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EGFR Tyrosine Kinase Targeted *In Silico* Design and Synthesis of Novel Quinazoline Derivatives

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Abstract

Quinazolines are medicinally important as anti-convulsant, anti-cancer, anti-microbial, and anti-tubercular properties etc. They target epidermal growth factor receptors on tumor cells. This work is aimed to synthesize a new series of quinazoline derivatives which could deliver drug specifically to the EGFR over expressing tumors. A series of novel Schiff's base analogues were developed by *in silico* screening methods. The drug likeness of the analogues was analyzed by using Molinspiration software. Biological activities of these analogues were evaluated by using PASS software. The candidates which obeyed Lipinski rule of five and having suitable anti-cancer and anti-microbial activity were taken for docking studies using Schrodinger software. All the proposed derivatives were docked with various protein targets obtained from PDB, using GLIDE software and satisfactory docking energy scores were obtained. Selected 10 derivatives of quinazoline have been synthesized by anthranilamide and benzaldehyde as starting materials. These analogues were purified by analyzing its melting point, Rf value. These were further characterized by FT-IR, ^{1}H NMR and MASS spectral studies. The anti-cancer activity of these derivatives was done by MTT assay against HeLa cell lines, LD_{50} values were calculated by Brine Shrimp Lethality Assay. From these experiments it is clearly revealed that these analogues possess good anti-cancer activity and are good lead compounds against tumors.

Keywords: Quinazolinone; EGFR tyrosine kinase; *In silico* design; Anti-cancer

Introduction

Quinazolinone is a heterocyclic compound with molecular formula $C_8H_6N_2O$. These are crystalline solids, insoluble in water and dilute acids and soluble in aqueous alkali. Quinazolinones are one of the most important core structures present in many natural products as well as synthetic drugs [1].

They are medicinally important as anti-cancer, antimicrobial, anti-tubercular and they show prevention of blood platelet aggregation. Recently quinazolinone chemistry has got new direction due to some resemblance with folic acid. Studies on modification of its chemistry have been increased because of its association in cancer chemotherapy. The analogs were slightly more potent than methotrexate as inhibitors of dihydrofolate reductase in human leukemia cells. They target EGFR and VEGFR receptors on tumor cells [2].

Current approaches in the treatment of cancer involve surgery, radiation therapy and chemotherapy. Major drawbacks of chemotherapy include toxicity towards normal cells, associated adverse effects and multi drug resistance. Therefore, to overcome the short-comings of the present cancer chemotherapy, an antitumor drug with a new mechanism of action, capable of discriminating tumor cells from normal cells and exhibiting selective toxicity against cancer is the subject of this research [3].

The epidermal growth factor receptor (EGFR) is a 170 kDa membrane glycoprotein composed of an extracellular domain, an intermembrane region, and an intracellular domain which presents protein kinase activity. The binding of EGF to its receptor (EGFR) activates a cascade in which several proteins are phosphorylated and processes of regulation, maintenance and cell survival occur. The over expression of EGFR was found in a number of cancers [4].

Owing to the increase in knowledge about cancer pathways, there is a growing interest in finding novel potential drugs. Quinazoline is one of the most widespread scaffolds amongst bioactive compounds. The literature review reveals that quinazoline analogues exhibit excellent anticancer, anti-HIV, antibacterial, and antifungal activities. A number of patents and papers appear in the literature regarding the discovery and development of novel promising quinazoline compounds for cancer chemotherapy. Although there is a progressive decrease in the number of patents filed, there are an increasing number of biochemical targets for quinazoline compounds. Similarly, acetidinones are also endowed with wide range of pharmacological activities, especially antibacterial activities [5]. The present research envisaged to design, synthesis and EGFR targeting of novel acetidino-quinazoline derivatives as potential anticancer agents with additional pharmacological effects such as anti-fungal, anti-bacterial activities, etc.

Another important aim is to carry out a highly environmentally benign protocol for the synthesis of 2-substituted quinazolinones from anthranilamides and aldehydes via aerobic oxidative cyclization in an open flask without the use of metal co-catalysts and bases.

Materials and Methods

In silico studies were carried out using structures were drawn in ACD Chemsketch 12.0. Prediction of Biological activity and Analysis of Lipinski's rule of five were done by using PASS ONLINE and Molinspiration. The docking studies were carried out using Schrödinger under Maestro (Glide). The ADME prediction were carried out using Schrödinger under Maestro (Qikprop). The toxicity predictions of

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synthesized compounds were carried out using Osiris, Lazar, and Gussar. Conventional synthesis of Quinazolinone was carried out from anthranilamide and benzaldehyde by open air oxidation method. After the synthesis of individual products in each synthetic step the products were purified by recrystallization using appropriate solvents. The newly synthesized compounds were characterized by Melting point, Vibrational spectra (IR), ^{1}H NMR spectra and Mass spectral analysis. The melting points of the newly synthesized compounds were determined in open capillaries and were uncorrected. The compounds synthesized were confirmed for their purity on silica gel G TLC glass plates of 2 mm thickness and also on precoated plates by using suitable solvent system, getting single spot in TLC and consistency in Rf value. IR Spectra of the synthesized compounds were recorded using KBr pellets in the range of 4000-400 cm^{-1} on Agilent Cary 630 FT-IR spectrometer. ^{1}H NMR Spectra of the synthesized compounds were recorded using TMS (Tetra Methyl Silane) as internal standard in Bruker Avance AV 500 at 300 MHz. Mass spectra of the synthesized compounds were done by using GC-MS.

Chemistry

All the chemicals and reagents used were of analytical or synthetic grades. The structures were drawn in ACD Chemsketch 12.0. Prediction of Biological activity and Analysis of Lipinski's rule of five were done by using PASS ONLINE and Molinspiration. The docking studies were carried out using Schrödinger under Maestro (Glide). The ADME prediction were carried out using Schrödinger under Maestro (Qikprop). The toxicity predictions of synthesized compounds were carried out using Osiris, Lazar, and Gussar.

Quinazolinone was synthesized using anthranilamide and benzaldehyde by open air oxidation method followed by conventional and microwave synthesis of ethyl oxy acetate derivatives, acetohydrazide derivatives, and novel Schiff bases of quinazoline. After that Schiff bases were cyclized to get novel Acetidino-quinazoline derivatives and Thiazolidino- quinazoline derivatives. After the synthesis of individual products in each synthetic step the products were purified by recrystallization using appropriate solvents. The newly synthesized compounds were characterized by Melting point, Vibrational spectra (IR), ^{1}H NMR spectra and Mass spectral analysis. The melting points of the newly synthesized compounds were determined in open capillaries and were uncorrected. The compounds synthesized were confirmed for their purity on silica gel G TLC glass plates of 2 mm thickness and also on precoated plates by using suitable solvent system, getting single spot in TLC and consistency in Rf value. IR Spectra of the synthesized compounds were recorded using KBr pellets in the range of 4000-400 cm^{-1} on Agilent Cary 630 FT-IR spectrometer. ^{1}H NMR Spectra of the synthesized compounds were recorded using TMS (Tetra Methyl Silane) as internal standard in Bruker Avance AV 500 at 300 MHz. Mass spectra of the synthesized compounds were done by using GC-MS.

Procedure for the formation of 2-phenylquinazolin-4 (3H) -one: This is a highly environmentally benign protocol for the synthesis of quinazolinones from anthranilamides and aldehydes via aerobic oxidation in DMSO. To the solution of 2-aminobenzamide (0.01 mole, 1.36 g) and benzaldehyde (0.01 mole, 1.019 ml) in 10 ml Dimethyl sulfoxide, catalytic amount of acetic acid was added. The solution was heated in an open flask at 120°C for 16 hrs. The progress of reaction was monitored using TLC 15% ethyl acetate in chloroform. After completion of reaction, the reaction mixture was cooled to room temperature and the product obtained was filtered washed with water and crystallized from absolute ethanol [9]. Yield 90%, melting point: 256°C IR (KBr): 3,303 cm^{-1} (NH), 1,667 cm^{-1} (C=O), and 1,614 cm^{-1} (C=N), rf 0.66.

