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DESIGN AND SYNTHESIS OF NONPEPTIDAL ENDOTHELIN RECEPTOR ANTAGONISTS BASED ON THE STRUCTURE OF A CYCLIC PENTAPEPTIDE

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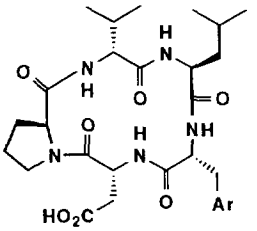
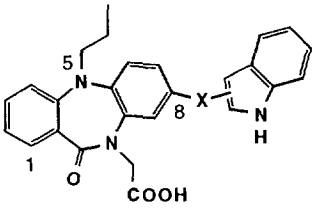
Abstract: A series of dibenzodiazepine-10-acetic acid derivatives were synthesized as prototypes to mimic the structural features of the cyclopentapeptide endothelin antagonist **1**. Some of the analogs showed moderate affinity for both the ET_A and ET_B receptors.

The endothelins (ETs) are a family of 21 amino acid peptides, with ET-1 being one of the most potent vasoconstrictors identified to-date.[1] Although originally isolated from endothelial cells, these peptides are now known to be produced by a number of other cell types. Numerous reports have described the diversity of physiological effects elicited by the ETs and they exert their biological effects through interaction with specific guanine nucleotide coupled, membrane bound cellular receptors of which two subtypes, ET_A and ET_B, have been cloned and characterized.[2,3] It is widely expected that the discovery of receptor subtype selective as well as non-selective ET antagonists will clarify the physiological and pathological roles of ET and may also lead to useful therapeutic agents.[4]



1a 3-indole (BQ-123)
1b 1-napthyl
1c phenyl



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Recently a number of groups have reported their efforts in the development of selective and non-selective ET antagonists.[5-8] Of particular interest to us was the cyclic pentapeptide BQ-123 (**1a**, ET_A K_i = 18 nM; ET_B K_i = 7 μM), which was derived by chemical modification of the naturally occurring antagonist, BE 18257B.[9] We[10] and others[11-13] have determined the solution conformations of this peptide using NMR as well as molecular modeling techniques. These studies revealed useful information regarding the relative position of

the amino acid side chains as well as the existence of a β-turn involving the Val-Leu-Trp-Asp residues. We used this information as the basis on which to design non-peptide ligands.

One of our design goals was to append the critical amino acid side chain pharmacophores to a central scaffold so as to mimic the structural as well as conformational features of the peptide. Structure-activity studies of BQ-123 revealed that the Asp and Trp side chains were the critical pharmacophores and the lipophilic side chains of Leu and Val also contributed to overall activity. Molecular modeling studies indicated that a rigid tricyclic template, such as the dibenzodiazepine nucleus, could mimic the peptide backbone as well as the β-turn. The nitrogens of the diazepine ring span the cyclic pentapeptide core and offer attachment points for a Val and an Asp side chain surrogate, while the fused aryl rings offer attachment points for the Ile and Trp surrogates. Guided by Sybyl-based modeling techniques, we introduced an acetic acid side chain onto the amide nitrogen and an n-propyl group onto the amine nitrogen. Modeling studies suggested that these side chain placements required that the Trp surrogate be introduced at C-8 of the dibenzodiazepine ring. We felt that attachment of the indole surrogate through a linker group of 1-3 atoms would maximize the chances of delivering this critical side chain to its binding site (Figure 1). We thus arrived at the generic structure **2** as our prototype non-peptide mimic of the cyclic peptide **1**.

