Bioorganic & Medicinal Chemistry 18 (2010) 2872–2879

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and anti-HIV activity of alkylated quinoline 2,4-diols

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ARTICLE INFO

Article history:
Received 14 January 2010
Revised 7 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Keywords:
Quinoline 2,4-diol
Alkylation
Anti-HIV activity
CEM-GFP cells

ABSTRACT

Naturally occurring quinolone alkaloids, buchapine **(1)** and compound **2** were synthesized as reported in literature and evaluated for anti-HIV potential in human CD4+ T cell line CEM-GFP, infected with HIV-$1_{NL4.3}$ virus by p24 antigen capture ELISA assay. The compounds **1** and **2** showed potent inhibitory activity with IC_{50} value of 2.99 and 3.80 μM, respectively. Further, 45 alkylated derivatives of quinoline 2,4-diol were synthesized and tested for anti-HIV potential in human CD4+ T cell line CEM-GFP. Among these, 13 derivatives have shown more than 60% inhibition. We have identified three most potent inhibitors **6**, **9** and **23**; compound **6** was found to be more potent than lead molecule **1** with IC_{50} value of 2.35 μM and had better therapeutic index (26.64) as compared to AZT (23.07). Five derivatives **7**, **19a**, **19d**, **21** and **24** have displayed good noticeable anti-HIV activity. All active compounds showed higher CC_{50} values which indicate that they have better therapeutic indices.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is one of the greatest challenges to mankind in the history of Biomedical Science. It is estimated that 33.4 million people were living with HIV, 2.7 million became newly infected and 2.0 million lost their lives as a result of AIDS (UNAIDS 2009 Report).[1] Two major types of HIV have been identified, HIV-1 and HIV-2. HIV-1 is the cause of worldwide epidemic of AIDS whereas HIV-2 is less pathogenic and found mostly in West Africa. HIV-1 is classified into three major groups M, O and N (non-M and non-O). There are ten subtypes within group M from subtype A to subtype K, of which presently subtype C is most prevalent worldwide. Group O contains distinct group of heterogeneous viruses. Group N viruses exhibit distinct sequence of envelop protein from group M and group O. HIV-1 causes loss of CD4+T cells by direct killing of the cells or indirectly by impairing function of cells consequently leading to the cell destruction by apoptosis.[2] Though successful use of synthetic Reverse Transcriptase (RT) inhibitors and protease inhibitors in combination (Highly active antiretroviral therapy, HAART) have effectively suppressed the virus replication and significantly prolonged the life of AIDS patients, the appearance of mutant viruses that are resistant to current drugs require the development of new types of anti-HIV drugs, from the point of view of structure and mechanism.[3] HIV infection represent a global health hazard necessitating identification of novel targets and new lead molecules for drug discovery.[4] Therefore, there is an urgent need to develop new compounds with potent anti-HIV activity and with novel modes of action.

Natural Products are an important source of new drug candidates. Many natural products, semi-synthetic or NP derived candidates having variety of biological activities are in clinic or in clinical trials.[5] Anti-HIV agents from natural resources belonging to several classes including terpenoids, coumarins, alkaloids, polyphenols, tannins and flavonoids have been elaborately reviewed by our group.[6] Bevirimat, a semi-synthetic derivative of the plant metabolite betulinic acid, is in Phase IIb trials in HIV-infected patients.[5] Bevirimat blocks HIV maturation by inhibiting the final step of the HIV Gag protein processing.[7] Batzelladines,[8] harmine,[9] michellamine B,[10] calanolide A and B, calceolarioside B,[11] mallotojaponin,[12] macrocarpals[13] are few other examples of anti-HIV natural products. The buchapine **(1)**, quinolone alkaloid, was isolated from methanolic extract of the epigeal part of *Haplophyllum bucharicum*,[14] *Haplophyllum. tuberculatum*[15] and *Euodia roxburghiana*.[16] Compound **2** was also isolated from *H. tuberculatum* and CH_2Cl_2/MeOH (1:1) extract of *E. roxburghiana* along with **1**. The natural products **1** and **2** (Fig. 1) exhibit anti-HIV activity against HIV-1 in cultured human lymphoblastoid CEM-SS cells (EC_{50} 0.94 μM, IC_{50} 29.0 μM and EC_{50} 1.64 μM, IC_{50} 26.9 μM), respectively.[16] These molecules are not yet explored in order to obtain the new lead molecules for anti-HIV activity. As a part of our ongoing work to identify newer anti-HIV agents,[17] we selected natural products **1** and **2** for our study. Here, we report synthesis and

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doi:10.1016/j.bmc.2010.03.015

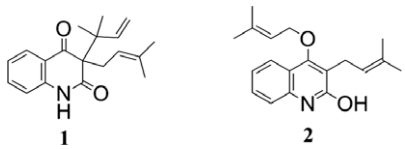


Figure 1. Anti-HIV natural products.

anti-HIV activity of alkylated analogues of quinoline 2,4-diol based on the natural products **1** and **2**.

2. Results and discussion

2.1. Chemistry

The key intermediates quinoline 2,4-diol (**3**) and substituted quinoline 2,4-diol (**4**) were synthesized[18] in excellent yields by condensation of aniline or substituted aniline and diethyl malonate under microwave irradiation using 3–5 drops of DMF for 5–10 min as outlined in Schemes 1 and 2. Synthesis of natural products **1** and **2** was achieved as reported in the literature.[19,20] As shown in Scheme 1, alkylation of **3** was performed by heating with K_2CO_3 and prenyl bromide in DMF at 60 °C to give natural product **2**, which underwent Claisen rearrangement when heated at 150 °C in presence of *N*-methyl 2-pyrolidone (NMP) to form buchapine **(1)** in 40% yield. To obtain potential anti-HIV agent, we designed three series of compounds for synthesis.

2.1.1. Series 1

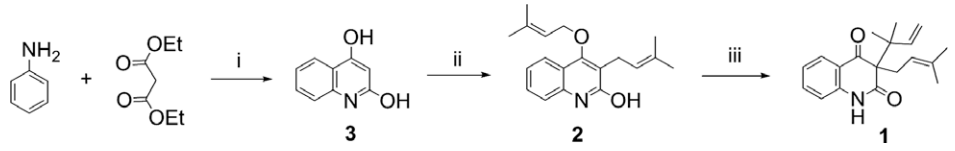
Based on natural product **1**, compounds **5–17** having dialkyl substitutions on C-3 of **3** or **4** were synthesized as depicted in Scheme 2. The C-3 di-alkylation of **3** or **4** was achieved in a single step by heating with lithium hydroxide and excess of alkyl halides in water at 80 °C. Alkylation in polar protic solvent like water gives more C/O selectivity. After optimization, this method was very efficient for di-alkylation by using lithium hydroxide as base in water. The products were obtained in good 60–80% yields.[21]

