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Bioorganic & Medicinal Chemistry Letters 14 (2004) 3971–3974
Bioorganic & Medicinal Chemistry Letters

Lysine sulfonamides as novel HIV-protease inhibitors: *N*ε-disubstituted ureas

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Received 17 March 2004; revised 21 May 2004; accepted 21 May 2004
Available online 17 June 2004

Abstract—A series of lysine sulfonamide analogues bearing a *N*ε-benzylic ureas was synthesized using both solution-phase and solid-phase approaches. A novel synthetic route of *N*α-(alkyl)-*N*α-(sulfonamides)lysinol using α-amino-caprolactam was developed. Evaluation of these novel protease inhibitors revealed compounds with high potency against wild-type HIV virus.

Human immunodeficiency virus (HIV) aspartyl protease inhibitors are a major component of anti-HIV chemotherapy, the current treatment for acquired immunodeficiency syndrome (AIDS).[1–3] This class of compounds inhibits the formation of mature viral particles and thus the infectious process. 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. 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 the most important issue in the treatment of HIV infection.[4] Most currently available drugs are peptidomimetics containing the hydroxyethylene moiety, which mimic the hydrolytic transition state of the protease substrate.[5,6] We recently discovered HIV protease inhibiting compounds devoid of the hydroxyethylene moiety of general structure as shown in Figure 1, which also demonstrated interesting anti-viral activity.[7–9] We had previously found that lysine or lysinol could serve as an efficient backbone scaffold for the synthesis of such inhibitors.[10]

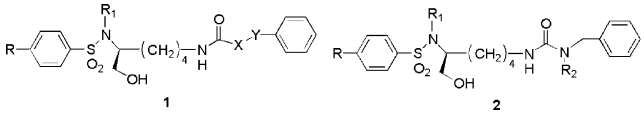
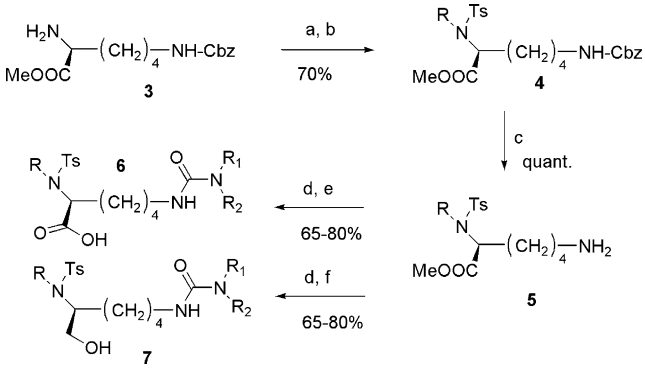


Figure 1. General structure of lysine sulfonamide HIV protease inhibitors.

Moreover several types of *N*ε-linkages led to different classes of compounds, which retained excellent potencies.

One such class, *N*ε-ureas will be discussed in this letter. A synthetic route was devised using *N*ε-(Cbz)-lysine methyl ester **3** as a starting point (Scheme 1). Reductive



Scheme 1. Reagents and conditions: (a) RCHO, $NaCNBH_3$, MeOH, pH 4; (b) TsCl, DIPEA, DCM; (c) H_2 Pd/C, EtOH; (d) CDI, DMF, **17** or H_2NR_1; (e) NaOH 1 M/THF 1:1, HCl 1 M; (f) $LiAlH_4$ THF.

Keywords: Lysine; Ureas; HIV protease inhibitors.
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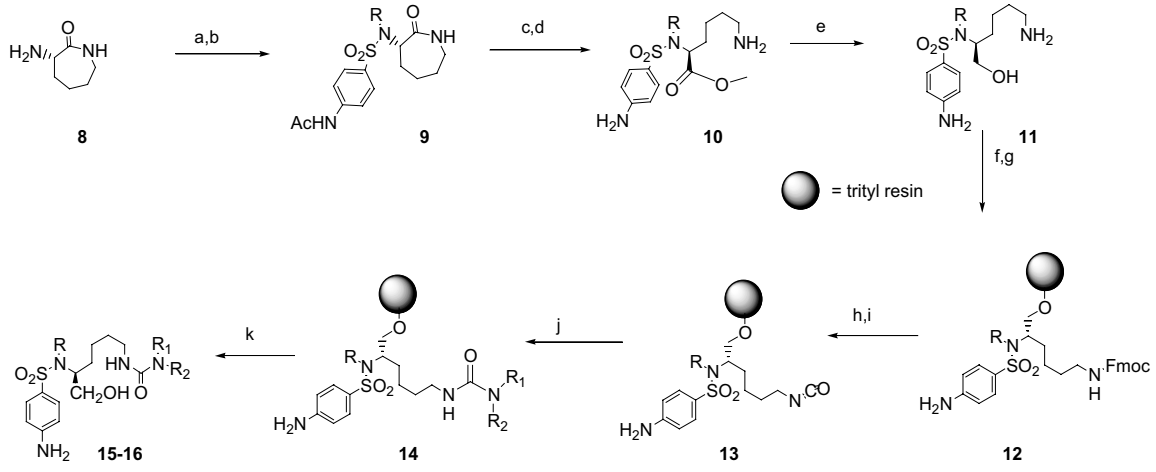
doi:10.1016/j.bmcl.2004.05.049

