SPECTRAL ELUCIDATION, ANTIMICROBIAL AND ANTIOXIDANT STUDY OF NEWLY SYNTHESIZED PYRAZOLINE DERIVATIVES

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ABSTRACT
The preparation of Chalcones and Pyrazoline derivatives from different aromatic ketones and substituted aldehydes is detailed. Chalcones were produced by reacting aldehydes with substituted aromatic ketones, such as acetophenones, in the Claisen-Schmidt condensation reaction. Cyclohexenone derivatives are produced when ethyl aceto aromatic ketones undergo base-catalyzed cyclo-condensation to Chalcones under microwave radiation. The synthesized compounds are characterized using spectrum techniques from 1H NMR, 13C NMR, and IR. These are examined for their antioxidant qualities as well as their antibacterial action against Staphylococcus aureus, Escherichia coli, Aspergillus niger, and Aspergillus flavus.

Keywords: Chalcones, Pyrazoline, Antibacterial Activity, Antifungal Activity, Spectral Elucidation.

INTRODUCTION
Because of their numerous potential uses in scientific fields like synthetic and medicinal chemistry, natural Chalcones and their synthetic derivatives have garnered a lot of interest.¹,² These are useful intermediates in organic synthesis from a chemical perspective, and when basic catalysis is present, they can function as activated unsaturated systems in the conjugated addition reaction of carbanions.³,⁴ Utilizing this kind of reaction could lead to the production of derivatives of cyclohexenone that are highly functionalized.⁵,⁶ Chalcones have a remarkable range of biological activities linked to them, such as blood platelet anti-aggregating activity and antitubercular, antiparkinson, anti-inflammatory, anticancer, antiasthmatic, antibacterial, and antihypertensive properties. Chalcones and their derivatives are important biologically, but they also have a wide range of uses in cosmetic compositions and dyes.⁷-¹⁸ Another class of cyclic compounds with intriguing biological activities are cyclohexenone derivatives of chalcones; among these, its antimicrobial activity is well-documented.¹⁸,¹⁹ Cyclohexenone are therefore highly desirable target molecules in synthetic organic chemistry. Chalcones are used more frequently in the synthesis of cyclohexenone, which is an effective precursor for the synthesis of isoxazolones, benzisoxazoles, carbazole derivatives, and indazoline pyrazoline. Derivatives of cyclohexenone are well-known lead compounds for the management of autoimmune disorders and inflammation.²⁰-²⁴ Under microwave radiation, chemical reactions can be sped up and the resulting products' selectivity can be achieved.²⁵ Compared to traditional heating, this method has a number of benefits, including fast and instantaneous heating, high temperature homogeneity, and selective heating.²⁶ Because microwave-assisted synthesis delivers on its promise of being a quick synthesis technique, synthetic organic chemistry has made extensive use of the microwave heating technique. Given the significance of Chalcones' cyclohexenone derivatives both chemically and biologically, a report has been made on the high yield synthesis of these derivatives using the microwave technique.
In vitro antimicrobial activity against Staphylococcus aureus, Escherichia coli, Aspergillus Niger, and Aspergillus flavus is assessed after the synthesized compounds undergo structural elucidation. Because of the encouraging outcomes, we decided to further test them for antioxidant activity.27

EXPERIMENTAL

Chemicals
All chemical reagents were procured from Sigma Aldrich and the reputable Merck pharmaceutical company located in Mumbai, India. Glacial acetic acid and solvents were provided by S.D. Fine (Mumbai, India) and were of LR mark. Spectrochem, India, supplied the ethanol solvent. The following were obtained from Sigma-Aldrich and CDH (New Delhi, India): Cyanuric chloride, 4-amino acetanilide, 1,3-benzodioxole-5-carbaldehyde(piperonal), 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, thiophene-2-carbaldehyde, malononitril, and hydrazine hydrate. The entire set of reagents and solvents used in this investigation's heterocyclic derivative synthesis were purified using conventional methods. As developing solvents, benzene, ethyl acetate, and chloroform were employed.28

Thin Layer Chromatography (TLC)
A vision apparatus was used to record the melting points. Using silica gel G (Merck) as the immobile phase and an iodine chamber and UV lamp to visualize the TLC points, reaction improvement was monitored on TLC. The exposure to iodine gas, heating the plates dipped in diluted KMnO4 stain, and ultraviolet lighting all contributed to the visualization of points on TLC plates. Highly pure silica gel for the Column Chromatography (CC) technique was acquired from Sigma Aldrich & Co. Absolute ethanol was used to recrystallize every compound that was synthesized.

IR Spectra
Using a KBr pellet and a JASCO spectrophotometer, the solid-state IR spectra were recorded using Perkin-Elmer Spectrum IR version 10.6.0. FT-IR data were also obtained with a Jasco-400 spectrometer for the synthetic organic mixes.

