Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2673–2676
Bioorganic & Medicinal Chemistry Letters

Design, synthesis and evaluation of potential inhibitors of HIV gp120–CD4 interactions

Cyrille Boussard,[a] Thomas Klimkait,[b] Naheed Mahmood,[c] Martin Pritchard[d] and Ian H. Gilbert[a,*]

[a]*Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK*
[b]*Institute for Medical Microbiology, University of Basel, CH-4003 Basel, Switzerland*
[c]*Turner Building, St Batholomews and The Royal London School of Medicine and Dentistry, Turner Street, London E1 2AD, UK*
[d]*Parke–Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Road, Cambridge, UK*

Received 24 November 2003; revised 2 February 2004; accepted 4 February 2004

Abstract—This paper describes an approach to prevent HIV-cell fusion by disrupting the interaction between HIV protein gp120 and CD4 receptor. The CD4 residues Phe43 and Arg59 make important interactions with gp120. Small molecule analogues were made to mimic the crucial features of these residues. The analogues were assayed using a cellular 'FIGS' assay to measure inhibition of cell fusion and caused some inhibition.

1. Introduction

HIV-AIDS is a major cause of mortality and due to the potential for development of resistance to existing therapies,[1] discovery of new therapeutic agents is crucial. While current therapies are based on inhibition of reverse transcriptase and protease,[2] we were interested in investigating the fusion step as a potential drug target. Entry of the virus into the cell is a complex process. Initially, the viral surface protein gp120 interacts with the cellular receptor CD4.[3] This causes a change in the conformation of gp120,[4] which binds to a second cellular receptor of the family of chemokine receptors,[5] allowing the HIV protein gp41 to penetrate the cell membrane, leading to membrane fusion.[6]

A number of groups have been working on developing inhibitors of fusion.[5,7] Much of this work has concentrated on development of antagonists of chemokine receptors CCR5[8] and CXCR4.[2,9,10] More recently, new compounds targeting the fusion process, have shown promising results in clinical trials, leading to the approval of the first entry inhibitor (Enfuvirtide, a linear 36 amino acid peptide mimetic of the HR2 region of gp41) by the US FDA in March 2003.[11,12] As an alternative approach, we decided to see if we could design small compounds, which bind to HIV gp120 and prevent interaction with CD4.

The gp120–CD4 interaction is an example of a protein–protein interaction. Binding occurs often over a relatively large surface area and identifying specific binding sites is crucial in the development of a new protein antagonist.[13] The crystal structure of gp120 bound to CD4 has been solved[14] and indicates that two residues from CD4 seem to be of crucial importance in interaction with gp120; these are Phe43 and Arg59. For these residues, which are spatially in close proximity, the important interactions are through the phenyl ring and the guanidine group, respectively.[15]

In a previous paper, the identification of lead peptides as potential inhibitors of gp120–CD4 interactions was described.[16] A series of tri- and tetra-peptides based around the amino acids arginine and phenylalanine and designed to mimic the original spatial arrangement of the gp120 residues Arg59 and Phe 43, were shown to have moderate inhibition of this interaction in a cell-free assay. Biological testing of these libraries led to IC_{50} values from 0.17 mM for HPhe-Asp-$ArgNH_2$ to 9.75 mM for HPhe-Gly-Phe-$ArgNH_2$. It has been noticed that shorter peptides showed higher activity and that constrained and hydrophobic residues enhanced the inhibitory properties of these peptides.

