1,5-Benzothiazepinone and arylazepinone dipeptide mimetics as local constraints in peptidomimetic design

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Introduction

Herein, the application of novel conformationally constrained amino acids, 4-amino-8-bromo-2-benzazepin-3-one (8-Br-Aba), 3-amino-3,4-dihydroquinolin-2-one (Dhq), and regioisomeric 4-aminonaphthoazepinones (1- and 2-Ana), is described. Introduction of these constricted scaffolds into the N-terminal tetrapeptide of dermorphin (i.e., H-Tyr-D-Ala-Phe-Gly-NH₂) induced significant shifts in binding affinity, selectivity, and *in vitro* activity at the μ - and δ -opioid receptors (MOP and DOP, respectively). A reported constrained μ / δ -opioid lead tetrapeptide H-Dmt-D-Arg-Aba-Gly-NH₂ was modified through application of various constrained building blocks to identify optimal spatial orientations in view of activity at the opioid receptors. Interestingly, when the aromatic moieties were turned towards the C-terminus of the peptide sequences, (partial) (ant)agonism at MOP and weak (ant)agonism at DOP were noticed, whereas the incorporation of the 1-Ana residue (with the aromatic moiety shifted towards the N-terminus) led towards balanced low nanomolar MOP/DOP binding and *in vitro* agonism.[1]

Additionally, a 3-step methodology for the synthesis of 1,5-benzothiazepin-4(5H)-one dipeptidomimetics has been elaborated *via* an Ugi-4CR followed by a S-trityl deprotection and an intramolecular Cu(I)-catalyzed Ullmann condensation with moderate to good yields. *In silico* and NMR conformational studies showed that the lowest energy conformers stabilize γ - and β -turn structures.[2]

Figure 1: 8-Br-Aba, Dhq, and 1/2-Ana scaffolds incorporated in the N-terminal tetrapeptide of dermorphin

Results and Discussion

The insertion of constricted 4-amino-8-bromo-2-benzazepin-3-one (8-Br-Aba), 3-amino-3,4-dihydroquinolin-2-one (Dhq), and regioisomeric 4-aminonaphthoazepinones 7a-c (1/2-Ana) (1-4, Figure 1) dipeptidomimetics into an optimized dermorphin tetrapeptide lead sequence has resulted in compact and high affinity MOP/DOP opioid receptor ligands. Whereas insertion of the conformationally constrained 8-Br-Aba-Gly, Dhq-Gly, or 2-Ana-Gly dipeptides resulted in decreased DOP recognition, application of the regioisomeric 1-Ana-Gly building block led to excellent low nanomolar MOP and DOP binding affinities and *in vitro* functional activities (Table 1). The described cyclic constrained aromatic amino acids can be regarded as additional tools to modulate receptor selectivity and activity, but they also provide a way to enhance proteolytic stability and bioavailability of lead peptides. Bulky naphthylalanine residues often provide a powerful means of modulating receptor selectivity. The current building blocks present the additional feature to control χ dihedral angles, thus presenting specific ligand topologies. The present study clearly showcases the advantages of such constrained residues for improving ligand potency and opioid receptor selectivity. It is expected that these building blocks will rapidly find application in other biologically active peptides.

Table 1: In vitro opioid receptor affinity and activity of dermorphin tetrapeptides

Compound	GPI ^a (IC ₅₀ nM)	MVD ^a (IC ₅₀ nM)	MOP ^b (IC ₅₀ nM)	DOP ^b (IC ₅₀ nM)	Selectivity IC ₅₀ DOP / IC ₅₀ MOP
H-Dmt-D-Arg-[8-Br-Aba-Gly]-NH ₂	22.0±2.4 ^c	8.51±0.70	0.76±0.49	60.7±13.7	80
H-Dmt-D-Arg-[Dhq-Gly]-NH ₂	33.7±3.6 ^d	90.5±14.4	1.62±0.03	630±65.0	389
H-Dmt-D-Arg-[1-Ana-Gly]-NH ₂	0.252±0.014	1.42±0.18	0.33±0.01	1.00±0.45	3
H-Dmt-D-Ala-[1-Ana-Gly]-NH ₂	1.13±0.13	0.86±0.16	0.39±0.07	2.07±0.30	5
H-Dmt-D-Arg-[2-Ana-Gly]-NH ₂	21.4±1.9	114±7.0	2.19±0.70	153±12.0	70
H-Dmt-D-Ala-[2-Ana-Gly]-NH ₂ major	2.88±0.10	306±12.0	1.78±0.40	167±38.0	94
H-Dmt-NMe-D-Ala-[1-Ana-Gly]-NH ₂	0.43±0.06	1.09±0.04	0.35±0.04	1.47±0.04	4

Additionally, a short synthetic strategy towards constrained thiazepinones was developed which comprised an Ugi-4CR and an intramolecular Cu(I)-catalyzed C-S cyclization reaction as the two key synthetic steps (Scheme 1). Our goal was to generate a diverse set of substituted 1,5-benzothiazepin-4(5H)-one-based dipeptide mimetics 10, wherein the C α -position of the exocyclic amino acid was substituted with different alkyl and aryl substituents. Starting from commercially available Boc-L-Cys(Trt)-OH 5, different aldehydes 6, o-iodoaniline 7, and tert-butylisocyanide 8, linear Ugi dipeptides of type 9 were constructed. Orthogonal S-trityl deprotection, was followed by a CuI-catalyzed C-S Ullmann condensation, yielding the desired (R)-1,5-benzothiazepin-4(5H)-one-containing dipeptide mimetics of type 10.

Scheme 1: Optimized synthetic pathway towards (R)-1,5-benzothiazepin-4(5H)-one dipeptide mimetics via subsequent Ugi-4CR, S-trityl deprotection and CuI-catalyzed C-S ullman condensation

The effect of the second stereocenter on the overall turn-inducing properties of the dipeptidomimetics was explored *via* structural in silico calculations. Molecular modeling indicated that all lowest energy conformers of (R,S)- and (R,R)-benzothiazepinones possessed a hydrogen bond-stabilized γ -turn conformation. However, the expected β -turn conformations were also observed within a range of 1.95 to 5.60 kcal mol⁻¹ above the energetically lowest conformer. The conformational preferences and overall turn-inducing properties of these dipeptidomimetics can potentially be influenced by the steric bulk of the C-terminal tert-butylamide moiety. Therefore, structural calculations were repeated for and both (R,S)- and (R,R) with less sterically hindering primary and secondary amides (i.e. -CONH₂, -CONHMe, -CONHiPr), and indeed, significantly lower energy differences were recorded between the lowest energy γ -turn conformers and the first β -turn structures if the bulky C-terminal tert-butylamide group was replaced.

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