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Research Article

DESIGN AND SYNTHESIS OF NOVEL 2, 3-DISUBSTITUTED QUINAZOLINES: EVALUATION OF *IN VITRO* ANTICANCER ACTIVITY AND *IN SILICO* STUDIES

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ABSTRACT

Objective: In this study, a series of novel 2,3-disubstituted quinazolines (4a-4l) were synthesized using standard procedures and elucidated through different spectroscopic techniques.

Methods: Obtained compounds were evaluated for their cytotoxicity against human breast cancer (MDA-MB-231) and ovarian cancer (SK-O-V3) cell lines using MTT assay. Docking studies with JAK2 protein were performed to elucidate the possible mechanistic insights into these novel quinazoline derivatives.

Results: Moderate-to-good *in vitro* cytotoxic potentials of the newly synthesized molecules were reported against selected human cancer cell lines. Among the tested molecules, compound 4e showed good cytotoxic activity against MD-AMB-231 (14.2 ± 0.86 μM) and against SK-O-V3 (17.7 ± 0.62 μM).

Conclusion: The *in vitro* studies of the newly synthesized quinazoline derivatives reported considerable cytotoxic potentials against both breast and ovarian cancer cell lines and SAR studies suggest that quinazoline derivatives with heterocyclic benzothiazole nucleus with hydrophilic acetamide linkage at the 3rd position could probably increase the cytotoxic potentials and the presence of chlorine substitution could add more benefit. With the reported bioactivities of these derivatives, further studies on the derivatization could elucidate the broader cytotoxic potentials.

Keywords: Quinazoline, cytotoxic activity, MTT assay, breast cancer, JAK2.

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INTRODUCTION

Cancer is a major public health problem worldwide and is the leading cause of deaths to 606,880 alone in the USA in 2019 alone. Majority of cancer deaths are due to cancer of lungs, breast, genital system, digestive system, lymphoma, leukemia, etc. Breast cancer is one of the major problems in women leads to enhance mortality rate. In 2019, the American Cancer Society estimated 271,270 cases of the invasive breast cancer diagnosed and 42,260 deaths in the U. S. women [1]. Breast cancer treatment includes different approaches, such as chemotherapy (single agent or combination therapy), surgery, and radiotherapy. Advancement in chemotherapeutic strategies is currently not efficient in treating the malignancy, due to the lack of selectivity between normal cell and cancer cell, due to the development of drug resistance resulting in poor clinical benefits. Even though there is a progress in many aspects of cancer research, still have so many disadvantages like high toxicity and low efficacy. Hence, there is a necessity to develop novel molecules to treat wide variety of cancers that are occurring.

Since from long time, heterocycles are considered as an important core moiety for the development of newer generation cytotoxic agents. Quinazoline derivatives have made the researchers attention due to their number of pharmacological activities. Hence, many therapeutic activities of quinazoline derivatives were already determined by the researchers including anticancer [2-5], anti-inflammatory [6,7], antibacterial [8-11], analgesia [6,10], antivirus [12], anticytotoxic [13], antispasmodic [10,14], antituberculosis[15], antioxidant[16], antimalarial[17], antihypertension[18], antiobesity [19], antipsychotic [20], antidiabetic [21], anticoagulant [22], and VEGFR2 inhibitors [23]. Due to these many wide number of activities, researchers made efforts, led to the development of quinazoline as a new lead compound in the inhibition of a variety of cancers with less toxic effects.

In this present work, a novel series of quinazoline derivatives were synthesized accommodating a substituted phenyl group (hydrophobic moiety) at position-2 and introduction of benzothiazole group (heterocycle) at 3-position with acetamide linkage (hydrophilic) to 2-substituted quinazolinones. These novel quinazolines are screened for cytotoxicity potentials against human breast and ovarian cancer cell lines.

