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6,7-Dihydroxyisoindolin-1-one and 7,8-Dihydroxy-3,4-Dihydroisoquinolin-1(2*H*)-one Based HIV-1 Integrase Inhibitors

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Abstract

Integrase (IN) is an essential viral enzyme required for HIV-1 replication, which has been targeted by anti-AIDS therapeutics. Integrase strand transfer inhibitors (INSTIs) represent a new class of antiretroviral agents developed for the treatment of HIV-1 infections. Important structural features that are shared by many INSTIs include a coplanar arrangement of three heteroatoms that chelate two catalytic Mg^{2+} ions in the IN active site and a linked halophenyl ring that binds in the hydrophobic pocket formed by the complex of IN with viral DNA. We recently reported bicyclic 6,7-dihydroxyoxoisoindolin-1-one-based IN inhibitors. In the current study, we modified these inhibitors in three ways. First, we increased the spacer length between the metal-chelating triad and the halophenyl group. Second, we replaced the indoline [5,6] bicycle with a fused dihydroxyisoquinolinones [6,6] ring system. Finally, we prepared bis-6,7-dihydroxyisoindolin-1-one-4-sulfonamides as dimeric HIV-1 IN inhibitors. These new analogues showed low micromolar inhibitory potency in *in vitro* HIV-1 integrase assays.

Keywords

HIV-1 integrase; Inhibitor; Metal-chelating; Sulfonamide

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV), has become one of the world's most serious diseases. More than 33 million people are currently living with HIV/AIDS, and over 25 million people have died of AIDS worldwide since 1981 [1]. Integrase (IN) is an essential viral enzyme that is required for productive replication. This protein catalyzes insertion of a DNA copy of the viral genome into the host genome. The overall reaction occurs in two steps, termed "3'-processing" (3'-P), in which a GT dinucleotide is cleaved from the 3'-ends of the viral DNA and "strand transfer" (ST), in which the processed viral DNA ends are inserted into the host DNA. Integrase belongs to the superfamily of polynucleotidyl transferases that have similar metal-dependent catalytic mechanisms. In the case of IN, two catalytic Mg^{2+} ions are held in place by three acidic residues within the active site [D64, D116 and E152 (the "DDE motif")] [2–5]. Integrase inhibitors are a new class of antiretroviral agents that have been approved for the treatment of HIV-1 infections. Merck's Raltegravir (**1**, RAL, Isentress®, 2007) [6–8], was the first FDA-approved IN inhibitor (Fig. 1). Because RAL selectively blocks the strand transfer step, it has been designated as "IN strand transfer inhibitor" (INSTI). Among the important structural features shared by many INSTIs is a coplanar arrangement of three heteroatoms, which chelate the two catalytic Mg^{2+} ions (shown in red in Fig. 1). A halophenyl ring, which binds within a hydrophobic pocket formed by the complex of IN with viral DNA, is usually present [3,9,10]. Recently, crystal structures have been reported of the full-length IN of prototype foamy virus (PFV IN) in complexes with DNA substrates, and in some cases, these complexes also include bound INSTIs [11]. These structures reveal that the binding of RAL, and similar INSTIs, involves displacing the dA at 3'-end of the viral DNA from the active site, thereby disarming the viral nucleoprotein complex. These findings define the structural basis of retroviral DNA integration, and allow homology modeling of HIV-1 IN based on the PFV structure [12,13].

The development of IN inhibitors has continued since the appearance of RAL. Certain of these "2^{nd}-generation" INSTIs contain additional structural features that are not present in RAL [14,15]. GlaxoSmithKline's Dolutegravir (**2**, DTG, Tivicay®, 2013), a 2^{nd}-generation INSTI was recently approved by the FDA [16–19], contains a more extended and flexible carboxylamide spacer as compared to RAL. Alternate next-generation INSTIs differ from RAL in other ways. For example, Merck's MK-0536 (**3**) has a dihydronaphthyridinone [6,6] fused ring system, in contrast to the pyrimidi-none monocycle found in RAL [20, 21].

Based on the presence of a 1-dimethyl carboxamide group in **3**, we previously modified our bicyclic 6,7-dihydroxyoxoisoindolin-1-one-based inhibitors (**4a**) [22–24] by introducing a sulfonamido functionality [25]. This yielded a series of 6,7-dihydroxy-1-oxoisoindoline-4-sulfonamide-based inhibitors (**4b**) that have nanomolar antiviral potencies against both wild-type and RAL-resistant mutant forms of IN in cell-based viral infection assays [25, 26]. In the work described here, we modified compound **4** in three ways. First, we extended the linker between the halophenyl ring and the metal-chelating heteroatom triad (compound **5**, Fig. 1). Second, we transformed the [5,6] ring system of **4** into a [6,6] ring system (**6**). Finally, we prepared dimeric inhibitors (**7**) in an attempt to increase inhibitory potency by

creating compounds that can potentially simultaneously bind to the active sites of the two catalytically relevant INs in the pre-integration complex (also called the intasome).

