5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Enhancing anticancer activity of spiropyrazoline oxindoles by disrupting p53-MDMs PPIs

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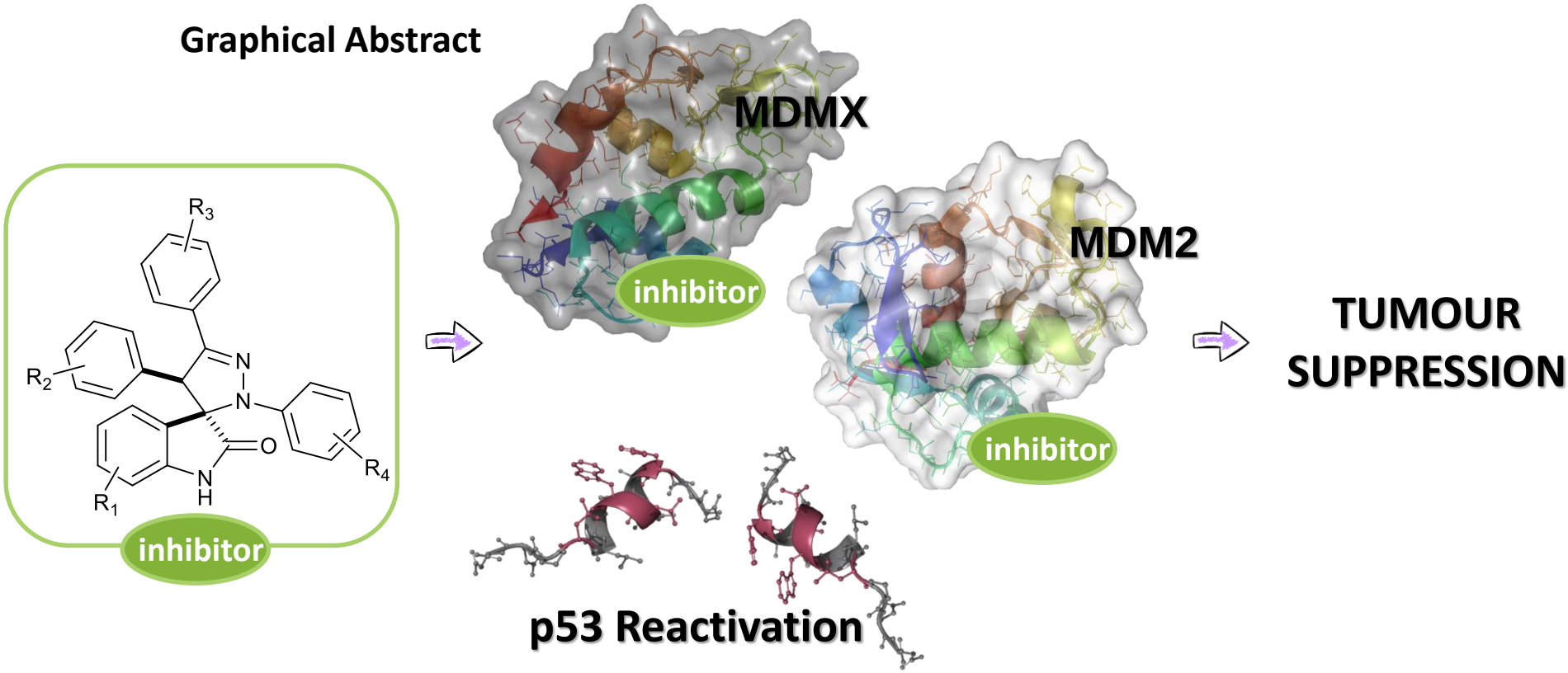
Enhancing anticancer activity of spiropyrazoline oxindoles by disrupting p53-MDMs PPIs

Graphical Abstract

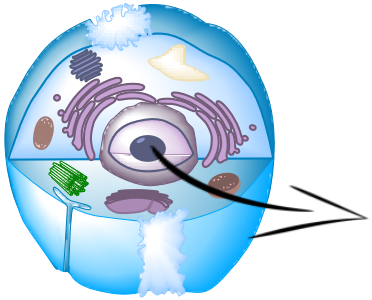


Abstract:

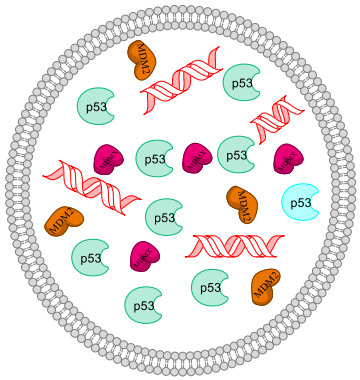
The protein p53 is an attractive target in oncology because it can modulate several additional cellular processes that are relevant for the suppression of tumour development. In all types of human cancers, the p53 tumour suppressor function is inactivated by mutation or gene deletion or by negative regulators. For the full reactivation of wild-type p53, both interactions of p53 with the negative regulators have to be inhibited. Due to the lack of dual p53-MDM2/X PPIs inhibitors in clinical trials, it is urgent to develop small molecules that inhibit these interactions. Our research team has been working on the development of spiropyrazoline oxindoles to obtain dual p53-MDM2/X PPIs inhibitors. Hence, we have already developed derivatives with good antiproliferative activities in HCT-116 $p53^{(+/+)}$ human colorectal carcinoma cell line, which induce apoptosis and cell cycle arrest at G0/G1 phase, and lead to a decrease of MDM2 levels. In this communication, we report the *hit-to-lead* optimization of this chemical family for the development of novel p53-MDM2/X interactions inhibitors. Furthermore, we report our most recent optimization of the synthesis of these new spiropyrazoline oxindoles derivatives and the first preliminary biological results.

Keywords: p53 activation, spiropyrazoline oxindoles, MDM2, MDMX

Introduction – *p53 tumour suppressor*

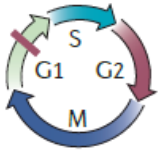


Cell under stress

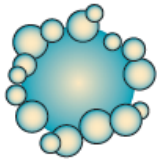


p53 is activated

triggers

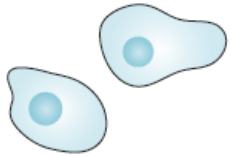


Cell cycle arrest

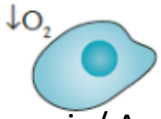


Apoptosis

DNA repair



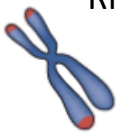
Senescence



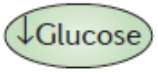
Hypoxia/ Anoxia

Oxidative stress

Ribonucleotide depletion



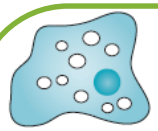
Telomere erosion



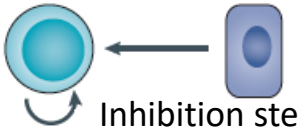
Nutrient deprivation

DNA damage

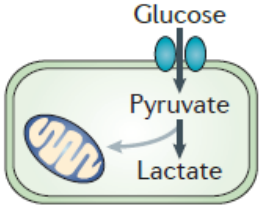
Replication stress



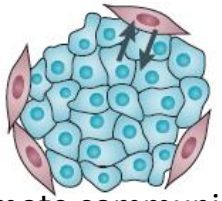
Activation autophagy



Inhibition stem cell self-renewal



Opposing metabolic reprogramming



Promote communication within tumour microenvironment signalling

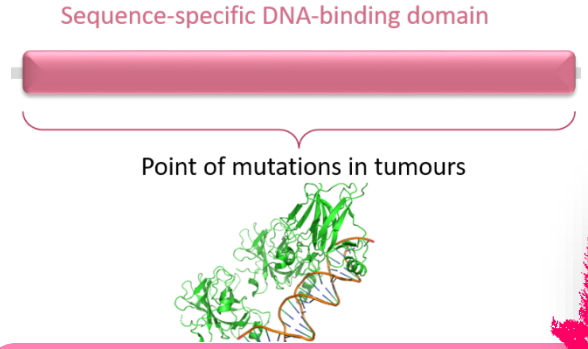
Restrain invasion and metastasis

TUMOUR SUPPRESSION

Nature Reviews. Cancer **2014**, 14, 359-370.

Introduction – *p53 inactivation in tumours*

The p53 **tumour suppressor** function is **inactivated in all** tumours



mutation or deletion of TP53 50%

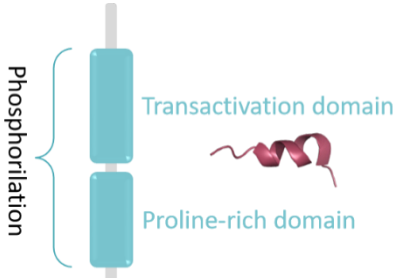
negative regulators 50%

Strategies to reactivate:

Give p53 original conformation

Strategies to reactivate:

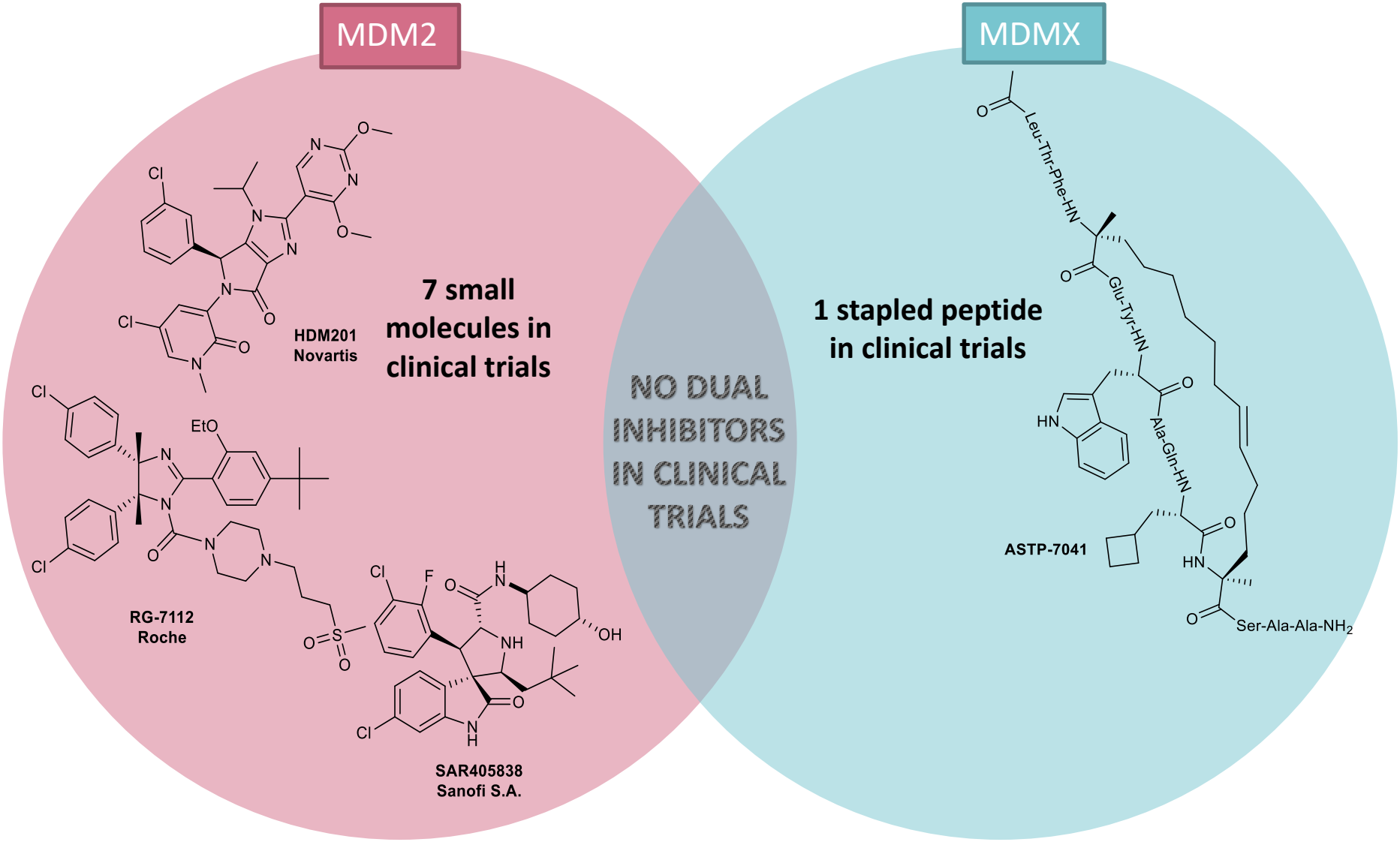
Inhibit p53-MDM2/X interactions



Full reactivation of p53 can only be achieved by inhibition of dual p53-MDM2 and p53-MDMX PPIs

Curr. Topics Med. Chem. **2018**, 18, 647-660.

