Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 2. Sulfonamide-Based ET_A/ET_B Mixed Antagonists

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When the *N*,*N*-dialkylacetamide side chain of the highly ET_A-selective endothelin antagonist ABT-627 (**1**; [2*R*,3*R*,4*S*]-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(*N*,*N*-dibutylamino)-carbonyl]methyl]pyrrolidine-3-carboxylic acid; A-147627) is replaced by *N*,*S*-dialkylsulfonamidoethyl, the resultant analogs retain ET_A affinity, but exhibit substantial ET_B affinity as well. Structure−activity studies reveal that modifications in the length of the two alkyl groups, and in the substitution on the anisyl ring, are important in optimizing this "balanced" antagonist profile. In particular the combination of an *N*-*n*-propyl group, an *S*-alkyl chain between four and six carbons in length, and a fluorine atom *ortho* to the aromatic OCH_3 provides compounds with sub-nanomolar affinities for both receptor subtypes, and with ET_A/ET_B ratios close to 1. A number of these compounds also exhibit oral bioavailabilities (in rats) in the 30−50% range and have substantial plasma half-lives. The balanced receptor-binding profile of these potent and orally bioavailable compounds complements the ET_A selectivity observed with **1**.

Introduction

In recent articles in this journal,[1−4] our group has described the identification and optimization of several novel classes of endothelin receptor antagonists. In particular[4−6] we have reported on a family of pyrrolidine-based compounds, exemplified by ABT-627 (**1**; [2*R*,3*R*,4*S*]-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(*N*,*N*-dibutylamino)carbonyl]methyl]pyrrolidine-3-carboxylic acid; A-147627; Figure 1), which are highly potent and selective for the ET_A receptor subtype. While we have hypothesized[7] that ET_A selectivity may confer an advantage for the treatment of ET-induced constrictive and/or proliferative disorders, the relative merits of selective vs nonselective therapy is still the subject of debate; in fact, many of the compounds currently under development[8] exhibit a "balanced" profile of ET_A and ET_B activity.

In order to maximize our chances for success in the field of endothelin-blocking therapeutics, and because we believe that differing receptor profiles might be optimally effective for the treatment of particular disease states, we hoped to develop a series of mixed ET_A/ET_B antagonists to complement our earlier ET_A-selective candidate. A fortuitous observation during the studies leading to **1** has provided us access to such a series of compounds; the results of our explorations in this area are reported below.

Critical Observation

Structure−activity studies during the development of **1** indicated to us the critical importance of the side chain amide carbonyl; we suspect that this carbonyl is involved in a hydrogen-bonding interaction with the ET_A receptor which serves to orient the butyl groups into appropriate hydrophobic binding pockets. Our studies also indicated that the presence of *both* alkyl groups are required to maintain high ET_A selectivity. We were curious to explore the effect of "reorienting" these two alkyl groups by moving the H-bond donor to different positions on the side chain. A notable effect was observed when the carbonyl group of the amide linkage of **1** was transposed along the chain (compound **2**, Table 1; see also Scheme 1). This modification led to a large decrease in ET_A affinity (from 0.36 to 20 nM) with little change in ET_B affinity (from 520 to 850 nM); the net result is a 35-fold change in ET_A selectivity, from 1400× to 40×. Intrigued by this observation, we experimented with other amide surrogates, and quickly discovered that the corresponding sulfonamide (**3**) was substantially more potent at both ET_A and ET_B receptors, while retaining the reduced ET_A/ET_B activity ratio of **2**. The potency/selectivity profile of **3** is comparable to those of a number of "balanced" compounds which have recently been described in the literature;[8] more importantly, it has served as a valuable lead for further optimization.

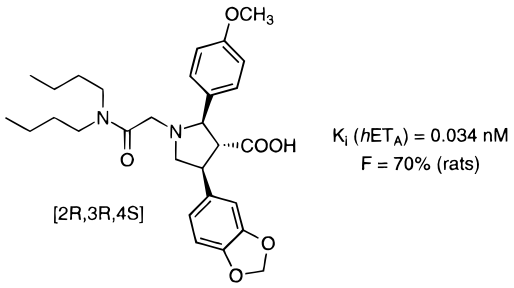

Figure 1. ABT-627, an ET_A-selective antagonist.

Chemistry

The core pyrrolidines employed in this study have been assembled (Scheme 2) by direct analogy to our earlier work.[4] Briefly, the Michael reaction between *β*-keto esters **7** and nitrostyrene **5** provides a mixture of diastereomeric nitro ketones. The keto esters them-

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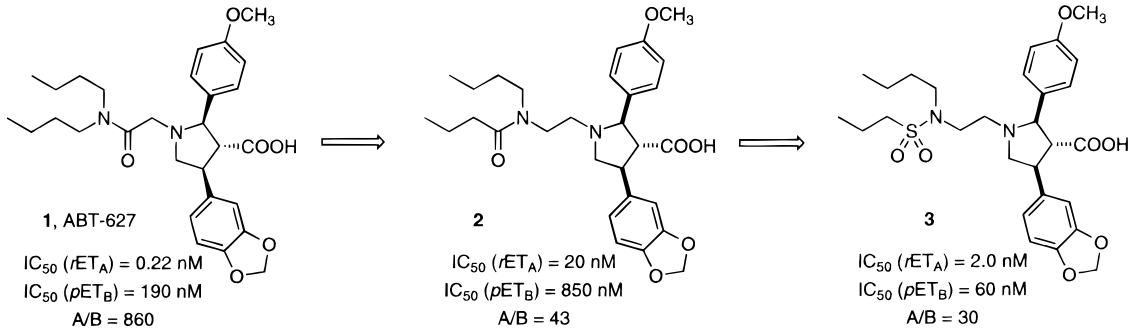
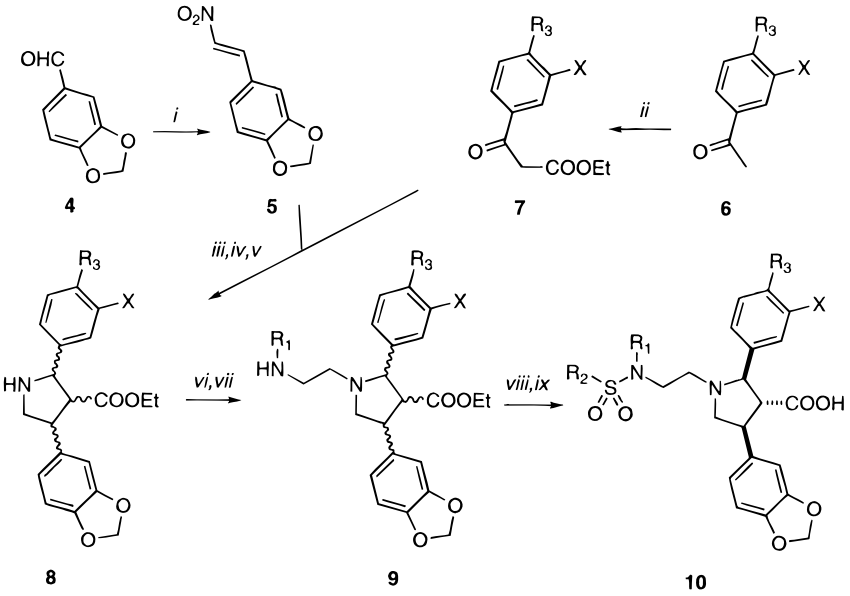
Scheme 1. Conceptual Development of Sulfonamide-Based Mixed Antagonists



Scheme 2. General Synthetic Route to Sulfonamidoethyl-Substituted Pyrrolidines[a]



[a] (*i*) CH_3NO_2, NH_4OAc, HOAc, heat; (*ii*) NaH, $(EtO)_2CO$, THF; (*iii*) DBU, THF−iPrOH; (*iv*) H_2, Ra-Ni, THF−HOAc; $NaBH_3CN$, THF−EtOH, pH 5; (*v*) DBU, THF, heat; (*vi*) $Br(CH_2)_2Br$, cat. NaI, heat; (*vii*) R_1NH_2, EtOH, cat. NaI, heat; (*viii*) R_2SO_2Cl, iPr_2NEt, CH_3CN; (*ix*) NaOH, H_2O−EtOH.

selves are prepared by carbethoxylation of the corresponding ketones **6**, which are either purchased (e.g. as in the case of *p*-methoxyacetophenone) or assembled through Friedel−Crafts acetylation. Nitrostyrene **5** derives from Henry reaction of piperonal **4**.

The diastereomeric mixture of nitro ketones is reductively cyclized in two steps (Raney nickel reduction to provide a cyclic imine, which is further reduced using cyanoborohydride) to give pyrrolidines **8**, generally as a mixture of three diasteromers in which the desired *trans,trans* isomer predominates. An additional portion of *trans,trans* material may be derived by epimerization (DBU, toluene) of the *cis,cis* isomer, which is conveniently separated by chromatography.

