# 2,4-Diarylpyrrolidine-3-carboxylic Acids—Potent $ET_A$ Selective Endothelin Receptor Antagonists. 1. Discovery of A-127722

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We have discovered a novel class of endothelin (ET) receptor antagonists through pharmacophore analysis of the existing non-peptide ET antagonists. On the basis of this analysis, we determined that a pyrrolidine ring might replace the indan ring in SB 209670. The resultant compounds were readily prepared and amenable to extensive SAR studies. Thus a series of N-substituted *trans,trans*-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylic acids (**8**) have been synthesized and evaluated for binding at $ET_A$ and $ET_B$ receptors. Compounds with *N*-acyl and simple *N*-alkyl substituents had weak activity. Compounds with *N*-alkyl substituents containing ethers, sulfoxides, or sulfones showed increased activity. Much improved activity resulted from compounds where the N-substituents were acetamides. Compound **17u** (A-127722) with the *N,N*-dibutylacetamide substituent is the best of the series. It has an $IC_{50} = 0.36$ nM for inhibition of ET-1 radioligand binding at the $ET_A$ receptor, with a 1000-fold selectivity for the $ET_A$ vs the $ET_B$ receptor. It is also a potent inhibitor ($IC_{50} = 0.16$ nM) of phosphoinositol hydrolysis stimulated by ET-1, and it antagonized the ET-1-induced contraction of the rabbit aorta with a $pA_2 = 9.20$. The compound has 70% oral bioavailability in rats.

The endothelins (ET-1 and ET-3), 21-residue amino acid bicyclic peptides discovered in 1988,[1] are the most potent peptide vasoconstrictors known and are also potent mitogens. There has been a great effort to discover endothelin receptor antagonists which may be of benefit in diseases with a significant vasoconstrictive or proliferative component. Pharmacological studies have suggested the value of ET antagonists in the prevention of restenosis following coronary angioplasty, the treatment of myocardial infarction, renal failure, hypertension, and subarachnoid hemorrhage.[2,3]

There are two endothelin receptors: one that is selective for ET-1 ($ET_A$) and another that does not discriminate between ET-1 and ET-3 ($ET_B$).[4,6,7] In the vascular wall, smooth muscle cells express $ET_A$ receptors which mediate the vasoconstrictive and mitogenic effects of ET-1. Endothelial cells express $ET_B$receptors which may act as a buffer to the vasoconstrictive effects of local ET-1 by mediating production of nitric oxide[5] and clearing of ET-1 from the plasma.[8] $ET_B$-mediated constrictor responses in some tissues have been reported.[9−11] Benigni[12] reported that FR139317 (a peptide based $ET_A$ selective antagonist) limited the progression to end-stage renal failure after $^7/_8$ renal ablation in the rat. BQ-123 (another peptidic $ET_A$ selective antagonist) has beneficial effects in models of pulmonary hypertension.[13,14] FR139317 and BQ-123 are effective in decreasing infarct size following coronary ligation[15] and in suppressing cerebral vasospasm following subarachnoid hemorrhage.[16] These findings suggest that an $ET_A$ selective agent may be useful as a therapeutic agent.

At the start of our work, we analyzed the structures

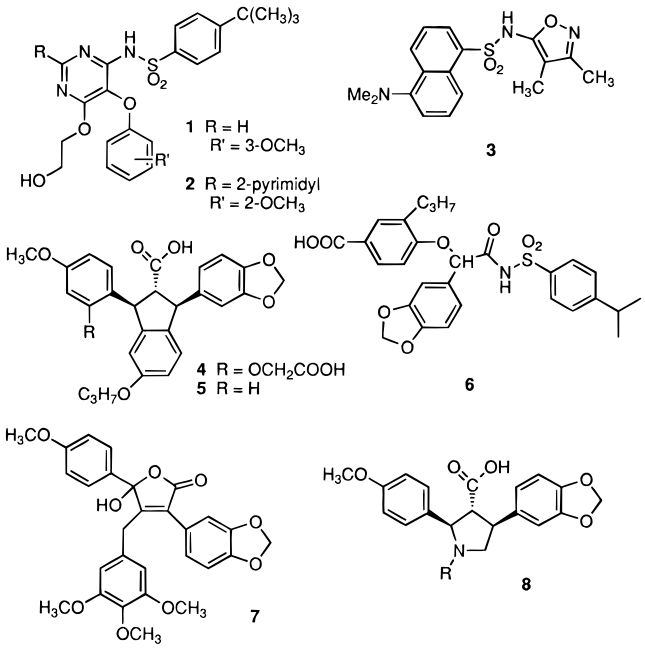


**Figure 1.** Non-peptide endothelin antagonists.

of three non-peptide endothelin antagonists: Ro 46−2005 (**1**),[17] BMS-182874 (**3**),[18,19] and SB-209670 (**4**)[20] (Figure 1). The common feature of **1**, **3**, and **4** is an acidic group positioned between two aromatic rings. Since then the structures of three other non-peptide antagonists, L-749,329 (**6**),[21,22] PD-156707 (**7**),[23] and bosentan (**2**),[24] have been revealed which also have this pharmacophore. Compound **4** has a third aromatic ring, the benzene ring of the indan, to which is connected the propoxy side chain. Few SAR around this indan side chain have been reported, probably because any variation has to be introduced early in the synthesis. If one

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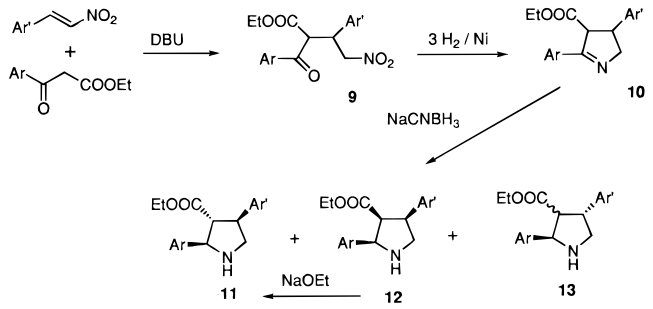
**Scheme 1.** Synthesis of the Ethyl 3,5-Diarylpyrrolidine-3-carboxylate




could substitute a pyrrolidine for the indan, a large number of side chain analogs could be prepared rapidly by acylating and alkylating the nitrogen. Thus our primary target became *trans*−*trans N*-substituted pyrrolidines (**8**). We chose 4-methoxy and 3,4-(methylenedioxy) for the phenyl ring substituents from work of the SmithKline Beecham group, since the SKB compound **5** was active ($IC_{50}$ = 9.68 nM at the $ET_A$ receptor).[21]

## Chemistry

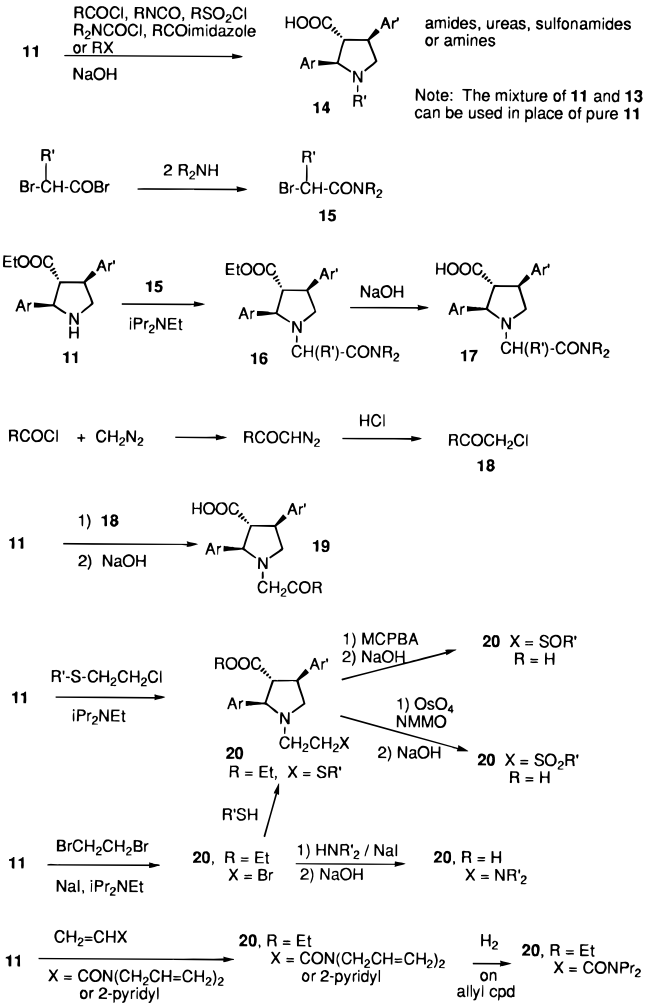
The Michael reaction between 3,4-(methylenedioxy)-2′-nitrostyrene and ethyl (4-methoxybenzoyl)acetate gives two isomers of the adduct **9** in good yield (Scheme 1 and Table 5). This nitro ketone was hydrogenated over Raney nickel to give the dihydropyrrolidine **10**. NMR analysis of this product showed one major product with two minor products. The major product was crystallized and shown by X-ray analysis as structure **10** with the ester and methylenedioxy phenyl group having a *trans* configuration.

This mixture of dihyropyrrolidine products was reduced with sodium cyanoborohydride to give three pyrrolidine isomers. NMR analysis of this mixture suggests that it consists of a 7:6:5 ratio of *trans*−*trans* isomer **11**, *cis*−*cis* isomer **12**, and *cis*−*trans* isomer **13**. Upon chromatography on silica gel, compounds **11** and **13** coeluted. Heating the original pyrrolidine mixture with sodium ethoxide in ethanol causes the *cis*−*cis* compound **12** to isomerize completely to the *trans*−*trans* compound **11**.