METHODS

Chemistry

The required chemicals were procured from Sigma-Aldrich Ltd., India, and thin-layer chromatography (TLC) was used to monitor the reactions and chromatographic separation. Silica gel (60–120 mesh, SRL chemicals) coated glass plates were used for TLC. Purification of the compounds was carried out by column chromatography using an increasing percentage of ethyl acetate in hexanes (3:7) as a solvent. SISCO instrument was used to measure all the melting points and uncorrected using open-ended capillary tubes. Nicolet-6700 spectrometer was used to record IR spectra of the compounds on KBr pellets. Tetramethylsilane as internal standard and DMSO-d6 solvent used to dissolve the compound. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were recorded for the synthesized compounds on a Bruker-400 spectrometer. ^{1}H-NMR data are reported as follows: Chemical shifts and in support of the structure; ^{13}C-NMR were also recorded. Mass spectra of the synthesized compounds were recorded on a Shimadzu liquid chromatography–mass spectrometry (LCMS)-8030 using ESI mode.

General procedure for the synthesis of anthranilic acid

To 4.2 ml of bromine, add 15 g of sodium hydroxide (NaOH) and dissolve it thoroughly, then add 12 g of phthalimide, [Scheme 1] stir

the solution until it gets dissolved completely. To this, add cooled solution of 11 g of NaOH in 40 ml of H_2O (Temperature 70°C) and maintain the solution temperature around 70°C–80°C for 5 min. To this, add 30 ml of concentrated HCl and 10–12 ml of glacial acetic acid to get anthranilic acid. This was recrystallized using water (H_2O). The obtained product was already reported which was confirmed by measuring the melting point which was around 146°C–148°C.

General procedure for the synthesis of 2-(4-substituted phenyl) quinazoline-4(3H)-ones: (2a-2l)

To 20 ml of ethanol, add equimolar quantities (0.01 mol) of anthranilic acid and substituted benzaldehyde then add 0.05 mol of $CuCl_2$. Stir the mixture well until all the compounds will get dissolved. Then reflux this mixture for about 1–3 h. Determine the completion of the reaction by TLC analysis. Cool the reaction mixture and add ice-cold water to get the crude compounds 2a-2l. The crude products are recrystallized by ethanol to get the pure product. The synthesized compounds were confirmed by their melting points, mass, ^{1}H-NMR, and ^{13}C-NMR spectroscopy.

General procedure for the synthesis of (N-(1,3-benzothiazol-2-yl)-2-chloroacetamide: (3)

To 30 ml of glacial acetic acid, add 10 ml of ethanol then add (0.016 mol) of 2-amino benzothiazole. Stir the reaction mixture thoroughly until it gets dissolved. Then dropwise add 0.032 mol of chloroacetyl chloride with constant stirring. Then reflux the reaction mixture for about 5 h, then the reaction mixture was poured on to the crushed ice to get the precipitate. The obtained precipitate was filtered off, washed with cold water and dried. The obtained product was recrystallized using aqueous ethanol to get the compound 3. The synthesized compounds were confirmed by their melting points, mass, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. All the synthesized compounds were correlated with the spectral data which confirm the product formation.

General procedure for the synthesis of N-(benzo[d]thiazol-2-yl)-2-(4-oxo-2-[4-substituted phenyl] quinazoline-3[4H] yl acetamide: (4a-4l)

