Azole Endothelin Antagonists. 1. A Receptor Model Explains an Unusual Structure−Activity Profile

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The pseudotetrapeptide FR-139317 is a potent and highly selective antagonist of the endothelin-A (ET_A) receptor; however, its peptidic nature leads to poor oral absorption characteristics which make it an unlikely drug candidate. In an attempt to improve these properties, we have replaced a portion of the amide bond framework of FR-139317 with a heterocyclic surrogate. The resultant analogs are also ET_A-selective antagonists, but show a structure−activity profile substantially different from that of the peptidic series, particularly with regard to the requirements for the side chain group that has been incorporated into the heterocycle. The nature of the heterocycle itself also has profound effects on the activity of the compounds. Both of these surprising results can be rationalized through examination of a 3D model of ET ligand−receptor binding that has previously been developed in our laboratories.

Introduction

Endothelin (ET),[1] a 21-amino acid bicyclic peptide, is the most powerful peptidic constrictor of vascular smooth muscle reported to date, as well as a potent mitogen. Produced predominantly by endothelial cells, it acts in both an autocrine and paracrine fashion as a mediator of vascular function. It has been implicated as a pathogenic factor for a variety of disease states,[2a] including asthma,[2b] coronary vasospasm and myocardial infarction,[2c] pulmonary hypertension,[2d] restenosis,[2e] and atherosclerosis,[2f] in which excessive vasoconstriction or smooth muscle proliferation play a role.

Endothelin acts by binding to a family of membrane-associated, G-protein-coupled receptors (GPCRs).[3] Binding to the ET_A receptor subtype, which predominates in vascular smooth muscle cells, triggers a cascade of events which leads, via the hydrolysis of inositol phosphates, to the observed vasoconstrictive and proliferative responses. The results of binding to ET_B, which is the major receptor on endothelial cells, are less clearly understood; while this receptor mediates constriction in some tissue beds, it has also been linked to the production of nitric oxide and to the clearance of endogenous ET. Because these latter effects might be beneficial in a number of the diseases described above, it has been suggested[2a] that a selective ET_A antagonist may provide some therapeutic advantage over a nonselective agent.

The unique role of endothelial cells as the "gatekeeper" controlling the exchange of information between blood and tissue has led to a significant interest in agents which might modulate their function. In particular endothelin has attracted attention in the recent literature; a wide variety of structures exhibiting ET-antagonist properties have been described,[4] including a number which are both highly potent and well-absorbed. In this and the following articles we describe our group's first attempts to develop a series of orally-active, ET_A-selective antagonists.

Antagonist Design

Our own interest in the area of endothelin receptor antagonism began with the report by Ishikawa et al.[4a] at Banyu of BQ-123 and its congeners. BQ-123, derived from the screening lead BE-18257, is a potent and highly selective blocker of the ET_A receptor (IC_{50} = 22 nM; ET_A/ET_B > 800); it was also the first low molecular weight inhibitor to be disclosed. Chemical modification studies of this cyclic pentapeptide structure quickly led both Banyu and Fujisawa to develop the linear pseudotetrapeptides BQ-485[4b] and FR-139317.[4c] Unfortunately these early compounds, which have excellent biochemical properties, are unlikely drug candidates due to their highly peptidic nature. In fact, we have shown (unpublished results) that when FR-139317 is dosed intraduodenally to rats at 10 mg/kg, circulating drug levels are lower than our 10 ng/mL HPLC detection limit.

We hoped to be able to modify the polyamide backbone of these peptidic antagonists in such a way as to maintain receptor affinity while improving physical properties such as gut transport and plasma stability. We chose to focus on the linear FR-139317/BQ-485 series as the starting point for modification. Believing that a dramatic departure from the amide linkage would be required to achieve our goals, we examined the heterocycles shown in Scheme 1 as dipeptide mimics. While the replacement of the CO−N−C_α−C_β network with a five-membered ring is formally an isosteric substitution, the change results in significant restrictions of the flexibility of the dipeptide. This rigidification is likely (*vide infra*) to affect receptor interactions.

Chemistry

Our synthetic strategy for preparing the heterocyclic dipeptide surrogates is modelled on the work of Gordon and co-workers at Sterling-Winthrop.[5] We have prepared mimics of both Leu-Trp and Trp-X dipeptides; the successful routes are described in Schemes 2 and 3, respectively. In brief, key intermediate **4** (or **7**) arises from sequential C- and N-acylation of glycine anion equivalent **3**. Because of the instability of the interme-

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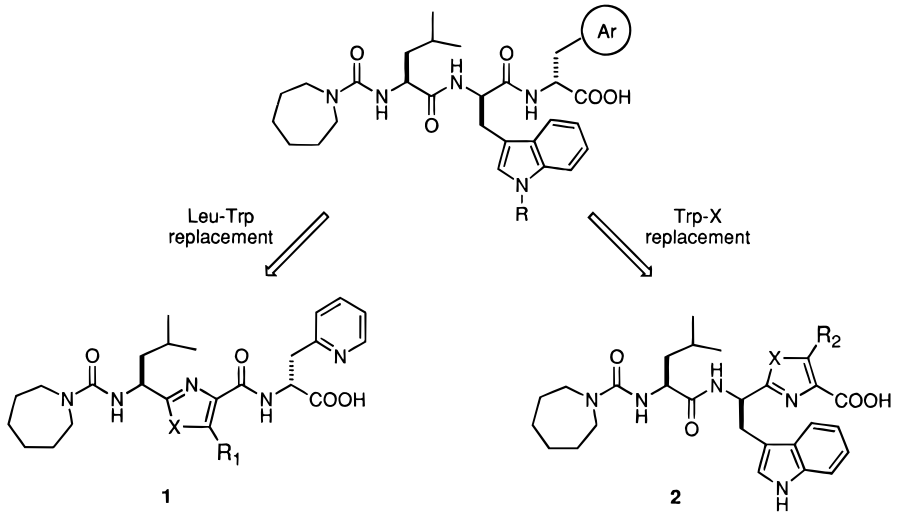
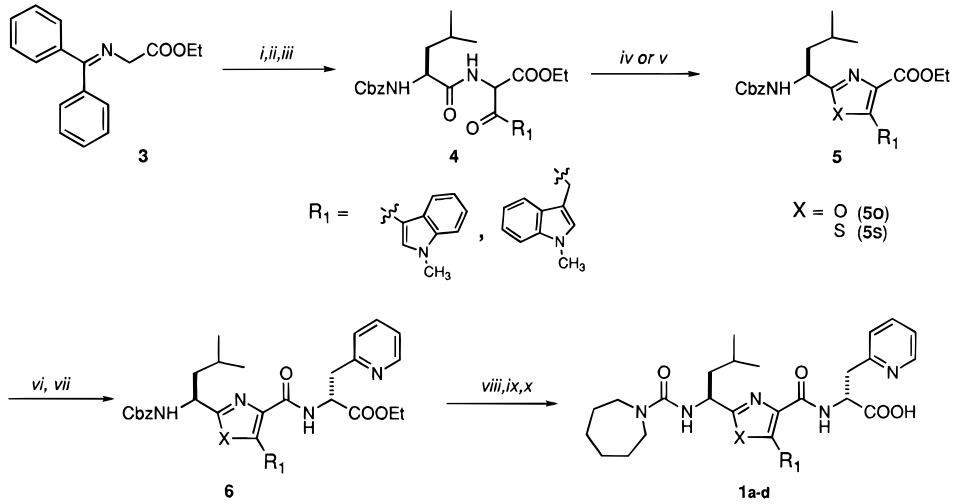
Scheme 1. Design Strategy

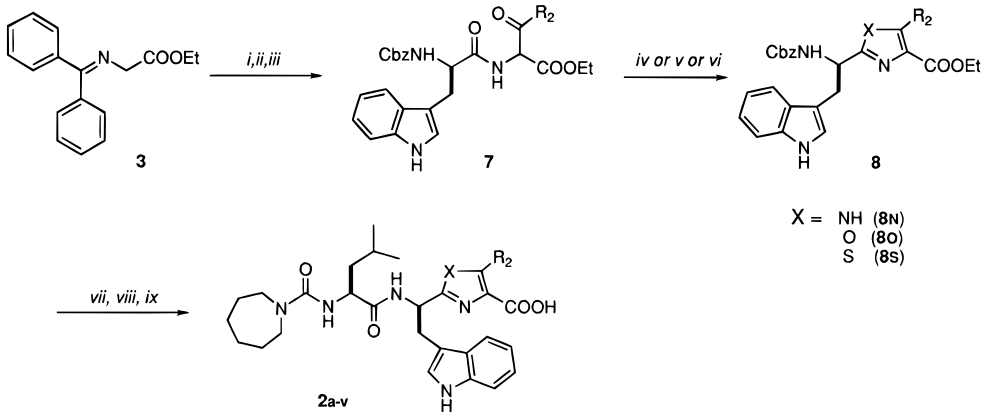


Scheme 2. Synthesis of Central Dipeptide Surrogates[a]



[a] (i) LiHMDS, THF, −78 °C; R_1COCl; (ii) HCl/H_2O; (iii) Cbz-Leu-OCOOiBu, THF, −20 °C; NMM (dropwise); (iv) PPh_3, CCl_4, pyr, CH_3CN; (v) Lawesson's reagent, THF, reflux; (vi) LiOH, H_2O, THF (heat); (vii) D-Pal-OEt, EDC, HOBt, NMM, THF, DMF; (viii) H_2/10% Pd−C, EtOH (X = O) or HBr/HOAc (X = S); (ix) CDI, Et_3N, THF; Pha; (x) LiOH, H_2O, THF.

