

## Synthesis of 5-Aryl-3-(5-Bromo-3-Benzofuran-2-yl)-1-Pyrazole as Antimicrobial Agent.

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**Abstract:** In this work, we evaluate the biological activities of some new derivatives of benzofuran which can be used as effective anti-microbial agents. The recent reviews of literature have highlighted the attention of medicinal chemists because of their diverse biological activities and profound efficacy. Clinically potent benzofurans generated interest to construct a system which possesses anti-bacterial and anti-fungal activity. Furan and benzofuran are associated with wide spectrum of biological activity. In the view of these, an effort was made to check some synthesized compounds for their anti-microbial activity.

In present study, pyrazoline derivatives were synthesized; 5-bromo-3-methyl acetophenone undergoes ring formation in presence of chloroacetone to form benzofuran which further forms chalcone on treatment with substituted benzaldehyde. This intermediate on treatment with hydrazine hydrate results into pyrazoline. Further it reacts with various benzoyl chlorides to form the titled product. Synthesized compounds have been confirmed on the basis of spectral studies and analytical data. All the compounds were screened for their *in-vitro* anti-bacterial activity against Gram positive *Staphylococcus aureus* ATCC3750 and Gram negative *Salmonella typhi* NCTC786, anti-fungal strains of *Candida albicans* ATCC10233 and *Aspergillus niger* ATCC 16404 using tube dilution method showing moderate activity.

**Keywords :** Anti-microbial activity, Benzofuran, Pyrazole.

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### I. INTRODUCTION

Benzofuran<sup>1</sup> compounds are ubiquitous in nature, particularly among plant kingdom. Often such natural products possessing benzofuran nucleus are endowed with useful pharmacological properties. This has generated enormous interest in synthetic products containing benzofuran nucleus and has resulted in the development of benzofuran chemistry during the last several years. Benzofurans are bicyclic ring system with multiple applications. The Literature indicates that compounds having Benzofuran<sup>2</sup> nucleus possess broad range of biological activities, like Griseofulvin as antifungal; Amiodarone as antiarrhythmic; Benzbromarone as uricosuric; Cloridarol as vasodilator; Oxetorone as antimigraine agent.

Pyrazolines are nitrogen-containing heterocyclic compounds, well known for their pronounced biological activity. These biological activities include antibacterial<sup>3</sup>, antifungal<sup>4</sup>, herbicidal<sup>5</sup> and insecticidal activities<sup>6</sup>. It was demonstrated that the combination of pyrazole with azole ring, linked to each by one sigma bond, led to more biologically active targets; for example, pyrazolylthiazoles showed excellent antimicrobial activities<sup>7</sup>.

Pyrazole<sup>8</sup> derivatives have a long history of application in Agrochemicals and Pharmaceutical industry as herbicides and active pharmaceuticals. The wide variety of Nitrogen containing heterocyclic compounds that have been exploited to develop pharmaceutically important molecules such as Pyrazole and their derivatives are important due to their diverse biological activities such as anti-fungal<sup>9</sup>, anti-bacterial<sup>10</sup> and others.

The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead. These findings encourage us to undertake the synthesis of pyrazolene-benzofuran ring system, in the hope that they could have some promising biological interest.

### II. MATERIALS AND METHODS

The synthesized compounds were screened for their anti-bacterial and anti-fungal activities. The various screenings carried out include the *in vitro* study against Gram positive *Staphylococcus aureus* ATCC 3750, Gram negative *Salmonella typhi* NCTC 786 and fungal strain *Candida albicans* and *Aspergillus niger*. The Minimum Inhibitory Concentration (MIC) was determined using tube dilution method according to the standard procedure<sup>11</sup>. DMSO was used as a solvent with appropriate controls. Ampicillin (MIC = 0.01 µg/ml against Gram positive *S. aureus*), Trimethoprim (MIC = 1 µg/ml against Gram negative *S. typhi*) were used as standard drugs for anti-bacterial screening and Miconazole was used as anti-fungal standard drug (MIC = 0.01 µg/ml).

