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BP-C4: A new diarylpentanoid with Potential Activation of the p53 Pathway

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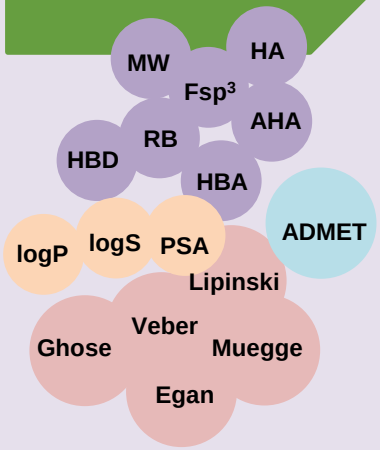
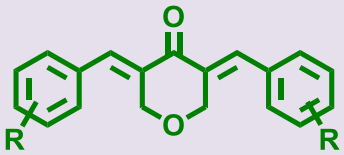
BP-C4: A new diarylpentanoid with Potential Activation of the p53 Pathway

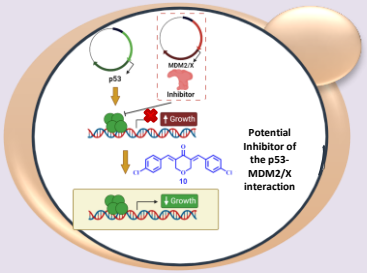
In silico studies



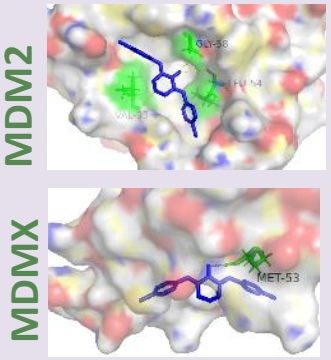
Synthesis



Biological activity



Docking studies



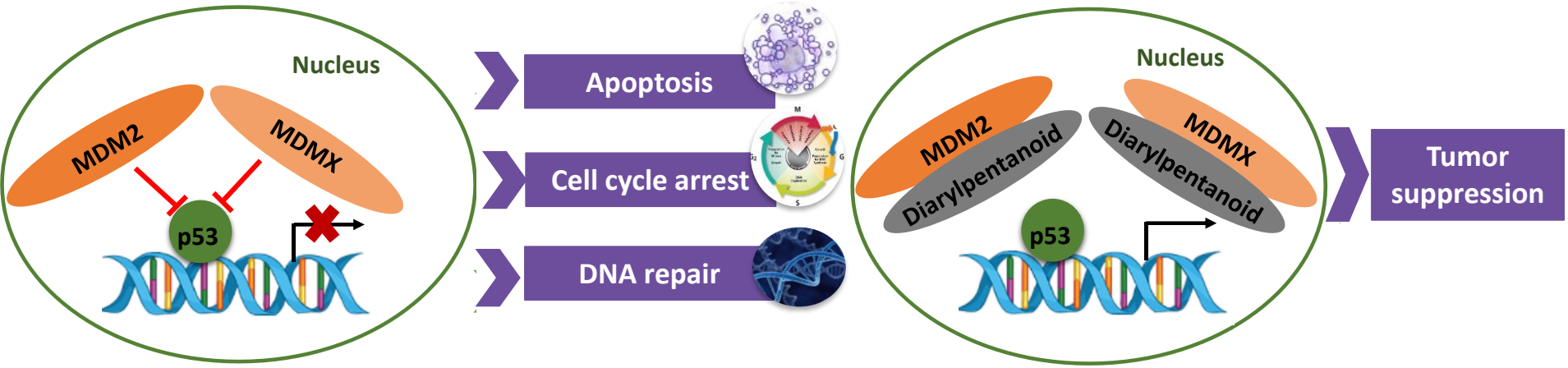
Abstract: The p53 protein is one of the most important tumor suppressors. In about half of human cancers retaining wild-type (wt) p53, its pathway is inactivated due to the overexpression of endogenous negative regulators, namely murine double minute 2 (MDM2) and X (MDMX). Therefore, the disruption of p53-MDM2/X interactions represent an efficient and selective therapeutic strategy against wt p53-expressing tumors.[1] Diarylpentanoids are a promising antitumor agents[2] with two aromatic rings connected by a five-carbon bridge. Although the underlying molecular mechanism by which these compounds suppress cancer cell growth is still unclear, the interference with the p53 pathway has been described.[3, 4] However, the interference with p53-MDM2/X interactions was never explored. Thus, in silico studies of a library of diarylpentanoids led us to the identification of potential new MDM2/X ligands. The diarylpentanoids with the best docking scores obeying the druglikeness and ADMET prediction properties were synthesized. Their antiproliferative activity on colon cancer HCT116 and fibroblasts HFF-1 cells was evaluated, being the most potent and selective compounds further studied to explore their effect as inhibitors of p53–MDM2/X interactions. BP-C4 was identified as potential dual inhibitor. Additionally, in absence of p53 and in cells expressing a mutant p53 form the growth inhibitory effect was significantly reduced. Furthermore, the growth inhibitory effect of BP-C4 was associated with induction of cell cycle arrest and apoptosis. Computational docking studies were performed in order to predict docking poses and residues involved in the inhibition of p53-MDM2/X interactions.

