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Design, Synthesis, and Biological Evaluation of 3,4-Dihydroquinolin-2(1*H*)-one and 1,2,3,4-Tetrahydroquinoline-Based Selective Human Neuronal Nitric Oxide Synthase (nNOS) Inhibitors

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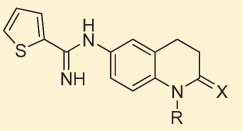
Supporting Information

ABSTRACT: Neuronal nitric oxide synthase (nNOS) inhibitors are effective in preclinical models of many neurological disorders. In this study, two related series of compounds, 3,4-dihydroquinolin-2(1*H*)-one and 1,2,3,4-tetrahydroquinoline, containing a 6-substituted thiophene amidine group were synthesized and evaluated as inhibitors of human nitric oxide synthase (NOS). A structure–activity relationship (SAR) study led to the identification of a number of potent and selective nNOS inhibitors. Furthermore, a few representative compounds were shown to possess druglike properties, features that are often difficult to achieve when designing nNOS inhibitors. Compound (*S*)-**35**, with excellent potency and selectivity for nNOS, was shown to fully reverse thermal hyperalgesia when given to rats at a dose of 30 mg/kg intraperitonieally (ip) in the L5/L6 spinal nerve ligation model of neuropathic pain (Chung model). In addition, this compound reduced tactile hyperesthesia (allodynia) after oral administration (30 mg/kg) in a rat model of dural inflammation relevant to migraine pain.

General Structure



X = O or H,H
R = alkyl or cycloalkyl amino

INTRODUCTION

Nitric oxide (NO) is a reactive free radical gas with diverse physiological roles, ranging from blood pressure regulation to neurotransmission.[1] In most cases, this essential signaling molecule functions by activating soluble guanylate cyclase (sGC), an enzyme that catalyzes the conversion of guanosine-5′-triphosphate (GTP) to the intramolecular second messenger 3,5-cyclic guanosine monophosphate (cGMP).[2] However, due to its reactivity, nitric oxide can participate in responses that are not mediated by the NO/cGMP signaling pathway.[3]

Three distinct isoforms of nitric oxide synthase (NOS) catalyze the five electron oxidation of L-arginine to L-citrulline to produce NO.[4] These include the neuronal or brain NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3), which are constitutively expressed, and the inducible isoform (iNOS or NOS2), which is expressed under conditions of stress or upon the release of inflammatory mediators (IM) such as TNFα, IL-1, or lipopolysaccharides (LPS). The NOS enzymes are homodimeric proteins, consisting of a C-terminal reductase domain that transfers electrons from NADPH through two prosthetic groups, FAD and FMN, to the N-terminal oxygenase domain, which binds L-arginine, (1′*R*,2′*S*,6*R*)-5,6,7,8-tetrahydrobiopterin (BH4), and heme.[5] While the two constitutive isoforms are activated by increases in Ca^{2+} concentration and binding of a Ca^{2+}/calmodulin complex, the activity of iNOS appears to be independent of Ca^{2+} concentration due to a tight binding of the complex at the dimer interface.[6] The active dimeric form of NOS is stabilized by a structural zinc binding at the oxygenase dimer interface. The reduction of BH4 and heme iron allows the activation of O_2 followed by the oxidation of L-arginine to N^{ω}-hydroxy-L-arginine and finally to L-citrulline, ultimately releasing NO.

The overproduction of NO by each isoform has been associated with many disease states. In particular, excess NO in the central nervous system by nNOS has been associated with the spinal transmission of pain, migraine and chronic tension-type headaches, neurodegeneration during stroke, Parkinson's disease, and Alzheimer's disease.[7−11] Consequently, the inhibition of nNOS has the potential to be therapeutic in these diseases. The selective inhibition of nNOS is essential because of the important functions of the other NOS isoforms; for example, eNOS plays a crucial role in the regulation of blood pressure,[12] and the inhibition of eNOS by a nonselective inhibitor has been shown to cause vasoconstriction in humans.[13] Accordingly, selectively inhibiting nNOS has been the goal of many research groups in the last two decades.[14−16] Although there have been many different classes of compounds reported in the literature, the most common mode of inhibiting NOS is to mimic the natural substrate L-arginine.

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The general pharmacophore model for competitively inhibiting NOS at the substrate binding site is depicted by **1**, which contains a guanidine isosteric group and a basic group attached to a central linker.[17] It should be noted, however, that some inhibitors only contain a guanidine isostere attached to a central linker. By varying the guanidine isostere, the central linker, or the basic group, potent and selective nNOS inhibitors have been obtained (for example, compounds **2**–**6**) (Chart 1).[16] We recently reported a series of potent and selective small molecule 2-aminobenzothiazole-based thiophene amidine inhibitors of nNOS exemplified by compound **6**.[17] In continuation of our work on designing selective nNOS inhibitors for treating CNS disorders, we now describe the synthesis and structure–activity relationships (SAR) of 3,4-dihydroquinolin-2(1*H*)-one and 1,2,3,4-tetrahydroquinoline-based nNOS inhibitors that led to the identification of compound (*S*)-**35**. This compound is a potent and selective nNOS inhibitor with druglike properties, and it is shown to be efficacious in two rat pain models, one of which employs an oral dosing protocol.

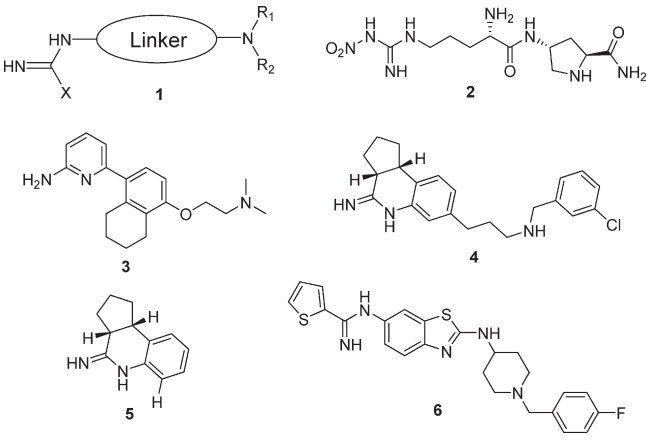
RESULTS AND DISCUSSION

Chemistry. Compounds **26**–**31**, which are from the 3,4-dihydroquinolin-2(1*H*)-one series, were prepared according to Scheme 1. The alkylation of **7** with a series of commercially available chloroalkylamine hydrochloride salts **9**–**13** in DMF with potassium carbonate at room temperature gave compounds **14**–**18**. In a similar fashion, fluoro compound **8** was alkylated with **9** to give **19**. The nitro groups of **14**–**19** were reduced to the corresponding anilines **20**–**25** with either catalytic Pd on carbon under a H_2 atmosphere or catalytic Raney nickel using hydrazine hydrate. These amines were converted to the products **26**–**31** by coupling with thiophene-2-carbimidothioate hydroiodide under mild conditions in ethanol at room temperature.

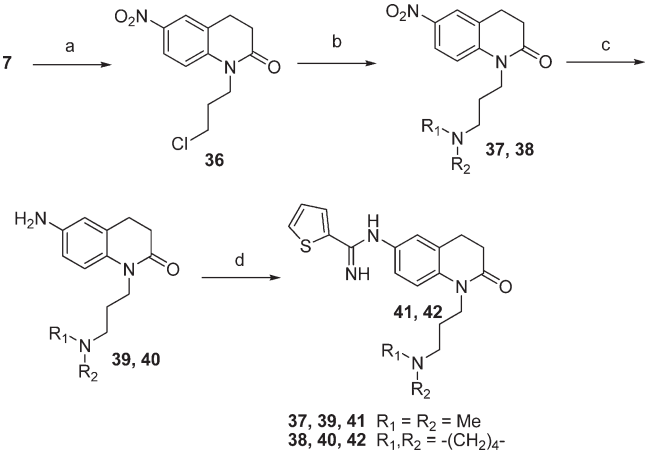
Two compounds (**41** and **42**) with a 3-carbon linker to the basic amine in the 3,4-dihydroquinolin-2(1*H*)-one series were prepared as outlined in Scheme 2. Treatment of **7** with NaH in DMF followed by reaction with chloroiodopropane provided the chloropropyl compound **36**. Nucleophilic displacement of the chloride with dimethylamine and pyrrolidine in aqueous acetonitrile using a catalytic amount of potassium iodide at 60 °C gave **37** and **38**, respectively. These compounds were reduced and then coupled with the 2-thiophene thioimidate reagent to give the final compounds.

We have utilized two methods for the synthesis of compounds in the 1,2,3,4-tetrahydroquinoline series with an acyclic side chain. In the first method (Scheme 3), anilines **20** and **24** were reduced with $LiAlH_4$ in THF to give compounds **32** and **33**. Coupling of these compounds with the 2-thiophene thioimidate provided compounds **34** and **35**. Compound **35** was easily separated into its enantiomers by chiral column chromatography.

Chart 1. Pharmacophore Model and Literature Examples of Selective nNOS Inhibitors

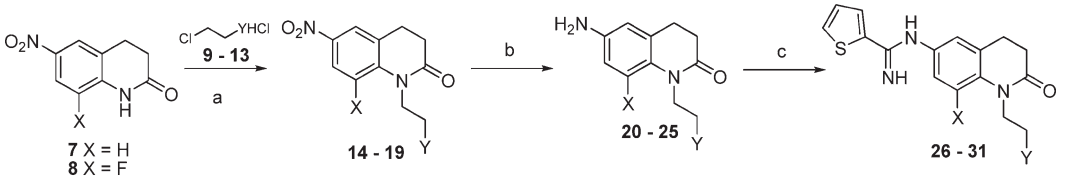


Scheme 2[a]



[a] Reagents and conditions: (a) $Cl(CH_2)_3I$, NaH, DMF, 25 °C, 5 h; (b) R_1R_2NH, K_2CO_3, KI, ACN, H_2O, 60 °C, 16 h; (c) Pd/C, H_2, EtOH, 3–17 h or Raney Ni, $NH_2NH_2.H_2O$, MeOH, reflux, 15 min; (d) thiophene-2-carbimidothioate HI, EtOH, 24 h.

Scheme 1[a]



9, 14, 20, 26 X = H, Y = *N*Me$_2$
10, 15, 21, 27 X = H, Y = *N*Et$_2$
11, 16, 22, 28 X = H, Y = *N*-piperdine
12, 17, 23, 29 X = H, Y = *N*-pyrrolidine
13, 18, 24, 30 X = H, Y = *N*-Me-2-pyrrolidine
19, 25, 31 X = F, Y = *N*Me$_2$

[a] Reagents and conditions: (a) K_2CO_3, DMF, 25 °C, 24 h; (b) Pd/C, H_2, EtOH, 3–17 h or Raney Ni, $NH_2NH_2 \cdot H_2O$, MeOH, reflux, 15 min; (c) thiophene-2-carbimidothioate HI, EtOH, 24 h.

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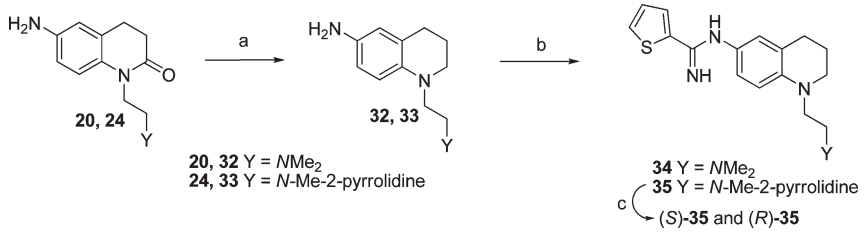
Scheme 3[a]



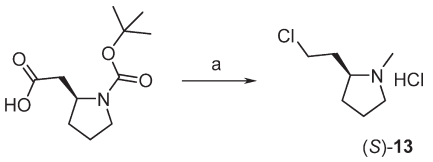
[a] Reagents and conditions: (a) $LiAlH_4$, THF, 24 h; (b) thiophene-2-carbimidothioate HI, EtOH, 24 h; (c) SFC chiral column chromatographic separation.

