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4-Substituted 2-Hydroxyisoquinoline-1,3(2*H*,4*H*)-diones as a Novel Class of HIV-1 Integrase Inhibitors

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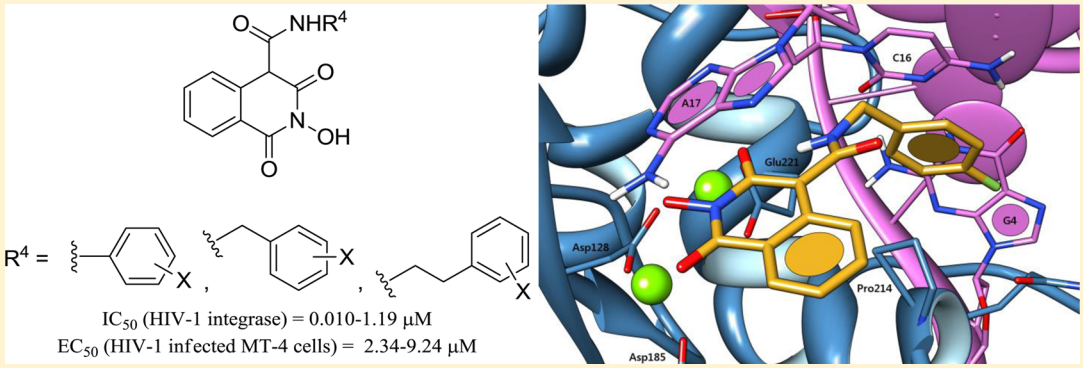
Supporting Information



ABSTRACT: A series of 2-hydroxy-1,3-dioxoisoquinoline-4-carboxamides featuring an *N*-hydroxyimide chelating functionality was evaluated for their inhibitory properties against human immunodeficiency virus type 1 integrase (HIV-1 IN). Several derivatives displayed low nanomolar IC_{50} values comparable to that of the clinically used raltegravir. A marked effect of one compound on both primary IN-catalyzed reactions, strand transfer (ST), and 3′ processing (3′-P), emphasizes a novel IN inhibition mechanism establishing it as a potential new generation IN inhibitor. Substitution of the 2-hydroxyisoquinoline-1,3-dione scaffold at position 4 by carboxamido chains was beneficial for antiviral activity since reproducible low micromolar anti-HIV activities were obtained for the first time within this scaffold.

KEYWORDS: *HIV, antiretroviral, integrase, 3′ processing, 2-hydroxy-1,3-dioxoisoquinoline-4-carboxamide*

HIV-1 integrase (IN) is a 32 kDa protein that plays a crucial role in HIV infection by incorporating the retrotranscribed viral DNA into the host chromosomal DNA. IN has been extensively studied as a therapeutic target in the field of AIDS antiretroviral therapy since it establishes irreversible infection and has no cellular equivalent, which limits toxicity.[1,2] The integration process involves a sequence of 2 reactions, which both require the presence of metallic cofactors: in the cytoplasm, a DNA-IN complex is formed that catalyzes the endonucleolytic cleavage of a dinucleotide at each 3′-end of the dsDNA (the 3′-processing step, 3′-P) After transport into the nucleus, the strand transfer step (ST), catalyzed by the intasome (a specific tetramer of IN and viral DNA ends) then joins each 3′-end of this recessed DNA to a 5′-end in the host DNA.

The first FDA-approved drug acting as a strong selective ST inhibitor[3] is raltegravir (Isentress). Elvitegravir, also recently approved, can be given once daily when combined with a booster (as part of the fixed-dose combination tablet Stribild),[4] but cross-resistance rules out treatment of patients failing on raltegravir therapy.[5,6] Dolutegravir (S/GSK1349572)[7] is currently in phase III clinical trials. Although superior to raltegravir, it also exhibits significant resistance overlap.[8] It can therefore be stated that IN still remains an orphan in terms of marketable drugs and an attractive and scientifically challenging target. One of the most innovative strategies so far was the design of inhibitors targeting the Lens Epithelium Derived Growth Factor (LEDGF)/p75 binding site on integrase (LEDGINs). These small molecules hinder the interaction of IN with the cellular cofactor LEDGF/p75. In addition LEDGINs stabilize integrase dimers and inhibit IN allosterically.[9] Dual inhibitors against IN and reverse transcriptase (RT) have also been investigated. Very recently, a non-nucleoside pyrimidine-2,4-dione RT inhibitor was 3-N hydroxylated,

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leading to a *N*-hydroxyimide derivative capable of inhibiting IN.[10]

We previously designed and studied several series of 2-hydroxyisoquinoline-1,3-diones as potential dual inhibitors of HIV-1 IN and RT associated RNase H activities. A few hits displaying high selectivity for IN or the RT associated RNase H function with submicromolar IC_{50} values were discovered.[11−13] Unfortunately nearly all tested compounds exhibited high cellular cytotoxicity in cell culture, which limited their applications as antiviral agents.

