Diketo Acids Derivatives as Dual Inhibitors of Human Immunodeficiency Virus Type 1 Integrase and the Reverse Transcriptase RNase H Domain

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Abstract: The HIV-1 integrase (IN) and reverse transcriptase (RT) are essential enzymes in the virus cycle. RT is crucial for the retrotranscription of the RNA viral genome, while IN is involved in the insertion in host chromosome of the proviral double strand DNA produced by RT. This enzyme has two associated functions: the RNA- and DNA-dependent DNA polymerase (RDDP and DDDP) and the ribonuclease H (RNase H). The RNase H function catalyzes the selective hydrolysis of the RNA strand of the RNA:DNA heteroduplex replication intermediate. Since the discovery that catalytic cores of both HIV-1 RNase H and IN are folded in a very similar way, have very similar active site geometries, and show the same DDE triad absolutely required for catalytic activity, some researches were devoted to study IN and RNase H dual inhibitor. Our decennial interest in design and synthesis of IN inhibitors led us to study the activity of our compounds also on RNase H activity. The results of the activities showed by pyrrolyl and quinolonyl diketo acids are reported and discussed.

Keywords: HIV-1, integrase, reverse transcriptase, ribonuclease H, RNase H, RNase H inhibitor, IN inhibitor, dual inhibitors, diketo acids, pyrrole derivatives, quinolinone drivatives.

Human immunodeficiency virus type 1 (HIV-1) integrase (IN) is an enzyme essential for viral replication that mediates the insertion of viral DNA within the host cell genome *via* a multistep process occurring in the following biochemical stages: (i) assembly of a stable DNA-enzyme complex with specific DNA sequences at the end of the HIV-1 long terminal repeat regions, (ii) 3′-processing that consists of the endonucleolytic cleavage of the 3′-ends of the viral DNA, (iii) transport through the nuclear envelope of the multimeric complex, called pre-integration complex (PIC), including viral proteins, IN and the processed DNA, (iv) strand transfer, which consists of the ligation of the viral 3′-OH DNA ends (generated by 3′-processing) to the 5′-DNA phosphate of a host chromosome, (v) removal of the two unpaired nucleotides at the 3′-ends of the viral DNA, and (vi) gap filling, probably accomplished by cellular enzymes, Fig. (**1**). In the past decade, IN has emerged as an attractive target; in fact, it is essential for retroviral replication, and no host-cell equivalent of IN is known, so that IN inhibitors should not interfere with normal cellular processes, and therefore have a high therapeutic index. HIV IN is a 32-kDa protein comprising three structural domains: the amino-terminal domain, the carboxy-terminal domain and the catalytic core domain that contains a triad of invariant carboxylate residues required for catalysis: D64, D116, and E152, called DDE motif. A Mg^{2+} ion is coordinated by D64 and D116 along with water molecules, whereas E152 that lies close to D64 does not participate in metal binding [1, 2].

Whereas structural studies of IN reveal a single binding site for Mg^{2+}, the number of metal ions present and required in the active site during the process remains controversial. Since a second metal has been observed in an avian sarcoma virus IN crystal structure [3], and because of the two-metal structure for polynucleotide transferases [4, 5], it has been proposed that a second metal (Mg^{2+} or Mn^{2+}) can be coordinated between D116 and E152 once HIV-1 IN binds its DNA substrate(s) [6, 7]. It is therefore likely that the metal(s) coordinate(s) IN and the phosphodiester backbone of the DNA substrate(s) during the 3′-processing and strand-transfer steps.

A great number of HIV-1 IN inhibitors with metal binding properties have been described, including diketo acids (DKAs) and their bioisosters, and numerous reviews have been published [8-18]. Among them, two compounds, Elvitegravir [19] and Raltegravir [20, 21], demonstrated promising clinical trial results and advanced into later stage trials, the latter being the first drug approved as IN inhibitor in 2007, Fig. (**2**).

It is generally believed that IN inhibitors such as DKA (A), or their bioisoster dihydroxypyrimidine carboxamide class (B), including raltegravir (**1**) bind these two metal ions in the active site while the hydrophobic aryl group participates in a specific interaction with an adjacent hydrophobic pocket or surface, Fig. (**3**) [22, 23].

Reverse transcriptase (RT) is another enzyme that is essential in the early steps of the HIV-1 life cycle for the conversion of the viral single-stranded RNA genome into a double-stranded DNA, which is subsequently translocated into the cell nucleus and integrated into the cell host DNA [24, 25]. RT is a multifunctional enzyme that possesses several distinct associated activities including RNA- and DNA-dependent DNA polymerase (RDDP and DDDP, respectively), ribonuclease H (RNase H), strand transfer, strand displacement synthesis and nucleotide excision, Fig. (**4**) [24-26].

RNase H activity, which degrades the RNA of the RNA-DNA hybrid molecules, is required at several steps during the reverse transcription process, and a functional RNase H is essential for retroviral replication. HIV IN and RNase H belong to a broader class of nucleotide-related enzymes including nucleases, polymerases, and polynucleotidyl transferases [3, 27].

Phosphoryl transfer is a common chemical transformation, which is essential for a number of basic cellular functions such as DNA replication and transcription, signal transduction and metabolism. HIV-1 RNase H catalyzes phosphoryl transfer through nucleophilic substitution reactions on phosphate esters, requiring the concerted action of a general base activating the nucleophile and a general acid protonating the leaving group. In particular, catalysis occurs by deprotonation of a water molecule to form a nucleophilic hydroxide group that attacks the scissile phosphate group on RNA [28, 29]. In order to accomplish this reaction, the HIV-1 RT-associated RNase H function requires either Mg^{2+} or Mn^{2+}, thus being classified as a metal dependent transposases, Fig. (**5**) [30]. However, differently from the other RNase Hs such as the *E. coli* RNase H that needs a single divalent cation in the active site [31], the structure of the HIV-1 RNase H domain has been proposed to need two divalent cations that, consistently with the phosphoryl transfer geometry, seem to be coordinated by the active site carboxylates D443, E478, D498 and D549, Fig. (**5**) [29]. These residues compose a three-amino-acid DDE motif, highly conserved in many HIV-1 strains in the core domain of the RNase H active site: mutations in any of the D443, D498 and E478 residues abolish enzyme activity [32, 33].

