Synthesis and Evaluation of 2-Pyridinone Derivatives as Specific HIV-1 Reverse Transcriptase Inhibitors. 3. Pyridyl and Phenyl Analogs of 3-Aminopyridin-2(1*H*)-one

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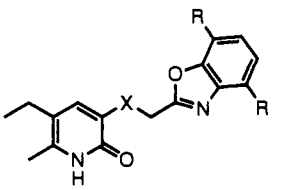
In an ongoing effort to develop novel nonnucleoside, specific human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) inhibitors, a series of 3-[(pyridylmethyl)amino]- and 3-[(phenylmethyl)amino]-2-pyridinone derivatives was synthesized and tested for HIV-1 RT inhibitory activity. The more potent compounds have a 2′-methoxy group and 4′- and/or 5′-aliphatic substituents on the pyridyl and phenyl rings. Several of the more potent compounds were also evaluated for antiviral activity in MT-4 cell culture. From this series of compounds, 3-[*N*-[(5-ethyl-2-methoxy-6-methyl-3-pyridyl)methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (**6**) was selected for clinical evaluation.

Human immunodeficiency virus type 1 (HIV-1) has been identified as the etiological agent of acquired immunodeficiency syndrome (AIDS). The unique nature of the replicative cycle of HIV-1 provides many potential targets for chemotherapeutic intervention. One of these is the viral reverse transcriptase (RT) which catalyzes the conversion of the viral genomic RNA into a double-stranded DNA copy. Nucleoside analog inhibitors of RT, such as 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (ddI) have been shown to be of benefit in treatment of HIV-infected patients. However, the utility of these nucleoside analogs is compromised by serious side effects which may be related to nonspecific inhibition of cellular DNA polymerases. The limitations of nucleoside analogs have encouraged an intensive search for nonnucleoside specific HIV-1 RT inhibitors.[1-4] Unfortunately, the clinical utility of these nonnucleosides as monotherapy may be compromised by early emergence of viral resistance.[5] Recently, we reported the discovery of 2-pyridinone derivatives that are effective nonnucleoside specific HIV-1 RT inhibitors.[6] Two of the molecules (**1** and **2**), having in common a benzoxazole moiety linked by a two-atom spacer to the 2-pyridinone unit, were selected for clinical evaluation as antiviral agents. Therefore, it was important to determine whether or not these molecules could be further optimized for antiviral activity by structural modification.

Kinetic and radioligand displacement data suggested that **1** and **2** were preferentially targeted at the enzyme template–primer complex.[6a] Further structural detail was not available beyond knowledge that active HIV-1 RT is a p66/p51 heterodimer[8] derived through asymmetric processing of a p66/p66 homodimer by HIV-1 protease.[9] This limited knowledge about the target enzyme, however, stimulated thinking about symmetry as a design element for optimizing these inhibitors of HIV-1 RT.[10] Earlier attempts to increase potency through structural modifications on the 2-pyridinone moiety had not been successful.[11,12] Thus, N-methylation, O-methylation, deletion



1 X = NH R = Cl IC_{50} = 19 nM[7]
2 X = CH_2 R = H IC_{50} = 20 nM

of one or both C-5 or C-6 alkyl groups, and substitution of the 5-ethyl with 5-methyl resulted in analogs with substantially reduced activity. These results focused attention on replacement of the benzoxazole moiety with a 2-pyridinone as a means of testing symmetry as a design element. Compound **3** was selected as the primary target for synthesis (Scheme I); however, available chemistry and intermediates provided rapid access to **4**, **5**, and **6**, as well as **3**. The structure–activity relationship (SAR) data obtained for this set of compounds differed in detail from that observed for the benzoxazole-based inhibitors **1** and **2**. These findings prompted further investigation of this new SAR pattern as set forth herein.

Chemistry

Results from enzyme assays suggested a preference for the aminomethyl linker (**4** and **6**) over the carba linker (**3** and **5**) as a potency-enhancing element in constructions of this type, 3-aminopyridin-2(1*H*)-ones will be the focus of this work. The general synthetic route to this series of 3-aminopyridin-2(1*H*)-one derivatives is illustrated in Scheme II. Reaction of 2-ethyl-3-oxobutanol (**7**) (sodium salt) with nitroacetamide in water at ambient temperature in the presence of piperidinium acetate afforded the nitropyridinone **8** in 51% yield.[11,13] Heating the reaction mixture led to decomposition of product. Catalytic reduction of **8** gave almost quantitatively aminopyridinone **9**,[11] the common intermediate. Reductive alkylation of **9** with an appropriate aldehyde and sodium borohydride was the method of choice to generate the desired pyridinone derivative **10**. The required benzaldehydes, nicotinaldehydes, and picolinaldehydes were prepared as described in the following.

