REVIEW OF LITERATURE

QUINAZOLINE OF PHARMACOLOGICAL INTEREST

The chemistry and pharmacology of quinazoline have been of great interest because quinazoline derivatives possess various biological activities. This include antimicrobial, anticonvulsant, antineoplastic, analgesic and antiinflammatory etc.

Therefore in present work we have prepared quinazoline incorporate with fluoro substituted benzothiazole.

1) U.L. Narayan, A.G. Nerkar and C.S. Panda\(^1\) have reported synthesis and screening of 6,6'-methylene bis-2-methyl-3-[2'-aryl-thiazolin-4'one] / [2'-aryl-imidazolindin-4'one]quinazolin-4-(3H)-ones for antibacterial and antifungal activities.

\[
\begin{align*}
\text{Ar} &= \text{P-Cl-C}_6\text{H}_5, \text{O-NO}_2\text{-C}_6\text{H}_4, \text{P-NO}_2\text{C}_6\text{H}_4 \text{ etc}
\end{align*}
\]

2) S.R. Pattan, V.V.K. Reddy, J.S. Pattan, N.V.Venkataramana, P.N. Prajapati, A.R. Bhat and B.M. Hemashettar\(^2\) have reported synthesis and microbiological evaluation of N'-3-(4-(4-chloro-phenyl)thiazolo-2-yl) quinazoline-4-(3H)-ones.

\[
\text{Where } R = \text{Cl, F, CH}_3 \text{ etc}
\]
3) Histesh D. Patel, B.D. Mistry\(^3\) and K.R. Desai have reported synthesis and antimicrobial activity of imidazole quinazoline.

\[
\begin{align*}
\text{N} & \quad \text{N} \\
| & \quad | \\
\text{O} & \quad \text{O} \\
\text{R} & \quad \text{R} \\
\text{NO}_2 & \quad \text{NO}_2
\end{align*}
\]

Where \( R = \text{Phenyl, Acetyl phenyl, 3 nitrophenyl etc.} \)

4) Marzoog S Al-Thebeiti and Maher F.El-Zohry\(^4\) have reported synthesis of some new spirothiazolidinone and spirozetidinone derivatives incorporated with quinazoline.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{N} \\
\text{\textbullet} & \quad \text{\textbullet} \\
n & \quad n \\
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R}
\end{align*}
\]

5) R.L. Sharma, Surinder Kumar, Anand Sachar, Jasbir Singh and Daljeet Kour\(^5\) have reported synthesis of 1,2,4-triazolo, tetrazolo and pyrazolylquinazolines.

\[
\begin{align*}
\text{C}_6\text{H}_5 \\
\text{N} & \quad \text{N} \\
\text{R} & \quad \text{R} \\
\text{R'} & \quad \text{R'} \\
\text{R} = & \text{H, CH}_3, \text{C}_6\text{H}_5 \\
\text{R'} = & \text{H, CH}_3, \text{C}_6\text{H}_5
\end{align*}
\]
6) V. Bhardwaj, V.N. Gupta and O.P. Suri have reported a new synthesis of 11 H-Pyrido [2,1-b] quinazolin-11-ones.

![Chemical Structure](image)

\[ R^1 = H, -OCH_3, -NO_2, -H, -Cl \]
\[ R^2 = -H, -H, -H, -Cl, -H \]

7) U.L. Narayan, A.G. Nerkar and C.S. Panda have reported synthesis and screening of 6,6'-methylene bis-2-phenyl-3-[2'-aryl-thiazolidin-4'-one]/[2-aryl-oxazolidin-4'-one]. Quinazolin-4-(3H)-ones for antibacterial and antiplatelet activities.

![Chemical Structure](image)

\[ Ar = 3-Cl-C_6H_4, 4-NO_2 -C_6H_4, 2-Cl -C_6H_4 \]

8) B. Shivarama Holla*, M.T. Padmaja, M.K. Shivananada and P.M. Akbarali have reported the synthesis and antibacterial activity of nitro furylvinyl quinazolinones.

![Chemical Structure](image)

\[ R = H, P-bromo, P-methyl, O-methoxy, etc. \]
9) N.A. Gangwal, U.R. Kothawade, A.D. Galande, D.S. Pharanda, A.S. Dhake\(^9\) have reported synthesis of 1-substituted-2-chloromethyl-4-(1H)-quinazolinones as antimicrobial agents.

\[
\begin{align*}
\text{Ar} &= \text{2-Chlorophenyl, 4-nitrophenyl, 2-hydroxy phenyl etc.}
\end{align*}
\]

10) A.R. Bhat*, G. Gautham Shenoy, Mohan Kotian\(^{10}\) have reported synthesis and biological activities of Mannich bases of 7-nitro-2-methyl-4-(3H)-quinazolinone.

\[
\begin{align*}
\text{Ar} &= \text{2-Chlorophenyl, 4-nitrophenyl, 2-hydroxy phenyl etc.}
\end{align*}
\]

11) P. Rajendra Kumar and M. Satyanarayana Reddy\(^{11}\) have reported synthesis of 2-substituted benzo[g] quinazolin-4(3H)-ones and 2,3 dihydro-2,2-disubstituted benzo[g] quinazolin-4-(1H)-ones.
12) R.H. Udupi, B. Ramesh\textsuperscript{12} have reported synthesis and biological activity of some quinazolinone derivatives.

\[
\begin{align*}
\text{R} & = 2\text{hydroxy-3-methylphenyl, 4-chlorophenylamino} \\
& \quad 2\text{hydroxy phenyl amino etc}
\end{align*}
\]

13) V.K. Pandey, Mukesh and meenal tandon\textsuperscript{13} have reported synthesis and antiviral activity of quinazolinyl sydnones.

\[
\begin{align*}
\text{Ar} & = \text{C}_6\text{H}_5, \text{C}_6\text{H}_5 \text{CH} = \text{CH}, 2\text{-Cl -C}_6\text{H}_4 \text{ etc}
\end{align*}
\]

14) Ashok Kumar, Shalabh Sharma, Kiran Bajaj, Deepti Bansal, Shipra Sharma, K.K. Sexena, S. Lata\textsuperscript{14} have reported synthesis and antiinflammatory, analgesic, ulcerogenic and cyclooxygenase activities of novel quinazolinyl-$\Delta^2$-pyrazoline.

\[
\begin{align*}
\text{X} & = \text{H, 6Br,} \\
\text{R} & = R' = R'' = 2\text{-Cl /4 -Cl /2 -OCH}_3 /4 -\text{OCH}_3
\end{align*}
\]
15) R.D. Chakole, N.D. Amnerkar, P.B. Khedekar and K.P. Bhusari\textsuperscript{15} have reported synthesis of substituted benzothiazole derivatives of thioquinazoline as anticonvulsant agents.

