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Full Length Research Paper

Synthesis, antiviral and cytotoxic activities of some novel 2-Phenyl-3-Disubstituted Quinazolin-4(3H)-ones

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The 2-phenyl-benzoxazin-4-ones were condensed with primary amine to form the 2, 3-disubstituted quinazolin-4(3H)-ones. Their chemical structure was elucidated by means of spectral (FT-IR, ^{1}H- NMR, MS) and elemental analysis. The antiviral activity and cytotoxicity of the compounds were tested in HeLa cells (vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus), HEL cells [herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus], Vero cells (parainfluenza-3, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus). Among the new derivatives evaluated, specific antiviral activity was noted with compound QAA against vaccinia virus, parainfluenza-3 virus and Punta Toro virus, compound QOPD against HSV-1, HSV-2 and vaccinia virus, and compounds QONA and PD-NFIN against Coxsackie virus B4.

Key words: Quinazoline, antiviral, HSV, vaccinia, sulphanamides.

INTRODUCTION

Quinazolin-4-(3H)-One is a versatile lead molecule for the design of potential bioactive agents. 2-Phenyl-3-Substi-tuted Quinazolin-4-(3H)-ones were reported to have anti-HIV, some of their derivatives have also shown signi-ficant anti-HIV activity (Shah et al.,1995; Alagarsamy et al., 2000; Desai et al.,1998), anti-cancer activity were studied for 2,3-disbstituted quinazolinones derivatives and they showed promising anticancer potential (Raffa et al., 1999; Murugan et al., 2003; Girija et al., 2005). Quinazolinones derivatives were screened for their wide spectrum anti-viral activity and they were found to be potential derivatives for further studies (Manoj et al., 2001; Selvam et al., 2004; Pandey et al., 1996). A large number of quinazolines derivatives have been synthe-sized and studied for wide range of anti-viral activity, but the anti- viral activities of quinazolines against viruses have 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 cyclisation. 2-phenyl-3-substituted quina-zolin-4(3*H*)-one derivatives were synthesized by conden-sation of the compounds containing primary aromatic amine with 2- phenyl-1,3-benzoxazine-4-one.Synthesized compounds were screened for antiviral activity against panel of human pathogenic viruses

MATERIALS AND METHODS

Melting points were determined using an open ended capillary tube method and are uncorrected. FT-IR spectra were recorded on a Perkin Elmer–1605 series FT- IR in KBr disc. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker FT-NMR spectrophotometer using TMS as internal standard. Completion of reaction and purity of the compounds were checked by TLC using silica gel G as stationary phase using chloroform and methano l(9:1) and spot is visualized by iodine vapour.

Synthesis of 6-bromo/6,8-dibromo-2- phenyl 1,3-benzoxazin-4-one (1): Anthranilic acid, 6-bromo- or 3, 5-dibromoanthranilic 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 recrystalized from ethanol (amount). General synthetic method for preparation of compounds: An equimola mixture (0.01 mol) of 2-phenyl-substituted benzo[1,3]oxazin-4-one and compounds with primary aromatic amino functioned compounds group was taken mixed and the mixture was refluxed for 6 h in 10 ml of pyridine. Upon cooling, the mixture was poured onto crushed ice. The precipitated solid was collected and recrystalized from ethanol to give the desired title compounds. The yields and the melting points of the compounds are given in Table 1.

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Table 1. Physical data of synthesized compounds

