Butenolide Endothelin Antagonists with Improved Aqueous Solubility

William C. Patt,*,† Xue-Min Cheng,† Joseph T. Repine,† Chet Lee,† Bill R. Reisdorph,† Mark A. Massa,† Annette M. Doherty,† Kathleen M. Welch,‡ John W. Bryant,‡ Michael A. Flynn,‡ Donnelle M. Walker,‡ Richard L. Schroeder,‡ Stephen J. Haleen,‡ and Joan A. Keiser‡

Departments of Chemistry and Vascular and Cardiac Diseases, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Received August 31, 1998

Continued development around our ET_A-selective endothelin (ET) antagonist **1** (CI-1020) has led to the synthesis of analogues with improved aqueous solubility profiles. Poor solubility characteristics displayed by **1** required a complex buffered formulation in order to conduct iv studies. To overcome the use of specific iv formulations for preclinical studies on additional drug candidates, analogues with improved aqueous solubility were desired. Several analogues were synthesized with substitution patterns that allowed for the formation of either acid or base addition salts. These derivatives had dramatically improved aqueous solubility. In addition, these analogues retained equivalent or improved ET_A receptor selectivity and antagonist potency, versus **1**, both in vitro and in vivo. Compound **29**, which contains as a substituent the sodium salt of a sulfonic acid, has an ET_A $IC_{50} = 0.38$ nM, ET_A selectivity of 4200-fold, and ET_A functional activity of $K_B = 7.8$, all of which are similar or superior to those of **1**. Compound **29** also has vastly superior aqueous solubility and solubility duration, compared to **1**. Furthermore, **29** after iv infusion displays improved activity to **1** in preventing acute hypoxia-induced pulmonary hypertension in rats with an $ED_{50} = 0.3$ μg/kg/h.

Introduction

The endothelins (ETs) are a family of peptides that display a variety of physiological activities. These peptides exert their actions via activation of two distinct receptor subtypes, ET_A and ET_B. Antagonists of these receptors have received numerous citations in the recent pharmaceutical literature.[1−3] Both ET_A- and ET_B-selective, as well as nonselective, receptor antagonists demonstrating wide therapeutic potential have been described.[4−6] Our first clinical candidate in this field, **1** (CI-1020), exemplifies an ET_A-selective antagonist.[4]

Compound **1** is an extremely potent antagonist of the human recombinant ET_A receptor with an $IC_{50} = 0.30$ nM. It is also 2600-fold selective for the ET_A receptor over the ET_B receptor (ET_B $IC_{50} = 780$ nM). Chemically **1** is rather interesting in that it exists, in solution, as an equilibrium mixture of two tautomers, Figure 1. At acidic pH's the equilibrium resides predominantly in the γ-hydroxy butenolide structure, **1**, while at basic pH's the equilibrium favors the ring-opened γ-keto acid salt structure, **1a**. This equilibrium allows for isolation of either form as a stable solid. However in aqueous solution, at physiological pH, both tautomers are present. In fact, due to solution-phase equilibration, both tautomers are always present, to some extent, regardless of pH. While one can easily synthesize and isolate the water-soluble salts of the keto acid forms, once they are placed in aqueous solutions the equilibrium determines how much of each tautomer is present. In fact, if the γ-hydroxy butenolide tautomer is sufficiently water-insoluble, it can precipitate out of solution and the equilibrium can drive the complete precipitation of the compound. While **1** has good oral activity,[4] displaying the ability to counteract the effects of an exogenous dose of the ET agonist ET-1, its iv use is limited by the insolubility of the closed-form butenolide tautomer, which precipitates out of aqueous solutions, without the use of a specific and complex buffered formulation.

Our goal in this project was to develop water-soluble closed-form γ-hydroxy butenolides that would not require specific formulations for parenteral uses. Toward that end, two general series of analogues were synthesized which retained the desired ET_A antagonist selectivity and potency while greatly improving the aqueous solubility of the drug candidates at physiological pH.

Chemistry

The synthetic route to these compounds has been previously published,[4,7] and the general approach is depicted in Scheme 1. Noncommercial aldehydes **8** employed in step iv were obtained by the alkylation of the corresponding phenols **7** (3-hydroxybenzaldehyde and 3,4-dimethoxy-5-hydroxybenzaldehyde) with a haloalkylamine, using standard methodology. The crude aldehydes were generally used without extensive purification in the subsequent condensation reaction.

Results and Discussion

Two series of compounds are shown in Table 1, and both contain the *p*-methoxyphenyl substituent at the γ-position and the 3,4-(methylenedioxy)phenyl moiety at the α-position on the butenolide structure. These two substituents impart high ET_A potency to the butenolide series.[4] The SAR presented here has been restricted to the "central" benzylic group. This position is known to accept a wide variety of substitution patterns while retaining potency.[4]

† Department of Chemistry.
‡ Department of Vascular and Cardiac Diseases.

10.1021/jm980504w CCC: $18.00
Published on Web 05/21/1999

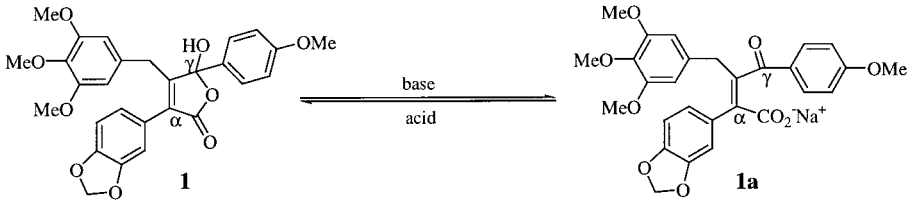
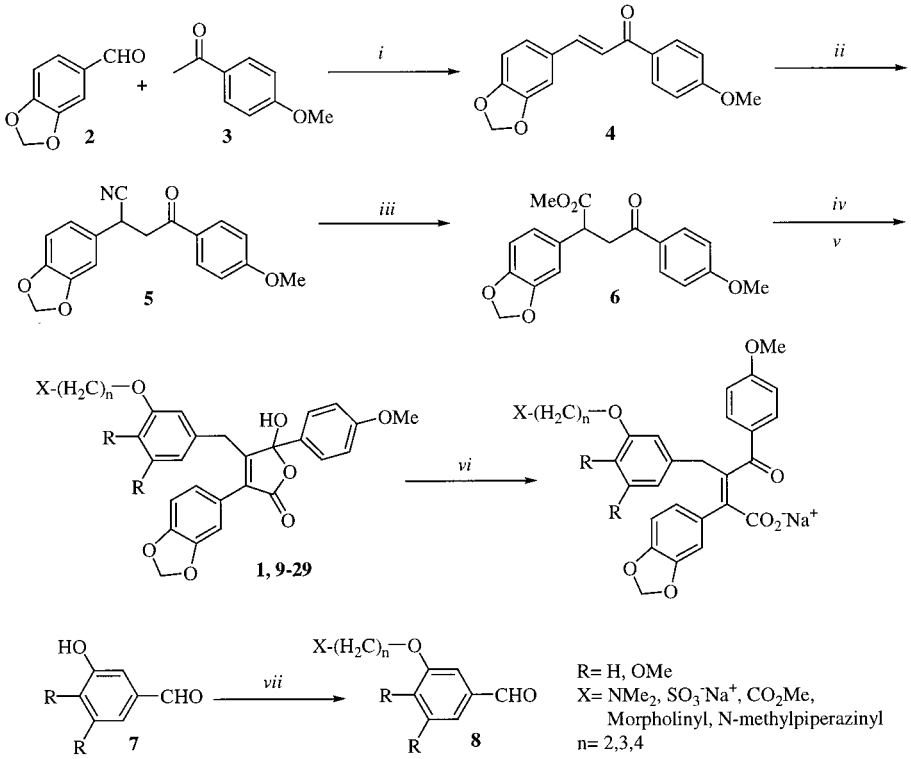


