

## Synthesis and HIV-1 Integrase Inhibitory Activities of 4-Hydroxy-5-azacoumarin 3-Carboxamides

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Recently, it has been reported that the inhibition of the strand transfer function of HIV-1 integrase is necessary to obtain significant antiviral activity. Accordingly, several compounds typified by aryl 1,3-diketo acids that can inhibit strand transfer reaction of HIV-1 IN have been identified. In this work, we synthesized new 4-hydroxy-5-azacoumarin-3-carbox(thio)amides (**1a-h**) and evaluated for the inhibition of HIV-1 IN strand transfer reaction with a brief SAR. Among synthesized, compound **1e** was the most potent HIV-1 IN inhibitor with equipotent activity to that of L-708,906. Therefore, the 4-hydroxy-5-azacoumarin ring can be considered as a new scaffold in designing more potent of HIV-1 IN inhibitors for treatment of AIDS.

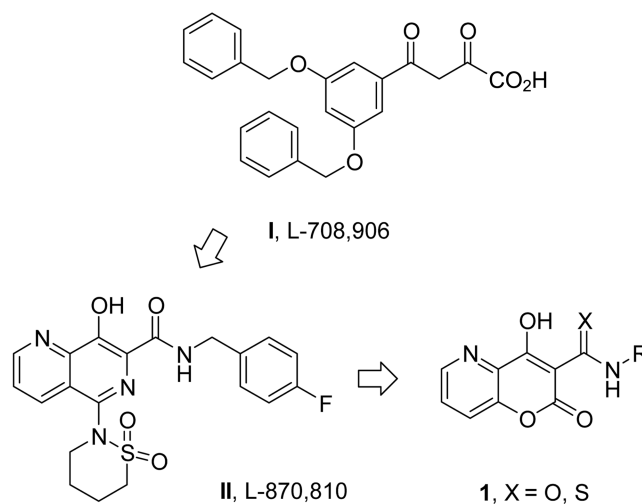
**Key Words** : HIV-1 integrase inhibitor, Strand transfer, 4-Hydroxy-5-azacoumarin

### Introduction

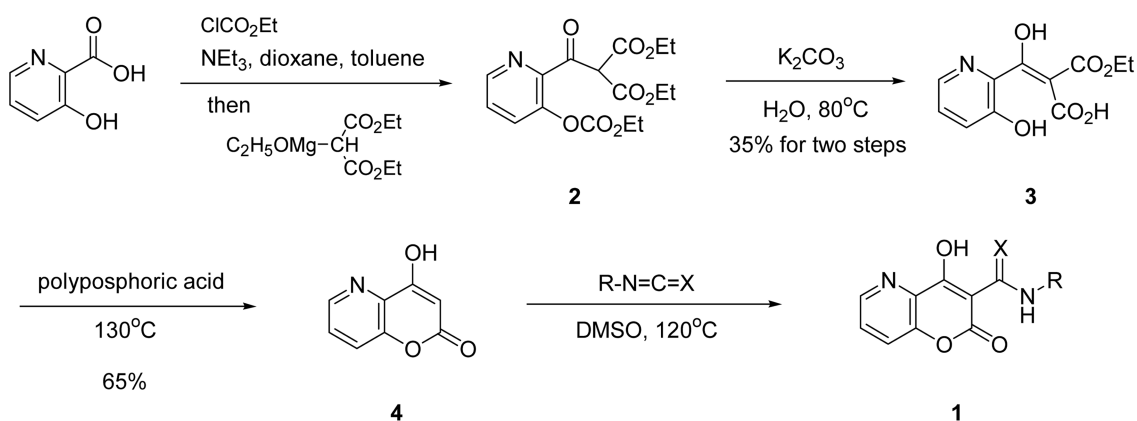
Human immunodeficiency virus (HIV) is the probable causative agent of acquired immune deficiency syndrome (AIDS), which is one of the world's most serious health problems.<sup>1</sup> The number of people living with HIV/AIDS is estimated to 38 million and approximately 3 million AIDS death were reported in 2003. Several biological processes in the life cycle of this virus have been targeted for anti-HIV therapy. Accordingly, a number of anti-HIV drugs targeting key enzymes for viral replication; HIV reverse transcriptase and HIV protease, has been approved for the treatment of HIV infected patients. However, since the efficiency of these drugs is limited by the emergency of HIV mutants and adverse side effects further development of different type of drug is continuously required. Another important step in the replication of HIV is integration of the viral DNA into the host cell DNA.<sup>3</sup> This step is catalyzed by the viral enzyme HIV integrase (IN). HIV IN catalyzes two distinct reactions, known as terminal cleavage at each 3' end of the proviral DNA removing a pair of bases and strand transfer which results in the joining of each 3' end to 5'-phosphates in the target DNA. Such integration is essential for the production of progeny viruses. As these reactions are essential for the life cycle of viruses, integrase represents an attractive target for treatment of HIV infections.<sup>4-6</sup> HIV IN has also been recognized as a safe target against HIV because there are no similar enzymes involved in human cellular function.<sup>7</sup> Recently, several aryl 1,3-diketo acids as exemplified by compounds **I** that can inhibit strand transfer reaction of HIV-1 IN have been identified as potent anti-HIV agent (Figure 1).<sup>8</sup> The 1,3-diketo acid moiety of the compound **I** has been

