Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2421–2425

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Structure-Based Design of Six Novel Classes of Nonpeptide Antagonists of the Bradykinin $B_2$ Receptor

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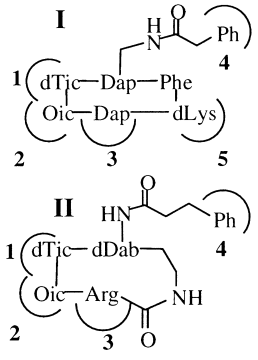
Received 12 June 2000; accepted 15 August 2000

**Abstract**—Six classes of nonpeptide bradykinin antagonists were designed using a template derived from structural studies of peptide antagonists. Several compounds from each class were synthesized and assayed for binding to the human bradykinin $B_2$ receptor. Each family showed compounds active at the level of the smallest template peptide; three classes contained compounds with $K_d < 8\,\mu M$. These results provide diverse leads for a medicinal chemistry-based optimization program. 

The nonapeptide hormone bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) acts as a mediator of inflammation under conditions of tissue injury or trauma.[1] Bradykinin is produced by the cleavage of kininogens by certain kallikreins, and are potent effectors at a family of G-protein coupled 7-transmembrane receptors. At the time this work was begun, a large body of data concerning the activities of peptide agonists and antagonists was available, but nonpeptide antagonists, of interest as novel anti-inflammatory therapeutics, had not been reported.[2] Subsequent to the completion of this work, advances in the pursuit of such compounds have recently been realized.[3–5]

The structure-based design of bioactive small molecules from peptides has proved to be a powerful technique in new lead discovery.[6] The derivation of small molecule $II_bIII_a$ antagonists from a structured peptide template core has highlighted the utility of peptide structure–function data in peptidomimetic design.[7] The body of available biological data for bradykinin-related peptides suggested that, given an appropriate structural template, such a strategy might well prove successful in the creation of small nonpeptide bradykinin antagonists.

Mutagenesis data on the bradykinin $B_2$ receptor had indicated that peptide antagonists, such as HOE-140[8,9] (d-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-dTic-Oic-Arg), bind the receptor in a fashion distinct from bradykinin.[10] In order to develop the structural template from which to design potential synthetic targets, we decided to center our studies on three classes of peptide antagonists. As representatives, we chose the linear compounds represented by HOE-140, and two cyclic peptide antagonists containing a hexa- (**I**) and tetrapeptide (**II**) core.[11] Compounds **I** and **II** bind with $K_d$s in the 0.2–16 μM range, and contain many of the features of HOE-140 in a more constrained framework. The SAR of these cyclic peptides had revealed a pharmacophore that could contain five features around the backbone core (labeled at right). These included the hydrophobic groups at (**1**) the dTic residue and (**2**) the Oic residue following 1; (**3**) a positively charged residue immediately following 2 (**4**) an aromatic residue proceeding 1 and (**5**) a second positive charge, which could be displayed from different portions of the scaffold.




We first chose to examine the structure of HOE-140 using NMR spectroscopy in water, DMSO and in SDS

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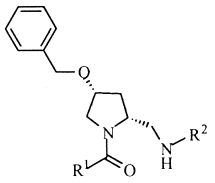
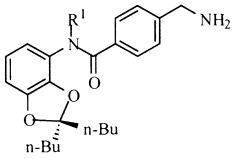
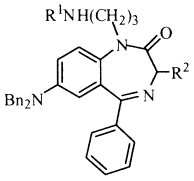
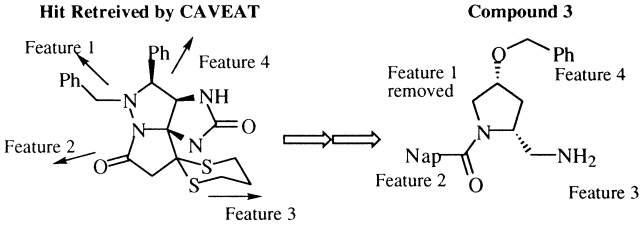
0960-894X/00/$ - see front matter 
PII: S0960-894X(00)00482-0

