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Original Article

Design, Synthesis and Docking studies of New Quinazolinone Derivatives as Anti-HIV-1 Agents

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Abstract

The Human Immunodeficiency Virus (HIV) infection is a global health challenge that creates an urgent need to develop new therapeutic agents. In this work, a new group of quinazolinone derivatives were designed and synthesized and evaluated their anti-HIV activity. The antiviral assay revealed that some analogues inhibited HIV replication in the cell culture. A docking study using the later crystallographic data available for PFV integrase including its complexes with Mg^{2+} and dolutegravir, showed that the designed compounds bind into the active site of integrase enzyme such that both carbonyl groups chelate Mg^{2+} ions. Interestingly, all of the synthesized compounds were found to present no significant cytotoxicity at a concentration of 100 μM. According to the anti-HIV evaluation results, the compound **10f** was found as the most active with the inhibition rate of 38%. Therefore, these compounds can provide a very good basis for the development of new anti-HIV agents.

*Keywords***:** Anti-HIV-1, Design, Synthesis, Quinazolinone, Benzamide, Docking studies.

1. Introduction

Human immunodeficiency virus (HIV) infection is a life-threatening disease necessitating serious drug therapy. Combinations of anti-HIV drugs have been developed for therapeutic intervention, but most of them have limited efficacy because of costs and development of resistances [1]. Therefore, there is a strong need to discover new anti-HIV compounds with different structural scaffolds [2, 3]. HIV integrase enzyme plays a key role in the growth and

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replication cycle of the virus and because of lacking any counterpart in mammalian cells has been regarded as a promising therapeutic target [4-7]. Integrase (IN) catalyzes the integration process of viral DNA into the human DNA. First, IN removes a GT dinucleotide from the 3' ends of viral DNA in the cytoplasm (3'-processing reaction). Subsequently, in the nucleus the 3′ end of the viral DNA attacks a phosphodiester bond in the target DNA, covalently joining the viral to target DNA (strand transfer reaction) [8, 9]. FDA-approved integrase inhibitors including Raltegravir (**1**), Elvitegravir (**2**), Dolutegravir (**3**) and Bictegravir (**4**) are shown in Figure 1 [4]. IN inhibitors specifically block the strand transfer step. All well-known HIV IN inhibitors commonly contain two structural features essential for the anti-IN activity: a chelating motif to interact with Mg^{2+} ions in the enzyme's active site and a properly oriented hydrophobic moiety [10, 11].

We have previously developed some compounds featuring quinazolinone core (**5**) (Figure 2) that effectively inhibited HIV-1 replication in cell culture [12]. In addition, quinazolinones are very attractive in anti-HIV drug discovery due to their well-known biochemical properties and having no significant cytotoxic effects [13-15]. As part of our research program, aimed at discovering new anti HIV-1 agents, we focused on the design, synthesis, and HIV-1 replication inhibitory activity of a series of quinazolinone derivatives containing benzamide moiety and various substituted phenyl moiety at C-2 (Figure 2).

