7[a]



[a] Reagents: (a) **21a**, Et_3N, EtOH; (b) MeOH, Δ; (c) morpholinecarbonyl chloride, Et_3N, THF; (d) H_2, 10% Pd/C, EtOAc; (e) **21b**, EtOH; (f) BOC_2O, satd $NaHCO_3$, THF; (g) HBr/AcOH, dioxane.

Scheme 8[a]



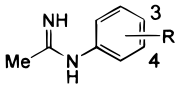
[a] Reagents: (a) BOC_2O, DMAP, ^{t}BuOH; (b) H_2, 10% Pd/C, EtOAc; (c) **21b**, EtOH; (d) HX/dioxane/AcOH; (e) EDC, morpholine, DMAP, CH_2Cl_2.

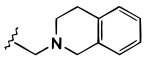
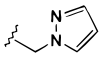
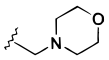
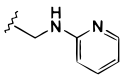
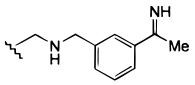
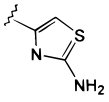
To gain insight into the amidine binding site, a limited number of modified acetamidine analogues were prepared and tested for NOS inhibition in vitro (Table 2). N-Methylation to give **71** resulted in a complete loss of inhibition of all isoforms of NOS. Incorporation of an α-amino group (**72**) at the α-carbon of the acetamidine functionality resulted in a slight reduction in inhibition potency on all isoforms. In contrast, addition of an α-thiomethyl functionality (**73**) or an α-fluoro atom (**74**) increased potency on all isoforms of NOS. This demonstrated that NOS isoform selectivity and inhibition potency could be attenuated with subtle changes at the α-carbon of the acetamidine moiety. Replacement of the acetamidine with a 2-pyridylamidine (**76**) resulted in decreased inhibition of all isoforms of NOS relative to **14**. However, replacement of the pyridyl ring with a furan or thiophene ring system resulted in a substantial increase in potency. Furan **77** and thiophene **78** exhibited a 5−6-fold increase in nNOS inhibition while maintaining modest selectivity over the inducible and endothelial isoforms.

The limited data in Table 2 indicated a combination of electronic and steric effects may be influencing inhibition potency and isoform selectivity with alteration of the acetamidine moiety. Relative to **14**, α-fluoroacetamidine **74** exhibited increased inhibition and was the second most selective compound in this series. A similar increase in nNOS inhibition potency was also observed upon addition of an α-fluoro atom to hydroxy analogue **46** leading to **75**. Since **74** is isosteric with **14**, the increased potency was most likely due to an electronic effect. Given the electronegativity of the α-fluorine, one plausible conclusion is that lowering the pK_a of the acetamidine NH may result in increased NOS inhibition potency. Substitution of the acetamidine in **14** with aryl amidines **76**−**78** provided potent but less selective nNOS inhibitors. The observation that **77** and **78** were more potent nNOS inhibitors than **76** would suggest that incorporation of a basic pyridylamine has a detrimental effect on nNOS inhibition; however, incorporation of a more basic α-amino group (**72**) did not decrease nNOS inhibition relative to **76**. This may suggest that subtle steric changes rather than electronics are attenuating nNOS activity with **76**−**78**. Interestingly, the data in Table 2 revealed that substitution of the acetamidine moiety consistently had a greater effect on iNOS inhibition than nNOS or eNOS inhibition. While potent, selective nNOS inhibitors were attained, this area may prove fruitful for discovery of other more potent and selective NOS inhibitors.

Compounds **14** and **74** were chosen for evaluation in a whole cell assay based on a combination of their nNOS inhibition potency and selectivity over eNOS and iNOS. These two compounds, along with L-NMMA (**1**) and L-NNA (**2**), were incubated in a whole cell assay with intact rat brain slices as previously described.[20] The cell-based data in Figure 1 correlates with the in vitro nNOS inhibition data (Tables 1 and 2) for L-NMMA (**1**),

Table 1. Nitric Oxide Synthase Inhibition of Substituted *N*-Phenylacetamidines



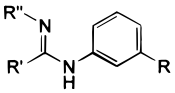
				K_i (μM)[a]			selectivity[b]	
compd	R	isomer	salt	iNOS	eNOS	nNOS	eNOS/nNOS	iNOS/nNOS
1	see Chart 1			0.86	0.40	0.84	0.5	1
2	see Chart 1			0.67	0.03[c]	0.015[c]	2	45
13	see Chart 3		2HCl	0.14[d]	75	2	38	0.1
14	CH_2NH_2	3	2HCl	2.0	6.2	0.04	155	50
33	CH_2NH_2	4	2HBr	2.5	>50	1.5	>33	1.7
34	NH_2	3	2HBr	46	27	14	1.9	3.3
35	$CH_2CH_2NH_2$	3	2HCl	10	33	1	33	10
36	CH_2NHMe	3	2HBr	2.5	1.9	0.056	34	44
37	CH_2NMe_2	3	2HBr	6.1	9.1	0.17	54	36
38		3	2HBr	>50	>50	4.6	>11	>4.5
39		3	2HBr	>50	46	14	3.3	>3.6
40		3	2HBr	10	13	2.8	4.6	3.6
41		3	2HBr	>50	>50	11	>4.5	>4.5
42	CH_2NHOH	3	2HBr	6.3	>50	2.1	>24	3
43	$CH_2NHC(NH)Me$	3	2HBr	2.0	9.5	0.37	26	5.4
44		3	3HBr	1.6	3.0	0.042	71	38
45	$CH_2SC(NH)NH_2$	3	2HBr	4	17	0.36	47	11
46	CH_2OH	3	HCl	>50	15	0.6	25	>83
47	CH(OH)Me	3	HBr	>50	17	39	0.4	>1.3
48	CH_2CO_2H	3	HBr	>50	>50	>50		
49	CH_2CO_2Me	3	HCl	>50	>50	>50		
50a	CO_2tBu	3	HCl	>50	>50	>50		
51	$CH_2C(O)$-*N*-morpholinyl	3	HBr	>50	>50	>50		
52	$CH_2NHC(O)$-*N*-morpholinyl	3	HBr	>50	46	46	>1	>1
53	H		HBr	>50	11	6	1.8	>8
54	F	3	HCl	>50	26	8	3.3	>6
55	Br	3	HCl	>50	>50	13		>3.8
56	NO_2	3	HCl	>50	>50	13		>3.8
57	CN	3	HCl	>50	43	10	4.3	>5
58	NMe_2	3	2HBr	>50	>50	33		>1.5
59	SMe	3	HCl	>50	39	13	3.0	>3.8
60	OMe	3	HCl	>50	38	>50	<0.8	
61	OH	3	HBr	20	14	>50	0.3	<0.3
62	C(O)Me	3	HCl	>50	44	5.6	7.9	7.9
63	CO_2H	3	HCl	>50	>50	>50		
64	$C(O)NH_2$	3	HCl	>50	>50	7.2	>6.9	>6.9
65	SO_2NH_2	3	HBr	46	8	29	0.3	1.6
66		3	2HBr	>50	>50	7.5	>6.7	>6.7
67		3	HBr	>50	27	4.6	5.9	>10
68	$NHNH_2$	3	2HBr	4.6	7.9	1.1	7.2	4.2
69	$C(NH)NH_2$	3	2HCl	15	36	0.57	63	26
70	NHC(NH)Me	3	2HBr	17	38	1.5	25	11

[a] Inhibition constants were obtained by measuring percent inhibition on the human isoforms of NOS with at least three concentrations of inhibitor at 10 min as described in ref 21. Values had a standard deviation of ≤10% ($n \geq 3$). [b] Defined as the ratio of K_i eNOS or K_i iNOS to K_i nNOS. [c] Inhibition of eNOS and nNOS by **2** has been shown to be time-dependent.[17] The values of 0.03 and 0.015 μM were determined at 10 min and are shown for comparison. [d] Inhibition of iNOS by **13** was shown to be time-dependent and essentially irreversible.[26] The value of 0.14 μM was determined at 10 min and is shown for comparison.

L-NNA (**2**), and **74**. However, **14** is a much weaker nNOS inhibitor in the brain slice assay as compared to the in vitro data which may be a result of poor cellular uptake.

Prior to in vivo evaluation, we were interested in determining (1) the pharmacokinetic properties of **14** and **74** and (2) if this structual class of molecules can penetrate the blood−brain barrier. The pharmacoki-

Table 2. Effect of Amidine Structure on Nitric Oxide Synthase Inhibition[a]



					K_i (μM)			selectivity[b]	
compd	R	R′	R″	salt	iNOS	eNOS	nNOS	eNOS/nNOS	iNOS/nNOS
14	CH_2NH_2	Me	H	2HCl	2.0	6.2	0.04	155	50
71	CH_2NH_2	Me	Me	2HBr	>50	>50	>50		
72	CH_2NH_2	CH_2NH_2	H	3HBr	3.4	13	0.19	68	18
73	CH_2NH_2	CH_2SMe	H	2HBr	0.021	0.32	0.011	29	2
74	CH_2NH_2	CH_2F	H	2HBr	0.48	1.1	0.011	100	44
75	CH_2OH	CH_2F	H	2HBr	17	3.8	0.30	13	57
76	CH_2NH_2	2-pyridyl	H	2HBr	37	13	0.33	39	112
77	CH_2NH_2	2-furanyl	H	2HBr	0.16	0.35	0.0063	56	25
78	CH_2NH_2	2-thienyl	H	2HBr	0.12	0.40	0.0087	46	14

[a] Inhibition constants were obtained as described in Table 1 using the human isoforms of NOS.

