SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL COMPOUNDS OF 3-(((1H-BENZO [D]IMIDAZOL-2-YL)METHYL)AMINO)-1-(2,5-DIFLUOROBENZoyl)-4-(2-PHENYLHYDRAZONO)-1H-PYRAZOL-5(4H)-ONE

M. Swarna Kumari¹*, K. Udaya Kumari³, K. Sudhakar Babu² and L. K. Ravindhranath²

¹Santhiram Engineering College, Nandyal, Kurnool (Dist) (AP) India.
²Dr. K. V. Subba Reddy Womens Engineering College Kurnool, (AP) India.
³Department of Chemistry, Sri Krishnadeveraya University, Anantapur, (AP) India.

ABSTRACT

New novel derivatives of 3-(((1H-benzo[d]imidazol-2-yl)methyl)amino)-1-(2,5-difluorobenzoyl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (4a-g) were prepared by refluxing of ethyl 2-(4-(2-(4-substituted methyl)phenyl) hydrazono)-1-(2,5-difluoro benzoyl)-4,5-dihydro-5-oxo-1H-pyrazol-3-yl)amino Carboxylic acid. (3a-g)Orthophenylene diamine, Cyclo hexane and ethyl acetate. The synthon 3a-g were synthesized by refluxing a mixture of ethyl 2-((1-(2, 5-difluorobenzoyl)-5-oxo-4-(2-phenyl hydrazano)-4, 5-dihydro-1H-pyrazol-3-yl) amino) acetate and tetra hydro furan. The newly synthesized compounds were characterized by IR, ¹H-NMR, ¹³C-NMR, mass spectra & Elemental analysis. The newly synthesized compounds were screened for their Biological activity.

KEYWORDS: Benzimidazoles, Ortho phenylene diamine, Antibacterial and Antifungal activity, spectral data.
INTRODUCTION

Benzimidazoles are a class of heterocyclic, aromatic chemical compounds which share a fundamental structural characteristic of six-membered benzene fused to five-membered imidazole.\[^{[1]}\]

The purines.\[^{[2]}\] Among the members of this group are several very well known and important biomolecules, such as adenine\[^{[3]}\] and guanine,\[^{[4]}\] two of the four nucleic acid bases, caffeine\[^{[5]}\] and uric acid.\[^{[6][1]}\]

A brief review of literature survey regarding the synthesis and biologically potent benzimidazole derivatives were described in the following few pages.

Benzimidazole derivatives have been found to possess high biological activity. The nucleus was found in vitamin B12 and a variety of antimicrobial,\[^{[7,8]}\] antiparasitic,\[^{[9]}\] and even antitumor\[^{[10]}\] agents.

**Benzimidazole**

Benzimidazoles are a class of heterocyclic, aromatic chemical compounds which share a fundamental structural characteristic of six-membered benzene fused to five-membered imidazole.\[^{[1]}\]

This basic ‘6+5’ heterocyclic structure is shared by another class of chemical compounds, the purines.\[^{[2]}\] Among the members of this group are several very well known and important biomolecules, such as adenine\[^{[3]}\] and guanine,\[^{[4]}\] two of the four nucleic acid bases, caffeine,\[^{[5]}\] and uric acid.\[^{[6][1]}\]
A brief review of literature survey regarding the synthesis and biologically potent benzimidazole derivatives were described in the following few pages.

Benzimidazole derivatives have been found to possess high biological activity. The nucleus was found in vitamin B12 and a variety of antimicrobial,\(^7,8\) antiparasitic,\(^9\) and even antitumor\(^10\) agents.\(^1,2\)
The review of literatures describes that the benzimidazole derivatives possess a variety of biological activities.\textsuperscript{[3]}