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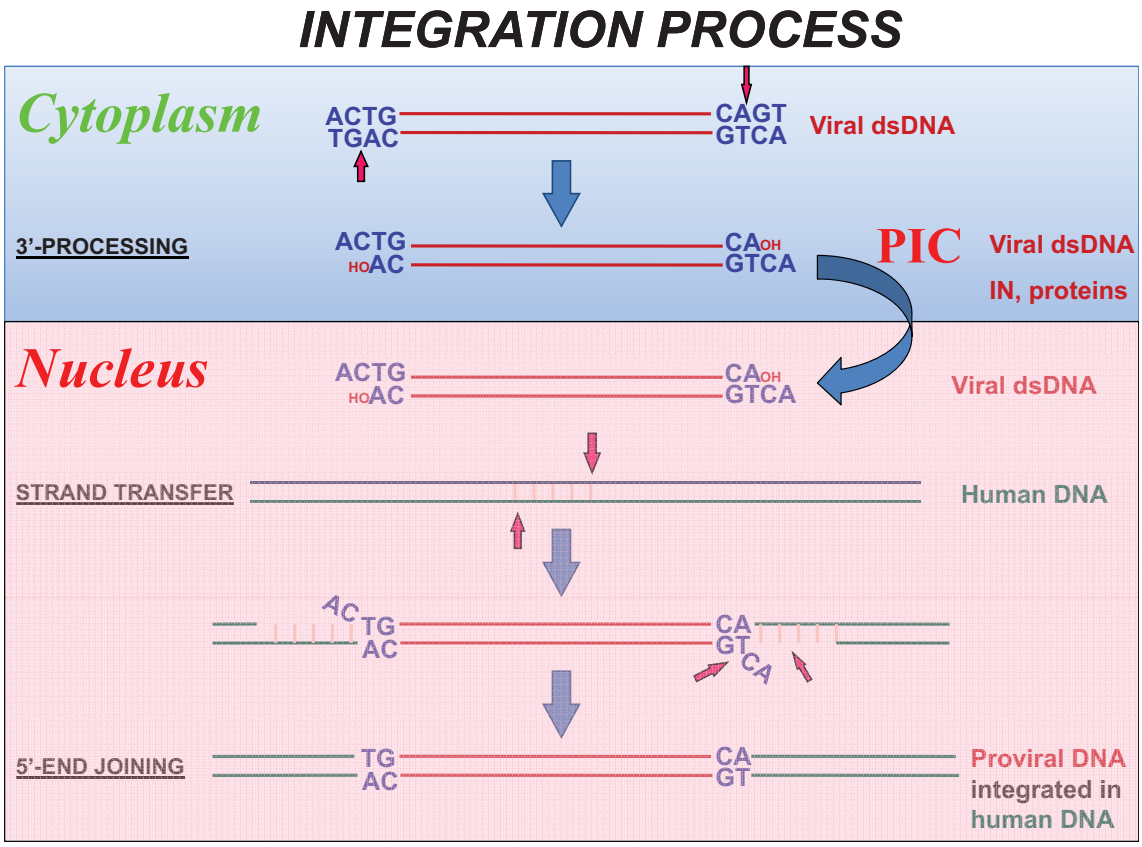


Fig. (1). Steps of integration process performed by HIV-1 IN.

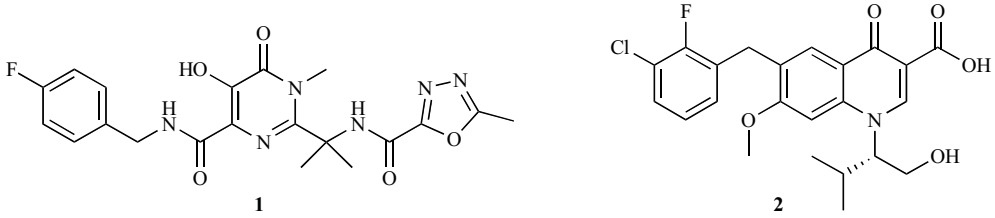


Fig. (2). Chemical structures of a HIV-1 IN inhibitors Raltegravir (**1**) and Elvitegravir (**2**).

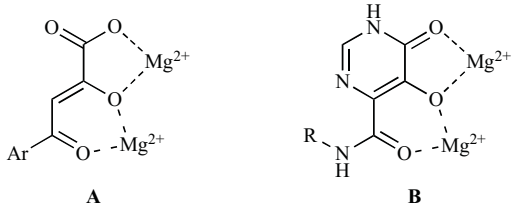


Fig. (3). Metal binding ability of DKA (A) and dihydroxypyrimidine carboxamide (B).

Surprisingly, determination of the three-dimensional structure of the catalytic core of HIV-1 RNase H led to the finding that HIV-1 RNase H and HIV-1 IN are folded in a very similar way and have very similar active site geometries. Catalytic cores of both HIV-1 RNase H and IN share a similar αβ-fold containing a central five-stranded mixed β-sheet surrounded by α-helices on both sides in the same topological order [34-36]. Moreover, RNase H and IN have remarkably similar active sites with the same DDE triad absolutely required for catalytic activity.

The pivotal role of RT in the HIV-1 life cycle has made this enzyme a key target in the AIDS chemotherapy leading to the development of several classes of RT inhibitors (RTI), which have been discovered and approved for the treatment of HIV-1 infected patients [37, 38]. However, although RT is a multifunctional enzyme, all approved RTI actually target, only the RT-associated polymerase function. Inhibitors of RT-associated RNase H function were less studied if compared with both RT-associated polymerase function and IN inhibitors. However, in the last five years more attention has been given to the HIV-1 RT-associated RNase H activity as drug target. As a result, several compound screenings were performed by different teams and diverse classes of inhibitors were found to be able to inhibit the HIV-1 RNase H activity. Among them, natural products, hydrazones, naphthalene sulfonic acids, DKAs, *N*-hydroxyimides, hydroxypyrimidine carboxylates, nitrofuran-2-carboxylic acid carbamoylmethyl ester derivatives, vinylogous ureas, and thiocarbamates and triazoles, were reported, the most part of which having metal-chelating ability [39].

Within the above cited class of RNase H inhibitors, some DKAs inhibitors of HIV-1 IN have shown anti-RNase H activities [40, 41] whereas DNA aptamers first described as inhibitors of RNase H have been reported to inhibit HIV-1 integrase [42]. Recently, tropolones [43-45] and madurahydroxylactone derivatives [46] have been reported to inhibit both enzymes.

