SYNTHESIS AND BIOLOGICAL ACTIVITIES OF OXADIAZOLE CLUBBED PYRAZOLE DERIVATIVES

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ABSTRACT
Pyrazole and oxadiazole moiety are individually widely used as a precursor of antidepressant1-3, antipsychotic4, anti-inflammatory5, anticancer6, and analgesic agent7. Zibptantane, Nesapidil, Thiadazosin, Raltegravir drugs contain 1, 3, 4-oxadiazole as a core part8. Novalgin, Celecoxib, Crizotinib, and purazofuran possessing pyrazole as base moiety are also readily available in the market9. Nowadays, many antimicrobial/antibiotic drugs are losing their therapeutic effect on microbes and bacteria due to resistance10. Therefore oxadiazole-doped pyrazole scaffolds are getting attention to resolve the resistance issue. In search of novel potent molecules, we tried to put oxadiazole, and pyrazoline in one framework via S-linkage.

In present work, we have synthesized a series of 2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(4,5-dihydro-3,5-substituted diphenylpyrazol-1-yl)ethanone derivatives from 5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio (3). Our work also focuses on screening against gram-positive S.aureus and gram-negative bacteria E.coli to confirm its potential.

EXPERIMENTAL
Material and Methods
All starting materials were acquired from Merck. All reactions were monitored through TLC (Hexane: ethyl acetate; 80: 20). FTIR spectra were carried out through Bruker spectrophotometer. 1H NMR 13C NMR spectra were carried out at 500 MHz through Bruker spectrophotometer.

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Synthesis of 5-benzyl-1-oxa,3,4-diazacycloPentadiene-2-ylthio\(^{(3)}\)
Phenylacetic acid (0.15 mol) in 100 ml \(\text{CH}_3\text{OH}\) with a catalytic amount of \(\text{H}_2\text{SO}_4\) was refluxed for 7 hours. Then it was poured on ice water and ethyl 2-phenylacetate \((2)\) was collected. Further \((2)\) was refluxed for 8 hours with \((0.12 \text{ mol})\) hydrazine hydrated. After getting a single spot of the reaction mixture, it was poured into ice water to get 2-phenylacetohydrazide \((2)\).

5-benzyl-1-oxa,3,4-diazacyclo Pentadiene-2-ylthio \((3)\) was synthesized by mixing \((0.06 \text{ mol})\) of \((2)\) in \((0.10 \text{ mol})\) \(\text{KOH}\) and 100 ml methanol in RBF. Then \((0.10 \text{ mol})\) \(\text{CS}_2\) was added portion and the reaction mixture was refluxed with stirring for 11 hours. The reaction mixture was poured into ice water and treated with dil. HCl to get oxadiazol at 3-4 PH. Oxadiazol was collected through filtration and re-crystallized with ethanol. Yield 78%, M.P. 1180 C, FTIR (cm\(^{-1}\), KBr) 2948 (C-H str), 1617 (C=N), 1230 (C-O).

Synthesis of Chalcones \(^{(4a-j)}\)
Acetophenone \((0.06 \text{ mol})\) and aldehydes \((0.06 \text{ mol})\) were dissolved in 100 ml methanol. Then 40% aq. \(\text{NaOH}\) was added dropwise to the reaction mixture at 10\(^0\) C and stirred for 3 hours. The mixture was left overnight in the refrigerator. The reaction mixture was poured in cold water to get solid chalcones. Solids were filtered off and recrystallized with ethanol. Yield 75% - 85% FTIR (cm\(^{-1}\), KBr) 1656 (C=O), 1323 (C=C).

Synthesis of Chloro Acetyl Pyrazoline Derivatives \(^{(5a-j)}\)
Chalcone \((0.04 \text{ mol})\) and hydrazine hydrate \((0.04 \text{ mol})\) were dissolved in ethanol in RBF. It was refluxed for 12 hours. After the completion of the reaction, chloroacetyl chloride was added and stirred for 7 to 8 hours. Solid were obtained through vacuum distillation and re-crystallized by ethanol. Yield: 61% -72%, FTIR (cm\(^{-1}\), KBr) 1680 (C=O), 1265 (CH\(_2\)Cl), 3035 (C-H).

Synthesis of Novel Title Compound 6a-j
\(3\) \((0.002 \text{mol})\) and \((5a-l)\) \((0.002 \text{mol})\) were dissolved in 20 ml DMF in a conical flask. Anhydrous \(\text{K}_2\text{CO}_3\) was added and stirred for 8 hours at 30 -35\(^0\) C. Solids were filtered off and re-crystallized with ethanol.

