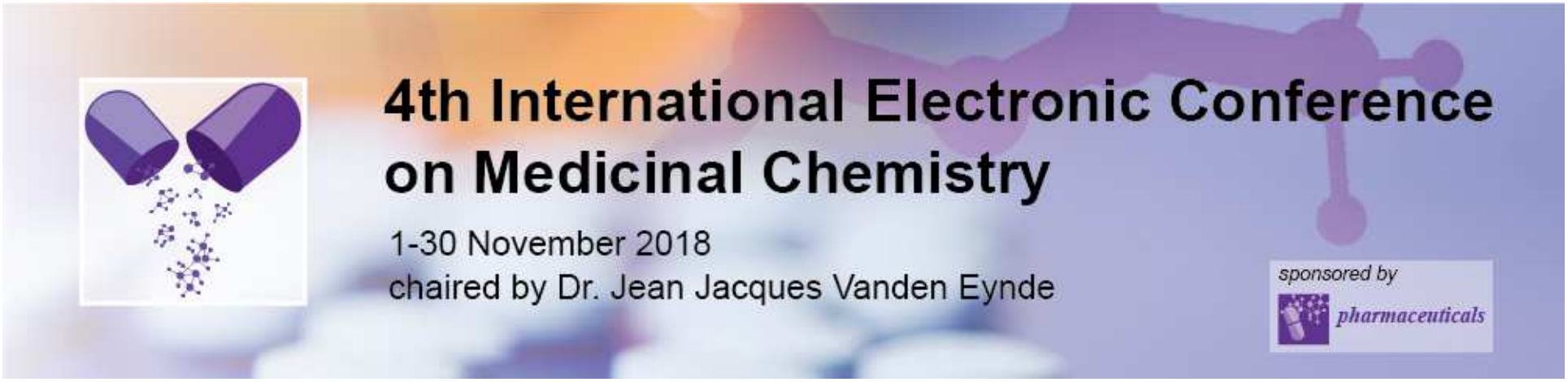

New prenylchalcones targeting the MDM2-p53 protein-protein interaction: synthesis and evaluation of antitumor activity

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#Authors contributed equally to this work.

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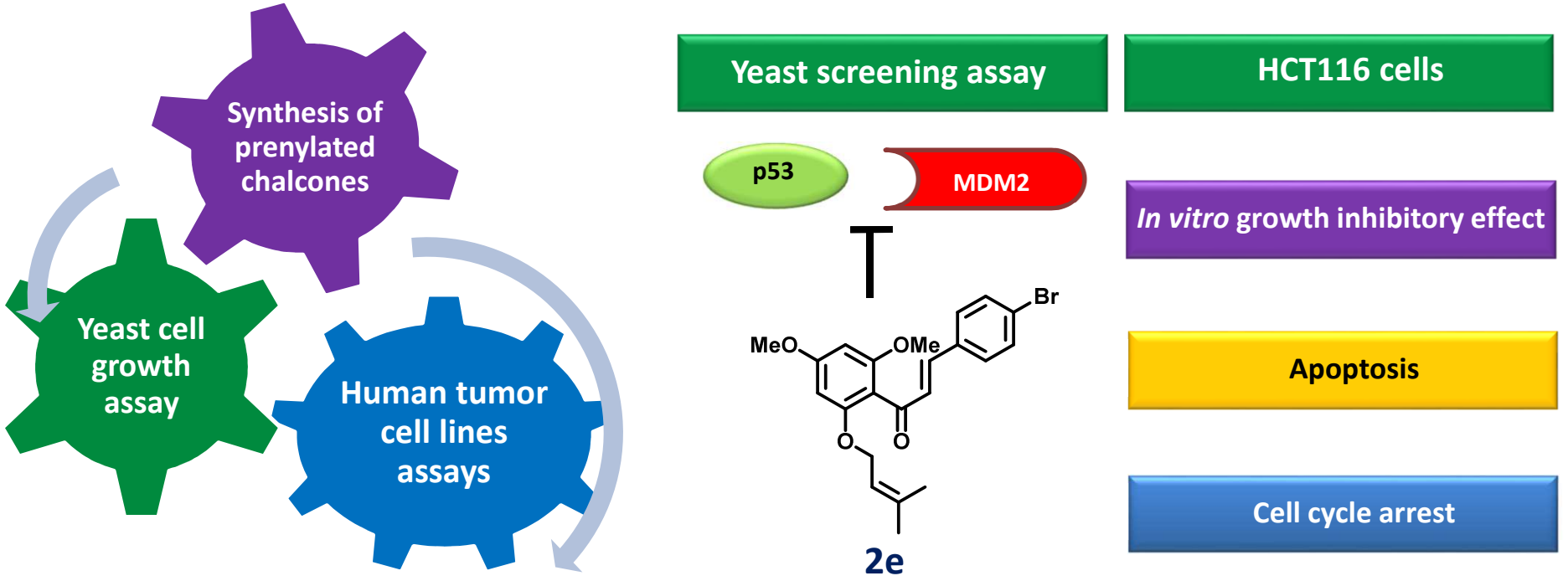
New prenylchalcones targeting the MDM2-p53 protein-protein interaction: synthesis and evaluation of antitumor activity

