In vitro cytotoxicity and druglikeness of pyrazolines and pyridines bearing benzofuran moiety

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ABSTRACT

A series of pyrazolines and pyridines bearing benzofuran moiety (M1–M10) were synthesized for evaluation of their in vitro cytotoxicity against MCF-7 and HepG2 cell lines. Furthermore, in silico drug-likeness study was carried out. The result of the cytotoxicity of M1–M10 showed that some compounds displayed cytotoxic activity against MCF-7 and HepG2 cells. An assessment of in silico drug-likeness study of M1–M10 illustrates that some compounds showed an agreement to the Lipinski, Ghose, Veber, Egan, and Muegge rules with orally bioavailable.

INTRODUCTION

Pyrazolines were known to exhibit various biological activities (Havrylyuk et al., 2016; Ozgun et al., 2019; Pandey et al., 2016). For example, N-acetyl pyrazoline (1), 1-(5-(4-bromophenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone, was selective and cytotoxic for leukemic cells (Stefanes et al., 2019). N-benzenesulfonylamide pyrazoline (2), 4-[5-(4-methoxyphenyl)-1′-phenyl-3′-(p-toly)-3,4-dihydro-1′H,2′H-[3,4′-bipyrrozol]-2-yl]benzenesulfonyamide, acts as an antimalarial activity through the inhibition of β-hematin (Kumar et al., 2018). N-formyl-pyrazoline (3), 1-formyl-3-phenyl-5-(4-isopropylphenyl)-2-pyrazoline, showed a promising antimicrobial activity (Sid et al., 2016).

Pyridines are important N-heterocyclic compounds due to their biological activities (Abdel-Galil et al., 2006; Klimešová et al., 1999; Xu et al., 2017), such as compound (4), 1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(6-(4-methoxyphenyl)-2-methylpyridin-3-yl)urea, which displayed anticancer activities against NCI-USA cell lines (El-Naggar et al., 2018a). Furthermore, compound (5), ethyl 4-methyl-1,7,8,9-tetrahydropyrano[2,3-b]pyrrolo[2,3-d]pyridine-3-carboxylate, showed antibacterial activities against Escherichia coli and Staphylococcus aureus (Elkanzi et al., 2019).

Benzofuran moiety was very interesting O-heterocycle which was found in biologically active compounds (Atta et al., 2010; Wang et al., 2019; Zhu et al., 2013). Compound (6), 1-(7-allyl-6-hydroxy-4-methoxy-1-benzofuran-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one, exhibited an antiproliferative activity (Ragab et al., 2014). Compound (7), (4-hydroxy-3-methylphenyl) (2-(4-methoxyphenyl)-5-methylbenzofuran-3-yl)methanone, exhibited antibacterial activities (Jiang et al., 2011) (Figure 1).

In view of these above facts with the program for discovery biologically active compounds (Abd El-All et al., 2016; Elgemeie et al., 2008, 2009; Elgiushy et al., 2018; El-Naggar et al., 2018b; Hafez et al., 2013; Hassan and Hafez, 2018; Hassan et al., 2015a, 2015b, 2015c, 2016, 2017a, 2017b, 2017c, 2018a, 2018b, 2019),
2019; Khatab et al., 2019; Magd-El-Din et al., 2018; Osman et al., 2014), this research involves: (i) synthesization of pyrazolines and pyridines bearing benzofuran moiety (M1–M10), (ii) in vitro cytotoxicity evaluation of compounds M1–M10 against MCF-7 and HepG2 cell lines, and (iii) we performed in silico druglikeness of compounds M1–M10.

MATERIALS AND METHODS

Chemistry

The target compounds [1H-pyrazolines (M1–M3), N-acetylpyrazolines (M4–M6), N-phenylpyrazolines (M7 and M8), and pyridines (M9 and M10)] were synthesized according to the reported procedure (Osman et al., 2012).

Cytotoxicity evaluation

An in vitro cytotoxic activity (IC_{50} µg/ml) of pyrazolines (M1–M8) and pyridines (M9 and M10) against two cell lines (breast MCF-7 and liver HepG2) was measured by using the sulforhodamine B stain (SRB) assay (Skehan et al., 1990).

