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[Research article]

SYNTHESIS, ANTI-HIV ACTIVITY AND CYTOTOXICITY OF 2-PHENYL, 3-SULPHONAMIDO QUINAZOLIN-4 (3*H*) -ONES

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ABSTRACT

A series of novel 2,3-disubstitutedquinazolin-4(3H)-ones have been synthesized by condensation of 2-substituted benzo [1,3] oxazine-4-ones and primary amines. Their chemical structures were assigned by means of spectral analysis (FT-IR and ^{1}H-NMR). Synthesized compounds were screened for *in vitro* antiviral activity against HIV-1 and -2 replication in MT-4 cells. Cytotoxicity was also investigated in uninfected MT-4 cells (C-type Adult T Leukemia cells). All the synthesized compounds exhibited cytotoxicity in MT-4 cells (CC_{50}: 1.93-87 µg/ml) and compound SPB-III displayed marked cytostatic properties in MT-4 cells (CC_{50}:1.93± 0.29µg/ ml).

Key words: Quinazoline, HIV, MT-4 Cells, MTT assay

INTRODUCTION

Quinazolin-4-(3*H*)-one is a versatile lead molecule for the design of potential bioactive agents and its derivatives were reported to possess broad spectrum activities. 2-Phenyl -3 - Substituted Quinazolin-4-(3H)-ones were reported to have anti-HIV, some of their derivatives have also shown significant anti-HIV activity[1,2,3], anti-cancer activity were studied for 2,3-di substituted quinazolinones derivatives and they showed promising anticancer potential[4,5,6]. Quinazolinones were screened for their board spectrum anti-viral activity and they were found to be potential derivatives for further studies[7,8,9].

Anthranilic acid reaction with benzoyl chloride yields 2-phenyl-1,3-benzoxazin-4-one by N-acylation via dehydrative cyclization[8]. A series of some novel 2,3-disubstituted quinazolin-4(3H)-one derivatives have been synthesized by condensation of primary aromatic amino group of sulphonamides with 2-substituted-1,3 - benzoxazine-4 - one[11] (Scheme-1). These compounds were previously investigated for antiviral activity against bio defence viruses and some of these derivatives inhibits the influenza viruses[11]. Present work is to investigate the anti-HIV activity and cytotoxicity of Novel 2-phenyl-3-sulphonamido quinazolin-4(3*H*)-one derivatives (Table 1)

EXPERIMENTAL

Melting points were determined using open ended capillary tube method and are uncorrected. FT-IR

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recorded on Perkin Elmer–1605 series FT-IR in KBr disc. ^{1}H NMR Spectra was recorded at 400 MHz on Bruker FT-NMR spectrophotometer using TMS as internal standard. Mass spectra was recorded on a Varian Atlas CH-7 Mass Spectrophotometer at 70 eV.

Synthesis of 2-phenyl-3-substituted quinazolin-4-*(3H)*-one derivatives

An equimolar (0.01 mol) mixture of 2-phenyl-1,3-benzoxazine-4-one and aromatic primary amine sulphonamide derivatives (Sulphanilic acid, sulphanilamide, sulphadiazine, sulphadimidine, sulphamoxole and sulphamethoxazole) was refluxed for 6 hours with 10 ml of acetic acid[11]. The mixture was cooled to room temperature and poured over crushed ice, filter and then washed with water. The solid thus obtained was recrystallised from ethanol.

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 cells[8]. 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-IIIB/LAI) strain and HIV-2 ($LAV\text{-}2_{ROD}$) 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.

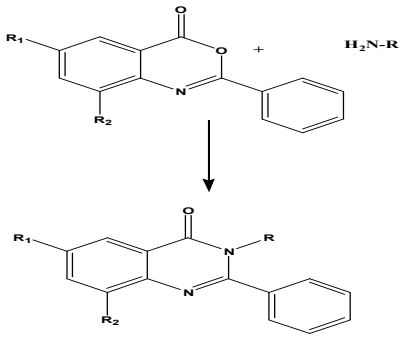
Inhibitory effects of the compounds on HIV-1 and HIV-2 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 µl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) was added to a flat-bottomed MT–4 cells ($6x10^5$ cells/ml). After 5 days of incubation, at 37°C the number of viable cells were determined by the 3 - (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium 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 standard AZT were also performed by a similar method in MT-4 cells. The anti-HIV activity and cytotoxicity data are presented in Table 2.

RESULTS

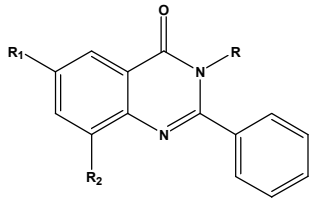
2-phenyl-3-sulphonamido quinazolin-4(*3H*)-one derivatives were synthesized by condensation of the compounds containing primary aromatic amino group of sulphonamide derivatives with 2-phenyl-1,3-benzoxazin-4-one[11]. These compounds were previously investigated for antiviral activity against bio-defense viruses and some of these derivatives inhibited the influenza viruses[11].

