Bioorganic & Medicinal Chemistry Letters 19 (2009) 2763–2765

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Low molecular weight non-peptide mimics of ω-conotoxin GVIA

Peter J. Duggan [a], Richard J. Lewis [b], Y. Phei Lok [a,*], Natalie G. Lumsden [b], Kellie L. Tuck [c], Aijun Yang [b]

[a] CSIRO Molecular and Health Technologies, Clayton, VIC 3168, Australia
[b] Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Qld 4072, Australia
[c] School of Chemistry, Monash University, Clayton, VIC 3800, Australia

ARTICLE INFO

Article history:
Received 12 January 2009
Revised 21 March 2009
Accepted 25 March 2009
Available online 28 March 2009

Keywords:
Conotoxin
GVIA
Peptidomimetic
Calcium channel blocker
Pain

ABSTRACT

We report the synthesis and biological activity of a low molecular weight non-peptidic mimic of the analgesic peptide ω-conotoxin GVIA. The molecular weight of this compound presents a reduction by 193 g/mol compared to a previously reported lead. This compound exhibits an EC_{50} of 5.8 μM and is accessible in only six synthetic steps compared to the original lead (13 steps). We also report several improvements to the original synthetic route.

Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.

The key aim of peptidomimetics is to discover small molecules which mimic peptide function without the drawbacks associated with large molecular weight peptides. In general, peptides cannot be orally administered[1] and have a higher tendency to elicit an immunological response. Reduction in molecular weight is associated with better oral bioavailability,[2] with smaller lead compounds also being more amenable to further optimization. For compounds targeting neuronal $Ca_v2.2$ channels, lower molecular weights are especially important to address blood–brain barrier permeability. With this aim in mind, we explored the structure–activity relationship of a previously designed non-peptide mimetic and concurrently sought to reduce the molecular weight of our lead compound.

ω-Conotoxin GVIA is a calcium channel ($Ca_v2.2$) blocker isolated from the marine snail *Conus geographus*.[3,4] ω-Conotoxins that target this voltage-gated channel exhibit analgesic properties. A related peptide, ω-conotoxin MVIIA (Ziconotide or Prialt)[5,6] has recently been approved for the treatment of chronic pain. Of note, the analgesic properties are effective in patients who are tolerant to opioids and Ziconotide is amenable for long-term use without causing dependence. Although Ziconotide is reported to be well-accepted as an intrathecally administered drug,[7,8] issues have arisen with the poor pharmacokinetic profile associated with this peptide.[9,10] Since $Ca_v2.2$ channel blockers are primarily targeted for the treatment of chronic pain, a drug which is administered orally is ideal for this application. In order to facilitate oral delivery and the subsequent requirement of blood–brain barrier permeability, it is therefore of interest to develop a mimetic which is less peptide-like and of molecular weight lower than 500 g/mol. Several small molecule Ziconotide mimics are known.[11–13] One recent example of an orally active $Ca_v2.2$ blocker is NMED-160.[5,9] Although development of this compound has been halted,[14] there is still strong interest in developing small molecule $Ca_v2.2$ blockers.[6,15,16] Using the strongly binding ω-conotoxin GVIA as a starting point, we have previously reported the design and syntheses of two classes of mimetics.[17–20] The benzothiazole class of mimetics **1a–c** are shown in Figure 1.

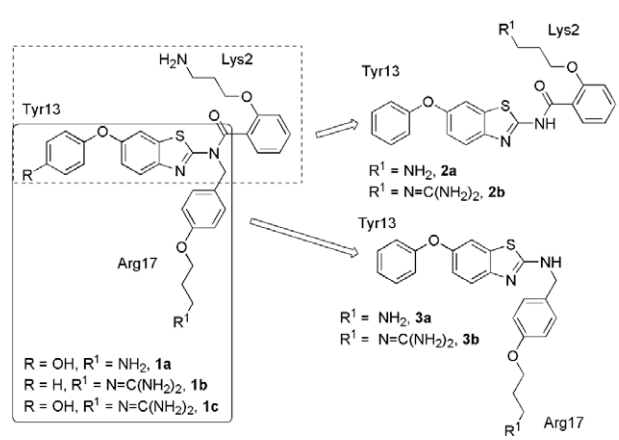


Figure 1. Non-peptide mimics of ω-conotoxin GVIA **1a–c** previously reported and low molecular weight, 'truncated' analogues **2a–b**, **3a–b**.

