Discovery of a Novel Series of Orally Active Non-Peptide Endothelin-A (ET_A) Receptor-Selective Antagonists

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The potent vasoconstrictor endothelin (ET) is implicated in several human disease states including hypertension, congestive heart failure, renal failure, pulmonary hypertension, ischemia, and cerebral vasospasm.[1–9] Two subtypes of ET receptors known as ET_A and ET_B have been cloned and characterized in animal and mammalian systems.[10–13] A third endothelin receptor subtype has been cloned from *Xenopus* dermal melanophores and heart,[14,15] although this subtype has not yet been described in mammalian tissues.

Both ET_A and ET_B receptors are widely distributed in animal and human tissues.[16–26] In a wide variety of animal tissues, vasoconstriction occurs *via* activation of ET_A and/or ET_B receptors depending upon the species and vascular bed under study.[17–26] However, there is some controversy as to whether ET_B receptors play an important role in mediating vasoconstrictor responses in mammalian tissues.[20–23] Davenport et al. have reported that ET_A-mediated vasoconstriction plays a major role in some human vessels, such as coronary artery, but have been unable to demonstrate ET_B receptor-mediated contractions in human tissues using ET_B-selective agonists such as [Ala 1,3,11,15]ET-1 and BQ 3020.[20] However, Luscher et al. have reported that ET_B receptor mRNA was detected by Northern blot analysis in human internal mammary artery and aortic smooth muscle cells.[23] Several groups have shown that the ET_B receptor agonist SRTX-6c can elicit vasoconstriction in human vessels although the magnitude of the response has been found to be considerably less than that observed for ET-1 itself.[24–26] It is possible that downregulation of ET_B receptors in isolated tissues is responsible for these observations.

A number of peptide ET antagonists have been reported including BQ-123[27,28] and FR 139317,[29] which are potent ET_A-selective antagonists; balanced ET_A/ET_B antagonists including PD 142893[30] and PD 145065[31] and the recently reported ET_B-selective antagonist, BQ-788.[32] A number of non-peptide endothelin antagonists have also been reported. These include the Shionogi steroid analog 97-139[33] and several balanced ET_A/ET_B non-peptide antagonists, including Ro 46-2005,[9] Ro 47-0203 (bosentan),[34] SKF 209670,[35] CGS 27830,[36] and L-749,329,[37] have been described, in addition to the more ET_A-selective antagonist BMS 182874.[38]

The development of non-peptide, low molecular weight antagonists with high potency, oral activity and reasonable duration of action is an important objective for defining adequately the potential role of ET and its isopeptides in both acute and chronic human diseases.[3] To this end, we screened our chemical library of about 168 000 compounds for their capacity to inhibit specific [^{125}I]ET-1 binding in rabbit renal artery vascular smooth muscle cells (VSMC), known to express only the ET_A receptor using an assay system previously described.[39,40] Using this approach, we discovered several series of non-peptide antagonists, and this report will describe one particular series of compounds, namely the butenolides.

We have optimized the potency of an initial lead structure, PD 012527, compound **1**, to discover potent orally active ET_A-selective antagonists.[41,42] Compound **1** showed μM binding affinity for the rabbit ET_A receptor[39,40] (IC_{50} = 2 μM) and also inhibited [^{125}I]ET-3 specific binding to rat cerebellum (ET_B)[39,40] with an IC_{50} of 5 μM. The compound also inhibited ET-1-induced arachidonic acid release in rabbit renal artery VSMC with an IC_{50} of 5 μM, showing that it was an ET_A functional antagonist.[43] Compound **1** exhibited very weak inhibitory activity against ET-1-induced vasoconstriction in the ET_A-specific rabbit femoral artery (pA_2 = ca. 5), and no activity was observed in inhibiting SRTX-6c-induced vasoconstriction in rabbit pulmonary artery, an assay that evaluates ET_B functional activity.[40,44]

In this report, we describe the lead optimization and structure–activity relationships of this novel series of ET_A selective receptor antagonists. Binding affinity of the compounds was evaluated in rabbit (ET_A) and rat (ET_B) assays described previously[39,40] and subsequently was also evaluated using cloned human receptors.[45,46] The oral activities and pharmacokinetic properties of two compounds, PD 155080 (**7b**) and PD 156707 (**20b**), are highlighted.[38,39,43]

