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Pyridoxine hydroxamic acids as novel HIV-integrase inhibitors

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ABSTRACT

A series of pyridoxine hydroxamic acid analog bearing a 5-*aryl*-spacers were synthesized. Evaluation of these novel HIV integrase complex inhibitors revealed compounds with high potency against wild-type HIV virus.

Human immunodeficiency virus (HIV) integrase inhibitors are a new component to the anti-HIV chemotherapy pharmacy, the current treatment for acquired immunodeficiency syndrome (AIDS).[1] This class of compounds inhibits the integration of the viral genome into the host's and thus the infectious process. Although integrase inhibitors have been well received, new problems have recently been identified.[2,3] The rapid emergence of several viral strains resistant to one or more of the drugs currently available or in trials for the treatment of AIDS has now become the most important issue in the treatment of HIV infection.[4] We recently discovered HIV integrase inhibiting compounds of general structure as shown in Figure 1. We had previously found that pyridoxine or pyridoxal phosphates could serve as an efficient backbone scaffold for the synthesis of such inhibitors albeit by an unknown mechanism of action.[5]

The inhibitors discussed in the present letter were designed to bind the active site of the HIV integrase complex, known to bear a two-divalent metal ion catalytic motif. Furthermore, in order to account for possible mutational changes in the active site, two different binding modes for the molecules were devised; in the first mode, the metal chelating ligand would involve the C-3 phenolic ligand while in the second mode would engage the pyridyl moiety directly.

One of the concerns about using a heterocyclic hydroxamic acid was the association of this functional group with toxicity. Studies in the ability of the hydroxamic acid ability to induce host DNA mutations have show this to proceed through formation of isocyanates resulting from Lossen rearrangement reaction.[6] This isocyanate is presumed to acylate genetic material causing the mutations. We surmised that using a ortho hydroxyl group would scavenge this putative isocyanate to form an azabenzoxazolone.

Our first approach to explore new designs was to build a structure activity relation on spacers and aryl groups at the 5 position of the pyridoxin binding ligand.[7,8] Scheme 1 shows the different synthetic routes taken to create the diversity of this class of molecules from a pivotal core intermediate. The core intermediate I was synthesized according to literature precedent[9] in good yields, giving us a benzylic alcohol as a point of entry. The coupling to phenols using Mitsunobu conditions to yield a variety of aryl ethers II. Oxidation of the benzylic alcohol to the corresponding aldehyde III was performed using MnO_2 in $CHCl_3$ with high yield of crystalline compound. This aldehyde could then be further coupled to activated carbanions to yield alkenes IV and alkanes V, or amines to give anilides and alkylamines VI. Mild oxidation of the aldehyde to yield the carboxylic acid VII was effected using phosphate buffered

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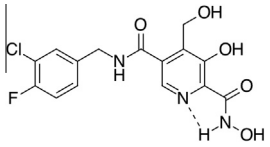
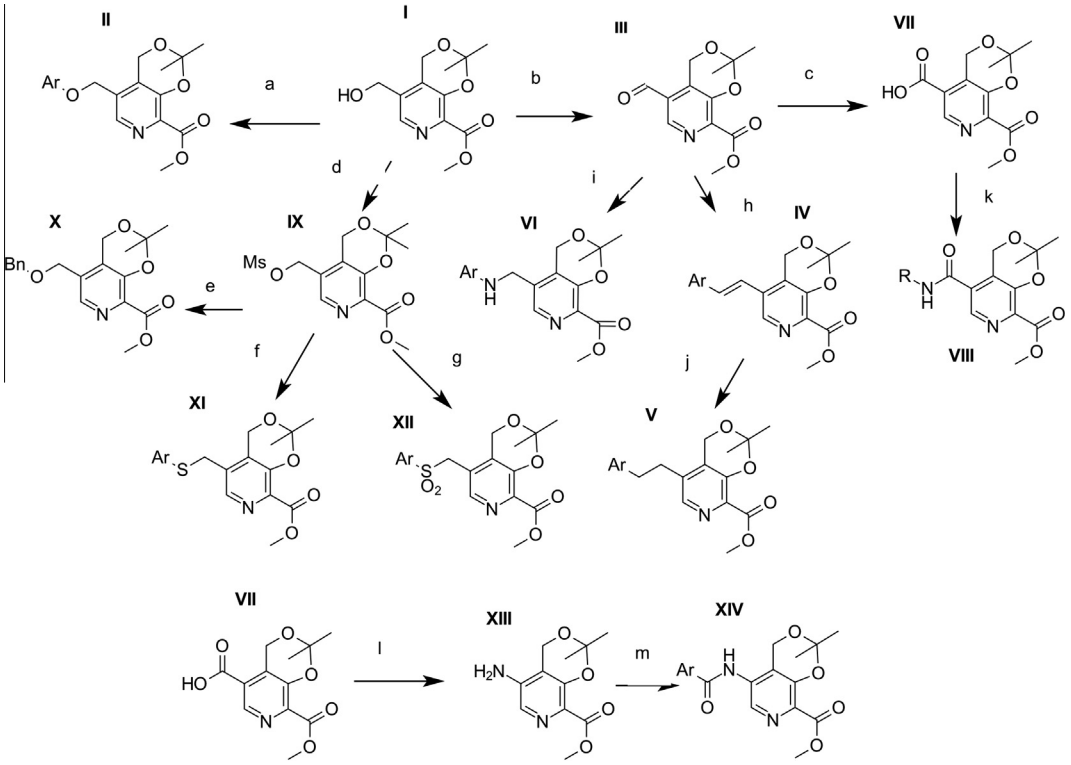


