4.1 INTRODUCTION

Flavonoids represent a large class of plant natural products, exhibiting multiple biological activities. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory actions.\textsuperscript{1-8}

The hypervalent iodine reagents also find interesting applications in developing new synthetic methods for the synthesis of flavonoids. This area has primarily been developed by us. Various useful transformations effected by iodine(III) mediated oxidative approach include transformation of (a) flavones to 3-hydroxyflavones, (b) flavanones to flavones, dihydrobenzofurans and isoflavones, (c) flavonols to 2,3-dimethoxy-3-hydroxyflavanones (d) 7-hydroxyflavone to 8-iodo-7-phenoxyflavone, (e) 6-hydroxyflavone to 5-alkoxy-6-hydroxyflavone, etc. The work reported in this chapter is mainly aimed at the scope of our I(III) mediated methods for:

(i) Hypervalent iodine oxidation of 3-(3-(aryl)-1-phenyl-1\textsubscript{H}-pyrazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-ones: Cleavage of carbon-carbon double bond

(ii) Synthesis and biological evaluation of 2-(3-(4-aryl)-1-phenyl-1\textsubscript{H}-pyrazol-4-yl)chroman-4-ones and their oxidation to \textit{cis}-3-hydroxy-2-[(1-phenyl-3-(aryl)-4-pyrazolyl)]chromanone dimethylacetals

Before an account of the actual results on these studies is presented, it will be appropriate to give a summary of some of the important transformations in flavonoids by using hypervalent iodine(III) and other oxidizing reagents. For understanding the nomenclature of flavonoids, Chart 4.1 is given is given separately.
Chart 4.1. Nomenclature of flavonoids and related pyrazolyl analogues

Oxidation with Hypervalent Iodine Reagents

A novel and convenient synthesis of isoflavones (2) has been reported from our laboratory by the oxidation of flavanones (1) with HTIB in acetonitrile or iodosobenzene in dichloromethane in the presence of methanesulphonic acid (CH₃SO₃H) or
p-toluenesulphonic acid (p-TsOH). The oxidation of flavanones (1) with HTIB in boiling acetonitrile or propionitrile does not afford the expected α-functionalized products, 3-tosyloxyflavanones (3). Instead, 1,2-shift of C(2)-aryl group to C(3) occurs, thus providing a novel route for isoflavones (Scheme 4.1).  

![Scheme 4.1](image)

Isoflavones (2) are also synthesized in one-pot reaction by treating o-benzoyloxychalcones (5) with the hypervalent iodine (III) reagent HTIB in methanol, the corresponding rearranged acetals (6) formed which upon addition of water-methanol solution containing sodium hydroxide undergo cyclization to give isoflavones (2). A combined use of iodobenzene diacetate (IBD)/p-toluenesulfonic acid (p-TsOH) is also effective for the same purpose (Scheme 4.2).  

![Scheme 4.2](image)
Thermal or ultrasound waves induced oxidation of several 2,2-dialkyl substituted chromanones (7, 9) with HTIB in acetonitrile involves 1,2-alkylshift. The reaction provides a convenient route for synthesis of chromones (8), tetrahydroxathones (10) and their higher homologs (Scheme 4.3).\textsuperscript{11}

\begin{center}
\begin{tikzpicture}
\node[compound] (a) at (0,0) {\includegraphics[width=1cm]{7.png}};
\node[compound] (b) at (2,0) {\includegraphics[width=1cm]{8.png}};
\node[compound] (c) at (0,-1) {\includegraphics[width=1cm]{9.png}};
\node[compound] (d) at (2,-1) {\includegraphics[width=1cm]{10.png}};
\draw[->, >=latex] (a) -- node[below] {HTIB/CH\textsubscript{3}CN} node[above] {ultrasound 45 °C or Reflux} (b);
\draw[->, >=latex] (c) -- node[below] {HTIB/CH\textsubscript{3}CN} node[above] {ultrasound 45 °C or Reflux} (d);
\end{tikzpicture}
\end{center}

Scheme 4.3

The synthesis of flavonols (13) by the IBD induced oxidation of flavones (11) in methanol in basic medium involves the intermediacy of 2-methoxy-3-hydroxyflavanone dimethylacetal (12), which on hydrolysis with conc. HCl in acetone leads to the formation of the corresponding flavonol. Overall reaction may be referred to as C(3)-hydroxylation of flavones (Scheme 4.4).\textsuperscript{12}

\begin{center}
\begin{tikzpicture}
\node[compound] (a) at (0,0) {\includegraphics[width=1cm]{11.png}};
\node[compound] (b) at (2,0) {\includegraphics[width=1cm]{12.png}};
\node[compound] (c) at (4,0) {\includegraphics[width=1cm]{13.png}};
\draw[->, >=latex] (a) -- node[below] {IBD-KOH/ MeOH} node[above] {} (b);
\draw[->, >=latex] (b) -- node[below] {Con. HCl Acetone} node[above] {} (c);
\end{tikzpicture}
\end{center}

Scheme 4.4

HTIB induced oxidation of flavanones (1) in methanol leads to dehydrogenation with the formation of flavones (11). This transformation can also be effected by using IBD in methanol or acetonitrile at room temperature (Scheme 4.5).\textsuperscript{13,14}
Oxidation of flavanones (1) with IBD or HTIB in trimethyl orthoformate (TMOF) in the presence of an acid leads to the formation of methyl 2-aryl-2,3-dihydrobenzofuran-3-carboxylates (14) by the contraction of the pyran ring of flavanones (Scheme 4.6).\textsuperscript{15}

Oxidation of 6-hydroxyflavone (15) and 6-hydroxyflavanone (16) with iodobenzene diacetate (IBD) in acetic acid leads to regioselective acetyloxylation, thereby providing a novel and convenient route for the synthesis of 5-acetoxylated products 17 & 18, respectively (Scheme 4.7).\textsuperscript{16}
Oxidation of 7-hydroxyflavone (19) with IBD in methanol or IBD-KOH/MeOH proceeds to afford iodonium ylide (20), which undergoes 1,4-sigmatropic rearrangement giving o-iodophenyl ether (21) (Scheme 4.8).\(^\text{17}\)

![Scheme 4.8]

Oxidation of flavonols (11) with HTIB or IBD in methanol results in the introduction of two methoxy groups to the carbon-carbon double bond to generate 2,3-dimethoxy-3-hydroxyflavanones (22) (Scheme 4.9).\(^\text{18,19}\)

![Scheme 4.9]

