Discovery, Modeling, and Human Pharmacokinetics of *N*-(2-Acetyl-4,6-dimethylphenyl)-3-(3,4-dimethylisoxazol-5-ylsulfamoyl)-thiophene-2-carboxamide (TBC3711), a Second Generation, ET_A Selective, and Orally Bioavailable Endothelin Antagonist[1]

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Sitaxsentan (**1**) (Wu et al. *J. Med. Chem.* **1997**, *40*, 1690) is our first endothelin antagonist being evaluated in clinical trials. It has demonstrated biological effects in an acute hemodynamic study in CHF (Givertz et al. *Circulation* **2000**, *101*, 2922), an open-label 20-patient pulmonary hypertension trial (Barst et al. *Chest* **2002**, *121*, 1860−1868), and a 31-patient trial in essential hypertension (Calhoun et al. AHA Scientific Sessions 2000). In a phase 2b/3 pulmonary arterial hypertension trial, once a day treatment of 100 mg of sitaxsentan statistically significantly improved 6-min walk distance and NYHA class at 12 weeks (Barst et al. *Am. J. Respir. Crit. Care Med.* **2004**, *169*, 441). We have since reported on our efforts in generating follow-up compounds (Wu et al. *J. Med. Chem.* **1999**, *42*, 4485) and recently communicated that an ortho acyl group on the anilino ring enhanced oral absorption in this category of compounds (Wu et al. *J. Med. Chem.* **2001**, *44*, 1211). Here we report an expansion of this work by substituting a variety of electron-withdrawing groups at the ortho position and evaluating their effects on oral bioavailability as well as structure−activity relationships. As a result, TBC3711 (**7z**) was identified as our second endothelin antagonist to enter the clinic due to its good oral bioavailability (∼100%) in rats, high potency (ET_A IC_{50} = 0.08 nM), and optimal ET_A/ET_B selectivity (441 000-fold). Compound **7z** has completed phase-I clinical development and was well tolerated with desirable pharmacokinetics in humans ($t_{1/2}$ = 6−7 h, oral availability > 80%).

Introduction

The 21 amino acid peptide endothelin-1 (ET-1) is a potent and long acting vasoconstrictive and mitogenic peptide.[9] Its structure features two disulfide bridges and a carboxyl terminal side chain conserved across ET isoforms,[10] and ET-1 has the same amino acid sequence across mammalian species. ET-1 has been implicated in a variety of diseases including chronic heart failure, hypertension, atherosclerosis, pulmonary hypertension, and coronary artery disease.[11] ET-1 exerts its biological effects via binding to its two G-protein-coupled receptors: ET_A, selective for ET-1, ET-2 over ET-3, and the nonselective ET_B.[9,10] ET_A and ET_B receptors are expressed on smooth muscle cells and mediate the vasoconstrictive effects of ET-1.[9,10] ET_B receptors are found also on endothelial cells where they clear ET-1 and mediate vasodilation through the release of NO and thromboxane.[12−14]

The potentially wide application of endothelin antagonists has spurred intensive medicinal research and a number of chemical entities are being evaluated in the clinic. The first oral dual ET_A/ET_B antagonist bosentan has been approved for pulmonary arterial hypertension. Although ET_B receptor disruption has been shown to cause hypertension,[15] both selective ET_A antagonist and dual antagonists are useful. Our laboratories have been involved in researching selective ET_A antagonists, and compound **1**[2] (sitaxsentan, Thelin) is our first such compound identified for clinical development. Compound **1** is an orally active ET_A selective endothelin antagonist that attenuates pulmonary vascular hypertension and cardiac hypertrophy[16] and prevents matrix metalloproteinase activation late post MI in rats.[17] It confers hemodynamic changes including reductions in pulmonary artery pressures and pulmonary vascular resistance in patients with congestive heart failure[3] and pulmonary arterial hypertension.[4] When administered orally at 2 mg/kg to humans (N = 4), sitaxsentan has an $AUC_{0-\infty}$ of 33.02 ± 7.08 h·μg/mL with C_{max} of 16.43 ± 5.86 μg/mL. The elimination half-life is 6.46 ± 1.52 h with a T_{max} of 1.75 ± 0.96 h.[18] In a phase 2b/3 STRIDE trial in 178 pulmonary arterial hypertension patients, once a day treatment of 100 mg and 300 mg of sitaxsentan statistically significantly improved 6-min walk distance and NYHA class at 12 weeks.[6,19] Both doses resulted in significant improvements in hemodynamics in these NYHA class II−IV patients.[20] The 300 mg dose of sitanxsentan also showed statistically significant improvement in change in percent of predicted peak VO_2 at 12 weeks.[6,19]

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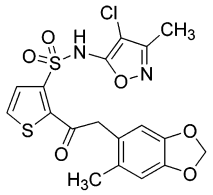
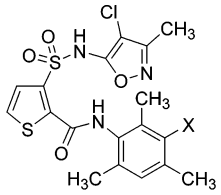
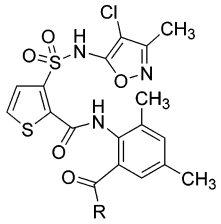
Chart 1. Maximal Plasma Concentration of Selected ET Antagonists in Rats (po 50 mg/kg)



	C_{max} (μg/mL)
1	122.8 ± 61.2 (5)



2a X = H	35.9 ± 24.9 (7)
2b X = CH_2OH	2.4 ± 0.9 (2)
2c X = CH_2OAc	3.8 ± 1.4 (2)
2d X = OCH_3	4.0 ± 1.4 (3)
2e X = OCH_2(cyclo-Pr)	6.1 ± 6.6 (4)
2f X = $N(CH_3)_2$	3.2 ± 0.1 (2)
2g X = $NHSO_2CH_3$	2.8 ± 0.4 (3)
2h X = SO_2NH_2	2.9 ± 1.8 (3)
2i X = CN	17.3 ± 15.8 (3)
2j X = CH_2CN	42.0 ± 55.1 (6)
2k X = OH	48.2 ± 61.6 (12)



3a R = CH_3	57.6 ± 13.5 (15)
3b R = *i*-Pr	59.2 ± 15.1 (3)

Efforts continued in our laboratories to generate a second clinical candidate that is more potent and more ET_A selective than **1** while maintaining the oral pharmacokinetic profile of **1**. We have reported progress[7] toward these goals by replacing the benzodioxolylacetyl group in **1** with a substituted mesitylcarboxamide, keeping the rest of the molecule constant. Within this scaffold the three methyl groups collectively are important for increasing potency of ∼10-fold over **1**. An additional 3-substitutent improves selectivity for ET_A receptors on average 10-fold, exerting minimal effect on potency. Accordingly, the strategy was to manipulate the physicochemical properties of this series of compounds by varying the functionality at the 3-position to achieve optimal oral pharmacokinectics.

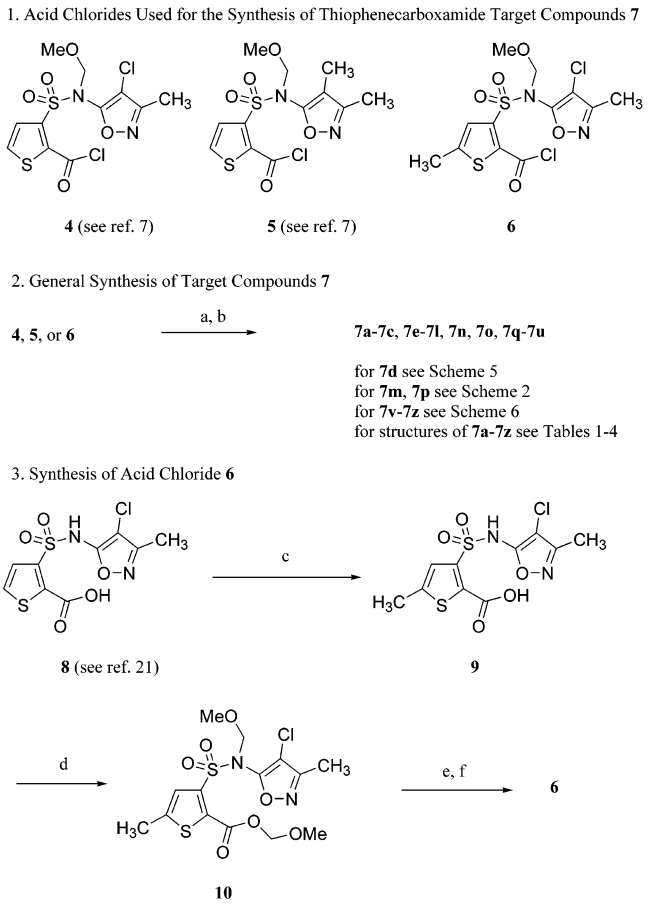
The maximal plasma concentrations in rats following oral administration of 50 mg/kg of selected compounds (**1**, **2a**−**k**, and **3a**,**b**) are listed in Chart 1. The parent, unsubstituted mesityl amide **2a** had a reasonable C_{max} of 35.9 μg/mL but to increase its aqueous solubility and lower its hemolytic activity, a polar group was required in the amide moiety of the molecule. Therefore, a range of groups with varying degree of polarity were screened at the 3-postion only to give ∼10-fold reduction in C_{max} value: Benzyl alcohol **2b** had a C_{max} value of 2.4 μg/mL while less polar benzyl acetate **2c**, methyl ether **2d**, and cyclopropylmethyl ether **2e** afforded C_{max} of 3.8, 4.0, and 6.1 μg/mL, respectively. The basic dimethylamino compound **2f** was also poor with C_{max} of 3.2 μg/mL whereas the sulfonamides **2g** and **2h** provided similarly unsatisfactory values of 2.8 and 2.9 μg/mL, respectively. The exceptions were cyanide **2i** (17.3 μg/mL), benzyl cyanide **2j** (42.0 μg/mL), and phenol **2k** (48.2 μg/mL), regaining close to or surpassing the level of the parent compound **2a** (35.9 μg/mL), but still comparing unfavorably with the C_{max} value of **1** (122.8 μg/mL).

It was concluded at this point that although the mesityl amide series offered improved potency and selectivity for ET_A receptors over **1**, they probably would not match the oral properties seen in **1**. A different scaffold of aryl amide would need to be designed that could maintain the high ET_A potency, ET_A receptor selectivity of the mesityl amide series, with a polar group in the amide arm of the molecule, yet match the oral characteristics of **1**. One such amide scaffold, 2-acyl-4,6-dimethylphenyl amide, offered possibility to meet all these criteria. We have recently reported[8] two leads in this series, the methyl ketone **3a** and the isopropyl ketone **3b**. Both compounds were highly potent (ET_A IC_{50} = 0.04 and 0.12 nM for **3a** and **3b**, respectively), highly selective for ET_A receptors (442 000- and 144 000-fold), with oral C_{max} values of 57 and 59 μg/mL, respectively. The methyl ketone **3a** has oral bioavailability of 25% in rats, 42% in cats, and 70% in dogs.[8] In addition, due to the electron-withdrawing effect of the ortho acyl group, the linker amide became carboxylic acid like and therefore served as the required polar group. In this paper, we report expanded structure−activity/rat oral pharmacokinetics relationships around **3a** and the identification of our second endothelin antagonist **7z** which has concluded phase I clinical trials.

Synthetic Chemistry. The synthesis of most of the target compounds **7** consisted of (1) the stockpiling of common advanced methoxymethyl-protected sulfonamide acid chlorides **4**,[7] **5**,[7] or **6** (Scheme 1), (2) the generation of prerequisite anilines (Schemes 2−5), and (3) the coupling of an aniline with **4**, **5**, or **6** and a subsequent cleavage of the methoxymethyl group to reveal the sulfonamide (Scheme 1). The coupling of electronically deficient anilines with these acid chlorides was accomplished using either 2 equiv of an aniline or, when the aniline was precious, 1 equiv of the aniline plus 1 equiv of 4-dimethylaminobenzonitrile to scavenge in situ generated hydrogen chloride. Heating with methanolic hydrochloric acid then liberated the sulfonamide group, the protection of the sulfonamide group being necessitated by its interference with acid chloride formation and the coupling. The 5-methylthiophene acid chloride **6** was obtained by saponification of ester **10** followed by treatment with oxalyl chloride. The bis-(methoxymethyl) compound **10** was in turn generated by a sequence of lithiating thiophenecarboxylic acid **8**,[21] immediate quenching of the resultant trianion with iodomethane, and a double alkylation of **9**[22] with methoxymethyl bromide.

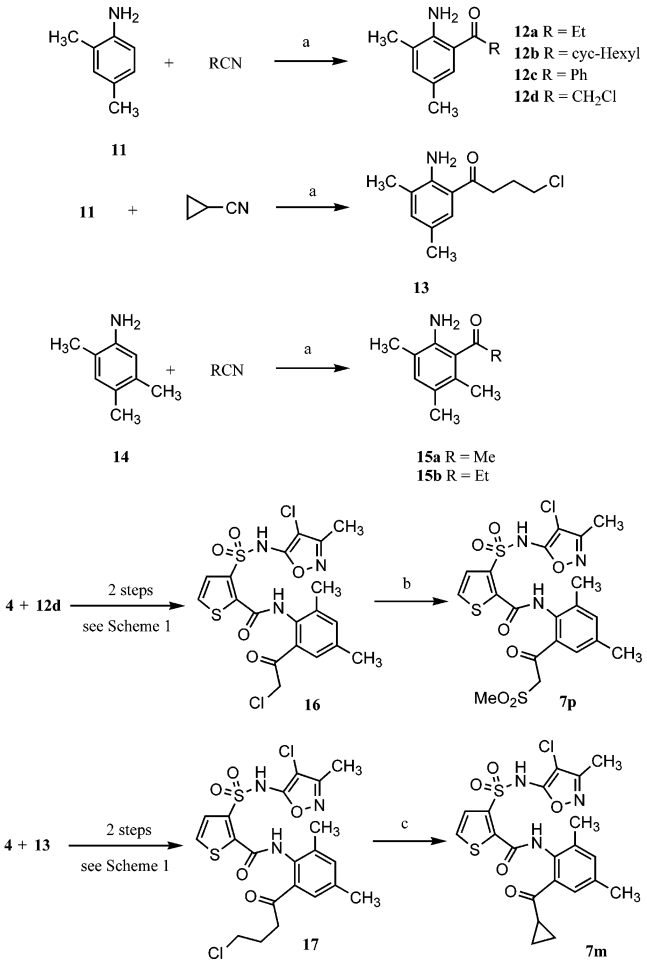
The synthesis of *o*-acylanilines is depicted in Scheme 2. Boron trichloride mediated Friedel−Crafts acylation reactions[23,24] between anilines **11** or **14** with alkyl cyanides were uneventful to furnish required *o*-acyl anilines **12a**−**d**, **15a**, and **15b**, respectively. However, a similar attempt using cyclopropylnitrile gave the ring-opened 4′-chlorobutyronylaniline **13** as the sole product. Fortunately, the cyclopropyl ring could be regenerated under strong basic conditions[25] to yield target compound **7m** from **17**. Target compound sulfone **7p** was accessed via a S_N2 displacement of the chloride in **16** with sodium

Scheme 1. Outline of General Synthesis[a]



[a] Reagents: (a) aniline/THF with or without 4-dimethylaminobenzonitirle; (b) MeOH/concentrated HCl (2:1), 70 °C 2 h; (c) ^{n}BuLi/THF/−78 °C/MeI; (d) Hunig base/THF/bromomethyl methyl ether; (e) 1N NaOH; (f) oxalyl chloride.

Scheme 2. Synthesis of 2-Acylanilines and **7m**, **7p**[a]



[a] Reagents: (a) BCl_3/RCN/1,2-dichloroethane, HCl/H_2O/MeOH; (b) $MeSO_2Na$/DMF/rt; (c) KOH/MeOH.

