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Novel HIV-1 Protease Inhibitors Active against Multiple PI-Resistant Viral Strains: Coadministration with Indinavir

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Abstract—HIV-1 protease inhibitors (PI) with an *N*-arylpyrrole moiety in the P_3 position afforded excellent antiviral potency and substantially improved aqueous solubility over previously reported variants. The rapid in vitro clearance of these compounds in human liver microsomes prompted oral coadministration with indinavir to hinder their metabolism by the cyctochrome P450 3A4 isozyme and allow for in vivo PK assessment.

HIV-1 Protease inhibitors (PI), including indinavir, have demonstrated efficacy as a component of multi-drug therapy in the treatment of AIDS.[1] The emergence of strains of HIV that are resistant to current anti-retroviral therapy[2] has prompted intensive research toward more broadly active chemotherapeutic agents.[3] The next generation of compounds must posses greater intrinsic potency against both the wild-type virus and PI-resistant variants. They must also achieve high and sustained drug concentration in vivo for more complete suppression of viral replication.[4] Finally, these compounds must be developed as a component of a multi-drug regimen. It is therefore imperative that potential metabolic interactions between chemotherapeutic agents be well controlled.

Previously we described the synthesis of protease inhibitors based on the indinavir scaffold with an arylfuran substituent in the P_3 position (Fig. 1).[5] These compounds afforded substantially increased potency against both wild type and PI-resistant viral strains. However, the most bioavailable compounds (where X = N) were found to be inhibitors of multiple P450 enzymes. Transposition of the pyridyl nitrogen to the P_1' position (where Y = N) afforded compounds with a more favorable P450 inhibition profile in vitro, but with poor solubility and very low bioavailability.[6] We sought to increase the aqueous solubility of this class of compounds, while maintaining the lipophilicity required for compound cell penetration,[7] by replacing the furanyl heterocycle in the P_3 biaryl substituent with a pyrrole moiety (Fig. 1).

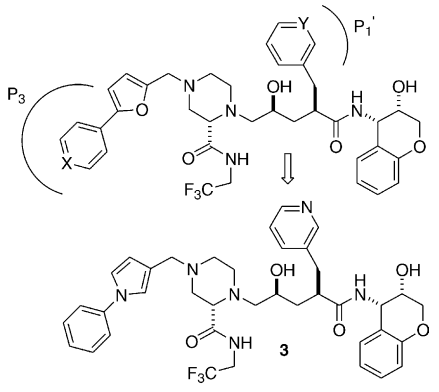

Figure 1. The P_3 biaryl substituents: either arylfuran, where X or Y = N, or *N*-arylpyrrole, as in **3**.

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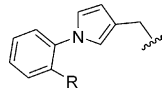
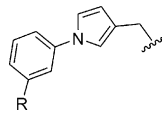
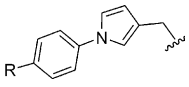
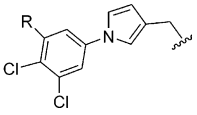
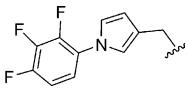
The synthesis of a representative of this class of compounds is illustrated in Scheme 1. A modified Paal–Knorr condensation with aniline afforded the aldehyde substituent **I**.[8] The aldehyde was condensed with the 3-pyridyl penultimate intermediate **II**[6] by reductive amination, affording the fully elaborated product **3**.

The compounds synthesized were tested for the ability to inhibit HIV-1 protease. The enzymes employed were derived from both the wild-type (NL4-3) virus and from a panel of clinical viral isolates from patients infected with highly PI-resistant strains of HIV-1 (K-60, Q-60, and V-18). The HIV-1 strains employed were those that we have observed to exhibit the highest resistance to indinavir, and the genotype and phenotype of these strains have been reported.[5,9] The compounds were also tested for the ability to inhibit the spread of viral infection in MT4 human T-lymphoid cells in culture using viral constructs derived from the strains described above. The concentrations required to inhibit viral spread by 95% (CIC_{95}) are presented.[10]

