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P1′ oxadiazole protease inhibitors with excellent activity against native and protease inhibitor-resistant HIV-1

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Abstract—HIV-1 protease inhibitors (PI's) bearing 1,3,4-oxadiazoles at the P1′ position were prepared by a novel method involving the diastereoselective installation of a carboxylic acid and conversion to the P1′ heterocycle. The compounds are picomolar inhibitors of native HIV-1 protease, with most of the compounds maintaining excellent antiviral activity against a panel of PI-resistant strains.

For nearly a decade, HIV-1 protease inhibitors (PI's) have served as a cornerstone of multi-drug antiretroviral therapy.[1] With increased frequency and duration of treatment, however, the rate of resistance toward antiretroviral agents, including PI's, has risen alarmingly, fueling the search for next-generation drugs with broad efficacy against PI-resistant mutants.[2] Previous reports from our group have described the replacement of the P3, P2, P1′, and P3′ subsites of indinavir (Fig. 1) to provide compounds with improved pharmacokinetic profiles and greatly increased potency against native HIV-1 and a broad spectrum of clinically derived PI-resistant strains.[3] More recently, we have replaced the P1′ phenyl ring of indinavir and related analogs with pyridyl groups to provide compounds with excellent potency against wild-type (wt) and PI-resistant HIV-1.[4] Transposition of the pyridyl group from P3 to P1′ was also shown to improve inhibitory profiles against cytochrome P-450 isoforms. Continuing this line of exploration, we describe herein the preparation and in vitro activity of a series of HIV protease inhibitors containing 1,3,4-oxadiazoles at the P1′ position. The compounds were synthesized by a novel approach involving the stereospecific introduction of a P1′ carboxylic acid, which can be used to install a variety of heterocycles at that position. All of the compounds prepared are picomolar inhibitors of native HIV-1 protease. A subset of compounds was tested against enzyme obtained from highly PI-resistant HIV-1 variant V-18, with most maintaining picomolar activity. The oxadiazoles were also screened for their ability to inhibit the viral spread of native HIV-1 and several clinically isolated PI-resistant strains[5] in MT4 human T-lymphoid cells,[6] with most of the compounds displaying nanomolar activity (IC_{95}) against the entire panel.

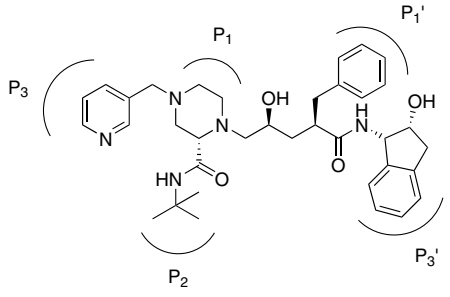

Figure 1. Binding sites of indinavir (**12**).

Keywords: Protease inhibitor; HIV.
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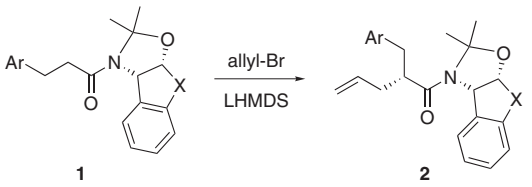
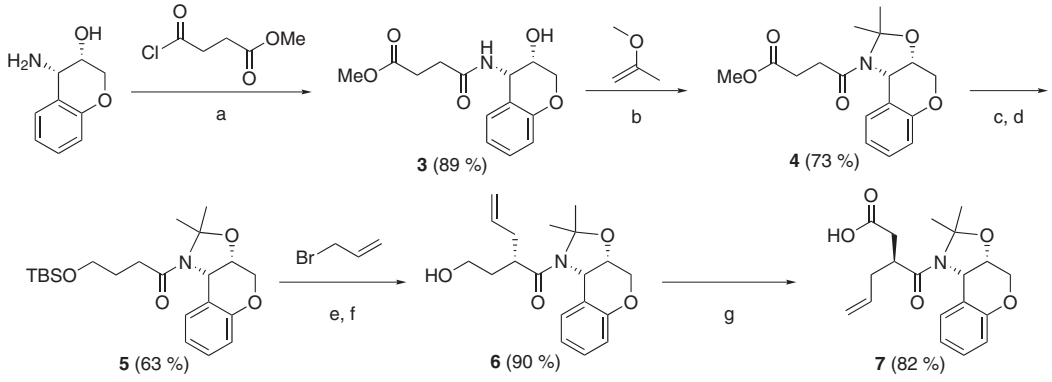


