FLAVONOIDS AS INHIBITORS OF HUMAN DPP III

Dejan Agić[1], Hrvoje Brkić[2], Sanja Tomić[3], Zrinka Karačić[3], Drago Bešlo[1], Miroslav Lisjak[1], Marija Špoljarević[1]

[1] Faculty of Agriculture, Osijek

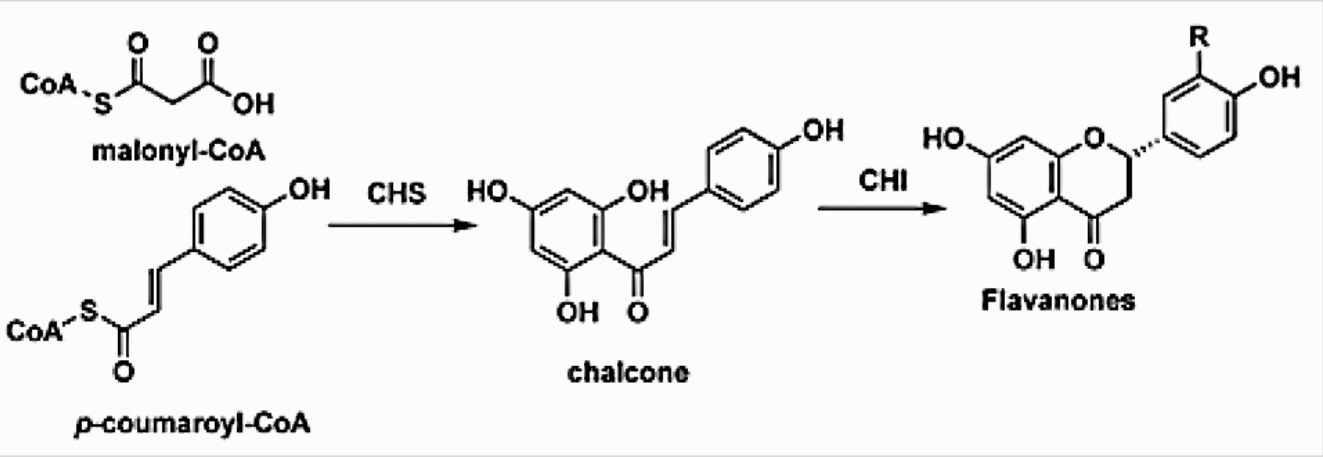
[2] Faculty of Medicine, Osijek

[3] Rudjer Bošković Institute, Zagreb

DPP III Minisymposium, Zagreb March 21st 2016

Flavonoids

- class of 9000 hydroxylated polyphenolic compounds
- found in all vascular plants
- synthesis:



- important functions in plants: attracting pollinating insects, combating environmental stresses, regulating cell growth…
- constituents of the human diet (fruits and vegetables)

