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1,2,3,4-Tetrahydroquinoline-Based Selective Human Neuronal Nitric Oxide Synthase (nNOS) Inhibitors: Lead Optimization Studies Resulting in the Identification of *N*-(1-(2-(Methylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide as a Preclinical Development Candidate

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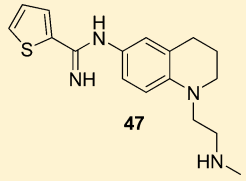
ABSTRACT: Numerous studies have shown that selective nNOS inhibitors could be therapeutic in many neurological disorders. Previously, we reported a series of 1,2,3,4-tetrahydroquinoline-based potent and selective nNOS inhibitors, highlighted by **1** (*J. Med. Chem.* **2011**, *54*, 5562−5575). Despite showing activity in two rodent pain models, **1** suffered from low oral bioavailability (18%) and moderate hERG channel inhibition (IC_{50} = 4.7 μM). To optimize the properties of **1**, we synthesized a small focused library containing various alkylamino groups on the 1-position of the 1,2,3,4-tetrahydroquinoline scaffold. The compounds were triaged based on their activity in the NOS and hERG manual patch clamp assays and their calculated physicochemical parameters. From these studies, we identified **47** as a potent and selective nNOS inhibitor with improved oral bioavailability (60%) and no hERG channel inhibition (IC_{50} > 30 μM). Furthermore, **47** was efficacious in the Chung model of neuropathic pain and has an excellent safety profile, making it a promising preclinical development candidate.



nNOS IC_{50} = 0.176 μM
eNOS IC_{50} = 40.7 μM
iNOS IC_{50} > 100 μM

hERG patch clamp IC_{50} > 30 μM
Oral bioavailability = 60%

INTRODUCTION

The discovery of nitric oxide (NO) as a mediator of vascular tone in the early 1980s[1,2] has led to the unraveling of the diverse biological functions of NO.[3,4] This small, short-lived molecule is synthesized from L-arginine by a family of three enzymes called nitric oxide synthase (NOS).[5] Two isoforms, endothelial NOS (eNOS) and neuronal NOS (nNOS), are constitutively expressed and depend on increases in external calcium and binding of a calcium/calmodulin complex for activation. The third isoform is inducible NOS (iNOS), requiring an external stimulus for production and independent of calcium activation due to the tight binding of a calcium/calmodulin complex at the dimer interface. Under normal conditions, these enzymes produce NO in a delicate balance to control diverse physiological functions such as blood pressure regulation, platelet aggregation, inflammation, and neurotransmission.[6] However, overproduction of NO by the different isoforms can lead to many pathological conditions. In particular, a large body of evidence indicates that overproduction of NO by nNOS can lead to many neurological disease states including neurodegeneration during Alzheimer's and Parkinson's diseases,[7] altered spinal transmission of neuropathic pain,[8,9] and progression of migraine and chronic tension-type headaches.[10] Consequently, the inhibition of nNOS has the potential to be therapeutic in many disease states, but in order to maintain the important roles of eNOS in regulating blood pressure and iNOS in immune responses, the selective inhibition of nNOS is crucial.[11]

The design and development of selective nNOS inhibitors have challenged both academic and industrial researchers alike for over 2 decades.[12−14] The early inhibitors of NOS were nonselective L-arginine-based compounds such as *N*-monomethyl-L-arginine (L-NMMA) and *N*-nitro-L-arginine methyl ester (L-NAME). The lack of selectivity prevented these compounds from being therapeutically useful, primarily because of the effects of eNOS inhibition;[15] for example, L-NMMA has been shown to increase blood pressure in human clinical trials.[16] Using traditional medicinal chemistry efforts and structure-guided drug discovery techniques, researchers have identified many structurally diverse compounds that inhibit nNOS potently and selectively (structures **1**−**4** are some recent examples, Figure 1).[17−20] Despite this progress, a major challenge still exists in designing CNS druglike molecules with favorable physicochemical properties to target the relatively polar active site of nNOS.[21]

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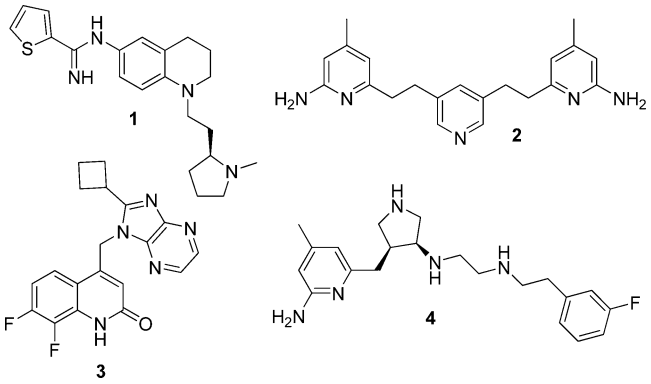


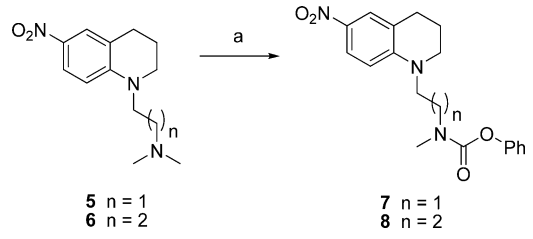
Figure 1. Examples of recently published nNOS inhibitors.

Our research group has been interested in designing and developing druglike selective nNOS inhibitors for treating CNS disorders, and we have reported a number of structurally diverse nNOS inhibitors.[17,22−25] Recently, we identified **1** as a druglike potent and selective human nNOS inhibitor that was efficacious in the rat Chung model of neuropathic pain and also in a rodent model of dural inflammation relevant to migraine pain.[17] However, because of the low oral bioavailability of this compound (18%), we could not advance it further into preclinical development. Later, we discovered that compound **1** was moderately active at the hERG ion channel (vide infra), which further hampered its development. To address these drawbacks, we now describe the lead optimization studies of **1** that led to the identification of *N*-(1-(2-(methylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (**47**), which is a potent and selective human nNOS inhibitor with the desirable features of a preclinical development candidate.

RESULTS AND DISCUSSION

Chemistry. We developed a general method for synthesizing the target compounds in this study from either a bromo or nitro intermediate (Scheme 5). The synthesis of intermediates **7**, **8**, **13**, **15**, **17**, **23**, **24**, and **28** are outlined in Schemes 1−4,

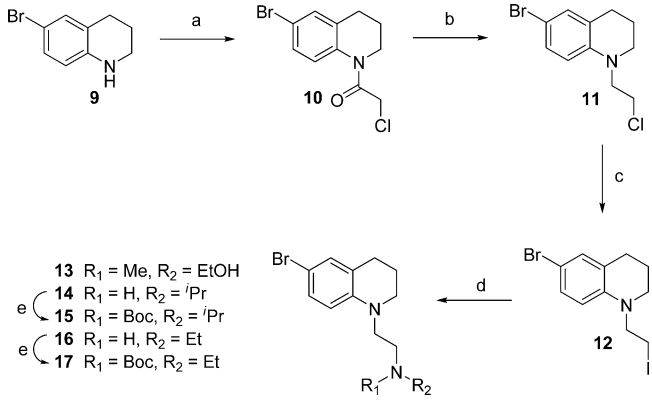
Scheme 1. Synthesis of Intermediates 7 and 8[a]



[a]Reagents and conditions: (a) PhOCOCl, CH_2Cl_2, rt, 17 h.

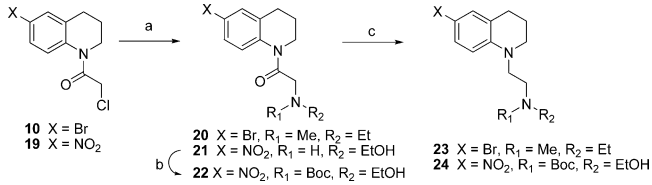
while the synthesis of intermediate **18** was described previously.[17] The nitro intermediates **7** and **8** were prepared according to Scheme 1 from compounds **5** and **6**, respectively, by N-demethylation with phenyl chloroformate and concomitant protection as the phenyl carbamate. To synthesize compounds **13**, **15**, and **17**, compound **9** was treated with chloroacetyl chloride to give the chloroamide **10**, which was reduced with borane in THF to give the alkyl chloride **11**. In order to efficiently couple a series of amines to this scaffold, it was necessary to convert **11** to the corresponding alkyl iodide **12** via a Finkelstein reaction. Reaction of iodide **12** with a series of amines gave compounds **13**, **14**, and **16**. The last two compounds were then Boc protected to give bromo intermediates **15** and **17**. The synthesis of intermediates **23** and **24** followed synthetic paths from **10** and **19**, respectively (Scheme 3). Nucleophilic displacement of the chloride **10** with methylethylamine gave **20**, while displacement of chloride **19** with ethanolamine gave **21**, which was Boc protected to give compound **22**. Compounds **20** and **22** were reduced with borane in THF to give **23** and **24**, respectively. Compared to Scheme 2, this route is much more efficient and provided a

Scheme 2. Synthesis of Intermediates 16, 17, and 18[a]



[a]Reagents and conditions: (a) chloroacetyl chloride, toluene, 95 °C, 30 min; (b) 1 M BH_3·THF, THF, rt, 17 h; (c) NaI, acetone, reflux, 2 days; (d) amine, K_2CO_3, ACN, reflux, 17 h; (e) Boc_2O, Et_3N, dioxane, rt, 17 h.

Scheme 3. Synthesis of Intermediates 23 and 24[a]



[a]Reagents and conditions: (a) chloroacetyl chloride, toluene, 105 °C, 15 min; (b) Boc_2O, Et_3N, dioxane, rt, 17 h; (c) 1 M BH_3·THF, THF, rt, 17 h.

simpler route for synthesizing the intermediates. The final intermediate **28** was synthesized according to Scheme 4 from **25**.[17] Nucleophilic displacement of the chloride **25** with ethanolamine gave **26**, and this compound was Boc protected to give **27**, which was converted to **28** after reduction with borane in THF.

