1-(3-ACETYL PHENYL)-4-(4-ARYLIDENE)-2-PHENYL-1H-IMIDAZOL-5(4H)-ONES: SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, ANTIMICROBIAL AND MOLECULAR DOCKING STUDIES

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ABSTRACT
Imidazole rings are privileged moieties in heterocyclic chemistry since they permit functional group and structural modifications to synthesize abundant substituted heterocycles. Thus, the pursuit of exploring many more alterations on imidazole moiety needs to be continued. In the present work an array of the new imidazolone derivatives was prepared as per the expedient method. The moieties were characterized by FTIR, 1H NMR, 13C NMR and Mass spectra. Docking was studied to exemplify the binding connections of intended scaffolds with the crystal structure of protein reductase of Staphylococcus aureus pdbid:4NZ9. The synthesized compounds were tested for antibacterial activity by the agar diffusion method. All the compounds showed potent activity against diverse bacterial species.

Keywords: Oxazolone, Glacial Acetic Acid, Imidazolone, Molecular Docking, Acetyl phenyl.

INTRODUCTION
Amid aromatic heterocycles, predominant moieties are five-membered rings. The role and efficacy of heterocycles in organic synthesis surfaced the way to develop precursors for aminoacids, medicinal drugs and other chemical components. For any organic moiety, the effectiveness is measured based on its nontoxic nature, lower dosage and inhibition of microbial growth. The research activity in heterocyclic compounds is intensified on the expansion of biologically active scaffolds, likewise on formulating novel molecules for assessing their potency as drugs, pharmaceuticals, special chemicals and agrochemicals. In this view, the synthesis of new drugs with a different mode of action is desirable to be explored. Imidazolone-5-ones form a significant class of heterocyclic moieties and are acknowledged to display pharmacological and biological actions such as CNS depressant, antifungal, antihelmintic, anticancer, anticonvulsant, anti-parkinsonian, anti-inflammatory and monoamine oxidase inhibitory agents. Few new disubstituted imidazolones were proved as anticonvulsant, monoamine oxidase suppressive and succinate dehydrogenase suppressive agents. Imidazoloquinazolinone derivatives which are synthesized from aryldiene-2-phenyl-oxaolone derivatives, showed promising Antibacterial activity. Imidazolidones are first-rate crucial ligands in Organometallic chemistry as their complexes of Cu2+ displayed anti-E.coli non-pathogenic strain activity. Thus, the pursuit to explore modifications on imidazole ring desiries to be sustained. The present work comprises of synthesis of novel imidazolinones(6a-j) and is evaluated for their antimicrobial activity. In the direction of elucidating the antibacterial assay, molecular docking studies on staphylococcus aureus were performed.

EXPERIMENTAL
Material and Methods
The experimental ingredients that were used are of analytical grade. The compounds there were prepared and checked in open-glass capillaries on Staurt-SMP10 melting point apparatus and were uncorrected.


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The purity of the sample was checked by (TLC)plates DC 0.25mm,60F254 coated sheets procured from merck, and spots were envisaged by iodinevapor(or)U.Vlight.Ten novel imidazolones were synthesized from azlactones by reported procedures.\textsuperscript{15} Molecular docking studies were done on Linux operating system using Schrodinger suite 2009. Crystal structure of reductaseprotein of Staphylococcus aureus was downloaded from the protein data bank pdbid:4NZ9. The protein fragments were set by protein groundwork wizard applying OPLS2005 forcefield, and a grid was prepared around the bindingsite by choosing the co-crystallizedligand. Imidazole derivatives were built in Maestro-build-panel and outfitted by Ligprep-module. These were docked in requisite-site using Glide-5.6. Ligand interaction diagrams were obtained using Maestro academic-version and figures were obtained using DS4.0 viewer.

**General Procedure for Synthesis 4-benzylidene-2-phenyl-2-oxazoline-5-one (4a-j)**

4-benzylidene-2-phenyl-2-oxazoline-5-ones (4a-j) are prepared by condensation of aromatic aldehydes with benzoyl derivative of glycine in the presence of (CH\textsubscript{3}CO)\textsubscript{2}O and fused anhydrous sodium acetate.0.008mol of benzyol derivative of glycine prepared by stirring glycine(0.33mol) with benzoyl chloride(0.385mol),1gram of freshly fused sodium acetate and 2-3 ml of acetic anhydride was added with slow stirring and stirring continued for 30 seconds. Then immediately, 0.008mol aromatic aldehyde was added and refluxed on a hot waterbath for about 1 hour. Continuation of the reaction was observed by using TLC.7ml ethanol was added to it and kept overnight, which resulted in the yellow crystalline precipitate, which was purified by washing with cold ethanol followed by boiling water.

