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Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

From Multitarget Directed Ligands to Molecular Degraders:

The Contribution of the University of Perugia to Spoke 8

Antimo Gioiello

September 9, 2025

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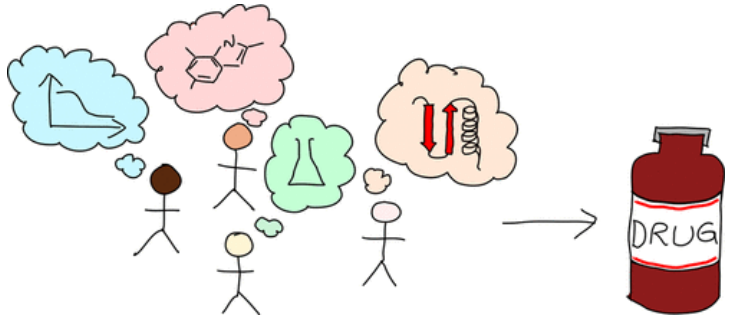
A multidisciplinary collaboration to foster preclinical drug discovery



UNIPG	UNIURB	External collaborators
Antimo Gioiello (CHEM-07/A)	**Giovanni Bottegoni (CHEM-07/A)**	Stefano Di Bona (Molecular Horizon, Italy)
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Andrea Carotti (CHEM-07/A)	Giovanni Piersanti (CHEM-05/A)	Daniela Passeri (Tes Pharma, Italy)
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Laura Goracci (MEDS-08/C)		
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Ciriana Orabona (BIOS-11/A)		
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WP1: Development of a third party accessible, enabling platform for efficient preclinical drug discovery

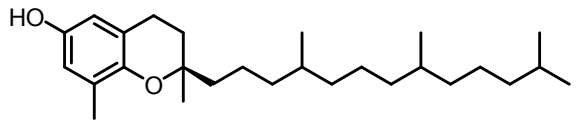
UNIPG Projects

Title: Discovery and characterization of Vitamin E derivatives as multitarget modulators for the treatment of chronic neurological diseases and cancer

PI: Antimo Gioiello (Dept. Pharmaceutical Sciences)

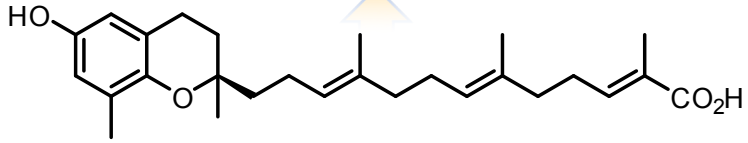
Title: Rationale for targeting unconventional IDO1 activity in cancer: Design and validation of IDO1 degraders

PI: Ciriana Orabona (Dept. Medicine and Surgery)



Vitamin E
(α-Tocopherol)

Hepatic ω-oxidations



Garcinoic acid
(trans-13′-Carboxy-δ-tocotrienol)

Garcinia kola

- Deficiency of **vitamin E** causes dysfunctional immune responses and degenerative diseases (e.g., atherosclerosis, Alzheimer disease) - *JAMA* **2014**, *311*, 33.
- Dietary intake of vitamin E prevent **NAFLD**, particularly in individuals devoid of hyperlipidemia - *J Gastroenterol Hepatol* **2021**, *36,* 311.

Garcinoic acid is a vitamin E derivative and a major component extract of *Garcinia Kola*

- ***Garcinia kola*** is traditionally used in African medicine by people who believe that it has purgative, antiparasitic, and antimicrobial properties.
- The seeds are used for treating bronchitis, throat infections, colic, head or chest colds, cough and **liver disorders**.

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Biological activity of Garcinoic acid

Compared to other LCM Vitamin E derivatives, there is only a limited number of studies on garcinoic acid. The major biological effects/activity include:

- **Antioxidant and anticancer**
- Improves **heart function** in myocardial infarction
- Exerts **anti-inflammatory** activity
- Reduces deposition of Ab particles in brain in mouse model of **Alzheimer's disease**

NB: Human clinical studies of GA are not yet available.

Disease/model	Animal/organ	Efficacious dose/mode of administration	Positive control	Major findings	References
Cardiovascular disorders					
Antiatherosclerotic effect	High fat diet fed ApoE $^{-/-}$ mice	1 mg/kg i.p	N/A	GA diminished lipopolysaccharide-induced upregulation of iNOS and COX-2 expression, decreased intra-plaque inflammation and changed NK and CD4 positive cell populations	Wallert et al. (2019)
Inflammation and pain					
Carrageenan-induced oedema model	Rats	50 mg/kg (N/A)	Indomethacin (10 mg/kg)	GA showed anti-inflammatory activity at comparable level to positive control	Tchimene et al. (2015a)
Anaesthesia	Guinea pigs	0.33, 0.66, 1.00 mg/kg i.d	Xylocaine (0.33, 0.66, 1.00 mg/kg)	GA induced local anaesthesia at comparable levels to xylocaine	Tchimene et al. (2015b)
Suppression of SARS-CoV-2 spike glycoprotein S1-induced hyper-inflammation	Human PBMC cells	0.5, 1.1, 2.1 µg/mL	N/A	GA reduced SARS-CoV-2 spike protein S1-induced secretion of TNFα, IL-6, IL-1β, and IL-8 in PBMCs	Olajide et al. (2021)
Cancer					
Brain	Glioma C6 cancer cells	4.3 µg/mL	N/A	GA showed in vitro antiproliferative effect on glioma C6 cancer cells	Mazzini et al. (2009)
Central nervous system					
Alzheimer's disease	mice	5, 10, 25 mg/kg p.o	N/A	GA reduced Aβ aggregation and accumulation in mouse cortical astrocytes	Marinelli et al. (2020)
In vitro antibacterial activity	*Porphyromonas gingivalis*	MIC = 13.4 µg/mL	N/A	GA exhibited antimicrobial activity against both of these microorganisms	Hioki et al. (2020)
	Streptococcus sobrinus	MIC = 13.4 µg/mL	N/A		