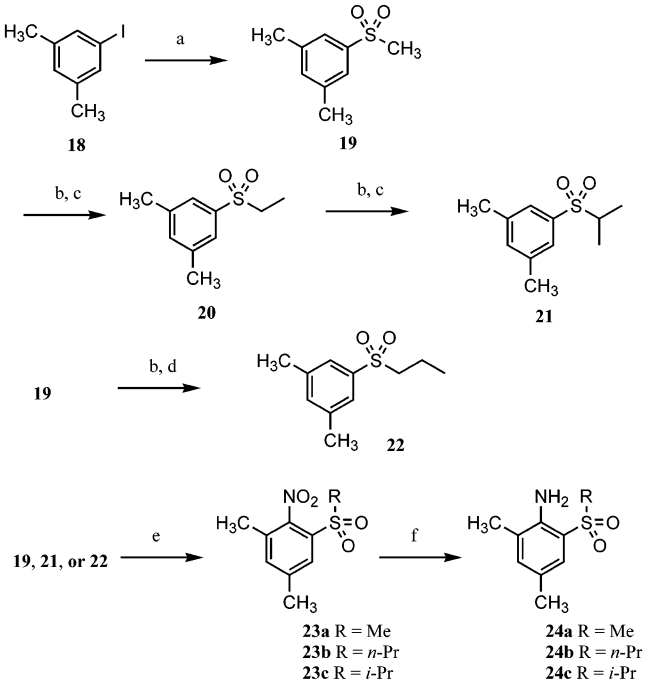
salt of methanesulfinic acid. The precursor compounds **16** and **17** were generated using the two-step protocol shown in Scheme 1 with acid chloride **4** and anilines **12d**, **13**, respectively.

Scheme 3 delineates the synthesis of ortho-sulfonylanilines **24a**−**c**. A cuprous iodide-mediated coupling[26] of 5-iodo-*m*-xylene with methanesulfinic acid, sodium salt afforded aryl sulfone **19**, a common intermediate for all three sulfonylanilines. Thus, lithiation of **19** followed by quenching with iodomethane (two iterations) or iodoethane provided isopropyl sulfone **21**, or *n*-propyl sulfone **22**, respectively. Nitration of **19**, **21**, or **22** generated only the desired regioisomers **23a**−**c**, which upon reduction with zinc were converted to the ortho-sulfonylanilines **24a**−**c**, respectively.

The synthesis of *o*-oxazolylaniline **30** required introduction of the oxazole ring first, followed by amination of the benzene ring, whereas a reversal of this sequence was necessitated to access *p*-oxazolylaniline **38** (Scheme 4). The installation of oxazolyl group consisted three steps: (1) coupling of acid chlorides derived from thionyl choride treatment of benzoic acids **25** or **32** with ethanolamine; (2) ring forming condensation of the resultant hydroxyethylamides **26** or **34**, with thionyl chloride[27] or PPA, respectively; and (3) aromatization of oxazolines **27** or **36** to the corresponding oxazoles **28** or **37**, respectively, using nickel dioxide.[28] The amination of benzenes was accomplished via standard nitration of oxazolylbenzene **28** and reduction of the nitro group of **29** or **37** with tin chloride.[29] The oxidation of a benzylic methyl group in nitromesitylene (**31**) proceeded to give both isomers **32** and **33**, the separation of which was strategically postponed until when the formation of the oxazoline ring was completed.

Some additional anilines with an ortho cyano, amido, or phenyl substituent were prepared as demonstrated in Scheme 5. Cuprous cyanide treatment[30] of bromoanilines **40a**, **40b**, provided the desired cyanoanilines **41a** and **41b**, respectively, whereas a Suzuki reaction[31] of **40a** with phenylboronic acid furnished aminobiphenyl **42**. The bromoanilines **40a** and **40b** were readily accessible via bromination of anilines **39a** and **39b**. A standard sequence of nitration, EDCI/HOBt-mediated coupling, methylation, and tin chloride reduction afforded neopentylamide **47**, while a CDI-mediated coupling reaction of anthranilic acid **48** and dimethylamine furnished dimethyl anthranilic amide **49**. For the synthesis of the final target compounds using cyanoanilines **41a** and **41b**, the acidic conditions employed to unmask the sulfonamide group proved harsh enough to hydrolyze the cyano group to a primary amide. An interesting steric effect on the nitrile hydrolysis was observed. If the cyano group was flanked with only one amino group (**41a**), it was completely converted to the corresponding primary amide (**7d**); however, the more hindered cyano group with an

Scheme 3. Synthesis of 2-Sulfonylanilines[a]



[a] Reagents: (a) $MeSO_2Na$/CuI/DMF/100 °C; (b) ^nBuLi/THF; (c) MeI; (d) EtI; (e) KNO_3/H_2SO_4/0 °C; (f) Zn/NH_4Cl/MeOH/H_2O.

additional ortho methyl (**41b**) partially survived the MOM deprotection to give a 1:1 mixture of nitrile **7s** and amide **43**.

The derivatization of methyl ketone **3a** is presented in Scheme 6. The keto group was amenable to standard ketone chemistry such as reduction to alcohol **7v** and oxime formation to give **7w**, methyloxime **7x**. The methylation of the 5-position of thiophene in **3a** required a lithiation/immediate quenching with iodomethane step[22] at the very beginning of the synthesis (Scheme 1, part 3). Similarly, a chloro to methyl switch at the 4-position of the isoxazole ring in **3a** also necessitated the use of dimethylisoxazole instead of chloromethylisoxazole at the outset of the synthetic scheme. Standard coupling of acid chlorides **6** and **5** with the aminoacetophenone **50** followed by MOM group cleavage furnished **3a** derivatives **7y** and **7z**.

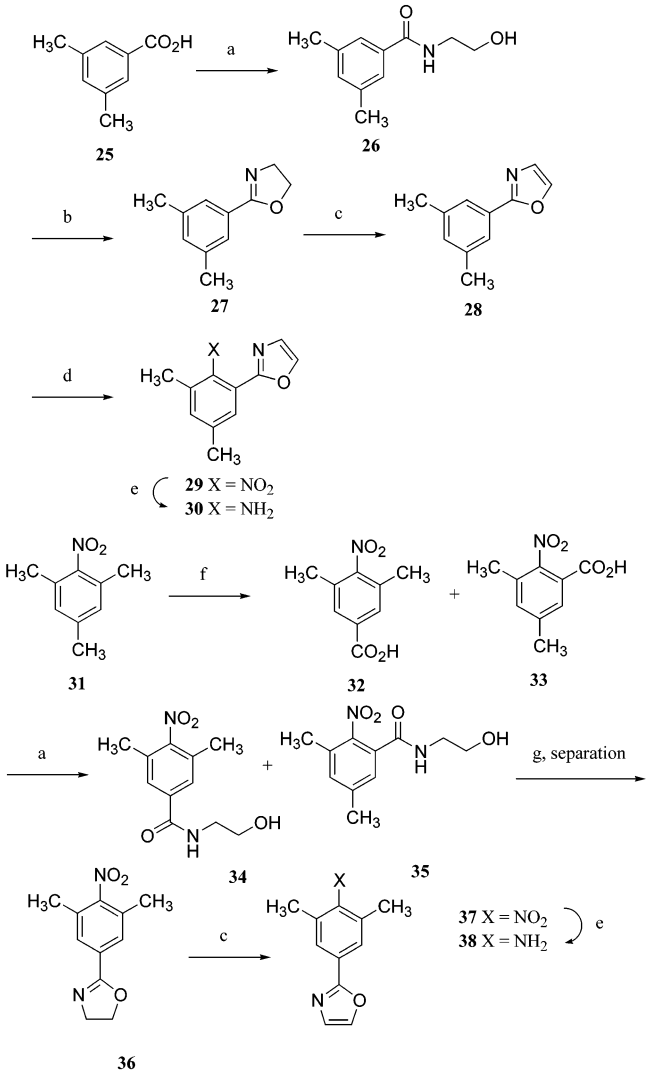
The synthetic methods, yields of last steps, melting points, and the molecular formulas of all target compounds (**7a**−**z**) are listed in Table 5

Results and Discussion

Sitaxsentan (**1**) binds competitively to human ET_A and ET_B receptors with an IC_{50} of 1.4 nM and 9.8 μM, respectively.[2] Our goals have been to generate second generation endothelin antagonists with significantly increased ET_A potency and ET_A/ET_B selectivity versus **1**. The inhibition of endothelin binding to ET_A and ET_B receptors was measured using ^{125}I-labeled ET-1 competition assays. Selectivity for ET_A over ET_B was expressed as the ratio of ET_B IC_{50} value over that of ET_A. ET_A binding potency and ET_A/ET_B selectivity are presented in Tables 1−4.

Selected compounds from Tables 1−4 with high ET_A potency and selectivity were administered orally at 50 mg/kg to rats. Area under the curve (AUC), number of rats, maximal plasma concentration (C_{max}), and plasma

Scheme 4. Synthesis of Oxazolylanilines[a]

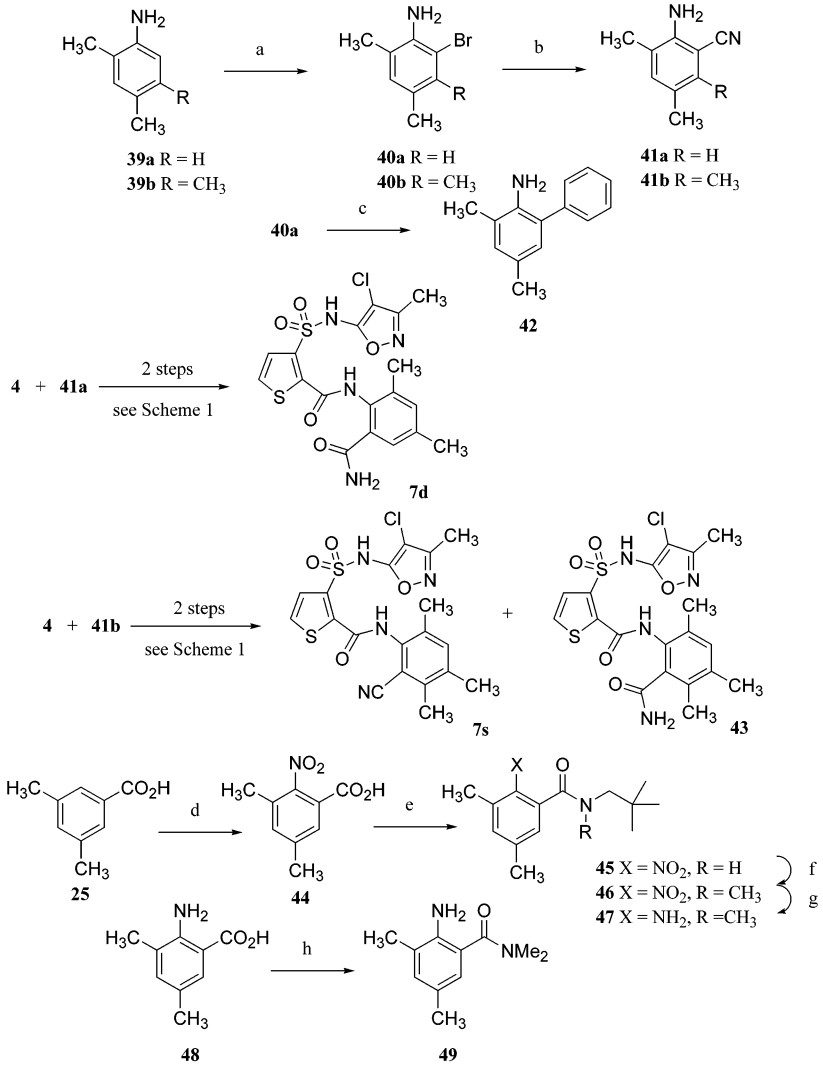


[a] Reagents: (a) $SOCl_2$/ethanolamine; (b) $SOCl_2$; (c) NiO_2/PhH; (d) KNO_3/H_2SO_4; (e) $SnCl_2$/HCl;(f) CrO_3/HOAc/H_2SO_4; (g) PPA.

half-life ($t_{1/2}$) are reported in Table 6. Those compounds from Table 6 with a decent oral pharmacokinetics profile were subjected to a full rat pharmacokinetic study to measure their oral bioavailability. Their in vivo effects were then evaluated in an acute hypoxia-induced pulmonary hypertension model in rats.[16] Oral bioavailability and the oral dose to effect 50% inhibition of mean pulmonary arterial pressure (MPAP) are shown in Table 7.

The first aryl system studied (Table 1) was 2,4-xylenyl with a different 6-position substituent, mostly electron-withdrawing groups. Comparing to **1**, a methyl group (**2a**) at 6-position caused a 10-fold increase of ET_A binding affinity with minimal effect on receptor subtype selectivity. A methyl to chloro switch (**2a** to **7a**) had a modest effect on potency (0.15 to 0.09 nM), whereas selectivity was significantly enhanced (7000 to 166 000-fold). A phenyl group (**7b**) reduced both potency (1.47 nM) and selectivity (1900-fold). Further erosion of ET_A potency and selectivity was caused by a carboxyl group (**7c**) with IC_{50} of 2.62 nM and selectivity of only 600-fold. The primary amide **7d** exhibited similar activity (0.14 nM) and selectivity profiles to **2a**, whereas the *N*,*N*-dimethylamide **7e** was more potent (0.07 nM) and

Scheme 5. Synthesis of 2-Cyano-, Amido-, and Phenylanilines[a]



[a] Reagents: (a) NBS/DCM; (b) CuCN/DMF/heat; (c) $PhB(OH)_3/Pd(PPh_3)_4/Na_2CO_3/EtOH$; (d) HNO_3/H_2SO_4; (e) EDCI/HOBt/neopentylamine; (f) NaH/MeI; (g) $SnCl_2/HCl$; (h) CDI/THF/dimethylamine.

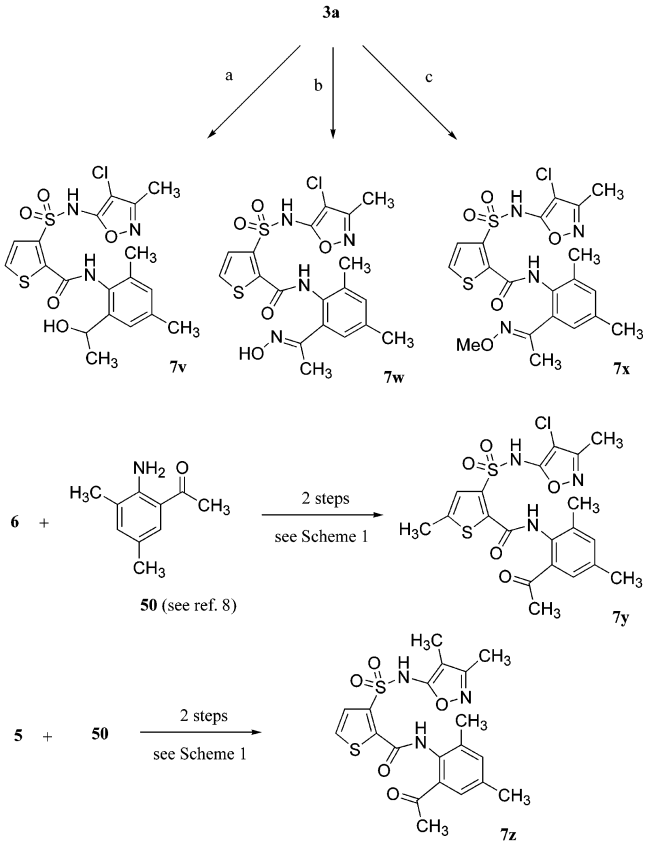
more selective for ET_A receptors (12 300-fold) than **2a**. Replacing one of the two methyl groups on amide nitrogen in **7e** with neopentyl resulted in a 10-fold loss of potency (0.07 to 0.68 nM). The amide groups seemed to have a size dependent effect on selectivity going from 8100 to 12 300 and to 15 900-fold for unsubstituted- **7d**, dimethyl- **7e**, and *N*-methyl-*N*-neopentylamide **7f**, respectively. Sulfonyl groups (**7g**−**i**) at the 6-position generally increased ET_A potency by 2−5-fold comparing with **2a** without much size effect. On the other hand, the small methyl sulfone **7g** improved selectivity significantly to 325 000-fold whereas a longer propyl (**7h**) and a branched isopropyl (**7i**) sulfone had little effect on selectivity. An aromatic 2-oxazolyl group in **7j** improved potency ~18-fold vs a phenyl group in **7b**, with much higher receptor subtype selectivity. However, a positional switch of the *o*-oxazole and the *p*-methyl group in **7j** completely eliminated those enhancements (**7k**).