**Table 4.0 Biologically active benzimidazole derivatives.**

<table>
<thead>
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<th>S. no</th>
<th>Compound</th>
<th>Activity</th>
<th>Reference</th>
</tr>
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<td>1</td>
<td><img src="image1" alt="Compound 1" /></td>
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<td>Anton smith\textsuperscript{[4]}</td>
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<tr>
<td>2</td>
<td><img src="image2" alt="Compound 2" /></td>
<td>Antimicrobial</td>
<td>K.F. Ansari \textit{et al.}\textsuperscript{[5]}</td>
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<tr>
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<td>Brumagniez \textit{et al.}\textsuperscript{[7]}</td>
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<td><img src="" alt="Chemical Structure" /></td>
<td>Anti-viral</td>
<td>A.K. Tiwari et al. [8]</td>
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<tr>
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<td>--------------------------------</td>
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<td>----------------------</td>
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<td>Yusuf Ozkay et al. [9]</td>
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<tr>
<td>7</td>
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<td>Malleshappa Noolvi [10]</td>
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<td>8</td>
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<td>Ozden Ozel Guuven et al. [11]</td>
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<tr>
<td>9</td>
<td><img src="" alt="Chemical Structure" /></td>
<td>Angiotensin II receptor antagonist</td>
<td>Mukesh C. Sharma et al. [12]</td>
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<td><img src="" alt="Chemical Structure" /></td>
<td>Anti-inflammatory</td>
<td>Gummadi et al. [13]</td>
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**Scheme I**: The synthetic route was depicted in scheme. The title compounds 4(a-g) were synthesised in two sequential steps using different reagents and reaction conditions the 4(a-g) were obtained in moderate yields. The structure were established by spectral (IR, 1H-NMR, 13C-NMR and mass) and analytical data.
MATERIALS AND METHODS

All the chemicals used in the present investigation were purchased from Sigma-Aldrich Chemicals Company, Inc. USA. And used without further purification. TLC was performed on aluminum sheet of silica gel 60F254, E-Merk, Germany using iodine as visualizing agent. Melting point was determined in open capillary tubes on Mel-Tempapparatus and is uncorrected. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The IR Spectra were recorded as KBr pellets on Perkin-Elmer 1000 units, instruments. All 1H and 13C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 400MHz for 1H-NMR and 75 MHz for 13C-NMR were recorded on a Varian XL-spectrometer operating at 161.89MHz. The compounds were dissolved in DMSO-d6 and Chemical shifts were referenced to TMS (1H and 13C-NMR). Mass spectral data were recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analysis was recorded on a Carlo Erba 1108 elemental Analyzer, Central Drug Research Institute, Lucknow, India.

RESULTS AND DISCUSSION

Synthesis of ethyl 2-(4-(2-(4-substituted methyl) phenyl) hydrazono)-1-(2,5-difluoro benzoyl)-4,5-dihydro-5-oxo-1H-pyrazol-3-yl)amino Carboxylic acid

To a solution of ester(2a,1eq) in tetra hydro furan/MeOH/H2O(1:1:1) ratio aq NaOH(2N) was added and stirred (room temp) or reflux for 4-6 hrs. After completion of the reaction as indicated by TLC using Mobile Phase as cyclo hexane and ethyl acetate (7:3). The a residue
was washed with EtOA (removing impurities). The solvent was evaporated under vacuum to afford 3a-g. After the residue was acidified with 1N HCl up to pH-2 to give solid suspension, which filtered extracted with EtOAc (2x30ml) twice. The organic layer was collected washed with water brain dried over anhydrous Na2SO4 filtered and evaporated under vacuum to give the crude acid product (4-(2-(4-substituted)phenyl) hydrazono)-1-(2,5-difluorobenzoyl)-4,5-dihydro-5-thioxo-1H-pyrazole-3-yl)amino carboxylic acid(3a).

**Synthesis of 3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-(4-Substituted)phenyl)hydrazono)-1H-pyrazol-5(4H)-one (4a-g)**

A mixture of (4-(2-(4-phenyl )hydrazono)-1-(2,5-difluoro benzoyl)-4,5-dihydro-5-oxo-1H-pyrazol-3-yl)amino Carboxylic acid (3a) and ortho phenylene diamine in 1:1 equivalent ratio was refluxed for 2h at 100°C in presence of 6N HCl. The progress of the reaction was monitored by TLC using 9:1 hexane and ethyl acetate solvent mixture as Mobile phase. After completion of the reaction, the reaction mixture was neutralized by NaHCO3. The crude product 3-(((1H-benzo[d]imidazol-2-yl)methyl)amino)-1-(2,5-difluorobenzoyl)-4-(2-(Phenylhydrazono)-1H-pyrazol-5(4H)-one(4a) was purified by using silica gel 60-120 mesh and Chloroform was used as an elutent. The yield of 4a was found to 70%, mp 160-162°C. The similar procedure was adopted to Synthesize 4 b-g from 3 b-g and O-phenyldiamine.

