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# Optimizing the oxazoloisoindolinone family: identification and biological evaluation of a potent and selective indole-based p53 activator in human colorectal cancer

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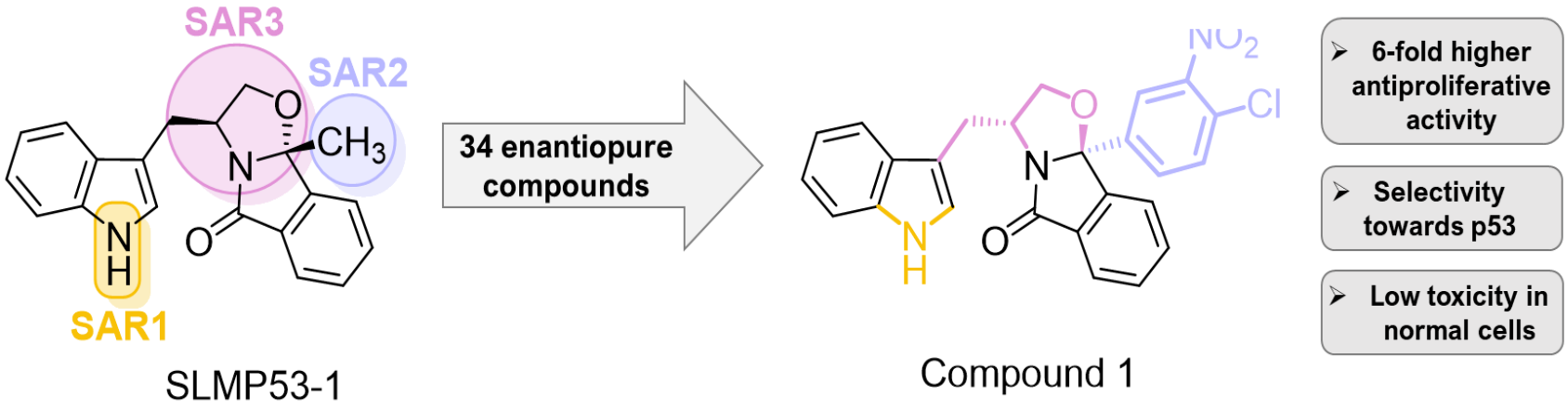
# Optimizing the oxazoloisoindolinone family: identification and biological evaluation of a potent and selective indole-based p53 activator in human colorectal cancer




**Graphical Abstract**

**Abstract:**

With 1.8 million new cases in 2018, Colorectal Cancer (CRC) is considered one of the most malignant cancers due to its frequency and high rate of mortality. The molecular pathogenesis of CRC involves in 40-50% of cases mutation of protein p53, a long-established tumor suppressor which inactivation occurs in all human cancers either by protein-protein interaction with its main inhibitors MDM2/X or by hotspot mutation. Unfortunately, patients with mutated p53 gene are likely to develop multidrug resistance, thus likely leading to therapy failure. Our group of research is actively involved in the identification of new p53 modulators and in this work, we want to share our latest results in the identification of **compound 1**, an optimized (*R*)-tryptophanol-derived oxazoloisoindolinone that was found to be six-fold more active than our hit compound SLMP53-1 in colon carcinoma HCT116 cell line (see Graphical abstract). Interestingly, *in vitro* results show that compound 1 was found to have increased selectivity for HCT116 cells with p53, and with low toxicity in normal cells. Molecular docking simulations and binding assays, reported in this study, give important insights about why this novel enantiopure oxazoloisoindolinone analogue cannot be consider a MDM2 inhibitor.

