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Orally bioavailable highly potent HIV protease inhibitors against PI-resistant virus

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Abstract—Efforts directed to identifying potent HIV protease inhibitors (PI) have yielded a class of compounds that are not only very active against wild-type (NL4-3) HIV virus but also very potent against a panel of PI-resistant viral isolates. Chemistry and biology are described.

The fast emerging resistance to the first generation of HIV protease inhibitors, such as indinavir, nelfinavir, saquinavir, and ritonavir, has been a substantial and persistent problem in the treatment of AIDS. The need for more potent and bioavailable protease inhibitors to achieve more complete viral suppression has become urgent. Furthermore, the alarming expression of viral cross-resistance to multiple protease inhibitors underscores the necessity for chemotherapeutic agents that are broadly active against a wide variety of HIV mutations.[1]

Our efforts in this area have been focused on reengineering the structure of indinavir that preserves its excellent biological profile while improving the antiviral activity against various resistant viral isolates. Previous work highlighted the modifications at the S2 pocket by introducing N-CF_3CH_2 amide for a *tert*-butyl amide[2] and at the S3′ pocket with aminochromanol moiety for aminoindanol.[3] In this communication, we report that modifications focused on the S1′ and the S3 pockets have afforded compounds with excellent potency against PI-resistant virus. Figure 1 shows R^1 and R^2 are the focus area under investigation.

The synthesis of these analogs is exemplified with the best compound **9** (Scheme 1). Treatment of **1** with LDA in THF at −78 °C followed by trapping with ethyl

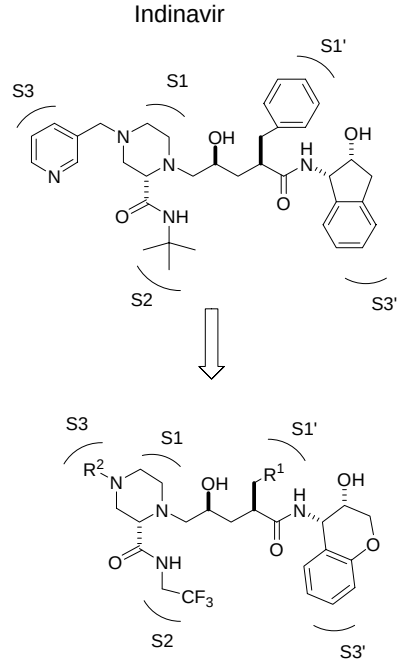

Figure 1. Modifications of indinavir for R^1 and R^2 may lead to improved potency against PI-resistant viral strains.

