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THE DESIGN OF NON-PEPTIDE HUMAN BRADYKININ B_2 RECEPTOR ANTAGONISTS EMPLOYING THE BENZODIAZEPINE PEPTIDOMIMETIC SCAFFOLD

Edward K. Dziadulewicz*[,a], Michael C. Brown[a,†], Andrew R. Dunstan[a,‡], Wai Lee[a], Najeeb B. Said[b,§] and Peter J. Garratt[b]

[a]*Novartis Institute for Medical Sciences, 5 Gower Place, London WC1E 6BN, U.K.*
[b]*Department of Chemistry, University College, 20 Gordon Street, London WC1H 0AJ, U.K.*
Current Addresses:-
†*NEN Life Science Products, DuPont UK Ltd., Wedgewood Way, Stevenage, Hertfordshire, SG1 4QN, U.K.*
‡*Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex, RH12 4AB, U.K.*
§*Instituto de Quimica Organica, CSIC, Juan de la Cierva 3, E-28006, Madrid, Spain.*

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Abstract: The Bradykinin B_2 receptor antagonist HOE 140 (D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg) has been used as a template for the *de novo* design and synthesis of a small number of non-peptide lead compounds based on the 1,4-benzodiazepin-2-one framework. Two of the compounds have been found to exhibit moderate K_i values of 8.9 and 9.2 μM at the human Bradykinin B_2 receptor.

Elevated levels of the endogenous hormonal nonapeptide Bradykinin (BK; Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]-Ser[6]-Pro[7]-Phe[8]-Arg[9]) have been implicated in numerous pathophysiological processes[1] many of which are believed to be mediated by the B_2 class of BK receptors. An antagonist of the BK B_2 receptor would therefore offer great potential for therapeutic intervention, particularly in pain and inflammation.[2,3]

There has been significant progress in the development of potent peptidic B_2 antagonists, the best example being HOE 140 (Icatibant; D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Thi[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]) a second-generation antagonist from the Hoechst group.[4] However, the well-documented disadvantages of peptides as therapeutic agents[5] have prompted a search for low-molecular weight non-peptide antagonists,[6] with superior oral bioavailability and metabolic stability properties.

A rational approach toward the *de novo* design of a non-peptide antagonist may be envisaged if the biologically relevant conformation of a peptide antagonist were known. The conformations of HOE 140[7] and BK[8] in a membrane-like surrounding have been determined *via* NMR spectroscopy in SDS micelles, and are characterized by C-terminal β-turns comprising residues 6-9. The β-turn is a structural motif that has been postulated in many other cases for the biologically active form of linear peptides.[9] As a putative recognition element,[10] it can be assumed that the precise spatial orientation of the side chains is important in the presentation of pharmacophoric information to the receptor.[11]

* Corresponding author. Email: ed.dziadulewicz@pharma.novartis.com; Fax: +44 (0)171 387 4116
Dedicated to Professor Dieter Seebach on the occasion of his 60th birthday

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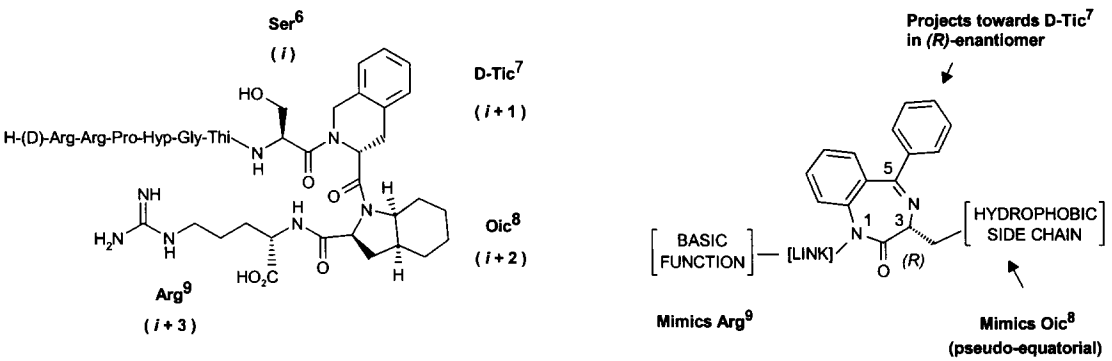
With its similar molecular dimensions, the benzodiazepine (BZD) nucleus has been shown to be a good mimetic of a number of β-turn types,[12] and often incorporates a second aromatic ring (in effect, an embedded diphenylmethyl or benzhydryl group). In the literature, there is precedent for a three-point binding model for small molecule interactions with peptide receptors,[13] and Ariens has proposed[14] that one of these sites recognizes the "double-ring" motif exemplified by the benzhydryl group, which is often conformationally constrained from intramolecular hydrophobic clustering or hydrophobic collapse.[15] Coupled with the fact that, as a drug class, BZDs are known to have good oral bioavailability and to be well tolerated, we determined to combine these observations and herein report our initial results in the *de novo* design of novel BZD-based lead compounds displaying affinity for the human BK B_2 receptor.

