Research Article

In-Silico Design, Synthesis and Anti-Proliferative Evaluation of Acetidino-Quinazoline Derivatives

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ABSTRACT

Cancer, the second cause of mortality in the world, is continuing to be major health problem worldwide. In order to overcome draw backs of nonspecific traditional chemotherapeutic agents, identification of specific tumor targets and design of novel analogues are very important. The EGFR, a tyrosine kinase receptor is one of the most suitable targets for cancer. Agents which could inhibit EGFR are directly related to blockade of regulatory processes of cellular proliferation. Our aim was to develop novel Acetidino-quinazoline derivatives inhibiting EGFR leads to potent anticancer agents. *In-silico* design of novel analogues were carried out using ACD labs ChemSketch 12.0. Molinspiration software was used to analyse 'Lipinski Rule of Five' and drug likeness properties. Biological activity was predicted by PASS software. Preliminary docking study was carried out using GLIDE software by SCHRODINGER. Five derivatives which obeyed rule of five and having predicted antitumor activity on EGFR were synthesized by five step process. After the completion of reaction in each step, the compounds were isolated, recrystallised by using suitable solvents, purified by TLC and column chromatography. Analogues were characterized by FT-IR, H^1NMR, C^{13}NMR and Mass Spectroscopy. The Biological evaluation was done by MTT assay using human cervical cancer cell lines (HeLa). The results were compared with standard anticancer drug paclitaxel. The results of present research work showed that novel acetidino-quinazoline derivatives have comparable antiproliferative effect with that of standard anticancer drug paclitaxel. This will lead to the development of promising lead compounds for target specific anticancer therapy and encourage further optimization to develop potent antiproliferative agents.

Keywords: Acetidino-quinazoline, in-silico, HeLa cells, antiproliferative agent.

INTRODUCTION

Cancer is emerging as a first major health problem in developing as well as developed countries[1-5]. Surpassing cardiac diseases, it is taking number one killer worldwide due to various social, economic and lifestyle factors. There are many chemotherapeutic strategies for cancer treatment have been proposed, tested and in some cases implemented in the past two decades, these diseases still remains deadly. Therefore, there is a desperate need to develop treatments with new chemical entities with novel mechanism of action to combat this disease. One of the most important receptor which is overexpressed in majority of solid tumors is epidermal growth factor receptor. It is a 170KDa membrane glycoprotein composed of an extracellular domain, an intermembrane region and an intracellular domain which presents protein kinase activity[6]. The binding of EGF to this EGFR activates a cascade in which several proteins are phosphorylated and processes of regulation, maintenance and cell survival occur[7,8]. The potential inhibitors of EGFR definitely prevent this enzyme cascade mechanism and blocks cell division[9].

Quinazolines are most important heterocyclic nucleus and its derivatives were found to be effective inhibitors of EGFR[10-15]. Inhibitors of the EGFR-TK are expected to have great potential in the treatment of malignant and nonmalignant epithelial diseases. Azetidinone ring which is otherwise known as β-Lactam is a cyclic amide. The biological activity of β-Lactam is believed to be associated with the chemical reactivity of the ring and on the substituents especially at nitrogen of 2-acetidinone ring. The rationale behind this research work is that on combining these two therapeutically significant heterocyclic moiety results into a new chemical nucleus which could specifically inhibit EGFR receptor, which ultimately leads to the prevention of growth and proliferation of cancerous tissue. So, we aimed to *in-silico* design and development of acetidino-quinazoline derivatives as potential anticancer agents.