p.o. orally, *i.p.* intraperitoneally, *i.d.* intradermal application

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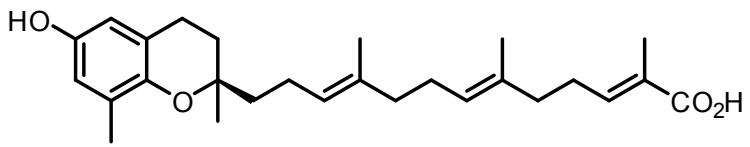
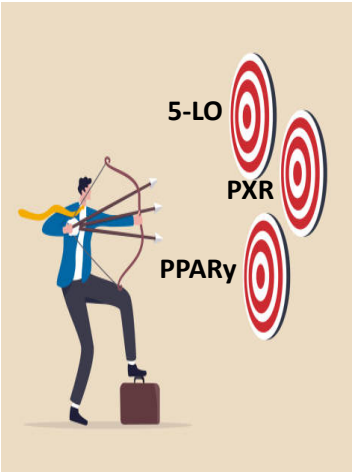


Garcinoic acid
(trans-13'-carboxy-δ-tocotrienol)

Garcinoic acid is a multitarget modulator

- Inhibitor of 5-lipoxygenase (**5-LO**) - Lorkowski *et al. Redox Biol* ***2019***, 24, 101166.
- Agonist of the Pregnane X receptor (**PXR**) - Gioiello, Galli *et al. J Med Chem* **2020**, *63*, 3701.
- Agonist of the Peroxisome Proliferator-activate receptor g (**PPARγ**) - Merk, Knapp *et al. Cell Chem Biol* **2021**, *28*, 1489.



Project AIMS:

A. Investigate the pharmacological profile of garcinoic acid in CNS, cancer and metabolic diseases

B. Develop novel semi-synthetic garcinoic acid analogs with improved properties, biological activity and metabolism

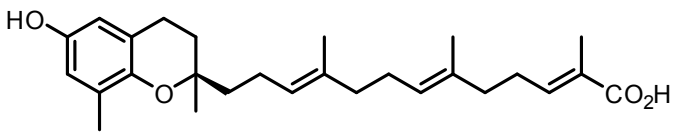
C. Discover new synthetic ('mimicking') compounds using enabling platforms (e.g., flow chemistry)

➢ Set-up an efficient method of extraction from seeds

Garcinia kola

1.2 Kg > 8.5 g (ca. 0.7%)

Extraction



Garcinoic acid

Naviglio extractor

Beyond the preparation of novel GA derivatives, the isolated natural product has been used to investigate its pharmacological profile in CNS, cancer and metabolic diseases:

- 'Garcinoic acid prevents β-amyloid (Aβ) deposition in the mouse brain' - *J. Biol. Chem.* **2020**, *295*, 11866.
- 'Anticancer effect of the vitamin E metabolite garcinoic acid in mouse models of Her2/neu positive breast cancer' - *Free Rad. Biol. Med.* **2022**, 189, 25.
- 'Garcinoic acid enhances inflammation resolution against colitis by activating Nrf2 dependent efferocytosis' - *Free Rad. Biol. Med.* **2025**, *237*, 37.
- 'Nanoparticles containing alpha-13'-COOH and garcinoic acid selectively target macrophages and improve inflammatory response' *manuscript in preparation*

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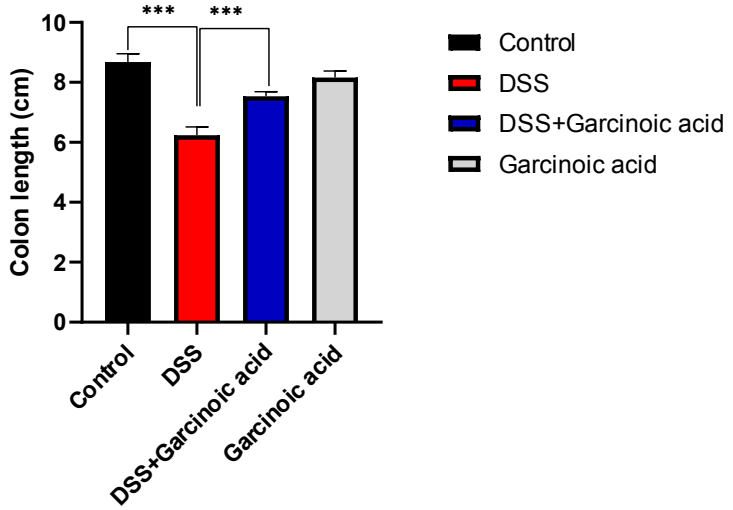
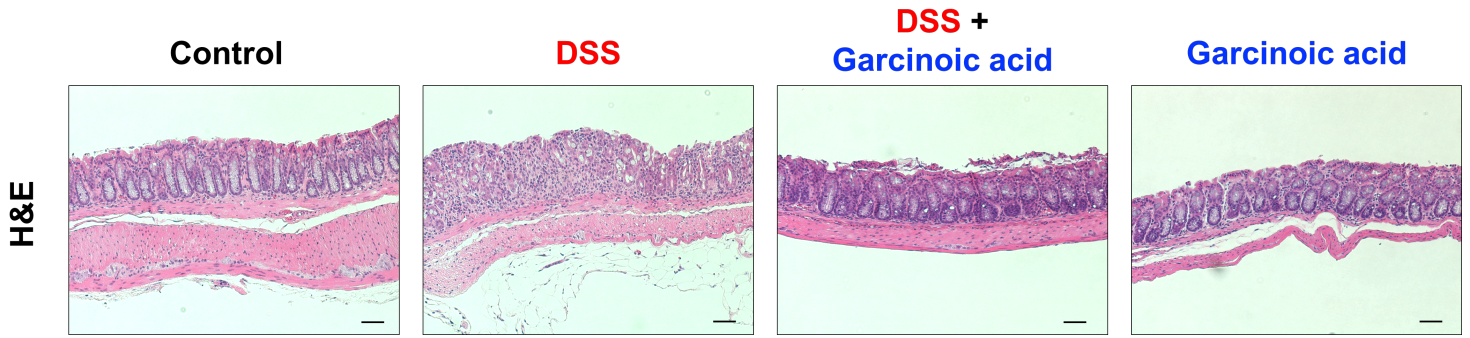
Garcinoic acid attenuates experimentally induced colitis





- Pre-administration of GA prevented the reduction of colon length (and body weight) in mice receiving DSS.
- Histological evaluation of the distal colon evidenced that DSS exposure resulted in epithelial degeneration, inflammatory cell infiltration and crypt loss, leading to colitis and impaired colonic tissue integrity.
- In contrast, the beneficial effect of GA restored this structure.

