Structure−Activity Relationships in a Series of Orally Active γ-Hydroxy Butenolide Endothelin Antagonists

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The design of potent and selective non-peptide antagonists of endothelin-1 (ET-1) and its related isopeptides are important tools defining the role of ET in human diseases. In this report we will describe the detailed structure−activity relationship (SAR) studies that led to the discovery of a potent series of butenolide ET_A selective antagonists. Starting from a micromolar screening hit, PD012527, use of Topliss decision tree analysis led to the discovery of the nanomolar ET_A selective antagonist PD155080. Further structural modifications around the butenolide ring led directly to the subnanomolar ET_A selective antagonist PD156707, IC_{50}'s = 0.3 (ET_A) and 780 nM (ET_B). This series of compounds exhibited functional activity exemplified by PD156707. This derivative inhibited the ET_A receptor mediated release of arachidonic acid from rabbit renal artery vascular smooth muscle cells with an IC_{50} = 1.1 nM and also inhibited the ET-1 induced contraction of rabbit femoral artery rings (ET_A mediated) with a pA_2 = 7.6. PD156707 also displayed in vivo functional activity inhibiting the hemodynamic responses due to exogenous administration of ET-1 in rats in a dose dependent fashion. Evidence for the pH dependence of the open and closed tautomerization forms of PD156707 was demonstrated by an NMR study. X-ray crystallographic analysis of the closed butenolide form of PD156707 shows the benzylic group located on the same side of the butenolide ring as the γ-hydroxyl and the remaining two phenyl groups on the butenolide ring essentially orthogonal to the butenolide ring. Pharmacokinetic parameters for PD156707 in dogs are also presented.

Introduction

Since the 1985 discovery of endothelin (ET),[1] as the peptidic endothelial derived constricting factor, over 2500 papers have been published describing various aspects of the biochemistry, pharmacology, and physiology of ET.[2−3] The biochemical pathway leading to the production of ET has been elucidated. It is derived in several steps from a 203 amino acid precursor known as prepro-ET. Prepro-ET is cleaved at two sites by dibasic pair specific endopeptidases to produce a 39 amino acid peptide known as big ET. Big ET is then further cleaved by the ET converting enzyme (ECE), giving rise to the mature peptide ET. The bovine and mammalian ECE enzymes have recently been cloned and identified. ECE is a zinc metalloproteinase, and two different isoforms have been identified, ECE1a and ECE1b. The two forms of the enzyme differ only in the size of the N-terminus and in their location. ECE1a is a cytosolic intracellular enzyme located almost exclusively in the golgi and ECE1b is a membrane bound extracellular enzyme.

The human genome identified for ET was subsequently found to encode for three separate isoforms of the peptide, known as ET-1, ET-2, and ET-3.[4] These isoforms contain several amino acid changes in the "ring" portion of the peptide while the C-terminal hexapeptide chain is identical in all three peptides. The endothelins are closely related to a series of snake venom toxins, the sarafotoxins. ET is a potent and prolonged vasoconstrictor and mitogen in a variety of cell types. In addition ET has potent renal, pulmonary, and neuroendocrine actions and has been implicated in a wide variety of human disease states including ischemia,[5] cerebral vasospasm,[6] stroke,[7] renal failure,[8] hypertension,[9] heart failure,[10] pulmonary hypertension,[11] and restenosis.[12]

ET exerts its physiological effects by acting upon specific G protein coupled receptors. Two distinct ET receptors, ET_A and ET_B, have been cloned and expressed in mammalian species.[13−16] There is some pharmacological evidence regarding the existence of ET_B receptor subtypes; however none have been cloned in mammalian species.[17] A third ET receptor, ET_C, has been isolated and identified in *Xenopus* dermal melanophores but has not been described in mammalian tissues.[18]

The two mammalian ET receptors are defined by their affinity for the ET isoforms. The ET_A receptor exhibits an affinity order of ET-1 = ET-2 ≫ ET-3 and Sarafotoxin 6C (S6C). The ET_B receptor shows essentially equal binding affinity for all three ET isoforms and S6C. Both receptors are widely distributed in both animal and human tissues. The ET_A and/or ET_B receptors have been shown to mediate vasoconstriction in animal tissues depending upon the species and vascular bed under study.[19−28] Furthermore, the ET_B receptor has

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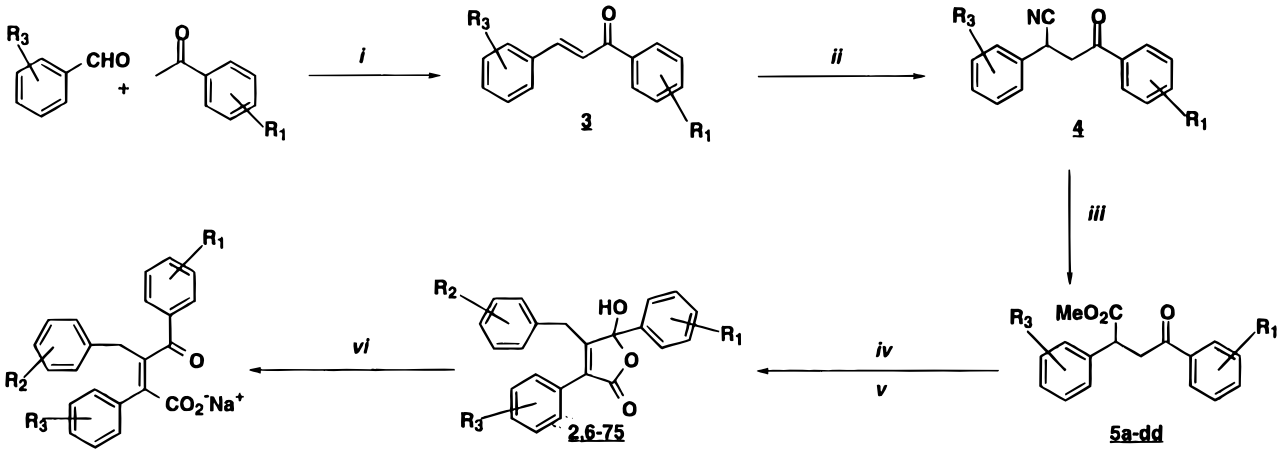
Scheme 1[a]



[a] (i) EtOH, NaOH, room temperature, 1.5 h; (ii) KCN, HOAc, reflux, 30 min; (iii) HCl/MeOH or $TsOH \cdot H_2O$, reflux; (iv) R_2PhCHO, Na, MeOH, reflux; (v) HOAc, reflux; (vi) NaOH.

also been shown to mediate vasodilatation via release of endothelin-derived relaxing factor from endothelial cells.

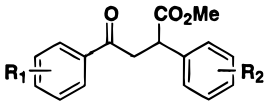
A large number of peptide and more recently non-peptide ET antagonists have been described in the literature. Early small peptide inhibitors included BQ-123[29,30] and FR 139317,[31] both which are potent ET_A selective antagonists. The hexapeptide antagonists PD142893[32] and PD145065[33] with a more balanced ET_A/ET_B receptor affinity have also been described. Recently an ET_B selective peptidic antagonist has been described, BQ-788.[34] In addition several non-peptide non-selective antagonists have appeared in the literature including Ro 46-2005,[35] Ro 47-0203 (Bosentan),[36] SB 209670,[37] CGS 27830,[38] and L-749,329,[39] as well as the more ET_A selective antagonists BMS 182874,[40] PD156707,[41] A-127722,[42] TBC-12519,[43] and LU127043.[44]

The discovery of small non-peptide receptor antagonists may represent a new group of therapeutic agents to treat a variety of human diseases. In this paper we will describe the full structure−activity relationships of our previously disclosed non-peptide ET antagonist PD 156707, **1**, derived from a compound library screening lead PD 012527, **2**.

Chemistry

Most of the compounds in this report have been synthesized via the route shown in Scheme 1.[45] The first step involved an aldol condensation of an acetophenone with an aldehyde in ethanolic hydroxide, giving the chalcones **3** in 50−98% yield. The chalcones were then reacted with cyanide in hot ethoxyethanol containing acetic acid producing the β-cyano ketones **4** in 40−90% yields. The cyano group was then hydrolyzed with either methanolic HCl (saturated) or *p*-toluenesulfonic acid hydrate (1 equiv) in methanol at reflux to afford the keto esters **5a**−**dd** in 30−90% yield (Table 1). The next series of steps was initiated by reaction of the keto ester **5** and an aldehyde with sodium methoxide in hot methanol. One possible mechanism for this series of reactions is depicted in Figure 1 and involves an aldol condensation, an elimination, a double bond isomerization, and an ester hydrolysis in one pot. Acidification of the reaction medium with acetic acid afforded cyclization to give the readily purified γ-hydroxy butenolides **1**−**2** and **6**−**75** (Tables 2−7) in 6−85% yields.