Keywords: HIV; Protease; Inhibitors.
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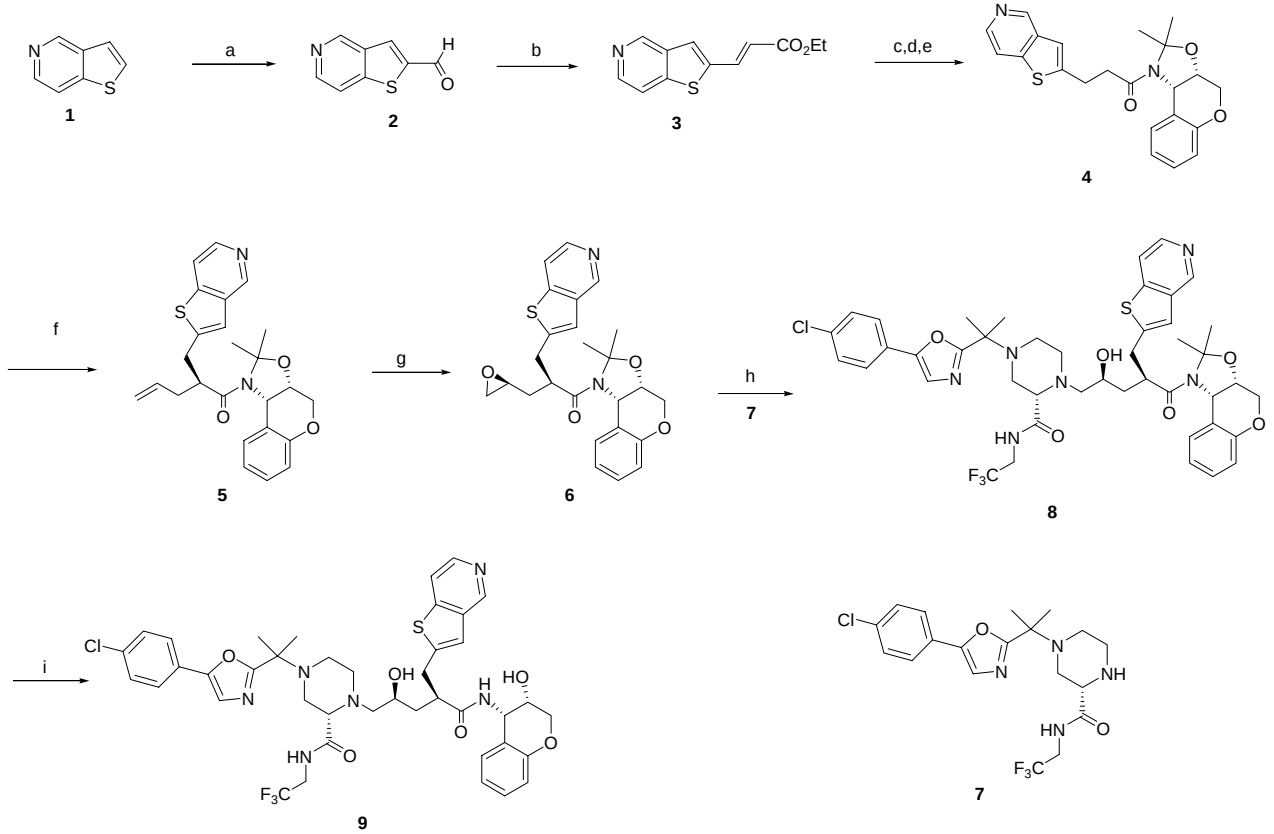
formate gave aldehyde **2** in 82% yield. Wittig–Horner reaction of **2** afforded ester **3** in excellent yield. Hydrogenation of **3** gave a saturated ester which was subsequently hydrolyzed to an acid. The acid was coupled with aminochromanol and the resulting coupling product was protected to give compound **4** in 51% overall yield. Compound **4** was treated with 1 M $LiN(TMS)_2$ and trapped with allyl bromide at −25 °C to afford **5** in 75% yield. The epoxide **6** was obtained in 58% yield by treatment of **5** with NIS in a mixture of EtOAc and aqueous $NaHCO_3$ followed by NaOMe in EtOAc at room temperature. The epoxide ring opening with compound **7** in *i*-PrOH under reflux conditions afforded compound **8** in 33% yield. Final deprotection of **8** with HCl in ether and MeOH gave compound **9** in 47% yield. The synthesis of all analogs described herein, was carried out in a similar fashion as illustrated in Scheme 1. All final products were isolated by preparative TLC and characterized by ^1H NMR and LC–MS (ESI). The preparation of the intermediate **7** was similar to the chemistry described elsewhere.[4]