6-Hydroxyflavone (15) on oxidation with IBD-MeOH undergoes nuclear oxidation to afford ortho (23) and para quinone monoacetals (24). p-Quinone monoacetals (24) when refluxed in dry methanol yields regioselective alkylated product, 6-hydroxy-5-methoxyflavone (25) (Scheme 4.10).\(^\text{20}\)
Various 3-haloflavones (26) have been prepared by the reaction of the corresponding flavone derivatives with iodobenzene diacetate and trimethylsilyl halide under mild reaction conditions. The iodobenzene diacetate can be replaced by the polymersupported iodobenzene diacetate without the decreasing activity (Scheme 4.11).21,22

\[
\begin{align*}
\text{R}^1, \text{R}^2 &= \text{H, OCH}_3 \\
\text{PhI(OAc)}_2 &\xrightarrow{T\text{MSX, DCM}, 0^\circ C} \text{X} = \text{Cl, Br}
\end{align*}
\]

Scheme 4.11

**Oxidation with other reagents:**

Synthesis of aurones (28) have been achieved by 2'-hydroxychalcones (27) with molar amount of mercury(II)acetate in pyridine and in catalytic amount of CuBr\(_2\) in DMSO (dimethylsulfoxide) in good yields (Scheme 4.12).23
Thakkar and Cushman synthesized aurones (30) by oxidative cyclization of 2'-hydroxychalcones (29) using Thallium(III)nitrate in MeOH (Scheme 4.13).^{24}

Treatment of o-hydroxychalcones (4) with acetic anhydride followed by addition of bromine results in the formation of o-acetoxychalcone dibromides (31), which upon heating with ethanolic potassium hydroxide give flavones (11) (Scheme 4.14).^{25}

Though this procedure, which constitutes the first synthesis of a flavone is of wide application, in certain cases intermediate yield 2-arylidene coumaran-3-ones (33), which on treatment with potassium cyanide results in the formation of flavones (Scheme 4.15).^{26-28}
Scheme 4.15

\[ \text{o-Hydroxychalcones as well as flavanones are converted into flavones on treatment with SeO}_2 \text{ in isoamyl alcohol e.g., 5,7-dimethoxyacacetin (36) is prepared by the action of SeO}_2 \text{ on 4',5,7-trimethoxyflavanone (34) or 2'-hydroxy-4',6',4-trimethoxychalcone (35) (Scheme 4.16).}^{29} \]

Scheme 4.16

Shankar et al. have shown that flavanones (1) can be easily dehydrogenated into flavones (11) on treatment with DDQ in dioxane (Scheme 4.17).^{30}
Fatma et al. reported that oxidation of o-hydroxychalcones (4) using I$_2$/DMSO leads to the formation of flavones (11), thus providing a versatile method for the preparation of flavones (Scheme 4.18).\textsuperscript{31}

Isoflavanones (37) upon oxidation with dimethylsulphoxide-iodine in the presence of sulphuric acid result in the formation of the corresponding isoflavones (2) in excellent yields (Scheme 4.19).
4.2 RESULTS AND DISCUSSION

4.2.1 Hypervalent iodine oxidation of 3-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-ones: Cleavage of carbon-carbon double bond

Previous investigations from our laboratory have shown that iodine(III) reagents, in particular, IBD, HTIB, etc find interesting applications in various synthetically useful transformations.

Oxidation of 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-ones (49) with [hydroxy(tosyloxy)iodo]benzene (HTIB) in DCM has been reported to provide a novel and convenient route for the synthesis of 3-aryl-7-methyl-pyran[4,3-b]pyran-4H,5H-diones (50) (Scheme 4.20).

![Scheme 4.20](image)

Encouraged by this result, o-hydroxychalcones 4 were treated with HTIB under similar conditions. Surprisingly, the reaction affords corresponding 5'-tosyloxy-2'-hydroxychalcones (40) in good yields (Scheme 4.21).

![Scheme 4.21](image)

The plausible mechanism suggested in this study is outlined in Scheme 4.22 and involves oxidation of phenolic group.
As we have discussed earlier that 2'-hydroxychalones contain both phenolic and $\alpha,\beta$-unsaturated carbonyl group, which are susceptible to react with I(III) reagents. On the basis of previous results from our laboratory, we became interested to study the reactions of hypervalent iodine with $o$-hydroxychalcone derived from 3-aryl-1-phenylpyrazole-4-carbaldehydes. In the reaction of $o$-hydroxychalcone of pyrazole analogues with HTIB, there is possibility of formation of various products as shown in Scheme 4.23.

Scheme 4.22
To determine which of the product(s) is actually formed, we conducted oxidation of 2'-hydroxychalcone derived from 3-(4-tolyl)-1-phenylpyrazolyl aldehyde (41a) with HTIB in dichloromethane (DCM). The progress of the reaction was monitored by TLC. After completion of the reaction, usual work-up of the reaction mixture afforded a solid product. The structure of the product obtained was confirmed by analyzing its spectral and elemental data. Surprisingly, the characterization data was not in favour of the any expected product. Instead, the data gave a clear indication of formation of aurone derivative 58a (Scheme 4.24)
Scheme 4.24

Encouraged by this result, we studied the scope of this approach to other derivatives (41b-g). Accordingly various 2'-hydroxychalcone analogues derived from different 3-aryl-1-phenylpyrazolyl aldehydes (41b-g) were subjected to oxidative cyclization with HTIB (1.1 equiv) in dichloromethane (DCM) under similar conditions. However reaction did not follow the same trend for other chalcone derivatives (41b-g). Only one compound i.e., 41e afforded aurone type product 56e.

Surprisingly, the reaction underwent smooth oxidative cleavage of C=C bond giving pyrazole-4-carboxaldehydes (49) in high yield, in other cases 41 b-d, 41 f, and 41 g (Scheme 4.25). The compounds were fully characterized by analyzing their spectral data and comparing their m.ps. with those reported in the literature. The $^1$H NMR showed a characteristic singlet at ~ $\delta$10 ppm for the presence of CHO group.
Mechanism

A plausible mechanism for the formation of aurone analogues (48a, e) is outlined in Scheme 4.26. The mechanistic explanation for C-C double bond cleavage is yet not clear.

Scheme 4.26

The mechanism outlined in Scheme 4.26 involves the intermediate ditosylate 45. Although the mechanism for cleavage is not certain, yet a plausible mechanism for the formation of 45 is shown in Scheme 4.27.
All the compounds 49, are known in the literature. So, these are confirmed by their melting points (Table 4.1).