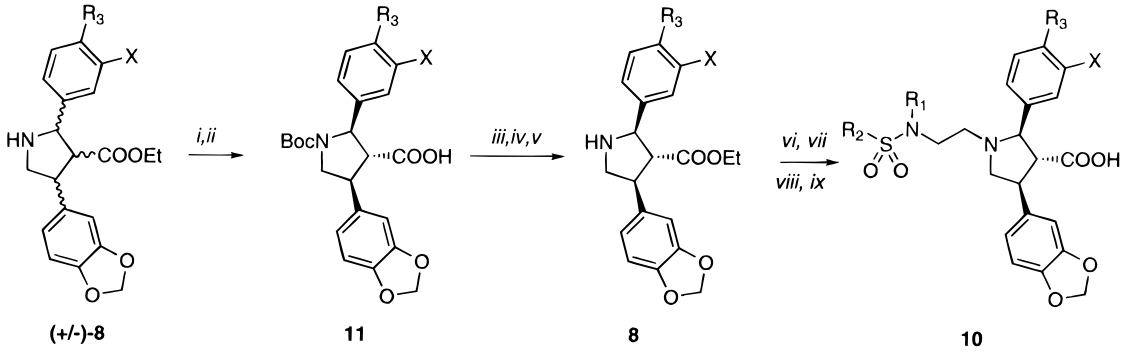
To attach the sulfonamidoethyl side chains, amine **8** (as a mixture of stereoisomers) is reacted with 1,2-dibromoethane under iodide catalysis to give a 1-(bromoethyl)pyrrolidine. Displacement of the second bromine atom (also catalyzed by I^-) with a primary amine gives **9**. Sulfonylation of the resultant secondary amine provides the fully elaborated side chain. Under standard saponification conditions (NaOH, MeOH, room temperature, overnight), the hydrolysis of the *trans,trans* ester is dramatically faster than that of any other isomer, providing the pure *trans,trans* acid **10**. This valuable selective hydrolysis was first observed during the preparation of our ET_A-selective analogs.[4]

In several cases the compounds have been prepared in optically pure form. The preparation of **10** as a single enantiomer is accomplished through resolution of the core pyrrolidine **8** prior to side chain assembly (Scheme 3). In practice, the racemic pyrrolidine ester is converted in two steps to the corresponding Boc acid; as described above, this hydrolysis step selects for the *trans,trans* isomer. Formation of a chiral salt of racemic acid **11** using (*R*)-(+)-α-methylbenzylamine provides a practical and general resolution strategy. In each case a single recrystallization produces material of >99.5% ee, as evaluated by chiral HPLC. The diasteromerically pure salt is washed with acid to remove the chiral amine (which may be recovered); the resultant optically active **11** is reconverted to amino ester **8** under Fischer esterification conditions. Optically pure antagonists **10** (see Table 4 for characterization) are prepared by the steps already described in Scheme 2.

Compound Evaluation

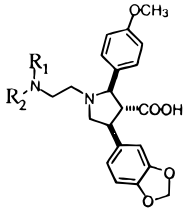
The first line of biochemical analysis for the compounds described in this study is a measurement of their ability to displace endothelin from its receptor. For the purpose of screening, we employ a rodent ET_A receptor derived from MMQ cells (rET_A); ET_B receptor (pET_B) is derived from porcine cerebellar tissue. IC_{50} data are

Scheme 3. Preparation of Enantiomerically-Enriched Analogs[a]



[a] (*i*) Boc_2O, CH_2Cl_2; (*ii*) NaOH, H_2O−EtOH; (*iii*) (*S*)-(−)-α-methylbenzylamine, EtOAc−Et_2O, 1 recrystallization, >99.5% ee; (*iv*) wash with 1 N H_3PO_4; (*v*) sat. HCl/EtOH; (*vi*) $BrCH_2CH_2Br$, cat. NaI, heat; (*vii*) R_1NH_2, cat. NaI, EtOH, heat; (*viii*) R_2SO_2Cl, iPr_2NEt, CH_3CN; (*ix*) NaOH, H_2O−EtOH.

Table 1. Exploration of Substituents on the Sulfonamidoethyl Side Chain



			IC$_{50}$ (nM)[a]			
compd	R_1	R_2	*r*ET$_A$ binding	*p*ET$_B$ binding	A/B ratio	formula
2	C_4H_9	C_3H_7CO	20	850	43	$C_{29}H_{38}N_2O_6$
3	C_4H_9	$C_3H_7SO_2$	2.0	60	30	$C_{28}H_{38}N_2O_7S$
12a	CH_3	$C_3H_7SO_2$	10	260	26	$C_{25}H_{32}N_2O_7S$
12b	C_3H_7	$C_3H_7SO_2$	1.6	36	22	$C_{27}H_{36}N_2O_7S$
12c	*i*-C_4H_9	$C_3H_7SO_2$	8.1	62	7.7	$C_{28}H_{38}N_2O_7S$·0.2TFA
12d	C_3H_7	$C_2H_5SO_2$	1.2	95	79	$C_{26}H_{34}N_2O_7S$·0.3TFA
12e	C_3H_7	$C_4H_9SO_2$	2.0	9.4	4.7	$C_{28}H_{38}N_2O_7S$
12f	C_3H_7	$C_5H_{11}SO_2$	0.57	4.1	7.2	$C_{29}H_{40}N_2O_7S$
12g	C_3H_7	$C_6H_{13}SO_2$	0.43	4.5	10	$C_{30}H_{42}N_2O_7S$·0.75H_2O
12h	C_3H_7	$C_7H_{15}SO_2$	5.1	57	11	$C_{31}H_{44}N_2O_7S$·0.3TFA
12i	C_3H_7	*i*-$C_4H_9SO_2$	0.78	5.2	6.6	$C_{28}H_{38}N_2O_7S$
12j	C_3H_7	$Cl(CH_2)_3SO_2$	2.0	6.3	3.1	$C_{27}H_{35}N_2O_7ClS$
12k	C_3H_7	$CH_3O(CH_2)_2SO_2$	5.2	77	15	$C_{27}H_{36}N_2O_8S$
12m	C_3H_7	$C_6H_5CH_2SO_2$	10	34	3.4	$C_{31}H_{36}N_2O_7S$
12n	C_3H_7	$C_6H_5SO_2$	1.4	210	150	$C_{30}H_{34}N_2O_7S$·0.5H_2O
12o	C_3H_7	4-$ClC_6H_4SO_2$	6.3	41	6.5	$C_{30}H_{33}N_2O_7ClS$
12p	C_3H_7	4-$CH_3C_6H_4SO_2$	8.3	85	10	$C_{31}H_{36}N_2O_7S$
12q	C_3H_7	4-$CH_3OC_6H_4SO_2$	0.53	66	120	$C_{31}H_{36}N_2O_8S$

[a] IC$_{50}$s calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

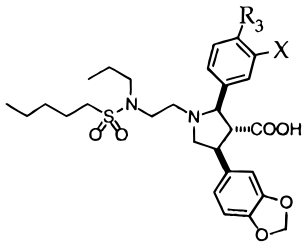
recorded by measuring the displacement of [^{125}I]ET-1 from ET$_A$, or of [^{125}I]ET-3 from ET$_B$. Confirmatory binding studies are performed in a similar fashion, using human ET$_A$ and ET$_B$ receptors (*h*ET$_A$, *h*ET$_B$) permanently expressed in CHO cells.

Analogs of particular interest are examined for their ability to block the ET-1-induced hydrolysis of inositol phosphates in MMQ cells (ET$_A$-receptor mediated) or in ET$_B$-CHO. In both experiments the compounds are also tested for the ability to function as agonists. This same subset of compounds of particular interest has also been examined for their pharmacokinetic properties using a standard protocol which compares the time course of plasma drug levels after dosing in rats by intravenous injection and oral gavage.

Results and Discussion

The lengths of the side chain *N*- and *S*-alkyl substituents in **3** (or **2**) were originally chosen to make the modified side chain roughly isosteric with the original *N*,*N*-dibutylacetamide of **1**. The observation of increased ET$_B$ affinity in this series suggested that our assumptions regarding the overlay of these alkyl substituents were likely to be inaccurate. The suspicion that one of these alkyl groups might be finding a new hydrophobic site which we had not previously explored prompted a reevaluation of the side chain structure−activity profile (Table 1). A brief exploration of the N-substituent led to the choice of an *n*-propyl group (as in **12b**) as one providing an optimal combination of ET$_A$ and ET$_B$ activity. Additional decreases in the length of this substituent (**12a**, $R_1 = CH_3$) or the presence of a branch in the side chain (viz., **12c**) are detrimental to ET$_A$ binding affinity and offer no improvement in ET$_A$/ET$_B$ ratio over the original *n*-butyl group. The strong analogy between this result and that of our earlier study[4] which determined the preferred amide *N*-alkyl substituents in the **1** series suggests that the sulfona-

Table 2. Modification of Position 2 Aryl Substituent



			IC_{50} (nM)[a]			
compd	R_3	X	rET_A binding	pET_B binding	A/B ratio	formula
12f	OCH_3	H	0.57	4.1	7.2	
13a	OCH_2OCH_3	H	0.62	5.2	8.4	$C_{30}H_{42}N_2O_8S{\cdot}0.25H_2O$
13b	$O(CH_2)_2CH_3$	H	48	610	13	$C_{31}H_{44}N_2O_7S$
13c	OCH_3	OCH_3	2.3	73	32	$C_{30}H_{42}N_2O_8S{\cdot}0.6TFA$
13d	$-O(CH_2)_2O-$		0.66	7.0	10	$C_{30}H_{40}N_2O_8S{\cdot}1.0H_2O$
13e	OCH_3	F	0.52	0.85	1.6	$C_{29}H_{39}N_2O_7FS{\cdot}0.25H_2O$
13f	OCH_2CH_3	F	1.3	2.6	2.0	$C_{30}H_{41}N_2O_7S{\cdot}0.25H_2O$
13g	F	F	18	68	3.8	$C_{28}H_{36}N_2O_6F_2S$

[a] IC_{50}s calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

mide *N*-alkyl is sharing one of these previously-described binding sites.

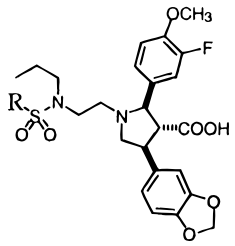
It *is* possible to substantially improve ET_B affinity (and thereby decrease the A/B ratio) through modification of the S-substituent. As the length of this alkyl chain varies from two (**12d**) through six carbons (**12g**), the affinity of the resultant analog for the ET_B receptor steadily improves, with little change in ET_A binding. This combination of events translates into better receptor balance; compounds **12e**−**h** have A/B ratios in the 5−10 range. The optimal chain length appears to be five or six atoms; further extension (as in **12h**) results in decreases in affinity for *both* receptors. This structure−activity profile is markedly different than any observed in the ABT-627 series; it suggests to us that this S-substituent has accessed a new hydrophobic domain that was not available to the amide series. Importantly, it appears that, unlike the "second" amide *N*-alkyl domain of the ET_A-selective series, this *S*-alkyl binding site is present on both ET_A and ET_B receptors.

