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ACE INHIBITORS AS A TEMPLATE FOR THE DESIGN OF BRADYKININ B_2 RECEPTOR ANTAGONISTS

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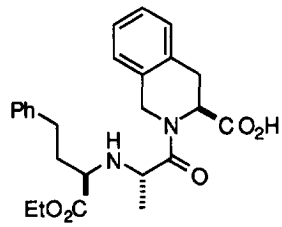
Abstract: Angiotensin converting enzyme (ACE) degrades both angiotensin II and bradykinin. Accordingly, we hypothesize that ACE inhibitors can serve as models to design antagonists for the bradykinin receptor. The potent ACE inhibitor Quinapril was modified to serve as a spacer separating two lipophilic positive charges required for bradykinin binding. The synthesis and bradykinin receptor activity of a series of antagonists **2-10** based on this hypothesis is described in this report.

The nonapeptide bradykinin, RPPGFSPFR, is a tissue and plasma hormone released by the proteolytic action of kallikrein.[2] Antagonists of the bradykinin B_2 receptor have been suggested as potential therapeutic agents for the treatment of pain and inflammation.[3] Potent and selective bradykinin receptor peptide antagonists are documented.[4] However, with the exception of our laboratories,[5] there are no reports of nonpeptide antagonists active in both bradykinin radioligand binding and functional assays. The SAR for peptide receptor antagonists demonstrates a crucial requirement of an aromatic residue at position 8 or an aromatic residue with the (D)-configuration at position 7 for high affinity binding. Anneal molecular dynamics modeling of the conformation of bradykinin suggests there is a positive charge separation for the two terminal arginines of approximately 10 Å.[6]

Angiotensin-converting enzyme (ACE) degrades bradykinin by cleaving it at the Pro^7-Phe^8 amide bond.[5] This suggested to us that ACE inhibitors may display features or conformations similar to those of bradykinin at this cleavage site enabling them to bind to the bradykinin receptor. To test this hypothesis, we modified the ACE inhibitor Quinapril[8] **1** by introducing two end-terminal lipophilic positive charges, removing the carboxyl function from the phenylbutyroyl fragment, while retaining the tetrahydroisoquinoline (Tic) residue.[9] These modifications to **1** led to a new series of compounds **2-10** which display bradykinin receptor



1 (Quinapril)

antagonist activity. The synthesis of these compounds starting from (±)-Tic ethyl ester **11** is presented in Scheme I.[10] The corresponding receptor binding affinities at the human IMR-90 bradykinin B_2 receptor[11] are presented in Table I.

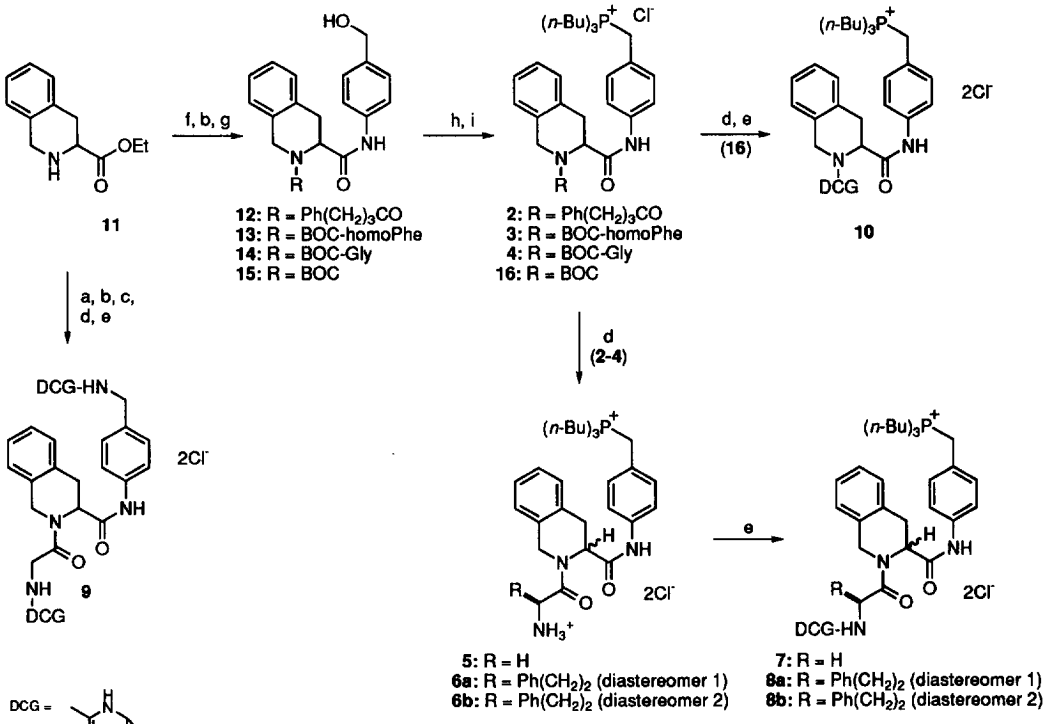
One of the first compounds prepared in this series was the phosphonium salt **2**. This inhibitor, containing a phenylbutyryl group on the Tic nitrogen and a single positive charge as linked through the Tic carboxylate, exhibits a K_i = 10 μM. In an attempt to enhance binding affinity by extending the phenyl ring further from the Tic nucleus (more analogous to **1**), the phenylbutyryl group was replaced with a BOC-homoPhe residue (**2** → **3**). This modification did not lead to a significant decrease in K_i. The importance of the phenylalkyl group for binding was demonstrated as the BOC-Gly analogue **4** is much less active than inhibitor **3** (K_i = 8 μM for **3** versus 18% inhibition at 10 μM for **4**).