S.NO	Compound Code	Mol. Formula	Yield (%)	M.P(˚C)	R_f Value#
1.	QSM	$C_{24}H_{18}N_4O_4S$	57.6	78-82	0.648
2.	MSM	$C_{24}H_{17}N_4O_4SBr$	66.3	165-172	0.51
3.	QSN	$C_{27}H_{18}N_4O_6S$	92.6	70-73	0.512
4.	QAA	$C_{21}H_{14}N_2O_3$	72.7	148-154	0.118
5.	QPABA	$C_{21}H_{14}N_2O_3$	51.6	135-139	0.384
6.	QPP	$C_{22}H_{18}N_2O_2$	62.8	134-140	0.213
7.	QOPD	$C_{20}H_{15}N_3O$	55.1	150-156	0.407
8.	QAP	$C_{19}H_{13}N_3O$	68.1	72-75	0.424
9.	DSM	$C_{24}H_{16}N_4O_6SBr$	59.5	154-158	0.361
10.	MSN	$C_{27}H_{17}N_4O_6SBr$	80.2	106-112	0.661
11.	DSN	$C_{27}H_{16}N_4O_6SBr_2$	55.3	80-85	0.574
12.	QSB	$C_{27}H_{20}N_3O_3S$	63.5	107-110	0.176
13.	QONA	$C_{20}H_{13}N_3O_3$	48.7	86-89	0.385
14.	QPNA	$C_{20}H_{13}N_3O_3$	85.8	70-74	0.417
15.	QPH	$C_{20}H_{15}N_3O$	55.1	110-118	0.381
16.	DSB	$C_{27}H_{19}N_3O_3SBr$	66.1	124-130	0.385
17.	QPAP	$C_{20}H_{14}N_2O_2$	80.7	156-162	0.271

Purity of the compounds were checked by TLC using solvent system $CHCl_3$:CH_3OH (9:1) and spot is visualized by iodine vapour

QAA: IR (KBr) cm^{-1}: 1697 (C=O), 1661 (C=N), 1537 (C=C), 3128 (OH): ^{1}H NMR (DMSO-d_6): 8.1-7.1 (m, 13H, Ar-H), 11.6(s, 1H, COOH); EI-MS (m/z):342.QPP: IR (KBr)cm^{-1}:1603(C=O),1511(C=N), 1325(C=C),1046(C-O-C),3277(Alkyl); ^{1}H NMR (DMSO-d_6): 8.1-6.9 (m, 13H, Ar-H), 1.4 (t,2H,CH_2), 4.0(q, 3H, CH_3).EI-MS (m/e):342.QOPD: IR (KBr) cm^{-1}: 1303 (NH), 1654 (C=O), 1590 (C=N), 1514 (C=C), ^{1}H NMR (DMSO- d_6): 8.2-7.2 (m, 13H, Ar-H), 4.0(s, 1H, Ar-C-NH). EI-MS (m/e):313.

QPABA: IR (KBr) cm^{-1}: 3279 (OH), 3063(Ar-H), 1700 (C=O),1652 (C=N), 1593 (C=C); ^{1}H NMR (DMSO-d_6): 7.2-8.1 (m, 13H, Ar-H), 10.5 (s, 1H, COOH) EI-MS (m/e): 342.

MSM: IR (KBr) cm^{-1}: 3094 (NH), 1701 (C=O), 1601 (C=N), 1520 (NO_2), 1097 (SO_2), 510(Br); ^{1}H NMR (DMSO-d_6): 8.4-7.3 (m, 17H, Ar-H), 8.6 (s,1H,NH) EI-MS(m/e):536.

QSN: IR (KBr) cm^{-1}: 3067 (Ar-H), 1765 (C=O), 1313 (C=N), 1610 (C=C), 1473(NO_2); ^{1}H NMR (DMSO-d_6): 8.2-7.4 (m,17H, Ar-H), 8.4 (d, 1H,NH). EI-MS (m/e):526.

QSM: IR (KBr) cm^{-1}: 1615 (C=O), 1311 (C=N), 1519 (C=C), 1019 (C-O-C); ^{1}H NMR (DMSO-d_6): 8.3-7.4 (m, 14H, Ar-H), 3.4 (s, 1H, NH), 2.5(s, 3H, methyl); EI-MS (m/e):458.QAP: IR (KBr) cm^{-1}: 1671 (C=O), 1374 (C=N), 1537 (C=C), 3266(Ar H); ^{1}H NMR (DMSO-d_6): 8.5-7.1 (m, 13H, Ar-H). EI-MS (m/e):299.

