Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1509–1513
Bioorganic & Medicinal Chemistry Letters

Lysine derivatives as potent HIV protease inhibitors. Discovery, synthesis and structure–activity relationship studies

Abderrahim Bouzide,[a,*] Gilles Sauvé[a] and Jocelyn Yelle[b]

[a]*Pharmacor Inc., 535, Cartier West Blvd., Laval, Que, Canada H7V 3S8*
[b]*Procyon Biopharma Inc., 1650, Trans Highway, Suite 200, Dorval, Que, Canada H9P 1H7*

Received 3 December 2004; revised 20 December 2004; accepted 21 December 2004
Available online 25 January 2005

Abstract—A screening assay program on HIV-protease was carried out on more than fifty commercially available N-protected amino acids and has revealed that those with a long side chain such as lysine, ornithine and arginine exhibited significant inhibition of HIV protease enzyme. The presence of an Fmoc group was found to be essential to obtain micromolar inhibitors and the addition of an alkyl group at the Nα-position resulted in the discovery of the lead compound **11** displaying a 5 nM inhibition constant. Although this new inhibitor series is not categorized among those mimicking the substrate with a non-hydrolyzable transition-state isoster, it was found very specific to inhibit HIV protease enzyme in comparison to the mammalian aspartyl proteases pepsin, renin and cathepsin. Furthermore, these inhibitors did not show any cytotoxicity at a concentration below 75 μM.

1. Introduction

Since its discovery two decades ago, acquired immunodeficiency syndrome (AIDS) has increased to epidemic proportions throughout the world, affecting more than 40 million people today and killing so far more than 22 million (UNAIDS, 2004).[1] Among various strategies to combat this devastating disease, therapeutic inhibition of the virally encoded human immunodeficiency virus (HIV) protease became an attractive target. Its inactivation leads to the formation of immature and non-infectious virions.[2] A number of reports on the design and synthesis of HIV protease inhibitors have been published.[3] In general, in these inhibitors, the scissile bond has been replaced by a non-hydrolyzable transition-state isoster. Unfortunately most of these peptidomimetic HIV protease inhibitors retain a substantial amount of peptide character, and as a result, their oral bioavailability is low and a short plasma half-time is observed.

Despite the success of the FDA-approved HIV protease inhibitors,[4] saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir, there is an urgent need for new and improved drugs to target HIV protease due to increasing viral resistance, a matter that is now of great concern.[5] The high cost of synthesis is today a barrier to the widespread use of the currently approved protease inhibitors, notably in the less developed countries. It is thus important that new improved protease inhibitors accessible at low cost are developed.[6]

2. Lead compound discovery

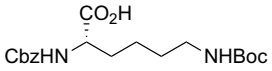
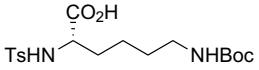
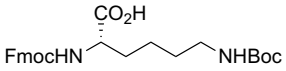
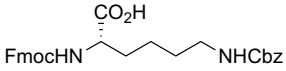
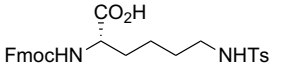
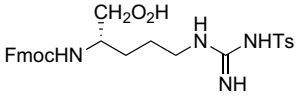
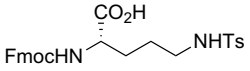
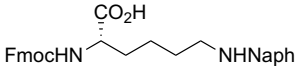
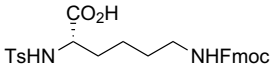
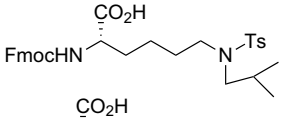
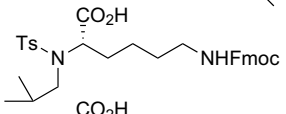
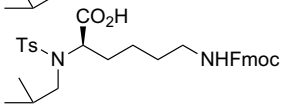
During a continuous effort in our laboratory to develop small molecules with low cost as potent HIV protease inhibitors, we initiated a broad screening program by testing more than 50 commercially available N-protected amino acids.[7] It was noticed that those with a long side chain, such as lysine, ornithine and arginine, showed decent micromolar inhibition constants (K_i), especially when they are protected with bulky protecting groups such us an Fmoc carbamate.