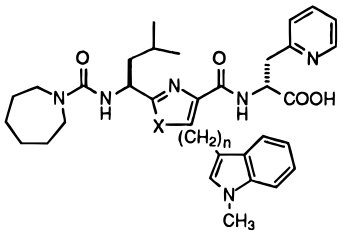
Scheme 3. Synthesis of Terminal Dipeptide Surrogates[a]



[a] (i) LiHMDS, THF, −78 °C; R_2COCl; (ii) HCl/H_2O; (iii) Cbz-D-Trp-OCOOiBu, THF, −20 °C; NMM (dropwise); (iv) NH_4OAc, HOAc, reflux; (v) PPh_3, CCl_4, pyr, CH_3CN; (vi) Lawesson's reagent, THF, reflux; (vii) H_2/10% Pd−C, EtOH (X = NH, O) or HBr/HOAc (X = S); (viii) Pha-Leu-OH, EDC, HOBt, NMM, THF, DMF; (x) LiOH, H_2O, THF.

diate amino ketone, care is taken to isolate this material directly as a hydrochloride salt from the imine hydrolysis step; this salt is slowly neutralized in the presence of a preformed active ester of Cbz-L-Leu (or Cbz-D-Trp) in order to maximize the production of the desired **4** (or **7**). Strategically, compounds **4** and **7** are key to our efforts. Their rapid assembly from a number of readily-available starting materials allows the exploration of a variety of substituents on the heterocyclic ring. Additionally, the α-amido ketone moiety allows ready

Table 1. Central Dipeptide Surrogates



compd	X	*n*	IC_{50} (ET_A), μM[a]	IC_{50} (ET_B), μM[a]	formula	solvate	characterization
1a	O	0	>100	>100	$C_{33}H_{40}N_6O_5$	1.3 TFA	NMR, MS, CHN
1b	S	0	>90	>100	$C_{33}H_{40}N_6O_4S$	2.0 TFA	NMR, MS, CHN
1c	O	1	68	>100	$C_{34}H_{42}N_6O_5$	1.5 TFA	NMR, MS, CHN
1d	S	1	>90	>100	$C_{34}H_{42}N_6O_4S$	1.5 TFA	NMR, MS, CHN

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

access from a common intermediate to three different heterocyclic cores. Thus, for example, reaction of **7** with an ammonium salt results in cyclization to imidazole **8N**; dehydration under Mitsonobu's conditions leads to the corresponding oxazole **8O**; and treatment with Lawesson's thiation reagent provides thiazole **8S**.

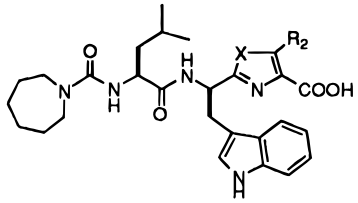
Assembly of the dipeptide mimics into potential ET antagonists occurs in straightforward fashion. Leu-Trp mimics **5** (Scheme 2) are saponified and coupled with D-2-pyridylalanine ethyl ester (D-Pal-OEt) to give pseudotripeptides **6**. The amino terminus of **6** is deprotected and converted to the corresponding urea; hydrolysis of the ester provides "internal" heterocyclic analogs **1a**−**d**. Similarly, deprotection of Trp-X mimic **8** (Scheme 3), followed by coupling with L-leucine perhydroazepinyl urea (Pha-Leu-OH) and hydrolysis, leads to "terminal" heterocycles **2a**−**v**.

In some cases the final saponification step is problematic. While hydrolysis of the oxazole and thiazole esters generally occurs smoothly at ambient or slightly elevated temperatures, imidazoles require significantly more vigorous conditions to effect this transformation. The hydrolysis reaction is also impeded by the presence of bulky substituents at the position adjacent to the ester. When these factors act in combination (e.g. compounds **2q** and **2t**) we find that we are unable to hydrolyze the ester without a significant loss of stereochemical integrity.

Receptor Binding Profile

Compounds **1a**−**d** and **2a**−**v** were evaluated for their ability to compete with ET-1 for ET_A binding sites in MMQ cell membranes (a rat pituitary cell line known to contain ET_A receptors) and with ET-3 for ET_B sites in porcine cerebellar membranes. Several points are apparent from an examination of the IC_{50} data reported in Tables 1 and 2. With regard to the Leu-Trp linkage, the results of Table 1 suggest clearly that our hetero-

Table 2. Terminal Dipeptide Surrogates



compd	X	R_2	IC_{50} (ET_A), μM[a]	IC_{50} (ET_B), μM[a]	formula	solvate	characterization
2a	NH	Ph	51	>100	$C_{33}H_{40}N_6O_4$	1.25 TFA	NMR, MS, CHN
2b	O	Ph	7.3	>85	$C_{33}H_{39}N_5O_5$		NMR, MS, HRMS
2c	S	Ph	51	>100	$C_{33}H_{39}N_5O_4S$	1.0 TFA	NMR, MS, CHN
2d	NH	Bn	8	69	$C_{34}H_{42}N_6O_4$	1.75 TFA	NMR, MS, CHN
2e	O	Bn	16	90	$C_{34}H_{41}N_5O_5$	0.6 TFA	NMR, MS, CHN
2f	S	Bn	39	>90	$C_{34}H_{41}N_5O_4S$		NMR, MS, HRMS
2g	NH	Me	0.57	>100	$C_{28}H_{38}N_6O_4$	1.5 TFA	NMR, MS, CHN
2h	O	Me	1.43	>100	$C_{28}H_{37}N_5O_5$	0.4 TFA	NMR, MS, CHN
2i	S	Me	41	>100	$C_{28}H_{37}N_5O_4S$	1.0 TFA	NMR, MS, CHN
2j	NH	Et	1.5	>100	$C_{29}H_{40}N_6O_4$	1.6 TFA	NMR, MS, CHNb
2k	O	Et	1.9	>80	$C_{29}H_{39}N_5O_5$	0.7 TFA	NMR, MS, CHNc
2l	S	Et	41	>100	$C_{29}H_{39}N_5O_4S$	0.6 TFA	NMR, MS, CHN
2m	NH	*n*-Pr	7.8	>100	$C_{30}H_{42}N_6O_4$	1.2 TFA	NMR, MS, CHN
2n	O	*n*-Pr	7.7	>100	$C_{30}H_{41}N_5O_5$	0.3 TFA	NMR, MS, CHN
2p	S	*n*-Pr	36	>100	$C_{30}H_{41}N_5O_4S$	0.4 TFA	NMR, MS, CHN
2q	NH	*i*-Pr	0.73	>100	$C_{30}H_{42}N_6O_4$	1.6 TFA	NMR, MS, CHN
2r	O	*i*-Pr	2.5	>60	$C_{30}H_{41}N_5O_5$	0.9 TFA	NMR, MS, CHN
2s	S	*i*-Pr	11	>100	$C_{30}H_{41}N_5O_4S$		NMR, MS, HRMS
2t	NH	c-Pr	5.2	>100	$C_{30}H_{40}N_6O_4$		NMR, MS, HRMS
2u	O	c-Pr	3.7	65	$C_{30}H_{39}N_5O_5$	0.8 TFA	NMR, MS, CHN
2v	S	c-Pr	24	>100	$C_{30}H_{39}N_5O_4S$	1.2 TFA	NMR, MS, CHN

[a] IC_{50}'s calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M. [b] N calculated 11.69, observed 11.23. [c] N calculated 11.34, observed 11.92.

cycles are poor replacements for the amide bond framework; analogs **1a**−**d** are all essentially inactive. The data reported in Table 2 indicate that these dipeptide "mimetics" are also less-than-perfect surrogates for the C-terminal dipeptide. Studies by Banyu[4b] have determined that an aromatic side chain on the C-terminal amino acid provides optimal activity in the pseudotetrapeptide series, and that there is wide latitude in the variety of substitution which is tolerated at this position. Compounds **2a**−**c**, containing an aryl side chain consistent with this optimal substitution pattern, show little affinity for the ET_A or ET_B receptor. Our attempts to impart flexibility to the system by adding a methylene group as a spacer between the heterocycle and the aryl group (**2d**−**f**) met with little success. On the other hand, when the size of this side chain was reduced dramatically, leaving just a methyl group, the resultant imidazole **2g** and oxazole **2h** improved significantly in their ability to bind to the ET_A receptor. Affinity for the ET_B receptor remains low. To explore this result further, we prepared a series of analogs having larger aliphatic substituents on the heterocycle. While an ethyl group is also tolerated at this position with only a slight loss of ET_A affinity (**2j**,**k**), longer (**2m**−**p**) or branched (**2q**−**v**) chains result in large decreases in activity.

The nature of the core heterocycle also has a significant effect on potency in the binding assay. When the substituent is sufficiently small, both imidazoles and oxazoles are active, with the former tending to be slightly more potent than the latter. The corresponding thiazoles, however, are less active by several orders of magnitude. This surprising result likely reflects the expansion of the azole ring which occurs upon introduction of the larger sulfur atom. Importantly, none of the compounds in the series show any significant affinity for the ET_B receptor, suggesting that it may be possible to develop selective antagonists based upon these azole lead structures.

It is apparent that the flexible backbone of the Banyu−Fujisawa pseudotetrapeptides allows their C-terminal side chains to access a portion of the ET_A receptor which is inaccessible to our conformationally restricted analogs. The difference in steric requirements at these formally analogous positions is substantial; while groups as large as indole or naphthyl are favored in the earlier series,[4b] only the smallest alkyl substituents are tolerated in the analogs of the present study. The steric restrictions around the heterocyclic ring in compounds **2** are also emphasized by the lack of activity of the subtly larger thiazoles. To attempt to understand both of these surprising results, we turned to our model of the endothelin receptors.

Modeling Endothelin Receptor−Ligand Interactions

A three-dimensional model of the ET_A receptor has been developed by analogy with our earlier work on the dopamine D_1 and D_2 receptors.[6] Briefly, we began with the prediction that the receptor will associate with cell membranes to produce a bundle of seven transmembrane hydrophobic helical segments connected by three intracellular and three extracellular loops. This prediction is in accord with the current understanding of GPCR tertiary structure, as supported by high-resolution electron diffraction studies on the bacteriorhodopsin receptor.[7] The seven transmembrane helices, 20−25 residues in length, were identified through hydrophobicity analysis[8] of the primary human ET_A receptor sequence,[9] accounting for the presence of specific residues which serve as helix initiators or terminators. A series of highly-conserved sequences (e.g. $G_{97}N_{98}$ in helix I, $L_{122}AXXD_{126}$ in helix II, $D_{182}RY_{184}$ in helix III, W_{210} in helix IV, $C_{318}WXP_{321}$ in helix VI, and $N_{365}P_{366}$ in helix VII) facilitate the alignment of ET_A with other GPCR.

