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Docking, Synthesis and Biological Evaluation of Novel Diketoquinoline Analogues as HIV-1 Integrase Inhibitor

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A series of novel diketoquinoline acid derivatives as potential anti-HIV-1 Integrase inhibitors were docked, synthesized and characterized by IR, NMR , CHN and MS spectral analysis. Many compounds were identified and docked in integrase pocket. The target diketoquinolines were prepared from substituted oxoquinoline-3-carboxylate. *In vitro* biological evaluation revealed that some of the titled compounds exhibited moderate to good anti-HIV-1 Integrase inhibitory activity in comparison with the reference drugs *i.e.* raltegravir and nevirapine. The cytotoxicity of most of testing compounds on C8166 were very low, the CC_{50} value of them were higher than 200 µM, except the few compounds. Compounds **1-5** showed weak anti-HIV-1 activity, its therapeutic index was 457, 531, 583, 869 and 909 respectively. As a positive control drug, Nevirapine has the best anti-HIV-1 activity (EC_{50} = 0.015-0.016 µM) *in vitro* and the CC_{50} of was higher than 200 µM, its therapeutic index was higher 12418.50. In integrase assay compound **6** and **7** showed EC_{50} value 0.08 µM as compared with standard drug raltegravir.

Keywords: Elvitegravir, Diketoquinoline, Docking, HIV-1 integrase, Raltegravir, Nevirapine, Syncytium.

INTRODUCTION

Integrase (IN) is a key enzyme for HIV-1 replication, catalyzing the integration of reverse transcribed DNA into the host cell genome. In the past decade, integrase has emerged as an attractive target. Whereas structural studies of integrase reveal a single binding site for Mg^{2+}, the number of metal ions present and required in the active site during the process remains controversial. A great number of HIV-1 integrase inhibitors with metal binding properties have been described and numerous reviews have been published [1-4].

Among all reported integrase inhibitors, the β-diketo acid (DKA) class of compounds has emerged as the most potent and the most promising. Raltegravir is the first approved integrase inhibitor whereas Elvitegravir and GSK364735 reached clinical development Fig. 1. Like other well-known DKA inhibitors, these also share two common structural chemotypes essential for the anti-integrase activity: a diketo acid chain able to interact with Mg^{2+} metal ions and a properly oriented hydrophobic benzyl moiety. They selectively inhibit ST (strand transfer) reaction, suggesting that they bind at the IN/DNA interface, acting as "interfacial inhibitors". Elvitegravir binds to magnesium cations and inhibits the strand transfer reaction. Designing such drug targeting integrase may give rise to newer ideal drug to treat AIDS and overcome the side effects of previous compounds and may generate second generation integrase inhibitors [5-7].

The target diketoquinolines were first selected from zinc database and few active compounds with their derivatives were prepared from the carboxylate compounds reacted with substituted piperazine, benzoic acid, 2-phenoxyacetic acid and benzene-1-sulfonyl chloride to form diketoquinoline series. In the present study, structures were docked in integrase pocket. In this context, we synthesized new diketoquinoline derivatives (**1-7**) by the replacement of various substituent's present on elvitegravir. All these compounds were evaluated for their anti-integrase activity.

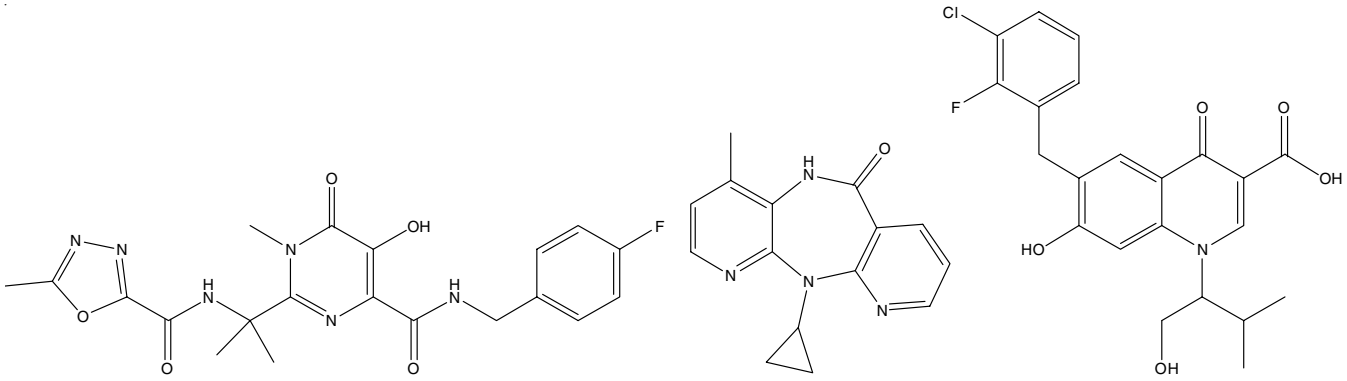


Fig. 1. Representative integrase inhibitors

EXPERIMENTAL

All commercially obtained reagents and solvents were used as received. Reactions were monitored by TLC with silica gel plates. Column chromatography was performed with silica gel (100-200 mesh) as stationary phase. The melting points determined were uncorrected. MS spectra of the synthesized compounds were recorded on Shimadzu QP-5050 spectrophotometer. ^{1}H NMR spectra were acquired on a Varian-300 (300 MHz NMR) spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvent. The infrared spectra were obtained using a Perkin Elmer Spectrum ES Version 10.5.3 Fourier-transform infrared spectrometer. Elemental analysis was performed on FLASH EA 1112 CHN Elemental analyzer, Thermofinnigen, Italy.