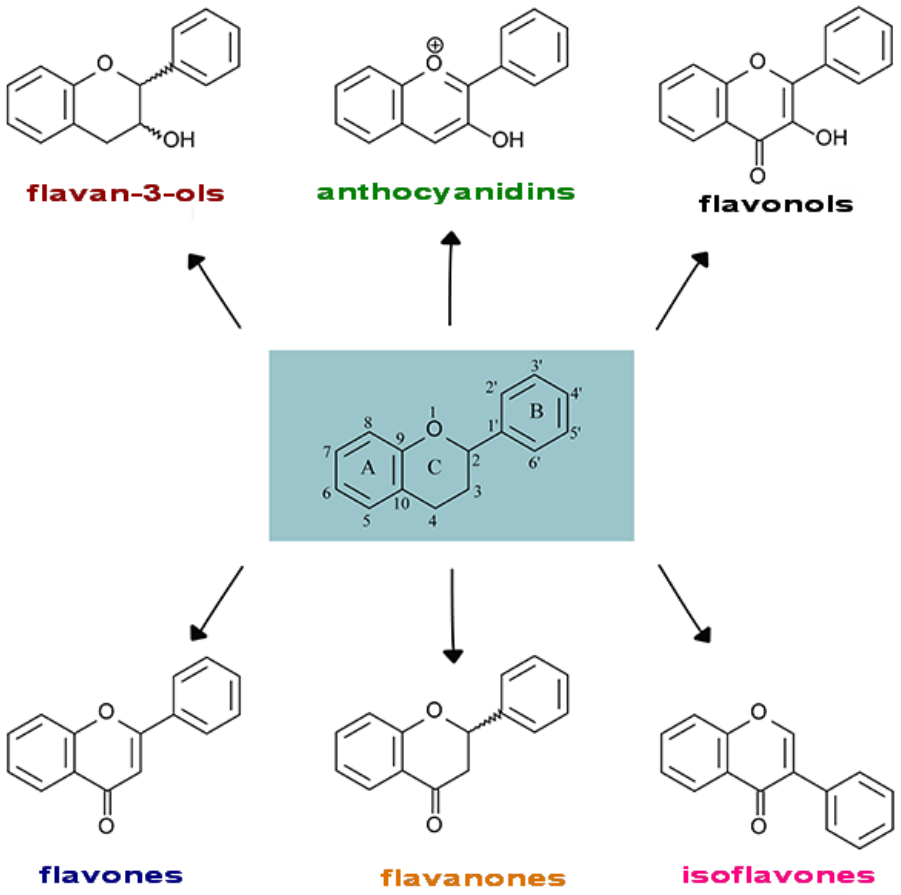
Dietary flavonoids

- six major subclasses based on their structural differences:

CATEHIN
EPICATEHIN
FISETINIDOL
MESQUITOL
ROBINITENIDOL…

AURANTINIDIN
CAPENSINIDIN
CYANIDIN
DELPHINIDIN
EUROPINIDIN
HIRSUTIDIN
MALVIDIN
PELARGONIDIN
PETUNIDIN
ROSINIDIN…

6-HYDROXYFLAVONE
APIGENIN
CHRYSIN
DIOSMIN
FLAVONE
LUTEOLIN
TANGERITIN…



3-HYDROXYFLAVONE
3,6-DIHYDROXYFLAVONE
FISETIN
GALANGIN
GOSSYPETIN
KAEMPFEROL
MORIN
MYRICETIN
QUERCETIN
RHAMNETIN…

FLAVANONE
HESPERIDIN NARINGENIN
NARINGENIN
PINOCEMBRIN
PONCIRIN
SAKURANETIN
SAKURANIN
STERUBIN…

2ˈ-HYDROXYGENISTEIN
DAIDZEIN
GENISTEIN
GLYCITEIN
WIGHTEONE…

Biological activities of dietary flavonoids

- **antiinflammatory**
  - reduce inflammation by suppressing the expression of pro-inflammatory mediators like nuclear factor NF-κB
- **antidiabetic**
  - improving insulin secretion and viability of pancreatic β-cells under glucotoxic conditions
- **anticancer**
  - preventing the activation of procarcinogenic chemicals and promoting their excretion from the body
- **neuroprotective**
  - promote neurogenesis and synaptic growth by stimulating the production of neurotrophins like brain-derived neurotrophic factor, BDNF
- **vascular protection**
  - induce arterial dilation by increasing nitrogen oxide bioavailability

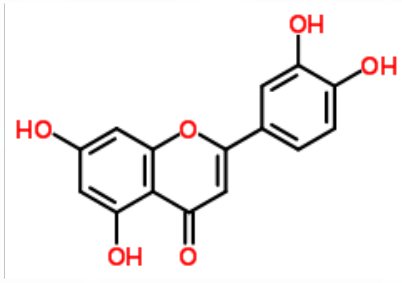
Inhibition of metallopeptidases by flavonoids

- **angiotensine-converting enzyme, ACE**
  - luteolin inhibit ACE from rabbit lung (IC_{50} 23 µM)*

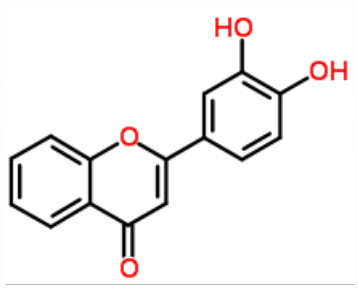
- **carboxypeptidase A2, CP-A2**
  - luteolin inhibit CP-A2 from bovine pancreas (61% I at 150 µM)**

