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6-Aryl-2,4-dioxo-5-hexenoic acids, novel integrase inhibitors active against HIV-1 multiplication in cell-based assays☆

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Abstract—A series of 6-aryl-2,4-dioxo-5-hexenoic acids, were synthesized and tested against HIV-1 in cell-based assays and against recombinant HIV-1 integrase (rIN) in enzyme assays. Compound **8a** showed potent antiretroviral activity ($EC_{50} = 1.5$ μM) and significant inhibition against rIN (strand transfer: $IC_{50} = 7.9$ μM; 3′-processing: $IC_{50} = 7.0$ μM). A preliminary molecular modeling study was carried out to compare the spatial conformation of **8a** with those of L-731,988 (**4**) and 5CITEP (**7**) in the IN core.

Acquired immunodeficiency syndrome (AIDS) is the consequence of the infection with human immunodeficiency virus type 1 (HIV-1). Among retroviral targets useful for chemotherapeutic intervention, reverse trascriptase (RT) and protease (PR) play a fundamental role and many potent and selective inhibitors of these enzymes are actually used in the clinical practice. Drugs approved so far include nucleoside (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs),[1] such as AZT, ddI, d4T, nevirapine, delavirdine, efavirenz, and protease inhibitors (PRIs)[2] such as saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, and lopinavir.

Due to failure of monotherapy with the above inhibitors, combination protocols were designed with both RTIs and PRIs in order to increase the clinical efficacy and reduce the emergence of resistant variants. However, also the combination therapy has failed to provide long-term suppression of HIV-1 replication in infected individuals. Rapid development of drug resistance and toxicity problems make urgent the need to investigate new targets in the replicative cycle of HIV-1 to develop inhibitors different from RTIs and PRIs.[3,4]

Besides RT and PR, another target useful for chemotherapeutic intervention is the HIV-1 integrase (IN), an enzyme which catalyzes the insertion of the viral DNA into the genome of the host cell through a multistep process. This includes: (i) assembly of integrase and viral DNA; (ii) endonucleolytic processing of viral DNA; (iii) covalent insertion of viral DNA into the host cell DNA.[5–9]

Many different classes of compounds[5–8] have been reported to inhibit the HIV-1 IN in enzyme assays. Among them, natural and synthetic polyhydroxylated molecules have emerged as potent IN inhibitors. However, only very few compounds of this class have been shown to possess specific anti-IN activity in enzyme

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assays and proved active in cell-based assays. Notable examples of natural products are L-chicoric acid (L-CCA) (**1**)[10] and 3,5-dicaffeoylquinic acid (3,5-DCQA) (**2**)[11] which, although capable of specific anti-IN activity in enzyme and PIC assays, have been proven to target the viral adsorption step.[12] Among the synthetic products, some properly substituted styrylquinolines (**3**)[13,14] have been reported to possess specific anti-IN activity and to be active in cell-based assays in the low micromolar concentration range.

More recently, compounds containing a distinct dioxobutanoic acid moiety (**4–6**) have been identified as potent and specific inhibitors of HIV-1 multiplication targeting the integration process (Chart 1).[7,15–17] The anti-IN activity was also retained when (i) the terminus carboxylic function was masked by a tetrazole ring (**7**) (Chart 2);[8] (ii) the dioxobutanoic group was shortened into oxopropanoic moiety.[18] On the contrary, to our knowledge, no attempts were found to length the dioxobutanoic group.

Because of the rising interest in dioxobutanoic acids as potent integrase inhibitors we were pulsed to explore how elongation of the diketoacid chain would affect anti-integrase activity. As a first attempt we designed some dioxohexenoic acids, whose acid side chain was conceived as the result of a partial superimposition between the cinnamoyl group of various natural and synthetic anti-IN substances (that is **1–3**) and the dioxobutanoic acid moiety of the recently reported inhibitors **4–6** (Chart 3).

Therefore, taking Merck derivative **4** as a lead compound we planned the synthesis of its vinylog 1-[(4-fluorophenylmethyl)-2-pyrrolyl]-1,4-dioxo-5-hexenoic acid **8c** and some related derivatives **8a**,**b**, **d–f** and **11a–f** (Scheme 1).