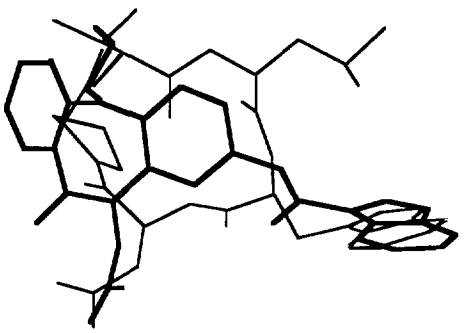


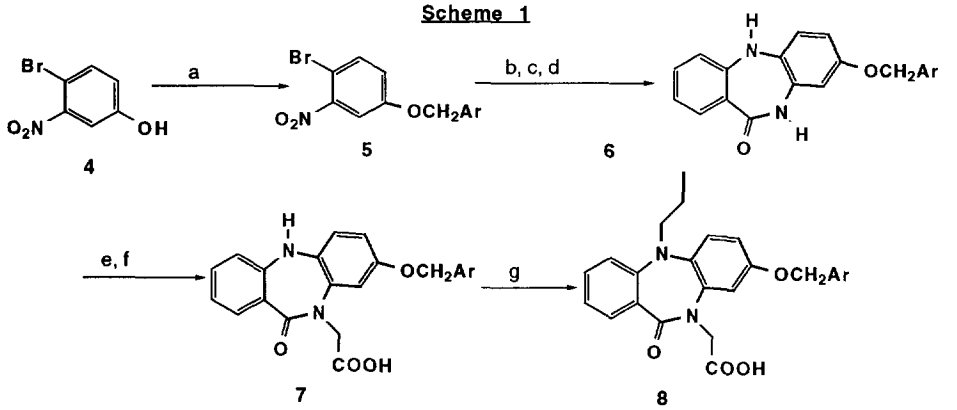
Figure 1. Superimposition between BQ-123 (**1a**) and Compound **18** (bold).

The synthesis of the D-Phe and D-Nal analogs of BQ-123 have been described but no biological activity was reported.[9] We prepared these analogs to compare their activity to the parent indole containing analog and found that successively lower ET_A binding affinities were produced by the indole to naphthalene to phenyl substitution (**1a** (BQ-123), $K_i = 18 \pm 4.2$ nM; **1b**, $K_i = 224 \pm 35$ nM; **1c**, $K_i = 2400 \pm 49$ nM). While the simple aromatic derivatives had lower affinity for ET_A receptors than BQ-123, their still substantial affinity led us to initially target dibenzodiazepines in which the indole ring was replaced by a phenyl or a 1-naphthyl. The simpler synthesis of these analogs was expected to quickly provide proof of our design principle.

The synthetic route used to make compounds **7** and **8** is shown in Scheme 1. Alkylation of 4-bromo-3-nitrophenol **4** with 1-bromomethylnaphthalene afforded compound **5** which was then converted to the dibenzodiazepine derivative **6** using a known sequence of reactions.[14] Alkylation of the amide nitrogen with methyl bromoacetate followed by saponification of the ester afforded compound **7**. Furthur alkylation of **7** with two equivalents of sodium hydride and n-propyl iodide provided the target compound **8**. Analogs **12** and **13** in

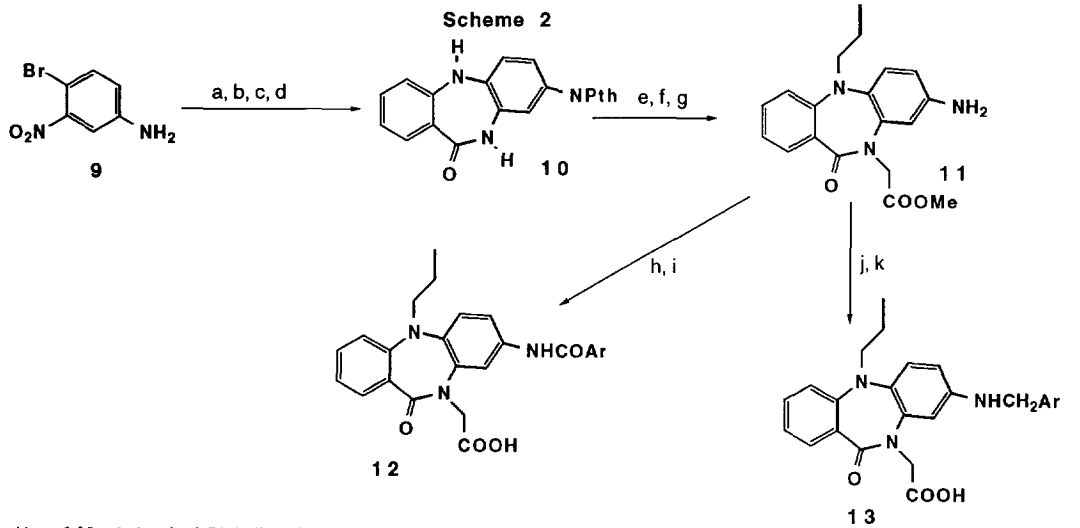
which the dibenzodiazepine moiety is linked to the naphthalene ring by nitrogen based linkers were synthesized as shown in Scheme 2. The N-phthaloyl derivative **10** was obtained using a similar sequence of reactions as descibed above. Deprotection of the phathaloyl group followed by reductive amination with 1-naphthaldehyde and subsequent hydrolysis of the ester group gave compound **13**. Acylation of **11** with 1-naphthoyl chloride followed by saponification provided analog **12**.

