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HIV Protease Inhibitors with Picomolar Potency Against PI-Resistant HIV-1 by Extension of the P_3 Substituent

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Abstract—A biaryl pyridylfuran P_3 substituent on the hydroxyethylene isostere scaffold affords HIV protease inhibitors (PI's) with picomolar (IC_{50}) potency against the protease enzymes from PI-resistant HIV-1 strains. Inclusion of a gem-dimethyl substituent afforded compound **3** with 100% oral bioavailability (dogs) and more than double the $t_{1/2}$ of indinavir. Inhibition of multiple P450 isoforms is dependent on the regiochemistry of the pyridyl nitrogen in these compounds.

The use of HIV protease inhibitors (PI's) in antiretroviral therapy has demonstrated remarkable success over the past six years by decreasing the mortality rate associated with HIV-1 infection.[1] However, as the duration of this treatment is extended an alarming increase has been observed in the occurrence of viral resistance to currently approved therapies.[2] To address this problem the next generation of PI's must meet several criteria. They must achieve more effective suppression of the wild-type virus through increased potency and higher plasma levels.[3] They must be broadly active against PI-resistant variants of HIV-1. Finally, they must have outstanding patient tolerability as a component of a multi-drug regimen. Many protease inhibitors are currently in development to address these issues.[4]

In order to meet these criteria we have been engaged in a program to further optimize the hydroxyethylene isostere class of PI's such as indinavir, which has resulted in compound **2** (Fig. 1).[5] We now report that extension of the P_3 pyridylfuran moiety in **2** to a biaryl system affords compounds with similar in vitro potency against the wild-type virus, but greatly improved potency against a panel of clinically isolated PI-resistant strains of HIV-1.

For those compounds from Figure 1 where R = H, the synthesis may be accomplished by direct analogy to the syntheses of indinavir (**4**, **5**, and **6** from Table 1).[6] For compound **3**, which contains the gem-dimethyl moiety, an alternate strategy has been employed as illustrated in Scheme 1. The biaryl substituent **I** was assembled by Stille coupling of 3-bromopyridine with the 2-stanylfuran. Acylation of this intermediate with *N*-methoxy-*N*-methylacetamide afforded the ketone **II**. The piperazine intermediate **III** was then condensed with this ketone in neat TMSCN,[7] resulting in the aminonitrile intermediate **IV**. Deprotection of the proximal nitrogen on this intermediate, followed by addition of methylmagnesium bromide, yielded the gem-dimethyl piperazine fragment **V**. Addition of this fragment to the lactone **VI**[8] afforded the cyclic intermediate **VII**. The lactone was opened under basic conditions, then treated with excess silylating agent, followed by selective hydrolysis of the silyl ester under neutral conditions, which gave the acid intermediate **VIII**. This intermediate was then coupled with the aminochrominol.[9] Final removal of the silyl protecting group afforded the fully elaborated molecule **3**.

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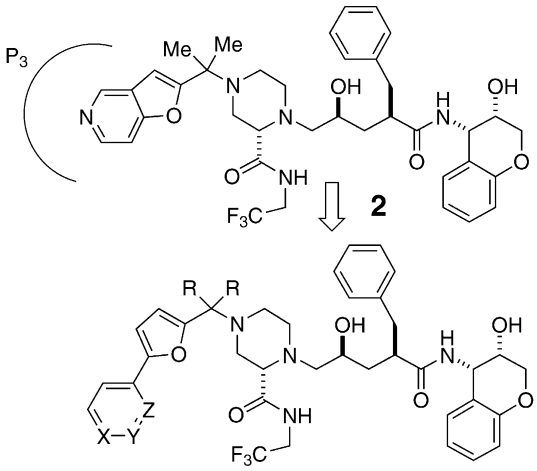


Figure 1. Extension of the P_3 pyridylfuran substituent to a biaryl moiety where R = H or Me, and X, Y, or Z = N.