SYNTHESIS

We synthesized dihydroxylisoindolinones **4** and **5**, having either one or two methylenes between the halophenyl and the isoindolinone ring systems, starting from methyl 2-chloromethyl-3,4-dimethoxybenzoate **8** (Scheme 1) [22, 27]. Coupling of appropriate halo-substituted benzylamines or phenylethylamines with **8** provided a series of lactams (**9a**, **10a**). Chlorosulfonation of these lactams with chlorosulfonic acid, followed by amination of the resulting sulfonyl chlorides with dimethylamine, afforded sulfonamides **9b** and **10b-1**, respectively. Using a similiar procedure for lactam **10a-2** gave bis-sulfonylation at both the 4-position of the indoline ring and the 3'-position of the 4'-fluorophenyl ring to yield bis-sulfonamide **10b-2**. Demethylation of the resulting lactams (**9a**, **10a-1**) and sulfonamides (**9b**, **10b-1**, **2**) with boron tribromide in dichloromethane provided the corresponding dihydroxyisoindolinones (**4, 5)**.

We synthesized dihydroxyisoquinolinones **6**, having a fused [6,6] isoquinolinone ring system, starting from methyl 2-chloromethyl-3,4-dimethoxybenzoate **8** (Scheme 2). Displacement of the benzylic chloride in **7** using trimethylsilanecarbonitrile gave nitrile **11**. Hydrogenation of **11** with platinum (IV) oxide afforded an intermediate phenylethylamine, which was directly cyclized in refluxing benzene to yield the isoquinolinone **12** [28]. Coupling of **12** with 3-chloro-4-fluorobenzylbromide afforded **13a**. Sulfonation of lactam **13a** with chlorosulfonic acid, followed by amination of the resulting sulfonyl chloride with dimethylamine, gave sulfonamide **13b**. Demethylation of **13a** and **13b** with boron tribromide in dichloromethane provided the final product dihydroxyisoquinolinones **6a** and **6b**.

It is known that Mg^{2+}-chelating heteroatoms are a key part of the binding of INSTIs to HIV-1 IN and that the HIV-1 intasome contains an IN tetramer that is composed of two IN dimers. Each of these IN dimers has a single enzymatically relevant IN, and each of the catalytically relevant INs inserts one of the two ends of the viral DNA into the host genome. This suggests that it might be possible for a single tethered inhibitor to bind both of the catalytically relevant IN active sites in the intasome. There have been reports of linker-dependency in symmetrically-joined dimeric IN inhibitors, although the basis for the dependency has not been clearly explained [29, 30]. The PFV co-crystal structure that contains **4b** shows that the sulfonamide group projects outward from the catalytic site [26]. This exposed moiety potentially offers a point that could be exploited by further structural modification. Therefore, we designed and synthesized a series of bis-dihydroxyloxoisoindolinesulfonamides having both hydrophorbic and hydrophilic linkers [**7**(**a-d**) and **7e**, respectively]. Sulfonation of 2-(3-chloro-4-fluorobenzyl)-6,7-dimethoxyisoindolin-1-one **9a** [22] with chlorosulfonic acid, followed by amination with different-length alkyl diamines in dichloromethane, and demethylation of the resulting bis-sulfonamide with boron tribromide, gave a series of bis-(dihydroxyloxoisoindoline) sulfonamides **7(a-d)** (Scheme 4). Bis-dihydroxyloxoisoindolinesulfonamide **7e** was prepared from 2-(2-(3-chloro-4-fluorobenzyl)-6,7-dihydroxy-*N*-methyl-1-oxoisoindoline-4-

sulfonamido)acetic acid **14** [25] by coupling with commercially available 3'-((oxybis(ethane-2,1-diyl))bis(oxy))bis(propan-1-amine) (Scheme 3).

BIOCHEMICAL AND BIOLOGICAL EVALUATION

The synthetic compounds described above were evaluated in biochemical assays using radiolabeled oligonucleotides to measure their inhibitory potencies in 3'-P and ST reactions (Table 1) [22]. Addition of a 4-sulfonamide substituent to **4a** resulted in a two-fold increase of the ST inhibitory potency (**4b**) [25, 26]. Increasing the distance separating the metal-chelating triads and the halophenyl ring by one methylene (**5a-1** and **5a-2**) resulted in significant loss of potency in both the 3'-P and ST inhibition assays. Addition of a 4-sulfonamide group to **5a-1** to give **5b-1** resulted in a threefold increase in potency in the ST assay. Compound **5b-2**, in which the chloro group on halophenyl ring of **5b-1** has been replaced by a dimethyl sulfonamide moiety, showed a 9-fold loss of potency in the ST assay (**5b-2** related to **5b-1**). With a fused [6,6] ring system, the 7,8-dihydroxy-3,4-dihydroisoquinolin-1(2*H*)-one (**6a**) showed a 13-fold loss of potency in the ST assay as compared to the fused [6,5] ring-containing system 6,7-dihydroxyisoindolin-1-one (**4a**). Consistent with these findings, addition of a 4-sulfonamide group, which converted **6a** to **6b**, resulted in an increase in potency in the ST assay (Table 1). In cell-based assays, **6b** showed sub-micromolar antiviral potency (EC_{50} = 552 nM), with cytotoxicity that was sufficiently high (CC_{50} = 8.9 μM) for the "selectivity index" (SI = CC_{50}/EC_{50}) to be relatively modest 16 (Table 2).

