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THE RATIONAL DESIGN AND SYNTHESIS OF NON-PEPTIDE 'RHEGNYLOGUES' OF CCK-26-33 - A NOVEL SERIES OF CCK-A SELECTIVE LIGANDS

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Abstract : A novel series of CCK-A receptor ligands has been prepared based on the appendage of modified amino acid side chain mimetics onto the α-carbon atom of tryptophan.

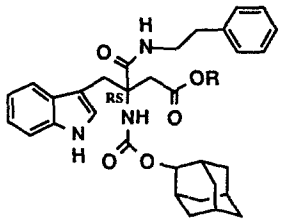
INTRODUCTION

An elusive goal for the medicinal chemist has been, and remains, the rational design of non-peptide topographical mimics ('peptoids')[1] of bioactive peptides. This objective becomes of special importance when the molecular recognition informational elements of the peptide of interest are rhegnylogically organised, i.e. in a discontiguous manner.[1,2] In this instance, pseudopeptidic analogue design, i.e. minor modifications of a linear, contiguous sequence of residues in the peptide, is unlikely to lead to an effective drug due to potential problems of poor oral bioavailability and metabolic degradation. Although the opiates are notable example of topographical mimics of the endorphins, there are very few rationally designed examples.[3] Several research groups have tackled this intriguing design problem by using various 'scaffolding' templates from which appropriate amino acid side chains have been appended. Examples of this template approach include the cyclohexane motifs of Farmer and Ariens[1] and Olson and colleagues,[4] the ß-D-glucose template of Nicolaou, Hirschmann and co-workers[5] and the ß-turn mimetics of Olson.[6] However, we considered the most simple and synthetically versatile template to be a single carbon atom.[1,2] This template also has an added potential advantage in allowing a significantly higher degree of conformational flexibility of the amino acid side chains than the above cited cyclic templates.

A recent example of the application of a single carbon atom template is the α,α-disubstituted tryptophan derivative (**1b**).[7] The α-carbon on the Trp residue represents the

single carbon atom template from which four side chains have been appended as a topographical mimic of the C-terminal octapeptide [CCK-26-33 (sulfated)] of the gut/brain peptide cholecystokinin.

This paper describes the synthesis and SAR of a series of related α,α-disubstituted Trp derivatives which have been shown to be a novel class of rationally designed CCK-A selective ligands.



1a R=CH_2Ph
1b R=H

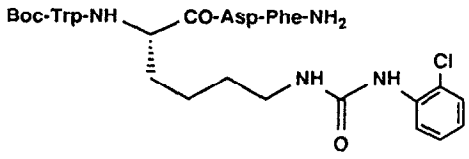


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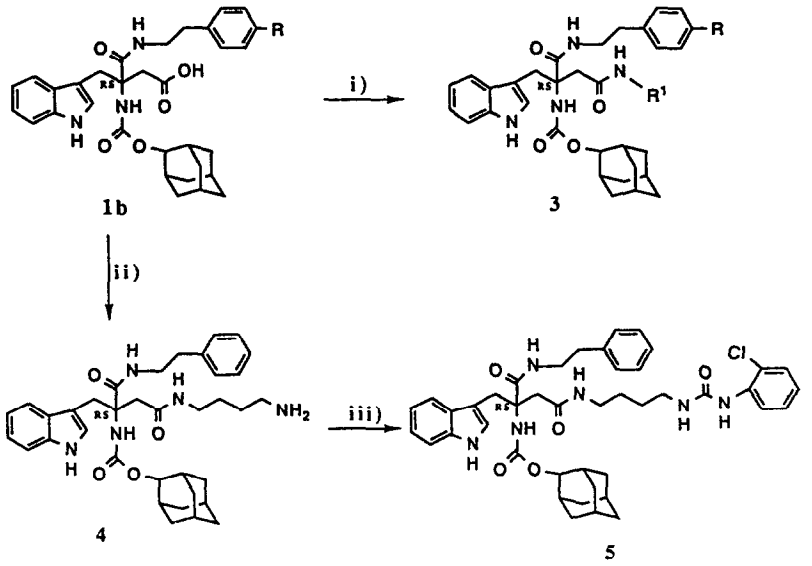
FIGURE

RESULTS AND DISCUSSION

The immediate synthetic precursor of the acid (**1b**) was the benzyl ester (**1a**).[7] This compound displayed submicromolar affinity for the CCK-A receptor subtype (IC_{50} = 0.7 μM) and was selective over the CCK-B receptor where it was inactive up to micromolar concentrations (Table). We have previously published[3,8] on the α-methyl tryptophan phenethylamide core of this molecule which was designed as a topographical mimic of the Trp and Phe residues of the CCK-B receptor selective CCK-30-33 fragment. As a consequence of the CCK-A receptor selectivity of (**1a**), we hypothesized that the phenyl ring of the benzyl ester ring may be accessing the same binding site as the aryl ring of the Tyr[SO_3H] residue of CCK-26-33. This Tyr[SO_3H] residue has often been cited as important for high CCK-A receptor affinity.[9] To further investigate this hypothesis we synthesized a series of derivatives of (**1a**) (Table and Scheme) where the length of the α-side chain has been varied and the ester replaced by a potentially, more metabolically stable, amide linkage.