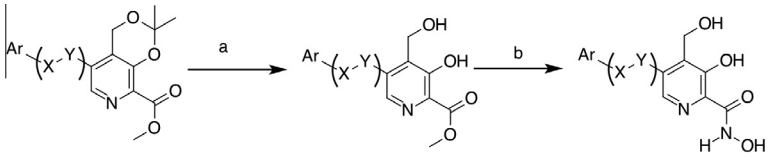
Figure 1. General structure of inhibitors.

(pH 9) NaOCl in phase-transfer conditions. This could then be coupled to a variety of amines to yield amides VIII. The benzylic alcohol was converted to a leaving group using methanesulfonyl chloride IX and reacted with alkanoates, sulfides, sulfinic acid salts to give compounds X, XI and XII. The acid VII was reacted in a Curtius reaction to give the 5-amino compound XIII which provided a point of entry for reversed amides XIV. Scheme 2 shows the method used to convert the intermediates discussed above to the phenolic hydroxamates. First deprotection was effected using 70% formic acid neat, followed by reaction of aqueous hydroxylamine in pyridine. The resulting compounds were isolated in purified form by either recrystallizing from acetonitrile water mixtures or by chromatography. All compounds were tested in the IN strand transfer assay as well as a cell-based antiviral assay using the MT-4 cell line. The data are summarized in Table 1. Our SAR study clearly shows that the spacer geometry between the pyridoxine hydroxymate binding moiety and a fluorobenzene are critical determinants for the activity of these inhibitors. Table 1 compares HIV integrase inhibition results found through varying the spacer geometry between the pyridoxin hydroxamate binding moiety and a fluorobenzene. The table lists results on the purified enzyme, and cell based assay. Enzyme inhibition strand-transfer assays (IC_{50}) were performed using a well known methodology using 3′ processed oligonucleotides.[12,13] Anti-viral activity (EC_{50}) was measured initially by a single cycle infection assay in MT-4 cells followed by a 7 day multi-cycle assay in parallel with the Cytotoxicity assay (CC_{50}).[14]

A good correlation is found between these for each compound. The comparison of the saturated dimethylene spacer and the styryl analog shows that a rigid planar spacer does not place the compound in a favorable position. Extension of the fluorophenyl by one or more atoms also seems to be unfavorable for binding. Two or three atom spacers containing sulfur gave

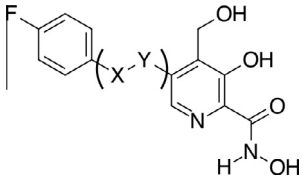


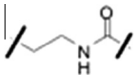
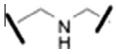
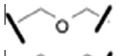
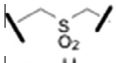
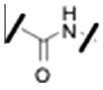
Scheme 1. Reagents and conditions: (a) ArOH, Ph_3P, DEAD; (b) MnO_2 $CHCl_3$ 99%; (c) $NaOCl_{aq}$ tetrabutylammonium iodide, DCM; (d) MS-Cl, DCM TEA rt 95%; (e) $ArCH_2OH$, NaH 85–95%; (f) ArSH, base 75%; (g) $ArSO_2$–Na, DMF; (h) Ar–CH_2–$P(Ph)_3$, THF reflux; (i) Ar–NH_2, $NaCNBH_4$, MeOH 60–80%; (j) $H_2/Pd_{quant.}$; (k) CDI, R–NH_2; (l) $(PhO)_3PN_3$, toluene reflux; (m) ArCO–X, base.



Scheme 2. Reagents and conditions: (a) HCOOH; (b) NH_2OH, Py.

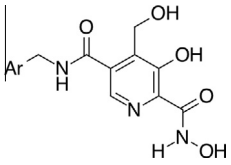
Table 1
HIV integrase inhibition constants for compounds **1**–**14**

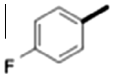
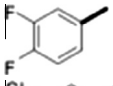
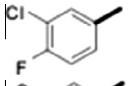
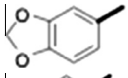
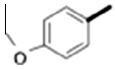
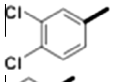
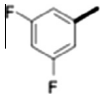


Compd		IC_{50}[a] (nM)	EC_{50}[b] (nM)	CC_{50} (μM)
1		327	76	21
2		>100,000	>10,000	50
3		25	20	50
4		41	7	27
5		10,000	9300	50
6		2300	2000	23
7		328	40	51
8		250		
9		550	500	2
10		550	695	20
11		80	15	11
12		2300	4350	16
13		300	6300	50

[a] Mean of at least three experiments.
[b] Anti-viral activity using a single-cycle infection where the cells were infected with a luciferase-bearing envelope defective (env−) NL-4.3 virus pseudotyped with HIV-1 env (HXBc2).

