5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Tryptophanol-derived oxazoloisoindolinones: Novel small molecule p53 activators with promising antitumor activity

Valentina Barcherini [1], Sara Gomes [2], Margarida Espadinha [1], Joana Soares [2], Liliana Raimundo [2], Célia Gomes [3], Flávio Reis [3], Alexandra Antunes [4], Lucília Saraiva [2], and Maria M. M. Santos [1,*]

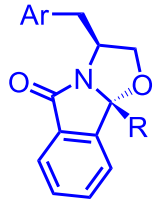
[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal;
[2] LAQV/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;
[3] Institute of Pharmacology & Experimental Therapeutics, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, CNC.IBILI Consortium & CIBB Consortium, University of Coimbra, Coimbra, Portugal;
[4] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049 001, Lisboa, Portugal.

* Corresponding author: mariasantos@ff.ulisboa.pt

Tryptophanol-derived oxazoloisoindolinones: Novel small molecule p53 activators with promising antitumor activity

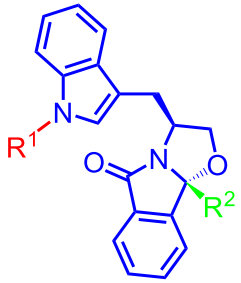
Phenotypic screening using yeast cell models

Search for new scaffolds that inhibit the p53-MDM2 interaction

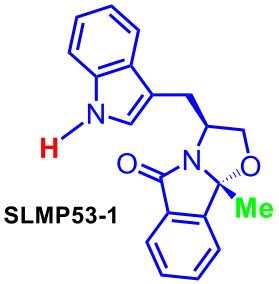


Phenylalaninol-derived oxazoloisoindolinones

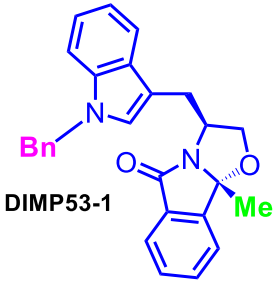
Hit optimization to obtain Selective p53 activators



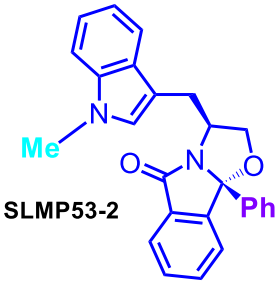
Tryptophanol-derived oxazoloisoindolinones



SLMP53-1



DIMP53-1



SLMP53-2

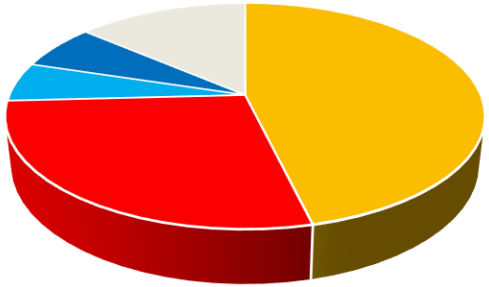
Abstract:

The tumour suppressor p53 is a pivotal target in cancer therapy as this protein is inactive in all human cancers. In the last years, our research group has been working on the design and synthesis of novel small molecules that are able to reactivate p53. Of these, novel scaffolds containing the oxazoloisoindolinone moiety in their chemical structure emerged with very promising anti-cancer properties.
In this communication an overview about the therapeutic potential of a tryptophanol-derived oxazoloisoindolinone chemical library as selective p53 activators will be given. Based on the hit tryptophanol-derived small molecule SLMP53-1, identified as a wild-type and mutant p53 reactivator, a second series of compounds was prepared leading to DIMP53-1 (a p53-MDM2/X interactions dual inhibitor) and to SLMP53-2 (small molecule able to restore the wild-type function of mut p53Y220C). The tryptophanol-derived oxazoloisoindolinone chemical family was prepared by a stereoselective cyclocondensation reaction of enantiopure aminoalcohol tryptophanol with several commercially available oxoacids. From the screening of this library, several very promising molecules emerged with potent anticancer activity against aggressive cancers. The anticancer activity and mechanism of action of the target molecules was studied in human colon adenocarcinoma HCT116 cells with wild-type p53 (HCT116 p53+/+) and the corresponding p53-null isogenic derivative cells (HCT116 p53-/-), as well as in several cancer cell lines with different p53 status. The most promising molecules were also evaluated in vivo.

Keywords: cancer; MDMs; oxazoloisoindolinone; p53; tryptophanol.

