Synthesis, Antimicrobial, Antioxidant and Docking Study of Novel Isoxazoline Derivatives

Ghosoun Laftaa Mohsen¹, Ahmed Mutanabbi Abdula¹ *, Abdulkadir Mohammed Noori Jassim¹

¹Mustansiriyah University, Department of Chemistry, Baghdad, Iraq.

ABSTRACT
A novel series of isoxazoline derivatives (3-10) was synthesized and characterized by using spectral analysis. The synthesized derivatives were in vitro screened against several bacterial species as well as Candida albicans and exhibit moderate to potent activity. All the synthesized products were screened qualitatively for their antioxidant property by using TLC technique and the percent DPPH radical scavenging activity of the potent derivatives (4-6,8) were evaluated. Docking study of isoxazoline derivatives 7 and 8 against L-Glutamine: D-fructose-6-phosphate aminotransferase (glucosamine-6-phosphate synthase), the target enzyme in antimicrobial chemotherapy, was evaluated to explore the interactions of the synthesized hits inside the amino acid residues of the enzyme active site. The docking outcomes strongly supported the in vitro assay of new derivatives against several microbial species.

Keywords: Isoxazoline, antimicrobial, antioxidant, docking study.

INTRODUCTION
Synthesis and evaluation of efficacious antimicrobial, due to limited number of effective drugs¹ and antioxidant agents to protect human body from free radicals² represent the main goal for the several research groups worldwide. Recently, considerable attention has been given to isoxazoline derivatives due to their varied pharmacological activities like antimicrobial³, anti-inflammatory⁴, anti-tubercular⁵, antidepressant⁶, antioxidant⁷, antitumor and DNA Methyltransferase 1 Inhibitors⁸. Therefore, our attempts to find novel antimicrobial and antioxidant agents focus on the isoxazoline derivative. A novel series of isoxazolines (3-10) were synthesized and characterized by spectral data. Isoxazoline derivatives (3-
10) were In vitro screened against several bacterial species (gram positive and gramm negative) as well as Candida albicans and found to exhibit moderate to potent activity. The synthesized derivatives screened for their reducing properties with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical by the TLC autographic. The percent of DPPH scavenging by using spectrophotometric assay for isoxazoline derivatives (4-6, 8) which exhibited strong yellow or blue fluorescence under UV light in TLC autographic was evaluated. Docking study of the potent antimicrobial derivatives (7, 8) against L-Glutamine: D-fructose-6-phosphate amidotransferase, the specific target for antibacterial and antifungal agents, was achieved to explore and explain the interactions of the discovered hits within the amino acid residues of the enzyme active pocket. The docking results enhanced the activity of new derivatives as promising antimicrobial agents. Autodock 4.2, the efficacious tool for examining the binding alliance of small ligand to enzyme binding pocket was applied to explore the interactions between the isoxazoline derivatives and the glucosamine-6-phosphate synthase active site.

METHODOLOGY

General Synthesis

All starting materials and solvents were obtained from Sigma-Aldrich and used without any additional purification. Melting points were measured on a electro-thermal capillary apparatus and are uncorrected; FT-IR spectrum were recorded on a Shimadzu model FTIR-8400S. Mass spectra were obtained on a Shimadzu GCMS-QP2010 Ultra apparatus. 1H NMR spectra were achieved with a Bruker spectrophotometer model ultra-shield at 300 MHz in CDCl3 or DMSO-d6 solution with the TMS as internal standard.

Synthesis of (E)-1-(4-aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (1)

This compound was synthesized according to the procedure described in the published work. Sodium hydroxide (1ml, 40%) was added to a solution of p-anisaldehyde (1 mmol) in methanol (10 ml), isoxazoline compound 2 (1 mmol) was added. The reaction crude was refluxed for 10-12 h and the reaction was checked by a thin layer chromatography using ethyl acetate: hexane (1:1). The starting material was precipitate by adding crushed ice then it was filtered, washing with water, dried and purified using ethyl acetate as recrystallized solvent.

Orange powder, yield 90%, m.p 110-113 °C; IR (cm⁻¹): 3365 (NH), 3099 (aliphatic C-H), 1604 (C=N), 1510 (C=C). 1HNMR (300 MHz, CDCl3) δ (ppm): 3.24-3.33 (dd, j = 8.7, 16.5 Hz, CH- isoxazoline), 3.64-3.70 (dd, j = 10.7, 16.5 Hz, CH-isoxazoline), 3.88 (s, 2H, NH), 3.94 (s, 3H, OCH3), 3.96 (s, 3H, OCH3). Mass (NCI) m/e: 298 M⁺ For C₁₇H₁₈N₂O₃. Rf = 0.72 (5.5, Hexane:Ethy acetate).

