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Design, Synthesis, Antiviral and Cytotoxicity Studies of Some 2-Phenyl-3-substituted quinazolin-4-(3*H*)-ones

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A series of novel 2-phenyl-3-substituted quinazolin-4-(3*H*)-one derivatives were synthesized and screened for antiviral activity against panel of human pathogenic viruses. The structures of the synthesized compounds were characterized by means of their IR, ^{1}H NMR data. The anti HIV activities of the new compounds were screened *in vitro* antiviral activity against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells using AZT-as standard. All the compounds displayed cytostatic properties in T-lymphocytes MT-4 cells. The compound 4-[(4-oxo-2-phenylquinazolin-3(4*H*)-yl-amino]methyl amino benzoic acid (QPAB) (CC_{50} = 11.90 µg/mL) was found to be more toxic in this series. 2-Amino-3-phenylquinazolin-4(3*H*)-one (BN) exhibited antiviral activity against Herpes Simplex virus-1,2 and vaccinia virus in HEL cells at the concentration of 10 and 12 µg/mL, whereas cytotoxicity was found to be 100 µg/mL (SI = 10). Among these compounds, compounds (QIS and QMB) exhibited antiviral activity against vesicular stomatitis virus in HeLa cells at the concentration of 12 µg/mL, whereas cytotoxicity was found to be 100 µg/mL (SI = 9).

Key Words: Cytotoxicity, Antiviral.

INTRODUCTION

Quinazolin-4-(3*H*)-one is a versatile lead molecule for the design of potential bioactive agents. 2-Phenyl-3-substituted quinazolin-4-(3*H*)-ones were reported to have anti HIV[1-3] anticancer[4-6] and antiviral[7-12] properties. A large number of quinazolines have been synthesized and studied for wide range of antiviral activity, but the antiviral activities of quinazolines against viruses has not been well explored.

Anthranilic acid reacts with benzoyl chloride to form 2-phenyl-1,3-benzoxazin-4-one by N-acylation followed by dehydrative cyclization. 2-Phenyl-3-amino quinazolin-4(3*H*)-one derivatives were synthesized by condensation of the compounds containing hydrazine hydrate with 2-phenyl-1,3-benzoxazine-4-one. A series of 2-phenyl-3-substituted quinazoline-4(3*H*)-one derivatives were synthesized by condensation of the compounds containing primary aromatic amino group and formaldehyde with 2-phenyl-3-amino quinazolin-4(3*H*)-one by Mannich reaction.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a (Shimadzu-8400s) FT-IR spectrophotometer, ^{1}H NMR spectra were determined Bruker AMX 400 MHZ with tetramethyl silane as an internal standard. The sample is dissolved in DMSO-d_6 and the ^{1}H NMR value is measured in δ ppm.

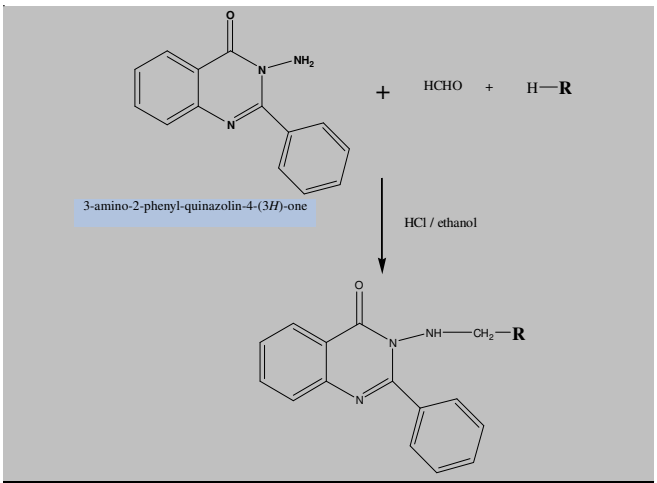
Synthesis of 2-phenyl-3-substituted quinazolin-4-(3*H*)-one derivatives: An equimolar (0.01 mol) mixture of quinazoline, different substituents and formaldehyde was refluxed for 6 h with 10 mL of ethanol in acidic condition. The mixture was cooled to room temperature and poured into crushed ice, filter and then washed with water. The solid thus obtained was recrystallised from ethanol. The yield and melting point was predicted in Table-1.