**Table 4.1 Physical data for the compounds 48 and 49**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48a</td>
<td>77</td>
<td>189-191</td>
</tr>
<tr>
<td>2</td>
<td>49b</td>
<td>90</td>
<td>120(122)</td>
</tr>
<tr>
<td>3</td>
<td>49c</td>
<td>86</td>
<td>121-123(121)</td>
</tr>
<tr>
<td>4</td>
<td>49d</td>
<td>85</td>
<td>150(149-151)</td>
</tr>
<tr>
<td>5</td>
<td>48e</td>
<td>79</td>
<td>164-166</td>
</tr>
<tr>
<td>6</td>
<td>49f</td>
<td>83</td>
<td>131(133)</td>
</tr>
<tr>
<td>7</td>
<td>49g</td>
<td>89</td>
<td>166(165)</td>
</tr>
</tbody>
</table>

*a Melting points are uncorrected and compared with literature reports.*

2'-Hydroxychalcone analogues 41 were prepared by the condensation of 2-hydroxyacetophenone (52) with 1-phenyl-3-aryl-pyrazole-4-carboxaldehydes (49) using
THF-MeOH as solvent, which dissolved pyrazole-4-carbaldehyde easily at room temperature (Scheme 4.28).\(^{36}\)

![Scheme 4.28](image)

All the compounds 41 are known in the literature and their melting points are compared with literature melting points (Table 4.2).\(^{36}\)

**Table 4.2 Physical data of 2'-hydroxychalcone analogues of pyrazole 41 prepared according to Scheme 4.28**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>M.p.</th>
<th>Lit. M.p. (°C)</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41a</td>
<td>C(_6)H(_5)</td>
<td>161-162</td>
<td>162-163</td>
<td>82</td>
</tr>
<tr>
<td>41b</td>
<td>4-CH(_3)C(_6)H(_4)</td>
<td>143-144</td>
<td>143-145</td>
<td>80</td>
</tr>
<tr>
<td>41c</td>
<td>4-OCH(_3)C(_6)H(_4)</td>
<td>153-154</td>
<td>150-152</td>
<td>85</td>
</tr>
<tr>
<td>41d</td>
<td>4-FC(_6)H(_4)</td>
<td>183-185</td>
<td>184-186</td>
<td>82</td>
</tr>
<tr>
<td>41e</td>
<td>4-ClC(_6)H(_4)</td>
<td>165-166</td>
<td>165-166</td>
<td>87</td>
</tr>
<tr>
<td>41f</td>
<td>4-BrC(_6)H(_4)</td>
<td>170-173</td>
<td>171-173</td>
<td>86</td>
</tr>
<tr>
<td>41g</td>
<td>4-NO(_2)C(_6)H(_4)</td>
<td>193-195</td>
<td>195-197</td>
<td>77</td>
</tr>
</tbody>
</table>

The pyrazole-4-carboxaldehydes (49) were accessible through the Vilsmeier-Haack reaction of acetophenone phenylhydrazones as outlined in Scheme 4.29 (Table 4.3).
![Scheme 4.29](image)

**Table 4.3. Physical data of pyrazole-4-carboxaldehydes 49 prepared according to Scheme 4.29**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>Mp.</th>
<th>Lit. mp.</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49a</td>
<td>C₆H₅</td>
<td>138-139</td>
<td>140</td>
<td>82</td>
</tr>
<tr>
<td>49b</td>
<td>4-CH₃C₆H₄</td>
<td>121-122</td>
<td>122</td>
<td>78</td>
</tr>
<tr>
<td>49c</td>
<td>4-OCH₃C₆H₄</td>
<td>119-120</td>
<td>121</td>
<td>81</td>
</tr>
<tr>
<td>49d</td>
<td>4-FC₆H₄</td>
<td>143-145</td>
<td>146</td>
<td>74</td>
</tr>
<tr>
<td>49e</td>
<td>4-ClC₆H₄</td>
<td>112-113</td>
<td>113</td>
<td>76</td>
</tr>
<tr>
<td>49f</td>
<td>4-BrC₆H₄</td>
<td>130-132</td>
<td>133</td>
<td>80</td>
</tr>
<tr>
<td>49g</td>
<td>4-NO₂C₆H₄</td>
<td>163-164</td>
<td>164</td>
<td>85</td>
</tr>
</tbody>
</table>

Finally, iodine(III) mediated approach offers an easy and simple method for synthesizing new 2-(((1,3-diphenyl-1H-pyrazol-4-yl)methylene)benzofuran-3(2H)-one (48a) and 2-(((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)benzofuran-3(2H)-one (48e). However, the major drawback of this approach is that the method is successful only for 48a and 48e and in other cases it leads to C=C double bond cleavage. The reason for this is not clear and more detailed investigations are required to solve such problems.
4.2.2 Synthesis and biological evaluation of 2-(3-(4-aryl)-1-phenyl-1H-pyrazol-4-yl)chroman-4-ones (55) and their conversion to corresponding cis-3-hydroxy-2-[(1-phenyl-3-(aryl)-4-pyrazolyl)]chromanone dimethylacetal (57)

4.2.2.1 Chemistry

Among various classes of heterocyclic compounds, flavonoids constitute an important component of pharmacologically active compounds. On the other hand, pyrazole and its derivatives, a class of well-known nitrogen containing heterocyclic compounds, occupy an important position in the medicinal and pesticide chemistry with having a wide range of bioactivities such as antimicrobial, anti-inflammatory, antibacterial, antifungal, and herbicidal. A literature survey revealed that the title compounds 2-(3-(4-aryl)-1-phenyl-1H-pyrazol-4-yl)chroman-4-ones (55a-g) remain unknown. Led by these observations, the synthesis of some new 2-(3-(4-aryl)-1-phenyl-1H-pyrazol-4-yl)chroman-4-ones (55a-g) was undertaken with a view to evaluate their antibacterial and antifungal activities.

The synthesis of 55a-g was effected by the cyclization of 3-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-ones (41) with conc. HCl in AcOH (Scheme 4.30).

![Scheme 4.30](image)

The structures of all the synthesized Chromanones (55a-g) were confirmed by their spectral (IR, $^1$H NMR and mass) and elemental analytical data. The lack of absorption band at 1640 cm$^{-1}$ in the IR spectra of compounds 55a-g and appearance of characteristic absorption band at 1680-1690 cm$^{-1}$ due to carbonyl group showed the absence of $\alpha,\beta$-unsaturated carbonyl group, thereby suggesting the cyclic structure. The $^1$H NMR spectra of compounds 55a-g
showed three characteristic doublets of doublet due to C(2) and C(3) protons in the regions $\delta$ 2.90-3.16 and 3.13-3.74, 5.56-5.68, respectively. The C(5)–H of pyrazole ring appeared as a singlet at $\delta$ 8.12 (Fig. 4.1).