Antiviral activity

Viruses and cells: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G); vaccinia virus, vesicular stomatitis virus, Coxsackievirus type B4, Sindbis virus, measles virus, and poliovirus type 1; reovirus type 1 (ATCC VR-230), and para-influenza virus type 3 (ATCC VR-93) (American Type Culture Collection, Rockville, MD. Antiviral assays were carried out in human embryonic lung (HEL) cells (herpes simplex virus types 1 and 2, vaccinia virus, and vesicular stomatitis virus), Vero cells (parainfluenza virus, reovirus, Sindbis virus, Coxsackie B virus and Punta Toro virus), and HeLa cells (vesicular stomatitis virus, Coxsackie B virus, and respiratory syncytial virus). The human embryonic lung (HEL), Vero and HeLa cell lines used in this study were regularly examined for mycoplasma contamination and found to be mycoplasma-free.

Inhibition of virus-induced cytopathogenicity *in vitro*: Confluent cell cultures in microtiter trays were inoculated with 100 CCID50 (1 CCID50 corresponding to the virus stock dilution that proved infective for 50% of the cell cultures). After 1 h of virus adsorption to the cells, residual virus was removed and replaced by cell culture medium (Eagle's minimal essential medium) containing 3% fetal calf serum and various concentrations of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus- infected cell cultures, that is, at 1 to 2 days for vesicular stomatitis virus; at 2 days for Coxsackie virus, at 2 to 3 days for vaccinia virus, herpes simplex virus types 1 and 2, and Sindbis virus; and at 6 to 7 days for reovirus and parainfluenza virus. The antiviral activity of the compounds is expressed as the concentration required to inhibit viral cytopathogenicity by 50%.

Cytotoxicity: Cytotoxicity measurements were based on an alteration of normal cell morphology. To evaluate cell morphology, confluent cell cultures which had not been infected but were treated with various concentrations of the test compounds were incubated in parallel with the virus-infected cell cultures and examined microscopically at the same time as viral cytopathogenicity was recorded in the virus-infected cell cultures. A disruption of the cell monolayer, e.g., rounding up or detachment of the cells, was considered as evidence for cytotoxicity. Cytotoxicity of the compounds was determined as the minimum concentration required to cause a microscopically detectable alteration of normal cell morphology. Antiviral and cytotoxicity data were presented in Tables 2, 3 and 4.

RESULTS

Anthranilic acid reaction with benzoyl chloride yielded 2-phenyl-1,3-benzoxazin-4-one by N-acylation via dehyd-rative cyclization. A series of novel 2,3-disubstituted qui

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Table 2. Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
QAA	20	>4	>4	>4
QAP	>100	>100	>100	>100
QSM	100	>20	>20	>20
QSN	100	>20	>20	>20
QPP	20	>4	>4	>4
QPN	100	>20	>20	>20
QOPD	100	>20	>20	>20
QONA	100	>20	>20	>20
QPNA	100	>20	>20	>20
QPABA	>100	>100	>100	>100
QANSA	100	>20	>20	>20
PAP	100	>20	>20	>20
MSN	100	>20	>20	>20
DSM	>100	>100	>100	>100
MSM	100	>20	>20	>20
Braved (µM)	>250	>250	>250	>250
(S)-DHPA (µM)	>250	150	>250	250
Ribavirin (µM)	>250	10	150	30

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

Table 3. Cytotoxicity and antiviral activity of compounds in vero cell cultures.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
QAA	**20**	**2.4**	>4	>4	>4	**4**
QAP	>100	>100	>100	>100	>100	>100
QSM	20	>20	>20	>20	>20	>20
QSN	100	>20	>20	>20	>20	>20
QPP	20	>4	>4	>4	>4	>4
QPN	100	>20	>20	>20	>20	>20
QOPD	100	20	>20	>20	>20	>20
QONA	**20**	>20	>20	**12**	**4**	>20
QPNA	20	>20	>20	>20	>20	>20
QPABA	>100	>100	>100	>100	>100	>100
QANSA	>100	>100	>100	>100	>100	>100
PAP	20	>4	>4	>4	>4	>4
MSN	100	>20	>20	>20	>20	>20
DSM	>100	60	>100	>100	>100	>100
MSM	100	>20	>20	>20	>20	>20
Brivudin (µM)	>250	>250	>250	>250	>250	>250
(S)-DHPA (µM)	>250	>250	>250	>250	>250	>250
Ribavirin (µM)	>250	150	>250	>250	>250	250