![Chemical structure](image)

$R_1 = H, Br$
$R_2 = H, Br$
$R_3 = H, Cl, OCH_3$
$R_4 = H, OCH_3, NO_2$

16) V. Murugan\textsuperscript{*}, M. Vijaya Baskaran, G.V.S. Rama Sharma, M. Ramanathan and B. Suresh\textsuperscript{16} have reported synthesis and esterogenic screening of some new quinazolinonyl thiazoles.

![Chemical structure](image)

$R = H, NO_2$
$R' = H, Br, OCH_3, CH_3$

17) Archana, V.K. Srivastava, Ramesh Chandra and Ashok Kumar\textsuperscript{17} have reported synthesis of potential quinazolinoyl pyrazolines and quinazolinyl isoxazoline as anticonvulsant agents.

![Chemical structure](image)

$X = H, 6$-Br
$R = H, P$-OCH_3, $P$-OH, $M$-OCH_3
18) V. Murugan, N.J. Shah, E.P. Kumar, B. Suresh and V.M. Reddy\textsuperscript{18} have reported synthesis of 2-\{(Bis(2-chloroethyl)amino\]-methyl\}-1-substituted quinazolinones as possible alkylating agents.

![Chemical structure image](image)

\[ R = \text{H, Br, Cl, NO}_2, \text{OCH}_3 \]

19) Ashok Kumar, Mirdula Tyagi and V.K. Srivastava\textsuperscript{19} have reported synthesis of some newer potential quinazolinones as hypotensive agents.

![Chemical structure image](image)

20) V. Murugan, N.P. Padmavathy, G.V.S. Ramasarma, Sunil V. Sharma and B. Suresh\textsuperscript{20} have reported synthesis of some quinazolinone derivatives as possible anticancer agents.

![Chemical structure image](image)

\[ X = \text{H, Br, Cl, I} \\
X' = \text{H, Br, Cl} \]
21) S.G. Abdel-Hamid\textsuperscript{21} have reported synthesis of some new quinazoline derivatives.

\[
\begin{array}{c}
\text{I} \\
\text{Ph} \\
\text{I}
\end{array}
\]

22) Varsha Jatav, S.K. Jain, S.K. Kashaw and P. Mishra\textsuperscript{22} have reported synthesis and antimicrobial activity of novel 2-methyl-3-(1'3'4'-thiadiazoyl)-4-(3H) quinazolinones.

\[
\begin{array}{c}
\text{6} \\
\text{5} \\
\text{9} \\
\text{1} \\
\text{2} \\
\text{11}
\end{array}
\]

23) Amar R. Desai and Kishor R. Desai\textsuperscript{23} have reported Niementowski reaction: microwave induced and conventional synthesis of quinazolinones and 3-methyl-1\textit{H}-5-pyrazolones and their antimicrobial activity.

\[
\begin{array}{c}
\text{Y} \\
\text{R} \\
\text{X}
\end{array}
\]

\[
\begin{array}{c}
\text{Y = H, Br} \\
\text{R = CH}_3, \text{C}_6\text{H}_5 \\
\text{X = 4, C}_6\text{H}_4, \text{CH}_2
\end{array}
\]

24) S.A. Afsah, Jawaid Ahmad*, R. Purbey and A. Kumar\textsuperscript{24} have reported synthesis of some new heterocyclic systems bearing 2-methyl quinazolin-4(3H)-ones and their antimicrobial effect.

\[
\begin{array}{c}
\text{X} \\
\text{COOH} \\
\text{X} \\
\text{NH}_2
\end{array}
\] reflux

\[
\text{X} \\
\text{CH}_3
\]

\[
\text{X} \\
\text{Ar} \\
\text{CH}_3
\]
Objective Of The Present Work

The literature survey reveals that 2-amino benzothiazole were reported to possess various pharmacological activities including anti cancer, anti-inflammatory, anti tubercular, anti oxidant, anti microbial, anti convulsant, and analgesic activities. Benzothiazole with substitution at 7th position has been reported to be associated with various activities.

In continuation of this work on benzothiazole, above observations promoted we to synthesise the title compounds with presumption that incorporation of amino moiety would produce new compounds with potent biological activities.
**Steps Involved In Plan Of Work**

- Synthesis of 2-amino-7-chloro-6-fluoro benzothiazole.
- Synthesis of 2, 3-dihydroquinoline-4(1H)-one.
- Synthesis of mannich bases of 2, 3-dihydroquinoline-4(1H)-one.
- Synthesis of title compounds
- Identification and characterization
- Melting point, $R_f$ values, Solubility
- Spectral studies

The present work was characterized by IR, NMR and Mass spectral analysis data

**Pharmacological Evaluation**

- Evaluation of Anti-oxidant activity
- Anti-bacterial activity
- Anti-fungal activity
SYNTHETIC SCHEME

R=H, o,m,p-CH₃, m,p OCH₃, NH₂
R'=NH(CH₃), NH(C₂H₅), NH(C₆H₅), NH₂(CH₂CH₂C₆H₅), Morpholine, Pyrrolidine, Tyrosine,
EXPERIMENTAL WORK

Materials And Methods

The following experimental methods were used for the characterization of the syntheised compounds.

- Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected.
- IR spectra were recorded on ABB BOMEM FTIR spectrometer using potassium bromide pellets.
- \(^1\)H-NMR spectra of the compounds in deuteriated dimethyl sulfoxide was recorded on BRUKER Av 400 spectrometer.
- Mass spectra were recorded on GCMS QP 5000 Shimadzu.

Thin layer chromatography was performed using pre-coated aluminium plates, coated with silica gel GF\(_{254}\) [E.Merck]. n-Butanol: Ethyl acetate: Benzene [1: 2: 1] was used as the eluent. The spots were visualized in the iodine chamber.

Method Of Synthesis

Step 1: Synthesis of 2-amino-7-chloro -6-fluoro- (1, 3) benzothiazole.

To glacial acetic acid (20mL) cooled below room temperature were added 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01 mol) of chloro fluoro aniline. The mixture was placed in freezing mixture of ice and salt and mechanically stirred while 1.6mL of bromine in 6mL of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rose beyond room temperature. After all the bromine was added (105min), the solution was stirred for 2 hrs below room temperature and at
room temperature for 10 hrs, it was then allowed to stand overnight, during which period an orange precipitate settle at the bottom, water (6mL) was added quickly and slurry was heated at 85°C and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85°C and filtered hot. The combined filtrate was cooled and neutralized with ammonia solution to the pH range 6.0 A dark yellow precipitate was collected. Recrystallized from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow crystals of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole. After drying in an oven at 80°C, the dry material (1gm 51.02%) melted at 210-212°C.

\[
\begin{align*}
\text{F} & \quad \text{Cl} & \quad \text{NH}_2 \\
\text{KSCN} / \text{Br}_2 & \quad \xrightarrow{\text{CH}_3\text{COOH} / \text{NH}_3} & \quad \text{H}_2\text{N} - \text{S} - \text{N} & \quad \text{F} \\
\text{Cl} & \quad \text{F}
\end{align*}
\]

**Step 2: Synthesis of 3-(7-chloro-6-fluoro benzothiazol-2-yl)-2-methyl-2, 3 dihydroquinazolin-4(1H)-one**

Anthranilic acid (0.01 M) and acetic anhydride were refluxed under anhydrous condition for 4 h. Excess of acetic anhydride was distilled off under reduced pressure. To the mixture obtained, 2-amino-7-chloro-6-fluoro benzothiazole (0.01 M) in glacial acetic acid was added and refluxed for 4h and the obtained reaction mixture was poured into crushed ice and kept overnight. The solid which separated out was filtered, thoroughly washed with cold distilled water, dried and recrystallized from hot ethanol (95%).
Step 3: Synthesis of 3-(7-chloro-6-fluoro benzothiazol-2-yl)-1-((diethyl amino) methyl)-2-methyl-2, 3-dihydroquinazolin-4(1H)-one.

