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Natural and synthetic compounds as inhibitors of human DPP III

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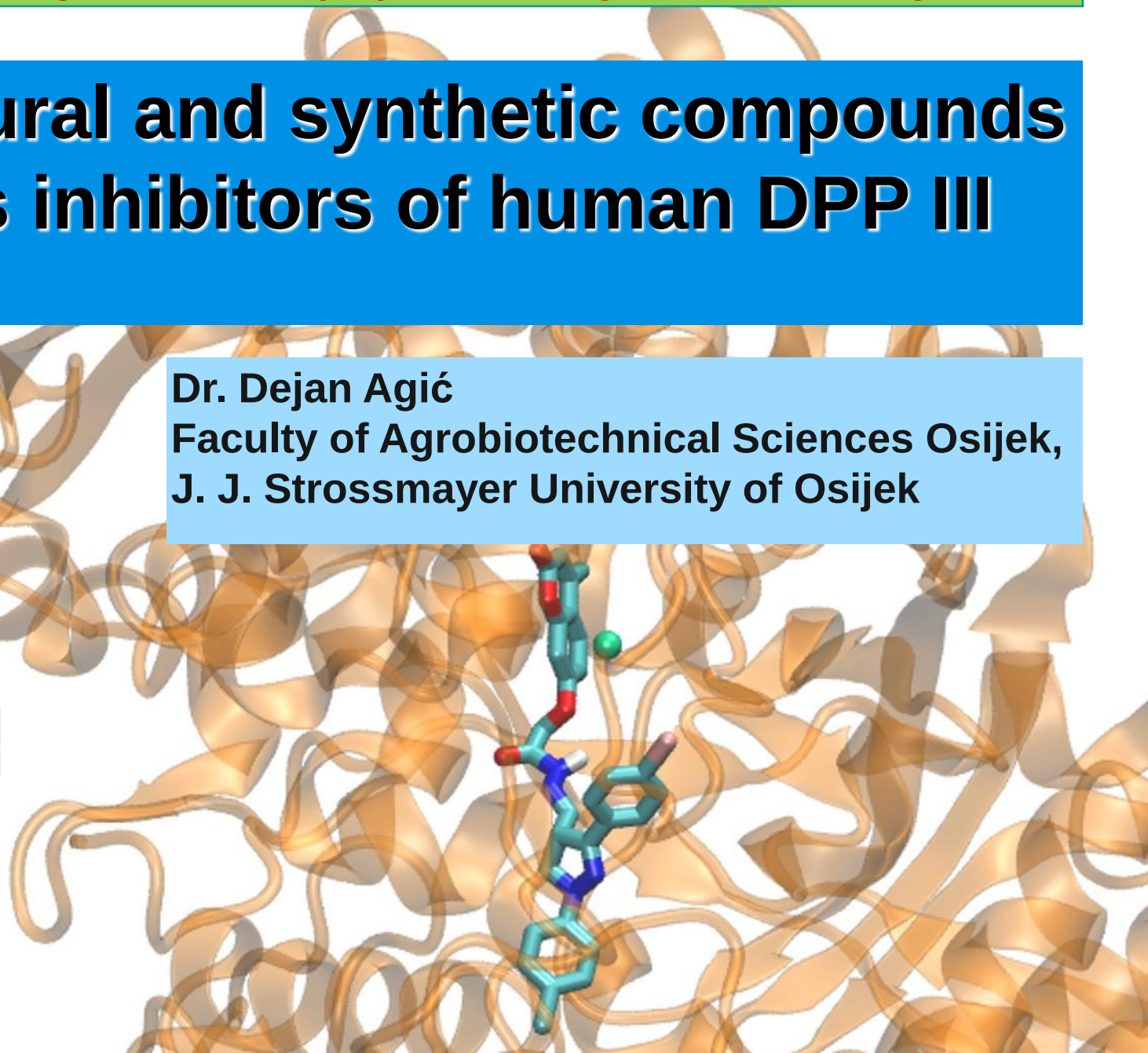

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An *in vitro* and *in silico* evaluation of bioactive potential of cornelian cherry (*Cornus mas* L.) extracts rich in polyphenols and iridoids

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In vitro

- **- fruit extract preparation in solvent (water / etOH = 1:1)**
- **- HPLC PDA analysis**
- **- antioxidant activity (FC, FRAP, DPPH)**
- **- antiproliferative activity on human colon cancer HT29 cell line**
- **- inhibition of α amylase, α glucosidase and recombinant hDPP III**

In silico

- **- molecular docking (feasibility of selected compounds from fruit extract to form complex with α amylase, α glucosidase and hDPP III)**

Determination of iridioid and phenolic constituens

Iridoid and phenolic compounds in cornelian cherry fruit extracts.

