New prenylchalcones targeting the MDM2-p53 protein-protein interaction: synthesis and evaluation of antitumor activity

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Graphical Abstract
Abstract:

Among the chemical world of flavonoids, prenylated derivatives have been attracting the attention because of the myriad of their biological activities, with chalcones being widely reported for their antitumor activity against a variety of tumor cell lines. In fact, it has been demonstrated that isoprenylation of flavonoids significantly increased their growth inhibitory effect on human tumor cell lines. A series of prenylchalcones was synthesized and evaluated for the ability to inhibit the MDM2-p53 interaction using a yeast-based assay. The capacity of all synthesized prenylchalcones and their non-prenylated precursors to inhibit the growth of human colon tumor HCT116 cells was evaluated and compared. The overall results led to the identification of a hit compound, which behaved as potential inhibitor of the MDM2-p53 interaction in yeast, and showed improved cytotoxicity against human tumor cells expressing wild-type p53. In HCT116 cancer cells, it was also shown that the growth inhibitory effect of this prenylchalcone was associated with the induction of cell cycle arrest, and apoptosis.

Keywords: Prenylated chalcones; MDM2-p53 inhibitors; antitumor activity
p53 acts as a transcription factor, inducing the expression of downstream targets with a central role in regulation of several cellular processes.


Introduction

p53 - tumor suppressor protein

Upon cellular stress signals, the activation of the p53 pathway may compromise the tumor development and growth, preventing the proliferation of damaged cells with oncogenic potential.

Regulation of p53 activity by MDM2

The oncoprotein MDM2 binds p53 and negatively regulates its activity by inhibiting p53 transcriptional activity and translocation to the cytoplasm, and by enhancing p53 degradation.

All types of cancers have inactivated p53, either by mutation or inhibition due to the overexpression of the endogenous negative regulators such as MDM2

Inhibition of the p53-MDM2 interaction is an important therapeutic strategy for activating wt p53 in tumors.

Wang et al., Top Med Chem, 2012, 8, 57-80.
Introduction

Chalcones

Diversity of substitution patterns

Wide range of biological activities

Xanthohumol

Anti-inflammatory

Antidiabetic

Antioxidant

Antimalarial

Antimicrobial

**Introduction**

**Prenylated chalcones with improved antitumor activity**

Enhanced cytotoxicity of prenylated chalcone against tumour cells via disruption of the p53–MDM2 interaction

Mariana Leão\(^{a1}\), Joana Soares\(^{a1}\), Sara Gomes\(^{a1}\), Liliana Raimundo\(^{a1}\), Helena Ramos\(^{a}\), Cláudia Bessa\(^{a}\), Glória Queiroz\(^{a}\), Sofia Domingos\(^{H^C}\), Madalena Pinto\(^{H^C}\), Alberto Inga\(^{a}\), Honorina Cidade\(^{H^C, a}\), Lucília Saraiva\(^{H^C, a}\).


\[\text{HCT116 p53}^{+ +} : G_{50} = 65 \, \mu M\]

\[\text{HCT116 p53}^{+ +} : \text{Human colon adenocarcinoma expressing wt p53}\]

\[\text{PC2} : G_{50} = 4 \, \mu M\]

\[\text{MDM2-p53 inhibitor}\]
Discovery of new inhibitors of MDM2-p53 interaction with promising antitumor activity
Results and discussion

Synthesis


1a and 2a: $R_1=R_5=H; R_2=R_3=R_4=OCH_3$
1b and 2b: $R_1=R_4=R_5=H; R_2=R_3=OCH_3$
1c and 2c: $R_4=R_5=H; R_3=OCH_3$
1d and 2d: $R_1=R_2=R_4=R_5=H; R_3=F$
1e and 2e: $R_4=R_5=H; R_3=Br$
1f and 2f: $R_3=R_4=R_5=H; R_1=R_2=Cl$
1g and 2g: $R_1=R_3=R_4=R_5=H; R_2=OCH_3$
1h and 2h: $R_1=R_3=R_5=H; R_2=R_4=OCH_3$
1i and 2i: $R_3=R_4=R_5=H; R_4=R_2=OCH_3$
1j and 2j: $R_1=R_3=R_5=H; R_2=R_4=Cl$
Results and discussion

**Biological activity evaluation**

Screening for potential inhibition of the MDM2-p53 interaction using yeast cell assay