The essence of our model, and the way in which it differs from many other models of GPCR binding, is in the positioning of ligands within the putative binding domain. It is our hypothesis that, throughout the family of GPCR and regardless of whether an individual ligand functions as an agonist or antagonist, all ligands bind within the cleft formed between helices II, III, VI, and VII. This binding mode is exemplified in Figure 1 for the specific case of ET-1 in the ET_A receptor. The disulfide-linked portion of ET-1 remains in the extracellular space, where it may be involved in interactions with the extracellular loop regions or the N-terminal tail of the receptor (these regions are not specifically located in our model); only the C-terminal hexapeptide makes contact with the interior of the receptor.

A series of receptor−ligand interactions serve to stabilize the structure as indicated. $Lysine_{166}$, on helix III, forms a salt bridge with the carboxylate side chain of aspartate-18 of ET-1. This lysine residue is almost unique to the endothelin family of GPCR but is conserved among ET receptors. An adjacent residue, $glutamine_{165}$ on helix III, is involved in a series of important hydrogen bonds with the main chain of the native ligand ET-1; specifically, this residue appears to form good H-bonding interactions with the amide oxygens of the Cys-His and Asp-Ile amide bonds. The C-terminal carboxylate of ET interacts with the side chain carboxyl of Asp_{126} on helix II. Mutagenesis of this residue has been demonstrated[10] to disrupt function with little effect on the binding properties of the receptor. We believe that it is a conformational change in the side chain of this residue upon agonist binding that serves as the "trigger" to induce the signal transduction cascade. The two isoleucyl residues fit into well-defined hydrophobic pockets. Isoleucine-19 tucks between helices II and VII into a space defined by Val_{130}, Leu_{134}, Gly_{354}, Leu_{355}, and Ala_{357}. Isoleucine-20 fits between helices II and III, where it is bounded by Tyr_{129}, Val_{130}, and Val_{169}. The Trp-21 indole is bound between helices VI and VII in a hydrophobic region which includes an edge−face interaction with Phe_{320}, and the Leu-17 side chain fits into a large space between helices VI and VII near the top of the receptor.

Our model of the conformation of FR-139317 with the ET_A receptor (Figure 2) takes advantage of many of the same interactions as are described above for ET-1. The lysine residue on helix III forms a salt bridge with the terminal carboxylate of the Fujisawa compound; the adjacent glutamine again provides a lattice of critical backbone H-bonds, specifically with the urea C═O and the Leu-Trp amide NH. The leucyl side chain fills the hydrophobic pocket which is occupied by Ile-19 of ET-1, and the indole moiety of the D-tryptophyl residue replaces the Trp-21 indole in the VI-VII "aromatic" pocket, albeit with a significantly different orientation.

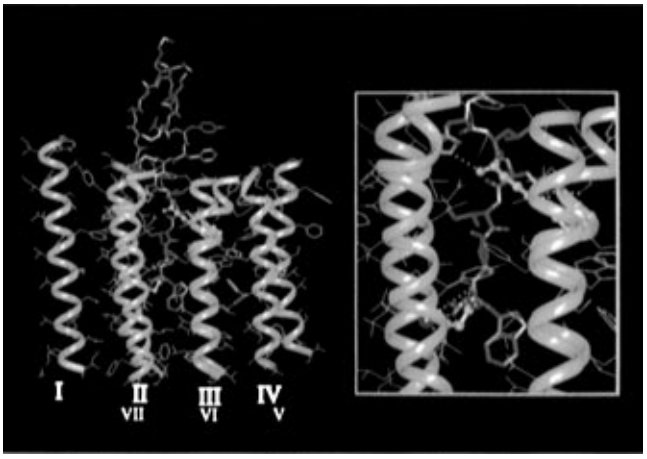


Figure 1. ET-1 in ET_A receptor.

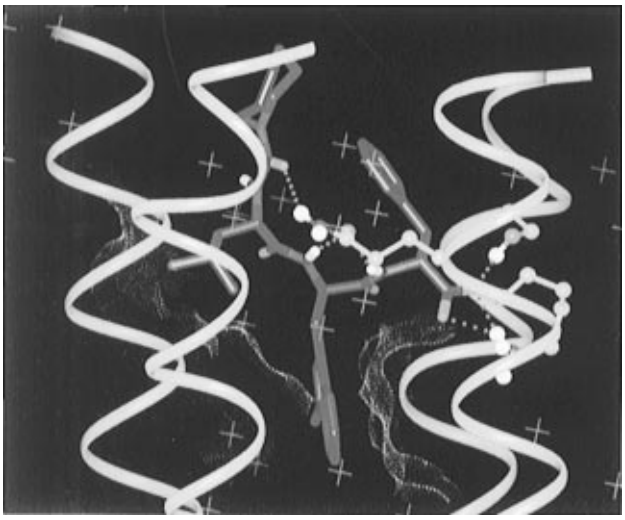


Figure 2. FR-139317 modeled into the II−III−VI−VII binding pocket, indicating critical ligand−receptor contacts.

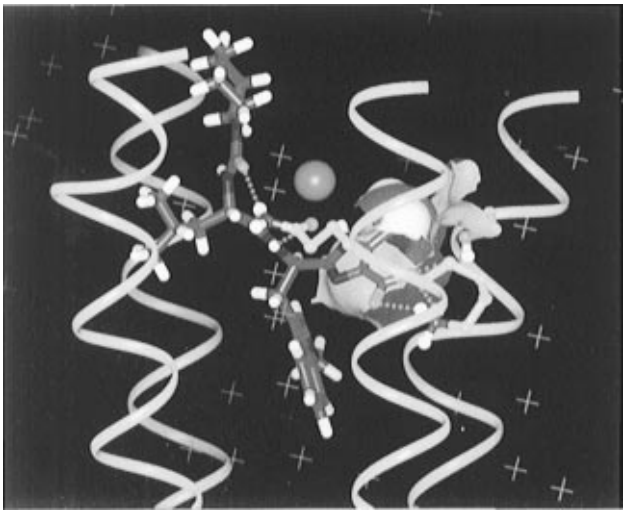


Figure 3. Imidazole **2g** modeled into the binding pocket. The C5-methyl group nests neatly against helix VI; the highlighted water molecule is within H-bonding distance of the imidazole NH.

The hydrophobic group of the urea positions itself in a nondescript hydrophobic cavity near the top of helix VII. Two consequences of this alignment are worthy of note. FR-139317 fails to penetrate deeply enough into the II−II−VI−VII pocket to interact with the side chain of Asp_{126}; thus, we would predict that the compound would function as an antagonist, as in fact it does. Our model also provides an explanation for the latitude of substitution which is permitted at the C-terminal amino acid in the Banyu−Fujisawa antagonist series. The orientation of this terminal C_β−Cγ bond positions a γ-substituent into the large open area at the extracellular interface of the receptor and directly between the four helices. Such a substituent encounters relatively few interactions with the receptor, either of a beneficial or detrimental nature.

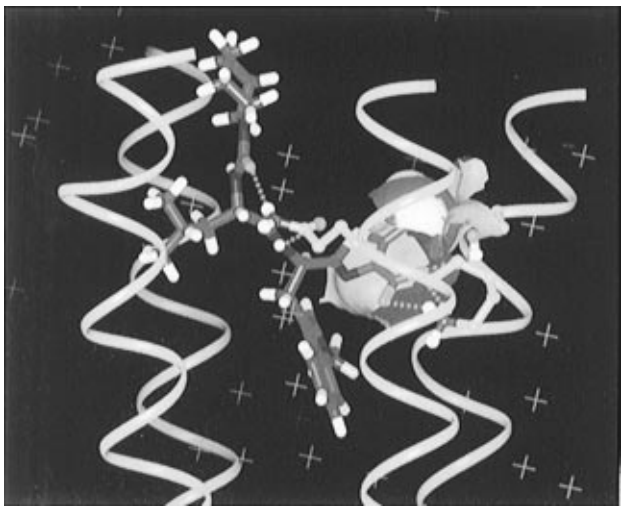


Figure 4. Thiazole **2i** modeled into the binding pocket. The methyl group no longer fits into the helix VI pocket; the water molecule has been displaced by the sulfur atom.

Rationalizing the Structure−Activity Profile

Figure 3 provides the corresponding view of our azole antagonists (specifically imidazole **2g**) in the ET_A receptor. To position this compound in the binding pocket, it was first overlayed with our earlier model of FR-139317 in regions where the two structures are homologous; the heterocycle was rotated to create a salt bridge between the terminal carboxylate and Lys_{166}, and the resultant receptor−ligand system was energy-minimized. Two aspects of this model-development process are important in evaluating the relevance of any predictive ability it might demonstrate: first, that this model of azole binding is merely an extension of the model developed for the Banyu−Fujisawa series; second, that *none of the information generated during our preliminary structure−activity study has been used in making this extension*.

The conversion of the Trp-X dipeptide to a heterocyclic surrogate has only subtle effects on the positioning of the amino-terminal functionality between the two series. While there is some shift of the backbone framework toward helices II and III in the azole series, corresponding minor adjustments in backbone and side chain torsion angles serve to locate the isobutyl and indolyl moieties and the perhydroazepinyl substituent into the same hydrophobic binding sites. The effects of rigidification on the position of the C-terminal residue are profound, however. In particular, it is worthwhile to evaluate these conformational restrictions in light of the unusual structure−activity profile observed in the azole antagonist series. The azole 5-methyl substituent, which is formally analogous to the γ-carbon of the C-terminal residue of FR-139317, is no longer able to

fold back into the large open space between the four helices. Rather, it is forced into direct contact with helix VI. As a rule such a close interaction would be strongly disfavored; however, in this case the methyl group finds a small hydrophobic "nest" comprised of the backbone and side chain atoms of Leu_{324} and Ser_{325} on helix VI. This hydrophobic pocket not only provides a set of beneficial contacts which help to stabilize a small alkyl substituent at this position on the azole ring but also its size and shape clearly indicate why larger 5-substitutients are *not* tolerated. Thus, it appears that our model of the ET_A receptor, and of the binding of compound **2g** within that receptor, is capable of "predicting" this surprising substituent effect with admirable accuracy.