**Physical, analytical and spectral data for the compounds**

3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2, 5-difluorobenzoyl)-4-(2-(trifluoromethyl) phenyl) hydrazono)-1H-pyrazol-5(4H)-one (4a)

Yield 70%.

mp: 160-162°C.

IR (KBr): 3381(-NH str.) 3040(Ar-Hstr.) 1694(exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm-1 (>C=N- group) and 1100cm-1 (C-F str.)

1H-NMR (400 MH2 DMSO-d6): δppm 2.0(s, H, NH attached to pyrazoline-5-one ring), 3.91(t,2H, -CH2 of 1H benzo [d] imidazole group), 5.0(s,1H, -NH of imidazole group) 10.15 (s,1H,Ar-NH-N= Group) and 6.81-7.59(m,12H C6H5 C6H4 and C6H3), respectively.
$^{13}$CNMR(75MHz,DMSO-d6): at 143.0,113.9, 129.5, 122.4,136.8, 162.1,142.7, 170.2,126.7,154.9,118.7,120.5, 158.6,113.9, 120.9,117.5, 37.5, 141.5,123,115.2 and 123.7. Corresponding to C$_1$,C$_2$ & C$_6$,C$_3$ & C$_5$,C$_4$,C$_7$, C$_8$, C$_9$, C$_{10}$,C$_{11}$, C$_{12}$,C$_{13}$,C$_{14}$, C$_{15}$, C$_{16}$,C$_{17}$,C$_{18}$,C$_{19}$ & C$_{24}$, C$_{20}$ & C$_{23}$, and C$_{21}$ & C$_{22}$. Respectively., Anal. Calcd. For C$_{24}$H$_{17}$F$_2$N$_2$O$_2$ C 57.72%, H 2.25% and N14.39%. Found: C 57.42 %, H 1.93% and N 14.02%.

3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-p-tolyl)hydrazono)-1H-pyrazol-5(4H)-one(4b)

Yield 73%.

mp: 163-166$^\circ$C.

IR (KBr): 3381(-NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm-1 (>C=N- group) and 1100cm-1 (C-F str.)

$^1$H-NMR (400 MHz, DMSO-d6): δppm 2.0(s, H, NH attached to pyrazoline-5-one ring), 3.91(t,2H, -CH2 of 1H benzo [d] imidazole group),5.0(s, $^1$H,-NH of imidazole group)10.15 (s,1H,Ar-NH-N= Group) and 6.81-7.59(m,12H C$_6$H$_5$ C$_6$H$_4$ and C$_5$H$_3$),respectively.

$^{13}$CNMR(75MHz,DMSO-d6): at 143.0,113.9,129.5,122.4,136.8, 162.1,142.7, 170.2,126.7,154.9,118.7,120.5, 158.6,113.9, 120.9,117.5, 37.5, 141.5,123,115.2 and 123.7. Corresponding to C$_1$,C$_2$ & C$_6$,C$_3$ & C$_5$,C$_4$,C$_7$, C$_8$, C$_9$, C$_{10}$,C$_{11}$, C$_{12}$,C$_{13}$,C$_{14}$, C$_{15}$, C$_{16}$,C$_{17}$,C$_{18}$,C$_{19}$ & C$_{24}$, C$_{20}$ & C$_{23}$, and C$_{21}$ & C$_{22}$. Respectively., Anal. Calcd. For C$_{24}$H$_{17}$F$_2$N$_2$O$_2$ C 57.72%, H 2.25% and N14.39%. Found: C 57.42 %, H 1.93% and N 14.02%.

3-(((1H-benzo[d]imidazol-2-yl)methyl)amino)-1-(2,5-difluorobenzoyl)-4-(2-(4-methoxyphenyl)hydrazono)-1H-pyrazol-5(4H)-one(4c)

Yield 70%.

mp: 160-162$^\circ$C.