Chemistry. The syntheses of compounds **1** and **7** are illustrated in Scheme 1 and are exemplary for the series of analogs. The route to **1** has previously been described by Allen et al.,[47] and we followed a similar route to **7** and analogs (Tables 1–3). Briefly, the piperonal **2** is employed in an aldol condensation with *p*-chloroacetophenone (for **1**) or *p*-methoxyacetophenone (for **7**) to afford the chalcone derivatives **3a** and **3b** in good yields (90% and 97%, respectively). Addition of sodium or potassium cyanide to **3a** or **3b** under refluxing acetic acid in ethoxyethanol afforded the product, β-cyano ketone **4a** or **4b**. Compounds **4a** and **4b** were hydrolyzed in acidic methanol to afford the β-carbomethoxy ketones **5a** or **5b** in yields of 60% and 94%, respectively. Subsequent base-catalyzed aldol reaction with benzaldehyde or a suitable aldehyde (for other analogs) followed by reflux under acidic conditions affected elimination, isomerization, and cyclization to the butenolides **1** or **7** in 50–60% yield. The order of reactions and mechanism(s) of this final step may vary according to the substituents at the R_1 and R_2 positions. All other analogs **6–20** were prepared by a similar synthetic sequence.

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Scheme 1

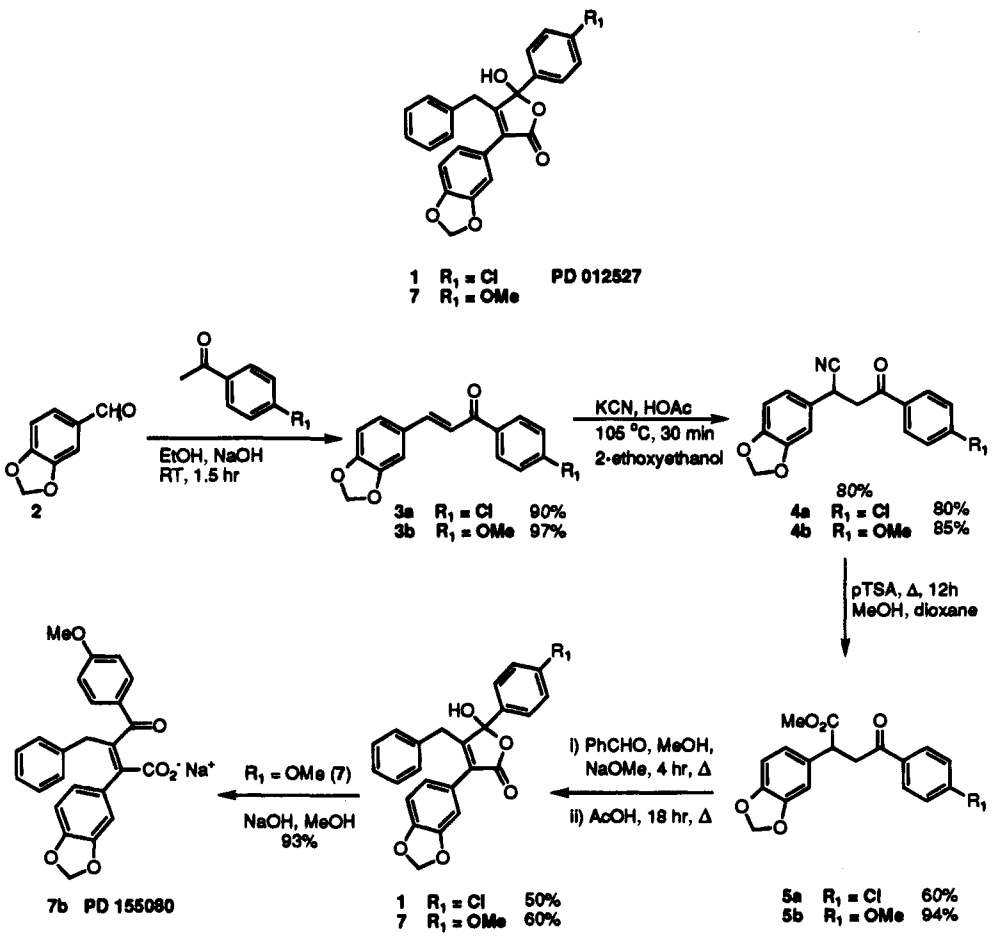
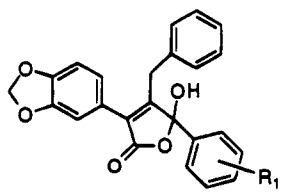


