Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2018, 10 [7]: 18-31
[http://scholarsresearchlibrary.com/archive.html]

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

4-Substituted Benzylideneisoquinoline-1,3(2*H*, 4*H*)-dione Derivatives: Synthesis and Biological Evaluation as Potential HIV-1 Integrase Inhibitors

Pankaj Wadhwa[1], Priti Jain[1], Jadhav HR[1*] and Santosh Rudrawar[2,3]

[1]*Department of Pharmacy, Birla Institute of Technology and Science Pilani, Pilani Campus, Rajasthan, India*

[2]*Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia*

[3]*School of Pharmacy, Griffith University, Gold Coast 4222, Australia*

***Corresponding author:** Jadhav HR, Department of Pharmacy, Birla Institute of Technology and Science Pilani, Pilani Campus, Rajasthan, India. Tel: 911596 515506: E-mail: hemantrj@pilani.bits-pilani.ac.in*

ABSTRACT

Herein, we report the synthesis, evaluation for HIV integrase (IN) inhibition and docking studies for a series of 4-substituted-benzylideneisoquinoline-1,3(2H,4H)-dione derivatives (11a-11p). All the derivatives were found to inhibit HIV-1 IN enzyme in vitro and most active compound (11f) showed IC50 value of 1.83 µM. Molecular docking studies were also performed to justify the IN inhibition and in vitro-in silico correlation was drawn. However, in cell culture studies, these compounds did not show HIV-1 and HIV-2 inhibition below their cytotoxic concentration indicating that these compounds must be structurally modified for anti-HIV activity.

Keywords: *HIV-1 integrase, Docking studies, Benzylideneisoquinoline-1,3(2H,4H)-dione, Anti-HIV*

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is a serious health condition caused by human immunodeficiency virus (HIV) infection. Although triple therapy or highly active antiretroviral therapy (HAART) has improved the longevity of affected population, due to high mutation rate, rapid emergence of resistance is a serious concern. Currently, around 28 antiretroviral (ARV) drugs are used in clinic; mainly reverse transcriptase, integrase and protease inhibitors. However, there is a need for development of new anti-HIV drugs to tackle the problems of resistance, toxicity and drug availability [1]. HIV IN is a 32kDa protein essential for viral replication as it integrates viral DNA into host genome through two different reactions: 3`-processing (3P) and strand transfer (ST) [2]. It has garnered interest because there is no human equivalent and thus can limit the toxicity [3]. So far, three IN inhibitors viz. raltegravir [1], elvitegravir [2] and dolutegravir [3] have been approved for clinical use while many such as BI-224436, MK-0536, MK-2048, GS-9160, Cabotegravir and Bictegravir are in clinical trials [4-7]. Hence, being an attractive and challenging target, there is lot of scope for development of HIV IN inhibitors [3].

The catalytic core of HIV IN contains a highly conserved catalytic triad of DD(35)E motif, comprising residues Asp 64, Asp116, and Glu152 which coordinate divalent magnesiums (Mg^{2+}). For being an inhibitor of HIV IN, compound must possess a hydrophobic moiety to occupy hydrophobic cavity and bind with Mg^{+2} ion in hydrophilic zone of the enzyme active site [8]. Various scaffolds such as hydroxylated aromatics, diketo acids, naphthyridine carboxamides, pyrroloquinolones, dihydroxypyrimidine carboxamides, 6,7-dihydroxy-1-oxoisoindolines, quinolone-3-carboxylic acids, etc have been explored so far [1]. Isoquinoline scaffold is considered druggable and hence has been explored widely for biological activities such as kinase inhibition [9], hepatitis B virus inhibition [10], HIV-1 non-nucleoside reverse transcriptase inhibition [11], tyrosyl DNA phosphodiesterase II inhibition [12], anticancer [13], antimicrobial [14], anti-hypertensive [15] and antifungal [16]. Most of the work on isoquinolinediones as HIV IN inhibitors is done by Billiamboz *et. al.* [2-3, 17-19]. Since diketo acids were reported to inhibit HIV-1 IN as well as RNase H activities, they planned to synthesize dual inhibitors. Initially, their group reported 7-substituted 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione derivatives [17], followed by 4-substituted 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones [18] and later 4-alkylated 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones [2]. Based on these, they studied the effect of phenyl or benzyl carboxamido side chain at position 4 of the 2-hydroxyisoquinoline-1,3-dione scaffold [3]. Almost all the compounds in these papers had limited application because of cytotoxicity. Suchaud *et. al.* took clue from these and studied the effect of substitution at 7^{th} position on HIV IN inhibition of hydrophobic carboxamido substituted 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones, expecting it to reduce the cytotoxic nature. However, the compounds, although less cytotoxic, showed reduced potency due to low cell permeability and high protein binding [20]. Same group also reported that phenyl or benzyl substituents on 4-carboxamido function can be efficiently replaced by an n-alkyl group. But it was found that the compounds lacked significant antiviral activities due to unfavorable tautomeric equilibrium (keto form), that is, failure to achieve enol form. They postulated that displacement of the keto-enol equilibrium towards enol will lead to higher lipophilicity and hence improved anti-HIV activity [19]. Recently, dihydroisoquinolines substituted with nucleophiles having differential ability to chelate with Mg^{2+} ions have been reported. These compounds do not have keto functionality at all on isoquinoline ring and showed HIV IN inhibition with reduced cytotoxicity [21].

Considering these reports, we planned to work on 4-substituted-benzylideneisoquinoline-1,3(2*H*,4*H*)-diones based on following assumptions:

1. We have previously worked on substituted 1,3-isoindolinediones as potent HIV IN inhibitors (unpublished results from our laboratory). Our aim was to study the effect of ring expansion (5 membered to 6 membered) with no substituent on aromatic benzene ring but on 4th position.

