Acyl Substitution at the Ortho Position of Anilides Enhances Oral Bioavailability of Thiophene Sulfonamides: TBC3214, an ET_A Selective Endothelin Antagonist[1]

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Sitaxsentan (**3**, TBC11251) (Wu et al. *J. Med. Chem.* **1997**, *40*, 1690) is an orally active ET_A selective endothelin antagonist that attenuates pulmonary vascular hypertension and cardiac hypertrophy in rats (Tilton et al. *Pulm. Pharmacol. Ther.* **2000**, *13*, 87). It has demonstrated efficacy in a phase II clinical trial for congestive heart failure (Givertz et al. *Circulation* **2000**, *101*, 2922). During the discovery of **3**, we observed several structure−oral bioavailability relationships. To investigate whether there is any generality in these trends, we synthesized some similar pairs of compounds in the latest series (Wu et al. *J. Med. Chem.* **1999**, *42*, 4485) and evaluated their oral properties. In both series, an acyl group at the 2-position of the anilide of these thiophene sulfonamides improved oral bioavailability. As a result of this exercise, TBC3214 (**17**) was identified as a sitaxsentan follow-on candidate. It is very potent (IC_{50} for ET_A = 40 pM) and highly selective for ET_A vs ET_B receptors (400 000-fold), with a half-life of >4 h and oral bioavailability of 25% in rats, 42% in cats, and 70% in dogs.

Introduction

The vaso-constrictive peptide endothelin has been implicated as a causative factor in a number of disease states, and as such it has become a popular target among drug discovery groups.[6] Mimicking binding of a peptide to its receptor or blocking of such binding by small molecule antagonists is an essential first step in drug discovery, but the successful advancement of such small molecule antagonists to clinical candidates often depends on building appropriate drug metabolism and pharmacokinetics (DMPK) properties into the lead scaffolds. In this report we present some oral bioavailability structure−activity relationships (SAR) which we hope will be useful to the scientific community in the discovery of therapeutics.

For successful oral action, the candidate molecule must not only be adsorbed but additionally it must partition to the appropriate target tissue. Lipophilicity has long been recognized as a major determinant of absorption,[7] but more recently, the importance of other molecular descriptors such as polar surface area (PSA) has been recognized.[8,9]

For endothelin antagonists, receptors on the surface of vascular endothelial cells are the target, and thus, plasma levels of test compounds have shown a good correlation with in vivo biological activity. Thus, measuring plasma levels of test compounds after oral administration have proven a useful technique for directing the synthesis of analogues.

We have reported the synthesis, biological assays, and pharmacokinectics of a series of benzodioxole thiophene sulfonamides **1**−**6**,[10,2] **3** (sitaxsentan or TBC11251) being in phase II trials for congestive heart failure[4] and pulmonary hypertension.[11] An analysis of the oral bioavailability data revealed the following trends in relationship with structure (Chart 1): (A) Changing the linker between thiophene and benzodioxole from amide to ketomethylene increased oral bioavailability greatly (from 0% for **1** to 30% for **2**). This was assuming that change from Br to Cl on the isoxazole ring did not influence significantly on oral bioavailability. (B) Changing the 4-chloroisoxazole to 4-methylisoxazole doubled oral bioavailability (from 60% for **3** to ∼100% for **4**). (C) Third, an acetyl group on the 6-position of the benzodioxozole afforded compound **6** good oral bioavailability (27%), comparing with unsubstituted **1** (0%) or the cyano compound **5** (8%).

We needed to ascertain if the three above-mentioned trends are general for this series or only compound-specific, not only for our drug optimization purpose but also for gaining a deeper understanding of medicinal chemistry. Therefore, we synthesized three groups of compounds (**19**, **20**; **21**−**23**; **24**, **25**, and **16**−**18**) where the benzodioxole moiety was replaced by a phenyl group.