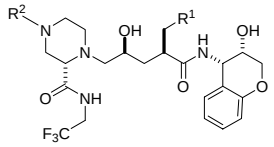
The biological activity of the synthesized compounds is shown in Tables 1 and 2. The compounds were tested for their ability to inhibit the protease enzyme (IC_{50}) and to inhibit the spread of viral infection in MT4 human T-lymphoid cells using NL4-3 virus (CIC_{95}).[5] The results indicate that all compounds **9**–**22** are very potent against wild-type protease enzyme and NL4-3 virus, and generally they are 5–30-fold more potent than indinavir. The similar potencies of these compounds have made it hard to differentiate them from each other by just comparing their wild-type IC_{50} and CIC_{95} (Table 1). Our discussion for the SAR therefore will be focused instead on the results from their viral spread assay[6] in Table 2. Among the compounds tested (**9**–**22**), compounds **9** and **16** are extremely potent and they are the best against all mutant viruses listed (CIC_{95} < 7.8 nM cross the board). These two compounds are the regio-isomers of the pyridylthiophene at R^1. With the same R^1, compounds **9**–**13** present an interesting SAR at R^2 site. All five groups at R^2 were used before in different series.[4,7] We try to examine how these groups respond to the pyridylthiophene moiety at the R^1. *gem*-Dimethyl-2-(4-chlorophenyl)-1,3-oxazol-5-yl moiety (**9**) is the best in all five groups. *gem*-Dimethyl-2-(4-chlorophenyl)-1,3-thiazol-2-yl group (**10**) also displays excellent antiviral activity and is only slightly less potent against mutant V-18C than compound **9**. The other three, furan analog **11**, thiophene analog **12**, and thiazole analog **13**, are active against mutant virus but less potent than **9** and **10**. It is worth noting that a CF_3 group in the thiazole analog **13** causes a loss of potency for both IC_{50} and CIC_{95} (Tables 1 and 2). Compound **15** is another very potent compound against mutant virus.

Introduction of a chlorine atom at the pyridylthiophene ring (**17**) resulted in a potent protease inhibitor with



Scheme 1. Reagents and conditions: (a) LDA, THF, −78 °C, ethylformate, 82%; (b) triethyl phosphonoacetate, NaH, THF, 0 °C, 92%; (c) H_2, Pd/C, MeOH, rt; (d) NaOH, $MeOH/H_2O$; (e) aminochromanol, EDC, HOBT, Et_3N, CH_2Cl_2, rt, 2-methoxy propene, 10-camphorsulfonic acid, CH_2Cl_2, 51% overall; (f) 1 M $LiN(TMS)_2$, allyl bromide, THF, −25 °C, 75%; (g) NIS, $EtOAc/NaHCO_3$, 0 °C to rt; NaOMe, EtOAc, rt, 58% overall; (h) *i*-PrOH, reflux, 33%; (i) HCl in ether, MeOH, 47%.

Table 1. Influence of substituents on in vitro potency of HIV protease inhibitors



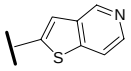
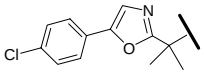
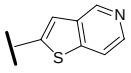
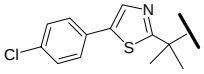
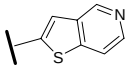
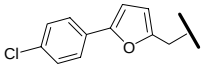
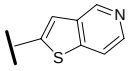
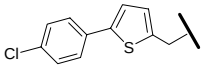
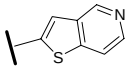
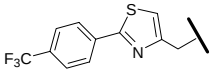
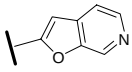
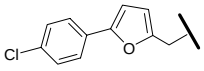
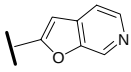
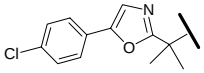
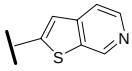
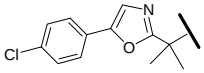
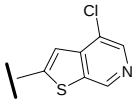
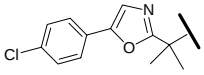
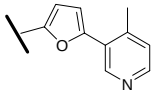
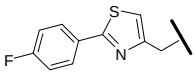
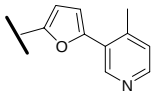
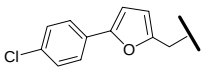
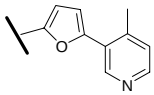
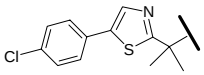
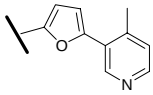
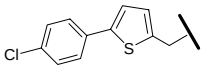
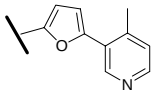
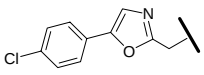
Compound	R^1	R^2	IC_{50} (nM)	CIC_{95} (nM) NL4-3
9			0.02	<7.8
10			0.04	<7.8
11			0.03	15.6
12			0.05	15.6
13			0.07	26.0
14			0.05	<7.8
15			0.02	<7.8
16			<0.02	<7.8
17			<0.02	<7.8
18			0.11	<7.8
19			0.08	15.6
20			0.03	15.6
21			0.04	<7.8
22			0.03	<7.8
Indinavir			0.59	50