Figure 1. Tautomerization of butenolides.

Scheme 1[a]



[a] (i) NaOH, EtOH; (ii) KCN, HOAc, 2-ethoxyethanol, 100 °C; (iii) *p*-TsOH, MeOH, reflux; (iv) NaOMe, MeOH, **8**; (v) HOAc; (vi) NaOH; (vii) Cs_2CO_3, X-$(CH_2)_n$-Cl, DMF.

Compounds **9**−**22** in Table 1 contain basic groups that allow the formation of acid addition salts, expected to improve the aqueous solubility of the closed butenolide form. The compounds were tested for their ability to inhibit specific [^{125}I]ET-1 binding to recombinant human ET_A and ET_B receptors as previously described.[8]

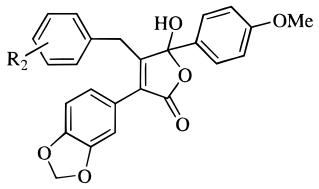
The first seven analogues, **9**−**15**, are monosubstituted with aminoalkoxy groups. From previous work, we know that long chains at the para position have a negative effect on ET receptor potency while meta substitution is unaffected. This is borne out in this series as exemplified by compounds **9** and **11**. The *p*-(dimethylamino)-propoxy compound **9** was essentially inactive, while the otherwise identical meta derivative **11** had an ET_A IC_{50} = 28 nM.

Chain length at the meta position, while not explored to a great extent, appears to be unimportant, with regard to binding potency. The (dimethylamino)ethoxy and -propoxy analogues **10** and **11**, as well as the morpholinylethoxy and -propoxy compounds **12** and **13**, were both equipotent within their own series. The cyclic amines were more potent than the dimethylamino derivatives. The morpholinyl (**13**), methylpiperazinyl (**14**), and pyrrolidinyl (**15**) analogues all had improved activity over the dimethylamino compounds. The morpholinylpropoxy compound **13** is the most potent of the monosubstituted basic compounds with an ET_A IC_{50} = 2.0 nM.

Compounds **16**−**20** contain 3,4-dimethoxy substitutions, on the benzyl, in addition to the *m*-aminoalkoxy moiety. This trialkoxy substitution pattern induces a 10−50-fold improvement in receptor binding affinity, as expected from previous work.[4] The (dimethylamino)-ethoxy and -propoxy compounds **16** and **17** have 2.8 and 1.0 nM binding affinities, respectively, at the ET_A receptor. Again the morpholinyl compounds are more potent as exemplified by compound **19** which has a binding affinity of IC_{50} = 0.2 nM (ET_A) which is just slightly more potent than **1**. Compounds **21** and **22** contain two morpholinylethoxy substituents, and both are substantially less active indicating some steric limitations around the central benzylic site. Few of these compounds had measurable ET_B inhibition, which is consistent with our earlier report in that most butenolides are extremely ET_A-selective.

Compounds **23**−**29**, in Table 1, contain acidic substitutions as well as some of their immediate ester precursors. The acidic functionalities on these analogues allowed for the formation of "metal" salts to attempt to improve the aqueous solubility of the butenolides. The three carboxylic acid compounds **24**, **26**, and **28** were

Table 1. Biological Data for Butenolides



compd	R_2	IC_{50} (nM) ET_A[a]	IC_{50} (nM) ET_B[a]	K_B ET_A[b]
1	3,4,5-$(OMe)_3$	0.30	780	7.5
9	4-O-$(CH_2)_3$-NMe_2	>25000	>2500	
10	3-O-$(CH_2)_2$-NMe_2	27	2400	
11	3-O-$(CH_2)_3$-NMe_2	28	>250	
12	3-O-$(CH_2)_2$-(*N*-morpholinyl)	4.0	>2500	6.2
13	3-O-$(CH_2)_3$-(*N*-morpholinyl)	2.0	>2500	
14	3-O-$(CH_2)_3$-(*N*′-Me-*N*-piperazinyl)	12	>2500	
15	3-O-$(CH_2)_2$-(*N*-pyrrolidinyl)	10	>2500	
16	3,4-$(OMe)_2$-5-O-$(CH_2)_2$-NMe_2	2.8	>2500	7.0
17	3,4-$(OMe)_2$-5-O-$(CH_2)_3$-NMe_2	1.0	>2500	
18	3,4-$(OMe)_2$-5-O-$(CH_2)_3$-(*N*′-Me-*N*-piperazinyl)	2.0	>2500	
19	3,4-$(OMe)_2$-5-O-$(CH_2)_2$-(*N*-morpholinyl)	0.2	2300	6.9
20	3,4-$(OMe)_2$-5-O-$(CH_2)_3$-(*N*-morpholinyl)	0.7	>250	6.8
21	4-OMe-3,5-[O-$(CH_2)_2$-(*N*-morpholinyl)]$_2$	2000	>2500	
22	3,5-[O-$(CH_2)_2$-(*N*-morpholinyl)]$_2$	>10	>2500	
23	3,4-$(OMe)_2$-5-OCH_2CO_2Me	0.2	600	
24	3,4-$(OMe)_2$-5-OCH_2CO_2H	8.5	4500	
25	3,4-$(OMe)_2$-5-O$(CH_2)_3CO_2Me$	0.09	200	7.5
26	3,4-$(OMe)_2$-5-O$(CH_2)_3CO_2H$	0.29	1200	7.4
27	3,4-$(OMe)_2$-5-O$(CH_2)_4CO_2Me$	0.05	900	7.8
28	3,4-$(OMe)_2$-5-O$(CH_2)_4CO_2H$	0.30	2100	
29	3,4-$(OMe)_2$-5-O$(CH_2)_3SO_3Na$	0.38	1600	7.8

