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Design, synthesis and biological evaluation of heteroaryl diketohexenoic and diketobutanoic acids as HIV-1 integrase inhibitors endowed with antiretroviral activity

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Abstract

Highly active anti-retroviral therapy (HAART) using reverse transcriptase (RT) and protease (PR) inhibitors and, more recently, inhibitors of the fusion is currently the best clinical approach in combating acquired immunodeficiency syndrome (AIDS), caused by infection from human immunodeficiency virus type 1 (HIV-1). However, this therapy does not completely eradicate the virus, so that resistant strains easily emerge. The above problem calls urgently for research on inhibitors of further viral targets such as integrase (IN), the third enzyme produced by HIV. Recently, our research group was engaged in studies on conformationally restrained cinnamoyl compounds related to curcumin as anti-IN agents. Compounds containing both a 3,4,5-trihydroxyphenyl group and a carboxylic acid function were potent IN inhibitors active against viral replication. More recently, a promising new class of inhibitors synthesized by Merck Company has emerged, which contain aryldiketoacid (ADK) functionality. The ADKs selectively inhibited the stand transfer (ST) step of integration and were proven to be effective IN inhibitors in vivo. Our interest in the field of IN inhibitors led us to designe pyrrole and indole derivatives containing both a cinnamoyl moiety and a diketoacid group. A number of the cited derivatives were proven potent IN inhibitors, which selectively inhibited the ST step at submicromolar concentrations and were effective against virus replication in HIV-1 infected cells.

Keywords: Integrase inhibitors; Anti-HIV agents; Pyrrole derivatives; Indole derivatives; Diketoacid derivatives

1. Introduction

Three different classes of chemotherapeutic agents are actually available to block the replication of human immunodeficiency virus (HIV, responsible of acquired immunodeficiency syndrome (AIDS)), namely reverse transcriptase inhibitors (RTI) [1] protease inhibitors (PRI) [2] and, more recently, inhibitors of the fusion [3]. The combination therapy based on the use of above drugs (highly active anti-retroviral therapy (HAART)) effectively inhibits the replication cycle of HIV.

In the recent years, the advent of this therapy has made possible to suppress HIV replication to such an extent that the virus becomes undetectable in many infected persons, leading to a reduction of both mortality and morbidity [4]. However, despite chemotherapy, these treatments fail to eradicate viral replication, which persists at a lower level. In such conditions HIV-1 escapes the therapeutic protocol via gen-

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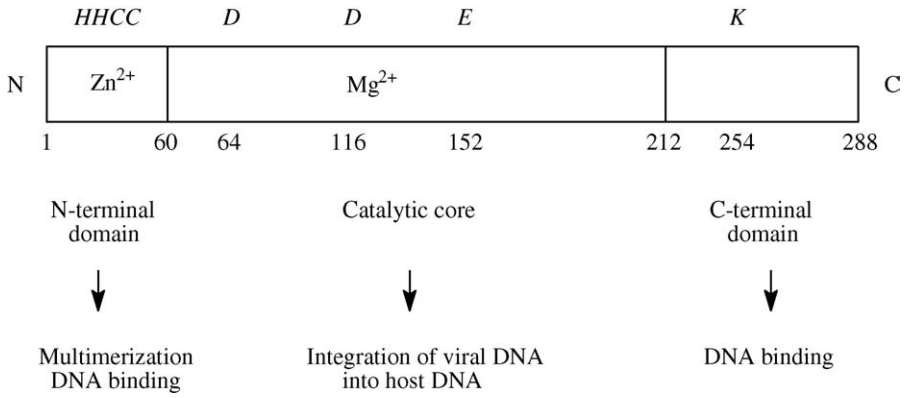


Fig. 1. Structure of HIV-1 integrase.

eration of resistant mutant strains. The reasons of this evolution are i) the suboptimal regimens due to the high toxicity of drugs, ii) the low compliance of patients in long-term therapies.

Therefore, it is essential to develop drugs targeted at alternative steps of the viral replication cycle. The integration of retro-transcribed viral DNA into the host chromosome is a vital step in the replication cycle of retroviruses performed by integrase (IN) [5] enzyme. After integration the proviral DNA is replicated and genetically transmitted as part of the cellular genome. Thus, integration defines a point of no return in the life cycle of HIV.

HIV-1 IN is a very attractive target to develop new anti-HIV drugs because: i) it plays a vital role in replication cycle; ii) it has no cellular counterpart [6] and iii) actually, no IN inhibitor is used in clinical practice. Recently, anti-IN agents in combination with RT and PR inhibitors have been found to be synergistic in in vitro assays [7] and a combination therapy, which use inhibitors of all three enzymes at the same time, could result in a real breakthrough in the HIV-1 therapy. Unfortunately, no inhibitor of HIV-1 integrase is currently used in clinical practice, and this fact led us to make great efforts for developing researches in this field.