![Chemical structure](image)

**Fig. 4.1. The $^1$H NMR spectrum of 2,3-Dihydro-2-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)chromen-4-one (55c)**

As stated in the introduction of this chapter, the use of hypervalent iodine reagents has evolved versatile methodology in organic synthesis. Moriarty and Prakash reported the synthesis of cis-3-hydroxy-2-[(1-phenyl-3-(aryl)-4-pyrazolyl)]chromanone dimethylacetals (56) from oxidation of flavanone (1) using iodobenzene diacetate in methanol (IBD-KOH/MeOH) (Scheme 4.30).\(^{45}\)

![Scheme](image)

**Scheme 4.31**

A literature survey reveals that the title compounds, cis-3-hydroxy-2-[(1-phenyl-3-(aryl)-4-pyrazolyl)]chromanone dimethylacetal (57) have not been synthesized earlier by this route. These observations, coupled with the diverse biological properties associated with pyrazole
and flavanone derivatives, prompted us to study the scope of the synthetic route outlined in Scheme 4.31 on the oxidation of 2-pyrazolyl chromanones (55). In continuation of our efforts to explore the utility of iodine (III) reagents in the synthesis of a wide variety of flavonoids possessing various biological activities and with a view to compare the oxidative behaviour of hypervalent iodine reagents toward flavonols with that of other reagents used by earlier workers, we herein report the synthesis of some cis-3-hydroxy-2-[(1-phenyl-3-(aryl)-4-pyrazolyl)]chromanone dimethylacetals (57) by the oxidation of chromanones analogues of pyrazole 55 using iodobenzene diacetate (IBD) in methanol.

The reaction of 2,3-dihydro-2-(1,3-diphenyl-1H-pyrazol-4-yl)chromen-4-one (55a) was carried out by treatment with 1.1 equivalents of iodobenzene diacetate (IBD) in KOH-methanol by stirring at room temperature for overnight. Usual work-up of the reaction afforded the pure crystalline product 4,4-dimethoxy-2-(1,3-diphenyl-1H-pyrazol-4-yl)chroman-3-ol (57a) in 78% yield. Encouraged by the feasibility of our strategy for 55a, we studied oxidation of a wide range of substituted 2-(3-(aryl)-1H-pyrazol-4-yl)-2,3-dihydrochromen-4-ones (55b-55g) under similar conditions. The reaction, indeed, afforded the desired dimethyl acetals (56b-56g) (Scheme 4.32).

Scheme 4.32

The structures of all compounds 57 were confirmed by their spectral (IR, 1H NMR and mass) and elemental analytical data. 1H NMR are instructive particularly on C(2)-H and C(3)-H coupling constants (J2,3 = 4-5 Hz) agree with the assigned cis stereochemistry (Fig. 4.2). In 1H NMR of dimethylacetals, the C(3) proton couple with hydroxy proton split into doublet and C(2) proton has a broad singlet. On shaking with D2O, hydroxy proton
disappeared and the C(2)-C(3) proton-proton couple together split into doublet of coupling constant 4-5 Hz which agrees with the assigned cis stereochemistry.\textsuperscript{47} The mass spectrum of 57a also supported its structure. It showed an intense molecular ion peak at m/z 456 (100%).

\begin{center}
\includegraphics[width=0.5\textwidth]{fig42.png}
\end{center}

**Fig. 4.2.** The $^1$H NMR of cis-3-Hydroxy-2-(1,3-diphenyl-4-pyrazolyl)chromanone dimethylacetal 57a

The stereochemistry of dimethylacetals may also be arrived at from its mechanism of formation, which is considered to involve (a) enolate anion formation 55 $\rightarrow$ 57 (b) addition of 58 to C$_6$H$_5$(OMe)$_2$ (formed in situ) at the face of the molecule anti to the C(2) phenyl ring (58 $\rightarrow$ 59) (c) Addition of CH$_3$O$^-$ to the carbonyl group 59 $\rightarrow$ 60 with intramolecular reductive elimination of PhI by the thus formed alkoxide anion (with inversion at C(3)), and finally, (d) by CH$_3$O$^-$ ring opening of the epoxide with a second inversion of configuration 60 $\rightarrow$ 57 (Scheme 4.33).\textsuperscript{48}
4.2.1.2 Antimicrobial screening

All the tested compounds 55a-55g possessed variable antibacterial activity against both Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, P. aeruginosa) bacteria. On the basis of maximum inhibitory activity shown against Gram-positive bacteria, compounds 55d, 55e and 55g were found to be most effective against S. aureus with zone of inhibition of 25.1, 24.1 and 24.2 mm and in case of B. subtilis, compound 55d, 55e and 55g were most effective with zone of inhibition 22.6, 22.6 and 23.6 mm respectively. However in case of Gram-negative bacteria, compounds 55a-55g displayed moderate to low activity against E. coli and P. aeruginosa (Table 4.4, Fig.4.1).

The Minimum inhibitory concentration (MIC) of compounds ranged between 8 and 64 µg/ml against Gram-positive bacteria, compounds 55d, 55e and 55g showed highest MIC of 8 µg/ml against S. aureus whereas compounds 55e, 55g also showed highest MIC of 8 µg/ml against B. subtilis. However in case of Gram-negative bacteria, MIC of compounds ranged between 64 and 128 µg/ml and compounds 55c, 55d, 55f and 55g showed highest
MIC of 64 µg/ml against *E. coli* and compound 55c, 55d and 55e showed highest MIC of 64 µg/ml against *P. aeruginosa* (Table 4.4, Fig.4.1). All the newly synthesized compounds showed low activity against gram-negative bacteria as compared to gram-positive bacteria.

All the seven compounds 55a-55g were also screened for antifungal activity. Compounds 55c, 55d, 55g displayed good antifungal activity against *Aspergillus flavus*, whereas compounds 55a, 55c, 55d, 55g showed good activity against *Aspergillus niger* when compared with commercially available antifungal Fluconazole (Table 4.5, Fig.4.2).

From above discussion, it can be concluded that presence of substituents such as fluoro, chloro and nitro in the aryl ring of pyrazole moiety of compounds 55a-55g enhance antibacterial activity.

