# Substituted *N*-Phenylisothioureas: Potent Inhibitors of Human Nitric Oxide Synthase with Neuronal Isoform Selectivity

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*S*-Ethyl *N*-phenylisothiourea (**4**) has been found to be a potent inhibitor of both the human constitutive and inducible isoforms of nitric oxide synthase. A series of substituted *N*-phenylisothiourea analogues was synthesized to investigate the structure−activity relationship of this class of inhibitor. Each analogue was evaluated for human isoform selectivity. One analogue, *S*-ethyl *N*-[4-(trifluoromethyl)phenyl]isothiourea (**39**), exhibited 115-fold and 29-fold selectivity for the neuronal isoform versus the inducible and endothelial derived constitutive isoforms, respectively. Studies have shown the substituted *N*-phenylisothiourea **39** binds competitively with L-arginine.

## Introduction

Nitric oxide (NO) has been recognized as an important mediator of diverse physiological processes including blood pressure homeostasis, platelet aggregation, neurotransmission, and immunological defense mechanisms.[1] The endogenous synthesis of NO is derived from the oxidation of L-arginine to L-citrulline. The L-arginine−nitric oxide pathway is mediated by a family of enzymes known as nitric oxide synthase (NOS).[2] Structurally distinct NOS enzymes have been identified which are generally classified as constitutive (cNOS) or inducible (iNOS) isoforms based on mechanism of regulation.[3] Two distinct constitutive NOS isoforms have been distinguished in the vascular endothelium (eNOS) and in the brain (nNOS). All NOS isoforms are heme-containing enzymes sharing similiar cofactor requirements for NADPH, FAD, FMN, and tetrahydrobiopterin for activity. The constitutive NOSs, however, are tightly regulated by $Ca^{2+}$ concentrations and are responsible for the low, transient bursts of NO generation involved in cellular communication processes. In contrast, iNOS is regulated transcriptionally and is expressed in response to various cytokines and endotoxin. The activation of iNOS results in prolonged elevated levels of NO production.

The distinct roles demonstrated by NO in normal physiology are attributed to the different NOS isoforms. The uncontrolled production of NO, however, has been implicated in numerous disease states[4] including septic shock,[5,6] inflammatory arthritis,[7] neurodegenerative diseases,[8] chronic ileitus,[9] and insulin-dependent diabetes mellitus.[10] Thus, selective NOS isoform inhibition to regulate NO synthesis has potential therapeutic benefits.

Despite the immense interest in nitric oxide, disclosures of novel NOS inhibitors have been limited. Inhibitors with high isoform selectivity are required to further define the role of each NOS isoform in various biological processes. The majority of described inhibitors are modified arginine analogues. One such analogue, $N^G$-methyl-L-arginine (L-NMA, **1**), has been shown to normalize blood pressure in animal models of cytokine-induced and septic shock.[11,12] Recently, we and the laboratories of Narayanan and Griffith independently reported the amino acid derived *S*-methyl and *S*-ethyl isothiourea derivatives **2** and **3** (Figure 1) as a new class of potent NOS inhibitor.[13,14]

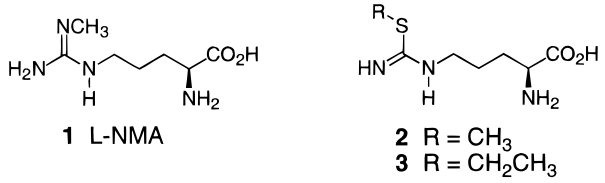
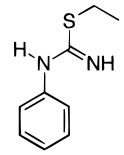



**Figure 1.** Arginine-based NOS inhibitors.




**4**

**Figure 2.** Structure of *S*-ethyl *N*-phenylisothiourea.

Prior to these reports, we discovered a class of structurally unrelated isothiourea analogues to be potent NOS inhibitors. We found simple *S*-alkyl isothioureas to be potent NOS inhibitors during our initial search for isoform selective NOS inhibitors.[15] Nakane and co-workers have also reported independent detailed studies identifying *S*-ethyl isothiourea as a potent and selective inhibitor of iNOS.[16] Further structural investigation of our compound series identified *S*-ethyl *N*-phenylisothiourea (**4**) (Figure 2) as a potent NOS inhibitor exhibiting selectivity for the neuronal isoform.[17] This selectivity differs from the iNOS selectivity observed for simple *S*-alkyl isothioureas. We initiated synthetic efforts to investigate the potential of this class of nonamino acid based inhibitor.[18] This communication reports the synthesis of a limited series of substituted *N*-phenylisothiourea analogues and their ability to inhibit purified human NOS isoforms.

