Design, Synthesis and Docking Studies of New 4-hydroxyquinoline-3-carbohydrazide Derivatives as Anti-HIV-1 Agents

Authors
Z. Hajimahdi[1], R. Zabihollahi[2], M. R. Aghasadeghi[2], A. Zarghi[1]

Affiliations
[1] Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Hepatitis and AIDS department, Pasteur institute of Iran, Tehran, Iran

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Abstract

A new class of 4-hydroxyquinoline-3-carbohydrazide derivatives was prepared and evaluated for its anti-HIV activity. The primary bioassay results indicated that most of tested compounds possess moderate inhibitory properties against HIV-1 virus (NL4-3) in Hela cells cultures. Our results also indicated that compounds **6d** and **7e** were the most potent anti-HIV agents among the synthesized compounds with inhibition rate of 32 and 28% at concentration of 100µM, respectively. A docking study using the later crystallographic data available for PFV integrase including its complexes with Mg^{2+} and raltegravir, showed that the designed compounds bind into the active site of integrase such that carboxylic and hydroxyl groups of 4-hydroxyquinoline-3-carbohydrazide chelate the Mg^{2+} ion. Interestingly, all of the synthesized compounds were found to present no significant cytotoxicity at concentration of 100µM. Therefore, these compounds can provide a very good basis for the development of new anti-HIV-1 agents.

Introduction

The causative agent of acquired immune deficiency syndrome (AIDS) is the human immunodeficiency virus type 1 (HIV-1). During the past 3 decades, the combination of antiretroviral drugs in HAART (highly active antiretroviral therapy) regimens has transformed the management of HIV infection from a fatal disease to a manageable chronic condition [1,2]. However, resistance to marketed anti-HIV drugs is increasing at an alarming rate. Thus, there is a need to develop new agents which work by different mechanisms. In this regard, there has been considerable interest in HIV integrase (IN) function as an attractive alternative target that may lead to the next generation of anti-HIV drug [3,4]. HIV-1 integrase is one of the 3 most important enzymes required for viral replication. It catalyzes the insertion and the integration of the proviral DNA into the genome of the host cell in 2 steps: 3′-processing (3′-P), the endonucleolytic sequence-specific hydrolysis of 3′-ends of the viral cDNA, and strand transfer (ST), the ligation of the viral 3′-OH cDNA ends to the phosphate backbone of the host DNA acceptor. This enzymatic process is dependent on an active site containing dual Mg^{2+} metal ions held in place with a highly conserved triad of carboxylate amino acid residues (asp64/asp116/glu152) commonly referred to as a DD(35)E triad [5–7]. Thus the chelation of the critical Mg^{2+} cofactors can cause the functional impairment of IN. In fact, all of the small molecule HIV-1 IN inhibitors commonly contain a structural motif that coordinates the 2 divalent magnesium ions in the enzyme's active site [8]. Typically, the most developed and promising β-diketoacid, naphthyridine carboxamide, pyrimidinone and quinolone carboxylic acid classes of IN inhibitors belong to this category [9]. Among these chelation inhibitors, raltegravir has been approved by the FDA, GS-9137 (Elvitegravir) and S/GSK-1349572 (**Fig. 1**) have reached clinical development [10,11]. Moreover, emergence of resistance against raltegravir due to viral mutations demands exploration of novel scaffolds for the treatment of HIV infection [12]. In this research, aimed at the discovery of new compounds as anti-HIV-1 agents, we selected the HIV-1 integrase inhibitors as a lead to design new analogues. In order to target the catalytic site of HIV-1 integrase, we designed new IN inhibitors by merging the pharmacophores of the salicylhydrazide and 4-quinolone-3-carboxylic acid to generate novel chemical scaffolds. The salicylhydrazide and polyhydroxyl aromatics (as exemplified by the structures (**1** & **2**) in **Fig. 1**) were previously reported as potent IN inhibitors, but the inherently high cytotoxicity in these compounds limited their therapeutic application as

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Correspondence
A. Zarghi
Department of Pharmaceutical Chemistry
School of Pharmacy
Shahid Beheshti University of Medical Sciences
P.O.Box: 14155-6153
Tehran
Iran
Tel.: +98/21/8820 0096
Fax: +98/21/8866 5341
zarghi@sbmu.ac.ir

