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γ-CARBAMATE BUTENOLIDE ANALOGUES AS POTENT ET_A SELECTIVE ENDOTHELIN RECEPTOR ANTAGONISTS AND PRODRUGS

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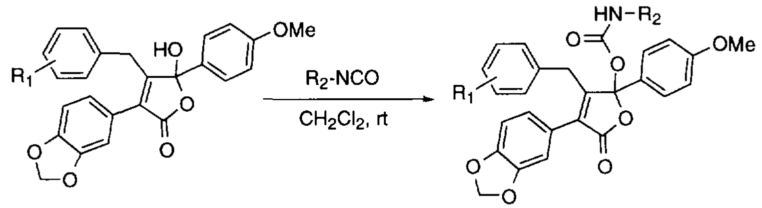
Abstract: Continued SAR around our ET_A selective series of butenolide antagonists, for example PD156707 (**1**) has yielded a new series of subnanomolar ET_A selective antagonists. Depending upon solution pH, **1** exists as the ring closed butenolide form (shown) or as the tautomeric open chain keto-acid salt. Reaction of the butenolide γ-hydroxyl with isocyanates yields carbamates with essentially identical ET_A binding affinity and with improved ET_A selectivity. As carbamates these derivatives may undergo facile hydrolysis, reverting back to their parent butenolides, and therefore may be useful as prodrugs of **1**. Stability studies of PD163140 (**7**) indicate that the compound is stable in the binding assay conditions and hence has intrinsic activity. In addition **7** is readily hydrolyzed by rat intestinal perfusate to yield the parent compound **1**.

Introduction: Endothelin (ET) is one of the most potent vasoconstricting agents isolated from mammalian cells. The biology and characterization of ET has been the subject of more than 6500 literature reports including many recent reviews.[1-3] ET is known to exert its pharmacological effects by acting upon two specific G-coupled protein receptors, ET_A and ET_B, both of which are widely distributed in mammalian tissues. Activation of either receptor has been shown to mediate vasoconstriction depending upon the species and tissue bed under study. ET has been implicated in a wide range of human disease states including hypertension, cerebral vasospasm, ischemia, renal failure, and pulmonary hypertension. The development of potent and selective antagonists of the ET receptors has been instrumental in helping define the role that ET plays in human diseases.

Numerous ET_A and ET_B selective antagonists have been reported and have been reviewed.[4-6] Herein, we wish to report a new series of ET_A selective analogues derived from our previously reported γ-hydroxy-butenolide ET_A selective antagonist PD156707, **1**.[7] These new analogues (**4-13**) contain a carbamate moiety attached to the γ-oxygen of the parent γ-hydroxy butenolides. This class of compounds has intrinsic activity equal to that of the parent γ-hydroxy butenolides. Additionally, being carbamates these compounds are prone to hydrolysis and potentially can revert to their parent γ-hydroxy butenolides, thereby acting as prodrugs in vivo. One representative example (**7**) has been shown to readily convert to the parent compound (**1**) in rat intestinal perfusate.

Chemistry: All carbamates were synthesized from commercially available isocyanates and the previously reported γ-hydroxy butenolides according to Scheme 1. Addition of catalytic 4-dimethylamino pyridine was used in some cases. The yields for the reaction varied according to the isocyanate utilized (10-65%).

Scheme 1: Synthesis of Carbamates

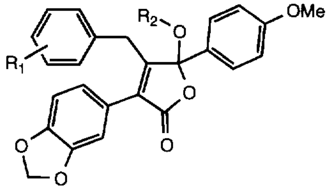


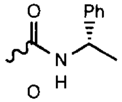
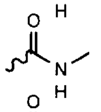
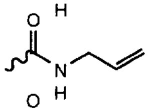
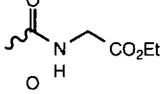
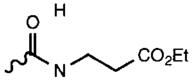
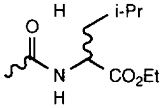
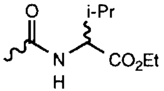
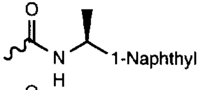
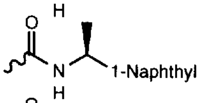
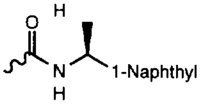
Results and Discussion: In an attempt to develop a UV assay for plasma level detection of compounds from the γ-hydroxy butenolide series, in particular compound **2**, we were interested in increasing the UV absorbance of **2**. Toward that end, we examined some derivatization procedures including the synthesis of carbamates. The initial carbamate derivative synthesized was **4**, Table 1. This compound was not expected to have potent receptor binding affinity based upon earlier work carried out with ethers at the γ-site on the butenolide.[7] The methyl ether, **3**, exhibited a significant loss of ET_A binding activity (IC_{50} = 300 nM) versus its parent compound **2** (IC_{50} = 7.4 nM). However, **4** retained significant ET_A receptor binding activity with an IC_{50} = 18 nM. Due to the activity of compound **4** we chose to examine carbamate derivatives of PD156707, **1** (IC_{50} = 0.3 nM at the ET_A receptor), which is a more potent ET_A selective receptor antagonist than **2**.