The intermediates were converted to the target compounds according to Scheme 5. The nitro intermediates **7**, **8**, **24**, and **28** were reduced under catalytic hydrogenation conditions to give the corresponding anilines **29**, **36**, **32**, and **37**, respectively, and the bromo intermediates **13**, **15**, **17**, **18**, and **23** were converted to the anilines **35**, **31**, **30**, **33**, and **34**, respectively, by a Buchwald−Hartwig amination reaction using LiHMDS as an ammonia surrogate. These anilines were coupled with thiophene-2-carbimidothioate hydroiodide at room temperature in ethanol to give either the target compounds **43** and **44** or the protected compounds **38**−**42**, **45**, and **46**. The carbamate protected compounds **38** and **45** were converted

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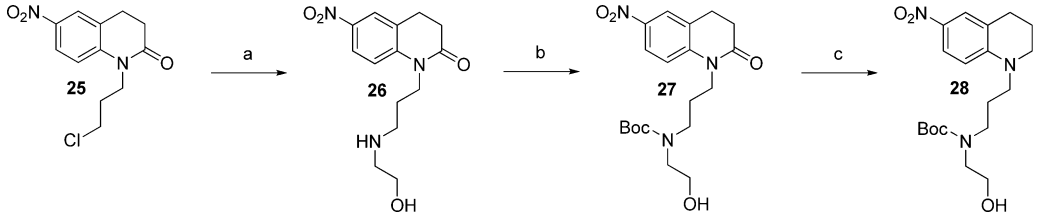
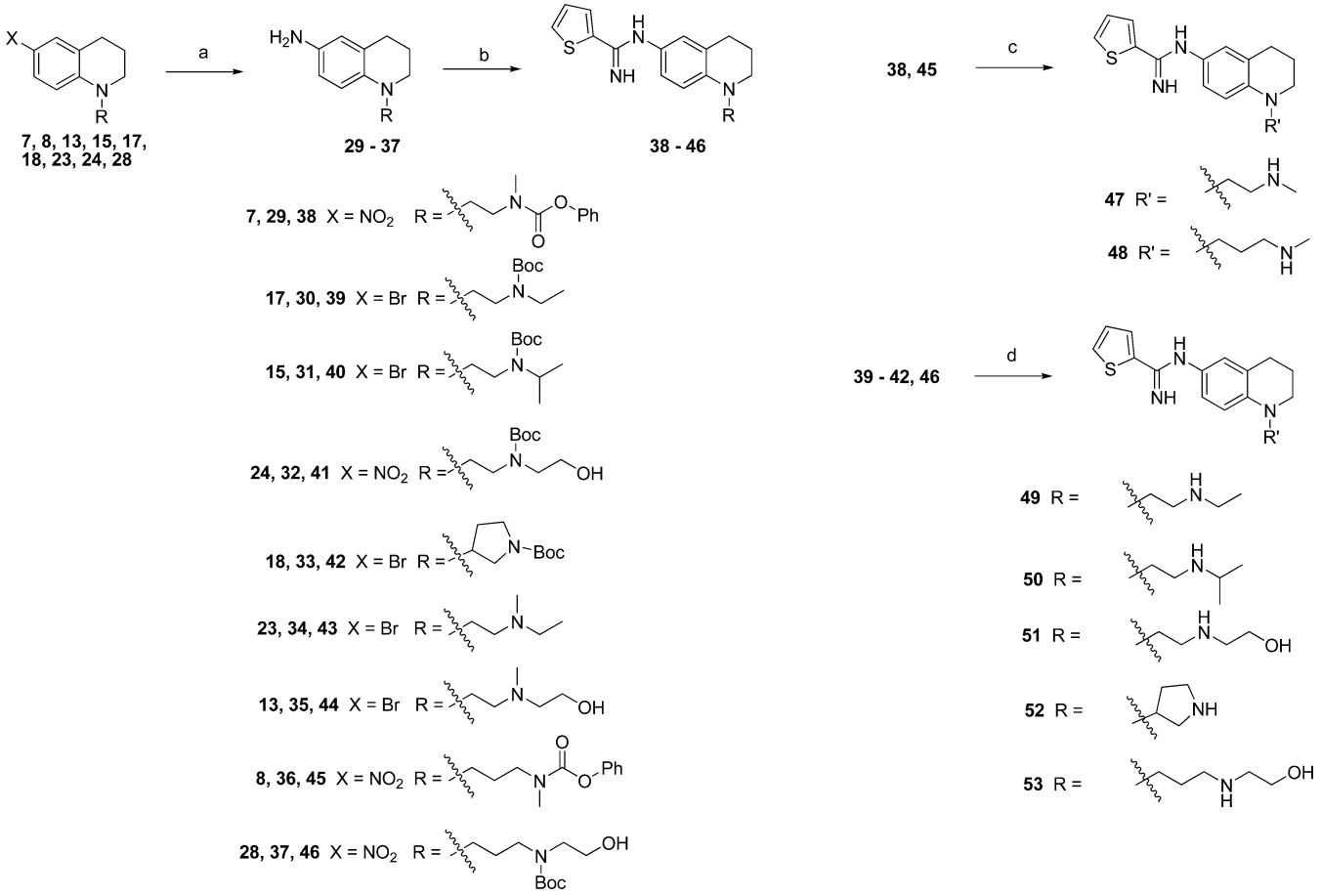
Scheme 4. Synthesis of Intermediate 28[a]



[a]Reagents and conditions: (a) ethanolamine, K_2CO_3, ACN, reflux, 17 h; (b) Boc_2O, Et_3N, dioxane, rt, 17 h; (c) 1 M BH_3·THF, THF, rt, 17 h.

Scheme 5. Synthesis of Target Compounds 43, 44, 47−53[a]



[a]Reagents and conditions: (a) Pd/C, H_2, EtOH, rt (X = NO_2) or LiHMDS, $Pd_2(dba)_3$, $PtBu_3$(X = Br), THF, reflux, 2 h; (b) thiophene-2-carbimidothioate·HI, EtOH, rt; (c) 1 N NaOH, ethylene glycol, 100 °C; (d) 3 N HCl in MeOH.

to target compounds **47** and **48** by base hydrolysis, whereas the Boc protected compounds **39**−**42** and **46** were converted to target compounds **49**−**53** under standard acidic conditions.

Human NOS Inhibition Studies. Our design strategy was based on targeting the L-arginine binding site of nNOS using a pharmacophore model described by us and others.[14,17] In brief, this model featured a guanidine isosteric group and a basic amine side chain attached to a central scaffold. In our previous publication, we prepared a number of analogues based on the 1,2,3,4-tetrahydroquinoline core with a thiophene amidine group attached at the 6-position and a variety of acyclic and cyclic alkylamino side chains attached at the 1-position of this scaffold.[17] This study led to the identification of a series of potent and selective inhibitors of nNOS. From this series, the most potent and selective nNOS inhibitor was compound **1**. Although compound **1** was active in two rodent pain models, it was not an optimal compound to advance further into preclinical development. As mentioned in the Introduction, we sought to improve the oral bioavailability and decrease the hERG inhibition properties of **1** while retaining or improving the nNOS inhibitory potency and selectivity. To achieve this objective, a small focused library was prepared by varying the nature of the aminoalkyl side chain while keeping the 1,2,3,4-tetrahydroquinoline core and thiophene amidine group constant. From our previous work using the 1,2,3,4-tetrahydroquinoline scaffold, the nature of the aminoalkyl side chain did not play a major role in the nNOS inhibitory potency and selectivity.[17] Consequently, we felt that this would be an ideal moiety to modify in order to optimize the ADME properties of the compounds.

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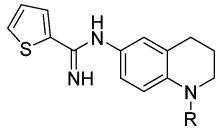
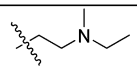
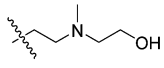
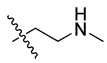
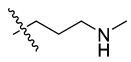
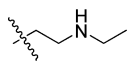
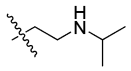
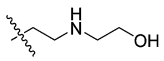
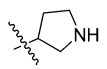
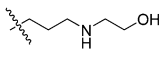
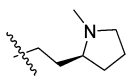
Table 1. In Vitro NOS Inhibitory and hERG Manual Patch-Clamp Data along with Calculated Ligand Efficiencies for nNOS Inhibition[a]



Compound	R	IC_{50} (μM)[a]			Selectivity		hERG, EC50 (μM)	Ligand Efficiency
		nNOS	eNOS	iNOS	e/n	i/n		
43		0.097 (0.063-0.150)	33.3 (22.2-50.0)	>100	343	>1000	4.5	0.37
44		0.133 (0.098-0.181)	63.9 (40.3-101.2)	>100	480	>700	5.8	0.38
47		0.176 (0.13 - 0.237)	40.7 (32.2-51.3)	>100	231	>500	>30	0.42
48		0.235 (0.098-0.563)	22.5 (12.0-42.1)	>100	96	>400	>30	0.40
49		0.139 (0.089-0.217)	46.5 (21.5 – 100.4)	>100	335	>700	>30	0.38
50		0.267 (0.204-0.350)	30.2 (11.3-80.8)	89.4	113	334	27.5	0.35
51		0.115 (0.039-0.334)	32.9 (13.7-79.3)	73.0	286	635	>30	0.40
(±)-**52**		0.248 (0.174-0.354)	23.5 (13.6-40.8)	NT	95	-	>30	0.36
53		0.169 (0.102-0.279)	39.2 (9.8-156.2)	>100	232	591	>30	0.34
1		0.098 (0.05-0.19)	45.6 (8.6-242)	25.7 (17.0-39.0)	262	92	4.7	0.37
L-NMMA	-	0.95 (0.63-1.4)	0.65 (0.45-0.94)	1.8 (0.47-6.7)	0.7	2	NT	0.64

[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. NT: not tested. Selectivity ratios for nNOS are defined as e/n = IC_{50}(eNOS)/IC_{50}(nNOS) and i/n = IC_{50}(iNOS)/IC_{50}(nNOS).

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.[5,26] 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 by the enzymatic conversion in the absence of compound. The IC_{50} 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 Table 1.