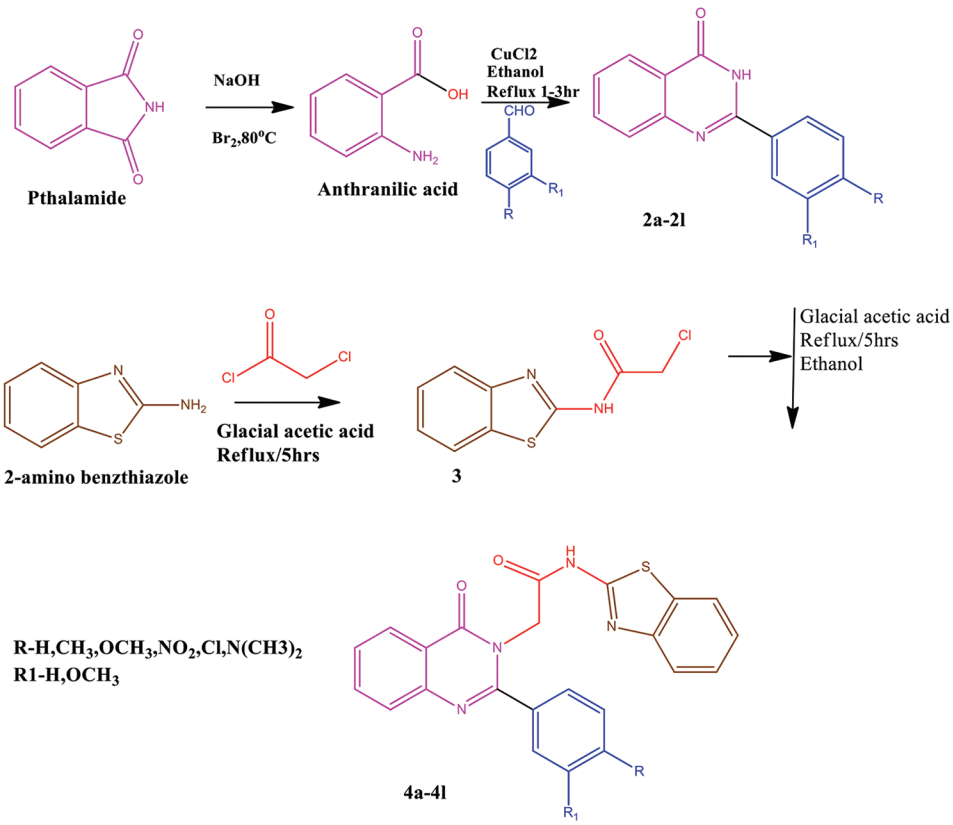
To 5 ml of ethanol and 15 ml of the glacial acetic acid solution, add equimolar quantities (0.01 mol) of compounds 2a-2l with compound 3, stir thoroughly until the compounds get completely dissolved. Then reflux the reaction mixture for about 3 h and then it was poured onto the crushed ice to get the precipitate. Filter the obtained precipitate. The obtained product was recrystallized by ethanol. The synthesized compounds were confirmed by their melting points, IR, mass, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. All the synthesized compounds were correlated with the spectral data which confirm the product formation.

N-(benzo[d]thiazol-2-yl)2-(4-oxo-2-phenylquinazolin-3(4H)-yl) acetamide: (4a)

Pale brown solid, yield – 73%, M. W – 412 g/mol, M. P – 267°C–269°C, Mass (LC–MS): M/z 412 (M), 413 (M + 1, 100%).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 9.59 (s,-NH, acetamide), 7.78-7.73 (d, 2H, Ar-H), 7.59-7.56 (d, 2H, Ar-H), 7.48-7.47 (d, 2H, Ar-H), 7.44-7.43 (t, 4H, Ar-H), 7.39-7.38 (t, 3H, Ar-H), 4.18-4.17 (s, 2H,-CH_2-); IR spectroscopy of the compound represents 3380 (-NH *Str*, Acetamide), 3050 (C-H *Str*, Ar), 2909, 2839 (C–H *Str*, Aliphatic), 2309 (C-S-C *Str*), 1737 (C = O *Str*, quinazoline), 1688 (C = O *Str*, acetamide), 1537 (C = CH *Str*), 1435 (C = C *Str*, Ar), 1162 (C-N *Str*). Analysis calculated for M. F $C_{23}H_{16}N_4O_2S$ (412.10): C, 66.97; H, 3.91; N, 13.58%. Found: C, 66.78; H, 3.99; N, 13.96%.

N-(benzo[d]thiazol-2-yl)-2-(4-oxo-2-(p-tolyl) quinazolin-3(4H)-yl) acetamide:(4b)

Light yellow solid, yield – 75%, M. W – 426 g/mol, M. P – >300°C, Mass (LC–MS): M/z 426 (M), 427 (M + 1, 100%);^{1}H-NMR (DMSO) $\delta\delta$ ppm: 9.82 (s, 1H, acetamide), 8.18-7.87 (d, 2H, Ar-H), 7.85-7.80 (d, 2H, Ar-H), 7.80-7.79 (d, 2H, Ar-H), 7.78-7.76 (t, 4H, Ar-H), 7.69-7.67 (t, 2H, Ar-H), 7.66-7.64 (t, 2H, Ar-H), 4.37-4.35 (s, 2H,-CH_2-), 2.09-2.05 (S, 3H,-CH_3;IR spectroscopy of the compound represents 3382 (-NH *Str*, Acetamide), 3025 (C-H *Str*, Ar), 2925, 2825 (C–H *Str*, Aliphatic), 2320 (C-S-C *Str*),