General procedure for synthesis of compounds 1-3

Methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-chlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate 2g (5.80 mmol) in *N,N*-dimethylformamide (20 mL), 3-chlorobenzoic acid (0.90 g, 5.80 mmol) and DIPEA (2.26 mL, 17.40 mmol) were added and stirred at room temperature for 20 min, hexafluorophosphate azabenzotriazole tetramethyl uranium (HATU, 3.30 g, 8.70 mmol) was added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was quenched with water, extracted with ethyl acetate, the combined organic layer was dried over sodium sulfate and concentrated under reduced pressure, the obtained crude product was purified by column chromatography using silica gel (100-200 mesh) and 5 % methanol in dichloromethane as eluent, desired organic fractions were distilled under reduced pressure to get methyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-chlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (39 %, 1.09 g), m.p. 212-14 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.06 (s, 1H, cyclopropyl C<u>H</u>), 1.32-1.34 (d, 4H, cyclopropyl C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.60 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.92 (s, 3H, OC<u>H</u>$_3$), 7.42-7.62 (m, 5H, Ar), 7.90-7.97 (d, 1H, Ar), 8.66 (s, 1H, N-C=C-<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 2924 (C-H Ar), 1720 (C=OOCH$_3$), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1469 (C=C Ar), 1380 (C-N st), 1260 (C-O-C), 974 (C-H cyclopropyl).

Methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-5-dichlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate 2g (5.80 mmol) in *N,N*-dimethylformamide (20 mL), 3-5 dichlorobenzoic acid (1.10 g, 5.80 mmol) and DIPEA (2.26 mL, 17.40 mmol) were added and stirred at room temperature for 20 min, HATU (3.30 g, 8.70 mmol) was added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was quenched with water, extracted with ethyl acetate, the combined organic layer was dried over sodium sulfate and concentrated under reduced pressure, the obtained crude product was purified by column chromatography using silica gel (100-200 mesh) and 5 % methanol in dichloromethane as eluent, desired organic fractions were distilled under reduced pressure to get methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-5-dichlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (45 %, 1.35 g), m.p. 232-34 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.06 (s, 1H, cyclopropyl C<u>H</u>), 1.32-1.34 (d, 4H, cyclopropyl C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.60 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.91 (s, 3H, OC<u>H</u>$_3$), 7.42-7.62 (m, 5H), 7.90-7.98 (d, 1H), 8.68 (s, 1H, N-C=C-<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 2922 (C-H Ar), 1710 (C=OOCH$_3$), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1469 (C=C aromatic), 1265 (C-O-C), 1380 (C-N st), 974 (C-H cyclopropyl).

Methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxybenzoyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate 2g (5.80 mmol) in *N,N*-dimethylformamide (20 mL), 4-methoxybenzoic acid (0.88 g, 5.80 mmol) and DIPEA (2.26 mL, 17.40 mmol) were added and stirred at room temperature for 20 min, HATU (3.30 g, 8.70 mmol) was added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was quenched with water, extracted with ethyl acetate, the combined organic layer was dried over sodium sulfate and concentrated under reduced pressure, the obtained crude product was purified by column chromatography using silica gel (100-200 mesh) and 5 % methanol in dichloromethane as eluent, desired organic fractions were distilled under reduced pressure to get methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methoxybenzoyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (45 %, 1.25 g), m.p. 244-46 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ

ppm: 1.07 (s, 1H, cyclopropyl C<u>H</u>), 1.32-1.34 (d, 4H, cyclopropyl C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.60 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.80 (s, 3H, Ph OC<u>H</u>$_3$), 3.90 (s, 3H, COOC<u>H</u>$_3$), 7.42-7.62 (m, 5H, Ar H), 7.90-7.98 (d, 1H, Ar H), 8.66 (s, 1H, N-C=C-<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 2926 (C-H Ar), 1710 (C=OOCH$_3$), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1468 (C=C aromatic), 1262 (C-O-C), 1380 (C-N st), 974 (C-H cyclopropyl).

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-chlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylic acid (1): A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-chlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate 4g (8.27 mmol) in methanol (55 mL) and water (25 mL) LiOH·H$_2$O (0.68 mL, 16.20 mmol) were added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was concentrated under reduced pressure, neutralized with 1 N HCl extracted with dichloromethane, the combined organic layer was dried over sodiumsulphate and concentrated under vacuum to get 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-chlorobenzoyl)-piperazin-1-yl)quinoline-3-carboxylicacid. Yield (68 %, 2.63 g), m.p. 198-200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.09 (s, 1H, cyclopropyl C<u>H</u>), 1.31-1.33 (d, 4H, cyclopropyl C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.57 (br-s, 4H, piperazine C<u>H</u>$_2$), 7.42-7.60 (m, 5H, Ar H), 7.90-7.95 (d, 1H, Ar H), 8.66 (s, 1H, N-C=C-<u>H</u>), 15.18 (s, 1H, COO<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 2924 (C-H Ar), 1712 (C=O, COOH), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1469 (C=C aromatic), 1380 (C-N st), 974 (C-H cyclopropyl), 3550 (OH st, COOH). MS: *m/z* (rel int %): 470 (22) [M$^+$], 430 (29), 337 (28), 320 (41), 297 (100), 278 (42), 270 (48), 245 (56), 234 (64), 223 (73), 205 (66), 194 (51), 149 (48). CHN analysis: $C_{24}H_{21}N_3O_4FCl$ Calcd. (%): C 61.29, H 4.46, N 8.93, Found (%): C 61.33, H 4.47, N 8.95.

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3,5-dichlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylic acid (2): A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3,5-dichlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylate 4g (8.27 mmol) in methanol (55 mL) and water (25 mL) LiOH·H$_2$O (0.68 mL, 16.20 mmol) were added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was concentrated under reduced pressure, neutralized with 1 N HCl extracted with dichloromethane, the combined organic layer was dried over sodium sulphate and concentrated under vacuum to get 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(3-5-dichlorobenzoyl)piperazin-1-yl)quinoline-3-carboxylic acid. Yield (68 %, 2.64 g), m.p. 218-20 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.06 (s, 1H, cyclopropyl C<u>H</u>), 1.32-1.34 (d, 4H, cyclopropyl 2C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.60 (br-s, 4H, piperazine 2C<u>H</u>$_2$), 7.42-7.62 (m, 5H, Ar H), 7.90-7.97 (d, 1H, Ar H), 8.68 (s, 1H, N-C=C-<u>H</u>), 15.20 (s, 1H, COO<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 3550 (OH st, COOH), 2922 (C-H Ar), 1714 (C=O, COOH), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1469 (C=C Ar), 1380 (C-N st), 974 (C-H cyclopropyl). MS: *m/z* (relint %): 504 (18) [M$^+$], 465 (22), 420 (28), 395 (32), 370 (36), 330 (39), 320 (45), 296 (100), 280 (48), 272 (61), 248 (68), 230 (56), 221 (66), 204 (78), 194 (72), 148 (82). CHN analysis: $C_{24}H_{20}Cl_2FN_3O_4$ Calcd. (%): C 57.16, H 4.00, N 8.33, Found (%): C 57.17, H 4.04, N 8.36.