MATERIALS AND METHODS

Materials and instrumentation

All the chemicals and reagents used in this research work were of analytical or synthetic grade from Sigma Aldrich, E-Merck (Germany) and S D Fine Chemicals (India). All the chemicals were dried and purified according to standard methods before use, wherever necessary. Software used for this study include ACD Labs Chemsketch, Chemdraw Ultra 8.0, Molinspiration, PASS and Discovery studio 4.1, and. All the reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. All the reaction courses and product mixtures were routinely monitored by aluminium coated TLC plates 60 F245 (E Merck) and visualized with UV light or iodine chamber. Melting point of synthetic compounds was determined on a Labindia MR-VIS visual melting point apparatus and is uncorrected. Absorbance values against wavelength were taken on a Systronic double

beam UV-166 spectrophotometer. The FT-IR spectra were recorded using FT-IR (Agilent Cary 630 FT-IR spectrophotometer using KBr pellet). ^{1}H NMR spectra were recorded using NMR spectrophotomer (Bruker 400 ultra schield DPX 400) and chemical shifts are expressed as δ (ppm) using TMS as an internal standard in DMSO-*d6*. Mass spectra of the compounds were done with mass spectrometer (micromass-O-TOF-MS ES+). Anticancer evaluation was done by MTT assay using the EGFR overexpressing Cervical cancer (A549) cell lines.

In-silico methods

In-silico molecular modeling

In-silico methods used helped to identify and quantify the physico-chemical descriptors and to analyse whether any of these properties have significant effect on drug's biological activity. These methods could help in identifying drugs' possible targets and predict its activity using various bioinformatics tools. These methods can also used to analyze target structures for possible binding or active sites, generate candidate molecules, check for their drug likeness, and dock these molecules to improve binding characteristics. The Physico-chemical properties of the molecule were calculated by different software. The electronic, lipophilic and various steric parameters can be determined by ACD Labs Chemsketch. Drug likeness and analysis of Lipinski rule of five were carried out using Molinspiration software Maestro software. Prediction of Activity Spectra for Substances (PASS) is based on the suggestion that Activity is a function of software. Thus, by comparing the structure of a new compound with structures of well-known biologically active substance it is possible to estimate if a new compound may have a particular effect and predict whether the molecule could be developed further for *in-vitro* and *in-vivo* studies.

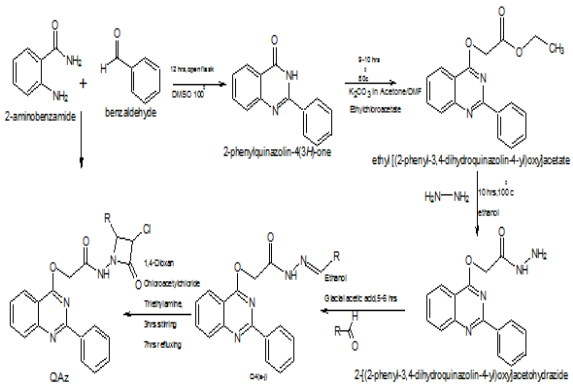
Docking studies

Docking is the computational simulation of a ligand binding to a receptor, which helps to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and the activity of the small molecule. Docking is very important tool in the rational design of drugs. Schrodinger is a comprehensive software suite for analyzing and modeling molecular structures, biological macromolecules (proteins and nucleic acid). The selected analogues were docked onto the binding pocket of Epidermal Growth Factor Receptor (EGFR, PDB ID. 1M17). These docking studies gives best matching between two molecules: designed acetidino quinazolines and the binding pocket of target protein. Different steps involved in docking studies include; preparation of ligand and protein, docking methods, scoring of docking results and analysis, refinement and filtering tools.

Preparation of protein and ligands is essential for performing molecular docking which was done with LigPrep. This software application is designed to prepare high quality, all atom 3-D structures for larger numbers of drug like molecules. This operation is aided with several tools in order to identify binding sites. The protein selected for the study is Epidermal Growth Factor Receptor (EGFR) and its x-ray crystallographic structure was obtained from protein data bank (1M17). Receptor Grid generation requires a "prepared" protein structure: an all atom structure with appropriate bond disorders and formal charges. The receptor gride can be set up and generated from the Receptor Grid Generation panel under GLIDE. Ligand docking jobs cannot be performed until the receptor grids have been generated. The Glide Ligand Docking Panel is used to set up and run docking jobs using previously calculated receptor grids. Ligprep or Macro Model can be used to prepare ligands. Docking results in the workspace was done using Glide XP Visualizer panel of the application menu.