Mice received 2.0% DSS in drinking water for 7 days to induce colitis. GA was administered intraperitoneally at a concentration of 1 mg/kg starting 7 days before. *DSS: Dextran Sodium Sulfate*

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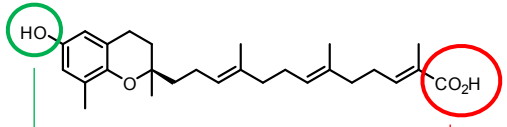
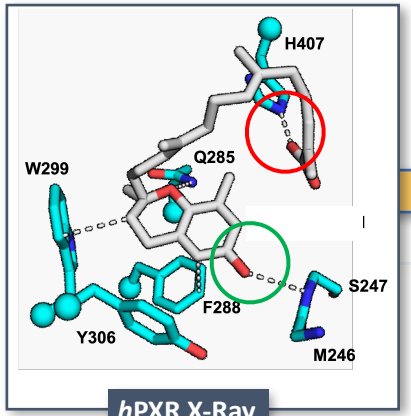


Garcinoic acid

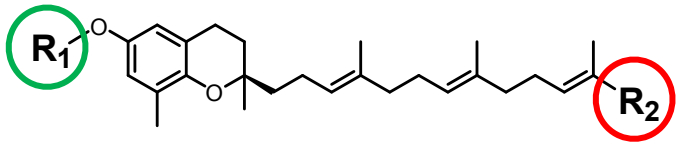
Key hot-spots

PXR activity
Off-target (selectivity): PPARγ, 5-LO
Metabolism



*h*PXR X-Ray

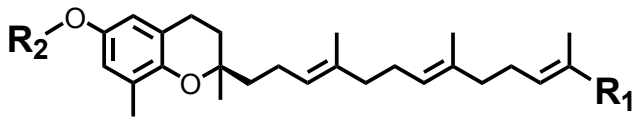
Lead optimization

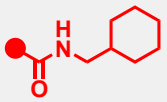
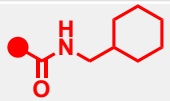
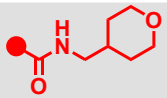
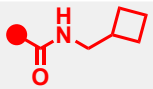


26 Novel Derivatives

R_1	R_2								
UPF-2724	UPF-2711	UPF-2730	UPF-2799	UPF-2858
UPF-2764	UPF-2725	UPF-2712	UPF-2797	UPF-2859
UPF-2763	UPF-2762	UPF-2767	UPF-2806	UPF-2860
UPF-2765	UPF-2731	UPF-2796	UPF-2804	UPF-2861
UPF-2766	UPF-2732	UPF-2798	UPF-2805	UPF-2862
UPF-2808				

Best molecules (so far…) to be further characterized



Compound Index	R1	R2	PXR (μM) (AlphaScreen, LanthaScreen)	PPARγ (AlphaScreen)	Citotoxicity (HepG2, μM)	HLM Stability (min)	Solubility (μM)
Garcinoic acid	**-H**	**-CO_2H**	1.3 (103%)	Mixed	135	33.7	24
UPF-2799	**-H**		8 (40%) 0.88	Allosteric	>300	161	< LOQ
UPF-2766		**-CO_2H**	>100 (IC_{50}= 2.8)	Mixed	>300	42	185.3
UPF-2858	**Na**		5.6 (65%)	Allosteric	>300	t.b.d.	t.b.d.
UPF-2860	**-H**		5.2 (60%)	Allosteric	258	t.b.d.	t.b.d.
UPF-2861	**-H**		3 (80%)	Allosteric	>300	t.b.d.	t.b.d.

Project activities (State-of-the-art)

A | Set-up an efficient extraction method of garcinoic acid from seeds ✓

(Gioiello, Cerra)

B | Development of novel garcinoic acid derivatives

- ✓ - Design and synthesis *(Gioiello, Cerra)*
- ✓ - Target screening assays (AlphaScreen, LantaScreen, cellular) *(Passeri, Tes Pharma)*
- ongoing - Computational analysis *(Bottegoni, Coricello)*
- ✓ - Gene expression and toxicity (HepG2) of selected molecules *(Galli, Bartolini, Passeri, Tes Pharma)*
- ongoing - Metabolic and solubility test *(Goracci)*
- Biophysical study (PPARγ) *(Macchiarulo, Bianconi)*

C | Profiling and in vivo evaluation of selected molecules

- ongoing - Activity at 5-LO *(Passeri, Tes Pharma)*
- Off-targets and gene expression profile *(Galli, Bartolini, Passeri, Tes Pharma)*
- Additional toxicity tests (endothelial cells (HUVEC), glial cells (HMC-3), primary astrocytes) *(Galli, Bartolini)*
- Assays on co-culture models and organoids *(Galli, Zelante, Bartolini)*
- Evaluation on mouse models of intestinal diseases *(Zelante, Galli)*

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WP1: Development of a third party accessible, enabling platform for efficient preclinical drug discovery