**Keywords:** Colorectal cancer; p53; anticancer drug; oxazoloisoindolinones.

# Introduction: Colorectal cancer at a glance



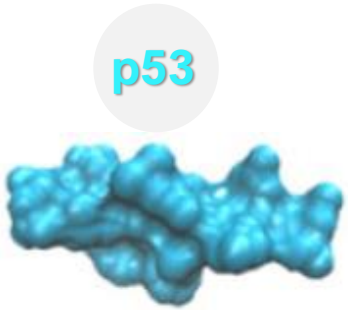
Colorectal cancer (CRC) is the third most common malignancy worldwide



With 1.8 M new cases and 881.000 deaths is the fourth cause of death

By 2030, the incidence of CRC could soar by 90% and 124 % for patients with age 20 to 34 years old

# p53 and colorectal cancer

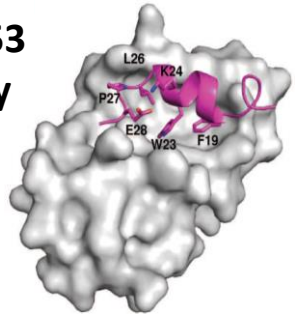



**It's the most frequently inactivated tumor suppressor**

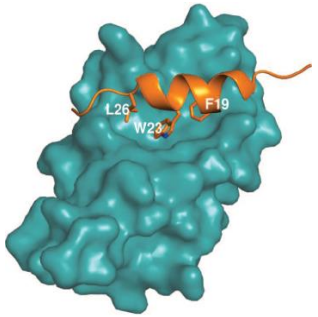
**When active p53 regulates**

- ❑ Apoptosis
- ❑ Cell cycle arrest
- ❑ DNA repair
- ❑ Senescence
- ❑ Autophagy

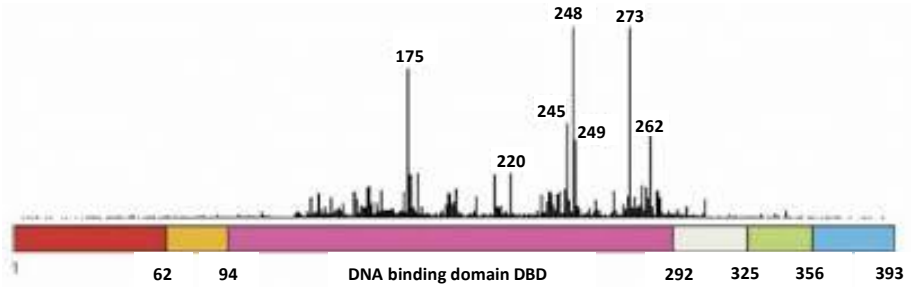
- ❑ **50% of cases p53 is inactivated by overexpression of its endogenous negative regulators**




**MDM2**




**MDMX**

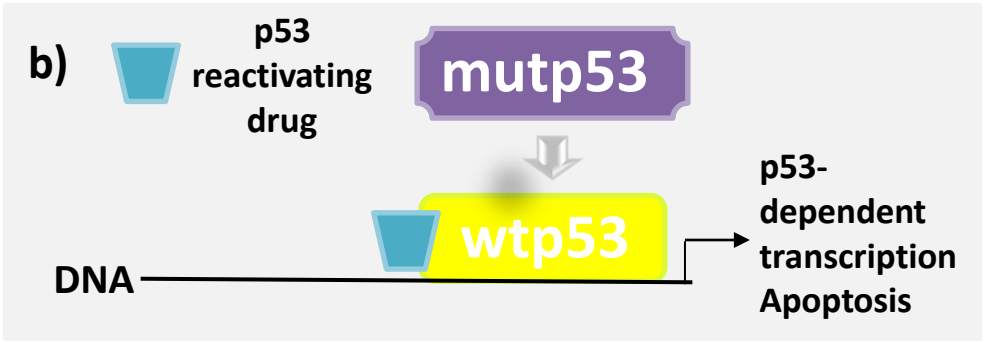



- ❑ **50% of cases p53 is inactivated by hotspot mutations on its DNA-binding domain**

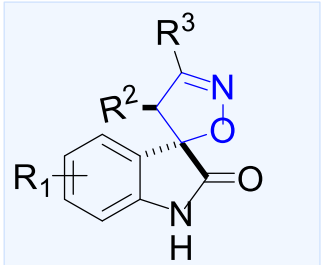
**p53 mutation develops in about 40-50% of CRC. Patients with mutated p53 gene gain multidrug resistance**

# Hunting p53 activators

- To date, most strategies of reactivation of p53 are focused on the inhibition of its main negative regulator MDM2 (a), or on the pharmacological restoration of wild-type-like activity to mutant p53 forms (b).