Careful examination of known potent IN strand transfer inhibitors (INSTIs) like raltegravir and dolutegravir points out the key presence of a magnesium chelating triad of oxygen atoms. Studies have also revealed the crucial importance of a hydrophobic side chain bearing a mono- or polyhalogenated benzyl group in the pharmacophore (Figure 1).[6] With the

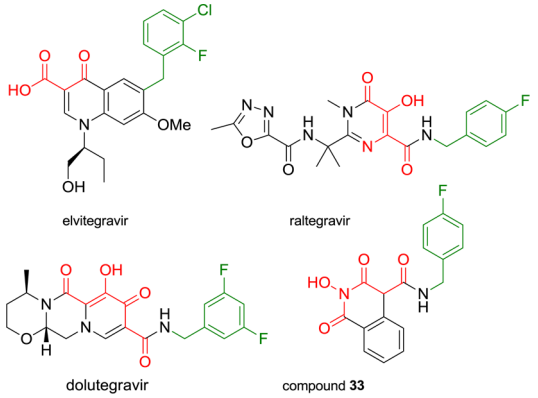


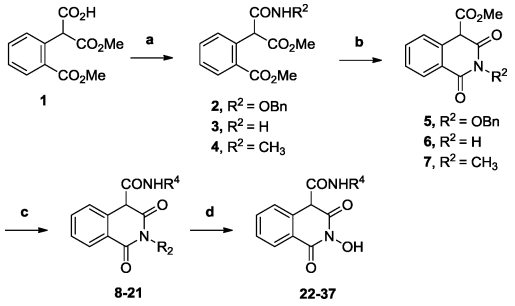
Figure 1. Structures of raltegravir, elvitegravir, dolutegravir, and compound **33** pointing out the key components of the HIV-1 IN inhibitory pharmacophore: the magnesium chelating moiety (red) and the hydrophobic halogenobenzyl group (green).

publication of their PFV intasome crystal structures, Hare et al. recently demonstrated that this aromatic component invades a pocket at the protein−DNA interface, which is natively occupied by the 3′-terminal base of viral DNA.[14] This led us to substitute our scaffold at position 4 with aromatic side chains, as exemplified by compound **33**. This could enhance not only the magnesium-chelating properties of our molecules by extending the coplanar arrangement of oxygen atoms but also their affinity for the IN catalytic center through additional hydrophobic contacts, namely, π-stacking interactions with retroviral DNA bases.

Herein, we propose further insight into the design of HIV IN inhibitors based on the 2-hydroxyisoquinoline-1,3-dione scaffold. The design, synthesis, and pharmacological evaluation of a novel series of 2-hydroxy-1,3-dioxoisoquinoline-4-carboxamides bearing diverse substituted phenyl, benzyl, or phenethyl side chains was carried out. The carboxamide link was chosen not only for its greater stability in physiological conditions, but also for its propensity to create an intramolecular hydrogen bond with the oxygen at position 3, thus orienting the aromatic side chain toward the desired hydrophobic cavity, as demonstrated by preliminary molecular modeling and docking studies (data not shown/see Supporting Information). Non-hydroxylated analogues **8** and **9** were also synthesized to validate the *N*-hydroxyl function as a key structural feature for magnesium complexation. The overall in vitro HIV-1 IN inhibitory potency, the anti-HIV activity in MT-4 cells, and cytotoxicity of the compounds were evaluated. Additionally, the in vitro inhibitions of HIV-1 IN 3′-P activity and RT associated RNase H function were also measured for the most interesting compounds.

