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6,7-Dihydroxy-1-oxoisindoline-4-sulfonamide-containing HIV-1 Integrase Inhibitors

Xue Zhi Zhao^{a,*}, Kasthuraiah Maddali^b, Steven J. Smith^c, Mathieu Métifiot^b, Barry C. Johnson^c, Christophe Marchand^b, Stephen H. Hughes^c, Yves Pommier^b, and Terrence R. Burke Jr.^{a,*}

^aChemical Biology Laboratory, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD 21702, United States

^bLaboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States

^cHIV Drug Resistance Program, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD 21702, United States

Abstract

Although an extensive body of scientific and patent literature exists describing the development of HIV-1 integrase (IN) inhibitors, Merck's raltegravir and Gilead's elvitegravir remain the only IN inhibitors FDA-approved for the treatment of AIDS. The emergence of raltegravir-resistant strains of HIV-1 containing mutated forms of IN underlies the need for continued efforts to enhance the efficacy of IN inhibitors against resistant mutants. We have previously described bicyclic 6,7-dihydroxyoxoisindolin-1-ones that show good IN inhibitory potency. This report describes the effects of introducing substituents into the 4- and 5- positions of the parent 6,7-dihydroxyoxoisindolin-1-one platform. We have developed several sulfonamide-containing analogs that enhance potency in cell-based HIV assays by more than two orders-of-magnitude and we describe several compounds that are more potent than raltegravir against the clinically relevant Y143R IN mutant.

Keywords

HIV-1 integrase; Inhibit; Raltegravir; Sulfonamide

Integrase (IN) is an HIV-1 encoded polynucleotidyl transferase that inserts viral cDNA into the host genome through a process involving two sequential enzymatic steps, termed 3'-processing (3'-P) and strand transfer (ST).¹ Integration is required for viral replication and IN is a validated antiviral target for the treatment of AIDS.^{2,3} There is an extensive body of scientific and patent literature describing the development of IN inhibitors. Reviews 4,5

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*Corresponding authors. Tel.: +1 301 846 5906 (T.R.B.); Tel: +1 301 846 5907 (X.Z.Z.); Fax: +1 301 846 6033 xuezhi.zhao@nih.gov (X.Z. Zhao), burkete@helix.nih.gov (T.R. Burke).

Supplementary data

Supplementary data (synthetic experimental procedures and analytical data for synthetic products) associated with this article can be found in Electronic supplementary information.

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Raltegravir (**1**) is the first IN inhibitor FDA-approved for the treatment of HIV/AIDS (Fig. 1).⁶ Raltegravir binds at the interface of the macromolecular complex formed by IN and the viral DNA substrate, blocking the active site and preventing the insertion of the viral DNA into the host genome.^{Review 7} Because it more potently blocks the second enzymatic step, it is referred to as an IN strand transfer inhibitor, or INSTI. All anti-HIV drugs select for resistant strains of the virus and raltegravir is no exception. Because there are clinical strains of HIV-1 that exhibit reduced sensitivity to raltegravir,⁸ “second-generation” inhibitors are being developed to treat patients failing raltegravir-based treatment.⁹ However, in *in vitro* experiments exposure to such compounds selected IN mutants showing reduced susceptibility,^{10,11} suggesting that additional work needs to be done to understand the underlying mechanisms of resistance, and to develop new compounds that will be more effective against resistant strains.

Raltegravir shares with other well-described INSTIs, the ability to chelate two catalytic divalent metal ions (Mn^{2+} or Mg^{2+}) through a triad arrangement of heteroatoms (Fig. 1). We have previously described structurally simple bicyclic 6,7-dihydroxyoxoisindolin-1-one-based IN inhibitors (**2**) that show good potency and strand transfer selectivity *in vitro* in the presence of Mg^{2+} cofactor.¹² Our earlier reports focused primarily on the arylamide “right side” of the molecules.^{13,14} This paper describes the effects of substitutions at the two free aryl positions on the “left side” of the molecule (Fig. 1). In designing new analogs we were guided by the fact that Merck’s bicyclic second-generation inhibitor MK-0536 (**3**)^{15,16} has carboxamido and isopropyl substituents at what would be equivalent to positions 4 and 5 of our 1-oxoisindoline ring system (Fig. 1). We applied a variation of this theme to the dihydroxyoxoisindoline nucleus by placing several different alkyl groups at the 5-position (**4**) and employing sulfonamido rather than carboxamido functionality at the 4-position (**5**, Fig. 1).