Keywords: Antitumor activity; p53; diarylpentanoids; p53-MDM2/X inhibitors; *in silico* studies

1. Lemos, A. et al., Med. res. rev. 2016, 36, (5), 789-844; 2. Moreira, J. et al., Eur. J. Med. Chem., 2020, 112177; 3. Selvendiran, K. et al., J. Biol. Chem. 2007, 282, (39), 28609-28618; 4. Modzelewska, A. et al., Bioorg. Med. Chem. 2006, 14, (10), 3491-3495.

Introduction

- ✓ In human tumors retaining wild-type (wt) p53, the p53 pathway is inactivated due to the overexpression of **MDM2** and **MDMX**
- ✓ The **disruption of p53-MDM2/X interactions** represents an efficient and selective therapeutic strategy against wt p53-expressing tumors[1]
- ✓ The interference of **diarylpentanoids** with the p53 pathway has been described. [2,3,4] Nevertheless, their effect on **p53-MDM2/X interactions** has never been explored

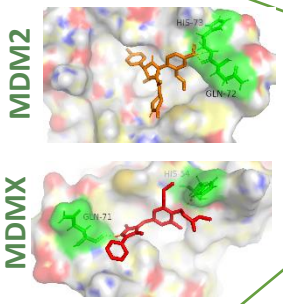


Aim

Discovery of **new diarylpentanoids** with **p53-MDM2/X dual inhibitory activity** and adequate **drug-likeness** and **ADME** profile.

1. Lemos, A. et al., Med. res. rev. 2016, 36, (5), 789-844; 2. Moreira, J. et al., Eur. J. Med. Chem., 2020, 112177; 3. Selvendiran, K. et al., J. Biol. Chem. 2007, 282, (39), 28609-28618; 4. Modzelewska, A. et al., Bioorg. Med. Chem. 2006, 14, (10), 3491-3495.

Results and discussion _ *In silico studies*



Lipinski's pre-filter [1]

Virtual screening MDM2/X [2]

Drug-likeness prediction [3]