## 1.1 EXPERIMENTAL

### 1.1.1 Synthesis of 5- Bromo-3-methyl-2acetyl benzofuran (I)

To stirred mixture of 2.15g (0.01 mole) of 5-Bromo-2-hydroxy acetophenone and 3.45g (0.025 mole) of anhydrous  $K_2CO_3$  in dry acetone for 30 mins. 1.0 ml (0.01 mole) of chloroacetone was added drop wise over a period of 10 mins. Reaction mixture was stirred for 3hours. Allow cooling and pour into crushed ice. Solid thus separated was filtered washed with 20ml cold water, crystallized with ethanol.

Yield: 60%, M.P: 183°C, Color & Nature: white crystalline solid. ,CHN Found C%: 52.45 H%: 3.73. Calculated C%:52.20 H%:3.58; IR(KBr)  $cm^{-1}$  : 1724 (CO stretching), 585 (CBr stretching), 2892 (CH<sub>3</sub> aliphatic stretching), 2078 (Aromatic C-H stretching), 1640 (alkene C=C stretching) ;<sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 7.12-7.84 (3H Ar-H, m), 2.0 ( 3H,s), 3.2 (3H, s)

### 1.1.2 Synthesis of 5-bromo-2-cinnamoyl-3-methyl Benzofuran (II)

5-Bromo-2-acetylbenzofuran (I) 2.52g (0.01 mole) was stirred in 10ml of anhydrous ethyl alcohol. 1.40g (0.01 mole) of 3-chlorobenzaldehyde was added to the reaction mixture at room temperature. This reaction mixture was cooled for 3 hours and contents allowed standing overnight. The solution was diluted with water and neutralized with dilute HCl. The off-white colored solid thus separated was collected, dried and crystallized from ethanol.

Yield: 55%, M.P: 135°C, Color & Nature: off white crystalline solid;CHN Found C%: 57.68 H%: 2.94. Calculated C%: 57.55 H%: 3.22; IR (KBr)  $cm^{-1}$ ; 585 (C-Br stretching), 594 (C-Cl stretching), 1640 (alkene C=C stretching), 1122 (C=O stretching), 2010 (Ar stretching); <sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 7.12-7.84 (3H, Ar-H, m), 2.7 (1H, d), 2.9 (1H, d)

### 1.1.3 Synthesis of 5-(3-Chlorophenyl)-3-(5-bromo-3-methylbenzofuran-2-yl)-1H-pyrazolines (III)

A mixture of 3.91g (0.01 mole) of 5-Chloro-2-cinnamoyl-3-methyl benzofuran (II) and 2.5g (0.15 mole) of hydrazine hydrate in 50ml anhydrous ethanol was refluxed for 3 hours. Excess of solvent was distilled off. Pour on to crush ice, solids collected. Product crystallized from ethanol.

Yield: 75%, M.P: 146°C, Color & Nature: off white crystalline solid;CHN Found C%: 55.68 H%: 3.95 N%: 7.43. Calculated C%:55.48 H%: 3.62 N%:7.19 ; IR (KBr)  $cm^{-1}$ : 585 ( C-Br stretching ), 594 (C-Cl stretching), 1175 (C=O stretching), 1690 ( C=N stretching), 2010 (Ar stretching), 1100 (CN stretching), 1620 (NH Bending); <sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 6.17- 7.58 (7H, Ar-H stretching, 2.4 (3H,s) 5.2 (1H,s), 5.0 (2H,s)

### 1.1.4 Synthesis of 5-(3-Chlorophenyl)-3-(5-bromo-3-methyl benzofuran-2-yl)-1-(Substituted benzoyl)-pyrazoline. (IV)

A mixture of 1.94g (0.005 mole) of 1H-pyrazoline (III) and 0.6ml (0.005 mole) of substituted benzoyl chloride in anhydrous pyridine (10ml) was refluxed for 1 hour. Allow cooling reaction mixture neutralized with cold dilute HCl. The solid separated out; product was filtered off and crystallized from ethanol.