NMR Spectra
Every NMR spectrum recorded from the instruments was recorded using a Bruker AMX 400 MHz instrument and five-millimetre PABBO/BB-13H pipes. Proton (1H) NMR spectra were recorded at frequencies between 300 and 400 MHz using 0.03 M solutions in CDCl3 or d6-DMSO, with Tetramethylsilane serving as the internal reference chemical shift. Using Tetra-methylsilane as the inner reference chemical shift, 13C NMR spectra were recorded at 75 MHz or 100 MHz using approximately 0.05 M solutions in CDCl3 or d6-DMSO.

Mass Spectra
The electron spray ionization-MS electrode WATERS-Q-TOF premier-HAB213 was used to perform the mass spectrum.

Elemental Analysis
A thermo fanning Flash EA1112 CHN (STIUJA) elemental analyser was used to obtain the elemental analysis. The yield that is reported is the isolated yield following compound purification.

General Procedure for Pyrazoline Derivative Synthesis(a-e)
Synthesis of Derivatives of Pyrazoline Standard protocol for N1-(4, 6-dichloro-1,3,5-triazin-2-yl) synthesis -N4-benzene-1,4-diamine (a-f) (4,5-dihydro-5-substituted-1H-pyrazol 3yl)
Equimolar amounts of diluted substituted hydrazine hydrate and substituted benaldehydes (0.01 M) in 40 millilitres of ethanol. For eight hours, this mixture was refluxed. After that, it was added to crushed ice and vented. After being separated, strained, cleaned with distilled water, and recrystallized from Power
alcohol, the resulting products were deemed to be (a-f). TLC was used to track the reaction's progress while n-hexane (1:3) and ethyl acetate served as the solvents. After ethanol was used to recrystallize the resultant product, it was dried. Six final compounds and all six intermediate compounds were synthesized. Melting point values, IR spectral data, H$^1$ NMR data, C$^{13}$ NMR data, mass spectra data, and elemental analysis were used to describe the structures of the synthesized compounds.

![Fig.-2: Synthesis of Derivatives of Pyrazoline (1a – 1f)](image)

Table-1: Antibacterial Activity of Pyrazoline Derivatives

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>Bacillus subtilis (mm)</th>
<th>Staphylococcus aureus (mm)</th>
<th>Salmonella typhi (mm)</th>
<th>Escherichia coli (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-a</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>1-b</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1-c</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>1-d</td>
<td>6</td>
<td>9</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>1-e</td>
<td>5</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>1-f</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>Ciprofloxacin (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-2: Antifungal Activity of Pyrazoline Derivatives

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>Aspergillus niger (mm)</th>
<th>Candida Albicans (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-a</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1-b</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1-c</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1-d</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>1-e</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1-f</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>Amphotericin-B (mm)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Larvicidal Activity

Larvae were separated into five collections of 20 and placed in 249 millilitres of distilled water and 1.0 millilitres of the intended chemical extract concentration for the bioassay tests. After a 24-hour exposure, the number of dead larvae was counted, and the percentage of mortality was calculated using the mean of five replicates (Table-3).28-30

Mosquito Larvae Consumed

Larvae of Anopheles subpictus were acquired from Pondicherry's Vector Control Research Centre. The larvicidal action was measured using the World Health Organization's (WHO) methodology with minor modifications.31, 32

Table-3: Larvicidal Activity of Pyrazoline Derivatives

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds designation</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-a</td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>1-b</td>
<td>68%</td>
</tr>
<tr>
<td>3</td>
<td>1-c</td>
<td>79%</td>
</tr>
</tbody>
</table>
Antioxidant Activity
Hi Media Laboratory Pvt. Ltd. provided ascorbic acid and DPPH (1, 1-diphenyl-2-picrylhydrazyl), and all other reagents, chemicals, and solvents used in the study were of analytical grade.

**In-vitro Anti-Oxidant Activity**

**DPPH-Scavenging Activity**
To evaluate the 1, 3-diphenyl-2-propene-1-one derivatives' ability to scavenge free radicals, the most stable radical, 2-diphenyl-picrylhydrazyl (DPPH), was used. 1 ml of 1, 3 diphenyl 2-propene-1-one derivatives (0.1 ml & 0.2 ml) prepared in DMSO was added to 1 ml of DPPH (1x10^-4 ml) solution made in DMSO, and the resultant compound was allowed to complete suspension with vigorous agitation. The DPPH solution served as the reference sample. The aluminium test tubes were used to seal the sample test tubes before adding the DPPH radical. The amount of organic matter was measured using a UV-visible spectrometer at a wavelength of 517 nm using DMSO as the blank solution. The following formula was used to evaluate the 1, 3-diphenyl-2-propene-1-one derivatives' 2, 2-diphenylpicrylhydrazyl (DPPH) scavenging abilities.

