Research Article

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SYNTHESIS, ANTI-HIV AND CYTOTOXIC ACTIVITIES OF 2-PHENYL, 3-SUBSTITUTEDQUINAZOLIN-4(3H)-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, ^{1}H-NMR). Synthesized compounds were screened for *in vitro* antiviral activity against HIV -1 and -2 in MT-4 cells. Cytotoxicity of test compounds against mock-infected MT-4 cells (C type adult T leukemia cells) was also assessed by the MTT method. Anticancer activity also tested against Human liver cancer cells by MTT assay. Compound Q-2ABT (CC_{50}:10.07±0.60) was found to be more toxic in this series in MT-4 cells. All the tested compounds exhibited significant cytotoxicity against liver cancer cells (CTC_{50}: 150-206 µg/ml). Among all the nine compounds tested, Q-2ABT (158.81±3.46 µg/ml) showed better cytotoxicity against HepG2 (human liver cancer cells), where as standard Cis-platin was found to be 11.09±0.59 µg/ml (15 fold higher). Compound Q-2ABT merits further investigation to screen for their anticancer property.

Keywords: Quinazoline, Anthranilic acid, 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[1,2,3,4], anti-cancer activity were studied for 2,3-disubstituted quinazolinones derivatives and they showed promising anticancer potential[5,6,7]. Quinazolinones were also screened for their wide spectrum anti-viral activity and they were found to be potential derivatives for further studies[8,9,10].

Anthranilic acid reaction with benzoyl chloride yields 2-phenyl-1,3-benzoxazin-4-one by N-acylation via dehydrative cyclization[9]. A series of some novel 2,3-disubstituted quinazolin-4(3H)-one derivatives have been synthesized by condensation of primary aromatic amino group of anthanilic acid with 2-substituted-1,3-benzoxazine-4-one to afford 2,3-disubstituted quinazolin-4(3H)-one derivatives (Scheme 1). Synthesized compounds were screened for antiviral activity against HIV -1 and -2 in MT-4 cells and cytotoxicity against mock-infected MT-4 cells. Anticancer activity also investigated against Human liver cancer cells (HepG2 cells) by using MTT assay.

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Experimental

Melting points were determined using open ended capillary tube method and are uncorrected. FT-IR recorded on Perkin Elmer–1605 series FT-IR in KBr disc. ^{1}H 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.

Synthesis of 6-bromo/6, 8-dibromo-2-phenyl-3-substituted quinazolin-4-*(3H)*-ones

An equimolar (0.01 mol) mixture of 6-bromo/6, 8-dibromo-2-phenyl-1,3-benzoxazine-4-one[9] and aromatic primary amine (2-amino-4-phenylthiazole, sulphaguanidine, 2-amino benzthiazole, 2-aminopyridine, sparfloxacin, 2,6-dichloro aniline and hydrazine hydrate) was refluxed for 6 hrs with 10 ml of acetic acid. The mixture was cooled to room temperature and poured into crushed ice, filter and then washed with water. The solid thus obtained was recrystallized from ethanol. The crude drug obtained was recrystallized from ethanol. The yields and the melting points of the compounds are given in Table 1.

N-Guanido-4-(4-oxo-2-phenyl-4*H*-quinazolin-3-yl)-benzenesulfonamide(Q-SG): IR (KBr) (cm^{-1}): 3450 (NH), 1600(C=O), 1500 (C=N), 1490 (C=C), 700 (Ar-H); ^{1}H NMR (DMSO-d_6): 6.5-8.2 (m, 9 H, Ar-H), 5.5(s, 1H, NH), 5.7(b, 1H, NH), 2.0(b, 1H, NH).

2-Phenyl-3-(4-phenyl-thiazol-2-yl)-3*H* quinazolin-4-one(Q-2A4PT): IR (KBr) (cm^{-1}), 1650 (C=O), 1575 (C=N), 1500 (C=C), 700 (Ar-H), ^{1}H NMR (DMSO-d_6):7 .0(t, 1 H, thiazole-3 H), 7.28-8.052(m, 14 H , Ar-H).

2-Phenyl-3-pyridin-2-yl-3*H*-quinazolin-4-one(Q-2AP): IR (KBr) (cm^{-1}), 1675 (C=O), 1600 (C=N), 1375 (C=C), 725 (Ar-H), ^{1}H NMR (DMSO-d_6): 8.06 (d, 1H, Q-8H), 7.20 (t, 2H, pyridine-H), 7.56-7.67(m , 8 H, Ar-H), 8.06(d, 1H, Q-8H), 8.7(d, 1H, pyridine-H).