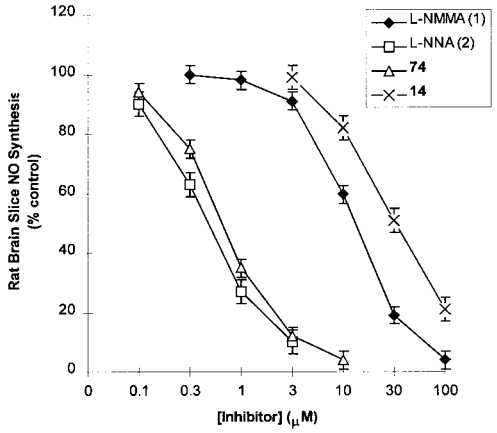


Figure 1. Effect of NOS inhibitors L-NMMA (**1**), L-NNA (**2**), **74**, and **14** on nitric oxide synthase activity in rat cerebral cortical slices. Enzyme activity was measured as described in ref 20. Results are mean ± SEM (n = 3).

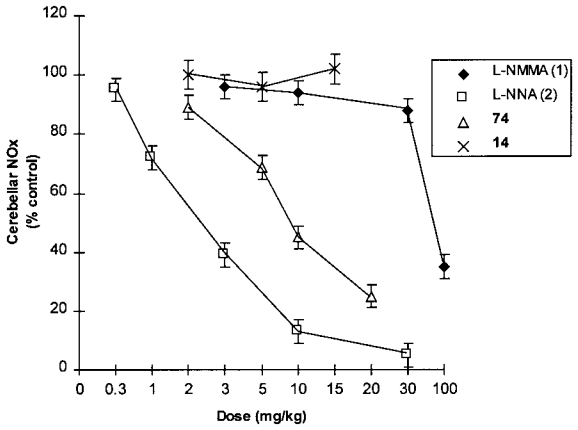


Figure 2. Effect of NOS inhibitors on brain (cerebellar) nitrite/nitrate in vivo following iv administration (4 mL/kg of rat body weight) of L-NMMA, L-NNA, **74**, and **14** to rats at the indicated doses. Method of ex vivo measurement of brain nitrite/nitrate was according to ref 33 and reflects nitric oxide synthase activity in vivo. Results are mean ± SEM (n = 3). All data were calculated as the percent of their own experimental control (i.e., vehicle only). L-NMMA was administered as the HCl salt. L-NNA was placed in a sonicating bath to solubilize it prior to administration.

netic properties of acetamidine **14** and **74** were determined in male Harlan Sprague−Dawley rats. After intravenous administration at 10 mg/kg, compounds **14** and **74** were rapidly cleared (4.4 and 4.7 L/kg/h, respectively) and had terminal elimination half-lives of 0.76 and 1.7 h, respectively. Although the high steady-state volumes of distribution (2.1 and 8.0 L/kg, respectively) suggested good tissue distribution, brain penetration of **14** was limited with brain concentrations decreasing in parallel with plasma concentrations. However, brain penetration of **74** was rapid and extensive. Interestingly, brain concentrations of compound **74** were maintained while plasma levels declined. Following oral administration at 50 mg/kg, the estimated oral bioavailability of **14** was 17% while that of α-fluoro derivative **74** was 80%.

Since compounds **14** and **74** were potent NOS inhibitors against purified enzyme, demonstrated nNOS inhibition in a whole cell assay, and possessed suitable pharmacokinetic properties, these compounds were chosen for evaluation in male Wistar rats in an ex vivo assay of in vivo brain (cerebellum) NOS activity.[33] Intravenous administration of **1**, **2**, **14**, and **74** at varying concentrations to male Wistar rats and ex vivo measurement of brain (cerebellar) nitrite/nitrate production as previously described provided the data in Figure 2. L-Nitroarginine (**2**) was the most potent nNOS inhibitor in this assay. Compound **74**, though slightly less potent than L-nitroarginine (**2**), inhibited brain (cerebellar) nitrite/nitrate production in a dose-dependent manner thus demonstrating an ability to inhibit neuronal NOS activity in vivo. L-NMMA (**1**) was less efficacious than **2** and **74** in the in vivo model. Compound **14** did not inhibit NOS activity in vivo at the doses given in this assay. This lack of inhibition can readily be explained by the relatively poor brain penetration observed in the tissue distribution studies and the poor cellular uptake suggested by the brain slice experiments.

Summary

Herein, substituted *N*-phenylamidines have been shown to be potent inhibitors of NOS that are selective for the neuronal isoform. Relative to substituted *N*-benzylamidines (cf. **13**), selectivity for nNOS vs iNOS has changed over 500-fold, with a large part of this change being due to the change in iNOS inhibition from time-dependent inactivation (*N*-benzylamidines) to relatively weak, rapidly reversible inhibition (*N*-phenylamidines). This investigation reveals some interesting trends with respect to inhibition of the different isoforms of NOS by substituted *N*-phenylacetamidines. First,

substitution at the meta position with a number of functional groups that possess various steric and electronic properties provides inactive or weakly active NOS inhibitors. One exception is the presence of a monosubstituted aminomethyl moiety at the 3-position that consistently exhibits selective, submicromolar inhibition of the neuronal isoform of NOS over the endothelial and inducible isoforms. The corresponding acetamidine binding site also appears to have both steric and electronic components that can greatly influence inhibition potency and isoform selectivity. Of the compounds tested, maximal inhibition potency and selectivity for nNOS were achieved with *N*-(3-(aminomethyl)phenyl)-2-furanylamidine (**77**) ($K_{i-nNOS} = 0.006\ \mu M$; $K_{i-eNOS} = 0.35\ \mu M$; $K_{i-iNOS} = 0.16\ \mu M$) and *N*-(3-(aminomethyl)-phenyl)acetamidine (**14**) ($K_{i-nNOS} = 0.04\ \mu M$; $K_{i-eNOS} = 6.2\ \mu M$; $K_{i-iNOS} = 2.0\ \mu M$), respectively. Functionalized α-fluoroacetamidine derivative **74** inhibited nNOS activity in a rat brain slice assay and demonstrated brain nNOS inhibitory activity in vivo in male Wistar rats. In light of the physiological importance of NO production, this class of potent nNOS inhibitor should prove useful for deciphering the role of excess NO production in certain disease states.

Experimental Section

Proton (^{1}H) nuclear magnetic resonance spectra were recorded on Varian XL-200 MHz or XL-300 MHz spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (δ 0.0) with peak multiplicities abbreviated as singlet, s; broad singlet, brs; doublet, d; triplet, t; multiplet, m. Coupling constants are reported in hertz. Mass spectra were recorded on a Hewlett-Packard Series 1050 automated mass spectrometer. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Capillary zone electrophoresis (16 kV, 210-nm absorbance) was performed on a fused silica capillary column (75 μm × 40 cm) using 50 mM HAS, 20 mM phosphoric acid, pH 2.1. All reagents and solvents used were reagent grade and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using E. Merck precoated silica gel 60 F_{254} (0.25-mm thickness) plates. The plates were visualized by 254-nm UV light, iodine chamber, and/or immersion in ninhydrin or phosphomolybdic acid stain and warming on a hot plate. Merck silica gel 60 (230−400 mesh) was utilized for flash chromatography. *S*-Benzylthioacetimidate (**21a**) hydrochloride or hydrobromide and *S*-(2-naphthylmethyl)thioacetimidate (**21b**) hydrobromide were prepared as described.[28] Phenylacetamidine hydrobromide (**53**) was prepared as described.[28]

***S*-Benzyl-*N*-methylthioacetimidate·HBr (21c).** To a stirred solution of *N*-methylthioacetamide (4.74 g, 53.2 mmol) in $CHCl_3$ was added benzyl bromide (9.20 g, 53.8 mmol). The mixture was heated to reflux for 1.5 h, cooled to room temperature, and poured into Et_2O (350 mL). The resulting white precipitate was collected and dried in vacuo to give **21c** (13.2 g, 95%) as a white solid: mp 174−175 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.35 (m, 5 H), 4.60 (s, 2 H), 3.17 (s, 3 H), 2.66 (s, 3 H). Anal. ($C_{10}H_{13}NS$·HBr) C, H, N, S, Br.

The following compounds were prepared in an analogous manner.

***S*-Benzyl-2-fluorothioacetimidate·HBr (21d):** prepared as an orange solid from α-fluorothioacetamide[34] (5.09 g, 54.6 mmol) in 49% yield; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.37 (m, 5 H), 5.66 (d, 1 H), 4.81 (s, 2 H).

***S*-Benzyl-2-[*N*-(*tert*-butoxycarbonyl)amino]thioacetimidate·HBr (21e):** prepared as a white solid from *N*-(*tert*-butoxycarbonyl)thioglycinamide[35] (1.50 g, 7.88 mmol) in 45% yield; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.40 (s, 9 H), 7.79 (t, 1 H), 7.42 (m, 5 H), 4.56 (s, 2 H), 4.27 (d, 2 H), 1.40 (s, 9 H).