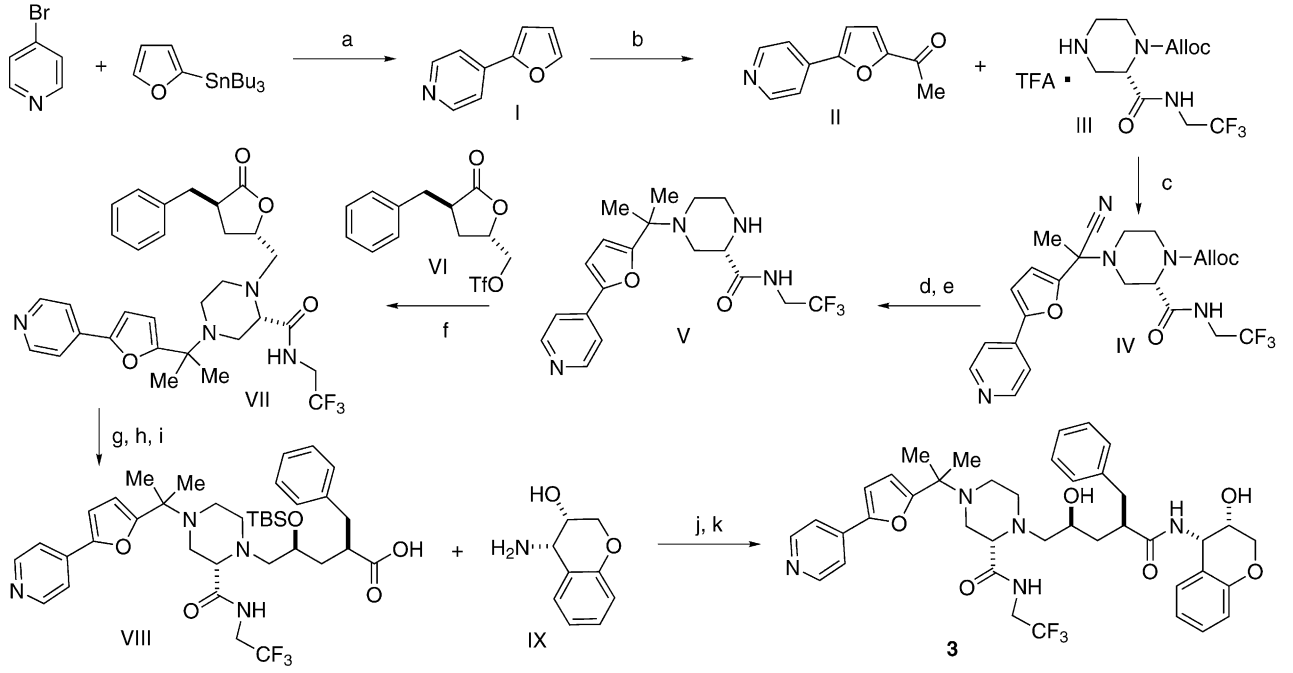
The compounds synthesized were tested for the ability to inhibit HIV-1 protease. The enzymes employed were derived from both the wild-type (NL4-3) virus, and a series of clinical viral isolates from patients infected with multiply PI-resistant HIV-1 (K-60, Q-60, and V-18). The genotype and phenotype of these isolates has been reported,[10] and the protease sequences for these strains are presented in Table 1. The activity (IC_{50}) of the compounds against this panel of HIV-1 proteases is presented in Table 2. The compounds were also tested for their ability to inhibit 95% of the spread of viral infection in MT4 human T-lymphoid cells (CIC_{95}) using viral constructs derived from the strains described above, and these values are also listed in Table 2.[11] It must be noted that the most highly cross-resistant viral phenotypes we have identified[10a] were chosen for optimization of the lead class without regard to the amino acid substitution pattern of their protease enzyme.

As is evident from Table 2, the resistant viral strains K-60C, V-18C, and Q-60C have lost almost all sensitivity to indinavir in the viral spread assay. Compound **2**,[5a] with substitutions for the aminoindanol, carboxamide, and pyridylmethyl moieties shows improved inhibitory potency against the enzymes from wild-type and resistant strains of HIV-1, and this translates directly into improved potency in the viral spread assays as well. Extension of the P_3 pyridylfuran substituent to the biaryl system in **3** afforded additional gains in potency, with **3** being at or near the potency limit of detection of the spread assay for each of the resistant strains tested. We further found that the exclusion of the gem-dimethyl substituent afforded similar or slightly improved potency in the enzyme inhibition assays, achieving

Table 1. Sequence mutations from wild-type HIV-1 protease for resistant viral strains

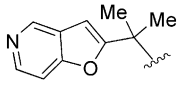
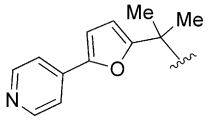
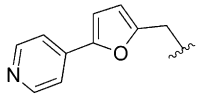
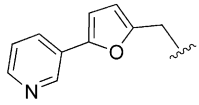
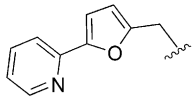
NL4-3[a]	Leu-10	Met-36	Asn-37	Arg-41	Met-46	Ile-54	Arg-57	Gln-58	Ile-62	Leu-63	Ile-64	Ala-71	Gly-73	Val-77	Val-82	Ile-84	Leu-90	Ile-93
K-60	Ile				Ile	Val		Glu		Pro	Val			Ile	Phe	Val	Met	Leu
Q-60	Ile		Ser	Lys	Ile	Val			Val	Pro		Val		Ile	Ala		Met	Leu
V18	Ile	Ile	Asp		Ile		Lys			Pro		Val	Ser			Val	Met	Leu

[a]For the complete sequence of the NL4-3 virus, see ref 10c.



