Azole Endothelin Antagonists. 3. Using Δ log *P* as a Tool To Improve Absorption

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Received August 8, 1995[⊗]

The oral absorption profile of a family of azole-based ET_A-selective antagonists has been improved through a rational series of structural modifications which were suggested by analysis of the physicochemical parameter Δ log P. Comparison of urea **2** with a series of well-absorbed compounds using Δ log *P* analysis suggested that **2** has an excess capacity for forming hydrogen bonds with solvent. A series of urea modifications were explored as a means of reducing H-bonding capacity while maintaining affinity for the ET_A-receptor. The correlation between Δ log *P* values and absorption in an intraduodenal (id) bioavailability model was good; this strategy uncovered replacements for *each* of the urea NH groups which simultaneously improve both potency and drug absorption. A combination of these optimized modifications produces carbamate **16h**, a highly-selective ET_A antagonist with a potency/bioavailability profile consistent with an oral route of administration.

Introduction

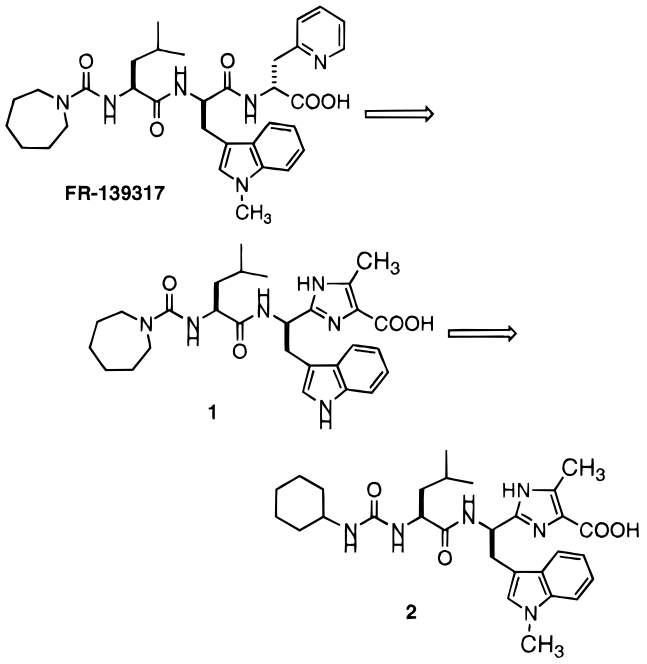
In the previous articles in this series,[1,2] we have described a strategy for modifying the backbone of the potent and selective endothelin A-receptor (ET_A) antagonist FR-139317. It was our hope that such a strategy would lead to the development of an agent with a biochemical profile similar to that of the pseudotetrapeptide, but with substantially improved *in vivo* stability and oral pharmacokinetics. In fact, beginning with **1** as a lead, structure−activity studies led quickly to compound **2**, which retains the desired biochemical properties of FR-139317 (Scheme 1). At this point we considered it appropriate to evaluate our original hypothesis that backbone modification might lead to improved oral absorption.

We compared the absorption of **2** with that of FR-139317 upon intraduodenal (id) injection into rats (this model is discussed in detail in the Experimental Section). The results (Figure 1) clearly indicate that our hypothesis was correct; the absorption profile of **2** is dramatically superior to that of the Fujisawa compound. At the same time, however, it is equally clear that portal blood levels achieved with **2** fall well short of what might be desirable for an orally-deliverable agent. We thus felt it necessary to embark on a structure−absorption study, employing compound **2** as an initial lead structure.

Physicochemical Correlations with Absorption

In order to guide and to facilitate our search for improvements in gut permeability within this family of compounds, we hoped to identify a physicochemical marker which could readily be determined for a large series of compounds, and which might be used to predict the absorption profile of a given class of analogs. There is an extensive literature attempting such correlations.[3] As a rule the parameter that has been employed in such studies is log *P*, the octanol/water partitioning coefficient, which is generally considered to be a measure of hydrophobicity. However, attempts to correlate log

Scheme 1. Evolution of Azole ET Antagonist Series



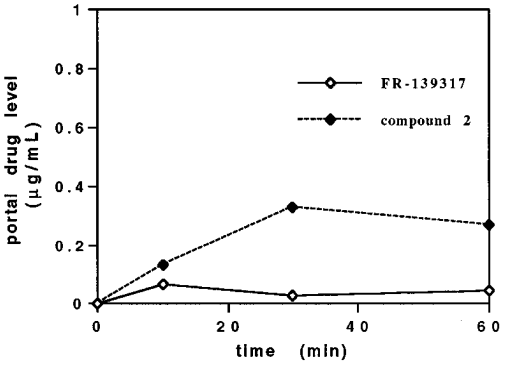


Figure 1. Drug levels upon id administration of azole **2** are substantially improved over FR-139317, but further improvements are still necessary.

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⊗ Abstract published in *Advance ACS Abstracts,* January 15, 1996.

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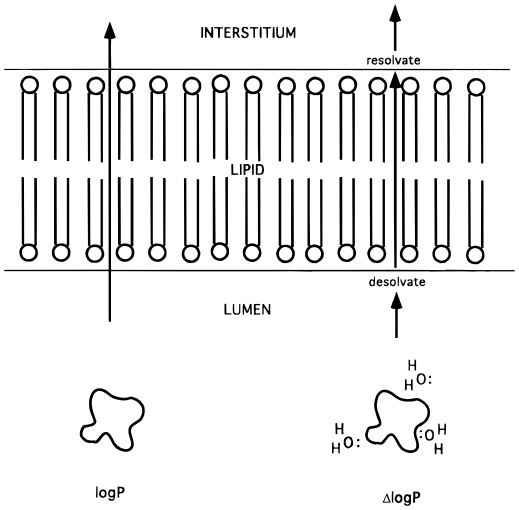


Figure 2. The world according to log *P* and Δ log *P*.

	Theophylline	A-81988 (AII antagonist)	Ro-46,2005 (Roche ET antagonist)	Compound **2**
logD (pH 6.5)	- 0.12	- 0.85	1.98	1.83
ΔlogP	1.53	2.45	2.37	5.92

Figure 3. Physicochemical comparison of **2** with benchmark compounds.

P with absorption parameters have met with mixed success; it appears that, while log *P* values may correlate with absorption rates within a given structural class, such correlations often fall apart when structures change significantly, and as a rule have more descriptive than predictive power.

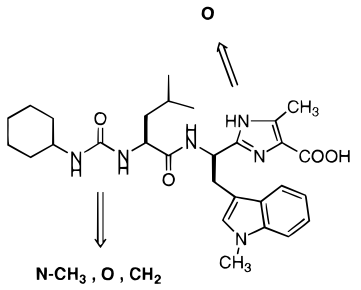
The failure of log *P* based absorption correlations may reflect the fact that this parameter provides an inaccurate view of compounds in the biologic milieu (see Figure 2). In an aqueous environment the effective size and polarity of a molecule depends not only upon its intrinsic character, but also upon interactions with its surroundings, for example through hydrogen-bonding. These latter interactions may be particularly relevant in describing a situation like intestinal transport, in which desolvation is presumably a prerequisite to crossing the membranes. For this reason we felt that it might be more relevant to examine an alternative physicochemical parameter, Δ log P. The Δ log *P* value,[4] described by the formula

$$\Delta \log P = \log P(\text{octanol/water}) - \log P(\text{cyclohexane/water})$$

still serves as a measure of hydrophobicity. However, by comparing the ability of a compound to partition into two organic solvents, one of which does and one of which does not have the ability to form hydrogen bonds, Δ log *P* analysis tends to emphasize the H-bonding capacity of molecules. Since its initial description by Seiler in 1974,[4] Δ log *P* has been employed by Burton and his co-workers[3,5] to correlate absorption with substitution in a family of tripeptides and has also been found to be useful as an indicator of blood−brain barrier penetration.[6] To the best of our knowledge, its power as a predictor of oral absorption has never been investigated.