Next, we evaluated the *in vitro* inhibitory potencies of dimeric analogues [**7(a-d)** and **7e**] (Table 2). Because the *in vitro* assays we performed do not depend on a IN being in tetrameric form, we would not expect that making a dimer would have significant impact on the potencies of the compounds in these assays. In fact, the *in vitro* inhibitory potencies of the dimeric analogues were largely unchanged relative to the monomeric parent (**4b**). In contrast, the intasome in the viral integration in the cell-based assays does involve a tetrameric form of IN and we would expect a significant increase in antiviral potencies for dimeric inhibitors if they were able to span, and simultaneously bind to, the two catalytically revelant IN active sites. Using a homology model of HIV-1 IN, generated as previously described [31], we placed sulfonamide-containing monomeric inhibitors within each of the two catalytic centers of the active tetrameric intasome and observed that a distance of approximately 20 Å separated the sulfonyl groups of each inhibitor. We then manually constructed the linking segment of dimeric inhibitor **7e** to demonstrate its ability to span the required separation (Fig. 2). However, in cell-based antiviral assays, the dimeric inhibitors showed a greater than 11-fold and 400-fold loss of potency when compared to the parental monomer **4a** and **4b**, respectively. This loss of potency could arise from a number of factors. The dimers may not be a good fit for the IN tetramer, either because they cannot span two catalytically relvant active sites, or there may be other constraints that prevent them from binding to the relevant IN active sites at the same time. The dimeric coumpounds may also have poor cellular uptake. It is of note that the retention of *in vitro* ST inhibitory potency by the dimeric inhibitors relative to the monomeric parent (**4b**) indicates that, in terms of the ability of the compounds to bind to IN, that the region of the molecule that was modified to

create the dimeric compounds will tolerate significant structural elaboration. This is consistent with the compound binding to HIV-1 IN in a fashion similar to the binding orientation shown in the PFV•**4b** co-crystal structure [26].

CONCLUSION

Development of IN inhibitors has continued since the appearance of RAL. Certain of these 2nd-generation inhibitors contain structural features not found in RAL. For DTG, this includes a flexible and more extended carboxamide spacer between the metal-chelating heteroatom triad and the halophenyl ring, and alternate INSTIs differ in other ways. For example MK-0536 includes a 1-carboxamido group and fused [6,6] ring system. Based on these differences, we modified our bicyclic 6,7-dihydroxyoxoisoindolin-1-one-based IN inhibitors (**4**) to yield new analogues: dihydroxyisoindolinones (**5**) and dihydroxyisoquinolinones (**6**), respectively. We also prepared bis-6,7-dihydroxyisoindolin-1-one-4-sulfonamides (**7**) as dimeric HIV-1 IN inhibitors. These new analogues showed low micromolar inhibitory potency in *in vitro* HIV-1 integrase assays. Our current work extends the SAR that we had previously reported for our series **4** compounds.

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Biography

X.Z. Zhao

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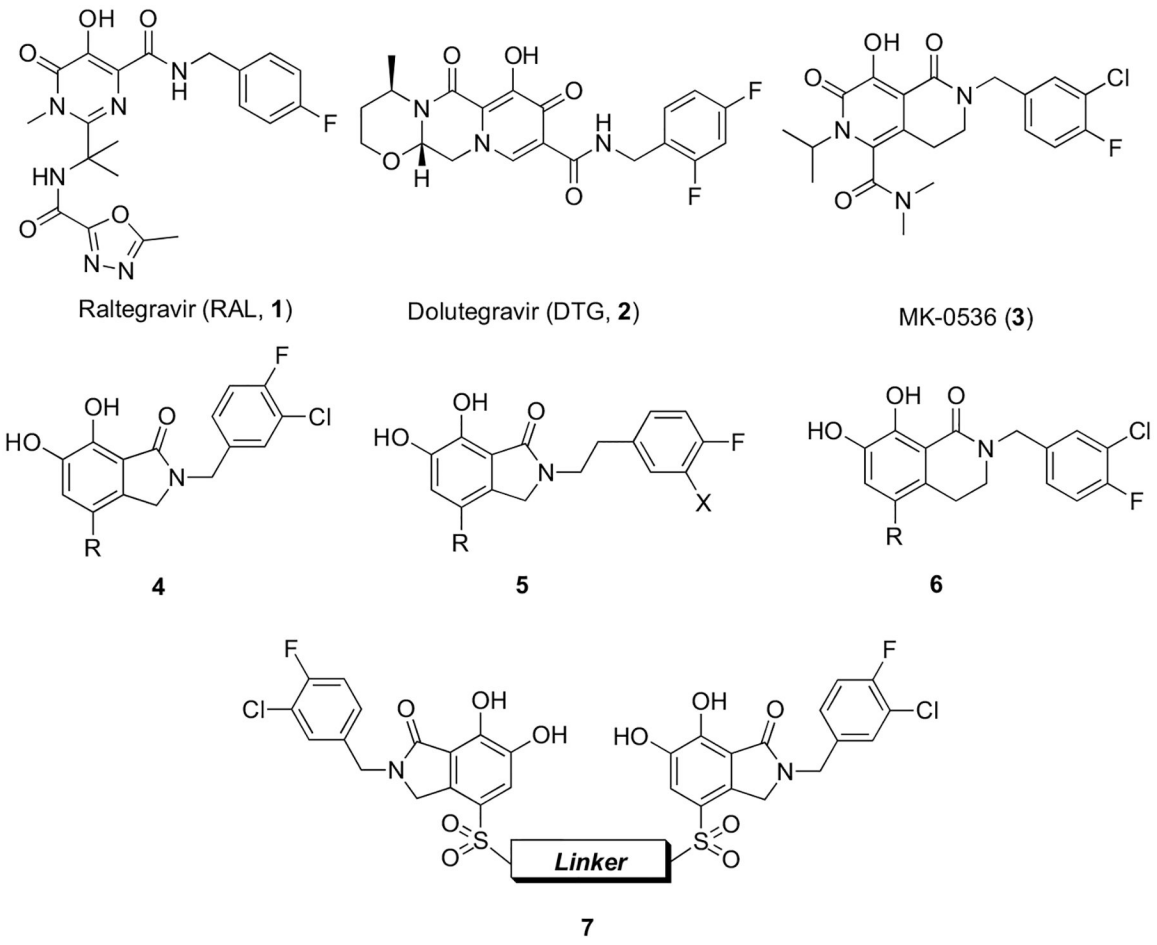


Fig. (1).