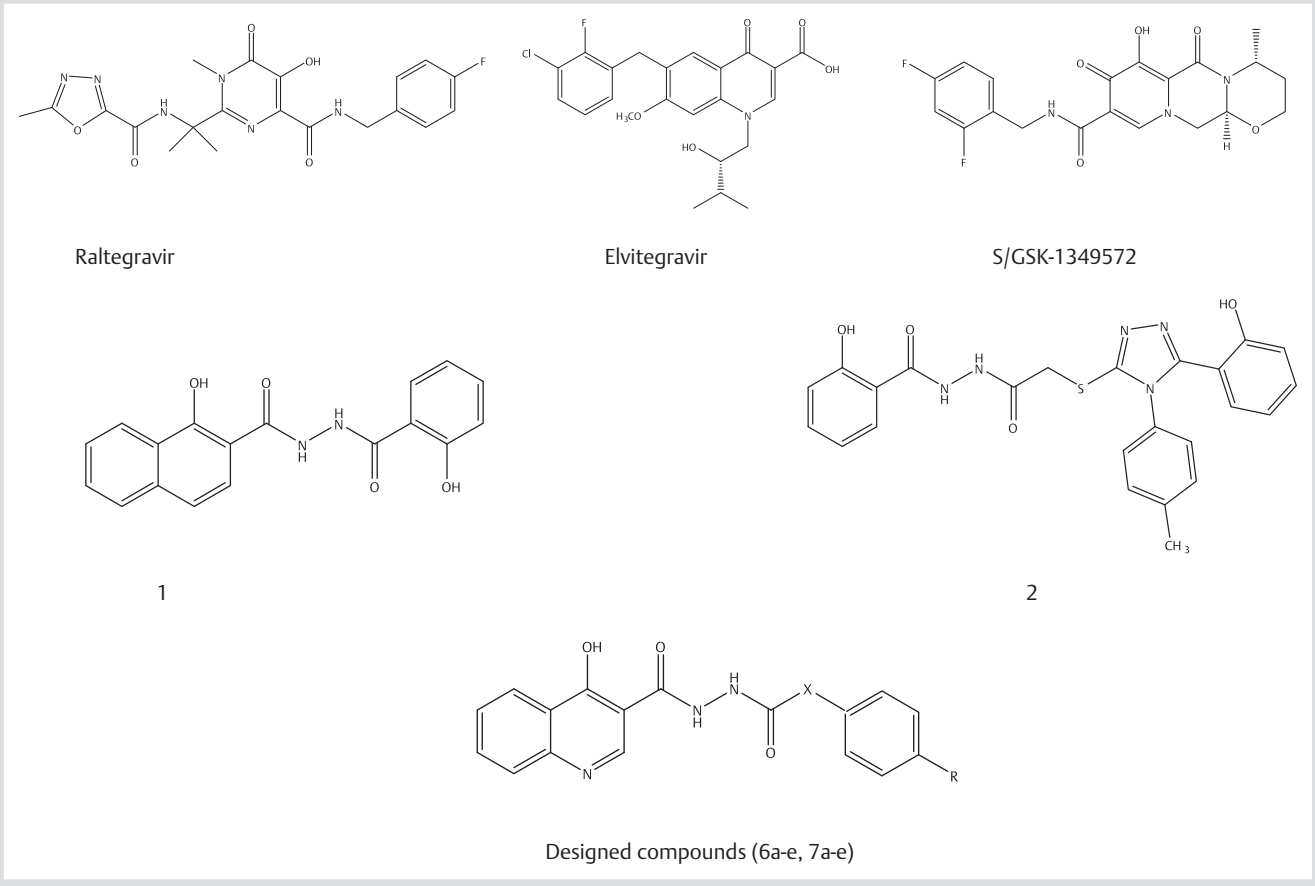


Fig. 1 HIV-1 integrase inhibitors (Raltegravir, Elvitegravir, S/GSK-1349572), lead compounds (**1**, **2**) and designed scaffold.

antiretroviral agents [13–16]. On the other hand, 4-quinolone-3-carboxylic acid derivatives are attractive in anti-HIV-1 drug discovery due to their extremely versatile nature, well-known biochemical properties and having no significant cytotoxic effects [17, 18]. Based on these facts supported by literature and as a part of our research program aimed at discovering new anti-HIV-1 agents, we designed a novel noncytotoxic class of 4-hydroxyquinoline-3-carbohydrazide derivatives with various substituted aromatic moiety by integration of fragments of salicylhydrazide and 4-quinolone-3-carboxylic acid. Actually, according to HIV-1 integrase inhibitors structure activity relationship, the adjacent carboxylic and hydroxyl groups on 4-hydroxyquinoline-3-carbohydrazide could serve as the metal binding pharmacophore. Furthermore, the aryl group might serve as the pharmacophore to interact with the hydrophobic binding surface of the IN. We also performed docking studies to predict the interaction of new synthesized compounds into the active site of integrase and their probable mechanism of action.

Material and Methods

Materials

All chemicals, reagents and solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points were determined with a Thomas–Hoover capillary apparatus. A Synthos 3000 microwave oven (Anton Paar, Austria) was used for synthesis of compounds. Infrared spectra were acquired using a Perkin Elmer Model 1420 spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, USA) was used to acquire ^{1}HNMR spectra with TMS as internal standard. Chloroform-D and DMSO-D_6 were used as solvents. The mass spectral measurements were performed on an 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface. Microanalyses, determined for C and H, were within ±0.4% of theoretical values.