![Scheme-1](image)

**Synthesis of Novel-1H-imidazole-5(4H)-ones**

1 mol of 4-aryliden-2-phenyl-2-oxazoline-5-ones (4a-j),0.008mol of 3-amino acetonaphone (5), 15 ml glacial acetic acid and CH\textsubscript{3}COONa were refluxed for 3 hrs at 100°C in aRB flask. After checking the continuity of reaction monitored by TLC, the reaction mass was transferred into crumpled ice. The precipitate was dried and run by column chromatography(Scheme-2).

**1-(3-acetylphenyl)-4-imidazolderivative(6a)**

FT-IR(KBr cm\textsuperscript{-1}) 3421, 2922, 1800, 1633, 1448, 1295, 1170, 877, 759, 698 cm\textsuperscript{-1}.\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \( \delta \) 8.28 (s,1H,aromatic), 8.11 (s,1H,CH\textsubscript{3}), 7.68 (t, \( J = 7.81 \) Hz, 2H, aromatic), 7.51 (t, \( J = 7.44 \) Hz, 2H, aromatic), 7.42 (d, \( J = 7.23 \) Hz, 2H, aromatic), 7.12 (d, \( J = 7.55 \) Hz, 2H, aromatic), 6.83 (d, \( J = 7.14 \) Hz, 1H, aromatic), 2.36 (s,3H,CH\textsubscript{3}) ppm. MS-ESIm/z 368 [M + 2H]\textsuperscript{+}.

**1-(3-acetylphenyl)-4-(2-chlorobenzylidene)-imidazol-one(6b)**

FT-IR(KBr cm\textsuperscript{-1}) 3447, 2923, 2849, 1784, 1634, 1550, 1289, 878, 694 cm\textsuperscript{-1}.\textsuperscript{1}H NMR : 8.15 (s,1H,aromatic), 8.10 (s,1H,CH\textsubscript{3}), 7.62 (t, \( J = 7.5 \) Hz, 2H, aromatic), 7.53 (m, 6H, aromatic), 7.42 (d, 2H, aromatic), 7.22 (d, \( J = 7.6 \) Hz, 2H, aromatic), 2.38(s,3H,CH\textsubscript{3}) ppm. MS-ESIm/z 401 [M + H]\textsuperscript{+}.

**1-(3-acetylphenyl)-4-(4-chlorobenzylidene)-1H-imidazolone(6c)**

FT-IR (KBr cm\textsuperscript{-1}) 3449, 2920, 2849, 1784, 1634, 1550, 1289, 878, 694 cm\textsuperscript{-1}.\textsuperscript{1}H NMR : 8.15 (s,1H,aromatic), 8.10 (s,1H,CH\textsubscript{3}), 7.62 (t, \( J = 7.5 \) Hz, 2H, aromatic), 7.53 (m, 6H, aromatic), 7.42 (d, 2H, aromatic), 7.22 (d, \( J = 7.6 \) Hz, 2H, aromatic), 2.38(s,3H,CH\textsubscript{3}) ppm. MS-ESIm/z 401 [M + H]\textsuperscript{+}.
Scheme-2: Synthetic Reactions of Imidazolones 6a-j

1-(3-acetylphenyl)-4-(2,4-benzylidenedichloro)-2-phenyl-imidazol-5(4H)-one(6d)

FT-IR (KBr cm\(^{-1}\)) 3557, 3313, 2923, 1644, 1563, 1267, 828, 780, 536 cm\(^{-1}\);

\(\text{\(1\text{H NMR}\)} \delta 8.15 (s, 1H, aromatic), 8.10 (s, 1H, CH), 7.62 (t, \(J = 7.36\) Hz, 2H, aromatic), 7.53 (m, 5H, aromatic), 7.42 (d, 3H, aromatic), 7.12(d, \(J = 7.52\) Hz, 1H, aromatic), 2.38 (s, 3H, CH\(_3\)) ppm.

