Bioorganic & Medicinal Chemistry Letters 23 (2013) 4892–4895

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

De novo design and synthesis of a μ-conotoxin KIIIA peptidomimetic

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ARTICLE INFO

Article history:
Received 25 March 2013
Revised 14 June 2013
Accepted 27 June 2013
Available online 6 July 2013

Keywords:
Conotoxin
Peptidomimetic
Sodium channel blocker
KIIIA
Pain

ABSTRACT

μ-Conotoxin KIIIA blocks voltage-gated sodium channels and displays potent analgesic activity in mice models for pain. Structure–activity studies with KIIIA have shown that residues important for sodium channel activity are presented on an α-helix. Herein, we report the de novo design and synthesis of a three-residue (Lys7, Trp8, His12) peptidomimetic based on a novel diketopiperazine (DKP) carboxamide scaffold.

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Voltage-gated sodium channels (VGSCs) initiate action potentials in excitable cells by regulating the influx of sodium ions and thus play a vital role in neuronal function. In humans, nine distinct isoforms ($Na_V1.1$–1.9) have been identified, each with different tissue distributions and biophysical properties. Several VGSC isoforms have been implicated in the perception of pain and chronic pain states.[1,2] Moreover, genetic studies have established strong links between mutations in the gene SCN9A, coding for the $Na_V1.7$ isoform and pain related conditions. Individuals with loss of function truncation mutations of SCN9A result in congenital insensibility for pain stimuli.[3] As such, sodium channels are recognized as the molecular targets for clinically relevant analgesics. However, the analgesic activity of many of these agents is a result of nonselective blockade, resulting in a narrow therapeutic index. Subtype-selective sodium channel blockers, on the other hand, have potential as therapeutics for the treatment of pain and indeed notable advances have been made in this area.[4–8]

Conotoxin μ-KIIIA from *Conus kinoshati* is a 16-residue peptide that displays potent blockade of several VGSC isoforms.[9] Furthermore, in mice models for pain, μ-KIIIA has displayed potent analgesic activity.[9] Structure–activity studies have identified five of the six residues important for functional activity (Lys7, Trp8, Arg10, Asp11, His12) occurred in an α-helical region of the peptide, and the sixth (Arg14) was located immediately C-terminal to the helix.[10] Truncated and lactam-stabilized analogues of μ-KIIIA have been prepared and found to retain sodium channel blockade.[11] This is particularly significant for a peptide mimetic approach, suggesting the pharmacophore can be potentially minimized without significantly compromising activity. Incorporating the μ-KIIIA pharmacophore within a nonpeptidic scaffold is a novel approach to discovering sodium channel blockers. Herein, we describe the computer-aided de novo design of a μ-KIIIA peptidomimetic based on a novel diketopiperazine (DKP) carboxamide scaffold.

We have successfully applied the de novo design strategy to create molecules that mimic peptide epitopes.[12–17] The key basis of this approach is the interactive design and energy minimization of organic scaffolds that mimic the projection of the C_α–C_β bond vectors of amino acid side chains in targeted peptides, utilizing a molecular modeling program such as Sybyl (Tripos associates). When applied to other conotoxins, successful design was based on the peptide solution structure, without knowledge of precise side chain orientation in the bound state.[15,17,18] This represents a notable advantage of the de novo design approach since high-resolution structural information is lacking for many bioactive peptides in complex with their targets. It is thought that by mimicking C_α–C_β bond vectors, the side chains attached to the mimetic scaffold can occupy the same conformational space as those in the parent peptide. Ultimately, the same binding mode should be adopted without energetic penalty.

The design of peptidomimetic **1** was based on the solution structure of μ-KIIIA,[10,11] along with SAR studies reported elsewhere.[9,11] Several iterations of in silico building and energy minimization began with superimposition of a DKP core on the Trp8

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http://dx.doi.org/10.1016/j.bmcl.2013.06.086

α-carbon (Fig. 1). *N*-Alkylation of the DKP nitrogen extends mimicry to the Lys7 C_α–C_β bond vector. Attachment of a carboxamide to the DKP using an amino acid results in a hydrogen-bond stabilized pseudo six membered ring. In this conformation the amino acid α-carbon in the scaffold provides mimicry of the His12 bond vector in μ-KIIIA. An RMSD of 0.321 Å was obtained using the fit atom function in Sybyl with superimposition of the Lys7, Trp8 and His12 alpha carbons. The designed scaffold **1** can be functionalized with an imidazole and indole by incorporating the His and Trp amino acid derivatives, respectively, while addition of an alkyl amine side chain gives rise to the three-residue μ-KIIIA peptidomimetic **2** (Fig. 2).

As a complement to in silico design, X-ray crystal structures are valuable for validating scaffold conformation. To this end, the Cambridge crystallography database (CCD) serves as a useful tool for analysis of structurally related compounds. Although no structures existed bearing a 2,5-DKP carboxamide, a related 2,3-DKP carboxamide **3** was retrieved from the CCD.[19]

Overlay of **3** with the μ-KIIIA solution structure with the Lys7, Trp8 and His12 α-carbon atoms set as constraints shows the spatial orientation of the scaffold resembles that in μ-KIIIA. Since the mimetic core is based on a 2,5-DKP ring, the crystal structure of compound **4** was retrieved from the CCD and used to validate the design.[20] Superimposition of **4** on the mimetic scaffold, shows very good agreement with the in silico predicted conformation (RMSD = 0.015 Å, Fig. 3D). However, it is well known that DKPs with aromatic side-chains form a folded conformation, stabilized by the interaction of the amide dipoles with the polarisable π electrons, as a dipole–induced-dipole interaction.[21] It is believed that the energy difference between the folded and extended conformations is in the vicinity of 3 kcal/mol.[21]

As shown in Figure 1, the indole side chain in μ-KIIIA is orientated away from the mimetic scaffold. In order for the indole in **2** to mimic Trp8 in μ-KIIIA, an extended conformation may be required (Fig. 3). However, since the precise side-chain orientation for binding is not known from the solution structure and either conformer can be adopted in the mimetic, we reasoned that this could be a potential point of optimization following evaluation of the mimetic **1**.

