ACS Medicinal Chemistry Letters

Letter

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Design, Synthesis, and Biological Evaluation of 1,2-Dihydroisoquinolines as HIV-1 Integrase Inhibitors

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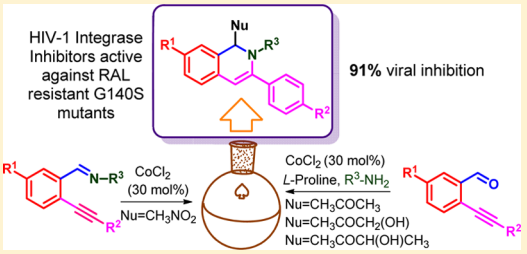
Supporting Information

ABSTRACT: 6-*Endo-dig*-cyclization is an efficient method for the synthesis of 1,2-dihydroisoquinolines. We have synthesized few 1,2-dihydroisoquinolines having different functionality at the C-1, C-3, C-7, and N-2 positions for evaluation against HIV-1 integrase (HIV1-IN) inhibitory activity. A direct nitro-Mannich condensation of *o*-alkynylaldimines and dual activation of *o*-alkynyl aldehydes by inexpensive cobalt chloride yielded desired compounds. Out of 24 compounds, **4m** and **6c** came out as potent integrase inhibitors in *in vitro* strand transfer (ST) assay, with IC_{50} value of 0.7 and 0.8 μM, respectively. Molecular docking of these compounds in integrase revealed strong interaction between metal and ligands, which stabilizes the enzyme−inhibitor complex. The ten most active compounds were subjected to antiviral assay. Out of those, **6c** reduced the level of p24 viral antigen by 91%, which is comparable to RAL in antiviral assay. Interestingly, these compounds showed similar ST inhibitory activity in G140S mutant, suggesting they can act against resistant strains.



KEYWORDS: *Multicomponent reaction, integrase, integrase inhibitors, molecular docking*

In the area of medicinal research efforts, the discovery, development, and evolution of novel and potent HIV-1 integrase (IN) inhibitors[1] remains a significant scientific endeavor, as the high mutation ability of this virus results in resistance against drugs. HIV-1 IN is a 32 kDa protein that catalyzes the incorporation reaction of a viral genome inside the host cell and can then be taken as an absolute target for treating HIV-1 inhibition.[2,3] Highly conserved DDE (D64, D116, and E152 amino acids) motif in catalytic core domain (CCD) of HIV-1 IN bind to divalent metal cofactors (Mg^{2+} and Mn^{2+}).[1,4,5] These metal ions present at the catalytic active site of an enzyme form a ligand−Mg^{2+}−IN complex with the inhibitors. This ligand−Mg^{2+}−IN complex would subsequently blocks the transition state of the IN−DNA complex by competing with the target DNA substrate, acting as an "interfacial inhibitor".[1] Treatment with FDA-approved raltegravir (RAL, Isentress or MK-0518)[6] demonstrated significant and sustained suppression of viral RNA levels to less than 50 copies/mL, along with a substantial increase in CD4 immune cell counts.[7] RAL has shown failure in the selection of mutations at integrase position Y143, Q148, or N155.[8] Elvitegravir (EVG) (GS-9137)[9] and dolutegravir (DTG) (S/GSK-1349572)[10] are recently approved drugs along with INSTIs, which show improved efficacies against RAL resistant strains (Figure 1).[11−13] However, viral strains that are highly resistant against EVG[14] and DTG[15] have been recently reported demonstrating multiple mutations in integrase.

Earlier it was reported that 2-hydroxyisoquinoline-1,3-(2*H*,4*H*)-dione scaffold chelates Mg^{2+} causing inhibition of

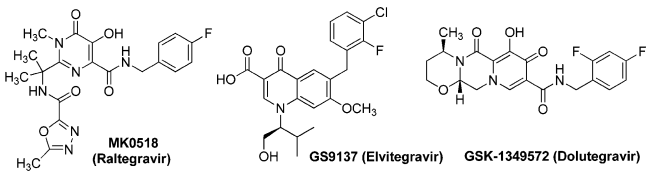


Figure 1. Structures of the best known HIV-1 integrase strand transfer inhibitors.

