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DESIGN AND SYNTHESIS OF A TARGETED SET OF AROMATIC AMINO ACID DERIVATIVES FOR IDENTIFICATION OF NEW LEAD COMPOUNDS.

D.C. Horwell, C.O. Kneen*, M.C. Pritchard, G.S. Ratcliffe, E. Roberts, N. Suman-Chauhan K. Steiner# and H.J. Betche#

Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way Cambridge, CB2 2QB, U.K. and #Godecke AG, Mooswaldallee 1, D-79090 Freiburg, Germany.

Abstract: A targeted set of 256 aromatic α-amino acid derivatives is described to provide new lead compounds for biological screening. The utility is exemplified by identification of ligands for the human neuromedin B (hNMB) receptor, such as compound 3 (Ki hNMB 37nM).

The medicinal chemist has traditionally used a variety of approaches to identify new leads. The endogenous ligand for a target receptor may be used as the initial lead. Screening of natural products has provided new leads generally with novel chemical structures. More recently there has been considerable interest in generating libraries of chemically diverse structures for screening, including those generated by combinatorial chemistry. In all cases further chemical modification is generally required to obtain ligands with high affinity and selectivity which can be evaluated as potential drug candidates.

We have recently published the design of a dipeptide library[1] with the aim of utilising the library to screen at peptide receptors. This has proven successful as a means of generating moderately active (micromolar) leads which have been chemically modified to provide high affinity (nanomolar) ligands and potential new drugs. High affinity ligands for membrane bound receptors have been developed from protected dipeptides e.g. the CCK-A and CCK-B receptor antagonists PD 140548[2] and CI 988[3] respectively from Boc-Trp-Phe-NH_2, tachykinin ligands from N-benzyloxy-S-Trp-S-phenylalanide[4], and, in the enzyme field, the development of captopril from Ala-Pro[5]. We wished to extend this concept to design a set of diprotected amino acids. These

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e.mail: Clare.Kneen@camb.wl.com fax: (44) 1223 249106

compounds would contain three potential binding moieties, have no peptide bonds and low molecular weight. The amino acids were selected as racemic aromatic amino acids Trp, Phe, His and Tyr, because of the importance of these aromatic residues in binding[6,7]. They are linked as amides at the carboxylic acid terminus and as carbamates at the amino terminus. The potential binding moieties that are represented by the protecting groups have been selected from the side chains of the naturally occurring amino acids, by analogy to the previous dipeptide library[1]. The compounds have the general formula shown below (see table 1) making four sets of 64 compounds.

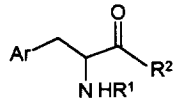


Table 1

R^1	Ar	R^2
(1) HO_2CCH_2OCO-		(9) -$NH(CH_2)_2CO_2H$
(2) $H_2N(CH_2)_4OCO$-		(10) -$NH(CH_2)_5NH_2$
(3) MeOCO-	3-indolyl	(11) -$NH(CH_2)_2OH$
(4) $PhCH_2OCO$-	4-imidazolyl	(12) -$NH(CH_2)_2Ph$
(5) Me_2CHCH_2OCO-	4-hydroxyphenyl	(13) -$NH(CH_2)_2CHMe_2$
(6) (3-indolyl)-$(CH_2)_2OCO$-	phenyl	(14) -$NH(CH_2)_2$-(3-indolyl)
(7) Me_2CHOCO-		(15) -$NHCH_2CHMe_2$
(8) (4-imidazolyl)-CH_2OCO-		(16) -$NH(CH_2)_2$-(3-imidazolyl)

Some modifications to the theoretically derived compounds were made for synthetic ease. The replacement of the serine side chain with the alanine side chain for the amine protecting group results in only a minimal change in the principal properties but provides compounds of much greater chemical stability. An additional methylene was introduced into the tryptophan side chain for the amine protecting group since the original target compounds proved to be unstable, but retention of a tryptophan mimic in the library was desirable. Histidine was added to the amino acids previously chosen for the dipeptide library to include a ligand with proton transfer capability into the dataset.