iMed. ULisboa
Instituto de Investigação do Medicamento

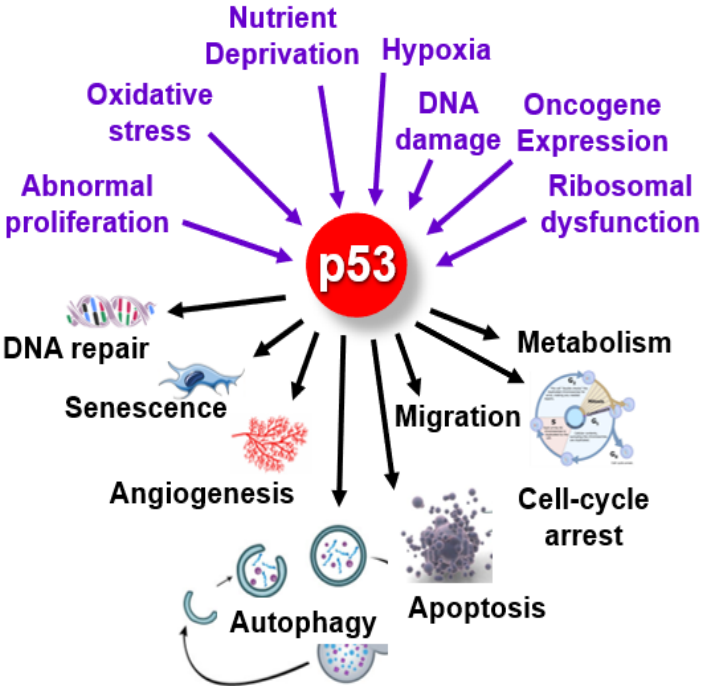
Cancer and p53



18.1 million new cases

9.6 million cases

World Health Organization, September **2018**

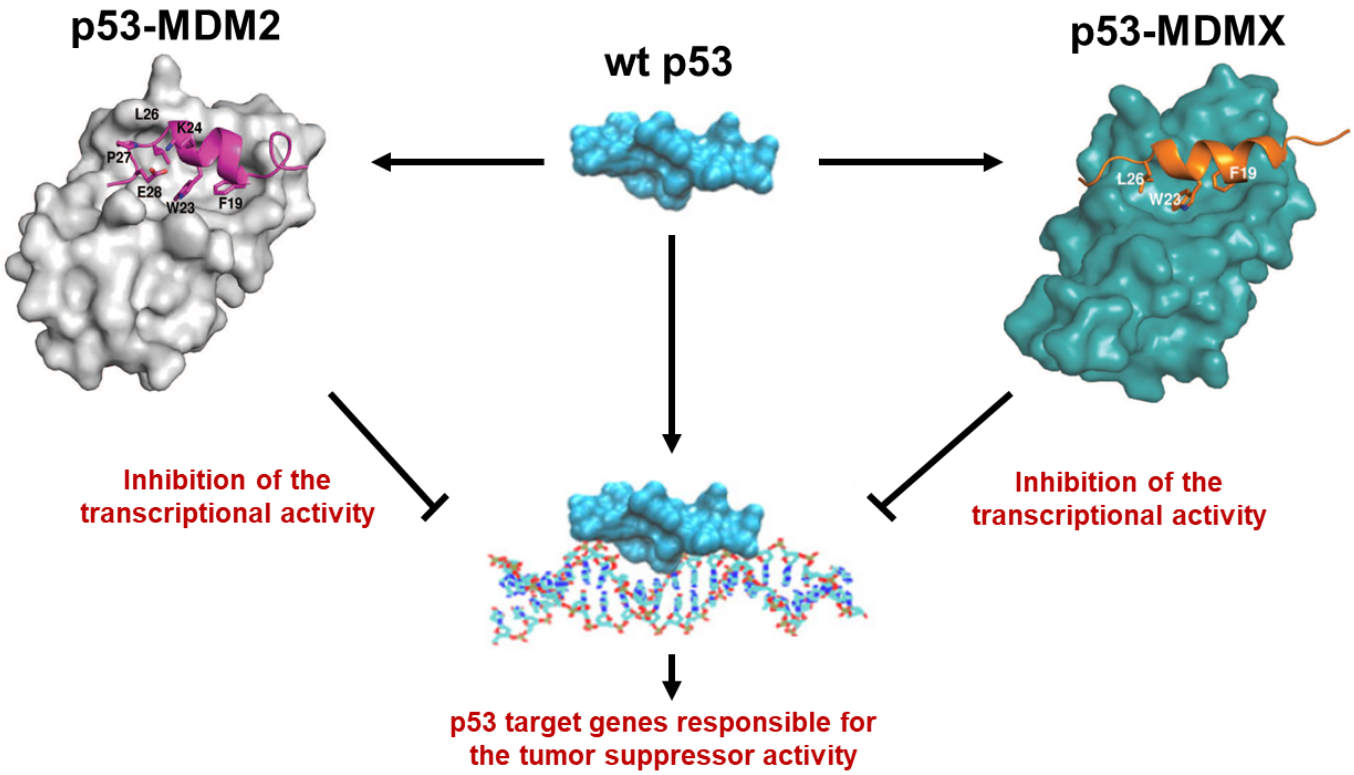


Second Leading Cause of Death

Functional inactivation of the p53 pathway is observed in most human tumors

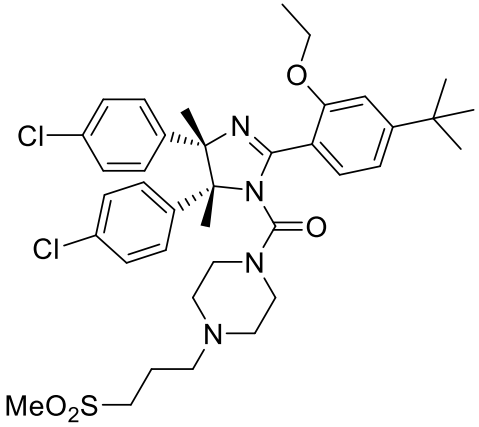
When DNA repair is not accessible, p53 orchestrates the induction of cell death by acting as a **tumor suppressor protein**

p53 Inactivation



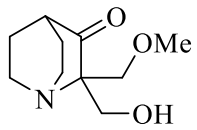
In 50% of cases the tumor suppressor function of p53 is inactivated by mutation or deletion of its gene. In the remainder the pathway is inactivated by reversible inhibition

p53 activators and clinical trials



RG7112 (Hoffmann-La Roche)

