Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3459–3462

Lysine sulfonamides as novel HIV-protease inhibitors: *N*ε-Acyl aromatic α-amino acids

Brent R. Stranix,* Jean-François Lavallée,† Guy Sévigny,‡ Jocelyn Yelle, Valérie Perron, Nicholas LeBerre,§ Dominik Herbart and Jinzi J. Wu

Ambrilia Biopharma Inc., 1000 chemin du Golf, Verdun, QC, Canada H3E 1H4

Received 16 February 2006; revised 31 March 2006; accepted 3 April 2006
Available online 27 April 2006

Abstract—A series of lysine sulfonamide analogues bearing *N*ε-acyl aromatic amino acids were synthesized using an efficient synthetic route. Evaluation of these novel protease inhibitors revealed compounds with high potency against wild-type and multiple-protease inhibitor-resistant HIV viruses.

Protease inhibitors (PIs) along with reverse transcriptase inhibitors (RTIs) are the basis of the highly active anti-retroviral therapy (HAART), which has led to a significant reduction in HIV-associated morbidity and mortality since its widespread availability in 1996.[1] HIV-1 protease is an excellent therapeutic target since its inhibition prevents proteolytic processing of the Gag and Gag-Pol polyproteins, thereby blocking viral maturation.[2–4] Unfortunately, long-term use of HAART often leads to the development of a high proportion of drug resistance in AIDS patients.[5,6] One of the highest priorities in anti-retroviral drug research today is the development of new HIV inhibitors for the treatment of patients infected by multi-resistant viral strains through combination therapy.

During the course of our research, we established that a novel scaffold base on lysine sulfonamides could generate potent compounds for both enzyme inhibition and anti-viral effects.[7] Furthermore, cross-resistance studies of one such compound suggested that this class of molecules was promising.[8] We had noted from our previous studies that potent inhibitors could be obtained through the acylation of the ε-terminal amine of the lysine scaffold with a second amino acid moiety, in particular those with aromatic side chains. We further ventured to develop more potent compounds based on this scaffold through a rational approach consisting of modulating the ε-terminal acylated aromatic amino acid[9] (Fig. 1).

Using (*S*)-α-amino-caprolactam **1** as starting material (Scheme 1), reductive alkylation followed by sulfonamidation of the free amine gave excellent yields of enantiomerically pure crystalline intermediate lactam. A novel and mild procedure was developed in order to effect a reductive cleavage of the lactam quantitatively and without any detectable racemization. Reaction of the intermediate diamide with Boc_2O/DMAP in acetonitrile[10] gave a quantitative conversion to the bis–boc intermediate **2**. Reduction of the resulting activated lactam was effected by $NaBH_4$ in EtOH, yielding a bis–boc-protected lysinol derivative **3**. This procedure concomitantly

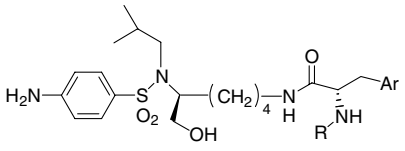
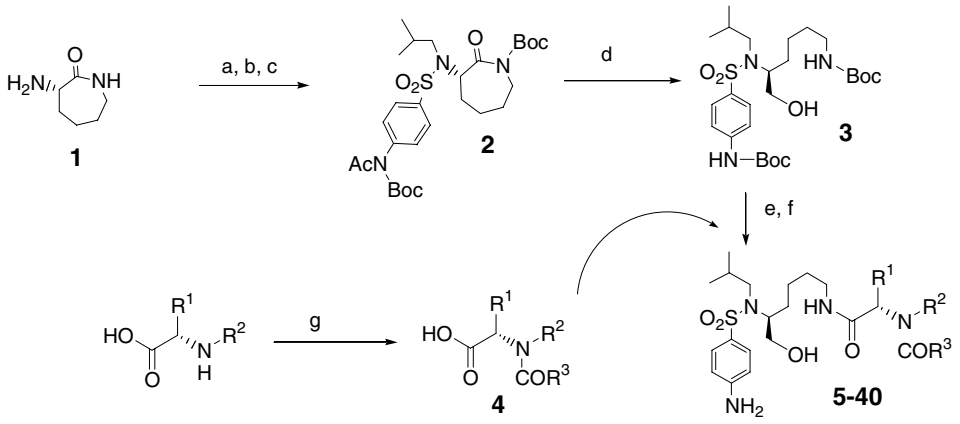


Figure 1. General Structure of lysine sulfonamide HIV protease inhibitors.