Preparation of diethyl 2-((phenylamino)methylene) malonate (3)

A mixture of aniline (0.9 ml, 10 mmol) and diethyl ethoxymethylenemalonate (EMME) (2 ml, 10 mmol) was heated at 120 °C for 1 h. The reaction mixture was cooled to room temperature. The solid thus formed was taken in n-hexane and stirred for 15 min and filtered to afford compound **3**. Yield, 62%; white powder; mp 44–45 °C [19]; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1660, 1690 (C=O), 3300 (N-H); LC-MS (ESI) m/z: 286.12 (M+23, 100).

Preparation of ethyl 4-hydroxyquinoline-3-carboxylate (4)

A diphenyl ether solution of **3** containing catalytic 2-chlorobenzoic acid was heated by microwave irradiation (250 °C, 180 W) for 2 h. The reaction mixture was cooled to room temperature and added n-hexane (50 ml) and stirred for 15 min, the precipitated solid was filtered and dried to get compound **4**. The final product was crystallized in ethanol. Yield, 75%; cream powder; mp 261–262 °C [19]; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1695 (C=O), 3150 (N-H); LC-MS (ESI) *m/z*: 218.08 (M+1, 100).

Preparation of 4-hydroxyquinoline-3-carbohydrazide (5)

A mixture of **4** (2 g, 4.5 mmol) and hydrazine hydrate (6 ml, 45 mmol) in DMF (5 ml) was stirred at room temperature for 12 h. Completion

of the reaction was monitored by TLC. After reaction completion, water (50 ml) was added and the solid product obtained was filtered, washed with water to give **5**. The final product was crystallized in ethanol. Yield, 55%; white powder; mp 290–292 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1730 (C=O), 2500–3400 (O-H), 3310 (N-H), 3370 (NH_2); LC-MS (ESI) m/z: 205.07 (M+1, 100).

General procedure for preparation of 4-hydroxy-N′-(4-substituted-benzoyl) quinoline -3-carbohydrazide (6a–e)

A mixture of **5** (0.3 g, 1.5 mmol), 4-substituted benzoyl chlorides (0.2 ml, 1.5 mmol) and a catalytic amount of Na_2CO_3 (0.05 g, 0.5 mmol) was dissolved in dry DMF (10 ml) and stirred at room temperature for 16 h. The reaction mixture was slowly poured over crushed ice and kept for 2 h. The solid thus separated out was filtered, washed with water and recrystallized from ethanol to give compounds (**6a–e**) (Yield, 30–40%).

N′-Benzoyl-4-hydroxyquinoline-3-carbohydrazide (6a)

Yield, 40%; white powder; mp 288–290 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1630–1680 (C=O), 2800–3200 (O-H), 3210 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 7.46–7.51 (m, 3H, benzoyl H_3-H_5), 7.54–7.57 (t, 1H, quinoline H_6, *J*=7.26 Hz), 7.71 (d, 1H, quinoline H_8, *J*=8.07 Hz), 7.76–7.79 (m, 1H, quinoline H_7), 7.88 (d, 2H, benzoyl H_2 & H_6, *J*=7.16 Hz), 8.27 (d, 1H, quinoline H_5, *J*=8.09 Hz), 8.77 (s, 1H, quinoline H_2), 10.75 (d, 1H, N-H, *J*=2.7 Hz), 11.70 (d, 1H, N-H, *J*=2.7 Hz), 12.83 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 307.80 (M+1, 100); Anal. Calcd. for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.26; N, 13.67. Found: C, 66.64; H, 4.49; N, 13.60.

N′-(4-Fluorobenzoyl)-4-hydroxyquinoline-3-carbohydrazide (6b)

Yield, 30%; white powder; mp 280 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1640–1675 (C=O), 2900–3400 (O-H), 3400 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 7.30–7.33 (t, 2H, 4-F-benzoyl H_3 & H_5, *J*=8.84 Hz), 7.47–7.50 (t, quinoline H_6, *J*=7.88 Hz), 7.71 (d, 1H, quinoline H_8, *J*=8.14 Hz), 7.76–7.79 (t, 1H, quinoline H_7, *J*=7.04 Hz), 7.94–7.97 (m, 2H, 4-F-benzoyl H_2 & H_6), 8.28 (d, 1H, quinoline H_5, *J*=7.47 Hz), 8.76 (s, 1H, quinoline H_2), 10.80 (s, 1H, N-H), 11.72 (s, 1H, N-H), 12.83 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 325.81 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}FN_3O_3$: C, 62.77; H, 3.72; N, 12.92. Found: C, 62.54; H, 3.56; N, 13.01.

