



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

Amy M. Bunker*, Jeremy J. Edmunds, Kent A. Berryman, Donnelle M. Walker, Michael A. Flynn, Kathy M. Welch, and Annette M. Doherty

Departments of Chemistry and Cardiovascular Therapeutics, *Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, Ann Arbor, Michigan 48105*

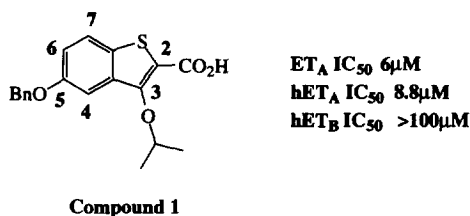
Abstract: Endothelin-1 is a potent vasoconstrictor that is thought to be involved in many human disease states. We have developed a series of indole non-peptide endothelin antagonists including PD 159110 (**31**), an ET_A selective antagonist, and PD 159020 (**37**), a non-selective ET_A/ET_B antagonist. The discovery, synthesis, and structure-activity relationships of this series of compounds are described. Copyright © 1996 Elsevier Science Ltd

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).



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 R₁, R₂ and R₅, R₆ afforded potent compounds in combination with an appropriate substituent at R₇. In fact, as compounds **22-25** indicate, the optimal substituent at R₇ was the propyloxy group. Increasing electron donation by substituting R₇ and R₈ 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 R₇ and R₈ substituents. Compounds **27-32** demonstrated that a propyloxy substituent at either R₇ or R₈ afforded active compounds. Interestingly, dual substitution of R₇ and R₈ (**36-38**) indicated that ET_A selective (**36**) and non-selective (**37**) compounds could be prepared by modification of the R₈ substituent.

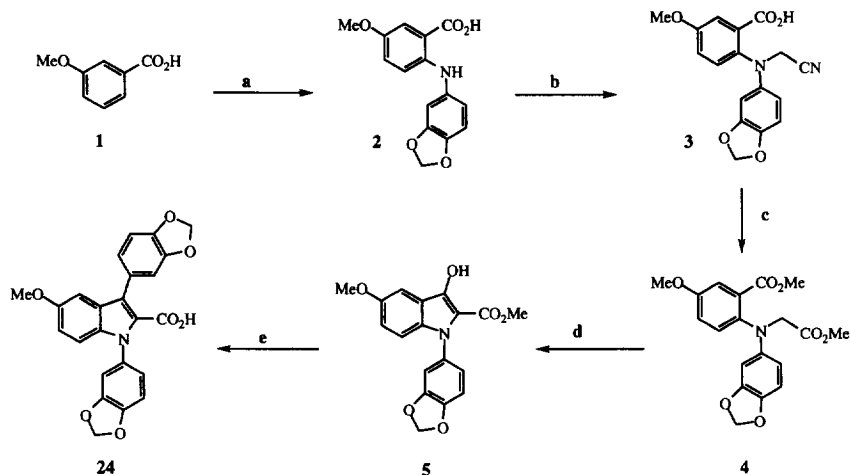
Synthesis: The initial synthetic route to compounds **23** and **24**, the first two indoles synthesized in this series of compounds, followed a classical route, as depicted in Scheme 1.¹⁸ However, this initial synthetic route required some modification to allow a more flexible approach for the incorporation 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

