Bioorganic & Medicinal Chemistry Letters 27 (2017) 3412–3416

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Urotensin core mimics that modulate the biological activity of urotensin-II related peptide but not urotensin-II

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ARTICLE INFO

Article history:
Received 28 April 2017
Accepted 30 May 2017
Available online 31 May 2017

Keywords:
Urotensin-II related peptide
Urotensin-II
Allosteric modulator
Head-to-tail cyclization
Peptide mimicry
SAM-linker

ABSTRACT

A novel approach for the synthesis of head-to-tail cyclic peptides has been developed and used to prepare two mimics of the urotensin II-related peptide (URP) cyclic core. Mimics **1** and **2** (c[Trp-Lys-Tyr-Gly-ψ(triazole)-Gly] and c[Phe-Trp-Lys-Tyr-Gly-ψ(triazole)-Gly]) were respectively prepared using a combination of solid- and solution-phase synthesis. The silyl-based alkyne-modifying (SAM) linker enabled installation of *C*-terminal alkyne and *N*-terminal azide moieties onto linear peptide precursors, which underwent head-to-tail copper-catalyzed azide-alkyne cycloaddition (CuAAC) in solution. In an aortic ring contraction assay, neither **1** nor **2** exhibited agonist activity; however, both inhibited selectively URP- but not UII-mediated vasoconstriction. The core phenylalanine residue was shown to be important for enhancing modulatory activity of the urotensinergic system.

Introduction

Peptide hormones and their related receptors are prominent starting points for developing probes and modulators of signaling important for homeostasis and diseases in the search for improved pharmaceuticals.[1] Selective modulation of specific signaling pathways is an important challenge in modern medicinal chemistry, because receptor activation typically triggers multiple interconnected signal cascades leading to downstream events. Towards this aim, peptide-based structures have demonstrated promise for selective modulation of receptor-ligand interactions and downstream signaling.[2] Applications of cyclic peptide analogues have been particularly relevant in this light due to the ability of the cycle to restrain conformation in ways that favor selectivity, biological activity and metabolic stability.[3]

Urotensin II (UII, Fig. 1) and urotensin II-related peptide (URP) are two cyclic disulfide bridged peptides, which both bind as endogenous ligands to the urotensin II receptor (UT), a class 1A (rhodopsin) G protein-coupled receptor (GPCR).[4] Found ubiquitously in vertebrates,[5] UII and URP contain a conserved c(Cys-Phe-Trp-Lys-Tyr-Cys) core sequence that is crucial for their biological activity among all species.[6] The so-called urotensinergic system, which features UII, URP and UT, is associated with cardiovascular, nervous and renal functions in humans,[7] and garners interest for therapeutic intervention in a variety of pathophysiological processes: *e.g.* hypertension,[8] artherosclerosis[9] and vascular hypertrophy.[10] Limited success in the development of drugs that target UT has however evoked a reassessment of the importance of the specific interactions of the endogenous ligands with the receptor and their respective signaling cascades.[11]

Selective modulators of UII and URP activity have emerged as valuable probes to understand their respective importance in normal function and pathological conditions.[12] For example, the peptide modulators urocontrin ([Bip^4]URP, Bip = 4,4′-biphenylalanine) and urocontrin A ([Pep^4]URP, Pep = (phenylethynyl)phenylalanine) possess the ability to decrease the maximal contractile response of human UII (hUII) without significantly changing its potency nor causing noticeable effects on URP-induced vasoconstriction.[12c] Introduction of *N*-aminosulfamide surrogates of the Trp^7 and Lys^8 residues in UII(4–11) has also generated ligands (e.g., **3**) that reduced UII and URP-induced contraction without affecting potency.[12a] Furthermore, small molecule pyrrolo[3,2-*e*][1,4]diazepin-2-ones (e.g., **4**) were shown to inhibit as well as potentiate selectively UII and URP activity.[12b] Noteworthy, these small molecule modulators were designed to mimic the side-chain functional groups and turn orientation of the shared Trp-Lys-Tyr sequence in the rings of UII and URP, and have furthered the notion that specific spatial arrangements of this