N′-(4-Chlorobenzoyl)-4-hydroxyquinoline-3-carbohydrazide (6c)

Yield, 40%; white powder; mp 290 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1640–1670 (C=O), 2800–3300 (O-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 7.47–7.50 (t, quinoline H_6, *J*=7.76 Hz), 7.56 (d, 2H, 4-Cl-benzoyl H_3 & H_5, *J*=8.52 Hz), 7.71 (d, 1H, quinoline H_8, *J*=8.14 Hz), 7.75–7.79 (t, 1H, quinoline H_7, *J*=8.24 Hz), 7.90 (d, 2H, 4-Cl-benzoyl H_2 & H_6, *J*=8.60 Hz), 8.27 (d, 1H, quinoline H_5, *J*=7.84 Hz), 8.76 (d, 1H, quinoline H_2, *J*=6.7 Hz), 10.87 (d, 1H, N-H, *J*=2.7 Hz), 11.75 (d, 1H, N-H), 12.83 (d, 1H, enolic OH, *J*=6.45 Hz); LC-MS (ESI) m/z: 342.04 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30. Found: C, 59.99; H, 3.36; N, 12.57.

4-Hydroxy-N′-(4-methylbenzoyl)quinoline-3-carbohydrazide (6d)

Yield, 30%; white powder; mp 299–301 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1640–1690 (C=O), 2900–3300 (O-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 2.34 (s, 3H, CH_3), 7.28 (d, 2H, p-Toluoyl H_3 & H_5, *J*=8.01 Hz), 7.47–7.50 (t, quinoline H_6, *J*=7.17 Hz), 7.71 (d, 1H, quinoline H_8, *J*=8.07 Hz), 7.75–7.80 (m, 3H, quinoline H_7 and 4 p-Toluoyl H_2 & H_6), 8.27 (d, 1H, quinoline H_5, *J*=8.06 Hz), 8.76 (s, 1H, quinoline H_2), 10.66 (s, 1H, N-H), 11.68 (s, 1H, N-H), 12.82 (s, 1H, enolic OH); LC-MS (ESI) m/z: 321.92 (M+1, 100); Anal. Calcd. for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.44; H, 4.87; N, 13.17.

4-Hydroxy-N′-(4-methoxybenzoyl)quinoline-3-carbohydrazide (6e)

Yield, 30%; white powder; mp 260–262 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1630–1685 (C=O), 2800–3200 (O-H), 3370 & 3200 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 3.79 (s, 3H, OCH_3), 7.00 (d, 2H, 4-OCH_3-benzoyl H_3 & H_5, *J*=8.76 Hz), 7.47–7.50 (t, quinoline H_6, *J*=7.79 Hz), 7.71 (d, 1H, quinoline H_8, *J*=8.18 Hz), 7.75–7.79 (t, 1H, quinoline H_7, *J*=8.22 Hz), 7.87 (d, 2H, 4-OCH_3-benzoyl H_2 & H_6, *J*=8.73 Hz), 8.27 (d, 1H, quinoline H_5, *J*=7.90 Hz), 8.75 (d, 1H, quinoline H_2, *J*=6.61 Hz), 10.59 (s, 1H, N-H), 11.65 (s, 1H, N-H), 12.81 (d, 1H, enolic OH, *J*=6.3 Hz); LC-MS (ESI) m/z: 337.82 (M+1, 100); Anal. Calcd. for $C_{18}H_{15}N_3O_4$: C, 64.09; H, 4.48; N, 12.46. Found: C, 64.24; H, 4.31; N, 12.56.

General procedure for preparation of N-(4-substituted-phenyl)-2-(4-hydroxy quinoline-3-carbonyl) hydrazinecarboxamide (7a–e)

a mixture of **5** (0.3 g, 1.5 mmol) and 4-substituted phenyl isocyanates (0.2 ml, 1.5 mmol) was dissolved in acetonitrile (10 ml) and stirred at room temperature for 16 h. The reaction mixture was slowly poured over crushed ice and kept for 2 h. The solid thus separated out was filtered, washed with water and recrystallized from ethanol to give compounds (**7a–e**) (yield, 30–40%)

2-(4-Hydroxyquinoline-3-carbonyl)-N-phenylhydrazinecarboxamide (7a)

Yield, 35%; white powder; mp 250–252 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1645–1670 (C=O), 2800–3500 (O-H), 3290, 3340 & 3470 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 6.89–6.92 (t, 1H, N-phenyl H_4, *J*=7.36 Hz), 7.20–7.23 (t, 2H, N-phenyl H_3 & H_5, *J*=7.59 Hz), 7.41 (d, 2H, N-phenyl H_2 & H_6, *J*=7.71 Hz), 7.46–7.49 (t, quinoline H_6, *J*=7.14 Hz), 7.69 (d, 1H, quinoline H_8, *J*=8.06 Hz), 7.74–7.77 (t, 1H, quinoline H_7, *J*=6.98 Hz), 8.26 (d, 1H, quinoline H_5, *J*=7.02 Hz), 8.40 (s, 1H, N-H), 8.73 (s, 1H, quinoline H_2), 8.83 (s, 1H, N-H), 11.39 (s, 1H, N-H), 12.83 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 322.91 (M+1, 100); Anal. Calcd. for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38. Found: C, 63.44; H, 4.29; N, 17.52.