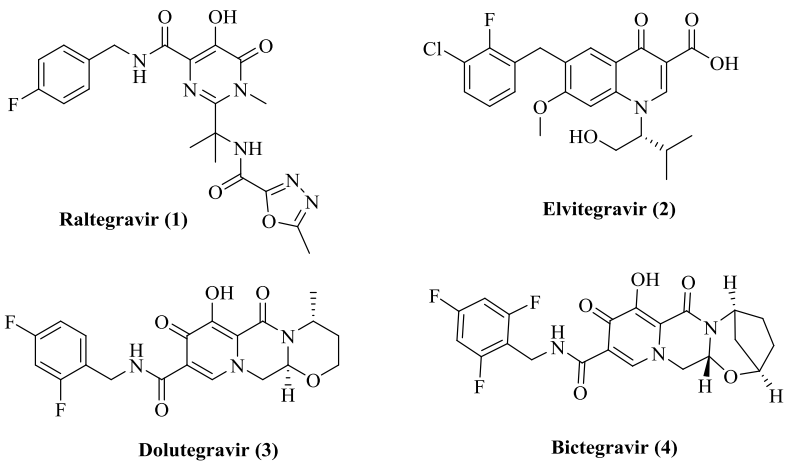


Figure 1. Chemical structure of FDA-approved integrase inhibitors

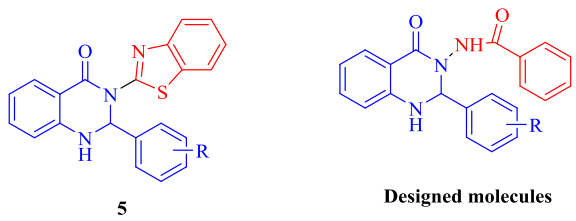


Figure 2. Chemical structure of compound **5** and designed molecules

2. Materials and Methods

2.1. General

All of the materials and solvents used in this research were reagent-grade and obtained from Sigma-Aldrich (Merck KGaA) Chemical. The MP (melting points) of the compounds were determined using an Electrothermal-IA9300 melting point apparatus. Infrared spectra were obtained using a PerkinElmer Infrared spectrometer (Model 1420). A Bruker FT-500 MHz (500 MHz) NMR spectrometer was used to record the ^{1}H-NMR spectra with tetramethyl silane (TMS) as the internal standard. As a solvent, DMSO-d_6 was utilized. The coupling constant (*J*) was represented in hertz (Hz), and values of chemical shifts (δ) were calculated as parts per million (ppm).

2.2. General procedure for the synthesis of benzohydrazide (7)

A mixture of methyl benzoate (**6**) (60 mmol, 8.7 mL) and hydrazine hydrate (600 mmol, 34 mL) in ethanol (40 mL) was refluxed for 5 hours. After completion of the reaction, the mixture was cooled to room temperature and the precipitated solid was filtered and crystallized in ethanol. Yield, 53%; yellow powder; mp: 110-113 °C; IR (KBr): υ (cm^{-1}) 1400–1600 (Aromatic), 1660 (C=O), 3300 (N-H).

2.3. General procedure for the synthesis of N-(2-(substituted phenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl) benzamide (10a-m)

A mixture of benzohydrazide (**7**) (7.35 mmol, 1.01 g), isatoic anhydride (**8**) (7.35 mmol, 1.2 g) and $KAl(SO_4)_2.12H_2O$ (alum) (7.35 mmol, 3.3 g) were dissolved in ethanol and refluxed for 6 hours. Then substituted benzaldehydes (7.35 mmol) (**9**) was added to the solution. After 18 hours the solid was filtered, washed with hexane and recrystallized in ethanol to give compounds (**10a-m**) (yield, 19-36%).

2.3.1. N-(4-oxo-2-phenyl-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10a)

Yield: 21%; white powder; mp: 164-165 °C; IR (KBr): υ (cm^{-1}) 1410-1600 (Aromatic), 1650 (C=O), 3400 (N-H); MS m/z (%): 343.39 (M^+, 70), 341.2 (100), 313.2 (40), 223.2 (100), 180.1 (22), 152.1 (11), 105.1 (89), 77.1 (55), 51.1 (11); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.18 (s, 1H, H_2), 6.79-6.84 (m, 2H, H_6 & H_8), 7.42-7.73 (m, 7H, NH, H_7, pheny $H_{3''}$, $H_{4''}$, $H_{5''}$ & benzamide $H_{3'}$, $H_{5'}$), 7.50-7.58 (m, 5H, phenyl $H_{2''}$, $H_{6''}$ & benzamide $H_{2'}$, $H_{6'}$, $H_{4'}$) , 7.71 -7.73 (d, 1H, *J* = 7.0 Hz, H_5), 10.43 (s, 1H, benzamide NH); Anal. Calcd. For $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.65; H, 5.20; N, 12.01.

2.3.2. N-(2-(2-fluorophenyl)-4-oxo-1,4-dihydro-quinazolin-3(2H)-yl)benzamide (10b)

Yield: 19%; white powder; mp: 187-188 °C; IR (KBr): υ (cm^{-1}) 1490-1600 (Aromatic), 1650 (C=O), 3300 (N-H); MS m/z (%): 361.38 (M^+, 38), 359.2 (100), 341.2 (13), 327 (6), 313.3 (38), 299.3 (19), 241.2 (100), 198.1 (19), 119.1 (31),77.1 (38); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.33 (s, 1H, H_2), 6.76-6.80 (m, 2H, H_6 & H_8), 7.19-7.22 (t, 1H, 2-fluorophenyl $H_{3''}$), 7.25-7.28 (t, 1H, 2-fluorophenyl $H_{5''}$), 7.35-7.39 (m, 2H, NH & H_7), 7.42-7.46 (m, 3H, 2-fluorophenyl $H_{4''}$ & benzamide $H_{3'}$, $H_{5'}$), 7.53-