We began our physicochemical analysis by comparing imidazole **2** with a number of compounds known to have excellent pharmacokinetic profiles (Figure 3). Theophylline is the prototype of a well-absorbed drug;[7] similarly the Abbott AII antagonist A81988[8] and Roche's first-generation ET antagonist Ro46,2005[9] have both been demonstrated to be highly orally bioavailable. While the octanol−water partition coefficients for this structurally diverse set of compounds span almost 3 orders of magnitude, the corresponding Δ log *P* measurements fall into a tighter range; more importantly, it appears that regardless of structure these well-absorbed species are all characterized by having Δ log $P < 3$. This result is consistent with previous reports.[3]

Scheme 2. Removing H-Bonding Character



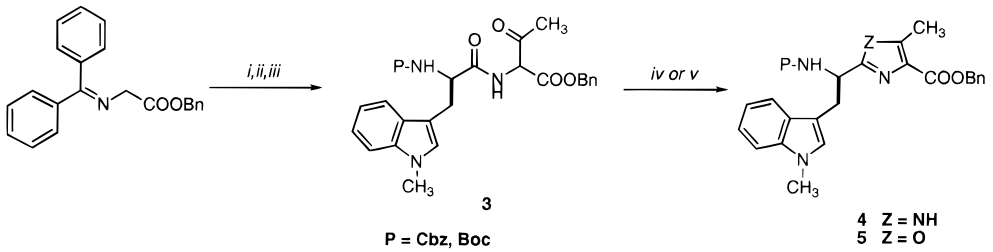
Under similar experimental conditions the Δ log *P* of compound **2** is 5.92. As Δ log *P* values increase, the amount of material present in the organic phase of the cyclohexane/buffer partition becomes vanishingly small; in practice a value of ∼6 represents the limit of our ability to detect material using an HPLC-based assay. In any event the message of this analysis is clear; it appears that **2** has a greater ability to form hydrogen bonds than would be expected for a well-absorbed compound.

On the basis of the synthetic strategy we have developed for this class of compounds, two approaches to decreasing H-bonding capacity (and thereby presumably decreasing Δ log *P* and improving absorption) seemed particularly straightforward to examine (Scheme 2). First, we might expect that switching from an imidazole nucleus to an oxazole would decrease Δ log *P* values. Unfortunately, our previous SAR studies suggest that such a shift will also result in a significant decrease in ET_A receptor binding. Alternatively we should be able to modify the urea moiety to substantially decrease its H-bonding potential. To probe the effects of these various modifications, we have synthesized a series of analogs in which each urea NH is replaced by three nondonating alternatives, NCH_3, O, and CH_2. Additionally this set of seven modified ureas (including the parent) has been prepared using both imidazole and oxazole cores.

Synthesis

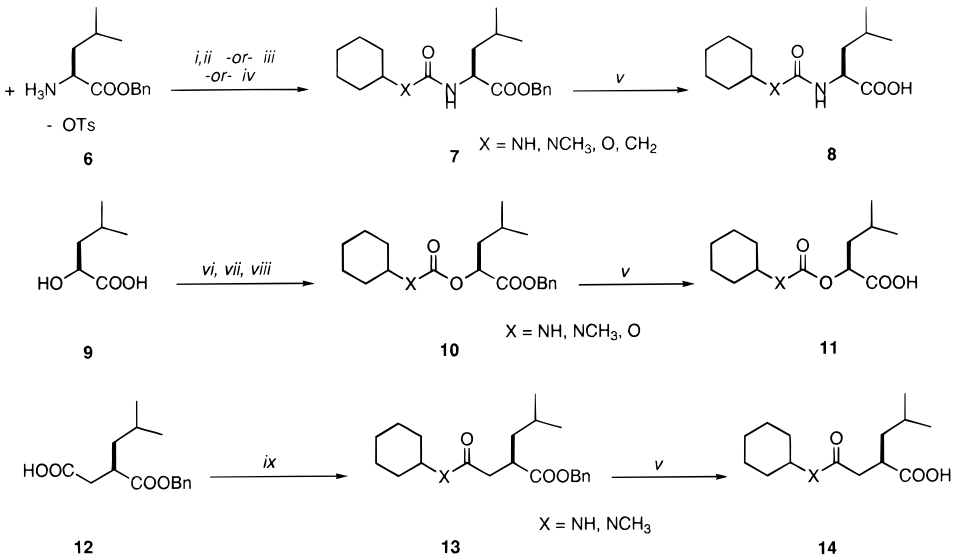
The compounds described here are prepared in a manner analogous to that reported in our previous articles.[1,2] In particular the preparation of the heterocyclic dipeptide surrogates (Scheme 3) remains unchanged; compounds **15** and **16** differ only through modification of the leucylurea and in the choice of an azole core. The synthesis of the requisite modified ureas is described in Scheme 4. The adduct of leucine benzyl ester with 1,1′-carbonyldiimidazole (CDI) can be condensed with amines to produce substituted ureas like **8** after hydrogenolysis; alternatively this same starting material may be condensed with a chloroformate (to produce carbamates) or with an acid (to give amides). Similar reaction of benzyl L-leucate with CDI gives an intermediate which, after further activation with methyl triflate, reacts rapidly with amines to provide "inverse"

Scheme 3.[a] Core Synthesis



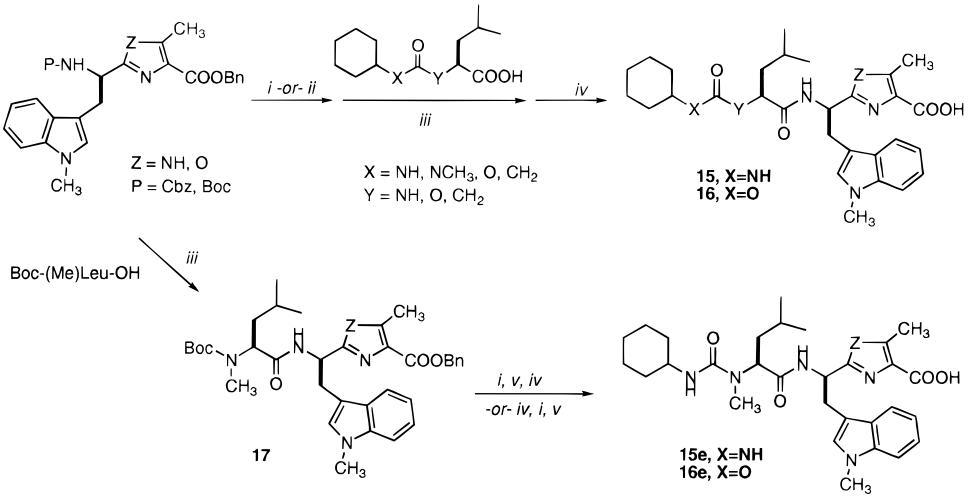
[a] (i) LiHMDS, THF, −78 °C; CH_3COCl; (ii) HCl/H_2O; (iii) P-D-Trp(Me)-OCOOiBu, THF, −20 °C; NMM (dropwise); (iv) NH_4OAc, HOAc, reflux; (v) PPh_3, CCl_4, pyr, CH_3CN.

Scheme 4.[a] Synthesis of N-Terminal Urea Replacements



[a] (i) CDI, Et_3N, THF; (ii) c-$HexNH_2$ or c-$HexNHCH_3$; (iii) c-HexOCOCl, Et_3N, THF; (iv) c-$HexCH_2COOH$, EDC, HOBt, NMM, THF/DMF; (v) H_2/10% Pd/C, EtOH; (vi) BnBr, Cs_2CO_3, DMF; (vii) CDI, THF; (viii) (a) MeOTf, THF, 0 °C, (b) c-$HexNH_2$ or c-$HexNHCH_3$ or c-HexOH; (x) c-$HexNH_2$ or c-$HexNHCH_3$, EDC, HOBt, NMM, THF/DMF.

Scheme 5.[a] Analog Assembly



[a] (i) TFA; (ii) 30% HBr/HOAc; (iii) EDC, HOBt, NMM, THF/DMF; (iv) H_2, 10% Pd/C, EtOH; (v) c-HexNCO, Et_3N, THF/DMF.

carbamates **10**. Finally, "inverse amide" derivatives **14** are readily assembled from acid **12**.[10]

In general the final products can be assembled through standard coupling protocols, followed by ester hydrogenolysis (Scheme 5). In the case of analogs containing an N-methylated leucine residue, activation of the corresponding *N*-methylurea leads to rapid hydantoin formation, so that no coupling occurs. This difficulty may be circumvented by first coupling core azole **4** or **5** with a protected *N*-methylleucine derivative (see Scheme 5) and (after deprotection) forming the urea as the penultimate step of the synthesis.