Results and Discussion

Synthetic Chemistry. The synthesis of **16**−**18** is delineated in Scheme 1 starting from commercially available 2,4-dimethylaniline **7**. A boron trichloride mediated Friedel−Crafts acylation reaction[12] converted **7** to acetophenone **8** or isobutyrophenone **9** in good yields, using acetonitrile or isobutyronitrile, respectively. After some unsuccessful attempts to reduce the keto group directly to a methylene, compound **11** was obtained via stepwise reduction of **8** to the alcohol **10** with borane and then a further reduction of **10** with triethylsilane in TFA. The anilines **11**, **8**, and **9** were

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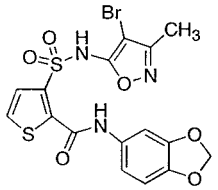
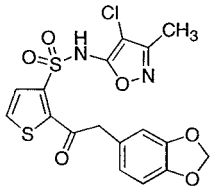
Chart 1. Structure−Oral Bioavailability (*F*) Relationships of Benzodioxole Endothelin Antagonists

A. Amide vs Ketomethylene Linker

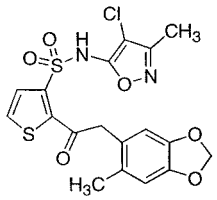


1
ET_A IC_{50} = 19 nM
ET_A Selectivity = 500
F = 0%

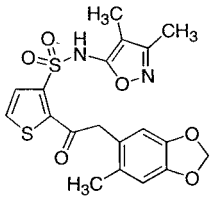


2
ET_A IC_{50} = 21 nM
ET_A Selectivity = 300
F = 30%

B. Dimethyl vs Chloro Methyl Isoxazole

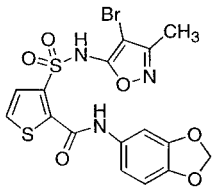


3 (sitaxsentan or TBC11251)
ET_A IC_{50} = 1.4 nM
ET_A Selectivity = 7,000
F = 60%

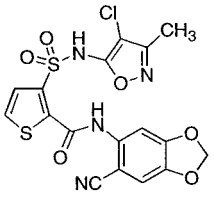


4
ET_A IC_{50} = 3.3 nM
ET_A Selectivity = 10,000
F = ~100%

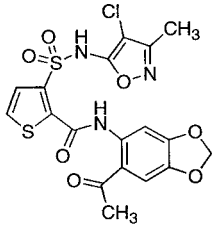
C. Ortho-Substituent: H vs Cyano vs Acetyl



1
ET_A IC_{50} = 19 nM
ET_A Selectivity = 500
F = 0%



5
ET_A IC_{50} = 3.4 nM
ET_A Selectivity = 12,000
F = 8%

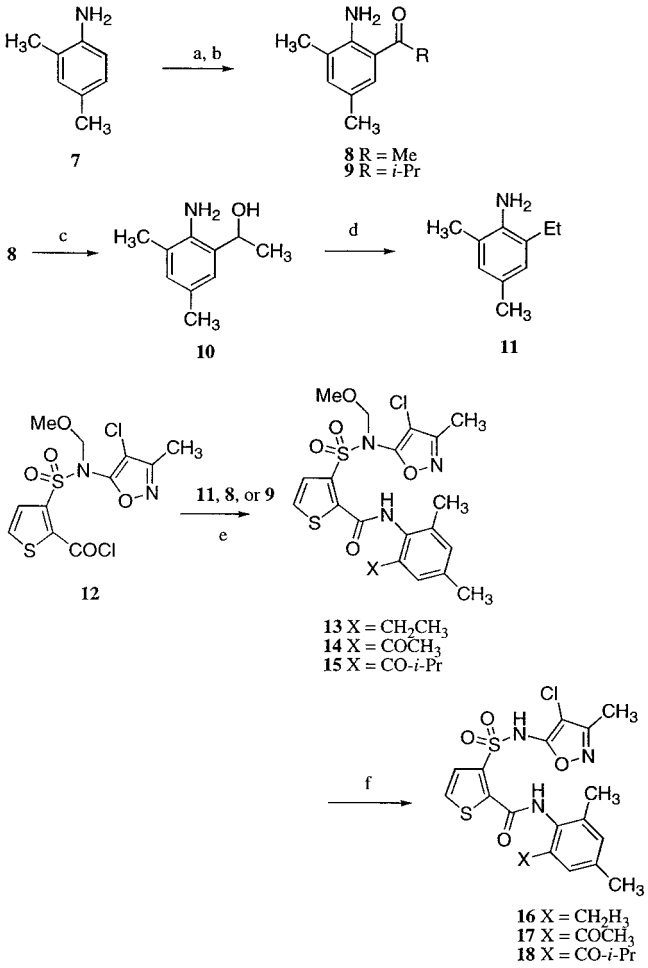


6
ET_A IC_{50} = 10 nM
ET_A Selectivity = 3,000
F = 27%

then independently coupled with thiophene acid chloride **12**[5] using 4-dimethylaminobenzonitrile as the base to give amides **13**−**15**, respectively. The reaction proceeded well using 2 equiv of the anilines **8**, **9**, or **11**, but addition of strong bases such as triethylamine or aqueous inorganic bases afforded no coupling products, giving back the aniline and decomposed acid chloride. The deprotection of the methoxymethyl group was accomplished with heating in a 2:1 mixture of methanol and concentrated HCl to reveal the desired sulfonamides **16**−**18**.