3-Benzothiazol-2-yl-2-phenyl-3*H*-quinazolin-4-one (Q-2ABT): IR (KBr) (cm^{-1}), 1770 (C=O), 1530 (C=N), 1446 (C=C), 703 (Ar-H), ^{1}H NMR (DMSO-d_6):8.19 (d, 1H, benzothiazole), 8.21 (d, 1 H, benzthiazole), 6.98-8.21 (m, 13H, Ar-H).

6-Bromo-2-phenyl-3-(4-phenyl-thiazol-2-yl)-3*H*-quinazolin-4-one (Q-M2A4PT): IR (KBr) (cm^{-1}), 1530 (C=N), 1376 (C=C), 615 (C-Br), 703 (Ar-H); ^{1}H NMR (DMSO-d_6):7.00-7.947(m, 13 H , Ar-H).

1-Cyclopropyl-5-(6,8-difluoro-4-oxo-2-phenyl-4*H*-quinazolin-3-yl)-7-(3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (Q-DSF): ^{1}H NMR (DMSO-d_6):0.825 (m, 3H, cyclopropane), 1.11(s, 6H, 2.CH_3), 3.067(2H, piperazinyl), 3.999(s, NH, piperazinyl), 4.460 (s, 2H, piperazinyl),7.28-8.518(m, 7H, Ar-H),7.98(d, 1H, Quinone-CH), 10.25 (s, NH-piperazinyl).

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[11]. 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 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 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-diphenyltetrazolium 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.

***In vitro* cytotoxicity studies**

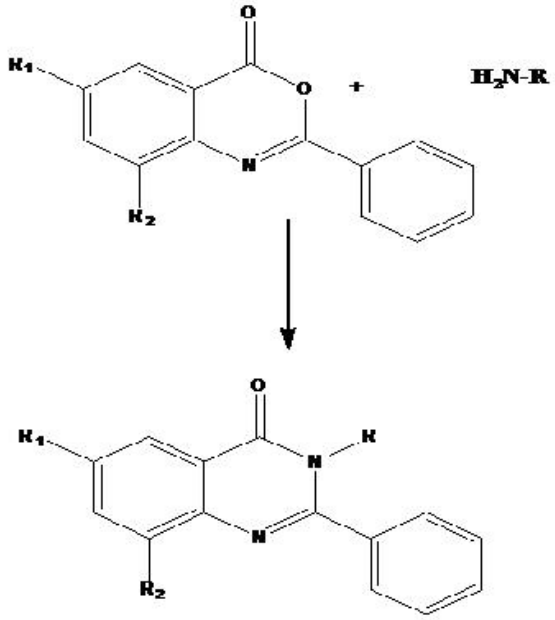
In–vitro cytotoxicity studies of the synthesised compounds were determined by MTT assay in Human liver cancer cells23 (Hep G2 cells)[12]. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium salt into a

blue coloured product-formazan. The monolayer cell culture was trypsinized and the cell count wasadjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100µl of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT in MEM was added toeach well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO_2atmosphere. The supernatant was removed and 50µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the formula below:

$$\%\text{Growth inhibition} = 100 - \frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control group}} \times 100$$

50% cytotoxic concentration (CTC_{50}) of the compounds was then calculated from the plot of concentration v/s % growth inhibition. The cytotoxicity data are presented in table 3.