Synthesis of 4-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl) aniline (2)

This compound was prepared as described in reference. To the starting material 1 (1mmol) in ethanol (10ml), a mixture of sodium hydroxide (40%, ml) and NH₂OH.HCl (1.5 mmol) was added and the crude reaction was refluxed for 15 h. The completion of reaction was monitored by a thin layer chromatography using ethyl acetate: hexane system (1:1). The starting material was precipitate by adding crush ice then it was filtered, washing with water, dried and purified using ethanol as recrystallized solvent.

Yellow off-white powder, yield 50%, m.p 132-134 °C; IR (cm⁻¹): 3059 (aromatic C-H), 1670 (C=O), 1626 (C=N), 1521 (C=C). 1HNMR (300 MHz, CDCl3) δ (ppm): 3.72-3.81 (dd, j = 8.9, 16.5 Hz, 1H, CH-isoxazoline), 3.89 (s, 3H, OCH3), 3.96 (s, 3H, OCH3). Mass (NCI) m/e: 283 M⁺ For C₁₇H₁₇NO₃. Rf = 0.78 (1:1, Hexane: Ethyl acetate).

Synthesis of Schiff bases (3-7)

These compounds were prepared according to the procedure described in the published reference. To solution of substituted benzaldehyde (1mmol) in methanol (10ml) with few drops of glacial acetic acid, isoxazoline compound 2 (1mmol) was added. The reaction crude was refluxed for 10-12 h and the reaction was checked by a thin layer chromatography (ethyl acetate:hexane ,1:1 and 3:7). The precipitate was filtered and washed with methanol, dried and recrystallized from ethanol.

1-(4-(dimethoxymethyl)phenyl)-N-(4-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)methanimine (3)

Yellow off-white powder, yield 50%, m.p 124-126 °C; IR (cm⁻¹): 3445, 3347 (NH2), 1642 (C-H), 1579 (C=N), 1598 (C=C). Rf = 0.72 (1:1, Hexane: Ethyl acetate).

Orange powder, yield 90%, m.p 173-175 °C; IR (cm⁻¹): (N-H), 3095 (aromatic C-H), 1670 (CH=N), 1593 (C≡N), 1512 (C=C). 1HNMR (300 MHz,
**N-(4-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-1-(4-nitrophenyl)methanimine (5)**

Yellow powder, yield 76%, m.p 139-141 °C; IR (cm⁻¹): 3005 (aromatic C=C), 1610, 1668 (C=C), 1606 (C=N), 1518 (C=C). Mass (NCI) m/e: 428 M⁺ For C₂₁H₁₈N₂O₅, Rf = 0.48 (3:7, hexane: ethyl acetate).

**2-(4-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)isoindoline-1,3-dione (9)**

Gray powder, yield 76%, m.p 220-222 °C; IR (cm⁻¹): 3009 (aromatic C-H), 2939 (aliphatic C-H), 1716, 1668 (C=C), 1604 (C=N), 1518 (C=C). Mass (NCI) m/e: 378 M⁺ For C₂₂H₂₁N₃O₅, Rf = 0.13 (3:7, hexane: ethyl acetate).

**Antimicrobial Studies**

The synthesized isoxazoline derivatives (3-10) were screened for their antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa (Gram negative), Staphylococcus aureus, Streptococcus Spp (Gram positive) as well as Candida albicans by using the well diffusion method (Table 1)⁹. DMSO was used as a control and the test was performed at 2 mg/mL concentration by using DMSO solvent. Amoxicillin was used as standard drugs. An experiment for each compound was made in triplicate and the average reading was recorded.

**Antioxidant study (DPPH. radical scavenging assay)**

TLC autographic assay: Few milligrams of isoxazoline derivatives (3-10) dissolved in methanol were added to the TLC plate by extremely small capillary. After drying, TLC plates were sprayed with methanolic solution of 0.2 % DPPH. The plates were examined half hour after spraying. Potent derivative appears as yellow or blue spots against a purple background⁹.

**Spectrophotometric (DPPH) assay:** At first 1.0 mL of the samples at concentr-
A concentration of 100, 300 and 500 µg/mL was mixed with 0.5 mL of DPPH solution (1.3 mg DPPH/mL methanol) and the volume was complete to 3 mL with methanol. The reaction mixture was left to stand for 30 min in dark place. The control contained all reagents without the sample while gallic acid was used as standard. The DPPH radical scavenging activity was determined by reading the absorbance at 517 nm against the blank. The capability to scavenge the DPPH radical was calculated by using the following equation: DPPH scavenging effect (%) = \( \frac{A_0 - A_1}{A_0} \times 100 \) where \( A_0 \) is the absorbance of the control reaction, and \( A_1 \) is the absorbance in the presence of compounds or standards.