To summerize for this series, small to mid-sized electron-withdrawing groups such as halogen, amide, sulfonyl, or oxazole are more effective in potency enhancement than phenyl and carboxyl, affording compounds with ET_A potency similar to that of methyl ketone **3a**.

Five analogues in this were selected for oral pharmacokinetics (PK) evaluation (50 mg/kg) in rats (Table 6). The chloro compound **7a** had an AUC value of 172.3 h·μg/mL and a $t_{1/2}$ of 2.6 h, with an acceptable C_{max} of 47.5 μg/mL which was better than the corresponding methyl compound **2a** (35.9 μg/mL) and comparable to the methyl ketone **3a** (57.6 μg/mL). Both the dimethylamide **7e** and methyl sulfone **7g** had low values of AUC (27.2 and 27.8 h·μg/mL), C_{max} (10.9 and 10.3 μg/mL), and $t_{1/2}$ (1.2 and 1.7 h, respectively). A higher homologue of **7g**, *n*-propyl sulfone **7h**, gave even more discouraging results: AUC, 5.9 h·μg/mL; C_{max} 2.9 μg/mL; and $t_{1/2}$, 1.3 h. In contrast, the 2-oxazolyl anilide **7j** exhibited the best oral profile of this mini-series with AUC, C_{max}, and $t_{1/2}$ values of 362.5 h·μg/mL, 66.0 μg/mL, and 5.3 h, respectively. The C_{max} of **7j** (66.0 μg/mL) was higher than that of the methyl analogue **2a** (35.9 μg/mL) and acetophenone **3a** (57 μg/mL), but still only half of that of **1** (122.8 μg/mL) disqualifying it as a follow-up clinical candidate.

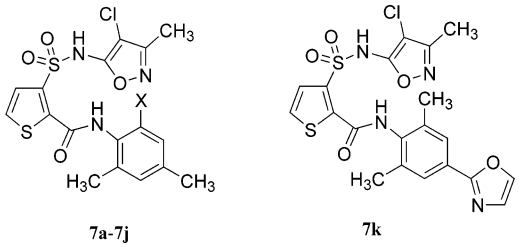
Efforts were then focused on expanding methyl ketone **3a** into a series where the aryl side of the ketone

Scheme 6. Modification of **3a**[a]



[a] Reagents: (a) $NaBH_4$; (b) $NH_2OH{\cdot}HCl$, NaOH (aq), 60 °C, 3 h; (c) $NH_2OMe{\cdot}HCl$, Na_2CO_3, EtOH, 60 °C.

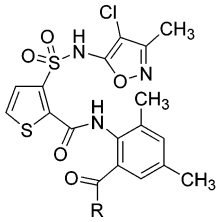
Table 1. Effect of Ortho Substituents on ET_A Affinity and Selectivity



entry	X	IC_{50} ET_A (nM) (*n*)	selectivity for ET_A
1		1.4 ± 0.5 (5)	6500
2a	CH_3	0.15 (1)	7000
7a	Cl	0.09 (1)	165600
7b	C_6H_5	1.47 ± 0 (3)	1900
7c	CO_2H	2.62 (1)	600
7d	$CONH_2$	0.14 (1)	8100
7e	$CON(CH_3)_2$	0.07 (1)	12300
7f	$CONCH_3$(neopentyl)	0.68 (1)	15900
7g	SO_2CH_3	0.03 (1)	325000
7h	SO_2-*n*-Pr	0.06 (1)	6100
7i	SO_2-*i*-Pr	0.05 (1)	8800
7j	oxazol-2-yl	0.08 (1)	118900
7k	see structure	1.27 (1)	1900
3a	$COCH_3$	0.04 ± 0 (5)	442000

carbonyl was maintained while different alkyl groups and a phenyl were screened at the other side of the keto group (Table 2). Small alkyl groups such as methyl (**3a**), ethyl (**7l**), or cyclopropyl (**7m**) afforded similarly potent compounds (ET_A IC_{50} = 0.03−0.06 nM) with comparable ET_A/ET_B selectivity (214 000−442 000-fold). A branched

Table 2. Effect of Ortho Keto Groups on ET_A Affinity and Selectivity



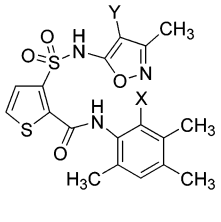
entry	R	IC_{50} ET_A (nM) (*n*)	selectivity for ET_A
3a	Me	0.04 ± 0 (5)	442000
7l	Et	0.06 (1)	214000
3b	*i*-Pr	0.11 (1)	144000
7m	*cyclo*-Pr	0.03 (1)	331400
7n	*cyclo*-Hex	1.37 (1)	13000
7o	Ph	0.39 (1)	46000
7p	$CH_2SO_2CH_3$	0.10 (1)	66500

isopropyl group (**3b**) and a much more polar methanesulfonylmethyl group (**7p**) reduced potency by approximately 2-fold vs **3a** with slightly diminished but still good selectivity (144 000- and 66 500-fold). Potency was decreased to the level of **1** with a bulkier cyclohexyl group (**7n**) with selectivity slightly better than **1** (13 000-fold). Comparing to **3a**, both binding affinity and subtype selectivity were lowered by ∼10-fold (0.39 nM and 46 000-fold, respectively) by a phenyl group (**7o**).

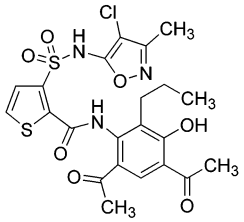
Three compounds (cyclopropyl ketone **7m**, phenyl ketone **7o**, and sulfonyl ketone **7p**) from this small series were selected for rat pharmacokinetic studies (Table 6). Comparing to methyl ketone **3a**, cyclopropyl compound **7m** had a similar C_{max} (53.4 vs 57.6 μg/mL), but a longer $t_{1/2}$ (5.4 vs 4.0 h) and hence a larger value of AUC (346.6 vs 272.4 h·μg/mL). Replacing the ketonic methyl group in **3a** with a phenyl (**7o**) lowered C_{max} (33.4 vs 57.6 μg/mL) and AUC (141.5 vs 272.4 h·μg/mL) values, with a similar $t_{1/2}$ of 3.95 h. Interestingly, the keto sulfone **7p**, with a C_{max} comparable to phenyl ketone **7o** (37.3 vs 33.4 μg/mL), had an extended $t_{1/2}$ of 12.2 h and an AUC 2.3-fold higher than **3a**. Full rat pharmacokinetics was conducted with **7p** and its oral bioavailability was determined to be 16% (Table 7) vs 25% for **3a**. In an acute hypoxia-induced pulmonary hypertension rat model, **7p** was much less efficacious than **1** or **3a** with an EC_{50} of >5 mg/kg vs 2.5, and <1 mg/kg for **1** and **3a**, respectively.

We next investigated 3,4,6-trimethylanilides (Table 3) where the 2-position was either an alkyl keto or a cyano group. The three examples of this series, methyl ketone **7q**, ethyl ketone **7r**, and cyanide **7s**, all were equipotent to the trisubstituted anilide **3a** (ET_A IC_{50} = 0.02−0.05 vs 0.04 nM for **3a**). Compounds **7r** and **7s** exhibited similar magnitude of receptor subtype selectivity to **3a** (200 000−600 000-fold) whereas **7q** had the highest selectivity (∼1 million-fold) in our thiophene sulfonamide series. A chloro to methyl switch at the 4-position of the isoxazole ring (**7s** to **7t**) caused a small decrease of ET_A potency without much effect on ET_A/ET_B selectivity (608 000 vs 328 000-fold). The last entry in Table 3 shows a highly functionalized tetrasubstituted anilide system (**7u**) where there are two acetyl groups, a hydroxyl, and a propyl group at the 2-, 4-, 5-, and 6-positions, respectively. Compound **7u** was synthesized, taking advantage of the commercial avail-

Table 3. Effect of Ortho Substituents on Trimethylphenyl System



7q-7t



7u

entry	X	Y	IC_{50} ET_A (nM) (*n*)	selectivity for ET_A
3a			0.04 ± 0 (5)	442000
7q	$COCH_3$	Cl	0.02 (1)	1180000
7r	$COCH_2CH_3$	Cl	0.03 (1)	215700
7s	CN	Cl	0.05 ± 0.02 (2)	608400
7t	CN	CH_3	0.08 (1)	328000
7u			12.36 (1)	1000

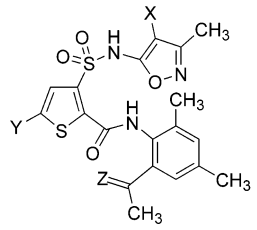
ability of the required aniline, but it was 300-fold less potent than **3a** with reduced selectivity (1000-fold).

All four potent analogues (**7q**−**t**) in this miniseries were subjected to rat oral pharmacokinetic studies and the data was shown in Table 6. The addition of a 3-position methyl on the acetophenone ring (**3a** to **7q**) had a substantial damaging effect on oral properties: C_{max} dropped from 57.6 to 10.8 μg/mL; AUC dwindled from 272.4 to 53.8 h·μg/mL; and $t_{1/2}$ was shortened from 4.0 to 1.7 h. The ethyl analogue of **7q** (**7r**) exhibited a comparably poor oral profile to **7q** with C_{max}, AUC, and $t_{1/2}$ values of 13.0 μg/mL, 24.3 h·μg/mL, and 1.5 h, respectively. Delightfully, the two cyano compounds **7s** and **7t** were endowed with good oral PK parameters comparing to **3a**. Accordingly, the operation of changing the methyl ketone group to a cyano plus adding another methyl to the 3-position (**3a** to **7s**) maintained the value of C_{max} (57.6 to 45.7 μg/mL) and AUC (272.4 to 373.4 h·μg/mL), with a slightly longer $t_{1/2}$ (4.0 to 5.1 h). The chloro to methyl switch at the 4-position of the isoxazole (**7s** to **7t**) achieved approximately 2-fold increase in C_{max} and AUC values with a similar $t_{1/2}$ of 5.3 h. However, the good in vitro potency/oral PK did not translate into good in vivo efficacy. The oral EC_{50} of **7s** and **7t** (1−5 mg/kg for both) in the acute hypoxia-induced rat pulmonary hypertension model did not compare favorably to **3a** (<1 mg/kg) or **1** (2.5 mg/kg).

Finally, the methyl ketone **3a** was subjected to a variety of synthetic modifications which included derivatization of the ketone carbonyl, methyl substitution at the thiophene ring, and chloro to methyl switch at the 4-position of the isoxazole ring (Table 4). Derivatization of the ketonic carbonyl group in **3a** did not have much effect on ET_A affinity with an IC_{50} range of 0.02−0.04 nM for racemic alcohol **7v**, oxime **7w**, and *O*-methyl oxime **7x**. In contrast, selectivity was reduced by an order of magnitude to range from 24 500 to 56 000-fold. Methyl substitution at the 5-position of the thiophene ring (**3a** to **7y**) resulted in a 5-fold decrease of potency (0.04 to 0.21 nM) and a 100-fold reduction of selectivity. Consistent with results in earlier series,[2,7,21] the chloro to methyl change at the 4-position of the isoxazole (**3a** to **7z**) reduced ET_A potency by 2-fold with little effect on selectivity.

Oral pharmacokinetic studies were conducted on **7w**-**7z** in rats and the data are shown in Table 6. Oxime

Table 4. Effect of Modifications on **3a**



entry	X	Y	Z	IC_{50} ET_A (nM) (*n*)	selectivity for ET_A
3a	Cl	H	=O	0.04 ± 0 (5)	442000
7v	Cl	H	OH, H	0.04 (1)	42000
7w	Cl	H	=NOH	0.02 (1)	24500
7x	Cl	H	$=NOCH_3$	0.04 (1)	56000
7y	Cl	CH_3	=O	0.21 ± 0.09 (2)	4500
7z	CH_3	H	=O	0.08 ± 0.02 (4)	441000

Table 5. Synthetic and Physical Data

entry	synth method	% yield[a]	mp, °C	formula[e]
7a[b]	Scheme 1	73	174−176	$C_{17}H_{14}Cl_2N_3NaO_4S_2$
7b	Scheme 1	24	178−181	$C_{23}H_{20}ClN_3O_4S_2$
7c	Scheme 1	33	171−174	$C_{18}H_{16}ClN_3O_6S_2$
7d	Scheme 5	6	40−43	$C_{18}H_{17}ClN_4O_5S_2$
7e[b]	Scheme 1	7	170−175	$C_{20}H_{21}ClN_4NaO_5S_2$
7f[b]	Scheme 1	83	174−176	$C_{24}H_{28}ClN_4NaO_5S_2$
7g[b]	Scheme 1	53	208−210	$C_{18}H_{17}ClN_3NaO_6S_3$
7h[b]	Scheme 1	26	152−155	$C_{20}H_{21}ClN_3NaO_6S_3$
7i[b]	Scheme 1	39	190−192	$C_{20}H_{21}ClN_3NaO_6S_3$
7j	Scheme 1	67	176−178	$C_{20}H_{17}ClN_4O_5S_2$
7k[b]	Scheme 1	31	205−207	$C_{20}H_{16}ClN_4NaO_5S_2$
7l[b]	Scheme 1	76	111−120	$C_{20}H_{19}ClN_3NaO_5S_2$
7m[b]	Scheme 2	36[c]	154−162	$C_{21}H_{19}ClN_3NaO_5S_2$
7n[b]	Scheme 1	44	162−166	$C_{24}H_{25}ClN_3NaO_5S_2$
7o[b]	Scheme 1	61	169−174	$C_{24}H_{19}ClN_3NaO_5S_2$
7p[b]	Scheme 2	26[c]	172−175	$C_{20}H_{19}ClN_3NaO_7S_3$
7q[b]	Scheme 1	76	223−225	$C_{20}H_{19}ClN_3NaO_5S_2$
7r[b]	Scheme 1	74	166−170	$C_{21}H_{21}ClN_3NaO_5S_2$
7s[b]	Scheme 1	57	218−220	$C_{19}H_{16}ClN_4NaO_4S_2$
7t[b]	Scheme 1	31	175−180	$C_{20}H_{19}ClN_4NaO_4S_2$
7u[b]	Scheme 1	46	163−167	$C_{22}H_{21}ClN_3NaO_7S_2$
7v[b]	Scheme 6	67[d]	147−154	$C_{19}H_{19}ClN_3NaO_5S_2$
7w[b]	Scheme 6	53[d]	136−142	$C_{19}H_{18}ClN_4NaO_5S_2$
7x[b]	Scheme 6	38[d]	140−145	$C_{20}H_{20}ClN_4NaO_5S_2$
7y[b]	Scheme 6	61	158−162	$C_{20}H_{19}ClN_3NaO_5S_2$
7z[b]	Scheme 6	29	158−160	$C_{20}H_{20}N_3NaO_5S_2$

[a] Yield of the last two steps of coupling and deprotection. [b] Data are for the corresponding sodium salt. [c] Yield of the last three steps. [d] Yield of the last step. [e] Formulas are based on high-resolution MS (experimental differs from calculated <5 mDa). Purity determined by two diverse HPLC systems.

formation (**3a** to **7w**) adversely affected oral C_{max} (57.6 to 6.9 μg/mL) and AUC (272.4 to 39.46 h·μg/mL) with a slightly extended $t_{1/2}$ (4.0 to 5.2 h). The *O*-methyl oxime **7x**, designed to moderate the polarity of the oxime group in **7w**, achieved ∼2-fold increase of oral C_{max} value vs **7w**, but its $t_{1/2}$ was shortened to 1.2 h, and thus an AUC (33.5 h·μg/mL) comparable to that of **7w**. All three pharmacokinetic parameters were improved with a methyl substitution at the 5-position of thiophene ring (**3a** to **7y**): C_{max}, 57.6 to 68.9 μg/mL; AUC, 272.4 to 313.0 h·μg/mL; and $t_{1/2}$, 4.0 to 5.1 h. Compound **7y** also boasted oral availability of 45% vs 25% for **3a** in rats; however, its mediocre potency (0.19 nM) and selectivity (4500-fold) prevented it from in vivo studies and further development. The dimethylisoxazole **7z** had a very high oral C_{max} of 179.5 μg/mL which was approximately 50% higher than that of **1**, with long $t_{1/2}$ of 5.3 h and AUC of