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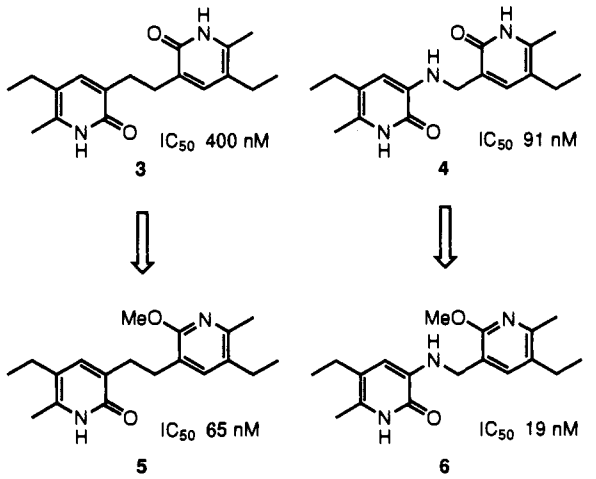
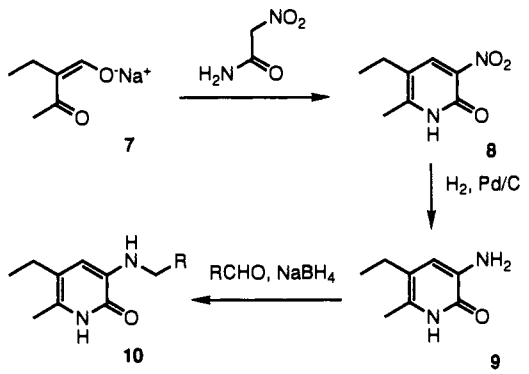
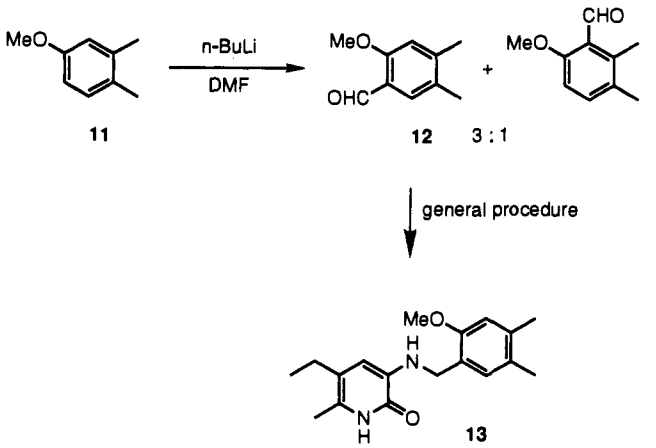
Scheme I



Scheme II



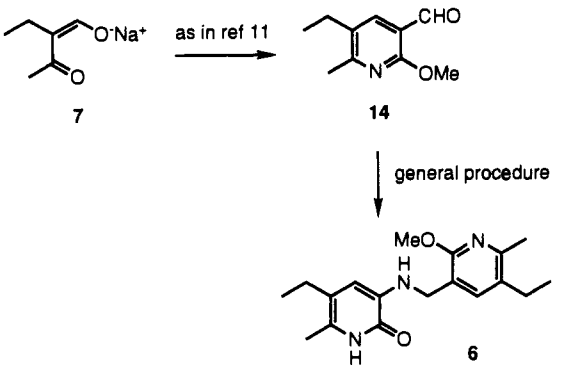
Scheme III



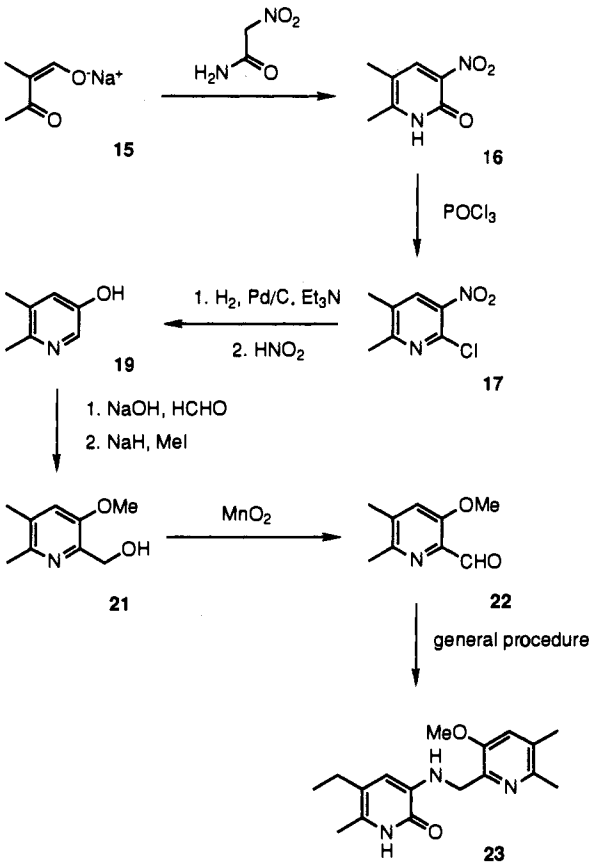
Substituted 2-methoxybenzaldehydes were obtained by treatment of the corresponding *ortho*-lithiated methoxyphenyl derivatives with dimethylformamide in ether at 0 °C (Scheme III). Substituted methoxynicotinaldehydes were obtained as previously described from ketoaldehydes[11,13] (Scheme IV).