Structures of HIV-1 integrase inhibitors described in the text. [X = Cl or SO_2NMe_2; R = H **(a)** or sulfonamide **(b)**].

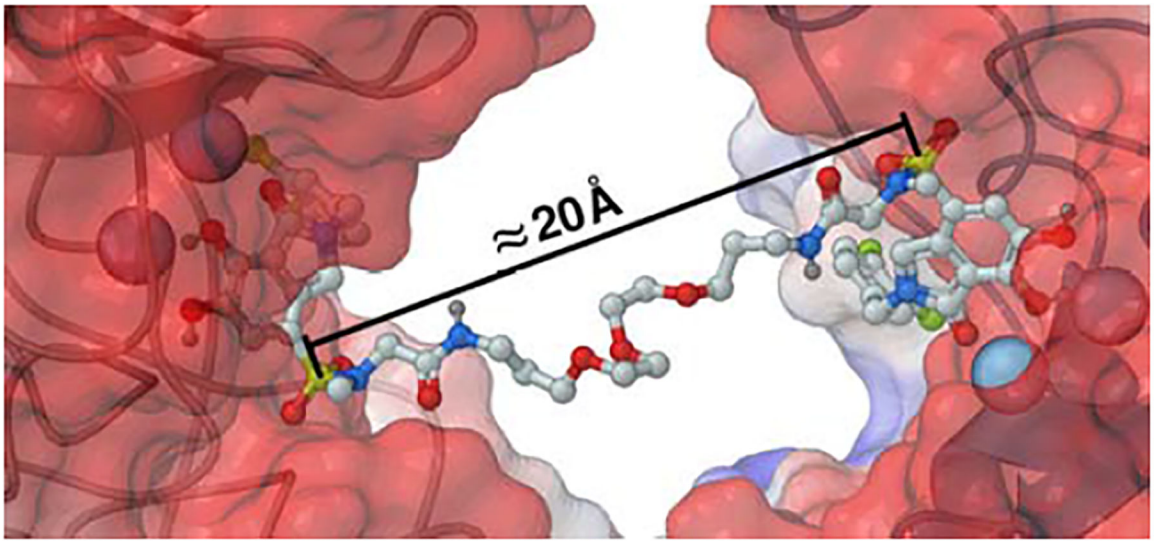
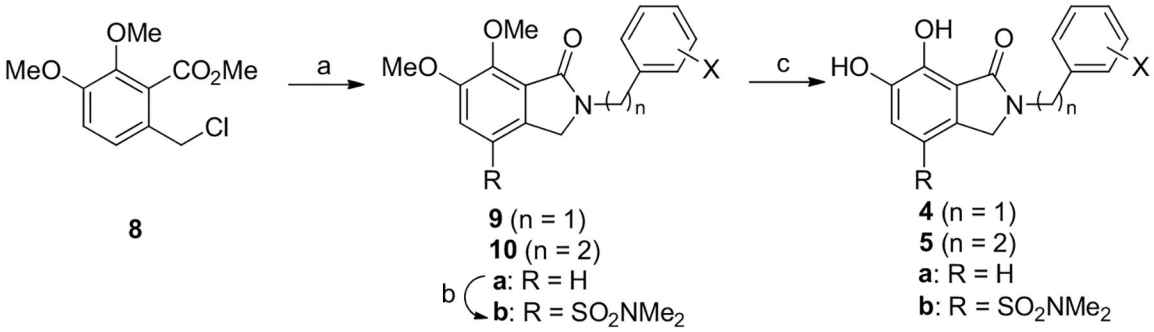