First p53-MDM2 interaction inhibitor to enter Clinical trials



APR-246 (Aprea)

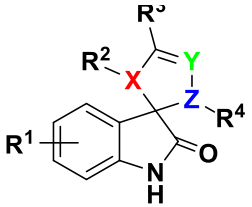
First compound targeting mutant p53 to enter Clinical trials

Curr. Top. Med. Chem. **2018**, 18, 647; *Curr. Med. Chem.* **2019**, 26, 1

iMed. ULisboa
Instituto de Investigação do Medicamento

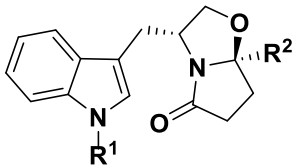
Our contribution to the p53 field

Chemical library design and synthesis of novel scaffolds of p53 activators



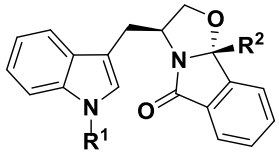
Spirooxindoles
MedChemComm, **2016**, 7, 420
Eur J Med Chem, **2017**, 140, 494
Frontiers in Chemistry **2019**

ECMC presentation of **E. Lopes**: Enhancing anticancer activity of spiropyrazoline oxindoles by disrupting p53-MDMs PPIs



Oxazolopyrrolidones
British. J. Pharmacol., **2018**, 175, 3947

ECMC presentation of **L. Raimundo**: Improving colon cancer therapy with a new promising small-molecule activator of the p53-pathway through disruption of p53-MDM2/MDMX interactions

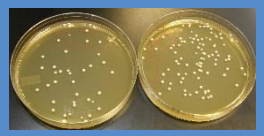


Oxazolosoindolinones
Oncotarget, **2016**, 7, 4326
Molecular Oncology **2017**, 11, 612
Cancers **2019,** 11, 1151