Procedure for the formation of ethyl [(2-phenylquinazolin-4-yl) oxy]acetate: In two necked 500 ml Round bottom flask, take 15-20 ml dry DMF. To this add 2-phenylquinazolin-4 (3H) -one (0.01 mole, 2.22 g), and ethylchloroacetate (0.01 mole, 1.25 ml) and anhydrous potassium carbonate (0.1 mole, 1.38 g). The resultant mixture was stirred and refluxed for 9-10 hrs at 80°C. After completion of reaction, which was monitored by in situ TLC, the reaction mixture was filtered and poured into large amount of water. The solid separated was filtered and washed with water, the solid was dried and recrystallized from ethanol. Yield 87%. Melting point 181°C, rf value 0.69, IR (KBr): IR (cm^{-1}): 3302-2922 (NH, NH2), 2852 (C-H alip.), 1653 (CO) carboxamide, 1511 (C=N), 1026 (C-O-C).

Procedure for the formation of 2-[(2-phenylquinazolin-4-yl) oxy]acetohydrazide: Ethyl [(2-phenylquinazolin-4-yl) oxy]acetate (0.05M) and hydrazine hydrate 99% (0.15 M, 7.29 ml) was dissolved in sufficient quantity of ethanol (50 ml) to give clear solution and refluxed for 10 hrs at 100°C. the excess solvent was removed by distillation, allowed to cool, the solid mass that separated on cooling was washed with small amount of ice cooled ethanol, dried and recrystallized from ethanol.*yield 80%. Melting point 181°C, rf value 0.69, IR (KBr): IR (cm^{-1}): 3302-2922 (NH, NH_2), 2852 (C-H alip.), 1653 (CO) carboxamide, 1511 (C=N), 1026 (C-O-C).

General procedure for the formation of Schiff's bases of 2-[(2-phenylquinazolin-4-yl) oxy]acetohydrazide (6a-6j): To a solution of appropriate substituted benzaldehyde (1mmol, 3.5 gm) in ethanol (15 ml), 2-[(2-phenylquinazolin-4-yl) oxy]acetohydrazide (1 mmol, 3 gm) were added. Make PH around 4.5 by adding 2-3 drops of glacial acetic acid. The reaction was refluxed for 5-6 hours and the course of reaction was monitored by TLC to its completion*. The reaction mixture was cooled by keeping it in room temperature. A solid mass separated out, which was filtered and washed with water [10].

(Z) -2- (2-phenylquinazolin-4-yloxy) -N'- (4-methoxybenzylidene) acetohydrazide (6a): Aldehyde: 4-methoxy benzaldehyde (1 mmol), 6 hours refluxing at 100°C, white solid. Melting point 160°C, Yield 75% Rf 0.64 IR (KBr) cm^{-1}: 3384.23 (N-H str.), 3045.45 (Ar C-H str.),

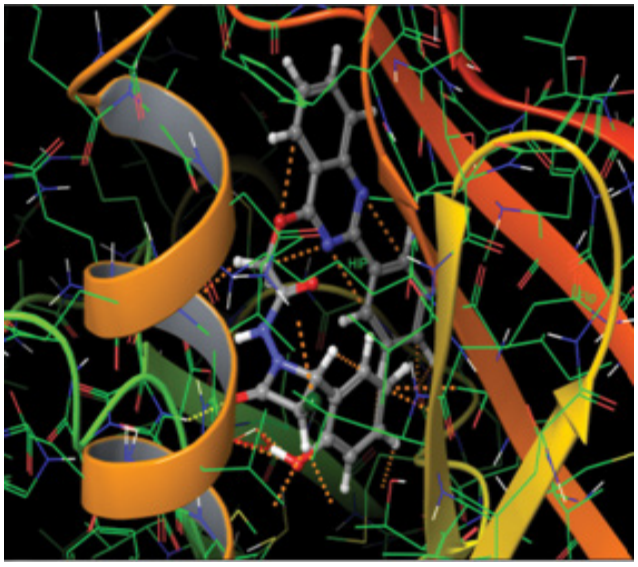


Figure 1: Docking image of QAz13 and interactions with EGF.

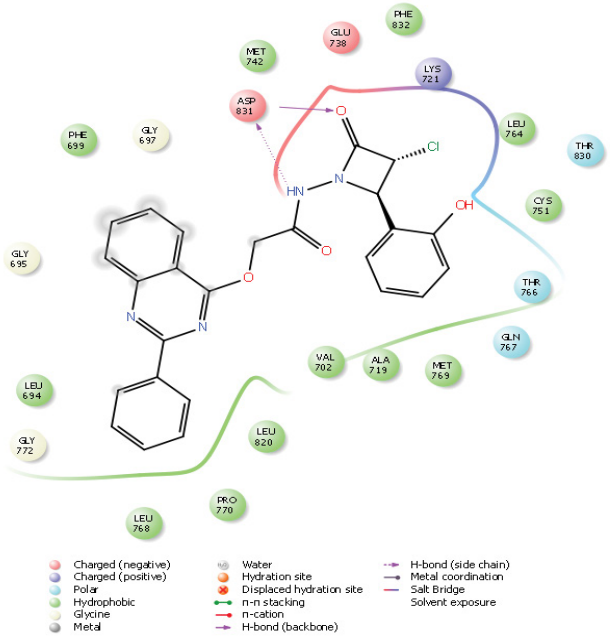


Figure 2: Acetidino-quinazoline derivatives Docked to the Epidermal Growth Factor Receptors (EGFR).