postulated to be an essential part for the inhibitory activity of HIV-1 IN strand transfer since these part is believed to interact with catalytically important Mg<sup>2+</sup> in the active site of HIV-1 IN step.<sup>9</sup> Accordingly, the variations of structural features of aryl 1,3-diketo acids have been made leading to 8-hydroxy-1,6-naphthyridine **II**, which mimic the metal cation interaction site of the 1,3-diketo acid pharmacophore.<sup>10,11</sup>

In our continued study on the development of HIV-1 IN inhibitors,<sup>12</sup> we here disclose the synthesis of 4-hydroxy-5-azacoumarin carboxamides or carboxthioamides **2**, which have an azacoumarin ring as a new scaffold of HIV-1 IN inhibitors. We envisioned that 4-hydroxy-5-azacoumarin



**Figure 1.** Evolution of coumarin HIV-1 integrase inhibitor from 1,3-diketo acid.



**Scheme 1.** Synthesis of 4-hydroxy-5-azacoumarin-3-carbox(thio)amides.

ring can mimic HIV-1 IN inhibitory 8-hydroxy-1,6-naphthyridines **II** since they have the same functional groups that may bind with  $Mg^{2+}$ . Furthermore, it is anticipated that additional hydrogen bonds may be possible *via* interaction of carbonyl oxygen of lactone ring in **2** with polar residues in the active site of HIV-1 IN. To investigate the influence of substituents on inhibitory effect we set the variations at amide part in the design of inhibitors.

### Results and Discussion

**Chemistry.** The chemistry used to prepare the 4-hydroxy-

5-azacoumarin derivatives **1a-h** is illustrated in Scheme 1. The target compounds were synthesized by reaction of 4-hydroxy-5-azacoumarin (**4**) with aryl isocyanate or aryl isothiocyanates. The required intermediate **4** was prepared by the slight modification of literature procedure.<sup>13</sup> Reaction of 2-hydroxypicolinic acid with ethyl chloroformate followed by condensation with ethyl ethoxymagnesium malonate afforded compound **2**. This compound was directly subjected to partial hydrolysis condition: treatment of potassium carbonate in water to provide a hemiester of hydroxypicolnoylmalonic acid (**3**) in 35% yield for two steps. 5-Azacoumarin ring was constructed by heating **3** at 130 °C in

**Table 1.** HIV-1 IN inhibitory activity of 4-hydroxy-5-azacoumarin-3-carbox(thio)amides (**1a-h**)

Inhibitors	Structures	Strand transfer ( $IC_{50}$ , $\mu M$ )	Inhibitors	Structures	Strand transfer ( $IC_{50}$ , $\mu M$ )
<b>1a</b>		15.9	<b>1f</b>		>300
<b>1b</b>		6.7	<b>1g</b>		>300
<b>1c</b>		35.7	<b>1h</b>		>300
<b>1d</b>		12.3	<b>1</b>		5.5 (L-708,906)
<b>1e</b>		>300			

polyphosphoric acid to give **4** in 65% yield. The 3-carbox-amides or thioamides of azacoumarin (**1a-h**) were obtained by the thermal reaction of **4** with aryl isocyanates or aryl isothiocyanates at 120 °C in DMSO containing triethylamine.<sup>14</sup>

