EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 3
209-214
Year: 2022

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEWER QUINAZOLINE BASED DERIVATIVES

Subodh Kumar*, Vivek Thakur, Deepak Basedia and Balkrishna Dubey

Technocrats Institute of Technology-Pharmacy, Bhopal.

***Corresponding Author: Subodh Kumar**
Technocrats Institute of Technology-Pharmacy, Bhopal.

Article Received on 09/01/2022 **Article Revised on 29/01/2022** **Article Accepted on 20/02/2022**

ABSTRACT
In the present work newer antibacterial agents based on Quinalzoline scaffold were synthesized and evaluated. 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 57-67% yields using the optimized reaction conditions. The compounds were insoluble in water, slightly soluble in methanol, soluble in chloroform and DMSO. Antibacterial activity of the synthesized compounds against four tested microorganisms was determined. The zone of inhibition was obtained as the primary indicator of the antibacterial action and the MIC value of the compounds was established using serial dilution technique. The antibacterial activity of **QZSB 4** was the maximum of all the compounds. On the other hand, **QZSB 1 and QZSB 5** exhibited the least zone of inhibitions and the highest MIC values.

KEYWORDS: Quinalzoline, antibacterial, zone of inhibition, MIC, serial dilution.

INTRODUCTION
Quinazoline is a bicyclic structure containing two fused six-membered rings; one is benzene ring another one is a pyrimidine ring. It is isomeric with the other diazanaphthalenes of the benzodiazine subgroup, cinnoline, quinoxaline and phthalazine (Figure 1).

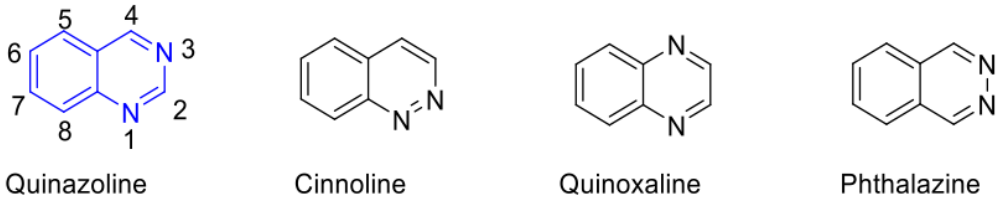


Figure 1: Quinazoline and its isomers.

Quinazoline scaffold has attracted significant attention due to their existence in several drugs and naturally occurring alkaloids.[1] Compounds containing 4(3H)-quinazolinone ring system have showed antitumor, anticonvulsant, antitubercular activities, anti-inflammatory, analgesic, antimicrobial and anticoccidal activities.[2-6]

Quinazoline are known to possess several biological actions including antimicrobial, anticancer, antihypertensive etc. The ease of synthesis of the quinazoline molecules has motivated us to design new molecules based on the quinazoline scaffold and evaluate them for their antimicrobial action.

MATERIAL AND METHODS
Anthranilic acid, ethanol, hydrazine hydrate, sodium hydroxide, 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. Melting point was determined using electrically heated melting point apparatus, FTIR was determined on Bruker FTIR spectrophotometer, Mass spectra was recorded on API Microsystems LC MS instrument and proton NMR spectra were recorded on Jeol system.

The scheme for synthesis[7,8] of the title compounds is presented in figure 2.