Received: June 9, 2015
Accepted: August 9, 2015
Published: August 10, 2015

DOI: 10.1021/acsmedchemlett.5b00230
ACS Med. Chem. Lett. 2015, 6, 1065−1070

HIV-1 IN and/or the HIV-1 reverse transcriptase ribonuclease H domain.[16,17] Billamboz et al. further established that this class of compounds inhibit viral replication of HIV-1 in MT-4 cells.[18] In line with continuous search for a better integrase inhibitor, we have selected dihydroisoquinoline as pharmacophore with a combination of nucleophiles having differential ability to chelate with Mg^{2+} ions. Based on these facts, we designed and synthesized three types of 1,2-dihydroisoquinolines derivatives bearing different functional groups at the C-1, C-3, C-7, and N-2 positions (Figure 2).

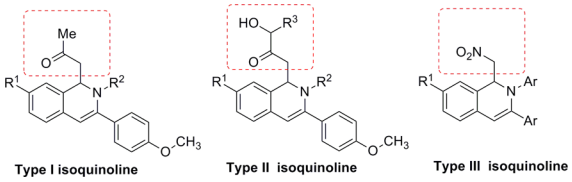


Figure 2. Designed isoquinolines as potential HIV-1 integrase strand transfer inhibitors.

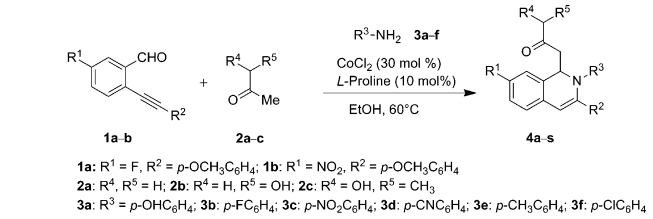
Molecular docking of these compounds to integrase protein showed strong interactions between both. The antiviral and strand transfer inhibition assay suggested good biological and biochemical responses. At last, the combined efforts proved **6c** as one of the most potent molecules in the antiviral assay among the ten compounds **4e**, **4f**, **4m**, **4s**, **4p**, **6a**−**d**.

The type I 1,2-dihydroisoquinolines **4a**−**h** were synthesized by the reaction of *o*-alkynyl aldehyde **1a**−**b** with ketones **2a**−**c** and amines **3a**−**f** via dual activation process as per the reported literature.[19−21] Under the effective catalysis of inexpensive anhydrous $CoCl_2$, intermolecular attack of enamine (generated by the reaction L-proline with ketones) onto *o*-alkynylaldimines (formed by the reaction of *o*-alkynylaldehyde with amine) resulted in the desired 6-*endo*-*dig* cyclized products **4a**−**h** (Scheme 1). Reactions of 2-alkynylbenzaldehydes **1a**−**b** with propanone (**2a**) and 4-aminophenol (**3a**) afforded the desired products **4a** and **4h** in 65 and 68% yields, respectively, after 12 h; whereas reaction of substrate **1b** with propanone and *p*-toluidine **3e** provided the product **4f** in 87% yield in less reaction time (4 h). In the case of 4-aminophenol **3a**, the presence of a hydroxy group at the *p*-position of the ring increases the possibility of the intermolecular hydrogen bonding and decreases the nucleophilicity of the NH_2 group as well as the formation of imine; however, in the case of *p*-toluidine **3e** the presence of an electron-releasing methyl group at the 4-position of the ring increases the nucleophilicity (+R effect) of the NH_2 group, which facilitates the formation of the key intermediate imine.