Synthesis of derivatives of compound **4** modified at the 5-position employed methodologies similar to those used to prepare the unsubstituted congeners.^{12–14} Substituents that would ultimately occupy the 5-position of the 6,7-dihydroxyoxoisindolines final products were derived from appropriately substituted methyl dimethoxybenzyl ethers (**6**, Scheme 1). Methyl-substituted **6b** was obtained from vanillin using 4-hydroxy-3-methoxy-5-methylbenzaldehyde¹⁷ as an intermediate (see Supporting Information Scheme SI1). The *n*-butyl-substituted compound (**6c**) was obtained serendipitously in an attempt to prepare the isopropyl congener (**6d**). The synthesis started from 5-bromovanillin and there was an unintended introduction of the *n*-butyl group when *n*-butyl lithium was used for metalation of the aryl bromide of intermediate 1-bromo-2,3-dimethoxy-5-(methoxymethyl)benzene (see Supporting Information Scheme SI2). The desired isopropyl containing analog (**6d**) was synthesized through the known 5-bromo-1-isopropyl-2,3-dimethoxybenzene¹⁸ (see Supporting Information Scheme SI3). The phenyl-substituted compound **6e** was obtained through a route involving Suzuki coupling of phenylboronic acid with the above-mentioned 1-bromo-2,3-dimethoxy-5-(methoxymethyl)benzene (see Supporting Information Scheme SI4). Finally, the trimethoxyphenyl-containing analog **6f** was obtained from commercially-available (3,4,5-trimethoxyphenyl)methanol by methylation using iodomethane and sodium hydride (see Supporting Information Scheme SI5).

Treatment of the benzyl methyl ethers **6(a – f)** with *n*-butyl lithium followed by methyl chloroformate provided the corresponding methyl esters **7(a – f)** (Scheme 1). Transformation to the benzyl chlorides **8(a – f)** was then accomplished using acetyl chloride in the presence of a catalytic amount of zinc chloride. Subsequent ring closure to the isoindolin-1-ones **9(a – f)** was achieved by coupling with 3-chloro-4-fluorobenzylamine. The final 5-substituted 6,7-dihydroxyisoindolin-1-ones **2, 4(b – e, g, h)** were obtained by demethylation using boron tribromide in dichloromethane (Scheme 1).

Introduction of sulfonyl functionality at the indolin-1-one 4-position was achieved by reacting the methyl ether-protected intermediates **9(a – f)** with chlorosulfonic acid gave the sulfonyl chlorides **10(a – f, h)** (Scheme 2). Further treatment with a variety of primary and secondary amines yielded the corresponding sulfonamides **11(a-n) – 11(f-n)** and **11(h-n)**, where “n” designates variation in the sulfonamido group. Finally, deprotection of the methyl ethers was accomplished by cleavage with boron tribromide in dichloromethane to produce the final products **5(a-n) – 5(e-n)**, **5(g-n)** and **5(h-n)** (Scheme 2).

Our previous structural studies on 6,7-dihydroxyoxoisoindolin-1-one-based IN inhibitors (**2**) were limited to analogs that were unsubstituted at the 4- and 5-positions.^{12–14} An important objective of our current work was to explore the effects of incorporating functionalities at the 5-position of the isoindolinone ring system (compounds **4**). MK-0536 (**3**) is one example of a diverse range of IN inhibitors that have substituents in this region.⁵ We were also interested in determining the effects of placing sulfonamido groups at the 4-position. Highly potent IN inhibitors have been reported that have sulfonamide groups located in this region. However, in almost all cases the sulfonamides are either in “reversed” orientations, having the amine rather than the sulfur attached to the metal-chelating aryl ring (for example^{19,20}), or the sulfonamide is attached indirectly through intervening structures (for example²¹). In some instances, carboxamide rather than sulfonamide functionality is employed, as in MK-0536 (**3**).