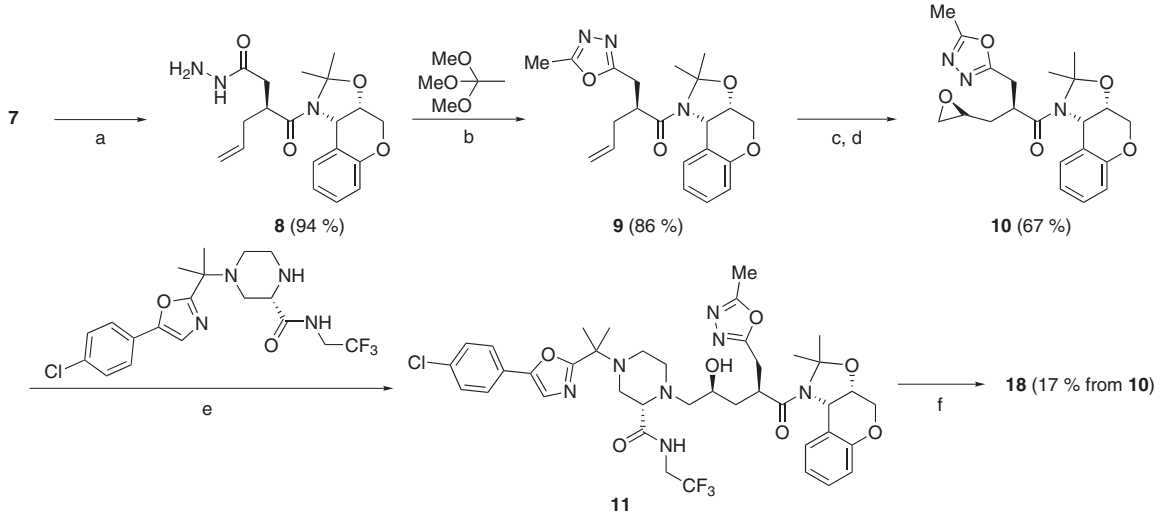
Scheme 1.

A key step in the synthesis of indinavir and related analogs is the diastereoselective allylation of a chiral phenylpropionamide **1**, which takes place alpha to the carbonyl group to provide intermediate **2** (Scheme 1).[7] We have found with some P1′ heterocycle analogs, however, that allylation proceeds alpha to the heterocycle, giving rise to the undesired regioisomer. To circumvent this problem, we settled on the strategy shown in Scheme 2, involving diastereoselective allylation of TBS-protected hydroxybutyramide **5**, followed by TBS deprotection to afford alcohol **6** and oxidation to the carboxylic acid **7**, which can be utilized to generate a variety of P1′ heterocycles. We chose to prepare 1,3,4-oxadiazoles due to their polarity, flexibility toward diversification, and to further explore the transposition of a heterocyclic nitrogen from P3 to P1′. The arylheterocycle P3 substituents were developed previously in our group and have been shown to significantly improve the activity of indinavir analogs against native and PI-resistant HIV-1.[3c,4a]

Synthesis of representative 1,3,4-oxadiazole **18** is depicted in Scheme 3. Carboxylic acid **7** was converted to hydrazide **8** using typical peptide coupling conditions. Due to the presence of the acetonide, heterocycle formation under nonacidic conditions was required. To that end, reaction of the hydrazide with the appropriate trimethoxy or triethoxy orthoester[8] afforded the oxadiazole **9** in good yield. The final product was assembled using established procedures,[7a,3d] involving stereoselective conversion of the allyl group to the epoxide **10** via the iodohydrin, followed by reaction with the appropriate piperazine to provide aminoalcohol **11**. Removal of the acetonide using TFA in methylene chloride provided protease inhibitor **18**.[9] All products were isolated by reverse-phase LC and characterized by ^{1}H NMR and LC–MS (ESI).

