ANTIMICROBIAL \textit{IN VITRO} PHARMACOLOGICAL STUDIES OF SOME PYRAZOLYL QUINAZOLIN-4(3H) ONE DERIVATIVES

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ABSTRACT
Some pyrazolylquinazolin-4(3H) one derivatives were synthesized by the base catalysed cyclisation of acrylamide with 3,5-dinitrophenyl hydrazine hydrate. The overall reaction was carried out by multistep process. The structural confirmation of the synthesized compounds was carried out on the basis of elemental analysis, IR and NMR spectra results. The title compounds were screened for antibacterial and antifungal pharmacological studies \textit{in vitro} by cup plate method. The potency of synthesized compounds focus on the strength compared with standard drug.

KEYWORDS: Antibacterial, Antifungal, Acrylamide, Quinazolin-4(3H) one.

INTRODUCTION
Heterocyclic derivatives are pharmaceutically very important class of compounds which were developed better result in the medicinal chemistry. Quinazolin-4(3H) one and its nitrogen containing precursors had diversified biological properties. Quinazolin-4(3H) one with its pyrazoline analogs has extensively used as anti-inflammatory, anticancer[1,2,3] Moreover, a large number of Quinazolin-4(3H) ones derivatives have been reported as a potential against alzheimer disease and analgesics agents.[4] Quinazolin-4(3H) one with pyrazole moiety have been reported as a very good antifungal and antimicrobial agents.[5,6] In addition triquinazolinone derivatives and quinazolin-4(3H) one bearing quinoline moiety reported to anti-hyperglycemic[7] activity and HIV-1 integrase inhibitor.[8]

Pyrazoline derivatives of quinazolin-4(3H) one have important therapeutic properties, among many derivatives are biologically active scaffold and important constituent of many pharmaceutical product and used as a Cox-II inhibitor.[9,10] Some precursor of quinazolin-4(3H) one has reported to anti convulsant activity and CNS depressant agent.[11,12]

Encourage by the wide spectrum of therapeutic activities exhibited and literature survey of quinazoline derivatives revealed that in this study, we have synthesized quinazolin-4(3H) one incorporating two heterocyclic moieties pyrazoline at C-3 and quinoline at C-2 respectively and studied its antibacterial and antifungal activities. The potency (Edwin and Marion 1945) of these compounds was calculated and compare with standard drugs to observe the strength of these compounds.
**Scheme I Synthetic Pathway for Target Molecule**

![Scheme I Synthetic Pathway for Target Molecule](image)

**EXPERIMENTAL SECTION**

**2-[3-(6-chloro-2-phenyl)quinolin-3-yl]methyl-6,8-dibromo-3,1-benzoxazin-4(3H) one 2**

To a solution of 3-(6-chloro-2-phenylquinolin)acetyl chloride (3.16 g, 0.01 mol) in pyridine (25 ml) kept on an ice bath at 0-5°C. Add small portion of 3:5-dibromo anthranilic acid (2.95 g, 0.01 mol) and stirred for 1 h. to keep the temperature between 0-5°C. Further reaction mixture was stirred 1 h. at room temperature. A pasty mass thus obtained was washed thoroughly with sodium bicarbonate (5%) to remove unreacted acid. Thus solid separated was filtered, dried and recrystallised from methanol.

M.P.: 145⁰C. Yeild: 79% IR(KBr): 3073, 2861(C-H), 1725(C=O), 1616(C=N), 1327(C-Cl), 580(C-Br). ¹HNMR(CDCl₃): 2.11(s, 2H, -N-NH₂), 6.42-7.96(m, 11H, Ar-H), 7.96-8.12(s, 2H, -OH). Anal. (%) for C₂₃H₁₁N₂O₂Br₂Cl Calcd; C, 50.93; H, 2.77; N, 9.14. Found; C, 50.49; H, 2.64; N, 9.83.

To a mixture of 2-[3-(6-chloro-2-phenyl)quinolin-3-yl]methyl-6,8-dibromo-3,1-benzoxazin-4(3H) one (5.565 g, 0.01 mol) and hydrazine (99%) (0.50 g, 0.01 mol) in 25.0 ml pyridine was heated at 180-200°C in an oil bath for 5-6 h. The oily mass was obtained, cooled and slowly poured onto crushed acidic (HCl, 25 ml) ice cold water with occasional stirring. The product obtained was filtered and washed several times with water. The crushed product was dried and recrystallized from methanol.