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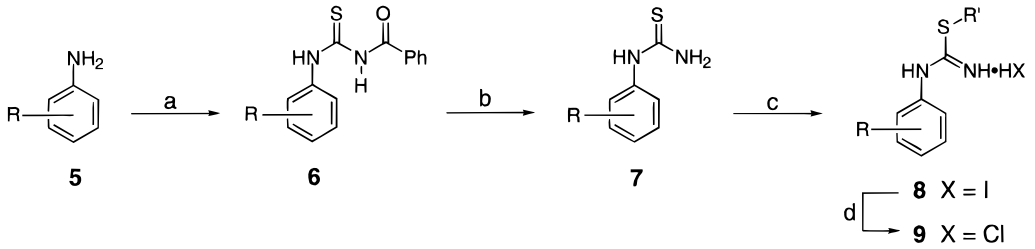
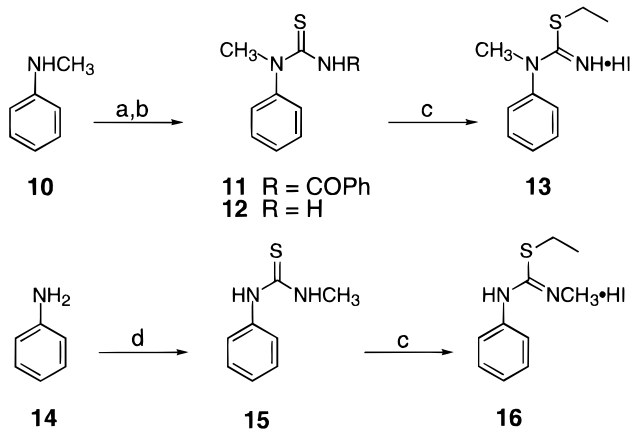
**Scheme 1**[a]




[a] (a) PhCONCS, acetone, room temperature; (b) NaOH, THF−$H_2O$, reflux; (c) R′I, acetone, reflux; (d) (i) $NaHCO_3$, $Et_2O$−$H_2O$; (ii) HCl, $Et_2O$.

**Scheme 2**[a]




[a] (a) PhCONCS, THF, 0 °C to room temperature; (b) $K_2CO_3$, EtOH−$H_2O$, reflux; (c) EtI acetone, reflux; (d) $CH_3NCS$, EtOH, reflux.

## Chemistry

The synthesis of our general series is outlined in Scheme 1. Many of the *N*-phenylthioureas employed in this study were available from commercial sources. The remaining thioureas were readily accessible from substituted anilines **5**. Treatment of substituted anilines **5** with benzoyl isothiocyanate and subsequent hydrolysis of the benzoyl group provided the requisite thioureas **7**.[19] Sulfur alkylation of the substituted thioureas **7** with alkyl iodides efficiently afforded the targeted substituted *N*-phenylisothioureas **8**. Crystallization of the isothiourea hydroiodide salts, however, often proved to be difficult. Purification was simplified by conversion to the corresponding hydrochloride salts **9** as illustrated in Scheme 1. Additionally, the N,S-disubstituted *N*-phenylisothiourea analogues **13** and **16** were synthesized in a similiar manner as depicted in Scheme 2.

## Results and Discussion

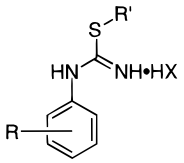
The ability of substituted *N*-phenylisothioureas to inhibit human NOS isoforms was determined by monitoring the conversion of L-[$^{14}$C]arginine to L-[$^{14}$C]citrulline as previously described.[15,20−23] Table 1 summarizes these results. Several general structure−activity relationship (SAR) observations regarding these analogues can be derived from evaluation of the results presented in Table 1. First, inhibition potency is directly related to the steric bulk of the *S*-alkyl substituent. Optimal potency is observed for the *S*-ethyl isothioureas. The analogous *S*-methyl isothioureas **20**, **38**, and **44** display a decrease in inhibition potency, and compounds **40**−**43** show inhibition rapidly diminishes with larger or branched alkyl substitution on sulfur. This trend is similiar to SAR evidence reported for simple alkyl isothioureas.[15] Second, N-methylation of either isothiourea nitrogen as in derivatives **13** and **16** (Scheme 2) abolishes NOS inhibition. The role of the isothiourea NH groups in hydrogen-bonding interactions may be crucial for NOS inhibition. Steric and conformational considerations, however, cannot be dismissed. Additionally, the *N*-phenyl ring is a key component for potent inhibition. Heteroaromatic replacement of the phenyl group with a pyridyl ring results in a significant decrease in NOS inhibition as evidenced by analogues **47**−**49**. Despite their reduced potency, these compounds retain selectivity for the neuronal isoform.

