Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3193–3196
Bioorganic & Medicinal Chemistry Letters

A three-residue, continuous binding epitope peptidomimetic of ShK toxin as a Kv1.3 inhibitor

Andrew J. Harvey,[a] Robert W. Gable[b] and Jonathan B. Baell[a,*]

[a]*The Walter and Eliza Hall, Institute of Medical Research, Biotechnology Centre, 4 Research Avenue, La Trobe R&D Park, Bundoora 3086, Australia*
[b]*School of Chemistry, University of Melbourne, Vic. 3010, Australia*

Received 14 April 2005; revised 27 April 2005; accepted 3 May 2005

Abstract—The ShK toxin is a polypeptide that blocks the Kv1.3 potassium channel in T-lymphocytes and has been identified as a potential therapeutic for multiple sclerosis. ShK is well characterised in terms of structure and binding, offering an attractive target for the design of structural and functional mimetics. Building on our previous success in developing rationally designed peptidomimetics of ShK, we report a novel mimetic of the K22–Y23–R24 residues of the peptide. The mimetic was shown to inhibit the Kv1.3 channel with moderate activity.

Although the voltage-gated potassium ion channel, Kv1.3, occurs as a monomer throughout the body, the potential therapeutic value of Kv1.3 arises from its presence as a homotetramer in selected cell types, most notably in T-cells.[1] Kv1.3 is involved in setting the membrane potential in T-cells. Blockade of Kv1.3 causes depolarisation across the membrane, disabling the cell's ability to increase intracellular calcium levels to a point sufficient for proliferation.[2,3] Thus, blockade of the Kv1.3 ion channel has been identified as a potential means for immunosuppression.[4]

Recent findings show that myelin-reactive T-cells from multiple sclerosis patients express extremely high levels of the Kv1.3 channel.[5] Such T-cells do not proliferate when treated with specific blockers of Kv1.3. These findings were applied to the treatment of autoimmune disease by showing that ShK-Dap22, a selective Kv1.3 blocker, ameliorates the symptoms of adoptive experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis.[6] Links between Kv1.3 blockade and immunosuppressive activity have also been established for small molecule blockers of Kv1.3 including natural product correolide, various derivatives of khellinone and molecules based around a psoralen core.[7–11]

The ShK peptide is a component of the venom of the sun anemone (*Stichodactyla helianthus*) that blocks the Kv1.3 ion channel in the picomolar range.[12] Being a peptide, ShK itself has limited promise as a therapeutic. ShK and its synthetic analogue, ShK-Dap22, have limited oral bioavailability and have short half-lives within the body,[13] although ongoing efforts to modify ShK aim to improve its pharmacokinetics and are meeting with some success.[14] The structure of ShK has been determined by NMR and its binding interactions with Kv1.3 have been mapped. These data, together, allow for the design of peptidomimetics that have more drug-like properties than the peptide. We have an interest in the design of binding-epitope mimetics,[15–17] and have previously reported a discontinuous binding epitope mimetic of ShK.[18] Here, we report a novel, continuous binding epitope mimetic of ShK.

Information about the binding interactions between Kv1.3 and ShK has been provided by two studies.[19,20] Alanine scanning from the latter study (Table 1) revealed that an elliptical 'patch' comprised the binding surface and a series of thermodynamic cycles showed that this patch sat deep in the extracellular vestibule of Kv1.3. The pharmacophore comprises both discontinuous (R11 and F27) and continuous (K22, Y23 and R24)

Keywords: Peptidomimetic; Indole; Kv1.3; Ion channel; Multiple sclerosis.
* Corresponding author. Tel.: +61 3 93452108; fax: +61 3 93452211; e-mail: jbaell@wehi.edu.au

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.05.014

Table 1. Approximate decrease in free energy of binding, $\Delta\Delta G_{obs}$ (kJ/mol), to Kv1.3 of single residue substituted analogues of ShK relative to wild type ShK[20]

