Articles

Endothelin Antagonists: Substituted Mesitylcarboxamides with High Potency and Selectivity for ET_A Receptors[1]

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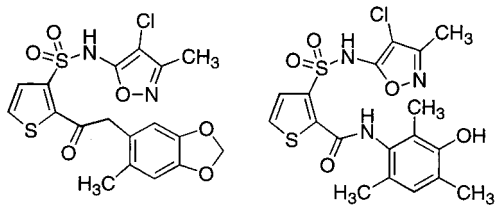
We have previously disclosed the discovery of 2,4-disubstituted anilinothiophenesulfonamides with potent ET_A-selective endothelin receptor antagonism and the subsequent identification of sitaxsentan (TBC11251, **1**) as a clinical development compound (Wu et al. *J. Med. Chem.* **1997**, *40*, 1682 and 1690). The orally active **1** has demonstrated efficacy in a phase II clinical trial of congestive heart failure (Givertz et al. *Circulation* **1998**, *98*, Abstr. #3044) and was active in rat models of myocardial infarction (Podesser et al. *Circulation* **1998**, *98*, Abstr. #2896) and acute hypoxia-induced pulmonary hypertension (Chen et al. *FASEB J.* **1996**, *10* (3), A104). We now report that an additional substituent at the 6-position of the anilino ring further increases the potency of this series of compounds. It was also found that a wide range of functionalities at the 3-position of the 2,4,6-trisubstituted ring increased ET_A selectivity by ∼10-fold while maintaining in vitro potency, therefore rendering the compounds amenable to fine-tuning of pharmacological and toxicological profiles with enhanced selectivity. The optimal compound in this series was found to be TBC2576 (**7u**), which has ∼10-fold higher ET_A binding affinity than **1**, high ET_A/ET_B selectivity, and a serum half-life of 7.3 h in rats, as well as in vivo activity.

Introduction

The endothelins are 21-amino acid residue peptides with strong vaso-active properties.[7,8] The endothelins function by binding to transmembrane G-protein-coupled receptors of which two major subtypes, ET_A and ET_B, have been identified.[9−13] Elevated levels of endothelins have been associated with a number of physiological[14,15] and pathological processes including: hypertension, congestive heart failure, renal failure, cerebral vasospasm, atherosclerosis, restenosis, myocardial infarcation, pulmonary disorders, and subarachnoid hemorrhage.[16−26] These studies have shown that the functions of ET-1 as related to the aforementioned pathological conditions are mediated via ET_A receptors, while some beneficial effects may be mediated by ET_B receptors. Accordingly, selective ET_A receptor antagonists may have clinical benefits for patients with those disease states. A number of non-peptide ET_A-selective antagonists have been reported: Ro61-1790,[27] BMS-182874,[28] TBC11251 (sitaxsentan),[3] PD156707,[29] PD180988,[30] SB217242,[31] SB247803,[32] and Z1611.[33] We have reported previously the discovery of TBC11251 (sitaxsentan, **1**; Chart 1),[3] which showed efficacy in a phase II clinical trial for congestive heart failure.[4] Efforts have continued in our laboratories to identify compounds with enhanced pharmacological properties.

Chart 1. TBC11251 and **7u**



1 (sitaxsentan, TBC11251) **7u** (TBC2576)

Chart 2 generalizes the drug design and optimization thought process. We have established a unique pharmacophore framework for selective ET_A antagonism[2] that contains a central thiophene ring, with a carboxamide and sulfonamide group at the 2- and 3-positions, respectively. The sulfonamide group in turn is substituted with a chloromethylisoxazole on the nitrogen, whereas the carboxamide nitrogen is substituted with an aryl group. Monosubstitution of the aryl group is most effective at the *para* position with a methyl substituent being the optimal equivalent to the 4,5-methylenedioxy group of **1**.[2] With a 4-methyl or 4,5-methylenedioxy group in place, substitution at the 2-position was most useful for improving binding affinity with methyl and cyano groups being the most desirable.[2]

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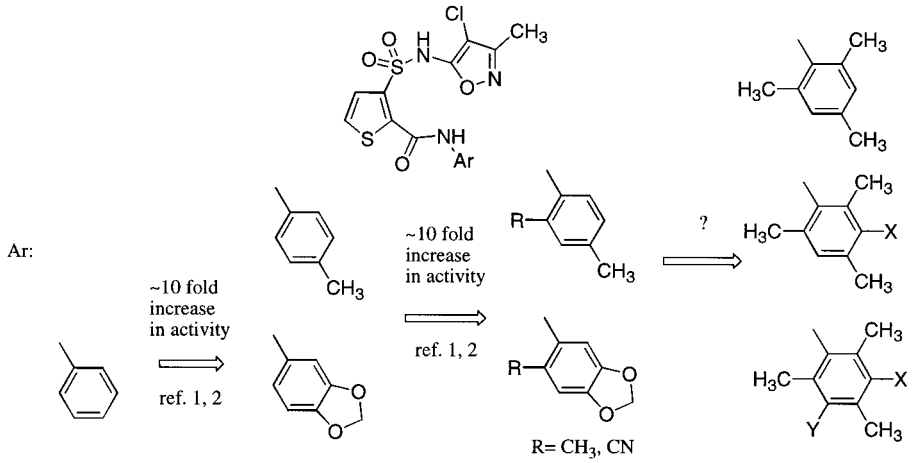
Chart 2. Evolution of Arylcarboxamides of the Thiophenesulfonamide Isoxazole

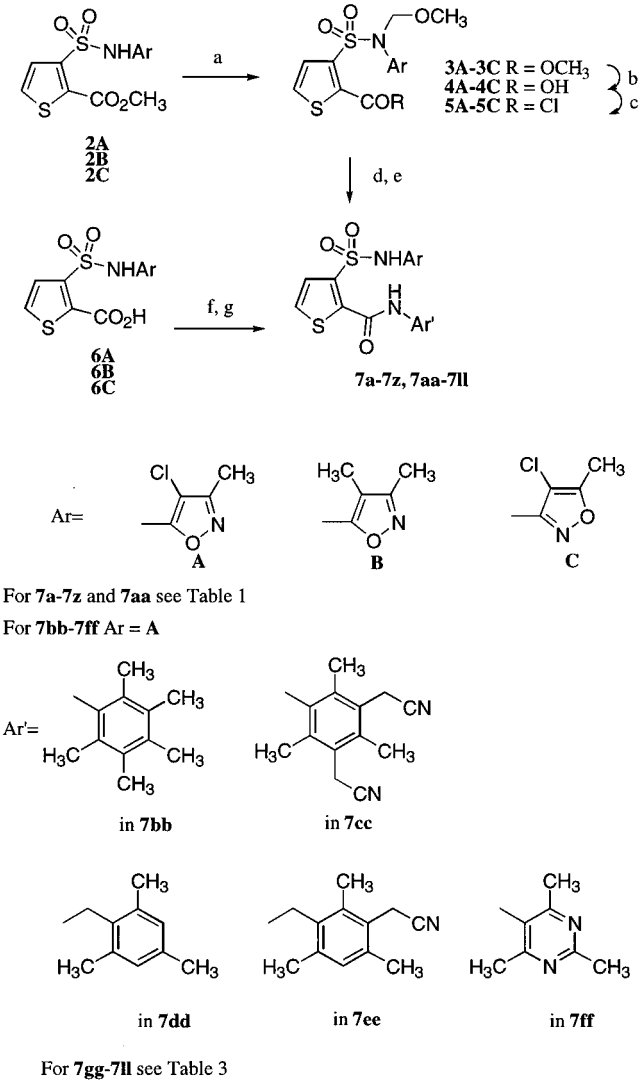


In this paper, we report that (1) with 2,4-dimethyl-substituted ring, an additional substitution at the 6-position, particularly methyl, increased binding affinity approximately 10-fold and (2) with 2,4,6-trimethyl-trisubstituted ring, substitution at the 3-position has little effect on ET_A binding potency but increases its selectivity by 1 order of magnitude, therefore providing a tool for manipulating the pharmacological properties of these antagonists.

Synthetic Chemistry

These sulfamoylthiophenecarboxamides **7a**−**7z**, **7aa**−**7ll** were synthesized generally in one of two ways (Scheme 1). If the aniline was sufficiently nucleophilic, a 1,1′-carbonyldiimidazole-mediated direct coupling to the thiophenecarboxylic acid **6** in hot DMF was applicable. For less nucleophilic anilines, it was necessary to employ the methoxymethyl (MOM)-protected thiophenecarbonyl chloride **5**, followed by removal of the MOM group under hot acidic conditions. The acid chloride **5** was made in three steps from the thiophenecarboxylate **2** by: (1) alkylation of the sulfonamido nitrogen with bromomethyl methyl ether in the presence of diisopropylethylamine; (2) saponification of the resulting methyl ester **3**; and (3) conversion of the resulting acid **4** to the corresponding acid chloride **5** with oxalyl chloride. Some of the resulting carboxamides were further modified as illustrated in Scheme 6. The synthesis of the required anilines is shown in Schemes 2−5. The trimethoxylcyanoaniline **9a** and the dimethylanilinocarboxylate **9b** were synthesized by nitration of the corresponding arenes **8a** and **8b** with nitric acid/sulfuric acid/acetic acid mixture, followed by zinc reduction (Scheme 2). Anilines **14**, **16**, **17**, and **20** required lengthier synthetic routes and started from the commercially available trimethylbenzyl chloride **10**. Chloride **10** was heated with sodium cyanide in DMSO to give trimethylphenylacetonitrile **11**. The nitrile **11** was nitrated, and the resulting nitro compound **12** was reduced using zinc powder in an aqueous methanolic solution of ammonium chloride to give the desired aniline **13**. The anilinonitrile **13** was converted to the methyl phenylacetate **14** by heating under reflux in a mixture of methanol and concentrated sulfuric acid. Direct nitration of the benzyl chloride **10** could be effected with nitronium tetrafluoroborate in dichloromethane to afford **15**. Displacement

Scheme 1. General Synthesis of Anilinosulfonamides[a]



For **7a-7z** and **7aa** see Table 1

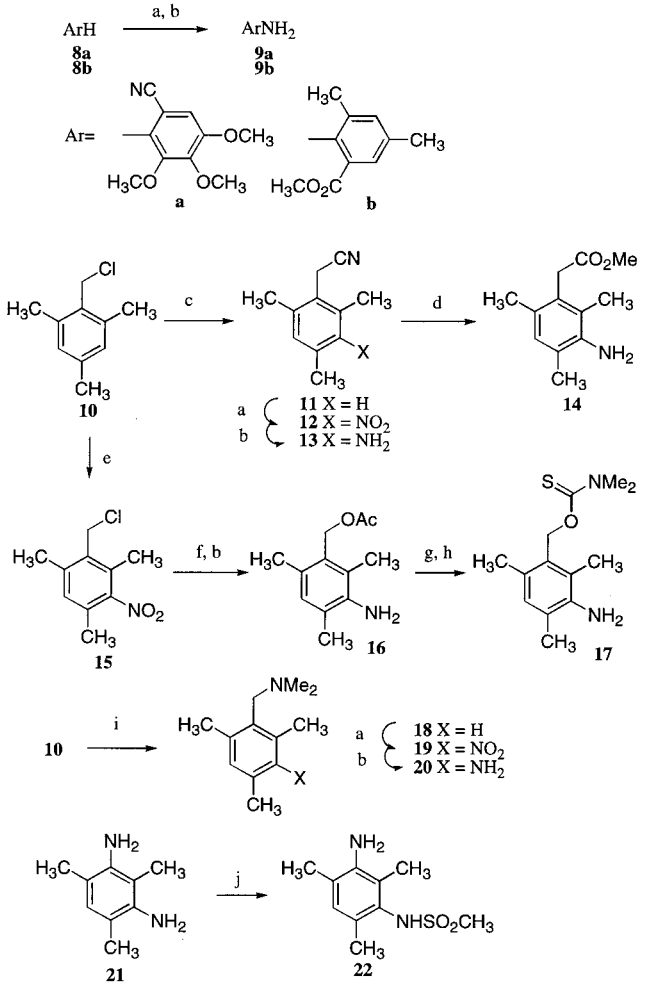
For **7bb-7ff** Ar = **A**

For **7gg-7ll** see Table 3

[a] Reagents: (a) $BrCH_2OMe/(i\text{-}Pr)_2NEt/THF$; (b) 1 N NaOH; (c) oxalyl chloride; (d) aniline/THF; (e) concentrated HCl/THF or MeOH or CH_3CN/reflux; (f) CDI/DMF; (g) aniline/80 °C.