Yield: 55%, M.P: 205°C, Color & Nature: off white amorphous solid.: CHN Found C%: 56.67 H%: 3.36 N%: 5.48. Calculated C%: 56.92 H%: 3.22 N%: 5.31; IR (KBr)  $cm^{-1}$ : 3144 ( C-H stretching Ar), 1689 ( C=O stretching), 1555 (N=C stretching pyrazoline), 1263 (C=O=C stretching benzofuran), 1065 ( C-F stretching), 722 ( C-Cl stretching); <sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s).

#### 1.1.4.1 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](4-chlorophenyl) methanone

Yield:52%, M.P:218°C, Color & Nature: brown amorphous soild.: CHN Found C%: 56.59 H%: 3.06 N%: 5.56. Calculated C%: 56.92 H%: 3.22 N%: 5.31;IR (KBr)  $cm^{-1}$ : 585 (C-Br stretching), 2078 (Ar stretching), 1060 (-C-O- stretching), 2950 (CH<sub>3</sub> stretching), 1705 (C=O stretching), 560 (C-Cl).; <sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s)

#### 1.1.4.2 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](3-fluorophenyl) methanone

Yield: 67%, M.P: 189°C, Color & Nature: brown amorphous solid : CHN Found C%: 58.25 H%: 3.54 N%: 5.63 Calculated C%: 58.65 H%:3.32 N%:5.47; IR (KBr)  $cm^{-1}$  : 585 (C-Br stretching), 2078 (Ar stretching), 1060 (-C-O- stretching), 2950 (CH<sub>3</sub> stretching), 1705 (C=O stretching), 560 (C-Cl), 1300 (C-F).; <sup>1</sup>H NMR (DMSO)  $\delta$ ppm : 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s)

**1.1.4.3 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](4-Hydroxyphenyl) methanone**

Yield: 65%, M.P: 193°C, Color & Nature: off white amorphous solid: CHN Found C%:58.92 H%: 3.37 N%:5.61 Calculated C%: 58.88 H%: 3.53 N%:5.49 ; IR (KBr)  $\text{cm}^{-1}$  : 585 (C-Br stretching), 2078 (Ar stretching), 1060 (-C-O- stretching), 2950 ( $\text{CH}_3$  stretching), 1705 (C=O stretching), 560 (C-Cl), 2951 (O-H stretching);  $^1\text{H}$  NMR (DMSO)  $\delta$ ppm: 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s), 3.1 (1H,s)

**1.1.4.4 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](2,4-dimethoxyphenyl) methanone**

Yield: 47%, M.P: 198°C, , Color & Nature: white amorphous solid: CHN Found C%: 58.75 H%: 3.99 N%: 5.41 Calculated C%: 58.64 H%: 3.80 N%:5.06; IR (KBr)  $\text{cm}^{-1}$  : 585 (C-Br stretching), 2078 (Ar stretching), 1060 (-C-O- stretching), 2950 ( $\text{CH}_3$  stretching), 1705 (C=O stretching), 560 (C-Cl), 1044 (O- $\text{CH}_3$ );  $^1\text{H}$  NMR (DMSO)  $\delta$ ppm: 7.18 – 8.00 (10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s), 5.2 (6H,s).

**1.1.4.5 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(4-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](3-hydroxyphenyl)methanone**

Yield: 63%, M.P: 88°C, Color & Nature: White amorphous solid: CHN Found C%: 59.65, H%: 4.20, N%: 5.41 Calculated C%:58.70 H%: 3.00 N%: 4.90 ; IR (KBr): 3144 ( C-H stretching Ar), 1689 ( C=O stretching), 1555 (N=C stretching pyrazoline), 1263 (C=O=C stretching benzofuran), 722 ( C-Cl stretching), 2950(OH stretching);  $^1\text{H}$  NMR (DMSO)  $\delta$ ppm : 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s), 2.7(1H,s)