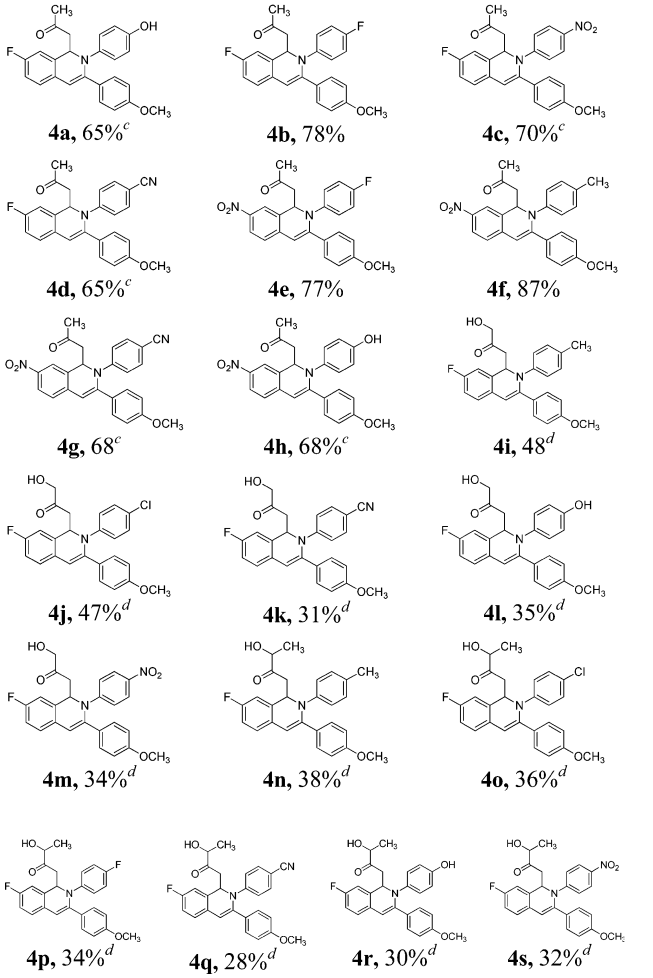
It is notable that the electron-withdrawing (−CN, $-NO_2$, −F) groups are present at the *p*-position of the phenyl ring of amines, providing the desired products **4b**−**e** and **4g** in comparatively low yields, which could be due to reduced nucleophilicity (−R effect) of the NH_2 group. Dihydroisoquinolines **4i**−**s** with a requisite hydroxyl group at the *α*-position of the keto group (Figure 2, type II isoquinolines) were synthesized in 28−48% yield by the reaction of substrate **1a** with respective amines, hydroxyacetone (**2b**), and 3-hydroxybutan-2-one (**2c**). The low yields might be attributed to the low reactivity of ketones (able to form intramolecular hydrogen bond). All of the dihydroisoquinolines were obtained as racemic mixtures (*R* and *S*).

Type III, 1,2-dihydroisoquinolines (Figure 2) were prepared by nitro-Mannich condensation by our reported procedure.[19] Dihydroisoquinolines **6a**−**e** were synthesized in 58−90% yields

Scheme 1. Synthesis of 1,2-Dihydroisoquinolines 4a−s[ab]



1a: R^1 = F, R^2 = *p*-$OCH_3C_6H_4$; **1b**: R^1 = NO_2, R^2 = *p*-$OCH_3C_6H_4$
2a: R^4, R^5 = H; **2b**: R^4 = H, R^5 = OH; **2c**: R^4 = OH, R^5 = CH_3
3a: R^3 = *p*-OHC_6H_4; **3b**: *p*-FC_6H_4; **3c**: *p*-$NO_2C_6H_4$; **3d**: *p*-CNC_6H_4; **3e**: *p*-$CH_3C_6H_4$; **3f**: *p*-ClC_6H_4