2.1.2. Series 2

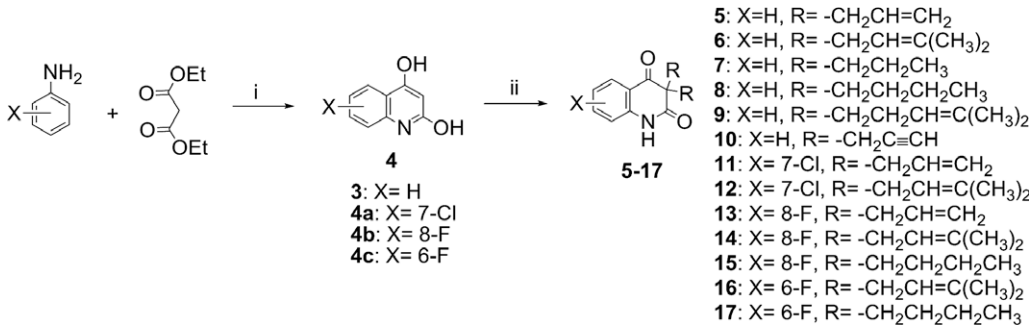
The compounds **19a–e** were synthesized based on natural product **2** as illustrated in Scheme 3. The C-3 alkyl quinoline 2,4-diol derivatives (**18a–e**) were obtained by treatment of **3** with alkyl halides and LiOH in water at 80 °C in 50–70% yield. The **18a–e** were then refluxed with alkyl halides and acetone containing anhydrous K_2CO_3 to yield C-3, O-4 di-alkyl quinoline 2,4-diol derivatives (**19a–e**). It is worth to note that excess of alkyl halides were used for the preparation of series 1 compounds while only slight excess (1.2 equiv) of alkyl halides were used for series 2 compounds.

2.1.3. Series 3

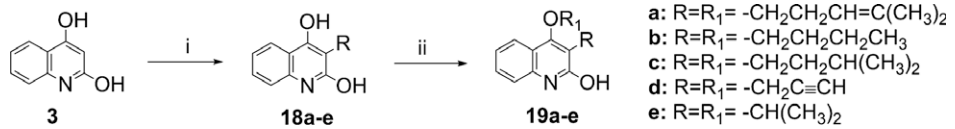
The hydroxyl groups at 2 and 4 positions of **3** or **4** were modified to investigate the role of these groups on anti-HIV activity. The *O*-2,4-di-alkyl quinoline 2,4-diol analogues of **3** or **4** were synthesized following the strategy shown in Scheme 4. The O-alkylation of **3** or **4** was done at 4-OH group by refluxing with alkyl halides and K_2CO_3 in acetone to afford **20–29** in 60–80% yields. Alkylation at 4-OH position was confirmed by 2D NMR study viz. HMBC experiment; for example, in case of compound **24**, methylene protons (δ 5.02) of propargyl moiety attached at 4-OH of quinoline 2,4-diol showed correlation with carbon at 4 position (δ 161.51) as depicted in Figure 2. Subsequently, O-2 alkylation of compounds **20–29** was accomplished by using similar reaction condition with varying alkyl groups to yield compounds **30–47** in 50–70% yields (Scheme 4).



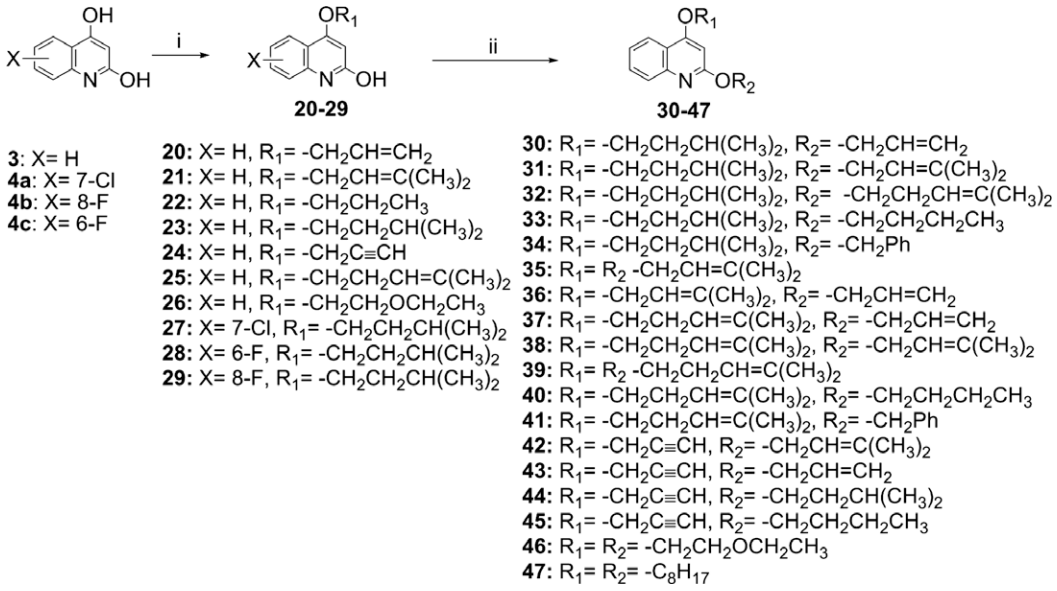
Scheme 1. Reagents and conditions: (i) MW, 850 W, 3–5 drops DMF, 5–10 min, 95%; (ii) prenyl bromide, K_2CO_3, DMF, 60 °C, 12 h, 60%; (iii) *N*-methyl 2-pyrolidone, reflux, 2 h, 35%.



Scheme 2. Reagents and conditions: (i) MW, 850 W, 3–5 drops DMF, 5–10 min, 95–98%; (ii) LiOH, H_2O, excess of RX′ (X′ = Br, 1), 80 °C, 24 h, 60–80%.



Scheme 3. Reagents and conditions: (i) RX, LiOH, H_2O, 80 °C, 12 h, 50–70%; (ii) R_1X, K_2CO_3, acetone, reflux, 12 h, 50–70%.



Scheme 4. Reagents and conditions: (i) R_1X, K_2CO_3, acetone, reflux, 12 h, 60–80%; (ii) R_2X, K_2CO_3, acetone, reflux, 12 h, 50–70%.

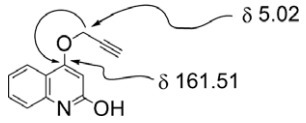


Figure 2. Selected HMBC correlation of **24**.

2.2. Biological evaluation

CEM-GFP is a human CD4+ reporter T cell line, which expresses GFP (green fluorescent protein) upon HIV infection due to transactivation by Tat protein of stably integrated long terminal repeat regulated GFP gene. This cell line is used widely for determination of anti-HIV activity due to easy visualization of infected cells.[22]

All synthesized analogues were first evaluated for their cytotoxicity in MTT based cell viability assay[23] in CEM-GFP cells. Further non-cytotoxic concentration of each analogue was used for determination of in vitro anti-HIV activity in CEM-GFP cells. Out of various compounds tested, 13 derivatives have shown more than 60% inhibition of HIV-1 replication as shown in Table 1. The lead natural product **1** was potent inhibitor of HIV-1 in our assay with IC_{50} of 2.99 μM. Based on this background, we designed series 1 and synthesized compounds **5–17**. Compound **6** having prenyl substitution is more potent than **1** with IC_{50} value of 2.35 μM and has larger CC_{50} of 62.6 μM as compared to AZT (CC_{50} 40 μM). Compound **6** has better therapeutic index (26.64) as compared to AZT (23.07). The introduction of CH_2 group to prenyl moiety led to reduction in activity. Compound **9** showed IC_{50} value of 3.23 μM. Decrease in length of alkyl chain to C-3, increase in length of alkyl chain to C-6 or more caused decrease in activity (e.g., compounds **5** and **7** in Table 1). These results indicated that substitution by prenyl group leads to compounds with better anti-HIV activity. To understand the effect of substitution on ring B of quinoline 2,4-diol, substituted derivatives **10–17** having chlorine and fluorine substitution at different positions were tested. It is revealed from the data in Table 1 that substitution on ring B led to complete loss of anti-HIV activity. Thus unsubstituted ring B is essential for inhibition of HIV-1.