***S*-(2-Naphthylmethyl)-2-(thiomethyl)thioacetamide·HBr (21f):** prepared from 2-(thiomethyl)thioacetamide[36] (1.10 g, 9.09 mmol) and 2-(bromomethyl)naphthylene (2.01 g, 9.09 mmol) in 27% yield; ^{1}H NMR (DMSO-d_6, D_2O, 200 MHz) δ 7.91−7.44 (m, 7 H), 4.67 (s, 2 H), 3.89 (s, 2 H), 2.09 (s, 3 H).

***S*-Benzyl-2-pyridylthioacetimidate·HBr (21g):** prepared as a pale-orange solid from pyridine-2-thiocarboxamide[37] (324 mg, 2.34 mmol) in 73% yield; mp 186 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.80 (d, 1 H), 8.26 (d, 1 H), 8.14 (t, 1 H), 7.78 (m, 1 H), 7.34 (m, 5 H), 4.61 (s, 2 H). Anal. ($C_{13}H_{12}N_2S$·HBr) C, H, N, S, Br.

***S*-(2-Naphthylmethyl)-2-thienylthioacetimidate·HBr (21h):** prepared as a white solid from thiophene-2-thiocarboxamide (1.53 g, 10.7 mmol) and 2-(bromomethyl)naphthylene (2.37 g, 10.7 mmol) in 90% yield; mp 200−201 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.31−7.40 (m, 10 H), 4.91 (s, 2 H). Anal. ($C_{16}H_{13}NS_2$·HBr) C, H, N, S, Br.

***S*-Benzyl-2-furfurylthioacetimidate·HBr (21i):** prepared as a white solid from furan-2-thiocarboxamide[38] (2.00 g, 18.0 mmol) in 82% yield; mp 172−174 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.99 (s, 1 H), 7.72 (d, 1 H), 7.44 (m, 5 H), 6.79 (d, 1 H), 4.63 (s, 2 H). Anal. ($C_{12}H_{11}NOS$·HBr) C, H, N, S, Br.

***N*-(3-Fluorophenyl)acetamidine·HCl (54).** A solution of 3-fluoroaniline (1.0 g, 9.0 mmol) in 15 mL of absolute EtOH at room temperature was treated with thioimidate **21a**·HCl (1.8 g, 9.0 mmol). After stirring overnight, the reaction mixture was concentrated to approximatley 5 mL and diluted with Et_2O. A solid was collected by filtration and recrystallized from MeOH/Et_2O to give 816 mg (48%) of the title compound as a white solid: mp 175−177 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.60−7.44 (m, 1 H), 7.28−7.05 (m, 3 H), 2.37 (s, 3 H); low-resolution MS (CI) 153 (MH^+). Anal. ($C_8H_9FN_2$·HCl·0.15NH_4Cl) C, H, N, Cl.

The following compounds were prepared in an analogous manner.

***N*-(3-(Aminoethyl)phenyl)acetamidine·2HCl (35):** prepared from 3-(*N*-(benzyloxycarbonyl)aminoethyl)aniline and thioimidate **21a**·HCl; deprotection [MeOH/3 M HCl in EtOH (3:1), 10% palladium on carbon, H_2] provided 950 mg of title compound as a gold solid; ^{1}H NMR (300 MHz, D_2O) δ 7.40−7.10 (m, 4 H), 3.14 (t, 2 H, J = 7.1), 2.89 (t, 2 H, J = 7.1), 2.25 (s, 3 H); low-resolution MS (CI) 178 (MH^+). Anal. ($C_{10}H_{15}N_3$·2HCl·1.25H_2O·0.6NH_4Cl) Calcd: C, 37.15; H, 6.98; N, 19.93; Cl, 28.51. Found: C, 37.38; H, 7.19; N, 13.05; Cl, 28.63. Capillary zone electrophoresis on a fused silica column (75 μm × 40 cm) at pH = 2, t_R = 5.06 min, 96.2% organic purity.

***N*-(3-(Hydroxymethyl)phenyl)acetamidine·HCl (46):** prepared from 3-aminobenzyl alcohol and thioimidate **21a**·HCl to give 2.0 g (100% yield) of title compound as a white solid; ^{1}H NMR (300 MHz, D_2O) δ 7.40 (t, 1 H, J = 7.7), 7.30 (d, 1 H, J = 7.4), 7.18 (s, 1H), 7.13 (d, 1 H, J = 7.9), 4.52 (s, 2 H), 2.26 (s, 3 H); low-resolution MS (CI) 165 (MH^+). Anal. ($C_9H_{13}N_2O$·HCl·0.25H_2O·0.35NH_4Cl) C, H, N, Cl. Capillary zone electrophoresis on a fused silica column (75 μm × 40 cm) at pH = 2, t_R = 7.78 min, 99.3% purity.

(+)-*N*-(3-(1-Hydroxyethyl)phenyl)acetamidine·HBr (47): prepared from (±)-3-(1-hydroxyethyl)aniline using thioimidate **21b**·HBr to give 2.1 g (88%) of title compound as an off-white solid; mp 132−135 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.45−7.28 (m, 2 H), 7.19 (s, 1 H), 7.12 (br d, 1 H, J = 7.8), 4.80 (q, 1 H, J = 6.6), 2.26 (s, 3 H), 1.32 (d, 3 H, J = 6.6); low-resolution MS (CI) 179 (MH^+). Anal. ($C_{10}H_{14}N_2O$·HBr) C, H, N, Br.

***tert*-Butyl 3-(acetimidoylamino)benzoate·HCl (50a):** prepared from *tert*-butyl 3-aminobenzoate and thioimidate **21a**·HCl; recrystallization from MeOH/Et_2O provided 1.78 g (51%) of title compound as a white solid; mp 172−173 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.92 (d, 1 H, J = 7.6), 7.75 (s, 1 H), 7.60−7.35 (m, 2 H), 2.29 (s, 3 H), 1.44 (s, 9 H); low-resolution MS (CI) 235 (MH^+). Anal. ($C_{13}H_{18}N_2O_2$·HCl·0.35NH_4Cl) C, H, N, Cl.

***N*-(3-Bromophenyl)acetamidine·HCl (55):** prepared from 3-bromoaniline using thioimidate **21a**·HCl. Recrystallization from MeOH/Et_2O gave 800 mg of a solid. The solid was dissolved in Et_2O/saturated $NaHCO_3$, and the aqueous layer was extracted with Et_2O. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was treated with Et_2O and a small amount of HCl/ethanol. The resulting white solid was collected to give 778 mg (54%) of title compound: mp 208−210 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.51 (d, 1 H, J = 7.5), 7.43 (br s), 7.31 (t, 1 H, J = 8.0), 7.18 (d, 1 H, J = 7.5), 2.26 (s, 3 H); low-resolution MS (CI) 213 (M^+), 215 (M^+). Anal. ($C_8H_9BrN_2$·HCl) C, H, N.

***N*-(3-Nitrophenyl)acetamidine·HCl (56):** prepared from 3-nitroaniline using thioimidate **21a**·HCl. Recrystallization from MeOH/Et_2O gave 513 mg of a solid. The solid was dissolved in CH_2Cl_2/saturated $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in 2-propanol and treated with a small amount of HCl/ethanol. Et_2O was added, and the resulting white solid was collected to give 310 mg (20%) of title compound: mp 226−228 °C; ^{1}H NMR (D_2O, 300 MHz) δ 8.24−8.16 (m, 1 H), 8.12 (br s, 1 H), 7.70−7.58 (m, 2 H), 2.31 (s, 3 H); low-resolution MS (CI) 180 (MH^+). Anal. ($C_8H_9N_3O_2$·HCl) C, H, N, Cl.

***N*-(3-Cyanophenyl)acetamidine·HCl (57):** prepared from 3-aminobenzonitrile using thioimidate **21a**·HCl. Recrystallization from MeOH/Et_2O gave 814 mg of a solid. The solid was dissolved in Et_2O/saturated $NaHCO_3$, and the aqueous layer was extracted with Et_2O. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in Et_2O and treated with a small amount of HCl/ethanol. The resulting white solid was collected to give 576 mg (35%) of title compound: mp 228−230 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.72 (dt, 1 H, J = 6.0, 1.8), 7.64 (br s, 1 H), 7.62−7.48 (m, 2 H), 2.28 (s, 3 H); low-resolution MS (CI) 160 (MH^+). Anal. ($C_9H_9N_3$·HCl) C, H, N, Cl.

***N*-(3-(Dimethylamino)phenyl)acetamidine·2HBr (58):** prepared from *N*,*N*-dimethyl-1,3-phenylenediamine using thioimidate **21a**·HBr. After removal of EtOH, the reaction was diluted with 10 mL of 1,4-dioxane and treated with 2 mL of 30% HBr/AcOH. After the mixture stirred for 5 min Et_2O was added, and stirring was continued for 30 min. The mixture was filtered, and the collected solid was recrystallized from MeOH/EtOAc to give 1.47 g (72%) of title compound as a white solid: mp 227−230 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.65−7.32 (m, 4 H), 3.15 (s, 6 H), 2.30 (s, 3 H); low-resolution MS (CI) 178 (MH^+). Anal. ($C_{10}H_{15}N_3$·2HBr) C, H, N, Br.

