DESIGN AND SYNTHESIS OF 1,4-DIAZABICYCLO[4.3.0]NONANE PEPTIDOMIMETIC ENDOTHELIN ANTAGONISTS

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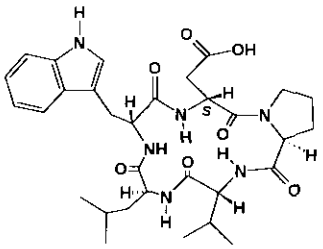
Abstract - The design and synthesis of a series of 2-(3-indolylmethyl)-4-(*tert*-butoxycarbonyl)-1,4-diaza-2-oxobicyclo[4.3.0]nonane-9-carboxylic acid peptidomimetics based on the peptide endothelin antagonists BQ-123 and FR139317 were described. The stereochemistry of the active (3*R*,6*S*,9*R*) isomer was determined by X-Ray crystallography.

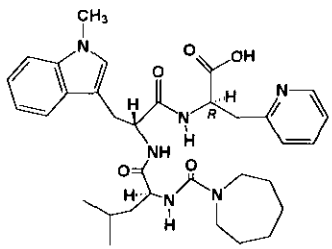
The endothelins (ET-1, ET-2 and ET-3) are potent vasoconstrictive peptides consisting of 21 amino acid residues and two disulfide bonds.[1] They exert their action through interaction with cell surface receptors. Currently two major receptor subtypes, ET_A and ET_B, are known.[3] The ET_A receptor has high affinity for ET-1 whereas the ET_B receptor has high affinity for all three peptides. Since the endothelins have been implicated in various disease states such as hypertension, renal failure, and congestive heart failure,[2] ET receptor antagonists are thus potentially valuable therapeutic agents. BQ-123[4] and FR139317[5] are two of the earliest ET antagonists reported in the literature. Both compounds are peptidic in nature and selective for the ET_A receptor. Due to the difficulties in administering peptide drugs, non-peptide analogues of these compounds are highly desirable. In this communication, we report our effort in designing and synthesizing a series of 1,4-diaza-2-oxobicyclo[4.3.0]nonane-9-carboxylic acid peptidomimetic endothelin antagonists based on BQ-123 and FR139317.

The solution conformation of BQ-123 has been studied extensively by NMR and molecular modeling.[6] The major solution conformation consists of a loose β-turn with Leu and D-Trp at the corner and a γ-turn centered at Pro (Figure 1). This conformation is thought to be a significant contributor to the biological activity. Structural-activity studies revealed that the Leu, D-Trp and D-Asp residues of BQ-123 are essential for binding to the ET_A receptor.[4b] We therefore focused our attention on the Leu-D-Trp-D-Asp moiety of BQ-123 for peptidomimetic design. Furthermore, the tripeptide endothelin antagonist FR139317 also adopts a similar β-turn-like conformation at the Leu and D-Trp residues.[5b] (Figure 1) In this conformation, the carboxylic acid group of the unnatural amino acid D-3-(2-pyridyl)alanine (2-Pya) would be oriented in a similar fashion to the D-Asp carboxylic acid in BQ-123 but with opposite stereochemistry (*vide infra*). Based on these observations, a series of 1,4-diaza-2-oxobicyclo[4.3.0]-nonane-9-carboxylic acids (**1**) was designed as potential ET antagonists. These peptidomimeitcs (**1**) were chosen due to their ready accessibility from Trp and pyrrolidine-2,5-dicarboxylic acid.[7] The requisite indole and carboxylic acid sidechains are built into the system and the hydrophobic residue from Leu can

be mimicked by a suitable nitrogen substituent (e.g. a Boc group). Furthermore, the peptide backbone of BQ-123 and FR139317 will be partially mimicked by the organic framework. Stereochemical considerations suggest that D-Trp derived peptidomimetics should best match the D-Trp residues of BQ-123 and FR139317. Since the carboxyl groups of these peptides have different stereochemical configurations (*vide supra*), we decided to synthesize all 4 isomers of **1** derived from D-Trp in order to study the effect of the carboxyl group stereochemistry on the biological activity.



BQ-123



FR139317

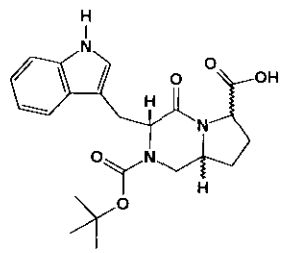


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Figure 1

The peptidomimetics (**1**) were synthesized as outlined in Scheme 1. Dimethyl *N*-Cbz-*trans*-pyrrolidine-2,5-dicarboxylate (**3b**), obtained from the corresponding *N*-benzyl compound (**2b**)[7] in two steps, was reduced with $LiBH_4$ in THF to give, rather unexpectedly, the monoester (**4b**) as the major (**4b**:**5b** 9:1) product. Under similar conditions, the *cis* isomer (**3a**) gave the diol (**5a**) (oil; IR: 3614, 1674 cm^{-1}) as the exclusive product. This difference in activity can be rationalized by the coordination of borohydride with the first alcohol formed which then facilitates the reduction of the second ester in the *cis* isomer through neighboring group participation (Figure 2). Such coordination is not possible in the *trans* isomer. Accordingly, selective reduction of the *cis* isomer (**3a**) should be possible with a less reactive reducing agent. Indeed, $NaBH_4$ reduction of **3a** led to a 1:1 mixture of the monoester (**4a**) (oil; IR: 3612, 1701, 1743 cm^{-1}) and diol (**5a**) which were readily separated by column chromatography (R_f 0.13 and 0.06, respectively, 40% EtOAc/hexanes).