To confirm the stereochemistry of compound (*S*)-**35**, it was synthesized using the known optically pure alkylating agent (*S*)-**13**,[18] which was obtained from the commercially available optically pure (*S*)-*N*-Boc-L-homoproline according to Scheme 4. Scheme 5 depicts a second method for preparing compounds in the 1,2,3,4-tetrahydroquinoline series. This method is potentially broader in scope because it uses a milder reducing agent that is compatible with many functional groups. Reduction of amides **15**, **19**, **37**, and **38** with 1 M borane in THF at room temperature gave compounds **43**−**46**, which were converted to products **50**−**53** in a similar fashion as described for all preceding target compounds.

To synthesize compounds with a cyclic side chain in the 1,2,3,4-tetrahydroquinoline series, we employed the route outlined in scheme 6. Reductive amination of **54** with ketones **55**−**57** gave the desired compounds **58**−**60**. It should be noted that reactions of **54** with piperidinone derivatives **55** and **56** were sluggish and low yielding. Compounds **58**−**60** were brominated under neutral conditions with NBS in DMF to give the corresponding 6-substituted bromides. The *N*-methylpyrrolidine analogue **64** was obtained by deprotecting the Boc group of **63** followed by reductive amination with formaldehyde. The arylbromides **61**, **62**, and **64** were converted to the anilines by a Buchwald−Hartwig amination procedure using LiHMDS as an ammonia surrogate.[19] The anilines **65**−**67** were coupled with the 2-thiophene thioimidate reagent to provide compounds **68** and **71** and the Boc protected **69**, which was converted to compound **70** by heating in 3N HCl.

Structure−Activity Relationships (SAR). The 3,4-dihydroquinolin-2(1*H*)-one scaffold, a member of the benzo-fused lactam skeleton, is found in many natural products and drug candidates.[20,21] We envisioned using this scaffold as a central linker with the amide nitrogen serving as an attachment point for a number of different basic amine side chains. In addition, we selected the 2-thiophene amidine group to function as the guanidine isostere because it is present in many structurally diverse NOS inhibitors.[17,22] The compounds prepared were tested as the dihydrochloride salts for inhibitory activity against all three human NOS isoforms. The inhibitory activities of these compounds were measured by following the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline in the presence of the requisite cofactors.[23,24] The enzymatic reaction was carried out in the presence or absence of varying concentrations of the compound. Following that, the negatively charged [^{3}H]-L-citrulline was separated from the positively charged [^{3}H]-L-arginine using resin beads. Inhibition of enzyme activity by the compound is measured by dividing the enzymatic conversion in the presence of compound divided by the enzymatic conversion in the absence of compound. IC_{50} value is the concentration of compound that gives rise to 50% inhibition. The observed NOS IC_{50} values and the selectivity ratios for nNOS, defined as IC_{50}(eNOS)/IC_{50}(nNOS) and IC_{50}(iNOS)/IC_{50}(nNOS), are shown in Tables 1 and 2.

Scheme 4[a]



[a] Reagents and conditions: (a) (i) $LiAlH_4$, THF, rt, (ii) $SOCl_2$, $CHCl_3$.

Our initial effort focused on the length of the side chain from the scaffold to the basic amine and on the nature of these terminal amines. Table 1 shows the results of the NOS inhibition assays for compounds in the 3,4-dihydroquinolin-2(1*H*)-one series. In the 2-carbon alkyl side chain series (compounds **26**−**29**), the inhibitory potency for nNOS is dependent on the nature of the terminal amines. The most potent nNOS inhibitor is the pyrrolidine derivative **29** (160 nM), which is also the most selective for the neuronal isoform over the endothelial isoform (180-fold). In contrast to compounds in the 2-carbon alkyl side chain series, compounds with a 3-carbon alkyl side chain ((±)-**30**, **41**, and **42**) do not display any significant differences in nNOS inhibitory potencies among the various terminal amines. In addition, these compounds are much less potent, albeit they still retain good to excellent selectivity over the eNOS and iNOS isoforms; for example, compound **42** is 7-fold less potent than compound **29** (1.22 μM versus 160 nM).

Compound **31**, which incorporates an 8-fluoro substituent, is about 6-fold less potent against nNOS than the corresponding unsubstituted **26** (3.36 versus 0.58 μM). This suggests that having a substituent at the 8-position on the 3,4-dihydroquinolin-2(1*H*)-one scaffold might restrict the flexibility of the alkyldimethylamino side chain, thus preventing it from adopting a favorable binding orientation. To increase the flexibility of this side chain, compound **51** was prepared by reducing the lactam. The nNOS inhibitory potency for this compound is 93 nM, which is 36-fold more potent than **31**. Encouraged by this result, we prepared a number of 1,2,3,4-tetrahydroquinoline analogues (Table 2). In this series, compounds with 2- and 3-carbon acyclic linkers as well as those with cyclic linkers show submicromolar activities against nNOS. The most selective (nNOS over eNOS) compound is (*S*)-**35**, which is also one of the most potent nNOS inhibitors. Interestingly, its enantiomer, (*R*)-**35**, was less selective against nNOS over eNOS, displaying 3-fold more potency against

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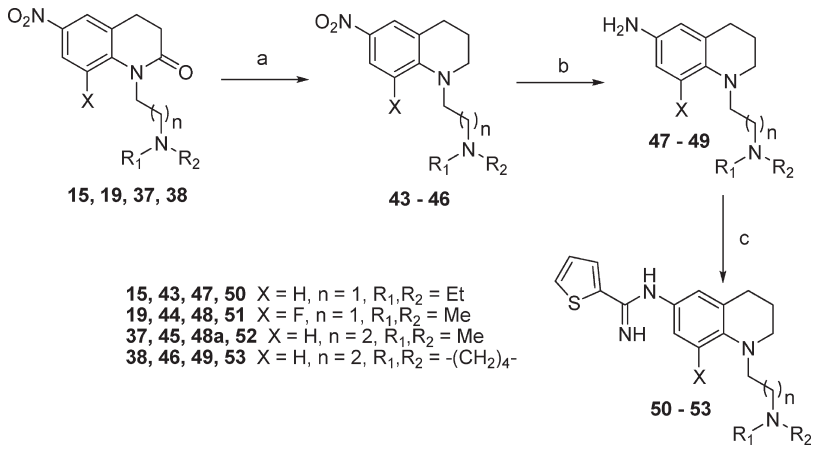
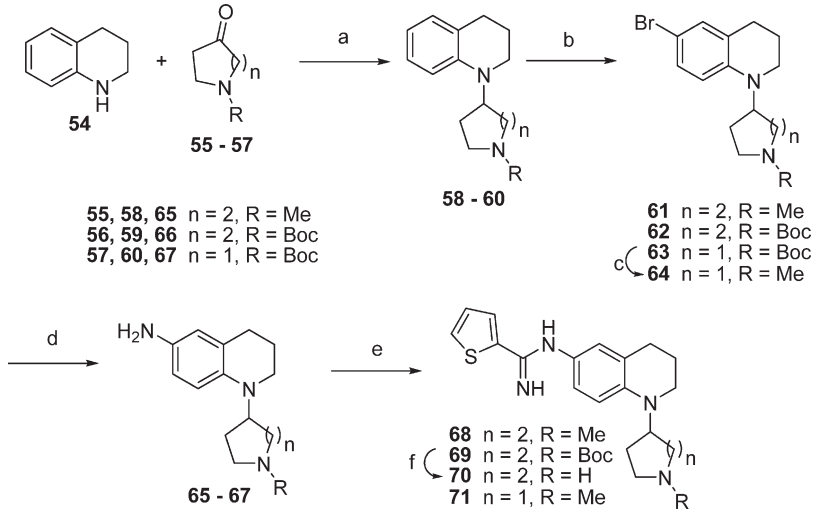
Scheme 5[a]



[a] Reagents and conditions: (a) $BH_3 \cdot THF$, 25 °C, 24 h; (b) Pd/C, H_2, EtOH, 3−17 h or Raney Ni, $NH_2NH_2.H_2O$, MeOH, reflux, 15 min; (c) thiophene-2-carbimidothioate HI, EtOH, 24 h.

Scheme 6[a]



[a] Reagents and conditions: (a) $NaBH(OAc)_3$, HOAc, DCE, 25 °C, 24 h; (b) NBS, DMF, 25 °C, 2 h; (c) (i) 1N HCl, MeOH, reflux, 30 min, (ii) 37% formaldehyde in H_2O, $NaBH_3CN$, HoAc, MeOH, 3 h; (d) LiHMDS, $Pd_2(dba)_3$, $PtBu_3$, THF, reflux, 2 h; (e) thiophene-2-carbimidothioate HI, EtOH, 24 h; (f) 3N HCl, MeOH, reflux, 30 min.

the eNOS enzyme. In general, the nNOS potency and selectivity over eNOS are better in the 1,2,3,4-tetrahydroquinoline series than those in the 3,4-dihydroquinolin-2(1*H*)-one series. Although there are some differences in nNOS inhibitory activities among the various terminal amines in the 1,2,3,4-tetrahydroquinoline series, the differences are not as pronounced as those in the 2-carbon 3,4-dihydroquinolin-2(1*H*)-one series. In general, all compounds inhibit nNOS selectively over both eNOS and iNOS. The compounds in both series do not inhibit iNOS to any appreciable extent. Of the compounds tested, the most selective nNOS over iNOS compound is compound **51** (over 1000-fold).

Physicochemical Properties of 3,4-Dihydroquinolin-2(1*H*)-one and 1,2,3,4-Tetrahydroquinoline-Based nNOS Inhibitors. Because the active site of nNOS is polar and acidic, compounds that mimic the natural substrate L-arginine are, in general, polar and basic.[25] However, these features are not appropriate when designing nNOS inhibitors to treat CNS diseases because these compounds would have to cross the blood−brain barrier and the cell membrane in order to be effective. In recent years, several seminal papers have established the importance of physicochemical properties on the in vivo behavior of drugs.[26−29] To assess the druglike characteristics of these new selective nNOS inhibitors, the physicochemical properties for a few representative compounds were determined (Table 3). All compounds follow Lipinski's rule of five[24] (log *P*, hydrogen bond donor/acceptor properties, and MW). Tertiary amines **34** and (*S*)-**35** have more favorable properties (log *P*, TPSA) for biomembrane penetration than that of lactam **26** and secondary amine **68**.[26,28] The pK_a was determined for compounds **34** and (*S*)-**35** in order to assess their basicity. The average pK_a of the tertiary amine group is 10.3, while that of the amidine group is 8.5. At physiological pH (7.4), these compounds are expected to be doubly protonated to an appreciable extent. This is corroborated with the negative experimental log *D* values.

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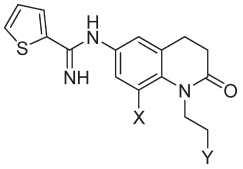
Table 1. In Vitro NOS Inhibitory Data for 3,4-Dihydroquinolin-2(1*H*)-one Analogues



			IC_{50} (μM)[a]			Selectivity[b]	
Compound	X	Y	nNOS	eNOS	iNOS	e/n	i/n
26	H	$N(Me)_2$	0.58 (0.37-0.91)	41.1(31.2-54.0)	35.0 (32.0-39.0)	71	60
27	H	$N(Et)_2$	2.21(1.53-3.19)	73.6 (46.9-115.0)	12.0 (1.0-15.0)	33	6
28	H		0.78 (0.40-1.52)	25.2 (10.1-62.6)	>100	32	>128
29	H		0.16(0.09-0.32)	29.8 (19.1-46.5)	11.0 (0.6-20.0)	181	69
(±)-30	H		1.05 (0.47-2.36)	68.9 (48.7-97.5)	>100	66	>91
31	F	$N(Me)_2$	3.36 (1.82-6.20)	194 (43.1-872)	NT	58	-
41	H	$-CH_2-N(Me)_2$	1.14 (0.66-1.98)	154 (87.8-269)	10.0 (0.7-13.0)	135	9
42	H		1.22 (0.82-1.81)	24.5 (15.0-40.1)	>100	20	>83

[a] Inhibitory activities were measured by following the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline. All assays were performed at least in duplicate. Values in parentheses are the 95% confidence intervals. [b] Selectivity ratios for nNOS, defined as e/n = IC_{50}(eNOS)/IC_{50}(nNOS) and i/n = IC_{50}(iNOS)/IC_{50}(nNOS). NT: not tested.