N-(4-Fluorophenyl)-2-(4-hydroxyquinoline-3-carbonyl) hydrazinecarboxamide (7b)

Yield, 35%; white powder; mp 309–310 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1620–1680 (C=O), 2900–3500 (O-H), 3290 & 3450 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 7.03–7.07 (t, 2H, N-4-fluorophenyl H_3 & H_5, *J*=8.86 Hz), 7.42–7.44 (m, 2H, N-4-fluorophenyl H_2 & H_6), 7.46–7.49 (t, quinoline H_6, *J*=7.60 Hz), 7.69 (d, 1H, quinoline H_8, *J*=8.22 Hz), 7.74–7.77 (t, 1H, quinoline H_7, *J*=7.59 Hz), 8.26 (d, 1H, quinoline H_5, *J*=8.00 Hz), 8.39 (s, 1H, N-H), 8.73 (s, 1H, quinoline H_2), 8.87 (s, 1H, N-H), 11.35 (s, 1H, N-H), 12.78 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 340.92 (M+1, 100); Anal. Calcd. for $C_{17}H_{13}FN_4O_3$: C, 60.00; H, 3.85; N, 16.46. Found: C, 60.14; H, 3.99; N, 16.67.

N-(4-Chlorophenyl)-2-(4-hydroxyquinoline-3-carbonyl) hydrazine carboxamide (7c)

Yield, 40%; white powder; mp 279–280 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1650–1690 (C=O), 2900–3500

(O-H), 3210 & 3380 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 7.25 (d, 2H, N-4-chlorophenyl H_3 & H_5, *J* = 8.85 Hz), 7.43–7.47 (m, 3H, quinoline H_6 & N-4-chlorophenyl H_2 & H_6), 7.68 (d, 1H, quinoline H_8, *J* = 8.06 Hz), 7.71–7.74 (t, 1H, quinoline H_7, *J* = 7.14 Hz), 8.25 (d, 1H, quinoline H_5, *J* = 7.56 Hz), 8.43 (br s, 1H, N-H), 8.73 (s, 1H, quinoline H_2), 8.98 (s, 1H, N-H), 11.46 (s, 1H, N-H); LC-MS (ESI) m/z: 356.85 (M + 1, 100); Anal. Calcd. for $C_{17}H_{13}ClN_4O_3$: C, 57.23; H, 3.67; N, 15.70. Found: C, 57.46; H, 3.88; N, 15.79.

2-(4-Hydroxyquinoline-3-carbonyl)-N-p-tolylhydrazinecarboxamide (7d)

Yield, 40%; white powder; mp 320 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1620–1670 (C=O), 2900–3500 (O-H), 3220 & 3280 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 2.19 (s, 3H, CH_3), 7.00 (d, 2H, N-*p*-tolyl H_3 & H_5, *J* = 8.11 Hz), 7.29 (d, 2H, *p*-tolyl H_2 & H_6, *J* = 8.26 Hz), 7.46–7.49 (t, quinoline H_6, *J* = 7.40 Hz), 7.69 (d, 1H, quinoline H_8, *J* = 8.21 Hz), 7.74–7.77 (t, 1H, quinoline H_7, *J* = 7.27 Hz), 8.26 (d, 1H, quinoline H_5, *J* = 8.04 Hz), 8.33 (s, 1H, N-H), 8.72–8.75 (d, 2H, quinoline H_2 & N-H), 11.36 (s, 1H, N-H), 12.78 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 356.82 (M + 1, 100); Anal. Calcd. for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66. Found: C, 64.39; H, 4.66; N, 16.77.

2-(4-Hydroxyquinoline-3-carbonyl)-N-(4-methoxyphenyl) hydrazine carboxamide (7e)

Yield, 35%; white powder; mp 265–266 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1650–1680 (C=O), 2800–3500 (O-H), 3300, 3360 & 3460 (N-H); ^{1}HNMR (DMSO-*d6*, 500 MHz): δ 3.66 (s, 3H, OCH_3), 6.80 (d, 2H, N-4-methoxyphenyl H_3 & H_5, *J* = 8.94 Hz), 7.31 (d, 2H, N-4-methoxyphenyl H_2 & H_6, *J* = 8.94 Hz), 7.46–7.49 (t, quinoline H_6, *J* = 7.84 Hz), 7.69 (d, 1H, quinoline H_8, *J* = 8.20 Hz), 7.74–7.77 (t, 1H, quinoline H_7, *J* = 8.26 Hz), 8.26 (d, 1H, quinoline H_5, *J* = 8.09 Hz), 8.29 (s, 1H, N-H), 8.65 (s, 1H, N-H), 8.73 (s, 1H, quinoline N-H), 11.34 (s, 1H, N-H), 12.78 (br s, 1H, enolic OH); LC-MS (ESI) m/z: 352.83 (M + 1, 100); Anal. Calcd. for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.44; H, 4.46; N, 15.87.

