Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6472–6475
Bioorganic & Medicinal Chemistry Letters

Discovery of novel non-cytotoxic salicylhydrazide containing HIV-1 integrase inhibitors

Laith Q. Al-Mawsawi,[a] Raveendra Dayam,[a] Laleh Taheri,[a] Myriam Witvrouw,[b] Zeger Debyser[b] and Nouri Neamati[a,*]

[a]*Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA*
[b]*Molecular Medicine, Katholieke Universiteit Leuven and IRC KULAK, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium*

Received 12 July 2007; revised 26 September 2007; accepted 28 September 2007
Available online 10 October 2007

Abstract—The previously discovered salicylhydrazide class of compounds displayed potent HIV-1 integrase (IN) inhibitory activity. The development of this class of compounds as antiretroviral agents was halted due to cytotoxicity in the nanomolar to sub-micromolar range. We identified a novel class of non-cytotoxic hydrazide IN inhibitors utilizing the minimally required salicylhydrazide substructure as a template in a small-molecule database search. The novel hydrazides displayed low micromolar IN inhibitory activity and are several hundred-fold less cytotoxic than previously disclosed salicylhydrazide IN inhibitors.

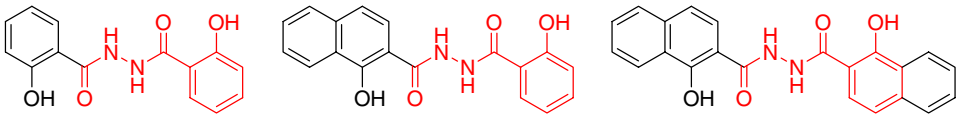
HIV-1 integrase (IN) is a critical enzyme for viral replication. The protein catalyzes the insertion of the reverse-transcribed proviral cDNA into the host cell nuclear genome, a prerequisite for virion production and propagation. Although efficient integration in vivo is a complex interplay between both cellular and viral proteins, IN enzymatic activity consists of two DNA reaction events that unfold in a 'cut and paste'-like process. Initially, IN hydrolytically cleaves a GT dinucleotide from each conserved CAGT 3′-terminal sequence of the reverse-transcribed viral DNA. This catalytic step is termed 3′-processing, occurs in the cytosol of the cell, and results in two 3′-recessed hydroxyl groups, which are then utilized for a nucleophilic attack in the second step of the integration process. This is followed by strand transfer, where the processed DNA product is inserted within the cellular genome.[1] Host cell nuclear enzymes presumably execute the removal of the two unpaired nucleotides on the 5′-viral DNA ends, DNA repair, gap filling, and ligation to complete the integration process. IN catalytic activities can be mimicked in vitro using isolated systems that contain purified IN, a DNA oligonucleotide substrate with ends corresponding to the U3 or U5 viral DNA termini, and Mg^{2+} or Mn^{2+} as a cofactor.[2]

Great strides have been achieved in the design and discovery of IN inhibitors as antiviral agents.[3] The development of different IN inhibitors, which each display strand transfer specific inhibition of the viral enzyme, have emerged as the most promising to provide a genuine FDA approved drug targeting IN. These include the β-diketoacid, naphthyridine carboxamide, pyrimidinone, and quinolone carboxylic acid class of IN inhibitors.[4] Indeed, two strand transfer specific inhibitors, the pyrimidinone MK-0518[5,6] and the quinolone carboxylic acid GS-9137,[7] have advanced to late-stage clinical studies.

Although the success of specific strand transfer IN inhibitors is a great achievement, the fact remains that therapeutics targeting crucial enzymes of HIV-1, a pathogen characterized by a high mutational rate, will inevitably lead to the development of drug resistant viral strains. The clinical use of first generation IN inhibitors will undoubtedly warrant the need for second generation IN inhibitors to treat patients harboring IN resistant HIV strains. Additionally, HIV-1 strains that exhibit cross-resistance to IN inhibitors belonging to different strand transfer specific chemical classes have been documented in pre-clinical and clinical development studies.[4]

Keywords: Substructure; SAR; HIV-1 integrase; Cytotoxicity.
* Corresponding author. Tel.: +1 323 442 2341; fax: +1 323 442 1390; e-mail: neamati@usc.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.102



	1	2	3
3'-Proccessing (IC_{50}, μM):	2.07 ± 0.75	2.3 ± 0.3	Not Tested
Strand transfer (IC_{50}, μM):	0.73 ± 0.13	1.1 ± 0.15	Not Tested
Cytotoxicity (CC_{50}, μM):	0.1	0.035 ± 0.007	0.24 ± 0.028

Figure 1. HIV-1 integrase inhibitory activity and cytotoxicity of previously discovered hydrazides **1**–**3**.