* Corresponding author. Fax: +44-29-2087-4149; e-mail: gilbertih@cardiff.ac.uk

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.02.091

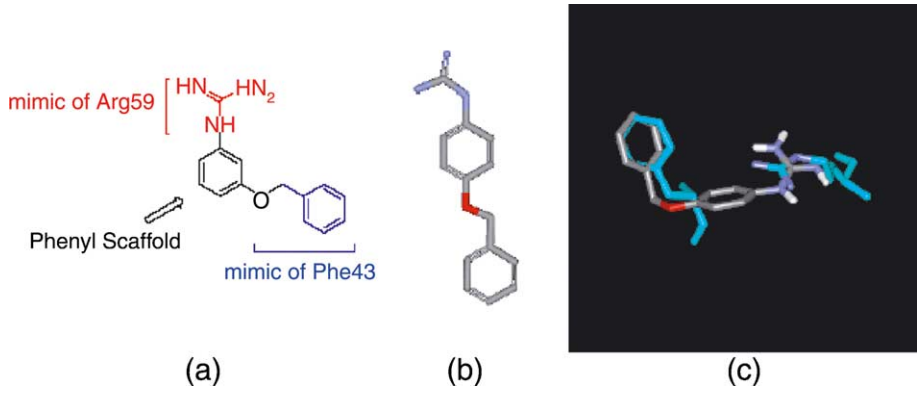


Figure 1. (a) Molecules designed to mimic the relative orientation of Phe43 and Arg59 in their binding to gp120. (b) The structure of the designed molecule A minimised using Monte Carlo conformational searching, followed by minimisation (Macromodel 6.0). (c) Superimpositions of original CD4 residues Phe43 and Arg59 (original spatial arrangement, in blue) and designed peptoid structure A (in grey) after minimisation (using Macromodel®).

As peptides are limited for use as drugs because of their susceptibility to peptidase degradation, poor oral bioavailability and their difficulty in crossing the blood–brain barrier, development of nonpeptidic compounds by a peptoid approach was envisaged. This paper describes the design, synthesis and biological evaluation of new potential inhibitors of the HIV gp120–CD4 interaction by using the previous reported work.

2. Compound design

To inhibit the gp120–CD4 interaction, a crucial feature of the designed compounds was to hold the phenyl ring and the guanidine groups in their correct stereochemical alignment. A good mimic of the stereochemical orientation of these groups was obtained by attaching the phenyl and the guanidine groups to a benzene ring. (Fig. 1a). This was confirmed in silico by performing Monte–Carlo conformational searching followed by minimisation (Fig. 1b). The lowest energy structure was then superimposed onto the coordinates Phe43 and Arg59 as found in CD4 in the gp120–CD4 complex (Fig. 1c) and gave a good fit.

Owing to the preliminary activity of the lead compounds (compound **3** and **12a**), a number of variations of the structure of the lead compound were proposed; the guanidine moiety could be replaced by the amidine group, which is a potential bio-isostere[17] and various substituents could be added to the benzyloxy moiety to change electronic and steric properties.

3. Chemistry[18]

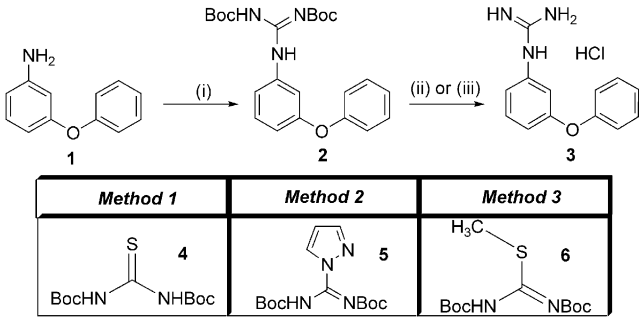
3.1. Synthesis of the guanidine series

Investigation of potential methods for guanylation were performed on the commercially available 3-phenoxyaniline. The use of three guanylating reagents on the 3-phenoxyaniline **1** gave, respectively, moderate yields with the diprotected thiourea **4**[19] and the bis-Boc protected guanyl pyrazole **5**;[20] and quantitative yields with the bis-Boc protected *S*-methyl isothiourea **6**[21] (Scheme 1).