ShK analogue	$\Delta\Delta G_{obs}$ (kJ/mol)
R11A	4
K22A	8
Y23A	8
R24A	11
F27A	3

binding elements. We have previously disclosed a rationally designed R11–K22–Y23 ShK mimetic, which binds to Kv1.3 with an affinity/K_d of 95 μM.[18] In the work discussed herein, we target K22, Y23 and R24. Residues K22 and Y23 have been retained as targets for the new mimetic since this diad appears as a common element across Kv binding peptide toxins and consistently rates as an important binding feature to Kv1.3.[1] We chose R24 as the third residue to target because, as shown in Table 1, it might interact more strongly with Kv1.3 than does R11 and could thus give rise to a more potent mimetic. Also, the contiguous nature of the surface of the K22–Y23–R24 binding epitope would allow for a smaller mimetic that would correspondingly be more drug-like than our previously reported R11–K22–Y23 mimetic. Furthermore, choice of a continuous epitope is attractive because the peptide backbone can aid in the design of the peptidomimetic scaffold.

In designing the peptidomimetic, the α–β bond vectors of the K22 and Y23 side chains in ShK were set as constraints. These vectors were chosen so that the peptidomimetic side chains would have at least the same degree of rotational freedom as in the peptide. Various molecular scaffolds were trailed during the interactive design process to meet the K22 and Y23 bond vector constraints.[21] Scaffold requirements included ease of synthesis, versatility and drug-likeness. An N-alkylated indole-7-carboxamido scaffold appeared to satisfy the criteria. Furthermore, such an indole suitably derivatised at the 4-position would conveniently reach into the space sampled by R24 in ShK. The indole **1** is an example of the type of compound afforded by this process (Fig. 1). The methyleneguanidinyl group attached to C4 mimics R24 and the aminobutyl group mimics the K22 residue. It has been shown that Y23F analogues of ShK have Kv1.3 activity in the same order of magnitude as wild type ShK.[19] Correspondingly, we have found that the phenolic OH of Y23 in ShK mimetics is not necessary for activity.[18] Thus for ease of synthesis, a phenethyl substituent on the indole nitrogen was used to mimic the side chain of ShK Y23F rather than ShK itself.

The synthesis is premised on the preparation of a symmetrically 4,7-disubstituted indole **6** and the subsequent differentiation of the equivalent functional groups. The key intermediate **6** was synthesised according to the methodology of Jones et al. wherein a 4,7-disubstituted indole is prepared from two successive Diels–Alder reactions of a vinylpyrrole with two equivalents of a dienophile followed by a retro-Diels–Alder reaction.[22] Thus,