The synthetic pathway was based upon a formerly reported route for the synthesis of 4-alkoxycarbonylisoquinoline-1,3-diones.[15] Acid precursor **1** was obtained in two steps from commercial homophthalic acid. After coupling of *O*-benzylhydroxylamine, ammonia, or *N*-methylamine, the corresponding amides **2**−**4** were quantitatively cyclized in mild alkaline conditions into ester precursors **5**−**7** (Scheme 1). Diversification of the side chain was then obtained via addition−elimination of various primary or secondary amines on the ester moiety to afford protected compounds **8**−**21**.The *O*-benzyl protecting group was finally removed with boron tribromide or boron trichloride to afford 2-hydroxy-1,3-dioxoisoquinoline-4-carboxamides **22**−**37**. The target compounds were obtained either as the keto form or as a keto/enol mixture with a large preference for the keto form (measured by ^{1}H NMR).

Scheme 1[a]



[a]Reagents and conditions: (a) $BnONH_2$ or CH_3NH_2, BOP, NMM, CH_2Cl_2, rt; $SOCl_2$ then AcOEt, NH_3 g; (b) 2.5 M KOH/MeOH, rt; (c) R_4NH_2, toluene, reflux; (d) BCl_3 or BBr_3, CH_2Cl_2, rt then H_2O.

Table 1 reports the enzyme inhibitory activities of this series. First, it can be observed that compounds **8** and **9** are completely devoid of inhibitory activity against HIV-1 IN. This strongly suggests that the N-hydroxyl function participating in the chelating pharmacophore is indeed crucial to IN binding. The introduction of a phenylcarboxamido side chain (compounds **22**−**25**) led to strong HIV-1 IN inhibition with IC_{50} values in the nanomolar range similar to that of raltegravir. The overall tendency seems to indicate that the linker's length between the amide function and the aromatic ring does not have a drastic influence on activity since compounds **31**, **32**, and **33** bearing benzylcarboxamido side chains display IC_{50} values similar to those of their respective homologues **35**, **36**, and **34** with phenethylcarboxamido side chains. Even though halogen substitution of the aromatic ring seems favorable, its *m*,*p*-disubstitution with donating oxygenated substituents (−OH or -OMe) had a dramatic effect on the HIV-1 IN inhibition with a one-log collapse of the IC_{50} values (**31**, **32**, **35**, and **36**) when compared to the *o*,*p*-disubstituted compounds **29** and **30**. This may be explained by unfavorable steric hindrance preventing optimal π-stacking with the DNA cytosine in the hydrophobic cavity rather than by electronic effects. Moreover, replacing the amide proton by a methyl group (compound **37**) provoked a 17-fold decrease of the HIV-1 IN inhibition, which stresses the importance of the side chain orientation and of the supposed intramolecular H-bond within the complex. Apart from overall HIV-1 IN inhibition, careful

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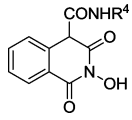
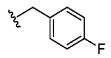
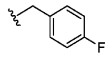
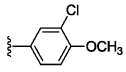
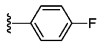
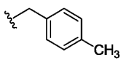
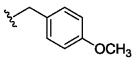
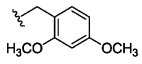
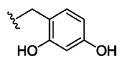
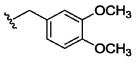
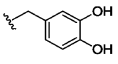
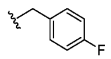
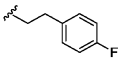
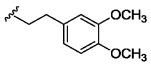
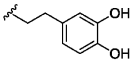
Table 1. Inhibitory Activities of Compounds 8 and 9 and of the 2-Hydroxy-1,3-dioxoisoquinoline-4-carboxamides 22−37



Comp.	R_4	IC_{50} (μM)				
		Overall IN[a]	ST[b]	3′ P[c]	3′ P/ST[d]	RNase H[e]
8(R^2 = H, not OH)		199	79			
9 (R^2 = CH_3, not OH)		> 250	> 250			
22		0.31	0.73			
23		0.020	0.277	1.58	5.7	16.6
24		0.010	0.110			7.31
25		0.8	1.12			
26		0.08	0.03			
27		0.076	0.084			14.0
28		1.19	1.03			
29		0.035	0.254			0.63
30		0.035	0.572	2.35	4.1	0.52
31		0.235	0.307			
32		0.205	0.258			0.90
33		0.056	0.099	0.06	0.7	0.36
34		0.030	0.020			
35		0.495	0.126			3.85
36		0.673	0.296			0.13
37	 ($R^{4'}$ = CH_3)	5.21	5.6			
Raltegravir		0.010	0.007	0.90	128	