Graphical Abstract



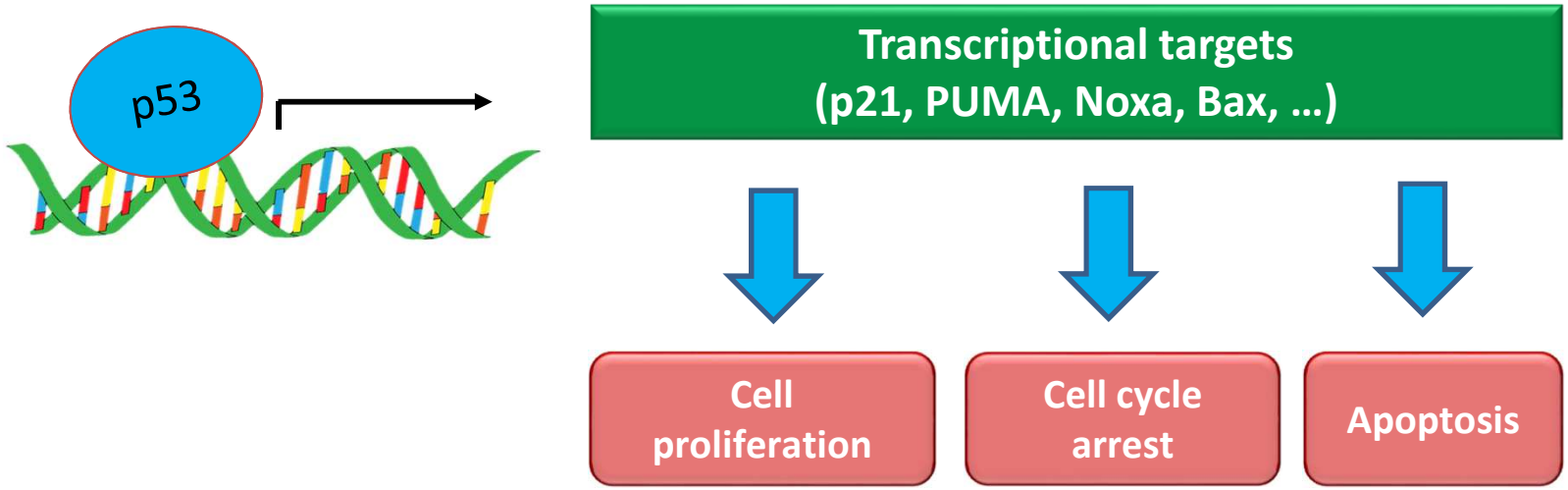
Abstract:

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 - tumor suppressor protein

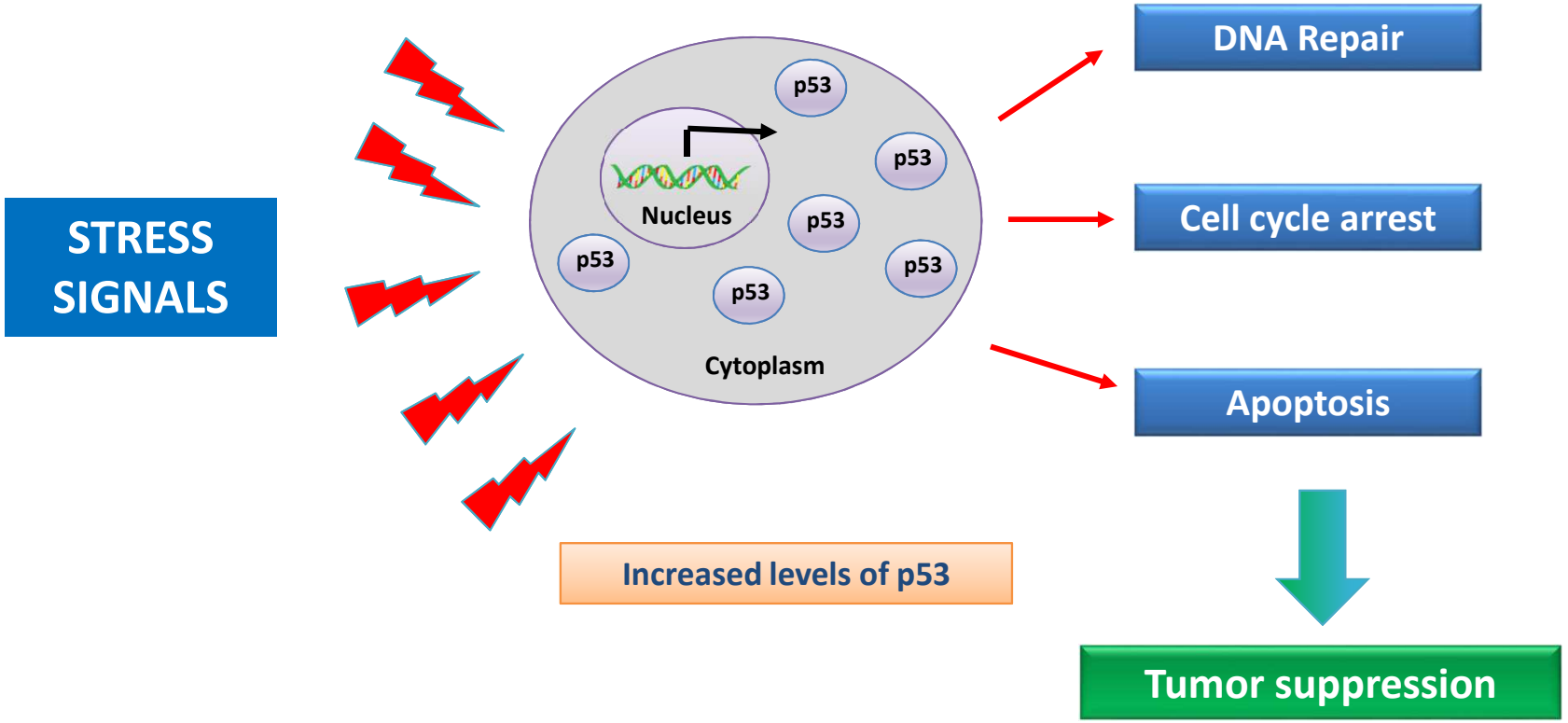
p53 acts as a transcription factor, inducing the expression of downstream targets with a central role in regulation of several cellular processes.



Moll, U. M.; Petrenko, O. *Mol. Cancer Res.*, **2003**, *1* (14), 1001–1008.
Hong, B. et al. *Curr. Drug Targets*, **2014**, *15* (1), 80–89.