The full length HIV-1 integrase (288 amino acids) has three domains: the catalytic core, the C-terminal, and the N-terminal domains (Fig. 1). It is thought that the catalytic core contains the active site responsible for the catalysis of all the reactions of integration. Three amino acids (Asp64, Asp116 and Glu152) in the catalytic core domain are highly conserved among retrotransposon and retroviral integrases. Mutation of such residues generally leads to a loss of all the catalytic activities of these proteins; therefore Asp64, Asp116 and Glu152 are essential components of the integrase active site [8].

The catalytic core domain soacked with 5CITEP (a potent inhibitor of IN reported by Shionogi & Co.) was resolved by Goldur et al. [9]. The crystal structure shows a dimeric model, in which two monomers interact with each other, but actually it is not clear whether IN works in vivo as a monomer, a dimer or a tetramer.

Multiple steps in the integration process are catalyzed by HIV-1 integrase, as shown schematically in Fig. 2.

The integration of HIV-1 DNA into the host chromosome is achieved by IN, which performs a series of DNA cutting and joining reactions. The first step in the integration is 3′-processing in which the enzyme removes two nucleotides from each 3′ end of the proviral DNA, leaving recessed CA OH's at the 3′ ends. In the second step, termed strand transfer, IN joins the previously processed 3′-OH ends to the target DNA in a transesterification reaction (Fig. 2). The integration sites are 5 bases apart on each strand of the target

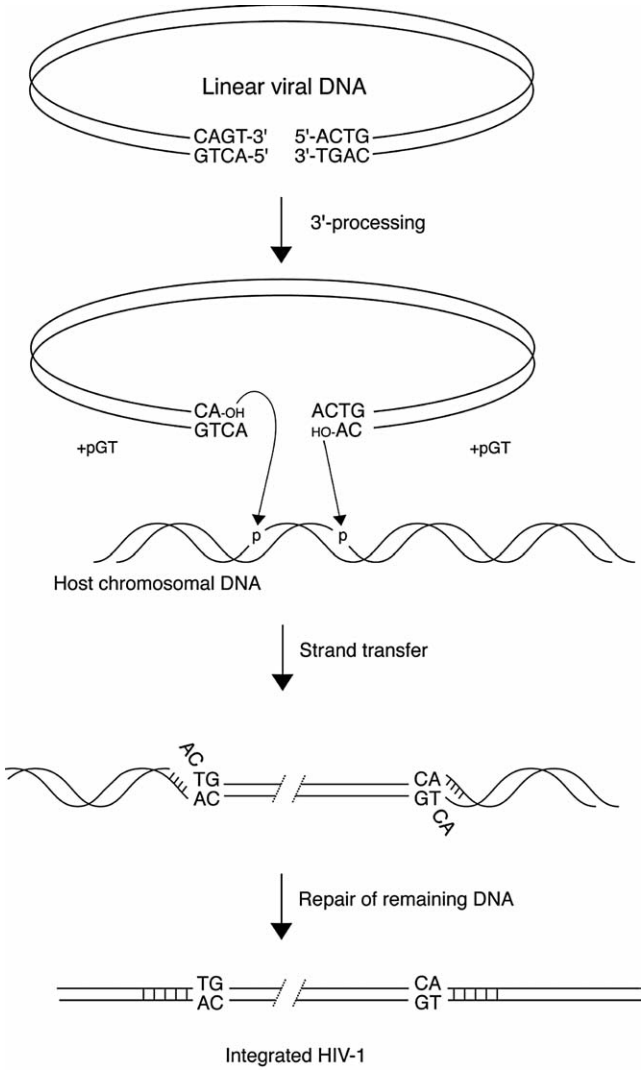


Fig. 2. Outline of the integration reaction in vivo.

DNA [10]. Finally, removal of the two unpaired nucleotides at the 5′-end of the viral DNA and gap-filling process lead to a fully repaired integrated product (Fig. 2). These events are probably accomplished by cellular components.

2. Polyhydroxylated aromatic inhibitors

In the last few years, a number of natural (caffeic acid phenethyl ester (CAPE) [11], quercetin [12], chicoric acid [13], curcumin [14] and 3,5-dicaffeoylquinic acids [15]) or synthetic (catechols [16], flavones [17]) polyhydroxylated compounds have proven to inhibit HIV-1 IN in in vitro assays, but failed to provide anti-viral efficacy in HIV-1 infected cells. Notable exceptions were chicoric acid and curcumin, which were active in cell-based assays (Fig. 3).