Physicochemical Analysis

We have recorded Δ log *P* values for analogs **15** and **16** using a modification of the reported procedures. Samples of test compounds are taken up in 0.05 M PIPES buffer (pH 6.5), and their ability to partition into an organic solvent is evaluated through HPLC analysis of both phases after 2 h of thorough agitation. Octanol

is chosen as the typical organic solvent which is capable of forming hydrogen bonds with a solute molecule, while cyclohexane provides an organic medium without such H-bonding capacity. After determination of the individual log *D* values, Δ log *P* is calculated using the formula described above. For the purpose of these calculations we may use log *D* and log *P* values interchangeably; for acidic compounds these are related by the formula

$$\log P = \log D + \log(1 + 10^{\mathrm{pH}-\mathrm{p}K_a})$$

and the correction term, which is constant for a given compound and pH, cancels out.

Pharmacokinetic Studies

We have evaluated the intestinal absorption of compounds **15a**−**g** and **16a**−**i** using the id rat model developed previously to evaluate a series of renin inhibitors.[11] Briefly, blood samples are collected from the portal vein of an anesthetized rat at several time points after the test compound is injected into its duodenum. Plasma drug levels are quantitated using an HPLC-based assay after the samples have undergone organic extraction. The individual data points may be used to create a time course for drug absorption (as in Figure 1); alternatively they may be used to estimate the total drug absorption ("id AUC") over the 1-h period of the experiment. These portal drug levels provide an indication of the ability of the analogs to cross from the gut into the circulation.

A more detailed analysis of pharmacokinetic behavior of **16h** is accomplished through an oral dosing protocol in rats. The plasma concentration profile of a test compound administered at a dose of 10 mg/kg by gavage is compared with an intravenous dose of the same analog. Systemic blood samples, sequentially collected from a tail vein of each animal over a 12-h period, are analyzed by HPLC for drug levels after an extraction. These plasma concentration values permit the estimation of maximum blood levels (C_{max} and T_{max}), plasma half-lives, and oral bioavailability (*F*).

Results and Discussion

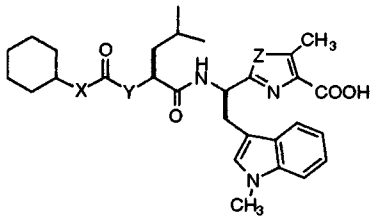
Table 1 lists the Δ log *P* values measured for analogs **15a**−**g** and **16a**−**i**. Several important trends are apparent from these data. The "parent" imidazole **15a** and oxazole **16a** both have Δ log *P* values near 6, suggesting not only that the presence of the intact urea is problematic, but that simply changing the nature of the heterocycle is not enough to bring Δ log *P* into the desired range (nearer to a value of 3). Conversely, the presence of an imidazole as the heterocyclic core seems to present an insurmountable problem; imidazoles **15a**−**g** all have high Δ log *P* values regardless of the modifications made to the urea group. However, in the series of analogs **16a**−**g** which have oxazole cores, it is clear that changes in the urea which reduce H-bonding character simultaneously produce lower Δ log *P* values. While these values are still outside of the range that we consider to be optimal, we might expect, if our predicted correlation holds, to see improvements in the absorption profile for these compounds.

The results of blood level analysis of the same series of analogs is summarized in the table. Once again, several trends emerge. The family of imidazoles **15b**−**g** are characterized by poor to modest absorption, with portal AUCs ranging from 4.9 to 20.8 μg min/mL. It is clear, nonetheless, that the modification of the urea moiety has led to improved intestinal uptake in this series, as all of these values are significantly better than that recorded for the parent imidazole **15a** (0.26 μg min/mL). Replacement of the imidazole with oxazole also boosts absorption (viz. **15a** vs **16a**), a trend that persists throughout the series of urea modifications. In fact, oxazoles **16b**−**g** represent dramatic improvements over **15a**, suggesting that it is this oxazole series that is likely to provide a compound for further development. These results are all consistent with our expectations on the basis of analysis of Δ log *P* values in the series. Specifically, when Δ log *P* analysis predicts a large excess of H-bonding capacity, the compounds are poorly absorbed; as Δ log *P* values decrease, id absorption improves. This relationship is depicted graphically in Figure 4. It thus appears that, at least for the case at hand, this new physicochemical parameter provides a useful qualitative assessment of gut transport.

An additional factor which is important to evaluate in "optimizing" a urea replacement is the effect of NH modification on the receptor affinity of the resultant analogs. Binding data for compounds **15** and **16** are included in Table 1. As anticipated from our previous studies, compounds containing an imidazole core are generally more potent than the corresponding oxazoles. Pairwise comparison suggests that the imidazole:oxazole potency ratio varies from 0.5 (**15c** vs **16c**) to 20 (**15a** vs **16a** or **15e** vs **16e**), with a ratio of 5 being typical. This result is somewhat unfortunate, since our earlier results in the id absorption model suggest that oxazoles will be preferred as the heterocyclic nucleus. On the positive side, our urea substitution studies have identified replacements that not only are tolerated by the receptor but actually lead to improvements in ET_A binding affinity. For example, while replacement of the first NH of the urea with a methylene group results in a loss of potency, N-methylation leads to a substantial improvement in ET_A activity (compare **15b** vs **15a** or **16b** vs **16a**). Substitution with oxygen at this position is beneficial for the oxazole series, but detrimental for the imidazoles. In a similar fashion we observe that an oxygen atom is a beneficial replacement for the second urea NH; on the other hand, NCH_3 or CH_2 substitution appears to produce a slight decrease in binding affinity. Fortuitously, the effects of these beneficial modifications appear to be enhanced in the oxazole series, with **16b** (IC_{50} = 25 nM) and **16f** (IC_{50} = 21 nM) each better than the parent urea by roughly a factor of 10.

Through this multifactorial analysis we have been able to identify a replacement for *each* of the potential H-bond donors of the urea (NCH_3 for the first, O for the second) that is able simultaneously to improve the efficiency of intestinal transport and increase affinity for the ET_A receptor. To further optimize these critical parameters, we next prepared carbamate **16h** which contains both of the beneficial substitutions. While the effects of the individual changes are not completely additive, compound **16h** is nonetheless more potent (IC_{50} = 4.6 nM) and better absorbed (id AUC = 110 μg min/mL) than any of the second-generation oxazoles. More importantly, the pharmacokinetic profile of **16h** upon oral delivery (Figure 5) suggests that the compound could be successfully administered by this route.

Table 1. Urea Replacements



compound	X	Y	Z	ET_A binding IC_{50} (μM)[a]	ET_B binding IC_{50} (μM)[a]	ΔlogP	i.d. AUC (μg-min/mL)	formula	solvate	characterization
15a (=2)	NH	NH	NH	0.011	34	5.92	0.26	$C_{29}H_{40}N_6O_4$	1.7 TFA	NMR,MS,CHN
15b	NCH_3	NH	NH	0.0018	20†	5.40	6.2	$C_{30}H_{42}N_6O_4$	2.0 H_2O	NMR,MS,CHN
15c	O	NH	NH	0.076	>100	> 6.2	10.1	$C_{29}H_{39}N_5O_5$	1.3 TFA	NMR,MS,CHN
15d	CH_2	NH	NH	0.47	>90	5.66		$C_{30}H_{41}N_5O_4$	1.7 TFA	NMR,MS,CHN
15e	NH	NCH_3	NH	0.022	>100	> 6.0	14.8	$C_{30}H_{42}N_6O_4$	---	NMR,MS,HRMS
15f	NH	O	NH	0.0093	54	> 6.1	20.8	$C_{29}H_{39}N_5O_5$	1.2 TFA	NMR,MS,CHN
15g	NH	CH_2	NH	0.029	>100	> 6.2	4.9	$C_{30}H_{41}N_5O_4$	3.0 TFA	NMR,MS,CHN
16a	NH	NH	O	0.22	>100	> 6.1	11.6	$C_{29}H_{39}N_5O_5$	0.6 TFA	NMR,MS,CHN
16b	NCH_3	NH	O	0.025	>100	4.05	50.0	$C_{30}H_{41}N_5O_5$	1.0 H_2O	NMR,MS,CHN
16c	O	NH	O	0.041	>100	4.66	81.5	$C_{29}H_{38}N_4O_6$	0.3 TFA	NMR,MS,CHN
16d	CH_2	NH	O	0.68	>100	3.78		$C_{30}H_{40}N_4O_5$	0.5 TFA	NMR,MS,CHN[b]
16e	NH	NCH_3	O	0.45	>100	4.59	59.0	$C_{30}H_{41}N_5O_5$	0.55 TFA; 1.0 H_2O	NMR,MS,CHN
16f	NH	O	O	0.021	48	4.04	48.9	$C_{29}H_{38}N_4O_6$	0.3 TFA	NMR,MS,CHN
16g	NH	CH_2	O	0.31	>100	4.16	17.5	$C_{30}H_{40}N_4O_5$	1.0 TFA; 1.5 H_2O	NMR,MS,CHN
16h	NCH_3	O	O	0.0046	90	3.82	110.3	$C_{30}H_{40}N_4O_6$	0.9 TFA	NMR,MS,CHN
16i	O	O	O	0.017	79	3.71	105.8	$C_{29}H_{37}N_3O_7$	0.3 TFA	NMR,MS,CHN