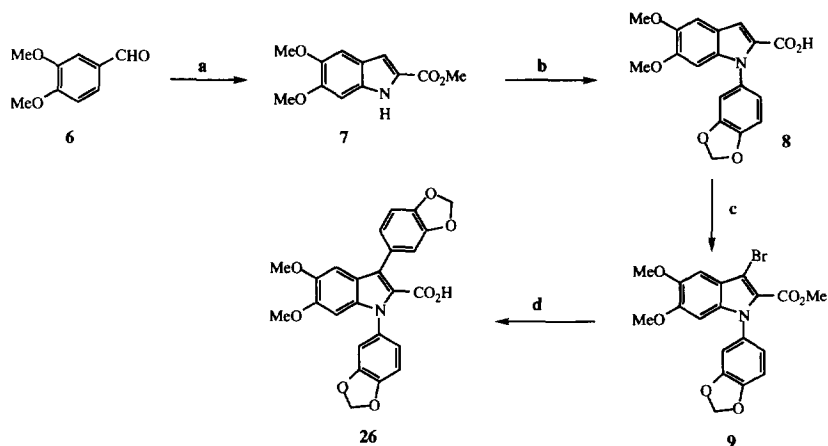
CHO-K1 cells expressing recombinant human receptors (hET_B).^{9,21} Selected compounds were evaluated for antagonist activity by measuring the ability of these compounds to reduce ET-1 stimulated arachidonic acid release (AAR) in cultured rabbit renal vascular smooth muscle cells.^{9,22} In addition, in vitro antagonism of ET-1 stimulated vasoconstriction was carried out in rabbit femoral artery, ET_A(pA₂), 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(pA₂).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 IC₅₀ of 0.48 μ M and effectively inhibited the contraction of ET-1 induced rabbit femoral artery, which are known to express predominantly ET_A receptors, with a pA₂ of 6.9. Compound **37** also demonstrated excellent inhibition of arachidonic acid release, AAR-B, with an IC₅₀ of 0.23 μ M and thereby demonstrating functional ET_B activity. These compounds should prove useful in elucidating the physiological and pathophysiological role of endothelin.

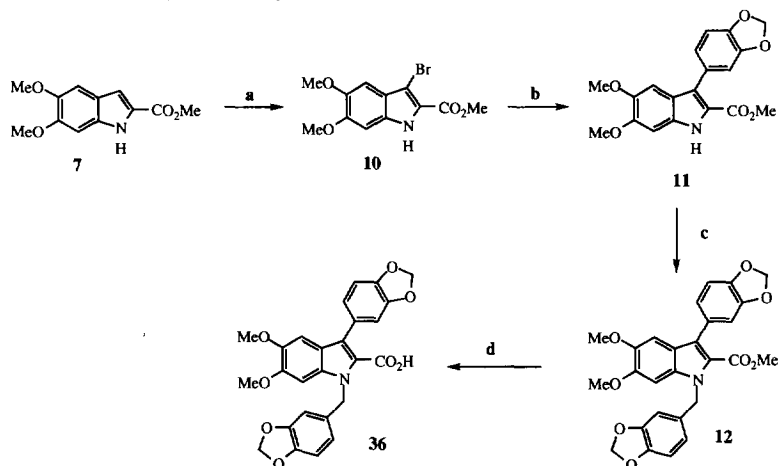
Scheme 1: 1,3-diaryl-2-carboxyindoles



(a) i. 1.0 equiv. KOH, 1.10 equiv. Br₂, 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. Et₃N, 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. TMSCHN₂, 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, CH₂Cl₂, 0°C, 86% crude yield; ii. 1.5 equiv. (3,4-methylenedioxy)phenyl boronic acid, 1.5 equiv. K₂CO₃, 0.1 equiv. Pd(PPh₃)₄, toluene/DMF 5:1, 55% yield; iii. 15.0 equiv. LiOH, THF/MeOH/H₂O 3:1:1, 84% yield.

