Journal of
Medicinal
Chemistry

Volume 41, Number 9 April 23, 1998

Communications to the Editor

(−)-6-Chloro-2-[(1-furo[2,3-*c*]pyridin-5-yl-ethyl)thio]-4-pyrimidinamine, PNU-142721, a New Broad Spectrum HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitor

Donn G. Wishka, David R. Graber, Laurice A. Kopta, Robert A. Olmsted, Janice M. Friis, John D. Hosley, Wade J. Adams, Eric P. Seest, Thomas M. Castle, Lester A. Dolak, Barbara J. Keiser, Yoshihiko Yagi, Azhwarsamy Jeganathan, Stephen T. Schlachter, Michael J. Murphy, Gary J. Cleek, Richard A. Nugent, Susan M. Poppe, Steven M. Swaney, Fusen Han, William Watt, Wendy L. White, Toni-Jo Poel, Richard C. Thomas, Richard L. Voorman, Kevin J. Stefanski, Randall G. Stehle, W. Gary Tarpley, and Joel Morris*

Pharmacia & Upjohn, Inc., Kalamazoo, Michigan 49001

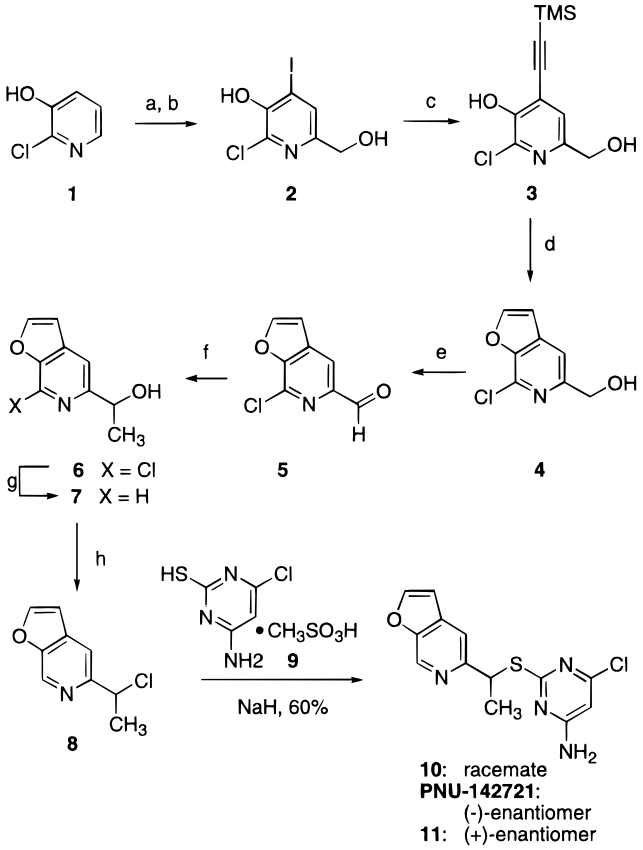
Received February 17, 1998

Considerable effort has been focused over the past decade on the development of the HIV-1 specific non-nucleoside reverse transcriptase inhibitors (NNRTIs), due to their desirable safety, selectivity, and antiviral profiles, for the treatment of HIV-1 infection and AIDS.[1] Such efforts at Pharmacia & Upjohn (PNU) have led to the discovery of the bis(heteroaryl)piperazine (BHAP) class of NNRTIs and clinical development of delavirdine.[2] The recent FDA approval of delavirdine (RESCRIPTOR) was based on its safety profile and, importantly, its effectiveness in combination with the NRTI AZT.[3] However, a more complete picture of the clinical utility of this NNRTI class of agents awaits the results from triple combination studies.

Although the observed clinical benefits of triple combination regimens of NRTIs and protease inhibitors currently being utilized are promising, the emergence of drug-resistant variants remains a major concern. The rapidity with which these variants of HIV-1 are selected in vivo by NRTIs and NNRTIs, as well as viral protease inhibitors, can be explained by extremely high rates of virus replication and turnover.[4] High levels of replication combined with the infidelity of the RT DNA polymerase inevitably lead to the generation of the viral quasispecies from which drug-resistant variants are selected during drug therapy.