The solution of 3-(7-chloro-6-fluoro benzothiazol-2-yl)-2-methyl-2, 3-dihydroquinazolin-4(1H)-one (0.01 mol), formaldehyde (40%, 1.5 ml) and secondary amine (diethyl amine) (0.01 mol) and ethanol (20 ml) was stirred for 3 hrs. and left overnight at room temperature. The solid mass separated was collected by filtration, washed with ethanol, dried and recrystallized using ethanol.
Step 4: Synthesis of various substituted 3-(7-chloro-6-fluoro benzothiazol-2-yl)-1-
((diethyl amino) methyl)-2-methyl-2, 3-dihydroquinazolin-4(1H)-one.

To 0.0025 mol of 3-(7-chloro-6-fluoro benzothiazol-2-yl)-1-diethylamino) methyl)-2-methyl-2, 3-dihydroquinazolin-4(1H)-one was treated with equimolar quantities of various aromatic amines, refluxed for 2 hrs in presence of N, N'-dimethyl formamide (DMF). The mixture was cooled and poured in to crushed ice. The solid separated was filtered off, dried and recrystallized from alcohol and benzene.
<table>
<thead>
<tr>
<th>SL.No</th>
<th>COMPOUND CODE</th>
<th>STRUCTURE AND CHEMICAL NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q₁</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(phenylamino) benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>2</td>
<td>Q₂</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(o-toluidino) benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>3</td>
<td>Q₃</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(m-toluidino) benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-(1H)-one</td>
</tr>
<tr>
<td>4</td>
<td>Q₄</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(p-toluidino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>Q₅</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(m-anisidino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>6</td>
<td>Q₆</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(p-anisidino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>7</td>
<td>Q₇</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(dimethylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>8</td>
<td>Q₈</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(diethylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>----</td>
<td>-----</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9</td>
<td>Q₉</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(diphenylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>10</td>
<td>Q₁₀</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(2-aminophenylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
<tr>
<td>11</td>
<td>Q₁₁</td>
<td>1-((diethylamino)methyl)-3-(6-fluoro-7-(N-β-phenylethylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one</td>
</tr>
</tbody>
</table>
12. $Q_{12}$

1-((diethylamino)methyl)-3-(6-fluoro-7-(pyrrolidin-1-yl)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one

13. $Q_{13}$

1-((diethylamino)methyl)-3-(6-fluoro-7-(N-tyrosinyl)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one

14. $Q_{14}$

1-((diethylamino)methyl)-3-(6-fluoro-7-morpholino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one
<table>
<thead>
<tr>
<th>COMPOUND CODE</th>
<th>AMINES USED</th>
<th>QUANTITY TAKEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q 1</td>
<td>Aniline</td>
<td>0.93mL</td>
</tr>
<tr>
<td>Q 2</td>
<td>o-Toludine</td>
<td>1.08ml</td>
</tr>
<tr>
<td>Q 3</td>
<td>m-Toludine</td>
<td>0.9mL</td>
</tr>
<tr>
<td>Q 4</td>
<td>p-Toludine</td>
<td>0.54gm</td>
</tr>
<tr>
<td>Q 5</td>
<td>m-Anisidine</td>
<td>0.84gm</td>
</tr>
<tr>
<td>Q 6</td>
<td>p-Anisidine</td>
<td>0.25ml</td>
</tr>
<tr>
<td>Q 7</td>
<td>Dimethyl amine</td>
<td>0.25ml</td>
</tr>
<tr>
<td>Q 8</td>
<td>Diethyl amine</td>
<td>0.5ml</td>
</tr>
<tr>
<td>Q 9</td>
<td>Diphenyl amine</td>
<td>0.84gm</td>
</tr>
<tr>
<td>Q 10</td>
<td>o-Phenylene diamine</td>
<td>0.54gm</td>
</tr>
<tr>
<td>Q 11</td>
<td>β-Phenyl ethyl amine</td>
<td>1.25ml</td>
</tr>
<tr>
<td>Q 12</td>
<td>Pyrrolidine</td>
<td>0.41ml</td>
</tr>
<tr>
<td>Q 13</td>
<td>Tyrosine</td>
<td>0.9gm</td>
</tr>
<tr>
<td>Q 14</td>
<td>Morpholine</td>
<td>0.86ml</td>
</tr>
</tbody>
</table>
IDENTIFICATION AND CHARACTERIZATION

Introduction

The identification and characterization of the prepared compounds were carried out by the following procedure to ascertain that all the prepared compounds have different chemical nature than the respective parent compounds.

1. Melting Point,
2. Solubility,
3. Thin layer chromatography,
4. Ultra violet-visible spectroscopy [U.V-Vis],
5. Infrared spectroscopy [I.R],
6. Nuclear Magnetic resonance spectroscopy [N.M.R.] and

1. Melting Point Determination

The melting points of the organic compounds were determined by open capillary tube method.

Melting point is a valuable criterion of purity for an organic compound as a pure crystal is having definite and sharp melting point\(^{29-35}\). The synthesized compounds showed a minute change in melting point after re-cristallization.

2. Solubility:

The solubility of synthesized compounds were tested in arious solvents. The solubility characters were listed
3. Thin Layer Chromatography

Chromatography is an important technique to identify the formation of new compounds and also to determine the purity of the compound. The Rf value is characteristic for each of the compound.

a. Preparation of Chromatoplate:

Cleaned and dried glass plates were taken. Uniform slurry of silica Gel-G in alcohol was prepared. The slurry was then poured into the chamber of the TLC applicator, which was fixed and the thickness was set to 0.5mm. Glass plates were moved under the applicator smoothly to get a uniform coating of slurry on the plates.

The plates were dried first at room temperature and then kept in an oven for activation at 110°C for 1 hour.

b. Preparation of solvent system and saturation of chamber:

The solvent system used for the development of chromatogram was prepared carefully by mixing n-Butanol: Ethyl acetate: Benzene [1:4:1]

c. Application of sample:

The solution of the parent compounds and its target molecule were taken in small bored capillary tube and spotted at 2 cm from the base end of the plate. After spotting the plate were allowed to dry at room temperature and plates were transferred to chromatographic chamber containing solvent system for development.

d. Development of Chromatogram:

Plates were developed by ascending technique when solvent front had reached a distance of 10-12cm, they were taken out and dried at room temperature.

e. Detection of spots.

