Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5499–5503
Bioorganic & Medicinal Chemistry Letters

Synthesis and activity of *N*-acyl azacyclic urea HIV-1 protease inhibitors with high potency against multiple drug resistant viral strains

Chen Zhao,* Hing L. Sham, Minghua Sun, Vincent S. Stoll, Kent D. Stewart, Shuqun Lin, Hongmei Mo, Sudthida Vasavanonda, Ayda Saldivar, Chang Park, Edith J. McDonald, Kennan C. Marsh, Larry L. Klein, Dale J. Kempf and Daniel W. Norbeck

GPRD, Abbott Laboratories, Abbott Park, IL 60064-6123, USA

Received 26 April 2005; revised 25 August 2005; accepted 29 August 2005
Available online 3 October 2005

Abstract—As part of our efforts to identify potent HIV-1 protease inhibitors that are active against resistant viral strains, structural modification of the azacyclic urea (**I**) was undertaken by incorporating acyl groups as P_1' ligands. The extensive SAR study has yielded a series of *N*-acyl azacyclic ureas (**II**), which are highly potent against both wild-type and multiple PI-resistant viral strains.
© 2005 Elsevier Ltd. All rights reserved.

Inhibition of human immunodeficiency virus (HIV) protease is one of the most important approaches for the intervention of HIV infection.[1] A novel series of highly potent azacyclic urea HIV-1 protease inhibitors (**I**)[2] were designed and synthesized by Abbott chemists based on the linear aza protease inhibitors[3] and the DuPont Merck's *C*-2 symmetric cyclic ureas.[4] However, the bioavailability of this series is very low, seldom exceeding 10%. The metabolism studies revealed that efficient hepatic oxidation of the carbon atoms α to the aza linkage may be contributing to the poor pharmacokinetics. To prevent this unfavorable oxidation, an acyl group was utilized instead of the benzyl or alkyl group as P_1' ligands. The extensive SAR study has yielded a series of *N*-acyl azacyclic ureas (**II**), which exhibits excellent protease inhibition and antiviral activity against wild type HIV. Furthermore, we have recently found that this series of compounds also retain high activity against some highly resistant strains. For example, inhibitor **II-15** holds EC_{50} values of 54 and 65 nM against the indinavir-resistant (Ind-R)[5] and lopinavir-resistant (A17)[6] viruses. This degree of activity represents a more than 10-fold improvement over lopinavir (see Fig. 1).

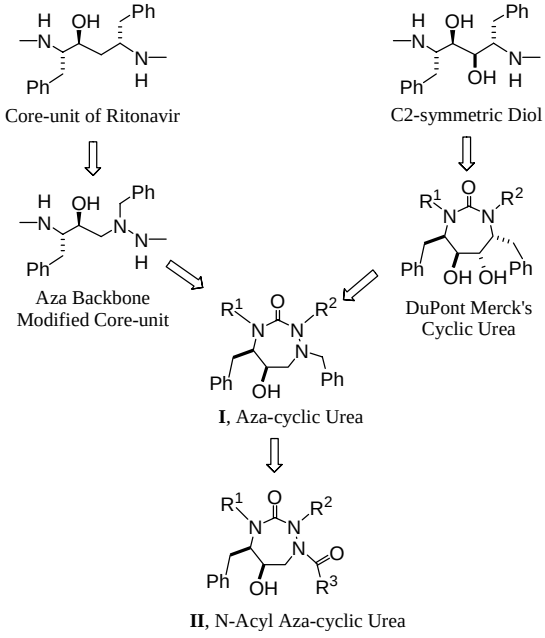
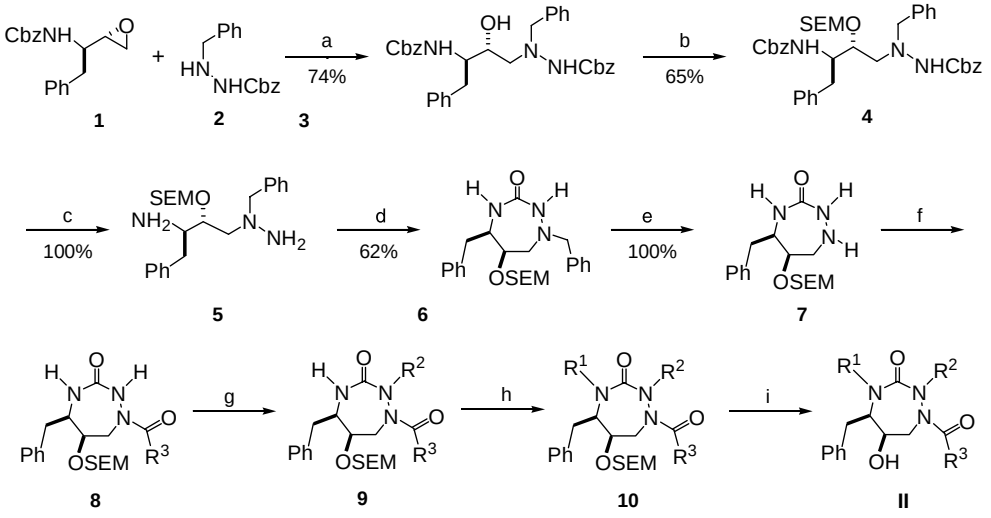


