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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF SUBSTITUTED ACETIDINO-QUINAZOLINE DERIVATIVES

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ABSTRACT

In the era of emerging bacterial resistance to traditional antibacterial agents, there is rapid need of development of novel antibacterial agents which could specifically identify new targets and exert its biological action. This work is aimed to develop novel Acetidino-quinazoline derivatives inhibiting 2-DHFR leads to potent antibacterial agents. In-silico design of novel analogues were carried out using ACD labs Chem Sketch 12.0 and Marvin sketch software. Molinspiration software was used to analyze '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 antibacterial activity 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 antimicrobial activity was done against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) organisms by agar diffusion assay. Among the synthesized compounds, two compounds (QAz3 and QAz8), showed significant anti-bacterial activity against gram negative E coli. These novel analogues could specifically target bacterial DHFR and can be considered as suitable lead compounds for further research and development.

KEY WORDS

Acetidino-quinazoline, Dihydrofolate reductase, E coli, Antimicrobial activity.

INTRODUCTION

The alarming increase in resistance to antimicrobial agents is widespread; there is urgency for identification of novel structure leads to the development of new, potent and less toxic antimicrobial agents. The multiple pharmacological actions of unique synthetic compounds are a prerequisite for classifying a drug as highly potent, and efficacious, because these actions lead to offer possibility of treating various diseases [1-3]. Therefore success in designing antimicrobial agents which are distinct from those classical antibiotics is the key for treating infectious diseases known for their choronicity and failure to treat traditional antibiotics will lead to death eventually. Consequently, there is a vital need for the development of novel antibacterials with potent activity against drug resistant microorganisms [4-6].

Different heterocyclic systems have been explored for developing therapeutically important agents. Among these Quinazolines played significant role in medicinal chemistry, polymer sciences and building blocks for construction of different biologically active molecules [7]. Many of these quinazoline conjugated structures display remarkable biological activities such as antimicrobial, anti-inflammatory, anticonvulsant, anticancer, anti-HIV and genotoxic activities [8-12]. The unique chemotherapeutic properties of β–lactam antibiotics continue to attract the attention of the chemical community. One important application of the β-lactam moiety in synthesis involves the production of natural and non-natural α-amino acids. The simplest β-lactam is 2-azetidinone. The biological activity of β-lactam skeleton is believed to be associated with the chemical reactivity of the ring and on the substituents especially at nitrogen of 2-azetidinone ring. The present research work aim to design novel chemical nuclei coupled with quinazoline and azetidinone moiety which could target Dihydrofolate reductase as potent antibacterial agents.

MATERIALS AND METHODS

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 and Schrodinger. 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+). Antibacterial screening was done with *Staphylococcus aureus* (Gram Positive) and *Escherichia coli* (Gram negative) microorganisms. The method adopted was approved by National Committee for Clinical Laboratory Standards, 1993.

EXPERIMENTAL 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 to be 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.

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 bacterial dihydrofolate reductase protein. 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 dihydrofolate reductase and its x-ray crystallographic structure was obtained from protein data bank (2DHFR). Receptor Grid generation requires a "prepared" protein structure: an all atom structure with appropriate bond disorders and formal charges. The receptor grid 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

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

To the solution of 2-aminobenzamide (0.01mole, 1.36g) and benzaldehyde (0.01 moles, 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^{0}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 80^{o}C. After completion of reaction, which was monitored by *in situ* TLC in 15% ethyl, 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^{o}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, QAz15)

Antibacterial screening

Antibacterial screening was done by Agar well Diffusion method on synthesized Acetidino-quinazoline analogues selected according to PASS value (QAz3, QAz8, QAz15). The bacterial strains, *Staphylococcus aureus* (Gram+ve) and *Escherichia coli* (Gram –ve) procured from Microbiology laboratory, Govt. Medical College, Thiruvananthapuram was used for the study. Gentamicin was used as standard for both gram positive and gram negative organisms. The responses of the organisms to the synthesized compounds were measured and compared with the responses of the standard drug. All the synthesized compounds were dissolved in DMSO to make the concentrations of 1000µg/mL. Three different concentrations were used for the study 100 µg/ml, 50 µg/mL and 25 µg/mL.

RESULLTS AND DISCUSSION

***In-silico* molecular modelling**

The *In-silico* molecular modeling studies of 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 30 analogues of acetidino-quinazolines by means of ACD Lab ChemSketch 12.0, Chem Draw 8.0, Molinspiration, PASS and Schrodinger.

Among the 30 designed analogues, five analogues were found to obey Lipinski rule of five and their drug likeness were predicted by Molinspiration software. These analogues which are having desired physico-chemical properties and predicted antibacterial activity were chosen for wet lab synthesis **(Table 1, 2, 3, 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
QAz8	$C_{26}H_{21}ClN_4O_3$	974.7 ±6.0	332.8 ±5.0	51.27±0.5	129.35 ±0.4
QAz15	$C_{25}H_{19}ClN_4O_4$	951.7±6. 0	314.0 ±5.0	50.05 ± 0.5	126.25 ±0.4

Table 2. Analysis of Lipinski rule of five for selected 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
QAz8	4.75	472.93	7	1	6	0
QAz15	3.82	474.90	8	2	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	-0.06
QAz8	-0.11	-0.40	-0.22	-0.51	-0.23	-0.10
QAz15	-0.04	-0.32	-0.16	-0.39	-0.18	-0.02

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

Compound	Activity	Pa	Pi
QAz1	Antibacterial	0.166	0.113
QAz3	Anti bacterial	0.139	0.114
QAz5	Anti bacterial	0.184	0.133
QAz8	Anti bacterial	0.148	0.115
QAz15	Anti bacterial	0.266	0.260

Molecular docking

All the proposed derivatives were subjected to flexible docking on the bacterial protein Dihydro Folate Reductase (2DHF) using GLIDE Programe of Schrodinger. The docking scores were calculated with Glide score **(Figure 1, Table 5)**.