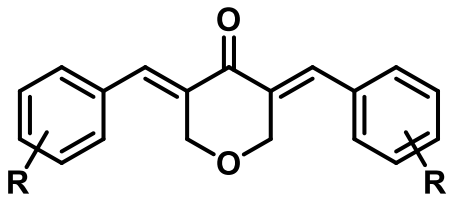
ADMET prediction

Molecular descriptors: HA, Fsp^3, AHA, RB

Physicochemical properties: PSA, logP, logS

Rules of Drug-likeness: Veber, Muegge, Ghose, Egan

22 diarylpentanoids

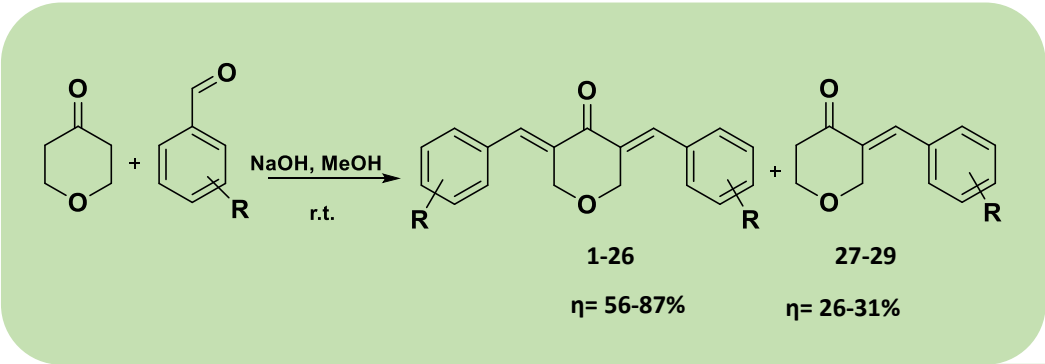


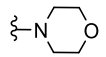
[1] using SwissADME web server; [2] using MDM2 and MDMX as targets; program PyRx; [1] using SwissADME and PreADME web server.

Results and discussion _ Synthesis

- ✓ **Diarylpentanoids** with the **best docking** scores complying with the **drug-likeness** and **ADMET properties** were subsequently prepared

- ✓ In addition, four **structure-related diarylpentanoids** with docking scores lower than positive controls, were also synthesized, in order to **understand the relationship** between the docking scores, docking poses, and % of p53-MDM2/X inhibition activity



Compound ID	R_2	R_3	R_4	R_5
1	Br	H	H	H
2	Cl	H	H	H
3	F	H	H	H
4	OCH_3	H	H	H
5	CH_3	H	H	H
6	H	Br	H	H
7	H	OCH_3	H	H
8	H	CH_3	H	H
9	H	H	Br	H
10	H	H	Cl	H
11	H	H	F	H
12	H	H	OCH_3	H
13	H	H	CH_3	H
14, 27	H	H	$N(CH_3)_2$	H
15, 28	H	H	$N(CH_2CH_3)_2$	H
16, 29	H	H		H
17	-OCH_2O-		H	H
18	OCH_3	OCH_3	H	H
19	OCH_3	H	OCH_3	H
20	OCH_3	H	H	OCH_3
21	H	-OCH_2O-		H
22	H	OCH_3	OCH_3	H
23	H	OCH_3	H	OCH_3
24		H	H	F
25	OCH_3	H	OCH_3	OCH_3
26	H	OCH_3	OCH_3	OCH_3

Results and discussion _ Biological activity

- *Screening assay based on in vitro growth of human cell lines*

Table 1: GI_{50} values of compounds **1-29** in human cancer HCT116 $p53^{+/+}$ and normal fibroblasts HFF-1 cells.

Compound ID	GI_{50} (μM) HCT116 $p53^{+/+}$	GI_{50} (μM) HFF-1	SI*
1	> 25	-----	-----
2	> 25	-----	-----
3	0.69 ± 0.21	0.77 ± 0.30	1.12
4	2.45 ± 0.07	1.44 ± 0.12	0.59
5	3.8 ± 0.42	3.24 ± 0.01	0.85
6	0.9 ± 0.14	0.63 ± 0.008	0.7
7	0.99 ± 0.01	0.55 ± 0.15	0.56
8	1.21 ± 0.01	3.88 ± 0.022	3.21
9	10.1 ± 4.85	9.45 ± 2.62	0.94
10	**6.25 ± 1.18**	**36.20 ± 5.54**	**5.79**
11	0.71 ± 0.08	0.55 ± 0.01	0.77
12	> 25	-----	-----
13	> 25	-----	-----
14	> 25	-----	-----
15	> 25	-----	-----