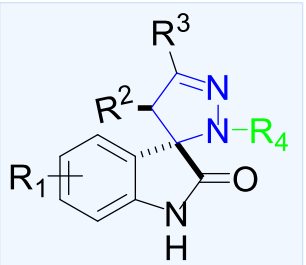




**Our research group have identified several small molecules that are able to activate the p53 pathway**



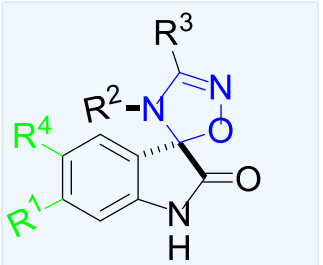

**Spiroisooxazoline oxindoles**

*Bioorg. Med. Chem.* **2014**, *22*, 577



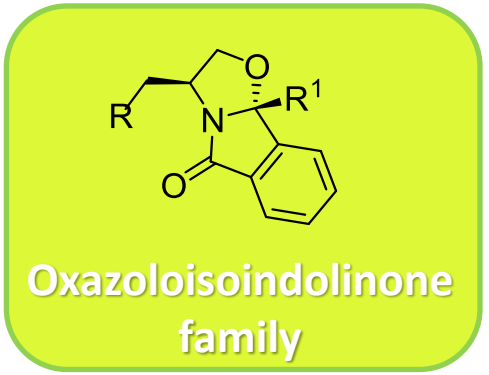

**Spiropyrazoline oxindoles**

*Eur. J. Med. Chem.* **2014**, *79*, 266




**Spirooxadiazoline oxindoles**

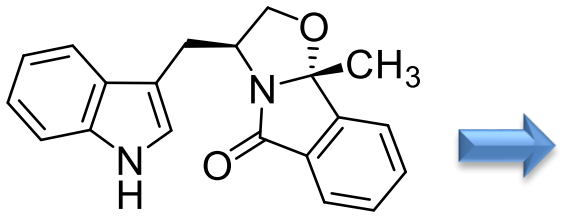
*MedChemComm* **2016**, *7*, 420




*Eur. J. Pharm. Sci.* **2015**, *66*, 138
*Oncotarget* **2016**, *7*, 4326
*Mol. Oncol.* **2017**, *11*, 612
*Cancers* **2019**, *11*, 1151