a IC_{50}s calculated using a mean of at least 2 measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted

b N calculated 9.44, observed 9.02 .

Oral dosing of the compound at 10 mg/kg provides modest but sustained drug concentrations in the blood; comparison of oral and iv time course graphs provides an estimated oral bioavailability of 13.5%. Compound **16i**, another third-generation antagonist produced by replacing each urea NH with an oxygen atom, is less potent than **16h** (IC_{50} = 17 nM), but has similar id and oral (not shown) pharmacokinetic profiles. Both **16h** and **16i** have Δ log *P* values < 4.

Conclusions

The physicochemical parameter Δ log *P* has been successfully employed as part of a strategy to improve the pharmacokinetic profile of a series of azole-based endothelin antagonists. A preliminary Δ log *P* analysis of compound **2** suggested a strategy for improving absorption by removing hydrogen-bonding capability from several portions of the molecule. On the basis of this analysis a series of modified analogs were prepared and evaluated. Within this series a strong correlation exists between Δ log *P* values and drug absorption. By combining absorption data with the results of receptor binding analysis, we have been able to identify optimal replacements for for both the heterocyclic core and the urea moiety of **2**. These optimized replacements may be combined together to produce carbamate **16h**, which unlike any of its predecessors has a pharmacokinetic profile that might be compatible with an oral dosing regimen. While **16h** is neither the most potent ET antagonist reported to date nor the best absorbed, it is to the best of our knowledge the most highly ET_A-selective compound to have a potency/bioavailability profile consistent with an oral route of administration. If our hypothesis regarding the importance of such

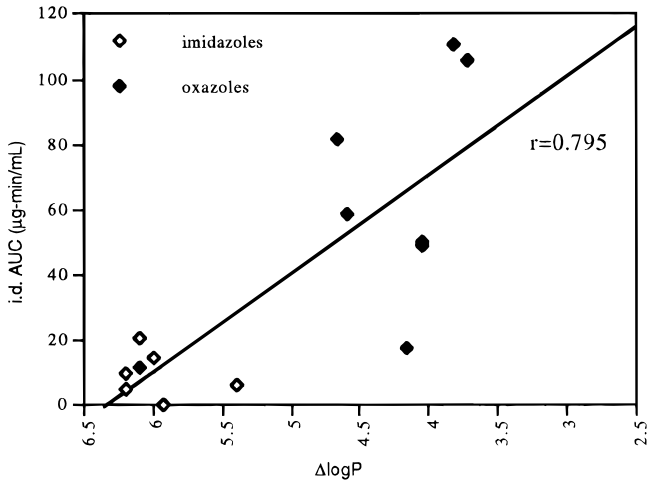


Figure 4. Relationship between Δ log *P* and absorption.

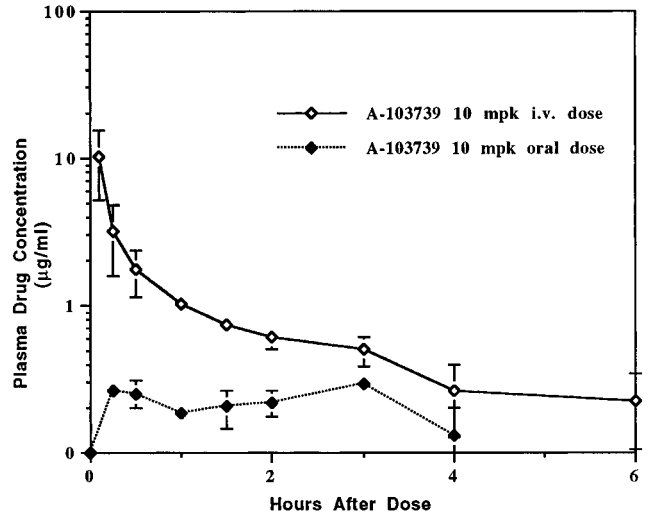


Figure 5. Pharmacokinetic profile of compound **16h** (A-103739).

receptor selectivity proves to be correct, an agent with this profile might offer significant advantages as a therapeutic agent.

Experimental Section

Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. THF was dried over sodium and purified by distillation. All reactions were performed under nitrogen atmosphere unless specifically noted. All final products are analyzed for purity by analytical HPLC using a 25-cm Vydac Protein and Peptide C18 column, and are >95% pure unless otherwise stated. ^{1}H-NMR spectra were recorded at 300 MHz; all values are referenced to tetramethylsilane as internal standard and are reported as shift (multiplicity, coupling constants). Mass spectral analysis is accomplished using fast atom bombardment (FAB-MS) or direct chemical ionization (DCI-MS) techniques. All elemental analyses are consistent with theoretical values to within ±0.4% unless indicated.

Abbreviations: CDI, 1,1′-carbonyldiimidazole; c-Hex, cyclohexyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, dimethylformamide; EDCI, 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; NMM, *N*-methylmorpholine; PIPES, 1,4-piperazinebis(ethanesulfonic acid); PPh_3, triphenylphosphine; pyr, pyridine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; *p*-TsOH, *p*-toluenesulfonic acid.

Synthesis of Core Heterocycles. Compounds **4** (P = Cbz) and **5** (P = Boc) were prepared from the appropriate starting materials using the procedures described in the first article in this series.[1]

Synthesis of Acidic Partners. *N*-((Cyclohexylamino)-carbonyl)leucine (8, X = NH). Leucine benzyl ester−*p*-TsOH (100 mg) was dissolved in $CHCl_3$ (2 mL). Et_3N (51 mg, 75 μL) was added and the solution cooled to 0 °C in an ice bath. Carbonyldiimidazole (41 mg) was added and the solution stirred at 0 °C for 1 h. The bath was removed, and the solution was stirred an additional 1 h at room temperature. Cyclohexylamine (44 mg, 50 μL) was added and the solution stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried with $MgSO_4$, and evaporated under reduced pressure to give a white solid which was purified by flash chromatography on silica gel eluting with 25% EtOAc−hexane to give *N*-((cyclohexylamino)-carbonyl)leucine benzyl ester (75 mg, 88%). The ester was dissolved in EtOH (5 mL), the solution was purged of oxygen, 10% Pd/C (0.10 g) was added, and the mixture was stirred under hydrogen for 2 h. The solvent was removed *in vacuo* and the residue taken up in EtOAc and filtered through Celite to remove the catalyst. The solvent was evaporated *in vacuo* to give the carboxylic acid as a white solid (50 mg, 90%).

***N*-((*N*-Cyclohexyl-*N*-methylamino)carbonyl)leucine (8, X = NCH_3)** was prepared as described above, substituting *N*-methylcyclohexylamine.

