Antimicrobial evaluation and molecular properties prediction of
pyrazolines incorporating benzofuran and pyrazole moieties

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ABSTRACT

A series of chalcones 3–5, 1H-pyrazolines 6–8, N-phenylpyrazolines 9–11, and N-acetylpyrazolines 12–14 incorporating benzofuran and pyrazole moieties were synthesized and screened for their in vitro antimicrobial activity against some of pathogenic microorganisms. Among the screened compounds, 7 and 13 showed the most promising antibacterial activity against Escherichia coli (G). Compound 11 displayed broad spectrum antibacterial activity against Bacillus subtilis (G'). Moreover, compounds 10 and 4 were found to be the most potent antifungal agent against Candida albicans and Aspergillus niger, respectively. Also, the molecular properties prediction and drug-likeness model score (DLS) of all the synthesized compounds were calculated by SwissADME and MolSoft websites, respectively. The two compounds 7 and 13 were found to be maximum DLS of 0.75 and 0.83, respectively.

INTRODUCTION

An antimicrobial is an agent that kills microorganisms or stops their growth. There are many types of antimicrobial drugs in the markets, e.g., penicillins, cycloserine, aminoglycosides, chloramphenicol, quinolones, tetracyclines, and glycopeptides, but the resistance of microorganisms to antimicrobial drugs by internal resistance or acquired resistance decreased the activities of these drugs. Therefore, the development of newer antimicrobial compounds for the treatment of the resistance of microorganisms has become a major objective of medicinal chemists.

Literature survey revealed that substituted pyrazolines could act as anticancer, antiviral, antioxidant, anti-inflammatory, antimicrobial, antidepressant, antiprotozoal, and antidiabetic agents (Havrylyuk et al., 2016; Marella et al., 2013; Silva et al., 2018). Derivative A showed potent antibacterial profile against the tested Gram-positive [Minimal Inhibition Concentration (MIC) = 8 µg/ml] and Gram-negative (MIC= 32 µg/ml) bacterial strains (Sharma et al., 2010). Compound B exhibit good activities against Staphylococcus aureus [inhibition zone (IZ)= 21 mm] and Candida albicans (IZ = 24 mm) (Sharhira et al., 2012). Compound C exhibited the most potent antimicrobial activities against S. aureus, Pseudomonas Aeruginosa, and C. albicans with MIC= 3.12 µg/ml (Ahmad et al., 2016). Also, some drugs bearing a pyrazoline moiety in their structures, e.g., phenazone and propyphenazone have analgesic and antiinflammatory effects. Metamizole is a spasm reliever, fever reliever, and it has anti-inflammatory effects (Fig. 1).

Further literature survey revealed that benzofuran or pyrazole moieties have been implemented as anticancer, antiviral, antioxidant, antimicrobial, anti-inflammatory, and antimalarial agents (Chand et al., 2017; Karrouchi et al., 2018; Shamsuzzaman et al., 2015). Moreover, the compound D bearing benzofuran and pyrazole moieties showed excellent antimicrobial activities for Ralstonia solanacearum, Klebsiella pneumoniae,
Fusarium oxysporum and Aspergillus flavus (Lingaraju et al., 2017). Compound E bearing benzofuran and pyrazoline moieties exhibited excellent antimicrobial activities in comparison with the standard drug used (Rangaswamy et al., 2012) (Fig. 1).

Based on above information and in continuation of our research program to find new potent antimicrobial and anticancer agents (Abd El-All et al., 2016; Abo-Ghalia et al., 2017; Al-Salem et al., 2017; Amr et al., 2018; El-Naggar et al., 2018; Elgemeie et al., 2008; Hafez et al., 2013; Hassan and Hafez, 2018; Hassan et al., 2019; 2015a; 2017a; 2018a; 2017b; 2015b; 2017c; 2015c; Kassem et al., 2019; Khatab et al., 2019; Moustafa et al., 2018; 2019; Naglah et al., 2017; 2013; Osman et al., 2014; 2009), a series of pyrazolines 6–14 incorporating benzofuran and pyrazole moieties have been synthesized to evaluate their antimicrobial activity against some of pathogenic microorganisms. Also, the calculation of the pharmacokinetic properties and drug-likeness of all compounds were studied (Fig. 2).