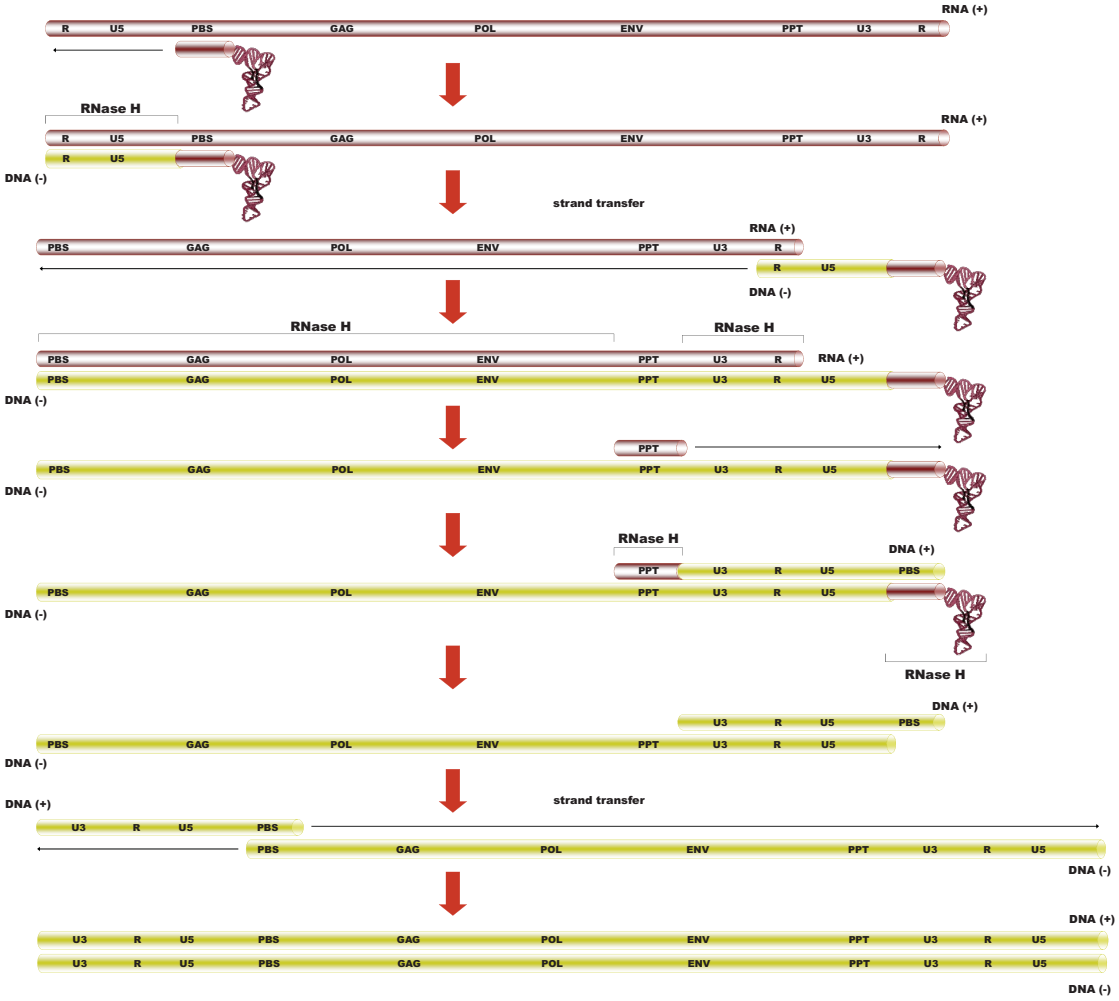


Fig. (4). HIV-1 RNA genome conversion into DNA by RT.

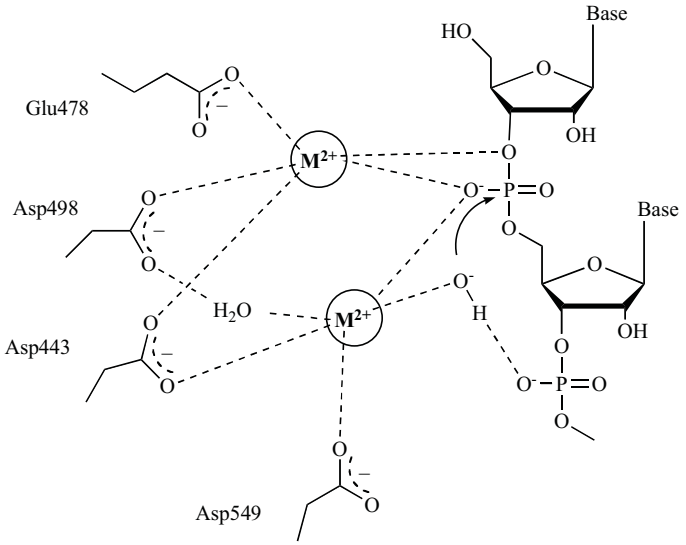


Fig. (5). Schematic representation of the two-metal ion mechanism of catalysis for the RNase H activity of HIV-1 reverse transcriptase.

Since the hypermutability of the HIV-1 genome easily results in drug-resistant strains, in time this leads to an overall reduction of drug efficacy. Therefore, the identification of new RTI that target other essential RT-associated functions such as the RNase H activity could be an attractive approach to enhance the anti-HIV-1 drug armamentarium effectiveness [47]. Further, the simultaneous inhibition of HIV-1 IN, RT polymerase, and RT RNase H activities by metal-chelating compounds appears as a novel and attractive therapeutic, way which could contribute to overcome the problems occurring during combination therapy.

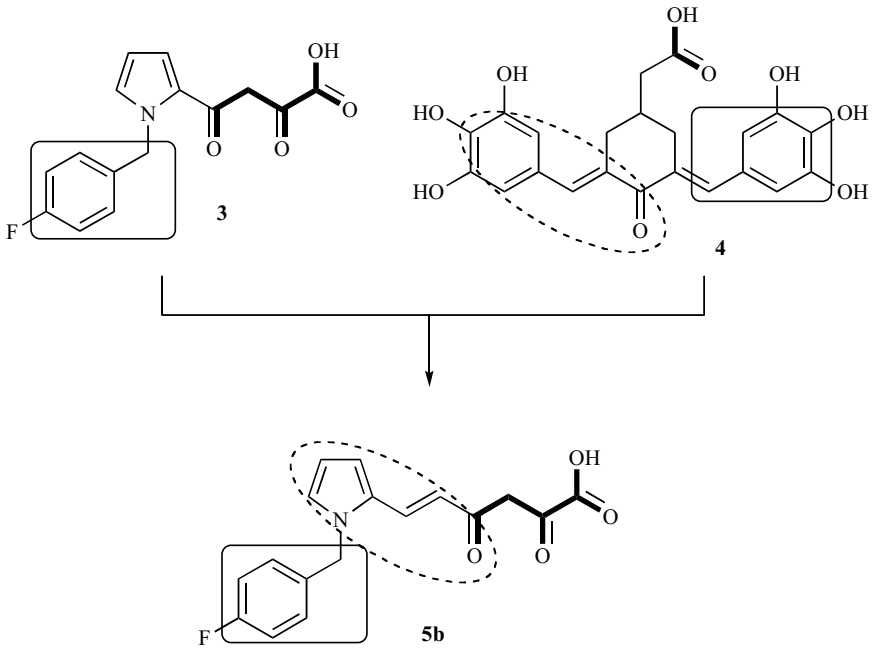


Fig. (6). Design of dikeyo hexenoic acids (i.e. **5b**) and reference structures **3** and **4**. The cinnamoyl and the benzyl portions are highlighted with dashed and full line, respectively, while diketo portion and carboxylic function are pointed out with bold bonds.