**Biological activity.** The resulting 4-hydroxy-5-azacoumarin-3-carbox(thio)amides (**1a-h**) were assayed *in vitro* for inhibition of HIV-1 IN strand transfer (Table 1).<sup>15,16</sup> To compare the inhibitory activity, L-708,906 (**I**) in Figure 1 was prepared by using a known procedure<sup>8</sup> and the activity data was included as a reference in the table. *N*-Benzyl-carboxamide derivatives of azacoumarin (**1a-d**) showed moderate to good inhibitory activities for HIV-1 IN strand transfer reaction. Among tested, 4-fluorobenzyl-substituted azacoumarin (**1b**) showed the most potent inhibitory activity ( $IC_{50} = 6.7 \mu M$ ) comparable to that of L-708,906 (**I**,  $IC_{50} = 5.5 \mu M$ ). On the other hand, *N*-phenyl-carboxamide (**1e**) and thiocarboxamide derivatives (**1g-h**) exhibited no activity under the concentration of 300  $\mu M$  indicating that amide carbonyl and benzyl substituent in amide part are important for inhibition of HIV-1 IN strand transfer reaction.

In conclusion, 4-hydroxy-5-azacoumarin-3-carbox(thio)-amides (**1a-h**) as novel HIV-1 IN inhibitors were synthesized and evaluated for the inhibition of HIV-1 IN strand transfer reaction with a brief SAR. Among synthesized, compound **1b** was the most potent HIV-1 IN inhibitor with equipotent activity to that of L-708,906. Therefore, the 4-hydroxy-5-azacoumarin ring can be considered as a new scaffold in designing more potent of HIV-1 IN inhibitors for treatment of AIDS.

## Experimental Section

**Chemistry: general.** All reactions were carried out under nitrogen atmosphere. Flash column chromatographies were performed with Merck Kiesegel 60 Art 9385 (230-400 mesh). All solvents used were purified according to standard procedures. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Gemini Varian-300 (300 and 75 MHz, respectively): chemical shifts are expressed value (ppm) and coupling constants (*J*) in Hz.

**Ethyl hemiester of 3-hydroxypicolinoylmalonic acid (3).** A solution of 3-hydroxypicolinic acid (13 g, 93.5 mmol) and Et<sub>3</sub>N (26.1 mL, 187.2 mmol) in the mixed solvent of 1,4-dioxane and toluene (1:1, 160 mL) was stirred for 30 min. This solution was cooled to -5 °C and treated with a solution ethyl chloroformate (17.9 mL, 187.5 mmol) in toluene (40 mL). After stirring at 0 °C for 2 h, a solution of ethyl ethoxymagnesium malonate (32 g, 140.3 mmol) in toluene (50 mL) was added to the mixture. The mixture was further stirred overnight at rt and then acidified to pH 3 by addition of 3*N* HCl. The aqueous solution was stirred 30 min and extracted with a mixture of benzene and chloroform (1:1). The organic layer was dried over MgSO<sub>4</sub>, evaporated under reduced pressure to give a brown oily residue. This residue (*ca.* 33 g) was dissolved in water (100 mL) and treated with K<sub>2</sub>CO<sub>3</sub> (19 g, 140.3 mmol). The reaction

mixture was stirred at 80 °C for 4 h. After the mixture was completely cooled to rt and the resulting orange colored precipitate was filtered. The filter cake was washed with a small quantity of methanol and acetone. The washed filter cake was dissolved in water (100 mL) and acidified to pH 1-2 by addition of 3*N* HCl. An orange colored precipitate of flakes was filtered, washed with water, and dried *in vacuo* to give **3** (8.28 g, 35%) as a yellow solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.59 (1H, d, *J* = 5.7 Hz, pyridine-*H*6), 8.12 (1H, d, *J* = 8.4 Hz, pyridine-*H*4), 7.98 (1H, dd, *J* = 5.7, 8.4 Hz, pyridine-*H*7), 4.12 (2H, q, *J* = 6.6 Hz, -OCH<sub>2</sub>CH<sub>3</sub>), 1.22 (2H, t, *J* = 6.6 Hz, -OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  209.0, 179.6, 163.4, 163.1, 152.5, 139.3, 129.9, 125.4, 84.2, 58.4, 15.0.