Viral strains displaying resistance to strand transfer specific inhibitors are expected to be susceptible to structurally novel classes of IN inhibitors. One approach to this problem is to revive previously identified IN inhibitor chemical classes, which displayed potent IN inhibition, but were developmentally halted due to unwanted pharmacokinetic, pharmacodynamic, or toxicological characteristics. Here we present a series of novel non-cytotoxic salicylhydrazide-containing IN inhibitors. The developmental progress of the salicylhydrazide class of IN inhibitors was halted due to cytotoxicity issues. The disclosed novel salicylhydrazides retained potent IN inhibition in vitro, but are several hundred-fold less toxic in cell culture as compared to previously discovered salicylhydrazide IN inhibitors greatly enhancing their therapeutic potential as antiretroviral agents.

The salicylhydrazine **1** (Fig. 1) was first discovered as being the active component in a mixture of compounds retrieved using three-dimensional pharmacophore searches from the open compounds listed in the National Cancer Institute (NCI) Drug Information System (DIS) database. The three-dimensional pharmacophore models were developed and utilized to identify potential IN inhibitors from the NCI DIS database that did not contain a catechol moiety.[8,9] Previously identified catechol-containing IN inhibitors were highly cytotoxic since this moiety was susceptible to oxidation to a quinone species, which has a propensity to cross-link with cellular proteins.[10] A thorough structure–activity relationship (SAR) study was carried out on the hydrazide **1**. Structural modifications that abolished IN inhibitory activity of **1** included the substitution of one or both hydroxyl groups with amine groups, or deletion of the hydroxyl(s) altogether.[11] Addition of a second aryl ring to phenol of the hydrazide **1** resulted in an unsymmetrical naphthalenol/phenol hydrazide **2** (one aryl ring) and a symmetrical naphthalenol hydrazide **3** (two aryl rings). Interestingly, the unsymmetrical naphthalenol/phenol hydrazide **2** showed similar IN inhibitory potency. Furthermore, elimination of one salicyloyl moiety from hydrazide **1** gave an IN inhibitor with reduced inhibitory potency. The detailed SAR study around the hydrazide **1** demonstrated that a salicyloylhydrazide moiety is the minimally required substructure for IN inhibitory potency of the compounds. In addition, the salicylhydrazides are proposed to inhibit IN catalytic activities through chelation of the active site Mg^{2+}. Even though the salicylhydrazides lacked the catechol moiety, the majority of active compounds in this class exhibited cytotoxicity in the nanomolar range.[12] The most potent salicylhydrazides, for example compounds **1** –**2** (Fig. 1), showed strong cytotoxicity in the cell-based assays, limiting their therapeutic application as antiretroviral agents.[13,14]

Considering the previously observed SAR and cytotoxicity profiles of the salicylhydrazide class of IN inhibitors, we designed an optimization program utilizing the minimally required substructure (Fig. 2a) to use as a two-dimensional search query to identify potent salicylhydrazide IN inhibitors with diverse structural scaffolds. Potent salicylhydrazide IN inhibitors exhibiting a wide therapeutic window without cytotoxic properties would provide a diverse structural class of lead compounds to develop antiretroviral drugs for the treatment of HIV/AIDS. Using the minimally required salicylhydrazide substructure (Fig. 2a) as a search query, we retrieved a series of novel salicylhydrazides **4**–**20** from a commercial database that contains over 300,000 small-molecule compounds (Asinex Corp.). The IN inhibitory activity and cellular toxicity of the salicylhydrazides **4**–**20** are given in Table 1. IN inhibitory activity and cytotoxicity of each compound was determined as described previously.[14,15] Several of these compounds inhibited IN catalytic activities in a similar range to the previously discovered hydrazides **1**–**2**, and are remarkably non-cytotoxic. Except compounds **5** and **13**, none of the novel salicylhydrazides showed significant cellular toxicity at a maximum tested concentration of 20 μM. Compounds **5** and **13** displayed CC_{50} values of 20 and 15 μM in HCT116 (colon cancer cell-line) cells, respectively. Compound **13** displayed a CC_{50} value of 20 μM in MDA-MB-435 (breast cancer cell-line) cells. Overall,

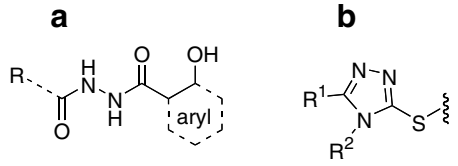
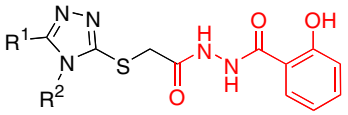
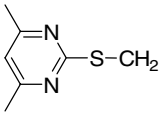
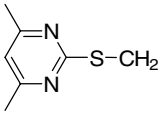
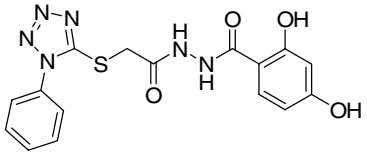
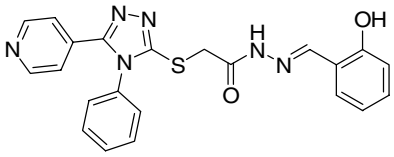
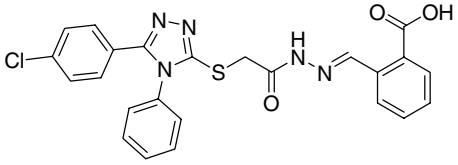


