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1,3-DIARYL-2-CARBOXYINDOLES AS POTENT NON-PEPTIDE ENDOTHELIN ANTAGONISTS

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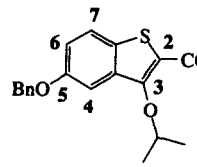
Abstract: Endothelin-1 is a potent vasoconstrictor that is thought to be involved in many human disease states. We have developed a series of indole non-peptide endothelin antagonists including PD 159110 (**31**), an ET_A selective antagonist, and PD 159020 (**37**), a non-selective ET_A/ET_B antagonist. The discovery, synthesis, and structure-activity relationships of this series of compounds are described.

Introduction: The family of endothelin peptides consist of endothelin-1 (ET-1), endothelin-2 (ET-2), endothelin-3 (ET-3), and the vasoactive intestinal contractor (VIC). The biological effects of the endothelins has been shown to occur through the interaction of the peptides with specific receptor subtypes. The ET_A receptor subtype, which is selective for ET-1, is predominantly found in the vascular smooth muscle. The ET_B receptor subtype is non-selective, binding ET-1, ET-2, and ET-3 with similar affinity, and has been found in a variety of tissues including human cultured umbilical vein and human mammary arteries and veins.[1,2,3,4] The ET_C receptor subtype, which is selective for ET-3, has recently been cloned from dermal melanophores of *Xenopus laevis*, but no mammalian homolog is known.[5]

Endothelin has been implicated in a number of disease states including renal failure, pulmonary hypertension, cerebral ischemia and vasospasm, endotoxic shock, and congestive heart failure.[6] The ET_A receptor is known to mediate a major portion of the vasoconstrictor activity of endothelin in human vessels.

There have been a number of peptide and non-peptide endothelin antagonists reported in the literature. These include potent ET_A-selective antagonists such as BQ-123 [7,8] and PD 156707;[9] non-selective ET_A/ET_B antagonists such as PD 142893,[10] PD 145065,[11] SKF 209670,[12] L- 749,329,[13] as well as, Ro 47-0203 (bosentan);[14] and the ET_B-selective antagonist BQ-788.[15] The synthesis of potent, orally active, non-peptide endothelin antagonists with differing selectivity for the ET_A and ET_B receptors was the objective of this research.

Results and Discussion: Screening of the Parke-Davis compound library afforded several moderately active compounds when tested in rabbit renal artery vascular smooth muscle cells expressing the ET_A receptors.[9,16] We selected the lead structure, compound **1**, for synthetic modification to define the structure-activity relationships and to enhance potency. The compound exhibited micro molar binding affinity for the rabbit ET_A receptor and was inactive at the rat ET_B receptor (rat cerebellum).



ET_A IC_{50} 6μM
hET_A IC_{50} 8.8μM
hET_B IC_{50} >100μM

Compound 1

Preliminary SAR revealed that an acidic substituent at C-2, a lipophilic substituent at C-3, and a benzyloxy substituent at C-5 were essential for receptor binding activity. However, with the disclosure of a series of indanes[12,17] as endothelin antagonists, we directed our attention to a series of N1-substituted indoles. While incorporating substituents at C-2, C-3, and C-5 that had been found to be important for activity in the benzothiophene series, we decided to synthesize the two indoles **23** and **24**.[9] These two compounds both displayed moderate ET_A binding affinity and encouraged us to systematically investigate the effect of electron donating substituents on each aromatic ring.

Two series of indoles were prepared. The first series, the N-aryl analogs, are summarized in Table 1. From these compounds it was evident that a methylenedioxy substituent at both R_1, R_2 and R_5, R_6 afforded potent compounds in combination with an appropriate substituent at R_7. In fact, as compounds **22-25** indicate, the optimal substituent at R_7 was the propyloxy group. Increasing electron donation by substituting R_7 and R_8 with methoxy groups afforded compound **26** which demonstrated a binding affinity approaching that of the optimal substitution.

A second series of indoles incorporated a benzyl substituent at N-1 as exemplified in Table 2. While it was apparent that methylenedioxy substituted phenyl rings again afforded active compounds, optimal activity was achieved by modifications of the R_7 and R_8 substituents. Compounds **27-32** demonstrated that a propyloxy substituent at either R_7 or R_8 afforded active compounds. Interestingly, dual substitution of R_7 and R_8 (**36-38**) indicated that ET_A selective (**36**) and non-selective (**37**) compounds could be prepared by modification of the R_8 substituent.