**Table 1.** CAVEAT-derived bradykinin antagonists

| | $R^1$ | $R^2$ | $K_d$ (μM)±S.E. |
|---|---|---|---|
|  | | | |
| (**1**) | Ph | H | Inactive |
| (**2**) | Ph | $C(=NH)NH_2$ | Inactive |
| (**3**) | β-Naphthyl | H | 66±4.2 |
| (**4**) | β-Naphthyl | $C(=NH)NH_2$ | 15±0.79 |
|  | | | |
| (**5**) | H | | 13±1.4 |
| (**6**) | $(CH_2)_3NH_2$ | | 14±.63 |
|  | | | |
| (**7**) | $C(=NH)NH_2$ | $CH_2NHR^1$ | 12±.37 |
| (**8**) | H | $CH_2NH_2$ | 4.3±.32 |
| (**9**) | H | $CH_2CH_2NH_2$ | 15±1.3 |
| (**10**) | $C(=NH)NH_2$ | $CH_2CH_2NHR^1$ | 4.5±.41 |

micelles.[10] These studies led to the conclusion that the major structural motif of HOE-140 consisted of a type II β-turn at residues 2–5 and a type II′ β-turn at residues 6–9, with a highly flexible 'hinge' region separating the turns. Cyclic peptides **I** and **II** were also examined in DMSO solution via NMR, and found to retain a turn centered on the dTic-Oic substructure identical to that observed for HOE-140. The structures of HOE-140 and peptides **I** and **II** were next subjected to runs of 50–100 ps of unconstrained room temperature in vacuo dynamics,[12] in order to explore the areas of uncertainty in the template. Two different starting positions were used for HOE-140, both with the experimentally observed β-turns, but with different extended structures in the hinge regions. Although collapsed structures were often observed, the type II′ β-turn centered on the dTic-Oic pair persisted throughout the simulations, consistent with the NMR data obtained for these molecules. These studies provided several different models of the 'core' structural unit of the peptide inhibitors. This structural template provided a fairly high degree of confidence in the dTic-Oic-Arg or dTic-Oic-Dap regions, and significantly higher variability in most other structural aspects of the pharmacophore.

Our design strategy focused on replacing the structurally well-defined 'core' of our cyclic peptides with a new non-peptide scaffold, preserving a degree of flexibility in the areas of our molecule where we had little structural information. The variable precision in our template made it well suited for use with the program CAVEAT.[13] This program may be used to search molecular databases for compounds that can accommodate the geometrical relationship between specific bonds in a template molecule. In this case, the search templates were derived from minimized structures extracted from low energy regions of the molecular dynamics trajectory for each peptide. These structures were used to define a set of bond vectors which were used to carry out a CAVEAT search of the Cambridge Structural Database.[14] We ultimately utilized the bond vectors from the amide nitrogen to the Cε or Cδ for the dTic and Oic residues, and from the Cα–Cβ bond for the following residue in the various peptides, leaving the incorporation of the functionality of features 4 and 5 (see above) to the post-search design process. This choice of vectors dramatically enhanced the diversity of the compound classes returned from the searches and avoided most of the less desirable peptide and steroid systems normally found using an all Cα–Cβ vector search. Families of compounds were extracted using the *class* module of the CAVEAT suite, and examined for potential suitability as organic linkers.



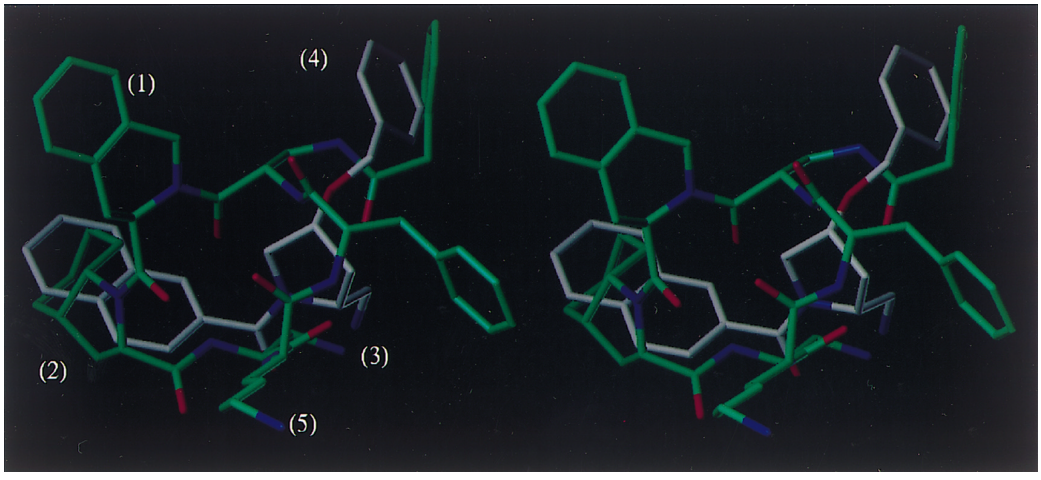



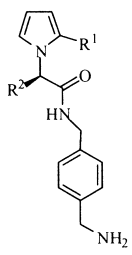
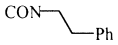
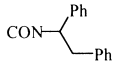
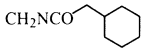
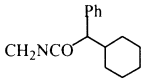
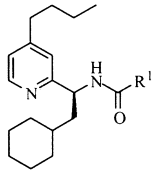
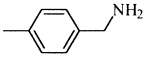
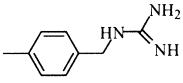
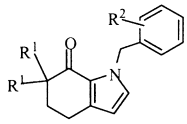