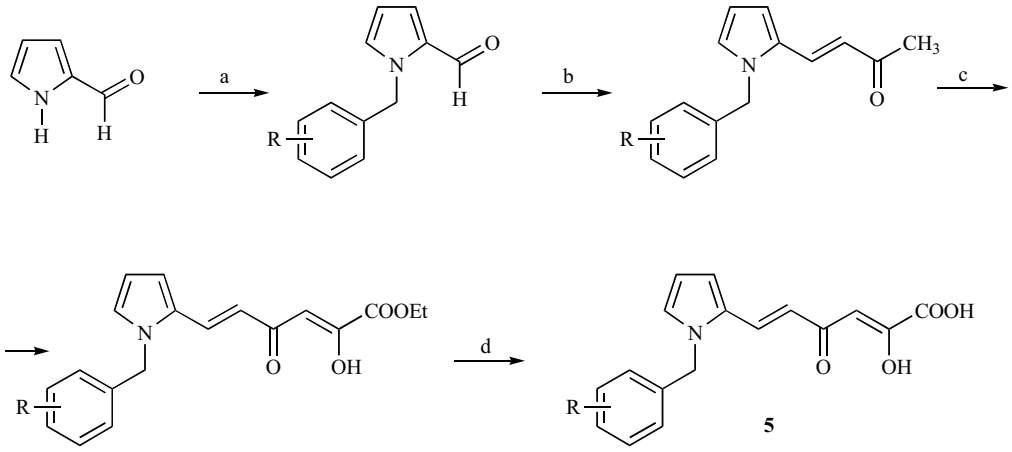
Our efforts to find HIV-1 IN inhibitors, started in the last part of 90s, time in which we designed and synthesized conformationally restrained cinnamoyl derivatives belonging to the polyhydroxylate aromatic compound class [48,49]. Then, after the demonstration that polyhydroylated aromatic derivatives were inhibitors of HIV-1 entry in cell-based assays [50] and did not target the IN *in vivo*, we decided to focus our attention on the aryl diketoacid compounds, which were proven to be effective HIV-1 IN inhibitors both in cell-based assays and *in vivo* [51, 52].

Starting form the structure of the *i*) pyrrole derivative L-731,988 (**3**) reported by Hazuda as anti-IN agent [51] and *ii*) polyhydroxy cinnamoyl derivatives (such as **4**) that we previously identified as potent IN inhibitors in enzyme assays [48, 49], we designed new pyrrolyl diketohexenoic acid derivatives as potential IN inhibitors (i.e. **5b**), Fig. (**6**). Pyrrolyl diketohexenoic acids (i.e. **5b**) are characterized by i) the cinnamoyl moiety of polyhydroxy aromatic derivatives, in which the phenyl ring is replaced by the bioisosteric pyrrole moiety; ii) the diketo acid group of DKA series; iii) the carboxylic function found both in DKAs (i.e. **3**) and in our derivatives (i.e. **4**); iv) an benzyl portion typical for both DKAs and cinnamoyl derivatives (i.e. 1-benzylpyrrole or trihydroxybenzylidene group, respectively), Fig. (**6**). The newly designed *N*-benzylpyrrolyl diketohexenoic acids (**5**) were a promising tool to explore how the elongation of the diketobutanoic chain would affect anti-IN activity [53-55].

Compound **5b** and 75 congeners were synthesized and tested against both recombinant IN in enzyme assays and HIV-1 infected cells in cell-based assays. The synthetic pathway to obtain derivatives **5** is reported in Scheme **1**.

Data of cytotoxicity and antiviral activities in enzyme (IN) and cell-based assays of selected pyrrolyl diketohexenoic acids **5** and a few related congeners **6** and **7** are reported in Table **1**, using **3** as the reference compound.

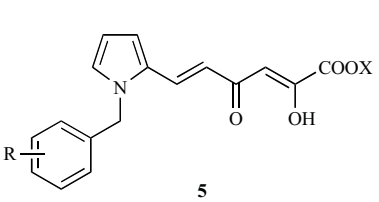
In general, compounds **5**-**7** were found to be potent ST inhibitors, with activities within the range 0.026-110 μM. These DKA



Scheme 1. Synthetic pathway to obtain pyrrolyl diketohexenoic acids **5**.

(a) Benzylhalide, K_2CO_3, DMF, 18 h, 90°C; (b) 2-propanone, 5N NaOH, 24 h, 25 °C: (c) diethyl oxalate, NaOEt, THF, 1h, 45 min, 25 °C; (d) 1N NaOH, THF, MeOH, 1.5 h, 25 °C.

Table 1. Cytotoxicity and Antiviral Activities in Enzyme (IN - Related to ST, and RNase H) and Cell-Based Assays of Pyrrolyl Diketo Acids 5-7



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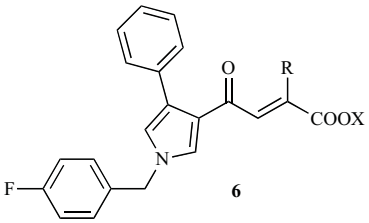

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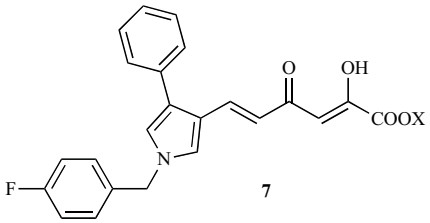

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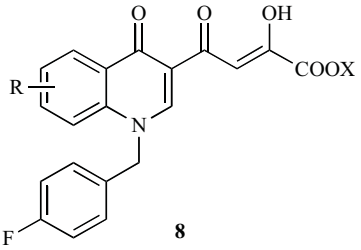
			IC_{50}[a] (μM)				
Compd	**R**	**X**	**ST**	**RH**	EC_{50}[b] (μM)	CC_{50}[c] (μM)	**SI**[d]
5a	H	Et	15	-	0.25	>50	>200
5b	H	H	0.09	15	0.35	>50	>143
5c	2-Cl	Et	8.0	9.8	3	>50	>17
5d	2-Cl	H	4.0	5.0	1.3	>50	>38
5e	2-CN	Et	9.00	32	17	32	>2
5f	2-CN	H	0.26	6	>50	>50	-
5g	2-F	Et	0.98	6.3	0.31	>50	>161
5h	2-F	H	0.98	6.4	0.35	>50	>143
5i	2-Me	Et	32	3.0	1.4	>50	>36
5j	2-Me	H	0.17	26	3.7	>50	>14
5k	2-OEt	Et	12	>100	0.6	33	55
5l	2-OEt	H	0.31	64	>0.2	33	>165
5m	2-OMe	Et	23	> 100	0.2	39	195
5n	2-OMe	H	0.53	16	0.2	>50	>250
5o	3-Cl	Et	6	19	0.79	>50	>63
5p	3-Cl	H	0,31	9	>50	>50	-
5q	3-CN	Et	-	5	7.1	>50	>7
5r	3-CN	H	0.75	5	>50	>50	-
5s	3-F	Et	11	9.0	2.2	>50	>23
5t	3-F	H	0.92	14	5.1	>50	>10
5u	3-Me	Et	8.0	9.6	2.4	>50	>21
5v	3-Me	H	1.3	4.6	1.9	>50	>26
5w	3-OMe	Et	60	20	0.35	>50	>143
5x	3-OMe	H	0.49	>100	1.3	>50	>38
5y	4-Cl	Et	42	8.0	26.3	>50	>2
5z	4-Cl	H	≥4.1	5.0	>50	>50	-
5aa	4-CN	Et	>111	8	>50	>50	-
5ab	4-CN	H	1.70	6	>50	>50	-
5ac	4-F	Et	2.3	8.0	<0.2	>50	>250
5ad	4-F	H	0.026	2.5	0.63	>50	>79
5ae	4-Me	Et	≥111	7.3	17.4	>50	>3
5af	4-Me	H	1.2	17	>50	>50	-
5ag	4-OMe	Et	110	6.7	>50	>50	-
5ah	4-OMe	H	4.1	3.0	17.4	>50	>3
6a	=O	H	0.232	7.5	0.66		
6b	NH_2	H	0.204	2.0	0.63		
7	-	H	0.73	7.0	1.4		
3			0.057		1.5	54	36
BTDBA			1.9	3.2	>50		