No.	Compound	t_R (min)	λ_{max}	Calculated as	Apatinski rani	Bačka	KIC1	R1	Elegantnyi	Semen	Svetlyachok
1	Loganic acid (LA)	13.5	240	LA	852.04 ± 19.68^{c}	1111.30 ± 22.46^{a}	499.67 ± 14.42^{d}	962.84 ± 22.24^{b}	553.45 ± 15.98^{d}	789.03 ± 20.50^{c}	1054.78 ± 21.31^{a}
2	Sweroside (Sw)	19.2	239	Ln	29.05 ± 0.59^{d}	65.77 ± 1.52^{a}	14.49 ± 0.42^{e}	45.65 ± 1.32^{b}	25.60 ± 0.52^{d}	36.01 ± 0.83^{c}	62.21 ± 1.62^{a}
3	Secoxyloganin (Sec)	27.5	240	Ln	1.06 ± 0.03^{e}	4.01 ± 0.08^{b}	1.01 ± 0.02^{e}	3.69 ± 0.11^{b,c}	1.84 ± 0.04^{d}	3.45 ± 0.10^{c,d}	4.63 ± 0.11^{a}
4	Loganin (Ln)	22.1	239	Ln	1.95 ± 0.05^{e}	4.83 ± 0.10^{d}	0.47 ± 0.01^{f}	10.09 ± 0,29^{a}	6.27 ± 0.14^{c}	8.47 ± 0.24^{b}	4.91 ± 0.11^{d}
5	Cornuside (Cn)	42.0	239, 272	Cn	69.35 ± 1.60^{d,e}	125.87 ± 3.27^{b}	59.41 ± 1.37^{e}	71.42 ± 1.44^{d}	113.37 ± 2.29^{c}	162.51 ± 4.69^{a}	121.02 ± 3.49^{b,c}
6	Delphinidin 3-*O*-galactoside (Dp3gal)	13.9	525	Cy3glu	1.42 ± 0.03^{b,c}	0.93 ± 0.02^{b,c}	0.88 ± 0.02^{b,c}	0.19 ± 0.00^{c}	2.19 ± 0.04^{b}	1.99 ± 0.06^{b}	29.84 ± 0.86^{a}
7	Cyanidin 3-*O*-galactoside (Cy3gal)	15.7	518	Cy3glu	21.89 ± 0.63^{f}	105.20 ± 2.43^{d}	77.10 ± 1.56^{d,e}	36.63 ± 0.85^{e,f}	153.46 ± 3.99^{c}	257.48 ± 5.20^{b}	968.20 ± 27.95^{a}
8	Pelargonidin 3-*O*-galactoside (Pg3gal)	18.4	505	Pg3glu	106.11 ± 2.14^{d}	52.08 ± 1.50^{e}	130.83 ± 3.02^{c}	55.31 ± 1.44^{e}	121.66 ± 3.51^{c}	144.33 ± 2.92^{b}	211.06 ± 4.87^{a}
9	Pelargonidin 3-*O*-robinobioside (Pg3rob)	21.7	503	Pg3glu	3.76 ± 0.10^{d}	3.21 ± 0.06^{d,e}	8.84 ± 0.18^{b}	2.88 ± 0.08^{e}	7.46 ± 0.17^{c}	6.93 ± 0.20^{c}	10.09 ± 0.23^{a}
10	Kaempferol 3-*O*-galactoside (Kf3gal)	41.2	342	Q3gal	0.38 ± 0.01^{a}	0.13 ± 0.00^{d}	0.32 ± 0.01^{b}	0.13 ± 0.00^{d}	0.25 ± 0.01^{c}	0.25 ± 0.01^{c}	0.26 ± 0.01^{c}
11	Gallic acid der (GA der)	3.6	275	GA	0.31 ± 0.01^{d,e}	0.38 ± 0.01^{c,d,e}	0.26 ± 0.01^{e}	1.70 ± 0.05^{b}	0.46 ± 0.01^{c}	0.43 ± 0.01^{c,d}	2.86 ± 0.07^{a}
12	Gallic acid (GA)	5.0	270	GA	0.18 ± 0.00^{d}	0.36 ± 0.01^{c}	0.10 ± 0.00^{e}	0.62 ± 0.02^{b}	0.61 ± 0.01^{b}	0.61 ± 0.02^{b}	0.77 ± 0.02^{a}
13	*cis*-Caftaric acid (*c*CftA)	10.3	327	CA	0.16 ± 0.00^{c}	0.12 ± 0.00^{d}	0.12 ± 0.00^{d}	0.19 ± 0.00^{b}	0.11 ± 0.00^{d}	0.15 ± 0.00^{c}	0.23 ± 0.01^{a}
14	*trans*-Caftaric acid (*c*CftA)	13.6	327	CA	0.91 ± 0.03^{b}	0.37 ± 0.01^{d,e}	0.40 ± 0.01^{d}	0.66 ± 0.02^{c}	0.30 ± 0.01^{e}	0.44 ± 0.01^{d}	1.22 ± 0.04^{a}
15*	*p*-Coumaric acid derivatives (*p*CoAsum)	10.6–17.7	308–310	*p*CoA	0.21 ± 0.00 ^{g}	0.26 ± 0.00^{f}	0.36 ± 0.00^{d}	0.27 ± 0.00^{e}	0.41 ± 0.01^{c}	0.44 ± 0.00^{b}	0.67 ± 0.00^{a}
16	Ellagic acid (EA)	38.3	254	EA	1.09 ± 0.03^{e}	2.80 ± 0.06^{c}	0.43 ± 0.01^{f}	2.06 ± 0.05^{d}	1.33 ± 0.03^{e}	3.32 ± 0.07^{b}	5.40 ± 0.16^{a}
	TOTAL				1089.98 ± 18.74^{d}	1477.64 ± 19.44^{b}	794.76 ± 17.01^{f}	1194.36 ± 22.19^{c}	988.82 ± 6.99^{e}	1415.90 ± 14.80^{b}	2478.24 ± 5.19^{a}