Synthesis of 2-Phenyl-3-Substituted Quinazolin-4(3H)-one derivatives

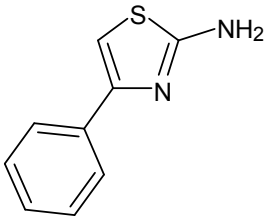
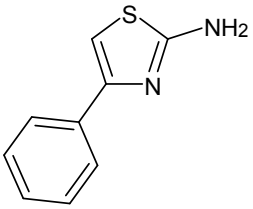
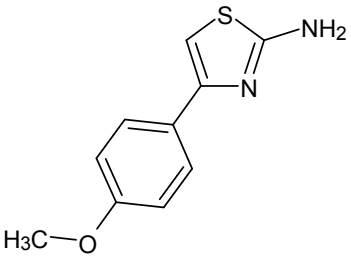
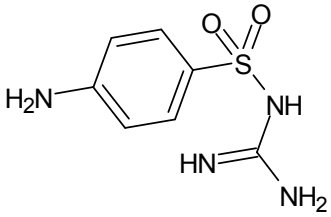
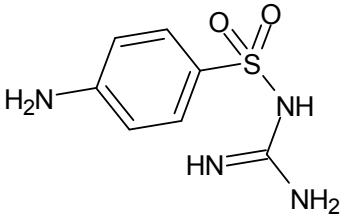
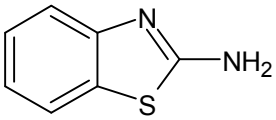
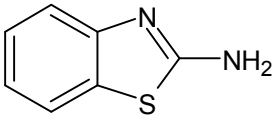
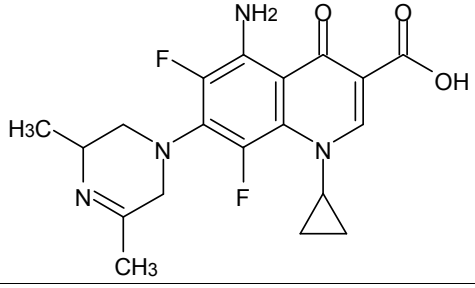


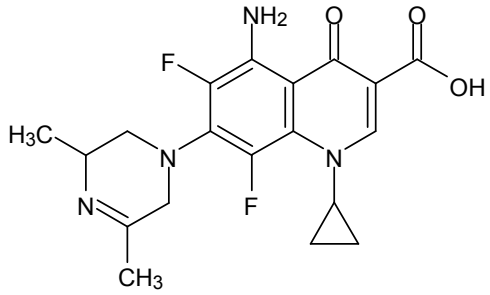
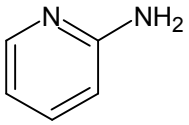
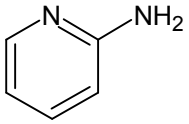
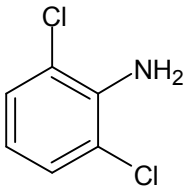
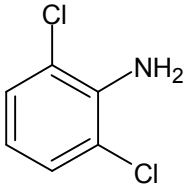
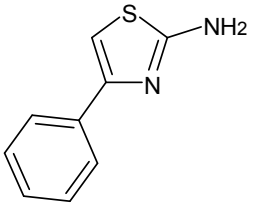
Where, R_1 = H, Br, R_2 = H, Br: R = Different substitution

Results

6-bromo/6,8-dibromo-2-phenyl-3-aminoquinazolin -4(*3H*)-one derivatives were synthesized by condensation of the compounds containing primary aromatic amino group (2-amino-4-phenylthiazole, sulphaguanidine, 2-aminobenzthiazole, 2-amino pyridine, sparfloxacin, 2,6-dichloroaniline) with 6-bromo/6,8-dibromo 2-phenyl-1, 3-benzoxazin -4-one. All the synthesized compounds show the yield of 25 - 80% and structure of the synthesized compounds were elucidated by using Spectral analysis (FT-IR and NMR). The inhibitory effect of anti-viral drugs on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cells was determined by the MTT (tetrazolium) assay method. Cytotoxicity of test compounds against mock-infected MT-4 cells (C type adult T leukemia cells) was assessed by MTT method. Compound Q-ABT (CC_{50}:10.07 ±0.60) was found to be more toxic in this series in MT-4 cells.

Table No. 1

COMPOUND CODE	R_1	R_2	R-NH_2
Q-2A4PT	H	H	
Q-M2A4PT	Br	H	
Q-2A4PT-O-Me	H	H	
Q-SG	H	H	
Q-DSG	Br	Br	
Q-2ABT	H	H	
Q-D2ABT	Br	Br	
Q-SF	H	H	