2. It has been reported that the cytotoxicity and probably hepatotoxicity is the result of α-diketo or diketo acid functionality [22]. We hypothesized that by removing 2-hydroxyl substitution from 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione scaffold, the cytotoxicity can be reduced. This is supported by anti-HIV IN potential of dihydroisoquinoline compounds [21]. Further, this hydroxyl removal will also shift the keto-enol equilibrium towards enol and hence would lead to improved lipophilicity and anti-HIV activity as proposed by Billamboz et al. [19].

3. Lipophilic substitution at 4th position is essential to occupy the hydrophobic cavity. It interacts by π stacking with retroviral DNA bases and other non-aromatic residues [3]. We tried to reduce the polarity further by replacing substituted carboxamido function with benzylidene. We expected that by this replacement, there would be better burying of compound in hydrophobic pocket and improved activity. Also, the effect of varying substitution on this benzylidene was also studied.

The designed structure is given in Figure 1. After synthesis, HIV IN inhibition was studied at 10 µM in enzyme inhibition assay. To verify our hypotheses, we performed docking analysis with Schrodinger suite. Further, cell based anti-HIV activity was done to evaluate the effect on HIV infection and cell viability.

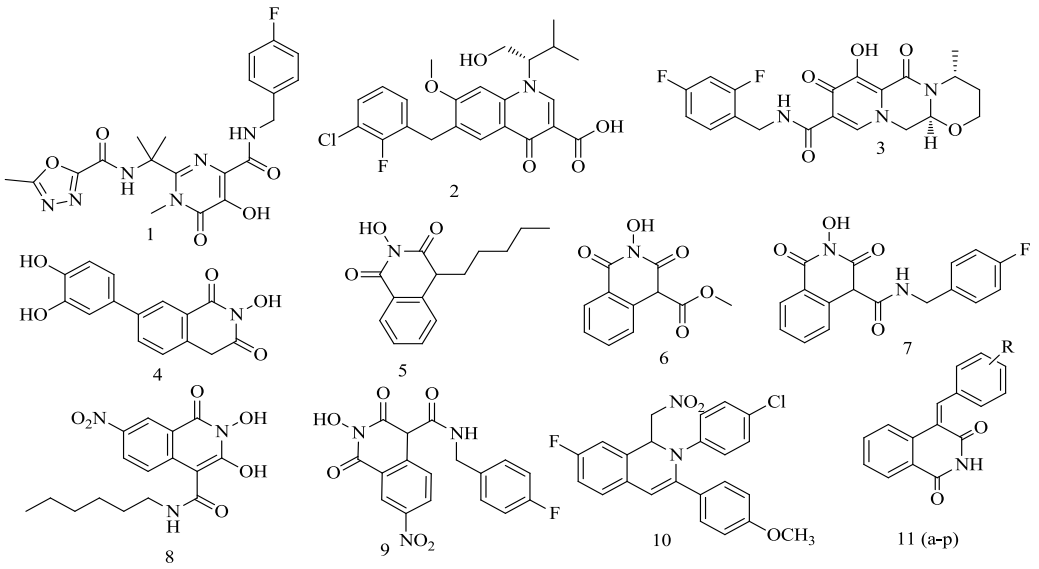
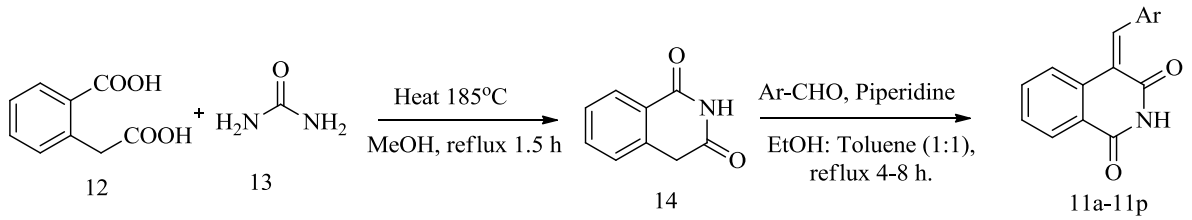


Figure 1: Literature reported HIV-1 IN inhibitors. Compounds 1-3 are FDA-approved raltegravir, elvitegravir, and dolutegravir, Compounds 4-9 were reported by Billiamboz et al. and structure 10 is reported by Tandon *et. al*. Compound 11 is the general structure of designed molecule (11a-11p).

MATERIALS AND METHODS

Chemicals used in synthetic work were purchased from Sigma Aldrich, used without purification. Analytical TLC was carried out with plates precoated with silicagel 60 F254 (0.25 mm thick) by using mobile phase ethyl acetate and hexane in suitable portion. Melting points were determined on a Precision Buchi B530 and are given not corrected. The IR and NMR spectra of the solid samples were recorded using Shimadzu FT-IR spectrophotometer and BRUKER DPX-400 spectrometer, respectively. ESI-MS were recorded on MICROMASS Quattro-II LCMS system. All the products obtained were purified by column chromatography using silica gel (100-200 mesh).



Scheme 1: Protocol for the synthesis of substituted benzylideneisoquinoline-1,3(2H,4H)-diones

Synthesis of isoquinoline-1,3(2H,4H)-dione 14

In mortar pestle 2-(carboxymethyl)benzoic acid (also known as homophthalic acid) **12** (0.0832 mol) and urea **13** (0.0989 mol) was ground to a fine powder. The powder was transferred to 250 ml RBF and heated at 100 °C in microwave for about 8 minute at 720 watt. The mixture was further cooled to ambient temperature and methanol (150 mL) was added. The reaction mixture was then refluxed for 30 minutes, filtered, and allowed to cool to ambient temperature. The solvent was removed under vacuum and resulted solid was treated with hexane (40 ml), collected by filtration, and dried under vacuum to give isoquinoline-1,3(2H,4H)-dione (also known as homophthalimide) **14**.

Yield: 84%; M.p. 237-239 °C; IR (KBr) V_{max} (cm^{-1}): 3324, 2947, 1722, 1714; ^{1}H NMR (400 MHz, $CdCl_3$): 3.65 (s, 2H, CH_2), 7.24-7.28 (d, 2H, ArH), 7.35-7.43 (t, 2H, ArH), 8.68 (s, 1H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 40.2, 124.5, 126.7, 128.2, 129.6, 133.9, 136.3, 158.8, 172.4; MS $[M+1]^+$: m/z 162.08.