Some commercially available amino acids that showed inhibitions are presented in Table 1. Amino acids protected with small protecting groups such as Boc- or Cbz-carbamates did not exhibit any activity (**1** and **2**). However, the presence of an Fmoc group whether at the Nα-position or at the end of the side chain was always accompanied with some activity. The first

* Corresponding author. Present address: Neurochem, 275, Armand Frappier Blvd., Laval, Que, Canada H7V 1B7; e-mail: abouzide@neurochem.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.068

Table 1. HIV protease inhibition data for N-protected amino acids[8]

No	N-Protected amino acid	K_i (μM)[a]
1		>100
2		>100
3		28.0
4		21.5
5		17.4
6		14.2
7		26.2
8		10.5[b]
9		8.0
10		2.0
11		0.005
12		0.33

[a] All values are the average of at least two runs.
[b] Naph = 1-naphtyl–SO_2.

encouraging result was exhibited by the commercially available Nα-Fmoc-*Nε*-Boc-L-lysine **3**, which displayed a 28 μM K_i. Compounds with Nε-Cbz carbamate **4** or tosylamide **5** instead of Boc group displayed the same potency. The Nα-Fmoc-*Nω*-tosylarginine **6**, which was reported during the progress of this project by Ortiz de Montellano and co-workers[9] showed a similar potency than **5** and slight loss in activity was noticed in the ornithine analogue **7** (vide infra). On the other hand, the 10 μM potency displayed by inhibitor **8** containing a naphtylsulfonamide moiety on the side chain was a worthy indication that compounds attached with bulkier groups at the Nε-position might exhibit some optimization. In fact, compound **9** where the Fmoc group was switched from the Nα- to the Nε-position exhibited an affinity 2-fold higher than its analogue **5**. Based on these valuable indications revealed by the commercially available amino acids, we started the preparation of our 'home-made' inhibitors. First we prepared compound **10**, which is a derivative of **5** bearing an additional isobutyl group. Interestingly, this inhibitor exhibited an 8-fold increase in potency compared to **5**. After several modifications, we gratifyingly found that the addition of an isobutyl group at the Nα-position while keeping the Fmoc group at Nε-position led to a dramatic enhancement in potency resulting in the discovery of the lead candidate **11**.[10] To the best of our knowledge, this is the first example of a single amino acid that inhibit HIV protease in nanomolar range. As we anticipated and hoped,[11] the D-isomer **12** displayed a potency 67 fold lower than **11** indicating that the natural L-stereochemistry is the preferred one.

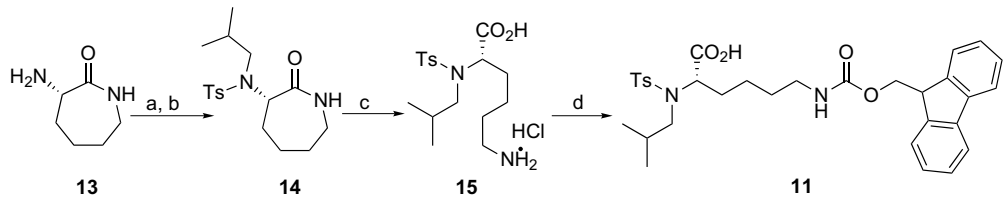
The synthesis of **11** was completed in four steps starting from the commercially available α-amino-caprolactam **13** (Scheme 1).[12] Reductive N-alkylation of **13** in the presence of isobutyraldehyde followed by N-tosylation afforded the corresponding sulfonamide **14** in excellent yield. Ring opening hydrolysis proceeded quantitatively and without any detectable racemization to afford amino acid **15**. Treatment of **15** with FmocOSuc under aqueous conditions gave the desired product **11** in high yield. Initial attempts to use FmocCl reagent were unfruitful and poor yields were obtained.

3. Structure–activity relationship

To investigate the structure–activity relationships (SAR) of this new HIV protease inhibitor series, we synthesized and examined various analogues of **11** by modifying the Nα- and Nε-substituents as well as the side chain length and the carboxylic acid function.

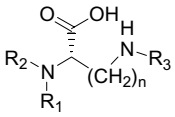
3.1. Modifications of Nα-substituents

Compounds **16**–**28** in Table 2 were prepared in the same manner as for **11**. Replacement of the isobutyl group in **11** with benzyl (**16**), 3-ethyl-butyl (**17**) or 3-methyl-butyl



Scheme 1. Reagents and conditions: (a) *i*-PrCHO, MeOH, $NaCNBH_3$, pH 4; (b) TsCl, NEt_3, CH_2Cl_2, 88% (two steps); (c) 6 M HCl, reflux; (d) FmocOSuc, K_2CO_3 (1 M), THF 82% (two steps).