Scheme 1. (a) $Pd(PPh_3)_4$, DIEA, DMF, 100 °C; (b) BuLi, *N*-Methoxy-*N*-methylacetamide, THF, −78 °C; (c) TMSCN (neat), 60 °C; (d) Thiosalicilic acid, $Pd_2(dba)_3$, DPPB, THF; (e) MeMgCl, DME, −20 °C; (f) DIEA, *i*PrOH; (g) 1.0 N aq LiOH, DME, 0 °C; (h) TBSOTf, DIEA, CH_2Cl_2, 0 °C; (i) 30% H_2O in THF; (j) HBTU, HOBT, DIEA, NMP; (k) TBAF, THF, 45 °C.

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

Compd	P_3 substituent	HIV-1 Protease inhibition IC_{50} (nM)				Viral Spread CIC_{95} (nM)[a]			
		NL4-3	K-60C	V-18C	Q-60C	NL4-3	K-60C	V-18C	Q-60C
1	Indinavir	0.60	61.2	43.6	20.1	50	>1000	>1000	>1000
2		0.07	5.8	5.2	1.6	≤8	250	62	62
3		0.05	0.3	1.2	0.1	≤8	≤8	12	≤8
4		0.08	0.6	1.3	0.8	≤8	15	31	31
5		0.03	0.3	0.7	n.d.	≤8	15	15	≤8
6		0.05	0.5	0.7	0.4	≤8	31	15	15

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

picomolar inhibition against all enzymes for compound **6**. The activity of **3**–**6** were comparable in the viral spread assays, and the regiochemistry of the pyridyl nitrogen appeared to have little effect on antiviral potency.

The pharmacokinetic properties of **3**–**6** were examined by dosing the compounds both orally and IV (Table 3).[12] Compound **3** exhibited extraordinary bioavailability when dosed at 5 mpk PO in dogs (~100%). A follow-up experiment was performed in rhesus macaques that confirmed the favorable PK profile of this compound. Removal of the gem-dimethyl moiety as in **4** caused a substantial decrease in the A.U.C. of the compound. The 3-pyridyl isomer **5** exhibited a further dramatic decrease in A.U.C. The corresponding 2-pyridyl isomer **6** was similarly poor, even at twice the oral dose (10 mpk). Thus the bioavailability of this series of compounds is critically dependent on the location of the pyridyl nitrogen.

The source of SAR surrounding the bioavailability of these compounds is apparent from the in vitro metabolism assays in Table 4. The bioavailability of the compounds when dosed orally in dogs (Table 3) is inversely proportional to the stability of the compounds when incubated in human liver microsomes, as measured by their intrinsic clearance (CL_{int}, Table 4). Thus, com-

Table 3. Pharmacokinetics of HIV protease inhibitors dosed in vivo

Compd	Species	Dose PO/IV (mpk)	Cmax (μM)	$t_{1/2}$ (min)	A.U.C. (μM*h)	CL_p (mL/min/kg)	%F
Indinavir	Dog	10/2	11.4	28	12.5	16.0	72
3	Dog	5/2	7.3	70	11.3	22.5	100
4	Dog	5/2	5.3	60	2.6	24.5	55
5	Dog	5/2	0.2	41	0.1	25.2	2
6	Dog	10/2	0.9	50	0.4	33.5	4
3	Rhesus	10/2	4.4	91	9.9	20.4	90

Table 4. In vitro metabolism assays for 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
3	19	0.01	0.36	0.41
4	133	0.32	0.53	5.50
5	233	0.44	6.40	>30.00
6	655	5.20	29.60	>30.00

[a]Determined in human liver microsomes.

pound **3** was remarkably stable in this assay, while **5** and **6** were rapidly metabolized.

It is equally apparent from Table 4 that the microsome stability of these compounds mirrors their potency as inhibitors of the P450 isoform CYP3A4. In previous investigations we have established that CYP3A4 is the isoform primarily responsible for the metabolism of indinavir, and that indinavir is an inhibitor of this isoform.[13] Compound **3** is 10-fold more potent than indinavir as an inhibitor of CYP3A4, but is also a potent inhibitor of the related isoforms 2D6 and 2C9. It should be noted, however, that **3** is not a comprehensive P450 inhibitor, as it is essentially inactive against the isoforms 2C19, 1A2, and 2A6 ($IC_{50} > 50\ \mu M$). The des-methyl derivative **4** remains a sub-micromolar inhibitor of 3A4 and 2D6, but is a micromolar inhibitor of 2C9. The corresponding 3-pyridyl isomer **5** loses all activity as an inhibitor of 2C9, and was only a sub-micromolar inhibitor of 3A4. Finally, the 2-pyridyl isomer **6** had lost substantial activity as an inhibitor of all of the isoforms. Thus it may be possible to achieve greater selectivity among the P450 isoforms by modification of the pyridy moiety.