All of the compounds prepared inhibited nNOS in the submicromolar range and were highly selective over both eNOS and iNOS. The most potent nNOS inhibitor was compound **43** (IC_{50} = 97 nM), while the most selective compound against eNOS was compound **44**, with over 400-fold selectivity. As observed previously,[17] despite the diverse nature of the aminoalkyl side chains, the differences in inhibitory potencies against nNOS for all compounds are within 0.5 log unit, demonstrating that the aminoalkyl substituents on the 1-

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Table 2. Calculated Physicochemical Properties and CNS MPO Scores of the Compounds[a]

compd	clogP	PSA	clogD (pH 7.4)	pK_a (most basic)	Hb-A	Hb-D	MW	FRB	CNS MPO
43	3.59	70.6	0.54	9.1	4	2	342.5	6	4.7
44	2.71	90.83	0.46	9.1	5	3	358.5	8	4.6
47	2.77	79.4	−0.7	9.52	4	3	314.45	5	4.4
48	3.06	79.4	−0.82	10.28	4	3	328.48	6	4.1
49	3.28	79.4	−0.26	9.52	4	3	328.48	6	4.3
50	3.63	79.4	0.17	9.52	4	3	342.5	6	4.1
51	2.07	98.5	−0.72	9.1	5	4	344.47	8	4.2
(±)-**52**	2.83	79.4	−0.66	9.46	4	3	326.46	3	4.4
53	2.36	99.6	−1.06	9.3	5	4	358.5	9	4.0
1	3.97	70.6	0.42	10.26	4	2	368.54	5	4.0
L-NMMA	−0.08	111.2	−3.55	14.06	6	6	188.23	6	3.3

[a]Hb-A: sum of H-bond acceptors. Hb-D: sum of H-bond donors. MW: molecular weight. FRB: number of freely rotating bonds. PSA: polar surface area. CNS MPO: central nervous system multiple parameter optimization.

position of the 1,2,3,4-tetrahydroquinoline core occupy a fairly tolerant region in the nNOS enzyme. Furthermore, the ligand efficiencies[27] (nNOS potency/non-H atoms) of all compounds prepared were within a narrow range of 0.42−0.34. The compounds do not inhibit iNOS to any appreciable extent, and inhibitory activity against eNOS was fairly weak for these compounds. A number of compounds showed similar or better nNOS/eNOS selectivity than compound **1**, and all compounds were more nNOS/iNOS selective than **1**. On the basis of this primary screen, we advanced nNOS inhibitors that were highly selective over eNOS (>200-fold) and iNOS (>500-fold). Accordingly, compounds **48**, **50**, and (±)-**52** were given lower priority.

hERG Channel Inhibition Studies. Inhibition of the human ether-a-go-go-related gene (hERG) channel is known to cause torsade de pointes cardiac arrhythmias via a prolongation of cardiac QT action potential, and it is a major reason for compound attrition and withdrawal from the market.[28] We evaluated these compounds in the hERG manual patch clamp functional assay, and the results are presented in Table 1. It is clear that compounds containing a tertiary amine (**1**, **43**, and **44**) inhibit the channel moderately in the single digit micromolar range. The corresponding secondary amines for **43** (**47** and **49**) and **44** (**47** and **51**) do not inhibit the channel to any extent. The other secondary amines are devoid of hERG activity as well, with only compound **50** showing weak activity (IC_{50} = 27.5 μM). The relationship between physicochemical properties, particularly lipophilicity, and hERG channel activity is well established and explains the current trend we observe.[29] The calculated log *D* (Table 2) for compounds **1**, **43**, and **44** are 0.54, 0.45, and 0.42, respectively, which are significantly higher than the calculated log *D* for all other compounds. It is noteworthy that the secondary amine **50**, which has a positive calculated log *D* (0.17), showed some weak inhibition of the hERG ion channel, further supporting the key role lipophilicity plays in hERG channel blockage. By use of these results, compounds **43** and **44** were not progressed further because of their inhibition of the hERG channel. These two compounds along with **1** have over 40-fold selectivity of nNOS inhibition over hERG channel inhibition. Nevertheless, the other compounds are much weaker (>30 μM) hERG channel blockers.

Physicochemical Properties and CNS Druglikeness of the nNOS Inhibitors. Because of the polar and acidic nature of the nNOS active site, compounds that mimic the substrate L-arginine are likely to be polar and basic, which poses a major challenge when designing nNOS inhibitors to treat CNS disorders. In the past decade, several seminal papers have established the importance of physicochemical properties on the in vivo behavior of drugs.[30,31] More recently, Wager et al. examined and utilized the interplay among six of these physicochemical properties to create a druglikeness central nervous system multiparameter optimization (CNS MPO) algorithm.[32] Using this prospective design tool, which is based on a scale of 0−6, they evaluated a large number of drugs for CNS disorders and showed that most marketed CNS drugs (74%) have a CNS MPO score of ≥4. To assess the druglikeness of these new selective nNOS inhibitors, the physicochemical properties and CNS MPO scores were calculated (Table 2).[33] All new compounds follow Lipinski's rule of five[30] (log *P*, hydrogen bond donor/acceptor properties, and MW) and had better CNS MPO scores than **1**. Interestingly, the tertiary amines **43** and **44** had the highest CNS MPO scores while the nonselective amino acid based L-NMMA, not surprisingly, had the lowest CNS MPO scores. Using the CNS MPO as a final selection criterion for the remaining five compounds, we selected compound **47** for further evaluation.

L5/L6 Spinal Nerve Ligation (SNL) or Chung Model of Neuropathic Pain in Rats. To evaluate the in vivo behavior of **47**, its efficacy was assessed in the rat Chung model of neuropathic pain (Figure 2).[34] This model serves as a preliminary in vivo screen to assess the druglikeness of our selective nNOS inhibitors. The inhibitory activity of **47** against

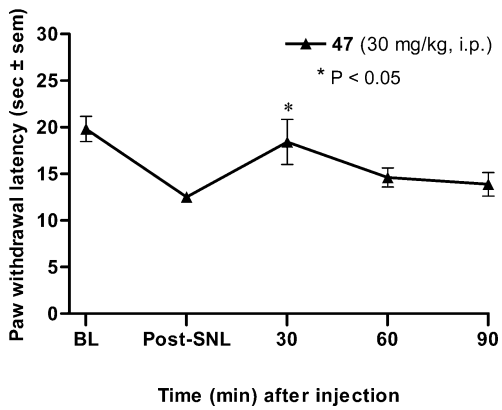

Figure 2. Compound **47** reverses thermal hyperalgesia in the L5/L6 spinal nerve ligation (Chung) model of neuropathic pain in rats.

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Table 3. Comparing Rat Pharmacokinetics of Compounds 1 and 47[a]

compd	C_{max} (μM)	T_{max} (h)	$t_{1/2}$ (h)[b]	AUC (μg·h/mL)	V_{dss} (L/kg)	Cl_p (L h^{-1} kg^{-1})	F_{po} (%)
1	0.24	1.7	9.4	0.55	94.7	4.02	18.4
47	0.88	2.0	8.1	1.16	83.9	5.26	59.9

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

Table 4. Experimental Conditions for hERG K^+ Channel Conventional Patch-Clamp Assay

cells	solution	incubation	detection
HEK-293 cell line stably expressing hERG	extracellular solution: 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-(+)-glucose, 10 mM HEPES (pH 7.4 by NaOH)	5−10 min for concentration at rt (22−24 °C) cumulatively	conventional whole-cell patch-clamp (by Axopatch 200 B or HEKA EPC9)
	intracellular solution: 130 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 10 mM EGTA, 5 mM MgATP, 10 mM HEPES (pH 7.2 by KOH)		

rat nNOS was similar to that of human nNOS (192 nM versus 176 nM). As shown in Figure 2, this compound reverses thermal hyperalgesia when given to rats at a dose of 30 mg/kg intraperitoneally with a maximum effect at 30 min after administration. The activity of **47** in this in vivo model demonstrated that this compound possesses druglike properties.

Comparison of Rat Pharmokinetics of Compounds 47 and 1. Rat pharmacokinetics was carried out on compound **47**, and the results were compared to those obtained with compound **1** (Table 3).[17] Compound **47** showed a significant improvement in oral exposure over **1** with an oral bioavailability of 60%. Also, AUC (10 mg/kg po) was more than twice as high for **47**, and compound **47** showed a 3-fold increase in C_{max}. The AUC and C_{max} are higher than the nNOS inhibitory activity (0.2 μM).

Off-Target Activitiy of Compound 47. Having identified a compound with improved oral bioavailability and decreased hERG inhibition over **1**, we evaluated **47** for off-target activity. First, compound **47** did not exhibit any inhibitory activity against the five major human CYP P450 isoforms, making it less likely to have any drug−drug interactions. In addition, compound **47** had a relatively low human plasma protein binding of only 40%, further reducing the likelihood of drug−drug interactions. Second, the selectivity profile of **47** was determined against a panel of 80 targets comprising G-protein-coupled receptors, ion channels, transporters of biogenic amines, and enzymes (CEREP Screen) at a test concentration of 10 μM. Compound **47** only showed significant inhibition (>70%) at the following targets: rat α-2 adrenergic receptor (80%) and human muscarinic receptor (M_1, 72%; M_2, 74%; M_3, 89%; M_4, 94%). As a follow-up to the high throughput profile, the cellular functional assays of these targets were evaluated and the EC_{50} values were greater than 40 μM. Overall, **47** possesses an excellent safety profile and has been advanced for further in vivo preclinical studies.