[a]Compound concentration required to reduce rIN strand transfer (ST) and rRNase H activity (RH) by 50%. [b] Compound concentration required to achieve 50% protection of MT-4/KB cells from the HIV-1 induced cytopathicity, as determined by MTT method. [c]Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50%. [d]Selectivity index: CC_{50}/EC_{50} ratio.

Table 2. Cytotoxicity and Antiviral Activities in Enzyme (IN - Related to ST and RNase H) and Cell-Based Assays of Quinolonyl Diketo Acids 8 and 9



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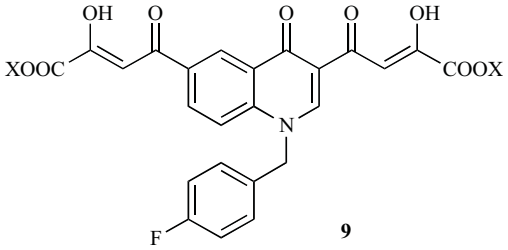

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Compd	R	X	IC_{50}[a] (μM) ST	inhib %[b] (10 μM) RH	EC_{50}[c] (μM)	CC_{50}[d] (μM)	SI[e]
8a	7-Py[f]	Et	2.3	55.6	4.1	>200	>49
8b	7-Py[f]	H	0.028	70.7	0.17	>200	>1176
8c	6-F	Et	0.58	21.5	>50	>200	-
8d	6-F	H	0.030	61.6	>50	>200	-
8e	7-F	Et	1.3	23.9	>50	>200	-
8f	7-F	H	0.020	6	>50	>200	-
8g	6-Cl	Et	21	24.5	nt[g]	nt[g]	
8h	6-Cl	H	0.028	-10.3	46.1	>200	>4
8i	7-Cl	Et	14	-19.5	nt[g]	nt[g]	
8j	7-Cl	H	0.019	-10.1	>50	>200	-
9a		Et	0.34	11.8	0.91	191	210
9b		H	0.012	80.4	4.29	>200	>47

[a]Compound concentration required to reduce rIN strand transfer (ST) by 50%. [b]Percentage of inhibition of RNase H (RH) activity at 10 μM. [c]Compound concentration required to reduce the exponential growth of MT-4/KB cells by 50%. [d]Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50%. [e]Selectivity index: CC_{50}/EC_{50} ratio. [f]Py = pyrrolidin-1-yl. [g]nt = not tested.

derivatives were also endowed of antiretroviral activity against HIV-1 infected cells (EC_{50} from 0.2 to 26.3 μM), and good selectivity indexes (up to >250), with few exceptions (**5f,p,r,z,aa,ab,af,ag**). However, an accurate analysis of the data led us to think that further targets could be involved in the inhibition of the life cycle of HIV-1 in infected cells, since no direct correlation could be found between the anti-IN data and the antiviral activities. In particular, a number of compounds **5-7** were more potent in cell-based than in enzyme assays against rIN (see compounds **5a,c,d,g-i,k,m-o,s,u,w,y,ac,ae** and **7**). First we thought that RNase H function of the RT could be inhibited besides IN, based on *i*) the above cited similarities of the active sites of IN and RNase H [34-36], and *ii*) the reports on the anti-RNase H activity of 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid (BTDBA), a DKA discovered by Merck [40]. Thus, we decided to test all pyrrolyl diketo acids synthesized in our laboratories, against RNase H in enzyme assays. The results of this screening are reported for selected derivatives **5-7** in Table **1**, using BTDBA as the reference compound. Very interestingly, the majority of tested compounds were active in inhibiting the RNase H activity of RT. The data of activity were within the range 2-64 μM, and only a few compounds (**5a,k,m,x**) were inactive in these assays. So we decided to study more deeply the properties of **5ac**, one of the most potent derivatives against RNase H. Compound **5ac** (RDS 1643) selectively inhibits the RNase H function of the HIV-1 RT, without affecting neither its DNA polymerase associated activity, nor the RNase H activity associated to the Avian Myeloblastosis Virus (AMV) RT or the *E. coli* RNase H, while it barely affected the HIV-1 IN activity [41]. We also performed a partial characterization of the interaction between RDS 1643 and the HIV-1 RT by time of addition, kinetic and drug association studies. Finally, we demonstrated that RDS 1643 inhibits in cell-based assays the replication of wild type and HIV-1 strains resistant to RTIs currently used in clinical practice [41]. This interesting results led us to pursue the research in this field and actually, new compounds as RNase H inhibitors are in development that will be publish soon.

In parallel to the development of pyrrolyl diketo acids, we designed also quinolonyl diketo acids [56-62] characterized by one (**8**) [56] or two (**9**) DKA chains [57], as IN inhibitors. The monofunctional derivatives **8** were selective ST inhibitor, while the bifunctional compounds **9** were active also against 3'-P. Thus IN inhibitors **8** and **9** were useful tools to study the mechanism of action of IN. In particular, we demonstrated through cross-linking experiments between IN and its DNA substrate that derivatives **9** bind to both DNA acceptor and donor sites [63].

Based on the results obtained in the enzyme assays against RNase H with pyrrolyl diketo acids **5-7**, we decided to test also our quinolonyl diketo acids against the RNase H function of the RT. A first group of quinolonyl DKAs were tested against rRNase H at 10 μM. Compound **8** and **9** in general showed good activity against the RNase H function with % that ranged from 6 to 80.4. In general, both monofuntional and bifunctional derivatives were good RNase H inhibitors (Table **2**). Interestingly in a few cases (see **8h-j**) we found increased activity of RNase H activity. This issue will be further studied and discussed in the future.