Scheme 1



(Ar = 1-Naphthalene) a) 1-Bromomethylnaphthalene, K_2CO_3, DMF, rt, 92%. b) Anthranilic acid, Cu, K_2CO_3, AmOH, reflux, 6h, 95%. c) Sodium hydrosulfite, 2N aq. NH_4OH, 88%. d) Xylenes, reflux, 48%. e) Methyl bromoacetate, NaH, DMF, rt, 69%. f) 1N aq. NaOH, MeOH, rt, 77%. g) 2 eq. NaH, 1-Iodopropane, DMF, rt, 42%.

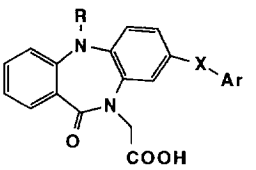
Scheme 2



(Ar = 1-Napthalene) a) Phthalic anhydride, Xylene, reflux, 88%. b) Anthranilic acid, Cu, K_2CO_3, AmOH, reflux, 7h. c) Sodium hydrosulfite, 2N aq. NH_4OH. d) Xylenes, reflux, 30% (for three steps). e) Methyl bromoacetate, NaH, DMF, rt, 65%. f) propionaldehyde, $Na(OAc)_3BH$, AcOH, 87%. g) NH_2NH_2, MeOH, 90%. h) 1-Naphthoyl chloride, $(C_2H_5)N$, CH_2Cl_2, 92%. i)1N aq. NaOH, MeOH, 86%. j) 1-Naphthaldehyde, $Na(OAc)_3BH$, AcOH, 88%. k) 1N aq. NaOH, MeOH, 87%.

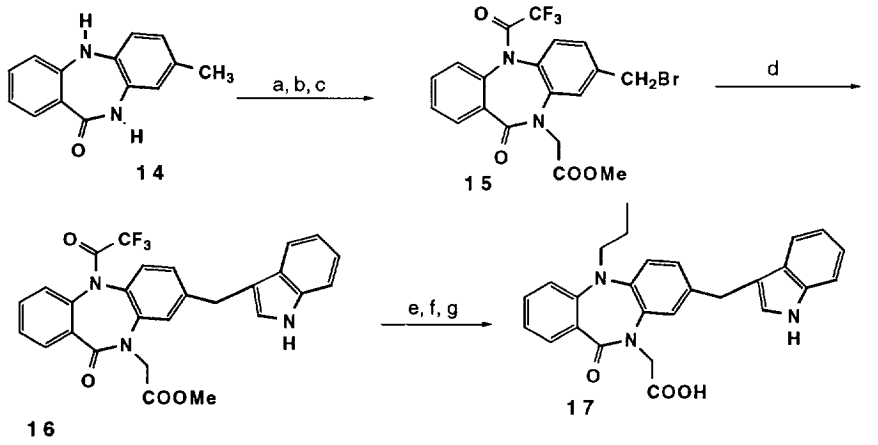
Compounds **3, 7, 8, 12** and **13** showed affinities for both ET_A and ET_B receptors in the micromolar range (Table 1).[15] More importantly, the relative affinities of these analogs were in the order expected from our understanding of the structure activity relationships of BQ-123. For example, the naphthyl analog **7** was more potent than the corresponding phenyl analog **3** at the ET_A receptor and the addition of the propyl group to **7** to pick up the hydrophobic binding interaction of the valine pocket led to the most potent compound at the ET_A receptor (Compound **8**, 7 μM). The micromolar affinity of these molecules for the ET_B receptor is similar to that of BQ-123.