of the chloride of **15** with sodium acetate in hot DMSO followed by routine reduction produced the acetoxyaniline **16**. Conversion of the chloride **10** to dimethylaminomethylaniline **20** was realized by sequential treatment with dimethylamine, followed by nitration and

Scheme 2. Synthesis of Required Anilines—Part 1[a]

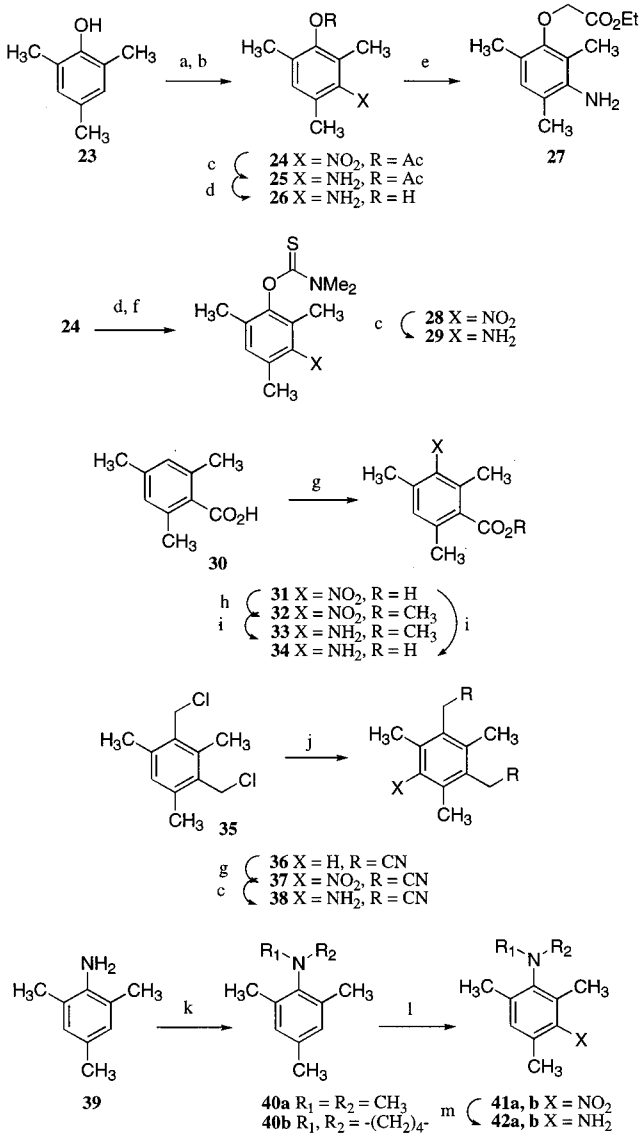


[a] Reagents: (a) HNO_3/H_2SO_4/HOAc; (b) Zn/NH_4Cl/MeOH/H_2O; (c) NaCN/DMSO; (d) H_2SO_4/MeOH/reflux; (e) NO_2BF_4; (f) NaOAc/DMSO; (g) NaOH/MeOH; (h) NaH/ClCSNMe$_2$; (i) NHMe$_2$/THF/H_2O; (j) $MeSO_2Cl/Et_3N$, recrystallization.

zinc reduction. The methanesulfonamidoaniline **22** was derived from trimethylphenylenediamine **21** by reaction with methanesulfonyl chloride.

The synthesis of anilines **25**−**27**, **29**, **33**, **34**, **38**, **42a**, and **42b** is outlined in Scheme 3. Since the direct nitration of trimethylphenol **23** proved problematic, **23** was acetylated prior to nitration and then reduced to the acetoxyaniline **25**. Selective *O*-alkylation of aminophenol **26**, generated by hydrolysis of **25**, was accomplished using cesium carbonate and ethyl bromoacetate to produce aminophenoxyacetate **27**. The aminothiourethane **29** was obtained in three steps from the nitroacetate **24** by sequential hydrolysis of the acetate, acylation of the phenol with dimethylthiocarbamoyl chloride, and reduction of the nitro functionality of **28** using iron in hot acetic acid.[34] Trimethylbenzoic acid **30** was nitrated with nitric acid/sulfuric acid to form **31**, which in turn was esterified with 2,2-dimethoxypropane[35] to afford nitrobenzoate **32**. Nitro compounds **31** and **32** were separately reduced to the corresponding anilines **34** and **33**, which were coupled with **5A** and **6A** to give carboxamides **7e** and **7c** (Table 1), respectively. Attempts to derive **7e** from **7c** were not successful due to the methyl ester's surprising resistance to basic hydrolysis and cleavage reactions using iodotrimethyl-

Scheme 3. Synthesis of Required Anilines—Part 2[a]

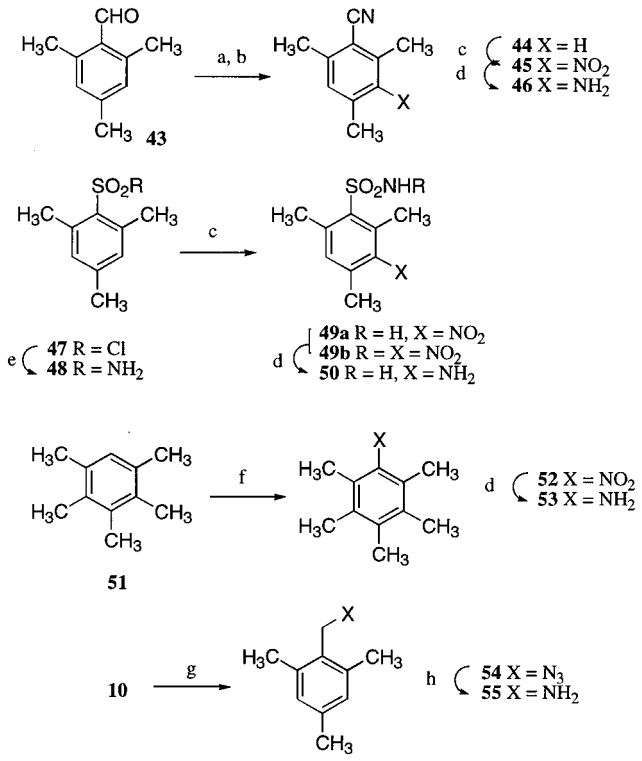


[a] Reagents: (a) AcCl/Et_3N; (b) HNO_3/HOAc; (c) Fe/AcOH, 90−110 °C/1 h; (d) 1 N NaOH/MeOH; (e) $Cs_2CO_3/BrCH_2CO_2Et$; (f) NaH/ClCSNMe$_2$; (g) HNO_3/H_2SO_4/HOAc; (h) $(MeO)_2CMe_2$/MeOH/AcCl; (i) Zn/NH_4Cl/MeOH/H_2O; (j) NaCN/DMSO; (k) (i) KN(SiMe$_3$)$_2$/THF, (ii) CH_3I or 1,4-dibromobutane; (l) HNO_3/H_2SO_4; (m) HCO_2NH_4/Pd−C/MeOH.

silane. The fully substituted aniline **38** was synthesized from the bis(chloromethyl)benzene **35** by sequential displacement with sodium cyanide in DMSO, nitration, and zinc reduction. The dimethylaminoaniline **42a** and the pyrrolidinoaniline **42b** were accessed via dialkylation of trimethylaniline **39** with iodomethane or 1,4-dibromobutane followed by nitration and reduction with ammonium formate in the presence of palladium.[36]

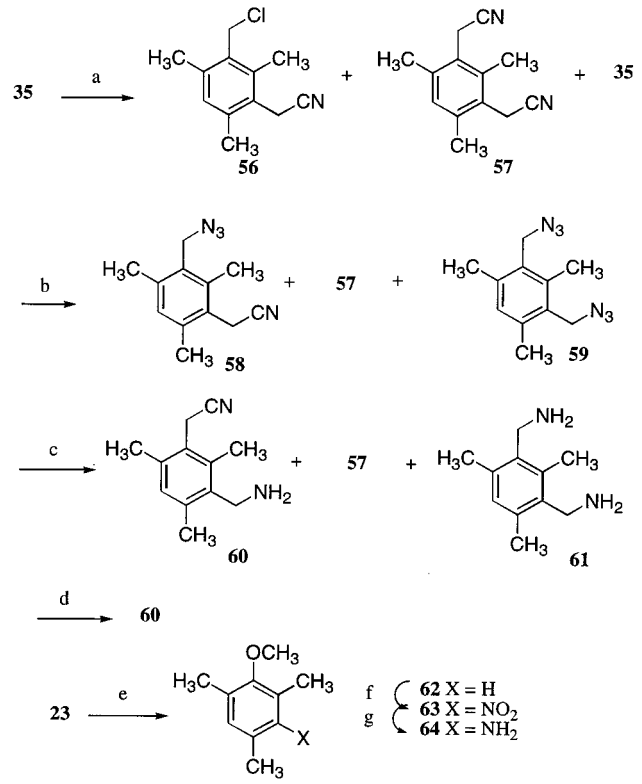
Scheme 4 delineates methodologies for the synthesis of anilines **46**, **50**, and **53** and benzylamine **55**. Mesitaldehyde (**43**) was first converted to benzonitrile **44** via oxime formation and dehydration in refluxing acetic anhydride,[37] followed by the routine nitration/reduction sequence to produce cyanoaniline **46**. Nitration of mesitylenesulfonamide **48**, obtained from the sulfonyl chloride **47** and ammonia, gave a 1:1 mixture of the desired product **49a** and dinitro compound **49b**. Zinc reduction of this mixture resulted in anilinosulfonamide **50** as a single product with the nitrosulfonamide group in **49b**

Scheme 4. Synthesis of Required Anilines—Part 3[a]



[a] Reagents: (a) NH_2OH; (b) Ac_2O/reflux; (c) HNO_3/H_2SO_4/HOAc; (d) $Zn/NH_4Cl/MeOH/H_2O$; (e) NH_4OH; (f) NO_2BF_4/DCM; (g) NaN_3/DMSO; (h) $Ph_3P/THF/H_2O$/reflux.

Scheme 5. Synthesis of Required Anilines—Part 4[a]