A need remains for the discovery and development of more potent NNRTIs targeting drug-resistant variants

Scheme 1[a]



[a] (a) $NaHCO_3$, aqueous formaldehyde, 81%; (b) I_2, $NaHCO_3$, H_2O, 74%; (c) TMS-acetylene, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, $CHCl_3$/THF, 92%; (d) (i) CuI, Et_3N, EtOH; (ii) NaOH, MeOH; 80%; (e) oxalyl chloride, DMSO, −60 °C, 95%; (f) CH_3MgBr, THF, 80%; (g) cyclohexene, 20% $Pd(OH)_2$, MeOH, reflux, 77%; (h) $SOCl_2$, CH_2Cl_2, 0 °C to rt, 96%.

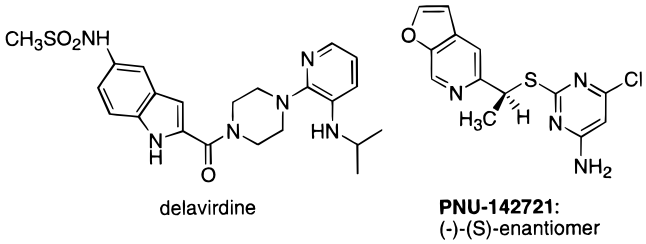
S0022-2623(98)00104-6 CCC: $15.00
Published on Web 04/04/1998

Table 1. Inhibitory Activity (IC_{50}, μM) of Furo[2,3-*c*]pyridine Pyrimidine Thioethers against WT-RT and a Panel of Mutant RT Enzymes[a,b]

	WT	P236L	Y181C	E233V	L100I	Y188H
PNU-142721	0.02	0.022	0.179	0.017	0.039	0.077
10	0.024	0.022	0.36	0.044	0.044	0.055
11	0.085	0.025	>50	ND	ND	ND
delavirdine	0.26	18.0	8.3	ND	ND	ND

[a] Inhibitory assays were performed as previously described in ref 12. IC_{50} values were determined by nonlinear least-squares fit of data from duplicate points at six drug concentrations. [b] ND = not determined.

of specific genotypes. By producing a more potent viral suppression, the use of agents of this type in combination therapy may prolong the time frame for the selection of variants resistant to the NNRTIs.[5] The search at PNU to identify compounds with broad activity against several NNRTI-resistant variants of HIV-1 has led to the discovery of the furo[2,3-*c*]pyridine thiopyrimidine class of NNRTIs.[6] Intensive evaluation of this class has led to the recent selection of PNU-142721 as a candidate for clinical development. Herein, the antiviral and initial pharmacokinetic profile of PNU-142721 is described.



Chemistry. The title compound was initially prepared as its racemate **10** in nine steps beginning with 2-chloro-3-hydroxypyridine (**1**). The synthetic plan for the synthesis of this agent called for the alkylation of 4-amino-6-chloro-2-thiopyrimidine (**9**)[7] with (chloroethyl)furopyridine **8**. The preparation of **8** began with the hydroxymethylation[8] of **1** followed by iodination of the resultant hydroxypyridine to afford **2** in 60% overall yield (Scheme 1). Construction of the furan ring was accomplished first by the Pd−Cu coupling of **2** with TMS-acetylene to generate a 92% yield of **3**.[9] The efficient cyclization of **3** with CuI followed by in situ desilylation afforded the furo[2,3-*c*]pyridine **4** in 80% yield.[10] The efficient elaboration of the required hydroxyethyl side chain in two steps and subsequent reductive removal of the 7-chloro substituent generated a 58% overall recovery of **7**. Finally, treatment of **7** with thionyl chloride afforded chloride **8** in 96% yield. The coupling of **8** and **9** producing **10** was accomplished in 60% yield, utilizing the sodium anion produced from the mesylate salt of **9** and 2 equiv of NaH.[7] Resolution of racemate **10** into its (+)- and (−)-enantiomers (**11** and PNU-142721, respectively) was achieved using a preparative Chiralcel OD-H column, eluting with 20% 2-propanol/hexane (0.5 mL/min). The absolute configuration of the (−)-enantiomer, PNU-142721, was established as the (*S*)-stereoisomer by X-ray crystallographic analysis.[11]

Results and Discussion. The furopyridine pyrimidine thioethers **10**, **11**, and PNU-142721 were evaluated for inhibitory activity against wild-type reverse transcriptase (WT-RT) and a panel of mutant RT enzymes (Table 1).[12] All three derivatives proved to be extremely potent against WT-RT, with IC_{50} values ranging from 20 to 85 nM. Similarly, the compounds were exceptionally potent against P236L RT, a mutant enzyme that has been genotypically identified in virus resistant to the NNRTI delavirdine (IC_{50}s from 22 to 25 nM).[13] Interestingly, activity levels against the Y181C enzyme, a mutant RT observed under pressure by several known NNRTIs, including L-697,661[14] and α-APA,[15] were dependent on the stereochemistry of this thiopyrimidine substrate. Whereas racemate **10** and its (−)-enantiomer PNU-142721 displayed submicromolar IC_{50}s against the mutant Y181C RT, the corresponding (+)-enantiomer **11** proved to be inactive. Inhibitory potency levels for PNU-142721 against several other variant RT, including E233V, L100I, and Y188H, were also quite strong.