Our hypothesis that the insertion of a double bond between the phenylmethylpyrrol-1-yl and 2,4-dioxobutanoic acid moieties would afford compounds capable of retaining the anti-rIN activity of the lead compound **4**, was supported by a three-dimensional (3D) structure-based drug design (SBDD) and a molecular modeling (MM) investigation aimed at understanding the possible binding mode of the newly synthesized derivatives **8–11**

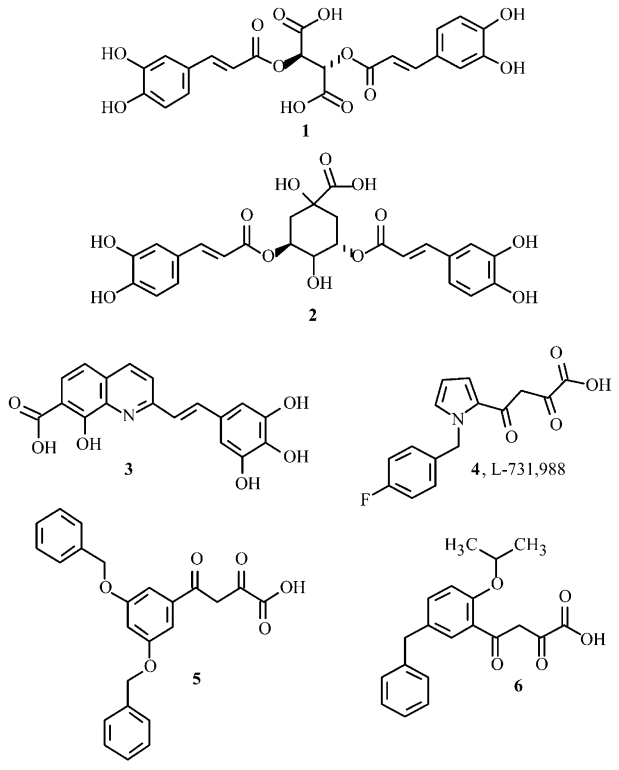


Chart 1. Anti-IN inhibitors and related derivatives.

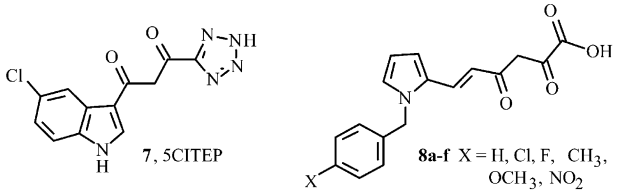


Chart 2. 5CITEP and newly synthesized anti-IN derivatives.

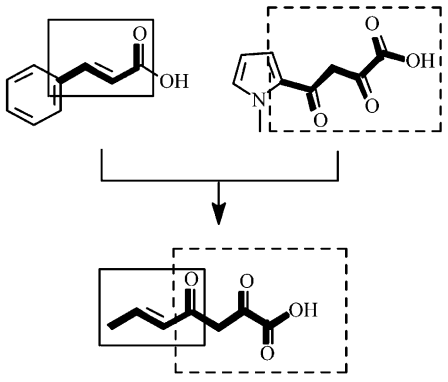
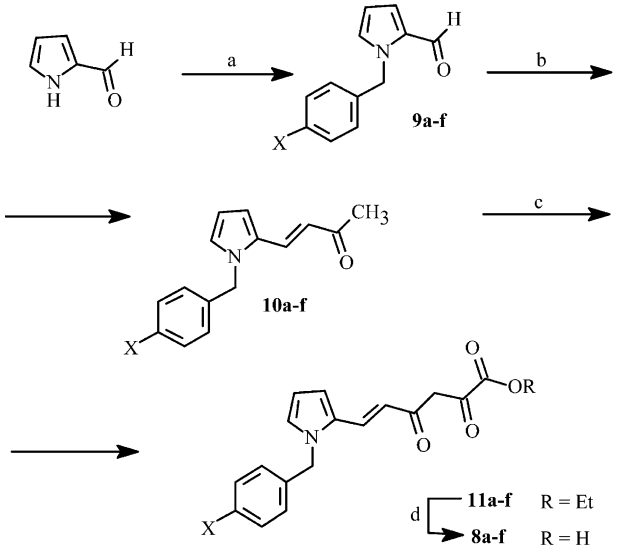


Chart 3. Design of dioxohexenoic acids.