Scheme V outlines the preparation of 5,6-dimethyl-3-methoxy-2-picolinaldehyde (**22**). Other methoxypicolinaldehydes were prepared similarly. Coupling of 3-keto aldehyde **15** (sodium salt) with nitroacetamide afforded the nitropyridinone **16**. Treatment of **16** with $POCl_3$ at 110 °C provided 2-chloro-3-nitropyridinone **17**, which was hydrogenated in methanol in the presence of 5% palladium on charcoal and triethylamine to give the corresponding 3-aminopyridine **18** as a hydrochloride salt. The free base of **18** was obtained by suspending the salt in chloroform

Scheme IV



Scheme V



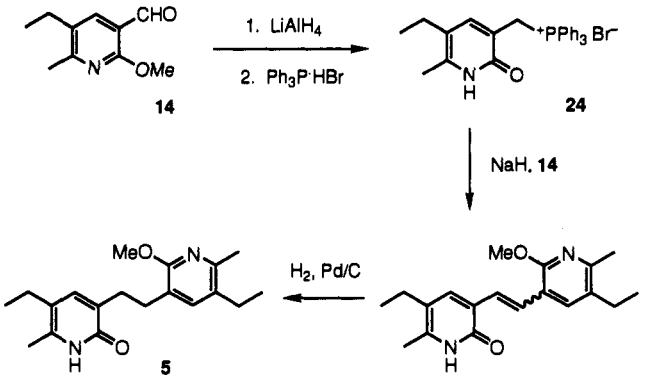
saturated with ammonia gas. Diazotization of the free base, followed by acid-catalyzed hydrolysis of the diazonium salt yielded 3-hydroxypyridine **19**. The hydroxypyridines are very soluble in aqueous acid and isolation of product was facilitated by neutralization and saturation of the product solution with sodium chloride. Treatment of **19** with formaldehyde in aqueous sodium hydroxide[14] gave the 2-hydroxymethylated pyridine **20**, which was selectively methylated with sodium hydride and iodomethane at 0 °C. Manganese(IV) oxide oxidation of the resultant product afforded **22**.

Phosphonium salt **24**, prepared by successive treatment of 2-methoxy-5-ethyl-6-methylnicotinaldehyde (**14**) with lithium aluminum hydride and triphenylphosphine hydrobromide, coupled with nicotinaldehyde **14** to provide a mixture of cis and trans olefins. Hydrogenation of the mixture gave the carba analog **5** (Scheme VI).

Results and Discussion

The in vitro HIV-1 RT assay using rC·dG template primer was used as the primary screen for these compounds

Scheme VI



as previously described.[7] The results are presented in Tables I–III. The concentration that produced 50% inhibition (IC_{50}) is stated as the mean of at least three experiments. Selected compounds were evaluated for inhibiting the spread of HIV-1 infection in MT-4 cell culture (Table IV). Ninety-five percent cell culture inhibitory concentrations (CIC_{95}) are defined as those which inhibited by ≥95% the spread of HIV-1 infection, which is assessed by HIV-1 p24 core antigen ELISA.[6a]

Synthesis of compounds **3–6** and determination of each analog's IC_{50} value for inhibition of HIV-1 RT focused attention on the 2′-methoxypyridine moiety as a bioisosteric replacement for benzoxazole. These results also suggested a preference for the aminomethyl linker (**4** and **6**) over the carba linker (**3** and **5**) as a potency-enhancing element in constructions of this type. As illustrated in Table I, the 2′-methoxy group had a significant impact (**25** vs **26** and **27** vs **28**) on enzyme inhibitory potency while the 3-pyridyl nitrogen (**25** vs **27**) had little effect on this parameter. Analogs **29–40** were synthesized and tested to determine whether this potency enhancement was general for 2′-substituents or related to some chemical or structural property of the substituent. While a variety of 2′-substituents (CN, F, Cl, MeS, Me) exhibited a modest potency-enhancing effect relative to the unsubstituted parent compound **28**, only the 2′-ethoxy (**29**) and 2′-nitro (**30**) substituents gave results comparable to those obtained with **27**. The CF_3 (**38**), NH_2 (**39**), and $MeOCH_2$ (**40**) substituents were clearly detrimental to potency. The significant contribution to activity observed with an appropriately placed ether oxygen is suggestive of a hydrogen bonding and/or chelation interaction with the enzyme and/or the metal cations which are essential for its functions (analogs **37**, **40**, **41**, **42**). As illustrated in Table II, the position of the nitrogen atom in the pyridyl series affects potency. The adverse impact of nitrogen in positions 4′ and 5′ (**43** and **44**) on activity may reflect a requirement for hydrophobic binding elements at these positions as observed with **6** and discussed below.

The enzyme inhibitory activity of **6** vs **25** indicated that the alkyl substituents on the pyridine ring also were contributing to potency in a significant way. This contribution was analyzed first in the phenyl series with the analogs **13**, **46–53** (Table III). Placing a methyl group at the 4′- or 5′-position (**47** and **48**) significantly enhanced inhibitory potency while comparable substitution at the 3′- or 6′-position (**46** and **49**) decreased activity. Presumably the methyl substituent at the 4′- or 5′-position is binding to a hydrophobic pocket. Extending the methyl group at the 4′- or 5′-position to an ethyl group (**50** and **51**) was not beneficial. A further increase in potency was achieved by placing methyl groups in both 4′- and 5′-positions (**13**). 4′-Methyl-5′-ethyl substitutions (**52**) and 4′,5′-trimethylene (**53**) led to decreases in potency relative to the 4′,5′-dimethyl analog (**13**). Similar substituent effects were observed with the 3-pyridyl analogs (**6**, **54**, **55**, **56** compared to **25**). 2-Pyridyl derivatives (**23**, **57**, **58**, **59**) showed a slightly different pattern and are consistently less potent than the corresponding phenyl and 3-pyridyl analogs. The difference in enzyme inhibition between the two classes of pyridyl analogs parallels that observed for the 4′,5′-unsubstituted pyridyl compounds (**25** and **45**).