The IN inhibitory IC₅₀ values of the synthetic analogs against both 3'-P and ST reactions were determined using an *in vitro* assay employing Mg²⁺ cofactor as previously described.¹² Introduction of substituents at the 5-position uniformly decreased ST inhibitory potency relative to the unsubstituted parent (**2**; ST IC₅₀ = 0.16 μM), in most cases by more than one order of magnitude (Table 1). The least deleterious substituents were phenyl (**4e**; ST IC₅₀ = 0.87 μM) and 4-dimethylsulfonamidophenyl (**4h**; ST IC₅₀ = 0.4 μM). With the exception of the 5-hydroxy analog (**4g**; 3'-P IC₅₀ = 36.0 μM), all the substituents we tested reduced the 3'-P inhibitory potency by at least an order of magnitude (3'-P IC₅₀ > 111 μM) relative to the parent **2** (3'-P IC₅₀ = 13.2 μM).

In contrast, introducing sulfonamide groups at the 4-position of parent compound **2** had little effect on inhibitory potencies in 3'-P reactions relative to **2** (Table 2). However, ST inhibitory potencies were generally increased (**5a-1** to **5a-3** and **5a-9** to **5a-12**) with ST IC₅₀ values ranging from 0.047 μM to 0.19 μM). These results are similar to what has been reported for the effects of sulfonamido functionality in this general structural region of other IN inhibitors.^{19–21} A recent report showed that introduction of 5-sulfonamido groups onto *N*-(4-fluorobenzyl)-2,3-dihydroxybenzylamide increased inhibitory potency.²² This is of note, since we had originally designed the 6,7-dihydroxyisoindolin-1-ones as conformationally constrained variants of *N*-(4-fluorobenzyl)-2,3-dihydroxybenzylamide.¹² We found that this constraint increased the inhibitory potency of the compounds in the ST reaction by approximately ten-fold. The enhancing effects of sulfonamido substituents in our current conformationally constrained analogs is consistent with enhancing effects reported for the ring-open compounds.²²

Although substituents at the 5-position (**4b – 4h**) decreased inhibitory potencies in the ST reactions compared to the parent compound **2**, these potentially represented biased examples, since the compounds lacked functionalities at the adjoining 4-position (Table 1). IN inhibitors often have substituents at both the 4 and 5-position, as exemplified by raltegravir (**1**) and MK-0536 (**3**). Therefore, a series of analogs was prepared that combined sulfonamido functionality at the 4-position with various substituents at the 5-position (Table 3). Adding a 4-sulfonamido group increased the inhibitory potencies in the ST reaction relative to the compounds described in Table 1. The increase was most pronounced in the

case of **5d-2** (ST IC₅₀ = 0.19 μM), which has an *i*-Pr group at the 5-position. This led to a 20-fold enhancement in the ST reaction relative to the parent *i*-Pr-containing **4d** (Table 1 ST IC₅₀ = 5.4 μM). However, for a given 4-sulfonamido group, introducing substituents at the 5-substituent decreased ST inhibitory potency (Table 3).