**Figure 1.** Overlap of CAVEAT-derived compound **3** with template peptide **I** (relaxed stereoview).

Systems composed of a core of two or three rings, or which could be simplified down to one or two rings without leaving the necessary vectors in a high-energy conformational state, were selected. These structures were then simplified by replacing saturated ring systems with aromatic systems to eliminate unnecessary stereocenters, especially when diequatorial substitution was present and needed to be retained. Hybridization and conjugation were also modified to affect the in-plane/out-of-plane conformation of particular functional groups. The initial targets were then examined for ease of synthesis, changes in the structures were made as required, and the compounds reanalyzed structurally to insure that reasonable overlap with the template was maintained. Based on these efforts, three series of molecules were selected for synthesis. The resulting compounds are shown in Table 1.

While these design efforts were fundamentally structure-based, the emphasis was placed on maximizing the diversity and synthetic accessibility of the different series of molecules, rather than on displaying all of the template features in any one compound. For instance, in Figure 1 the functionality associated with feature 1 of the pharmacophore was omitted to facilitate a simple synthesis of a compound class based on hydroxyproline, while retaining substitution for features 2 and 3, and adding the functionality of feature 4.

Nonpeptidic compounds were also designed from the peptide templates based on a direct graphical analysis. Novel organic linkers were created, each replacing a small region of the peptide structure, in a fashion to allow the display of some of the desired functional groups. The iterative process of modifying the initial design was similar to that employed with the CAVEAT-derived scaffolds. Three series of compounds were selected for synthesis, the results of which are shown in Table 2. The synthetic approaches to the molecules in Tables 1 and 2 are shown in Scheme 1, save for the benzodiazepine class of compounds. The SAR for these compounds was examined in greater detail, and these results will be discussed in a subsequent manuscript.[15]

**Table 2.** Model-derived bradykinin antagonists

| | | $R^1$ | $R^2$ | $K_d$(μM) ±S.E |
|---|---|---|---|---|
|  | **(11)** |  | $-(CH_2)_4NH_2$ | Inactive |
| | **(12)** |  | $-(CH_2)_4NH_2$ | 28±2.6 |
| | **(13)** |  | $CH_2NH_2$ | 101±12 |
| | **(14)** |  | $CH_2NH_2$ | 17±2.7 |
| | **(15)** | $(CH_2)_4NH_2$ | | 62±2.6 |
|  | **(16)** | $(CH_2)_4NBu_2$ | | 23±3.1 |
| | **(17)** |  | | 7.4±3.1 |
| | **(18)** |  | | 5.0±.29 |
|  | **(19)** | Bn | 3-$CH_2NH_2$ | 7.6±.3 |
| | **(20)** | Bn | 2-CO-Ornithine | Inactive |

Compounds were assayed for binding against the human bradykinin $B_2$ receptor expressed in CHO cells.[10] Each family yielded compounds with activity close or comparable to the smallest peptide lead. In addition, many families were found to contain both

