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Synthesis of novel HIV protease inhibitors (PI) with activity against PI-resistant virus

Subharekha Raghavan,[a,*] Zhijian Lu,[a] Teresa Beeson,[a] Kevin T. Chapman,[a] William A. Schleif,[b] David B. Olsen,[b] Mark Stahlhut,[b] Carrie A. Rutkowski,[b] Lori Gabryelski,[b] Emilio Emini[b] and James R. Tata[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Antiviral Research, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—A series of HIV protease inhibitors with modifications on the P3 position have been designed and synthesized. These compounds exhibit excellent antiviral activity against both the wild type enzyme and PI-resistant clinical viral isolates. The synthesis and biological activity of the compounds are described.

Human immunodeficiency virus (HIV) protease cleaves the *gag* and *gag-pol* polyproteins required by the infectious virus to mature. Inhibition of HIV protease results in immature virons that are incapable of replication.[1] Protease inhibitors (PIs) used in combination with reverse transcriptase inhibitors form the basis of HAART therapy that has led to a significant reduction in HIV-related morbidity. However, despite their clinical success, prolonged use of PIs has resulted in emergence of PI-resistant virus.[2] Thus, there remains a need to identify and develop next generation of drugs that are effective against a broad panel of PI-resistant mutant viruses.

In a recent paper, we described a hybrid analog that incorporated P1′P3′ portion of indinavir and the P1P2P3 of JE-2147 that resulted in a new class of potent HIV protease inhibitors exemplified by (**1**).[3] This novel class of compounds exhibited excellent antiviral activity against wild type enzyme (NL4-3) in both the enzyme inhibition assay (IC_{50}), and the viral spread assay (CIC_{95}). In addition, this series also showed good antiviral activity against a panel of HIV-resistant mutants. Extensive SAR conducted around the P2 portion of the molecule, while keeping the P1′, P3′, and P3 groups constant, identified the 2,6-dimethyl benzyl group to be optimal for antiviral activity. In order to explore the SAR of the P3 portion of the molecule, we undertook a study to find a surrogate for the thiazolidine ring moiety. In this communication, we report that the thiazolidine ring at P3 can be replaced with a spiro oxazolidine moiety (Fig. 1).

The compounds described herein were prepared using two different synthetic routes (Schemes 1 and 2). The synthesis of the compounds of the structure **10** is outlined in Scheme 1. The epoxide **3**[4] was treated with NaH and *p*-methoxy benzyl alcohol to give the mono-

	HIV-1 protease inhibition IC_{50}(nM)	Viral Spread Assay CIC_{95} (nM)
	NL4-3	NL4-3
Indinavir	0.60	45
1	0.03	15

Figure 1. Hybrid of indinavir and JE-2147.

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* Corresponding author. Tel.: +1 732 594 7153; fax: +1 732 594 9473; e-mail: subha_raghavan@merck.com