**4-Hydroxy-5-azacoumarin (4).** A mixture of **3** (7.5 g, 29.6 mmol) and polyphosphoric acid (45.0 g) were heated at 127 °C until no more gas evolved. After cooling to rt, the mixture was treated with water and stirred to dissolve polyphosphoric acid. Sodium hydrogen carbonate was slowly added to the mixture with stirring until the solution become *ca.* pH 3. The resulting precipitate was filtered and the filtered cake was washed with water and MeOH, and dried to give **4** (3.19 g, 65%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.63 (1H, d, *J* = 4.5 Hz, *H*6), 7.89 (1H, d, *J* = 8.7 Hz, *H*8), 7.72 (1H, dd, *J* = 8.7, 4.5 Hz, *H*7), 5.83 (1H, s, *H*3); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 166.1, 161.8, 150.9, 146.1, 133.9, 128.0, 125.4, 95.0.

**General procedure for the synthesis of compounds 1a-h.** Solutions of **4** (300 mg, 1.84 mmol), triethylamine (0.77 mL, 5.52 mmol) and 1 equivalent of arylisocyanate or arylisothiocyanate in dimethylsulfoxide (DMSO, 5 mL) were heated at 95 °C overnight. After cooling to rt, the mixture was treated with 3*N* HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO<sub>4</sub>, concentrated, and purified by recrystallization from methanol-chloroform and/or flash column chromatography (CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> = 1:10) to provide **1a-h**.

**3-(*N*-Benzylcarbamoyl)-4-hydroxy-5-azacoumarin (1a):** The treatment of **4** (300 mg, 1.84 mmol) with benzylisocyanate (0.23 mL, 1.84 mmol) according to the general procedure provided the desired product **1a** (81 mg, 15%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.76 (1H, t, *J* = 4.6 Hz, -CONHCH<sub>2</sub>-), 8.73 (1H, d, *J* = 3.3 Hz, *H*6), 7.98 (1H, d, *J* = 8.4 Hz, *H*8), 7.83 (1H, dd, *J* = 8.4, 3.3 Hz, *H*7), 7.39 (5H, m, phenyl-*H*), 4.63 (2H, d, *J* = 4.6 Hz, -NHCH<sub>2</sub>Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  176.3, 170.6, 161.2, 151.5, 147.7, 138.6, 133.3, 130.0, 129.2, 128.3, 128.0, 126.0, 94.7, 43.4.

**3-(*N*-(4-Fluorobenzyl)carbamoyl)-4-hydroxy-5-azacoumarin (1b):** The treatment of **4** (150 mg, 0.92 mmol) with 4-fluorobenzylisocyanate (0.12 mL, 0.92 mmol) according to the general procedure provided the desired product **1b** (122 mg, 42%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.54 (1H, s, CO-NH-CH<sub>2</sub>), 8.80 (1H, d, *J* = 3.9 Hz, *H*6), 7.75 (1H, d, *J* = 8.4 Hz, *H*8), 7.68 (1H, dd, *J* = 8.4, 4.5 Hz, *H*7), 7.38 (2H, two d, *J* = 8.4, 5.4 Hz, phenyl-*H*), 7.10 (2H, two d, *J* = 8.7, 9.0 Hz, phenyl-*H*), 4.67 (2H, two s, -NHCH<sub>2</sub>-phenyl); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  164.6, 164.2, 150.1, 144.6, 132.3, 128.5, 126.3, 100.7, 60.9, 14.0.

**3-(N-(4-Bromobenzyl)carbamoyl)-4-hydroxy-5-azacoumarin (1c):** The treatment of **4** (250 mg, 1.53 mmol) with 4-bromobenzylisocyanate (0.21 mL, 1.53 mmol) according to the general procedure provided the desired product **1c** (154 mg, 27%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  9.80 (1H, br t,  $J = 6$  Hz, -CONHCH $_2$ -), 8.73 (1H, d,  $J = 4.2$  Hz,  $H_6$ ), 7.98 (1H, d,  $J = 8.4$  Hz,  $H_8$ ), 7.84 (1H, dd,  $J = 8.4, 4.2$  Hz,  $H_7$ ), 7.57 (2H, d,  $J = 8.1$  Hz, phenyl- $H$ ), 7.36 (2H, t,  $J = 8.1$  Hz, phenyl- $H$ ), 4.59 (2H, d,  $J = 6$  Hz, -NHCH $_2$ -);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  176.20, 170.7, 161.1, 151.5, 147.7, 138.2, 133.3, 132.1, 130.6, 130.0, 126.0, 121.0, 94.9, 42.8.