In summary, the following conclusions can be drawn about the structure–activity relationship involved in the variation of the pyridyl moiety of **6**. Maximum enzyme inhibition is obtained with compounds containing a 2′-methoxy group on either the phenyl or pyridyl ring. There is no significant difference between the 2-methoxyphenyl and 2-methoxy-3-pyridyl derivatives. However, in the pyridine subclass, relative position of the pyridine nitrogen is important to activity. Small lipophilic groups at 4′- and 5′-positions are required for optimum activity: analogs with larger alkyl groups are less potent. Methyl substitution at the 3′- and 6′-positions is detrimental. These results suggest that 2-fold symmetry is not a requirement for potency with this class of enzyme inhibitors.

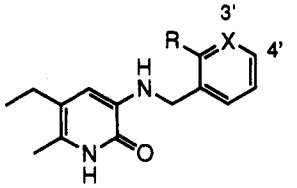
Compounds **6**, **13**, **54**, and **23** were found to effectively inhibit the spread of HIV-1 infection in MT-4 cell culture (Table IV) and showed no sign of cytotoxicity at concentrations as high as 50 μM. A direct correlation was observed for inhibition of enzymatic activity and spread of infection in cell culture (Table IV, compounds **6**, **13**, **54**, **23**, **27**, **25**), suggesting that the mechanism for the antiviral activity is inhibition of HIV-RT. Pyridinone **6**, and in general the whole class of compounds, specifically inhibited HIV-1 RT. Little enzyme inhibition was observed with RT from HIV-2, simian immunodeficiency virus, avian myeloblastosis virus, and Moloney murine leukemia virus at concentrations up to 300 μM. Furthermore, at the same concentrations, no inhibitory activity was observed with mammalian DNA polymerases α, β, γ, δ, Klenow fragment, *M. luteus* DNA polymerase, and *E. coli* RNA polymerase. Of these compounds, **6** exhibited the best oral bioavailability in rat and monkey and was selected for further development.

Experimental Section

^1H NMR spectra were obtained either on a Varian XL 300 or a Varian Unity 300 spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses results are within ±0.4% of the theoretical values except where otherwise stated. Mass spectra were taken on a VG Micromass MM7035 spectrometer. Flash chromatography was performed on E. Merck silica gel 60 (230–400 mesh).

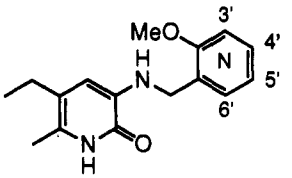
General Procedure: Reductive Coupling of 3-Amino-5-ethyl-6-methylpyridin-2(1*H*)-one (9) with 2-Methoxybenzaldehydes, Nicotinaldehydes, and Picolinaldehydes. A solution of 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one (**9**,[11] 1 equiv), aldehyde (1 equiv), and glacial acetic acid (1 drop) in methanol (0.1–0.05 M) was stirred at room temperature for 2 h. The yellow precipitate was filtered and dissolved in a mixture of methanol and chloroform. To the resultant yellow solution, sodium borohydride (1–2 equiv) was added until the solution became colorless. The product solution was then concentrated and diluted with ethyl acetate. The organic extract was washed with water, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel eluting with methanol in chloroform.

Table I. Inhibition of HIV-1 RT by 2-Pyridinones: Pyridyl and Phenyl Derivatives



no.	X	R	recryst solvent	mp, °C	formula	IC_{50} (nM)
25	N	MeO	EtOAc/hexane	178–179	$C_{15}H_{19}N_3O_2$	680
26	N	H	MeOH/H_2O	185–186	$C_{14}H_{17}N_3O$	300000
27	CH	MeO	EtOH	167–169	$C_{16}H_{20}N_2O_2$	265
28	CH	H	MeOH	202–204	$C_{15}H_{18}N_2O$	5300
29	CH	EtO	EtOH	167–169	$C_{17}H_{22}N_2O_2$	350
30	CH	NO_2	MeCN	176–179	$C_{15}H_{17}N_3O_3$	530
31	CH	CN	MeCN	190–193	$C_{16}H_{17}N_3O$	790
32	CH	F	EtOH	199–202	$C_{15}H_{17}FN_2O$	1400
33	CH	Cl	MeOH	206–210	$C_{15}H_{17}ClN_2O$	1450
34	CH	MeS	MeOH	167–171	$C_{16}H_{20}N_2OS$	1500
35	CH	Me	MeOH	237–239	$C_{16}H_{20}N_2O$	2200
36	CH	HO	EtOH/$CHCl_3$	194–196	$C_{15}H_{18}N_2O_2$	4600
37	CH	Et	EtOH	183–185	$C_{17}H_{22}N_2O$	6900
38	CH	CF_3	MeOH	170–172	$C_{16}H_{17}N_2F_3O$	12500
39	CH	NH_2	EtOH	234–237	$C_{15}H_{19}N_3O$	28000
40	CH	$MeOCH_2$	EtOH	162–164	$C_{17}H_{22}N_2O_2$	35500
41	CH	H, 3′-MeO	EtOH	183–185	$C_{16}H_{20}N_2O_2$	1250
42	CH	H, 4′-MeO	EtOH	151–153	$C_{16}H_{20}N_2O_2$	7500