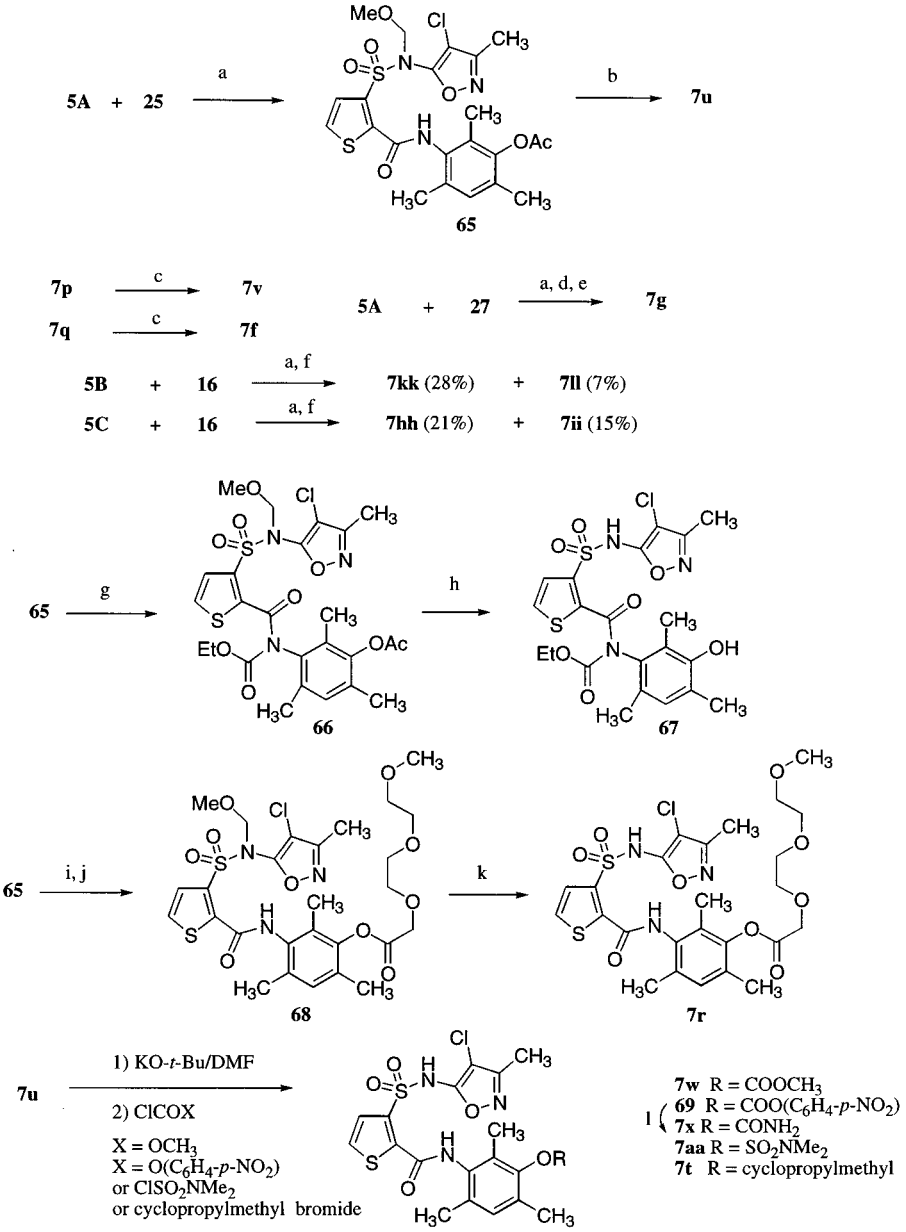
[a] Reagents: (a) 1 equiv NaCN/DMSO/rt; (b) NaN_3/DMSO/same pot as (a); (c) $Ph_3P/THF/H_2O$/reflux; (d) acid/base extraction/filtration, see Experimental Section; (e) MeI/NaH/THF; (f) NO_2BF_4/DCM; (g) $Zn/NH_4Cl/MeOH/H_2O$.

being reduced back to sulfonamide. The pentamethylaniline **53** was accessed via zinc reduction of pentamethylnitrobenzene **52**, which in turn was obtained by nitration of the commercially available pentamethylbenzene (**51**) using nitronium tetrafluoroborate. Standard nitration conditions were unsuccessful in this instance. Thus, the trimethylbenzyl chloride **10** was converted to the corresponding benzylamine **55** by displacement with sodium azide to give **54** followed by its reduction with triphenylphosphine in moist tetrahydrofuran.[38]

The synthesis of anilines and a benzylamine concludes in Scheme 5. The cyanomethyltrimethylbenzylamine **60** was derived from bis(chloromethyl)trimethylbenzene **35**. Treatment of **35** with 1 equiv of sodium cyanide in DMSO at room temperature gave a 2:1:1 mixture of the desired cyanide **56**, the dicyanide **57**, and the starting material **35**. This reaction mixture was subjected to excess sodium azide in hot DMSO to generate a 2:1:1 mixture of the cyanoazide **58**, the carried over dicyanide **57**, and the diazide **59**. This mixture in turn was heated with triphenylphosphine in moist THF to afford a mixture of the cyanoamine **60**, the diamine **61**, and the carried over dicyanide **57** in the same ratio. The desired product **60** was isolated from the mixture by acid—base extractions. The methoxyaniline **64** was accessed from **23** by sequential methylation of **62**, nitronium tetrafluoroborate-mediated nitration (**63**), and subsequent zinc reduction.

The synthesis of **7u**, **7hh**, **7ii**, **7kk**, and **7ll** and the derivatization of **7u** are shown in Scheme 6. Coupling of aniline **25** with acid chloride **5A** proceeded smoothly, and the acetoxy group of **65** was removed simultaneously during acid-catalyzed MOM cleavage to give **7u**. Direct coupling using the corresponding free aminophenol **26** with acid chloride **5A** generated intractable products. Similarly, acetoxymethylaniline **16** was coupled with **5B** or **5C**, and the MOM groups of the resulting intermediates were cleaved with 2 N sulfuric acid in acetic acid. The milder conditions allowed partial liberation of the benzyl alcohol to produce **7kk** and **7ll** or **7hh** and **7ii**, respectively. Base-catalyzed hydrolysis of **7p** and **7q** in methanol generated alcohol **7v** and acid **7f**, respectively. Compound **7g** was accessed by a sequence of coupling between **7A** and **27**, cleavage of the MOM group with BCl_3, and hydrolysis of the ethyl ester. The lability of the acetate side chain to hot acidic conditions necessitated the use of BCl_3 to deprotect MOM. It was possible to acylate the amide group in **65** with ethyl chloroformate to afford **66**. Acidic hydrolysis deprotected both the sulfonamide and the phenolic oxygen in **66** to yield the *N*-acylated analogue of **7u** (**67**). The acetoxy group in **65** could be removed with potassium carbonate in methanol, and the resulting phenoxy group could then be selectively acylated with 2-[2-(2-methoxyethoxy)-ethoxy]acetyl chloride to generate **68**. Removal of the MOM group then produced analogue **7r**. The hydroxyl group in **7u** could be selectively acylated using potassium *tert*-butoxide as the base, and carbonates **7w** and **69** were synthesized using methyl chloroformate and *p*-nitrophenyl chloroformate as the quenching reagents, respectively. Conversion of **69** to the corresponding carbamate **7x** was effected with ammonia. The sulfamate **7aa** and the cyclopropylmethyl ether **7t** were obtained when *N*,*N*-dimethylsulfamoyl chloride and

Scheme 6. Synthesis and Derivatization of Some Thiophenecarboxamides[a]



[a] Reagents: (a) THF/rt; (b) concentrated HCl/THF/reflux; (c) NaOMe/MeOH/rt/30 min/91%; (d) BCl_3/DCM/−78 °C; (e) LiOH/THF/H_2O; (f) HOAc/H_2O/2 N H_2SO_4/75−80 °C/3.5 h; (g) KHMDS/THF, EtOCOCl; (h) concentrated HCl/EtOH/heat, 42%; (i) K_2CO_3/CH_3OH; (j) DMAP/DCM/$ClCOCH_2(OCH_2CH_2)_2OCH_3$/70%; (k) 1 N HCl/THF/reflux/4 h, 61%; (l) $NH_3 \cdot H_2O$.

cyclopropylmethyl bromide were utilized as quenching reagents, respectively.

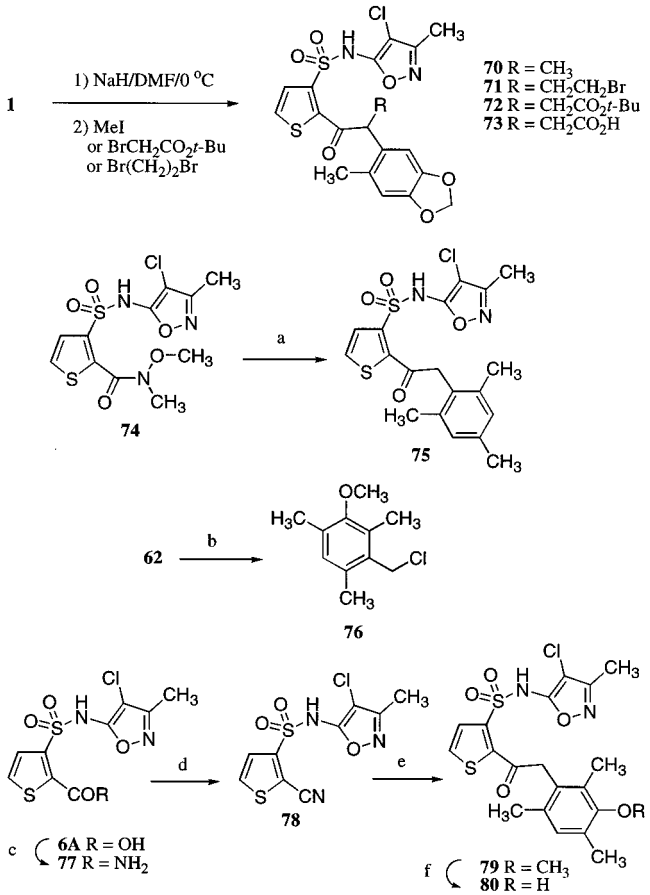
The synthesis of some ketones is described in Scheme 7. Monoalkylation at the α-position of ketone **1** was accomplished using sodium hydride as the base. Accordingly, alkylation with iodomethane, *tert*-butyl bromoacetate, or 1,2-dibromoethane afforded **70**, **72**, and **71**, respectively. Treatment of **72** with TFA in dichloromethane generated acid **73**. The Weinreb amide **74**[39] was treated with trimethylbenzylmagnesium bromide in THF followed by acidic workup to yield ketone **75**. The 3-hydroxy ketone **80** was obtained via a Grignard reaction of the benzylmagnesium chloride, generated freshly from **76**, with nitrile **78**, followed by demethylated with BBr_3.[40] The required benzyl chloride **76** was accessed by chloromethylation of methoxymesitylene **62**. The nitrile **78** was in turn generated by 1,1′-carbonyldiimidazole-mediated coupling of the acid **6A** with ammonia to give the primary amide **77**, which was subsequently dehydrated in hot $POCl_3$.[41]

The synthetic methods, yield of the last step, melting points, and formulas as established by elemental analysis for all target compounds are summarized in Table 5.

Discussion

Structure−Activity Relationships. TBC11251 (**1**) binds competitively to human ET_A receptors with an IC_{50} of 1.7 nM (IC_{50} for ET_B = 9800 nM).[3] Our efforts have been to identify second-generation compounds with significantly increased ET_A potency and selectivity versus **1**. Accordingly, the relative IC_{50} for ET_A was defined as ET_A IC_{50} value of a compound divided by that of **1**. Selectivity for ET_A was expressed as the ratio of ET_B IC_{50} value over that of ET_A. The inhibition of endothelin binding to ET_A and ET_B receptors was

Scheme 7. Synthesis of Analogues with a Ketone Linker[a]



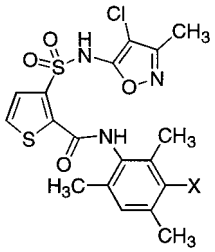
[a] Reagents: (a) 2,4,6-trimethylbenzylmagnesium chloride/THF; (b) HCHO/concentrated HCl/DCM/*n*-Bu_4NBr; (c) CDI/NH_4OH/DMF; (d) $POCl_3$/60 °C; (e) (i) **76**, Mg/THF, (ii) H_3O^+; (f) BBr_3/DCM.

measured using ^{125}I-labeled ET-1 competition assays. Relative ET_A binding potency and selectivity are presented in Tables 1−3.

The prototypical mesitylcarboxamide **7a** had a relative ET_A IC_{50} of 0.1 and was 10-fold more potent than **1** (Table 1). The structural differences between **1** and **7a** are the following: (1) the ketone linkage in **1** was replaced by its bioisotere amide tether that has previously been shown to be an equipotent change;[3] (2) the methylenedioxy group at the 4,5-position of the phenyl in **1** was replaced with a 4-methyl group that has also been previously demonstrated not to affect binding affinity;[2] and (3) the only change that caused this 10-fold increase in potency appeared to be the additional methyl substitution at the 6-position. Another 2,4,6-trisubstituted arylcarboxamide **7d** (the coupling product of acid **6A** and aniline **9b**) with a methyl ester at the 2-position also showed a large potency improvement over **1**. When a methyl ester was substituted at the 3-position of the mesityl group (**7c**), the binding affinity was not much affected consistent with our earlier findings, but the ET_A/ET_B selectivity was increased ~6-fold as compared to **7a**. The cyanotrimethoxy compound **7b** (synthesized by coupling **6A** and **9a**) was almost 2 orders of magnitude more potent than **1**.