M.P.: 173⁰C. Yeild: 69% IR(KBr): 3407(NH), 3069, 2863(C-H), 1718(C=O), 1614(C=N), 1325(C-N), 779(C-Cl), 580(C-Br). ¹HNMR(CDCl₃): 2.12(s, 2H, -N-NH₂), 6.42-7.96(m, 11H, Ar-H), 7.96-8.12(s, 2H, -OH). Anal. (%) for C₂₃H₁₇N₂O₂Br₂Cl Calcd; C, 50.93; H, 2.77; N, 9.14. Found; C, 50.49; H, 2.79; N, 9.16.

R₁ = H, 2-Cl, 3-Cl, 4-Cl, 2-OH, 3-OH, 4-OH, 2-NO₂, 3-NO₂, 4-NO₂, 2-OCH₃, 4-OCH₃
2-[3-(6-chloro-2-phenyl)quinolin-3-yl]methyl-3-substituted phenyl acryl amido 6,8-dibromo quinazolin-4(3H)-one 5₁

To a solution of 2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-acetamido-6,8-dibromo quinazolin-4(3H)-one (6.125g, 0.01 mol) in absolute ethanol (50 ml) and add benzaldehyde (1.06g, 0.01 mol) in 2% NaOH was refluxed for 10-12 h., cooled and poured into ice cold water. The solid thus obtained was filtered, washed with water and recrystallized from methanol.

M.P.: 151°C. Yeild: 74% IR(KBr): 3409(NH), 3062, 2859(C-H), 1719(=C =O), 1641(=C =O of –COCH₃), 1578 (CH =CH), 1318(C-N), 778(C-Cl), 579(C-Br). ¹H NMR(CDCl₃): 2.11(s, 1H, =CH=Ar). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 51.14; H, 2.62; N, 12.24; Found: C 51.16; H, 2.64; N, 12.25.

The remaining 5₂-12 compounds were prepared by the above mention similar method.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[1-(3,5-dinitrophenyl)-5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl amino]-6,8-dibromo quinazolin-4(3H)-one 6₁

To a solution of 2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-substituted phenyl acryl amido-6, 8-dibromoquinazolin-4(3H)-one (7.005 g, 0.01 mol) in methanol, add 3-dinitrophenyl hydrazine hydrate (99%) (4.36g, 0.02mol) and few drops of glacial acetic acid. The reaction mixture was refluxed for 8-10 h., distilled the excess methanol and cooled. Thus the solid separated was filtered, washed with water and recrystallized from methanol.

M.P.: 147-148°C. Yeild: 76% IR(KBr); 3372(N-H), 3063,2858(C=H), 1728(C=O),1616(C=N), 1566, 1361(NO₂),1319 (C=N), 779(C-Cl), 581(C-Br). ¹H NMR(CDCl₃): 2.13 (d, 1H, =N-NH), 3.62 (s, 2H, =CH₂), 3.06 (d,1Ha), 3.45 (d,1Hb), 6.51(t,1Hx), 6.42-7.96 (m,19H,Ar-H). ¹³C NMR: 31.4(-CH₂), 36.5, 41.1, 161.2(pyrazol-C), 162.2 (>C=O), 173.1 (immine aromatic-C) 109.21-143.20(atomic-33C). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 51.15; H, 2.63; N, 12.26.

The remaining 6₂-12 compounds were prepared by the above mention similar method.

2-[3-(6-chloro-2-phenyl)quinolin-3-yl]methyl-3-[5-(2-chloro)phenyl-1-(3,5-dinitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl amino]-6,8-dibromo quinazolin-4(3H)-one 6₂

M.P.: 173-174°C. Yeild: 74% IR(KBr); 3368(N-H), 3062,2860(C=H), 1727(C=O),1616(C=O), 1564, 1362(NO₂), 1318(C-N), 780 (C=Cl), 574 (C-Br). ¹H NMR(CDCl₃): 2.12(d,1H, =N-NH), 3.63(s,2H, =CH₂), 3.05 (d,1Ha), 3.47(d,1Hb), 6.52(t,1Hx), 6.42-7.96(m,18H,Ar-H). ¹¹C NMR: 31.3(=CH₃), 36.4, 41.6, 160.9 (immine pyrazol-C),162.2 (>C=O),173.1 (immine aromatic-C) 109.21-143.20(atomic-33C). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 51.14; H, 2.62; N,12.24; Found: C 51.16; H, 2.63; N, 12.25.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[5-(3-chloro)phenyl-1-(3,5-dinitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl amino]-6,8-dibromo quinazolin-4(3H)-one 6₃