In Vitro Antibacterial Evaluation
The antibacterial potential of Novel compounds was investigated against gram-positive \(\text{S. aureus}\) and gram-negative \(\text{E. coli}\) through the Disc Diffusion method. \(^{14}\) 5mm Discs were saturated with 100 \(\mu\text{g/ml}\) solution in DMSO and placed on media inoculated with bacteria. It was incubated at 35\(^0\) C for 24 hours. Zone inhibition diameters were measured.

RESULTS AND DISCUSSION
All novel molecules have been synthesized as mentioned in scheme-1 and confirmed with characterization.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(4,5-dihydro-5-(4-methoxyphenyl)-3-phenylpyrazol-1-yl)ethanone \((6a)\)
White solid, yield 78%, M.P. 201\(^0\) C; M. Formula: \(\text{C}_{27}\text{H}_{24}\text{N}_{4}\text{O}_{3}\text{S}\); MW (Grams /mole): 484; FT-IR(cm\(^{-1}\), KBr): 1664 (C-O amide), 2833 (C-H- methoxy), 1514 (C=C str- aromatic ring ) 1582 (C=N str – oxadiazole), 691 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 3.64 ppm (s, OCH\(_3\)), 4.06 ppm (s, CH\(_2\)), 5.42 (t, CH), 6.78 CH (5H,m, Ar-H of benzyl), 7.0-7.6(9H, m, Ar-H); 13CarbonNMR (500 MegaHz,δ ppm ): 31.82, 38.35, 42.32,55.39, 60.16, 114.38 -130.11, 155.69 -166.23 Mass: \(M^+\) 485.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(4,5-dihydro-3-(4-methoxyphenyl)-5-(3-nitrophenyl) pyrazol-1-y1)ethanone \((6b)\)
White solid, yield 75%, M.P. 230\(^0\) C; M. Formula: \(\text{C}_{27}\text{H}_{23}\text{N}_{5}\text{O}_{5}\text{S}\); MW(Grams /mole): 629; FT-IR(cm\(^{-1}\), KBr): 1657 (C=O amide), 2839 (C-H- methoxy), 1526 (C=C str- aromatic ring ) 1608 (C=N str – oxadiazole), 689 (str of S-C), 1344 str of NO\(_2\)HNR(500 MegaHz,δ ppm ): 3.72 ppm (s,OCH\(_3\)), 4.06 ppm (s, CH\(_2\)), 5.42 (t, CH), 6.78 CH (5H,m, Ar-H of benzyl), 7.0-7.6(9H, m, Ar-H); 13CarbonNMR (500 MegaHz,δ ppm ): 31.78, 35.36, 42.35,55.49, 59.8, 114.33 -133.48, 142.52-166.32; Mass: \(M^+\) 530.
2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(4,5-dihydro-5-phenyl-3-p-toly pyrazol-1-yl)ethanone (6c)
White solid, yield 77%. M.P. 218° C; C:39.24, H:2.40, N:4.44, O:2.07; MW (Grams/mole):468; FT-IR(cm⁻¹, KBr): 1658 (C=O amide), 1530 (C=C str- aromatic ring) 1590 (C=N str – oxadiazole), 687 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.45 ppm (s,CH₂), 5.53 (t,CH), 7.12 (5H,m, Ar-H of benzyl),7.49 -7.92 (9H, m, Ar-H); 1³CarbonNMR(500 MegaHz,δ ppm ): 32.10, 35.38,42.35,59.82,114.43-149.98 -167.22; Mass: M⁺467.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(5-(4-chlorophenyl)-4,5-dihydro-3-p-toly pyrazol-1-yl)ethanone (6d)
