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Synthesis and Antimicrobial Activity of Some Novel 6-Bromo-2-methyl/phenyl-3-(sulphonamido) quinazolin-4(3H)-ones

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A series of novel 2-substituted-3-(sulphonamido) quinazolin-4(3H)-ones have been synthesized by condensing the primary amino group of sulphamoxole and sulphadimidine with 2-substituted benzoxazin-2-one. The compounds synthesized were screened for antibacterial activity (*Escherichia coli* and *Staphylococcus aureus)* antiviral activity *(Herpes simplex virus* type 1 and 2 in E_6SM cells, HIV-1 and 2 in MT-4 cells), all the test compounds exhibited comparable antibacterial activity with that of standards sulphamoxole and sulphadimidine.

Quinazolin-4(3H)-one is a versatile lead molecule for designing potential bioactive agents. Quinazolin-4(3H)-one derivatives have been evaluated for a wide spectrum of biological activities such as sedative[1], anticonvulsant[2], antifungal and antibacterial[3], antiHIV[4,5] and anticancer[5] activities. The objective of the study was to synthesize a series of hitherto unreported 2-substituted and 6-bromo-2-substituted-3-(sulphonamido)quinazolin-4(3H)-ones and these compounds were also evaluated for antibacterial (*Escherichia coli* and *Staphylococcus aureus)* and antiviral (*Herpes simplex virus* type 1 and 2 in E_6SM cells, HIV-1 and 2 in MT-4 cells) activities. C log P calculation was performed based on the structure of the synthesized compounds by Biobyte software for measuring the hydrophobic parameter[6].

Anthranilic acid/6-bromo anthranilic acid reacts with acetic anhydride and benzoyl chloride to form corresponding 2-methyl/-phenyl benzoxazin-4-one by N-acylation, followed by dehydrative cyclization mechanism[7]. The 2-methyl/2-phenyl and 6-bromo derivatives of benzoxazin-4-one were condensed with the primary amino group of sulphamoxole to afford 6-bromo and 2-methyl/phenyl-[N(4,5-dimethyl-oxazol-2-yl)sulphonamido)]quinazolin-4-(3H)-ones; similarly, 2-methyl/2-phenylbenzoxazin-4-one reacted with sulphadimidine to form 2-methyl/phenyl-3[N-4,6-dimethyl-pyrimidin-2-yl)sulphonamido)]quinazolin-4(3H)-one (Scheme 1 and 2). All the compounds (Table 1) gave satisfactory elemental analysis. IR and NMR spectra were consistent with the assigned structure.

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MATERIALS AND METHODS

Melting points were determined by using Thomas melting point apparatus and were uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated by using Perkin-Elmer FT-IR in KBr disc and ^{1}H NMR was taken on a Bruker AMX (500 MHz) FT-NMR. Mass spectra were obtained on a Varian Atlas CH-7 mass spectrometer at 70 eV. Microanalyses were performed by the microanalytical unit of the Central Drug Research institute, Lucknow.

Synthesis of 6-bromo and 2-methyl/phenyl-3[N-(4,5-dimethyl-oxazol-2-yl) sulphonamido)]quinazolin-4(3H)-one(I-IV):

An equimolar (0.01 mol) mixture of 2-substituted-1,3-

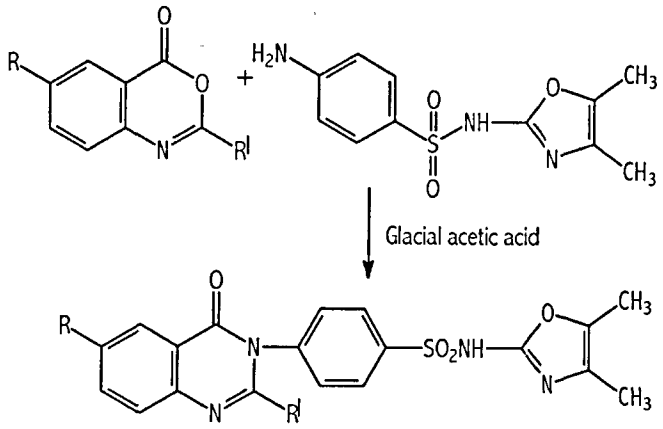

Fig. 1: Synthetic protocol of the compounds (I-IV).