The sulfonamide S-substituent is not limited to linear alkyl groups. Branching is well tolerated (**12i**), as is heteroatom substitution (see in particular **12j**; also **12k**). A benzyl group decreases the activity at both ET_A and ET_B receptors by about 1 order of magnitude (**12m**), retaining a low A/B ratio. A phenyl substituent also reduces affinity for both receptors (**12n**); through placement of substituents on the aromatic ring (**12o**−**q**), we are able to substantially improve the ET_A activity, but not ET_B. This leads the more active compounds in this *S*-aryl series (e.g. **12q**) to have unacceptably high (>100×) A/B ratios.

With optimal lengths established for the N- and S-substituents on the side chain sulfonamide, compound **12f** was chosen as a standard to evaluate modifications of the pyrrolidine core. In particular we focused (Table 2) on changes in the anisyl ring at position 2 of the pyrrolidine. Structure−activity studies of compounds related to **1** had suggested to us that the replacement of the 4-methoxy substituent with a group other than alkoxy was detrimental to ET_B activity (these studies are unpublished, but will be reported in due course) and led us to focus our attention on substituted 4-alkoxyaryls. Replacement of the 4-OCH_3 group with 4-(methoxymethoxy) is well tolerated (compound **13a**), but offers no improvement in either potency or A/B balance; additionally, the acid lability of this group might prove to be disadvantageous. The stable isosteric 4-propoxy replacement (**13b**) is at least 50 times less potent. The latter result suggested that the second oxygen atom in **13a** might be playing an important role and led us to find other means for introducing such a moiety. Substitution at the position adjacent to the methoxy group provides a useful means for accomplishing this purpose and for modulating A/B selectivity. The 3,4-dimethoxyphenyl analog **13c** exhibits a slight decrease in ET_A affinity and a larger decrease in ET_B; the net result is a higher A/B ratio. When these two alkoxy groups are tied together to form a 3,4-benzodioxane moiety (**13d**), both ET_A and ET_B affinity improve, as does the A/B ratio. The critical observation in this study results from introduction of a fluorine atom at the aryl 3-position. This change produces a substantial improvement in ET_B affinity with little change in ET_A binding; the resultant analog **13e** is the first in the series (and the first reported to date) to exhibit subnanomolar potency against both receptor subtypes and in addition has an exceptionally good balance between ET_A and ET_B. As follow-up to this result we have subsequently prepared analogs having a slightly larger alkoxy substituent (**13f**) as well as one in which the 4-OCH_3 group is completely replaced by a second fluorine atom (**13g**). These studies confirm that a methoxy group is the optimal 4-substituent. The 3-fluoro-4-ethoxyphenyl analog **13f** is slightly less active (2−4×) at both receptors, retaining an A/B ratio near unity; with the 3,4-difluorophenyl replacement, the loss in binding affinity for ET_A and ET_B is between 20- and 100-fold.

This second SAR study served to establish **13e** as a new standard for the sulfonamide series. The combination of 3-fluorination on the anisyl ring with a longer (five-carbon) *S*-alkyl chain has led to a compound with IC_{50} values of 0.52 and 0.88 nM for ET_A and ET_B receptors, respectively. Since our earlier study of side chain substitution had suggested that several other alkyl groups were well-tolerated as replacements for

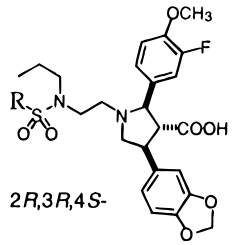
Table 3. Second-Generation Analogs



compd	R	IC$_{50}$ (nM)[a] rET$_A$ binding	IC$_{50}$ (nM)[a] pET$_B$ binding	A/B ratio	formula
13e	C_5H_{11}	0.52	0.85	1.6	
14a	C_4H_9	0.54	1.0	1.9	$C_{28}H_{37}N_2O_7FS$
14b	C_6H_{13}	0.46	1.5	3.3	$C_{30}H_{41}N_2O_7FS$
14c	*i*-C_4H_9	0.21	1.6	7.6	$C_{28}H_{37}N_2O_7FS{\cdot}0.25H_2O$
14d	*i*-C_5H_{11}	0.46	1.2	2.6	$C_{29}H_{39}N_2O_7FS$
14e	$Cl(CH_2)_3$	0.40	1.0	2.5	$C_{27}H_{34}N_2O_7ClFS$
14f	$CF_3(CH_2)_3$	0.55	0.98	1.8	$C_{28}H_{34}N_2O_7F_4S$
14g	$C_6H_5CH_2$	2.7	2.9	1.1	$C_{31}H_{35}N_2O_7FS{\cdot}0.5H_2O$

[a] IC$_{50}$s calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

Table 4. Summary Data for Single Enantiomers



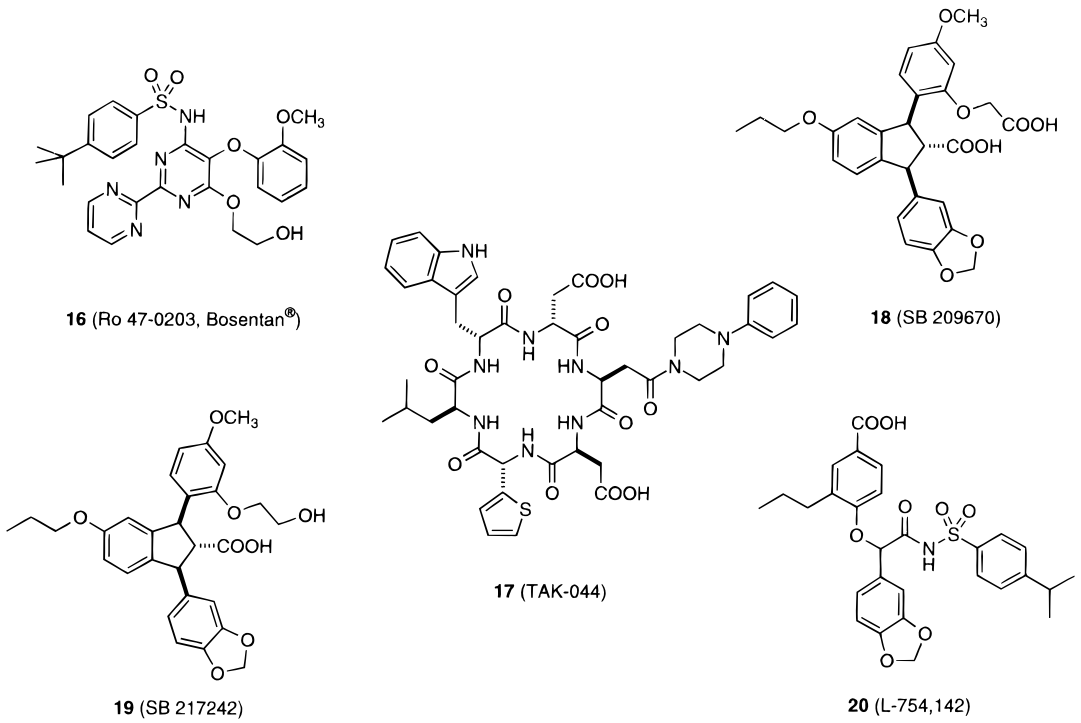
compd	R	binding IC$_{50}$, nM (SEM, n)[a,b] rET$_A$	pET$_B$	hET$_A$	hET$_B$	functional IC$_{50}$, nM (n)[a,c] rET$_A$	hET$_B$	pharmacokinetic profile[c] (rats) $T_{1/2}$, iv (h)	$T_{1/2}$, oral (h)	C_{max} (μg/mL)	AUC (μg h/mL)	F (%)
15a	C_4H_9	0.10 (0.018, 7)	0.32 (0.055, 7)	0.044 (3)	0.47 (3)	0.38 (2)	0.60 (2)	6.9	7.3	1.1	3.7	31
15b (A-182086)	C_5H_{11}	0.13 (0.015, 6)	0.29 (0.029, 6)	0.078 (3)	0.28 (3)	0.48 (4)	1.28 (3)	5.0	8.1	2.4	11.2	54
15c	C_6H_{13}	0.11 (0.016, 5)	0.34 (0.046, 5)	0.077 (2)	0.48 (2)	0.14 (1)	1.12 (1)	3.3	6.4	0.7	2.8	40
15d	$Cl(CH_2)_3$	0.094 (0.021, 5)	0.20 (0.024, 5)	0.070 (2)	0.14 (2)	0.55 (1)	0.83 (1)	14.0	9.6	0.4	2.2	25
15e	$CF_3(CH_2)_3$	0.22 (0.055, 5)	0.32 (0.059, 5)	0.055 (2)	0.20 (2)	1.66 (1)	1.46 (1)	4.3	8.9	1.2	4.3	25

[a] IC$_{50}$s calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted. [b] Binding assays recorded as described in Experimental Section, using MMQ cells (rET$_A$), porcine cerebellar tissue (pET$_B$), or clonal CHO cell lines (hET$_A$ and hET$_B$). [c] Measurements of phosphoinositide (PI) hydrolysis as described in Experimental Section, using MMQ cells (rET$_A$) or CHO cells containing a human ET$_A$ receptor clone (hET$_B$). [d] $T_{1/2}$ iv, half-life after intravenous dosing. $T_{1/2}$ oral, C_{max}, AUC, and F are half-life, maximum drug concentration, total drug exposure (area under the curve) and oral bioavailability after oral dosing in rats. Calculated from raw pharmacokinetic data as described in the Experimental Section.