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http://dx.doi.org/10.1016/j.bmcl.2017.05.088
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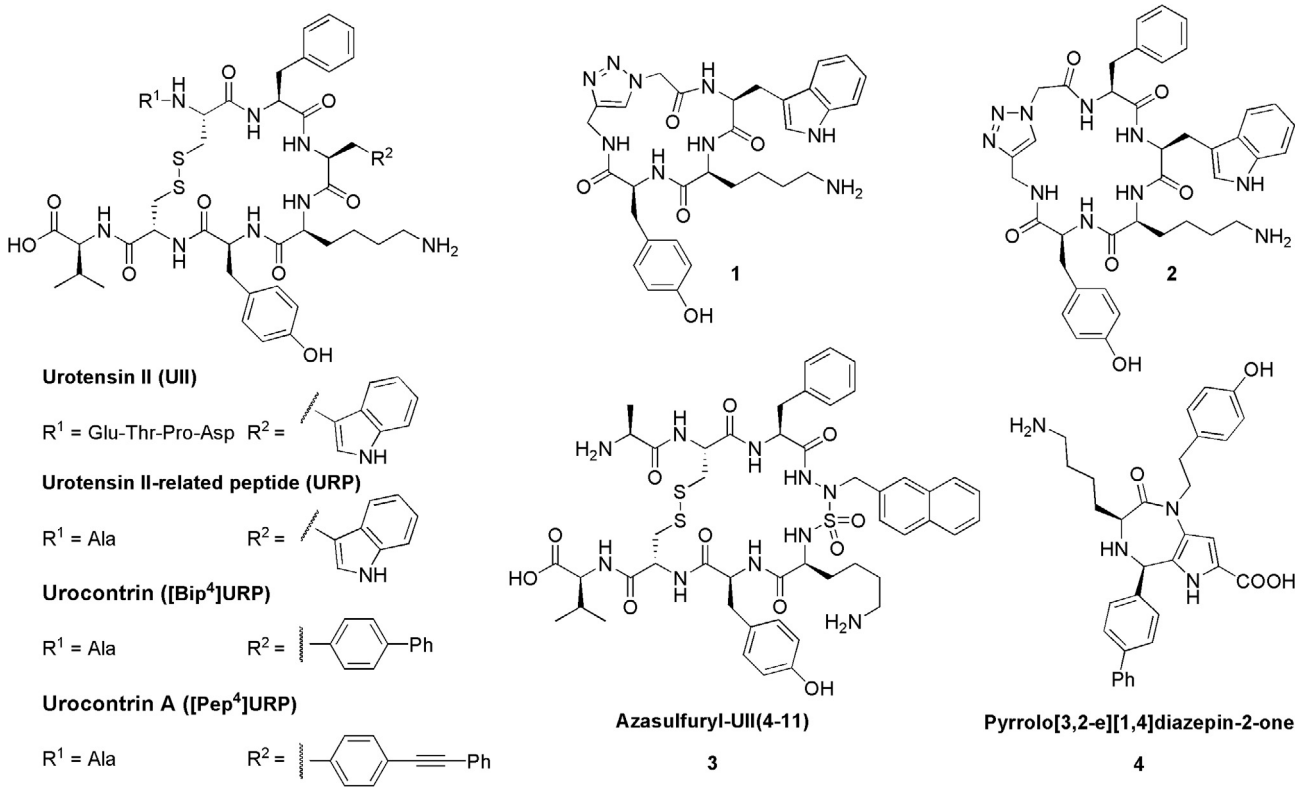
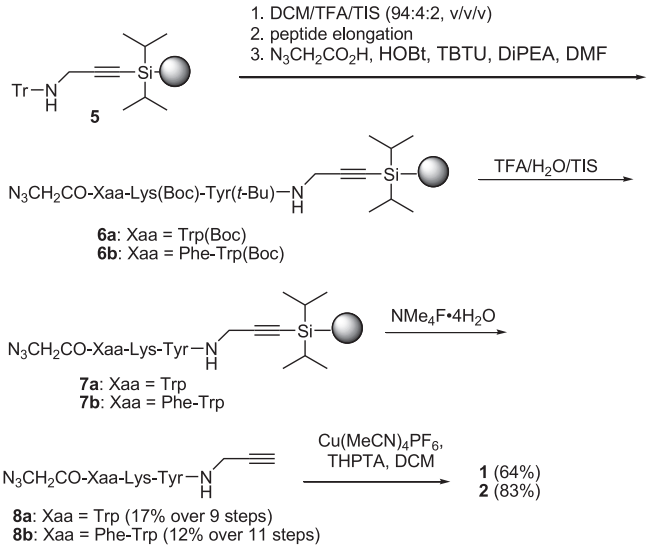