- **leucine aminopeptidase, LAP**
  - 3ˈ,4ˈ-dihydroxyflavone inhibit LAP from porcine kidney (80% I at 50 µM)**

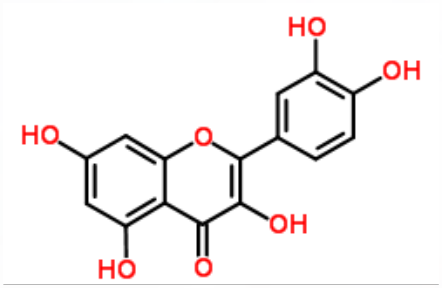
- **aminopeptidase M, AP-M**
  - quercetin inhibit AP-M from porcine kidney microsomes (35% I at 100 µM)**



LUTEOLIN



3ˈ,4ˈ-DIHYDROXYFLAVONE

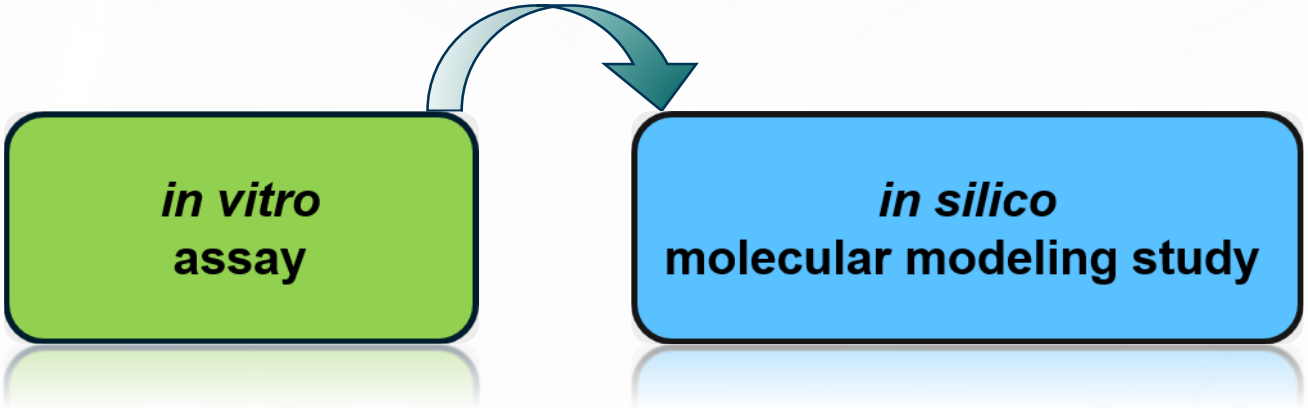


QUERCETIN

*L.Guerrero et al,(2012) *PLOS ONE*, 7,e49493, **J. Parellada, et al,(1998) J. Enzyme Inhib. 13, 347-59.

Objectives

- investigate 15 flavonoids (four structural subclasses) for their potential inhibitory activity towards human DPP III
- study the influence of the most potent inhibitor on the structure and flexibility of the protein
- elucidate interactions between the most potent inhibitor and the human DPP III binding site



In vitro assay

➢ **IC_{50} determination**

- C-terminal His-tagged recombinant human DPP III (0.29 nM)
- Tris/HCl buffer, pH 7.4 (50 mM)
- flavonoids (Luteolin, Galangin, Fisetin, Kaempferol, Genistein, Apigenin, 3,6-Dihydroxyflavone, Quercetin, 6-Hydroxyflavone, Morin, Flavanone, Chrysin, 3,7-Dihydroxyflavone,3-Hydroxyflavone, and Flavone) in DMSO

preincubation → 1 min 25 ° C + 3 min 37 ° C

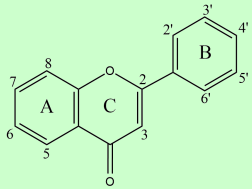
Arg-Agr-2NA (40 µM)

incubation → 15 min 37 ° C

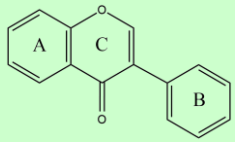
Fast blue B salt → A_{530} nm

$$\% \text{ inh.} = \frac{\text{normal activity} - \text{inhibited activity}}{\text{normal activity}} \text{ x100}$$