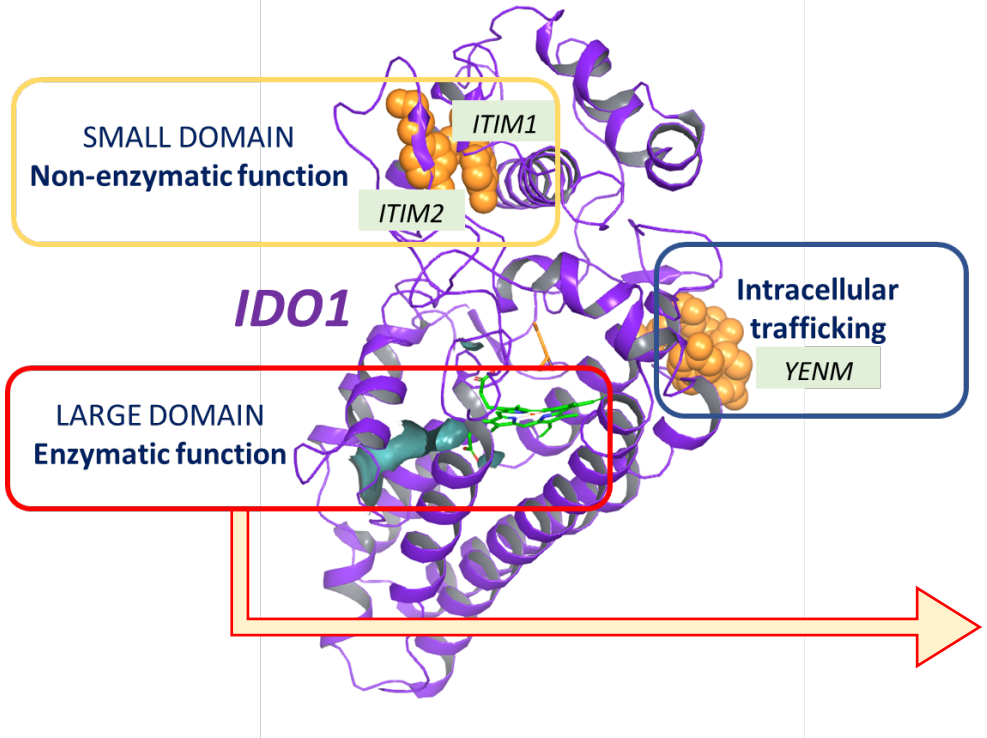
UNIPG Projects

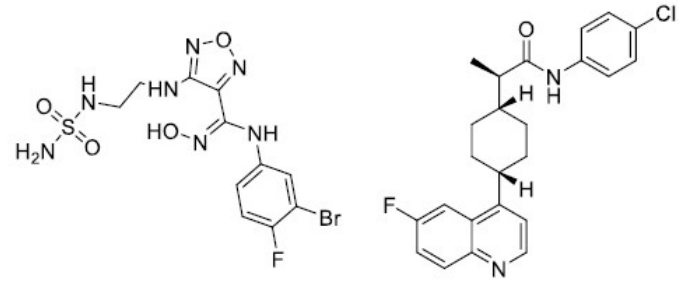
Title: Discovery and characterization of Vitamin E derivatives as multitarget modulators for the treatment of chronic neurological diseases and cancer

PI: Antimo Gioiello

Title: Rationale for targeting unconventional IDO1 activity in cancer: Design and validation of IDO1 degraders

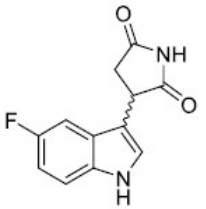
PI: Ciriana Orabona



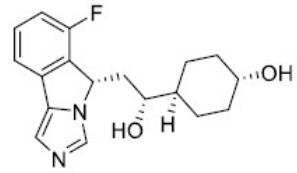


Epacadostat (Phase 3)
IDO1 IC_{50} = 73 nM
HeLa IDO1 IC_{50} = 7.4 nM

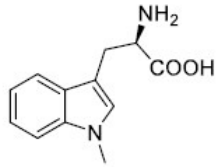
BMS-986205 (Phase 3)
IDO1 IC_{50} = 0.5 nM



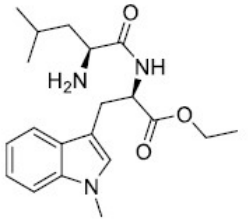
PF-06840003 (Phase 1)
IDO1 IC_{50} = 0.41 μM
HeLa IDO1 IC_{50} = 1.8 μM



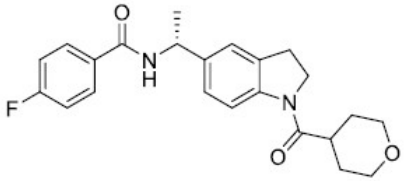
Navoximod (Phase 1)
IDO1 IC_{50} = 28 nM



Indoximod (Phase 3)
IDO1 K_i = 34 μM



NLG802 (Phase 1)