Scheme 1: Synthesis of 2, 3-disubstituted quinazolines

1729 (C = O *Str*, quinazoline), 1682 (C = O *Str*, acetamide), 1519 (C = CH *Str*), 1437 (C = C *Str*, Ar), 1160 (C-N *Str*). Analysis calculated for M. F $C_{24}H_{18}N_4O_2S$ (426.12): C, 67.59; H, 4.25; N, 13.14%. Found: C, 67.78; H, 4.39; N, 13.06%.

N-(benzo[d]thiazol-2-yl)-2-(2-(4-methoxyphenyl)-4-oxoquinazolin-3(4H)-yl)acetamide:(4c)

Dark brown solid, yield – 77%, M. W – 442 g/mol, M. P – 237°C–239°C, Mass (LC–MS): M/z 442 (M), 443 (M + 1, 100%). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 9.53 (s, 1H, acetamide), 8.28-8.23 (d, 2H, Ar-H), 8.17-8.12 (d, 2H, Ar-H), 8.05-8.02 (d, 2H, Ar-H), 7.88-7.87 (d, 2H, Ar-H), 7.78-7.75 (t, 2H, Ar-H), 7.73-7.61 (t, 2H, Ar-H), 4.127-4.125 (s, 2H,-CH_2-), 3.69-3.61 (S, 3H,-OCH_3; ^{13}C-NMR (DMSO-d6) 173 (-CO, Quinazoline), 170 (-CO, acetamide), 162 (C = N), 143-110 (Ar-C), 49.12 (-CH_2-), 46.31 (-OCH_3); IR spectroscopy of the compound represents 3388 (-NH *Str*, Acetamide), 3060 (C-H *Str,* Ar), 2960, 2869 (C–H *Str*, Aliphatic), 2353 (C-S-C *Str*), 1701 (C = O *Str*, quinazoline), 1667 (C = O *Str*, acetamide), 1524 (C = CH *Str*), 1439 (C = C *Str*, Ar), 1153 (C-N *Str*). Analysis calculated for M. F $C_{24}H_{18}N_4O_3S$ (442.11): C, 65.14; H, 4.10; N, 12.66%. Found: C, 65.28; H, 4.16; N, 12.86%.

N-(benzo[d]thiazol-2-yl)-2-(2-(4-nitrophenyl)-4-oxoquinazolin-3(4H)-yl)acetamide: (4d)

Brownish yellow solid, yield – 69%, M. W – 457 g/mol, M. P – 213°C–216°C, Mass (LC–MS): M/z 457 (M), 458 (M + 1, 100%); ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 9.30 (s, 1H, acetamide), 7.79-7.78 (d, 2H, Ar-H), 7.77-7.75 (d, 2H, Ar-H), 7.69-7.66 (d, 2H, Ar-H), 7.62-7.60 (d, 2H, Ar-H), 7.58-7.50 (t, 4H, Ar-H), 4.26-4.24 (s, 2H,-CH_2-); ^{13}C-NMR (DMSO-d6) 181 (-CO, Quinazoline), 171 (-CO, acetamide), 169 (C = N), 151-125 (Ar-C), 42.12 (-CH_2-); IR spectroscopy of the compound represents 3388 (-NH *Str*, Acetamide), 3059 (C-H *Str,* Ar), 2911 (C–H *Str*, Aliphatic), 2359 (C-S-C *Str*), 1709 (C = O *Str*, quinazoline), 1693 (C = O *Str*, acetamide), 1584 (-NO_2 *Str*), 1584 (C = CH *Str*), 1484 (C = C *Str*, Ar), 1159 (C-N *Str*). Analysis calculated for M. F $C_{23}H_{15}N_5O_4S$ (457.46): C, 60.39; H, 3.31; N, 15.31%. Found: C, 60.68; H, 3.46; N, 15.46%.