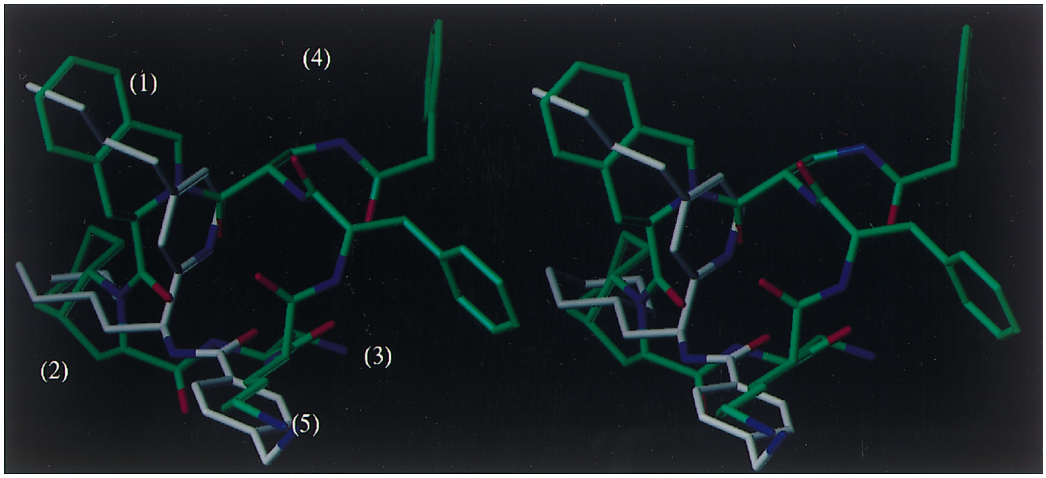
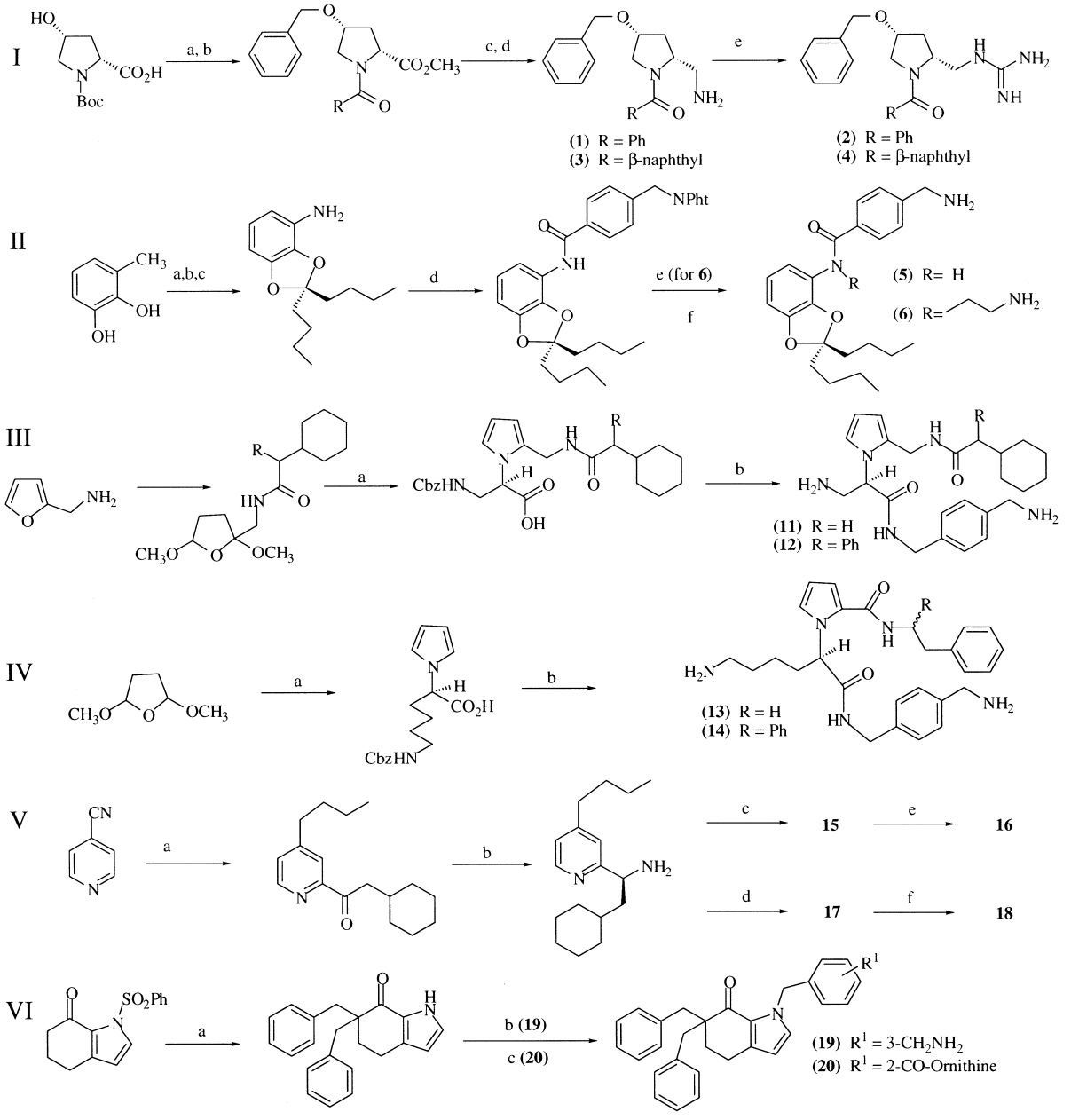



**Figure 2.** Overlap of compound **17** with template peptide **I** (relaxed stereoview).

active and inactive members, suggesting the presence of a nascent SAR. The general synthetic strategy utilized in this project allowed for the short synthesis of multiple members of each family, and has provided the basis for future synthetic efforts. Both design approaches employed in the study appeared to be equally successful, and the six families of antagonists together form a highly diverse set of compounds.