Clinical resistance to raltegravir (**1**) is associated primarily with IN mutations at amino acids Y143, Q148 and N155.²³ In order to examine the effects of these resistance mutations on the potency of the new compounds, antiviral efficacy (EC₅₀ values) were determined for select inhibitors using HIV vectors that replicated using either WT IN or vectors carrying Y143R, N155H, or the G140S-Q148H double mutant (designated as “SH”) (Table 4).^{24,25} Sub-micromolar EC₅₀ values were obtained against the wild-type vector for several of the sulfonamide derivatives that lacked a substituent at the 5-position (“**5a**” compounds, Table 4) and for certain compounds, nanomolar potencies (17 ~ 40 nM) were observed. Comparing *in vitro* IC₅₀ values obtained for inhibition of ST reactions with the cellular EC₅₀ values revealed differing degrees of concordance. Compounds **5a-1**, **5a-2**, **5a-3** and **5a-10** gave EC₅₀ values (0.04 μM, 0.017 μM, 0.93 μM and 0.022 μM, respectively, Table 4) that were slightly better than the corresponding *in vitro* ST IC₅₀ data (0.12 μM, 0.054 μM, 0.11 μM and 0.047 μM, respectively, Table 2). Compound **5a-8** showed a 10-fold enhancement in its cellular antiviral EC₅₀ value relative to its *in vitro* ST IC₅₀ data (0.043 μM and 0.4 μM, respectively). This is somewhat unexpected, because **5a-8** contains a piperazine nitrogen that should be protonated at physiological pH, and this charged species would be expected to reduce cell membrane transit.

It is not uncommon for cell-based antiviral efficacy to be greater than the IC₅₀ data obtained *in vitro* in ST inhibition assays.^{12-14,26} This may be due in part to the fact that IC₅₀ values obtained *in vitro* in ST inhibition assays depend on factors, such as the concentration of substrate DNA, that may be different in the cellular environment.²⁷ Two compounds (**5a-11** and **5a-12**) provided antiviral EC₅₀ values that were higher than their corresponding *in vitro* ST IC₅₀ values. These compounds contained anionic carboxylic acid functionality that would be expected to reduce cellular bioavailability.

In cell-based assays performed with HIV vectors carrying the Y143R mutant, raltegravir (**1**) has been reported to exhibit an approximate 40-fold loss of potency relative to the WT enzyme.^{24,25} This loss of potency can be understood on the basis of X-ray crystallographic studies with the prototype foamy virus (PFV) intasome, which show that the oxadiazole ring of raltegravir makes extensive interactions through π-stacking with the side chain aryl ring of Y212 of PFV IN (which is equivalent to Y143 of HIV-1 IN). Replacement of Tyr with Arg eliminates these interactions and is generally understood to account for the reduced potency of raltegravir against the Y143R mutant.²⁸ The retention of relative good potency against the Y143R mutant shown by all sulfonamides in Table 4 is consistent with the absence of aromatic functionality equivalent to raltegravir’s oxadiazole ring;²⁹ none of these compounds rely on a π-π interaction with Y143.

Raltegravir has also been reported to exhibit an approximate 40-fold loss of antiviral potency in cellular studies employing HIV vectors bearing the N155H mutant.²⁵ Crystallographic studies with the PFV intasome show that the side chain of N224 (equivalent to N155 of HIV-1 IN) forms a hydrogen bond with the carboxylate group of E221, which is part of the catalytic “DDE” triad chelating the divalent Mg²⁺ ions. In the case of N224H mutant, the His imidazole ring makes electrostatic interactions with the phosphate group of the 3’ adenosine of the viral DNA, causing a shift in the position of the DNA backbone and the catalytic Asp and Glu carboxylates (PFV D128 and E221).²⁸ Binding of INSTIs requires the energetically unfavorable breaking of the His-phosphate interaction. The sulfonamides shown in Table 4 exhibit a range of sensitivities to the N155H

mutation that are both greater and less than what has been reported for raltegravir. Interestingly, compounds having bulky *n*-butyl or *i*-propyl groups at the 5-position were significantly less affected by the mutation (**5c-2**, **5d-2** and **5d-10**, Table 4). Although the absolute potencies of these latter compounds against the WT enzyme is not as good as some of the other inhibitors shown in Table 4, their ability to retain inhibitory efficacy against the N155H mutant may reflect an enhanced capacity to break the H155 imidazole – phosphate interaction and re-order the DNA needed to accommodate ligand binding.

