Potent and Selective Non-Benzodioxole-Containing Endothelin-A Receptor Antagonists

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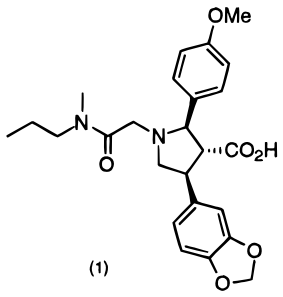
The benzodioxole ((methylenedioxy)benzene) group is present in a number of endothelin (ET) receptor antagonists thus far reported. As part of our own endothelin antagonist program we have developed (2*R**,3*R**,4*S**)-1-(*N*,*N*-dibutylacetamido)-4-(1,3-benzodioxol-5-yl)-2-(4-methoxyphenyl)pyrrolidine-3-carboxylic acid (A-127722). This is a potent antagonist, binding to the ET_A and ET_B receptor subtypes with affinities (IC_{50}) of 0.4 and 520 nM, respectively, and also contains the aforementioned benzodioxole. While this compound was seemingly optimized at its N-terminus, no effort had been directed toward understanding the contributions to binding affinity or receptor subtype selectivity conferred by the benzodioxole. Substitution by 1- or 2-naphthyl yielded weak antagonists. Oxygenated benzenes, such as *p*-anisyl, were potent compounds with IC_{50}s in the low-nanomolar range. Simple deletion of either of the two oxygen atoms (dihydrobenzofurans) yielded extremely potent agents, possessing subnanomolar affinity for the ET_A receptor. Additionally, the compounds showed enhanced selectivity, binding to the ET_B receptor subtype in the micromolar range. This paper describes the development of this novel class of compounds.

Introduction

Endothelins (ET) are 21-amino acid bicyclic peptides originally isolated from the supernatant of cultured porcine endothelial cells.[1] ET-1 is one member of a family of isopeptides encoded by three distinct genes; ET-2 and ET-3 are also members of this class.[2] Some 10-fold more potent than angiotensin II, ET-1 is the most powerful pressor peptide isolated to date and also a potent mitogen. These diverse actions are attributed to the existence of multiple receptor subtypes with discrete cellular distributions and functions. This suggests an unusual role for these hormones in the pathogenesis of cardiovascular diseases. Thus far, two subtypes of the ET receptor have been characterized.[3] The ET_A receptor is found principally in vascular smooth muscle and binds the isopeptides with affinities in the order ET-1 ∼ ET-2 > ET-3. It regulates vascular tone[4] and plays a role in smooth muscle cell proliferation.[5] The ET_B receptor has equal affinity for the three peptide isoforms. ET_B was originally considered the 'vasodilator receptor' due to its mediation of nitric oxide release.[6] It is now apparent that ET_B receptors are involved in smooth muscle contraction in blood vessels such as the rabbit saphenous vein[7] and the guinea pig bronchus.[8] Numerous reports exist in the literature describing both peptidic and nonpeptidic antagonists. ET_A-selective peptidic agents include BQ 123[9] and FR 139317.[10] BQ 788[11] and IRL 1038[12] are specific for the ET_B receptor. In addition, a hexapeptide (PD 142893)[13] displays nonselective antagonism at both receptors. Nonselective, nonpeptidic structures include Ro 47-0203,[14] SB 209670,[13] and L-749,329.[16] The first potent and highly selective ET_A antagonist, PD 156707, was disclosed recently.[17] We[18] recently reported a series of potent endothelin antagonists based on pyrrolidinecarboxylic acids (Chart 1).

Chart 1



(1) ET_A 6 nM; ET_B 2900 nM

Cytochrome P450 is essential to the phase I metabolism of foreign compounds.[19] This enzyme system is a ubiquitous monooxygenase that effects numerous oxidative processes required by the organism. In certain instances the benzodioxole group can inhibit and induce cytochrome P450, its subsequent degradation following either of two pathways: It can undergo oxidation to yield formate or carbon monoxide and a catechol, which is then redox active and may enter into quinone-reactive oxygen-based toxicity, or alternatively, it may complex with the heme function by way of a metallocarbene. In certain instances these complexes—characterized by their absorption at 455 nm—exhibit high stability and inhibit the catalytic cycle of the enzyme. This phenomenon has been used to advantage with the insecticide synergist piperonyl butoxide.

With the exception of Ro 47-0203 and BMS-182,874,[20] each series of the nonpeptide endothelin antagonists reported to date contains the benzodioxole group. Seeking to avoid possible complications arising from P450 oxidation, we synthesized a number of compounds with benzodioxole replacements. These compounds were potent and displayed high ET_A selectivity.

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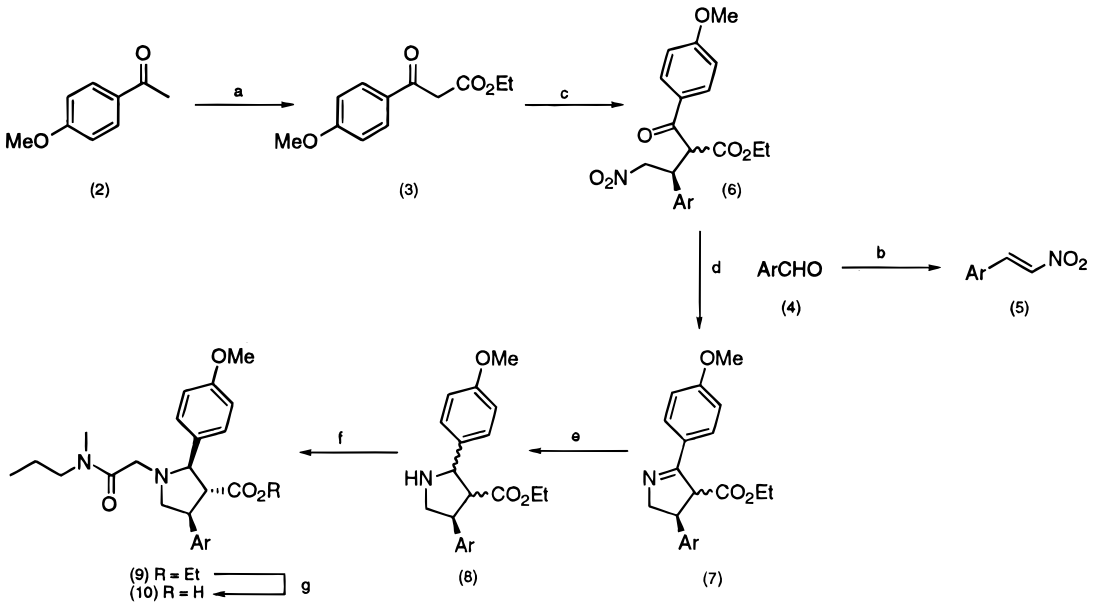
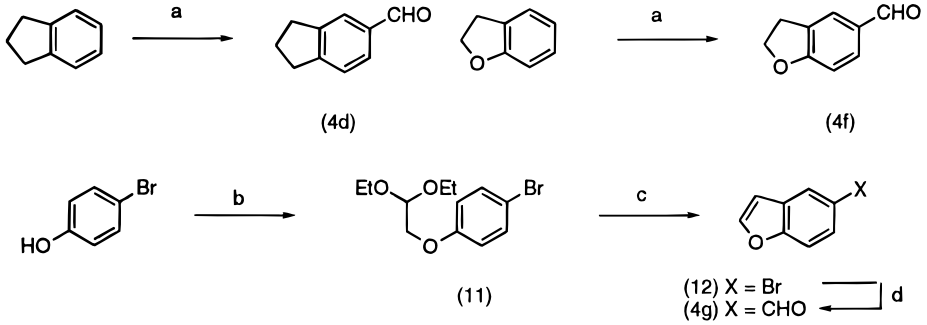
Scheme 1[a]