# Results and discussion: Hit-to-lead optimization strategy

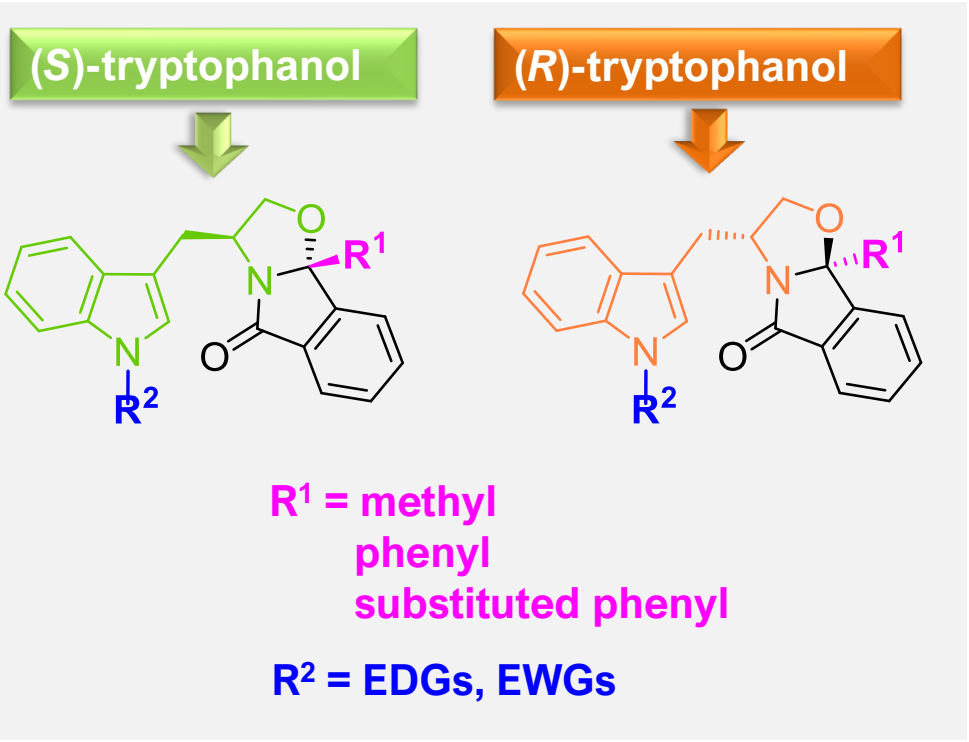
- **SLMP53-1 was selected as hit compound to optimize**




SLMP53-1

HCT116 $p53^{+/+}$ = 16 μM
HCT116 $p53^{-/-}$ = 34 μM

*Oncotarget* 2016, *7*, 4326

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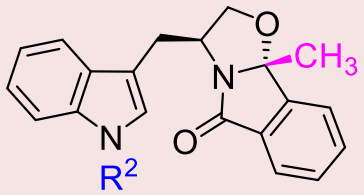
- **The synthetic approach involves the use of enantiopure forms of the aminoalcohol tryptophanol which control the stereochemistry of the products**

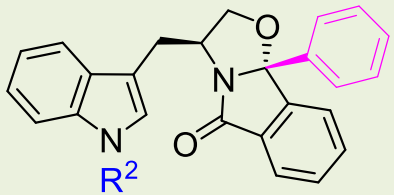
# Screening the oxazoloisoindolinone family in HCT-116 cell line