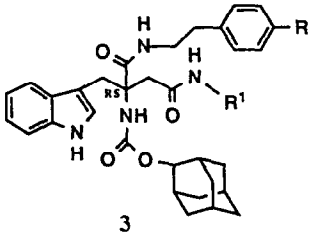
In increasing the side chain length from the phenylamide (**3a**) to the



SCHEME

Regents and Conditions : i, HOBt, DCCI, R^1NH_2, EtOAc; ii, Pentafluorophenol, DCCI, $H_2N(CH_2)_4NH_2$, EtOAc, O°C, iii, *o*-chlorophenylisocyanate, EtOAc, O°C.

Table. CCK-A and CCK-B Receptor Binding Affinities of α,α-Disubstituted Tryptophan Derivatives.



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No	R	R^1	IC_{50}, μM[14] CCK-A	CCK-B	B/A ratio
1a	-	-	0.70	IA at 10^{-6}M	>14
3a	H	Ph	0.23	0.81	3.5
3b	H	CH_2Ph	2.7	8.2	3.0
3c	H	$(CH_2)_2Ph$	0.14	2.2	16
3d	H	$(CH_2)_3Ph$	1.4	13	9.3
3e	H	$(CH_2)_2Ph$-4-OH	3.1	1.3	0.42
3f	H	$(CH_2)_2Ph$-4-OSO_3H	0.19	0.55	2.9
3g	OH	$(CH_2)_2Ph$	0.057	0.54	9.5
3h	OSO_3H	$(CH_2)_2Ph$	1.2	0.33	0.28
5	-	-	0.075	18	240
2	-	-	0.0038	1.9	500
R-Lorglumide			0.05	3.0	60
Devazepide[15]			0.1 nM	31 nM	310
CCK-26-33(S)			0.1 nM	0.3 nM	3.0

phenylpropylamide (**3d**) it is apparent that the phenethylamide (**3c**) is the optimum compound for CCK-A receptor affinity (IC_{50} = 0.14 μM) and selectivity. We subsequently synthesized the corresponding tyramine (**3e**) and sulfated tyramine (**3f**) derivatives in an attempt to more closely mimic the sulfated Tyr residue in CCK-26-33 and further test our hypothesis. Although the sulfated derivative (**3f**) is tolerated by the CCK-A receptor (IC_{50} = 0.19 μM), it remains unclear whether this side chain mimics the corresponding Tyr residue in CCK-26-33 since (**3f**) has only similar affinity to the parent unsubstituted phenethylamide (**3c**).

In order to test whether the C-terminal phenyl ring is contributing to the enhanced CCK-A affinity of (**3c**), we prepared the corresponding tyramine (**3g**) and sulfated tyramine (**3h**) at this position. The tyramine derivative (**3g**) showed significantly improved CCK-A receptor affinity (IC_{50} = 0.057 μM) compared with (**3c**) although the CCK-A receptor selectivity over the CCK-B receptor was not so great. The sulfated derivative (**3h**) however, displayed poor affinity for the CCK-A receptor (IC_{50} = 1.2 μM) indicating that the C-terminal phenyl group is unlikely to be mimicking the Tyr residue in CCK-26-33.

Recent publications on a series of phenyl N-substituted lysine derivatives of CCK-30-33 have shown them to be potent and selective CCK-A receptor agonists.[10] A representative compound from this series is (**2**). It is apparent that the substituted Lys residue is responsible for the high CCK-A receptor affinity and selectivity since the parent tetrapeptide, having a methionine residue in place of the Lys, is CCK-B selective. We appended the corresponding o-chlorophenyl urea moiety of (**2**) onto (**1b**) maintaining the same through bond distance from the phenyl ring to the α-carbon of the Trp residue as that found with (**2**). The resulting compound (**5**)[11] also showed high CCK-A receptor affinity (IC_{50} = 0.075 μM) comparable with compound (**3g**) but, significantly, maintained excellent selectivity over the CCK-B receptor (IC_{50} = 18 μM). Although the through bond distance between the phenyl ring and α-carbons of (**3g**) and (**5**) differ, it is possible that the phenyl rings are accessing the same binding site at the receptor since both side chains have several rotatable bonds and are consequently highly flexible.

We are currently investigating the chiral synthesis of both enantiomers of (**3g**) and (**5**) using synthetic methodology that we have previously published.[12] The agonist/antagonist profile will be evaluated on each enantiomer independently.

