Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4289–4292

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Lysine Sulfonamides as Novel HIV-Protease Inhibitors: Optimization of the *Nε*-Acyl-Phenyl Spacer

Brent R. Stranix,*,† Gilles Sauvé,‡ Abderrahim Bouzide, Alexandre Coté, Guy Sévigny† and Jocelyn Yelle†

Pharmacor Inc., 535 Cartier West, blvd., Laval, Quebec, Canada H7V 3S8

Received 18 July 2003; revised 24 September 2003; accepted 27 September 2003

Abstract—A series of *N*α-isobutyl-*N*α-arylsulfonamido-(*N*ε acyl) lysine and lysinol derivatives were prepared and evaluated as inhibitors of HIV protease and wild type virus. A simple original synthesis was devised to form *N*α-(arylsulfonamide)-*N*α-isobutyl lysine, which could be easily acylated with carboxylic acids at the *N*ε position. A two-atom spacer was found to be optimal between this acyl group and a phenyl yielding compounds of sub-nanomolar potency on purified enzyme.

One of the main strategies applied to address the AIDS pandemic consists of the inhibition of the virally encoded enzyme human immunodeficiency virus (HIV) aspartyl protease.[1–3] In particular, much success has been obtained through the use of small molecules, which mimic the hydrolytic transition state of the enzyme's natural peptide substrate. The most commonly encountered motif for these peptidomimetics is the hydroxyethylene bioisostere.[4] Although protease inhibitors have radically improved the life of AIDS patients and contributed in large part to the success of highly active anti-retroviral therapy (HAART), new problems have recently been identified. Most importantly, the rapid emergence of several viral strains resistant to one or more of the drugs currently available for the treatment of AIDS has now become a major issue in the treatment of HIV infection.[5] This drawback, along with long term toxicity, has generated a pressing need for a next generation of protease inhibitors. Some of the requirements include designs containing original structures and simple means of preparation. Very few HIV protease inhibitors containing a non-peptidic amino acid backbone are known.[6–8] In the course of our studies to design and develop inhibitors of HIV-protease,[9,10] we screened commercially available protected amino acids and found two distinct series of amino acids, which included lysine, that gave significant enzymatic inhibition.[11] Further development of this lysine series permitted us to identify several promising low molecular weight leads with a general structure as shown in Figure 1. A synthetic route for preparing the enantiomerically pure[12] trisubstituted L-lysine derivatives was devised using commercially available L-Lys(ε-Cbz)-OBn (Scheme 1). Reductive alkylation with, in particular, isobutyraldehyde, under mild reaction conditions gave good yields of the monoalkylated compounds. Addition of aryl sulfonyl chloride gave intermediate aryl sulfonamide **2a**, which after flash chromatography, was easily deprotected using Pd/C catalyzed hydrogenation to give intermediates **3a**. Acylation of the liberated ε-amino acid using Schotten–Baumann conditions was easily accomplished with a series of activated carboxylic acids. For those activated carboxylic acids that were not already commercially available, activation of the carboxylic acid was done with 1,1′-carbonyldiimidazole (CDI), DCC/HOSu or EDAC/HOBt prior to the addition to the aqueous amino acid solution. The same conditions were used to obtain the 4-aminobenzenesulfonyl derivatives, using the 4-nitrobenzenesulfonyl

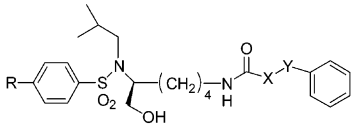
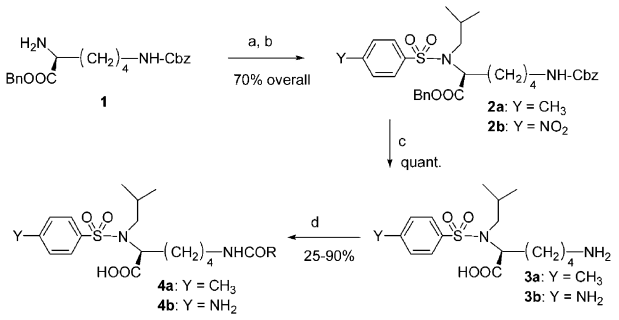


Figure 1.