[a] IC_{50} values were determined as described using six concentrations of inhibitor (0.01−2500 nM), depending on inhibitor and $n = 2$. [b] Inhibition of ET-1-induced contraction in rabbit femoral artery rings; K_B determined from $n = 2$.

derived from their corresponding esters **23**, **25**, and **27** by base hydrolysis. The acids were less potent at the ET_A receptor than their esters by 5−40-fold but still retained significant activity since the esters were some of the most potent analogues we have synthesized in this program. Ester derivative **27** has an ET_A IC_{50} = 50 pM, 6 times more potent than that of **1**. These esters are, unfortunately, virtually insoluble in aqueous systems and were unsuitable for in vivo testing, and consequently further development was prohibited. The most potent of the carboxylic acids, **26**, has an ET_A IC_{50} = 0.3 nM, equivalent to that of **1**. The final acidic analogue in this study, **29**, contains a sulfonic acid group which was isolated solely as a sodium salt due to the strongly acidic nature of the sulfonic acid moiety. This derivative is also rather potent with an ET_A IC_{50} = 0.38 nM, identical to that of **1**.

Functional activity due to ET_A receptor antagonism was then determined, for selected compounds from Table 1. This arterial ring contraction assay was run in isolated rabbit femoral arteries, an ET_A tissue, using ET-1 as the agonist as previously disclosed.[8,9] As expected, antagonist activity correlated with receptor binding affinity. The clinical lead **1** has a K_B value of 7.5 in this assay. The base-containing compounds have K_B values of 6.2−7.0, while the more potent esters and acids had potencies equivalent with the potency of **1**. The sulfonic acid analogue **29** had a K_B value of 7.8, slightly more potent than that of **1**.

In Table 2 the solubility data for several of the compounds in pH 7.4 phosphate buffer is presented. The parent ring-closed butenolide version of **1** has essentially

Table 2. Solubility Data for Selected Butenolides

compd	salt form	aqueous solubility[a]	solubility duration[b]
1	parent	<0.01	not determined
1a	Na	≥125	precipitates[c]
12	parent	<0.25	not determined
12	isethionic	<0.25	not determined
16	isethionic	≥250	soluble for >120 h
20	isethionic	≥250	soluble for >120 h
26a	di Na	≥250	soluble for >120 h
29	Na	≥250	soluble for >120 h

[a] In mg/mL, determined in pH 7.4 phosphate buffer. [b] Visual observation of a 50 mg/mL solution in pH 7.4 phosphate buffer. [c] Turns cloudy within minutes, and a noticeable precipitate is observed within 2 h.

no solubility, while the sodium salt **1a** has good immediate solubility (≥125 mg/mL), but exposure to physiological pH quickly drives the equilibrium to the poorly soluble closed-form butenolide. Thus, in 10 min an initial 50 mg/mL solution of **1a** starts to turn hazy, and after 2 h a definite precipitate is observed. It should be noted that even a 1.0 mg/mL solution of **1a** precipitates out of solution rapidly.

Numerous acid addition salts were examined (e.g., citric acid, hydrochloric acid, acetic acid, methanesulfonic acid...) for their ability to increase aqueous solubility of the base-containing derivatives, and the isethionic acid salt was found to give the greatest degree of improvement. All of the γ-hydroxy butenolides (closed form) containing basic groups had very poor aqueous solubility without an acid addition salt. The monoaminoalkoxy-substituted derivative **12** has low aqueous solubility, <0.25 mg/mL, as both a free base and its

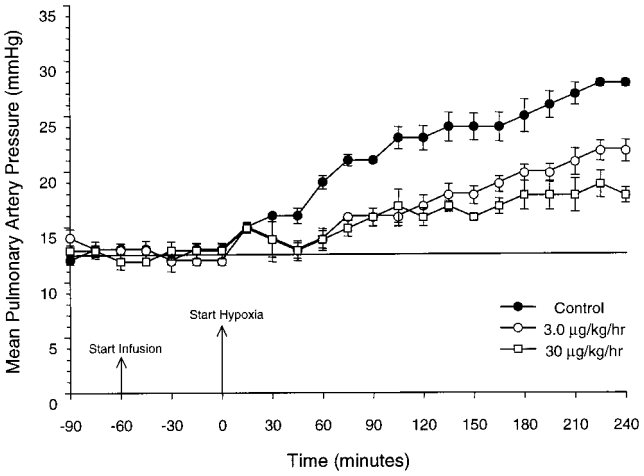


Figure 2. Pulmonary hypertensive response for **16**.

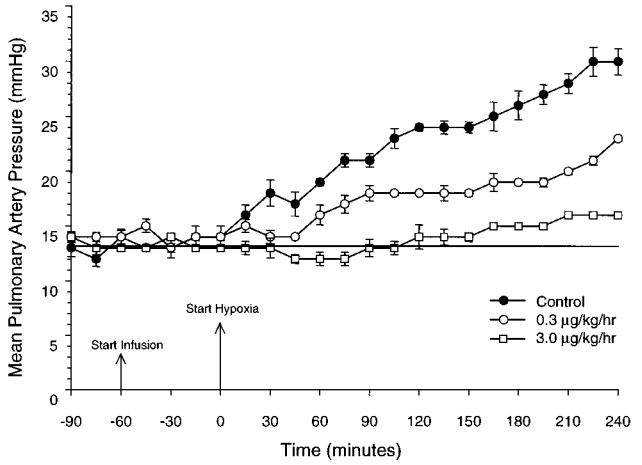


Figure 3. Pulmonary hypertensive response for **29**.

isethionic acid salt. Salts of the more potent dimethoxy derivatives had greatly improved aqueous solubility characteristics. The isethionic acid salts of **16** and **20** had excellent aqueous solubility (≥250 mg/mL respectively). More importantly a 50 mg/mL solution of either did not cause precipitation for >120 h after dissolution.

