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Synthesis of C-2 and C-3 substituted quinolines and their evaluation as anti-HIV-1 agents

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ABSTRACT

A plenty of natural products and synthetic derivatives containing quinoline moiety have been reported to possess various pharmacological activities. Quinolines such as 2-styrylquinolines and 8-hydroxyquinolines are extensively studied for their anti-HIV-1 activity and found to act mainly through HIV-1 integrase enzyme inhibition. In continuation of our efforts to search for newer anti-HIV-1 molecules, thirty-one quinoline derivatives with different linkers to ancillary phenyl ring were synthesized and evaluated for *in vitro* anti-HIV-1 activity using TZM-bl assays. Compound **31** showed higher activity in TZM-bl cell line against HIV-1_{VB59} and HIV-1_{UG070} cell associated virus (IC_{50} 3.35 ± 0.87 and 2.57 ± 0.71 μM) as compared to other derivatives. Compound **31** was further tested against cell free virus HIV-1_{VB59} and HIV-1_{UG070} (IC_{50} 1.27 ± 0.31 and 2.88 ± 1.79 μM, TI 42.20 and 18.61, respectively). This lead molecule also showed good activity in viral entry inhibition assay and cell fusion assay defining its mode of action. The activity of compound **31** was confirmed by testing against HIV-1_{VB51} in activated peripheral blood mononuclear cells (PBMCs). Binding interactions of **31** were compared with known entry inhibitors.

1. Introduction

A large number of biologically important natural products and synthetic derivatives containing quinoline moiety have been reported to possess anti-bacterial, anti-fungal, anti-parasitic, anti-viral, anti-protozoal, anti-neoplastic, cytotoxic, anti-inflammatory and immunosuppressive activities [1]. Amongst the anti-viral profile, quinoline derivatives with various substituents have been reported for *in vitro* anti-HIV activity through various mechanisms [2–9]. Some of the natural products with 8-oxygenated quinoline as substructure possessing anti-HIV-1 activity are depicted in Fig. 1.

γ-Fagarine **(1)** and haplopine **(2)** are furoquinoline class of alkaloids isolated from root bark of *Zanthoxylum ailanthoides*. γ-Fagarine has been reported to possess potent anti-HIV activity with EC_{50} and TI values of < 0.44 μM and > 231, respectively. Haplopine, which has a hydroxy (−OH) group at C-7 position, showed EC_{50} and TI values of 2.58 μM and 36.7, respectively [10]. Marine alkaloids aaptamine **(3)**, isoaaptamine **(4)** and demethyl(oxy)aaptamine **(5)**, containing a rare 1*H*-benzo[*de*]-1,6-naphthyridine skeleton, have been reported for anti-HIV-1 activity with EC_{50} of 1.3, 0.6 and 0.34 μM, respectively These aaptamines were isolated from sea sponge *Aaptos aaptos* [11,12].

Quinoline derivatives such as 2-styrylquinolines and 8-hydroxyquinolines are extensively studied for their anti-HIV-1 activity and found to act through inhibition of HIV-1 integrase enzyme [13–17]. Some of them (**6**, **7**, **8**) are shown in Fig. 2. Our previous efforts in the search of anti-HIV agents with styrylquinolines also led to the identification of two lead molecules (**9** and **10**, Fig. 2) [9]. Schiff bases have also been reported for anti-HIV activity [18].

In continuation of search for newer anti-HIV molecules, 2,3-disubstituted quinoline derivatives (**16–22**) were synthesized using differently substituted anilines, leading to imine moiety at C-3 position and substituted amine moiety at C-2 position. However, these derivatives did not show any effect against both the studied HIV-1 strains. Further based on the literature, 8-methoxyquinoline scaffold was explored by modifying its C-2 position. Substituted amide derivatives of quinoline (**11** and **12**, Fig. 2) are reported to exhibit anti-HIV activity by inhibiting HIV-1 integrase [2]. Recently, oxadiazoles containing molecules have also been reported for anti-HIV activity and being privileged structures, can be used as linkers [19]. Here, combining the above structural features, C-2 position of 8-methoxyquinoline was

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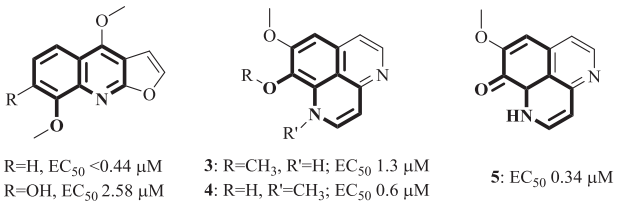


Fig. 1. Naturally occurring 8-oxygenated quinolines with anti-HIV-1 activity.

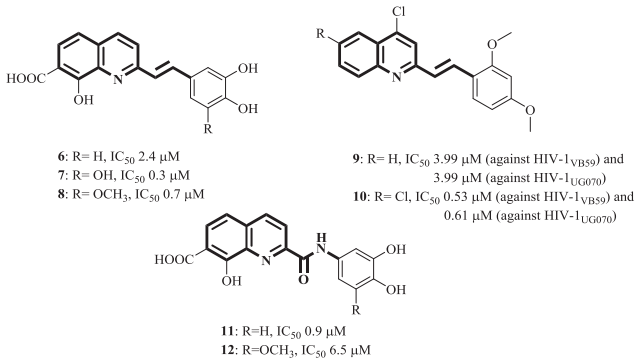


Fig. 2. Synthetic quinoline derivatives containing styryl and amide linkers possessing anti-HIV-1 activity.

substituted with *α,β*-unsaturated amides (**27–31**) and 2,5-disubstituted-1,3,4-oxadiazoles (**34–47**) by replacing the styryl functionality to investigate the effect on the anti-HIV-1 potential.