p53 - tumor suppressor protein

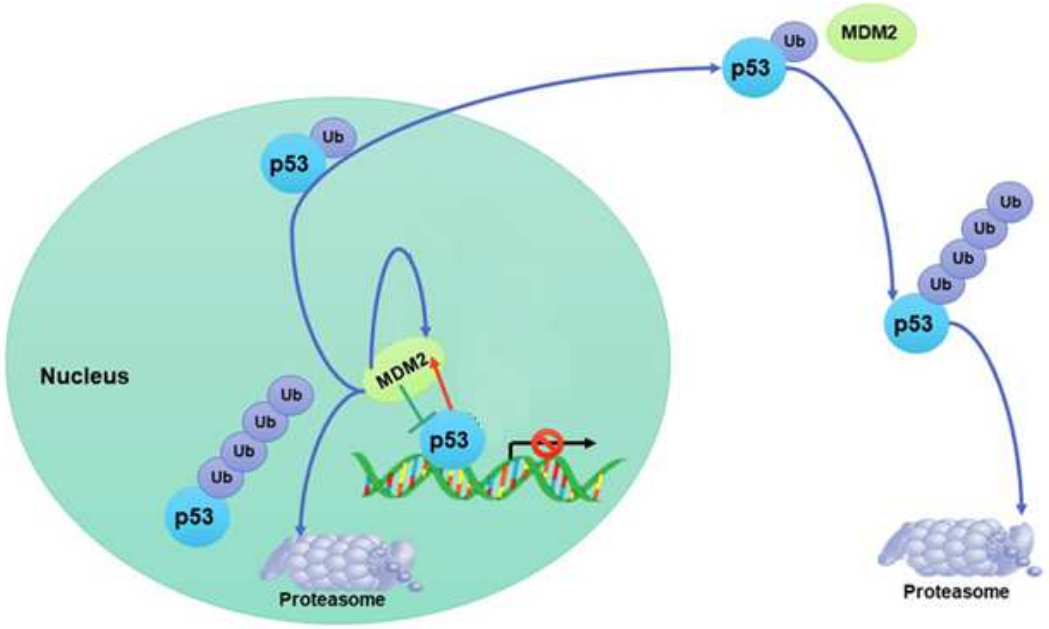
Upon cellular stress signals, the activation of the p53 pathway may compromise the tumor development and growth, preventing the proliferation of damaged cells with oncogenic potential



Soares, J. et al., *Advances in Drug Discovery and Development*, **2017**, pp 2–87.

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.

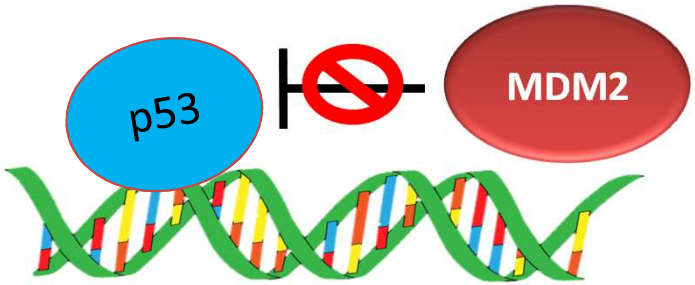


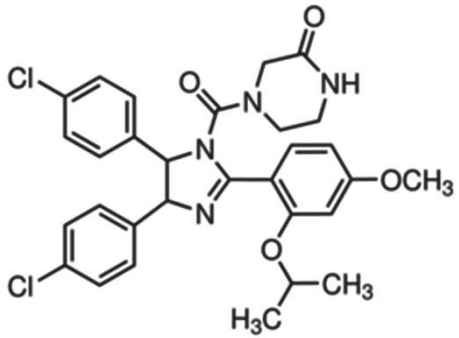
All types of cancers have inactivated p53, either by mutation or inhibition due to the overexpression of the endogenous negative regulators such as MDM2

Soares, J. et al., L. *Advances in Drug Discovery and Development*, **2017**, pp 2–87.

MDM2 - p53 interaction inhibition

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





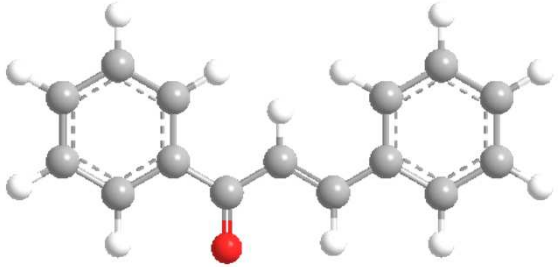
Nutlin-3a

Inhibits MDM2 activity and blocks p53-MDM2 interaction

Wang and Hu, *Med Res Rev*, **2011**, DOI: 10.1002/med.20236
Wang et al., *Top Med Chem*, **2012**, *8*, 57-80.