Finally, two of the derivatives containing acidic substituents were examined. The carboxylic acid **26a** was tested as a bis-sodium salt in the open form similar to **1**. This salt had initial solubility similar to that of **1**, ≥250 mg/mL. Fortunately, the duration of solubility was greatly enhanced versus that of **1**. A 50 mg/mL solution showed no evidence of precipitation after >120 h in solution. The more potent antagonist **29** was tested as its monosodium salt in the closed form, and it had excellent aqueous solubility (≥250 mg/mL). This derivative also showed no evidence of precipitating out of solution even upon standing for >120 h.

Two of the potent water-soluble salts, **16** and **29**, were then tested in vivo for activity against acute hypoxic pulmonary hypertension.[10,11] In this model male Sprague−Dawley rats were surgically instrumented with pulmonary artery and femoral vein cannulae. The day after surgery the instrumented rats were given either vehicle or drug by iv infusion from 1 h before and throughout 4 h of hypoxia (10% oxygen). This hypoxic regimen produced more than a 2-fold increase in pulmonary arterial pressure (13 ± 1 to 30 ± 4 mmHg) in vehicle-treated rats.

The data obtained for **16** and **29** are displayed graphically in Figures 2−4. Figure 2 gives the actual blood pressure response observed with **16**, and Figure 3 is the raw data obtained with **29**. Both compounds gave a good dose response, and both lowered pulmonary arterial pressure versus vehicle-treated rats over the entire infusion period. Figure 4 depicts the potency of **16** and **29** using area under the curve (AUC) methodology. The ED_{50}'s that were determined via the AUC method are displayed in Table 3 and compared to the published data for **1**. These two novel analogues bracket the potency of **1** (ED_{50} = 1.5 μg/kg/h). The single-digit nanomolar receptor binding compound **16** gave an ED_{50} = 6.0 μg/kg/h, while the more potent subnanomolar binding analogue **29** gave an ED_{50} = 0.3 μg/kg/h, 5 times as potent as that of **1**.

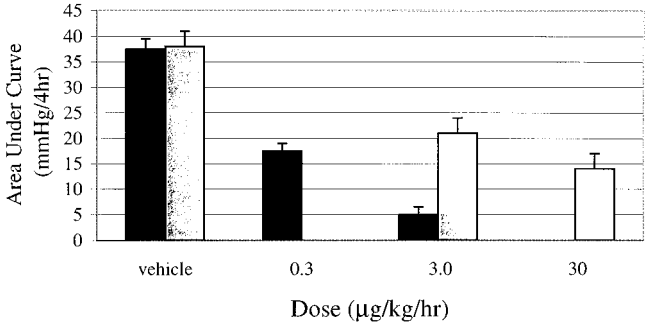


Figure 4. Pulmonary hypertension data for **29** (black) and **16** (gray). Pulmonary arterial hypertensive response in conscious Sprague−Dawley rats to hypoxia (10% O_2). This area-under-the-curve representation is the hypertensive response above prehypoxic baseline levels accumulated over 4 h.

Table 3. Acute Pulmonary Hypertension Data

compd	ED_{50} (μg/kg/h)[a]
1	1.5
16	6.0
29	0.3

[a] Determined using the AUC data from Figure 4.

Conclusions

We have been able to synthesize analogues closely related to **1** that retain similar ET_A receptor potency and selectivity. Several analogues containing salts of either acidic or basic groups had increased aqueous solubility and solution duration, a prerequisite for parenteral administration. Of particular note are the isethionic acid salt of the (dimethylamino)ethoxy analogue **16** and the sodium salt of the sulfonic acid **29**. Compound **16** had an ET_A IC_{50} = 2.8 nM (>900-fold selective) and a K_B value of 7.0 in the in vitro ET_A functional assay, while **29** had an ET_A IC_{50} = 0.38 nM (4200-fold selective) and a K_B value of 7.8 in the in vitro ET_A functional assay. Both compounds exhibit high aqueous solubility and did not precipitate out of solution upon prolonged exposure to aqueous solutions at physiological pH. Additionally, **16** and **29** were active in preventing acute hypoxia-induced pulmonary hypertension in the conscious rat. Compound **16** was slightly less potent than **1**, in this assay, with an ED_{50} = 6.0 μg/kg/h, while **29** was slightly more potent with an ED_{50} = 0.3 μg/kg/h. Several of these compounds are undergoing preclinical evaluation.

Experimental Section

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 a Varian Unity 300- or 400-MHz spectrometer and are referenced to internal TMS. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 500 resolution, a Finnigan MAT 900Q spectrometer, or a Micromass platformLC using MassLynx 2.3 data system and OpenLynxLC open access software. Microanalyses were determined under contract with Robertson Analytical Services.

General Example for Compounds 1 and 9−29. 3-Benzo[1,3]dioxol-5-yl-4-[3-(2-(dimethylamino)-ethoxy)-4,5-dimethoxybenzyl]-5-hydroxy-5-(4-methoxy-phenyl)-5*H*-furan-2-one, 16. In DMF (45 mL) were stirred 3,4-dimethoxy-5-hydroxybenzaldehyde (1.82 g, 10 mmol) and cesium carbonate (8.14 g, 25 mmol). To this was added (dimethylamino)ethyl chloride hydrochloride (1.73 g, 12 mmol). The mixture was stirred at 50 °C for 18 h. The mixture was filtered free of insolubles and evaporated in vacuo to give an oil. This was partitioned between ether (100 mL) and water (100 mL). The ether layer was dried over magnesium sulfate and evaporated in vacuo to give 1.75 g (69%) of crude 3,4-dimethoxy-5-[(dimethylamino)ethoxy]benzaldehyde, which was used as is. In methanol (10 mL) was dissolved sodium metal (97 mg, 4.2 mmol). To this solution were added **6** (2-benzo[1,3]dioxol-5-yl-4-(4-methoxyphenyl)-4-oxobutyric acid methyl ester[7]) (1.37 g, 4.0 mmol) and 3,4-dimethoxy-5-[(dimethylamino)ethoxy]benzaldehyde (1.04 g, 4.1 mmol). The solution was warmed to 60 °C and stirred for 24 h. The mixture was treated with acetic acid (1 mL) and stirred at reflux for an additional 24 h. The mixture was cooled to room temperature and evaporated free of solvents. The residue was partitioned between ethyl acetate (75 mL) and water (50 mL). The organic phase was separated and the aqueous phase extracted with methylene chloride (2 × 50 mL). The methylene chloride washes were combined and dried over magnesium sulfate. The solvents were evaporated in vacuo to give a foam. The foam was purified by flash chromatography (150 g silica gel, 10−15% methanol in methylene chloride). The appropriate fractions were evaporated in vacuo to give 1.05 g (47%) of pure compound. ^{1}H NMR: (DMSO-d_6) δ 2.34 (s, 6H), 2.79 (d, J = 5.8 Hz, 2H), 3.64−3.67 (m, 2H), 3.66 (s, 3H), 3.69 (s, 3H), 3.77 (s, 3H), 4.06 (t, J = 5.8 Hz, 2H), 5.93 (s, 2H), 6.08 (s, 1H), 6.36 (s, 1H), 6.75−6.94 (m, 5H), 7.45−7.49 (m, 2H), butenolide OH missing. Microanalysis ($C_{31}H_{33}NO_9$) C, H, N. MS: (CI) M^+ = 564.

