Synthesis, Antiviral and Cytotoxic Activities of 2-(2-Phenyl carboxylic acid)-3-Phenylquinazolin -4(3H)-one Derivatives

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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 anthranilic acid. Synthesized compounds were evaluated for *in vitro* antiviral activity against HIV, HSV and vaccinia viruses. 5-Bromo-2-(6-bromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (MBR2) exhibited distinct antiviral activity against Herpes simplex and vaccinia viruses.

Key words: Quinazoline, anthranilic acid, HIV, MT-4 Cells, MTT assay, vaccinia virus

Quinazolin-4-(3*H*)-one is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess broad spectrum activities. 2-Phenyl-3-substitutedquinazolin-4-(3*H*)-ones were reported to have antiHIV activity[1-3] and anticancer activity[4-6]. Quinazolinones were screened for their wide spectrum antiviral activity and they have rich potential for further studies[7-9]. A series of some novel 2,3-disubstituted quinazolin-4(3*H*)-one derivatives has been synthesized by condensation of primary aromatic amino group of anthranilic acid with 2-substituted-1,3-benzoxazine-4-one[8] to afford 2,3-disubstituted quinazolin-4(3*H*)-ones (Scheme 1).

Melting points were determined using open ended capillary tube method and are uncorrected. IR spectrum were recorded In KBr on a Perkin

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TABLE 1: PHYSICAL DATA FOR SYNTHESIZED COMPOUNDS

Compound code	Mol. formula	Yield (%)	M.P(°)	R^f Value#
QAA1	$C_{21}H_{14}N_2O_3$	72.7	148	0.118
QAA2	$C_{21}H_{13}N_2O_3Br$	66.3	165	0.51
QAA3	$C_{21}H_{12}N_2O_3Br_2$	92.6	70	0.512
MBR1	$C_{21}H_{14}N_2O_3Br$	72.7	154	0.118
MBR2	$C_{21}H_{13}N_2O_3Br_2$	51.6	135	0.384
DBR1	$C_{21}H_{12}N_2O_3Br_2$	62.8	140	0.213
DBR2	$C_{21}H_{11}N_2O_3Br_3$	55.1	150	0.407
DBR3	$C_{21}H_{10}N_2O_3Br_4$	68.1	72	0.424

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

TABLE 2: ANTI-HIV ACTIVITY OF QUINAZOLIN-4(3H)-ONES

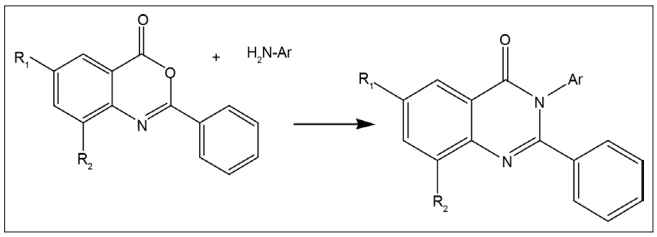
Compound	Strain	IC_{50}[a](µg/ml)	IC_{50}[b](µg/ml)
AA1	III_B	>125	>125
	ROD	>125	>125
AA2	III_B	>88.20	88.20
	ROD	>88.20	88.20
AA3	III_B	101	>125
	ROD	>125	>125
MBR1	III_B	74.8	>125
	ROD	>125	>125
MBR2	III_B	>60.40	60.40
	ROD	>60.40	60.40
DBR1	III_B	>83.15	83.15
	ROD	>83.15	83.15
DBR2	III_B	>64.18	64.18
	ROD	>64.18	64.18
DBR3	III_B	>125	>125
	ROD	>125	>125
AZT	III_B	0.0012	65.90
	ROD	0.00062	65.90

[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%.

Elmer-1605 grating spectrometer (cm^{-1}). ^1H NMR Spectra were recorded at 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 by adopting electron impact ionization.