Cytochrome P450 Inhibition Studies. The closest related enzymes to NOS are the cytochrome P450 enzymes.[5] Therefore, it is crucial to test for inhibition of these enzymes by NOS inhibitors because inhibition can result in the potential for drug−drug interactions. Moreover, compounds that bind to heme iron have been shown to be potent inhibitors of cytochrome P450.[30] Table 4 shows the activity of a number of compounds against the five major human cytochrome P450 enzymes. The inhibitory activity ranges from moderately weak to no significant interaction, indicating that cytochrome P450 inhibition will not be a factor in developing these compounds.

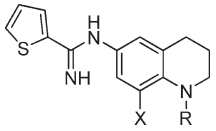
Because of its excellent nNOS inhibitory potency and selectivity, its favorable physicochemical parameters for membrane permeability, and its weak inhibition of the five major human cytochrome P450 subtypes, compound (*S*)-**35** was selected for further in vivo characterization. Rat pharmacokinetics was carried out on compound (*S*)-**35**, showing that it is orally bioavailable with AUC (10 mg/kg po) of 0.6 μM, a value ~2.6-fold higher than the rat nNOS IC_{50} value (0.23 μM) (Table 5). Despite the high plasma clearance (Cl_p >100 L/min/kg), compound (*S*)-**35** has a high volume of distribution (V_{dss} = 94.7 L/kg), resulting in a long half-life.

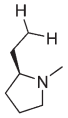
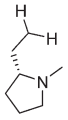
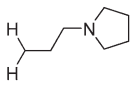
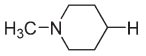
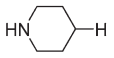
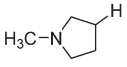
L5/L6 Spinal Nerve Ligation and Migraine Models of Pain in Rats. The ability of compound (*S*)-**35** to reduce pain behaviors in two rat models of pain was investigated. In Figure 1, compound (*S*)-**35** was shown to fully reverse thermal hyperalgesia when given to rats at a dose of 30 mg/kg intraperitoneally (ip) in the L5/L6 spinal nerve ligation model of neuropathic pain (Chung model).[31] Given the positive result in the Chung model, compound (*S*)-**35** was investigated in a rat model of migraine. This model involves the application of a mixture of IM onto the dura via a guide cannula, followed by monitoring of hindpaw sensory thresholds of the animals to measure development of cutaneous tactile allodynia.[32] After administration of the IM, animals develop signs of facial and hindpaw allodynia, which peaks approximately 3 h after dural stimulation. However, oral administration of (*S*)-**35** 15 min prior to the IS (30 mg/kg) attenuated the development of allodynia between 1 and 4 h postdosing, with a maximum effect at 3 h after dosing (Figure 2).

CONCLUSIONS

In summary, two related series of compounds, 3,4-dihydroquinolin-2(1*H*)-one and 1,2,3,4-tetrahydroquinoline, were synthesized and evaluated as inhibitors of human nitric oxide synthase (NOS). Structure−activity relationship analysis using a 6-substituted thiophene amidine group as a guanidine isostere led to the identification of a number of potent and selective nNOS inhibitors, some of which were shown to possess druglike properties. In the 3,4-dihydroquinolin-2(1*H*)-one series, compounds with a 2-carbon linker to the basic side chain are more potent than those with the corresponding 3-carbon linker. Reducing the amide on the 3,4-dihydroquinolin-2(1*H*)-one scaffold led to the identification of the related 1,2,3,4-tetrahydroquinoline series of compounds, which are generally more potent and selective for nNOS. Several compounds from these two series were profiled for their interaction with the five major CYP

Table 2. In Vitro NOS Inhibitory Data for 1,2,3,4-Tetrahydroquinoline Analogues



Compound	X	R	IC_{50} (μM)[a]			Selectivity[b]	
			nNOS	eNOS	iNOS	e/n	i/n
34	H	$-CH_2CH_2N(Me)_2$	0.43 (0.25-0.76)	58.7 (45.2-76.2)	NT	137	-
(*S*)-**35**	H		0.098 (0.05-0.19)	45.6 (8.6-242)	25.7 (17.0-39.0)	465	92
(*R*)-**35**	H		0.13 (0.08-0.18)	14.3 (11.1-18.6)	33.2 (25.0-44.0)	110	255
50	H	$-CH_2CH_2N(Et)_2$	0.26 (0.14-0.48)	64.4 (35.8-116)	>100	253	>333
51	F	$-CH_2CH_2N(Me)_2$	0.093 (0.07-0.13)	24.3 (15.8-37.6)	>100	261	>1000
52	H	$-CH_2CH_2CH_2N(Me)_2$	0.27 (0.20-0.38)	22.4 (12.2-41.3)	NT	81	-
53	H		0.086 (0.06-0.11)	9.5 (4.6-19.8)	NT	110	-
68	H		0.82 (0.41-1.63)	42.7 (29.8-61.2)	>100	52	>121
70	H		0.54 (0.35-0.83)	119.0 (56.5-249.3)	>100	220	>232
(±)-**71**	H		0.36 (0.28-0.48)	27.5 (24.4-31.0)	NT	76	-

[a] Inhibitory activities were measured by following the conversion of [^{3}H]-L-arginine into [^{3}H]-L-citrulline. All assays were performed at least in duplicate. Values in parentheses are the 95% confidence intervals. [b] Selectivity ratios for nNOS, defined as $e/n = IC_{50}(eNOS)/IC_{50}(nNOS)$ and $i/n = IC_{50}(iNOS)/IC_{50}(nNOS)$. NT: not tested.

P450 and shown to be essentially inactive, suggesting a low probability of drug−drug interactions. Finally, these new compounds possess favorable physicochemical properties, allowing good brain penetration and suitable oral bioavailability as demonstrated by the activity of (*S*)-**35** in the SNL model of neuropathic pain and in a model of migraine after oral administration. These compounds represent some of the first examples of highly selective and druglike nNOS inhibitors that demonstrate activity in rodent pain models.

EXPERIMENTAL SECTION

Chemistry. *General Procedures.* All reactions were conducted under an atmosphere of argon and stirred magnetically unless otherwise noted. Commercial reagents and anhydrous solvents were used as received without further purification. When necessary, Sure/Seal anhydrous solvents were utilized. Reactions were monitored by analytical TLC using precoated silica gel aluminum plates (Sigma-Aldrich, 0.2 mm, 60 Å) and were visualized with UV light or stained where appropriate. Flash column chromatography was performed using Silicycle Siliaflash F60 (40−63 μm) silica gel. The ^{1}H NMR spectra were performed at York University on a Bruker 300 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CDCl_3$ (7.26 ppm), CD_3OD (4.87 ppm), or DMSO-d_6 (2.50 ppm). Coupling constants (J values) are given in hertz (Hz). Low and high resolution MS were performed at the University of Toronto AIMS (Mass Spectrometry Laboratory) on an Applied Biosystems/MDS Sciex QstarXL hybrid quadrupole/TOF instrument using electrospray ionization except where indicated. Analytical HPLC spectra were collected on an Agilent 1100 HPLC system using a reverse phase column. All final compounds

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Table 3. Physicochemical Data Related to the Absorption and Biomembrane Permeability of Selected Compounds[a]

compd	ClogP	TPSA	log *D* (pH 7.4)	pK_a	Hb-A	Hb-D	MW	RB
26	2.26	59.43	−0.93	ND	4	2	342.46	6
29	3.03	59.43	ND	ND	4	2	368.49	6
34	3.59	42.36	ND	10.55 8.71	3	2	328.48	6
(*S*)-**35**	4.44	42.36	−0.63	10.12 8.35	3	2	368.54	6
70	2.99	51.15	−1.57	ND	3	3	340.49	4

[a] Hb-A: sum of H-bond acceptors. Hb-D: sum of H-bond donors. MW: molecular weight. RB: number of freely rotating bonds. TPSA: topological molecular polar surface area. ND: not determined.

Table 4. Cytochrome P450 Inhibition Values of Selected Compounds

	CYP450 IC_{50} (μM)				
compd	CYP 1A2	CYP 2C9	CYP 3A4	CYP 2D6	CYP 2C19
26	>100	>100	>100	12.2	>100
34	>100	>100	>100	>100	>100
(*S*)-**35**	>100	>100	>100	>33.3	>100
(*R*)-**35**	>100	>100	>100	>33.3	>100
70	>100	>100	>100	>100	47.1

Table 5. Rat Pharmacokinetics of Compound (*S*)-35[a]

C_{max} (μM)	T_{max} (h)	$t_{1/2}$ (h)[b]	AUC (μg·h/mL)	V_{dss} (L/kg)	Cl_p (L/min/kg)	F_{po} (%)
0.24	1.7	9.4	0.55	94.7	131	18.4

[a] 3 mg/kg iv; 10 mg/kg po. [b] po $t_{1/2}$.

were >95% purity. Preparative chiral HPLC separations were performed at Lotus Separations (Princeton, NJ). No attempts were made to optimize yields.

*1-(2-(Dimethylamino)ethyl)-6-nitro-3,4-dihydroquinolin-2(1H)-one (**14**).* A suspension of 6-nitro-3,4-dihydroquinolin-2(1*H*)-one **7** (500 mg, 2.60 mmol), (*N*,*N*-dimethyamino)ethyl chloride hydrochloride **9** (412 mg, 2.86 mmol), and potassium carbonate (1.07 g, 7.74 mmol) in 8 mL of DMF was stirred at room temperature for 48 h. After this time, the reaction was transferred to a separatory funnel and diluted with cold water and ethyl acetate. The aqueous layer was extracted twice more with ethyl acetate, and the combined organic fractions were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was subjected to flash chromatography on silica gel using 5% 2 M NH_3 in MeOH/CH_2Cl_2 to give a yellow solid. Yield: 370 mg (54%). ^{1}H NMR ($CDCl_3$) δ: 8.15 (dd, J = 2.7, 9.0 Hz, 1H), 8.06 (d, J = 2.7 Hz, 1H), 7.17 (d, J = 9 Hz, 1H), 4.09 (t, J = 7.2 Hz, 2H), 3.00 (t, J = 6.6 Hz, 2H), 2.71 (t, J = 7.5 Hz, 2H), 2.52 (t, J = 7.5 Hz, 2H), 2.32 (s, 6H). MS (ESI): 264.1 (M + 1).

*1-(2-(Diethylamino)ethyl)-6-nitro-3,4-dihydroquinolin-2(1H)-one (**15**).* Prepared as described for compound **14** using compounds **7** and **10**. Yield: 96.5%. ^{1}H NMR ($CDCl_3$) δ: 8.16 (dd, J = 2.5,9 Hz, 1H), 8.06 (d, J =2.5 Hz, 1H), 7.23 (d, J = 9.0 Hz, 1H), 4.07 (t, J = 7.0 Hz, 2H), 3.00 (t, J = 7.0 Hz, 2H), 2.73−2.55 (m, 8H), 1.01(t, J = 7.0 Hz, 6H). MS (ESI): 292.2 (M + 1, 100%).

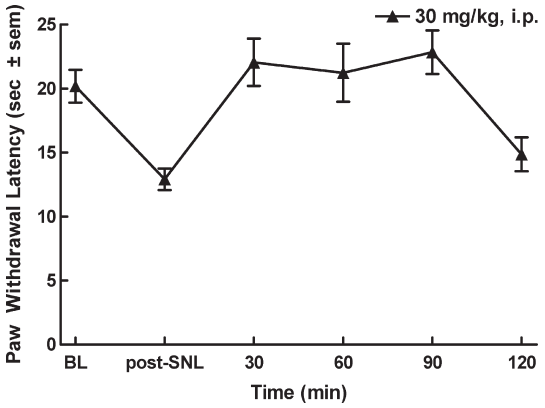

Figure 1. Compound (*S*)-**35** fully reverses thermal hyperalgesia in the L5/L6 spinal nerve ligation model of neuropathic pain.

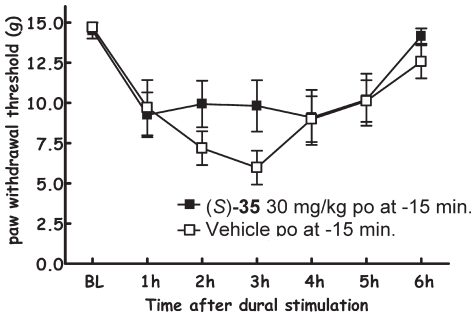

Figure 2. Compound (*S*)-**35** reduces tactile hyperesthesia (allodynia) after oral administration in a rat model of dural inflammation relevant to migraine pain.