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxy benzoyl)piperazin-1-yl)quinoline-3-carboxylic acid (3): A solution of methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methoxy benzoyl)piperazin-1-yl)quinoline-3-carboxylate 4g (8.27 mmol) in methanol (55 mL) and water (25 mL) LiOH·H$_2$O (0.68 mL, 16.20 mmol) were added and stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was concentrated under reduced pressure, neutralized with 1N HCl extracted with dichloromethane, the combined organic layer was dried over sodiumsulphate and concentrated under vacuum to get 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methoxy-benzoyl)piperazin-1-yl)quinoline-3-carboxylic acid. Yield (72 %, 2.73 g), m.p. 226-28 °C. ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 1.08 (s, 1H, cyclopropyl C<u>H</u>), 1.32-1.34 (d, 4H, cyclopropyl 2C<u>H</u>$_2$), 3.40 (br-s, 4H, piperazine C<u>H</u>$_2$), 3.60 (br-s, 4H, piperazine 2C<u>H</u>$_2$), 3.70 (s, 3H, OCH$_3$), 7.42-7.62 (m, 5H, Ar H), 7.90-7.97 (d, 1H, Ar H), 8.68 (s, 1H, N-C=C-<u>H</u>), 15.20 (s, 1H, COO<u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 3550 (OH st, COOH), 2922 (C-H Ar), 1717 (C=O, COOH), 1700 (C-CO-N), 1680 (C=O quinoline), 1564, 1469 (C=C Ar), 1380 (C-N st), 974 (C-H cyclopropyl). MS: *m/z* (relint %): 466 (21) [M$^+$], 433 (28), 334 (26), 320 (28), 299 (100), 280 (31), 270 (38), 245 (41), 236 (44), 224 (45), 202 (48), 194 (72), 164 (78), 154 (58). CHN analysis: $C_{25}H_{24}FN_3O_5$ Calcd. (%): C 64.51, H 5.20, N 9.00, Found (%): C 64.54, H 5.22, N 9.04.

General procedure for synthesis of compounds 4-5

Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-phenoxyacetyl)piperazin-1-yl)quinoli ne-3-carboxylate: A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate 1g (3.00 mmol) in DMF (10 mL), 2-phenoxyacetic acid (1.97 g, 3.00 mmol), DIPEA (0.5 mL), HATU (1.70 g, 4.50 mmol) were added at room temperature and stirred for 6h. TLC shows completion of starting material. The reaction mixture was quenched with water, extracted with ethyl acetate, dried over sodium sulfate and concentrated under reduced pressure to get crude compound. The obtained crude was purified by silica gel column chromatography by using 5 % methanol in dichloromethane as a eluent to get Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylateas. Yield (55 %, 0.77 g), m.p. 205 °C. ^{1}H NMR (300 MHz, DMSO) δ 1.40-1.46 (t, 3H, C<u>H</u>$_3$), 2.86-2.90 (S, 4H, piperazine C<u>H</u>$_2$), 3.15-3.17 (d, 1H, -C=C-<u>H</u>), 3.70 (s, 4H, piperazine C<u>H</u>$_2$), 3.75 (q, 2H, C<u>H</u>$_2$), 3.82 (s, 3H, OC<u>H</u>$_3$), 4.89 (s, 2H, -C<u>H</u>$_2$OPh), 7.12-7.15 (d, 1H, Ar <u>H</u>), 7.94.7.98 (d, 1H, Ar <u>H</u>) 8.97 (s, 5H, Ar <u>H</u>). IR (KBr, ν_{max}, cm^{-1}): 2924 (C-H Ar), 1710 (C=O, COOH), 1700 (C=O, CCON), 1680 (C=O quinoline), 1625, 1566, 1451 (C=C Ar), 1380, 1327 (C-N st), 1263 (C-O-C).

Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-4-chloro phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate 1g (3.00 mmol) in DMF (10 mL), 2-(4-chloro phenoxy) acetic acid (0.55 g, 3.00

mmol), DIPEA (0.5 mL), HATU (1.70 g, 4.50 mmol) were added at room temperature and stirred for 6 h. TLC shows completion of starting material. The reaction mixture was quenched with water, extracted with ethyl acetate, dried over sodium sulfate and concentrated under reduced pressure to get crude compound. The obtained crude was purified by silica gel column chromatography by using 5 % methanol in dichloromethane as a eluent to get Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-2-(4-chloro phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (62 %, 0.93 g), m.p. 241-43 °C. ^{1}H NMR (300 MHz, DMSO) δ: 3.84 (s, 3H, OC$\underline{H}_3$), 1.40-1.46 (t, 3H, C$\underline{H}_3$), 2.86-2.90 (S, 4H, piperazine $\underline{H}$ e, f), 3.70 (s, 4H, piperazine H c, d), 4.59-4.61 (q, 2H, C$\underline{H}_2$), 7.12-7.15 (d, 1H, aromatic Ha), 7.94.7.98 (d, 1H, aromatic Hb), 3.15-3.17 (d, 1H, -C=C-H), 4.90 (s, 2H, -C$\underline{H}_2$OPh), 8.98 (s, 5H, aromatic H). IR (KBr, ν_{max}, cm^{-1}): 2924 (C-H Ar), 1710 (C=O, COOH), 1700 (C=O, CCON), 1680 (C=O quinoline), 1625, 1566, 1451 (C=C Ar), 1380, 1328 (C-N st), 1264 (C-O-C).