An equimolar mixture (0.01 mol) of 2-phenyl-substituted benzo[1,3]oxazin-4-one and compounds with primary aromatic amino functional group (anthranilic acid, 6-bromo- or 3,5-dibromo anthranilic acid) was 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) Spectral data as follows



Scheme 1: Synthesis of 2-phenyl-3-substituted quinazolin-4(3H)-ones For QAA1, R_1 is H, R_2 is H, Ar is -phenyl carboxylic acid; for QAA2, R_1 is Br, R_2 is H, Ar is -4-bromo-2-phenyl carboxylic acid; for QAA3, R_1 is Br, R_2 is Br, Ar is -4,6-dibromo-2-phenyl carboxylic acid; for MBR1, R_1 is Br, R_2 is H, Ar is -2-phenyl carboxylic acid; for MBR2, R_1 is Br, R_2 is H, Ar is -4-bromo-2-phenyl carboxylic acid; for DBR1, R_1 is Br, R_2 is Br, Ar is -2-phenyl carboxylic acid; for DBR2, R_1 is Br, R_2 is Br, Ar is -4-bromo-2 phenyl carboxylic acid; for DBR3, R_1 is Br, R_2 is Br, Ar is -4,6-dibromo-2-phenyl carboxylic acid.

2-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (QAA1) IR (KBr) cm^{-1}: 1537 (C=C), 1661 (C=N), 1697 (C=O), 3128 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 13H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):342. 5-Bromo-2-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (QAA2) IR (KBr) cm^{-1}: 1530 (C=C), 1665 (C=N), 1692 (C=O), 3130 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 12H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):432. 3,5-dibromo-2-(4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (QAA3) IR (KBr) cm^{-1}: 1532 (C=C), 1665 (C=N), 1691 (C=O), 3125 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 11H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):522. 2-(6-Bromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (MBR1) IR (KBr) cm^{-1}: 1529 (C=C), 1658 (C=N), 1685 (C=O), 3129 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 12H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):432. 5-Bromo-2-(6-bromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (MBR2) IR (KBr) cm^{-1}: 1531 (C=C), 1659 (C=N), 1687 (C=O), 3125 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 11H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):522. 2-(6,8-Dibromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (DBR1): IR (KBr) cm^{-1}: 1537 (C=C), 1661 (C=N), 1697 (C=O), 3122 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 12H, Ar-H), 11.6 (s, 1H,

TABLE 3: ANTIVIRAL ACTIVITY OF QUINAZOLIN-4(3H)-ONES DERIVATIVES

Compound	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]
AA1	>100	100	58	>100	100
AA2	>100	50	58	100	58
AA3	>100	50	100	100	100
DBR1	>100	58	50	100	100
DBR2	100	>20	>20	>20	>20
DBR3	>100	>100	>100	>100	>100
MBR1	100	>20	>20	>20	>20
MBR2	100	12	12	12	12
Brivudin	>250	0.04	22	6	10
Ribavirin	>250	>250	>250	>250	>250
Cidofovir	>250	0.8	0.8	10	1

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

COOH); EI-MS(m/z):522. 5-Bromo-2-(6,8-dibromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (DBR2) IR (KBr) cm^{-1}: 1537 (C=C), 1661 (C=N), 1697 (C=O), 3125 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 11H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):612. 3,5-Dibromo-2-(6,8-dibromo-4-oxo-2-phenyl-4H-quinazolin-3-yl)-benzoic acid (DBR3) IR (KBr) cm^{-1}: 1537 (C=C), 1661 (C=N), 1697 (C=O), 3126 (OH): ^1H NMR (DMSO-d_6): 8.1-7.1 (m, 10H, Ar-H), 11.6 (s, 1H, COOH); EI-MS (m/z):702.

The compounds were tested for antiHIV 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). Human immunodeficiency virus-1 (HTLV-IIIB/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° 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 (6×10^5 cells/ml). After 5 d of incubation, at 37° 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 was assessed by the MTT method. AntiHIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The antiHIV activity and cytotoxicity data are presented in (Table 2).