Table 6. Rat Oral Pharmacokinetics of Endothelin Antagonists at 50 mg/kg

entry	$AUC_{0-infinite}$ (h·μg/mL)	C_{max} (μg/mL)	$t_{1/2}$ (h)
7a	172.3 ± 75.0 (4)	47.5 ± 28.1	2.6 ± 0.9
7e	27.2 ± 10.03 (4)	10.9 ± 4.14	1.19 ± 0.02
7g	28.2 ± 10.5 (4)	10.3 ± 2.1	1.7 ± 0.4
7h	5.9 ± 3.04 (4)	2.9 ± 0.92	1.3 ± 0.26
7j	362.5 ± 58 (4)	66.0 ± 9.0	5.3 ± 1.2
7m	364.6 ± 95.9 (3)	53.4 ± 5.4	5.4 ± 0.4
7o	141.46 ± 8.7 (3)	33.42 ± 14.4	3.95 ± 0.3
7p	636.0 ± 160.0 (4)	37.3 ± 12.8	12.2 ± 3.2
7q	53.8 ± 21.47 (3)	10.8 ± 2.91	1.68 ± 0.29
7r	24.3 ± 14.70 (4)	13.0 ± 12.78	1.47 ± 0.38
7s	373.4 ± 48.40 (4)	45.7 ± 15.24	5.11 ± 0.46
7t	667.1 ± 206.1 (3)	85.8 ± 16.1	5.3 ± 0.4
7w	39.46 ± 14.79 (3)	6.92 ± 2.21	5.20 ± 2.77
7x	33.5 ± 9.30 (3)	13.8 ± 3.12	1.20 ± 0.59
7y	327 ± 84.27 (6)	68.90 ± 26.88	2.7 ± 0.31
7z	1309.6 ± 80.31(4)	179.5 ± 68.0	5.3 ± 0.9

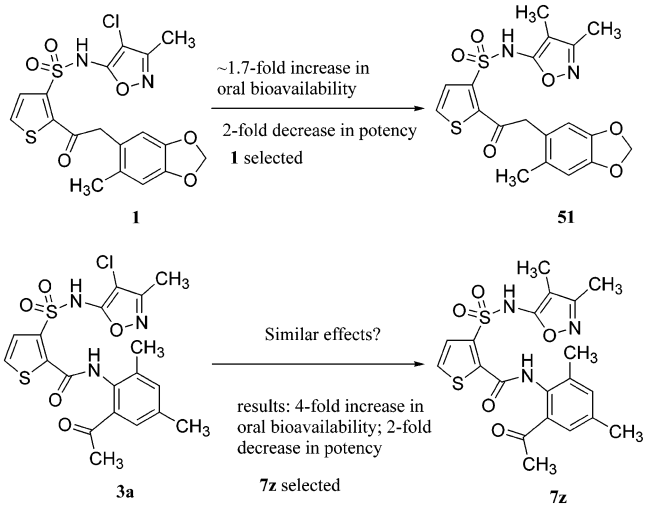
Table 7. Oral Bioavailability and in Vivo Potency of Selected Compounds in Acute Pulmonary Hypertension Rat Model

entry	oral bioavailability in rats (%)	oral dose to effect 50% inhibition of MPAP increase (mg/kg)
1	60	2.5
3a	25	<1
7p	16	>5
7s		1 to 5
7t		1 to 5
7y	49	
7z	~100	<1

1309.6 h·μg/mL. The oral availability of **7z** was determined to be ~100% in rats, and it was efficacious in the hypoxia-induced pulmonary hypertension rat model with an EC_{50} of <1 mg/kg.

It had been our strategy to conduct structure−activity relationships studies only on 4-chloroisoxazole series, and when highly promising analogues were identified, their corresponding 4-methylisoxaoles were then synthesized. This had proved to be more efficient and practical than making both 4-chloro- and 4-methylisoxazole compounds in parallel for every structural modification in other parts of the molecule. It had been established that the 4-chloroisoxazoles were generally more potent than their 4-methyl counterparts but with inferior oral availability.[2,7,21] This dichotomy played a pivotal role in the selection of our first and second generation clinical candidates (Scheme 7). Accordingly, in the ketomethyl linked benzodioxole series, the 4-chloroisoxazole **1** was evaluated against its corresponding 4-methylisoxaole **51**.[2] Compound **1** was 2.4-fold more potent than **51** (ET_A IC_{50} = 1.4 vs 3.3 nM) with comparable ET_A/ET_B selectivity (7000 vs 10 000-fold), but **51** was more orally available than **1** (~100% vs 60% in rats).[2] Given the high oral availability of both **1** and **51**, potency was the determining factor; therefore, the more potent 4-chloroisoxazole **1** was selected for clinical development. In the current amide-tethered dimethyl acetophenone series, again the 4-chloroisoxazole **3a** was judged against the corresponding 4-methyl compound **7z**. Going from 4-methyl to 4-chloroisoxazole caused a similar 2-fold potency increase (0.04 vs 0.08 nM for **3a** and **7z**, respectively) with little effect on selectivity. On the other hand, **7z** was 4-fold more orally available (~100%) than its chloro analogue **3a** (25%) in rats. Considering the very high level of potency of **3a** and **7z** (0.04 and 0.08 nM) and the more dramatic difference in oral availability (25% vs 100%), the 4-methylisoxazole **7z** was nominated to enter the clinic.

Scheme 7. 4-Methyl- vs 4-Chloroisoxazole: Selection of Clinical Candidates



Computer Modeling. The model structure of ET_A was constructed with the homology module in InsightII 2000 (Accelerys, Inc., San Diego, CA), using the crystal structure of bovine rhodopsin GPCR[32] as the template. Sequences of ET_A and bovine rhodopsin GPCR were first aligned to determine the structurally conservative regions (SCR) using the mutation score function. For the non-SCR, or loops, database searches were conducted to select their local templates. After alignment, the coordinates of the backbone atoms in the templates were copied to construct the backbone of the model protein, and side chains of templates were replaced by those in the model protein. The initial model had poor resolution and was refined with a stepwise optimization protocol. At first, all side chains, then loops, and then SCRs of the intra- and extracellular parts, and finally the whole model protein were relaxed by minimizations in sequence. The structures were further relaxed by performing a 300 ps molecular dynamics (MD) simulation. All the minimization and MD simulations were carried out using the CHARMm force field[33] without solvent. The dielectric constant was set to 4 to mimic the electrostatic environment of a GPCR.

Since the receptor structure is only a homology model and the currently available docking programs may not work very well for a peptide like ET-1, manual docking was conducted for both ET-1 and **7z**. The conformation of **7z** used in docking was the putative active conformation that gave the best matching score to the pharmacophore model derived separately from the active analogue analysis,[34] while the crystal structure (pdb1edn) was used as the docking conformation of ET-1. The following criteria were employed to achieve meaningful docking modes: (1) No steric crashes would happen between any two atoms. (2) Structure−activity relationships data[2,7,8,21] of our thiophenesulfonamide series could be well interpreted by the docked structure. (3) Three key residues (Leu17, Asp18, and Trp25) of ET-1 had favorable interactions with the receptor.

It is shown in Figure 1 that **7z** and ET-1 can indeed bind to the same binding site of ET_A composed of 10

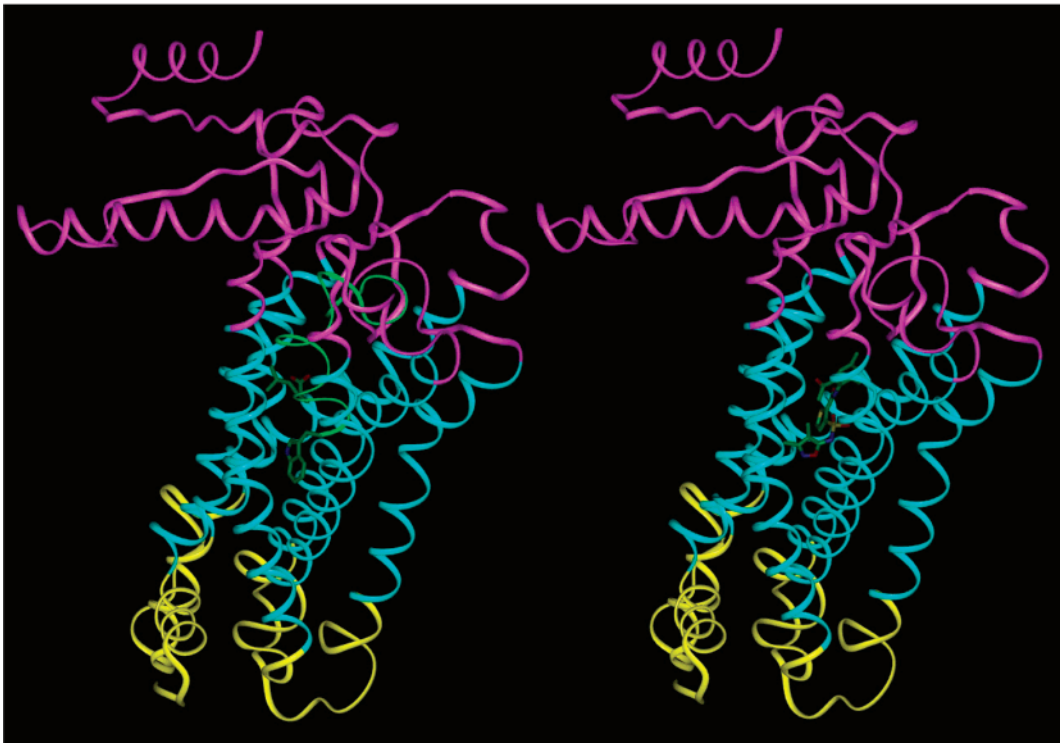


Figure 1. Comparison of ET-1/ET_A and **7z**/ET_A complexes. ET_A structure shown here is a homology model based on the crystal structure (pdb1hzx) of bovine rhodopsin GPCR. Color and render schemes: ET_A extracellular (magenta ribbon), transmembrane (cyan ribbon), and intracellular (yellow ribbon); ET-1 (green ribbon) and **7z** (colored by atom types, sticks). Side chains of key residues (Leu17, Asp18, and Trp25) of ET-1 are also shown.

key residues in four transmembrane helices (Ile86 in Helix I; Asp126, Tyr129, Leu134, and Asn137 in Helix II; Lys166 in Helix III; Ile355, Thr359, and Ser362 in Helix VII). Both ET-1 and **7z** enjoy several favorable interactions with the receptor as demonstrated in Figures 2 and 3. For instance, residue Leu17 in ET-1 or the methyl of the acetyl group in TBC3711 can fit nicely into a hydrophobic pocket formed by Ile86, Leu134, and Thr359 of the receptor. Additionally, Asp18 in ET-1 and the sulfonamide group in **7z** may interact favorably with Lys166 of ET_A. Furthermore, the indole nitrogen in Trp25 of ET-1 or the isoxazole nitrogen and oxygen of **7z** are positioned so that they form multiple hydrogen bonds with Ser362 and Asp126 of ET_A. Beside the above critical interactions with ET_A, the 3-methyl group in dimethyl isoxazole and the phenyl group of **7z** also enjoy favorable hydrophobic interactions with Val93 and Ile355 of ET_A, respectively. Finally, the thiophene ring of **7z** and the benzene ring of Tyr129 in the receptor form a favorable PI−PI interaction. A clearer view of the docking of **7z** into ET_A is furnished by Figure 4 where the active site residues of ET_A are collectively represented by a molecular surface.

A schematic summary of **7z** structural motifs interacting with ET_A active site residues is highlighted in Figure 5. Hydrophobic interactions include the two methyl groups of the isoxazole with Val93, Leu134; the two methyls on the benzene ring with Ile355 and the side chain carbons of Lys166; the methyl of the acetyl group with Ile86, Leu134; and a $\pi-\pi$ interaction of thiophene with Tyr129. Hydrogen bonds form between the following pairs of donors/acceptors: N−O of the isoxazole/Ser362, Asp126; sulfonamide NH/carbonyl of Tyr129; sulfonyl oxygens/OH of Tyr129, side chain nitrogen of Lys166, and the NH of Leu134; the acetyl oxygen/Asn137; and the amide O, N/Thr359.

To summerize, our ET_A homology model and its binding studies provided us with structural insights that helped explain the experimental binding data on ET-1, as well as the structure−activity relationships analysis of **7z** compound series. In the absence of crystal structures of ET-1/ET_A or **7z**/ET_A complexes, the modeling work tried to establish that **7z** binds to the same site of ET_A as the natural ligand ET-1 does. It may be worthwhile to confirm this binding model with site-directed mutagenesis.

Human Pharmacokinectics. A phase I, single-center, randomized, placebo-controlled, double-blind, ascending dose study was conducted using healthy male volunteers. The dose escalation design (0.5, 1, 2, 4, 8, and 12 mg/kg body weight) was carried out in six treatment blocks ($N = 5$ subjects in each). Within a treatment block, four subjects were to be randomly allocated to receive a single dose of **7z** and one subject received a single dose of placebo.

Compound **7z** exhibited linear pharmacokinetics after oral administration over the range of doses from 0.5 mg/kg to 8 mg/kg. Maximum plasma concentrations were reached at a median T_{max} of 2.5 to 3 h, and the mean terminal elimination half-life was 6.6−6.7 h (at a dose of 8 mg/kg). Figure 6 depicts the average concentration vs time profile for each dose level.

As depicted in Figure 7, **7z** exhibited linear, proportional increases in C_{max} and AUC over the range of doses from 0.5 to 8.0 mg/kg.

Single dose administration of oral doses of **7z** was tolerated well up to a dose level of 4.0 mg/kg. Therefore,

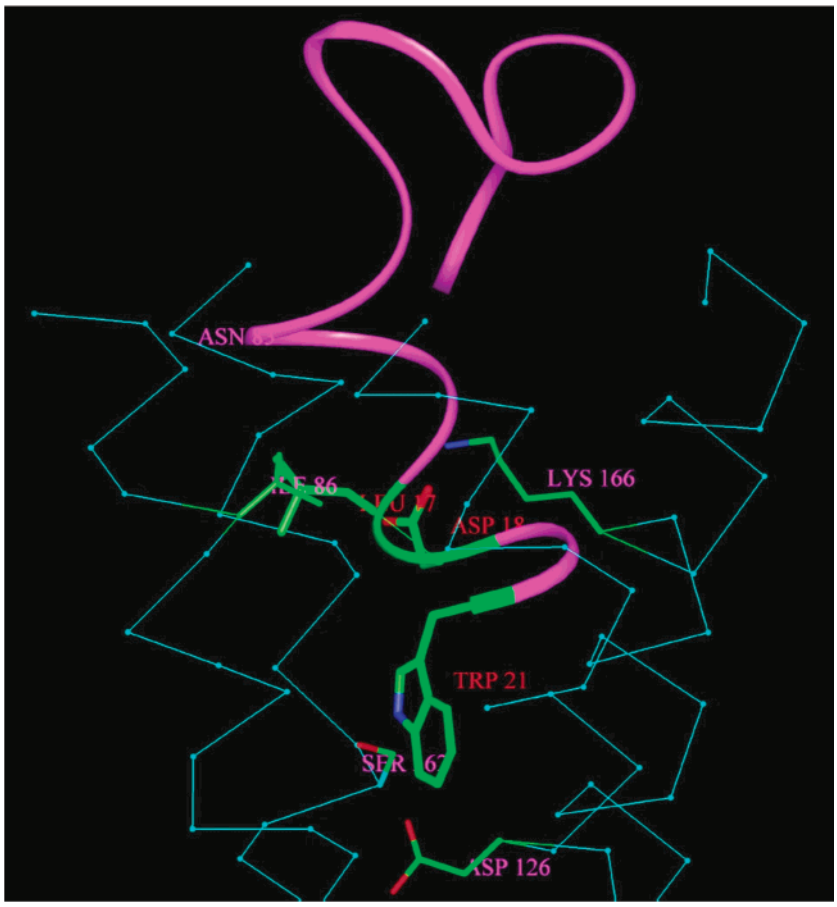


Figure 2. A detailed representation of ET-1 binding to ET_A. Key residues of ET_A and ET-1 are represented in sticks and colored by atom types (carbon: green, oxygen: red, nitrogen: blue, sulfur: yellow). Key residues are labeled in red and magenta for ET_A and ET-1, respectively.

the maximal tolerated dose in this study was considered to be 4 mg/kg.

