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SYNTHESIS AND ANTI-HIV ACTIVITY OF SOME NOVEL 2, 3-DISUBSTITUTED QUINAZOLIN-4(3H)-ONES

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ABSTRACT

Three of novel 2, 3-disubstituted quinazolin-4(3H)-one derivatives, have been synthesized by condensation of 2-substituted benzo[1,3]oxazine-4-ones and primaquine. Their chemical structures were assigned by means of spectral analysis (FT-IR, ^{1}H - NMR, MS). Synthesized compounds were screened for *in vitro* antiviral activity against HIV-1 in MT-4 cells. Compound 6-bromo-3-(4-(6-methoxyquinolin-8-ylamino)pentyl)-2-phenylquinazolin-4(3H)-one (PY-QZ MBR) exhibited 15 percent maximum protection against replication of HIV-1 (IIIB) in acutely infected MT-4 cells .

Key words: Anti – HIV, Quinagolines, Syntheais.

INTRODUTION

Quinazolin-4(3H)-one is a versatile lead molecule for the design of potential bioactive agents, and 2,3-disubstituted quinazolin-4(3H)-ones were reported to possess anti-HIV[1-4], anticancer[4-7]and antiviral[8-10] activities. A large number of quinazolines have been synthesized and studied for wide range of antiviral activity, but the antiviral activity against HIV virus of quinazolines is relatively less explored and therefore, some new 6-bromo/6,8-dibromo-2,3-disubstitutedquinazolinones were synthesized **(Scheme 1)** and screened for anti-HIV activity.

Reaction of anthranilic acid with benzoyl chloride yielded 2-phenyl-1,3-benzoxazin-4-one by N-acylation via dehydrative cyclization[12]. Three of novel 2,3-disubstituted quinazolin-4(3H)-one derivatives were synthesized by condensation of the

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primary aromatic amino group of primaquine with 2-phenyl-1,3-benzoxazine-4-one to afford 2,3-disubstituted quinazolin-4(3H)-one derivatives **(Scheme 1).**

EXPERIMENTAL

Melting points were determined using an open end capillary tube method and are uncorrected. FT-IR were recorded on Perkin Elmer–1605 series FT-IR in KBr disc. ^{1}H NMR spectra were recorded with 400 MHz on a Bruker FT-NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded on a Varian Atlas CH-7 Mass spectrophotometer at 70 eV.

Synthesis of 6-bromo/6,8-dibromo2-phenyl 1,3-benzoxalin-4-one (1)

Anthranilic acid, 5-bromo or 3, 5-dibromoanthanilic acid (0.1 mol) was dissolved in 50 mL of dry pyridine. To this solution, benzoyl chloride (0.2 mol) was added drop wise with constant stirring, at low temperature. The reaction mixture was cooled. When the addition of benzoyl chloride was completed, the resultant reaction mixture was treated with 10% sodium bicarbonate. The reaction mixture was filtered and washed repeatedly with water to remove inorganic materials. The crude product obtained was recrystallized from ethanol.

General synthetic method for preparation of compounds (3)

An equimolar (0.01 mol) of 2-phenyl benzo[1,3]oxazine-4-one **(1)** and primaquine were mixed and the mixture was refluxed for 6 h in 10 mL of ethanol. Upon cooling, the mixture was poured onto crushed ice. The precipitated solid was collected and recrystalized from ethanol to give the desired title compounds.

3 - (4 - (6 - Methoxyquinolin - 8 - ylamino) pentyl) - 2 - phenylquinazolin - 4(3H) - one (3a) (PY - QZ)

(R_1 = H ; R_2 = H): yield 60%, solid, mp 127ºC, R_f 0.324, IR (KBr) (cm^{-1}), 1747 (C=O), 1623 (C=N), 1457 (C=C); ^{1}H NMR (DMSO-d_6): 1.23(s, 3H, CH_3),1.48 (q, 2H, CH_2) 1.58 (q, 2H, CH_2) 3.20 (q, 2H, CH_2) 3.70 (s, 3H, CH_3) 4.0(s, 1H, NH), 6.4 - 8.6 (m, 13H, Ar-H); MS (m/z): 464.56 .

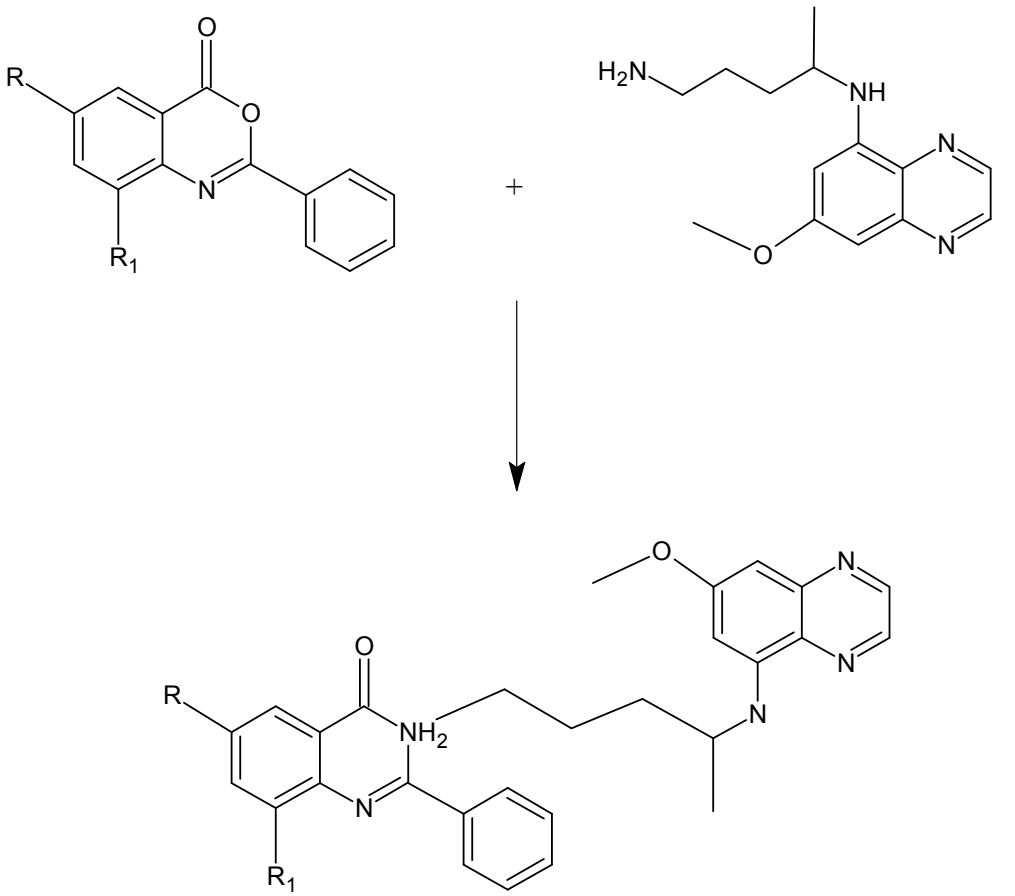
6-Bromo-3-(4-(6-methoxyquinolin-8-ylamino)pentyl)-2-phenylquinazolin-4(3H)-one (PY-QZ-MBR)