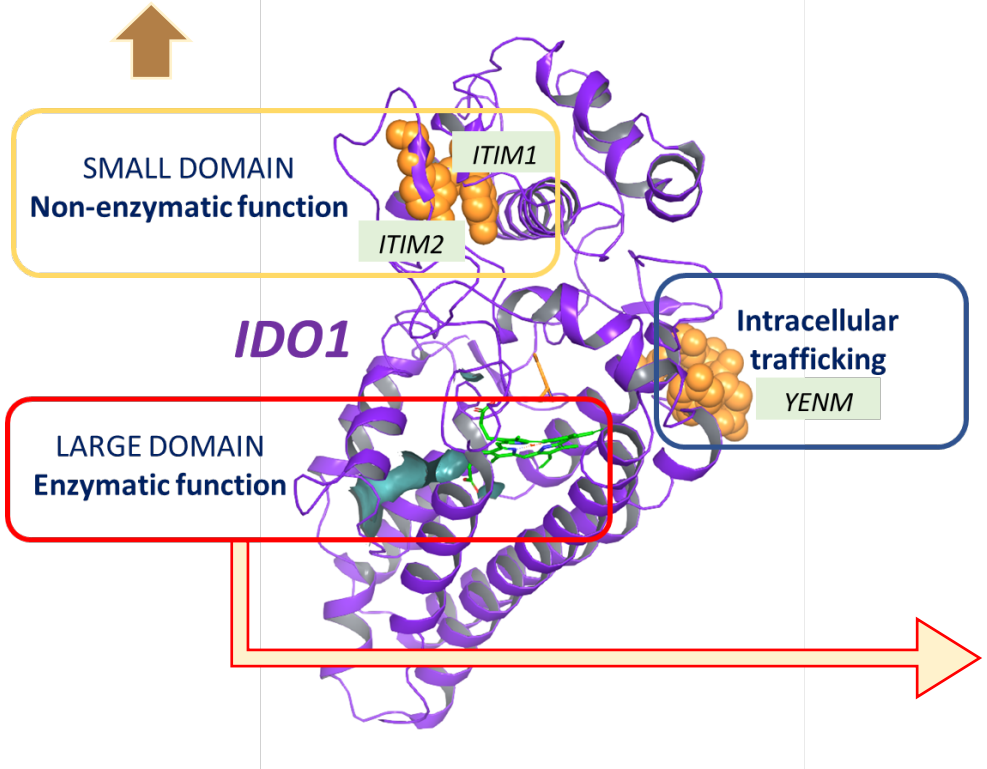


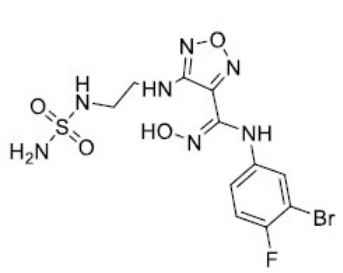
LY3381916 (Phase 1)
IDO1 IC_{50} = 7 nM

IDO1 catalytic inhibitors failed as anti-cancer drugs

A NOVEL APPROACH

IDO1-mediated signaling pathways that promotes tumor proliferation





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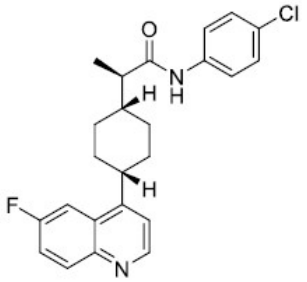
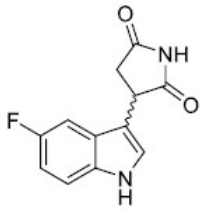


BMS-986205 (Phase 3)
IDO1 IC_{50} = 0.5 nM



PF-06840003 (Phase 1)
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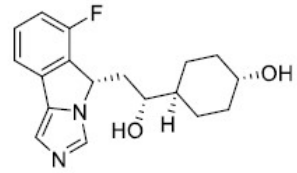

Navoximod (Phase 1)
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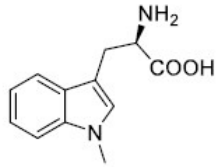
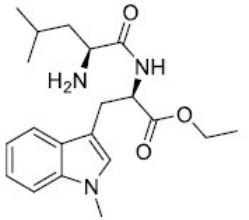
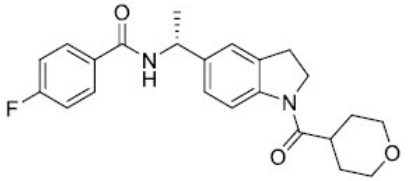


Indoximod (Phase 3)
IDO1 K_i = 34 μM



NLG802 (Phase 1)



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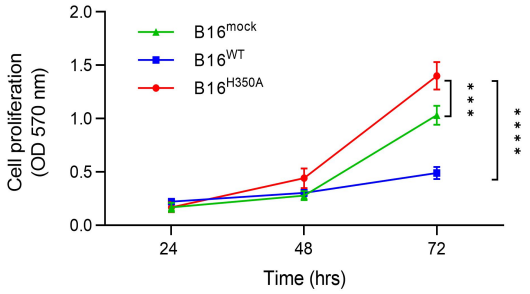
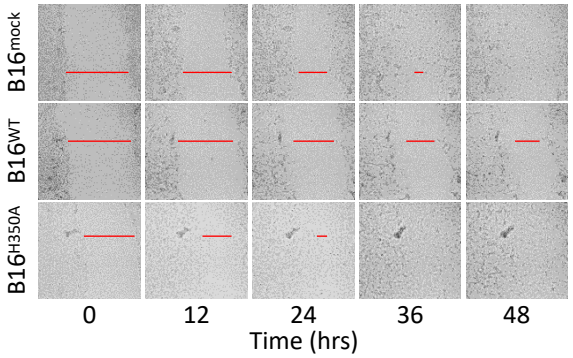
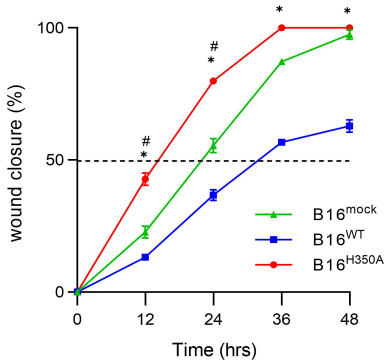
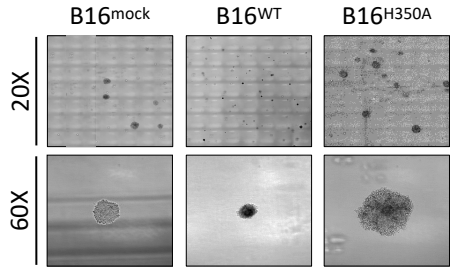
The non-enzymatic IDO1 confers a pro-tumorigenic phenotype to B16 melanoma cells