The simple methyl carbamate **5** was the least potent analogue derived from **1** with an IC_{50} = 9 nM, at the ET_A receptor. Increasing the alkyl group chain length led to more potent derivatives. The allyl carbamate (**6**) had an ET_A receptor potency of, IC_{50} = 0.8 nM. A series of carbamates derived from amino acids were then synthesized, **7**-**10**. All these compounds had subnanomolar ET_A binding affinity, essentially equivalent to that seen with the parent γ-hydroxy butenolide. Particularly potent is **7**, where the carbamate is derived from glycine. It has an IC_{50} = 0.35 nM and >5000 fold selectivity for the ET_A receptor.

The last three compounds in Table 1 were synthesized in an attempt to separate the optical isomers formed at the butenolide γ-position after formation of the carbamates. The relatively large (S)-α-1-naphthylethyl carbamate derivative (**11**) was synthesized and found to have an ET_A potency of 1 nM. The diastereomers were readily separated by column chromatography, **12** and **13**. We had expected to find a

Table 1: Carbamate SAR



#	$\underline{R_1}$	$\underline{R_2}$	Receptor Binding IC$_{50}$ (nM)	
			$\underline{ET_A}$	$\underline{ET_B}$
1, PD156707	3,4,5-(OMe)$_3$	H	0.37	480
2, PD155080	H	H	7.4	4550
3	H	CH_3	300	>25000
4	H		18	>2500
5	3,4,5-(OMe)$_3$		9.0	>2500
6	3,4,5-(OMe)$_3$		0.8	>2500
7, PD163140	3,4,5-(OMe)$_3$		0.35	1800
8	3,4,5-(OMe)$_3$		0.4	>2500
9	3,4,5-(OMe)$_3$		0.5	2500
10	3,4,5-(OMe)$_3$		0.6	1300
11	3,4,5-(OMe)$_3$		1.0	>2500
12, fast isomer	3,4,5-(OMe)$_3$		1.0	>2500
13, slow isomer	3,4,5-(OMe)$_3$		2.0	>2500

difference in binding affinity with these diastereomers. However, within experimental error all three analogs had identical ET_A and ET_B binding affinity. These results may be due to chiral instability of these analogs in the assay buffer although this has not yet been determined.

Several analogues were further examined in our functional assays and compared to **1**, Table 2. Compound **7** had an IC_{50} = 3.4 nM in inhibiting ET-1 induced arachidonic acid release in vascular smooth muscle cells and a pA_2 = 7.3 in inhibiting ET-1 induced contraction in rabbit femoral artery rings. These values compare favorably with **1** which has an IC_{50} = 1.0 nM in the arachidonic assay and a pA_2 of 7.5 in the artery ring contraction assay.

Table 2: Functional Data

#	Inhibition of ET-1 induced Arachidonic acid release (IC_{50} in nM)	Inhibition of ET-1 induced Contraction in femoral artery (pA_2)
1	1.0	7.5
7	3.4	7.3
8	5.3	7.7
12	12.0	6.8

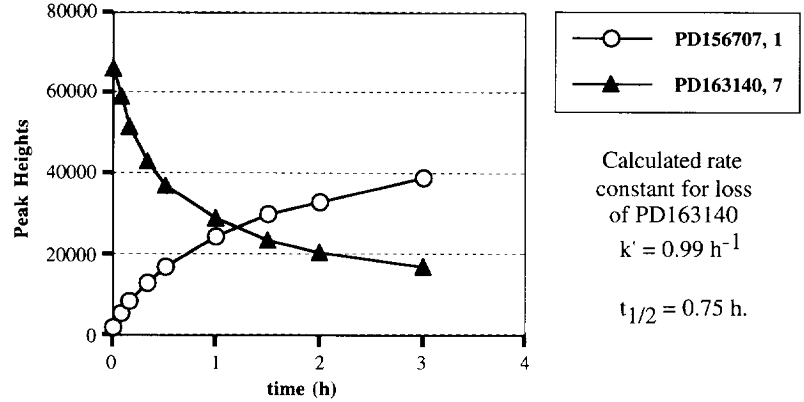
Since the ET_A binding affinity and functional data for the carbamate derivatives were essentially equivalent to that seen with the parent butenolides we evaluated whether carbamate hydrolysis to the parent was occurring. Providing some support for carbamate stability, it was noted that the carbamate analogues were significantly less active at the ET_B receptor versus the parent. The stability of **7** was therefore evaluated in the buffer solution employed in the binding assays. In the time frame used in the binding assay, 2-3 h, only minimal conversion of **7** to **1** was observed via HPLC analysis (less than 5% conversion). This indicated that compound **7** has intrinsic receptor binding activity.