3-Benzo[1,3]dioxol-5-yl-4-[3-(2-(dimethylamino)ethoxy)-4,5-dimethoxybenzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 16, Isethionic Acid Salt. To **16** (3.62 g, 6.4 mmol) in methanol (75 mL) was added isethionic acid (15.2 mL of 0.42 M solution in water, 6.4 mmol). The mixture was stirred for 30 min and evaporated in vacuo to give a foam. This was treated with methanol (2 × 25 mL) and again evaporated. The residue was triturated with ether (50 mL) and the resultant solid collected by filtration to give 3.95 g (90%) of the pure salt. ^{1}H NMR: (DMSO-d_6) δ 2.60 (t, J = 6.8 Hz, 2H), 2.89 (s, 6H), 3.43−3.49 (m, 2H), 3.53 (s, 3H), 3.54 (s, 3H), 3.56−3.58 (m, 4H), 3.73 (s, 3H), 3.98−4.04 (m, 2H), 6.01 (s, 2H), 6.08 (s, 2H), 6.87−6.95 (m, 5H), 7.36 (d, J = 7.7 Hz, 2H), 8.2 (br.s, ex, 1H), 9.53 (br.s, ex, 1H), butenolide OH missing. Microanalysis ($C_{31}H_{33}NO_9 \cdot C_2H_6SO_4 \cdot 0.6H_2O$) C, H, N, S, H_2O-(KF). MS: (CI) M^+ = 564.

3-Benzo[1,3]dioxol-5-yl-4-[4-(3-(dimethylamino)propoxy)-benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 9. ^{1}H NMR: (DMSO-d_6) δ 2.03−2.08 (m, 2H), 2.75 (s, 6H), 3.11−3.15 (m, 2H), 3.55−3.60 (m, 2H), 3.75 (s, 3H), 3.89−3.92 (m, 2H), 6.02 (s, 2H), 6.59 (d, J = 8.6 Hz, 2H), 6.71 (d, 8.6 Hz, 2H), 6.88−6.94 (m, 5H), 7.32 (d, J = 8.8 Hz, 2H), 8.11 (s, 1H), 10.31 (br.s, 1H). Microanalysis ($C_{30}H_{31}NO_7 \cdot HCl \cdot 1.3H_2O$) C, H, N, Cl^-. MS: (CI) M^+ = 518.

3-Benzo[1,3]dioxol-5-yl-4-[3-(2-(dimethylamino)ethoxy)-benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 10. ^{1}H NMR: (DMSO-d_6) δ 2.23 (s, 6H), 2.66 (t, J = 5.5 Hz, 2H), 3.71 (s, 2H), 3.75 (s, 3H), 3.89 (t, J = 5.5 Hz, 2H), 5.95 (s, 2H), 6.47 (d, J = 7.4 Hz, 1H), 6.58 (m, 2H), 6.78 (m, 3H), 6.96 (m, 3H), 7.41 (m, 2H), butenolide OH missing. Microanalysis ($C_{29}H_{29}NO_7 \cdot 1.3H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 504.

3-Benzo[1,3]dioxol-5-yl-4-[3-(3-(dimethylamino)propoxy)benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 11. ^{1}H NMR: (DMSO-d_6) δ 1.93 (m, 2H), 2.38 (s, 6H), 2.69 (t, J = 7.6 Hz, 2H), 3.62 (s, 2H), 3.75 (s, 3H), 3.85 (t, J = 6.0 Hz, 2H), 5.92 (s, 2H), 6.58 (m, 3H), 6.74 (d, J = 8.0 Hz, 1H), 6.81 (m, 2H), 6.95 (m, 3H), 7.4 (m, 2H), butenolide OH missing. Microanalysis ($C_{30}H_{31}NO_7 \cdot 2.2H_2O$): C, H, N. MS: (CI) $M^+ + 1$ = 518.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-[3-(2-morpholin-4-ylethoxy)benzyl]-5*H*-furan-2-one, 12. ^{1}H NMR: (DMSO-d_6) δ 2.62 (m, 4H), 2.78 (t, J = 5.5 Hz, 2H), 3.69 (s, 2H), 3.73 (m, 4H), 3.80 (s, 3H), 4.02 (t, J = 5.5 Hz, 2H), 5.96 (s, 2H), 6.53 (m, 2H), 6.64 (m, 1H), 6.77 (m, 1H), 6.85 (m, 2H), 6.96 (m, 2H), 7.06 (t, J = 8.0 Hz, 1H), 7.40 (m, 2H), butenolide OH missing. Microanalysis ($C_{31}H_{31}NO_8 \cdot 1.5H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 546.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-[3-(2-morpholin-4-ylethoxy)benzyl]-5*H*-furan-2-one, 12, Isethionic Acid Salt. ^{1}H NMR: (DMSO-d_6) δ 2.50 (m, 4H), 2.60 (t, J = 7.0 Hz, 2H), 3.19 (m, 1H), 3.72−3.40 (m, 6H), 3.74 (s, 3H), 3.96 (m, 2H), 4.5 (m, 2H), 6.02 (s, 2H), 6.48 (s, 1H), 6.50 (d, J = 7.7 Hz, 1H), 6.67 (m, 1H), 6.91 (m, 6H), 7.36 (m, 2H), 8.16 (s, 1H), 9.83 (br.s, 1H), acid protons missing. Microanalysis ($C_{30}H_{31}NO_7 \cdot C_2H_6SO_4 \cdot 0.57H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 546.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-[3-(3-morpholin-4-ylpropoxy)benzyl]-5*H*-furan-2-one, 13. ^{1}H NMR: (DMSO-d_6) δ 2.10 (m, 2H), 2.50 (m, 4H), 3.05 (m, 2H), 3.35 (br.s, 1H), 3.42 (m, 2H), 3.64 (m, 2H), 3.75 (s, 3H), 3.81 (m, 2H), 3.96 (m, 2H), 6.03 (s, 2H), 6.29 (s, 1H), 6.42 (d, J = 7.7 Hz, 1H), 6.58 (m, 1H), 6.88 (m, 5H), 7.35 (d, J = 9.2 Hz, 2H), 8.17 (s, 1H). Microanalysis ($C_{32}H_{33}NO_8 \cdot 2.3H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 560.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-{3-[3-(4-methylpiperazin-1-yl)propoxy]benzyl}-5*H*-furan-2-one, 14. ^{1}H NMR: (DMSO-d_6) δ 1.85 (m, 2H), 2.22 (s, 3H), 2.48 (m, 10H), 3.68 (s, 2H), 3.77 (s, 3H), 3.79 (m, 2H), 5.95 (s, 2H), 6.48 (m, 2H), 6.61 (m, 1H), 6.81(m, 3H), 6.94 (m, 2H), 7.00 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 8.9 Hz, 2H), butenolide OH missing. Microanalysis ($C_{33}H_{36}N_2O_7 \cdot 0.65H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 573.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-5-(4-methoxyphenyl)-4-[3-(2-pyrrolidin-1-ylethoxy)benzyl]-5*H*-furan-2-one, 15. ^{1}H NMR: (DMSO-d_6) δ 1.89 (br.m, 4H), 2.96 (m, 4H), 3.06 (t, J = 5.3 Hz, 2H), 3.68 (s, 2H), 3.78 (s, 3H), 4.7 (t, J = 5.3 Hz, 2H), 5.92 (s, 2H), 6.57 (m, 2H), 6.73 (m, 2H), 6.83 (m, 2H), 6.93 (m, 2H), 7.0 (t, J = 7.9 Hz, 1H), 7.45 (m, 2H), butenolide OH missing. Microanalysis ($C_{31}H_{31}NO_7 \cdot 1.7H_2O$) C, H, N. MS: (CI) $M^+ + 1$ = 530.

