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1-BENZYL-3-THIOARYL-2-CARBOXYINDOLES AS POTENT NON-PEPTIDE ENDOTHELIN ANTAGONISTS

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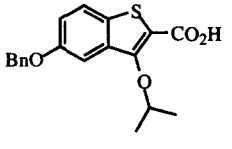
Abstract: Endothelin-1 is a potent vasoconstrictor which is thought to be involved in many human disease states. We have developed a series of indole non-peptide endothelin antagonists which display nanomolar receptor affinity. The representative compound from this series is PD 159433 (**22**), an ET_A selective antagonist with an IC_{50} of 2 nM. 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). Endothelin-1, a 21 amino acid bicyclic peptide isolated from cultured porcine endothelial cells, was the first of this series to be identified.[1,2] Since this discovery, endothelin-2 and endothelin-3, which differ by 2 and 6 amino acids respectively, have been reported and characterized.[3,4] 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.[5,6,7,8]

The vasoactive and mitogenic actions of endothelin has implicated this peptide in the pathogenesis of a number of disease states including renal failure, pulmonary hypertension, cerebral ischemia and vasospasm, endotoxic shock, and congestive heart failure.[9] The ET_A receptor is known to mediate a major portion of the vasoconstrictor activity of endothelin in human vessels.[10,11]

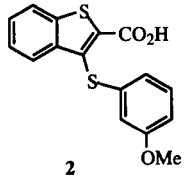
There have been a number of peptide and non-peptide endothelin antagonists reported in the literature.[12-20] These include a related series of 1,3-diaryl-2-carboxyindoles previously disclosed from these laboratories[21] and others.[22]

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.[14,23] We selected the lead structure, compound **1**, for synthetic modification and soon discovered that compound **2** showed moderate activity, confirming that an acidic substituent at C-2 and a lipophilic substituent at C-3 were essential for receptor binding activity. Compound **3**, being 4-fold less potent than the benzothiophene, **2**, demonstrated that the 1 position played some role in receptor binding activity.



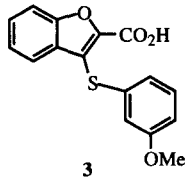
1

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



2

ET_A IC_{50} 5.9μM
hET_B IC_{50} >25μM



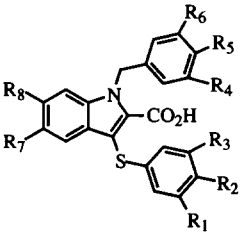
3

ET_A IC_{50} 23μM
hET_B IC_{50} >25μM

Therefore, following the disclosure of a series of indanes[17,22] as endothelin antagonists we directed our attention to a series of N1-substituted indoles. Since previous SAR studies revealed that, in general, electron donating substituents on the aromatic rings favored ET_A binding affinity,[14] we decided to synthesize 1-benzyl-3-thioaryl-indoles that incorporated these substituents.

Structure-Activity Relationships:

Table 1 - 1-benzyl-3-thioaryl-2-carboxyindoles



Ex	R1	R2	R3	R4	R5	R6	R7	R8	hET_A I C_{50} (μM)	hET_B IC_{50} (μM)	AAR-A (μM)	AAR-B (μM)	ET_A (pA_2)	ET_B (pA_2)
4	OMe	H	H	H	H	H	H	H	4.6	15	-	-	-	-
5	OMe	H	H	H	H	H	OMe	OMe	0.16	0.87	-	-	-	-
6	OMe	H	H	H	-(OCH_2O)-		H	H	0.086	7.4	-	-	-	-
7	OMe	H	H	H	-(OCH_2O)-		OMe	OMe	0.02	1.1	0.033	0.21	7.0	
8	OMe	H	H	H	-(OCH_2O)-		H	OPr	0.59	6.7	-	-	-	-
9	OMe	H	H	H	-(OCH_2O)-		OBn	OMe	0.081	0.69	-	-	-	-
10	OMe	H	H	H	-(OCH_2O)-		OMe	OBn	2.0	1.6	-	-	-	-
11	OMe	H	H	H	-(OCH_2O)-		-(OCH_2O)-		0.084	0.76	-	-	-	-
12	OMe	H	H	OMe	-(OCH_2O)-		-(OCH_2O)-		0.38	2.9	-	-	-	-
13	OMe	H	H	OMe	-(OCH_2O)-		OMe	OMe	0.058	1.1	-	-	-	-
14	OMe	OMe	H	OMe	-(OCH_2O)-		-(OCH_2O)-		0.17	0.42	-	-	-	-
15	OMe	OMe	H	H	-(OCH_2O)-		-(OCH_2O)-		0.041	0.17	0.065	0.17	6.3	6.2
16	-(OCH_2O)-		H	H	-(OCH_2O)-		H	H	0.38	4.5	-	-	-	-
17	-(OCH_2O)-		H	H	-(OCH_2O)-		-(OCH_2O)-		0.1	0.42	-	-	-	-
18	-(OCH_2O)-		H	H	-(OCH_2O)-		OMe	OBn	0.62	0.49	3.4	0.74	-	-
19	-(OCH_2O)-		H	H	-(OCH_2O)-		OMe	OMe	0.005	0.28	0.1	1.1	7.3	6.2
20	OMe	OMe	OMe	H	-(OCH_2O)-		-(OCH_2O)-		0.007	0.23	0.064	0.17	6.9	-
21	OMe	OMe	OMe	H	-(OCH_2O)-		OMe	OBn	>2.5	>2.5	-	-	-	-
22	**OMe**	**OMe**	**OMe**	**H**	**-(OCH_2O)-**		**OMe**	**OMe**	**0.002**	**0.38**	**0.0046**	**0.44**	**7.5**	**5.5**