The developed spots were detected by exposing them to iodine vapours.
f. **Calculation of R<sub>f</sub> Values**:

The R<sub>f</sub> values of compounds were calculated using the formula.

\[
R_f = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent front}}
\]

In all the cases the distance travelled by the sample was found to be different from that of the parent compound spotted along with it. Thus it confirms the fact that the compounds formed were entirely different from that of the parent compound. Since, the sample gave a single spot; the compounds were taken to be free from impurities. The R<sub>f</sub> value of compounds were reported.
<table>
<thead>
<tr>
<th>S. No</th>
<th>Comp. code</th>
<th>Molecular Formula</th>
<th>Mol.Wt.</th>
<th>M.P. °C</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q₁</td>
<td>C₂₇H₂₈OSN₅F</td>
<td>489.61</td>
<td>212-214</td>
<td>71%</td>
</tr>
<tr>
<td>2</td>
<td>Q₂</td>
<td>C₂₉H₃₀OSN₅F</td>
<td>503.63</td>
<td>208-210</td>
<td>69%</td>
</tr>
<tr>
<td>3</td>
<td>Q₃</td>
<td>C₂₉H₃₀OSN₅F</td>
<td>503.63</td>
<td>213-215</td>
<td>65%</td>
</tr>
<tr>
<td>4</td>
<td>Q₄</td>
<td>C₂₉H₃₀OSN₅F</td>
<td>503.63</td>
<td>207-209</td>
<td>70%</td>
</tr>
<tr>
<td>5</td>
<td>Q₅</td>
<td>C₂₉H₃₀O₂SN₅F</td>
<td>519.21</td>
<td>213-215</td>
<td>66%</td>
</tr>
<tr>
<td>6</td>
<td>Q₆</td>
<td>C₂₉H₃₀O₂SN₅F</td>
<td>519.21</td>
<td>212-215</td>
<td>70%</td>
</tr>
<tr>
<td>7</td>
<td>Q₇</td>
<td>C₂₄H₃₁OSN₅F</td>
<td>456.22</td>
<td>198-200</td>
<td>65%</td>
</tr>
<tr>
<td>8</td>
<td>Q₈</td>
<td>C₂₆H₃₅OSN₅F</td>
<td>484.25</td>
<td>216-218</td>
<td>66%</td>
</tr>
<tr>
<td>9</td>
<td>Q₉</td>
<td>C₃₅H₃₈OSN₅F</td>
<td>595.77</td>
<td>215-217</td>
<td>50%</td>
</tr>
<tr>
<td>10</td>
<td>Q₁₀</td>
<td>C₂₇H₂₉OSN₅F</td>
<td>504.62</td>
<td>203-205</td>
<td>40%</td>
</tr>
<tr>
<td>11</td>
<td>Q₁₁</td>
<td>C₂₉H₃₂OSN₅F</td>
<td>505.65</td>
<td>210-212</td>
<td>48%</td>
</tr>
<tr>
<td>12</td>
<td>Q₁₂</td>
<td>C₂₅H₃₀OSN₅F</td>
<td>467.6</td>
<td>211-213</td>
<td>50%</td>
</tr>
<tr>
<td>13</td>
<td>Q₁₃</td>
<td>C₂₉H₃₀O₂SN₅F</td>
<td>483.6</td>
<td>215-217</td>
<td>52%</td>
</tr>
<tr>
<td>14</td>
<td>Q₁₄</td>
<td>C₃₂H₃₈O₄SN₅F</td>
<td>607.74</td>
<td>207-209</td>
<td>55%</td>
</tr>
<tr>
<td>S. No</td>
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<td>Water</td>
<td>Ethanol</td>
<td>Methanol</td>
<td>Acetone</td>
</tr>
<tr>
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<td>------------</td>
<td>-------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>Q₁</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Q₂</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Q₃</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</tr>
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<td>Q₅</td>
<td>-</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Q₆</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>-</td>
<td>+</td>
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<td>+</td>
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<td>Q₈</td>
<td>-</td>
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</tr>
<tr>
<td>9</td>
<td>Q₉</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Q₁₀</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Q₁₁</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Q₁₂</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Q₁₃</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Q₁₄</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++: freely soluble, +: soluble, -: insoluble
Table No.2.3: TLC DATA OF THE SYNTHESISED COMPOUNDS

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Compound Code</th>
<th>Solvent system for developing</th>
<th>Proportion of Components</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Q&lt;sub&gt;1&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.65</td>
</tr>
<tr>
<td>2.</td>
<td>Q&lt;sub&gt;2&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.71</td>
</tr>
<tr>
<td>3.</td>
<td>Q&lt;sub&gt;3&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.74</td>
</tr>
<tr>
<td>4.</td>
<td>Q&lt;sub&gt;4&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.73</td>
</tr>
<tr>
<td>5.</td>
<td>Q&lt;sub&gt;5&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.64</td>
</tr>
<tr>
<td>6.</td>
<td>Q&lt;sub&gt;6&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.67</td>
</tr>
<tr>
<td>7.</td>
<td>Q&lt;sub&gt;7&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.62</td>
</tr>
<tr>
<td>8.</td>
<td>Q&lt;sub&gt;8&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.59</td>
</tr>
<tr>
<td>9.</td>
<td>Q&lt;sub&gt;9&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.73</td>
</tr>
<tr>
<td>10.</td>
<td>Q&lt;sub&gt;10&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.64</td>
</tr>
<tr>
<td>11.</td>
<td>Q&lt;sub&gt;11&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.72</td>
</tr>
<tr>
<td>12.</td>
<td>Q&lt;sub&gt;12&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.69</td>
</tr>
<tr>
<td>13.</td>
<td>Q&lt;sub&gt;13&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.62</td>
</tr>
<tr>
<td>14.</td>
<td>Q&lt;sub&gt;14&lt;/sub&gt;</td>
<td>n-Butanol: Ethyl acetate: Benzene</td>
<td>1:2:1</td>
<td>0.65</td>
</tr>
</tbody>
</table>
SPECTRAL STUDIES

Ultra Violet Spectra

Molecular absorption in the UV-vis region of spectrum is characteristic of structures of the molecules. The UV-vis scanning of the compounds was carried and 3-chloro-4-fluoroaniline exhibited $\lambda_{\text{max}}$ at 265nm. The UV-vis spectra of 2-amino-6-fluoro-7-chloro benzothiazole exhibited $\lambda_{\text{max}}$ 303 and 288nm. This clearly indicates that the bathochromic shift of the compounds.

IR Spectra

The peaks in IR spectrum give an idea about the probable structure of the compound. IR region ranges between 4000-666 cm\(^{-1}\). Quanta of radiation from this region of the spectrum correspond to energy differences between different vibration levels of molecules.