Synthesis of 4-substituted-benzylideneisoquinoline-1,3(2H,4H)-dione (11a-11p)

In 100 ml RBF isoquinoline-1,3(2H,4H)-dione **14 (**0.0043 mol**)** was dissolved in a mixture of ethanol and toluene (1:1, 10 ml) at room temperature. Then base piperidine (0.00645 mol) was added to reaction mixture. The resulting mixture was stirred for 10-15 minutes. The appropriate aldehydes were slowly added to the mixture and the mixture was refluxed for 4-8 hours. The progress of reaction was monitored by TLC and after completion of reaction, reaction mass was cooled to room temperature. The solvents were removed by rota evaporator and the residue was diluted with ethyl acetate (50 ml) and washed successively with aqueous water (60 ml). Concentration of solvent under vacuum gave crude solids (**11a-11p**). The compounds were further purified by column chromatography.

4-benzylideneisoquinoline-1,3(2H,4H)-dione (11a)

Yield: 66%; M.p. 212-214 °C; IR (KBr) V_{max} (cm^{-1}): 3420, 3115, 3098, 3021, 1696, 1687; ^{1}H NMR (400 MHz, $CdCl_3$): 7.28 (s, 1H, C=CH), 7.42-8.24 (m, 9H, Ar-H), 8.30 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 122.6, 123.3, 124.7, 126.2, 128.5, 129.1, 133.2, 135.2, 136.4, 136.7, 137.3, 138.4, 139.6, 140.1, 159.2, 168.6; MS $[M+1]^+$: m/z 250.18.

4-(2-nitrobenzylidene)isoquinoline-1,3(2H,4H)-dione (11b)

Yield: 50%; M.p. 228-230 °C; IR (KBr) V_{max} (cm^{-1}): 3460, 3129, 3054, 3014, 1690, 1684; 1H NMR (400 MHz, CdCl3): 6.39 (s, 1H, C=CH), 6.45-7.72 (m, 8H, Ar-H), 7.92 (s, I H, NH); ^{13}C NMR (100 MHz, CdCl3, δ, assignment): 124.2, 124.9, 125.6, 126.8, 127.9, 130.3, 132.2, 134.8, 135.3, 137.1, 137.5, 139.2, 139.9, 141.6, 161.3, 169.1; MS [M+1]+ : m/z 295.20.

4-(4-nitrobenzylidene)isoquinoline-1,3(2H,4H)-dione (11c)

Yield: 56%; M.p. 223-225 °C; IR (KBr) V_{max} (cm^{-1}): 3454, 3114, 3059, 3027, 1697, 1689; ^{1}H NMR (400 MHz, $CdCl_3$): 7.33 (s, 1H, C=CH), 7.43-8.14 (m, 8H, Ar-H), 8.31 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 124.5, 125.3, 125.8, 126.2, 127.5, 130.6, 132.9, 133.7, 134.6, 136.8, 138.5, 139.6, 140.2, 141.3, 158.5, 172.9; MS $[M+1]^+$: m/z 295.17.

4-(2-chlorobenzylidene)isoquinoline-1,3(2H,4H)-dione (11d)

Yield: 58%; M.p. 217-219 °C; IR (KBr) V_{max} (cm^{-1}): 3461, 3119, 3062, 3031, 1694, 1683; ^{1}H NMR (400 MHz, $CdCl_3$): 7.28 (s, 1H, C=CH), 7.37-8.29 (m, 8H, Ar-H), 8.32 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 123.8, 124.9, 125.2, 126.4, 127.1, 128.6, 129.3, 131.8, 132.6, 134.5, 136.1, 138.4, 140.6, 140.9, 160.9, 170.2; MS $[M+1]^+$: m/z 284.04.

4-(3-chlorobenzylidene)isoquinoline-1,3(2H,4H)-dione (11e)

Yield: 61%; M.p. 216-218 °C; IR (KBr) V_{max} (cm^{-1}): 3467, 3123, 3064, 3036, 1692, 1685; ^{1}H NMR (400 MHz, $CdCl_3$): 7.28 (s, 1H, C=CH), 7.30-8.24 (m, 8H, Ar-H), 8.27 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 123.1, 123.5, 125.2, 126.8, 127.9, 129.6, 131.3, 133.4, 134.2, 136.9, 138.1, 139.5, 141.2, 142.5, 161.3, 171.1; MS $[M+1]^+$: m/z 284.15.

4-(4-chlorobenzylidene)isoquinoline-1,3(2H,4H)-dione (11f)

Yield: 67%; M.p. 220-222 °C; IR (KBr) V_{max} (cm^{-1}): 3451, 3127, 3069, 3032, 1699, 1682; ^{1}H NMR (400 MHz, $CdCl_3$): 7.28 (s, 1H, C=CH), 7.32-8.20 (m, 8H, Ar-H), 8.27 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 125.4, 125.9, 126.3, 127.0, 128.2, 129.1, 130.9, 132.5, 135.3, 136.7, 138.9, 140.2, 141.7, 142.3, 162.4, 173.7; MS $[M+1]^+$: m/z 284.09.

4-(2,4-dichlorobenzylidene)isoquinoline-1,3(2H,4H)-dione (11g)

Yield: 53%; M.p. 239-241 °C; IR (KBr) V_{max} (cm^{-1}): 3464, 3135, 3047, 3024, 1687, 1680; ^{1}H NMR (400 MHz, $CdCl_3$): 7.28 (s, 1H, C=CH), 7.32-7.85 (m, 7H, Ar-H), 8.31 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 124.2, 126.3, 127.1, 128.5, 129.4, 130.7, 131.9, 133.1, 134.5, 136.0, 138.2, 141.1, 143.4, 143.9, 158.8, 170.1; MS $[M+1]^+$: m/z 318.05.