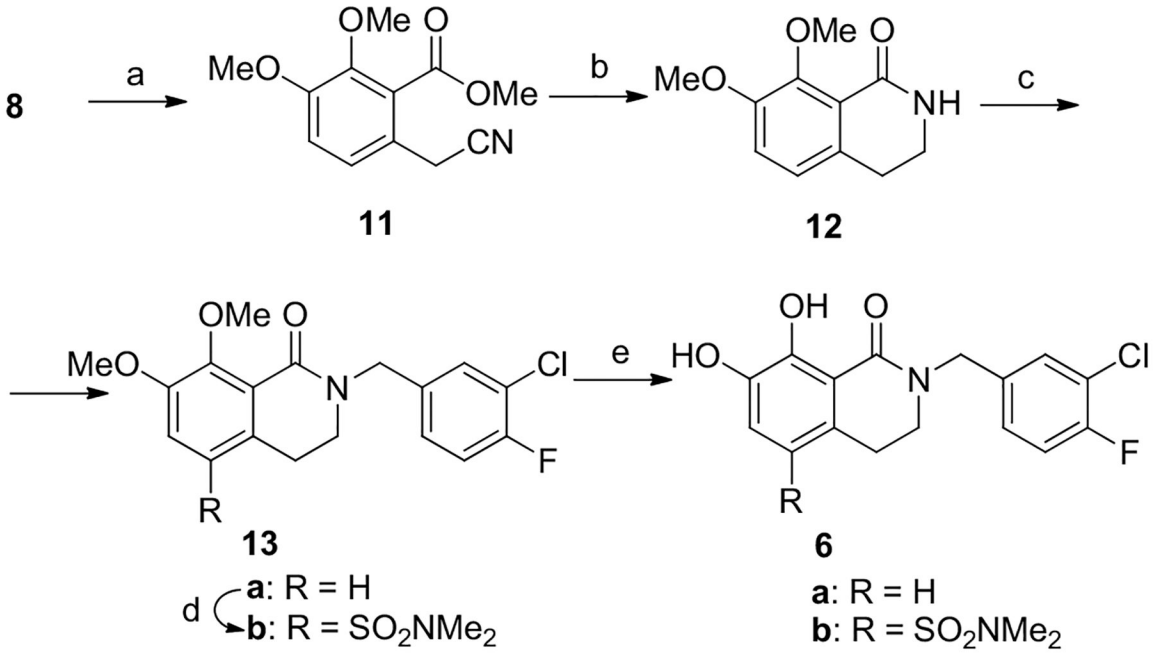
Fig. (2).

Theortical simultaneous occupation of both catalytic centers of tetrameric IN by inhibitor **7e**, showing the calculated distance separating the sulfonlyl groups of each monomeric inhibitor. The graphic was generated by manual docking using a homology model of HIV-1 IN, generated as previously described [31].



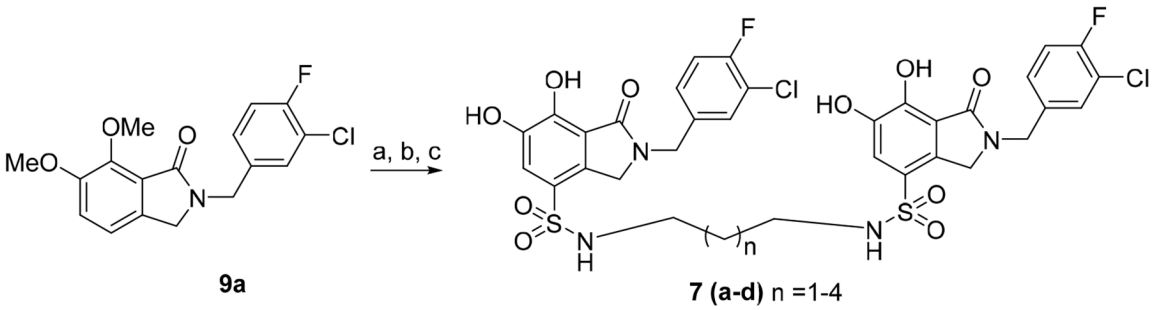
Scheme 1.

Synthesis of dihydroxyisoindolin-1-ones **4** and **5**. *Reagents and conditions*: a) 3-chloro-4-fluorobenzylamine, 2-(3-chloro-4-fluorophenyl)ethanamine, or 2-(4-fluorophenyl)ethanamine, Et_3N, CH_3CN; b) i. $ClSO_3H$; ii. Me_2NH, Et_3N, CH_2Cl_2; c) BBr_3, CH_2Cl_2.



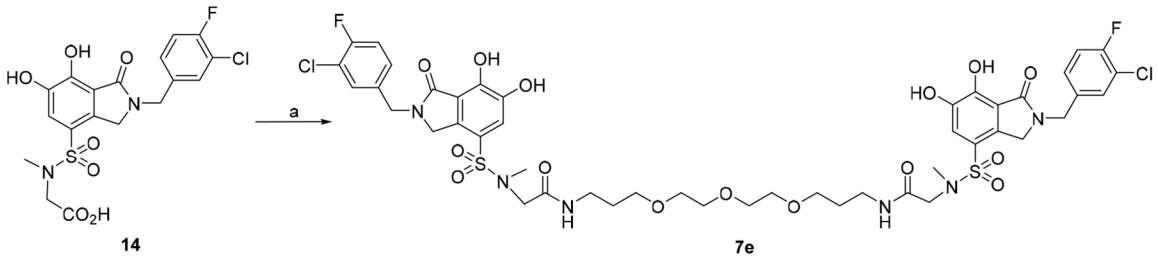
Scheme 2.

Synthesis of dihydroxyisoquinolinones **6**. *Reagents and conditions*: a) TMSCN, TBAF, rt, o/n, 80%; b) H_2, PtO_2; c) NaH, 3-chloro-4-fluorobenzylbromide; d) i. $ClSO_3H$; ii. Me_2NH, Et_3N, CH_2Cl_2; e) BBr_3, CH_2Cl_2.



Scheme 3.

Synthesis of bis-6,7-dihydroxyisoindolin-1-one-4-sulfonamides **7 (a-d)**. *Reagents and conditions*: a) $ClSO_3H$; b) $NH_2CH_2(CH_2)_nCH_2NH_2$, Et_3N, CH_2Cl_2; c) BBr_3, CH_2Cl_2.