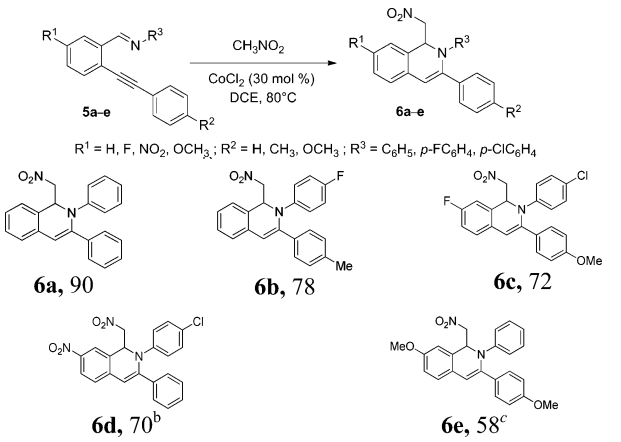
[a]Reactions were performed using 1.08 mmol of 2-alkynylbenzaldehyde **1**, 5.0 equiv of ketone **2**, 1 equiv of amine **3**, 30 mol % $CoCl_2$, 10 mol % L-proline in 5.0 mL of EtOH at 60 °C for 4 h unless otherwise noted. [b]Isolated yield. [c]Reaction time 12 h. [d]Reaction time 16 h.

via attack of aci-nitromethane on *o*-alkynylaldimines under cobalt-catalysis (Scheme 2). Substrate **5c** and **5d** bearing electron-withdrawing groups at the C-5 position provided the desired products in 72 and 70% yields, respectively; however, substrate **5e** with an electron-releasing group yielded the desired product **6e** in a 58% yield and required a longer reaction time.

The synthesized compounds were examined for anti-integrase activity through *in vitro* biochemical assays, adapted from previously described methods.[22] The strand transfer IC_{50} value (*μ*M) and cell-based (TZM-bl) cytotoxicity CC_{50} value (*μ*M) at 48 h were determined for all derivatives (Table 1). It was observed that dihydroisoquinolines having 7-fluoro function **4a**−**d** were found to be less potent while the IC_{50} and CC_{50} values of 7-nitro derivatives of 1,2-dihydroisoquinoline **4e**−**h** showed a

DOI: 10.1021/acsmedchemlett.5b00230
ACS Med. Chem. Lett. 2015, 6, 1065−1070

Scheme 2. Synthesis of Nitro-Substituted 1,2-Dihydroisoquinolines[a,b]



[a]The reaction was carried out using **5a–e** (1 equiv) and nitromethane (2 equiv) in the presence of 30 mol % $CoCl_2$ in 1,2-dichloroethane at 80 °C for 6 h. [b]Isolated yield. [c]Reaction time 10 h.

Table 1. Inhibition of HIV-1IN, Cytotxicity, and Antviral Activity of Substituted 1,2-Dihydroxyquinolines

entry	%ST[a] inhibition at 10 μM dose in WT	IC_{50} of ST activity in μm	IC_{50} of ST[b] activity in G140S mutant	EC_{50} (μM) In TZM-bl cells	CC_{50} on TZM-bl cells (μm)	TI
RAL	94.7	0.007	0.05	0.15	>200	>1300
4a	65.7	12.0	14.0	21.4	60.5	2.8
4b	44.2	ND	ND	ND	ND	–
4c	40.2	ND	ND	ND	11.2	–
4d	4.4	ND	20.0	ND	ND	–
4e	62.4	0.46	0.64	36.3	7.7	0.2
4f	69.9	0.6	0.65	26.3	10.6	0.4
4g	67.5	2.9	2.8	22.5	15.4	0.6
4h	65.0	6.6	7.6	31.4	23.5	0.7
4i	57.7	15.1	14.1	28.4	23.7	0.8
4j	55.1	17.9	14.9	26.4	8.5	0.3
4k	71.9	7.6	7.0	29.1	39.7	1.3
4l	72.5	22.1	20.1	24.4	47.7	1.9
4m	90.6	0.82	46.4	10.41	46.4	4.4
4n	60.9	10.6	12.7	ND	12.7	–
4o	78.3	11.8	10.8	ND	9.9	–
4p	78.7	7.9	8.0	11.7	14.0	1.1
4q	62.2	22.2	22.0	18.7	24.1	1.2
4r	78.0	25.2	25.0	19.7	50.9	2.5
4s	88.2	5.2	5.5	12.7	10.1	0.7
6a	40.2	ND	ND	15.6	10.3	0.6
6b	55.2	ND	ND	16.5	21.5	1.3
6c	91.5	0.7	0.8	0.03	32.2	1073
6d	71.5	3.1	3.0	1.15	26.0	22.6
6e	55	ND	ND	10.5	29.4	2.8