**Docking study**

AutoDock 4.2 tool was used to specify the affinity of the potent isoxazoline derivatives (7, 8) to the binding site of GlcN-6-P synthase as described by the reported reference. The pdb enzyme file of receptor was downloaded from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a fixed molecule. All the water molecules were eliminated and hydrogens were added to the amino acid residues. ChemDraw ultra 7.0 software was used to construct the chemical structure of examined derivatives as mol format, while the open Babel 2.3.1 software was used to build the pdb file. The docking study was achieved by using grid dimensions 30.5, 17.5 and -2.2, respectively. Docking algorithm using Lamarckian Genetic was employed with 150 population size, 10 runs and 2,500,000 maximum number of energy evaluations, while the maximum number of generations was 27,000.

**RESULTS AND DISCUSSION**

**Organic Synthesis**

Chalcone derivative 1 and the isoxazoline compound 2 were prepared and characterized as described by our previous work. Shiff bases (3-7) were synthesized from the reaction of isoxazoline derivative 2 with different aromatic aldehydes in acidic methanolic solution (Scheme 1).

**Scheme 1.** (a) benzaldehyde-dimethylacetal, MeOH (b) pyrrole-2-carboxaldehyde, EIOH (c) p-nitrobenzaldehyde, ETOH (d) 4-N,N-dimethylbenzaldehyde, EIOH (e) 2,4-dinitrobenzaldehyde, MeOH (f) methyl chloroformate, acetone (g) phthalic anhydride or maleic anhydride, glacial acetic acid

The structures of the obtained compounds were confirmed by spectral analysis (see experimental section). The FT-IR spectra of compounds (3-7) showed the absorption bands at 1670-1618 cm\(^{-1}\), 1606-1593 cm\(^{-1}\) regions due to the stretching vibrations of the \(\text{CH}=\)N and \(\text{C}=\)N groups. The disappearance of the \(\text{NH}\) stretching frequencies strongly enhances the elucidation of prepared compounds. The mass spectra are consistent with the molecular ion peak values of the prepared compounds. The \(\text{HNMR}\) spectra of compound 4 and 5 showed singlet within the 8.89, 8.91 ppm regions due to \(\text{CH}=\)N protons with the absent of the singlet signal at 3.88 related to \(\text{NH}\) group in compound 2. The amide derivative 8 was obtained by the reaction of isoxazoline compound (2) with methyl chloroformate in glacial acetic acid. IR spectrum showed broad peak at 3329 cm\(^{-1}\) of NH stretching and characteristic peak at 1718 cm\(^{-1}\) due to \(\text{C}=\text{O}\) group. \(\text{HNMR}\) spectra showed two singlet signals at 3.68 and 9.96 ppm related to \(\text{CH}_3\text{OCO}\) and \(\text{NH}\) protons. The reaction between the isoxazoline derivative 2 with phthalic and maleic anhydride was carried out (Scheme 1) and purified by recrystallization from ethanol to yield N-substitutedphthalimide 9 and N-substitutedmaleimide 10 in high yield. The structures of the N-substitutemide derivatives were confirmed by using IR spectroscopy. The stretching of two carbonyl groups appeared at 1741, 1712 and 1716, 1668 cm\(^{-1}\) for compound 9 and 10, respectively. Further elucidation of molecular ion was confirmed via Mass spectroscopy.

**Antimicrobial Activity**

The *in vitro* assay of the isoxazoline derivatives (3-10) against several microbial species was achieved by using 2 mg/mL concentration as illustrated in Table 1. The tested compounds displayed auspicious activity against different species. Compound 7 and 8 were the potent agents against *gram positive*, *gram negative* as well as *Candida Albicans*. 
The scavenging properties of all the synthesized derivatives (3-10) were evaluated against DPPH radical by using TLC autographic assay. The isoxazoline derivatives dissolved in methanol were transferred to the one end of a TLC plate by using spotting capillary. After drying and spraying the DPPH solution, the active compounds (4-6, 8) appeared as yellow or blue spots with purple background. The scavenging activity of the lead derivatives (4-6, 8) was determined by using spectroscopic method as described in the indicated reference. The relationships between the in vitro percentage inhibition and the concentration of the potent hits (100, 200 and 300 µg/mL) are summarized in Figure 1.