4-(2-hydroxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11h)

Yield: 56%; M.p. 231-233 °C; IR (KBr) V_{max} (cm^{-1}): 3478, 3041, 3020, 1692, 1702; ^{1}H NMR (400 MHz, $CdCl_3$): 5.47 (s, 1H, OH), 7.27 (s, 1H, C=CH), 7.30-8.12 (m, 8H, Ar-H), 8.32 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 121.8, 122.5, 125.9, 127.2, 129.3, 131.8, 133.1, 134.7, 135.5, 138.3, 139.7, 143.8, 145.1, 145.9, 164.2, 173.2; MS $[M+1]^+$: m/z 266.12.

4-(4-methoxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11i)

Yield: 68%; M.p. 242-244 °C; IR (KBr) V_{max} (cm^{-1}): 3462, 3129, 3031, 3023, 2958, 1687, 1690; ^{1}H NMR (400 MHz, $CdCl_3$): 3.83 (s, 3H, OCH_3), 7.29 (s, 1H, C=CH), 7.50-8.10 (m, 8H, Ar-H), 8.38 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 58.1, 120.9, 123.7, 124.0, 128.5, 131.6, 131.9, 133.7, 135.4, 136.8, 141.3, 142.2, 145.4, 147.9, 158.4, 160.5, 169.7; MS $[M+1]^+$: m/z 280.11.

4-(3,4-dimethoxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11j)

Yield: 71%; M.p. 223-225 °C; IR (KBr) V_{max} (cm^{-1}): 3445, 3124, 3039, 3026, 2947, 1682, 1695; ^{1}H NMR (400 MHz, $CdCl_3$): 3.83-3.87 (s, 6H, OCH_3), 7.27 (s, 1H, C=CH), 7.40-7.79 (m, 7H, Ar-H), 8.37 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 57.5, 57.9, 121.5, 124.8, 126.1, 128.7, 130.5, 132.4, 134.0, 136.9, 139.1, 144.6, 146.4, 149.3, 157.4, 158.4, 161.3, 171.3; MS $[M+1]^+$: m/z 310.34.

4-(2,5-dimethoxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11k)

Yield: 73%; M.p. 221-223 °C; IR (KBr) V_{max} (cm^{-1}): 3452, 3132, 3042, 3033, 2952, 1678, 1681; ^{1}H NMR (400 MHz, $CdCl_3$): 4.05 (s, 6H, OCH_3), 7.13 (s, 1H, C=CH), 7.04-8.20 (m, 7H, Ar-H), 8.33 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 58.3, 58.5, 123.1, 124.4, 127.6, 129.3, 130.9, 132.8, 133.8, 137.4, 139.3, 142.6, 146.7, 149.1, 156.8, 158.7, 166.3, 172.9; MS $[M+1]^+$: m/z 310.29.

4-(3,4,5-trimethoxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11l)

Yield: 65%; M.p. 236-238 °C; IR (KBr) V_{max} (cm^{-1}): 3477, 3139, 3048, 3042, 2963, 1672, 1689; ^{1}H NMR (400 MHz, $CdCl_3$): 3.83-3.88 (s, 9H, OCH_3), 7.29 (s, 1H, C=CH), 7.46-7.81 (m, 6H, Ar-H), 8.39 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 55.6, 56.2, 57.1, 124.6, 124.9, 126.3, 128.7, 131.4, 132.9, 134.1, 135.8, 139.9, 143.7, 145.1, 155.7, 157.9, 158.4, 169.7, 171.5; MS $[M+1]^+$: m/z 340.21.

4-(4-hydroxy-3-methoxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11m)

Yield: 59%; M.p. 241-243 °C; IR (KBr) V_{max} (cm^{-1}): 3485, 3441, 3124, 3038, 3029, 2954, 1708, 1696; ^{1}H NMR (400 MHz, $CdCl_3$): 3.86 (s, 3H, OCH_3), 5.54 (s, 1H, OH), 7.30 (s, 1H, C=CH), 7.42-8.14 (m, 7H, Ar-H), 8.38 (s, 1H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 56.6, 120.8, 122.4, 123.9, 127.5, 131.6, 133.4, 134.8, 135.2, 137.4, 141.6, 149.6, 159.3, 159.5, 161.3, 168.6, 172.3; MS $[M+1]^+$: m/z 296.18.

4-(3-ethoxy-4-hydroxybenzylidene)isoquinoline-1,3(2H,4H)-dione (11n)

Yield: 65%; M.p. 240-242 °C; IR (KBr) V_{max} (cm^{-1}): 3466, 3458, 3129, 3042, 3038, 2958, 2914, 1698, 1686; ^{1}H NMR (400 MHz, $CdCl_3$): 1.52 (t, 3H, CH_3) 2.89 (q, 2H, OCH_2), 6.25 (s, 1H, OH), 6.78 (s, 1H, C=CH), 7.44-8.16 (m, 7H, Ar-H), 8.32 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 16.8, 65.2, 121.7, 123.9, 124.5, 125.8, 127.9, 132.8, 134.2, 136.7, 137.3, 139.7, 140.8, 144.7, 152.8, 158.5, 162.7, 169.1, 170.8; MS $[M+1]^+$: m/z 310.21.

4-(4-(dimethylamino)benzylidene)isoquinoline-1,3(2H,4H)-dione (11o)

Yield: 74%; M.p. 218-220 °C; IR (KBr) V_{max} (cm^{-1}): 3472, 3136, 3052, 3047, 2969, 2942, 1692, 1676; ^{1}H NMR (400 MHz, $CdCl_3$): 2.83-2.85 (s, 6H, CH_3), 7.29 (s, 1H, C=CH), 7.42-7.89 (m, 8H, Ar-H), 8.34 (s, I H, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 42.5, 42.6, 117.5, 118.2 122.2, 124.8, 125.3, 126.4, 127.2, 130.7, 135.4, 138.6, 139.8, 142.7, 149.5, 154.4, 159.8, 163.7, 168.3, 172.4; MS $[M+1]^+$: m/z 293.31.