Introduction

Chalcones

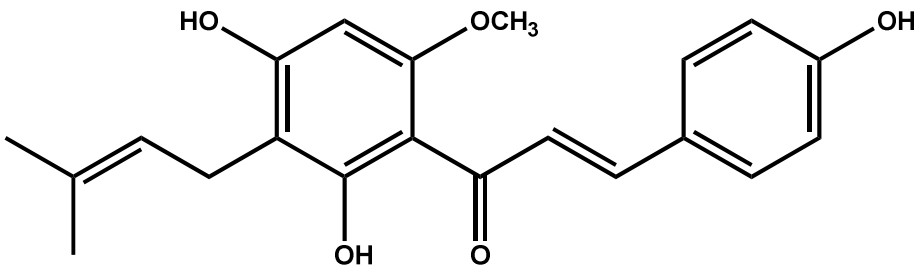


Diversity of substitution patterns

Wide range of biological activities

- Anti-inflamatory
- Antidiabetic
- Antioxidant
- Cardiovascular agents
- **Antitumor**
- Antimalarial
- Antimicrobial

Xanthohumol



Zhuang, C. et al., Chemical Reviews, **2017**, *117*(12), 7762–7810.
Jiang, C. H. et al., Front. Pharmacol., **2018**, *9*:530,Doi: 10.3389/fphar.2018.00530

Introduction

Prenylated chalcones with improved antitumor activity

Life Sciences 142 (2015) 60–65

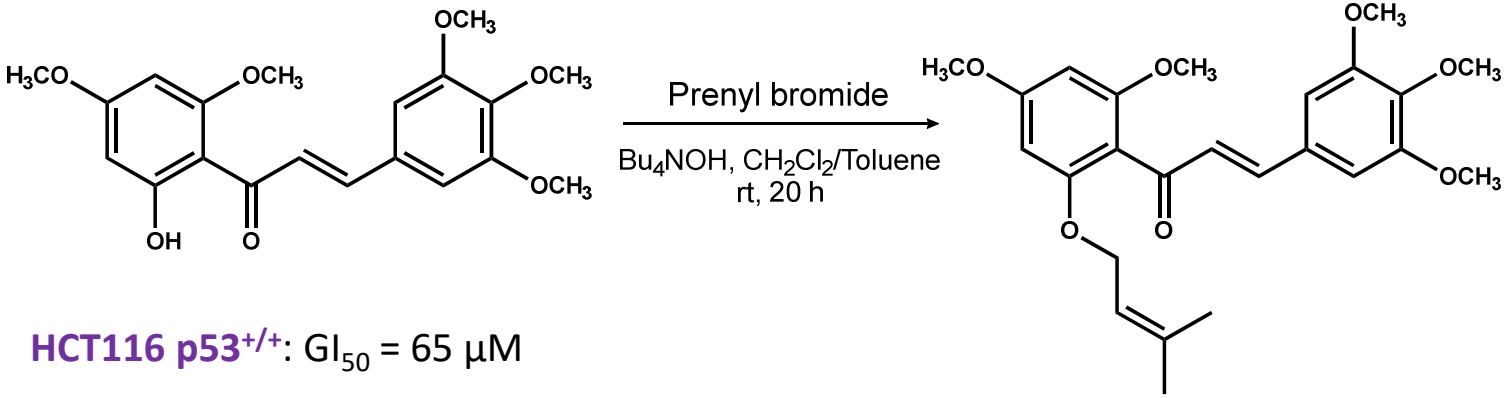
Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

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

Mariana Leão [a,1], Joana Soares [a,1], Sara Gomes [a,1], Liliana Raimundo [a,1], Helena Ramos [a], Cláudia Bessa [a], Glória Queiroz [a], Sofia Domingos [b,c,d], Madalena Pinto [b,c,d], Alberto Inga [e], Honorina Cidade [b,c,d,*], Lucília Saraiva [a,**]