7.55 (m, 1H, benzamide $H_{4'}$), 7.64-7.66 (d, 2H, *J* = 7.3 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.74 (m, 2H, H_5 & 2-fluorophenyl $H_{6''}$), 10.55 (s, 1H, benzamide NH); Anal. Calcd. For $C_{21}H_{16}FN_3O_2$: C, 69.80; H, 4.46; N, 11.63. Found: C, 69.60; H, 4.23; N, 11.53.

2.3.3. N-(2-(3-fluorophenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10c)

Yield: 15%; white powder; mp: 124-125 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1650 (C=O), 3400, 3500 (N-H); MS m/z (%): 361.38 (M^+,73), 359.2 (100), 349.2 (52), 335.2 (5), 241 (100), 198.1 (15), 105.1 (85), 51.1 (15); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.20 (s, 1H, H_2), 6.79-6.84 (m, 2H, H_6 & H_8), 7.22-7.25 (d, 1H, *J* = 8.5 Hz, 3-fluorophenyl $H_{2''}$), 7.35-7.45 (m, 7H, H_7, NH, benzamide $H_{3'}$, $H_{5'}$ & 3-fluorophenyl $H_{4''}$, $H_{5''}$, $H_{6''}$), 7.53-7.57 (t, 1H, benzamide $H_{4'}$), 7.62-7.63 (d, 2H, *J* = 7.3 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.73 (d, 1H, *J* = 7.1 Hz, H_5), 10.51 (s, 1H, benzamide NH); Anal. Calcd. For $C_{21}H_{16}FN_3O_2$: C, 69.80; H, 4.46; N, 11.63. Found: C, 69.99; H, 4.61; N, 11.45.

2.3.4. N-(2-(4-fluorophenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10d)

Yield: 20%; white powder; mp: 248-249 °C; IR (KBr): υ (cm^{-1}) 1430-1600 (Aromatic), 1650 (C=O), 3300 (N-H); MS m/z (%): 361.38 (M^+,40), 359.2 (100), 349.2 (26), 344.2 (20), 328.1 (13), 241.2 (100), 198.1 (30), 170.1 (15), 105.1 (46), 77.1 (50); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.27 (s, 1H, H_2), 6.87-6.92 (m, 2H, H_6 & H_8), 7.29-7.32 (t, 2H, 4-fluorophenyl $H_{3''}$ & $H_{5''}$), 7.42-7.45 (m, 2H, NH & H_7), 7.48-7.51 (t, 2H, *J* = 7.5 Hz, benzamide $H_{3'}$, $H_{5'}$), 7.59-7.60 (t, 1H, benzamide $H_{4'}$), 7.66-7.68 (m, 4H, 4-fluorophenyl $H_{2''}$, $H_{6''}$ & benzamide $H_{2'}$, $H_{6'}$), 7.79-7.81 (d, 1H, *J* = 7.8 Hz, H_5), 10.51 (s, 1H, benzamide NH); Anal. Calcd. For $C_{21}H_{16}FN_3O_2$: C, 69.80; H, 4.46; N, 11.63. Found: C, 70.01; H, 4.61; N, 11.66.