More recently, our research group was engaged in studies on conformationally restrained cinnamoyl compounds related to curcumin as anti-IN agents [18] proving that: i) the catechol is a potent pharmacophore, giving the maximum anti-IN potency; ii) removal of OH groups, or their methylation abated the activity; iii) the cinnamoyl group is a very good pharmacogenic moiety to obtain potent IN inhibitors. So we synthesized a number of polyhydroxybenzylidenecycloalkanones active against IN enzyme at nanomolar concentrations, but devoid of antiviral activity in cell-based assays at subcytotoxic concentrations (Fig. 4).

The evidence that the cytotoxicity of the above compounds was due to the catechol group [19], led us to replace this moiety with bioequivalent molecular portions. In particular the 3,4,5-trioxyphenyl (Fig. 5), gave the best results. In such way, we obtained for the first time IN inhibitors active against viral replication. Moreover, the introduction of a carboxylic function (found in natural derivatives, i.e. chicoric or rosmarinic acid, or synthetic compounds, i.e. styrylquinolines [20]) gave a further increase in the potency of these inhibitors [21,22].

However, recent reports about the possible alternative mechanism of inhibition of the viral cycle involving the viral entry and not the integration as predominant antiviral target in cell culture assays [3,23,24], led us to start new research in this field.

3. Aryldiketoacids

More recently a promising new class of inhibitors has emerged, which contain aryldiketoacid (ADK) functionality. In general, this family is characterized by an ability to afford preferential inhibition of stand transfer (ST) versus 3′-P reactions. Additionally, ADK class not only selectively inhibits ST in extra cellular assays using recombinant IN, but also provide anti-viral protection in HIV-infected cells by mechanisms consistent with inhibition of IN. In particular, drug-

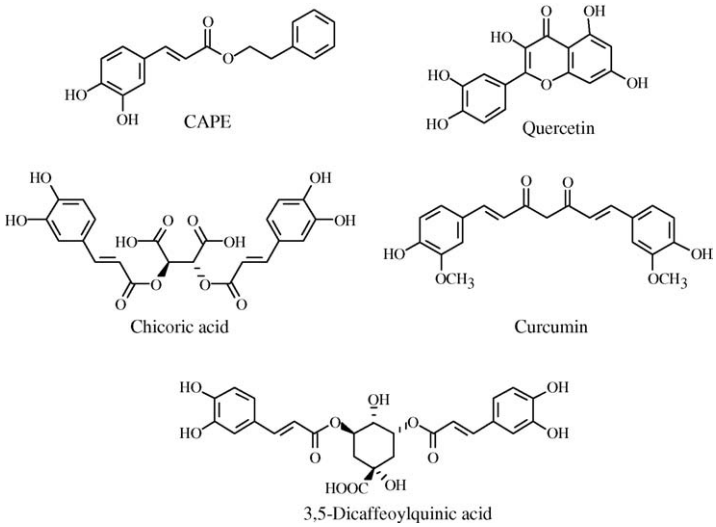


Fig. 3. Polyhydroxylated integrase inhibitors.

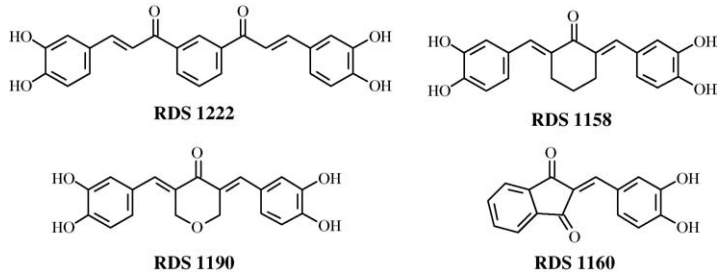


Fig. 4. Conformationally restrained cinnamoyl compounds related to curcumin as integrase inhibitors in vitro: examples of the most potent derivatives.

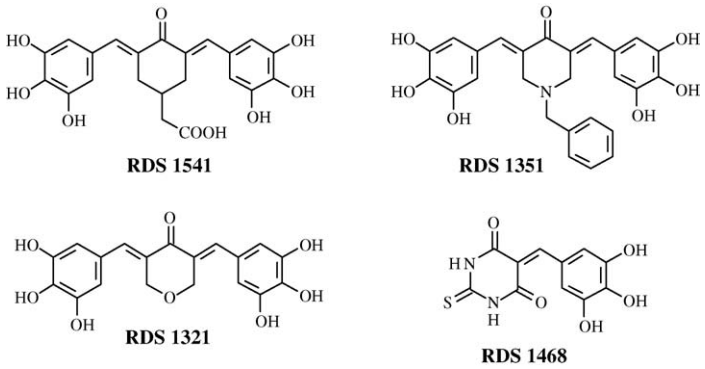


Fig. 5. Trihydroxycinnamoyl derivatives as integrase inhibitors in vitro endowed with antiviral activity in cell-based assays: examples.

resistant HIV strains were selected and shown to carry mutations in integrase gene [25].