*Corresponding author. Tel.: +1-514-496-1735; fax: +1-514-496-4857; e-mail: bstranix@procyonbiopharm.com
†Present address: Procyon Biopharma Inc., 6100 Royalmount, Montreal, Qc, Canada H4P 2R2.
‡Present address: INRS-Institut Armand-Frappier, 531, des Prairies Blvd, Building 27, Office D-103, Laval, Quebec, Canada H7V 1B7.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.09.058

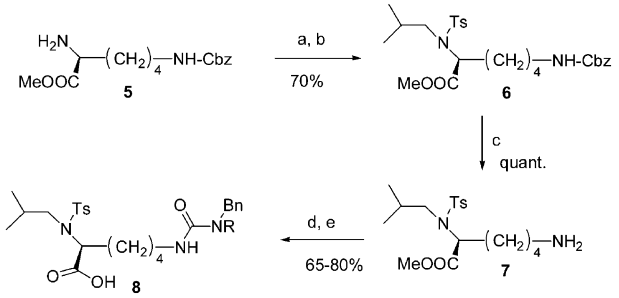


Scheme 1. (a) Isobutyraldehyde, $NaCNBH_3$, MeOH, pH 4; (b) $ArSO_2Cl$, DIPEA, DCM; (c) H_2 Pd/C, EtOH; (d) R-CO-X, THF/1 M K_2CO_3.

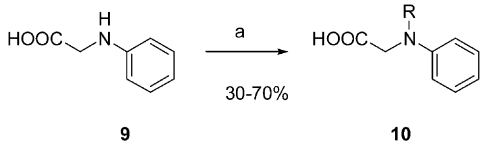
(nosyl) group, in step b to give **2b**, which was subsequently reduced in step c to the aniline **3b**. The non-desired acylation of this aniline during the coupling reaction was rarely observed giving compounds of general structure **4b** exclusively. A slight modification to the synthetic route was made in order to synthesize the benzylic ureas.

L-Lys(Cbz)-OMe was used as the starting material, which underwent identical chemical modifications as in the Scheme 1 for the first three steps. Activation with CDI gave a pendant isocyanate, which could be modified with benzylic amines to give, after alkaline hydrolysis of the methyl ester **8**, compounds **11l**, **11n** and **11p** (Scheme 2). *N*-Substituted-*N*-phenylglycines were synthesized by reacting three equivalents of *n*-BuLi with *N*-phenyl glycine in THF followed by an alkylating agent such as benzyl-bromide (Scheme 3). Attempts at reductive aminations of *N*-Ph-Gly were unsuccessful. Coupling of amino acids was effected with the Boc protected L-Phe and L-*N*-Me-Phe, followed by deprotection using TFA to yield compounds **11q** and **11r**.

The resulting compounds, purified to >95% by preparative RP-HPLC, were characterized by NMR and



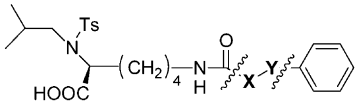
Scheme 2. (a)–(c) See Scheme 1; (d) CDI, amine DMF; (e) 1:1 1 M NaOH/THF.



Scheme 3. (a) 3 equiv *n*-BuLi, R-Cl (Br).

LC–MS for structure confirmation and enantiomeric purity.[13] The novel derivatives were evaluated as HIV protease inhibitors. The activities were measured using purified HIV protease[14] in vitro, and are tabulated in Table 1. The anti-protease activity of compounds **11a**–**11d**, suggest that the spacer length between the carboxamide and the aryl group is optimal at two methylenes. Locking the conformation with rigid groups such as *trans*-ethylene and *trans*-cyclopropane, such as in **11e**–**f**, appears to diminish the inhibitory properties. The addition of two aryl groups also appears to be deleterious (**11g**). By adding a bulky *tert*-butyl group in this alpha position led to a complete loss of activity (**11h**). We then proceeded to replace the dimethylene spacer with isosteres containing N, O and S heteroatoms. Insertion of a –*O* –CH_2 –spacer between the carboxamide and the phenyl group, as in **11i**, abolishes the in vitro activity whereas, conversely, the –CH_2 –*O* –spacer of **11j** retains a significant amount of activity. The addition of a hydroxyl group in the alpha position was obtained by the CDI mediated condensation of L-phenyl lactic acid did not significantly change the K_i and the addition of a ketone, through a similar condensation of pyruvic acid led to a slight loss of activity as shown for **11k** and **11l**. Similarly, the –CH_2 –S –spacer of **11m** shows identical activity as well as its oxidized analogue **11n**. In the case of the nitrogen analogues, a similar yet not as significant pattern is seen. Substitution of the alpha carbon by