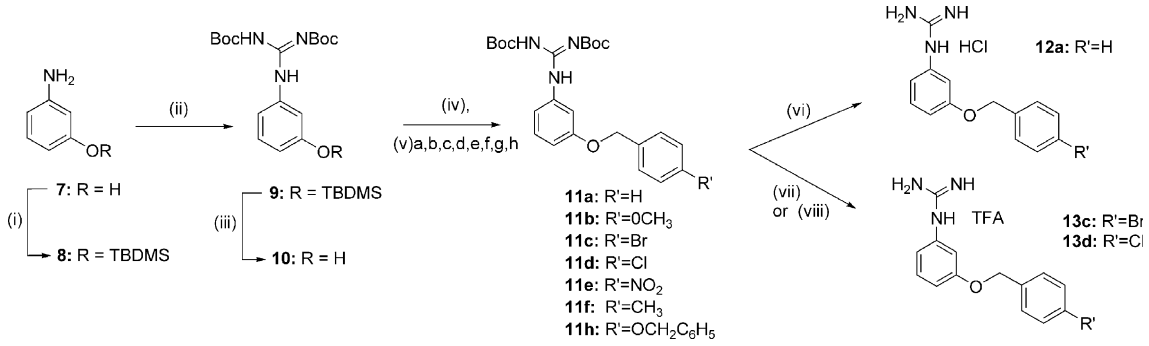
The final step of the guanidine synthesis was the deprotection of the guanidine moiety. Boc deprotection in acidic conditions using trifluoroacetic acid (TFA) in DCM[22] was carried out and led to partial cleavage of the Boc groups, giving a mixture of the mono-protected form and other impurities. However, the method of Miel and Rault[23] using tin(IV) chloride followed by trituration with ether, yielded the desired product **3** without any of the mono-Boc guanidine intermediate.

3.2. SAR studies

Having established a suitable pathway for the preparation of the phenoxy guanidine derivatives, this methodology was applied to the corresponding benzyl derivatives. In order to probe interactions of the benzyl moiety with the protein, it was decided to prepare a broad series of compounds with different substituents on the benzyl ring.



Scheme 1. Reagents and conditions: (i) method 1: **4**/Mukaiyama's reagent/TEA/DCM, 46%; method 2: **5**/anhydrous THF, 55%; method 3: **6**/THF, 95%; (ii) $SnCl_4$/AcOEt, MeOH/Et_2O, 50%; (iii) TFA/DCM, partial cleavage.



Scheme 2. Reagents and conditions: (i) TBDMSCl/anhyd pyridine, 90%; (ii) method 3: Guanylating reagent **6**/THF, 95%; (iii) TBAF/DCM, 95%; (iv) NaH/DMF; (v) a: benzyl chloride; b: 4-methoxybenzyl bromide, 80%; c: 4-bromobenzyl bromide, 40%; d: 4-chlorobenzyl chloride, 88%; e: 3-nitrobenzyl bromide, 35%; f: 4-methylbenzyl bromide, 46%; g: 4-chloromethyl phenyl acetate, no yield available; h: 4-benzyloxybenzyl chloride, 86%; (vi) $SnCl_4$/AcOEt, MeOH/Et_2O, 95% over two steps for **12a**; (vii) for **13c**: 2 equiv TFA/DCM, quantitative; (viii) for **13d**: 10 equiv TFA/DCM, 89%.

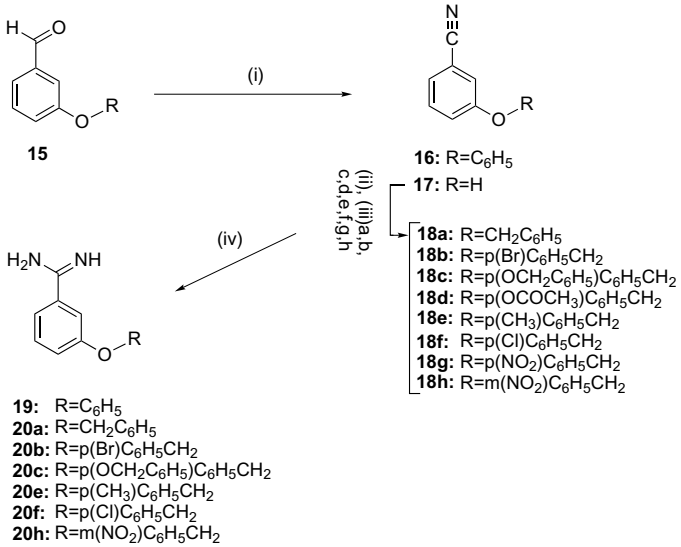
The most efficient synthesis would proceed via a common intermediate, which would allow attachment of a variety of substituted benzyl groups. Starting from the commercially available 3-aminophenol **7**, the protection of the hydroxyl in position 3 was achieved by action of TBDMSCl in pyridine. Then the bis-Boc protected guanidine derivative **9** was prepared via the use of the bis-Boc protected *S*-methyl isothiourea **6** in high yield (95%). The last step of the preparation of compound **10** was the cleavage of the *tert*-butyldimethylsilyl group, achieved by using TBAF in DCM (Scheme 2).