CONCLUSIONS

In this paper we have outlined the rational design and synthesis of non-peptide CCK-A selective ligands. The optimum compounds in this series, (3g) and (5), display similar CCK-A receptor binding affinities to the clinically useful CCK-A antagonist lorglumide.[13] This novel series of compounds represent non-peptide topographical mimics of CCK-26-33 based on the utilisation of a single carbon atom template. These results demonstrate the feasibility of this methodology to design non-peptide ligands for 'rhegnylogically' arranged peptides. We are currently investigating the application of this single carbon atom template approach to other neuropeptide targets.

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REFERENCES AND NOTES

1. Farmer, P.S.; Ariens, E.J. Trends Pharmacol. Sci. **1982**, 3, 362.
2. Farmer, P.S. Drug Design Vol. X, Ariens, E.J. editor, Academic Press, 1980.
3. Horwell, D.C.; Hughes, J.; Hunter, J.C.; Pritchard, M.C.; Richardson, R.S.; Roberts, E.; Woodruff, G.N. J. Med. Chem. **1991**, 34, 404.
4. Olson, G.L.; Cheung, H.C.; Voss, M.E.; Hill, D.E.; Kahn, M.; Madison, V.S.; Cook, C.M.; Sepinwall, J.; Vincent, G. Proc. Biotechnol. (USA), Conference Management Corporation, Norwalk, CT, 1989, S.348.
5. Nicolaou, K.C.; Salvino, J.M.; Raynor, K.; Pietranico, S.; Reisine, T.; Freidinger, R.M.; Hirshmann, R. Pept. Chem. Struct. Biol. Proc. Am. Pept. Symp. 11th 1989, **1990**, 881.
6. Olson, G.L.; Voss, M.E.; Hill, D.E.; Kahn, M.; Madison, V.S.; Cook, C.M. J. Am. Chem. Soc. **1990**, 112, 323.
7. Bourne, G.T.; Horwell, D.C.; Pritchard, M.C. Tetrahedron **1991**, 47, 4763.

8. Birchmore, B.; Boden, P.R.; Hewson, G.; Higginbottom, M.; Horwell, D.C.; Ho, Y-P.; Hughes, J.; Hunter, J.C.; Richardson, R.S. Eur. J. Med. Chem. **1990**, 25, 53.

9. Sawyer, T.K.; Jensen, R.T.; Moran, T.H.; Wang, L.H.; Yu, D.H.; Staples, D.J.; Devaux, A.E.; Bradford, V.S. (1990) in Peptides; Chemistry, Structure and Biology, Proc. 11th American Peptide Symposium (Rivier, J.E. and Marshall, G.R. eds.), pp143-145, ESCOM, Leiden.

10 Shiosaki, K.; Lin, C.H.; Kopecka, H.; Tufano, M.D.; Bianchi, B.R.; Miller, T.R.; Witte, D.G.; Nadzan, A.M. J. Med. Chem. **1991**, 34, 2837.

11. Physical data for compound (5) : m.p. 98-106°C; υ_{max} (film)/cm^{-1} 3321 (NH), 3029 (arom-H). 2921 (ali-H), 1732 br (CO urethane, urea and amide); δ_H ($CDCl_3$) (300 MHz) 1.50-2.00 (18 H, m), 2.56 (1 H, d, J 14 Hz), 2.73 (2 H, t, J 7 Hz), 2.92 (1 H, d, J 14 Hz), 3.22 (4 H, m), 3.45 (3 H, m), 3.60 (1 H, d, J 14 Hz), 4.77 (1 H, s), 5.73 (1 H, br s), 6.36 (1 H, br s), 6.69 (1 H, s), 6.84 1 H, s), 6.91 (1 H, t, J 8 Hz), 7.04-7.34 (11 H, m), 7.49 (1 H, d, J 8 Hz), 7.95 (1 H, s), 8.19 (1 H, s); m/z (FAB) 676.3688 (M+1, 100%, $C_{43}H_{52}N_6O_5Cl$ requires 767.3688).

12. Bourne, G.T.; Crich, D.; Davies, J.W.; Horwell, D.C. J. Chem. Soc., Perkin Trans. I, **1991**, 1693.

13. Makovec, F.; Peris, W.; Revel, L.; Giovanetti, R.; Mennuni, L.; Rovati, L. J. Med. Chem. **1992**, 35, 28.

14. IC_{50} represents the concentration (μM) producing half-maximal inhibition of specific binding of [^{125}I] Bolton-Hunter CCK-26-33 to CCK receptors in the mouse cerebral cortex (CCK-B) or rat pancreas (CCK-A). Values given represent the geometric mean from at least three separate experiments. See reference 3 for full experimental details.

15. Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whittler, W.L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; Kling, P.J.; Kunkel, K.A.; Springer, J.P.; Hirshfield, J. J. Med. Chem. **1988**, 31, 2235.