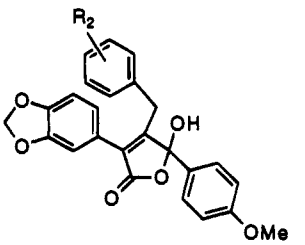
Table 1. Variation of R_1



compd	R_1	IC_{50}, nM ET_A	IC_{50}, nM ET_B
1	4-Cl	2200[a]	5200[c]
		430[b]	27000[d]
6	4-H	1300[a]	22000[c]
		600[b]	30000[d]
7a	4-OMe	50[a]	500[c]
		7.4[b]	4550[d]
8	4-Me	1200[a]	4500[c]
		4900[b]	>25000[d]
9	3,4-Cl_2	560[a]	
		400[b]	>25000[d]
10	3-Me, 4-OMe	12[a]	
		12[b]	1750[d]

[a] Rabbit renal artery vascular smooth muscle cells. [b] Human ET_A (Ltk-expressed). [c] Rat cerebellum. [d] Human ET_B (CHO expressed).

Table 2. Variation of R_2



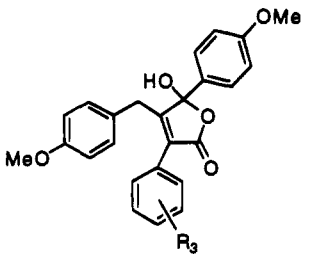
compd	R_2	IC_{50}, nM ET_A	IC_{50}, nM ET_B
7a	4-H	50[a]	500[c]
		7.4[b]	4550[d]
11	4-Cl	140[a]	720[c]
		110[b]	>2500[d]
12	4-OMe	9[a]	220[c]
		1.8[b]	1550[d]
13	4-Me	50[a]	4500[c]
		51[b]	390[d]
14	3-Me, 4-OMe	6[a]	
		2.4[b]	1140[d]

[a] Rabbit renal artery smooth muscle cells. [b] Human ET_A (Ltk-expressed). [c] Rat cerebellum. [d] Human ET_B (CHO expressed).

Results and Discussion. Preliminary enhancement of the receptor binding affinity of compound **1** was achieved *via* application of the Topliss "decision tree" approach for lead optimization based upon QSAR principles.[48,49] Topliss developed a nonmathematical, nonstatistical, and noncomputerized guide to the use of basic Hansch principles, including electronic, lipophilic, and steric considerations, for the optimization of activity of a lead structure containing benzene rings. We first applied this approach for optimization of the substituents R_1, R_2, and R_3 on each of the phenyl rings in the butenolide structure (Tables 1, 2, and 3).

Compound **1** with 4-Cl substitution at the R_1 position was slightly less active than the unsubstituted R_1 phenyl ring analog **6** in binding to both ET_A (rabbit) and

Table 3. Variation of R_3



compd	R_3	IC_{50}, nM ET_A	ET_B
12	3,4-OCH_2O	9[a]	
		1.8[b]	1550[d]
15	4-H	2000[a]	
		2200[b]	>2500[d]
16	4-Cl	960[a]	
		700[b]	>2500[d]
17	4-OMe	310[a]	
		190[b]	>2500[d]
18	4-Me	750[a]	
		570[b]	>2700[d]
19	3,4-Cl_2	310[a]	
		170[b]	>2500[d]

[a] Rabbit renal artery vascular smooth muscle cells. [b] Human ET_A (Ltk-expressed). [c] Rat cerebellum. [d] Human ET_B (CHO expressed).