**3-(N-(4-Methylbenzyl)carbamoyl)-4-hydroxy-5-azacoumarin (1d):** The treatment of **4** (300 mg, 1.84 mmol) with 4-methylbenzylisocyanate (0.27 mL, 1.84 mmol) according to the general procedure provided the desired product **1d** (80 mg, 14%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  9.70 (1H, br t,  $J = 6.0$  Hz -CONHCH $_2$ -), 8.73 (1H, d,  $J = 4.2$  Hz,  $H_6$ ), 7.97 (1H, d,  $J = 8.4$  Hz,  $H_8$ ), 7.83 (1H, dd,  $J = 8.4, 4.2$  Hz,  $H_7$ ), 7.28 (2H, d,  $J = 7.6$  Hz, phenyl- $H$ ), 7.18 (2H, d,  $J = 7.6$  Hz, phenyl- $H$ ), 4.58 (2H, d,  $J = 6.0$  Hz, -NHCH $_2$ -phenyl), 2.30 (3H, s, CH $_3$ phenyl-);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  176.4, 170.5, 161.2, 151.5, 147.7, 137.2, 135.5, 133.3, 130.0, 129.8, 128.4, 126.0, 94.6, 43.1, 21.4.

**3-(N-Phenylcarbamoyl)-4-hydroxy-5-azacoumarin (1e):** The treatment of **4** (300 mg, 1.84 mmol) with phenylisocyanate (0.21 mL, 1.84 mmol) according to the general procedure provided the desired product **1e** (110 mg, 21%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.10 (1H, s, -CONH-phenyl), 8.77 (1H, d,  $J = 4.5$  Hz,  $H_6$ ), 8.06 (1H, d,  $J = 8.4$  Hz,  $H_8$ ), 7.88 (1H, dd,  $J = 8.4, 4.5$  Hz,  $H_7$ ), 7.69 (2H, d,  $J = 7.8$  Hz, phenyl- $H_{2,6}$ ), 7.45 (2H, t,  $J = 7.5$  Hz, phenyl- $H_{3,5}$ ), 7.24 (1H, t,  $J = 7.4$  Hz, phenyl- $H_4$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  174.4, 168.0, 147.7, 137.1, 130.2, 129.9, 126.2, 126.0, 121.8, 97.0.

**3-(N-Phenylthiocarbamoyl)-4-hydroxy-5-azacoumarin (1f):** The treatment of **4** (300 mg, 1.84 mmol) with phenylisothiocyanate (0.22 mL, 1.84 mmol) according to the general procedure provided the desired product **1f** (120 mg, 22%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  13.02 (1H, s, -CSNH-phenyl), 8.68 (1H, d,  $J = 4.4$  Hz,  $H_6$ ), 7.92 (1H, d,  $J = 8.3$  Hz,  $H_8$ ), 7.75 (1H, dd,  $J = 8.3, 4.4$  Hz,  $H_7$ ), 6.75-7.12 (5H, m, phenyl- $H$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  178.4, 164.6, 158.8, 150.4, 147.2, 137.2, 136.5, 129.2, 129.0, 126.6, 126.0, 124.2, 91.6.

**3-(N-Benzylthiocarbamoyl)-4-hydroxy-5-azacoumarin (1g):** The treatment of **4** (300 mg, 1.84 mmol) with benzylisothiocyanate (0.25 mL, 1.84 mmol) according to the general procedure provided the desired product **1g** (180 mg, 31%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.15 (1H, br t,  $J = 5.4$  Hz, -CSNHCH $_2$ -), 8.71 (1H, d,  $J = 4.5$  Hz,  $H_6$ ), 7.99 (1H, d,  $J = 8.4$  Hz,  $H_8$ ), 7.81 (1H, dd,  $J = 8.4, 4.5$  Hz,  $H_7$ ), 7.36 (5H, m, phenyl- $H$ ), 4.94 (2H, d,  $J = 5.4$  Hz, -NHCH $_2$ -phenyl);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  190.3, 162.7, 159.9, 149.8, 146.6, 137.3, 133.5, 129.1, 128.8, 128.4, 128.0, 125.7, 109.0, 48.6.