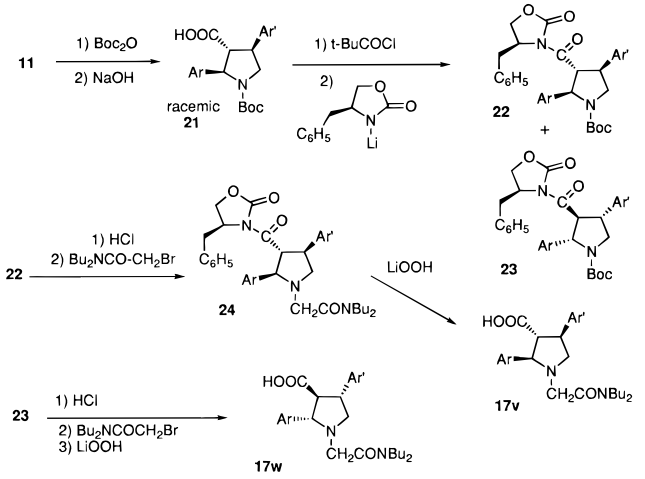
N-substituted *trans*−*trans* derivatives may be prepared either from the pure *trans*−*trans* **11**, obtained by isomerizing **12**, or alternatively from the mixture of *trans*−*trans* and *cis*−*trans* obtained in the chromatography (Scheme 2). After N-alkylation of this mixture, the hydrolysis of the esters is carried out with sodium hydroxide in water/ethanol at room temperature. Under these conditions only the *trans*−*trans* ester is hydrolyzed. The unreacted *cis*−*trans* ester is removed by ether extraction. Hydrolysis of the *cis*−*trans* ester is much slower, requiring refluxing with sodium hydroxide in an ethanol−water mixture for 2 days.

The structure of compound **11** was determined by converting it to compound **23** (Scheme 3) whose structure was determined by X-ray crystallographic analysis. The structure of the *cis*−*trans* 1-(*N*,*N*-dibutylacetamide)-3-carboxylic acid derivative **17y** (Table 2) was determined by X-ray analysis, but this structure cannot be used to assign the relative stereochemistry of the *cis*−*trans* isomer **13**, since base was used in the synthesis of **17y**. This could have resulted in isomerization; thus the relative stereochemistry of **13** remains unknown.

**Scheme 2**

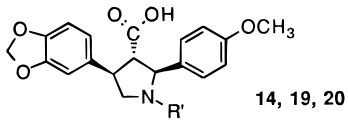



**Scheme 3.** Resolution of A-127722




In the NMR spectra of **11**, the chemical shifts of the ethoxy group fall in the expected range ($CH_3$ at 1.12 ppm and $CH_2$ at 4.07 ppm). The NMR of **12** showed that the chemical shift of the $CH_3$ of the ethoxy group was shifted upfield to 0.79 ppm and the $CH_2$ was shifted upfield to 3.67 ppm. The *cis*−*trans* **13** compound also showed upfield shifts of the ethoxy peaks ($CH_3$ at 0.84 ppm and $CH_2$ at 3.72 ppm) slightly less than those of the *cis*−*cis* compound. These shifts are consistent with the indicated structural assignments. When the ethoxy group is *cis* to the benzene ring, it is situated above the

**Table 1.** SAR of Alkyl and Acyl Side Chains—Radioligand Binding




14, 19, 20

| | | $ET_A$ binding [a] | | | $ET_B$ binding [a] | | |
|---|---|---|---|---|---|---|---|
| no. | R′ | $IC_{50}$ (nM) | (range) | *n* | $IC_{50}$ (nM) | (range) | *n* |
| | | | Hydrocarbons | | | | |
| **14a** | $C_6H_{13}$ | 2870 | (2330−3540) | 2 | 23300 | (19400−27900) | 2 |
| **14b** | $Me_2CHCH_2CH{=}CHCH_2$ | 1630 | (1620−1640) | 2 | 27300 | (22900−32500) | 2 |
| **14c** | $CH_3(CH_2)_2C{\equiv}CCH_2$ | 765 | (551−1060) | 2 | 3590 | (3530−3660) | 2 |
| **14d** | 4-F-$C_6H_4CH_2$ | 4320 | (1520−12300) | 2 | 7930 | (3870−16260) | 2 |
| | | | Acyl | | | | |
| **14e** | 2-furoyl-CO | 5220 | (2969−9164) | 2 | 40300 | (33900−47900) | 2 |
| **14f** | 4-F-$C_6H_4OCH_2CO$ | 14100 | (5190−38500) | 2 | 12700 | (5640−28400) | 2 |
| **14g** | $C_6H_5NHCO$ | 2430 | (1850−3190) | 2 | 3510 | (1930−6390) | 2 |
| **14h** | 4-$CH_3O$-$C_6H_5NHCO$ | 781 | (660−925) | 2 | 3790 | (3410−4200) | 2 |
| **14i** | $C_4H_9NHCO$ | 684 | (530−881) | 2 | 11100 | (10500−11700) | 2 |
| **14j** | $C_4H_9SO_2$ | 1240 | (1090−1410) | 2 | 6180 | (5300−7220) | 2 |
| **14k** | $O(CH_2CH_2)NCO$ | 1080 | (830−1570) | 3 | 5020 | (2830−11300) | 3 |
| **14m** | $(C_4H_9)_2NCO$ | 396 | (248−700) | 3 | 1470 | (775−2800) | 2 |
| **14n** | $(C_4H_9)_2NCH_2CO$ | 6810 | (2630−12200) | 3 | 29400 | (10200−70000) | 3 |
| | | | Ethers | | | | |
| **14p** | $C_2H_5OCH_2CH_2$ | 520 | (304−887) | 2 | 9010 | (5390−15100) | 2 |
| **14q** | $C_3H_7OCH_2CH_2$ | 108 | (50.1−360) | 4 | 4970 | (2770−16300) | 4 |
| **14r** | $C_4H_9OCH_2CH_2$ | 220 | (112−508) | 3 | 3830 | (3740−3910) | 2 |
| **14s** | $(CH_3)_2CHCH_2OCH_2CH_2$ | 437 | (354−540) | 2 | 8380 | (7160−9820) | 2 |
| **14t** | $MeOCH_2CH_2OCH_2CH_2$ | 682 | (205−2270) | 2 | 3390 | (2510−4570) | 2 |
| **14u** | $C_6H_5OCH_2CH_2$ | 885 | (873−892) | 2 | 3400 | (3150−3670) | 2 |
| | | | Sulfoxide and Sulfone | | | | |
| **20a** | $C_2H_5SOCH_2CH_2$ | 44 | (25.1−77.6) | 2 | 834 | (580−1206) | 2 |
| **20b** | $C_3H_7SO_2CH_2CH_2$ | 37 | (16.7−79.5) | 3 | 750 | (489−959) | 3 |
| | | | Amines, Amides, and Ketones | | | | |
| **20c** | 2-pyridyl-$CH_2CH_2$ | 900 | (609−1330) | 2 | 2360 | (2160−2580) | 2 |
| **20d** | $(C_4H_9)_2NCH_2CH_2$ | 794 | (311−2560) | 3 | 1590 | (1300−2050) | 3 |
| **20e** | $(C_3H_7)_2NCOCH_2CH_2$ | 121 | (114−128) | 2 | 4200 | (3070−5760) | 2 |
| **19a** | $C_4H_9COCH_2$ | 163 | (112−237) | 2 | 2430 | (1730−3430) | 2 |
| **19b** | $(C_3H_7)_2CHCOCH_2$ | 85 | (46−155) | 2 | 283 | (204−392) | 2 |

[a] Average. [b] *n* = number of determinations.

center of the benzene ring and is thus exposed to a shielding effect.

Compound **17u** (A-127722) was prepared in an optically active form according to the method of Scheme 3. The racemic **11** was first converted in two steps to the *N*-Boc carboxylic acid **21**. This was used to acylate (*S*)-(−)4-benzyl-2-oxazolidinone to give **22** and **23**, which were separated by chromatography. The slower moving diastereoisomer **23** was crystallized and its structure was determined by X-ray analysis. The Boc group was removed from both **22** and **23** and replaced by $CH_2$-$CONBu_2$ as previously described, and the oxazolidine was cleaved to give the two enantiomers of A-127722. Compound **23**, for which we have the X-ray structure, was converted to the less active enantiomer of structure **17w**. Compound **24** was converted to the more active enantiomer, which must have the structure **17v**. In an alternative strategy, racemic **21** was resolved by crystallization of its salt with (*R*)-(+)-α-methylbenzylamine. This was reconverted to resolved **11** by treatment with HCl in ethanol and then to resolved (+)-A-127722 **17v** as before.

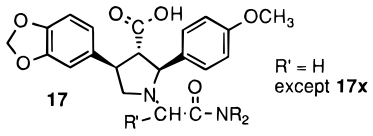
## Structure−Activity Relationship

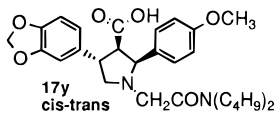
Activity at the $ET_A$ receptor was measured by inhibition of $^{125}$I-labeled ET-1 binding to rat MMQ cells.[25] Activity at $ET_B$ receptors was measured by inhibition of [$^{125}$I]ET-3 binding to porcine cerebellum. Functional activity was measured as inhibition of ET-1-stimulated phosphinositol hydrolysis in MMQ cells and by inhibition of ET-1-stimulated contraction in isolated rat aorta.

Acyl groups were attached to the nitrogen of the pyrrolidine, giving compounds **14e**−**k** (Table 1) that would not be protonated at the physiological pH of 7.4. They showed modest activity at the $ET_A$ receptor, the best being the *n*-butyl (**14i**) and anisylurea (**14h**) derivatives. Alkylation of the pyrrolidine nitrogen with hydrocarbon chains gave **14a**,**b**,**d**, with weak activity. Speculating that **14a**,**b**,**d** were weak because the pyrrolidine nitrogen was protonated at pH of 7.4, an acetylene was introduced into the molecule which would reduce the basicity of this pyrrolidine nitrogen. The activity of this target compound (**14c**) was only marginally improved. Ethers were attached to the pyrrolidine nitrogen, since **5** has an ether side chain. This improved the activity; the propoxyethyl compound (**14q**) had an $IC_{50}$ at the $ET_A$ receptor of 108 nM. Compounds with sulfoxide (**20a**) or sulfone (**20b**) groups showed a further improvement in activity.