Table 1. Intermediate Keto−Esters



5	R_1	R_2	molecular formula
a	4-Cl	3,4-OCH_2O−	$C_{18}H_{15}ClO_5$
b	H	3,4-OCH_2O−	$C_{18}H_{16}O_5$
c	4-CH_3	3,4-OCH_2O−	$C_{19}H_{18}O_5$
d	3,4-Cl_2	3,4-OCH_2O−	$C_{18}H_{14}Cl_2O_5$
e	3-CH_3,4-OCH_3	3,4-OCH_2O−	$C_{20}H_{20}O_6$
f	4-OCH_3	3,4-OCH_2O−	$C_{19}H_{18}O_6$
g	4-OCH_3	H	$C_{18}H_{18}O_4$
h	4-OCH_3	4-Cl	$C_{18}H_{17}ClO_4$
i	4-OCH_3	4-CH_3	$C_{19}H_{20}O_4$
j	4-OCH_3	4-OCH_3	$C_{19}H_{20}O_5$
k	4-OCH_3	3,4-Cl_2	$C_{18}H_{16}Cl_2O_4$
l	4-OCH_3	3,4-$(OCH_3)_2$	$C_{20}H_{22}O_6$
m	4-OCH_3	3-OCH_3,-4,5-OCH_2O−	$C_{20}H_{20}O_7$
n	2,4-$(OCH_3)_2$	3,4-OCH_2O−	$C_{20}H_{20}O_7$
o	3,4-$(OCH_3)_2$	3,4-OCH_2O−	$C_{20}H_{20}O_7$
p	3,4,5-$(OCH_3)_3$	3,4-OCH_2O−	$C_{21}H_{22}O_8$
q	3,4-OCH_2O−	3,4-OCH_2O−	$C_{19}H_{16}O_7$
r	4-C_2H_5	3,4-OCH_2O−	$C_{20}H_{20}O_5$
s	4-SCH_3	3,4-OCH_2O−	$C_{19}H_{18}O_5S$
t	4-O-*i*-C_3H_7	3,4-OCH_2O−	$C_{21}H_{22}O_6$
u	4-OCH_2Ph	3,4-OCH_2O−	$C_{25}H_{22}O_6$
v	4-CO_2CH_3	3,4-OCH_2O−	$C_{20}H_{18}O_7$
w	4-OCH_3	3,5-$(OCH_3)_2$	$C_{20}H_{22}O_6$
x	4-OCH_3	2,4-$(OCH_3)_2$	$C_{20}H_{22}O_6$
y	4-OCH_3	2,5-$(OCH_3)_2$	$C_{20}H_{22}O_6$
z	4-OCH_3	3,4,5-$(OCH_3)_3$	$C_{21}H_{24}O_7$
aa	3-CH_3,4-OCH_3	3-OCH_3,-4,5-OCH_2O−	$C_{21}H_{22}O_7$
bb	2-OCH_3	3,4-OCH_2O−	$C_{19}H_{18}O_6$
cc	3-OCH_3	3,4-OCH_2O−	$C_{19}H_{18}O_6$
dd	4-OCH_3	3-OCH_3	$C_{19}H_{20}O_5$

These compounds can be converted with a suitable base to the open chain keto−acid form furnishing stable, isolable, water soluble salts.

The open form keto−acid salts and the closed form butenolides exist in a pH dependent equilibrium in solution, and at physiological pH both forms exist and the biological activity might reside with either or both forms (Figure 2). The extent of tautomerization and the reversibility of tautomerization can be estimated by examination of the ^1H NMR spectra at various pH*'s.[46] PD155080, **8**, was studied in 1:1 (DMSO-d_6:D_2O) at pH*'s varying from 2.65 to 9.05. At acidic pH* the compound exists essentially completely in the closed

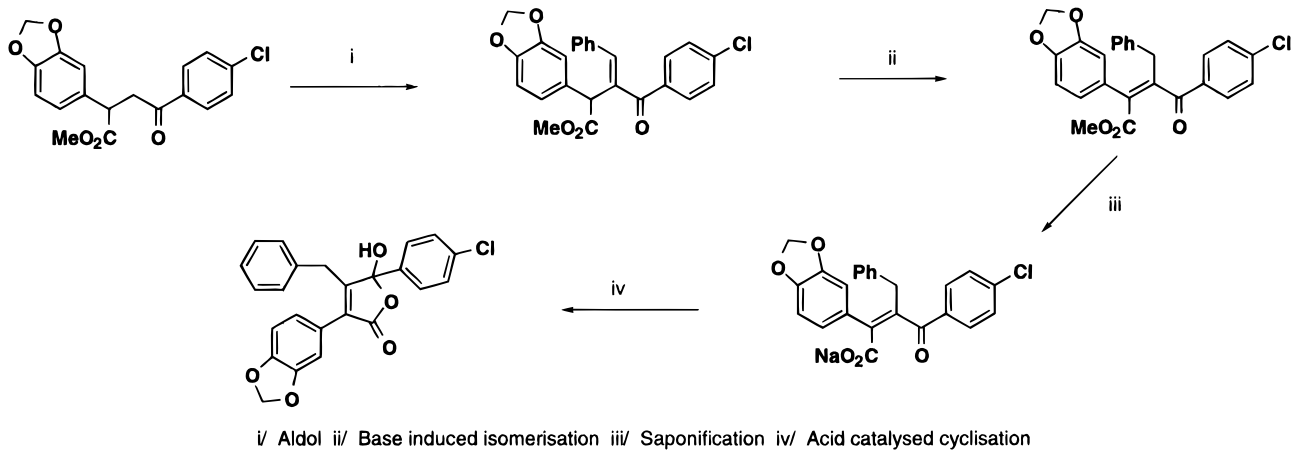


Figure 1. Possible mechanism.

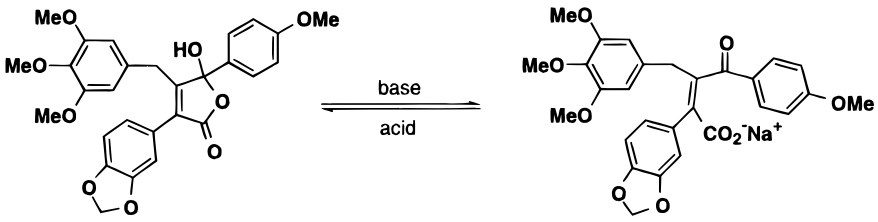


Figure 2. Butenolide tautomerization.

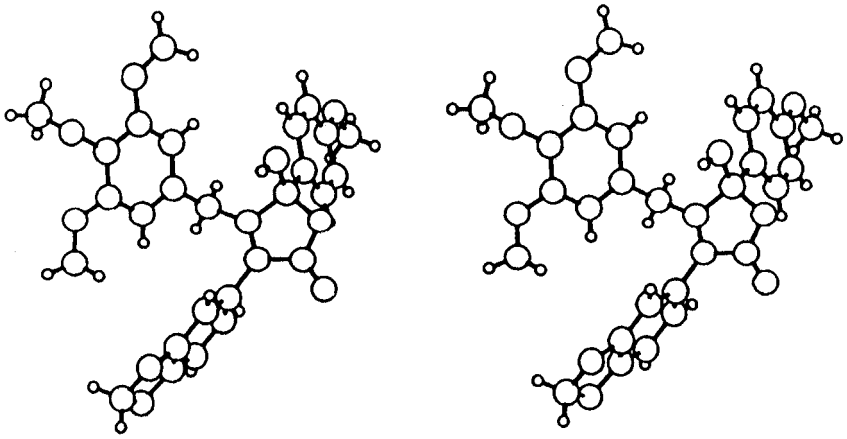


Figure 3. X-ray structure of **1** (closed form), stereoview.

butenolide form. As the pH* is slowly raised by addition of 1.0 N NaOD, the compound starts to exhibit properties associated with the open form keto−acid, and at basic pH* the compound is essentially all in the open form. Of particular note is the coupling pattern shown by the benzylic protons. At acidic pH* the benzylic protons exhibit an AB quartet pattern consistent with the ring-closed structure. As the pH* is raised this pattern coalesces to a singlet, broad at neutral pH*, and sharp at basic pH* as would be expected with the open form keto−acid structure. After the pH was basic, addition of DCl to acidify the solution caused the spectra to return to its original appearance, proving that the tautomerization is clean and reversible. As expected, identical biological results have been obtained with the salt and the parent in all pharmacological assays.

