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SYNTHESIS CHARACTERIZATION AND ANTIBACTERIAL ACTION OF FEW QUINAZOLINONE DERIVATIVES

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ABSTRACT: The objective of the present investigation was to develop newer antibacterial molecules based on quinazolinone scaffold. It was accomplished by preparing schiffs base of a quinazolinone scaffold. The synthesized compounds presented anti bacterial activity comparable to that of the standard drug norfloxacin. The compound QS3 was able to reduce the growth of both gram negative and gram positive bacteria in low concentration. The study lead to the conclusion that lead molecules could be designed from quinazolinone nucleus by utilizing computer aided methods.

IndexTerms **– Docking, Quinazolinone, Antibacterial activity**

INTRODUCTION

Quinazolinone and their derivatives are building block for approximately 150 naturally occurring alkaloids isolated from a number of families of the plant kingdom, from microorganisms and animals[1-4]. In light of the growing number of applications in recent years, there has been an enormous increase in the interest among biologists and chemists in their synthesis and bioactivity of quinazolinone derivatives. Compounds containing 4(3H)-quinazolinone ring system have showed antitumor, anticonvulsant, antitubercular activities, anti-inflammatory, analgesic, antimicrobial and anticoccidal activities[5-9]. Quinazolinones have been frequently used in medicine[10-12], such as quinethazone and metolazone and are used in medicine as diuretics while prazosin is a vasodilator, which is also used as an antihypertensive drug. Quinazolinones are also a class of drugs which function as hypnotic/sedatives that contain a 4-quiazolinone core. Their use has also been proposed in the treatment of cancer[13].

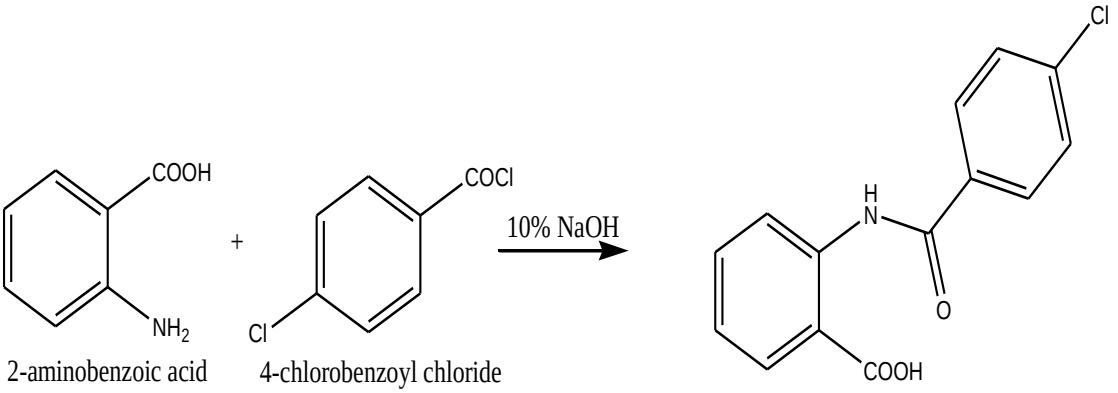
RESEARCH ENVISAGED

Antibiotic (antibacterial) resistance is a serious global problem and the need for new treatments is urgent. The current antibiotic discovery model is not delivering new agents at a rate that is sufficient to combat present levels of antibiotic resistance. Heterocyclic compounds have been widely investigated for their antimicrobial potential. Quinazolinones, pyrimidines, pyridines, quionolines etc have been the center point of designing of antimicrobial compounds. The synthesis of this newer quinazolinone derivatives is expected to provide a novel structural modification effective as antimicrobial molecule which may be converted to lead compound for managing even the antibiotic resistant infections.