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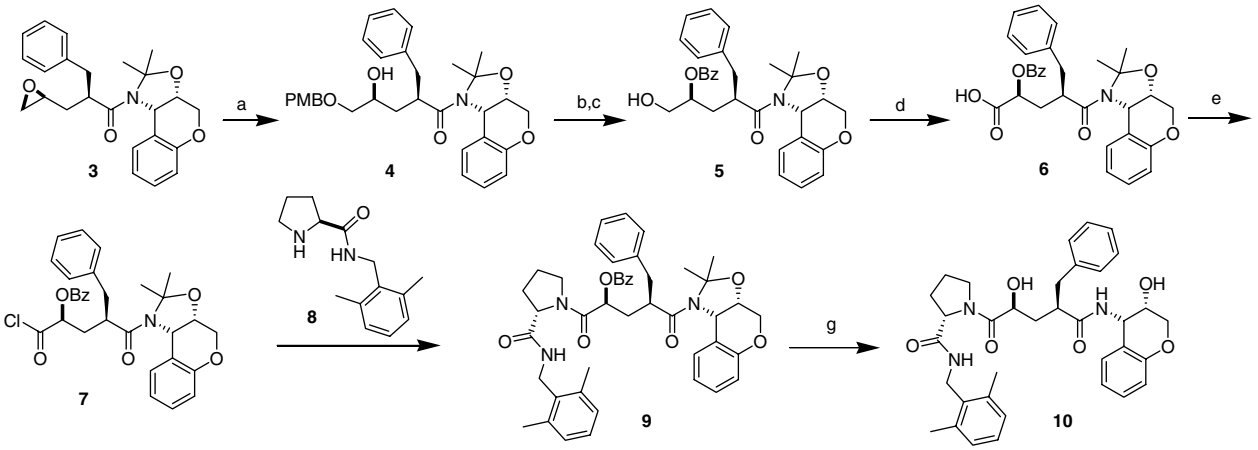
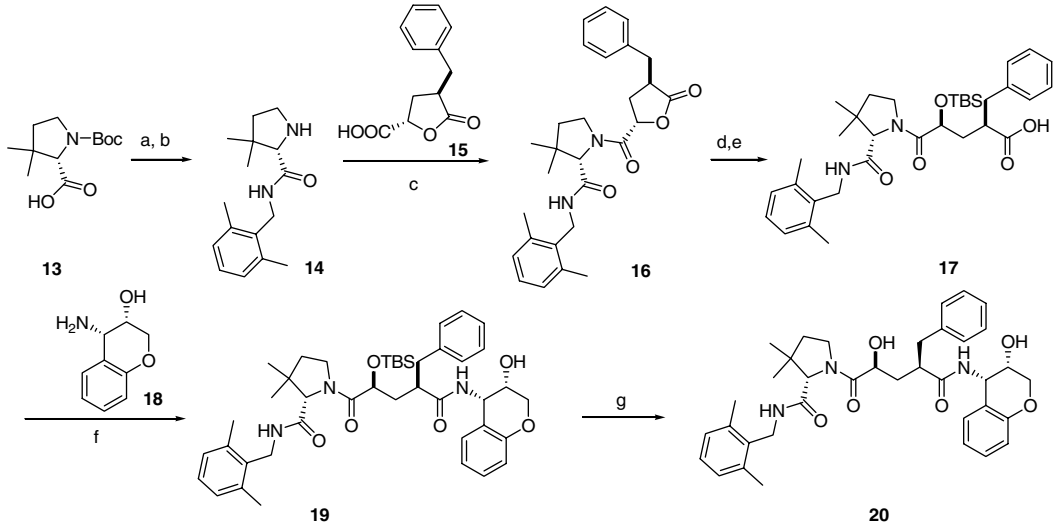


Scheme 1. Reagents and conditions: (a) $4MeOC_6H_5CH_2OH$, NaH, THF reflux, 60%; (b) BzCl, py., 84%; (c) DDQ, THF-DCM, 78%; (d) PDC, DMF, 70%; (e) $(COCl)_2$, C_6H_6, DMF (cat.); (f) amine **8**, TEA, DCM; (g) HCl, dioxane; 1 N NaOH, dioxane.



Scheme 2. Reagents and conditions: (a) pyBrop, 2,6-dimethyl benzyl amine (91%); (b) TFA, DCM (100%); (c) pyBOP, HOAt, DIEA, **15**, DCM, rt (81%); (d) 1 N LiOH, H_2O/THF, rt; (e) TBSOTf, DIEA, EtOAc, rt; H_2O/THF; (f) 4-amino-3-chromanol, pyBOP, HOAt, DIEA, 59%; (g) TBAF, THF, rt, 3 h, 75%.

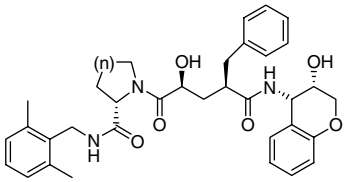
protected diol **4**. The secondary alcohol was protected as the benzoate ester using BzCl/pyridine. Next, the PMB ether was de-protected under oxidative conditions with DDQ to give the primary alcohol **5**, which was oxidized to the acid **6** with PDC in a 60% yield. The acid **6** was converted to the acid chloride **7** under standard conditions using oxalyl chloride. Commercially available Boc-protected L-proline was coupled to 2,6-dimethyl benzyl amine using bromo-tris-pyrrolidino-phosphonium hexafluoro phosphate (pyBrop) and DIEA in 90% yield. Removal of the Boc group with TFA yielded **8**, which upon treatment with the acid chloride **7** in DCM gave the desired amide **9** in 50% yield. Removal of the acetal group using HCl/dioxane followed by saponification of the benzoate ester gave the final product **10**.

