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Synthesis, anti-microbial and molecular docking studies of some 2,3-disubsttuted quinazolinone analogs

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ABSTRACT

A series of some 2{(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]amino}-N-(substituted phenyl) acetamides were synthesized and characterized on the basis of IR, NMR and Mass Spectral (MS) data, the compounds have been subjected to molecular docking studies. The title compounds were subjected to in-vitro antibacterial screening against both the stains viz. Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeuroginosa. The binding affinity of the compounds with Sortase A of Staphylococcus aureus protein (1T2W) was analyzed by molecular docking.

Key words: Quinazolin-4(3*H*)-one, antibacterial activity, molecular docking study.

INTRODUCTION

Quinazolinone derivatives have been found to possess potent and wide spectrum of activities like antitubercular [1,2], antibacterial [3], antimicrobial[4], anti-inflammatory[5], and CNS depressant[6]. It has been reported that substitution of various functional group, atom or heterocyclic ring at C-2 or C-3 position of quinazolinone nucleus modulates the biological activity. It is a versatile lead molecule for the design of potential bioactive agents [7]. The ever growing resistance to antibiotics led to continuous screening for new biologically effective compounds of either natural or synthetic origin. Quinazolinone derivatives are extensively used in pharmaceutical industry, medicine and in agriculture for their wide scope of biological activities. These analogs have been reported for various biological activities such as antimicrobial, anticancer and antioxidant activities. The quinazolinone moiety is a building block for approximately 150 naturally occurring alkaloids and drugs.

Molecular docking has been a focus of attention for many years. In the current scenario, the flexible docking program is able to predict protein ligand complex structures with reasonable accuracy and speed. The present investigation was to analyze the binding affinity of the synthesized compounds 2{[2-(4-chlorophenyl)-4-oxoquinazolin-3(4*H*)-yl] amino}-N-(substituted phenyl) acetamides (4a-m) against the enzyme Sortase A. This enzyme involves in the pathogenesis of variety of bacterial infections, including respiratory tract, bloodstream, skin and tissue infection which serve to bind to some proteins responsible for virulence mainly by Gram +ve bacteria. Sortase A has attracted great interest as potential drug targets since decades[8]. The inhibition of *Sortase A* activity results in the separation of *S. aureus* from the host cells and ultimately alleviation of the infection. We used the reported compounds to identify their binding affinity by performing molecular docking studies against *Staphylococcus* aureus Sortase A using autodock 4.2.5 program.

RESULTS AND DISCUSSION

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on a Fourier Transform IR spectrophotometer (Shimadzu 8700) using KBr disc method. ^{1}H NMR spectra were recorded on a DPX 300 MHz Bruker FT-NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm) using tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin –Elmer 2400 CHN analyzer and values were within the acceptable limits of the calculated values. The purity of the test compounds was determined by thin layer chromatography. Physical data of the synthesized compounds are shown in **Table 1**.

Table 1: Physical properties and antibacterial activity of synthesized compounds

Comp Code	Ar	m.p°C	Mol Formula	Mol Wt	Rf	Yield	MIC value against S.aureus
4a	H	192-93	$C_{23}H_{18}ClN_3O_2$	403	0.75	42.3	>12.5
4b	2-Cl	180-82	$C_{23}H_{17}Cl_2N_3O_2$	437	0.82	48.56	>25
4c	3-Cl	201-03	$C_{23}H_{17}Cl_2N_3O_2$	437	0.81	37.1	>25
4d	4-Cl	188-90	$C_{23}H_{17}Cl_2N_3O_2$	437	0.80	50.1	>25
4e	2-CH_3	190-92	$C_{24}H_{20}ClN_3O_2$	417	0.80	38.8	>12.5
4f	3-CH_3	175-77	$C_{24}H_{20}ClN_3O_2$	417	0.54	51.9	>25
4g	4-CH_3	185-87	$C_{24}H_{20}ClN_3O_2$	417	0.65	57.42	>12.5
4h	2-OCH_3	201-03	$C_{24}H_{20}ClN_3O_3$	433	0.59	54.21	>25
4i	3-OCH_3	197-99	$C_{24}H_{20}ClN_3O_3$	433	0.50	50.18	>50
4j	4-OCH_3	210-13	$C_{24}H_{20}ClN_3O_3$	433	0.58	56.87	>50
4k	2-Br	178-80	$C_{23}H_{17}ClBrN_3O_2$	482	0.71	54.25	>25
4l	3-Br	190-94	$C_{23}H_{17}ClBrN_3O_2$	482	0.61	49.7	>25
4m	4-Br	183-85	$C_{23}H_{17}ClBrN_3O_2$	482	0.86	45.1	>25