[a] (a) NaH, $CO(OEt)_2$; (b) $MeNO_2$, 50% NaOH, 15 °C, then pour into 6 N aq HCl; (c) nitrostyrene **5**, 5 mol % DBU, ^iPrOH, THF; (d) W-2 Raney Ni, 4 atm of H_2, EtOAc; (e) $NaBH_3CN$, HCl, EtOH, THF, pH 3−4; (f) $BrCH_2C(O)N(Me)Pr$, $^iPr_2NEt$, MeCN; (g) 50% aq NaOH, EtOH.

Scheme 2[a]



[a] (a) $MeOCHCl_2$, $SnCl_4$, CH_2Cl_2; (b) NaH, DMF, 0 °C, then $BrCH_2CH(OEt)_2$; (c) 1.5 equiv of PPA, PhH, reflux; (d) 2 equiv of ^tBuLi, Et_2O, −78 °C, then DMF.

Chemistry

All compounds were prepared in a racemic form as previously described[18] and depicted in Scheme 1. Carbethoxylation of 4′-methoxyacetophenone (**2**) with sodium hydride in diethyl carbonate yielded the β-keto ester **3**. Condensation[21] of nitromethane with the requisite aldehyde **4** (*vide infra*) afforded the nitrostyrene **5**. Treatment of an equimolar mixture of **3** and **5** with a catalytic amount of DBU yielded nitro ketone **6** as a mixture of diastereomers. The nitro group was reduced with Raney nickel to form an intermediate amino ketone that underwent spontaneous cyclization to pyrroline **7**. This compound was rapidly reduced with sodium cyanoborohydride at pH 3−4 to give pyrrolidine **8** as a mixture of four diastereomers. The *cis*−*cis* isomer was readily separable by flash chromatography. This compound, however, usually accounted for only 20% of the material and was routinely discarded. The remaining inseparable mixture of *cis*−*trans*, *trans*−*trans*, and *trans*−*cis* was alkylated with *N*-methyl-*N*-propylbromoacetamide to yield ester **9** and subsequently hydrolyzed. We were extremely fortunate in that the *cis*−*trans* epimers were resistant to hydrolysis under the conditions employed (50% aqueous sodium hydroxide in an equal volume of ethanol, room temperature, 3−4 h), and the pure *trans*−*trans* acid **10** was isolated by simple extractive workup.

Aldehydes were synthesized by various routes as delineated in Scheme 2. Indan-5-carboxaldehyde (**4d**) was prepared by the method of Mathison,[22] employing stannic chloride in place of titanium tetrachloride. Indole-6-carboxaldehyde (**4e**) was prepared by the method of Rapoport.[23] Stannic chloride- catalyzed condensation of α,α-dichloromethyl methyl ether with 2,3-dihydrobenzofuran afforded almost exclusively the 5-carboxaldehyde **4f**. The 7-isomer impurity, estimated at ∼5% by proton NMR, was readily removed in the subsequent Henry reaction, at which point the nitrostyrene was purified by recrystallization. Benzofuran-5-carboxaldehyde (**4g**) was synthesized following a procedure adapted from Barker.[24] Reaction of 2-bromoacetaldehyde diethyl acetal with 4-bromophenol afforded ether **11.** Subsequent intramolecular Friedel−Crafts reaction with polyphosphoric acid in refluxing benzene gave 5-bromobenzofuran **12**. The crude product was subjected to halogen−metal exchange and subsequent formylation with DMF in the manner described for **4f** to yield the aldehyde **4g**.

Benzofurans bearing the aldehyde function at the 4- or 6-position were prepared in an analogous fashion

starting with 3-bromophenol. The resultant mixture of 4- and 6-bromobenzofurans was carried on as described (*vide supra*) to yield the aldehydes **4h,i**, which were readily separated by flash chromatography. Fluorobenzaldehydes and anisaldehydes are commercially available. The final diarylpyrrolidinecarboxylic acid **10j** bearing the 2,3-dihydro-6-benzofuran pendant was prepared by catalytic hydrogenation[25] of the unsaturated analogue **10i** (H_2 at 60 psi, Pd(C), AcOH, 16 h, 78% yield). The 4-substituted benzofuran **10h**, proved resistant to reduction. 2-(3-Pyridyl)nitroethylene (**5a**) was prepared by the procedure of Bourguignon.[26]