All compounds were tested for their ability to inhibit the wild type enzyme (NL4-3, IC_{50}).[5a] In addition, the compounds were tested for their ability to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected with the NL4-3 isolate (CIC_{95}).[5b] A subset of compounds were also tested against highly PI-resistant viral constructs that were engineered from viral isolates of patients infected with multiple PI-resistant HIV.[6]

We first examined the effect of ring size on activity. Accordingly, four-, five-, and six-membered-ring analogs were synthesized (Table 1). Proline analog **10** was the most active compound with an IC_{50} = 14 nM compared to either **11** or **12**. However, compared to the thiazolidine analog **1**, it was significantly less potent. This result suggested to us, the importance of the *gem*-dimethyl groups present on the thiazolidine ring for activity.

We reasoned that the *gem*-dimethyl group could impart a conformational constraint that helps orient the entire benzyl amide group at the P2 position in the enzyme binding pocket. In addition, it was felt that the *gem*-di-

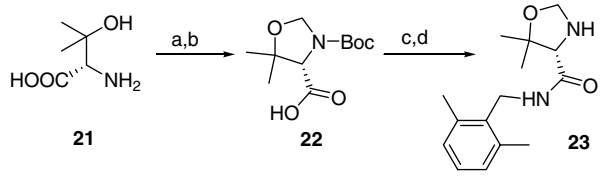
Table 1. Antiviral activity as a function of ring size

	n	Enzyme inhibition IC_{50} (nM) NL4-3	Viral spread assay CIC_{95} (nM) NL4-3
Indinavir		0.6	45
	0, **11**	90	1000
	1, **10**	14	1000
	2, **12**	42	1000

methyl group might have some positive van der Waals interactions, accounting for the high affinity to the enzyme. To study this further the corresponding carbon (**20**) and oxygen analogs (**24**) of the thiazolidine were synthesized.

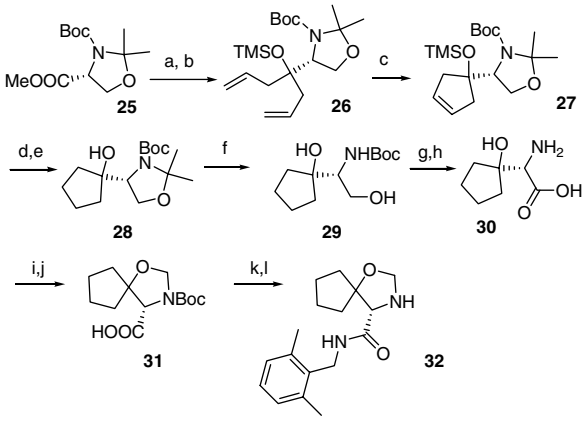
Scheme 2 outlines the synthesis of these compounds. (2*S*)-3,3-Dimethyl-*N*-(Boc)proline **13** was prepared using a literature procedure.[7] The carboxyl group was coupled to 2,6-dimethyl benzyl amine with pyBrop/DIEA in 91% yield (Scheme 2). After removal of the Boc group with TFA, **14** was coupled to the acid **15**[3,8] using pyBOP to give the desired amide **16** in 81% yield. Hydrolysis of the lactone **16** with aqueous 1 N LiOH in dioxane followed by protection of the alcohol as the *tert*-butyl dimethyl silyl ether with TBSOTf/DIEA gave the desired acid **17**. This was coupled with *cis* amino chromanol[9] **18** to afford **19**. Removal of the TBS group with 1 M TBAF solution provided the final product **20** in 75% yield.

Synthesis of the oxazolidine intermediate **23** is outlined in Scheme 3. Commercially available (*S*)-(−)-2-amino-3-hydroxy-3-methyl-butanoic acid (β-hydroxy-L-valine) **21** upon treatment with aqueous formaldehyde gave the desired oxazolidine.[10] The nitrogen was protected with Boc group to give **22**. This was elaborated using the chemistry described in Scheme 2 to **24**.



Scheme 3. Reagents and conditions: (a) HCHO (37% aqueous), 2N NaOH, 24 h, rt; (b) Boc_2O; 70% over 2 steps; (c) 2,6-dimethyl benzyl amine, pyBrop, DIEA, 67%; (d) CH_3SO_3H, DCM, 93%.