\(\text{MS-ESIm/z 437 [M + H}\)\(^{+}\).}

4-(2,6-dichlorobenzylidene)-1-(3-acetylphenyl)-2-phenyl-1H-imidazol-5(4H)-one(6e)

FT-IR (KBr cm\(^{-1}\)) 3300, 2927, 1707, 1619, 1457, 1151, 955, 777, 637 cm\(^{-1}\);

\(\text{\(1\text{H NMR}\)} \delta 8.15 (s, 1H, aromatic), 8.10 (s, 1H, CH), 7.62 (t, \(J = 7.42\) Hz, 2H, aromatic), 7.51 (m, 5H, aromatic), 7.42 (d, \(J = 7.61\) Hz, 2H, aromatic), 7.11 (d, \(J = 7.52\) Hz, 2H, aromatic), 2.38 (s, 3H, CH\(_3\)) ppm.

\(\text{MS-ESIm/z 437 [M + H}\)\(^{+}\).}
4-(2-hydroxybenzylidene)-1-(3-acetylphenyl)-2-phenyl-1H-imidazol-5(4H)-one (6f)
FT-IR (KBr cm\(^{-1}\)) 3499, 2923, 2853, 1643, 1539, 1429, 1397, 1099, 712, 668 cm\(^{-1}\). 1\(^H\) NMR δ 8.28 (s, 1H, CH), 8.10 (s, 1H, aromatic), 7.64 (t, \(J = 7.42\) Hz, 2H, aromatic), 7.61 (t, \(J = 7.53\) Hz, 2H, aromatic), 7.51 (m, 3H, aromatic), 6.82 (d, \(J = 7.10\) Hz, 1H, aromatic), 6.63 (d, \(J = 7.33\) Hz, 1H, aromatic), 4.82 (s, 1H, OH), 2.41 (s, 3H, CH\(_3\)) ppm. MS-ESI m/z 383 [M + H]\(^+\).

4-(4-hydroxybenzylidene)-1-(3-acetylphenyl)-2-phenyl-1H-imidazol-5(4H)-one (6g)
FT-IR (KBr cm\(^{-1}\)) 3419, 2924, 1629, 1550, 1321, 891, 750, 676 cm\(^{-1}\). 1\(^H\) NMR: 8.11 (s, 1H, aromatic), 7.86 (s, 1H, CH), 7.61 (t, \(J = 7.44\) Hz, 2H, aromatic), 7.51 (m, 3H, aromatic), 6.82 (d, \(J = 7.10\) Hz, 2H, aromatic), 4.87 (s, 1H, OH), 2.36 (s, 3H, CH\(_3\)) ppm. MS-ESI m/z 383 [M + H]\(^+\).

1-(3-acetylphenyl)-4-(4-nitrobenzylidene)-2-phenyl-1H-imidazolone (6h)
FT-IR (KBr cm\(^{-1}\)) 3010, 2925, 1665, 1622, 1548, 1444, 1307, 1053, 703, 678 cm\(^{-1}\). 1\(^H\) NMR δ: 8.25 (t, \(J = 7.46\) Hz, 3H, aromatic), 8.09 (d, \(J = 7.22\) Hz, 2H, aromatic), 7.93 (s, 1H, CH), 7.59 (t, \(J = 7.44\) Hz, 2H, aromatic), 7.51 (m, 3H, aromatic), 7.41 (d, \(J = 7.64\) Hz, 2H, aromatic), 7.29 (t, \(J = 7.10\) Hz, 1H, aromatic), 2.39 (s, 3H, CH\(_3\)) ppm. MS-ESI m/z 412 [M + H]\(^+\).

1-(3-acetylphenyl)-2-phenyl-4-(3,4,5-trimethoxybenzylidene)-imidazol-5(4H)-one (6i)
FT-IR (KBr cm\(^{-1}\)) 3449, 2920, 1784, 1638, 1550, 1289, 1164, 817, 694 cm\(^{-1}\). 1\(^H\) NMR δ 8.20 (s, 1H, aromatic), 8.09 (s, 1H, CH), 7.61 (t, \(J = 7.48\) Hz, 2H, aromatic), 7.50 (d, \(J = 7.77\) Hz, 2H, aromatic), 6.45 (s, 2H, aromatic), 3.82 (s, 9H, OCH\(_3\)), 2.46 (s, 3H, CH\(_3\)) ppm. MS-ESI m/z 457 [M + H]\(^+\).