[a]ST, % inhibition of strand transfer activity of integrase enzyme. [b]ST, dose determination of molecules at which 50% of integrase activity was inhibited. RAL, raltegravir. EC, effective concentration. TI, therapeutic index (CC_{50}/EC_{50}). ND is not determined.

significant range from 0.4 to 6.6 μM and 7.7 to 23.5 μM, respectively. Furthermore, the 1,2-dihydroisoquinoline bearing hydroxyl acetone **4i–m** and hydroxyl butanone **4n–s** functionality at the C-1 position showed an IC_{50} ranging from 0.82 to 22.1 μM and 5.2 to 25.2 μM, respectively. Additionally, the CC_{50} values were in the range of 8.5 to 50.9 μM for both earlier mentioned derivatives. However, in the case of the **6a–e** derivatives (nitromethane group at the C-1 position) IC_{50} of 0.7 to 3.1 μM and CC_{50} 10.3 to 32.2 μM were observed, which gave a lead to move on to the next step. The inhibitory activity of the compounds was also evaluated on one of the RAL resistant IN mutants G140S.[23] Interestingly our compounds showed almost similar activity against mutant strain compared with the WT strain (Table 1), suggesting these compounds can act against RAL resistant IN. Furthermore, these molecules were screened in an antiviral assay using TZM-bl cell (Hela cell clone engineered to express CD4 and CCR5 receptors).[24] Compounds **4m** and **6c** had shown good antiviral activity and a safety index of 4- and 1073-fold, respectively. p24 is a viral protein that is not present in normal uninfected cells. The early diagnosis of HIV infection can be measured by a p24 antigen assay, which is standard screening assay for HIV testing. TZM-bl cells may or may not be correlated with infectivity of other target cell types, e.g., PBMC, whereas p24 directly correlates with infectivity of cells. Hence we subjected ten potent compounds **4e**, **4f**, **4m**, **4s**, **4p**, and **6a–e** to antiviral assays on VSV-G pseudotyped virus-pNL4–3(WT-HIV-1) using a p24 antibody[25–27] (Figure 3). At a concentration of 10 μM, compound **6c** exhibited 91% reduction in the band intensity of p24 (calculated by densitometry analysis shown in Figure 3 and Figure S1), while for raltegravir, the band was observed to be reduced by 92%, and **4m** exhibited 70.9% reduction in band intensity of p24 at 10 μM. Thus, taking into account cytotoxicity and the antiviral properties of **6c**, having a nitro group as a metal coordinating unit showed promising potency in antiviral assays compared to corresponding

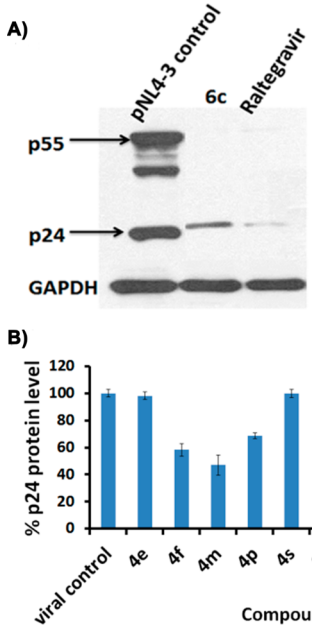


Figure 3. (A) One representative Western blot of jurkat cells treated with **6c** or with no ligand (lane pNL4–3) and then infected at an MOI of **1** with VSV-G pseudotyped virus–pNL4–3 (WT–HIV-1). Cell supernatants were pelleted, solubilized in lysis buffer, detected, and analyzed using a p24 antibody. (B) Percent p24 viral protein level after treatment by compounds, calculated by densitometry analysis. *$p < 0.05$ compared to the control. (C) Cytotoxicity of the Jurkat cell line of the indicated compounds.