The fact that the 2,4,6-trimethylphenyl system afforded a 10-fold increase in potency while additional substitution at the 3-position increases ET_A selectivity while maintaining binding affinity provided ample

Table 1. Effect of Mesitylene Substitution on [^{125}I]ET-1 Binding

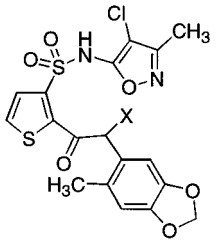


entry	X	relative ET_A IC_{50}[a] (*n*)	selectivity for ET_A[b]
1	see Chart 1	1	3 211
7a	H	0.10 (1)	7 047
7b	see SAR text	0.01 ± 0 (4)	29 208
7c	$-CO_2CH_3$	0.21 (1)	41 944
7d	see SAR text	0.16 (1)	36 204
7e	$-CO_2H$	0.79 (1)	ND
7f	$-CH_2CO_2H$	0.12 ± 0.02 (2)	59 864
7g	$-OCH_2CO_2H$	0.05 (1)	23 014
7h	$-CH_2N(CH_3)_2$	0.88 (1)	ND
7i	$-N(CH_3)_2$	0.07 (1)	36 644
7j	$-N(-CH_2(CH_2)_2CH_2-)$	0.15 (1)	
7k	$-CN$	0.10 ± 0.02 (3)	37 672
7l	$-CH_2CN$	0.08 ± 0.04 (4)	32 881
7m	$-SO_2NH_2$	0.06 ± 0.04 (2)	27 825
7n	$-NHSO_2CH_3$	0.11 ± 0.02 (3)	41 693
7o	$-OCOCH_3$	0.11 (1)	6 966
7p	$-CH_2OCOCH_3$	0.06 ± 0.01 (3)	849 570
7q	$-CH_2CO_2CH_3$	0.07 ± 0.01 (2)	113 510
7r	$-(OCOCH_2(OCH_2CH_2)_2OCH_3)$	0.50 (1)	
7s	$-OCH_3$	0.06 (1)	56 364
7t	$-OCH_2CH(CH_2)_2$	0.07 (1)	59 429
7u	$-OH$	0.11 ± 0.05 (4)	10 900
7v	$-CH_2OH$	0.04 ± 0.01 (3)	111 390
7w	$-OCO_2CH_3$	0.05 (1)	22 233
7x	$-OCONH_2$	0.34 (1)	6 700
7y	$-OCSN(CH_3)_2$	0.09 (1)	22 594
7z	$-(CH_2OCSN(CH_3)_2)$	0.21 (1)	16 181
7aa	$-OSO_2N(CH_3)_2$	2.30 (1)	1629
7bb	see Scheme 1	1.97 (1)	1083
7cc	see Scheme 1	3 111 (1)	3
7dd	see Scheme 1	1 153 119 (1)	0.004
7ee	see Scheme 1	56.35 (1)	95
7ff	see Scheme 1	0.25 (1)	9 162
67	see Scheme 6	5.56 (1)	1 528

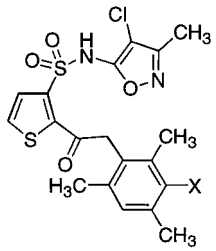
[a] Relative ET_A IC_{50} was calculated as IC_{50} of compound/IC_{50} of TBC11251; ET_A IC_{50} for TBC11251 is 1.7 nM. [b] Expressed as ET_B IC_{50}/ET_A IC_{50}.

opportunity to manipulate the physical and pharmacological properties of this class of compounds by varying functionality at the 3-position. It was preferable to have a polar group at the 3-position as this was observed to both increase aqueous solubility (Table 4) and, more importantly, lower the hemolytic activity observed with some compounds, such as **7a**.[42] We first investigated cases where a carboxyl group was tethered to the 3-position via 0, 1, and 2 atom linkers (**7e**−**g**). Compared with the parent compound **7a**, the benzoic acid **7e** was 7-fold less active while the phenoxyacetic acid **7g** was 2-fold more active. The phenylacetic acid **7f** was equipotent. A dimethylamino group attached via a methylene group (**7h**) or directly (**7i**) to the 3-position was then examined. The more distal amino group (**7h**) lowered binding affinity by 8-fold while **7i** maintained activity. This is in contrast with a more removed carboxyl group being preferred for in vitro activity. A bulkier pyrrolidino group (**7j**) was essentially equipotent to **7a**. A cyano (**7k**) or cyanomethyl (**7l**) substitution at the 3-position

Table 2. [^{125}I]ET-1 Binding of Ketone Analogues



1, 70, 71, 73

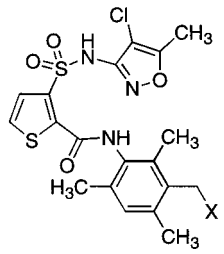


75, 79, 80

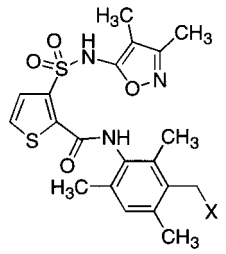
entry	X	relative ET_A IC_{50}[a] (*n*)	selectivity for ET_A[b]
1	−H	1	3 211
70	$-CH_3$	5.63 (1)	ND
71	$-CH_2CH_2Br$	38.36 (1)	59
73	$-CH_2CO_2H$	5.36 (1)	ND
75	−H	0.24 ± 0.18 (2)	9 390
79	$-OCH_3$	0.13 (1)	7 638
80	−OH	0.08 (1)	10 814

[a] Relative ET_A IC_{50} was calculated as IC_{50} of compound/IC_{50} of TBC11251; ET_A IC_{50} for TBC11251 is 1.7 nM. [b] Expressed as ET_B IC_{50}/ET_A IC_{50}.

Table 3. Effect of Isoxazoles on [^{125}I]ET-1 Binding



7gg-7ii



7jj-7ll

entry	X	relative ET_A IC_{50}[a] (*n*)	selectivity for ET_A[b]
7gg	−CN	0.06 ± 0 (2)	8 554
7hh	$-OCOCH_3$	0.03 ± 0.03 (2)	38 358
7ii	−OH	0.06 (1)	3 499
7jj	−CN	0.10 ± 0.02 (2)	14 558
7kk	$-OCOCH_3$	0.06 (1)	160 325
7ll	−OH	0.07 (1)	30 409

[a] Relative ET_A IC_{50} was calculated as IC_{50} of compound/IC_{50} of TBC11251; ET_A IC_{50} for TBC11251 is 1.7 nM. [b] Expressed as ET_B IC_{50}/ET_A IC_{50}.

did not have much effect on binding, nor did a sulfamoyl (**7m**) or methanesulfonamido group (**7n**). As expected, small to medium sized nonpolar groups such as acetoxy (**7o**), acetoxymethyl (**7p**), methyl acetate (**7q**), methoxy (**7s**), and cyclopropylmethoxy (**7t**) all had negligible effects on potency. Note that even the lengthy methoxyethoxyethoxyacetoxy group (**7r**) only caused a 5-fold loss of activity. Other groups of various polarity such as hydroxyl, carbonate, carbamate and thiocarbamate were also studied giving mixed results. The phenol **7u** was equipotent to **7a**, while the benzyl alcohol **7v** showed slightly increased potency. Although the carbonate **7w** increased potency by 2-fold, the carbamate **7x** and thiocarbamate **7z** decreased activity by 2−3-fold, while the thiocarbamate **7y** was equipotent. The sulfamate **7aa** lost 23-fold of potency and 4-fold selectivity as compared to **7a**. Compound **7aa** was the only compound to significantly deviate from the general trend of 3-substitution: increasing ET_A/ET_B selectivity while maintaining ET_A potency.

Table 4. po Half-Lives, Aqueous Solubility, and Effective Oral Dose in Acute Pulmonary Hypertension Rat Model

entry	po $t_{1/2}$ (h)	aqueous solubility (mg/mL)[a]	effective oral dose (mg/kg)[b]
1	4.1	<10	5
7a	2.5	23[c]	ND[d]
7b	<1	43	ND
7i	1.0	50	ND
7k	1.8	37	ND
7l	2.3	<10	5
7m	1.5	30	5
7n	2.1	<10	ND
7p	0.5	ND	5
7s	1.8	<10	ND
7u	7.3	83	5
7v	1.4	65	15
7w	NM[e]	ND	ND
7x	NM	ND	ND
7y	NM	ND	ND
7gg	3.1	70[c]	29% inhibn at 5
7jj	1.8	<10	64% inhibn at 5
79	3.86	ND	ND
80	3.9	ND	ND

[a] Measured in 0.2 M phosphate buffer of pH 7.4. [b] Administered orally 2 h prior to experiment; lowest tested dose to cause 90−100% inhibition of mean pulmonary arterial pressure; no significant effects on mean systemic arterial pressure and heart rate. [c] Measured using preformed sodium salt of compound in saline (9 mg/mL). [d] ND, not determined. [e] NM, not measurable; only **7u** detected.

Accordingly, for the 3-position, most groups did not have a significant effect on in vitro binding of this class of compounds. The effect of an additional substitution at the 5-position was explained with the two pentasubstituted arylcarboxamides **7bb** and **7cc**. The pentamethylphenyl compound **7bb** caused a 18-fold loss of activity, while the analogue of **7l** with a cyanomethyl group at the 5-position (**7cc**) was even more deleterious, giving micromolar activity. As a result, no more pentasubstituted arylcarboxamides were pursued. The effect of moving the mesityl group away from the amide by a carbon unit was also examined. Such an analogue of **7a** (**7dd**) was devoid of any significant activity, while the analogue of **7l** (**7ee**) was >700-fold less potent. One example incorporating a heterocyclic aniline unit, the pyrimidine analogue of **7a** (**7ff**), was 2.5-fold less active than **7a**. Compound **67**, an analogue of **7u** with the amide nitrogen substituted with an ethoxycarbonyl group, was 50-fold less active than **7u**.

Some thiophenesulfonamides with a ketone linkage, either as derivatives of **1** (**70**, **71**, **73**) or as analogues of **7a** (**75**) or **7u** (**79**, **80**), were also studied, and the data is summarized in Table 2. The bioisosterism of the ketone and amide linkages was again demonstrated by equipotencies of mesitylenes **75** (relative ET_A IC_{50} = 0.24) and **7a** (0.10 nM), methoxymesitylenes **79** (0.08) and **7s** (0.06), and trimethylphenols **80** (0.08) and **7u** (0.11). However, when the α-position of ketone **1** was substituted with methyl (**70**), bromoethyl (**71**), or acetic acid (**73**), the binding affinity was lowered by 5.6-, 37-, or 5.4-fold, respectively. This is comparable to a similar deleterious effect in the amide linker series as seen in **67** (Table 1).

We then replaced the 4-chloro-3-methyl-5-isoxazole moiety on the sulfonamide nitrogen with its isomeric 4-chloro-5-methyl-3-isoxazole (**7gg**−**ii**) or 3,4-dimethyl-