Keywords: HIV protease inhibitor; Lysine; Amino acids; Resistant viral strains.
* Corresponding author. Tel.: +1 514 732 3218; fax: +1 514 751 2709; e-mail: bstranix@ambrilia.com
† Present address: Gemin-X Biotechnologies Inc., 3576 Avenue du Parc, Montreal, QC, Canada H2X 2H7.
‡ Present address: Chronogen Inc., 521 Rachel E Suite 22, Montreal, QC, Canada H1W 4A4.
§ Present address: INRS-Institut Armand-Frappier, 531, des Prairies Blvd, Building 27, Laval, Que., Canada H7V 1B7.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.04.011

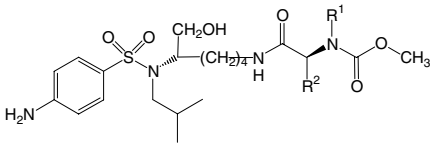


Scheme 1. Reagents and conditions: (a) *i*-PrCHO, STAB, DCM 89%; (b) 4-(AcNH)PhSO$_2$Cl, TEA, EtOAc 88%; (c) Boc$_2$O, MeCN, DMAP$_{cat.}$ 80–99%; (d) NaBH$_4$, EtOH 6 h; (e) HCl, EtOH, rt 95%; (f) EDAC HOBt, DMF, 35–95%; (g) R$_3$ COX, acetone:1 M, K$_2$CO$_3$ 1:1.

removed the acetyl group protecting the aniline moiety. An acidic deprotection of the Boc groups liberated the free amines. The amino alcohol obtained could be selectively acylated with a variety of activated substituted amino acids. Acylation of the pendant aniline was not observed. The N-substituted amino acids **4** were obtained by Schotten–Baumann type acylations of commercially available amino acids with commercial anhydrides, acid chlorides or active esters. The biphenyl derivatives were obtained by the reaction of 2-Br Phe derivatives with phenyl boronic acids through Suzuki-type coupling. The final products **5**–**40** were then subjected to a preparative RPHPLC purification followed by LC/MS and NMR characterization.[9] The products were then evaluated as protease inhibitors in an in vitro enzyme assay[11] and in cell based assays.[12] The effect of spacer length between the ε-amide and a phenyl group has been discussed previously,[7] along with the effect of an α-amino group. The encouraging results prompted us to examine more closely the effects of the phenyl group's distance and geometry using non-natural amino acids. Table 1 describes the inhibition constants (IC_{50}) of the compounds **5**–**13** on the activity of purified HIV protease, and their anti-viral activity (EC_{50}) determined in whole-cell cytopathic assay.

The Moc-Phe derivative **5**, closely related to a compound from a previous article, gave a respectable IC_{50} of 12 nM and some moderate anti-viral activity in whole-cell assays of 2 μM. As in our previous approach the spacer length between the phenyl and ε-amide was varied in the amino acid series using Moc-Phg **6**, placing the phenyl directly on the carbon and Moc-HomoPhe, **7** increasing the distance by one methylene unit. The effect of decreasing or increasing the distance was similar in that a marked decrease in anti-protease and anti-viral activity was noted. In order to explore the conformational preference of the phenyl moiety, we synthesized a conformationaly locked phenylalanine analogue using a Moc-Tic **8**, which places the phenyl in a (-g) gauche position.[13] This resulted in a >10-fold decrease in enzyme inhibition potency. Conversely, favouring the *trans* conformation by placing a methyl group onto the α′-amino group (Moc-N-Me-Phe) **9** increased the potency ~2-fold with respect to the Moc-Phe. Increasing

Table 1. Inhibition constants of compounds for HIV aspartyl protease and whole-cell anti-viral inhibition

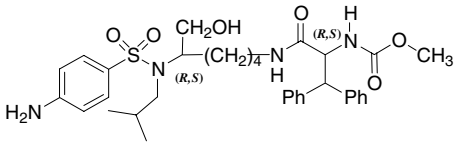


Compound	R^1	R^2	IC_{50}[a] (nM)	EC_{50}[a,b] (nM)
5	H	$C_6H_5CH_2$	12	2000
6	H	Ph	>300	NR
7	H	$C_6H_5CH_2CH_2$	40	9000
8	CH_2–	C_6H_4CH (TIC)	130	NR
9	CH_3	$C_6H_5CH_2$	6.5	1000
10	H	Naphthyl-1-CH_2	1.2	120
11	H	Naphthyl-2-CH_2	1.2	258
12	H	Biphenyl-2-CH_2	0.521	200
13	H	$(C_6H_5)_2CH$	0.500	16