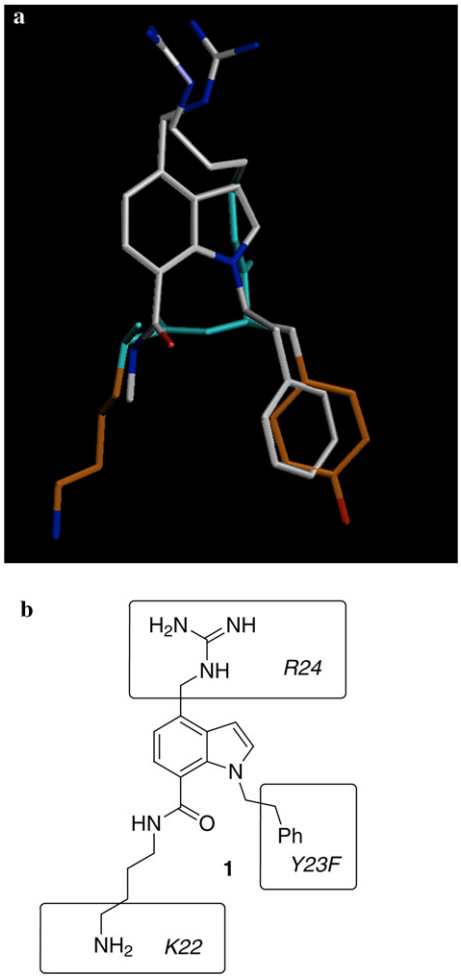
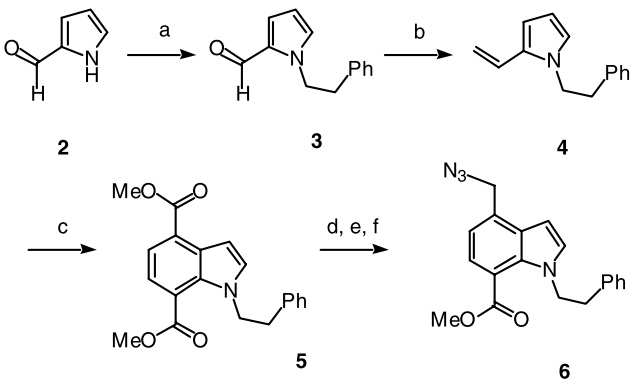
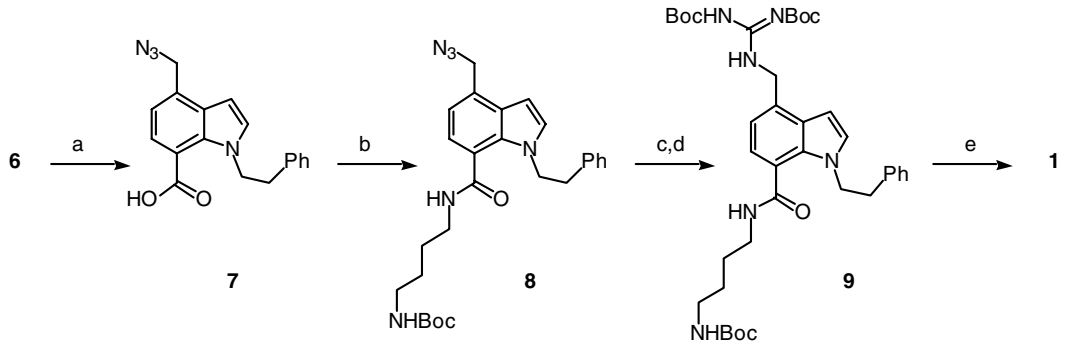


Figure 1. (a) The design rationale for **1**: a superimposition of the active residues of ShK with the minimised conformation of the proposed peptidomimetic. The K22–Y23–R24 residues of ShK, taken from the NMR structure of the peptide, are shown with cyan on the backbone and orange on the Lys and Tyr side chains. (b) The indole peptidomimetic labelled with the ShK side chains to be mimicked.



Scheme 1. Synthesis of intermediate **6**: (a) $PhCH_2CH_2Br$, tetrabutylammonium bromide, Cs_2CO_3, DMF, 80 °C, 9 h, 76%. (b) Ph_3PCH_3Br, NaH, THF, 70 °C, 2 h, 93%. (c) Methyl propiolate, hydroquinone, PhMe, dark, 80 °C, 66 h, 68%. (d) LiOH, MeOH/H_2O, rt, 16 h. (e) $BH_3 \cdot$ THF, THF, rt, 4 h. (f) PPh_3, DIAD, DPPA, THF, rt, 16 h, 25% over three steps.



Scheme 2. Synthesis of peptidomimetic **1**: (a) LiOH, MeOH/H_2O, 90 °C, 5 h, 84%. (b) $NH_2(CH_2)_4NHBoc$, CDI, THF, rt, 16 h, 37%. (c) PPh_3, H_2O, THF, rt, 16 h, 76%. (d) $CF_3SO_2N{=}C(NHBoc)_2$, NEt_3, DCM, rt, 24 h, 32%. (e) Trifluoroacetic acid, DCM, rt, 30 min, 90%.

pyrrole-2-carboxaldehyde **2** was N-alkylated with phenethylbromide to give **3** in 76% yield by the returned starting material (Scheme 1).[23] The aldehyde underwent a Wittig reaction to furnish the vinyl pyrrole **4**. The reaction of **4** with two equivalents of methyl propiolate in the presence of hydroquinone gave intermediate **5**.