A phase I, single-center, randomized, double-blind, placebo-controlled, ascending multiple-dose study in healthy male volunteers was also conducted. Five escalating oral dose levels of **7z** (25 mg once-per-day (QD), 25 mg twice-per-day (BID), 50 mg QD, 100 mg QD, and 200 mg QD) were administered for 7 days.

Each of the five treatment groups was exposed to one dose level with six subjects in each treatment group. Two subjects were randomized to receive placebo, and four subjects were randomized to receive active drug (**7z**) in each treatment group. Figure 8 depicts the concentration vs time profiles after the initial oral dose and after the final dose on day seven.

Maximum plasma concentrations were reached between 2 and 4 h after dosing, independent of either the dose or duration of dosing. Consistent with the half-life and the dosing frequency, there was no apparent accumulation during once-daily dosing.

Under the multiple oral dose regimen administered in this study (once daily for 7 days at doses of 25 mg, 50 mg, 100 mg, and 200 mg, and twice daily for 7 days at a dose of 25 mg), **7z** exhibited linear pharmacokinetics (Figure 9).

Compound **7z** was tolerated well across the dose ranges studied based on a 7 day multiple dose regimen.

Conclusion

ET-1 is a potent vasoconstrictor peptide implicated in serious diseases such as pulmonary hypertension, congestive heart failure, and prostate cancer. Drug discovery efforts for this well validated target continued in our laboratories to identify a follow-up clinical candidate to sitaxsentan (**1**). Structure−activity relationships along with oral pharmacokinetic studies were performed on the following three substitution patterns of the anilide, keeping the rest of the thiophene isoxazole sulfonamide scaffold unchanged. They include (1) 2,4-dimethyl-6-substituent, (2) 2,4-dimethyl-6-alkyl/aryl-carbonyl, and (3) 2,4,5-trimethyl-6-substituent. This exercise established that 2-acetyl-4,6-dimethylphenyl was the optimal group on the amide nitrogen in overall profile of in vitro/in vivo potency, receptor subtype selectivity, and oral pharmacokinetic properties. On top of this, using 3,4-dimethylisoxaole instead of 4-chloro-3-methylisoxazole on the sulfonamide nitrogen afforded our second clinical compound **7z**.

Medicinal chemistry research has been quite successful in the endothelin area, and at least 14 lead antagonists generated from various laboratories have been evaluated preclinically or clinically. Their names/codes, potency (IC_{50}, K_i), selectivity, oral availability, half-lives, and references[35−46] are listed in Table 8. Compound **7z** compares very well with other lead compounds in terms of in vitro potency, selectivity, and oral availability. It

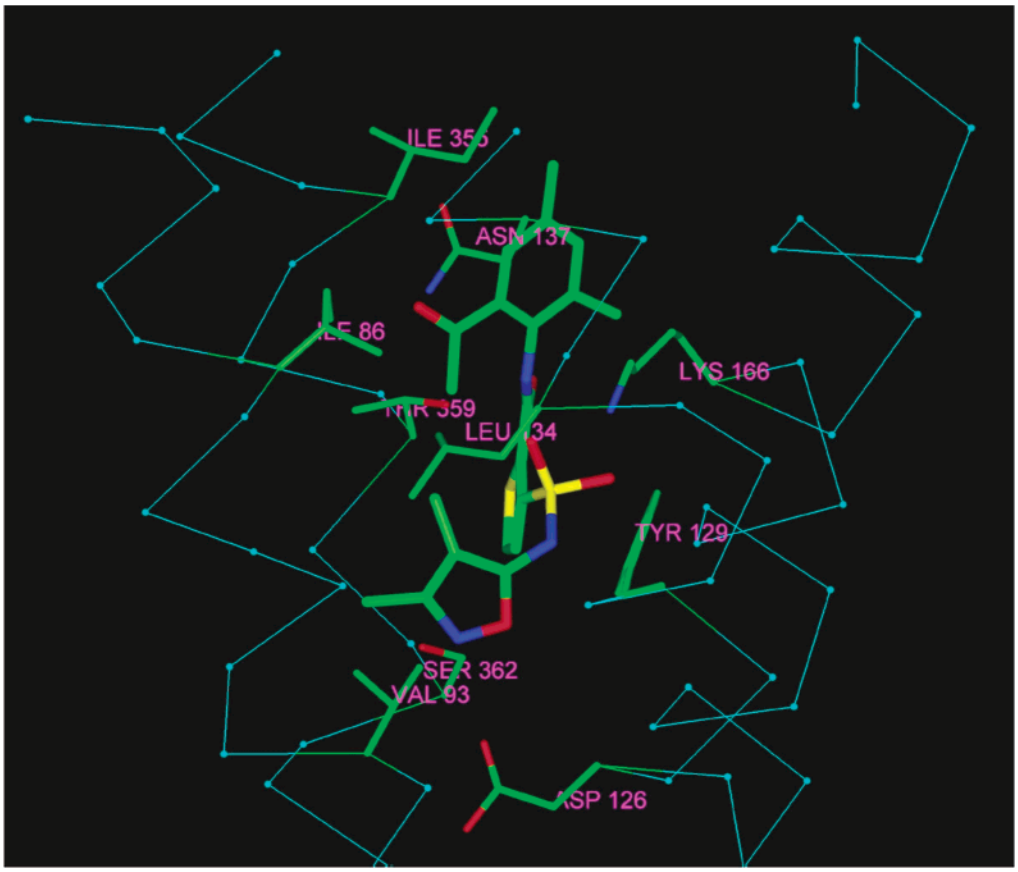


Figure 3. A detailed representation of docking **7z** to ET_A. Compound **7z** and the key residues of ET_A are represented in sticks and colored by atom types (carbon: green, oxygen: red, nitrogen: blue, sulfur: yellow).

is among the most potent (IC_{50} = 0.08 nM) vs atrasentan (0.31 nM), Cl-1034 (0.46 nM), Z1611 (0.2 nM), the peptide TAK044 (0.24 nM), and J-104132 (K_i = 0.034 nM). Selectivity of **7z** was in the lead (441000-fold) compared with other ET_A selective compounds such as BMS193884, atrasentan, Cl-1034, Z1611 and sitaxsentan (going from 1400- to 7000-fold). Except for peptides BQ123, TAK-044, and the iv drug tezosentan, most small molecule antagonists are generally >50% orally available, with **7z** being close to 100% in rats and 80% in humans.[1c]

Compound **7z** was effective in acute hypoxia-induced pulmonary hypertension in rats[47] and piglets.[48] and phase I trials of **7z** established safety and linear pharmacokinetics in humans.

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. Exact mass analysis was performed by LC/MS using a Waters 2690 HPLC with a dual wavelength detector (Waters 2487) coupled on-line to a Micromass QTOF API Ultima in positive ESI mode. The column utilized was Phenomenex Primesphere C18-MC, 300 Å, 250 × 4.6 mm. 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) and preparative HPLC on Dynamax-60A (83−241-c) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. The detection wavelength was 254 nm.

3-{[*N*-(4-Chloro-3-methylisoxazol-5-yl)(methoxymethyl)-amino]sulfonyl}-5-methylthiophene-2-carbonyl Chloride (6). To a solution of **8**[21] (6.0 g, 18.6 mmol) in anhydrous THF (240 mL) at −78 °C under nitrogen atmosphere was added dropwise ^{n}BuLi (2.5 M in hexanes, 30 mL, 74.4 mmol). The mixture was stirred at −78 °C for 20 min before the addition of iodomethane (10.9 g, 77.1 mmol). The resulting mixture was immediately poured into ice (∼400 g) and acidified with concentrated HCl to a final pH of ∼1. The mixture was extracted with EtOAc (100 and 300 mL), and the combined organic layers were dried over $MgSO_4$. The solids were filtered off, and the filtrate was concentrated to give a 2:1 mixture of **9** (5.75 g, 92% yield) and **8** (2.75 g). For compound **9**: ^{1}H NMR (DMSO-d_6) δ: 7.20 (s, 1H), 2.48 (s, 3H), 2.15 (s, 3H). The mixture was used in the next step without separation. To a solution of this mixture of **8** and **9** (8.5 g) in THF (150 mL) were sequentially added *N*,*N*-diisopropylethylamine (9.62 g, 74.4 mmol) and bromomethyl methyl ether (90% pure, 7.75 g, 55.80 mmol). The mixture was stirred overnight before the volatiles were stripped off on a rotavap. The residue was diluted with EtOAc (200 mL) and washed with water (2 × 150 mL). The organic layer was dried over $MgSO_4$, the solids were filtered, and the filtrate was concentrated on a rotavap. The residue was purified on a Biotage column using 10% EtOAc in hexanes as the eluent to afford **10** (3.40 g, 51% yield). ^{1}H NMR ($CDCl_3$) δ: 7.17 (q, J = 0.9 Hz, 1H), 5.42 (s, 2H), 5.27 (s,

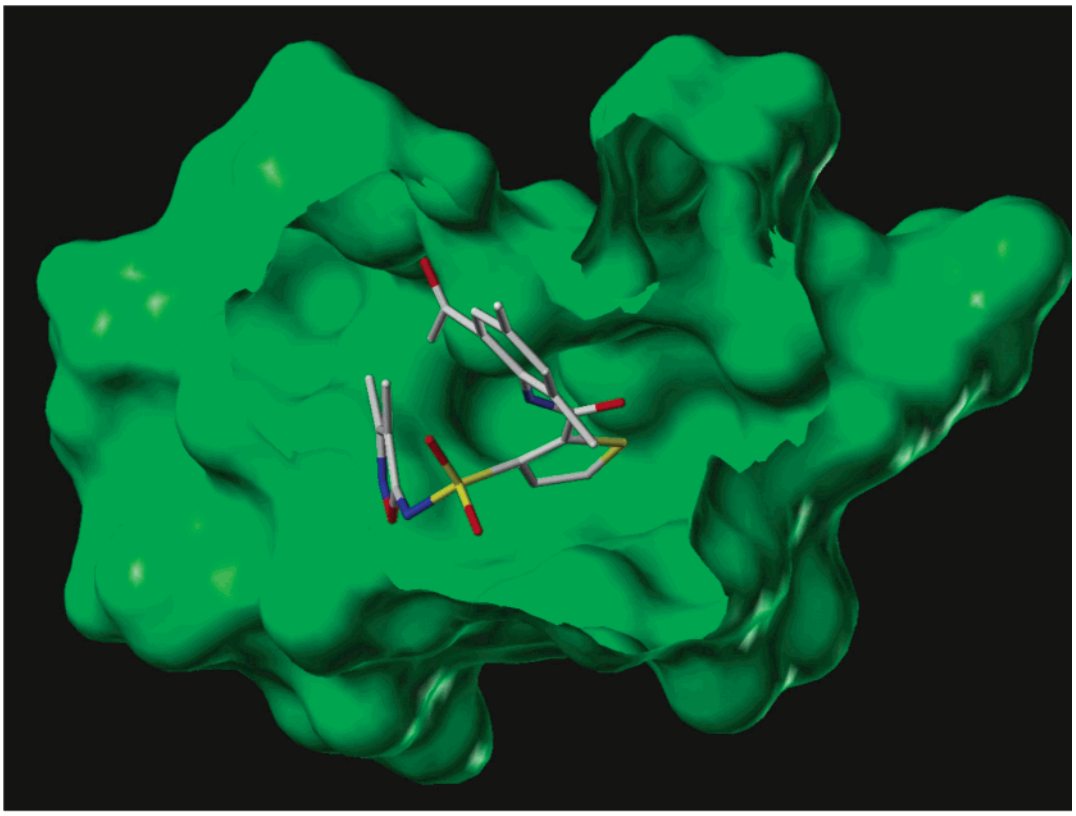


Figure 4. Connelly molecular surface of ET_A for the residues within 4 Å of the mass center of **7z**. Seven residues (Asn137, Lys140, Pro162, Phe163, Thr238, Cys239, and Met240) that do not have close interactions with the inhibitor are blanked for clarity. Compound **7z** is represented in sticks and colored by atom types.

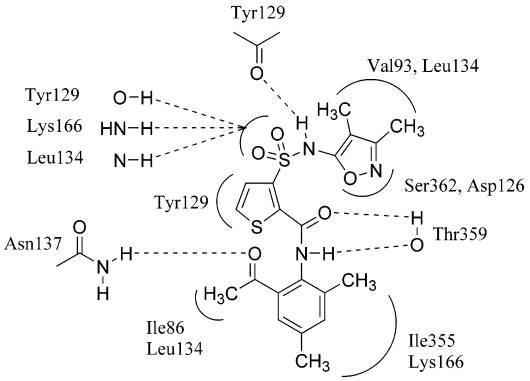


Figure 5. Putative binding interaction of **7z** and ET_A.

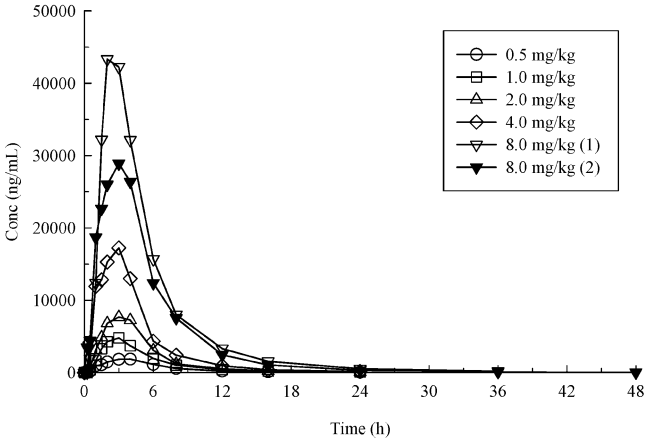


Figure 6. Human plasma concentration of **7z** vs time profile at different single ascending dose levels.

2H), 3.54(2) (s, 3H), 3.53(7) (s, 3H), 2.48 (d, J = 0.9 Hz, 3H), 2.26 (s, 3H). To a solution of **10** (3.4 g, 8.0 mmol) in THF (24 mL) was added 1 N NaOH (24 mL, 24 mmol). The mixture was stirred at room temperature for 4 h before the volatiles were removed on a rotavap. The residue was diluted with water (50 mL) and extracted with ether (3 × 20 mL). The aqueous layer was cooled to 0 °C, acidified to pH 2 using 4 N HCl, and extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over $MgSO_4$/charcoal, the solids were filtered, and the filtrate was concentrated to give the corresponding carboxylic acid as an oil. ^{1}H NMR ($CDCl_3$) δ: 7.20 (q, J = 1.0 Hz, 1H), 5.19 (s, 2H), 3.51 (s, 3H), 2.51 (d, J = 1.0 Hz, 3H), 2.28 (s, 3H). To a solution of this oil (2.38 g, 6.22 mmol) in a 1:1 mixture of THF/$CHCl_3$ (30 mL) at 0 °C was added oxalyl chloride (2 M in dichloromethane, 15.5 mL, 31.1 mmol) and two drops of pyridine. The mixture was stirred at room-temperature overnight and then heated at 50 °C for 3 h. The volatiles were evaporated followed by iterative addition of hexanes/dichloromethane (1:1) and concentration on a rotavap to give **6** as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 1H), 5.22 (s, 2H), 3.54 (s, 3H), 2.53 (s, 3H), 2.25 (s, 3H).