Results are given as mg of the compound per 100 g lyophilized fruit; mean value ± SEM. Different small superscripts within the same column denote values that differ significantly according to Duncan's test ($p < 0.01$).

Biological activities of cornelian cherry fruit extracts

Biological activities of cornelian cherry fruit extracts - antioxidant activity (FC, FRAP, DPPH), antiproliferative activity on HT29 cells, inhibition of α-amylase, α-glucosidase and DPP III enzymes.

Activity	Calculated as	Apatinski rani	Bačka	KIC1	R1	Elegantnyi	Semen	Svetlyachok
FC	mg GAE/g LF	10.15 ± 0.33^{e}	15.01 ± 0.34^{b}	$10.99 \pm 0.09^{d,e}$	11.80 ± 0.29^{d}	11.25 ± 0.05^{d}	14.08 ± 0.03^{c}	21.68 ± 0.11^{a}
FRAP	mg AAE/g LF	7.83 ± 0.34^{f}	$14.37 \pm 0.16^{c,d}$	9.11 ± 0.13^{f}	11.71 ± 0.96^{e}	$12.22 \pm 0.66^{d,e}$	$15.63 \pm 0.67^{b,c}$	23.95 ± 0.32^{a}
DPPH	IC_{50} (mg/mL)*	3.17 ± 0.01^{f}	1.83 ± 0.02^{b}	3.59 ± 0.03^{g}	1.94 ± 0.01^{c}	2.79 ± 0.04^{e}	2.10 ± 0.01^{d}	1.76 ± 0.01^{a}
HT29	EC_{50} (mg/mL)*	12.69 ± 0.26^{c}	9.93 ± 0.23^{b}	13.97 ± 0.32^{c}	13.68 ± 0.28^{c}	13.07 ± 0.38^{c}	13.48 ± 0.31^{c}	9.14 ± 0.21^{a}
α-Amy	IC_{50} (mg/mL)*	$8.00 \pm 0.23^{c,d}$	3.56 ± 0.15^{a}	13.81 ± 0.49^{e}	7.71 ± 0.52^{c}	11.65 ± 1.21^{e}	$10.34 \pm 0.85^{d,e}$	4.31 ± 0.12^{b}
α-Gls	IC_{50} (mg/mL)*	0.29 ± 0.01^{b}	0.37 ± 0.01^{c}	0.19 ± 0.00^{a}	0.28 ± 0.01^{b}	0.37 ± 0.01^{c}	0.33 ± 0.01^{c}	0.19 ± 0.00^{a}
DPP III	IC_{50} (μg/mL)**	ND	40.45 ± 0.09^{b}	180.12 ± 4.63^{d}	42.82 ± 0.10^{b}	$101.62 \pm 0,89^{c}$	44.12 ± 0.22^{b}	33.86 ± 0.17^{a}

