EXPLORING THE PHARMACOLOGICAL POTENTIAL OF QUINAZOLINONE DERIVATIVES: ANTIBACTERIAL EFFICACY

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INTRODUCTION

In the realm of modern medicine and drug discovery, the pursuit of novel pharmacological agents to combat infectious diseases remains an ever-evolving and crucial endeavor. As humanity grapples with the relentless adaptability of bacterial pathogens and the growing concern of antibiotic resistance, the search for innovative therapeutic compounds has never been more urgent. In this context, quinazolinone derivatives have emerged as a promising class of compounds with the potential to revolutionize antibacterial drug development.

The rise of antibiotic-resistant bacterial strains poses a significant global health threat, leading to increased mortality rates and healthcare costs. Traditional antibiotics, once hailed as miracle drugs, are becoming less effective against a growing number of pathogens. This alarming trend necessitates a paradigm shift in the approach to antibacterial drug discovery. Quinazolinones, with their diverse chemical structures and unique pharmacological properties, have recently garnered substantial attention as a potential solution to this crisis[1].

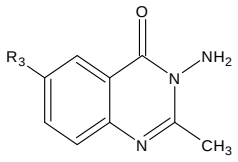
The quinazolinone scaffold is a versatile chemical framework that has been explored in the context of various therapeutic areas, including anticancer, anti-inflammatory, and central nervous system disorders. However, it is their promising antibacterial efficacy that has ignited particular interest among researchers and pharmaceutical developers. Quinazolinone derivatives, through systematic structural modifications and optimization, offer a versatile platform to design and synthesize compounds with potent antibacterial activity. Their mechanisms of action, interactions with bacterial targets, and potential synergy with existing antibiotics present exciting avenues for exploration.

This paper embarks on a comprehensive exploration of the pharmacological potential of quinazolinone derivatives as antibacterial agents. We delve into the synthesis, characterization, and evaluation of these compounds, shedding light on their antibacterial efficacy against a range of clinically relevant bacterial strains. By examining the molecular mechanisms underpinning their antibacterial activity and assessing their potential for synergy with existing antibiotics, we aim to contribute to the development of novel therapeutic strategies to combat antibiotic-resistant infections[2].

In the subsequent sections, we will delve into the synthesis and structural diversity of quinazolinone derivatives, discuss their mechanisms of action, present experimental findings on their antibacterial activity, and explore the implications of our research for the future of antibacterial drug discovery. Through this exploration, we hope to emphasize the significance of quinazolinone derivatives as a promising avenue in the ongoing battle against bacterial infections, ultimately paving the way for innovative therapeutic solutions to safeguard public health.

Therefore, a strategy was employed to synthesize Quinazolinone derivatives on the laboratory scale and evaluate their antibacterial efficiency. These compounds were synthesized as per the chemical reactions of **Schemes I (Table 1).**

Table 1: Synthetic schemes for the synthesis of Quinazolinone derivatives

Scheme I	Synthesis of 4(3*H*)-Quinazolinone derivatives from 2-amino benzoic acid (R_3= NO_2, Br, OCH_3) 

EXPERIMENTAL SECTION

Various substituted 2-amino benzoic acid, hydrazine hydrate, ethanol, chloroform was purchased from commercial suppliers like Loba Chemie PVT. LTD, S. D. Fine chemicals, and Sigma-Aldrich. All chemicals and reagents were used of an analytical grade for the current study.

1.1 Molecular Docking study[3]

Design of Quinazolinone compounds

The Quinazolinone compounds were designed using Molecular modeling software Autodock 4.0 and docking analysis by Discovery Studio Visualizer. The design of molecules was done using the following steps:

I. Preparation of protein structures

Protein preparation involves the following steps:

- The DNA gyrase enzyme was selected and downloaded from the Research Collaboratory for Structural Bioinformatics Protein data bank (RCSB's PDB).
- The downloaded enzyme was refined.