The synthesis, in vitro binding, in vivo activity, and serum half-lives for compounds **19**−**23** and **25** have been recently reported.[5]

Scheme 1. Synthesis of Endothelin Antagonists **16**−**18**[a]



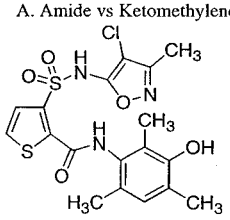
[a] (a) BCl_3/RCN/1,2-dichloroethane; (b) HCl/H_2O/MeOH; (c) BH_3·THF; (d) TFA/Et_3SiH; (e) THF/4-dimethylaminobenzonitrile; (f) concentrated HCl/MeOH/70 °C.

Structure−Oral Absorption Relationships. The structure−oral absorption comparisons are presented in Chart 2. The oral absorption in rats is expressed as (i) peak plasma concentration (C_{max}) of said compounds after oral dosing at 50 mg/kg and (ii) the area under the curve extrapolated to infinity (AUC_{inf}). Although these parameters are not equivalent to oral bioavailability, for comparison purposes they should offer accurate enough insights.

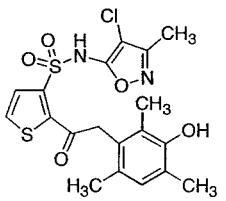
The beneficial effect of changing an amide linker to the corresponding ketone tether (from **1** to **2**) did not show in this trimethylphenol series. Actually such a change (from **19** to **20**) decreased oral absorption approximately by an order of magnitude. The effects of different isoxazoles were then studied in a trimethylbenzyl cyanide grouping. In contrast to the promoting effect on oral bioavailability going from 4-chloro (**3**) to 4-methylisoxazole (**4**), a similar switch (from **22** to **21**) lowered the C_{max} while AUC did not change much. It is interesting to note that when the oxygen and nitrogen atoms in the chloroisoxazole ring were swapped positions (**22** to **23**), a modest increase in C_{max} was observed while AUC was 3-fold higher. The effects of ortho substituents were then investigated. The compound without any ortho substitutents (**24**)[10] was not orally available at all, while the mesitylanilide (**25**), with two methyl groups on the two ortho positions, respectively,

Chart 2. Structure–Oral Absorption Relationships of Phenyl Endothelin Antagonists

A. Amide vs Ketomethylene Linker

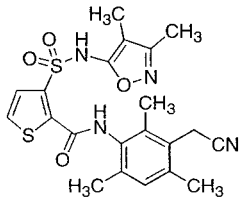


19
ET_A IC_{50} = 0.19 nM
ET_A Selectivity = 10,900
C_{max} = 48.2 μg/mL
AUC_{inf} = 117.9 hr μg/mL

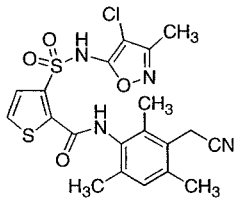


20
ET_A IC_{50} = 0.14 nM
ET_A Selectivity = 10,800
C_{max} = 2.6 μg/mL
AUC_{inf} = 15.8 hr μg/mL

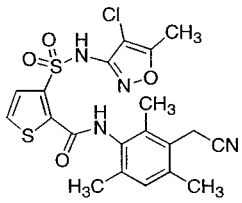
B. Dimethyl vs Chloro Methyl Isoxazole



21
ET_A IC_{50} = 0.17 nM
ET_A Selectivity = 14,600
C_{max} = 13.0 μg/mL
AUC_{inf} = 45.8 hr μg/mL

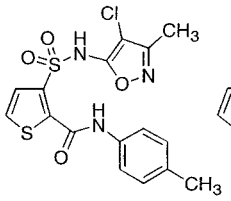


22
ET_A IC_{50} = 0.14 nM
ET_A Selectivity = 32,900
C_{max} = 42.0 μg/mL
AUC_{inf} = 59.9 hr μg/mL

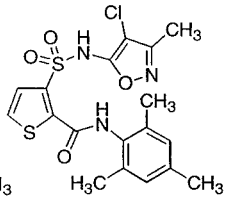


23
ET_A IC_{50} = 0.10 nM
ET_A Selectivity = 8,500
C_{max} = 53.9 μg/mL
AUC_{inf} = 170.7 hr μg/mL

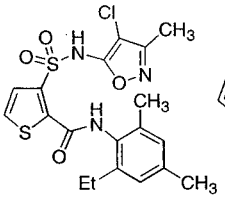
C. Ortho-Substituent: H vs Methyl vs Acetyl