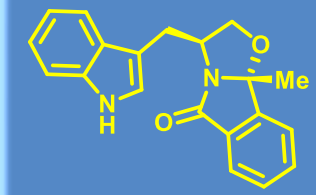
Searching for p53 activators

Chemical Libraries

Search for new scaffolds using yeast cell models

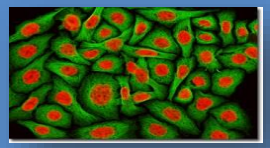


Hit identification

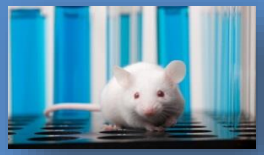


Hit optimization

Evaluation on cancer cell lines



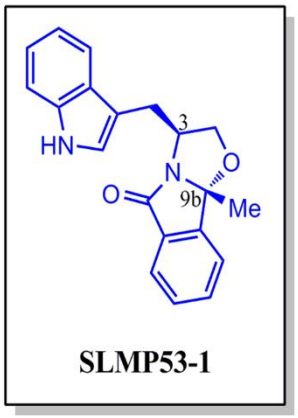
Evaluation on animal models

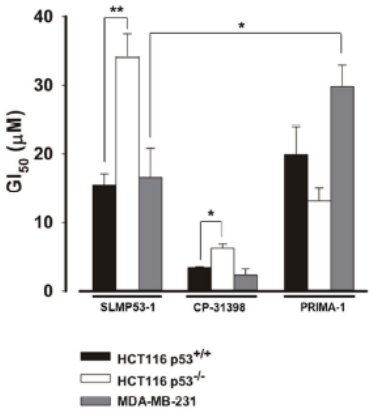


iMed. ULisboa
Instituto de Investigação do Medicamento

Hit identification

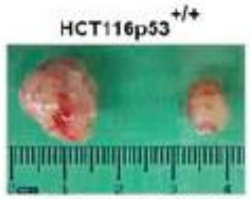
Yeast target-directed Screening assay

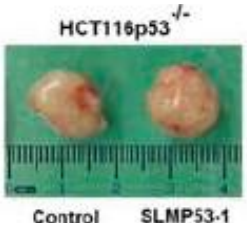




reactivator of wt p53 and mut p53R280K

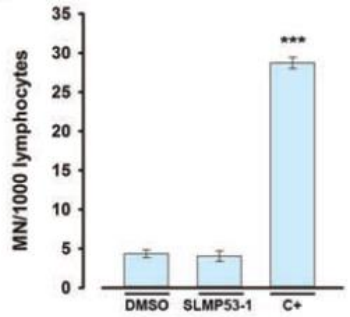
Oncotarget, Vol. 7, No. 4





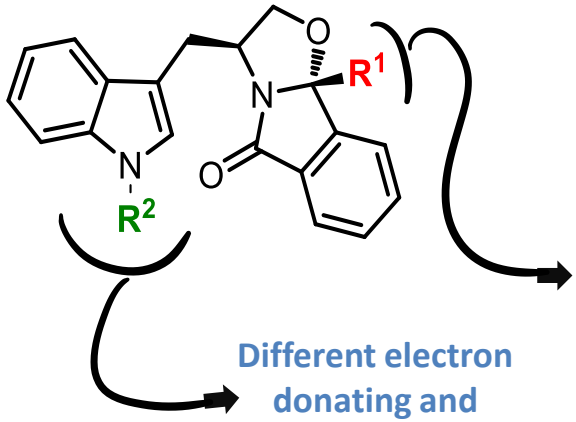
- Selective for HCT116 p53$^{+/+}$ and MDA-MB-231 cells
- induces cell cycle arrest in HCT116 p53$^{+/+}$ and MDA-MB-231 cells
- induces apoptosis in HCT116 p53$^{+/+}$ and MDA-MB-231 cells
- Not cytotoxic against wt p53-expressing normal MCF-10A cells
- Increased expression levels of several p53 target genes
- Has potent in vivo antitumor activity

potently suppresses the growth of wt/mut p53-expressing tumours, but not of p53-null tumours, in xenograft mice models



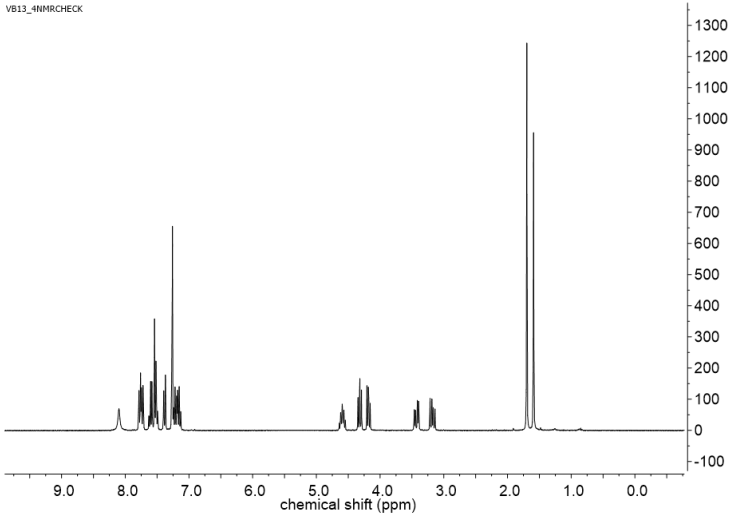
iMed. ULisboa
Instituto de Investigação do Medicamento

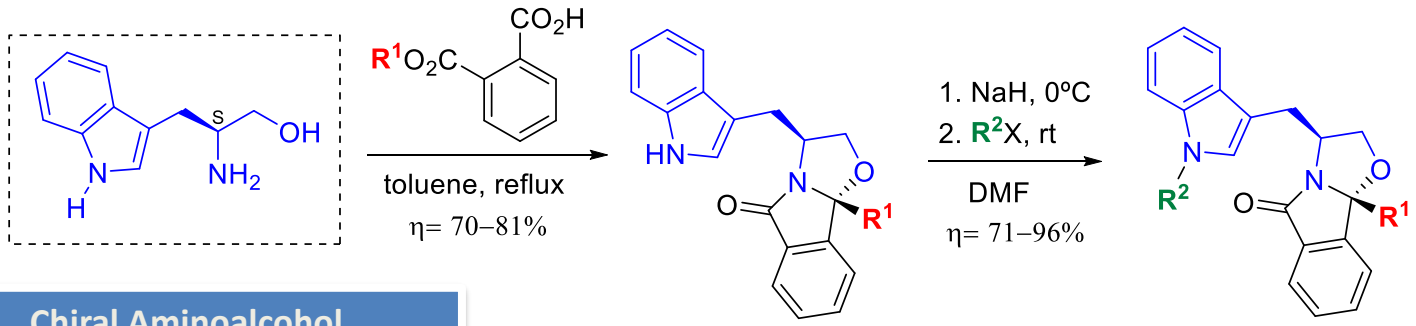
Synthesis of chemical library



Alkyl and aromatic groups at position 9b

Different electron donating and withdrawing groups inserted





Chiral Aminoalcohol
Source of chirality

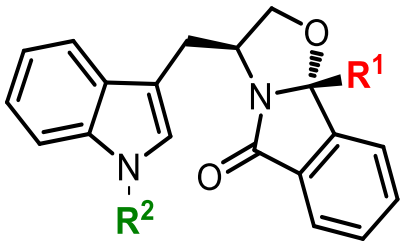
34 compounds; both enantiomers

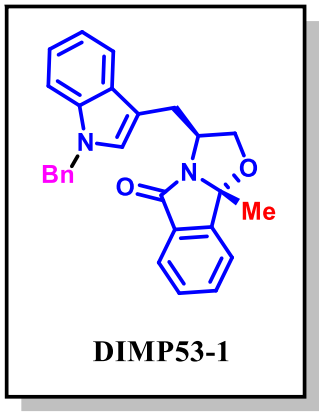
Structure-activity relationship studies

34 compounds evaluated in HCT116 cells

2 compounds with GI_{50} lower than SLMP53.1 in HCT 116 p53 $^{(+/+)}$ (15.5 μM) selective for p53

