Nano Innovation 2016

Rome, 20-23 September

Virtual Screening of Nutraceutical Products

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SUMMARY

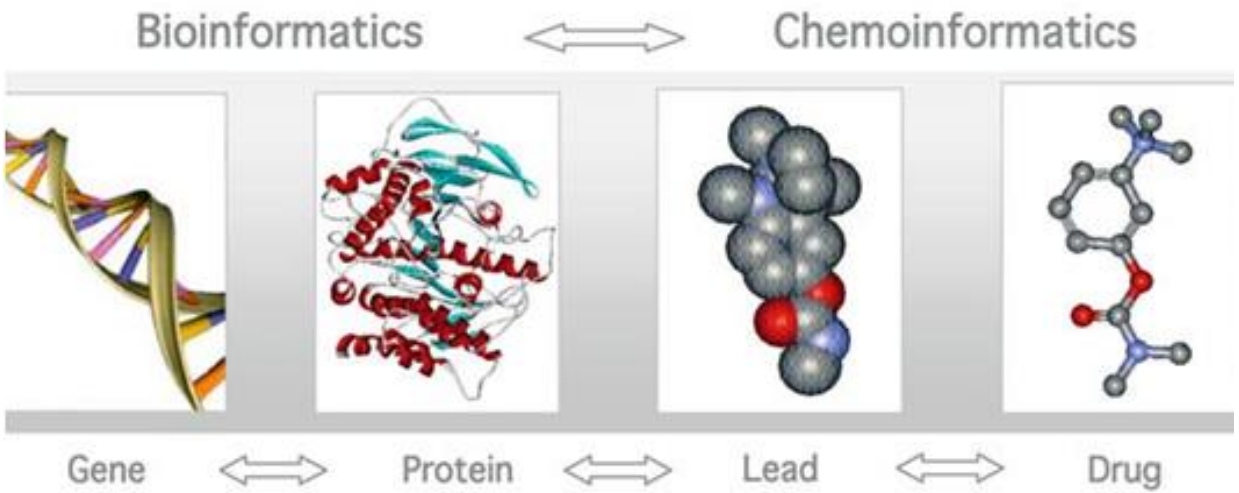
- INTRODUCTION
  - Drug discovery process
  - Nutraceuticals
  - Chemo Cataloging Process
  - Virtual Screening
- PURPOSE OF THE WORK
- MATERIALS AND METHODS
- RESULTS AND DISCUSSION
- CONCLUSION
- ACKNOWLEDGMENTS

Drug discovery process

Drug development is the process of bringing a new pharmaceutical drug to the market once a lead compound has been identified through the process of drug discovery.

[1]Newmann DJ, Cragg GM. *J Nat Prod* **2012**; *75*:311-

Bioinformatics and Chemoinformatics



- A strategy for the discovery of new bioactive compounds is the cooperation between chemoinformatics and bioinformatics.
- Scientific disciplines that have evolved in recent decades at the interface between chemistry, biology and computer science.

[2]Rollinger JM, Stuppner H, Langer T. *Prog Drug Res*. **2008**, 65, 213-49.

Nutraceuticals

Nutraceutical, a fusion of the words "**nutrition**" and "**pharmaceutical**", was coined in 1989 by Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine. The term is applied to products that range from isolated nutrients, dietary supplements and herbal products, specific diets and processed foods such as cereals, soups, and beverages.

Nutraceuticals are products derived from food sources that are supposed to **provide extra health benefits**, in addition to the basic nutritional value found in foods. Depending on the jurisdiction, products may claim to **prevent chronic diseases**, **improve health**, delay the aging process, **increase life expectancy**, or support the structure or function of the body

Nutraceuticals

September 2011

December 2013

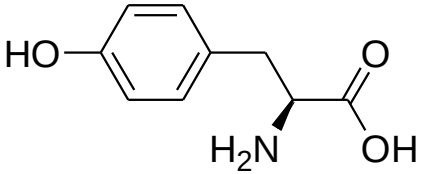
June 2014

September 2014

In University "Magna Græcia" of Catanzaro in recent years there is increasing discussion of nutraceuticals. There are several publicized events, to which also the group of Medicinal Chemistry (COI lab) took part

Chemo cataloging process

Systematic name	L-tyrosine
IUPAC name	(2S)-2-amino-3-(4-hydroxyphenyl)propanoic acid
Empirical formula	C9H11NO3
WNL	QVYZ1R DQ
Canonical SMILE	C1=CC(=CC=C1CC(C(=O)O)N)O
Isomeric SMILE	C1=CC(=CC=C1C[C@@H](C(=O)O)N)O
SLN	OHC(=O)CH(NH2)CH2C[1]=CHCH=C(OH)CH=CH@1
ROSDAL	1O-2=3O,2-4-5N,4-6-7=-12-7,10-13O
InChI	InChI=1S/C9H11NO3/c10-8(9(12)13)5-6-1-3-7(11)4-2-6/h1-4,8,11H,5,10H2,(H,12,13)/t8-/m0/s1
InChI Key	OUYCCCASQSFEME-QMMMGPOBSA-N
CAS RN	60-18-4



1D chemical structure of L-tyrosine with different line notations

The enormous increase in the number of compounds and related data led in the past decades to inefficient data-handling; the only way to fix this, was by electronic means using chemoinformatics

Chemo cataloging process

```
177
  -OEChem-09191510062D
  7  6  0     0  0  0  0  0  0999 V2000
    3.7320    0.5600    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
    2.0000    0.5600    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    2.8660    0.0600    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    2.3100    1.0969    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
    1.4631    0.8700    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
    1.6900    0.0231    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
    2.8660   -0.5600    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
  2  3  2  0  0  0  0
  2  7  1  0  0  0  0
  2  1  1  0  0  0  0
  1  4  1  0  0  0  0
  1  5  1  0  0  0  0
  1  6  1  0  0  0  0
M  END

> <PUBCHEM_IUPAC_TRADITIONAL_NAME>
acetaldehyde

$$$$
```

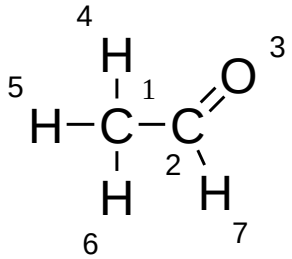
Header block

Counts line

Atom block

Bond block

Properties block

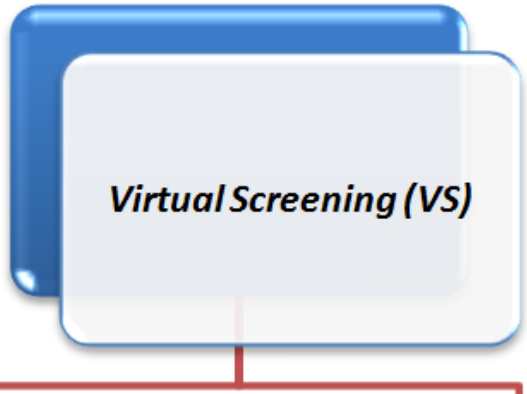


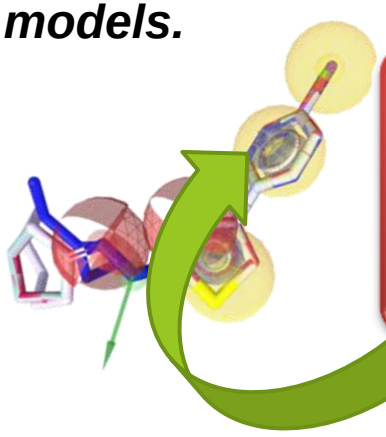
2D acetaldehyde chemical structure with a part of the SDfile

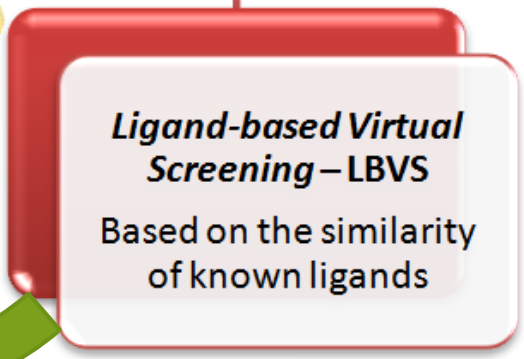
Virtual Screening. LBVS & SBVS

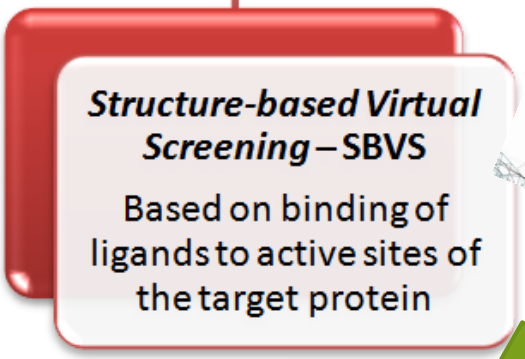
Identify molecules of novel chemical structure that bind to the macromolecular target of interest