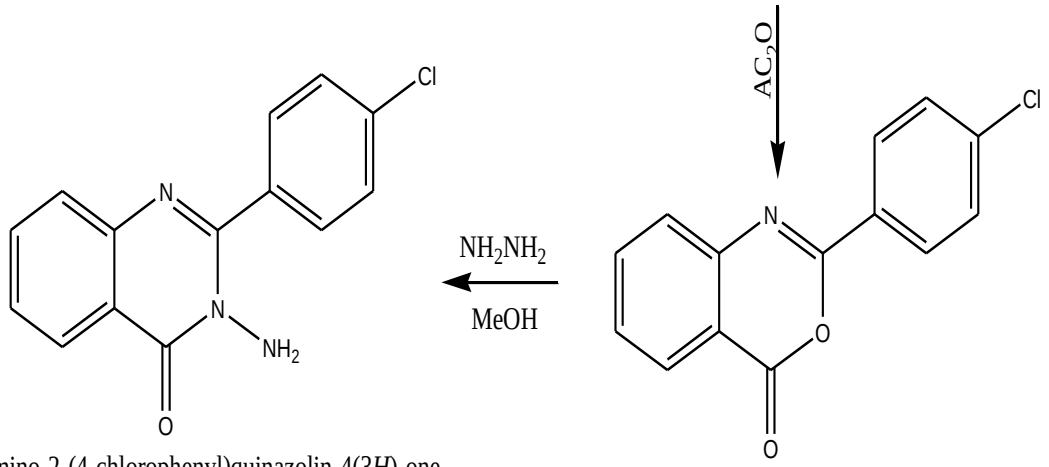
EXPERIMENTAL :- The scheme for the synthesis of the quinazolinone derivatives was adapted from the procedures reported by Rahman et al[21], Dash et al[22] and Hussain et al[34] utilizing the scheme as depicted in Figure 4.1.

Materials :- Anthranilic acid, ethanol, hydrazine hydrate, sodium hydroxide, chloro benzoyl chloride, glacial acetic acid and various aromatic aldehyde were procured from Oxford Fine Chemicals LLP, and were used as obtained without any further purification or treatment. All other chemicals used in the study were of laboratory grade.

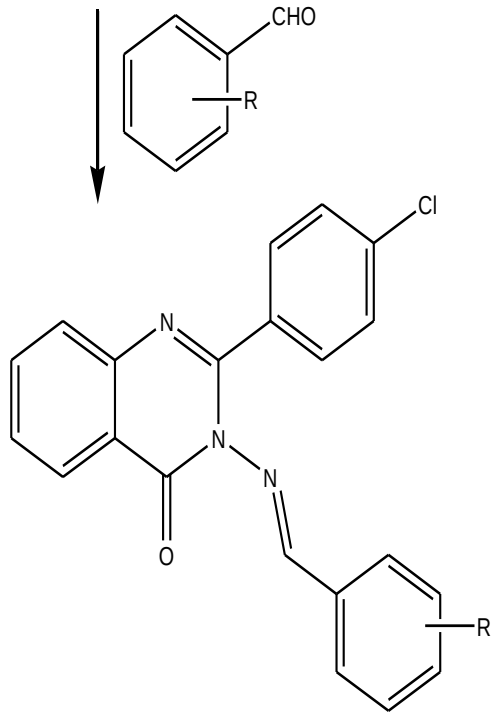
Synthetic Procedure The entire scheme comprises of 4 steps leading to the formation of the titlecompounds.



