New chalcone derivatives with suitable drug-like lipophilicity targeting mitosis

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1. Synthesis

2. Human Tumor Cell lines

Compounds 3, 5, 9, 11, 15-19 $\text{GI}_{50} < 10 \, \mu\text{M}$

15-17 exhibited antimitotic activity

3. Lipophilicity evaluation

Most potent compounds ($\text{GI}_{50} < 8 \, \mu\text{M}$)

3.30 $< \text{lop Kp} < 3.68$
Abstract:
Chalcones are natural flavonoid precursors that have been reported for their wide range of biological activities, namely antitumor [1-2]. In addition, the presence of an α,β-unsaturated ketone moiety makes these compounds a valuable chemical substrate for the synthesis of bioactive derivatives, such as pyrazoles [3]. Our research group has reported two synthetic chalcone derivatives with antimitotic effect [4-5]. Hence, in continuation of our efforts to obtain new chalcone derivatives with improved antitumor and antimitotic activity, a small library of chalcone derivatives, including pyrazole and α,β-epoxide, was synthesized and evaluated for their cell growth inhibitory activity in three human tumor cell lines. Additionally, their lipophilicity using liposomes as a biomimetic membrane model was determined. From this work, nine chalcones showing suitable drug-like lipophilicity with antimitotic effect were identified. Moreover, one of the compounds was able to enhance chemosensitivity of tumor cells to paclitaxel in NCI-H460 cells.

Keywords: Chalcone derivatives; lipophilicity; mitosis
Introduction: Microtubules Targeting Agents (MTAs)

Stabilizers
- Taxane binding site
  - Paclitaxel

Destabilizers
- Colchicine binding site
  - Colchicine
- Vinca alkaloids binding site
  - Vincristine

Disadvantages of MTAs
- Hematopoietic toxicity
- Neurologic toxicity
- Drug resistance

New Antimitotic agents
Introduction: Chalcones

Biological Activities

- Anti-inflammatory
- Anti-tuberculosis
- Antidiabetic
- Antioxidant
- Antimicrobial
- Cardiovascular agents
- Antiallergic
- Antimicrobial
- Antileishmanial
- Antimalarial
- Antiulcer
- Antitumor

Molecular targets
- p53/MDM2 interaction
- Sex hormones
- mTOR pathway
- NF-Kb pathway
- Oxireductases
- ABC transporters
- Microtubules – Tubulin Polymerization

Introduction: Chalcones with Antimitotic Effect

Chalcones with antimitotic effect previously reported by our research group:

Caused abnormal spindle apparatus assembly

Prolonged mitotic arrest followed by cell death

Introduction

Aims

To obtain new chalcone derivatives with promising antimitotic effect with suitable drug-like lipophilicity

- Synthesis of a small library of chalcones, structure related with 1 and PC2 (2)
- Synthesis of pyrazole derivatives
- Evaluate the growth inhibitory effect of all synthesized chalcone derivatives
- Assess the antimitotic effect of the most promising chalcone derivatives
- Determine lipophilicity of all synthesized chalcone derivatives
Results and Discussion

Synthesis of Chalcones

\[ \text{Ar} \text{CHO} + \text{R}_2 \text{R}_3 \text{R}_4 \text{R}_5 \text{H} \rightarrow \text{Ar} \text{CH=CH} \text{R}_2 \text{R}_3 \text{R}_4 \text{R}_5 \text{O} \]

40% NaOH, MeOH
MW, 180 W, 2-3 h

| Ar = | R_2 = H, R_3, R_4, R_5 = OCH_3; n = 71% | 3 |
| Ar = | R_3 = H, R_2, R_4, R_5 = OCH_3; n = 24% | 4 |
| Ar = | R_2, R_4 = H, R_3, R_5 = OCH_3; n = 44% | 5 |
| Ar = | R_2, R_5 = H, R_3, R_4 = OCH_3; n = 44% | 6 |
| Ar = | R_2, R_4 = H, R_3, R_5 = Cl; n = 46% | 7 |
| Ar = | R_2, R_5 = H, R_3, R_4 = Cl; n = 66% | 8 |
| Ar = | R_2 = H, R_3, R_4, R_5 = OCH_3; n = 52% | 9 |
| Ar = | R_3 = H, R_2, R_4, R_5 = OCH_3; n = 23% | 10 |
| Ar = | R_2, R_4 = H, R_3, R_5 = OCH_3; n = 46% | 11 |
| Ar = | R_2, R_5 = H, R_3, R_4 = OCH_3; n = 28% | 12 |
| Ar = | R_2, R_4 = H, R_3, R_5 = Cl; n = 65% | 13 |
| Ar = | R_2, R_5 = H, R_3, R_4 = Cl; n = 67% | 14 |
| Ar = | R_2 = H, R_3, R_4, R_5 = OCH_3; n = 16% | 15 |
| Ar = | R_2 = H, R_3, R_4, R_5 = OCH_3; n = 38% | 16 |
| Ar = | R_2 = H, R_3, R_4, R_5 = OCH_3; n = 71% | 17 |
Results and Discussion