2.3.5. N-(2-(2-chlorophenyl)-4-oxo-1, 4-dihydroquinazolin-3(2H)-yl) benzamide (10e)

Yield: 35%; white powder; mp: 224-225 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1640 (C=O), 3200, 3300 (N-H); MS m/z (%): 377.83 (M^+, 26), 368.5 (100), 353.4 (22), 340 (48), 327.3 (20), 313 (80), 303.1 (13), 257.1 (100), 180.1 (13), 105.1 (45), 77.1 (54); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ ppm 6.69 (s, 1H, H_2), 6.79-6.83 (m, 2H, H_6 & H_8), 7.35-7.45 (m, 7H, NH, H_7, benzamide $H_{3'}$, $H_{5'}$ & 2-chlorophenyl $H_{4''}$, $H_{5''}$, $H_{6''}$), 7.52-7.55 (t, 1H, J = 7.2 Hz, benzamide $H_{4'}$), 7.66-7.68 (d, 2H, *J* = 7.6 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.75-7.76 (d, 1H, *J* = 7.7 Hz, 2chlorophenyl $H_{3''}$), 7.86-7.88 (d, 1H, *J* = 7.3 Hz, H_5), 10.53 (s, 1H, benzamide NH). Anal. Calcd. For $C_{21}H_{16}ClN_3O_2$: C, 66.76; H, 4.27; N, 11.12. Found: C, 66.56; H, 4.45; N, 11.22.

2.3.6. N-(2-(3-chlorophenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10f)

Yield: 26%; white powder; mp: 212-213 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1650 (C=O), 3200 (N-H); MS m/z (%): 377.83 (M^+, 70), 375.2 (100), 368.4 (20), 339.3 (10), 313.3 (40), 257.1 (100), 214(10), 152 (20), 105 (80), 77.1 (65); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.18 (s, 1H, H_2), 6.81-6.83 (m, 2H, H_6 & H_8), 7.35-7.48 (m, 7H, H_7, NH, benzamide $H_{3'}$,

$H_{5'}$ & 3-chlorophenyl $H_{4''}$, $H_{5''}$, $H_{6''}$), 7.53-7.54 (t, 1H, *J* = 7.6 Hz, benzamide $H_{4'}$), 7.62-7.64 (m, 3H, 3-chlorophenyl $H_{2''}$, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.73 (d, 1H, *J* = 7.7 Hz, H_5), 10.53 (s, 1H, benzamide NH); Anal. Calcd. For $C_{21}H_{16}ClN_3O_2$: C, 66.76; H, 4.27; N, 11.12. Found: C, 66.52; H, 4.41; N, 11.38.

2.3.7. N-(2-(4-chlorophenyl)-4-oxo-1,4-dihydro-quinazolin-3(2H)-yl)benzamide (10g)

Yield: 37%; white powder; mp: 236- 237 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1650 (C=O), 3300 (N-H); MS m/z (%): 377.83 (M^+, 13), 350.2 (100), 335.1 (47), 323.3 (28), 309.4 (30), 165.0 (100), 138 (30), 111.0 (55), 75 (50); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 6.18 (s, 1H, H_2), 6.79-6.83 (m, 2H, H_6 & H_8), 7.34-7.47 (m, 6H, NH, H_7, benzamide $H_{3'}$, $H_{5'}$ & 4-chlorophenyl $H_{2''}$, $H_{6''}$), 7.52-7.57 (m, 3H, benzamide $H_{4'}$, 4chlorophenyl $H_{3''}$ & $H_{5''}$), 7.62-7.63 (d, 2H, *J* = 7.7 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.73 (d, 1H, *J* = 7.6 Hz, H_5), 10.46 (s, 1H, NH); Anal. Calcd. For $C_{21}H_{16}ClN_3O_2$: C, 66.76; H, 4.27; N, 11.12. Found: C, 66.52; H, 4.12; N, 11.33.

2.3.8. N-(4-oxo-2-(o-tolyl)-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10h)

Yield: 19%; white powder; mp: 226-228 °C; IR (KBr): υ (cm^{-1}) 1416-1600 (Aromatic), 1640 (C=O), 3328 (N-H); MS m/z (%): 357.41 (M^+, 18), 356.2 (21), 341.2 (100), 321.2 (64), 306.1 (36), 237.2 (100), 194.1 (13), 165.1 (6), 119.1 (13), 77.1 (35); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 2.30 (s, 3H, CH_3), 6.50 (s, 1H, H_2), 6.80-6.83 (m, 2H, H_6 & H_8), 7.19-7.42 (m, 7H, NH, H_7, benzamide $H_{3'}$, $H_{5'}$ & *o*-toly $H_{3''}$, $H_{4''}$, $H_{5''}$), 7.50-7.62 (m, 3H, benzamide $H_{2'}$, $H_{6'}$ & $H_{4'}$), 7.73-7.75 (d, 1H, *J* = 7.8 Hz, *o*-toly $H_{6''}$), 7.94-7.95 (d, 1H, *J* = 7.0 Hz, H_5), 10.43 (s, 1H, NH). Anal. Calcd. For $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 73.85; H, 5.26; N, 12.02.