The lead compound of this novel class of IN inhibitors is the pyrrolyldiketobutanoic acid derivative L-731,988 synthesized by Merck [25]. At the same time an indole derivative, namely 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)propenone (5CITEP) [26] was produced by Shionogi, in which the carboxylic function is masked by a tetrazole ring (Fig. 6). Since the discovery of L-731,988 and 5CITEP a number of new ADKs were reported (L-708,906, S-1360 etc.) as IN inhibitors endowed with antiviral activity. The relevance of the ADK inhibitors as anti-HIV-1 agents is confirmed by the recent submission of S-1360 to II phase of clinical trials. Interestingly, the anti-IN activity of ADKs was also retained when the diketobutanoic group was shortened into oxopropanoic moiety [27]. On the contrary, to our knowledge, no attempts were found to elongate the diketobutanoic group.

3.1. a) Pyrrolyldiketohexenoic acids and related derivatives

An examination of chemical structures of the polyhydroxylated compounds and the aryldiketo derivatives led us to design a new class of IN inhibitors, the 1-arylmethyl-1*H*-pyrrol-2-yl-dioxo-5-hexenoic acids [28,29], characterized by the following features: i) the cinnamoyl moiety of natural products and synthetic derivatives, in which the phenyl ring is replaced by the bioisosteric pyrrole moiety; ii) the diketo group of ADK series; iii) the carboxylic function of natural products (i.e. chicoric acid), in diketo acids series (i.e. L-731,988) and in our derivatives; iv) the aromatic portion of ADKs and cinnamoyl derivatives (i.e. 1-benzylpyrrole, indole, or trihydroxyphenyl group).

In particular, the above diketohexenoic acids are characterized by a side chain conceived as the result of a partial superimposition between the cinnamoyl group of various natural and synthetic anti-IN substances and the diketobutanoic acid moiety of the recently reported ADKs (Fig. 7). Moreover, the newly synthesized aryldiketohexenoic acids, seem a promising tool to explore how elongation of the diketoacid chain would affect anti-integrase activity.

The design of such structures was supported by preliminary molecular modeling (MM) studies about the possible binding mode of the newly synthesized derivatives in the IN enzymatic core. The 5CITEP structure extracted from the corresponding soaked complex [9], was used as a template to model the 3-D structure of our derivatives, which were also compared to L-731,988. Inspection of the minimized complexes with IN of 5CITEP, L-731,988 and our prototypes **RDS 1699** and **RDS 1698** (Table 1) reveals that these inhibitors share a common binding mode (Fig. 8).

The synthesis of arylmethylpyrrolyldiketohexenoic acids is reported in Scheme 1. 1*H*-Pyrrole-2-carboxaldehyde was treated with substituted phenylmethylhalides to afford 1-arylmethyl-1*H*-pyrrole-2-carboxaldehydes, which were then reacted with 2-propanone to yield 4-(1-arylmethyl-1*H*-pyrrol-2-yl)-3-buten-2-ones. Claisen condensation of the last compounds with diethyl oxalate in the presence of sodium ethoxide led to the formation of 6-(1-arylmethyl-1*H*-pyrrol-

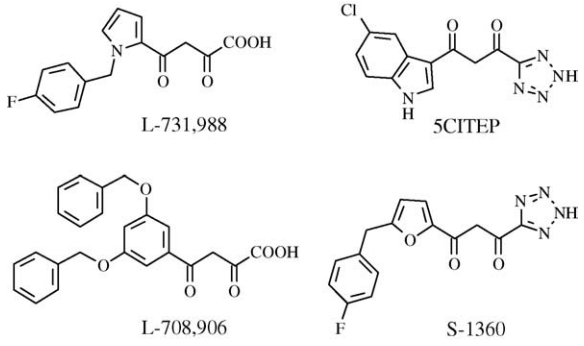


Fig. 6. Aryldiketoacid (ADKs) derivatives which inhibit selectively strand transfer reaction and virus replication.

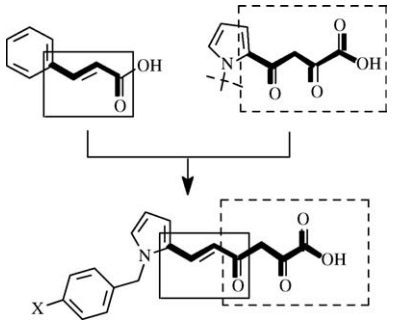
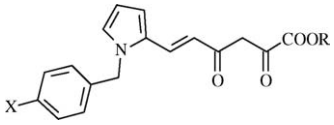


Fig. 7. Design of diketohexenoic acids.