3-Benzo[1,3]dioxol-5-yl-4-[3-(3-(dimethylamino)-propoxy)-4,5-dimethoxybenzyl]-5-hydroxy-5-(4-methoxy-phenyl)-5*H*-furan-2-one, 17. ^{1}H NMR: (DMSO-d_6) δ 2.17−2.24 (m, 2H), 2.75 (s, 6H), 3.11−3.15 (m, 2H), 3.61 (s, 2H), 3.65 (s, 3H), 3.69 (s, 3H), 3.79 (s, 3H), 3.97−4.00 (m, 2H), 5.93 (s, 2H), 6.03 (s, 1H), 6.33 (s, 1H), 6.75−6.93 (m, 5H), 7.42−7.45, (m, 2H), butenolide OH missing. Microanalysis ($C_{32}H_{35}NO_9$) Calcd: C, 66.54; H, 6.11; N, 2.42. Found: C, 60.99; H, 5.94; N, 2.17. High-resolution ESI-MS (resolution 19 000 FWHH): measured, 578.2360; theory, 578.2390; difference, −5.2 ppm. MS: (CI) M^+ = 578.

3-Benzo[1,3]dioxol-5-yl-4-{3,4-dimethoxy-5-[3-(4-methylpiperazin-1-yl)propoxy]benzyl}-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 18. ^{1}H NMR: (DMSO-d_6) δ 1.89−1.96 (m, 2H), 2.27 (s, 3H), 2.50−2.70 (m, 10H), 3.63 (m, 2H), 3.66 (s, 3H), 3.74 (s, 3H), 3.80 (s, 3H), 3.88−3.92 (m, 2H), 5.96 (s, 2H), 6.06 (s, 1H), 6.26 (s, 1H), 6.79−6.96 (m, 5H), 7.44−

7.46 (m, 2H), butenolide OH missing. Microanalysis ($C_{35}H_{40}N_2O_9 \cdot H_2O$) C, H, N. MS: (CI) $M^+ = 633$.

3-Benzo[1,3]dioxol-5-yl-4-[3,4-dimethoxy-5-(2-morpholin-4-ylethoxy)benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 19. ^{1}H NMR: (DMSO-d_6) δ 2.55−2.57 (m, 4H), 2.74 (t, $J = 5.8$ Hz, 2H), 3.65 (s, 3H), 3.65−3.73 (m, 6H), 3.74 (s, 3H), 3.79 (s, 3H), 3.92 (t, $J = 5.8$ Hz, 2H), 5.97 (s, 2H), 6.04 (s, 1H), 6.10 (s, 1H), 6.79−6.97 (m, 5H), 7.39−7.42 (m, 2H), butenolide OH missing. Microanalysis ($C_{33}H_{35}NO_{10} \cdot 0.6H_2O$) C, H, N. MS: (CI) $M^+ = 606$.

3-Benzo[1,3]dioxol-5-yl-4-[3,4-dimethoxy-5-(3-morpholin-4-ylpropoxy)benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 20. ^{1}H NMR: (DMSO-d_6) δ 1.89−1.96 (m, 2H), 2.47−2.54 (m, 6H), 3.66 (s, 3H), 3.66−3.72 (m, 6H), 3.75 (m, 3H), 3.80 (m, 3H), 3.83−3.87 (m, 2H), 5.97 (s, 2H), 6.04 (s, 1H), 6.12 (s, 1H), 6.80−6.98 (m, 5H), 7.38−7.42 (m, 2H), butenolide OH missing. Microanalysis ($C_{34}H_{37}NO_{10} \cdot 0.3H_2O$) C, H, N. MS: (CI) $M^+ = 620$.

3-Benzo[1,3]dioxol-5-yl-4-[3,4-dimethoxy-5-(3-morpholin-4-ylpropoxy)benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 20, Isethionic Acid Salt. ^{1}H NMR: (DMSO-d_6) δ 2.0−2.1 (m, 2H), 2.58−2.62 (m, 2H), 3.05−3.18 (m, 2H), 3.18−3.25 (m, 2H), 3.3−3.6 (m, 4H), 3.51 (s, 3H), 3.52 (s, 3H), 3.6−3.85 (m, 6H), 3.74 (m, 3H), 3.99−4.02 (m, 2H), 5.95 (s, 1H), 6.02 (s, 2H), 6.88−6.95 (m, 5H), 7.35−7.37 (m, 2H), 8.17 (br.s, ex, 1H), 9.5 (br.s, ex, 1H), butenolide OH missing. Microanalysis ($C_{34}H_{37}NO_{10} \cdot C_2H_6SO_4 \cdot 0.5H_2O$): C, H, N, S, H_2O(KF). MS: (CI) $M^+ = 620$.