*6-Nitro-1-(2-(piperidin-1-yl)ethyl)-3,4-dihydroquinolin-2(1H)-one (**16**).* Prepared as described for compound **14** using compounds **7** and **11**. Yield: 88.7%. ^{1}H NMR ($CDCl_3$) δ: 8.14 (dd, J = 2.7, 9 Hz, 1H), 8.06−8.05 (m, 1H), 7.24 (d, J = 9.0 Hz, 1H), 4.11 (t, J = 7.2 Hz, 2H), 3.02−2.95 (m, 2H), 2.73−2.67 (m, 2H), 2.57−2.48 (m, 6H), 1.59−1.44 (m, 6H). MS (ESI): 304.2 (M + 1, 100%).

*6-Nitro-1-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinolin-2(1H)-one (**17**).* Prepared as described for compound **14** using compounds **7** and **12**. Yield: 71%. ^{1}H NMR ($CDCl_3$) δ: 8.14 (dd, J = 2.7, 9 Hz, 1H), 8.06 (d, J = 2.4 Hz, 1H), 7.20 (d, J = 9.0 Hz, 1H), 4.13 (t, J = 7.5 Hz, 2H), 3.00 (t, J = 6.9 Hz, 2H), 2.73−2.68 (m, 4H), 2.63−2.60 (m, 4H), 1.82−1.78 (m, 4H). MS (ESI): 290.2 (M + 1, 100%).

*(±)-1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-6-nitro-3,4-dihydroquinolin-2(1H)-one (**18**).* Prepared as described for compound **14** using compounds **7** and (±)-**13**. Yield: 73.7%. ^{1}H NMR ($CDCl_3$) δ: 8.13(dd, J = 2.7, 9 Hz, 1H), 8.05 (d, J = 2.4 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 4.15−4.05 (m, 1H), 3.97−3.87 (m, 1H), 3.05−3.01 (m, 4H), 2.72−2.70 (m, 2H), 2.28 (s, 3H), 2.17−1.60 (m, 7H). MS (EI): 303 (M+).

*1-(2-(Dimethylamino)ethyl)-8-fluoro-6-nitro-3,4-dihydroquinolin-2(1H)-one (**19**).* Prepared as described for compound **14** using compounds **8** and **9**. Yield: 44.8%. ^{1}H NMR (DMSO-d_6) δ 8.13−8.07 (m, 2H), 4.06−4.00 (m, 2H), 3.02−2.96 (m, 2H), 2.63−2.57 (m, 2H), 2.41−2.35 (m, 2H), 2.01 (s, 6H). MS-ESI: 282 (MH+, 100), 262 (19), 237 (41).

*6-Amino-1-(2-(dimethylamino)ethyl)-3,4-dihydroquinolin-2(1H)-one (**20**).* A suspension of 1-(2-(dimethylamino)ethyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (320 mg, 1.215 mmol) in dry methanol (10 mL) was treated with Raney nickel (~0.05 g) followed by hydrazine hydrate (0.38 mL, 12.2 mmol) at room temperature, and the resulting

mixture was refluxed for 20 min. The colorless reaction was cooled to room temperature, filtered through a Celite pad, and washed with methanol (2 × 10 mL). The combined methanol layer was evaporated and the crude was purified by column chromatography (2 M NH_3 in MeOH:CH_2Cl_2, 5:95). Yield: 280 mg of colorless oil (98%). ^{1}H NMR ($CDCl_3$) δ: 6.86 (d, J = 8.4 Hz, 1H), 6.57 (dd, J = 2.7, 8.4 Hz, 1H), 6.51 (d, J = 2.1 Hz, 1H), 4.01 (t, J = 7.5 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 7.2 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 2.31 (s, 6H). MS (ESI): 234.2 (M + 1).

*6-Amino-1-(2-(diethylamino)ethyl)-3,4-dihydroquinolin-2(1H)-one (**21**).* Prepared as described for compound **20** using compound **15**. Yield: 81.2%. ^{1}H NMR ($CDCl_3$) δ: 6.88 (d, J = 8.4 Hz, 1H), 6.58 (d, J = 2.7 Hz, 1H), 6.51(dd, J = 2.7,9 Hz, 1H), 3.98 (t, J = 7.8 Hz, 2H), 3.54 (br s, 2H), 2.78 (t, J = 7.8 Hz, 2H), 2.66−2.55 (m, 8H), 1.04 (t, J = 7.2 Hz, 6H). MS (ESI): 262.2 (M + 1, 100%).

*6-Amino-1-(2-(piperidin-1-yl)ethyl)-3,4-dihydroquinolin-2(1H)-one (**22**).* Prepared as described for compound **20** using compound **16**. ^{1}H NMR (DMSO-d_6) δ 6.81 (d, J = 8.2 Hz, 1H), 6.46−6.41 (m, 2H), 4.84 (br s, 2H), 3.87 (t, J = 7.1 Hz, 2H), 2.66 (t, J = 6.5 Hz, 2H), 2.40−2.32 (m, 8H), 1.46−1.35 (m, 6H). MS (ESI): 274.2 (M + 1, 100%).

*6-Amino-1-(2-(pyrrolidin-1-yl)ethyl)-3,4-dihydroquinolin-2(1H)-one (**23**).* Prepared as described for compound **20** using compound **17**. Yield: 98%. ^{1}H NMR ($CDCl_3$) δ: 6.89 (d, J = 8.4 Hz, 1H), 6.56 (dd, J = 2.7, 8.4 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 4.04 (t, J = 7.5 Hz, 2H), 2.81−2.76 (m, 2H), 2.71−2.66 (m, 2H), 2.63−2.56 (m, 6H), 1.81−1.77 (m, 4H). MS (ESI): 260.2 (M + 1, 100%).

*6-Amino-1-(2-(1-methylpyrrolidin-2-yl)ethyl)-3,4-dihydroquinolin-2(1H)-one (**24**).* A suspension of 1-(2-(1-methylpyrrolidin-2-yl)ethyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (2.25 g, 7.42 mmol) and palladium on activated carbon (10 wt %, 100 mg, 0.09 mmol) in 50 mL of ethanol was stirred under a balloon of hydrogen for 2 days. The suspension was filtered through a pad of Celite. The filter pad was rinsed with 50 mL of ethanol, and the filtrate was concentrated to give a viscous oil. The crude product was subjected to Biotage flash chromatography on silica gel using 0−5% 2 M NH_3 in MeOH/CH_2Cl_2 to give a yellow foam (1.48 g, 72.9%). ^{1}H NMR ($CDCl_3$) δ: 6.82 (d, J = 8.4 Hz, 1H), 6.55 (dd, J = 3.0, 8.4 Hz, 1H), 6.52 (d, J = 3.0 Hz, 1H), 4.09−3.99 (m, 1H), 3.86−3.76 (m, 1H), 3.54 (br s, 2H), 3.04−3.01 (m, 1H), 2.81−2.76 (m, 2H), 2.61−2.56 (m, 2H), 2.29 (s, 3H), 2.17−1.60 (m, 8H). MS (ESI): 274.2 (M + 1, 100%).

*6-Amino-1-(2-(dimethylamino)ethyl)-8-fluoro-3,4-dihydroquinolin-2(1H)-one (**25**).* Prepared as described for compound **24** using compound **19**. The crude product was used in the next step without further purification. EI-MS: 251 (M+, 3%), 180 (18%), 71 (12%), 58 (100%).

*N-(1-(2-(Dimethylamino)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**26**).* A solution of 6-amino-1-(2-(dimethylamino)ethyl)-3,4-dihydroquinolin-2(1*H*)-one (0.280 g, 1.20 mmol) in absolute ethanol (5 mL) was treated with methyl thiophene-2-carbimidothioate hydroiodide (0.684 g, 2.40 mmol) at room temperature, and the resulting mixture was stirred overnight (18 h). The reaction was diluted with diethyl ether (45 mL) and the precipitate collected by vacuum filtration. The precipitate was washed from the filter with methanol, and the solvent was evaporated. The residue was diluted with 1N sodium hydroxide solution (5 mL), and product was extracted into ethyl acetate (3 × 10 mL). The combined ethyl acetate layer was washed with brine and dried (Na_2SO_4). Solvent was evaporated and crude was purified by column chromatography (2 M ammonia in methanol:dichloromethane, 1:19). Product was dried under high vacuum. Yield: 230 mg of yellow oil (56%). ^{1}H NMR ($CDCl_3$) δ: 7.44 (dd, J = 1, 5.4 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.09 (t, J = 4.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.88 (dd, J = 2.1, 8.4 Hz, 1H), 6.83 (d, J = 2.1 Hz, 1H), 4.87 (br s, 2H), 4.06 (t, J = 7.5 Hz, 2H), 2.86 (t, J = 7.2 Hz, 2H), 2.63 (t, J = 7.2 Hz, 2H), 2.55 (t, J = 7.2 Hz, 2H), 2.33 (s, 6H). MS (ESI): 357.2 (M + 1). ESI-HRMS calculated for $C_{18}H_{23}N_4SO$ (MH^+), 343.1587; observed, 343.1598. HPLC purity: 99.2%.

*N-(1-(2-(Diethylamino)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**27**).* A solution of 6-amino-1-(2-(diethylamino)ethyl)-3,4-dihydroquinolin-2(1*H*)-one **21** (275 mg, 1.05 mmol) in 10 mL of EtOH was treated with methyl thiophene-2-carbimidothioate hydroiodide (600 mg, 2.10 mmol) and stirred for 1 day at room temperature. Argon was bubbled through the mixture for 20 min, and then the mixture was partitioned between CH_2Cl_2 (50 mL) and saturated sodium bicarbonate (10 mL). The aqueous layer was extracted with an additional 20 mL of CH_2Cl_2. The combined organic layers were dried over sodium sulfate and concentrated to give a yellow residue which was subjected to flash chromatography on silica gel using 5% MeOH/CH_2Cl_2 then 5% 2 M NH_3 in MeOH/CH_2Cl_2 to give a yellow solid (170 mg, 43.7%). ^{1}H NMR (DMSO-d_6) δ: 7.73 (d, J = 3.6 Hz, 1H), 7.60 (d, J = 4.8 Hz, 1H), 7.11−7.04 (m, 2H), 6.76−6.74 (m, 2H), 6.42 (br s, 2H), 3.92 (t, J = 7 Hz, 2H), 2.80 (t, J = 7 Hz, 2H), 2.56−2.47 (m, 8H), 0.94 (t, J = 7 Hz, 6H). MS (ESI): 371.2 (M + 1). ESI-HRMS calculated for $C_{20}H_{27}N_4SO$ (MH^+), 371.1900; observed, 371.1906. HPLC purity: 100%.

*N-(2-Oxo-1-(2-(piperidin-1-yl)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide Dihydrochloride (**28**).* Prepared as described for compound **27** using compound **22**. This compound was converted to the dihydrochloride salt by dissolving in 10 mL of a 10% MeOH/CH_2Cl_2 solution, cooled to 0 °C, and treated with 0.5 mL of a 1 M HCl in Et_2O solution. The solution was stirred for 20 min and then concentrated to give a yellow−brown oil. A yellow solid was obtained after drying under high vacuum overnight. ^{1}H NMR (CD_3OD) δ: 8.08−8.05 (m, 2H), 7.40−7.38 (m, 4H), 4.42 (t, J = 6.7 Hz, 2H), 3.77−3.73 (m,2H), 3.41 (t, J = 6.7 Hz, 2H), 3.32−3.02 (m, 4H), 2.76−2.72 (m, 2H), 2.00−1.53 (m, 6H). MS (ESI): 383.2 (M + 1), 30%, 192.1 (M + 2), 100%. ESI-HRMS calculated for $C_{21}H_{27}N_4OS$ (MH^+), 383.1900; observed, 383.1908. HPLC purity: 98.7%.