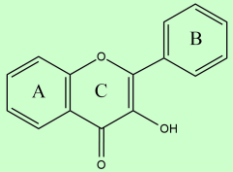
IC_{50} values



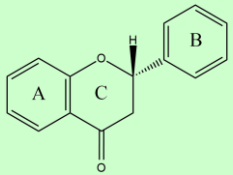
Flavone



Isoflavone

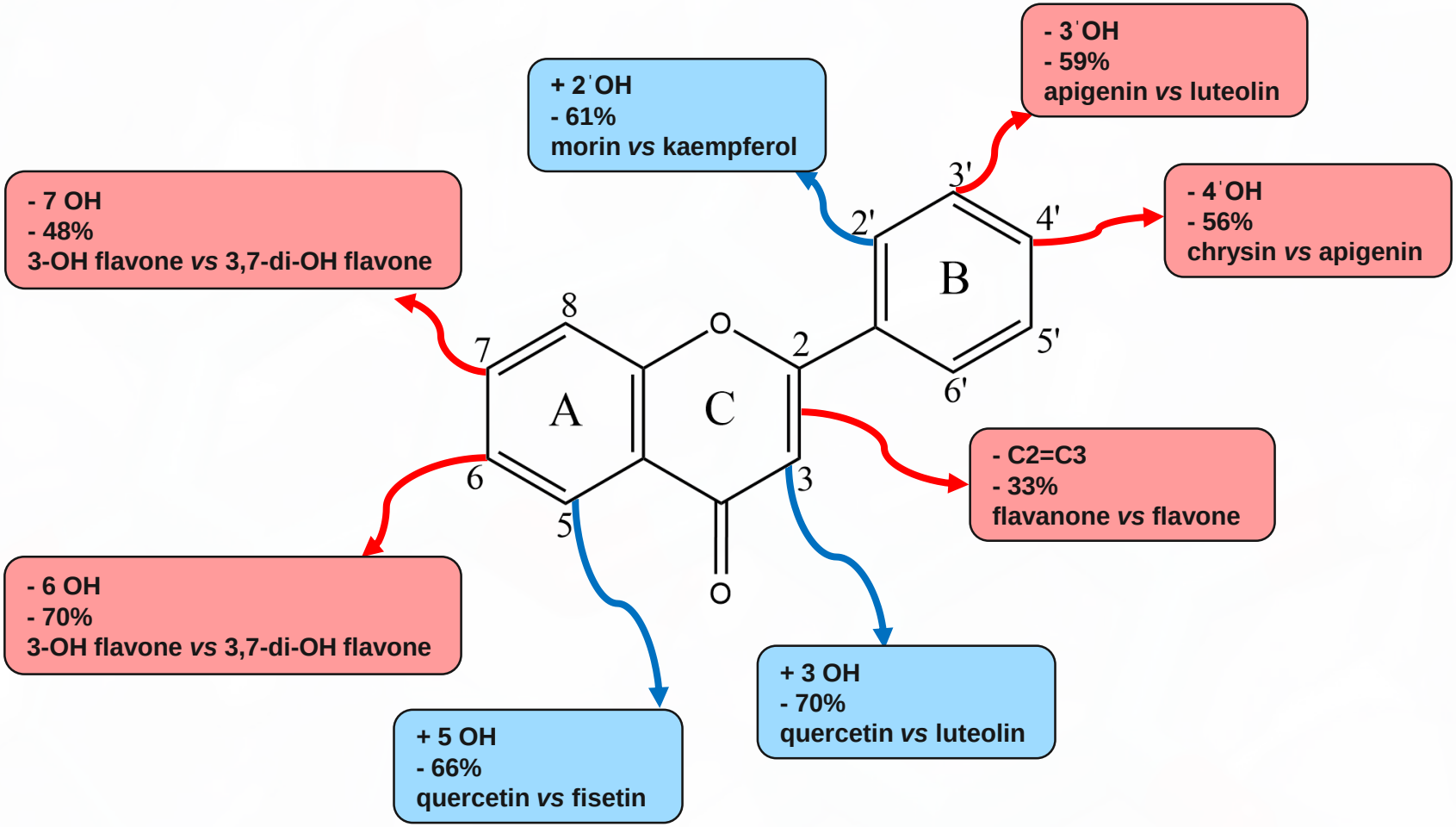


Flavonol



Flavanone

Group	Name	Substitution						IC_{50} (µM)
		2'	3'	4'	5	6	7	
Flavone	**Luteolin**	**H**	**OH**	**OH**	**OH**	**H**	**OH**	**22.0(±2)**
Flavonol	Galangin	H	H	H	OH	H	OH	23.4(±3)
Flavonol	Fisetin	H	OH	OH	H	H	OH	24.9(±2)
Flavonol	Kaempferol	H	H	OH	OH	H	OH	32.9(±3)
Isoflavone	Genistein	H	H	OH	OH	H	OH	36.6(±4)
Flavone	Apigenin	H	H	OH	OH	H	OH	54.0(±4)
Flavonol	3,6-Dihydroxyflavone	H	H	H	H	OH	H	56.4(±3)
Flavonol	Quercetin	H	OH	OH	OH	H	OH	74.1(±6)
Flavone	6-Hydroxyflavone	H	H	H	H	OH	H	82.2(±4)
Flavonol	Morin	OH	H	OH	OH	H	OH	85.0(±11)