**Table 4.4: In vitro antibacterial activity of synthesized compounds 55a-55g**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of growth of inhibition zone (mm)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>55a</td>
<td>22.6 (16)</td>
</tr>
<tr>
<td>55b</td>
<td>19.3 (64)</td>
</tr>
<tr>
<td>55c</td>
<td>20.6 (32)</td>
</tr>
<tr>
<td>55d</td>
<td>25.1 (8)</td>
</tr>
<tr>
<td>55e</td>
<td>24.1 (8)</td>
</tr>
<tr>
<td>55f</td>
<td>20.6 (32)</td>
</tr>
<tr>
<td>55g</td>
<td>24.2 (8)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26.0 (5)</td>
</tr>
</tbody>
</table>

ᵃValues, including diameter of the well (8mm), are means of three replicates

MIC values are given in parenthesis
Table 4.4: *In vitro* antifungal activity of synthesized compounds 55a-55g

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mycelial growth inhibition (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aspergillus niger</em></td>
<td><em>Aspergillus flavus</em></td>
</tr>
<tr>
<td>55a</td>
<td>55.5</td>
<td>61.1</td>
</tr>
<tr>
<td>55b</td>
<td>55.5</td>
<td>55.5</td>
</tr>
<tr>
<td>55c</td>
<td>66.6</td>
<td>66.6</td>
</tr>
<tr>
<td>55d</td>
<td>61.1</td>
<td>66.6</td>
</tr>
<tr>
<td>55e</td>
<td>33.3</td>
<td>50</td>
</tr>
<tr>
<td>55f</td>
<td>38.8</td>
<td>55.5</td>
</tr>
<tr>
<td>55g</td>
<td>66.6</td>
<td>61.1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>81.1</td>
<td>77.7</td>
</tr>
</tbody>
</table>
Fig. 4.1: Diameter of growth of inhibition zone (mm) of synthesized compounds 55a-55g.

Fig. 4.2: Mycelial growth inhibition (%) of synthesized compounds 55a-55g.
CONCLUSIONS

i) The present study offers an easy access to new 2-(3-(4-aryl)-1-phenyl-1H-pyrazol-4-yl)chroman-4-ones (55), which are associated with strong antimicrobial activity.

ii) The oxidation of chromanones analogues of pyrazole 55 using iodine (III) offers an easy and simple method for synthesis of 2,3-dimethoxy-3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromanones (57).

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4.3 EXPERIMENTAL

4.3.1 Chemistry

Melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded on Perkine Elmer IR1800 spectrophotometer. The $^1$H NMR spectra were recorded in CDCl$_3$ on Bruker 300 MHz instrument with TMS as an internal standard. The data are reported as follows: chemical shift in parts per million (δ) and coupling constant (Hz). Elemental analyses were carried out in PerkineElmer 2400 instrument.

3-Aryl-1-phenylpyrazolyl aldehydes (49)/ 2-((3-(Aryl)-1-phenyl-1H-pyrazol-4-yl)methylene)benzofuran-3(2H)-one (48)

**General procedure:** To a stirred solution of 2'-hydroxychalcone (57, 10 mmol) in dichloromethane (50 ml) was added HTIB (12 mmol) in one portion. The resulting mixture was allowed to stir at room temperature for about 30-40 min. HTIB was highly insoluble in dichloromethane, gradually disappeared as the reaction proceeded. After completion of the reaction (as monitored by TLC), the crude product was purified by column chromatography on silica gel using 1:9 EtOAc- Hexane as eluent. As mentioned in results and discussion, compounds 57a and 57e gave aurone analogues, 48a and 48e, whereas other derivatives underwent carbon-carbon cleavage thereby giving corresponding pyrazole-4-carbaldehydes. The characterization data for the products is given below:

1-Phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde (49b)

IR (KBr, cm$^{-1}$): 1682;
\textsuperscript{1}H NMR (CDCl\textsubscript{3}, \(\delta\)): 2.45 (s, 3H), 7.32-7.43 (m, 3H), 7.50-7.56 (m, 2H), 7.72-7.83 (m, 4H), 8.55 (s, 1H), 10.07 (s, 1H).

3-(4-Methoxyphenyl)-1-phenyl-1\textit{H}-pyrazole-4-carbaldehyde (49c)

\[
\begin{array}{c}
\text{Ph} \\
\text{N} \equiv 	ext{N} \\
\text{CHO} \\
\text{OMe}
\end{array}
\]

IR (KBr, cm\textsuperscript{-1}): 1684;
\textsuperscript{1}H NMR (CDCl\textsubscript{3}, \(\delta\)): 3.902 (s, 3H), 7.38-7.43 (m, 2H), 7.50-7.55 (m, 3H), 7.99-7.82 (m, 4H), 8.54 (s, 1H), 10.06 (s, 1H).

3-(4-Fluorophenyl)-1-phenyl-1\textit{H}-pyrazole-4-carbaldehyde (49d)

\[
\begin{array}{c}
\text{Ph} \\
\text{N} \equiv 	ext{N} \\
\text{CHO} \\
\text{F}
\end{array}
\]

IR (KBr, cm\textsuperscript{-1}): 1680;
\textsuperscript{1}H NMR (CDCl\textsubscript{3}, \(\delta\)): 7.17-7.25 (m, 2H), 7.40-7.45 (m, 1H), 7.51-7.56 (m, 2H), 8.55 (s, 1H), 10.05 (s, 1H).

3-(4-Bromophenyl)-1-phenyl-1\textit{H}-pyrazole-4-carbaldehyde (49f)

\[
\begin{array}{c}
\text{Ph} \\
\text{N} \equiv 	ext{N} \\
\text{CHO} \\
\text{Br}
\end{array}
\]

IR (KBr, cm\textsuperscript{-1}): 1684;
\textsuperscript{1}H NMR (CDCl\textsubscript{3}, \(\delta\)): 7.40-7.45 (m, 1H), 7.51-7.57 (m, 2H), 7.64-7.66 (m, 2H), 7.77-7.82 (m, 4H), 8.55 (s, 1H), 10.06 (s, 1H).
3-(4-Nitrophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (49g)

IR (KBr, cm⁻¹): 1690;
¹H NMR (CDCl₃, δ): 7.44-7.49 (m, 1H), 7.54-7.60 (m, 2H), 7.81-7.84 (d, 2H, J = 7.8 Hz), 8.18-8.20 (d, 4H, J = 8.7 Hz), 8.36-8.38 (d, 1H, J = 8.7 Hz), 8.60 (s, 1H), 10.11 (s, 1H).