4-((1H-indol-3-yl)methylene)isoquinoline-1,3(2H,4H)-dione (11p)

Yield: 68%; M.p. 252-254 °C; IR (KBr) V_{max} (cm^{-1}): 3476, 3466, 3142, 3014, 3017, 1686, 1675; ^{1}H NMR (400 MHz, $CdCl_3$): 7.53-7.56 (s, 2H, C=CH), 7.59-8.81 (m,8H, Ar-H), 8.41 (s, 1H, NH), 8.52 (s, IH, NH); ^{13}C NMR (100 MHz, $CdCl_3$, δ, assignment): 112.4, 113.8, 118.7, 120.4, 121.8, 123.4, 124.6, 127.5, 129.6, 131.4, 132.2, 133.8, 134.7, 135.4, 141.8, 149.4, 168.2, 171.8; MS $[M+1]^+$: m/z 289.06.

Docking study

The X-ray crystal structure of protein (PDB Code: 1QS4) was retrieved from Protein data bank (PDB) [23]. The refinements of protein and 3D energy minimization of designed ligands was done by using protein preparation wizard and Lig-prep 2.3 module, respectively. After Grid generation, the prepared molecules were docked. Validation of docking protocol was done by co-crystallized ligand [24]. The RMSD value found by superimposing docked pose over co-crystallized ligand was 0.5, which suggested the reliability of docking protocol. The best-docked poses were visually analyzed for comparing the percentage inhibition values with interactions in the active site.

Biological evaluation

HIV-IN inhibition activity

In-vitro HIV-1 IN inhibitory activity of the synthesized compounds was evaluated using Xpress Bio kit in accordance with the kit protocol (Xpressbio Life Science Products, USA). The working procedures involves addition of 100 µL double-stranded HIV-1 LTR U5 donor substrate (DS) DNA was to the wells of streptavidin-coated 96-well microtiter plates. Following incubation at 37 ºC for 30 min and a wash step, 100 µL purified HIV-1 IN was added onto the pre-processed donor DNA and incubated for 30 min at 37 °C. Following a wash step, compounds **11a-11p** and dolutegravir were added at a final concentration of 10 µM into individual wells. The microtiter plates were incubated for 5 min at room temperature, washed and the strand transfer reaction was commenced through the addition of 50 µL double stranded target substrate (TS) DNA. After an incubation at 37 °C for 30 min,

the plates were washed and then 100 µL HRP antibody solution per well was titrated. Finally, the plates were washed and 100 µL TMB peroxidase substrate solution followed by incubation period for 10 min at room temperature and 100 µL TMB stop solution was added to allow for detection at 450 nm using a microplate reader. All inhibition values are the average of duplicate experiments [25].

Anti-HIV assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed for evaluating the antiviral activity of the compounds by measuring the cell proliferation rate. The yellow tetrazolium MTT (Acros Organics, Geel, Belgium) is reduced to blue-purple formazan by metabolically active cells. The absorbance was read in an eight-channel computer-controlled photometer (Infinite M1000, Tecan, Mechelen, Belgium), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells.

Briefly, stock solutions (10x final concentration) of test compounds in 25 µl volumes to two series of triplicate wells were added to flat-bottomed 96-well micro titer trays using Biomek 3000 robot (Beckman instruments, Fullerton, CA). Further, untreated control HIV and mock-infected cell samples followed by HIV-1 (IIIB) or HIV-2 (ROD) stock (50 µl) at 100–300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium were added for each sample. Cytotoxicity effect of test compounds was examined by evaluating mock-infected cells. It comprises centrifugation of exponentially growing MT-4 cells for 5 min at 220 g. Then the obtained supernatant was thrown away and MT-4 cells were re-suspended at 6 x 10^5 cells/ml and followed by transfer of 50 µl volumes to the microtiter tray wells. After five days of infection, the viability of mock and HIV infected cells were examined spectrophotometrically.

Here, EC_{50} (50% effective antiviral concentration) is distinct as the concentration of the tested compound achieving 50% protection from virus-induced cytopathic effect and CC_{50} (50% cytotoxic concentration) was defined as the compound concentration that reduced the viability of mock-infected cells by 50%.

RESULT AND DISCUSSION

Chemistry

The synthetic route for 4-substituted-benzylideneisoquinoline-1,3(2*H*,4*H*)-dione derivatives is summarized in Scheme 1. It involves reaction of 2-(carboxymethyl) benzoic acid (12) with urea (13) to form intermediate by loss of a water molecule. The intermediate further cyclizes to produce isoquinoline-1,3(2*H*,4*H*)-dione (14). In second step, it (14) was stirred with base piperidine (to abstract a proton from α carbon), and reacted with different aromatic aldehydes (which attack the carbanion) to give desired molecules (50-64%, 11a-11p). The compounds were purified by column chromatography followed by recrystallization. The compounds were characterized using spectroscopic techniques.

HIV-1 Inhibition studies

All synthesized compounds (**11a-11p**) were tested for their HIV-1 integrase inhibitory potential in duplicate using protocol supplied with kit (XpressBio Life Science Products, USA). Second-generation HIV IN inhibitor, Dolutegravir was used as

standard to compare the inhibitory potency of tested derivatives. From amongst those showing maximum inhibitory potential, few were subjected to IC_{50} value determination. To justify the results obtained, *in silico* docking study was performed using Glide (maestro version 9.3, Schrödinger suite) in extra precision mode. The results of *in vitro* evaluation as well as *in silico* binding energy (Gscore) are given in Table 1.

In general, there was good correlation between docking scores and HIV-1 IN inhibition. It was observed that all compounds showed metal coordination with Mg 1001 by C3-carbonyl of isoquinoline ring, indicating that the isoquinoline ring occupied same cavity. Also, it was observed that more than the substituents - electron donating or withdrawing- on phenyl ring, the position of substitution affected the docking scores and enzyme inhibition as it affected the interactions of benzylidine with active site.