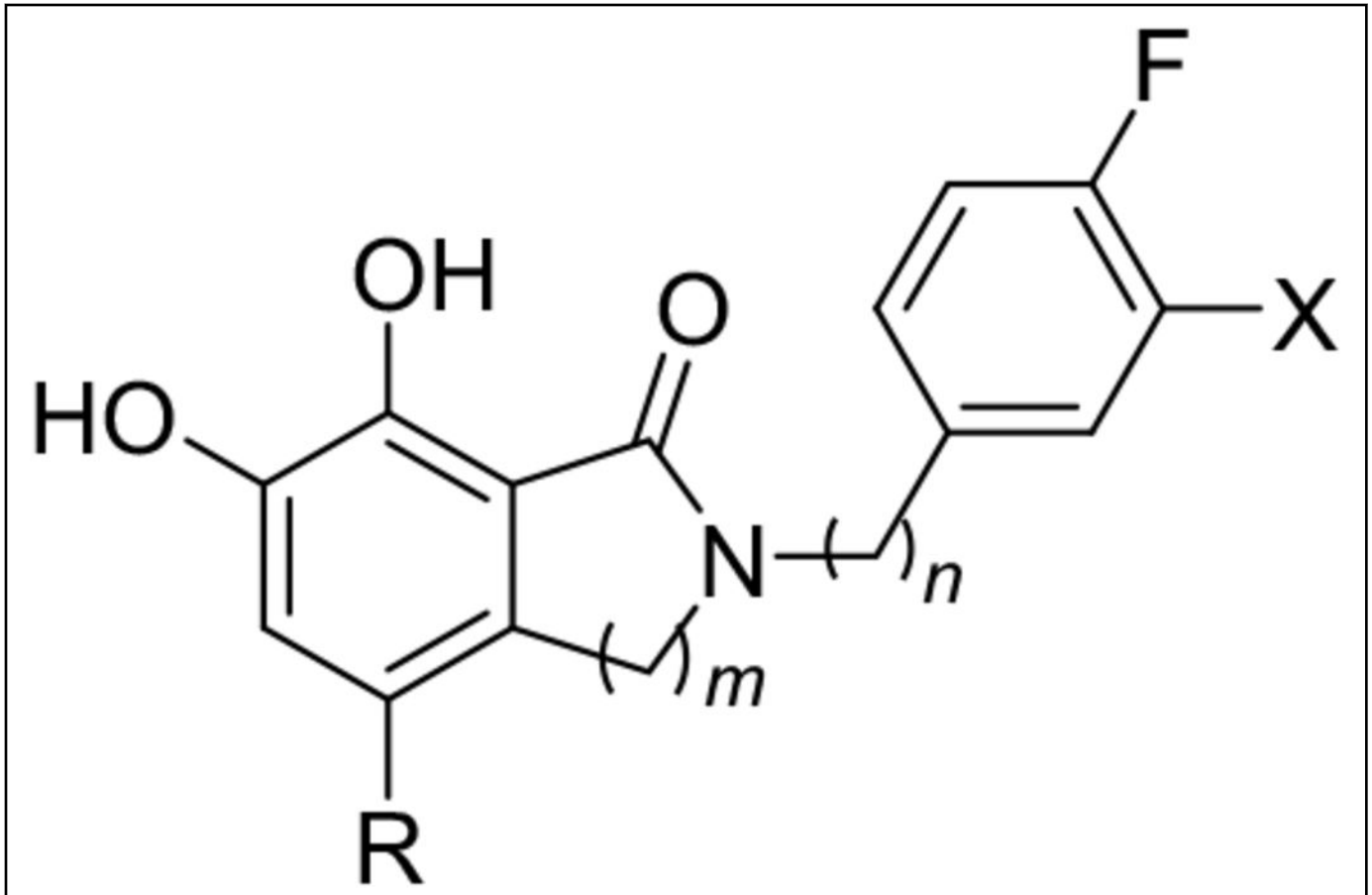


Scheme 4.