DOI: 10.1021/acsmedchemlett.5b00230
ACS Med. Chem. Lett. 2015, 6, 1065–1070

hydroxyacetone bearing dihydroisoquinoline; **4m** with a nitro group at the C-7 can also be considered a potent molecule.

Molecular docking was carried out *in silico* with both *R* and *S* stereoisomers to understand the molecular recognition interactions. A two-metal model for HIV-1 IN CCD in complex with the small molecule, 1-(5-chloroindol-3-yl)-3- (tetrazoyl)-1,3-propandione-ene (5-CITEP), was used as a surrogate for an IN/viral DNA complex, as this model explains the molecular recognition interactions.[28] The molecular docking poses along with interactive sites of the compounds with amino acids are provided (Supporting Table S1). The molecular docking study of **4m**, **6c**, and **6d** demonstrated that nitro groups chelate with both the active site magnesium ions. Additionally these compounds show cation−π interaction with Mg^{2+} (Figure 4). Compound **4m**

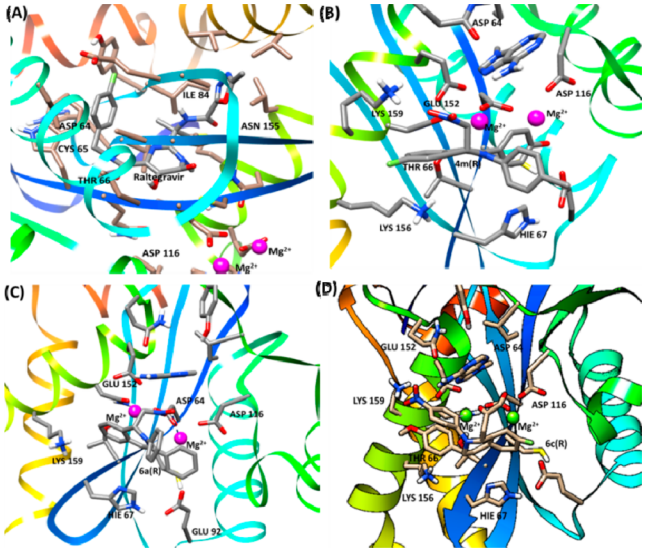


Figure 4. Docked poses of ligand-HIV IN CCD in complexes of (A) raltegravir, (B) **4m**(R), (C) **6a**(R), and (D) **6c**(R). The chelation of two Mg^{2+} ions with the ligands is shown here. The most interactive residues in the docked complexes were found to be Asp116, Glu92, Lys159, Hie67, and Asp64.

has been found to be the best among the compounds **4a**−**s** in *in vitro* as well as antiviral assays. The molecular docking scores of these compounds were in agreement with their activity. Additionally, the *p*-fluorophenyl group of compound **4m** interacts with Lys159 and Lys156 to form hydrogen bonding networks (Figure 4b). Compound **6c** perfectly fits in the active site, chelating both active site Mg^{2+} so that it blocks access of Mg^{2+} to the substrate. Additionally, it forms hydrogen bonds between the phenolic methoxy group and Ser119. Asn117, Asn120, and Lys159 were involved in interactions with the target DNA. Alves et al.[29] reported the importance of Lys159 in anti-HIV IN activity by a quantum mechanics/molecular mechanics (QM/MM) study of the protein−ligand interaction for HIV-1 integrase inhibitors. Recently, Ser119 contribution to target site preferences was reported in the CCD of IN.[30] Our results corroborate with the literature reports in terms of substrate−enzyme interactions.