Incorporation of a second positive charge into **2** was then carried out to more closely mimic the Arg^1 and Arg^9 residues present in bradykinin. This led to the synthesis and HPLC separation of diastereomers **8a** and **8b**. As anticipated, these compounds possess binding affinities some 8-fold higher than the mono-charged analogue **2** (**8a**: K_i = 1.0 μM; **8b**: K_i = 0.8 μM). Compounds **8a** and **8b** are true receptor antagonists since they dose-dependently inhibit Lys-bradykinin stimulated $^{45}Ca^{2+}$ efflux from IMR-90 cells.[11] The compounds do not demonstrate agonist activity in this system at a concentration of 100 μM (data not shown).

By analogy with the inhibitor pair **3** and **4**, removing the phenylalkyl moiety in antagonists **8a,b** affords a weakly binding inhibitor **6** (K_i >10 μM). The inactivity of **9** and **10** may also be due to the absence of this appendage. Finally, the fact that amines **6a,b** have decreased receptor affinity versus **8a,b** is consistent with the design requirement of a diffuse, lipophilic positive charge rather than a point positive charge for potent antagonist activity.[5a]

These data demonstrate that it is possible to design bradykinin B_2 receptor antagonists based on the structure of the ACE inhibitor Quinapril. Receptor binding in this limited series **2-10** is observed with compounds having two lipophilic charges separated by approximately 10 Å with homoPhe-Tic residues. The novel antagonists **8a,b** embody these structural features.

Scheme I. Synthesis of Quinapril-Based Bradykinin B_2 Receptor Antagonists **2-10**



Reagents and conditions: (a) BOC-Gly, HBTU, $(iPr)_2EtN$, CH_3CN, 6 h, 0 °C to 25 °C, 82%; (b) LiOH, THF-H_2O (4:1), 5 h, 65 °C, 86%; (c) *p*-amino-*t*-butylbenzylcarbamate, HBTU, $(iPr)_2EtN$, DMF, 16 h, 25 °C, 83%; (d) 2 M aq HCl-THF (1:1), 12 h, 25 °C, 95%; (e) dicyclohexylcarbodiimide, MeCN, 7 days, 25 °C, 36%; (f) RCO_2H, HBTU, $(iPr)_2EtN$, DMF, 72 h, 25 °C, or as in the case of **15**, $(BOC)_2O$, $(iPr)_2EtN$, DMF, 24 h, 25 °C, 75%; (g) *p*-aminobenzyl alcohol, HBTU, $(iPr)_2EtN$, DMF, 16 h, 25 °C, 100%; (h) MsCl, collidine, DMF, LiCl, 16 h, 0 °C to 25 °C, 100%; (i) $(n\text{-}Bu)_3P$, toluene, 48 h, 120 °C, 92%. HBTU = O-benzotriazolyl-N, N, N', N'-tetramethyluronium hexafluoro-phosphate: Dourtoglou, V.; Gross, B. *Synthesis* **1984**, 572.

Table I. Bradykinin B_2 Receptor Affinities[12]

Compd no.	% Inhibition (10 μM)	K_i (μM)	Compd no.	% Inhibition (10 μM)	K_i (μM)
2	-[a]	10	**7**	33	-
3	-	8	**8a**	-	1
4	18	-	**8b**	-	0.8
5	36	-	**9**	12	-
6a	26	-	**10**	34	-
6b	26	-			

[a]In cases where the % inhibition was <50% at 10 μM, the K_i was not determined. Similarly, where >50% inhibition was observed, only the K_i is reported.

References and Notes

1. Present address: Diagnostic Imaging, Sterling Winthrop Pharmaceutical Research Division, 1250 S. Collegeville Road, P.O. Box 5000, Collegeville, PA 19426-0900.

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9. Removal of the carboxyl function would be expected to eliminate zinc binding, leading to weak ACE inhibition.

10. All new compounds exhibited physical and structural properties consistent with their structure.

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12. The binding affinities for each compound were determined in [^{3}H]-bradykinin binding studies using human IMR-90 fetal lung fibroblasts as previously described.[11] The presented data are means of duplicate determinations for either percent inhibition of binding at a single test concentration (10 μM) or true ligand binding affinities (K_i) calculated from IC_{50} values determined from competitive inhibition dose response curves.

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