Swern oxidation[8] of the *cis*-monoester (**4a**) gave an unstable aldehyde (**6a**) (oil; IR: 1707, 1739 cm^{-1}) which was immediately reacted with D-Trp-OBn and $NaBH_3(CN)$[9] to give a diastereomeric mixture of amines (**7a**) and (**7b**). These diastereomers were readily separated by column chromatography (R_f 0.19 and 0.25, 40% EtOAc/hexanes). The amines (**7a**) and (**7b**) were individually protected as the Boc derivatives (**8a**) and (**8b**). The $CbzCl_2$ and Bn groups in the less polar isomer (**8a**) (oil, R_f 0.26, 40% EtOAc/hexanes; IR: 3470, 1741, 1697 cm^{-1}) were simultaneously deprotected by catalytic hydrogenation over 10% Pd/C catalyst to give the corresponding amino acid (**9a**) [gum; IR: 3398 (br), 1743, 1695 cm^{-1}] which was immediately cyclized with BOP reagent to give the esters (**10a**) [structure **1a**; R^1=CO_2Me, R^2=H; mp 155-158 °C; IR: 3325(br), 1749, 1689, 1656 cm^{-1}]. Saponification of **10a** (LiOH, aqueous THF) gave **1a** [10] which was determined to be the (3*R*,6*S*,9*R*) isomer by X-Ray crystallography (*vide infra*). The acid(**1b**)(mp 92-96 °C) was obtained in a similar manner from **8b** . The other diastereomeric pairs (**1c**) (mp 213-217 °C) and (**1d**) (foam) was similarly obtained from D-Trp and **4b**.