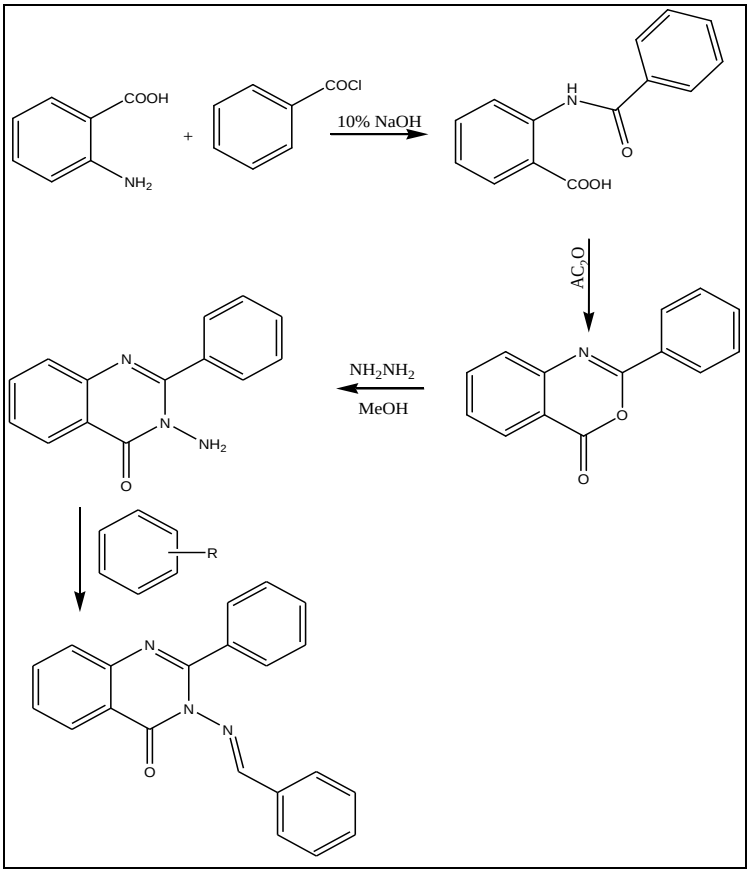


Figure 2: Scheme for synthesis of quinazolines.

Step 1: Synthesis of 2-benzamidobenzoic acid

To the anthranilic acid (2 mmol) dissolved in 10% sodium hydroxide (10 mL), 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.

Step 2: Synthesis of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one

A solution of 2-benzamidobenzoic 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.

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

A mixture of benzoxazine (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.

Step 4: General procedure for synthesis of Quinazoline based schiffs base

10 mmol of 3-amino-2-phenylquinazolin-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.[9]

Chemical Characterization

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR). The melting points were determined by open capillary method and are uncorrected using a electrically heated melting point determination apparatus. The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates. Iodine vapors were used for development of the chromatogram. The solvent system used for running the compounds was chloroform: acetone in the ratio 4:6.

Antimicrobial activity

Origins of the bacterial strains used in this study are *E. coli* MTCC 3261, *P. aeruginosa* MTCC 647 as Gram negative bacteria and *B. subtilis* MTCC 1134, *S. aureus* MTCC 3382 as the Gram positive strains. The MTCC strains of microorganisms used in this study was obtained from Institute of microbial technology, Chandigarh, India.

Preparation of test solutions

The synthesized quinazolines were dissolved in dimethyl sulfoxide (DMSO) and the further dilutions of the test compounds were prepared at the required quantities of 100, 50, 25, 12.5 and 6.25 µg/mL concentrations with Mueller-Hinton broth medium.[10]

Antimicrobial Assay

All the synthesized compounds were evaluated for minimum inhibitory concentration (MIC). The cultures for all the bacterial strains were obtained from Mueller-Hinton broth after 24 h of incubation at 37 ± 1°C. Primary antimicrobial evaluation of the compounds was carried out using cup and plate method. The determination of MIC was carried out in Mueller-Hinton broth at pH 7.4 and the two-fold serial dilution technique was applied.[11] The final inoculum size was maintained to 10^5 CFU/mL. A set of tubes containing only the inoculated broth was used as the negative control, and one containing only the broth was used to ensure the sterility of the medium. Ampicillin was used as the positive control. Different concentrations of each compound were incubated with 1 mL broth and 1 mL of the bacterial culture for 24 h at 37 ± 1°C. The last tube with no growth of microorganism was recorded to represent the MIC expressed as µg/mL (table 3).

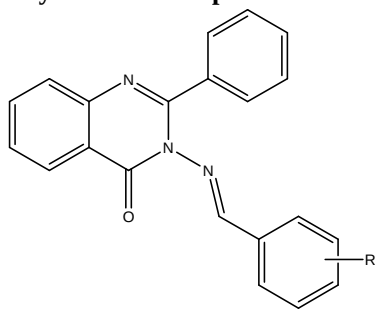
RESULTS AND DISCUSSION

The synthesis of all the compounds was achieved using the scheme depicted in figure 2. The results of characterization of the synthesized compounds are presented in the present section.