2.3.9. N-(4-oxo-2-(m-tolyl)-1,4-dihydro-quinazolin- 3(2H)-yl)benzamide (10i)

Yield: 15%; white powder; mp: 176-178 °C; IR (KBr): υ (cm^{-1}) 1450-1600 (Aromatic), 1650 (C=O), 3400, 3500 (N-H); MS m/z (%): 357.41 (M^+,33), 356.2 (83), 350.2 (100), 335.2 (50), 307.2 (17), 237.2 (73), 105.1 (100), 51.1 (13); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 2.30 (s, 3H, CH_3), 6.14 (s, 1H, H_2), 6.78-6.84 (m, 2H, H_6 & H_8), 7.20-7.21 (d, 1H, *J* = 7.4 Hz, *m*-tolyl $H_{4''}$), 7.25-7.43 (m, 7H, NH, H_7, benzamide $H_{3'}$, $H_{5'}$ & *m*-tolyl $H_{2''}$, $H_{5''}$, $H_{6''}$), 7.52-7.55 (t, 1H, *J* = 7.5 Hz, benzamide $H_{4'}$), 7.59 -7.61 (d, 2H, *J* = 7.6 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.72-7.73(d, 1H, *J* = 7.7 Hz, H_5), 10.40 (s, 1H, benzamide NH); Anal. Calcd. For $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 74.13; H, 5.66; N, 11.56.

2.3.10. N-(4-oxo-2-(p-tolyl)-1,4-dihydro-quinazolin-3(2H)-yl)benzamide (10j)

Yield: 25%; white powder; mp: 222-223 °C; IR (KBr) υ (cm^{-1}) 1480-1600 (Aromatic), 1650 (C=O), 3305 (N-H); MS m/z (%): 357.41 (80), 355.2 (100), 341.3 (38), 327.3 (10), 313.3 (47), 237 (100), 194.1 (15),105.1 (43), 51.1 (7); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 2.30 (s, 3H, CH_3), 6.14 (s, 1H, H_2), 6.77-6.83 (m, 2H, H_6 & H_8), 7.19-7.20 (d, 2H, *J* = 7.9 Hz, *p*-tolyl $H_{3''}$ & $H_{5''}$), 7.29 (s, 1H, NH), 7.33-7.34 (t, 1H,

J = 7.5 Hz, H_7), 7.40-7.43 (m, 4H, *p*-tolyl $H_{2''}$, $H_{6''}$ & benzamide $H_{3'}$, $H_{5'}$), 7.51-7.54 (t, 1H, benzamide $H_{4'}$), 7.60-7.62 (d, 2H, *J* = 7.2 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71 -7.72 (d, 1H, *J* = 7.8 Hz, H_5), 10.30 (s, 1H, benzamide NH); Anal. Calcd. For $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 74.02; H, 5.26; N, 11.91.

2.3.11. N-(2-(2-methoxyphenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10k)

Yield: 36%; white powder; mp: 224-225 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1640 (C=O), 3340 (N-H); MS m/z (%): 373.15 (M^+, 37), 368.4 (100), 353.3 (20), 339.3 (37), 327.3 (25), 313.3 (100), 253 (100), 132 (21), 105 (47), 77.1 (52); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 3.65 (s, 3H, OCH_3), 6.51 (s, 1H, H_2), 6.73-6.76 (d, 1H, *J* = 7.5 Hz, H_8), 6.77-6.79 (t, 1H, *J* = 8.1 Hz, H_6), 6.97-7.01 (m, 2H, 2-methoxyphenyl $H_{3''}$ & $H_{5''}$), 7.12 (s, 1H, NH), 7.29-7.35 (m, 2H, H_7 & 2-methoxyphenyl $H_{4''}$), 7.42-7.45 (t, 2H, *J* = 7.6 Hz, benzamide $H_{3'}$ & $H_{5'}$), 7.52-7.55 (t, 1H, *J* = 7.3 Hz, benzamide $H_{4'}$), 7.60-7.61 (d, 1H, *J* = 7.6 Hz, 2-methoxyphenyl $H_{6''}$), 7.66-7.68 (d, 2H, *J* = 7.7 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.69-7.71 (d, 1H, *J* = 7.7 Hz, H_5), 10.45 (s, 1H, benzamide NH); Anal. Calcd. for $C_{22}H_{19}N_3O_3$:C, 70.76; H, 5.13; N, 11.25. Found: C, 70.55; H, 5.22; N, 11.43.