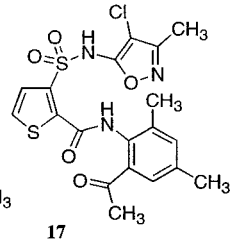
24
ET_A IC_{50} = 15 nM
ET_A Selectivity = 940
C_{max} = ~0 μg/mL
AUC_{inf} = ~0 hr μg/mL



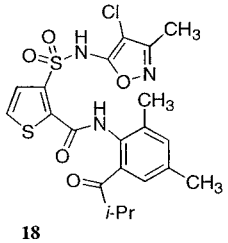
25
ET_A IC_{50} = 0.17 nM
ET_A Selectivity = 7,000
C_{max} = 35.9 μg/mL
AUC_{inf} = 102.9 hr μg/mL



16
ET_A IC_{50} = 0.43 nM
ET_A Selectivity = NT
C_{max} = 21.8 μg/mL
AUC_{inf} = 47.0 hr μg/mL



17
ET_A IC_{50} = 0.04 nM
ET_A Selectivity = 442,000
C_{max} = 57.6 μg/mL
AUC_{inf} = 272.4 hr μg/mL
$t_{1/2}$ = 4 h
F = 25% in rat
F = 42% in cat
F = 70% in dog



18
ET_A IC_{50} = 0.12 nM
ET_A Selectivity = 14,000
C_{max} = 59.2 μg/mL
AUC_{inf} = 297.5 hr μg/mL

had decent oral absorption. When one of the two ortho substituents was ethyl (**16**), the oral absorption was still respectable but considerably lower than the *o*-dimethyl compound **25**. Interestingly, when the ethyl group in **16** was changed to acetyl group, the resulting compound **17** had a marked increase in oral absorption as reflected by a 2.6-fold increase in C_{max} and a 5.8-fold increase in AUC. The oral bioavailability of **17** was confirmed in three species: 25% in rats, 42% in cats, and 70% in dogs.

Since a comparison between **16** and **17** is the fairest in terms of both size of substituents and substitution pattern, it appeared that an *o*-acyl group did help increase oral bioavailability as established in both series of compounds. To further substantiate this favorable acyl effect on oral bioavailability in this series, an additional compound (**18**) was synthesized where the 2-position of the anilide was substituted with an isopropylcarbonyl group. Indeed, **18** (IC_{50} for ET_A = 0.12 nM with a selectivity for ET_A over ET_B = 14 000) has an oral bioavailability as good as that of **17** as demonstrated by C_{max} of 59.2 μg/mL and AUC of 297.5 h μg/mL.

One plausible explanation may be that the acyl carbonyl and the amide NH can form an internal hydrogen bond. This would increase permeability[13] of these compounds across the intestinal mucosa, resulting in increased oral availability. The fact that a cyano group on the ortho position (**5**) only increased oral bioavailability marginally seems to support such argument: the nitrile group probably can increase in vivo aqueous solubility by merit of both its own polarity and its effect of making the amide more acid-like. However, being a straight triple bond, the cyano group cannot form an internal hydrogen bond, therefore, making it less permeable to membranes. Second, the acyl group is highly electron withdrawing and, therefore, makes the linker amide behave as a carboxyl group, resulting in increased aqueous solubility of the compound.