In cell culture,[16] PNU-142721 demonstrated superior activity (IC_{90} = 1 nM) against the wild-type HIV-1 strain

Table 2. Antiviral Activity (IC_{90}, μM) of Furo[2,3-*c*]pyridine Pyrimidine Thioethers against in Vitro Selected NNRTI-Resistant HIV-1 Variants[a,b]

	IIIB (WT)	DLV^R-MF (P236L)[c]	L-697661^R-IIIB (Y181C)[c]	DLV^R-IIIB (L100I)[c]	R88703^R-IIIB (Y181C)[c]
PNU-142721	0.001	0.008	1.1	0.07	0.17
11	ND	>0.01 (0%)[d]	>3.0 (0%)[d]	ND	ND
10	0.002	0.01	2.4	0.06	0.17
delavirdine	0.05	>10	5.2	>10	1.0
L-697,661	0.11	0.43	>10	4.0	7.7
R88703	0.15	0.08	>10	6.5	>10

[a] IC_{90}, concentration of drug that inhibited p24 production by 90% in infected MT4 cells. Antiviral assays were performed as previously described in ref 16. The IC_{90} values represent averages from at least two IC determinations. [b] ND = not determined. [c] Primary resistance conferring mutation at the designated codon of HIV-1 RT. [d] Percent inhibition of p24 production at the indicated drug concentration.

Table 3. Single-Dose Pharmacokinetics of PNU-142721 in the Sprague−Dawley Rat

route	dose[a] (mg/kg)	C_{max} (μM)	t_{max} (h)	$t_{1/2}$[b] (h)	V_{ss} (L/kg)	CL/*F* (mL/min/kg)	*F*[c] (%)
IV	15	123 ± 19	0.033	0.70 ± 0.05	0.55 ± 0.05	6.7 ± 1.6	
PO	30	52 ± 14	1.4 ± 1.1	0.78 ± 0.24		7.1 ± 2.3	94 ± 40
PO	80	103 ± 23	3.8 ± 0.5	1.5 ± 0.4		7.1 ± 1.5	94 ± 30

[a] n = 3. Drug dose in free base equivalents. The compound was formulated as an acidified solution (methansulfonic acid) in PEG 400 for intravenous administration. For oral administration, an acidified propylene glycol:water (9:1, v:v) formulation was utilized. [b] Harmonic mean apparent terminal disposition half-life. [c] Absolute oral bioavailability.

IIIB (50-fold more potent than delavirdine) (Table 2). In addition, PNU-142721 was found to have potent inhibitory activity against a panel of NNRTI-resistant HIV-1 variants, including the delavirdine resistant HIV-1_{MF} variant, DLV^R -MF, carrying the P236L substitution.[13] A second delavirdine resistant virus having substitutions at amino acids 100 and 230 remained susceptible to submicromolar concentrations of PNU-142721 as well. The two variants resistant to L-697,-661 and R88703, both of which encode the broad NNRTI cross-resistance conferring mutation Y181C RT, demonstrated more modest sensitivity to the antiviral effects of PNU-142721.[17] As expected, the (+)-enantiomer **11** proved to be relatively inactive against variant virus carrying the Y181C mutation. However, in contrast to the results of the in vitro enzyme assay, **11** displayed a significant loss of potency against variant virus selected for by delavirdine.

The pharmacokinetics of PNU-142721 were determined following single iv and oral dose administration of the compound in male Sprague−Dawley rats (Table 3). The mean systemic clearance of PNU-142721 was low (6.7 ± 1.6 mL/min/kg). The mean steady-state volume of distribution was moderate (0.55 ± 0.05 L/kg, ≥ 9 times plasma volume), and the harmonic mean terminal disposition half-life was short (0.70 ± 0.05 h). Following oral dose administration in the rat, systemic concentrations of PNU-142721 were high and increased in proportion to dose for doses ranging from 30 to 80 mg/kg. The absolute oral bioavailability was 94 ± 40% at 30 mg/kg dose and remained unchanged at the 80 mg/kg dose. Furthermore, in a single iv dosed rat (15 mg/kg), brain levels of PNU-142721 were found to be 75% of simultaneous plasma concentrations. Consistent with this finding is the conclusion that CNS penetration for this compound will be favorable in clinical studies.