Results are given as mean value ± SEM. Different small superscripts within the same column denote values that differ significantly according to Duncan's test ($p < 0.01$). ND = not detected.

* mg of lyophilized fruit (LF)/mL of the added extract.

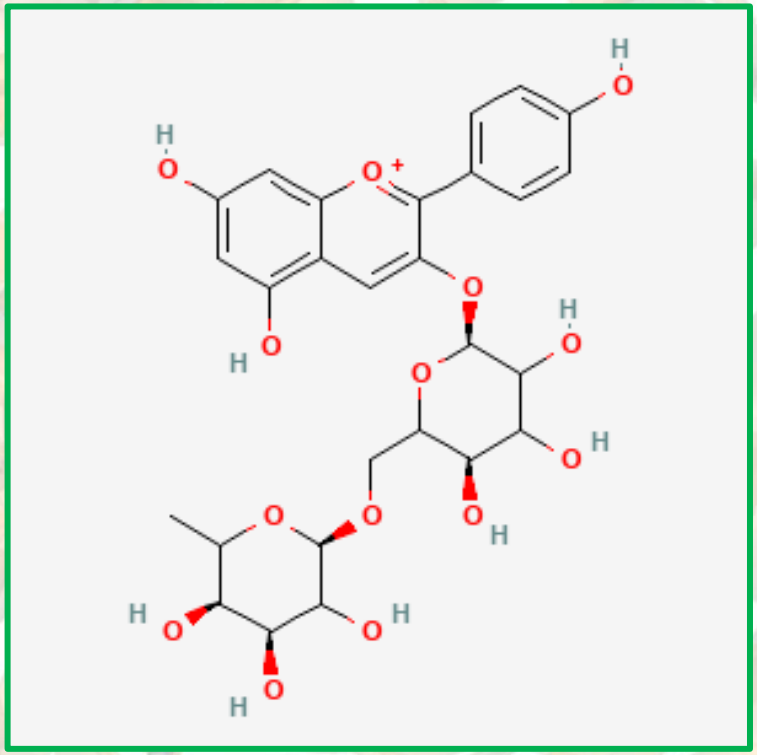
** μg of lyophilized fruit (LF)/mL of the reaction medium.

Abbreviations: FC, Folin-Ciocalteu reducing capacity; FRAP, ferric reducing antioxidant power; DPPH, scavenging capacity of DPPH radical; HT29, antiproliferative effect on human colon cancer cells; α-Amy, α-amylase inhibition activity; α-Gls, α-glucosidase inhibition activity; DPP III, dipeptidyl peptidase III inhibition activity; LF, lyophilized fruit; GAE, gallic acid equivalents; AAE, ascorbic acid equivalents; EC_{50}, effective sample concentration necessary to decrease 50% of the cellular viability; IC_{50}, concentration that inhibits 50% of the enzyme activity, or neutralizes 50% of the DPPH radical.