**Series 1**



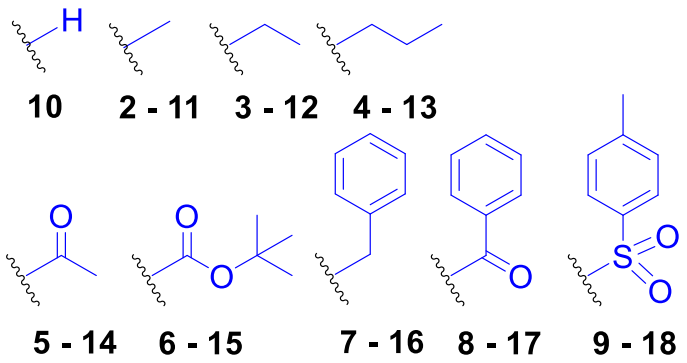

**2-9**

**Series 2**



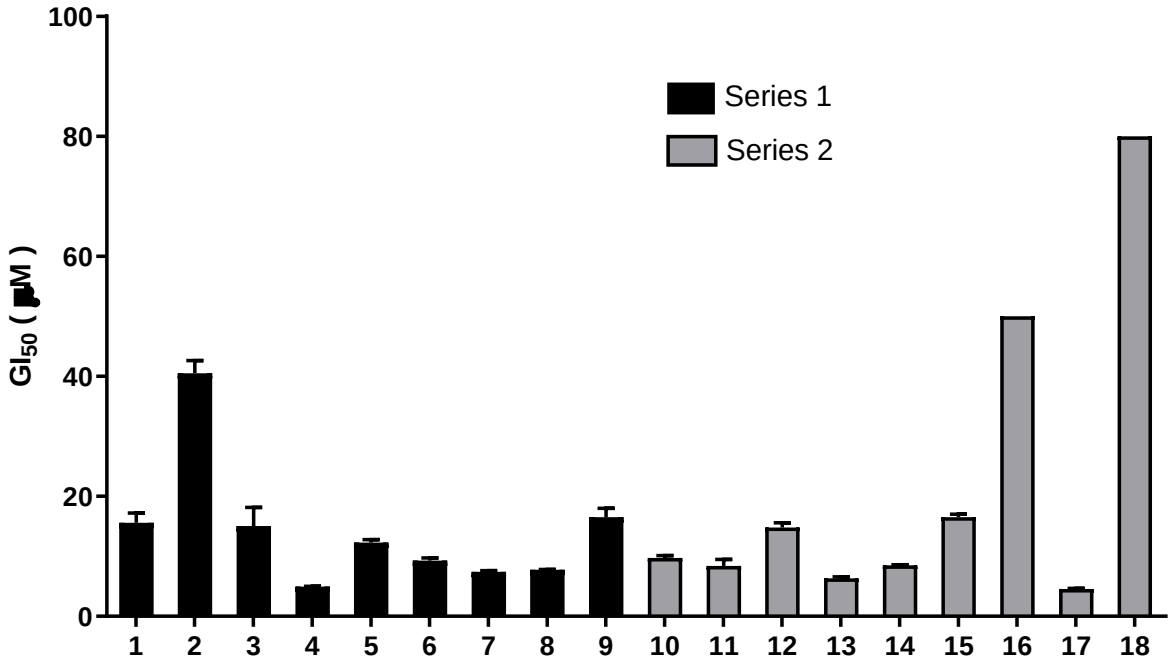

**10-18**

$R^2$=




**10** **2 - 11** **3 - 12** **4 - 13**

**5 - 14** **6 - 15** **7 - 16** **8 - 17** **9 - 18**

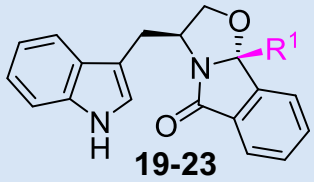



- **Four Series for a total of 34 new enantiopure tryptophanol-derived oxazoloisoindolinones were prepared and the antiproliferative effect of the compounds was evaluated in human colon adenocarcinoma HCT116 cell line**

*ChemMedChem* 2020, doi: 10.1002/cmdc.202000522

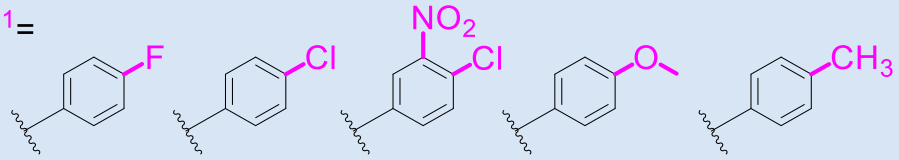
# Screening the oxazoloisoindolinone family in HCT-116 cell line