The design, synthesis, and assay phases of this project were completed with a modest allocation of resources over approximately nine months. These efforts yielded a variety of active compound classes with well-elaborated synthetic approaches. The results described herein highlight the benefits of a structure-based approach to new lead discovery of peptidomimetics that can complement existing screening techniques.




**Scheme 1.** Synthesis of bradykinin antagonists. I Reagents: (a) (i) NaH, (ii) benzylbromide, (iii) $SOCl_2$, MeOH; (b) RCOCl, $DCM/H_2O$; (c) $LiBH_4$, cat. B-OMe-9-BBN; (d) (i) $Ph_3P$, DEAD, *t*-$BuO_2CNHP(OEt)_2$, (ii) HCl, toluene; (e) $H_2NC(=NH)SO_3H$. II Reagents: (a) (i) 4-nonanone, TsOH, (ii) $KMnO_4$, pyridine, $H_2O$; (b) $(PhO)_2P(=O)N_3$, $PhCH_2OH$; (c) $H_2$, Pd/C; (d) (i) 4-(chloromethyl)benzoyl chloride, (ii) KNPht, DMF; (e) (i) NaH, (ii) $PhtN(CH_2)_3Br$; (f) $NH_2NH_2{\cdot}H_2O$, MeOH. III Reagents: (a) D-N-β-Cbz-diaminopropionic acid, HOAc, NaOAc, 100 °C; (b) (i) CDI, (ii) α,α′-diamino-*p*-xylene, (iii) $H_2$, Pd/C. IV Reagents: (a) D-N-ε-Cbz-lysine, HOAc, NaOAc, 100 °C; (b) (i) CDI, (ii) α,α′-diamino-*p*-xylene; (c) (i) Cbz-Cl, (ii) $COCl_2$, toluene; (d) (i) $PhCH_2CH(R)NH_2$, DCC, (ii) $H_2$, Pd/C. V Reagents: (a) (i) BuBr, Mg, ether, (ii) $H^+$, (iii) $NH_2NH_2$, KOH, (iv) $NaNH_2$, cumene, 150 °C, (v) $Br_2$, 48% HBr, $NaNO_2$, (vi) NaOH; (b) (i) *n*-BuLi, −78 °C, (ii) $C_6H_{11}CH_2CON(OCH_3)CH_3$, (iii) (*R*)-(−)-2-phenylglycinol, (iv) $H_2$, Pd/C, (v) $NaIO_4$, $H_2O$, THF; (c) (i) $BocNH(CH_2)_4CO_2H$, DCC, (ii) TFA; (d) (i) $BocNHCH_2(C_6H_4)CO_2H$, DCC, (ii) TFA; (d) *n*-PrCHO, $NaCNBH_3$; (e) $NH_2C(=NH)SO_3H$. VI Reagents: (a) (i) KH, (ii) $PhCH_2I$; (b) (i) NaH, (ii) 3-(phthalimidomethyl)benzylbromide, (iii) $NH_2NH_2{\cdot}H_2O$, MeOH; (c) (i) NaH, (ii) 2-(carbomethoxy)benzyl bromide, (iii) NaOH, (iv) N-∂-Boc-L-ornithine methyl ester, Pybop, (v) TFA, (vi) LiOH.

## References and Notes

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9. Three letter abbreviations for unnatural amino acids used in the text correspond to the following: Hyp, hydroxyproline; Thi, thienylalanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Oic, octahydroindole-2-carboxylic acid; Dap, 2,3-diaminopropionic acid; Dab, 2,4-diaminobutyric acid.
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12. Computational studies were carried out using Sybyl 5.5 (Tripos, St Louis, MO) and the Tripos and AMBER 3.0 force fields (for nonpeptide and peptidic compounds, respectively). The in vacuo molecular dynamics simulations were carried out using charges scaled to 10%, and a 4*r dielectric constant. Parameters and charges for the nonstandard peptide residues were developed as needed, and are available on request.
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15. Benzodiazepine-based bradykinin antagonists with quite different substitution patterns have recently also been disclosed, see ref 5.