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Novel nevirapine-like inhibitors with improved activity against NNRTI-resistant HIV: 8-heteroarylthiomethyldipyridodiazepinone derivatives

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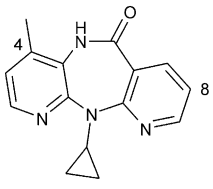
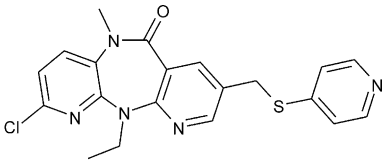
Abstract—A series of 8-heteroarylthiomethyldipyridodiazepinone derivatives were prepared and evaluated for their antiviral profile against wild type virus and the important K103N/Y181C mutant as an indicator for broad activity. 2,6-Dimethylpyridine derivative **16** was found to have a good pharmacokinetic profile in spite of poor metabolic stability in rat liver microsomes.

Reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) is a key target for the inhibition of viral replication.[1] RT inhibitors are divided into two groups: nucleoside reverse transcriptase inhibitors (NRTIs), as exemplified by zidovudine (AZT, Retrovir®), and non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine (Viramune®). Although chemically diverse, all NNRTIs exert their antiviral effect through a non-competitive mechanism by interacting with an allosteric site adjacent to the active site of the enzyme.[2,3]

Nevirapine was the first NNRTI to receive regulatory approval for the treatment of HIV-1 infection. However, antiretroviral therapy with nevirapine, like all NNRTI-containing regimens, results in the development of resistance due to mutation of the RT enzyme.[4] A challenge for the next generation of NNRTIs is the design of compounds with higher antiviral potency and a broader spectrum of activity against clinically prevalent NNRTI-resistant variants, combined with favorable pharmacokinetic properties. It was previously reported that the addition of a substituent at C-8 of nevirapine provided inhibitors with potency against a large number of clinically relevant mutants.[5,6] We have used inhibitor **1**[6] as a starting point for further optimization of these substituted analogues for potency and biopharmaceutical properties.



Nevirapine



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Early work on a series of compounds such as **1** has demonstrated that *N*-demethylation of the tertiary amide was rapid when incubated with rat liver microsomes. Thus, we have decided to initiate our work with the C-4 methyl derivatives. The synthetic procedure which allows rapid access to inhibitors is exemplified in Scheme 1. Key intermediate **2** was prepared using an analogous procedure reported previously.[6] Palladium-mediated coupling using vinyltributyltin gave intermediate **3**, which was subjected to ozonolysis followed by a reductive workup to produce the primary alcohol (**4**). Alcohol **4** was converted to the benzylic bromide **5**, which gave inhibitor **6** upon treatment with 2,6-dimethyl-4-mercaptopyridine under basic conditions.

Most thiols used in this study were commercially available or accessed using published procedures. Substituted

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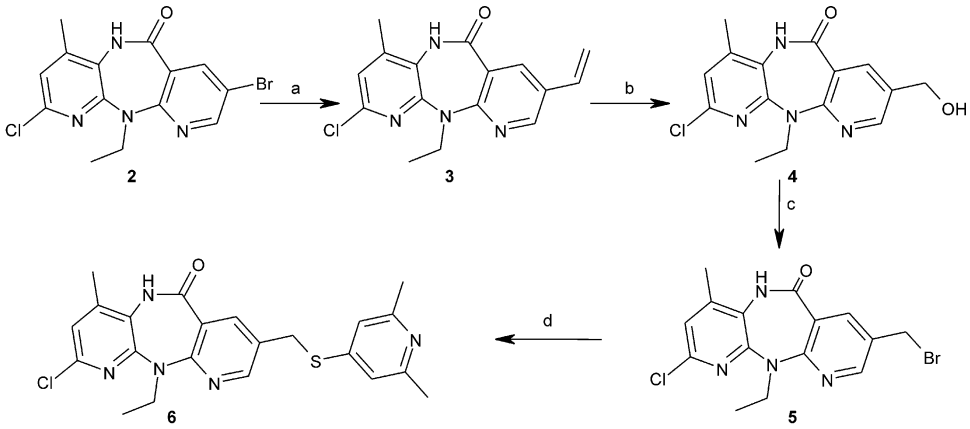
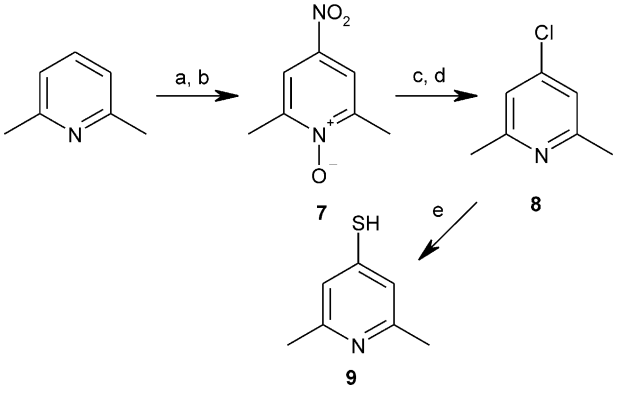