2-((3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)benzofuran-3(2H)-one (48e)

IR (KBr, cm⁻¹): 1743;
1H NMR (CDCl3, δ): 8.13 (s, 1H), 7.95-7.99 (dd, 1H, J = 1.5, 8.1 Hz), 7.73-7.78 (m, 4H), 7.42-7.56 (m, 6H), 7.32 (s, 1H) 7.08-7.11 (m, 2H);
13C NMR (CDCl3, δ): 176.29, 156.03, 153.72, 150.08, 139.81, 133.76, 130.31, 130.20, 129.46, 129.36, 128.34, 126.70, 126.31, 125.43, 124.10, 119.19, 118.19, 117.16, 115.94, 115.65, 110.94.
MS (m/z): 399.14 (M+1), 401.08 (M+3).

4.3.2 Chemistry

Pyrazole-4-carboxaldehydes (49)

Step I: Synthesis of Hydrazones (54)

General procedure: Acetophenones (53, 0.02 mol) was dissolved in ethanol (50 ml) by warming and phenylhydrazine (0.02 ml) was added in it while shaking. The contents were stirred for 10 min. and allowed to stand at room temperature for 2 hrs. The solid so obtained was filtered, crystallized from ethanol.

Step II: Synthesis of 3-aryl-1-phenylpyrazole-4-carboxaldehydes (49)

General procedure: To a cold solution of dimethylformamide (10 ml) and phosphorus oxychloride (0.5 ml, 6 mmol), was added acetophenone phenylhydrazone (54, 4 mmol). The mixture was stirred at 50-60 °C for 5-6 hrs, cooled to room temperature and then poured into ice cold water. A saturated solution of sodium bicarbonate was added to neutralize the mixture to give pyrazole-4-carboxaldehyde 49, isolated by filtration followed by washing with water.
1-(2-Hydroxyphenyl)-3-(1-phenyl-3-aryl-4-pyrazolyl)prop-2-en-1-ones (41)

General procedure: To a solution of KOH (1.12 g, 0.02 mol) in methanol (50 ml) was added 2-hydroxyacetophenone (52, 1.36 g, 0.01 mol) and 1-phenyl-3-(4-methylphenyl) pyrazole-4-carboxaldehydes (49, 2.62 g, 0.01 mol) at 0-5°C. The reaction mixture was stirred overnight at room temperature. Then, this reaction mixture was poured over crushed ice and acidified with dil. HCl. The yellow solid thus obtained was filtered, washed with water and dried. The crude product was crystallized with chloroform-ethanol to afforded pure 2'-hydroxychalcones 41.

2.1. 2-(3-(4-Aryl)-1-phenyl-1H-pyrazol-4-yl)chroman-4-ones (55)

General procedure: To a solution of 2'-hydroxychalcone (41, 0.01 mol) in acetic acid (20 ml) was added 1 ml of conc. HCl. The reaction mixture was refluxed for 6-8 hrs and was then poured on to crushed ice with vigorous stirring. The yellow solid thus obtained was filtered, washed with water and dried. The crude product was crystallized with chloroform-methanol to afford pure chromanones 55a-55g.
2,3-Dihydro-2-(1,3-diphenyl-1H-pyrazol-4-yl)chromen-4-one (55a)

Mp: 170 °C; Yield: 68%;
IR (KBr, cm\(^{-1}\)): 1682;
\(^1\)H NMR (CDCl\(_3\), \(\delta\)): 3.04-3.05 (dd, 1H, \(J = 3.3, 13.9\) Hz), 3.18-3.27 (dd, 1H, \(J = 12.7, 13.9\) Hz), 5.65-5.70 (dd, 1H, \(J = 3.3, 12.1\) Hz), 8.11 (s, 1H), 8.25-28 (dd, 1H, \(J = 1.5, 7.8\) Hz), 7.87-7.90 (d, 2H, \(J = 7.8\) Hz), 7.30-7.32 (d, 2H, \(J = 7.8\) Hz), 7.61-7.72 (m, 5H), 7.36-7.40 (m, 3H);
MS (m/z): 367.10.

2,3-Dihydro-2-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)chromen-4-one (55b)

Mp: 206 °C; Yield: 79%;
IR (KBr, cm\(^{-1}\)): 1682;
\(^1\)H NMR (CDCl\(_3\), \(\delta\)): 2.32 (s, 3H), 2.89-2.95 (dd, 1H, \(J = 3.3, 16.8\) Hz), 3.08-3.18 (dd, 1H, \(J = 16.8, 12.1\) Hz), 5.55-5.60 (dd, 1H, \(J = 3.3, 12.1\) Hz), 8.04 (s, 1H), 7.892-7.898 (d, 1H, \(J = 1.8\) Hz), 7.67-7.70 (m, 2H), 7.58-7.61 (d, 2H, \(J = 8.1\) Hz), 7.37-7.46 (m, 4H), 7.25-7.27 (m, 2H), 6.98-7.03 (m, 2H);
MS (m/z): 381.15.
2,3-Dihydro-2-(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)chromen-4-one (55c)

Mp: 175 °C; Yield: 72%;
IR (KBr, cm⁻¹): 1687.4;
¹H NMR (CDCl₃, δ): 3.48 (s, 3H), 3.03-3.05 (dd, 1H, J = 3.3, 16.8 Hz), 3.14-3.18 (dd, 1H, J = 12, 16.8 Hz), 5.64-5.69 (dd, 1H, J = 3.3, 12 Hz), 8.12 (s, 1H), 7.96-7.99 (dd, 1H, J = 1.5, 7.9 Hz), 7.75-7.77 (d, 2H, J = 8 Hz), 7.73-7.74 (d, 2H, J = 8.1 Hz), 7.43-7.55 (m, 6H), 7.05-7.10 (m, 2H);
MS (m/z): 397.15.

2-(3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,3-dihydrochromen-4-one (55d)

Mp: 190 °C; Yield: 74%;
IR (KBr, cm⁻¹): 1685.1;
¹H NMR (CDCl₃, δ): 3.03-3.05 (dd, 1H, J = 3.3, 16.2 Hz), 3.16-3.18 (dd, 1H, J = 12, 16.2 Hz), 5.60-5.64 (dd, 1H, J = 3.3, 12 Hz), 8.12 (s,1H), 7.97-7.98 (dd, 1H, J = 1.5, 7.8 Hz), 7.75-7.82 (m, 4H), 7.47-7.55 (m, 3H), 7.35-7.38(m, 1H), 7.05-7.19 (m, 4H);
MS (m/z): 385.13.
2-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,3-dihydrochromen-4-one (55e)

Mp: 226 °C; Yield: 78%;
IR (KBr, cm⁻¹): 1685.3;
¹H NMR (CDCl₃, δ): 3.05-3.06 (dd, 1H, J = 3.3, 16.6 Hz), 3.18-3.22 (dd, 1H, J = 11.7, 16.6 Hz), 5.65-5.69 (dd, 1H, J = 3.3, 11.7 Hz), 8.12 (s, 1H), 7.96-7.99 (dd, 1H, J = 1.5, 8.1 Hz), 7.75-7.78 (m, 4H), 7.43-7.55 (m, 6H), 7.05-7.10 (m, 2H);
MS (m/z): 403.11, 401.13.