Table 2. Structure and HIV Protease inhibitory activities of N-functionalized natural and unnatural amino acids[8]



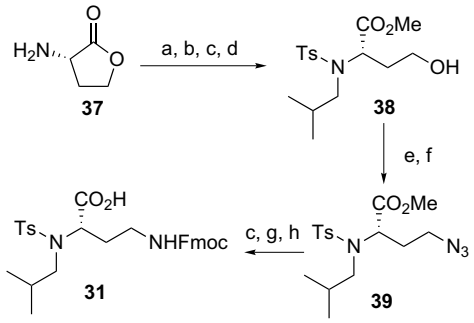
No	R_1	R_2	R_3	n	K_i (nM)[a]
11	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	4	5.0
16	$C_6H_5CH_2$–	4-$MeC_6H_4SO_2$	Fmoc	4	19.6
17	Et_2CHCH_2–	4-$MeC_6H_4SO_2$	Fmoc	4	15.2
18	$MeEtCHCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	4	13.5
19	*c*-$C_3H_5CH_2$–	4-$MeC_6H_4SO_2$	Fmoc	4	3.9
20	*c*-$C_5H_9CH_2$–	4-$MeC_6H_4SO_2$	Fmoc	4	5.2
21	*i*-$PrCH_2$–	$C_6H_5SO_2$	Fmoc	4	18.7
22	*i*-$PrCH_2$–	COC_6H_5	Fmoc	4	>300
23	*i*-$PrCH_2$–	4-*t*-$BuC_6H_4SO_2$	Fmoc	4	257
24	*i*-$PrCH_2$	1-Naphtyl-SO_2	Fmoc	4	54.9
25	*i*-$PrCH_2$–	4-$FC_6H_5SO_2$	Fmoc	4	17.4
26	*i*-$PrCH_2$–	2-ThiopheneSO_2	Fmoc	4	42
27	*i*-$PrCH_2$–	4-$NO_2C_6H_4SO_2$	Fmoc	4	7.2
28	*i*-$PrCH_2$–	4-$NH_2C_6H_4SO_2$	Fmoc	4	2.1
29	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	3	180
30	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	5	>250
31	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	2	>250
32	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Fmoc	1	>250
33	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	CO–CH_2–9-Fluorene	4	89.7
34	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	CO–9-Fluorene	4	105
35	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	*t*-Boc	4	>250
36	*i*-$PrCH_2$–	4-$MeC_6H_4SO_2$	Cbz	4	>250

[a] All values are the average of at least two runs.

(**18**) moieties resulted in an alteration in activity, while slight improvement was noticed in the case of cyclopropylmethyl group (**19**). Of particular interest, the comparison between the benzenesulfonamide **21** and benzenecarboxamide **22** showed that while the first maintain some potency, the second lost completely its activity, suggesting that in addition to its hydrogen bond acceptor character, –SO_2– group may provide an appropriate conformation essential for the potency of this series. The utilization of more sterically demanding sulfonamide such as *tert*-butyl benzenesulfonamide **23** or naphtylsulfonamide **24** resulted in substantial loss in activity. An 8-fold decrease in affinity was noticed when the tosyl was replaced with a thiophene group as in **26** while 2-fold improvement in potency was attained by replacing the 4-Me group of **11** with 4-NH_2 as in **28**.

3.2. Modification of the side chain length

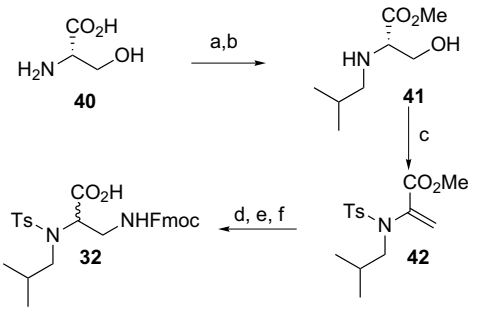
Compounds **29** and **30** were prepared, respectively, from ornithine and racemic homolysine[13] as described in Ref. 12. Compound **31** was prepared as described in Scheme 2. Reductive N-alkylation of the commercially available γ-lactone **37** followed by sulfonamidation, ring opening hydrolysis and esterification in the presence of diazomethane afforded **38**. Tosylation of the alcohol and displacement of the resulting tosylate with sodium azide gave the corresponding azide **39**. Alkaline hydrolysis of the methyl ester followed by a catalytic hydrogenation gave the corresponding amino acid, which in the presence of FmocOSuc afforded **31** in good yield.



Scheme 2. Reagents and conditions: (a) *i*-PrCHO, MeOH, $NaCNBH_3$; (b) TsCl, NEt_3, CH_2Cl_2, 82% (two steps); (c) NaOH (1 M), THF; (d) CH_2N_2, Et_2O (77% two steps); (e) TsCl, NEt_3, CH_2Cl_2; (f) NaN_3, DMF 66% (two steps); (g) H_2, Pd/C, EtOAc; (h) FmocOSuc, K_2CO_3 (1 M), THF 73% (three steps).