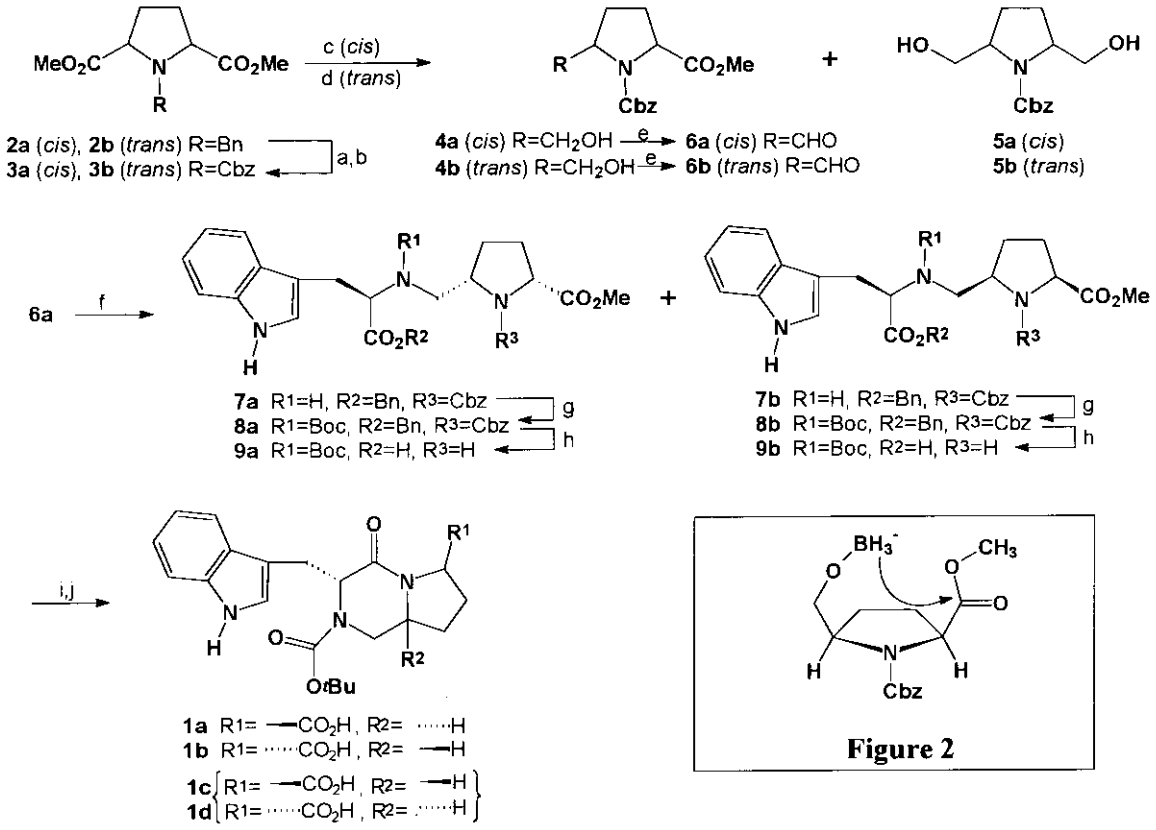

Figure 2

Scheme 1

Reagents and conditions: (a) H_2 (60 psi), 10% Pd/C, MeOH, 100%; (b) $PhCH_2OCOCl$, $NaHCO_3$, EtOAc/H_2O, 75-100%; (c) $NaBH_4$, MeOH/THF, 25 °C, 60 % (1:1 **4a**:**5a**); (d) $LiBH_4$, THF, 25 °C, 82% (9:1 **4b**:**5b**); (e) $(COCl)_2$, DMSO, Et_3N, -78 to 25 °C; (f) H-D-Trp-OBn·HCl, $NaBH_3CN$, MeOH/AcOH (98:2), 25 °C, 54-66% for 2 steps; (g) $(Boc)_2O$, MeCN, 25 °C, 78-79%; (h) H_2 (55 psi), 10% Pd/C, MeOH, 100%; (i) BOP, CH_2Cl_2, 0-25 °C, 50-60%; (j) LiOH, THF, 25 °C, 90-95%.

Of the four peptidomimetics (**1a-d**) synthesized, only isomer (**1a**) derived from *cis*-2,5-pyrrolidinedicarboxylic acid showed an IC_{50} of 10 μM for the ET_A receptor.[11] The other isomers did not show appreciable binding to either the ET_A or ET_B receptors at concentrations up to 100 μM. Due to the presence of rapidly interconverting conformational isomers, the stereochemistry of these compounds cannot be readily determined by ^1H NMR. The stereochemistry of the active isomer (**1a**) was thus determined by X-Ray crystallography and was found to be (3*R*,6*S*,9*R*) (Figure 3).[12] The stereochemical configuration of the carboxyl sidechain in **1a** thus resembles that of FR139317 rather than BQ-123.

In conclusion, we have designed and synthesized a series of peptidomimetic endothelin antagonists based on BQ-123 and FR139317. Compound (**1a**) showed moderate affinity for the ET_A receptor and should serve as a good lead for designing other peptidomimetic ET antagonists. The dependence of the biological activity on the stereochemistry of the peptidomimetics (**1a-d**) suggests that the 1,4-diaza-2-oxobicyclo[4.3.0]nonane-9-carboxylic acid framework is relatively rigid and may be a suitable scaffold for other types of peptidomimetics.

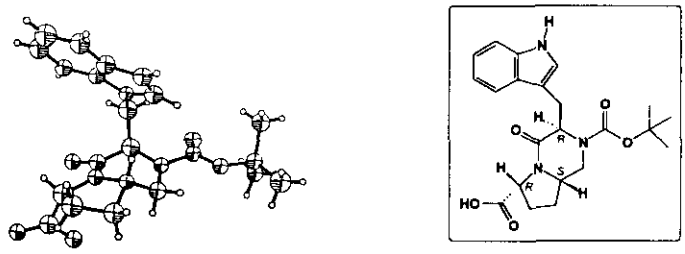

Figure 3. X-Ray structure of **1a**

ACKNOWLEDGMENT

We thank Dr. Ilya Okun for performing the endothelin receptor binding assays.

REFERENCES AND NOTES

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10. Data for **1a**: mp 180-184 °C (purified by preparative HPLC). IR(KBr): 3314, 2940, 1709, 1692, 1611, 1381, 1169 cm^{-1}; ^{1}HNMR (300 MHz, 100 °C, DMSO-d_6): δ 12.1 (br s, 1H), 10.56 (br s, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 6.93-7.07 (m, 3H), 4.38 (t, J = 5 Hz, 1H), 4.09 (d, J = 8.7 Hz, 1H), 3.73 (d, J = 8.6 Hz, 1H), 3.33 (A of ABX, J = 6.4, 14 Hz, 1H), 3.13 (B of ABX, J = 4.5, 14 Hz, 1H), 2.84 (br s, 2H), 1.2-1.9 (m, 4H), 1.38 (s, 9H). HRMS(FAB): m/z 413.1952; calcd for $C_{22}H_{28}N_3O_5$ $[M+1]^+$: 413.1951. *Anal.* Calcd for $C_{22}H_{27}N_3O_5$: C, 63.91; H, 6.58; N, 10.16. Found: C, 63.76; H, 6.75; N, 10.09.
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12. **1a** was crystallized from H_2O/MeOH as orthorhombic crystals, space group $P2_12_12_1$, a=9.55(2), b=12.25(4), c=19.58(6) Å with one molecule in the asymmetric unit. The diffraction data to a 2Θ limit of 45° were measured with a Siemens R3m/V diffractometer. The structure was solved and refined with the SHELXTL package to a R-factor of 9.74% using a total of 710 reflections. Detailed X-Ray crystallographic data will be published elsewhere.

Received, 21st August, 1998