Compounds	Molecular formula	Molar refractivity (cm^3)	Molecular volume (cm^3)	Parachor (cm^3)	Polarizability (cm^3) (10-24)
QAz1	$C_{26}H_{21}ClN_4O_4$	131.09 ± 0.4	338.8 ± 5.0	995.0 ± 6.0	51.97 ± 0.5
QAz2	$C_{22}H_{21}ClN_4O_3$	113.90 ± 0.4	307.0 ± 5.0	882.8 ± 6. 0	45.15 ± 0.5
QAz3	$C_{25}H_{18}Cl_2N_4O_3$	129.55 ± 0.4	327.9 ± 5.0	973.6 ± 6. 0	51.36 ± 0.5
QAz4	$C_{22}H_{19}ClN_4O_3$	133.81 ± 0.4	300.3 ± 5.0	870.4 ± 6. 0	45.12 ± 0.5
QAz5	$C_{25}H_{18}BrClN_4O_4$	133.97 ± 0.4	326.6 ± 5.0	1002.7 ± 6. 0	53.11 ± 0.5
QAz6	$C_{25}H_{19}ClN_4O_4$	126.25 ± 0.4	314.0 ± 5.0	951.7 ± 6.0	50.05 ± 0.5
QAz7	$C_{25}H_{18}ClN_5O_5$	130.76 ± 0.4	328.0 ± 5.0	993.5 ± 6.0	51.83 ± 0.5
QAz8	$C_{26}H_{21}ClN_4O_3$	129.35 ± 0.4	332.8 ± 5.0	974.7 ± 6.0	51.27 ± 0.5
QAz9	$C_{27}H_{24}ClN_5O_3$	137.86 ± 0.4	355.1 ± 5.0	1041.1 ± 6.0	54.65 ± 0.5
QAz10	$C_{19}H_{15}ClN_4O_3$	100.03 ± 0.4	257.9 ± 5.0	764.6 ± 6.0	39.65 ± 0.5
QAz11	$C_{23}H_{17}ClN_4O_4$	117.21 ± 0.4	299.7 ± 5.0	891.7 ± 6.0	46.46 ± 0.5
QAz12	$C_{21}H_{19}ClN_4O_5$	112.32 ± 0.4	286.0 ± 5.0	874.7 ± 6.0	44.53 ± 0.5
QAz13	$C_{25}H_{219}ClN_4O_4$	126.25 ± 0.4	314.0 ± 5.0	951.7 ± 6. 0	50.05 ± 0.5
QAz14	$C_{26}H_{21}Cl_2N_4O_5$	135.62 ± 0.4	335.5 ± 5.0	1010.3 ± 6. 0	52.57 ± 0.5
QAz15	$C_{25}H_{19}ClN_4O_4$	126.25 ± 0.4	314.0 ± 5.0	951.7 ± 6. 0	50.05 ± 0.5
QAz16	$C_{25}H_{18}BrClN_4O_3$	132.45 ± 0.4	329.8 ± 5.0	987.5 ± 6. 0	52.5. ± 0.5
QAz17	$C_{25}H_{20}ClN_5O3$	128.34 ± 0.4	320.2 ± 5.0	964.4 ± 6.0	50.88 ± 0.5
QAz18	$C_{28}H_{23}ClN_4O_5$	140.46 ± 0.4	367.2 ± 5.0	1078.6 ± 6.0	55.68 ± 0.5
QAz19	$C_{33}H_{35}ClN_4O_4$	163.30 ± 0.4	442.4 ± 5.0	1261.2 ± 6.0	64.73 ± 0.5
QAz20	$C_{27}H_{23}ClN_4O_5$	137.45 ± 0.4	360.3 ± 5.0	1053.7 ± 6.0	54.49 ± 0.5
QAz21	$C_{25}H_{18}ClN_5O_5$	130.76 ± 0.4	328.0 ± 5.0	993.5 ± 6.0	51.83 ± 0.5
QAz22	$C_{25}H_{18}ClN_5O_5$	130.76 ± 0.4	328 ± 5.0	993.5 ± 6.0	51.83 ± 0.5
QAz23	$C_{27}H_{23}ClN_4O_6$	138.98 ± 0.4	357.1 ± 5.0	1068.9 ± 6.0	55.09 ± 0.5
QAz24	$C_{26}H_{21}ClN_4O_5$	132.62 ± 0.4	335.5 ± 5.0	1010.3 ± 6. 0	52.57 ± 0.5
QAz25	$C_{25}H_{18}ClFN_4O_3$	124.84 ± 0.4	321.6 ± 5.0	943.8 ± 6. 0	49.49 ± 0.5
QAz26	$C_{27}H_{23}ClN_4O_5$	137.45 ± 0.4	360.3 ± 5.0	1053 ± 6. 0	54.49 ± 0.5
QAz27	$C_{28}H_{25}ClN_4O_6$	143.82 ± 0.4	381.9 ± 5.0	1112.3 ± 6. 0	57.01 ± 0.5
QAz28	$C_{25}H_{17}Cl_3N_4O_3$	134.38 ± 0.4	338.7 ± 5.0	1010.7 ± 6.0	53.27 ± 0.5
QAz29	$C_{25}H_{18}BrClN_4O_4$	133.97 ± 0.4	326.6 ± 5.0	1002.7 ± 6.0	53.11 ± 0.5
QAz30	$C_{26}H_{21}ClN_4O_3$	129.35 ± 0.4	332.8 ± 5.0	974.7 ± 6.0	51.27 ± 0.5

Table 1: Molecular descriptors of molecules generated from ACD Lab Chemsketch 12.0.

Compound	GPCR Ligand	Ion Channel modulator	Kinase inhibitor	Nuclear receptor Ligand	Protease Inhibitors	Enzyme Inhibitor
QAz1	-0.10	-0.40	-0.21	-0.49	-0.22	-0.09
QAz2	-0.02	-0.36	-0.20	-0.49	-0.13	0.00
QAz3	-0.08	-0.35	-0.20	-0.50	-0.21	-0.08
QAz4	-0.04	-0.34	-0.26	-0.43	-0.14	0.02
QAz5	-0.15	-0.40	-0.24	-0.48	-0.30	-0.10
QAz6	-0.08	-0.36	-0.20	-0.50	-0.19	-0.06
QAz7	-0.18	-0.40	-0.28	-0.53	-0.29	-0.14
QAz8	-0.11	-0.40	-0.22	-0.51	-0.23	-0.10
QAz9	-0.07	-0.38	-0.16	-0.45	-0.21-	-0.07
QAz10	-0.07	-0.471	-0.19	-0.58	-0.20	-0.03
QAz11	-0.18	-0.52	-0.41	-0.64	-0.41	-0.17
QAz12	0.03	-0.30	-0.12	-0.43	-0.00	0.13
QAz13	-0.08	-0.34	-0.20	-0.42	-0.20	-0.04
QAz14	-0.08	-0.39	-0.18	-0.44	-0.24	-0.06
QAz15	-0.04	-0.32	-0.16	-0.39	-0.18	-0.02
QAz16	-0.15	-0.40	-0.23	-0.57	-0.27	-0.12
QAz17	-0.05	-0.31	-0.12	-0.54	-0.13	-0.00
QAz18	-0.15	-0.50	-0.33	-0.51	-0.29	-0.15
QAz19	-0.08	-0.59	-0.35	-0.41	-0.17	-0.16
QAz20	-0.11	-0.44	-0.24	-0.45	-0.24	-0.10
QAz21	-0.17	-0.37	-0.35	-0.51	-0.31	-0.14
QAz22	-0.19	-0.41	-0.28	-0.54	-0.29	-0.15
QAz23	-0.10	-0.45	-0.17	-0.45	-0.22	-0.05
QAz24	-0.10	-0.37	-0.20	-0.48	-0.25	-0.06
QAz25	-0.07	-0.36	-0.17	-0.47	-0.21	-0.07
QAz26	-0.11	-0.44	-0.20	-0.48	-0.24	-0.09
QAz27	-0.10	-0.52	-0.22	-0.45	-0.22	-0.12
QAz28	-0.08	-0.37	-0.28	-0.48	-0.26	-0.12
QAz29	-0.16	-0.40	-0.25	-0.50	-0.29	-0.09
QAz30	-0.09	-0.38	-0.28	-0.48	-0.26	-0.11

Table 2: Analysis of drug likeness score for selected analogues.

Compound.	Horal Abs	% Horal Abs	QP logKhsa	QP PCaco	QP logBB	QP logKp	QP log HERG	#meta b	QPP MDCK
QAz 1	1	100	1.039	1036.021	-0.628	-1.05	-7.936	4	1016.834
QAz 3	1	100	1.137	1425.97	-0.388	-1.171	-7.893	3	2537.096
QAz 6	1	100	1.014	1425.246	-0.536	-0.746	-7.672	4	1397.798
QAz 8	1	100	1.182	1427.295	-0.569	-1.218	-7.634	4	1003.092
QAz 11	1	100	0.765	1072.186	-0.75	-1.09	-7.63	4	915.075
QAz13	1	94.209	0.88	562.257	-1.065	-1.544	-7.522	5	401.738
QAz15	1	91.831	0.892	431.629	-1.223	-1.799	-7.552	4	479.017
QAz17	1	100	0.841	371.902	-1.302	-1.938	-7.503	4	407.682
QAz25	1	100	1.059	1425.152	-0.437	-0.872	-7.557	3	2533.23
QAz30	1	100	1.106	1327.347	-0.514	-0.838	-7.865	4	1839.98

Table 3: Prediction of ADME profile of selected Acetidino-quinazoline analogues.

TARGET	PDB ID	COMPOUND CODE	G SCORE
EGFR	1M17	QAz13	-8.9
		QAz 15	-7.4
		QAz 30	-6.8
		QAz6	-6.66
		QAz17	-6.5
		QAz33	-6.5
		QAz11	-6.3
		QAz1	-6.2
		QAz25	-5.8
		QAz 8	-5.3

Table 4: Glide scores for docking with various proteins.

