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HIV-1 Protease Inhibitors with Picomolar Potency against PI-Resistant HIV-1 by Modification of the P_1' Substituent

Joseph L. Duffy,[a,*] Brian A. Kirk,[a] Nancy J. Kevin,[a] Kevin T. Chapman,[a] William A. Schleif,[b] David B. Olsen,[c] Mark Stahlhut,[c] Carrie A. Rutkowski,[c] Lawrence C. Kuo,[d] Lixia Jin,[e] Jiunn H. Lin,[e] Emilio A. Emini[b] and James R. Tata[a]

[a]*Department of Basic Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Virus and Cell Biology, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[d]*Department of Structural Biology, Merck Research Laboratories, West Point, PA 19486, USA*
[e]*Department of Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—Transposition of the pyridyl nitrogen from the P_3 substituent to the P_1' substituent in HIV-1 protease inhibitors (PI) affords compounds such as **3** with an improved inhibitory profile against multiple P450 isoforms. These compounds also displayed increased potency, with **3** inhibiting viral spread (CIC_{95}) at <8 nM for every strain of PI-resistant HIV-1 tested. The poor to modest bioavailability of these compounds may correlate in part to their aqueous solubility.

Since their emergence in 1995, HIV-1 protease inhibitors (PI), including indinavir, have demonstrated efficacy in the treatment of HIV-1 infection as a component of antiretroviral therapy.[1] The subsequent emergence of drug resistant strains of HIV-1 has necessitated further development of this treatment class with an emphasis on the development of agents with broad activity against PI-resistant variants of the virus.[2]

We have reported that modifications of the indinavir scaffold afforded compounds such as those illustrated in Figure 1 where X is nitrogen, and both Y and Z are C–H.[3] These compounds afforded exceptional antiviral potency against several resistant strains of HIV-1. Although high levels of plasma exposure were achieved in both dogs and rhesus monkeys by oral dosing of one of these compounds, an unacceptable profile of inhibition of P450 isoforms was observed. In order to improve this metabolic profile we have investigated the transposition of the pyridyl nitrogen from the P_3 substituent to the P_1' position. This afforded compounds in Figure 1 where either Y or Z are nitrogen, and X is C–H.[4]

The synthesis of the P_1 pyridyl derivatives was accomplished by direct analogy to the synthesis of indinavir developed by Askin and coworkers.[5] A representative example is the synthesis of **3**, which is presented in Scheme 1. The aminoindanol chiral auxiliary was coupled with 3-pyridylpropionic acid affording intermediate **I**. Acetonide formation and diastereoselective allylation provided the α-substituted intermediate **II**. This material was treated with iodine in the presence of acid, which favored the formation of the iodolactone **III**. The iodide was displaced with the piperazine **IV**,[6] which afforded the lactone **V**. 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 **VI**.

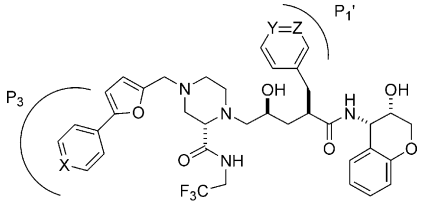

Figure 1. Transposition of the pyridyl nitrogen from P_3, where X = N, to the P_1' position, where Y or Z = N.