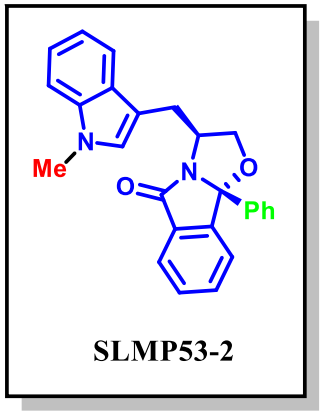
(*S*)-Tryptophanol-derived compounds are more active than the corresponding enantiomers





DIMP53-1

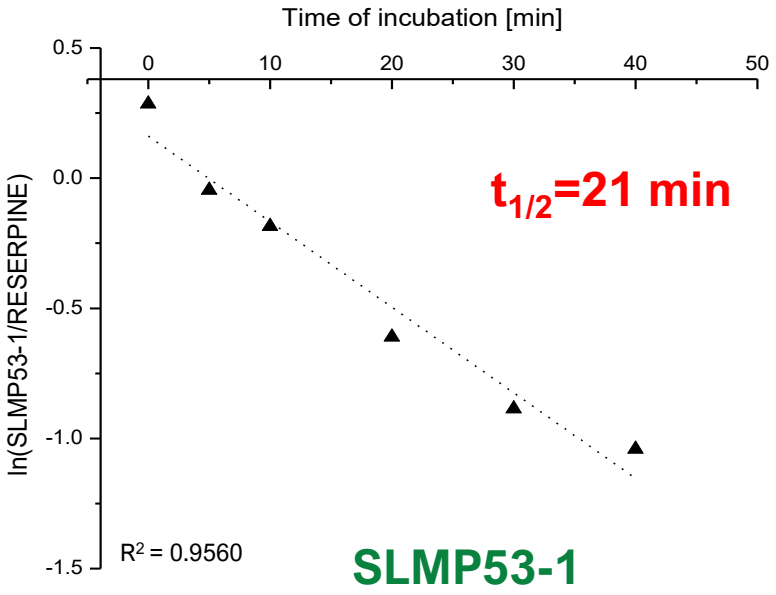
GI_{50} HCT116 p53$^{+/+}$ = 7.4 μM
GI_{50} HCT116 p53$^{-/-}$ = 17.3 μM

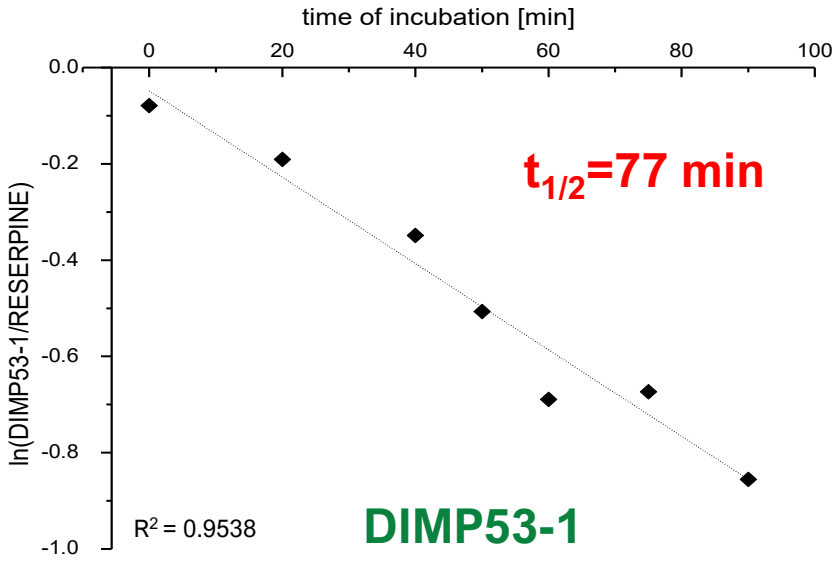


SLMP53-2

GI_{50} HCT116 p53$^{+/+}$ = 8.4 μM
GI_{50} HCT116 p53$^{-/-}$ = 17.7 μM

Stability Studies in human microsomes



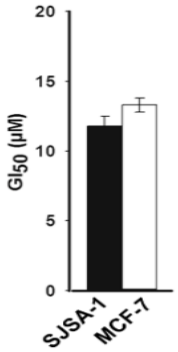


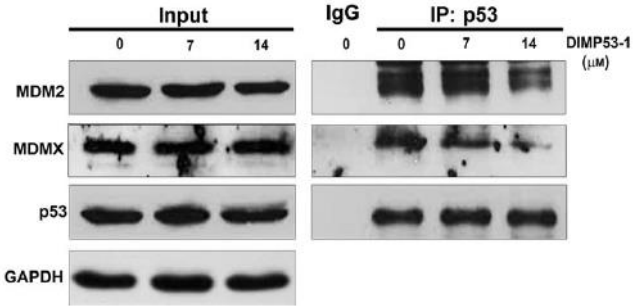
The $t_{1/2}$ of DIMP53-1 increased compared to the hit compound SLMP53.1