Discovery Studio Analyzer was used to refine the enzyme docking with ligands. Further, water molecules were deleted, hydrogen bonds were added wherever required and unnecessary hetero groups were deleted from the enzyme 4WUC and structure was saved in .pdb format. Finally, the structure of enzyme 4WUC (Fig. 1) was refined by optimizing the orientations of H-bonded groups and minimizing the structure's energy.

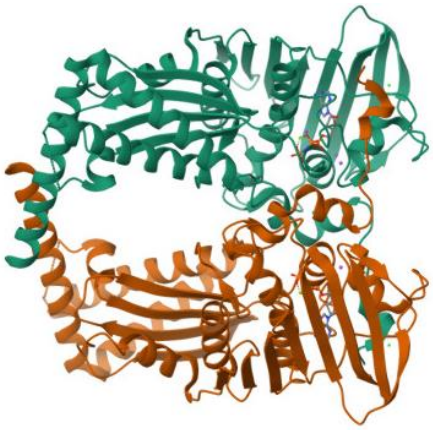


Fig 1. Structure of enzyme 4WUC

II. Energy minimization of all 3D structures of proteins

CHIMERA 1.6.1 program was used to minimize all 3D structures of protein. *Preparation of ligand structures*

Molecules of interest from the classes of Quinazolinone were built using Marvin sketch. These built molecules were converted to 3D structures using the program *LigPrep* and then optimized using the OPLS-3 force field.

Validation of docking protocol

Autodock used interaction maps for docking. The program auto grid computes these maps before the docking process itself. The interaction energy between each kind of ligand atom and the

receptor was computed for the entire binding site, which was discretized using a grid for each ligand atom type. With a grid box size of 60 by 60 by 60 and grid points spaced 0.375 inches apart,

Molecular docking

All AutoDock docking operations were carried out using a 2.20 GHz IBM Centrino Core2Duo CPU and 2 GB of DDR2 RAM. The Microsoft Windows XP operating system was used to compile and execute AutoDock 4.0. The ligand-receptor complexes were examined using the Discovery studio analyzer program after docking.

The Ligand Poses procedure determined the RMSD value, hydrogen bond created between the receptor and the docked conformation, and close contact (van der Waals collision). All ligand conformations in the molecule window were displayed from the input ligand box. Every hydrogen bond parameter was calculated from the input receptor box. In the docking process, optimized ligands derivatives of 4(3H)-Quinazolinone were docked in the binding pocket of enzyme, 4WUC.

Results of molecular docking

A total of 3 docked ligands from the derivatives of 4 (3H)-Quinazolinone displayed good binding energy, H- bonding, and pi interactions (Table 1)

The evaluation of docking results was done based on the following parameters.

I. **Binding energy:** The compounds from the series A1-A3 showed binding energy from -7 to -8 kcal/mol. Thus, the compounds exhibited good interaction with the enzyme 4WUC in silico.

II. **Number of H-bonds:** Compounds showed formation of 1to 3 hydrogen bonds with various amino acid like Leu115, Asn46, His116, Gly102, Lys103, of the enzyme 4WUC (Fig. 2).