[a] Mean of at least two experiments.
[b] Anti-viral activity using the molecular clone HIV-1 NL4-3 in MT-4 cells.

the bulk of the R_2 group in the terminal amino acid also gave significant increase in IC_{50} potency. Both 1 and 2 naphthylalanines (**10** and **11**) gave similar results in IC_{50}, 1.2 nM and a very potent 100–200 nM in the whole-cell anti-viral assay. Our strategy then focused on combining both the geometric aspect and the hydrophobic bulk by using an *o*-biphenyl alanine **12** and a diphenylalanine **13**. This approach, using the steric bulk of the second phenyl group to enrich the *trans* conformation population, yielded highly potent compounds with an IC_{50} of 0.5 nM, and anti-viral EC_{50} values of 200 and 16 nM for both compounds, respectively. Encouraged by an impressive activity of 16 nM for compound **13** we addressed the stereochemistry of the diastereomeric compound. We synthesized the stereoisomers through similar chemistry as discussed using the commercially available D-amino acids, and tested the purified compounds **13SR**, **13RR** and **13RS** (>97% purity, de >95%) against the purified enzyme and in whole-cell anti-viral assay. We confirmed the *S*,*S* stereoisomer **13** to be the most active compound as shown in Table 2.

Table 2. The effect of stereoisomers on the inhibition constants of compounds for HIV aspartyl protease and whole-cell anti-viral assays



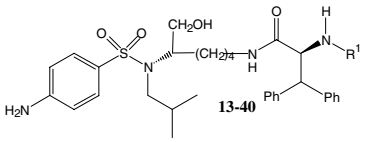
Compound	Absolute configuration	IC_{50}[a]	EC_{50}[a,b]
13	*S*,*S*	0.500	16
13SR	*S*,*R*	25	3,000
13RR	*R*,*R*	> 300	> 100,000
13RS	*R*,*S*	13	850

[a] Mean of at least two experiments.
[b] Anti-viral activity using the molecular clone HIV-1 NL4-3 in MT-4 cells.

We also explored the effect of various acyl groups on the α′-amino of the L-diphenylalanine portion of the molecule (Table 3). Both IC_{50} and K_i were assessed experimentally in order to evaluate the impact of the changes at the enzyme inhibition level. IC_{50} values were determined from a dose–response curve, whereas K_i values were obtained by fitting initial rates to a tight-binding inhibition equation.[14] Also, EC_{50} were determined for laboratory adapted wild-type strain NL4-3, multi-PI-resistant strain 4596 and Saquinavir-resistant strain (Saqr strain)[15–19] to assess their potential against resistant viral strains.

The non-acylated compound **14** (obtained by acid catalysed Boc deprotection of **18**) gave an IC_{50} of 0.5 nM and an excellent anti-viral activity of 44 nM against NL4-3 strain. Small alkyl amides such as acetyl (**15**) and cyclopropyl (**16**) were first examined. The acetyl gave a reduction in enzyme inhibition but retained a potent 66 nM on the wt whole-cell assay. Cyclopropyl amide was more potent with a K_i of 0.45 nM and EC_{50} of 25 and 46 nM for wt and 4596 strains, respectively. A more bulky amide bearing a cyclohexyl ring (**17**) showed a decrease in potency for both enzymatic and whole-cell assays. A

Table 3. Inhibition constants of compounds **13**–**40** for HIV aspartyl protease and anti-viral assays