Introduction – *p53-MDM2 and p53-MDMX PPIs inhibitors in clinic*



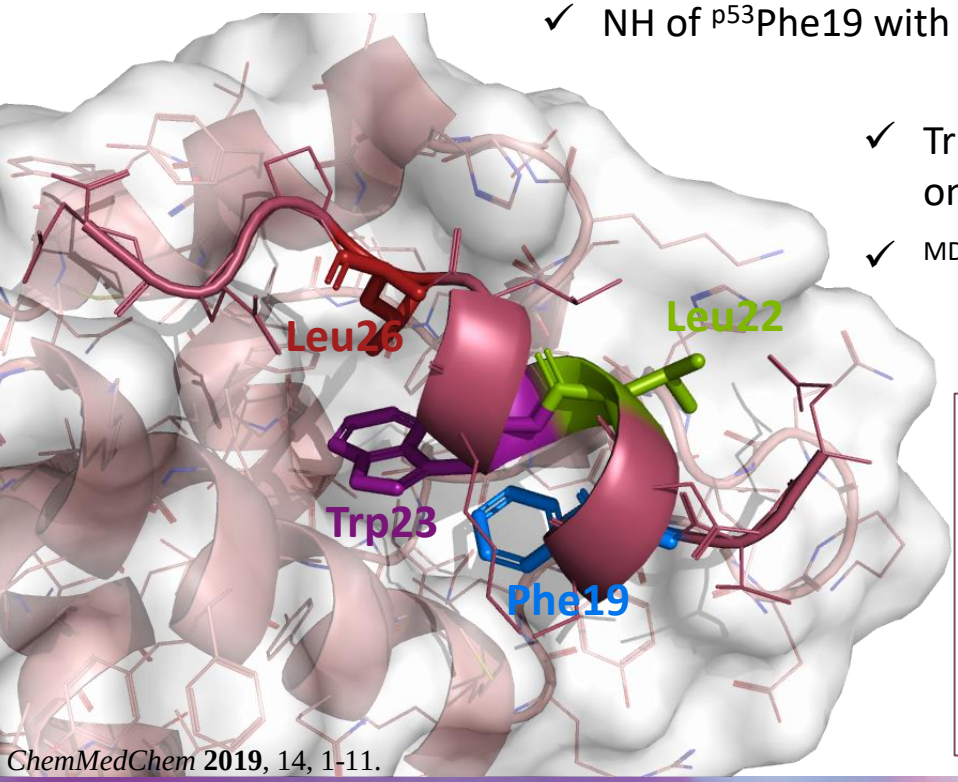
Curr. Topics Med. Chem. **2018**, 18, 647-660.

Introduction – *Understanding MDM2 and MDMX pockets*

MDM2

- ✓ Interacts with p53 by 4 amino acid residues: ^{p53}Phe19, ^{p53}Leu22, ^{p53}Trp23, ^{p53}Leu26
- ✓ Intermolecular van der Waals contacts
- ✓ Two hydrogen bonds
  - ✓ NH of ^{p53}Trp23 with ^{MDM2}Leu54 (2.8Å)
  - ✓ NH of ^{p53}Phe19 with ^{MDM2}Gln72 (3.0Å)

Important pharmacophore feature



ChemMedChem **2019**, 14, 1-11.

- ✓ Trp23 sub-pocket can adapt its side chain depending on the groups in the Trp23 and Leu26 sub-pockets
- ✓ ^{MDM2}His96 (Leu26 sub-pocket) can adapt its side chain

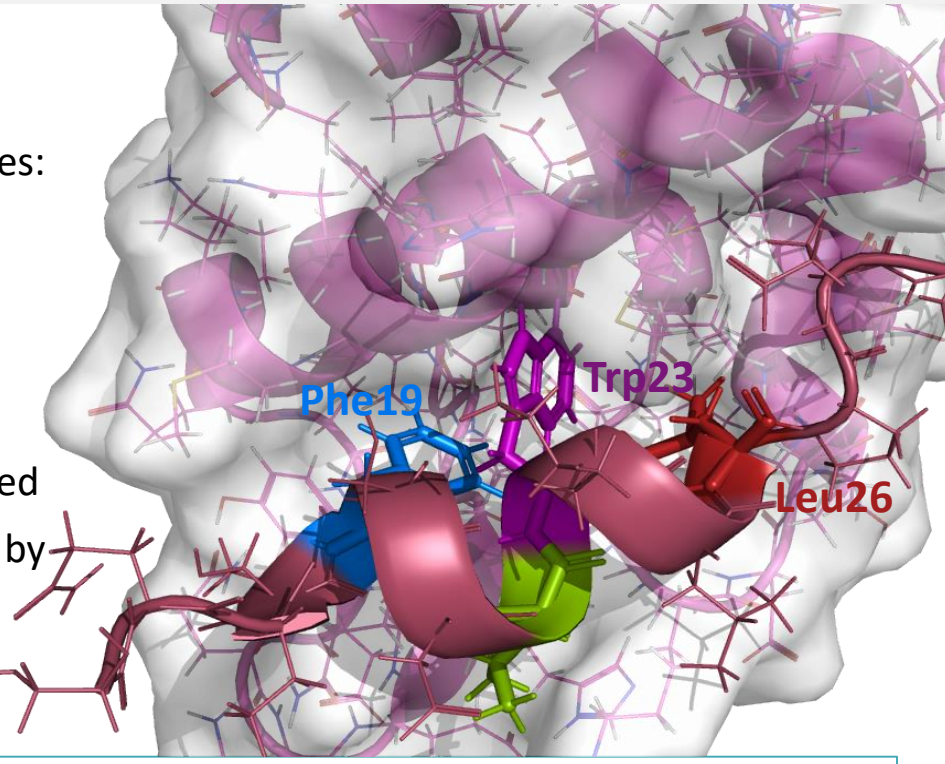
Other important features:

- ✓ π-π stacking interaction with ^{MDM2}His96 (enthalpic based binding)
- ✓ ^{MDM2}Tyr67 "out conformation" of Phe19 sub-pocket enlarges the space and leads to higher affinity