Further SAR investigation of the phenyl ring led to enhanced nNOS selectivity. The improvement in selectivity was achieved by selectively decreasing both iNOS and eNOS inhibition potency without markedly effecting nNOS activity. Para substitution of the aromatic ring has the greatest influence upon selectivity. The inducible enzyme appears to be the most sensitive isoform to *N*-phenylisothioureas structurally modified at the *p*-phenyl position. The unsubstituted *N*-phenylisothiourea analogue **4** is a potent inhibitor of all three isoforms ($K_i$ < 1 $\mu$M) displaying highest activity toward nNOS. In general, those analogues possessing a para electron-withdrawing group (EWG) exhibit a significant decrease in iNOS inhibition. This trend is supported by the weak iNOS inhibition ($K_i$ >35 $\mu$M) displayed by the *p*-$OCF_3$, *p*-$CO_2Et$, *p*-$CF_3$, and *p*-$NO_2$ derivatives **25**, **29**, **39**, and **45**, respectively. The inhibition of eNOS also decreased ($K_i$ > 1 $\mu$M) but to a lesser extent than observed for iNOS. In contrast, the inhibition of nNOS by these para-EWG-substituted *S*-ethyl *N*-phenylisothioureas remained submicromolar with the exception of analogue **29**.

Incorporation of electron-releasing groups (ERGs) para to the isothiourea functionality also influences inhibition selectivity as exemplified by comparing compounds **24**, **26**−**28**, and **32**−**35** with unsubstituted analogue **4**. These modified derivatives display a decrease in nNOS inhibition similiar in magnitude to the para-EWG-substituted derivatives. The magnitude of decrease in eNOS and iNOS inhibition for these analogues, however, was significantly lower relative to the observed inhibitions of the para-EWG-substituted derivatives. As a result, the effect of the para-ERGs upon selectivity in this reported series was less pronounced than those observed for the analogues possessing EWGs.

Steric factors associated with *p*-alkyl substituents also affect inhibition potency. Replacement of the small *p*-methyl substituent in compound **32** with the larger branched isopropyl or cyclohexyl groups to give ana-