PNU-142721 offers a significantly improved antiviral profile relative to PNU's currently marketed NNRTI, delavirdine. In addition to being 50-fold more potent against a laboratory strain of wild-type HIV-1, PNU-142721 is extremely potent against variant viruses selected for by delavirdine and other NNRTIs. The demonstration of broad, potent antiviral activity and a favorable pharmacokinetic profile for PNU-142721 has led to the selection of this compound for future study in a clinical setting. Resistance patterns and more detailed antiviral profiling of the furopyridine pyrimidine thioethers are under study with other members of this class of NNRTIs and will be reported in due course.

Supporting Information Available: Full experimental and spectroscopic data for all new compounds and ORTEP drawing and atomic coordinate information for PNU-142721 (12 pages). Ordering information is given on any current masthead page.

References

(1) (a) Romero, D. L. Advances in the Development of HIV Reverse Transcriptase. *Annu. Rep. Med. Chem.* **1994**, *29*, 123−32. (b) Tucker, T. J.; Lumma, W. C.; Culberson, J. C. Development of nonnucleoside HIV reverse transcriptase inhibitors. *Methods Enzymol.* **1996**, *275*, 440−472. (c) Spence, R. A.; Johnson, K. A. Therapeutic potential of nonnucleoside reverse transcriptase inhibitors in the treatment of HIV infection. *Expert Opin. Invest. Drugs* **1996**, *5*, 985−1001. (d) De Clercq, E. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs). *Exp. Opin. Invest. Drugs* **1994**, *3*, 253−271.

(2) Romero, D. L.; Morge, R. A.; Biles, C.; Berrios-Pena, N.; May, P. D.; Palmer, J. R.; Johnson, P. D.; Smith, H. W.; Busso, M.; Tan, C.-K.; Voorman, R. L.; Reusser, F.; Althaus, I. W.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G.; Aristoff, P. A. Discovery, Synthesis, and Bioactivity of Bis(heteroaryl)piperazines. 1. A Novel Class of Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors. *J. Med. Chem.* **1994**, *37*, 999−1014.

(3) (a) Freimuth, W. W. Delavirdine mesylate, a potent non-nucleoside HIV-1 reverse transcriptase inhibitor. *Adv. Exp. Med. Biol.* **1996**, *394* (Antiviral Chemotherapy 4), 279−289. (b) Davey, R. T., Jr.; Chaitt, D. G.; Reed, G. F.; Freimuth, W. W.; Herpin, B. R.; Metcalf, J. A.; Eastman, P. S.; Falloon, J.; Kovacs, J. A.; Randomized, controlled phase I/II trial of combination therapy with delavirdine (U-90152S) and conventional nucleosides in human immunodeficiency virus type 1-infected patients. *Antimicrob. Agents Chemother.* **1996**, *40*, 1657−1664.

(4) Molla, A.; Kohlbrenner, W. E. Resistance to antiretroviral drug therapy. *Annu. Rep. Med. Chem.* **1997**, *32*, 131−140.

(5) De Clercq, E. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. *Med. Res. Rev.* **1996**, *16*, 125−57.

(6) (a) Morris, J. Non-nucleoside reverse transcriptase inhibitors for the treatment of AIDS. Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13−17, 1997, BIOC-056. (b) Nugent, R. A.; Wishka, D. G.; Cleek, G. J.; Graber, D. R.; Schalachter, S. T.; Murphy, M. J.; Morris, J.; Thomas, R. C. Preparation of 2-pyrimidino alkyl ethers and thioethers as inhibitors of viral reverse transcriptase. WO/9635678.

(7) 4-Amino-6-chloro-2-thiopyrimidine (**9**) was prepared in four steps starting with 4,6-dihydroxy-2-thiopyrimidine by modification of the methodology described in the following: d'Atri, G.; Gomarasca, P.; Resnati, G.; Tronconi, G.; Scolastico, C.; Sirtori, C. R. Novel Pyrimidine and 1,3,5-Triazine Hypolipidemic Agents. *J. Med. Chem.* **1984**, *27*, 1621−1629.

(8) Baldo, M. A.; Chessa, G.; Marangoni, G.; Pitteri, B. Synthesis of 2,6-Diformyl-3-hydroxypyridine Bis[*N*-methyl-*N*-(2′-pyridyl)-hydrazone]: A Pentadentate *N*, *N*, *N*, *N*, *N* Ligand for the Preparation of Chelating Resins. *Synthesis* **1987**, 720−723.