Compounds were synthesised using standard protecting group chemistry[8], and standard peptide coupling procedures[9]. This type of chemistry is eminently amenable to solid phase and further compounds could therefore be synthesised in a combinatorial fashion.

The compounds were screened in several neuropeptide receptor binding assays which all identified at least one compound with micromolar affinity (see table 2).

Table 2. Binding affinities of selected R^1-Trp-R^2 at neuropeptide receptors

Receptor	R^1	R^2	IC_{50} (μM)
NK_1	5	14	0.43
NK_2	4	12	3.27
NK_3	7	14	9.42
CCK-A	4	14	0.45
CCK-B	4	13	2.12

IC_{50} values represent binding to human NK_1 receptors expressed in human IM9 cells[4], NK_2 receptors in hamster urinary bladder[10], NK_3 receptors in CHO (chinese hamster ovary) cells[11], CCK-A receptors in rat pancreas[3] and CCK-B receptors in mouse cerebral cortex[3].

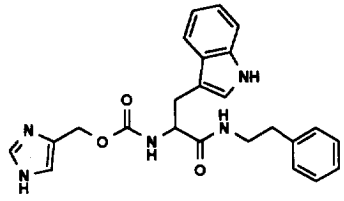
Table 3. Binding at Human NMB Receptors (K_i μM) for R^1-Trp-R^2

Trp	$R^2 = 9$	$R^2 = 10$	$R^2 = 11$	$R^2 = 12$	$R^2 = 13$	$R^2 = 14$	$R^2 = 15$	$R^2 = 16$
$R^1 = 1$	ns	ia	ia	ia	ia	ia	ia	ns
$R^1 = 2$	ia	ia	ia	ia	ia	**8.9**	ia	ia
$R^1 = 3$	ia	ia	ia	ia	ia	ia	ia	ia
$R^1 = 4$	ia	ia	ia	**2.6**	**6.1**	**2.8**	ia	ia
$R^1 = 5$	ia	ia	ia	**4.3**	ia	**4.7**	ia	ia
$R^1 = 6$	ia	**7.8**	ia	**1.1**	**2.7**	**0.98**	**3.5**	**5.0**
$R^1 = 7$	ia	ia	ia	ia	ia	ia	ia	ia
$R^1 = 8$	ns	ns	ns	**2.7**	**3.9**	**3.7**	ia	ns

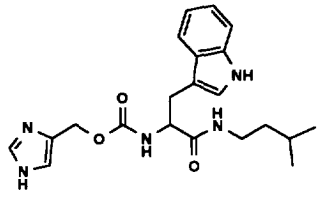
Note: ia represents binding affinity $K_i > 10\mu M$. ns represents compounds not synthesised.

We also routinely assayed compounds for binding to human neuromedin B (hNMB or BB_1) receptors expressed in CHO cells[12]. For the tryptophan set fifteen compounds with micromolar affinity were identified to serve as potential new leads for this receptor (table3).

These fifteen compounds e.g. compounds 1 and 2 (K_i 2.6 and 3.9μM respectively) provided initial leads.

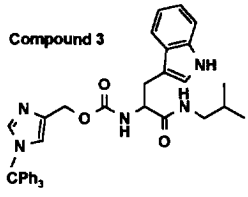


Compound 1



Compound 2

Based on the identification of these leads substructure searching of the Parke-Davis compound collection identified congeneric compounds. These were therefore assayed for human NMB receptor binding. From this study compound 3 was identified as a chemically novel example with high affinity (K_i 37nM) at the human NMB receptor.



Conclusions

We have described the design of a small set of aromatic α-amino acid derivatives. This set was used to identify lead compounds with micromolar affinity at several neuropeptide receptors. Chemical modification of the human NMB receptor lead provided a non-peptide high affinity ligand (K_i hNMB 37nM). This set therefore offers the potential to provide novel non-peptide leads for a range of receptor targets.