In an effort to reduce the basicity of the pyrrolidine nitrogen, acetamide side chains were incorporated ($pK_a$ = 2.5 for **17u**). See Table 2. The unsubstituted acetamide (**17a**) had weak activity, but mono *N*-alkyl compounds showed improved activity, with the *N*-isobutyl

**Table 2.** SAR of Acetamide Side Chains—Radioligand Binding and Phosphoinositol Hydrolysis Inhibition (PI)




| | | $ET_A$ binding | | | $ET_B$ binding | | | |
|---|---|---|---|---|---|---|---|---|
| no. | $NR_2$ | $IC_{50}$ (nM) | (range) | *n* | $IC_{50}$ (nM) | (range) | *n* | PI[a] $IC_{50}$ (nM) |
| **17a** | $NH_2$ | 2030 | (811−5060) | 2 | 3274 | (3270−15100) | 2 | |
| **17b** | $NHC_3H_7$ | 17.2 | (11.2−26.7) | 4 | 398 | (298−492) | 3 | 24.6 |
| **17c** | $NHC_4H_9$ | 43.0 | (34.9−51.1) | 3 | 1259 | (946−1470) | 3 | 10.0 |
| **17d** | $NHCH_2CHMe_2$ | 8.20 | (7.45−8.87) | 3 | 184 | (85.8−478) | 3 | 9.22 |
| **17e** | $NHCH_2CH{=}CH_2$ | 34.2 | (19.9−54.1) | 3 | 889 | (750−986) | 3 | 16.5 |
| **17f** | $NHCH_2CH_2OCH_3$ | 178 | (154−205) | 2 | 3730 | (3210−4330) | 2 | |
| **17g** | NH-cyclopentyl | 16.2 | (13.0−19.0) | 3 | 307 | (145−554) | 3 | 23.8 |
| **17h** | $N(Me)C_3H_7$ | 6.63 | (3.04−9.53) | 5 | 2900 | (1210−4280) | 5 | 9.12 |
| **17i** | $N(Me)C_4H_9$ | 4.71 | (3.65−6.09) | 3 | 1390 | (621−2590) | 3 | 0.97 |
| **17j** | $N(Me)C_5H_{11}$ | 5.78 | (2.39−14.0) | 2 | 624 | (444−877) | 2 | 5.64 |
| **17k** | $N(Me)C_6H_{11}$ | 7.61 | (6.08−9.53) | 2 | 404 | (273−598) | 2 | 1.00 |
| **17m** | $N(Me)CH_2C{\equiv}CH$ | 106 | (71−160) | 2 | 4000 | (1850−8640) | 2 | |
| **17n** | $N(Me)CH_2CHMe_2$ | 1.88 | (0.94−3.88) | 3 | 1050 | (939−1180) | 3 | 0.28 |
| **17o** | $N(Me)\ CH_2CMe_3$ | 112 | (56.6−224) | 2 | 1800 | (1150−2830) | 2 | |
| **17p** | N(Me)cyclohexyl | 23.8 | (16.4−34.6) | 2 | 806 | (415−1567) | 2 | 2.06 |
| **17q** | N(Me)phenyl | 50.2 | (22.6−112) | 2 | 1180 | (605−2313) | 2 | |
| **17r** | N(Me)benzyl | 769 | (620−954) | 2 | 1680 | (1640−1720) | 2 | |
| **17s** | $N(Et)C_4H_9$ | 0.85 | (0.64−1.21) | 3 | 531 | (434−680) | 3 | 0.33 |
| **17t** | $N(Pr)C_4H_9$ | 0.22 | (0.14−0.36) | 3 | 40 | (31−47) | 3 | 0.25 |
| **17u** | $N(C_4H_9)_2$ (A127722) | 0.36 | (±0.04)[b] | 10 | 515 | (±51.9)[b] | 10 | 0.16 |
| **17v** | $N(C_4H_9)_2$ (*RRS*) | 0.31 | (0.24−0.43) | 3 | 191 | (111−387) | 3 | 0.04 |
| **17w** | $N(C_4H_9)_2$ (*SSR*) | 171 | (134−240) | 3 | 17730 | (8117−33260) | 2 | 46.8 |
| **17x** | $N(C_4H_9)_2$ R′ = Me, mixt | 0.46 | (.233-.921) | 2 | 315 | (266−372) | 2 | 1.34 |
| **17y** | $N(C_4H_9)_2$ cis−trans isomer | 4.62 | (4.02−5.30) | 2 | 14000 | (12600−15500) | 2 | |
| **17z** | $N(C_4H_9)_2$ $CONH_2$ deriv | 58 | (38−83) | 3 | inact | | | |
| **17aa** | $N(C_2H_5)_2$ | 32.9 | (25.2−42.9) | 2 | 5250 | (3656−7527) | 2 | 2.26 |
| **17bb** | $N(C_3H_7)_2$ | 1.23 | (0.59−2.55) | 2 | 598 | (510−702) | 2 | 0.24 |
| **17cc** | $N(C_5H_{11})_2$ | 0.56 | (.352-.894) | 3 | 313 | (281−386) | 3 | |
| **17dd** | $N(CH_2CH_2OCH_3)_2$ | 4.45 | (3.50−5.67) | 2 | 1070 | (847−1350) | 2 | 4.91 |
| **17ee** | $N(CH_2CHMe_2)_2$ | 16.2 | (9.51−27.4) | 2 | 1820 | (922−3610) | 2 | 0.60 |
| **17ff** | $N(CH_2CH_2CHMe_2)_2$ | 0.43 | (.363-.578) | 3 | 41 | (29−62) | 3 | |
| **17gg** | $NBuC_6H_5$ | 0.81 | (0.69−0.93) | 2 | 306 | (181−516) | 2 | |
| **17hh** | piperidine | 350 | (286−427) | 2 | 3040 | (1980−4650) | 2 | |

[a] Inhibition of phosphoinositol hydrolysis. [b] Standard error of the mean.

compound (**17d**) having binding activity at the $ET_A$ receptor equal to that of the corresponding indan **5**. Substitution of oxygen for methylene as in **17f** reduced activity, while cyclization as in **17g** was tolerated. It was found that N-methylation gave us another boost in activity when **17h**−**r** were synthesized. Of these *N*-methyl-*N*-alkyls, the butyl (**17i**), pentyl (**17j**), and isobutyl (**17n**) were optimal groups. Compounds with N-substituents containing a benzene ring (**17q**), a quaternary center (**17o**), or an acetylene (**17m**) were less active. Increasing the size of the *N*-methyl on **17i** to ethyl (**17s**), propyl, (**17t**), and butyl (**17u**) gave a large increase in activity, with the propyl butyl (**17t**) and dibutyl (**17u** A-127722) compounds being the most potent analogs. These compounds are approximately 30 times more potent than the indan **5**. Substitution of oxygen for two of the carbons in the dibutyl amide gave **17dd** and reduced activity by a factor of 12. The dipentyl **17cc** and diisopentyl amides **17ff** were almost as active, but the diisobutyl amide **17ee** was similar to monoisobutyl amide **17d**. The phenyl butyl analog **17gg** was 2-fold less potent than A-127722. This was in contrast to the *N*-methyl compounds, where substitution of phenyl for butyl (**17q** vs **17i**) reduced activity by a factor of 10. Cyclization to give **17hh** reduced activity by 700.

Homologating the acetamide **17bb** to the propionamide **20e** (Table 1) reduced $ET_A$ binding by 100. Addition of an α-methyl to the side chain (**17x**, mix of isomers) had no effect on the activity. Changing the amides into the corresponding ketones (**19a**,**b** Table 1) caused a reduction in binding activity. This could be due to the fact that the butyls in **19b** extend into a different direction in space from the butyls of **17u**. Replacement of the carbonyl for a methylene, affording the diamine **20d** (Table 1) also caused reduced activity. This could be due to the importance of the carbonyl or the fact that **20d** would be positively charged at physiologic pH. Removing the methylene gave the urea **14m**, and reversing the acetamide gave **14n**. Both are relatively inactive compounds, consistent with earlier observations on the *N*-acylpyrrolidines. The *cis*−*trans* isomer of A-127722 (**17y**) was 13 times less active than the *trans*−*trans* one. The importance of the carboxylic acid is shown by a 160-fold loss in activity in the carboxamide analog **17z**. All of the compounds in the acetamide series were very selective for $ET_A$ over $ET_B$ receptors.

The activity of A-127722 resides in the *R*,*R*,*S* isomer, which had a similar relative and absolute configuration to SB-209670. $K_i$ values were determined, being a more sensitive method for measuring the activity of such potent compounds. The *R*,*R*,*S* isomer ($K_i$ = 0.047 ± 0.009 nM, $n$ = 3), and the racemate ($K_i$ = 0.105 ± 0.009

**Table 3.** Radioligand Binding of Selected Compounds to Human Receptors in Chinese Hamster Ovary Cells

| | $ET_A$ binding | | | $ET_B$ binding | | |
|---|---|---|---|---|---|---|
| no. | $IC_{50}$ (nM) | (range) | *n* | $IC_{50}$ (nM) | (range) | *n* |
| **17b** | 45.3 | (31.2−65.8) | 2 | 579 | (552−608) | 2 |
| **17h** | 21.3 | (11.5−39.5) | 2 | 4570 | (2440−8560) | 2 |
| **17i** | 4.88 | (4.57−5.20) | 2 | 1080 | (665−1750) | 2 |
| **17t** | 0.81 | | 1 | 211 | | 1 |
| **17u** (A-127722) | 0.11 | (±0.05)[a] | 5 | 98 | (±11.5)[a] | 5 |
| **17bb** | 0.55 | (0.45−0.70) | 2 | 397 | (370−423) | 2 |

[a] SEM.