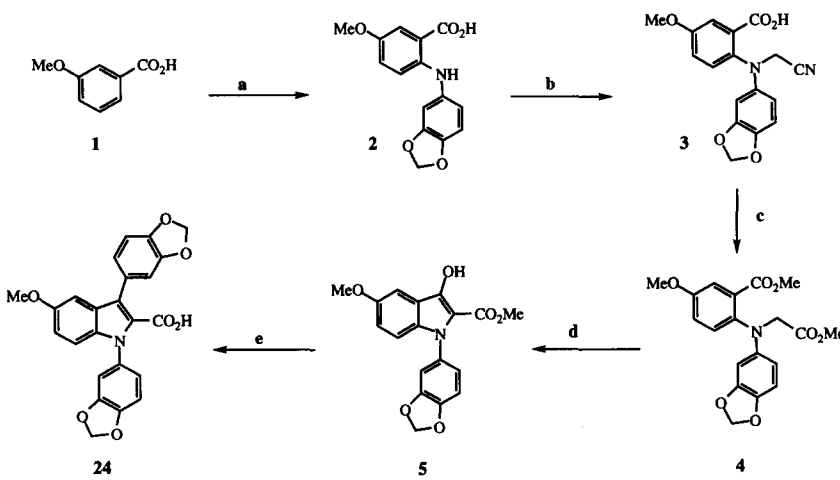
Synthesis: The initial synthetic route to compounds **23** and **24**, the first two indoles synthesized in this series of compounds, followed a classical route, as depicted in Scheme 1.[18] However, this initial synthetic route required some modification to allow a more flexible approach for the incorporation of a variety of substituents at N-1 and C-3. Furthermore, since the SAR studies indicated that a variety of C-5 and C-6 substituents were important for activity, we chose a variety of benzaldehydes as starting materials for the synthesis of substituted indoles. This synthetic route, as outlined in Scheme 2, allowed a variety of substituents to be introduced at C-3, C-5 and C-6. Unfortunately, the introduction of substituents at N-1 posed a significant problem. SAR studies revealed that electron rich arenes were required at N-1, but the copper catalyzed arylation of nitrogen was not efficient with these substrates. Thus, while bromobenzene efficiently arylates indoles, the use of 1-bromo-3,4-methylenedioxy benzene simply led to decarboxylation in addition to some arylation. The use of 1-iodo-3,4-methylenedioxy benzene improved the yield of product substantially but decarboxylation was still a problem. Fortunately, we discovered that benzyl substituents were well tolerated at N-1 and hence, Scheme 3 demonstrated a remarkably simple approach allowing the exploration of N-1 and C-3.[19,20]

Biological Evaluation: Structure-activity relationships were investigated using IC_{50} values obtained from receptor binding in Ltk-cells expressing recombinant human receptors (hET_A), and

CHO-K1 cells expressing recombinant human receptors (hET_B).[9,21] Selected compounds were evaluated for antagonist activity by measuring the ability of these compounds to reduce ET-1 stimulated arachidonic acid release (AAR) in cultured rabbit renal vascular smooth muscle cells.[9,22] In addition, in vitro antagonism of ET-1 stimulated vasoconstriction was carried out in rabbit femoral artery, $ET_A(pA_2)$, to demonstrate a functional response to antagonism of ET_A in this isolated tissue. Inhibition of sarafotoxin-6c stimulated vasoconstriction was carried out in rabbit pulmonary artery, $ET_B(pA_2)$.[21]