*Corresponding author. Fax: +1-732-594-5350; e-mail: joseph_duffy@merck.com

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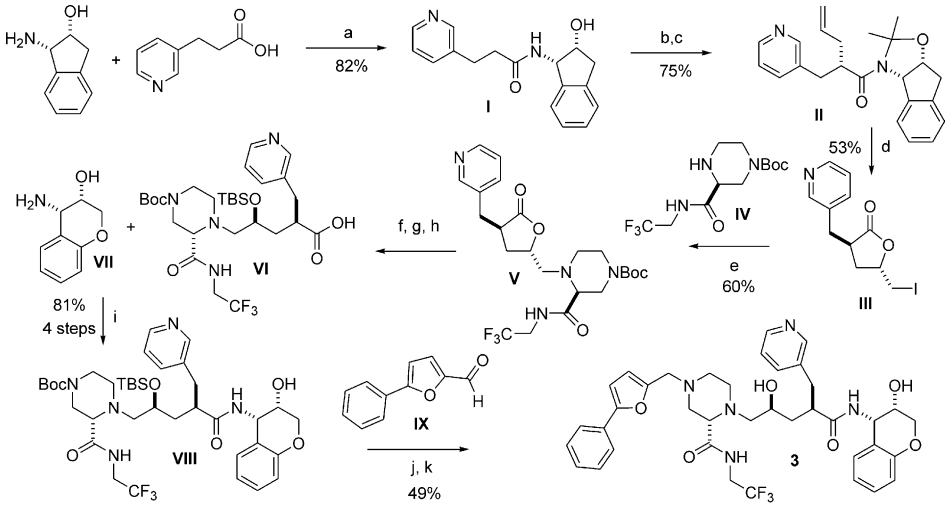
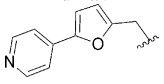
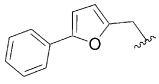
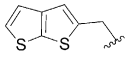
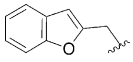
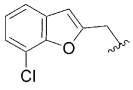


Scheme 1. (a) EDC, HOBT, CH_2Cl_2/DMF, 4 h; (b) 2-methoxypropene, CSA, CH_2Cl_2, 1 h; (c) allyl bromide, LHMDS, THF, −25 °C, 1 h; (d) I_2, MsOH, THF/H_2O, 12 h; (e) DIEA, DMF, 90 °C, 21 h; (f) 1.0 N LiOH (aq), DME, 0 °C, 0.3 h; (g) TBSCl, Imidazole, DMF, 0.3 h; (h) 30% H_2O in THF, 8 h; (i) HBTU, HOBT, DIEA, DMF, 16 h; (j) 10 N HCl (aq), *i*PrOH, 0 °C, 1 h; (k) **IX**, $NaBH(OAc)_3$, DMF, HOAc, 0.5 h.

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

Compd	P_3 substituent	P_1' substituent	HIV-1 protease inhibition IC_{50} (nM)				Viral spread CIC95 (nM)[a]			
			NL4-3	K-60C	V-18C	Q-60C	NL4-3	K-60C	V-18C	Q-60C
1	Indinavir	Benzyl	0.60	61.2	43.6	20.1	50	>1000	>1000	>1000
2		Benzyl	0.08	0.6	1.3	0.8	≤8	15	31	31
3		3-Pyridyl	0.02	0.06	0.15	0.07	≤8	≤8	≤8	≤8
4		4-Pyridyl	0.04	0.11	0.34	0.20	15	≤8	31	31
5		3-Pyridyl	0.03	5.0	0.43	3.2	≤8	250	15	125
6		4-Pyridyl	0.07	20.0	1.2	4.8	≤8	250	15	250
7		3-Pyridyl	0.04	3.7	1.3	2.7	15	125	31	125
8		4-Pyridyl	0.10	10.0	2.2	n.d.	15	250	62	250
9		3-Pyridyl	0.02	n.d.	0.12	n.d.	10	31	≤8	31
10		4-Pyridyl	0.03	0.92	0.14	n.d.	≤8	62	≤8	125

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

This intermediate was then coupled with the aminochrominol.[7] Final removal of the carbamate and silyl protecting groups, followed by reductive amination with the appropriate aldehyde, afforded the fully elaborated 3-pyridylmethyl substituted compound **3**. The analogous 4-pyridylmethyl compounds were synthesized in a similar fashion.