X = H (**a**), Cl (**b**), F (**c**), Me (**d**), OMe (**e**), NO_2 (**f**)

Scheme 1. (a) Benzylchloride (**b**,**e**,**f**) or bromide (**a**,**c**,**d**,), K_2CO_3, DMF, 18 h, 90 °C; (b) 2-Propanone, 5N NaOH, 24 h, 25 °C; (c) Diethyl oxalate, NaOEt, 1 h 45 min, 25 °C; (d) 1 N NaOH, 1.5 h, 25 °C.

in the HIV-1 IN enzymatic core. Compound **8a** was chosen as the prototype of the novel 2,4-dioxo-5-hexenoic acid class.

In the 1999, Goldgur et al.[8] reported the structure of the HIV-1 integrase catalytic domain complexed with the reference structure **7**. In this structure the 5CITEP seems to mimic the DNA substrate/integrase interaction lying in the middle of the active site of enzyme subunit A between the three catalytic acidic residues, Asp-64, Asp-116 and Glu-152, without displacing the bound magnesium ion which remains complexed to the two aspartates. During further inspections of the 5CITEP binding mode, Sotriffer et al.[6] pointed out that the 5CITEP bound conformation might be influenced by the crystal packing and that a more reliable binding mode could be represented by their docking calculations. More recently Barreca et al.[19] reported a further different binding mode for the 5CITEP obtained by means of molecular dynamics studies.

Although we were aware of some discrepancies between the theoretical models and Goldgur studies, we believed that the experimental data, taken from Brookhaven Protein Data Bank[20,21] (entry code 1qs4), could be more reliable for supporting our docking experiments.

Therefore, we used the 5CITEP structure (**7**), extracted from the corresponding HIV-1 soaked complex,[9] as a template to model the 3D structure of **8a** and **11a**. For direct comparison purposes, the Merck compound **4** was modeled in the same way. Monte Carlo type conformational searches were carried out on isolated IN inhibitors (MCMM routine with continuum solvent simulation). Superimposition of the obtained global minima structures of **4**, **8a** and **11a** to the structure of 5CITEP (**7**) via atom by atom procedure displayed a good overlapping (not shown).[22]

The above 3D resemblance suggested that these structures could share a common binding site for the diketoacid and tetrazolyldioxopropane portions. In pursuing this hypothesis, the global minima structures of **4**, **8a** and **11a** were inserted in turn within the IN receptor site in place of **7**, with formation of three new complexes: IN/**4**, IN/**8a** and IN/**11a**. The latter were structurally refined by the aid of molecular mechanic geometry optimization software (MACROMODEL 6.5, AMBER all atom force field).[23] Relaxation of the 8 Å receptor core around the inhibitor was allowed during minimization. Again, good sterical agreement was observed among the three structures (Fig. 1).[24] Inspection of the minimized complexes reveals that the pharmacophoric features of **4**, **7**, **8a** and **11a** share a common ligand/receptor interaction pattern. Such a receptor-based structure alignment could be used: (i) as a rule in future 3D quantitative structure activity relationship (QSAR) studies;[25]; (ii) to define the pharmacophore to be used in 3D data-base searches.[26]

On the basis of the above preliminary MM study, both **8a** and **11a** were expected to show anti-rIN activity in enzyme assays.

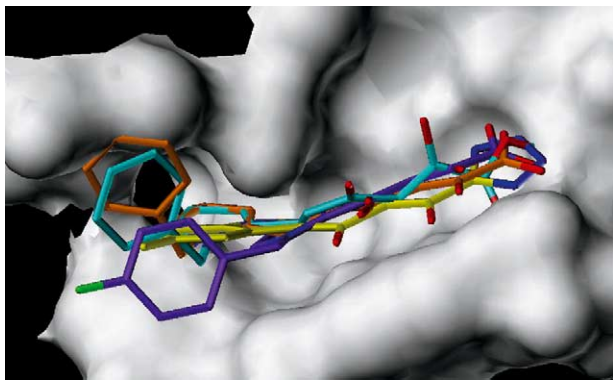


Figure 1. Superimposition **4** (purple), **7** (yellow), **8a** (orange) and **11a** (cyan) after their minimization in the IN catalytic core (white).

