Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2589–2594

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PD 176252 - The First High Affinity Non-peptide Gastrin-Releasing Peptide (BB_2) Receptor Antagonist

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Received 26 June 1998; accepted 6 August 1998

Abstract : In this paper we describe the development of a novel series of non-peptide, "balanced" neuromedin-B preferring (BB_1)/gastrin-releasing peptide preferring (BB_2) receptor ligands as exemplified by PD 176252. PD 176252, which exhibits nanomolar affinity for both the BB_1 (K_i=0.15nM) and BB_2 (K_i=1.0nM) receptors, has been demonstrated to be a competitive antagonist at these bombesin receptor subtypes.

INTRODUCTION

We have previously reported on a novel class of high affinity, non-peptide neuromedin-B preferring (BB_1) receptor selective antagonists.[1] These compounds were developed *via* the application of a "peptoid" drug design strategy[2] that had previously proved successful in the identification of non-peptide ligands for cholecystokinin[3] and tachykinin receptors.[4] The basic premise behind the peptoid structure-based approach to drug design is the development of non-peptide receptor ligands starting from, and utilising the information contained within, the endogenous peptide of the targeted receptor. Figure 1 highlights how we were able to identify the novel non-peptide BB_1 receptor selective antagonist PD 165929 by employing such a methodology.[1]

In this paper we describe how we have been able to develop the SAR of the BB_1 receptor ligand PD 165929 (**1**) to yield related ligands with nanomolar affinity for both BB_1 and BB_2 bombesin receptor types.

BOMBESIN, NEUROMEDIN-B AND GASTRIN-RELEASING PEPTIDE

The amphibian tetradecapeptide bombesin (BB) belongs to a class of peptides that share structural homology within their C-terminal sequences.[5] The decapeptides neuromedin-B (NMB) and neuromedin-C (NMC) and a 27 residue amino acid, gastrin-releasing peptide (GRP), are the three mammalian bombesin-like peptides to have thus far been identified.[6] NMB and GRP peptides are believed to mediate a variety of peripheral and centrally mediated

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0960-894X/98/$ - see front matter
PII: S0960-894X(98)00462-4

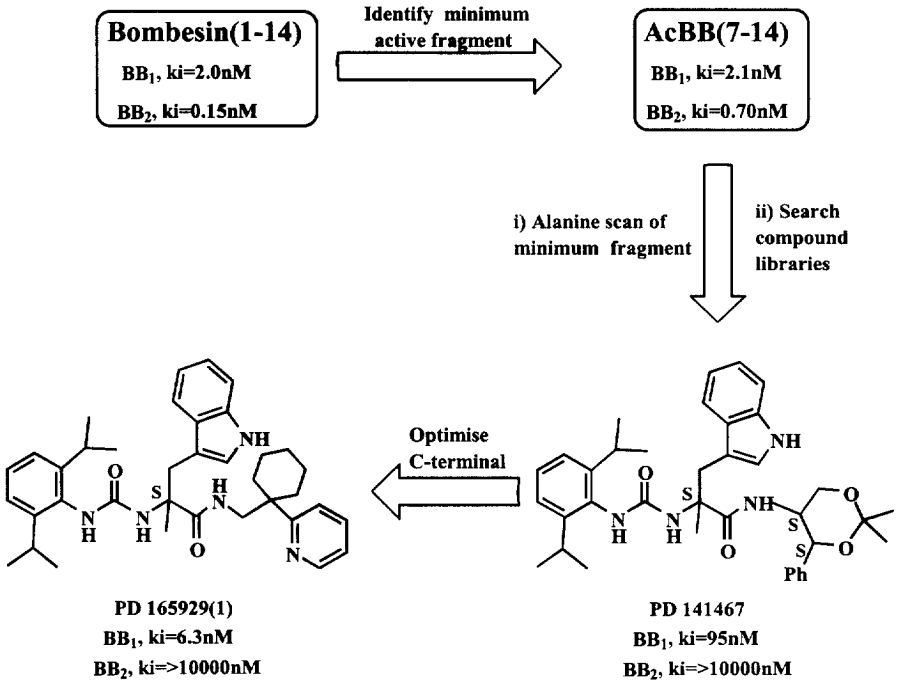


Figure 1. Development of the high affinity, non-peptide BB_1 receptor selective antagonist PD 165929(1). See reference 1 for further details.