Sample	Conc. (µg/ml)	Log conc.	Average number of deaths (n=10)	% Mortality	Corrected %	P value
QAz1	100	2	0	0	0.24	3.04
	200	2.3	3	30	30	4.48
	400	2.6	5	50	50	5.00
	800	2.9	7	70	70	5.72
	1600	3.2	10	100	97.5	6.28
QAz3	100	2	0	0	0.24	3.04
	200	2.3	2	20	20	4.16
	400	2.6	6	60	60	5.52
	800	2.9	8	80	80	5.84
	1600	3.2	10	100	97.5	6.28
QAz6	100	2	0	0	0.24	3.04
	200	2.3	2	20	20	4.16
	400	2.6	5	50	50	5.00
	800	2.9	8	80	80	5.84
	1600	3.2	10	100	97.5	6.28
QAz8	100	2	0	0	0.24	3.04
	200	2.3	4	40	40	4.75
	400	2.6	6	60	60	5.52
	800	2.9	8	80	80	5.84
	1600	3.2	9	90	90	6.28
QAz11	100	2	1	10	10	3.72
	200	2.3	3	30	30	4.48
	400	2.6	5	50	50	5.00
	800	2.9	8	80	80	5.84
	1600	3.2	10	100	97.5	6.28
QAz13	100	2	0	0	0.25	3.04
	200	2.3	2	20	20	4.16
	400	2.6	4	40	40	4.75
	800	2.9	7	70	70	5.52
	1600	3.2	10	100	97.5	6.28
QAz15	100	2	0	0	0.25	3.04
	200	2.3	2	20	20	4.16
	400	2.6	4	40	40	4.75
	800	2.9	8	80	80	5.84
	1600	3.2	10	100	97.5	6.28
QAz17	100	2	0	0	0.25	3.04
	200	2.3	2	20	20	4.16
	400	2.6	5	50	50	5.00
	800	2.9	7	70	70	5.52
	1600	3.2	10	100	97.5	6.28
QAz25	100	2	0	0	0.25	3.04
	200	2.3	3	30	30	4.48
	400	2.6	6	60	60	5.25
	800	2.9	8	80	80	5.84
	1600	3.2	10	100	100	6.28
QAz30	100	2	0	0	0.25	3.04
	200	2.3	2	20	20	4.16
	400	2.6	4	40	40	4.75
	800	2.9	6	60	60	5.25
	1600	3.2	10	100	97.5	6.28
Vehicle Control (DMSO)	1600	-	0	0	-	-
Negative Control (Sea Water)	1600	-	0	0	-	-
Positive control ($K_2Cr_2O_7$)	100		10	100		

Table 5: Determination of LD50 of the synthesized quinazoline analogues.

Compound	LD_{50} (µg)
QAz1	436.5
QAz3	398.1
QAz6	467.7
QAz8	380.2
QAz11	478.6
QAz13	501.2
QAz15	467.7
QAz17	457.1
QAz25	398.1
QAz30	501.2

Table 6: LD_{50} value of synthesized quinazoline analogue.

Sample Concentration (µg/ml)	Absorbance @ 540nm	Percentage Viability
Control	**0.9968**	
6.25	0.9527	95.57584
12.5	0.8396	84.22953
25	0.6613	66.3423
50	0.4201	42.14486
100	0.3774	37.86116

Table 7: Anticancer activity of QAz 3 on human cervical carcinoma cell lines (HELA).

Sample Concentration (µg/ml)	Absorbance @ 540 nm	Percentage Viability
Control	**0.9968**	
6.25	0.9589	96.19783
12.5	0.8497	85.24277
25	0.6615	66.3623
50	0.4111	41.24197
100	0.3772	37.84109

Comparative Evaluation percentage of inhibition and percentage viability of QAz3 and QAz6

Table 8: Anticancer activity of QAz 6 on human cervical carcinoma cell lines (HELA).

Concentration (µg/ml)	% Inhibition		%Viability	
	QAz3	QAz6	QAz3	QAz6
6.25	4.42416	3.80217	95.57584	96.19783
12.5	15.77047	14.75723	84.22953	85.24277
25	33.6577	33.6377	66.3423	66.3623
50	57.85514	58.75803	42.14486	41.24197
100	62.13884	62.15891	37.86116	37.84109

Table 9: Comparative Evaluation percentage of inhibition and percentage viability of QAz3 and QAz6.

Concentration (µg/ml)	Paclitaxel	QT4a
1	62	31.06
10	71	35.55
100	75	39.92
250	78	44.76
500	79.01	49.19

Comparative evaluation percentage of inhibition of synthesized compounds and standard drugs.

Table 10: Comparative evaluation of standard drug and QT4a at different concentration in SiHa cell Line.

Concentration (µg/ml)	% Inhibition		
	QAz3	QAz6	Paclitaxel
6.25	4.42416	3.80217	22.89
12.5	15.77047	14.75723	59.12
25	33.6577	33.6377	60.15
50	57.85514	58.75803	61.75
100	62.13884	62.15891	63.70

Acetidino-quinazoline derivatives docked to the epidermal growth factor receptors (EGFR).

Table 11: Comparative evaluation percentage of inhibition of synthesized compounds and standard drugs.

Components	Weight (g/ml)
Sodium Chloride L R	24.0
Calcium Chloride L R	1.5
Potassium Bromide L R	0.1
Potassium Chloride L R	0.7
Sodium Sulphate L R	4.0
Sodium bicarbonate L R	0.2
Magnesium Chloride L R	11.0
Total Salt	41.5

The pH should be adjusted to pH 7-8 with sodium bicarbonate

Table 12: Composition of artificial sea water.

1676.52 (C=O str.), 1545.37 (C=N str.), 1600.08 (Ar C-C str.), 1325.52 (C-N str.), 1193.85 (aliphatic C-H str. of N=CH-), 1072.31 (C-O-C str.).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- (4-chlorobenzylidene) acetohydrazide (6b): Aldehyde: p-chlorobenzaldehyde (1mmol). 6 hours refluxing at 100°C, light yellow solid, Melting point 185°C Yield 64% Rf 0.61 IR (KBr) vcm^{-1}: 3302.60 (N-H str.), 3062.51 (Ar C-H str.), 1657.82 (C=O str.), 1538.07 (C=N str.), 1566.09 (Ar C-C str.), 1292.23 (C-N str.), 890.91 (aliphatic C-H str. of N=CH-).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- benzylideneacetohydrazide (6c): Aldehyde: Benzaldehyde (1mmol). 5.30 hour refluxing at 110°C, white solid, Melting point 188°C Yield 74% Rf 0.65 IR (KBr) vcm^{-1}: 3315.68 (N-H str.), 3064.68 (Ar C-H str.), 1657.82 (C=O str.), 1538.07 (C=N str.), 1566.09 (Ar C-C str.), 1292.23 (C-N str.), 890.91 (aliphatic C-H str. of N=CH-).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- (4-methylbenzylidene) acetohydrazide (6d): Aldehyde: p-tolualdehyde. 5.30 hour refluxing at 110°C, M.F., $C_{21}H_{15}N_3O_2$, white solid, Melting point 159°C Yield 63% Rf 0.58 IR (KBr) vcm^{-1}: 3201.45 (O-H), 3023.42 (Ar-H), 1709.02 (C=O), 1676.82 (C=N), 1180.49 (C-N).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- ((furan-2-yl) methylene) acetohydrazide (6e): Aldehyde: furfur aldehyde (1mmol). 6 hour refluxing at 120°C, M.F. $C_{21}H_{14}N_4O_3$, white solid. Melting point 195°C Yield 70% Rf 0.62 IR (KBr) vcm^{-1}: 3215.68 (N-H str.), 3062.68 (Ar C-H str.), 1647.82 (C=O str.), 1527.07 (C=N str.), 1566.09 (Ar C-C str.), 1292.23 (C-N str.), 890.27 (aliphatic C-H str. of N=CH-), 1185.04 (C-O-C).