Synthesis of 2-(4-chlorophenyl)-3-amino quinazolin-4-one (2)

To a solution of 2-(4-chlorophenyl) benzoxazin-4-one (2.57g, 0.01 mol) in alcohol (30 ml) was added hydrazine hydrate (1.02ml, 0.02mol) with shaking. The mixture was then refluxed for 4h . The excess of alcohol was removed and the reaction mixture was cooled. The solid obtained was filtered, washed with water and recrystallised from alcohol to obtain needle shaped crystals. IR (KBR) (cm^{-1}): 3311 (Ar NH_2), 3217 (Ar str), 1664 (C=O str), 1596 (C=N str); ^{1}H NMR ($CDCl_3$) : δ(ppm) 7.2-7.9 (m,8H,ArH), 12.07 (s,2H,NH_2); Mass: 271 (M^+) 34.17% , 273 (M+2).

Synthesis of 2{[2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]amino}-N-(4-chlorophenyl) acetamide (4g)

A mixture of 2-(4-chlorophenyl)-3-amino quinazolin-4-one (2.71g, 0.01 mol) and 4-chlorophenyl chloro acetamide (0.243g, 0.012 mol) were refluxed in dry pyridine (20 ml) for 5h. The reaction mixture was then poured in to a beaker containing concentrated Hydrochloric acid in ice cold water, the solid obtained was filtered, washed with water and recrystallised from alcohol to yield yellow coloured crystals of 2{[2-(4-chlorophenyl)-4-oxoquinazolin-3(4*H*)-yl]amino}-N-(4-chlorophenyl) acetamide (4g).

IR (KBR) (cm^{-1}): 3215 (Ar str), 1672 acyclic CONH and 1637 cyclic CONH str; ^{1}H NMR ($CDCl_3$) : δ(ppm) 9.7 (s,1H,NH), 7.2-8.3 (m,12H,ArH), 5.0 (s,1H,NH), 1.6 (s,2H,CH_2); Mass: 438 (M^+) 21.34%; C H N Analysis: Calcd: C 60.27, H 3.65, N 12.78, Found: C 60.22, H 3.66, N 12.82.

All other compounds in Table-1 were synthesized following the similar procedure with various substituted acetamides.