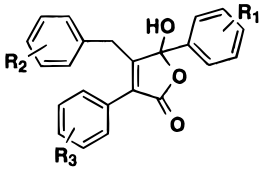
The X-ray crystal structure of the closed form of compound **1**, PD156707, has been determined (Figure 3). In the crystal structure the butenolide ring hydroxyl and the benzylic ring are on the same side of the butenolide ring and the two phenyl rings are essentially orthogonal to the butenolide ring (the coordinates are included as supplementary material).

Compounds **76**−**79**, in Table 8, were synthesized from the parent butenolides **8** and **1**. The two methoxy analogues, **76** and **78**, were synthesized by treating the parent butenolides with saturated hydrochloric acid in refluxing methanol, giving the methoxy derivative in 55−72% yield. The reaction can proceed through the intermediate γ-chloro butenolides, which although unstable and reactive, may be isolated by treating the parent hydroxy butenolide with hydrochloric acid in a nonprotic solvent such as methylene chloride. The deshydroxy analogues **77** and **79** were prepared in 70−85% yield by treatment of the parent with either sodium borohydride in trifluoroacetic acid at room temperature or triethylsilane in trifluoroacetic acid at 0 °C.[47]

Biological Testing

Initially our screening assay for ET_A receptor affinity evaluated [^{125}I]ET-1 binding inhibition in rabbit renal artery vascular smooth muscle cells.[48] ET_B affinity was

Table 2. Initial SAR of Butenolide Series, Topliss Tree Approach



				receptor binding affinity, IC_{50} (nM)[a]	
compd	R_1	R_2	R_3	ET_A[b]	ET_B[c]
2	4-Cl	H	3,4-OCH_2O−	430	27000
6	H	H	3,4-OCH_2O−	600	30000
7	4-CH_3	H	3,4-OCH_2O−	4900	>25000
8	4-OCH_3	H	3,4-OCH_2O−	7.4	4550
9	3,4-Cl_2	H	3,4-OCH_2O−	400	>25000
10	3-CH_3, 4-OCH_3	H	3,4-OCH_2O−	12	1750
11	4-OCH_3	4-CH_3	3,4-OCH_2O−	51	>2500
12	4-OCH_3	4-Cl	3,4-OCH_2O−	110	>2500
13	4-OCH_3	4-OCH_3	3,4-OCH_2O−	1.8	1550
14	4-OCH_3	3,4-Cl_2	3,4-OCH_2O−	150	>2500
15	4-OCH_3	3-CH_3, 4-OCH_3	3,4-OCH_2O−	2.4	1140
16	4-OCH_3	4-OCH_3	H	2200	>2500
17	4-OCH_3	4-OCH_3	4-Cl	700	>2500
18	4-OCH_3	4-OCH_3	4-CH_3	570	>2500
19	4-OCH_3	4-OCH_3	3,4-Cl_2	170	2700
20	4-OCH_3	4-OCH_3	4-OCH_3	190	9000

[a] IC_{50} values were measured as described,[50] using six concentrations of inhibitor (0.08−2500 nM, depending on inhibitor); $n = 2$. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor.

determined similarly in rat cerebellum tissue preparations.[49] More recently compounds have been evaluated for their affinity toward cloned human ET_A and ET_B receptors expressed in Ltk^- cells and CHO-K1 cells, respectively.[50] Protocols for these binding assays have been previously reported.[48−50] All data presented in this report are those obtained using cloned human receptors unless specifically noted.

Cell-based antagonist activity and potency were measured using an ET receptor activated arachidonic acid release assay. Rabbit renal artery vascular smooth muscle cells and ET-1 (as agonist) were used in the ET_A assay while recombinant human receptors expressed in CHO-K1 cells and ET-3 (as agonist) were used in the ET_B assay.[51,52] Functional antagonism was demonstrated by ET-1-induced vasoconstriction in rabbit femoral artery (ET_A mediated) or S6C-induced vasoconstriction in rabbit pulmonary artery (ET_B mediated).[49,53]

In vivo antagonism was determined by measuring the ability of compounds to block the hemodynamic response to an exogenous ET-1 challenge in rats.[41,54] In this procedure rats were given an oral dose of the antagonist by gavage followed 15 min later by ganglionic blockade using mecamylamine. This gave a baseline MABP of 80−90 mmHg, both in antagonist and vehicle-treated animals. After an additional 15 min the rats were administered an iv dose of ET-1 (0.3 nM/kg). In vehicle-treated animals, ET-1 caused a rapid albeit transient depressor response of approximately 18 mmHg. ET-1 then caused a prolonged pressor response in control animals with an averaged 44 mmHg peak response 5 min post ET-1 dose. The ET_A selective antagonist BQ-123 failed to block the initial depressor response and blocked only approximately 60% of the pressor response, while the nonselective antagonist PD 145065 blocked 100% of both the depressor and pressor responses. This indicated that in this assay the depressor response is mediated only by the ET_B receptor and the pressor response is mediated by both the ET_A (60%) and ET_B (40%) receptors.

Results and Discussion

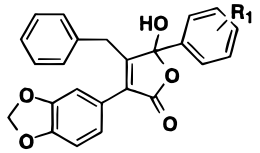
The Parke-Davis compound library, of approximately 165 000 compounds, was screened for ET_A binding affinity using the rabbit receptor assay. From a number of micromolar hits, compound **2** ($ET_A = 2.2\ \mu M$ (rabbit), 430 nM (human) and $ET_B = 5.2\ \mu M$ (rat), 4.5 μM (human)) was chosen for further development. This analogue was subsequently found to have modest activity in our functional assays. Compound **2** inhibited ET-1-induced arachidonic acid release in rabbit renal vascular smooth muscle cells with an IC_{50} of 3.8 μM and had modest activity in the inhibition of ET-1-induced vasoconstriction in rabbit femoral artery with a K_B of 1.4×10^{-5} M.

Initial attempts to improve the ET receptor binding affinity of **2** employed the Topliss "decision tree" approach (Table 2). The Topliss approach takes into account the electronic, lipophilic, and steric factors for substitution on a phenyl ring using basic Hansch principles in a noncomputerized manner.[55,56] For optimization at the R_1 site, we kept the unsubstituted benzyl at the R_2 site and the 3,4-(methylenedioxy)phenyl moiety at R_3, **5**−**10**. From this we discovered compound **8** (PD 155080) with an IC_{50} of 7.4 nM at the human ET_A receptor.

Optimization at the R_2 site via the Topliss tree, **11**−**15**, was performed with the *p*-OMe substituent at R_1 and the 3,4-(methylenedioxy)phenyl moiety at R_3. This yielded compound **13** with an additional 6-fold increase in ET_A activity, $IC_{50} = 1.8$ nM, versus **8**. At both the R_1 and R_2 sites the optimal substituents had small $-\pi$ values and $-\sigma$ values.

We also attempted to optimize activity at the R_3 position via the Topliss approach keeping optimized substituents at both R_1 and R_2, **16**−**20**. While the

Table 3. Expanded Butenolide SAR at the R_1 Site



		receptor binding affinity, IC_{50} (nM)[a]			
compd	R_1	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
21	2-OCH_3	480	>2500	$C_{25}H_{20}O_6 \cdot 0.2H_2O$	C,H,N
22	3-OCH	500	18000	$C_{25}H_{20}H_6$	C,H,N
23	4-C_2H_5	630	>2500	$C_{26}H_{22}O_5$	C,H,N
24	4-SCH_3	170	>2500	$C_{25}H_{20}O_5S$	C,H,N[e]
25	4-O-*i*-C_3H_7	1150	11000	$C_{27}H_{24}O_6$	C,H,N
26	4-OCH_2Ph	950	5400	$C_{31}H_{24}O_6$	C,H,N
27	4-CO_2H	>25000	>2500	$C_{25}H_{18}O_7$	C,H,N
28	4-CO_2CH_3	400	>2500	$C_{26}H_{20}O_7$	C,H,N
29	3,4-$(OCH_3)_2$	11	18000	$C_{26}H_{22}O_7$	C,H,N
30	2,4-$(OCH_3)_2$	13	>2500	$C_{26}H_{22}O_7 \cdot 0.4H_2O$	C,H,N,H_2O(KF)
31	3,4-OCH_2O−	19	2000	$C_{25}H_{18}O_7 \cdot 0.35H_2O$	C,H,N
32	3,4,5-$(OCH_3)_3$	25	4500	$C_{27}H_{24}O_8 \cdot 0.35H_2O$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values. [e] Calcd: C, 69.43; H, 4.66. Found: C, 68.57; H, 4.63.