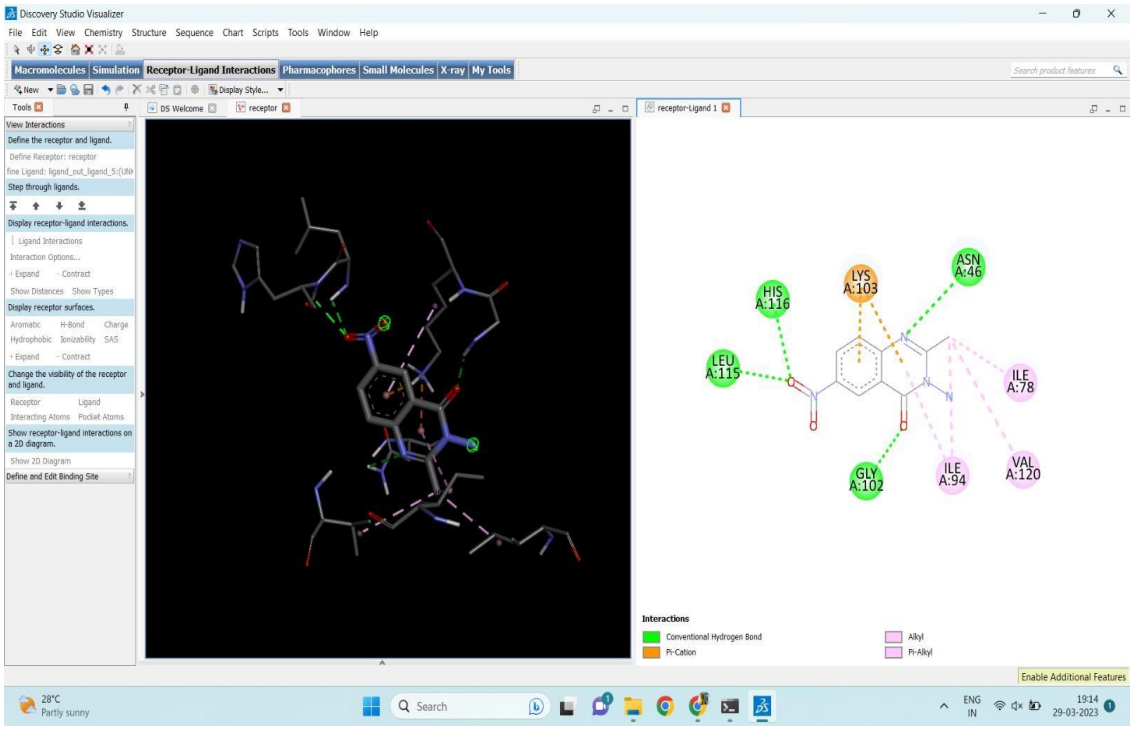


Fig 2. Interaction of compound with Hydrogen bonds

Table 1 Binding energy, important amino acids of the enzyme involved in binding with the compounds A1-A3

Compound	**Estimated free energy of binding (kcal/mol)**	**Hydrogen bond**
A1.	-7.8	His116, Asn46, Leu115, Gly102
A2.	-8.3	Asn46, Gly103, Lys103, Gly117
A3.	-7.0	Gly117, Gly102

Molecular properties and drug-likeness of synthesized compounds

Molsoft's ICM suite was used to calculate and predict parameters such as logP, HBA, HBD, CNS, Polar surface area and drug-likeness of small molecules. These tools include the calculation of

various drug-likeness filters and rules based on Lipinski's Rule of Five and other guidelines (Table 2).

Table 2 Molecular properties and drug-likeness of synthesized compounds

Sr. No	Compound code	HBA	HBD	Log P	MolPSA (A^2)	CNS	Drug Likeness score
1.	A1	4	2	0.51	48.71	3.06	0.41
2.	A2	5	2	0.63	79.42	2.66	0.26
3.	A3	3	2	1.33	41.16	3.98	0.06

1.2 Synthesis and qualitative analysis of designed EGFR TK inhibitors

A. Synthesis of designed Quinazolinone derivatives

Quinazolinone derivatives were synthesized on the laboratory scale. These compounds were synthesized as per the chemical reactions shown in **Schemes I.**

Scheme I: Synthesis of 4(3*H*)-Quinazolinone derivatives from 2-amino benzoic acid (R= NO2, Br, OCH3) (Compounds A1-A3)

The synthesis of compounds A1-A3 is divided into 2 steps:

Step 1: Synthesis of 2-methyl-3, 1 benzoxazine-4-one

The solution of 2-aminobenzoic acid: ($R=NO_2$) 5-nitro-2- aminobenzoic acid/ (R=Br) 5-bromo-2-amino benzoic acid/ ($R=OCH_3$) 5-methoxy-2-amino benzoic acid (0.1 mol) and acetic anhydride (0.2 mo1) was refluxed for 4-6 hours in anhydrous conditions. TLC analyzed product formation. The excess acetic anhydride was distilled off and cooled to room temperature to obtain a pale-yellow crude product which was purified in methanol.