**Table 1.** Substituted *N*-Phenylisothioureas and Their Inhibition of Human NOS[a]




| | | | | | $K_i$, ($\mu$M) | | | selectivity[c] | |
|---|---|---|---|---|---|---|---|---|---|
| compd[b] | R | R′ | X | mp, °C | iNOS | eNOS | nNOS | iNOS/ nNOS | eNOS/ nNOS |
| **4**[d] | H | Et | I | | 0.87 | 0.40 | 0.12 | 7.3 | 3.3 |
| **17** | 2-Br | Et | I | 100 | 4.7 | 2.0 | 0.25 | 19 | 8.0 |
| **18** | 2-Cl | Et | Cl | 87−90 | 2.9 | 1.8 | 0.17 | 17 | 11 |
| **19** | 3-Cl | Et | Cl | 95−96 | 2.4 | 1.8 | 0.45 | 5.3 | 4.0 |
| **20** | 4-Cl | Me | I | 172−174 | 42 | 2.6 | 0.57 | 74 | 4.6 |
| **21** | 4-Cl | Et | Cl | 154−157 | 4.2 | 0.9 | 0.14 | 30 | 6.4 |
| **22** | 2-$OCH_3$ | Et | Cl | 75−78 | 1.4 | 1.6 | 0.17 | 8.2 | 9.4 |
| **23** | 3-$OCH_3$ | Et | I | 75−77 | 3.2 | 3.1 | 0.56 | 5.7 | 5.5 |
| **24** | 4-$OCH_3$ | Et | I | 127−128 | 4.9 | 2.7 | 0.29 | 16 | 9.3 |
| **25** | 4-$OCF_3$ | Et | Cl | 96−97 | 55 | 17 | 0.70 | 79 | 24 |
| **26** | 4-OPh | Et | I | 140−143 | 3.1 | 4.0 | 0.19 | 16 | 21 |
| **27** | 4-$OCH_2Ph$ | Et | I | 172−174 | 6.6 | 2.8 | 0.21 | 31 | 13 |
| **28** | 4-OH | Et | I | 135 | 2.5 | 2.7 | 0.34 | 7.4 | 7.9 |
| **29** | 4-$CO_2Et$ | Et | I | 80−85 | 63 | 12 | 1.6 | 39 | 7.5 |
| **30** | 4-$CO_2H$ | Et | I | 214−215 | 0%[e] | 14%[e] | 58 | | |
| **31** | 3-$CO_2H$ | Et | I | 149−152 | 44 | 14 | 11 | 4.0 | 1.3 |
| **32** | 4-$CH_3$ | Et | Cl | 138−141 | 1.9 | 1.0 | 0.18 | 11 | 5.6 |
| **33** | 2-iPr | Et | Cl | 66−68 | 10 | 6.5 | 1.1 | 9.1 | 5.9 |
| **34** | 4-iPr | Et | Cl | 113−116 | 25 | 7.7 | 0.33 | 76 | 23 |
| **35** | 4-c-$C_6H_{11}$ | Et | I | 174−175 | 28 | 14 | 1.5 | 19 | 9.3 |
| **36** | 2-$CF_3$ | Et | Cl | 75−77 | 22 | 18 | 1.4 | 16 | 13 |
| **37** | 3-$CF_3$ | Et | Cl | 102 | 3.2 | 28 | 1.1 | 2.9 | 26 |
| **38** | 4-$CF_3$ | Me | I | 125−127 | 0%[e] | 31 | 4.6 | | 6.7 |
| **39** | 4-$CF_3$ | Et | Cl | 126−127 | 37 | 9.4 | 0.32 | 115 | 29 |
| **40** | 4-$CF_3$ | nPr | Cl | 169−170 | 4%[e] | 0%[e] | 64 | | |
| **41** | 4-$CF_3$ | iPr | Cl | 67−70 | 0%[e] | 0%[e] | 29 | | |
| **42** | 4-$CF_3$ | Bu | Cl | 151 | 0%[e] | 0%[e] | 0%[e] | | |
| **43** | 4-$CF_3$ | $CH_2Ph$ | Cl | 71−72 | 0%[e] | 0%[e] | 11%[e] | | |
| **44** | 4-$NO_2$ | Me | I | 195−198 | 0%[e] | 17 | 3.3 | | 5.2 |
| **45** | 4-$NO_2$ | Et | I | 161−162 | 54 | 9.1 | 0.66 | 82 | 14 |
| **46** | 4-$N(CH_3)_2$ | Et | I | 138−141 | 29 | 9.0 | 1.4 | 21 | 6.4 |
| **47** | Ar = 2-pyridyl | Et | I | 127−128 | 23 | 17 | 4.8 | 4.8 | 3.5 |
| **48** | Ar = 3-pyridyl | Et | Cl | 96−97 | 23 | 7.0 | 1.0 | 23 | 7.0 |
| **49** | Ar = 4-pyridyl | Et | f | 138−141 | 22 | 16 | 0.8 | 28 | 23 |

[a] Inhibition constants were obtained by measuring percent inhibition with at least three concentrations of inhibitor as described in ref 15. Values had a standard deviation of ≤10% ($n \geq 3$). [b] All compounds gave satisfactory analyses for C, H, N, S, X (±0.4%). [c] Defined as the ratio of $K_i$ iNOS or $K_i$ eNOS to $K_i$ nNOS. [d] Purchased from Aldrich Chemical Co. [e] Percent inhibition using 25 $\mu$M of test compound. [f] Not isolated as a HX salt.

logues **34** and **35** resulted in reduced inhibition of all three isoforms.

For a given substituent, para substitution imparts the greatest selectivity as shown by comparison of the trifluoromethyl analogues **36**, **37**, and **39**, methoxy derivatives **22**−**24**, and isopropyl analogues **33** and **34**. The *p*-$CF_3$ analogue **39** proved to be the the most selective compound. The selectivity of this inhibitor for nNOS versus iNOS and eNOS is 115-fold and 29-fold, respectively.