Natural product **2** had shown potent inhibitory activity in our assay with IC_{50} value of 3.88 μM. On this background, we designed series 2 and synthesized various compounds. Among these, **19a–e** exhibited good anti-HIV activity with **19a** having IC_{50} value of 8.76 μM. However, no compound had shown better activity than **2** indicating that prenyl group is best for HIV-1 inhibitory activity. The chain lengths up to four carbons are required for activity. Ser-

Table 1
Anti-HIV activity of synthesized compounds

Compounds	Non-cytotoxic concn μM	% Inhibition p24 ELISA	IC_{50}[c] μM	CC_{50}[d] μM	T.I.[e]
1	23.51	80.09	2.99	55.89	18.69
2	20.20	87.52	3.80	71.17	18.72
5	31.12	44.3	N.D.[a]	—	—
6	8.41	96	2.35	62.62	26.64
7	24.48	74.65	16.73	113.46	6.78
8,19e,47	—	N.S.[b]	—	—	—
9	12.30	82	3.23	67.38	20.86
10–17,22	—	0	—	—	—
19a	23.07	68.3	8.76	103.38	11.80
19b	65.93	81.04	34.43	191.72	5.56
19c	59.80	88.63	34.88	160.13	4.59
19d	27.42	92.38	11.60	146.83	12.65
21	30.56	95.49	8.951	77.37	8.64
25–29,33,35	—	0	—	—	—
23	43.29	76.74	3.89	114.67	29.47
24	35.17	65.06	19.34	102.51	5.3
30	1.84	23	—	—	—
31	1.68	31.2	—	—	—
32	1.59	15	—	—	—
38,40,41,46	—	0	—	—	—
34	23.36	47	N.D.	N.D.	—
36	22.30	35	—	—	—
37	21.20	16	—	—	—
39	12.30	32.68	N.D.	N.D.	—
42	14.98	2.56	—	—	—
43	16.73	16.08	—	—	—
44	18.58	56.4	N.D.	N.D.	—
45	19.60	56.4	N.D.	N.D.	—
AZT	4.98	89.75	1.04	24.0	23.07

[a] N.D. = not done.
[b] N.S. = not soluble.
[c] IC_{50} = concentration of compound required to achieve 50% protection of CEM-GFP cells ($n = 3$) from virus, as determined by p24 ELISA assay.
[d] CC_{50} = concentration of compound required to reduce proliferation of CEM-GFP cells ($n = 3$) by 50%, as determined by MTT assay.
[e] T.I. = therapeutic index.

ies 1 and 2 indicated that C-3 di-substituted quinoline 2,4-dione derivatives are more potent than C-3, O-4 di-substituted quinoline 2,4-diol derivatives.

To determine the role of free hydroxyl groups, several O-mono and O-dialkylated derivatives of quinoline 2,4-diol (series 3) were evaluated. Among mono *O*-alkyl derivatives, few analogues (**21**, **23** and **24**) had exhibited good activity. Compound **23** having isoamyl group on 4-OH showed IC_{50} value of 3.89 μM and CC_{50} of 114.67 μM with best therapeutic index value of 29.47 amongst all derivatives. Based on potent derivative **23**, we synthesized analogues **27–29**. As shown in Table 1, a similar pattern to series 1 was observed. The chloro and fluoro substitution on any of positions on ring B of quinoline 2,4-diol led to loss of activity.

The potent inhibitors **21**, **23** and **24** that possessed excellent to good potency were chosen for further modification along with others. Among O-dialkylated derivatives (**30–47**), none showed promising activity. It indicated that O-4 substituted quinoline 2,4-diol derivatives possessed better activity than O-2,4 di-substituted quinoline 2,4-diols. Further, free 2-OH of quinoline 2,4-diol is essential for anti-HIV activity.

3. Conclusion

In conclusion, 47 derivatives of quinoline 2,4-diol were synthesized based on anti-HIV natural products **1** and **2** and evaluated for in vitro anti-HIV activity in HIV-$1_{NL4.3}$ infected CEM-GFP cells. The structure activity relationship of quinoline 2,4-diol was studied for anti-HIV activity. It indicated that the unsubstituted ring B and free 2-OH group are essential for anti-HIV activity. Further, the chain length upto four carbons and prenyl group at C-3 and/or O-4 is required for HIV-1 inhibitory activity. The O-2,4-di-substituted quinoline 2,4-diols were inactive against HIV-1. The C-3-di-alkyl quinoline 2,4-dione derivatives are more potent than C-3,O-4-di-alkyl quinoline 2,4-diol derivatives. Moreover, this study led to identification of potent inhibitors **6**, **9** and **23**. Compound **6** was found to be more potent than lead molecule **1** with IC_{50} value of 2.35 μM and had higher therapeutic index of 26.64 which was better than AZT (23.07). The compound **23** showed best therapeutic index value of 29.47 amongst all derivatives. Thus compounds **6** and **23** are safer than AZT. Few derivatives like **7**, **19a**, **19d**, **21** and **24** were showed good inhibitory activity. All active compounds showed higher CC_{50} values which indicate that they have broad safety indices.

4. Experimental

4.1. Chemistry

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. Nuclear magnetic resonance spectra were recorded on Brukers avance (DPX 300 MHz) and (400 MHz) with tetramethyl silane (TMS) as internal standard. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to TMS. Mass spectra were recorded on GCMS-QS Shimadzu (QP-500) and LCMS waters (Micromass ZQ). IR spectra were recorded on Nicolet spectrometer. The purity of samples was analysed by HPLC (Waters INDIA Ltd) using C18 Merck column (4.6 × 250 mm). Isocratic elution was carried out with mobile phase as methanol/water (7:3). The combustion analyses were carried out using Vario EL Elementar elemental analyzer. TLC was performed on Merck 0.25 mm Kieselgel 60 F254 plates. Column chromatography was performed using either silica gel-60 (60–120 mesh). Details of %purity of compounds are available as Supplementary data.

4.1.1. Synthesis of 3-(3-methylbut-2-enyl)-4-(3-methylbut-2-enyloxy) quinolin-2-ol (2)

To a solution of **3** (200 mg, 1.25 mmol) in DMF (20 ml) and potassium carbonate (1 g, 3.1 mmol) was added prenyl bromide (0.33 ml, 3.1 mmol) at 30 °C and mixture was heated at 60 °C under reflux for 12 h. The reaction mixture was cooled to 30 °C, diluted with water (10 ml) and extracted with ethyl acetate (3 × 20 ml). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give crude product, which was subjected to column chromatography (silica gel #60–120, hexane/EtOAc-gradient) to afford **2** (220 mg, 60% yield) as white solid. IR, ^{1}H NMR and ^{13}C NMR ($CDCl_3$) data were consistent with the literature.[15] Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. Found: C, 76.87; H, 8.02; N, 4.76.