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 series of clinical viral isolates from patients infected with highly PI-resistant strains of HIV-1 (K-60, Q-60, and V-18). The genotype and phenotype of these isolates has been reported, and the protease sequences for each strain have also been presented.[3,8] The most highly indinavir-resistant viral phenotypes we have identified were used in these investigations without regard to the amino acid substitution pattern of their protease enzymes. The potencies (IC_{50}) of the compounds against this panel of HIV-1 protease enzymes are presented in Table 1. The compounds were also tested for their 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 also listed in Table 1.[9]

Compound **2**, reported previously,[3] affords substantial improvements over indinavir (**1**) in potency against both the wild type and resistant strains of HIV-1. Transposition of the nitrogen from the P_3 position to the P_1' position as in the 3-pyridylmethyl isomer **3** resulted in an additional tenfold increase in potency in the protease inhibition assays, with picomolar activity observed against the protease enzyme from every strain investigated. The

Table 2. Solubility and pharmacokinetic properties of HIV protease inhibitors dosed in dogs both PO (5 mpk) and IV (2 mpk)

P_1' substituent	Compd	Solubility pH 5.2 (mg/mL)	C_{max} (μM)	$t_{1/2}$ (min)	A.U.C. (μM h)	CLp (mL/min/kg)	% F
Benzyl	**2**	0.67	5.3	60	2.58	24.5	55
3-Pyridyl	**3**	0.01	0.11	74	0.08	20.5	1
	5	0.18	1.82	55	1.29	16.7	19
	7	0.31	3.36	52	1.94	15.0	24
	9	0.01					
4-Pyridyl	**4**	0.04	0.21	64	0.16	17.4	2
	6	0.15	0.30	127	0.18	23.2	4
	8	0.28	0.38	102	0.22	22.5	4
	10	<0.01					

Table 3. Cytochrome P450 isoform inhibition IC_{50} (μM)[a]

S_1' substituent	Compd	P450 Isoform		
		CYP3A4	CYP2D6	CYP2C9
Benzyl	Indinavir	0.15	>30.00	>30.00
	2	0.32	0.53	5.50
3-Pyridyl	**3**	0.43	15.6	83.9
	5	0.31	11.1	19.7
	7	0.48	19.0	48.8
	9	0.50	8.80	27.3
4-Pyridyl	**4**	1.50	14.9	
	6	0.47	3.20	8.59
	8	2.20	3.60	

[a]Measured in human liver microsomes. See ref 10a.

increased potency in this compound is evident in the viral spread assay as well, where the observed CIC_{95} values for each of the PI-resistant viral strains were below the minimum concentration tested in our assays. The 4-pyridylmethyl regioisomer **4** maintained substantial potency against each viral strain, although it was generally less potent than the corresponding 3-pyridymethyl compound.

Despite these gains in antiviral potency, **3** and **4** were poorly bioavailable. The compounds were dosed in dogs both orally (5 mpk) and IV (2 mpk) and the pharmacokinetic parameters are presented in Table 2.[10] The clearance and half-life of these compounds compares favorably with the benzyl substituted compound **2**, but the maximum plasma levels obtained with **3** and **4** were over 10-fold lower. A correlation has been observed between the aqueous solubility and oral absorption of protease inhibitors in the indinavir lead class,[4b,11] and both **3** and **4** were found to be substantially less soluble than the more bioavailable compound **2** at pH 5.2 (Table 2).

In order to improve the solubility of this class of compounds our efforts turned to replacement of the lipophilic P_3 moiety. This substituent was replaced with the previously identified[3,2-*b*]thienothiophene (**5** and **6**),[4] or with the corresponding fused-ring benzofuran (**7** and **8**, Table 1). These substitutions afforded similarly potent compounds in the viral spread assay against the NL4-3 virus, however a loss in potency was observed with the PI-resistant strains. Much of this potency was restored in the 8-chlorobenzofuran substituted compounds **9** and **10**.[12]

The thienothiophene and benzofuran substituents afforded substantially higher aqueous solubility, as illustrated in Table 2. However, this greater solubility only translated into higher C_{max} values in the 3-pyridylmethyl series when dosed orally. Thus, while all of the compounds dosed had similar metabolic clearance, only **5** and **7** achieved appreciable bioavailability. None of the 4-pyridylmethyl isomers resulted in significant plasma exposure, despite a similar clearance rate and similar solubility to the 3-pyridylmethyl series. The reason for the lack of correlation between aqueous solubility and C_{max} values in the 4-pyridylmethyl series is not clear at present. The poor aqueous solubility of the chlorobenzofuran substituted compounds **9** and **10** precluded their inclusion in the oral dosing experiments, despite their relatively higher potency.[13]