N-(benzo[d]thiazol-2-yl)-2-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)acetamide: (4e)

Light orange solid, yield – 83%, M. W – 446 g/mol, M. P – 207°C–209°C, Mass (LC–MS): M/z 446 (M), 447 (M + 1, 100%), 448 (M + 2, 30%); ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 9.33 (s, 1H, acetamide), 7.69-7.68 (d, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 7.63 (d, 2H, Ar-H), 7.38-7.35 (d, 2H, Ar-H), 7.29-7.28 (t, 4H, Ar-H), 4.1271-4.1272 (s, 2H,-CH_2-). ^{13}C-NMR (DMSO-d6) 171 (-CO, Quinazoline), 169 (-CO, acetamide), 157 (C = N), 153-106 (Ar-C),54.10 (-CH_2-). IR spectroscopy of the compound represents 3415 (-NH *Str*, Acetamide), 3027 (C-H *Str,* Ar), 2927, 2826 (C–H *Str*, Aliphatic), 2368 (C-S-C *Str*), 1737 (C = O *Str*, quinazoline), 1669 (C = O *Str*, acetamide), 1588 (C = CH *Str*), 1437 (C = C *Str*, Ar), 1118 (C-N *Str*), 814 (-Cl *Str*). Analysis calculated for M. F $C_{23}H_{15}N_4O_2SCl$ (446.91): C, 61.81; H, 3.38; N, 12.54%. Found: C, 61.78; H, 3.46; N, 12.36%.

***In vitro* cytotoxic activity**

MTT assay [24] was carried out for determining the cytotoxic activity of the selected compounds in terms of *in vitro* growth inhibition. The cytotoxicity of the selected compounds was evaluated against both human and non-human cell lines: MDA-MB-231 (human breast cancer), SK-O-V3 (human ovarian cancer). The exponential growth of healthy cells and plated (1×10^4) in 96-well microliter plates and grown for a period of 24 h. These cells were treated against selected compounds at varying concentrations and then incubated for 48 h. Then, these cells were treated with 250 µg/ml of MTT and incubated again for 2 h. The medium was replaced with 100 µL of DMSO and the absorbance was measured at 570 nm. IC_{50} values of the test compounds were calculated from the dose-response curves using doxorubicin as standard drug. The experiment was carried out in triplicates and IC50 values are expressed in the standard error of the mean.

Molecular docking and binding energy calculations

Structures of all the synthesized quinazoline derivatives (4a-4l) were drawn using ChemSketch and optimized/energy minimized using Ligprep module of Schrodinger suite, employing OPLS-2005 (optimized potentials for liquid simulations) force field [25]. A 3D structure of the target kinase domain of JAK2 protein was retrieved from the Protein Data Bank website (PDB Id: 3RVG). Protein structure was optimized using Protein Preparation Wizard, by adding hydrogen atoms to satisfy the valences and optimized using OPLS-2005 force field. Protein-ligand interactions and conformational changes were examined using Glide XP docking protocol [26]. Glide score is a combination of hydrophilic, hydrophobic, metal-binding groups, Van der Waals energy, freezing rotatable bonds, and polar interactions with receptor. MM/GBSA calculations were performed for the docked protein-ligand complexes to determine the binding energy. The total free energy of binding is then expressed in the form below mentioned equation:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

Where ΔG_{bind} is ligand binding energy.

In other terms,

$$\Delta G_{bind} = \Delta E_{gas} + \Delta G_{solvation} - T\Delta S$$

where gas-phase molecular mechanical energy is ΔE_{gas}, solvation free energy is $\Delta G_{solvation}$ (polar and non-polar contributions), and $T\Delta S$ is the entropy [27].