* Corresponding author. Tel.: +61 3 9545 2573; fax: +61 3 9545 2446.
E-mail address: pheilok@yahoo.com (Y. P. Lok).

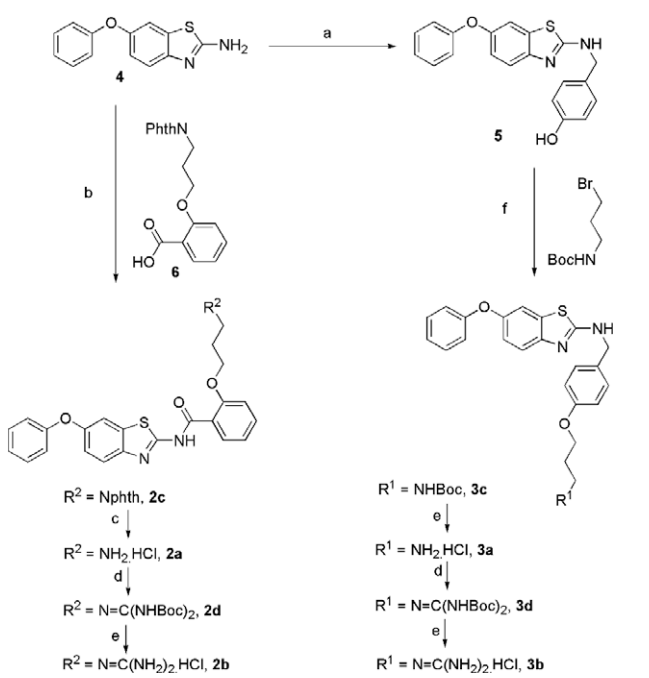
0960-894X/$ - see front matter Crown Copyright © 2009 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.130

Compounds **1a**, **1b** and **1c** (Fig. 1)[17] inhibit rat neuronal $Ca_v2.2$ channels with EC_{50} of 3.18, 3.08 and 1.78 μM, respectively. Although valid leads for further development, these compounds possess high molecular weights in the range of 599–628 g/mol. We report here the synthesis of low molecular weight, biologically active analogues of **1b** as well as several improvements to the original synthetic route which will facilitate the high-throughput parallel synthesis of other analogues.

Compound **1c** was designed to mimic the Lys2, Tyr13 and Arg17 side chains of ω-Conotoxin GVIA. However, compound **1b** showed greater selectivity for N-type ($Ca_v2.2$) versus P/Q-type ($Ca_v2.1$) channels while retaining reasonable activity[17] and hence was chosen as a lead. The omission of a hydroxyl group in **1b** was advantageous from a synthetic point of view. In this work, we designed and synthesized four 'truncated' analogues of **1b**, generating mimics of two amino acid residues instead of three. Truncation of **1b** gives compound **3b** which mimics the Tyr13 and Arg17 residues, and **2a** which mimics the Tyr13 and Lys2 residues of ω-conotoxin GVIA (Fig. 1).

Previous molecular modeling studies on the benzothiazole core of **1a–c** revealed that rotation around the *N*-benzyl bond results in two conformations.[21] The Arg17-mimicking substituent can point upwards or downwards relative to the plane of the benzothiazole ring and hence may interact in the space intended for the Lys2 mimicking substituent. Therefore, we prepared the guanidine substituted analogue **2b** which represents one possible conformation. By similar reasoning, compound **3a** was prepared. Compound **3a** also represents an analogue of **1a** where Arg17-mimicking substituent was replaced with a propylamine chain (Fig. 1). Compounds **2a–b** contain an amide bond attached to the 2-aminobenzothiazole and an *ortho*-substituted ring, whereas compounds **3a–b** are *para*-substituted benzylamines. Comparisons between **2a–b** and **3a–b** will uncover any significant effects due to these differences.