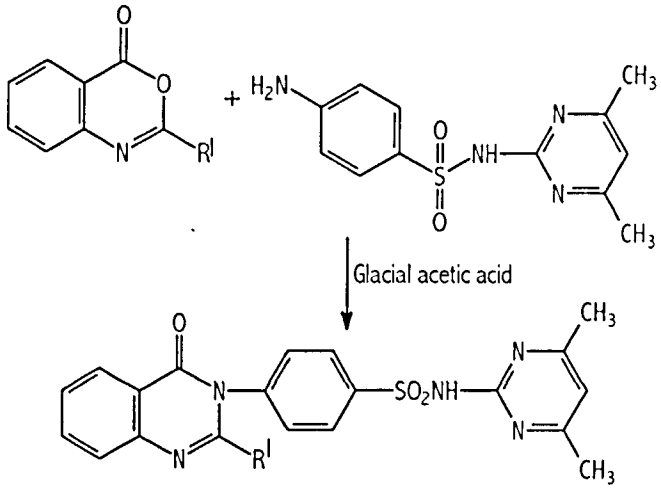

Fig. 2: Synthetic protocol of the compounds (V).

benzoxazon-4-one and sulphamoxole was refluxed for 6 h with 10 ml of glacial acetic acid. The mixture was cooled to room temperature and poured into crushed ice; the solid thus obtained was recrystallized from ethanol. The yield and melting points are given in Table 1.

(I): IR (KBr, υ_{max} in cm^{-1}): 3260 (NH), 1664 (C=O), 1556 (C=N), 1159 (SO_2); PMR (DMSO-d_6) δ ppm: 2.1 (m, 9H, 3 x CH_3), 7.2 (t, 1H,Q-7H), 7.4 (s,1H,Q-6H), 8.1-7.6 (m, 7H,Ar-H), 8.5 (d,1H,Q-5H), 10.3 (b,1H, SO_2NH); EI-MS (m/z):410.447. (II): IR (KBr, υ_{max} in cm^{-1}): 3260 (NH), 1665 (C=O), 1594 (C=N), 1148 (SO_2); PMR (DMSO-d6) δ ppm: 2.05 (s, 3H, CH_3), 2.1 (d, 6H, 2xCH_3), 7.3 (t,1H,Q-7H), 7.5-7.8 (m,

TABLE 1: PHYSICAL DATA FOR 6- BROMO- AND 2- SUBSTITUTED-3-(SULPHONAMIDO) QUINAZOLIN-4(3H)-ONES (I-VI)

Comp.	R	R^1	Molecular Formula	Yield (%)	MP (°)	C log P Value	R_f[a] value	Elemental Analysis Calculated / Found		
								C	H	N
I	-H	$-CH_3$	$C_{20}H_{18}N_4O_4S$	74	SS	1.768	0.71	58.54	4.39	13.66
								58.51	4.12	13.14
II	-Br	$-CH_3$	$C_{20}H_{17}BrN_4O_4S$	65	245	2.639	0.81	49.08	3.48	11.45
								49.10	3.35	11.42
II	-H	$-C_6H_5$	$C_{25}H_{20}N_4SO_4$	75	196	3.367	0.80	63.56	4.24	11.86
	-							63.22	4.16	11.74
IV	Br	$-C_6H_5$	$C_{25}H_{20}BrN_4SO_4$	65	242	4.238	0.84	54.45	3.45	10.16
								54.42	3.36	10.08
V	-H	$-CH_3$	$C_{21}H_{19}N_5SO_3$	75	183	2.208	0.85	59.86	4.51	16.63
								59.42	4.48	16.58
VI	-H	$-C_6H_5$	$C_{26}H_{21}N_5SO_3$	67	186	3.807	0.82	64.60	4.35	14.50
								64.32	4.28	14.38

SS – semisolid [a] Eluent used in TLC was chloroform: methanol(9:1)