In-vitro anti-HIV and cytotoxicity assays

The inhibitory effect of compounds against HIV-1 was studied by single cycle replication assay as previously described [20]. In brief, Hela cells (6×10^3 per well of 96-wells plate) were infected with single cycle replicable HIV NL4–3 virions (200 ng P_{24}) in the presence of different concentrations of compounds (1, 10 and 100 µM). Addition of compounds to the cells environment was simultaneous with viral infection. The supernatants were collected 72 h postinfection and evaluated for P_{24} antigen load by capture ELISA (Biomerieux, France). The inhibition rate (%) of P_{24} expression was calculated. The cellular toxicity was evaluated by XTT (sodium 3-[1(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid) proliferation assay (Roche, Germany) according to the kit instruction [21,22]. The HIV replication assay plates were directly considered for cytotoxicity assay after determination of P_{24} load.

Molecular modeling (docking) studies

The active compounds were selected for docking studies against HIV-1 IN. 3OYA is used for binding mode analysis of HIV-1 IN inhibitory activity. All the compounds were built using ChemDraw and subsequently minimized. The protein structure was prepared for docking using AUTODOCK Tool. Docking was performed by AutoDock 4.0 program, using the implemented empirical free energy function and the Lamarckian Genetic Algorithm (LGA) [23]. Co-crystallized ligand and all water molecules were removed from crystal protein (3OYA) while magnesium ions (Mg^{2+}) at the active site of HIV-1 IN were maintained. Polar hydrogens were added and non polar hydrogens were merged, finally Kallman united atom charge and atom type parameter was added to 3OYA. Grid map dimensions (20 × 20 × 20) were set surrounding active site. Lamarckian genetic search algorithm was employed and docking run was set to 50.

Results and Discussion

Chemistry

The target 4-hydroxyquinoline-3-carbohydrazide derivatives were synthesized via the route outlined in **Fig. 2**. The Gould–Jacob cyclization reaction is regarded as the most fundamental and versatile method to form the core structure of 4-hydroxyquinoline-3-carboxylate [19]. The compounds 4-hydroxy-N′-(4-substituted-benzoyl) quinoline-3-carbohydrazides **6a–e** and N-(4-substituted-phenyl)-2-(4-hydroxyquinoline-3-carbonyl) hydrazinecarboxamide **7a–e** were prepared according to Gould–Jacob cyclization reaction, starting from aniline in 4 steps. Condensation of aniline with ethoxymethylene malonate diethyl ester (EMME) yielded diethyl 2-((phenylamino)-methylene) malonate **3**. Compound **3** was converted to ethyl 4-hydroxyquinoline-3-carboxylate **4** in Ph_2O containing catalytic 2-chlorobenzoic acid. Compound **4** was subsequently treated with hydrazine in DMF to form the corresponding hydrazide intermediate **5**. Compound **5** was used as a key intermediate for obtaining 2 different series of compounds. In the first series, to synthesize 4-hydroxy-N′-(4-substituted-benzoyl)quinoline-3-carbohydrazides **6a–e**, compound **5** reacted with 4-substituted benzoyl chlorides in presence of catalytic amount of Na_2CO_3 in DMF. In the second series, compounds **7a–e** were synthesized by reaction of hydrazide intermediate **5** with 4-substituted phenyl isocyanate in MeCN. All compounds were stable and pure and their chemical structures were confirmed by IR, ^{1}HNMR and ESI-MS.

Biological evaluation

The anti-HIV activity of the all compounds was measured by determining their ability to general inhibition of the replication of HIV-1 in Hela cells cultures. For comparative purposes, nucleoside reverse transcriptase inhibitor, AZT, was assayed in the same cells. All synthesized compounds were also tested for their cytotoxicity by MTT assay. As shown in **Table 1**, all synthesized compounds displayed no significant cytotoxicity at concentration of 100 µM. Most of the tested compounds produced inhibitory effects at 100 µM concentration with a percentage ranging from 5 to 32%. However, in all cases, the measured activities were lower than that of AZT. These results indicated that chelation of the 2 Mg^{2+} cations by adjacent carboxylic and hydroxyl groups as chelation motif are not strong enough to allow for a strong anti-HIV-1 activity by these compounds. 4-Hydroxyquinoline-3-carbohydrazide compounds **6a–e** containing 4-substituted benzoyl groups as aromatic ring, showed moderate activity against HIV ranging from 21–32%, except for compound **6c**, which exhibited 8% inhibition rate. However, compounds **7a–e** having 4-substituted N-phenyl moiety displayed no considerable anti-HIV activity (0–5%) except for compound **7e** (having methoxy substitution) with inhibition rate of 28%. The structural difference between **6a–e** and **7a–e** lies in the incorporation of N-H between substituted phenyl ring