References and Notes

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8. Greene, T.W., "Protecting Groups in Organic Synthesis" Wiley Interscience: New York, 1981; pp. 152-187, 218-248 and 272-3. For example, isobutyl chloroformate (2.95g, 21.6mmol) was added to (RS)-tryptophan (4.08g, 20.0mmol) and sodium bicarbonate (5.04g, 60.0mmol) in water (100mls) and dioxan (100mls). After stirring at room temperature overnight the mixture was diluted with water and washed with ether. The aqueous phase was acidified and extracted with ethyl acetate. The extracts were washed (water), dried (magnesium sulphate), filtered and evaporated to give the required acid as a pale yellow solid (quantitative yield). A portion was recrystallised from ethyl acetate/ether to give colourless crystals mp 144-6°C; H NMR (400MHz, d_6-DMSO, 70°C. At 20°C two rotamers are observed.) δ 0.85(d, J=6.3Hz, 6H, Me_2), 1.70-1.90(m, 1H, CH), 3.00-3.25(m, obscured by water, $C\underline{H}_2$indole), 3.70(d, J=6.3Hz, 2H, CH_2), 4.20-4.30(m, 1H, CH), 6.98(t, J=7.6Hz, 2H, CH + CONH), 7.07(t, J=7.8Hz, 1H, CH), 7.14(s, 1H, CH), 7.34(d, J=8.1Hz, 1H, CH), 7.53(d, J=7.8Hz,1H, CH), 10.68(s, 1H, indoleNH), 12.4(bs, 1H, CO_2H). Anal. calc'd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.25; H, 6.62; N, 9.20.

9. The amine (1mmol) is added to a stirred mixture of the acid (1mmol), HBTU (2-(1H-Benzotriazolyl)-1,1,3,3-tetramethyl uronium hexafluorophosphate) (1mmol), and di-isopropylethylamine (2mmol) in DMF. After stirring overnight the mixture is either evaporated to dryness or diluted with ethyl acetate, washed as appropriate with sodium bicarbonate solution, dilute hydrochloric acid and water, and then dried, filtered and evaporated. The product is then purified by chromatography.
For example the acid obtained as above (Ref 8, 0.61g, 2.0mmol) was coupled with histamine dihydrochloride (0.37g, 2.0mmol) using HBTU (0.76g, 2.0mmol) and di-isopropylethylamine (1.09g, 8.4mmol) in DMF (50mls). After stirring overnight at room temperature the mixture was diluted with ethyl acetate and water. The organic phase was washed with water and sodium bicarbonate solution, dried (magnesium sulphate), filtered and evaporated. Purification by reverse phase chromatography gave the required product (0.30g, 38%) mp 85°C. 400MHz NMR (d_6-DMSO, two rotamers observed) δ 0.70(s) and 0.78(d, J=6.6Hz), and 0.79(d, J=6.6Hz, 6H, $CH(C\underline{H}_3)_2$), 1.72(h, J=6.6Hz, 1H, $CHMe_2$), 2.54(t, J=6.6Hz, 2H, CH_2), 2.84(dd, J=14.3, 9.4Hz,

1H, CHH-indole), 2.99(dd, J=14.4, 4.4Hz, 1H, CHH-indole), 3.15-3.25(m, obscured by water, CH_2), 3.55-3.65(m, 2H, CH_2), 4.10-4.20(m, 1H, CH), 6.6(OCONH one rotamer), 6.73(bs, 1H, NCHNH), 6.92(t, J=7.1Hz, 1H, CH), 7.00(t, J=7.6Hz, 1H, CH), 7.05-7.10(m, 2H, CH and OCONH), 7.27(d, J=8.1Hz, 1H, CH), 7.46(s, 1H, CH), 7.55(d, J=7.8Hz, 1H, CH), 7.98(bt, 1H, CONHCH$_2$), 10.73(s, 1H, indole NH). Anal calc'd for $C_{21}H_{27}N_5O_3.0.5H_2O$: C, 62.04; H, 6.94; N, 17.23. Found: C, 62.34; H, 6.80; N, 17.23.

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