*N-(2-Oxo-1-(2-(pyrrolidin-1-yl)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**29**).* Prepared as described for compound **27** using compound **23**. Yield: 68%. ^{1}H NMR ($CDCl_3$) δ: 7.44 (d, J = 5.4 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.09 (t, J = 4.2 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.84 (d, J = 4.2 Hz, 1H), 4.86 (br s, 2H), 4.12−4.06 (m, 2H), 2.88−2.83 (m, 2H), 2.75−2.70 (m, 2H), 2.65−2.60 (m, 6H), 1.82−1.78 (m, 4H). MS (ESI): 369.2 (M + 1). ESI-HRMS calculated for $C_{20}H_{25}N_4SO$ (MH^+), 369.1743; observed, 369.1731. HPLC purity: 96.3%.

*(±)-N-(1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**30**).* Prepared as described for compound **27** using compound **24**. Yield: 6%. ^{1}H NMR (DMSO-d_6) δ: 7.73 (d, J = 3.0 Hz, 1H), 7.60 (d, J = 5.4 Hz, 1H), 7.10−7.02 (m, 2H), 6.72−6.75 (m,2H), 6.45 (br s, 2H), 3.91−3.80 (m, 2H), 2.96−2.90 (m, 1H), 2.82−2.78 (m, 2H), 2.19 (s, 3H), 2.08−1.80 (m, 6H), 1.66−1.49 (m, 4H). MS (ESI): 383.2 (M + 1). ESI-HRMS calculated for $C_{21}H_{27}N_4OS$ (MH^+), 383.1900; observed, 383.1902. HPLC purity: 97.4%.

*N-(1-(2-(Dimethylamino)ethyl)-8-fluoro-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**31**).* Prepared as described for compound **27** using compound **25**. Yield: 69%. ^{1}H NMR (DMSO) δ 7.75 (m, 1H), 7.62 (m, 1H), 7.09 (m, 1H), 6.61 (m, 4H), 3.95 (t, 2H, J = 6.5 Hz), 2.80 (t, 2H, J = 6.6 Hz), 2.49 (m, 2H, masked by DMSO), 2.44 (t, 2H, J = 6.7 Hz), 2.14 (s, 6H). ESI-MS: 361 (MH+, 100). ESI-HRMS calculated for $C_{18}H_{22}N_4OFS$ (MH^+), 361.1492; observed, 361.1508. HPLC purity: 95.9%.

*1-(2-(Dimethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine (**32**).* A solution of 6-amino-1-(2-(dimethylamino)ethyl)-3,4-dihydroquinolin-2(1*H*)-one **20** (1.3 g, 5.57 mmol) in 10 mL of anhydrous THF was added dropwise to a cooled suspension of 1 M $LiAlH_4$ in THF (22.3 mL, 22.3 mmol). The suspension was stirred at room temperature for 1 day. After this time, the mixture was cooled to 0 °C and treated with 5 mL of 1N NaOH

dropwise with rapid stirring. After stirring for 30 min, the suspension was treated with Na_2SO_4 and filtered. The filter cake was rinsed with 10% 2 M NH_3 in MeOH/CH_2Cl_2 (100 mL total). The filtrate was concentrated, and the dark residue was subjected to flash chromatography on silica gel using 5−10% 2 M NH_3 in MeOH/CH_2Cl_2 to give a dark viscous oil (930 mg, 76.2%). ^{1}H NMR ($CDCl_3$) δ: 6.49 (br s, 2H), 6.40 (s, 1H), 3.34−3.30 (m, 2H), 3.30 (br s, 2H), 3.21 (t, J = 5.7 Hz, 2H), 2.68 (t, J = 5.7 Hz, 2H), 2.49−2.44 (m, 2H), 2.28 (s, 6H), 1.95−1.87 (m, 2H). MS (ESI): 220.2 (M + 1).

*(±)-1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine (**33**).* Prepared as described for compound **32** using compound (±)-6-amino-1-(2-(1-methylpyrrolidin-2-yl)ethyl)-3,4-dihydroquinolin-2(1*H*)-one(1*H*)-one **24**. Yield: 52.0%. ^{1}H NMR ($CDCl_3$) δ 6.48 (br s, 2H), 6.41 (br s, 1H), 3.29−3.03 (m, 8H), 2.68 (t, J = 6.6 Hz, 2H), 2.30 (s, 3H), 2.18−1.42 (m, 9H). MS (ESI): 260.2 (M + 1, 100%).

*N-(1-(2-(Dimethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**34**).* Prepared as described for compound **27** using 1-(2-(dimethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine **32**.

^{1}H NMR (DMSO-d_6) δ: 7.66 (d, J = 3.6 Hz, 1H), 7.55 (d, J =4.8 Hz, 1H), 7.08−7.05 (m, 1H), 6.57−6.48 (m, 3H), 6.25 (br s, 2H), 3.29−3.21 (m, 4H), 2.65 (t, J = 6.3 Hz, 2H), 2.39 (t, J = 6.3 Hz, 2H), 2.19 (s, 6H), 1.85−1.82 (m, 2H). MS (ESI): 329.2 (M + 1). ESI-HRMS calculated for $C_{18}H_{24}N_4S$ (MH^+), 329.1794; observed, 329.1804. HPLC purity: 99.5%.

*Chiral Column Chromatographic Separation of (±)-N-(1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**35**).* Preparative chiral chromatographic (supercritical fluid chromatography, SFC) separation was performed using a chiralpak AD-H column (3 cm × 15 cm) with 50% isopropyl alcohol/CO_2 (0.2% diethylamine) at 100 bar, flow rate = 50 mL/min, 254 nm collection wavelength. The sample was dissolved in ethanol/diethylamine (0.2%) at a concentration of 5.75 g/L, and the injection volume was 3 mL. The first eluting enantiomer was (*S*)-*N*-(1-(2-(1-methylpyrrolidin-2-yl)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (RT = 2.42 min; ee =99.86%). The absolute stereochemistry was assigned based on an independent synthesis as described for (±)-**35** using (S)-**13**, which was derived from the corresponding homoproline analogue according to Scheme 3. The second eluting enantiomer was (*R*)-*N*-(1-(2-(1-methylpyrrolidin-2-yl) ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (RT = 3.30 min; ee =100%).

The compounds were converted to the dihydrochloride salts under standard conditions.

*(S)-N-(1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide dihydrochloride ((S)-**35**).* Optical rotation: $[\alpha]^{25}_{589}$ = −18.0°, c = 0.5 in MeOH. ^{1}H NMR (CD_3OD) δ 8.02−7.99 (m, 2H), 7.34 (pseudo t, J = 4.5 Hz, 1H), 7.11−7.03 (m, 2H), 6.85 (d, J = 8.4 Hz, 1H), 3.70−3.65 (m, 1H), 3.51−3.37 (m, 4H), 3.20−3.11 (m, 2H), 2.93 (s, 3H), 2.84−2.80 (m, 2H), 2.50−1.75 (m, 8H). MS (ESI): 369.2 (M + 1). ESI-HRMS calculated for $C_{21}H_{29}N_4S_1$ (MH^+), 369.2107; observed, 369.2118. HPLC purity: 99.3%.

*(R)-N-(1-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide Dihydrochloride ((R)-**35**).* Optical rotation: $[\alpha]^{25}_{589}$ = +17.0°, c = 0.48 in MeOH. ^{1}H NMR (CD_3OD) δ 8.03−7.99 (m, 2H), 7.34 (pseudo t, J = 4.5 Hz, 1H), 7.12−7.03 (m, 2H), 6.85 (d, J = 8.7 Hz, 1H), 3.73−3.65 (m, 1H), 3.51−3.39 (m, 4H), 3.20−3.11 (m, 2H), 2.93 (s, 3H), 2.84−2.80 (m, 2H), 2.50−1.75 (m, 8H). MS (ESI): 369.2 (M + 1). ESI-HRMS calculated for $C_{21}H_{29}N_4S_1$ (MH^+), 369.2107; observed, 369.2113. HPLC purity: 98.9%.

*1-(3-Chloropropyl)-6-nitro-3,4-dihydroquinolin-2(1H)-one (**36**).* 6-Nitro-3,4-dihydroquinolin-2(1*H*)-one **7** (1.50 g, 7.81 mmol) was dissolved in anhydrous DMF (30 mL) in an argon purged round-bottom flask. The reaction was stirred in an ice−water bath, and 60% sodium hydride in mineral oil (1.25 g, 31.25 mmol) was added in one portion. The reaction became dark red−orange. This solution was transferred using a cannulating needle to a solution of 1-chloro-3-iodopropane (2.52 mL, 23.47 mmol) in DMF (20 mL). The reaction was stirred at room temperature for 5 h. The reaction was quenched with brine (25 mL), transferred to a separatory funnel, and partitioned with ethyl acetate (30 mL). The aqueous was extracted twice more with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried with sodium sulfate, decanted, and concentrated to afford a yellow solid. Purification by flash column chromatography afforded a yellow solid (ethyl acetate:hexanes, 30:70−100:0). Yield: 1.58 g (75%). ^{1}H NMR (DMSO) δ: 8.16 (s, 1H), 8.13 (d, J = 2.7 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 4.09−4.04 (m, 2H), 3.71 (t, J = 6.3 Hz, 2H), 3.01 (t, J = 7.5 Hz, 2H), 2.66−2.61 (m, 2H), 2.04−1.99 (m, 2H). MS (ESI): 291.0 and 293.0 (M + 1).

*1-(3-(Dimethylamino)propyl)-6-nitro-3,4-dihydroquinolin-2(1H)-one (**37**).* 1-(3-Chloropropyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one **36** (300 mg, 1.12 mmol), dimethylamine hydrochloride (911 mg, 11.16 mmol), potassium iodide (1.85 g, 11.16 mmol), and potassium carbonate (1.54 g, 11.16 mmol) were weighed into an argon purged vial fitted with a magnetic stirbar. Anhydrous acetonitrile was added, and the yellow suspension stirred at room temperature for 18 h. The reaction was placed in a heating block at a temperature of 60 °C for 2 h. After cooling to room temperature, the reaction was filtered through Celite and the Celite pad washed with methanol and the filtrate concentrated to afford a yellow solid. No further purification was performed. Yield: 520 mg crude material. ^{1}H NMR (DMSO) δ: 8.11 (d, J = 2.4 Hz, 1H), 8.06 (dd, J = 2.7 Hz, 9.3 Hz, 1H), 7.41 (d, J = 9 Hz, 1H), 3.94 (t, J = 7.2 Hz, 2H), 2.99 (t, J = 6.9 Hz, 2H), 2.73 (s, 6H), 2.59 (t, J = 8.1 Hz, 2H), 2.45 (t, J = 1.5 Hz, 2H), 1.86 (t, J = 7.5 Hz, 2H). MS (ESI): 278.1 (M + 1).

*6-Nitro-1-(3-(pyrrolidin-1-yl)propyl)-3,4-dihydroquinolin-2(1H)-one (**38**).* Prepared as described for compound **37** using compound **36** and pyrrolidine. Yield: 91%. ^{1}H NMR ($CDCl_3$) δ: 8.13 (dd, J = 2.7, 9 Hz, 1H), 8.06 (d, J = 2.4 Hz, 1H), 7.23 (d, J = 9.0 Hz, 1H), 4.05 (t, J = 7.5 Hz, 2H), 3.00 (t, J = 7.2 Hz, 2H), 2.73−2.68 (m, 2H), 2.56−2.51 (m, 6H), 1.92−1.77 (m, 6H). MS (EI): 303 (M+).

*6-Amino-1-(3-(dimethylamino)propyl)-3,4-dihydroquinolin-2(1H)-one (**39**).* Prepared as described for compound **20** using compound **37**. Yield: 20%. ^{1}H NMR ($CDCl_3$) δ: 6.86 (d, J = 8.4 Hz, 1H), 6.56 (dd, J = 2.7, 8.4 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 3.92 (t, J = 7.2 Hz, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 2.35 (t, J = 7.2 Hz, 2H), 2.23 (s, 6H), 1.85−1.75 (m, 2H). MS (ESI): 248.2 (M + 1).