2-aminobenzoic acid 4-chlorobenzoyl chloride 2-(4-chlorobenzamido)benzoic acid



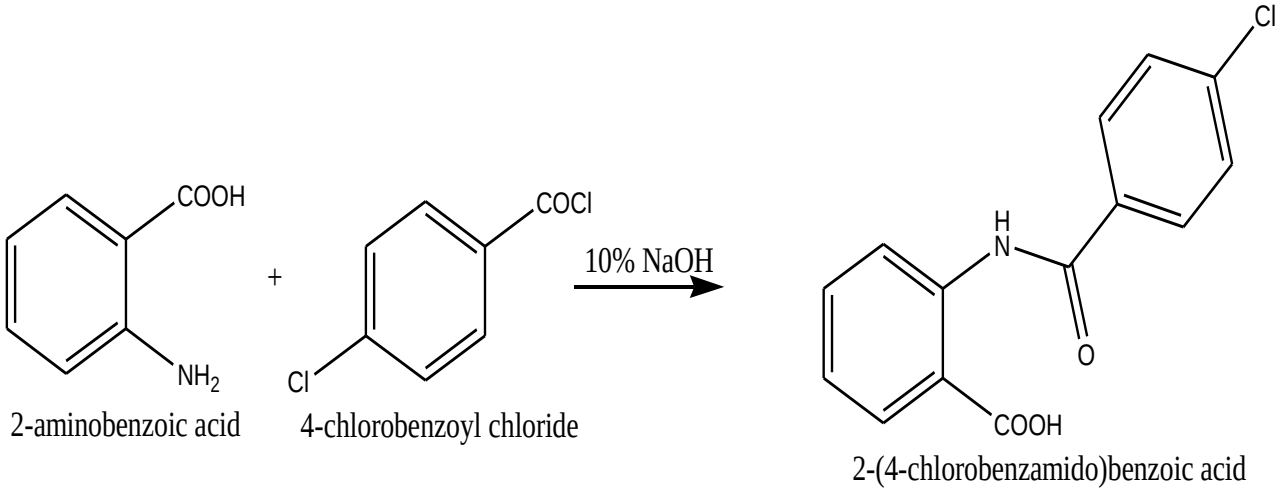
3-amino-2-(4-chlorophenyl)quinazolin-4(3*H*)-one 2-(4-chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one



(*E*)-3-(benzylideneamino)-2-(4-chlorophenyl)quinazolin-4(3*H*)-one

Synthesis of 2-(4-chlorobenzamido)benzoic acid

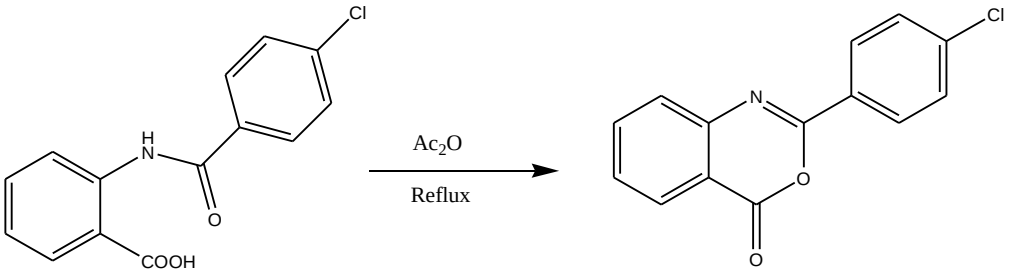
To the anthranilic acid (2 mmol) dissolved in 10% sodium hydroxide (10 mL), chloro benzoyl chloride (2.2 mmol) was added with stirring at room temperature for over 1 h. Upon completion, reaction mixture was quenched with cold water to obtain solid residue, which was washed with dilute HCl followed by water and recrystallized from ethanol.