Fig. 1. Structures of UII, URP and known modulators.

triad of residues lead to selective modulation of endogenous ligand activity.[12d]

Considering an alternative constraint of the spatial arrangement of the Trp-Lys-Tyr triad, we have pursued cyclic penta- and hexapeptide mimics **1** and **2** (c[Trp-Lys-Tyr-Gly-ψ(triazole)-Gly] and c[Phe-Trp-Lys-Tyr-Gly-ψ(triazole)-Gly], Fig. 1). Head-to-tail cyclization was employed to replace the side chain-to-side chain disulfide bridge by a backbone 1,4-disubstituted 1,2,3-triazole cross-link using a copper-catalyzed azide alkyne cycloaddition (CuAAC).[13] Application of a triazole amide isostere was explored in part to improve metabolic stability relative to the disulfide.[14] Moreover, the synthesis of mimics **1** and **2** was conceived to demonstrate further the utility of the Silyl-based Alkyne Modifying (SAM)-linker, which has enabled solid-phase construction of *C*-terminal peptide alkynes for bio-conjugate chemistry.[15] By affixing an *N*-terminal azido acetamide on the *C*-terminal peptide alkyne, an effective solid-phase approach was conceived for making linear peptide precursors for subsequent CuAAC chemistry in solution to furnish the requisite cyclic peptides. Mimics **1** and **2** were subsequently evaluated for their ability to induce contraction or modulate UII and URP mediated vasoconstriction.



Scheme 1. Synthesis of head-to-tail cyclic URP mimics **1** and **2**.

Results and discussion

Peptide propargylamides **8** were synthesized on the SAM2 solid support (0.26 mmol/g initial loading, Scheme 1).[15c] The core sequences Trp-Lys-Tyr and Phe-Trp-Lys-Tyr were assembled onto propargylamide resin **5** using standard Fmoc-based solid-phase peptide synthesis (SPPS) procedures.[16] After Fmoc group removal both peptides were *N*-capped with azido acetate (4 equiv.) using HOBt/TBTU/DiPEA (4 equiv./3.8 equiv./8 equiv.) in DMF, and treated with a cocktail of TFA/H_2O/TIS (90:5:5) to remove all side-chain protection. The resin was cleaved using tetramethylammonium fluoride and the resulting *N*-terminal azido peptide *C*-terminal alkynes **8a** and **8b** were respectively isolated in 17% and 12% overall isolated yield after HPLC purification.

With linear sequences **8** in hand, head-to-tail cyclization was studied to identify conditions that mitigated competing formation of dimer and higher aggregates. High dilution[17] and specialized catalyst systems[18] have been previously employed to favor CuAAC reactions.[19] In our hands, only at a higher dilution (0.1 vs 3.0 mM) cyclic peptide monomer was obtained. For example, trace amounts of cyclic monomer were isolated using a catalyst system of CuI (2 equiv.) and DiPEA (8 equiv.). Cyclization was achieved more efficiently using $Cu(MeCN)_4PF_6$ (0.95 equiv.) and tris(3-hydrox-