The compounds were recorded on ELICO FTIR-8400 spectrophotometer shows different vibration levels of molecules by using KBr pellet technique.
<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>COMP. CODE</th>
<th>Ar (C=C) (in cm$^{-1}$)</th>
<th>Ar (C-H) (in cm$^{-1}$)</th>
<th>Ar –NH$_2$ (in cm$^{-1}$)</th>
<th>C-S (in cm$^{-1}$)</th>
<th>C-F (in cm$^{-1}$)</th>
<th>-CONH$_2$ (in cm$^{-1}$)</th>
<th>C=N (in cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Q1</td>
<td>1546</td>
<td>870</td>
<td>3472</td>
<td>1207</td>
<td>1070</td>
<td>1694</td>
<td>1305</td>
</tr>
<tr>
<td>2.</td>
<td>Q2</td>
<td>1548</td>
<td>819</td>
<td>3471</td>
<td>1211</td>
<td>1078</td>
<td>1648</td>
<td>1297</td>
</tr>
<tr>
<td>3.</td>
<td>Q3</td>
<td>1548</td>
<td>819</td>
<td>3471</td>
<td>1211</td>
<td>1078</td>
<td>1648</td>
<td>1297</td>
</tr>
<tr>
<td>4.</td>
<td>Q4</td>
<td>1548</td>
<td>819</td>
<td>3471</td>
<td>1211</td>
<td>1078</td>
<td>1648</td>
<td>1297</td>
</tr>
<tr>
<td>5.</td>
<td>Q5</td>
<td>1608</td>
<td>852</td>
<td>3405</td>
<td>1175</td>
<td>1080</td>
<td>1651</td>
<td>1247</td>
</tr>
<tr>
<td>6.</td>
<td>Q6</td>
<td>1608</td>
<td>852</td>
<td>3405</td>
<td>1175</td>
<td>1080</td>
<td>1651</td>
<td>1247</td>
</tr>
<tr>
<td>7.</td>
<td>Q7</td>
<td>1609</td>
<td>800</td>
<td>3471</td>
<td>1200</td>
<td>1070</td>
<td>1686</td>
<td>1285</td>
</tr>
<tr>
<td>8.</td>
<td>Q9</td>
<td>1587</td>
<td>867</td>
<td>3475</td>
<td>1161</td>
<td>1071</td>
<td>1646</td>
<td>1301</td>
</tr>
<tr>
<td>9.</td>
<td>Q11</td>
<td>1545</td>
<td>849</td>
<td>3475</td>
<td>1198</td>
<td>1069</td>
<td>1645</td>
<td>1281</td>
</tr>
<tr>
<td>10.</td>
<td>Q14</td>
<td>1542</td>
<td>801</td>
<td>3474</td>
<td>1238</td>
<td>1068</td>
<td>1611</td>
<td>1289</td>
</tr>
</tbody>
</table>
NMR spectroscopy enables us to record differences in magnetic properties of the various magnetic nuclei present, and to deduce in the large measure about the position of these nuclei are within the molecule. We can deduce how many different kinds of environment are there in the molecules and also which atoms are present in neighbouring groups.

The proton NMR spectra enable us to know different chemical and magnetic environments corresponding to protons in molecules.

The samples are analyzed on BRUKER 300 MHz spectrometer.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound Code</th>
<th>No of Protons</th>
<th>Hydrogen</th>
<th>$\delta$ (ppm)</th>
<th>Multiplicity</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Q4</td>
<td>30</td>
<td>Ar H N-CH$_2$ Ar NH -CH$_3$</td>
<td>6.3-7.9 2.41 4.08 2.35</td>
<td>Multiplet Singlet Singlet Singlet</td>
<td>DMSO</td>
</tr>
<tr>
<td>2.</td>
<td>Q5</td>
<td>30</td>
<td>Ar H N-CH$_2$ Ar NH OCH$_3$</td>
<td>6.35-7.77 2.97 4.00 3.73</td>
<td>Multiplet Singlet Singlet Singlet</td>
<td>DMSO</td>
</tr>
<tr>
<td>3.</td>
<td>Q10</td>
<td>29</td>
<td>Ar H N-CH$_3$ Ar NH</td>
<td>6.21-7.77 2.41 4.05</td>
<td>Multiplet Singlet Singlet</td>
<td>DMSO</td>
</tr>
<tr>
<td>4.</td>
<td>Q11</td>
<td>34</td>
<td>Ar H Ar NH N-CH$_2$</td>
<td>6.77-7.9 4.12 2.97</td>
<td>Multiplet Singlet Singlet</td>
<td>DMSO</td>
</tr>
</tbody>
</table>
MASS SPECTROSCOPY

Mass spectroscopy enables us to know

a) Relative molecular masses (molecular weights) with very high accuracy, from this exact molecular formula can be deduced.

b) To detect within the molecule the places at which it prefers fragmentation, from this we can deduce the presence of recognizable groups with molecule.

c) As a method of identifying analytes by comparison of their mass spectra with libraries of digitalised mass spectra of known compounds.

Mass spectra of title compounds are recorded on LCMS-2010A

**Spectral data:**

Compound code: Q6

Molecular Formula: C_{28}H_{30}O_{2}SN_{5}F

Calculated Molecular Weight: 519.21

Observed Molecular Weight: 519.0

M^+ ion peak = M/Z peak = 519.0

Mass Spectrum of the compound (Q6) peak= 519 for which the molecular weight of Q6 1-((diethylamino)methyl)-3-(6-fluoro-7-(dimethylamino)benzo[d]thiazol-2-yl)-2-methyl-2,3-dihydroquinazolin-4(1H)-one was 519.
PHARMACOLOGICAL EVALUATION

Evaluation Of Anti-Inflammatory Activity (In-Vitro)\textsuperscript{29-33}

Introduction

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair. It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations.

Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis.

In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophils chemo taxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short - lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin -1).


In vitro models of anti inflammatory activity

- Inhibition of albumin denaturation
- Membrane stabilization test
- Proteinase inhibitory action

**Experimental Protocol**

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique.

The standard drug and test compounds were dissolved in minimum amount of dimethyl sulfoxide (DMSO) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMSO in all solutions was less than 2.0%. Test solution (1 mL) containing different concentrations of drug was mixed with 1 mL of 1% mM egg albumin solution in phosphate buffer and incubated at 27°±1°C in incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60°±1°C in water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The Diclofenac sodium was used as standard drug.

\[
\text{% of inhibition} = \left( \frac{V_c - V_t}{V_c} - 1 \right) \times 100
\]

- **Preparation of solution:**
- **Preparation of Phosphate buffer solution:**
  - Dissolve 6.8 gm of potassium dihydrogen orthophosphate into 250 ml of distilled water.
• Dissolve 2 gm sodium hydroxide into 250 ml of distilled water. Take 173.5 ml of this solution and add it into above prepared 250 ml solution of potassium dihydrogen orthophosphate.

• Make up the volume up to 1000 ml with distilled water.

• pH of the solution was measured with digital pH meter and adjusted by using 0.2M NaOH.