[a,b,c]Concentration required to inhibit by 50% the in vitro overall, strand transfer, and 3′ processing integrase activities, respectively. [d]IC_{50} 3′-P/IC_{50} ST ratio. [e]Concentration required to inhibit by 50% of the in vitro RNase H activity.

examination of the compounds' effects on HIV-1 IN 3′-P and ST activities was also very instructive. A glance at the data of raltegravir evidence its well-known selectivity for ST over 3′-P, measured here at 128-fold. Close values of the IN overall and ST inhibitions were also observed for compounds **25**−**28** and **31**−**36**, which may infer ST selectivity. In contrast, there was a

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discrepancy between overall IN and ST inhibition for compounds **23**, **24**, **29**, and **30**. The ST IC_{50} values are 10- to 15-fold higher than the overall inhibition, suggesting either a synergistic effect on both primary IN functions or the intervention of an unknown mechanism of IN inhibition. The 3′-P inhibition of compounds **23**, **30**, and **33** was thus examined, and the calculated ratios IC_{50}(3′-P)/IC_{50}(ST) were measured at 5.7, 4.1, and 0.7, respectively. Unlike raltegravir, ST selectivity is not retained for these isoquinoline-4-carboxamides, and to the best of our knowledge, compound **33** is the first low molecular IN inhibitor that displays inhibition of both IN catalytic steps in the low nanomolar range. We also tested some compounds against HIV-1 RT associated RNase H activity (Table 1), and submicromolar IC_{50} values were obtained for the most active compounds **29**−**30**, **32**−**33**, and **36**.

Finally, this series was evaluated in a cell-based antiviral assay against HIV-1 (Table 2). As expected, the non-N-2 hydroxylated compounds **8** and **9** were not active, suggesting that the antiviral potencies may strongly be related to the metal-chelating ability and integrase inhibition. The amide proton also proved essential for antiviral activity (EC_{50} > 107 μM for compound **37**). Unfortunately, compounds **28**, **31**, **32**, **35**, and **36** were either poorly active or cytotoxic. Compounds **22**−**27**, **33**, and **34** ,however, displayed low micromolar EC_{50} values ranging from 1.75 to 5.08 μM. Although these are modest in comparison to that of the ST selective raltegravir (EC_{50} = 6.0 nM), it is still a very promising result since it is the first time that cytotoxicity is overcome on this scaffold. Compound **33** displayed a good aqueous solubility of 190.0 μM and a partition coefficient, log *D* of 0.56. At a 10 μM concentration, a mean permeability coefficient P_{app} (Caco-2 cells, pH 6.5/7.4) below 0.2×10^{-6} cm/s was determined, and high human plasma protein binding (mean of 99.9% protein bound) was observed, which might account for the lack of strong antiviral activity. This issue is currently being addressed through further pharmacomodulation. It must be stressed that, although we previously designed several compounds based on this scaffold, it is the first time that a reproducible low micromolar antiviral activity is attained in conjunction with a low nanomolar integrase inhibition. Moreover, compound **33** showed a profile similar to that of raltegravir and elvitegravir in time of addition experiments.[24] This attests for the cellular inhibition of integrase by this scaffold, whereas inhibition of the RT associated RNase H function remained a side activity. Half of the series showed an advantageous window between antiviral efficacy and cellular toxicity (21- to 86-fold).

Table 2. Anti-HIV Activities of Compounds 8 and 9 and of the 2-Hydroxy-1,3-dioxoisoquinoline-4-carboxamides 22−37

compd	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
8	>250	>250	
9	>250	>250	
22	4.95	105.5	21.3
23	3.34	12.3	3.7
24	2.47	64.0	25.9
25	1.75	114.5	65.4
26	3.12	130	41.7
27	5.7	>125	>22
28	17.63	118.5	6.7
29	9.24	60.4	6.5
30	7.94	>125	>16
31	>125	125	
32	>11	11	
33	2.34	202	86.3
34	5.08	123.5	24
35	70.77	>125	>1.8
36	>63	63	
37	>107.2	107.2	
raltegravir	0.006	>8.0	>1333

[a]Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells. [b]Cytotoxic concentration required to reduce MT-4 cell viability by 50%. [c]Therapeutic index, defined by CC_{50}/EC_{50}.