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

nazolin-4(3H)-one derivatives were synthesized by condensation of compounds containing a primary aromatic amino group with 2-phenyl-substituted-1,3-benzo-xazine-4-one to afford 2,3-disubstituted quinazolin-4(3*H*)-one

Table 4. Cytotoxicity and antiviral activity of compounds in HEL cell cultures.

Compound	Minimum cytotoxic concentration [a] (µg/ml)	EC_{50} [b] (µg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
QAA	**100**	**20**	**20**	**4**	**>20**	**20**
QAP	>100	>100	>100	>100	>100	>100
QSM	100	>20	>20	>20	>20	>20
QSN	100	>20	>20	>20	>20	>20
QPP	100	>100	>100	>100	>100	>100
QPN	>100	>100	100	>100	>100	>100
QOPD	**100**	**20**	**20**	**12**	**>20**	**>20**
QONA	100	>20	>20	>20	>20	>20
QPNA	100	>20	>20	>20	>20	>20
QPABA	>100	>100	>100	>100	>100	>100
QANSA	>100	100	100	>100	>100	>100
PAP	20	>20	>20	>20	>20	>20
MSN	100	>100	>100	>100	>100	>100
DSM	100	>100	>100	>100	>100	>100
MSM	100	20	20	>20	>20	>20
Brivudin (µM)	>250	0.08	30	10	>250	150
Ribavirin (µM)	>250	50	250	150	150	250
Acyclovir (µM)	>250	0.4	0.08	>250	>250	50
Ganciclovir (µM)	>100	0.032	0.032	>100	>100	0.8

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

derivatives (Figure 1) The spectral data of synthesized compounds were consistent with the assigned structure and the synthesized compounds were obtained in 48 - 92 % yield (Table 1).

2- (o-phenyl carboxylic acid)-3-phenyl quinazolin-4(3H)-one (QAA) exhibited anti-viral activity against Vaccinia virus, Herpes Simplex virus-1,2 in HEL cells at the concentration of 4 and 20 µg/ml respectively, whereas cytotoxicity was found to be 100 µg/ml (SI = 25 for vaccinia virus).QAA also inhibited the replication of para influenza 3 and Punta Toro virus Vero cells at the concentration of 2.6 and 4 µg/ml respectively, whereas cytotoxicity was found to be 20 µg/ml. 2-(o-aminophenyl)-3-phenyl quinazolin-4(3H)-one (QOPD) exhibited anti-viral activity against Vaccinia virus, Herpes Simplex virus-1,2 in HEL cells at the concentration of 12 and 20 µg/ml respectively, whereas cytotoxicity was found to be 100 µg/ml. The compound (QOPD) also inhibited the replication of Coxsackie B4 virus at the concentration of 4 µg/ml whereas cytotoxicity was found to be 20 µg/ml. 2-(o-nitrophenyl)-3-phenyl quinazolin-4(3H)-one (QONA) inhibited the replication of Coxsackie B4 virus at the concentration of 4 µg/ml whereas cytotoxicity was found to be 20 µg/ml.

DISCUSSION

We have previously reported the antiviral activity of novel quinazolinones against vaccinia virus, and many of those compounds also exhibited marked cytostatic properties in lymphocytes (Selvam et al., 2003). Though a variety of quinazoline derivatives had been synthesized and studied for wide range of antiviral activity, the antiviral activities of quinazolines against panel of pathogenic viruses have not been extensively explored. In this study we synthesized 17 derivatives of quinazolinones and evaluated them for antiviral activity against panel of human pathogenic viruses

2- (o-phenyl carboxylic acid)-3-phenyl quinazolin-4(3H)-one inhibited the replication of HSV -1 and 2 vaccinia viruses below the cytotoxic concentration. Two newly synthesized compounds (QONA and QOPD) effectively inhibited the replication of the vaccinia and para-influenza 3 and Coxsacke B4 viruses. These lead molecules are suitable for designing newer derivatives against HSV and Vaccinia based upon promising antiviral activity seen.