![Figure 1.](image)

**Figure 1.** a- TLC autographic assay of the isoxazoline derivatives (3-10), b- Comparison of DPPH scavenging assay of potent compounds (4-6, 8)

### Docking Study

The docking study of the potent active isoxazoline derivatives (7, 8) toward antimicrobial species inside the active pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase, the active target for antimicrobial agents was explored. As described by the X-ray study, the binding pocket of target enzyme including the following subsequent residues, cysteine 300, glycine 301, threonine 302, serine 303, serine 347, glutamine 348, serine 349, threonine 352, valine 399, serine 401, alanine 602 and lysine 603 as shown in Figure 2.

![Figure 2.](image)

**Figure 2.** The binding of glucosamine-6-phosphate inside the active site of target enzyme.

The binding energy of active compounds inside the known three-dimensional structure of the specific enzyme was explored by using Autodock 4.2. The binding of the best building conformers for compounds 7 and 8 inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase is illustrated in Figure 3.

![Figure 3.](image)

**Table 1.** In vitro antimicrobial inhibition zone (mm) of the synthesized compounds

<table>
<thead>
<tr>
<th>Isoxazoline derivatives</th>
<th>Gram positive</th>
<th>Gram negative</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td><em>P.aeruginosa</em></td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>20</td>
<td>15</td>
<td>33</td>
</tr>
</tbody>
</table>

(-) exhibit no activity at specific concentration

**Antioxidant Activity**

The scavenging properties of all the synthesized derivatives (3-10) were evaluated against DPPH radical by using TLC autographic assay. The isoxazoline derivatives dissolved in methanol were transferred to the one end of a TLC plate by using spotting capillary. After drying and spraying the DPPH solution, the active compounds (4-6, 8) appeared as yellow or blue spots with purple background. The scavenging activity of the lead derivatives (4-6, 8) was determined by using spectroscopic method as described in the indicated reference. The relationships between the in vitro percentage inhibition and the concentration of the potent hits (100, 200 and 300 µg/mL) are summarized in Figure 1.
As indicated by molecular docking parameters (Table 1), the high-ranking binding energies of the generated conformer was -5.31 and -5.68 kcal mol\(^{-1}\) for compound 7 and 8, respectively. The docking results of all generated conformers of compounds within the binding pocket are strongly enhancing antibacterial and antifungal activities as depicted in Table 1. Furthermore, the inhibition constant Ki, intermolecular energy and hydrogen bonds were also determined and recorded in Table 2.

### Table 2. Docking parameters of isoxazoline compounds (7 and 8)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Energy (Kcal mol(^{-1}))</th>
<th>Inhibition constant (µM)</th>
<th>Intermolecular energy (kcal mol(^{-1}))</th>
<th>H-bonds Bonding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-5.31</td>
<td>128.24</td>
<td>-7.7</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>-5.24</td>
<td>144.17</td>
<td>-7.63</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>-5.00</td>
<td>214.77</td>
<td>-7.39</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>-4.85</td>
<td>280.90</td>
<td>-7.23</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>-4.77</td>
<td>317.38</td>
<td>-7.16</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>-4.58</td>
<td>440.84</td>
<td>-6.96</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-4.39</td>
<td>602.48</td>
<td>-6.87</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>-4.28</td>
<td>723.19</td>
<td>-6.67</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>-3.87</td>
<td>1460.00</td>
<td>-6.23</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>-3.55</td>
<td>2490.00</td>
<td>-5.94</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-5.68</td>
<td>68.83</td>
<td>-7.47</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>-5.68</td>
<td>68.30</td>
<td>-7.47</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>-5.63</td>
<td>75.14</td>
<td>-7.42</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>-5.53</td>
<td>88.24</td>
<td>-7.23</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>-5.19</td>
<td>157.24</td>
<td>-6.98</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>-5.13</td>
<td>173.35</td>
<td>-6.92</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>-5.11</td>
<td>180.95</td>
<td>-6.90</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>-5.09</td>
<td>187.11</td>
<td>-6.88</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>-4.95</td>
<td>233.34</td>
<td>-6.74</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>-4.67</td>
<td>380.49</td>
<td>-6.46</td>
<td>1</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The present work summarized the synthesis of novel isoxazoline derivatives as promising antimicrobial agents. The scavenging activity of the potent antioxidant derivatives was estimated by using DPPH radical. On the other hand, docking study using Autodock 4.2 was achieved to illustrate the bound state of ligand-enzyme complex for the potent discovered hits.
ACKNOWLEDGMENT

The authors would like to thank, Mustansiriya University (www.uomustansiriya.edu.iq) Baghdad, Iraq for its support in the present work.

REFERENCES