Scheme 1. (a) $CH_2CHSnBu_3$, $(PhP)_4Pd$, DMF 80 °C (80%); (b) O_3, CH_3Cl/MeOH followed by $NaBH_4$ (81%); (c) Ph_3P, CBr_4 (80%); (d) 2,6-dimethyl-4-mercaptopyridine, $CsCO_3$, MeCN/THF/H_2O (59%).



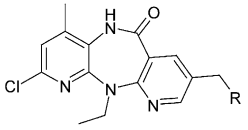
Scheme 2. (a) mCPBA, CH_2Cl_2, 85%; (b) H_2SO_4, HNO_3, 80 °C, 59%; (c) HCl (12 N), 200 °C, pressure flask, 86%; (d) PCl_3, $CHCl_3$, quant; (e) NaSH, DMF, 140 °C, 52%.

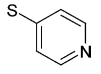
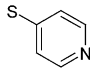
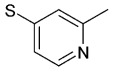
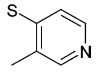
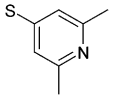
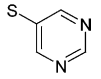
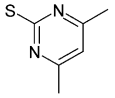
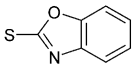
pyridines such as 2,6-dimethyl-4-mercaptopyridine were obtained as outlined in Scheme 2. Intermediate **8** was prepared, as previously reported,[7] in multi-gram scale starting from 2,6-dimethylpyridine. Chlorine displacement with sodium hydrogen sulfide[8] provided the desired thiol **9**.

For the early SAR studies, we used the enzymatic activity (IC_{50})[9] against wild-type RT (WT RT) and the key K103N/Y181C mutant as an indicator of broad activity. The choice of this critical double mutant stems from early work which indicated that this was a challenging mutant for such nevirapine-based inhibitors. In addition, we have evaluated the antiviral activity (EC_{50})[10] for wild type virus.

As shown in Table 1, moving the methyl group from N-5 (**1**) to C-4 (**10**) resulted in similar activity against the WT enzyme. However, a 3-fold loss in potency was observed against the K103N/Y181C mutant. Selected examples of a large number of heterocyclic thioethers investigated are shown in Table 1. Pyrimidine (**13**–**14**) and benzoxazole derivatives (**15**) were found to be less potent than the pyridine analogues. Interestingly, addition of a methyl group at the 2 (**11**) or 3 (**12**) position of the pyridine ring led to compounds significantly less potent then the parent compound **10**. In contrast, the 2,6-dimethyl analogue **6** showed a good level of potency. Indeed only a 3-fold loss in intrinsic activity was observed against WT RT which was reflected in the antiviral activity (EC_{50} = 1.2 nM). However, the enzymatic activity against the double mutant RT was only marginally improved (60 nM versus 80 nM).

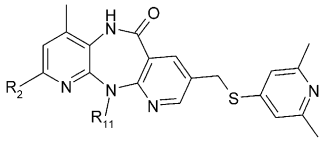
Table 1. SAR at C-8: heteroaryl derivatives



Compd	R	IC_{50} (nM)[9]		EC_{50} (nM)[10]
		WT	K103N/Y181C	WT
1[a]		3.0	26	0.31
10		2.2	80	0.48
11		27	310	3.4
12		11	1640	3.1
6		7.0	60	1.2
13		26	5000	7.8
14		6.0	320	1.5
15		8.0	230	2.7

[a] N-5 Methyl derivative.