ET_B (rat) receptors. The subsequent course of action called for synthesis of the 4-OMe analog, compound **7a**. The 4-Me (**8**) and 3,4-Cl_2 (**9**) analogs were also synthesized to check the validity of the approach. As can be seen from Table 1, the 4-OMe analog, compound **7a**, was considerably more potent than the unsubstituted and 4-Cl-, 4-Me-, and 3,4-Cl_2-containing analogs (compounds **6**, **1**, **8**, and **9**) as expected for favorable substitution with a small $-\pi$ value and reasonably large $-\sigma$ value.[48,49]

In order to enhance the $-\sigma$ effect still further, the 4-NMe_2 analog is next suggested from the Topliss tree approach; however, this was not accessible *via* the outlined synthetic route. However, the 3-Me, 4-OMe analog (**10**) was synthesized and, as expected, was found to be slightly more potent than the 4-OMe analog (**7a**) (Table 1). It should be noted that in general compounds were more potent against cloned human ET_A receptors compared with the rabbit ET_A receptors. In contrast, compounds tended to be less potent against the human ET_B receptor compared with the rat ET_B receptor. The net result of these species differences was increased selectivity for ET_A versus ET_B in the human receptor systems.

We applied the same approach to optimize the substituents on the remaining two phenyl rings utilizing 4-OMe at the R_1 position (Tables 2 and 3). Since the 4-Cl (as R_2) analog was again less active than the unsubstituted analog, the 4-OMe, 4-Me and 3-Me, 4-OMe analogs were synthesized (Table 2). It is clear that optimization of the lipophilicity and electronic character of the phenyl ring substituents proved successful in optimizing potency with compound **14**, the 3-Me, 4-OMe analog exhibiting strongest binding affinity at the ET_A receptor. Thus at both the R_1 and R_2 positions, optimization of potency was achieved by increasing the lipophilicity and electron-donating power of the substituents on the aromatic ring. The same trends in species differences were observed.

At the R_3 position, with 4-OMe at R_1 and R_2, the SAR

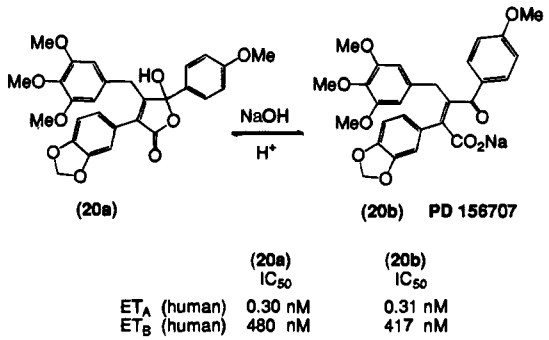


Figure 1. Tautomerization of butenolides.

followed the Topliss decision tree analysis (Table 3, compounds **15**–**19**) with electron-rich aromatic rings preferred. However, the specific substitution pattern was important and the 3,4-methylenedioxy moiety afforded the most active compounds (compound **12**).

Having elucidated the importance of electron-donating substituents at R_2, we explored further ring substitutions to discover the potent trimethoxy analog **20a** (and its sodium salt **20b**) with subnanomolar affinity for the ET_A receptor (Figure 1).

Compounds **7a** and **20a** were converted to their water-soluble tautomeric keto acid sodium salts **7b** and **20b**, PD 155080 and PD 156707, respectively,[41,42,46] by treatment with sodium hydroxide in methanol (Scheme 1 and Figure 1). Activities for the butenolides **7a** and **20a** were very similar to those of their open form, tautomeric sodium salts **7b** and **20b**, respectively (Figure 1 for **20a** and **20b**). Further pharmacological characterization was carried out with the sodium salts **7b** and **20b** due to their substantially increased aqueous solubility.

Compound **7b** is a potent competitive inhibitor of [^{125}I]-ET-1 and [^{125}I]ET-3 binding to human cloned ET_A and ET_B receptors with IC_{50}'s of 7.8 nM and 3.5 μM, respectively. The compound also antagonizes ET-1-induced arachidonic acid release in rabbit renal artery VSMC with an IC_{50} of 0.15 μM. Compound **20b** is approximately 10-fold more potent in binding to human cloned ET_A and ET_B receptors with IC_{50}'s of 0.3 nM and 0.42 μM, respectively, and antagonizes ET-1-induced arachidonic acid release in rabbit renal artery VSMC with an IC_{50} of 1.1 nM.