In the design of their β-turn peptidomimetic scaffold, Ripka, De Lucca, and co-workers[12] placed the benzodiazepinone core in the (i + 2)th corner of a number of β-turn types. Acting as an internal β-turn mimetic,[16] the BZD core is then able to radiate its substituents along the corresponding peptide side chain trajectories, with the lactam moiety more or less superimposed onto the peptide bond between amino acid residues i + 2 and i + 3. Figure 1 illustrates the overlay with respect to our peptide template, HOE 140, and highlights the amino acid residues we chose to mimic assuming that this conformation is maintained in the receptor-bound state.[17]



HOE 140 C-terminus β-turn

Fig.1. *Peptidomimetic design rationale.*

By utilizing the phenyl substituent at C5 as the i + 1 binding determinant (retaining the "double-ring" motif[14]), the subsequent introduction of additional functionality would be immediately facilitated. A C3-hydrophobic mimic of the i + 2 residue would easily be incorporated by use of an appropriate amino acid, whereas a mimic of the Arg[9] side chain could conceivably be elaborated from N1 by alkylation with a suitable electrophile. Alternatively, the i + 3 position could comprise a carboxylic acid function in accordance with peptide structure-activity requirements.[18]

The correct orientation of the BZD substituents is also an important design aspiration. In order to accommodate the C3-substituent in a *pseudo*-equatorial position,[12] the seven-membered ring is puckered in such a way that the C5-substituent is projected on one side of the lactam plane in the (*S*)-enantiomer and on the opposite side in the antipode. Only the (*R*)-BZD enantiomer is revealed by molecular modelling to be capable of maintaining all three binding determinants in the appropriate positions. Despite the aforementioned stereochemical bias in this paradigm, it was prudent and expedient to evaluate racemic mixtures at the outset of the lead finding process. In this way the (*S*)-enantiomers would not be disqualified from revealing any antagonistic activity arising from an alternative mode of binding. Finally, the benzyl side chain of phenylalanine was initially chosen as the C3-substituent. Use of an aromatic amino acid to represent the residue Oic[8], an aliphatic analogue of proline, was deemed acceptable given the occurrence of the former at the corresponding $i + 2$ position in the first-generation BK antagonists.[19]

Racemic 1,4-benzodiazepin-2-one compounds **3**, **4b-c**, **5b-c**, **7** and **9** were synthesized according to Schemes 1 and 2. In order to mimic the Arg[9] basic side chain of HOE 140, a four-carbon atom chain length was maintained for the distance separating the BZD lactam nitrogen atom and the basic terminal group. Table 1 summarizes the binding affinities of this initial set of compounds against the human BK B_2 receptor expressed as K_i values.

Table 1. Human Bradykinin B_2 Receptor Affinities (K_i) of BZD Ligands.[a]

compd.[b]	K_i (μM)	compd.[b]	K_i (μM)	compd.[b]	K_i (μM)
3[c]	>100	**5b**	>100	**(*S*)-5c**[e]	15.8 ± 7.6
4b	(26 ± 5)[d]	**5c**	8.9 ± 4.3	**7**	>100
4c	21.3 ± 4.0	**(*R*)-5c**[e]	11.9 ± 3.8	**9**	9.2 ± 5.4

[a] [^{3}H]-BK binding assay. K_i values were determined from concentration-response curves at the human B_2 BK receptor (membranes from Cos-7 cells expressing the human B_2 receptor). The values shown are the mean ± s.e.m. ($n \geq 3$).