Synthesis of the mimetic is detailed in Scheme 1. The azide in **6** was introduced by activating the commercially available alcohol **5** as the mesylate and was then subsequently displaced with sodium azide. Treatment with anhydrous 4 N HCl in dioxane resulted in Boc-deprotection, which was followed by reaction with ethyl bromoacetate to afford the *N*-alkyl glycine derivative **7**. Coupling **7** with *N*-Fmoc-N^{In}-Boc-L-trp, using the reagent HBTU, allowed efficient formation of the amide **8**. Removal of the Fmoc group in the presence of 50% piperidine/DMF solution was followed by

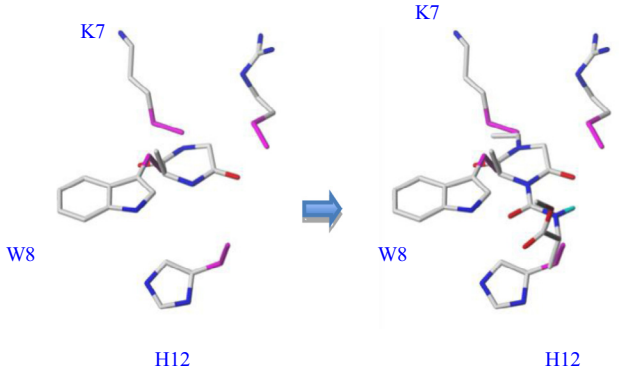

Figure 1. In silico de novo design process from a DKP superimposed on the Trp8 C_α–C_b bond vector leading to a three-residue KIIIA peptidomimetic.

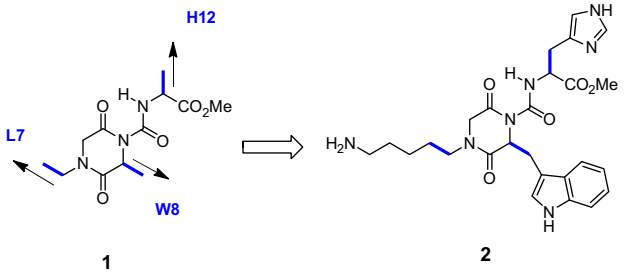

Figure 2. Functionalisation of the designed scaffold **1**, affording the Lys7, Trp8, His12 three-residue peptidomimetic **2**.

cyclization and formation of the DKP **9**. Reduction of the azide, followed by boc deprotection afforded the two residue peptidomimetic **10** which could serve as a preliminary probe into the relative importance of the Lys and Trp residues to sodium channel binding.

We have developed a very efficient protocol for synthesizing acylureas from secondary amides via a carbamoyl chloride intermediate.[22,23] Accordingly, treatment of the DKP **9** with TMSOTf and phosgene, followed by reaction of the resultant carbamoyl chloride with *N*-(im)-Boc-L-His methyl ester rapidly afforded the acylurea **11** (Scheme 2). Hydrogenolysis of the azide, followed by boc deprotection gave the μ-KIIIA peptidomimetic **2**. In addition analogue **12** was prepared using histamine as the His12 side chain mimic, in order to investigate the importance of the imidazole ring orientation.

The ability of the mimetic **2** and related analogues to block $Na_V1.7$ was determined by voltage-clamp protocols. Oocytes expressing the VGSC $Na_V1.7$ isoform were expressed and two-electrode voltage clamped as described previously.[9] Preliminary

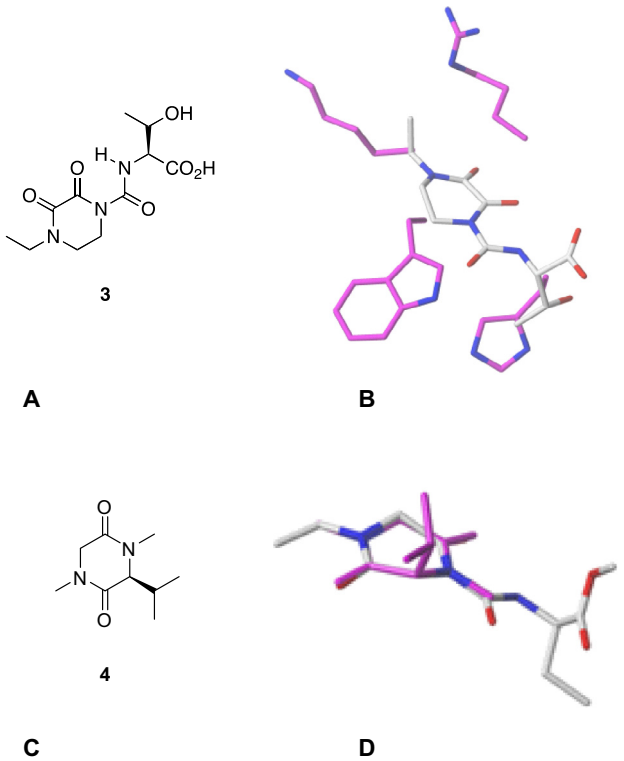

Figure 3. (A) A structurally related CCD compound **3**. (B) Overlay of **3** with the Lys7, Trp8 and His12 α-carbons set as constraints. (C) DKP **4** superimposed with the energy-minimized mimetic scaffold (D).

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