**Table 4.** Comparison of A-127722 to Literature Standards in Binding to Human Endothelin Receptors in Chinese Hamster Ovary Cells

| | | $K_i$[a] | |
|---|---|---|---|
| no. | name | $ET_A$ | $ET_B$ |
| **2** | Bosentan | 6.5 | 343 |
| **3** | BMS-182874 | 48 | >50000 |
| **4** | SB-209670 | 0.43 | 14.7 |
| **6** | L-749,329 | 0.13 | 5.4 |
| **7** | PD-156707 | 0.17 | 139 |
| **17u** | A-127722 | 0.069 | 139 |
| **17v** | A-127722 (*RRS*) | 0.034 | |
| **17w** | A-127722 (*SSR*) | 63 | |

[a] Data for compounds **2**−**7** are literature values. See the text for references.

nM, $n = 3$) showed a ratio close to two. The SSR isomer had $K_i = 37.1$ nM, $n = 2$. From Scatchard analysis, increasing concentrations of A-127722 impacted [$^{125}$I]-ET-1 binding by causing successive increases in the $K_d$ value without having significant effect on the $B_{max}$ value, suggesting that A-127722 is a competitive antagonist of ET-1 binding to its receptor.[26]

Binding to human $ET_A$ and to $ET_B$ receptors expressed in Chinese hamster ovary cells was determined for key compounds. The $IC_{50}$'s for several pyrrolidine-N-acetamides are shown in Table 3, with A-127722 (**17u**) being the most potent among these analogs. The compound and its enantiomers has also been compared to a series of literature benchmarks (Table 4). Clearly A-127722 is among the most potent ET antagonists reported to date.

The inhibition of ET-1-stimulated phosphoinositol hydrolysis was determined in rat MMQ cells, to show that these compounds were indeed functional antagonists. This activity roughly paralleled the binding activity, with A-127722 being the most potent compound, with an $IC_{50}$ of 0.16 nM. A-127722 also blocked the contractions caused by ET-1 in isolated rat aorta with a $pA_2 = 9.20$ (SEM = 0.27, $n = 4$, slope = 1.05, $r = 0.84$). For comparison, in our assay, L-749329 had a $pA_2 = 8.16$ (SEM = 0.13, $n = 4$, slope = 1.04, $r = 0.95$) and PD-156707 had a $pA_2 = 8.13$ (SEM = 0.47, $n = 5$, slope = 0.84, $r = 0.80$).

## Pharmacokinetics

Pharmacokinetics in rats showed that at an iv dose of 5 mg/kg A-127722 gave an area under the curve (Figure 2) of 7.96 $\mu$g h/mL, a half-life of 3.5 h, a volume of distribution of the central compartment (dose divided by the plasma concentration extrapolated to time zero) = 0.26 L/kg, and a total plasma clearance (dose divided by the area under the curve) = 0.7 mL/min. At an oral dose of 10 mg/kg, A-127722 had an area under the curve of 11.2 $\mu$g h/mL, a half-life of 4.5 h, a peak plasma concentration of 3.24 $\mu$g/mL, and the time of peak plasma concentration = 0.7 h. The calculated oral bioavailability was 70%.

## Conclusions

Replacement of the indan ring of SB209670 with a pyrrolidine ring led to the discovery of a new class of

**Table 5.** Synthetic and Physical Data

| no. | synth method | % yield[a] | mp | formula[b] | no. | synth method | % yield[a] | mp | formula[b] |
|---|---|---|---|---|---|---|---|---|---|
| **14a** | C | 75 | 85−86 | $C_{25}H_{31}NO_5 \cdot 0.25H_2O$ | **17m** | A,B | 87 | | $C_{25}H_{26}N_2O_6$ |
| **14b** | C | 81 | | $C_{26}H_{31}NO_5$ | **17n** | A,B | 79 | | $C_{26}H_{32}N_2O_6$ |
| **14c** | C | 83 | | $C_{25}H_{27}NO_5$ | **17o** | A,B | 74 | | $C_{27}H_{34}N_2O_6H_2O$ |
| **14d** | C | 79 | | $C_{26}H_{24}FNO_5$ | **17p** | A,B | 81 | | $C_{28}H_{34}N_2O_6$ |
| **14e** | E | 76 | | $C_{24}H_{21}NO_7$ | **17q** | A,B | 80 | | $C_{28}H_{28}N_2O_6$ |
| **14f** | E | 63 | | $C_{27}H_{24}FNO_7$ | **17r** | A,B | 71 | | $C_{29}H_{30}N_2O_6 \cdot 0.5H_2O$ |
| **14g** | F | 83 | 209−211 | $C_{26}H_{24}N_2O_6$ | **17s** | A,B | 81 | | $C_{27}H_{34}N_2O_6 \cdot 0.5H_2O$ |
| **14h** | F | 83 | 185−187 | $C_{27}H_{26}N_2O_7$ | **17t** | A,B | 80 | 160−162 | $C_{28}H_{36}N_2O_6$ |
| **14i** | F | 71 | 220−221 | $C_{24}H_{28}N_2O_6$ | **17u** | A,B | 77 | 122−124 | $C_{29}H_{38}N_2O_6$ |
| **14j** | E | 75 | 67−69 | $C_{23}H_{27}NO_7S$[c] | **17v** | O | | | $C_{29}H_{38}N_2O_6 \cdot 0.5H_2O$ |
| **14k** | E | 77 | 244−246 | $C_{24}H_{26}N_2O_7$ | **17w** | O | | | $C_{29}H_{38}N_2O_6 \cdot 0.25H_2O$ |
| **14m** | E | 75 | 120−122 | $C_{28}H_{36}N_2O_6$ | **17x** | A,B | 61 | | $C_{30}H_{40}N_2O_6 \cdot 0.5H_2O$ |
| **14n** | G | 73 | | $C_{29}H_{38}N_2O_6$ | **17y** | A,B | 64 | 165−166 | $C_{29}H_{38}N_2O_6$ |
| **14p** | C | 70 | 88−90 | $C_{23}H_{27}NO_6$ | **17z** | N | 83 | 194−196 | $C_{29}H_{39}N_3O_5$ |
| **14q** | C | 58 | | $C_{24}H_{29}NO_6$ | **17aa** | A,B | 62 | 132−134 | $C_{25}H_{30}N_2O_6$ |
| **14r** | C | 60 | 53−56 | $C_{25}H_{31}NO_6$[c] | **17bb** | A,B | 73 | 170−172 | $C_{27}H_{34}N_2O_6$ |
| **14s** | C | 65 | 68−70 | $C_{25}H_{31}NO_6$[c] | **17cc** | A,B | 73 | | $C_{31}H_{42}N_2O_6$ |
| **14t** | C | 57 | | $C_{24}N_{29}NO_7$ | **17dd** | A,B | 73 | 120−122 | $C_{27}H_{34}N_2O_8$ |
| **14u** | C | 38 | | $C_{27}H_{27}NO_6$ | **17ee** | A,B | 81 | 141−143 | $C_{29}H_{38}N_2O_6$ |
| **17a** | A,B | 72 | 246−248 | $C_{21}H_{22}N_2O_6$ | **17ff** | A,B | 68 | | $C_{31}H_{42}N_2O_6$ |
| **17b** | A,B | 75 | 151−153 | $C_{24}H_{28}N_2O_6$ | **17gg** | A,B | 48 | 70−74 | $C_{31}H_{34}N_2O_6$ |
| **17c** | A,B | 70 | 105−107 | $C_{25}H_{30}N_2O_6$ | **17hh** | A,B | 82 | | $C_{26}H_{30}N_2O_6$ |
| **17d** | A,B | 72 | 175−177 | $C_{25}H_{30}N_2O_6$ | **19a** | L | 39 | | $C_{25}H_{29}NO_6$ |
| **17e** | A,B | 72 | 138−140 | $C_{24}H_{26}N_2O_6$ | **19b** | L | 46 | | $C_{28}H_{35}NO_6$ |
| **17f** | A,B | 80 | 107−109 | $C_{24}H_{28}N_2O_7$ | **20a** | H | 65 | 61−63 | $C_{23}H_{27}NO_6S \cdot 0.5H_2O$ |
| **17g** | A,B | 73 | 137−139 | $C_{26}H_{30}N_2O_6$ | **20b** | J | 90 | 73−75 | $C_{24}H_{29}NO_7S$ |
| **17h** | A,B | 72 | | $C_{25}H_{30}N_2O_6$ | **20c** | D | 56 | 77−80 | $C_{26}H_{26}N_2O_5 \cdot 2.75H_2O$ |
| **17i** | A,B | 79 | | $C_{26}H_{32}N_2O_6$ | **20d** | K | 78 | | $C_{29}H_{40}N_2O_5$ |
| **17j** | A,B | 90 | | $C_{27}H_{34}N_2O_6$ | **20e** | M | 66 | | $C_{28}H_{36}N_2O_6$ |
| **17k** | A,B | 83 | | $C_{28}H_{36}N_2O_6$ | | | | | |

[a] Combined yield of last two steps. [b] Analysis for C, H, and N are within 0.4% of theory. [c] C, H, N not done. High-resolution MS within 0.004 of theory.