**Series 3**



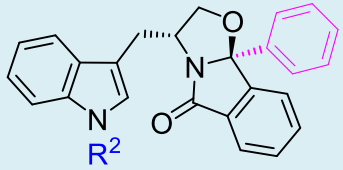

19-23

$R^1$=

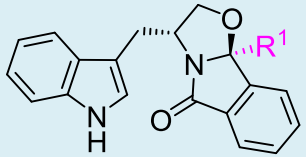



19 20 21 22 23

**Series 4**

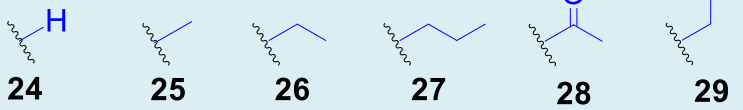



24-29




30-34

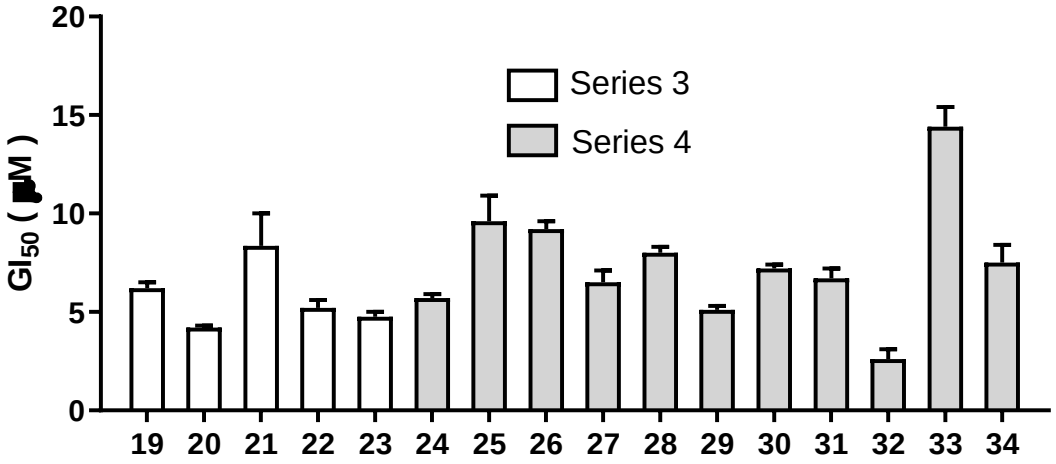
$R^2$=




24 25 26 27 28 29

$R^1$=


30 31 32 33 34




- **25 Oxazoloisoindolinones present a $GI_{50}$ lower than the hit SLMP53-1**

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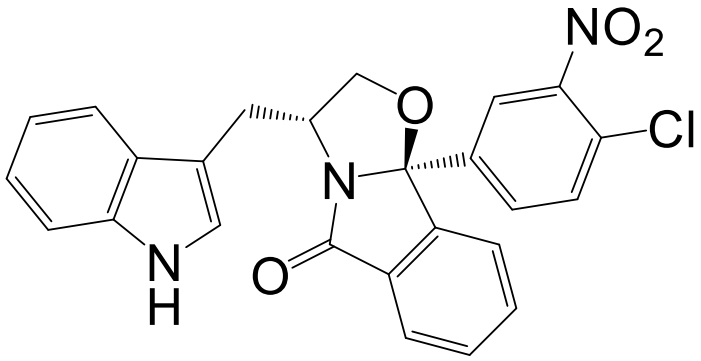
# Selectivity in HCT-116 cell line for the p53 pathway

Table 1. Phenotypic activity of tryptophanol-derived isoindolinones against HCT116 $p53^{+/+}$ and $p53^{-/-}$ cell growth.

| Compound | HCT116 $p53^{+/+}$ $GI_{50}$ [μM] | HCT116 $p53^{-/-}$ $GI_{50}$ [μM] | SI[a] |
|---|---|---|---|
| 1 | 15.5 ± 1.6[30] | 34.0 ± 3.5[30] | 2.2 |
| 10 | 9.7 ± 0.4 | 9.7 ± 0.3 | 1.0 |
| 11 | 8.4 ± 1.1[32] | 17.7 ± 2.3[32] | 2.1 |
| 13 | 6.35 ± 0.2 | 7.0 ± 1.1 | 1.1 |
| 14 | 8.5 ± 0.1 | 9.5 ± 1.5 | 1.1 |
| 17 | 4.55 ± 0.1 | 3.8 ± 0.3 | 0.8 |
| 19 | 6.2 ± 0.3 | 7.4 ± 0.1 | 1.2 |
| 20 | 4.2 ± 0.1 | 4.8 ± 0.3 | 1.1 |
| 21 | 8.35 ± 1.65 | 8.95 ± 0.75 | 1.1 |
| 22 | 5.2 ± 0.4 | 5.6 ± 0.7 | 1.1 |
| 23 | 4.75 ± 0.05 | 6.3 ± 0.4 | 1.3 |
| 24 | 5.7 ± 0.2 | 7.0 ± 0.5 | 1.2 |
| 25 | 9.6 ± 1.3 | 16.7 ± 1.9 | 1.7 |
| 26 | 9.2 ± 0.4 | 7.3 ± 0.9 | 0.8 |
| 27 | 6.5 ± 0.6 | 10.0 ± 1.3 | 1.5 |
| 28 | 8.0 ± 0.3 | 5.4 ± 0.4 | 0.7 |
| 29 | 5.1 ± 0.2 | 3.6 ± 0.2 | 0.7 |
| 30 | 7.2 ± 0.2 | 9.9 ± 1.3 | 1.4 |
| 31 | 6.7 ± 0.5 | 9.0 ± 0.4 | 1.3 |
| 32 | 2.6 ± 0.5 | 8.6 ± 0.4 | 3.3 |
| 34 | 7.5 ± 0.9 | 10.2 ± 0.8 | 1.4 |