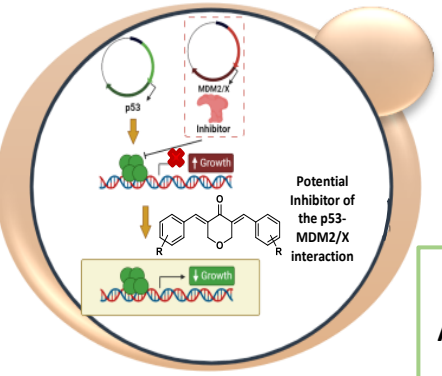
Compound ID	GI_{50} (μM) HCT116 $p53^{+/+}$	GI_{50} (μM) HFF-1	SI*
15	> 25	-----	-----
16	> 25	-----	-----
17	0.64 ± 0.03	0.59 ± 0.005	0.92
18	0.68 ± 0.02	0.89 ± 0.07	1.31
19	1.65 ± 0.64	2.21 ± 0.08	1.34
20	0.87 ± 0.03	0.63 ± 0.03	0.72
21	4.55 ± 0.95	14.76 ± 2.21	3.24
22	1.75 ± 0.10	0.69 ± 0.04	0.39
23	0.63 ± 0.01	0.49 ± 0.01	0.78
24	0.22 ± 0.02	0.33 ± 0.06	1.5
25	> 25	---	-----
26	**0.17 ± 0.01**	**1.21 ± 0.07**	**7.12**
27	> 25	-----	-----
28	> 25	-----	-----
29	> 25	-----	-----

*SI = selective index (GI_{50} of HFF-1/GI_{50} of HCT116 $p53^{+/+}$)

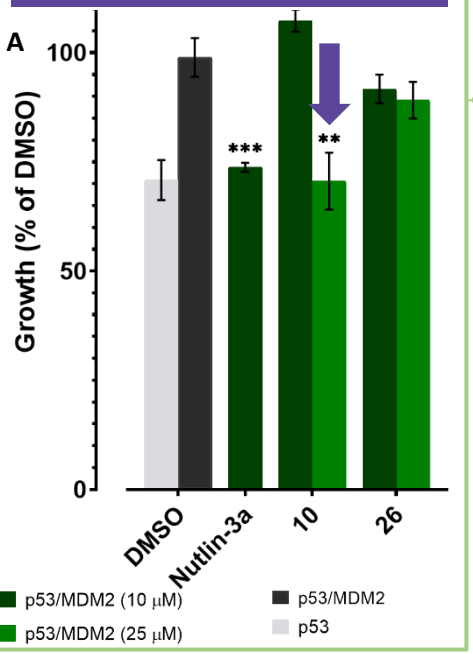
Compounds 10 and 26 showed potent and seletive growth inhibitory effect on HCT116 cells

Results and discussion _ Biological activity

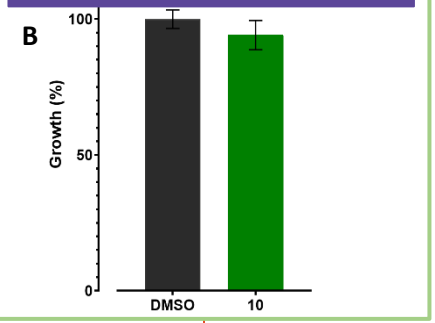
- *Yeast-based assay to screen for inhibitors of p53-MDM2/X interactions*



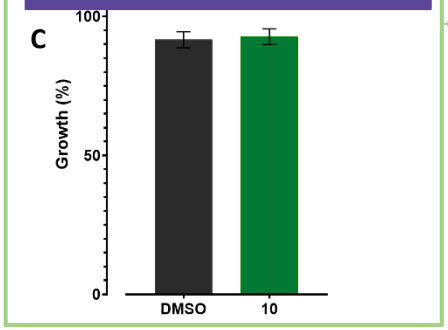
Compound 10 showed to be potential inhibitor of the p53-MDM2 interaction in the yeast-based assay



Compound 10 did not interfere with the growth of yeasts transformed with the empty vector



Compound 10 did not interfere with the growth of yeasts expressing p53 only



Compound 10 showed to be potential inhibitor of p53-MDMX in the yeast-based assay