Synthetic methodology

Synthetic Scheme



Scheme 1: Synthesis of Acetidino-quinazoline derivatives (QAz1-QAz10)

Step 1: Synthesis of 2-phenylquinazolin-4(3*H*)-one

To the solution of 2-aminobenzamide (0.01mole, 1.36g) 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 h. 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.

Step 2: Synthesis of ethyl [(2-phenylquinazolin-4-yl)oxy]acetate

In 500 mL Round bottom flask, take 15-20mL dry DMF. To this add 2-phenylquinazolin-4(3H)-one (0.01 mole, 2.22g), and ethylchloroacetate (0.01mole, 1.25mL) and anhydrous potassium carbonate (0.1 mole, 1.38g). The resultant mixture was stirred and refluxed for 9-10 hrs at 80ºC. 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.

Step 3: Synthesis of 2-[(2-phenylquinazolin-4-yl)oxy] acetohydrazide

Ethyl [(2-phenylquinazolin-4-yl)oxy]acetate (0.05M) and hydrazine hydrate 99% (0.15M, 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.

Step 4: Synthesis of Schiff's bases of 2-[(2-phenylquinazolin-4-yl)-oxy]-acetohydrazide

To a solution of appropriate substituted benzaldehyde (1mmol, 3.5g) in ethanol (15mL), 2-[(2-phenylquinazolin-4-yl) oxy] acetohydrazide (1mmol, 3g) were added. Make pH around 4.5 by adding 2-3 drops of glacial acetic acid. The reaction was refluxed for 5-6 h 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. The crude product was recrystallised from ethanol.

Step 5: Synthesis of substituted *N*-(3-chloro-2-oxoazetidin-1-yl)-2-[(2-phenylquinazolin-4-yl) oxy]-acetamide

A mixture of Schiff's base [4a-4h] (0.01 mol) and triethylamine (5-6 drops) was dissolved in 1,4-dioxane (50mL), cooled and stirred. To this well-stirred cooled solution, chloroacetyl chloride (0.015mole, 1.68mL) 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. The solid thus obtained was recrystallized from acetone to yield desired 2-azetidinone derivatives (QAz1, QAz3, QAz5, QAz8, QAz10)

Antiproliferative evaluation (MTT Assay)

Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents and screening other biologically active compounds. MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g. Dimethylsulfoxide) and the released, solubilized formazan reagent is measured spectrophotometrically at 540 nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells were washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT- 5mg/mL dissolved in PBS). It was then incubated at 37°C for 3h. MTT was removed by washing with 1x PBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a ELISA microplate reader.

% viability = (OD of Test/ OD of Control) X 100

Percentage mortality= 100 - %viability

RESULTS AND DISCUSSION

In-silico molecular modeling studies

The *In-silico* molecular modeling studies of novel acetidino-quinazoline derivatives were carried out successfully with the aid of different software for selection of suitable drug candidates prior to wet lab synthesis. *In-silico* studies were performed on designed 30 acetidino-quinazoline derivatives by means of ACD Lab ChemSketch 12.0, Chem Draw 8.0, Molinspiration, PASS, Schrodinger software. Among the 30 designed analogues, five analogues were found to obey Lipinski rule of five and their drug likeness were predicted by Molinspiration software. The analogues which are having desired physico-chemical properties and predicted antibacterial activity were chosen for wet lab synthesis **(Table 1, Table 2, Table 3, Table 4).**

Table 1: Molecular descriptors for designed analogues generated by ACD Labs Chemsketch 12.0