Introduction – *Understanding MDM2 and MDMX pockets*

MDMX

- ✓ Interacts with p53 by 4 amino acid residues: ^{p53}Phe19, ^{p53}Leu22, ^{p53}Trp23, ^{p53}Leu26
- ✓ Phe19 sub-pocket similar to MDM2
- ✓ Trp23 sub-pocket is 2.0 Å shorter
- ✓ ^{MDM2}His96 is replaced by ^{MDMX}Pro96 residue
  - ✓ No longer π-π stacking interaction is needed
- ✓ ^{MDM2}Leu54 and ^{MDM2}Ile99 are replaced by ^{MDMX}Met54 and ^{MDMX}Leu99
  - ✓ Leu26 pocket is more shallow and deeper



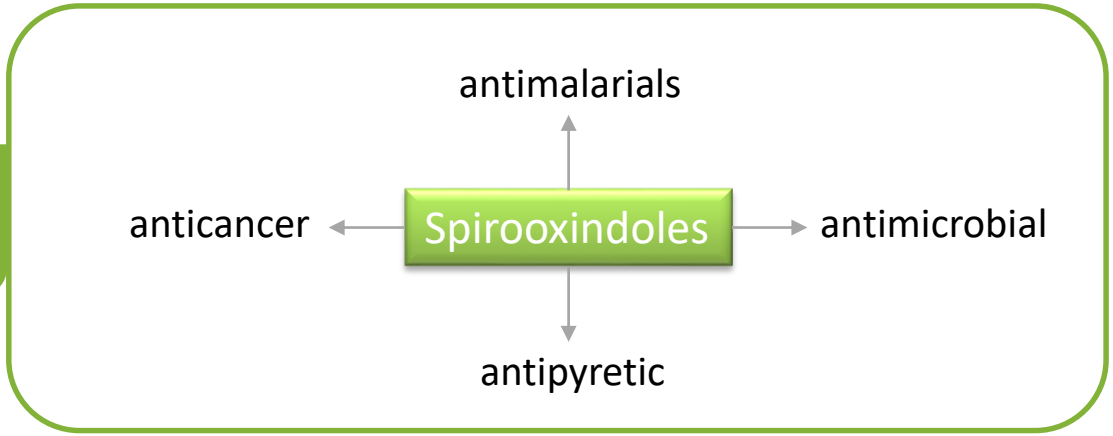
Other important features:

- ✓ Carboxylate group can induce a flip of His55 enabling a strong hydrogen bond
  - ✓ Water-mediated hydrogen bond with Lys51
- ✓ Interactions with His55 can compensate weaker interactions in the Leu26 and Trp23 sub-pockets
- ✓ Induction of dimerization of MDMX complexes

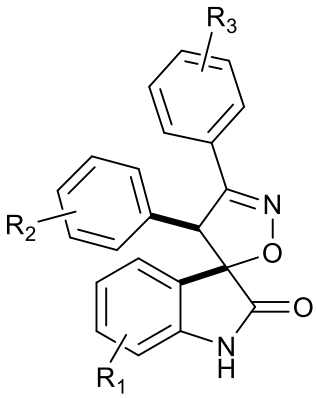
ChemMedChem **2019**, 14, 1-11.

Introduction – *Spirooxindoles*

Biological activities of spirooxindoles



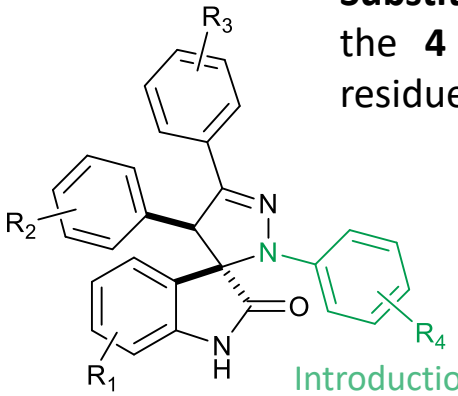
First spirooxindole derivative synthetized by our group



Spiroisoxazoline oxindoles

- ✓ Library of 18 new compounds;
- ✓ Tested in human colorectal carcinoma cell lines
- ✓ p53-MDM2 interaction inhibitor.