**3-(N-(4-Fluorobenzyl)thiocarbamoyl)-4-hydroxy-5-azacoumarin (1h):** The treatment of **4** (300 mg, 1.84 mmol) with 4-fluorophenylisothiocyanate (0.32 mL, 1.84 mmol) according to the general procedure provided the desired

product **1h** (240 mg, 40%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  11.12 (1H, br t,  $J = 5.4$  Hz, -CSNHCH $_2$ -), 8.71 (1H, d,  $J = 4.5$  Hz,  $H_6$ ), 7.99 (1H, d,  $J = 8.1$  Hz,  $H_8$ ), 7.81 (1H, dd,  $J = 8.1, 4.5$  Hz,  $H_7$ ), 7.48 (2H, m, phenyl- $H$ ), 7.22 (2H, m, phenyl- $H$ ), 4.93 (2H, d,  $J = 6.0$  Hz, -NHCH $_2$ -phenyl);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  190.4, 163.8, 162.5, 160.5, 159.8, 149.8, 146.6, 133.5, 130.4, 130.3, 128.8, 125.7, 116.0, 115.7, 109.1, 47.8.

### Biological Test

**Purification of HIV-1 integrase:** Recombinant human immunodeficiency virus type 1 (HIV-1) integrase was expressed in *Escherichia coli* and purified using a nickel-chelated column in one-step manner, as described previously.<sup>15</sup> Aliquots of HIV-1 integrase of 0.5 mg/mL as stock solutions were stored at  $-70$  °C until used.

**Oligonucleotide substrates:** Two 20-mer and one 18-mer oligonucleotides whose sequences resemble the end of the U5 LTR of HIV-1 viral DNA were obtained from Bioneer Inc., namely K16 (U5 LTR, +strand), 5'-TGTTGGAAAA-TCTCTAGCAGT-3', K17 (U5 LTR, -strand), 5'-ACTGCTAGAGATTTTCCACA-3', K16-2 (U5 LTR, +strand), 5'-TGTTGGAAA, and ATCTCTAGCA-3'. The oligonucleotides were purified using 20% polyacrylamide gel before use. In order to construct the substrate for endonucleolytic reaction, the oligonucleotide K16 of 50 pmol was labeled at the 5' end, using of 50  $\mu\text{Ci}$  [ $\gamma$ - $^{32}\text{P}$ ]-ATP (3,000 Ci/mmol; 1 Ci = 37 GBq; Amersham Life Science, Illinois, U.S.A.) and T4 polynucleotide kinase (T4 PNK, New England Biolabs, Beverly, Massachusetts, U.S.A.). The labeling reaction was subjected to 10 mM EDTA, and heated to 75 °C for 15 min to inactivate T4 PNK. After addition of complementary oligonucleotide K17 of 60 pmol, the reaction mixture was boiled for 3 min and cooled down slowly. Labeled substrate was separated from unincorporated nucleotide by passage through a Biospin 6 (Bio-Rad, Hercules, California, U.S.A.). The substrate for strand transfer reaction was prepared by labeling the oligonucleotide, K16-2, with T4 PNK and [ $\gamma$ - $^{32}\text{P}$ ]-ATP. The labeled K16-2 was annealed with the complementary oligonucleotide K17. The subsequent procedures are same as described above.

**In vitro assay:** A standard assay for strand transfer activity was carried out in the presence of potential inhibitor containing 0.1 pmol of duplex oligonucleotide substrate (K16-2/K17) and 15 pmol of HIV-1 integrase in 15 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM MnCl $_2$ , 2 mM 2-mercaptoethanol, 2.5 mM CHAPS, 0.1 mM EDTA, 0.1 mM PMSF, 1% glycerol, and 10 mM imidazole in a total volume of 10  $\mu\text{L}$ . Inhibitors or drugs are initially dissolved in 100% DMSO or ethanol, and added to the reaction mixture at 5% concentration of solvent in the final volume. Reaction mixtures were incubated at 37 °C for 60 min and stopped by the addition of 4  $\mu\text{L}$  of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF. The reactions were heated to 90 °C for 3 min and analyzed on a 20% denaturing polyacrylamide gel. Reaction products were visualized by autoradiography of the dried gel in a phosphoimage analyzer (GS-525, Bio-Rad). The strand transfer

activity was evaluated by studying conversion of the 18-mer oligonucleotides to the bigger ones.

Percent inhibition was calculated using the equation  $100 \times [1 - (D-C)/(N-C)]$  where C, N, and D are the fractions of 20-mer substrate converted to strand transfer products for DNA alone, DNA plus integrase, and integrase plus drug, respectively. The IC<sub>50</sub> value was determined by plotting the drug concentration versus percent inhibition and determining the concentration that produced 50% inhibition.<sup>16</sup>

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