Molecular Modeling. Molecular modeling was initiated to investigate if indeed an intramolecular hydrogen bond exists in the lowest energy conformation. The first question was should the sulfonamide be treated as a charged or neutral group? Since absorption usually takes place in the intestine where pH is close to 7, and the pK_a value of the chloroisoxazolylsulfonamide hydrogen is estimated to be around 4.5, the compounds should exist to a large extent in ionized form. On the

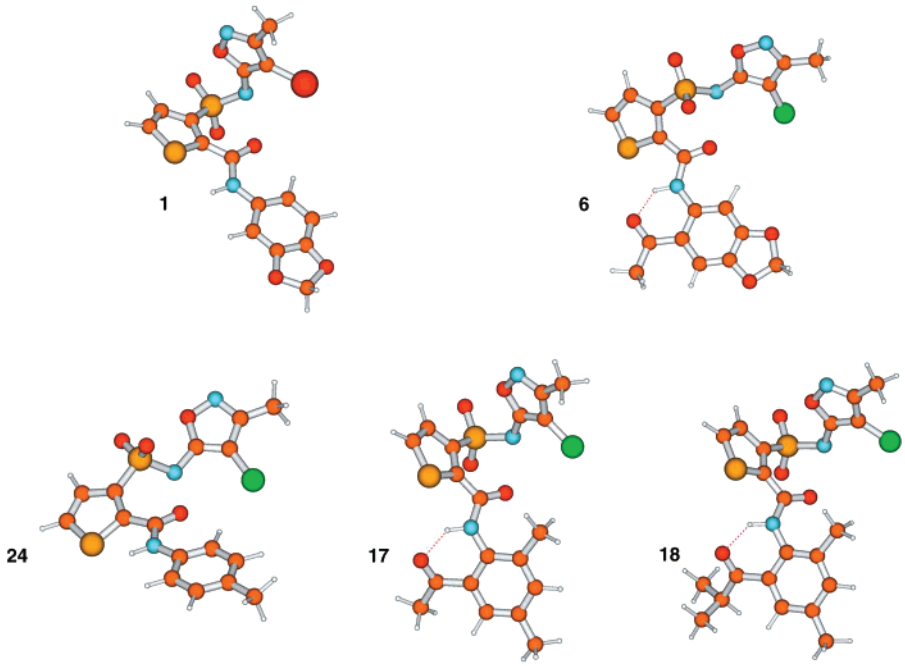


Figure 1. Lowest energy conformations at pH 7.5 for selected compounds.

Table 1. Calculated Physicochemical Properties Descriptors

entry	dynamic polar surface area (Å^2)	dynamic averaged dipolar moment (D)	SCHD[a] (e Å^2)
1	120	7.8	1.16
2	112	7.4	0.92
3	111	6.6	1.11
4	111	7.7	0.91
5	147	7.0	0.56
6	131	9.5	0.36
16	91	7.9	0.79
17	103	9.3	0.62
18	103	8.9	0.60
19	111	6.3	1.46
20	107	9.2	1.42
21	120	8.9	0.87
22	121	7.2	0.80
23	118	6.8	0.83
24	95	7.5	0.98
25	91	7.4	0.74

[a] Sum of (surface area × charge of H-bond donor atom)/number of H-bond donor atoms.

other hand, passively transported compounds are suspected to cross the membrane more efficiently in their uncharged state. Molecular modeling of previous compounds of this class utilizing the charged or neutral form of sulfonamides yielded essentially identical bioactive conformations.[14] Therefore, for this study, the lowest energy conformation of the ionized form of compounds **1**, **6**, **17**, **18**, and **24** were selected and are shown in Figure 1. A Conformation search using SYBYL 6.5 package[15] with Merck molecular force field (MMFF)[16] confirmed that an intramolecular hydrogen bond forms between the acyl carbonyl and the NH of the linker amide in compounds **6**, **17**, and **18**. This H-bond may play an important role in increasing permeability as desolvation of the polar bonds in a molecule is a determinant of permeability.[13] Due to the strong electron withdrawing effects of the acyl group, the linker amide NH group becomes more polar. The calculated MOPAC[17] electrostatic potential (ESP) charges for N and H atoms change from −0.21 and 0.20 for compound **1** to −0.42 and 0.36 for compound **6**, and from −0.20 and 0.19 for compound **24**, to −0.31 and 0.29 for compound **17**. We also calculated several physicochemical property descriptors (Table 1) for compounds **1**−**6** and **16**−**25**, such as dynamic polar surface area (PSA_d), dynamic averaged dipolar moment (D_d), and surface area multiplying charge per hydrogen bond donor atom (SCHD). These descriptors have been proven useful in predicting human intestinal absorption of drug compounds.[18] For our series of compounds, there is no correlation between PSA_d or D_d and oral bioavailability. This is probably due to the fact that our compounds exist as charged species at physiological pH, while PSA_d and D_d are more suited for neutral species. On the other hand, some trend can be observed between SCHD values and measured oral bioavailability F or C_{max}: With decreasing of SCHD, F or C_{max} increases. For example, the SCHD values are 1.16, 0.56, and 0.36 for compounds **1**, **5**, and **6** respectively, correlating to the F values of 0%, 8%, and 27%. For compounds **24**, **16**, **25**, **17**, and **18**, the SCHD values are 0.98, 0.79, 0.74, 0.62, and 0.60, and C_{max} values are 0, 21.8, 35.9, 57.6, and 59.2 μg/mL, respectively. The same trend holds for other compound series shown in Chart 1: the SCHD value decreases from 1.16 to 0.92 and F increases from 0 to 30% for **1** and **2**, respectively. Similarly, going from compound **3** to **4**, SCHD drops from 1.11 to 0.91 while F doubles. Oral bioavailability escalates from 0 to 8% to 27% for **1**, **5**, and **6**, whereas SCHD decreases from 1.16 to 0.56 to 0.36. Since SCHD reflects the capacity of forming an external hydrogen bond for a compound, a smaller SCHD value might be indicative of better permeability or absorption and, in turn, better oral bioavailability.