The next step was to introduce the substituted benzyl groups. A variety of substituents were introduced onto the phenyl ring, mainly in the *para*-position. These were selected to give a variety of types of substituent—electron withdrawing, electron donating, bulky and allowing further additional chemical modifications such as reduction or acylation. Starting from compound **10**, derivatives **11a**–**h** were prepared by alkylation of the hydroxyl using either the appropriately substituted benzyl bromide or chloride[22] in 35–88% (Scheme 2).

The Boc-protected guanidines were deprotected. Initially tin(IV) chloride was used and yielded **12a** but **11b**,**c**,**e** decomposed under these conditions. Use of excess of TFA (10 equiv) in DCM yielded the chloroderivative **13d** but led to decomposition of **11b**,**f** and **h**. Finally 2 equiv of TFA yielded the bromoderivative **13c** but led to decomposition of **11b**,**e**,**f** and **h** (Scheme 2).

3.3. Amidine synthesis (Scheme 3)

Amidines are an isostere of the guanidine group. They were prepared by treatment of aldehyde **15** with hydroxylamine hydrochloride in ethanol in presence of HCl to give a good yield (75%) of the nitrile **16**.[24] The nitriles **16** were reacted with the etherate complex of a lithium salt of hexamethyldisilazane ($LiN(SiCH_3)_2$



Scheme 3. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$/EtOH/HCl/heat, 75%; (ii) NaH/DMF; (iii) a: benzyl bromide, 93%; b: 4-bromobenzyl bromide, 75%; c: 4-benzyloxybenzyl chloride, 84%; d: 4-chloromethyl phenyl acetate, 91%; e: 4-methylbenzyl bromide, 59%; f: 4-chlorobenzyl chloride, 68%; g: 4-nitrobenzyl bromide, 15%; h: 3-nitrobenzyl bromide, 92%; (iv) 4 equiv of corresponding compounds **16**, **18a**–**c**,**e**,**f** and **h** and 2 equiv of $LiN(SiCH_3)_2Et_2O$ prepared in situ by addition of *n*-BuLi to a solution of hexamethyldisilazane in Et_2O at 0 °C, HCl, NaOH, 90%–95%.

Et_2O)[25] to give the free amidine **19** pure with excellent yield (90%, Scheme 3).[25]

Again the SAR of the phenyl ring was probed using substituted benzyl derivatives. These were prepared by alkylation of 3-cyanophenol **17** with various substituted benzyl chlorides followed by treatment with lithium hexamethyldisilazane. The acetyl **18d** and *para*-nitro **18g** derivatives did not yield the required amidines.

4. Biological assays

Compounds were principally evaluated using a cellular assay to study cell fusion, the so-called FIGS assay.[26] Essentially in this assay HIV infected cells are cultured in the presence of a reporter cell. The reporter cell expresses a β-galactosidase when fusion occurs with an HIV-infected cell. However, when fusion is prevented, the expression is prevented. The levels of expression of β-galactosidase are measured using a colourimetric assay. Encouragingly, compounds **3** and **12a** showed inhibition of fusion with IC_{50} of 167 and 131 μM, respectively. However the remaining compounds showed cytotoxicity at levels where activity was found, minimising a 'therapeutic window'.

Compounds **3** and **12a** were then investigated further and assayed using an ELISA assay[16] to detect the inhibition of the HIV-specific CD4–gp120 interaction using recombinant protein. The compounds showed weak inhibition at concentrations of 5 mM. They were also tested for their activity against HIV-1 IIIB infections of C8166 cells.[27] In this last assay the compounds gave some anti-HIV activity ($EC_{50} \sim 4$–8 μM), although with a low therapeutic index ($TC_{50} = 20$ and 40 μM, respectively).

Compounds **13c**,**d**, **19**, **20a**–**c**,**e**,**f** and **h** were all found to be inactive at 10 μM and toxic at 100 μM in the FIGS assay and did not show any antiviral activity.

5. Conclusion

In this paper we have focused on preparation of small, nonpeptide analogues that contain functionality corresponding to the side chains of Phe43 and Arg59. Two of the compounds prepared showed some activity in a reporter cell assay for prevention of HIV—cellular fusion and weak activity in an ELISA assay to measure CD4–gp120 interaction using recombinant protein. These compounds could be a starting point for further synthesis.