Figure 2. (a) Minimally required substructure for HIV-1 integrase inhibitory activity of the salicylhydrazide class of compounds. Replacement of one of the two phenol groups in the *N*,*N*′-bis-salicylhydrazide **1** with a substituted heterocyclic moiety (b) rendered a novel class of salicylhydrazide integrase inhibitors without cytotoxicity.

Table 1. Inhibition of HIV-1 integrase catalytic activities and cellular toxicity of a novel class of hydrazides



Compound	R^1	R^2	IC_{50} (μM)		CC_{50} (μM)[a]	
			3′-processing	Strand transfer	HCT116	MDA-MB-435
4	2-OHPh	4-MePh	5 ± 2	3 ± 1	>20 (26%)[b]	>20 (12%)
5	2-OHPh	2-OMePh	8 ± 1	6 ± 1	20	>20 (40%)
6	2-OHPh	Bn	15 ± 3	9 ± 2	>20 (8%)	>20 (15%)
7	Ph	Bn	21 ± 6	9 ± 7	>20 (34%)	>20 (22%)
8	Bn	3-ClPh	9 ± 1	8 ± 4	>20 (11%)	>20 (5%)
9	Bn	4-OMePh	18 ± 1	17 ± 1	>20 (0%)	>20 (10%)
10	Bn	C_6H_{11}–	21 ± 2	17 ± 1	>20 (11%)	>20 (5%)
11	4-ClPh	Ph	11 ± 3	5 ± 1	>20 (36%)	>20 (30%)
12	3,4-diOMePh	Ph	22 ± 1	21 ± 1	>20 (24%)	>20 (13 %)
13	Ph	4-MePh	23 ± 6	12 ± 8	15	20
14	4-Pyridine	4-FPh	49 ± 1	31 ± 2	>20 (22%)	>20 (14%)
15	Ph	Me	64 ± 4	58 ± 2	>20 (39%)	>20 (32%)
16		Ph	51 ± 5	27 ± 8	>20 (0%)	>20 (3%)
17		Me	>100	>100	>20 (21%)	>20 (16%)
18			68 ± 3	61 ± 6	>20 (15%)	>20 (3%)
19			>100	>100	>20 (40%)	>20 (44%)
20			>100	>100	>20 (0%)	>20 (8%)

[a] Concentration of compound resulting in 50% cellular toxicity (cell death).
[b] Percent cytotoxicity at highest tested compound concentration of 20 μM.

these compounds are several hundred-fold less cytotoxic as compared to the hydrazides **1**–**3**. The replacement of one of the two phenols in hydrazide **1** with an optimally substituted heterocyclic group (Fig. 2b) rendered a novel class of non-cytotoxic salicylhydrazides with low micromolar IN inhibitory activity (Table 1). SAR analysis on the retrieved compounds illustrates that variation in the substitution pattern on the 1, 2, 4 triazole core (Fig. 2b) is well correlated with the IN inhibitory profile of the compounds (Table 1). Compounds with a bulky substituted aromatic ring (a cyclohexyl ring in compound **10**) on N4 position of the triazole core showed moderate IN inhibitory activity. On the other hand, compounds **15** and **17** with a methyl group on N4 position of the triazole core showed decreased to no activity against both the 3′-processing and strand transfer reactions of IN. Interestingly, compounds **4**–**6** containing a 2-hydroxy phenyl substitution on the 5th position of the triazole core are moderately strong inhibitors with activity against both the strand transfer as well as 3′-processing reactions of IN. Similarly, compounds **8**–**10** containing a benzyl substitution on the 5th position of the triazole core also showed moderate inhibitory activity against both 3′-processing and strand transfer activities of IN. Compound **11** with a 4-chlorophenyl substitution on the 5th position and a phenyl group at the N4 position

of the triazole core inhibited both the 3′-processing and strand transfer activities of IN with IC_{50} values of 11 ± 3 and 5 ± 1 μM, respectively. Compound **18**, the only compound containing a tetrazole core instead of the triazole core (Table 1), displayed weak IN inhibitory activity. It inhibited both the 3′-processing and strand transfer activities of IN with IC_{50} values of 68 ± 3 and 61 ± 6 μM, respectively. We also tested compounds **19** and **20**, without the salicyloyl carbonyl, to evaluate the role of the salicyloyl group in conjunction with the heavily substituted triazole core in this novel class of hydrazide IN inhibitors. Interestingly, compounds **19** and **20** showed no activity against both IN catalytic reactions at a maximum tested concentration of 100 μM. This result reaffirms the importance of the salicyloyl group for IN inhibitory activity of this novel class of hydrazides.