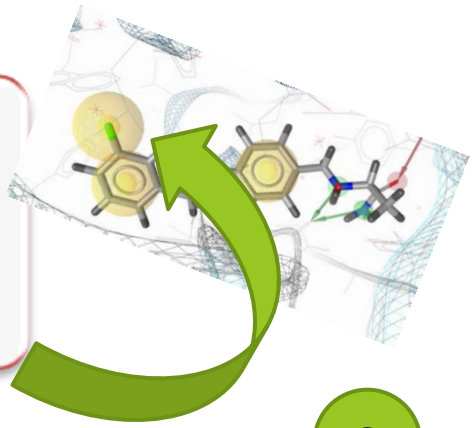
Given a set of structurally diverse ligands that binds to a receptor, a model of the receptor can be built by using the collective information contained in such set of ligands. These are ***known as pharmacophore models.***









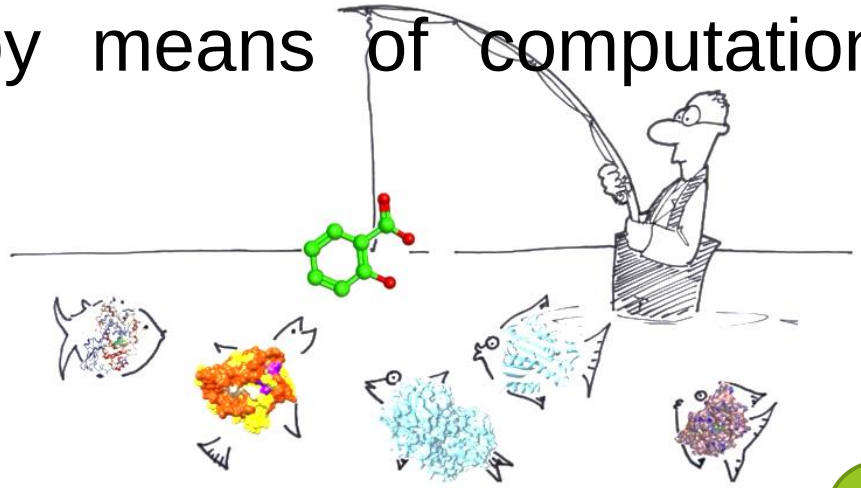


Structure-based virtual screening involves **docking** of candidate ligands into a protein target followed by applying a scoring function to estimate the possibility that the ligand will bind to the protein with high affinity.

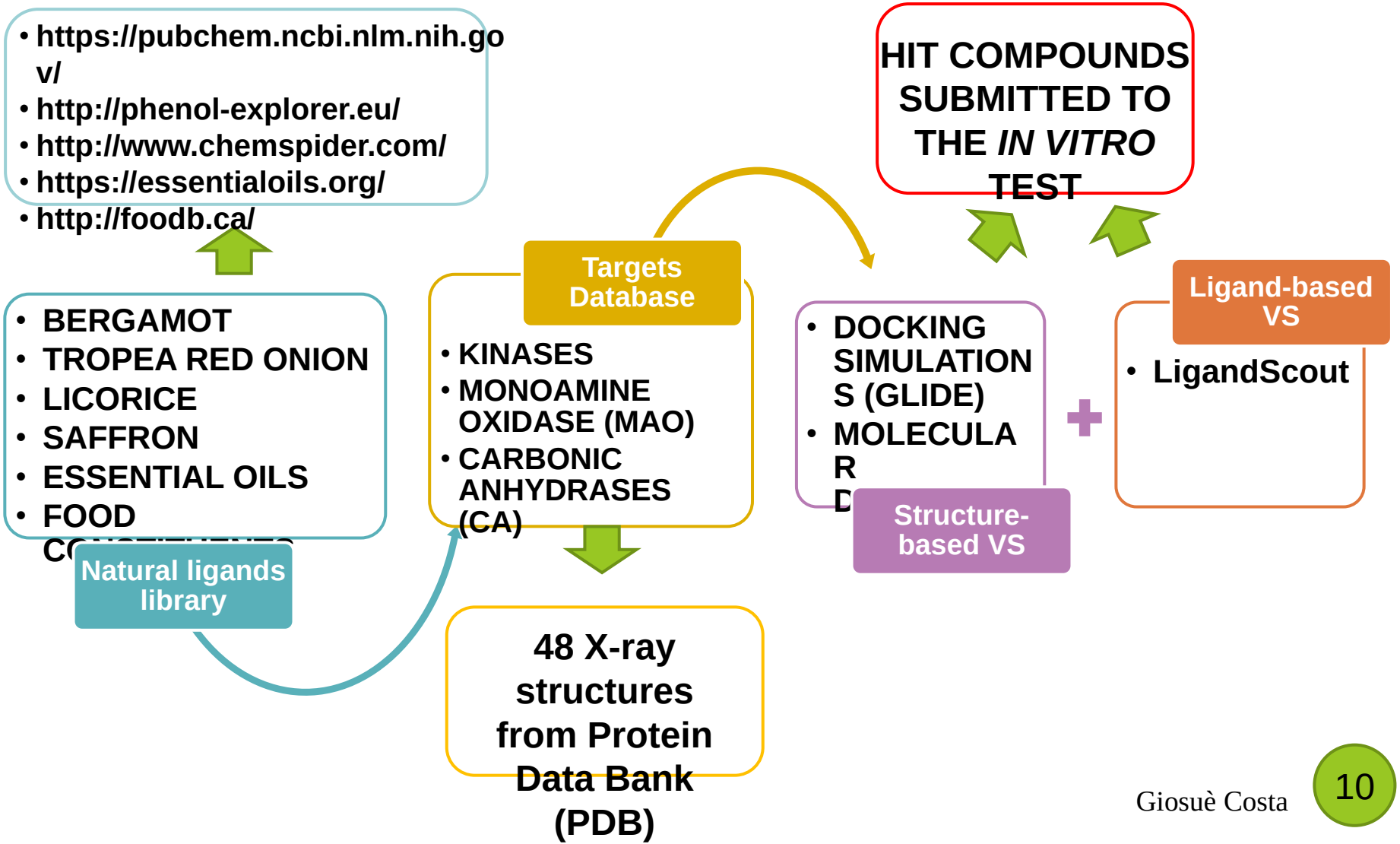
[5]Alcaro S et al. *J Med Chem* **2013**; *56*: 843−855

PURPOSE OF THE WORK

1. Chemo-cataloging process starting from **Natural products**
2. **Identification of novel hits** from natural sources against several macromolecular targets (MultiTagLig) selected on the basis of their roles in relevant pathologies by means of computational meth



MATERIALS AND METHODS

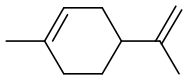


NATURAL LIGANDS LIBRARY: EXAMPLES

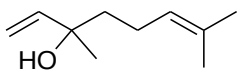
Citrus bergamia
Risso
(Bergamot)

Protected Denomination Origin (PDO)

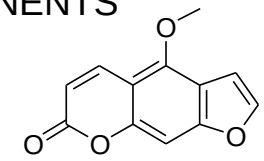
ESSENTIAL OI L COMPONENTS



limonene



linalool



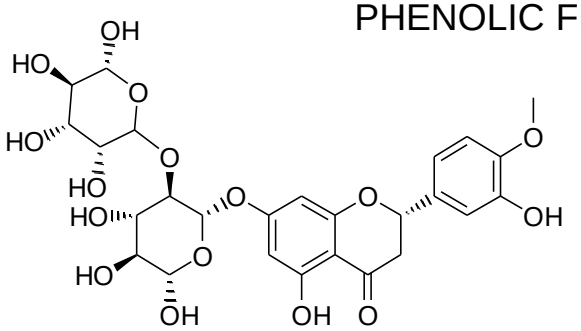
bergapten

With a unique profile of flavonoid and flavonoid glycosides

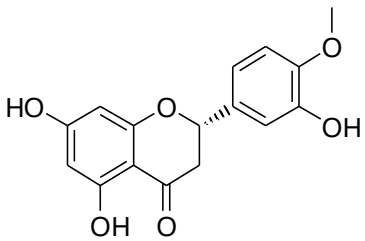
anti-proliferative, anti-aging and immune modulating effect.

chemo cataloging process of 92 compounds

PHENOLIC FRACTION



brutieridin

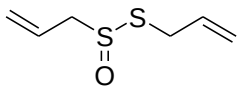


hesperetin

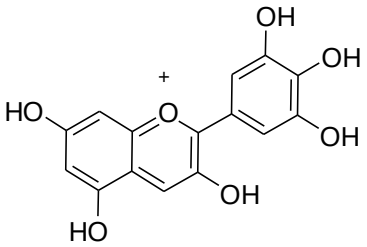
NATURAL LIGANDS LIBRARY: EXAMPLES

Allium cepa
L. var. Tropea
(Red Onion)