Conclusion

In summary, we have established the *o*-acyl effect on oral bioavailability of a specific series of anilides. Exploitation of such an effect conferred good oral bioavailability to our second-generation endothelin antagonists which are generally >10-fold more potent and

>10-fold more selective for ET_A than sitaxsentan. This effort expeditiously generated our clinical development candidate **17** (TBC3214) with an IC_{50} for ET_A of 40 pM (equipotent to ET-1), selectivity for ET_A vs ET_B of 442 000-fold, serum half-life of >4 h, and oral bioavailability of 25−70% depending on the species.

It would be interesting to investigate if other acyl groups have the same effect as acetyl on oral bioavailability. Efforts are being spent to synthesize compounds with acyl or other electron withdrawing groups on the ortho position, and results will be reported in due course.

Experimental Section

General. Melting points were determined using a Fisher-Johns hot stage apparatus and are uncorrected. Proton NMR (^{1}H NMR) spectra were recorded on a JEOL 400 MHz spectrometer. Chemical shifts were reported in parts per million as δ units relative to a residual solvent as internal standard. Infrared spectra were recorded on a Bruker IFS-25 instrument as KBr pellets. Elemental analyses were performed by Oneida Research Services, Inc. (Whitesboro, NY) and were within 0.4% of the theoretical values unless otherwise indicated. Anhydrous solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI) in Sure-Seal bottles. Unless otherwise stated, reagents and chemicals were of the highest grade from commercial sources and were used without further purification. ET-1 was obtained from Clinalfa Co. (Laufelfingen, Switzerland) and ET-3 from American Peptide Co. (Sunnyvale, CA). [^{125}I]ET-1 was obtained from Amersham (Arlington Heights, IL). Flash chromatography was performed on silica gel 60 (230−400 mesh, E. Merck). Thin-layer chromatography was performed with E. Merck silica gel 60 F-254 plates (0.25 mm) and visualized with UV light, phosphomolybdic acid, or iodine vapor. Analytical HPLC was performed on a Dynamax-300A column (C18, 4.6 × 250 mm) preparative HPLC on Dynamax-60A (83-241-c) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. The detection wavelength was 254 nm.

2-Amino-3,5-dimethylacetophenone (8) and 2-Amino-3,5-dimethylisobutyrophenone (9). Compounds **8** and **9** were synthesized using a literature procedure.[12a] ^{1}H NMR ($CDCl_3$) δ for **8**: 7.41 (d, J = 0.8 Hz, 1H), 7.05 (d, J = 0.8 Hz, 1H), 6.41 (br s, 2H), 2.58 (s, 3H), 2.24 (s, 3H), 2.15 (s, 3H). ^{1}H NMR ($CDCl_3$) δ for **9**: 7.48 (br s, 1H), 7.06 (br s, 1H), 6.71 (br s, 2H), 3.62 (m, 1H), 2.25 (s, 3H), 2.18 (s, 3H), 1.20 (d, J = 6.6 Hz, 6H).