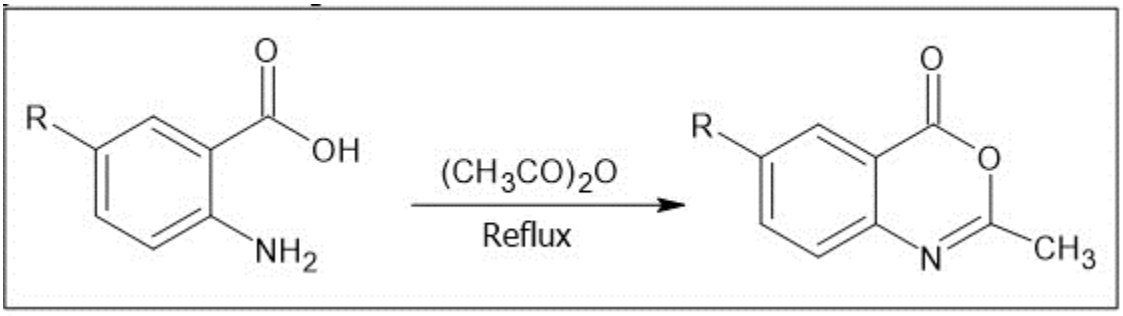


Fig 3: Synthesis of 2-methyl-3, 1 benzoxazine-4-one.

Step 2: Synthesis of substituted 4(3H)-Quinazolinone

Substituted Benzoxazine (0.15 mol) and excess hydrazine hydrate were mixed and refluxed for 2 hours in the presence of ethanol, and TLC monitored the reaction progress (Chloroform: Methanol – 9.5:0.5) collected substituted Quinazolin-4-one as crystal. The purification was carried out by recrystallization.

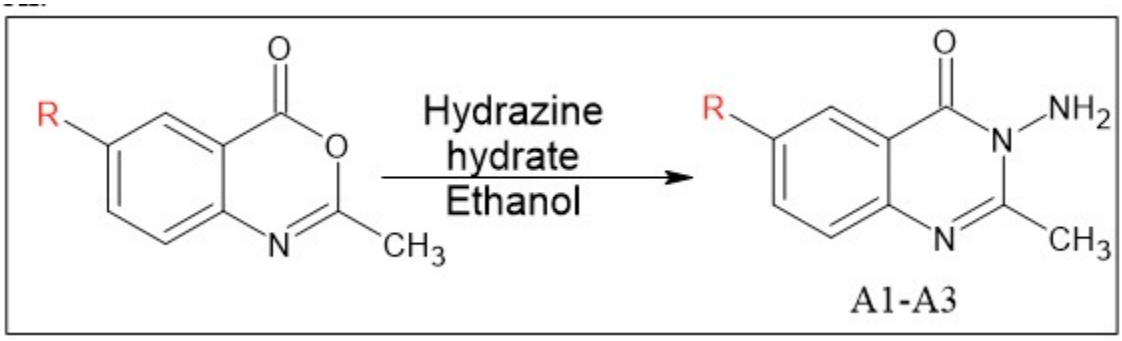
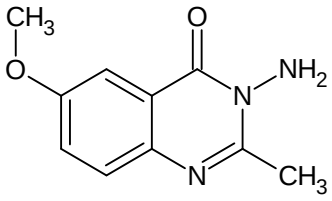
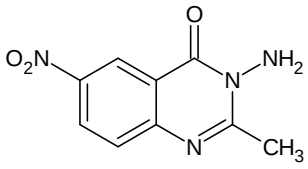
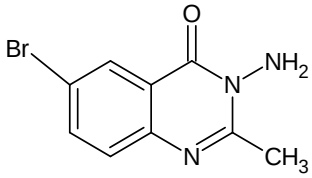


Fig.4: Synthesis of compounds A1-A3.