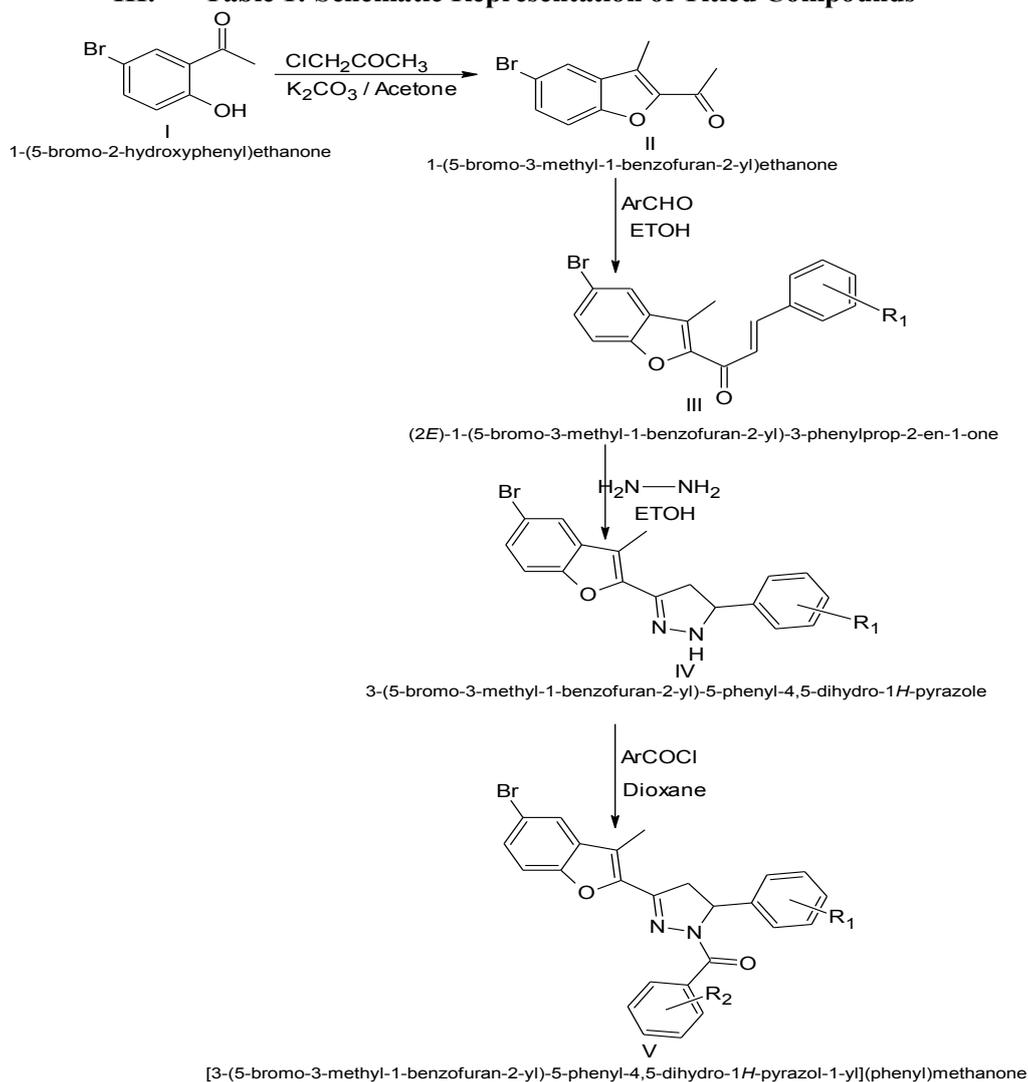
**1.1.4.6 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(3-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](4-hydroxyphenyl)methanone**

Yield: 66%, M.P: 128°C, Color & Nature: Off-white amorphous solid: CHN Found: C%: 59.12 H%: 4.26 N%: 5.50 Calculated: C%: 58.30 H%: 3.98 N%: 5.00; IR (KBr): 3144 ( C-H stretching Ar), 1689 ( C=O stretching), 1555 (N=C stretching pyrazoline), 1263 (C=O=C stretching benzofuran), 722 ( C-Cl stretching), 2950(OH stretching);  $^1\text{H}$  NMR (DMSO)  $\delta$ ppm : 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s), 2.7(1H,s)