❖ Preparation of 0.01mM of albumin solution:

• Dissolve 0.660gm of Bovine albumin into the 100ml of freshly prepared phosphate buffer solution.

❖ Preparation of test solution (stock solution):

• Dissolve 5mg of synthesized drug into the 5ml of DMF solution.

• Preparation of 0.25 mg solution: Take 0.25ml of solution from the above prepared stock solution into the test tube. Add 1ml of albumin solution and make up the volume up to 10ml with phosphate buffer.

• Preparation of 0.50 mg solution: Take 0.50ml of solution from the above prepared stock solution into the test tube. Add 1ml of albumin solution and make up the volume up to 10ml with phosphate buffer.

• Preparation of 0.75 mg solution: Take 0.75ml of solution from the above prepared stock solution into the test tube. Add 1ml of albumin solution and make up the volume up to 10ml with phosphate buffer.
<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of the compounds</th>
<th>Absorbance value (Mean ± SE)</th>
<th>Inhibition of denaturation (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Control</td>
<td>0.083</td>
<td>-</td>
</tr>
<tr>
<td>02</td>
<td>Diclofenac Sodium</td>
<td>0.153</td>
<td>84.33</td>
</tr>
<tr>
<td>03</td>
<td>Q 1</td>
<td>0.120</td>
<td>44.57</td>
</tr>
<tr>
<td>04</td>
<td>Q 2</td>
<td>0.121</td>
<td>45.78</td>
</tr>
<tr>
<td>05</td>
<td>Q 3</td>
<td>0.122</td>
<td>46.98</td>
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<tr>
<td>06</td>
<td>Q 4</td>
<td>0.129</td>
<td>55.42</td>
</tr>
<tr>
<td>07</td>
<td>Q 5</td>
<td>0.130</td>
<td>56.62</td>
</tr>
<tr>
<td>08</td>
<td>Q 6</td>
<td>0.131</td>
<td>57.83</td>
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<td>09</td>
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<td>0.124</td>
<td>49.39</td>
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<tr>
<td>10</td>
<td>Q 8</td>
<td>0.121</td>
<td>45.78</td>
</tr>
<tr>
<td>11</td>
<td>Q 11</td>
<td>0.125</td>
<td>50.60</td>
</tr>
<tr>
<td>12</td>
<td>Q 14</td>
<td>0.122</td>
<td>46.98</td>
</tr>
</tbody>
</table>
Inhibition of Denaturation

Compound Code

Diluted Sodium Q.1 Q.2 Q.3 Q.4 Q.5 Q.6 Q.7 Q.8 Q.11 Q.14

Inhibition of Denaturation
Introduction

Antioxidants are gaining a lot of importance as a panacea for a large number of life-style diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases etc. owing to our sedentary way of life and stressful existence. Added to these are the deleterious effects of pollution and exposure to harmful chemicals. All the above cause an accumulation of harmful free radicals.

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive oxygen containing molecules. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by the free radicals appears to be a major contribution to aging and degenerative disease of aging such as cancer, cardiovascular diseases, cataract, immune system decline, liver disease, diabetes mellitus, inflammation, renal failure, brain dysfunction.

In vitro models for evaluation of antioxidant activity are

- Conjugated diene assay
- DPPH Method (1, 1-diphenyl-2- picryl hydrazyl) Method
- Super oxide radical scavenging activity
- Nitric oxide radical inhibition activity
- Reducing Power Method
- Phosphomolybdenum Method
- Peroxynitrite radical scavenging activity
- ABTS (2, 2-azinobis (3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt) Method
- DMPD (N, N-dimethyl-p-phenylene diamine dihydrochloride) Method
- Oxygen Radical Absorbance Capacity (ORAC)
- β-Carotene Linolate model
- Xanthine oxidase Method
- FRAP (Ferric Reducing Ability of Plasma)
- Total Peroxy Radical Trapping Potential (TRAP) Method
- Cytochrome C test
- Erythrocyte ghost system
- Microsomal lipid peroxidation or Thiobarbituric acid (TBA) assay

**Experimental Protocol**

**Method:** HYDROGEN PEROXIDE SCAVENGING METHOD

- **Reagents:**
  - Hydrogen peroxide
  - DMSO
  - Phosphate buffer saline (pH-7.4)

- **Standard:** Ascorbic acid

**Procedure:**

All the compounds and the standard were dissolved in DMSO as a solvent - stock solution (100µg/100ml) and from stock solution various concentrations (two fold dilutions) of 50, 100, 200, 400 and 800µg/ml were prepared in different volumetric flasks. To each solution 2 ml hydrogen peroxide was added and the volume was made to 10 ml with phosphate buffer saline (pH-7.4). A control solution was prepared with DMSO in phosphate buffer saline without drug. The absorbance at 230nm was recorded using U.V spectrophotometer against blank (Phosphate buffer saline). The % inhibition by hydrogen peroxide scavenging activity was calculated using the following formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]
Table No.2.7: *In Vitro* ANTI OXIDANT ACTIVITY

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>1mg/ml</th>
<th>2mg/ml</th>
<th>3mg/ml</th>
<th>4mg/ml</th>
<th>5mg/ml</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q 1</td>
<td>3.1</td>
<td>5.9</td>
<td>10.3</td>
<td>14.9</td>
<td>19.8</td>
<td>12.19</td>
</tr>
<tr>
<td>Q 2</td>
<td>1.83</td>
<td>2.99</td>
<td>10.58</td>
<td>19.9</td>
<td>28.4</td>
<td>8.21</td>
</tr>
<tr>
<td>Q 3</td>
<td>18.8</td>
<td>29.5</td>
<td>50.1</td>
<td>59.7</td>
<td>65.4</td>
<td>4.67</td>
</tr>
<tr>
<td>Q 4</td>
<td>2.3</td>
<td>10.5</td>
<td>16.3</td>
<td>28.9</td>
<td>36.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Q 5</td>
<td>10.23</td>
<td>13.36</td>
<td>24.6</td>
<td>38.3</td>
<td>49.3</td>
<td>5.22</td>
</tr>
<tr>
<td>Q 6</td>
<td>4.6</td>
<td>9.7</td>
<td>15.7</td>
<td>19.7</td>
<td>26.9</td>
<td>9.35</td>
</tr>
<tr>
<td>Q 7</td>
<td>3.1</td>
<td>7.9</td>
<td>12.3</td>
<td>17.9</td>
<td>24.6</td>
<td>9.95</td>
</tr>
<tr>
<td>Q 8</td>
<td>11.8</td>
<td>19.1</td>
<td>25.7</td>
<td>31.8</td>
<td>42.5</td>
<td>7.29</td>
</tr>
<tr>
<td>Q 9</td>
<td>14.4</td>
<td>20.2</td>
<td>29.5</td>
<td>34.8</td>
<td>47.4</td>
<td>6.83</td>
</tr>
<tr>
<td>Q 10</td>
<td>19.4</td>
<td>27.4</td>
<td>38.5</td>
<td>46.4</td>
<td>57.3</td>
<td>6.04</td>
</tr>
<tr>
<td>Q 11</td>
<td>20.9</td>
<td>31.7</td>
<td>45.2</td>
<td>56.5</td>
<td>66.9</td>
<td>5.06</td>
</tr>
<tr>
<td>Q 12</td>
<td>7.4</td>
<td>14.8</td>
<td>39.3</td>
<td>47.2</td>
<td>57.8</td>
<td>4.25</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>39.6</td>
<td>45.7</td>
<td>53.9</td>
<td>59.5</td>
<td>68.3</td>
<td>2.55</td>
</tr>
</tbody>
</table>
ANTI MICROBIAL ACTIVITY

Introduction

The chemical substances which act against the microorganism are known as “antimicrobial agents”, whereas the substances which act against bacteria are called antibacterial agents and those which act against fungi are called anti-fungal agents. Antimicrobial agents can be obtained from both natural and synthetic methods. The production of these synthetic agents is a lengthy and expensive process.