*6-Amino-1-(3-(pyrrolidin-1-yl)propyl)-3,4-dihydroquinolin-2(1H)-one (**40**).* Prepared as described for compound **20** using compound **38**. Yield: 73%. ^{1}H NMR ($CDCl_3$) δ: 6.86 (d, J = 8.4 Hz, 1H), 6.55 (dd, J = 2.7, 8.4 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 3.94 (m, 2H), 2.81−2.76 (m, 2H), 2.61−2.56 (m, 2H), 2.53−2.49 (m, 6H), 1.87−1.82 (m, 2H), 1.79−1.75 (m, 4H). MS (ESI): 274.2 (M + 1).

*N-(1-(2-(Dimethylamino)ethyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**41**).* Prepared as described for compound **27** using compound **39**. Yield: 58%. ^{1}H NMR ($CDCl_3$) δ 7.44 (dd, J = 1, 5.4 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.09 (t, J = 4.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.88 (dd, J = 2.1, 8.4 Hz, 1H), 6.83 (d, J = 2.1 Hz, 1H), 4.87 (br s, 2H), 3.98 (t, J = 7.2 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.63 (t, J = 7.2 Hz, 2H), 2.37 (t, J = 7.2 Hz, 2H), 2.25 (s, 6H), 1.89−1.79 (m, 2H). MS (ESI): 357.2 (M + 1). ESI-HRMS calculated for $C_{19}H_{25}N_4SO$ (MH^+), 357.1743; observed, 357.1752. HPLC purity: 97.1%.

*N-(1-(3-(Pyrrolidin-1-yl)propyl)-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**42**).* Prepared as described for compound **27** using compound **40**. Yield: 42%. ^{1}H NMR ($CDCl_3$) δ: 7.44 (d, J = 5.4 Hz, 1H), 7.41 (d, J = 3.3 Hz, 1H), 7.09 (t, J = 4.2 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.84 (d, J = 4.2 Hz, 1H), 4.91

(br s, 2H), 4.05−4.00 (m, 2H), 2.89−2.85 (m, 2H), 2.70−2.64 (m, 8H), 2.02−1.97 (m, 2H), 1.90−1.82 (m, 4H). MS (ESI): 383.2 (M + 1). ESI-HRMS calculated for $C_{21}H_{27}N_4SO_2$ (MH^+), 383.1900; observed, 383.1895. HPLC purity: 98.9%.

*N,N-Diethyl-2-(6-nitro-3,4-dihydroquinolin-1(2H)-yl)ethanamine (**43**).* A solution of 1-(2-(diethylamino)ethyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (1 g, 3.43 mmol) and 1 M BH_3 in THF (17.16 mL, 17.16 mmol) was stirred at room temperature overnight. After this time, the reaction was cooled to 0 °C and then treated with MeOH (25 mL) dropwise with caution. The mixture was stirred at 0 °C for 10 min and then concentrated on the rotovap to give a yellow solid. This compound was dissolved in 40 mL of methanol and heated at reflux for 3 h. After cooling, the solvent was evaporated and the resulting yellow−brown residue was subjected to flash chromatography on silica gel using 5% 2 M NH_3 in MeOH/95% CH_2Cl_2 to give a bright-yellow residue (0.87 g, 91%). ^{1}H NMR ($CDCl_3$) δ 7.96 (dd, J = 2.7, 9.0 Hz, 1H), 7.85−7.84 (m, 1H), 6.51 (d, J = 9.0 Hz, 1H), 3.47 (t, J = 5.4 Hz, 4H), 2.74 (t, J = 6.3 Hz, 2H), 2.68−2.59 (m, 6H), 1.99−1.91 (m, 2H), 1.05 (t, J = 6.9 Hz, 6H). MS (ESI): 278.2 (M + 1).

*2-(8-Fluoro-6-nitro-3,4-dihydroquinolin-1(2H)-yl)-N,N-dimethylethanamine (**44**).* Prepared as described for compound **43** using compound **19**. Yield: 64%. ^{1}H NMR ($CDCl_3$) δ 7.77 (dd, 1H, J = 9 Hz, 2.7 Hz), 7.69 (m, 1H), 3.41 (m, 4H), 2.77 (m, 2H), 2.56 (m, 2H), 2.27 (s, 6H), 1.95 (m, 2H). ESI-MS: 268.1 (MH+, 100).

*N,N-Dimethyl-3-(6-nitro-3,4-dihydroquinolin-1(2H)-yl)propan-1-amine (**45**).* Prepared as described for compound **43** using compound **37**. ^{1}H NMR (DMSO-d_6) δ 7.87 (dd, J = 9.3, 3 Hz, 1H), 7.77 (d, J = 3 Hz, 1H), 6.68 (d, J = 9.6 Hz, 1H), 3.43−3.38 (m, 4H), 2.74 (t, J = 6 Hz, 2H), 2.23 (t, J = 6.5 Hz, 2H), 2.13 (s, 6H), 1.85 (quint, J = 6 Hz, 2H), 1.68 (quint, J = 7 Hz, 2H). ESI-MS (m/z, %) 264 (MH+, 100).

*6-Nitro-1-(3-(pyrrolidin-1-yl)propyl)-1,2,3,4-tetrahydroquinoline (**46**).* Prepared as described for compound **43** using compound **38**. Yield: 56.6%. ^{1}H NMR (DMSO-d_6) δ 7.87 (dd, J = 9.3, 2.7 Hz, 1H), 7.77 (d, J = 2.7 Hz, 1H), 6.70 (d, J = 9.3 Hz, 1H), 3.46−3.38 (m, 4H), 2.74 (t, J = 6 Hz, 2H), 2.43−2.38 (m, 6H), 1.86−1.82 (m, 2H), 1.74−1.67 (m, 6H).

*1-(2-(Diethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine (**47**).* Prepared as described for compound **24** using compound **43**. Yield: 90%. ^{1}H NMR ($CDCl_3$) δ 6.63−6.32 (m, 3H), 3.37−3.23 (m, 4H), 2.74−2.56 (m, 8H), 1.93 (t, J = 5.7 Hz, 2H), 1.08 (t, J = 7.2 Hz, 6H). MS (ESI): 248.2 (M + 1).

*1-(2-(Dimethylamino)ethyl)-8-fluoro-1,2,3,4-tetrahydroquinolin-6-amine (**48**).* Prepared as described for compound **24** using compound **44**. Yield: 89%. ESI-MS: 238 (MH+, 100), 193 (37), 147 (31).

*1-(3-(Dimethylamino)propyl)-1,2,3,4-tetrahydroquinolin-6-amine (**48a**).* Prepared as described for compound **20** using compound **45**. Yield: 92.2% . ^{1}H NMR (DMSO-d_6) δ 6.37−6.34 (m, 1H), 6.30−6.26 (m, 1H), 6.22−6.20 (m, 1H), 4.17 (br s, 2H), 3.12−3.03 (m, 4H), 2.55 (t, J = 6.45 Hz, 2H), 2.20 (t, J = 6.9 Hz, 2H), 2.11 (s, 6H), 1.79 (quint, J = 6 Hz, 2H), 1.56 (quint, J = 7.12 Hz, 2H). ESI-MS (m/z, %) 234 (MH+, 100), 161 (60).

*1-(3-(Pyrrolidin-1-yl)propyl)-1,2,3,4-tetrahydroquinolin-6-amine (**49**).* Prepared as described for compound **24** using compound **46**. Yield: 98%. MS (ESI): 360.2 (M + 1).

*N-(1-(2-(Diethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**50**).* Prepared as described for compound **27** using compound **47**. Yield: 69.4%. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.0 Hz, 1H), 7.54 (d, J = 4.8 Hz, 1H), 7.07 (dd, J = 3.6, 4.5 Hz, 1H), 6.57−6.48 (m, 3H), 5.76 (br s, 2H), 3.32−3.25 (m, 4H), 2.67−2.63 (m, 2H), 2.52−2.48 (m, 6H), 1.86−1.82 (m, 2H), 0.97 (t, J = 6.9 Hz, 6H). MS (ESI): 357.2 (M + 1). ESI-HRMS calculated for $C_{20}H_{29}N_4S$ (MH^+), 357.2107; observed, 357.2110. HPLC purity: 99.6%.

*N-(1-(2-(Dimethylamino)ethyl)-8-fluoro-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**51**).* Prepared as described for compound **27** using compound **48**. Yield: 15%. ^{1}H NMR (DMSO) δ 1.75 (m, 2H), 2.16 (s, 6H), 2.46 (m, 2H), 2.66 (m, 2H), 3.12 (m, 4H), 6.40 (m, 4H), 7.07 (m, 1H), 7.57 (m, 1H), 7.71 (m, 1H). ESI-MS: 347 (MH+, 33), 276 (100), 143 (12). ESI-HRMS calculated for $C_{18}H_{24}N_4FS$ (MH^+), 347.1700; observed, 347.1700. HPLC purity: 95.5%.

*N-(1-(3-(Dimethylamino)propyl)-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**52**).* Prepared as described for compound **27** using compound **48**. Yield: 85%. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3 Hz, 1H), 7.55 (d, J = 5.1 Hz, 1H), 7.07 (dd, J = 5.1, 3 Hz, 1H), 6.55 (m, 2H), 6.48 (m, 1H), 6.31 (br s, 2H), 3.24−3.17 (m, 4H), 2.66 (t, J = 6.3 Hz, 2H), 2.25 (t, J = 6.9 Hz, 2H), 1.88−1.82 (m, 2H), 1.67−1.60 (m, 2H). ESI-MS (m/z, %) 343 (MH+, 89), 258 (100), 135 (48), 127 (60). ESI-HRMS calculated for C19H27N4S (MH+) calculated, 343.1963; observed, 343.195. HPLC purity: 97.1%.

*N-(1-(3-(Pyrrolidin-1-yl)propyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**53**).* Prepared as described for compound **27** using 1-(3-(pyrrolidin-1-yl)propyl)-1,2,3,4-tetrahydroquinolin-6-amine, which was prepared by reducing compound **46**. Yield: 72.8%. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.6 Hz, 1H), 7.55 (d, J = 5 Hz, 1H), 7.06 (dd, J = 5, 3.6 Hz, 1H), 6.58 (br s, 2H), 6.48 (s, 1H), 6.27 (br s, 2H), 3.26−3.17 (m, 4H), 2.66 (t, J = 6.3 Hz, 2H), 2.45−2.40 (m, 6H), 1.89−1.81 (m, 2H), 1.70−1.62 (m, 6H). ESI-MS (m/z, %) 369 (MH+, 47), 185 (100). ESI-HRMS calculated for C21H29N4S (MH+) calculated, 369.2122; observed, 369.2107. HPLC purity: 95.4%.

*1-(1-Methylpiperidin-4-yl)-1,2,3,4-tetrahydroquinoline (**58**).* A solution of 1,2,3,4-tetrahydroquinoline **54** (1.0 mL, 7.94 mmol) in 20 mL of 1,2-dichloroethane was treated with 1-methylpiperidin-4-one **55** (2.76 mL, 23.8 mmol) followed by sodium triacetoxyborohydride (8.4 g, 39.7 mmol) and then acetic acid (2.25 mL). The suspension was stirred at room temperature for 1 day. After this time, the mixture was cooled to 0 °C, quenched with 5 mL of 1N NaOH, and stirred for 20 min. The suspension was extracted with 100 mL of CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a light residue which was subjected to flash chromatography on silica gel using 5% MeOH/CH_2Cl_2 then 5% 2 M NH_3 in MeOH/CH_2Cl_2. A yellow oil was obtained (440 mg, 24.1%). ^{1}H NMR ($CDCl_3$) δ: 7.07−7.02 (m,1H), 6.95 (d, J = 7.5 Hz, 1H), 6.65 (d, J = 8.4 Hz, 1H), 6.55 (pseudo t, J = 7.8 Hz, 1H), 3.65−3.55 (m, 1H), 3.20 (t, J = 5.7 Hz, 2H), 2.99−2.95 (m, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.31(s, 3H), 2.11−2.05(m, 2H), 1.93−1.73 (m, 6H). MS (ESI): 231.2 (M + 1, 100%).