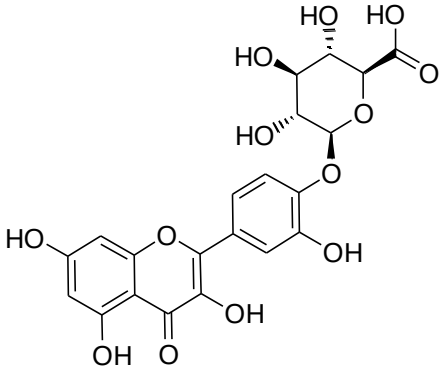
Protected Geographical Indication (PGI)



allicin



delphinidin



quercetin 4′-glucoside

Chemo cataloging process
of 30 compounds

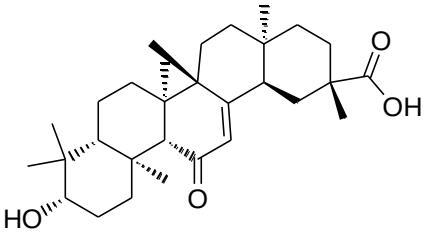
TROPEA RED ONION COMPONENTS	
FLAVONOIDS	**AMINO ACIDS**
isorhamnetin	alanine
kaempherol	arginine
quercetin	aspartic acid
taxifolin	cystine
GLYCOSIDES FLAVONOIDS	glutamic acid
7-O-β-glucopyranoside	glycine
isorhamnetin 3,4'-diglucoside	histidine
isorhamnetin 4'-glucoside	leucine
isorhamnetin 7-glucoside	lysine
quercetin 3-O-glucoside	phenylalanine
quercetin 7-4'-diglucoside	proline
quercetin 3,4,'-diglucoside	serine
quercetin 3,7,4'-triglucoside	threonine
quercetin 4'-O-glucoside	tyrosine
taxifolin 4'-O-glucoside	valine
taxifolin 7-glucoside	

NATURAL LIGANDS LIBRARY: EXAMPLES

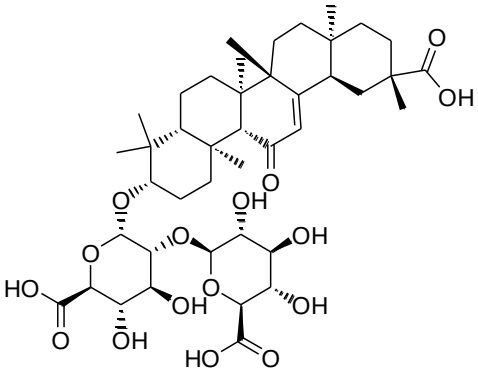
Glycyrrhiza Glabra
(Licorice)

Protected Denomination Origin (PDO)

chemo cataloging process
of 300 compounds

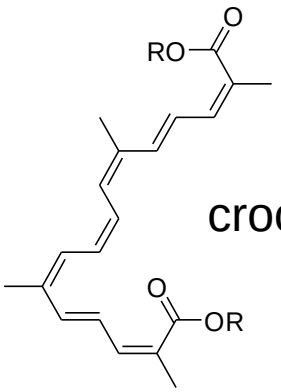


glycyrrhetic acid



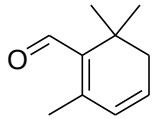
glycyrrhizic acid

Crocus Sativus
(Saffron)



crocin

R= β-D-gentiobiosyl



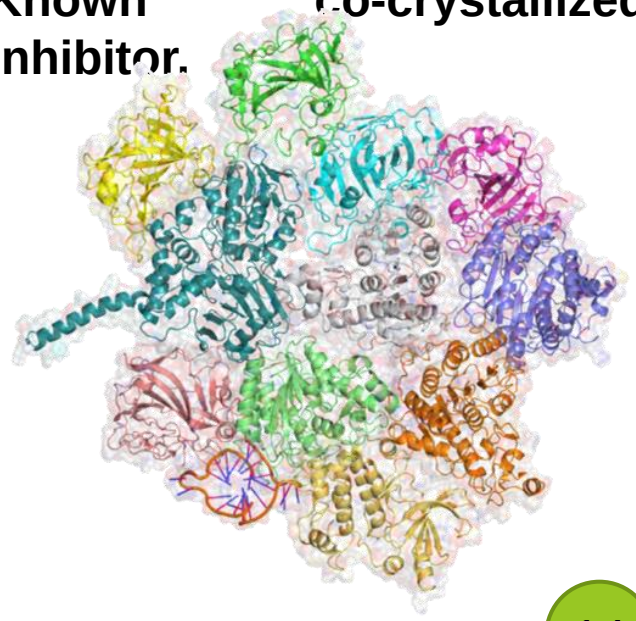
safranal

Semi-synthetic
derivatives

TARGETS DATABASE

#	TARGET	PDB code	#	TARGET	PDB code
1	AKT	4GV1	25	GLP1R	3C5T
2	ALK	3AOX	26	GSK3β	4ACC
3	ALK**	2YFX	27	HcK	2HCK
4	Alpha-Glucosidase	3TOP	28	HcK (Stem Cells)	3VRY
5	Aurora A Kinase	2X81	29	HDAC2	4LXZ
6	Aurora B Kinase	2VRX	30	HDAC4	2VQM
7	BMX	3SXR	31	HDAC7	3C10
8	BRAF	1UWH	32	HDAC8	1T67
9	BRAF**	3OG7	33	IGF-R1	3I81
10	CA I	1AZM	34	MAO-A	2Z5X
11	CA II	4CQ0	35	MAO-B	2V5Z
12	CA VA*	1DMY	36	MEK1	4ARK
13	CA IX	3IAI	37	Pantothenate syntetase*,**	3LE8
14	CA XII	4HT2	38	P-glycoprotein*	3G61
15	CDK2	4KD1	39	PI3K	3DBS
16	c-MET	2WGJ	40	PL	1LPB
17	DPP-IV	4LKO	41	Proteosoma 20S*	3MG0
18	EGFR-K**	4I22	42	Regulator Transcriptional*,**	3V78
19	ERβ	1X7J	43	RET	2IVU
20	ERK1	2ZOQ	44	ROCK1	3TWJ
21	ERK5	4B99	45	SIRT1	4I5I
22	FES	4E93	46	SIRT3	4JSR
23	FGF-R2	2FGI	47	VEGFR2	3CJF
24	FYN	2DQ7	48	ZAP70	1U59

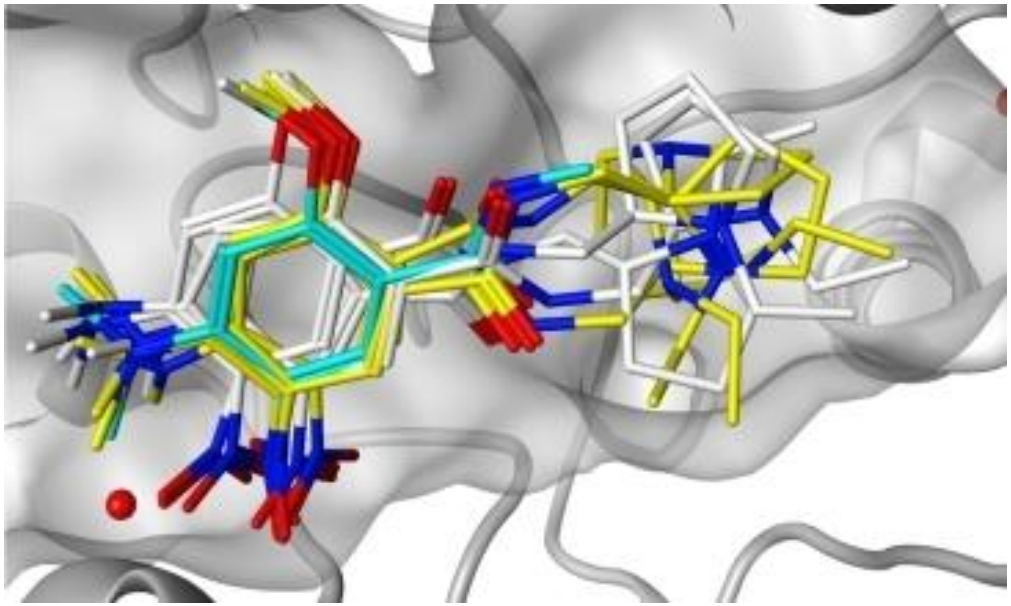
- **Protein Data Bank site (PDB);**
- **48 Crystallographic X-ray structures;**
- **Human Target;**
- **Resolution < 2.5 Å;**
- **Known co-crystallized Inhibitor.**

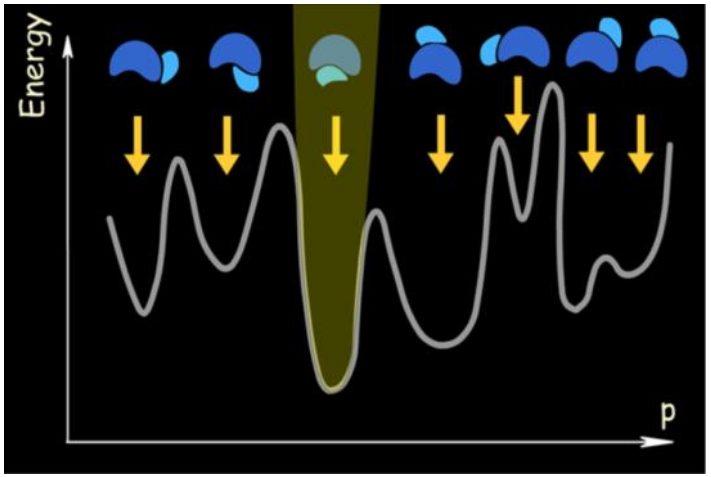


- **PROTEIN PREPARATION WIZARD function in MAESTRO 9.7;**
- **Hydrogen atom were added; All water molecules were deleted;**

SBVS: *MOLECULAR DOCKING*

Docking is used to perform an exhaustive search of the positional, orientational and conformational space available to the ligand.