M.P.: 162-163°C. Yeild: 78% IR(KBr); 3372(N-H), 3061,2859(C=H), 1729(C=O),1616(C=O), 1566, 1361(NO₂), 1317(C-N), 782 (C=Cl), 575 (C-Br). ¹H NMR(CDCl₃): 2.13(d,1H, =N-NH), 3.63 (s,2H, =CH₂), 3.06 (d,1Ha), 3.48 (d,1Hb), 6.54(t,1Hx), 6.42-7.96(m,18H,Ar-H). ¹³C NMR: 31.6(-CH₃), 36.7, 41.3, 161.1 (immine pyrazol-C),162.2 (>C=O),173.1 (immine aromatic-C) 109.21-143.20(atomic-33C). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 51.14; H, 2.62; N,12.24; Found: C 51.15; H, 2.64; N, 12.25.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[5-(4-chloro)phenyl-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl amino]-6,8-dibromo quinazolin-4(3H)-one 6₄

M.P.: 178-179°C. Yeild: 76% IR(KBr); 3369(N-H), 3062,2861(C=H), 1727(C=O),1616(C=O), 1566, 1361(NO₂), 1319(C-N), 781 (C=Cl), 578(C-Br). ¹H NMR(CDCl₃): 2.13 (d,1H, =N-NH), 3.63(s,2H, =CH₂), 3.07 (d,1Ha), 3.48(d,1Hb), 6.55(t,1Hx), 6.42-7.96(m,18H,Ar-H). ¹³C NMR: 31.5(-CH₃), 36.3, 41.5, 161.2 (immine pyrazol-C),162.2 (>C=O),173.1 (immine aromatic-C) 109.21-143.20(atomic-33C). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 51.14; H, 2.62; N,12.24; Found: C 51.16; H, 2.63; N, 12.26.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[5-(2-hydroxy)phenyl-1-(3,5-dinitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl amino]-6,8-dibromo quinazolin-4(3H)-one 6₅

M.P.: 155-156°C. Yeild: 73% IR(KBr); 3543(O-H), 3369(N-H), 3063,2861(C=H), 1729(C=O), 1616(C=O), 1565, 1361(NO₂), 1318(C-N), 780 (C=Cl), 576(C-Br). ¹H NMR(CDCl₃): 2.12 (d,1H, =N-NH), 3.62(s,2H, =CH₂), 3.06(d,1Ha), 3.48(d,1Hb), 6.54(t,1Hx), 6.42-7.96(m,18H,Ar-H), 10.38(s,1H,OH). ¹³C NMR: 31.4(-CH₃), 36.4, 41.3, 161.1(pyrazol-C),162.2 (>C=O),173.1 (immine aromatic-C) 109.21-143.20(atomic-33C). Anal; (%) C₉H₇N₂O₂Br₂Cl Calcd: C 52.20; H, 2.78; N,12.49; Found: C 52.21; H, 2.79; N, 12.51.
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CH₃, 36.4, 41.6, 161.2 (imine pyrazol-C), 162.2 (>=C=O), 173.1 (imine aromatic-C), 109.21-143.20 (aromatic-33C). Anal; (%) C₃H₃N₂O₂BrCl Calcd: C, 52.20; H, 2.78; N,12.49; Found: C, 52.21; H, 2.79; N, 12.51.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[5-(4-hydroxy-phenyl-1)-3,5-dinitrophenyl]-4,5-dihydro-1H-pyrazol-3-yl amino-6,8-dibromoquinazolin-4(3H)-one 6₉

M.P.: 191-192°C. Yield: 74% IR(KBr): 3375(N=O), 3063,2859(C-H),1729(C=O),1616 (C=N), 1567, 1361(-NO₂),1319(C-N), 779(C-Cl), 577(C-Br).¹H NMR(CDCl₃): 2.13 (d,1H=NH-2), 3.62 (s,2H-CH₂), 3.07(d,1H), 3.46(d,1H), 6.53(t,1H), 6.42-7.96(m,18H-Ar-H). ¹¹C NMR: 31.5(-CH₂), 36.3, 41.7, 161 (imine pyrazol-C), 162.2 (>C=O), 173.1 (imine aromatic-C), 109.21-143.20 (aromatic-33C). Anal; (%) C₃H₃N₂O₂BrCl Calcd: C, 50.56; H, 2.59; N,13.61; Found: C, 50.58; H, 2.60; N, 13.63.