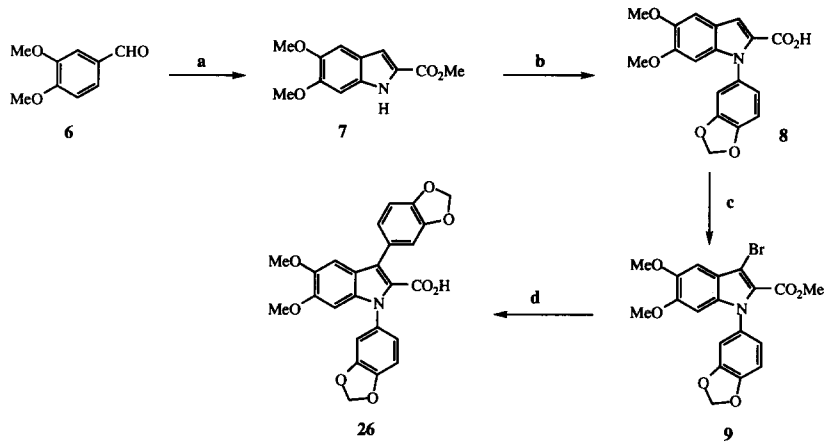
Conclusions: Extensive investigation of electron donating substituents on all three aromatic rings led to the discovery of ET_A selective indoles, such as compound **31**, and compounds that demonstrated affinity to both ET_A and ET_B receptors, such as compound **37**. Selected compounds were evaluated for their ability to inhibit the release of arachidonic acid in rabbit renal artery vascular smooth muscle cells (ET_A) upon stimulation with ET-1. Compound **31**, for example, demonstrated an AAR-A IC_{50} of 0.48 μM and effectively inhibited the contraction of ET-1 induced rabbit femoral artery, which are known to express predominantly ET_A receptors, with a pA_2 of 6.9. Compound **37** also demonstrated excellent inhibition of arachidonic acid release, AAR-B, with an IC_{50} of 0.23 μM and thereby demonstrating functional ET_B activity. These compounds should prove useful in elucidating the physiological and pathophysiological role of endothelin.

Scheme 1: 1,3-diaryl-2-carboxyindoles



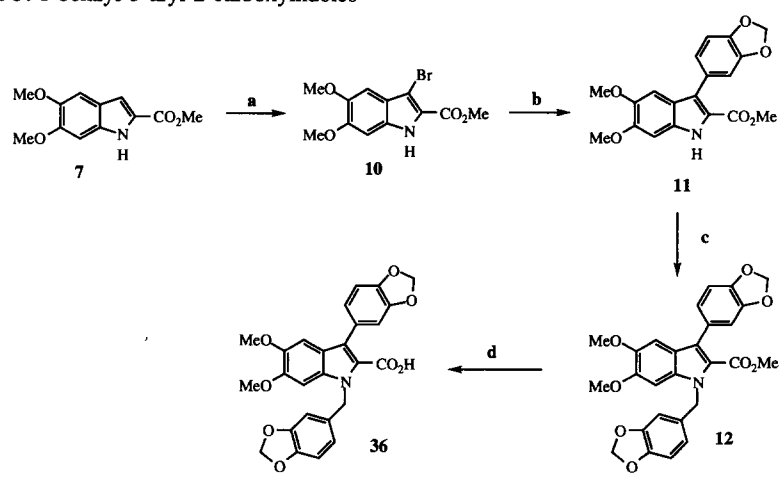
(**a**) i. 1.0 equiv. KOH, 1.10 equiv. Br_2, 21.5 equiv. HOAc, reflux, 95% yield; ii. 3.0 equiv. 3,4-methylenedioxyaniline, 1.10 equiv. KOAc, 0.002 equiv. cupric acetate, 1.10 equiv. Et_3N, isopropyl alcohol, 85% yield; (**b**) i. 37% formaldehyde, ethanol, reflux, 74% yield; ii. 1.0 equiv. NaCN, 7.1 equiv. DMF, 42°C, 94% yield; (**c**) i. 4.0 equiv. 50% NaOH (aq), reflux, 77% yield; ii. 3.0 equiv. $TMSCHN_2$, toluene/methanol 5:1, 93% yield; (**d**) 1.4 equiv. Na metal, methanol, 89% yield; (**e**) i. 5.0 equiv. pyridine, 1.2 equiv. trifluoromethanesulfonic anhydride, CH_2Cl_2, 0°C, 86% crude yield, ii. 1.5 equiv. (3,4-methylenedioxy)phenyl boronic acid, 1.5 equiv. K_2CO_3, 0.1 equiv. $Pd(PPh_3)_4$, toluene/DMF 5:1, 55% yield; iii. 15.0 equiv. LiOH, THF/MeOH/H_2O 3:1:1, 84% yield.