The compounds showed higher potency in an infection assay, than in the fusion assay characterised by less stringent conditions. However, these data taken together suggest that the main mode of antiviral action of the compound is not by inhibition of CD4–gp120 interaction.

Acknowledgements

We would like to acknowledge Ingrid Ziekau and Doris Löw for excellent technical assistance, Parke–Davies and the Welsh School of Pharmacy for funding and the EPSRC National Mass Spectrometry Centre at Swansea for accurate mass spectrometry.

References and notes

1. http://www.WHO.org
2. Cooley, L. A.; Lewin, S. R. *J. Clin. Virol.* **2002**, 1–12.
3. Dalgeish, A. G.; Beverley, P. C.; Clapham, P. R.; Crawford, D. H. *Nature* **1984**, *312*, 763–767.
4. Wyatt, R.; Kwong, P. D.; Desjardins, E.; Sweet, R. W.; Robinson, J.; Hendrickson, W. A.; Sodroski, J. G. *Nature* **1998**, *393*, 705–711.
5. LaBranche, C. C.; Galasso, G.; Moore, J. P.; Bolognesi, D. P.; Hirsch, M. S. *Antiviral Res.* **2001**, *50*, 95–115.
6. Yachou, A.; Sékaly, R. P. *Biochem. Biophys. Res. Commun.* **1999**, *265*, 428–433.
7. Jiang, S. B.; Zhao, Q.; Debnath, A. K. *Curr. Pharm. Des.* **2002**, *8*, 563–580.
8. Tremblay, C. L.; Giguel, F.; Kollmann, C.; Guan, Y. B.; Chou, T. C., et al. *Antimicrob. Agents Chemother.* **2002**, *46*, 1336–1339.
9. Tamamura, H.; Omagari, A.; Oishi, S.; Kanamoto, T.; Yamamoto, N.; Peiper, S. C.; Nakashima, H.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2633–2637.
10. Ruff, M. R.; Melendez-Guerrero, L. M.; Yang, Q.; Ho, W.; Mikovits, J. W., et al. *Antiviral Res.* **2001**, *52*, 63–75.
11. Fletcher, C. V. *The Lancet* **2003**, *361*, 1577–1578.
12. Labonte, J.; Lebbos, J.; Kirkpatrick, P. *Nature Rev. Drug Discov.* **2003**, *2*, 345–346.
13. Toogood, P. L. *J. Med. Chem.* **2002**, *45*, 1543–1558.
14. Kwong, P. D.; Wyatt, R.; Robinson, J.; Sweet, R. W.; Sodroski, J.; Hendrickson, W. A. *Nature* **1998**, *393*, 648–659.
15. Moore, J. P.; Binley, J. *Nature* **1998**, *393*, 630–631.
16. Boussard, C.; Doyle, V. E.; Mahmood, N.; Klimkait, T.; Pritchard, M.; Gilbert, I. H. *Eur. J. Med. Chem.* **2002**, *37*, 883–890.
17. Magalhes, A.; Monteiro, M. R.; Magalhes, H. P.; Mares-Guia, L.; Rogana, E. *Toxicon.* **1997**, *35*, 1549–1559.
18. Compounds were characterised by ^{1}H, ^{13}C, mass spectrometry and accurate mass analysis.
19. Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540–1542.
20. Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389–3392.
21. Miel, H.; Rault, S. *Tetrahedron Lett.* **1998**, *39*, 1565–1568.
22. Greene, T. W.; Peter, G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley-Interscience: New York, USA, 1991.
23. Miel, H.; Rault, S. *Tetrahedron Lett.* **1997**, *38*, 7865–7866.
24. Findlay, J. A.; Tang, C. S. *Can. J. Chem.* **1967**, *45*, 1014–1015.
25. Lappert, M. F.; Slade, M. J.; Singh, A. *J. Am. Chem. Soc.* **1983**, *105*, 302–304.
26. Klimkait, T.; Stauffer, F.; Lupo, E.; Sonderegger-Rubli, C. *Arch. Virol.* **1998**, *143*, 2109.
27. *HIV, Biochemistry, Molecular Biology and Drug Discovery*; Karn, J., Ed.; Oxford University Press: New York, USA, 1995; Vol. 2.