Molecular docking

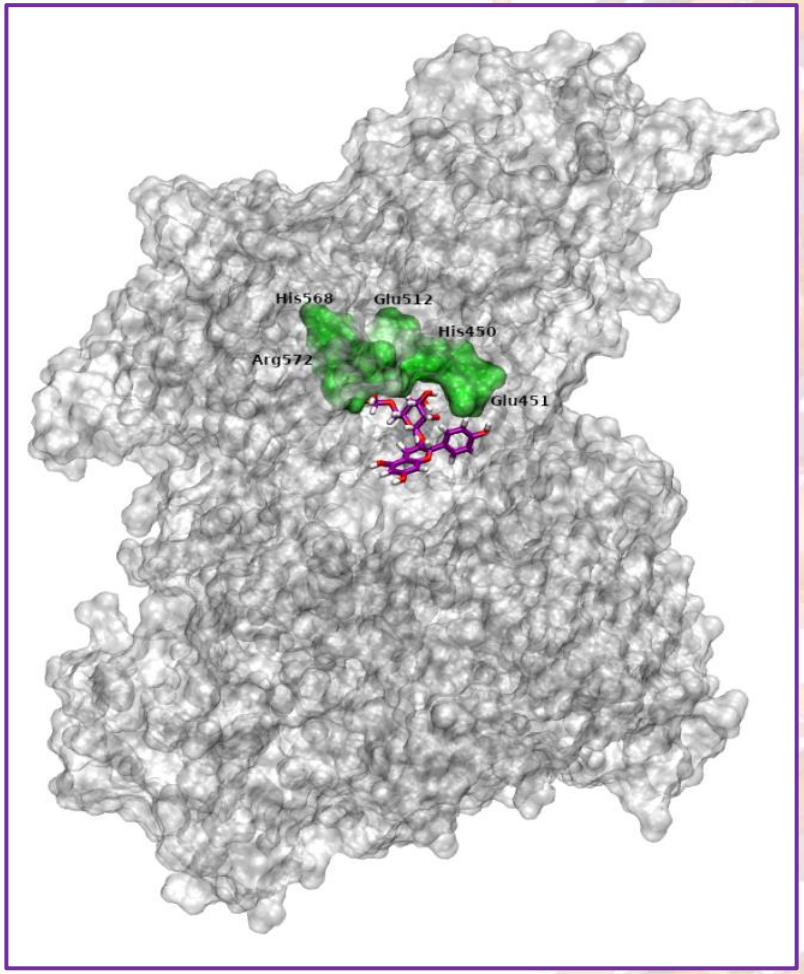
- - 3D structure of hDPP III (PDB ID: 3FVY, resolution 1.9 Å)
- - Compounds (PubChem)
- - AutoDock Vina 1.1.2
- - VMD 1.9.3 & LigPlot+ (visualization & ligand-receptor interactions)

