Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2423–2426

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Activity of Novel HIV Protease Inhibitors with Improved Potency Against Multiple PI-Resistant Viral Strains

Joseph L. Duffy,[a,*] Nancy J. Kevin,[a] Brian A. Kirk,[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*

Received 26 March 2002; accepted 17 May 2002

Abstract—Substitution of the *t*-butylcarboxamide substituent in analogues of the HIV protease inhibitor (PI) Indinavir with a trifluoroethylamide moiety confers greater potency against both the wild-type (NL4-3) virus and PI-resistant HIV. The trifluoroethyl substituent also affords a slower clearance rate in vivo (dogs); however, this may be due to more potent inhibition of at least two P450 isoforms.

The advent of chemotherapeutic agents to suppress human immunodeficiency virus (HIV-1) infection has proven to be beneficial in the treatment of acquired immune deficiency syndrome (AIDS). Several HIV protease inhibitors (PIs), including Indinavir (Fig. 1), have been approved for use in combination with other therapies for the treatment of HIV infection.[1] However, the clinical emergence of drug-resistant variants of the HIV virus underscores the need for more potent and bioavailable protease inhibitors to achieve sustained viral suppression in vivo. Furthermore, the alarming increase in the number of viral strains that are resistant to multiple protease inhibitors highlights the necessity for compounds that are active against a wide variety of HIV mutations.[2]

Recently, we reported that modification of the aminoindanol moiety and the pyridylmethyl substituent on Indinavir afforded compounds, such as compound **1** (Fig. 1), with both improved bioavailability and increased potency against PI-resistant viral strains.[3] In this communication we report that modification of the piperazine *tert*-butylcarboxamide moiety in Indinavir analogues affords compounds with further increased potency against PI-resistant virus, as well as improvements in pharmacokinetic properties.

The synthesis of the carboxamide analogues was designed so as to differentiate the amide position as late as possible in the synthetic sequence (Scheme 1). The bis-protected piperazine-2-(*S*)-carboxylic acid **I**[4] was first protected as the benzyl ester under standard conditions. Deprotection of the distal nitrogen, followed by copper mediated alkylation, afforded intermediate **II**.[5] Cyclization of this intermediate with 4-hydroxy-3,5-diiodopyridine with the Castro–Stevens reaction afforded the pyridylfuran **III**.[6] Protecting group manipulation, followed by hydrogenolysis, afforded the free acid **IV**. Amide coupling with a variety of amine nucleophiles under standard conditions (EDC and HOAT) afforded the desired amides **V**. Deprotection of the piperazine, followed by nucleophilic epoxide opening with intermediate **VII**[7] and final deprotection under acid conditions, gave the desired products **VIII**.

The biological activity of the compounds synthesized is shown in Table 1. The compounds were tested for the ability to inhibit the protease enzyme (IC_{50}) and to