1-(-3-acetylphenyl)-4-(2,5-dimethoxybenzylidene)-2-phenyl-imidazolone (6j)
FT-IR (KBr cm\(^{-1}\)) 3436, 2925, 1787, 1638, 1599, 1555, 1451, 1332, 1188, 882, 696 cm\(^{-1}\). 1\(^H\) NMR δ 8.21 (s, 1H, aromatic), 8.10 (s, 1H, CH), 7.60 (t, \(J = 7.52\) Hz, 2H, aromatic), 7.50 (m, 6H, aromatic), 6.45 (s, 3H, aromatic), 3.80 (s, 6H, OCH\(_3\)), 2.48 (s, 3H, CH\(_3\)) ppm. MS-ESI m/z 427 [M + H]\(^+\).

Detection Method
The prepared derivatives were checked in open glass capillaries on Stuart SMP10 melting point instrument and were uncorrected. TLC was used to identify the purity of the sample, and the single spot was visualized under U.V light. The IR spectra were obtained with IR AFFINITY-1 FTIR Shimadzu spectrometer. 1\(^H\) NMR spectra (ppm) were noted in CDCl\(_3\) and DMSO-d\(_6\) on a varianmercury 300MHz spectrometer. 13\(^C\) NMR spectra have been recorded in CDCl\(_3\) and DMSO-d\(_6\) on a Bruker Avance II 400 spectrometer at 400MHz. All derivatives were regularly checked by TLC by means of CHCl\(_3\):CH\(_3\)OH (9:1) solvent system and checked under UV light.

Analytical Discussion
As a representative case, the spectral analysis of (6a) is considered. In the IR spectrum of (6a), the stretching frequency at 3421 cm\(^{-1}\) and 2922 cm\(^{-1}\) indicates absorption due to the aliphatic (C-H) group. The characteristic (C=N) absorption was observed at 1448 cm\(^{-1}\) and (C-N) group absorption at 1295 cm\(^{-1}\). The 1\(^H\)NMR spectrum (400MHz,CDCl\(_3\), ) (6a) showed multiplet between δ 7.12-8.11 due to aromatic protons and δ 8.28 due to arylidene proton. ESI-MS mass spectra of derivative (6a) m/z 368 (M+2H)\(^+\) appeared as base peak.

RESULTS AND DISCUSSION

Proposed Mechanism
The mechanism involves Erlenmeyer plochl reaction which involves perkin condensation where acetic anhydride reacts with substituted aromatic aldehydes to undergo cyclization and
condensation reaction in presence of weak base. Intermolecular acetylation reaction occurs via alkoxide anhydride intermediate in this mechanism.

![Chemical Structure](image)

Table-1: Physical Characterization of Compounds (6a-j)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Systemic Name</th>
<th>Molecular Formula</th>
<th>Yield %</th>
<th>M.P (°C)</th>
<th>Color of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>1-(3-acetyl phenyl)-4-benzylidene-2-phenyl-1H-imidazol-5(4H)-one</td>
<td>C_{24}H_{18}N_{2}O_{2}</td>
<td>80</td>
<td>232°C</td>
<td>Orange solid</td>
</tr>
<tr>
<td>6b</td>
<td>1-(3-acetylphenyl)-4-(2-chlorobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one</td>
<td>C_{24}H_{17}ClN_{2}O_{2}</td>
<td>82</td>
<td>195°C</td>
<td>Yellowish Orange solid</td>
</tr>
<tr>
<td>6c</td>
<td>1-(3-acetylphenyl)-4-(4-chlorobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one</td>
<td>C_{24}H_{17}ClN_{2}O_{2}</td>
<td>85</td>
<td>256°C</td>
<td>Yellowish Orange solid</td>
</tr>
<tr>
<td>6d</td>
<td>1-(3-acetylphenyl)-4-(2,4-dichlorobenzylidene)-2-phenyl-imidazol-5(4H)-one</td>
<td>C_{24}H_{16}Cl_{2}N_{2}O_{2}</td>
<td>78</td>
<td>278°C</td>
<td>light orange solid</td>
</tr>
<tr>
<td>6e</td>
<td>1-(3-acetylphenyl)-4-(2,6-dichlorobenzylidene)-2-phenyl-imidazolone</td>
<td>C_{24}H_{16}Cl_{2}N_{2}O_{2}</td>
<td>82</td>
<td>280°C</td>
<td>Yellowish orange solid</td>
</tr>
</tbody>
</table>
Antibacterial Assay
The antibacterial assay of synthesized moieties were checked against gram positive bacteria B. subtilis and S. aureus and two gram negative bacteria E. coli and S. typhi. A stock solution of 2mg/ml was prepared. Wells were made in the agar plate using a borer. The sample solution is pipetted into the wells. And the plates were incubated for around 24hrs. Then the region of restrained and recorded as shown in Table-2. All compounds exhibited moderate to potent activity against gram(+) and gram(-) bacteria.