3-Benzo[1,3]dioxol-5-yl-5-hydroxy-4-[4-methoxy-3,5-bis(2-morpholin-4-ylethoxy)benzyl]-5-(4-methoxyphenyl)-5*H*-furan-2-one, 21, Diisethionic Acid Salt. ^{1}H NMR: (D_2O) δ 3.16 (t, $J = 6.5$ Hz, 4H), 3.4−3.6 (m, 8H), 3.63−3.66 (m, 4H), 3.71 (s, 3H), 3.7−3.8 (m, 2H), 3.81 (s, 3H), 3.96 (t, $J = 6.5$ Hz, 4H), 3.9−4.15 (m, 8H), 4.15−4.2 (m, 4H), 5.99 (s, 2H), 6.08 (s, 2H), 6.83−6.93 (m, 5H), 7.41−7.43 (m, 2H), both hydroxys, both acid OH's, and butenolide OH exchanged. Microanalysis ($C_{38}H_{44}N_2O_{11} \cdot 2(C_2H_6SO_4) \cdot 0.8H_2O$) C, H, N, S, H_2O(KF). MS: (CI) $M^+ = 705$.

3-Benzo[1,3]dioxol-5-yl-4-[3,5-bis(2-morpholin-4-ylethoxy)benzyl]-5-hydroxy-5-(4-methoxyphenyl)-5*H*-furan-2-one, 22. ^{1}H NMR: (DMSO-d_6) δ 2.53 (m, 8H), 2.71 (t, $J = 5.6$ Hz, 4H), 3.66 (s, 2H), 3.71 (m, 8H), 3.79 (s, 3H), 3.91 (t, $J = 5.6$ Hz, 4H), 5.97 (s, 2H), 6.07 (d, $J = 2.2$ Hz, 2H), 6.22 (t, $J = 2.2$ Hz, 1H), 6.81 (m, 1H), 6.83 (dd, $J = 2.0$, 6.9 Hz, 2H), 6.97 (m, 2H), 7.40 (dd, $J = 2.0$, 6.9 Hz, 2H), butenolide OH missing. Microanalysis ($C_{37}H_{42}N_2O_{10} \cdot 0.5H_2O$) C, H, N. MS: (CI) $M^+ + 1 = 675$.

{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}acetic Acid Methyl Ester, 23. ^{1}H NMR: ($CDCl_3$) δ 3.54−3.79 (m, 2H), 3.65 (s, 3H), 3.76 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 3.90 (s, 1H), 4.50−4.51 (m, 2H), 5.98 (s, 2H), 6.02 (s, 1H), 6.06 (s, 1H), 6.80−6.95 (m, 5H), 7.34−7.38 (m, 2H). Microanalysis ($C_{30}H_{28}O_{11}$) C, H, N. MS: (CI) $M^+ = 564$.

{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}acetic Acid, 24. ^{1}H NMR: ($CDCl_3$) δ 3.56−3.79 (m, 2H), 3.64 (s, 3H), 3.79 (s, 6H), 4.49 (s, 2H), 5.96 (s, 2H), 6.07 (s, 2H), 6.78−6.92 (m, 5H), 7.33−7.37 (m, 2H), butenolide OH and acid proton missing. Microanalysis ($C_{29}H_{26}O_{11} \cdot 1.4H_2O$) C, H, N. MS: (CI) $M^+ = 551$.

4-{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}butyric Acid Methyl Ester, 25. ^{1}H NMR: ($CDCl_3$) δ 1.98−2.05 (m, 2H), 2.47−2.50 (m, 2H), 3.58−3.80 (m, 2H), 3.68 (s, 3H), 3.71 (s, 3H), 3.74 (s, 3H), 3.82 (s, 3H), 3.85−3.88 (m, 2H), 5.96 (s, 2H), 6.09 (s, 1H), 6.23 (s, 1H), 6.78−6.80 (m, 1H), 6.87−6.89 (m, 2H), 6.94−6.97 (m, 2H), 7.41−7.43 (m, 2H), butenolide OH missing. Microanalysis ($C_{32}H_{32}O_{11}$) C, H, N. MS: (CI) $M^+ = 551$.

4-{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}butyric Acid, 26. ^{1}H NMR: ($CDCl_3$) δ 2.04−2.11 (m, 2H), 2.52−2.55 (m, 2H), 3.57−3.80 (m, 2H), 3.67 (s, 3H), 3.74 (s, 3H), 3.81 (s, 3H), 3.85−3.88 (m, 2H), 5.96 (s, 2H), 6.07 (s, 1H), 6.17 (s, 1H), 6.78−6.96 (m, 5H), 7.39−7.41 (m, 2H), butenolide OH and acid proton missing. Microanalysis ($C_{31}H_{30}O_{11} \cdot 0.5$EtOAc (by NMR)) C, H, N. MS: (CI) $M^+ = 578$.

2-Benzo[1,3]dioxol-5-yl-3-[3-(3-carboxypropoxy)-4,5-dimethoxybenzyl]-4-(4-methoxyphenyl)-4-oxobut-2-enoic Acid, 26, Disodium Salt. ^{1}H NMR: (D_2O) δ 190−1.94 (m, 2H), 2.27−2.31 (m, 2H), 3.48 (s, 2H), 3.60 (s, 3H), 3.64 (s, 3H), 3.84 (s, 3H), 3.75−3.79 (m, 2H), 6.04 (s, 2H), 6.06 (s, 1H), 6.10 (s, 1H), 6.89−6.93 (m, 4H), 7.00−7.02 (m, 1H), 7.61−7.63 (m, 2H), butenolide OH exchanged. Microanalysis ($C_{31}H_{28}O_{11}Na_2 \cdot 2.1H_2O$) C, H, N, Na, H_2O(KF). MS: (CI) $M^+ = 577$.

