



SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME NOVEL 6-BROMO/6, 8-DIBROMO, N-BENZOYL-4-(4-OXO-2-PHENYL-4H-QUINAZOLIN-3-YL)-BENZENESULPHONAMIDE

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ABSTRACT

A series of novel 2, 3-disubstituted quinazolin-4(3H)-one derivatives have been synthesized by condensation of 2-substituted 1, 3-benzoxazin-4-ones and sulphabenzamide. Their chemical structures were assigned by means of spectral analysis (FT-IR, ¹H-NMR, MS). Synthesized compounds were screened for antiviral activity against HIV 1 and 2 in MT-4 cells. Compound **SPB** exhibited 47 percent maximum protection against replication of HIV-1 in acutely infected MT-4 cells.

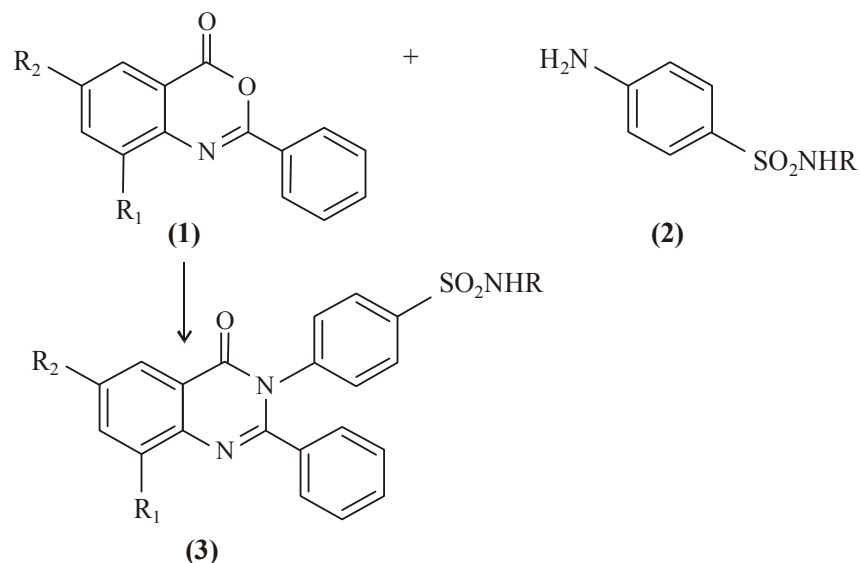
Key word: HIV, MT-4 Cells, Quinazoline, Sulphabenzamide, MTT assay.

INTRODUCTION

Quinazolin-4(3H)-one is a versatile lead molecule for the designing of potential bioactive agents and 2, 3-disubstituted quinazolin-4(3H)-ones were reported to possess anti-HIV¹⁻³, anticancer⁴⁻⁸ and antiviral⁹⁻¹² activities. We have previously synthesized novel heterocyclic compounds and screened for antiviral activity. Some of derivatives exhibited significant activity against HIV and vaccinia viruses¹³⁻¹⁶. A large number of quinazolines have been synthesized and studied for wide range of antiviral activity, but the antiviral activity against HIV of quinazolines is relatively unexplored. Based on these findings, some new 6-bromo/6, 8-dibromo-2, 3-disubstituted quinazolinones were synthesized and screened for antiviral activity against HIV. Anthranilic acid reaction with benzoyl chloride yields 2-phenyl-1, 3-benzoxazin-4-one by N-acylation via dehydrative cyclization¹⁷ (**1**). A series of some novel 2, 3-disubstituted quinazolin-4(3H)-one derivatives have been synthesized by condensation of primary aromatic amino group of sulphabenzamide with 2-substituted-1, 3-

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benzoxazine-4-one to afford 2, 3-disubstituted quinazolin-4(3H)-one (**3**) derivatives (**4**) (Scheme 1).