Compound	Molecular Formula	Parachor (cm^3)	Molar Volume (cm^3)	Polarisability (10^{-24} cm^3)	Molar Refractivity (cm^3)
QAz1	$C_{26}H_{21}ClN_4O_4$	995.0 ± 6.0	338.8 ± 5.0	51.97 ± 0.5	131.09 ± 0.4
QAz3	$C_{25}H_{18}Cl_2N_4O_3$	973.6 ± 6. 0	327.9 ± 5.0	51.36 ± 0.5	129.55 ± 0.4
QAz5	$C_{25}H_{18}BrClN_4O_4$	1002.7 ± 6. 0	326.6 ± 5.0	53.11 ± 0.5	133.97 ± 0.4
QAz6	$C_{25}H_{18}ClN_4O_4$	951.7 ± 6. 0	314.0 ± 5.0	50.05 ± 0.5	126.25 ± 0.4
QAz8	$C_{26}H_{21}ClN_4O_3$	974.7 ± 6.0	332.8 ± 5.0	51.27 ± 0.5	129.35 ± 0.4

Table 2: Analysis of Lipinski rule of five for selected acetidino-quinazoline analogues

Compound	Log P	Mol. Wt.	No. of Hydrogen bond acceptors	No. of Hydrogen bond donors	No. of Rotatable bonds	Violations
QAz1	4.36	488.93	8	1	7	0
QAz3	4.98	493.35	7	1	6	0
QAz5	5.03	553.80	8	2	6	2
QAz6	4.30	458.90	7	1	6	0
QAz8	4.75	472.93	7	1	6	0

Table 3: Analysis of drug likeness score for selected derivatives

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
QAz3	-0.08	-0.35	-0.20	-0.50	-0.21	-0.08
QAz5	-0.08	-0.36	-0.20	-0.50	-0.19	-0.10
QAz6	-0.08	-0.36	-0.20	-0.50	-0.19	-0.06
QAz8	-0.11	-0.40	-0.22	-0.51	-0.23	-0.10

Table 4: Prediction of Biological activity of proposed analogues using PASS software

Compound	Activity	Pa	Pi
QAz1	Anticancer	0.799	0.012
QAz3	Anticancer	0.772	0.015
QAz5	Anticancer	0.654	0.010
QAz6	Anticancer	0.822	0.009
QAz8	Anticancer	0.789	0.013

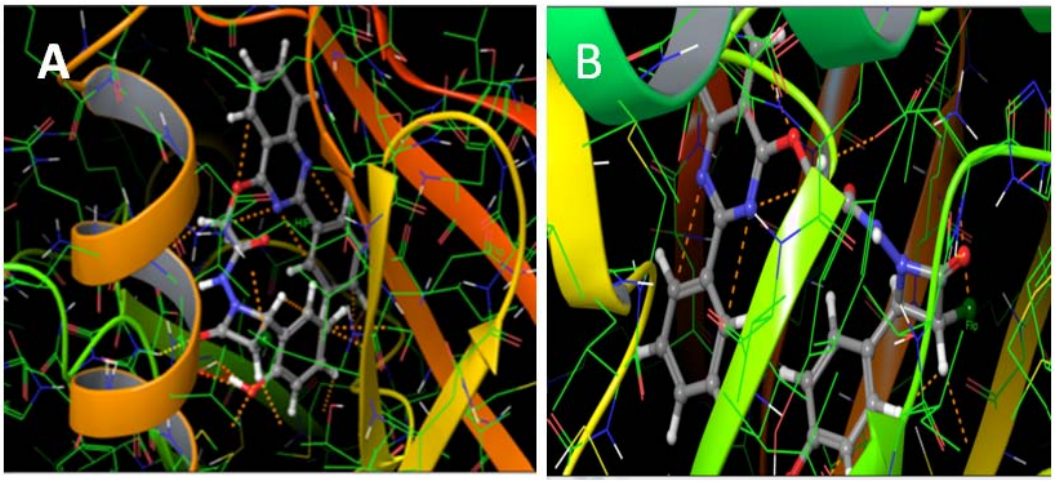


Figure 1: Docking images of Acetidino-quinazoline derivatives (A. QAz3 & B. QAz8) on binding pocket of EGFR