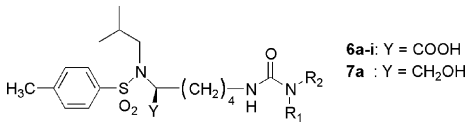
alkylation and a slow sulfonamidation with toluenesulfonyl chloride (TsCl) gave intermediate **4**. Flash chromatography purification permitted isolation of this intermediate in moderate yield. The elimination of the Cbz protecting group was effected using catalytic hydrogenation, which yielded the desired free amino ester **5** in quantitative yields. This intermediate was then converted to an isocyanate using carbonyldiimidazole (CDI) in DMF,[11] which could be kept stable in solution for several weeks. The urea portion was then simply formed by adding primary or secondary amines **17** to the pendant isocyanate solution to give the corresponding urea esters in moderate to good yields. Finally, the ester was selectively hydrolyzed with NaOH or reduced with $LiAlH_4$ to yield the desired protease inhibitors **6** or **7**. However, the final hydrolysis step with NaOH was often accompanied by partial or complete racemization of the chiral centre. An alternative synthetic route was devised to eliminate certain time consuming reactions, low yields, protecting groups, chromatography and frequent racemization of the intermediates.

This procedure used α-amino-caprolactam **8** as a starting material (Scheme 2). Reductive alkylation and sulfonamidation of the free amine gave excellent yields of enantiomerically pure crystalline intermediate **9**. Hydrolysis of the lactam proceeded quantitatively and without any detectable racemization.[12] Reduction of the resulting carboxyl was effected by esterification to form **10** and reduction with $LiAlH_4$ to yield **11**. Once again, crystalline intermediates were obtained without racemization. The amino alcohol obtained could be selectively acylated with a variety of activated acids, isocyanates, or chloroformates. When required the amino ester obtained was used to form urea compounds as in Scheme 1, on larger scale (1–10 g). We then adapted a solid-phase technique for a rapid generation of compounds bearing ureas and thioureas of both primary and secondary amines. This route also enabled us to avoid the use of $LiAlH_4$ to generate each final compound. A Fmoc protected[13] derivative of **11** was stirred with a suspension of freshly activated trityl resin (1 mmol/g) and reacted with the pendant alcohol to yield a resin **12** with 0.4 mmol/g degree of substitution. Standard deprotection[13] of the amine followed by activation with CDI or thiocarbonyldiimidazole, gave pendant isocyanates[11,14] **13** or isothiocyanates, which were reacted with primary and secondary amines to yield, after mild cleavage, the desired *N*ε-ureas **15**–**16**. Acylation of the pendant aniline was not observed. The secondary benzylic amines **17**, were synthesized using two standard procedures, reductive amination of aldehydes and nucleophilic substitution of alkyl or benzylic halides both of which gave good yields of desired products (Scheme 3). The final products were then subjected to a preparative RPHPLC purification followed by LC/MS and NMR characterization. The products were then evaluated as protease inhibitors in an in vitro enzyme assay and in cell based assays.[15–17] Table 1 describes the inhibition of the compounds on the activity of purified HIV protease. The library produced using the above methodologies revealed that *N*-benzyl urea of the lysine sulfonamide **6a** inhibits HIV protease with sixfold greater efficacy than the longer phenethyl urea **6b** or the heterocyclic 2-picolyl urea **6c**. The addition of a second substituent such as the aliphatic methyl (**6d**) and isopropyl (**6g**) groups gave no improvement. Ureas substituted with two aliphatic substituents such as **6i** or with the phenethyl group **6e**, gave inactive compounds. However, much more effective, was the *N*,*N*-dibenzyl urea system **6f**, which improved the potency by fivefold. Also noteworthy is the *N*-(benzyl)-N-4-picolyl urea **6h**, which showed some twofold improvement in inhibition over **6f**. Compound **7a**, the lysinol based analogue of **6f** showed a twofold loss of potency in the enzyme based assay. However, these lysine-based compounds did not show any significant anti-viral activity in the whole-cell based assay. The substituted *N*-benzyl urea system was explored further using the *N*α-alkyl-Nα-(4-amino-