4.1.2. Synthesis of buchapine (1)

Compound **2** (100 mg, 0.33 mmol) was dissolved in *N*-methyl 2-pyrrolidone (15 ml) and solution was refluxed at 150 °C for 2–3 h. It was then diluted with water and extracted with ethyl acetate (3 × 15 ml). The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo to give crude product which was subjected to column chromatography (silica gel #60–120, hexane/EtOAc-gradient) to afford **1** (35 mg, 35% yield) as light yellow solid. ^{1}H NMR and ^{13}C NMR ($CDCl_3$) data were closely consistent with the literature.[15]

4.2. General procedure for C-3 alkylation of 3

4.2.1. 3-Butylquinoline-2,4-diol (18b)

To a suspension of **3** (200 mg, 1.25 mmol) in water (20 ml) was added lithium hydroxide (63 mg, 1.5 mmol) and stirred at 30 °C for 1 h. to give homogeneous solution. *n*-Butyl iodide (0.21 ml, 1.8 mmol) was added dropwise and reaction mixture was heated at 80 °C under reflux for 12 h. Reaction mixture was cooled to 30 °C and extracted with ethyl acetate (3 × 30 ml). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give crude product, which upon column chromatography (silica gel #60–120, hexane/EtOAc-gradient) gave **18b** as white solid (200 mg, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (t, J = 6.3, 12.8 Hz, 3H), 1.10 (m, 2H), 1.24 (m, 2H), 2.2 (t, J = 7.8, 14.8 Hz, 2H), 6.90 (d, J = 7.1 Hz, 1H), 7.17 (t, J = 7.1, 14.2 Hz, 1H), 7.59 (t, J = 7.7, 15.2 Hz, 1H), 7.91 (d, J = 7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14, 22.3, 23.1, 33.8, 115.6, 118.2, 119.3, 124.3, 128.2, 132.1, 143.8, 175.8, 198.7; MS (APCI) *m/z* 218 $[M+1]^+$; Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.23; H, 6.60; N, 6.39.

4.3. General procedure for O-4 alkylation of 3-alkyl-quinoline 2,4-diol (18a–e)

4.3.1. 4-Butoxy-3-butylquinolin-2-ol (19b)

To a suspension of **18b** (100 mg, 0.4 mmol) in dried acetone (10 ml) and K_2CO_3 (95 mg, 0.6 mmol) was added *n*-butyl iodide (0.1 ml, 0.6 mmol) dropwise at 30 °C and reaction mixture was then refluxed for 12 h. Reaction mixture was cooled to 30 °C and filtered; filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, washed with water (2x15 ml) and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give crude product, which upon column chromatography (silica gel #60–120, hexane/EtOAc-gradient) gave **19b** as white solid (80 mg, 66% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.2, 16.3 Hz, 3H), 0.99 (t, J = 7.4, 13.8 Hz, 3H), 1.43 (q, J = 7.6, 15.7 Hz, 2H), 1.92 (q, J = 7.2, 14.6 Hz, 2H), 2.69 (t, J = 8.3, 17.2 Hz, 2H), 4.0 (t, J = 7.7, 15.6 Hz, 2H), 7.20 (t, J = 6.9, 14.6 Hz, 1H), 7.34 (d, J = 9.2 Hz, 1H), 7.46 (t, J = 8.4, 18.1 Hz, 1H), 7.75 (d, J = 8.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.5, 19.8, 20.8, 23.5, 24.9,

30.3, 31.2, 31.5, 33, 68.8, 97.4, 114.6, 116.1, 121.8, 123.5, 130.3, 137.7, 157.4, 163.9; MS (APCI) *m/z* 274 $[M+1]^+$.

4.3.2. 3-(4-Methylpent-3-enyl)-4-(4-methylpent-3-enyloxy) quinolin-2-ol (19a)

White solid, 72% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (s, 3H), 1.66 (s, 3H), 1.70 (s, 3H), 1.76 (s, 3H), 2.36 (q, *J* = 7.4, 15.9 Hz, 2H), 2.59 (q, *J* = 7.5, 14.7 Hz, 2H), 2.73 (t, *J* = 8.2 Hz, 2H), 4.0 (t, *J* = 6.5, 13.2 Hz, 2H), 5.29 (m, 2H), 7.20 (t, *J* = 8.2, 19.3 Hz, 1H), 7.37 (d, *J* = 10.4 Hz, 1H), 7.46 (t, *J* = 9.4, 23.4 Hz, 1H), 7.77 (d, *J* = 8.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.2, 19.1, 22.4, 24.7, 28.3, 30.2, 69.4, 112.5, 116.1, 119.3, 120.9, 123.1, 127.4, 129.3, 135.4, 137.3, 163.2, 169.1; MS (APCI) *m/z* 326.1 $[M+1]^+$; Anal. Calcd for $C_{21}H_{27}NO_2$: C, 77.50; H, 8.36; N, 4.30. Found: C, 77.63; H, 8.69; N, 4.31.

4.3.3. 3-Isopentyl-4-(isopentyloxy)quinolin-2-ol (19c)

White solid, 60% yield; IR (KBr, cm^{-1}): 3436, 3168, 2930, 2872, 1650, 1610, 1466, 1369; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0 (t, *J* = 7.7, 15.2 Hz, 12H), 0.99 (t, *J* = 7.4 Hz, 3H), 1.55 (q, *J* = 7.4, 15.1 Hz, 2H), 1.71 (m, 1H), 1.81 (q, *J* = 7.7, 16.2 Hz, 2H), 1.93 (m, 1H), 2.70 (t, *J* = 6.6, 13.6 Hz, 2H), 4.04 (t, *J* = 7.2, 15.2 Hz, 2H), 7.20 (t, *J* = 8.2, 19.5 Hz, 1H), 7.32 (d, *J* = 8.9 Hz, 1H), 7.47 (t, *J* = 8.7, 18.7 Hz, 1H), 7.72 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 23.1, 23.4, 25.5, 29, 36.8, 38.3, 39.7, 73.7, 97.5, 114.5, 16.1, 118.1, 121.8, 122.6, 123.4, 124.5, 130.3, 137.7, 149.4, 163.4; MS (APCI) *m/z* 302 $[M+1]^+$.

4.3.4. 3-(Prop-2-ynyl)-4-(prop-2-ynyloxy)quinolin-2-ol (19d)

Slight yellow solid, 70% yield; IR (KBr, cm^{-1}): 3295, 2952, 2868, 1646, 1602, 1498, 1367, 1228; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (s, 1H), 2.63 (s, 1H), 3.7 (d, *J* = 2.6 Hz, 2H), 4.91 (d, *J* = 2.3 Hz, 2H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.26 (t, *J* = 8.7, 15.7 Hz, 1H), 7.53 (t, *J* = 9.7, 18.1 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2, 57.3, 70.2, 76.3, 80.3, 81.1, 100.3, 116.5, 121.3, 122.9, 129.6, 138.5, 159.4, 161.7; MS (ESI) *m/z* 238 $[M]^+$; Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.87; H, 4.70; N, 5.88.

4.3.5. 4-Isopropoxy-3-isopropylquinolin-2-ol (19e)

White solid, 58% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.8, 14.9 Hz, 6H), 1.42 (t, *J* = 7.3, 15.1 Hz, 6H), 3.4 (m, 1H), 4.46 (m, 1H), 7.17 (m, 2H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.41 (t, *J* = 8.6, 20.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.3, 22.8, 23.5, 70.4, 99.2, 117.3, 120.5, 125, 128.1, 130.2, 139.1, 158, 163.8; MS (ESI) *m/z* 245 $[M]^+$.