The data in our hand led us to conclude that both classes of compounds pyrrolyl and quinolonyl DKAs, are dual inhibitors of IN and RNase H function of the RT. Further studies will be devoted to develop potent agents active against both enzymes (dual inhibitors). These dual inhibitors could be excellent antiretroviral agents useful to overcome the resistance of the virus.

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ABBREVIATIONS

HIV-1	=	Human immunodeficiency virus type 1
IN	=	integrase
PIC	=	pre-integration complex
RT	=	reverse transcriptase
RDDP	=	RNA-dependent DNA polymerase
DDDP	=	DNA-dependent DNA polymerase
RNase H	=	ribonuclease H
DKA	=	diketo acid
RTI	=	RT inhibitors
BTDBA	=	4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid
AMV	=	Avian Myeloblastosis Virus

REFERENCES

[1] Goldgur, Y.; Dyda, F.; Hickman, A.B.; Jenkins, T.M.; Craigie, R.; Davies, D.R. Three new structures of the core domain of HIV-1 integrase: an active site that binds magnesium. *Proc. Natl. Acad. Sci. U.S.A.,* **1998**, *95*, 9150-9154.

[2] Maignan, S.; Guilloteau, J.P.; Zhou-Liu, Q.; Clément-Mella, C.; Mikol, V. Crystal structures of the catalytic domain of HIV-1 integrase free and complexed with its metal cofactor: High level of similarity of the active site with other viral integrases. *J. Mol. Biol.,* **1998**, *282*, 359-368.

[3] Bujacz, G.; Alexandratos, J.; Wlodawer, A.; Merkel, G.; Andrake, M.; Katz, R.A.; Skalka, A.M. Binding of different divalent cations to the active site of avian sarcoma virus integrase and their effects on enzymatic activity. *J. Biol. Chem.,* **1997**, *272*, 18161-18168.

[4] Yang, W.; Steitz, T.A. Recombining the structures of HIV integrase, RuvC and RNase H. *Structure,* **1995**, *3*, 131-134.

[5] Beese, L.S.; Steitz, T.A. Structural basis for the 3'-5'exonuclease activity of *Escherichia coli* DNA polymerase I: a two metal ion mechanism. *EMBO J.,* **1991**, *10*, 25-33.

[6] Grobler, J.A.; Stillmock, K.; Hu B.; Witmer, M.; Felock, P.; Espeseth, A.S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J.S.; Young, S.; Vacca. J.; Hazuda, D.J. Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 6661-6666.

[7] Marchand, C.; Johnson, A.A.; Karki, R.G.; Pais, G.C.; Zhang, X.; Cowansage, K.; Patel, T.A.; Nicklaus, M.C.; Burke, T.R., Jr.; Pommier, Y. Metal-dependent inhibition of HIV-1 integrase by β-diketo acids and resistance of the soluble double-mutant (F185K/C280S). *Mol. Pharmacol.,* **2003**, *64*, 600-609.

[8] Pommier, Y.; Johnson, A.A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discovery,* **2005**, *4*, 236-248.

[9] Johnson, A.A.; Marchand, C.; Pommier, Y. HIV-1 integrase inhibitors: A decade of research and two drugs in clinical trial. *Curr. Top. Med. Chem.,* **2004**, *4*, 1059-1077.

[10] Dayam, R.; Deng, J.; Neamati, N. HIV-1 integrase inhibitors: 2003-2004 update. *Med. Res. Rev.*, **2006**, *26*, 271-309.

[11] Makhija, M.T. Designing HIV integrase inhibitors: Shooting the last arrow. *Curr. Med. Chem.,* **2006**, *13*, 2429-2441.

[12] Lataillade, M.; Kozal, M.J. The hunt for HIV-1 integrase inhibitors. *AIDS Patient Care STDS,* **2006**, *20*, 489-501.

[13] Maurin, C.; Bailly, F.; Cotelle, P. Structure-activity relationships of HIV-1 integrase inhibitors: enzyme-ligand interactions. *Curr. Med. Chem.,* **2003**, *10*, 1795-1810.

[14] Cotelle, P. Patented HIV-1 integrase inhibitors (1998-2005). *Recent Patents Anti-Infect. Drug Discovery,* **2006**, *1*, 1-15.

[15] Dayam, R.; Neamati, N. Small-molecule HIV-1 integrase inhibitors: the 2001-2002 update. *Curr. Pharm. Des.,* **2003**, *9*, 1789-1802.

[16] Neamati, N. Patented small molecules inhibitors of HIV-1 integrase: a 10-year saga. *Expert Opin. Ther. Patents,* **2002**, *12*, 709-724.

[17] Witvrouw, M.; Van Maele, B.; Vercammen, J.; Hantson, A.; Engelborghs, Y.; De Clercq, E.; Pannecouque, C.; Debyser, Z. Novel inhibitors of HIV-1 integration. *Curr. Drug. Metab.,* **2004**, *5*, 291-304.

[18] Young, S.D. Inhibition of HIV-1 integrase by small molecules: the potential for a new class of AIDS chemotherapeutics. *Curr. Opin. Drug Discov. Devel.*, **2001**, *4*, 402-410.

[19] Dejesus, E.; Berger, D.; Markowitz, M.; Cohen, C.; Hawkins, T.; Ruane, P.; Elion, R.; Farthing, C.; Zhong, C.L.; Cheng, A.K.; McColl, D.; Kearney, B.P. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. *J. Acquired Immune Defic. Syndr.,* **2006**, *43*, 1-5.

[20] 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. Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: A phase II randomised controlled trial. *Lancet,* **2007**, *369*, 1261-1269.

[21] Markowitz, M.; Morales-Ramires, 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. Antiretroviral activity, pharmacokinetics, and tolerability of MK-0518, a novel inhibitor of HIV-1 integrase, dosed as monotherapy for 10 days in treatment-naive HIV-1-infected individuals. *J. Acquired Immune Defic. Syndr.,* **2006**, *43*, 509-515.

[22] Bacchi, A.; Biemmi, M.; Carcelli, M.; Carta, F.; Compari, C.; Fisicaro, E.; Rogolino, D.; Sechi, M.; Sippel, M.; Sotriffer, C.A.; Sanchez, T.W.; Neamati, N. From ligand to complexes. Part 2. Remarks on human immunodeficiency virus type 1 integrase inhibition by β-diketo acid metal complexes. *J. Med. Chem.*, **2008**, *51*, 7253-7264.

[23] Sechi, M.; Bacchi, A.; Carcelli, M.; Compari, C.; Duce, E.; Fisicaro, E.; Rogolino, D.; Gates, P.; Deruda, M.; Al-Mawsawi, L.Q.; Neamati, N. From ligand to complexes: inhibition of human immunodeficiency virus type 1 integrase by beta-diketo acid metal complexes. *J. Med. Chem.,* **2006**, *49*, 4248-4260.