HCT116 p53$^{+/+}$: GI_{50} = 65 µM

HCT116 p53$^{+/+}$: Human colon adenocarcinoma expressing wt p53

PC2

HCT116 p53$^{+/+}$: GI_{50} = 4 µM

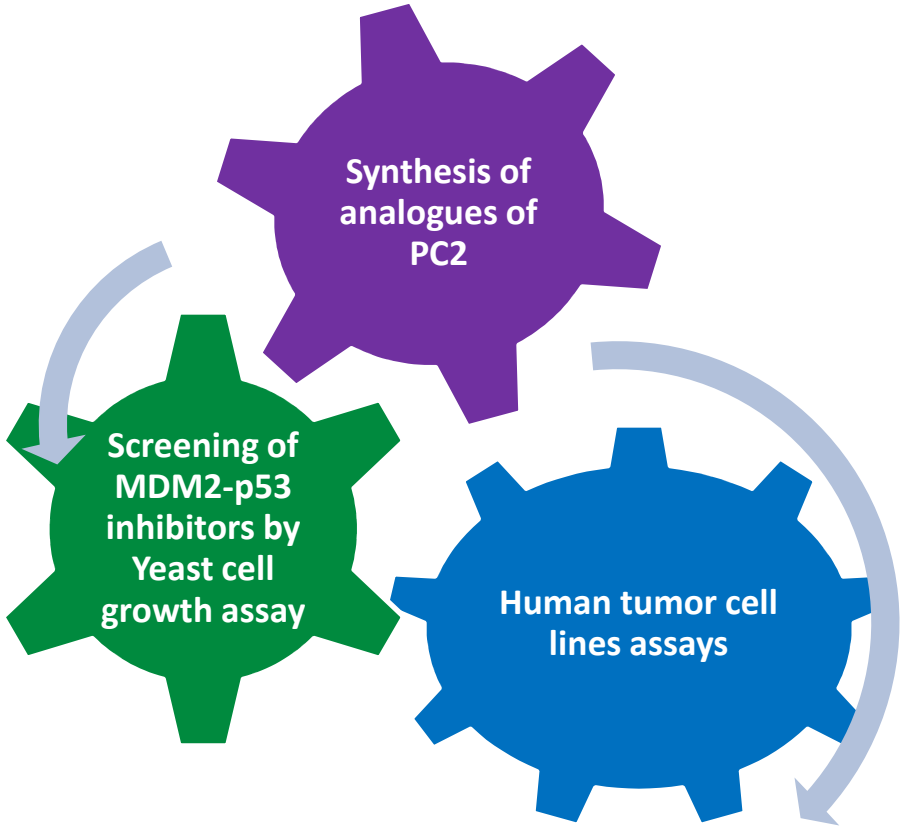
MDM2-p53 inhibitor

Leão, M. et al., *Life Sciences*, **2015**, *142*, 60-65.

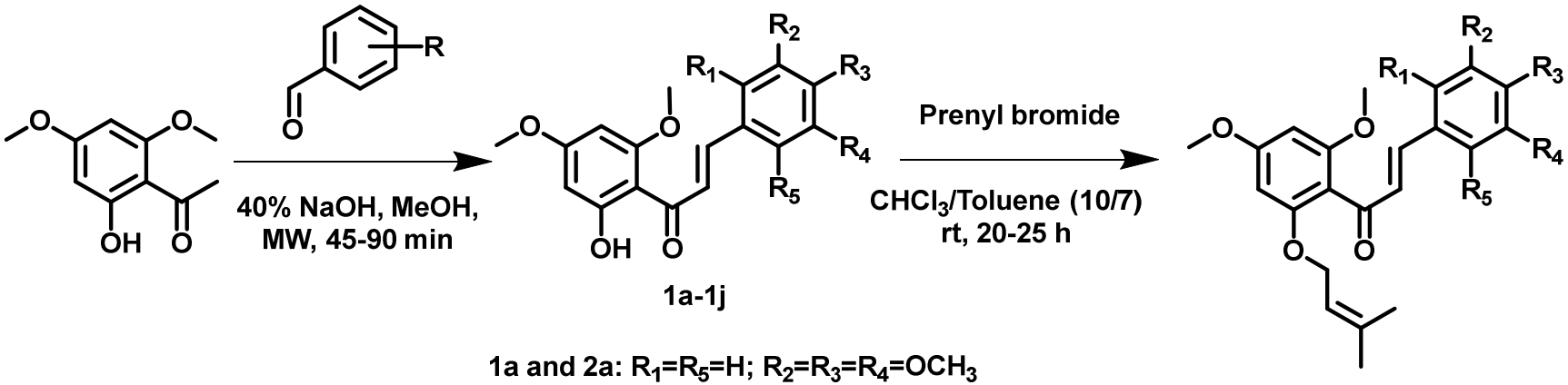
Neves, M. P. et al., *Chem Biodivers* **2012**, *9*, 1133-1143.