Results and Discussion

At the outset of this work we were concerned over the potential of the benzodioxole moiety in certain compounds to undergo metabolism by cytochrome P450. The presence of a methylene bonded by two oxygens is apparently critical for this process to occur. Our initial studies focused on replacing this group with simple carbocyclic analogues. Naphthalene, bonded at either the 1- or 2-position (Table 1, entries **10a**,**b**), resulted in a dramatic reduction in binding affinity; this was equally true for the phenyl analogue **10c**. A better substitution was the isosteric 5-indan **10d**, which was 50-fold less potent than benzodioxole **1**. The hydrogen bond-donating 6-indole **10e** was exceptionally deleterious. We felt, therefore, that a hydrogen-bond acceptor was necessary for good potency. Simple deletions of each oxygen atom in turn yielded a series of dihydrobenzofurans: The 5-substituted dihydrobenzofuran **10f** and the 6-isomer **10j** were the most potent compounds with IC_{50}s of 130 and 137 nM, respectively. Benzofuran **10i** displayed somewhat lower affinity. Driven by these results, we sought to design simpler compounds. The *p*-methoxyphenyl analogue **10k** possessed activity midway between the benzofurans and their saturated analogues. A surprising result is observed with the *m*-methoxyl **10l**: It would appear the binding pocket has rather strict steric requirements, and the freely rotating methyl group suffers an unfavorable interaction with the receptor. Adding an additional hydrogen bond acceptor at the *para*-position (entries **10m,n**) restores the binding affinity. Especially interesting is the 3,4-difluorobenzene **10o**. This substituent is barely larger than the phenyl **10c** yet has a binding affinity almost 10-fold higher. The benzodioxan **10p** yields surprising results: It is somewhat isosteric with the 2-naphthyl yet displays greatly increased binding affinity. Furthermore, the compound shows increased ET_B activity. All the analogues described are, however, significantly weaker antagonists than the benzodioxole **1**.

During this work it was discovered[18] that increasing the side chain length from *N*-methyl-*N*-propyl to *N*,*N*-dibutyl enhanced the potency of **1** 15-fold, and yields compound **13** (A-127722, Chart 2) that displays 1000-fold selectivity with receptor binding affinities of 0.4 and 520 nM for the ET_A and ET_B subtypes, respectively. Accordingly, the most potent compounds from the *N*-methyl-*N*-propylamide series were resynthesized incorporating the extended chain. Table 2 summarizes the biological data. The increased binding affinity is more striking for the non-benzodioxoles: Dihydrobenzofurans **14b**,**d** show a 300-fold increase in binding affinity, and both are extremely potent ET_A receptor antagonists with IC_{50}s of 0.6 and 1 nM, respectively. Moreover, the 5-isomer **14b** displays higher selectivity, with an ET_B/ET_A ratio of 4300. Interestingly, the 6-substituted analogue **14d** is almost 5-fold less selective. A similar picture holds for the unsaturated analogues **14a**,**c**: Overall activity increases greatly. Reducing the Lewis basicity of the oxygen atom, however, yields a compound with enhanced selectivity for the ET_A receptor. In contrast to their saturated analogues, the unsaturated 5- and 6-benzofurans **14a**,**c** have essentially the same binding profile. The trend continues with the fluoro- and methoxy-substituted analogues. All display high affinity (1−10 nM) and selectivities in the 3000−10000-fold range. Again the benzodioxan **14l** follows the same pattern, showing increased binding affinity for both receptor subtypes. The indan **14m** possessing no hydrogen-bonding capability is only 2-fold weaker with an IC_{50} of 1.5 nM and shows great selectivity (5300-fold). This illustrates that the presence of an electron-rich aromatic ring is desirable but not essential for activity. These results suggest the presence of a hydrogen-bonding site in the ET_B receptor situated to interact with an acceptor at the *meta*-position of the C(4) phenyl substituent.

Pharmacokinetic studies in conscious rats were performed on a number of the most potent compounds. The results are summarized in Table 3. All compounds were well absorbed and had oral bioavailabilities in the 30−65% range.

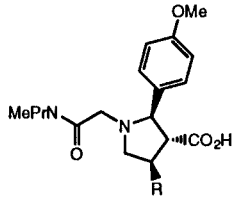
Conclusions

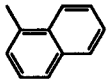
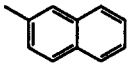
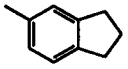
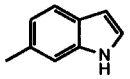
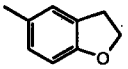
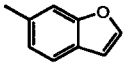
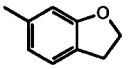
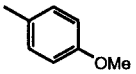
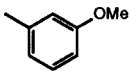
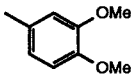
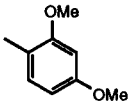
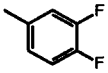
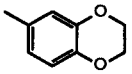
At the outset of this work we wanted to examine the role played by the benzodioxole moiety in endothelin receptor binding. Additionally we were concerned with the propensity of benzodioxole-containing compounds to undergo cytochrome P450-mediated metabolism, wherein the substrate becomes irreversibly bound to the enzyme. This type of metabolism may lead to drug−drug interactions or nonlinear pharmacokinetics. With this in mind we sought to develop compounds devoid of this functionality. We have since attempted to induce A-127722 to form such P450−carbene complexes (detected by a shift in the ultraviolet absorbance from 450 to 455 nm) and have been unable to find any direct evidence for such an event. Nonetheless, these benzodioxole replacement studies have proved to be interesting for other reasons. We have developed a series of agents that function as orally active, highly potent antagonists for the ET_A receptor; more importantly several of these compounds display increased selectivity over the ET_B receptor. Compounds lacking the *meta*-oxygen on the C(4) phenyl group provide this increased selectivity without sacrificing potency.

Experimental Section

General. Compounds were prepared in a fashion analogous to ref 18 substituting the appropriate aldehyde. All solvents and reagents were reagent grade unless otherwise specified and were purchased from Aldrich Chemical Co. Flash chromatography was performed on silica gel (230−400 mesh) from E. M. Science. Proton NMR spectra were recorded on a General Electric QE300 instrument. Chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard. Elemental analyses were obtained from Robertson Microlit Laboratories, Madison, NJ. Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran.