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylic acid (4): A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylate (1g, 2.14 mmol) in Methanol (16 mL), Water (6 mL). LiOH·H_2O (0.26 mL, 6.42 mmol) was added stirred at room temperature for 6 h, TLC shows completion of starting material. The reaction mixture was quenched wit 1 N HCl, extracted with dichloromethane, dried over sodium sulphate, concentrated under reduced pressure, washed with hexane to get 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylic acid, Yield (65 %, 0.63 g), m.p. 196-98 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.40-1.47 (t, 3H, C$\underline{H}_3$), 2.86-2.92 (S, 4H, piperazine CH_2), 3.14-3.17 3.70 (s, 4H, piperazine CH_2), 4.59-4.63 (q, 2H, C$\underline{H}_2$), 7.12-7.17 (d, 1H, Ar $\underline{H}$), 7.96.7.98 (d, 1H, Ar $\underline{H}$), (d, 1H, -C=C-H), 4.90 (s, 2H, -C$\underline{H}_2$OPh), 8.96 (s, 5H, Ar$\underline{H}$). 15.34 (s, 1H, COO$\underline{H}$). IR (KBr, ν_{max}, cm^{-1}): 3638 (O-H, COOH), 2924 (C-H Ar), 1710 (C=O, COOH), 1680 (C=O quinoline), 1625, 1566, 1451 (C=C Ar), 1380, 1327 (C-N st), 1263 (C-O-C). MS: *m/z* (relint %) 454 (11) [M$^+$], 430 (18), 402 (21), 388 (15), 356 (29), 309 (24), 297 (41), 278 (40), 270 (62), 245 (54), 234 (24), 223 (44), 205 (100), 194 (72), 149 (58). CHN analysis: $C_{24}H_{24}N_3O_5F$ Calcd. (%): C 63.57, H 5.33, N 09.27, Found (%): C 63.62, H 5.38, N 9.34.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-4-chloro phenoxyacetyl)piperazin-1-yl)quino line-3-carboxylic acid (5): A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-4-chloro phenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylate (1g, 2.14 mmol) in methanol (16 mL), water (6 mL). LiOH·H_2O (0.26 mL, 6.42 mmol) was added stirred at room temperature for 6 h. TLC shows completion of starting material. The reaction mixture was quenched wit 1 N HCl, extracted with dichloromethane, dried over sodium sulphate, concentrated under reduced pressure, washed with hexane to get 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-4-chlorophenoxyacetyl)piperazin-1-yl)quinoline-3-carboxylicacid, Yield (70 %, 0.72 g), m.p. 227-29 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.40-1.46 (t, 3H, C$\underline{H}_3$), 2.86-2.90 (S, 4H, piperazine CH_2), 3.15-3.17 (d, 1H, -C=C-H), 3.70 (s, 4H, piperazine CH_2), 4.59-4.61 (q, 2H, C$\underline{H}_2$), 4.90 (s, 2H, -C$\underline{H}_2$OPh), 7.12-7.15 (d, 1H, Ar $\underline{H}$), 7.94.7.98 (d, 1H, Ar$\underline{H}$), 8.98 (s, 5H, Ar$\underline{H}$), 15.30 (s, 1H, COO$\underline{H}$). IR (KBr, ν_{max}, cm^{-1}): 3550 (O-H st, COOH), 2924 (C-H Ar), 1710 (C=O, COOH), 1680 (C=O quinoline), 1625, 1566, 1451 (C=C Ar), 1380, 1328 (C-N st), 1264 (C-O-C). MS: *m/z* (relint %) 488 (17) [M$^+$], 474 (21), 445 (12), 432 (21), 405 (22), 384 (27), 342 (42), 312 (32), 293 (48), 277 (41), 251 (20), 222 (41), 208 (10), 194 (100), 168 (50), 144 (42). CHN analysis: $C_{24}H_{23}N_3O_5ClF$ Calcd. (%): C 59.08, H 4.75, N 08.61, Found (%): C 59.10, H 4.82, N 8.65.

General procedure for synthesis of compounds 6-7

Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate (1g, 3.00 mmol) in dichloromethane (20 mL), DIPEA (0.2 mL, 15.15 mmol), 4-methoxybenzene-1-sulfonyl chloride (0.62 g, 3.0 mmol) and HATU (1.70 g, 4.50 mmol) were added stirred at room temperature for 6 h. TLC shows the completion of starting material. The reaction mixture quenched with water extracted with dichloromethane, dried over sodium sulfate and concentrated under reduced pressure to get crude. The obtained crude was purified by silica gel column chromatography by to get methyl1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (75 %, 1.31 g), m.p. 186-88 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.38 (t, 3H, C$\underline{H}_3$), 3.05 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.36 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.85 (s, 3H, OC$\underline{H}_3$), 3.88 (s, 3H, OC$\underline{H}_3$), 4.53 (q, 2H, C$\underline{H}_2$CH$_3$), 7.17-7.20 (d, 3H, Ar $\underline{H}$), 7.71-7.75 (d, 2H, Ar $\underline{H}$), 7.87-7.91 (d, 1H, Ar $\underline{H}$), 8.94 (s, 1H, C$\underline{H}$=C). IR (KBr, ν_{max}, cm^{-1}): 2924 (C-H Ar), 2852 (C-H alkyl), 1710 (C=O, COOCH$_3$), 1684 (C=O quinoline), 1596, 1565 (C=C Ar), 1346, 1376 (C-N st), 1259 (C-O-C).