Compound	R^1	IC_{50}[a] (nM)	K_i[a,c] (nM))	EC_{50}[a,b] (nM) NL4-3	EC_{50}[a,d] (nM) 4596	EC_{50}[a,e] (nM) Saq^r
13	CH_3O–CO	0.50	0.04	16	38	19
14	H	0.50	0.56	44	43	
15	Ac	1.5		66	66	
16	Cyclopropyl-CO	0.43	0.45	25	46	17
17	Cyclohexyl-CO	<3.8		93	186	
18	$(CH_3)_3CO$–CO	0.40	0.50	41	290	
19	$(CH_3)_2N$–CO	0.44	0.58	62	56	
20	4-Morpholine-CO	0.51	0.55	78		
21	Pyrazine-CO	0.52	0.60	19	46	
22	Pyrrole-2-CO	0.43	0.47	34	51	
23	2-Pyridyl-CO	0.46	0.44	68	161	
24	3-Pyridyl-CO	0.68	0.55	39	25	
25	4-Pyridyl-CO	0.65	0.66	32	26	
26	2-CH_3-3-pyridyl-CO	0.40	0.40	42	36	
27	6-CH_3-3-pyridyl-CO	0.40	0.50	24	34	
28	3-HO-2-pyridyl-CO	0.90	0.70	153	186	
29	6-H_2N-3-pyridyl-CO	0.40	0.50	84	72	
30	6-HO-3-pyridyl-CO	0.50	0.40	>	>	
31	3-Picolyl-CO	0.24	0.34	194	128	
32	2-Picolyl-O-CO	0.12	0.40	17	18	14
33	3-Picolyl-O-CO	0.48	0.36	18	18	
34	4-Picolyl-O-CO	0.39	0.02	20	13	4
35	2-HO-phenyl-CO	0.63	0.58	64	134	
36	3-HO-phenyl-CO	0.40	0.40	64	30	
37	4-HO-phenyl-CO	0.53	0.47	88	34	
38	3-HO-4-NO_2-phenyl-CO	0.33	0.33	16	10	
39	4-HO-3-NO_2-phenyl-CO	0.56	0.48	55	35	
40	4-HO-3-CH_3O-phenyl-CO	0.5	0.45	14	23	

[a] Mean of at least three experiments.
[b] Anti-viral activity using the molecular clone HIV-1 NL4-3 in MT-4 cells.
[c] The K_i is determined using XLfit3 software (Version: 3.0.3 Build: 09) according to the following equation (Inhibition)[14]: $Y = V_o/(2 * E)\{((K + x - E)^2 + 4 * K * E)^{0.5} - (K + x - E)\}$; where Y is the enzyme rate (RFU/min), x is the concentration of the compound (ηM), V_o is the maximum rate (initial rate measured from 200 to 600 s) (RFU/min), E is the protease concentration (ηM) and K is the K_i of the compound.
[d] 4596; **46I**, **82T**, **84V**, **10R**, **63P**.
[e] SaqR; **48V**, **90M**.

similar pattern was observed for terminal carbamates and ureas, where smaller moieties were more potent than bulky groups. This is exemplified in the case of carbamate compounds Moc (**13**) and Boc (**18**), K_i 0.04 and 0.5 nM, EC_{50} wt 16 and 41 nM, respectively. Similar K_is were obtained for both dimethyl and morpholyl ureas **19** and **20**. However, a slight increase is noted for the dimethyl urea whole-cell EC_{50} value. Heterocyclic amides and phenolic amides were introduced as R_1 in order to assess the influence of H-bond acceptors and donors at this position. 2-pyrrole carboxamide **22**, 2-pyridyl and pyrazinoyl amides (**23** and **21**) gave very potent compounds with the pyrazinoyl compound showing EC_{50}s for NL4-3 and 4596 strains of 19 and 46 nM, respectively. The nicotinoyl and 4-picoloyl derivatives **24** and **25** were added and a small regiomeric effect was observed, especially significant on the 2-pyridyl derivative **23**, where a significant drop in potency was observed in the whole-cell assays. The addition of substituents CH_3, NH_2 and OH to the nicotinamide and picoloyl amide moieties (**26**–**30**) showed some influence on EC_{50}. Increasing the distance between the terminal amine and the heterocycles was assessed by compounds 3-picolyl-CO **31** (2 atoms) and carbamates **32**–**34** (3 atoms). Of these, the three carbamates **32**–**34** gave highly potent compounds with EC_{50}s of 17–20 nM for NL4-3 and 13–18 nM for the multi-resistant strain 4596. The K_i of compound **34** gave an excellent 0.02 nM on the purified enzyme. Phenolic amides **35**–**37** were synthesized and very similar Kis were obtained for the 2, 3 and 4 OH regiomers, with the 3′-OH derivative **36** showing the best activity in the whole-cell assays. Increasing the acidity by addition of a nitro group to the phenyl ring had the effect of lowering both the K_i and EC_{50} for the 3′-phenol **39**. Addition of an electron-withdrawing group or an electron-donating group to the 4′-OH (**38** and **40**) did not affect the K_i however a marked improvement was noted on the whole-cell assay with an excellent 14 nM being recorded for compound OCH_3. Also tabulated in Table 3 are results for whole-cell assays for strain Saq^r. Only 4 compounds (**13**, **16**, **32** and **34**) were evaluated which in all cases showed a similar or greater potency on this strain than on the wild-type strain.