Table 1. Biological Data of *N*-Methyl-*N*-propyl-Substituted Pyrrolidine Amides

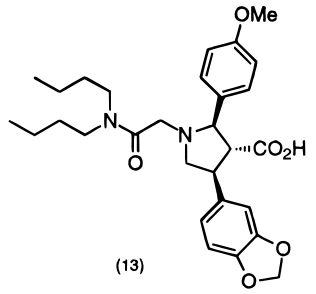


Entry	R	formula	analysis[a]	IC_{50}[b] μM ET_A	IC_{50}[b] μM ET_B
10a		$C_{28}H_{32}N_2O_4 \cdot 0.5$ AcOH	C,H,N	2.3 (1)	32 (1)
10b		$C_{28}H_{32}N_2O_4 \cdot 0.5$ H_2O	C,H,N	2.3 (1)	25 (1)
10c		$C_{24}H_{30}N_2O_4 \cdot 2$ H_2O	C,H,N	2.0 (1)	>100 (1)
10d		$C_{27}H_{34}N_2O_4 \cdot 2.5$ H_2O	C,H,N	0.310 (1)	11 (1)
10e		$C_{26}H_{31}N_3O_4 \cdot 0.75$ H_2O	C,H,N	5.1 (1)	35 (1)
10f		$C_{26}H_{32}N_2O_5 \cdot 0.25$ H_2O	C,H,N	0.130 (1)	19 (1)
10h		$C_{26}H_{30}N_2O_5 \cdot 1$ AcOH	C,H,N	0.255 (1)	34 (1)
10i		$C_{26}H_{30}N_2O_5 \cdot 0.5$ H_2O	C,H,N	0.258 (1)	13 (1)
10j		$C_{26}H_{32}N_2O_5 \cdot 1.25$ TFA	C,H,N	0.137 (1)	6.4 (1)
10k		$C_{25}H_{32}N_2O_5 \cdot 0.5$ H_2O	C,H,N	0.176 (1)	16 (1)
10l		$C_{25}H_{32}N_2O_5 \cdot 0.5$ H_2O	C,H,N	1.7 (1)	35 (1)
10m		$C_{26}H_{34}N_2O_6 \cdot 0.5$ H_2O	C,H,N	0.730 (1)	31 (1)
10n		$C_{26}H_{34}N_2O_6 \cdot 0.75$ H_2O	C,H,N	0.407 (1)	72 (1)
10o		$C_{24}H_{28}F_2N_2O_4 \cdot 1.8$ H_2O	C,H,N	0.31 (1)	35 (1)
10p		$C_{26}H_{32}N_2O_6 \cdot$	C,H,N	0.13 (1)	35 (1)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations unless otherwise stated. [b] Number of determinations in parentheses.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(1-naphthyl)pyrrolidine-3-carboxylic acid (10a):** substituting naphthalene-1-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (rotamer) δ 8.29 (1H, bd, *J* = 8 Hz), 7.86 (2H, d, *J* = 8 Hz), 7.75 (1H, d, *J* = 8 Hz), 7.49 (3H, m), 7.34 (2H, dd, *J* = 3, 9 Hz), 6.83 (2H, dd, *J* = 9, 2 Hz), 4.50 (1H, m), 3.94 (1H, dd, *J* = 9, 2 Hz), 3.78 (3H, s), 3.65 (1H, m), 3.49 (1H, d, *J* = 14 Hz), 3.40−2.93 (5H, m), 2.91 (2.83) (3H,

Chart 2



(13)