4.4. General procedure for O-4 alkylation of 3 or 4

4.4.1. 4-Propoxyquinolin-2-ol (22)

To a suspension of **3** (100 mg, 0.6 mmol) in dried acetone (15 ml) and K_2CO_3 (290 mg, 0.9 mmol) was added *n*-propyl bromide (0.1 ml, 0.9 mmol) dropwise at 30 °C and reaction mixture was then refluxed for 12 h. Reaction mixture was cooled to 30 °C and filtered; filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, washed with water (2x15 ml) and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give crude product, which upon column chromatography (silica gel #60–120, hexane/EtOAc-gradient) gave **22** (100 mg, 79% yield). IR (KBr, cm^{-1}): 3435, 2964, 2840, 1642, 1611, 1376, 1227; ^{1}H NMR (300 MHz, CD_3OD) δ 1.12 (t, *J* = 7.2, 14.7 Hz, 3H), 1.95 (m, 2H), 4.13 (t, *J* = 7.7, 15.1 Hz, 2H), 5.94 (s, 1H), 7.24 (t, *J* = 8.1, 17.3 Hz, 1H), 7.34 (d, *J* = 9.3 Hz, 1H), 7.55 (t, *J* = 8.2, 17.9 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 10.1, 22.5, 71, 96.2, 116.3, 122.5, 123.4, 127.1, 131.9, 165.3, 176.9; MS (APCI) *m/z* 204.1 $[M+1]^+$; Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.85; H, 6.49; N, 6.82.

4.4.2. 4-(3-Methylbut-2-enyloxy) quinolin-2-ol (21)

White solid, 80% yield; IR (KBr, cm^{-1}): 3282, 2965, 2929, 1649, 1608, 1437, 1228; ^{1}H NMR (300 MHz, CD_3OD) δ 1.81 (s, 3H), 1.84 (s, 3H), 4.27 (d, *J* = 7.3 Hz, 2H), 5.55 (t, *J* = 3.5, 8.2 Hz, 1H), 5.96 (s, 1H), 7.23 (t, *J* = 8.3, 16.4 Hz, 1H), 7.33 (d, *J* = 9.4 Hz, 1H), 7.54 (t, *J* = 8.1, 16.2 Hz, 1H), 7.89 (d, *J* = 9.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.5, 24.3, 66, 96, 116.3, 119.1, 122.8, 123.4, 130.1, 137.3, 165.3, 177.1; MS (APCI) *m/z* 230.1 $[M+1]^+$; Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.38; H, 6.56; N, 5.78.

4.4.3. 4-(Isopentyloxy) quinolin-2-ol (23)

Slight yellow solid, 80% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 1.01 (d, *J* = 6.4 Hz, 6H), 1.80 (q, *J* = 6.1, 13.4 Hz, 2H), 1.87 (m, 1H), 4.20 (t, *J* = 6.9, 14.1 Hz, 2H), 5.96 (s, 1H), 7.24 (t, *J* = 7.6, 15.6 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.55 (t, *J* = 8.1, 15.9 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 22.2, 25.8, 37.8, 68.1, 96, 116.1, 122.9, 123.2, 131.9, 164.9, 176.2; MS (APCI) *m/z* 232 $[M+1]^+$; Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.43; H, 7.69; N, 6.03.

4.4.4. 4-(Prop-2-ynyloxy) quinolin-2-ol (24)

White solid, 75% yield; IR (KBr, cm^{-1}): 3436, 2925, 2840, 1650, 1608, 1498, 1411, 1260; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.15 (s, 1H), 4.98 (s, 2H), 6.09 (s, 1H), 7.26 (t, *J* = 9.9, 19.2 Hz, 1H), 7.35 (d, *J* = 11.1 Hz, 1H), 7.55 (t, *J* = 9.8, 20 Hz, 1H), 7.93 (d, *J* = 10.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 57, 69.5, 74.4, 96.3, 114.5, 117.5, 121.6, 125.5, 134.5, 138.9, 170.5; MS (APCI) *m/z* 200 $[M+1]^+$; Anal. Calcd for $C_{12}H_9NO_2$: C, 72.35; H, 4.55; N, 7.03. Found: C, 72.38; H, 4.60; N, 7.00.

4.4.5. 4-(4-Methylpent-3-enyloxy) quinolin-2-ol (25)

White solid, 75% yield; ^{1}H NMR (300 MHz, CD_3OD) δ 1.72 (s, 3H), 1.74 (s, 3H), 2.66 (q, *J* = 7.3, 14.2 Hz, 2H), 4.13 (t, *J* = 7.5, 15.1 Hz, 2H), 5.29 (t, *J* = 6.2, 12.5 Hz, 1H), 5.94 (s, 1H), 7.24 (t, *J* = 8.3, 16.5 Hz, 1H), 7.31 (d, *J* = 9.3 Hz, 1H), 7.55 (t, *J* = 7.5, 15.6 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.1, 23.9, 27.5, 65.4, 96.1, 116.4, 118,8, 122.3, 123.9, 130.7, 133.9, 165, 177.2; MS (APCI) *m/z* 244.1 $[M+1]^+$; Anal. Calcd for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.98; H, 7.15; N, 5.38.

4.4.6. 4-(2-Ethoxyethoxy) quinolin-2-ol (26)

Colourless oil, 65% yield; IR (KBr, cm^{-1}): 3392, 2974, 2868, 1650, 1608, 1503, 1436, 1405, 1232; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, 3H, *J* = 7.4, 14.2 Hz), 3.66 (q, 2H, *J* = 6.3, 12.1 Hz), 3.9 (t, 2H, *J* = 1.6, 3.1 Hz), 4.2 (t, 2H, *J* = 1.4, 2.9 Hz), 5.97 (s, 1H), 7.24 (t, 1H, *J* = 7.9, 15.8 Hz), 7.34 (d, 1H, *J* = 7.8 Hz), 7.54 (t, 1H, *J* = 8, 17.3 Hz), 7.98 (d, 1H, *J* = 7.4 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 16, 66.3, 69.1, 96.8, 114.2, 119.4, 121.2, 126.1, 131.3, 137.1, 159, 163.1; MS (APCI) *m/z* 234.1 $[M+1]^+$; Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.81; H, 6.51; N, 5.91.

4.4.7. 7-Chloro-4-(isopentyloxy) quinolin-2-ol (27)

White solid, 78% yield; IR (KBr, cm^{-1}): 3363, 2954, 2900, 1677, 1592, 1528, 1419, 1169; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (d, *J* = 6.8 Hz, 6H), 1.58 (q, *J* = 6.3, 10.3 Hz, 2H), 2.09 (m, 1H), 3.5 (t, *J* = 6.1, 11.4 Hz, 2H), 6.21 (s, 1H), 7.14 (d, *J* = 7.4, 13.9 Hz, 1H), 7.43 (d, *J* = 7.6, 12.8 Hz, 1H), 7.8 (s, 1H), 9.2 (s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2, 27.7, 31.8, 56.7, 96.3, 118.1, 120.3, 125, 30, 134.7, 138.3, 169.6, 180; MS (APCI) *m/z* 366 $[M+1]^+$.

4.4.8. 6-Fluoro-4-(isopentyloxy) quinolin-2-ol (28)

White solid, 73% yield; IR (KBr, cm^{-1}): 3269, 2958, 1673, 1618, 1507, 1406, 1215; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 7.2 Hz, 6H), 1.57 (q, J = 5.3, 9.8 Hz, 2H), 2.09 (m, 1H), 3.34 (t, J = 6.3, 10.4 Hz, 2H), 6.31 (s, 1H), 7.01–7.07 (m, 2H), 7.55 (d, J = 5,6 Hz, 1H), 9.0 (s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 27.7, 31.6, 56.7, 97.3, 115.6, 115.8, 122, 122.1, 133.2, 133.3, 160.8, 169.4; MS (APCI) m/z 250 $[M+1]^+$.