Table II. Inhibition of HIV-1 RT by 2-Pyridinones: Pyridyl Derivatives



no.	position of N	recryst solvent	mp, °C	formula	IC_{50} (nM)
25	3′	EtOAc/hexane	178–179	$C_{15}H_{19}N_3O_2$	680
43	4′	EtOAc/hexane	211–213	$C_{15}H_{19}N_3O_2$	110000
44	5′	MeOH	200–202	$C_{15}H_{19}N_3O_2$	10000
45	6′	MeOH/$CHCl_3$	208–209	$C_{15}H_{19}N_3O_2$	1550

Collection and concentration of appropriate fractions, and recrystallization yielded the desired product.

3-[2-(5-Ethyl-2-methoxy-6-methyl-3-pyridyl)ethyl]-5-ethyl-6-methylpyridin-2(1*H*)-one (5) and 1,2-Bis(1,2-dihydro-5-ethyl-6-methyl-2-oxopyridin-3-yl)ethane (3). To a solution of 5-ethyl-2-methoxy-6-methylnicotinaldehyde (14) (2.28 g, 10.9 mmol) in tetrahydrofuran (50 mL), lithium aluminum hydride (0.77 g, 20 mmol) was added. The resultant slurry was refluxed overnight, cooled with an ice–water bath, and quenched with saturated aqueous sodium sulfate. The organic solution was dried over sodium sulfate, filtered, and concentrated under vacuo to provide the corresponding alcohol. Without further purification, the alcohol (363 mg, 2.0 mmol) obtained was heated with triphenylphosphine hydrobromide (690 mg, 2.0 mmol) in acetic acid (3 mL) at reflux for 24 h. The resultant mixture was diluted with diethyl ether and crude [(1,2-dihydro-5-ethyl-6-methyl-2-oxopyridin-3-yl)methyl]triphenylphosphonium bromide (24) was obtained as a gummy solid.

A mixture of the phosphonium salt 24 (815 mg) and sodium hydride (60% dispersion in mineral oil, 95 mg, 2.4 mmol) in anhydrous THF (10 mL) was warmed at 50 °C for 15 min. 5-Ethyl-2-methoxy-6-methylnicotinaldehyde (14,[11] 323 mg, 1.8 mmol) was added and the resultant mixture refluxed for 5 h. The reaction was cooled, diluted with chloroform, and washed with water. The organic solution was separated, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with 0–2% methanol–chloroform gradient to give 106 mg (19%) of an oil as a mixture of cis and trans olefins.

A solution of the above olefins (106 mg, 0.34 mmol) in a mixture of methanol (8 mL) and THF (8 mL) containing 5% palladium on charcoal (92 mg) was hydrogenated (1 atm) at room temperature for 20 h. The resultant mixture was filtered and concentrated in vacuo. The residue was subjected to column chromatography on silica gel eluting with 0–1.25% methanol–chloroform gradient, followed by recrystallization from diethyl ether–hexane to yield 38 mg (36%) of 5 (mp 130–1 °C): ^{1}H NMR ($CDCl_3$) δ 7.09 (s, 1 H), 6.96 (s, 1 H), 3.91 (s, 3 H), 2.72–2.87 (m, 4 H), 2.48 (AB q, 2 H, J = 7.6 Hz), 2.39 (s, 3 H), 2.33 (AB q, 2 H, J = 7.6 Hz), 2.30 (s, 3 H), 1.08 (t, 3 H, J = 7.5 Hz), and 1.03 (t, 3 H, J = 7.6 Hz). Anal. ($C_{19}H_{26}N_2O_2$) C, H, N.

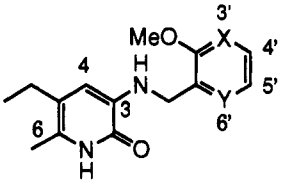
A mixture of 5 (18.5 mg, 59 μmol) and pyridine hydrochloride (168 mg, 1 mmol) was heated at 150 °C for 10 min. The resultant mixture was cooled to room temperature, quenched with water, and filtered. The solid obtained was washed successively with water, methanol, and diethyl ether and dried to give 10 mg (56%) of 3: ^{1}H NMR (Me_2SO-d_6) δ 7.00 (s, 2 H), 2.55 (s, 4 H), 2.26 (AB q, 4 H, J = 7.5 Hz), 2.10 (s, 6 H), 0.98 (AB q, 6 H, J = 7.6 Hz); HRMS calcd for $C_{18}H_{24}N_2O_2$ (M^+) 301.1916, found 301.1923.