In silico docking studies were also performed in order to determine a possible binding mode with the target. Although our previously reported method was originally based on the PDB:3L2T crystallographic structure of PFV-IN intasome in complex with raltegravir,[14] we decided to adapt it to the more recent 3S3M X-ray structure of the PFV intasome bound to dolutegravir (see Supporting Information).[16] Whereas the invariant 3′-deoxyadenosine is flipped out of the active site in the case of elvitegravir and MK-0536, a raltegravir-derived INSTI with improved resistance profile,[17] it seems to participate in additional π-stacking interactions with the core of dolutegravir in the 3S3M structure. In a similar manner, the extended planar aromatic nature of our *N*-hydroxyisoquinoline-1,3-dione core bearing the metal chelating pharmacophore infers a great propensity to interact with this 3′-deoxyadenosine via π-stacking contacts.

As expected, two possible binding modes were obtained for compound **33** using this model (Figure 2), both of which show similar statistical significance and high overall fitness function scoring. Both poses involve (a) dual magnesium complexation, (b) π-stacking of the fluorobenzyl side chain with the invariant deoxycytosine C16, and (c) π-stacking of the central isoquinoline moiety with the invariant terminal 3′-deoxyadenosine A17. Although pose 2B involving the exocyclic oxygen in the chelation pharmacophore is not to be excluded, we strongly think that pose 2A is more likely to occur in reality. A closer look at the weighed terms of the CHEMPLP fitness function indeed reveals that despite a slightly better metal chelation score, the ligand conformation in pose 2B requires significant internal torsion and close steric contacts in the carboxamide linkage. Conversely, not only does pose 2A allow a more favorable dihedral angle at this linkage but it also involves an additional intramolecular hydrogen bond between the amide proton of the 4-(4-fluorobenzylcarboxamido) side chain with the oxygen at position 3, which may direct and maintain the aromatic ring toward the desired hydrophobic pocket. If this docking model may only reflect the ST inhibition mechanics of our molecules, we cannot yet provide a theoretical explanation for the activity on 3′-processing.

To our knowledge, it is the first time that such cumulative and synergistic effects on both integrase primary functions leading to strong integrase inhibition are observed. Little is known about 3′-P inhibition mechanism since only ST selective IN inhibitors have been cocrystallized with PFV IN so far. Crystal structures of PFV IN bound to unprocessed viral DNA prior to 3′-P were recently reported. As stipulated by Hare et al.,[18] the selectivity of known IN inhibitors for ST may be explained by the fact that their binding to the catalytic site in pre-3′-P configuration would require the displacement of the 3′-terminal AAT trinucleotide, involving the rupture of phosphate−metal and phosphate−amide interactions, as

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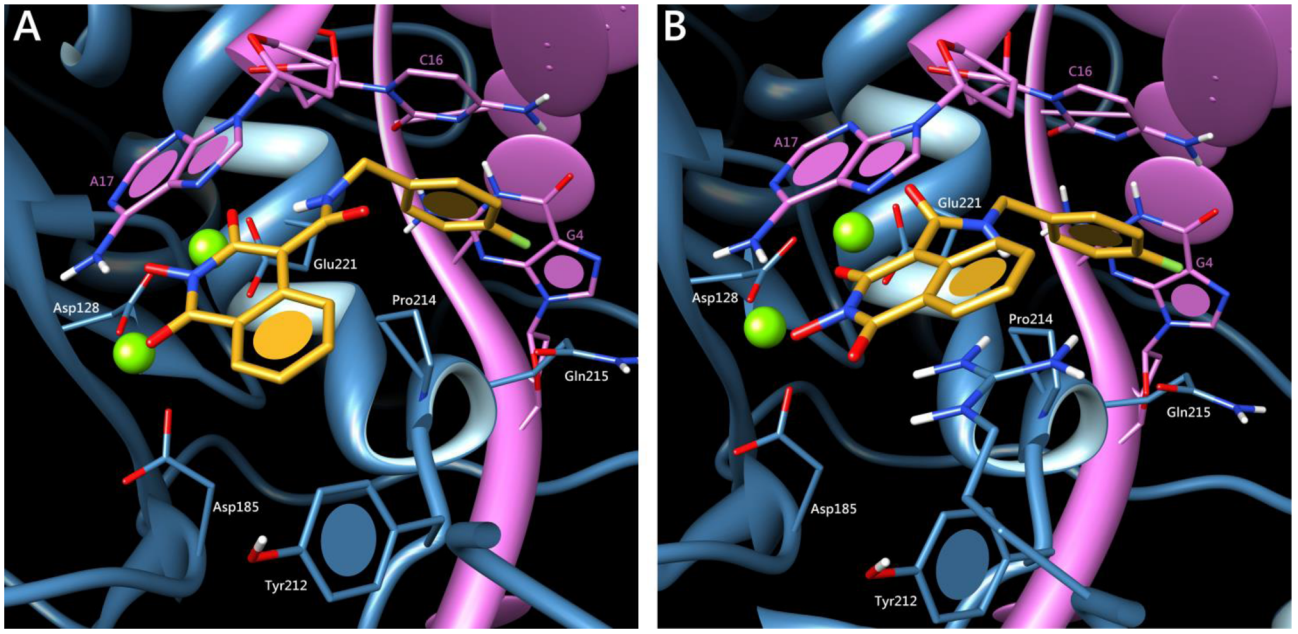