Table 5: Docking scores of selected derivatives with target protein EGFR

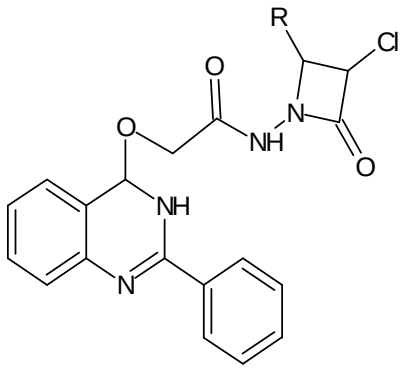
Target	PDB ID	Compound Name	GLIDE Score
EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)	1M17	QAz1	-7.1
		QAz3	-8.4
		QAz5	-5.5
		QAz6	-6.4
		QAz8	-7.3

Molecular docking

All the proposed derivatives were subjected to flexible docking on to the binding pocket of Epidermal Growth Factor Receptor (Pdb ID: 1M17) using GLIDE Programe of Schrodinger. The docking scores were calculated on the basis of Glide score **(Figure 1, Table 5).**

Synthetic methods

The analogues which were designed by *in-silico* studies were selected for wet lab synthesis based on Lipinski rule of five, PASS value and docking energy score. The synthetic scheme involved was a five step reaction. After the isolation of product in each step the products were recrystallised and purified by TLC and column chromatography. The structure of proposed analogue is shown in **Figure 2.** Five new derivatives were synthesized by conventional method (QAz1, QAz3, QAz5, QAz6, QAz8). The percentage yield of the reaction, melting point, and R_f value of each compounds were calculated and shown in **Table 6.**



QAz

Figure 2: General structure of Acetidino-quinazoline derivatives

Table 6: Characterization data of synthesized acetidino-quinazoline derivatives

Code	R	Molecular Formula	Molecular Weight	Melting Point	Yield	R_f
QAz1	C6H5 p-OCH_3	$C_{26}H_{21}ClN_4O_4$	488.93	124	65	0.46
QAz3	C_6H_5 p-Cl	$C_{25}H_{18}Cl_2N_4O_3$	493.35	130	68	0.61
QAz5	C_4H_3O	$C_{23}H_{17}ClN_4O_4$	448.87	141	61	0.62
QAz6	C_6H_5	$C_{25}H_{19}ClN_4O_4$	458.8	118	64	0.56
QAz8	C_6H_5 p-CH_3	$C_{26}H_{21}ClN_4O_3$	472.93	120	75	0.59

Table 7: Characteristic FT-IR, 1HNMR and Mass spectral analysis of synthesized analogues

Compound	IR (KBr υ cm^{-1})
Step 1	3,303 cm^{-1} (N-H str), 1,667 cm^{-1} (C=O), and 1,614 cm^{-1} (C=N)
Step 2	1,653 cm–1 (C=O), 1,609 cm^{-1} (C=N) and 1,152 cm^{-1} (C-O, ether), 2851(CH aliphatic)
Step 3	3302-2922 (NH, NH2), 2852 (C-H alip.), 1653 (CO) carboxamide, 1511 (C=N), 1026 (C-O-C).
Step 4	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-).
Step 5 **(QAz3)**	3302 (N-H str.), 3061.68 (Ar C-H str.), 1651.04 (C=O str.), 1613.96 (C=N str.), 1659.54(lactone), 1886.68cm^{-1} (-NCO, stretch.), 1148cm^{-1}(CH–Cl, stretch.), 860.32cm^{-1} (aromatic C=C)
^{1}HNMR QAz3	8.5(s,1H,NH), 7.06-8.01(m, 13H, aromatic ring), 7.409-7.430 (t, 1H, Ar-H), 6.244(s, 1H, Ar H), 5.417(s, 2H,CH_2), 2.50(s, CH_3)
Mass spectral Analysis	
QAz3	493.017 (Molecular ion peak), 102.345 (Base peak)