Flavonol	3,7-Dihydroxyflavone	H	H	H	H	H	OH	98.0(±5)
Flavone	Chrysin	H	H	H	OH	H	OH	123.1(±10)
Flavonol	3-Hydroxyflavone	H	H	H	H	H	H	188.2(±7)
Flavanone	Flavanone	H	H	H	H	H	H	292.5(±18)
Flavone	Flavone	H	H	H	H	H	H	437.2(±14)


+ 2'OH
- 61%
morin vs kaempferol
- 3'OH
- 59%
apigenin vs luteolin
- 7 OH
- 48%
3-OH flavone vs 3,7-di-OH flavone
- 4'OH
- 56%
chrysin vs apigenin
- C2=C3
- 33%
flavanone vs flavone
- 6 OH
- 70%
3-OH flavone vs 3,7-di-OH flavone
+ 5 OH
- 66%
quercetin vs fisetin
+ 3 OH
- 70%
quercetin vs luteolin
A
B
C
O
O
2
3
5
6
7
8
2'
3'
4'
5'
6'

In silico molecular modeling study

- **Docking**

  - 3D structure of human DPP III (PDB_code 3FVY, resolution 1.9 Å)

  - luteolin (3',4',5,7-tetrahydroxyflavone) structure (PubChem)

  - AutoDock 4.2.6(5)