BN: IR (KBr, ν_{max}, cm^{-1}): 3212 (NH), 1598 (C=N), 1606 (C=C), 1664 (C=O); ^{1}H NMR (DMSO-d_6): 6-8.7 (m, 9H, Ar-H), 5.9 (s, 2H, NH_2).

QSD: IR (KBr, ν_{max}, cm^{-1}): 3397 (NH), 1508 (C=N), 1603 (C=O), 1080 (C-alkyl) , 1432 (SO_2); ^{1}H NMR (DMSO-d_6): 6.3-8.7 (m, 13H, Ar-H), 4.7 (s, 1H, NH), 3.4 (s, 2H, -CH_2-).

QPH: IR (KBr, ν_{max}, cm^{-1}): 3206 (NH), 1528 (C=N), 1729 (C=O), 1603 (C=C)); ^{1}H NMR (DMSO-d_6) 6.9-8.1 (m, 13H, Ar-H), 11.2 (s, 1H, NH), 4.4 (s, 2H, -CH_2-).

QBA: IR (KBr, ν_{max}, cm^{-1}): 3305 (NH), 1723 (C=O), 1573 (C=N), 1633 (C=C); ^{1}H NMR (DMSO-d_6): 6.9 - 8.1 (m, 14H, Ar-H), 3.2 (s, 2H, -CH_2-), 4.9 (s, 1H, NH).



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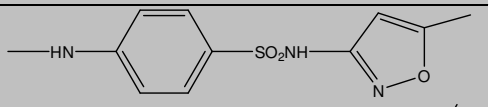
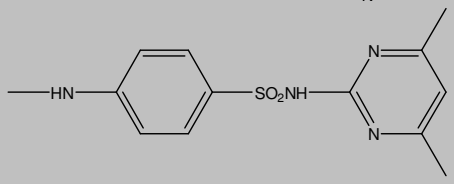
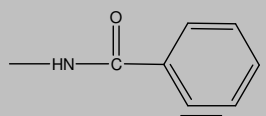
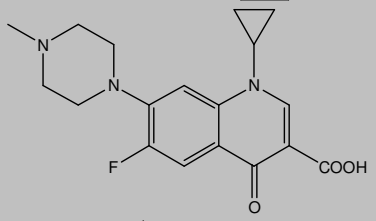
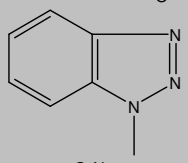
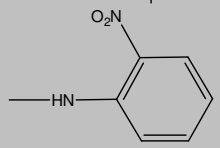
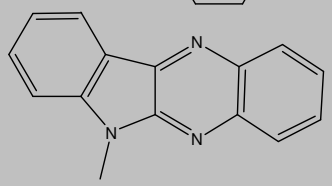
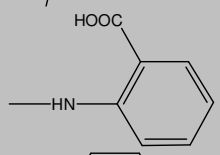
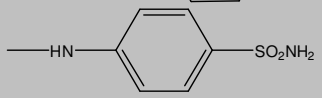
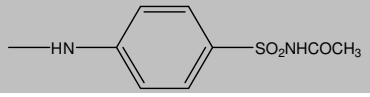
QSM	
QSD	
QBA	
QCP	
QBT	
QNA	
QIP	
QAA	
QSA	
QSS	

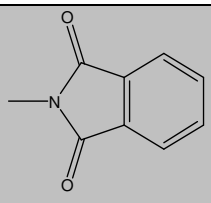
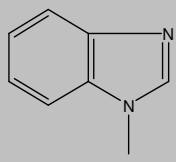
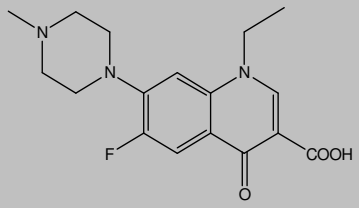
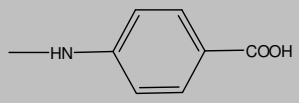
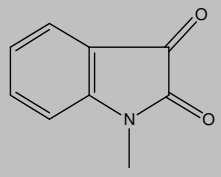
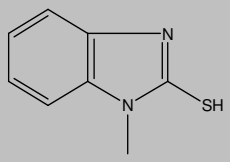
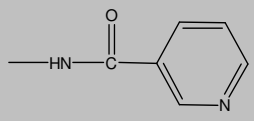
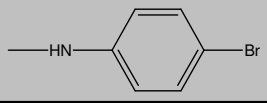
QPH	
QBI	
QNF	
QPAB	
QIS	
QMB	
QNI	
QBR	