To evaluate whether compound **7** is a prodrug for compound **1** we performed an in vitro assay in rat intestinal perfusate, Figure 1. Rapid conversion of **7** to **1** was observed with a rate constant of 0.990 h^{-1} (59.4 min^{-1}) and a half life of 0.75 h. This confirms that **7** is indeed an effective prodrug for PD156707.

Conclusions: A series of potent γ-carbamate butenolide antagonists selective for the ET_A receptor were synthesized. The majority of these carbamate derivatives had ET_A binding and functional activity equal to that of the parent butenolides. Due to the possibility of hydrolytic instability of the carbamates PD163140 (**7**) was examined for stability in the receptor binding assay buffer. Under conditions that mimicked the conditions used in the binding assay, **7** was shown to be stable indicating the carbamates have intrinsic receptor binding activity.

Compound **7** was then further examined to determine if it is converted to its parent butenolide in an in vitro milieu. Compound **7** was indeed readily converted to PD156707 in rat intestinal perfusate with a half-life of 0.75 h. This demonstrates that compound **7** can definitely act as a prodrug in the presence of reconversion enzymes in vitro.

Figure 1: Rat Intestinal Perfusate Hydrolysis of PD163140, 7



Calculated rate constant for loss of PD163140
$k' = 0.99\ h^{-1}$

$t_{1/2} = 0.75$ h.

Pharmacology Assays: Receptor binding was evaluated using human cloned ET_A and ET_B receptors. The ET_A receptor was expressed in Ltk⁻ cells and the ET_B receptor was expressed in CHO-K1 cells. The specific binding protocols have been previously reported.[8] ET_A functional activity was demonstrated in two assays. Inhibition of ET-1 induced arachidonic acid release in rabbit renal artery vascular smooth muscle cells (ET_A) and inhibition of ET-1 induced contraction in rabbit femoral arterial rings (ET_A) were used. Both of these assays have been previously reported.[9]

Pharmacokinetic Study: The perfusate was generated by oscillating MES buffer (~12 mL) through rat jejunum in situ for 90 min at 30 mL/min. The perfusate was then centrifuged at 2 K rpm for five minutes at room temperature to obtain clear perfusate. The perfusate was assayed for activity of the intestinal enzyme leucine aminopeptidase (LAP), using a colorimetric assay as an indicator of brush border membrane (BBM) enzyme activity. Ten microliters of drug stock was spiked into 90 microliters perfusate at the beginning of the incubation at 37 °C. Samples of the incubation mixture were removed, combined with two volumes of acetonitrile, vortexed, then centrifuged five minutes at 14 K rpm and analyzed by HPLC for loss of parent and appearance of breakdown products.[10]

General Synthetic Route: [4-Benzo[1,3]dioxol-5-yl-2-(4-methoxy-phenyl)-5-oxo-3-(3,4,5-trimethoxy-benzyl)-2,5-dihydro-furan-2-yloxycarbonylamino]-acetic acid ethyl ester, **7**.

In methylene chloride (15 mL) was stirred the butenolide, **1**, (1.0 g, 1.97 mmol). To this was added ethyl isocyanatoacetate (284 mg, 2.2 mmol) and dimethylamino pyridine (10 mg). The mixture was stirred at room temperature for 18 h. The solution was washed with 1 N HCl (25 mL), water (25 mL), and brine (25 mL). The organic phase was separated, dried over magnesium sulfate, and evaporated in vacuo to give a foam. The foam was passed through a column of flash silica gel (150 g) and eluted with 10% ethyl acetate in methylene chloride to give 122 mg (10%) of pure carbamate derivative. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.0 Hz, 3H), 3.55-3.8 (m, 2H), 3.65 (s, 6H), 3.7-3.95 (m, 2H), 3.73 (s, 3H), 3.81 (s, 3H), 4.21 (q, J = 7.0 Hz, 2H), 5.32 (t, ex., 1H), 5.95 (s, 2H), 5.99 (s, 2H), 6.79 (d, 1H), 6.86 (d, 2H), 6.95-6.99 (m, 2H), 7.36 (d, 2H). MS: (APCI+) 636.1 Da. Microanalysis: $C_{33}H_{33}NO_{12}$, Calculated: C, 62.36; H, 5.23; N, 2.20; Found: C, 62.35; H, 5.26; N, 1.97.

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