4H, Ar-H), 8.1 (d, 1H, Q-8H), 8.4 (d, 1H, Q-5H), 10.3 (b,1H,-SO_2NH); EI-MS (m/z): 489.343. (III): IR (KBr, υ_{max} in cm^{-1}): 3290 (NH), 1650 (C=O), 1606 (C=N), 1146 (SO_2); PMR (DMSO-d_6) δ ppm: 2.1 (d, 6H, 2xCH_3), 7.2 (t, 1H, Q-6H), 7.5 (m, 4H, Ar-H), 7.9 (m, 6H, Ar-H), 8.3 (d, 1H, Q-5H), 11.7 (b, 1H,-SO_2NH); EI-MS (m/z); 472.517. (IV): IR (KBr, υ_{max} in cm^{-1}): 3295 (NH), 1672 (C=O), 1643 (C=N), 1146 (SO_2); PMR (DMSO-d_6) δ ppm: 2.0 (d, 6H, 2 x CH_3), 7.5 (t, 1H, Q-7H), 7.82 (m, 9H, Ar-H), 7.8 (d, 1H, Q-8H), 8.2 (d, 1H, Q-5H), 10.8 (b, 1H,-SO_2NH); EI-MS (m/z): 551.414.

Synthesis of 2-methyl/phenyl-3[N-(4,6-dimethyl-pyrimidin-2-yl)sulphonamido)] quinazolin-4(3H)-one (VandVI):

An equimolar (0.01 mol) mixture of 2-substituted-1,3-benzoxazin-4-one and sulphadimidine was refluxed for 6 h with 10 ml of glacial acetic acid. The mixture was cooled to room temperature and poured onto crushed ice; the solid thus obtained was recrystallized from ethanol. The yield and melting points are given in Table 1.

(V): IR (KBr, υ_{max} in cm^{-1}): 3290 (NH), 1590 (C=O), 1540 (C=N), 1151 (SO_2); PMR (DMSO-d_6) δ ppm: 2.1 (s, 3H, CH_3), 2.3 (s, 6H, 2xCH_3), 6.6 (d, 1H, Q-7H), 7.1 (t, 1H, Q-6H), 7.5-7.8 (m, 4H, Ar-H) 7.9 (d,1H,Q-8H), 8.0 (d, 1H, Pyrimidinyl), 8.5 (d, 1H,Q-5H), 10.3 (b, 1H, -SO_2NH); EI-MS (m/z): 421.473. (VI):IR (KBr, υ_{max} in cm^{-1}): 3290 (NH), 1649 (C=O), 1583 (C=N), 1158 (SO_2); PMR (DMSO-d_6) δ ppm: 2.2 (s, 6H, 2xCH3), 7.2 (d, 1H, Q-7H) 7.3 (t, 1H, Q-6H), 7.5-7.8 (m, 5H, Ar-H), 7.8-8 (m, 4H, Ar-H) 8.1 (d, 1H, Pyrimidinyl), 8.3 (d, 1H, Q-8H), 8.7 (d, 1H, Q-5H), 10.3 (b, 1H, -SO_2NH); EI-MS (m/z): 483.544.

C log P calculation:

Log P is a hydrophobic parameter used in QSAR studies for correlation of lipophilic character of molecules with their biological activities. C log P calculation of the synthesized compounds was performed with the Biobyte software and the results are given in Table 1.

TABLE 2: ANTIBACTERIAL ACTIVITY OF COMPOUNDS (I-VI)

Comp.	Minimum Inhibitory Concentration(MIC)	
	E. coli	*S. aureus*
I	10	15
II	12	17
III	10	15
IV	11	14
V	14	14
VI	14	16
SM	11	16
SD	13	15

MIC in μg/ml, SM: Sulphamoxole, SD: Suphadimidine.

TABLE 3: ANTIVIRAL ACTIVITY AND CYTOTOXICITY OF COMPOUNDS IN E_6SM CELLS

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)	
		HSV-1	HSV-2
I	>400	>400	240
II	>400	240	240
III	>80	>80	>80
IV	80	>80	>80
V	>400	>400	>400
VI	80	>80	>80
BVDU	>400	0.0153	80

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

Antibacterial activity:

The antibacterial activity was determined by the agar dilution method[8] against *E. coli* and *S. aureus.* The medium was prepared as per the instructions of the Manufacturer of dry Mueller Hinton agar powder (Hi-Media). The concentrations of the test samples used were from 100 μg/ml to lower concentrations made by serial double dilutions with DMF. The minimum inhibitory concentration (MIC) was taken as the lowest concentration (highest dilution) without visible growth. The study was simultaneously performed with the pure standard drugs (sulphamoxole and sulphadimidine). The MICs are reported in Table 2.