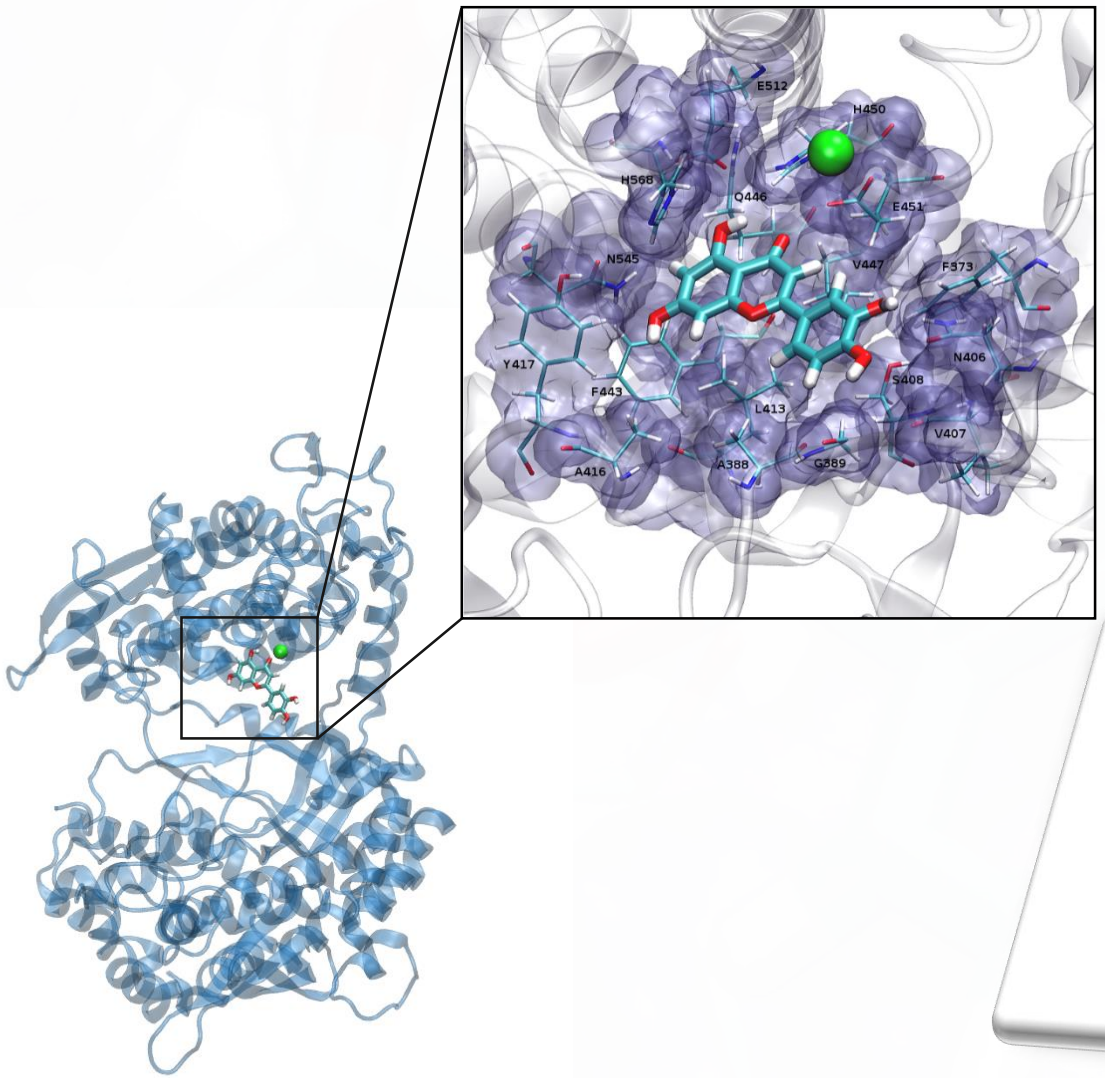
- **Molecular dynamics simulations**

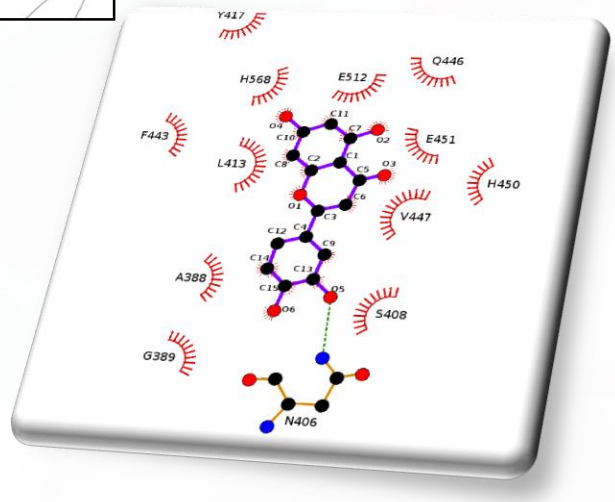
  - AMBER 12 and AMBERTools14

  - parameterization by antechamber and tleap using GAFF and ff12SB

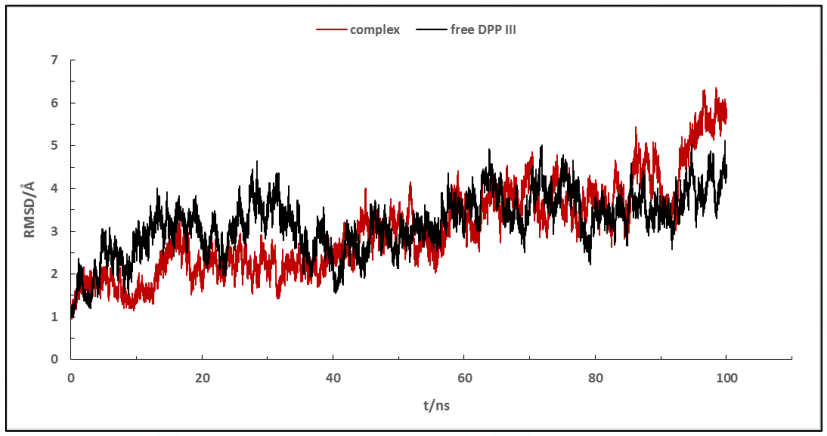
  - MD simulations (100 ns)