Table 5. Synthetic and Physical Data

entry	synth method	% yield	mp, °C	formula[a]
7a[b]	A	27	45−48	$C_{18}H_{17}ClN_3NaO_4S_2\cdot0.35EtOAc\cdot1.1H_2O$
7b	A	11	88−90	$C_{19}H_{17}ClN_4O_7S_2\cdot0.3EtOAc\cdot0.2TFA$
7c	A	3	66−70	$C_{20}H_{20}ClN_3O_6S_2$[c]
7d	A	10	152−154	$C_{19}H_{18}ClN_3O_6S_2$[c]
7e	B	56	179−181	$C_{19}H_{18}ClN_3O_6S_2$
7f	Scheme 6	∼100	110−113	$C_{20}H_{20}ClN_3O_6S_2\cdot1.18C_4H_8O$
7g	B	50	187−188	$C_{20}H_{20}ClN_3O_7S_2\cdot0.45TFA$
7h	A	6	92−94	$C_{21}H_{25}ClN_4O_4S_2\cdot1.3TFA$
7i[b]	B	77	185−188	$C_{20}H_{22}ClN_4NaO_4S_2\cdot NaHCO_3\cdot H_2O$
7j[b]	B	17	178−180	$C_{22}H_{24}ClN_4NaO_4S_2\cdot1.2NaHCO_3\cdot1.2H_2O$
7k	B	49	72−75	$C_{19}H_{16}ClN_4O_4S_2\cdot1.5H_2O$
7l[b]	A	10	100−103	$C_{20}H_{18}ClN_4NaO_4S_2\cdot2H_2O$
7m	B	74	214−217	$C_{18}H_{19}ClN_4O_6S_3$
7n	A	9	130−133	$C_{19}H_{21}ClN_4O_6S_3\cdot0.1TFA$
7o[b]	B	23	192−195	$C_{20}H_{19}ClN_3NaO_6S_2$[c]
7p[b]	A	3	90−93	$C_{21}H_{21}ClN_3NaO_6S_2\cdot1.5H_2O$
7q	A	8	75−78	$C_{21}H_{22}ClN_3O_6S_2\cdot0.35C_6H_{14}$
7r[b]	Scheme 6	61	130−133	$C_{25}H_{29}ClN_3NaO_9S_2\cdot H_2O$
7s	B	16	34−39	$C_{19}H_{20}ClN_3O_5S_2$[c]
7t[b]	ref 49		155−158	$C_{22}H_{23}ClN_3NaO_5S_2\cdot0.35NaHCO_3\cdot0.35H_2O$
7u	Scheme 6	65	75−78	$C_{18}H_{17}ClN_3O_5S_2\cdot2.5H_2O$
7v	Scheme 6	93	117−120	$C_{19}H_{20}ClN_3O_5S_2$
7w[b]	ref 49		155−165	$C_{20}H_{19}ClN_3NaO_7S_2\cdot0.4NaHCO_3\cdot0.6H_2O$
7x[b]	ref 49		175−182	$C_{19}H_{18}ClN_4NaO_6S_2$[c]
7y[b]	B	82	188−190	$C_{21}H_{22}ClN_4NaO_5S_3\cdot0.3NaHCO_3\cdot1.5H_2O$
7z[b]	B	26	198−200	$C_{22}H_{23}ClN_4NaO_5S_3\cdot0.5NaHCO_3\cdot1.6H_2O$
7aa[b]	ref 49		169−174	$C_{20}H_{22}ClN_4NaO_7S_3\cdot0.3NaHCO_3\cdot1.0H_2O\cdot0.3EtOAc$
7bb	B	38	196−198	$C_{20}H_{22}ClN_3O_4S_2$
7cc	B	4	185−187	$C_{22}H_{20}ClN_5O_4S_2\cdot0.5TFA$
7dd	B	83	175−177	$C_{19}H_{20}ClN_3O_4S_2\cdot0.3TFA$
7ee	B	30	76−79	$C_{21}H_{21}ClN_4O_4S_2\cdot0.1TFA$
7ff[b]	B	12	170−175	$C_{16}H_{15}ClN_5NaO_4S_2\cdot0.5NaHCO_3\cdot1.5H_2O$
7gg[b]	A	30	187−205	$C_{20}H_{18}ClN_4NaO_4S_2\cdot1.5H_2O$
7hh	B	21	190−210	$C_{21}H_{22}ClN_3O_6S_2\cdot1.5H_2O$
7ii	B	15	120−135	$C_{19}H_{20}ClN_3O_5S_2\cdot0.5H_2O$
7jj	B	32	105−108	$C_{21}H_{22}ClN_4O_4S_2\cdot0.8H_2O$
7kk	B	28	75−77	$C_{22}H_{25}ClN_3O_6S_2\cdot0.1TFA$
7ll	B	7	110−115	$C_{20}H_{23}ClN_3O_5S_2\cdot0.42CH_3CN\cdot0.37EtOAc$
67[b]	Scheme 6	42	161−163	$C_{21}H_{21}ClN_3NaO_7S_2\cdot0.3NaHCO_3\cdot H_2O$
70	ref 49		65−68	$C_{19}H_{17}ClN_2O_6S_2$
71	ref 49		60−63	$C_{20}H_{18}BrClN_2O_6S_2$
73	ref 49		76−79	$C_{20}H_{17}ClN_2O_8S_2\cdot0.6TFA\cdot0.6HOAc\cdot0.7CH_3CN$
75	Scheme 7	31	42−46	$C_{19}H_{19}ClN_2O_4S_2$[c]
79	Scheme 7	53	175−182	$C_{19}H_{19}ClN_4O_6S_2$[c]
80	Scheme 7	81	82−85	$C_{19}H_{19}ClN_2O_5S_2$[c]

[a] Analysis for C, H, N was within 0.4% of theory. [b] Data are for the corresponding sodium salt, prepared according to Blok et al.[48] [c] C, H, N not done due to insufficient sample; homogeneity established by two diverse analytical HPLC systems.

5-isoxazole (**7jj**−**ll**) for compounds **7l**, **7p**, and **7v** to see what effects such a change would have (Table 3). In the 3-isoxazole series, the cyano (**7gg**) and acetoxy (**7hh**) compounds were marginally more potent than their 5-isoxazole counterparts **7l** and **7p**, and the alcohol (**7ii**) was slightly less active than **7v**. On the other hand, in the dimethylisoxazole series, the cyano (**7jj**), acetoxy (**7kk**), and hydroxy (**7ll**) all were slightly less potent. The previously established trend that chloro-3-isoxazolesulfonamides are ∼2-fold more potent than chloro-5-isoxazoles, which in turn are ∼5-fold more active than dimethylisoxazoles,[43] was not repeated in these much more potent antagonists.

Pharmacokinetics and in Vivo Efficacy. Selected compounds from Tables 1 and 3 with high potencies were administered orally at 50 mg/kg to rats, and serum half-lives were measured (see Experimental Section for details) and are summarized in Table 4. Our reference compound **1** had an oral half-life of 4.5 h, as did the prototypical mesitylcarboxamide **7a** (2.5 h). In contrast, the trimethoxycyanide **7b** had a very short half-life probably due to enzymatic demethylation reactions.[42] As a result we did not further pursue this methoxy series. Likewise, demethylation reactions perhaps also result in short half-lives of the dimethylamino compound **7i** and the methoxy compound **7s**, having half-lives of 1.0 and 1.8 h, respectively. However, some analogues containing a cyano group did not show increased elimination, as exemplified by **7k** and its next higher homologue **7l** having half-lives of 1.8 and 2.3 h, respectively, as compared with the corresponding cyano-free compound **7a** (2.5 h). Incorporation of the rather polar sulfonamide group as in **7m** caused increased elimination, and **7m** had a half-life of 1.5 h. The less polar reversed sulfonamide **7n** was slightly more stable toward metabolism as shown by its serum half-life of 2.1 h. Analogue **7p**, which contains a nonpolar ester group, had a short half-life (0.5 h). As expected it was enzymatically hydrolyzed to its corresponding hydroxy compound **7v**, which had a short half-life (1.4 h). The lower homologue of **7v** (**7u**), however, exhibited enhanced stability toward metabolism with a half-life of 4.1 h, twice as long as that of **7a**. The fact that both the two *ortho*- and *para*-positions of the phenolic hydroxy group of **7u** are substituted with a methyl group seemed to have effectively protected it from being oxidized to *o*-

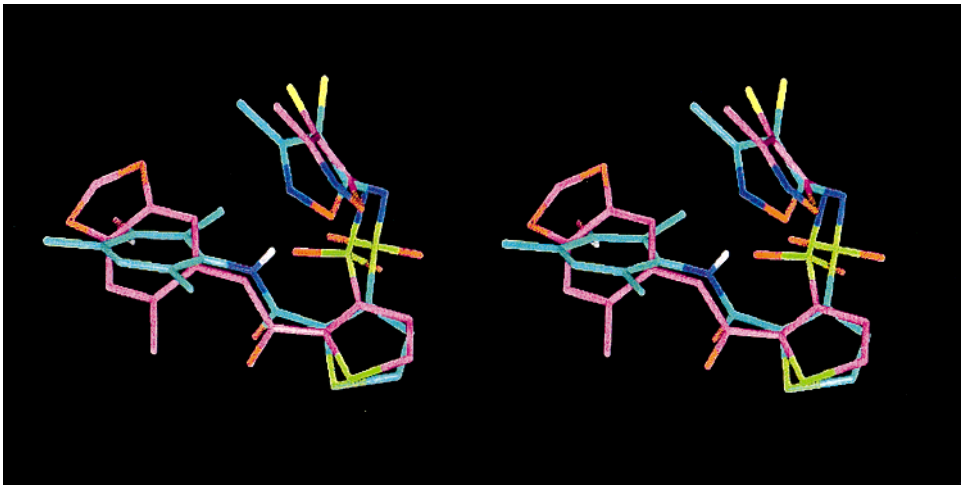


Figure 1. Stereoview of energy-minimized structures of **1** and **7u**. The hydrogen atoms are not shown except for the amide and phenol hydrogens (in white). The oxygen atoms are colored red, nitrogen blue, chlorine yellow, and sulfur green. The carbon atoms are shown in magenta for **1** and in cyan for **7u**, respectively.

or *p*-quinones, albeit the aryl ring is so electron rich. Designed as prodrugs of **7u**, compounds **7w**−**y** were rapidly metabolized into **7u**. Since **7u** was the only detectable compound by HPLC analysis, oral half-lives for **7w**−**y** could not be measured. The chloro-3-isoxazole **7gg** had a slightly longer half-life (3.1 h) than the chloro-5-isoxazole **7l** (2.3 h), while the half-life of dimethylisoxazole **7jj** was 2-fold shorter than that of **7l**. The analogue of **7s** with a ketone linker (**79**) showed a 2.2-fold extension of serum half-life relative to the amide linked **7s**, indicating that the ketone provided additional protection for an already quite hindered amide against hydrolytic enzymes. However, a similar amide to ketone linker change (**7u** to **80**) had an opposite impact on their half-lives (7.3 h for **7u** and 3.9 h for **80**).

Those compounds with an acceptable half-life were then tested in a rat model of acute hypoxia-induced pulmonary hypertension.[43] The compounds were orally administered 2 h prior to hypoxia, and the lowest dose tested that could effect 90−100% inhibition of pulmonary hypertension is reported in Table 4. At the doses tested, systemic arterial pressure and heart rate were not significantly affected. Compounds **7l**, **7gg**, and **7jj** all had a cyanomethyl group on the aniline ring but with chloro-5-isoxazole, chloro-3-isoxazole, and dimethylisoxazole, respectively, on the sulfonamide group. They were selected to test the isoxazole effect on in vivo potency. Having the standard isoxazole and an acceptable half-life of 2.3 h, compound **7l** showed good activity at 5 mg/kg via oral dosing. The dimethylisoxazole analogue **7jj**, with a slightly shorter half-life, was also very effective at 5 mg/kg. Surprisingly, the 3-isoxazole **7gg** with a half-life of 3.1 h did not show activity at the same dose. Although the precise reason for this unexpected result is unknown, it might be explained by the extremely high degree of protein binding of **7gg**,[42] so that the free concentration of the compound was too low to be effective. Compound **7m**, with a desirable polar sulfamoyl group on the aniline ring, prevented pulmonary hypertension at 5 mg/kg effectively. Compound **7p** was designed as a prodrug of the benzyl alcohol **7v**, although **7p** itself had as good binding affinity as **7v**. Esterification of the hydroxyl group proved to be useful in increasing duration of action: **7v** with a rather short half-life of 1.4 h showed activity only at 15 mg/kg, while the prodrug **7p**, with a half-life of 0.5 h with **7v** being its active metabolite, was very effective at 5 mg/kg. More excitingly, the lower homologue of **7v**, phenol **7u** with a good half-life of 7.3 h, demonstrated good effectiveness at 5 mg/kg. The polar phenolic hydroxyl rendered **7u** with good aqueous solubility and stability compared with an ester functionality.