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

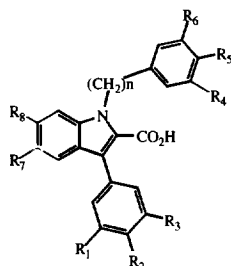
(a) i. 2.5 equiv. $\text{N}_3\text{CH}_2\text{CO}_2\text{CH}_3$, 2.5 equiv. Na metal, MeOH, -19 to 0°C , 49% yield; ii. toluene, reflux, 51% yield; (b) i. 15 equiv. LiOH, THF/MeOH/ H_2O 3:1:1, 50°C , 95% yield; ii. 1.3 equiv. 1-iodo-3,4-methylenedioxy benzene, 1.0 equiv. copper oxide, 2.0 equiv. KOH, 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. $\text{Pd}(\text{PPh}_3)_4$, 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-methylenedioxy)phenyl boronic acid, 0.01 equiv. $\text{Pd}(\text{PPh}_3)_4$, 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	-OCH ₂ O-	OMe	OMe	H	1.1	>25
14	0	H	OMe	H	-OCH ₂ O-	OBn	OBn	H	2.4	21
15	0	OMe	OMe	H	-OCH ₂ O-	OMe	OMe	H	1.5	>25
16	0	OMe	H	H	-OCH ₂ O-	OMe	OMe	H	5.5	>25
17	0	-OCH ₂ O-	H	H	H	H	OMe	H	12	>25
18	0	-OCH ₂ O-	H	H	OMe	H	H	H	2.3	>25
19	0	-OCH ₂ O-	H	H	OMe	H	OMe	H	3.5	>25
20	0	-OCH ₂ O-	H	H	H	H	Cl	H	10	>25
21	0	-OCH ₂ O-	H	H	H	H	H	OMe	7.4	>25
22	0	-OCH ₂ O-	H	H	-OCH ₂ O-	H	H	H	4.1	22
23	0	-OCH ₂ O-	H	H	-OCH ₂ O-	OBn	OBn	H	1.3	14
24	0	-OCH ₂ O-	H	H	-OCH ₂ O-	OMe	OMe	H	0.26	15
25	0	-OCH ₂ O-	H	H	-OCH ₂ O-	OPr	OPr	H	0.043	12
26	0	-OCH ₂ O-	H	H	-OCH ₂ O-	OMe	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	-OCH ₂ O-	H	H	-OCH ₂ O-	H	H	H	1.5	3.3
28	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OBn	OBn	H	0.8	2.5
29	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OMe	OMe	H	0.35	2.4
30	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OPr	OPr	H	0.08	0.85
31	1	-OCH ₂ O-	H	H	-OCH ₂ O-	H	H	OPr	0.047	5.6
32	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OPr	OPr	H	0.13	1.1
33	1	-OCH ₂ O-	H	H	H	H	H	H	11	14.5
34	1	-OCH ₂ O-	H	H	OMe	H	OBn	H	2.7	4.4
35	1	OMe	OMe	H	-OCH ₂ O-	OMe	OMe	OMe	0.06	0.85
36	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OMe	OMe	OMe	0.06	1.4
37	1	-OCH ₂ O-	H	H	-OCH ₂ O-	OMe	OMe	OBn	0.03	0.05
38	1	-OCH ₂ O-	H	H	-OCH ₂ O-	-OCH ₂ O-	-OCH ₂ O-	-OCH ₂ O-	0.24	0.67

References:

- (1) Arai, H.; Hori, S.; Aramori, I.; Ohkubo, H.; Nakanashi, S. *Nature* (London) **1990**, *348*, 730.
- (2) Sakuri, T.; Yanagisawa, M.; Takuwa, Y.; Miyazaki, H.; Kimura, S.; Goto, K.; Masaki, T. *Nature* (London) **1990**, *348*, 732.
- (3) Davenport, A. P.; O'Reilly, G.; Molenaar, P.; Maguire, J. J.; Kuc, R. E.; Sharkey, A.; Bacon, C. R.; Ferro, A. *J. Cardiovasc. Pharmacol.* **1993**, *22*, S22.
- (4) Seo, B.; Oemar, B. S.; Siebenmann, R.; Von Segesser, L.; Luscher, T. F. *Circulation* **1994**, *89*, 1203.
- (5) Karne, S.; Jayawickreme, C. K.; Lerner, M. R. *J. Biol. Chem.* **1993**, *268*, 19126.
- (6) Doherty, A. M.; Endothelin: A New Challenge, *J. Med. Chem.* **1991**, *35*, 1493.
- (7) Ishikawa, K.; Fukami, T.; Nagase, T.; Fujita, K.; Hayama, T.; Niyama, K.; Mase, T.; Ihara, M.; Yano, M. *J. Med. Chem.* **1992**, *35*, 2139.
- (8) Ihara, M.; Noguchi, K.; Saeki, T.; Fukuroda, T.; Tsuchida, S.; Kimura, S.; Fukami, T.; Ishikawa, K.; Nishikibe, M.; Yano, M. *Life Sci.* **1991**, *50*, 247.
- (9) 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. M.; Haleen, S. J.; Keiser, J. A.; Flynn, M. A.; Welch, K. M.; Hallak, H.; Taylor, D. G.; Reynolds, E. E. *J. Med. Chem.* **1995**, *38*, 1259.
- (10) Cody, W. L.; Doherty, A. M.; He, J. X.; Depue, P. M.; Rapundalo, S. T.; Hingorami, G. A.; Major, T. C.; Panek, R. L.; Haleen, S.; LaDouceur, D.; Reynolds, E. E.; Hill, K. E.; Flynn, M. A. *J. Med. Chem.* **1992**, *35*, 3301.
- (11) Cody, W. L.; Doherty, A. M.; He, J. X.; Depue, P. M.; Wait, L. A.; Topliss, J. G.; Haleen, S. J.; LaDouceur, D.; Reynolds, E. E.; Hill, K. E.; Flynn, M. A. *Med. Chem. Res.* **1993**, *3*, 154.
- (12) Elliott, J.; Lago, M. A.; Cousins, R.; Gao, A.; Leber, J.; Erhard, K.; Nambi, P.; Elshourbagy, N.; Kumar, C.; Lee, J.; Bean, J.; DeBrosse, C.; Eggleston, D.; Brooks, D.; Feuerstein, G.; Ruffolo, R.; Weinstock, J.; Gleason, J.; Peishoff, C.; Ohlstein, E. 1,3-Diarylindan-2-carboxylic Acids, *J. Med. Chem.* **1994**, *37*, 1553.
- (13) Walsh, T. F.; Fitch, K. J.; Chakravarty, K.; Williams, D. L.; Murphy, K. A.; Nolan, N. A.; O'Brien, J. A.; Lis, E. V.; Pettibone, D. J.; Kivlighn, S. D.; Gabel, R. A.; Zingaro, G. J.; Krause, S. M.; Siegl, P. K. S.; Clineschmidt, B. V.; Greenlee, W. J. Discovery of L-749,329, a highly potent, orally active antagonist of endothelin receptors. ACS National Meeting, Washington D. C., August 1994, MEDI 145.
- (14) Roux, S. P.; Clozel, M.; Sprecher, U.; Gray, G.; Clozel, J. P. *Circulation* **1993**, *88*, I-170.
- (15) Ishikawa, K.; Ihara, M.; Noguchi, K.; Mase, T.; Mino, N.; Saeki, T.; Fukuroda, T.; Fukami, T.; Ozaki, S.; Nagase, T.; Nishikibe, J.; Yano, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4892.
- (16) Connor, D. T.; Cetenko, W. A.; Mullican, D.; Sorenson, R. J.; Unangst, P. C.; Weikert, R. J.; Aldolphson, R. L.; John, A.; Thueson, D. O.; Wright, C. D.; Conroy, M. C. *J. Med. Chem.* **1992**, *35*, 958.
- (17) Cousins, R.; Elliott, J.; Lago, M.; Leber, J.; Peishoff, C. Endothelin Receptor Antagonists, Smith-Kline Beecham Corporation, **WO 93/08799**
- (18) Unangst, P. C.; Connor, D. T.; Stabler, R. S.; Weikert, R. J. *J. Heterocycl. Chem.* **1987**, *24*, 811.
- (19) Moody, C. J. *J. Chem. Soc. Perkin Trans., 1*, **1984**, 1333.
- (20) Noland, W. E.; Baude, F. J. *Org. Synth. Coll.* **1973**, *5*, 567.
- (21) 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. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1410.
- (22) Reynolds, E. E.; Mok, L.; Kurokawa, S. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 868.

(Received in USA 9 February 1996; accepted 8 April 1996)