ET_A 0.4 nM

ET_B 520 nM

s), 1.48 (2H, sept, J = 7 Hz), 0.83 (0.77) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 461 (MH^+), 415, 348, 302, 279. Anal. ($C_{28}H_{32}N_2O_4$·0.5AcOH) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(2-naphthyl)pyrrolidine-3-carboxylic acid (10b):** substituting naphthalene-2-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (rotamer) δ 7.82 (4H, m), 7.69 (1H, m), 7.47 (2H, m), 7.37 (2H, dd, J = 7.5, 2 Hz), 6.85 (2H, dd, J = 7.5, 2 Hz), 3.90 (1H, d, J = 8 Hz), 3.78 (3H, s), 3.57 (1H, m), 3.52−2.97 (6H, m), 2.93 (2.85) (3H, s), 2.90 (1H, m), 1.52 (2H, m), 0.86 (0.76) (3H, t, J = 7.5 Hz); MS (DCI/NH_3) m/z 461 (MH^+). Anal. ($C_{28}H_{32}N_2O_4$·0.5H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(2-phenyl)pyrrolidine-3-carboxylic acid (10c):** substituting benzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.53 (4H, d, J = 6 Hz), 7.40−7.20 (3H, m), 6.88 (2H, d, J = 8 Hz), 3.90 (1H, m), 3.79 (3H, s), 3.70−2.95 (8H, m), 2.90 (2.79) (3H, s), 1.50 (2H, sept, J = 7 Hz), 0.87 (0.72) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 411 (MH^+). Anal. ($C_{24}H_{30}N_2O_4$·2.00H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propyl)acetamido-2-(4-methoxyphenyl)-4-(5-indanyl)-pyrrolidine-3-carboxylic acid (10d):** substituting indan-5-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.25−7.1 (5H, m), 6.78 (2H, d, J = 8 Hz), 3.89 (1H, d, J = 8 Hz), 3.75 (3H, s), 3.50−2.90 (6H, m), 2.88 (6H, t, J = 6 Hz), 2.82 (2.80) (3H, s), 2.04 (2H, t, J = 8 Hz), 1.48 (2H, sept, J = 7 Hz), 0.83 (0.73) (3H, t, J = 7 Hz); MS (FAB) m/z 473 (MNa^+), 451 (MH^+). Anal. ($C_{27}H_{34}N_2O_4$·2.5H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(6-indolyl)pyrrolidine-3-carboxylic acid (10e):** substituting indole-6-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 8.43 (1H, bs), 7.57 (1H, d, J = 8 Hz), 7.43 (1H, s), 7.31 (2H, dd, J = 6, 3 Hz), 7.22 (1H, d, J = 8 Hz), 7.1 (1H, t, J = 3 Hz), 6.78 (2H, dd, J = 6, 3 Hz), 6.45 (1H, m), 3.93 (1H, dd, J = 6, 3 Hz), 3.80 (1H, m), 3.73 (3H, s), 3.60−2.90 (6H, m), 2.86 (2.82) (3H, s), 1.47 (2H, sept, J = 7 Hz), 0.83 (0.73) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 450 (MH^+). Anal. ($C_{26}H_{31}N_3O_4$·0.75H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(5-benzo-2,3-dihydrofuranyl)pyrrolidine-3-carboxylic acid (10f):** substituting 2,3-dihydrobenzofuran-5-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.33 (1H, d, J = 8 Hz), 7.28 (1H, m), 7.19 (1H, m), 6.87 (1H, d, J = 8 Hz), 6.73 (1H, d, J = 8 Hz), 4.56 (1H, t, J = 8 Hz), 3.83 (1H, d, J = 10 Hz), 3.80 (3H, s), 3.63 (1H, m), 3.4−3.0 (9H, m), 2.87 (2.84) (3H, s), 1.51 (2H, sept, J = 7 Hz), 0.88 (0.78) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 453 (MH^+), 426, 352, 294. Anal. ($C_{26}H_{32}N2O_5$·0.25H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(4-benzofuranyl)pyrrolidine-3-carboxylic acid (10h):** substituting benzofuran-4-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.59 (1H, t, J = 3 Hz), 7.4−7.2 (6H, m), 6.8 (2H, d, J = 8 Hz), 4.03 (1H, m), 3.94 (1H, dd, J = 8, 3 Hz), 3.77 (3H, s), 3.61 (1H, dd, J = 8 7 3 Hz), 3.42 (1H, dd, J = 11, 5 Hz), 3.40−2.90 (5H, m), 2.82 (2.81) (3H, s), 1.50 (2H, sept, J = 7 Hz), 0.82 (0.75) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 451 (MH^+), 336, 160. Anal. ($C_{26}H_{30}N_2O_5$·AcOH) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(6-benzofuranyl)pyrrolidine-3-carboxylic acid (10i):** substituting benzofuran-6-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.65 (1H, bd), 7.60 (1H, d, J = 2 Hz), 7.55 (1H, d, J = 8 Hz), 7.35 (3H, m), 6.85 (2H, dd, J = 8, 3 Hz), 6.75 (1H, dd, J = 3, 2 Hz), 3.83 (2H, m), 3.79 (3H, s), 3.60−3.0 (7H, m), 2.91 (2.83) (s, 3H), 1.51 (2H, sept, J = 7 Hz), 0.83 (0.78) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 451 (MH^+), 350, 263. Anal. ($C_{26}H_{30}N_2O_5$·0.5H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(6-benzo-2,3-dihydrofuranyl)pyrrolidine-3-carboxylic acid (10j)**: prepared by catalytic hydrogenation of **9i** (4 atm of H_2, AcOH, followed by preparative HPLC); ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.49 (7.47) (2H, d, J = 8 Hz), 7.19 (1H, d, J = 8 Hz), 7.00 (1H, m), 7.82 (3H, m), 5.40 (1H, dd, J = 11, 7 Hz), 4.58 (2H, t, J = 8 Hz), 4.18 (1H, m) 4.10 (1H, m), 3.88 (1H, m), 3.79 (3H, s), 3.60 (1H, m), 3.35 (1H, m), 3.19 (2H, t, J = 8 Hz), 3.00 (4H, m), 2.91 (2.78) (s, 3H), 1.53 (1.40) (2H, sept, J = 7 Hz), 0.88 (0.78) (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 453 (MH^+), 352. Anal. ($C_{26}H_{32}N_2O_5$·1.25TFA) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2,4-bis-(4-methoxyphenyl)pyrrolidine-3-carboxylic acid (10k):** substituting 4-methoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.37 (2H, d, J = 7.5 Hz), 7.32 (2H, d, J = 7.5 Hz), 6.86 (4H, m), 3.83 (1H, m), 3.81 (3H, s), 3.79 (3H, s), 3.64 (1H, m), 3.48−2.97 (6H, m), 2.87 (2.83) (3H, s), 2.85 (1H, m), 1.45 (2H, m), 0.84 (0.74) (3H, t, J = 7.5 Hz); MS (DCI/NH_3) m/z 441 (MH^+). Anal. ($C_{25}H_{32}N_2O_5$·0.5H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(3-methoxyphenyl)pyrrolidine-3-carboxylic acid (10l):** substituting 3-methoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.33 (2H, d, J = 7.5 Hz), 7.24 (1H, t, J = 7.5 Hz), 7.05 (2H, m), 6.85 (2H, dd, J = 7.5, 2 Hz), 6.76 (1H, m), 3.83 (1H, m), 3.81 (3H, s), 3.79 (3H, s), 3.64 (1H, m), 3.48−2.97 (6H, m), 2.87 (2.83) (3H, s), 2.85 (1H, m), 1.45 (2H, m), 0.84 (0.74) (3H, t, J = 7.5 Hz); MS (DCI/NH_3) m/z 441 (MH^+). Anal. ($C_{25}H_{32}N_2O_5$·0.5H_2O) C, H, N.

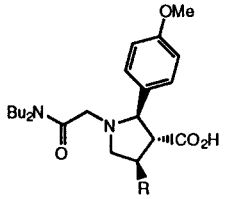
(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(3,4-dimethoxyphenyl)pyrrolidine-3-carboxylic acid (10m):** substituting 3,4-dimethoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.33 (2H, d, J = 7.5 Hz), 7.07 (1H, d, J = 2.0 Hz), 6.98 (1H, m), 6.85 (1H, d, 7.5 = Hz), 6.82 (2H, d, 7.5 = Hz), 3.91 (3H, s), 3.86 (3H, s), 3.83 (1H, m), 3.79 (3H, s), 3.64 (1H, m), 3.50−2.95 (6H, m), 2.87 (1H, m), 2.85 (2.83) (3H, s), 1.45 (2H, m), 0.84 (0.74) (3H, t, J = 7.5 Hz); MS (DCI/NH_3) m/z 471 (MH^+). Anal. ($C_{26}H_{34}N_2O_6$·0.5H_2O) C, H, N.