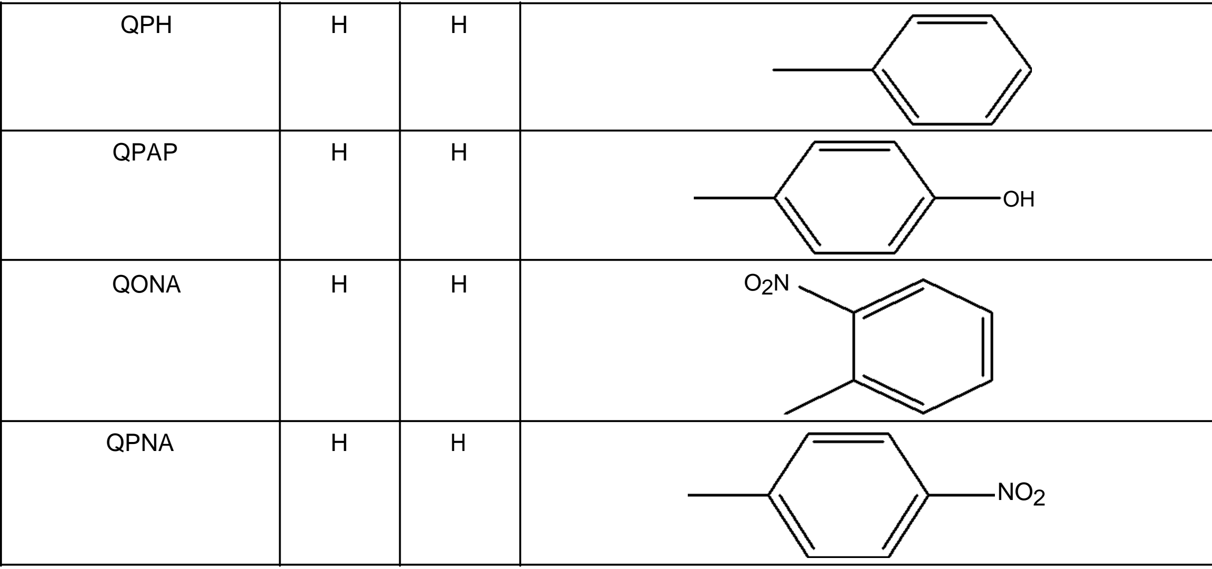
Recently we reported the activities of certain quinazolinone against biodefence viruses in cell culture (Selvam et al., 2007). The potencies of some of them exceeded

Figure 1. Synthetic protocol of studied compounds.

O
R_1
N
R_2
\+ H_2N R_3 →
O
R_1
N
N
R_3
R_2

Compound Code	R1	R2	R3
QSM	H	H	SO_2NH O N CH_3
MSM	Br	H	SO_2NH O N CH_3
DSM	Br	Br	SO_2NH O N CH_3
QAP	H	H	N
QAA	H	H	HOOC
QPABA	H	H	COOH
QPP	H	H	OC_2H_5
QOPD	H	H	H_2N

Figure 1. Continued



QPH	H	H	
QPAP	H	H	OH
QONA	H	H	O_2N
QPNA	H	H	NO_2

those of the present quinazolinone series. The compounds were found to inhibit virus replication as a result of interfering with virus adsorption (Selvam et al., 2006). There is a need to discover new compounds that are inhibitory to HSV and vaccinia viruses due to the emergence of potentially pandemic virus strains and viral resistance against approved drugs. The methodology reported here allows for rapid synthesis of a number of quinazolinone derivatives that can be tested for antiviral activity, as well as for activity against other viruses of concern to the medical community.

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