[24] Telesnitsky, A.; Goff, S.P. in *Retroviruses*, Coffin, J.M.; Hughes, H; Varmus, H.E. Eds., Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, **1997**, pp. 121-160.

[25] Hughes, S.H.; Arnold, E.; Hostomsky, Z. In *Ribonucleases H*, Crouch, R.J; Toulmé, J.J. Eds. Les Editions Inserm: Paris, **1998**; pp.195-224.

[26] Nikolenko, G.N.; Palmer, S.; Maldarelli, M.; Mellors, J.W.; Coffin, J.M.; Pathak, V.K. Mechanism for nucleoside analog-mediated abrogation of HIV-1 replication: Balance between RNase H activity and nucleotide excision. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 2093-2098.

[27] Haren, L.; Ton-Hoang, B.; Chandler, M. Integrating DNA: Transposases and retroviral integrases. *Annu. Rev. Microbiol.,* **1999**, *53*, 245-281.

[28] Klumpp, K.; Hang, J.Q.; Rajendran, S.; Yang, Y.; Derosier, A.; Wong Kai, P.; Overton, H.; Parkes, K.E.; Cammack, N.; Martin, J.A. Two-metal ion mechanism of RNA cleavage by HIV RNase H and mechanism-based design of selective HIV RNase H inhibitors. *Nucleic Acids Res.*, **2003**, *31*, 6852-6859.

[29] Klumpp, K.; Mirzadegan, T. Recent progress in the design of small molecule inhibitors of HIV RNase H. *Curr. Pharm. Drug*, **2006**, *12*, 1909-1922.

[30] Keck, J.L.; Goedken, E.R.; Marqusee, S. Activation/attenuation model for RNase H. A one-metal mechanism with second-metal inhibition. *J. Biol. Chem.*, **1998**, *273*, 34128-34133.

[31] Katayanagi, K.; Okumura, M.; Morikawa, K. Crystal structure of *Escherichia coli* RNase HI in complex with Mg2+ at 2.8 A resolution: proof for a single Mg(2+)-binding site. *Proteins,* **1993**, *17*, 337-347.

[32] Mizrahi, V.; Usdin, M.; Harington, A.; Dudding, L. Site-directed mutagenesis of the conserved Asp-443 and Asp-498 carboxy-terminal residues of HIV-1 reverse transcriptase. *Nucleic Acids Res.*, **1990**, *18*, 5359-5363.

[33] Mizrahi, V.; Brooksbank, R.; Nkabinde, N. Mutagenesis of the conserved aspartic acid 443, glutamic acid 478, asparagine 494, and aspartic acid 498 residues in the ribonuclease H domain of p66/p51 human immunodeficiency virus type I reverse transcriptase. Expression and biochemical analysis. *J. Biol. Chem.*, **1994**, *269*, 19245-19249.

[34] Andréola, M.L. Closely related antiretroviral agents as inhibitors of two HIV-1 enzymes, ribonuclease H and integrase: "Killing two birds with one stone". *Curr. Pharm. Des.,* **2004**, *10*, 3713-3723.

[35] Yang, W.; Hendrickson, W.A.; Crouch, R.J.; Satow, Y. Structure of ribonuclease H phased at 2 Å resolution by MAD analysis of the selenomethionyl protein. *Science,* **1990**, *249*, 1398-1405.

[36] Davies, J.F., II; Hostomska, Z.; Hostomsky, Z.; Jordan, S.R.; Matthews, D.A. Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. *Science,* **1991**, *252*, 88-95.

[37] Jochmans, D. Novel HIV-1 reverse transcriptase inhibitors. *Virus Res.*, **2008**, *134*, 171-185.

[38] De Clercq, E. Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. *Int. J. Antimicrob. Agents*, **2009**, *33*, 307-320.

[39] Tramontano, E.; Di Santo, R. HIV-1 RT-associated RNase H function inhibitors: recent advances in drug development. *Curr. Med. Chem.*, **2010**, *17*, 2837-2853.

[40] Shaw-Reid, C.A.; Munshi, V.; Graham, P.; Wolfe, A.; Witmer, M.; Danzeisen, R.; Olsen, D.B.; Carroll, S.S.; Embrey, M.; Wai, J.S.; Miller, M.D.; Cole, J.L.; Hazuda, D.J. Inhibition of HIV-1 ribonuclease H by a novel diketo acid, 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid. *J. Biol. Chem.,* **2003**, *278*, 2777-2780.

[41] Tramontano, E.; Esposito, F.; Badas, R.; Di Santo, R.; Costi, R.; La Colla, P. 6-[1-(4-Fluorophenyl)methyl-1H-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester a novel diketo acid derivative which selectively inhibits the HIV-1 viral replication in cell culture and the ribonuclease H activity *in vitro*. *Antiviral Res.,* **2005**, *65*, 117-124.

[42] de Soultrait, V.; Lozach, P.; Altmeyer, R.; Tarrago-Litvak, L.; Litvak, S.; Andréola, M.L. DNA aptamers derivated from HIV-1 RNase H inhibitors are strong anti-integrase agents. *J. Mol. Biol.,* **2002**, *324*, 195-203.

[43] Didierjean, J.; Isel, C.; Querré, F.; Mouscadet, J.F.; Aubertin, A.M.; Valnot, J.Y.; Piettre, S.R.; Marquet, R. Inhibition of human immunodeficiency virus type 1 reverse transcriptase, RNase H, and integrase activities by hydroxytropolones. *Antimicrob. Agents Chemother.,* **2005**, *49*, 4884-4894.

[44] Budihas, S.; Gorshkova, I.; Gaidamakov, S.; Wamiru, A.; Bona, M.; Parniak, M.; Crouch, R.; McMahon, J.; Beutler, J.; Le Grice, S. Selective inhibition of HIV-1 reverse transcriptase-associated ribonuclease H activity by hydroxylated tropolones. *Nucleic Acids Res.,* **2005**, *33*, 1249-1256.

[45] Semenova, E.A.; Johnson, A.A.; Marchand, C.; Davis, D.A.; Yarchoan, R.; Pommier, Y. Preferential inhibition of the magnesiumdependent strand transfer reaction of HIV-1 integrase by α-hydroxytropolones. *Mol. Pharmacol.,* **2006**, *69*, 1454-1460.

[46] Marchand, C.; Beutler, J.A.; Wamiru, A.; Budihas, S.; Mollmann, U.; Heinisch, L.; Mellors, J.W.; Le Grice, S.F.; Pommier, Y. Madurahydroxylactone derivatives as dual inhibitors of human immunodeficiency virus type 1 integrase and RNase H. *Antimicrob. Agents Chemother.,* **2008**, *52*, 361-364.

[47] Tramontano, E. HIV-1 RNase H: recent progress in an exciting, yet little explored, drug target *Mini-Rev. Med. Chem.*, **2006**, *6*, 727-737.