The inhibitory effect of 2-phenyl-3-sulphonamido quinazolin-4 (*3H*)-ones on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT assay. Cytotoxicity was also investigated in uninfected MT-4 cells (C-type Adult T Leukemia cells). All the synthesized compounds exhibited cytotoxicity in MT-4 cells (CC_{50}: 1.93-87 µg/ml) and Compound SPB-III displayed marked cytostatic properties in MT-4 cells (CC_{50}: 1.93±0.29 µg/ ml).

Synthesis of 2-Phenyl-3-substitutedQuinazolin-4(3*H*)-one derivatives



Where
R_1= H, Br
R_2= H, Br
R= Different substitution



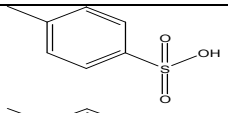
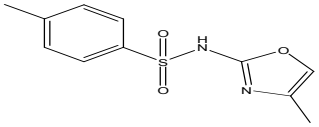
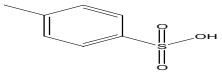
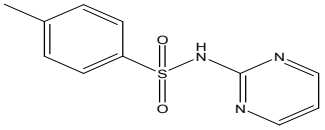
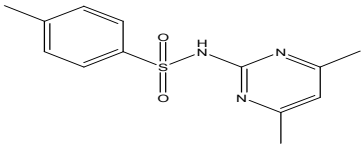
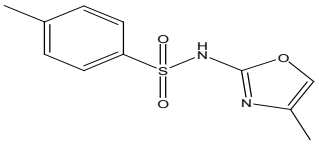
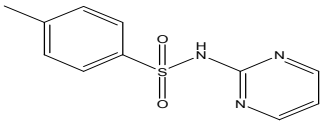
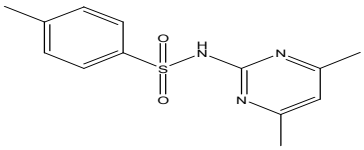
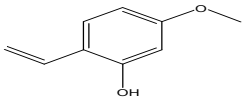
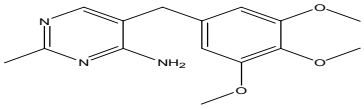
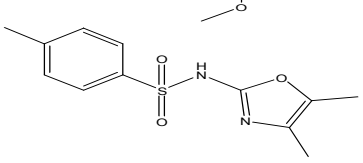
COMPOUND CODE	R_1	R_2	R-NH_2
PB-SA	H	H	
PB-SM	H	H	
MBR-SA	Br	H	
MBR-SD	Br	H	
MBR-SDM	Br	H	
MBR-SM	Br	H	
DBR-SD	Br	Br	
DBR-SDM	Br	Br	
PSAVA	H	H	
SPB-III	H	H	
SPF-IIIBr	Br	H	

Table 1: Anti-HIV activity and cytotoxicity of Quinazolin-3(4H)-one derivatives

Compounds	Strain	IC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Maximum Protection
PB-SA	IIIB	>67.58	67.58±10.88	16
	ROD	>67.58	67.58±10.88	12
PB-SM	IIIB	>60.88	60.88±10.46	8
	ROD	>60.88	60.88±10.46	1
MBR-SA	IIIB	>87.15	87.15±24.70	3
	ROD	>87.15	87.15±24.70	5
MBR-SD	IIIB	>70.73	70.73±9.17	14
	ROD	>70.73	70.73±9.17	12
MBR-SDM	IIIB	>58.45	58.45±12.18	5
	ROD	>58.45	58.45±12.18	4
MBR-SM	IIIB	>58.08	58.08±11.76	3
	ROD	>58.08	58.08±11.76	5
DBR-SD	IIIB	>72.20	≥72.20	6
	ROD	>72.20	≥72.20	2
DBR-SM	IIIB	>71.70	70.73±19.40	5
	ROD	>71.70	70.73±19.40	8
PSA-VA	IIIB	>89.08	89.08±14.20	4
	ROD	>89.08	89.08±14.20	8
SPB-III	**IIIB**	**>1.93**	**1.93±0.29**	**2**
	ROD	**>1.93**	**1.93±0.29**	**1**
SPF-IIIBr	IIIB	>96.20	≥96.20	1
	ROD	>96.20	≥96.20	1
SP-II	IIIB	>36.63	36.63±9.14	1
	ROD	>36.63	36.63±9.14	2
S-II	IIIB	>87.20	87.20±22.75	4
	ROD	>87.20	87.20±22.75	5
AZT (STD)	IIIB	0.0015±0.0002	>25	96
Zidovudine	ROD	0.0016±0.0003	>25	76

[a]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%.
HIV I -(HTLV IIIB): HIV-2 (ROD)

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