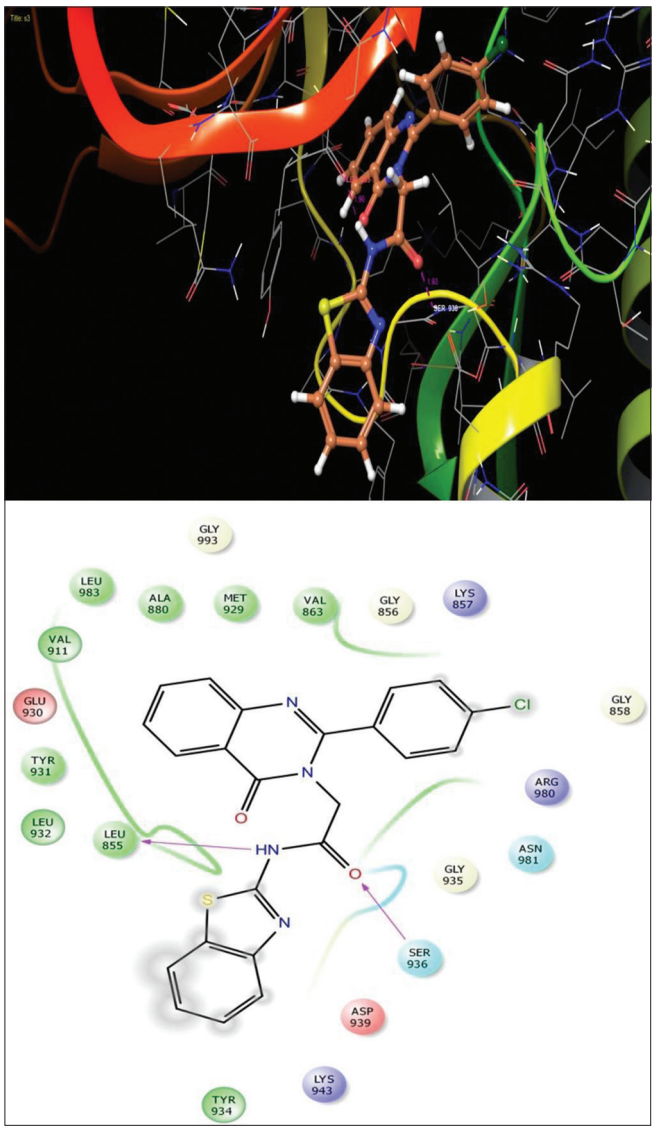
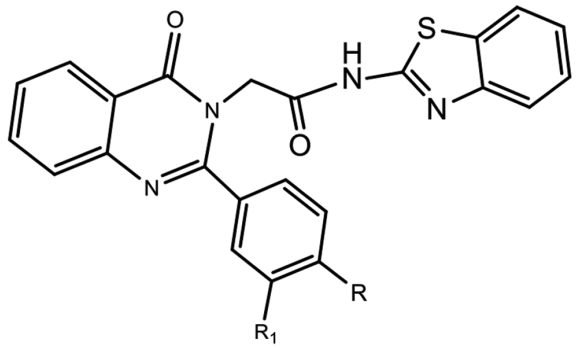


Fig. 1: Protein-ligand interactions of compound 4e with JAK2 protein

Table 1: Physical characterization of novel 2,3-disubstituted quinazolines



4a-4l

Compound name	*R*	R_1	Molecular formula	M.W (g/mol)	M.P (°C)	Yield (%)	Rf values
4a	H	H	$C_{23}H_{16}N_4O_2S$	412.10	267–269	73	0.62
4b	CH_3	H	$C_{24}H_{18}N_4O_2S$	426.12	>300	75	0.72
4c	OCH_3	H	$C_{24}H_{18}N_4O_3S$	442.11	237–239	77	0.59
4d	NO_2	H	$C_{23}H_{15}N_5O_4S$	457.46	213–216	69	0.71
4e	Cl	H	$C_{23}H_{15}N_4O_2SCl$	446.91	207–209	83	0.60
4f	N $(CH_3)_2$	H	$C_{25}H_{21}N_5O_2S$	455.53	280–283	81	0.62
4g	H	OCH_3	$C_{24}H_{18}N_4O_3S$	442.49	272–275	76	0.81
4h	CH_3	OCH_3	$C_{25}H_{20}N_4O_3S$	456.52	293–295	65	0.79
4i	OCH_3	OCH_3	$C_{25}H_{20}N_4O_4S$	472.52	289–290	62	0.65
4j	NO_2	OCH_3	$C_{24}H_{17}N_5O_5S$	487.49	224–226	74	0.58
4k	Cl	OCH_3	$C_{24}H_{17}ClN_4O_3S$	476.93	200–203	85	0.75
4l	N $(CH_3)_2$	OCH_3	$C_{26}H_{23}N_5O_3S$	485.56	279–281	77	0.63