In vitro antiviral activity for the compounds is summarized in Table 2. As anticipated, we observed a 100-fold improvement in activity for **20** compared to **10** for the wild type enzyme. More significant was the superior



Scheme 4. Reagents and conditions: (a) AllylMgBr, THF, −20 °C to rt, 75%; (b) TMSOTf, Et_3N, DCM, 55%; (c) Schrock's catalyst, C_6H_6, reflux 95%; (d) $HCOONH_4$, HCOOH, EtOH, Pd/C, rt, 87%; (e) 1 M TBAF, THF, 83%; (f) TsOH, MeOH rt, 100%; (g) Jones oxidation, −15 °C to rt, 74%; (h) HCl, 1,4-dioxane; (i) HCHO (37% aq), 2 N NaOH; (j) Boc_2O, NaOH 48% over three steps; (k) pyBrop, DIEA, 2,6-dimethyl benzyl amine, 69%, (l) CH_3SO_3H, DCM, 78%.

Table 2. Influence of the *gem*-dimethyl group on antiviral activity

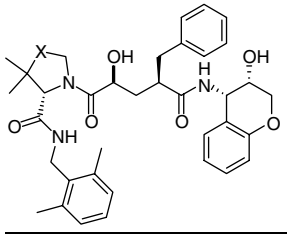
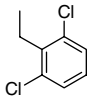
		HIV protease inhibition IC_{50} (nM)			Viral spread assay CIC_{95} (nM)		
		WT	K-60C	V-18C	WT	K-60C	V-18C
Indinavir		0.6	61	43.6	45	1000	890
	X=CH_2, **20**	0.14	1.6	0.4	15	500	31
	X=O, **24**	0.17	2.2	1.3	15	500	62

Table 3. Antiviral activity of spiro cyclopentane-oxazolidine analogs with different P2 substituents

		HIV-1 protease inhibition IC_{50} (nM)			Viral spread assay CIC_{95} (nM)		
		WT	K-60C	V-18C	WT	K-60C	V-18C
Indinavir		0.6	61	43.6	45	1000	890
	**33**	0.1	—	0.5	8	125	62
	**34**	0.11	0.5	1.0	62.5	250	500
	**35**	0.2	1.5	3.2	250	500	1000
	**36**	0.1	0.4	0.8	125	250	250

antiviral activity observed for both **20** and **24** against mutant enzymes, especially V18-C.

Encouraged by these findings we examined the effect of placing a larger group at the P3 position. Thus, the *gem*-dimethyl group was replaced with a spiro-cylopentane moiety (**33**) (Scheme 4).

Treatment of commercially available D-serine methyl ester **25** with an excess of allylmagnesium bromide in THF gave the desired tertiary alcohol in 75% yield, which was protected as the TMS ether to give **26**. Upon treatment of **26** with Schrock's catalyst in refluxing benzene, it smoothly underwent olefin metathesis to give the cyclopentene **27** in excellent yield.[11] Hydrogenation of the double bond followed by de-protection of the silyl group afforded the alcohol **28**. Next, the acetonide group was removed under acid catalyzed conditions with *p*-toluene sulfonic acid to give the diol **29**. Jones oxidation followed by removal of the Boc group gave the amino acid **30**. Treatment with formaldehyde followed by Boc protection as described in Scheme 3 gave the desired amino acid. This material was transformed to **33** using the chemistry described in Scheme 1. We also synthesized three additional analogs that varied the groups at the P2 position. The biological results are summarized in Table 3.

Consistent with our proposition, placing a large group at the P3 position resulted in the wild type enzyme activity being maintained. We also observed an improvement in the activity against the PI-resistant strains of the virus. Interestingly, replacing the 2,6-dimethyl benzyl group at P2 position with 2,6-dichloro benzyl group resulted in a 2-fold decrease in activity. However, replacing the benzyl group at the P2 position with the smaller 5-membered heterocycle such as the isoxazole **35** or the isothiazole **36** resulted in a significant loss in activity particularly against PI-resistant virus isolates. This result was surprising to us because earlier observations by our group had shown that these groups synergized well with the thiazolidine ring.[3a] The decrease in activity of **35** and **36** in the viral spread assay could be due to poor cell permeability.

In summary, we designed and synthesized a new series of HIV protease inhibitors that were active against HIV-resistant clinical isolates. In particular, we found that a spiro cyclopentane-oxazolidine moiety to be an ideal replacement for the thiazolidine ring while maintaining the activity against both wild type and PI-resistant isolates.

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