Compound **11a** having unsubstituted phenyl ring showed, apart from metal coordination with C-3 carbonyl oxygen, hydrogen bonding interactions with Asp 64 (by -NH of isoquinoline ring) and Asn 155 (by carbonyl group at C-1 position of isoquinoline ring) and hydrophobic interactions with Cys 65, His 67 and Lys 159 were also observed. It showed 50.43% inhibition of IN and G Score was -5.40.

Substitution of electron withdrawing nitro group, either at ortho position (**11b**) or at para position (**11c**), showed hydrogen bonding with Asp 64 (by -NH of isoquinoline ring), Cys 65 (by C-3 carbonyl group), Asn 155 (by carbonyl group at C-1 position of isoquinoline ring) and hydrophobic interactions with Glu 92, Glu 152 and Lys 156. Although the% inhibition profile of both compounds was better than **11a**, substitution at ortho position (compound **11b**) was favored, as compound **11c** lacked of hydrogen bonding interactions with Thr 66 and π-π stacking interaction with His 67, which were present in **11b**.

On substitution of chloro group at ortho, meta and para positions (Compounds **11d** to **11f**), it was found that meta substitution is not favored. These derivatives showed hydrogen bonding with Cys 65 and hydrophobic interactions with Thr 66, His 67, Glu 92, Glu 152, Lys 156 and Lys 159; as also coordinate with magnesium. Compound **11d** (ortho chloro) showed, in addition, hydrogen bonding interactions with Thr 66, His 67 (by carbonyl group at C-1 position of isoquinoline ring), But it lacks interactions with Asp 64 ((by -NH of isoquinoline ring)) which was shown by para chloro substituted compound **11f**, which was also most active of these three (G score -7.76 and% inhibition 84.12). Substitution of chlorine at both ortho and para positions (compound **11g**) showed similar interactions as **11d.** It showed absence of docking interactions with Asp 64 (G score -6.19) leading to reduction in Gscore as well as activity as compared to **11f**.

On Substitution of electron donating hydroxy group at 2 position (**11h**) showed highest docking interactions (G score -7.86) as well significant HIV-1 IN inhibition (% inhibition 87.30) activity. It showed hydrogen bonding interactions with Asp 64 (by -NH of isoquinoline ring), Cys 65 (by carbonyl group of C-3 position of isoquinoline ring), Asn 155 (by carbonyl group of C-1 position of isoquinoline ring). The ortho hydroxyl group also participated in hydrogen bonding interactions with Cys 65, Thr 66 and His 67. The hydrophobic interactions bond with amino acids Asp 64, Cys 65, His 67, Lys 159. The 2-hydroxyl substitution also showed metal coordination with Mg 1001 in addition to by carbonyl group of C-3 position of isoquinoline ring.

O-alkylation (4-methoxy substituted **11i**) or di- and tri- substitution (compounds **11j** to **11n**) with electron donating groups was found to decrease the interactions and hence lower docking and% inhibition scores were obtained for these. Specifically, **11i**

showed reduction in docking score because 4-methoxy group was found to be solvent exposed. Among dimethoxy substituted compounds, 3,4-dimethoxy (**11j**) was found to have better interactions than 2,5-dimethoxy (**11k**) as **11k** did not show interaction with Asp 64 but **11j** did. 3,4,5-trimethoxy substitution (**11l**) showed similar interactions as that of **11j** such as hydrogen bonding interactions with Asp 64, Cys 65 (by -NH of isoquinoline ring), Thr 66, Asn 155 (by carbonyl group of C-1 position of isoquinoline ring).

It was found that compound substituted with 4-hydroxy-3-methoxy phenyl (**11m**) also shows similar interactions like **11j** and hence docking score and% inhibition values of **11j**, **11l** and **11m** were similar. However, replacing methoxy in **11m** with ethoxy (**11n**) was not favored as benzylidene ring comes out of active site and only two hydrogen bonding interactions with Asp 64 (by NH of isoquinoline ring) and Asn 155 (by carbonyl group of C-1 position of isoquinoline ring) were observed. Substitution of *N*,*N*-dimethyl (**11o**) at para position of aryl ring or replacement of substituted phenyl ring with fused ring like indole (**11p**) did not show any difference than **11j**.

Thus, it was observed that the HIV-1 IN inhibitory potency of designed compounds altered with variation in nature as well as position of substituents. Since compounds having 2-nitro (**11b**), 4-chloro (**11f**) and 4-hydroxy substitution (**11h**) exhibited significant IN inhibitory activity (more than 75%), these were further tested for calculation of IC_{50} values (Table 1). It was observed that compounds **11b** and **11f** with electron withdrawing substituent showed IC_{50} value of 5.96 µM and 3.83 µM respectively. Compound **11h** had IC_{50} value of 1.90 µM.

After analyzing the *in silico* and *in vitro* results, all the compounds were examined for anti-HIV activity against HIV-1 (III_B) and HIV-2 (ROD). First, cytotoxicity was studied using MTT based cell viability assay against HeLa cell line and then anti-HIV activity against both HIV-1 (IIIB) and HIV-2 (ROD) at/below their cytotoxic concentration was evaluated. It was however observed that none of the compound showed anti-HIV activity below their cytotoxic concentration (Table 2). The compounds showed very good potential *in vitro* however, in cell culture assay the activity was not seen. This indicates that the cytotoxicity rather increases by removing 2-hydroxy substitution and these results support the necessity to have N-hydroxy substitution as proposed by Billiamboz et al. [17]. Next, we will be studying the effect of substitution on isoquinoline ring on anti-HIV potential (Figure 2).

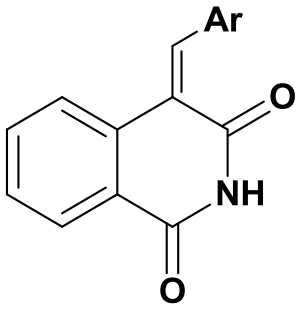


Figure 2: N-hydroxy substitution

Table 1: Results of *in-vitro* HIV-1 IN inhibition and docking studies.