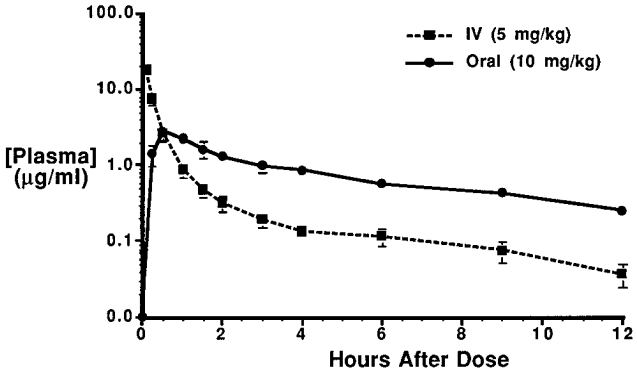



**Figure 2.** Pharmacokinetics of A-127722 in rats.

endothelin antagonists. The pyrrolidine nitrogen was readily substituted, allowing exploration of an area of space not easily accessed in the Smith-Kline series. The nature of the N substituent was crucial for endothelin receptor antagonism, with N,N-disubstituted acetamides being optimal. A-127722 (**17u**) the *N*,*N*-dibutyl analog, was discovered to be a very potent $ET_A$ antagonist with an $IC_{50}$ in radioligand binding of 0.36 nM.

## Experimental Section

**General.** Flash chromatography was done using silica gel (230−400 mesh) from E. M. Science. Proton NMR spectra were recorded on a General Electric QE300 instrument with $Me_4Si$ as an internal standard. Structure determination by X-ray crystallography was done on a Rigaku ASC-5 instrument. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ. Melting points were measured on a Thomas Hoover apparatus and are uncorrected.

**Ethyl 2-(4-Methoxybenzoyl)-3-(nitromethyl)-1,3-benzodioxol-5-propionate (9).** Ethyl (4-methoxybenzoyl)acetate[27] (35.0 g, 0.158 mol) and 5-(2-nitrovinyl)-1,3-benzodioxole[28] (37.9 g, 0.196 mol) were dissolved in 210 mL of THF and 35 mL of 2-propanol. One gram of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) was added and the mixture stirred at room temperature for 2 h, during which time additional DBU was added after 20 min (0.65 g) and 1 h (0.25 g). After 2 h TLC (95% $CH_2Cl_2$−5% EtOAc) showed that most of the starting materials were gone. The solvents were concentrated in vacuum and 500 mL of toluene was added. The organic phase was extracted with dilute hydrochloric acid, and then with NaCl solution. After drying ($Na_2SO_4$) the solution was concentrated and the residue chromatographed on silica gel, eluting with 3:1 hexane−EtOAc to give 58.14 g (78%) of the desired product as a mixture of isomers.

**Ethyl 2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-4,5-dihydro-3*H*-pyrrole-3-carboxylate (10).** The nitro ester **9** (21 g) in 500 mL of ethanol was hydrogenated at 4 atm of pressure with 51 g of Raney nickel 2800 (washed with ethanol three times before use). After the catalyst was filtered the solution was concentrated and the residue chromatographed on silica gel eluting with 8.5% EtOAc in $CH_2Cl_2$ to give 12.34 g of the desired product. This compound must be kept away from air as it is easily hydroxylated at the 3-position and this byproduct could not be separated by chromatography. NMR ($CDCl_3$): major isomer 1.18 (t, $J$ = 7 Hz, 3H), 3.72−3.80 (m, 1H), 3.84 (s, 3H), 4.08−4.20 (m, 4H), 4.52−4.63 (m, 1H), 5.92 (s, 2H), 6.6−6.75 (m, 3H), 6.92 (d, $J$ = 9, 2H), 7.74 (d, $J$ = 9, 2H).

**Ethyl 2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-pyrrolidine-3-carboxylate as a Mixture of *cis*−*cis* (12), *trans*−*trans* (11), and *cis*−*trans* (13) Isomers.** The imine **10** (11.89 g, 0.324 mol) was dissolved in 27 mL of THF and 54 mL of ethanol. Sodium cyanoborohydride (2.35 g, 0.374 mol) and 5 mg of bromocresol green were added. To this blue solution was added dropwise a solution of concentrated HCl in ethanol (1:2), at such a rate that the color was kept at light yellow-green. After the yellow color persisted without additional HCl, the solution was stirred an additional 20 min. The solution was concentrated in vacuum and then partitioned between $CHCl_3$ and $KHCO_3$ isolution. The organic phase was separated, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed on silica gel eluting with 85% EtOAc−15% hexane to give 5.96 g of a mixture of 64% *trans*−*trans* compound (**11**) and 36% *cis*−*trans* compound (**13**). Further elution with pure ethyl acetate gave 3.044 g of pure *cis*−*cis* compound (**12**). If the starting imine had some 3-hyroxy impurity, there will be some 3-hydroxypyrrolidine eluting between these fractions. NMR ($CDCl_3$): *cis*−*cis* **12**, 0.79 (t, $J$ = 7 Hz, 3H), 3.49−3.56 (m, 2H), 3.67 (q, $J$ = 7 Hz, 2H), 3.71 (dd, $J$ = 10 Hz, 10 Hz, 1H), 3.79 (s, 3H), 3.80−3.88 (m, 1H), 4.60 (d, $J$ = 5 Hz, 1H), 5.92 (s, 2H), 6.73 (s, 2H), 6.79 (s, 1H), 6.85 (d, $J$ = 8 Hz, 2H), 7.28 (d, $J$ = 8 Hz, 2H); *cis*−*trans* **13**, 0.84 (t, $J$ = 7 Hz, 3H), 3.03 (dd, $J$ = 9 Hz, 9 Hz, 1H), 3.28 (dd, $J$ = 8 Hz, 9 Hz, 1H), 3.55−3.78 (m, 2H), 3.72 (t, $J$ = 7 Hz, 2H), 3.80 (s, 3H), 4.67 (d, $J$ = 9 Hz, 1H), 5.94 (s, 2H), 6.71−6.80 (m, 3H), 6.85 (d, $J$ = 8 Hz, 2H), 7.75 (d, $J$ = 8 Hz, 2H); for *trans*−*trans* **11**, see below.

***trans,trans*-Ethyl 2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate (11).** The above described pure *cis*−*cis* compound (**12**) (3.02 g) was dissolved in 10 mL of ethanol, and 20 drops of a solution of 21% sodum ethoxide in ethanol was added. The solution was refluxed overnight. TLC (EtOAc) showed no starting material. The excess NaOEt was neutralized with HCl in ethanol and the solution concentrated in vacuum. The residue was taken up in toluene and extracted with $KHCO_3$ in water. The toluene was dried ($Na_2SO_4$) and concentrated to give 2.775 g of **11** which was pure by TLC (EtOAc). NMR ($CDCl_3$): 1.12 (t, $J$ = 7 Hz, 3H), 2.90 (dd, $J$ = 9 Hz, 9 Hz, 1H), 3.10 (dd, $J$ = 6 Hz, 10 Hz, 1H), 3.51−3.66 (m, 2H), 3.80 (s, 3H), 4.07 (q, $J$ = 7 HZ, 2H), 4.48 (d, $J$ = 8 Hz, 1H), 5.92 (s, 2H), 6.72 (s, 2H), 6.82 (s, 1H), 6.88 (d, $J$ = 8 Hz, 2H), 7.37 (d, $J$ = 8 Hz, 2H).

***N*,*N*-Dibutylbromoacetamide (14, $NR_2 = NBu_2$, $R' = H$).** Using the method of Weaver,[29] bromoacetyl bromide (45.9 g, 0.227 mol) was dissolved in 50 mL of dichloroethane and added over a 2 min period to 61.74 g (0.478 mol) of dibutylamine dissolved in 250 mL of dichloroethane cooled to −45 °C. The mixture was stirred at −30 for 20 min and at room temp for 30 min. Additional (500 mL) dichloroethane was added and the solution was washed with 100 mL of water twice. The solvents were removed in vacuum, and the heptane (600 mL) was added to the residue. Some insoluble material was removed by filtration, and the solution was concentrated in vacuum to yield 54.31 g (95%) of the desired product. Other bromoacetamides were prepared similarly. The monosubstituted amides were insoluble in heptane.

**Method A. *trans,trans*- and *cis,trans*-Ethyl 2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutylaminocarbonyl]methyl]pyrrolidine-3-carboxylate (16, $NR_2 = NBu_2$, $R' = H$).** The mixture of 64% **11** and 36% **13** (26.33 g, 0.714 mol), 21.50 g (0.086 mol) of *N*,*N*-dibutylbromoacetamide, 18.30 g (0.142 mol) of diisopropylethylamine, and 55 mL of acetonitrile was heated at 50 °C for 1 h. TLC (EtOAc) showed no NH starting material. Toluene was added and the mixture was washed with $KHCO_3$ solution, dried ($Na_2SO_4$), and concentrated. Additional toluene was added and again concentrated in vacuum. This was repeated until the odor of diisopropylethylamine was gone (yield, 42.02 g; theory, 38.4 g). This material was used directly in the next step.