Target compounds **7a**−**z** were synthesized using our published procedure[6] unless a specific procedure is provided.

***N*-(2-Chloro-4,6-dimethylphenyl)-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7a).** Compound **7a** (sodium salt) is a white solid: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ: 11.25 (s, 1H), 7.74 (d, J = 5.3 Hz, 1H), 7.42 (d, J = 5.3 Hz, 1H), 7.20 (s, 1H), 7.08 (s, 1H), 2.29 (s, 3H), 2.22 (s, 3H), 2.00 (s, 3H); IR (KBr pellet): 3477, 3238, 1642, 1598, 1533, 1489 cm^{-1}.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(3,5-dimethylbiphenyl-2-yl)thiophene-2-carboxamide (7b). Compound **7b** is a light brown solid: mp 178−181 °C; ^{1}H NMR (DMSO-d_6) δ: 10.83 (br s, 1H), 7.63 (d, J = 5.1 Hz, 1H), 7.19−7.34 (m, 6H), 7.11 (s, 1H), 7.00 (s, 1H), 2.33 (s, 3H), 2.25 (s, 3H), 2.02 (s, 3H). HRMS Calcd for $C_{23}H_{20}ClN_3O_4S_2$: 501.0584. Found: 501.0610.

2-(3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carbonylamino)-3,5-dimethylbenzoic Acid

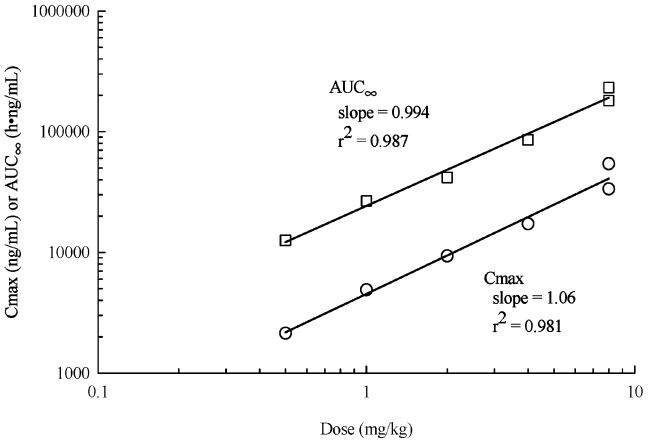


Figure 7. Human AUC and maximal plasma concentration of **7z** vs dose (single dosing).

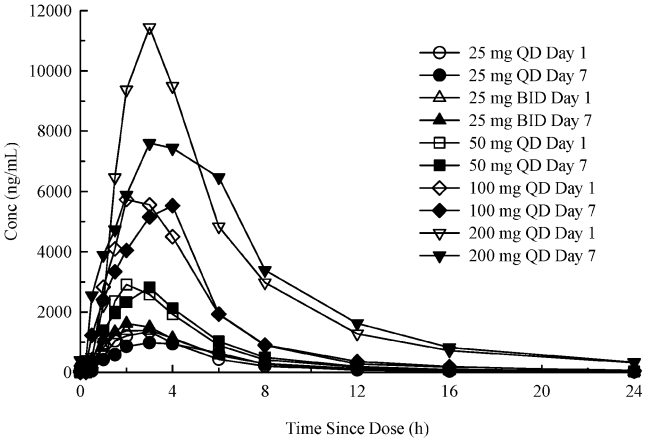


Figure 8. Human plasma concentration of **7z** vs time profile at different multiple ascending dose levels.

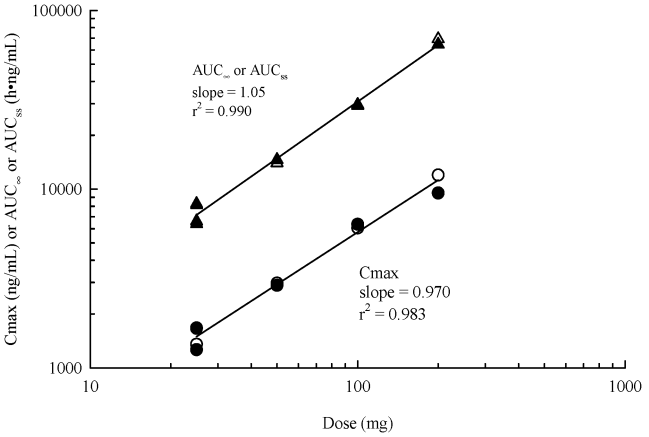


Figure 9. Human AUC and maximal plasma concentration of **7z** vs dose (multiple dosing)].

(7c). Compound **7c** is a white solid: mp 171−174 °C; ^{1}H NMR (DMSO-d_6) δ: 10.87 (br s, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.45 (d, 1H), 7.38 (d, J = 5.1 Hz, 1H), 7.28 (d, 1H), 2.32 (s, 3H), 2.29 (s, 3H), 2.03 (s, 3H); IR (KBr pellet): 3112, 1737, 1690, 1627 cm^{-1}. HRMS Calcd for $C_{18}H_{16}ClN_3O_6S_2$: 469.0169. Found: 469.0156.

***N*-(2-Aminocarbonyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7d).** Compound **7d** is a light brown solid: mp 40−43 °C; ^{1}H NMR (DMSO-d_6) δ: 10.81 (s, 1H), 7.76 (d, J = 5.5 Hz, 1H), 7.39 (d, J = 5.5 Hz, 1H), 7.26 (s and s, 2H), 7.24 (br s, 1H), 7.19 (br s, 1H), 2.31 (s, 3H), 2.20 (s, 3H), 2.05 (s, 3H); IR (KBr pellet): 3457, 3355, 1660, 1527, 1491 cm^{-1}. HRMS Calcd for $C_{18}H_{17}ClN_4O_5S_2$: 468.0329. Found: 468.0332.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2-(dimethylamino)carbonyl-4,6-dimethylphenyl]thiophene-2-carboxamide (7e). Compound **7e** (sodium salt) is a white solid: mp 170−175 °C; ^{1}H NMR (DMSO-d_6) δ: 11.02 (s, 1H), 7.67 (d, J = 5.3 Hz, 1H), 7.36 (d, J = 5.3 Hz, 1H), 7.12 (s, 1H), 6.89 (s, 1H), 2.84 (s, 3H), 2.76 (s, 3H), 2.30 (s, 3H), 2.20 (s, 3H), 1.98 (s, 3H); IR (KBr pellet): 3447, 3235, 1641, 1598, 1542 cm^{-1}. HRMS Calcd for $C_{20}H_{21}ClN_4O_5S_2$: 496.0462. Found: 496.0630.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-{2-[*N*-(2,2-dimethylpropyl)-methylamino]carbonyl-4,6-dimethylphenyl}thiophene-2-carboxamide (7f). Compound **7f** (sodium salt) is a white solid: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ: 10.88 (s, 1H), 7.67 (d, J = 5.1 Hz, 1H), 7.36 (d, J = 5.1 Hz, 1H), 7.12 (s, 1H), 6.89 (s, 1H), 3.14 (s, 2H), 2.83 (s, 3H), 2.31 (s, 3H), 2.24 (s, 3H), 1.98 (s, 3H), 0.82 (s, 9H); IR (KBr pellet): 3441, 1605, 1497 cm^{-1}. HRMS Calcd for $C_{24}H_{29}ClN_4O_5S_2$: 552.1268. Found: 552.1256.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2-methanesulfonyl-4,6-dimethylphenyl)thiophene-2-carboxamide (7g). Compound **7g** (sodium salt) is a white solid: mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ: 11.17 (s, 1H), 7.74 (d, J = 5.5 Hz, 1H), 7.62 (s, 1H), 7.50 (s, 1H), 7.36 (d, J = 5.5 Hz, 1H), 3.14 (s, 3H), 2.40 (s, 3H), 2.24 (s, 3H), 1.99 (s, 3H); IR (KBr pellet): 3583, 3261, 1732, 1659, 1608, 1535 cm^{-1}. HRMS Calcd for $C_{18}H_{18}ClN_3O_6S_3$: 503.0046. Found: 503.0025.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2,4-dimethyl-6-(propane-1-sulfonyl)phenyl]thiophene-2-carboxamide (7h). Compound **7h** (sodium salt) is an off-white solid: mp 152−155 °C; ^{1}H NMR (DMSO-d_6) δ: 11.20 (s, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.60 (s, 1H), 7.50 (s, 1H), 7.42 (d, J = 5.1 Hz, 1H), 2.39 (s, 3H), 2.22 (s, 3H), 1.99 (s, 3H), 1.47 (m, 2H), 0.86 (t, J = 7.3 Hz, 3H); IR (KBr pellet): 3597, 1646, 1603, 1638, 1502 cm^{-1}. HRMS Calcd for $C_{20}H_{22}ClN_3O_6S_3$: 531.0359. Found: 531.0316.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2,4-dimethyl-6-(propane-2-sulfonyl)phenyl]thiophene-2-carboxamide (7i). Compound **7i** (sodium salt) is an off-white solid: mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ: 11.20 (s, 1H), 7.73 (d, J = 5.3 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.41 (d, J = 5.3 Hz, 1H), 3.52 (septet, J = 6.6 Hz, 1H), 2.39 (s, 3H), 2.21 (s, 3H), 1.99 (s, 3H), 1.07 (d, J = 6.6 Hz, 6H); IR (KBr pellet): 3582, 3362, 3249, 1740, 1655, 1598, 1542, 1506 cm^{-1}. HRMS Calcd for $C_{20}H_{22}ClN_3O_6S_3$: 531.0359. Found: 531.0388.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2,4-dimethyl-6-oxazol-2-ylphenyl)thiophene-2-carboxamide (7j). Compound **7j** is a white solid: mp 176−178 °C; ^{1}H NMR (DMSO-d_6) δ: 11.35 (s, 1H), 7.87 (d, J = 0.7 Hz, 1H), 7.67 (d, J = 5.1 Hz, 1H), 7.65 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 7.26 (d, J = 2.0 Hz, 1H), 7.15 (d, J = 0.7 Hz, 1H), 2.36 (s, 3H), 2.30 (s, 3H); IR (KBr pellet): 3405, 3293, 1646, 1591, 1535, 1504 cm^{-1}. HRMS Calcd for $C_{20}H_{17}ClN_4O_5S_2$: 492.0329. Found: 492.0302.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2,6-dimethyl-4-oxazol-2-ylphenyl)thiophene-2-carboxamide (7k). Compound **7k** is an off-white solid: mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ: 11.32 (s, 1H), 8.20 (d, J = 0.8 Hz, 1H), 7.73 (s and s, 2H), 7.72(5) (d, J = 5.2 Hz, 1H), 7.43 (d, J = 5.2 Hz, 1H), 7.37 (d, J = 0.8 Hz, 1H), 2.27 (s and s, 6H), 1.99 (s, 3H); IR (KBr pellet): 3448, 3231, 1722, 1598, 1540, 1497 cm^{-1}. HRMS Calcd for $C_{20}H_{17}ClN_4O_5S_2$: 492.0329. Found: 492.0315.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2,4-dimethyl-6-propionylphenyl)thiophene-2-carboxamide (7l). Compound **7l** (sodium salt) is an off-white solid: mp 111−120 °C; ^{1}H NMR (DMSO-d_6) δ: 11.35 (s, 1H), 7.71 (d, J = 1.1 Hz, 1H), 7.41 (d, J = 1.1 Hz, 1H), 7.23 (s, 1H), 7.17 (s, 1H), 2.76 (q, J = 6.6 Hz, 2H), 2.31 (s and s, 6H), 1.98 (s, 3H), 2.92 (t, J = 6.6 Hz, 3H); IR (KBr pellet): 3478, 3253, 1685, 1639, 1603 cm^{-1}. HRMS Calcd for $C_{20}H_{20}ClN_3O_5S_2$: 481.0533. Found: 481.0517.

Table 8. Comparisons of **7z** with Lead Endothelin Antagonists

generic name/code	ET_A IC_{50} (nM)	ET_A K_i (nM)	selectivity for ET_A over ET_B (fold)	oral availability (%)	$t_{1/2}$ (h)	references
BQ123	22		818	peptide		35
bosentan		6.5	53	30−80		36
tezosentan	23		56	iv drug	0.13	37
atrasentan	0.31	0.034	2000	60, rat	3.5	38
darusentan		6	60			39
enrasentan		1.1	100	60, rat		40
BMS-193884		1.4	1400	43, rat	2, rat	41
				71, monkey	9, monkey	
J-104132		0.034	3	40, rat		42
Cl-1034	0.46		4500	77, rat	8.5, rat	43
				100, dog	2.2, dog	
Z1611	0.2		5000		4.8	44
TAK-044	0.24		540	peptide	0.5−1	45
YM598	3.1		387	89, rat	2.5, rat	46
				97, dog	7.4, dog	
1	1.4	0.43	7000	50−60, rat	5.9−7.5, rat	2
				90−100, dog	4−4.8, dog	
				∼80, human	6.46, human	
7z	0.08		441000	∼100, rat	6.0, rat	
				>80, human	6−7, human	

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2-cyclopropanecarbonyl-4,6-dimethylphenyl)thiophene-2-carboxamide (7m). Compound **17** was synthesized according to our general procedure[6] using acid acid chloride **4** and aniline **13**. To a solution of **17** (1.0 g, 1.89 mmol) in methanol (20 mL) was added KOH pellets (85%, 5.3 g) without cooling. The resulting hot mixture was stirred until it cooled to room temperature. The mixture was poured into ice (150 g), acidified with concentrated HCl to pH 1, and extracted with EtOAc (200 mL). The organic layer was concentrated on a rotavap, and the residue was subjected to reverse phase HPLC purification and sodium salt formation to afford **7m** (sodium salt, 480 mg, 49% yield). Compound **7m** (sodium salt) is an off-white solid: mp 154−162 °C; ^{1}H NMR (DMSO-d_6) δ: 11.38 (s, 1H), 7.72 (d, J = 5.1 Hz, 1H), 7.43 (d, J = 5.1 Hz, 1H), 7.28 (d, J is very small, 1H), 7.10 (d, J is very small, 1H), 2.42 (m, 1H), 2.31 (s and s, 6H), 1.96 (s, 3H), 0.79 (m, 2H), 0.50 (m, 2H); IR (KBr pellet): 3447, 3253, 1726, 1665, 1598 cm^{-1}. HRMS Calcd for $C_{21}H_{20}ClN_3O_5S_2$: 493.0533. Found: 493.0499.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2-cyclohexanecarbonyl-4,6-dimethylphenyl)thiophene-2-carboxamide (7n). Compound **7n** (sodium salt) is a yellow solid: mp 162−166 °C; ^{1}H NMR (DMSO-d_6) δ: 11.40 (s, 1H), 7.72 (d, J = 5.3 Hz, 1H), 7.40 (d, J = 5.3 Hz, 1H), 7.23 (s, 1H), 7.04 (s, 1H), 2.96 (tt, J = 7.7, 3.3 Hz, 1H), 2.30(2) (s, 3H), 2.29-(5) (s, 3H), 1.98 (s, 3H), 1.45−1.61 (m, 5H), 0.97−1.18 (m, 5H); IR (KBr pellet): 3447, 3232, 2925, 2854, 1680, 1644, 1598 cm^{-1}. HRMS Calcd for $C_{24}H_{26}ClN_3O_5S_2$: 535.1002. Found: 535.1018.

