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The Design, Synthesis and Evaluation of Novel HIV-1 Protease Inhibitors with High Potency Against PI-Resistant Viral Strains

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Abstract—Replacement of the pyridylmethyl moiety in indinavir with a pyridyl oxazole yielded HIV-1 protease inhibitors (PI) with greatly improved potency against PI-resistant HIV-1 strains. A *meta*-methoxy group on the pyridyl ring and a *gem*-dimethyl methyl linkage afforded compound **10** with notable in vitro antiviral activity against HIV-1 viral strains with reduced susceptibility to the clinically available PIs. Compound **10** also demonstrated favorable in vivo pharmacokinetics in animal models.

The introduction of HIV-1 protease inhibitors (PIs) into the clinic in 1996 was a great advance in the treatment of AIDS.[1] However, there is an increasing incidence of viral resistance to these agents.[2] Therefore, there exists a need for PIs that possess increased potency against the wild-type virus as well as against viral strains with reduced PI susceptibility.

Recently, we have reported modifications of the indinavir (**1**) scaffold that have afforded compounds with increased potency against resistant HIV-1 viral strains, as exemplified by compound **2** (Fig. 1).[3] Although compound **2** has desirable antiviral activity and a good pharmacokinetic profile, it is a potent inhibitor of CYP isomers 3A4, 2D6 and 2C9, a property that would dramatically complicate its clinical use due to the potential for serious drug–drug interactions. This work details our efforts to modify this P450 inhibition profile while maintaining antiviral potency and bioavailability. These efforts have resulted in compound **10**.

The synthesis of compounds **4**, **5** and **6** (Table 1), which lack the *gem*-dimethyl substituent, was similar to that of indinavir.[4] The aldehydes used for the final reductive amination were prepared by formylation of the corresponding pyridyl furans. After much experimentation, a unique synthetic strategy for the incorporation of a *gem*-dimethyl linkage between the piperazine and the oxazole was developed. Scheme 1 illustrates this successful strategy for the preparation of compound **10**. The bis-protected piperazine I was first deprotected with TFA.[3] Silver triflate induced substitution with 2-bromo-2-methylpropionic acid afforded amino acid II. In a separate route, 3,5-dibromopyridine was converted into 3-bromo-5-methoxypyridine with sodium methoxide.[5] Treatment of III with isopropylmagnesium chloride followed by addition to the Weinreb amide of BOC-glycine provided aminoketone V. PyBOP mediated coupling of II and V gave ketoamide VI, which was treated with fuming sulfuric acid to afford oxazole VII with concomitant deprotection of the piperazine. Epoxide opening of VIII[6] with VII, followed by the final deprotection under acid conditions gave the desired product **10**.

The compounds, including indinavir, were tested for their ability to inhibit the protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells infected either by wild-type HIV-1

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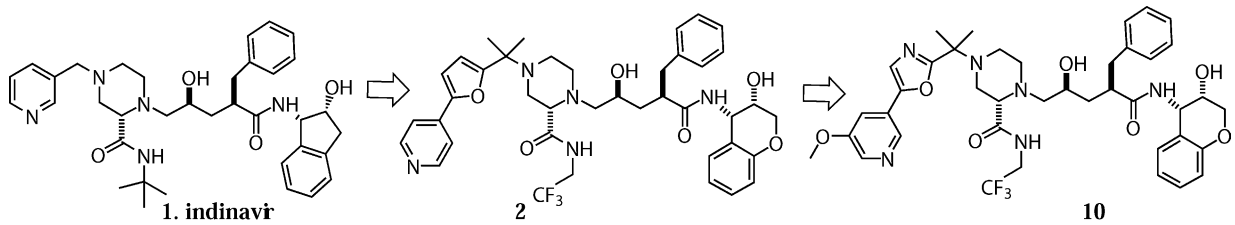
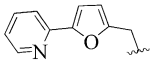
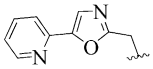
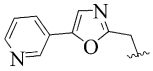
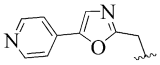
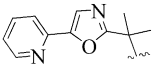
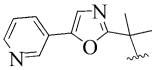
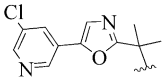
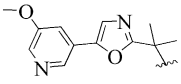
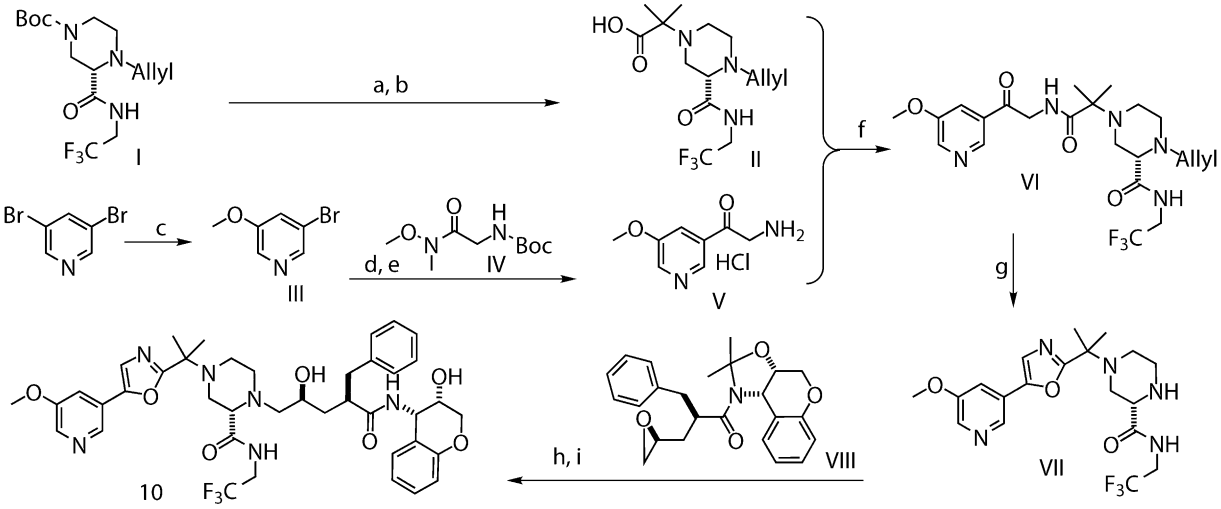