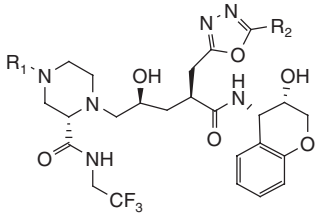


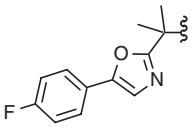
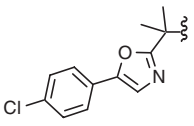
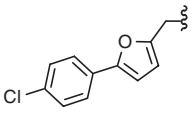
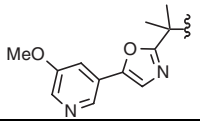
Scheme 2. Reagents and conditions: (a) Et_3N, CH_2Cl_2, 0 °C; (b) TsOH, CH_2Cl_2, 16 h; (c) $NaBH_4$, MeOH, 0 °C; (d) TBS–Cl, imidazole, CH_2Cl_2; (e) LHMDS, THF, −20 to 0 °C; (f) TBAF, THF; (g) PDC, DMF, 4 h.



Scheme 3. Reagents and conditions: (a) hydrazine, EDC, HOBt, CH_2Cl_2/DMF; (b) neat, 115 °C, 24 h; (c) NIS, $NaHCO_3$, EtOAc/H_2O, 16 h; (d) NaOMe/MeOH, EtOAc, 15 min; (e) *t*-amyl alcohol, 75 °C, 16 h; (f) 10% TFA/CH_2Cl_2, 15 min.

Table 1. Enzyme inhibition and intiviral activity of HIV protease inhibitors



Entry	R1	R2	HIV-1 protease IC_{50} (nM)		Viral spread CIC_{95} (nM)[a]				
			NL4-3	V-18	NL4-3	V-18	4X	K-60	Q-60
12	Indinavir		0.60	61	50	>1000	400	>1000	>1000
13		H	0.024	0.065	125	125	31	125	31
14		Me	0.027	0.19	125	63	31	125	31
15		Pr	0.016	0.19	16	63	16	16	⩽8
16		Ph	0.020		⩽8	63	16	⩽8	16
17		H	<0.015	0.043	125	63	31	63	31
18		Me	0.016	0.060	250	63	16	31	⩽8
19		Pr	<0.015	0.12	⩽8	63	⩽8	16	⩽8
20		Ph	<0.015	0.18	⩽8	125	16	⩽8	16
21		H	0.015	0.099	63	125	31	63	31
22		Me	<0.015		63	63	31	31	31
23		Pr	<0.015		⩽8	125	31	⩽8	16
24		Ph	0.019		15.6	250	125	⩽8	63
25		H	0.24	7.4					
26		Me	0.40	15	>1000	>1000	>1000	>1000	>1000
27		Pr	0.32		500	>1000	500	500	500
28		Ph	0.48		125	>1000	250	250	500

[a] The < and > values denote the lower and upper concentrations tested in our assays.

The oxadiazoles were evaluated for their ability to inhibit native HIV-1 protease (NL4-3). As shown in Table 1, all of the compounds prepared inhibit the enzyme with picomolar activity (IC_{50}) and are more potent than indinavir. Five compounds (**17**, **19**, **20**, **22**, and **23**) exhibit activities below the limit of the assay ($IC_{50} < 15$ pM). Increasing polarity at the P3 position results in decreased activity, as seen somewhat in fluorophenyl oxadiazoles **13**–**16**, and more so in methoxypyridyl derivatives **25**–**28**. Excepting the limit of the assay, the oxadiazole substituents do not strongly influence potency, with all the oxadiazoles within a given series being of similar activity.

A subset of compounds was also tested against HIV-1 protease obtained from highly PI-resistant clinical isolate V-18. In all cases, the oxadiazoles are less potent against the mutant protease than against native enzyme, though compounds with a terminal phenyl group at P3 maintain excellent activity ($IC_{50} < 0.2$ nM). Interestingly, the oxadiazole substituents more strongly modulate activity against the mutant protease, with the more polar 5-H oxadiazoles **13** and **17** being better inhibitors than their corresponding propyl and phenyl derivatives. In addition, the 5-H and 5-Me oxadiazoles (with the exception of **25** and **26**) show only a 3–10-fold decrease in activity against V-18 protease compared to native enzyme, compared to a 100-fold loss by indinavir. P3 methoxypyridyl derivatives **25** and **26** lose most activity against V-18, being 30-fold less active than against native protease.