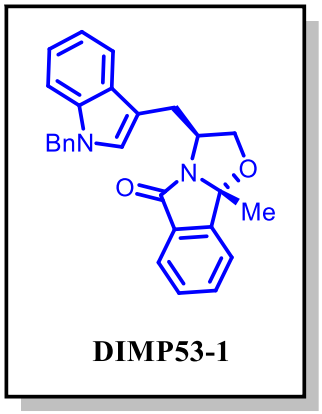
DIMP53-1 blocks the p53-MDM2/X PPIs

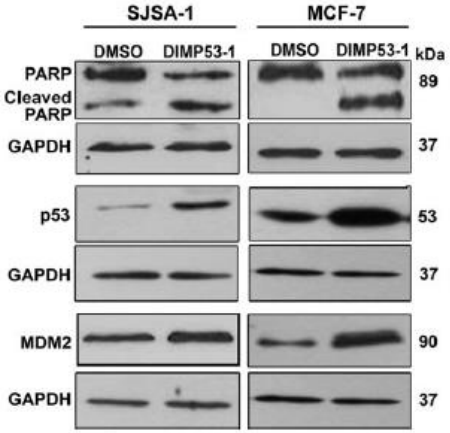
Molecular Oncology **2017**, 11, 6, 612

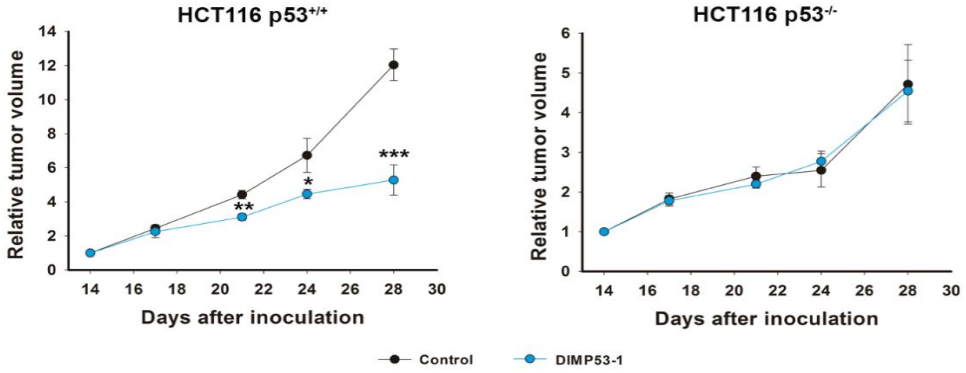
- induces cell cycle arrest in HCT116 p53$^{+/+}$, SJSA-1 and MCF-7 cells
- induces apoptosis in HCT116 p53$^{+/+}$, SJSA-1 and MCF-7 cells
- Increased expression levels of several p53 target genes







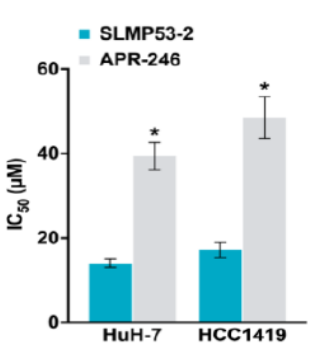


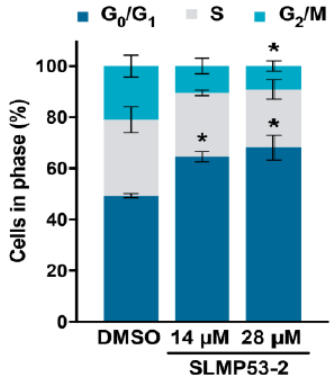


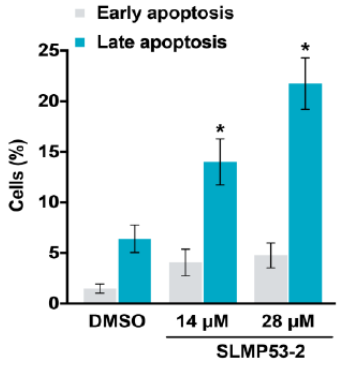
iMed. ULisboa
Instituto de Investigação do Medicamento

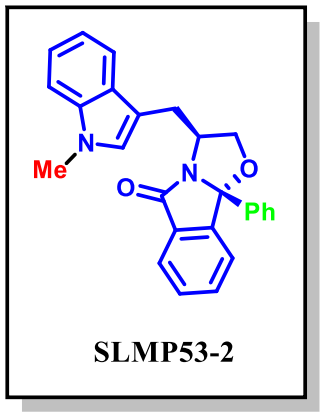
Restores wt-like function to mutp53-Y220C

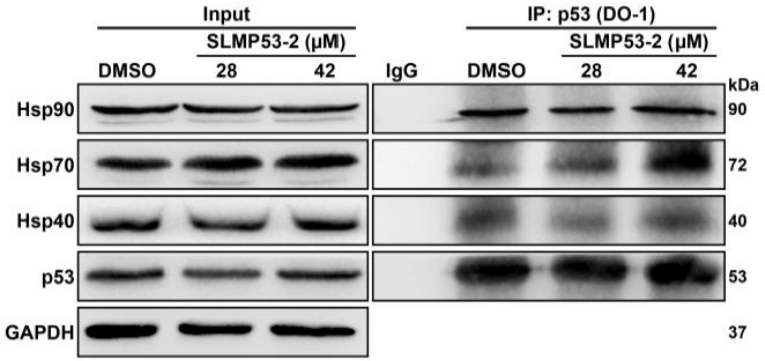
- Leads to growth inhibition of mutp53-Y220C-expressing HCC cells
- Induces cell cycle arrest and apoptosis in HuH-7 cells