M.W: Molecular weight, M.P: Melting point

Table 2: Cytotoxic activity of novel 2,3-disubstituted quinazolines

Compound	Cell lines, IC_{50} (μM)±SEM	
	MDAMB231[a]	SKOV3[b]
4a	40.2±0.66	37.4±0.44
4b	51.2±0.92	46.8±0.72
4c	48.2±0.56	44.4±0.84
4d	28.2±0.53	24.5±0.79
4e	14.2±0.86	17.7±0.62
4g	34.9±0.69	38.9±0.71
4h	41.5±0.82	45.4±0.74
Doxorubicin	0.7±0.23	0.8±0.22

[a]MDAMB231: Human breast cancer, [b]SKOV3: Human ovarian cancer,
SEM: Standard error of the mean, IC_{50}: Half-maximal inhibitory concentration

RESULTS AND DISCUSSION

Chemistry

Twelve new compounds of 2-substituted quinazolines and 2,3-disubstituted quinazolines were synthesized and the reaction sequence for the synthesis of these compounds is outlined in Scheme 1. Physical characterization of the newly synthesized compounds is outlined in Table 1. Phthalimide on treatment with NaOH, bromination with Br2 produces anthranilic acid. The anthranilic acid on treatment with $CuCl_2$ and substituted benzaldehyde gives 2-substituted quinazolines produces the series of compounds 2a-2l. Then, benzothiazole treated with chloroacetyl chloride in the presence of glacial acetic acid produces an intermediate compound N-(benzo[d] thiazol-2-yl)-2-chloroacetamide (3). Now, fusion of the compound 2a-2l with intermediate of compound 3 produces final 2,3-disubstituted quinazolines (4a-4l). The spectral data for all the newly synthesized compounds were completely in agreement with the proposed structures.

Fourier transform infrared spectra reveal that the peaks in the range of 3382 cm^{-1}, 3050 cm^{-1}, 2909, 2839 cm^{-1}, 2309 cm^{-1}, 1737 cm^{-1}, 1688 cm^{-1}, 1537 cm^{-1}, 1435 cm^{-1}, and1162 cm^{-1} represent –NHstr, Aromatic C-Hstr, Aliphatic C-Hstr, C-S-C str, C = O str, C = CH str, C = C str, and C-N str groups, respectively, indicated the formation of the substituted quinazoline compounds. In the ^{1}H-NMR spectra, the signals of the respective protons of the synthesized quinazoline derivatives were confirmed by the presence of peaks δ ppm 7.38–7.78 for aromatic protons, δ ppm 9.59 represents –NH, acetamide, and δ ppm 3.69 represents OCH_3. Additional support for the structures of these title compounds was provided by the ^{13}C-NMR: δ ppm 143.55-δ ppm 110.74 represent aromatic carbons; δ ppm 173.95 and 170.26 represent C-O quinazoline and C-O acetamide; δ ppm 162.41, C = N; δ ppm 49.12 (CH_2); and δ ppm 46.31 (OCH_3).

The appearance of molecular ion peaks as M+H confirms the title compounds. The mass spectra of all the title compounds were in accordance with the proposed structures.

Cytotoxic activity

All the synthesized compounds were evaluated for their cytotoxic activity against such as human breast cancer (MDA-MB-231) and human ovarian cancer (SK-O-V3) using MTT assay. The tested cell lines were incubated with varying concentrations (0.5–100 μM) of each selected compound (standard drug doxorubicin) and created concentration versus growth inhibition curves.