Molecular Modeling. As exemplified by **7u** (Figure 1), the molecular modeling studies revealed that the compounds studied here can adopt the same conformation that was proposed to be the bioactive conformation of **1** and its analogues.[45] The three-dimensional quantitative structure−activity relationships (3D-QSAR) of **1** and its analogues were studied using the comparative molecular field analysis (CoMFA).[46] The bioactive conformation was suggested in conjunction with the common conformation analysis on the active compounds. It is mainly stabilized by the interactions between the isoxazole and phenyl moieties. The calculation and graphic works were done using the SYBYL 6.4 molecular modeling program.[47]

The stereoview of energy-minimized structures of **1** and **7u** is shown in Figure 1. The structures were superimposed to each other using the heavy atoms of three rings and the sulfonamide bond. Two compounds overlap very well with each other as indicated by the small root-mean-square deviation of the superimposition (0.8 Å). This allows two compounds to interact with the ET_A receptor in the same binding mode. As expected, little 3D-structural difference is observed on the right-hand side of the molecules, which have identical 2D-structure. However, **7u** differs from **1** by having an additional *ortho*-substituent, smaller groups in 4- and 5-positions of the phenyl ring, and an amide linker. As a result, two phenyl ring planes form an angle of ∼35°, placing the corresponding substituents at slightly different locations. The CoMFA results suggested that the steric interactions around the *ortho*-position of the phenyl ring might improve both the potency and selectivity,[45] consistent with current experimental results (Table 1). The activity enhancements of **7u** vs **1** are 10-fold for potency and 30-fold for selectivity, respectively. The disruptive effect of a complete substitution of the

phenyl group may be due to an excluded volume effect around the 5-position because **7bb** and **7cc** could still adopt the tentative bioactive conformation. On the contrary, **7dd** and **7ee** lost their activity because they would not adopt the bioactive conformation due to the additional methylene group in the linker.

Conclusion

We have synthesized and assayed a number of 3-isoxazolylsulfamoyl-2-thiophenecarboxamides with a 2,4,6-tri-, 2,3,4,6-tetra-, or pentasubstituted phenyl group on the amide nitrogen. The class of compounds containing 2,4,6-trisubstituted aryl groups exhibited ~10-fold increase of binding affinity to the ET_A receptor over **1**. Additional substituents in the 3-position increased ET_A/ET_B selectivity but did not have a pronounced effect on binding. Complete substitution of the aryl group was very disruptive to binding and resulted in a significant loss of activity. Accordingly, changing the fourth substituent to various polar groups or their biological precursors allowed for manipulation of the pharmacological properties of the mesitylcarboxamides. Compounds **7a**, **7k**, **7l**, **7n**, **7s**, and **7jj** had oral half-lives of ~2 h in rats, **7gg**, **79**, and **80** 3−4 h. In a rat model of hypoxia-induced pulmonary hypertension, compounds **7l**, **7m**, **7p**, **7u**, and **7jj** were all active when administered orally at 5 mg/kg. Compound **7u** (ET_A $IC_{50} = 0.19$ nM, $t_{1/2} = 4.1$ h, ET_A/ET_B selectivity > 10 000, with in vivo activity) is suitable for further preclinical studies.

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- or 300-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) or Desert Analytics (Tucson, AZ) 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 silical 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.

Methyl 3-{[*N*-(4-Chloro-3-methyl-5-isoxazolyl)-*N*-(methoxymethyl)amino]sulfonyl}-2-thiophenecarboxylate (3A). To a solution of **2A** (3.3 g, 10.0 mmol) in anhydrous THF (50 mL) at 0 °C were sequentially added *N*,*N*-diisopropylethylamine (1.9 g, 15.0 mmol) and bromomethyl methyl ether (1.5 g, 12.0 mmol). The reaction was stirred for 2 h at 0 °C and 6 h at room temperature before the addition of morpholine (0.87 g, 10.0 mmol) to scavenge the excess and toxic bromomethyl methyl ether. After being stirred at room temperature for another hour, the mixture was concentrated and the residue was dissolved in ethyl acetate (200 mL) and washed with 1 N HCl (2 × 150 mL). The organic layer was dried ($MgSO_4$) and concentrated to give **3A** as a greenish oil (3.5 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, $J = 4.4$ Hz, 1H), 7.44 (d, $J = 4.4$ Hz, 1H), 5.28 (s, 2H), 3.92 (s, 3H), 3.54 (s, 3H), 2.25 (s, 3H).

3-{[*N*-(4-Chloro-3-methyl-5-isoxazolyl)-*N*-(methoxymethyl)amino]sulfonyl}-2-thiophenecarboxylic Acid (4A). To a solution of **3A** (3.0 g, 7.8 mmol) in THF (30 mL) was added 1 N NaOH (30 mL) and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was partitioned between 1 N HCl (200 mL) and ethyl acetate (200 mL). The organic layer was dried ($MgSO_4$) and concentrated to give **4A** as an oil (2.90 g, ~100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, $J = 5.5$ Hz, 1H), 7.51 (d, $J = 5.5$ Hz, 1H), 5.20 (s, 2H), 3.50 (s, 3H), 2.27 (s, 3H).

3-{[*N*-(4-Chloro-3-methyl-5-isoxazolyl)-*N*-(methoxymethyl)amino]sulfonyl}-2-thiophenecarbonyl Chloride (5A). To a solution of **4A** (1.5 g, 4.1 mmol) in a mixture of THF (10 mL) and chloroform (5 mL) at 0 °C were sequentially added a catalytic amount of pyridine and oxalyl chloride (2 M in dichloromethane, 4.5 mL, 9.0 mmol). After being stirred at room temperature for 15 h, the mixture was concentrated under reduced pressure to afford **5A** as a viscous oil which solidified upon standing (1.7 g, ~100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, $J = 5.5$ Hz, 1H), 7.58 (d, $J = 5.5$ Hz, 1H), 5.23 (s, 2H), 3.53 (s, 3H), 2.25 (s, 3H).

Methods for Thiophenecarboxamide Synthesis. Method A. 3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(2,4,6-trimethylphenyl)-2-thiophenecarboxamide (7a). To a solution of **6A** (1.0 g, 3.1 mmol) in anhydrous DMF (10 mL) was added 1,1′-carbonyldiimidazole (553 mg, 3.41 mmol). After gas evolution ceased (~10 min), 2,4,6-trimethylaniline (4.2 g, 31.0 mmol) was added. The reaction was heated at 100 °C for 15 h before it was cooled to room temperature and poured into iced water (~100 g). The resulting precipitate was collected by filtration and then purified by reverse-phase HPLC to give **7a** (360 mg, 27%) as a brownish powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (br s, 1H), 7.74 (d, $J = 5.4$ Hz, 1H), 7.40 (d, $J = 5.4$ Hz, 1H), 6.90 (s, 2H), 2.24 (s, 3H), 2.13 (s, 6H), 2.02 (3H, s); IR (KBr pellet) 3227, 3106, 1782, 1636, 1530, 1358 cm^{-1}.

Method B. 3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(3-cyano-2,4,6-trimethylphenyl)-2-thiophenecarboxamide (7k). To a solution of **46** (1.63 g, 10.15 mmol) in anhydrous THF (30 mL) was added **5A** (1.0 g, 2.54 mmol). The mixture was stirred at room temperature for 15 h before it was concentrated under vacuum. The residue was partitioned between 1 N HCl (200 mL) and ethyl acetate (200 mL). The organic layer was concentrated, the residue was dissolved in methanol (20 mL), and concentrated HCl (10 mL) was added. The reaction was heated at 70 °C for 2 h before it was cooled to room temperature and then poured into iced water (250 mL). The resulted brown precipitate was collected on filtration and then purified by reverse-phase HPLC to give **7k** (0.90 g, 76%) as a yellow powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (br s, 1H), 7.76 (d, $J = 5.5$ Hz, 1H), 7.42 (d, $J = 5.5$ Hz, 1H), 7.23 (s, 1H), 2.45 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 2.02 (s, 3H); IR (KBr pellet) 3434, 3266, 2218 (CN), 1784, 1638, 1528, 1349 cm^{-1}.

Typical Methods for Nitrating Arenes. Method a. 2,4,6-Trimethyl-3-nitrophenylacetonitrile (12). To a suspension of **11** (5 g, 31 mmol) in acetic acid (20 mL) were added dropwise sequentially nitric acid (70%, 50 mL) and concentrated sulfuric acid (3 mL). The mixture was stirred for 1 h before it was poured into iced water. The aqueous mixture was then extracted with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), and concentrated to give **12** as a yellow oil (5.4 g, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (s, 1H), 3.63 (s, 2H), 2.38 (s, 3H), 2.67 (s, 3H), 2.23 (s, 3H).

Method b. 2,4,6-Trimethyl-3-nitroanisole (63). To a solution of **62** (6.65 g, 44.3 mmol) in dichloromethane (200 mL) at 0 °C was quickly added nitronium tetrafluoroborate (5 g, 85%, 44.3 mmol). The mixture was stirred at 0 °C for 2 h and at room temperature for 6 h. The reaction was quenched at 0 °C by careful addition of water (200 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a ~1:1 mixture (NMR ratio) of **63** and **62** (7.4 g combined wt) which was used

in the next step without further purification. Compound **63**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (s, 1H), 3.70 (s, 3H), 2.28 (s, 3H), 2.22 (s, 3H), 2.21 (s, 3H).

Typical Method for Reducing Nitroarenes to Anilines. 3-Amino-2,4,6-trimethylphenylacetonitrile (13). To a solution of **12** (5.0 g, 24.5 mmol) in methanol (200 mL) were sequentially added a solution of ammonium chloride (5.0 g, 93 mmol) in water (50 mL) and, in portions, zinc dust (5 g, 77 mmol). The mixture was vigorously stirred for 4 h before the solids were filtered and washed with methanol. The filtrate was concentrated to remove methanol and the aqueous residue was partitioned between ethyl acetate and 1 N NaOH. The organic layer was dried ($MgSO_4$) and concentrated to give **13** (3.4 g, 79%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (s, 1H), 3.62 (s, 2H), 3.56 (br s, 2H), 2.27 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H).

2,4,6-Trimethylphenylacetonitrile (11). To a mixture of **10** (5.0 g, 29.6 mmol) and sodium cyanide (5.8 g, 118.6 mmol) was added DMSO (16 mL). The exothermic reaction was stirred until it cooled to room temperature. The mixture was then heated at 80 °C for 30 min before the mixture was poured into water (200 mL). The resulting white precipitate was filtered, washed with water, and dried under vacuum to afford **11** (4.8 g, ~100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 2H), 3.60 (s, 2H), 2.35 (s, 6H), 2.26 (s, 3H).

Methyl 3-Amino-2,4,6-trimethylphenylacetate (14). To a solution of **13** (4.5 g, 25.86 mmol) in methanol (50 mL) was added concentrated sulfuric acid (50 mL). The mixture was heated under reflux for 15 h before it was poured into ice (~200 g). The aqueous mixture was basified with sodium bicarbonate until gas evolution ceased. The resulting precipitate was filtered and dissolved in ethyl acetate. The organic solution was dried ($MgSO_4$) and concentrated to yield **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 3.68 (s, 2H), 3.66 (s, 3H), 3.23 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H).

3-Amino-2,4,6-trimethylbenzyl Acetate (16). To a solution of **15** (6.0 g, 28.1 mmol) in DMF (30 mL) was added sodium acetate (6.0 g, 73.1 mmol). The mixture was heated at 100 °C for 8 h before it was poured into water (200 mL). The resulting precipitate was filtered, washed with water, and then subjected to the typical reduction method as for **13** to afford **16** (3.3 g, 57% for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (s, 1H), 5.17 (s, 2H), 3.52 (br s, 2H), 2.28 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H).

***O*-(3-Amino-2,4,6-trimethylbenzyl)-*N*,*N*-dimethylthiocarbamate (17).** To a solution of **16** (1.55 g, 7.48 mmol) in THF (15 mL) were added 2 N NaOH (5 mL) and methanol (5 mL). The reaction was stirred for 15 min before it was acidified with 2 N HCl (5 mL). The mixture was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was dried ($MgSO_4$) and concentrated to give a solid (1.23 g). To a solution of this solid (183 mg, 1.11 mmol) in DMF (4 mL) under nitrogen was added NaH (49 mg, 1.22 mmol, 60% dispersion in mineral oil). The mixture was stirred for 30 min before the addition of a solution of dimethylthiocarbamoyl chloride (151 mg, 1.22 mmol) in DMF (4 mL). The reaction was stirred for 18 h, diluted with 1:1 mixture of hexanes and ethyl acetate, and then washed with saturated sodium bicarbonate and water. The organic layer was dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated. The residue was chromatographed eluting with 40−50% ethyl acetate in hexanes to give **17** (84 mg, 30% for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 1H), 5.48 (s, 2H), 3.38 (s, 3H), 3.01 (s, 3H), 2.31 (s, 3H), 2.19 (s, 3H), 2.20 (s, 3H).