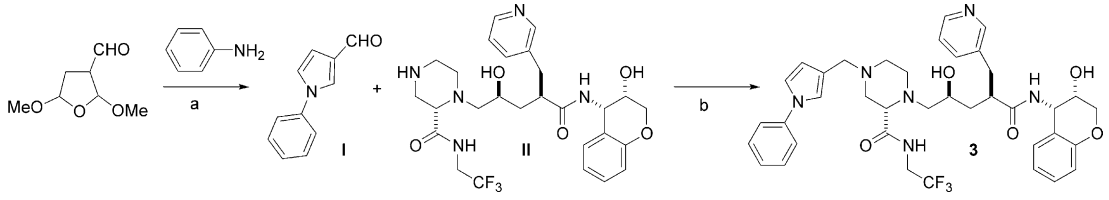
As we have reported previously, the furanyl analogue **2** afforded substantial improvements in inhibitory potency (IC_{50}) over indinavir (**1**) against all viral strains investigated (Table 1). These improvements translated directly into improved antiviral potency (CIC_{95}). The analogous pyrrole-substituted compound **3** was substantially less potent than **2** in the enzymatic assays, particularly against the enzymes derived from resistant strains of HIV-1. However, the compound remained a remarkably potent antiviral in the viral spread assays, affording a CIC_{95} of <40 nM against each strain investigated. The aqueous solubility of **3** (0.74 mg/mL) was similar to indinavir at pH 5.2, and was substantially improved over **2**.

We sought to optimize the phenyl moiety in **3** by parallel solution-phase library syntheses (6–12 compounds per batch) using the route from Scheme 1. The modified Paal–Knorr reactions were carried out in test tubes on a heating block and monitored by HPLC-MS. These mixtures were extracted in parallel with saturated $NaHCO_3$ and dichloromethane. The biphasic mixtures were passed by gravity filtration through disposable polypropylene reaction vessels fitted with 20 micron frit filters, which afforded passage of only the dichloromethane

Table 2. Enzyme inhibitory concentrations (IC_{50}) and viral spread inhibitory concentrations (CIC_{95}) of HIV protease inhibitors

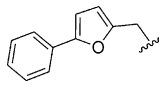
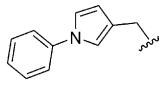
PI	P_3-Substituent	R	IC_{50} (nM)	Viral spread CIC_{95} (nM)[a]			
			NL4-3	NL4-3	K-60C	V-18C	Q-60C
4		$-CF_3$	1.32	38	500		500
5		–CN	0.46	46	250		62
6		–F	0.16	15	62		31
7		$-CH_3$	0.16	≤8	125	31	31
8		–Cl	0.18	≤8	62	31	31
9		$-CF_3$	0.05	15	31		≤8
10		–CN	0.15	62	62		31
11		–F	0.05	15	31		15
12		$-CH_3$	0.10	≤8	31	≤8	≤8
13		–Cl	0.05	≤8	≤8	≤8	≤8
14		$-CF_3$	0.04	15	15		≤8
15		–CN	0.10	62	125		31
16		–F	0.05	15	15		15
17		$-CH_3$	0.06	15	15	15	≤8
18		–Cl	0.03	≤8	15	≤8	≤8
19		–H	0.02	15	31	15	≤8
20		–Cl	0.02	≤8	31	15	≤8
21			0.05	≤8	15	≤8	≤8

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



Scheme 1. (a) AcOH, 90 °C, 30 min (89%); (b) $NaBH(OAc)_3$, DMF, AcOH, 1 h, (66%).

Table 1. Enzyme inhibitory concentrations (IC_{50}) viral spread inhibitory concentrations (CIC_{95}), and aqueous solubility of HIV protease inhibitors

PI	P_3 Substituent	P_1' Substituent	HIV-1 Protease inhibition IC_{50} (nM)				Viral spread CIC_{95} (nM)[a]				Solubility at pH 5.2 (mg/mL)
			NL4-3	K-60C	V-18C	Q-60C	NL4-3	K-60C	V-18C	Q-60C	
1	Indanivir	Benzyl	0.60	61.2	43.6	20.1	50	>1000	>1000	>1000	0.69
2		3-Pyridyl	0.02	0.06	0.15	0.07	≤8	≤8	≤8	≤8	0.01
3		3-Pyridyl	0.11	2.70	2.49	n.d.	15	31	≤8	≤8	0.74

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

layers. These filtered solutions were then applied directly to disposable silica gel columns and purified using a parallel chromatography apparatus.[11] The purified pyrrole carboxaldehydes thus obtained were condensed with **II** by parallel reductive amination, and the crude reaction mixtures were again applied directly to the disposable silica gel columns for similar parallel purification.