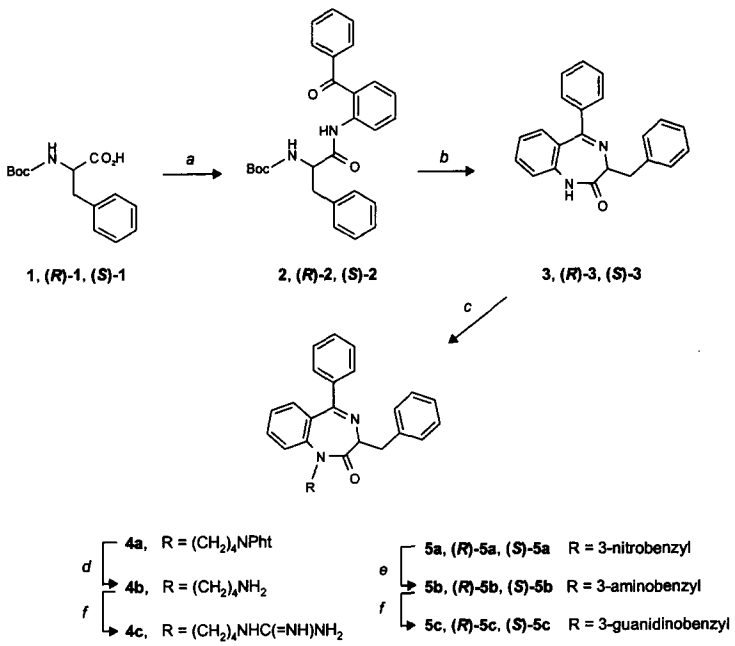
[b] All the compounds gave satisfactory ^{1}H NMR, mass spectra, and elemental microanalyses.

[c] Apart from **3**, all compounds were isolated as amorphous, TFA-containing solids after preparative HPLC.

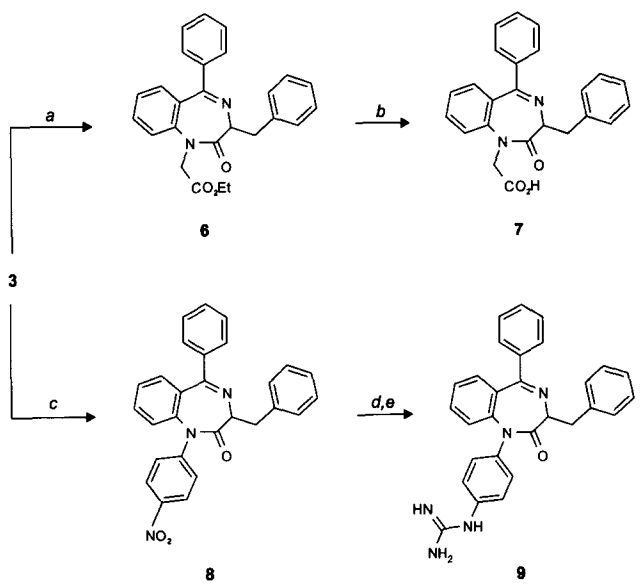
[d] Figure in parentheses is the % inhibition at 10 μM (in this case the K_i was not determined).

[e] **(*R*)-5c**: $[\alpha]_D$ = +8.9° (c = 1.007 in MeOH); **(*S*)-5c**: $[\alpha]_D$ = -8.7° (c = 1.01 in MeOH).