2-(3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,3-dihydrochromen-4-one (55f)

Mp: 220 °C; Yield: 81%;
IR (KBr, cm⁻¹): 1682.3;
¹H NMR (CDCl₃, δ): 3.05-3.06 (dd, 1H, J = 3.3, 18.0 Hz), 3.18-3.22 (dd, 1H, J = 12.0, 18.0 Hz), 5.61-5.66 (dd, 1H, J = 3.3, 12.0 Hz), 8.12 (s, 1H), 7.96-7.99 (dd, 1H, J = 1.5, 7.8 Hz), 7.75-7.77 (d, 2H, J = 8.1 Hz), 7.70-7.73 (d, 2H, J = 8.1 Hz), 7.47-7.61 (m, 5H), 7.33-7.38 (m, 1H), 7.04-7.13 (m, 2H);
MS (m/z): 446.01, 448.05.
2-(3-(4-Nitrophenyl)-1-phenyl-1\textit{H}-pyrazol-4-yl)-2,3-dihydrochroman-4-one (55g)

![Chemical structure](image)

Mp: 214 °C; Yield: 65%;
IR (KBr, cm$^{-1}$): 1689.3;
$^1$H NMR (CDCl$_3$, δ): 3.00-3.05 (d, 1H, $J = 16.2$ Hz), 3.14-3.24 (dd, 1H, $J = 16.5$, 12.6 Hz), 5.69-5.73 (dd, 1H, $J = 11.1$ Hz), 8.26 (s, 1H), 8.37-8.40 (d, 2H, $J = 8.4$ Hz), 7.97-8.05 (m, 3H), 7.78-7.81 (m, 2H), 7.48-7.59 (m, 4H), 7.06-7.14 (m, 2H);
MS (m/z): 412.12.

cis-3-Hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl)chramanone dimethylacetals (57)

![Chemical structure](image)

**General procedure:** Chromanone (55, 0.001 mol) was dissolved in 100 mL of absolute methanol and added dropwise to a stirred solution of potassium hydroxide (0.003 mol) in 50 mL of methanol over a period of 10-15 min at 5-10 °C. After the solution had stirred for an additional 10 min, iodobenzene diacetate (0.0011) was added in small portions during 15 min and the resulting mixture was allowed to stir overnight. Then most of the methanol was removed in vacuo and to the residue was added 100 ml of water. A solid was filtered, washed with cold water and dried. The crude product 57 was crystallized with methanol. Melting points are uncorrected and compared with the literature reports.$^{48}$
cis-3-Hydroxy-2-(1, 3-diphenyl-4-pyrazolyl)chromanone dimethylacetal (57a)

Mp: 154-156 °C; Yield: 72%;
IR (v_max, in KBr): 3480 cm⁻¹ (-OH str.);
¹H NMR (CDCl₃, 300 MHz, δ): 2.19 (d, 1H, OH, J = 5.1 Hz), 4.20 (d, 1H, C₃-H, J = 5.1 Hz), 5.53 (bs, 1H, C₂-H), 3.22 (s, 3H, OCH₃), 3.46 (s, 3H, OCH₃), 7.34-7.47 (m, 7H), 7.01-7.04 (m, 2H), 7.80 (m, 5H), 8.57 (s, 1H);
Elemental analysis: Calculated for C₂₆H₂₄N₂O₄: C 72.90, H 5.61, N 6.54; Found: C 72.04, H 5.77, N 6.50;
MS (m/z): 429.07 (M⁺⁺⁺).

cis-3-Hydroxy-2-[(1-phenyl-3-(p-tolyl)-4-pyrazolyl)]chromanone dimethylacetal (57b)

Mp: 178-180 °C; Yield: 72%;
IR (v_max, in KBr): 3491 cm⁻¹ (-OH str.);
¹H NMR (CDCl₃, 300 MHz, δ): 2.16 (d, 1H, OH, J = 5.1 Hz), 4.21 (d, 1H, C₃-H, J = 5.1 Hz), 5.53 (bs, 1H, C₂-H), 2.42 (s, 3H, CH₃), 3.22 (s, 3H, OCH₃), 3.46 (s, 3H, OCH₃), 7.82 (d, 2H, J = 7.7 Hz), 7.74 (d, 2H, J = 7.7 Hz), 7.62 (m, 1H), 7.45-7.51 (m, 2H), 7.27-7.32 (m, 4H), 7.01-7.04 (m, 2H), 8.55 (s, 1H);
Elemental analysis: Calculated for C$_{27}$H$_{26}$N$_2$O$_4$: C 73.30, H 5.88, N 6.33; Found: C 72.19, H 6.01, N 6.50;
MS (m/z): 443.13 (M$^+$+1).

* cis-3-Hydroxy-2-[(1-phenyl-3-(p-anisyl)-4-pyrazolyl)]chromanone dimethylacetal (57c) *

![Chemical Structure](image)

Mp: 172-173 °C; Yield: 70%;

IR ($\nu_{max}$, in KBr): 3523 cm$^{-1}$ (-OH str.);

$^1$H NMR (CDCl$_3$, 300 MHz, $\delta$): 2.16 (d, 1H, OH, $J = 5.0$ Hz), 4.21 (d, 1H, C$_3$-H, $J = 5.0$ Hz), 5.52 (bs, 1H, C$_2$-H), 3.88 (s, 3H, OCH$_3$), 3.20 (s, 3H, OCH$_3$), 3.45 (s, 3H, OCH$_3$), 7.91 (d, 2H, $J = 7.7$ Hz), 6.98 (d, 2H, $J = 7.7$ Hz), 7.63 (dd, 1H), 7.36-7.54 (m, 6H), 7.01-7.05 (m, 2H), 8.53 (s, 1H);

Elemental analysis: Calculated for C$_{27}$H$_{26}$N$_2$O$_5$: C 70.74, H 5.67, N 6.11; Found: C 70.78, H 5.75, N 6.18;

MS (m/z): 459.17 (M$^+$+1).