Table 1
Cytotoxicity and antiviral activities in cell-based assays of 4-substituted phenylmethyl pyrrolyldiketo hexenoic acids and ethyl esters [a]



Compound	X	R	CC_{50} [b] (μM)	EC_{50} [c] (μM)	S.I.[d]
RDS 1699	H	H	61	1.5	41
RDS 1698	H	Et	17	1.5	11
RDS 1714	Cl	H	95	> 95	
RDS 1713	Cl	Et	52	> 52	
RDS 1644	F	H	80	11	7.3
RDS 1643	F	Et	55	12	4.4
RDS 1693	Me	H	41	> 41	
RDS 1692	Me	Et	7	> 7	
RDS 1716	OMe	H	78	> 78	
RDS 1715	OMe	Et	27	> 27	
RDS 1704	NO_2	H	33	> 33	
RDS 1703	NO_2	Et	31	> 31	
L-731,988			54	1.5	36

[a] Data represent mean values for three independent determinations.
[b] Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50% as determined by the MTT method.
[c] Compound concentration required to reduce the exponential growth of MT-4/KB cells by 50%.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

2-yl)-2,4-dioxo-5-hexenoic acid ethyl esters, which were hydrolyzed in alkaline medium to give the required acids.

In addition to the unsubstituted derivatives **RDS 1699** and **RDS 1698**, a few substituents were also inserted at *para*-position of the phenyl ring (X = F, Cl, Me, OMe, NO_2).

The newly synthesized arylmethylpyrrolyldiketohexenoic acids and the ester intermediates were all screened to provide information on the inhibition of the HIV-1 IN either in cell culture or in enzyme assays. The assays were made in parallel with those of L-731,988 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.

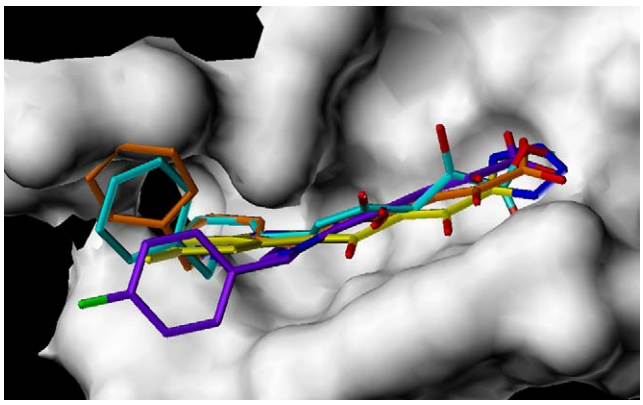
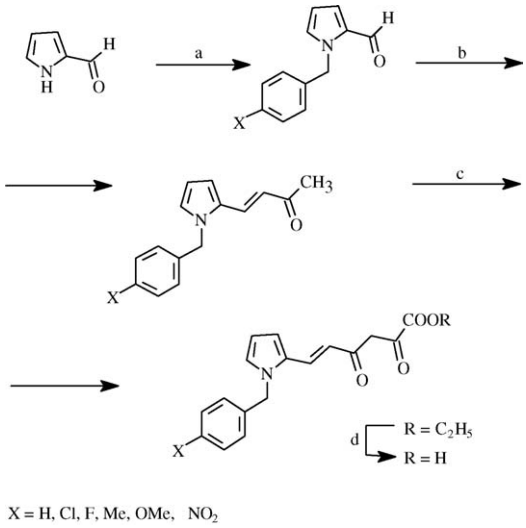


Fig. 8. Superimposition L-731,988 (purple), 5CITEP (yellow) **RDS 1699** (orange) and **RDS 1698** (cyan) after their minimization in the IN catalytic core (white).



Scheme 1. Synthetic pathway to obtain 1-arylmethyl-1*H*-pyrrol-2-yl-2,4-dioxo-5-hexenoic acids.
(a) Benzylhalide, K_2CO_3, DMF, 18 h, 90 °C; (b) 2-propanone, 5 N NaOH, 24 h, 25 °C; (c) diethyl oxalate, NaOEt, THF, 1 h 45 min, 25 °C; (d) 1 N NaOH, THF, MeOH, 1.5 h, 25 °C.

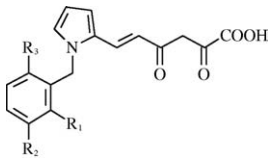
The cytotoxicity and antiviral activity are reported in Table 1. **RDS 1699** and its ethyl ester **RDS 1698** showed anti-HIV-1 potency (EC_{50} = 1.5 μM) comparable to that of L-731,988 (EC_{50} = 1.5 μM). The antiviral data reveal that the best performing inhibitors were **RDS 1699** and **RDS 1698**, unsubstituted at the *para*-position of the phenyl ring. Contrary to expectations, the 4-fluoro derivatives (**RDS 1644** and **RDS 1643**) were less potent than the unsubstituted counterparts. The introduction of chlorine, methyl, methoxy or nitro substituents in 4-position led to totally inactive products. Taken together these results suggest that steric hindrance at 4-position of the arylmethyl moiety is not tolerated. The above derivatives were proven to inhibit HIV-1 integrase in enzyme assays. Derivatives **RDS 1699** and **RDS 1698** 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 inhibited IN at higher concentrations ranging from 22 to 95 μM (data not shown).