5-{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}pentanoic Acid Methyl Ester, 27. ^{1}H NMR: ($CDCl_3$) δ 1.76−1.78 (m, 4H), 2.37−2.41 (m, 2H), 3.56−3.75 (m, 2H), 3.63 (s, 3H), 3.66 (s, 3H), 3.66−3.75 (m, 2H), 3.73 (s, 3H), 4.33 (s, 1H), 5.96 (s, 2H), 6.00 (s, 1H), 6.79−6.95 (m, 5H), 7.37−7.41 (m, 2H), butenolide OH missing. Microanalysis ($C_{33}H_{34}O_{11}$): C, H, N. MS: (CI) $M^+ = 606$.

5-{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}pentanoic Acid, 28. ^{1}H NMR: ($CDCl_3$) δ 1.77−1.80 (m, 4H), 2.42−2.46 (m, 2H), 3.47−3.75 (m, 2H), 3.64 (s, 3H), 3.73 (s, 3H), 3.75−3.85 (m, 2H), 3.79 (s, 3H), 5.96 (s, 2H), 6.01 (s, 1H), 6.07 (s, 1H), 6.78−6.94 (m, 5H), 7.37−7.41 (m, 2H), butenolide OH and acid proton missing. Microanalysis ($C_{32}H_{32}O_{11} \cdot 0.25H_2O$) C, H, N, H_2O(KF). MS: (CI) $M^+ = 592$.

3-{5-[4-Benzo[1,3]dioxol-5-yl-2-hydroxy-2-(4-methoxyphenyl)-5-oxo-2,5-dihydrofuran-3-ylmethyl]-2,3-dimethoxyphenoxy}propane-1-sulfonic Acid, 29. ^{1}H NMR: (DMSO-d_6) δ 2.10−2.14 (m, 2H), 2.99−3.29 (t, 2H), 3.43 (s, 2H), 3.53 (s, 3H), 3.58 (s, 3H), 3.73−3.76 (t, 2H), 3.78 (s, 3H), 5.80 (s, 2H), 5.97 (s, 2H), 6.78 (s, 1H), 6.78−6.89 (m, 4H), 7.18 (s, 1H), 7.38−7.40 (d, 2H). Microanalysis ($C_{30}H_{29}O_{12}SNa \cdot 1.0H_2O$) C, H, N, S, Na, H_2O(KF). MS: (ES) $M^+ = 613$.

References

(1) Lago, M. A.; Luengo, J. I.; Peishoff, J. D.; Elliott, J. D. Chapter 9. Endothelin Antagonists. In *Annual Reports in Medicinal Chemistry;* Bristol, J. A., Ed.; Academic Press: New York, 1996; Vol. 31, pp 81−90.

(2) Peishoff, C. E.; Lago, M. A.; Ohlstein, E. H.; Elliott, J. D. Endothelin receptor antagonists. *Curr. Pharm. Des.* **1995**, *1*, 425−440.

(3) Webb, M. L.; Meek, T. D. Inhibitors of Endothelin. *Med. Res. Rev.* **1997**, *17*, 17−67.

(4) Patt, W. C.; Edmunds, J. J.; Repine, J. T.; Berryman, K. A.; Reisdorph, B. R.; Lee, C.; Plummer, M. S.; Shahripour, A.; Haleen, S. J.; Keiser, J. A.; Flynn, M. A.; Welch, K. M.; Reynolds, E. E.; Rubin, R.; Tobias, B.; Hallak, H.; Doherty, A. M. Structure−activity relationships in a series of orally active γ-hydroxy butenolide endothelin antagonists. *J. Med. Chem.* **1997**, *40*, 1063−1074.

(5) Ishikawa, K.; Ihara, M.; Noguchi, K.; Mase, T.; Mino, N.; Saeki, T.; Fukuroda, T.; Fukami, T.; Ozaki, S.; Nagase, T.; Nishikibe, J.; Yano, M. Biochemical and Pharmacological profile of a potent and selective endothelin B-receptor antagonist BQ-788. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4892−4896.

(6) Raju, B.; Okun, I.; Stavros, F.; Chan, M. F. Search for surrogates: a study of endothelin receptor antagonist structure activity relationships. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 933−938.

(7) Doherty, A. M.; Patt, W. C.; Edmunds, J. J.; Berryman, K. A.; Reisdorph, B. R.; Plummer, M. S.; Shahripour, A.; Lee, C.; Cheng, X.; Walker, D.; Haleen, S. J.; Keiser, J. A.; Flynn, M. A.; Welch, K. M.; Hallak, H.; Taylor, D. G.; Reynolds, E. E. Discovery of a novel series of orally active non-peptide endothelin-A (ETA) receptor-selective antagonists. *J. Med. Chem.* **1995**, *38*, 1259−1263.

(8) Reynolds, E. E.; Keiser, J. A.; Haleen, S. J.; Walker, D. M.; Davis, L. S.; Olszewski, B.; Taylor, D. G.; Hwang, O.; Welch, K. M.; Flynn, M. A.; Thompson, D. M.; Edmunds, J. J.; Berryman, K. A.; Lee, C.; Reisdorph, B. R.; Cheng, X.-M.; Patt, W. C.; Doherty, A. M. Pharmacological characterization of PD156707, an orally active ETA receptor antagonist. *J. Pharmacol. Exp. Ther.* **1994**, *273*, 1410−1417.

(9) Doherty, A. M.; Cody, W. L.; He, J.; DePue, P. L.; Waite, L. A.; Leonard, D. M.; Leitz, N. L.; Dudley, D. T.; Rapundalo, S. T.; Hingorani, G. P.; Haleen, S. J.; LaDouceur, D. M.; Hill, K. E.; Flynn, M. A.; Reynolds, E. E. Design of C-terminal peptide antagonists of endothelin: structure−activity relationships of ET-1[16,21,D-His]. *J. Med. Chem.* **1993**, *36*, 2585−2594.

(10) Keiser, J.; Schroeder, R.; Hallak, H.; Uprichard, A. C. G.; Doherty, A. M.; Haleen, S. J. Pharmacodynamics of PD156707, A selective endothelin receptor-A (ET_A) antagonist, in acute hypoxic pulmonary hypertension (PH). *FASEB J.* **1997**, *11 (3)*, A36.

(11) Haleen, S.; Schroeder, R.; Walker, D.; Quenby-Brown, E.; Welch, K.; Hallak, H.; Uprichard, A.; Keiser, J. Efficacy of **1**, an endothelin-A receptor antagonist, in hypoxic pulmonary hypertension. *J. Cardiol. Pharmacol.* **1998**, *31*, S331−S335.

JM980504W