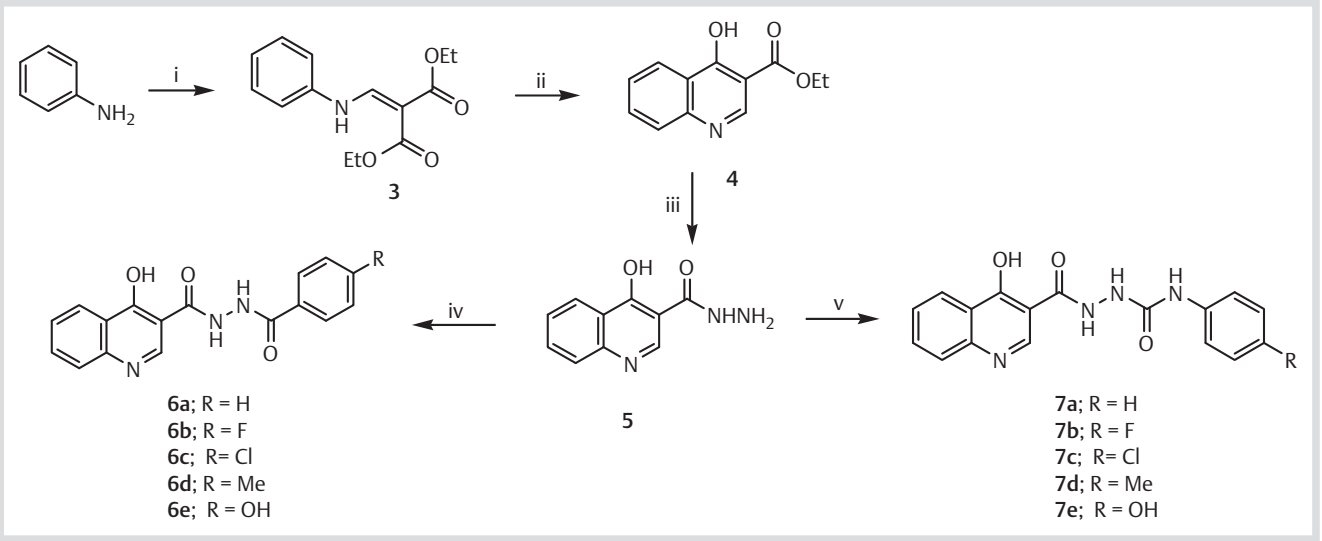


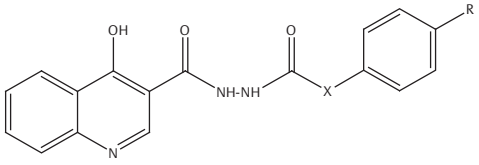
Fig. 2 Reagents and conditions: (**i**) EMME, 120 °C, 1 h (**ii**) 2-chlorobenzoic acid, Ph_2O, 250 °C, 2 h (**iii**) NH_2NH_2.OH, DMF, rt, 12 h (**iv**) 4-substituted benzoyl chloride, Na_2CO_3, DMF, rt, 16 h (**v**) 4-substituted phenyl isocyanate, MeCN, rt, 16 h.

and second carbonyl group in compounds **7a–e**. These results demonstrated that specific orientation of phenyl ring to the chelator group is a requirement for high anti-HIV-1 activity. According to our results compound 6d having methyl group at para position of phenyl ring showed the highest anti-HIV-1 activity (32%) among the synthesized compounds. These findings conform to the common pharmacophore of HIV-1 integrase inhibitors in which a hydrophobic aromatic group with a specific spatial arrangement to a chelator is a required structural determinant.

Molecular modeling studies

Docking studies were performed to predict the binding mode of designed compounds and to rationalize the observed structure activity relationships. Based on these results, a mechanism of inhibition for these compounds is proposed. Due to the lack of HIV-1 IN/DNA experimental structures, the building of a reliable model is essential for predicting docking of integrase inhibitors. Hare et al. solved the crystal structures of full-length prototype foamy virus (PFV) integrase DNA complexes with various HIV-1 integrase inhibitors, exhibiting 2-metal ions in the strand transfer active site (Mg^{2+} or Mn^{2+}) [24]. As shown by Hare et al. PFV integrase can be considered as a good model for the development of HIV-1 integrase strand transfer inhibitors. The secondary structures of PFV IN (PDB: 3OYA) and HIV-1 IN catalytic core domain (PDB: 1BL3) have highly conserved architectures, with a calculated RMSD of 1.04 Å. 2 Mg^{2+} ions in PFV integrase are coordinated by Asp128, Asp185 and Glu221 which are corresponded to HIV-1 integrase Asp64, Asp116 and Glu152, respectively. On the basis of these considerations, we decided to undertake docking studies of our molecules based on the 3OYA X-ray crystallographic structure of PFV IN**.** Docking of the most potent compounds (**6d** and **7e**) in the newly built model was performed with the AUTODOCK 4.2 program. All these compounds showed a similar interfacial-binding mode in which the adjacent carboxylic and hydroxyl group at position of C-3 and C-4 of quinoline ring moiety chelate the Mg^{2+} ions (see docking result of **6d** and **7e**, **Fig. 3**). The displaced 3′-adenosine terminal base (A17) was involved in a π-stacking interaction with the quinoline ring. The *p*-substituted-phenyl group fitted within a tight pocket formed by cytosine 16 (C16), guanine 4 (G4). Further docking study revealed that active compounds (**6d** and **7e**) occupied same space near to Mg^{2+} ions as Raltegravir (**Fig. 4**). These docking results were in agreement with the common structure activity relationships of HIV-1 integrase inhibitors.