Figure 1. Modifications of indinavir that lead to compound **10**.

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

Compd	P_3 moiety	IC_{50} (nM)	Viral speed CIC_{95} (nM)				
			NL4-3	4X Virus	K-60C	V-18C	Q-60C
Indinavir		0.60	50	400	>1000	>1000	>1000
3		0.05	≤8	≤8	31	15	15
4		0.13	23	31	—	125	125
5		0.14	27	62	125	250	125
6		0.10	23	62	125	125	250
7		0.04	≤8	≤8	31	62	15
8		0.04	≤8	≤8	31	125	31
9		0.02	≤8	≤8	16	31	≤8
10		0.02	≤8	≤8	15	15	31



Scheme 1. (a) TFA, CH_2Cl_2; (b) 2-bromo-2-methylpropionic acid, silver trifluoromethane sulfonate, TEA, THF; (c) $NaOCH_3$, MeOH, DMF, 90 °C, 6 h; (d) *N*-(*tert*-butoxycarbonyl)glycine *N*′-methoxy-*N*′-methylamide, isopropylmagnesium chloride, THF; (e) HCl, MeOH; (f) PyBop, HOAt, DIEA, DMF; (g) fuming sulfuric acid, 55 °C; (h) EtOH, reflux; (i) MeOH, HCl.

(strain NL4-3) or by various PI-resistant strains.[7,8a] The results, presented in Table 1, are given as the concentration of compound that inhibits at least 95% of infectious viral spread in the cell culture relative to the untreated virus control culture (CIC_{95}). Each of the PI-resistant strains (4X, K-60C, V-18C, Q-60C) represent engineered viral constructs that express varying patterns of multiple PI resistance as described in our previous reports.[3,8]

As indicated in Table 1, indinavir expressed no activity against all of the resistant viral strains whereas the other tested compounds expressed varying degrees and patterns of activity. When the furan moiety in compound **3** was replaced with the oxazole (compound **4**), a significant loss in potency was observed. Some of this potency was regained in the *gem*-dimethyl analogues (compare **4** with **7** and **5** with **8**). A further increase in potency was realized with the addition of a *meta* substituent to the pyridyl ring. Chloro or methoxy substituents at this position (compounds **9** and **10**) provided a substantial increase in activity against all of the viral variants.

Previous studies have shown that CYP3A4 is the primary P450 enzyme that is responsible for the metabolism of indinavir.[9] Furthermore, a correlation between inhibition of 3A4 and metabolic stability has been demonstrated for indinavir related analogues.[10] Although the inhibition of 3A4 could result in drug–drug interactions, clinical experience with both indinavir and ritonavir has shown this to be manageable.[11] In fact, treatment strategies have been devised to take advantage of this property; thus the inhibition of 3A4 without significant inhibition of either 2D6 or 2C9 is considered acceptable.[12]

As expected, the CYP inhibition profile for the pyridyl–oxazole series (Table 2) parallels the pyridyl–furan series.[3] Once again, the *meta*-pyridine provided the best selectivity for CYP3A4 over 2D6 and 2C9. The trend toward better metabolic stability (shown in Table 2 as CL_{int}) with increasing 3A4 inhibition also holds here, with the most potent inhibitor, compound **10**, also being the most metabolically stable.