TABLE-1
CHARACTERIZATION DATA OF COMPOUNDS

Comp. code	m.f.	Yield (%)	m.p. (ºC)	R_f Value	m.w.
QSM	$C_{25}H_{22}N_6O_4S$	67.6	180-182	0.64	302
QSS	$C_{23}H_{21}N_5O_4SNa$	70.7	160-164	0.60	486
QSD	$C_{27}H_{25}N_7O_3S$	89.5	206-210	0.56	527
QPH	$C_{23}H_{16}N_4O_3$	57.3	155-158	0.44	396
QBA	$C_{22}H_{18}N_4O_2$	61.3	120-122	0.38	370
QCF	$C_{31}H_{29}N_6O_4F$	58.1	221-226	0.62	581
QNF	$C_{31}H_{27}N_6O_4F$	73.6	210-215	0.53	579
QBI	$C_{22}H_{17}N_5O$	80.4	100-104	0.47	367
QBT	$C_{21}H_{16}N_6O$	53.4	166-170	0.40	368
QPAB	$C_{22}H_{18}N_4O_3$	58.2	180-184	0.47	366
QNA	$C_{21}H_{17}N_5O_3$	53.4	110-115	0.84	367
QIS	$C_{23}H_{16}N_4O_3$	48.7	140-146	0.47	376
QIP	$C_{29}H_{20}N_6O$	55.7	100-104	0.76	468
QMB	$C_{22}H_{17}N_5OS$	85.8	170-176	0.55	379
QAA	$C_{22}H_{18}N_4O_3$	61.3	231-235	0.60	366
QNI	$C_{21}H_{17}N_5O_2$	62.8	132-136	0.49	371
QSA	$C_{21}H_{19}N_5O_3S$	53.4	223-227	0.63	391
QBR	$C_{21}H_{17}N_4OBr$	55.3	106-110	0.86	401

QNF: IR (KBr, ν_{max}, cm^{-1}): 1481 (C=N), 1630 (C=C), 1718 (C=O), 1092 (C-F); ^1H NMR (DMSO-d_6): 6.9-8.0 (m, 11H, Ar-H), 9.4 (s, 1H, NH), 4.3 (s, 2H, -CH_2-), 9 (s, 1H, COOH).

QBI: IR (KBr, ν_{max}, cm^{-1}): 3249 (NH), 1591 (C=N), 1676 (C=O), 1591 (C=C); ^1H NMR (DMSO-d_6): 6.6-8.7 (m, 13H, Ar-H), 4.2 (s, 2H, -CH_2-), 4.8 (s, 1H, NH).

QBT: IR (KBr, ν_{max}, cm^{-1}): 3143 (NH), 1370 (C=N), 1655 (C=O), 1521 (C=C); ^1H NMR (DMSO-d_6): 6.0-8.8 (m, 13H, Ar-H), 3.4 (s, 2H, -CH_2-), 4.7 (s, 1H, NH).

QNA: IR (KBr, ν_{max}, cm^{-1}): 3377 (NH), 1509 (C=N), 1672 (C=O), 1445 (C=C); ^1H NMR (DMSO-d_6): 6.5-8.8 (m, 13H, Ar-H), 4.2 (s, 2H, -CH_2-), 4.8 (s, 1H, NH).

Biological investigation

Anti HIV assay: Anti HIV assay compounds were tested for their inhibitory effects against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells. The MT-4 cells were grown and maintained in RPMI-1640 DM medium supplemented with 10 % (v/v) heat-inactivated fetal calf serum (FCS), 2 mM-glutamine, 0.1 % sodium bicarbonate and 20 mg^3/mL gentamycin (culture medium). Inhibitory effect of test 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 mL of HIV-1 and HIV-2 (100-300 $CCID_{50}$) were added to a flat-bottomed microtiter tray with 50 mcl of medium containing various concentrations of compounds. MT-4 cells were added at a final concentration of 6×10^5 cells/mL. After 5 days of incubation at 37 ºC, the number of viable cells were determined by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Cytotoxicity of test compounds against mockinfected MT-4 cells were also assessed by the MTT method. Compounds were evaluated for their inhibitory effect on the replication of HIV-1 and HIV-2 in human MT-4 cells. The anti HIV and cytotoxicity datas are presented in Table-2.