Raltegravir has also been reported to exhibit an approximate 500-fold loss of antiviral potency in cellular studies employing HIV vectors having the 140S/Q148H [designated as “SH”] double mutant.²⁵ G140 and Q148 correspond to PFV residues S209 and S217, respectively, which occupy the termini of the “flexible loop” bearing the Y212 residue (Y143 of HIV-1 IN), which is critical for raltegravir binding. Crystallographic studies with the PFV intasome show that the S217H mutation requires a shift in the His side chain to accommodate binding of the fluorbenzyl ring of raltegravir.²⁸ Due to their positions at the termini of the flexible loop, the side chains of the S140 and H148 residues are in close proximity. In the “SH” double mutant, movement of the bulky H148 side chain is sterically impeded by the side chain of S140. In antiviral studies using the SH mutant, sulfonamides lacking substituents at the 5-position exhibited two-orders-of-magnitude reductions in potency (**5a-n**, Table 4). The 5-substituted sulfonamides showed less relative loss of potency. However, similar to what was observed against the N155H mutant, in most cases the absolute efficacy of these inhibitors against wildtype IN was also reduced.

Except for those compounds bearing free carboxylic acid groups (**5a-11** and **5a-12**), which may adversely affect cellular uptake, cytotoxicity CC_{50} values for all sulfonamides in Table 4 were uniformly in the low micromolar range and very similar to the value of 5.2 μM reported for the parent 6,7-dihydroxyisindolinone (**2**). Although cytotoxicity remained unchanged, several of the sulfonamide-containing analogs exhibit cellular EC_{50} values that are approximately two orders-of-magnitude better than the parent **2**. The overall effect was to improve the “selectivity index” ($SI = CC_{50} / EC_{50}$) for several sulfonamides by two orders-of-magnitude relative to **2**.