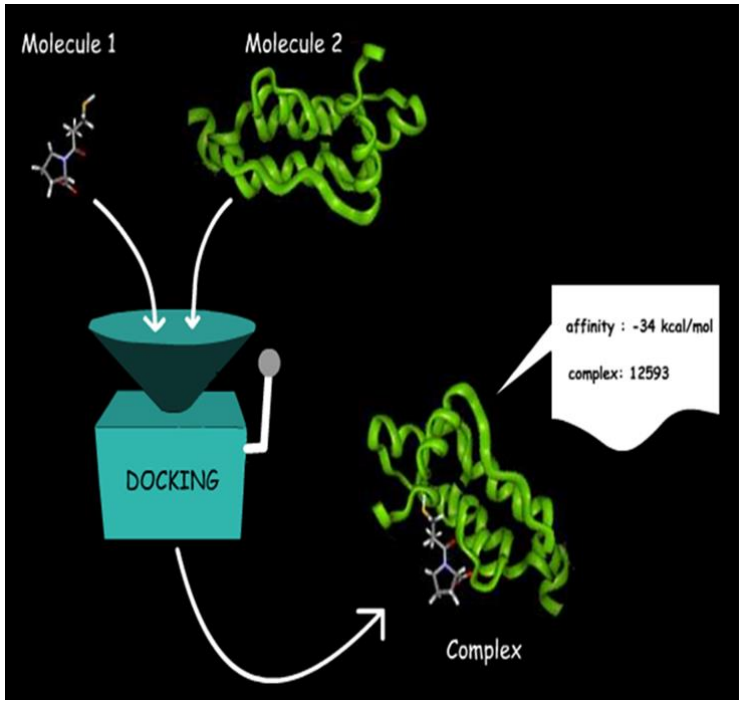


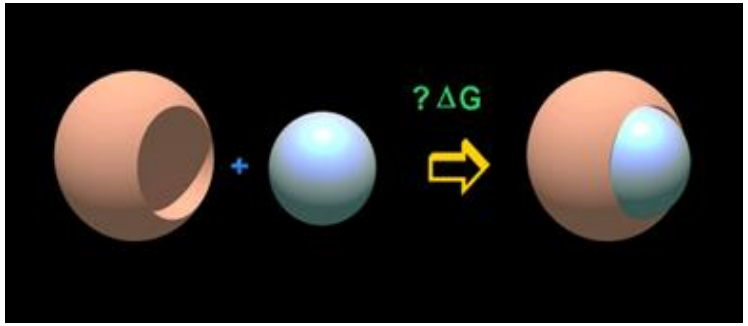
$$\Delta G_{bind} = \Delta G_{solv}^{complex} - \Delta G_{solv}^{prot} - \Delta G_{solv}^{lig} + \Delta G_{\text{int}} - T\Delta S + \Delta\lambda$$

A goal of docking is to find the lowest energy ligand-target complex

[6]Artese A et al. *Future Med Chem* **2013**; *5*:907-

MOLECULAR DOCKING: Glide Score





$$\Delta G_{bind} = C_{lipo-lipo}\sum f(r_{lr}) + C_{hbond-neut-neut}\sum g(\Delta r)\, h(\Delta\alpha) + C_{hbond-neut-charged}\sum g(\Delta r)\, h(\Delta\alpha) + C_{hbond-charged-charged}\sum g(\Delta r)\, h(\Delta\alpha) + C_{max-metal-ion}\sum f(r_{lm}) + C_{rotb}H_{rotb} + C_{polar-phob}V_{polar-phob} + C_{coul}E_{coul} + C_{vdW}E_{vdW} + \text{solvation terms}$$

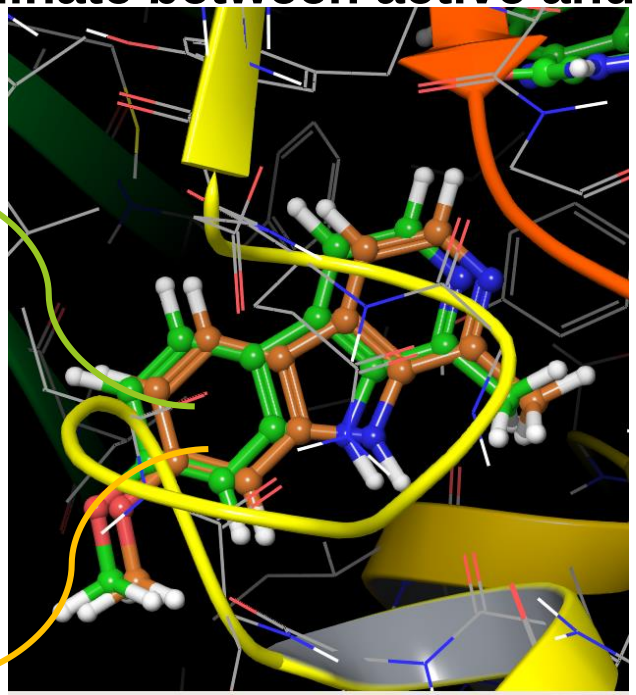
A score is assigned to the complex formed, taking into account all the binding contributions that are established in the complex.

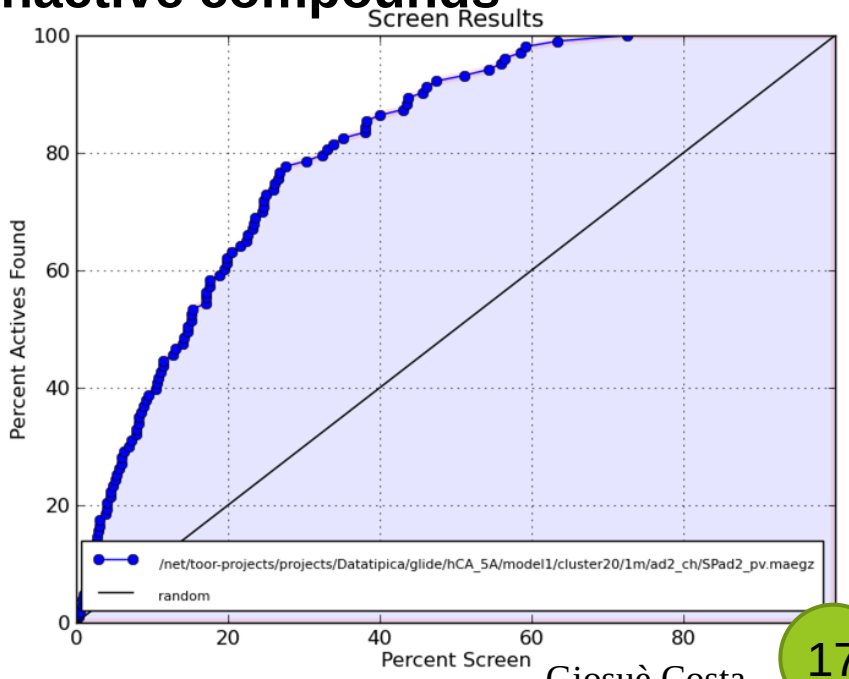
[7]Friesner RA et al. *J Med Chem* **2004**;

MOLECULAR DOCKING – Method Validation (Redocking)

The accuracy of Glide has been shown to:

1. **successfully reproduce experimentally observed binding modes of co-crystallized inhibitors, in terms of root-mean squared deviation (RMSD).**
2. **discriminate between active and inactive compounds**