Synthesis of Pyrazole Derivatives

H$_2$O$_2$, 5% NaOH, CH$_3$COCH$_3$ : CH$_3$OH (3:2), r.t., 2-3 h;

η = 61 \%

η = 58 \%

NH$_2$NH$_2$H$_2$O
p-toluenesulfonic acid, Xylenes and dichloromethane, 100 ºC, 3-5 h

η = 4 \%

η = 1 \%
## Evaluation of the Antiproliferative Activity

<table>
<thead>
<tr>
<th></th>
<th>A375-C5 (µM)</th>
<th>MCF-7 (µM)</th>
<th>NCI-H460 (µM)</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>3.63 ± 0.58</td>
<td>5.95 ± 0.88</td>
<td>5.06 ± 0.20</td>
</tr>
<tr>
<td>4</td>
<td>11.12 ± 0.96</td>
<td>12.60 ± 2.68</td>
<td>13.62 ± 2.61</td>
</tr>
<tr>
<td>5</td>
<td>4.15 ± 0.85</td>
<td>7.70 ± 2.32</td>
<td>7.12 ± 0.20</td>
</tr>
<tr>
<td>6</td>
<td>17.77 ± 5.08</td>
<td>23.92 ± 7.18</td>
<td>17.76 ± 2.97</td>
</tr>
<tr>
<td>7</td>
<td>5.37 ± 1.47</td>
<td>11.65 ± 4.57</td>
<td>8.34 ± 2.02</td>
</tr>
<tr>
<td>8</td>
<td>7.25 ± 2.97</td>
<td>12.12 ± 2.33</td>
<td>8.44 ± 2.13</td>
</tr>
<tr>
<td>9</td>
<td>3.21 ± 0.45</td>
<td>3.26 ± 0.11</td>
<td>3.02 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>6.96 ± 0.65</td>
<td>10.06 ± 3.70</td>
<td>7.48 ± 0.41</td>
</tr>
<tr>
<td>11</td>
<td>3.33 ± 1.18</td>
<td>4.28 ± 2.17</td>
<td>4.44 ± 0.87</td>
</tr>
<tr>
<td>12</td>
<td>11.27 ± 1.30</td>
<td>10.78 ± 4.44</td>
<td>15.28 ± 2.85</td>
</tr>
<tr>
<td>13</td>
<td>7.14 ± 1.87</td>
<td>12.17 ± 2.79</td>
<td>11.85 ± 3.46</td>
</tr>
<tr>
<td>14</td>
<td>12.14 ± 1.87</td>
<td>22.54 ± 1.84</td>
<td>15.50 ± 5.66</td>
</tr>
<tr>
<td>15</td>
<td>5.70 ± 1.45</td>
<td>5.56 ± 1.51</td>
<td>6.28 ± 0.31</td>
</tr>
</tbody>
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<tr>
<td>16</td>
<td>6.90 ± 1.10</td>
<td>6.89 ± 0.41</td>
<td>6.61 ± 0.63</td>
</tr>
<tr>
<td>17</td>
<td>8.57 ± 1.06</td>
<td>9.75 ± 1.24</td>
<td>8.35 ± 0.31</td>
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<tr>
<td>18</td>
<td>2.89 ± 0.19</td>
<td>3.97 ± 0.82</td>
<td>5.60 ± 1.20</td>
</tr>
<tr>
<td>19</td>
<td>7.10 ± 0.62</td>
<td>8.52 ± 1.03</td>
<td>8.74 ± 1.03</td>
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<tr>
<td>20</td>
<td>3.45 ± 0.54</td>
<td>6.49 ± 0.30</td>
<td>10.84 ± 1.92</td>
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<tr>
<td>21</td>
<td>3.81 ± 0.765</td>
<td>13.15 ± 0.44</td>
<td>8.95 ± 1.01</td>
</tr>
<tr>
<td>22</td>
<td>4.51 ± 1.30</td>
<td>8.41 ± 3.63</td>
<td>9.61 ± 2.54</td>
</tr>
<tr>
<td>23</td>
<td>4.14 ± 0.70</td>
<td>15.10 ± 0.39</td>
<td>27.68 ± 1.91</td>
</tr>
<tr>
<td>24</td>
<td>38.50 ± 4.26</td>
<td>59.92 ± 12.70</td>
<td>61.78 ± 2.04</td>
</tr>
<tr>
<td>25</td>
<td>6.63 ± 3.37</td>
<td>14.01 ± 1.73</td>
<td>16.88 ± 3.48</td>
</tr>
<tr>
<td>26</td>
<td>&gt; 37.5</td>
<td>&gt; 37.5</td>
<td>&gt; 37.5</td>
</tr>
<tr>
<td>27</td>
<td>16.08 ± 2.94</td>
<td>16.34 ± 1.40</td>
<td>16.15 ± 0.56</td>
</tr>
</tbody>
</table>