Our current report describes the effects of introducing substituents into the 4- and 5-positions of the parent 6,7-dihydroxyisindolinone platform. We found that several sulfonamide-containing analogs exhibit potencies in cell-based HIV assays that are enhanced by more than two orders-of-magnitude. The overall effect of these efforts has been to generate several compounds exhibiting better absolute efficacy than raltegravir against the clinically relevant Y143R IN mutant and with SI values exceeding 100. Although cytotoxicity remains an issue, our current work has advanced the development of the 6,7-dihydroxyisindolinone platform.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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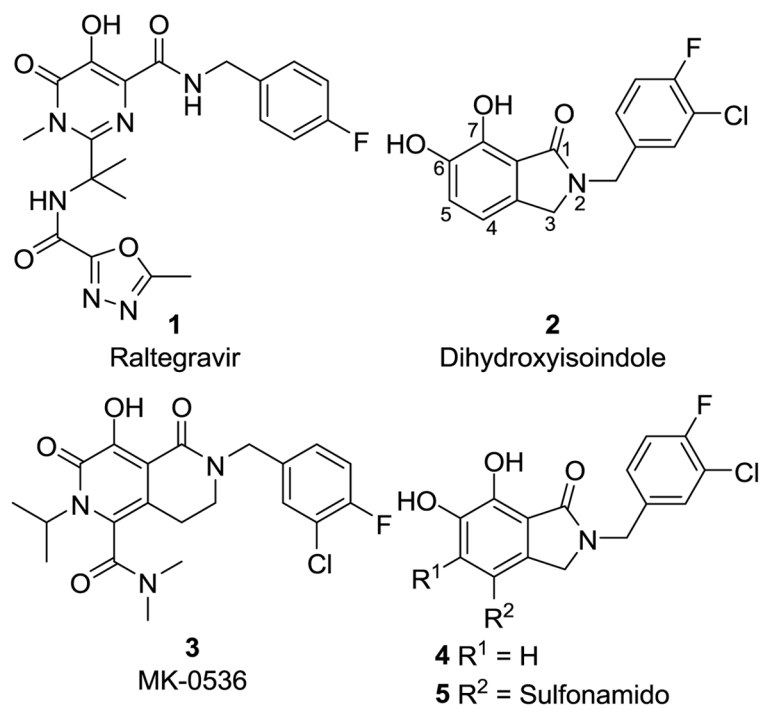
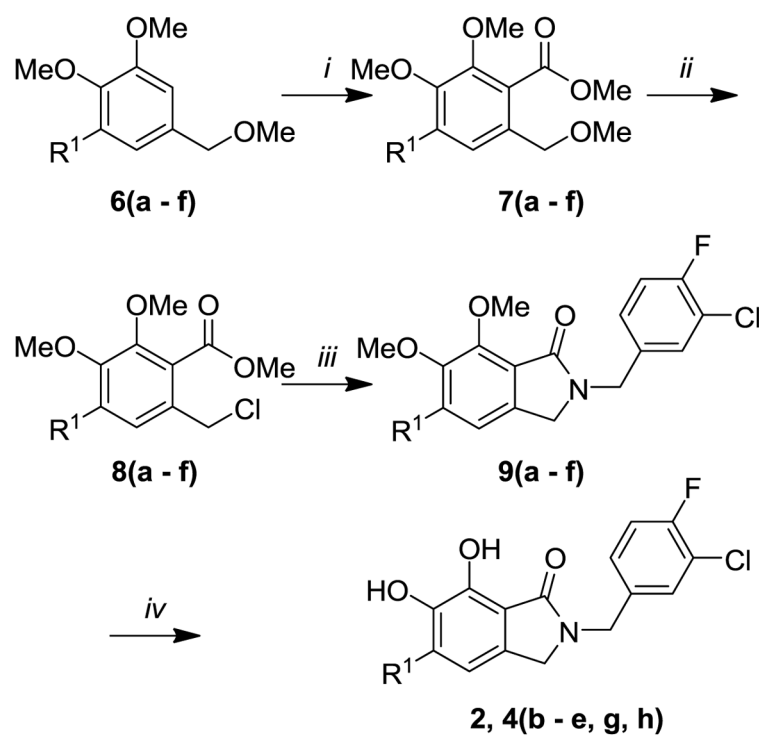
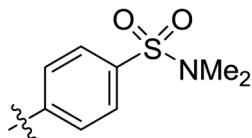


Figure 1.
Structures of IN inhibitors discussed in the text.