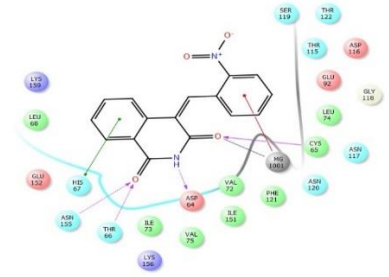
Code	Ar	Docking score	% IN Inhibition*	IC_{50} value**
Dolutegravir	-	-7.30	95.00	nd
11a	-Ph	-5.40	50.43	nd
11b	2-NO_2-Ph	-7.64	77.24	5.96 ± 1.0
11c	4-NO_2-Ph	-6.71	69.04	nd
11d	2-Cl-Ph	-7.13	70.72	nd
11e	3-Cl-Ph	-6.10	63.75	nd
11f	4-Cl-Ph	-7.76	84.12	3.83 ± 0.27
11g	2,4-diCl-Ph	-6.19	67.19	nd
11h	2-OH-Ph	-7.86	87.80	1.90 ± 0.15
11i	4-OMe-Ph	-5.46	54.49	nd
11j	3,4-diOMe-Ph	-6.26	64.55	nd
11k	2,5-diOMe-Ph	-5.72	57.85	nd
11l	3,4,5-triOMe-Ph	-5.87	61.92	nd
11m	4-OH-3-OMe-Ph	-6.08	62.13	nd
11n	4-OH-3-OEt-Ph	-5.28	44.97	nd
11o	4-(N,N-diCH_3)-Ph	-6.03	60.19	nd
11p	1H-indol-3-yl	-6.18	62.48	nd
Note: *% inhibition at 10 µM concentration, values are mean of duplicate experiment performed independently, nd = not done; ** in µM				

Table 2: Anti-HIV activity of the synthesized compounds.

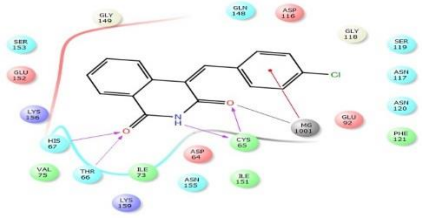
Compound code	HIV-1 (III_B)			HIV-2 (ROD)		
	EC_{50}[1] (µM)	CC_{50}[2] (µM)	HIV-1[3] SI	EC_{50}[1] (µM)	CC_{50}[2] (µM)	HIV-2[3] SI
Dolutegravir	0.0006 ± 0.000001	1.275 ± 0.3	2110	0.00232 ± 0.00061	1.495 ± 0.165	597
11a	>56.45 ± 2.25	56.45 ± 2.25	<1	>50.1 ± 2.5	50.1 ± 2.5	<1
11b	>45.25 ± 1.75	45.25 ± 1.75	<1	>45.5 ± 1.1	45.5 ± 1.1	<1
11c	>12.35 ± 0.75	12.35 ± 0.75	<1	>11.75 ± 0.75	11.75 ± 0.75	<1
11d	>0.14 ± 0.06	0.14 ± 0.06	<1	>0.14 ± 0.05	0.14 ± 0.05	<1
11e	>15.75 ± 2.35	15.75 ± 2.35	x1	>17.6 ± 2.1	17.6 ± 2.1	<1
11f	>11.5 ± 0.9	11.5 ± 0.9	<1	>11.55 ± 0.75	11.55 ± 0.75	<1
11g	>0.685 ± 0.315	0.685 ± 0.315	<1	>0.7 ± 0.3	0.7 ± 0.3	<1
11h	>51.5 ± 1.45	51.5 ± 1.45	<1	>52.2 ± 2.2	52.2 ± 2.2	<1
11i	>53.1 ± 0.3	53.1 ± 0.3	<1	>48.6 ± 5.5	48.6 ± 5.5	<1
11j	>63.1 ± 2.6	63.1 ± 2.6	<1	>61.6 ± 1.1	61.6 ± 1.1	<1

11k	>12.3 ± 0.5	12.3 ± 0.5	<1	>6.5 ± 5.5	6.5 ± 5.5	<1
11l	>58.5 ± 7.4	58.5 ± 7.4	<1	>58.9 ± 3.3	58.9 ± 3.3	<1
11m	>38.25 ± 7.65	38.25 ± 7.65	<1	>35 ± 4.5	35 ± 4.5	<1
11n	>56.65 ± 5.85	56.65 ± 5.85	<1	>58.8 ± 1.3	58.8 ± 1.3	<1
11o	>42.85 ± 3.65	42.85 ± 3.65	<1	>45.1 ± 1.5	45.1 ± 1.5	<1
11p	>11.35 ± 3.85	11.35 ± 3.85	<1	>10.5 ± 2.6	10.5 ± 2.6	<1

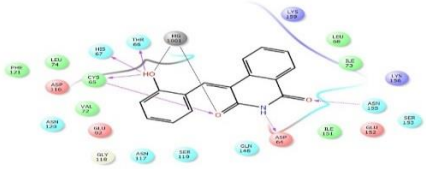
Notes:
1 EC50: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV induced cytotoxicity, as determined by MTT method.
2 CC50: concentration required to reduce the viability of mock-infected cells by 50%, as determined by MTT method.
3 SI: selectivity index (CC50/EC50).



(A)



(B)



(C)

Figure 2: Docking poses of few representative compounds A: 2D interaction plot of 11b; B: Docking pose of 11f; C: Docking pose of 11h.

CONCLUSION

In summary, sixteen 4-Substituted-Benzylideneisoquinoline-1,3(2*H*,4*H*)-dione Derivatives were synthesized and evaluated for anti-HIV activity using isolated enzyme (IN) assay, *in silico* and in cell culture assay against HIV-1 and HIV-2. Three compounds **11b, 11f** and **11h** exhibited significant percentage inhibition of HIV-1 IN with IC_{50} value less than 5.96 µM. There was reasonably good *in vitro-in silico* correlation. However, none of the derivative was active against HIV-1 and HIV-2 below their cytotoxic concentration. This indicates that these types of compounds can be excluded from further exploration for anti-HIV activity. Future course of action could be placing hydroxyl at NH of isoquinoline ring or by replacing phenyl ring with furan ring so that it can bind with catalytical site.