The modern era of antimicrobial chemotherapy began in 1929 with Fleming’s discovery of powerful bactericidal substance penicillin, and Domagk’s discovery of synthetic sulphonamides with broad antimicrobial activity in 1935.

In the 1940’s World War II as surplus amount of antibacterial agents were required. Penicillin was isolated purified and injected into animals, where it was found to cure infections. This fact ushered into being the age of antibiotic chemotherapy and similar antimicrobial agents of toxicity to animals that might prove useful in the infectious disease.

Antimicrobial agents produced by microorganisms that kill or inhibit the microorganism are known as antibiotics. A more broadened definition included any chemical of natural origin (from any type of cell) which has the effect on the growth of other type cells. Since most clinically-useful antibiotics are produced by microorganisms and are used to kill or inhibit infections bacteria.

The increasing incidence of systematic fungal infections in hospitalized patients, coupled with the shortage of effective and safe antifungal agents have stimulated renewed research interest in search for broad spectrum antifungal agents.
Currently, soil microbes remain as the richest source of new antibiotic agents. In 1990, the world consumed literally tons of antibiotics valued in excess of 7 billion dollars more than half of these antibiotics were of the \( \beta \)-lactam type.

**Antibacterial Activity**

The following conditions must be accomplished for the determination of proper antibacterial activity.

1. There should be intimate contact between the test organism and substance to be evaluated.

2. Microorganism should be provided with the required condition for growth.

3. Measurement of activity should be done correctly.

4. Aseptic environment should be maintained.

5. Condition should be maintained unchanged throughout the study.

Various methods with their own advantages and limitations have been used from time to time to evaluate the antimicrobial activity of the drug. The antimicrobial activity can be evaluated by the following techniques.

1. Agar streak dilution method

2. Serial dilution method

3. Agar diffusion method
   a. Cup plate method
   b. Cylinder method
   c. Paper disc method

4. Turbidimetric method

In the present study, the well diffusion method was used to evaluate the antimicrobial activities of the synthesized compounds in vitro. The well diffusion method is one the methods that may be used for determining the relative effectiveness of the
antibacterial activity. The result obtained by this method depends not only on the toxicity of the antimicrobial agent but also on its liability to diffuse through the medium. The standard antibiotic used in the present study was ciprofloxacin.

**Sensitive microorganisms are**

1. Gram –ve: *E.coli* and various species of *Salmonella, Shigella, Enterobacter, Campylobacter* and *Neisseria*.

2. Gram +ve (less sensitive): *Streptococci, Staphylococci* and *Histeria* species.

In the present study the following bacteria were used

1. *Bacillus subtilis* (ATCC 6051)
2. *Staphylococcus aureus* (ATCC 12600)
3. *Klebsiella pneumonia* (ATCC 13883)
4. *Escherichia coli* (ATCC 11775)

The antibacterial activity of compounds (Q1-Q14) was studied by disc plate method. Compounds were used in the concentration of 50µg/ml, 100µg/ml and 150µg/ml using a solvent DMSO. Ciprofloxacin same concentrations as test were used as standards.

**Media used**

Nutrient Agar was used as the media for the study.

**Nutrient Agar Composition**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>INGREDIENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Beef Extract</td>
<td>3 g</td>
</tr>
<tr>
<td>2.</td>
<td>Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>4.</td>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>5.</td>
<td>Distilled Water</td>
<td>Up to 1000mL</td>
</tr>
</tbody>
</table>
The zone of inhibition of various concentrations of the synthesized compounds against gram positive and gram negative bacteria was measured and were tabulated. All the compounds possess potent to moderately potent activity against Gram-positive and Gram-negative bacteria.

**Materials and Methods:**

**Media used** - Nutrient Agar 1.5%

**Media Sterilization**

All the culture media were sterilized by autoclaving at 15 lbs/inch² corresponding to 20 min.

**Method** - Agar streak dilution method

**Preparation of agar plates with different concentrations of test compounds**

5µg/ml stock solution of the test compound were made using DMSO as the solvent. From these stock solutions, required quantities of drug solutions were mixed with the known quantities of molten sterile agar media aseptically to provide the following concentrations 50 and 100µg/ml.

About 20ml of the media containing the drug was dispensed into each sterile Petri-dish (diameter about 10cm). Then the media were allowed to get solidified.

**Streaking of microorganisms**

Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated in the incubator, set at 37± 1°C for 24 hrs. Then the plates were observed for the growth of the microorganisms.

**Preliminary Screening**

The antibacterial activities are performed by disc plate method. The fresh culture of bacteria are obtained by inoculating bacteria nutrient broth media and incubated at 37 ±
2°C for 18 – 24 hours. This culture mixed with nutrient agar media and poured into Petri dishes by following aseptic techniques. After solidification of the media, the plates were placed in a refrigerator for 2 hours. After two hours of cold incubation, four discs are made at equal distance by using sterile what man paper (5 mm diameter).

Dip these discs in to different concentrations. Dimethyl sulphoxide was used as a control. After introduction of standard drugs and synthesized compounds, the plates were placed in a refrigerator for 2hrs for proper dipping of drug into the media. After 2hrs the plate were placed in an incubator and maintained at 37°C ± 2°C for 18-24 hours. After the incubation period, over mean the Petri plates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs. The results are the mean value of zone of inhibition measured in millimetre of two sets. The results are tabulated in the Table. The standard drug was dissolved in Dimethyl sulphoxide and the compounds were dissolved in minimum quantity of DMSO. The Ciprofloxacin used as a standard drug.
Table No.2.8: ANTI-BACTERIAL ACTIVITY

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>ANTI BACTIRIAL ACTIVITY (Mean zone of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. Subtilis</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
</tr>
<tr>
<td>Q₁</td>
<td>11</td>
</tr>
<tr>
<td>Q₂</td>
<td>7</td>
</tr>
<tr>
<td>Q₃</td>
<td>11</td>
</tr>
<tr>
<td>Q₄</td>
<td>9</td>
</tr>
<tr>
<td>Q₅</td>
<td>11</td>
</tr>
<tr>
<td>Q₆</td>
<td>10</td>
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<td>Q₇</td>
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<td>Q₉</td>
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<td>Q₁₀</td>
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<tr>
<td>STANDARD</td>
<td>13</td>
</tr>
<tr>
<td>CONTROL</td>
<td>8</td>
</tr>
</tbody>
</table>
ANTIFUNGAL ACTIVITY

The antifungal activity of the synthesized compounds was studied by disc diffusion method.

