Bioorganic & Medicinal Chemistry Letters 26 (2016) 1233–1236

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pyridoxine hydroxamic acids as novel HIV-integrase inhibitors

Brent R. Stranix [a,*], Jinzi J. Wu [a,†], Guy Milot [a], Françis Beaulieu [a,§], Jean-Emanuel Bouchard [a], Kristine Gouveia [a], André Forte [a,‡], Seema Garde [a], Zhigang Wang [a], Jean-François Mouscadet [b,¶], Olivier Delelis [b], Yong Xiao [a]

[a] Ambrilia Biopharma Inc., 1000 Ch. du Golf, Verdun, Montreal, QC H4P 2R2, Canada
[b] BPA, ENS de Cachan, CNRS, Cachan, France

ARTICLE INFO

Article history:
Received 5 November 2015
Revised 7 January 2016
Accepted 11 January 2016
Available online 20 January 2016

Keywords:
HIV integrase inhibitor
Pyridoxine
Hydroxamic acid
Active site binding

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

* Corresponding author at present address: Champlain Exploration Inc., 1100-2500 Rene-Levesque W., Montreal, QC H3B C6L, Canada.
† Present address: Ascletis, 1785 Jianghai Road, Building No. 1, Suite 804, Binjiang District, Hangzhou, China.
‡ Present address: Cirion Biopharma Inc., 3150 Delaunay, Laval, QC H7L 5E1, Canada.
§ Present address: Omegachem, 480, rue Perreault Lévis, QC G6W 7V6, Canada.
¶ Present address: Bio-Rad Laboratories, Marnes-la-Coquette, France.

http://dx.doi.org/10.1016/j.bmcl.2016.01.028
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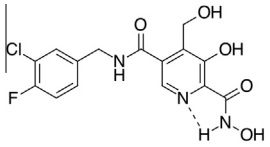
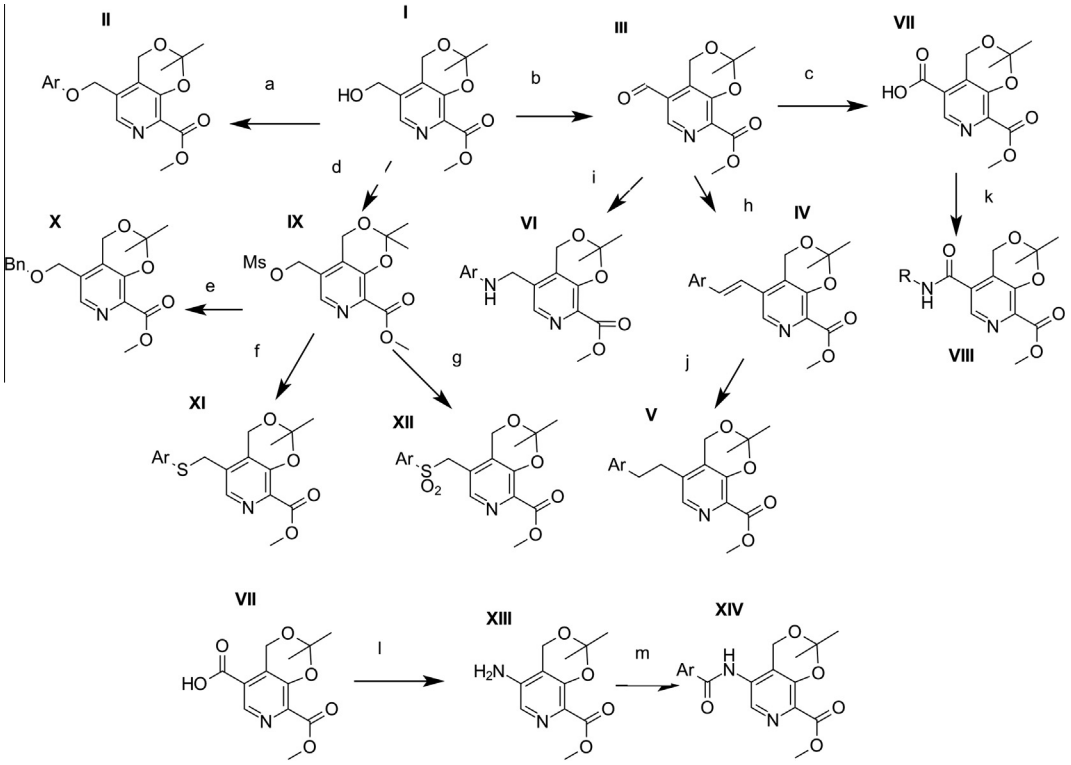


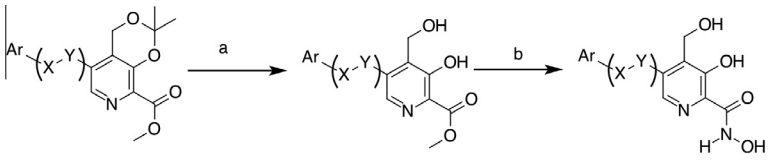
Figure 1. General structure of inhibitors.

(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.