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

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00425-0

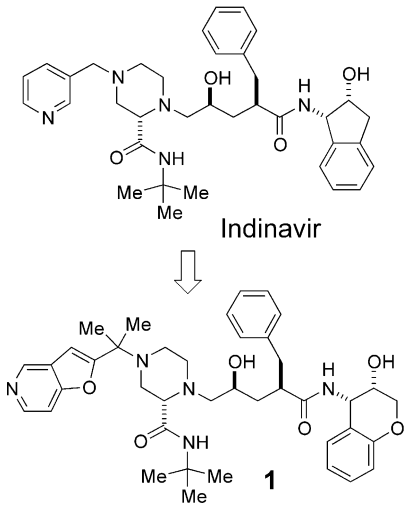


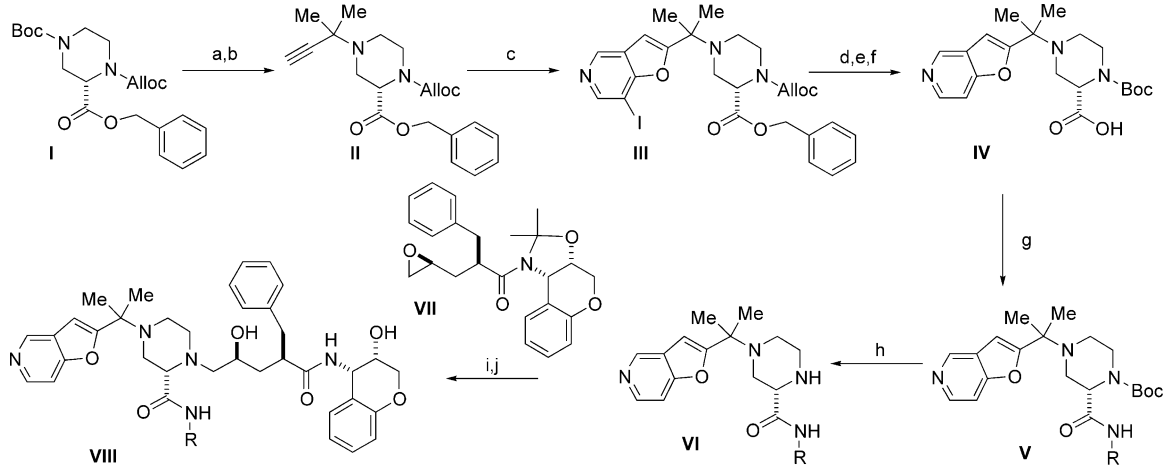
Figure 1. Aminoindanol and pyridylmethyl replacements that afford improved potency and bioavailability.

inhibit the spread of viral infection in MT4 human T-lymphoid cells using NL4-3 virus (CIC_{95}).[8] In addition, the compounds were tested using the 4X mutant virus model system, as an initial indication of their potential potency against PI-resistant HIV.[9] The results indicate that the *t*-butylamide substituent (**1**, Table 1) is highly optimized for potency in this series, and all modifications with aliphatic amides (**2**–**7**) resulted in a loss of activity. Furthermore, the arylamides and arylalkylamides (**8**–**10**) were all substantially less potent than the *t*-butylamide in this series of compounds. The fluoroalkyl compounds (**11**–**13**) exhibited variable effects on potency depending on their size, with the optimum substituent being the trifluoroethylamide (**11**).[10] This substituent afforded increased potency in both the enzyme inhibition assay and the inhibition of viral spread assay. In addition, a 4-fold increase in potency over the *t*-butylamide substituted compound was observed against the 4X mutant virus.

The impact of the trifluoroethylamide substituent on the potency of Indinavir analogues was further investigated by testing a set of compounds against PI cross-resistant viral strains (Table 2). The viral constructs employed were engineered from clinical viral isolates of patients infected with multiply PI-resistant HIV, and the genotype and phenotype of these isolates have been reported elsewhere.[11] While Indinavir remains largely inactive against the resistant viral strains, the substitution of the trifluoroethylamide moiety (**14**, Table 2) affords a limited increase in potency against the 4X virus and the V-18 and Q-60 viral constructs. Similarly, while compound **1** represents a substantial improvement in potency over Indinavir, the trifluoroethyl analogue (**11**) affords a further 2-fold increase in potency for each viral construct.

The pharmacokinetic impact of the trifluoroethylamide substituent was next investigated by dosing compound **11** in dogs, both orally (5 mg/kg) and iv (2 mg/kg) (Table 3).[12] Remarkably high plasma levels of compound **11** were observed after oral dosing, with a maximum concentration of 17.0 μM. In fact, the trifluoroethylamide substituent appears to confer substantially greater plasma exposure than the analogous *t*-butylamide compound (compound **1**), as measured by the A.U.C. In addition, the overall clearance (CL_p) of the trifluoroethylamide analogue was less than a third the rate of the corresponding *t*-butylamide. Both compounds compared quite favorably with the pharmacokinetic profile of Indinavir in dogs.[12]

The substantially lower in vivo clearance observed for compound **11** versus **1** in dogs was further probed by comparative studies in a series of in vitro metabolic assays. In human liver microsomes the trifluoroethylamide substituent conferred slightly greater metabolic stability to the compound than the corresponding *t*-butylamide, as measured by the intrinsic clearance (CL_{int}, Table 4). However, both of the investigated compounds exhibited greater in vitro metabolic stability than Indinavir.[13] The metabolic stability of Indinavir may be attributed to the competitive inhibition of the cyctochrome P450 isoform 3A4 (CYP3A4), which is the isoform primarily responsible for metabolism of this compound in vivo.[14] While