Spiropyrazoline oxindoles



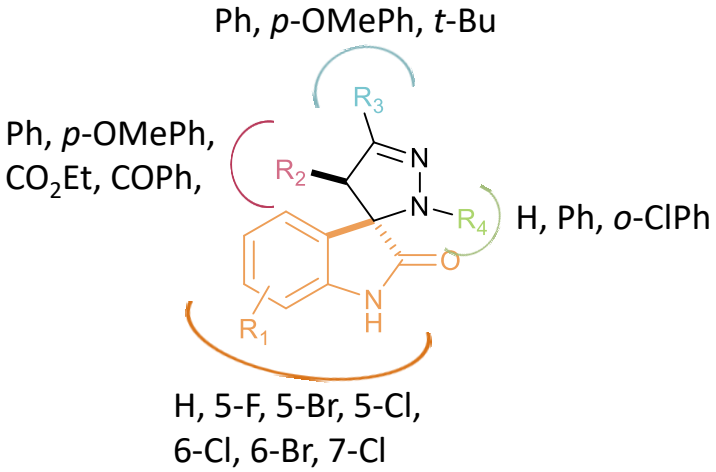
Substituents mimic the **4 amino acid** residues of p53:

- ✓ Phe19
- ✓ Leu22
- ✓ Trp23
- ✓ Leu26

Introduction of a fourth substituent

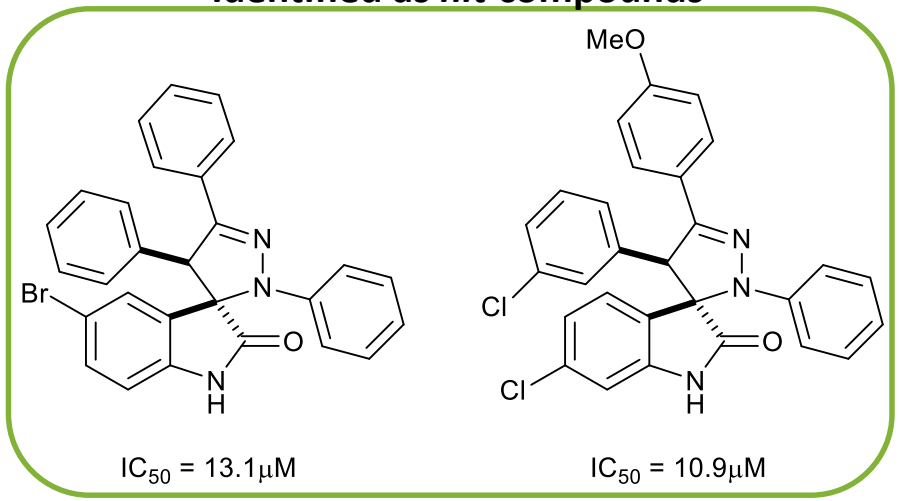
Bioorg. Med. Chem. **2014**, 22, 577-584

Introduction – *Spiropyrazoline oxindoles*



- ✓ Library of 32 new compounds
- ✓ Evaluated in human colorectal carcinoma cell line HCT-116 *p53*$^{(+/+)}$ (*wild-type* p53)
- ✓ Non-cytotoxic against HEK293T cell line up to 100 µM

Identified as *hit* compounds



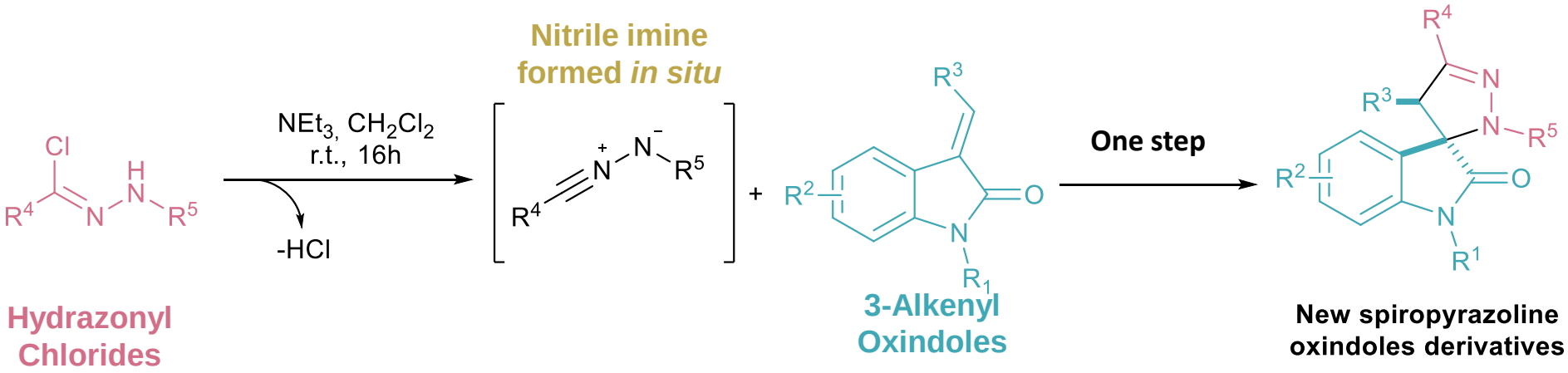
Selected for further studies, including molecular docking

- ✓ Disrupt p53-MDM2 PPI (BiFC assay)
- ✓ Decrease MDM2 level
  - ✓ Other mecanisms besides p53-dependent effects
- ✓ Induce apoptosis
- ✓ Induce cell cycle arrest in G0/G1 phase in a time-dependent manner

Eur. J. Med. Chem. **2014**, 79, 266-272. *Eur. J. Med. Chem.* **2017**, 139, 168-179.