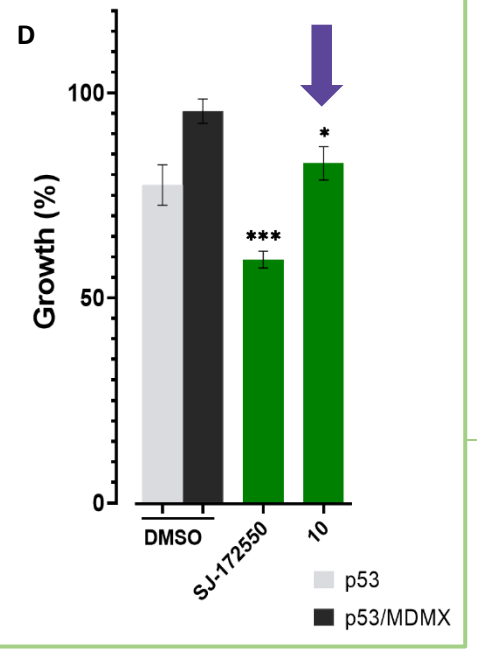


Figure 1. (**A**) Effect of 10 μM nutlin-3A and compounds **10** and **26** (10 μM and 25 μM) on the percentage of growth of yeasts expressing p53 and MDM2, after 42 h treatment. (**B**) Effect of 25 μM compound **10** on the growth of yeast cells transformed with the empty vector, after 42 h. (**C**) Effect of 25 μM compound **10** on the growth of yeast cells expressing p53, after 42 h (**D**) Effect of 25 μM SJ-172550 (SJ) and compound **10** on the growth of yeasts expressing p53 and MDMX, after 42 h. Data are mean ± SEM of at least five independent experiments; values significantly different from DMSO are indicated (***p<0.001, **p<0.01, *p<0.05).

Results and discussion _ Biological activity

- *Study of the mechanism of action of compound 10*

The inhibitory effect was significantly reduced in HCT116 p53 $^{-/-}$ cells and on MDA-MB-231, which expresses mutp53

Table 2: Growth inhibitory effect of **10** on **HCT116**$^{-/-}$ and MDA-MB-231 tumor cells.

	IC_{50} µM	
Compound ID	**HCT116 p53 $^{-/-}$**	**MDA-MB-231**
10	**10.13 ± 0.47***	**> 50***

Data represent mean ± SEM of three independent experiments; values significantly different from HCT116 p53+/+ cells are indicated (*p<0.01)

Results suggest a potential selectivity of compound 10 towards the wt p53 pathway.

Effect of compound 10 on cell cycle, apoptosis and protein levels of p53 and its transcriptional targets in HCT116 p53$^{+/+}$ cells

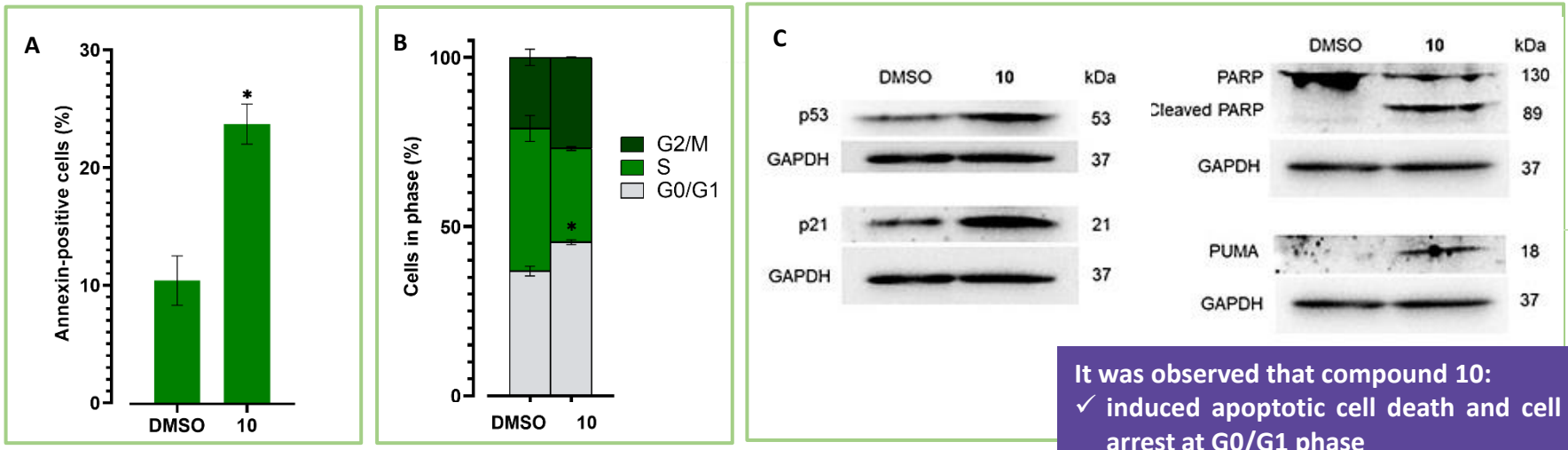


Figure 2. Effect of **10** on cell cycle, apoptosis, and protein levels of p53 and its transcriptional targets, in HCT116 p53$^{+/+}$ cells. (**A**) Effect of 12 µM **10** on apoptosis after 48 h treatment. (**B**) Effect of 12 µM **10** on cell cycle progression after 48 h treatment. (**C**) Protein levels of p53 and its transcriptional targets after 48 h treatment with 12 µM **10** or DMSO. Values significantly different from DMSO are indicated (*p<0.05).