1-(Cyclohexyloxycarbonyl)leucine (8, X = O). Cyclohexanol (0.53 mL) was dissolved in THF (10 mL) and the mixture cooled to 0 °C. Phosgene (2.6 mL, 1.97 M in toluene) was added and the solution stirred for 90 min. A solution of Leu-OBn·TsOH (1.97 g) and Et_3N (0.8 mL) in THF (20 mL) was added slowly over 5 min, followed by additional Et_3N (0.8 mL). The mixture was allowed to warm to ambient temperature over 2 h. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc and washed with 1:1 saturated $NaHCO_3$ solution/water, 1 N H_3PO_4, and brine. The organic phase was dried over Na_2SO_4, filtered through Celite, and concentrated to give a yellow oil, which was purified by flash chromatography eluting with 6:1 hexanes−EtOAc. The product was dissolved in ethanol (50 mL), 10% palladium on carbon (100 mg) was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through Celite. The solvents were removed *in vacuo* to give the title compound as a white solid.

1-(Cyclohexylacetyl)leucine (8, X = CH_2). Leucine benzyl ester *p*-tosylate (1.97 g, 5.0 mmol) and cyclohexylacetic acid (0.72 g, 1.0 equiv) were combined in a mixture of 10 mL of THF and 5 mL of DMF; 1 mL of NMM was added, followed by 0.96 g of EDCI. The resultant solution was stirred at ambient temperature for 15 h. The solvents were removed *in vacuo*; the residue was taken up in EtOAc and washed with 1:1 saturated sodium bicarbonate solution−water, 1 N H_3PO_4, and brine. The organic phase was dried over Na_2SO_4, filtered through Celite, and concentrated to give a yellow oil. This crude product was dissolved in ethanol (50 mL), 10% palladium on carbon (100 mg) was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through Celite. The solvents were removed *in vacuo* to give the title compound as a white solid.

(2*S*)-(*N*-Cyclohexylamino)-2-carboxyisovaleric acid (11, X = NH). A mixture of $CsCO_3$ (10 g) and Na_2CO_3 (15 g) was suspended in 80 mL of THF; L-leucic acid (10.0 g) was added, and the mixture was stirred for 20 min. Benzyl bromide (9.5 mL, 1.05 equiv) was added, and the mixture was stirred for 15 h at ambient temperature. DMF (20 mL) was added, and the mixture was heated at 40 °C for 3 h. The mixture was taken up in 200 mL of water and extracted with 100 mL of EtOAc. The organics were washed twice with water and then once each with 1 N H_3PO_4 and brine. The organic extracts were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo*. A sample of the resultant ester (6.40 g) was combined in 75 mL of THF with 4.63 g (1.0 equiv) of carbonyldiimidazole. The solution was stirred at ambient temperature for 6 h. The solvents were removed *in vacuo*; the residue was taken up in EtOAc and then washed sequentially with 0.2 N H_3PO_4 and brine. The organic layer was dried over

Na_2SO_4, filtered through Celite, and evaporated *in vacuo*. A sample of this material (400 mg) was dissolved in 10 mL of THF and cooled to 0 °C. Methyl trifluoromethanesulfonate (0.14 mL, 1.0 equiv) was added dropwise, and the solution was allowed to stir for 30 min. Cyclohexylamine (0.3 mL) was added, and the solution was allowed to warm to ambient temperature and stir for 12 h. The solvents were removed *in vacuo*; the residue was taken up in EtOAc and washed sequentially with 1:1 saturated $NaHCO_3$ solution/water, 1 N H_3PO_4, and brine. The organic phase was dried over Na_2SO_4, filtered through Celite, and concentrated to give a yellowish oil. The crude product was purified by flash chromatography on silica gel, eluting with 3:1 hexanes/ether, to give 300 mg of a colorless oil. This material was dissolved in 30 mL of ethanol; 80 mg of 10% palladium-on-carbon was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through a pad of Celite; the solvents were removed *in vacuo* to provide 210 mg (65% overall yield) of the title compound.

(2*S*)-(*N*-Cyclohexyl-*N*-methylamino)-2-carboxyisovaleric acid (11, X = NCH_3) was prepared as described above, substituting *N*-methylcyclohexylamine.

(2*S*)-(Cyclohexyloxy)-2-carboxyisovaleric acid (11, X = O) was prepared as described above, substituting cyclohexanol.

***N*-Cyclohexyl-(3*R*)-3-carboxy-5-methylhexanamide (14, X = NH).** To a solution of (3*R*)-3-(benzyloxycarbonyl)-5-methylhexanoic acid (464 mg; prepared by the method of Plattner et al. *J. Med. Chem.* **1988**, *31*, 2277−88, but substituting isocaproyl chloride for 3-phenylpropionyl chloride) and 0.23 mL (1 equiv) of cyclohexylamine in 20 mL of 3:1 THF/DMF were added sequentially 270 mg (1 equiv) of HOBt, 1.2 mL of NMM, and 384 mg (1 equiv) of EDC. The resultant solution was stirred at ambient temperature for 15 h; the solvents were removed *in vacuo*, and the residue was taken up in EtOAc and washed sequentially with 1:1 saturated $NaHCO_3$ solution−water, 1 N H_3PO_4 and brine. The organic phase was dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo*. The crude ester was dissolved in 50 mL of ethanol; 100 mg of 10% palladium-on-carbon was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through a pad of Celite; the solvents were removed *in vacuo* to provide the title compound.

Assembly of Analogs: 2-{(1*R*)-1-[*N*-((Cyclohexylamino)carbonyl)leucylamino]-2-(1-methyl-indol-3-yl)ethyl}-5-methylimidazole-4-carboxylic Acid (15a). Compound **4** (P = Cbz, R = Bn; 100 mg) was suspended in 1.5 mL of 30% HBr−HOAc. The resulting solution was stirred at ambient temperature for 60 min. The solvents were removed *in vacuo*; the residue was taken up in saturated $NaHCO_3$ solution and extracted with EtOAc. The organic extracts were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo*. This crude amine was dissolved in THF (10 mL). HOBt (36 mg), *N*-((cyclohexylamino)carbonyl)leucine (64 mg), and EDC (50 mg) were added. *N*-Methylmorpholine (20 μL) and DMF (1.5 mL) were added, and the mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure and the residue taken up in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried with $MgSO_4$, and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel eluting with 50% EtOAc−hexane. This benzyl ester was combined in 10 mL of ethanol with 30 mg of 10% palladium on carbon. The flask was fitted with a three-way stopcock connected to a hydrogen-filled balloon and a nitrogen/vacuum manifold. The flask was evacuated, filled with nitrogen, evacuated again, and then put under a hydrogen atmosphere. The mixture was stirred at ambient temperature for 14 h. The hydrogen was evacuated and the flask filled with nitrogen. The catalyst was removed by filtration through a pad of Celite and the solvent removed *in vacuo*. The crude product was purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The appropriate fraction was lyophilized to give the product as a white solid (76 mg, 62%): ^{1}H NMR (CD_3OD, 300 MHz) of major tautomer δ 0.75 (d, 3H, *J* = 7 Hz), 0.78 (d, 3H, *J* = 7 Hz), 1.1−1.4 (m, 8H), 1.5−1.9 (m, 6H), 2.53 (s, 3H), 3.33 (m, 1H), 3.50 (m, 2H), 3.77 (s, 3H), 4.03 (t, 1H, *J* = 8 Hz), 5.40 (dd, 1H, *J* = 6, 10 Hz), 7.04 (ddd, 1H, *J* = 1, 7, 8 Hz), 7.06 (s, 1H), 7.18 (ddd, 1H, *J* = 1, 7, 8 Hz), 7.35 (d, 1H, *J* = 8 Hz), 7.47 (d, 1H, *J* = 8 Hz); MS (FAB/NBA) *m*/*e* 599 $(M + Cu)^+$. Anal. for $C_{29}H_{40}N_6O_4 \cdot 1.7TFA$: C, H, N.