Most of the tested quinazoline derivatives were active against all the cell lines. For each cell line, the IC50 values (response parameter) were calculated and tabulated in Table 2. The results, the compounds 4a, 4b, 4c, 4d, 4e, 4g, and 4 h, have shown higher cytotoxic activity against all the tested cell lines. The growth of inhibition was further enhanced due to the substitution of acetamide linked benzothiazole at the third position of the quinazoline moiety. The presence of chlorine on the benzene

Table 3: *In silico* inhibition of JAK2 protein by substituted quinazoline derivatives

Compound	Dock score	#h-bonds	Interacted amino acids	Bond distance	Binding energy
4a	−5.652	1	LEU 855	2.32	−37.412
4b	−5.935	1	SER 936	2.72	−48.901
4c	−7.415	1	SER 936	2.38	−53.861
4d	−8.110	2	SER 936 ARG 980	2.54	−56.367
4e	−8.831	3	SER 936 LEU 855 VAL911	2.49	−63.786
4f	−3.789	2	VAL 911 SER 936	2.52	−28.931
4g	−4.115	1	ARG 980	2.46	−43.761
4h	−6.176	1	LEU 855	2.01	−37.412
4i	−3.512	1	VAL 863	2.68	−48.901
4j	−6.205	2	ARG 980 LEU 855	2.94	−53.861
4k	−3.100	3	VAL 863 SER 936 ARG 980	2.26	−56.367
4l	−4.992	1	ARG 980	2.87	−61.327

ring at the 2nd position of the quinazoline moiety played an important role in the cytotoxic activity of a molecule. The cytotoxic activity of chlorosubstituted aromatic moiety at the 2nd position of quinazoline (4e) (14.2 ± 0.86, 17.7 ± 0.62) showed the highest activity against all the cell lines when compared to that of remaining substituted aromatic moiety at the 2nd position of quinazoline against human cancers. The most active compound among the series was 4e against all the tested cancer cell lines which had shown comparable cytotoxic activity to that of doxorubicin.

Overall, the SAR of the screened compounds against these cell lines seems comparable to that of activity against MDA-MB231 and SK-O-V3 cell lines.

Molecular docking and binding energy calculations

The association of JAK2 in the human breast and ovarian cancers was observed from the previous reports. By considering this association, the protein and ligand interactions of the molecules that were tested for *in vitro* JAK2 inhibition are listed in Table 1 with the kinase domain of JAK2 protein which is elucidated. The dock scores of the tested compounds were ranged between −8.405 and −3.241. Compound 4e which has been reported as the most effective cytotoxic agent among the series also reported the highest dock score of −8.405 and showed hydrogen bond interaction with SER-936, LEU-855, and VAL-911 (Fig. 1). Ligand binding energy with protein suggested that compound 4e has shown the highest binding energy of −63.786 kcal/mol, then compound 4l with the binding energy of −61.327 kcal/mol. Protein-ligand interactions along with binding energies of quinazoline derivatives with JAK2 are shown in Table 3.

CONCLUSION

We have designed a convenient synthetic method for the synthesis of (benzo[d]thiazol-2-yl)-2-[4-oxo-2-(4-substituted phenyl) quinazoline-3(4H) yl acetamide derivatives and screened for human cell lines to achieve better potential cytotoxic agents. The results suggest that the newly synthesized ligands reported considerable cytotoxic activity against all the evaluated cancer cell lines. Moreover, from the SAR studies, it is evident that quinazoline derivatives with benzothiazole heterocycle nucleus with hydrophilic acetamide linkage could probably increase the cytotoxic potentials and the presence of chlorine or nitrosubstitution could add more benefit. *In silico* studies reveal the JAK2 inhibitory potential of the synthesized compounds and their mechanistic insights. It is noteworthy that the compound [benzo[d]thiazol-2-yl]-2-[4-oxo-2-(4-chlorophenyl) quinazoline-3(4H) yl acetamide used as a new scaffold for further designing of multifunctional compounds.

AUTHORS' CONTRIBUTIONS

Siva Jyothi Buggana contributed to the preparation of manuscript, Dr. Mani Chandrika Paturi supervised the manuscript preparation, and Dr. V. V. S. Rajendra Prasad organized and reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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