4.4.9. 8-Fluoro-4-(isopentyloxy) quinolin-2-ol (29)

White solid, 75% yield; IR (KBr, cm^{-1}): 3264, 2952, 2866, 1678, 1595, 1531, 1451, 1261, 1162; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, J = 6.4 Hz, 6H), 1.62 (q, J = 6.2, 11.3 Hz, 2H), 2.13 (m, 1H), 3.45 (t, J = 6.3, 10.4 Hz, 2H), 6.23 (s, 1H), 7.09–7.17 (m, 2H), 8.24 (t, J = 8.1, 18.2 Hz, 1H), 8.58 (s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 27.8, 31.4, 57.3, 97, 114.9, 115.1, 122.1, 124.5, 125, 125.6, 154, 169.7; MS (APCI) m/z 233 $[M+1]^+$.

4.5. General procedure for O-2 alkylation of O-4 alkyl 2,4-dihydroxy quinoline (20–29)

4.5.1. 2-(Allyloxy)-4-(isopentyloxy) quinoline (30)

To a suspension of **20** (100 mg, 0.4 mmol) in dried acetone (10 ml) and K_2CO_3 (200 mg, 0.6 mmol) was added allyl bromide (0.05 ml, 0.6 mmol) dropwise at 30 °C and mixture was then refluxed for 12 h. Reaction mixture was cooled to 30 °C and filtered; filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, washed with water (2 × 20 ml) and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give crude product, which upon column chromatography (silica gel #60–120, hexane/EtOAc-gradient) gave **30** as white solid (75 mg, 65% yield). ^1H NMR (300 MHz, CD_3OD) δ 1.01 (d, J = 6.3 Hz, 6H), 1.78 (q, J = 6.7, 13.1 Hz, 2H), 1.9 (m, 1H), 4.12 (t, J = 6.9 Hz, 2H), 4.90 (d, J = 7.1 Hz, 2H), 5.04 (dd, J = 0.7, 16.2 Hz, 1H), 5.17 (dd, J = 0.8, 14.7 Hz, 1H), 5.90 (m, 1H), 6.04 (s, 1H), 7.20 (t, J = 8.2, 16.7 Hz, 1H), 7.26 (d, J = 9.2 Hz, 1H), 7.55 (t, J = 8.4, 17 Hz, 1H), 7.97 (d, J = 9.9 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 23.1, 24.4, 39.4, 66.1, 71.7, 96.2, 117.9, 118.5, 122.1, 123.8, 130.2, 133.5, 138, 163.6, 164.9; MS (APCI) m/z 272 $[M+1]^+$.

4.5.2. 4-(Isopentyloxy)-2-(3-methylbut-2-enyloxy) quinoline (31)

White solid, 70% yield; ^1H NMR (300 MHz, CD_3OD) δ 1.0 (d, J = 6.4 Hz, 6H), 1.71 (s, 3H), 1.87 (s, 3H), 1.79 (q, J = 6.1, 12.7 Hz, 2H), 1.92 (m, 1H), 4.11 (t, J = 6.7 Hz, 2H), 4.90 (d, J = 6.7 Hz, 2H), 5.12 (t, J = 1.7, 3.2 Hz, 1H), 6.02 (s, 1H), 7.19 (t, J = 8.4, 17 Hz, 1H), 7.28 (d, J = 9.2 Hz, 1H), 7.53 (t, J = 8.6, 17.2 Hz, 1H), 7.98 (d, J = 9.4 Hz, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 18.2, 23.2, 24.2, 38.6, 66, 69.4, 97.2, 117.5, 118.3, 122, 123.4, 130.7, 139, 164.6, 165; MS (APCI) m/z 200 $[M+1]^+$; Anal. Calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68. Found: C, 76.54; H, 8.70; N, 4.63.

4.5.3. 4-(Isopentyloxy)-2-(4-methylpent-3-enyloxy) quinoline (32)

White solid, 65% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (d, J = 6.5 Hz, 6H), 1.60 (s, 3H), 1.62 (s, 3H), 1.82 (q, J = 6.8, 14 Hz, 2H), 1.92 (m, 1H), 2.39 (q, J = 6.3, 13.1 Hz, 2H), 4.11 (t, J = 6.9, 13.9 Hz, 2H), 4.21 (t, J = 7.1, 14.4 Hz, 2H), 5.24 (t, J = 6.8, 13.5 Hz, 1H), 6.01 (s, 1H), 7.20 (t, J = 8.5, 17.1 Hz, 1H), 7.36 (d, J = 9.5 Hz, 1H), 7.55 (t, J = 8.2, 16.6 Hz, 1H), 7.99 (d, J = 9.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.1, 22.8, 23.2, 28.1, 37.6, 66.4, 70.3, 96.4, 116.4, 121.6, 123.8, 127.5, 130.9, 133.7, 163.8, 165.1; MS (APCI) m/z 314.5 $[M+1]^+$.

4.5.4. 2-Butoxy-4-(isopentyloxy) quinoline (33)

White solid, 56% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (d, J = 2.2, 5 Hz, 6H), 1.0 (t, J = 1.9, 3.1 Hz, 3H), 1.46 (m, 2H), 1.66 (m, 2H), 1.75 (q, J = 4.8, 9.1 Hz, 2H), 1.87 (m, 1H), 4.11 (t, J = 7.4, 13.2 Hz, 2H), 4.27 (t, J = 7.1, 14.2 Hz, 2H), 6.01 (s, 1H), 7.20 (t, J = 9.9, 19.3 Hz, 1H), 7.31 (d, J = 10.3 Hz, 1H), 7.55 (t, J = 10.1, 19.8 Hz, 1H), 7.99 (d, J = 11.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.8, 17.7, 20, 23.1, 25.9, 38.2, 51.3, 70, 97.7, 115.3, 117.4, 120.9, 124.9, 131.9, 138.2, 162.8; MS (APCI) m/z 288 $[M+1]^+$.

4.5.5. 2-(Benzyloxy)-4-(isopentyloxy) quinoline (34)

Colourless oil, 59% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (d, J = 34.1 Hz, 6H), 1.84 (q, J = 4.9, 9.6 Hz, 2H), 1.94 (m, 1H), 4.15 (t, J = 6.3, 12.5 Hz, 2H), 5.52 (s, 2H), 6.11 (s, 1H), 7.21 (m, 7H), 7.45 (t, J = 10.2, 20.1 Hz, 1H), 7.99 (d, J = 10.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.7, 23.1, 37.2, 49.3, 68.4, 96.8, 115.1, 117.3, 120.3, 121.2, 121.9, 125.9, 129.9, 139.2, 164.1; MS (APCI) m/z 322 $[M+1]^+$.