The synthesized compound indicated off-white to yellowish and dark brown, single spot-on TLC, percent yield=70- 80 % with melting points (M.P) = 190-230°C.

Table 3: Physical properties of compounds A1-A3

S. No.	R	Name	Structure	Melting point (0^0c)	Mobile phase	Rf value
1.	OCH_3	3-amino-6-methoxy-2-methylquinazoline-4(3H)-		116	Hexane: Ethyl acetate (3:2)	0.71

		one				
2.	NO_2	3-amino-6-nitro-2-methylquinazoline-4(3H)-one		220-225	Hexane: Ethyl acetate (3:2)	0.68
3.	Br	3-amino-6-bromo-2-methylquinazoline-4(3H)-one		189	Hexane: Ethyl acetate (3:2)	0.72

B. Qualitative analysis of the synthesized compound

Structures of synthesized compounds A1-A3 were confirmed by spectral analysis FTIR spectra were recorded on a Spectrophotometer (Shimadzu IRAffinity-1S) from SVKMs Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle, Mumbai.

Spectral characteristics data of synthesized compounds A1-A3 are depicted in Table 6

Table 4: Spectral characteristics data of compounds A1-A3

S.No.	Compound Code	IR range
1.	A1	Ar-CH – 3188.39, CH in CH_3 – 2973, C=O – 1658.81 N-H – 1374, Ar N-H – 3333.05
2.	A2	Ar-CH – 3188.39, CH in CH3 – 2973, C=O – 1658.81 C=C – 1436.99, N-H – 1374, N-O – 1513, ArN-H – 3333.05
3.	A3	Ar-CH – 3187.42, CH in CH3 – 2972.36, C=O – 1795.76 C=C – 1440.85, N-H – 1374.30, ArN-H – 3333.05

1.3 Evaluation of Antimicrobial Activity

Spread plate technique was performed for determination of antimicrobial characters of compounds

1000 µg/mL - stock solution was used for performing this activity.

Antimicrobial properties of derivatives were determined on different gram positives such as S. aureus and B. subtilis and gram negatives such as E. coli and S. typhi

The evaluation was done by taking standard drugs ciprofloxacin and gentamycin. Activity of derivatives were compared against percent activity against the standards.

I. E. coli (Gram Negative)

Table.5: Zone of Inhibition against E.coli in mm and percentage activity against standard

Derivative	Zone of inhibition	% activity against Ciprofloxacin	%activity against Gentamycin
A	25mm	92.50%	104%
B	27mm	100%	112%
C	23mm	85.18%	95.80%
Ciprofloxacin	27mm		
Gentamycin	24mm		