Scheme 1. (a) TFA, CH_2Cl_2; (b) $HC{\equiv}C(CH_3)_2Cl$, CuCl, Cu(0), Et_3N; (c) 4-hydroxy-3,5-diiodopyridine, Cu_2O, pyridine, 115 °C; (d) $Pd_2(dba)_3$, DPPB, thiosalicylic acid, THF; (e) di-*tert*-butyldicarbonate, DMAP, CH_2Cl_2; (f) H_2, Pd/C, Et_3N, MeOH; (g) RNH_2, EDC, HOAT, Et_3N, CH_2Cl_2; (h) TFA, CH_2Cl_2; (i) **VII**, *i*PrOH, 85 °C; (j) HCl (g), MeOH.

Table 1. Influence of carboxamide substituents on in vitro potency of HIV-protease inhibitors of structure **VIII**

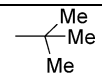
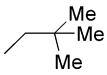
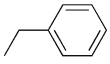
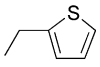
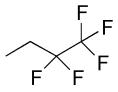
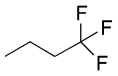
Compd	R	IC_{50} (nM)	CIC_{95} (nM) NL4-3	CIC_{95} (nM) 4X
1		0.08	≤8.0	31
2		1.16	31	500
3		0.61	31	62
4		0.40	15	125
5		0.60	62	250
6		0.50	46	125
7		0.37	≤8.0	31
8		13.8	250	>1000
9		2.19	125	250
10		0.65	94	125
11		0.07	≤8.0	≤8.0
12		0.14	≤8.0	62
13		1.60	62	500

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

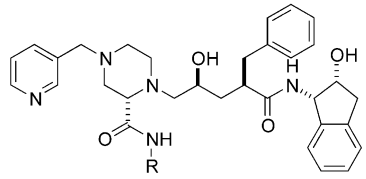
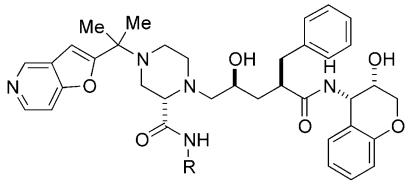
Compd	Scaffold	Amide substituent	NL4-3	4X Virus	Viral constructs		
					K-60C	V-18C	Q-60C
Indinavir		$R = C(CH_3)_3$	50	400	>1000	>1000	>1000
14		$R = CH_2CF_3$	62	125	>1000	500	62
1		$R = C(CH_3)_3$	9.2	16	500	125	125
11		$R = CH_2CF_3$	≤8.0	≤8.0	250	62	62

Table 3. Pharmacokinetics of HIV-protease inhibitors in dogs po (5 mg/kg) and iv (2 mg/kg)

Compd	R	C_{max} (μM)	$t_{1/2}$ (min)	A.U.C. (μM h)	CL_p (mL/min/kg)	% F
1	$C(CH_3)_3$	8.8	62	7.4	14.2	81
11	CH_2CF_3	17.0	52	18.8	4.01	65

Table 4. In vitro metabolic assays of investigational HIV-protease inhibitors

Compd	CL_{int}[a] (mL/min/kg)	CYP3A4 IC_{50} (μM)	CYP2D6 IC_{50} (μM)
Indinavir[b]		0.15	>30.0
1	34 (64)	0.31	2.43
11	22 (50)	0.03	0.52

[a]Measured in human liver microsomes.
[b]Clearance values for Indinavir measured in tandem experiments are given in parentheses.

the *t*-butylamide analogue (**1**) is a potent inhibitor of CYP3A4 in its own right (IC_{50} = 0.31 μM), the corresponding trifluoroethylamide substituent confers over a 10-fold increase in potency against this metabolic enzyme. This same increase in potency was observed in the related metabolic enzyme CYP2D6. Indinavir is a very weak inhibitor of this P450 isoform,[15] while the *t*-butylamide (**1**) exhibits micromolar inhibition potency, and the analogous trifluoroethylamide affords almost a further 5-fold increase in potency (0.52 μM). Although the P450 inhibitory properties of compound **11** may afford slower clearance of this compound in vivo, they also greatly increase the likelihood of complicating drug–drug interactions if this compound were employed as part of a clinical regimen.