4.5.6. 2,4-Bis(3-methylbut-2-enyloxy) quinoline (35)

White solid, 67% yield; IR (KBr, cm^{-1}): 3459, 2970, 2914, 1645, 1591, 1498, 1455,1378; ^1H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3H), 1.71 (s, 3H), 1.82 (s, 3H), 1.87 (s, 3H), 4.62 (d, J = 7.4 Hz, 2H), 4.88 (d, J = 7.3 Hz, 2H), 5.11 (t, J = 5.4, 9.4 Hz, 1H), 5.53 (t, J = 5.8, 11 Hz, 1H), 6.03 (s, 1H), 7.19 (t, J = 8.1, 17 Hz, 1H), 7.22 (d, J = 10.2 Hz, 1H), 7.53 (t, J = 9.3, 17.3 Hz, 1H), 8.01 (d, J = 9.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.8, 26.1, 26.3, 40.9, 66, 97.7, 115, 118.8, 120.5, 121.8, 124.1, 131.4, 136, 139.7, 164.1, 166.5; MS (APCI) m/z 298 $[M+1]^+$; Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. Found: C, 76.67; H, 8.00; N, 4.71.

4.5.7. 2-(Allyloxy)-4-(3-methylbut-2-enyloxy) quinoline (36)

Light yellow solid, 63% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.76 (s, 3H), 1.82 (s, 3H), 4.62 (d, J = 6.6 Hz, 2H), 4.91 (t, J = 2.6, 5.1 Hz, 2H), 5.10 (dd, J = 0.3, 17.2 Hz, 1H), 5.20 (dd, J = 0.3, 16.8 Hz, 1H), 5.53 (t, J = 5.7, 11.9 Hz, 1H), 5.92 (m, 1H), 6.05 (s, 1H), 7.18 (t, J = 8.3, 17.5 Hz, 1H), 7.28 (d, J = 10.2 Hz, 1H), 7.51 (t, J = 9, 19.1 Hz, 1H), 7.99 (d, J = 11.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.3, 23.9, 66.7, 71.2, 97.6, 116.2, 119.5, 122.8, 123.8, 130.3, 138.9, 162.7, 164.9; MS (APCI) m/z 270.1 $[M+1]^+$.

4.5.8. 2-(Allyloxy)-4-(4-methylpent-3-enyloxy) quinoline (37)

White solid, 69% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.61 (s, 3H), 1.64 (s, 3H), 2.60 (q, J = 4.7, 9.2 Hz, 2H), 4.23 (t, J = 5.3, 11.4 Hz, 2H), 4.91 (t, J = 3.2, 6.4 Hz, 2H), 5.04 (dd, J = 0.2, 19.2 Hz, 1H), 5.17 (dd, J = 0.2, 20 Hz, 1H), 5.26 (m, 1H), 5.91 (m, 1H), 6.03 (s, 1H), 7.20 (t, J = 7.9, 15.5 Hz, 1H), 7.29 (d, J = 9.3 Hz, 1H), 7.53 (t, J = 8.1, 16 Hz, 1H), 8.0 (d, J = 9.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.7, 28.2, 30.2, 44.6, 60.9, 68.8, 97.3, 115.2, 117.1, 119.5, 122.1, 123.9, 131.9, 132.7, 159.8, 163.2; MS (APCI) m/z 284.4 $[M+1]^+$.

4.5.9. 2-(3-Methylbut-2-enyloxy)-4-(4-methylpent-3-enyloxy) quinoline (38)

White solid, 70% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.70 (s, 3H), 1.74 (s, 3H), 2.59 (q, J = 4.9, 9.7 Hz, 2H), 4.04 (t, J = 6.8, 13.4 Hz, 2H), 4.88 (t, J = 5.1, 11.2 Hz, 2H), 5.12 (t, J = 5.9, 12.2 Hz, 1H), 5.23 (t, J = 7.2, 13.2 Hz, 1H), 6.01 (s, 1H), 7.20 (t, J = 8.9, 17.5 Hz, 1H), 7.28 (d, J = 10.2 Hz, 1H), 7.53 (t, J = 8.3, 17.7 Hz, 1H), 7.99 (d, J = 10.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.4, 18.8, 22.8, 23.8, 26.2, 40.9, 68.5, 97.4, 115, 119.6, 120.5, 121.9, 132.9, 124, 131.4, 136, 139.7, 164.1; MS (APCI) m/z 312.3 $[M+1]^+$.

4.5.10. 2,4-Bis(4-methylpent-3-enyloxy) quinoline (39)

White solid, 68% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 1.59 (s, 6H), 1.62 (s, 6H), 2.23 (m, 4H), 4.07 (t, J = 7.1, 14.3 Hz, 2H), 4.25 (t, J = 6.8, 12.5 Hz, 2H), 5.01 (t, J = 5.4, 11.9 Hz, 1H), 5.23 (t, J = 6.2,

12.2 Hz, 1H), 6.0 (s, 1H), 7.20 (t, J = 8.4, 17.2 Hz, 1H), 7.36 (d, J = 11 Hz, 1H), 7.54 (t, J = 9.2, 18 Hz, 1H), 7.97 (d, J = 11.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.2, 18.6, 22.2, 22.9, 26.3, 39.9, 67.9, 97.2, 115.2, 117.3 119.2, 121.5, 121.9, 133.9, 124.8, 132.5, 139.3, 139.7, 163.1; MS (ESI) m/z 325 $[M]^+$; Anal. Calcd for $C_{21}H_{27}NO_2$: C, 77.50; H, 8.36; N, 4.30. Found: C, 77.28; H, 8.25; N, 4.29.

4.5.11. 2-Butoxy-4-(4-methylpent-3-enyloxy) quinoline (40)

Light yellow solid, 53% yield; IR (KBr, cm^{-1}): 3364, 2984, 2868, 1663, 1591, 1488, 1401, 1222; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, J = 7.4, 14.1 Hz, 3H), 1.70 (s, 3H), 1.74 (s, 3H), 1.43 (m, 2H), 2.0 (m, 2H), 2.59 (q, J = 5.2, 10.1 Hz, 2H), 4.04 (t, J = 6.3, 11.9 Hz, 2H), 4.27 (t, J = 6.9, 13.1 Hz, 2H), 5.23 (t, J = 7.1, 13.3 Hz, 1H), 6.0 (s, 1H), 7.23 (t, J = 9.2, 18.2 Hz, 1H), 7.34 (d, J = 10.1 Hz, 1H), 7.56 (t, J = 9.8, 18.2 Hz, 1H), 8.0 (d, J = 11.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2, 18.2, 19.3, 22.2, 26.9, 34.3, 65.9, 69.4, 97.6, 116.1, 117.7, 121.5, 122.3, 124.2, 130.9, 135.5, 138.1, 163.1; MS (APCI) m/z 300 $[M+1]^+$.

4.5.12. 2-(Benzyloxy)-4-(4-methylpent-3-enyloxy) quinoline (41)

Colourless oil, 55% yield; IR (KBr, cm^{-1}): 3262, 2898, 1632, 1535, 1348, 1270, 1143; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (s, 3H), 1.75 (s, 3H), 2.66 (q, J = 5.4, 11 Hz, 2H), 4.08 (t, J = 7.3, 13.6 Hz, 2H), 5.25 (t, J = 7.3, 14.3 Hz, 1H), 5.5 (s, 2H), 6.10 (s, 1H), 7.12 (m, 6H), 7.29 (d, J = 9.3 Hz, 1H), 7.40 (t, J = 9.9, 18.9 Hz, 1H), 7.96 (d, J = 11.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.7, 22.1, 27.3, 51.3, 68.3, 97, 116.3, 117.1, 120.1, 121.4, 124.9, 131.9, 136.3, 137.2, 165.8; MS (APCI) m/z 334 $[M+1]^+$.