Physicochemical properties and druglikeness

The physicochemical properties and druglikeness of pyrazolines (M1–M8) and pyridines (M9 and M10) were predicted by using the SwissADME website (http://swissadme.ch).

RESULTS AND DISCUSSION

The chemistry

The synthetic route is outlined in Scheme 1. The target compounds [1H-pyrazolines (M1–M3), N-acetylpyrazolines (M4–M6), or N-phenylpyrazolines (M7 and M8)] were synthesized by the condensation of chalcones with hydrazine hydrate in ethanol, hydrazine hydrate in glacial acetic acid, or phenylhydrazine in ethanol, respectively. Furthermore, pyridines M9 and M10 were synthesized by the reaction of 3c with 2-cyano(thio)acetamide 4a, b (Osman et al., 2012).

In vitro cytotoxic activity

The cytotoxicity (IC_{50} µg/ml) of pyrazolines (M1–M8) and pyridines (M9 and M10) was measured by using the sulforhodamine B stain (SRB) assay (Skehan et al., 1990) against two cell lines (breast MCF-7 and liver HepG2). The result of the cytotoxicity values is shown in Table 1, and we could see that:

- The compounds, M1 (IC_{50} = 12.08 ± 1.20 µg/ml), M2 (IC_{50} = 12.22 ± 2.80 µg/ml), M3 (IC_{50} = 10.43 ± 1.12 µg/ml), M4 (IC_{50} = 10.65 ± 4.61 µg/ml), and M6 (IC_{50} = 10.50 ± 0.60 µg/ml), show cytotoxic activity against MCF-7 cancer cells, but this activity is less than the positive control [doxorubicin (IC_{50} = 4.70 ± 0.55 µg/ml)].
For HepG-2 cancer cells, the compounds M1–M4 and M6 show IC₅₀ values in the range from 10.41 ± 2.71 to 12.75 ± 0.60 µg/ml.

The results revealed that the compounds M5 and M7–M10 did not exert any activity against MCF-7 and HepG-2 cancer cell lines.

Physicochemical properties and druglikeness

We predicted the physicochemical properties and druglikeness of the compounds (M1–M10) by using the SwissADME website (http://swissadme.ch).

The physicochemical properties give a global description of the structures of compounds (M1–M10) including molecular
weight, molecular refractivity, topological polar surface area, number of rotatable bonds, heavy atoms, and hydrogen bond acceptors and donors (Table 2).

The bioavailability radar of the compounds, pyrazolines (M1–M8) and pyridines (M9 and M10), displayed a rapid evaluation of druglikeness. The bioavailability radar includes the following six physicochemical properties:

1. Lipophilicity (XLOGP3 between −0.7 and +5.0).
2. Size (molecular weight between 150 and 500 g/mol).
3. Polarity (the total polar surface area between 20 and 130 Å²).
4. Solubility (log S not higher than 6).
5. Saturation (fraction Csp3 not less than 0.25).
6. Flexibility (the number of rotatable bonds not more than 9).

The bioavailability radar of the compounds, pyrazolines (M1–M8) and pyridines (M9 and M10), is shown in Figure 2. The pink area represents the optimal range of these properties (Lovering et al., 2009; Ritchie et al., 2011), and the red line represents the properties of pyrazolines (M1–M8) and pyridines (M9 and M10).

In Figure 2, the red lines of four compounds (M1, M3, M4, and M6) are in the range of the pink area. Therefore, we can conclude that these compounds are predicted orally bioavailable.

Furthermore, druglikeness was established based on the physicochemical properties to find oral drug candidates (Daina et al., 2017). There are five different rule-based filters which are defined as follows:

1. Lipinski’s filter includes molecular weight ≤ 500, MLOGP (lipophilicity) ≤ 4.15, hydrogen bond acceptors 

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ar</th>
<th>X</th>
<th>Human cancer cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MCF-7</td>
</tr>
<tr>
<td>M1</td>
<td>4-CH₃O-C₆H₄</td>
<td>–</td>
<td>12.08 ± 1.20</td>
</tr>
<tr>
<td>M2</td>
<td>1-naphthyl</td>
<td>–</td>
<td>12.22 ± 2.80</td>
</tr>
<tr>
<td>M3</td>
<td>5-methyl-2-furyl</td>
<td>–</td>
<td>10.43 ± 1.12</td>
</tr>
<tr>
<td>M4</td>
<td>4-CH₃O-C₆H₄</td>
<td>–</td>
<td>10.65 ± 4.61</td>
</tr>
<tr>
<td>M5</td>
<td>1-naphthyl</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>M6</td>
<td>5-methyl-2-furyl</td>
<td>–</td>
<td>10.50 ± 0.60</td>
</tr>
<tr>
<td>M7</td>
<td>4-CH₃O-C₆H₄</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>M8</td>
<td>1-naphthyl</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>M9</td>
<td>5-methyl-2-furyl</td>
<td>O</td>
<td>NA</td>
</tr>
<tr>
<td>M10</td>
<td>5-methyl-2-furyl</td>
<td>S</td>
<td>NA</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>–</td>
<td>–</td>
<td>4.70 ± 0.55</td>
</tr>
</tbody>
</table>

N.A. is no activity

The bioavailability radar of the compounds for six physicochemical properties is shown in Figure 2. The pink area represents the optimal range of these properties (Lovering et al., 2009; Ritchie et al., 2011), and the red line represents the properties of pyrazolines (M1–M8) and pyridines (M9 and M10).

In Figure 2, the red lines of four compounds (M1, M3, M4, and M6) are in the range of the pink area. Therefore, we can conclude that these compounds are predicted orally bioavailable.

Furthermore, druglikeness was established based on the physicochemical properties to find oral drug candidates (Daina et al., 2017). There are five different rule-based filters which are defined as follows:

1. Lipinski’s filter includes molecular weight ≤ 500, MLOGP (lipophilicity) ≤ 4.15, hydrogen bond acceptors

### Table 1. In vitro cytotoxicity (IC₅₀ µg/ml) of pyrazolines (M1–M8) and pyridines (M9 and M10).

| Compounds | Ar       | X       | MCF-7                  | HepG2       |
|-----------|----------|---------|-------------------------|
| M1        | 4-CH₃O-C₆H₄ | –       | 12.08 ± 1.20            | 11.05 ± 3.52 |
| M2        | 1-naphthyl | –       | 12.22 ± 2.80            | 12.73 ± 1.50 |
| M3        | 5-methyl-2-furyl | –       | 10.43 ± 1.12            | 10.41 ± 2.71 |
| M4        | 4-CH₃O-C₆H₄ | –       | 10.65 ± 4.61            | 12.75 ± 0.60 |
| M5        | 1-naphthyl | –       | NA                     | NA          |
| M6        | 5-methyl-2-furyl | –       | 10.50 ± 0.60            | 11.25 ± 5.65 |
| M7        | 4-CH₃O-C₆H₄ | –       | NA                     | NA          |
| M8        | 1-naphthyl | –       | NA                     | NA          |
| M9        | 5-methyl-2-furyl | O       | NA                     | NA          |
| M10       | 5-methyl-2-furyl | S       | NA                     | NA          |
| Doxorubicin | –       | –       | 4.70 ± 0.55            | 4.20 ± 0.40 |