S-pentyl, we deemed it worthwhile to reevaluate a number of these side chain possibilities with the fluorine-containing core. The results of this fine-tuning (Table 3) largely confirm our initial observations. As the length of the linear *S*-alkyl chain varies from four to six carbon atoms, receptor affinities are largely unchanged, and the A/B ratio remains low. Branching is tolerated at the S-substituent (**14c**,**d**) and does not appear to distinguish between ET$_A$ and ET$_B$. Halo substitution is also acceptable, with both 3-chloropropyl and 4,4,4-trifluorobutyl chains (compounds **14e** and **14f**, respectively) resulting in analogs with potencies similar to **13e**. In fact, not only **13e** but also **14a**−**f** seem likely (when prepared as single, active enantiomers) to meet our goal of providing subnanomolar affinities for both receptors. Only benzylsulfonamide **14g** appears to be significantly less active.

A more detailed evaluation was undertaken on a smaller set of compounds prepared in optically enriched form (Table 4). This subset (compounds **15a**−**e**) was first evaluated in our standard screening assay, as well as in a second set of binding assays employing human ET$_A$ and ET$_B$ receptors expressed in CHO cells. As we have observed in previous studies,[3,4] the rET$_A$ and pET$_B$ receptors used in the screening assays accurately predict the relative and absolute affinities observed against human receptors, with some tendency for this class of compounds to exhibit higher affinities for the human genotypes. In fact, **15a**−**e** all have subnanomolar potencies against both hET$_A$ and hET$_B$ receptors; A/B

Chart 1. Representative "Balanced" $ET_{A/B}$ Antagonists



ratios vary from 2 (**15d**) to 10 (**15a**). To confirm that the compounds are antagonists, **15a**−**e** have been evaluated in a set of functional assays measuring ET_R-mediated hydrolysis of inositol phosphates. The potency in these assays correlates well with receptor affinity, and the compounds show no agonist effect at high doses (data not shown), confirming that as a class they are pure, functional antagonists at both ET_A and ET_B.

Because it was difficult to identify an optimal compound in this series based purely on receptor binding properties, pharmacokinetic profiles (in rats) were acquired for all analogs in Table 4. These studies suggest that, in addition to their superior biochemical profiles, this class of balanced ET antagonists may be readily deliverable through an oral route. Oral bioavailabilities for **15a**−**e** are generally high, and the compounds have plasma half-lives in the range 5−10 h. While it is difficult to find a clear distinction between compounds **15**, it appears that **15b** (A-182086) may offer the best combination of potency, ET_A/ET_B balance, and pharmacokinetic properties.

It is instructive to evaluate **15b** against other balanced ET antagonists which have appeared in the recent literature (Chart 1; comparison data in Table 5). Roche's Bosentan[8a] (**16**) has been extensively studied and is currently under evaluation in human subjects. Takeda's TAK-044[8b] (**17**) is another nonselective peptide; balanced nonpeptide antagonist series have been reported by SmithKline Beecham (SB209670, **18**; and SB217242, **19**),[8c] and by Merck (L-754,142; **20**),[8d] among others. The most potent of these are **17**, **18**, and **20**, which exhibit subnanomolar affinities for human ET_A receptor.

While the first two of these are useful only as parenteral agents, **20** is well-absorbed orally (60% in rats) and is also the best "balanced" agent (17-fold difference between ET_A and ET_B activities) reported to date. By comparison **15b** appears to be substantially more potent at the ET_A receptor than any of the above

Table 5. Comparison Data for Representative Mixed Antagonists

	IC_{50} (nM)[a]				
compd	*h*ET_A binding	*h*ET_B binding	A/B ratio[b]	iv half-life[c] (h)	F^c (%)
16[8a]	6.5 (K_i)	340 (K_i)	53	4−8	30−80
17[8b]	0.24	130	540	0.5−1.0	
18[8c(1)]	0.20 (K_i)	18 (K_i)	90		
19[8c(2)]	1.1 (K_i)	110 (K_i)	100	2.0	60
20[8d]	0.26	4.4	17	2.0	60
15b	0.078	0.28	3.6	5.0	54

[a] Human receptor data acquired in a variety of systems; IC_{50} values are given unless K_i is explicitly stated. [b] Expressed as $IC_{50}(ET_B)/IC_{50}(ET_A)$. [c] $T_{1/2}$ and oral bioavailability (F) data, measured in rats, collected from a variety of sources.

antagonists; it also shows a balance between receptors closer than has previously been described. The subnanomolar ET_B potency of this compound is particularly noteworthy; to our knowledge, **15b** is the most potent ET_B antagonist reported to date. The compound is also well absorbed (oral bioavailability = 54%) and has a substantially longer iv half-life (5 vs 2 h) than **20**. It is difficult to make direct comparisons between data recorded by different groups under differing assay conditions (for example, it is known[9] that the presence or absence of serum albumin in binding assay buffer can substantially impact the binding of various ET antagonist molecules to ET_R); however, it is clear that **15b** has superior properties as a balanced antagonist of endothelin receptors.

Conclusions

When the *N*,*N*-dialkylacetamide side chain of the highly ET_A-selective endothelin antagonist ABT-627 (**1**) is replaced by (*N*,*S*-dialkylsulfonamido)ethyl, the resultant analogs retain ET_A affinity, but exhibit substantially improved ET_B activity as well. Structure−activity studies reveal that modifications in the length of the two alkyl groups, and in the substitution on the anisyl

ring, are important in optimizing this "balanced" antagonist profile. In particular, the combination of an *N*-*n*-propyl group, an *S*-alkyl chain between four and six carbons in length, and a fluorine atom *ortho* to the aromatic OCH_3 provides compounds with sub-nanomolar affinities for both receptor subtypes, and with ET_A/ET_B ratios close to unity. A number of these compounds also exhibit oral bioavailabilities (in rats) in the 30−50% range and have relatively long plasma half-lives. Of these, **15b** exhibits the best combination of physical and biochemical properties.

Our SAR studies suggest that there is no direct mapping of the *N*- and *S*-alkyl groups of the sulfonamide onto the two amide *N*-butyl substituents of **1**. Instead, while it is likely that the sulfonamide *N*-alkyl *does* share a hydrophobic domain with one of the amide *N*-butyls, the sulfonamide moiety serves to position the longer *S*-alkyl into a domain which (unlike the binding site for the second amide *N*-butyl) is present in both ET_A and ET_B receptor subtypes.

The balanced receptor-binding profile of these potent and orally-bioavailable compounds complements the ET_A selectivity observed with **1**. It remains to be seen whether this balanced profile will prove to be an advantage (or disadvantage) in treating diseases in which endothelin plays a pathogenic role. Studies to evalute this question are underway in our labs, and will be reported in due course.

Experimental Section

Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. THF was dried over sodium and purified by distillation. All reactions were performed under nitrogen atmosphere unless specifically noted. Flash chromatography was done using silica gel (230−400 mesh) from E. M. Science. ^{1}H-NMR spectra were recorded at 300 MHz; all values are referenced to tetramethylsilane as internal standard and are reported as shift (multiplicity, coupling constants, proton count). Mass spectral analysis is accomplished using fast atom bombardment (FAB-MS) or direct chemical ionization (DCI-MS) techniques. All elemental analyses are consistent with theoretical values to within ±0.4% unless indicated. Analytical HPLC chromatographs are recorded on a Beckman system using a Vydac "Peptide & Protein" C18 column, eluting with gradients of acetonitrile in 0.1% aqueous TFA. Melting points were measured on a Thomas Hoover apparatus and are uncorrected.

Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; EtOAc, ethyl acetate; TFA, trifluoroacetic acid.

General Synthesis of Core Compounds. Ethyl 2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate as a Mixture of *Trans,Trans* and *Cis,Trans* Diastereomers (8, $R_3 = OCH_3$, X = F). This compound was prepared from ethyl (3-fluoro-4-methoxybenzoyl)acetate and 3,4-(methylenedioxy)-2′-nitrostyrene as starting materials according to procedures described in our previous article.[4] The mixture was refluxed in toluene (5 mL/g) and DBU (1.2 equiv) overnight to convert the *cis,cis* isomer in the mixture to *trans,trans*. Other compounds **8** were prepared in a similar fashion from the appropriate starting materials.

Resolution of Compound 8 ($R_3 = OCH_3$, X = F). To racemic compound **8** (15.0 g, 38.8 mmol), dissolved in 75 mL of dichloromethane and cooled in an ice bath, was added di-*tert*-butyl dicarbonate (9.30 g, 42.7 mmol). After 2 h of stirring at room temperature, the solution was concentrated *in vacuo*; the residue was dissolved in 50 mL of ethanol and treated with a solution of 3.75 g of NaOH in 19 mL water. The solution was warmed until homogeneity was achieved. After 2 h of stirring at room temperature, the solution was concentrated and redissolved in 200 mL of water. The resultant mixture was extracted with 75 mL of diethyl ether; the ether layer was extracted with 40 mL of water. The combined aqueous phases were acidified with 7.5 g of acetic acid; the mixture was stirred until a solid formed. The solid was filtered, washed with water, and dissolved in dichloromethane. After being dried with Na_2SO_4, the solution was concentrated and the residue crystallized from 1:1 ether−hexanes to get 16.0 g of product, mp 200−203 °C (90% yield). The crude acid was suspended in 80 mL of EtOAc and treated with 4.00 g (33.1 mmol) of (*S*)-(−)-α-methylbenzylamine. After being heated to dissolve the acid, 80 mL of ether was added. Scratching with a glass rod caused the product to crystallize. The solids were filtered and washed with ether−EtOAc solution to give 8.22 g (81% yield based on 50% maximum recovery) of salt, mp 165−168 °C. After one recrystallization, chiral HPLC analysis, using a Regis Whelk-O column, indicated >99.5% ee. The salt was dissolved in 500 mL of 36% HCl in ethanol; a white solid forms. The resultant suspension was warmed for 16 h at 50 °C. After the suspension was concentrated *in vacuo*, the residue was combined with toluene and stirred with potassium bicarbonate in water for 30 min. The toluene was separated, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel, eluting with 1:2 hexane−EtOAc to get 6.9 g (99%) of the resolved amino ester.