* cis-3-Hydroxy-2-[(1-phenyl-3-(p-chlorophenyl)-4-pyrazolyl)]chromanone dimethylacetal (57d) *

![Chemical Structure](image)

Mp: 166-168° C; Yield: 65%;
IR (v_max, in KBr): 3470 cm⁻¹ (-OH str.);

\(^1\)H NMR (CDCl\textsubscript{3}, 300 MHz, δ): 2.18 (d, 1H, OH, J = 5.1 Hz), 4.21 (d, 1H, C\textsubscript{3}-H, J = 5.1 Hz), 5.48 (bs, 1H, C\textsubscript{2}-H), 3.24 (s, 3H, OCH\textsubscript{3}), 3.49 (s, 3H, OCH\textsubscript{3}), 7.88 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.60 (dd, 1H), 7.41-7.49 (m, 2H), 7.30-7.34 (m, 4H), 7.03-7.07 (m, 2H), 8.54 (s, 1H);

Elemental analysis: Calculated for C\textsubscript{26}H\textsubscript{23}N\textsubscript{2}O\textsubscript{4}Cl: C 67.53, H 4.98, N 6.06; Found: C 67.40, H 5.12, N 6.19;

MS (m/z): 463.18 (M\textsuperscript{+}+1).

cis-3-Hydroxy-2-[(1-phenyl-3-(p-fluorophenyl)-4-pyrazolyl)chromanone dimethylacetal (57e)

Mp: 163-165 °C; Yield: 68%;

IR (v_max, in KBr): 3491 cm⁻¹ (-OH str.);

\(^1\)H NMR (CDCl\textsubscript{3}, 300 MHz, δ): 2.17 (d, 1H, OH, J = 5.1 Hz), 4.18 (d, 1H, C\textsubscript{3}-H, J = 5.1 Hz), 5.46 (bs, 1H, C\textsubscript{2}-H), 3.20 (s, 3H, OCH\textsubscript{3}), 3.46 (s, 3H, OCH\textsubscript{3}), 8.54 (s, 1H), 7.84-7.96 (m, 4H), 7.46-7.65 (m, 5H), 7.12-7.15 (m, 2H), 7.02-7.04 (m, 2H);

Elemental analysis: Calculated for C\textsubscript{26}H\textsubscript{23}N\textsubscript{2}O\textsubscript{4}F: C 69.95, H 5.16, N 6.28; Found: C 70.06, H 5.18, N 6.41;

MS (m/z): 447.13 (M\textsuperscript{+}+1).
cis-3-Hydroxy-2-[(1-phenyl-3-(p-bromophenyl)-4-pyrazolyl)chromanone dimethylacetal (57f)

Mp: 160-161°C; Yield: 72%;
IR (ν_max, in KBr): 3487 cm⁻¹ (-OH str.);
¹H NMR (CDCl₃, 300 MHz, δ): 2.21 (d, 1H, OH, J = 5.1 Hz), 4.20 (d, 1H, C₁-H, J = 5.1 Hz), 5.48 (bs, 1H, C₂-H), 3.23 (s, 3H, OCH₃), 3.48 (s, 3H, OCH₃), 7.88 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.60 (dd, 1H), 7.42-7.48 (m, 2H), 7.33-7.34 (m, 4H), 7.05-7.08 (m, 2H), 8.57 (s, 1H);
Elemental analysis: Calculated for C₂₆H₂₃N₂O₄Br: C 61.55, H 4.57, N 5.25; Found: C 61.47, H 4.51, N 6.10;
MS (m/z): 507.06 (M⁺+1).

cis-3-Hydroxy-2-[(1-phenyl-3-(p-nitrophenyl)-4-pyrazolyl)chromanone dimethylacetal (57g)

Mp: 151-153 °C; Yield: 66%;
IR (ν_max, in KBr): 3464 cm⁻¹ (-OH str.); ¹H NMR (CDCl₃, 300 MHz, δ): 2.22 (d, 1H, OH, J = 5.1 Hz), 4.20 (d, 1H, C₁-H, J = 5.1 Hz), 5.48 (bs, 1H, C₂-H), 3.24 (s, 3H, OCH₃), 3.46 (s,
3H, OCH₃), 8.31 (d, 2H, J = 9.0 Hz), 8.07 (d, 2H, J = 9.0 Hz), 7.77-7.82 (m, 2H), 7.32-7.46 (m, 5H), 7.01-7.06 (m, 2H), 8.57 (s, 1H);
Elemental analysis: Calculated for C₂₆H₂₃N₃O₆: C 65.96, H 4.86, N 8.88; Found: C 66.01, H 4.97, N 9.06;
MS (m/z): 474.09 (M⁺+1).

4.3.2.1 Antimicrobial assay

The antibacterial activity of fourteen new compounds 55a-g was evaluated by agar well diffusion method. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 ×10⁸ cfu/ml. 20ml of Mueller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 4.0mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 hrs. Antibacterial activity of compounds 55a-g was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism.⁴⁹,⁵⁰

Minimum inhibitory concentration (MIC) of the various compounds 55a-g against bacterial strains was tested through a macrodilution tube method as recommended by NCCLS. In this method, various test concentrations of synthesized compounds 55a-g were made from 128 to 0.25µg/ml in sterile tubes No.1 to 10. 100 µl sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 µl test compound in tube 1. Two fold serial dilutions were carried out from the tube 1 to the tube 10 and excess broth (100 µl) was discarded from the last tube No.10. To each tube, 100 µl of standard inoculum (1.5 ×10⁸ cfu/ml) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 hrs.⁵¹

The antifungal activity of the synthesized compounds 55a-g was evaluated by poison food technique. The moulds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7 days and used as inocula. The 15ml of molten SDA (45 °C) was poisoned by the addition of 100
µl volume of each compound having concentration of 4.0mg/ml reconstituted in the DMSO and poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8mm diameter) obtained from the actively fungus growing on margins of the SDA plates and incubated at 25 °C for 7 days. DMSO was used as the negative control whereas Fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition by applying the formula.52

\[
\text{Percentage inhibition of mycelial growth} = \frac{(dc-dt)}{dc} \times 100
\]

dc = average diameter of fungal colony in negative control sets
dt = average diameter fungal colony in experimental sets
4.4 REFERENCE


49. Ahmad, I.; Beg, A. J. J. Ethnopharmacol. 2001, 74, 113.