Compound **3** shows high potency against PI resistant HIV-1 strains, and outstanding bioavailability in two species.[14] Therefore it would likely meet the first two criteria for a second generation protease inhibitor. However it would not meet our third criteria, which is tolerability as a component of a multi-drug antiretroviral regimen. The profile of P450 isoform inhibition observed with **3** increases the likelihood of drug-drug interactions with other medications metabolized by these P450 isoforms, and this precluded further development of this molecule. It is also apparent that the P450 isoform inhibition profile is exquisitely sensitive to the regiochemistry of the pyridyl substituent, indicating that further modification of the P_3 region of the molecule may afford a more optimal PK profile.

References and Notes

1. Kempf, D. J.; Molla, A.; Hsu, A. In *Antiretroviral Therapy*; De Clercq, E. Ed.; ASM: Washington D. C., 2001; pp 147–174.
2. Romano, L.; Venturi, G.; Giomi, S.; Pippi, L.; Valensin, P. E.; Zazzi, M. *J. Med. Virol.* **2002**, *66*, 143.
3. 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.
4. Rusconi, S.; Catamancio, S. *Exp. Opin. Investig. Drugs* **2002**, *11*, 387.
5. (a) Duffy, J. L.; Kevin, N. J.; Kirk, B. A.; 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.* **2002**, *12*, 2423. (b) Cheng, Y. C.; Zhang, F.; Rano, T. A.; Lu, Z.; Schleif, W. A.; Gabrylski, L.; Stahlhut, M.; Lin, J. H.; Jin, L.; Chapman, K. T.; Tata, J. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2419.
6. Askin, D. *Curr. Opin. Drug Disc. Dev.* **1998**, *1*, 338.
7. Bacque, E.; Paris, J. M.; Lebitoux, S. *Synth. Commun.* **1995**, *25*, 803. CAUTION: Trimethylsilyl cyanide is highly toxic and should only be handled by those familiar with its safe use and disposal.
8. Herdeis, C.; Leutch, K.; Waibel, D. *Arch. Pharm.* **1994**, *327*, 41.
9. Hansen, K. B.; Rabbat, P.; Springfield, S. A.; Devine, P. N.; Grabowski, E. J. J.; Reider, P. J. *Tetrahedron Lett.* **2001**, *42*, 8743.
10. (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. (c) Myers, G.; Wain-Hobson, S.; Henderson, L. E.; Korber, B.; Jeang, K.-T.; Pavlakis, G. N. *Human Retroviruses and AIDS 1993*; Los Alamos National Laboratory: Los Alamos, N. Mex, 1993.
11. 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. U.S.A.* **1994**, *91*, 4096.
12. 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.
13. Chiba, M.; Hensleigh, M.; Nishime, J. A.; Balani, S. K.; Lin, J. H. *Drug Metab. Disp.* **1996**, *24*, 307.
14. Data for compound **3**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.29 (t, $J = 6.4$ Hz, 1H), 8.62 (dd, $J = 1.6$ Hz, $J = 4.8$ Hz, 1H), 7.47 (dd, $J = 1.6$ Hz, $J = 4.4$ Hz, 1H), 7.29 (m, 5H), 7.10 (t, $J = 8.8$ Hz, 1H), 7.06 (d, $J = 7.6$ Hz, 1H), 6.34 (d, $J = 3.2$ Hz, 1H), 5.95 (d, $J = 8.0$ Hz, 1H), 5.16 (dd, $J = 4.4$ Hz, 1H), 4.13 (m, 1H), 4.07 (d, $J = 12.4$ Hz, 1H), 4.00 (dd, $J = 5.2$ Hz, $J = 12.0$ Hz, 1H), 3.81 (m, 1H), 3.75 (t, $J = 10.0$ Hz, 1H), 3.65 (m, 1H), 3.35 (s, 1H), 3.10 (d, $J = 11.6$ Hz, 1H), 2.86 (m, 4H), 2.64 (m, 2H), 2.39 (m, 2H), 1.89 (t, $J = 10.8$ Hz, 1H), 1.65 (t, 1H), 1.56 (s, 3H), 1.53 (s, 3H); HPLC-MS (ES) 750.4 (M + 1).