The other significant surprise encountered in our early structure−activity studies was the dramatic decrease in binding which resulted when the imidazole (or oxazole) heterocyclic core was replaced with a thiazole (e.g., **2g** or **2h** → **2i**). To explore the effects of such a modification computationally, we began with the model developed for imidazole **2g**, directly substituted a sulfur atom for the imidazole NH, and reminimized the resultant receptor/ligand system. The results of this analysis are shown in Figure 4. The expansion of the azole ring which results from the introduction of the larger sulfur atom produces two effects, each of which is detrimental. First, the C5-methyl group is pushed further into the Leu_{324}-Ser_{325} pocket on helix VI, such that a beneficial interaction is no longer possible. This is obviously an unacceptable situation, and so, upon minimization, the alkyl group tips out of this pocket, with the backbone flexing to accommodate the new conformation. Secondly, the larger sulfur atom protrudes further into the solvated interior of the II−III−VI−VII pocket, displacing a putative water molecule and disrupting the internal solvation of the analog. The importance of such an effect is difficult to predict *a priori*. To estimate the magnitude of energy gained or lost through this perturbation, we have compared solvation energies for **2g** and **2i** as modeled, using three different solvation parameter sets. The results (see the Experimental Section for details) suggest that, on average, thiazole analog **2i** may have approximately 1.7 kcal/mol less solvation stabilization than the corresponding imidazole. It is clear that a combination of these two effects can readily account for the difference in binding energy (70× difference in binding affinity ∼2.6 kcal/mol) we observe between these two species. Once again, it appears that the model provides a neat explanation for an apparently surprising result.

Conclusions

We have developed a novel family of antagonists selective for the ET_A receptor by replacing the C-terminal dipeptide of the known peptidic antagonist FR-139317 with a heterocyclic dipeptide surrogate. While this replacement is formally isosteric, the resultant series exhibits a markedly different structure−activity profile than was previously reported for the parent structure. We have simultaneously developed a computer model of the ET_A receptor by analogy with our earlier work in the area of G-protein-coupled receptors and have applied this model to examine both the peptidic and peptidomimetic analog structures. Despite the fact that no structure−activity information was employed in these modeling efforts, the resultant model not only helps to explain why the two SAR profiles are different but also accounts for several surprising aspects of the profile of the new series. This "pseudopredictive" ability is noteworthy and suggests the possibility that the model may accurately reflect the details of ET/ET-R binding.

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; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, dimethylformamide; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; LiHMDS, lithium hexamethyldisilazide; NMM, *N*-methylmorpholine; Pal, 2-pyridylalanine; Pha, hexamethyleneimine (perhydroazepine); PPh_3, triphenylphosphine; pyr, pyridine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

General Synthesis of Compounds 1. Cbz-L-Leucyl-2-[(1-methylindolyl)-3-carbonyl]glycine Ethyl Ester (4, R_1 = 3-[1-methylindole]). 1-Methylindole-3-carboxylic acid (1.75 g) was suspended in 10 mL of CH_2Cl_2; 1.05 mL of oxalyl chloride was added (gas evolves), and the resultant solution was stirred at room temperature for 90 min. The solvents were removed *in vacuo*, and the residue was dried under vacuum to remove traces of reagent from the crude acid chloride, which was used without further purification. Simultaneously, *N*-(diphenylmethylene)glycine ethyl ester (2.68 g) was dissolved in THF (15 mL), and the solution was cooled to −78 °C. LiHMDS (10 mL, 1 N solution in THF) was added dropwise, and the resultant yellow slurry was stirred at −78 °C for 30 min. The slurry was transferred *via* cannula to a solution of the above acid chloride in THF (10 mL) at −78 °C. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirring was continued for 2 h. The reaction was then quenched with 2 N HCl (120 mL) and allowed to stir at room temperature for 1 h. The THF was evaporated, and the resultant aqueous solution was extracted with EtOAc (2 × 10 mL). The organic phases were reextracted with 2 N HCl and then discarded; the combined aqueous phase was concentrated *in vacuo*. The resultant slurry was treated with EtOH (20 mL) and filtered. The filtrate was concentrated *in vacuo* to give 2-[(1-methylindol-3-yl)carbonyl]glycine ethyl ester hydrochloride as a pinkish solid which was used without further purification. (Note: this material may be stored at −20 °C as the salt, but is unstable as the free base.) Cbz-L-leucine (2.65 g) was dissolved in THF (15 mL), and the solution was cooled to −20 °C. *N*-Methylmorpholine (1.11 mL, 1.0 equiv) was added, followed by the dropwise addition of isobutylchloroformate (1.30 mL). After the addition was complete, the reaction mixture was stirred for 30 min at −20 °C. The cooling bath was removed; the above salt was dissolved in DMF (20 mL) and added to the mixed anhydride. *N*-Methylmorpholine (1.2 mL) was added dropwise via syringe pump over a 1 h period. After the addition was complete, the reaction mixture was allowed to stir at room temperature for 15 h. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc. The organic layer was washed sequentially with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give an orange oil which was

purified by flash chromatography on silica gel eluting with a gradient of 1:2 → 1:1 EtOAc/hexane. The title compound was isolated as a yellowish oil (3.45 g, 68% overall yield).

2-[1(*S*)-[(Benzyloxycarbonyl)amino]-3-methylbutyl]-5-[3-(1-methylindolyl)]oxazole-4-carboxylic Acid Ethyl Ester (5O, R_1 = 3-[1-methylindole]). Compound **4** (500 mg) was dissolved in 3 mL of 1:1 CH_3CN/pyr; 0.2 mL of CCl_4, 0.5 mL of DBU, and 285 mg of PPh_3 were added sequentially, and the resultant solution was allowed to stir at room temperature for 18 h. The solvents were evaporated under reduced pressure, and the residue was dissolved in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated to give a yellowish oil which was purified by flash chromatography on silica gel eluting with 25% EtOAc/hexane to give the title compound (208 mg, 43% yield).

2-[1(*S*)-[(Benzyloxycarbonyl)amino]-3-methylbutyl]-5-[3-(1-methylindolyl)]thiazole-4-carboxylic Acid Ethyl Ester (5S, R_1 = 3-[1-methylindole]). Compound **4** above (300 mg) was dissolved in 4 mL of THF; 300 mg of Lawesson's reagent were added, and the resultant solution was heated at 80 °C for 4 h. The solvents were evaporated under reduced pressure, and the residue was dissolved in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated to give a yellowish oil which was purified by flash chromatography on silica gel eluting with 25% EtOAc/hexane to give the title compound (248 mg, 83% yield).

{**[2-[1(*S*)-[(Perhydroazepin-1-ylcarbonyl)amino]-3-methylbutyl]-5-[3-(1-methylindole)]oxazol-4-yl]carbonyl**}-D-**2-pyridylalanine (1a).** Compound **5O** described above (100 mg) was dissolved in 2 mL of THF; a solution of 50 mg of LiOH in 1 mL of water was added, and the resultant mixture was heated at 80 °C for 6 h. The mixture was acidified with 1 N H_3PO_4 and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude acid was taken up in 4 mL of 1:1 THF/DMF; 50 mg of *D*-Pal-OEt·HCl was added, followed by 27 mg of HOBt, 0.2 mL of NMM, and 39 mg of EDC. The resultant solution was stirred overnight. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc. The organic layer was washed sequentially with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give an orange oil which was purified by flash chromatography on silica gel eluting with a gradient of 2:1 → 3:1 EtOAc/hexane. The product (45 mg) was dissolved in 4 mL of EtOH; 20 mg of 10% Pd−C was added, and the mixture was purged with nitrogen. A balloon of hydrogen was placed on the reaction vessel, and the mixture was stirred at room temperature for 2 h. The vessel was again purged with nitrogen, and the mixture was filtered through Celite to remove the catalyst. The solvents were removed *in vacuo*, and the crude product was taken up in 1 mL of THF. Et_3N (0.1 mL) was added, followed by 20 mg of CDI; the reaction mixture was stirred at room temperature for 2 h. Perhydroazepine (0.2 mL) was added, and stirring was continued for 50 h. EtOAc was added; the reaction mixture was extracted with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated to give a yellowish oil. The crude product was dissolved in 1 mL of THF; a solution of 25 mg of LiOH in 0.5 mL water was added, and the resultant mixture was stirred at room temperature for 3.5 h. The solution was acidified with 1 N H_3PO_4, and the product was purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The desired fractions were lyophilized to give the product as a white solid: 21 mg; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.04 (t, 3H, J = 7), 1.07 (t, 3H, J = 7), 1.55 (m, 4H), 1.76 (m, 5H), 1.95 (m, 2H), 3.50 (m, 5H), 3.70 (dd, 1H, J = 6, 15), 3.87 (s, 3H), 5.07 (dd, 1H, J = 5, 9), 5.18 (d, 1H, J = 7, 9), 7.18 (ddd, 1H, J = 1, 7, 8), 7.28 (ddd, 1H, J = 1, 7, 8), 7.45 (d, 1H, J = 8), 7.73 (ddd, 1H, J = 1, 7, 8), 7.85 (d, 1H, J = 8), 8.12 (d, 1H, J = 8), 8.26 (dt, 1H, J = 1, 8), 8.55 (s, 1H), 8.67 (ddd, 1H, J = 1, 2, 6); MS (FAB/NBA) m/e 601 $(M + H)^+$. Anal. for $C_{33}H_{40}N_6O_5$·1.3TFA: C, H, N.