Topliss tree was followed at this site, the activities achieved in this study were significantly poorer than with the 3,4-(methylenedioxy)phenyl-substituted compound, **13**.

These initial studies led quickly to potent and ET_A selective antagonists. All the compounds, **2** and **6**−**20**, were selective for ET_A receptor binding, over ET_B receptor binding, with the majority being >500-fold selective.

Having elucidated that electron-donating substituents seemed to be beneficial to receptor binding we then started to expand our SAR to further optimize potency. At the R_1 position, Table 3, we compared the methoxy substitution pattern and its effect upon receptor affinity. The ortho, **21**, and meta, **22**, methoxy analogues of **8** both suffered about a 50-fold decrease in ET_A binding activity. Next we synthesized the alkyl steric equivalent of PD155080, (*p*-Et) **23**, and lost significant affinity, indicating the importance of the heteroatom. Substituting the methoxy with thiomethoxy (*p*-SMe), **24**, also led to a decrease in ET_A receptor activity. Increasing the alkyl chain length or bulk on the alkoxy groups at R_1 also led to a lowering of binding affinity, **25** and **26**.

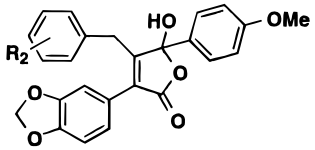
Replacement of the *p*-methoxy group with either a carboxylic acid or an ester group at R_1 led to a dramatic loss of binding affinity, **27** and **28**. An additional electron donating alkoxy group in the R_1 ring, **29**−**31**, gave equipotent derivatives, to the *p*-OMe derivative **8**, while addition of a third methoxy, **32**, was slightly detrimental to ET_A binding affinity.

Exploration of the SAR at the R_2 site led to many highly potent compounds, **1** and **33**−**60**, Tables 4 and 5. While the Topliss approach was accurate in predicting the most active substitution patterns at the R_2 site, the degree of improvement was in general moderate. Most R_2 para-monosubstituted phenyl groups had binding affinities between 1−50 nM at the ET_A receptor, **11**−**15** and **33**−**44**. Electron-donating groups such as *p*-methoxy, **13**, and *p*-hydroxy, **33**, gave potent analogues. However, when the substituent was strongly electron withdrawing the correlation was less distinct. The *p*-nitro derivative **39** had dramatically lower potency whereas the *p*-methylsulfonyl compound **36** had very good potency. The hydrophobic π parameters seem to be more influential than the electronic σ values with large negative π values and small negative or positive σ values being preferred.

When the substituent was moved to the meta position, all compounds seemed to be potent regardless of electronic or hydrophobic characteristics. The 3-carbomethoxy compound **40** was potent IC_{50} = 8.5 nM, the 3-nitro compound **42** was less potent IC_{50} = 70 nM, and the 3-methoxy derivative **44** was also potent with nanomolar ET_A binding affinity, IC_{50} = 3.0 nM. The addition of a second methoxy substituent gave four derivatives, **45**−**48**, all with good ET_A binding affinity although not significantly improved over the monomethoxy compound **13**. In an attempt to link the two alkoxy substituents together in a ring structure we synthesized two cyclic 3,4-derivatives, **49** and **50**, both of which were potent compounds. Addition of a third methoxyl gave some interesting results. The first two trimethoxy compounds, **51** and **52,** were found to be equally potent to the monomethoxy derivative **13**. However, the 3,4,5-trimethoxy derivative **1** was about 10-fold more potent than any of the previous substitution patterns, giving an IC_{50} value of 0.3 nM at the ET_A receptor. Increasing the alkyl portion of the three alkoxy groups on **1** to ethoxy gave compound **53**, which was even more potent than **1** and somewhat more ET_A selective. The effects of further changes in alkyl size at this site have been previously reported and are the subject of a recent publication.[57]

The R_2 pocket clearly tolerated a wide range of substitution patterns, and this was extended to explore some larger aromatic and heteroaromatic substituents (Table 5). The two naphthylmethyl compounds, **55** and **56**, and the two heteroaryl derivatives **57** and **58** retained reasonable ET_A binding affinity. Substitution with even larger aromatic groups such as the biphenyl derivative **59** and the dibenzosuberone **60** led to significant losses of ET_A binding affinity. Alkyl substitution at the R_2 site, such as the ethyl compound **61**, led to a loss of ET_A potency. When the alkyl substituent more closely mimicked the benzyl in size and shape, such as cyclopentylmethyl, cyclohexylmethyl, and cy-

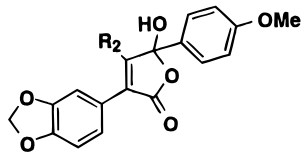
Table 4. Expanded Butenolide SAR at the R_2 Site



		receptor binding affinity, IC_{50} (nM)[a]			
compd	R_2	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
33	4-OH	8.0	>250	$C_{25}H_{20}O_7 \cdot 0.28CH_2Cl_2$	C,H,N
34	4-$N(CH_3)_2$	28	1800	$C_{27}H_{25}NO_6$	C,H,N[e]
35	4-SCH_3	3.9	>2500	$C_{26}H_{22}O_6S \cdot 0.3H_2O$	C,H,N,H_2O(KF)
36	4-SO_2CH_3	4.0	3000	$C_{26}H_{22}O_8S \cdot 0.95H_2O$	C,H,N,H_2O(KF)
37	4-NHAc	1.2	880	$C_{27}H_{23}NO_7 \cdot H_2O$	C,H,N
38	4-CO_2CH_3	11	2000	$C_{27}H_{22}O_8$	C,H,N
39	4-NO_2	800	17000	$C_{25}H_{19}NO_8$	C,H,N
40	3-CO_2CH_3	8.5	6000	$C_{26}H_{20}O_8 \cdot 0.18EtOAc$	C,H,N
41	3-CO_2H	50	>2500	$C_{26}H_{22}O_7 \cdot 0.28EtOAc$	C,H,N
42	3-NO_2	70	>25000	$C_{25}H_{19}O_7 \cdot 0.4H_2O$	C,H,N
43	3-CF_3	43	>2500	$C_{26}H_{19}F_3O_6$	C,H,N[f]
44	3-OCH_3	3.0	260	$C_{26}H_{22}O_7$	C,H,N
45	2,3-$(OCH_3)_2$	11	2200	$C_{27}H_{24}O_8 \cdot 0.35EtOAc$	C,H,N
46	2,5-$(OCH_3)_2$	2.1	1000	$C_{27}H_{24}O_8$	C,H,N
47	3,4-$(OCH_3)_2$	1.4	950	$C_{27}H_{24}O_8 \cdot 0.4\ H_2O$	C,H,N,H_2O(KF)
48	3,5-$(OCH_3)_2$	1.7	760	$C_{27}H_{24}O_8 \cdot 0.4H_2O$	C,H,N,H_2O(KF)
49	3,4-OCH_2O-	16	1500	$C_{26}H_{20}O_8$	C,H,N
50	3,4-OCH_2CH_2O-	7.5	1600	$C_{27}H_{22}O_8$	C,H,N
51	2,3,4-$(OCH_3)_3$	12	2000	$C_{28}H_{29}O_9 \cdot 0.25EtOAc$	C,H,N
52	2,4,5-$(OCH_3)_3$	1.8	850	$C_{28}H_{26}O_9 \cdot 0.25EtOAc$	C,H,N
1	3,4,5-$(OCH_3)_3$	0.3	780	$C_{28}H_{26}O_9$	C,H,N
53	3,4,5-$(OC_2H_5)_3$	0.12	445	$C_{31}H_{32}O_8 \cdot 0.3EtOAc$	C,H,N
54	3-OCH_3,4,5-OCH_2O-	14	16000	$C_{27}H_{22}O_9$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values. [e] Calcd: C, 69.71; H, 5.59; N, 2.23. Found: C, 70.58; H, 5.48; N, 3.05. [f] Calcd: C, 63.75; H, 3.96. Found: C, 64.47; H, 3.95.