Figure 1. Design of rational and structures of ritonavir, azacyclic ureas, and *N*-acyl azacyclic ureas.

The synthesis of *N*-acyl azacyclic ureas is outlined in Scheme 1. Opening of 2*S*,3*R*-epoxide **1**[7] with Cbz-protected benzylhydrazine **2**[8,9] provided compound **3** in

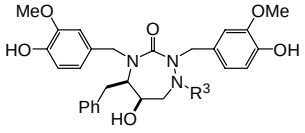
Keywords: HIV-1 protease inhibitor; Resistant viral strain; *N*-Acyl azacyclic urea.
* Corresponding author. Tel.: +1 847 938 5292; fax: +1 847 937 9195; e-mail: chen.zhao@abbott.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.093



Scheme 1. Reagents and conditions: (a) *i*-PrOH, reflux, 48 h; (b) SEM-Cl, $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt, overnight; (c) H_2, 10% Pd–C, MeOH, rt, 4 h; (d) CDI, CH_2Cl_2, rt, 6 days; (e) H_2, 10% $Pd(OH)_2/C$, MeOH, rt, 2 h; (f) R^3COCl, pyridine, CH_2C_{l2}, rt; (g) R^2X, KO^{t}Bu, THF, rt; (h) R^1X, NaH, DMF, rt; (i) 4 N HCl, MeOH, rt.

Table 1. HIV-1 protease inhibitory and antiviral activities of *N*-acyl azacyclic ureas-R^3modifications



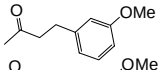
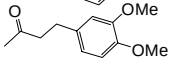
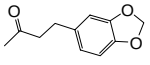
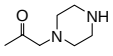
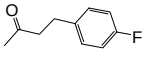
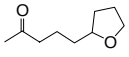
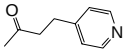
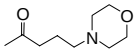
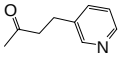
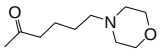
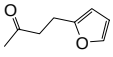
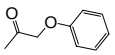
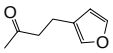
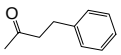
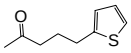
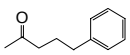
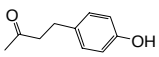
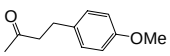
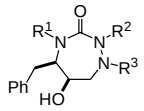
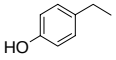
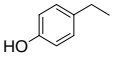
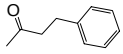
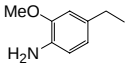
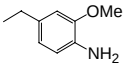
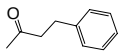
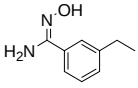
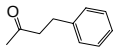
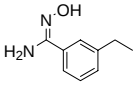
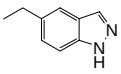
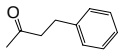
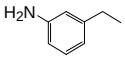
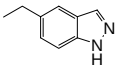
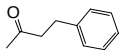
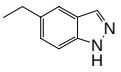
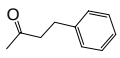
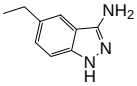
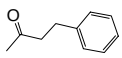
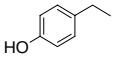
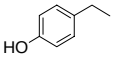
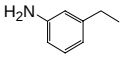
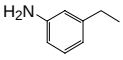
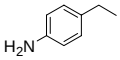
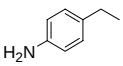
Compound	R^3	IC_{50} (nM) or % inhibition at 0.5 nM	EC_{50} (μM)		Compound	R^3	IC_{50} (nM) or % inhibition at 0.5 nM	EC_{50} (μM)	
			0% HS	50% HS				0% HS	50% HS
II-1		73%	0.009	0.027	**II-13**		67%	0.004	0.062
II-2		66%	0.003	0.060	**II-14**		72%	0.015	0.047
II-3		7.9	0.572	0.490	**II-15**		73%	0.004	0.056
II-4		6.1	1.49	3.22	**II-16**		67%	0.004	0.028
II-5		76%	0.009	0.077	**II-17**		1.2	0.212	0.546
II-6		3.8	0.308	0.576	**II-18**		58%	0.054	0.353
II-7		2.1	0.086	0.480	**II-19**		73%	0.003	0.016
II-8		53%	0.929	5.15	**II-20**		68%	0.009	0.018
II-9		55%	0.004	0.028	**II-21**		64%	0.004	0.056
II-10		75%	0.005	0.027	**I-1**		81%	0.004	0.029
II-11		73%	0.061	0.165	Ritonavir		79%	0.022	0.483
II-12		67%	0.006	0.048	Lopinavir		93%	0.016	0.067