Compounds **2a–b**, and **3a–c** were prepared according to the route shown in Scheme 1. The 2-aminobenzothiazole **4**[17] was coupled with acid **6** to give the precursor **2c**. The use of a phthalimide protecting group in **6** gave a crystalline compound which was more stable and easier to handle compared to the BOC-protected analogue previously used.[17]



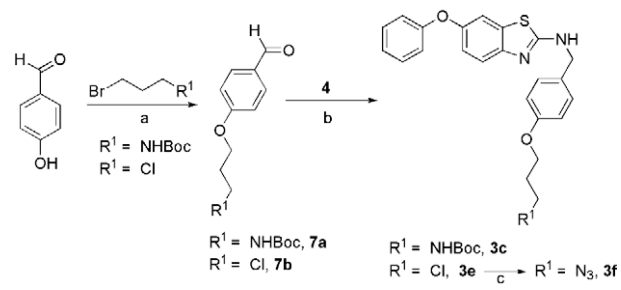
Scheme 1. Synthesis of truncated analogues **2a–b** and **3a–c**. Reagents and conditions: (a) (i) 4-hydroxybenzaldehyde, dry toluene, 4 Å molecular sieves, reflux (ii) ethanol, $NaBH_4$, reflux, 70%; (b) EDCI, HOBt, NMM, DMF, **6**, 74 °C, 66%; (c) $N_2H_4.H_2O$, EtOH, THF, reflux, 56%; (d) *N,N'*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, DCM, Et_3N, 81%; (e) 2 M HCl/EtOAc, rt, 53%; (f) K_2CO_3, DMF, 50 °C, 54%.

A significant improvement to our earlier work is the preparation of the key precursor phenol **5**, prepared from commercially available 4-hydroxybenzaldehyde. Functionalization of this precursor allows the attachment of various substituents that may not survive the reductive amination step. For example, we previously prepared the analogue of **3a** where the amine was protected with a phthalimide group.[17] Under non-anhydrous conditions, the phthalimide ring was partially cleaved. This did not occur with the BOC protecting group as shown in Scheme 2 for an alternative method of preparing **3a**.

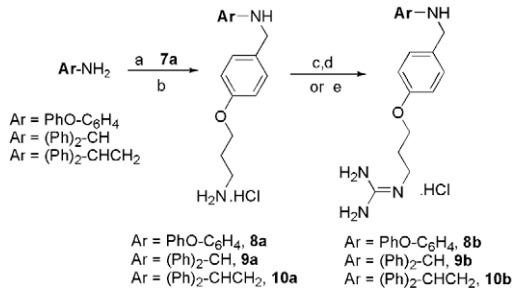
Compounds **3e** and **3f** were prepared by the methods outlined in Scheme 2. These compounds are precursors that should enable the generation of a large number of analogues.

In an effort to further simplify the structure of the mimic and investigate the role of the benzothiazole ring system, we prepared analogues of **3a–b** where the benzothiazole group was replaced with different aromatic ring systems as shown in Scheme 3. Aldehyde **7a**, (Scheme 2) an alternative precursor to compound **3c**, was utilized as the precursor to compound series **8–10** (Scheme 3). The use of the BOC group is significant for the preparation of compounds **8–10** as the aromatic amines used caused ring-opening of the phthalimide group. Compounds **8a** and **8b** are simplified analogues of **3a** and **3b**, respectively, which do not contain the benzothiazole ring system. Analogues **9b–c** and **10b–c** were designed to investigate the effects of the diphenylmethylene group as this moiety is prevalent in several calcium channel blockers, including NMED-160. For this series of compounds we investigated the use of guanidinium carboxamidine hydrochloride reagent to install the guanidinium group for **8d** and **10b**. This reagent was used instead of the bis-BOC protected guanidinium as this reagent avoids the need for a final acidic deprotection step which may cleave the *N*-benzyl bond. In general, we found that the deprotection of both BOC groups to give **8b–10b** required prolonged treatment with 4 M HCl in dioxane.