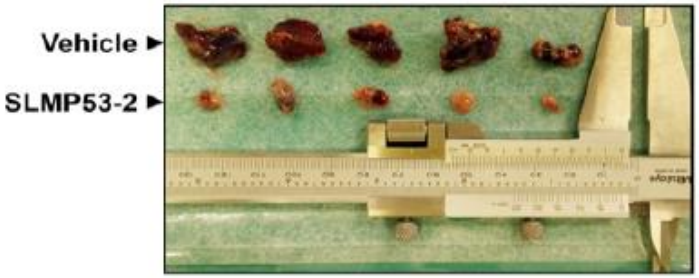




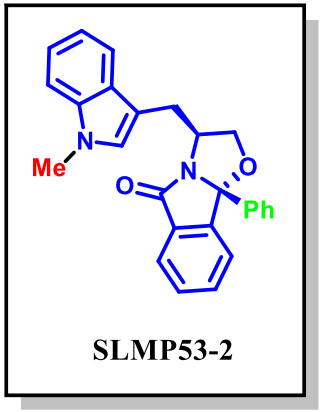
Restores wild-type-like conformation and DNA-binding ability of mutp53-Y220C leading to the reestablishment of p53 transcriptional activity

Cancers **2019**, 11, 1151

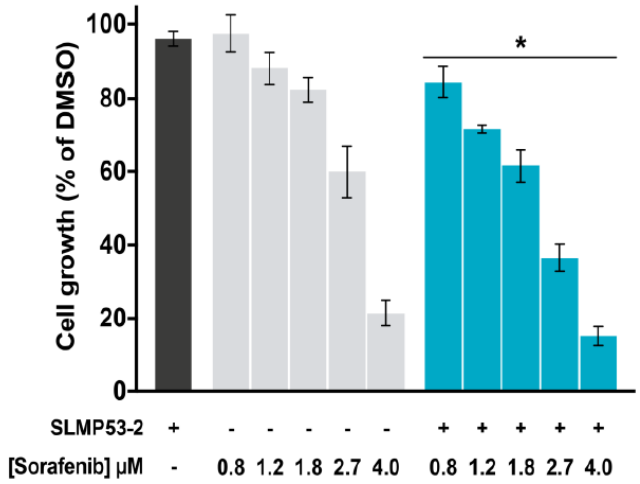
Potent antitumor activity

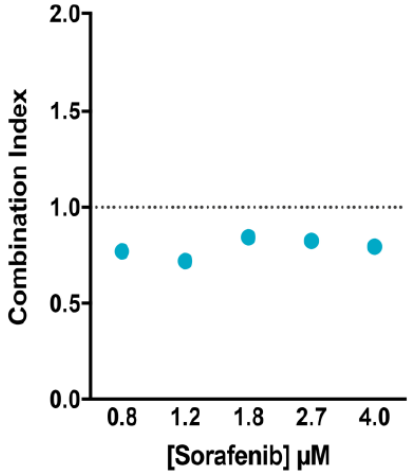


Potent antitumor activity in human HCC xenograft mice models



SLMP53-2

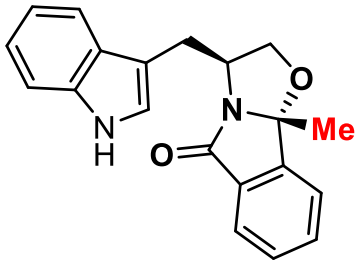




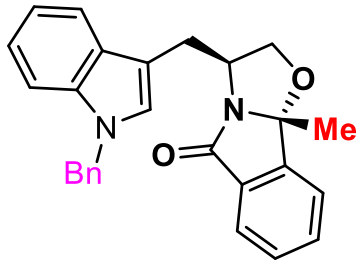
Has synergistic effect with sorafenib

Cancers **2019**, 11, 1151

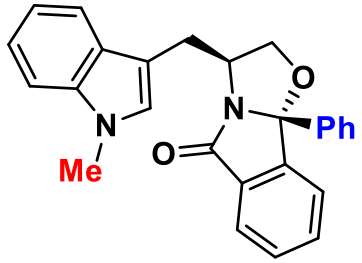
Conclusions



reactivator of wt p53 and mut p53R280K



p53-MDM2/X interactions dual inhibitor



reactivator of mut p53Y220C

- ✓ **Together, the results obtained in HCT116 tumor cells indicate that tryptophanol-derived oxazoloisoindolinones reactivate p53, subsequently increasing the expression levels of p53 target genes**
- ✓ **These compounds represent promising lead structures for the development of novel antitumor agents.**

iMed. ULisboa
Instituto de Investigação do Medicamento

Acknowledgments

ULisboa, Lisboa
Valentina Barcherini
Margarida Espadinha
Elizabeth Lopes
Nuno Pereira
Ângelo Monteiro
Daniel Santos
Alexandra Antunes