Table 2. HIV-1 protease inhibitory and antiviral activities of *N*-acyl azacyclic ureas-R^1 and R^2 modifications



Compound	R^1	R^2	R^3	% inhibition at 0.5 nM	EC_{50} (μM) 0% HS
II-22				76[a]	0.017
II-23				88[a]	0.053
II-24				73[a]	0.045
II-25				90[a]	0.074
II-26				74[a]	0.013
II-27				66[a]	0.045
II-28				48[a]	0.050
II-29				65[b]	0.030
II-30				66[b]	0.037
II-31				56[b,c]	0.150

[a] At pH 7.0.
[b] At pH 4.5.
[c] At 1.0 nM.

74% yield. The secondary hydroxy group was protected as its SEM-ether **4**. After deprotecting the Cbz groups via hydrogenolysis, the resulting diamine **5** was cyclized with 1,1′-carbonyldiimidazole (CDI) in methylene chloride to give the azacyclic urea **6** in 62% yield. The N-benzyl group was successfully removed by palladium hydroxide catalyzed hydrogenolysis in quantitative yield to afford the key intermediate **7**. Acylation of the same nitrogen provided *N*-acyl azacyclic urea core **8**. Unsymmetrical alkylation of the urea nitrogens was accomplished in two steps. Deprotonation with potassium *tert*-butoxide in THF followed by addition of halide provided mono alkylated product **9**. Compound **9** was then reacted with sodium hydride and second halide in DMF to afford the diakylated product **10**. The compounds with identical R^1 and R^2 substituents were made by deprotonation with sodium hydride followed by addition of 2 equiv of halide. Finally, the SEM ether was removed with 4 N hydrochloric acid in methanol at room temperature to give the series of acyl azacyclic ureas **II**.[12]

The acyl azacyclic ureas were tested in enzyme inhibition and whole cell antiviral assays.[10] The results are shown in Tables 1 and 2. Compared to the most potent azacyclic urea (**I-1**)[2], the acyl analogs displayed similar cellular antiviral activities. Acyl azacyclic ureas retained excellent activity in the presence of 50% of human serum. For most of acyl azacyclic ureas, the serum effect is modest and the activity is about 3- to 8-fold decreased (Table 1).

In addition to antiviral potency against wild type HIV, the activity against multiple drug resistant viral strains is a very important criterion for selecting next generation protease inhibitors. Despite the efficacy of current protease inhibitors containing antiretroviral regimens, some failures do occur due to the development of viral resistance. Selected acyl azacyclic ureas were tested against multiple drug resistant strains IND-R[5] and A17.[6] A number of compounds displayed excellent activity, with up to 15-fold improved potency over Lopinavir (Table 3).