3-[N-[(1,2-Dihydro-5-ethyl-6-methyl-2-oxopyridin-3-yl)-methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (4). Following the general procedure, 2-(benzyloxy)-5-ethyl-6-methylnicotinaldehyde[11] (0.31 g, 1.21 mmol) and 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one (9, 0.184 g, 1.21 mmol) gave, after chromatography with 3% methanol in chloroform, 0.43 g (91%) of 3-[*N*-[5-ethyl-2-(benzyloxy)-6-methyl-3-pyridylmethyl]-amino]-5-ethyl-6-methylpyridin-2(1*H*)-one: mp 167–9 °C (methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 7.49 (d, 2 H, J = 7 Hz), 7.4–7.2 (m, 4 H), 6.14 (s, 1 H), 5.43 (s, 2 H), 5.29 (s, 2 H), 2.48 (q, 2 H, J = 7.5 Hz), 2.42 (s, 3 H), 2.27 (q, 2 H, J = 7.5 Hz), 2.19 (s, 3 H), 1.11 (t, 3 H, J = 7.5 Hz), and 1.00 (t, 3 H, J = 7.5 Hz).

A solution of 3-[*N*-[[2-(benzyloxy)-5-ethyl-6-methyl-3-pyridyl]-methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (0.40 g, 1.02 mmol) in a 1:1 mixture of ethanol and ethyl acetate (150 mL) was hydrogenated over 5% palladium on charcoal (0.30 g) at 30 psi at room temperature for 5 h. The resultant mixture was filtered and concentrated in vacuo. The residue was subjected to chromatography over silica gel eluting with 15% methanol in chloroform to give 90 mg (29%) of 4: mp 258–62 °C (methanol–ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 7.16 (s, 1 H), 6.06 (s, 1 H), 5.42 (t, 1 H, J = 6.3 Hz), 3.94 (d, 1 H, J = 6.3 Hz), 2.30–2.15 (m, 4 H), 2.11 (s, 3 H), 2.00 (s, 3 H), 0.96 (t, 6 H, J = 7.5 Hz). Anal. ($C_{17}H_{23}N_3O_2$) C, H, N.

3-[*N*-[(5-Ethyl-2-methoxy-6-methyl-3-pyridyl)methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (6). Following the

Table III. Inhibition of HIV-1 RT by 2-Pyridinones: Alkyl-Substituted 2′-Methoxy Pyridyl and Phenyl Derivatives



no.	X	Y	substituents	recryst solvent	mp, °C	formula	IC_{50} (nM)
27	CH	CH		EtOH	167–169	$C_{16}H_{20}N_2O_2$	265
46	CH	CH	3′-Me	EtOH	161–163	$C_{17}H_{22}N_2O_2$	3800
47	CH	CH	4′-Me	MeOH	168–169	$C_{17}H_{22}N_2O_2$	32
48	CH	CH	5′-Me	EtOH	167–169	$C_{17}H_{22}N_2O_2$	23
49	CH	CH	6′-Me	MeOH	233–236	$C_{17}H_{22}N_2O_2$	1300
50	CH	CH	4′-Et	MeOH	147–149	$C_{16}H_{24}N_2O_2$	510
51	CH	CH	5′-Et	EtOH	152–154	$C_{16}H_{24}N_2O_2$	32
13	CH	CH	4′,5′-Me_2	MeOH	175–177	$C_{16}H_{24}N_2O_2$	8
52	CH	CH	4′-Me, 5′-Et	EtOH	183–185	$C_{19}H_{26}N_2O_2$	33
53	CH	CH	4′,5′-$(CH_2)_3$	EtOH	188–191	$C_{19}H_{24}N_2O_2$	40
54	N	CH	4′,5′-Me_2	MeOH/EtOAc	177–179	$C_{17}H_{23}N_3O_2$	9
6	N	CH	4′-Me, 5′-Et	MeOH/hexane	165–167	$C_{16}H_{25}N_3O_2$	19
55	N	CH	4′,5′-$(CH_2)_4$	EtOH/$CHCl_3$	194–196	$C_{19}H_{25}N_3O_2$	35
56	N	CH	4′,5′-$(CH)_4$	EtOH/$CHCl_3$	243–244	$C_{19}H_{21}N_3O_2$	34
23	CH	N	4′,5′-Me_2	EtOH/$CHCl_3$	234–236	$C_{17}H_{23}N_3O_2$	68
57	CH	N	4′-Me, 5′-Et	MeOH/$CHCl_3$	215–217	$C_{16}H_{25}N_3O_2$	70
58	CH	N	4′,5′-$(CH_2)_3$	MeOH/hexane	229–231	$C_{16}H_{23}N_3O_2$	40
59	CH	N	4′,5′-$(CH_2)_4$	MeOH/hexane	250–253	$C_{19}H_{25}N_3O_2$	47

Table IV. Antiviral Properties of Selected Compounds in MT-4 Cell Culture[15]

compound	IC_{50} (nM)	CIC_{95} (nM, MT-4 Cells)	compound	IC_{50} (nM)	CIC_{95} (nM, MT-4 Cells)
6	19	13	TIBO R82150	70	200
13	8	13	Nevirapine	73	100
54	9	50	E-EPU	32	100
23	70	100	U-87201	1100	1500
27	265	400	1 (L-697,661)	19	50
25	680	800			

general procedure, 5-ethyl-2-methoxy-6-methylnicotinaldehyde (14,[11] 15.0 g, 83.2 mmol) and 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one (9[11] 12.66 g, 83.2 mmol) gave, after chromatography with 5% methanol in chloroform, 10.5 g (40%) of 6: mp 165–7 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 7.28 (s, 1 H), 6.17 (s, 1 H), 4.23 (s, 2 H), 3.96 (s, 3 H), 2.51 (q, 2 H, J = 7.6 Hz), 2.42 (s, 3 H), 2.31 (q, 2 H, J = 7.6 Hz), 2.22 (s, 3 H), 1.11 (t, 3 H, J = 7.6 Hz), and 1.04 (t, 3 H, J = 7.6 Hz). Anal. ($C_{18}H_{25}N_3O_2$) C, H, N.