Synthesis

The synthesized compounds were subjected to determination of yield, melting point, solubility and structure elucidation. The physicochemical properties are shown in Table 1. All the compounds were found to be slightly soluble in methanol, soluble in DMSO and chloroform and insoluble in water.

Table 1: Yield, color and melting point of synthesized compounds.



Compound code	Aldehyde Used	Yield (%)	Melting point (°C)	Color
QZSB 1	1-allyl	57	280-282	Yellow
QZSB 2	2-OH	63	226-228	Brownish Yellow
QZSB 3	4-OCH_3	67	249-251	Yellow
QZSB 4	3-OCH_3, 4-OH	62	273-275	Yellow
QZSB 5	4-N-$(CH_3)_2$	59	268-271	Brown

The structure elucidation of the synthesized compounds was confirmed by interpretation of the IR, ^{1}HNMR and Mass spectra of the compounds. 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.

2-phenyl-3-((E)-((E)-3-phenylallylidene)amino)quinazolin-4(3H)-one

IR (cm-1): 3202.53 (C-H Str), 3042.73 (C-C Str), 1678.80 (C=N Str), 1510.55 (N=N Str), 1428.04 (C-N Str);
^{1}HNMR (δ ppm): 7.2-7.9 Ar H, 8.1 imine H, 5.3-6.5 H of C=C;
m/z: 351.4 (M^+)

(E)-3-(2-hydroxybenzylideneamino)-2-phenylquinazolin-4(3H)-one

IR (cm-1): 3705.25 (O-H str), 3104.67 (C-H Str), 2970.38 (Ar C-C Str), 1639.00 (C=N Str), 1456.90 (N=N Str), 1289.63 (C-N Str), 1082.70 (C-O Str);
^{1}HNMR (δ ppm): 7.2-7.9 Ar H, 8.1 imine H, 6.8 H adj to OH, 5.0 OH;
m/z: 342.2 (M++1)

(E)-3-(4-methoxybenzylideneamino)-2-phenylquinazolin-4(3H)-one

IR (cm-1): 3100.40 (C-H Str), 2970.97 (C-C Str), 1639.54 (C=N Str), 1456.90 (N=N Str), 1289.17 (C-N Str);
^{1}HNMR (δ ppm): 7.2-7.9 Ar H, 8.1 imine H, 8.2 H adj to OCH3;
m/z: 356.1 (M++1)

(E)-3-(4-hydroxy-3-methoxybenzylideneamino)-2-phenylquinazolin-4(3H)-one

IR (cm-1): 3733.07 (O-H Str), 3107.54 (C-H Str), 2967.05 (C-C Str), 1653.56 (C=N Str), 1477.79 (N=N Str), 1289.54 (C-N Str), 1082.15 (C-O Str);
^{1}HNMR (δ ppm): 7.2-7.9 Ar H, 8.1 imine H, 6.8 H adj to OH, 5.0 OH;
m/z: 371.0 (M+)

(E)-3-(4-(dimethylamino)benzylideneamino)-2-phenylquinazolin-4(3H)-one

IR (cm-1): 3112.47 (C-H Str), 2933.32 (C-C Str), 1651.13 (C=N Str), 1477.24 (N=N Str), 1284.08 (C-N Str);
^{1}HNMR (δ ppm): 7.2-7.69 Ar H, 8.1 imine H;
m/z: 371.0 (M+)

Antibacterial action

Antibacterial activity of the synthesized compounds against four tested microorganisms was determined. The zone of inhibition was obtained as the primary indicator of the antibacterial action. Table 2 presents the zone of inhibition obtained for each of the quinazoline derivatives in different bacterial species. Figure 3 represents the comparative zone of inhibition obtained from the test compounds at the four different concentrations in different bacteria.

Table 2: Zone of inhibition exhibited by the quinazoline derivatives.