Taking **RDS 1699** as a reference structure, lead optimization efforts focused on derivatives bearing one or two substituents at *ortho* and/or *meta* position of the phenyl ring. The cytotoxicity and the antiretroviral activities of the most interesting derivatives are reported in Table 2.

The newly synthesized derivatives did not show any significant improvement in the potency if compared with the lead **RDS 1699**, except the 2,6-difluoro derivative **RDS 1764**, which exhibited a fourfold increase in potency (EC_{50} = 0.3 μM) as well as a 2.5-fold in selectivity (SI = 123). This compound can be considered one of the most potent antiretroviral inhibitors of IN described in literature so far.

These results pulsed us to further investigations on aryldiketo acids as inhibitors of HIV-1 integrase. In particular, the findings of Merck researchers that the optimum relative orientation between the diketoacid chain and the benzyl

Table 2
Cytotoxicity and antiviral activities of pyrrolyldiketohexenoic acids in cell-based assays: examples[a]



Compound	R_1	R_2	R_3	CC_{50}[b] (μM)	EC_{50}[c] (μM)	S.I.[d]
RDS 1684	F	H	H	49	1.0	49
RDS 1760	Cl	H	H	52	4.2	12
RDS 1688	Me	H	H	35	0.7	50
RDS 1819	OMe	H	H	56	2	28
RDS 1827	OEt	H	H	32.5	2.4	13.5
RDS 1744	H	F	H	57	7	8
RDS 1779	H	Cl	H	54	7	7.7
RDS 1737	H	Me	H	46	7.4	6
RDS 1823	H	OMe	H	54	6.5	8.3
RDS 1764	F	H	F	37	0.3	123
L-731,988				54	1.5	36

[a] Data represent mean values for three independent determinations.
[b] Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50% as determined by the MTT method.
[c] Compound concentration required to reduce the exponential growth of MT-4/KB cells by 50%.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

moiety of ADKs was about 120°, corresponding to a 1,3-disubstitution on the central ring, and the presence of two benzyloxy groups on the phenyl of L-708,906, pulsed us to design pyrrolyl derivative **RDS 1611** (Fig. 9). In fact, this compound shows the following chemical features: i) a phenyl ring on 4-position, ii) a benzyl group in 1-position of pyrrole moiety, and ii) a diketoacid chain arranged in 1-3-position relative to benzyl group.

The following further modifications were conceived on the **RDS 1611** derivative: i) the elongation of diketobutanoic chain to afford diketohexenoic derivative **RDS 1712**; iii) the replacement of the diketobutanoic chain with a keto-iminoacid moiety (**RDS 1628**), and iv) the masking of the diketo group with a pyrazole ring (**RDS 1936**).

As an example, the synthetic pathway to obtain derivative **RDS 1712** is reported in Scheme 2. The crucial step of the synthesis is the selective annulation of TosMIC on the γ,δ-double bond of the dienone system to obtain (4-phenyl-1*H*-pyrrol-3-yl)-3-buten-2-one.

Derivatives described above were tested either in enzyme or in cell-based assays and the results are reported in Table 3.

The shift of diketoacid chain and the introduction of the phenyl ring on L-731,988, gave compound **RDS 1611** endowed with potent anti-IN activity selective against ST (26 nM). It is worthy of note that **RDS 1611** was two times more potent than Merck derivative, either in ST or in 3′-processing steps. Interestingly, the masking of diketobutanoic acid chain of **RDS 1611** in a pyrazole ring led to completely inactive derivative **RDS 1936**, while its replacement with imino-keto or diketohexenoic acid moieties led to **RDS 1628** and **RDS 1712**, which were two and 10 time less potent than parent compound **RDS 1611**.