IR (KBr): 3381(-NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one),1617 cm-1 (>C=N- group) and 1100cm-1 (C-F str.)

$^1$H-NMR (400 MHz, DMSO-d6): δppm 2.0(s, H, NH attached to pyrazoline-5-one ring), 3.91(t,2H, -CH2 of 1H benzo [d] imidazole group),5.0(s, $^1$H,-NH of imidazole group)10.15 (s,1H,Ar-NH-N= Group) and 6.81-7.59(m,12H C$_6$H$_5$ C$_6$H$_4$ and C$_5$H$_3$),respectively.
Corresponding to C₁₁C₂₂ & C₆C₇ & C₅C₄C₇, C₈, C₉, C₁₀C₁₁, C₁₂C₁₃C₁₄, C₁₅, C₁₆C₁₇C₁₈C₁₉ & C₂₄, C₂₀ & C₂₃, and C₂₁ & C₂₂. Respectively., Anal.Calcd. For C₂₄H₁₇F₃N₇O₂ C 57.72%, H 2.25% and N14.39%. Found: C 57.42 %, H 1.93% and N 14.02%.

3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-4-(2-(4-chlorophenyl)hydrazono)-1-(2,5-difluorobenzoyl)-1H-pyrazol-5(4H)-one (4d)

Yield 74%.

m p: 164-167°C.

IR (KBr): 3381(NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm⁻¹ (>C=N-group) and 1100cm⁻¹ (C-F str.)

¹H-NMR (400 MH₂ DMSO-d₆): δppm 2.0(s, H, NH attached to pyrazoline-5-one ring), 3.91(t,2H, -CH2 of 1H benzo[d]imidazole group), 5.0(s,¹H, -NH of imidazole group) 10.15 (s,1H,Ar-NH-N= Group) and 6.81-7.59(m,12H C₆H₅C₆H₄ and C₆H₃),respectively.

¹³CNMR(75MHz DMSO-d₆): at 143.0,113.9,129.5,122.4,136.8, 162.1,142.7, 170.2,126.7,154.9,118.7,120.5,158.6,113.9,120.9,117.5,37.5,141.5,123,115.2 and 123.7

Corresponding to C₁C₂&C₆&C₇&C₈, C₉, C₁₀C₁₁, C₁₂C₁₃C₁₄, C₁₅, C₁₆C₁₇C₁₈C₁₉&C₂₄, C₂₀&C₂₃, andC₂₁&C₂₂. Respectively., Anal.Calcd. For C₂₄H₁₇F₃N₇O₂ C 57.72%, H 2.25% and N14.39%. Found: C 57.42 %, H 1.93% and N 14.02%.

3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-4-(2-(4-bromophenyl) hydrazono)-1-(2,5-difluorobenzoyl)-1H-pyrazol-5(4H)-one (4e)

Yield 70%.

m p: 160-162°C.

IR (KBr): 3381(NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm⁻¹ (>C=N-group) and 1100cm⁻¹ (C-F str.)

¹H-NMR (400 MH₂ DMSO-d₆): δppm 2.0(s, H, NH attached to pyrazoline-5-one ring ), 3.91(t,2H, -CH2 of 1H benzo[d]imidazole group), 5.0(s,¹H, -NH of imidazole group) 10.15 (s,1H,Ar-NH-N= Group) and 6.81-7.59(m,12H C₆H₅C₆H₄ and C₆H₃),respectively.

¹³CNMR(75MHz DMSO-d₆): at 143.0,113.9,129.5,122.4,136.8, 162.1,142.7, 170.2,126.7,154.9,118.7,120.5,158.6,113.9,120.9,117.5,37.5,141.5,123,115.2 and 123.7
Corresponding to $C_1$, $C_2$, $C_6$, $C_3$, $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{23}$, and $C_{21}$. Respectively., Anal.Calcd. For $C_{24}H_{17}F_2N_7O_2$ C 57.72%, H 2.25% and N 14.39%. Found: C 57.42%, H 1.93% and N 14.02%.