The inhibitory potencies (IC_{50}) and antiviral activity (CIC_{95}) of a representative subset of compounds synthesized in this fashion are presented in Table 2. It is evident that *N*-arylpyrroles bearing an *ortho*-substituent (**4**–**8**) are suboptimal in antiviral activity against resistant strains of HIV-1. The meta-substituted aryl moieties (**9**–**13**) and *para*-substituted compounds (**14**–**18**) afforded generally higher levels of antiviral potency. Of these compounds the chloro-substituted *N*-arylpyrroles **13** and **18** exhibited the greatest antiviral potency. The effect of these chloro-substituents was not synergistic, as **19** and **20** exhibited decreased antiviral activity. However, the polyfluorinated *N*-arylpyrrole **21** was equipotent with the mono-chlorinated species.

The most potent antiviral compounds synthesized were investigated further to determine their aqueous solubility and metabolic profile in vitro, and these results are presented in Table 3. The halogenated *N*-arylpyrrole substituted compounds **13**, **18**, and **21** exceeded the gains in aqueous solubility afforded by **3** and were significantly more soluble than indinavir itself. The pyrrole-substituted compounds were also found to be competitive inhibitors of the metabolic P450 isoform CYP3A4, in a similar fashion to indinavir and all other currently available PI.[12] We have previously established that CYP3A4 is the isoform primarily responsible for the metabolism of indinavir.[13] Thus the inhibition of this isoform contributes to the slower clearance of indinavir, both in human liver microsomes and in vivo. The pyrrole-substituted compounds show no apparent correlation between CYP3A4 inhibitory potency and clearance rate in human liver microsomes. One possible explanation may be that these compounds are metabolized by additional or alternate P450 isoforms. However, this hypothesis has not been rigorously investigated.

Table 3. In vitro metabolism assays for HIV protease inhibitors

PI	Solubility pH 5.2 (mg/mL)	P450 Isoform IC_{50} (μM)		CL_{int}[a] (mL/min/kg)
		CYP3A4	CYP2D6	
Indinavir	0.69	0.150	≥30	49.5
2	0.01	0.430	15.6	238
3	0.74	0.400	12.0	332
13	1.20	0.080	n.d.	307
18	0.98	0.050	n.d.	229
21	1.00	0.050	n.d.	218

[a]Determined in human liver microsomes.

Clinical investigations have clearly established the utility of coadministration of a PI substrate of CYP3A4 with a more potent inhibitor of CYP3A4. This strategy, known as duel PI therapy,[14] may afford substantially enhanced oral exposure of the more metabolically labile compound. Given the relatively high rate of in vitro clearance observed with the compounds presented (Table 3), we reasoned that there was a poor likelihood of achieving high and sustained plasma levels of these compounds following oral dosing. We therefore pursued a strategy of coadministration of these compounds in dogs with an equal dose of indinavir. The results are presented in Table 4.[15] The poor pharmacokinetic profile for **2** when dosed alone (5 mpk) has been published[6] and is presented for comparison. Coadministration of a 10 mpk dose of indinavir with **2** (10 mpk) resulted in substantially improved plasma levels of **2**. Indeed, the enhanced A.U.C. of **2** with coadministration (10.0 μM h) is comparable to that achieved with indinavir alone. The more soluble N-arylpyrrole compounds **3** and **21** afforded lower plasma exposure when dosed with indinavir. This further supports the possibility that isoforms other than CYP3A4 are responsible for the metabolism of **3** and **21**. Conversely, coadministration of the potent CYP3A4 inhibitor **21** with indinavir did afford significantly enhanced exposure of indinavir. The A.U.C. of indinavir was more than doubled by this coadministration (26.6 μM·h) as compared to an equal dose of indinavir alone.

The *N*-arylpyrrole substituted HIV-1 PI are substantially more soluble than the phenylfuran derivative **2** while affording comparable antiviral potency. However, the high metabolic clearance of these compounds in human liver microsomes necessitated coadministration with the CYP3A4 inhibitor indinavir for pharmacokinetic studies. In these investigations higher plasma levels were achieved with **2** than any of the more soluble pyrrole substituted variants. This is possibly due to the involvement of other CYP isoforms in the metabolism of these compounds.