Gratifyingly, 24 compounds were successfully synthesized using an operationally simple, direct approach. The cell based cytotoxicity assay in TZM-bl and Jurkat cells (Figure 3C) along with ST inhibition suggest that **4e**, **4f**, **4m**, **4s**, **4p**, and **6a**−**e** have good therapeutic efficacy *in vitro*. The probable reason for the diminished activity of **4e**, **4f**, **4m**, **4s**, **4p**, **6a**, **6b**, **6d**, and **6e** in antiviral assays could be high protein binding. According to the molecular docking study, the addition of a nitro moiety instead of an acetone chain is an attractive approach to increase the inhibitory activity against HIV-1 IN, as the nitro group is a stronger nucleophile in which oxygen atoms can chelate with the active site Mg^{2+} ions in a more effective manner. In light of the above observations, undesired intramolecular hydrogen bonding could be a reason for the diminished activity of these acetone, hydroxyacetone, and hydroxybutanone analogues. Compounds **4a**−**s** contain a number of oxygen atoms in the vicinity and had a maximum opportunity to chelate Mg^{2+} to inhibit enzymatic activity (due to keto−enol tautomerism) and form intramolecular hydrogen bonds, which drastically reduce the availability of oxygen atoms to chelate Mg^{2+}. Hence, the introduction of nitro in the 1,2-dihydroisoquinoline derivatives is an attractive idea for the generation of new drug entities. Compound **6c** showed a noteworthy 91% reduction of viral antigens as indicated in Figure 3B comparable to that of RAL. Interestingly, these compounds showed excellent inhibition of ST activity against RAL-resistant IN mutant G140S. The G140S mutation causes a conformational change that overcomes the ability of Q148H and other RAL-resistant mutants to replicate in cells. As these compounds have comparable activity against G140S, they are better substitutes to overcome the limitations developed against RAL. The docking posture of these compounds simplifies the presence of oxygen atoms in the vicinity of both the active site Mg^{2+} to form metal coordination. These compounds show cation−π interactions with the active site Mg^{2+} ions. Furthermore, the binding of these compounds at the active site of HIV-IN is stabilized by various electrostatic and van der Waals interactions with the active site amino acids (docking table). The CCD of IN is essential for 3′-processing of the viral DNA and ST reactions, and the substitution of any of the residues in the DDE motif dramatically inhibits the activity of IN. We propose that newly synthesized INTIs **6a**−**e** primarily act through this mechanism.

In summary, a series of differently substituted 1,2-dihydroisoquinolines were synthesized in one-pot via a nitro-Mannich reaction from easily accessible *o*-alkynylal dehydes under mild reaction conditions and cobalt catalysis as an inexpensive methodology. Screening of the designed compounds on HIV-1 IN inhibition have confirmed 1,2-dihydroisoquinoline derivatives as potential HIV-1 INSTIs. The lead compounds disclosed in this study demonstrated (i) significant inhibition against the strand transfer processes of HIV-1 IN; (ii) significant antiviral activity, and (iii) comparatively reduced cytotoxicity in the TZM-bl cell line. Among 24 compounds screened, **4m** and **6c** stood out as potent HIV-1 inhibitors with significant ST inhibition. Dihydroisoquinoline **6c** showed significant viral inhibition with better ST inhibition making it a potent HIV-1 inhibitor. The cross resistance of elvitegravir[31] and dolutegravir[32] with raltegravir cannot be ruled out. Hence, novel agents can prove to be an effective alternative to FDA approved INSTIs. The potential newer molecules presented in our study are indeed toxic compared to raltegravir but will provide a backbone to develop next-generation inhibitors against integrase.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00230.

DOI: 10.1021/acsmedchemlett.5b00230
ACS Med. Chem. Lett. 2015, 6, 1065−1070

Experimental procedures, biological assays, characterization of compounds, and docking table (PDF)

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Author Contributions

The manuscript was written via contributions of all authors. All authors have given approval of the final version of the manuscript.

Funding

The authors acknowledge the Department of Science and Technology for financial support and the Delhi University Instrumentation Centre for providing NMR facilities. P.Y. is thankful to DST-WOS B for financial support. S.S. and V.Ti. are thankful to CSIR, India for SRF.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

RAL, raltegravir; IN, integrase; INSTI, Integrase strand transfer inhibitor

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ACS Med. Chem. Lett. 2015, 6, 1065−1070