Figure 2. Putative binding modes of compound **33** in the PFV IN catalytic site obtained by molecular docking using the GOLD docking suite and the CHEMPLP fitness function. The ligand is depicted in orange, magnesium cations in green, IN in blue, and viral DNA in pink. Pose A: the three oxygens on the heterocyclic core contribute to Mg^{2+} chelation, allowing an intramolecular H-bond within the ligand. π-stacking interactions occur with deoxycytosine C16 and deoxyadenosine A17. Pose B: both π-stacking interactions occur as well. The exocyclic amide oxygen contributes to the metal chelation pharmacophore, at the expense of internal ligand torsion.

opposed to the displacement of only one deoxyadenosine at the ST stage. Considering the apparent mobility of the unprocessed terminal 3′-dinucleotide in the catalytic site, such a mechanism might be envisaged. However, the energetic barrier needed for such a displacement might be difficult to reach, and a compound would need to establish additional contacts in the pre-3′-P complex in order to balance the energetics of binding and maintain its potency.

Compounds inhibiting 3′-P and ST catalytic activities in the same range are rare. They are exemplified by some conjugates of single-stranded oligonucleotides with hydrophobic molecules acting in the low nanomolar range[19] and by low molecular compounds like numerous polyphenols, salicylhydrazides,[20] pyranodipyrimidines,[21] and styrylquinolines.[22,23] The latter were very instructive, as a few compounds inhibited HIV-1 replication at low micromolar concentrations. After being characterized as metal chelating inhibitors, further cellular mechanistic investigations showed that they exert their IN inhibitory activity by interacting with the viral DNA or target DNA binding regions of IN and/or by interfering with the nuclear import mechanism of IN. In our case, the loss of the N-hydroxyl function strongly impaired inhibitory properties, suggesting that our compounds may target the HIV-1 IN catalytic site. However, at this stage, another mechanism of inhibition cannot entirely be ruled out. The alternative would consist of an allosteric mechanism through interactions with a specific region of IN prior to DNA binding. No effect of compound **33** was observed on IN/LEDGF binding ($IC_{50IN/LEDGF} \gg 100\ \mu M$), excluding the interference with the cellular cofactor LEDGF/p75.

In summary, the introduction of a carboxamido side chain at position 4 of the 2-hydroxyisoquinoline-1,3-dione scaffold proved to be highly beneficial since low nanomolar anti-integrase activities close to that of raltegravir were obtained. In vitro enzymatic assays highlighted the unique behavior of these molecules: the typical ST selectivity of raltegravir and elvitegravir was not retained as a marked inhibition of the 3′-P step was observed. This pharmacomodulation had a positive effect on the antiviral activities as well since the limiting cytotoxicity of this scaffold was overcome and, for the first time, some compounds demonstrated low micromolar anti-HIV activities. Last but not least, compound **33** retained full activity against all common INSTI resistant strains tested (E92Q, Q148H, N155H, and G140S/Q148H) indicating a lack of cross-resistance with first-generation INSTIs and encouraging further clinical development.[24]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis of **8**−**9** and **22**−**37**, experimental details, docking protocol, compound characterization (^1H, ^{13}C NMR, and HRMS) for final products, and biological assay methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS

HIV-1 IN, human immunodeficiency virus type 1 integrase; ST, strand transfer; 3′-P, 3′ processing; AIDS, acquired immune deficiency disease; FDA, food and drug administration; LEDGF, lens epithelium derived growth factor; LEDGIN, inhibitor of the lens epithelium derived growth factor binding site in integrase; RT, reverse transcriptase; INSTI, integrase strand transfer inhibitor; PFV, prototype foamy virus

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