Also prepared by a similar procedure was the following.

{**[2-[1(*S*)-[(Perhydroazepin-1-ylcarbonyl)amino]-3-methylbutyl]-5-[[3-(1-methylindolyl)methyl]oxazol-4-yl]carbonyl**}-D-**2-pyridylalanine (1c):** ^{1}H NMR (CD_3OD, 300 MHz) δ 0.88 (d, 3H, J = 7), 0.92 (d, 3H, J = 7 Hz), 1.47 (m, 4H), 1.64 (m, 5H), 1.74 (m, 2H), 3.36 (m, 4H), 3.47 (dd, 1H, J = 9, 15 Hz), 3.71 (dd, 1H, J = 6, 15 Hz), 3.75 (s, 3H), 4.39, (AB, 2H, J = 15 Hz), 4.97 (dd, 1H, J = 6, 9 Hz), 5.08 (d, 1H, J = 7, 9 Hz), 6.99 (ddd, 1H, J = 1, 7, 8 Hz), 7.01 (s, 1H), 7.16 (ddd, 1H, J = 1, 7, 8 Hz), 7.29 (d, 1H, J = 8 Hz), 7.45 (d, 1H, J = 8 Hz), 7.70 (ddd, 1H, J = 1, 7, 8 Hz), 7.84 (d, 1H, J = 8 Hz), 8.24 (dt, 1H, J = 1, 8 Hz), 8.65 (ddd, 1H, J = 1, 2, 6 Hz); MS (FAB/NBA) m/e 615 $(M + H)^+$, 637 $(M + Na)^+$. Anal. for $C_{34}H_{42}N_6O_5$·1.5TFA: C, H, N.

{**[2-[1(*S*)-[(Perhydroazepin-1-ylcarbonyl)amino]-3-methylbutyl]-5-[3-(1-methylindolyl)]thiazol-4-yl]carbonyl**}-D-**2-pyridylalanine (1b).** Compound **5S** described above (90 mg) was dissolved in 2 mL of THF; a solution of 50 mg of LiOH in 1 mL of water was added, and the resultant mixture was heated at 80 °C for 4 h. The mixture was acidified with 1 N H_3PO_4 and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude acid was taken up in 4 mL of 1:1 THF/DMF; 50 mg of *D*-Pal-OEt·HCl was added, followed by 27 mg of HOBt, 0.2 mL of NMM, and 39 mg of EDC. The resultant solution was stirred overnight. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc. The organic layer was washed sequentially with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give an orange oil which was purified by flash chromatography on silica gel eluting with a gradient of 2:1 → 3:1 EtOAc/hexane. The product (64 mg) was dissolved in 2 mL of 30% HBr/HOAc and stirred at room temperature for 2 h. The solvents were removed *in vacuo*; the residue was taken up in saturated $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvents were removed *in vacuo*, and the crude product was taken up in 2 mL of THF. Et_3N (0.1 mL) was added, followed by 25 mg of CDI; the reaction mixture was stirred at room temperature for 2.5 h. Perhydroazepine (0.2 mL) was added, and stirring was continued for 15 h. EtOAc was added; the reaction mixture was extracted with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over Na_2SO_4, and evaporated to give a yellowish oil. The crude product was dissolved in 1 mL of THF; a solution of 40 mg of LiOH in 0.5 mL water was added, and the resultant mixture was stirred at room temperature for 3 h. The solution was acidified with 1 N H_3PO_4, and the product was purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The desired fractions were lyophilized to give the product as a white solid: 19 mg; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.05 (t, 6H, J = 7), 1.61 (m, 4H), 1.77 (m, 4H), 1.90 (m, 3H), 3.50 (m, 5H), 3.68 (dd, 1H, J = 6, 15), 3.83 (s, 3H), 4.98 (dd, 1H, J = 3, 9), 5.23 (d, 1H, J = 6, 9), 7.13 (ddd, ^{1}H, J = 1, 7, 8), 7.26 (ddd, 1H, J = 1, 7, 8), 7.44 (d, 1H, J = 10), 7.64 (d, 1H, J = 9), 7.73 (ddd, 1H, J = 1, 7, 8), 7.77 (s, 1H), 7.87 (d, 1H, J = 8), 8.28 (dt, 1H, J = 1, 8), 8.62 (ddd, 1H, J = 1, 2, 6); MS (FAB/NBA) m/e 617 $(M + H)^+$. Anal. for $C_{33}H_{40}N_6O_4S$·2.0TFA: C, H, N.

Also prepared by a similar procedure was the following.

{**[2-[1(*S*)-[(Perhydroazepin-1-ylcarbonyl)amino]-3-methylbutyl]-5-[[3-(1-methylindolyl)methyl]thiazol-4-yl]carbonyl**}-D-**2-pyridylalanine (1d).** ^{1}H NMR (CD_3OD, 300 MHz) δ 0.95 (t, 6H, J = 7 Hz), 1.43 (m, 4H), 1.60 (m, 4H), 1.76 (m, 3H), 3.33 (m, 4H), 3.52 (dd, 1H, J = 9, 15 Hz), 3.72 (dd, 1H, J = 6, 15 Hz), 3.75 (s, 3H), 4.58 (s, 2H), 5.06 (dd, 1H, J = 1, 7 Hz), 5.09 (d, 1H, J = 7 Hz), 6.97 (ddd, 1H, J = 1, 7, 8 Hz), 7.04 (s, 1H), 7.15 (ddd, 1H, J = 1, 7, 8 Hz), 7.32 (d, 1H, J = 10 Hz), 7.35 (d, 1H, J = 9 Hz), 7.74 (ddd, 1H, J = 1, 7, 8 Hz), 7.87 (d, 1H, J = 8 Hz), 8.30 (dt, 1H, J = 1, 8 Hz), 8.69 (ddd, 1H, J = 1, 2, 6 Hz); MS (FAB/CH_3OH) m/e 631 $(M + H)^+$, 669 $(M + K)^+$. Anal. Calcd for $C_{34}H_{42}N_6O_4S$·1.5TFA: C, H, N.

General Synthesis of Compounds 2. Cbz-D-tryptophanyl-2-acetylglycine Ethyl Ester (7, R_2 = Me). *N*-(Diphenylmethylene)glycine ethyl ester (30.0 g) was dissolved in THF (125 mL) and the solution cooled to −78 °C. LiHMDS (100 mL, 1 N solution in THF) was added slowly over 10 min, and

the resultant yellow slurry was stirred at −78 °C for 45 min. The slurry was transferred *via* cannula to a solution of acetyl chloride (8.4 mL) in THF (50 mL) at −78 °C. Additional THF (250 mL) was added to the anion solution to facilitate transfer to the acetyl chloride solution. After the addition was complete, the reaction mixture was allowed to warm to room temperature, and stirring was continued for 4 h. The reaction was then quenched with 2 N HCl (115 mL). The THF was evaporated, and the resultant aqueous solution was extracted with EtOAc (2 × 100 mL). The organic phases were discarded, and the aqueous phase was concentrated *in vacuo*. The resultant slurry was treated with EtOH (150 mL) and filtered. The filtrate was concentrated *in vacuo* to give 2-acetylglycine ethyl ester hydrochloride as a yellow solid which was used without further purification. (Note: this material may be stored at −20 °C as the salt, but is unstable as the free base.) Cbz-D-tryptophan (40.6 g) was dissolved in THF (100 mL), and the solution was cooled to −20 °C. *N*-Methylmorpholine (13 mL, 1.0 equiv) was added, followed by the dropwise addition of isobutyl chloroformate (15.6 mL). After the addition was complete, the reaction mixture was stirred for 30 min at −20 °C. The cooling bath was removed; the above salt was dissolved in DMF (50 mL) and added to the mixed anhydride. *N*-Methylmorpholine (13 mL) was added dropwise via syringe pump over a 1 h period. After the addition was complete, the reaction mixture was allowed to stir at room temperature for 1 h. Water (200 mL) was added, and the layers were separated. The organic layer was washed sequentially with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give an orange oil which was purified by flash chromatography on silica gel eluting with 15% EtOAc/hexane. The title compound was isolated as an orange oil (30.7 g, 59% yield overall). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (dt, 3H, J = 1, 7 Hz), 2.24 (s, 1.5H), 2.30 (s, 1.5H), 3.20 (m, 1H), 3.35 (m, 1H), 4.22 (dq, 2H, J = 1, 7 Hz), 4.50 (m, 1H), 5.06 (dd, 1H, J = 7, 8 Hz), 5.12 (s, 2H), 5.45 (m, 1H), 6.82 (m, 1H), 7.23 (m, 9H), 7.65 (m, 1H), 8.10 (s, 1H); MS (DCI/NH_3) m/e 466 (M + H)$^+$, 483 (M + NH_4)$^+$.

(Perhydroazepin-1-ylcarbonyl)leucine. Leucine benzyl ester *p*-toluenesulfonate (7.88 g) was dissolved in THF (40 mL). Et_3N (2.92 mL) was added, and the solution was cooled to 0 °C in an ice bath. Carbonyldiimidazole (3.40 g) was added and the solution stirred at 0 °C for 1 h. The cooling bath was removed, and the solution was stirred an additional 1 h at room temperature. Perhydroazepine (2.68 mL) was added, and the resultant solution was stirred overnight at room temperature. The reaction mixture was poured onto 400 mL of water; the mixture was stirred for 30 min, and a white solid was collected by filtration. The crude ester was dissolved in MeOH (150 mL), 10% Pd/C (0.9 g) was added, and the mixture was purged with a stream of nitrogen gas and then placed under a ballon of hydrogen. After stirring at room temperature for 2 h, the reaction mixture was again purged with nitrogen. The solvent was removed i*n 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 (5.06 g, 98%).