*tert-Butyl 4-(3,4-Dihydroquinolin-1(2H)-yl)piperidine-1-carboxylate (**59**).* Prepared as described for compound **58** using compound **54** and *tert*-butyl 4-oxopiperidine-1-carboxylate **56**. The crude product was purified by silica gel chromatography using 15% EtOAc/85% hexanes. Yield: 37.4%. ^{1}H NMR ($CDCl_3$) δ 7.09−7.03 (m, 1H), 6.97−6.94 (m, 1H), 6.65 (d, J = 8.4 Hz, 1H), 6.60−6.55 (m, 1H), 4.30−4.19 (m, 2H), 3.80−3.70 (m, 1H), 3.17 (t, J = 5.7 Hz, 2H), 2.84−2.71 (m, 2H), 2.73 (t, J = 6.3 Hz, 2H), 1.93−1.85 (m, 2H), 1.78−1.63 (m, 4H), 1.48 (s, 9H). MS (ESI): 317.2 (M + 1).

*tert-Butyl 3-(3,4-Dihydroquinolin-1(2H)-yl)pyrrolidine-1-carboxylate (**60**).* Prepared as described for compound **59** using compound **54** and compound **57**. Yield: 78.8%. ^{1}H NMR ($CDCl_3$) δ 7.07 (pseudo t, J = 7.2 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 6.62 (pseudo t, J = 7.5 Hz, 1H), 4.44−4.40 (m, 1H), 3.63−3.20 (m, 6H), 2.75 (t, J = 6.3 Hz, 2H), 2.13−2.08 (m, 2H), 1.94−1.90 (m, 2H), 1.48 (s, 9H). MS (ESI): 303.2 (M + 1).

*6-Bromo-1-(1-methylpiperidin-4-yl)-1,2,3,4-tetrahydroquinoline (**61**).* A solution of 1-(1-methylpiperidin-4-yl)-1,2,3,4-tetrahydroquinoline **58** (500 mg, 2.17 mmol) in 7 mL of DMF was cooled to 0 °C and then treated dropwise with NBS (386 mg, 2.17 mmol) in 7 mL of DMF. The reaction was stirred at 0 °C for 2 h and then treated with 30 mL of H_2O. The suspension was extracted with 100 mL of EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a dark residue which was filtered through a short plug of silica gel using 5% 2 M NH_3 in

MeOH/CH_2Cl_2 (100 mL). The filtrate was concentrated and subjected to flash chromatography on silica gel using 5% MeOH/CH_2Cl_2 then 5% 2 M NH_3 in MeOH/CH_2Cl_2. A light-yellow oil was obtained (490 mg, 73.0%). ^{1}H NMR ($CDCl_3$) δ: 7.10 (dd, J = 2.1, 10.8 Hz), 7.04−7.03 (m,1H), 6.50 (d, J = 9.0 Hz, 1H), 3.57−3.47 (m, 1H), 3.18 (t, J = 5.7 Hz, 2H), 2.98−2.94 (m, 2H), 2.68 (t, J = 6.0 Hz, 2H), 2.31 (s, 3H), 2.10−2.02 (m, 2H), 1.91−1.69 (m, 6H). MS (ESI): 309.1 and 311.1 (M + 1, 100%).

*tert-Butyl 4-(6-Bromo-3,4-dihydroquinolin-1(2H)-yl)piperidine-1-carboxylate (**62**).* Prepared as described for compound **61** using compound **59**. The crude product was purified by silica gel chromatography using a gradient of 0−20% EtOAc/100−80% hexanes. Yield: 80.4%. ^{1}H NMR ($CDCl_3$) δ 7.10 (dd, J = 2.4 Hz, 8.7 Hz, 1H), 7.05−7.04 (m, 1H), 6.51 (d, J = 9.0 Hz, 1H), 4.33−4.19 (m, 2H), 3.71−3.64 (m, 1H), 3.14 (t, J = 6.0 Hz, 2H), 2.82−2.74 (m, 2H), 2.69 (t, J = 6.3 Hz, 2H), 1.91−1.77 (m, 2H), 1.61−1.57 (m, 4H), 1.47 (s, 9H). MS (ESI): 395.1 and 377.1 (M + 1).

*tert-Butyl 3-(6-Bromo-3,4-dihydroquinolin-1(2H)-yl)pyrrolidine-1-carboxylate (**63**).* Prepared as described for compound **62** using compound **60**. Yield: 63.8%. ^{1}H NMR ($CDCl_3$) δ 7.13 (d, J = 9.0 Hz, 1H), 7.07 (br s, 1H), 6.55 (d, J = 9.0 Hz, 1H), 4.35−4.33 (m, 1H), 3.58−3.18 (m, 6H), 2.71 (t, J = 6.3 Hz, 2H), 2.11−2.04 (m, 2H), 1.91−1.87 (m, 2H), 1.47 (s, 9H). MS (ESI): 325.1 and 327.1 (M + 1, 100%).

*6-Bromo-1-(1-methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinoline (**64**).* A solution of *tert*-butyl 3-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)pyrrolidine-1-carboxylate **63** (700 mg, 1.83 mmol) in 10 mL of methanol was treated with 12 mL of 1N HCl. A precipitate formed upon addition of HCl. To solubilize the mixture, additional methanol (10 mL) was added. The solution was then heated at reflux for 30 min. The solution was concentrated and extracted with CH_2Cl_2 (2 × 50 mL). The aqueous layer was basified with saturated Na_2CO_3 and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic fractions were rinsed with brine, dried over Na_2SO_4, filtered, and concentrated to give 6-bromo-1-(pyrrolidin-3-yl)-1,2,3,4-tetrahydroquinoline (yield: 404 mg, 78.4%). ^{1}H NMR ($CDCl_3$) δ 7.11 (dd, J = 2.7, 9.0 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.57 (d, J = 8.7 Hz, 1H), 4.36−4.27 (m, 1H), 3.21 (t, J = 5.4 Hz, 2H), 3.16−2.90 (m, 4H), 2.71 (t, J = 6.3 Hz, 2H), 2.11−2.00 (m, 1H), 1.93−1.78 (m, 4H). MS (EI): 281.1 and 283.1 (M + 1).

A solution of 6-bromo-1-(pyrrolidin-3-yl)-1,2,3,4-tetrahydroquinoline (200 mg, 0.71 mmol) in 7 mL of anhydrous methanol was treated with formaldehyde (37% aqueous solution, 79 μL, 1.07 mmol), followed by acetic acid (100 μL, 1.78 mmol). The solution was treated with sodium cyanoborohydride (67 mg, 1.07 mmol). The suspension was stirred at room temperature for 3 h. The mixture was concentrated to dryness and partitioned between 20 mL of 1N NaOH and 100 mL of CH_2Cl_2. After extraction, the organic layer was dried over Na_2SO_4, filtered, and concentrated to give a oily residue which was subjected to flash chromatography on silica gel using 5% 2 M NH_3 in MeOH/CH_2Cl_2. A yellow oil was obtained (153 mg, 72.9%). ^{1}H NMR ($CDCl_3$) δ 7.10 (dd, J = 2.4, 8.7 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.57 (d, J = 9.0 Hz, 1H), 4.47−4.38 (m, 1H), 3.26 (t, J = 5.7 Hz, 2H), 2.81−2.68 (m, 4H), 2.60−2.55(m, 1H), 2.40−2.24 (m, 1H), 2.35 (s, 3H), 2.24−2.13 (m, 1H), 1.92−1.79 (m, 3H). MS (EI): 295.1 and 297.1 (M + 1).

*1-(1-Methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinolin-6-amine (**65**).* A suspension of $Pd_2(dba)_3$ (22 mg, 0.024 mmol) in 2 mL of anhydrous THF was treated P^tBu_3 (285 μL of a 10 wt % in hexane solution, 0.094 mmol). The mixture was stirred at room temperature for 5 min, and then lithium hexamethyldisilizane (0.95 mL of a 1 M solution in THF, 0.95 mmol) was added. The resulting dark mixture was treated with 6-bromo-1-(1-methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinoline (140 mg, 0.47 mmol) in 8 mL of THF. The dark-brown suspension was heated at 95 °C in a sealed tube for 2 h. The mixture was concentrated and treated with 5 mL of a 1N HCl solution and then stirred at room temperature for 10 min. The mixture was partitioned between CH_2Cl_2 (100 mL) and 1N NaOH (20 mL). After extraction, the organic layer was separated and dried over Na_2SO_4, filtered, and concentrated to give a dark-brown residue. This residue was subjected to flash chromatography on silica gel using 2.5% MeOH/CH_2Cl_2 and then 5% 2 M NH_3 in MeOH/CH_2Cl_2 to give a dark-brown residue (95 mg, 87.2%). ^{1}H NMR ($CDCl_3$) δ 6.59 (d, J = 8.4 Hz, 1H), 6.47 (dd, J = 2.7, 8.7 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 4.45−4.38 (m, 1H), 3.28 (br s, 2H), 3.23−3.12 (m, 2H), 2.75−2.60 (m, 5H), 2.45−2.39 (m, 1H), 2.34 (s, 3H), 2.19−2.09 (m, 1H), 1.92−1.82 (m, 3H). MS (EI): 232.2 (M + 1).

*tert-Butyl 4-(6-Amino-3,4-dihydroquinolin-1(2H)-yl)piperidine-1-carboxylate (**66**).* A suspension of $Pd_2(dba)_3$ (29 mg, 0.032 mmol) in 2 mL of anhydrous THF was treated with $PtBu_3$ (400 μL of a 10 wt % in hexanes solution, 0.13 mmol) and stirred at room temperature for 5 min. To this mixture was added *tert*-butyl 4-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)piperidine-1-carboxylate (250 mg, 0.63 mmol), followed by lithium hexamethyldisilizane (1.3 mL of a 1 M solution in THF, 1.3 mmol). The resulting dark-brown suspension was heated at 95 °C for 3 h. The mixture was cooled to room temperature and treated with 5 mL of a 1 M tetrabutlyammonium fluoride solution in THF and then stirred at room temperature for 30 min. The mixture was partitioned between EtOAc (100 mL) and H_2O (20 mL). After extraction, the organic layer was separated, dried over Na_2SO_4, filtered, and concentrated to give a dark-brown residue. This residue was subjected to flash chromatography on silica gel using 2.5% 2 M NH_3 in MeOH/CH_2Cl_2 to give a viscous dark-brown residue (160 mg, 76.6%). ^{1}H NMR ($CDCl_3$) δ 6.56 (d, J = 8.4 Hz, 1H), 6.48 (dd, J = 2.7, 8.7 Hz, 1H), 6.43−6.42 (m, 1H), 4.25−4.21 (m, 2H), 3.69−3.59 (m, 1H), 3.24 (br s, 2H), 3.07 (t, J = 5.4 Hz, 2H), 2.78−2.72 (m, 2H), 2.66 (t, J = 6.6 Hz, 2H), 1.93−1.83 (m, 2H), 1.76−1.55 (m, 4H), 1.47 (s, 9H). MS (ESI): 332.2 (M + 1, 100%).

*1-(1-Methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinolin-6-amine (**67**).* Prepared as described for compound **65** using 6-bromo-1-(1-methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinoline (**64**). Yield: 87.2%. ^{1}H NMR ($CDCl_3$) δ 6.59 (d, J = 8.4 Hz, 1H), 6.47 (dd, J = 2.7, 8.7 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 4.45−4.38 (m, 1H), 3.28 (br s, 2H), 3.23−3.12 (m, 2H), 2.75−2.60 (m, 5H), 2.45−2.39 (m, 1H), 2.34 (s, 3H), 2.19−2.09 (m, 1H), 1.92−1.82 (m, 3H). MS (EI): 232.2 (M + 1).

*N-(1-(1-Methylpiperidin-4-yl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**68**).* Prepared as described for compound **27** using compound **65**. ^{1}H NMR (DMSO-d_6) δ: 7.67 (d, J = 3.6 Hz, 1H), 7.54 (d, J = 5.4 Hz, 1H), 7.06 (pseudo t, J = 4.2 Hz, 1H), 6.63−6.48 (m, 3H), 6.21 (br s, 2H), 3.54−3.47 (m, 1H), 3.11 (t, J = 5.7 Hz, 2H), 2.86−2.82 (m, 2H), 2.63 (t, J = 6.0 Hz, 2H), 2.17 (s, 3H), 2.03−1.96 (m, 2H), 1.84−1.57(m, 6H). MS (ESI): 355.2 (M + 1, 100%). ESI-HRMS calculated for $C_{20}H_{26}N_4S$ (MH^+), 355.1950; observed, 355.1938. HPLC purity: 99.7%.