**Method B. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutyl amino)carbonyl]methyl]-pyrrolidine-3-carboxylic Acid (17u).** The above mixture of esters was dissolved in 220 mL of ethanol. Sodium hydroxide (23.65 g) in 100 mL of water was added. This mixture was stirred for 3 h at room temperature. The solution was concentrated in vacuum using low (40 °C) heat. Water (300 mL) and ether (500 mL) were added. Three layers formed. The bottom two layers were separated, and the ether layer was extracted with two 50 mL portions of water. The combined aqueous mixture was back-extracted with 400 mL of ether and then neutralized with acetic acid. This mixture was extracted with ether twice. The ether was dried ($Na_2SO_4$) and concentrated. Toluene was added, and the resulting

solution was concentrated in vacuum. More toluene was added and the mixture reconcentrated until the odor of acetic acid was gone. Ether (150 mL) was added. Cooling gave 17.88 g of **17u**: mp 122−124 °C; yield 77% in two steps based upon 16.85 g of trans−trans **11**; TLC (5% HCOOH in EtOAc) was one spot of $R_f$ = 0.33. The ether extracts from the basic solution were concentrated to give recovered *cis*−*trans* ester. This was hydrolyzed as above (refluxing for two days was necessary) to give **17y**, the structure of which was determined by X-ray analysis. NMR ($CDCl_3$): **17u**, 0.79 (t, $J$ = 7 Hz, 3H), 0.85 (t, $J$ = 7 Hz, 3H), 1.00−1.50 (m, 8H), 2.74 (d, $J$ = 13 Hz, 1H), 2.90−3.09 (m, 4H), 3.23−3.50 (m, 3H), 3.38 (d, $J$ = 13 Hz, 1H), 3.52−3.62 (m, 1H), 3.75 (d, $J$ = 10 Hz, 1H), 3.78 (s, 3H), 5.93 (dd, $J$ = 2 Hz, 4 Hz, 2H), 6.71 (d, $J$ = 8 Hz, 1H), 6.81−6.89 (m, 2H), 7.03 (d, $J$ = 2 Hz, 1H), 7.30 ($J$ = 8 Hz, 2H); **17y**, 0.82 (t, $J$ = 7 Hz, 3H), 0.90 (t, $J$ = 7 Hz, 3H), 1.08−1.48 (m, 8H), 2.92−3.38 (m, 6H), 3.04 (d, $J$ = 13 Hz, 1H), 3.45 (d, $J$ = 13 Hz, 1H), 3.45−3.52 (m, 1H), 3.74 (s, 3H), 3.75−3.87 (m, 1H), 4.24 (d, $J$ = 10 Hz, 1H), 5.93 (s, 2H), 6.70−6.82 (m, 3H), 6.77 (d, $J$ = 8 Hz, 2H), 7.28 (d, $J$ = 8 Hz, 2H).

**Method C. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(2-methoxyethoxy)ethyl]pyrrolidine-3-carboxylic Acid (14t).** Compound **11** (250 mg, 0.677 mmol, pure *trans*−*trans*), 150 mg (0.820 mmol) of 2-(2-methoxyethoxy)ethyl bromide, 175 mg of (1.356 mmol) diisopropylethylamine, and 1 mL of acetonitrile were refluxed for 3 h, when TLC showed that no **11** was left. Toluene was added, and the mixture was washed with $KHCO_3$ solution, dried ($Na_2SO_4$), and concentrated. More toluene was added and concentrated to get rid of the diisopropylethylamine. The yield was 230 mg, which was used in the hydrolysis step (method B) to give 151 mg of **14t** as a white powder (57% in two steps). TLC (5% HCOOH in EtOAc) showed one spot of $R_f$ = 0.25. NMR ($CD_3OD$): 2.90−3.90 (m, 13H), 3.81 (s, 3H), 4.49 (d, $J$ = 10 Hz, 1H), 5.94 (s, 2H), 6.79 (d, $J$ = 8 Hz, 1H), 6.89 (dd, $J$ = 8 Hz, 1 Hz, 1H), 7.00 (d, $J$ = 9 Hz, 2H), 7.05 (d, $J$ = 1 Hz), 7.49 (d, $J$ = 9 Hz, 2H).

Other compounds synthesized by this method were **14a**−**d**,**p**−**u**. Either pure **11** or the mixture of **11** and **13** was used. Compound **14c** was synthesized by reacting 1-bromo-2-hexyne[30] and **11** for 1 h at 55 °C. Compound **14d** was synthesized from 4-fluorobenzyl bromide and **11** at room temp for 3 h. Compound **14b** was made from *trans*-1-bromo-5-methylhex-2-ene, the preparation of which is described below.

***trans*-1-Bromo-5-methylhex-2-ene.** Diisopropyl (ethoxycarbonylmethyl) phosphonate (5.0 mL) in 20 mL of THF was reacted with 0.85 g of NaH for 20 min. Isovaleraldehyde (2.0 mL) was added. After 18 h at room temperature the reaction was worked up giving 2.10 g of *trans*-5-methylhex-2-enoic acid, ethyl ester. This was reduced to *trans*-5-methylhex-2-en-1-ol with diisobutyl aluminum hydride, which was brominated with $PBr_3$ in ether to give the title compound.

**Method D. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(2-pyridyl)ethyl]pyrrolidine-3-carboxylic Acid (20c).** Compound **11** (250 mg, 0.677 mmol of pure *trans*−*trans*), 2-vinylpyridine (355 mg, 3.38 mmol), and one drop of acetic acid were dissolved in 1 mL of methoxyethanol and stirred at 100 °C for 2.5 h. Toluene was added and the solution was washed with $KHCO_3$, dried ($Na_2SO_4$), and concentrated. More toluene was added and the mixture concentrated to get rid of the excess 2-vinylpyridine. The residue was dissolved in heptane, filtered from a small amount of insoluble matter, and concentrated to yield 225 mg of the intermediate ester. This was hydrolyzed by method B to give 202 mg of **20c**, mp 77−80 (from water), as the dihydrate. NMR ($CD_3OD$): 2.8−3.3 (m, 6H), 3.55−3.70 (m, 2H), 3.76 (s, 3H), 3.99 (d, $J$ = 10 Hz, 1H), 5.92 (d, $J$ = 1 Hz, 2H), 6.72 (d, $J$ = 8 Hz, 1H), 6.80 (dd, $J$ = 8 Hz, 1 Hz, 1H), 6.85 (d, $J$ = 9 Hz, 2H), 6.92 (d, $J$ = 1 Hz, 1H), 7.20 (d, $J$ = 9 Hz, 2H), 7.20−7.32 (m, 2H), 7.70−7.80 (m, 2H), 8.40 (d, $J$ = 4 Hz, 2H).

**Method E. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(4-fluorophenoxyacetyl)pyrrolidine-3-carboxylic Acid (14f).** To compound **11** (300 mg, 0.813 mmol of pure *trans*−*trans*) and 165 mg of triethylamine in 2 mL of $CH_2Cl_2$ cooled in an ice bath was added 183 mg (0.973 mmol) of (4-flourophenoxy)acetyl chloride (prepared from the acid and oxalyl chloride) in 1 mL of $CH_2Cl_2$. After 2 h at room temperature, the solution was washed with bicarbonate and dried ($Na_2SO_4$) to give 394 mg of the intermediate ethyl ester. This was hydrolyzed by method B to give 222 mg of **14f** (63% yield). NMR (DMSO-$d_6$): rotational isomers, 2.92 and 3.10 (dd, $J$ = 11 Hz, 9 Hz, 0.5H), 3.35−3.58 (m, 2H), 3.72 and 3.76 (s, 3H), 4.05 and 4.22 (dd, $J$ = 10 Hz, 8 Hz, 0.5H), 4.78 and 4.90 (s, 2H), 5.00 and 5.20 (d, $J$ = 9 Hz, 1H), 6.00 (s, 2H), 6.6−7.2 (m, 9H), 7.32 and 7.41 (d, $J$ = 8 Hz, 2H).

Compounds synthesized by a similar manner were **14e**,**j**,**k**,**m**. For **14m**, dibutylcarbamoyl chloride[31] was reacted with **11** at 45 °C for 1 h.

**Method F. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[(phenylamino)carbonyl]pyrrolidine-3-carboxylic Acid (14g).** Phenyl isocyanate (75 mg, 0.630 mmol) in 0.5 mL of THF was added to 200 mg (0.542 mmol) of compound **11** in 0.5 mL of THF. After 1 h at room temperature, toluene was added, and the solvents were concentrated in vacuo to remove the excess phenyl isocyanate. This intermediate ester was hydrolyzed by method B to give 206 mg (83% of **14g**, mp 209−211 °C. NMR (DMSO-$d_6$): 3.03 (dd, $J$ = 11 Hz, 9 Hz, 1H), 3.50−3.62 (m, 1H), 3.65−3.80 (m, 1H), 3.75 (s, 3H), 4.15 (dd, $J$ = 10 Hz, 8 Hz) 5.13 (d, $J$ = 9 Hz, 1H), 6.82−6.92 (m, 5H), 7.07 (s, 1H), 7.12−7.30 (m, 2H), 7.30 (d, $J$ = 8 Hz, 2H), 7.38 (d, $J$ = 8 Hz, 2H), 8.20 (s, 1H).

**Method G. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl) −1-[(dibutylamino)acetyl]pyrrolidine-3-carboxylic Acid (14n).** To 152 mg (0.813 mmol) of *N*,*N*-dibutylglycine[32] in 0.75 mL of THF was added 138 mg (0.852 mmol) 1,1-carbonyldiimidazole. The solution was kept at 50 °C for 30 min. Compound **11** (250 mg, 0.678 mmol) was added and the solution kept at 45 °C for 1 h. The product was chromatographed on silica gel, eluting with 1:1 hexane−EtOAc to give 306 mg (84%) of the intermediate ethyl ester, which was hydrolyzed by method B to yield **14n** in 92% yield, as a white powder. TLC (5% HCOOH in EtOAc) showed one spot of $R_f$ = 0.15. NMR ($CDCl_3$): rotational isomers, 0.75 and 0.85 (t, $J$ = 7 Hz, 3H), 1.05−1.50 (m, 8H), 2.65−3.20 (m, 6H), 3.43−3.70 (m, 3H), 3.72 (s, 3H), 3.87 (d, $J$ = 15 Hz, 1H), 4.49 (dd, $J$ = 12 Hz, 6 Hz) and 5.23 (dd, $J$ = 12 Hz, 8 Hz, 2H), 5.90 (dd, $J$ = 2 Hz, 4 Hz, 2H), 6.63−6.78 (m, 3H), 6.86 and 7.04 (d, $J$ = 9 Hz, 2H), 7.22 (d, $J$ = 9 Hz, 2H).