Scheme 1 depicts the chemical pathway employed in the synthesis of arylmethylpyrrolyldiketohexenoic acids **8a–f**. 1*H*-Pyrrole-2-carboxaldehyde was treated with 4-substituted phenylmethylhalides to afford 1-arylmethyl-1*H*-pyrrole-2-carboxaldehydes **9a–f**, which were then reacted with 2-propanone to yield 4-(1-arylmethyl-1*H*-pyrrol-2-yl)-3-buten-2-ones **10a–f**. Claisen condensation of **10a–f** with diethyl oxalate in the presence of sodium ethoxide led to the formation of 6-(1-arylmethyl-1*H*-pyrrol-2-yl)-2,4-dioxo-5-hexenoic acid ethyl esters **11a–f**, which were hydrolyzed in alkaline medium to give title acids **8a–f**.

Title compounds were screened to provide informations on inhibition of the HIV-1 IN replication either in cell culture and in enzyme assays. The assays were made in parallel with those of **4** used as reference compound. Cytotoxicity of compounds, evaluated in parallel with their antiviral activity, was based on the viability of mock-infected cells, as monitored by the MTT method. Cell-based and enzyme assays were performed as previously described[27–29] and the related experimental procedures are reported in the Supporting Information section.

The antiretroviral activity of the unsubstituted derivative **8a** and its ethyl ester **11a** against HIV-1 in MT-4 cells and the effects on activity produced by introduction of substituents on the phenyl rings (**8b–f** and **11b–f**, respectively) were examinated to acquire preliminary SAR informations.

The results of assays are reported in Table 1. **8a** and its ethyl ester **11a** showed antiviral activities ($EC_{50} = 1.5$ μM) comparable to that of L-731,988 (**4**) ($EC_{50} = 1.5$ μM), one of the most promising integrase inhibitors described up to date in the literature. When tested in enzyme assays derivatives **8a** and **11a** were found the most potent inhibitors (3′-processing: 7.9 and 8.9 μM; strand transfer: 7.0 and 7.5 μM, respectively).

The remaining derivatives **8b–f** and **11b–f** inhibited integrase at higher concentrations.

An example of gel showing the inhibitory activities of **8a**,**b** and **11a** compared with those obtained for **4** in enzyme assays is reported in Figure 2.

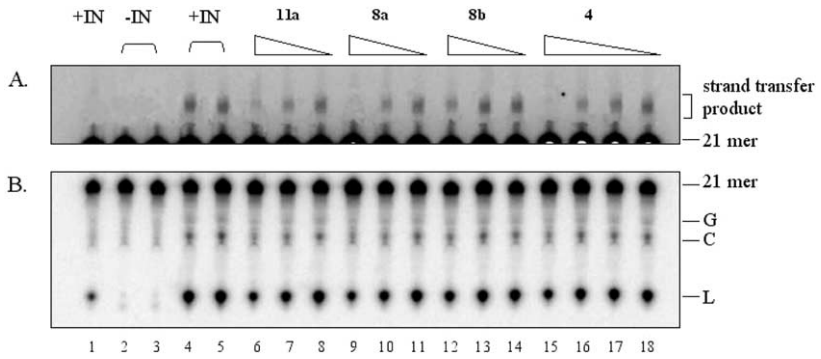
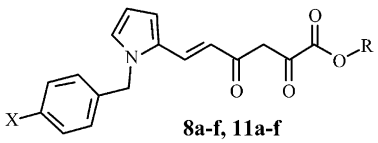


Figure 2. Inhibition of HIV-1 IN-catalyzed 3′-processing and strand-stransfer reactions by **8a**,**b** and **11a** derivatives compared with **4**. The strand-transfer products migrate slower than the 21-mer substrate (Panel A, darker exposure) and the 3′-processing products glycerol-GT (G), cyclic-GT (C) and linear-GT (L) (Panel B, lighter exposure). Lane 1, DNA and IN without drugs incubated for 10 min at 37 °C; lanes 2–3, DNA alone incubated for 1 h at 37 °C; lanes 4–5, DNA and IN without drugs incubated for 1 h at 37 °C, lanes 6–18 DNA, IN and a titration of: lanes 6–8, **11a** (100, 10, 1 μM); lanes 9–11, **8a** (100, 10, 1 μM), lanes 12–14, **8b** (100, 10, 1 μM); lanes 15–18 **4** (10, 1, 0.1, 0.01 μM). Samples of lanes 6–18 were incubated for 1 h at 37 °C.