The nNOS binding of the *p*-$CF_3$ analogue **39** was competitive with L-arginine as illustrated in Figure 3. The equation $V = V_{max}[S]/\{[S] + K_m(1 + [I]/K_i)\}$ was fitted to the data to give $K_m = 2$ $\mu$M and $K_i = 0.12$ $\mu$M. This result suggests that the enzyme-binding interactions of compound **39** and structurally related *N*-phenylisothioureas occur within the L-arginine binding site.

The potent and selective analogue **39** was selected for *in vivo* evaluation. The pharmacokinetic properties and the ability of isothiourea **39** to inhibit nNOS in intact cells within brain tissue were investigated as a preliminary predictor of potential *in vivo* efficacy. When

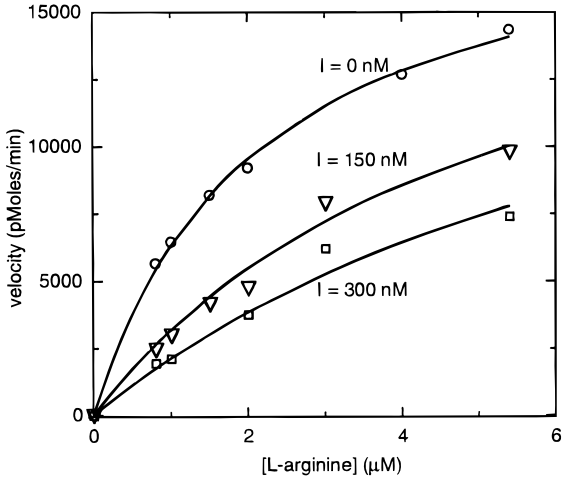



**Figure 3.** Competitive inhibition of nNOS by compound **39**. Velocity of nNOS catalyzed conversion of L-arginine to L-citrulline in the presence of 0, 150, and 300 nM concentrations of **39** was determined as described in ref 22. The solid lines represent the fit of velocity = $V_{max}$[arginine]/{[arginine] + $K_m$-(1 + [**39**]/$K_i$)} where $V_{max} = 19000 \pm 3000$ pmol/min, $K_m = 2.0 \pm 0.6$ $\mu$M, and $K_i = 0.12 \pm 0.03$ $\mu$M.

administered intravenously to mice at 25 mg/kg, compound **39** was found to readily penetrate the blood−brain barrier, immediately reaching concentrations in the brain equal to the plasma concentration. The ratio of brain to plasma concentration essentially remained equivalent throughout the experiment. This compound, however, was rapidly cleared (5.6 (L/kg)/h) in a biphasic manner ($t_{1/2}\alpha = 4$ min and $t_{1/2}\beta = 31$ min), and several unidentified plasma metabolites were observed immediately after dosing. Compound **39** disappointingly failed to inhibit nNOS activity in rat brain slices employing previously described assay methods.[14] Other potent isothiourea inhibitors of purified NOS enzymes have been shown to exhibit significantly decreased inhibition potency in whole cell assays due to poor cellular uptake.[15] The efficiency of substituted *N*-phenylisothioureas to penetrate into specific cells is unknown and may be a contributing factor to the result observed for compound **39**. Further detailed studies are required. On the basis of the above results, the *in vivo* evaluation of these *N*-phenylisothioureas was not pursued.

In summary, substituted *N*-phenylisothioureas are potent, competitive inhibitors of human NOS displaying selectivity for the neuronal isoform. Preliminary SAR evidence indicates that the constitutive isoforms nNOS and eNOS are more tolerable than iNOS toward structural modifications to this class of inhibitor. As a result, greater nNOS versus iNOS selectivity than nNOS versus eNOS selectivity was achieved. The further development of these and related agents for *in vivo* therapeutic evaluation is in progress.

## Experimental Section

Solvents and reagents were reagent grade and used without further purification. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All $^1$H NMR spectra were recorded on Varian Unity 300 MHz or Varian Gemini 200 MHz spectrometers. Chemical shifts ($\delta$) are reported downfield from tetramethylsilane ($Me_4Si$) in parts per million (ppm) of the applied field. Peak multiplicities are abbreviated: singlet, s; broad singlet, bs; doublet, d; triplet, t; quartet, q; multiplet, m. Coupling constants ($J$ values) are reported in hertz. Analytical thin-layer chromatography (TLC) was used to monitor reactions. Plates (2.5 × 10 cm) precoated with silica gel GHLF of 0.25-mm thickness, supplied by Analtech, were used. Combustion microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA.