2-aminobenzoic acid 4-chlorobenzoyl chloride 2-(4-chlorobenzamido)benzoic acid

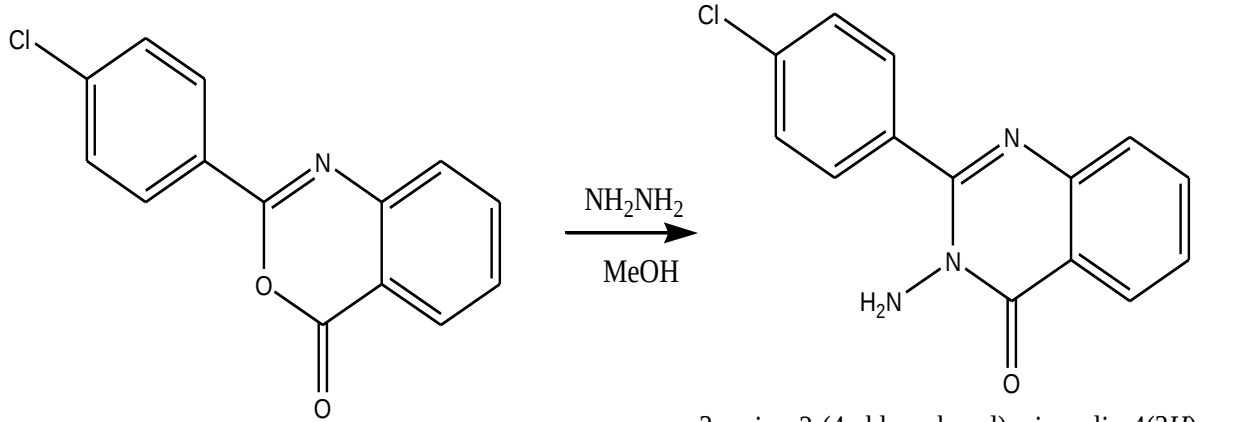
Synthesis of 2-(4-chlorophenyl)-4H-benzo[d][1,3]oxazin-4-one

A solution of 2-(4-chlorobenzamido)benzoic acid (2 mmol) in acetic anhydride (10 mL) was heated under reflux for 2 h and then poured into crushed ice. The solid residue thus obtained was filtered, dried, and recrystallized with ethanol.



2-(4-chlorobenzamido)benzoic acid 2-(4-chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one

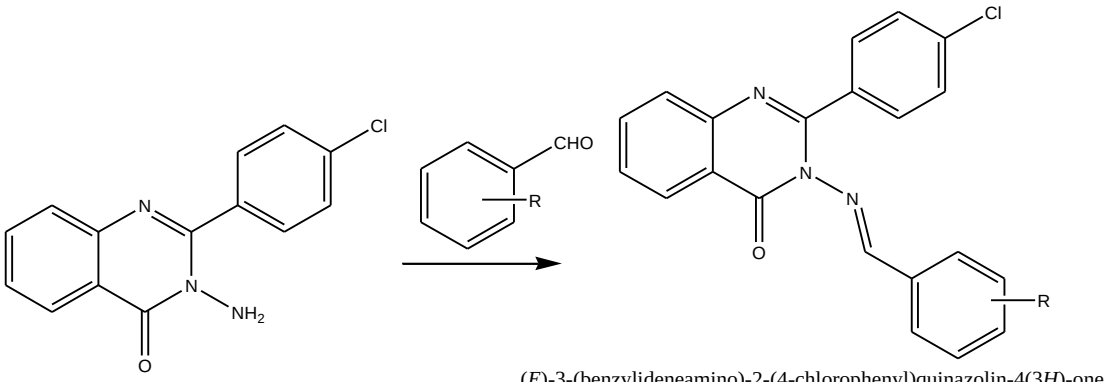
Synthesis of 3-amino-2-(4-chlorophenyl)quinazolin-4(3H)-oneA mixture of oxazine (2 mmol) and hydrazine hydrate (2 mmol) in glacial acetic acid was heated under reflux for 3 h. The completion of reaction was monitored by TLC. On cooling a solid separated that was collected by filtration, washed with water, dried, and recrystallized from ethanol



2-(4-chlorophenyl)-4*H*-benzo[*d*][1,3]oxazin-4-one 3-amino-2-(4-chlorophenyl)quinazolin-4(3*H*)-one

General procedure for synthesis of Quinazolinone based schiffs base

10 mmol of 3-amino-2-(4-chlorophenyl)quinazolin-4(3H)-one was dissolved in 25 ml of ethanol and to it was added 10 mmol of appropriate aromatic aldehyde. The mixture was stirred for 2-3h for the completion of reaction, as monitored by TLC and was evaporated under reduced pressure. The product obtained was filtered off and recrystallized from ethanol/acetone[34]



3-amino-2-(4-chlorophenyl)quinazolin-4(3*H*)-one

(*E*)-3-(benzylideneamino)-2-(4-chlorophenyl)quinazolin-4(3*H*)-one

Evaluation of antibacterial action35[35]

The antibacterial action of the synthesized compounds was evaluated against two gram positive (*Proteus mirabilis* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and Staphylococcus).