The methyl ester attached to C4 was preferentially hydrolysed with lithium hydroxide. The resulting mixture was treated with borane tetrahydrofuran complex to give a mixture of regioisomeric alcohols (6:1 by ^1H NMR) comprising predominantly the desired alcohol. Mitsunobu conditions (PPh_3, diphenylphosphorylazide (DPPA), diisopropylazodicarboxylate (DIAD)) were employed for the generation of the azide **6**, which was isolated pure after chromatography in 25% yield over three steps.

The relatively hindered second methyl ester was cleaved with heating in the presence of lithium hydroxide giving an acid, which was then coupled using 1,1′-carbonyldiimidazole (CDI) with *N*-Boc-1,4-diaminobutane to afford the amide **7** (Scheme 2). The azide was reduced using Staudinger conditions and the resulting amine was treated with a guanidinylating agent to give **13**.[24] Deprotection of **13** gave the target compound **1** as a trifluoroacetate salt.

A crystal structure of **7** was obtained to support the structure arrived at by molecular modelling.† Compound **7** is a 4,7-disubstituted *N*-phenethylindole and as such comprises the essential elements of the peptidomimetic scaffold. The crystal structure of **7** overlaid with the K22–Y23–R24 residues from the NMR structure of ShK shows high atom coincidence (see Fig. 2). The level of Kv1.3 blockade by **1** was determined by patch-clamp methods on L929 cells, which were expressing murine Kv1.3.[10] The peptidomimetic gave an EC_{50} of 75 μM based on preliminary data. This promising result is on par with our previously reported R11–K22–Y23 mimetic (EC_{50} = 95 μM),[18] further confirming the validity of this approach to it de novo designed three-point peptidomimetics based on side-chain bond constraints. In the future, using chemistry amenable to multiple parallel synthesis, such as the elegant methodology of Li et al.,[25] we aim to build a library around the indole scaffold to improve on the current activity.

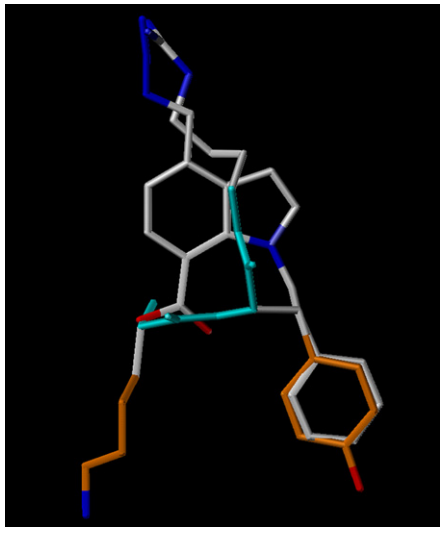


Figure 2. Overlay of the crystal structure of **7** with the K22-Y23-R24 residues from ShK.

Acknowledgments

The authors thank Assoc. Prof. Heike Wulff for performing the electrophysiological experiments.

References and notes

1. Kem, W. R.; Pennington, M. W.; Norton, R. S. *Perspect. Drug Discovery Des.* **1999**, *16*, 111.
2. Cahalan, M. D.; Chandy, K. G. *Curr. Opin. Biotechnol.* **1997**, *8*, 749.

† Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 266815. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