Molecular modeling and X-ray crystal structures were used to understand the SAR. The crystal structure of **II-11** (Fig. 2, PDB entry 2A4F)[11] reveals a binding mode essentially identical to that of **I-1** (PDB entry 1PRO).[2b]

Table 3. Antiviral activities of *N*-acyl azacyclic ureas against HIV wild type and resistant mutant virus

Compound	EC_{50} (μM)		
	WT	IND[a]	A17[b]
II-1	0.009	0.094	0.586
II-2	0.003	0.132	0.367
II-7	0.086	1.04	>10
II-9	0.004	0.049	0.102
II-12	0.006	0.088	0.133
II-15	0.004	0.054	0.065
II-16	0.004	0.164	0.189
II-19	0.003	0.081	0.455
II-20	0.009	0.227	0.562
II-22	0.017	0.014	0.192
II-23	0.053	0.504	0.464
II-24	0.045	—	2.02
II-25	0.074	0.058	0.084
II-26	0.013	0.023	0.098
II-27	0.045	0.821	1.07
II-28	0.050	0.102	0.897
II-29	0.030	0.171	0.312
II-30	0.037	—	2.07
II-31	0.150	—	—
I-1	0.004	—	—
Ritonavir	0.022	—	1.24
Lopinavir	0.016	0.385	1.06

[a] IND-R: L10R/M46I/L63P/V82T/I84V.
[b] A17: L10F/V32I/M46I/I47V/Q58E/I84V.

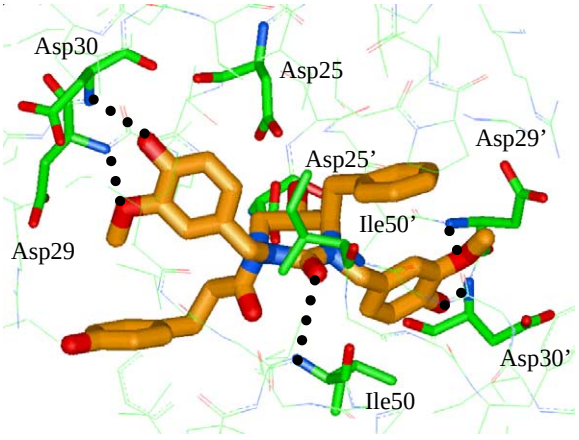


Figure 2. X-ray crystal structure of the *N*-acyl azacyclic urea **II-11** in complex with HIV-1 protease.

The 3-OCH_3/4-OH makes the same hydrogen bonds with the main chain N–H groups of Asp29, Asp30, Asp29′, and Asp30′. The hydroxyl group of the azacyclic urea is oriented between the catalytic aspartates of Asp25 and Asp25′. The carbonyl oxygen of the azacyclic urea core is hydrogen bonded to the main chain N–H of Ile50 and Ile50′. The phenyl ring makes van der Waals interactions with Gly49′, Val82, and Ile84. The difference between **II-11** and **I-1** is the P_1' side chain. The acyl oxygen is not making a direct contact to the protein, it is 3.8 Å from the main chain N–H of Ile50, the closest protein atom. The remainder of the acyl side chain makes van der Waals contact with Leu23′, Val82′, and Pro81′. The terminal *para*-hydroxyl group on the acyl side chain

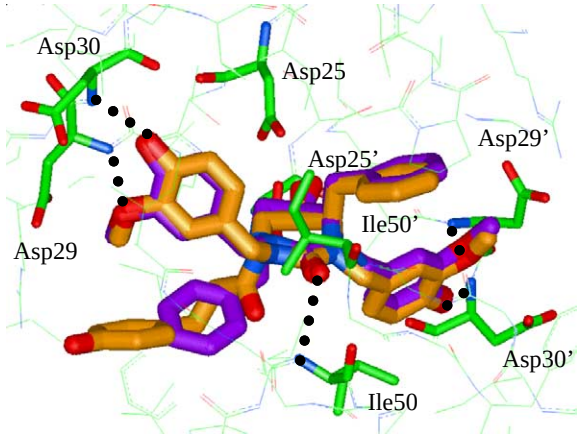


Figure 3. Overlap X-ray crystal structures of the *N*-acyl azacyclic urea **II-11**, and azacyclic urea **I-1** in complex with HIV-1 protease.

is solvent exposed, not making direct protein contacts. These interactions may contribute to the excellent activity of this series against the resistant viral strains (see Fig. 3).