Table 5. Expanded Butenolide SAR at the R_2 Site



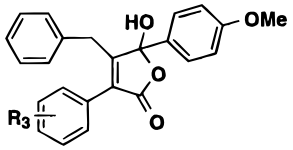
		receptor binding affinity, IC_{50} (nM)[a]			
compd	R_2	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
55	1-naphthylmethyl	18	>2500	$C_{29}H_{22}O_6 \cdot 0.4H_2O$	C,H,N,H_2O(KF)
56	2-naphthylmethyl	14	>2500	$C_{29}H_{22}O_6 \cdot 0.35EtOAc$	C,H,N
57	3-pyridylmethyl	40	>2500	$C_{24}H_{19}NO_6 \cdot 0.4H_2O$	C,H,N,H_2O(KF)
58	1-isoquinolylmethyl	10	3200	$C_{28}H_{21}NO_6 \cdot 0.3H_2O$	C,H,N
59	4′-phenylbenzyl	200	1900	$C_{31}H_{24}O_6 \cdot 0.45H_2O$	C,H,N
60	dibenzosuberonylmethyl	16000	10000	$C_{34}H_{28}O_6$	C,H,N
61	ethyl	320	15000	$C_{20}H_{18}O_6$	C,H,N
62	(c-C_5H_9)CH_2	10	630	$C_{24}H_{24}O_6$	C,H,N
63	(c-C_6H_{11})CH_2	13	225	$C_{25}H_{26}O_6$	C,H,N
64	(c-C_7H_{13})CH_2	35	1300	$C_{26}H_{28}O_6$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values.

cloheptylmethyl (**62**−**64**), the ET_A binding activity resembles that seen in **8**.

At the R_3 site, Table 6, the 3,4-methylenedioxy moiety was optimal in the initial set of analogues **13**. The 3,4-dimethoxy compound **65** was 10-fold less active than the 3,4-methylenedioxy **8** at this site. Comparison of the 3-methoxy, **66**, and the 4-methoxy, **20**, derivatives showed that the former was preferred by at least 2-fold (**20** contains a 4-methoxy substituent at the R_2 position, which is about 4 fold more potent than an R_2 unsubstituted compound, e.g. **66**). To explore alkoxy substitution patterns further, a series of dialkoxy analogues were made, compounds **65** and **67**−**69**. The 3,5-dimethoxy derivative **69** (IC_{50} of 46 nM, ET_A) was the most potent of the dialkoxy analogues synthesized and was still 5-fold less potent than **8**. The 3,4,5-trimethoxy derivative **70** was synthesized and was only weakly active. This study indicated that para substituents, larger than the methylenedioxy moiety, at the R_3 site were not well tolerated, probably due to steric factors. Combining both the 3,5-dimethoxy and the 3,4-methylenedioxy moiety gave the 3,4-(methylenedioxy)-5-methoxy analogue **71** with ET_A binding affinity, $IC_{50} = 6.7$ nM, equal to the 3,4-methylenedioxy analogue **8**.

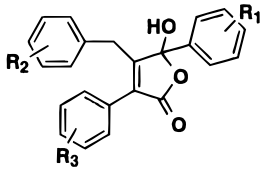
Table 6. Expanded Butenolide SAR at the R_3 Site



		receptor binding affinity, IC_{50} (nM)[a]			
compd	R_3	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
65	3,4-$(OCH_3)_2$	70	3900	$C_{26}H_{24}O_6$	C,H,N
66	3-OCH_3	90	12000	$C_{25}H_{22}O_5$	C,H,N
67	2,4-$(OCH_3)_2$	300	25000	$C_{26}H_{24}O_6$	C,H,N
68	2,5-$(OCH_3)_2$	240	>2500	$C_{26}H_{24}O_6 \cdot 0.6EtOAc$	C,H,N
69	3,5-$(OCH_3)_2$	46	5200	$C_{26}H_{24}O_6$	C,H,N
70	3,4,5-$(OCH_3)_3$	1300	1600	$C_{27}H_{26}O_7 \cdot 0.15H_2O$	C,H,N,H_2O(KF)
71	3-OCH_3, 4,5-OCH_2O-	6.7	640	$C_{26}H_{22}O_7 \cdot 0.5H_2O$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values.

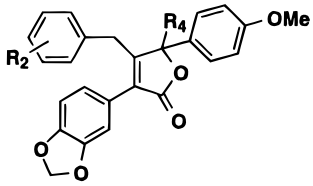
Table 7. Combination of Best R_1, R_2, and R_3 Groups



				receptor binding affinity, IC_{50} (nM)[a]			
compd	R_1	R_2	R_3	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
71	4-OCH_3	H	3-OCH_3,4,5-OCH_2O-	6.7	640	$C_{26}H_{22}O_7$	C,H,N
72	4-OCH_3	3,4,5-$(OCH_3)_3$	3-OCH_3,4,5-OCH_2O-	0.5	260	$C_{29}H_{28}O_{10} \cdot 0.4H_2O$	C,H,N
73	3-CH_3,4-OCH_3	3,4,5-$(OCH_3)_3$	3-OCH_3,4,5-OCH_2O-	0.9	180	$C_{30}H_{30}O_{10}$	C,H,N
74	3-CH_3,4-OCH_3	3,4,5-$(OCH_3)_3$	3,4-OCH_2O-	0.5	240	$C_{29}H_{28}O_9 \cdot 0.1EtOAc$	C,H,N
75	3-CH_3,4-OCH_3	H	3-OCH_3,4,5-OCH_2O-	8.2	1410	$C_{27}H_{24}O_7 \cdot 0.5H_2O$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values.

Table 8. Expanded SAR at the Butenolide Ring γ-Site



			receptor binding affinity, IC_{50} (nM)[a]			
compd	R_2	R_4	ET_A[b]	ET_B[c]	mol form	microanalysis[d]
8	H	OH	7.4	4550		
76	H	OCH_3	300	>25000	$C_{26}H_{22}O_6 \cdot 0.7H_2O$	C,H,N,H_2O(KF)
77	H	H	700	>2500	$C_{29}H_{28}O_9 \cdot 0.2H_2O$	C,H,N
1	3,4,5-$(OCH_3)_3$	OH	0.3	780		
78	3,4,5-$(OCH_3)_3$	OCH_3	600	>2500	$C_{29}H_{28}O_7$	C,H,N
79	3,4,5-$(OCH_3)_3$	H	30	>25000	$C_{28}H_{26}O_8$	C,H,N

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor. [d] Analyses for the elements indicated were within 0.4% of the theoretical values.

The five compounds in Table 7, **71**−**75**, exemplify combination of the best groups from all three sites, R_1, R_2, and R_3. As expected, these derivatives are all extremely potent and selective for the ET_A receptor, 170−520-fold selective.

The results tabulated in Table 8 demonstrate the importance of the ring hydroxyl in retaining potency. Conversion of **8** and **1** to their corresponding methyl ethers, **76** and **78**, led to a loss of potency. Similarly, removal of the hydroxy group to give the corresponding methines, **77** and **79**, also led to loss in binding affinity. These analogues, by design, cannot tautomerize to form the open chain keto−acids. Whether it is the loss of the hydroxy and any hydrogen-bonding interactions, it forms with the receptor, or the elimination of the open chain form that is responsible for the loss of potency is being examined.[58]

Many of the compounds were then examined in the functional assay for antagonist activity. In general as receptor binding potency improved we saw a corresponding improvement in the functional activity. Table 9 includes data for selected butenolides and their open

Table 9. Functional Data for Selected Butenolide ET Antagonists

	inhibition of ET-1 induced arachidonic acid release, IC_{50}(nM)[a]		inhibition of agonist-induced contraction in arterial rings, K_B(M)[b]	
compd	ET_A[c]	ET_B[d]	ET_A[e]	ET_B[f]
2	3800	ia	1.4×10^{-5} ($n = 2$)	ia at 10^{-5} M
8, choline salt	49	22000	5.6×10^{-7} ($n = 2$)	2.6×10^{-5}
37, K salt	4.9	8200	7.8×10^{-8} ($n = 1$)	nt
1	1.1	120	1.9×10^{-8} ($n = 2$)	nt
1, Na salt	1.1	130	2.9×10^{-8} ($pA_2 = 7.6$)[g]	3.4×10^{-5}
71, Na salt	10	15700	8.5×10^{-8} ($n = 1$)	nt
72, Na salt	0.34	5100	2.2×10^{-8} ($n = 1$)	nt
73, Na salt	0.78	68	2.5×10^{-8} ($n = 1$)	nt
74, Na salt	0.98	1900	2.8×10^{-8} ($n = 1$)	nt

[a] See Table 2. [b] IC_{50} values were measured as described,[51−52] using six concentrations of inhibitor (0.01−100 nM, depending on inhibitor); $n = 3$. [c] ET-1 used as the agonist, rabbit renal artery vascular smooth muscle cells. [d] ET-3 used as the agonist, human cloned receptors/CHO-K1 cells. [e] ET-1 used as the agonist, rabbit femoral artery. [f] S6C used as the agonist, rabbit pulmonary artery. [g] Schild regression analysis was run to give pA_2 value.