Preparation of test solutions

The synthesized derivatives (**QS1-QS5**) were dissolved in dimethyl sulfoxide (DMSO) and the further dilutions of the test compounds were prepared at the required quantities of 1000 µg/mL concentrations with Mueller-Hinton broth medium.

Preparation of Inoculum

Overnight culture of all four bacteria were prepared separately in nutrient broth, and used as a microbial source for the determination of MIC.

Determination of MIC

1. The sterile test tubes were numbered from 1 to 7 and all of the steps were carried out using aseptic technique.

2. 2.0 ml of nutrient broth was added to all the tubes.

3. 100 µl of drug sample was transferred from the first tube to the second tube.

4. Using a separate pipette, mixed the contents of this tube and transferred 100 µl to the third tube.

5. Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette.

6. 100 µl from tube 7 was pipetted out and discarded. The 8th tube, which serves as a control, receives no drug sample (only broth).

7. Norfloxacin (10µg/ml) was used as standard drug and diluted as per above procedure.

8. Suspended to an appropriate turbidity several colonies of the culture to be tested in 5.0 ml of nutrient broth to give a slightly turbid suspension.

9. Added 0.2 µl of the diluted culture suspension to each of the tubes. The final concentration of drug sample is now one-half of the original concentration in each tube.

10. All tubes were at 37°Covernight.

The optical density (absorbance) of each bacterial broth dilution was measured at 640 nm using UV-Visible spectrophotometer[36]

RESULT AND DISCUSSION

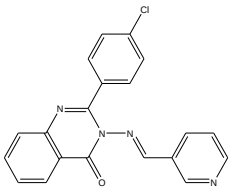
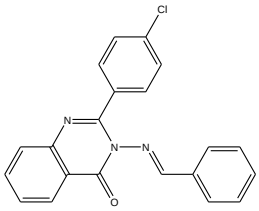
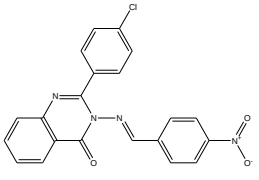
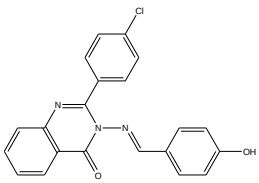
The synthesis of all the compounds was achieved using the scheme depicted in Figure 4.1. The results of characterization of the synthesized compounds are presented in this chapter.

RESULTS

Chemical Characterization

The synthesized compounds (**QS1-5**) were subjected to determination of yield, melting point, solubility and structure elucidation. The yield and color of the compounds are presented in Table 1.

Table 1.Yield and color of synthesized compounds

Compound code	**Aldehyde Used**	**Structure**	**Yield (%)**	**Color**
QS1	Nicotinaldehyde		62	Yellow
QS 2	Benzaldehyde		73	Brownish Yellow
QS 3	4-nitrobenzaldehyde		69	Yellow
QS 4	4-hydroxybenzaldehye		67	Yellow

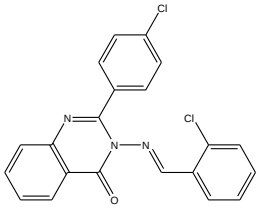
QS 5	2-chlorobenzaldehyde		61	Brown

Table 2. Physical Properties of the synthesized compounds

Compound code	Molecular Formula	Molecular Weight	R_f Value	Melting point (°C)
QS 1	$C_{20}H_{14}N_4O$	326.12	6.39	283-285
QS 2	$C_{21}H_{15}N_3O$	325.12	7.27	196-198
QS 3	$C_{21}H_{14}N_4O_3$	370.11	6.14	247-249
QS 4	$C_{21}H_{15}N_3O_2$	341.12	6.26	268-270
QS 5	$C_{21}H_{14}ClN_3O$	359.08	6.17	271-274