Table 1. HIV aspartyl protease inhibition constants for compounds **11a**–**v**

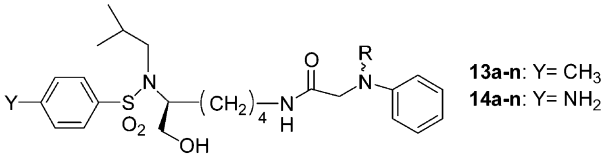


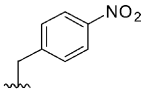
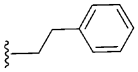
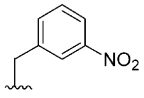
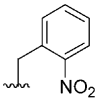
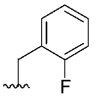
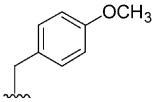
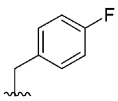
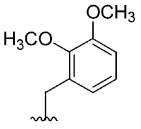
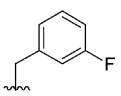
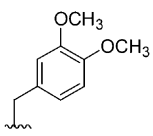
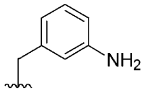
11

Compd	X Y	K_i (nM)	Compd	X Y	K_i (nM)
11a	$(CH_2)_0$	>300	**11l**	C=O	14
11b	$(CH_2)_1$	>300	**11m**	S	18
11c	$(CH_2)_2$	6.9	**11n**	SO_2	20
11d	$(CH_2)_3$	>300	**11o**	NH	7.8
11e	CH=CH	>300	**11p**	N(H)	32
11f	cyclopropyl	>300	**11q**	N(CH_3)	12
11g	CH(Ph)	171	**11r**	N(CH_3)	55
11h	C(CH$_3$)$_3$	>300	**11s**	N(Bn)	0.72
11i	O	>300	**11t**	N(Bn)	8.0
11j	O	18	**11u**	NH_2	1.5
11k	OH	6.2	**11v**	H_3C–NH	2.1

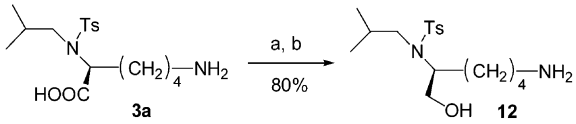
nitrogen leads to a compound of lesser activity than the beta-substituted carbon as seen in **11o–p**. Methylation of the nitrogen atoms in **11q–r** does not change significantly the activity, whereas benzylation dramatically improves the potency, especially in the case of **11s**. The addition of a pendant amino group in the alpha position also leads to some improvement, as well as the methylated derivative as seen for **11u** and **11v**. Although encouraged by the in vitro potency of **11s**, this compound did not show any activity when tested in vivo.[15,16] We hypothesized that removing the negative charge, inherent to the carboxyl group, could improve the potency in cell culture while retaining most of its anti-protease activity. We esterified intermediate **3a** and reduced this ester to the corresponding alcohol with $LiAlH_4$ without loss of enantiomeric purity.[17] This transformation led to a compound of lesser potency in vitro but which showed some modest anti-viral properties in cell culture. We carried out some slight modifications on **11o** and its 4-aminobenzene sulfonamide analogue by varying the R group and adding some substituents on the *N*-benzyl moiety. Extension of the spacer length in **13b** by one methylene resulted in a significant loss of activity. Deletion of this spacer gave product **13d** which retained the anti-protease and anti-viral properties. Replacement of the benzyl group with isopropyl in **13c** conserved some activity and potency in the whole-cell assay. Electron withdrawing substituents on the *N*-benzyl moiety such as nitro also improved potency on both the enzyme and viral culture as shown in **13h–j** and **14h–j**. Notably, the *para*-nitro compound **14h** showed a K_i of 0.6 nM. Also, both the *meta* and *ortho* substituted compounds showed good enzyme inhibition as well as good potency in the anti-viral assay. Compounds **14k–m** bearing fluoride substituents showed some improved inhibition of the enzyme but some loss of activity in cell culture. Addition of an electron donating substituent such as *para*-methoxy (**13e** and **14e**) showed no improvement both in vitro and in whole-cell assay. However, adding two methoxyls (**13f–g** and **14f–g**) yielded products of great potency, especially when the tosyl group was replaced by a more water soluble *para*-aminobenzenesulfonyl group. The *meta*-anilines derivatives **13n** and **14n** gave a slight loss

Table 2. HIV aspartyl protease inhibition constants and anti-viral potencies for compounds **13a–n** and **14a–n**



Compd	R	13		14		Compd	R	13		14	
		K_i (nM)	EC_{50} (nM)	K_i (nM)	EC_{50} (nM)			K_i (nM)	EC_{50} (nM)	K_i (nM)	EC_{50} (nM)
a		7.6	4200	2.4	ND	**h**		ND		0.59	540
b		52	$>10^4$	ND	ND	**i**		1.4	730	1.2	339
c		16	1100	ND	ND	**j**		3.9	3500	1.5	341
d		12	6700	ND	ND	**k**		ND	ND	2.3	2200
e		7.3	$>10^4$	2.4	ND	**l**		ND	ND	2.3	1000
f		2.4	760	1.6	340	**m**		ND	ND	1.1	1100
g		3.5	1300	2.9	366	**n**		7.6	3000	6.9	524

ND, not determined.