CONCLUSIONS

In a previous study, we identified a series of potent and selective nNOS inhibitors based on the 1,2,3,4-tetrahydroquinoline core. The most potent and nNOS selective compound (**1**) was selected for further evaluation to demonstrate the usefulness of selective nNOS inhibitors. Although this compound was efficacious in two in vivo rodent pain models, we could not advance this compound further because of its low oral bioavailability and low micromolar inhibition of the hERG ion channel. We undertook this current study to identify a compound that addressed these drawbacks. Using a previously described pharmacophore model, we prepared a small focused library centered around the 1-position alkylamino side chain of the 1,2,3,4-tetrahydroquinoline scaffold. This region was tolerant to modifications, since all compounds displayed nNOS inhibitory potencies in the submicromolar range. The compounds were triaged based on a number of experimental studies and calculated physicochemical parameters. In addition to the NOS inhibition assay, hERG channel inhibition was assessed using a manual patch clamp functional assay, which revealed that tertiary amines in this series were active with inhibitory potencies in the low micromolar range while secondary amines were essentially inactive. Further analysis revealed that the clogD value might be a key driver in the hERG channel blockage activity. The recently described CNS MPO parameter was used to further triage the compounds. On the basis of this parameter, all new compounds scored equal or better than the lead compound **1**, and by use of this parameter as a final selection criterion, compound **47** was selected for further evaluation. A preliminary screen in the Chung model of neuropathic pain showed that **47** was efficacious, demonstrating its druglikeness. Furthermore, the oral bioavailability of **47** (60%) was much better than that for **1** (18%). The off-target activity of **47** as assessed in a high throughput screen, CYP P450 inhibition studies, and plasma protein binding studies was minimal, making **47** a promising preclinical development selective nNOS inhibitor candidate.

EXPERIMENTAL SECTION

Chemistry. General Procedures. All reactions were conducted under an atmosphere of argon, and the mixtures were 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, Canada, 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) are given in hertz (Hz). Low and high resolution MS were performed at the University of Toronto AIMS (Mass Spectrometry Laboratory), Canada, 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

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Table 5. HPLC Purity of Final Compounds

compd	retention time (min)	purity (%)	HPLC method
43	11.94	96.7	C
44	11.36	95.0	C
47	8.65	96.0	A
48	7.92	96.3	B
49	15.20	96.3	C
50	17.02	96.5	C
51	7.66	95.5	B
(±)-**52**	6.82	98.1	A
53	7.95	98.4	B
	Method A[a]		
time (min)	solvent A (%)	solvent B (%)	
0	20	80	
20	80	20	
25	80	20	
30	20	80	
35	20	80	
	Method B[b]		
time (min)	solvent A (%)	solvent B (%)	
0	0	100	
5	30	70	
7	30	70	
7.5	50	50	
10	50	50	
12	30	70	
14	0	100	
15	0	100	
	Method C[c]		
time (min)	solvent A (%)	solvent B (%)	
0	18	82	
7	24	76	
15	37	63	
25	65	35	
26	18	82	
35	18	82	

[a]Method A: solvent A, acetonitrile; solvent B, aqueous buffer, pH 10.6 (ammonium carbonate/NaOH); column, Waters XTerra RP8, 5 μm, 4.6 mm × 150 mm; flow rate, 1 mL/min; concentration, 1 mg/mL in MeOH; injection volume, 10 μL; wavelength, 254 nm. [b]Method B: solvent A, acetonitrile/TFA (100:0.1); solvent B, water/TFA (100:0.1); column, Phenomenex Luna C18 2.5 μm, 100 mm × 3.0 mm; flow rate, 0.5 mL/min; concentration, 1 mg/mL in MeOH; injection volume, 5 μL; wavelength, 254 nm. [c]Method C: solvent A, acetonitrile; solvent B, buffer, pH 10.6 (water/triethylamine); column, Waters XTerra RP8, 5 μm, 4.6 mm × 150 mm; flow rate, 1.5 mL/min; concentration, 1 mg/mL in MeOH; injection volume, 5 μL; wavelength, 254 nm.

compounds were >95% purity. No attempts were made to optimize yields.

Phenyl Methyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)-ethyl)carbamate (7). A solution of *N*,*N*-dimethyl-2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethanamine (**5**) (0.5 g, 2.01 mmol) in CH_2Cl_2 (10 mL) was treated dropwise with phenyl chloroformate (0.37 mL, 3.01 mmol). The mixture was stirred at room temperature for 17 h and then partitioned between CH_2Cl_2 and 1 N NaOH solution. After extraction, the organic layer was separated, dried (Na_2SO_4), filtered, and concentrated to give a yellow residue. This residue was subjected to flash column chromatography (EtOAc/CH_2Cl_2, 1:4) to obtain the title compound (0.61 g, 85.4%). ^{1}H NMR ($CDCl_3$) δ 7.99–7.92 (m, 1H), 7.87 (d, J = 2.1 Hz, 1H), 7.39–7.33 (m, 2H), 7.23–7.19 (m, 1H), 7.06–7.01 (m, 2H), 6.69 (m, 1H), 3.67–3.52 (m, 4H), 3.48 (t, J = 5.7 Hz, 2H), 3.14 and 3.07 (2s, 3H), 2.79 (t, J = 6.3 Hz, 2H), 1.99–1.96 (m, 2H). ESI-MS (m/z, %): 356 (MH^+).

Phenyl Methyl(3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)-propyl)carbamate (8). **8** was prepared from *N*,*N*-dimethyl-3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propan-1-amine (**6**) (1 g, 3.80 mmol) and phenyl carbonochloridate (0.71 mL, 5.70 mmol) as described for **7** to obtain the title compound (1.02 g, 72.7%). ^{1}H NMR (DMSO-d_6) δ 7.88 (t, J = 7 Hz, 1H), 7.78 (s, 1H), 7.37 (t, J = 7 Hz, 2H), 7.20 (t, J = 7 Hz, 1H), 7.12–7.06 (m, 2H), 6.70 (d, J = 9 Hz, 1H), 3.50–3.33 (m, 6H), 3.06, 2.93 (2s, 3H), 2.76–2.71 (m, 2H), 1.92–1.81 (m, 4H). ESI-MS (m/z, %): 370 (MH^+, 100).

1-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)-2-chloroethanone (10). A solution of 6-bromo-1,2,3,4-tetrahydroquinoline (**9**) (2.09 g, 9.85 mmol) in toluene (55 mL) was treated with 2-chloroacetyl chloride (0.86 mL, 10.84 mmol) dropwise over 10 min. The resulting suspension was stirred at room temperature for 1.5 h and then heated at 95 °C for 30 min. After cooling to room temperature, the yellow solution was diluted with EtOAc and sat. bicarbonate solution. The mixture was poured into a separatory funnel and extracted. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexanes, 3:7) to obtain the title compound (2.4 g, 84%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.35–7.33 (m, 3H), 4.19 (s, 2H), 3.80 (t, J = 6.3 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.04–1.98 (m, 2H).

6-Bromo-1-(2-chloroethyl)-1,2,3,4-tetrahydroquinoline (11). A round-bottom flask was charged with 1-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-2-chloroethanone (**10**) (0.546 g, 1.89 mmol) and treated with borane–THF complex (1 M in THF, 18.9 mL, 18.9 mmol), and the resulting mixture was stirred overnight at room temperature. After the mixture was cooled to 0 °C, the reaction was quenched by dropwise addition of MeOH (5 mL) and the mixture was stirred for 10 min at 0 °C. The mixture was concentrated under reduced pressure and purified by column chromatography (EtOAc/hexanes, 1:9) to obtain the title compound (0.519 g, 100%) as a viscous residue. ^{1}H NMR ($CDCl_3$) δ 7.11 (dd, 1H, J = 8.7, 2.4 Hz), 7.08–7.04 (m, 1H), 6.42 (d, 1H, J = 8.7 Hz), 3.64–3.56 (m, 4H), 3.35 (t, 2H, J = 5.6 Hz), 2.73 (t, 2H, J = 6.3 Hz), 1.97–1.89 (m, 2H). ESI-MS (m/z, %): 274/276 (MH^+, 100).

6-Bromo-1-(2-iodoethyl)-1,2,3,4-tetrahydroquinoline (12). A suspension of 6-bromo-1-(2-chloroethyl)-1,2,3,4-tetrahydroquinoline (**11**) (1.80 g, 6.56 mmol) in acetone (50 mL) was heated to reflux for 2 days. A further portion of sodium iodide (19.66 g, 132.0 mmol) was added and the suspension refluxed for 5 more days. The mixture was cooled to room temperature, filtered through a pad of Celite, and concentrated to obtain the title compound (2.19 g, 91%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.12 (dd, 1H, J = 8.7, 2.5 Hz), 7.09–7.04 (m, 1H), 6.42 (d, 1H, J = 8.7 Hz), 3.65–3.60 (m, 2H), 3.33 (t, 2H, J = 5.6 Hz), 3.25–3.19 (m, 2H), 2.72 (t, 2H, J = 6.3 Hz), 1.98–1.90 (m, 2H). ESI-MS (m/z, %): 366/368 (MH^+, 100).

2-((2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)(methyl)-amino)ethanol (13). A solution of 6-bromo-1-(2-iodoethyl)-1,2,3,4-tetrahydroquinoline **12** (0.475 g, 1.298 mmol) in acetonitrile (19 mL) and water (1 mL) in a 50 mL pressure vessel fitted with a stir bar was treated with potassium carbonate (1.793 g, 12.98 mmol) and 2-(methylamino)ethanol (0.975 g, 12.98 mmol), and the sealed vessel was stirred at 80 °C overnight. After 18 h the mixture was partitioned between CH_2Cl_2 (100 mL) and water (20 mL) and transferred to a separatory funnel. The organic layer was separated and the aqueous layer (pH 12) extracted further with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to a brown residue. Purification on silica gel, eluting with 5% 2 M NH_3 in methanol/95% dichloromethane, yielded a pale yellow oil, **2** (341 mg, 84%). ^{1}H NMR (DMSO-d_6) δ 7.06 (dd, 1H, J = 8.8, 2.5 Hz), 6.99 (d, 1H, J = 2.4 Hz), 6.48 (d, 1H, J = 8.8 Hz), 4.34 (t, 1H, J = 5.3 Hz), 3.44 (q, 2H, J = 6.2 Hz), 3.32–3.25 (m, 4H), 2.64 (t, 2H, J = 6.2 Hz), 2.47–2.41 (m, 4H), 2.23 (s, 3H), 1.83–1.76 (m, 2H). ESI-MS (m/z, %): 313/315 (MH^+, 100).