Antibacterial Action

The antibacterial activity of the synthesized compounds was determined by determining the MIC value by serial dilution method. The optical density of each broth was measured at 640 nm to determine the MIC value of the compounds. The results obtained are presented in Table 5.15-5.20.

Table 3. Optical density of QS1 against tested bacteria

S. No.	Concentration (µg/ml)	Optical Density at 640 nm			
		Proteus mirabilis	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
1	**100**	0.121	0.123	0.109	0.111

2	**50**	0.159	0.155	0.147	0.151
3	**25**	0.192	0.190	0.178	0.179
4	**12.5**	0.221	0.225	0.203	0.209
5	**6.25**	0.278	0.283	0.256	0.262
6	**3.125**	0.336	0.345	0.311	0.324
7	**1.5625**	0.396	0.399	0.354	0.361

Table 4. Optical density of Norfloxacin against tested bacteria

S. No.	**Concentration (µg/ml)**	**Optical Density at 640 nm**			
		Proteusmirabilis	*Bacillussubtilis*	*Pseudomonasaeruginosa*	*Staphylococcusaureus*
1	**10**	0.051	0.046	0.037	0.028
2	**5**	0.093	0.082	0.052	0.050
3	**2.5**	0.116	0.111	0.069	0.063
4	**1.25**	0.133	0.127	0.083	0.081
5	**0.625**	0.156	0.142	0.102	0.099
6	**0.3125**	0.171	0.165	0.133	0.125
7	**0.15625**	0.192	0.183	0.151	0.148