Compound **32** was prepared in six steps from L-serine **40** as outlined in Scheme 3. Its esterification and reductive N-alkylation furnished **41**. Treatment of the amino alcohol in the presence of an excess of TsCl provided in one pot the acrylate **42** in moderate yield. Michael addition in the presence of dissolved ammonia in EtOH followed by saponification and reaction with FmocOsuc afforded racemate **32**.

The side chain length was found to be hypercritical for the activity of this series of *N*-Fmoc protected amino acid. In fact, when it was shortened by one carbon as in the ornithine derivative **29**, 36-fold loss in potency



Scheme 3. Reagents and conditions: (a) TMsCl, MeOH, Δ; (b) *i*-PrCHO, MeOH, $NaCNBH_3$, pH 4, 89% (two steps); (c) TsCl, NEt_3, CH_2Cl_2, 45%; (d) NH_3, MeOH; (e) NaOH (1 M), THF; (f) FmocOSuc, K_2CO_3 (1 M), THF, 33% (three steps).

compared to **11** was noticed. No activity, however, was noticed at a concentration below 250 nM when the side chain was shortened by two carbons as in **31** or by three carbons as in **32**. On the other hand, no inhibition was detected when the side chain was extended by one more carbon as in **30**, suggesting that the side chain length is optimal at four carbons as in the natural amino acid lysine. The role of the side chain remains an enigma in the absence of an X-ray of the enzyme–inhibitor complex. One may speculate that in the case where the inhibitor adopt an extended conformation in the active site, the side chain may act only as a spacer assisting the Fmoc to reach S_2' and S_3' subsites, while in the case of constrained conformation, the side chain may itself fit in S_1' subsite.

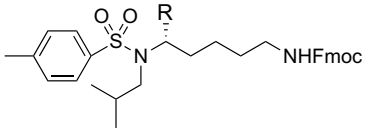
3.3. Modification of Nε-Fmoc

The carbamate oxygen atom, as well as the $-OCH_2-$ group of the Fmoc were essential for the high potency as it is substantiated by the loss in activity of **33** and **34** (18-fold and 21-fold less active than **11**). As we already mentioned, no inhibition was noticed when the Fmoc group was replaced with small Boc- and Cbz-carbamate (**35**, **36**). For more details on the modifications achieved on Nε-Fmoc, see Ref. 14.

3.4. Modification of the carboxylic acid

Finally the carboxylic acid group was modified by various functional groups, such as methyl ester **43**, carboxaldehyde **44**, alcohol **45**, carboxamide **46**, thiocarboxamide **47**, amidine **48**, *N*-hydroxyamide **49**, hydrazide **50**, tetrazol **51** and amine **52** (see Table 3). The carboxylic acid was found so far the best function to afford potent inhibition. Presumably the carbonyl and the OH groups of the carboxylic acid may interact with asp25 and asp25′ in the active site of the enzyme. In fact, its protection as a methyl ester **43** resulted in complete loss in activity indicating the primordial role of the OH group in the binding affinity. This was substantiated by the loss in activity of the aldehyde **44** lacking the OH moiety, whereas the lysinol counterpart **45** maintained some potency albeit its 20-fold decrease in activity compared to **11**. The presence of a hydrogen bond donor (HBD) was essential for the inhibitory

Table 3. Inhibition constant of lysine derivative modified on the carboxyl group[8]



No	R	K_i (nM)[a]
11	COOH	5.0
43	COOMe	>300
44	CHO	>300
45	CH_2OH	105
46	$CONH_2$	107
47	$CSNH_2$	127
48	$CNHNH_2$	280
49	CONHOH	245
50	$CONHNH_2$	254
51	Tetrazole	>300
52	$CH_2NH_2.HCl$	>300

[a] All values are the average of at least two runs.

Table 4. Specificity of **11** and **28** as inhibitors of different aspartyl proteases

Enzyme	IC_{50} of **11** (nM)	IC_{50} of **28** (nM)
HIV PR	5.0	2.1
Pepsin	5400	4100
Renin	6500	5200
Cathepsin	15,600	10,300

potency of this series as it was corroborated by the carboxamide **46** and thioamide **47**, where the NH_2 might be engaged in a similar kind of hydrogen bond interaction as an OH. In the case of compounds containing two HBDs as in the amidine **48**, *N*-hydroxyamide **49** and hydrazide **50**, moderate binding affinity was observed, which could be explained by the contrasting interaction induced by the second HBD. Although tetrazols are known to mimic the carboxylic acid, no activity was obtained in the case of **51**. Finally, the positively charged amine **52** did not show any activity.