The results shown in Table 3 suggest that the acylation with differing acyl groups of the L-diphenylalanine moiety yielded a library of compounds with a narrow range of biochemical and anti-viral activities. Most notably, the potencies are retained when comparing wild-type and mutated virus in the whole-cell assays.

In summary, the acylation of the ε-amine of *N*-isobutyl-*N*-aminophenylsulfonamido lysinol with substituted aromatic amino acids yields potent HIV protease inhibitors. In particular, compounds bearing the various *N*-acylated diphenylalanine moieties display exceptional enzyme inhibitory potencies and anti-viral activities in vitro on wild type and selected mutant viral strains. These compounds provide a versatile platform for the selection of orally dosed pre-clinical candidates.

Acknowledgments

We gratefully acknowledge A. Dubois, A. M. Lemieux, Y. Zhao and B. Tian for their participation in the biological analyses. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 mutated strains, SaqR (catalog no. 2948) from Dr. N. Roberts and P. Tomlinson, and 4596 (catalog no. 2840) from Dr. E. Emini and Dr. W. Schleif.

References and notes

1. Palella, F. J., Jr.; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. *N. Engl. J. Med.* **1998**, *338*, 853.
2. Debouck, C. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 153.
3. Katz, R. A.; Skalka, A. M. *Annu. Rev. Biochem.* **1994**, *63*, 133.
4. Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686.
5. Richman, D. D.; B. S.; Morton, S.; Chien, S.; Wrin, T.; Dawson, K.; Hellmann, N. 41st International Conference on Antimicrobial Agents and Chemotherapy, Chicago, December 18 [abstract LB-17]; **2001**.
6. Richman, D. D.; Morton, S. C.; Wrin, T.; Hellmann, N.; Berry, S.; Shapiro, M. F.; Bozzette, S. A. *AIDS* **2004**, *18*, 1393.
7. Stranix, B. R.; Sauvé, G.; Bouzide, A.; Côté, A.; Sévigny, G.; Yelle, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4289.
8. Sévigny, G.; Stranix, B. R.; Tian, B.; Dubois, A.; Sauvé, G.; Petropoulos, C.; Lie, Y.; Hellmann, N.; Conway, B.; Yelle, J. *Antiviral Res.* **2006**, in press.
9. Stranix, B. R.; Lavallee, J. F.; LeBerre, N.; Perron, V. US Patent 6,632,816, October 14 2003, 2003.
10. Grehn, L.; Gunnarsson, K.; Ragnarson, U. *Acta Chem. Scand.* **1986**, *40*, 745.
11. Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954.
12. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; Clercq, E. D. *J. Virol. Methods* **1988**, *20*, 309.
13. Kazmierski, K.; Hruby, V. J. *Tetrahedron* **1988**, *44*, 697.
14. Williams, J. W.; Morrison, J. F. *Methods Enzymol.* **1979**, *63*, 437.
15. Boden, D.; Markowitz, M. *Antimicrob. Agents Chemother.* **1998**, *42*, 2775.
16. Condra, J. H.; Schleif, W. A.; Blahy, O. M.; Gabryelski, L. J.; Graham, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M., et al. *Nature* **1995**, *374*, 569.
17. Jacobsen, H.; Yasargil, K.; Winslow, D. L.; Craig, J. C.; Krohn, A.; Duncan, I. B.; Mous, J. *Virology* **1995**, *206*, 527.
18. Kaltenbach, R. F., III; Trainor, G.; Getman, D.; Harris, G.; Garber, S.; Cordova, B.; Bacheler, L.; Jeffrey, S.; Logue, K.; Cawood, P.; Klabe, R.; Diamond, S.; Davies, M.; Saye, J.; Jona, J.; Erickson-Viitanen, S. *Antimicrob. Agents Chemother.* **2001**, *45*, 3021, DPC 681 and DPC 684.
19. Otto, M.; Garber, S.; Winslow, D.; Reid, C.; Aldrich, P.; Jadhav, P.; Patterson, C.; Hodge, C.; Cheng, Y. *Proc. Natl. Acad. Sci. U.S.A. PNAS* **1993**, *90*, 7543.