**GI<sub>50</sub> values** (concentration that causes 50% of growth inhibitory effect) in tumour cells. Cells were treated for 48 h and analysed with the sulforhodamine B assay.
Results and Discussion

NCI-H460 cells arrest in mitosis, in response to most potent compounds treatment

Figure 1. Mitotic Index graph showing accumulation of mitotic cells after 15 h of compound treatment with all selected compounds. Statistical significance of samples treated with the compounds when compared with control (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001). Data represent mean±SD of three independent experiments.

15, 16 and 17 exhibited the strongest antimitotic activity, with a mitotic index between 25.80% and 49.37%.
NCI-H460 cells arrest in mitosis, in response to 15, 16 and 17 treatment

**Figure 2.** Treatment with 15, 16 and 17 arrests NCI-H460 cells in mitosis. Phase contrast microscopy images showing an accumulation of rounded-mitotic cells (Bar= 20 μm).
Figure 3. 15, 16 and 17 treatment arrests NCI-H460 cells in mitosis, as shown with DAPI staining of DNA (Bar=5 μm).
Figure 4. (a) 15 treatment affects mitotic spindle morphology. Immunofluorescence staining with anti-α-tubulin antibody. DNA was stained with DAPI (blue). Bar = 5 μm. (b) Multipolar mitotic spindle graph showing the percentage of multipolar mitotic spindle in mitotic cells, by 15 hours treatment with 15. Statistical significance of samples with 15 when compared with control (*P<0.05). Data represent mean±SD of three independent experiments. The same result was obtained for 16 and 17 treatment.
Figure 5. The concentrations of Tx used were from 1 nM to 25 nM as indicated. As control were considered untreated cells. The concentration of Tx at 25 and 2.5 nM with 6.28 μM of 15 presented statistical significance (*p<0.05), Tx at 10 and 5 nM with 6.28 μM of 15 was significant (**p<0.005). Tx at 1 nM combined with 6.28 μM of 15 had statistical significance (****p<0.0001). Data are means ± SD from at least three independent experiments.
Prediction vs determination of Log P values

(a) Mean, median and standard deviation of the Log P predicted by different in silico methods.

(b) Experimentally obtained Log Kp values, and mean and median of predicted log P for the studied chalcones.

**Figure 6.**

- **Majority of chalcone derivatives:** Predicted log P < determined log Kp
- **Di-ortho chloro-substituted (8, 14 and 23):** Determined log Kp << predicted log P

**Results and Discussion**
Figure 7. Comparison between the GI\textsubscript{50} of the chalcone derivatives on NCIeH460 cell line and the log KP. The insert displays the comparison of the most potent compounds (GI\textsubscript{50} < 8 mM) and their molecular weight.

None compounds showing a GI\textsubscript{50} < 10 µM

Most potent compounds (GI\textsubscript{50} < 8 µM)

log KP below 3

3.30 < lop Kp < 3.68

Not share the same chemical features (MW)
Most potent compounds (GI$_{50}$ < 8 µM) have lop Kp values between 3.30 and 3.60

- Chalcones 3, 5, 9, 11, 15-19, and 22 demonstrated a potent antiproliferative activity
- Compounds 15-17 emerged as potent antimitotic agents by interfering with mitotic spindle assembly
- Chalcone 15 sensitizes human tumor cells to death by low doses of paclitaxel

- 25 chalcone derivatives were synthetized
- 7, 9, 10, 13-17 and 24-27 were described for the first time

- Similar lipophilicity do nor share same chemical proprieties (MW)
This research was partially supported by the Strategic Funding UID/Multi/04423/2019 and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds. This work was also supported through funds provided by CESPU, Crl under the project ComeTarget_CESPU_2017. Ana Henriques, Joana Moreira and José Soares acknowledge for their FCT grants (SFRH/BD/111365/2015 and SFRH/BD/135852/2018, and SFRH/BD/98105/2013, respectively).