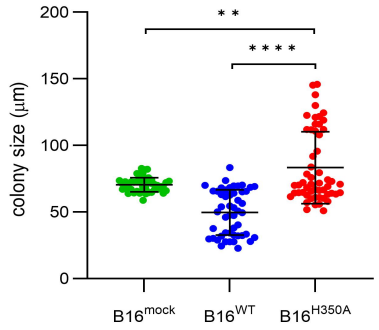
In vitro tumor growth and migration

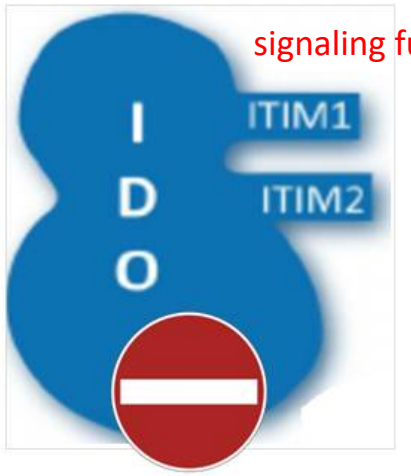








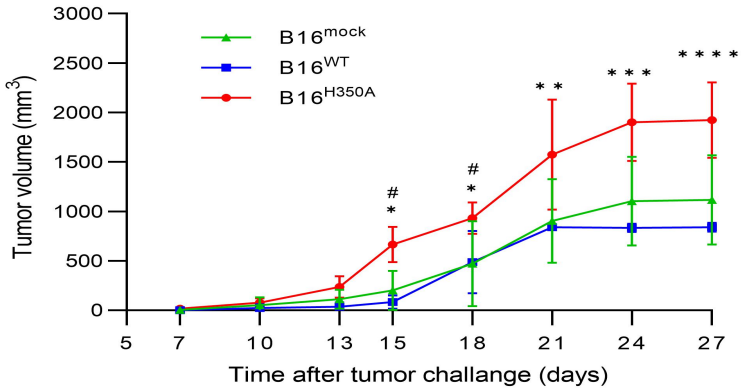


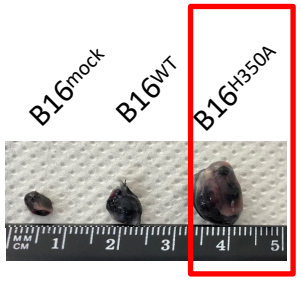


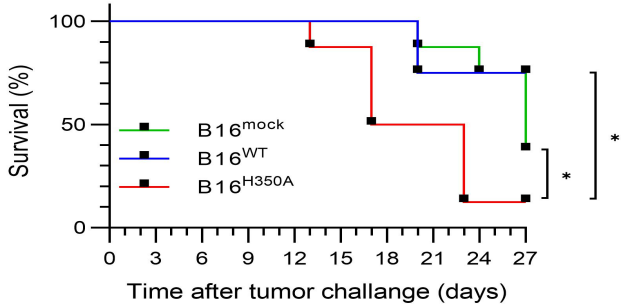
signaling function

catalytic function

In vivo tumor growth and survival



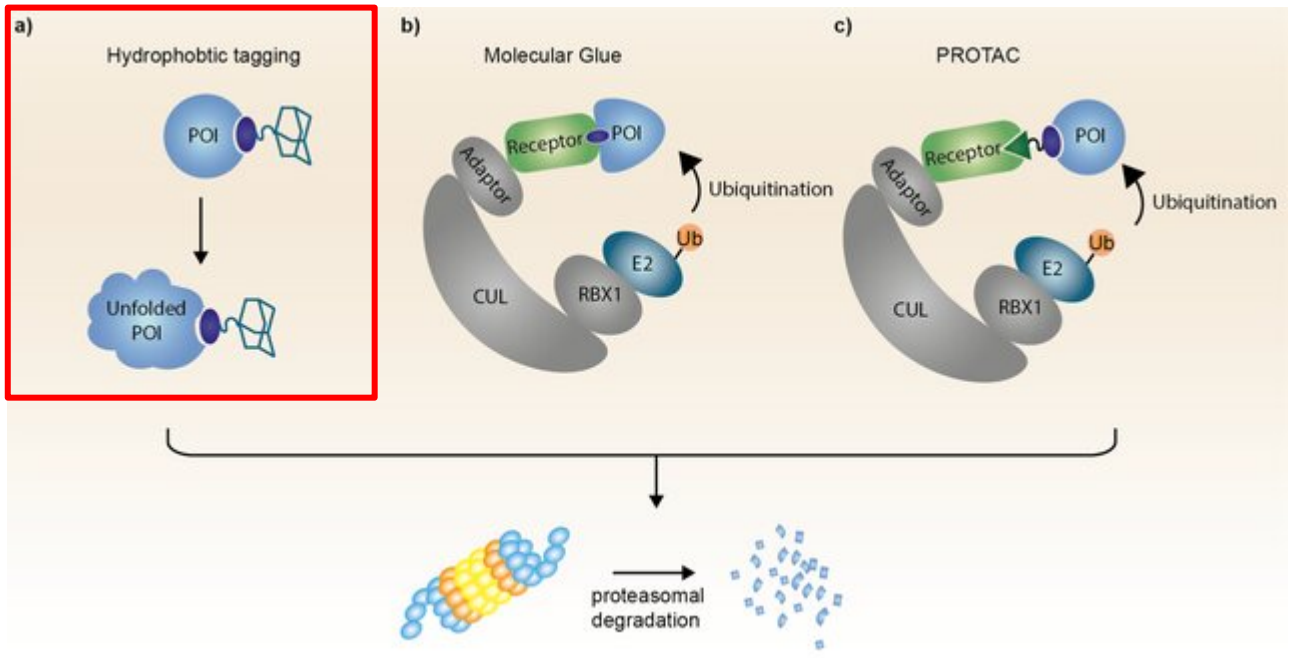




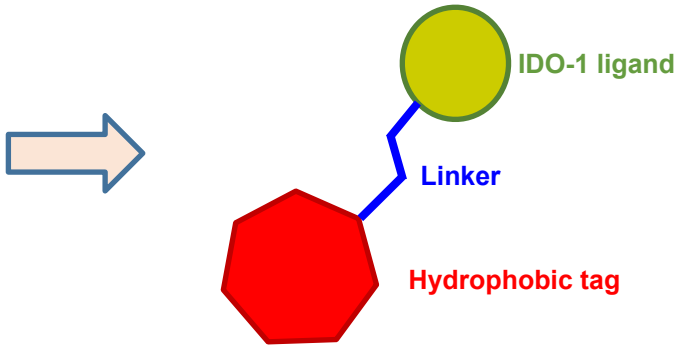
Orecchini E., Orabona, C. *et al., Oncoimmunology* **2023**