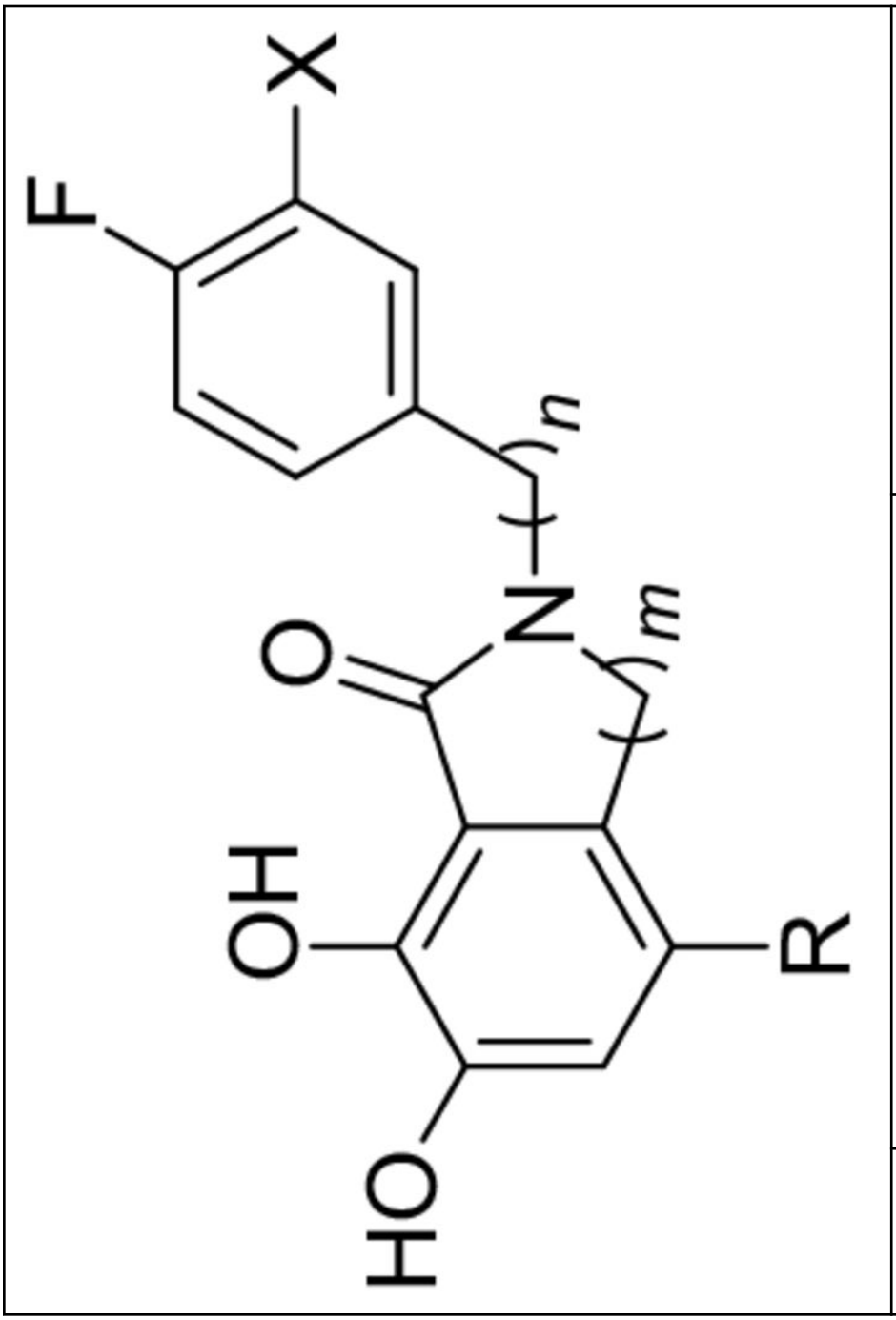
Synthesis of bis-6,7-dihydroxyisoindolin-1-one-4-sulfonamide **7e**. *Reagents and conditions*: a) $NH_2(CH_2)_3O(CH_2)_2O(CH_2)_2O(CH_2)_3NH_2$, HBTU, DIEA, DMF.

Table 1.

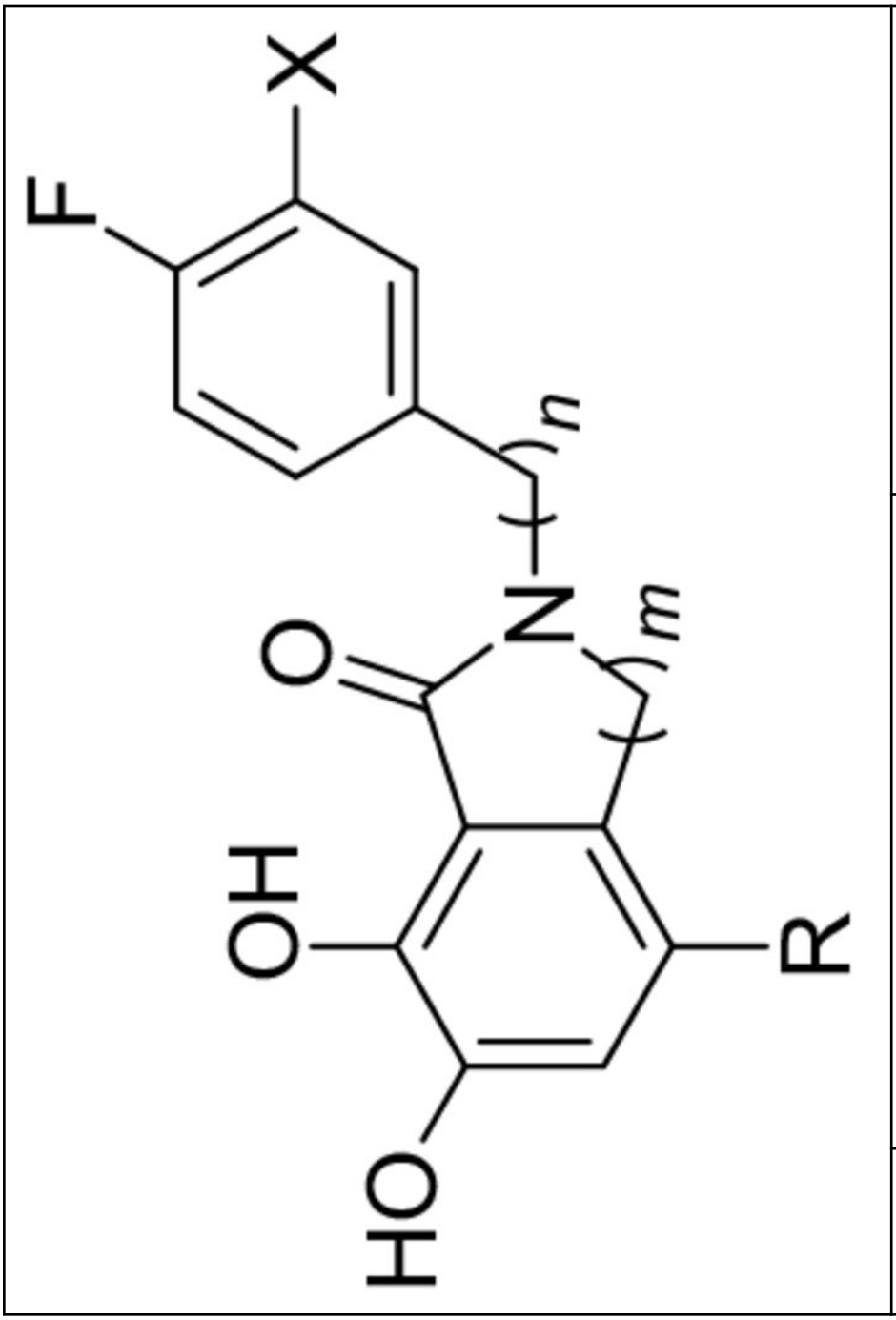
In vitro IN inhibitory potencies of 6,7-dihydroxyisoindolin-1-ones (4 and 5), and 7,8-dihydroxy-3,4-dihydroisoquinolin-1(2*H*)-ones (6).