*tert-Butyl-4-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2H)-yl)piperidine-1-carboxylate (**69**).* Prepared as described for compound **27** using compound **26**. Yield: 60.6%. ^{1}H NMR (DMSO-d_6) δ 7.40−7.38 (m, 2H), 7.06 (dd, J = 3.9, 1.2 Hz, 1H), 6.74 (dd, J = 2.4, 8.4 Hz, 1H), 6.67−6.65 (m, 2H), 4.33−4.18 (m, 2H), 3.77−3.68 (m, 1H), 3.14 (t, J = 5.7 Hz, 2H), 2.83−2.79 (m, 2H), 2.72 (t, J = 6.3 Hz, 2H), 1.94−1.82 (m, 2H), 1.69−1.61 (m, 4H), 1.48 (s, 9H). MS (ESI): 441.2 (M + 1, 100%).

*N-(1-(Piperidin-4-yl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide Dihydrochloride (**70**).* A solution of *tert*-butyl-4-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl) piperidine-1-carboxylate **69** (115 mg, 0.26 mmol) in 10 mL of methanol was treated with 1.5 mL of 2N HCl then heated at 90 °C for 30 min. The solution was concentrated and dried under reduced pressure to give a yellow−brown solid. This solid was triturated with 5% H_2O/95% acetone. The solid was collected and dried under reduced pressure. Yield: 78 mg (72.6%). ^{1}H NMR (CD_3OD) δ 7.98−7.94 (m, 2H), 7.28 (dd, J = 4.2, 5.1 Hz, 1H), 7.07 (dd, J = 2.4, 8.7 Hz, 1H), 7.01−6.98 (m, 2H), 4.14−4.03 (m, 1H), 3.49−3.45 (m, 2H), 3.28−3.25 (m, 2H),

3.20−3.10 (m, 2H), 2.77 (t, J = 6.3 Hz, 2H), 2.15−1.89 (m, 6H). MS (ESI): 341.2 (M + 1). ESI-HRMS calculated for $C_{19}H_{25}N_4S$ (MH^+), 341.1794; observed, 341.1800. HPLC purity: 95.6%.

*(±)-N-(1-(1-Methylpyrrolidin-3-yl)-1,2,3,4-tetrahydroquinolin-6-yl) thiophene-2-carboximidamide (**71**).* Prepared as described for compound **27** using compound **67**. Yield: 65.6%. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.3 Hz, 1H), 7.55 (d, J = 5.1 Hz, 1H), 7.08−7.06 (m, 1H), 6.70 (d, J = 8.7 Hz, 1H), 6.55−6.50 (m, 2H), 6.29 (br s, 2H), 4.46−4.37 (m, 1H), 3.21−3.15 (m, 2H), 2.74−2.69 (m, 4H), 2.50−2.43 (m, 2H), 2.25 (s, 3H), 2.15−2.04 (m, 1H), 1.86−1.67 (m, 3H). MS (ESI): 341.2 (M + 1). ESI-HRMS calculated for $C_{19}H_{25}N_4S_1$ (MH^+), 341.1794; observed, 341.1788. HPLC purity: 100%.

General Procedure for the Conversion of the Free Base to the Dihydrochloride Salt. To a solution of the free base (1.0 equiv) in methanol was added 1 M HCl in diethyl ether (3.0 equiv). The solution was stirred at room temperature for 10 min and then concentrated to dryness. The residue was dried under reduced pressure for 2 days to give a solid. In all cases, the HPLC purity of the salt is similar to that of the free base.

NOS Inhibition Assay. Recombinant human inducible NOS (iNOS), human endothelial constitutive NOS (eNOS), or human neuronal constitutive NOS (nNOS) were produced in Baculovirus-infected Sf9 cells (ALEXIS). In a radiometric method, NO synthase activity was determined by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline. To measure iNOS, 10 μL of enzyme is added to 100 μL of 100 mM HEPES, pH = 7.4, containing 1 mM $CaCl_2$, 1 mM EDTA, 1 mM dithiotheitol, 1 μM FMN, 1 μM FAD, 10 μM tetrahydrobiopterin, 120 μM NADPH, and 100 nM CaM. To measure eNOS or nNOS, 10 μL of enzyme is added to 100 μL of 40 mM HEPES, pH = 7.4, containing 2.4 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mg/mL BSA, 1 mM EDTA, 1 mM dithiothreitol, 1 μM FMN, 1 μM FAD, 10 μM tetrahydrobiopterin, 1 mM NADPH, and 1.2 μM CaM.

To measure enzyme inhibition, a 15 μL solution of a test substance was added to the enzyme assay solution, followed by a preincubation time of 15 min at RT. The reaction was initiated by addition of 20 μL of L-arginine containing 0.25 μCi of [^{3}H] arginine/mL and 24 μM L-arginine. The total volume of the reaction mixture was 150 μL in every well. The reactions are carried out at 37 °C for 45 min. The reaction was stopped by adding 20 μL of ice-cold buffer containing 100 mM HEPES, 3 mM EGTA, 3 mM EDTA, pH = 5.5. [^{3}H]L-citrulline was separated by DOWEX (ion-exchange resin DOWEX 50 W X 8−400, SIGMA), and the DOWEX is removed by spinning at 12000g for 10 min in the centrifuge. A 70 μL aliquot of the supernatant was added to 100 μL of scintillation fluid, and the samples were counted in a liquid scintillation counter (1450 Microbeta Jet, Wallac). Specific NOS activity is reported as the difference between the activity recovered from the test solution and that observed in a control sample containing 240 mM of the inhibitor L-NMMA. All assays were performed at least in duplicate. Standard deviations are 10% or less.

Chung Model of Injury-Induced Neuropathic-Like Pain. Nerve ligation injury was performed according to the method described by Kim and Chung.[29] This technique produces signs of neuropathic dysesthesias, including tactile allodynia, thermal hyperalgesia, and guarding of the affected paw which begins on day 1 of the surgery and peaks on day 16. Rats were anesthetized with halothane, and the vertebrae over the L4 to S2 region were exposed. The L5 and L6 spinal nerves were exposed, carefully isolated, and tightly ligated with 4−0 silk suture distal to the DRG. After ensuring homeostatic stability, the wounds were sutured, and the animals allowed to recover in individual cages. Sham-operated rats were prepared in an identical fashion except that the L5/L6 spinal nerves were not ligated. Any rats exhibiting signs of motor deficiency were euthanized. After a period of recovery following the surgical intervention, rats showed enhanced sensitivity to painful and normally nonpainful stimuli.

Migraine Model[30]. *Animals.* Male, Sprague−Dawley rats (275−300 g) were purchased from Harlan Sprague−Dawley (Indianapolis, IN). Animals were given free access to food and water. Animals were maintained on a 12 h light (7 a.m. to 7 p.m.) and 12 h dark cycle (7 p.m. to 7 a.m.). All procedures were in accordance with the policies and recommendations of the International Association for the Study of Pain and the National Institutes of Health guidelines and use of laboratory animals as well as approved by the Animal Care and Use Committee of the University of Arizona.

Surgical Preparation. *Migraine Cannulation.* Male Sprague−Dawley rats were anesthetized using ketamine/xylazine (80 mg/kg, ip), the top of the head was shaved using a rodent clipper (Oster Golden A5 w/size 50 blade), and the shaved area was cleaned with betadine and 70% ethanol. Animals were placed into a stereotaxic apparatus (Stoelting model 51600), and the body core temperatures of 37 °C were maintained using a heating pad placed below the animals. Within the shaved and cleaned area on the head, a 2 cm incision was made using a scalpel with a no. 10 blade and any bleeding was cleaned using sterile cotton swabs. Location of bregma and midline bone sutures were identified as references and a small hole 1 mm in diameter was made using a hand drill without breaking the dura but deep enough to expose the dura. Two additional holes (1 mm in diameter) 4−5 mm from the previous site were made in order to mount stainless steel screws (Small Parts no. A-MPX-080-3F) securing the cannula through which an inflammatory soup could be delivered to induce experimental migraine. A modified intracerebroventricular (ICV) cannula (Plastics One no. C313G) was placed into the hole without penetrating into or through the dura. The ICV cannula was modified by cutting it to a length of 1 mm from the bottom of the plastic threads using a Dremel mototool and a file to remove any steel burrs. Once the modified migraine cannula was in place, dental acrylic was placed around the migraine cannula and stainless steel screws in order to ensure that the cannula was securely mounted. Once the dental acrylic was dry (i.e., after 10−15 min), the cap of the cannula was secured on top to avoid contaminants entering the cannula and the skin was sutured back using 3−0 silk suture. Animals were given an antibiotic injection (Amikacin C, 5 mg/kg, im) and removed from the stereotaxic frame and allowed to recover from anesthesia on a heated pad. Animals were placed in a clean separate rat cage for a 5 day recovery period.

Injections. *Migraine Cannula Injections.* An injection cannula (Plastics One, C313I cut to fit the modified ICV cannulas) connected to a 25:l Hamilton syringe (1702SN) by tygon tubing (Cole-Palmer, 95601−14) was used to inject 10:l of the IM solution onto the dura.

Behavioral Testing. Naïve animals prior to the day of migraine surgery were placed in suspended plexiglass chambers (30 cm L × 15 cm W × 20 cm H) with a wire mesh bottom (1 cm^2) and acclimated to the testing chambers for 30 min.

Hindpaw Sensory Thresholds to Non-noxious Tactile Stimuli in Rats. The paw withdrawal thresholds to tactile stimuli were determined in response to probing with calibrated von Frey filaments (Stoelting, 58011). The von Frey filaments were applied perpendicularly to the plantar surface of the hind paw of the animal until it buckled slightly and was held for 3 to 6 s. A positive response was indicated by a sharp withdrawal of the paw. The 50% paw withdrawal threshold was determined by the nonparametric method of Dixon.[9] An initial probe equivalent to 2.00 g was applied, and if the response was negative the stimulus was increased one increment, otherwise a positive response resulted in a decrease of one increment. The stimulus was incrementally increased until a positive response was obtained and then decreased until a negative result was observed. This "up-down" method was repeated until three changes in behavior were determined. The pattern of positive and negative responses was tabulated. The 50% paw withdrawal threshold was determined as $(10^{[Xf+kM]})/10000$, where Xf = the value of the last von Frey filament employed, k = Dixon value for the positive/

negative pattern, and M = the mean (log) difference between stimuli. Only naïve animals with baselines of 11−15 g were used in the experiment. Fifteen grams was used as the maximal cutoff. Five days post migraine surgery, animals paw withdrawal thresholds were retested using the same habituation and von Frey procedure as stated above. Data were converted to % "antiallodynia" by the formula: % activity = 100((postmigraine value − baseline value)/(15 g − baseline value)). Only animals that demonstrated no difference in their tactile hypersensitivity as compared to their premigraine surgery values were used in all studies.

After establishing baseline paw withdrawal thresholds, individual animals were removed from the testing chamber, the cap of the migraine cannula was removed, and animals received an injection of either a mixture of IM (1 mM histamine, 1 mM 5-HT [serotonin], 1 mM bradykinin, 1 mM PGE_2) or vehicle at 10 μL volume via the migraine cannula over a 5−10 s period. The inflammatory mediator (IM) cocktail was made fresh on the day of each experiment. The cap of the migraine cannula was replaced, individual animals were placed back into their corresponding testing chamber, and paw withdrawal thresholds were measured at 1 h intervals over a 6 h time course. Data were converted to % "antiallodynia" by the formula: % activity = 100((post-IM value − pre-IM baseline value)/(15 g − pre-IM baseline value)).

■ ASSOCIATED CONTENT

Supporting Information. Full details on the HPLC methods and purity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED:

NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; SAR, structure−activity relationship; sGC, soluble guanylyl cyclase; GTP, guanosine-5′-triphosphate; cGMP, 3′,5′-cyclic guanosine monophosphate; TNFα, tumor necrosis factor-α; IL-1, interleukin-1; LPS, lipopolysaccharide; NADPH, nicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; BH4, (1′*R*,2′*S*,6*R*)-5,6,7,8-tetrahydrobiopterin; ip, intraperitonieal; IM, inflammatory mediators

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