**General Procedure for the Preparation of Substituted 1-Benzoyl-3-phenylthioureas 6.** The procedure for **1-benzoyl-3-[4-(trifluoromethoxy)phenyl]thiourea (6, R = 4-OCF$_3$)** is representative. To a stirred solution of 4-(trifluoromethoxy)aniline (5.24 g, 29.6 mmol) in 100 mL of acetone was added benzoyl isothiocyanate (5.46 g, 33.5 mmol). After being stirred overnight, the mixture was concentrated at reduced pressure, giving a yellow solid. Recrystallization from ethyl acetate−hexane afforded 9.29 g (92%) of the desired substituted phenylthiourea as a pale yellow solid: mp 124−125 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 7.39−7.73 (5H, m), 7.83 (2H, d, $J$ = 9.0 Hz), 8.01 (2H, d, $J$ = 7.2 Hz), 11.67 (1H, s,), 12.60 (1H, s). Anal. ($C_{15}H_{11}N_2O_2SF_3$) C, H, N, S.

**General Procedure for the Preparation of Substituted *N*-Phenylthioureas 7.** The procedure for ***N*-[4-(trifluoromethoxy)phenyl]thiourea (7, R = 4-OCF$_3$)** is representative. To a stirred solution of 1-benzoyl-3-[4-(trifluoromethoxy)-phenyl]thiourea (**6**, R = 4-OCF$_3$) (7.80 g, 22.9 mmol) in 100 mL of THF was added 2.0 N aqueous NaOH (25.0 mL, 50.0 mmol). The mixture was heated to reflux for 3 h, cooled to room temperature, and concentrated at reduced pressure. The residue was suspended in water and extracted repeatedly with $CH_2Cl_2$. The combined organic layers were washed with brine and dried over $MgSO_4$. Removal of solvent at reduced pressure and recrystallization from ethyl acetate−hexane afforded 3.77 g (70%) of the desired thiourea **7** (R = 4-OCF$_3$) as a white solid: mp 136−137 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 7.33 (2H, d, $J$ = 9.0 Hz), 7.57 (2H, d, $J$ = 9.0 Hz), 9.81 (1H, bs). Anal. ($C_8H_{17}N_2OSF_3$) C, H, N, S.

**General Procedure for the Preparation of Substituted *N*-Phenylisothiourea Hydroiodides 8.** The procedure for ***S*-ethyl-*N*-(4-phenoxyphenyl)isothiourea (26)** is representative. To a stirred solution of 1-(4-phenoxyphenyl)thiourea (**7**, R = 4-OPh) (2.00 g, 8.19 mmol) in 20 mL of acetone was added iodoethane (7.61 g, 48.7 mmol). The mixture was heated to reflux for 3 h, cooled to room temperature, and concentrated at reduced pressure, giving a viscous oil. Crystallization from acetone−pentane afforded 2.00 g (61%) of the desired *N*-phenylisothiourea hydroiodide **26** as a beige solid. mp 140−143 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 1.34 (3H, t, $J$ = 7.4 Hz), 3.29 (2H, q, $J$ = 7.4 Hz), 7.17 (5H, m), 7.42 (4H, m). Anal. ($C_{15}H_{16}N_2OS$·HI) C, H, N, S, I.

**General Procedure for the Preparation of Substituted *N*-Phenylisothiourea Hydrochlorides 9.** The procedure for ***S*-ethyl-*N*-[4-(trifluoromethoxy)phenyl]isothiourea (25)** is representative. To a stirred solution of 1-[4-(trifluoromethoxy)phenyl]thiourea (**7,** R = 4-OCF$_3$) (3.00 g, 12.7 mmol) in 100 mL of acetone was added iodoethane (5.85 g, 37.5 mmol). The mixture was heated to reflux and left overnight. After being cooled to room temperature, the mixture was concentrated at reduced pressure, giving an oil. This oil was dissolved in water and washed with pentane. The aqueous layer was poured into saturated aqueous $NaHCO_3$ (75 mL) and extracted with $Et_2O$. The organic layer was dried over $MgSO_4$, concentrated to a volume of approximately 200 mL, and treated with 1 N HCl in $Et_2O$ (15.0 mL, 15.0 mmol). After being stirred for 20 min, the mixture was concentrated at reduced pressure and placed *in vacuo* overnight to give a gummy foam. Trituration with pentane provided 3.35 g (88%) of the desired *N*-phenylisothiourea hydrochloride **25** as a white solid: mp 96−97 °C; 200 MHz $^1$H NMR ($D_2O$) $\delta$ 1.38 (3H, t, $J$ = 7.4 Hz), 3.20 (2H, q, $J$ = 7.4 Hz), 7.44 (4H, s). Anal. ($C_{10}H_{11}N_2OSF_3$·HCl) C, H, N, S, Cl.