Table 10. In Vivo Effects of Orally Dosed Butenolide ET Antagonists

compd	*N*	MABP response (% ET-1)[a]
8, choline salt	4	80 ± 9
1, Na salt	5	50 ± 8
71, Na salt	6	76 ± 7
72, Na salt	6	52 ± 9
73, Na salt	6	86 ± 12
74, Na salt	6	53 ± 9

[a] Effect of antagonists at 10 mg/kg on MABP in anesthetized, ganglionic-blocked rats versus an ET-1 (0.3 nmol/kg, iv) baseline.

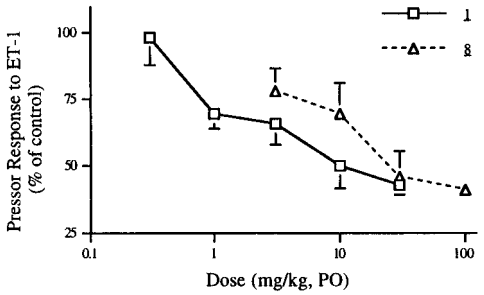


Figure 4. Dose−response curve of the inhibition (**1** and **8**) of the pressor response due to ET-1 (0.3 nmol/kg, iv) in anesthesized, ganglionic-blocked rats. The antagonists were administered at doses from 0.3 to 100 mg/kg (po) by oral gavage.

chain keto−acid salt forms. The data, as expected, shows a very good correlation between the tautomeric forms. The initial screening hit **2** inhibited ET-1-induced arachidonic acid release in rabbit renal vascular smooth muscle cells with an IC_{50} of 3.8 μM. Compound **8** ($ET_A = 7.4$ nM, $ET_B = 4500$ nM) with an approximate 100-fold increase in binding affinity over **2** inhibited ET-1-induced arachidonic acid release with an IC_{50} of 150 nM and inhibited ET-1-induced vasoconstriction with a K_B of 5.6×10^{-7} M. Compounds with subnanomolar ET_A receptor binding affinity showed corresponding increases in functional activity. Compound **1** inhibited ET-1-induced arachidonic acid release with an IC_{50} of 1.1 nM and inhibited ET-1-induced vasoconstriction with a K_B of 2.9×10^{-8} M (pA_2 of 7.6). This compound further has ET_B functional activity with an IC_{50} of 120 nM in the ET-3-induced arachidonic acid release and a K_B of 3.4×10^{-5} M in the S6C-induced vasoconstriction in rabbit pulmonary artery. The most potent ET_A selective antagonist in this study, functionally (overall), is compound **72**. Compound **72** inhibited ET-1-induced arachidonic acid release with an IC_{50} of 0.34 nM and inhibited ET-1-induced vasoconstriction with a K_B of 2.2×10^{-8} M. This compound also had modest ET_B functional activity with an IC_{50} of 5.1 μM for inhibition of ET-3-induced arachidonic acid release.

Many of the open chain salt forms of the compounds in Table 9 were further examined for their ability after oral administration, to inhibit the increase in mean arterial blood pressure (MABP) due to administration of exogenous ET-1 to conscious ganglionic blocked rats (Table 10). Compound **8** at 10 mg/kg shows a 20% reduction in the peak pressor response to ET-1. The more potent compounds **1**, **72**, and **74** show, at 10 mg/kg, the maximal effect seen with an ET_A selective antagonist in this screen, approximately 50% reductions in peak pressor response. Figure 4 shows graphically the dose response curves for **8** and **1** in this assay.

Table 11. ET_A Receptor Selectivity

	receptor binding affinity, IC_{50} (nM)[a]		ET receptor selectivity ET_B/ET_A
compd	ET_A[b]	ET_B[c]	
1	0.3	780	2600
8	7.4	4550	615
53	0.12	445	3700
72	0.5	260	520
74	0.5	240	480

[a] See Table 2. [b] Human cloned ET_A receptor. [c] Human cloned ET_B receptor.

Compound **8** is essentially inactive at 3 mg/kg and maximally effective at 30 mg/kg. Compound **1**, the more potent derivative, shows moderate activity at a 1 mg/kg dose and maximal effect at the 10 mg/kg dose.

A selection of compounds in Table 11 demonstrate that, as a class, the γ-hydroxy butenolides are extremely ET_A potent and selective antagonists. All of the butenolide compounds, Tables 2−8, were ET_A selective, and of those in Table 11, compounds **1** and **53** were greater than 2500-fold selective and had sub-nanomolar ET_A receptor binding affinity. Additionally compound **1** was tested by Panlabs against a battery of 28 receptor binding and seven enzyme inhibition assays and was found to be inactive against all (at 10 μM) except the endothelin receptors.[59]

The pharmacokinetics parameters for compound **1**, initially reported in rats[41] have now been characterized in dogs (Table 12). In a three-way crossover study design, four male beagle dogs were dosed with a single 2.5 mg/kg intravenous dose to fed animals, or 5 mg/kg oral capsule to fed or fasted animals. Serial heparinized plasma samples were collected up to 24 h postdose. Plasma concentrations were measured by a specific HPLC assay.

Table 12. Mean PD156707 Pharmacokinetic Parameters in Dogs ($n = 4$)[a]

parameter	intravenous	oral fasted	oral fed
dose (mg/kg)	2.5	5.0	5.0
$t_{1/2}$ (h)[b]	0.62	1.2	1.9
C_{max}[c]		1.52	0.48
t_{max}[d]		0.69	2.5
AUC (0−∞)[e]	1.5	2.0	0.59
F (%)[f]		67	20

[a] Following a single intravenous or oral dose. [b] Apparent terminal phase elimination half-life; arithmetic mean. [c] Maximum observed concentration (μg/mL). [d] Time to reach C_{max} (h). [e] Area under the concentration−time curve from time zero to infinity (μg at h/mL). [f] Absolute oral bioavailability.

Following intravenous administration, PD 156707, **1**, plasma concentrations declined in a biexponential manner. PD 156707 was rapidly absorbed following oral dosing. Food appeared to slow oral absorption and decrease oral bioavailability: fasted 66%, fed 20%. The mean plasma terminal elimination half-life was longer following oral (1.9 h) than intravenous dosing (0.62 h).

Conclusion

Starting from the modestly active compound library screening hit, **2**, we were able to develop, using the structured Topliss tree approach, a number of potent nanomolar ET_A selective ET antagonists. Further lead optimization via examination of the electron and structural requirements led to a series of sub-nanomolar affinity ET_A antagonists, represented by **1**, with oral activity against an exogenous ET-1 challenge. Compounds **1** and **8** are being evaluated in a number of preclinical disease models, and efficacy in stroke for **1** has been demonstrated.[7]

Experimental Section

Pharmacokinetics Methods. PD156707 was isolated from dog plasma by reverse-phase solid phase extraction. Liquid chromatographic separation was achieved on a C18, Spherex 5μ (3.2 × 150 mm), Phenomenex reverse phase column. The mobile phase consisted of 40% acetonitrile/60% ammonium acetate buffer (50 mM, pH 3.5). Column effluent was monitored by a fluorescence detector with an excitation wavelength of 312 nm and emission of 430 nm. Assay limit of quantitation ranged from 10 to 5000 ng/mL. Plasma samples contained more than 5000 ng/mL PD156707 and were diluted with blank dog plasma and reanalyzed. Minimum limit of quantitation for PD156707 was 10 ng/mL from a 200 μL dog plasma sample aliquot.