Table 1 Anti-HIV activity of synthesized compounds.



Compound	X	R	100 µM (%) inhibition rate of P_{24} expression	% Cell viability
6a	–	H	21.1	97.3
6b	–	F	22.7	79.1
6c	–	Cl	7.9	70.0
6d	–	CH_3	31.7	81.1
6e	–	OCH_3	22.9	76.9
7a	NH	H	–	81.3
7b	NH	F	5.8	73.1
7c	NH	Cl	–	86.7
7d	NH	CH_3	ND*	84.0
7e	NH	OCH_3	28.0	98.0
AZT			100	100
Ralvitegravir			100	100

*ND: Not determined

Conclusions

In conclusion, we have designed and synthesized a novel class of 4-hydroxyquinoline-3-carbohydrazide derivatives by merging 2 well-known anti-HIV-1 scaffolds, salicylhydrazide and 4-quinolone-3-carboxylic acid. The major significance of this work as compared to previous reports on salicylhydrazide structure is that the synthesized compounds displayed no considerable cytotoxicity in cells cultures. So, this novel scaffold is suitable for the

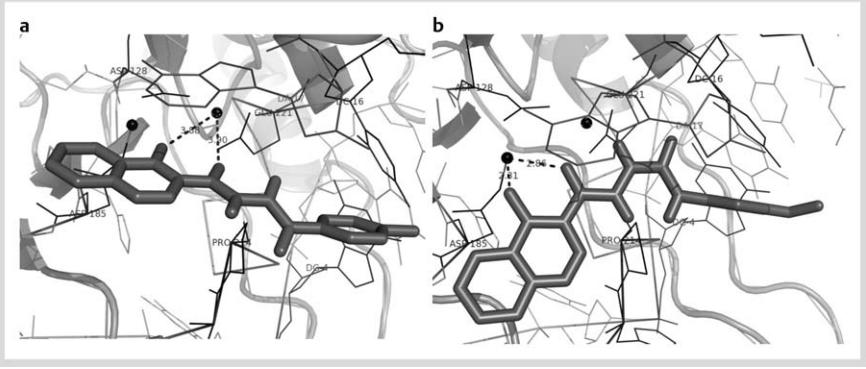


Fig. 3 Mode of binding of **6d (a)** and **7e (b)** in the active site of HIV-1 IN.

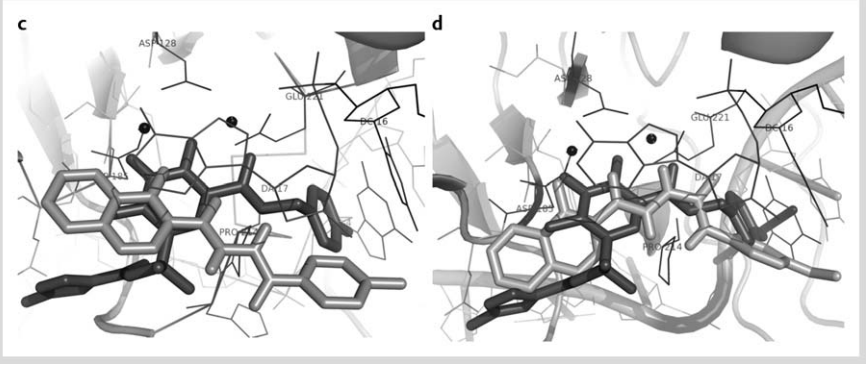


Fig. 4 Compound **6d** and **7e** superimposed on the Raltegravir molecule (**c** and **d** respectively).

development of new hits. Compounds **6d** and **7e** were found to be the most active in cell based anti-HIV assay with inhibition rates of 32 and 28% at concentration of 100µM, respectively. The molecular modeling studies suggested that the anti-HIV activity of these compounds might involve a metal chelating mechanism.

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Conflict of Interest

The authors have declared no conflict of interest.

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