***N*-(2,4,6-Trimethylbenzyl)dimethylamine (18).** To a mixture of THF (20 mL) and dimethylamine (20 mL, 40 wt % in water) at 0 °C was added **10** (5.0 g, 29.7 mmol). The reaction was stirred at 0 °C for 30 min and at room temperature for 3 h. The mixture was concentrated and the aqueous residue was partitioned between ethyl ether and 1 N NaOH. The organic layer worked up as usual to give **18** (5.2 g, ~100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 1H), 3.35 (s, 2H), 2.34 (s, 6H), 2.25 (s, 3H), 2.21 (s, 6H).

***N*-(3-Amino-2,4,6-trimethylphenyl)methanesulfonamide (22).** To a solution of **21** (5.8 g, 38.8 mmol) in ethyl acetate (100 mL) at 0 °C were sequentially added triethylamine (2.6 g, 25.8 mmol) and methanesulfonyl chloride (3.0 g, 25.8 mmol). The reaction was stirred at 0 °C for 30 min and at room temperature for 1 h. The mixture was concentrated under vacuum and the residue was recrystallized from methanol to produce **22** (3.7 g, 41%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (s, 1H), 5.78 (br s, 1H), 3.54 (br s, 2H), 3.00 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H).

Ethyl 3-Amino-2,4,6-trimethylphenoxyacetate (27). To a solution of **26** (0.77 g, 5 mmol) in acetonitrile (20 mL) were sequentially added cesium carbonate (1.95 g, 6.0 mmol) and ethyl bromoacetate (0.87 g, 5.2 mmol). The mixture was stirred for 3 h and partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to give **27** (1.2 g, ~100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (s, 1H), 4.33 (s, 2H), 4.29 (q, J = 7.0 Hz, 2H), 2.18 (s, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 1.32 (t, J = 7.0 Hz, 3H).

***O*-(3-Nitro-2,4,6-trimethylphenyl)-*N*,*N*-dimethylthiocarbamate (28).** Compound **28** was synthesized in the same manner as for **17** except that **24** was used instead of **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (s, 1H), 3.47 (s, 3H), 3.39 (s, 3H), 2.27 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H).

2,4,6-Trimethylphenylene-1,3-diacetonitrile (36). Compound **36** was synthesized in the same manner as for **11** except that **35** was used instead of **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 3.65 (s, 4H), 2.42 (s, 3H), 2.36 (s, 6H).

***N*-(2,4,6-Trimethylphenyl)pyrrolidine (40b).** To a solution of **39** (1.0 g, 7.4 mmol) in THF (20 mL) under nitrogen and at 0 °C was added potassium bis(trimethylsilyl)amide (15 mL, 7.5 mmol, 0.5 M in toluene). The mixture was stirred at 0 °C for 30 min and at room temperature for 1 h before the addition of 1,4-dibromobutane (1.75 g, 8.1 mmol) at 0 °C. The reaction was stirred for 2 h at room temperature, cooled to 0 °C, followed by the addition of more potassium bis(trimethylsilyl)amide (15 mL, 7.5 mmol) at 0 °C. The reaction mixture was stirred overnight before it was diluted with ethyl acetate (150 mL) and washed with water (2 × 30 mL) and brine (2 × 30 mL). The organic layer was dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated. The residue was chromatographed eluting with 1% ethyl acetate in hexanes to give **40b** (862 mg, 62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (s, 2H), 3.15 (m, 4H), 2.24 (s, 3H), 2.21 (s, 6H), 1.95 (m, 4H).

3-Amino-2,4,6-trimethylphenylacetonitrile (60). To a solution of **35** (10 g, 46 mmol) in DMSO (30 mL) was added sodium cyanide (2.25 g, 46 mmol). The mixture was stirred at room temperature for 12 h. A small aliquot of the mixture was added to water and the resulting precipitate was filtered, washed with water, and dried under vacuum to give a 2:1:1 mixture (NMR ratio) of **56**, **57**, and **35**. **56**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1H), 4.66 (s, 2H), 3.65 (s, 2H), 2.44 (s, 3H), 2.36 (s, 3H), 2.35 (s, 3H). **57**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 3.65 (s, 4H), 2.42 (s, 3H), 2.39 (s, 6H).

To the bulk of the aforementioned mixture was added sodium azide (12 g, 184 mmol) and the reaction was heated at 80 °C for 3 h. The mixture was poured into water and the resulting precipitate was filtered, washed with water, and dried under vacuum to produce a 2:1:1 mixture (NMR ratio) of **58**, **57**, and **59**. **58**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (s, 1H), 4.42 (s, 2H), 3.66 (s, 2H), 2.41 (s, 3H), 2.36 (s and s, 6H). **59**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 4.42 (4H), 2.42 (s, 3H), 2.37 (s, 6H).

To a solution of the mixture of **57**, **58**, and **59** in THF (300 mL) and water (10 mL) was added triphenylphosphine (18 g, 69 mmol). The mixture was heated under reflux for 4 h before it was concentrated under vacuum. The residue was partitioned between 1 N HCl (200 mL) and ethyl ether (200 mL). The aqueous layer was treated with potassium carbonate until gas evolution ceased and extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated to give **60** (2.4 g, 29% for 3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 1H), 3.87 (s, 2H), 3.62 (s, 2H), 2.43 (s, 3H), 2.35 (s, 3H), 2.33 (s, 3H).

2,4,6-Trimethylanisole (62). To a solution of **23** (10 g, 73.4 mmol) in anhydrous THF (200 mL) at 0 °C were sequentially added NaH (3.23 g, 60% dispersion in mineral oil, 80.8 mmol) and iodomethane (12.5 g, 88.1 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature for 12 h before water (20 mL) was added at 0 °C to quench the reaction. The mixture was concentrated under vacuum and the residue was chromatographed eluting with hexanes to give **62** (10 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (s, 2H), 3.69 (s, 3H), 2.25 (s, 6H), 2.24 (s, 3H).

Ethyl *N*-[(3-{[*N*′-(4-Chloro-3-methyl-5-isoxazolyl)-*N*′-(methoxymethyl)amino]sulfonyl}-2-thienyl)carbonyl]-*N*-(3-acetoxy-2,4,6-trimethylphenyl)carbamate (66). To a solution of **65** (350 mg, 0.68 mmol) in anhydrous THF (2.7 mL) at 0 °C under nitrogen was added potassium bis(trimethylsilyl)amide (1.5 mL, 0.5 M in toluene, 0.75 mmol). The mixture was stirred at 0 °C for 30 min before the addition of ethyl chloroformate (71 μL, 0.75 mmol). The reaction was stirred at 0 °C for 15 min and at room temperature for 2 h. The mixture was partitioned between ethyl acetate and 2 N HCl. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed eluting with 25−33% ethyl acetate in hexanes to give **66** (250 mg, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, J = 4.40, 1.12 Hz, 1H), 7.28 (dd, J = 4.40, 1.12 Hz, 1H), 7.04 (s, 1H), 5.02 (d, J = 1.12 Hz, 2H), 4.11 (m, 2H), 3.38 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 2.17 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.07 (dt, J = 1.48, 7.36 Hz, 3H).

3-{*N*-[(3-{[*N*′-(4-Chloro-3-methyl-5-isoxazolyl)-*N*′-(methoxymethyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl 2-[2-(2-Methoxyethoxy)ethoxy]-acetate (68). To a solution of **65** (820 mg, 1.51 mmol) in methanol (7.6 mL) was added potassium carbonate (626 mg, 4.5 mmol). The mixture was stirred for 30 min before it was diluted with ethyl acetate and washed with 2 N HCl, water, and brine. The organic layer was dried ($MgSO_4$), the solids were filtered, and the filtrate was concentrated. The residue was chromatographed eluting with 50% ethyl acetate in hexanes to afford a light yellow solid (552 mg). To a solution of this solid (197 mg) in dichloromethane (0.75 mL) were sequentially added 4-(dimethylamino)pyridine (102 mg, 0.84 mmol) and 2-[2-(2-methoxyethoxy)ethoxy]acetyl chloride (0.76 mL, 1 M in dichloromethane, 0.76 mmol). The reaction was stirred for 90 min before it was diluted with dichloromethane and washed sequentially with 2 N HCl, water, saturated aqueous sodium bicarbonate, water, and brine. The organic layer was dried ($MgSO_4$) and concentrated to yield **68** (222 mg, 62% for 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.52 (d, J = 5.5 Hz, 1H), 7.42 (d, J = 5.5 Hz, 1H), 6.98 (s, 1H), 5.11 (s, 2H), 4.47 (s, 2H), 3.83 (m, 2H), 3.73 (m, 2H), 3.67 (m, 2H), 3.55 (m, 2H), 3.37(2) (s, 3H), 3.36(8) (s, 3H), 2.25 (s, 3H), 2.22 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl 2-[2-(2-Methoxyethoxy)ethoxy]acetate (7r). To a solution of **68** (222 mg, 0.335 mmol) in THF (4.4 mL) was added 1 N HCl (2.2 mL). The resulting mixture was heated under reflux for 4 h before it was cooled to room temperature and diluted with ethyl acetate. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed eluting with 10−20% methanol in chloroform to give **7r** (127 mg, 61%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 5.5 Hz, 1H), 7.31 (d, J = 5.5 Hz, 1H), 6.89 (s, 1H), 4.45 (s, 2H), 3.73 (m, 2H), 3.60 (m, 2H), 3.56 (m, 2H), 3.48 (m, 2H), 3.27 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(6-cyano-2,3,4-trimethoxyphenyl)-2-thiophenecarboxamide (7b): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.56 (br s, 1H), 7.79 (d, J = 5.4 Hz, 1H), 7.41 (d, J = 5.4 Hz, 1H), 7.33 (s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 2.01 (s, 3H); IR (KBr pellet) 2232 cm^{-1} (CN).

Methyl 3-{({3-[[(4-chloro-3-methyl-5-isoxazolyl)amino]-sulfonyl]-2-thienyl}carbonyl)amino}-2,4,6-trimethylbenzoate (7c): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.04 (br s, 1H), 7.75 (d, J = 5.4 Hz, 1H), 7.41 (d, J = 5.4 Hz, 1H), 7.05 (s, 1H), 3.84 (s, 3H), 2.23 (s, 3H), 2.18 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H); IR (KBr pellet) 1728 cm^{-1}.

Methyl 2-{({3-[[(4-chloro-3-methyl-5-isoxazolyl)amino]-sulfonyl]-2-thienyl}carbonyl)amino}-3,5-dimethylbenzoate (7d): ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.99 (br s, 1H), 7.76 (d, J = 5.4 Hz, 1H), 7.43 (s, 1H), 7.39 (d, J = 5.4 Hz, 1H), 7.32 (s, 1H), 3.64 (s, 3H), 2.32 (s, 3H), 2.29 (s, 3H), 2.04 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylbenzoic acid (7e): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (br s, 1H), 7.73 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 7.01 (s, 1H), 2.44 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.01 (s, 3H).

2-(3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]-sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl)-acetic acid (7f): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (br s, 1H), 7.72 (d, J = 5.1 Hz, 1H), 7.40 (d, J = 5.1 Hz, 1H), 6.93 (s, 1H), 3.59 (s, 2H), 2.23 (s, 3H), 2.11 (s & s, 6H), 2.00 (s, 3H); IR (KBr pellet) 1708 cm^{-1}.

2-(3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]-sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenoxy)-acetic acid (7g): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.74 (d, J = 5.1 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 6.94 (s, 1H), 4.32 (s, 2H), 2.21 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H); IR (KBr pellet) 1736 cm^{-1}.

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-{3-[(dimethylamino)methyl]-2,4,6-trimethylphenyl}-2-thiophenecarboxamide (7h): ^{1}H NMR (400 MHz, DMSO-d_6 for its TFA salt) δ 11.19 (s, 1H), 8.83 (m, 1H), 7.74 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.1 Hz, 1H), 7.11 (s, 1H), 4.39 (d, J = 5.5 Hz, 2H), 2.82 (br d, 6H), 2.40 (s, 3H), 2.29 (s, 3H), 2.16 (s, 3H), 1.99 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(dimethylamino)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7i): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 7.69 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 6.86 (s, 1H), 2.76 (s, 6H), 2.23 (s, 3H), 2.10 (s and s, 6H), 1.99 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[2,4,6-trimethyl-3-(1-pyrrolidinyl)phenyl]-2-thiophenecarboxamide (7j): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.68 (d, J = 5.1 Hz, 1H), 7.40 (d, J = 5.1 Hz, 1H), 6.90 (s, 1H), 3.11 (m, 4H), 2.19 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.95 (m, 4H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(cyanomethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7l): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (br s, 1H), 7.75 (d, J = 5.2 Hz, 1H), 7.42 (d, J = 5.2 Hz, 1H), 7.03 (s, 1H), 3.90 (s, 2H), 2.21 (s, 3H), 2.33 (s, 3H), 2.14 (s, 3H), 2.02 (s, 3H); IR (KBr pellet) 2258 cm^{-1} (CN).