***N*-(3-(Methylthio)phenyl)acetamidine·HCl (59):** prepared from 3-(methylmercapto)aniline using thioimidate **21a**·HCl. Recrystallization from MeOH/Et_2O gave 1.11 g of a purple solid. The resulting solid was dissolved in Et_2O/saturated $NaHCO_3$, and the aqueous layer was extracted with Et_2O. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in Et_2O and treated with a small amount of HCl/ethanol. The resulting solid was collected to give 531 mg (34%) of title compound: mp 134−135 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.33 (t, 1 H, J = 7.8), 7.24 (dd, 1 H, J = 7.8, 1.5), 7.09 (d, 1 H, J = 1.5), 6.97 (d, 1 H, J = 7.8), 2.36 (s, 3 H), 2.25 (s, 3 H); low-resolution MS (CI) 181 (MH^+). Anal. ($C_9H_{12}N_2S$·HCl) C, H, N, Cl, S.

***N*-(3-Methoxyphenyl)acetamidine·HCl (60):** prepared from 3-methoxyaniline using thioimidate **21b**·HBr. The resulting solid was dissolved in Et_2O/saturated $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in Et_2O and treated with a small amount of HCl/ethanol. The resulting solid was recrystallized from MeOH/Et_2O to give 267 mg (16%) of title compound: mp 185−188 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.40−7.20 (m, 1 H), 7.00−6.65 (m, 3 H), 3.70 (s, 3 H), 2.25 (s, 3 H); low-resolution MS (CI) 165 (MH^+). Anal. ($C_9H_{12}N_2O$·HCl) C, H, N, Cl.

***N*-(3-Hydroxyphenyl)acetamidine·HBr (61):** prepared from 3-aminophenol using thioimidate **21a**·HBr; trituration with MeOH/Et_2O provided 1.9 g (90%) of title compound as a white solid; mp 115−118 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.25 (dt, 1 H, J = 8.0, 2.1), 6.81 (d, 1 H, J = 8.0), 6.73 (d, 1 H, J = 8.0), 6.66 (br s, 1 H), 2.24 (s, 3 H); low-resolution MS (CI) 151 (MH^+). Anal. ($C_8H_{10}N_2O$·HBr·0.35H_2O) C, H, N, Br.

***N*-(3-Acetylphenyl)acetamidine·HCl (62):** prepared from 3-aminoacetophenone using thioimidate **21a**·HCl. Recrystallization from MeOH/Et_2O gave 1.16 g of a white solid. The solid was dissolved in Et_2O/saturated $NaHCO_3$, and the aqueous layer was extracted with Et_2O. The combined organics were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in Et_2O and treated with a small amount of HCl/ethanol. The resulting white solid was collected to give 325 mg (21%) of title compound: mp 204−206 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.94 (d, 1 H, J = 7.8), 7.77 (br s, 1 H), 7.55 (t, 1 H, J = 7.8), 7.46 (br d, 1 H, J = 7.8), 2.52 (s, 3 H), 2.29 (s, 3 H); low-resolution MS (CI) 177 (MH^+). Anal. ($C_{10}H_{12}N_2O$·HCl) C, H, N, Cl.

3-(Acetimidoylamino)benzamide·HCl (64): prepared from 3-aminobenzamide and thioimidate **21a**·HCl; recrystallization from MeOH/Et_2O provided 1.1 g (70%) of title compound as a white solid; mp 290−292 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.74 (d, 2 H, J = 7.8), 7.62 (s, 1 H), 7.53 (t, 1 H, J = 7.8), 7.43 (d, 1 H, J = 7.8), 2.29 (s, 3 H); low-resolution MS (CI) 178 (MH^+). Anal. ($C_9H_{12}N_3O$·HCl) C, H, N.

3-(Acetimidoylamino)benzamidine·HCl (69): prepared from 3-aminobenzamidine·2HCl, **21a**·HCl, and Et_3N (1 equiv); recrystallization from MeOH/Et_2O gave 1.48 g (82%) of title compound as an off-white solid; mp 263−265 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.80−7.60 (m, 4 H), 2.29 (s, 3 H); low-resolution MS (CI) 177 (MH^+). Anal. ($C_9H_{12}N_4$·2HCl·0.45NH_4-Cl) C, H, N, Cl.

***N*,*N*′-(1,3-Phenylene)diacetamidine·2HBr (70):** prepared from 1,3-phenylenediamine using 2 equiv of thioimidate **21b**·HBr; recrystallization from MeOH/EtOAc gave 1.55 g (53%) of title compound as an off-white solid; mp 256−258 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.56 (t, 1 H, J = 8.4), 7.36−7.27 (m, 2 H), 7.23 (br s, 1 H), 2.28 (s, 3 H); low-resolution MS (CI) 191 (MH^+). Anal. ($C_{10}H_{14}N_4$·2HBr·0.5H_2O) C, H, N, Br.

***N*-(4-(Aminomethyl)phenyl)acetamidine·2HBr (33).** A solution of 4-nitrobenzylamine·HCl (1.15 g, 6.1 mmol) in 76 mL of THF at 25 °C was treated with 38 mL of saturated $NaHCO_3$ solution followed by BOC_2O (1.33 g, 6.1 mmol). After stirring overnight, the reaction was diluted with H_2O, and the product was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was dissolved in 50 mL of EtOAc and treated with 10% palladium on carbon (150 mg). The reaction was placed on a Parr shaker under hydrogen atmosphere overnight and then filtered through a pad of Celite washing with EtOAc. The filtrate was concentrated in vacuo. The residue was dissolved in 23 mL of EtOH and treated with thioimidate **21b**·HBr (1.81 g, 6.1 mmol). After stirring overnight the reaction was concentrated in vacuo, diluted with 10 mL of 1,4-dioxane, and treated with 1.2 mL of 48% HBr in AcOH. After the mixture stirred for 5 min Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOAc provided 1.2 g (61%) of title compound as a white solid; mp 256−258 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.46 (d, 2 H, J = 8.4), 7.29 (d, 2 H, J = 8.4), 4.10 (s, 2 H), 2.28 (s, 3 H); low-resolution MS (CI) 164 (MH^+). Anal. ($C_9H_{13}N_3$·2HBr·H_2O) C, H, N, Br.

The following compound was prepared in an analogous manner.

***N*-(3-(Aminomethyl)phenyl)acetamidine·2HBr (14):** prepared from 3-nitrobenzylamine hydrochloride using thioimidate **21b**·HBr; recrystallization from MeOH/EtOAc provided the title compound as a white solid: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.60−7.50 (m, 3 H), 7.30 (m, 1 H), 4.03 (s, 2 H), 2.36 (s, 3 H); low-resolution MS (CI) 164 (MH^+). Anal. ($C_9H_{13}N_3$·2HBr) C, H, N, Br.

***N*-(3-Aminophenyl)acetamidine·2HBr (34).** A solution of 3-nitroaniline (10.0 g, 72.4 mmol) in 200 mL of CH_2Cl_2 at 25 °C was treated sequentially with 10 mL (71.4 mmol) of Et_3N, 17.4 g (72.4 mmol) of BOC_2O, and a catalytic amount of

DMAP. After stirring for 72 h, the reaction was filtered through a pad of silica gel washing with hexanes/EtOAc (1:1), and the filtrate was concentrated in vacuo. Purification by medium-pressure silica gel column chromatrography using hexanes/EtOAc (3:1 → 1:1) provided 4.4 g (26%) of 3-*N*-(*tert*-butoxycarbonyl)nitroaniline. A solution of 1.5 g (6.3 mmol) of the nitroaromatic in 50 mL of EtOAc was treated with 150 mg of 10% palladium on carbon and placed on a Parr hydrogenator under hydrogen atmosphere for 2 h. The reaction was filtered through a pad of silica gel washing with EtOAc, and the filtrate was concentrated in vacuo to give 1.3 g (100%) of intermediate aniline. To 1.3 g (6.3 mmol) of the aniline in 22 mL of EtOH at 25 °C was added 1.9 g (6.3 mmol) of thioimidate **21b**·HBr. After stirring overnight, the reaction was concentrated in vacuo, and the residue was diluted with 10 mL of 1,4-dioxane and treated with 2 mL (7.6 mmol, 1.2 equiv) of 3.7 M HBr in AcOH. After the mixture stirred for 15 min, Et_2O was added, and a solid was collected by filtration. The solid was recrystallized from MeOH/EtOAc to give 1.15 g (59%) of title compound as a white solid: mp 288−289 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.51 (t, 1 H, J = 8.1), 7.33−7.23 (m, 2 H), 7.20 (t, 1 H, J = 1.8), 2.27 (s, 3 H); low-resolution MS (CI) 150 (MH^+). Anal. ($C_8H_{11}N_3$·2HBr) C, H, N, Br.