Table 2
Effect of Aryl substituents



Compd	Ar	EC_{50}[a,b] (nM)	CC_{50} (μM)
14		100	54
7		40	50
15		100	51
16		25	25
17		1860	50
18		1500	25
19		4500	32
20		40	50
21		11,000	50
22		400	31

Mean of at least three experiments.
[a] Luciferase-bearing envelope defective (env−) NL-4.3 virus pseudotyped with HIV-1 env (HXBc2).
[b] Anti-viral activity using a single-cycle infection where the cells were infected with HIV-1 env (HXBc2).

weak binding inhibitors. Indeed the best spacers appear to be the aminomethylene, amides, oxymethylene and the benzylic ethers.

Table 2 compares the effect of varying the aryl group on a series of 5-amides. The effect of electron withdrawing groups on the phenyl ring is notable. Methoxy and methylene dioxy compounds showed a major loss of activity in comparison to the phenyl ring, whereas the halogenated rings displayed the most activity. A significant loss of activity was also found when replacing the phenyl by a cyclohexane ring.

All the compounds were tested in several assays however we chose to show the results on the most promising candidate, compound **16**. Table 3 shows the favorable p*K* profile and selectivity of this compound in animal models and in vitro tests. Compound **16** was selected due to its excellent therapeutic index (CC_{50}/EC_{50}) of >1000. The HIV integrase strand-transfer inhibition of this compound was measured bypassing 3′ processing activity as 3′ processing inhibitors are known to remain tightly complexed and do not favor a high catalytic turnover making inhibitors of this mechanism less desirable as therapeutics. This compound showed specificity when compared to assays involving HCV polymerase and HIV Reverse Transcriptase. Two distinct assays were used to determine the mechanism of action of these compounds. The first was accumulation of 2-LTR circles observed when inhibitors were added during infection and is considered as a hallmark of these inhibitors. Non-integrated genomes form episomic 2-LTR circles.[10,11] The second method used active site mutation N155H which displayed a 10-fold drop in inhibition. We also tested these compounds in protein binding assays and found a relatively low binding in general. For example we found a protein binding value of 89% for compound **16**. Animal tests showed this family of compounds to have favorable pharmacokinetics in rats. The bioavailability for the example compound **16** was 25% over 24 h with a $t_{1/2}$ of 0.73 h. The compound plasma concentration after a 50 mg/kg dosage remained above the EC_{50} for 18 h.

In conclusion, we report the design, synthesis and SAR of a new series of pyridoxine hydroxamic acids as potent anti-HIV agents. The SAR illustrated that anti-viral potency of these IN inhibitors depended on the aryl substitution and aryl-spacer at the 5-position of core pyridoxine heterocycle. Amongst them, compound **18** showed favorable pharmacological data to warrant further development. Further studies are ongoing to assess the inhibitory activity against resistant HIV strains.

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Table 3
Potency and pharmacokinetics of **16**

IC_{50}	29 nM	Reverse transcriptase	>50 μM	Mutagenicity	Negative Ames test
EC_{50} (single cycle assay	25 nM	Log*P*	0.6	Accumulation of 2-LTR at EC_{50}	7 fold
EC_{50} (multicycle assay)	128 nM	Protein binding	89%	Accumulation of 2-LTR at EC_{90}	10 fold
CC_{50}	22 μM	*F*% (24 h)	25%	N155H mutant EC_{50}	>300 nM
HCV EC_{50}	>50 μM	$T_{1/2}$	0.73 h		

[a] Mean of at least three experiments.
[b] Anti-viral activity using the molecular clone HIV-1 NL4-3 in MT-4 cells.
Anti-viral activity using a single-cycle infection where the cells were infected with a luciferase-bearing envelope defective (env−) NL-4.3 virus pseudotyped with HIV-1 env (HXBc2).
Protein binding was tested by Rapid Equilibrium Dialysis (RED) method (The RED (Rapid equilibrium Dialysis) (Pierce, Rockford Ill.)).
Female Sprague-Dawley rats were randomly selected and assigned to two groups. A group of 13 rats were administered 5 mg/kg of compound **2** intravenously. A second group of 8 rats were administered 50 mg/kg of compound **2** orally. Following the dosing, blood was collected at 7 different time points. Plasma samples obtained from the blood samples were analyzed by LC/MS/MS and the bioavailability of compound **16** was determined.

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