2-{(1*R*)-1-[(Benzyloxycarbonyl)amino]-2-(indol-3-yl)-ethyl}-5-methylimidazole-4-carboxylic Acid Ethyl Ester (8N, X = NH, R_2 = Me). Compound **7** described above (5.0 g) was dissolved in acetic acid (15 mL). Ammonium acetate (4.0 g) was added, and the mixture was heated at reflux for 16 h. After cooling, the solvent was evaporated under reduced pressure and the residue was taken up in saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The resultant orange oil was purified by flash chromatography on silica gel eluting with 25% EtOAc/hexane to afford 3.60 g (73%) of the title compound. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, 3H, J = 7 Hz), 2.43 (s, 3H), 3.37 (m, 1H), 3.47 (m, 1H), 4.27 (q, 2H, J = 7 Hz), 5.03 (s, 2H), 5.07 (m, 1H), 5.26 (br s, 1H), 6.82 (s, 1H), 7.04 (t, 1H, J = 8 Hz), 7.16 (t, 1H, J = 8 Hz), 7.30 (m, 5H), 7.50 (d, 1H, J = 8 Hz), 8.05 (s, 1H); MS (DCI/NH_3) m/e 447 (M + H)$^+$.

2-{1(*R*)-[(Benzyloxycarbonyl)amino]-2-(indol-3-yl)ethyl}-5-methyloxazole-4-carboxylic Acid Ethyl Ester (8O, X = O, R_2 = Me). Compound **7** described above (4.44 g) was dissolved in acetonitrile (15 mL). Pyridine (25 mL), carbon tetrachloride (2 mL), DBU (2.90 g), and PPh_3 (2.75 g) were added, and the mixture was stirred for 16 h at room temperature. The solvents were evaporated under reduced pressure, and the residue was dissolved in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated to give an off-white semisolid which was purified by flash chromatography on silica gel eluting with 15% EtOAc/hexane to give the title compound (2.83 g, 66% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (t, 3H, J = 7 Hz), 2.50 (s, 3H), 3.40 (d, 2H, J = 8 Hz), 4.37 (q, 2H, J = 7 Hz), 5.08 (s, 2H), 5.28 (m, 1H), 5.48 (m, 1H), 6.89 (s, 1H), 7.05 (t, 1H, J = 8 Hz), 7.16 (t, 1H, J = 8 Hz), 7.35 (m, 7H), 8.06 (s, 1H); MS (DCI/NH_3) m/e 448 (M + H)$^+$, 465 (M + NH_4)$^+$.

2-{1(*R*)-[(Benzyloxycarbonyl)amino]-2-(indol-3-yl)ethyl}-5-methylthiazole-4-carboxylic Acid Ethyl Ester (8S, X = S, R_2 = Me). Compound **7** described above (0.335 g) was dissolved in THF (5 mL). Lawesson's reagent (0.45 g) was added, and the mixture was stirred at reflux for 5 h. The solvent was evaporated under reduced pressure, and the residue was taken up in EtOAc (20 mL). 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 yellow oil which was purified by flash chromatography eluting with 15% EtOAc/hexane to give the product as a white solid (155 mg, 46%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (t, 3H, J = 7 Hz), 2.65 (s, 3H), 3.42 (m, 1H), 3.55 (m, 1H), 4.43 (q, 2H, J = 7 Hz), 5.09 (s, 2H), 5.38 (m, 1H), 5.63 (br s, 1H), 6.90 (s, 1H), 7.07 (t, 1H, J = 8 Hz), 7.18 (t, 1H, J = 8 Hz), 7.32 (m, 6H), 7.52 (1H, d, J = 8 Hz), 8.01 (s, 1H); MS (DCI/NH_3) m/e 464 (M + H)$^+$, 481 (M + NH_4)$^+$.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-methylimidazole-4-carboxylic Acid (2g). Imidazole **8N** described above (90 mg) was dissolved in EtOH (50 mL). The solution was purged of oxygen, 10% Pd/C (0.5 g) was added, and the mixture was stirred at room temperature under an atmosphere of hydrogen. After 2 h the catalyst was removed by filtration, and the solvent was evaporated *in vacuo* to give a white solid. This crude material was dissolved in THF (2 mL). HOBt (30 mg), the above-described leucine derivative (55 mg), and EDC (42 mg) were added. *N*-Methylmorpholine (0.1 mL) and DMF (1 mL) were added, and the mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, and the residue was taken up in EtOAc. The solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over $MgSO_4$, and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography on silica gel eluting with 50% EtOAc/hexane. This material was dissolved in THF (2 mL), and a solution of LiOH (50 mg) in H_2O (1 mL) was added. The mixture was heated in a Carius tube at 100 °C for 15 h. The solvents were evaporated under reduced pressure, and the residue was taken up in 1 N H_3PO_4 (5 mL). The suspension was dissolved in water and acetonitrile, and the product was purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The desired fractions were lyophilized to give the product as a white solid: 40 mg; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.77 (d, 3H, J = 7 Hz), 0.82 (d, 3H, J = 7 Hz), 1.30 (m, 2H), 1.52 (m, 5H), 1.66 (m, 4H), 2.52 (s, 3H), 3.4 (m, 6H), 3.59 (dd, 1H, J = 7, 15 Hz), 4.07 (dd, 1H, J = 5, 9 Hz), 5.42 (dd, 1H, J = 6, 8 Hz), 7.01 (ddd, 1H, J = 1, 7, 8 Hz), 7.11 (ddd, 1H, J = 1, 7, 8 Hz), 7.12 (s, 1H), 7.36 (d, 1H, J = 8 Hz), 7.45 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 523 (M + H)$^+$. Anal. for $C_{28}H_{38}N_6O_4 \cdot 1.5$TFA: C, H, N.

The following compounds are prepared using the procedures described above for compound **2g**:

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-phenylimidazole-4-carboxylic acid (2a): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.80 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 6 Hz), 1.2−1.45 (m, 10H), 3.2 (m, 4H), 3.43 (dd, 1H, J = 8, 15 Hz), 3.52 (dd, 1H, J = 6, 7 Hz), 3.62 (dd,

1H, J = 6, 15 Hz), 4.13 (t, 1H, J = 7 Hz), 5.43 (dd, 1H, J = 6, 8 Hz), 6.96 (ddd, 1H, J = 1, 7, 8 Hz), 7.10 (ddd, 1H, J = 2, 7, 8 Hz), 7.14 (s, 1H), 7.36 (ddd, 1H, J = 1, 2, 8 Hz), 7.45 (m, 4H), 7.69 (dt, 1H, J = 2, 8 Hz), 7.57 (dd, 1H, J = 2, 7 Hz); MS (FAB + NBA) m/e 585 $(M + H)^+$, 647 $(M + Cu)^+$. Anal. for $C_{33}H_{40}N_6O_4 \cdot 1.25TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-benzylimidazole-4-carboxylic acid (2d): ^1H NMR (CD_3OD, 300 MHz) δ 0.81 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 6 Hz), 1.36 (m, 1H), 1.51 (m, 5H), 1.63 (m, 5H), 3.2−3.6 (m, 7H), 4.09 (dd, 1H, J = 6, 8 Hz), 4.24 (d, 1H, J = 15 Hz), 4.36 (d, 1H, J = 15 Hz), 5.37 (dd, 1H, J = 7, 8 Hz), 6.94 (ddd, 1H, J = 1, 7, 8 Hz), 7.04 (s, 1H), 7.1 (d, 2H, J = 8 Hz), 7.2−7.3 (m, 4H), 7.34 (dd, 2H, J = 1, 8 Hz). MS (FAB + G/SG) m/e 599 $(M + H)^+$. Anal. Calcd for $C_{34}H_{42}N_6O_4 \cdot 1.75TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-ethylimidazole-4-carboxylic acid (2j): ^1H NMR (CD_3OD, 300 MHz) δ 0.81 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 7 Hz), 1.15 (t, 3H, J = 8 Hz), 1.3−1.4 (m, 2H), 1.53 (m, 5H), 1.67 (m, 4H), 2.9 (m, 2H), 3.3−3.6 (m, 6H), 4.10 (dd, 1H, J = 6, 8 Hz), 5.36 (dd, 1H, J = 7, 8 Hz), 6.98 (ddd, 1H, J = 1, 7, 8 Hz), 7.10 (ddd, 1H, J = 1, 7, 8 Hz), 7.12 (s, 1H), 7.36 (d, 2H, J = 8 Hz); MS (DCI/NH_3) m/e 537 $(M + H)^+$. Anal. Calcd for $C_{29}H_{40}N_6O_4 \cdot 1.6TFA$: C, H; N: calcd, 11.69; found, 11.23.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-propylimidazole-4-carboxylic acid (2m): ^1H NMR (CD_3OD, 300 MHz) δ 0.79 (d, 3H, J = 6 Hz), 0.84 (d, 3H, J = 7 Hz), 0.88 (t, 3H, J = 8 Hz), 1.3−1.4 (m, 2H), 1.53 (m, 5H), 1.60 (m, 2H), 1.68 (m, 4H), 2.94 (m, 2H), 3.3−3.6 (m, 6H), 4.10 (dd, 1H, J = 7, 9 Hz), 5.35 (dd, 1H, J = 7, 8 Hz), 6.97 (ddd, 1H, J = 1, 7, 8 Hz), 7.07 (s, 1H), 7.10 (ddd, 1H, J = 1, 7, 8 Hz), 7.35 (d, 1H, J = 8 Hz), 7.40 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 551 $(M + H)^+$. Anal. for $C_{30}H_{42}N_6O_4 \cdot 1.2TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-isopropylimidazole-4-carboxylic acid (2q): ^1H NMR (CD_3OD, 300 MHz) δ 0.85 (d, 3H, J = 6 Hz), 0.87 (d, 3H, J = 6 Hz), 1.10 (d, 3H, J = 7 Hz), 1.21 (d, 3H, J = 7 Hz), 1.4−1.5 (m, 2H), 1.52 (m, 5H), 1.68 (m, 4H), 3.3−3.5 (m, 6H), 3.72 (m, 1H), 4.10 (dd, 1H, J = 6, 10 Hz), 5.29 (t, 1H, J = 6 Hz), 6.96 (ddd, 1H, J = 1, 7, 8 Hz), 7.09 (s, 1H), 7.12 (ddd, 1H, J = 1, 7, 8 Hz), 7.24 (d, 1H, J = 8 Hz), 7.35 (td, 1H, J = 1, 8 Hz); MS (FAB + NBA) m/e 551 $(M + H)^+$, 573 $(M + Na)^+$. Anal. Calcd for $C_{30}H_{42}N_6O_4 \cdot 1.6TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-cyclopropylimidazole-4-carboxylic acid (2t): ^1H NMR (CD_3OD, 300 MHz) δ 0.85 (d, 3H, J = 6 Hz), 0.88 (d, 3H, J = 6 Hz), 1.08 (d, 3H, J = 7 Hz), 1.13 (d, 3H, J = 6 Hz), 1.5−1.8 (m, 11H), 3.3−3.5 (m, 6H), 3.72 (m, 1H), 4.09 (dd, 1H, J = 6, 10 Hz), 5.38 (t, 1H, J = 7 Hz), 6.96 (ddd, 1H, J = 1, 7, 8 Hz), 7.07 (s, 1H), 7.09 (ddd, 1H, J = 1, 7, 8 Hz), 7.22 (d, 1H, J = 8 Hz), 7.35 (dd, 1H, J = 1, 8 Hz); MS (FAB + NBA) m/e 549 $(M + H)^+$; HRMS calcd for $C_{30}H_{41}N_6O_4$ 549.3189, found 549.3210.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-methyloxazole-4-carboxylic Acid (2h). Oxazole **8O** described above (90 mg) was dissolved in EtOH (5 mL), and 10% Pd/C (50 mg) was added. The mixture was purged of oxygen and stirred under a balloon of hydrogen for 5 h. The solvent was removed i*n vacuo* and the residue taken up in EtOAc and filtered through Celite to remove the catalyst. The solvent was evaporated to give the amino ester as a yellow oil. This crude material was dissolved in THF (2 mL). HOBt (30 mg), the above-described leucine derivative (55 mg), and EDCI (42 mg) were added. *N*-Methylmorpholine (0.1 mL) was added, and the resultant solution was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, and the residue was taken up in EtOAc. This solution was washed with saturated $NaHCO_3$ solution, 1 N H_3PO_4, and brine, dried over Na_2SO_4, and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography on silica gel eluting with 50% EtOAc−hexane. To this material dissolved in THF (2 mL) was added a solution of LiOH (50 mg) in H_2O (1 mL); the resultant mixture was stirred at room temperature for 15 h, at which point it had become homogeneous. The solvents were evaporated under reduced pressure, and the residue was neutralized with 1 N H_3PO_4 and purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The desired fractions were lyophilized to give the product as a white solid: 66 mg; ^1H NMR (CD_3OD, 300 MHz) δ 0.87 (d, 3H, J = 7 Hz), 0.88 (d, 3H, J = 7 Hz), 1.43 (m, 2H), 1.52 (m, 5H), 1.67 (m, 4H), 2.55 (s, 3H), 3.25−3.5 (m, 6H), 4.34 (dd, 1H, J = 6, 9 Hz), 5.40 (t, 1H, J = 7 Hz), 6.95 (ddd, 1H, J = 1, 7, 8 Hz), 6.99 (s, 1H), 7.07 (ddd, 1H, J = 1, 7, 8 Hz), 7.31 (td, 1H, J = 1, 8 Hz), 7.37 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 524 $(M + H)^+$, 541 $(M + NH_4)^+$. Anal. for $C_{28}H_{37}N_5O_5 \cdot 0.4TFA$: C, H, N.