Table 4. Pharmacokinetics of orally dosed HIV-1 PI (dogs), both alone or with indinavir

PI	Dose PI/indinavir (mg/kg)	PI		Indavir codose	
		C_{max} (μM)	A.U.C. (μM h)	C_{max} (μM)	A.U.C. (μM h)
Indinavir	0/10	—	—	11.4	12.5
2	5/0	0.1	0.08	—	—
2	10/10	6.1	10.0	6.5	11.2
3	10/10	5.3	7.0	12.1	18.9
21	10/10	1.9	4.4	13.2	26.6

References and Notes

1. Kempf, D. J.; Molla, A.; Hsu, A. In *Antiretroviral Therapy*; De Clercq, E. Ed.; ASM: Washington DC., 2001; p 147.
2. Romano, L.; Venturi, G.; Giomi, S.; Pippi, L.; Valensin, P. E.; Zazzi, M. *J. Med. Virol*. **2002**, *66*, 143.
3. Rusconi, S.; Catamancio, S. *Expert Opin. Investig. Drugs* **2002**, *11*, 387.
4. Dorsey, B. D.; McDoough, C.; McDaniel, S. L.; Levin, R. B.; Newton, C. L.; Hoffman, J. M.; Darke, P. L.; Zugay-Murphy, J. A.; Emini, E. A.; Schleif, W. A.; Olsen, D. B.; Stahlhut, M. W.; Rutkowski, C. A.; Kuo, L. C.; Lin, J. H.; Chen, I.-W.; Michelson, S. R.; Holloway, M. K.; Huff, J. R.; Vacca, J. P. *J. Med. Chem*. **2000**, *43*, 3386.
5. Duffy, J. L.; Rano, T. A.; Kevin, N. J.; Chapman, K. T.; Schleif, W. A.; Olsen, D. B.; Stahlhut, M.; Rutkowski, C. M.; Kuo, L. C.; Jin, L.; Lin, J. H.; Emini, E. A.; Tata, J. R. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2569.
6. Duffy, J. L.; Kirk, B. A.; Kevin, N. J.; Chapman, K. T.; Schleif, W. A.; Olsen, D. B.; Stahlhut, M.; Rutkowski, C. A.; Kuo, L. C.; Jin, L.; Lin, J. H.; Emini, E. A.; Tata, J. R. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 3323.
7. Dorsey, B. D.; McDaniel, S. L.; Levin, R. B.; Vacca, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Lin, J. H.; Chen, I.-W.; Holloway, M. K.; Anderson, P. S.; Huff, J. R. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 2769.
8. Sundberg, R. J. In *Comprehensive Heterocyclic Chemistry II*; Bird, C. W. Ed.; Elsevier Science, Tarrytown, NY, 1996, Vol. 2, p 149.
9. (a) Olsen, D. B.; Stahlhut, M. W.; Rutkowski, C. A.; Schock, H. B.; vanOlden, A. L.; Kuo, L. C. *J. Biol. Chem*. **1999**, *274*, 23699. (b) Condra, J. H.; Holder, D. J.; Schleif, W. A.; Blahy, O. M.; Danovich, R. M.; Gabyelski, L. J.; Graham, D. J.; Laird, D.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M.; Yang, T.; Chodakewitz, J. A.; Deutsch, P. J.; Leavitt, R. Y.; Massari, F. E.; Mellors, J. W.; Squires, K. E.; Steigbigel, R. T.; Teppler, H.; Emini, E. A. *J. Virol*. **1996**, *70*, 8270.
10. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Nat. Acad. Sci*. **1994**, *91*, 4096.
11. An early prototype of the QUAD3 apparatus from Biotage, Inc. was utilized for parallel chromatography.
12. Williams, G. C.; Sinko, P. J. *Adv. Drug Delivery Reviews* **1999**, *39*, 211.
13. Chiba, M.; Hensleigh, M.; Nishime, J. A.; Balani, S. K.; Lin, J. H. *Drug Metab. Disp*. **1996**, *24*, 307.
14. Kempf, D. J.; Akhterzzaman, M.; Hsu, A. In *Antiretroviral Therapy*; De Clercq, E., Ed.; ASM Press: Washington, DC, 2001, Chapter 8.
15. The use of animals was done under the purview of an Institutional Animal Care and Use Committee, and all applicable regulations and laws pertaining to the use of laboratory animals were followed. For experimental methods for both in vitro and in vivo pharmacokinetic investigations, see: Lin, J. H.; Chiba, M.; Balani, S. K.; Chen, I.-W.; Kwei, G. Y.-S.; Vastag, K. J.; Nishime, J. A. *Drug Metab. Disp*. **1996**, *24*, 1111.