Compound	*Conc (µg/mL)*	Zone of inhbition (mm)			
		E.coli	***B.subtilis***	***P.aeuroginosa***	***S.aureus***
QZSB 1	250	9	9	10	10
	500	10	10	10	10
	750	11	11	13	13
	1000	12	13	13	14
QZSB 2	250	11	10	12	11
	500	13	11	13	13
	750	14	13	14	14
	1000	16	13	14	14
QZSB 3	250	10	11	11	11
	500	11	12	12	11
	750	12	12	13	13
	1000	15	15	14	14
QZSB 4	250	13	13	14	14
	500	14	15	15	16
	750	14	15	16	16
	1000	17	17	16	16
QZSB 5	250	10	10	10	10
	500	10	11	10	10
	750	11	12	12	12
	1000	12	13	13	13

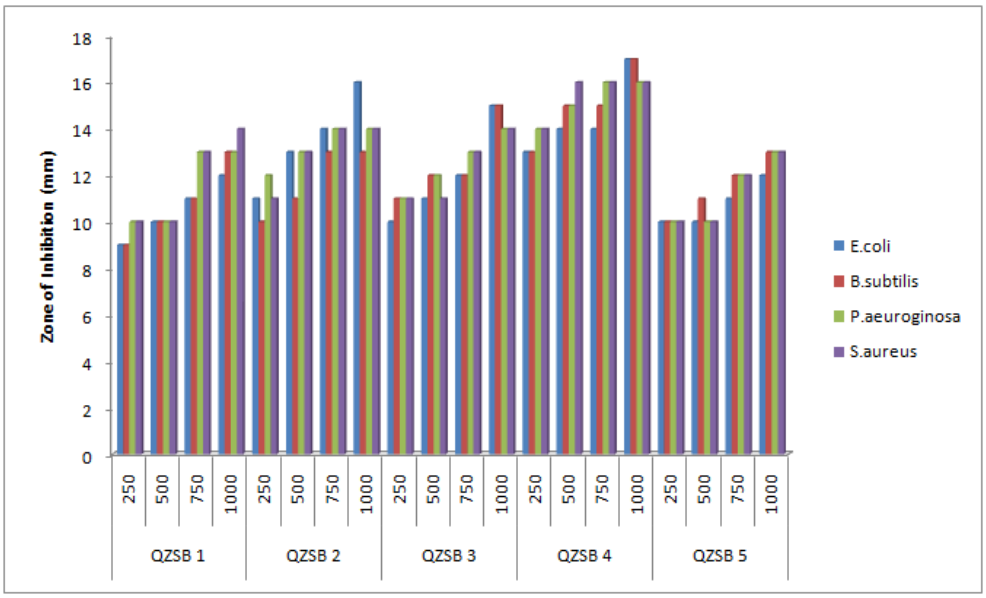


Figure 3: Zone of inhibitions (compared) at different doses for QZSB1-5.

The MIC value of the compounds was established using serial dilution technique and the results are presented in table 3.

Table 3: MIC of the synthesized compounds against gram positive and gram negative bacteria.

Compd. No.	MIC (µg/mL)[b]			
	E.coli	***B. subtilis***	***P. aeuroginosa***	***S. aureus***
QZSB 1	25	25	12.5	12.5
QZSB 2	12.5	12.5	6.25	6.25
QZSB 3	6.25	6.25	12.5	6.25
QZSB 4	6.25	6.25	6.25	6.25
QZSB 5	25	12.5	6.25	12.5
Ampicillin	2.56	1.28	1.28	1.28

[a] A set of tubes with only the inoculated broth was used as control to determine MIC
[b]MIC is expressed by measuring the turbidity of test and control dilution tubes. A 50% decrease in turbidity was taken as MIC.

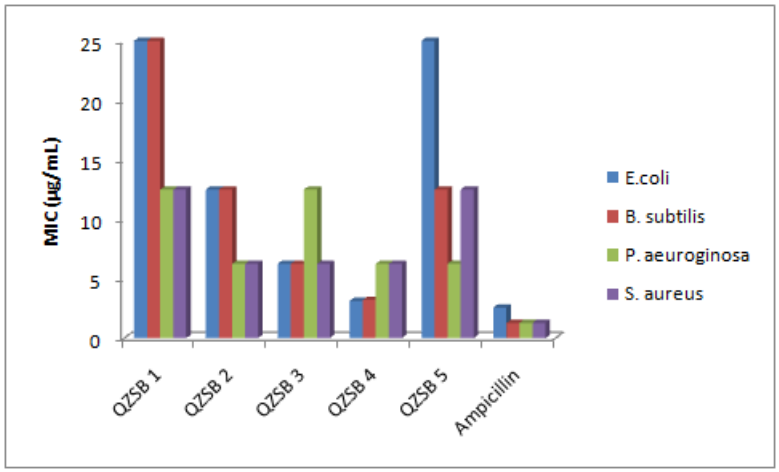


Figure 4: Comparison of MIC values of QZSB1-5.