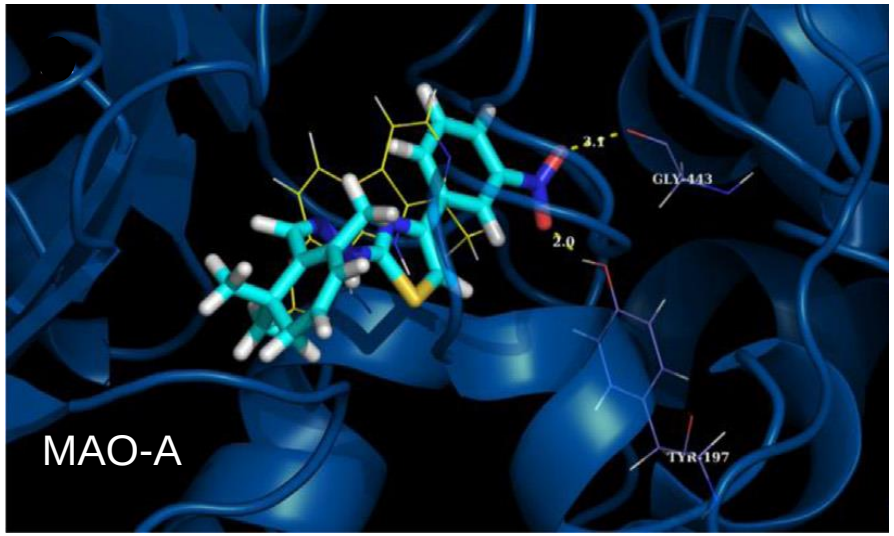


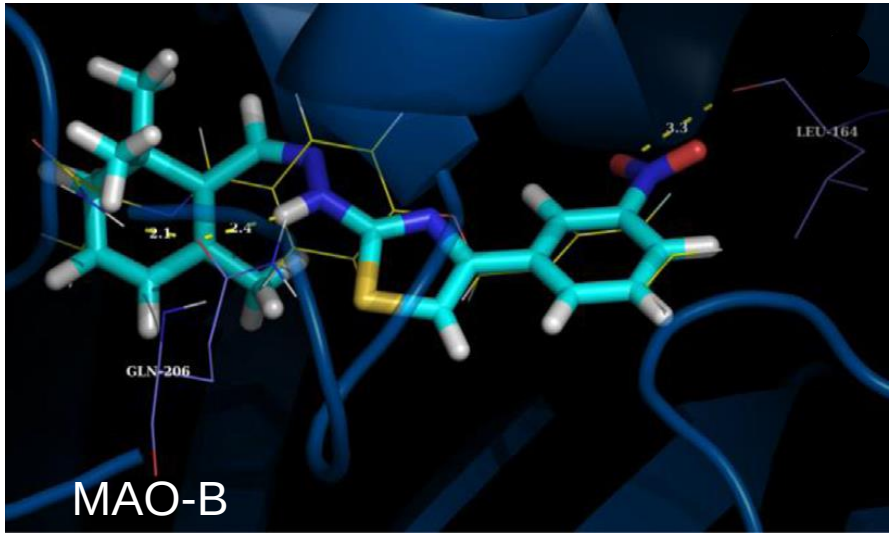


European Journal of Medicinal Chemistry 82 (2014) 164–171

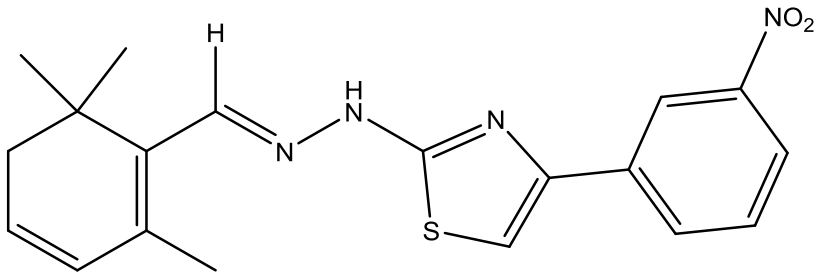
New insights into the biological properties of *Crocus sativus* L.: chemical modifications, human monoamine oxidases inhibition and molecular modeling studies

Celeste De Monte [a], Simone Carradori [a, *], Paola Chimenti [a], Daniela Secci [a], Luisa Mannina [a, d], Francesca Alcaro [b], Anél Petzer [c], Clarina I. N'Da [c], Maria Concetta Gidaro [b], Giosuè Costa [b], Stefano Alcaro [b], Jacobus P. Petzer [c, *]





***Crocus Sativus* (Saffron)**



Safranal derivate

IC_{50} MAO-A (μM)	IC_{50} MAO-B (μM)
9.93 ± 1.51	0.100 ± 0.010

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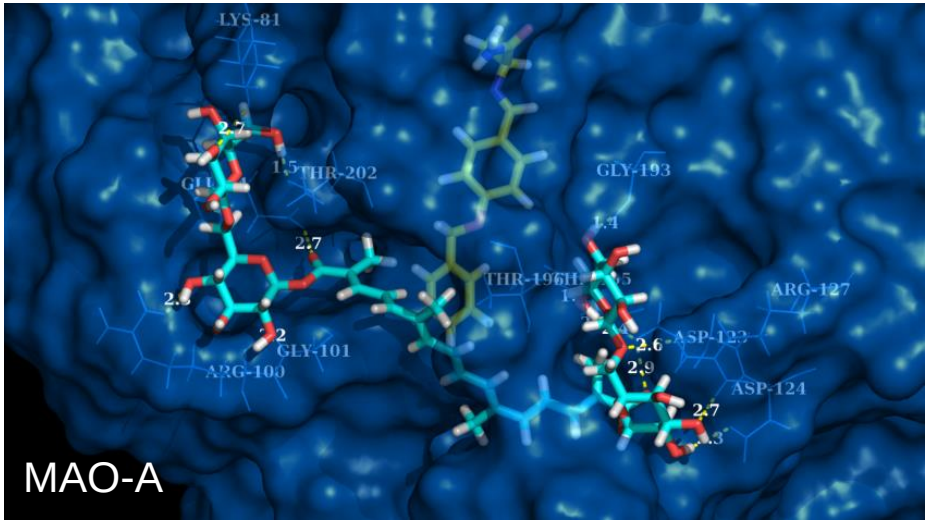
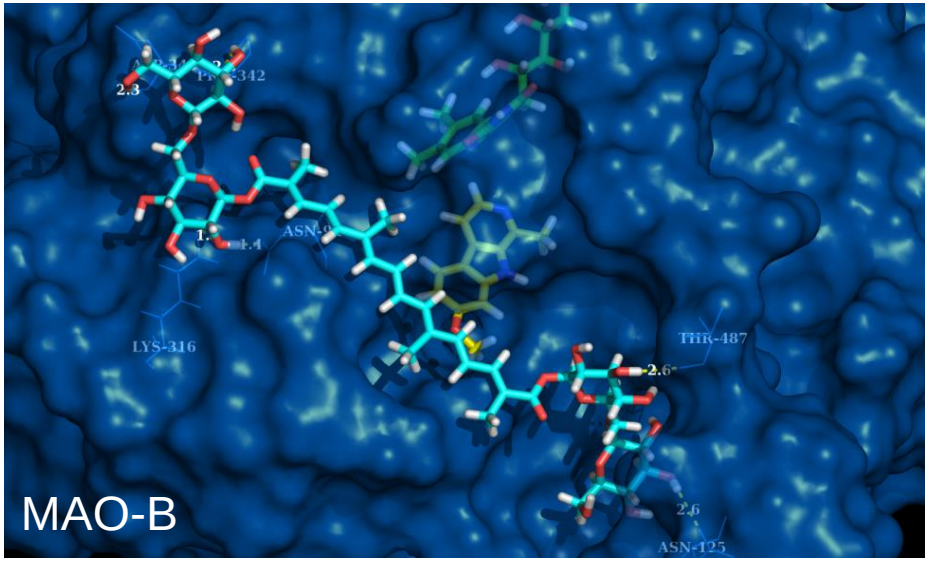
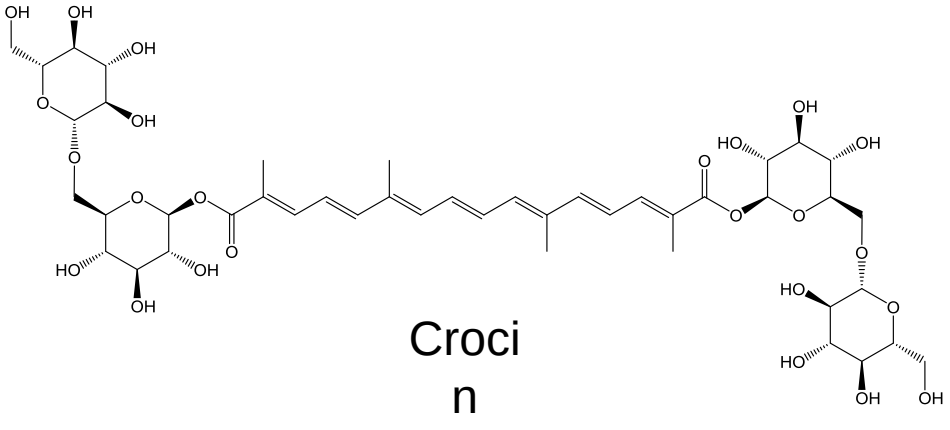




The results suggest that crocin may inhibit both hMAO isoform with non-competitive mechanisms by binding to allosteric sites on the surfaces of the proteins.