White solid, yield 73%. M.P. 180° C; C:39.13, H:2.30, N:4.44, O:2.07; MW (Grams/mole):503; FT-IR(cm⁻¹,KBr ): 1661 (C=O amide), 1527 (C=C str- aromatic ring) 1581 (C=N str – oxadiazole), 854 (str of C-Cl), 690 (str of S-C), 1HNMR (500 MegaHz,δ ppm ): 4.61 ppm ( s,CH₂), 5.48(t,CH), 6.74 (5H,m, Ar-H),7.35-7.87 (8H, m, Ar-H); 1³CarbonNMR: 31.52, 36.29, 42.46,59.90, 115.24 -135.66, 145.72 -166.46; Mass: M⁺505.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(5-(4-(dimethylamino)phenyl)-4,5-dihydro-3-p-toly pyrazol-1-yl)ethanone (6e)
White solid, yield 78%. M.P. 150° C; C:39.34, H:2.94, N:5.56, O:2.07; MW (Grams/mole):511; FT-IR(cm⁻¹,KBr): 1665 (C=O amide), 1525 (C=C str- aromatic ring) 1588 (C=N str – oxadiazole), 685 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.51 ppm (2H,s,CH₂), 5.66 (H,t,CH), 6.84 (5H,m, Ar-H of benzyl),7.36-7.75 (8H, m, Ar-H ); 1³CarbonNMR(500 MegaHz,δ ppm ): 31.56, 39.23, 41.40,61.09, 114.58-133.42, 145.36-166.50; Mass: M⁺512.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(4,5-dihydro-5-(3,4-dimethoxyphenyl)-3-p-toly pyrazol-1-yl)ethanone (6f)
White solid, yield 67%. M.P. 243° C; C:39.24, H:2.40, N:4.44, O:4.14; MW (Grams/mole):528; FT-IR(cm⁻¹,KBr): 1670.5 (C=O amide), 1513 (C=C str- aromatic ring) 2830 (Carbon –H; methoxy)1590 (C=N str – oxadiazole), 688 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.43 ppm (2H,s,CH₂), 5.64 (H,t,CH), 7.0(5H,m, Ar-H of benzyl),7.3.(8H, m, Ar-H); 1³CarbonNMR(500 MegaHz,δ ppm ): 31.49, 39.82,41.82,61.34, 114.86-134.47, 148.11-162.83; Mass: M⁺529.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(5-(4-(dimethylamino)phenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)ethanone (6g)
White solid, yield 77%. M.P. 228° C; C:39.13, H:2.30, N:5.56, O:2.07; MW (Grams/mole):497; FT-IR(cm⁻¹,KBr): 1659 (C=O amide), 1508 (C=C str- aromatic ring) 1587 (C=N str – oxadiazole), 693 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.45 ppm ( s,CH₂), 5.62 (t,CH) 6.98 (5H,m, Ar-H of benzyl),7.29-7.75(9H, m, Ar-H); 1³CarbonNMR(500 MegaHz,δ ppm ): 30.92, 38.75, 40.66,59.54, 115.62-138.37, 145.24-166.28; Mass: M⁺498.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(5-(4-(dimethylamino)phenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)ethanone (6h)
White solid, yield 71%. M.P. 251° C; M. Formula:C₂₈H₂₁N₅O₄S;MW (Grams /mole):499; FTIR(cm⁻¹, KBr ): 1665 (C=O amide), 1508 (C=C str- aromatic ring) 1587 (C=N str – oxadiazole), 693 (str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.51 ppm ( s,CH₂), 5.64 (t,CH), 6.84 (5H,m, Ar-H of benzyl),7.29-7.75(9H, m, Ar-H); 1³CarbonNMR(500 MegaHz,δ ppm ): 30.92, 38.75, 40.66,59.54, 115.62-138.37, 145.24-166.28; Mass: M⁺ 498.