Table-2: Antibacterial Activity of 4-(4-arylidene)-1-(3-acetyl phenyl)-2-phenyl-1H-imidazol-5(4H)-ones(6a-j)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition(mm) in 2µg/ml concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gram -ve</td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
</tr>
<tr>
<td>6a</td>
<td>12</td>
</tr>
<tr>
<td>6b</td>
<td>12</td>
</tr>
<tr>
<td>6c</td>
<td>13</td>
</tr>
<tr>
<td>6d</td>
<td>13</td>
</tr>
<tr>
<td>6e</td>
<td>12</td>
</tr>
<tr>
<td>6f</td>
<td>13</td>
</tr>
<tr>
<td>6g</td>
<td>12</td>
</tr>
<tr>
<td>6h</td>
<td>10</td>
</tr>
<tr>
<td>6i</td>
<td>9</td>
</tr>
<tr>
<td>6j</td>
<td>11</td>
</tr>
</tbody>
</table>

Molecular Docking Studies
Imidazole derivatives have accomplished much contemplation for their anti-microbial activity. Bacterial ENRs are believed to be fine explicit targets for antibacterial agents development and are one of the target proteins that are inhibited by imidazole derivatives.17,18 In order to explicate the mode of interaction and binding of synthesized imidazole derivatives, molecular docking studies were carried out on X-ray crystal structure of FabI of Staphylococcus aureus, pdbid:4NZ9 found from databank (www.rcsb.org).19-22 The docking results are provided in Table-3. The highest dock score of -10.318 kcal/mol and least dock score of -9.014 kcal/mol were obtained for molecules 6a and 6g, respectively. All molecules showed a good
dock score, indicating that all molecules are good inhibitors of enoylacylcarrier protein reductase (Fab I). Dock pose of molecule 6g in the active location of Fab I is shown in Fig.-1 and hydrophobic interaction of the molecules with active site amino acid residues is visible in Fig.-2. The hydrogen bonding interaction of ligand is shown in Fig.-3. The molecule showed 2 H-bond interactions with amino acid residue Asn156 and Ser197; the other major interaction with the protein was of hydrophobic nature with amino acid residues Met 99, Tyr147, Tyr157, Met160, Pro 192 and Ile207 in Fig.-3.

Fig.-1: Dock Pose of Molecule 6g in the Active Site of FABI

Fig.-2: Hydrophobic Interaction of the Molecules with Dynamic Site Amino Acid Residues

Fig.-3: Ligand Interaction Diagram representing the Hydrogen Bond Interaction.
Table-3: Dockscore of imidazole derivatives docked into the active site of FabI of S. aureus using extra precision (XP) docking protocol of Glide 5.6.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Entry</th>
<th>Dockscore (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6a</td>
<td>-10.318</td>
</tr>
<tr>
<td>2.</td>
<td>6b</td>
<td>-9.021</td>
</tr>
<tr>
<td>3.</td>
<td>6c</td>
<td>-9.648</td>
</tr>
<tr>
<td>4.</td>
<td>6d</td>
<td>-9.781</td>
</tr>
<tr>
<td>5.</td>
<td>6e</td>
<td>-9.159</td>
</tr>
<tr>
<td>6.</td>
<td>6f</td>
<td>-9.488</td>
</tr>
<tr>
<td>7.</td>
<td>6g</td>
<td>-9.014</td>
</tr>
<tr>
<td>8.</td>
<td>6h</td>
<td>-9.603</td>
</tr>
<tr>
<td>9.</td>
<td>6i</td>
<td>-9.121</td>
</tr>
<tr>
<td>10.</td>
<td>6j</td>
<td>-9.511</td>
</tr>
</tbody>
</table>

CONCLUSION

The synthesized novel imidazole scaffolds exhibited wide spectrum antibacterial activity. Compounds 6a, 6c, 6d, 6g and 6h displayed potent activity against gram+ve and gram −ve bacteria. Molecular docking studies with bacterial host DNA inferred confirmative outcomes for the binding affinity of derivatives in accord with experimental data. All the derivatives have shown moderate to good docking scores indicating that they are inhibitors of enoyl acyl carrier protein reductase. Among all the entries, 6a showed the least dock score of -9.014 kcal/mol and 6g maximum dock score of -10.318 kcal/mol. The twang on docking is to computationally conjure up the molecular recognition process.

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