Modification of the piperazine carboxamide substituent on Indinavir and a related series of compounds can significantly impact the inhibitory potency of the compounds against the HIV protease enzyme. The trifluoroethylamide substituent in compound **11** (Table 1) imparts greater potency in halting the viral spread of both the wild-type virus and a number of PI-resistant variants of HIV. This compound also exhibited more favorable pharmacokinetic properties in vivo as compared to the analogous *t*-butyalmide; however, this may be due to the striking increase in inhibitory potency of at least two important P450 isoforms, CYP3A4 and CYP2D6.

References and Notes

1. (a) Kempf, D. J.; Molla, A.; Hsu, A. In *Antiretroviral Therapy*; De Clercq, E., Ed.; ASM: Washington, DC, 2001; p 147. (b) Vacca, J. P.; Condra, J. H. *Drug Discov. Today* **1997**, *2*, 261.
2. Boden, D.; Markowitz, M. *Antimicrob. Agents Chemother.* **1998**, *42*, 2775.
3. Cheng, Y.; Zhang, F.; Rano, T. A.; Lu, Z.; Schleif, W. A.; Gabrylski, 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.
4. 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.
5. Hennion, G. F.; Manzel, R. S. *J. Am. Chem. Soc.* **1960**, *82*, 4908.
6. Castro, C. E.; Gaughan, E. J.; Owsley, D. C. *J. Org. Chem.* **1966**, *31*, 4071.
7. The epoxide **VII** may be synthesized in direct analogy to the aminoindanol derivative: Maligres, P. E.; Upadhyay, V.; Rossen, K.; Cianciosi, S. J.; Purick, R. M.; Eng, K. K.; Reamer, R. A.; Askin, D.; Reider, P. J. *Tetrahedron Lett.* **1995**, *36*, 2195.
8. 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. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
9. Schock, H. B.; Garsky, V. M.; Kuo, L. C. *J. Biol. Chem.* **1996**, *271*, 31957.
10. **Data for compound 11**. ^{1}H NMR ($CDCl_3$, 400 MHz) 9.42 (t, *J* = 4.8 Hz, 1H), 8.90 (s, 1H), 8.52 (d, *J* = 6.0 Hz, 1H), 7.37 (d, *J* = 5.6 Hz, 1H), 7.30 (m, 5H), 7.11 (t, *J* = 8.4 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 6.78 (m, 2H), 6.67 (s, 1H), 5.91 (d, *J* = 8.4 Hz, 1H), 5.15 (dd, *J* = 4.0 Hz, 1H), 4.27 (m, 1H), 4.06 (d, *J* = 10.4 Hz, 1H), 4.00 (dd, *J* = 4.8 Hz, *J* = 11.6 Hz, 1H), 3.76 (m, 3H), 3.46 (s, 1H), 3.37 (s, 1H), 3.11 (d, *J* = 11.6 Hz, 1H), 2.85 (m, 4H), 2.70 (m, 4H), 2.44 (m, 2H), 2.10 (d, *J* = 5.2 Hz, 1H), 1.90 (t, *J* = 11.2 Hz, 1H), 1.57 (s, 8H); HPLC-MS (ES) 724.6 (M + 1).
11. (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. The most highly cross-resistant viral isolates reported in ref 11b were chosen for this investigation without regard to their amino acid substitution pattern.
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.; Lin, J. H. *Biochem. Pharmacol.* **1997**, *53*, 1187.
14. Chiba, M.; Hensleigh, M.; Nishime, J. A.; Balani, S. K.; Lin, J. H. *Drug Metab. Disp.* **1996**, *24*, 307.
15. von Moltke, L. L.; Greenblatt, D. J.; Duan, S. X.; Daily, J. P.; Harmatz, J. S.; Shader, R. I. *J. Pharm. Sci.* **1998**, *87*, 1184.