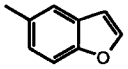
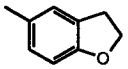
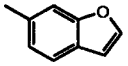
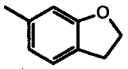
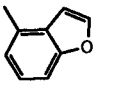
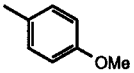
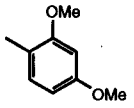
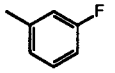
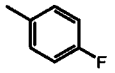
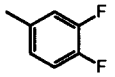
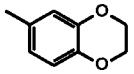
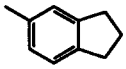
(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(2,4-dimethoxyphenyl)pyrrolidine-3-carboxylic acid (10n):** substituting 2,4-dimethoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$−CD_3OD) (minor rotamer) δ 7.61 (1H, d, J = 8Hz), 7.30 (2H, d, J = 8Hz), 6.82 (2H, d, J = 8Hz), 6.55 (1H, d, J = 8Hz), 6.45 (1H, d, J = 3Hz), 3.90 (1H, m), 3.81 (3H, s), 3.79 (3H, s), 3.77 (3H, s), 3.70−2.90 (8H, m), 2.85 (3H, s), 1.50 (2H, sept, J = 7 Hz), 0.87 (0.77) (3H, t, J = 7 Hz). MS (DCI/NH_3) m/z 471 (MH^+). Anal. ($C_{26}H_{34}N_2O_6$·0.75 H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*-Methyl-*N*-propylacetamido)-2-(4-methoxyphenyl)-4-(3,4-difluorophenyl)pyrrolidine-3-carboxylic acid (10o):** substituting 3,4-difluorobenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) (minor rotamer) δ 7.60−7.3 (4H, m), 7.13 (1H, q, J = 9 Hz), 6.90 (2H, d, J = 8 Hz), 3.90 (1H, m), 3.79 (3H, s), 3.60−2.95 (6H, m), 2.92 (2.78) (3H, s), 1.55 (2H, sept, J = 7 Hz), 0.88 (0.73) (3H, t, J = 7 Hz). MS (DCI/NH_3) m/z 447 (MH^+). Anal. ($C_{24}H_{28}F_2N_2O_4$·1.80H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(5-benzofuranyl)pyrrolidine-3-carboxylic acid (14a):** substituting benzofuran-5-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (1H, bd), 7.59 (1H, d, J = 2 Hz), 7.43 (2H, m), 7.33 (2H, d, J = 8 Hz), 6.85 (2H, d, J = 8 Hz), 6.73 (1H, dd, J = 3, 1 Hz), 3.82 (1H, d, J = 11 Hz), 3.89 (1H, d, J = 9Hz) 3.79 (3H, s), 3.53 (1H, dd, J = 10, 3 Hz), 3.44 (2H, m), 3.30 (1H, m), 3.20−2.95 (5H, m), 2.82 (1H, d, J = 14 Hz), 1.43 (3H, m), 1.23 (3H, m), 1.08 (2H, m), 0.87 (3H, t, J =

Table 2. Biological Data of *N,N*-Dibutyl-Substituted Pyrrolidine Amides



Entry	R	formula	analysis[a]	IC_{50}[b] μM ET_A	IC_{50}[b] μM ET_B
14a		$C_{30}H_{38}N_2O_5$	C,H,N	0.0005 (3)	5.1 (3)
14b		$C_{30}H_{40}N_2O_5 \cdot 0.25\ H_2O$	C,H,N	0.0006 (4)	2.6 (4)
14c		$C_{30}H_{38}N_2O_5 \cdot 0.75\ H_2O$	C,H,N	0.0007 (2)	5 (2)
14d		$C_{30}H_{40}N_2O_5 \cdot 0.85$ TFA	C,H,N	0.001 (3)	0.96 (3)
14e		$C_{30}H_{38}N_2O_5$	C,H,N	0.0020 (3)	11 (3)
14f		$C_{29}H_{40}N_2O_5$	C,H,N	0.0013 (2)	4.1 (2)
14g		$C_{30}H_{42}N_2O_6 \cdot 1.3$ TFA	C,H,N	0.0037 (3)	48(2)
14h		$C_{28}H_{37}FN_2O_4 \cdot 0.25\ H_2O$	C,H,N	0.005 (2)	16 (2)
14i		$C_{28}H_{37}FN_2O_4$	C,H,N	0.014 (3)	77 (3)
14j		$C_{28}H_{36}F_2N_2O_4 \cdot 1\ H_2O$	C,H,N	0.0015 (3)	17 (3)
14k		$C_{27}H_{37}N_3O_4 \cdot 1.65$ TFA	C,H,N	0.93 (2)	>100 (2)
14l		$C_{30}H_{40}N_2O_6 \cdot 1\ H_2O$	C,H,N	0.0008 (2)	0.93 (2)
14m		$C_{31}H_{42}N_2O_4$	C,H,N	0.0015 (2)	8 (2)

[a] Compounds gave satisfactory analyses within ±0.4% of theoretical calculations unless otherwise stated. [b] Number of determinations in parenthesis.

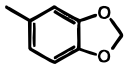
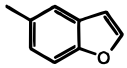
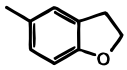
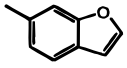
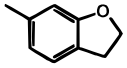
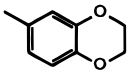
7 Hz), 0.82 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 507 (MH^+), 350, 336, 172. Anal. ($C_{30}H_{38}N_2O_5$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(5-benzo-2,3-dihydrofuranyl)pyrrolidine-3-carboxylic acid (14b):** substituting 2,3-dihydrobenzofuran-5-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (2H, d, J = 8 Hz), 7.27 (1H, d, J = 2 Hz), 7.18 (1H, dd, J = 7, 3 Hz), 6.86 (2H, d, J = 8 Hz), 6.72 (1H, d, J = 8 Hz), 4.56 (2H, t, J = 7 Hz), 3.78 (3H, s), 3.62 (1H, m), 3.50−3.25 (4H, m), 3.17 (2H, t, J = 7 Hz), 3.15−2.90 (5H, m), 2.79 (1H, d, J = 14 Hz), 1.43 (3H, m), 1.26 (3H, m), 1.08 (2H, m), 0.87 (3H, t, J = 7 Hz), 0.81 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 509 (MH^+). Anal. ($C_{30}H_{40}N_2O_5 \cdot 0.25H_2O$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(6-benzofuranyl)pyrrolidine-3-carboxylic acid (14c):** substituting benzofuran-6-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (1H, bd), 7.59 (1H, d, J = 2 Hz), 7.53 (1H, d, J = 8 Hz), 7.36 (3H, m), 6.85 (2H, d, J = 8 Hz), 6.73 (1H, dd, J = 3, 1 Hz), 3.82 (1H, d, J = 11 Hz), 3.89 (1H, d, J = 9 Hz) 3.79 (3H, s), 3.53 (1H, dd, J = 10, 3 Hz), 3.44 (2H, m), 3.30 (1H, m), 3.20−2.95 (5H, m), 2.80 (1H, d, J = 14 Hz), 1.43 (3H, m), 1.23 (3H, m), 1.08 (2H, m), 0.87 (3H, t, J = 7 Hz), 0.82 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 507 (MH^+), 336, 290, 172. Anal. ($C_{30}H_{38}N_2O_5 \cdot 0.75H_2O$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(6-benzo-2,3-dihydrofuranyl)pyrrolidine-**

Table 3. Pharmacokinetic Data of *N,N*-Dibutyl-Substituted Pyrrolidine Amides

			i.d. AUC, μg•min mL^{-1}		oral	
Entry	R	i.v. $t_{1/2}$, h	portal	carotid	C_{max}, μg mL^{-1}	F, %
13		3.5	640	350	3.2	68
14a		3.2	240	140	1.6	39
14b		1.1	530	180	1.5	33
14c		4.7	540	370	4.7	38
14d		4.7	730	370	4.0	68
14l		2.3	370	150	2.5	39