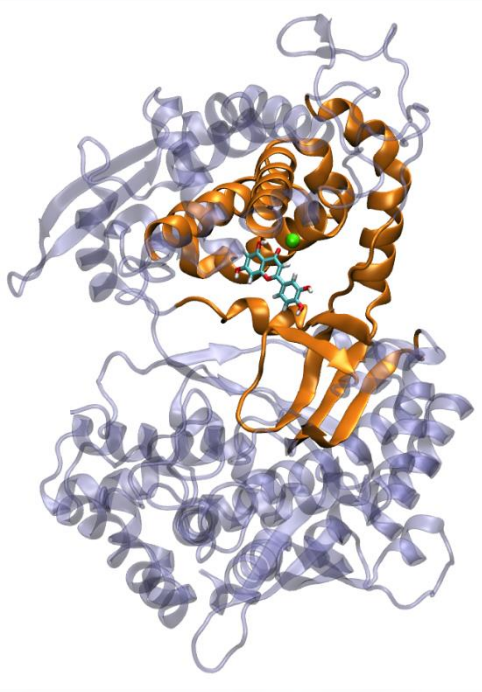
Best docked pose for luteolin at the DPP III binding site

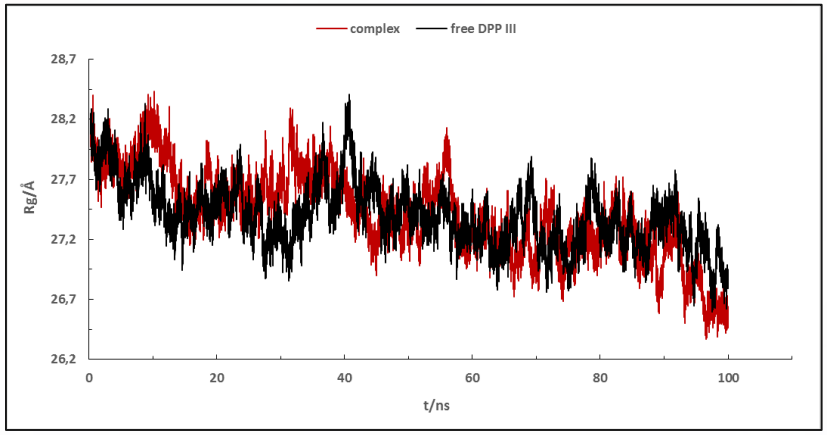


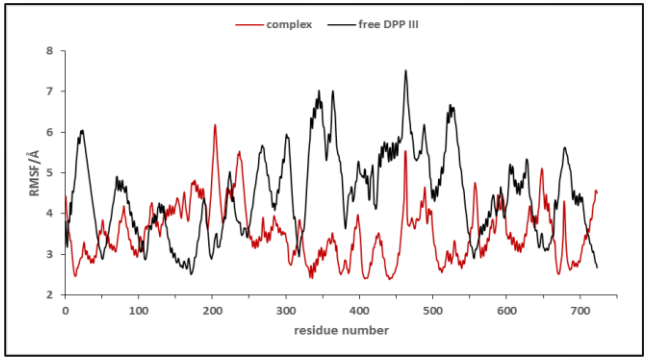


Molecular dynamics simulations

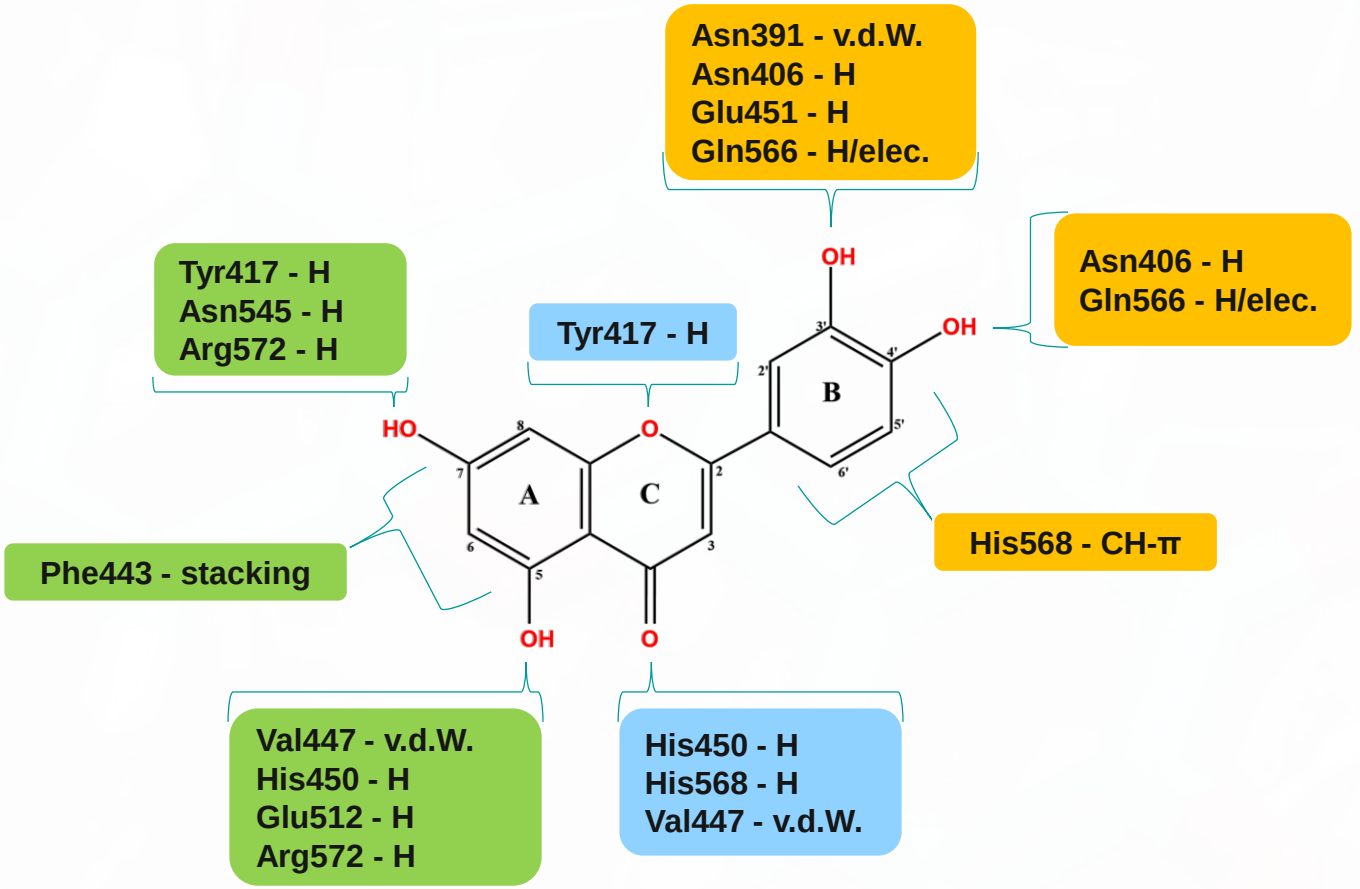








Luteolin - human DPP III interactions



Conclusions

- All analyzed flavonoids have shown inhibitory effects against human DPP III (IC_{50} 22 - 437 μM)

- Changes in the flavonoid active core affect its capacity to inhibit the enzyme (3-OH = 6-OH > 5-OH > 2'-OH ~ 3'-OH ~ 4'-OH > 7-OH > C_2=C_3)

- Luteolin binding to human DPP III induces:
  - protein stabilization (20-40 ns of MD simulations)
  - incrase in the protein globularity (last 10 ns of MD simulations)

- Hydroxyl moieties at C3', C4', C5 and C7, carbonyl at C4 and O1 oxigen contribute favorable hydrogen bonds, electrostatic and van der Waals interactions between luteolin and the human DPP III binding site