The pharmacokinetic profiles of compounds **8**, **9**, and **10** were evaluated in dogs dosed both IV and PO.[13] Compound **10** displayed reasonable PK parameters in dogs (Table 3).

Follow up studies were performed on compound **10** in both rat and rhesus. In the rat, compound **10** displayed non-linear pharmacokinetics with oral doses in the 10–160 mg/kg range. Both C_{max} and AUC values increased in the rat in a greater than dose-proportional manner (see Table 4), suggesting clearance via a saturable first pass metabolic pathway. Further evidence of this was obtained by comparing the steady-state drug concentrations in the systemic circulation during portal vein verses femoral vein infusion. The hepatic extraction ratio dropped from 90% at low dose (0.6 μg min) to 38% at high dose (12 μg.min).

In the rhesus, compound **10** displayed a high clearance with poor bioavailability (Table 4), a result that is consistent with its poor metabolic stability in rhesus liver microsomes.

The differences in in vivo clearance rate of compound **10** among species was further investigated by incubation of the compound in liver microsomes. As can be seen in Table 5, compound **10** is most stable in human microsomes and least stable in rhesus microsomes.

Assuming that compound **10** would be cleared primarily by hepatic metabolism in humans, as it is in rat and most likely in dog and rhesus, it would be expected to display non-linear pharmacokinetics in the clinic. Accordingly, at higher compound plasma concentrations, its clearance would be saturated enabling the maintenance of clinically relevant drug levels. This property, along with the compound's stability in human microsomes and its excellent antiviral potency, make the compound potentially suitable for further investigation.[14]

Table 2. In vitro metabolism assays of HIV protease inhibitors

Compd	CL_{int}[a] (mL/min/kg)	CYP3A4 IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP2C9 IC_{50} (μM)
Indinavir	50	0.15	>30.00	>30.00
7	500	5.0	22.2	28
8	27	0.11	10.5	33
9	35	0.04	4.17	21
10	13	0.04	8.7	30

[a]Measured in human liver microsomes.

Table 3. Pharmacokinetics of HIV protease inhibitors in dog

Compd	Dose PO/IV (mpk)	C_{max} (μM)	$T_{1/2}$ (min)	CL_p (mL/min/kg)	F (%)
8	10/2	3.8	94	21	10
9	10/2	1.0	92	19	8
10	10/2	4.3	61	18	30

Table 4. Pharmacokinetics of compound **10** dosed in rat and rhesus

Species	Dose (mpk)	C_{max} (μM)	$T_{1/2}$ (min)	AUC (μM-h)	CL_p (mL/min/kg)	F (%)
Rhesus	10/2	0.10	73	0.12	33	2.2
Rat	10/5	0.04	26	0.03	104	1.5
	20	0.37		0.38		9.2
	50	3.06		4.02		40.5
	160	6.88		15.67		47.2

Table 5. Incubation of compound 10 in liver microsomes CL_{int} (mL/min/kg)

Rat	Dog	Rhesus	Human
87	67	120	13

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14. Data for compound **10**. ^{1}H NMR (CD_3OD, 500 Hz) δ 8.49 (s, 1H), 8.22 (d, $J=1.6$ Hz, 1H), 7.66–7.67 (m, 1H), 7.20–7.25 (m, 4H), 7.14–7.17 (m, 1H), 7.06–7.10 (m, 2H), 6.80 (t, $J=7.6$ Hz, 1H), 6.71 (d, $J=8.0$ Hz, 1H), 5.13 (d, $J=3.8$ Hz, 1H), 4.04–4.06 (m, 2H), 3.92–3.98 (m, 1H), 3.94 (s, 3H), 3.78–3.82 (m, 1H), 3.72–3.77 (m, 2H), 3.06–3.10 (m, 1H), 2.96–3.03 (m, 2H), 2.88–2.94 (m, 1H), 2.85 (d, $J=11.2$ Hz, 1H), 2.70–2.77 (m, 2H), 2.63–2.67 (m, 1H), 2.44–2.50 (m, 1H), 2.34–2.44 (m, 4H), 2.00–2.04 (m, 1H), 1.60 (s, 3H); LC-MS ($M^+ + 1$) (EI) 781.5.