3-((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-(4-nitrophenyl)hydrazono)-1H-pyrazol-5(4H)-one (4f)

Yield 69%.

m p: 162-163°C.

IR (KBr): 3381(-NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm$^{-1}$ (>C=N group) and 1100cm$^{-1}$ (C-F str.)

$^1$H-NMR (400 MHz DMSO-d6): δppm 2.0 (s, H, NH attached to pyrazoline-5-one ring), 3.91 (t, 2H, -CH2 of 1H benzo [d] imidazole group), 5.0 (s, $^1$H,-NH of imidazole group) 10.15 (s, 1H,Ar-NH-N= Group) and 6.81-7.59 (m, 12H C$_6$H$_5$ C$_6$H$_4$ and C$_6$H$_3$), respectively

$^{13}$CNMR(75MHz,DMSO-d6): at 143.0, 113.9, 129.5, 122.4, 136.8, 162.1, 142.7, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 120.9, 117.5, 37.5, 141.5, 123.5, 115.2 and 123.7 Corresponding to $C_1$, $C_2$, $C_6$, $C_3$, $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{23}$, and $C_{21}$ & $C_{22}$. Respectively., Anal.Calcd. For $C_{24}H_{17}F_2N_7O_2$ C 57.72%, H 2.25% and N 14.39%. Found: C 57.42%, H 1.93% and N 14.02%.

3-((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2, 5-difluorobenzoyl)-4-(2-(4-(trifluoromethyl)phenyl)hydrazono)-1H-pyrazol-5(4H)-one (4g)

Yield 71%.

m p: 160-162°C.

IR (KBr): 3381(-NH str.)3040(Ar-Hstr.) 1694 (exocyclic >C=O), 1654 (>C=O of pyrazoline-5-one), 1617 cm$^{-1}$ (>C=N group) and 1100cm$^{-1}$ (C-F str.)

$^1$H-NMR (400 MHz DMSO-d6): δppm 2.0 (s, H, NH attached to pyrazoline-5-one ring), 3.91 (t, 2H, -CH2 of 1H benzo [d] imidazole group), 5.0 (s, $^1$H,-NH of imidazole group) 10.15 (s, 1H,Ar-NH-N= Group) and 6.81-7.59 (m, 12H C$_6$H$_5$ C$_6$H$_4$ and C$_6$H$_3$), respectively.

$^{13}$CNMR(75MHz,DMSO-d6): at 143.0, 113.9, 129.5, 122.4, 136.8, 162.1, 142.7, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 120.9, 117.5, 37.5, 141.5, 123.5, 115.2 and 123.7 Corresponding to $C_1$, $C_2$, $C_6$, $C_3$, $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{23}$, and $C_{21}$ & $C_{22}$. Respectively., Anal.Calcd. For $C_{24}H_{17}F_2N_7O_2$ C 57.72%, H 2.25% and N 14.39%. Found: C 57.42%, H 1.93% and N 14.02%.
C_{16}C_{17}C_{18}C_{19}&C_{24}, C_{20}C_{23}, and C_{21} & C_{22}. Respectively., Anal. Calcd. For C_{24}H_{17}F_{2}N_{2}O_{2} C 57.72%, H 2.25% and N14.39%. Found: C 57.42 %, H 1.93% and N 14.02%.

Table 1: Yield and Melting Points data of Compounds 4(a-g).

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<td>4b</td>
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<td>4c</td>
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<td>4g</td>
<td>160-163</td>
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Mass spectra
The electron impact mass spectrum of 3-((benzo[d]imidazol-2-ylmethyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-(4-(trifluoromethyl)phenyl)hydrazono)-1H-pyrazol-5(4H)-one(4a) was recorded and interpreted. The mass spectral data of compound (4a) showed the molecular ion [M^+] ion peak at m/z=473.10(100%) it appeared as a base peak and indicates the presence of odd number of nitrozen atoms. The molecular ion signal was obeying nitrozen rule, but primary fragmented ions derived from molecular ion may or may not obeying nitrozen rule. The mass data of primary fragmented ions derived from 4a was shown in the table IV.1.7. The primary fragmentation process of 4a was reported in the chart IV.1.2.