2.3.12. N-(2-(3-methoxyphenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10l)

Yield: 24%; white powder; mp: 161-163 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1650 (C=O), 3400, 3500 (N-H); MS m/z (%): 373.15 (M^+, 54), 371.2 (100), 357.2 (63), 120.1 (100), 77 (82); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 3.74 (s, 3H, OCH_3), 6.14 (s, 1H, H_2), 6.78-6.83 (m, 2H, H_6 & H_8), 6.94-6.96 (d, 1H, *J* = 6.0 Hz, 3-methoxyphenyl $H_{4''}$), 7.06-7.08 (d, 1H, *J* = 7.5 Hz, 3 methoxyphenyl $H_{6''}$), 7.14 (s, 1H, NH), 7.27-7.30 (t, 1H, *J* = 7.9 Hz, 3-methoxyphenyl $H_{5''}$), 7.34 7.37 (m, 2H, H_7 & 3-methoxyphenyl $H_{2''}$), 7.41-7.44 (t, 2H, *J* = 7.7 Hz, benzamide $H_{3'}$ & $H_{5'}$), 7.51-7.54 (t, 1H, *J* = 7.4 Hz, benzamide $H_{4'}$), 7.60-7.62 (d, 2H, *J* = 7.4 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.72 (d, 1H, *J* = 7.7 Hz, H_5), 10.42 (s, 1H, benzamide NH); Anal. Calcd. For $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: 70.52; H, 5.31; N, 11.40.

2.3.13. N-(2-(4-methoxyphenyl)-4-oxo-1,4-dihydroquinazolin-3(2H)-yl)benzamide (10m)

Yield: 42%; white powder; mp: 226-227 °C; IR (KBr): υ (cm^{-1}) 1400-1600 (Aromatic), 1650 (C=O), 3300, 3400 (N-H); MS m/z (%): 373.15 (M^+, 88), 371.2 (100), 354.3 (8), 339.3 (18), 327 (8), 313 (40), 299 (20), 253.2 (100), 167.1 (15), 130 (14), 105.1 (42), 77.1 (42); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ ppm 3.74 (s, 3H, OCH_3), 6.13 (s, 1H, H_2), 6.78-6.81 (t, 1H, *J* = 7.2 Hz, H_6), 6.82-6.84 (d, 1H, *J* = 8.1 Hz, H_8) , 6.93-6.95 (d, 2H, *J* = 8.7 Hz, 4-methoxyphenyl $H_{3''}$ & $H_{5''}$) , 7.27 (s, 1H, NH), 7.33-7.36 (t, 1H, *J* = 8.4 Hz, H_7) , 7.40-7.43 (t, 2H, *J* = 7.5 Hz, benzamide $H_{3'}$ & $H_{5'}$) , 7.46-7.47 (d, 2H, *J* = 8.6 Hz, 4-methoxyphenyl $H_{2''}$ & $H_{6''}$) , 7.51-7.54 (t, 1H, *J* = 7.4 Hz, benzamide $H_{4'}$) , 7.60-7.61 (d, 2H, *J* = 7.2 Hz, benzamide $H_{2'}$ & $H_{6'}$), 7.71-7.72 (d, 1H, *J* = 7.8 Hz, H_5), 10.37 (s, 1H, benzamide NH); Anal. Calcd. For $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.52; H, 5.26; N, 11.39.

2.4. In vitro anti-HIV-1 and cytotoxicity assay method

We utilized a single-cycle replication assay to evaluate our compounds, as described previously [16-18]. In this experiment, HIV-1 NL4-3 single-cycle replicating virions (200 ng p24) were injected into Hela cells along with other compounds at varying concentrations. The enzyme-linked immunosorbent assay (ELISA- Biomerieux, France) was used to determine the inhibition rate (percentage) of p24 expression after 72 hours. The inhibition rate of p24 was estimated as a percentage of the total inhibition of p24 expression in the treated culture. The XTT (sodium 3′-[1-(phenylaminocarbonyl)- 3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid) assay was selected for this study to evaluate the cellular toxicity. After determining the p24 load in the HIV-1 replication test plates, the cytotoxicity of compounds as cell viability was computed.