Table 2. SAR at position 2 and 11



Compd	R_2	R_{11}	IC_{50} (nM)[9]		EC_{50} (nM)[10]	
			WT	K103N/Y181C	WT	K103N/Y181C
6	Cl	Et	7.0	60	1.2	14
16	F	Et	6.9	120	1.0	21
17	H	Et	4.2	170	0.46	12
18	Cl	*c*-Propyl	12	280	2.2	104

Having on hand this interesting 2,6-dimethyl-4-mercaptopyridine derivative, we decided to further optimize positions 2 and 11 based on previous knowledge.[5,6] As shown in Table 2, 2-chloro (**6**), 2-fluoro (**16**) and 2-hydrogen (**17**) derivatives exhibited similar potencies against WT and K103N/Y181C RT. Compound **17** had the best antiviral activity against the WT virus ($EC_{50}=0.46$ nM). Although the chloro derivative **6** was the most potent in the enzymatic assay against K103N/Y181C mutant, this improved activity was not seen in the antiviral assay. Modifications at the N-11 position indicated that only small alkyl groups such as ethyl or cyclopropyl were tolerated. The cyclopropyl derivative (**18**) proved to be significantly less potent against the critical double mutant.

Antiretroviral treatment includes a triple combination therapy, thus drug–drug interaction is an important concern. One of the mechanisms underlying drug–drug interaction is cytochrome P450 inhibition. As highlighted in Table 3, all three inhibitors had IC_{50} values significantly greater than our minimum requirement of 1 μM. Compounds were also evaluated for first pass metabolism[11] using male human and rat liver microsomes.[12] All three compounds were found to be rapidly metabolized resulting in short half-life (see Table 3).

Due to the excellent intrinsic and antiviral activity against the K103N/Y181C mutant virus and, in spite of the disappointing metabolic stability results, compounds were selected for in vivo pharmacokinetic profiling in rats. Compound **6** was found to have the best profile. After oral gavage of **6** at 5 mg/kg we observed an AUC_{po} of 2 μM h and a C_{max} of 0.8 μM which corresponds to approximately ~700-fold higher then the EC_{50} against wild type HIV. Following intravenous administration (1 mg/kg), inhibitor **6** exhibited high clearance (57.0±0.9 mL/min/kg), a volume of distribution at steady state (V_{SS}) of 4.68±0.16 L/kg, and a short half life ($T_{1/2}=1.5$ h),[13] consistent with the observed metabolic rate. The apparent bioavailability was found to be 76%.

Table 3. Cyp450 inhibition and metabolic stability

	Cyp450 Inhibition IC_{50} (μM)						Metabolic rate $T_{1/2}$(min)	
Compd	1A2	2C9	2C19	2D6	3A4-BFC[a]	3A4-BQ[a]	Rat	Human
6	23	2.6	4.2	27	2.3	8.1	7	4
16	26	4.5	5.4	27	2.5	10	5	5
17	>30	7.4	26	>30	1.6	10	9	9

[a] 7-Benzyloxy-4-(trifloromethyl)-coumarin and 7-benzyloxyquinoline (BQ) are fluorogenic substrates for cyp3A4.

In summary, nevirapine-like inhibitors with excellent activity against wild type and the key K103N/Y181C mutant virus were identified. In particular, the 2,6-dimethylpyridine derivative (**6**) has shown a good pharmacokinetic profile in spite of poor metabolic stability in rat liver microsomes. Unfortunately, the metabolic instability of this series in a human liver microsome preparation precludes further development. Although not all metabolites were characterized, the two major metabolites were identified as the corresponding sulfoxide and sulfone. Unfortunately, upon synthesis and evaluation, these metabolites were found to be much less potent then the parent compounds. Based on these findings, the design of new derivatives having similar potency but with improved metabolic stability is in progress and the results will be reported shortly.

Acknowledgements

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References and notes

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9. Enzymatic assays: IC_{50} values for wild-type and mutant RTs were obtained from a scintillation proximity assay using poly rC/biotin-dG_{15} and ^{3}H-dGTP at 37 °C. Each value represents the mean of at least three determinations The reproducibility of the assay was gauged using an internal standard.
10. Replication assays: For EC_{50} determinations, C8166 cells

were acutely infected (MOI 0. 001) and incubated with compounds for 3 days at 37 °C. Viral replication was assessed by p24 ELISA of the culture supernatants.
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12. Liver microsome stability assay: $T_{1/2}$ values for phase I oxidative metabolic stability weas determined using male human or rat liver microsomes at a starting concentration of 2 μM for the compounds.
13. Half life ($t_{1/2}$)is calculated using the equation $t_{1/2} = 0.693$ (V_d/clearance) where V_d is the volume of distribution at pseudo terminal phase ($V_d = 7.3 \pm 0.1$).