N.A. is no activity

### Table 2. Physicochemical properties and lipophilicity of pyrazolines (M1–M8) and pyridines (M9 and M10).

<table>
<thead>
<tr>
<th>Properties</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
<th>M9</th>
<th>M10</th>
<th>Mean</th>
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<tr>
<td>Molecular weight</td>
<td>368.38</td>
<td>388.42</td>
<td>342.35</td>
<td>410.42</td>
<td>430.45</td>
<td>384.38</td>
<td>444.48</td>
<td>464.51</td>
<td>392.36</td>
<td>408.43</td>
<td></td>
</tr>
<tr>
<td># Heavy atoms</td>
<td>27</td>
<td>29</td>
<td>25</td>
<td>30</td>
<td>32</td>
<td>28</td>
<td>33</td>
<td>35</td>
<td>29</td>
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<tr>
<td># Arom. heavy atoms</td>
<td>15</td>
<td>19</td>
<td>14</td>
<td>15</td>
<td>19</td>
<td>14</td>
<td>21</td>
<td>25</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Fraction Csp3</td>
<td>0.25</td>
<td>0.17</td>
<td>0.28</td>
<td>0.27</td>
<td>0.20</td>
<td>0.30</td>
<td>0.19</td>
<td>0.14</td>
<td>0.14</td>
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<td>4</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td># H-Bond acceptors</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td></td>
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<tr>
<td># H-Bond donors</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Molar Refractivity</td>
<td>108.39</td>
<td>119.41</td>
<td>99.13</td>
<td>108.30</td>
<td>129.32</td>
<td>109.04</td>
<td>133.53</td>
<td>144.55</td>
<td>103.86</td>
<td>109.09</td>
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<tr>
<td>Total polar surface area Å²</td>
<td>85.45</td>
<td>76.22</td>
<td>89.36</td>
<td>93.73</td>
<td>84.50</td>
<td>97.64</td>
<td>76.66</td>
<td>67.43</td>
<td>121.88</td>
<td>140.45</td>
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<tr>
<td>MLOGP</td>
<td>1.21</td>
<td>2.22</td>
<td>0.52</td>
<td>1.20</td>
<td>2.17</td>
<td>0.53</td>
<td>2.54</td>
<td>3.50</td>
<td>0.06</td>
<td>0.44</td>
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<tr>
<td>XLOGP</td>
<td>3.29</td>
<td>4.57</td>
<td>2.82</td>
<td>2.66</td>
<td>3.94</td>
<td>2.19</td>
<td>4.98</td>
<td>6.26</td>
<td>3.88</td>
<td>3.83</td>
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</table>

The bioavailability radar of the compounds for six physicochemical properties is shown in Figure 2. The pink area represents the optimal range of these properties (Lovering et al., 2009; Ritchie et al., 2011), and the red line represents the properties of pyrazolines (M1–M8) and pyridines (M9 and M10).
Figure 2. The bioavailability radar of pyrazolines (M1–M8) and pyridines (M9 and M10).
≤ 10, and hydrogen bond donors ≤ 5 (Lipinski et al., 2001).
2) Ghose’s filter includes 160 ≤ molecular weight ≤ 480, –0.4 ≤ WLOGP (lipophilicity) ≤ 5.6, 40 ≤ the molar refractivity ≤ 130, and 20 ≤ number of atoms ≤ 70 (Ghose et al., 1999).
3) Veber’s filter includes the number of rotatable bonds ≤ 10 and the total polar surface area ≤ 140 (Veber et al., 2000).
4) Egan’s filter includes WLOGP (Lipophilicity) ≤ 5.88 and the total polar surface area ≤ 131.6 (Egan et al., 2000).
5) Muegge’s filter includes 200 ≤ molecular weight ≤ 600, –2 ≤ XLOGP3 (lipophilicity) ≤ 5, the total polar surface area ≤ 150, the number of rings ≤ 7, the number of carbon > 4, the number of heteroatoms > 1, the number of rotatable bonds ≤ 15, the hydrogen bond acceptors ≤ 10, and the hydrogen bond donors ≤ 5 (Muegge et al., 2001).

The result of drug-likeness evaluation of pyrazolines (M1–M8) and pyridines (M9 and M10) is shown in Table 3, and we can conclude that:

- All the compounds, pyrazolines (M1–M8) and pyridines (M9 and M10), are in agreement with the Lipinski’s rule.
- According to Ghose’s rule, all the compounds pass this rule excluding the two pyrazoline compounds (M7 and M8) due to the molar refractivity more than 130.
- In the cases of Veber’s rule and Egan’s rule, all the compounds are in agreement with the two rules excluding one compound, pyridine M10, due to the total polar surface area more than 140 and 131.6, respectively.
- According to Muegge’s rule, all the compounds are in agreement with this rule excluding pyrazoline M8 due to its lipophilicity (XLOGP3) more than 5.

CONCLUSION
In this work, a series of pyrazolines M1–M8 and pyridines M9 and M10 were synthesized for the evaluation of their in vitro cytotoxic activities against two cell lines such as MCF-7 and HepG2. In general, some of pyrazolines and pyridines displayed cytotoxicity. Furthermore, the drug-likeness study revealed that most of the compounds fulfill the requirements of Lipinski, Ghose, Veber, Egan, and Muegge rules, and four compounds (M1, M3, M4, and M6) are predicted orally bioavailable. These preliminary results provide the lead for the design of more potent and selective anticancer drugs.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

REFERENCES