Entry	Structure				IN Inhibition (μM)[a]	
	m	n	R	X	3'-P (IC_{50} ± SD)	ST (IC_{50} ± SD)
4a[b]	1	1	H	Cl	13.2 ± 3.1	0.16 ± 0.08



Entry	Structure				IN Inhibition (μM)[a]	
	m	n	R	X	3'-P (IC_{50} ± SD)	ST (IC_{50} ± SD)
4b[c]	1	1	SO_2NMe_2	Cl	11.3 ± 2.1	0.074 ± 0.01
5a-1	1	2	H	Cl	> 333	5.7 ± 1.1
5a-2	1	2	H	H	> 333	9.6 ± 2.1
5b-1	1	2	SO_2NMe_2	Cl	> 333	1.6 ± 0.3



Entry	Structure				IN Inhibition (μM)[a]	
	m	n	R	X	3'-P (IC_{50} ± SD)	ST (IC_{50} ± SD)
5b-2	1	2	SO_2NMe_2	SO_2NMe_2	> 333	14.8 ± 3.2
6a	2	1	H	Cl	> 333	2.1 ± 0.6
6b	2	1	SO_2NMe_2	Cl	69 ± 9	0.40 ± 0.08

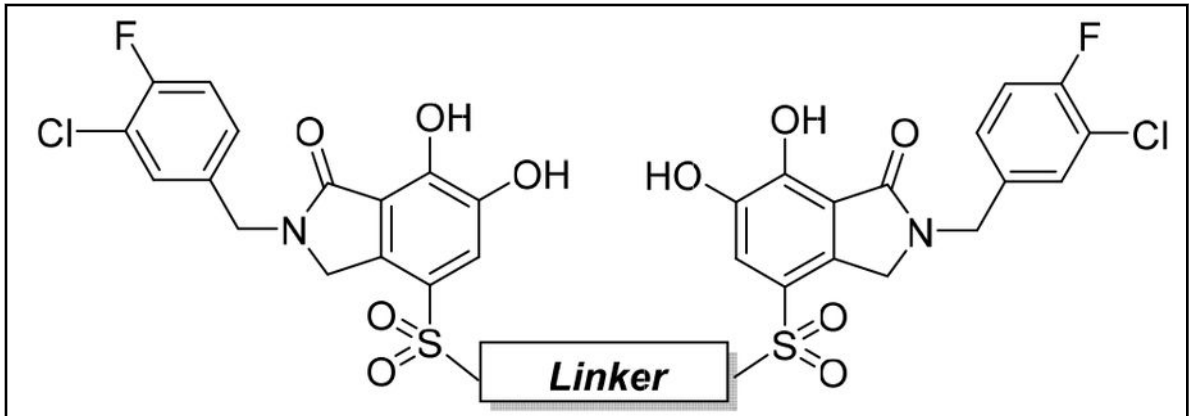
[a] Assays were performed using a gel-based protocol with Mg^{2+} cofactor [20];

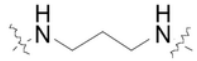
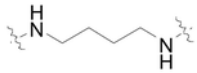
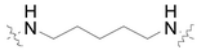
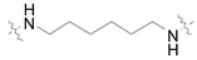
[b] Reported previously [22];

[c] Reported previously [25].

Table 2.

Integrase inhibitory potencies *in vitro* and antiviral potencies of bis-6,7-dihydroxyisoindolin-1-one-4-sulfonamides 7(a-e).



No.	Linker	IC_{50} (3'-P, μM)[a]	IC_{50} (ST, μM)[a]	CC_{50} (μM)[b]	EC_{50} (μM)[b]
7a		8.0 ± 0.9	0.095 ± 0.01	50	5.7
7b		13.5 ± 0.3	0.046 ± 0.007	35	4.9
7c		20.7 ± 2.3	0.07 ± 0.009	18.3	5.3
7d		52 ± 6	0.16 ± 0.05	13.1	5.5
7e		48 ± 15	0.14 ± 0.01	ND[c]	ND[c]

[a] Assays were performed using a gel-based protocol with Mg^{2+} cofactor [22];

[b] Assays were performed as describe previously [32];

[c] Not determined.