The following compounds are prepared using the procedures described above for compound **2h**:

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-phenyloxazole-4-carboxylic acid (2b): ^1H NMR ($CDCl_3$, 300 MHz) δ 0.84 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 6 Hz), 1.47 (m, 7H), 1.62 (m, 4H), 3.2−3.4 (m, 5H), 3.47 (d, 2H, J = 7 Hz), 4.40 (br q, 1H, J = 8 Hz), 4.87 (br d, 1H, J = 8 Hz), 5.60 (q, 1H, J = 8 Hz), 7.02 (d, 1H, J = 1 Hz), 7.07 (t, 1H, J = 8 Hz), 7.16 (t, 1H, J = 8 Hz), 7.33 (d, 1H, J = 8 Hz), 7.40 (m, 4H), 7.53 (d, 1H, J = 8 Hz), 7.94 (m, 2H), 8.13 (br s, 1H); MS (FAB + NBA) m/e 586 $(M + H)^+$; HRMS calcd for $C_{33}H_{40}N_5O_5$ 586.3029, found 586.3033.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-benzyloxazole-4-carboxylic acid (2e): ^1H NMR ($CDCl_3$, 300 MHz) δ 0.84 (d, 3H, J = 6 Hz), 0.85 (d, 3H, J = 6 Hz), 1.50 (m, 7H), 1.66 (m, 4H), 3.2−3.5 (m, 6H), 4.18 (d, 1H, J = 15 Hz), 4.32 (d, 1H, J = 15 Hz), 4.45 (br q, 1H, J = 8 Hz), 4.94 (br d, 1H, J = 8 Hz), 5.46 (q, 1H, J = 8 Hz), 6.70 (d, 1H, J = 2 Hz), 7.03 (dt, 1H, J = 1, 8 Hz), 7.14 (dt, 1H, J = 1, 8 Hz), 7.19 (m, 2H), 7.26 (m, 5H), 7.37 (br s, 1H), 7.46 (d, 1H, J = 8 Hz), 7.88 (br s, 1H); MS (FAB + NBA) m/e 600 $(M + H)^+$, 622 $(M + Na)^+$. Anal. for $C_{34}H_{41}N_5O_5 \cdot 0.6TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-ethyl-oxazole-4-carboxylic acid (2k): ^1H NMR (CD_3OD, 300 MHz) δ 0.87 (d, 3H, J = 7 Hz), 0.88 (d, 3H, J = 7 Hz), 1.14 (t, 3H, J = 8 Hz), 1.4 (m, 2H), 1.54 (m, 5H), 1.68 (m, 4H), 2.94 (dq, 2H, J = 2, 8 Hz), 3.2−3.5 (m, 6H), 4.37 (dd, 1H, J = 6, 10 Hz), 5.39 (t, 1H, J = 6 Hz), 6.94 (ddd, 1H, J = 1, 7, 8 Hz), 6.98 (s, 1H), 7.07 (ddd, 1H, J = 1, 7, 8 Hz), 7.33 (td, 1H, J = 1, 8 Hz), 7.34 (dd, 1H, J = 1, 8 Hz); MS (FAB/NBA) m/e 538 $(M + H)^+$. Anal. for $C_{29}H_{39}N_5O_5 \cdot 0.7TFA$: C, H; N: calcd 11.34, found 11.92.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-propyloxazole-4-carboxylic acid (2n): ^1H NMR (CD_3OD, 300 MHz) δ 0.85 (d, 3H, J = 7 Hz), 0.86 (d, 3H, J = 7 Hz), 0.90 (t, 3H, J = 8 Hz), 1.4−1.5 (m, 2H), 1.53 (m, 5H), 1.60 (m, 2H), 1.68 (m, 4H), 2.94 (dt, 2H, J = 2, 8 Hz), 3.3−3.6 (m, 6H), 4.36 (dd, 1H, J = 6, 10 Hz), 5.38 (t, 1H, J = 6 Hz), 6.95 (ddd, 1H, J = 1, 7, 8 Hz), 6.97 (s, 1H), 7.07 (ddd, 1H, J = 1, 7, 8 Hz), 7.30 (d, 1H, J = 8 Hz), 7.35 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 552 $(M + H)^+$, 569 $(M + NH_4)^+$. Anal. for $C_{30}H_{41}N_5O_5 \cdot 0.3TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-isopropyloxazole-4-carboxylic Acid (2r): ^1H-NMR (CD_3OD, 300 MHz) δ 0.87 (d, 3H, J = 6 Hz), 0.89 (d, 3H, J = 6 Hz), 1.15 (d, 3H, J = 7 Hz), 1.18 (d, 3H, J = 7 Hz), 1.4−1.5 (m, 2H), 1.52 (m, 5H), 1.68 (m, 4H), 3.3−3.5 (m, 6H), 3.69 (m, 1H), 4.38 (dd, 1H, J = 6, 10 Hz), 5.37 (t, 1H, J = 6 Hz), 6.93 (ddd, 1H, J = 1, 7, 8 Hz), 6.97 (s, 1H), 7.06 (ddd, 1H, J = 1, 7, 8 Hz), 7.30 (d, 2H, J = 9 Hz); MS (DCI/NH_3) m/e 552 $(M + H)^+$, 569 $(M + NH_4)^+$. Anal. for $C_{30}H_{41}N_5O_5 \cdot 0.9TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-cyclopropyloxazole-4-carboxylic acid (2u): ^1H NMR (CD_3OD, 300 MHz) δ 0.84 (d, 3H, J = 6 Hz), 0.86 (d, 3H, J = 6 Hz), 0.87 (m, 2H), 1.04 (dd, 2H, J = 3, 9 Hz), 1.4 (m, 2H), 1.53 (m, 5H), 1.68 (m, 4H), 2.65 (m, 1H), 3.3−3.6 (m, 6H), 4.34 (dd, 1H, J = 6, 10 Hz), 5.34 (t, 1H, J = 7 Hz), 6.95 (ddd, 1H, J = 1, 7, 8 Hz), 6.97 (s, 1H), 7.07 (ddd, 1H, J = 1, 7, 8 Hz), 7.32 (d, 1H, J = 8 Hz), 7.34 (dd, 1H, J = 1, 8 Hz); MS (DCI/NH_3) m/e 550 $(M + H)^+$, 567 $(M + NH_4)^+$. Anal. for $C_{30}H_{39}N_5O_5 \cdot 0.8TFA$: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-methylthiazole-4-carboxylic Acid (2i). Thiazole **8S** described above (72 mg, 0.15 mmol) was dissolved in 2 mL of 30% HBr in HOAc and stirred at ambient temperature for 3 h. The solvents were removed *in vacuo*; the residue was taken up in saturated sodium bicarbonate solution and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4. The solvents were removed *in vacuo*, and the crude product was dissolved in THF (2 mL). HOBt (30 mg), the above-described leucine derivative (55 mg), and EDCI (42 mg) were added. *N*-Methylmorpholine (0.1 mL) was 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 an orange oil which was purified by flash chromatography on silica gel eluting with 50% EtOAc/hexane. This material was dissolved in THF (2 mL), a solution of LiOH (50 mg) in H_2O (1 mL) was added, and the mixture was warmed at 80 °C for 10 h. The solvents were evaporated under reduced pressure; the residue was neutralized with 1 N H_3PO_4 and purified by preparative HPLC (Vydac μC18) eluting with a 10−70% gradient of CH_3CN in 0.1% TFA. The desired fractions were lyophilized to give the product as a white solid: 54 mg; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.80 (d, 3H, J = 8 Hz), 0.82 (d, 3H, J = 8 Hz), 1.25−1.45 (m, 3H), 1.51 (m, 4H), 1.68 (m, 5H), 2.68 (s, 3H), 3.2−3.45 (m, 6H), 4.29 (dd, 1H, J = 6, 8 Hz), 5.50 (dd, 1H, J = 6, 8 Hz), 6.97 (dt, 1H, J = 1, 7 Hz), 7.04 (s, 1H), 7.08 (dt, 1H, J = 1, 7 Hz), 7.31 (d, 1H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz); MS (DCI/NH_3) m/e 540 (M + H)$^+$, 557 (M + NH_4)$^+$. Anal. for $C_{28}H_{37}N_5SO_4$·1.0TFA: C, H, N.