2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-ylthio)-1-(3-(4-chlorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)ethanone (6i)
White solid, yield 69%. M.P. 165° C; M. Formula:C₂₈H₂₁ClN₅O₄S;MW (Grams /mole):488; FTIR(cm⁻¹, KBr ): 1665(C=O amide),1522(C=Cstr- aromatic ring), 1590(C=N str – oxadiazole),859 (str of C-Cl), 694(str of S-C), 1HNMR(500 MegaHz,δ ppm ): 4.51 ppm (s,CH₂), 5.53 (t, CH), 6.69 (5H,m, Ar-H of benzyl), 7.15-7.58 (9H, m, Ar-H); 1³CarbonNMR(500 MegaHz,δ ppm ): 32.05, 39.6, 41.15,62.5, 114.35 -134.78, 142.37-164.34; Mass: M⁺2 490.
2-(5-benzyl-1-oxa,3,4-diazacyclopentadiene-2-thlthio)-1-(3-(4-chlorophenyl)-4,5-dihydro-5-(3,4dimethoxyphenyl) pyrazol-1-yl)ethanone(6j)

Pale yellow solid, yield 63%, M.P. 205°C; M. Formula:C_{28}H_{25}ClN_{4}O_{4}S; MW (Grams /mole):548

FTIR(cm\(^{-1}\), KBr): 1662 (C=O amide), 1528 (C=C str- aromatic ring), 1593 (C=N str – oxadiazole), 862 (str of C-Cl), 687 (str of S-C), \(^{1}\)HNMR(500 MegaHz, δ ppm): 4.43 ppm (2H,s,CH\(_2\)), 5.51 (H, t, CH), 6.95 (5H,m, Ar-H of benzyl), 7.28-7.7(8H, m, Ar-H of phenyl); \(^{13}\)CarbonNMR (500 MegaHz,δ ppm): 31.82, 39.47, 40.59,61.12, 113.83 -132.64, 149.69-166.38; Mass: M\(^{+}\)550

\[
\begin{align*}
\text{(a)} & \text{CH}_{3}\text{OH}, \text{H}_{2}\text{SO}_{4} & \text{(b)} & \text{NH}_{2}\text{NH}_{2}, \text{2H}_{2}\text{O} & \text{(c)} & \text{CH}_{3}\text{OH}, \text{KOH} & \text{(d)} & \text{Ethanol, aq. NaOH} & \text{(e)} & (1)\text{NH}_{2}\text{NH}_{2}, \text{2H}_{2}\text{O},(2) \text{chloro acetyl chloride} & \text{(f)} & \text{DMF}, \text{K}_{2}\text{CO}_{3}
\end{align*}
\]

**Table-1: In-vitro Antibacterial Evaluation**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R)</th>
<th>(R_1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>4-OCH(_3)</td>
<td>H</td>
</tr>
<tr>
<td>6b</td>
<td>4-OCH(_3)</td>
<td>3-NO(_2)</td>
</tr>
<tr>
<td>6c</td>
<td>4-CH(_3)</td>
<td>H</td>
</tr>
<tr>
<td>6d</td>
<td>4-CH(_3)</td>
<td>4-Cl</td>
</tr>
<tr>
<td>6e</td>
<td>4-CH(_3)</td>
<td>4-N(CH(_3))(_2)</td>
</tr>
<tr>
<td>6f</td>
<td>4-CH(_3)</td>
<td>3,4-di OCH(_3)</td>
</tr>
<tr>
<td>6g</td>
<td>H</td>
<td>4-N(CH(_3))(_2)</td>
</tr>
<tr>
<td>6h</td>
<td>H</td>
<td>4-NO(_2)</td>
</tr>
<tr>
<td>6i</td>
<td>4Cl</td>
<td>H</td>
</tr>
<tr>
<td>6j</td>
<td>4-Cl</td>
<td>3,4 diOCH(_3)</td>
</tr>
</tbody>
</table>
All screened compounds show good antibacterial activity through the DD method as mentioned in Fig.-1(a) E. coli, (b) S. aureus, and Table-2.

Table-2: Inhibition Zone: 1-9 (mild), 10-15 (moderate), 15-20 (high)

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>6b</td>
<td>09</td>
<td>10</td>
</tr>
<tr>
<td>6c</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>6d</td>
<td>14</td>
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</tr>
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<td>6e</td>
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<td>6f</td>
<td>09</td>
<td>08</td>
</tr>
<tr>
<td>6g</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>6h</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>6i</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>6j</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

Zone of Inhibition (mm)

Compound 6d and 6i shows the highest activity among all compounds.
Chlorine-substituted compound (6i) is a highly potent molecule against S. aureus and E. coli. Methyl, dimethoxy, and dimethyl amine substituted compounds are moderately active in S. aureus and E. coli. Molecules bearing methoxy and nitro groups (comp. 6b and 6f) are mild/less active against both bacteria. These Molecules bear with amide group, oxadiazole, pyrazole, s-linkage as well as electron-withdrawing substitutions, which are responsible for their excellent activity. There are also possibilities of having other therapeutic activities.

CONCLUSION

We have synthesized a novel series of 2-(5-benzyl-1-oxa,3,4-diazaacyclo[2.2.1]heptane-2-ythio)-1-(4,5-dihydro-3,5-diphenylpyrazol-1-yl)ethanone. Further in-vitro antibacterial investigation indicates that oxadiazole clubbed pyrazole derivatives bearing a S-linkage having a higher potential as a pharmaceutical agent.

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CONFLICT OF INTERESTS

Authors express declaration of no conflicted interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:
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