The compounds were evaluated for their ability to inhibit the spread of native HIV-1 and clinically obtained PI-resistant strains[5] in MT4 human T-lymphoid cells in culture.[6,10] Listed in Table 1 are the compound concentrations required to inhibit infectious viral spread by 95% relative to untreated virus control culture (CIC_{95}) in the presence of 10% fetal bovine serum. Despite their similar protease inhibitory activities, oxadiazoles with a given P3 substituent differ greatly in their ability to block the spread of wt virus, with propyl and phenyl oxadiazoles being significantly more potent than their 5-Me and 5-H counterparts. This difference may reflect greater cell penetration of the more hydrophobic oxadiazoles, as has been reported previously with indinavir analogs,[11] though this possibility has yet to be examined.

In contrast to indinavir, which is ineffective at blocking the spread of PI-resistant strains, all of the oxadiazoles tested, with the exception of P3 methoxypyridyl analogs

26–**28**, maintain nanomolar activity against the entire panel of resistant variants. As with native HIV-1, propyl, and phenyl oxadiazoles are generally more active against PI-resistant strains than the corresponding Me and H analogs, though the trend is much less pronounced than is seen against wt virus. The decreased sensitivity of variant V-18 toward the oxadiazole substituents may in part reflect a balance between greater protease activity of the less hydrophobic oxadiazoles and superior cell penetration of the more hydrophobic oxadiazoles. Significantly, in all but one case, 5-H oxadiazoles **13**, **17**, and **21** and 5-methyl oxadiazoles **14**, **18**, and **22** are at least as effective at inhibiting the spread of all the PI-resistant strains tested as blocking the spread of native virus. Such is the case even with variant V-18, despite greater intrinsic activity of the compounds against native HIV-1 protease compared to V-18-derived enzyme.

The P1′ oxadiazoles described herein generally show excellent activity against both native and PI-resistant HIV-1. Markedly different trends are observed, however, against wild-type and mutant strains. While oxadiazoles with the same P3 substituent show similar activities against native HIV-1 protease, their ability to block the spread of wt virus in the cell-based assay vary greatly. Conversely, oxadiazoles within a series display a wider range of activity against mutant V-18 protease than against native enzyme, though differences in their ability to block the spread of PI-resistant strains are generally less pronounced than is seen against wt virus. Notably, compounds were prepared, which are more effective at blocking the spread of a panel of PI-resistant HIV-1 variants and native HIV-1. The differing responses of native and PI-resistant HIV-1 toward the oxadiazoles implicate the potential importance of the P1′ position for developing next-generation PI's of high potency and wide spectrum.

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9. Data for compound **18**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.33 (t, J = 6.4 Hz, 1H); 7.55 (m, 2H); 7.42 (m, 2H); 7.28 (s, 1H); 7.13 (d, J = 8.0 Hz, 1H); 7.10 (t, J = 7.8 Hz, 1H); 6.83 (d, J = 8.2 Hz, 1H); 6.77 (m, 1H); 5.25 (dd, J = 8.2, 4.1 Hz, 1H); 4.26 (dd, J = 7.7, 4.6 Hz, 1H); 4.20 (m, 1H); 4.14 (d, J = 11.7 Hz); 3.82 (m, 1H); 3.72 (m, 1H); 3.35 (s, 1H); 3.16–3.23 (m, 2H); 3.05 (d, J = 11.7 Hz, 1H); 2.92 (m, 1H); 2.81 (d, J = 4.1 Hz, 1H); 2.62–2.74 (m, 4H); 2.46 (d, J = 3.2 Hz, 1H); 2.42 (s, 3H); 1.87 (t, J = 11.3 Hz, 1H); 1.73 (m, 1H); 1.62 (s, 3H); 1.61 (s, 3H); 1.47 (m, 1H). MS (ESI) 812.5 (M + 23); 790.5 (M + 1).
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