Methyl-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-ethoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate: A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate (1g, 3.00 mmol) in dichloromethane (20 mL), DIPEA (0.2 mL, 15.15 mmol), 4-ethoxybenzene-1-sulfonyl chloride (0.66 mL, 3.0 mmol) and HATU (1.70 g, 4.50 mmol) were added stirred at room temperature for 6 h. TLC shows the completion of starting material. The reaction mixture quenched with water extracted with dichloromethane, dried over sodium sulfate and concentrated under reduced pressure to get crude. The obtained crude was purified by silica gel column chromatography by to get methyl1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-ethoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate. Yield (64 %, 0.99 g), m.p. 158-60 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.11 (t, 3H, C$\underline{H}_3$), 1.39-1.45 (t, 3H, C$\underline{H}_3$), 3.10 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.20 (q, 2H, OC$\underline{H}_2$), 3.38 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.86 (s, 3H, OC$\underline{H}_3$), 4.55-4.62 (q, 2H, C$\underline{H}_2$), 7.16-7.20 (d, 3H, Ar $\underline{H}$), 7.70-7.75 (d, 2H, Ar $\underline{H}$), 7.87-7.92 (d, 1H, Ar $\underline{H}$), 8.94 (s, 1H, C$\underline{H}$=C). IR (KBr, ν_{max}, cm^{-1}): 2922 (C-H Ar), 2854 (C-H alkyl), 1710 (C=O, COOCH$_3$), 1684 (C=O quinoline), 1596, 1565 (C=C Ar), 1346, 1378 (C-N st), 1260 (C-O-C).

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)quino line-3-carboxylic acid

(6): A solution of methyl 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate (0.8 g, 1.59 mmol) in THF (7 mL), methanol (1 mL) and water (3 mL). $LiOH·H_2O$ (0.19 g, 4.77 mmol) was added and stirred at room temperature for 6 h. TLC shows the completion of starting material. The reaction mixture neutralized with 1 N HCl extracted with dichloromethane, dried over sodium sulphate, concentrated under reduced pressure and washed with hexane to get 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylic acid, Yield (70 %, 0.53 g), m.p. 178-80 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.36 (t, 3H, C$\underline{H}_3$), 3.05 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.38 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.86 (s, 3H, OC$\underline{H}_3$), 4.55 (q, 2H, C$\underline{H}_2$CH$_3$), 7.18-7.20 (d, 3H, Ar $\underline{H}$), 7.71-7.74 (d, 2H, Ar $\underline{H}$), 7.87-7.90 (d, 1H, Ar $\underline{H}$), 8.93 (s, 1H, C$\underline{H}$=C), 15.27 (s, 1H, COOH). MS, M^+ 489.8. IR (KBr, ν_{max}, cm^{-1}): 3550 (OH, COOH), 2924 (C-H Ar), 2852 (C-H alkyl), 1714 (C=O, COOH), 1684 (C=O quinoline), 1596, 1565 (C=C Ar), 1346, 1376 (C-N st), 1259 (C-O-C). MS: *m/z* (relint %) 490 (12) [M^+], 472 (11), 451 (12), 432 (16), 404 (21), 375 (24), 348 (31), 326 (33), 302 (16), 280 (31), 255 (33), 218 (65), 202 (100), 184 (68), 166 (24), 145 (40), 125 (44), 102 (48). CHN analysis: $C_{23}H_{24}N_3O_6SF$ Calcd. (%): C 56.38, H 4.90, N 8.58, Found (%): C 56.42, H 4.94, N 8.62.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-ethoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylic acid (7): A solution of methyl1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-ethoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylate (0.8 g, 1.59 mmol) in THF (7 mL), methanol (1 mL) and water (3 mL). $LiOH·H_2O$ (0.19 g, 4.77 mmol) was added and stirred at room temperature for 6 h. TLC shows the completion of starting material. The reaction mixture neutralized with 1NHCl extracted with dichloromethane, dried over sodium sulphate, concentrated under reduced pressure and washed with hexane to get 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(4-ethoxyphenylsulfonyl)piperazin-1-yl)quinoline-3-carboxylic acid, Yield (58 %, 0.44 g), m.p. 150-52 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.22 (t, 3H, C$\underline{H}_3$), 1.38 (t, 3H, C$\underline{H}_3$), 3.10 (br-s, 4H, piperazine 2C$\underline{H}_2$), 3.20 (q, 2H, OC$\underline{H}_2$), 3.38 (br-s, 4H, piperazine 2C$\underline{H}_2$), 4.54 (q, 2H, C$\underline{H}_2$CH$_3$), 7.16-7.20 (d, 3H, Ar$\underline{H}$), 7.70-7.75 (d, 2H, Ar $\underline{H}$), 7.88-7.92 (d, 1H, Ar $\underline{H}$), 8.94 (s, 1H, C$\underline{H}$=C), 15.30 (s, 1H, COO$\underline{H}$). IR (KBr, ν_{max}, cm^{-1}): 3550 (O-H st, COOH), 2922 (C-H Ar), 2856 (C-H alkyl), 1682 (C=O quinoline), 1596, 1566 (C=C Ar), 1346, 1376 (C-N st), 1260 (C-O-C). MS: *m/z* (relint %) 504 (10) [M^+], 484 (8), 468 (16), 448 (21), 424 (20), 386 (20), 355 (36), 340 (38), 312 (18), 290 (38), 266 (38), 249 (48), 222 (82), 194 (100), 178 (28), 154 (41), 136 (48) 122 (48), 102 (51). CHN analysis: $C_{24}H_{26}N_3O_6SF$ Calcd. (%): C 57.25, H 5.20, N 8.34, Found (%): C 57.30, H 5.25, N 8.40.

Biological assays

Cells and viruses: Assay was performed in Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Science. Reagents were procured in laboratory as HEPES (N-2(2-hydroxyothyl)piperazine-N'-(2-ethanesufonic acid), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), DMF (*N,N'*-dimethyl formamine), penicillin, streptomycin sulfate, glutamine were purchased from Sigma; 2-ME (2-mercapto ethanol) was purchased from Bio-Rad. RPMI-1640 and fetal bovine serum (FBS) were purchased from Gibco.