\[
\text{DPPH-scavenging effect} = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Sample}}}\right) \times 100
\]

Table-4 lists the anti-oxidant activity of the standard and 1, 3-diphenyl-2-propene-1-one derivatives at different concentrations. Figure-6 and 7 show the plot of the percentage of inhibition against the different concentrations of the solutions and standard Ascorbic acid. Ascorbic acid was used as the standard.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>% of DPPH scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100µgml^-1</td>
</tr>
<tr>
<td>1.</td>
<td>6-a</td>
<td>69.13</td>
</tr>
<tr>
<td>2.</td>
<td>6-b</td>
<td>64.43</td>
</tr>
<tr>
<td>3.</td>
<td>6-c</td>
<td>40.32</td>
</tr>
<tr>
<td>4.</td>
<td>6-d</td>
<td>36.95</td>
</tr>
<tr>
<td>5.</td>
<td>6-e</td>
<td>42.37</td>
</tr>
<tr>
<td>6.</td>
<td>6-f</td>
<td>40.19</td>
</tr>
<tr>
<td>Standard</td>
<td>Ascorbic Acid</td>
<td>53.97</td>
</tr>
</tbody>
</table>

Results and Discussion
Compounds 1-a, 1-b, 1-d, and 1-e exhibit good to moderate potency, while compounds 6-c and 6-f exhibit moderate to low inhibition against all tested strains. Trials were conducted on compounds with low activity for enhanced concentration. The synthesized compounds exhibited moderate activity at higher
concentrations. Verified compounds' antimicrobial activity can be linked to structural variations and divergences of the corresponding compounds. Tables-1 and 2 provide statistics on antibacterial activity. Among these compounds, 1-b (-4-CH₃), 1-d (-4-F), and 1-f (pipernol) demonstrated exceptional activity against B. subtilis. Compounds 1-d (-4-F) showed the greatest activity against S. typhi. Good activity against E. coli was demonstrated by compounds 1-b (-4-CH₃), and 1-e (thiophene). C. albicans is demonstrated to be moderately active against 1-d (-4-F). Compounds 1-b, 1-d, 1-e, and 1-f) had moderate activity, while compounds 1-a, and 1-c demonstrated virtuous larvicidal activity. The findings of these investigations are presented in Table-4; remarkably, the majority of them demonstrated strong larvicidal activity. Compounds (1c-1f)) showed moderate activity. The screening results for the antimicrobial, larvicidal, and antioxidant properties suggest that all of the recently manufactured mixtures be moderately exposed to worthy activity against the indicated organisms (Figure 4).

![Anti-Bacterial Plate](image1)

**Aspergillus Niger**
Standard – Amphotericin-B
A1 - Compound 1-a
B1 – Compound 1-c
C1 - Compound 1-e

![Anti-fungal Activity Plate](image2)

**F Candida albicans**
Standard – Amphotericin-B
A1 - Compound 1-a
B1 - Compound 1-c
C1 - Compound 1-e

**CONCLUSION**

In the current work, various Chalcones types with therapeutic requests are made, and their extensive biological demands are further exploited through further modification. We have synthesized and evaluated the antioxidant, larvicidal, and antimicrobial properties of a few novel Chalcones with the core s-triazine moiety. Chalcones compounds, which are primarily pharmacologically active substances, are identified chemically as 1-3, diphenylprop 2-en-1-one byproducts. For this research project, we prepared eighteen bioactive s-triazine derivatives. Each synthetic derivative was successfully made in two or three steps. We discovered in this study that derivatives of Pyrazoline and cyanopyridine were useful substances. The newly prepared compounds’ ¹H NMR, ¹³C NMR, MS (ESI), IR spectral data, and melting point analysis all contributed to the explanation of their structure. Finally, we have successfully synthesized a new series of derivatives containing Pyrazoline and cyanopyridine, and some of these compounds also contain a bioactive heterocyclic moiety. Surprisingly, the majority of them demonstrated superior antibacterial and antifungal properties. Compounds 1c and 1f showed moderate and little inhibition against all of the strains tested, while compounds 1a, 1b, 1d, and 1e showed better and moderate activity. Low-activity compounds were confirmed to have higher concentrations. The fluoro substituent compound (1-d) exhibits good activity against the fungi under experimentation. The synthesized compounds showed reasonable activity at higher concentrations Compounds (1-a to 1-f) demonstrated good and moderate inhibition towards the compounds, according to their larvicidal activity. The most active compounds, however, were compounds [1-a & 1-b]. There was good and moderate inhibition towards the compounds (1-a to 1-f). It is recommended by the screening results for antioxidant, larvicidal, and antimicrobial activities that all newly synthesized compounds exhibit reasonable to good activity against the verified organisms.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of 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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