MATERIALS AND METHODS

Antimicrobial activities

The synthesized compounds (Chalcones 3–5, 1H-pyrazolines 6–8, N-phenylpyrazolines 9–11, and N-acetylpyrazolines 12–14) were evaluated their in vitro antimicrobial activities.
antimicrobial properties against *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (NRRL-B-4219), *Aspergillus niger* (ATCC 16888), and *C. albicans* (ATCC 10231) and comparison with antibiotic drugs (Negram, Vancomycin, and Nystatin) as standards by use of an agar well-diffusion method (MacLowry et al., 1970; Othman et al., 2011; Rocha et al., 1995; Valgas et al., 2007).

**RESULTS AND DISCUSSION**

**Chemistry**

The starting materials [khellinone 1 (Osman et al., 2012) and 3-substituted-1-phenyl-1H-pyrazole-4-carbaldehydes 2a–c (Jadhav et al., 2013)] were prepared according to the synthetic methods in Scheme 1.

The synthetic route used to synthesize the target series of pyrazolines incorporating benzofuran and pyrazole moieties is outlined in Scheme 2. Chalcones 3–5 have synthesized *via* the condensation of khellinone 1 with pyrazole aldehydes 2a–c. Then, described a synthesis of 1H-pyrazolines 6–8, N-phenylpyrazolines 9–11, and N-acetylpyrazolines 12–14 *via* the cyclocondensation of 3–5 with hydrazine hydrate or phenyl hydrazine in refluxing ethanol or glacial acetic acid (Hassan et al., 2016) (Scheme 2).

**Biological evaluations**

*In vitro antimicrobial activity*

The antibacterial and antifungal activities of the synthesized compounds 3–14 against a panel of pathogenic tested...
organisms are represented in Table 1 and Figure 3. The results revealed that some synthesized derivatives exhibited excellent to moderate inhibitory effect.

In case of \textit{B. subtilis} (G+), compound 11 [inhibition zone (IZ) = 20 mm] recorded excellent inhibitory effect and equipotent to the antibacterial reference drug (Vancomycin, IZ = 21 mm). Compounds \(4, 7, 8, 9, 10, \text{ and } 13\) showed a moderate inhibitory effect and recorded IZ diameter ranged from 12 to 16 mm. On the other hand, the rest of compounds \(3, 5, 6, 12, \text{ and } 14\) did not show any inhibitory effect.

In case of \textit{E. coli} (G-), the two compounds 7 and 13 (IZ = 20 mm) showed more potent inhibitory effect in comparison to the antibacterial reference drug (Negram, IZ = 16 mm). Compounds \(4, 9, 10, \text{ and } 11\) showed activity (IZ rang = 14–15 mm) nearly equal to the activity of the antibacterial drug used (Negram, IZ = 16 mm), while the other tested compounds have not any inhibition effect.

By testing the compounds against \textit{C. albicans}, compounds \(4, 7, 10, 11, \text{ and } 14\) were more potent (IZ rang = 15–17 mm) than antifungal drug used (Nystatin, IZ = 14 mm). The two compounds \(5 \text{ and } 8\) showed activity equal to antifungal drug used in this study (Nystatin, IZ = 14 mm). Also, the derivative 13 (IZ = 12 mm) showed a moderate inhibition effect.

In case of the pathogenic fungi, \textit{A. niger}, compound 4 showed excellent inhibitory effect (IZ = 18 mm) more than (Nystatin, IZ = 15 mm). The four compounds \(3, 5, 10, \text{ and } 11\) have activity equal to antifungal drug used (Nystatin, IZ = 15 mm).