Aims

Discovery of new inhibitors of MDM2-p53 interaction with promising antitumor activity



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_1=R_2=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_1=R_2=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_1=R_2=OCH_3
1j and 2j: R_1=R_3=R_5=H; R_2=R_4=Cl

P. Brandão et al., *Eur J Med Chem*, **2018**, *156*, 711-721.

Biological activity evaluation

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

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

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

P. Brandão et al., *Eur J Med Chem*, **2018**, *156*, 711-721.

Biological activity evaluation

In vitro human HCT116 colon adenocarcinoma cell lines growth effect

Comp	IC_{50} (µM)	Comp	IC_{50} (µM)
1b	27.6 ± 0.9	2b	5.7 ± 0.4
1c	10.6 ± 0.4	2c	7.6 ± 0.5
1d	4.4 ± 0.5	2d	3.2 ± 0.3
1e	14.0 ± 2.0	2e	2.1 ± 0.1
1f	2.7 ± 0.3	2f	1.9 ± 0.2
1g	3.1 ± 0.1	2g	7.7 ± 0.1
1h	50.0 ± 4.0	2h	3.5 ± 0,1
1i	3.5 ± 0.3	2i	11.7 ± 1.7
1j	1.9 ± 0.1	2j	24.5 ± 2.0

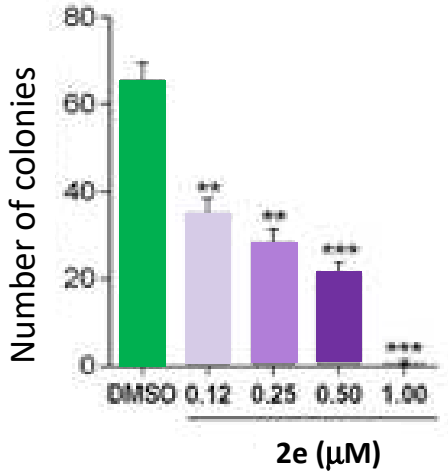
Growth inhibition was studied by SRB assay, after 48 h treatment; values correspond to the IC_{50} values and are mean ± S.E.M. of 3-4 independent experiments; growth obtained with solvent was set as 100%.

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).