Antiviral assay: Antiviral activity and cytotoxicity of the synthesized compounds were determined by an *in vitro* cell culture technique. The antiviral assays were based on inhibition of virus-induced cytopathicity in HeLa cells (VSV and RSV), HEL cells (HSV-1 and HSV-2), Vero cells (parainfluenza-3, reovirus-1, sindbis virus, coxsackie virus B4 and punta toro virus). Briefly, confluent cell culture in 96-wells microtiter plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50 % of the cell cultures. After 1 h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200 and 100 µg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were treated with the test compounds. The antiviral and cytotoxicity datas are presented in Tables 3-5.

RESULTS AND DISCUSSION

The results of study of 2-phenyl-3-substituted quinazoline-4(3*H*)-one derivatives are reported. The inhibitory effect of antiviral drugs on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay. Cytotoxicity of test compounds against

TABLE-2
ANTI HIV ACTIVITY AND CYTOTOXICITY OF SYNTHESIZED COMPOUNDS IN MT-4 CELLS

Compound code	Strain	EC_{50} [a] (µg/mL)	CC_{50} [b] (µg/mL)
QAA	IIIB	>57.18	57.18 ± 6.32
	ROD	>57.18	57.18 ± 6.32
QBA	IIIB	>106.10	106.10 ± 11.18
	ROD	>106.10	106.10 ± 11.18
QBI	IIIB	>71.85	71.85 ± 1.16
	ROD	>71.85	71.85 ± 1.16
QBR	IIIB	>60.63	60.63 ± 5.59
	ROD	>60.63	60.63 ± 5.59
QBT	IIIB	>61.33	61.33 ± 4.48
	ROD	>61.33	61.33 ± 4.48
QCF	IIIB	>52.48	52.48 ± 6.75
	ROD	>52.48	52.48 ± 6.75
QIP	IIIB	>74.20	74.20 ± 2.43
	ROD	>74.20	74.20 ± 2.43
QIS	IIIB	>27.93	27.93 ± 25.87
	ROD	>27.93	27.93 ± 25.87
QNA	IIIB	>68.70	68.70 ± 7.95
	ROD	>68.70	68.70 ± 7.95
QMB	IIIB	>66.65	66.65 ± 7.16
	ROD	>66.65	66.65 ± 7.16
QNF	IIIB	>61.80	61.80 ± 3.40
	ROD	>61.80	61.80 ± 3.40
QNI	IIIB	>125	>125
	ROD	>125	>125
QPAB	IIIB	>11.90	11.90 ± 0.42
	ROD	>11.90	11.90 ± 0.42
QPH	IIIB	>125	>125
	ROD	>125	>125
AZT	IIIB	0.00120	65.9 ± 6.1
	ROD	0.00062	65.9 ± 6.1

a: Concentrations required to inhibit the cytopathic effect of HIV-1(III_B) in MT-4 cells by 50 %. b: Concentrations required to cause cytotoxicity to 50 % of the MT-4 cells. All the values are SD of two independent experiments. IIIB-HIV-1, ROD-HIV-2.

TABLE-3
CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF COMPOUNDS IN HeLa CELL CULTURES

Comp. code	Minimum cytotoxic concentration [a] (µg/mL)	EC_{50} [b] (µg/mL)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
BN	100	>20	>20	>20
QAA	100	>20	>20	>20
QBA	≥100	>100	>100	>100
QBI	≥20	>20	>20	>20
QBR	100	>20	>20	>20
QBT	>100	>100	>100	>100
QCF	100	>20	>20	>20
QIP	≥20	>20	>20	>20
QIS	100	12	>20	>20
QNA	100	>20	>20	>20
QMB	100	12	>20	>20
QNF	100	>20	>20	>20
QNI	100	>20	>20	>20
QPAB	≥20	>20	>20	>20
QPH	100	>20	>20	>20
QSA	100	>20	>20	>20
QSD	100	>20	>20	>20
QSM	100	>20	>20	>20
QSS	>100	>100	>100	>100
DS-5000	>100	4	>100	4

(S)-DHPA (μM)	>250	112	>250	>250
Ribavirin (μM)	>250	12	146	10

a: Required to cause a microscopically detectable alteration of normal cell morphology, b: Required to reduce virus-induced cytopathogenicity by 50 %.