Pharmacokinetic parameter values were estimated by noncompartmental analysis of individual dog plasma PD156707 concentration−time data. Maximum PD156707 plasma concentrations (C_{max}) and times for these to occur (t_{max}) were recorded as observed. Apparent terminal elimination−rate constants (λ_z) were calculated by linear regression of the log−linear terminal phase of concentration−time profiles. Apparent terminal elimination half-life values were calculated from elimination−rate constants as $t_{1/2} = 0.69/\lambda_z$. Area under plasma concentration−time curve AUC(0−∞) values were calculated from time zero to time of last detectable concentration using the trapezoidal rule and were extrapolated to infinity. Absolute oral bioavailability $F\% = (\text{dose}_{iv} \times \text{AUC}(0-\infty)_{po}/\text{dose}_{po} \times \text{AUC}(0-\infty)_{iv}) \times 100$.

General Chemical Procedures. All reagents were either purchased from common commercial sources or synthesized according to literature references using commercial sources. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. Proton NMR (^{1}H NMR) were obtained on Bruker AC-200 or AM-400 or Varian Unity 400 MHz spectrometers and are referenced to TMS. Mass spectra (MS) were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer. Microanalyses were determined by the Parke-Davis Pharmaceutical Analytical Department or under contract with Robertson Analytical Services.

General Example of Synthesis of Compounds 5a−s and 5w−ff. 3-(1,3-Benzodioxol-5-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (3f). 4-methoxyacetophenone (200 g, 1.33 mol) was dissolved in absolute ethanol (300 mL), and the solution was treated with piperonal (200 g, 1.33 mol). The solution was then treated with 10% aqueous NaOH (41 mL) and the mixture stirred at room temperature for 1.5 h and then allowed to precipitate. The yellow solid was collected by filtration and washed with ethanol:water (1:1) (600 mL) and dried in vacuo overnight. The solid was used as in the next step: 360.15 g (96%); ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 6.02 (s, 1H), 6.84 (s, 2H), 6.97 (d, 2H), 7.16 (d, 2H), 7.38 (d, 1H), 7.72 (s, 1H), 8.02 (s, 1H); MS (EI^+): 310 Da; mp 127−131 °C.

2-(1,3-Benzodioxol-5-yl)-1-(4-methoxyphenyl)-4-oxobutyronitrile (4f). A solution of **3f** (360.15 g, 1.28 mol) in freshly distilled 2-ethoxyethanol (1 L) and acetic acid (91.9 g, 1.53 mol) at 105 °C was treated in portions with a solution of KCN (124.4 g, 1.91 mol) in water (217 mL). The mixture was stirred at 105 °C for 30 min and allowed to cool to room temperature. The mixture was allowed to stand overnight to precipitate. The solid was collected by filtration, washed with methanol:water (1:1) (1.6 L), and dried under high vacuum. This gave 338.8 g of a yellow/brown solid (85%); ^{1}H NMR ($CDCl_3$) δ 3.43 (m, 1H), 3.62 (m, 1H), 3.87 (s, 3H), 4.48 (m, 1H), 5.97 (s, 2H), 6.79 (d, 1H), 6.88 (m, 2H), 6.93 (m, 2H), 7.90 (d, 2H); MS (EI^+) 310 Da; mp 90−92 °C. Anal. ($C_{18}H_{15}NO_4 \cdot 0.2H_2O$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-1-(4-methoxyphenyl)-4-oxobutyric Acid Methyl Ester (5f). A suspension of **4f** and *p*-toluenesulfonic acid hydrate (208.3 g, 1.10 mol) in methanol (1 L) and dioxane (0.5 L) was heated to reflux overnight under nitrogen. The mixture was cooled to room temperature and the precipitate collected by filtration, washed with methanol, and dried to give 354.3 g of a white solid: ^{1}H NMR ($CDCl_3$) δ 3.20 (d of d, 1H), 3.70 (s, 3H), 3.82 (d of d, 1H), 3.86 (s, 3H), 4.18 (d of d, 1H), 5.95 (s, 2H), 6.80 (m, 3H), 6.92 (d, 2H), 7.94 (d, 2H); MS (EI^+) 343 Da; mp 113−114°C. Anal. ($C_{19}H_{18}O_6 \cdot 0.2H_2O$) C, H, N.

Nonstandard Intermediates. 3-(1,3-Benzodioxol-5-yl)-1-(4-(methylthio)phenyl)prop-2-en-1-one (3s). From 4′-(methylthio)acetophenone (15.5 g, 93.3 mmol) and piperonal (14 g, 93.3 mmol) in the same manner as **3f** was synthesized the chalcone **3s**. This gave 27 g (97%) of a yellow solid; ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H), 6.03 (s, 2H), 6.83−7.96 (m, 9H); MS (CI^+) 298 Da; mp 135−137 °C. Anal. ($C_{17}H_{14}O_3S$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-1-(4-(methylthio)phenyl)-4-oxobutyric Acid Ethyl Ester (5s). Triphenyl orthothioformate (6.8 g, 20 mmol) was treated with *n*-BuLi (2.1 M, 10 mL) at −78 °C under nitrogen and stirred for 30 min. To this was added the chalcone **3s** (5.4 g, 18.1 mmol) in THF (100 mL), and the mixture was stirred cold for another 30 min. The mixture warmed to room temperature and stirred for 30 min. The solution was treated with NH_4Cl (saturated) (5 mL), passed a short packed silica gel column (200 g), and evaporated to dryness. The crude product was recrystallized in ethyl acetate/ether to give the ortho thioester as a white solid, 7.2 g (62%), which was identified by ^{1}H NMR and used as is. The ortho thioester (3.7 g, 5.8 mmol), $HgCl_2$ (7.8 g, 28.7 mmol), and HgO (2.5 g, 11.5 mmol) were refluxed in 95% ethanol (150 mL) for 7 h under nitrogen. The mixture was then filtered and the filtrate diluted with water (75 mL) and extracted with methylene chloride (2 × 100 mL). The combined extracts were washed successively with 1 N HCl (200 mL) and brine (200 mL) and then dried over $MgSO_4$. Evaporation of the solvent gave 1.05 g (49%) of the ester: ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 3H), 2.53 (s, 3H), 3.20 (m, 1H), 3.83 (m, 1H), 4.07−4.21 (m, 4H), 5.95 (s, 2H), 6.75−7.90 (m, 7H). Anal. ($C_{20}H_{20}O_5S$) C, H, N (not run).

3-(1,3-Benzodioxol-5-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3ee). From 4′-hydroxyacetophenone (9.1 g, 69 mmol) and piperonal (10 g, 67 mmol) in the same manner as

3f was synthesized 16.5 g (91%) of the chalcone; ^{1}H NMR ($CDCl_3$) δ 6.10 (s, 2H), 6.87−8.09 (m, 9H), 10.38 (br s, 1H); MS (CI^+) 268 Da; mp 204−205 °C. Anal. ($C_{16}H_{12}O_4$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-1-(4-isopropoxyphenyl)-4-oxobutyronitrile (4t). The chalcone **3ee** (5.35 g, 19.7 mmol) was dissolved in DMF (100 mL) and treated with K_2CO_3 (3 g, 20 mmol) and isopropyl bromide (3 g, 24 mmol). The mixture warmed to 100 °C and stirred for 3 h. The mixture filtered and the filtrate evaporated in vacuo to give the crude solid. The material was purified by flash chromatography (1000 g of flash silica gel, chloroform:ethyl acetate (200:1)). Evaporation of the appropriate fractions gave 4.8 g (79%) of the chalcone. From this chalcone (4.0 g, 12.9 mmol) in the same manner as **4f** was isolated the nitrile: 3.85 g (89%); ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 6H), 3.51 (m, 2H), 4.49 (m, 1H), 4.65 (m, 1H), 5.99 (d, 2H), 6.78−8.03 (m, 7H); MS (CI^+) 338 Da. Anal. ($C_{20}H_{19}NO_4$) C, H, N (not run).

2-(1,3-Benzodioxol-5-yl)-1-(4-isopropoxyphenyl)-4-oxobutyric Acid Methyl Ester (5t). From the nitrile **4t** (3.0 g, 8.9 mmol) in the same manner as **4f** was synthesized the ester **4t**, giving 2.4 g (73%): ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 6H), 3.42−4.50 (m, 3H), 3.64 (s, 3H), 4.66 (m, 1H), 6.01 (s, 2H), 6.665−7.55 (m, 7H); MS (CI^+) 371 Da. Anal. ($C_{21}H_{22}O_2$) C, H, N (not run).