***N*-(2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)propan-2-amine (14).** **14** was prepared from 6-bromo-1-(2-iodoethyl)-

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1,2,3,4-tetrahydroquinoline (**12**) (0.4 g, 1.09 mmol), potassium carbonate (0.75 g, 5.46 mmol), and isopropylamine (0.64 g, 10.93 mmol) as described for **13** to obtain the title compound (0.27 g, 83%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.06 (dd, 1H, J = 8.8, 2.5 Hz), 6.99 (d, 1H, J = 2.5 Hz), 6.52 (d, 1H, J = 8.8 Hz), 3.31−3.20 (m, 4H), 2.78−2.67 (m, 1H), 2.67−2.59 (m, 4H), 1.84−1.76 (m, 2H), 0.96 (d, 6H, J = 6.2 Hz). ESI-MS (m/z, %): 297/299 (MH^+, 10), 238/240 (20), 159 (100).

***tert*-Butyl 2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(isopropyl)carbamate (15).** A solution of *N*-(2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)propan-2-amine **14** (0.140 g, 0.471 mmol) in anhydrous dioxane (10 mL) was treated with triethylamine (0.132 mL, 0.942 mmol) and di-*tert*-butyl dicarbonate (0.108 g, 0.495 mmol) and the resulting mixture stirred overnight at room temperature. The mixture was concentrated to residue and purified directly on silica gel, eluting with 10% EtOAc/90% hexanes to yield a colorless oil (0.150 g, 80%). ^{1}H NMR ($CDCl_3$) δ 7.09 (dd, 1H, J = 8.7, 2.0 Hz), 7.05−7.00 (m, 1H), 6.55 (d, 1H, J = 8.7 Hz), 4.50−4.00 (m, 1H), 3.42−3.27 (m, 4H), 3.24−3.11 (m, 2H), 2.70 (t, 2H, J = 6.2 Hz), 1.97−1.86 (m, 2H), 1.53−1.51 (2s, 9H), 1.13 (d, 6H, J = 6.8 Hz). ESI-MS (m/z, %): 397/399 (MH^+, 80), 341/343 (100).

2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)-*N*-ethylethanamine (16). **16** was prepared from 6-bromo-1-(2-iodoethyl)-1,2,3,4-tetrahydroquinoline (**12**) (0.1 g, 0.27 mmol), potassium carbonate (0.37 g, 2.73 mmol), and ethanamine hydrochloride (0.22 g, 2.73 mmol) as described for **13** to obtain the title compound (55 mg, 71.1%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.09 (dd, 1H, J = 8.7, 2.4 Hz), 7.06−7.01 (m, 1H), 6.51 (d, 1H, J = 8.7 Hz), 3.36 (t, 2H, J = 6.8 Hz), 3.29 (t, 2H, J = 5.5 Hz), 2.82 (t, 2H, J = 6.8 Hz), 2.80−2.65 (m, 4H), 1.96−1.88 (m, 2H), 1.11 (t, 3H, J = 7.1 Hz).

***tert*-Butyl 2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(ethyl)carbamate (17).** **17** was prepared from 2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-*N*-ethylethanamine (**16**) (0.25 g, 0.88 mmol), triethylamine (0.24 mL, 1.76 mmol), and di-*tert*-butyl dicarbonate (0.20 g, 0.92 mmol) as described for **15** to give the title compound (0.28 g, 83%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.09 (dd, 1H, J = 8.7, 2.3 Hz), 7.03 (brs, 1H), 6.50 (d, 1H, J = 8.7 Hz), 3.48−3.11 (m, 8H), 2.70 (t, 2H, J = 6.3 Hz), 2.05−1.87 (m, 2H), 1.47 (s, 9H), 1.10 (brs, 3H). ESI-MS (m/z, %): 383/385 (MH^+, 38), 327/329 (100).

1-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)-2-(ethyl(methyl)-amino)ethanone (20). A suspension of 1-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-2-chloroethanone (**10**) (1.0 g, 3.47 mmol) and potassium iodide (0.11 g, 0.69 mmol) in THF (10 mL) was treated with *N*-methylethanamine (1.78 mL, 20.79 mmol). The mixture was stirred at room temperature for 2 h and then heated at 65 °C for 1 h. The mixture was concentrated and partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. After extraction, the organic layer was separated, dried (Na_2SO_4), filtered, and concentrated to give a light brown residue. This residue was purified by flash column chromatography (2 M NH_3 in MeOH/CH_2Cl_2, 5:95) to obtain the title compound (1.06 g, 98%) as a light brown residue. ^{1}H NMR ($CDCl_3$) δ 7.52−7.40 (m, 1H), 7.30−7.27 (m, 2H), 3.80 (t, J = 6.0 Hz, 2H), 3.28 (s, 2H), 2.72 (t, J = 6.9 Hz, 2H), 2.50 (q, J = 7.2 Hz, 2H), 2.30 (s, 3H), 1.96 (quint, J = 6.6 Hz, 2H), 1.03 (t, J = 7.2 Hz, 3H). ESI-MS (m/z, %): 311 and 313 (MH^+, 100%).

2-(2-Hydroxyethylamino)-1-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (21). To a solution of 2-chloro-1-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**19**) (5.9 g, 23.17 mmol) in acetonitrile (75 mL) was added potassium carbonate (9.61 g, 69.5 mmol), followed by ethanolamine (14.01 mL, 232 mmol). The mixture was stirred at room temperature for 30 min. The mixture was diluted with water and extracted into CH_2Cl_2. The combined organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by column chromatography (EtOAc, followed by 2 M NH_3 in MeOH/CH_2Cl_2, 1:99 to 3:97) to obtain the title compound (3.1 g, 47.9%). ^{1}H NMR (DMSO-d_6) δ 8.07 (d, J = 2.4 Hz, 1H), 8.03 (dd, J = 9.0, 2.4 Hz, 1H), 7.96 (d, J = 9.3 Hz, 1H), 3.73 (t, J = 6.15 Hz, 2H), 3.45 (t, J = 5.7 Hz, 2H), 2.84 (t, J = 6.4 Hz, 2H), 2.61 (t, J = 5.5 Hz, 2H), 1.90 (quint, J = 6.2 Hz, 2H). ESI-MS (m/z, %): 280 (MH^+, 100).

***tert*-Butyl 2-Hydroxyethyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)-2-oxoethyl)carbamate (22).** **22** was prepared from 2-(2-hydroxyethylamino)-1-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)-ethanone (**21**) (3.1 g, 11.10 mmol), triethylamine (3.12 mL, 22.20 mmol), and di-*tert*-butyl dicarbonate (2.71 mL, 11.65 mmol) as described for **15** to obtain the title compound (4.2 g, 100%). ^{1}H NMR (DMSO-d_6) δ 8.09−7.90 (m, 3H), 4.65−4.59 (m, 1H), 3.76−3.71 (m, 2H), 3.56 (s, 2H), 3.52−3.47 (m, 2H), 3.31−3.25 (m, 2H), 2.88−2.83 (m, 2H), 1.95−1.89 (m, 2H), 1.39, 1.31 (2s, 9H). ESI-MS (m/z, %): 380 (MH^+, 17), 280 (100).

2-(6-Bromo-3,4-dihydroquinolin-1(2*H*)-yl)-*N*-ethyl-*N*-methylethanamine (23). **23** was prepared from 1-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-2-(ethyl(methyl)amino)ethanone (**20**) (1.05 g, 3.37 mmol) and 1 M borane in THF (33.7 mL, 33.7 mmol) as described for **11** to obtain the title compound (0.89 g, 89%) as a colorless residue. ^{1}H NMR ($CDCl_3$) δ 7.09 (dd, J = 1.8, 6.6 Hz, 1H), 7.02−7.01 (m, 1H), 6.44 (d, J = 6.6 Hz, 1H), 3.36 (t, J = 5.7 Hz, 2H), 3.29 (t, J = 4.2 Hz, 2H), 2.70 (t, J = 4.8 Hz, 2H), 2.53−2.43 (m, 4H), 2.28 (s, 3H), 1.94−1.88 (m, 2H), 1.06 (t, J = 5.4 Hz, 3H). ESI-MS (m/z, %): 397 and 399 (MH^+, 100%).

***tert*-Butyl 2-Hydroxyethyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (24).** **24** was prepared from *tert*-butyl 2-hydroxyethyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)-2-oxoethyl)-carbamate (**22**) (4.2 g, 11.07 mmol) and 1 M borane in THF (33.2 mL, 33.2 mmol) as described for **11** to obtain the title compound (3.39 g, 84%). ^{1}H NMR (DMSO-d_6) δ 7.84 (d, J = 9.0 Hz, 1H), 7.78 (d, J = 9.9 Hz, 1H), 6.74 (m, 1H), 4.73 (t, J = 4.8 Hz, 1H), 3.58−3.53 (m, 2H), 3.49−3.39 (m, 6H), 3.27−3.17 (m, 2H), 2.75−2.68 (m, 2H), 1.88−1.81 (m, 2H), 1.35 (s, 9H). ESI-MS (m/z, %): 366 (MH^+, 19), 310 (100).

1-(3-(2-Hydroxyethylamino)propyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (26). **26** was prepared from 1-(3-chloropropyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (**25**) (0.5 g, 1.86 mmol) and 2-aminoethanol (1.12 mL, 18.61 mmol) as described for **13** to obtain the title compound (0.27 g, 49.5%). ^{1}H NMR (DMSO-d_6) δ 8.10−8.05 (m, 2H), 7.37 (d, J = 8.7 Hz, 1H), 4.42 (brs, 1H), 3.93 (t, J = 7.4 Hz, 2H), 3.39 (t, J = 5.7 Hz, 2H), 2.96 (t, J = 7.35 Hz, 2H), 2.61−2.57 (m, 2H), 2.52−2.45 (m, 4H), 1.66−1.57 (m, 2H). ESI-MS (m/z, %): 294 (MH^+, 100), 233 (19).

***tert*-Butyl 2-Hydroxyethyl(3-(6-nitro-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (27).** **27** was prepared from 1-(3-(2-hydroxyethylamino)propyl)-6-nitro-3,4-dihydroquinolin-2(1*H*)-one (**26**) (0.27 g, 0.92 mmol), triethylamine (0.25 mL, 1.84 mmol), and di-*tert*-butyl dicarbonate (0.21 g, 0.96 mmol) as described for **15** to obtain the title compound (0.359 g, 99%). ^{1}H NMR (DMSO-d_6) δ 8.16−8.10 (m, 2H), 7.34 (d, J = 9.0 Hz, 1H), 4.67−4.64 (m, 1H), 3.98−3.91 (m, 2H), 3.47−3.41 (m, 2H), 3.28−3.16 (m, 4H), 3.01 (t, J = 7.4 Hz, 2H), 2.62 (t, J = 7.4 Hz, 2H), 1.81−1.72 (m, 2H), 1.39−1.30 (m, 9H). ESI-MS (m/z, %): 416 (100), 294 (77).