(Z) -N′- (2-hydroxybenzylidene) -2- (2-phenylquinazolin-4-yloxy) acetohydrazide (6f): Aldehyde: o-hydroxybenzaldehyde (1 mmol).5.30 hour refluxing at 110°C, M.F. $C_{21}H_{14}N_4O_3$, white solid, Melting point 176°C Yield 65% Rf 0.70 IR (KBr) vcm^{-1}: 3512.62 (O-H str.), 3410.53 (N-H str.), 3062.86 (Ar C-H str.), 1652.02 (C=O str.), 1581.47 (C=N str.), 1510.16 (Ar C-C str.), 1308.76 (C-N str.), 886.19 (aliphatic C-H str. of N=CH).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- (4-hydroxybenzylidene) acetohydrazide (6g): Aldehyde: 4-hydroxybenzaldehyde (1mmol). 5.45 hour refluxing at 110°C. M.F. $C_{21}H_{14}N_4O_3$, white solid, Melting point 164°C, Yield 61% Rf 0.69 IR (KBr) vcm^{-1}: 3538.74 (O-H str.), 3396.61 (N-H str.), 3057.35 (Ar C-H str.), 1659.94 (C=O str.), 1571.08 (C=N str.), 1541.48 (Ar C-C str.), 1346.62 (C-N str.), 1034.16 (aliphatic C-H str. of N=CH-).

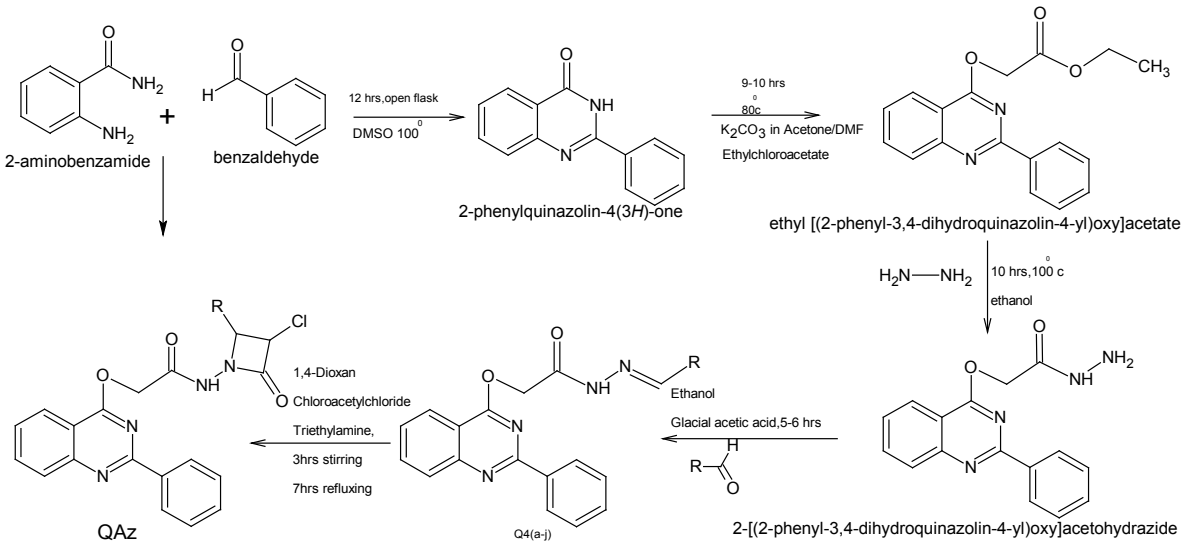
(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- (4-aminobenzylidene) acetohydrazide (6h): Aldehyde: p-aminobenzaldehyde (1mmol). 6 hour refluxing at 100°C, M.F. $C_{22}H_{17}N_3O_3$, white solid, Melting point 191°C Yield 62% Rf 0.64 IR (KBr) vcm^{-1}: 3310.10 (O-H), 3010.56 (Ar-H), 1710.34 (C=O), 1600 (C=N), 1201.09 (C-O-C), 1540.47 (Ar C-C str.), 1310.62 (C-N str.), 1034.16 (aliphatic C-H str. of N=CH-).

(Z) -2- (2-phenylquinazolin-4-yloxy) -N′- (4-fluorobenzylidene) acetohydrazide (6i): Aldehyde: p-flurobenzaldehyde (1 mmol). 6 hour refluxing at 100°C, M.F. $C_{19}H_{13}N_3O_2$, white needle crystals, Melting point 205°C Yield 60% Rf 0.73 IR (KBr) vcm^{-1}: 3171.25 (Ar-H), 1708.08 (C=O), 1640.66 (C=N), 1180.09 (C-O-C).

(Z) -N′- (2-chlorobenzylidene) -2- (2-phenylquinazolin-4-yloxy) acetohydrazide (6j): Aldehyde: 2-chloro benzaldehyde (1mmol). 6 hour refluxing at 100°C, M.F. $C_{21}H_{15}N_3O_2$, light yellow solid, Melting point 184°C Yield 60% Rf 0.32 IR (KBr) vcm^{-1}: 3397.59 (N-H str.), 3022.08 (Ar C-H str.), 1658.53 (C=O str.), 1583.13 (C=N str.), 1539.42 (Ar C-C str.), 1314.33 (C-N str.), 1139.58 (aliphatic C-H str. of N=CH-), 1065.48 (C-Br str.).

General procedure for the synthesis of substituted N- (3-chloro-2-oxoazetidin-1-yl) -2-[(2-phenylquinazolin-4-yl) oxy]acetamide (7a-7j)

A mixture of Schiff's base (0.01 mol) and triethylamine (5-6 drops) was dissolved in 1,4-dioxan (50 mL), cooled and stirred. To this well-stirred cooled solution, chloroacetyl chloride (0.015 mole, 1.68 ml) was added drop wise within a period of 30 minutes. The reaction mixture was then stirred for an additional 3 hours at room temperature and refluxed for 7 hours. The reaction mixture was filtered to remove triethylamine hydrogen chloride and the resultant solution was concentrated, cooled and poured into ice-cold water with stirring.



Scheme 1: General procedure for preparation of QAZ.