SUPPLEMENTARY MATERIAL

NMR and mass spectra of targeted compounds **11a-11p** are presented. Supplementary material is available on the publishers web site along with the published article. H. Li, J. Kim, L. Groy, *J. Am. Chem. Soc.*, **2001**, 123, 4867.

REFERENCES

1. Hajimahdi, Z., and Zarghi, A., Progress in HIV-1 integrase inhibitors: A review of their chemical structure diversity. *Iran J Pharm Re.*, **2011.** 15: 595.

2. Billamboz, M., et al, Magnesium chelating 2-hydroxyisoquinoline-1,3(2H,4H)-diones, as inhibitors of HIV-1 integrase and/or the HIV-1 reverse transcriptase ribonuclease H domain: discovery of a novel selective inhibitor of the ribonuclease H function, *J Med Chem***, 2011.** 54: 1812-1824.

3. Billamboz, M., et al. 4-Substituted 2-Hydroxyisoquinoline-1,3(2***H***,4***H***)-diones as a novel class of HIV-1 integrase inhibitors *ACS Med Chem Lett*, **2013**. 4: 606-611.

4. Li, X., and Krishnan, L., et al. Structural biology of retroviral DNA integration, *Virology*, **2011**. 411: 194-205.

5. Li, Y., et al. Mutations located outside the integrase gene can confer resistance to HIV-1 integrase strand transfer inhibitors, *Drug Discovery Today***, 2015.** 20: 435-449.

6. Fader, LD., et al. Minimizing the contribution of enterohepatic recirculation to clearance in rat for the NCINI class of inhibitors of HIV**.** *ACS Med Chem Lett*, **2014.** 5: 422-427.

7. Quinoline, coumarin and other heterocyclic analogs based HIV-1 integrase inhibitors.
8. Jadhav, RA., Debnath U, Kumar P, Agarwal A, N-hydroxy-substituted 2-aryl acetamide analogs: A novel class of HIV-1 integrase inhibitors, *Chem Biol Drug Des***, 2017.** 90: 527-534.
9. Tsou, HR., et al. Ethyl 4-methyl-1,3-dioxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate, *J Med Chem.*, **2008.** 51: 3507-3525.

10. Edwards, TC., et al., Inhibition of hepatitis B virus replication by N-hydroxyisoquinolinediones and related polyoxygenated heterocycles., *Antiviral Res*, **2017.** 143: 205-217.
11. Kang, D., and Huo, Z., et al. Neuroprotective effect of acute melatonin treatment on hippocampal neurons against irradiation by inhibition of caspase-3, *Expert Opin Ther Pat*, **2017.** 27: 383-391.
12. Kankanala J, Marchand C, et al., Discovery of selective inhibitors of tyrosyl-DNA phosphodiesterase 2 by targeting the enzyme DNA-binding cleft *J. Med. Chem*., **2016**, 59, 2734-2746.
13. . Kang B.R, Wang J et al., Synthesis and antibacterial activity of 1-(2-Fluorovinyl)-7-substituted-4-quinolone-3-carboxylic acid derivatives, conformational restricted analogues of fleroxacin. *Med Chem. Res.*, **2014**, 23 (3), 1340-1349.
14. Galan A., Moreno L, et al., Novel isoquinoline derivatives as antimicrobial agents. *Bioorg Med Chem.*, **2013**, 21, 3221-30.
15. Demerson, CA., et al, Short step synthesis of 4-substituted indoles and ergot alkaloids. L.G. Humber, M.J. Kraml, M.P. Charest, H. Tom, I. Vavra, *J Med Chem*, **1974.** 17: 1140-1145.
16. Zhang, W., et al., Antibacterial, antifungal and cytotoxic isoquinoline alkaloids from *Litsea cubeba Molecules*, **2012.** 17: 12950-60.
17. Billamboz, M., et al., Design, synthesis, and biological evaluation of a series of 2-hydroxyisoquinoline-1,3(2H,4H)-diones as dual inhibitors of human immunodeficiency virus type 1 integrase and the reverse transcriptase RNase H domain, *J Med Chem.*, **2008.** 51: 7717-7730.
18. Billamboz, M., and Bailly, F., 2-hydroxyisoquinoline-1,3(2H,4H)-diones as inhibitors of HIV-1 integrase and reverse transcriptase RNase H domain: influence of the alkylation of position 4, *Eur J Med Chem*., **2011.** 46: 535-546.
19. Billamboz, M., et al., 2-hydroxyisoquinoline-1,3(2H,4H)-diones (HIDs) as human immunodeficiency virus type 1 integrase inhibitors: Influence of the alkylcarboxamide substitution of position 4. *Eur J Med Chem.*, **2016**. 117: 256-268.
20. Suchaud V, et al. Investigation of a novel series of 2-hydroxyisoquinoline-1,3(2H,4H)-diones as human immunodeficiency virus type 1 integrase inhibitors. *J Med Chem.*, **2014.** 57: 4640-4660.
21. Tandon, V., et al., 4-Substituted 2-hydroxyisoquinoline-1,3(2***H***,4***H***)-diones as a novel class of HIV-1 integrase inhibitors. *ACS Med Chem Lett*, **2015.** 6: 1065-1070.
22. Hajimahdi, Z., and Zarghi, A., QSAR study on anti-HIV-1 activity of 4-Oxo-1,4-dihydroquinoline and 4-Oxo-4***H***-pyrido[1,2-***a***] pyrimidine derivatives Using SW-MLR. Artificial Neural Network and Filtering Methods, *Med Chem Res*, **2013**, 22: 2467-2475.
23. Goldgur, Y., and Craigie, R., Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design, *Proc Natl Acad Sci USA.*, **1999.** 96: 13040-13043.
24. Friesner, RA., and Murphy, R.B., Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem.*, **2006.** 49: 6177-6196.
25. Debyser, Z., et al., LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *Methods Mol Biol*, **2001.** 139-155.