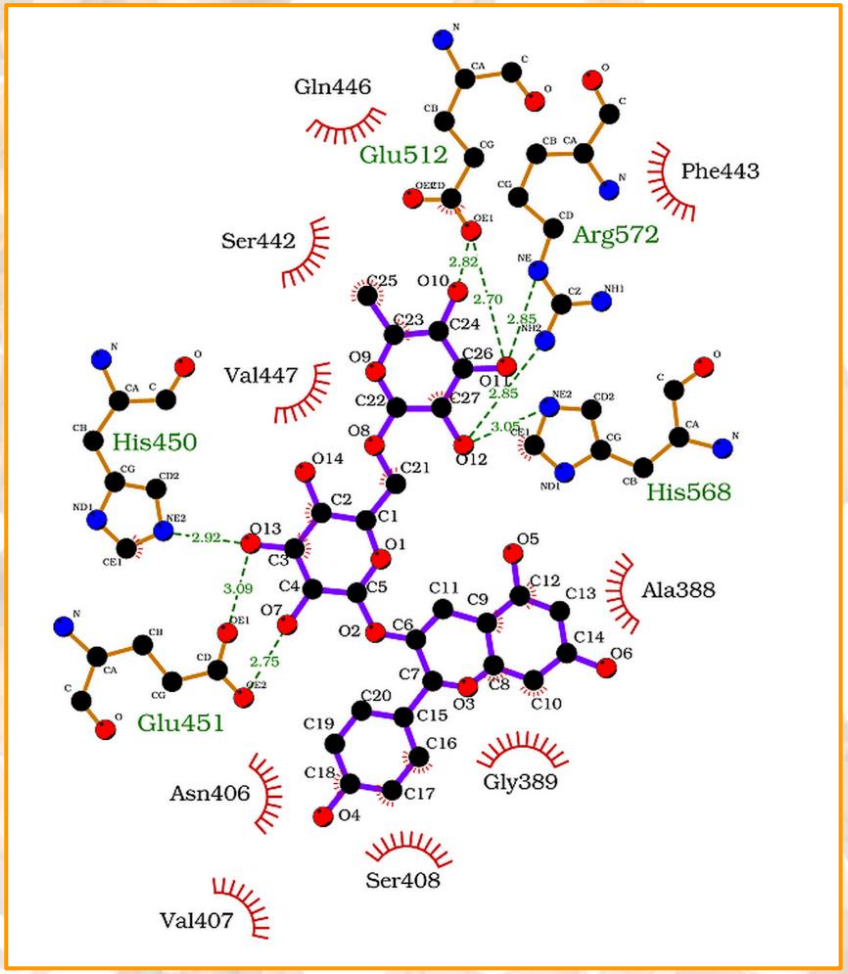
Constituents	Binding energy kcal/mol
Loganic acid (LA)	-7.3
Sweroside (Sw)	-7.8
Loganin (Ln)	-6.8
Cornuside (Cn)	-8.4
Secoxyloganin (Sec)	-6.4
Delphinidin 3-galactoside (Dp3gal)	-7.6
Cyanidin 3-galactoside (Cy3gal)	-7.8
Pelargonidin 3-galactoside (Pg3gal)	-7.1
Pelargonidin 3-robinobioside (Pg3rob)	-9.0
Gallic acid (GA)	-6.2
Ellagic acid (EA)	-7.5
cis-Caftaric acid (cCftA)	-7.4
trans-Caftaric acid (tCftA)	-7.2
Caftaric acid (CftA)	-7.3
Caffeic acid (CA)	-6.2
p-Coumaric acid (pCoA)	-5.9
4-p-Coumaroylquinic acid (4pCoQA)	-8.6
Kaempferol 3-galactoside (Kf3gal)	-7.9



Pelargonidin 3-robinobioside

Best docking pose for pelargonidin-3-robinobioside at the hDPP III binding site





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Exploring fruits from genus *Prunus* as a source of potential pharmaceutical agents – *In vitro* and *in silico* study

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In vitro

- **- fruit extract preparation in solvent (water / etOH = 1:1)**
- **- UPLC qTOF- MS/MS analysis**
- **- antioxidant activity (FC, FRAP, DPPH)**
- **- antiproliferative activity on human colon cancer HT29 cell line**
- **- inhibition of α amylase, α glucosidase and hDPP III**

In silico

- **- molecular docking (feasibility of selected compounds from fruit extract to form complex with α amylase, α glucosidase and hDPP III)**

Determination of phenolic compounds

Quantitative amounts of phenolic compounds found in *Prunus* fruits.