Scheme 2: 1,3-diaryl-2-carboxyindoles



(**a**) i. 2.5 equiv. $N_3CH_2CO_2CH_3$, 2.5 equiv. Na metal, MeOH, -19 to 0°C, 49% yield; ii. toluene, reflux, 51% yield; (**b**) i. 15 equiv. LiOH, THF/MeOH/H_2O 3:1:1, 50°C, 95% yield; ii. 1.3 equiv. 1-iodo-3,4-methylenedioxy benzene, 1.0 equiv. copper oxide, 2.0 equiv. KOH, DMF, 64% crude yield; (**c**) i. 3.0 equiv. $TMSCHN_2$, toluene/methanol 5:2, 61% yield; ii. 1.05 equiv. pyridinium bromide perbromide, pyridine, 77% yield; (**d**) i. 1.5 equiv. (3,4-methylenedioxy) phenyl boronic acid, 0.1 equiv. $Pd(PPh_3)_4$, toluene/MeOH/sat aq $NaHCO_3$; ii. 15 equiv. LiOH, THF/MeOH/H_2O 3:1:1, 50°C, 71% yield.

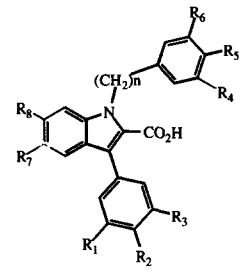
Scheme 3: 1-benzyl-3-aryl-2-carboxyindoles



(**a**) 1.05 equiv. pyridinium bromide perbromide, pyridine, 92% yield; (**b**) 1.5 equiv. (3,4-methylene-dioxy)phenyl boronic acid, 0.01 equiv. $Pd(PPh_3)_4$, toluene/methanol/sat. aq sodium bicarbonate 1:1:1, 80°C, 82% yield; (**c**) 1.3 equiv. NaH, 1.25 equiv. (3,4-methylenedioxy)benzyl chloride, DMF, 73% yield; (**d**) 15.0 equiv. LiOH, THF/MeOH/H_2O 3:1:1, 53°C, 98%yield.

Structure-Activity Relationships:

Table 1 - 1,3-diaryl-2-carboxyindoles



Ex	n	R1	R2	R3	R5	R6	R7	R8	hET_A IC_{50} (μM)	hET_B IC_{50} (μM)
13	0	H	OMe	H	-OCH2O-		OMe	H	1.1	>25
14	0	H	OMe	H	-OCH2O-		OBn	H	2.4	21
15	0	OMe	OMe	H	-OCH2O-		OMe	H	1.5	>25
16	0	OMe	H	H	-OCH2O-		OMe	H	5.5	>25
17	0	-OCH2O-		H	H	H	OMe	H	12	>25
18	0	-OCH2O-		H	OMe	H	H	H	2.3	>25
19	0	-OCH2O-		H	OMe	H	OMe	H	3.5	>25
20	0	-OCH2O-		H	H	H	Cl	H	10	>25
21	0	-OCH2O-		H	H	H	H	OMe	7.4	>25
22	0	-OCH2O-		H	-OCH2O-		H	H	4.1	22
23	0	-OCH2O-		H	-OCH2O-		OBn	H	1.3	14
24	0	-OCH2O-		H	-OCH2O-		OMe	H	0.26	15
25	**0**	**-OCH2O-**		**H**	**-OCH2O-**		**OPr**	**H**	**0.043**	**12**
26	0	-OCH2O-		H	-OCH2O-		OMe	OMe	0.08	7.0

Table 2 - 1-benzyl-3-aryl-2-carboxyindoles

Ex	n	R1	R2	R3	R5	R6	R7	R8	hET_A IC_{50} (μM)	hET_B IC_{50} (μM)
27	1	-OCH2O-		H	-OCH2O-		H	H	1.5	3.3
28	1	-OCH2O-		H	-OCH2O-		OBn	H	0.8	2.5
29	1	-OCH2O-		H	-OCH2O-		OMe	H	0.35	2.4
30	1	-OCH2O-		H	-OCH2O-		OPr	H	0.08	0.85
31	**1**	**-OCH2O-**		**H**	**-OCH2O-**		**H**	**OPr**	**0.047**	**5.6**
32	1	-OCH2O-		H	-OCH2O-		OPr	H	0.13	1.1
33	1	-OCH2O-		H	H	H	H	H	11	14.5
34	1	-OCH2O-		H	OMe	H	OBn	H	2.7	4.4
35	1	OMe	OMe	H	-OCH2O-		OMe	OMe	0.06	0.85
36	1	-OCH2O-		H	-OCH2O-		OMe	OMe	0.06	1.4
37	**1**	**-OCH2O-**		**H**	**-OCH2O-**		**OMe**	**OBn**	**0.03**	**0.05**
38	1	-OCH2O-		H	-OCH2O-		-OCH2O-		0.24	0.67

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