Q-DSF	Br	Br	
Q-2AP	H	H	
Q-D2AP	Br	Br	
Q-DCA	H	H	
Q-DDCA	Br	Br	
Q-D2A4PT	Br	Br	

Physical data of synthesized compounds

Compound code	Molecular Formula	Molecular Weight	Yield(%)	Melting point(^{0}C)	R_f Value
Q-2A4PT	$C_{27}H_{26}FN_5O_3S$	381.45	57.06	134-136	0.8
Q-M2A4PT	$C_{26}H_{26}FN_5O_3S$	460.35	57.3	140-142	0.5
Q-2A4PT-O-Me	$C_{29}H_{30}F_2N_6O_4S$	413.49	48.78	176-180	0.3
Q-SG	$C_{25}H_{28}FN_7O_5S$	419.46	53	280-282	0.5
Q-DSG	$C_{24}H_{28}FN_7O_5S$	577.25	53.1	270-272	0.6
Q-2ABT	$C_{27}H_{32}F_2N_8O_5S$	355.41	60.91	126-128	0.44
Q-D2ABT	$C_{27}H_{32}FN_7O_6S$	513.20	52	158-160	0.63
Q-SF	$C_{23}H_{25}FN_4O_6S$	579.62	35	144-146	0.9
Q-DSF	$C_{25}H_{24}FN_5O_3S$	737.41	28.5	140-142	0.3
Q-2AP	$C_{27}H_{25}Cl_2FN_8O_3$	299.32	50.8	148-150	0.4
Q-D2AP	$C_{26}H_{25}Cl_2FN_8O_3$	457.11	72.5	154-156	0.9
Q-DCA	$C_{31}H_{36}FN_7O_6$	367.22	43.71	110-110	0.8
Q-DDCA	$C_{32}H_{36}FN_7O_6$	525.02	69.44	108-112	0.7
Q-D2A4PT	$C_{23}H_{15}Br_2N_3OS$	541.25	50.5	120-123	0.5

Table No. 02: Anti-HIV activity and cytotoxicity of synthesized compounds in MT-4 cells

Compound code	Strain	IC_{50}[a] (µg/ml)	CC_{50}[b] (µg/ml)	Max. Protection
2A4PT	IIIB	>70.30	70.30±3.25	3
	ROD	>70.30	70.30±3.25	3
2ABT	IIIB	>11.50	11.50±0.71	1
	ROD	>11.50	11.50±0.71	2
Q-2A4PT	IIIB	>58.10	58.10±3.21	4
	ROD	>58.10	58.10±3.21	10
Q-M2A4PT	IIIB	>10.19	10.19±4.62	2
	ROD	>10.09	10.19±4.62	8
Q-D2A4PT	IIIB	>55.30	55.30±2.69	1
	ROD	>55.30	55.30±2.69	3
Q-SG	IIIB	>64.10	64.10±11.03	0
	ROD	>64.10	64.10±11.03	8
Q-DSG	IIIB	>99.00	99.00±9.90	7
	ROD	>99.00	99.00±9.90	24
Q-ABT	IIIB	>10.07	10.07±0.60	4
	ROD	>10.07	10.07±0.60	6
Q-2A4PT-OME	IIIB	>66.25	66.25±2.69	3
	ROD	>66.25	66.25±2.69	3
Q-DCA	IIIB	≥21.50	60.13±4.35	38
	ROD	≥11.20	60.13±4.35	70
Q-D2CA	IIIB	>61.23	61.23±4.87	25
	ROD	>61.23	61.23±4.87	3
Q-2AP	IIIB	>90.15	90.15±13.27	3
	ROD	>90.15	90.15±13.27	3
Q-D2AP	IIIB	>92.08	92.08	68
	ROD	>92.08	92.08	63
Q-SF	IIIB	>60	>60	68
	ROD	>60	>60	63
DDN/AZT	IIIB	0.0067±0.0002	>25	68
Retrovir (STD)	ROD	0.0020±0.0001	>25	63

[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 reduc reduce the viability of mock infected MT-4 cells by 50%.

Table No. 03: Determination of CTC_{50} by using MTT assay in HepG2 cells cell cultures

Extract	CTC_{50} in (µg/ml) MTT assay against HepG2 cells@
QM2A4PT	157.26 ± 4.31
QD2AP	184.10 ± 3.25
QDSG	192.18 ± 4.77
QD2ABT	177.85 ± 4.02
QSG	217.35 ± 4.10
QSF	206.23 ± 3.35
Q2AP	161.42 ± 3.05
Q2ABT	150.78 ± 2.45
Q2A4PT	158.81 ± 3.46
Cis-Platin (Standard)	11.09 ± 0.59

CTC_{50} = 50% cytotoxic concentration [@]Average of six independent determinations, values are mean ± S.E.M.

Nine compounds in this series were screened for anticancer activity against human liver cancer cells (HepG2) by using MTT assay using standard anticancer drug Cis-platin. All the tested compounds exhibited significant cytotoxicity against liver cancer cells (CTC_{50}: 150-206 µg/ml). Among the compounds tested, Q-2ABT (CTC_{50}: 150.78 ± 2.45 µg/ml) showed better cytotoxicity against HepG2 (human liver cancer cells), where as standard Cis-platin was found to be 11.09±0.59 µg/ml (15 fold higher). Compound Q-2ABT merits further investigation to screen for their anticancer property.

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