(9) Sonogashira, K.; Tohda, Y.; Hagihara, N. A Convenient Synthesis of Acetylenes: Catalytic Substitutions of Acetylenic Hydrogen with Bromoalkenes, Iodoarenes, and Bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4470.

(10) Houpis, I. N.; Choi, W. B.; Reider, P. J.; Molina, A.; Churchill, H.; Lynch, J.; Volante, R. P. Synthesis of functionalized furo-[2,3-b]pyridines via the Pd-catalyzed coupling of acetylenes to iodopyridones. Preparation of a key intermediate to a new HIV protease inhibitor L-754,394. *Tetrahedron Lett.* **1994**, *35*, 9355−8.

(11) ORTEP drawing of PNU-142721 and atomic coordinate information can be obtained from Supporting Information.

(12) Romero, D. L.; Olmsted, R. A.; Poel, T. J.; Morge, R. A.; Biles, C.; Keiser, B. J.; Kopta, L. A.; Friis, J. M.; Hosley, J. D.; Stefanski, K. J.; Wishka, D. G.; Evans, D. B.; Morris, J.; Stehle, R. G.; Sharma, S. K.; Yagi, Y.; Voorman, R. L.; Adams, W. J.; Tarpley, W. G.; Thomas, R. C. Targeting Delavirdine/Atevirdine Resistant HIV-1: Identification of (Alkylamino)piperidine-Containing Bis(heteroaryl)piperazines as Broad Spectrum HIV-1 Reverse Transcriptase Inhibitors. *J. Med. Chem.* **1996**, *39*, 3769−3789.

(13) (a) Dueweke, T. J.; Pushkarskaya, T.; Poppe, S. M.; Swaney, S. M.; Zhao, J. Q.; Chen, I. S. Y.; Stevenson, M.; Tarpley, W. G. A Mutation in Reverse Transcriptase of Bis(heteroaryl)piperazine Resistant Human Immunodeficiency Virus Type 1 that Confers Increased Sensitivity to other Nonnucleoside Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 4713−4717. (b) Fan, N.; Evans, D. B.; Rank, K. B.; Thomas, R. C.; Tarpley, W. G.; Sharma, S. K. Mechanism of resistance to U-90152S and sensitization to L-697,661 by a proline to leucine change at residue 236 of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *FEBS Lett.* **1995**, *359*, 233−8.

(14) Carroll, S. S.; Olsen, D. B.; Bennett, C. D.; Gotlib, L.; Graham, D. J.; Condra, J. H.; Stern, A. M.; Shafer, J. A.; Kuo, L. C. Inhibition of HIV-1 reverse transcriptase by pyridinone derivatives. Potency, binding characteristics, and effect of template sequence. *J. Biol. Chem.* **1993**, *268*, 276−81.

(15) Pauwels, R.; Andries, K.; Debyser, Z.; Van Daele, P.; Schols, D.; Stoffels, P.; De Vreese, K.; Woestenborghs, R.; Vandamme, A. M.; Janssen, C. G. M.; Anne, J.; Cauwenbergh, G.; Desmyter, J.; Heykants, J.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of α-anilinophenylacetamide derivatives targeted at HIV-1 reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1711−15.

(16) Olmsted, R. A.; Slade, D. E.; Kopta, L. A.; Poppe, S. M.; Poel, T. J.; Newport, S. W.; Rank, K. B.; Biles, C.; Morge, R. A.; Dueweke, T. J.; Yagi, Y.; Romero, D. L.; Thomas, R. C.; Sharma, S. K.; Tarpley, W. G. (Alkylamino)piperidine bis(heteroaryl)piperizine analogs are potent, broad-spectrum nonnucleoside reverse transcriptase inhibitors of drug-resistant isolates of human immunodeficiency virus type 1 (HIV-1) and select for drug-resistant variants of HIV-1_{IIIB} with reduced replication phenotypes. *J. Virol.* **1996**, *70*, 3698−3705.

(17) Genotypic analyses of the L-697, 661^{R}-IIIB and $R88703^{R}$-IIIB variants revealed multiple amino acid substitution patterns, including the Y181C mutation, in the RT genes from both viruses. However, the L-697,661 resistant variant harbored three additional substitutions not detected in the R88703 resistant variant. These substitutions may be responsible for the 10-fold difference in antiviral activity observed for PNU-142721 against these two NNRTI-resistant variants.

JM9801049