3-[*N*-(4,5-Dimethyl-2-methoxybenzyl)amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (13). To a solution of 3,4-dimethylanisole (9.74 g, 0.071 mol) in dry ether (150 mL) at 0 °C was added a solution of *n*-butyllithium in hexane (2.5 M, 28.6 mL). The resultant mixture was stirred at 0 °C for 1.5 h and then at room temperature for 16 h. The reaction was cooled to 0 °C and dimethylformamide (11 mL, 0.14 mol) was added. The reaction mixture was stirred at 0 °C for 1.5 h and treated with 10% aqueous hydrochloric acid. The ethereal solution was separated, washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel eluting with 5% ethyl acetate in hexane to give two products, R_f = 0.37 and R_f = 0.25 (same solvent system on silica gel TLC plate). The R_f = 0.37 material was 2,3-dimethyl-6-methoxybenzaldehyde (1.4 g, 11%): ^{1}H NMR ($CDCl_3$) δ 9.53 (s, 1 H), 7.29 (d, 1 H, J = 8 Hz), 6.74 (d, 1 H, J = 8 Hz), 3.88 (s, 3 H), 2.48 (s, 3 H), 2.24 (s, 3 H). The R_f = 0.25 material was 4,5-dimethyl-2-methoxybenzaldehyde (3.6 g, 30%): ^{1}H NMR ($CDCl_3$) δ 10.38 (s, 1 H), 7.57 (s, 1 H), 6.76 (s, 1 H), 3.89 (s, 3 H), 2.32 (s, 3 H), 2.22 (s, 3 H).

Following the general procedure, 4,5-dimethyl-2-methoxybenzaldehyde (240 mg, 1.45 mmol) and 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one (9, 220 mg, 1.45 mmol) gave 273 mg (63%) of 13: mp 176–7 °C (methanol); ^{1}H NMR (Me_2SO-d_6) δ 6.97 (s, 1 H), 6.78 (s, 1 H), 6.08 (s, 1 H), 5.3 (br, s, 1 H), 4.10 (s, 2 H), 3.77 (s, 3 H), 2.22 (q, 2 H, J = 7.6 Hz), 2.18 (s, 3 H), 2.09 (s, 3 H), 2.01 (s, 3 H), 0.97 (t, 3 H, J = 7.6 Hz); HRMS calcd for $C_{18}H_{24}N_2O_2$ (M^+) 301.1916, found 301.1913.

2-Chloro-5,6-dimethyl-3-nitropyridine (17). A solution of 3-nitro-5,6-dimethylpyridin-2(1*H*)-one (16,[11] 16.8 g, 0.1 mol) in phosphorus oxychloride (100 mL) was heated at 110 °C for 6 h. Excess $POCl_3$ was distilled off under reduced pressure. The residue was poured into vigorously stirred ice–water. The off-white solid that precipitated was collected by filtration and dissolved in methylene chloride. The resultant solution was washed with saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and passed through a column of silica gel, eluting with methylene chloride. Removal of solvent gave 17.2 g (92%) of 2-chloro-5,6-dimethyl-3-nitropyridine as a white solid. Recrystallization from hexane gave white needles (mp 72–3 °C): ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 2.58 (s, 3 H), 2.38 (s, 3 H). Anal. ($C_7H_7ClN_2O_2$) C, H, N.

3-Amino-5,6-dimethylpyridine (18). A mixture of 2-chloro-5,6-dimethyl-3-nitropyridine (17, 5 g, 26.8 mmol), triethylamine (10 mL), and 5% palladium on charcoal (0.4 g) in methanol (200 mL) was shaken under an atmosphere of hydrogen (45–40 psi) for 16 h. The resultant mixture was filtered through a plug of Celite and concentrated under reduced pressure. The residue was then treated with chloroform saturated with ammonia gas. The resultant slurry was filtered and concentrated to yield 3.2 g (100%) of 3-amino-5,6-dimethylpyridine. Recrystallization from chloroform–hexane gave a white solid (mp 75–6 °C): ^{1}H NMR ($CDCl_3$) δ 7.85 (d, 1 H, J = 2.5 Hz), 6.79 (d, 1 H, J = 2.5 Hz), 3.62 (br s, 2 H), 2.38 (s, 3 H), 2.20 (s, 3 H). Anal. ($C_7H_{10}N_2$) C, H, N.