C8166 cells and HIV-1_{IIIB} were kindly donated by Medical Research Council, AIDS Regent Project. The cells were maintained at 37 °C in 5 % CO_2 in RPMI-1640 medium supplemented with 10 % heat-inactivating FBS (Gibco). HIV-1_{IIIB} was prepared from the supernatants of H9/HIV-1_{IIIB} cells. The 50 % HIV-1 tissue culture infectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by Reed and Muench method [8,9]. Virus stocks were stored in small aliquots at -76 °C. The titer of virus stock was 1.0×10^8 $TCID_{50}$ per mL.

in vitro assays

Inhibition of syncytia formation: The inhibition effect of samples on acute HIV-1 infection was measured by the syncytia formation assay. In the presence or absence of various concentrations of samples, 4×10^4 C8166 cells were infected with HIV-1 at a multiplicity of infection (MOI) of 0.04 and cultured in 96-well plates at 37 °C in 5 % CO_2 for 72 h. NVP was used as a positive control. At 72 h post-infection, cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell) in each well of 96 well plates under an inverted microscope (100×). The inhibitory percentage of syncytia formation was calculated by the percentage of syncytia number in treated sample compared to that in infected control. 50 % effective concentration (EC_{50}) was calculated [10].

Cytotoxicity: The cellular toxicity of compounds on C8166 was assessed by MTT colorimetric assay [9]. Briefly, 100 μL of 4×10^5 cells were plated into 96-well plates, 100 μL of various concentrations of compounds was added and incubated at 37 °C in a humidified atmosphere of 5 % CO_2 for 72 h. Discard 100 μL supernatant, MTT reagent was added and incubated for 4 h, 100 μL 50 % DMF-15 % SDS was added. After the formazan was dissolved completely, the plates were analyzed by a Bio-Tek ELx 800 ELISA reader at 570 nm/630 nm. 50 % cytotoxicity concentration (CC_{50}) was calculated.

Integrase assays: The enzymatic integration reactions were carried out with minor modifications as described previously [11]. To determine the susceptibility of the HIV-1 integrase enzyme to different compounds, we used an enzyme-linked immunosorbent assay (ELISA) adapted from Hwang *et al.* [8]. The overall integration assay uses an oligonucleotide substrate for which one oligonucleotide (5'-ACTGCTAGAGATTTTCC ACACTGACTAAAAGGGTC-3') is labeled with biotin at the 3' end and the other oligonucleotide (5'-GACCCTTTTAGTCA GTGTGGAAAATCTCTAGCA$\underline{GT}$-3') is labeled with digoxigenin at the 5' end. For the strand transfer assay, a precleaved oligonucleotide substrate (the second oligonucleotide lacks GT [underlined] at the 3' end) was used. The integrase enzyme was diluted in 750 mM NaCl, 10 mM Tris (pH 7.6), 10 % glycerol and 1 mM β-mercaptoethanol. To perform the reaction, 4 μL of diluted integrase (corresponding to a concentration of 1.6 μM) and 4 μL of annealed oligonucleotides (7 nM) were added in a final reaction volume of 40 μL containing 10 mM $MgCl_2$, 5 mM dithiothreitol, 20 mM HEPES (pH 7.5), 5 % polyethylene glycol and 15 % dimethyl sulfoxide. As such, the final concentration of integrase in this assay was 160 nM. The reaction was carried out for 1 h at 37 °C. Reaction products

were denatured with 30 mM NaOH and detected by ELISA on avidin-coated plates. For determining the effect of compounds on the 3'processing activity a classical cleavage assay with detection of products by denaturating gelelectrophoresis was performed as described previousluy [10,11]. Briefly, 0.2 pmol of the radioactive labeled oligonucleotide substrate (INT1, ^{32}P-5' TGTGGAAAATCTCTAGCAGT 3'; INT2,5'-ACTGCTAGAGATTTTCCACA 3') and 10 nmol integrase in a final volume of 10 µL was incubated for 1 h at 37°C. The final reaction mixture contained 20 mM HEPES pH 7.5), 5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, 0.5 % (v/v) polyethylene glycol 8000, 15 % DMSO, integrase was diluted previosly in 750 mM NaCl, 10 mM Tris (pH 7.6), 10 % glycerol and 1 mM β-mercaptoethanol. The reactions were stopped by the addition of formamide loading buffer (95 % formamide, 0.1 % xylene cyanol, 0.1 % xylene cyanol, 0.1 % bromophenol blue and 0.1 % sodium dodecyl sulfate). Samples were loaded on a 15 % denaturating polyacrylamide/ureum gel. The extent of 3' processing or DNA strand transfer was based on measuring the respective amounts of -2 bands or strand transfer products relative to the intensity of the total radioactivity present in the lane. These data were determined using the Opti Quant Acquisition and Analysis software (Perkin Elmer Corporate, Fremont, CA).

Drug susceptibility assay: The inhibitory effect of antiviral drugs on the HIV-induced CPE in MT-4 cell culture was determined by the MTT-assay [9]. This assay is based on the reduction of the yellow coloured 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan derivative, which can be measured spectrophotometrically. The 50 % cell culture infective dose of the HIV strains was determined by titration of the virus stock using MT-4 cells. For the drug susceptibility assays, MT-4 cells were infected with 100 to 300 50 % cell culture infective doses of the HIV strains in the presence of fivefold serial dilution of the antiviral drugs. The concentration of the compound achieving 50 % protection against the CPE of HIV, which is defined as the 50 % effective concentration (IC_{50}), was determined. The concentration of the compound destroying 50 % of the MT-4 cells, which is defined as the 50 % cytotoxic concentration (CC_{50}), was determined as well.

Molecular modeling

Docking: The molecular docking tool, GLIDE was used for ligand docking studies into the HIV-1 Integrase pocket. The crystal structure was obtained from the protein data bank, PDB ID: 1qs4. The protein preparation was carried out using 'protein preparation wizard' in Maestro 8.0 in two steps, preparation and refinement. Grids were generated centering onco-crystalized ligand**.** The ligands were developed using maestro build panel and prepared by Ligprep 2.2 module that produces the low energy conformer of ligands using OPLS 2005 force field. The low energy conformation of the ligands was selected and docked into the grid generated from protein structures using standard precision (SP) docking mode.