Table 8: Comparative evaluation of Antiproliferative effect of QAz3 and QAz6 on HeLa cells

Concentration (µg/mL)	Percentage 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

Spectral characterization of Acetidino quinazoline derivatives

The newly synthesized novel Acetidino-quinazoline derivatives were further characterized by FT-IR, ^{1}HNMR, ^{13}CNMR and Mass spectral studies. The complete spectral analysis of prototype lead molecule QAz3 is shown in **Table 7.**

Evaluation of antiproliferative effect (MTT Assay)

After the preliminary in-silico molecular modeling studies followed by the docking studies on the binding pocket of EGFR receptor, five acetidino-quinazoline derivatives were selected for wet lab synthesis (QAz1, QAz3, QAz5, QAz6, QAz8). The synthesized compounds were purified and characterized by FT-IR, ^{1}HNMR and Mass spectral studies. Docking studies proved that the QAz3 and QAz6 were more effectively bind with the receptor based on glide score. Five concentrations of the test compounds (QAz3 and QAz6) were used for MTT assay. The results were compared with that of standard drug paclitaxel. The concentrations used were 100, 50, 25, 12.5, 6.25 µg/mL. The cell lines used was Human Cervical Cancer Cell lines (HELA). Test results showed that both QAz3 and QAz8 showed comparatively very good inhibitory effect on EGFR positive HeLa cell lines. At higher concentration of 100µg/mL its antiproliferative effect is almost equal to that of standard drug paclitaxel **(Table 8, Figure 3).**

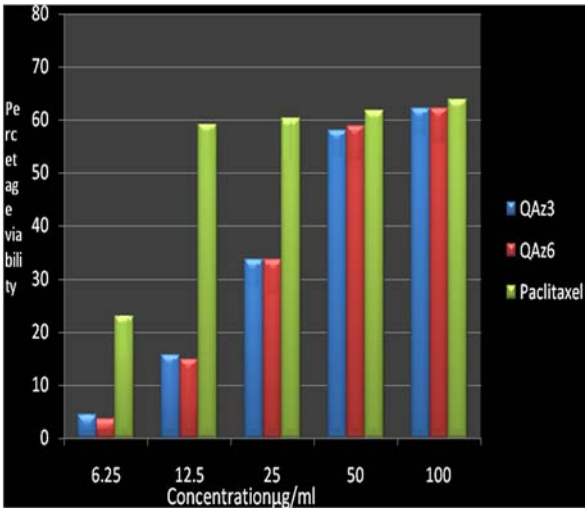


Figure 3: Comparative evaluation of antiproliferative effect of QAz3 and QAz6 with Paclitaxel on HeLa cells.

CONCLUSION

The present work led to the development of novel antitumor molecules containing acetidino-quinazoline pharmacophore. This research work was focused on the structure based drug design and development of novel Acetidino-quinazoline derivatives and their antiproliferative evaluation. We have designed 30 new analogues and after *in-silico* molecular modeling and docking studies, selected five analogues for wet lab synthesis (QAz1, QAz3, QAz5, QAz6, QAz8). These derivatives were spectrally characterized by FT-IR, ^{1}HNMR, mass spectroscopy. The antiproliferative evaluation of two derivatives was done against EGFR overexpressing human cervical cancer cell lines (HeLa). The compounds QAz3 and Qaz6 have shown significant activity against HeLa cells and compared with that of standard drug paclitaxel. Thus this work presents a potent antiproliferative effect of synthesized analogues. The Activity prediction by *in-silico* methods are very well correlated with biological activity. From this study, this can be concluded that the synthesized Acetidino-quinazoline derivatives can be lead candidate to be developed into useful antiproliferative agents that could lead further research work on this potent nucleus. An extensive study is also warranted to determine additional physiochemical and biological parameters to have deeper insight into SAR and optimize the effectiveness of these lead molecules.

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Conflict of interest

The authors declare that there is no conflict of interest

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