***N*-[3-(Aminosulfonyl)-2,4,6-trimethylphenyl]-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide (7m):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (br s, 1H), 7.75 (d, J = 5.5 Hz, 1H), 7.42 (d, J = 5.5 Hz, 1H), 7.25 (br s, 2H), 7.11 (s, 1H), 2.58 (s, 3H), 2.48 (s, 3H), 2.18 (s, 3H), 2.03 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-{2,4,6-trimethyl-3-[(methylsulfonyl)amino]phenyl}-2-thiophenecarboxamide (7n): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.84 (s, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 7.02 (s, 1H), 3.00 (s, 3H), 3.32 (s, 3H), 2.21 (s, 3H), 2.14 (s, 3H), 2.02 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl acetate (7o): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 7.73 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 7.02 (s, 1H), 2.34 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1,95 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylbenzyl acetate (7p): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (br s, 1H), 7.72 (d, J = 5.1 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 6.99 (s, 1H),

5.11 (s, 2H), 2.31 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H).

Methyl 2-(3-{[(3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl)acetate (7q): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (br s, 1H), 7.71 (d, J = 5.5 Hz, 1H), 7.40 (d, J = 5.5 Hz, 1H), 6.94 (s, 1H), 3.70 (s, 2H), 3.60 (s, 3H), 3.22 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.01 (s, 3H); IR (KBr pellet) 1735 cm^{-1}.

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(3-methoxy-2,4,6-trimethylphenyl)-2-thiophenecarboxamide (7s): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 5.2 Hz, 1H), 7.48 (d, J = 5.2 Hz, 1H), 7.45 (br s, 1H), 6.99 (s, 1H), 3.71 (s, 3H), 2.28 (s, 3H), 2.25 (s and s, 6H), 2.20 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(cyclopropylmethoxy)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7t): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 7.69 (d, J = 5.2 Hz, 1H), 7.41 (d, J = 5.2 Hz, 1H), 6.91 (s, 1H), 3.55 (d, J = 6.96 Hz, 2H), 2.21 (s, 3H), 2.09(4) (s, 3H), 2.08(5) (s, 3H), 1.99 (s, 3H), 1.22 (m, 1H), 0.56 (m, 2H), 0.29 (m, 2H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(3-hydroxy-2,4,6-trimethylphenyl)-2-thiophenecarboxamide (7u): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (br s, 1H), 7.72 (d, J = 5.48 Hz, 1H), 7.40 (d, J = 5.48 Hz, 1H), 6.79 (s, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 2.031 (s, 3H), 2.026 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(hydroxymethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7v): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (br s, 1H), 7.69 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 6.90 (s, 1H), 4.47 (s, 2H), 2.33 (s, 3H), 2.22 (s, 3H), 2.11 (s, 3H), 1.99 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl methyl carbonate (7w): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 7.71 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 7.04 (s, 1H), 3.86 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 1.99-(1) (s, 3H), 1.98(8) (s, 3H); IR (KBr pellet) 1754 cm^{-1}.

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl carbonate (7x): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 7.71 (d, J = 5.1 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 7.10 (s, 1H), 2.23 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H), 1.98 (s, 3H); IR (KBr pellet) 1752 cm^{-1}.

***O*-(3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl) *N*,*N*-dimethylcarbamothioate (7y):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 7.70 (d, J = 5.1 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 6.98 (s, 1H), 3.39 (s, 3H), 3.35 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H).

***O*-(3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylbenzyl) *N*,*N*-dimethylcarbamothioate (7z):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 7.71 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.1 Hz, 1H), 7.01 (s, 1H), 5.43 (s, 2H), 3.28 (s, 6H), 2.33 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H), 1.99 (s, 3H).

3-{[(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylphenyl *N*,*N*-dimethylsulfamate (7aa): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 7.71 (d, J = 5.2 Hz, 1H), 7.41 (d, J = 5.2 Hz, 1H), 7.05 (s, 1H), 3.03 (s, 6H), 2.32 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H), 1.99 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(2,3,4,5,6-pentamethylphenyl)-2-thiophenecarboxamide (7bb): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (br s, 1H), 7.52 (d, J = 5.5 Hz, 1H), 7.48 (br s, 1H), 7.47 (d, J = 5.5 Hz, 1H), 2.25 (s, 6H), 2.24 (s and s, 9H), 2.21 (s, 3H).

***N*-[3,5-Bis(cyanomethyl)-2,4,6-trimethylphenyl]-3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thiophenecarboxamide (7cc):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (br s, 1H), 7.75 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.1 Hz, 1H), 4.00 (s, 4H), 2.45 (s, 3H), 2.25 (s, 6H), 2.01 (s, 3H); IR (KBr pellet) 2250 cm^{-1} (CN).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(mesitylmethyl)-2-thiophenecarboxamide (7dd): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (br s, 1H), 7.43 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 6.92 (s, 2H), 6.04 (br t, 1H), 4.66 (d, J = 4.8 Hz, 2H), 2.39 (s, 6H), 2.32 (s, 3H), 2.23 (s, 3H).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(cyanomethyl)-2,4,6-trimethylbenzyl]-2-thiophenecarboxamide (7ee): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.52 (br s, 1H), 7.43 (d, J = 5.1 Hz, 1H), 7.37 (d, J = 5.1 Hz, 1H), 6.99 (s, 1H), 6.09 (br t, 1H), 4.69 (d, J = 4.8 Hz, 2H), 3.66 (s, 2H), 2.43 (s, 3H), 2.39 (s, 3H), 2.37 (s, 3H), 2.23 (s, 3H); IR (KBr pellet) 2240 cm^{-1} (CN).

3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-*N*-(2,4,6-trimethyl-5-pyrimidinyl)-2-thiophenecarboxamide (7ff): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 7.77 (d, J = 5.2 Hz, 1H), 7.43 (d, J = 5.2 Hz, 1H), 2.55 (s, 3H), 2.35 (s and s, 6H), 1.99 (s, 3H).

3-{[(4-Chloro-5-methyl-3-isoxazolyl)amino]sulfonyl}-*N*-[3-(cyanomethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7gg): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 7.62 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 7.01 (s, 1H), 3.88 (s, 2H), 2.32 (s, 3H), 2.21 (s, 3H), 2.17 (s, 3H), 2.13 (s, 3H); IR (KBr pellet) 2262 cm^{-1} (CN).

3-{[(3-{[(4-Chloro-5-methyl-3-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylbenzyl acetate (7hh): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 7.62 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 6.97 (s, 1H), 5.11 (s, 2H), 2.30 (s, 3H), 2.17 (s, 6H), 2.02 (s, 3H); IR (KBr pellet) 1733 cm^{-1}.

3-{[(4-Chloro-5-methyl-3-isoxazolyl)amino]sulfonyl}-*N*-[3-(hydroxymethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7ii): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (br s, 1H), 7.80 (d, J = 5.1 Hz, 1H), 7.43 (d, J = 5.1 Hz, 1H), 6.92 (s, 1H), 4.48 (s, 2H), 2.33 (s, 3H), 2.30 (s, 3H), 2.24 (s, 3H), 2.15 (s, 3H).

3-{[(3,4-Dimethyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(cyanomethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7jj): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (br s, 1H), 7.85 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 7.04 (s, 1H), 3.90 (s, 2H), 2.33 (s, 3H), 2.23 (s, 3H), 2.17 (s, 3H), 2.08 (s, 3H), 1.66 (s, 3H); IR (KBr pellet) 2243 cm^{-1} (CN).

3-{[(3-{[(3,4-Dimethyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]amino}-2,4,6-trimethylbenzyl acetate (7kk): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (br s, 1H), 7.85 (d, J = 5.1 Hz, 1H), 7.34 (d, J = 5.1 Hz, 1H), 7.00 (s, 1H), 5.12 (s, 2H), 2.31 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.65 (s, 3H).

3-{[(3,4-Dimethyl-5-isoxazolyl)amino]sulfonyl}-*N*-[3-(hydroxymethyl)-2,4,6-trimethylphenyl]-2-thiophenecarboxamide (7ll): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (br s, 1H), 7.84 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 6.92 (s, 1H), 4.48 (s, 2H), 2.33 (s, 3H), 2.23 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 1.65 (s, 3H).

Ethyl *N*-[(3-{[(4-chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)carbonyl]-*N*-(3-hydroxy-2,4,6-trimethylphenyl)carbamate (67): ^{1}H NMR (400 MHz, D_2O) δ 8.05 (d, J = 3.8 Hz, 1H), 7.84 (d, J = 3.8 Hz, 1H), 7.47 (s, 1H), 5.86 (s, 1H), 4.44 (m, 2H), 2.66 (s, 3H), 2.54 (s and s, 6H), 2.52 (s, 3H), 1.36 (t, J = 7.0 Hz, 3H).

***N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-(6-methyl-1,3-benzodioxol-5-yl)propanoyl]-3-thiophenesulfonamide (70):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (m, 1H), 7.31 (m, 1H), 6.73 (s, 1H), 6.54 (s, 1H), 5.92 (d, J = 3.0 Hz, 2H), 4.92 (m, 1H), 2.20 (s, 3H), 2.08 (s, 3H), 1.32 (d, J = 6.6 Hz, 3H).

2-[4-Bromo-2-(6-methyl-1,3-benzodioxol-5-yl)butanoyl]-*N*-(4-chloro-3-methyl-5-isoxazolyl)-3-thiophenesulfonamide (71): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, J = 5.1 Hz, 1H), 7.31 (d, J = 5.1 Hz, 1H), 6.76 (s, 1H), 6.56 (s, 1H), 5.94 (s, 2H), 4.92 (t, J = 5.8 Hz, 1H), 3.41 (t, J = 7.7 Hz, 2H), 2.21 (s, 3H), 2.16 (m, 2H), 2.09 (s, 3H).

4-(3-{[(4-Chloro-3-methyl-5-isoxazolyl)amino]sulfonyl}-2-thienyl)-3-(6-methyl-1,3-benzodioxol-5-yl)-4-oxobutanoic acid (73): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (d, J = 5.1 Hz, 1H), 7.30 (d, J = 5.1 Hz, 1H), 6.72 (s, 1H), 6.57 (s, 1H), 5.92 (s, 2H), 5.06 (m, 1H), 2.93 (dd, 1H), 2.70 (dd, 1H), 2.22 (s, 3H), 2.06 (s, 3H); IR (KBr pellet) 1711, 1680 cm^{-1}.

***N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-(2,4,6-trimethylphenyl)acetyl]-3-thiophenesulfonamide (75):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.87 (br s, 1H), 7.61 (s, 2H), 6.93 (s, 2H), 4.34 (s, 2H), 2.30 (s, 3H), 2.25 (s, 6H), 2.21 (s, 3H).

***N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-(3-methoxy-2,4,6-trimethylphenyl)acetyl]-3-thiophenesulfonamide (79):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.60 (AB system, 2H), 6.93 (s, 1H), 4.33 (s, 2H), 3.69 (s, 3H), 2.27 (s, 3H), 2.20 (s and s, 6H), 2.19 (s, 3H).

***N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-(3-hydroxy-2,4,6-trimethylphenyl)acetyl]-3-thiophenesulfonamide (80):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (br s, 1H), 7.52 (AB system, J_{AB} = 5.1 Hz, 1H), 7.47 (AB system, J_{AB} = 5.1 Hz, 1H), 7.44 (br s, 1H), 6.93 (s, 1H), 4.61 (br s, 2H), 2.23 (s and s, 6H), 2.22 (s, 3H), 2.21 (s, 3H).

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 immediatedly 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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