No		plum	'vlaškača'	damson plum	white damson	purple-leaf cherry plum	red cherry plum	white cherry plum	sweet cherry	sweet cherry, wild type	sour cherry	steppe cherry	mahaleb cherry	blackthorn	peach	apricot
1	3-*O*-Caffeoylquinic acid	3.39^{efg}	70.59^{b}	156.92^{a}	69.62^{b}	0.79 ^{g}	2.87 ^{fg}	7.88^{e}	1.38 ^{g}	7.27^{ef}	1.12 ^{g}	–	–	21.50^{d}	36.93^{c}	1.50 ^{g}
2	5-*O*-Caffeoylquinic acid	–	3.90^{d}	9.64^{b}	5.49^{c}	–	0.74^{f}	10.08^{b}	–	–	–	–	–	–	22.42^{a}	2.21^{e}
3	Methyl 3-*O*-caffeoylquinate	86.87^{d}	105.34^{c}	58.25^{e}	42.45^{f}	–	–	–	56.68^{e}	142.00^{b}	22.53 ^{g}	–	–	271.04^{a}	–	–
4	Ferulic acid hexoside	–	–	–	–	–	–	–	–	–	–	15.30^{a}	–	–	–	–
5	*m*-Coumaric acid hexoside	–	–	–	–	–	–	–	–	–	–	–	374.65^{a}	–	–	–
6	Methyl 3-*O*-*p*-coumaroylquinate	5.25^{e}	2.24^{f}	2.41^{f}	1.58^{f}	–	–	–	–	10.05^{d}	20.15^{c}	23.46^{b}	–	31.33^{a}	–	–
7	Methyl 4-*O*-caffeoylquinate	6.07^{d}	7.35^{c}	6.91^{c}	5.75^{de}	–	–	–	5.36^{e}	10.58^{b}	–	–	–	22.06^{a}	–	–
8	Ethyl 4-*O*-caffeoylquinate	3.79^{e}	3.95^{c}	2.08^{d}	2.52^{d}	–	–	–	6.59^{b}	–	–	–	–	17.48^{a}	–	–
9	Methyl 5-*O*-caffeoylquinate	3.81^{de}	6.18^{d}	2.29^{e}	2.18^{e}	–	0.25^{e}	3.54^{de}	3.48^{de}	10.13^{c}	43.17^{b}	147.10^{a}	–	2.10^{e}	–	–
10	Ferulic acid hexoside	–	–	–	–	–	–	–	–	–	–	65.93^{a}	–	–	–	–
11	Methyl 4-*O*-*p*-coumaroylquinate	–	–	–	–	–	–	–	0.75^{b}	–	0.90^{b}	25.37^{a}	–	–	–	–
12	Methyl 5-*O*-*p*-coumaroylquinate 1	–	–	–	–	–	–	–	–	–	Tr	25.37^{a}	–	–	–	–
13	Methyl 5-*O*-*p*-coumaroylquinate 2	–	–	–	–	–	–	–	–	–	–	8.32^{a}	–	–	–	–
	Total phenolic acids	**109.18** ^{g}	**199.56**^{d}	**238.51**^{d}	**129.59**^{f}	**0.79** ^{k}	**3.86**^{jk}	**21.50**^{j}	**74.24**^{gh}	**180.02**^{e}	**87.88** ^{h}	**310.86**^{c}	**374.65**^{b}	**365.50**^{a}	**59.35**^{i}	**3.71**^{jk}
14	Quercetin 3-*O*-(2″,6″-dirhamnosyl)hexoside	–	–	–	–	–	–	–	–	–	–	–	41.23^{a}	–	–	–
15	Quercetin 3-*O*-pentosyl-hexoside 1	–	–	–	–	–	–	–	–	–	–	–	7.09^{a}	–	–	–
16	Kaempferol 3-*O*-(2″,6″-dirhamnosyl)hexoside	–	–	–	–	–	–	–	–	–	–	–	6.39^{a}	–	–	–
17	Quercetin 3-*O*-rhamnosyl (1 → 6)hexoside	–	–	8.37^{a}	6.22^{b}	–	–	–	–	–	–	–	–	–	–	–
18	Quercetin 3-*O*-rutinoside	17.46^{f}	23.91^{e}	33.93^{d}	6.23 ^{h}	2.73^{ij}	3.05^{ij}	16.96^{f}	4.87^{hi}	25.28^{e}	12.83 ^{g}	42.03^{b}	52.69^{a}	36.50^{c}	2.10^{ij}	19.17^{f}
19	Quercetin 3-*O*-pentosyl-hexoside 2	–	–	–	–	–	–	–	–	–	–	–	–	3.41^{a}	–	–
20	Quercetin 3-*O*-glucoside	–	2.13^{f}	8.01^{d}	0.63 ^{h}	Tr	Tr	1.94 ^{fg}	–	8.86^{c}	0.84 ^{h}	3.73^{e}	26.53^{a}	12.12^{b}	1.91 ^{fg}	1.19^{gh}