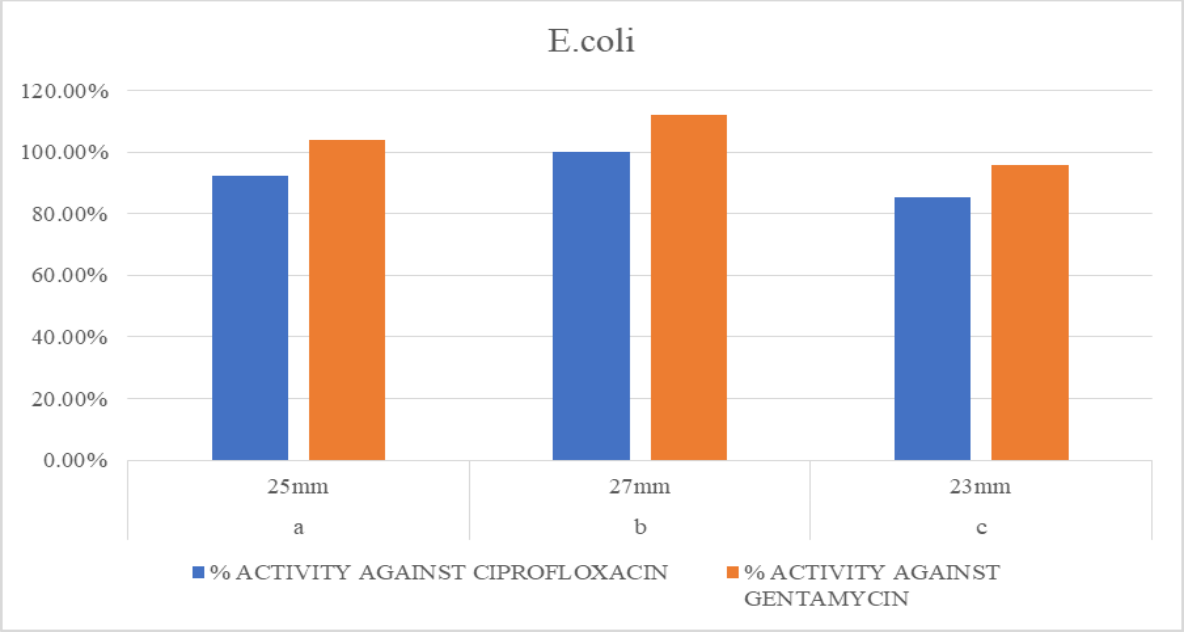


Fig.5: Graph of percentage activity against standards (E.coli)

II. S.typhi (Gram negative)

Table.6: Zone of Inhibition against S.typhi in mm and percentage activity against standard

Derivative	Zone of inhibition	% activity against Ciprofloxacin	%activity against Gentamycin
A	20mm	80%	95.00%
B	22mm	88%	104.00%
C	21mm	84.00%	100%
Ciprofloxacin	25mm		
Gentamycin	21mm		

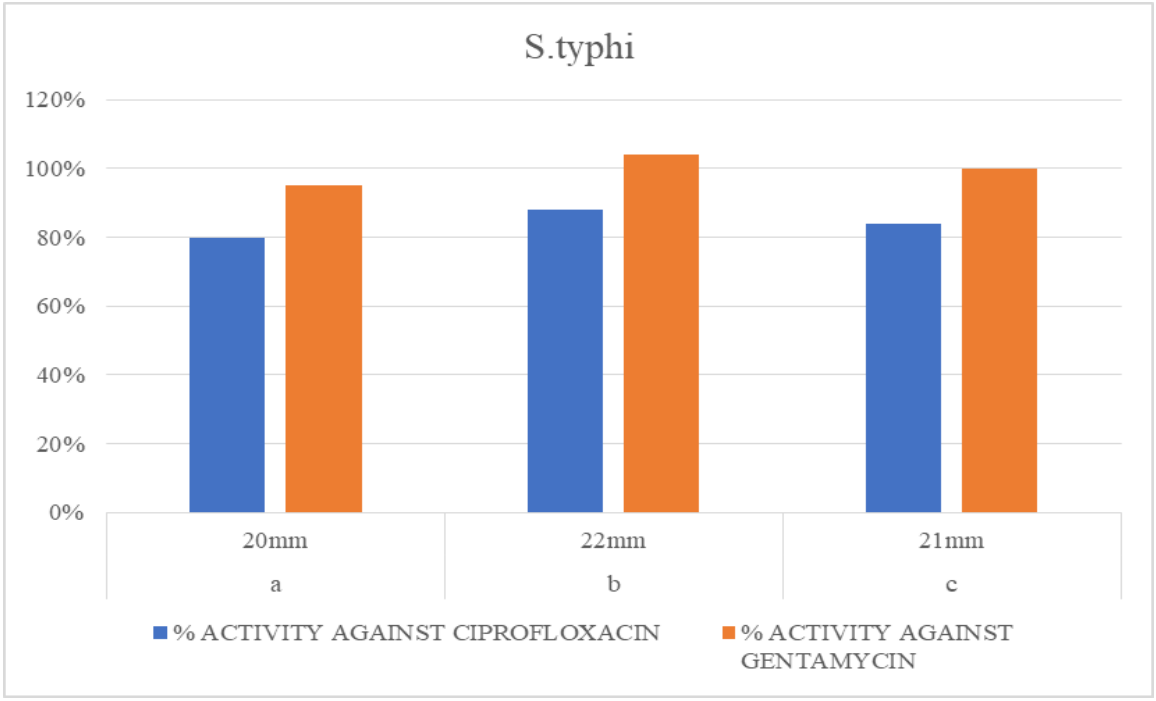


Fig.6: Graph of percentage activity against standards (S.typhi)