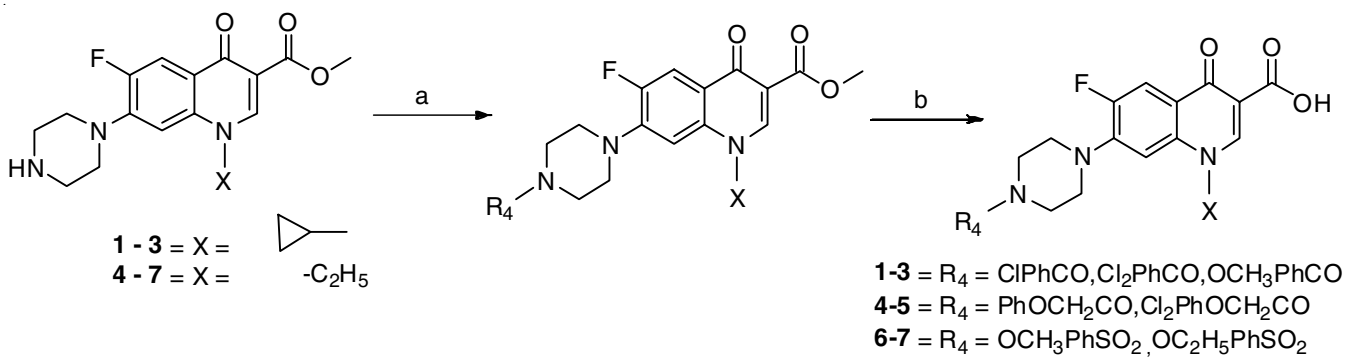
RESULTS AND DISCUSSION

Design of the compounds: The design for the series of compounds to be considered for study was performed using virtual screening protocol [12-14]. Considering the pharmacophoric requirements and the standard compound elvitegravir, the zinc database was explored. The *in silico* screened compounds were then tested for Lipinsky rule of five to evaluate drug likeness, which becomes an essential tool to facilitate drug discovery. The novelty of compounds in terms of HIV-1 Integrase inhibitory activity was checked over SciFinder. These virtually screened hits were synthesized along with its derivatives and evaluated for their inhibitory potential.

It was found that diketoquinolines have not been much explored for HIV-I integrase activity. Hence, we studied the diketoquinoline motif for integrase inhibition. A series of compound were synthesized as diketoquinoline analogues.

Chemistry: The target diketoquinolines (**1-7**) were prepared from the commercially procured intermediates such as methyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylate and 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylate [15-20]. The synthetic route is outlined in **Scheme-I**. All the carboxylate compounds were reacted with substituted benzoic acid, phenoxy acetic acid and benzene-1-sulfonyl chloride *via* substitution of hydrogen of piperazine in presence of DIPEA (*N,N*-di isopropyl ethyl amine) to produce intermediate compound in an excellent yield. The ester linkage in intermediate compound ($COOCH_3$) is hydrolyzed to carboxylic acid (COOH) in alkaline medium in the presence of aqueous lithium hydroxide to yield the target compounds **1-7**. The conformation of removal of C-Cl and attachment of substituted compounds and conversion of carboxylate to carboxylic acid verified by spectral analysis.

Biological activity: Diketoquinolines derivatives (**1-7**) were tested *in vitro* for their cytotoxicity assay and inhibition



Scheme-I: Reagents and conditions: (a) DMF/dichloromethane, DIPEA, substituted benzoic acid/2-phenoxy acetic acid/benzene sulphonyl chloride, HATU stirred 6 h; (b) methanol, water, LiOH·H_2O, stirred 6 h

of syncytia formation using a MTT and CPE method [8,9]. CC_{50} and EC_{50} values were generated from duplicate experiments in μM and selective index also calculated using dose response curves Table-1. As already observed for diketoquinolines derivative series [21], acidic derivatives were more potent with a high selectivity against integrase. The replacement of hydrophobic ring from C_6 of elvitegravir by flourine and C_7 by substituted piperazine group and further benzene groups does not lead to significant improvement in HIV-1 integrase inhibition. Substitution at quinoline 'N' by ethyl and piperazine 'N' by hydrophobic phenoxy carbonyl and sulphonyl group **4-7** showed EC_{50} of 0.10 and 0.08 μM against integrase enzyme respectively. This suggests that substitution of quinoline 'N' by alkyl and hydrophobic moiety at piperazine 'N' affect the ability of the inhibitors to bind with integrase enzyme. Other substitution does not make any significant interaction with HIV-1 integrase enzyme Table-2.

Molecular docking: The binding mechanisms of synthesized compounds were investigated using molecular docking studies. The docking studies for the designed and synthesized molecules were performed using Schrodinger Suite. The molecular docking tool, GLIDE was used for ligand docking studies into the HIV-1 integrase pocket [22-24]. The crystal structure of HIV-1 integrase was obtained from the protein data bank, PDB ID: 1QS4. The protein preparation was carried out using 'protein preparation wizard' in Maestro 8.0 in two steps, preparation and refinement. Grids were generated centering on co-crystallized ligand**.** The ligands were developed using maestro build panel and prepared by Ligprep 2.2 module that produces the low energy conformer of ligands using OPLS 2005 force field. The low energy conformation of the ligands was selected and docked into the grid generated from protein structures using standard precision (SP) docking mode.

As per the literature, the active site comprises of Thr 66, Lys 156, Lys 159 and DDE motif (Asp 64, Asp 116 and Glu 152). The docking poses revealed the interaction of few ligands with desired amino acids. The standard drug elvitegravir had docking score of -8.931402 and displayed interactions with lys156, Asn155, Lys159 and Thr66. When raltegravir and nevirapine were docked in the same active site, they displayed comparable docking scores and interaction patterns. Raltegravir revealed hydrogen bonding with Asp 116 while nevirapine showed hydrogen bonding with Asp 64. The synthesized compounds which displayed fair integrase inhibition were also docked to ascertain the interactions and were compared with standard pose. Compound **6, 7** had maximum potency with EC_{50} of 0.08 mM and 0.08 mM in enzyme inhibition and cell line assay respectively. The docking analysis also shows that though the compounds favoured interactions with desired amino acids but none of the compounds could show inter-