Antiviral activity:

Antiviral activity of the test compounds was tested against *Herpes simplex virus* type 1 and 2 in E_6SM cells. Cytotoxicity was verified in mock-infected E_6SM cells[9]. The antiviral activity assays were based on inhibition of virus-induced cytopathicity in E_6SM 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 completion in the control virus-infected cell cultures. Antiviral activity and cytotoxicity of synthesized compounds were compared with that of BVDU [brivudin (bromovinyldeoxyuridine)] evaluated under similar conditions. The results on antiHSV activity and cytotoxicity are shown in Table 3.

AntiHIV activity:

The compounds were tested for antiHIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells[10]. 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) and HIV-2 ($LAV\text{-}2_{ROD}$) were used in all experiments. 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.

The 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 the MTT method. Briefly, 50 μl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) were added to a flat-bottomed microtiter tray with 50 ml of medium containing various concentrations of the test compounds. MT-4 cells were added at a final concentration of $6x10^5$ 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 also assessed by the MTT method. AntiHIV activity and cytotoxicity of the standard compound AZT were also performed by a similar method in MT-4 cells.

TABLE 4. ANTIHIV ACTIVITY AND CYTOTOXICITY OF THE COMPOUNDS IN MT-4 CELLS

Compound	Strain	EC_{50}[a] (μg/ml	CC_{50}[b] (μg/ml)
I	HIV-1(III_B)	> 124	124
I	HIV-2(III_B)	> 125	>125
I	HIV-2(ROD)	> 125	>125
II	HIV-1(III_B)	> 80.4	80.4
II	HIV-1(III_B)	> 118	118
II	HIV-2(ROD)	> 125	125
III	HIV-1(III_B)	> 125	>125
III	HIV-1(III_B)	> 125	>125
III	HIV-2(ROD)	> 125	> 125
IV	HIV-1(III_B)	>17	17
IV	HIV-1(III_B)	> 46.3	46.3
IV	HIV-2(ROD)	> 48.6	48.6
V	HIV-1(III_B)	> 125	>125
V	HIV-1(III_B)	> 125	>125
V	HIV-2(ROD)	> 125	>125
VI	HIV-1(III_B)	> 68.3	68.3
VI	HIV-1(III_B)	>82.6	82.6
VI	HIV-2(ROD)	> 78.5	78.5
AZT	HIV-1(III_B)	0.0012	65.90
	HIV-2(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%.

The results on antiHIV activity and cytotoxicity are shown in Table 4.

RESULTS AND DISCUSSION

Synthesized compounds were screened for antibacterial activity against *E. coli* and S. *aureus* by agar dilution method. The test compounds were found to have comparable antibacterial activity with that of the parent drugs, sulphamoxole and sulphadimidine. C log P studies indicated that increase in ring size leads to increase in hydrophobicity of the compounds. Substitution of phenyl group for methyl group in the second position of quinazolin-4(3H)-one also leads to an increase in the hydrophobicity.

From results of the antiviral activity and cytotoxicity studies, it can be concluded that the compounds have no appreciable activity against HSV-1 or HSV-2 at non-cytotoxic concentrations, whereas the standard compound BVDU was found to have a MIC of 0.0153 and 80 μg/ml in the E_6SM cells against HSV-1 and 2, respectively.

The synthesized compounds were evaluated for antiHIV activity against HIV-1(III_B) and HIV-2 (ROD) replication in acutely infected MT-4 cells. The 50% effective concentration (EC_{50}) values of the synthesized compounds against the replication of HIV-1 and HIV-2 in acutely infected MT-4 cells were higher than the cytotoxic concentrations (CC_{50}), which means that the compounds did not exert any specific antiHIV activity.

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