***tert*-Butyl 2-Hydroxyethyl(3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (28).** **28** was prepared from *tert*-butyl 2-hydroxyethyl(3-(6-nitro-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)-propyl)carbamate (**27**) (0.35 g, 0.90 mmol) and 1 M borane in THF (2.69 mL, 2.70 mmol) as described for **11** to obtain the title compound (0.25 g, 73.8%). ^{1}H NMR (DMSO-d_6) δ 7.87 (dd, J = 9.3, 2.7 Hz, 1H), 7.78 (d, J = 2.7 Hz, 1H), 6.65 (d, J = 9.3 Hz, 1H), 4.68 (t, J = 5.0 Hz, 1H), 3.48−3.32 (m, 6H), 3.26−3.18 (m, 4H), 2.75 (t, J = 6.0 Hz, 1H), 1.88−1.76 (m, 4H), 1.36 (brs, 9H). ESI-MS (m/z, %): 402 (100), 380 (MH^+, 14), 280 (95).

Phenyl 2-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(methyl)carbamate (29). A suspension of phenyl methyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (**7**) (0.5 g, 1.41 mmol) and palladium on activated carbon (10%, 75 mg, 0.07 mmol) in a 1:1 mixture of THF/EtOH (20 mL) was stirred under a balloon of hydrogen for 4.5 h. The suspension was filtered through a pad of Celite. The pad was rinsed with methanol, and the filtrate was concentrated to obtain the title compound (0.46 g, quantitative) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ 7.39−7.34 (m, 2H), 7.22−7.17 (m, 1H), 7.12−7.08 (m, 2H), 6.61−6.42 (m, 3H), 3.61−3.45 (m, 4H), 3.26 (t, J = 6.0 Hz, 2H), 3.20 (brs, 2H), 3.13 and 3.06 (2s, 3H), 2.70

(t, J = 6.3 Hz, 2H), 1.96−1.87 (m, 2H). ESI-MS (m/z, %): 326 (MH^+).

***tert*-Butyl 2-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(ethyl)carbamate (30).** A suspension of tris(dibenzylideneacetone)-dipalladium(0) (66 mg, 0.07 mmol) in anhydrous THF (3 mL) was treated with tri-*tert*-butylphosphine in hexane (10 wt %) (0.43 mL, 0.14 mmol) and the mixture stirred for 5 min at room temperature. A solution of *tert*-butyl 2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(ethyl)carbamate (**17**) (0.27 g, 0.71 mmol) in THF (7 mL) was added followed by lithium bis(trimethylsilyl)amide (1 M in THF, 1.43 mL, 1.43 mmol) and the mixture heated in a sealed reaction vial at 90 °C for 3 h. The mixture was cooled to room temperature and treated with TBAF (1 M in THF, 4 mL, 4 mmol) and stirred for 30 min. The mixture was partitioned between water and EtOAc, transferred to a separatory funnel and the organic layer separated. The aqueous layer (pH 10) was further extracted with EtOAc and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give a dark brown residue. The crude was purified by column chromatography (2 M NH_3 in MeOH/CH_2Cl_2, 2.5:97.5) to obtain the title compound (171 mg, 74.6%) as a brown residue. ^{1}H NMR ((DMSO-d_6) δ 6.42 (d, 1H, J = 8.5 Hz), 6.31−6.26 (m, 1H), 6.22 (brs, 1H), 4.20 (brs, 2H), 3.26−3.08 (m, 8H), 2.55 (t, 2H, J = 6.3 Hz), 1.82−1.74 (m, 2H), 1.41 (s, 9H), 1.02 (t, 3H, J = 7.0 Hz). ESI-MS (m/z, %): 320 (MH^+, 90), 264 (100).

***tert*-Butyl 2-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl-(isopropyl)carbamate (31).** **31** was prepared from tris-(dibenzylideneacetone)dipalladium(0) (35 mg, 0.03 mmol), tri-*tert*-butylphosphine in hexane (10 wt %) (0.22 mL, 0.07 mmol), *tert*-butyl 2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl(isopropyl)carbamate (**15**) (0.15 g, 0.37 mmol), and lithium bis(trimethylsilyl)amide (1 M in THF, 0.75 mL, 0.75 mmol) as described for **30** to obtain the title compound (85 mg, 67.5%) as a dark brown residue. ^{1}H NMR (DMSO-d_6) δ 6.44 (d, 1H, J = 8.0 Hz), 6.28 (dd, 1H, J = 8.3, 2.1 Hz), 6.24−6.18 (m, 1H), 4.33−3.91 (m, 3H), 3.24−3.04 (m, 6H), 2.60−2.50 (m, 2H), 1.85−1.74 (m, 2H), 1.44 (s, 9H), 1.07 (d, 6H, J = 6.8 Hz). ESI-MS (m/z, %): 334 (MH^+, 100).

***tert*-Butyl 2-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl(2-hydroxyethyl)carbamate (32).** **32** was prepared from *tert*-butyl 2-hydroxyethyl(2-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)-carbamate (**24**) (3.3 g, 9.03 mmol) as described for **29** to obtain the title compound as a dark oil. ^{1}H NMR (DMSO-d_6) δ 6.43 (d, J = 8.4 Hz, 1H), 6.28 (d, J = 8.7 Hz, 1H), 6.22 (s, 1H), 4.35 (t, J = 5.1 Hz, 1H), 4.18 (s, 2H), 3.48−3.31 (m, 2H), 3.28−3.16 (m, 6H), 3.13−3.10 (m, 2H), 2.55 (t, J = 6.4 Hz, 2H), 1.80−1.76 (m, 2H), 1.41 (s, 9H). ESI-MS (m/z, %): 336 (MH^+, 100), 280 (100).

***tert*-Butyl 3-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)-pyrrolidine-1-carboxylate (33).** **33** was prepared from $Pd_2(dba)_3$ (46 mg, 0.05 mmol), P^tBu_3 (0.6 mL of a 10 wt % in hexanes solution, 0.2 mmol), *tert*-butyl 3-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-pyrrolidine-1-carboxylate (**18**) (0.38 g, 1.00 mmol), and lithium hexamethyldisilizane (2.0 mL, 1 M solution in THF, 2.0 mmol) as described for **30** to obtain the title compound (0.295 g, 93.1%) as a viscous dark brown residue. ^{1}H NMR ($CDCl_3$) δ 6.59−6.44 (m, 3H), 4.35−4.23 (m, 1H), 3.59−3.11 (m, 8H), 2.69 (t, J = 6.3 Hz, 2H), 2.09−2.04 (m, 2H), 1.93−1.87 (m, 2H), 1.47 (s, 9H). ESI-MS (m/z, %): 318 (MH^+, 100%).

1-(2-(Ethyl(methyl)amino)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine (34). **34** was prepared from 2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)-*N*-ethyl-*N*-methylethanamine (**23**) (0.5 g, 1.68 mmol), tris(dibenzylideneacetone)dipalladium(0) (77 mg, 0.08 mmol), tri-*tert*-butylphosphine in hexane (10 wt %) (0.61 mL, 0.20 mmol), and lithium hexamethyldisilazide (1 M in THF, 5.05 mL, 5.05 mmol) as described for **30** to obtain the title compound (0.35 g, 89%) as a dark brown residue. ^{1}H NMR ($CDCl_3$) δ 6.49−6.48 (2*H*), 6.41 (brs, 1H), 3.36−3.31 (m, 2H), 3.21 (t, J = 5.7 Hz, 2H), 3.20 (brs, 2H), 2.68 (t, J = 6.3 Hz, 2H), 2.56−2.43 (m, 4H), 2.28 (s, 3H), 1.95−1.87 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H). ESI-MS (m/z, %): 234 (MH^+).

2-((2-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)(methyl)-amino)ethanol (35). **35** was prepared from tris-(dibenzylideneacetone)dipalladium(0) (48 mg, 0.05 mmol), tri-*tert*-butylphosphine in hexane (10 wt %) (0.32 mL, 0.10 mmol), 2-((2-(6-bromo-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)(methyl)amino)ethanol (**13**) (0.16 g, 0.52 mmol), and lithium bis(trimethylsilyl)amide (1 M in THF, 1.58 mL, 1.58 mmol) as described for **30** to obtain the title compound (57 mg, 43.4%) as an orange-red residue. ^{1}H NMR (DMSO-d_6) δ 6.36−6.27 (m, 2H), 6.21 (brs, 1H), 4.31 (t, 1H, J = 5.4 Hz), 4.18 (brs, 2H), 3.44 (q, 2H, J = 6.3 Hz), 3.18 (t, 2H, J = 7.3 Hz), 3.10 (t, 2H, J = 5.3 Hz), 2.56−2.40 (m, 6H), 2.22 (s, 3H), 1.84−1.72 (m, 2H). ESI-MS (m/z, %): 250 (MH^+, 100).

Phenyl 3-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)propyl-(methyl)carbamate (36). **36** was prepared from phenyl methyl(3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (**8**) (1.02 g, 2.76 mmol) as described for **29** to obtain the title compound (0.83 g, 89%) as a dark oil. ESI-MS (m/z, %): 340 (MH^+, 100).

***tert*-Butyl 3-(6-Amino-3,4-dihydroquinolin-1(2*H*)-yl)propyl-(2-hydroxyethyl)carbamate (37).** **37** was prepared from *tert*-butyl 2-hydroxyethyl(3-(6-nitro-3,4-dihydroquinolin-1(2*H*)-yl)propyl)-carbamate (**28**) (0.23 g, 0.60 mmol) as described for **29** to obtain the title compound (0.21 g, 99%) as a dark oil. ^{1}H NMR (DMSO-d_6) δ 6.34−6.25 (m, 2H), 6.22 (m, 1H), 4.65−4.60 (m, 1H), 4.20 (brs, 2H), 3.48−3.41 (m, 2H), 3.22−3.15 (m, 4H), 3.06−3.01 (m, 4H), 2.59−2.50 (m, 2H), 1.38−1.78 (m, 2H), 1.72−1.62 (m, 2H), 1.38 (s, 9H). ESI-MS (m/z, %): 350 (MH^+, 100).