We additionally tested seven representative compounds for antiviral activity against HIV-1 in the human MT-4 T-cell line. Antiviral testing was conducted as described previously.[16,17] Compounds chosen for antiviral activity testing include **5**, **6**, **8**–**11**, and **13**. Unfortunately, none of the compounds displayed significant antiviral activity. Optimization efforts are underway to enhance the antiviral activity of this new class of hydrazides.

In conclusion, we have identified a series of novel non-cytotoxic salicylhydrazide IN inhibitors through substructure database search methods. As compared to earlier disclosed hydrazides, the retrieved compounds displayed comparable IN inhibitory activity, but are several hundred-fold less cytotoxic. Structurally, the replacement of one of the two phenol (1-naphthalenol in hydrazide **2**) rings in hydrazide **1** with heavily substituted triazole groups affords novel hydrazide-containing IN inhibitors with significantly improved cytotoxic profiles, greatly enhancing the therapeutic potential of this chemical class of IN inhibitors.

Acknowledgments

This work was supported by grants from The Campbell Foundation and the California HIV/AIDS Research Program (CHRP) to Nouri Neamati.

References and notes

1. Brown, P. O. Integration. In *Retroviruses*; Coffin, J. M., Hughes, S. H., Varmus, H. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1997; p 161.
2. Bushman, F. D.; Craigie, R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1339.
3. Nair, V.; Chi, G. *Rev. Med. Virol.* **2007**, *17*, 277.
4. Dayam, R.; Al-Mawsawi, L. Q.; Neamati, N. *Drugs R D* **2007**, *8*, 155.
5. Grinsztejn, B.; Nguyen, B. Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261.
6. Markowitz, M.; Morales-Ramirez, J. O.; Nguyen, B. Y.; Kovacs, C. M.; Steigbigel, R. T.; Cooper, D. A.; Liporace, R.; Schwartz, R.; Isaacs, R.; Gilde, L. R.; Wenning, L.; Zhao, J.; Teppler, H. *J. Acquir. Immune Defic. Syndr.* **2006**, *43*, 509.
7. Dejesus, E.; Berger, D.; Markowitz, M.; Cohen, C.; Hawkins, T.; Ruane, P.; Elion, R.; Farthing, C.; Zhong, L.; Cheng, A. K.; McColl, D.; Kearney, B. P. *J. Acquir. Immune Defic. Syndr.* **2006**, *43*, 1.
8. Hong, H.; Neamati, N.; Wang, S.; Nicklaus, M. C.; Mazumder, A.; Zhao, H.; Burke, T. R., Jr.; Pommier, Y.; Milne, G. W. *J. Med. Chem.* **1997**, *40*, 930.
9. Nicklaus, M. C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.; Chen, J.; Milne, G. W.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 920.
10. Stanwell, C.; Ye, B.; Yuspa, S. H.; Burke, T. R., Jr. *Biochem. Pharmacol.* **1996**, *52*, 475.
11. Zhao, H.; Neamati, N.; Sunder, S.; Hong, H.; Wang, S.; Milne, G. W.; Pommier, Y.; Burke, T. R., Jr. *J. Med. Chem.* **1997**, *40*, 937.
12. Neamati, N.; Hong, H.; Owen, J. M.; Sunder, S.; Winslow, H. E.; Christensen, J. L.; Zhao, H.; Burke, T. R., Jr.; Milne, G. W.; Pommier, Y. *J. Med. Chem.* **1998**, *41*, 3202.
13. Neamati, N.; Lin, Z.; Karki, R. G.; Orr, A.; Cowansage, K.; Strumberg, D.; Pais, G. C.; Voigt, J. H.; Nicklaus, M. C.; Winslow, H. E.; Zhao, H.; Turpin, J. A.; Yi, J.; Skalka, A. M.; Burke, T. R., Jr.; Pommier, Y. *J. Med. Chem.* **2002**, *45*, 5661.
14. Plasencia, C.; Dayam, R.; Wang, Q.; Pinski, J.; Burke, T. R., Jr.; Quinn, D. I.; Neamati, N. *Mol. Cancer Ther.* **2005**, *4*, 1105.
15. Al-Mawsawi, L. Q.; Sechi, M.; Neamati, N. *FEBS Lett.* **2007**, *581*, 1151.
16. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
17. Witvrouw, M.; Pannecouque, C.; Switzer, W.; Folks, T.; De Clercq, E.; Heneine, W. *Antiviral Ther.* **2004**, *9*, 57.