Where R= H, COC₆H₅

Scheme 1: Synthetic protocol of compounds

EXPERIMENTAL

Melting points were determined using open ended capillary tube method and are uncorrected. FT-IR spectra were recorded on Perkin-Elmer-1605 series FT-IR in KBr disc. ¹H NMR spectra were recorded on 400 MHz on 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-dibromo, N-benzoyl-4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzenesulphonamide (**3**).

An equimolar (0.01 mol) mixture of 6-bromo/6, 8-dibromo 2-substituted-1, 3-benzoxazin-4-one (**1**) and sulphabenzamide (**2**) was refluxed for 6 h with 10 mL of ethanol. The mixture was cooled to room temperature and poured into crushed ice; the solid obtained was recrystallized from ethanol.

N-Benzoyl-4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzenesulphonamide (SPA)

Yield 72%, mp 275°C, IR (KBr) (cm⁻¹): 3363 (NH), 1652 (C=O), 1590 (C=N), 1519

(C=C), 1150 (SO₂); PMR (DMSO-d₆): 7.2 - 8.0 (m, 18H, Ar-H), 10.7 (s, 1H, -SO₂NH); MS (m/z): 481 (M⁺).

6-Bromo-N-benzoyl-4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzenesulphonamide (SPB)

Yield 53%, mp 147°C, IR (KBr) (cm⁻¹): 3367 (NH), 1660 (C=O), 1590 (C=N), 1526 (C=C), 1157 (SO₂); PMR (DMSO-d₆): 7.3 - 8.0 (m, 17H, Ar-H), 10.6 (s, 1H, -SO₂NH).

6, 8-Dibromo, N-benzoyl-4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzene sulphonamide (SPC)

Yield 46%, mp 245°C, IR (KBr) (cm⁻¹): 3356 (NH), 1645 (C=O), 1600 (C=N), 1519 (C=C), 1146 (SO₂); PMR (DMSO-d₆): 7.2 - 7.9 (m, 16H, Ar-H), 10.7 (s, 1H, -SO₂NH).

Anti-HIV activity

The compounds were tested for anti-HIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells¹⁸. 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 µg/mL gentamicin (culture medium). HIV-1 (HTLV-III_B/LAI) strain and HIV-2 (LAV-2_{ROD}) strain were 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.

Inhibitory effects of the compounds on HIV-1 and HIV-2 replications were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 µL of HIV-1 and HIV-2 (100-300 CCID₅₀) was added to a flat-bottomed MT-4 cells were added at a final concentration of 6x10⁵ cells/mL. After 5th day of incubation, at 37°C, the number of viable cells were 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 were assessed by the MTT method. Anti-HIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The anti-HIV activity and cytotoxicity data are presented in Table 1.

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

Code	Strain	IC ₅₀ (µg/mL)	CC ₅₀ (µg/mL)	Max. Prot. (%)
SPA	III _B	>125	>125	20

Cont...

Code	Strain	IC ₅₀ (µg/mL)	CC ₅₀ (µg/mL)	Max. Prot. (%)
	ROD	>125	>125	24
SPB	III_B	120	>125	47
	ROD	>125	>125	33
SPC	III _B	>125	>125	16
	ROD	>125	>125	39
AZT	III _B	0.0012	65.90	126
	ROD	0.00062	65.90	148

^a50% Effective concentration required to reduce virus induced cytopathicity by 50%.

^b50% Cytotoxic concentration required to reduce host cell viability by 50%.

RESULTS AND DISCUSSION

From the results of antiviral activity and cytotoxicity, it was observed that all the compounds exhibit 16-47 percent maximum protection against replication of HIV-1 and 2 in acutely infected MT-4 cells (Table 1). 6-Bromo-benzoyl-4-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzene sulphonamide (SPB) inhibits the replication of HIV-1(III_B) with IC₅₀ of 120 µg/mL and cytotoxicity was found to have more than 125 µg/mL in MT-4 cells. Apparently, further bromination of the quinazolin-4(3H)-one is detrimental to the antiviral activity of the compounds (SPB). Further molecular modification in this series may help in optimizing antiviral activity.

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Revised : 07.12.2009

Accepted : 12.12.2009