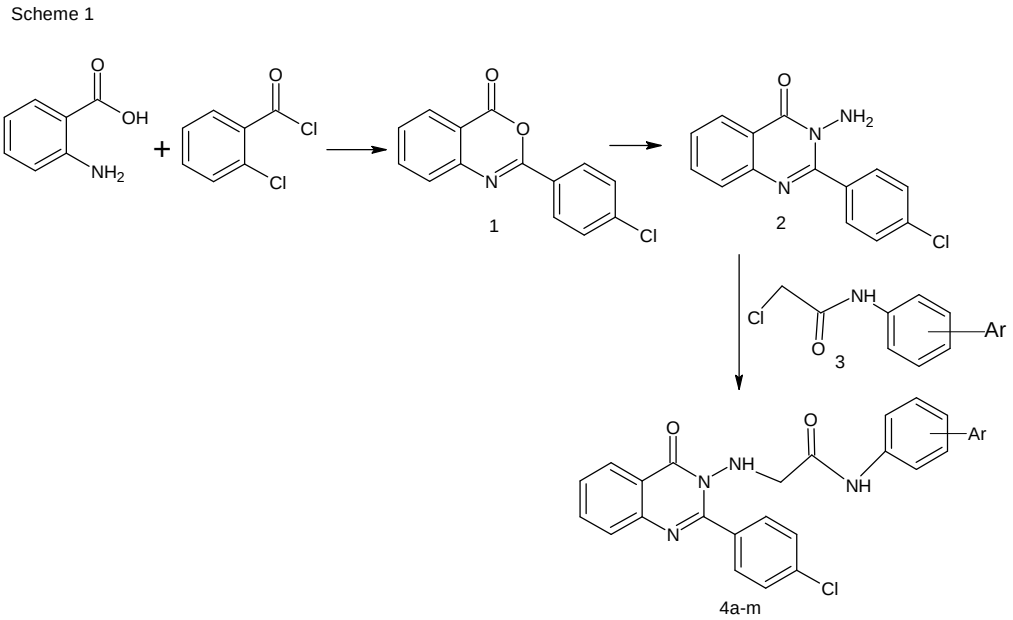

Figure 1: Scheme of synthesis

Antibacterial Activity

The antibacterial activity of the test compounds 4a-m against *S.aureus* (NCIM 2079) was determined by agar disc diffusion method[9]. The minimum inhibitory concentration (*MIC*) was determined by the serial dilution technique using dimethyl sulphoxide (DMSO) as solvent. DMSO was used as a negative control. Ciprofloxacin was used as the standard drug and the result of the antibacterial activity is presented in **Table 1**.

It was found that compounds 4a,4e and 4g showed profound antibacterial activity against *S.aureus* while the compounds 4i and 4j showed least activity and all other compounds showed moderate activity.

Molecular Docking Studies

The antimicrobial potency of all the newly synthesized compounds was further subjected for docking studies to explore the binding pattern against Sortase A of *Staphylcoccus aureus*. PDB entry code 1T2W with 1.80 Å resolution retrieved from Brookhaven Protein Data Bank.

The ligand structures were prepared in JME, the *pdb* files were obtained from PRODRG and converted to *pdbqt* by using ADT. The protein 1T2W was obtained from PDB site as *pdb* file, water molecules were removed along with the co-crystallised ligand molecule. The file was further converted to *.pdbqt* through Python Molecular Viewer program. All computations were performed on Linux operating system using autodock 4.2.5. All files were imported to autodock program and minimized by adding Kollmann charges and setting the charge residues on the active site to be flexible. The multi conformation library of all the 13 ligands was generated by exploring the torsional space of the ligands. A grid box was generated with three dimensional grid, 70 ºA grid size (x,y,z) with a spacing of 0.375 ºA. Then automated docking studies were carried out using Autodock 4.2.5 version. Among the various search algorithms the Lamarckian genetic algorithm was chosen to search the best conformers and the parameters were set using the software ADT (Autodock Tool Kit). For all docking parameters, default values were used with 10 independent docking runs for each docking study. The docking results are shown in Table 2. The docked complexes of the designed compounds along with the ligand receptor poses have been shown in the **Figure 2**.

The final evaluation is done with docking score and single best pose is generated as the output for particular ligand.
Gscore= vdw+Hbond+desol energy+electrostatic energy
where, vdW: Van der Waal energy; Hbond : hydrogen bond, desol energy: desolvation energy

Docking Results

The compounds 4a-m were docked on to the active site of 1T2W protein and the results are shown in Table. An identification of the binding pocket indicated that the compounds adopted a position in the protein in such a way that a hydrogen bond is generated between the ligand and protein. It was found that the acetamido NH group has more interation towards GLU 204 of the protein. All the compounds have shown similar type of hydrogen bond interaction; however the compound 4e with good binding energy and RMSD interacted with THR 203 and LYS 177 through NH and CO of acetamido group.

The residues including Glu 77, Glu 204, Lys 206, Lys 177, Thr 203, Asp 80 Tyr 143 and Val 205 were found to be in the active site of receptor, responsible for hydrogen bond interactions and Glu 204 was the hotspot residue showing significant hydrogen bond within the binding pocket for most of the compounds. The docking result is shown in **Table 2.**