2. Results and discussion

2.1. Synthesis

The overall synthetic strategies are depicted in Schemes 1 and 2. As shown in Scheme 1, aniline (**13**) was first acetylated using acetic anhydride to yield acetanilide (**14**) in 88% yield, which on refluxing with $POCl_3$ in DMF resulted in 2-chloro-quinoline-3-carbaldehyde (**15**) via Vilsmeier-Haack reaction [20]. 2-Chloro-quinoline-3-carbaldehyde (**15**) was used as penultimate compound for the synthesis of 2,3-disubstituted quinoline derivatives (**16–22**) using substituted anilines in moderate to good yields. All the derivatives (**16–22**) showed N—H stretching absorption band in the region of 3300–3100 cm^{-1} in IR spectra and N—H proton signal in the range of 11–12 ppm in ^{1}H NMR spectra. Apart from this, imine proton CH=N was observed in the range of 8–9 ppm in ^{1}H NMR spectra.

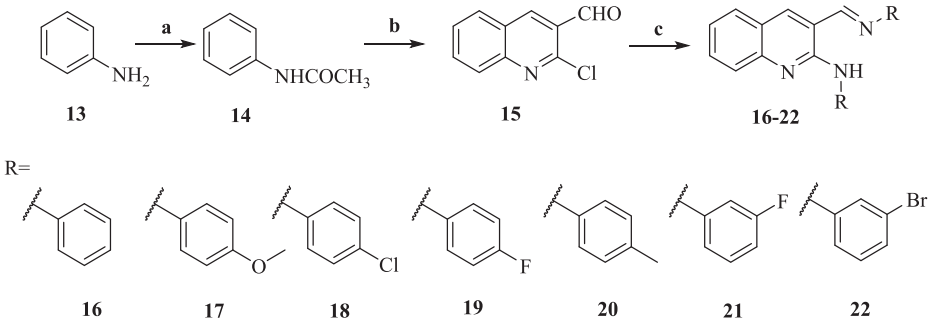
For the synthesis of C-2-substituted 8-methoxyquinoline derivatives (Scheme 2), 8-methoxy-2-quinaldine (**24**) was synthesized using modified Skraup quinoline synthesis, by refluxing *o*-anisidine (**23**) in 6 M HCl and crotonaldehyde in toluene [21]. This compound **24** was oxidised to 8-methoxyquinoline-2-carbaldehyde (**25**) by refluxing with selenium dioxide in anhydrous 1,4-dioxane [22], which upon reaction with malonic acid in pyridine yielded (*E*)-3-(8-methoxyquinolin-2-yl) acrylic acid (**26**). The acid (**26**) was further coupled with different substituted anilines using HOBt and DIC to yield various *α,β*-unsaturated amide derivatives (**27–31**). All the derivatives (**27–31**) showed an N—H stretching absorption band in the region of 3300–3100 cm^{-1} in IR spectra and N—H proton signal in the range of 7–9 ppm in ^{1}H NMR spectrum. Moreover, two protons with coupling constants > 15 Hz in ^{1}H NMR, revealed the presence of *trans* protons. An exceptional result was obtained with aniline, which yielded **32** in addition to **27**. This was due to Michael addition to the *α,β*-unsaturated carbonyl group. Compound **32** also showed N—H stretching absorption band in the region of 3300–3100 cm^{-1} in IR spectra and two N—H proton signals at 9.79 and 6.41 ppm in ^{1}H NMR spectrum.

For the synthesis of oxadiazoles, compound **25** was refluxed with various substituted acylhydrazines in ethanol to produce acyl hydrazides of 8-methoxyquinoline-2-carbaldehyde. These acyl hydrazides were directly converted to 1,3,4-oxadiazoles (**33–50**) using I_2/K_2CO_3 mediated oxidative cyclization [23]. All the derivatives (**33–50**) showed characteristic peaks of 1,3,4-oxadiazole *i.e.* 1650–1580 cm^{-1} (C=N), 1300–1200 cm^{-1} (C-O-C_{asymm}), 1050–950 cm^{-1} (C-O-C_{symm}) in IR spectra.

All the synthesized compounds were confirmed using ^{1}H NMR, ^{13}C NMR, HRESI-MS and IR. Percentage purity of all synthesized compounds were determined using HPLC and observed at their respective λ_{max} values.

2.2. In vitro evaluation for anti-HIV-1 activity

All the synthesized compounds were first screened for cytotoxicity in TZM-bl cell line using MTT assay and CC_{50} (Concentration showing 50% cytotoxicity) values were determined. The non-cytotoxic concentration of compounds were screened for *in vitro* anti-HIV-1 activity against cell-associated primary isolates HIV-1_{VB59} (R5, Subtype C) and HIV-1_{UG070} (X4, Subtype D) in TZM-bl cell line and IC_{50} (concentration showing 50% inhibition of HIV-1 replication in cell culture) values were determined [9]. In this assay, the TZM-bl cells were initially infected with HIV-1 and allowed for integration in the cell and subsequently the test compound was added. Therapeutic indices (TI) were calculated using CC_{50} and IC_{50} values. Azidothymidine was kept as a positive control throughout the study. Results are shown in Table 1. The primary aim of the study was to identify new molecules against global subtype which is majorly subtype C. Therefore, HIV-1 Indian subtype C strain was used for the study. For mechanistic study, due to non-availability of the X4 tropic Indian subtype C strain, a representative strain available with NIHARRRP (subtype D) was used.



Scheme 1. Synthesis of 2,3-disubstituted quinoline derivatives: **Reagents and conditions: (a)** acetic anhydride, 40 °C, 10 min, 88%; **(b)** $POCl_3$, DMF, 80 °C, 8 h, 65%; **(c)** substituted anilines, DMF, 75 °C, 7 h, 40–61%.