Modest B_2 receptor affinity was revealed for this compound class once a basic side chain was appended to the BZD core (compounds **4b** and **4c**). Replacement of the flexible butyl linker chain with the correspondingly constrained benzyl one abolishes activity completely (compound **5b**), presumably due to the reduced basicity of the terminal nitrogen atom which is rendered anilinic in this example. Elaboration of the aniline **5b** to the guanidine **5c** enhances inhibitory potency, although there is still little to distinguish the relative affinities of **4c**



Scheme 1. *Reagents and conditions:* (a) (i) *N*-methylmorpholine, THF, -15°C, isobutyl chloroformate, (ii) 2-aminobenzophenone; (b) (i) HCl(g), EtOAc, 0°C, (ii) MeOH, 1M NaOH; (c) (i) *t*-BuOK in THF (1M), THF, 0°C, N_2, (ii) *N*-(4-bromobutyl)phthalimide (for **4a**) or 3-nitrobenzyl bromide (for **5a**, **(*R*)-5a**, **(*S*)-5a**); (d) H_2NNH_2, EtOH; (e) $SnCl_2.2H_2O$, EtOAc, 70°C, 16h; (f) (i) *N,N'*-di-Boc-thiourea, $HgCl_2$, DMF, 18h, (ii) TFA, CH_2Cl_2.



Scheme 2. *Reagents and conditions:* (a) (i) *t*-BuOK in THF (1M), THF, 0°C, N_2, (ii) ethyl bromoacetate; (b) 1M KOH, EtOH, rt; (c) (i) 1-iodo-4-nitrobenzene, Cu powder, NaOAc, DMF, 150°C, 4h, (ii) 20h, rt; (d) $SnCl_2.2H_2O$, EtOAc, 70°C, 16h; (e) (i) *N,N'*-di-Boc-thiourea, $HgCl_2$, DMF, 20h, (ii) TFA, CH_2Cl_2.

and **5c**, despite the greater entropic penalty one might expect for the former. According to peptide structure-activity relationships, the presence of a free C-terminal carboxylic acid function is essential for high-affinity binding of the peptide ligands.[18] However, this observation did not extrapolate to a workable design feature in the present series of compounds (compound **7**), although other chain lengths would have to be assessed before discounting this avenue altogether.

Compound **9** represents the most conformationally restricted example, and is accorded a similar binding affinity to that of **5c** (9.2 μM and 8.9 μM respectively) suggesting a common orientation of the basic moieties in their interaction with complementary receptor components. Analysis of **5c** in a $^{45}Ca^{2+}$ efflux functional assay (SK-N-SH human neuroblastoma cells) revealed that this compound, active in a radioligand binding assay, was also able to antagonize the functional response to BK, with an IC_{50} of 2.3 ± 1.1 μM.

As mentioned earlier, adherence to the overlay model should manifest itself as preferential accommodation of one enantiomer, the higher *in vitro* activity expected to reside in the (*R*)-form. The individual enantiomers of **5c** were synthesized from (*R*)- and (*S*)-*N*-Boc phenylalanine (Scheme 1), monitoring stereochemical integrity by chiral HPLC. Unfortunately, **(*R*)-5c** and **(*S*)-5c** had similar affinity profiles for the B_2 receptor (Table 1), suggesting that the amino acid side chains are not differentially perceived by the immediate binding site environment. The degree of activity of **5c**, **(*R*)-5c** and **(*S*)-5c** is more reminiscent of a two-point binding interaction, and indicates that the spatial relationship between the β-turn-mimicking side chains is not optimal. It therefore remains an important objective of ours not only to advance *in vitro* activity, seeking an improvement in the eudismic ratio as confirmation of the current design requirements, but also to ascertain the selectivity of this compound class at a number of other receptors.[20]

We have successfully designed novel human Bradykinin B_2 receptor lead compounds (**5c** and **9**) using a generalized BZD scaffold as a β-turn mimetic. Although different G protein-coupled receptor antagonists may bind in different receptor regions, the presence of the guanidine residue and the hydrophobic BZD core bears a structural resemblance to the positively charged terminal arginine residue (Arg^9) and the prominent hydrophobic residues (D-Tic^7 and Oic^8) in HOE 140 which we have used as a template in the lead finding process.[21] In describing the direct conversion of a second generation peptide antagonist into a non-peptide lead,[22] this communication provides another example of certain "privileged structures" furnishing useful leads in the search for new receptor-binding ligands. Encouraged by these early results, further evaluation of various side chains and the BZD core architecture has been pursued and will form the basis of a future publication.

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