***N*-(3-((Methylamino)methyl)phenyl)acetamidine·2HBr (36).** A solution of *N*-BOC-3-nitrobenzylamine (2.0 g, 7.93 mmol), prepared as in **14**, in 32 mL of anhydrous THF at −78 °C was treated with a 1.5 M solution of LDA·THF in cyclohexane (6.34 mL, 9.51 mmol) dropwise. After stirring for 25 min the reaction was treated with iodomethane (0.59 mL, 9.51 mmol), then warmed to room temperature, and stirred for 4 h. The reaction was poured into water, and the product was extracted with diethyl ether. The combined organics were dried over anhydrous magnesium suflate, filtered, and concentrated in vacuo. Purification on 70 g of silica gel using hexanes/diethyl ether (2:1) gave 1.25 g (59%) of an oil. The residue was dissolved in 50 mL of EtOAc and treated with 10% palladium on carbon (140 mg). The reaction was placed on a Parr shaker under hydrogen atmosphere for 4 h and then filtered through a pad of silica gel washing with EtOAc. The filtrate was concentrated in vacuo to give an oil. The residue was dissolved in 17 mL of EtOH and treated with thioimidate **21b**·HBr (1.39 g, 4.69 mmol). After stirring overnight the reaction was concentrated in vacuo, and the residue was dissolved in 10 mL of 1,4-dioxane and treated with 5.9 M HBr/AcOH (1 mL). After the mixture stirred for 5 min Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOAc provided 950 mg (59%) of title compound as a white solid: mp 222−223 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.54−7.24 (m, 4 H), 4.12 (s, 2 H), 2.60 (s, 3 H), 2.28 (s, 3 H); low-resolution MS (CI) 178 (MH^+). Anal. ($C_{10}H_{15}N_3$·2HBr) C, H, N, Br.

***N*-(3-((Dimethylamino)methyl)phenyl)acetamidine·2HBr (37).** A solution of 3-nitrobenzyl bromide (1.0 g, 4.63 mmol) in 30 mL of toluene was treated with a 2.0 M solution of dimethylamine in THF (6.95 mL, 13.9 mmol). The reaction was heated to reflux and stirred for 2 h. Upon cooling to 25 °C, the reaction was diluted with EtOAc and washed with 1.0 N NaOH. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was dissolved in 50 mL of EtOAc and treated with 10% palladium on carbon (100 mg). The reaction was placed on a Parr shaker under a hydrogen atmosphere for 30 min and then filtered through a pad of Celite washing with MeOH. The filtrate was concentrated in vacuo, and the residue was dissolved in 17 mL of EtOH and treated with thioimidate **21b**·HBr (1.37 g, 4.63 mmol). After stirring overnight, the reaction was concentrated in vacuo. The residue was dissolved in 10 mL of 1,4-dioxane and treated with 5.9 M HBr/AcOH (0.9 mL). After the mixture stirred for 5 min Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOAc provided 960 mg (59%) of title compound as a white solid: mp 242−243 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.58−7.23 (m, 4 H), 4.23 (s, 2 H), 2.73 (s, 6 H), 2.28 (s, 3 H); low-resolution MS (CI) 192 (MH^+). Anal. ($C_{11}H_{17}N_3$·2HBr) C, H, N, Br.

The following compounds were prepared in an analogous manner.

***N*-(3-((1,2,3,4-Tetrahydroisoquinolin-2-yl)methyl)-phenyl)acetamidine·2HBr (38):** prepared as above from 3-nitrobenzylamine and neat tetrahydroisoquinoline in toluene; recrystallization from EtOH/EtOAc provided 366 mg (18%) of title compound as a brown solid; mp 260−262 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.64−6.95 (m, 8 H), 4.41 (br s, 2 H), 4.26 (br s, 2 H), 3.69 (br s, 1 H), 3.36 (br s, 1 H), 3.06 (br s, 2 H), 2.29 (s, 3 H); low-resolution MS (CI) 280 (MH^+). Anal. ($C_{18}H_{21}N_3$·2HBr·0.25H_2O·0.25EtOAc) C, H, N, Br.

***N*-(3-(1*H*-Pyrazol-1-ylmethyl)phenyl)acetamidine·2HBr (39):** prepared as above from 3-nitrobenzyl bromide and pyrazole; recrystallization from MeOH/EtOAc gave 960 mg (55%) of title compound as a white solid; mp 218−220 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.79 (s, 1 H), 7.63 (s, 1 H), 7.40 (t, 1 H, J = 7.8), 7.18 (m, 2 H), 7.03 (s, 1 H), 6.38 (br s, 1 H), 5.35 (s, 2 H), 2.23 (s, 3 H); low-resolution MS (CI) 215 (MH^+). Anal. ($C_{12}H_{14}N_4$·2HBr) C, H, N, Br.

***N*-(3-(Morpholinomethyl)phenyl)acetamidine (40):** prepared as above from 3-nitrobenzyl bromide and morpholine; recystallization from EtOH/EtOAc gave 938 mg (51%) of title compound as a white solid; mp 219−220 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.56−7.30 (m, 4 H), 4.28 (s, 2 H), 3.93 (br s, 2 H), 3.63 (br s, 2 H), 3.28 (br s, 2 H), 3.15 (br s, 2 H), 2.28 (s, 3 H); low-resolution MS (CI) 234 (MH^+). Anal. ($C_{13}H_{19}N_3O$·2HBr) C, H, N, Br.

***N*-(3-((2-Pyridylamino)methyl)phenyl)acetamidine·2HBr (41).** A solution of 3-nitrobenzyl bromide (1.0 g, 4.63 mmol) in 30 mL of toluene was treated with 2-aminopyridine (872 mg, 9.26 mmol). The reaction was heated to reflux and stirred for 4 h. Upon cooling to 25 °C, the solution was basified with 1.0 N NaOH, and the product was extracted with EtOAc. The combined organic layers were washed with 1.0 N NaOH, dried ($MgSO_4$), filtered, and concentrated to an oil. A solution of the residue in 30 mL of THF was treated with BOC_2O (3.0 g, 14 mmol) and stirred at 25 °C for 24 h. The reaction was treated with saturated $NaHCO_3$, and the product was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification on silica gel using hexanes/EtOAc (3:1) as eluent provided 411 mg (27%) of an oil. The oil was dissolved in 20 mL of EtOAc and treated with 10% palladium on carbon (100 mg). The reaction was placed on a Parr hydrogenator under hydrogen atmosphere for 3 h. Filtration through a pad of Celite washing with EtOAc and concentration provided a clear oil. The oil was dissolved in 9 mL of EtOH and treated with thioimidate **21b**·HBr (370 mg, 1.25 mmol). After stirring for 3 days the reaction was concentrated in vacuo. The residue was dissolved in 5 mL of 1,4-dioxane and treated with 5.9 M HBr/AcOH (0.30 mL). After the mixture stirred for 5 min Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOAc provided 270 mg (54%) of title compound as a white solid: mp 247−248 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.56 (m, 1 H), 7.65 (d, 1 H, J = 6.4), 7.41 (t, 2 H, J = 7.8), 7.34 (d, 1 H, J = 7.8), 7.22−7.13 (m, 2 H), 6.88 (d, 1 H, J = 9.0), 6.77 (t, 1 H, J = 6.4), 4.53 (s, 2 H), 2.25 (s, 3 H); low-resolution MS (CI) 241 (MH^+). Anal. ($C_{14}H_{16}N_4$·2HBr) C, H, N, Br.

***N*-(3-((Hydroxyamino)methyl)phenyl)acetamidine·2HBr (42).** A solution of 3-nitrobenzyl bromide (1.5 g, 6.94 mmol) in 30 mL of DMPU was treated with sodium carbonate (2.2 g, 20.8 mmol) followed by hydroxylamine·HCl (482 mg, 6.94 mmol). After the mixture stirred overnight, BOC_2O (1.51 g, 6.94 mmol) was added, and stirring was continued for 3 h. The reaction was diluted with water, and the product was extracted with hexanes/EtOAc (1:1). The combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography on 30 g of silica gel eluting with hexanes/Et_2O (2:1 → 1:1) provided 1.05 g (56%) of protected hydroxycarbamate that was used immediately. The intermediate was dissolved in 50 mL of EtOAc and treated with 10% palladium on carbon. The reaction was placed on a Parr hydrogenator under hydrogen atmosphere until the required amount of hydrogen was consumed. Filtration

through a pad of Celite washing with EtOAc and concentration of the filtrate provided an oil. The oil was dissolved in 20 mL of EtOH and treated with thioimidate **21b**·HBr. After stirring for 2 days, the reaction was concentrated in vacuo. The residue was dissolved in 10 mL of 1,4-dioxane and treated with 5.9 M HBr/AcOH (0.75 mL). After the mixture stirred for 30 min Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOH provided 600 mg (47%) of title compound as a white solid: mp 214−215 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.54−7.25 (m, 2 H), 7.36−7.28 (m, 2 H), 4.35 (s, 2 H), 2.28 (s, 3 H); low-resolution MS (CI) 180 (MH^+). Anal. ($C_9H_{13}N_3O$·2HBr) C, H, N, Br.

***N*-(3-(Acetimidoylamino)benzyl)acetamidine (43).** A solution of 3-nitrobenzylamine·HCl (1.2 g, 6.36 mmol) in absolute EtOH was placed on a Parr hydrogenator under a hydrogen atmosphere for 1 h. The reaction was filtered through a pad of Celite washing with EtOH, and the filtrate was concentrated in vacuo. The residue was dissolved in dichloromethane and basified with 1.0 N NaOH. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 600 mg of crude material that was used immediately. The residue was dissolved in 17 mL of EtOH and treated with thioimidate **21b**·HBr (2.9 g, 9.82 mmol). After stirring overnight, the reaction was treated with 17 mL of EtOH followed by additional thioimidate **21b**·HBr (1.45 g, 4.9 mmol). After stirring for 5 h, the solution was filtered, and the filtrate was concentrated in vacuo. The residue was diluted with MeOH/Et_2O, and a solid was collected. Recrystallization from MeOH/Et_2O gave 1.46 g (81%) of a white solid: mp 276−277 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.45 (t, 1 H, J = 7.8), 7.31 (d, 1 H, J = 7.8), 7.25−7.15 (m, 2 H), 4.41 (s, 2 H), 2.28 (s, 3 H), 2.15 (s, 3 H); low-resolution MS (CI) 205 (MH^+). Anal. ($C_{11}H_{16}N_4$·2HBr·0.35NH_4Br) C, H, N, Br.