<table>
<thead>
<tr>
<th>Comp</th>
<th>Reversion of MDM2 effect (%)*</th>
<th>Comp</th>
<th>Reversion of MDM2 effect (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>0.1 ± 2.7</td>
<td>2b</td>
<td>56.9 ± 2.6</td>
</tr>
<tr>
<td>1c</td>
<td>93.4 ± 2.6</td>
<td>2c</td>
<td>57.9 ± 10.1</td>
</tr>
<tr>
<td>1d</td>
<td>0.3 ± 3.2</td>
<td>2d</td>
<td>23.7 ± 7.1</td>
</tr>
<tr>
<td>1e</td>
<td>0.1 ± 1.9</td>
<td>2e</td>
<td>76.1 ± 6.8</td>
</tr>
<tr>
<td>1f</td>
<td>48.0 ± 6.5</td>
<td>2f</td>
<td>29.1 ± 6.0</td>
</tr>
<tr>
<td>1g</td>
<td>11.7 ± 7.6</td>
<td>2g</td>
<td>15.4 ± 5.6</td>
</tr>
<tr>
<td>1h</td>
<td>73.0 ± 4.4</td>
<td>2h</td>
<td>13.5 ± 2.6</td>
</tr>
<tr>
<td>1i</td>
<td>39.8 ± 3.1</td>
<td>2i</td>
<td>76.1 ± 6.8</td>
</tr>
<tr>
<td>1j</td>
<td>20.8 ± 3.8</td>
<td>2j</td>
<td>80.9 ± 3.0</td>
</tr>
</tbody>
</table>

Effect of 10 µM of compounds on the reversion of MDM2 effect, by reestablishment of p53-induced growth inhibition in yeast cells co-expressing p53 and MDM2, after 42 h of treatment; the ability of compounds to disrupt the MDM2-p53 interaction was evaluated considering the percentage of DMSO-treated cells expressing wtp53 as 100%; data are mean ± SEM of 4-5 independent experiments.

Chalcones 1c, 1h, 2e, 2i, and 2j revert the MDM2 inhibitory effect on p53-induced yeast growth inhibition

Biological activity evaluation

In vitro human HCT116 colon adenocarcinoma cell lines growth effect

<table>
<thead>
<tr>
<th>Comp</th>
<th>IC$_{50}$ (µM)</th>
<th>Comp</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>27.6 ± 0.9</td>
<td>2b</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>1c</td>
<td>10.6 ± 0.4</td>
<td>2c</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>1d</td>
<td>4.4 ± 0.5</td>
<td>2d</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>1e</td>
<td>14.0 ± 2.0</td>
<td>2e</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>1f</td>
<td>2.7 ± 0.3</td>
<td>2f</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>1g</td>
<td>3.1 ± 0.1</td>
<td>2g</td>
<td>7.7 ± 0.1</td>
</tr>
<tr>
<td>1h</td>
<td>50.0 ± 4.0</td>
<td>2h</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>1i</td>
<td>3.5 ± 0.3</td>
<td>2i</td>
<td>11.7 ± 1.7</td>
</tr>
<tr>
<td>1j</td>
<td>1.9 ± 0.1</td>
<td>2j</td>
<td>24.5 ± 2.0</td>
</tr>
</tbody>
</table>

Growth inhibition was studied by SRB assay, after 48 h treatment; values correspond to the IC$_{50}$ values and are mean ± S.E.M. of 3-4 independent experiments; growth obtained with solvent was set as 100%.

Among the compounds revealed by the yeast assay as potential p53-activating agents, the compound 2e exhibited the lowest IC$_{50}$ value (2.1 ± 0.1 µM).

Results and discussion

Cytotoxicity of compound 2e against HCT116 cells in the colony formation assay

Colony formation assay for HCT116 cells treated with 2e (or DMSO only) for 11 days; images correspond to a representative experiment of three; graphs represent mean ± SEM of three independent experiments; values significantly different from DMSO are indicated: **P < 0.01; ***P < 0.001.

\[ \text{IC}_{50} = 0.17 \pm 0.09 \, \mu \text{M} \]

**Results and discussion**

**Effect of compound 2e on cell cycle and apoptosis**

(A) Effect of 4.2 µM 2e on cell cycle progression of HCT116 cells, after 48 h treatment; cell cycle phases were analyzed by flow cytometry using PI; data are mean ± SEM of three independent experiments; values significantly different from DMSO are indicated: *P < 0.05. (B) Effect of 4.2 µM 2e on apoptotic cell death of HCT116 cells was evaluated by flow cytometer using FITC-Annexin V and PI, after 48 h treatment; values correspond to the increase in the percentage of Annexin V-positive cells (early and late apoptotic cells); data are mean ± SEM of three independent experiments; values significantly different from DMSO are indicated: **P < 0.01.

Chalcone 2e inhibits the growth of human tumor cells through induction of apoptosis, and cell cycle arrest.

2e may activate p53 through potential inhibition of its interaction with MDM2

Conclusions

In vitro growth inhibitory effect

Yeast screening assay

HCT116 cells

Synthesis

1c, 1h, 2e, 2i, and 2j

1a-1j

2a-2j

HCT116 cells

In vitro growth inhibitory effect

2e showed the lowest G1s0 and was selected for further studies

In vitro growth inhibitory effect

Apoptosis

Cell cycle arrest
Acknowledgments

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