Table 1



Compound	R	X	Ar	ET_A K_i (μM)	ET_B K_i (μM)
3	H	OCH_2	Phenyl	109 ± 14	>100
7	H	OCH_2	1-Napthyl	28 ± 4	8 ± 0.5
8	n-Propyl	OCH_2	1-Napthyl	7 ± 1	60 ± 1
12	n-Propyl	NHCO	1-Napthyl	83 ± 36	NT
13	n-Propyl	$NHCH_2$	1-Napthyl	17± 7	9 ± 0.1

Scheme 3

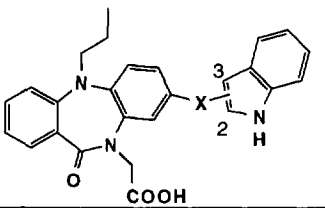


a) Methyl bromoacetate, NaH, DMF, rt, 63%. b) $(CF_3CO)_2O$, $(C_2H_5)N$, CH_2Cl_2, 0°C. c) NBS, benzoyl peroxide, CCl_4, 64%. d) Indole, AgO, dioxane, reflux, 24h, 38%. e)10% aq. K_2CO_3, MeOH, 100%. f) propionaldehyde, $Na(OAc)_3BH$, AcOH, 73%. g) 1N aq. NaOH, MeOH, 90%.

Encouraged by these results, we set out to synthesize analogs **17** - **20** in which the naphthalene ring was replaced by the more relevant indole ring. Our expectation was that analogs which more closely approximated the tryptophan interaction would exhibit higher affinity. The indole ring was attached to the dibenzodiazepine ring via different spacer groups containing one to three atoms. The synthetic route used to make compound **17** in which there is a methylene spacer between the two rings is shown in Scheme 3.

The known dibenzodiazepine **14**[14] was first alkylated using methyl bromoacetate. The free amine nitrogen was protected with a trifluroacetyl group prior to bromination of the methyl group to the bromomethyl derivative **15**. Reaction of **15** with indole in the presence of silver oxide afforded the indolylmethyl analog **16**. Hydrolysis of the trifluoroacetyl protecting group, reductive amination with propionaldehyde, followed by hydrolysis of the ester group then provided the target compound **17**. Analogs **18** - **20** were synthesized using the same protocol described in scheme 2 starting from the corresponding indole carboxylic acids.

Table 2



Compound	X	Substitution Position	ET_A K_i (μM)	ET_B K_i (μM)
17	CH_2	3	41 ± 10	12 ± 0.7
18	NHCO	3	80 ± 22	11 ± 0.7
19	NHCO	2	33 ± 5	6 ± 0.9
20	$NHCOCH_2$	3	14 ± 1	7 ± 0.1

As shown in Table 2, all of the compounds, regardless of spacer length or site of indole attachment, had micromolar affinity for the ET_A receptor. While a slight improvement in ET_A affinity was observed with the longest spacer length, none of the indole-containing targets showed substantially higher affinity than the simple naphthalene analogs. This contrasts dramatically with the structure activity relationships of BQ-123 which we report in this paper, where the naphthalene analog has 10-fold lower affinity than the indole analog. It is possible that the cyclic pentapeptides and dibenzodiazepines bind differently to the ET_A receptor in spite of their potential to similarly position the appropriate pharmacophores. As with the naphthalene analogs, the indole analogs showed substantial affinity for the ET_B receptor and in fact all were slightly selective for this subtype. The ET_B affinities of all of the dibenzodiazepines were similar to the ET_B binding constant we have determined for BQ-123 in rat cerebellum ($K_i = 7.0 \pm 0.1$ μM).

In conclusion, we have designed and synthesized a series of non-peptide mimics of the cyclic peptide BQ-123 using the dibenzodiazepine ring as a template and connecting the phamacophore side chains around this ring. Our initial set of target compounds, albeit much less active than BQ-123, showed moderate activity at both the ET_A and ET_B receptors. While a strict structure-activity correlation with BQ-123 was not observed with regards to the tryptophan side chain, our results nevertheless confirm the viability of our dibenzodiazepine template design. Further elaboration of the dibenzodiazepine to include an appropriate leucine pharmacophore might be expected to lead to higher affinity analogs.

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(15) The receptor binding assays were carried out as described in ref. 5.

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