Compounds **2a–b, 3a–f, 8a–10a** and **8b–10b** were tested for N-type calcium channel inhibition using radioligand-binding displacement assay as previously reported.[17] As shown in Table 1, the truncated Tyr13- Lys2 mimic **2a** and the corresponding guanidylated analogue **2b** were inactive. However, compound **3b** gave an EC_{50} of 5.8 μM which is only 2.7 μM lower than the preferred lead compound **1b**. This small reduction in activity is compensated by a significant drop in molecular weight by 193 g/mol to 448 g/mol compared to the original lead of 641 g/mol. In addition, the time and cost of synthesis has also been reduced as compound **3b** can be prepared in six steps, compared to 13 steps for compound **1c**. Interestingly, compound **3a**, which contained an amino



Scheme 2. An alternative method for the preparation of **3c**, and precursors **3e** and **3f**. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C, 91%; (b) (i) dry toluene, 4 Å molecular sieves, reflux; (ii) dry ethanol, $NaBH_4$, reflux, 87%; (c) Bu_4NF, $(CH_3)_4SiN_3$, THF, 50 °C, 88%.



Scheme 3. Synthesis of compounds **8–10**. Reagents and conditions: (a) (i) dry toluene, 4 Å molecular sieves, reflux (ii) dry ethanol, $NaBH_4$, reflux, 69–91%; (b) 2 M HCl/EtOAc, 81–100%; (c) *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, DCM, Et_3N, 58–62%; (d) 4 M HCl in dioxane, 0 °C, 81–98%; (e) guanidinium carboxamidine hydrochloride, DMF, Hunig's base, 47–85% (for **8a** and **10b**).

Table 1
N-type calcium channel binding results

Compound	EC_{50}[a] (μM)
1a	3.18
1b	3.08
1c	1.78
2a	>100
2b	33
3a	76
3b	5.8
8a	34
8b	21
9a	>100
9b	23
10a	>100
10b	12

[a] EC_{50} values were calculated from dose–response curves, with each data point recorded in triplicate.

group instead of a guanidyl group did not show appreciable activity. This highlights the importance of the guanidyl group and also may indicate that the higher basicity is required to retain activity. The comparison between **2b** and **3b** may indicate that less rigidity is required since **3b** contains a more freely-rotating *N*-benzyl bond instead of an amide bond. Furthermore, **3b** which has the propylguanidyl at the *para*-position of the ring gives a compound which is more extended than the *ortho*-substituted **2b**. The larger span of **3b** could be important as the compound is expected to mimic a very large peptide. The dose–response curves for compounds **2b** and **3a–b** are shown in Figure 2.

We also tested some of the precursor compounds. Compound **3c** was prepared in our previous work but not tested for activity.[17] The hydrochloride salt of this BOC-protected compound,

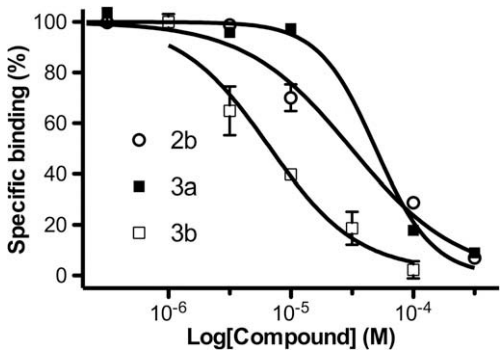


Figure 2. Dose–response curves for **2b**, **3a** and **3b**. 95% Confidence intervals are shown.

prepared by addition of 1 molar equivalent of HCl in EtOAc did not show any activity in the assay. The precursors **3e**, **3f** and **5** were also inactive.

The fact that none of the compounds with varied aromatic cores **8–10** were active suggests that the rigidity and/or hydrogen-bonding interactions of the benzothiazole ring system are important for activity. Additionally, the removal of the benzothiazole-fused system shortens the span of the molecule towards any putative binding pockets. An increase in bulk due to the large diphenylmethyl groups in **9a–b** or **10a–b** did not give any appreciable activity. However, the presence of a guanidyl side chain in **9b** and **10b** gave detectable micromolar activity compared to the amino analogues **9a** and **10a**. It could be argued that guanidylated substituent possesses higher basicity and a more delocalized charge that could enhance interaction at the binding site.