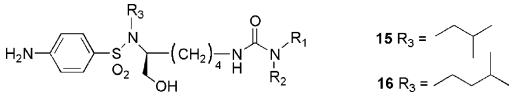
Scheme 2. Reagents and conditions: (a) RCHO, STAB, DCM 89%; (b) 4-(AcNH)$PhSO_2Cl$, TEA, EtOAc 88%; (c) 6 M HCl reflux 99%; (d) TMS-Cl, MeOH rt 95%; (e) $LiAlH_4$, ether 85–95%; (f) Fmoc-Osu, THF 1 M, K_2CO_3 1:1 75%; (g) trityl chloride resin, DMF, pyridine, then methanol 40% conversion; (h) 30% piperidine DMF rt quant; (i) CDI, (ThioCDI) DMF, TEA 80 °C, 0.5 h quant; (j) **17** excess, DMF; (k) 5% TFA, DCM 10–60% isolated yield.

Table 1. Inhibition constants of compounds for HIV aspartyl protease

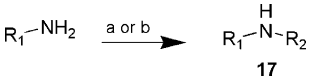


Compound	R_1	R_2	K_i (nM)
6a	H	$C_6H_5CH_2$	32
6b	H	$C_6H_5CH_2CH_2$	204
6c	H	2-Picolyl	>300
6d	$C_6H_5CH_2$	CH_3	55
6e	$C_6H_5CH_2$	$C_6H_5CH_2CH_2$	>300
6f	$C_6H_5CH_2$	$C_6H_5CH_2$	7.2
6g	$C_6H_5CH_2$	$(CH_3)_2CH$	19
6h	$C_6H_5CH_2$	4-Picolyl	3.7
6i	$(CH_3)_2CH$	$(CH_3)_2CH$	300
7a	$C_6H_5CH_2$	$C_6H_5CH_2$	15

Table 2. Inhibition constants of compounds for HIV aspartyl protease and Wild type virus in anti-viral assays



Compound	R_1	R_2	**15** K_i (nM)	EC_{50} (nM)	**16** K_i (nM)	EC_{50} (nM)
a	$C_6H_5CH_2$	$C_6H_5CH_2$	12	3000	5.8	$>10^4$
b	$C_6H_5CH_2$	3-Picolyl	34	8500		
c	$C_6H_5CH_2$	4-Picolyl	90	$>10^4$		
d	$C_6H_5CH_2$	2-Thiophene-CH_2	11	5000		
e	$C_6H_5CH_2$	2,3-$(OCH_2O)C_6H_3CH_2$	5.4	$>10^4$	4.0	$>10^4$
f	$C_6H_5CH_2$	2,4-$F_2C_6H_3CH_2$	12	3200		
g	$C_6H_5CH_2$	4-$FC_6H_4CH_2$	3.3	$>10^4$		
h	$C_6H_5CH_2$	3,4-$(OCH_2O)C_6H_3CH_2$	2.6	1400	4.1	871
i	3,4-$(OCH_2O)C_6H_3CH_2$	2-Thiophene-CH_2	2.0	4000	1.4	800
j	3,4-$(OCH_2O)C_6H_3CH_2$	3-Thiophene-CH_2	6.3	5000		
k	3,4-$(OCH_2O)C_6H_3CH_2$	4-$NO_2C_6H_4CH_2$	2.0	620	2.2	845
l	3,4-$(OCH_2O)C_6H_3CH_2$	4-$FC_6H_4CH_2$	2.0	488	4.9	1200
m	3,4-$(OCH_2O)C_6H_3CH_2$	4-$CF_3C_6H_4CH_2$	2.5	1600	3.0	$>10^4$
n	3,4-$(OCH_2O)C_6H_3CH_2$	4-$CH_3OC_6H_4CH_2$	2.2	500	2.9	700
o	3,4-$(OCH_2O)C_6H_3CH_2$	3,4-$(OCH_2O)C_6H_3CH_2$	1.7	150		



Scheme 3. Reagents and conditions: (a) RCHO, STAB, DCM; (b) R–Cl (Br) EtOH reflux.