Although this new series of inhibitors is not categorized among those mimicking the substrate with a non-hydrolyzable transition-state isoster, we were very pleased to find it very specific as HIV protease inhibitors in comparison to the mammalian aspartyl proteases pepsin, renin and cathepsin (see Table 4). Furthermore, inhibitors **11** and **28** for example, did not show any cytotoxicity at a concentration below 75 and 94 μM, respectively.

4. Conclusion

In summary, a screening assay program carried out on commercially available N-protected amino acids showed that *N*α-sulfonamide-*N*ε-Fmoc-L-lysine **9** displayed a decent 8 μM inhibition constant. Addition of an isobutyl group at Nα-position allowed the discovery of the lead candidate **11** exhibiting a 5.0 nM K_i. Furthermore this new inhibitor series is very specific to HIV protease

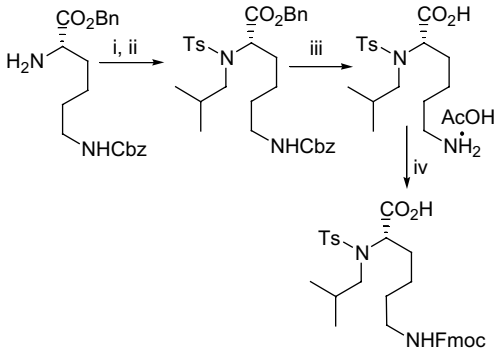
enzyme and did not show any cytotoxicity below a 75 μM concentration.[15]

Acknowledgements

The authors would like to thank Dr. Guy Sevigny and Mr. Patrick Soucy for assistance with the assay, Mr. Nicolas LeBerre and Dr. Fadil Dahhani for analysis of compound purity.

References and notes

1. http://www.unaids.org.
2. McQuade, T. J.; Tomaselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. A. *Science* **1990**, *247*, 454.
3. (a) Leung, D.; Abbenate, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305; (b) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
4. De Clercq, E. *J. Clin. Virol.* **2001**, *22*, 73.
5. (a) De Clercq, E. *Nat. Drug Discov.* **2002**, *1*, 13; (b) Molla, A.; Granneman, G. R.; Sun, E.; Kempf, D. *J. Antiviral Res.* **1998**, 1; (c) Flexner, C. J. *N. Eng. J. Med.* **1998**, *338*, 1281.
6. Sham, H. L.; Chen, X. *Exp. Opin. Invest. Drugs* **1996**, *5*, 977.
7. The K_i values were determined with a fluorometric assay according to Matayoshi test. Matayoshi, E. D.; Wang, G. T.; Kraft, G. A.; Erickson, J. *Science* **1990**, *247*, 957.
8. Inhibitory value for clinically used saquinavir[16] and amprenavir[17] in this assay are 0.18 and 0.25 nM, respectively.
9. Rutenber, E. E.; De Voss, J. J.; Hoffman, L.; Stroud, M. R.; Lee, K. H.; Alvarez, J.; McPhee, F.; Craik, C.; Ortiz de Montellano, P. R. *Bioorg. Med. Chem.* **1997**, *5*, 1311.
10. Bouzide, A.; Sauvé, G.; Stranix, B.; Sévigny, G.; Yelle, J. U.S. Patent 6,455,587 B1; Pharmacor, Inc., USA, 2002.
11. In terms of cost, 100 g of L-lysine cost $30 and 100 g of D-lysine cost $700 at Novabiochem.
12. The same compound was obtained readily from commercially available L-Lys(ε-Cbz)-OBn as described bellow.



(i) *i*-PrCHO, MeOH, $NaCNBH_3$. (ii) TsCl, NEt_3, CH_2Cl_2, 3 days 88% (two steps). (iii) H_2, Pd/C, AcOH. (iv) FmocOSuc, K_2CO_3 (1 M), THF 82% (two steps).

13. Payn, L. S.; Boger, J. *Synth. Commun.* **1985**, *15*, 1277.
14. (a) Stranix, B. R.; Sauvé, G.; Bouzide, A.; Cote, A.; Sévigny, G.; Yelle, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3601; (b) Stranix, B. R.; Sauvé, G.; Bouzide, A.; Cote, A.; Sévigny, G.; Yelle, J.; Perron, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3971. The first author in these two papers published hastily the results in which he was involved without waiting the preliminary work to be published.
15. All new compounds were characterized by ^1H NMR, ^{13}C NMR and HRMS.
16. Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncun, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Redchaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. *Science* **1990**, *248*, 358.
17. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. J.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181.