We have previously reported that **2** and related compounds would be suboptimal clinical development candidates due to their unacceptable metabolic profile.[3] While all of the currently approved HIV-1 protease inhibitors are competitive inhibitors of the cytochrome P450 isoform CYP3A4,[14] **2** is also an inhibitor of the related isoform 2D6, and a modest inhibitor of the 2C9 isoform, as shown in Table 3. This simultaneous inhibition of multiple P450 isoforms would increase the likelihood of harmful drug–drug interactions for patients on a multidrug antiretroviral regimen. All of the 3-pyridylmethyl substituted compounds resulted in a more favorable P450 isoform inhibitory profile, with submicromolar IC_{50} values observed only with the 3A4 isoform. In this respect the 3-pyridylmethyl series exhibited an in vitro metabolic profile similar to indinavir and other clinically approved HIV-1 protease inhibitors. The analogous 4-pyridylmethyl compounds afforded a less consistent profile, in which inhibition of the 3A4 isoform was compound specific. The corresponding 2-pyridylmethyl compounds were not pursued, owing to our experience that this regioisomer afforded no 3A4 inhibition and poor bioavailability for the related P_3 pyridyl series.[3]

Transposition of the pyridyl nitrogen from the P_3 to the P_1' substituent in HIV-1 protease inhibitors of the indinavir class afforded several advantages. The resulting

compounds, exemplified by **3**, exhibited substantially greater potency against PI-resistant strains of HIV-1. Furthermore, the 3-pyridylmethyl series afforded a more favorable inhibitory profile against several metabolic P450 isoforms than the analogous P_3 pyridyl compounds. At best only modest bioavailability was achieved in the P_1' 3-pyridylmethyl series, owing to the lower maximal plasma levels achieved with these compounds after oral dosing.

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. Duffy, J. L.; Rano, T. 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. and Tata, J. R. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2569.
4. (a) This strategy was successfully employed during the development of indinavir. See: 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. (b) Dorsey, B. D.; McDonough, 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. Maligres, P. E.; Weissman, S. A.; Upadhyay, V.; Cianciosi, S. J.; Reamer, R. A.; Purick, R. M.; Sager, J.; Rossen, K.; Eng, K. K.; Askin, D.; Volante, R. P.; Reider, P. J. *Tetrahedron* **1996**, *52*, 3327.
6. Askin, D.; Eng, K. K.; Rossen, K.; Purick, R. M.; Wells, K. M.; Volante, R. P.; Reider, P. J. *Tetrahedron Lett*. **1994**, *35*, 673.
7. Hansen, K. B.; Rabbat, P.; Springfield, S. A.; Devine, P. N.; Grabowski, E. J. J.; Reider, P. J. *Tetrahedron Lett*. **2001**, *42*, 8743.
8. (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.
9. 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.
10. (a) 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: Chiba, M.; Hensleigh, M.; Nishime, J. A.; Balani, S. K.; Lin, J. H. *Drug Metab. Disp*. **1996**, *24*, 307. (b) 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.
11. Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Lin, J. H.; Chen, I.-W.; Holloway, M. K.; Fitzgerald, P. M. D.; Axel, M. G.; Ostovic, D.; Anderson, P. S.; Huff, J. R. *J. Med. Chem*. **1994**, *37*, 3443.
12. Synthesis of the benzofuran derivatives was accomplished by modification of the corresponding 8-iodobenzofuran intermediate. See: Cheng, Y.; Zhang, F.; Rano, T. A.; Lu, Z.; Schleif, W. A.; Gabryelshi, L.; Olsen, D. B.; Stahlhut, M.; Rutkowski, C. A.; Lin, J. H.; Jin, L.; Emini, E. A.; Chapman, K. T.; Tata, J. R. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2419.
13. Prior investigations in this lead class have established that inclusion of more hydrophilic substituents in both the P_3 and P_1' sites simultaneously results in decreased efficacy in the viral spread assay. See ref 4a.
14. Williams, G. C.; Sinko, P. J. *Adv. Drug Deliv. Rev*. **1999**, *39*, 211.