Phenyl Methyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (38). A solution of phenyl 2-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl(methyl)-carbamate (**29**) (0.445 g, 1.37 mmol) in EtOH (20 mL) was treated with methyl thiophene-2-carbimidothioate hydroiodide (0.78 g, 2.73 mmol) and stirred overnight at room temperature. Argon was bubbled through the mixture for 20 min. Then it was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. After extraction, the organic layer was separated and the aqueous layer was extracted with an additional CH_2Cl_2. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated to give a dark oil which was purified by flash column chromatography (MeOH/CH_2Cl_2, 2.5:97.5, then 2 M NH_3 in MeOH/CH_2Cl_2, 2.5:97.5 to 7.5:92.5) to obtain the title compound (0.4 g, 67.2%) as a yellow-brown solid. ^{1}H NMR (DMSO-d_6) δ 7.68 (brs, 1H), 7.58 (d, J = 5.1 Hz, 1H), 7.42−7.34 (m, 2H), 7.24−7.18 (m, 1H), 7.11−7.03 (m, 3H), 6.68 (d, J = 9.0 Hz, 1H), 6.57−6.51 (m, 2H), 6.29 (brs, 2H), 3.58−3.44 (m, 4H), 3.32−3.27 (m, 2H), 3.09, 2.97 (2s, 3H), 2.73−2.65 (m, 2H), 1.90−1.83 (m, 2H). ESI-MS (m/z, %): 435 (MH^+, 100%).

***tert*-Butyl Ethyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (39).** **39** was prepared from *tert*-butyl 2-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl(ethyl)-carbamate (**30**) (0.165 g, 0.51 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.295 g, 1.03 mmol) as described for **38** to obtain the title compound (0.12 g, 54.2%) as a yellow-orange solid. ^{1}H NMR (DMSO-d_6) δ 7.69 (d, 1H, J = 3.2 Hz), 7.58 (d, 1H, J = 5.0 Hz), 7.08 (dd, 1H, J = 5.0, 3.7 Hz), 6.68−6.59 (m, 1H), 6.59−6.47 (m, 2H), 6.50−6.20 (brs, 2H), 3.33−3.16 (m, 8H), 2.66 (t, 2H, J = 6.2 Hz), 1.91−1.79 (m, 2H), 1.41 (s, 9H), 1.04 (t, 3H, J = 7.0 Hz). ESI-MS (m/z, %): 429 (MH^+, 100).

***tert*-Butyl Isopropyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (40).** **40** was prepared from *tert*-butyl-2-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)-ethyl(isopropyl) carbamate (**31**) (0.085 g, 0.25 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.127 g, 0.44 mmol) as described for **38** to obtain the title compound (0.054 g, 47.9%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.70 (d, 1H, J = 3.4 Hz), 7.59 (d, 1H, J = 5.0 Hz), 7.09 (dd, 1H, J = 5.0, 3.6 Hz), 6.71−6.51 (m, 3H), 6.51−6.28 (brs, 2H), 4.29−3.94 (m, 1H), 3.43−3.18 (m, 6H), 2.66 (t, 2H, J = 5.8 Hz), 1.91−1.80 (m, 2H), 1.46 (s, 9H), 1.11 (d, 6H, J = 6.8 Hz). ESI-MS (m/z, %): 443 (MH^+, 100).

***tert*-Butyl 2-Hydroxyethyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (41).** **41** was prepared from *tert*-butyl 2-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl(2-hydroxyethyl)carbamate (**32**) (3.61 g, 10.76 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (6.14 g, 21.52 mmol) as described for **38** to obtain the title compound (3.13 g,

65.4%). ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 3.3 Hz, 1H), 7.56 (d, J = 4.8 Hz, 1H), 7.07 (dd, J = 4.8, 3.3 Hz, 1H), 6.66−6.62 (m, 1H), 6.55−6.46 (m, 2H), 6.27 (brs, 2H), 4.71 (t, J = 4.8 Hz, 1H), 3.49−3.45 (m, 2H), 3.36−3.32 (m, 4H), 3.25−3.21 (m, 4H), 2.66 (t, J = 6.2 Hz, 2H), 1.86−1.82 (m, 2H), 1.42 (s, 9H). ESI-MS (m/z, %): 445 (MH^+, 100).

***tert*-Butyl 3-(6-(Thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)pyrrolidine-1-carboxylate (42).** **42** was prepared from *tert*-butyl 3-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)-pyrrolidine-1-carboxylate (**33**) (0.21 g, 0.66 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.37 g, 1.32 mmol) as described for **38** to obtain the title compound (0.168 g, 59.6%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 3.3 Hz, 1H), 7.57 (d, J = 5.1 Hz, 1H), 7.07 (dd, J = 3.9, 5.1 Hz, 1H), 6.73 (d, J = 8.7 Hz, 1H), 6.59−6.52 (m, 2H), 6.34 (brs, 2H), 4.46−4.34 (m, 1H), 3.51−3.10 (m, 6H), 2.69 (t, J = 6.3 Hz, 2H), 2.08−1.99 (m, 2H), 1.85−1.78 (m, 2H), 1.41 (s, 9H). ESI-MS (m/z, %): 427 (MH^+, 100%).

***N*-(1-(2-(Ethyl(methyl)amino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (43).** **43** was prepared from 1-(2-(ethyl(methyl)amino)ethyl)-1,2,3,4-tetrahydroquinolin-6-amine (**34**) (0.335 g, 1.43 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.81 g, 2.87 mmol) as described for **38** to obtain the title compound (0.36 g, 73.2%) as a dark yellow residue. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.6 Hz, 1H), 7.55 (dd, J = 0.9, 5.1 Hz, 1H), 7.07 (dd, J = 3.6, 4.8 Hz, 1H), 6.58−6.48 (3H), 6.30 (brs, 2H), 3.30−3.22 (m, 4H), 2.65 (t, J = 6.0 Hz, 2H), 2.46−2.36 (m, 4H), 2.20 (s, 3H), 1.87−1.80 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H). ESI-MS (m/z, %): 343 (MH^+). ESI-HRMS calculated for $C_{19}H_{27}N_4S$ (MH^+), 343.1950; observed, 343.1946.

***N*-(1-(2-((2-Hydroxyethyl)(methyl)amino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (44).** **44** was prepared from 2-((2-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)-(methyl)amino)ethanol (**35**) (52 mg, 0.209 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.119 g, 0.41 mmol) as described for **38** to obtain the title compound (44 mg, 58.9%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.67 (d, 1H, J = 3.0 Hz), 7.55 (dd, 1H, J = 5.0, 0.9 Hz), 7.07 (dd, 1H, J = 5.0, 3.7 Hz), 6.60−6.51 (m, 2H), 6.48 (brs, 1H), 6.29 (brs, 2H), 4.34 (t, 1H, J = 5.3 Hz), 3.46 (q, 2H, J = 6.2 Hz), 3.32−3.22 (m, 4H), 2.65 (t, 2H, J = 6.2 Hz), 2.50−2.44 (m, 4H), 2.25 (s, 3H), 1.88−1.80 (m, 2H). ESI-MS (m/z, %): 359 (MH^+, 100). ESI-HRMS calculated for $C_{19}H_{27}N_4OS$ (MH^+), 359.1900; observed, 359.1908.

Phenyl Methyl(3-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (45). **45** was prepared from phenyl 3-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)propyl-(methyl)carbamate (**36**) (0.83 g, 2.44 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (1.39 g, 4.89 mmol) as described for **38** to obtain the title compound (0.63 g, 57.4%). ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.0 Hz, 1H), 7.55 (d, J = 4.8 Hz, 1H), 7.41−7.35 (m, 2H), 7.23−7.21 (m, 1H), 7.13−7.05 (m, 3H), 6.56 (m, 2H), 6.49 (brs, 1H), 6.25 (brs, 2H), 3.50−3.45 (m, 2H), 3.38−3.32 (m, 2H), 3.23−3.19 (m, 2H), 3.06, 2.93 (2s, 3H), 2.69−2.64 (m, 2H), 1.89−1.81 (m, 4H).

***tert*-Butyl 2-Hydroxyethyl(3-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (46).** **46** was prepared from *tert*-butyl 3-(6-amino-3,4-dihydroquinolin-1(2*H*)-yl)propyl(2-hydroxyethyl)carbamate (**37**) (0.2 g, 0.57 mmol) and methyl thiophene-2-carbimidothioate hydroiodide (0.32 g, 1.14 mmol) as described for **38** to obtain the title compound (0.112 g, 42.7%). ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.6 Hz, 1H), 7.55 (d, J = 4.2 Hz, 1H), 7.07 (dd, J = 4.2, 3.6 Hz, 1H), 6.54−6.47 (m, 3H), 6.29−6.21 (brs, 2H), 4.68−4.62 (m, 1H), 3.50−3.42 (m, 2H), 3.27−3.12 (m, 8H), 2.69−2.63 (m, 2H), 1.89−1.81 (m, 2H), 1.78−1.69 (m, 2H), 1.39 (s, 9H). ESI-MS (m/z, %): 459 (MH^+, 100).