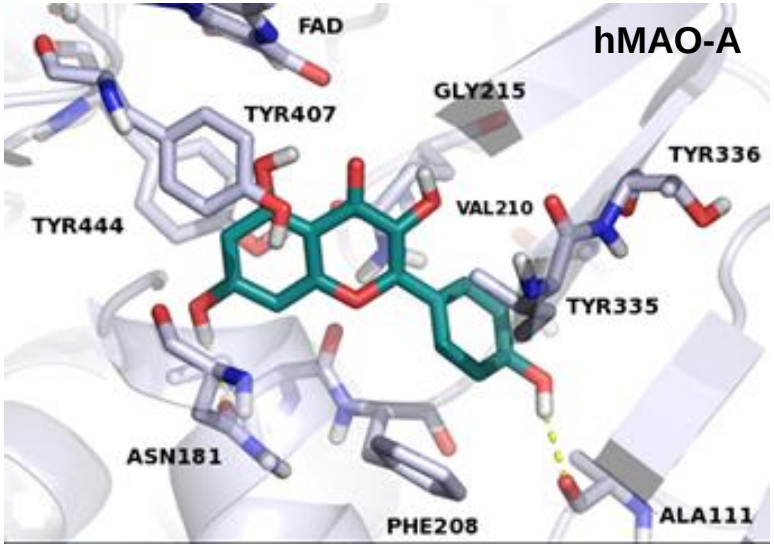
Croci
n

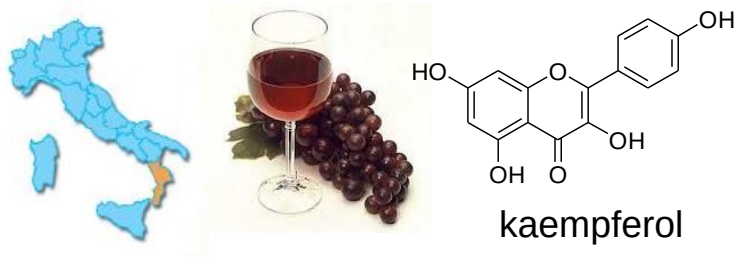
IC_{50} MAO-A (μM)	IC_{50} MAO-B (μM)
70.300 ± 22.426	28.300 ± 10.872

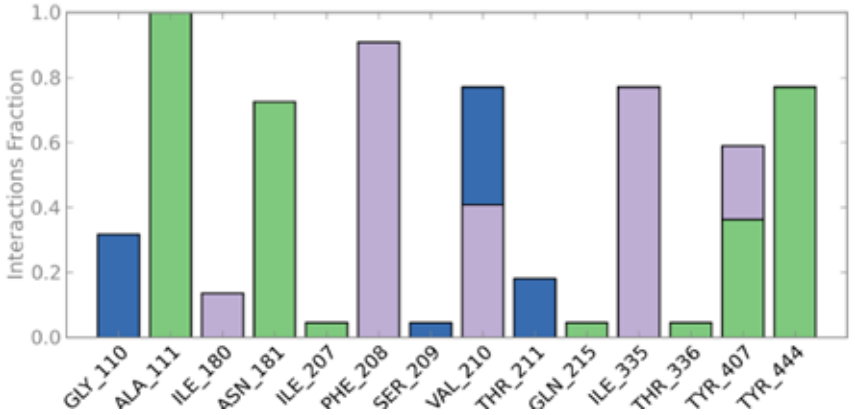
J. Agric. Food Chem. 2016, 64, 1394−1400

Kaempferol as Selective Human MAO-A Inhibitor: Analytical Detection in Calabrian Red Wines, Biological and Molecular Modeling Studies

Maria Concetta Gidaro,[†] Christian Astorino,[‡] Anél Petzer,[§] Simone Carradori,[⊥] Francesca Alcaro,[†] Giosuè Costa,[†] Anna Artese,[†] Giancarlo Rafele,[#] Francesco M. Russo,[‡] Jacobus P. Petzer,[§] and Stefano Alcaro*[,†]







The complexes were submitted to 100 ns of MDs to investigate the contributions of the amino acids of the catalytic site in their molecular recognition.

Kaempferol binding mode to the hMAO-A active site is stabilized by the hydrophobic interactions with these key residues for a longer time than in hMAO-B.

Compound	hMAO-A		hMAO-B	
	IC_{50} (μM)	dG Bind	IC_{50} (μM)	dG Bind
kaempferol	0.525 ± 0.035	-49.52	>100	-22.66
harmine	0.0029 ± 0.00042	-56.07	/	/
safinamide	/	/	0.0479 ± 0.00472	-53.70

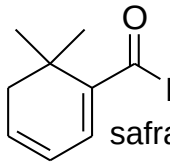
J Enzyme Inhib Med Chem, Early Online: 1–7

Journal of Enzyme Inhibition and Medicinal Chemistry

informa healthcare

Bioactive compounds of *Crocus sativus* L. and their semi-synthetic derivatives as promising anti-*Helicobacter pylori*, anti-malarial and anti-leishmanial agents

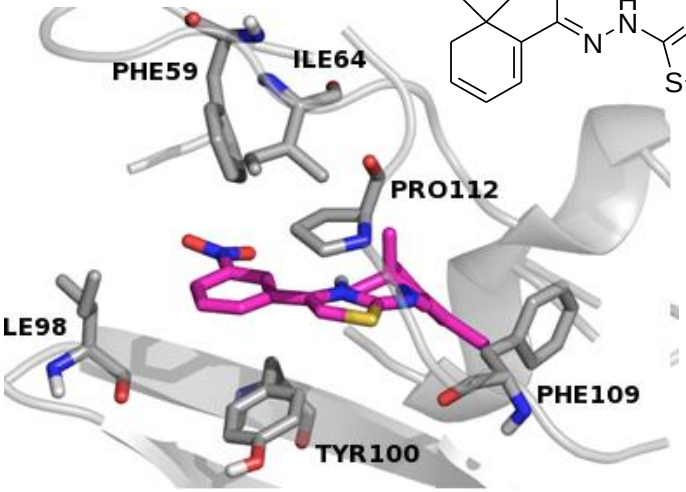
Celeste De Monte[1], Bruna Bizzarri[1], Maria Concetta Gidaro[2], Simone Carradori[3], Adriano Mollica[3], Grazia Luisi[3], Arianna Granese[1], Stefano Alcaro[2], Giosuè Costa[2], Nicoletta Basilico[4], Silvia Parapini[5], Maria Maddalena Scaltrito[4], Carla Masia[4], and Francesca Sisto[4]

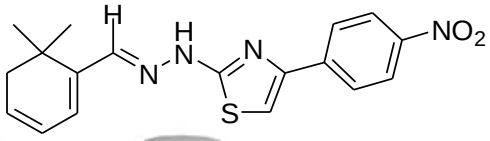


safranal

Docking experiments were performed using the X-ray crystallographic structures of six new strategic targets for the treatment of H. pylori infection, in order to **understand** their putative **mechanism of action**.

H. pylori target	PDB code	Resolution
DHQ2	2XD9	1.95 Å
FabZ	3CF8	2.40 Å
FBP aldolase	3C56	2.30 Å
Glutamate racemase	2W4I	1.87 Å
HpPDF	2EW5	2.20 Å
urease	1E9Y	3.00 Å





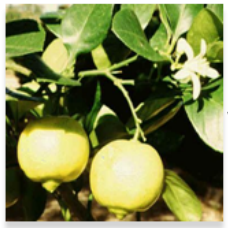
Compounds	Range	MIC_{50}	MIC_{90}
1	32–0.03	32	>32
2	32–0.03	>32	>32
3	32–0.03	32	>32
4	32–0.03	4	8
5	32–0.03	4	8
6	32–0.03	>32	>32
7	32–0.03	16	32
8	32–0.03	16	32
9	32–0.03	2	4
Metronidazole	32–0.03	0.5	>32
Claritromycin	32–0.06	<0.06	>32

Safranal derivative into the binging pocket of H. pylori β-hydroxyacyl-ACP (**FabZ**), an enzyme involved in the bacterial type II fatty acid synthetic

Eriocitrin and Apigenin as New Carbonic Anhydrase VA Inhibitors from a Virtual Screening of Calabrian Natural Products

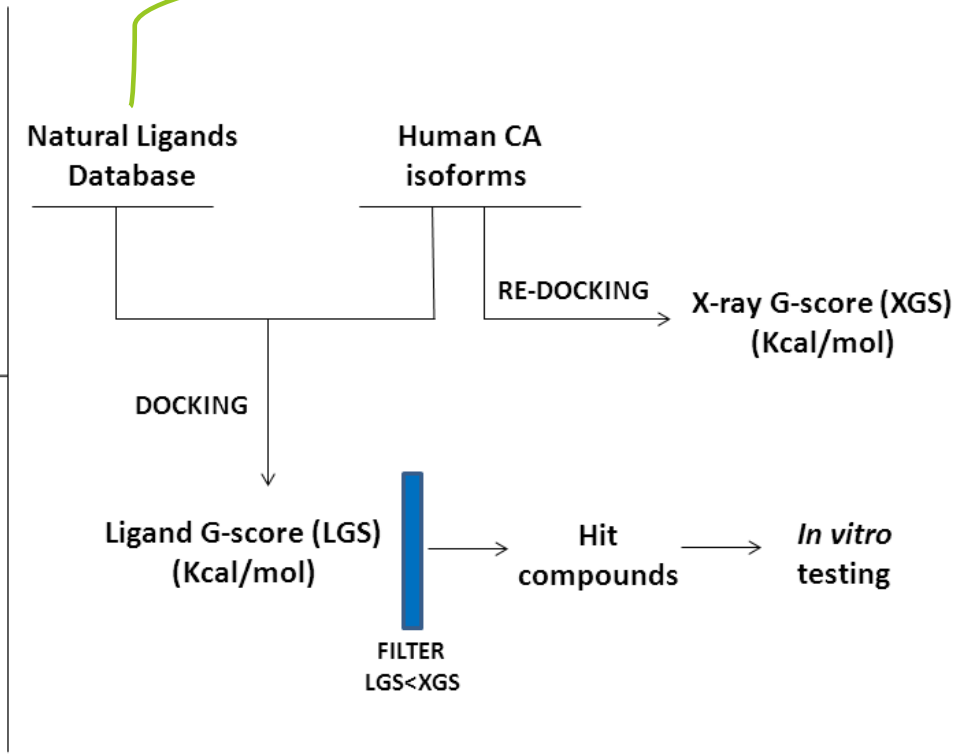
Planta Med 2015; 81: 533–540

Authors Maria Concetta Gidaro[1], Francesca Alcaro[1], Simone Carradori[2], Giosuè Costa[1], Daniela Vullo[3], Claudiu T. Supuran[3],



Citrus bergamia
Risso

Allium cepa
L. var. Tropea



The database was not only implemented with the glycoside compounds, but the aglycone structure for each of them was considered as well. In this way, the possible metabolites of the natural ligands after a hydrolytic reaction were also taken into account.