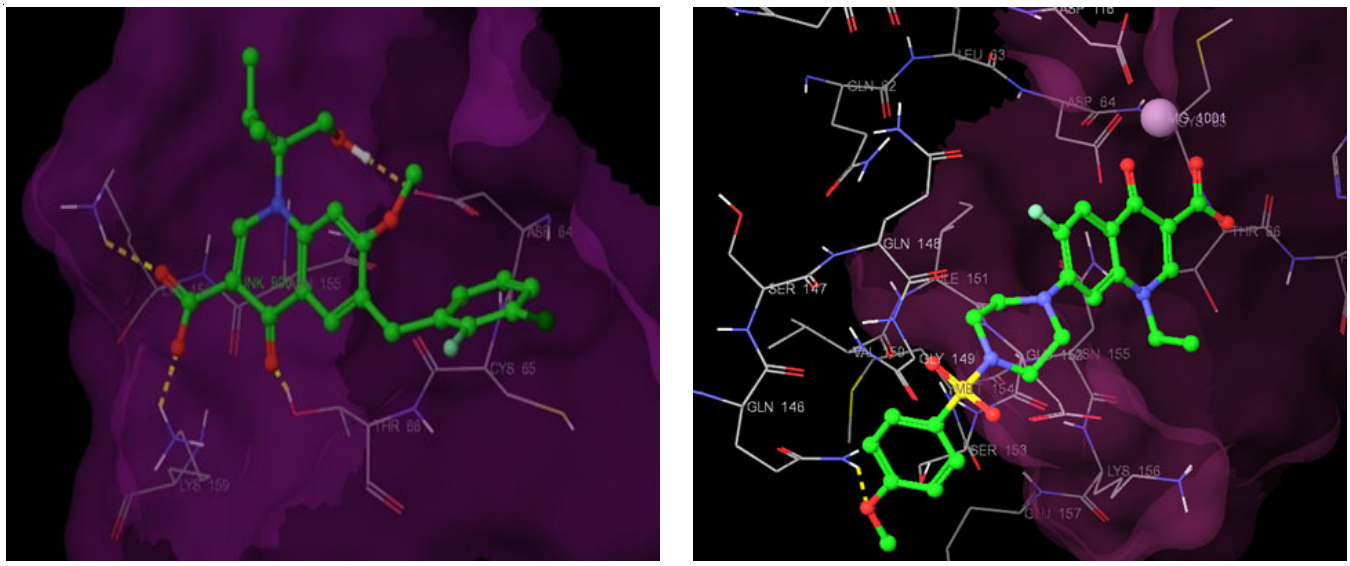
TABLE-1
CYTOTOXICITY AND INHIBITION OF SYNCYTIUM FORMATION ACTIVITIES OF COMPOUNDS **1-7**

Compd. No.	Experiment	Method	CC_{50} (μM)	EC_{50} (μM)	Therapeutic index (TI)
1	Cytotoxicity assay	MTT	160	–	457
	Inhibition of syncytium formation	CPE	–	0.35	
2	Cytotoxicity assay	MTT	170	–	531
	Inhibition of syncytium formation	CPE	–	0.32	
3	Cytotoxicity assay	MTT	175	–	583
	Inhibition of syncytium formation	CPE	–	0.30	
4	Cytotoxicity assay	MTT	> 200	–	869
	Inhibition of syncytium formation	CPE	–	0.23	
5	Cytotoxicity assay	MTT	> 200	–	909
	Inhibition of syncytium formation	CPE	–	0.22	
6	Cytotoxicity assay	MTT	> 200	–	1111
	Inhibition of syncytium formation	CPE	–	0.18	
7	Cytotoxicity assay	MTT	> 200	–	1176
	Inhibition of syncytium formation	CPE	–	0.17	
Nevirapine	Cytotoxicity assay	MTT	> 200	–	12500
	Inhibition of syncytium formation	CPE	–	0.016	

TABLE-2
CORRELATION OF DOCKING SCORE AND CYTOTOXICITY AND ANTI-INTEGRASE ACTIVITIES OF SYNTHESIZED COMPOUNDS **1-7**

Compd. No.	Docking score	EC_{50} (μM)	CC_{50} (μM)	Selective index
1	-8.839481	0.15	192	1280
2	-8.865308	0.14	188	1342
3	-8.884674	0.13	188	1446
4	-9.715626	0.10	>200	2000
5	-9.989743	0.10	>200	2000
6	-10.583468	0.08	>200	2500
7	-10.884390	0.08	>200	2500
Raltegravir	-4.291	0.0111	Not determined	Not determined

Note: When data indicate > 200 for EC_{50} and CC_{50} it means that the compounds were neither active not toxic at 200 μM, which is the highest concentration we can test to stay in the DMSO tolerance levels. When data indicate a number lower than 200 for EC_{50} and then the same number with the equality sign in the CC_{50} it means that we observed toxicity at this concentration.



Elvitegravir Compound **6**

Fig. 2. Docked view for quinolonyl diketo compounds

actions with the important Mg ions *i.e.* Mg1001 and Mg1002. The docking scores are given in Table-2 and Fig. 2 represents the docked view.

Conclusion

In order to elucidate the binding mode of diketoacid quinolonyl series of compounds, new derivatives were synthesized by replacing the 6-position with flourine atom in place of substituted hydrophobic benzyl moiety of elvitegravir and substitution at 7-position by substituted piperazine, benzoic acid, 2-phenoxyacetic acid and benzene-1-sulfonyl chloride. These compounds were evaluated for their enzymatic activity. The replacement of hydrophobic ring from C_6 of elvitegravir by F and C_7 by piperazine group does not lead to significant improvement in HIV-1 integrase inhibition. Substitution at quinoline N by ethyl and piperazine N by sulphonyl group **6, 7** showed IC_{50} value 0.08 and 0.08 μM against integrase enzyme. This result suggests that substitution of quinoline N by alkyl and hydrophobic moiety at piperazine N by phenyl carbonyl and sulphonyl effect on the ability of the inhibitors to bind with integrase enzyme. Other substitution does not make any significant interaction with HIV-1 integrase enzyme.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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