***N*-(2-Benzoyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7o).** Compound **7o** (sodium salt) is a light orange solid: mp 169−174 °C; ^{1}H NMR (DMSO-d_6) δ: 11.31 (s, 1H), 7.60−7.62 (m, 2H), 7.56 (d, J = 5.1 Hz, 1H), 7.42−7.46 (m, 1H), 7.28−7.32 (m, 3H), 7.26 (d, J = 5.1 Hz, 1H), 7.03 (d, J is very small, 1H), 2.43 (s, 3H), 2.30 (s, 3H), 2.01 (s, 3H); IR (KBr pellet): 3440, 3249, 1726, 1655, 1598 cm^{-1}. HRMS Calcd for $C_{24}H_{20}ClN_3O_5S_2$: 529.0533. Found: 529.0504.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2-(2-methanesulfonylacetyl)-4,6-dimethylphenyl]thiophene-2-carboxamide (7p). To a solution of **16** (0.4 g, 0.80 mmol) in DMF (20 mL) was added sodium methanesufinate (1.2 g, ∼11.7 mmol). The mixture was stirred at room-temperature overnight before it was poured into water (150 mL). The aqueous mixture was acidified to pH close to 1 at 0 °C, and the resulting precipitate was filtered and washed with cold 0.5 N HCl (2 × 50 mL). The solids were dissolved in EtOAc (100 mL), and the solution was shaken with saturated $NaHCO_3$ (100 mL). The organic layer was separated and dried over Na_2SO_4, the solids were filtered, and the filtrate was concentrated in vaccuo to give the sodium salt of **7p** (195 mg, 43% yield) as a solid: mp 172−175 °C. ^{1}H NMR (DMSO-d_6) δ: 11.67 (s, 1H), 7.75 (d, J = 5.3 Hz, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.34 (s, 1H), 7.32 (s, 1H), 4.75 (s, 2H), 3.06 (s, 3H), 2.35 (s, 3H), 2.33 (s, 3H), 2.00 (s, 3H); IR (KBr pellet): 3513, 2931, 1691, 1608, 1533, 1512 cm^{-1}.

***N*-(2-Acetyl-3,4,6-trimethylphenyl)-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7q).** Compound **7q** (sodium salt) is an off-white solid: mp 223−225 °C; ^{1}H NMR (DMSO-d_6) δ: 11.09 (s, 1H), 7.70 (d, J = 5.2 Hz, 1H), 7.39 (d, J = 5.2 Hz, 1H), 7.13 (s, 1H), 2.35 (s, 3H), 2.24 (s, 3H), 2.12 (s, 3H), 2.04 (s, 1H), 1.99 (s, 1H); IR (KBr pellet): 3431, 1633, 1598 cm^{-1}. HRMS Calcd for $C_{20}H_{20}ClN_3O_5S_2$: 481.0533. Found: 481.0523.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(3,4,6-trimethyl-2-propionylphenyl)thiophene-2-carboxamide (7r). Compound **7r** (sodium salt) is a white solid: mp 166−170 °C; ^{1}H NMR (DMSO-d_6) δ: 11.04 (s, 1H), 7.69 (d, J = 5.3 Hz, 1H), 7.39 (d, J = 5.3 Hz, 1H), 7.12 (s, 1H), 2.65 (q, J = 7.3 Hz, 2H), 2.23 (s, 3H), 2.13 (s, 3H), 2.00 (s and s, 6H), 0.90 (t, J = 7.3 Hz, 3H). HRMS Calcd for $C_{21}H_{22}ClN_3O_5S_2$: 495.0689. Found: 495.0653.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(2-cyano-3,4,6-trimethylphenyl)thiophene-2-carboxamide (7s). Compound **7s** (sodium salt) is a white solid: mp 218−220 °C; ^{1}H NMR (DMSO-d_6) δ: 11.67 (s, 1H), 7.75 (d, J = 5.3 Hz, 1H), 7.43 (d, J = 5.3 Hz, 1H), 7.41 (s, 1H), 2.39 (s, 3H), 2.28 (s, 3H), 2.26 (s, 3H), 1.99 (s, 3H); IR (KBr pellet): 3431, 2225, 1726, 1601 cm^{-1}. HRMS Calcd for $C_{19}H_{17}ClN_4O_4S_2$: 464.0380. Found: 464.0374.

***N*-(2-Cyano-3,4,6-trimethylphenyl)-3-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7t).** Compound **7t** (sodium salt) is a light yellow solid: mp 175−180 °C; ^{1}H NMR (DMSO-d_6) δ: 11.90 (br s, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.40 (s, 1H), 7.35 (d, J = 5.1 Hz, 1H), 2.39 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H), 1.95 (s, 3H), 1.55 (s, 3H); IR (KBr pellet): 3470, 3238, 2229, 1627, 1540, 1475 cm^{-1}. HRMS Calcd for $C_{20}H_{20}N_4O_4S_2$: 444.0926. Found: 444.0963.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-(4,6-diacetyl-3-hydroxy-2-propylphenyl)thiophene-2-carboxamide (7u). Compound **7u** (sodium salt) is an off-white solid: mp 163−167 °C; ^{1}H NMR (DMSO-d_6) δ: 13.05 (s, 1H), 11.89 (s, 1H), 8.00 (s, 1H), 7.78 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.1 Hz, 1H), 2.77 (t, J = 8.1 Hz, 2H), 2.71 (s, 3H), 2.35 (s, 3H), 2.29 (s, 3H), 1.99 (s, 3H), 1.46 (m, 2H), 0.84 (t, J = 7.3 Hz, 3H); IR (KBr pellet): 3473, 1639, 1603, 1532 cm^{-1}. HRMS Calcd for $C_{22}H_{21}ClN_3O_7S_2$: 539.0588. Found: 539.0594.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2-(1-hydroxyethyl)-4,6-dimethylphenyl]thiophene-2-carboxamide (7v). To a solution of the sodium salt of **3a** (100

mg, 0.20 mmol) in water (5 mL) was added sodium borohydride (100 mg), and the mixture was stirred at room temperature for 3 h. More sodium borohydride (200 mg) was added, and the mixture was stirred for another 10 h. The reaction was quenched by adding saturated NH_4Cl, and the resulting mixture was partitioned between saturated NH_4Cl (150 mL) and EtOAc (150 mL). The organic layer was shaken with saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated to give the sodium salt of **7v** (67 mg, 67% yield) as a brown solid: mp 147−154 °C; ^{1}H NMR (DMSO-d_6) δ: 10.92 (s, 1H), 7.69 (d, J = 5.5 Hz, 1H), 7.40 (d, J = 5.5 Hz, 1H), 7.21 (s, 1H), 6.94 (s, 1H), 4.86 (br s, 1H), 2.29 (s, 3H), 2.11 (s, 3H), 1.99 (s, 3H), 1.20 (d, J = 6.2 Hz, 3H); IR (KBr pellet): 3437, 3237, 1634, 1603, 1537, 1496 cm^{-1}. HRMS Calcd for $C_{19}H_{20}ClN_3O_5S_2$: 469.0533. Found: 469.0516.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2-(1-hydroxyimino)ethyl-4,6-dimethylphenyl]thiophene-2-carboxamide (7w). To a solution of the sodium salt of **3a** (500 mg, 1.02 mmol) in 2 N NaOH (40 mL) and EtOH (4 mL) was added hydroxylamine hydrochloride (4 g). The reaction was heated at 60 °C for 3 h before it was quenched with 2 N HCl (45 mL) at 0 °C. The precipitate was filtered, washed with dilute HCl, and partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated to afford the sodium salt of **7w** (275 mg, 53% yield) as an off-white solid: mp 136−142 °C; ^{1}H NMR (DMSO-d_6) δ: 10.98 (s, 1H), 10.82 (s, 1H), 7.68 (d, J = 5.3 Hz, 1H), 7.39 (d, J = 5.3 Hz, 1H), 7.10 (s, 1H), 6.98 (s, 1H), 2.30 (s, 3H), 2.20 (s, 3H), 1.99(1) (s, 3H), 1.98(6) (s, 3H); IR (KBr pellet): 3434, 1727, 1632, 1601, 1533 cm^{-1}. HRMS Calcd for $C_{19}H_{19}ClN_4O_5S_2$: 482.0485. Found: 482.0462.

3-{[(4-Chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-*N*-[2-(1-methoxyiminoethyl)-4,6-dimethylphenyl]thiophene-2-carboxamide (7x). Compound **7x** was synthesized in the same fashion as for **7w** from **3a** except that *O*-methylhydroxylamine hydrochloride (25% w/w in water) and Na_2CO_3 were used instead of hydroxylamine hydrochloride and 2 N NaOH, respectively. It was a mixture of two isomers in the ratio of 4.5:1, and the sodium salt is a gray solid (38% yield): mp 140−145 °C; ^{1}H NMR (DMSO-d_6) major isomer δ: 11.03 (s, 1H), 7.69 (d, J = 5.3 Hz, 1H), 7.39 (d, J = 5.3 Hz, 1H), 7.12 (s, 1H), 7.01 (s, 1H), 3.73 (s, 3H), 2.30 (s, 3H), 2.19 (s, 3H), 1.98(7) (s, 3H), 1.95 (s, 3H); minor isomer δ: 10.76 (s, 1H), 7.66 (d, J = 5.1 Hz, 1H), 7.36 (d, J = 5.1 Hz, 1H), 7.07 (s, 1H), 6.83 (s, 1H), 3.53 (s, 3H), 2.29 (s, 3H), 2.22 (s, 3H), 1.99 (5) (s, 3H), 1.97 (s, 3H); IR (KBr pellet): 3456, 1634, 1533, 1497 cm^{-1}. HRMS Calcd for $C_{20}H_{21}ClN_4O_5S_2$: 496.0462. Found: 496.0630.

***N*-(2-Acetyl-4,6-dimethylphenyl)-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}-5-methylthiophene-2-carboxamide (7y).** Compound **7y** (sodium salt) is an off-white solid: mp 158−162 °C; ^{1}H NMR (DMSO-d_6) δ: 11.35 (s, 1H), 7.24 (s, 1H), 7.20 (s, 1H), 7.15(1) and 7.16(4) (s and s, 1H), 2.44 (s, 3H), 2.33 (s, 3H), 2.31 (s, 3H), 2.29 (s, 3H), 1.99 (s, 3H); IR (KBr pellet): 3447, 3243, 2966, 2925, 1680, 1639, 1603, 1501 cm^{-1}. HRMS Calcd for $C_{20}H_{20}ClN_3O_5S_2$: 481.0533. Found: 481.0505.

***N*-(2-Acetyl-4,6-dimethylphenyl)-3-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (7z).** Compound **7z** is a yellow solid. Its sodium salt: mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ: 11.99 (s, 1H), 7.68 (d, J = 5.3 Hz, 1H), 7.35 (d, J = 5.3 Hz, 1H), 7.25 (s, 1H), 7.20 (s, 1H), 2.35 (s, 3H), 2.31 (s, 3H), 2.29 (s, 3H), 1.92 (s, 3H), 1.53 (s, 3H); IR (KBr pellet): 3455, 3245, 1685, 1627, 1540 cm^{-1}. HRMS Calcd for $C_{20}H_{21}N_3O_5S_2$: 447.0922. Found: 447.0899.

Compounds **12a**−**d** were synthesized according to our published procedure.[23]

1-(2-Amino-3,5-dimethylphenyl)propan-1-one (12a). ^{1}H NMR ($CDCl_3$) δ: 7.46 (d, J = 0.7 Hz, 1H), 7.04 (d, J = 0.7 Hz, 1H), 6.40 (br s, 2H), 2.98 (q, J = 7.3 Hz, 2H), 2.24 (s, 3H), 2.15 (s, 3H), 1.20 (t, J = 7.3 Hz, 3H).

1-(2-Amino-3,5-dimethylphenyl)-1-cyclohexylmethanone (12b). ^{1}H NMR ($CDCl_3$) δ: 7.47 (s, 1H), 7.07 (d, 1H), 3.29 (tt, J = 11.6, 2.9 Hz, 1H), 2.27 (s, 3H), 2.21 (s, 3H), 1.20−1.90 (m, 10H).

1-(2-Amino-3,5-dimethylphenyl)-1-phenylmethanone (12c). ^{1}H NMR ($CDCl_3$) δ: 7.40−7.70 (m, 5H), 7.12 (s, 1H), 7.07 (s, 1H), 6.26 (br s, 2H), 2.21 (s, 3H), 2.15 (s, 3H).

1-(2-Amino-3,5-dimethylphenyl)-2-chloroethanone (12d). ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 1H), 7.09 (s, 1H), 6.34 (br s, 2H), 4.71 (s, 2H), 2.24 (s, 3H), 2.16 (s, 3H).

1-(2-Amino-3,5-dimethylphenyl)-4-chlorobutan-1-one (13). The title compound was essentially the exclusive product when 2,4-dimethylaniline and cyclopropanecarbonitrile were subjected to the literature procedure.[23] ^{1}H NMR ($CDCl_3$) δ: 7.48 (s, 1H), 7.07 (d, 1H), 3.67 (t, J = 6.2 Hz, 2H), 3.16 (t, J = 7.0 Hz, 2H), 2.25 (s, 3H), 2.18 (s, 3H), 1.01 (m, 2H).

Compounds **15a** and **15b** were synthesized according to our published procedure.[23]

1-(2-Amino-3,5,6-trimethylphenyl)ethanone (15a). ^{1}H NMR (DMSO-d_6) δ: 6.81 (s, 1H), 4.49 (br s, 2H), 2.41 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H).

1-(2-Amino-3,5,6-trimethylphenyl)propan-1-one (15b). ^{1}H NMR ($CDCl_3$) δ: 7.22 (s, 1H), 2.99 (q, J = 7.3 Hz, 2H), 2.75 (s, 3H), 2.68 (s, 3H), 2.37 (s, 3H), 1.31 (t, J = 7.3 Hz, 3H).

***N*-[2-(2-Chloroacetyl)-4,6-dimethylphenyl]-3-{[(4-chloro-3-methylisoxazol-5-yl)amino]sulfonyl}thiophene-2-carboxamide (16).** Compound **16** was synthesized according to our published procedure[6] using acid chloride **4** and aniline **12d**. For **16** ^{1}H NMR (DMSO-d_6) δ: 11.26 (s, 1H), 7.76 (d, J = 5.1 Hz, 1H), 7.40 (d, J = 5.1 Hz, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 4.86 (s, 2H), 2.32(3) (s, 3H), 2.31(5) (s, 3H), 2.03 (s, 3H).

3,5-Dimethylphenyl Methyl Sulfone (19). A mixture of 5-iodoxylene (12.0 g, 51.7 mmol), sodium methanesufinate (20.8 g, 206.8 mmol), and cuprous iodide (14.8 g, 77.5 mmol) in DMF (150 mL) was heated at 140 °C for 36 h under nitrogen. To workup, the mixture was poured into water (300 mL), and the solids were filtered and washed with water (2 × 100 mL). The solids were then washed with EtOAc (6 × 50 mL), and the combined washings dried over $MgSO_4$ and concentrated on a rotavap to give **19** (8.0 g, 84% yield). ^{1}H NMR ($CDCl_3$) δ: 7.54 (s, 2H), 7.25 (s, 1H), 3.03 (s, 2H), 2.39 (s, 6H).

Ethyl (3,5-Dimethylphenyl) Sulfone (20). To a solution of **19** (200 mg, 1.09 mmol) in anhydrous THF (1 mL) at −78 °C under nitrogen was added ^{n}BuLi (1 M in hexanes, 0.52 mL, 1.30 mmol). The mixture was stirred for 15 min before the addition of iodomethane (616 mg, 4.34 mmol). The reaction was allowed to warm to room temperature and then poured into iced water (50 g). The aqueous mixture was extracted with EtOAc (2 × 25 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated to give **20** (150 mg, 70% yield). ^{1}H NMR (DMSO-d_6) δ: 7.50 (s, 2H), 7.37 (s, 1H), 3.24 (q, J = 7.4 Hz, 2H), 2.38 (s, 6H), 1.10 (t, J = 7.3 Hz, 3H).

3,5-Dimethylphenyl (2-propyl) sulfone (21). ^{1}H NMR (DMSO-d_6) δ: 7.47 (s, 2H), 7.38 (s, 1H), 3.36 (septet, J = 6.7 Hz, 1H), 2.38 (s, 6H), 1.15 (d, J = 6.7 Hz, 6H).

3,5-Dimethylphenyl (1-propyl) sulfone (22). ^{1}H NMR (DMSO-d_6) δ: 7.50 (s, 2H), 7.36 (s, 1H), 3.16−3.28 (m, 2H), 2.38 (s, 6H), 1.50−1.62 (m, 2H), 0.92 (t, J = 7.7 Hz, 3H).