2-{(1*R*)-1-[*N*-((*N*-Cyclohexyl-*N*-methylamino)carbonyl)-leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic acid (15b) was prepared according to the above procedures, substituting *N*-((*N*-cyclohexyl-*N*-methylamino)carbonyl)leucine. The crude product was triturated with 50% diethyl ether/hexane, dissolved in acetonitrile and water, and then lyophilized to give a white powder (26 mg): ^{1}H NMR (CD_3OD, 300 MHz) of major tautomer δ 0.72 (d, 3H, *J* = 7 Hz), 0.75 (d, 3H, *J* = 7 Hz), 1.1−1.5 (m, 8H), 1.5−1.8 (m, 5H), 2.48 (s, 3H), 2.72 (s, 3H), 3.18 (m, 1H), 3.58 (m, 2H), 3.72 (s, 3H), 4.05 (t, 1H, *J* = 8 Hz), 5.36 (dd, 1H, *J* = 6, 10 Hz), 6.95 (s, 1H), 7.0 (t, 1H, *J* = 8 Hz), 7.15 (t, 1H, *J* = 8 Hz), 7.30 (d, 1H, *J* = 8 Hz), 7.54 (d, 1H, *J* = 8 Hz); MS (FAB/NBA) *m*/*e* 551 $(M + H)^+$. Anal. for $C_{30}H_{42}N_6O_4 \cdot 2\ H_2O$: C, H, N.

2-{(1*R*)-1-[*N*-(Cyclohexyloxycarbonyl)leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic acid (15c) was prepared according to the above procedures, substituting 1-(cyclohexyloxycarbonyl)leucine. The crude material was purified by trituration with ether−EtOAc to give a white solid which was dissolved in 0.1% aqueous TFA in acetonitrile and lyophilized to give a white powder (106 mg): ^{1}H NMR (CD_3OD, 300 MHz) of major tautomer δ 0.78 (d, 3H, *J* = 6 Hz), 0.81 (d, 3H, *J* = 6 Hz), 1.2−1.9 (m, 13H), 2.45 (s, 3H), 3.3−3.5 (m, 2H), 3.76 (s, 3H), 4.02 (m, 1H), 4.59 (m, 1H), 5.23 (t, 1H, *J* = 8 Hz), 7.03 (dt, 1H, *J* = 1, 7 Hz), 7.04 (s, 1H), 7.17 (dt, 1H, *J* = 1, 7 Hz), 7.33 (d, 1H, *J* = 8 Hz), 7.45 (d, 1H, *J* = 8 Hz); MS (FAB/NBA) *m*/*e* 538 $(M + H)^+$, 560 $(M + Na)^+$. Anal. for $C_{29}H_{39}N_5O_5 \cdot 1.3TFA$: C, H, N.

2-{(1*R*)-1-[*N*-(Cyclohexylacetyl)leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic acid (15d) was prepared according to the above procedures, substituting 1-(cyclohexylacetyl)leucine. The final product was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give the title compound as a white powder (11 mg): ^{1}H NMR (CD_3OD, 300 MHz) of the major tautomer δ 0.76 (d, 3H, *J* = 7 Hz), 0.79 (d, 3H, *J* = 7 Hz), 0.95 (m, 2H), 1.1−1.5 (m, 5H), 1.7 (m, 7H), 2.07 (d, 2H, *J* = 8 Hz), 2.51 (s, 3H), 3.35 (m, 2H), 3.77 (s, 3H), 4.19 (dd, 1H, *J* = 6, 7 Hz), 5.35 (dd, 1H, *J* = 6, 8 Hz), 7.02 (m, 1H), 7.05 (s, 1H), 7.16 (m, 1H), 7.35 (d, 1H, *J* = 7 Hz), 7.45 (d, 1H, *J* = 7 Hz); MS (FAB/NBA) *m*/*e* 536 $(M + H)^+$. Anal. for $C_{30}H_{41}N_5O_4 \cdot 1.7TFA$: C, H, N.

2-{(1*R*)-1-(*N*-((2*S*)-((*N*-Cyclohexylamino)-2-carboxyisovaleryl)amino)-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic acid (15f) was prepared according to the above procedures, substituting (2*S*)-(*N*-cyclohexylamino-2-carboxyisovaleric acid. The crude material was purified by trituration with ether; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give a white powder (43.5 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.85 (d, 6H, *J* = 7 Hz), 1.1−1.9 (m, 13H), 2.50 (s, 3H), 3.3−3.5 (m, 4H), 3.76 (s, 3H), 5.34 (dd, 1H, *J* = 7, 8 Hz), 7.03 (s, 1H), 7.04 (dt, 1H, *J* = 1, 8 Hz), 7.17 (ddd, 1H, *J* = 1, 7, 8 Hz), 7.34 (d, 1H, *J* = 8 Hz), 7.43 (d, 1H, *J* = 8 Hz); MS (DCI/NH3) *m*/*e* 538 $(M + H)^+$. Anal. for $C_{29}H_{39}N_5O_5 \cdot 1.2TFA$: C, H, N.

2-{(1*R*)-1-(*N*-((2*R*)-2-(((*N*-Cyclohexylamino)carbonyl)-methyl)isovaleryl)amino)-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic acid (15g) was prepared according to above procedures, substituting *N*-cyclohexyl-(3*R*)-3-carboxy-5-methylhexanamide. The crude material was triturated with ether; the resultant material was purified by HPLC, using a gradient of 0 → 80% acetonitrile in 0.1% aqueous TFA to elute the product as a white solid (17 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.59 (d, 3H, *J* = 6 Hz), 0.61 (d, 3H, *J* = 6 Hz), 0.79 (m, 1H), 0.96 (ddd, 1H, *J* = 5, 10, 14 Hz), 1.1−1.4 (m, 6H), 1.6−1.9 (m, 5H), 2.25 (dd, 1H, *J* = 3, 15 Hz), 2.50 (dd, 1H, *J* = 14, 15 Hz), 2.56 (s, 3H), 2.63 (m, 1H), 3.2−3.4 (m, 2H), 3.65 (m, 1H), 3.77 (s, 3H), 5.52 (dd, 1H, *J* = 5, 11 Hz), 7.04 (ddd, 1H, *J* = 1, 7, 8 Hz), 7.08 (s, 1H), 7.18 (ddd, 1H, *J* =

1, 7, 8 Hz), 7.35 (d, 1H, J = 8 Hz), 7.55 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 536 $(M + H)^+$. Anal. for $C_{30}H_{41}N_5O_4 \cdot 3.0TFA$: C, H, N.

2-{(1*R*)-1-[(*N*-((*N*-Cyclohexylamino)carbonyl)-*N*-methylleucyl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methylimidazole-4-carboxylic Acid (15e). Compound **4** (P = Cbz, R = Bn; 80 mg) was suspended in 1 mL of 30% HBr−HOAc. The resulting solution was stirred at ambient temperature for 60 min. The solvents were removed *in vacuo*; the residue was taken up in saturated $NaHCO_3$ solution and extracted with EtOAc. The organic extracts were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo*. This crude amine was dissolved in THF (4 mL) and DMF (2 mL). HOBt (42 mg), *N*-Boc-*N*-methylleucine[13] (64 mg), and EDC (57 mg) were added, followed by *N*-methylmorpholine (8 drops), and the mixture was stirred at ambient temperature for 18 h. The solvent was evaporated, and the residue was taken up in EtOAc, washed with saturated sodium bicarbonate solution, 1 N H_3PO_4, and brine, and evaporated to give a yellow oil. This crude product was dissolved in TFA (5 mL). The solution was stirred at ambient temperature for 2 h. The solvent was evaporated and the residue taken up in saturated sodium bicarbonate solution and extracted with EtOAc. The organic layer was washed with brine and evaporated. The crude product was dissolved in THF (5 mL). *N*-Methylmorpholine (0.2 mL) and cyclohexyl isocyanate (5 drops) were added, and the solution was stirred at ambient temperature for 18 h. The solvent was evaporated, and the residue was taken up in EtOAc, washed with saturated sodium bicarbonate solution, 1 N H_3PO_4, and brine, and evaporated to give a yellow oil which was dissolved in EtOH (20 mL). Next, 10% palladium on carbon (30 mg) was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through Celite, and the solvents were evaporated. The crude product was triturated with 1:1 EtOAc−ether to give a white solid which was dissolved in 0.1% aqueous TFA in acetonitrile and lyophilized to give the title compound as a white powder (30 mg): ^{1}H NMR (CD_3OD, 300 MHz) of major tautomer δ 0.86 (d, 3H, J = 7 Hz), 0.89 (d, 3H, J = 7 Hz), 1.0−1.8 (m, 13H), 2.48 (s, 3H), 2.72 (s, 3H), 3.3−3.5 (m, 3H), 3.75 (s, 3H), 4.51 (dd, 1H, J = 7, 10 Hz), 5.29 (t, 1H, J = 7 Hz), 7.02 (s, 1H), 7.04 (dt, 1H, J = 1, 7 Hz), 7.18 (dt, 1H, J = 1, 7 Hz), 7.35 (d, 2H, J = 8 Hz); MS (DCI/NH_3) m/e 551 $(M + H)^+$; HRMS calcd for $C_{30}H_{43}N_6O_4$ 551.3346, found 551.3351.