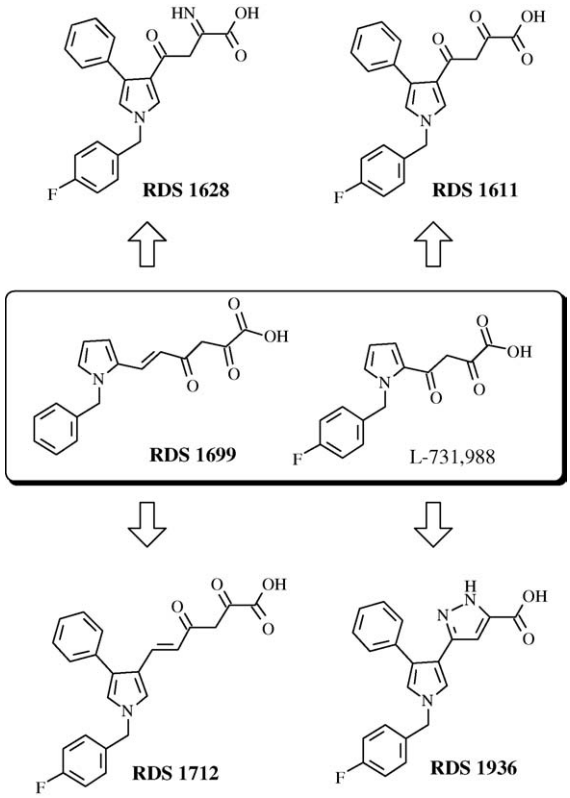


Fig. 9. Newly designed pyrrolyldiketoacid derivatives.

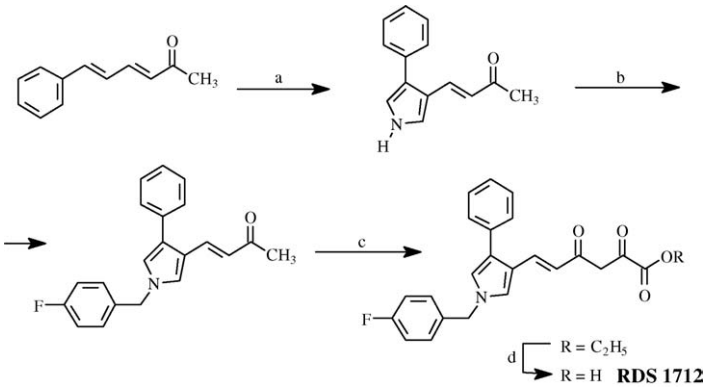
3.2. b) Indolyldiketohexenoic acids

As a further development in the searches of heteroaryldiketohexenoic acids as anti-IN agents, we planned the synthesis of 6-(5-chloro-1H-indol-3-yl)-2,4-dioxo-5-hexenoic acid (**RDS 1738**). This compound was conceived as the result of the assembling between the 5-chloroindole moiety of 5CITEP and the diketohexenoic chain of 1-arylmethyl-1*H*-pyrrol-2-yl-2,4-dioxo-5-hexenoic acid derivatives.

The synthesis of **RDS 1738** was achieved as depicted in Scheme 3. The condensation of the aldehyde function with 2-propenone in alkaline medium was obtained after masking of NH of 5-chloroindole-3-carboxaldehyde with benzenesulfonyl group. Then, indolylbutenone that formed underwent to condensation with diethyl oxalate using NaOEt as a catalyst, with simultaneous removal of protecting group to achieve ester **RDS 1707**. Finally, hydrolysis of the latter compound was performed with NaOH 1 N to give the required indolyldiketohexenoic acid derivative **RDS 1738**.

Similar pathway gave the ethyl ester of 6-(5-chloro-1-(4-fluorophenylmethyl)-1*H*-indol-3-yl)-2,4-dioxo-5-hexenoic acid (**RDS 1640**) via the corresponding ethyl ester **RDS 1639**.

Unfortunately, either derivatives **RDS 1707** and **RDS 1738** or the corresponding *N*-4-fluorobenzyl substituted counterparts **RDS 1639** and **RDS 1640** were unable to block the HIV-1 replication in cell-based assays at concentrations lower than 100 μM.



Scheme 2. Synthesis of derivative **RDS 1712**.
(a) TosMIC, NaH, DMSO/Et_2O, 20 min, r.t.; (b) 4-F-benzylbromide, K_2CO_3, DMF, 20 h, 90 °C; (c) diethyl oxalate, NaOEt, THF, 2 h, r.t.; (d) 1 N NaOH, THF/MeOH 3 h, r.t.

Table 3
Cytotoxicity and antiviral activities in enzyme (IN) and cell-based assays of derivatives of **RDS 1699** related to L,731,988[a]

Compound	CC_{50}[b] (μM)	EC_{50}[c] (μM)	S.I.[d]	IC_{50}[e] (μM) 3′-Proc.	IC_{50}[e] (μM) S.T.
RDS 1611	≥ 200	9	≥ 22	5.1	0.026
RDS 1628	182	8	23	8.8	0.044
RDS 1712	≥ 100	20	≥ 5	> 100	0.340
RDS 1936	ND	ND		> 333	> 333
L-731–988	54	1.5	36	10	0.057