A SBVS were performed against five CA isoform: ubiquitous hCA I-II; anti-obesity CA VA and cancer-related CA IX-XII.

Eriocitrin and Apigenin as New Carbonic Anhydrase VA Inhibitors from a Virtual Screening of Calabrian Natural Products

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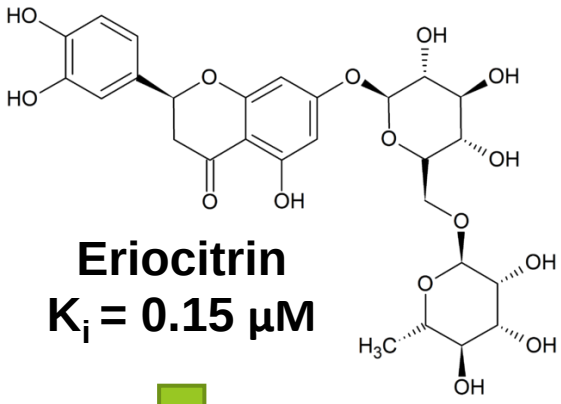
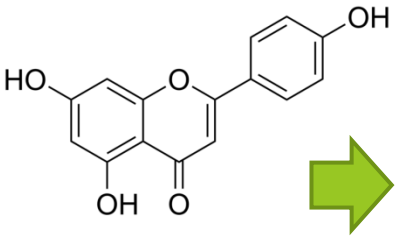
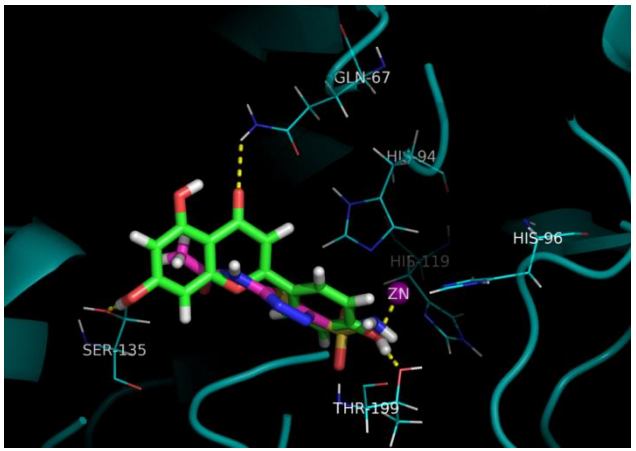
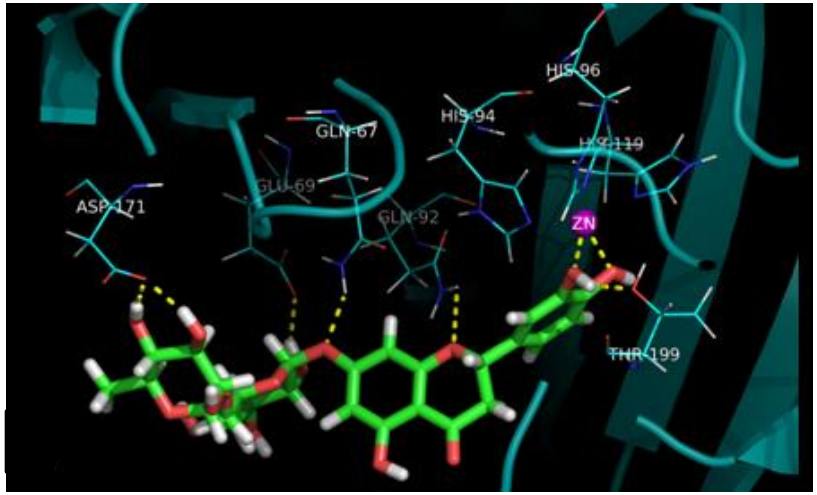


Eriocitrin
K_i = 0.15 µM

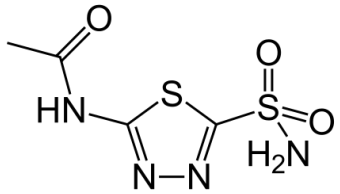


Apigenin
K_i = 0.30 µM





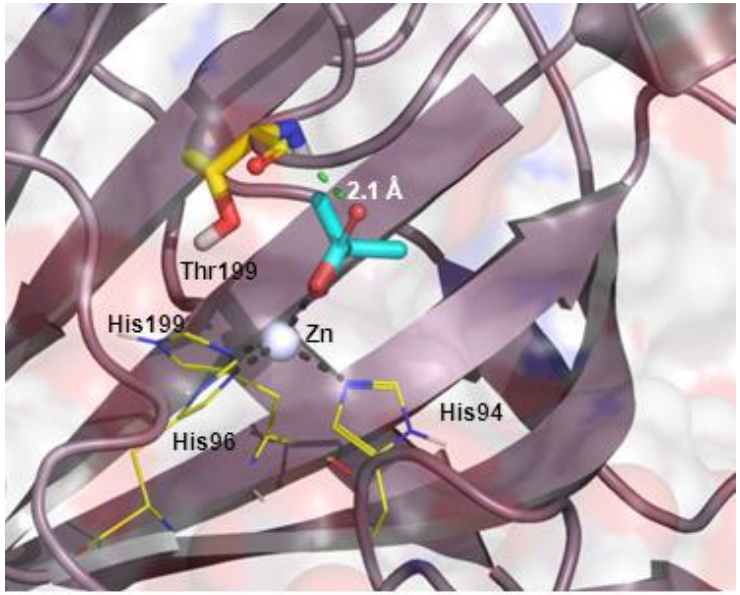
AZM positive control and co-crystallized inhibitor
K_i = 0.38 µM



VS techniques seems to confirm its important role with the aim to speed up the identification of bioactive compounds useful for both nutraceutical and drug discovery purposes.

Potential hit candidates for the obesity treatment and prevention

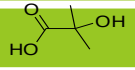
The Essential Oils as Resources of Anti-Obesity Potential Drugs investigated by *in silico* techniques



Active Components of Essential Oils as Anti-Obesity Potential Drugs Investigated by in Silico Techniques

Giosuè Costa†, Maria Concetta Gidaro*†, Daniela Vullo‡, Claudiu T. Supuran‡, and Stefano Alcaro†
† Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, Campus Universitario "S. Venuta", Viale Europa, Loc. Germaneto, 88100 Catanzaro, Italy
‡ Laboratorio di Chimica Bioinorganica, Polo Scientifico, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy

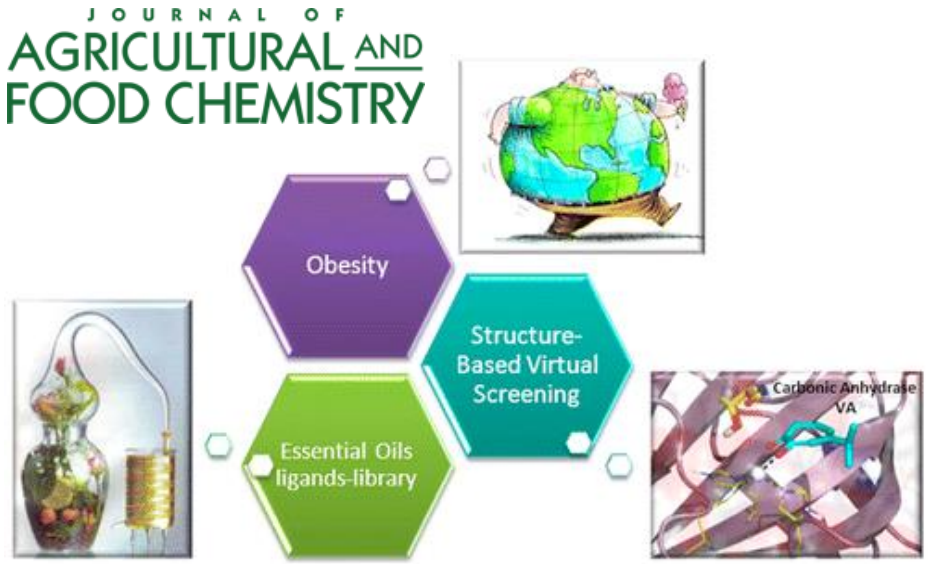
J. Agric. Food Chem., **2016**, *64* (26), pp 5295–5300
DOI: 10.1021/acs.jafc.6b02004