(R_1= Br ; R_2 = H): yield 53%, solid, mp 217ºC, R_f 0.455: IR (KBr) (cm^{-1}): 3469 (NH), 1747 (C=O), 1623 (C=N), 1457 (C=C), 687 (C-Br); 1.23(s, 3H, CH_3),1.48 (q, 2H,

CH_2) 1.58 (q, 2H, CH_2) 3.20 (q, 2H,CH_2), 3.70 (s,3H,CH_3) 4.0 (s,1H,NH), 6.4 - 8.6 (m, 12H, Ar-H); MS (m/z): 542.13 .

6,8-Dibromo-3-(4-(6-methoxyquinolin-8-ylamino)pentyl)-2-phenylquinazolin-4(3H)-one (PY-QZ-DBR)

(R_1 = Br ; R_2 = Br): yield 48%, solid, mp 140ºC, R_f 0.186 :IR (KBr) (cm^{-1}): 3363 (NH), 1652 (C=O), 1590 (C=N), 1519 (C=C), 1157 (SO_2); ^{1}H NMR (DMSO-d_6): 1.23(s, 3H, CH_3),1.48 (q, 2H,CH_2) 1.58 (q, 2H,CH_2) 3.20 (q, 2H,CH_2) 3.70 (s,3H,CH_3) 4.0(s,1H,NH), 6.4-8.6 (m, 11H, Ar-H); MS (m/z): 620.04.



R = H, Br, R_1 = H, Br

Scheme I

Anti-HIV activity

The compounds were tested for antiHIV activity against the replication of HIV-1(III_B) in MT-4 cells[11]. The cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat–inactivated fetal calf serum (FCS), 2 mM- glutamine, 0.1% sodium bicarbonate and 20 μgm/mL gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain was used in the experiment. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at -70°C until used.

Table 1. Anti-HIV activity of quinazoline-4(3H)-one derivatives in MT-4 cells

Code	Strain	IC_{50}[a] (μM)	CC_{50}[b](μM)	Maximum protection (%)
PY	III_B	>1.8	1.8	1.5
PY-QZ	III_B	>6.9590	6.9590	2
PY-QZ MBR	III_B	>166.93	166.93	15
PYQZ DBR	III_B	>25.76	25.76	0
AZT	III_B	0.0064	65.90	106

[a]50% Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.

[b]50% cytotoxic concentration of compound, required to reduce the viability of mock-infected MT-4 cells by 50%.

Inhibitory effects of the compounds on HIV-1 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells, as estimated by the MTT assay. Briefly, 50 μL of HIV-1(100-300 $CCID_{50}$) were added to MT–4 cells at a final concentration of $6x10^5$cells/mL. After 5 days of incubation, at 37°C the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock-infected MT-4 cells was assessed by the MTT method. Anti-HIV activity and cytotoxicity of AZT were also determined by a similar method in MT-4 cells. The anti-HIV activity and cytotoxicity data are presented in Table 1. Anti HIV activity of Quinazolin-4(3H)-one derivatives in MT-4 cells

RESULTS AND DISCUSSION

Primaquine is an analog of 8-aminoquinoline and found to inhibit HIV Integrase[13] and New Castle Virus by blocking the synthesis of its ribonucleic acid (RNA) and release of its progeny[14]. The aforementioned facts prompted us to look for antiviral activity of primaquine derivatives against HIV-1(IIIB) in MT-4 cell-line.

Among the tested compounds 6-bromo-3-(4-(6-methoxyquinolin-8-ylamino)pentyl)-2-phenylquinazolin-4(3H)-one (PY-QZ MBR) inhibited the replication of HIV-1 in acutely infected MT-4 cells with 15% maximum protection at sub toxic concentration, where as the compounds PY-QZ and PY-QZ DBR displayed cytotoxic properties in MT-4 cells. Also pyridine derivative exhibited the best cytostatic activities against colon carcinoma, human T-lymphocyte and murine leukemia. Further molecular modification in this series may help in optimizing antiviral activity. Our results indicate that the lead compound primaquine (PY) is not active against HIV-1 replication (EC50 > 1.8 μM CC50 =1.8 μM) though it is reported to be blocking agent for HIV-1 integrase (EC50 = 35μM).

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