P. Brandão et al., *Eur J Med Chem*, **2018**, *156*, 711-721.

Biological activity evaluation

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



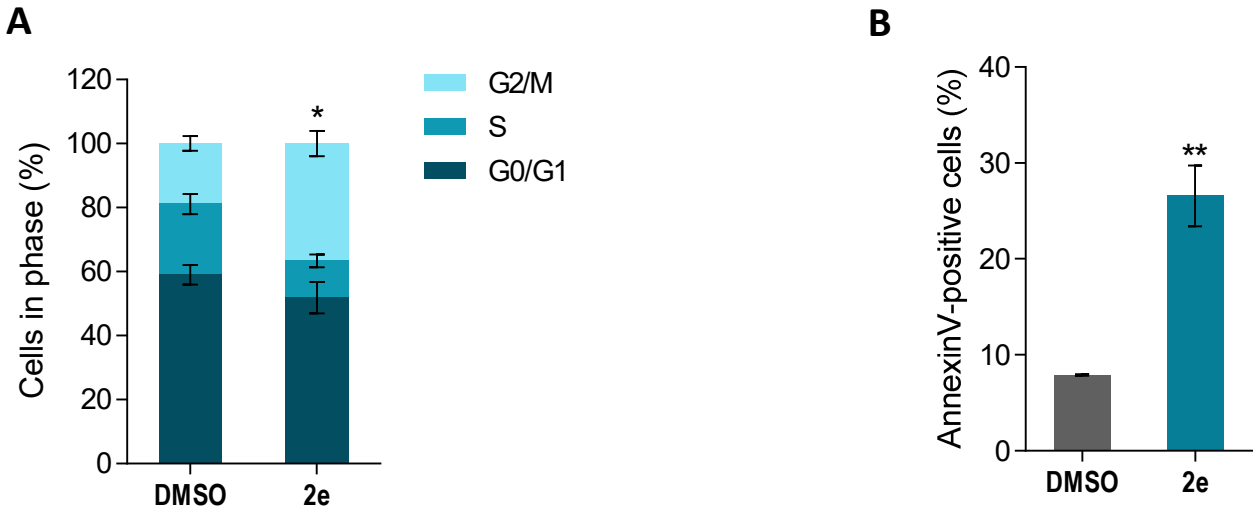
IC_{50} = 0.17 ± 0.09 μM

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.

P. Brandão et al., *Eur J Med Chem*, **2018**, *156*, 711-721.

Biological activity evaluation

Effect of compound 2e on cell cycle and apoptosis



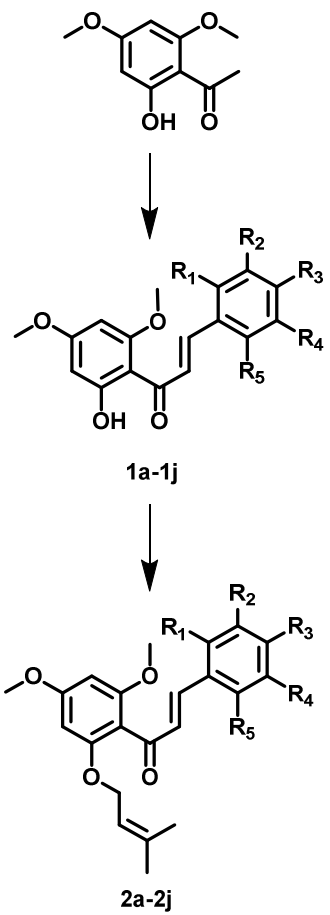
(A) Effect of 4.2 µM **2e** on cell cycle progression of HCT116 cells, after 48 h treatment; cell cycle phases were analyzed by flow cytometry using PI; data are mean ± SEM of three independent experiments; values significantly different from DMSO are indicated: **P* < 0.05. **(B)** Effect of 4.2 µM **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.

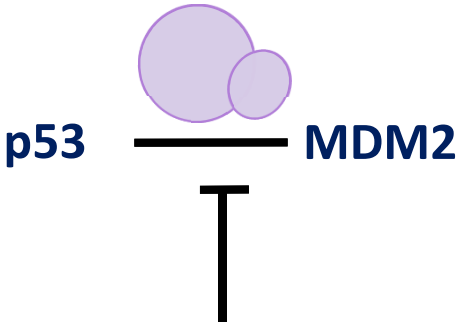
P. Brandão et al., *Eur J Med Chem*, **2018**, *156*, 711-721.

Conclusions

Synthesis



Yeast screening assay



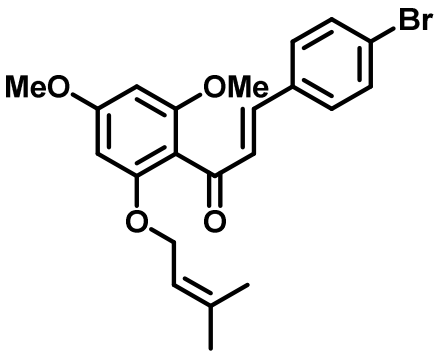
1c, 1h, 2e, 2i, and 2j

HCT116 cells

***In vitro* growth inhibitory effect**

2e showed the lowest GI_{50} and was selected for further studies

HCT116 cells



2e

***In vitro* growth inhibitory effect**

Apoptosis

Cell cycle arrest

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

Acknowledgments

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