[48] Artico, M.; Di Santo, R.; Costi, R.; Novellino, E.; Greco, G.; Massa, S.; Tramontano, E.; Marongiu, M.E.; De Montis, A.; La Colla, P. Geometrically and conformationally restrained cinnamoyl-compounds as inhibitors of HIV-1 integrase: synthesis, biological evaluation and molecular modeling. *J. Med. Chem.,* **1998**, *41*, 3948-3960.

[49] Costi, R.; Di Santo R.; Artico, M.; Massa, S.; Ragno, R.; Loddo, R.; La Colla, M.; Tramomtano, E.; La Colla, P.; Pani, A. 2,6-Bis(3,4,5-trihydroxybenzylidene) derivatives of cyclohexanone: novel potent HIV-1 integrase inhibitors that prevent HIV-1 multiplication in cell-based assays. *Bioorg. Med. Chem.,* **2004**, *12*, 199-215.

[50] Pluymers, W.; Neamati, N.; Pannecouque, C.; Fikkert, V.; Marchand, C.; Burke, T.R., Jr.; Pommier, Y.; Schols, D.; De Clercq, E.; Debyser, Z.; Witvrouw, M. Viral entry as the primary target for the anti-HIV activity of chicoric acid and its tetra-acetyl esters. *Mol. Pharmacol.*, **2000**, *58*, 641-648.

[51] Hazuda, D.J.; Felock, P.; Witmer, M.V.; Wolfe, A.; Stillmock, K.; Grobler, J.A.; Espeseth, A.; Gabryelski L.; Schleif, W.A.; Blau, C.; Miller, M.D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science*, **2000**, *287*, 646-650.

[52] Hazuda, D.J.; Young, S.D.; Guare, J.P.; Anthony, N.J.; Gomez, R.P.; Wai, J.S.; Vacca, J.P.; Handt, L.; Motzel, S.L.; Klein, H.J.; Dornadula, G.; Danovich, R.M.; Witmer, M.V.; Wilson, K.A.; Tussey, L.; Schleif, W.A.; Gabryelski, L.S.; Jin, L.; Miller, M.D.; Casimiro, D.R.; Emini, E.A.; Shiver, J.W. Integrase inhibitors and cellular immunity suppress retroviral replication in rehesus macaques. *Science*, **2004**, *305*, 528-532.

[53] Di Santo, R.; Costi, R.; Artico, M.; Tramontano, E.; La Colla, P.; Pani, A. HIV-1 integrase inhibitors that block HIV-1 replication in infected cells. Planning synthetic derivatives from natural products. *Pure Appl. Chem.,* **2003**, *75*, 195-206.

[54] Di Santo, R.; Costi, R.; Artico, M.; Ragno, R.; Greco, G.; Novellino, E.; Marchand, C.; Pommier, Y. Design, synthesis and biological evaluation of heteroaryl diketohexenoic and diketobutanoic acids as HIV-1 integrase inhibitors endowed with antiretroviral activity. *Il Farmaco,* **2005**, *60*, 409-417.

[55] Costi, R.; Di Santo R.; Artico, M.; Roux, A.; Ragno, R.; Massa, S.; Tramontano, E.; La Colla, M.; Loddo, R.; Marongiu, M.E.; Pani, A.; La Colla, P. 6-Aryl-2,4-dioxo-5-hexenoic acids, novel integrase inhibitors active against HIV-1 multiplication in cell-based assays. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 1745-1749.

[56] Di Santo, R.; Costi, R.; Roux, A.; Artico, M.; Lavecchia, A.; Marinelli, L.; Novellino, E.; Palmisano, L.; Andreotti, M; Amici, R.; Galluzzo, C.M., Nencioni, L.; Palamara, A.T.; Pommier, Y.; Marchand, C. Novel bifunctional quinolonyl diketo acid derivatives as HIV-1 integrase inhibitors: design, synthesis, biological activities and mechanism of action. *J. Med. Chem.*, **2006**, *49*, 1939-1945.

[57] Di Santo, R.; Costi R.; Roux, A.; Miele, G.; Cuzzucoli Crucitti, G.; Iacovo, A.; Rosi, F.; Lavecchia, A.; Marinelli, L.; Di Giovanni, C.; Novellino, E.; Palmisano, L.; Andreotti, M.; Amici, R.; Galluzzo, C.M.; Nencioni, L.; Palamara, A.T.; Pommier, Y.; Marchand, C. Novel quinolinonyl diketo acid derivatives as HIV-1 integrase inhibitors: design, synthesis and biological activities. *J. Med. Chem.,* **2008**, *51*, 4744-4750.

[58] Bona, R.; Andreotti, M.; Buffa, V.; Leone, P.; Galluzzo, C.M.; Amici, R.; Palmisano, L.; Mancini, M.G.; Michelini, Z.; Di Santo, R.; Costi, R.; Roux, A.; Pommier, Y.; Marchand, C.; Vella, S.; Cara, A. Development of a human immunodeficiency virus vector-based, single-cycle assay for evaluation of anti-integrase compounds. *Antimicrobial Ag. Chemother.,* **2006**, 50, 3407-3417.

[59] Cianfriglia, M.; Dupuis, M.L.; Molinari, A.; Verdoliva, A.; Costi, R.; Galluzzo, C.M.; Andreotti, M.; Cara, A.; Di Santo, R.; Palmisano, L. HIV-1 integrase inhibitors are substrates for the multidrug transporter MDR1-P-glycoprotein. *Retrovirology,* **2007**, *4*, 17.

[60] Terrazas-Aranda, K.; Van Herrewege, Y.; Hazuda, D.; Lewi, P.; Costi, R.; Di Santo, R.; Cara, A.; Vanham, G. Human immunodeficiency virus type 1 (HIV-1) integration: a potential target for microbicides to prevent cell-free or cell-associated HIV-1 infection. *Antimicrob. Agents Chemother.,* **2008**, *52*, 2544-2554.

[61] Jegede, O.; Babu, J.; Di Santo, R.; McColl, D. J.; Weber, J.; Quiñones-Mateu, M.E. HIV type 1 integrase inhibitors: from basic research to clinical implications. *AIDS Rev.,* **2008**, *10*, 172-189.

[62] Michelini, Z.; Galluzzo, C.M.; Negri, D.R.; Leone, P.R.; Amici, R.; Bona, R.; Summa, V.; Di Santo, R.; Costi, R.; Pommier, Y.; Marchand, C.; Palmisano, L.; Vella, S.; Cara, A. Evaluation of HIV-1 integrase inhibitors on human primari macrophages using a luciferase-based single-cycle phenotypic assay. *J. Virol. Methods,* **2010**, *168*, 272-276.

[63] Johnson, A.A.; Marchand, C.; Patil, S.S.; Costi, R.; Di Santo, R.; Burke, T.R., Jr.; Pommier, Y. Probing HIV-1 integrase inhibitor binding sites with position-specific integrase-DNA cross-linking assays. *Mol. Pharmacol.,* **2007**, *71*(3), 893-901.

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