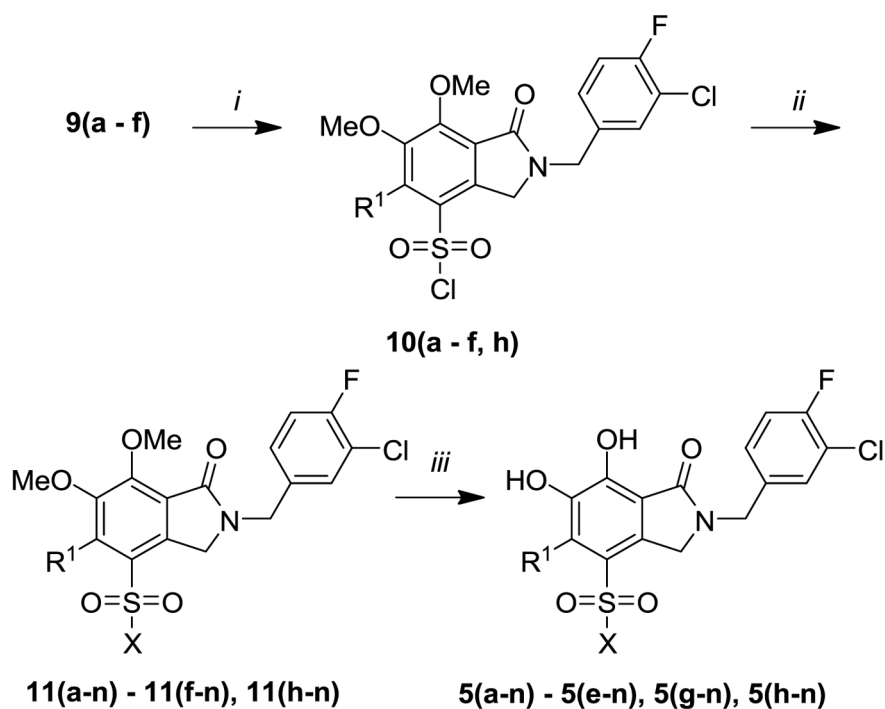


$R^1 =$ a, H; b, Me; c, n Bu; d, i Pr; e, Ph; f, OMe;
 g, OH; h,

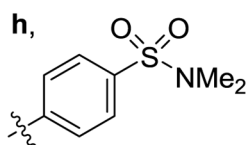


Scheme 1.

Reagents and conditions: *i*) n BuLi, ClCO₂Me; *ii*) AcCl, ZnCl₂, Et₂O; *iii*) 3-chloro-4-fluoro-benzylamine, Et₃N, CH₃CN; *iv*) BBr₃, CH₂Cl₂.

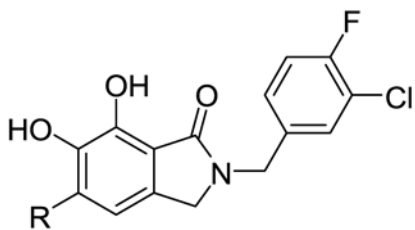


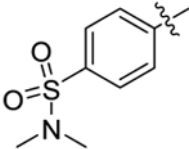
$R^1 = \mathbf{a}$, H; \mathbf{b} , Me; \mathbf{c} , ^nBu ; \mathbf{d} , ^iPr ; \mathbf{e} , Ph; \mathbf{f} , OMe; \mathbf{g} , OH;



Scheme 2.

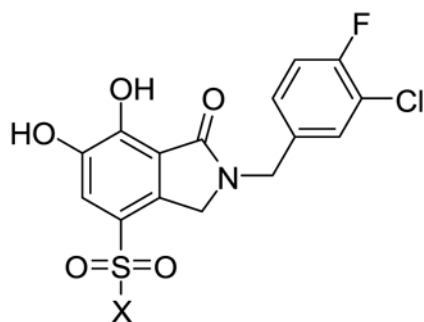
Reagents and conditions: *i*) ClSO_3H ; *ii*) $\text{R}^2\text{R}^3\text{NH}$, Et_3N , CH_2Cl_2 ; *iii*) BBr_3 , CH_2Cl_2 .

Table 1Inhibitory potencies using an *in vitro* IN assay.^a

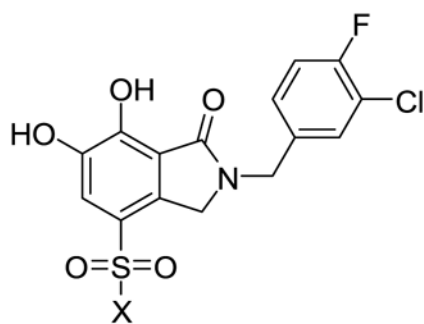
No.	R	IC ₅₀ Values (μM)	
		3'-Processing	Strand transfer
2 ^b	H	13.2 ± 3.1	0.16 ± 0.08
4b	Me	>111	11.5 ± 1.6
4c	^t Bu	>111	6.8 ± 0.9
4d	ⁱ Pr	>111	5.4 ± 0.6
4e	Ph	>111	0.87 ± 0.08
4g	OH	36.0 ± 6.0	1.5 ± 0.3
4h		>111	0.4 ± 0.04

^a Assays were performed using a gel-based protocol with Mg²⁺ cofactor as describe in reference 12;^b Reported previously in references 12–14.