The following compounds are prepared using the procedures described above for compound **2i**:

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-phenyl-thiazole-4-carboxylic acid (2c): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (d, 3H, J = 6 Hz), 0.87 (d, 3H, J = 6 Hz), 1.45 (m, 6H), 1.62 (m, 5H), 3.30 (m, 5H), 3.37 (dd, 1H, J = 7, 15 Hz), 3.48 (dd, 1H, J = 7 , 15 Hz), 4.32 (br q, 1H, J = 8 Hz), 4.70 (br d, 1H, J = 8 Hz), 5.60 (br q, 1H, J = 8 Hz), 7.06 (d, 1H, J = 2 Hz), 7.12 (dt, 1H, J = 1, 8 Hz), 7.20 (dt, 1H, J = 1, 8 Hz), 7.36 (m, 5H), 7.47 (m, 2H), 7.58 (d, 1H, J = 8 Hz), 8.12 (br s, 1H); MS (DCI/NH_3) m/e 602 (M + H)$^+$. Anal. for $C_{33}H_{39}N_5O_4S$·1.0TFA: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-benzylthiazole-4-carboxylic acid (2f): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (d, 3H, J = 6 Hz), 0.87 (d, 3H, J = 6 Hz), 1.48 (m, 6H), 1.63 (m, 5H), 3.24 (m, 4H), 3.34 (m, 3H), 4.32 (br q, 1H, J = 8 Hz), 4.52 (s, 1H), 4.57 (br d, 1H, J = 8 Hz), 5.50 (br q, 1H, J = 8 Hz), 6.93 (d, 1H, J = 2 Hz), 7.07 (dt, 1H, J = 1, 8 Hz), 7.18 (m, 2H), 7.26 (m, 5H), 7.34 (dd, 1H, J = 7, 8 Hz), 7.50 (d, 1H, J = 8 Hz), 8.03 (br s, 1H); MS (FAB + NBA) m/e 616 (M + H)$^+$, 638 (M + Na)$^+$, 678 (M + Cu)$^+$; HRMS calcd for $C_{34}H_{42}N_5O_4S$: 616.2958, found: 616.2943.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-ethylthiazole-4-carboxylic acid (2l): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.82 (d, 3H, J = 7 Hz), 0.83 (d, 3H, J = 7 Hz), 1.24 (t, 3H, J = 8 Hz), 1.4 (m, 2H), 1.52 (m, 5H), 1.67 (m, 4H), 3.18 (q, 2H, J = 8 Hz), 3.2−3.5 (m, 6H), 4.30 (dd, 1H, J = 6, 10 Hz), 5.49 (dd, 1H, J = 6, 8 Hz), 6.94 (ddd, 1H, J = 1, 7, 8 Hz), 7.04 (s, 1H), 7.07 (ddd, 1H, J = 1, 7, 8 Hz), 7.32 (d, 1H, J = 8 Hz), 7.47 (dd, 1H, J = 1, 8 Hz); MS (DCI/NH_3) m/e 554 (M + H)$^+$, 551 (M + NH_4)$^+$. Anal. for $C_{29}H_{39}N_5O_4S$·0.6TFA: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-propylthiazole-4-carboxylic acid (2p): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.82 (d, 3H, J = 7 Hz), 0.83 (d, 3H, J = 7 Hz), 0.93 (t, 3H, J = 8 Hz), 1.4 (m, 2H), 1.52 (m, 5H), 1.67 (m, 6H), 3.14 (t, 2H, J = 8 Hz), 3.2−3.5 (m, 6H), 4.30 (dd, 1H, J = 6, 10 Hz), 5.50 (dd, 1H, J = 6, 8 Hz), 6.95 (ddd, 1H, J = 1, 7, 8 Hz), 7.04 (s, 1H), 7.06 (ddd, 1H, J = 1, 7, 8 Hz), 7.31 (d, 1H, J = 8 Hz), 7.46 (dd, 1H, J = 1, 8 Hz); MS (DCI/NH_3) m/e 568 (M + H)$^+$. Anal. for $C_{30}H_{41}N_5O_4S$·0.4TFA: C, H, N.

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-isopropylthiazole-4-carboxylic acid (2s): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.83 (d, 3H, J = 7 Hz), 0.84 (d, 3H, J = 7 Hz), 1.24 (d, 3H, J = 8 Hz), 1.25 (d, 3H, J = 8 Hz), 1.3−1.4 (m, 2H), 1.53 (m, 5H), 1.68 (m, 4H), 2.94 (m, 2H), 3.3−3.5 (m, 6H), 4.09 (septet, 1H, J = 7 Hz), 4.30 (dd, 1H, J = 6, 10 Hz), 5.49 (dd, 1H, J = 6, 8 Hz), 6.94 (ddd, 1H, J = 1, 7, 8 Hz), 7.07 (s, 1H), 7.08 (ddd, 1H, J = 1, 7, 8 Hz), 7.30 (d, 1H, J = 8 Hz), 7.40 (dd, 1H, J = 1, 8 Hz); MS (DCI/NH_3) m/e 568 (M + H)$^+$; HRMS calcd for $C_{30}H_{42}N_5O_4S$ 568.2958, found 568.2958 .

2-{1(*R*)-[(Perhydroazepin-1-ylcarbonyl)leucylamino]-2-(indol-3-yl)ethyl}-5-cyclopropylthiazole-4-carboxylic acid (2v): ^{1}H NMR (CD_3OD, 300 MHz) δ 0.63 (m, 2H), 0.90 (d, 3H, J = 6 Hz), 0.91 (d, 3H, J = 6 Hz), 1.20 (m, 2H), 1.2−1.4 (m, 2H), 1.51 (m, 5H), 1.67 (m, 4H), 2.96 (m, 1H), 3.3−3.6 (m, 6H), 4.28 (dd, 1H, J = 6, 10 Hz), 5.44 (dd, 1H, J = 6, 8 Hz), 6.94 (ddd, 1H, J = 1, 7, 8 Hz), 7.05 (s, 1H), 7.08 (ddd, 1H, J = 1, 7, 8 Hz), 7.32 (d, 1H, J = 8 Hz), 7.46 (dd, 1H, J = 1, 8 Hz); MS (DCI/NH_3) m/e 566 (M + H)$^+$. Anal. for $C_{30}H_{39}N_5O_4S$·1.2TFA: C, H, N.

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

Binding assays were performed in 96-well microtiter plates pretreated with 0.1% BSA. Membranes were diluted ∼100-fold in buffer B (20 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4, with 0.2% BSA, 0.1 mM PMSF, 5 μg/mL Pepstatin A, 0.025% bacitracin, and 50 mM EDTA) to a final concentration of 0.2 mg/mL of protein. In competition binding studies, membranes (0.02 mg) were incubated with 0.1 nM of [^{125}I]ET-1 (for ET_A assay in MMQ) or [^{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 3 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), washing the filter strips 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.

Receptor Modeling Studies. Model development and refinement studies were performed using the InsightII modeling software package from Biosym Technologies, Inc. Energy minimizations and molecular dynamics calculations were performed using the Discover module and were accomplished using a modified version of the CVFF parameter set. Solvent water molecules were incorporated into the model by applying the SOAK subroutine in InsightII to the modeled receptor in the absence of ligand. All water molecules containing oxygen atoms within 2.0 Å of a non-hydrogen atom of the ligand were then removed. The receptor and ligand were held fixed during a short minimization (100 steps of conjugate gradient) and molecular dynamics (2 ps at 300 K) to allow the water molecules to adjust position. The entire complex was then subjected to a short minimization (100 steps of conjugate gradient) and molecular dynamics (2 ps at 300 K holding the backbone of the receptor fixed) procedure to relieve major stresses. Solvation calculations were performed using three different solvation models as implemented in the Solvation module of Insight. All three models[11−13] use solvation parameters corresponding to CFF91 force field atom types. The

results of these calculations are summarized below.

	Eisenberg–McLachlan[11]	Wesson–Eisenberg[12] (Kyte–Doolittle[8] parameterization)	Wesson–Eisenberg[12] (Sharp[13] parameterization)
imidazole **2g**	10.33 kcal/mol	−16.05 kcal/mol	−10.80 kcal/mol
thiazole **2i**	11.05	−14.03	−8.47
ΔE	0.72	2.02	2.33

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