**Method H. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodiolol-5-yl)-1-[2-(ethylsulfinyl)ethyl]pyrrolidine-3-carboxylic Acid (20a).** To the 64:36 mixture of **11** and **13** (100 mg, 0.27 mmol) in 6 mL acetonitrile were added 67.5 mg (0.50 mmol) of 2-chloroethyl ethyl sulfide, 375 mg of diisoproplyethyamine, and 10 mg of KI. The mixture was refluxed for 4 h. The solvents were removed in vacuum and the residue chromatographed on silica gel eluting with 4:1 hexane−EtOAc to give 93 mg of the intermediate 1-(ethylthio)ethyl compound. To this compound dissolved in 5 mL of $CH_2Cl_2$ was added 68 mg of 3-chloroperoxybenzoic acid. The mixture was stirred for 40 min in an ice bath and then for 3 h at room temperature. After washing with $NaHCO_3$ and drying, the solution was concentrated and the residue chromatographed on silica gel eluting with EtOAc to give 62 mg (65%) of the sulfoxide, ethyl ester. This was hydrolyzed by method B to give **20a** as a mixture of diastereoisomers. NMR ($CDCl_3$): 1.25 and 1.32 (t, $J$ = 7 Hz, 3H), 2.45−2.75 (m, 4H), 2.84−2.96 (m, 3H), 3.02−3.08 (m, 1H), 3.32 and 3.36 (d, $J$ = 3 Hz, 1H), 3.47−3.58 (m, 2H), 3.65 and 3.68 (d, $J$ = 8 Hz, 1H), 3.76 and 3.80 (s, 3H), 5.94 (s, 2H), 6.72 (d, $J$ = 8 Hz, 1H), 3.84−3.89 (m, 3H), 7.02 (d, $J$ = 6 Hz, 1H), 7.30 and 7.34 (d, $J$ = 8 Hz, 2H).

***trans,trans*-Ethyl 2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2-bromoethyl)pyrrolidine-3-carboxylate (20, R = Et, X = Br).** Compound **11** (2.00 g, 5.42 mmol), 2 mL of diisopropylethylamine, and 200 mg of NaI were stirred in 18 mL of 1,2-dibromoethane at 100 °C for l h. Toluene was added, and the mixture was washed with bicarbonate. The solvents were concentrated, and the black residue was chromatographed on silica gel, eluting with 4:1 hexane−EtOAc to yield 2.22 g (87%) of product. NMR ($CDCl_3$): 1.11 (t, $J$ = 7 Hz, 3H), 2.48−2.56 (m, 1H), 2.80−3.00 (m, 3H), 3.31−3.42 (m, 3H), 3.48−3.55 (m, 1H), 3.70 (d, $J$ = 9 Hz, 1H), 3.70 (s, 3H), 4.05 (q, $J$ = 7, 2H), 5.95 (s, 2H), 6.72 (d, $J$ = 7 Hz, 1H), 6.82 (d, $J$

= 2 Hz, 1H), 6.87 (d, *J* = 9 Hz, 2H), 7.08 (d, *J* = 2 Hz, 1H), 7.33 (d, *J* = 9 Hz, 2H).

**Method J. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(2-propylsulfonyl)ethyl]pyrrolidine-3-carboxylic Acid (20b).** To 1-propanethiol (3.5 g, 46.05 mmol) in 10 mL of THF was added 632 mg (26.32 mmol) of NaH in portions under a $N_2$ atmosphere. The mixture was heated at 65 °C for 1 h. To this mixture was added 180 mg (0.38 mmol) of the *N*-(2-bromoethyl)pyrrolidine (**20**, R = Et, X = Br). This mixture was heated at 65 °C for 2 h. The solvents were removed in vacuum, and the residue was chromatographed on silica gel eluting with 3:2 hexane−EtOAc to give 175 mg (95%) of the intermediate 1-(propylthio)ethyl compound. This was dissolved in 20 mL of acetone containing 93 mg (0.8 mmol) of *N*-methylmorpholine *N*-oxide, 5 mL of water, and 10 mg of osmium tetroxide in 0.3 mL of *tert*-butyl alcoholl.[33] This mixture was stirred overnight at room temperature and then concentrated in vacuum. The residue was dissolved in EtOAc, washed with NaCl, and dried ($Na_2SO_4$). Flash chromatography afforded 177 mg (98%) of the ethyl ester sulfone, which was hydrolyzed using method B to give **20b**, mp 73−75 °C. NMR ($CDCl_3$): 1.04 (t, *J* = 7 Hz, 3H), 1.78 (m, 2H), 2.59−2.66 (m, 1H), 2.84−3.08 (m, 7H), 3.43 (dd, *J* = 9 Hz, 3 Hz, 1H), 3.53−3.60 (m, 1H), 3.68 (d, *J* = 9 Hz, 1H), 3.82 (s, 3H), 5.96 (s, 2H), 6.75 (d, *J* = 8 Hz, 1H), 6.82 (dd, *J* = 8 Hz, 3 Hz, 1H), 6.88 (d, *J* = 9 Hz, 2H), 6.99 (d, *J* = 3 Hz, 1H), 7.32 (d, *J* = 9 Hz, 2H).

**Method K. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-(dibutylamino)ethyl]pyrrolidine-3-carboxylic Acid (20d).** The *N*-(2-bromoethyl)pyrrolidine (150 mg, 0.316 mmol of **20**, R = Et, X = Br), 150 mg (1.16 mmol) of dibutylamine, and 18 mg of NaI in 0.75 mL of ethanol were heated at 80 °C for one h. Toluene was added, and the mixture was washed with bicarbonate, dried ($Na_2SO_4$), and concentrated. More toluene was added and evaporated to get rid of the dibutylamine. The residue was dissolved in heptane and filtered from a small amount of insoluble material. Evaporation of the heptane gave 143 mg (87%) of the intermediate ethyl ester which was hydrolyzed to **20d** in 90% yield by method B. TLC (EtOAc−$H_2O$−HCOOH 9:1:1) showed one spot with $R_f$ = 0.5. NMR ($CD_3OD$, 300 MHz): 0.89 (t, *J* = 7 Hz, 6H), 1.16−1.30 (m, 4H), 1.44−1.56 (m, 4H), 2.48−2.57 (m, 1H), 2.80−3.08 (m, 8H), 3.14−3.25 (m, 1H), 3.31−3.38 (m, 1H), 3.59−3.60 (m, 1H), 3.74 (s, 3H), 3.75 (d, *J* = 10 Hz, 1H), 5.89 (s, 2H), 6.71 (d, *J* = 9 Hz, 1H), 6.81 (dd, *J* = 9 Hz, 2 Hz, 1H), 6.90 (d, *J* = 10 Hz, 2H), 6.96 (d, *J* = 2 Hz, 1H), 7.37 (d, *J* = 10 Hz, 2H).

**Method L. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[(4-heptylcarbonyl)methyl]pyrrolidine-3-carboxylic Acid (19b).** 1-Chloro-3-propyl-2-hexanone (**15**, R = 4-heptyl) was prepared by forming the acid chloride from 2-propylpentanoic acid [$(COCl)_2$], treating this with excess diazomethane in ether for 18 h, and treating the resulting diazoketone with HCl in dioxane. Compound **11** (295 mg, 0.80 mmol) was reacted with 0.100 mmol of 1-chloro-3-propyl-2-hexanone, 4.00 mmol of diisopropylethylamine, and 36 mg of NaI in 4 mL of acetonitrile for 47 °C for 18 h. Chromatography (9:1 hexane−EtOAc) gave 237 mg (46%) of the intermediate ethyl ester, which was hydrolyzed to **19b** by method B. NMR ($CDCl_3$): 0.73−0.97 (m, 6H), 1.03−1.33 (m, 6H), 1.36−1.58 (m, 2H), 2.46 (m, 1H), 2.80−2.98 (m, 3H), 3.38−3.64 (m, 3H), 3.75−3.90 (m, 1H), 3.79 (s, 3H), 5.94 (s, 2H), 6.75 (d, 1H), 6.86 (d, 2H), 6.92 (d, 1H), 7.12 (s, 1H), 7.32 (d, 2H).

**Method M. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2-[(dipropylamino)carbonyl]ethyl]-pyrrolidine-3-carboxylic Acid (20e).** Compound **11** (250 mg, 0.677 mmol), 205 mg (1.36 mmol) of diallylacrylamide (Polysciences, Inc.), and 10 mg of acetic acid were heated at 85 °C in 0.75 mL of methoxyethanol for 1 h. Toluene was added and the solution was washed with bicarbonate, dried, and concentrated. Chromatography (3:1 hexane−ethyl acetate) gave 283 mg (80%) of the diallyl compound. This was hydrogenated (10% Pd/C) in 100% yield to the dipropylamide ethyl ester, which was hydrolyzed to **20e** in 83% yield by method B. NMR ($CDCl_3$, 300 MHz): 0.82 and 0.83 (t, *J* = 7 Hz, 6H), 1.39−1.54 (m, 4H), 2.35−2.60 (m, 3H), 2.80−3.07 (m, 5H), 3.14−3.21 (m, 2H), 3.31−3.38 (m, 1H), 3.51−3.61 (m, 1H), 3.73 (d, *J* = 12H, 1H), 3.75 (s, 3H), 5.94 (s, 2H), 6.71 (d, *J* = 9 Hz, 1H), 6.79−6.85 (m, 3H), 7.04 (d, *J* = 2 Hz, 1H), 7.32 (d, *J* = 9 Hz, 2H).