***N*,*N*′-(3,3′-(Iminodimethylene)diphenyl)diacetamidine (44).** A solution of 3-nitrobenzyl bromide (1.0 g, 4.63 mmol) in 20 mL of DMPU at room temperature was treated with 3-nitrobenzylamine·HCl (0.87 g, 4.63 mmol) followed by sodium carbonate (1.5 g, 13.89 mmol). The reaction was heated to 100 °C and stirred for 1 h. After the mixture cooled to room temperature, BOC_2O (1.06 g, 4.63 mmol) was added to the reaction mixture, and stirring was continued for 2 days. The reaction was diluted with water and hexanes/EtOAc (1:1), and the layers were separated. The aqueous layer was extracted with hexanes/EtOAc (1:1), and the combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on 50 g of silica gel using hexanes/Et_2O (1:1) as eluent to give 1.26 g (70%) of intermediate bisnitroaromatic. A solution of the nitroaromatic in 50 mL of EtOAc was treated with 10% palladium on carbon (150 mg) and placed on a Parr hydrogenator under H_2 until gas consumption ceased. The reaction was filtered through a pad of Celite washing with EtOAc, and the filtrate was concentrated in vacuo to give 1.07 g (100%) of an oil that was used immediately. The oil was dissolved in 15 mL of EtOH and treated with thioimidate **21b**·HBr (1.93 g, 6.5 mmol). After stirring overnight the reaction was concentrated in vacuo. The residue was treated with 10 mL of 1,4-dioxane followed by 5.9 M HBr/AcOH (0.75 mL). After the mixture stirred for 5 min, Et_2O was added, and stirring was continued for 2 h. A solid was collected and recrystallized from MeOH to give 708 mg (40%) of title compound as a white solid: mp 282−283 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.54−7.25 (m, 8 H), 4.23 (s, 4 H), 2.28 (s, 6 H); low-resolution MS (CI) 310 (MH^+). Anal. ($C_{18}H_{23}N_5$·3HBr) C, H, N, Br.

***S*-(3-(Acetimidoylamino)benzyl)isothiourea·3HBr (45).** A solution of 3-nitrobenzyl bromide (1.0 g, 4.63 mmol) in 10 mL of acetone at room temperature was treated with thiourea (352 mg, 4.63 mmol) and stirred for 3 h. The solid was collected by filtration to give 1.29 g (96%) of a white solid. A solution of the solid (1.29 g, 4.41 mmol) in 50 mL of EtOH was treated with 10% palladium on carbon (200 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere overnight. The reaction mixture was filtered through a pad of Celite washing with EtOH, and the filtrate was concentrated in vacuo to give 1.26 g of an orange solid. The solid was immediately dissolved in 17 mL of EtOH and treated with thioimidate **21b**·HBr (1.31 g, 4.41 mmol). After stirring for 3 days, the reaction was concentrated to 5 mL and then triturated with Et_2O. A brown solid was collected and recrystallized from MeOH/EtOAc to give 672 mg (40%) of title compound as a brown solid: mp 228−230 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.46−7.12 (m, 4 H), 4.31 (s, 2 H), 2.27 (s, 3 H); low-resolution MS (CI) 223 (MH^+). Anal. ($C_{10}H_{14}N_4S$·2HBr) C, H, N, Br, S.

2-(3-(Acetimidoylamino)phenyl)acetic acid·HBr (48). A solution of 3-nitrophenylacetic acid (5.0 g, 27.6 mmol) in 55 mL of *t*-BuOH at 25 °C was treated with DMAP (1.0 g, 8.3 mmol) followed by BOC_2O (12.0 g, 55.2 mmol). After stirring for 2 h the reaction was concentrated in vacuo. The residue was filtered through a pad of silica gel washing with hexanes/EtOAc (1:1). The filtrate was concentrated in vacuo, immediately dissolved in 200 mL of EtOH, and treated with 10% palladium on carbon (800 mg). After placing on a Parr hydrogenator under hydrogen atmosphere for 1 h, the reaction was filtered through silica gel washing with EtOAc, and the filtrate was concentrated in vacuo to give 5.7 g (100%) of solid. To 3.0 g (14.5 mmol) of this solid in 40 mL of EtOH was added thioimidate **21b**·HBr (4.3 g, 14.5 mmol). After stirring overnight, the reaction was filtered washing with MeOH. The filtrate was concentrated in vacuo, then dissolved in 30 mL of AcOH, and treated with 5 mL of 30% HBr in AcOH. After the mixture stirred for 15 min Et_2O was added, and the solid was collected by filtration to give 3.6 g (91%) of title compound as an off-white solid: mp 192−194 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.42−7.00 (m, 4 H), 3.65 (s, 2H), 2.26 (s, 3H); low-resolution MS (CI) 193 (MH^+). Anal. ($C_{10}H_{12}N_2O_2$·HBr·0.3H_2O) C, H, N, Br.

Methyl 2-(3-(Acetimidoylamino)phenyl)acetate·HCl (49). A solution of 3-aminophenylacetic acid (1.0 g, 6.62 mmol) in 15 mL of EtOH was treated with thioimidate **21a**·HCl (2.6 g, 13.2 mmol) and triethylamine (0.921 mL, 6.62 mmol). After stirring for 48 h, the reaction was concentrated in vacuo and then treated with Et_2O and 1.0 N HCl. The aqueous layer was separated, concentrated in vacuo, and recrystallized three times from MeOH/Et_2O to give 428 mg (27%) of title compound as white crystals: mp 192−193 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.39 (t, 1 H, J = 8.1), 7.25 (d, 1 H, J = 8.1), 7.20−7.05 (m, 2 H), 3.67 (s, 2 H), 3.57 (s, 3 H), 2.26 (s, 3 H); low-resolution MS (CI) 207 (MH^+). Anal. ($C_{11}H_{14}N_2O_2$·HCl·0.05H_2O·0.5NH_4Cl) C, H, N, Cl.

***N*-(3-(2-Morpholino-2-oxoethyl)phenyl)acetamidine·HBr (51).** A solution of 3-nitrophenylacetic acid (1.8 g, 10.5 mmol) in 50 mL of CH_2Cl_2 was treated with triethylamine (1.46 mL, 10.5 mmol) followed by morpholine (0.92 mL, 10.5 mmol). The solution was treated with EDC (2.0 g, 10.5 mmol) followed by catalytic DMAP and stirred overnight. The solution was washed with 1 N HCl and 1 N NaOH, and the organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.37 g (55%) of intermediate amide. The amide in 50 mL of EtOAc was treated with 10% palladium on carbon (150 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere for 1.5 h. The mixture was filtered through a pad of Celite washing with EtOAc, and the filtrate was concentrated in vacuo to provide the desired aniline. This aniline in 21 mL of EtOH was treated with thioimidate **21b**·HBr (1.62 g, 5.47 mmol) and stirred for 48 h. Upon concentrating to 5 mL, Et_2O was added, and a solid was collected. Recrystallization from MeOH/EtOAc provided 1.12 g (60%) of title compound as a white solid: mp 185−186 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.50−6.95 (m, 4 H), 3.75 (s, 2 H), 3.65−3.40 (m, 8 H), 2.26 (s, 3 H); low-resolution MS (CI) 263 (MH^+). Anal. ($C_{14}H_{19}N_3O_2$·HBr·0.2H_2O) C, H, N, Br.

***N*-(3-(Acetimidoylamino)benzyl)-4-morpholinecarboxamide (52).** A solution of 3-nitrobenzylamine·HCl (1.0 g, 5.3 mmol) in 21 mL of THF was treated with triethylamine (2.7

mL, 19.4 mmol) followed by 4-morpholinecarbonyl chloride (0.65 mL, 5.57 mmol). After stirring for 48 h, the reaction was filtered, and the filtrate was washed with 1 N HCl, dried ($MgSO_4$), filtered, and concentrated in vacuo to give an intermediate urea. A solution of this urea in 50 mL of EtOAc was treated with 10% palladium on carbon (150 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere until gas consumption ceased. The mixture was filtered through a pad of Celite washing with EtOAc, and the filtrate was concentrated in vacuo to give an intermedate aniline. A solution of the aniline in 20 mL of EtOH was treated with thioimidate **21b**·HBr (1.57 g, 5.31 mmol) and stirred overnight. After stirring overnight, the reaction was concentrated to 5 mL and triturated with Et_2O. After the mixture stirred for 4 h, a solid was collected and recrystallized from EtOH/EtOAc to give 1.04 g (53%) of title compound as an off-white solid: mp undefined; ^{1}H NMR (D_2O, 300 MHz) δ 7.45−7.00 (m, 4 H), 4.23 (s, 2 H), 3.57 (t, 4 H, J = 4.9), 3.27 (t, 4 H, J = 4.9), 2.25 (s, 3 H); low-resolution MS (CI) 277 (MH^+). Anal. ($C_{14}H_{20}N_4O_2$·1.2HBr·0.5H_2O·0.4EtOAc) C, H, N, Br.