Compounds 9. Compound **8** was dissolved in 1,2-dibromoethane (10 mL per 1 g of starting material); diisopropylethylamine (1 mL per 1 g of starting material) and NaI (100 mg per 1 g of starting material) were added, and the mixture was stirred at 100 °C for 1 h. Toluene was added, and the mixture was washed with sodium bicarbonate solution. The solvents were concentrated, and the resultant black residue was chromatographed on silica gel, eluting with 4:1 hexanes−EtOAc to give the *N*-(2-bromoethyl)pyrrolidine (85−92%). This compound was combined with the appropriate amine (3.5 equiv) and NaI (10% by weight of bromide) in ethanol (5 mL per 1 g of bromide) and was heated at 80 °C for 2 h. Toluene was added, and the mixture was washed with sodium bicarbonate solution, dried (Na_2SO_4), and concentrated. More toluene was added, and removed *in vacuo*, to get rid of the primary amine. The residue was dissolved in heptane and filtered to remove a small amount of insoluble material. Evaporation of the solvent gave the desired product (86−93% yield), which was used for the next step without further purification.

Various Sulfonyl Chlorides. Most of the sulfonyl chlorides employed in this study are commercially available. Other sulfonyl chlorides were prepared using the procedures of Roemmele and Rapoport,[10] with the exception of 4,4,4-trifluorobutanesulfonyl chloride, which was synthesized by the method of Bunyagidi et al.[11]

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-butyl-*N*-*n*-butanoylamino)ethyl]pyrrolidine-3-carboxylic Acid (2).** To compound **9** (R_1 = *n*-Bu, $R_3 = OCH_3$, X = H) (211 mg, 0.45 mmol) dissolved in 8 mL of 1,2-dichloroethane was added 0.25 mL of diisopropylethylamine. The solution was cooled to −40 °C, butyryl chloride (53 mg, 1.1 equiv) was added, the bath was removed, and the mixture was allowed to warm to ambient temperature and stirred overnight. Solvents were removed *in vacuo*; the residue was taken up in EtOAc, washed sequentially with sodium bicarbonate solution, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel, eluting with a gradient of 1:1 EtOAc−hexane going to EtOAc and finally 10% methanol−EtOAc. The purified ester was dissolved in 2.5 mL of ethanol; 0.75 mL of a 17% aqueous NaOH solution was added, and the resultant mixture was stirred at ambient temperature for 3 h. The solvents were evaporated, and the residue was taken up in water and washed with ether. The aqueous phase was acidified with 1 N H_3PO_4 to pH 5 and extracted twice with ether. The combined organic extracts were washed with brine and dried over Na_2SO_4. The solvents were removed *in vacuo* to provide 153 mg (61%) of the title compound as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (m, 3H), 0.90 (t, J = 8 Hz, 3H), 1.45 (m, 4H), 1.6 (m, 2H), 2.20 (t, J = 8 Hz, 3H), 2.94 (br m, 2H), 3.10 (br m, 2H), 3.5 (br m, 3H), 3.80 (br m,

2H), 3.82 (s, 3H), 4.30 (br s, 1H), 5.95 (s, 2H), 6.75 (d, J = 8 Hz, 1H), 6.84 (m, 1H), 6.85 (d, J = 8 Hz, 2H), 7.04 (d, J = 1 Hz, 1H), 7.40 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 511 (M + H)$^+$; HRMS (FAB) calcd for $C_{29}H_{38}N_2O_6$ 511.2808, found 511.2809; analytical HPLC, 94% pure.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-butyl-*N*-(*n*-propylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic Acid (3).** Compound **9** (R_1 = *n*-Bu, R_3 = OCH_3, X = H) (200 mg, 0.43 mmol) was dissolved in 5 mL of CH_3CN; 110 mg (2 equiv) of *N*,*N*-diisopropylethylamine and 73 mg (1.2 equiv) of 1-propanesulfonyl chloride were added sequentially, and the resultant solution was allowed to stir at room temperature for 30 min. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc. The solution was washed with sodium bicarbonate solution, 1 N H_3PO_4, and brine, dried over Na_2SO_4, and evaporated to give a yellowish oil which was purified by flash chromatography on silica gel, eluting with 40% EtOAc/hexanes to give 220 mg of product (89%). This ester was dissolved in 5 mL of ethanol, to which was added NaOH (46 mg, 3 equiv) solution in 2 mL of water. This mixture was stirred for 3 h at room temperature. The solution was concentrated in vacuo using a low (<40 °C) bath temperature. Water (10 mL) and ether (50 mL) were added; the ether layer was extracted with 5 mL of water. The combined aqueous mixture was back-extracted with ether and then neutralized with acetic acid. This solution was extracted twice with ether. The acidic extracts were dried (Na_2SO_4) and concentrated in vacuo. EtOAc (1 mL) and ether (1 mL) were added to dissolve the product, and hexanes was added dropwise to produce a white solid. The solid was collected and dried in vacuo to give 126 mg of **3**: mp 64−65 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (t, J = 7 Hz, 3H), 0.98 (t, J = 7 Hz, 3H), 1.12−1.25 (m, 2H), 1.32−1.41 (m, 2H), 1.75 (sextet, J = 7 Hz, 2H), 2.23−2.31 (m, 2H), 2.72−3.32 (m, 8H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.53−3.59 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.83 (dd, J = 1, 8 Hz, 1H), 6.88 (d, J = 9 Hz, 2H), 7.02 (d, J = 1 Hz, 1H), 7.33 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 547 (M + H)$^+$; HRMS (FAB) calcd for $C_{28}H_{39}N_2O_7S$ 547.2474, found 547.2478; analytical HPLC, 95% pure.