The standard drug selected for antifungal activity was Fluconazole. It is orally active broad spectrum antifungal agent.

The antifungal activity of the synthesized compounds was studied against the following organisms.

1. *Aspergillus niger* (ATCC 9029)
2. *Aspergillus flavus* (ATCC 46645)

Compounds (were used in the concentrations 100 and 150µg/ml and using a solvent system consisting of DMSO. The standard used was Fluconazole against both the organisms.

The disc diffusion method was employed for the screening of antifungal activity

**Materials and Methods:**

**Media used**

Potato Dextrose Agar Medium

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>INGREDIENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>2.</td>
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</tr>
<tr>
<td>3.</td>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>4.</td>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>5.</td>
<td>Potato</td>
<td>200 g</td>
</tr>
<tr>
<td>6.</td>
<td>Distilled Water</td>
<td>Up to 1000mL</td>
</tr>
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</table>
Microorganisms used

1. *Aspergillus niger* (ATCC 9029)
2. *Aspergillus flavus* (ATCC 46645)

Media used

Potato Dextrose Agar Medium

Preparation of agar plates with different concentrations of test compounds

5mg/ml stock solution of the test compound were made using DMSO as the solvent. From these stock solutions, required quantities of drug solutions were mixed with the known quantities of molten sterile agar media aseptically to provide the following concentrations 100 and 150µg/ml for fungus.

About 20ml of the media containing the drug was dispensed into each sterile Petri dishes (diameter about 10cm). Then the media were allowed to get solidified.

Streaking of microorganisms

Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated in the incubator, set at 37± 1°C for 48 hrs. Then the plates were observed for the growth of the microorganisms. The lowest concentration of the test compounds showing no growth of given bacteria has been reported here as minimum inhibitory concentration (MIC) of the test compound against the name of the fungus. The MIC values of each compound against the name of the fungus were tabulated.

Preliminary Screening

The synthesized compounds are screened against two selected fungal strains *Aspergillus niger* and *Aspergillus flavus* by using diffusion method. The 48 hours old fungal culture inoculated into nutrient broth by following aseptic techniques and incubated
for 48 hours at $37^0\pm2^0C$ in an incubator. This culture mixed with well sterilized and cooled media like Potato-dextrose agar media and poured into Petri plates. After solidification five discs are made at equal distance by using sterile Whatman filter paper (5 mm in diameter). Into these place different concentrations of standard drug and synthesized compounds along with control (N, N’- Dimethyl Sulphoxide) are introduced.

After introduction of standard drug and compounds, these plates are placed in a refrigerator at $8^0 - 5^0C$ for 2hrs for proper diffusion after 2hr the Petri plates are transferred to incubator and maintained at $37^0\pm2^0C$ for 24-36 hours. After the incubation period, the plates were observed for zone of inhibition by using vernier scale. Results are evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug. The results are the mean value of zone of inhibition measured in millimetre of three sets. The results are tabulated in the Table No. The standard drug was dissolved in minimum quantity of distilled water and the compound was dissolved in minimum quantity of DMSO. The Fluconazole used as a standard drug. (Table No.2.8)
### Table No.2.8: ANTIFUNGAL ACTIVITY

<table>
<thead>
<tr>
<th>Sl. No</th>
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<td></td>
<td></td>
<td>Aspergillus flavus</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>1</td>
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<td>11</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
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<td>Q1</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Q2</td>
<td>7</td>
<td>8</td>
</tr>
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<td>5</td>
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<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Q5</td>
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</tr>
<tr>
<td>8</td>
<td>Q6</td>
<td>7</td>
<td>12</td>
</tr>
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<td>9</td>
<td>Q7</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>Q8</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>Q9</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>Q10</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

1. Anti-Inflammatory Activity

Compounds synthesised were screened for the anti inflammatory activity at the concentrations of 100 and 150 µg/mL by inhibition of protein denaturation method using Diclofenac sodium as standard.

Among the compounds screened for anti inflammatory activity Q6, Q5, Q4 and Q11 had shown better activity.

2. Anti-Oxidant Activity

Compounds synthesized were screened for anti oxidant activity. These compounds are screened for the activity at 1, 2, 3, 4 and 5 µg/ml using Ascorbic acid as standard. The % inhibition of the compounds at various concentrations is calculated from their absorbance values. Among the screened compounds, Q12, Q3, Q11 and Q5 had shown the better activity against the standard.

3. Anti-Bacterial Activity

Compounds synthesized were screened for anti bacterial activity using disc plate method at concentrations 50 and 100µg/ml using gram + ve and gram – ve strains. Such as Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escheria coli.

Among the synthesized screened compounds, Q10, Q4, Q8 and Q5 had shown promising anti bacterial activity against the standard.

4. Anti-Fungal Activity

Compounds synthesized were screened for anti fungal activity using disc plate method at concentrations 150 and 200µg/ml and the strains used for the screening are Aspergillus flavus, Aspergillus niger.

Among the synthesized screened compounds, Q1, Q3, Q6 and Q9 had shown the better activity against the standard.
SUMMARY AND CONCLUSION

In the present study, novel benzothiazole substituted quinazolinones moieties were synthesized and are characterized by IR, NMR and Mass spectral data.

All the synthesized compounds were screened for anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal activity.

Anti-inflammatory activity was screened for synthesized compounds using Diclofenac as standard. Compounds Q6, Q5, Q4 and Q11 had shown the better anti-inflammatory activity.

Evaluation of anti-oxidant activity was screened for synthesized compounds using Ascorbic acid as standard. Compounds Q12, Q3, Q11 and Q5 had shown the significant anti-oxidant activity.

Anti-bacterial activity of the synthesized compounds were tested against the micro-organisms, *E.coli, B.subtilis, S.aureus and K.pneumonia* using Ciprofloxacin as a standard. Compounds Q10, Q4, Q8 and Q5 had shown the better activity against the standard.

Anti-fungal activity of the synthesized compounds were tested against the micro-organisms, *A. flavus and A. niger* using Fluconazole as a standard. Compounds Q1, Q3, Q6 and Q9 had shown significant anti-fungal activity.

From this study, it may be concluded that benzothiazole substituted with morpholino, pyrolidine, -N(CH₃)₂, -N(CH₂CH₂(C₆H₅)), -N(C₆H₅) on seventh position enhances the anti-microbial, anti-inflammatory, anti-oxidant and anti mitotic activities and hence the study would deserve for future investigation and derivatisation.
REFERENCES


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