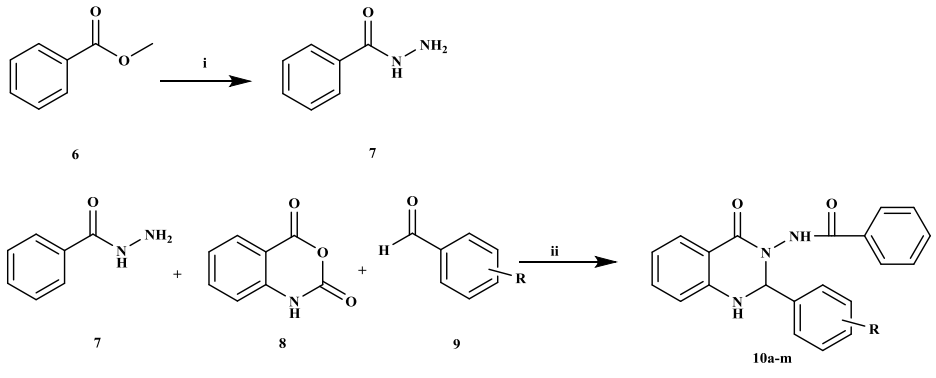
2.5. Molecular docking study

Autodock Vina software was used to perform molecular modeling study [19]. 3S3M was used for analysis of binding mode of compounds in IN active site. Autodock tools 1.5.6 from MGL Tools package was utilized to prepare the protein and ligands' structures [20]. At first, the co-crystallized dolutegravir and water molecules were removed from the protein structure, then, Kollman charges were calculated, nonpolar hydrogens were removed and AutoDock4 atom type was assigned to the protein structure. HyperChem 8.0 was used to create and optimize the ligand molecule [21]. The Grid box with 20×20×20 dimensions were defined around the crystallographic ligand, raltegravir and regarded as the active site. Autodock Vina was used to dock the molecule in the active site and produce the bioactive conformations.

3. Results and Discussion

3.1. Chemical synthesis

Quinazolinone derivatives were achieved according to the procedure indicated in Scheme 1. At first methyl benzoate (**6**) was refluxed with hydrazine hydrate in ethanol to obtain the benzohydrazide (**7**). Finally, the obtained benzohydrazide, isatoic anhydride (**8**) and substituted benzaldehydes (**9**) were condensed in the presence of $KAl(SO_4)_2.12H_2O$ (alum) in ethanol to afford final compounds (**10a-m**). The structure of the synthesized compounds was confirmed by IR, ^{1}H-NMR and MS.

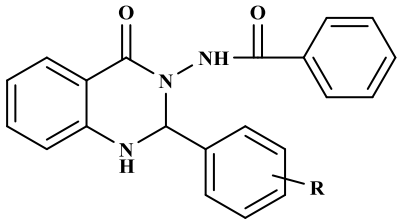


Scheme 1. Reagents and conditions: (**i**) hydrazine hydrate, ethanol, reflux, 5h; (**ii**) alum, ethanol, reflux, 6h

3.2. Biological evaluation

In vitro evaluation of quinazolinone derivatives was performed against Hela cells cultures infected by single-cycle replicable HIV-1. The synthesized compounds were tested in order to calculate their inhibitory activity against the replication of HIV-1 through determining of p24 expression inhibition rate quantitatively. AZT, nucleoside reverse transcriptase inhibitor, was tested as a positive control. The synthesized compounds were also assayed for their cytotoxicity and the results were reported as cell viability percentage. All results are listed in Table 1.