3-(Acetimidoylamino)benzoic Acid·HCl (63). A solution of *tert*-butyl ester **50** (1.0 g, 3.7 mmol) in 5 mL of 1,4-dioxane was treated with 4.0 M HCl in 1,4-dioxane (3 mL) and stirred for 4 h. The solution was treated with 5 mL of acetic acid and stirred for 28 h. Et_2O was added, and a solid was collected and washed with Et_2O to give 769 mg (97%) of a white solid: mp 230−232 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.94 (dd, 1 H, J = 7.9, 1.3), 7.81 (br s, 1 H), 7.60−7.35 (m, 2 H), 2.29 (s, 3 H); low-resolution MS (CI) 277 (MH^+). Anal. ($C_9H_{10}N_2O_2$·HCl·0.45NH_4Cl·0.2Et_2O) C, H, N, Cl.

3-(Acetimidoylamino)benzenesulfonamide·HBr (65). A solution of 3-nitrobenzenesulfonamide (1.5 g, 7.42 mmol) in 50 mL of EtOH containing 10% palladium on carbon (150 mg) was placed on a Parr hydrogenator under a hydrogen atmosphere for 1 h. The reaction was filtered through a pad of Celite washing with EtOH, and the filtrate was concentrated in vacuo to give 1.28 g (100%) of crude 3-aminobenzenesulfonamide. A solution this aniline (1.28 g, 7.43 mmol) in 40 mL of EtOH was treated with thioimidate **21b**·HBr (2.2 g, 7.43 mmol). The reaction was stirred overnight, concentrated to 5 mL, and triturated with Et_2O. A solid was collected and recrystallized from MeOH/Et_2O to give 1.46 g (67%) of title compound as an off-white solid: mp 240−242 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.86 (d, 1 H, J = 8.1), 7.75 (s, 1 H), 7.63 (t, 1 H, J = 8.1), 7.51 (d, 1 H, J = 8.1), 2.30 (s, 3 H); low-resolution MS (CI) 214 (MH^+). Anal. ($C_8H_{11}N_3O_2S$·HBr) C, H, N, Br, S.

***N*-(3-(2-Amino-4-thiazolyl)phenyl)acetamidine·2HBr (66).** A solution of α-bromo-3-nitroacetophenone (5.0 g, 20.5 mmol) in 20 mL of acetone at 25 °C was treated with thiourea (1.6 g, 20.5 mmol). After the mixture stirred overnight, 6.1 g (98%) of a white solid was collected by filtration. A solution of the solid (5.1 g, 16.9 mmol) in 200 mL of EtOH was treated with 10% palladium on carbon (500 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere for 2.5 h. The reaction was filtered through a pad of Celite washing with EtOH, and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and basified with 1 N NaOH. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.78 g (55%) of intermediate. A solution of this intermediate (1.03 g, 5.38 mmol) in 25 mL of EtOH was treated with thioimidate **21b**·HBr (1.6 g, 5.38 mmol) and stirred for 18 h. After concentrating in vacuo, 10 mL of 1,4-dioxane was added followed by 30% HBr/AcOH. After the mixture stirred for 30 min Et_2O was added, and a solid was collected by filtration. The solid was recrystallized from MeOH/Et_2O to give 1.09 g (51%) of title compound as a white solid: mp 252−254 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.62−7.40 (m, 3 H), 7.29 (br d, 1 H, J = 7.5), 6.91 (s, 1 H), 2.29 (s, 3 H); low-resolution MS (CI) 233 (MH^+). Anal. ($C_{11}H_{12}N_4S$·2HBr) C, H, N, Br, S.

***N*-(3-(1*H*-Pyrazol-3-yl)phenyl)acetamidine·1.8HBr (67).** A solution of 3-nitroacetophenone (5.0 g, 30.3 mmol) in 16 mL of DMF was treated with 4.8 mL of $(MeO)_2CHNMe_2$ and heated at 120 °C for 24 h. After cooling to 25 °C, the reaction was poured into 1 N HCl and extracted with hexanes/EtOAc (1:1). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.88 g (28%) of intermediate enamide. A solution of enamide (940 mg, 4.27 mmol) in 34 mL of MeOH and 8.5 mL of H_2O was treated with H_2NNH_2·H_2O (0.855 mL, 17.6 mmol) and heated at reflux for 2 h. After cooling to 25 °C, the reaction was poured into 1 N HCl and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 808 mg (100%) of intermediate pyrazole. The pyrazole (808 mg, 4.27 mmol) in 21 mL of CH_2Cl_2 was treated with a catalytic amount of DMAP followed by BOC_2O (918 mg, 4.27 mmol). After stirring overnight, the reaction mixture was concentrated in vacuo and used immediately in the next reaction. A solution of protected pyrazole (1.23 g, 4.27 mmol) in 50 mL of EtOAc was treated with 10% palladium on carbon (150 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere until gas consumption ceased. The reaction was filtered through a pad of Celite washing with EtOAc, and the filtrate was concentrated in vacuo to give an oil that was used immediately in the next reaction. The aniline (1.1 g, 4.27 mmol) in 20 mL of EtOH was treated with thioimidate **21b**·HBr (1.26 g, 4.27 mmol) and stirred overnight. After the mixture was concentrated in vacuo, 10 mL of 1,4-dioxane was added followed by 5.9 M HBr in AcOH (0.80 mL). After the mixture stirred for 30 min Et_2O was added, and a brown solid was collected. Recrystallization from EtOH/EtOAc/Et_2O provided 381 mg (25%) of title compound as a brown solid: mp 224−226 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.81 (d, 1 H, J = 2.4), 7.67 (dd, 1 H, J = 8.1, 1.2), 7.55 (br s, 1 H), 7.49 (t, 1 H, J = 8.1), 7.25 (br d, 1 H, J = 8.1), 6.75 (d, 1 H, J = 2.4), 2.28 (s, 3 H); low-resolution MS (CI) 201 (MH^+). Anal. ($C_{11}H_{12}N_4$·2.8HBr·1.0Et_2O) C, H, N, Br.

***N*-(3-Hydrazinophenyl)acetamidine·2HBr (68).** A solution of 3-nitrophenylhydrazine·HCl (1.0 g, 5.3 mmol) in 66 mL of THF was treated with 33 mL of saturated $NaHCO_3$ followed by BOC_2O (1.15 g, 5.3 mmol). After stirring overnight, the reaction was diluted with H_2O and extracted with Et_2O. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.33 g (100%) of a yellow solid that was used immediately. A solution of the protected hydrazine in 50 mL of EtOAc at 25 °C was treated with 10% palladium on carbon (200 mg) and placed on a Parr hydrogenator under a hydrogen atmosphere until gas consumption ceased. The reaction was filtered through a pad of Celite washing with EtOAc, and the filtrate was concentrated in vacuo to give an intermediate aniline. The aniline was dissolved in 17 mL of EtOH and treated with thioimidate **21b**·HBr (1.56 g, 5.27 mmol). After stirring overnight the reaction was concentrated in vacuo, diluted with 10 mL of 1,4-dioxane, and treated with 48% HBr in AcOH (1 mL). After the mixture stirred for 30 min, Et_2O was added and a solid was collected. Recrystallization from MeOH/EtOAc provided 780 mg (45%) of a brown solid: mp 218−220 °C; ^{1}H NMR (D_2O, 300 MHz) δ 7.38 (t, 1 H, J = 8.1), 6.96−6.88 (m, 2 H), 6.81 (t, 1 H, J = 2.1), 2.26 (s, 3 H); low-resolution MS (CI) 165 (MH^+). Anal. ($C_8H_{12}N_4$·2HBr) C, H, N, Br.

***N*-(3-(Aminomethylphenyl)-*N*′-methylacetamidine·2HBr (71).** To a stirred, cooled (0 °C) solution of 3-(*N*-(*tert*-butoxycarbonyl)aminomethyl)aniline (1.0 g, 4.5 mmol) in 30 mL of EtOH was added thioacetimidate **21c** (1.18 g, 4.52 mmol). The mixture was kept at 0 °C for 2 h and then allowed to warm slowly to room temperature while stirring overnight. Solvent was removed under reduced pressure, and the residue was partitioned between water and Et_2O. The aqueous layer was further washed with Et_2O, frozen, and lyophilized to provide the desired *N*-BOC-protected acetamidine·HBr (1.44 g, 89%) as a white solid. To a stirred solution of the solid (700 mg, 1.95 mmol) in 10 mL of anhydrous 1,4-dioxane was added 30% HBr in acetic acid (1 mL). After 3 h the mixture was diluted with pentane, and the precipitated solid was collected and dried in vacuo to provide 605 mg (92%) of title compound as a white solid: mp 223−225 °C; ^{1}H NMR (D_2O, 200 MHz) δ

7.45 (m, 4 H), 4.21 (s, 2 H), 2.97 (s, 3 H), 2.12 (s, 3 H); low-resolution MS (CI) 179 (MH^+). Anal. ($C_{10}H_{15}N_3$·2HBr) C, H, N, Br.