**1.1.4.7 Synthesis of [ 3-(5- bromo-3-methyl-1-benzofuran-2-yl)-5-(3-chlorophenyl)-4,5-dihydro-1H pyrazol-1-yl](4-hydroxyphenyl)methanone**

Yield: 51%, M.P: 116°C, Color & Nature: white amorphous solid : CHN Found: C%: 59.12 H%: 4.02 N%: 4.88 Calculated: C%: 58.50 H%: 3.90 N%: 4.70; IR (KBr):3144 ( C-H stretching Ar), 1689 ( C=O stretching), 1555 (N=C stretching pyrazoline), 1263 (C=O=C stretching benzofuran), 722 ( C-Cl stretching);  $^1\text{H}$  NMR (DMSO)  $\delta$ ppm: 7.18 – 8.00 ( 10H, Ar-H), 2.3 (3H, s), 5.1 (2H,s), 2.7(1H,s)

III. Table 1: Schematic Representation of Titled Compounds



3.1 Table 2: Anti-microbial activity of synthesized compound

Sr.no	Substituent's		Antibacterial		Antifungal	
	R	R'	<i>S.aureus</i>	<i>S.typhi</i>	<i>C.albicans</i>	<i>A.niger</i>
1	3-Chloro	4-Chloro	100	200	100	200
2	4-Chloro	4-Chloro	200	200	50	200
3	4-Chloro	3-Fluoro	100	100	100	100
4	4-Chloro	4-Hydroxy	50	200	200	200
5	4-Chloro	2,4-Dimethoxy	50	200	200	N.A
6	4-Chloro	3-Hydroxy	100	100	100	100
7	3-Chloro	4-Hydroxy	200	N.A	200	N.A
8	3-Chloro	3-Chloro	100	N.A	200	100

- Ampicillin (MIC- 0.04 µg/ml) used as standard *Staphylococcus aureus*
  - Trimethoprim (MIC- 0.01 µg/ml) used as standard *Salmonella typhi*
  - Miconazole (MIC- 6.25 µg/ml) used as standard against *Candida albicans* and *Aspergillus niger*.
- N.A.:- Not active at 200 µg/ml.

#### IV. RESULTS

All the eight synthesized compounds (Table No.2) showed moderate activity upto 200 µg/ml among which 4-Hydroxy and 2, 4-Dimethoxy (Sr. no. 4& 5) showed activity upto 50 µg/ml against *Staphylococcus aureus*. The 4-fluoro derivative (Sr. no. 3) exhibited activity at 100µg/ml while the other remaining compounds exhibited activity upto 200 µg/ml against *Salmonella typhi*.

All the compounds showed moderate activity against fungal species of *Candida albicans* upto 100 µg/ml exceptional of 2,4-Dimethoxy substitutions (Sr. no.5) with activity at 200 µg/ml. The compounds with 4-fluoro substitution (Sr. no 3) showed activity at 100 µg/ml while remaining others showed activity at 200 µg/ml against *Aspergillus niger* species except 2,4- Dimethoxy substitutions (compound no. 305) which is found inactive upto 200 µg/ml.

#### 4.1 DISCUSSION

The purpose of the present study was to examine whether molecular modifications might result in detection of new potential anti-microbial drugs. A series of compounds were prepared and assayed in variety of biological tests for anti-microbial activity. The data reported in table 2 shows that the effect of variation in chemical structure on activity was rather unpredictable. The substitution which appeared to be the most important for higher order of activity in the greatest number was test for p-chloroaryl group. The introduction of P-methoxy and P-hydroxy in aryl moiety of the pyrazole analogs produced compounds with anti-microbial properties.

#### V. CONCLUSION

In conclusion, the results of this investigation revealed that the observed substitution at Para position is better for enhancing anti-microbial activity. Obviously, the comparative evaluation of active compounds will require further studies; the data reported in this article may be a helpful guide for medicinal chemists.

#### ACKNOWLEDGEMENTS

The authors are thankful to SAIF, IIT, Powai, Mumbai for carrying out the CHN elemental analysis and also thankful to SAIF, Patiala University, Punjab for recording the NMR spectra.

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