5,6-Dimethyl-3-hydroxypyridine (19). To a cold (0 °C) solution of 3-amino-5,6-dimethylpyridine (18, 4 g, 32.8 mmol) in 5% aqueous sulfuric acid (100 mL) was added dropwise a solution of sodium nitrite (2.5 g, 36 mmol) in water (20 mL). The resultant solution was stirred at 0 °C for 30 min, transferred into an additional funnel maintained at 0 °C with external cooling, and added dropwise into boiling 5% aqueous sulfuric acid (70 mL) over a period of 30 min. The resultant solution was refluxed for an additional 15 min, cooled to 0 °C, neutralized with 40% aqueous sodium hydroxide, and saturated by the addition of solid sodium chloride. The product was extracted into methylene chloride. The organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to yield 3.5 g (86%) of crude 3-hydroxy-5,6-dimethylpyridine. Recrystallization from hexane–chloroform gave a white solid (mp 147–9 °C): ^{1}H NMR ($CDCl_3$) δ 9.71 (br s, 1 H), 7.95 (d, 1 H, J = 2.5 Hz), 7.12 (d, 1 H, J = 2.5 Hz), 2.42 (s, 3 H), 2.25 (s, 3 H). Anal. (C_7H_9NO) C, H, N.

5,6-Dimethyl-2-(hydroxymethyl)-3-hydroxypyridine (20). To a solution of 3-hydroxy-5,6-dimethylpyridine (19, 3.5 g, 28.4

mmol) and sodium hydroxide (1.2 g, 28.4 mmol) in water (12 mL) at 90 °C was added 38% aqueous formaldehyde in four 2.5-mL aliquots in 90-min intervals.[14] The resultant solution was heated at 90 °C for an additional 90 min, neutralized with acetic acid, and concentrated under reduced pressure. The residue was triturated with 10% methanol in chloroform saturated with ammonia, and the resultant mixture was filtered through a plug of Celite. The filtrate was concentrated and the residue subjected to column chromatography on silica gel eluting with 10% methanol in chloroform. Collection and concentration of appropriate fractions gave 2.1 g (48%) of 2-(hydroxymethyl)-3-hydroxy-5,6-dimethylpyridine. Recrystallization from hexane gave a white solid (mp 174–6 °C): ^{1}H NMR (Me_2SO-d_6) δ 9.41 (s, 1 H), 6.91 (s, 1 H), 4.81 (t, 1 H, J = 5.5 Hz), 4.45 (d, 2 H, J = 5.5 Hz), 2.28 (s, 3 H), 2.14 (s, 3 H). Anal. ($C_8H_{11}NO_2$) C, H, N.

2-(Hydroxymethyl)-5,6-dimethyl-3-methoxypyridine (21). To a solution of 2-(hydroxymethyl)-3-hydroxy-5,6-dimethylpyridine (**20**, 1.16 g, 7.6 mmol) and sodium hydride (0.18 g, 7.6 mmol) in dimethylformamide (20 mL) at 0 °C was added methyl iodide (1.08 g, 7.6 mmol). The mixture was stirred at 0 °C for 30 min and at room temperature for 1 h. The resultant solution was concentrated under reduced pressure, and the residue was subjected to column chromatography on silica gel eluting with 2% methanol in chloroform. Collection and concentration of appropriate fractions gave 0.7 g (55%) of 2-(hydroxymethyl)-3-methoxy-5,6-dimethylpyridine. Recrystallization from hexane gave a white solid (mp 73–5 °C): ^{1}H NMR ($CDCl_3$) δ 6.91 (s, 1 H), 4.66 (s, 1 H), 4.46 (br s, 1 H), 3.80 (s, 3 H), 2.43 (s, 3 H), 2.28 (s, 3 H). Anal. ($C_9H_{13}NO_2$) C, H, N.

5,6-Dimethyl-3-methoxy-2-picolinaldehyde (22). 2-(Hydroxymethyl)-3-methoxy-5,6-dimethylpyridine (**21**, 0.7 g, 4.2 mmol) and activated manganese(IV) oxide (8 g) in methylene chloride (40 mL) was stirred at room temperature overnight. The resultant mixture was filtered through a plug of Celite and the filtrate concentrated to give 0.4 g (58%) of 5,6-dimethyl-3-methoxy-2-picolinaldehyde. Recrystallization from methanol gave a white solid (mp 70–2 °C): ^{1}H NMR ($CDCl_3$) δ 10.28 (s, 1 H), 7.17 (s, 1 H), 3.94 (s, 3 H), 2.52 (s, 3 H), 2.38 (s, 3 H). Anal. ($C_9H_{11}NO_2$) C, H, N.

3-[*N*-[(5,6-Dimethyl-3-methoxypyridyl)methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one (23). According to the general procedure, 5,6-dimethyl-3-methoxy-2-picolinaldehyde (**22**, 200 mg, 1.21 mmol) and 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one (**9**, 184 mg, 1.21 mmol) gave, after chromatography with 8% methanol in chloroform, 200 mg (56%) of **23**: mp 234–6 °C (chloroform–ethanol); ^{1}H NMR (Me_2SO-d_6) δ 7.25 (s, 1 H), 6.22 (s, 1 H), 5.83 (t, 1 H, J = 5.2 Hz), 4.13 (d, 2 H, J = 5.2 Hz), 3.83 (s, 3 H), 2.38 (s, 3 H), 2.30 (q, 2 H, J = 7.5 Hz), 2.26 (s, 3 H), 2.05 (s, 3 H), 1.05 (t, 3 H, J = 7.5 Hz). Anal. ($C_{17}H_{23}N_3O_2$) C, H, N.

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(15) Activities of TIBO analog R82150,[1] Nevirapine (BI-RG-587),[2] E-EPU,[3] U-87201,[4] and L-697,661 (1)[6] in our cell culture assay are also included for reference (Table IV).