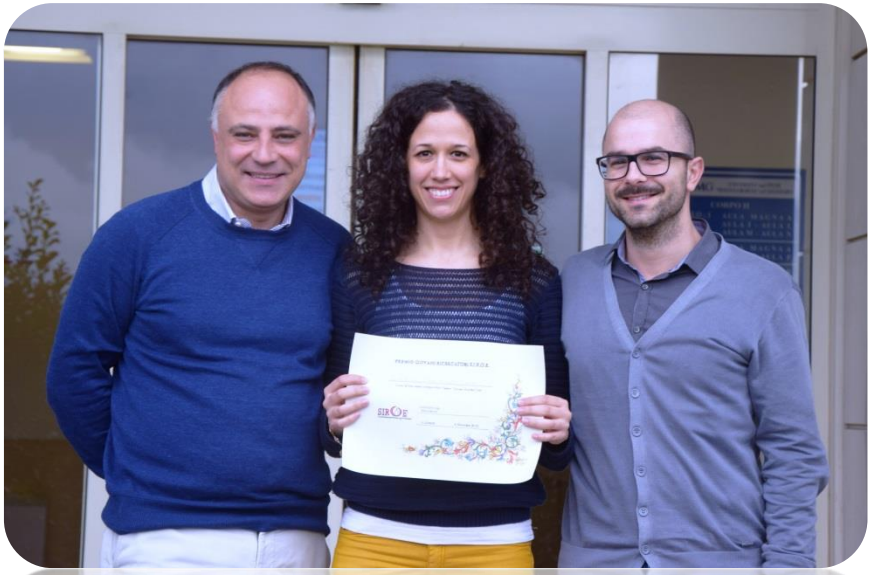
Eos	CA I (μM)	CA II (μM)	CA VA (μM)
	25.18	>100	3.80

The potential hit compounds were submitted to in vitro assays and experimental results, corroborated by molecular modeling studies, showed EOs components as a new class of CAIs with a competitive mechanism of action due to the zinc ion coordination within the active sites of these metallo-enzymes.

Best SIROE Award 2015

The work, published in a specialist journal of the American Chemical Society, was awarded the prize for the best oral presentation at Siroe national congress of 2015 in Rome.





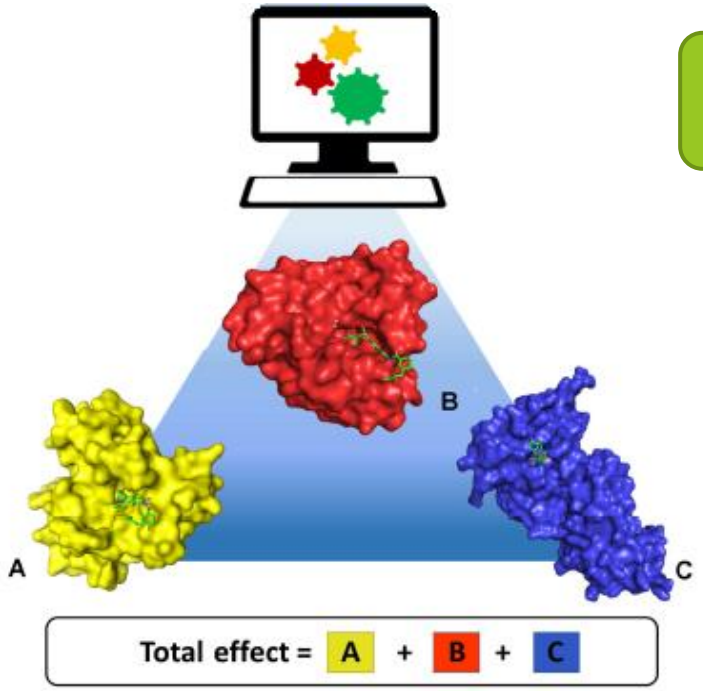
Giosuè Costa

CONCLUSION

- **Computational methods represent essential tools for the modern drug-discovery process;**
- **Potentially actives compounds are identified, and some of these are also experimentally tested, in term of enzyme inhibition;**
- **Biological activities against some target were confirmed trough studying molecular recognition.**

CONCLUSION. *Computational Polypharmacology*

Drug molecules typically bind to several targets, and their efficacy and safety is mostly dependent on their polypharmacological profile



1 **Side effects** caused by drug binding to unwanted off-targets (adverse polypharmacology) should be early identified

2 Potential synergistic effects arising from binding **multiple targets** (beneficial polypharmacology) should be taken into consideration

3 Polypharmacological approaches have the potential to redirect stalled drug discovery projects and to reposition valuable hits or leads (**drug repositioning**)

[3]Anighoro A. *Chem. Inf. Model*. **2015**, *55*, 676-686.
[4]Rastelli G. *Frontiers in pharmacology, **2015**, 6*, 1-4.

UMG
dubium sapientiae initium

IRC FSH
Interregional Research Center for Food Safety & Health

CCLab

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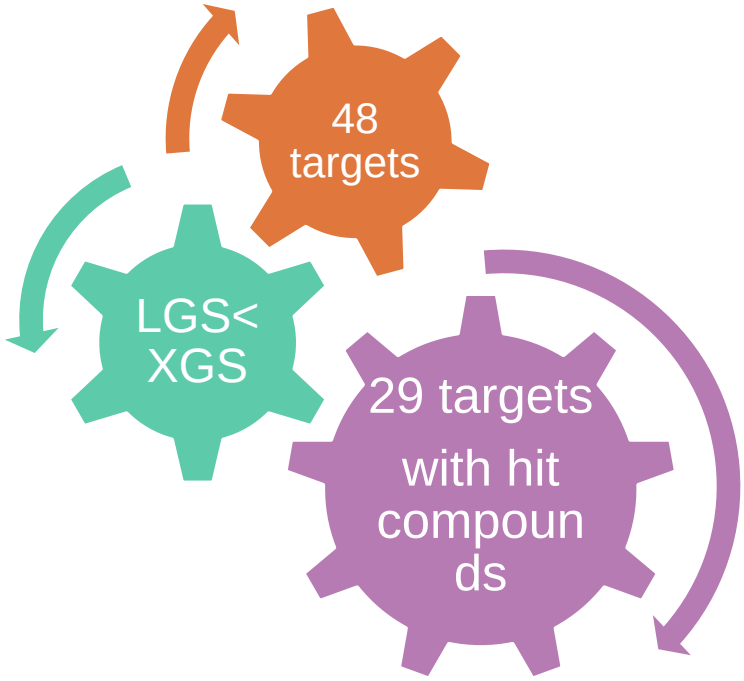
Dott.ssa D. Vullo

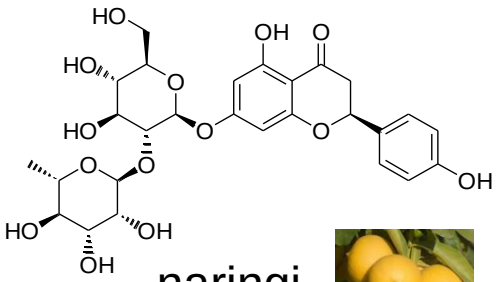
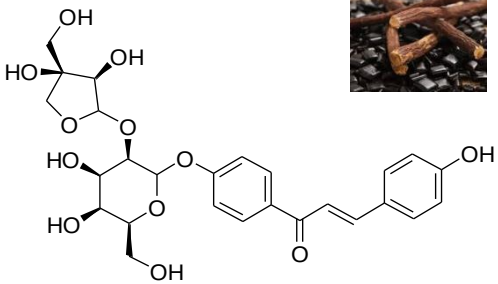
Thank you!

Nano Innovation 2016

Rome, 20-23 September

Potential anti-cancer agents and MultiTagLig



COMPOUND	TARGET	XGS	LGS	ΔGS
 naringin 	EGFR-K	-10.19	-11.21	-1.02
	GSK3β	-8.73	-9.54	-0.81
	HDAC2	-6.42	-7.57	-1.15
	HDAC7	-4.92	-7.27	-2.35
	MEK1	-8.08	-11.76	-3.68
	ROCK1	-8.71	-9.45	-0.74
	VEGFR2	-8.17	-8.72	-0.55
 Isoliquiritin apioside1	DPPIV	-7.83	-7.96	-0.13
	EGFR-K	-10,19	-10.59	-0.40
	GSK3β	-8.73	-8.88	-0.15
	HDAC2	-6.42	-8.77	-2.35
	HDAC7	-4.92	-8.95	-4.03
	MEK1	-8,08	-9.90	-1.82
	ROCK1	-8.71	-9.57	-0.86