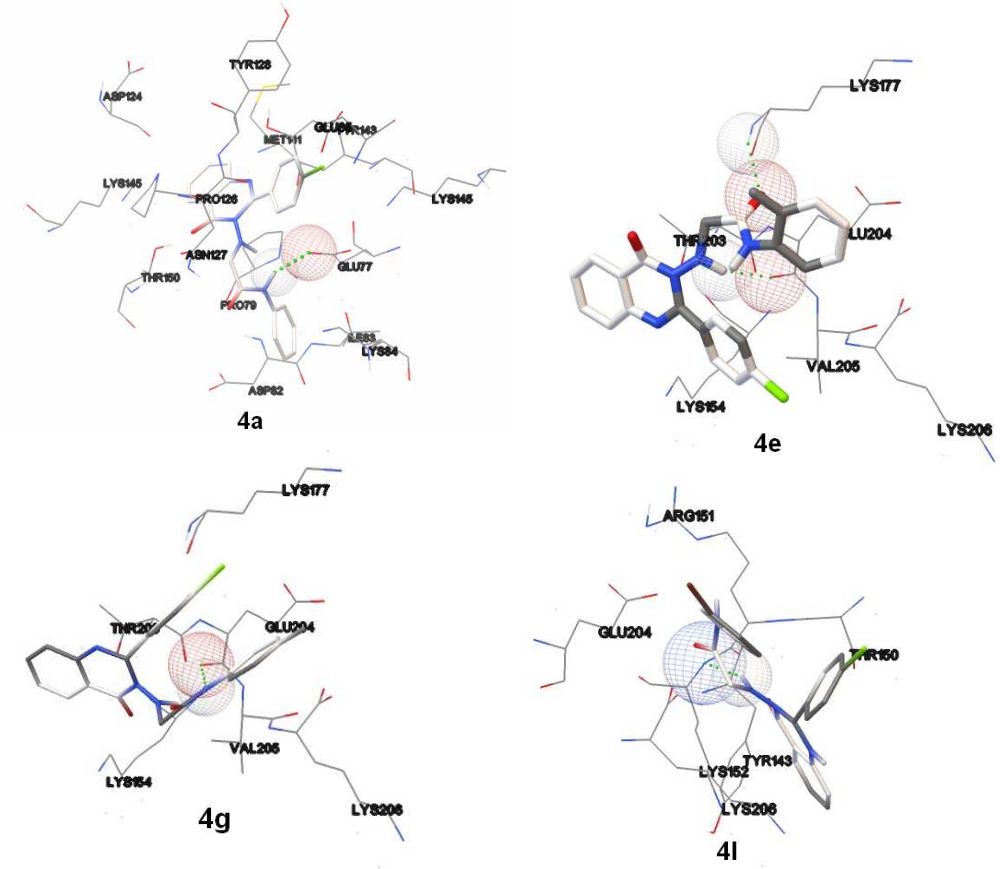

Figure 2: Molecular Docking Interaction

Table 2: The docking results based on the binding free energies () and inhibition constants (Ki) of compounds docked on 1T2W

Sl. no	Molecule Name	Binding Energy	kI	RMSD	Hydrogen bond Interaction	
					Aminoacid	Atom of compound
1.	R1	-10.66	15.41μM	28.49	GLU77	NH of acetamido group
2.	R2	-5.88	48.85μM	8.63	GLU204	NH of acetamido group
3.	R3	-5.77	59.06μM	8.86	GLU204	NH of acetamido group
4.	R4	-5.79	57.19μM	10.38	------	No interaction
5.	R5	-6.25	26.13μM	7.45	THR203 LYS177	NH and CO of acetamido group
6.	R6	-6.05	36.7μM	9.75	-----	No interaction
7.	R7	-5.21	150.56μM	7.36	GLU204	NH of acetamido group
8.	R8	-5.43	103.85μM	8.56	-------	No interaction
9.	R9	-4.9	254.77μM	8.35	-------	No interaction
10	R10	-4.88	263.38μM	8.56	ASP180	NH of acetamido group
11.	R11	-5.61	77.5μM	11.39	-------	No interaction
12.	R12	-6.04	37.41μM	10.53	THR143	NH of acetamido group
13.	R13	-5.23	147.24μM	10.56	VAL205 LYS177	NH of acetamido group

CONCLUSION

A set of thirteen new quinazolinone derivatives were synthesized and characterized by IR, ^{1}HNMR, Mass and elemental analysis. All the newly synthesized compounds were tested for antibacterial activity against *S.aureus* by agar disc diffusion method. Among the screened samples, the compounds 4a, 4e and 4g emerged as active against the microorganism compared to the standard drug.

Finally the molecular docking studies of the synthesized compounds were carried out and the results of such studies were reported. *In silico* studies revealed that all the synthesized compounds 4a-m have relatively lesser binding energy as compared to the standard drug and may be considered as a inhibitor of 1T2W. Hence, the reported molecules shall further be taken for further exploitation for designing of new antibacterial agents with quinazolinone moiety.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper. Also, we declare that this paper or part of it has not been published elsewhere in any journal.

CONTRIBUTION OF THE AUTHORS

Dr GopalKrishna Rao designed the scheme for synthesis of molecules. Dr S Rajasekaran collected data, synthesized the molecules, carried out docking studies. Dr GopalKrishna Rao analysed the data and drafted and revised the paper. Dr S Rajasekaran wrote the whole paper and revised the article. Both the authors read and approved the final version that is sent for publication.

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