[a] Selectivity index toward HCT116 $p53^{+/+}$, which is expressed by the ratio $GI_{50}$ HCT116 $p53^{-/-}$/$GI_{50}$ HCT116 $p53^{+/+}$.




**Compound 32**

- ❑ 6-fold higher antiproliferative activity than SLMP53-1
- ❑ Increased Selectivity towards p53

**Toxicity tested in normal CCD-18Co colon cells**
**$IC_{50}$ = 26.7±1.8 μM**
- ❑ **10-fold higher than the obtained in HCT116 $p53^{+/+}$ cells**
- ❑ **compound 32 is selective towards cancer cells**

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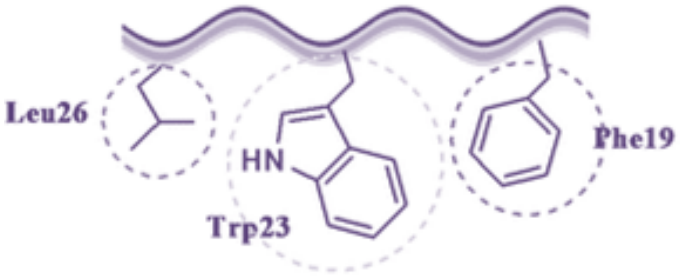
# Evaluation of compound 32 as MDM2 inhibitor

- p53-MDM2 protein-protein interaction consists of a steric complementary interface between the MDM2 cleft and the hydrophobic residues Phe19, Leu22, Trp23 and Leu26 of the α-helix of p53




- polarization (FP) competitive binding assay was used to evaluate if the molecular target for the activation of p53 observed for compound 32 is MDM2

- Compound 32 was not able to compete with the fluorescent probe molecule that binds potently to MDM2