3. Chandy, K. G.; Cahalan, M.; Pennington, M.; Norton, R. S.; Wulff, H.; Gutman, G. A. *Toxicon* **2001**, *39*, 1269.
4. Chandy, K. G.; Wulff, H.; Beeton, C.; Pennington, M.; Gutman, G. A.; Cahalan, M. D. *Trends Pharmacol. Sci.* **2004**, *25*, 280.
5. Wulff, H.; Calabresi, P. A.; Allie, R.; Yun, S.; Pennington, M.; Beeton, C.; Chandy, K. G. *J. Clin. Invest.* **2003**, *111*, 1703.
6. Beeton, C.; Wulff, H.; Barbaria, J.; Clot-Faybesse, O.; Pennington, M.; Bernard, D.; Cahalan, M. D.; Chandy, K. G.; Beraud, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13942.
7. Felix, J. P.; Bugianesi, R. M.; Schmalhofer, W. A.; Borris, R.; Goetz, M. A.; Hensens, O. D.; Bao, J. M.; Kayser, F.; Parsons, W. H.; Rupprecht, K.; Garcia, M. L.; Kaczorowski, G. J.; Slaughter, R. S. *Biochemistry* **1999**, *38*, 4922.
8. Bao, J. M.; Miao, S. W.; Kayser, F.; Kotliar, A. J.; Baker, R. K.; Dossa, G. A.; Felix, J. P.; Bugianesi, R. M.; Slaughter, R. S.; Kaczorowski, G. J.; Garcia, M. L.; Ha, S. N.; Castonguay, L.; Koo, G. C.; Shah, K.; Springer, M. S.; Staruch, M. J.; Parsons, W. H.; Rupprecht, K. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 447.
9. Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*, 1627.
10. Baell, J. B.; Gable, R. W.; Harvey, A. J.; Toovey, N.; Herzog, T.; Hansel, W.; Wulff, H. *J. Med. Chem.* **2004**, *47*, 2326.
11. Vennekamp, J.; Wulff, H.; Beeton, C.; Calabresi, P. A.; Grissmer, S.; Hansel, W.; Chandy, K. G. *Mol. Pharmacol.* **2004**, *65*, 1364.
12. Tudor, J. E.; Pallaghy, P. K.; Pennington, M. W.; Norton, R. S. *Nat. Struct. Biol.* **1996**, *3*, 317.
13. Norton, R. S.; Pennington, M. W.; Wulff, H. *Curr. Med. Chem.* **2004**, *11*, 3041.
14. Beeton, C.; Pennington, M. W.; Wulff, H.; Singh, S.; Nugent, D.; Crossley, G.; Khaytin, I.; Calabresi, P. A.; Chen, C.; Gutman, G. A.; Chandy, K. G. *Mol. Pharmacol.* **2005**, *67*, 1369.
15. Baell, J. B.; Forsyth, S. A.; Gable, R. W.; Norton, R. S.; Mulder, R. J. *J. Comput. Aid. Mol. Des.* **2001**, *15*, 1119.
16. Baell, J. B.; Duggan, P. J.; Forsyth, S. A.; Lewis, R. J.; Lok, Y. P.; Schroeder, C. I. *Bioorg. Med. Chem.* **2004**, *12*, 4025.
17. Baell, J. B.; Duggan, P. J.; Lok, Y. P. *Aust. J. Chem.* **2004**, *57*, 179.
18. Baell, J. B.; Harvey, A. J.; Norton, R. S. *J. Comput. Aid. Mol. Des.* **2002**, *16*, 245.
19. Pennington, M. W.; Mahnir, V. M.; Krafte, D. S.; Zaydenberg, I.; Byrnes, M. E.; Khaytin, I.; Crowley, K.; Kem, W. R. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 696.
20. Rauer, H.; Pennington, M.; Cahalan, M.; Chandy, K. G. *J. Biol. Chem.* **1999**, *274*, 21885.
21. Sybyl 7.0 Tripos Associates, Missouri. Software package was used both for graphics and minimization Tripos forcefield, default minimization and convergence settings, with Gasteiger–Huckel atomic charges and distance-dependent dielectric.
22. Jones, R. A.; Marriott, M. T. P.; Rosenthal, W. P.; Sepulvedaarques, J. *J. Org. Chem.* **1980**, *45*, 4515.
23. Attempted alkylation of pyrrole with phenethylbromide in the presence of various bases afforded mixtures of the desired N-alkylated pyrrole and styrene.
24. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804.
25. Li, L. H.; Martins, A. *Tetrahedron Lett.* **2003**, *44*, 5987.