3-carboxylic acid (14d): prepared by catalytic hydrogenation of **12c** (4 atm of H_2, AcOH, followed by preparative HPLC); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (2H, d, J = 8 Hz), 7.16 (1H, d, J = 8 Hz), 6.97 (1H, dd, J = 8, 2 Hz), 6.89 (3H, m), 5.90 (1H, bs), 4.57 (2H, t, J = 9 Hz), 4.93 (2H, m), 3.80 (3H, s), 3.70−3.58 (2H, m), 3.40 (1H, m), 3.30−2.90 (8H, m), 1.40 (2H, m), 1.29 (3H, m), 1.08 (2H, m), 0.92 (3H, t, J = 7 Hz), 0.82 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 509 (MH^+), 338. Anal. ($C_{30}H_{40}N_2O_5$·0.85TFA) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(4-benzofuranyl)pyrrolidine-3-carboxylic acid (14e):** substituting *N*,*N*-dibutyl bromoacetamide; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (1H, d, J = 3 Hz), 7.39 (1H, dt, J = 8, 2 Hz), 7.34 (3H, m), 7.26 (1H, d, J = 2 Hz), 7.23 (1H, d, J = 8 Hz), 6.84 (2H, d, J = 8 Hz), 4.02 (1H, ddd, J = 8, 6, 4 Hz), 3.89 (1H, d, J = 9 Hz), 3.79 (3H, s), 3.67 (1H, dd, J = 10, 3 Hz), 3.44 (2H, m), 3.35−3.15 (3H, m), 3.00 (2H, m), 2.84 (1H, d, J = 14 Hz), 1.43 (3H, m), 1.23 (3H, m), 1.08 (2H, m), 0.87 (3H, t, J = 7 Hz), 0.82 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 507 (MH^+), 350. Anal. ($C_{30}H_{38}N_2O_5$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2,4-bis(4-methoxyphenyl)pyrrolidine-3-carboxylic acid (14f):** substituting 4-methoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (2H, d, J = 8 Hz), 7.30 (2H, d, J = 8 Hz), 6.87 (4H, dd, J = 7, 3 Hz), 3.78 (3H, s), 3.76 (3H, s), 3.63 (1H, m), 3.50−3.20 (4H, m), 3.15−2.90 (5H, m), 2.78 (1H, d, J = 14 Hz), 1.43 (3H, m), 1.27 (3H, m), 1.09 (2H, m), 0.87 (3H, t, J = 7 Hz), 0.81 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 497 (MH^+). Anal. ($C_{29}H_{40}N_2O_5$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(2,4-dimethoxyphenyl)pyrrolidine-3-carboxylic acid (14g):** substituting 2,4-dimethoxybenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (2H, d, J = 8 Hz), 7.20 (1H, d, J = 8 Hz), 6.92 (2H, d, J = 8 Hz), 6.60 (1H, d, J = 3 Hz), 6.49 (1H, dd, J = 6, 2 Hz), 5.35 (1H, d, J = 8 Hz), 4.20 (3H, m), 4.10 (3H, s), 3.83 (3H, s), 3.81 (3H, s), 3.75 (3H, m), 3.17 (2H, hep, J = 7 Hz), 3.05 (2H, t, J = 7 Hz), 1.30 (4H, m), 1.07 (4H, m), 0.87 (3H, t, J = 7 Hz), 0.80 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 527 (MH^+). Anal. ($C_{30}H_{42}N_2O_6$·1.30TFA) C, H,N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(3-fluorophenyl)pyrrolidine-3-carboxylic acid (14h):** substituting 3-fluorocarboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (2H, d, J = 8 Hz), 7.22 (2H, m), 6.91 (1H, m), 6.86 (2H, d, J = 8 Hz), 3.79 (1H, m), 3.78 (3H, s), 3.68 (1H, m), 3.55−3.37 (3H, m), 3.29 (1H, m), 3.15−2.90 (5H, m), 2.78 (1H, d, J = 14 Hz), 1.43 (2H, m), 1.25 (4H, m), 1.07 (2H, m), 0.87 (3H, t, J = 7 Hz), 0.80 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 485 (MH^+). Anal. ($C_{28}H_{37}FN_2O_4$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(4-fluorophenyl)pyrrolidine-3-carboxylic acid (14i):** substituting 4-fluorocarboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (1H, m), 7.42 (1H, dd, J = 7, 3 Hz), 7.36 (2H, d, J = 8 Hz), 7.01 (3H, t, J = 8 Hz), 6.87 (1H, d, J = 8 Hz), 3.83 (1H, m), 3.8 (3H, s), 3.67 (1H, m), 3.47 (3H, m), 3.30−2.90 (5H, m), 2.82 (1H, d, J = 14 Hz), 1.43 (2H, m), 1.28 (4H, m), 1.08 (2H, m), 0.90 (3H, t, J = 7 Hz), 0.82 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 485 (MH^+). Anal. ($C_{28}H_{37}FN_2O_4$) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(3,4-difluorophenyl)pyrrolidine-3-carboxylic acid (14j):** substituting 3,4-difluorobenzaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (1H, m), 7.30 (2H, d, J = 8 Hz), 7.20−7.00 (2H, m), 6.87 (2H, d, J = 8 Hz), 3.78 (3H, s), 3.79 (1H, m), 3.62 (1H, m), 3.50−3.30 (3H, m), 3.23 (1H, m), 3.15−2.90 (4H, m), 2.78 (1H, d, J = 14 Hz), 1.43 (2H, m), 1.27 (4H, m), 1.08 (2H, m), 0.85 (3H, t, J = 7 Hz), 0.80 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 503 (MH^+). Anal. ($C_{28}H_{36}F_2N_2O_4$·1H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(3-pyridyl)-pyrrolidine-3-carboxylic acid (14k):** substituting pyridine-3-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (1H, bs), 8.73 (1H, bd, J = 9 Hz), 8.62 (1H, bd, J = 7 Hz), 7.78 (1H, bdd, J = 9, 3 Hz), 7.38 (2H, d, J = 10 Hz), 6.90 (2H, d, J = 10 Hz), 4.39 (1H, d, J = 12 Hz), 3.95 (1H, m), 3.80 (3H, s), 3.79 (1H, m), 3.68 (1H, d, J = 18 Hz), 3.50−3.30 (3H, m), 3.25−2.90 (6H, m), 1.47 (2H, m), 1.31 (4H, m), 1.20 (2H, m), 0.92 (3H, t, J = 7 Hz), 0.83 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 468 (MH^+). Anal. ($C_{27}H_{37}N_3O_4$·1.65 TFA) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(1,4-benzodioxan-6-yl)pyrrolidine-3-carboxylic acid (14l):** substituting benzodioxan-6-carboxaldehyde; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (1H, d, J = 9 Hz), 6.98 (1H, d, J = 3 Hz), 6.91 (1H, dd, J = 9, 3 Hz), 6.85 (2H, d, J = 9 Hz), 6.78 (1H, d, J = 9 Hz), 4.28 (4H, m), 3.79 (3H, s), 3.75 (1H, d, J = 9 Hz), 3.59−3.26 (5H, m), 3.09−2.95 (4H, m), 2.75 (1H, d, J = 12 Hz), 1.48−1.21 (6H, m), 1.08 (2H, m), 0.88 (3H, t, J = 7 Hz), 0.80 (3H, t, J = 7 Hz); MS (DCI/NH_3) m/z 468 (MH^+). Anal. ($C_{30}H_{40}N_2O_6$·0.25H_2O) C, H, N.