Table 2

Inhibitory potencies using an *in vitro* IN assay.^a

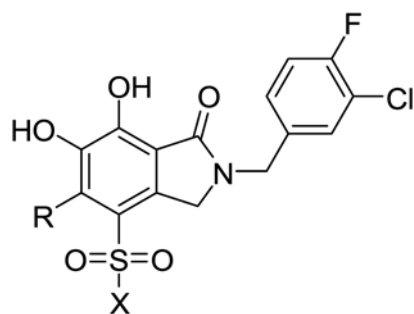
No.	X	IC ₅₀ Values (μM)	
		3'-Processing	Strand transfer
5a-1	NHMe	17.5 ± 3.0	0.12 ± 0.04
5a-2	NEt ₂	9.5 ± 1.2	0.054 ± 0.009
5a-3		14.7 ± 1.7	0.11 ± 0.01
5a-4		15.9 ± 1.3	0.24 ± 0.02
5a-5		30.2 ± 2.3	0.32 ± 0.05
5a-6		19.3 ± 0.6	0.31 ± 0.03
5a-7		23 ± 3	0.37 ± 0.04
5a-8		23 ± 4	0.4 ± 0.08

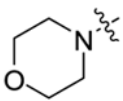
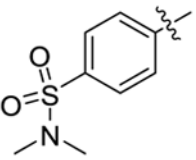


No.	X	IC ₅₀ Values (μM)	
		3'-Processing	Strand transfer
5a-9		21.2 ± 1.9	0.19 ± 0.08
5a-10		6.8 ± 1.1	0.047 ± 0.007
5a-11		11.7 ± 0.7	0.08 ± 0.01
5a-12		6.9 ± 0.6	0.058 ± 0.01

^a Assays were performed using a gel-based protocol with Mg²⁺ cofactor as described in reference 12.

Table 3

Inhibitory potencies using an *in vitro* IN assay.^a

No.	R	X	IC ₅₀ Values (μM)	
			3'-Processing	Strand transfer
5b-2	Me	NMe ₂	49.7 ± 4.0	2.8 ± 0.6
5c-2	ⁿ Bu	NMe ₂	>111	2.3 ± 0.4
5d-2	^t Pr	NMe ₂	>111	0.19 ± 0.03
5d-10	^t Pr		>111	0.41 ± 0.06
5g-2	OH	NMe ₂	7.6 ± 0.8	0.26 ± 0.08
5h-2		NMe ₂	35.2 ± 2.6	0.25 ± 0.06

^a Assays were performed using a gel-based protocol with Mg²⁺ cofactor as describe in reference 12.

Table 4

Antiviral potencies in cell infected with HIV-1 containing wild-type or mutant integrase enzymes.^a

No.	CC ₅₀ (μM) ^b	EC ₅₀ (μM) ^c	SI ^d	Integrase Mutants ^e		
				Y143R	N155H	SH ^f
2	5.2	0.447	12	1x	2x	8x
5a-1	4.3	0.040	108	1x	19x	95x
5a-2	8.4	0.017	494	3x	57x	300x
5a-3	4.6	0.093	49	1x	68x	132x
5a-8	6.2	0.043	144	1x	44x	430x
5a-10	5.3	0.022	241	2x	23x	218x
5a-11	106.6	0.929	115	3x	ND	ND
5a-12	95.2	0.140	680	2x	272x	ND
5b-2	6.3	0.322	20	2x	12x	30x
5c-2	7.0	3.8	2	1x	4x	1x
5d-2	12.1	3.6	3	3x	2x	4x
5d-10	5.9	3.4	2	4x	2x	3x
5g-2	4.6	0.159	29	2x	10x	ND
5h-2	2.8	0.132	21	4x	16x	25x

^a Assays were performed as described in reference 25;

^b Cytotoxic concentration resulting in 50% reduction in the level of ATP in human osteosarcoma (HOS) cells;

^c Values obtained from cells infected with lentiviral vector harboring WT IN;

^d Selectivity index calculated as the ration of CC₅₀ to EC₅₀;

^e Cells were infected with viral constructs carrying IN mutation as described in reference 25 and indicated values correspond to the fold-change in EC₅₀ relative to WT;

^f G140S-Q148H double mutant.