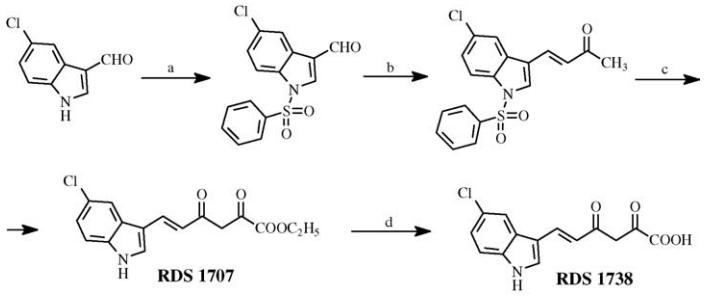
[a] Data represent mean values for three independent determinations.
[b] Cytotoxicity: compound dose required to reduce the viability of mock-infected cells by 50% as determined by the MTT method.
[c] Compound concentration required to reduce the exponential growth of MT-4/KB cells by 50%.
[d] Selectivity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

4. Molecular modeling studies

Docking studies were undertaken by the mean of the Autodock [30] program, with the aim of better understanding the binding mode of the above reported heteroaryldiketoacid derivatives to the biological target. The coordinates of the 5CITEP/IN[9] complex filed in the Brookheaven Protein Database (PDB entry code 1QS4) were retrieved, and subjected to minimization and molecular dynamics procedures using the AMBER [31] program.

Ninety-six pyrrolyl and indolyl derivatives were docked into the IN catalytic site, using a similar procedure to that reported by Sotriffer et al. [32]. These studies clearly demonstrated that the above derivatives generally share a common binding mode. Moreover, a good overlapping of the main chemical groups of RDS derivatives it could be observed (Fig. 10).

On the basis of this receptor-based alignment, a preliminary 3D-QSAR study was also started by the mean of the combination of the GRID [33] and GOLPE [34] programs. The 3D-QSAR model demonstrated the high correlation between the biological activity (EC_{50} values reported in Tables 1–3) and the chemical structures of the training set (52 RDS derivatives) displaying a conventional correlation coefficient value of 0.95 (r^2) and a five group cross-validated correlation coefficient value of 0.78 (q^2). In Fig. 11 is reported the PLS coefficient plot. The biggest polyhedra are arranged (disposed) around the arylmethylheteroaryl moiety, confirming that this part of the molecular scaffold could play a crucial role in the recognition mechanism. Further studies to better elucidate the binding mode of the RDS derivatives are currently in progress, and will be reported elsewhere.



Scheme 3. Chemical pathway to obtain indole derivative **RDS 1738**.
(a) Benzenesulfonylchloride, Bu_4NHSO_4, NaOH, CH_2Cl_2, 1.5 h, 0 °C; (b) 2-propanone, 5 N NaOH, 24 h, 25 °C; (c) diethyl oxalate, NaOEt, THF, 2 h 15 min, 25 °C; (d) 1 N NaOH, THF, MeOH, 3 h, 25 °C.

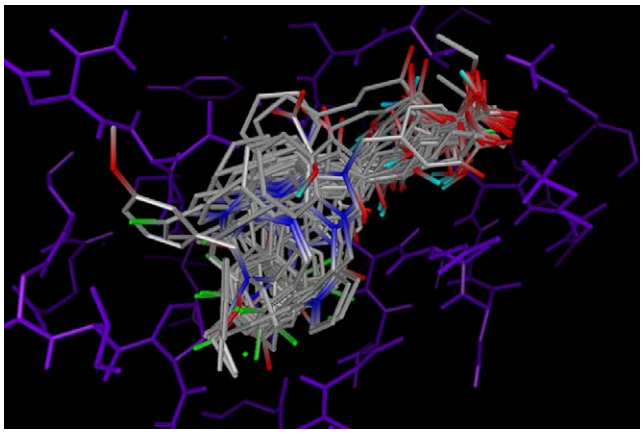


Fig. 10. The RDS derivatives docked into the IN catalytic core. In purple is also reported the IN. Hydrogen atom are omitted for sake of clarity.

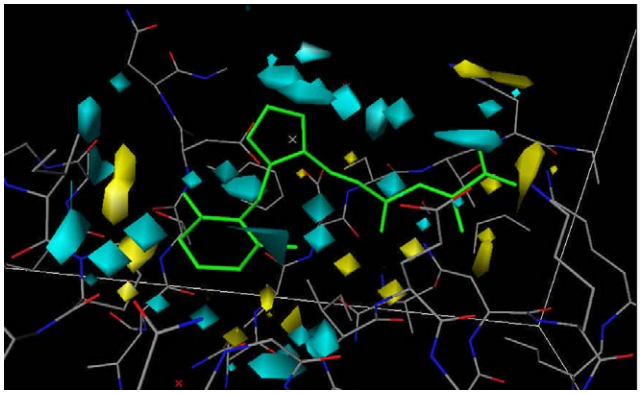


Fig. 11. PLS coefficient plot of the preliminary 3D QSAR model. Derivative **RDS 1764** is also reported for interpretation. The IN is also reported for polyhedron matching.

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