2-[3-(6-chloro-2-phenyl) quinolin-3-yl]methyl-3-[5-(3-nitrophenyl-1)-3,5-dinitrophenyl]-4,5-dihydro-1H-pyrazol-3-yl amino-6,8-dibromoquinazolin-4(3H)-one 6₈

M.P.: 182-183°C. Yield: 71% IR(KBr): 3371(N=O), 3062,2857(C-H),1725(C=O),1616 (C=N), 1566, 1361(-NO₂),1319(C-N), 779(C-Cl), 577(C-Br).¹H NMR(CDCl₃): 2.13 (d,1H=NH-2), 3.63 (s,2H-CH₂), 3.06(d,1H), 3.46(d,1H), 6.52(t,1H), 6.42-7.96(m,18H-Ar-H). ¹¹C NMR: 31.4(-CH₂), 36.5, 41.6,161.2 (imine pyrazol-C), 162.1 (>C=O),172.9 (imine aromatic-C), 109.21-143.20 (aromatic-33C). Anal; (%) C₃H₃N₂O₂BrCl Calcd: C, 50.56; H, 2.59; N,13.61; Found: C, 50.57; H, 2.61; N, 13.62.

Determination of Antimicrobial Activity

Cup plate Method

The in vitro antimicrobial activity of synthesized compounds was carried out by cup-plate method.¹⁴¹⁵ The cup was bored into the inoculated Petri dish. The cups were made (equidistance) by punching in to the agar surface with sterile cup borer and punching out the part of the agar. After punching a bore, in to these cups were added 0.01 ml portion of the test compound (0.01 g dissolved in 10 ml DMF solvent) in solvent with the help of sterile syringe. The solution was allowed to defuse for about an hour in to the medium.

Bacterial and Plant Pathogenic Stains Used

The in vitro antimicrobial activity of synthesized compounds was screened against two gram positive bacteria(Staphylococcus aureus ATCC 9144 and Bacillus Subtilis ATCC 6633) and two gram negative bacteria(Escherichia coli ATCC 25922 and Pseudomonas
aeruginosa ATCC 9027), whereas two plant pathogens for antifungal activity were tested against Candida albicans ATCC 10231 and Aspergillus niger ATCC 6275.

Measurement of the zone of Inhibition
After 2 h, for the diffusion of the substance in the agar medium and the plates were incubated at 37°C for 24 h. After incubation period observed the plate for zone of inhibition around the cups. Measure the diameter of each zone in mm.

A solvent control was also run to know the activity of the blank. This was carried out in DMSO at concentration of 0.05 ml in similar manner and the zone of the inhibition of the bacterial growth were measured in diameter and it was 0.0 mm. The standard drugs were also screened under similar condition.

The zone of inhibition measured for antibacterial activity at two different concentrations 100 and 50 μg/ml. Penicillin-G was used as standard, where as zone of inhibition measured for antifungal activity also at two different concentrations 20 and 10 μg/ml and Fluconazole was used as a standard.

RESULT AND DISCUSSION
The title compound 6, 8-dibromoquinazolin-4(3H) one incorporating pyrazoline and quinoline moiety 6_{1-12} were synthesized and structure was confirmed by the spectral results. The IR spectra showing strong stretching vibration at 1729 and 1646 cm\(^{-1}\) indicates the presence of C=O group of quinazolinone and acetamide respectively. This was further confirming by \(^1\)H NMR spectra which showed singlet at δ 2.73 ppm equivalent to three protons of acetamide group(4). The acrylamide 5_{1-12} which showed CH=CH stretching at 1578 cm\(^{-1}\) in IR spectrum while \(^1\)H NMR spectra showed doublet of these protons at δ 6.81 and δ 8.61 ppm with coupling constant \(J = 16.0-16.6\) Hz. The IR spectra of compounds 6_{1-12} showed C=O and C=N stretching of quinazolinone at 1725 and 1616 cm\(^{-1}\) respectively. The \(^1\)H NMR spectra of compounds 6a-l indicates that the –CH\(_2\) protons of the pyrazoline ring resonated as a pair of doublet of doublets (H\(_a\) and H\(_b\)) because of geminal and vicinal coupling. The CH proton appeared as a doublet of doublet (Hx) because of vicinal coupling with the two magnetically nonequivalent protons of methylene group at C-4 of pyrazoline ring. The Ha proton which is cis to Hx resonates up field in the range δ 3.01-3.08 ppm as a doublet of doublet while Hb, the other proton which is trans to Hx resonates downfield in the range of δ 3.45-3.51 ppm as a doublet of doublet. The Hx proton which is vicinal to two methylene protons (Ha and Hb) resonates as a doublet of doublet in the range of δ 6.45-6.53 ppm. In \(^1\)C NMR spectra, signals at δ 36.4 ppm, δ 41.1 ppm and δ 161.3 ppm confirms the presence of CH\(_2\), CH and C=N of pyrazoline ring respectively, whereas C=O and C=N signals of quinazolinone ring are appear at around δ 162.2 and δ 173.1 ppm respectively.[16,17,18,19]