The following compounds are prepared using the procedures described above for compound **3**.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-methyl-*N*-(*n*-propylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12a):** white solid; mp 69−70 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02 (t, J = 7.5 Hz, 3H), 1.78 (hexad, J = 7.5 Hz, 2H), 2.18−2.26 (m, 1H), 2.72 (s, 3H), 2.75−2.95 (m, 5H), 3.13−3.22 (m, 1H), 3.25−3.35 (m, 1H), 3.47−3.58 (m, 2H), 3.66 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.96 (s, 2H), 6.74 (d, J = 7.5 Hz, 1H), 6.84 (dd, J = 3, 7.5 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 7.04 (d, J = 3 Hz, 1H), 7.43 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 505 (M + H)$^+$; HRMS (FAB) calcd for $C_{25}H_{33}N_2O_7S$ 505.2015, found 505.2008; analytical HPLC, 91% pure.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-propylsulfonyl)amino)ethyl]-pyrrolidine-3-carboxylic acid (12b):** white solid; mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (t, J = 8 Hz, 3H), 0.98 (t, J = 8 Hz, 3H), 1.43 (sextet, J = 8 Hz, 2H), 1.75 (sextet, J = 8 Hz, 2H), 2.22−2.32 (m, 1H), 2.69−3.32 (m, 9H), 3.42 (dd, J = 3, 12 Hz, 1H), 3.52−3.58 (m, 1H), 3.64 (d, J = 12 Hz, 1H), 3.80 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 11 Hz, 1H), 6.83 (dd, J = 1, 11 Hz, 1H), 6.87 (d, J = 11 Hz, 2H), 7.0 (d, J = 2 Hz, 1H), 7.32 (d, J = 11 Hz, 2H); MS (DCI/NH_3) m/e 533 (M + H)$^+$. Anal. ($C_{27}H_{36}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-isobutyl-*N*-(*n*-propylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12c):** white solid; mp 73−74 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, J = 6 Hz, 6H), 0.98 (t, J = 8 Hz, 3H), 1.62 (sextet, J = 6 Hz, 1H), 1.74 (sextet, J = 8 Hz, 2H), 2.23−2.34 (m, 1H), 2.68−2.98 (m, 7H), 3.08−3.18 (m, 1H), 3.26-3.42 (m, 2H), 3.52−3.58 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.90 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.86 (d, J = 8 Hz, 2H), 6.98 (d, J = 1 Hz, 1H), 7.33 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 547 (M + H)$^+$; HRMS (FAB) calcd for $C_{28}H_{39}N_2O_7S$ 547.2478, found 547.2480. Anal. ($C_{28}H_{38}N_2O_7S$·0.2TFA) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(ethylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12d):** white solid; mp 70−72 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (t, J = 8 Hz, 3H), 1.28 (t, J = 7 Hz, 3H), 1.43 (q, J = 8 Hz, 2H), 2.22−2.30 (m, 1H), 2.71−2.80 (m, 1H), 2.82−3.10 (m, 6H), 3.18−3.32 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.53−3.60 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.96 (s, 2H), 6.73 (d, J = 7 Hz, 1H), 6.82 (dd, J = 1, 7 Hz, 1H), 6.88 (d, J = 8 Hz, 2H), 7.00 (d, J = 1 Hz, 1H), 7.32 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 519 (M + H)$^+$; HRMS (FAB) calcd for $C_{26}H_{35}N_2O_7S$ 519.2165, found 519.2165. Anal. ($C_{26}H_{34}N_2O_7S$·0.3TFA) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-butylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12e):** white solid; mp 65−66 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78 (t, J = 7 Hz, 3H), 0.92 (t, J = 7 Hz, 3H), 1.31−1.46 (m, 4H), 1.68 (quintet, J = 7 Hz, 2H), 2.21−2.32 (m, 1H), 2.70−3.08 (m, 7H), 3.12−3.23 (m, 2H), 3.42 (dd, J = 2, 9 Hz, 1H), 3.52−3.58 (m, 1H), 3.64 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.96 (s, 2H), 6.72 (d, J = 7 Hz, 1H), 6.83 (dd, J = 1, 7 Hz, 1H), 6.86 (d, J = 8 Hz, 2H), 7.00 (d, J = 1 Hz, 1H), 7.32 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 547 (M + H)$^+$. Anal. ($C_{28}H_{38}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12f):** white solid; mp 59−61 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (t, J = 7.5 Hz, 3H), 0.90 (t, J = 6 Hz, 3H), 1.26−1.32 (m, 4H), 1.43 (sextet, J = 7.5 Hz, 2H), 1.67−1.76 (m, 2H), 2.23−2.32 (m, 1H), 2.70−3.08 (m, 7H), 3.15−3.32 (m, 2H), 3.42 (dd, J = 3, 9 Hz, 1H), 3.52−3.57 (m, 1H), 3.63 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 7.5 Hz, 1H), 6.83 (dd, J = 1, 7.5 Hz, 1H), 6.87 (d, J = 8 Hz, 2H), 7.00 (d, J = 1 Hz, 1H), 7.32 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 561 (M + H)$^+$. Anal. ($C_{29}H_{40}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-hexylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12g):** white solid; mp 59−60 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (t, J = 7.5 Hz, 3H), 0.89 (t, J = 7 Hz, 3H), 1.25−1.36 (m, 6H), 1.53 (sextet, J = 7.5 Hz, 2H), 1.72 (quintet, J = 7 Hz, 2H), 2.23−2.32 (m, 1H), 2.72−3.08 (m, 7H), 3.15−3.32 (m, 2H), 3.43 (d, J = 9 Hz, 1H), 3.55−3.62 (m, 1H), 3.65 (d, J = 10 Hz, 1H), 3.80 (s, 3H), 5.96 (s, 2H), 6.74 (d, J = 7.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 7.01 (s, 1H), 7.32 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 575 (M + H)$^+$. Anal. ($C_{30}H_{42}N_2O_7S$·0.75H_2O) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-heptylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12h):** white solid; mp 58−59 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7 Hz, 3H), 1.23−1.36 (m, 8H), 1.94 (q, J = 7.5 Hz, 2H), 1.71 (quintet, J = 7 Hz, 2H), 2.23−2.32 (m, 1H), 2.70−3.09 (m, 7H), 3.13-3.32 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.52−3.58 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.96 (s, 2H), 6.73 (d, J = 7 Hz, 1H), 6.83 (dd, J = 1, 7 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 7.01 (d, J = 1 Hz, 1H), 7.32 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 589 M + H)$^+$; HRMS (FAB) calcd for $C_{31}H_{45}N_2O_7S$ 589.2947, found 589.2942. Anal. ($C_{31}H_{44}N_2O_7S$·0.3TFA) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(isobutylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12i):** white solid; mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7.5 Hz, 3H), 1.04 (d, J = 6 Hz, 6H), 1.44 (q, J = 7.5 Hz, 2H), 2.15−2.33 (m, 2H), 2.57−2.75 (m, 3H), 2.84−3.08 (m, 4H), 3.12−3.21 (m, 1H), 3.23−3.45 (m, 1H), 3.43 (d, J = 11 Hz, 1H), 3.55−3.62 (m, 1H), 3.66 (d, J = 9 Hz, 1H), 3.80 (s, 3H), 5.95 (s, 2H), 6.75 (d, J = 9 Hz, 1H), 6.83 (dd, J = 1, 9 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 7.02 (d, J = 1 Hz, 1H), 7.33 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 547 M + H)$^+$. Anal. ($C_{28}H_{38}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((3-chloropropyl)sulfonyl)amino)-ethyl]pyrrolidine-3-carboxylic acid (12j):** white solid; mp 75−76 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (t, J = 7 Hz,

3H), 1.45 (sextet, J = 7 Hz, 2H), 2.15−2.31 (m, 3H), 2.70−2.80 (m, 1H), 2.85−3.10 (m, 6H), 3.23−3.31 (m, 2H), 3.43 (bd, J = 9 Hz, 1H), 3.55−3.66 (m, 4H), 3.81 (s, 3H), 5.94 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.86 (d, J = 8 Hz, 2H), 7.00 (s, 1H), 7.33 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 567 $(M + H)^+$. Anal. ($C_{27}H_{35}N_2O_7ClS$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((2-methoxyethyl)sulfonyl)amino-)ethyl]pyrrolidine-3-carboxylic acid (12k):** white solid; mp 62−64 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78 (t, J = 7 Hz, 3H), 1.42 (sextet, J = 7 Hz, 2H), 2.23−2.32 (m, 1H), 2.72−2.79 (m, 1H), 2.86−3.05 (m, 4H), 3.10−3.27 (m, 4H), 3.32 (s, 3H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.53−3.58 (m, 1H), 3.65 (d, J = 9 Hz, 1H), 3.69 (t, J = 6 Hz, 2H), 3.80 (s, 3H), 5.94 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.82 (dd, J = 1, 8 Hz, 1H), 6.87 (d, J = 8 Hz, 2H), 7.02 (d, J = 1 Hz, 1H), 7.33 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 549 $(M + H)^+$. Anal. ($C_{27}H_{36}N_2O_8S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(benzylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12m):** white solid; mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.72 (t, J = 7 Hz, 3H), 1.32 (sextet, J = 7 Hz, 2H), 2.06−2.16 (m, 1H), 2.56−2.67 (m, 1H), 2.75−3.10 (m, 6H), 3.30 (dd, J = 2, 9 Hz, 1H), 3.52 (m, 1H), 3.58 (d, J = 9 Hz, 1H), 3.78 (s, 3H), 4.09 (d, J = 12 Hz, 1H), 4.15 (d, J = 12 Hz, 1H), 5.95 (s, 2H), 6.73 (d, J = 7 Hz, 1H), 6.80 (dd, J = 1, 7 Hz, 1H), 6.86 (d, J = 8 Hz, 2H), 6.97 (d, J = 1 Hz, 1H), 7.27−7.35 (m, 7H); MS (DCI/NH_3) m/e 581 $(M + H)^+$; HRMS (FAB) calcd for $C_{31}H_{37}N_2O_7S$ 581.2321, found 581.2330. Anal. ($C_{31}H_{36}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(phenylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12n):** white solid; mp 89−91 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (t, J = 6 Hz, 3H), 1.33 (sextet, J = 6 Hz, 2H), 2.20−2.30 (m, 1H), 2.62−2.72 (m, 1H), 2.85−3.05 (m, 4H), 3.12−3.22 (m, 2H), 3.38 (dd, J = 3, 9 Hz, 1H), 3.49−3.57 (m, 1H), 3.62 (d, J = 9 Hz, 1H), 3.82 (s, 3H), 5.96 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.84 (dd, J = 1, 8 Hz, 1H), 6.85 (d, J = 9 Hz, 2H), 7.02 (d, J = 1 Hz, 1H), 7.28 (d, J = 9 Hz, 2H), 7.39−7.54 (m, 3H), 7.70 (d, J = 7 Hz, 2H); MS (DCI/NH_3) m/e 567 $(M + H)^+$. Anal. ($C_{30}H_{34}N_2O_7S\cdot0.5H_2O$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((4-chlorophenyl)sulfonyl)amino)-ethyl]pyrrolidine-3-carboxylic acid (12o):** white solid; mp 105−106 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.72 (t, J = 7 Hz, 3H), 1.34 (sextet, J = 7 Hz, 2H), 2.56−2.62 (m, 1H), 2.78−2.86 (m, 1H), 2.96−3.03 (m, 3H), 3.13−3.26 (m, 3H), 3.51 (dd, J = 5, 9 Hz, 1H), 3.62−3.68 (m, 1H), 3.80 (s, 3H), 3.94 (d, J = 9 Hz, 1H), 5.92 (s, 2H), 6.75 (d, J = 8 Hz, 1H), 6.84 (dd, J = 2, 8 Hz, 1H), 6.94 (d, J = 8 Hz, 2H), 6.98 (d, J = 2 Hz, 1H), 7.36 (d, J = 8 Hz, 2H), 7.49 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 601 $(M + H)^+$. Anal. ($C_{30}H_{33}N_2ClO_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((4-methylphenyl)sulfonyl)amino-)ethyl]pyrrolidine-3-carboxylic acid (12p):** white solid; mp 78−79 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.73 (t, J = 7 Hz, 3H), 1.33 (sextet, J = 7 Hz, 2H), 2.20−2.30 (m, 1H), 2.40 (s, 3H), 2.61−2.72 (m, 1H), 2.83−3.05 (m, 4H), 3.08−3.19 (m, 2H), 3.48 (dd, J = 3, 9 Hz, 1H), 3.49−3.57 (m, 1H), 3.62 (d, J = 9 Hz, 1H), 3.81 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.87 (d, J = 8 Hz, 2H), 7.00 (s, 1H), 7.21 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 7.57 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 581 $(M + H)^+$. Anal. ($C_{31}H_{36}N_2O_7S$) C, H, N.

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((4-methoxyphenyl)sulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (12q):** white solid; mp 96−97 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.73 (t, J = 7 Hz, 3H), 1.34 (sextet, J = 7 Hz, 2H), 2.20−2.30 (m, 1H), 2.62−2.71 (m, 1H), 2.82−3.03 (m, 4H), 3.08−3.18 (m, 2H), 3.38 (dd, J = 3, 9 Hz, 1H), 3.48−3.56 (m, 1H), 3.62 (d, J = 9 Hz, 1H), 3.81 (s, 3H), 3.86 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.81−6.89 (m, 5H), 7.01 (d, J = 1 Hz, 1H), 7.28 (d, J = 8 Hz, 2H), 7.62 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 597 $(M + H)^+$. Anal. ($C_{31}H_{36}N_2O_8S$) C, H, N.