benzenesulfonyl)-lysinol moiety, which was shown to be more effective than the lysine based compounds.[10] A library of *N*,*N*-dibenzyl compounds **15** (*N*α-isobutyl) and **16** (*N*α-isovaleryl) containing various aromatic substituents and heterocycles were then synthesized in parallel. Table 2 reveals the dibenzyl urea analogue **15a** based upon the *N*α-isobutyl-*N*α-(4-aminobenzenesulfonyl) lysinol scaffold showed a similar K_i to the *N*α-isobutyl *N*α-(tosyl) analogue **7a**. Heterocycles such as picolines **15b–c** and thiophene **15d** did not significantly improve the activity against the enzyme as compared to the *N*,*N*-dibenzyl compound **15a**. The addition of electron donating substituents such as piperonyl **15h**, gave the best activities, increasing the enzyme inhibition sevenfold. Extending the isobutyl group of **15h** to an *N*α-isovaleryl group **16h**, showed an improvement in the whole-cell anti-viral assay. Since the piperonyl group appeared to be a better pharmacophore, a series of *N*ε-(piperonyl-arylsubstituted benzyl)ureas were prepared. Although this series of compounds gave inhibition constants on the purified enzyme ranging from 1.7 to 6.3 nM, the antiviral assay gave EC_{50} values ranging from 5 to 0.15 μM. Thiophene bearing derivatives such as **15i–j** showed poor antiviral properties, whereas

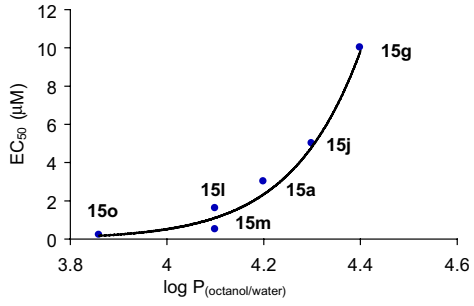
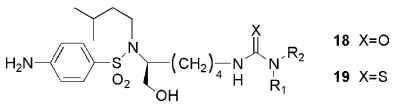


Figure 2. Whole-cell assay EC_{50} (μM) versus log *P*.

Table 3. The effect of urea versus thioureas on the inhibition constants of compounds for HIV aspartyl protease



Compound	R_1	R_2	X	
			18 K_i	**19** K_i
a	3,4-$(OCH_2O)C_6H_3CH_2$	3-$CH_3OC_6H_4CH_2$	2.2	2.6
b	3,4-$(OCH_2O)C_6H_3CH_2$	4-$CH_3OC_6H_4CH_2$	2.9	3.6
c	3,4-$(OCH_2O)C_6H_3CH_2$	3,4-$(CH_3O)C_6H_3CH_2$	3.0	2.9
d	3,4-$(O(CH_2)_2O)C_6H_3CH_2$	4-$FC_6H_4CH_2$	5.4	9.9

*N*α-isovaleryl derivatives **16i–j**, were an improvement over **15h**. Compounds **15–16m** bearing the *para*-trifluoro benzyl group also gave poor antiviral inhibition. Other electron withdrawing substituents such as *para*-fluoro and *para*-nitro groups as in **15–16k–l** in contrast to the previously mentioned compounds **15–16m**, gave good antiviral inhibition. By comparison with **15h**, compounds bearing electron rich moieties such as the *para*-methoxy benzyl **15–16n** and piperonyl **15o** showed an increase in anti-viral potency of three, two and ten fold, respectively. The role of the piperonyl substituent in improving the anti-protease activity is unclear, although we can speculate dioxolane oxygen atoms may form an additional hydrogen bond to the enzyme active site. The increase in anti-viral potency in the whole cell assay can also be partially explained by the improved water solubility of the molecules bearing substituents such as piperonyl, which lowered the $\log P_{(\text{octanol/water})}$ value. Figure 2 relates the effect of $\log P$ on the whole cell assay EC_{50} of a few representative compounds, and clearly show the beneficial effect of better water solubility. Furthermore, the replacement of urea (Series **18**) by a thiourea (Series **19**) showed no improvement (Table 3). In summary, a series of lysine sulfonamide analogues bearing a *N*ε-benzylic ureas was synthesized using both solution-phase and solid-phase approaches. A novel synthesis of *N*α-(alkyl), *N*α-(sulfonamides)lysinol using α-amino-caprolactam was developed. Evaluation of these novel protease inhibitors revealed highly potent compounds against wild-type HIV virus. Further studies are ongoing to assess the inhibitory activity against resistant HIV strains.

Acknowledgements

We gratefully acknowledge A. Dubois, P. Soucy, Y. Zhao and B. Tian for their participation in the biological analyses. We also acknowledge Drs. F. Dahani and A. Ahmed for their analyses of compound purity and $\log P$ determination.

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