Scheme 4. (a) MeOH, TMS–Cl; (b) $LiAlH_4$ THF.

of inhibitory properties on the protease in vitro but still showed modest anti-viral properties. The substitution pattern of the benzylic portion of this class of molecules would seem to be crucial to the anti-viral activity but retains very similar activities on the purified enzyme in vitro. This observation could be explained by the better accessibility of such substituted benzyl phenylglycines to the viral enzyme when tested in whole-cell assays, due to factors such as solubility and cell penetration (Table 2, Scheme 4).

In summary, simple and potent anti-HIV protease compounds were fashioned from the amino acid L-lysine. A straightforward and original synthesis was devised to form *N*α-(arylsulfonamide)-*N*α-isobutyl lysine, which could be easily acylated with carboxylic acids in the *N*ε position. A two-atom spacer was found to be optimal between this acyl group and a phenyl with or without aryl substituents. In particular, the *N*-benzylated derivatives of *N*-phenyl glycinamides gave highly potent derivatives in vitro. Reduction of the pendant carboxylate to the corresponding alcohol gave a novel class of compounds, which retained the in vitro inhibition and showed potent inhibition in anti-viral assays.

Acknowledgements

We thank A. Dubois, P. Soucy Y. Zhao and B. Tian for their participation in the biological analyses. We also acknowledge Dr. F. Dahani for his analysis of compound purity.

References and Notes

1. Tomasselli, A. G.; Heinrikson, R. L. *Biochim. Biophys. Acta* **2000**, *1477*, 189.
2. Vella, S. *Handb. Exp. Pharmacol.* **2000**, *140*, 23.
3. Eron, J. J., Jr. *Clin. Infect. Dis.* **2000**, *30*, S160.
4. Babine, R.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
5. Swanstrom, R.; Erona, J. *Pharmacol. Ther.* **2000**, *86*, 145.
6. Marastoni, M.; Bortolotti, F.; Salvadori, S.; Tomatis, R. *Arzneim.-Forsch.* **1998**, *48*, 709.
7. Marastoni, M.; Bazzaro, M.; Bortolotti, F.; Tomatis, R. *Arzneim.-Forsch.* **2000**, *50*, 564.
8. Rutenber, E. E.; De Voss, J. J.; Hoffman, L.; Stroud, R. M.; Lee, K. H.; Alvarez, J.; McPhee, F.; Craik, C.; Ortiz de Montellano, P. R. *Bioorg. Med. Chem.* **1997**, *5*, 1311.
9. Stranix, B. R.; Sauvé, G.; Bouzide, A.; Côté, A.; Bérubé, G.; Soucy, P.; Zhao, Y.; Yelle, J. US Patent No. 6,506,786; Pharmacor Inc: US, 2002.
10. Bouzide, A.; Sauvé, G.; Stranix, B. R.; Sévigny, G.; Yelle, J. US Patent No. 6,455,587; Pharmacor Inc.: US, 2002.
11. Sauve, G.; Bouzide, A. *unpublished results*.
12. Hiebert, C. K.; Kopp, W. C.; Richerson, H. B.; Barfknecht, C. F. *J. Med. Chem.* **1988**, *31*, 2022.
13. The carboxylic acid **11a** was derived with both *R* and *S*-1-phenethylamine to form the diastereomeric amides. ^1H NMR showed a diastereomeric purity of greater than 95%.
14. Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954.
15. Japour, A. J.; Mayers, D. L.; Johnson, V. A.; Kuritzkes, D. R.; Beckett, L. A.; Arduino, J. M.; Lane, J.; Black, R. J. P. S.R.; D'Aquila, R. T. *Antimicrob. Agents Chemother.* **1993**, *37*, 1095.
16. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; Clercq, E. D. *J. Virol. Methods* **1988**, *20*, 309.
17. Compound **14f** was analyzed by ^1H NMR (acetone-d_6) in the presence of 0.9 equiv $Eu(hfc)_3$ and showed an enantiomeric purity of $>95\%$.