In summary, we have designed and synthesized compounds **2a–b** and **3a–b** which are 'truncated' analogues of a previously reported lead compound.[17] Compound **3b** was found to retain significant activity despite the loss of nearly 200 g/mol in molecular weight compared to the original lead compounds **1b–c**. The discovery of this compound opens a new avenue for our research where future analogues of **3b** are not only more drug-like, but these analogues can be accessed in 5–6 steps instead of 12–13 steps (for **1a–c**). The reduction of molecular weight and reduction in number of synthetic steps are important and necessary advancements for the translation of this research into a pragmatic drug development setting. The various improvements to the previously published[17] original synthetic scheme that are reported here will also facilitate the efficient preparation of analogues of **3b**.

Supplementary data

Supplementary data (details of experimental procedures and spectroscopic data for all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.130.

References and notes

1. Morishita, M.; Peppas, N. A. *Drug Discovery Today* **2006**, *11*, 905.
2. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
3. Olivera, B. M. *J. Biol. Chem.* **2006**, *281*, 31173.
4. Schroeder, C. I.; Doering, C. J.; Zamponi, G. W.; Lewis, R. J. *Med Chem.* **2006**, *2*, 535.
5. McGivern, J. G. *Drug Discovery Today* **2006**, *11*, 245.
6. Miljanich, G. P. *Curr. Med. Chem.* **2004**, *11*, 3029.
7. Ellis, D. J.; Dissanayake, S.; McGuire, D.; Charapata, S. G.; Staats, P. S.; Wallace, M. S.; Grove, G. W.; Vercruysse, P. *Elan Study 95–002 Group, Neuromodulation* **2008**, *11*, 40.
8. Lynch, S. S.; Cheng, C. M.; Yee, J. L. *Ann Pharmacother.* **2006**, *40*, 1293.
9. Staats, P. S.; Yearwood, T.; Charapata, S. G.; Presley, R.; Wallace, M. S.; Byas-Smith, M.; Fisher, R.; Bryce, D. A.; Mangieri, E. A.; Luther, R. R.; Mayo, M.; McGuire, D.; Ellis, D. *J. Am. Med. Assoc.* **2004**, 63.
10. Wermeling, D.; Drass, M.; Ellis, D.; Mayo, M.; McGuire, D.; O'Connell, D.; Hale, V.; Cho, S. *J. Clin. Pharma.* **2003**, 624.
11. Dabak, K. *Turk. J. Chem.* **2002**, *26*, 955.
12. Menzler, S.; Bikker, J. A.; Horwell, D. C. *Tetrahedron Lett.* **1998**, *39*, 7619.
13. Menzler, S.; Bikker, J. A.; Suman-Chauhan, N.; Horwell, D. C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 345.
14. http://www.contractpharma.com/news/2007/08/08/neuromed%2c_merck_discontinue_pain_drug_candidate. Accessed 20 July 2008.
15. Cao, Y.-Q. *Pain* **2006**, *126*, 5.
16. http://www.neuromed.com, Accessed 1 Sept 2008.
17. Baell, J. B.; Duggan, P. J.; Forsyth, S. A.; Lewis, R. J.; Lok, Y. P.; Schroeder, C. I. *Bioorg. Med. Chem.* **2004**, *12*, 4025.
18. Baell, J. B.; Duggan, P. J.; Forsyth, S. A.; Lewis, R. J.; Lok, Y. P.; Schroeder, C. I. *Tetrahedron* **2006**, *62*, 7284.
19. Baell, J. B.; Duggan, P. J.; Lok, Y. P. *Aust. J. Chem.* **2004**, *57*, 179.
20. Duggan, P. J.; Faber, J. M.; Graham, J. E.; Lewis, R. J.; Lumsden, N. G.; Tuck, K. L. *Aust. J. Chem.* **2008**, *61*, 11.
21. Baell, J. B.; Forsyth, S. A.; Gable, R. W.; Norton, R. S.; Mulder, R. J. *J. Comput. Aided Mol. Des.* **2001**, *15*, 1119.