Design and development of innovative drugs for IDO1 degradation

TARGETED PROTEIN DEGRADATION TECHNOLOGIES



Degraders by Hydrophobic Tagging



- Lower molecular weight
- Enhanced drug-like properties

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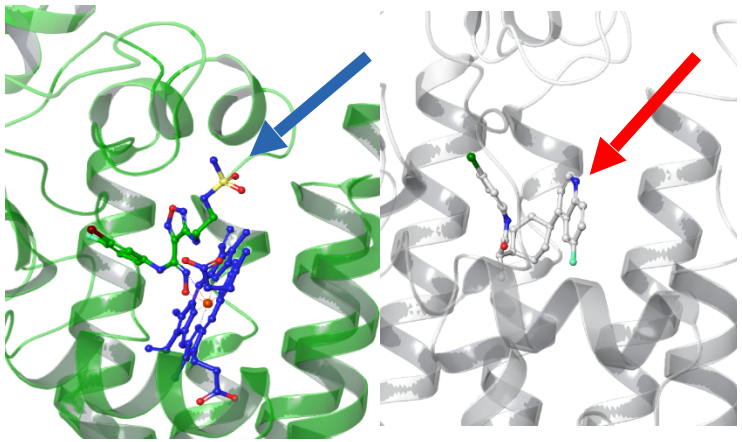
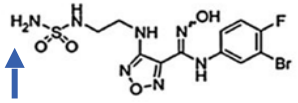
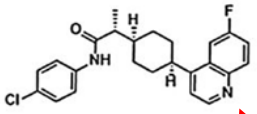
IDO1 hydrophobic tagging compounds: Drug design

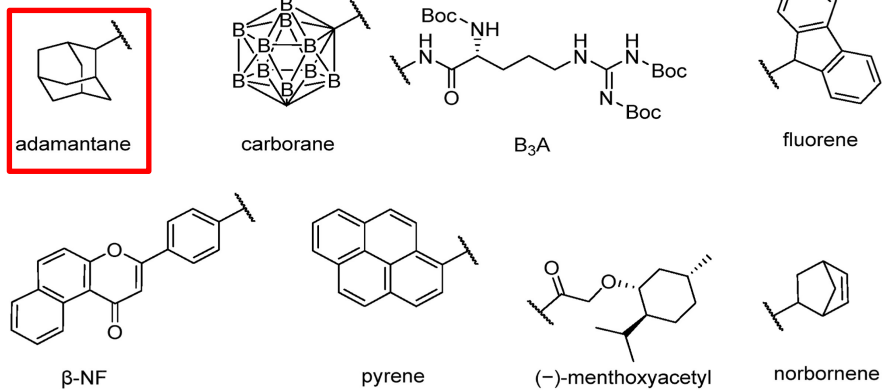


Features	Epacadostat	Linrodostat
PDB Code	6E40	6DPQ
Heme	Interaction	Displacement

IDO1 ligands

Incyte	BMS
Epacadostat	BMS-986205
INCB024360	*F001287*
	
Catalytic inhibitor	Suicide inhibitor
Tryptophan competitive	Irreversible
12 nmol/L	2 nmol/L
>100-fold	>100-fold
2012	2015
Phase III	Phase II
Epacadostat	Linrodostat

Hydrophobic Tags



Priority selection criteria

- ✓ Synthetic accessibility/feasibility
- ✓ Molecular modeling results (Docking/Molecular dynamics)
- ✓ PK/PD predicted profiles
- ✓ Intellectual Property

Putative regions to modify with hydrophobic tags in order to reach the protein surface and activate the proteasome degradation.

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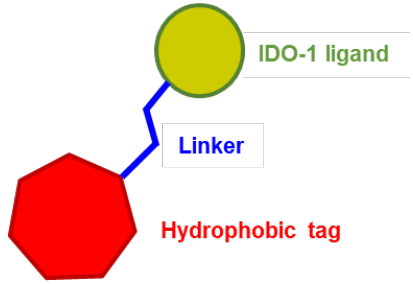
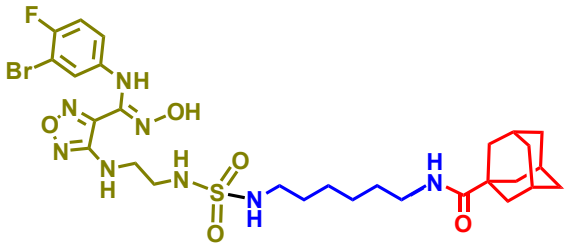
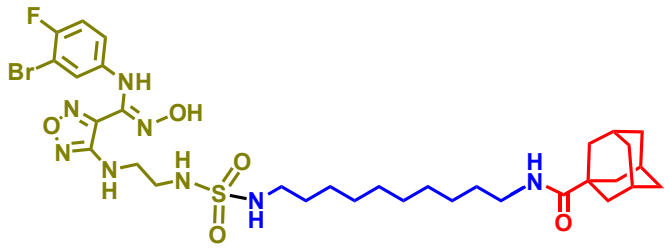
IDO1 hydrophobic tagging compounds: Synthesis