(2*R,3*R**,4*S**)-1-(*N*,*N*-Dibutylacetamido)-2-(4-methoxyphenyl)-4-(5-indanyl)pyrrolidine-3-carboxylic acid (14m):** substituting indan-5-carboxaldehyde; ^{1}H NMR (300M Hz, $CDCl_3$) δ 7.30 (3H, m), 7.28 (2H, m) 6.85 (2H, d, J = 8 Hz), 3.81 (1H, m), 3.79 (3H, s), 3.68 (1H, m), 3.40 (3H, m), 3.32 (1H, m), 3.14 (4H, m), 3.91−3.80 (6H, m), 2.08 (1H, t, J = 7 Hz), 2.07 (1H, t, J = 7 Hz), 1.43 (2H, m), 1.25 (4H, m),

1.11 (2H, m), 0.88 (3H, t, $J = 7$ Hz), 0.81 (3H, t, $J = 7$ Hz); MS (DCI/NH_3) m/z 525 (MH^+). Anal. ($C_{31}H_{42}N_2O_4$) C, H, N.

Receptor Binding Assays. Membranes prepared from MMQ cells (prolactin secreting rat pituitary cells known to contain predominantly ET_A receptors) or porcine cerebellar tissues (known to contain ET_B receptors) grown in 20 culture dishes (150 mm in diameter) were collected by washing and scraping the cells in phosphate-buffered saline (PBS) containing 50 mM EDTA. The cell pellet was then homogenized in 50 mL of 10 mM HEPES (pH 7.4) containing 0.25 M sucrose and protease inhibitors (50 mM EDTA, 0.1 mM PMSF, and 5 μg/mL pepstatin A) by a microultrasonic cell disrupter (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 buffer B (20 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4, containing the aforementioned inhibitors) and centrifuged again at 60000*g* for 60 min. During the preparation of cell membranes, the sample was kept at 4 °C to avoid degradation of membrane proteins. The final pellet was resuspended in buffer B and stored at −80 °C until used. Protein content was determined by the Bio-Rad dye-binding protein assay.

[^{125}I]ET-1 binding to the membrane receptor was performed in 96-well microtiter plates precoated with 0.1% BSA. Membranes prepared from cells (5−10 μg) were incubated with 0.1 nM [^{125}I]ET-1 in binding buffer (buffer B containing 0.2% BSA) at 25 °C for the indicated periods of time. The total incubation volume for each well was 0.2 mL. At the end of the incubation, unbound [^{125}I]ET-1 was separated from bound [^{125}I]ET-1 by vacuum filtration using glass-fiber filter strips in a PHD cell harvester (Cambridge Technology, Inc., MA) followed by washing the filter strips with saline (1 mL) three times. Nonspecific binding was determined in the presence of 1 μM ET-1. In a saturation binding study, various concentrations of [^{125}I]ET-1 (from 0.002 nM to 1 nM) were incubated with 10 μg membrane protein at 25 °C for 4 h. In the competition binding study, 10 μg/well of membrane proteins were incubated with 0.1 nM [^{125}I]ET-1 in the presence of increasing concentrations of unlabeled test ligands for 3 h at 25 °C.

Rat id Absorption Model. Male Sprague−Dawley rats weighing approximately 225 g were fasted overnight. After anesthetization with inactin (100 mg/kg, ip), the pyloric sphincter was ligated, and PE50 catheters were surgically implanted in the carotid artery and portal vein. The test compounds were dosed (10 mg/kg) by injection into the duodenum ($t = 0$), and portal and carotid blood samples were drawn at 10, 30, and 60 min. On completion of the protocol, the animals were sacrificed by inactin overdose.

Plasma samples (200 μL) and an equal volume of an internal standard were extracted with dichloromethane:ethanol (8:2, 5 mL). The organic phase was transferred to a 10 mL conical centrifuge tube, the solvent was evaporated using a stream of dry air, and the residue was redissolved in mobile phase. Recovery of samples following extraction varied 40−80%. Samples were analyzed by HPLC using a Regis Little Champ column (50 × 4.6 mm i.d., Spherisorb S3ODSII, 3 μm). Chromatography was performed using a 10 min linear gradient usually consisting of 38−48% acetonitrile, 5% methanol, and 57−47% 10 mM tetramethylammonium perchlorate (aqueous) containing 0.1% trifluoroacetic acid. UV detection of sample analyte was measured at 226 nm. Drug concentrations (μg/mL) were calculated from standard curves formulated in plasma from 0.01 to 50 μg/mL in triplicate. Portal blood drug levels were used to calculate the area under the curve (id AUC) using the trapezoidal rule. The limit of detection by these methods was 15−20 ng/mL.

Pharmacokinetics in Rats. The pharmacokinetic behavior of the test compound was evaluated in male Sprague−Dawley rats. The compound was prepared as a 10 mg/ml solution in an ethanol:propylene glycol:D5W (20:30:50, by vol) vehicle containing 1 mol equiv of sodium hydroxide. Groups of rats ($n = 4$/group) received either a 5 mg/kg (0.5 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 for NONLIN84[27] were obtained with the program CSTRIP.[28] AUC values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant (*b*) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an $AUC_{0-\infty}$ value. The plasma clearance (CLp) was calculated by dividing the dose by the AUC. 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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