***trans,trans*-2-[4-(Methoxymethoxy)phenyl]-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (13a):** white solid; mp 57−59 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78 (t, J = 7 Hz, 3H), 0.90 (t, J = 7 Hz, 3H), 1.28−1.36 (m, 4H), 1.93 (sextet, J = 7 Hz, 2H), 1.72 (t, J = 7 Hz, 2H), 2.20−2.32 (m, 1H), 2.72−3.10 (m, 7H), 3.18−3.41 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.48 (s, 3H), 3.52−3.59 (m, 1H), 3.68 (d, J = 9 Hz, 1H), 5.15 (s, 2H), 5.94 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.82 (dd, J = 1, 8 Hz, 1H), 6.98−7.02 (m, 3H), 7.32 (d, J = 9 Hz, 2H); MS (DCI/NH_3) m/e 591 $(M + H)^+$. Anal. ($C_{30}H_{42}N_2O_8S\cdot0.25H_2O$) C, H, N.

***trans,trans*-2-(4-Propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (13b):** white solid; mp 53−54 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.79 (t, J = 7 Hz, 3H), 0.89 (t, J = 7 Hz, 3H), 1.03 (t, J = 7 Hz, 3H), 1.24−1.34 (m, 4H), 1.43 (sextet, J = 7 Hz, 2H), 1.67−1.75 (m, 2H), 1.80 (sextet, 2H), 2.23−2.33 (m, 1H), 2.72−2.93 (m, 6H), 3.05 (septet, J = 7 Hz, 2H), 3.15−3.35 (m, 2H), 3.42 (d, J = 9 Hz, 1H), 3.54−3.62 (m, 1H), 3.67 (d, J = 9 Hz, 1H), 4.90 (t, J = 7 Hz, 2H), 5.95 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.85 (d, J = 8 Hz, 2H), 7.02 (s, 1H), 7.32 (d, J = 8 Hz, 2H); MS (DCI/NH_3) m/e 589 $(M + H)^+$. Anal. ($C_{31}H_{44}N_2O_7S$) C, H, N.

***trans,trans*-2-(3,4-Dimethoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]-pyrrolidine-3-carboxylic acid (13c):** white solid; mp 75−86 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.75 (t, J = 7 Hz, 3H), 0.82 (t, J = 7 Hz, 3H), 1.32−1.43 (m, 6H), 1.65−1.77 (m, 2H), 3.0−3.09 (m, 4H), 3.23−3.27 (m, 2H), 3.44 (t, J = 6 Hz, 1H), 3.47−3.56 (m, 2H), 3.78 (d, J = 9 Hz, 1H), 3.83−3.93 (m, 1H), 3.87 (s, 3H), 3.92 (s, 3H), 4.63 (d, J = 13 Hz, 1H), 5.97 (s, 2H), 6.82 (d, J = 7 Hz, 1H), 6.93 (d, J = 7 Hz, 1H), 7.06 (d, J = 7 Hz, 1H), 7.08 (d, J = 3 Hz, 2H), 7.16 (dd, J = 3, 7 Hz, 1H), 7.27 (d, J = 3 Hz, 1H); MS (DCI/NH_3) m/e 591 $(M + H)^+$; HRMS (FAB) calcd for $C_{30}H_{43}N_2O_8S$ 591.2740, found 591.2725. Anal. ($C_{30}H_{42}N_2O_8S\cdot0.6TFA$) C, H, N.

***trans,trans*-2-(Benzodioxan-6yl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (13d):** white solid; mp 68−69 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.89 (t, J = 6 Hz, 3H), 1.28−1.37 (m, 4H), 1.45 (quintet, J = 7 Hz, 2H), 1.73 (m, 2H), 2.28 (m, 1H), 2.65−2.95 (m, 5H), 3.0−3.12 (m, 2H), 3.15−3.32 (br, 2H), 3.41 (m, 1H), 3.50−3.64 (m, 2H), 4.25 (s, 4H), 5.95 (s, 2H), 6.73 (d, J = 7 Hz, 1H), 6.81−6.98 (m, 5H); MS (DCI/NH_3) m/e 589 $(M + H)^+$; HRMS (FAB) calcd for $C_{30}H_{41}N_2O_8S$ 589.2594, found 589.2573. Anal. ($C_{30}H_{40}N_2O_8S\cdot1.0H_2O$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (13e):** white solid; mp 66−68 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (t, J = 7.5 Hz, 3H), 0.89 (t, J = 7 Hz, 3H), 1.26−1.35 (m, 4H), 1.45 (sextet, J = 7.5 Hz, 2H), 1.68−1.76 (m, 2H), 2.25−2.33 (m, 1H), 2.72−2.92 (m, 5H), 2.97−3.12 (m, 2H), 3.16−3.33 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.53−3.60 (m, 1H), 3.66 (d, J = 10 Hz, 1H), 3.88 (s, 3H), 5.95 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (dd, J = 1, 8 Hz, 1H), 6.92 (t, J = 8 Hz, 1H), 6.97 (d, J = 1 Hz, 1H), 7.12 (d, J = 8 Hz, 1H), 7.18 (dd, J = 1, 12 Hz, 1H); MS (DCI/NH_3) m/e 579 $(M + H)^+$. Anal. ($C_{29}H_{39}N_2FO_7S\cdot0.25H_2O$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-ethoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)-ethyl]pyrrolidine-3-carboxylic acid (13f):** white solid; mp 65−66 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.90 (t, J = 7 Hz, 3H), 1.26−1.36 (m, 4H), 1.41−1.52 (m, 5H), 1.73 (quintet, J = 7 Hz, 2H), 2.23−2.33 (m, 1H), 2.69−2.96 (m, 5H), 2.97−3.12 (m, 2H), 3.16−3.37 (m, 2H), 3.43 (d, J = 9 Hz, 1H), 3.52−3.59 (m, 1H), 3.66 (d, J = 9 Hz, 1H), 4.08 (q, J = 7 Hz, 2H), 5.95 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.92 (t, J = 8 Hz, 1H), 6.97 (s, 1H), 7.07 (d, J = 8 Hz, 1H), 7.15 (d, J = 12 Hz, 1H); MS (DCI/NH_3) m/e 593 $(M + H)^+$. Anal. ($C_{30}H_{41}N_2O_7FS\cdot0.25H_2O$) C, H, N.

***trans,trans*-2-(3,4-Difluorophenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-pentylsulfonyl)amino)ethyl]-pyrrolidine-3-carboxylic acid (13g):** white solid; mp 71−