2-{(1*R*)-1-[*N*-((Cyclohexylamino)carbonyl)leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic Acid (16a). Compound **5** above (120 mg) was dissolved in 6 mL of trifluoroacetic acid and allowed to stir at ambient temperature for 1 h. The solvents were removed *in vacuo*, the residue was neutralized with bicarbonate solution, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was dissolved in THF (2 mL). HOBt (42 mg), *N*-((cyclohexylamino)-carbonyl)leucine, and EDC (57 mg) were added. *N*-Methylmorpholine (100 μL) was added and the mixture stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure and the residue taken up in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried with $MgSO_4$, and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography on silica gel eluting with 50% EtOAc−hexane. The resultant ester was dissolved in 30 mL of EtOH, 50 mg of 10% palladium on carbon was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through a pad of Celite; the solvents were removed *in vacuo*. The crude material was triturated with ether−hexanes, dissolved in 0.1% aqueous TFA−acetonitrile, and lyophilized to give the title compound as a white powder (96 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.70 (d, 3H, J = 7 Hz), 0.74 (d, 3H, J = 7 Hz), 1.1−1.9 (m, 13H), 2.50 (s, 3H), 3.40 (m, 1H), 3.76 (s, 3H), 3.90 (m, 1H), 4.35 (dd, 1H, J = 6, 7 Hz), 5.35(m, 1H), 6.91 (s, 1H), 7.02 (t, 1H, J = 8 Hz), 7.13 (t, 1H, J = 8 Hz), 7.32 (d, 1H, J = 8 Hz), 7.48 (d, 1H, J = 8 Hz); MS (FAB/NBA) m/e 538 $(M + H)^+$, 560 $(M + Na)^+$, 576 $(M + K)^+$. Anal. for $C_{29}H_{39}N_5O_5 \cdot 0.6TFA$: C, H, N.

2-{(1*R*)-1-[*N*-(*N*-Methyl-*N*-cyclohexylamino)carbonyl)-leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16b) was prepared according to the above procedure, substituting *N*-((*N*-cyclohexyl-*N*-methylamino)carbonyl)leucine. The crude product was triturated with diethyl ether−hexanes, dissolved in acetonitrile and water, and lyophilized to give the product as a white powder (72 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.71 (d, 3H, J = 7 Hz), 0.73 (d, 3H, J = 7 Hz), 1.1−1.8 (m, 13H), 2.54 (s, 3H), 2.71 (s, 3H), 3.45 (m, 1H), 3.72 (s, 3H), 3.90 (m, 1H), 4.32 (dd, 1H, J = 6, 7 Hz), 5.38 (m, 1H), 6.92 (s, 1H), 7.0 (t, 1H, J = 8 Hz), 7.13 (t, 1H, J = 8 Hz), 7.30 (d, 1H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz); MS (FAB/NBA) m/e 552 $(M + H)^+$. Anal. for $C_{30}H_{41}N_5O_5 \cdot H_2O$: C, H, N.

2-{(1*R*)-1-[*N*-(Cyclohexyloxycarbonyl)leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic Acid (16c). The title compound was prepared following the procedures described above, substituting 1-(cyclohexyloxycarbonyl)leucine. The crude final product was purified by trituration with ether−hexanes; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give the title compound as a white powder (60 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.83 (d, 6H, J = 7 Hz), 1.3−1.9 (m, 13H), 2.54 (s, 3H), 3.3−3.5 (m, 2H), 3.73 (s, 3H), 4.10 (m, 1H), 4.51 (m, 1H), 5.38 (dd, 1H, J = 7, 8 Hz), 6.96 (s, 1H), 7.01 (t, 1H, J = 7 Hz), 7.13 (dt, 1H, J = 1, 7 Hz), 7.29 (d, 1H, J = 8 Hz), 7.45 (d, 1H, J = 8 Hz); MS (FAB/NBA) m/e 539 $(M + H)^+$, 561 $(M + Na)^+$. Anal. for $C_{29}H_{38}N_4O_6 \cdot 0.3TFA$: C, H, N.

2-{(1*R*)-1-[*N*-(Cyclohexylacetyl)leucylamino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16d) was prepared according to the above procedures, substituting 1-(cyclohexylacetyl)leucine. The final product was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give the title compound as a white powder (33 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.79 (d, 3H, J = 7 Hz), 0.82 (d, 3H, J = 7 Hz), 0.9 (m, 3H), 1.1−1.5 (m, 6H), 1.65 (m, 5H), 2.04 (d, 2H, J = 8 Hz), 2.53 (s, 3H), 3.35 (m, 2H), 3.72 (s, 3H), 4.37 (dd, 1H, J = 6, 9 Hz), 5.40 (dd, 1H, J = 7, 8 Hz), 6.96 (s, 1H), 7.02 (ddd, 1H, J = 1, 7, 8 Hz), 7.14 (ddd, 1H, J = 1, 7, 8 Hz), 7.30 (d, 1H, J = 7 Hz), 7.45 (d, 1H, J = 7 Hz); MS (DCI/NH_3) m/e 537 $(M + H)^+$, 554 $(M + NH_4)^+$. Anal. for $C_{30}H_{40}N_4O_5 \cdot 0.5TFA$: C, H; N: calcd, 9.44; found, 9.02.

2-{(1*R*)-1-[((2*S*)-(*N*-Cyclohexylamino)-2-carboxyisovaleryl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16f) was prepared according to the above procedures, substituting (2*S*)-(*N*-cyclohexylamino)-2-carboxyisovaleric acid. The crude material was purified by trituration with 2:1 hexanes/ether; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give a white powder (103 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.86 (d, 3H, J = 7 Hz), 0.88 (d, 3H, J = 7 Hz), 1.0−1.9 (m, 13H), 2.55 (s, 3H), 3.3−3.5 (m, 2H), 3.73 (s, 3H), 4.92 (dd, 1H, J = 5, 10 Hz), 5.38 (dd, 1H, J = 7, 8 Hz), 6.93 (s, 1H), 7.00 (ddd, 1H, J = 1, 7, 8 Hz), 7.13 (dt, 1H, J = 1, 7 Hz), 7.28 (d, 1H, J = 8 Hz), 7.44 (d, 1H, J = 8 Hz); MS (FAB/NBA) m/e 539 $(M + H)^+$, 561 $(M + Na)^+$. Anal. for $C_{29}H_{38}N_4O_6 \cdot 0.3TFA$: C, H, N.

2-{(1*R*)-1-[*N*-((2*R*)-2-(((*N*-Cyclohexylamino)carbonyl)-methyl)isovaleryl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16g) was prepared according to above procedures, substituting *N*-cyclohexyl-(3*R*)-3-carboxy-5-methylhexanamide. The crude material was purified by trituration with hexanes−ether; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give a white powder (76 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.70 (d, 6H, J = 7 Hz), 1.0−1.4 (m, 8H), 1.5−1.8 (m, 5H), 2.12 (dd, 1H, J = 7, 14 Hz), 2.31 (dd, 1H, J = 8, 14 Hz), 2.54 (s, 3H), 2.71 (m, 1H), 3.2−3.4 (m, 2H), 3.50 (m, 1H), 3.73 (s, 3H), 5.45 (dd, 1H, J = 7, 9 Hz), 6.99 (s, 1H), 7.02 (dt, 1H, J = 1, 7 Hz), 7.13 (dt, 1H, J = 1, 7 Hz), 7.29 (d, 1H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 537 $(M + H)^+$, 554 $(M + NH4)^+$. Anal. for $C_{30}H_{40}N_4O_5 \cdot TFA \cdot 1.5H_2O$: C, H, N.