The quantitative antibacterial results are reported in terms of minimum inhibitory concentrations (MICs). The tube dilution test is the standard method for determining levels of resistance to an antibiotic. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC).

The result revealed that most of the tested compounds showed antibacterial activities with varying magnitudes. In this study the different tested compounds had a various inhibitory effects on the growth of the different tested microorganisms. This difference might be due to different structure of chemical substances and the efficiency of side chains in the reaction with other compounds. As it can be witnessed from figure 3 and 4, the antibacterial activity of **QZSB 4** was the maximum of all the compounds. On the other hand, **QZSB 1 and QZSB 5** exhibited the least zone of inhibitions and the highest MIC values. The suggests that the presence of electron donating group (N-$(CH_3)_2$) on the ring of the aromatic aldehyde used for schiffs base formation was detrimental for its antibacterial efficacy whereas electron withdrawing groups (OH, OCH_3) favored the antibacterial action as exhibited by QZSB2, QZSB 3 and QZSB 4.

CONCLUSION

The objective of the present investigation was to develop newer antibacterial molecules based on quinazoline scaffold. It was accomplished by preparing schiffs base of a novel quinazoline scaffold. The synthesized compounds presented anti-bacterial activity comparable to that of the standard drug ampicilllin in tested microorganisms (in vitro). It could be concluded from the study that quinazoline nucleus can be modulated in future for design of better antimicrobial agents using approaches of drug design.

REFERENCES

1. Tiwari AK, Singh VK, Bajpai A, Shukla G, Singh S, Mishra AK. Synthesis and biological properties of 4(3H)-quinazolone derivatives. European J Med Chem., 2007; 42: 1234-1238.
2. Cao SL, Feng YP, Jiang YY. Synthesis and in vitro antitumor activity of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains. Bio Org Med Chem., 2005; 15: 1915-1917.
3. Giri RS, Thaker HM, Giordano T, Williams J. Design, synthesis and characterization of novel 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3H-quinazoline-4-one derivatives as inhibitors of NF-kappaB and AP-1 mediated transcription activation

and as potential anti-inflammatory agents. European J Med Chem., 2009; 44: 2184–2189.
4. Helby, Abdel MH. Design and synthesis of some new derivatives of 3H-quinazolin-4-one with promising anticonvulsant activity. Acta Pharma, 2003; 53: 127–138.
5. Kadi AA, Azab AS, Alafeefy AM, Abdel SG. Synthesis and biological screening of some new substituted 2-mercapto-4(3H)quinazolinone analogues as anticonvulsant agents. J. Pharma. Sci., 2006; 34: 147-158.
6. Jatav V, Mishra P, Kashaw S. CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. European J Med Chem., 2008; 43: 1945-1951.
7. Rahman M, Rathore A, Siddiqui AA, Parveen G, Yar MS. Synthesis and characterization of quinazoline derivatives: search for hybrid molecule as diuretic and antihypertensive agents. J Enzyme Inhib Med Chem., 2013. DOI: 10.3109/14756366.2013.845820
8. Dash B, Dash S, Laloo D, Chakraborty J. Design, synthesis and in vivo antitumor activity of novel 3, 4 disubstituted quinazoline derivatives. Int J Pharm Chem., 2017; 7(1): 20-30.
9. Gautam S, Mishra D, Singh R and Pal DK (2012) Synthesis of some novel 4, 6-disubstituted derivatives and evaluation of their antimicrobial activity, International Journal of Pharmaceutical, Chemical and Biological Sciences, 2012; 2(1): 97-103.
10. Jaiswal M. Microwave-Assisted Synthesis of Pyrimidinethione Derivatives. Journal of Pharmacology and Biomedicine. 2017; 1(4): 115-118.
11. Mishra R, Jain S. Investigation of antimicrobial potential of some thiazolyl chalcone Derivatives. Pharmacologyonline. 2013; 1: 190-193.