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-methoxyphenyl) -4-oxoazetidin-1-yl) acetamide (7a): Aldehyde: 4-methoxy benzaldehyde.3 hour stirring, 7 hour refluxing, white shining crystals, Melting Point 124°C, Yield 65%, Rf 0.46 IR (KBr) vcm^{-1}: 1600.08 (Ar C-C), 3440 cm^{-1} (NH stretch), 3012.45 (Ar C-H str.), 1686 cm^{-1} (C=O), 1545.37 (C=N str.) 1755 cm^{-1} (-NCO, stretch.), 1285 cm^{-1} (CH-Cl, stretch.), 3244 cm^{-1} (-NH stretch), 854 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-chlorophenyl) -4-oxoazetidin-yl) acetamide (7b): Aldehyde: p-chlorobenzaldehyde,3 hrs stirring, 6 hour refluxing, light yellow solid, Melting Point 130°C, Yield 68%, Rf 0.61 IR (KBr) vcm^{-1}: 3302 (N-H str.), 3061.68 (Ar C-H str.), 1651.04 (C=O str.), 1613.96 (C=N str.), 1659.54 (lactone), 1886.68 cm^{-1} (-NCO, stretch.), 1148 cm^{-1} (CH-Cl, stretch.), 860.32 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-oxo-4-phenylazetidin-1-yl) acetamide (7c): Aldehyde: Benzaldehyde., Rf 0.56 IR (KBr) vcm^{-1}: 3046.25 (Ar C-H str.), 1649.23 (C=O str.), 1608.12 (C=N str.), 1635.52 (lactone), 1882.36 cm^{-1} (-NCO, stretch.), 1160 cm^{-1} (CH-Cl, stretch.), 852.55 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-oxo-4-p-tolylazetidin-1-yl) acetamide (7d): Aldehyde: p-tolualdehyde., 4 hour stirring. 5 hour refluxing, White solid, Melting Point 120°C, Yield 75%, Rf 0.59, IR (KBr) vcm^{-1}: 3330 (N-H str.), 3075.12 (Ar C-H str.), 1638.24 (C=O str.), 1612.53 (C=N str.), 1602.23 (lactone), 1810.86 cm^{-1} (-NCO, stretch.), 1120 cm^{-1} (CH-Cl, stretch.), 811.98 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2- (furan-2-yl) -4-oxoazetidin-1-yl) acetamide (7e): Aldehyde: Furfuraldehyde, 4 hour stirring, 5 hour refluxing, Light yellow solid, Melting Point 141°C, Yield 61%, Rf 0.62, IR (KBr) vcm^{-1}: 3380 (N-H str.), 3057.21 (Ar C-H str.), 1714.53 (C=O str.), 1586 (C=N str.), 1610.58 (lactone), 1826.25 cm^{-1} (-NCO, stretch.), 1215.46 cm^{-1} (CH-Cl, stretch.), 816.98 cm^{-1} (aromatic C=C), 1185.04 (C-O-C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(2-hydroxyphenyl) -4-oxoazetidin-1-yl) acetamide (7f): Aldehyde: 2-hydroxy benzaldehyde. 5 hour stirring, 5 hour refluxing, light yellow solid, Melting Point 145°C, Yield 69%, Rf 0.6, IR (KBr) vcm^{-1}: 3023.55 (Ar-H), 3270 (N-H str.), 3158.35 (Ar C-H str.), 3191.32 (O-H), 1690 (C=O str.), 1544.28 (C=N str.), 1670.58 (lactone), 1846.45 cm^{-1} (-NCO, stretch.), 1280.86 cm^{-1} (CH-Cl, stretch.), 820.76 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-hydroxyphenyl) -4-oxoazetidin-1-yl) acetamide (7g): Aldehyde: 4-hydroxybenzaldehyde. 5 hour stirring, 6 hour refluxing, light yellow solid, Melting Point 142°C, Yield 68%, Rf 0.63, IR (KBr) vcm^{-1}: 3300.86 (Ar-H), 3180.23 (N-H str.), 3158.19 (Ar C-H str.), 3110.24 (O-H), 1650 (C=O str.), 1543.57 (C=N str.), 1645.85 (lactone), 1816.92 cm^{-1} (-NCO, stretch.), 1291.30 cm^{-1} (CH-Cl, stretch.), 895.26 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (2- (4-aminophenyl) -3-chloro-4-oxoazetidin-1-yl) acetamide (7h): Aldehyde: 4-aminobenzaldehyde, 5 hour stirring, 5 hour refluxing, white solid, Melting Point 150°C, Yield 65%, Rf 0.65, IR (KBr) vcm^{-1}: 3375.28 (NH), 2900.44 (Ar-H), 1614.32 (C=O), 1590.86 (C=N), 1600.98 (lactone), 1814.74 cm^{-1} (-NCO, stretch.), 1278.36 cm^{-1} (CH-Cl, stretch.), 844.95 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-fluorophenyl) -4-oxoazetidin-1-yl) acetamide (7i): Aldehyde: p-flurobenzaldehyde, 5 hour stirring, 5 hour refluxing, yellowish brown crystals, Melting Point 136°C, Yield 63%, Rf 0.66 IR (KBr) vcm^{-1}: 3210.42 (Ar-H), 3118.95 (N-H str.), 3178.25 (Ar C-H str.), 1630.58 (C=O str.), 1528.86 (C=N str.), 1684.26 (lactone), 1978.54 cm^{-1} (-NCO, stretch.), 1288.27 cm^{-1} (CH-Cl, stretch.), 876.38 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(2-chlorophenyl) -4-oxoazetidin-1-yl) acetamide (7j): Aldehyde: 2-chlorobenzaldehyde. 5 hour stirring, 5 hour refluxing, white solid. Melting Point 126°C, Yield 69%, Rf 0.58, IR (KBr) vcm^{-1}: 3100.98 (N-H str.), 3028.38 (Ar C-H str.), 1600.56 (C=O str.), 1672.08 (C=N str.), 167.96 (lactone), 1812.96 cm^{-1} (-NCO, stretch.), 1170 cm^{-1} (CH-Cl, stretch.), 825.86 cm^{-1} (aromatic C=C).

General procedure for the synthesis of substituted N- (4-oxo-1,3-thiazolidin-3-yl) -2-[(2-phenylquinazolin-4-yl) oxy] acetamide (8a-8j)

The Schiff's base (0.011M) and thioglycollic acid (0.011 M) in ethanol (50 mL) in the presence of anhydrous $ZnCl_2$ were allowed to react at room temperature. The reaction mixture was first stirred on a magnetic stirrer for about 5 h followed by refluxing for about 5-7 h. The completion of the reaction was monitored by TLC plate. The product was filtered and cooled at room temperature and recrystallized from ethanol.

2- (2-phenylquinazolin-4-yloxy) -*N*- (2- (4-methoxyphenyl) -4-oxothiazolidin-3-yl) acetamide (8a): Aldehyde: 4-methoxy benzaldehyde. 5 hour stirring, 7 hour refluxing, white shining crystals, Melting Point 174°C, Yield 67%, Rf 0.51 IR (KBr) vcm^{-1}: 1600.08 (Ar C-C), 3440 cm^{-1} (NH stretch), 3012.45 (Ar C-H str.), 1686 cm^{-1} (C=O), 1545.37 (C=N str.) 1755 cm^{-1} (-NCO, stretch.), 3244 cm^{-1} (-NH stretch), 854 cm^{-1} (aromatic C=C), 2364.34 cm^{-1} (S-H).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-chlorophenyl) -4-oxoazetidin-yl) acetamide (8b): Aldehyde: p-chlorobenzaldehyde,3 hrs stirring, 6 hour refluxing, light yellow solid, Melting Point 130°C, Yield 68%, Rf 0.61 IR (KBr) vcm^{-1}: 3302 (N-H str.), 3061.68 (Ar C-H str.), 1651.04 (C=O str.), 1613.96 (C=N str.), 1659.54 (lactone), 1886.68 cm^{-1} (-NCO, stretch.), 1148 cm^{-1} (CH-Cl, stretch.), 860.32 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-oxo-4-phenylazetidin-1-yl) acetamide (8c): Aldehyde: Benzaldehyde, Rf 0.56 IR (KBr) vcm^{-1}: 3046.25 (Ar C-H str.), 1649.23 (C=O str.), 1608.12 (C=N str.), 1635.52 (lactone), 1882.36 cm^{-1} (-NCO, stretch.), 1160 cm^{-1} (CH-Cl, stretch.), 852.55 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-oxo-4-p-tolylazetidin-1-yl) acetamide (8d): Aldehyde: p-tolualdehyde, 4 hour stirring. 5 hour refluxing, White solid, Melting Point 120°C, Yield 75%, Rf 0.59, IR (KBr) vcm^{-1}: 3330 (N-H str.), 3075.12 (Ar C-H str.), 1638.24 (C=O str.), 1612.53 (C=N str.), 1602.23 (lactone), 1810.86 cm^{-1} (-NCO, stretch.), 1120 cm^{-1} (CH-Cl, stretch.), 811.98 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2- (furan-2-yl) -4-oxoazetidin-1-yl) acetamide (8e): Aldehyde: Furfuraldehyde, 4 hour stirring, 5 hour refluxing, Light yellow solid, Melting Point 141°C, Yield 61%, Rf 0.62, IR (KBr) vcm^{-1}: 3380 (N-H str.), 3057.21 (Ar C-H str.), 1714.53 (C=O str.), 1586 (C=N str.), 1610.58 (lactone), 1826.25 cm^{-1} (-NCO, stretch.), 1215.46 cm^{-1} (CH-Cl, stretch.), 816.98 cm^{-1} (aromatic C=C), 1185.04 (C-O-C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(2-hydroxyphenyl) -4-oxoazetidin-1-yl) acetamide (8f): Aldehyde: 2-hydroxy benzaldehyde. 5 hour stirring, 5 hour refluxing, light yellow solid, Melting Point 145°C, Yield 69%, Rf 0.6, IR (KBr) vcm^{-1}: 3023.55 (Ar-H), 3270 (N-H str.), 3158.35 (Ar C-H str.), 3191.32 (O-H), 1690 (C=O str.), 1544.28 (C=N str.), 1670.58 (lactone), 1846.45 cm^{-1} (-NCO, stretch.), 1280.86 cm^{-1} (CH-Cl, stretch.), 820.76 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-hydroxyphenyl) -4-oxoazetidin-1-yl) acetamide (8g): Aldehyde: 4-hydroxybenzaldehyde. 5 hour stirring, 6 hour refluxing, light yellow solid, Melting Point 142°C, Yield 68%, Rf 0.63, IR (KBr) vcm^{-1}: 3300.86 (Ar-H), 3180.23 (N-H str.), 3158.19 (Ar C-H str.), 3110.24 (O-H), 1650 (C=O str.), 1543.57 (C=N str.), 1645.85 (lactone), 1816.92 cm^{-1} (-NCO, stretch.), 1291.30 cm^{-1} (CH-Cl, stretch.), 895.26 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (2- (4-aminophenyl) -3-chloro-4-oxoazetidin-1-yl) acetamide (8h): Aldehyde: 4-aminobenzaldehyde,5 hour stirring, 5 hour refluxing, white solid, Melting Point 150°C, Yield 65%, Rf 0.65, IR (KBr) vcm^{-1}: 3375.28 (NH), 2900.44 (Ar-H), 1614.32 (C=O), 1590.86 (C=N), 1600.98 (lactone), 1814.74 cm^{-1} (-NCO, stretch.), 1278.36 cm^{-1} (CH-Cl, stretch.), 844.95 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(4-fluorophenyl) -4-oxoazetidin-1-yl) acetamide (8i): Aldehyde: p-flurobenzaldehyde, 5 hour stirring, 5 hour refluxing, yellowish brown crystals, Melting Point 136°C, Yield 63%, Rf 0.66 IR (KBr) vcm^{-1}: 3210.42 (Ar-H), 3118.95 (N-H str.), 3178.25 (Ar C-H str.), 1630.58 (C=O str.), 1528.86 (C=N str.), 1684.26 (lactone), 1978.54 cm^{-1} (-NCO, stretch.), 1288.27 cm^{-1} (CH-Cl, stretch.), 876.38 cm^{-1} (aromatic C=C).