III. S.aureus (Gram Positive)

Table.7: Zone of Inhibition against S.typhi in mm and percentage activity against standard

Derivative	Zone of inhibition	% activity against Ciprofloxacin	%activity against Gentamycin
A	24mm	92%	100%
B	25mm	96%	104%
C	23mm	88.00%	95.80%
Ciprofloxacin	26mm		
Gentamycin	24mm		

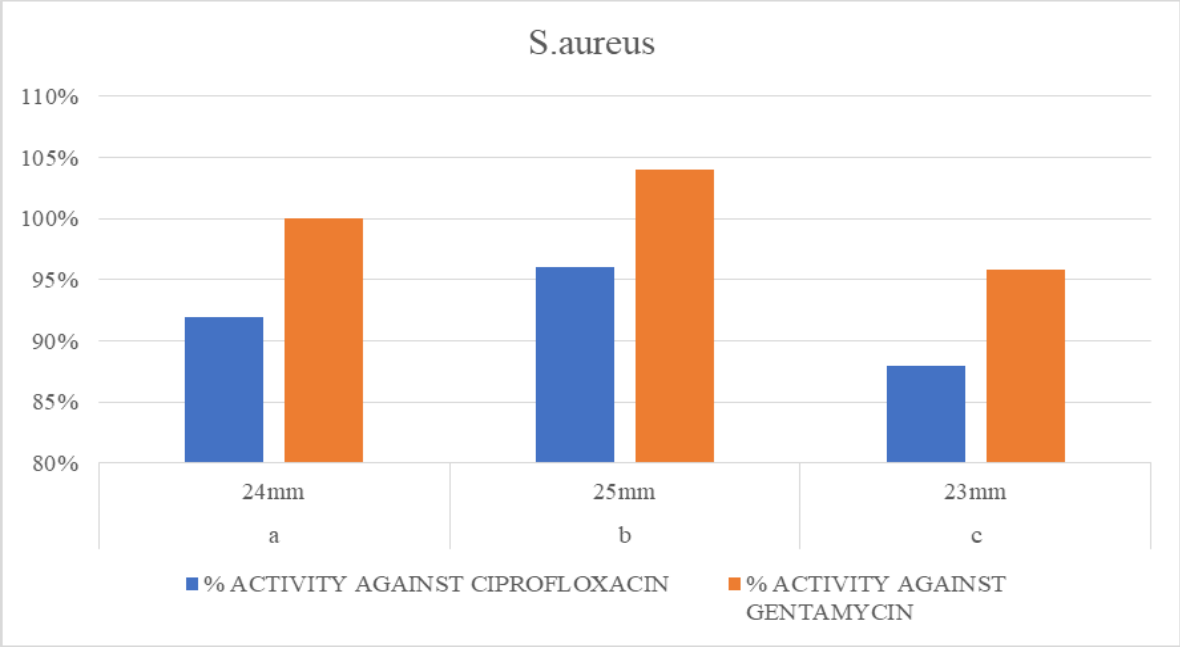


Fig.7: Graph of percentage activity against standards (S.aureus)

IV. B. subtilis (Gram Positive)

Table.8: Zone of Inhibition against S.typhi in mm

Derivative	Zone of inhibition	% activity against	% activity against

		Ciprofloxacin	Gentamycin
A	21mm	91%	87.50%
B	23mm	100%	95.80%
C	20mm	86.90%	83%
Ciprofloxacin	23mm		
Gentamycin	24mm		