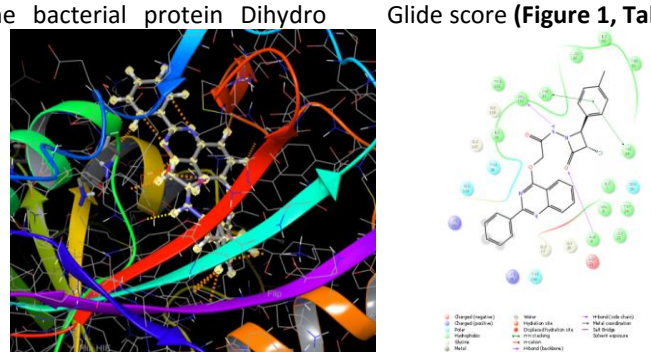


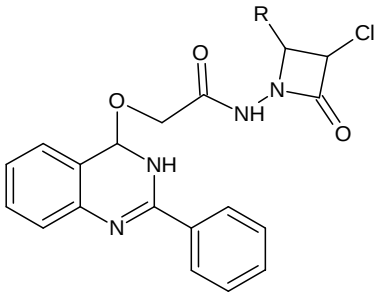
Figure 1. Docking image of QAz8 to 2DHFR

Table 5. Docking scores of selected derivatives with target protein 2DHFR

Target	PDB ID	Compound Name	GLIDE Score
DIHYDRO FOLATE REDUCTASE	2DHF	QAz1	-8.2
		QAz3	-8.4
		QAz5	-7.9
		QAz8	-12.3
		QAz15	-10.9

Synthetic methods

Those 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 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, QAz8, QAz15). 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 novel 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_6H_5	$C_{25}H_{19}ClN_4O_4$	458.9	118	64	0.56
QAz8	C_6H_5 p-CH_3	$C_{26}H_{21}ClN_4O_3$	472.93	120	75	0.59
QAz15	C_6H_5 p-OH	$C_{25}H_{19}ClN_4O_4$	474.9	142	68	0.63

Spectral Characterization of Acetidino-quinazoline derivatives

The newly synthesized Schiff bases of 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, 8, 9.**

Table 7. Characteristic IR absorption peaks 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	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)

Table 8. ^{1}HNMR data of synthesized prototype analogue

Compound	^{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),

Table 9. Mass spectral analysis of synthesized prototype analogue

Compound	Molecular ion peak	Base peak
QAz3	493.017	102.3445

Antibacterial evaluation of the compounds

Three analogues were selected for antibacterial evaluation by agar well diffusion method (QAz1, QAz3, and QAz8). Three different concentrations of the Acetidino-quinazoline derivatives were used for antibacterial evaluation (100, 50 and 25 µg/ml). The same concentrations of standard drug gentamicin were also used for comparative evaluation of candidate drugs. The analogues QAz3 and QAz8 were found to have profound activity against gram negative *E coli*. There is a graded increase in zone of inhibition with concentration of candidate drugs used. Antibacterial activity was highest with higher concentration i.e. 100 µg/ml **(Figure 3, Table 10).** The effect of both these analogues on gram positive *Staphylococcus aureus* was comparatively very less when compared to standard gentamicin.

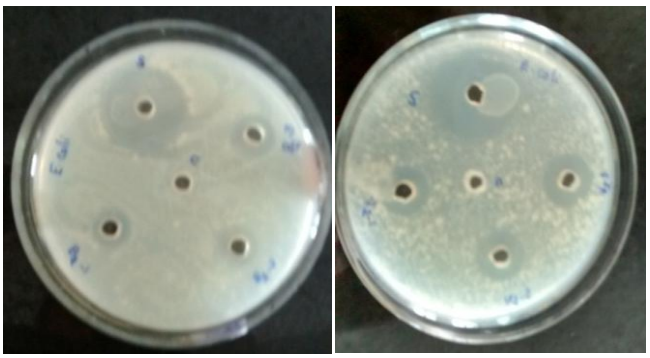


Figure 3. Antibacterial evaluation of QAz3 and QAz8 against *E.coli* (Agar diffusion assay)

Table 10. Antibacterial evaluation of Acetidino-quinazoline derivatives

Group	Drug	Zone of inhibition in mm	
		Escherichia coli	***Staphylococcus aureus***
Control	**DMSO**	--	--
Standard	**Gentamicin (100µg/ml)**	20	30
QAz3	**100µg/ml**	15	5
	50µg/ml	9	2
	25µg/ml	3	0
QAz8	**100µg/ml**	17	3
	50µg/ml	9	2
	25µg/ml	4	0

CONCLUSION

This research work was focused on the rational approach in design and development of novel Acetidino-quinazoline derivatives and their antimicrobial 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, QAz8, QAz15). These derivatives were spectrally characterized by FT-IR, ^{1}HNMR, mass spectroscopy. The biological

evaluations were done against both Gram positive and Gram negative bacterial strains. The compounds QAz3 and Qaz15 have shown significant activity against Gram negative *E coli* and compared with that of standard drug gentamicin. Thus this work presents a potent antimicrobial activity of synthesized analogues. The Activity prediction by *in-silico* methods are 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 antimicrobial 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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