General Procedure for Nitration of Alkyl Aryl Sulfones. To a mixture of **22** (5.06 g, 23.8 mmol) and concentrated H_2SO_4 (17 mL) at 0 °C was added KNO_3 (2.40 g, 23.8 mmol). The reaction was stirred at room-temperature overnight before the mixture was poured onto ice (150 g). The precipitate was collected via filtration and washed with water (200 mL) to give **23b** (5.60 g, 95% yield).

1-Methanesulfonyl-3,5-dimethyl-2-nitrobenzene (23a). ^{1}H NMR (DMSO-d_6) δ: 7.77 (s, 1H), 7.70 (s, 1H), 3.24 (q, J = 7.4 Hz, 2H), 2.38 (s, 6H), 1.10 (t, J = 7.3 Hz, 3H).

1,5-Dimethyl-2-nitro-3-(propane-1-sulfonyl)benzene (23b). ^{1}H NMR (DMSO-d_6) δ: 7.73 (s, 1H), 7.70 (s, 1H), 3.34 (s, 3H), 2.45 (s, 3H), 2.29 (s, 3H).

1,5-Dimethyl-2-nitro-3-(propane-2-sulfonyl)benzene (23c). ^{1}H NMR (DMSO-d_6) δ: 7.71(2) (s, 1H), 7.70(5) (s, 1H), 3.58 (septet, J = 6.6 Hz, 1H), 2.45 (s, 3H), 2.28 (s, 3H), 1.23 (d, J = 6.6 Hz, 6H).

2-Methanesulfonyl-4,6-dimethylaniline(24a). To solution of **23a** (5.0 g, 21.8 mmol) in methanol (200 mL) were sequentially added a solution of ammonium chloride (5.0 g, 93.0 mmol) in water (50 mL) and, in portions, zinc dust (5.0 g, 77.0 mmol). The mixture was vigorously stirred for 4 h before the solids were filtered and washed with methanol. The filtrate was concentrated, and the aqueous residue was partitioned between EtOAc and 1 N NaOH. The organic layer was dried over $MgSO_4$ and concentrated to give **24a** (3.5 g, 80% yield). ^{1}H NMR (DMSO-d_6) δ: 7.26 (s, 1H), 7.14 (s, 1H), 5.56 (br s, 2H), 3.09 (s, 3H), 2.18 (s, 3H), 2.17 (s, 3H).

2,4-Dimethyl-6-(propane-1-sulfonyl)aniline (24b). ^{1}H NMR (DMSO-d_6) δ: 7.22 (s, 1H), 7.13 (s, 1H), 5.57 (br s, 2H), 3.15 (m, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 1.57 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H).

2,4-Dimethyl-6-(propane-2-sulfonyl)aniline (24c). ^{1}H NMR (DMSO-d_6) δ: 7.17 (s, 1H), 7.14 (s, 1H), 5.60 (br s, 2H), 3.35(septet, J = 6.8 Hz, 3H), 2.18 (s, 3H), 2.13 (s, 3H), 1.16 (d, J = 6.8 Hz, 6H).

2-(3,5-Dimethylphenyl)-4,5-dihydrooxazole (27). To a solution of 3,5-dimethylbezenzoic acid (**25**, 10.0 g, 66.6 mmol) in thionyl chloride (24.5 g, 206 mmol) was added 1 drop of pyridine. The mixture was heated at 50 ° for 2 h before it was concentrated. The residue was diluted with dichloromethane (60 mL) followed by the addition of a solution of 2-aminoethanol (8.3 g, 135 mmol) in dichloromethane (50 mL). The mixture was stirred for 48 h before the resulting precipitate was filtered off, and the filtrate was concentrated to ~ 30 mL and diluted with EtOAc (150 mL). The resulting mixture was sequentially washed with 1 N HCl, water, brine, and saturated $NaHCO_3$ (100 mL each) and concentrated to give a white solid (**26**). To a solution of this solid (4.53 g) in dichloromethane (50 mL) was added thionyl chloride (8.4 g, 70.3 mmol), and the mixture was stirred for 1 h. The reaction was quenched by dropwise addition of water (50 mL) at 0 °C. The aqueous layer was separated, basified with NaOH pellets at 0 °C, and then extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to give **27** as an oil (2.7 g, 66% yield). ^{1}H NMR ($CDCl_3$) δ: 7.56 (s, 2H), 7.09 (s, 1H), 4.41 (t, J = 9.7 Hz, 2H), 4.03 (t, J = 9.7 Hz, 2H), 2.32 (s, 6H).

2-(3,5-Dimethylphenyl)oxazole (28). To a solution of **27** (1.22 g, 6.95 mmol) in benzene (50 mL) was added NiO_2 monohydrate (16.5 g, 140 mmol). The mixture was heated under reflux for 48 h with vigorous stirring before it was cooled to room temperature and filtered. The filtrate was concentrated, and the residue was chromatographed to give **28** (310 mg, 26% yield) along with recovered **27** (420 mg, 35%). ^{1}H NMR (DMSO-d_6) for **28** δ: 8.19 (s, 1H), 7.61 (s, 2H), 7.36 (s, 1H), 7.15 (s, 1H), 2.34 (s, 6H).

2-(3,5-Dimethyl-2-nitrophenyl)oxazole (29). To a solution of **28** (640 mg, 3.69 mmol) in concentrated H_2SO_4 (10 mL) was added KNO_3 (404 mg, 4.00 mmol). The mixture was stirred for 1 h before it was poured into iced water. The resulting aqueous mixture was basified with 25% NaOH at 0 °C and extracted with EtOAc (100 mL). The organic layer was washed with water and brine (75 mL each), dried over $MgSO_4$, and concentrated to give **29** as an oil (720 mg, 89% yield). ^{1}H NMR (DMSO-d_6) δ: 8.32 (d, J = 0.7 Hz, 1H), 7.77 (s, 1H), 7.47 (s, 1H), 7.44 (d, J = 0.7 Hz, 1H), 2.42 (s, 3H), 2.28 (s, 3H).

2,4-Dimethyl-6-(oxazol-2-yl)aniline (30). To a mixture of **29** (750 mg, 3.44 mmol) and concentrated HCl (20 mL) was added a mixture of $SnCl_2$ in concentrated HCl (5 mL). The reaction was heated at 55 °C for 1 h before it was cooled to 0 °C and basified with 25% NaOH, and the aqueous mixture was extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified via silica gel chromatography (20% EtOAc in hexanes) to furnish **30** as a yellow solid (520 mg, 80% yield). ^{1}H NMR (DMSO-d_6) δ: 8.13 (d, J = 1.0 Hz, 1H), 7.45 (s, 1H), 7.40 (d, J = 1.0 Hz, 1H), 6.95 (s, 1H), 6.46 (br s, 2H), 2.19 (s, 3H), 2.13 (s, 3H).

3,5-Dimethyl-4-nitrobenzoic Acid (32) and 3,5-dimethyl-2-nitrobenzoic Acid (33). A solution of **31** (21.3 g, 127 mmol) in acetic acid (100 mL) and concentrated H_2SO_4 (27 mL) was heated at 100 °C. A solution of CrO_3 (40 g) in water (133 mL) was then added dropwise over 20 min. The mixture was heated under reflux for another 30 min before it was poured into ice (250 g). The aqueous mixture was extracted with EtOAc, the organic layer washed with 1 N NaOH, and the combined washings were acidified to pH ~ 1. The aqueous mixture was stirred overnight, and the resulting solids were filtered, washed with dilute acid, and dried under high vacuum to give a 1:1 mixture of **32** and **33** (5.95 g, 24% yield). ^{1}H NMR (DMSO-d_6) for **32** δ: 7.88 (s, 2H), 2.36 (s, 6H). ^{1}H NMR (DMSO-d_6) for **33** δ: 7.74 (s, 1H), 7.34 (s, 1H), 2.43 (s, 3H), 2.32 (s, 3H).

***N*-(2-Hydroxyethyl)-3,5-dimethyl-4-nitrobenzamide (34) and *N*-(2-Hydroxyethyl)-3,5-dimethyl-2-nitrobenzamide (35).** ^{1}H NMR (DMSO-d_6) for **34** δ: 8.90 (t, J = 5.5 Hz, 1H), 7.76 (s, 2H), 3.75 (t, J = 5.5 Hz, 2H), 3.60 (dt, J = 5.5, 5.8 Hz, 2H), 2.31 (s, 6H). ^{1}H NMR (DMSO-d_6) for **35** δ: 9.00 (t, J = 5.5 Hz, 1H), 7.41 (s, 1H), 7.36 (s, 1H), 3.69 (t, J = 5.5 Hz, 2H), 3.51 (dt, J = 5.5, 5.8 Hz, 2H), 2.38 (s, 3H), 2.28 (s, 3H).

2-(3,5-Dimethyl-4-nitrophenyl)-4,5-dihydrooxazole (36). ^{1}H NMR (DMSO-d_6) δ: 7.78 (s, 2H), 4.44 (t, J = 9.7 Hz, 2H), 4.00 (t, J = 9.7 Hz, 2H), 2.30 (s, 6H).

2,6-Dimethyl-4-(oxazol-2-yl)aniline (38). ^{1}H NMR (DMSO-d_6) δ: 8.01 (s, 1H), 7.46 (s, 2H), 7.21 (s, 1H), 5.15 (br s, 2H), 2.19 (s, 3H), 2.14 (s, 6H).

2-Bromo-4,6-dimethylaniline (40a). To a solution of 2,4-dimethylaniline (9.80 g, 80.9 mmol) in dichloromethane (200 mL) at 0 °C was added *N*-bromosuccinimide (15.1 g, 84.9 mmol). The mixture was stirred 1 h at 0 °C and another 10 h at room temperature. To workup, the precipitate was filtered off and the filtrate was washed with 1 N NaOH (2 × 150 mL). The organic layer was dried over $MgSO_4$ and concentrated to give **40a** (15.5 g, 96% yield). ^{1}H NMR ($CDCl_3$) δ: 7.12 (s, 1H), 6.81 (s, 1H), 3.90 (br s, 2H), 2.22 (s, 3H), 2.18 (s, 3H).

2-Bromo-3,4,6-trimethylaniline (40b). ^{1}H NMR ($CDCl_3$) δ: 6.80 (s, 1H), 3.98 (br s, 2H), 2.32 (s, 3H), 2.23 (s, 3H), 2.17 (s, 3H).

2-Amino-3,5-dimethylbenzonitrile (41a). To a solution of **40a** (15.5 g, 77.7 mmol) in dimethylformamide (120 mL) was added cuprous cyanide (114.0 g, 155.3 mmol). The mixture was heated at reflux overnight before it was poured into ice (300 g). The aqueous mixture was treated with ferric chloride (27.7 g, 170.8 mmol) and extracted with EtOAc (200 mL). The organic layer was washed with water (3 × 150 mL), dried over $MgSO_4$, and concentrated to give **41a** as an oil (5.3 g, 47% yield). ^{1}H NMR ($CDCl_3$) δ: 7.06 (s, 1H), 7.04 (s, 1H), 4.20 (br s, 2H), 2.17 (s, 3H), 2.14 (s, 3H).

2-Amino-2,4,6-trimethylbenzonitrile (41b). ^{1}H NMR (DMSO-d_6) δ: 7.00 (s, 1H), 2.25 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H).

3,5-Dimethylbiphenyl-2-ylamine (42). To a solution of **40a** (7.56 g, 37.8 mmol) in ethanol (80 mL) were sequentially added phenylboronic acid (4.75 g, 37.8 mmol), 4 M Na_2CO_3 (aq. 80 mL), and $Pd(PPh_3)_4$ (500 mg). The mixture was heated under reflux for 10 h before it was concentrated. The aqueous residue was extracted with EtOAc, and the organic layer was filtered through Celite. The filtrate was dried over MgSO4 and concentrated to afford **42** as a red-brown oil (6.85 g, 92% yield). ^{1}H NMR ($CDCl_3$) δ: 7.43 (m, 4H), 7.35 (m, 1H), 6.90 (d, J = 1.5 Hz, 1H), 6.84 (d, J = 1.5 Hz, 1H), 3.71 (br s, 2H), 2.26 (s, 3H), 2.20 (s, 3H).

3,5-Dimethyl-2-nitrobenzoic acid (44). To a mixture of 3,5-dimethylbenzoic acid (1.5 g, 10.0 mmol), HNO_3 (70%, 3.0 mL), and acetic acid (10 mL) was added dropwise concentrated H_2SO_4 (0.5 mL). The reaction was stirred 30 min at 80 °C before it was poured into iced water. The precipitate was filtered, washed with water, and then dried under high vacuum to give **44** (1.5 g, 77%). ^{1}H NMR (DMSO-d_6) δ: 7.63 (s, 1H), 7.50 (s, 1H), 2.38 (s, 3H), 2.24 (s, 3H).

***N*-(2,2-Dimethylpropyl)-3,5-dimethyl-2-nitrobenzamide (45).** To a solution of **44** (1.95 g, 10.0 mmol) in DMF (5 mL) were sequentially added 2,2-dimethylpropylamine (0.86 g, 10.0 mmol), a catalytic amount of DMAP, and EDCI (1.92

g, 10.0 mmol). The mixture was stirred for 10 h before it was diluted with water and extracted with EtOAc. The organic layer was separated and washed sequentially with 1 N HCl, water, saturated $NaHCO_3$, and brine. Drying over $MgSO_4$ and concentration of the resulting organic mixture afforded **45** (1.15 g, 44% yield). ^{1}H NMR (DMSO-d_6) δ: 8.61 (t, J = 6.4 Hz, 1H), 7.36 (s, 1H), 7.33 (s, 1H), 3.01 (d, J = 6.4 Hz, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 2.36 (s, 3H), 0.89 (s, 9H).

***N*-(2,2-Dimethylpropyl)-3,5,*N*-trimethyl-2-nitrobenzamide (46).** To a slurry of NaH (60% in mineral oil, 48 mg, 1.2 mmol) in anhydrous THF (5 mL) was added dropwise a solution of **45** (263 mg, 1.0 mmol) in THF (5 mL). The mixture was stirred for 15 min at room temperature before the addition of iodomethane (426 mg, 3.0 mmol). The reaction was quenched with water, and the resulting mixture was partitioned between ether and water. The organic layer was separated, dried over $MgSO_4$, and concentrated to give **46** as a yellowish solid (130 mg, 47% yield). ^{1}H NMR (DMSO-d_6) δ: 7.33 (s, 1H), 7.20 (s, 1H), 3.27 (s, 2H), 2.94 (s, 3H), 2.38 (s, 3H), 2.36 (s, 3H), 0.96 (s, 9H).

2-Amino-*N*-(2,2-dimethylpropyl)-3,5,*N*-trimethylbenzamide (47). A mixture of **46** (2.54 g, 9.13 mmol) and $SnCl_2$ (5.70 g, 30.0 mmol) in a solvent mixture of ethanol (30 mL), dioxane (30 mL), and water (2 mL) was heated at reflux for 1 h. The volatiles were evaporated, and the residue was stirred with a mixture of 2 N NaOH (200 mL) and EtOAc (150 mL) for 1 h. The solids were filtered and washed with EtOAc (150 mL). The two layers of the filtrate and combined washings were separated, dried over $MgSO_4$, and concentrated to give **47** as a yellowish solid (1.55 g, 68% yield). ^{1}H NMR (DMSO-d_6) δ: 6.82 (s, 1H), 6.69 (s, 1H), 4.55 (br s, 2H), 3.34 (s, 2H), 2.94 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 0.97 (s, 9H).

Ligand Binding Studies. See ref 20.

Pharmacokinetic Assays. Adult Harlen Sprague Dawley rats ($\sim$ 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 0.5, 1, 2, 4, 6, 8, 12, 24, and 30 h 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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Note Added after ASAP Posting

In the version posted March 16, 2004, on the second page, left column, line 4, the reference citation was changed from 6 to 7. In Tables 3 and 4, "relative" was removed from the column headings "relative IC_{50} ET_A". The manuscript was reposted March 17, 2004.

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