Table 1. Cytotoxicity and antiviral activities in enzyme (IN) and cell-based assays of derivatives **8a–f** and **11a–f**[a]



8a-f, 11a-f

Compd[b]	X	R	CC^{c}_{50} (μM)	EC^{d}_{50} (μM)	S.I.[e]	IC_{50} (μM)[f]	
						3′-Proc.	S.T.
8a	H	H	61	1.5	41	7.9	7.0
11a	H	Et	17	1.5	11	8.9	7.5
8b	Cl	H	95	>95		50	65
11b	Cl	Et	52	>52		85	90
8c	F	H	80	11	7.3	61	72
11c	F	Et	55	12	4.4	87	95
8d	Me	H	41	>41		56	67
11d	Me	Et	7	>7		73	88
8e	OMe	H	78	>78		22	41
11e	OMe	Et	27	>27		38	50
8f	NO_2	H	33	>33		76	92
11f	NO_2	Et	31	>31		57	45
4			54	1.5	36	2.0	0.3

[a] Data represent mean values for thee independent determinations.
[b] See ref 30.
[c] Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50% as determined by the MTT method.
[d] Compound concentration required to reduce the exponential growth of MT-4/KB cells by 50%.
[e] Selectivity index: CC_{50}/EC_{50} ratio.
[f] Compound concentration required to reduce rIN 3′-proc. and S.T. of 3′-end-labeled 40mer substrate by 50%.

From data of antiviral assays compounds **8a** and **11a** turned out to be the most potent and selective in cell-based assays. The introduction of a fluorine atom at the 4-position of the phenyl ring (**8c** and **11c**) did not lead to an improvement of antiviral activity but, contrary to expectations, it caused a seven-fold loss of potency. In addition, the introduction of chlorine (**8b** and **11b**), methyl (**8d** and **11d**), methoxy (**8e** and **11e**) or nitro (**8f** and **11f**) substituents led to totally inactive products.

When tested in enzyme assays against HIV-1 rIN in both strand transfer and 3′-processing reactions, only compound **8a** and its ester **11a** showed inhibitory activity in the micromolar range (Table 1), whereas all 4-substituted phenylmethyl derivatives were active at concentrations ranging from 22 to 95 μM.

As a rule, acid derivatives (**8a–f**) were less cytotoxic than the corresponding esters (**11a–f**), whereas no difference in cell-based antiviral activities was observed by comparing the active acids (**8a** and **11a**) with the related esters (**8c** and **11c**).

The different steric and electronic arrangement of the phenylmethyl moieties of **8a** and **4** in the enzyme binding site (Fig. 1) could very likely account for: (i) the low inhibitory activity of **8a** compared with **4** in the strand transfer reaction; ii) the absence of selectivity between strand transfer and 3′-processing inhibitory activities of **8a** in enzyme assays. Furthermore, the steric hindrance along with differences in the electronic effects exerted by the substituents in the position 4 of the phenyl ring could be a determinant for the low activities of **8b–f**.

On the basis of the above results, we conclude that 6-aryl-2,4-dioxo-5-hexenoic acids are novel inhibitors of the HIV-1 rIN which show anti-HIV-1 activity in cell-based assays. Because of its favorable selectivity index, **8a** is a promising lead for further studies. These will clarify whether the higher activity shown by this compound in cell-based assays compared to that in enzyme assays, is related to the possibility that **8a** targets additional viral enzymes.

Further studies, such as 3D QSAR and docking, are ongoing to get an insight into the design of new, more potent derivatives and to prioritize their synthesis.

Acknowledgements

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30. All new compounds exhibited satisfactory spectroscopic and analytical properties. Data for compound **8a**, including chemical and physical data, are reported: yield 68%, mp 146–148 °C (from toluene). IR (nujol): cm^{-1} 3400 (OH), 1700 (CO acid) and 1590 (CO); ^1H NMR (200 MHz, DMSO-d_6): δ 5.43 (s, 2H, CH_2), 6,30 (m, 1H, pyrrole C4-H), 6.45 (s, 1H, C3-H), 6.69 (d, 1H, $J_t = 15.4$ Hz, C5-H), 7.01–7.40 (m, 7H, pyrrole C3-H and C5-H and benzene H), 7.65 (d, 1H, $J_t = 15.4$ Hz, C6-H), 15.50 (bs, 2H, OH). Anal. calcd for $C_{17}H_{15}NO_4$ (297.31): C, 68.68; H, 5.09; 4.71. Found: 68.63; 5.18; 4.74. Data for compounds **8b–f** and **11a–f** are reported in the Supplementary Information section.