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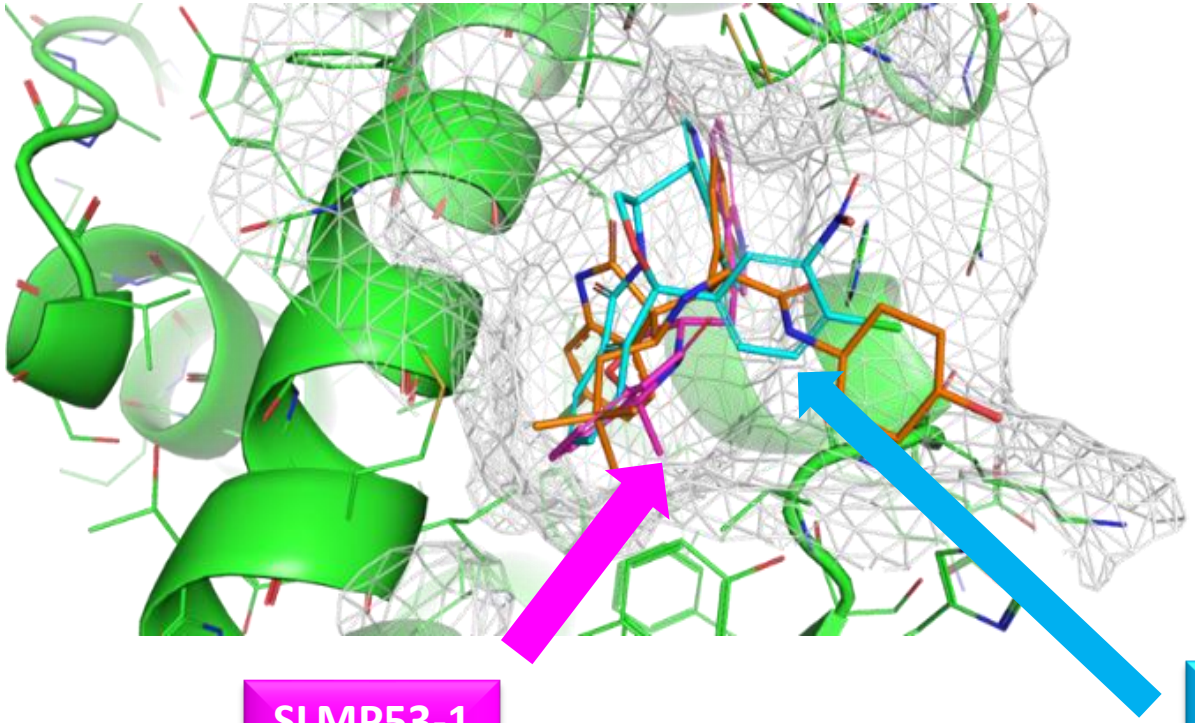
# Evaluation of compound 32 as MDM2 inhibitor: molecular docking



- ❑ indole moiety projected to the Leu26 pocket
- ❑ the oxazolidine moiety projected to the Phe19 pocket

- ❑ both compounds are unable to fill the four pockets of the MDM2 hydrophobic binding site

**SLMP53-1**

- ❑ does not fill the Trp23 and the Leu22 pockets
- ❑ only one hydrogen interaction with the carbonyl group of the backbone of His96

**Compound 32**

- ❑ 9b-phenyl group is projected to the Leu22 solvent-exposed pocket,
- ❑ the Trp23 pocket remains unfilled
- ❑ the nitro group establishes a hydrogen bond with the nitrogen of the Met6 residue

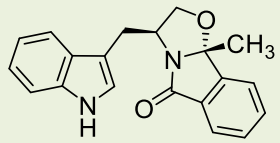
# Conclusions

In this presentation we briefly described:

- ❑ synthetic strategy
- ❑ structure-activity study

of a chemical library of 34 enantiopure tryptophanol-derived oxazoloisoindolinones as p53 activators

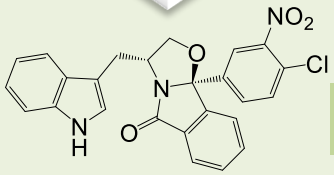
**Screening in human colon adenocarcinoma cell line leads to identification of compound 32**




SLMP53-1

HCT116

$p53^{+/+}$ = 16 μM
$p53^{-/-}$ = 34 μM




HCT116

$p53^{+/+}$ = 2.6 μM
$p53^{-/-}$ = 8.6 μM

**Compound 32**

- ❑ 6-fold higher antiproliferative activity than hit compound
- ❑ Increased Selectivity towards p53
- ❑ Low toxicity
- ❑ the mechanism of action as p53 activator does not involve the inhibition of the p53 main negative regulator (MDM2)

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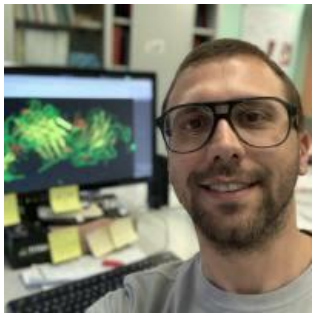

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