Table 2. Potency (CIC_{95}) (nM) against PI-resistant viral isolate constructs in the viral spread assay

Compound	NL4-3	4X Virus	Viral isolates		
			K-60C	V-18C	Q-60C
Indinavir	50	400	>1000	>1000	>1000
9	<7.8	<7.8	<7.8	<7.8	<7.8
10	<7.8	<7.8	<7.8	31.3	<7.8
11	15.6	15.6	<7.8	125	31.3
12	15.6	15.6	31.3	125	31.3
13	26.0	125	31.3	375	93.8
14	<7.8	31.3	<7.8	62.5	15.6
15	7.8	<7.8	<7.8	31	<7.8
16	<7.8	<7.8	<7.8	<7.8	<7.8
17	7.8	<7.8	<7.8	15.6	<7.8
18	7.8	15.6	125	62.5	125
19	15.6	31.3	<7.8	125	62.5
20	15.6	31.3	15.6	250	31.3
21	<7.8	15.6	125	62.5	62.5
22	<7.8	62.5	15.6	125	62.5

only a slight loss of potency against viral isolate V-18C (**17** vs **16**). The analogs of 3-(5-methyl-2-furyl)-4-methylpyridine moiety (**18**–**22**) are generally potent but less active than other compounds (**9**–**17**). We noticed that the V-18C viral isolate was one of the toughest viral isolates and most of our compounds performed less satisfactorily against it. Given their excellent antiviral activity against mutant virus, pyridylthiophene analog **9**, and pyridylfuran analog **15** were chosen to test the oral bioavailability in animal models. At the dose of 2 mpk po, compound **9** showed moderate bioavailability (7.3%) with a reasonable PK profile (Table 3). Additionally, compound **15** had decent oral bioavailability ($F = 42\%$ in dog and 14% in rat, see Tables 3 and 4 for PK data in dog and rat). Unfortunately, both **9** and **15** were potent ($IC_{50} < 1\mu M$) inhibitors of CYP 3A4, 2D6, and 2C9.

In summary, we have shown that replacement of phenyl group at the S1′ of Indinavir with various aryl heterocycles along with modifications at the S3 pocket can significantly impact the inhibitory potency of the compounds against the HIV protease enzyme. All compounds discussed above display much better antiviral activity against mutant isolates than does indinavir. Specifically, nearly a half dozen compounds (**9**, **10**, and **15**–**17**) show substantially better potency (low nanomolar) against the viral spread of both the wild-type virus (NL4-3) and a number of PI-resistant variants of HIV. One of the best compounds (**9**) shows moderate bioavailability in rats and another compound **15** shows a decent bioavailability in both dogs and rats.

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Table 3. Pharmacokinetics in rats for compounds **9** and **15** ($n = 4$)

Compound	Dose (po dose mpk)	C_{max} (μM)	AUC (μM h)	F (%)	$t_{1/2}$ (min)	CL (ml/min/kg)
9	2	0.052	0.36	7.3	48	111
15	10	0.18 (sd = 0.07)	0.29 (sd = 0.12)	14	48	98

Table 4. Pharmacokinetics in dogs for compound **15** ($n = 2$)

Compound	Dose (po dose mpk)	C_{max} (μM)	AUC (μM h)	F (%)	$t_{1/2}$ (min)	CL (ml/min/kg)
15	10	2.1 (sd = 1.51)	0.39 (sd = 0.31)	42	60	23