Results and discussion – *Synthesis*

1,3-dipolar cycloaddition



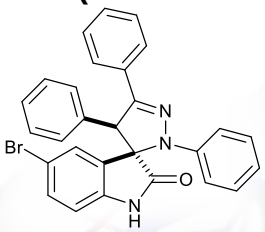
R^4 : Alkyl or Aromatic
R^5 : Aromatic

- ✓ High yields
- ✓ Commercially available starting materials

Eur. J. Med. Chem. **2014**, 79, 266-272. *Eur. J. Med. Chem.* **2017**, 139, 168-179. *Frontiers in Chemistry* **2019**, 7, article 15.

Results and discussion – *In silico design*

Docking pose of the binding of *hit compound* already identified and MDM2 (PDB ID 4WT2)



MDM2

cation-π interaction
Ph ring and aromatic ring of ^{MDM2}His96

π-π interaction
Ph ring and ^{MDM2}Tyr67

Hydrogen bond
NH of indole moiety and C=O of ^{MDM2}Leu54

Ligand energy minimization: SZYBKI (force field MMFF94S)
Software: Fred 3.2.0.2 (rigid receptor)
Validation with redocking of SAR405838

Results and discussion – *In silico design*

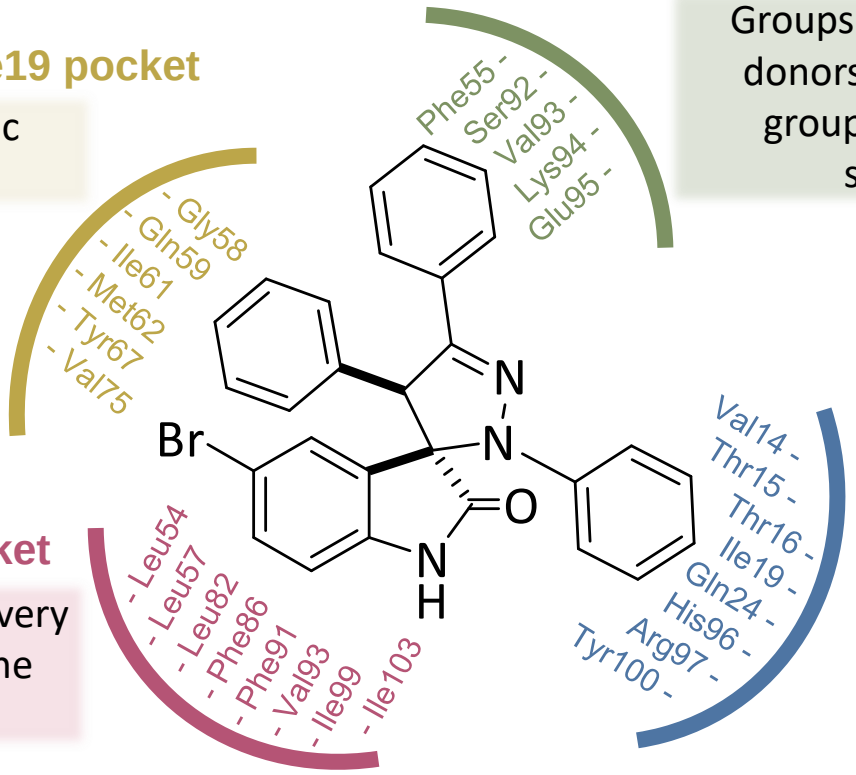
MDM2 optimizations

Leu22 pocket

Groups with hydrogen donors and chemical groups to increase solubility

Phe19 pocket

Highly hydrophobic pocket



Leu26 pocket

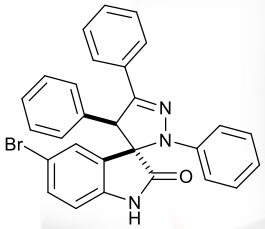
Very small pocket that only fits aromatic rings with small halogen atoms

Trp23 pocket

Small groups occupy a very small pocket inside the Trp23 pocket

Results and discussion – *In silico design*

Docking pose of the binding of *hit compound* already identified and MDMX (PDB ID 3LBJ)



MDMX

Mainly hydrophobic interactions
No hydrogen bonds

Ligand energy minimization: SZYBKI (force field MMFF94S)
Software: Fred 3.2.0.2 (rigid receptor)
Validation with redocking of WK298 (MDMX)