4.5.13. 2-(3-Methylbut-2-enyloxy)-4-(prop-2-ynyloxy) quinoline (42)

White solid, 64% yield; IR (KBr, cm^{-1}): 3322, 2945, 2811, 2319, 1611, 1549, 1412, 1302; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (s, 3H), 1.81 (s, 3H), 2.6 (s, 1H), 4.82 (s, 2H), 4.91 (d, J = 6.5 Hz, 2H), 5.13 (t, J = 1.2, 3.3 Hz, 1H), 6.15 (s, 1H), 7.21 (t, J = 7.9, 15.2 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.55 (t, J = 9.3, 18.4 Hz, 1H), 8.0 (d, J = 9.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.3, 24.1, 57.2, 69.2, 75.4, 97.3, 114.5, 117.5, 121.6, 125.5, 130.4, 132.2, 134.3, 136.9, 169.5; MS (APCI) m/z 268.1 $[M+1]^+$; Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.66; H, 6.26; N, 5.27.

4.5.14. 2-(Allyloxy)-4-(prop-2-ynyloxy) quinoline (43)

White solid, 71% yield; IR (KBr, cm^{-1}): 3233, 2991, 2858, 2396, 1710, 1599, 1470, 1305, 1200; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (s, 1H), 4.83 (s, 2H), 4.93 (d, J = 6.4 Hz, 2H), 5.11 (dd, J = 1.2, 10.5 Hz, 1H), 5.22 (dd, J = 1.3, 9.2 Hz, 1H), 5.92, (m, 1H), 6.15 (s, 1H), 7.22 (t, J = 8.9, 17.2 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.54 (t, J = 8.2, 18.6 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 57.3, 70.5, 74.1, 97.1, 114.4, 115.5, 119.6, 125.5, 131.9, 134.0, 137.4, 169.9; MS (APCI) m/z 240.1 $[M+1]^+$; Anal. Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.71; H, 5.56; N, 5.81.

4.5.15. 2-(Isopentyloxy)-4-(prop-2-ynyloxy) quinoline (44)

White solid, 61% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (s, 3H), 1.04 (s, 3H), 1.61 (q, J = 5.8, 12.5 Hz, 2H), 1.87 (m, 1H), 2.61 (s, 1H), 4.26 (t, J = 7.2, 13.7 Hz, 2H), 4.82 (s, 2H), 6.13 (s, 1H), 7.21 (t, J = 6.0, 14.3 Hz, 1H), 7.31 (d, J = 5.8 Hz, 1H), 7.58 (t, J = 6.7, 13.9 Hz, 1H), 8.0 (d, J = 7.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 23.1, 27, 30.2, 36, 56.8, 98.5, 114.5, 122, 124.1, 131.8, 139.5, 160.9, 163.6; MS (APCI) m/z 270.1 $[M+1]^+$; Anal. Calcd for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.98; H, 7.29; N, 5.24.

4.5.16. 2-Butoxy-4-(prop-2-ynyloxy) quinoline (45)

White solid, 53% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0 (d, J = 6.4 Hz, 3H), 1.43 (m, 2H), 1.79 (m, 2H), 2.58 (s, 1H), 4.20 (t, J = 6.1, 12.7, 2H), 4.73 (t, J = 6.5, 12.1 Hz, 2H), 6.0 (s, 1H), 7.20 (t, J = 6.2, 14.1 Hz, 1H), 7.34 (d, J = 5.2 Hz, 1H), 7.54 (t, J = 7.1, 14.3 Hz, 1H), 8.01 (d, J = 7.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.3, 19.9, 30.2, 56.4, 69, 75.2, 97, 116.2, 121, 126.1, 130.3, 139.2, 160, 162.6; MS (APCI): m/z 256.1 $[M+1]^+$; Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.53; H, 6.89; N, 5.52.

4.5.17. 2,4-Bis(2-ethoxyethoxy) quinoline (46)

Colourless oil, 67% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, J = 6, 11.9 Hz, 3H), 1.28 (t, J = 5.7, 10.9 Hz, 3H), 3.66 (q, J = 6.3, 14.1 Hz, 4H), 3.83 (t, J = 4.8, 9.2 Hz, 2H), 3.91 (t, J = 4.5, 9.7 Hz, 2H), 4.27 (t, J = 6.3, 13.1 Hz, 2H), 4.63 (t, J = 5.9, 10.6 Hz, 2H), 6.29 (s, 1H), 7.32 (t, J = 7.4, 15.1 Hz, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.75 (t, J = 6.9, 14.8 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 15.7, 65.5, 67.1, 68.5, 69, 69.5, 92.2, 122.5, 123.7, 127.3, 130.4, 163.2; MS (APCI) m/z 304 $[M-1]^+$.

4.5.18. 2,4-Bis(octyloxy) quinoline (47)

Colourless oil, 72% yield; IR (KBr, cm^{-1}): 3436, 2953, 2953, 2855, 1647, 1591, 1455,1230; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 4.2 Hz, 6H), 1.28 (m, 20H), 1.69 (q, J = 5.2, 9.5 Hz, 2H), 1.90 (q, J = 5.3, 10.1, 2H), 4.07 (t, J = 6.1, 12.6 Hz, 2H), 4.23 (t, J = 6.9, 11.9 Hz, 2H), 6.0 (s, 1H), 7.20 (t, J = 5.9, 10.9 Hz, 1H), 7.31 (d, J = 7.1 Hz, 1H), 7.56 (t, J = 7.2, 13.3 Hz, 1H), 8.0 (d, J = 6.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.6, 23.1, 26.6, 27.5, 28.2, 28.7, 29.2, 29.8, 29.9, 32.3, 33.3, 34.6, 42.4, 69.1, 97.4, 114.6, 117.4, 121.8, 124, 131.5, 139.5, 162.3, 164.2; MS (APCI) m/z 384 $[M+1]^+$; Anal. Calcd for $C_{25}H_{39}NO_2$: C, 77.87; H, 10.19; N, 3.63. Found: C, 77.76; H, 10.25; N, 3.65.

4.6. Biological methods

4.6.1. Cell cytotoxicity assay using MTT

The synthesized analogues were tested for their cytotoxicity in cell viability assay and non-toxic concentration of each analogue was used for further screening in cell based anti-HIV assay. The cytotoxicity was assessed by using MTT based cell viability assay Kit (Roche, Germany) in the CEM-GFP, according to the manufacturer's protocol. Briefly, 2×10^4 cells/well were seeded in 96-well plate; samples were then added into the wells at different concentrations keeping untreated wells as controls. After 48–72 h incubation, 10 μl of MTT Reagent (5 mg/ml) were added into the wells to allow the reaction to proceed. Formazan crystals produced during the reaction were solubilized and colour development was read at 540 nm.

4.6.2. Anti HIV screening in CEM-GFP cells

Human CD4+ T cell line, CEM-GFP cells were infected with HIV-$1_{NL-4.3}$ virus at a multiplicity of infection (MOI) of 0.05 using the standard protocol previously published from our laboratory.[24] The cells were then incubated with samples for up to 8 days post infection. Virus production was assayed in the culture supernatant on day 8 post infection by p24 antigen capture ELISA (Perkin–Elmer, USA).

Acknowledgement

Authors thank the Department of Biotechnology, Ministry of Science and Technology, Government of India for the financial support to this project (Grant No. BT/PR7020/Med/14/930/2005 Dt. 23/05/2006).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.015.

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