**Method N. *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutylamino)carbonyl]methyl]-pyrrolidine-3-carboxamide (17z).** Carbonyldiimidazole (150 mg, 0.926 mmol) was added to 300 mg (0.588 mmol) of **17u** in 0.8 mL of THF, and the mixture was heated for 40 min at 50 °C. A 25% solution of ammonia in methanol was then added to this solution with ice cooling. A solid formed immediately. After 30 min, the solid was filtered and washed with ethanol and finally with ether: yield, 250 mg (83%); mp 194−196 °C. NMR (DMSO-$d_6$): 0.75 (t, *J* = 7 Hz, 3H), 0.82 (t, *J* = 7 Hz, 3H), 1.00 (m, 2H), 1.10−1.40 (m, 6H), 2.65 (d, *J* = 13 Hz, 1H), 2.74 (dd, *J* = 7 Hz, 7 Hz), 2.85−3.04 (m, 2H), 3.12−3.32 (m, 4H), 3.26 (d, *J* = 13 Hz, 1H), 3.32 (s, 3H), 3.36−3.47 (m, 1H), 3.61 (d, *J* = 8, 1H), 5.99 (dd, *J* = 2 Hz, 1 Hz), 6.68−6.85 (m, 3H), 6.91 (d, *J* = 8 Hz, 2H), 7.02 (s, 2H), 7.23 (d, *J* = 8 Hz, 2H).

***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic Acid (21).** Compound **11** (2.08 g, 5.63 mmol) in 7 mL of $CH_2Cl_2$ was treated with 1.43 g (6.19 mmol) of di-*tert*-butyl dicarbonate for 1 h at room temperature. Chromatography on silica gel (3:1 hexane−EtOAc) gave 2.35 g (91%) of the ethyl ester, which was hydrolyzed to 2.11 g (95%) of **21** (mp 199−200 °C, dec). NMR ($CDCl_3$): rotational isomers, 1.15 and 1.40 (br s, 9H), 3.12 (m, 1H), 3.50−3.62 (m, 2H), 4.16−4.28 (br m, 1H), 4.90−5.05 (br m, 1H), 5.94 (s, 2H), 6.70−6.78 (m 3H), 6.86 (d, *J* = 9 Hz, 2H), 7.18 (d, *J* = 9H, 2H).

**Imides 22 and 23.** To 157 mg (1.28 mmol) of compound **21** in 10 mL of THF at −78 °C were added successively 0.232 mL (1.67 mmol) of $Et_3N$ and 0.175 mL of freshly distilled (1.41 mmol) pivaloyl chloride. The mixture was stirred at −78 °C for 15 min, 0 °C for 40 min, and then recooled to −78 °C. In a separate flask a solution of 1.44 mL (3.6 mmol) of 2.5 M *n*-butyllithium in hexane was added to 683 mg (3.85 mmol) of (*S*)-4-benzyl-2-oxazolidinone in 7 mL of THF at −78 °C and stirred for 15 min. This solution was transferred via cannula to the first solution. The reaction was kept at room temperature overnight. A sodium bisulfate solution was added, and the solvents were evaporated. The residue was partitioned between $CH_2Cl_2$ and water. The organic phase was dried ($MgSO_4$) and concentrated. Chromatography on silica gel (3:1 hexane−EtOAc) gave 89 mg of the faster moving **22** and 103 mg of slower moving **23**, which crystallized. The structure of **23** was determined by X-ray analysis.

**Imide 24.** To 189 mg (0.148 mmol) of *N*-Boc compound **22** in 0.2 mL of dioxane was added 1 mL of 4 N HCl in dioxane. After 1 h the solvents were removed in vacuum, and the residue was dissolved in acetonitrile. This was reacted with 0.78 mL of diisopropylethylamine and 44 mg of *N*,*N*-dibutylbromoacetamide. Chromatography (45% EtOAc in hexane) gave an 85% yield of **24**.

**Method O. (2*R*,3*R*,4*S*)-*trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutylamino)carbonyl]-methyl]pyrrolidine-3-carboxylic Acid (17v).** To a stirred solution of 64 mg (0.095 mmol) of imide **24** in 0.75 mL of THF was added successively 0.25 mL of water, 8 mg (0.19 mmol) of LiOH (after cooling to 0 °C), and one drop of 30% $H_2O_2$. After stirring for 30 min, 1.5 M $Na_2SO_3$ was added. The solvents were evaporated, and the residue was dissolved in 4 mL of 0.5 M NaOH. The solution was extracted with ether and the aqueous phase acidified with acetic acid giving 38 mg (78%) of **17v**.

**Resolved Acid 21.** Racemic **21** (2.858 g) in 10 mL of ethyl acetate was treated with 0.7833 g of (*R*)-(+)-α-methylbenzylamine. The ethyl acetate was evaporated and the residue was dissolved in ether. Ice cooling and scratching with a spatula gave crystals which were filtered. A second and third crop was obtained. The isomer ratio was analyzed by HPLC on a Regis (S,S) Whelk-01 column (25 cm × 4.6 mm), with 30% ethanol−70% (0.3% acetic acid in hexane) as solvent with a flow rate of 1.0 mL/min, using a Rainin HPXL solvent delivery system and a Dynamax model UV-1 absorbance detector. The

α-methylbenzylamine was removed before HPLC by extraction of a chloroform solution with 1 N phosphoric acid. The isomer with the same absolute configuration as **17v** elutes at 6.86 min and the isomer leading to **17w** elutes at 10.5 min. The three crops were combined, dissolved in THF, concentrated, and crystallized from ether. One more crystallization gave a yield of 1.315 g (77%) of 100% pure salt.

**Resolved Amino Ester 11.** The resolved salt described above (1.31 g) was dissolved in 100 mL of ethanol saturated with HCl gas and stirred for 19 h at 54 °C. The solvents were removed in vacuum, and the residue was dissolved in toluene and basified with $KHCO_3$ solution. The toluene was removed in vacuum and the residue was chromatographed on silica gel eluting with 75% EtOAc−25% hexane to yield 0.785 g of **11** (92%).

**Receptor Binding Assays.** 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 permanently transfected with human $ET_A$ or $ET_B$ receptors were homogenized in 25 mL of 10 mM Hepes (pH 7.4) containing 0.25 M sucrose and a protease inhibitor cocktail (3 mM EDTA, 0.1 mM PMSF, and 5 μg/mL pepstatin A) 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 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 3 mM EDTA) to a final concentration of 0.2 mg/mL of protein. The $IC_{50}$ was determined by competition studies. Briefly, membranes (0.02 mg) were incubated with 0.1 nM of [$^{125}$I]ET-1 (for $ET_A$ assay in MMQ) or [$^{125}$I]ET-3 (for $ET_B$ assay in porcine cerebellum) in buffer B (final volume, 0.2 mL) in the presence of increasing concentrations of the test compound for 4 h at 25 °C. $K_i$ values for A-127722 and related compounds were determined in saturation binding studies. Membranes were incubated with various concentrations of [125I]ET-1 in the absence or presence of A-127722 (0.05, 0.1, and 0.2 nM), the *R*,*R*,*S* isomer (0.025, 0.05, and 0.1 nM) or the *S*,*S*,*R* isomer (5, 20, and 40 nM) for 4 h at 25 °C. After incubation, unbound ligands were separated from bound ligands by a vacuum filtration method using glass-fiber filter strips in PHD cell harvesters (Cambridge Technology, Inc., MA), where the filter strips were washed three times with saline (1 mL). Nonspecific binding was determined in the presence of 1 μM ET-1. $IC_{50}$ values are calculated using an average of at least two separate determinations.

**Phosphoinositol Hydrolysis Assay.** 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 of as described by Berridge.[34] 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). $IC_{50}$ values are calculated using an average of at least two separate determinations.

**p$A_2$ Determination in Rat Aorta.** Male Sprague-Dawley rats (350−500 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip). The thoracic aorta was quickly removed and placed in Krebs−Henseleit buffer gassed with 95/5 $O_2/CO_2$ to maintain the pH at 7.4. The aorta was cleared of extraneous tissue and segmented into 4−5 mm wide rings which were then suspended in 2 mL jacketed tissue baths maintained at 37 °C. The tissue baths had been siliconized to prevent adsorption of the peptides to the glass. Vessels were attached via a gold chain to an isometric force transducer linked with a physiograph for monitoring tension changes. Baseline tension was set at 2.0 g and the tissues were allowed to equilibrate for 2.5 h. During this period, the tissues were washed every 5 min with fresh Krebs−Henseleit buffer, and the tension was continually adjusted to baseline. Thirty minutes into the equilibration period, tissues were maximally constricted with norepinephrine (NE, 1 μM) followed by a challenge with acetylcholine (ACh, 3 μM). A positive relaxant response to ACh confirmed the presence of vessel endothelium. NE and ACh were then completely washed out and the tissues were allowed to return to baseline. Baseline tension was readjusted if necessary prior to any subsequent manipulations.

**Constrictor Dose−Response Curves (DRCs).** ET-1 DRCs, 1E-11 to 1E-6 M, were performed in rat aorta to establish agonist-receptor potencies in those tissues. Upon completion of the DRCs, maximal constrictor efficacy was compared with constriction by $K^+$ depolarization (55 mM KCl).

**Antagonist Effects on Constrictor Responses.** Antagonists tested were equilibrated 15 min prior to the onset of the ET-1 DRCs. Concentrations of antagonist across experimental sets were in half-log increasing doses with a total of five different concentrations examined for each compound tested. ET-1 vehicle (control) curves were performed along side antagonist-treated curves.

**Drug Potency Determination and Analysis.** Tissues in each aorta subset were from the same animal and were treated with none or one of the concentrations in the antagonist test range—the data in aortic groups was thus paired for analysis. Agonist-induced tensions from control and antagonist-treated curves were calculated and normalized against maximal contraction in those curves and the effective concentration of agonist causing 50% maximum constriction ($EC_{50}$) was calculated (Allfit). Schild analysis of the antagonist-induced $EC_{50}$ shifts was performed yielding a −p$A_2$ value as the comparative index of antagonism.

**Pharmacokinetics in Rats.** The pharmacokinetic behavior of A-127722 was evaluated in male Sprague-Dawley rats. A-127722 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 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[35] were obtained with the program CSTRIP.[36] Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant ($\beta$) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an $AUC_{0-\infty}$ value. The plasma clearance ($CL_p$) 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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