1-(2-Amino-3,5-dimethylphenyl)ethanol (10). To a solution of **8** (200 mg, 1.22 mmol) in anhydrous THF (10 mL) under nitrogen was added borane·THF complex (1 M in THF, 3.67 mL, 3.67 mmol). The reaction was stirred at room temperature overnight before quenched with cold water and 1 N HCl. After being stirred for another 15 min, the mixture was basified with sodium carbonate and extracted with EtOAc. The organic layer was separated and dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated on rotavap to afford **10** (170 mg, ∼85% yield) as a solid: ^{1}H NMR (DMSO-d_6) δ 6.74 (d, J = 1.4 Hz, 1H), 6.67 (d, J = 1.4 Hz, 1H), 5.06 (d, J = 3.0 Hz, 1H), 4.75 (m, 1H), 4.53 (br s, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 1.33 (d, J = 1.7 Hz, 3H).

2-Ethyl-4,6-dimethylaniline (11). To a solution of **10** (170 mg, 1.04 mmol) in TFA (15 mL) was added triethylsilane (528 mg, 4.46 mmol). The mixture was heated under reflux overnight before poured into ice. After basification with sodium bicarbonate to pH 8−9, the aqueous mixture was extracted with EtOAc. The organic layer was separated and dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated on rotavap to afford **11** (126 mg, ∼80% yield) as a solid: ^{1}H NMR (DMSO-d_6) δ 6.62 (br s, 2H), 4.23 (br s, 2H), 2.44 (q, J = 7.4 Hz, 2H), 2.12 (s, 3H), 2.06 (s, 3H), 1.13 (t, J = 7.4 Hz, 3H).

Target compounds **16**−**18** were synthesized using a literature procedure.[5]

***N*-(2-Ethyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide (16) Sodium Salt.** The sodium salt of compound **16** is a yellowish solid: mp 152−154 °C; ^{1}H NMR (DMSO-d_6) δ 10.96 (br s, 1H), 7.68 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 6.91 (br s, 2H), 2.51 (q, J = 7.7 Hz, 2H), 2.28 (s, 3H), 2.13 (s, 3H), 1.99 (s, 3H), 1.05 (t, J = 7.7 Hz, 3H); IR (KBr pellet): 1637, 1604, 1546, 1494 cm^{-1}. Anal. Calcd for $C_{19}H_{19}ClN_3NaO_4S_2\cdot0.5H_2O$: C, 47.06; H, 4.16; N, 8.66. Found: C, 46.93; H, 4.36; N, 8.27.

***N*-(2-Acetyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide (17).** Compound **17** is a yellowish solid: mp 58−62 °C; ^{1}H NMR ($CDCl_3$) δ 10.44 (br s, 1H), 9.80 (br s, 1H), 7.52 (m, 3H), 7.32 (s, 1H), 2.64 (s, 3H), 2.40 (s, 3H), 2.32 (s, 3H), 2.22 (s, 3H); IR (KBr pellet): 3324, 3112, 1642, 1516, 1494 cm^{-1}. Anal. Calcd for the sodium salt $C_{19}H_{17}ClN_3NaO_5S_2\cdot2.2H_2O$: C, 43.09; H, 4.07; N, 7.93. Found: C, 42.94; H, 3.75; N, 7.71.

***N*-(2-Isobutyryl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide (18) Sodium Salt.** The sodium salt of compound **18** is a yellowish solid: mp 155−158 °C; ^{1}H NMR (DMSO-d_6) δ 11.36 (br s, 1H), 7.72 (d, J = 5.2 Hz, 1H), 7.42 (d, J = 5.2 Hz, 1H), 7.24 (s, 1H), 7.08 (s, 1H), 3.25 (m, 1H), 2.31 (br s, 6H), 2.00 (s, 3H), 0.89 (d, J = 7.0 Hz, 6H); IR (KBr pellet): 3478, 3232, 1685, 1598, 1537, 1291 cm^{-1}. Anal. Calcd for $C_{21}H_{21}ClN_3NaO_5S_2\cdot H_2O\cdot0.1$EtOAc: C, 47.18; H, 4.40; N, 7.71. Found: C, 47.33; H, 4.63; N, 7.34.

Pharmacokinetic Assays. Adult Harlen Sprague Dawley rats (∼200 mg) were used. The compound at a dose of 50 mg/kg was administered by gavage needle in 0.5% high viscosity carboxymethyl cellulose (5 mL/kg). Serial blood samples (200 μL) were taken at selected time points from the tail vein using heparin coated microhematocrit tubes. Red blood cells were removed immediately by centrifugation, and the plasma was stored at −80 °C until analyzed by HPLC following acetonitrile precipitation of the plasma proteins.

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