TABLE-4
CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF COMPOUNDS IN HeLa CELL CULTURES

Comp. code	Minimum cytotoxic concentration[a] (μg/mL)	EC_{50}[b] (μg/mL)			
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Herpes simplex virus-1 TK^- KOS ACV^r
BN	>100	10	10	12	10
QAA	100	>20	>20	>20	>20
QBA	>100	>100	>100	>100	>100
QBI	100	>20	>20	>20	>20
QBR	100	>20	>20	>20	>20
QBT	>100	>100	>100	>100	>100
QCF	100	>20	>20	>20	>20
GIP	100	>20	>20	>20	>20
QIS	20	>4	>4	>4	>4
QNA	>100	>100	>100	>100	>100
QMB	>100	>100	>100	>100	>100
QNF	100	>20	>20	>20	>20
QNI	100	>20	>20	>20	>20
QPAB	100	>20	>20	>20	>20
QPH	100	>20	>20	>20	>20
QSA	100	>20	>20	>20	>20
QSD	100	>20	>20	>20	>20
QSM	100	>20	>20	>20	>20
QSS	100	>20	>20	>20	>20
Brivudin (μM)	>250	0.04	50	10	>250
Ribavirin (μM)	>250	>250	>250	>250	>250
Acyclovir (μM)	>250	2	2	7	2
Ganciclovir (μM)	>100	0.06	0.1	>100	12

a: Required to cause a microscopically detectable alteration of normal cell morphology. b: Required to reduce virus-induced.

mock-infected MT-4 cells were also assessed by the MTT method. All the compounds displayed cytostatic properties in T-lymphocytes MT-4 cells. The compound 4-((4-oxo-2-phenyl-quinazolin-3(4*H*)-yl amino)methyl amino benzoic acid (QPAB) (CC_{50} = 11.90 μM) was found to be more toxic in this series. The synthesized compounds were tested for antiviral against HeLa Cells (VSV and RSV) HEL cells (HSV-1 and HSV-2) and Vero cells (parainfluenza-3, reovirus-1, sindbis virus, coxsackie virus B4 and punta toro virus. 2-Amino-3-phenyl quinazolin-4(3*H*)-one (BN) exhibited antiviral activity against herpes simplex virus-1,2 and vaccinia virus in HEL cells at the concentration of 10 and 12 μg/mL, whereas cytotoxicity was found to be 100 μg/mL (SI = 10). Among these compounds, compounds (QIS and QMB) exhibited antiviral activity against vesicular stomatitis virus in HeLa cells at the concentration of 12 μg/mL, whereas cytotoxicity was found to be 100 μg/mL (SI = 9).

ACKNOWLEDGEMENT

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TABLE-5
CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF COMPOUNDS IN VERO CELL CULTURES

Comp. code	Minimum cytotoxic concentration[a] (μg/mL)	EC_{50}[b] (μg/mL)				
		Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta toro virus
BN	>100	>100	>100	>100	>100	>100
QAA	100	>20	>20	>20	>20	>20
QBA	≥100	>100	>100	>100	>100	>100
QBI	>100	>100	>100	>100	>100	>100
QBR	100	20	>20	>20	>20	>20
QBT	>100	>100	>100	>100	>100	>100
QCF	100	>20	>20	>20	>20	>20
QIP	100	>20	>20	>20	>20	>20
QIS	100	>20	>20	>20	>20	>20
QNA	100	>20	>20	>20	>20	>20
QMB	100	>20	>20	>20	>20	>20
QNF	100	>20	>20	>20	>20	>20
QNI	100	>20	>20	>20	>20	>20
QPAB	100	>20	>20	>20	>20	>20
QPH	100	>20	>20	>20	>20	>20
QSA	100	>20	>20	>20	>20	>20
QSD	100	>20	>20	>20	>20	>20
QSM	>100	>100	>100	>100	>100	>100
QSS	100	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	>100	>100	>100
(S)-DHPA (μM)	>250	>250	250	>250	>250	>250
Ribavirin (μM)	>250	112	146	>250	>250	50

a: Required to cause a microscopically detectable alteration of normal cell morphology. b: Required to reduce virus-induced cytopathogenicity by 50 %.

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