It was observed that compound 10:

- ✓ induced apoptotic cell death and cell cycle arrest at G0/G1 phase
- ✓ increased the p53 expression levels, p21 and the pro-apoptotic protein PUMA
- ✓ induced PARP cleavage

Results and discussion _ Docking studies

Compound **10**, the **active diarylpentanoid** in the **yeast assay** and also one that was predicted to **bind more stably *in silico* to MDM2** (-7.1 Kcal.mol-1) and **MDMX** binding pocket (-6.4 Kcal.mol-1), were further analyzed in terms of docking poses and residues involved in the p53–MDM2/X potential interactions

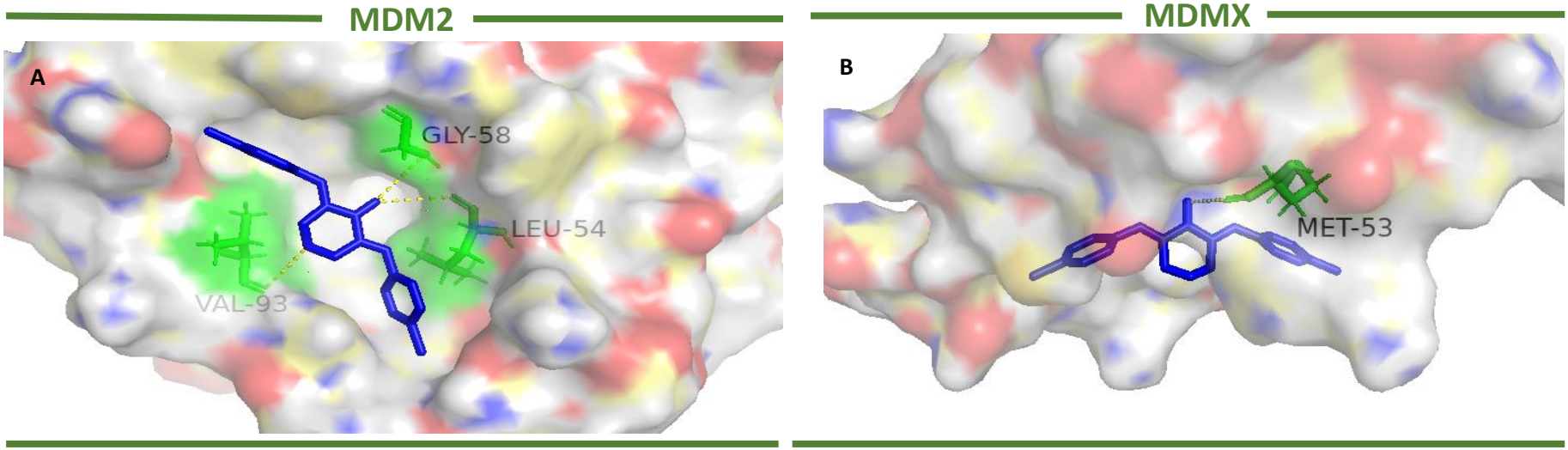
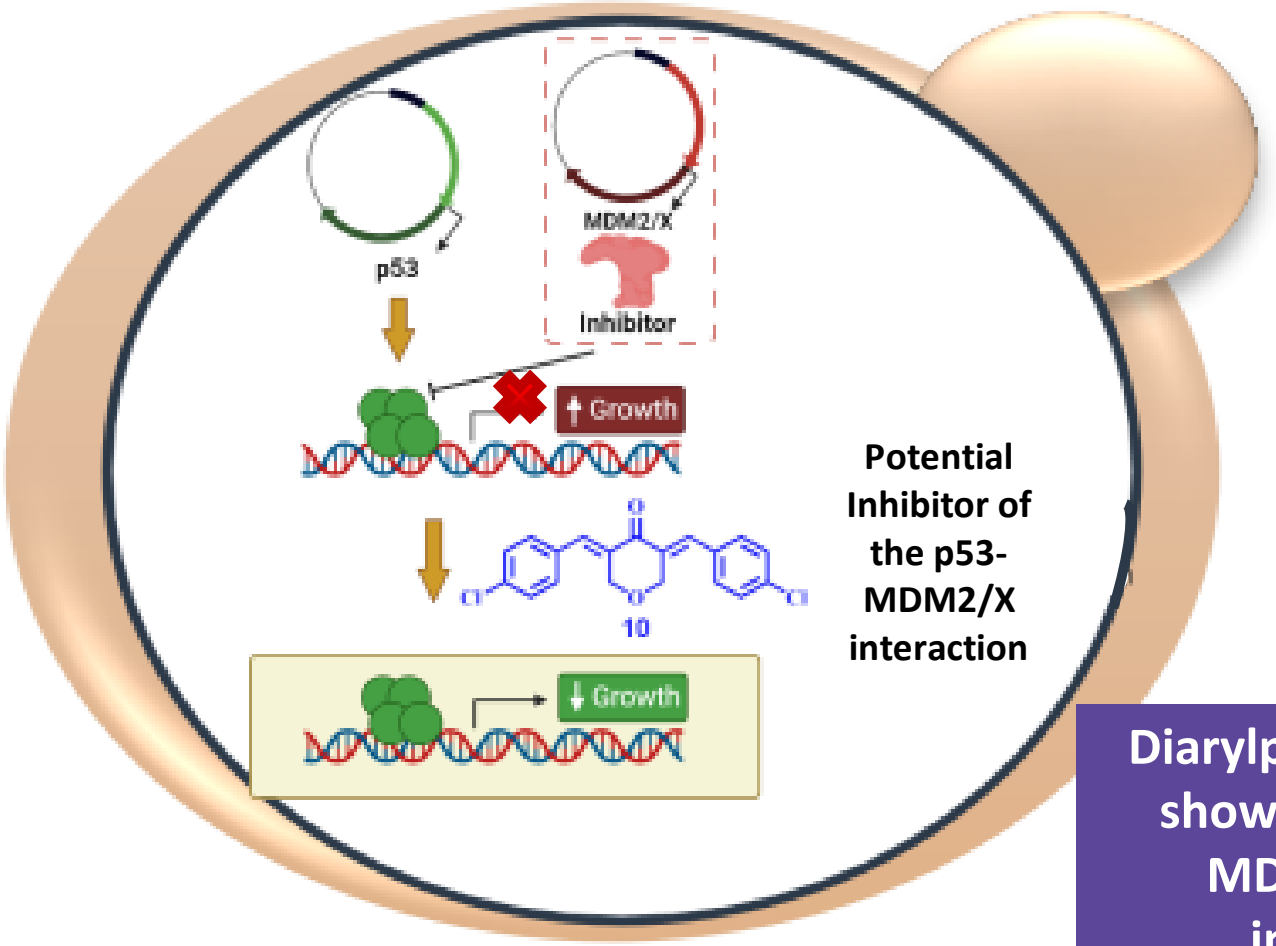


Figure 3. A) Predicted binding poses of **10** (represented in blue sticks) in the binding site of MDM2; (**B**) Predicted binding poses of **10** (represented in blue sticks) in the binding site of MDMX. MDM2/X is represented as surface, where carbon, oxygen, nitrogen, and sulfur are represented in, yellow, red, blue, and orange, respectively. Hydrogen interactions are depicted with a dashed yellow line. Residues involved on polar interactions are labelled and represented in green.

Compound 10 establishes three hydrogen interactions with Leu-54, Gly-58, and Val-93 and non-polar interactions with MDM2

Compound 10 establishes one hydrogen interaction with Met-53 and non-polar interactions with MDMX

Conclusions



Potential Inhibitor of the p53-MDM2/X interaction

Diarylpentanoid 10 (BP-C4) showed a potential p53-MDM2/MDMX dual inhibitory effect

Acknowledgments

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