Table 1. Anti-HIV activity and cell viability of compounds **10a-m** at 100 µM concentration



compound	R	% inhibition rate of p24 expression	%Cell viability
10a	H	<5	-
10b	2-F	ND*	-
10c	3-F	ND	-
10d	4-F	ND	-
10e	2-Cl	15	75
10f	3-Cl	38	80
10g	4-Cl	18	72
10h	2-Me	NA*	-
10i	3-Me	NA	-
10j	4-Me	NA	-
10k	2-OMe	<10	-
10l	3-OMe	24	52
10m	4-OMe	30	66
AZT	-	100	100

*ND: Not determined, NA: not active

As shown in Table 1, some of the tested compounds indicated the inhibitory effects at 100 µM concentration with a percentage ranging from15-38%. However, the measured activities were lower than that of AZT (100%). The cell viability percentage was calculated for active compounds which ranged from 52-80%. Thus, it can be concluded that the anti-HIV activity of the compounds was not a result of their cytotoxic effects. The results revealed that the anti-HIV activity of compounds was influenced by the existence of a substituent on the phenyl ring at C-2 position of the quinazolinone scaffold. Active compounds possessed an OMe (**10k-m**) or Cl (**10e-g**) group on the phenyl ring. The significant difference in potency was not observed between various positions of OMe group on the phenyl ring, while in compounds with Cl substituent, the better activity was found at meta position.

Overall, the best compounds in this series in terms of potency and cell viability was compound **10f** with an inhibition rate of 38% and cell viability of 80% at 100 µM concentration. Further structural modifications are needed to improve the anti-HIV activity profile of the synthesized quinazolinone derivatives.

3.3. Molecular modeling studies

To explore the binding mode of synthesized compounds at the active site of IN, the molecular modeling study was performed using AUTODOCK 4.2 program. The crystallographic structure of the prototype foamy virus (PFV) IN complexed with DNA strain, dolutegravir, two metal ions (Mg^{+2})

(PDB: 3S3M) was selected [22]. The most active compound, **10f** was selected to dock into the integrase enzyme active site. The results of docking studies were indicated in Figure 3.

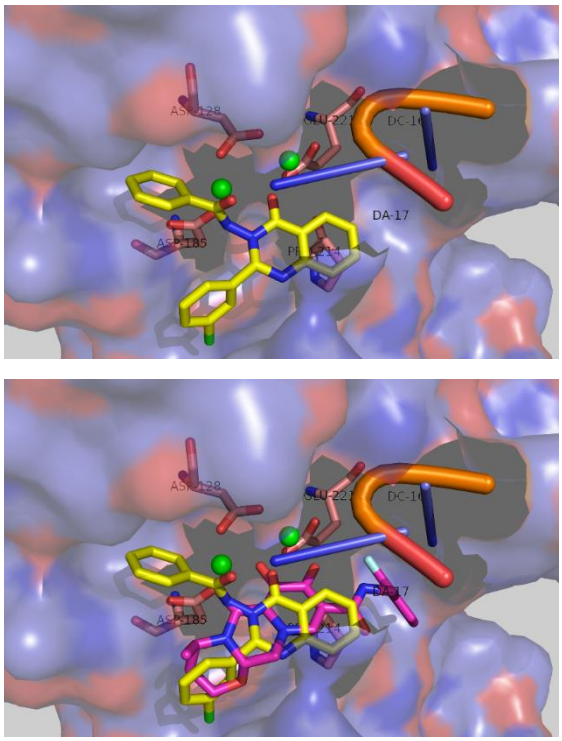


Figure 3. Top: Binding mode of compound **10f** (yellow) within the PFV IN active site. Down: Superimpose of compound **10f** on dolutegravir within the PFV IN active site.

According to the results, this compound imitated the binding mode of dolutegravir at IN/DNA interface. Figure 3 displays that the oxygen atoms of carbonyl groups belonging to benzamide side chain and quinazolinone core were located in close distance (less than 2 Å) of the Mg^{+2} ions coordinated by Asp128, Asp185 and Glu221 residues. Hydrophobic interaction of the central quinazolinone ring with Pro214 (less than 4 Å) is one of the main interactions of compound **10f** with IN active site. Overlay of the compound **10f** on the crystallographic ligand revealed that active compound **10f** embedded in the IN catalytic site as well as dolutegravir (Figure 3). Docking results suggested that the inhibitory property of synthesized compounds may be through IN inhibition.

4. Conclusion

Here in, the novel quinazolinone derivatives containing benzamide moiety were designed and synthesized. To investigate the binding mode of the designed compounds in the HIV integrase active site, molecular modeling study was carried out. According to the anti-HIV evaluation results, the synthesized compounds displayed no significant cytotoxicity effects and also compound **10f** was found as the most active with the inhibition rate of 38%.

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