From Table 1 it is evident that at positions R_4, R_5, and R_6 activity was enhanced from the unsubstituted compounds **4** and **5**, by the addition of a methylenedioxy group, as seen in compounds **6** and **7**. Since the methylenedioxy moiety was an improvement over the unsubstituted compounds, the substitution on this ring was kept constant.

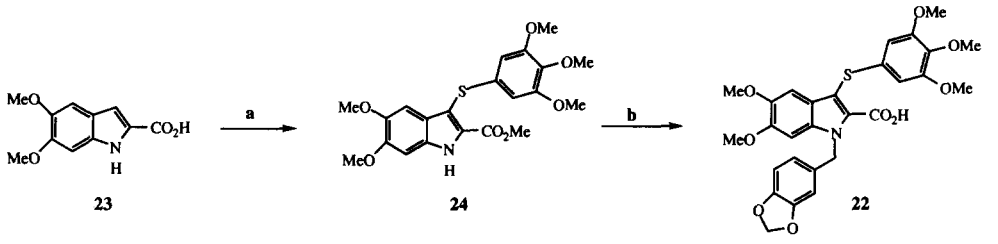
Substitution at the R_4 position with electron donating groups, such as a methoxy group, did not appear to enhance binding activity, as seen by comparing compounds **12-14** with their unsubstituted analogs compound **11**, **7**, and **15** respectively.

The addition of multiple electron donating groups at R_7 and R_8 improved activity significantly, as seen by comparing compounds **4** and **5**, compounds **6** and **7**, and compounds **16** and **19**. Further SAR involving compounds **7-11**, **12-13**, **16-19**, and **20-22** clearly demonstrated the need for methoxy substituents at R_7 and R_8.

Positions R_1, R_2, and R_3 were also substituted with a variety of electron donating groups. We synthesized several potent compounds incorporating the methylenedioxy moiety and methoxy substituents, exemplified by compounds **11**, **15**, **17**, and **20**. Substitution at each of the R_1, R_2, and R_3 positions with a methoxy group led to the discovery of PD 159433 (**22**), an ET_A selective endothelin antagonist. This compound potently inhibited the release of arachidonic acid from rabbit renal vascular smooth muscle cells stimulated by ET-1, and demonstrated functional antagonism of ET-1 stimulated vasoconstriction in isolated strips of rabbit femoral arteries.

Synthesis: Treatment of sodium salts of 2-carboxyindoles[21] with diaryldisulfides[24] and diazomethane work-up led to good yields of the 2-methoxycarbonyl-3-thioaryl-indoles. Benzylation of the indoles proceeded readily by treatment with the appropriate benzyl chlorides with hydrolysis affording the required target compounds, as seen in Scheme 1.

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



23 24 22

(**a**) i 3.0 equiv NaH, 1.0 equiv diaryldisulfide, DMF, 60 °C; ii 3.0 equiv $TMSCHN_2$, toluene:MeOH 4:1, 71% yield (**b**) i 1.25 equiv NaH, DMF, 1.1 equiv (3,4-methylenedioxy)benzyl chloride, 0 °C, 64% yield; ii 15.0 equiv LiOH, THF:MeOH:H_2O 5:1:1, 64% yield.

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).[14,25] 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.[14,26] 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, $ET_B(pA_2)$.[25,27]

Conclusions: Extensive investigation of electron donating substituents on all three aromatic rings led to the discovery of ET_A selective indoles, such as compounds **19**, **20**, and **22**. The ability of compound **22**, for example, to inhibit the release of arachidonic acid (AAR), and to inhibit the contraction of rabbit femoral artery, upon stimulation with ET-1 demonstrated that these compounds are functional antagonists of the ET_A receptor. These compounds should prove useful in elucidating the physiological and pathophysiological role of endothelin.

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