***P value is less than 0.05, it is statistically significant**

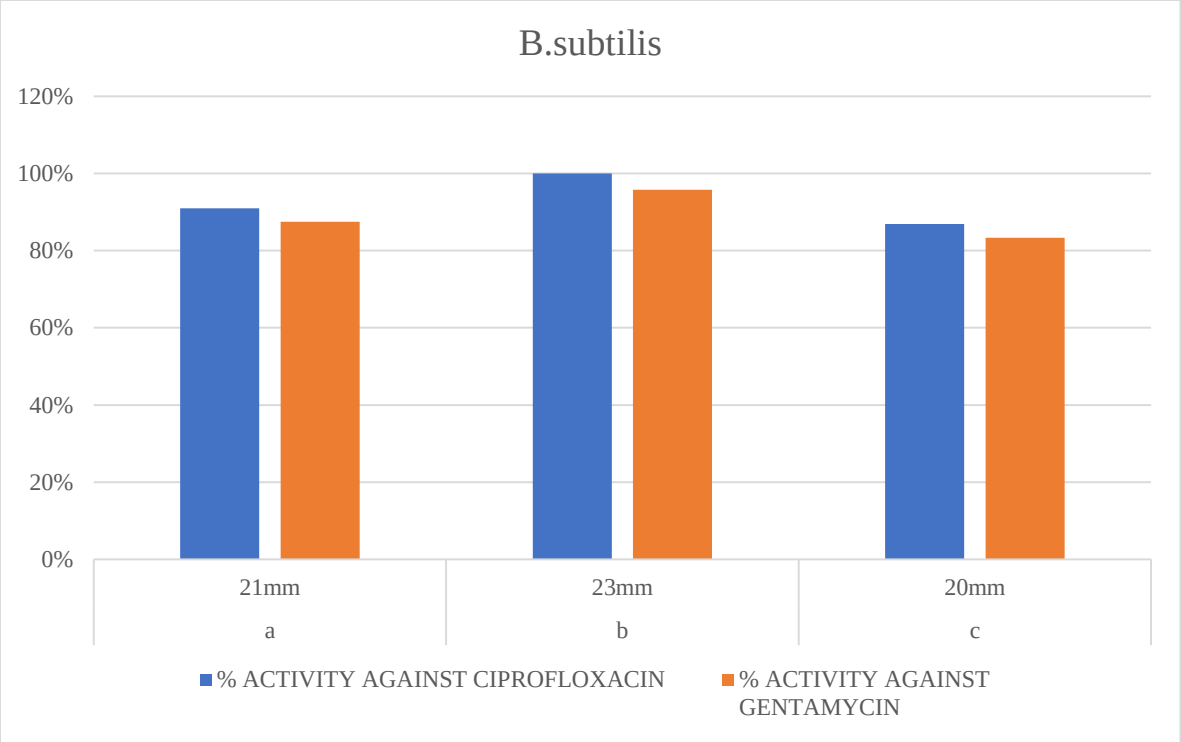


Fig.8: Graph of percentage activity against standards (B.subtilis)

Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that is bacteriostatic (prevents the visible growth of bacteria). MICs are used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentrations of drug over a defined period in terms of inhibition of microbial population growth.

Minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions.

Table.9: MIC and MBC of derivatives against organisms

Sr.No.	Organisms	Gram Stain	MIC (µg/ml)	MBC (µg/ml)
1	E.coli	Negative	175	250
2	S.typhi	Negative	175	250
3	S.aureus	Positive	175	250
4	B subtilis	Positive	175	250

Quinazolinone derivatives have shown promising antimicrobial activities against various microorganisms, including bacteria such as E.coli, S.typhi, B.subtilis and S.aureus. The synthesised compounds were tested in against gram-positive and gram-negative bacteria and antimicrobial activity of the compounds were tested in the study. Calculated MIC which is 175ug by using zone of inhibition and concentration of derivatives. Antimicrobial studies have shown that nitro substituted quinazolinone derivatives have potent antibacterial making them promising candidates for the development of new antimicrobial drugs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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