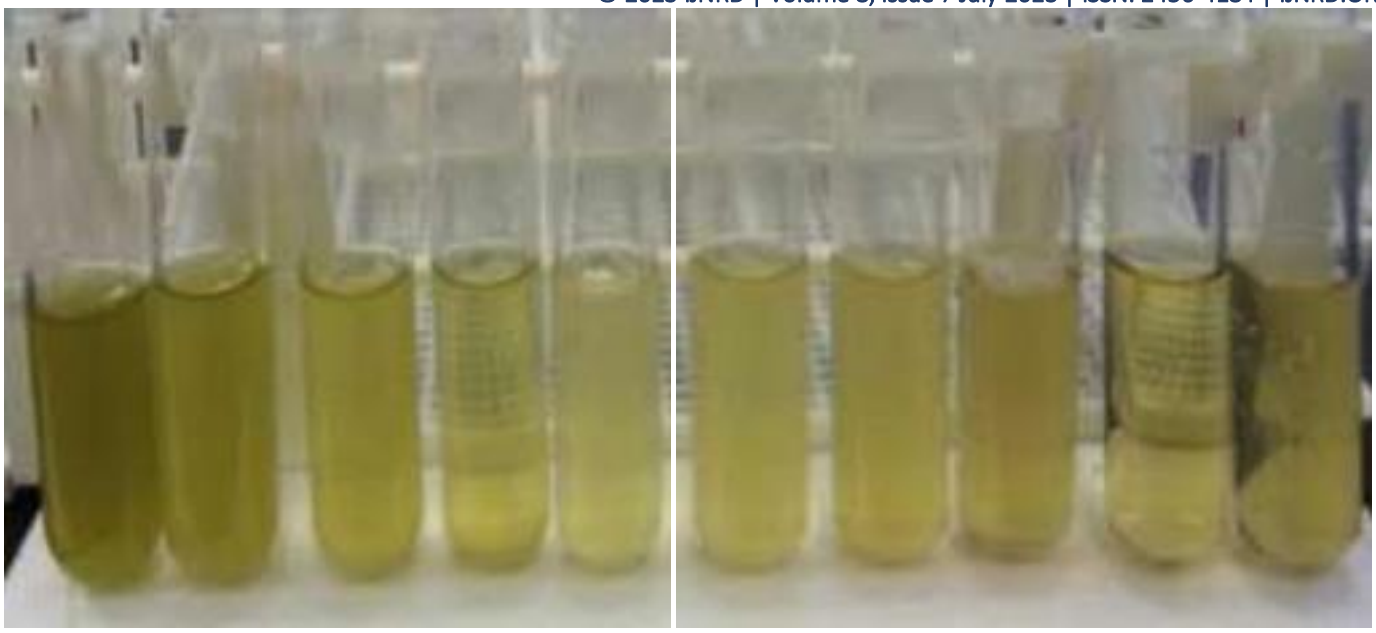


Figure 1 Broth dilution of QS1 Figure 2 Broth dilution of QS3

DISCUSSION

The IR spectra were observed for the characteristic peaks obtained due to the presence of the functional groups. All the compounds exhibited the peaks of aromatic C=C stretching, C-H stretching, C-N and C=N stretching and C=O stretching. The occurrence of absorption bands for C=O and C=N may occur at the same frequency and Fermi resonance peaks were the diagnostics of a carbonyl group in the compounds. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen and imine hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH. The mass spectra of the compounds were examined for the presence of molecular ion peak or the isotopic peaks to confirm the formation of the compounds.

Antibacterial activity

The results of the antibacterial suggested the importance of substitution on the benzylideneamino moiety. Compound **QS2** had poorest antibacterial activity while **QS3** exhibited the highest activity against the tested microbes. This signifies that the presence of a nitro substitution was beneficial for antibacterial action against both gram positive and gram negative bacteria while unsubstituted compound was having poor action. The introduction of nitrogen in the ring, as in **QS1** was also beneficial for its antibacterial activity. All the tested molecules where found to exhibit better antibacterial action against Gram negative bacteria in comparison to Gram positive bacteria.

SUMMARY AND CONCLUSIONS

The quest of development of newer treatments for existing diseases has been the threshold for the present research work. In the present work newer antibacteial agents based on Quinalzolinone scaffold were synthesized and evaluated. The scheme for the design of the molecules was based on the schemes reported by Rahman et al and Dash et al.

The synthesized compounds were characterized for the physicochemical properties such as melting point, colour and solubility. All the compounds were yellowish to brown in colour and were obtained in 63-73% yields using the optimized reaction cnditions. The compounds were insoluble in water, slightly soluble in methanol, soluble in chloroform and DMSO.

The confirmation of the structure of the synthesized compounds was done by IR, ^{1}HNMR and mass spectral studies. All the compounds exhibited the absorption bands of C=O, C=N, C-H, C=C stretching in the IR spectra. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen and imine hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH. The mass spectra of the compounds were examined for the presence of molecular ion peak or the isotopic peaks to confirm the formation of the compounds.

The results of the antibacterial suggested the importance of substitution on the benzylideneamino moiety. Compound **QS2** had poorest antibacterial activity while **QS3** exhibited the highest activity against the tested microbes. This signifies that the presence of a nitro substitution was beneficial for antibacterial action against both gram positive and gram negative bacteria while unsubstituted compound was having poor action. The introduction of nitrogen in the ring, as in **QS1** was also beneficial for its antibacterial activity.

Conclusion

The objective of the present investigation was to develop newer antibacterial molecules based on quinazolinone scaffold. It was accomplished by preparing schiffs base of a quinazolinone scaffold. The synthesized compounds presented anti-bacterial activity comparable to that of the standard drug norfloxacin. The compound QS3 was able to reduce the growth of both gram negative and gram positive bacteria in low concentration. The study led to the conclusion that lead molecules could be designed from quinazolinone nucleus by utilizing computer aided methods.

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