i3S, Porto
João Brás
Maria Almeida

CIBIO, Trento
Alberto Inga
Bartolomeo Bosco

MRC, Cambridge
Matthias Bauer
Alan R. Fersht

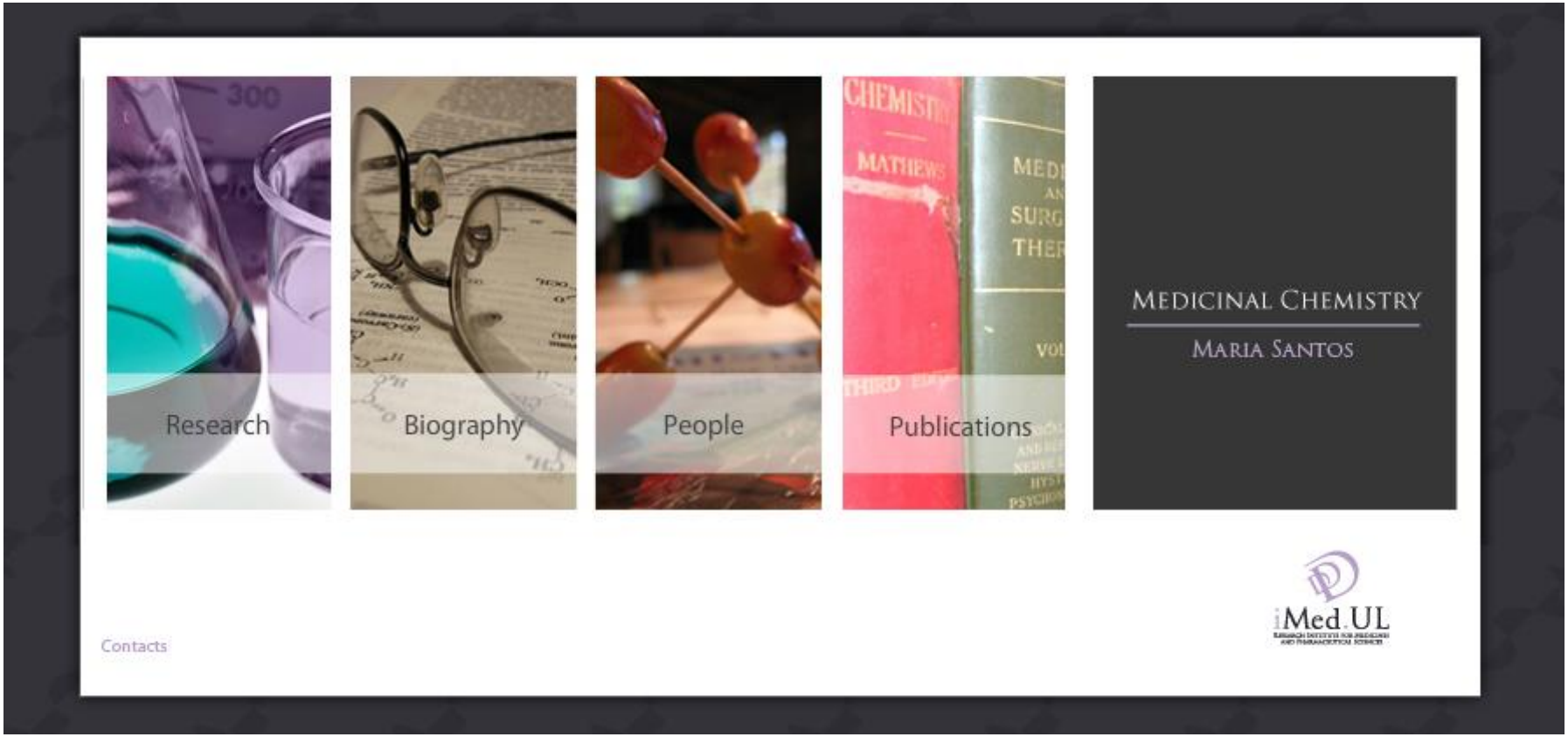
REQUIMTE, Porto
Lucília Saraiva
Sara Gomes
Joana Soares
Liliana Raimundo
Joana Loureiro
Helena Ramos

IBILI, Coimbra
Célia Gomes
Flávio Reis

CEB, Braga
Lucília Domingues
Carla Oliveira

UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional
COMPETE
PROGRAMA OPERACIONAL FACTORES DE COMPETITIVIDADE
FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
QREN
QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL
PORTUGAL 2007.2013

Projects and grants:
PTDC/DTP-FTO/1981/2014; PTDC/QUI-QOR/29664/2017; UID/DTP/04138/2019; UID/QUI/00100/2019; UID/QUI/50006/2019; CEECIND/02001/2017 (A. M. M. Antunes); CEECIND/01772/2017 (M. M. M. Santos); PD/BD/143126/2019 (V. Barcherini); SFRH/BD/96189/2013 (S. Gomes); SFRH/BD/117931/2016 (M. Espadinha)



e-mail: mariasantos@ff.ulisboa.pt
webpage: www.ff.ul.pt/~mariasantos
twitter: @SantosMMM_MChem