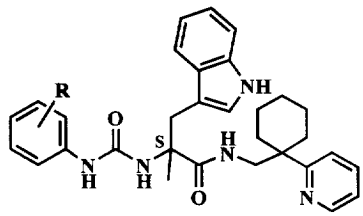
biological actions such as on autocrine growth, satiety, thermoregulation, stereotyped behaviour, stress and anxiety/depression by acting upon the corresponding NMB-preferring (BB_1) and GRP-preferring (BB_2) receptors.[7] More recently the existence of BB_3 and BB_4 receptors has been proven although their endogenous ligands remain to be identified.[8,9] Despite the development of a variety of peptide-based selective ligands for both the BB_1, BB_2 and BB_3 receptors,[10] the precise physiological roles of these neuropeptides remains unclear most likely as a consequence of the lack of high affinity non-peptide bombesin antagonists.[11]

RESULTS AND DISCUSSION

i) N-Terminal SAR:

Optimisation of the "C-terminal" segment of the initial non-peptide lead PD 141467 (see figure 1) was sufficient in itself to yield the nanomolar affinity BB_1 receptor selective lead PD 165929 (**1**).[1] However, we hoped to further improve upon the BB_1 receptor affinity of this compound by investigating the "N-terminal" SAR of this class of molecule. A selection of compounds prepared as part of this study are listed in table I. The SAR of the initial compounds in this series was developed in accordance with the Topliss decision tree scheme.[12] Initially encouraged by the minimal fall in BB_1 receptor affinity recorded in going from the diisopropylphenyl substituted parent (**1**; BB_1 K_i=6.3nM) to its unsubstituted derivative (**2**; BB_1

Table I : SAR of N-Terminal Diisopropyl Phenyl Moiety of PD 165929 (1).



Compound No.	R	BB_1, K_i(nM)[a]	BB_2, K_i(nM)[a]
1	2,6-Diisopropyl	6.3	>10000
PD 165929			
2	H	27	1096
3	4-Cl	2.8	149
4	3,4-diCl	2.1	85
5	4-CF_3	2.9	124
6	4-NO_2	0.15	17
PD 168368			
7	4-iPr	9.4	273
8	4-CN	0.32	37
9	3-NO_2	0.97	85
10	2-NO_2	184	1254
NMB		0.068	56
GRP		9.1	0.040
Bombesin		2.0	0.15
AcBB(7-14)		2.1	0.70

a) Values shown represent the geometric mean of at least 3 separate experiments carried out using [^{125}I][Tyr^4] bombesin to label cloned human BB_1 or BB_2 receptors stably expressed in CHO cells.[13]

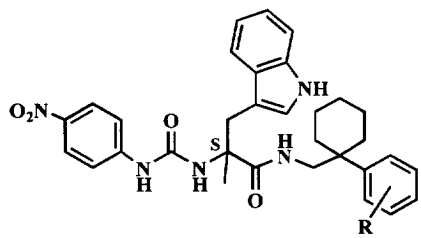
K_i=27nM), we were further heartened by the increase in affinity exhibited by the *para*-chloro derivative **3** (BB_1 K_i=2.8nM). Perhaps even more interesting was the discovery that this latter compound showed significant affinity for the BB_2 receptor type (**3**; BB_2 K_i=149nM) which was the first example from this class of compound to show any appreciable affinity for this receptor subtype. As a consequence of the *meta,para*-dichloro derivative **4** having only comparable BB_1 receptor affinity (K_i =2.1nM) to its corresponding mono-substituted precursor **3** (K_i=2.8nM), the *para*-trifluoromethyl (**5**) and *para*-nitro (**6**) derivatives were subsequently prepared as dictated by the Topliss scheme. Of these two latter compounds the *para*-nitro derivative (**6**; BB_1 K_i=0.15nM, BB_2 K_i=17nM) was clearly superior with respect to receptor affinity at both of the bombesin receptor types. The marked increase in both BB_1 and BB_2 receptor binding affinities may conceivably be a consequence of either the electron deficiency of the phenyl ring, the potential for the nitro moiety to be an H-bond acceptor or a more straightforward steric interaction. The markedly lower affinities exhibited by the isosteric *para*-isopropyl (**7**, BB_1 K_i=9.4nM) and electron withdrawing *ortho*-nitro (**10**, BB_1 K_i=184nM) derivatives together with

the comparable receptor binding profile witnessed with the *para*-cyano derivative (**8**, BB_1 K_i=0.32nM), suggests that an electron withdrawing, H-bond accepting *para*-substituent is necessary for optimal affinity at both bombesin receptor types.

ii) C-Terminal SAR:

Having optimised the N-terminal SAR to yield subnanomolar affinity BB_1 receptor selective ligands (>100-fold selective for BB_1 receptor) such as PD 168368 (**6**), we next turned our attention toward optimising the C-terminal. In this study our principal objective was to improve upon the BB_2 receptor affinity exhibited by PD 168368 (**6**) and related compounds. Table II lists a selection of compounds that were prepared as part of this study. All of the compounds synthesised were substituted phenyl rather than pyridyl derivatives due to the relative ease of synthesis. The derivatives prepared included examples of aryl *para*-substituents that were H-bond donating/acidic (**12**), H-bond accepting (**13, 16**), basic (**14**), alkyl (**15**), electron withdrawing (**13**) and electron donating (**16**) in nature. Optimal amongst these with respect to BB_2 receptor affinity was the H-bond accepting/electron donating *para*-methoxy substituted derivative **16** (BB_2 K_i=3.7nM). As a consequence of the increase in BB_2 receptor affinity seemingly induced by the *para*-methoxy substituent, the corresponding *para*-ethoxy (**17**), *ortho*-methoxy (**18**) and *para, meta*-methoxy (**19**) derivatives were prepared. However, none of these compounds were able to improve upon the excellent BB_2 receptor affinity exhibited by the *para*-methoxy derivative **16** (see table II). On the basis of these data the *para*-methoxy-2-pyridine derivative was subsequently prepared. This compound (PD 176252, **20**) proved to be optimal displaying a BB_2 receptor affinity of 1nM whilst retaining subnanomolar BB_1 receptor affinity (BB_1 K_i=0.17nM).

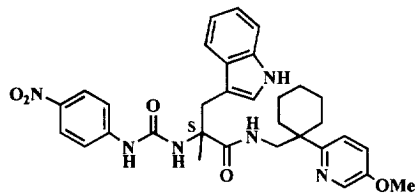
Table II : C-Terminal Derivatives of PD 168368 (6).



Compound No.	R	BB_1, K_i(nM)	BB_2, K_i(nM)
11	H	0.39	89
12	4-OH	0.62	32
13	4-NO_2	0.30	6.4
14	4-NMe_2	0.52	9.0
15	4-iPr	1.5	35
16	4-OMe	0.46	3.7
17	4-OEt	0.59	5.8
18	2-OMe	3.4	404
19	3,4-DiOMe	0.44	33

In vitro functional assays demonstrate that compound **20** (PD 176252) acts as a competitive antagonist at the human BB_1 and BB_2 receptors exhibiting $appK_B$ values in line with its binding affinities (Tables III).

Table III : Species Selectivity and *In Vitro* Functional Activity of 20 (PD 176252) at the BB_1 and BB_2 Receptor Type.



BB_1 Receptor Binding Affinities		In Vitro BB_1 Functional Assays	
Human	Rat[a]	Cytosensor[b]	$[Ca^{2+}]_i$[c]
Ki nM	IC_{50}, nM	$appK_B$, nM	$appK_B$, nM
0.17	0.66	4.0	2.3
(0.09-0.64)	(0.31-1.3)	(1.0-17)	(1.0-6.6)

BB_2 Receptor Binding Affinities		In Vitro BB_2 Functional Assays	
Human	Rat[a]	Cytosensor[b]	$[Ca^{2+}]_i$[c]
Ki, nM	IC_{50}, nM	$appK_B$, nM	$appK_B$, nM
1.0	16	13	36
(0.66-1.3)	(6.5-31)	(2.0-28)	(14-94)

a) Values shown represent the geometric mean of 3 separate experiments carried out using [^{125}I][Tyr^4] bombesin in the presence of [D-Phe^6] bombesin (6-13) ethyl ester to label BB_1 receptor binding sites in the rat olfactory bulb and in the presence of NMB to label BB_2 receptor binding sites in the rat cerebral cortex.[14]

b) Inhibition of acidification responses to NMB or NMC at the human BB_1 or BB_2 receptors expressed in CHO cells. Values (with ranges) are the means of at least 6 determinations.[15]

c) Inhibition of bombesin-evoked increases in intracellular calcium levels in CHO cells stably expressing human BB_1 or BB_2 receptors. Values represent the mean of at least three separate experiments.[16]

CONCLUSIONS

In this paper we have described the development of a novel series of high affinity, non-peptide BB_1/BB_2 receptor antagonists eg. PD 176252 (**20**). These compounds were developed from the previously published[1] BB_1 receptor selective ligand PD 165929 (**1**) which was identified *via* the application of a peptoid drug design strategy. To our knowledge these compounds represent the first known examples of non-peptide ligands that bind to the bombesin BB_1 and BB_2 receptor types with high affinity.

Detailed synthetic procedures and further *in vitro* and *in vivo* pharmacology on this class of compound will be published in full elsewhere.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. P. Daum, Mrs R. Franks and Mrs L. Webdale for their excellent technical assistance.

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