2- (2-phenylquinazolin-4-yloxy) -N- (3-chloro-2-(2-chlorophenyl) -4-oxoazetidin-1-yl) acetamide (8j): Aldehyde: 2-chlorobenzaldehyde. 5 hour stirring, 5 hour refluxing, white solid. Melting Point 126°C, Yield 69%, Rf 0.58., IR (KBr) vcm^{-1}: 3100.98 (N-H str.), 3028.38 (Ar C-H str.), 1600.56 (C=O str.), 1672.08 (C=N str.), 167.96 (lactone), 1812.96 cm^{-1} (-NCO, stretch.), 1170 cm^{-1} (CH-Cl, stretch.), 825.86 cm^{-1} (aromatic C=C).

After synthesis of novel analogues, preliminary biological screening should be conducted, which included the determination of LD_{50} by Brine Shrimp Lethality Assay and MTT Assay, Derivatives were selected according to the dock score and solubility. The activities of the screened analogues were comparable, reflecting the novelty of acetidino-quinazoline analogues as therapeutic agents.

Acute toxicity study

Brine shrimp lethality bioassay: The brine shrimp lethality bioassay was carried out on the various analogues using standard procedure.

Dried cysts (1 g cyst per litre) were hatched in a hatcher at 28-30°C with strong aeration, under a continuous light regime. Approximately 24 hr after hatching, the phototropic nauplii were collected with a pipette from the lighted side and concentrated in a small vial. Ten brine shrimp were transferred to each well using adequate pipette. The final volume of the solution in each test-tube were made up to 5 ml with artificial sea water, and a drop of dry yeast suspension (3 mg in 5 mL artificial sea water) as a food for shrimps. Each test consisted of exposing groups of 10 Artemia nauplii aged 24 h to various concentrations of the quinazolinothiazolidine analogues. The toxicity was determined after 24 hr and 48 hr of exposure. The numbers of survivors were counted and percentages of deaths were calculated. Composition of the artificial sea water is given in Table 4. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation.

The percentage of mortality (%M)=percentage of survival in the control-percentage of survival in the treatment. From all those analogues, various concentrations were prepared by serial dilution using DMSO as solvent. Each concentration was tested in triplicate, giving a total of 15 test-tubes. A control containing 5 ml of DMSO alone was used (vehicle treated). The final volume of the solution in each test-tube were made up to 5 ml with artificial sea water immediately after adding shrimp larvae (Table 12). The pH should be adjusted to pH 7-8 with sodium bicarbonate.

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values can be obtained from the best-fit line plotted between concentration and percentage lethality. The number of dead and live napulii in each

well was counted using a stereomicroscope. If deaths occurred in the solvent controls at the end of the treatment, the percentage of deaths were corrected using Abbott's formula:

Corrected mortality percentage=(m-M)/ × 100

Where, m=mean percentage of dead larvae in treated tubes, M=mean percentage of dead larvae in solvent controls, S=mean percentage of living larvae in solvent controls.

Correction factor is applied to 0 and 100 percent mortality group. The percent mortality values were converted to probit values. LD_{50} value was obtained from the best-fit line plotted between log of concentration and probit scale values.

Correction factor for 0% dead=100 (0.25/n)

Correction factor for 100% dead=100 (n-0.25/n)

MTT assay: Cells used: MCF-7 (breast cancer cell lines)

HeLa (cervical cancer cell lines)

SiHa (cervical cancer cell lines)

L929 (Fibrosarcoma cell lines)

Stain	: MTT assay
Standard	: Paclitaxel, Doxorubicin
Control	: DMSO
Compounds tested	: QT4a, QT4b, QT4c, QT4f and QT4h.

MTT assay-principle: Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Here yellow, MTT (3- (4,5-Dimethylthiazole-2yl) -2,5-diphenyltetrazolium bromide, a tetrazole) enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product in the mitochondria of the living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum depends on the solvent employed. The reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable cells.

Protocol: Short 96 well assay: Each condition should be done in triplicate or more. Day one: Trypsinise one T-25 flask and add 5 ml of complete media to trypsinized cells. Centrifuge in a sterile 15 ml falcon tube at 500 rpm in the swinging bucked rotor (~400 × g) for 5 min. Remove media and resuspend cells to 1.0 ml with complete media. Count and record cells per ml. Remember to remove the cells aseptically when counting. Dilute the cells (cv=cv) to 75,000 cells per ml. Use complete media to dilute cells. Add 100 µl of cells (7500 total cells) into each well and incubate overnight. Day two: Treat cells on day two with agonist, inhibitor or drug. If removing media, do very carefully. This is where most variation in data may occur. Final volume should be 100 µl per well. Day three: Add 20 µl of 5 mg/ml MTT to each well. Include one set of wells with MTT but no cells (control). All should be done aseptically. Incubate for 3.5 hours at 37°C in culture hood. Carefully remove media. Do not disturb cells and do not rinse with PBS. Add 150 µl MTT solvent. Cover with tinfoil and agitate cells on orbital shaker for 15 min. Read absorbance at 590 nm with a reference filter of 620 nm.

Procedure

Determination of *in-vitro* ant proliferative effect of compounds on MCF-7 breast cancer cell line, HeLa and SiHa cervical cell line and L929 fibro sarcoma T cells

Cells were purchased from NCCS Pune was maintained in Dulbecco's modified Eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5% CO_2 (NBS, EPPENDORF, Germany) in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025% Trypsin in PBS/0.5 mM EDTA solution (Himedia)) for 2 minutes and passed into T flasks in complete aseptic condition.

The effect of compounds on the proliferative capacity of the breast cancer cells (MCF7), Cervical cancer cells (HeLa and SiHa) and (L929) fibro sarcoma T cells were determined using MTT [3- (4, 5-dimethylthiazol-2-yl) -2, 5- diphenyl tetrazolium bromide] assay. Cells were seeded (5000 cells/well) in 96 well, flat bottom titer plates along with different concentrations of the compounds QT4a, QT4b, QT4c, QT4f and QT4h (1, 10, 100, 250 and 500 µg/ml) are incubated for 48 hours at 37°C in 5% CO_2 atmosphere. After incubation the medium was removed and wells washed with phosphate-buffered saline (PBS), 100 µl of the working MTT dye in Dulbecco's Modified Eagle Media (DMEM) was added and incubated for 2 hours. MTT lysis buffer (100 µl) was added and incubation was continued for 4 hours. The absorbance was measured at 570 nm and the proliferation rate (PR) was calculated using the formulae:

$$PR = \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Cytotoxicity of the compounds QT4a, QT4b, QT4c, QT4f and QT4h on the cells were calculated as cell growth inhibition rate (IR).