Results and discussion – *In silico design*

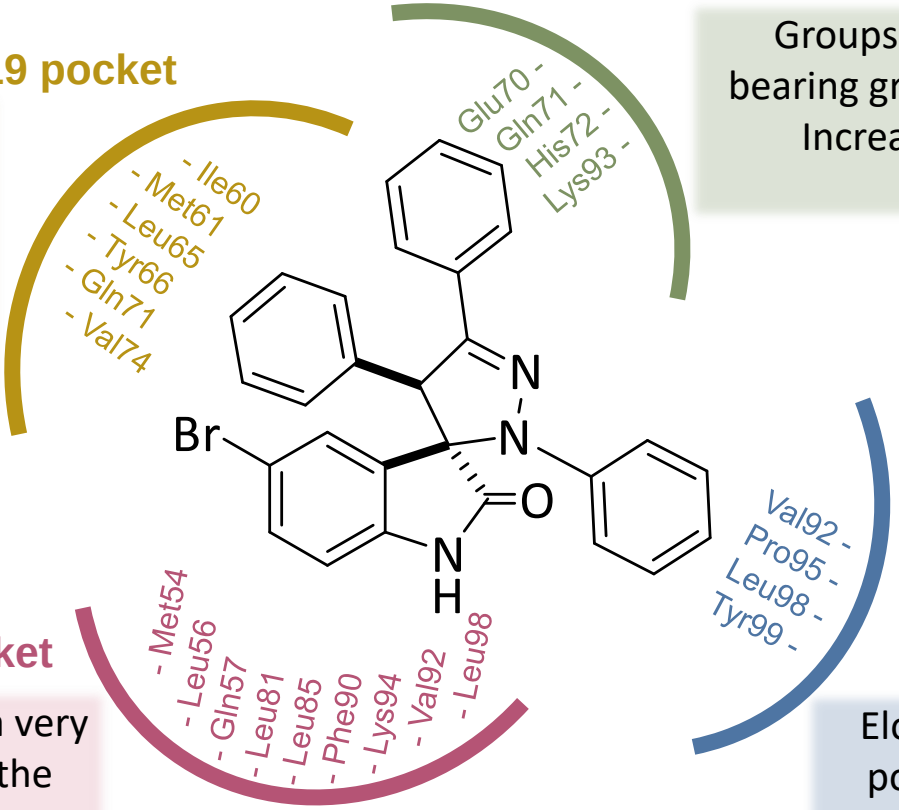
MDMX optimizations

Phe19 pocket

Highly hydrophobic pocket with possible hydrogen bonds in residues such as Tyr and Gln
Flexible groups

Leu22 pocket

Groups with hydrogen bearing groups and charges
Increases solubility



Leu26 pocket

Elongated hydrophobic pocket with hydrogen donor groups
Flexible groups

Trp23 pocket

Small groups occupy a very small pocket inside the Trp23 pocket

Results and discussion – *Biological evaluation*

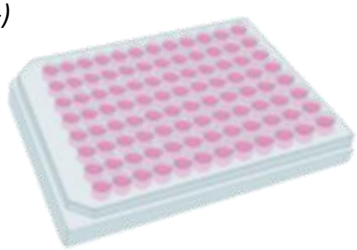
A NEW CHEMICAL LIBRARY OF 30 COMPOUNDS

- ✓ MDM2 fluorescence polarization (FP) competitive binding assay

Hit compound (IC_{50}=10µM)

- ✓ Screening of the new library in human colorectal carcinoma cell lines
  - ✓ HCT116 $p53^{(+/+)}$
  - ✓ HCT116 $p53^{(-/-)}$

Current work



2 new compounds more active than the positive control

Conclusions

Synthesis

- ✓ Spiropyrazoline oxindoles are synthetized by 1,3-dipolar cycloaddition between 2-indolinones and nitrile imines form *in situ* from hydrazonoyl chlorides.

In silico design

- ✓ Spiropyrazoline oxindoles establish the two important pharmacophore interactions: the hydrogen bond between ^{MDM2}Leu54 and NH of the indole moiety and π- π stacking interaction with ^{MDM2}His96
- ✓ Possible interactions around MDMX binding pocket can lead to extra interaction between MDMX and spiropyrazoline oxindoles and help to keep the ligand in the binding pocket.

Biological evaluation

- ✓ It was identified a *hit* compound by MDM2 fluorescence polarization (FP) competitive binding assay.
- ✓ Currently, it is being performed a screening in several cancer cell lines with *wild-type* p53.
- ✓ MDMX competitive binding assay – FUTURE WORK

Acknowledgments

Fundings:

UID/DTP/04138/2019
CEECIND/01772/2017
PTDC/QUI-QOR/29664/2017
SFRH/BD/117931/2016
SFRH/BD/137544/2018

STSM grant 39244

Dr Maria M. M. Santos
Margarida Espadinha, MSc
Prof Cecília Rodrigues

Prof Maurizio Botta
Dr Mattia Mori

Prof Shaomeng Wang