2-{(1*R*)-1-[*N*-((2*S*)-(*N*-Cyclohexyl-*N*-methylamino)-2-carboxyisovaleryl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16h) was prepared according to above procedures, substituting (2*S*)-2-((*N*-cyclohexyl-*N*-methylamino)carboxy)isovaleric acid. The crude final product was triturated with hexanes−ether; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give a white powder (62 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.85 (d, 6H, J = 7 Hz), 1.0−1.8 (m, 13H), 2.55 (s, 3H), 2.75 (bd s, 3H), 3.32 (m, 1H), 3.43 (dd, 1H, J = 7, 15 Hz), 3.74 (s, 3H), 3.84 (m, 1H), 4.89 (dd, 1H, J = 4, 10 Hz), 5.20 (dd, 1H, J = 7, 8 Hz), 6.94 (s, 1H), 7.02 (ddd, 1H, J = 1, 7, 8 Hz), 7.13 (dt, 1H, J = 1, 7 Hz), 7.29 (d, 1H, J = 8 Hz), 7.47 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 553 $(M + H)^+$, 570 $(M + NH_4)^+$. Anal. for $C_{30}H_{40}N_4O_6$·0.9TFA: C, H, N.

2-{(1*R*)-1-[*N*-((2*S*)-2-((Cyclohexyloxycarbonyl)oxy)-isovaleryl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic acid (16i) was prepared according to above procedures, substituting (2*S*)-2-((cyclohexyloxycarbonyloxy)isovaleric acid. The crude final product was triturated with hexanes−ether; the resultant material was dissolved in 0.1% aqueous TFA−acetonitrile and lyophilized to give a white powder (75 mg): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.84 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 6 Hz), 1.0−1.8 (m, 13H), 2.55 (s, 3H), 3.32 (m, 1H), 3.43 (dd, 1H, J = 7, 15 Hz), 3.74 (s, 3H), 4.47 (m, 1H), 4.83 (dd, 1H, J = 5, 9 Hz), 5.20 (dd, 1H, J = 7, 9 Hz), 6.96 (s, 1H), 7.02 (ddd, 1H, J = 1, 7, 8 Hz), 7.14 (dt, 1H, J = 1, 7 Hz), 7.30 (d, 1H, J = 8 Hz), 7.48 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 540 $(M + H)^+$, 557 $(M + NH_4)^+$. Anal. for $C_{29}H_{37}N_3O_7$·0.3TFA: C, H, N.

2-{(1*R*)-1-[*N*-(*N*-((Cyclohexylamino)carbonyl)-*N*-Methylleucyl)amino]-2-(1-methylindol-3-yl)ethyl}-5-methyloxazole-4-carboxylic Acid (16e). Compound **5** above (120 mg) was dissolved in 6 mL of trifluoroacetic acid and allowed to stir at ambient temperature for 1 h. The solvents were removed *in vacuo*, the residue was neutralized with saturated $NaHCO_3$ solution, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude amine was dissolved in THF (2 mL). HOBt (42 mg), *N*-Boc-*N*-methylleucine[13] (80 mg), and EDC (57 mg) were added, followed by *N*-methylmorpholine (100 μL), and the mixture was stirred at ambient temperature for 15 h. The solvent was evaporated, and the residue was taken up in EtOAc, washed with saturated sodium bicarbonate solution, 1 N H_3PO_4, and brine, and evaporated to give a yellow oil. This crude product was dissolved in EtOH (20 mL). Next, 10% palladium on carbon (30 mg) was added, and the mixture was purged with nitrogen. The nitrogen line was exchanged for a balloon of hydrogen, and the mixture was stirred at ambient temperature for 4 h. The catalyst was removed by filtration through Celite, and the solvents were evaporated. The resultant acid was dissolved in TFA (6 mL). The solution was stirred at ambient temperature for 1 h. The solvent was removed *in vacuo*; the residue was taken up in toluene and concentrated *in vacuo*. The resultant product was dissolved in DMF (5 mL). *N*-Methylmorpholine (0.2 mL) and cyclohexyl isocyanate (5 drops) were added, and the solution was stirred at ambient temperature for 15 h. The solvent was evaporated, and the residue was taken up in EtOAc, washed with 1 N H_3PO_4, and brine, and concentrated *in vacuo*. The crude product was triturated with 1:1 hexanes−ether to give a white solid which was dissolved in 0.1% aqueous TFA in acetonitrile and lyophilized to give the title compound as a white powder (122 mg): ^{1}H NMR (CD_3OD, 300 MHz) of major tautomer δ 0.85 (d, 3H, J = 7 Hz), 0.87 (d, 3H, J = 7 Hz), 1.0−1.9 (m, 13H), 2.50 (s, 3H), 2.66 (s, 3H), 3.3−3.4 (m, 2H), 3.47 (m, 1H), 3.74 (s, 3H), 4.78 (dd, 1H, J = 7, 9 Hz), 5.32 (dd, 1H, J = 6, 8 Hz), 6.99 (s, 1H), 7.00 (dt, 1H, J = 1, 7 Hz), 7.13 (dt, 1H, J = 1, 7 Hz), 7.29 (d, 1H, J = 8 Hz), 7.42 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 552 $(M + H)^+$, 569 $(M + NH_4)^+$. Anal. for $C_{30}H_{41}N_4O_5$·0.55TFA·1.5H_2O: C, H, N.

Receptor Binding Assays. All samples were assayed according to the protocols described in the first article in this series.[1] Representative analogs were also examined for their binding to human ET_A and ET_B receptors expressed in CHO cells, using similar protocols. The results are qualitatively similar, as indicated by the following data:

compound	IC_{50}, μM (hETA in CHO)	IC_{50}, μM (hETB in CHO)
16h	0.0053 (n = 2)	>100 (n = 2)
16i	0.029 (n = 2)	>100 (n = 2)

Physicochemical Determinations. All partition coefficients were measured using standard shake-flask techniques at 37 °C. A sample of test compound (~1 mg) was dissolved in 4.5 mL of 0.05 M PIPES buffer, pH 6.5. A 1-mL sample of the resultant solution was transferred to a screw cap test tube along with an equal volume of the appropriate organic phase; the samples were capped and shaken for 2 h on a reciprocating shaker at 100 cpm. Model organic phases were octanol (H-bonding) and cyclohexane (non-H-bonding). After centrifugation to separate the phases, the concentrations of a test compound in both organic and aqueous phases were determined by HPLC analysis. All values are the mean of at least two determinations.

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

Plasma samples (200 μL) and an equal volume of an internal standard were extracted with 5 mL of dichloromethane−ethanol (8:2). The organic phase was transferred to a 10 mL conical centrifuge tube, and the solvent was evaporated using a dry air stream. Samples were reconstituted in mobile phase. Recovery of samples after extraction varied between 40 and 80%. Samples were analyzed by HPLC using a Regis Little Champ column (50 × 4.6 mm id 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(aq) containing 0.1% trifluoroacetic acid. UV detection of 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 values were used to calculate area under the curve ("id AUC") using the trapezoidal rule. The limit of detection using thes methods was 15−20 ng/mL.

Oral Pharmacokinetic Analysis. The pharmacokinetic behavior of compound **16h** was evaluated in male Sprague−Dawley rats. Briefly, the test compound was prepared as a 10 mg/mL solution in an ethanol−propylene glycol−D5W (20:30:50, by volume) vehicle containing 1 molar equiv of sodium hydroxide. Groups of rats (n = 4 per group) received either a 10 mg/kg (1 mL/kg) intravenous dose administered as a slow bolus in the jugular vein or a 10 mg/kg (1 mL/kg) oral dose administered by gavage. Heparinized blood samples (~0.4 mL/sample) were obtained from a tail vein of each rat 0.1 (iv only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 h after dosing. The samples were analyzed by reverse phase HPLC following liquid−liquid extraction from the plasma. Initial estimates of the pharmacokinetic parameters for NONLIN84[14] were obtained with the program CSTRIP.[15] Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant (β) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an $AUC_{0-\infty}$ value. 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).

Acknowledgment. The authors thank the Abbott Analytical Department for assistance in acquiring ^{1}H-NMR and mass spectra.

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JM9505932