Preliminary pharmacokinetic evaluation indicated that compounds in this series still have relatively low bioavailability (0–23.2%), even upon co-dosing with ritonavir, in spite of the blockage of the putative oxidative N-dealkylation. The observed poor pharmacokinetics of the acyl azacyclic ureas may be a consequence of very low solubility rather than a result of metabolic pathways. Further experiments are underway to ascertain this hypothesis and results will be reported separately.

References and notes

1. Chrusciel, R. A.; Strohbagh, J. W. *Curr. Top. Med. Chem.* **2004**, *4*, 1097.
2. (a) Sham, H. L.; Zhao, C.; Marsh, K. C.; Betebenner, D. A.; Lin, S.; Rosenbrook, W.; Herrin, T.; Li, L.; Madigan, D.; Vasavanonda, S.; Molla, A.; Saldivar, A.; McDonald, E.; Wideburg, N. E.; Kempf, D. J.; Norbeck, D. W.; Plattner, J. J. *Biochem. Biophys. Res. Commun.* **1996**, *225*, 436; (b) Sham, H. L.; Zhao, C.; Stewart, K. D.; Betebenner, D. A.; Lin, S.; Park, C. H.; Kong, X.; Rosenbrook, W.; Herrin, T.; Madigan, D.; Vasavanonda, S.; Lyons, N.; Molla, A.; Saldivar, A.; Marsh, K. C.; McDonald, E.; Wideburg, N. E.; Denissen, J. F.; Robins, T.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. *J. Med. Chem.* **1996**, *39*, 392.
3. Sham, H. L.; Betebenner, D. A.; Zhao, C.; Wideburg, N. E.; Saldivar, A.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. *J. Chem. Soc., Chem. Commun.* **1993**, 1052.
4. Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Won, Y. N.; Chang, C. H.; Weber, P. C.; Jackson, D. A.; Sharp, T. R.; Erickson-Vittanen, S. *Science* **1994**, *263*, 380.
5. Resistant viral strain IND-R: L10R/M46I/L63P/ V82T/ I84V. See: (a) Javier Martinez-Picado, J.; Savara, A. V.; Sutton, L.; D'Aquila, R. T. *J. Virol.* **1999**, *73*, 3744; (b) Condra, J. H.; Schleif, W. A.; Blahy, O. M.; Gabryelski, L. J.; Graham, D. J.; Quintero, J. C.; Rhodes, A.; Shivaprakash, M.; Titus, D.; Yang, T.; Teppler, H.; Squires, K. E.; Deutsch, P. J.; Emini, E. A. *Nature* **1995**, *374*, 569.

6. Resistant viral strain A17: L10F/V32I/M46I/I47V/ Q58E/ I84V. See: Mo, H.; Lu, L.; Dekhtyar, T.; Stewart, K. D.; sun, E.; Kempf, D. J.; Molla, A. *Antiviral Res.* **2003**, *59*, 173.
7. 2*S*,3*R*-epoxide (**1**) was synthesized from Cbz-L-phenylalaninol. See Ref. 3.
8. Compound **2** was prepared from benzylhydrazine and *N*-(benzyloxycarbonyloxy)succinimide in CH_2Cl_2 at rt in 69% yield.
9. Dutta, A. S.; Morley, J. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1712.
10. Protease inhibition assay and cell culture assay see (a) Sham, H. L.; Zhao, C.; Marsh, K. C.; Betebenner, D. A.; Lin, S.; Rosenbrook, W.; Herrin, T.; Li, L.; Madigan, D.; Vasavanonda, S.; Molla, A.; Saldivar, A.; McDonald, E.; Wideburg, N. E.; Kempf, D. J.; Norbeck, D. W.; Plattner, J. J. *Biochem. Biophys. Res. Commun.* **1996**, *225*, 436; (b) Miller, M.; Jaskolski, M.; Rao, J. K. M.; Leis, J.; Wlodawer, A. *Nature* **1989**, *337*, 576.
11. Data were collected at beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science under Contract No. W-31-109-Eng-38.
12. All Compounds reported have NMR, MS, and analytical data consistent with the structures assigned.