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<th>Primary fragmented ion</th>
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<td>C_{7}H_{4}N_{2}•</td>
<td>C_{17}H_{12}F_{2}N_{6}O_{2}+ (XI)</td>
<td>357.10</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Biological activity
The antimicrobial profile of 3-((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (4 a-g)

The synthesis and characterization of Aryl hydrazono-Pyrazoline-5-ones bearing benzo[d]imidazol-2-yl) methyl) moiety (4a-g) were reported in Chapter-IV.1. The antibacterial and antifungal activity of 4(a-g) were studied and incorporated in the Chapter-V. The experimental results pertaining to the zone of inhibition (mm) of 4(a-g) were shown in the Table V.7.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Staphylococcus aureus NCCS2079</th>
<th>Bacillus cereus NCCS 2106</th>
<th>Escherichia coli NCCS2065</th>
<th>Aspergillus niger NCCS 1196</th>
<th>Candida albicans NCCS 2106</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>25 50</td>
<td>25 50</td>
<td>25 50</td>
<td>25 50</td>
<td>25 50</td>
</tr>
<tr>
<td>4b</td>
<td>- 09</td>
<td>- 08</td>
<td>- 07</td>
<td>- 09</td>
<td>- 10</td>
</tr>
<tr>
<td>4c</td>
<td>- 08</td>
<td>- 10</td>
<td>- 09</td>
<td>- 08</td>
<td>- 12</td>
</tr>
<tr>
<td>4d</td>
<td>06 10</td>
<td>09 10</td>
<td>04 08</td>
<td>06 10</td>
<td>08 09</td>
</tr>
<tr>
<td>4e</td>
<td>10 14</td>
<td>10 15</td>
<td>07 13</td>
<td>08 13</td>
<td>07 12</td>
</tr>
<tr>
<td>4f</td>
<td>09 12</td>
<td>10 14</td>
<td>06 12</td>
<td>07 12</td>
<td>06 15</td>
</tr>
<tr>
<td>4g</td>
<td>13 16</td>
<td>13 17</td>
<td>10 15</td>
<td>11 16</td>
<td>12 14</td>
</tr>
<tr>
<td>Chloromphenicol (5)</td>
<td>- 25</td>
<td>- 26</td>
<td>- 22</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Ketocanazole (50)</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
</tbody>
</table>
Each well contains 25 and 50 µg of compounds; Ch=Chloromphenicol 5 µg/mL, Ketocanazole 50 µg/mL.

RESULT AND DISCUSSION
The antibacterial activity of 3-(((1H-benzo[d]imidazol-2-yl)methyl)amino)-1-(2,5-difluorobenzoyl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (4a-g). was screened against the gram-positive bacteria *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106. The gram negative bacteria used was *Escherichia coli* 2065. The antibacterial results reveals that most of the compounds exhibit good antibacterial activity against both bacteria at the concentration of 50µg/mL. The presence of nitro (4f), tri fluoro methyl (4g), Chloro (4d) and Bromo (4e) were showed more activity than other substituted compounds. Here Chloromphenicol was used as reference compound to compare the activity.

The anti-fungal activity of 3-(((1H-benzo[d]imidazol-2-yl) methyl) amino)-1-(2,5-difluorobenzoyl)-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (4 a-g). was screened against the *Aspergillus niger* NCCS1196 and *Candida albicans* NCCS 2106. The antifungal results reveals that most of the compounds exhibit good anti-fungal activity against both fungi at the concentration of 50µg/mL. The presence of nitro (4f), tri fluoro methyl (4g), Chloro (4d) and Bromo (4e) were showed more activity than other substituted compounds. Here Ketocanazole was used as reference compound to Compare the activity.

The order of anti microbial activity (50µg/mL) 4f>4g >4d>4e>4c>4a>4b.

CONCLUSION
Hetero cyclic compound containing Benzimidazole nucleus plays most important role in Biological systems. Benzimidazoles and its derivatives are used for biological activities such as antiviral, antibacterial, antifungal and antitubercular. Vast number of Benzimidazole derivative compounds have been synthesized and evaluated for their biological activity.

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REFERENCES