Compound **7b** was evaluated for its ability to inhibit ET-1- and ET-3-induced vasoconstriction in rabbit femoral (ET_A) and pulmonary (ET_B) arteries and found to have pA_2 values of 6.3 and 4.8, respectively. In the same functional assays, compound **20b** was found to have pA_2 values of 7.5 (ET_A) and 4.5 (ET_B), respectively. Compound **20b** administered orally to ganglionic blocked rats[50] inhibited ET-1-induced pressor responses (ET_A receptor-mediated) in vivo (IC_{50} = 1 mg/kg po).

The pharmacokinetics of **7b** and **20b** were evaluated in male Wistar rats following a 15 mg/kg intravenous or oral gavage dose (three animals per dose).[51] Plasma concentrations of **7b** and **20b** were determined by a specific HPLC assay. The mean concentration time profiles are shown in Figures 2 and 3. The terminal elimination $t_{1/2}$ was 5 h for **7b** and 1 h for **20b**. After oral dose, both compounds were rapidly absorbed with an absolute oral bioavailability of 87% and 41% for compounds **7b** and **20b**, respectively.

In summary, optimization of a butenolide endothelin

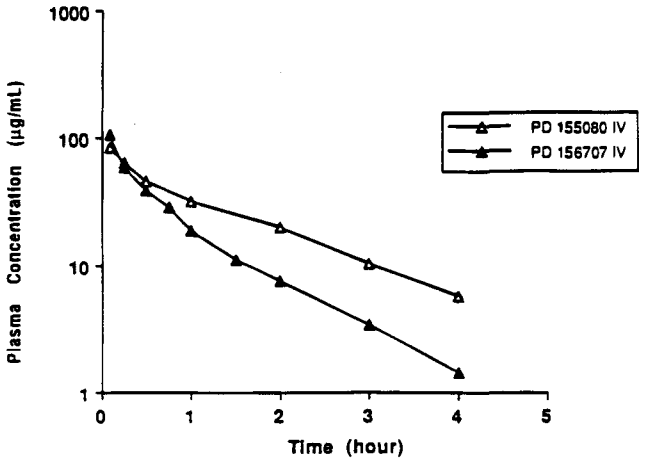


Figure 2. Mean concentration–time profiles of the non-peptide endothelin antagonists in male Wistar rats following a 15 mg/kg intravenous dose of **7b** or **20b** (three animals per dose).

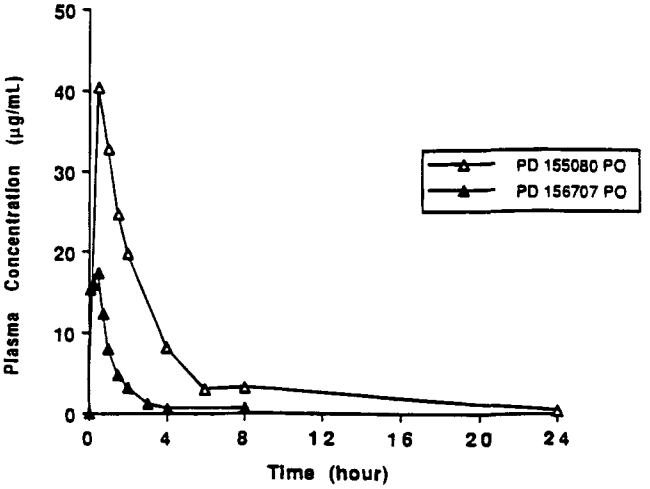


Figure 3. Mean concentration–time profiles of the non-peptide endothelin antagonists in male Wistar rats following a 15 mg/kg oral gavage dose of **7b** or **20b** (three animals per dose).

antagonist, compound **1**, discovered through a compound library screening program employing an ET_A receptor binding assay, led to the development of a potent series of ET_A-selective antagonists active against human receptors. Compounds **7b** and **20b** are orally active, non-peptide, highly ET_A selective receptor antagonists exemplified in this series. These compounds should prove useful in elucidating the role of endothelin in normal physiology and in the pathophysiology of human disease.

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Supplementary Material Available: Experimental procedures for the preparation of compounds **3b**, **4b**, **5b**, and **6–20** (5 pages). Ordering information is given on any current masthead page.

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