72 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.90 (t, J = 7 Hz, 3H), 1.25−1.38 (m, 4H), 1.46 (sextet, J = 7 Hz, 2H), 1.74 (quintet, J = 7 Hz, 2H), 2.26−2.36 (m, 1H), 2.72−2.95 (m, 5H), 2.98−3.12 (m, 2H), 3.15−3.34 (m, 2H), 3.45 (dd, J = 3, 9 Hz, 1H), 3.53−3.60 (m, 1H), 3.71 (d, J = 9 Hz, 1H), 5.96 (s, 2H), 6.75 (d, J = 9 Hz, 1H), 3.82 (dd, J = 2, 9 Hz, 1H), 5.96 (d, J = 2 Hz, 1H), 7.09−7.18 (m, 2H), 7.23−7.34 (m, 1H); MS (CDI/NH_3) *m/e* 567 (M + H)$^+$. Anal. ($C_{28}H_{36}N_2F_2O_6S$) H, N; C: calcd, 59.36; found, 59.92.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-butylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14a):** white solid; mp 65−66 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7.5 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H), 1.34−1.52 (m, 4H), 1.72 (quintet, J = 7.5 Hz, 2H), 2.25−2.35 (m, 1H), 2.72−2.94 (m, 5H), 2.97−3.12 (m, 2H), 3.19-3.46 (m, 2H), 3.44 (d, J = 9 Hz, 1H), 3.53−3.60 (m, 1H), 3.67 (d, J = 9 Hz, 1H), 3.89 (s, 3H), 5.95 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.92 (t, J = 9 Hz, 1H), 6.97 (s, 1H), 7.12 (d, J = 9 Hz, 1H), 7.18 (d, J = 12 Hz, 1H); MS (DCI/NH_3) *m/e* 565 (M + H)$^+$. Anal. ($C_{28}H_{37}N_2FO_7S$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(*n*-hexylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14b):** white solid; mp 63−65 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.88 (t, J = 6 Hz, 3H), 1.23−1.47 (m, 6H), 1.44 (sextet, J = 7 Hz, 2H), 1.71 (quintet, J = 6 Hz, 2H), 2.24−2.34 (m, 1H), 2.70−2.93 (m, 5H), 2.96−3.12 (m, 2H), 3.15−3.35 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.52−3.59 (m, 1H), 3.66 (d, J = 9 Hz, 1H), 3.87 (s, 3H), 5.95 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.42 (t, J = 8 Hz, 1H), 6.96 (s, 1H), 7.12 (d, J = 9 Hz, 1H), 7.17 (d, J = 12 Hz, 1H); MS (DCI/NH_3) *m/e* 593 (M + H)$^+$. Anal. ($C_{30}H_{41}N_2O_7FS$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(isobutylsulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14c):** white solid; mp 77−78 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (t, J = 7 Hz, 3H), 1.06 (d, J = 6 Hz, 6H), 1.45 (q, J = 7 Hz, 2H), 2.20 (septet, J = 6 Hz, 1H), 2.26−2.36 (m, 1H), 2.62−2.78 (m, 3H), 2.85−2.95 (m, 2H), 2.97−3.10 (m, 2H), 3.15−3.35 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.53−3.62 (m, 1H), 3.66 (d, J = 9 Hz, 1H), 3.88 (s, 3H), 5.95 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.92 (t, J = 8 Hz, 1H), 6.97 (d, J = 2 Hz, 1H), 7.12 (d, J = 9 Hz, 1H), 7.18 (dd, J = 2, 12 Hz, 1H); MS (DCI/NH_3) *m/e* 565 (M + H)$^+$. Anal. ($C_{28}H_{37}N_2O_7FS\cdot0.25H_2O$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((3-methyl-1-butyl)sulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14d):** white solid; mp 65−67 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.88 (d, J = 5 Hz, 6H), 1.46 (sextet, J = 7 Hz, 2H), 1.56−1.64 (m, 3H), 2.24−2.33 (m, 1H), 2.68−2.93 (m, 5H), 2.98−3.12 (m, 2H), 3.15−3.35 (m, 2H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.52−3.58 (m, 1H), 3.65 (d, J = 12 Hz, 1H), 3.87 (s, 3H), 5.95 (s, 2H), 6.73 (d, J = 8 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.92 (t, J = 8 Hz, 1H), 6.97 (d, J = 2 Hz, 1H), 7.10 (d, J = 9 Hz, 1H), 7.16 (dd, J = 2, 12 Hz, 1H); MS (DCI/NH_3) *m/e* 579 (M + H)$^+$. Anal. ($C_{29}H_{39}N_2FO_7S$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((3-chloropropyl)sulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14e):** white foam; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.80 (t, J = 7 Hz, 3H), 1.47 (bd hex, J = 8 Hz, 2H), 2.15 (pen, J = 7 Hz, 2H), 2.32 (m, 1H), 2.7−3.2 (m, 9H), 3.46 (dd, J = 4, 10 Hz, 1H), 3.57 (m, 1H), 3.64 (t, J = 6 Hz, 2H), 3.67 (d, J = 9 Hz, 1H), 3.86 (s, 3H), 5.92 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 6.84 (dd, J = 2, 8 Hz, 1H), 6.96 (d, J = 2 Hz, 1H), 7.06 (t, J = 9 Hz, 1H), 7.18 (m, 2H); MS (DCI/NH_3) *m/e* 585 (M + H; Cl_{35})$^+$, 587 (M + H; Cl_{37})$^+$. Anal. ($C_{27}H_{34}N_2O_7ClFS$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-((3,3,3-trifluoropropyl)-sulfonyl)amino)ethyl]pyrrolidine-3-carboxylic acid (14f):** white solid; mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 1.46 (sextet, J = 7 Hz, 2H), 2.03 (quintet, J = 7 Hz, 2H), 2.16−2.32 (m, 3H), 2.74 (m, 1H), 2.82−3.13 (m, 6H), 3.26 (m, 1H), 3.43 (dd, J = 3, 9 Hz, 1H), 3.56 (m, 1H), 3.67 (d, J = 9 Hz, 1H), 3.88 (s, 3H), 5.97 (s, 2H), 6.73 (d, J = 7 Hz, 1H), 6.82 (dd, J = 1, 7 Hz, 1H), 6.92 (t, J = 7 Hz, 1H), 6.96 (d, J = 1 Hz, 1H), 7.10 (d, J = 9 Hz, 1H), 7.17 (dd, J = 1, 12 Hz, 1H); MS (DCI/NH_3) *m/e* 619 (M + H)$^+$; HRMS (FAB) calcd for $C_{28}H_{35}N_2O_7F_4S_1$ 619.2101, found 619.2125. Anal. ($C_{28}H_{34}N_2O_7F_4S$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphhenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(*N*-*n*-propyl-*N*-(benzylsulfonyl)amino-)ethyl]pyrrolidine-3-carboxylic acid (14g):** white solid; mp 80−81 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.73 (t, J = 7 Hz, 3H), 1.34 (sextet, J = 7 Hz, 2H), 2.12 (m, 1H), 2.60 (m, 1H), 2.76−3.08 (m, 6H), 3.28 (dd, J = 3, 9 Hz, 1H), 3.48 (m, 1H), 3.58 (d, J = 9 Hz, 1H), 3.87 (s, 3H), 4.13 (dd, J = 9, 22 Hz, 2H), 5.95 (s, 2H), 6.72 (d, J = 7 Hz, 1H), 6.77 (dd, J = 1, 7 Hz, 1H), 6.88 (t, J = 9 Hz, 1H), 6.92 (d, J = 1 Hz, 1H), 7.05 (d, J = 9 Hz, 1H), 7.12 (dd, J = 1, 12 Hz, 1H), 7.32 (m, 5H); MS (DCI/NH_3) *m/e* 599 (M + H)$^+$. Anal. ($C_{31}H_{35}N_2O_7FS\cdot0.5H_2O$) C, H, N.

Receptor Binding Assays. All samples were kept at 4 °C throughout the process of membrane isolation. MMQ cells (prolactin secreting rat pituitary cells known to contain ET_A receptors), porcine cerebellar tissues (known to contain ET_B receptors), or chinese hamster ovary cells (CHO) permanently transfected with the human ET_A or ET_B receptor are homogenized in 25 mL of 10 mM Hepes (pH 7.4) containing 0.25 M sucrose and a protease inhibitor cocktail [50 mM EDTA, 0.1 mM PMSF, and 5 μg/mL Pepstatin A, and 0.025% Bacitracin] using a micro ultrasonic cell disruptor (Kontes). The mixture was centrifuged at 1000*g* for 10 min. The supernatant was collected and centrifuged at 60000*g* for 60 min. The precipitate was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitor cocktail and centrifuged again. The final membrane pellet was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitors and stored at −80 °C until used. Protein content was determined by the Bio-Rad dye-binding protein assay.

Binding assays were performed in 96-well microtiter plates pretreated with 0.1% BSA. Membranes were diluted ~100-fold in buffer B (20 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4, with 0.2% BSA, 0.1 mM PMSF, 5 μg/mL Pepstatin A, 0.025% bacitracin, and 50 mM EDTA) to a final concentration of 0.2 mg/mL of protein. In competition binding studies, membranes (0.02 mg) were incubated with 0.1 nM of [^{125}I]ET-1 (for ET_A assay in MMQ or CHO cells) or [^{125}I]ET-3 (for ET_B assay in porcine cerebellum or CHO cells) in buffer B (final volume: 0.2 mL) in the presence of increasing concentrations of the test compound for 3 h at 25 °C. After incubation, unbound ligand was separated from bound ligand by a vacuum filtration method using glass-fiber filter strips in PHD cell harvesters (Cambridge Technology, Inc., Cambridge, MA), washing the filter strips three times with saline (1 mL). Nonspecific binding was determined in the presence of 1 μM unlabeled ET-1. IC_{50} values are calculated using an average of at least two separate determinations.

Phosphoinositol Hydrolysis Assays. ET_A. MMQ cells (0.4 × 106 cells/mL) were labeled with 10 μCi/mL of [^{3}H]-myoinositol in RPMI for 16 h. The cells were washed with PBS and then incubated with buffer A (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, 25 mM Hepes, pH 7.4) containing protease inhibitors and 10 mM LiCl for 60 min. The cells were incubated with test compounds for 5 min and then challenged with 1 nM ET-1. ET-1 challenge was terminated by the addition of 1.5 mL of 1:2 (v/v) chloroform−methanol. Total inositol phosphates were extracted after adding chloroform and water to give final proportions of 1:1:0.9 (v/v/v) chloroform−methanol−water as described by Berridge.[12] The upper aqueous phase (1 mL) was retained, and a small portion (100 μL) was counted. The rest of the aqueous sample was analyzed by batch chromatography using anion-exchange resin AG1-X8 (Bio-Rad).

ET_B. Chinese hamster ovary cells (CHO) permanently transfected with the human ET_B receptor were grown to confluence in 24-well tissue culture plates and labeled with 5μCi/well of [^{3}H]myoinositol in F-12 media + 10%FBS + 1xP/S/F. The adherent cells were washed gently with PBS and then incubated in 200 μL of buffer A containing protease

inhibitors and 10 mM LiCl for 60 min at 37 °C in a CO_2 incubator. Test compounds were then added followed by the addition of 1 nM ET-1 and incubated for 30 min at 37 °C. The cells were then solubilized by the addition of 50 μL of 1 N NaOH and then neutralized by the addition of 50 μL of 1 N HCl. The solubilized cell suspension was transfered to glass tubes and extracted by the addition of 1.5 mL of 1:2 (v/v) chloroform−methanol. Total inositol phosphates were extracted and analyzed by batch chromatography on anion-exchange resin as above. All IC_{50} values are calculated using an average of at least two separate determinations.

Pharmacokinetic Analysis. The pharmacokinetic behavior of compound **16h** was evaluated in male Sprague-Dawley rats. Briefly, the test compound was prepared as a 10 mg/mL solution in an ethanol−propylene glycol−D5W (20:30:50, by volume) vehicle containing 1 molar equiv of sodium hydroxide. Groups of rats ($n = 4$ per group) received either a 10 mg/kg (1 mL/kg) intravenous dose administered as a slow bolus in the jugular vein or a 10 mg/kg (1 mL/kg) oral dose administered by gavage. Heparinized blood samples (~0.4 mL/sample) were obtained from a tail vein of each rat 0.1 (iv only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 h after dosing. The samples were analyzed by reverse phase HPLC following liquid−liquid extraction from the plasma. Initial estimates of the pharmacokinetic parameters (e.g. the maximum concentration C_{max}) for NONLIN84[13] were obtained with the program CSTRIP.[14] Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant (β) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an $AUC_{0-\infty}$ value, and in the calculation of $T_{1/2}$ values. Assuming dose proportionality and correcting for the differences in dosing, a comparison of the AUC following oral dosing with that obtained following an intravenous dose provided an estimate of the bioavailability (F).

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