***N*-(1-(2-(Methylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (47).** A solution of phenyl methyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (**38**) (0.38 g, 0.87 mmol) in ethanol (15 mL) was treated with NaOH (0.35 g, 8.70 mmol) followed by H_2O (8 mL). The mixture was heated at reflux for 6 h. The solution was concentrated and partitioned between CH_2Cl_2 and brine. After extraction, the organic layer was separated, and the aqueous layer was extracted once again with CH_2Cl_2. The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give a dark residue. This residue was purified by flash column chromatography (2 M NH_3 in MeOH/CH_2Cl_2, 5:95) to obtain the title compound (0.155 g, 56.8%) as a yellow solid. ^{1}H NMR (CD_3OD) δ 7.98−7.95 (m, 2H), 7.31−7.28 (m, 1H), 7.05 (dd, J = 2.4, 8.7 Hz, 1H), 6.97 (d, J = 2.4 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 3.62 (t, J = 6.6 Hz, 2H), 3.34 (t, J = 5.4 Hz, 2H), 3.20 (t, J = 6.9 Hz, 2H), 2.78 (t, J = 6.0 Hz, 2H), 2.72 (s, 3H), 1.99−1.92 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 156.39, 144.55, 134.30, 133.92, 129.17, 128.55, 126.13, 124.46, 123.38, 122.35, 111.24, 48.86, 46.88, 44.40, 32.57, 27.32, 21.17. ESI-MS (m/z, %): 315 (MH^+). ESI-HRMS calculated for $C_{17}H_{23}N_4S$ (MH^+), 315.1637; observed, 315.1629.

***N*-(1-(3-(Methylamino)propyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (48).** **48** was prepared from phenyl methyl(3-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (**45**) (0.63 g, 1.40 mmol) as described for **47** to obtain the title compound (0.13 g, 28.2%). ^{1}H NMR (DMSO-d_6) δ 7.66 (d, J = 3.3 Hz, 1H), 7.54 (d, J = 5.1 Hz, 1H), 7.06 (dd, J = 5.1, 3.6 Hz, 1H), 6.54 (brs, 2H), 6.47 (s, 1H), 6.21 (brs, 2H), 3.26−3.16 (m, 4H), 2.66 (t, J = 6.4 Hz, 2H), 2.53−2.48 (m, 2H), 2.28 (s, 3H), 1.85 (quint, J = 5.5 Hz, 2H), 1.64 (quint, J = 7.2 Hz, 2H).

***N*-(1-(2-(Ethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)-thiophene-2-carboximidamide (49).** A solution of *tert*-butyl ethyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (**39**) (0.115 g, 0.26 mmol) in dry MeOH (10 mL) was treated with 2 M HCl (1.35 mL, 2.7 mmol) and the mixture heated to reflux for 90 min. After cooling to room temperature, the solution was concentrated and dried briefly on a high-vacuum pump. The residue was purified by column chromatography (2 M NH_3 in MeOH/CH_2Cl_2, 1:9) to obtain the title compound (78 mg, 88%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.66 (d, 1H, J = 3.0 Hz), 7.54 (dd, 1H, J = 5.0, 0.9 Hz), 7.06 (dd, 1H, J = 5.0, 3.7 Hz), 6.59−6.52 (m, 2H), 6.47 (brs, 1H), 6.21 (brs, 2H), 3.32−3.20 (m, 4H), 2.70−2.64 (m, 4H), 2.56 (q, 2H, J = 7.1 Hz), 1.88−1.80 (m, 2H), 1.04 (t, 3H, J = 7.0 Hz). ESI-MS (m/z, %): 329 (MH^+, 100), 258 (100). ESI-HRMS calculated for $C_{18}H_{25}N_4S$ (MH^+), 329.1794; observed, 329.1798.

***N*-(1-(2-(Isopropylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (50).** **50** was prepared from *tert*-butyl isopropyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (**40**) (50 mg, 0.11 mmol) as described for **49** to obtain the title compound (40 mg, quantitative) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 7.66 (d, 1H, J = 3.1 Hz), 7.54 (d, 1H, J = 5.1 Hz), 7.06 (dd, 1H, J = 5.0, 3.7 Hz), 6.61−6.50 (m, 2H), 6.47 (brs, 1H), 6.21 (brs, 2H), 3.30−3.18 (m, 4H), 2.79−2.70 (m, 1H), 2.70−2.61 (m, 4H), 1.88−1.80 (m, 2H), 0.97 (d, 6H, J = 6.2 Hz). ESI-MS (m/z, %): 343 (MH^+, 100). ESI-HRMS calculated for $C_{19}H_{27}N_4S$ (MH^+), 343.1950; observed, 343.1953.

***N*-(1-(2-(2-Hydroxyethylamino)ethyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (51).** **51** was prepared from *tert*-butyl 2-hydroxyethyl(2-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)ethyl)carbamate (**41**) (3.13 g, 7.04 mmol) as described for **49** to obtain the title compound (1.65 g, 68.0%). ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.6 Hz, 1H), 7.54 (d, J = 4.8 Hz, 1H), 7.06 (dd, J = 4.8, 3.6 Hz, 1H), 6.60−6.52 (m, 2H), 6.48 (s, 1H), 3.45 (t, J = 5.7 Hz, 2H), 3.30−3.21 (m, 4H), 2.72−2.59 (m, 6H), 1.88−1.80 (m, 2H). ESI-MS (m/z, %): 345 (MH^+, 100). ESI-HRMS calculated for $C_{18}H_{25}N_4OS$ (MH^+), 344.1741; observed, 345.1743.

***N*-(1-(Pyrrolidin-3-yl)-1,2,3,4-tetrahydroquinolin-6-yl)-thiophene-2-carboximidamide (52).** A solution of *tert*-butyl-3-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)-pyrrolidine-1-carboxylate (**42**) (150 mg, 0.35 mmol) in methanol (5 mL) was treated with 1 N HCl (10 mL) and heated at 70 °C for 30 min. The solution was concentrated and dried under reduced pressure to give a yellow solid. The solid was triturated with MeOH/Et_2O (5:95) and dried under reduced pressure to obtain the dihydrochloride salt of the title compound (0.125 g 89.3%). ^{1}H NMR (DMSO-d_6) δ

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11.23 (s, 1H), 9.78 (brs, 1H), 9.65 (brs, 1H, 9.59 (brs, 1H), 8.61 (s, 1H), 8.15−8.14 (m, 2H), 7.36 (pseudo t, J = 4.5 Hz, 1H), 7.09−6.88 (m, 3H), 4.76−4.66 (m, 1H), 3.39−3.06 (m, 6H), 2.72 (t, J = 5.4 Hz, 2H), 2.16−2.01 (m, 2H), 1.87−1.83 (m, 2H). ESI-MS (m/z, %): 327 (MH^+). ESI-HRMS calculated for $C_{18}H_{23}N_4S$ (MH^+), 327.1637; observed, 327.1649.

***N*-(1-(3-(2-Hydroxyethylamino)propyl)-1,2,3,4-tetrahydroquinolin-6-yl)thiophene-2-carboximidamide (53).** **53** was prepared from *tert*-butyl 2-hydroxyethyl(3-(6-(thiophene-2-carboximidamido)-3,4-dihydroquinolin-1(2*H*)-yl)propyl)carbamate (**46**) (0.1 g, 0.21 mmol) as described for **49** to obtain the title compound (0.042 g, 53.7%). ^{1}H NMR (DMSO-d_6) δ 7.67 (d, J = 3.6 Hz, 1H), 7.54 (d, J = 4.8 Hz, 1H), 7.06 (dd, J = 4.8, 3.6 Hz, 1H), 6.55 (m, 2H), 6.47 (m, 1H), 6.22 (brs, 2H), 4.45 (t, J = 4.9 Hz, 1H), 3.45 (t, J = 5.2 Hz, 2H), 3.26−3.17 (m, 4H), 2.68−2.63 (m, 2H), 2.59−2.54 (m, 4H), 1.89−1.82 (m, 2H), 1.69−1.60 (m, 2H). ESI-HRMS calculated for $C_{19}H_{27}N_4OS$ (MH^+), 359.1907; observed, 359.19.

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.

Biology. NOS Inhibition Assay. Recombinant human inducible NOS (iNOS), human endothelial constitutive NOS (eNOS), or human neuronal constitutive NOS (nNOS) was produced in baculovirus-infected Sf9 cells (ALEXIS). In a radiometric method, NO synthase activity is 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 is added to the enzyme assay solution, followed by a preincubation time of 15 min at room temperature. The reaction is 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 is 150 μL in every well. The reactions are carried out at 37 °C for 45 min. The reaction is 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 is separated by DOWEX (ion-exchange resin DOWEX 50WX 8-400, Sigma), and the DOWEX is removed by spinning at 12000g for 10 min in the centrifuge. An 70 μL aliquot of the supernatant is added to 100 μL of scintillation fluid, and the samples are 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 inhibitor L-NMMA. All assays are 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 homeostatic stability was ensured, 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. Rats exhibiting signs of motor deficiency were euthanized. After a period of recovery following the surgical intervention, rats show enhanced sensitivity to painful and normally nonpainful stimuli.

hERG K$^+$ Channel Conventional Patch-Clamp Assay. Cultured cells (1−7 days) were used for patch-clamp assay. The cells were cultured in DMEM/GlutaMax-1 + 10% FBS and were planted on collagen-coated dishes at low density (~2 × 10^4 cells/dish). The cell was held at −80 mV. A 50 ms pulse to −40 mV was delivered to measure the leaking currents, which were subtracted from the tail currents online. Then the cell was depolarized to +20 mV for 2 s, followed by a second pulse to −40 mV for 1 s to reveal the tail currents. This paradigm was delivered once every 5 s to monitor the current amplitude. After the current amplitude was stabilized, the compound was delivered to the extracellular medium by a rapid solution changer perfusion system. During superfusion, the cell was repetitively stimulated with the protocol described above, and the current amplitude was continuously monitored. The experimental conditions are described in Table 4.

The degree of inhibition (%) was obtained by measuring the tail current amplitude before and after drug superfusion (the difference current was normalized to control and multiplied by 100 to obtain the percent of inhibition). Concentration (log) response curves were fitted to a logistic equation to generate estimates of IC_{50}. The concentration−response relationship of the compound was constructed from the percentage reductions of current amplitude by sequential concentrations.

HPLC Purity. Analytical HPLC spectra were collected on an Agilent 1100 HPLC system using a reverse phase column. All final compounds were >95% purity. Results are shown in Table 5.

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Notes

The authors declare no competing financial interest.

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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; L-NMMA, *N*-monomethyl-L-arginine; CNS MPO, central nervous system multiparameter optimization; hERG, human ether-a-go-go-related gene; SAR, structure−activity relationship; SNL, spinal nerve ligation

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