IR=100-PR

Results and Discussion

In silico design

In silico design, reveals the significance of rational design and development of azetidinone substituted quinazoline derivatives as good lead for anti-cancer drugs. The following *in silico* screening studies were carried out successfully with the aid of various software suits for selection of suitable drug candidates prior to wet lab synthesis: Analysis of Lipinski rule of five was carried out using Molinspiration software; all the proposed analogues except one were obeying the rule. Drug likeness profile of analogues was predicted by using Molinspiration software. Molecular descriptors that may have profound influence on the pharmacokinetic and receptor docking properties of the selected molecules like total polar surface area, molar volume etc., were determined using ACD Chemsketch. Biological activity spectrum for proposed analogues in which the probability to be active (Pa) and the probability to be inactive (Pi) were calculated using PASS Software. All the proposed analogues were subjected to flexible docking using GLIDE XP (Extra Precision) on various proteins which indicates the various biological activities. Selected analogues were subjected to *in silico* toxicity studies by using various web tools like Osiris, Lazar, Gusar and ADMETUS (Figure 1).

ADME properties of the proposed analogues are done by using Qikprop, an ADME prediction program provided by Schrodinger under Maestro. The tabulated results are shown below and which indicates

the proposed analogues has high oral absorption. *In silico* molecular modeling studies were carried out on different analogs using software like molinspiration, PASS, ACD Lab Chem Sketch and Schrodinger. Analysis of Lipinski's Rule of Five was carried out for the proposed analogues using molinspiration software and all the analogues obeyed the Rule of Five. Compounds which were predicted to have optimal activity by various drug design software like PASS were selected for wet lab synthesis and followed by pharmacological screening.

In silico molecular analysis of thirteen different Acetidino-quinazoline analogues has been done, all these compounds obeyed 'Lipinski rule of 5'. Out of these analogues, ten were taken for wet lab synthesis. These ten analogues possessed desired physicochemical properties with not any violations from Lipinski Rule of Five, optimal score in terms of activity prediction by (PASS) and docking (Tables 1 and 2).

ADME profile

Accurate prediction of ADME properties prior to expensive experimental procedures can eliminate unnecessary testing on compounds that will ultimately fail; ADME prediction can also be used to focus lead optimization efforts to enhance the desired properties of a given compound. Using QikProp, an ADME prediction program provided by Schrodinger under Maestro, all the proposed analogs were efficiently evaluated for pharmaceutically relevant properties within limited time fractions, making an indispensable lead generation and lead optimization tool (Table 3).

Molecular docking

Glide scores: All proposed derivatives were subjected to flexible docking using Schrodinger. The tabulated results are shown in Tables 4.

Anticancer activity: Refer Table 4.

Pharmacological evaluation

Brine shrimp toxicity assay: The preliminary cytotoxicity of the synthesized Acetidino-quinazoline derivatives were determined by Brine Shrimp lethality bioassay. As the brine shrimp method is primary investigation for assessment of cytotoxicity, all the synthesized derivatives were screened for this bioassay. Although the brine shrimb lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity. The LD_{50} of all the tested samples were showed to be lethal to brine shrimp nauplii. The degree of lethality was found to be directly proportional to the concentration of the analogues. The LD_{50} values of the compound were found to be in the range of 380 µg-501 µg (Tables 5 and 6; Figure 2).

Cytotoxicity study: Comparative Evaluation percentage of inhibition and percentage viability of QAz3 and QAz6. Comparative Evaluation percentage of inhibition of Synthesized Compounds and Standard Drugs (Tables 7-11).

Chemistry

After preliminary *in silico* screening techniques with the aid of commercially available and free download software, suitable drug candidates were selected. Of the proposed analogues, the candidates with desired physicochemical properties with no violation from Lipinski Rule of Five, maximum Pass value and optimal score in terms of docking were chosen for wet lab synthesis. All reactions were performed using reagents and solvents of analytical or synthetic grade. Solvents were dried by standard procedures.

The compounds were purified by recrystallization, and TLC, techniques. The compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

The reaction sequence for synthesis of target compounds is illustrated in Scheme 1. At first 2-aminobenzamide reacts with benzaldehyde to form 2-phenyl quinazolin-4 (3H) -one (3). 2-aminobenzamide reacts with benzaldehyde to form an intermediate. The quinazolinone could be obtained from direct 6-exo-tet cyclization of intermediate or 6-endo-trig cyclization of imine formed by removal of water from intermediate. This proposed mechanism also rationalized the significant increase in the yield of quinazolinone from anthranilamide and aldehyde. The formed quinazolinone exhibit resonance with quinazolin-4-ol [6].

2-phenyl quinazolin-4 (3H) -one (3) was treated with ethylchloroacetate in the presence of anhydrous potassium carbonate, ethyl [(2-phenyl-3,4-dihydroquinazolin-4-yl) oxy]acetate (4) was obtained. This is an example of nucleophilic substitution reaction in which hydroxyl group at 10^{th} position having an unshared pair of electrons is acting as a nucleophile. Chlorine atom is easily displaced by this nucleophile resulting in the formation of a new carbon oxygen bond. Next step is the base deprotonating the positive oxygen centre and the product obtained [7].

The reaction of hydrazine hydrate with ethyl [(2-phenyl-3,4-dihydroquinazolin-4-yl) oxy]acetate (4) results in the formation of 2-[(2-phenylquinazolin-4-yl) oxy]acetohydrazide (5). The reaction is carried out in alcohol in order to increase the yield.

2-[(2-phenylquinazolin-4-yl) oxy]acetohydrazide (5) react with aldehyde to generate azomethine compounds (6a - 6j). Under appropriate conditions, primary amines react with aldehyde to generate imines/azomethine. Imine formation is an example of a condensation reaction- where two molecules join together accompanied by the expulsion of a small molecule (usually water). The mechanism of imine formation starts with the basic addition of the amine to the carbonyl group. Protonation of the oxy anion and deprotonation of the nitrogen cation generates an unstable intermediate called a carbinolamine. The carbinolamine has its oxygen protonated and then water acts as the good leaving group [8].

This acid catalyzed dehydration creates double bond and the removal of the proton to produce the neutral imine product. The PH of the reaction mixture is crucial to successful formation of imine. The pH must be acidic to promote the dehydration step, yet if the mixture is too acidic, then the reacting amine will be protonated and therefore unnucleophilic and this should inhibit the first step. The best PH for imines formation is around 4.5.

The schiff's bases (6a-6j) were converted to 2-azetidinone derivatives (7a-7j) by the nucleophilic cycloaddition reaction. The mechanism of the ketene-imine cycloaddition reaction involves initial nucleophilic attack of the imine nitrogen on the ketone carbonyl carbon to form zwitter ionic intermediate.

Conclusion

The present work involved the preliminary *in-silico* screening of various analogues to analyze for their molecular descriptors using computational software. Derivatives with desired physicochemical properties, obeying Lipinski Rule of Five and those with no violations were chosen for wet lab synthesis. Synthesis of ten analogues was performed and the purity of the same was ascertained by consistency in melting point and Rf value. The compounds were characterized by IR, ^{1}H NMR and Mass spectral studies.

The synthesized compounds were subjected to *in-vitro* screening for anti-cancer, anti-bacterial and anti-fungal evaluation. The respective results of each were analyzed and evaluate the potency of the synthesized compounds.

The compounds QA6, QA7 and QA8 were studied for cytotoxicity studies against breast cancer and fibrosarcoma cell line. The compound QA7 showed better activity at the concentration of 500 µg/ml.

The significance of rational drug design approach by means of software like Schrodinger, PASS, Molinspiration and ACD Chemsketch in drug design was proved. Suitable molecular modifications can be experimented on these molecules to improve their activity for new leads.

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