Antimicrobial Assay
The in vitro antimicrobial screening results of synthesized compounds were recorded in the table I and 2. Potency[20] was calculated from the screening results and compares the strength of synthesized compounds with standard drug.

Table: I Anti-bacterial activity of compound 6_{1-12}

<table>
<thead>
<tr>
<th>Compd No.</th>
<th>R(_{1})</th>
<th>Zone of inhibition in (mm)</th>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>ATCC9144</td>
</tr>
<tr>
<td></td>
<td>Br. subtilis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.aeruginosa</td>
<td></td>
</tr>
<tr>
<td>C(_{H})</td>
<td>C(_{L})</td>
<td>Pot %</td>
</tr>
<tr>
<td>6(_1)</td>
<td>H</td>
<td>15</td>
</tr>
<tr>
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<td>2-Cl</td>
<td>20</td>
</tr>
<tr>
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<td>19</td>
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<td>6(_4)</td>
<td>4-Cl</td>
<td>22</td>
</tr>
<tr>
<td>6(_5)</td>
<td>2-OH</td>
<td>13</td>
</tr>
<tr>
<td>6(_6)</td>
<td>3-OH</td>
<td>12</td>
</tr>
<tr>
<td>6(_7)</td>
<td>4-OH</td>
<td>15</td>
</tr>
<tr>
<td>6(_8)</td>
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<td>14</td>
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<td>6(_9)</td>
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<td>15</td>
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<tr>
<td>6(_10)</td>
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<td>15</td>
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<td>6(_11)</td>
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</tr>
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<td>6(_12)</td>
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<tr>
<td>PenicillinG</td>
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C\(_{H}\) Zone of inhibition at concentration 100 μg/ml, C\(_{L}\) Zone of inhibition at concentration 50 μg/ml, potency of compound(%) as compared to penicillin-G.
CONCLUSION
The title compound 6, 8-dibromoquinazolin-4(3H) ones derivatives \(6_{1-12}\) were synthesized by well organized method. The active pharmacophore pyrazoline and quinoline present in a newly synthesized compounds possessed good antibacterial and antifungal activity in vitro. The chloro group in phenyl nucleus on ortho, meta and para position showed very good activity against gram positive bacteria while nitro analogues displayed very good activity against gram negative bacteria compared to standard. More over phenyl nucleus, ortho and para methoxy substituted phenyl compounds showed very good antifungal activity. From this work, we were able to identify a few active molecules which are capable to inhibiting the growth of some bacteria and fungus species in vitro.

ACKNOWLEDGEMENT
Author gracefully thanks to Mrs. Anandita Mehta, Head, microbiology department, ATIRA, for antibacterial and antifungal screening results and Director(SICART)Vallabhvidhyanagar, for spectral data.

REFERENCES

Table: 2 Antifungal activity of compound 61-12

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<th>Compd No</th>
<th>R (_{1})</th>
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<th>Aniger ATCC 6275</th>
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<td></td>
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<td>C(_{H})</td>
<td>C(_{L})</td>
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<td>16</td>
<td>70.63</td>
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</table>

Fluconazole

C\(_{H}\) Zone of inhibition at concentration 20 μg/ml, C\(_{L}\) Zone of inhibition at concentration 10 μg/ml, potency of compound(%) as compared to fluconazole.


20. Edwin JD, Marion BS. Assay of Antibiotic Substance, 1945; 459.