\begin{table}
\caption{In vitro antimicrobial (inhibition zone of growth IZ, mm) of chalcones 3–5 and pyrazolines 6–14 against panel of pathogenic tested organisms.}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Compounds} & \textbf{X} & \textbf{Bacteria} & \textbf{Fungi} \\
\hline
 & & \textit{B. subtilis} (G+) & \textit{E. coli} (G-) & \textit{C. albicans} & \textit{A. niger} \\
\hline
3 & H & 00 & 00 & 00 & 15 \\
4 & Cl & 14 & 15 & 16 & 18 \\
5 & Br & 00 & 00 & 14 & 15 \\
6 & H & 00 & 00 & 00 & 00 \\
7 & Cl & 13 & 20 & 16 & 00 \\
8 & Br & 15 & 00 & 14 & 00 \\
9 & H & 16 & 14 & 00 & 14 \\
10 & Cl & 15 & 15 & 20 & 15 \\
11 & Br & 20 & 15 & 17 & 15 \\
12 & H & 00 & 00 & 00 & 00 \\
13 & Cl & 12 & 20 & 12 & 00 \\
14 & Br & 00 & 00 & 15 & 00 \\
\hline
\textit{Negram} & & 00 & 16 & 00 & 00 \\
\textit{Vancomycin} & & 21 & 00 & 00 & 00 \\
\textit{Nystatin} & & 00 & 00 & 14 & 15 \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure3}
\caption{Antimicrobial activity of chalcones 3–5 and pyrazolines 6–14 against panel of pathogenic tested organisms.}
\end{figure}
Also, compound 9 (IZ = 14 mm) showed moderate activity. The compounds (6–8 and 12–14) have not any activity.

Finally, we recommend for using compounds 7 and 13 in the treatment of Gram-negative pathogenic microorganisms, compound 11 in the treatment of Gram-positive, compound 10 in the treatment of C. albicans and compound 4 in the treatment of A. niger.

**Pharmacokinetic properties and drug-likeness**

**Lipinski’s rule of five for the compounds, chalcones 3–5 and pyrazolines 6–14**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>MW*</th>
<th>MLogP*</th>
<th>nHBA</th>
<th>nHBD</th>
<th>nRB</th>
<th>n_violation/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rule</td>
<td>&lt;500</td>
<td>≤4.15</td>
<td>≤10</td>
<td>≤5</td>
<td>≤10</td>
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<tr>
<td>3</td>
<td>466.48</td>
<td>2.43</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>500.93</td>
<td>2.90</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>545.38</td>
<td>2.99</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>480.51</td>
<td>2.53</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>514.96</td>
<td>2.99</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>559.41</td>
<td>3.09</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
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<td>3.73</td>
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<td>7</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
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<td>1</td>
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<td>1</td>
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<td>14</td>
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<td>3.03</td>
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</tr>
</tbody>
</table>

* = Molecular weight, * = Calculated lipophylicity (MLogP), ' = Number of hydrogen bond acceptor, ' = Number of hydrogen bond donor, ' = Number of rotatable bond (nRB), / = Violations from Lipinski’s rule.

**Drug likeness calculations of the compounds, chalcones 3–5 and pyrazolines 6–14**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>TPSA*</th>
<th>Volume*</th>
<th>%ABS* = (109 − (0.345 × TPSA))</th>
<th>DLS</th>
</tr>
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</tr>
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<tr>
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<td>85.13</td>
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<tr>
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<td>81.90</td>
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<td>85.57</td>
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<td>85.57</td>
<td>0.34</td>
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<td>530.68</td>
<td>80.77</td>
<td>0.83</td>
</tr>
<tr>
<td>14</td>
<td>81.83</td>
<td>535.34</td>
<td>80.77</td>
<td>0.52</td>
</tr>
</tbody>
</table>


**CONCLUSION**

In conclusion, we have synthesized a series of chalcones 3–5, 1H-pyrazolines 6–8, N-acetylpyrazolines 12–14 9–11, and N-acetylpyrazolines incorporating benzofuran and pyrazole...
moieties. All the synthesized compounds were screened for their *in vitro* antimicrobial activity. The evaluations showed that compounds 4, 7, 10, 11, and 13 were the most active compounds against a panel of pathogenic tested organisms. Also, the pharmacokinetic properties and calculation of drug likeness exhibited the two compounds 7 and 13 were found to be maximum DLS of 0.75 and 0.83, respectively.

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

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**REFERENCES**