3-Benzo[1,3]-dioxo-5-yl-1-(4-cyanophenyl)prop-2-en-1-one (3ff). From 4′-cyanoacetophenone (9.7 g, 67 mmol) and piperonal (10 g, 67 mmol) was synthesized **3ff** in the same manner as **3f**. This gave 13.5 g (73%): ^{1}H NMR ($CDCl_3$) δ 6.05 (s, 2H), 6.85−8.08 (m, 9H); MS (CI^+) 277 Da. Anal. ($C_{17}H_{11}NO_3$) C, H, N (not run).

2-(1,3-Benzodioxol-5-yl)-1-(4-cyanophenyl)-4-oxobutyronitrile (4ff). From the chalcone **3ff** (12.5 g, 45.8 mmol) in the same manner as **4f** was synthesized the nitrile **4ff**, giving 8.6 g (62%): ^{1}H NMR ($CDCl_3$) δ 3.59 (m, 2H), 4.46 (m, 1H), 5.99 (s, 2H), 6.78−8.03 (m, 7H); MS (CI^+) 305 Da. Anal. ($C_{18}H_{12}N_2O_2$) C, H, N (not run).

2-(1,3-Benzodioxol-5-yl)-1-(4-(carboxymethyl)phenyl)-4-oxobutyric Acid Methyl Ester (5v). The nitrile **4ff** (6.2 g, 20.3 mmol) in methanol (50 mL) was saturated with HCl gas and warmed to 45 °C until no trace of starting material remained. The solution was cooled and treated with water (100 mL). The resultant solid was collected, washed with methanol:water (8:2) (50 mL), and dried in vacuo. This gave the diester: 6.1 g (81%); ^{1}H NMR ($CDCl_3$) δ 3.25 (m, 1H), 3.71 (s, 3H), 3.90 (m, 1H), 3.96 (s, 3H), 4.21 (m, 1H), 5.96 (m, 2H), 6.75−8.16 (m, 7H); MS (CI^+) 370 Da. Anal. ($C_{20}H_{18}O_7$) C, H, N (not run).

General Example for the Synthesis of Compounds 1, 2, and 6−75). 3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-(3,4,5-trimethoxybenzyl)-5*H*-furan-2-one (1). To methanol (6 mL) was added sodium metal (57 mg, 2.5 mmol), and the mixture was stirred to dissolve. To this was added **5f** (0.822 g, 2.4 mmol) followed by 3,4,5-trimethoxybenzaldehyde (0.50 g, 2.5 mmol). The mixture was warmed to reflux for 16 h. The solution was treated with acetic acid (1.5 mL) and refluxed an additional 6 h. The solvents were evaporated in vacuo to give a pasty residue. This was partitioned between ethyl acetate (20 mL) and water (20 mL). The organic phase was quickly separated, dried over $MgSO_4$, and evaporated to dryness. The crude product was purified by flash chromatography (150 g of flash silica gel, 20% ethyl acetate:methylene chloride). The product was isolated by evaporation of the appropriate fractions to give 0.719 g (59%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.64 (s, 6H), 3.69 (m, 2H), 3.74 (s, 3H), 3.80 (s, 3H), 5.97 (s, 2H), 6.01 (s, 2H), 6.79−6.98 (m, 5H), 7.39 (d, 2H); MS (CI^+) 507 Da; mp 73−75 °C. Anal. ($C_{28}H_{26}O_9$) C, H, N.

General Synthesis of Open Chain Form Salts. Sodium 2-(1,3-Benzodioxol-5-yl)-4-(4-methoxyphenyl)-4-oxo-3-(3,4,5-trimethoxybenzyl)-but-2-enoate (Na Salt of 1). The butenolide **1** (4.04 g, 7.97 mmol) was dissolved in methanol (100 mL) and treated with 0.493 N aqueous NaOH (15.8 mL, 7.8 mmol). The solution was stirred for 5 min and evaporated in vacuo to give a paste. The paste was partitioned between distilled water (100 mL) and ether (100 mL). The aqueous phase was evaporated free of residual ether, frozen, and lyophilized. This gave 3.65 g (88%) of a white lyophilate: ^{1}H NMR (D_2O) δ 3.21 (s, 2H), 3.54 (s, 3H), 3.55 (s, 6H), 3.83 (s, 3H), 5.94 (s, 2H), 6.03 (s, 2H), 6.85−6.90 (m, 4H), 6.98 (d, 1H), 7.66 (d, 2H); MS (CI^+) 505 Da. Anal. ($C_{28}H_{25}O_9Na\cdot0.8H_2O$) C, H, N, H_2O (KF), Na.

General Synthesis of γ-Methoxy Butenolides 76 and 78. 3-(1,3-Benzodioxol-5-yl)-4-benzyl-5-methoxy-5-(4-methoxyphenyl)-5*H*-furan-2-one (76). A solution of **8** (205 mg, 0.5 mmol) in methanol (15 mL) was saturated with HCl gas. The solution warmed to 50 °C and stirred for 18 h. The cooled solution was evaporated in vacuo to give a crude product. The crude was purified by flash chromatography (120 g of flash silica gel, hexane:ethyl acetate (3:1)). Evaporation of the appropriate fractions gave 119 mg (55%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 3.26 (s, 3H), 3.76 (q, 2H), 3.79 (s, 3H), 5.96 (s, 2H), 6.77−6.83 (m, 5H), 6.94−6.96 (m, 2H), 7.05−7.09 (m, 3H), 7.34 (d, 2H); MS (EI^+) 430 Da. Anal. ($C_{26}H_{22}O_6\cdot0.7H_2O$) C, H, N, H_2O (KF).

Synthesis of Deshydroxy Butenolides 77 and 79. 3-(1,3-Benzodioxol-5-yl)-4-benzyl-5-(4-methoxyphenyl)-5*H*-furan-2-one (77). In trifluoroacetic acid (8 mL), at 0 °C, under a nitrogen stream was added in parts a mixture of sodium borohydride (378 mg, 10 mmol) and **8** (416 mg, 1.0 mmol). The resultant deep green solution was stirred for 5 min and evaporated free of TFA. The residue was then carefully quenched with water (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL) and the organic phase washed with brine (25 mL). The organic phase then dried over $MgSO_4$ and evaporated in vacuo to give the crude product. The crude material was purified by flash chromatography (70 g of flash silica gel, 5% ethyl acetate:methylene chloride). The appropriate fractions were evaporated in vacuo to give 282 mg (70%) as an off-white solid: ^{1}H NMR ($CDCl_3$) δ 3.68 (q, 2H), 3.83 (s, 3H), 5.59 (s, 1H), 6.01 (s, 2H), 6.88−6.92 (m, 3H), 6.96−6.98 (m, 2H), 7.03−7.09 (m, 4H), 7.26−7.29 (m, 2H); MS (CI^+) 400 Da. Anal. ($C_{25}H_{20}O_5\cdot0.2H_2O$) C, H, N.

3-(1,3-Benzodioxol-5-yl)-4-(3,4,5-trimethoxybenzyl)-5-(4-methoxyphenyl)-5*H*-furan-2-one (79). In TFA (6.5 mL) frozen at −78 °C was added **53** (2.53 g, 5.0 mmol). The deep green solution was treated with triethylsilane (7.0 mL) and stirred for 1 h. The mixture was warmed to reflux for 3 h and stripped in vacuo to give an oil. The oil was triturated with petroleum ether and decanted. The insoluble residue was dissolved in ethyl acetate, washed successively with saturated $NaHCO_3$ and brine, and then dried over $MgSO_4$. The solvents were evaporated, and the resultant solid was recrystallized from ether. The solid dried in vacuo to give 2.08 g (85%) of **79**: ^{1}H NMR ($CDCl_3$) δ 3.63 (d of d, 2H), 3.75 (s, 6H), 4.02 (s, 6H), 5.63 (s, 1H), 6.02−6.03 (m, 2H), 6.10 (s, 2H), 6.89−6.92 (m, 3H), 7.05−7.09 (m, 4H); MS (CI^+) 400 Da. Anal. ($C_{28}H_{26}O_8$) C, H, N.

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Supporting Information Available: Spectral and general experimental information for all compounds which have either not previously been published or not been specifically mentioned in the preceding experimental, and X-ray coordinates for compound **1** (51 pages). Ordering information is given on any current masthead page.

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