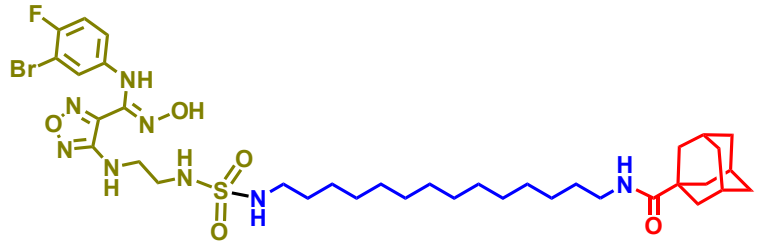




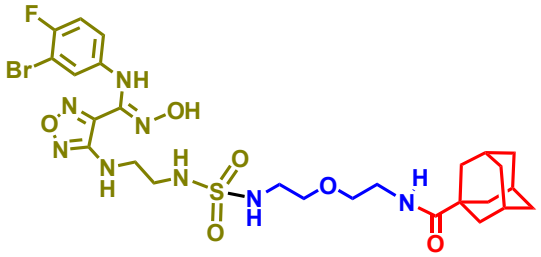
IDO1-Hytag1

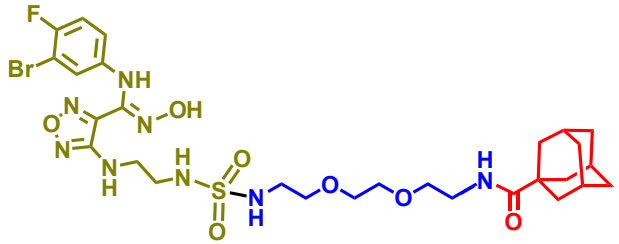


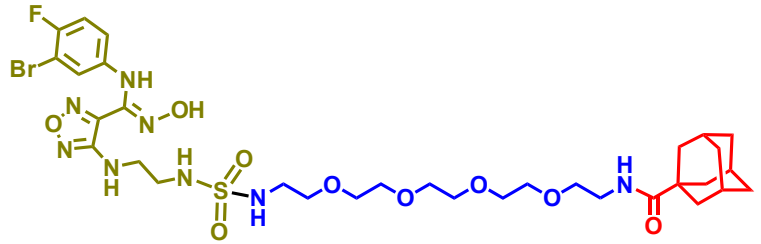
IDO1-Hytag2



IDO1-Hytag3







Project activities and state-of-the-art

A | **Characterization of IDO1-mediated signaling in tumor cells** *(Orabona)* ✅

B | **Development of of small molecules stabilizing IDO1 conformations suitable for the protein degradation** -

- Design and synthesis *(Gioiello, Carotti, Piersanti, Retini)* ongoing
- In vitro screening of IDO1 degraders *(Orabona)* ongoing
- Computational analysis *(Carotti)*
- Metabolic and solubility test *(Goracci)*

C | **Profiling and in vivo evaluation of selected IDO1 degraders**

- Biophysical PK/PD profiling of best IDO1 degraders *(Macchiarulo, Bianconi)*
- Scale-up optimization of preclinical candidates *(Gioiello, Piersanti, Retini)*
- In vivo validation of the best IDO1 degraders *(Orabona)*

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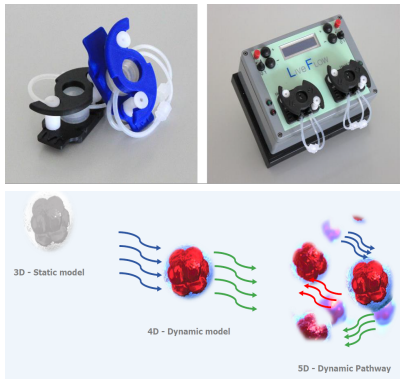
ADDITIONAL ONGOING COLLABORATIONS WITHIN SPOKE 8 NETWORK

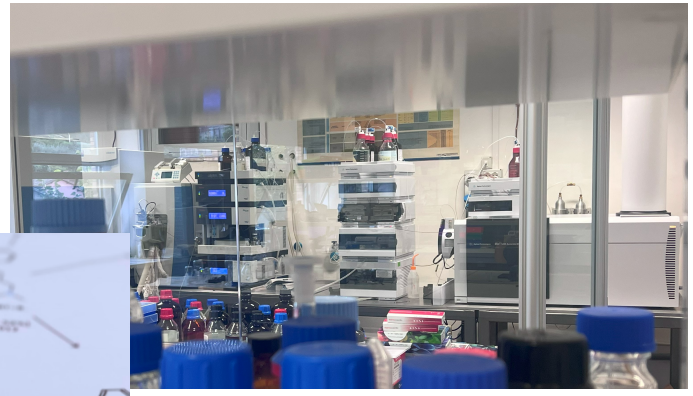
Orabona:

Screening of indole-based molecules on IDO1 enzymatic activity. (G. Favi and G. Mari – UniURB)

Screening of USP2 inhibitors on IDO1 protein stability (R. Crinelli – UniURB)



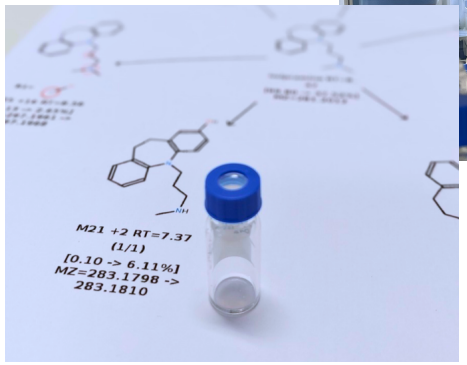
IVTech dynamic fluidic platform: 5D in-vitro models that mimicks a dynamic cell system for different applications, i.e., tissue cross modulation and drug effects.



Goracci:

Met-ID for I-152, a promising compound with antioxidant, anti-inflammatory, immunomodulatory and antiviral activities (G. Piersanti, F. Bartoccini, A. Paternale - UNIURB)

Kinetic solubility and chemical and Met-ID for antileishmania compounds (S. Lucarini, L. Galluzzi, G. Bottegoni - UNIURB)



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Discovery and characterization of Vitamin E derivatives as multitarget modulators for the treatment of chronic neurological diseases and cancer

Department of Pharmaceutical Sciences (UniPg)
Desiree Bartolini
Andrea Carotti
Bruno Cerra
Francesco Galli
Antonio Macchiarulo

Department of Medicine and Surgery (UniPg)
Prof. Teresa Zelante

Department of Chemistry, Biology and Biotechnology (UniPg)
Prof. Laura Goracci
Dr. Michela Eleuteri

Department of Biomolecular Science (UniUrb)
Prof. Giovanni Bottegoni
Adriana Coricello

Department of Biochemistry, Uskudar University (Turkey)
Prof. Nesrin K Ozer

Res. Institute of Pharmaceutical Sciences, Univ. Seoul (South Korea)
Prof. Seong Hoon Kim

Rationale for targeting unconventional IDO1 activity in cancer: Design and validation of IDO1 degraders

Department of Medicine and Surgery (UniPg)
Dr. Sofia Rossini
Dr. Sara Ambrosino
Prof. Maria Laura Belladonna
Prof. Maria Teresa Pallotta
Dr. Claudia Volpi
Dr. Eleonora Panfili
Dr. Chiara Suvieri

Department of Pharmaceutical Sciences (UniPg)
Prof. Antimo Gioiello
Prof. Andrea Carotti
Prof. Antonio Macchiarulo

Department of Biomolecular Science (UniUrb)
Prof. Giovanni Piersanti
Prof. Michele Retini