Antiviral activity and cytotoxicity of the synthesized compounds were determined by *in vitro* cell culture techniques[8]. The antiviral assays were based on inhibition of virus-induced cytopathicity in HEL (HSV-1 and HSV-2, VV, VSV) cultures. Briefly, confluent cell culture in 96-well 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 a 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 as completion in the control virus-infected cell cultures exposed to the test compounds. The antiviral activity and cytotoxicity data are presented in (Table 3.)

From the results the antiviral activity and cytotoxicity of the compound are arrived at the follows compound MBR2 was active against the replication of HSV-1, -2 and vaccinia virus with an IC_{50} of 12 µg/ml and cytotoxicity at 100 µg/ml. Those compounds displayed cytotoxicity in MT-4 cells were inactive against HIV-1 and -2 replication at non-cytotoxic concentrations. We have previously reported the antiviral activity of novel quinazolinones against vaccinia virus and many of these compounds also exhibited marked cytostatic properties in lymphocytes[8,10]. In this study we evaluated eight new derivatives of quinazolinones synthesized by us for their antiviral activity. This lead molecule is highly suitable for designing newer derivatives and molecular modification in them and may help in optimizing antiviral activity.

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REFERENCES

1. Shah BR, Bhatt JJ, Patel HH, Undavia NK, Trivedi PB, Desai NC. Synthesis of 2,3-disubstituted-3,1-quinazolin-4(3H)-ones as potential anticancer and anti-HIV agents. Indian J Chem 1995;34:201-8 .
2. Desai NC, Undavia NK, Trivedi PB, Dave D, Vyas GD. Synthesized and screened anti-HIV activity of some non-nucleoside 2,3-disubstitutedquinazoline derivatives. Indian J Exp Biol 1998;36:1280-3.
3. Alagarsamy V, Pathak US, Pandaya SN, Sriram D, De Clercq E. Anti-HIV and anti bacterial activities of some disubstituted quinazolones and their bio-isoster disubstituted thienopyrimidones. Indian J Pharm Sci 2000;66:433-7.
4. Raffa D, Daidone G, Maggio B, Schillaci D, Plescia F. Synthesis and antiproliferative activity of novel 3-(indazol-3-yl)-quinazolin-4(3H) one and 3-(indazol-3-yl)-benzotriazin-4(3H)one derivatives. Pharmazie 1999;332:317-20.
5. Murugan V, Padmavathy NP, Ramasarma GV, Sharma SV, Suresh B. Synthesis of some quinazolinone derivatives as possible anticancer agent. Indian J Heterocycl Chem 2003;13:143-6.
6. Girija K, Selvam P, Nagarajan R. Synthesis anticancer activity of 3-[5-Amino-6-(2,3-dichlorophenyl)-[1,2,4]triazin-3-yl]-6,8-dibromo-2-substituted-3H-quina-zolin-4-one. Asian J Chem 2005;17:1111-5.
7. Manoj K, Srivastava S, Bharati M, Nizamuddin N. Pharmacological studies of some 2-methyl-3-(arylthiocarbamido)quinazol-4(3H)-ones and antibacterial activity against *Bacillus cereus, S. aureus, S. lutae* and antiviral activity against *Gomphrena mosaic*. Indian J Chem 2001;40:342-4.
8. Selvam P, Dinakaran M, De Clercq E, Sridhar SK. Synthesis, antiviral and cytotoxic activity of 6-bromo-2,3-disubstituted-4(3H)-quinazolinones. Biol Pharm Bull 2003;26:1278-82.
9. Pandey VK, Synthesized 7-(2'-phenyl-3'-ethyl-4'-oxoquinazolinyl)-3,4-diphenyl isoquinolines and screened for antiviral activity against vaccinia virus. Indian Drugs 1996;26:168-71.
10. Selvam P, Chennama B, De Clercq E. Synthesis and antiviral activity of some novel 2-substituted 3-(6-ethyl, 4-amino,5-(4-chlorophenyl)-pyrimidin-2-yl) quinazolin-4(3H)ones. Int J Chem Sci 2004;2:627-31.

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