The following compounds were prepared in an analogous manner.

***N*-(3-(Aminomethyl)phenyl)(aminomethyl)acetamidine·3HBr (72):** prepared as a beige solid from thioacetimidate **21e** in 99% overall yield; mp 268−270 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.59 (m, 4 H), 4.25 (d, 4 H); low-resolution MS (CI) 179 (MH^+). Anal. ($C_9H_{14}N_4$·3HBr·0.5H_2O·0.7NH_4Br) Calcd: C, 21.68; H, 4.21; N, 13.21. Found: C, 21.76; H, 3.87; N, 13.24. Capillary zone electrophoresis on a fused silica column (75 μm × 40 cm) at pH = 2, t_R = 4.29 min, 98.0% organic purity.

***N*-(3-(Aminomethyl)phenyl)(thiomethyl)acetamidine·2HBr (73):** prepared as a light-beige solid from thioacetimidate **21f** in 48% overall yield; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.49 (m, 4 H), 4.12 (s, 2 H), 3.64 (s, 2 H), 2.30 (s, 3 H); low-resolution MS (CI) 210 (MH^+). Anal. ($C_{10}H_{15}N_3S$·2HBr·0.5CH_3CO_2H) C, H, N, Br.

2-Fluoro-*N*-(3-(aminomethyl)phenyl)acetamidine·2HBr (74). To a cooled solution of 3-nitrobenzylamine·HCl (30 g, 0.16 mol) in 100 mL of methanol, 100 mL of THF, and 48.8 mL of triethylamine was added a solution of BOC_2O (38.2 g, 0.18 mol) in 200 mL of THF over 60 min. After 1 h, the solution was concentrated, diluted with 100 mL of H_2O, and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by flash silica gel chromatography using hexanes/EtOAc (9:1 → 7:3) as eluent provided 38 g (95%) of protected amine. A solution of this intermedate (10 g, 39.6 mmol) in 100 mL of EtOAc was treated with 1 g of 10% palladium on carbon and placed under 55 psi of hydrogen for 1.5 h. After filtration and concentration of the filtrate, the residue was dissolved in 100 mL of absolute ethanol and treated with thioimidate **21d** (16 g, 50.8 mmol). After stirring for 16 h, the reaction was concentrated to ca. 15 mL and treated with 500 mL of Et_2O. The Et_2O was decanted, and the residue was treated with 75 mL of acetic acid followed by 15 mL of 30% HBr in acetic acid. After 15 min 500 mL of Et_2O was added, and the mixture was stirred 30 min. Filtration and recrystallization from MeOH/EtOAc provided 6 g (50%) of title compound as a white solid: mp 205 °C dec; ^{1}H NMR (D_2O, 300 MHz) δ 7.61−7.32 (m, 4 H), 5.5 (d, 2 H, J = 45.1), 4.08 (s, 2 H); low-resolution MS (CI) 182 (MH^+). Anal. ($C_9H_{12}N_3F$·2HBr) C, H, N, Br.

2-Fluoro-*N*-(3-(hydroxymethyl)phenyl)acetamidine·2HBr (75): prepared from 3-aminobenzyl alcohol using thioimidate **21d** to give a pale-yellow solid; recrystallization from MeOH/EtOAc provided the title compound (50%) as a white solid; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.55−7.20 (m, 4 H), 5.45 (d, 2 H, J = 45.1), 4.57 (s, 2 H); low-resolution MS (CI) 183 (MH^+). Anal. ($C_9H_{11}N_2FO$·HBr·0.25NH_4HBr) C, H, N, Br. Capillary zone electrophoresis on a fused silica column (75 μm × 40 cm) at pH = 2, t_R = 6.56 min, >99% purity.

***N*-(3-(Aminomethyl)phenyl)-2-pyridinecarboxamidine·2HBr (76):** prepared as a yellow solid from thioacetimidate **21g** in 86% overall yield; mp 267−268 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.60−8.80 (m, 8 H), 4.23 (s, 2 H); low-resolution MS (CI) 227 (MH^+). Anal. ($C_{13}H_{14}N_4$·3HBr) C, H, N, Br.

***N*-(3-(Aminomethyl)phenyl)-2-furancarboxamidine·2HBr (77):** prepared as a beige solid from thioacetimidate **21i** in 85% overall yield; mp 169−170 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.41−7.92 (m, 6 H), 6.80 (d, 1 H), 4.23 (s, 2 H); low-resolution MS (CI) 216 (MH^+). Anal. ($C_{12}H_{13}N_3O$·1.9HBr·1.2H_2O) C, H, N, Br. Capillary zone electrophoresis on a fused silica column (75 μm × 40 cm) at pH = 2, t_R = 6.06 min, 99.8% purity.

***N*-(3-(Aminomethyl)phenyl)-2-thiophenecarboxamidine·2HBr (78):** prepared as a beige solid from thioacetimidate **21h** in 90% overall yield; mp 174−176 °C; ^{1}H NMR (D_2O, 200 MHz) δ 7.97−7.31 (m, 7 H), 4.22 (s, 2 H); low-resolution MS (CI) 232 (MH^+). Anal. ($C_{12}H_{13}N_3S$·2HBr·H_2O) C, H, N, S.

Biology. 1. NOS Assay. The oxidation of L-arginine was monitored by the conversion of L-[^{14}C]arginine to L-[^{14}C]-citrulline as described by Schmidt.[39] Human iNOS,[30] eNOS,[31] and nNOS[17] were prepared as previously described. The enzyme was assayed at 37 °C in 20 mM Hepes, pH 7.4, with 2.5 mM dithiothreitol, 125 μM NADPH, 10 μM tetrahydrobiopterin, 10 μM FAD, and 0.2−20 μM L-[^{14}C]arginine. Calmodulin (10 μg/mL) and $CaCl_2$ (2.5 mM) were included when eNOS and nNOS were assayed. Apparent K_i was obtained by measuring percent inhibition at 0.5 μM L-arginine with at least three concentrations of inhibitor and by assuming competitive inhibition. K_m values for L-arginine were 2.2 ± 0.3 μM (iNOS), 0.9 ± 0.1 μM (eNOS), and 1.6 ± 0.1 μM (nNOS). The selectivity of an inhibitor is defined as the ratio of K_i values.

2. nNOS Rat Brain Slice Assay. Rat cortex was isolated and chopped into 0.2-mm cubes on an ice-cold block using a McIlwain tissue chopper. Tissue cubes were suspended in 5 mL (4 °C) of artificial cerebral spinal fluid (ACSF), which consisted of Krebs-Henseleit buffer, 11 mM glucose, and 1.24 mM $CaCl_2$. The tissue suspension was diluted 10-fold with ACSF, shaken on an orbital shaking water bath at 37 °C, and equilibrated with O_2/CO_2 (19:1) for 1 h. The tissue was centrifuged at 100*g* for 3 min at room temperature. The resulting pellet was suspended in 30 mL of ACSF with 54 mM KCl and 66 mM NaCl, centrifuged again, and finally suspended in 10 mL of ACSF with 54 mM KCl and 66 mM NaCl. Aliquots (300 μL) were gassed as described above in Suba-sealed tubes for 2 min in the presence of 20 μM L-[^{14}C]arginine (100 000 dpm), 1 mM L-citrulline, and plus or minus inhibitors. Tubes were then incubated for 2 h in a shaking water bath at 37 °C. Reactions were quenched with 4 mL of 50% (v/v) Dowex (Na^+ form), and the mixture was allowed to settle. Supernatant fluid (1 mL) was counted in 10 mL of Picofluor 40.

3. Ex Vivo Measurement of Nitrite/Nitrate after Drug Administration. Compounds were dissolved in 0.9% (w/v) saline and administered intravenously into the tail vein of male Wistar rats (200−250 g). At predetermined times animals were killed by decapitation, and the cerebellum was dissected at 4 °C and then rapidly frozen in a metal clamp cooled to equilibrium in liquid nitrogen. Nitrite and nitrate have been shown to be present even on new plastic- and glassware. Therefore, to minimize contamination, all apparatus coming into contact either with the sample or with administered drugs (directly or indirectly) were rinsed with Milli-Q water. In addition, all solutions used for the preparation of administered drugs, homogenization, and assay buffers were made up in Milli-Q water. Frozen tissue was homogenized in 15 volumes of Milli-Q water using a Ystral homogenizer and centrifuged (12000*g*, 10 min, 4 °C). To measure the combined levels of nitrite and nitrate, a nitrate reductase assay was performed. In brief, 100 μL of sample was incubated with 20 μL of buffer containing 50 mM potassium phosphate (pH 7.5), 0.6 mM NADPH, 5 mM FAD, and 20 mU of nitrate reductase (Sigma, U.K.) for 1 h at 37 °C. Samples (10 μL) were then injected into a sealed glass vessel containing sodium iodide (1.5%) in glacial acetic acid heated under reflux.[40] Under these conditions any nitrite present is reduced to NO, which is then carried on the constant stream of nitrogen gas to a photomultiplier tube and photon counter. Here the chemiluminescent reaction of NO with ozone is monitored. Chemiluminescent signals were quantified using a nitrite as well as a nitrate (that had first been reduced as described above) standard curve. Samples can be stored for at least several weeks at −20 °C without loss of signal.

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