**1-Benzoyl-3-methyl-3-phenylthiourea (11).** To a stirred, cooled (0 °C) solution of *N*-methylaniline (10.7 g, 100 mmol) in 60 mL of THF was added benzoyl isothiocyanate (19.6 g, 120 mmol). The mixture was allowed to warm to room temperature while stirring overnight and concentrated at reduced pressure giving a solid. Recrystallization from ethyl acetate−hexane afforded 22.7 g (84%) of **11** as a white solid: mp 136−137 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 3.69 (3H, s), 7.19−7.66 (11H, m), 10.8 (1H, bs). Anal. ($C_{15}H_{14}N_2OS$) C, H, N, S.

**1-Methyl-1-phenylthiourea (12).** To a stirred solution of 1-benzoyl-3-methyl-3-phenylthiourea (**11**) (10.0 g, 37.0 mmol) in EtOH (120 mL) was added 1.23 M aqueous $K_2CO_3$ (60 mL, 73.8 mmol). The resulting yellow mixture was heated to reflux. After 24 h, the mixture was cooled to room temperature, concentrated at reduced pressure, and extracted with EtOAc. The organic layer was dried over $MgSO_4$. Solvent was removed at reduced pressure and the crude product was recrystallized from ethyl acetate−hexane to afford 600 mg (10%) of pure **12** as a white solid: mp 98−103 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 3.47 (3H, s), 7.26−7.68 (5H, m). Anal. ($C_8H_{10}N_2S$) C, H, N, S.

***S*-Ethyl-1-methyl-1-phenylisothiourea Hydroiodide (13).** To a stirred solution of 1-methyl-1-phenylthiourea (**12**) (1.89 g, 11.4 mmol) in 20 mL of acetone was added iodoethane (5.90 g, 34.1 mmol). The mixture was heated to reflux for 4 h, cooled to room temperature, and concentrated at reduced pressure, giving a viscous oil. Crystallization from acetone−pentane afforded 3.10 g (86%) of the desired *N*-phenylisothiourea hydroiodide **13** as a white solid: mp 135−137 °C; 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 1.24 (3H, t, $J$ = 7.4 Hz), 3.16 (2H, q, $J$ = 7.4 Hz), 3.49 (3H, s), 7.54 (5H, m), 9.24 (1H, bs). Anal. ($C_{10}H_{14}N_2S$·HI) C, H, N, S, I.

**1-Methyl-3-phenylthiourea (15).** To a stirred solution of aniline (5.11 g, 54.9 mmol) in 100 mL of EtOH was added methyl isothiocyanate (4.70 g, 64.3 mmol). The mixture was heated to reflux overnight, cooled to room temperature, and

concentrated at reduced pressure, giving a dark yellow solid. Recrystallization from EtOH afforded 4.32 g (47%) of the desired thiourea **15** as a white solid. The mother liquor was concentrated at reduced pressure and recrystallized from ethyl acetate−hexane to provide an additional 3.70 g (41%) of **15** as a white solid: 200 MHz $^1$H NMR (DMSO-$d_6$) $\delta$ 2.93 (3H, bs), 7.14 (1H, m), 7.36 (4H, m), 7.71 (1H, bs), 9.54 (1H, bs).

***S*-Ethyl-1-methyl-3-phenylisothiourea Hydroiodide (16).** Compound **16** was prepared from 1-methyl-3-phenylthiourea (**15**) (1.60 g, 9.62 mmol) and iodoethane (1.76 g, 11.3 mmol) as described for compound **13** to afford 2.77 g (89%) of a white solid: mp 134 °C; 300 MHz $^1$H NMR ($D_2O$) $\delta$ 1.28 (3H, bs), 3.06 (5H, bs), 7.34 (2H, m), 7.48 (3H, m). Anal. ($C_{10}H_{14}N_2S\cdot$HI) C, H, N, S, I.

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