21	Quercetin pentoside	–	–	–	–	0.54^{e}	0.46^{e}	2.74^{b}	–	–	–	–	–	3.58^{a}	–	–
22	Kaempferol 3-*O*-rutinoside	0.92^{e}	–	Tr	–	–	–	–	–	6.04^{c}	1.99^{d}	22.96^{a}	6.77^{b}	–	–	–
23	Isorhamnetin 3-*O*-rutinoside	2.83^{d}	3.91^{c}	Tr	0.98^{e}	–	Tr	–	–	–	7.14^{b}	38.27^{a}	–	5.42^{b}	0.88^{e}	–
24	Quercetin pentoside	–	–	–	–	3.61^{b}	3.38^{c}	15.67^{a}	–	–	–	–	–	–	–	–
25	Quercetin rhamnoside	–	–	–	–	Tr	–	1.66^{b}	–	–	–	–	–	3.30^{a}	–	–
26	Quercetin	–	–	–	–	–	–	4.16^{b}	Tr	0.83^{e}	0.70^{e}	2.31^{c}	5.57^{a}	2.21^{d}	–	–
	Total flavonols	**21.21** ^{g}	**29.95**^{f}	**50.30**^{d}	**14.06** ^{h}	**6.88**^{i}	**6.89**^{i}	**43.13**^{e}	**4.87**^{i}	**41.01**^{e}	**23.50** ^{g}	**109.30**^{b}	**146.27**^{a}	**66.53**^{c}	**4.90**^{i}	**20.36** ^{g}
27	Cyanidin 3-*O*-sophoroside	–	–	–	–	–	–	–	–	–	9.92^{a}	4.68^{b}	–	–	–	–
28	Cyanidin 3-*O*-(2′-glucosyl)rutinoside	–	–	–	–	–	–	–	–	–	207.15^{a}	173.05^{b}	–	–	–	–
29	Cyanidin 3-*O*-glucoside	–	–	2.23^{d}	–	–	–	–	2.02^{d}	67.67^{b}	3.02^{d}	3.62^{d}	195.23^{a}	58.35^{c}	Tr	–
30	Cyanidin 3-*O*-(2′-xylosyl) rutinoside	–	–	–	–	–	–	–	–	–	5.47^{b}	2.14^{c}	86.16^{a}	–	–	–
31	Cyanidin 3-*O*-rutinoside	–	1.10^{f}	3.67^{f}	–	1.09^{f}	Tr	–	41.68^{e}	267.40^{a}	98.14^{d}	116.39^{c}	172.48^{b}	74.25^{d}	–	–
32	Pelargonidin 3-*O*-rutinoside	–	–	–	–	–	–	–	0.68^{b}	4.45^{a}	–	–	–	–	–	–
33	Peonidin 3-*O*-glucoside	–	–	Tr	–	–	–	–	–	–	–	–	–	8.34^{a}	–	–
34	Peonidin 3-*O*-rutinoside	Tr	2.65^{de}	2.32^{e}	–	–	–	–	2.76^{d}	12.38^{b}	9.95^{c}	10.77^{c}	–	30.36^{a}	–	–
	Total anthocyanins	**Tr**	**3.76** ^{g}	**8.22** ^{g}	**–**	**1.09** ^{g}	**Tr**	**–**	**47.14**^{f}	**351.90**^{b}	**333.64**^{c}	**310.65**^{d}	**453.88**^{a}	**171.29**^{e}	**Tr**	**–**
	Total	**130.39** ^{g}	**233.27**^{f}	**297.03**^{e}	**143.65** ^{g}	**8.76**^{i}	**10.75**^{i}	**64.63** ^{h}	**126.24** ^{g}	**572.94**^{c}	**445.01**^{d}	**730.81**^{b}	**974.79**^{a}	**603.33**^{c}	**64.24** ^{h}	**24.07**^{i}

Results are expressed as mg/100 g of liophylized fruit (LF). Values are means ($n = 3$), the relative standard deviations for all compounds were < 5%. Values in the same row with different letters are significantly different at $p < 0.01$. Abbreviation Tr – in traces.

Biological activities of Prunus fruits

Bioactive potential of *Prunus* fruits: Antioxidant capacity (FC, FRAP, DPPH), antiproliferative effect in HT29 cells, inhibition activity of α-amylase, α-glucosidase, and dipeptidyl peptidase III.

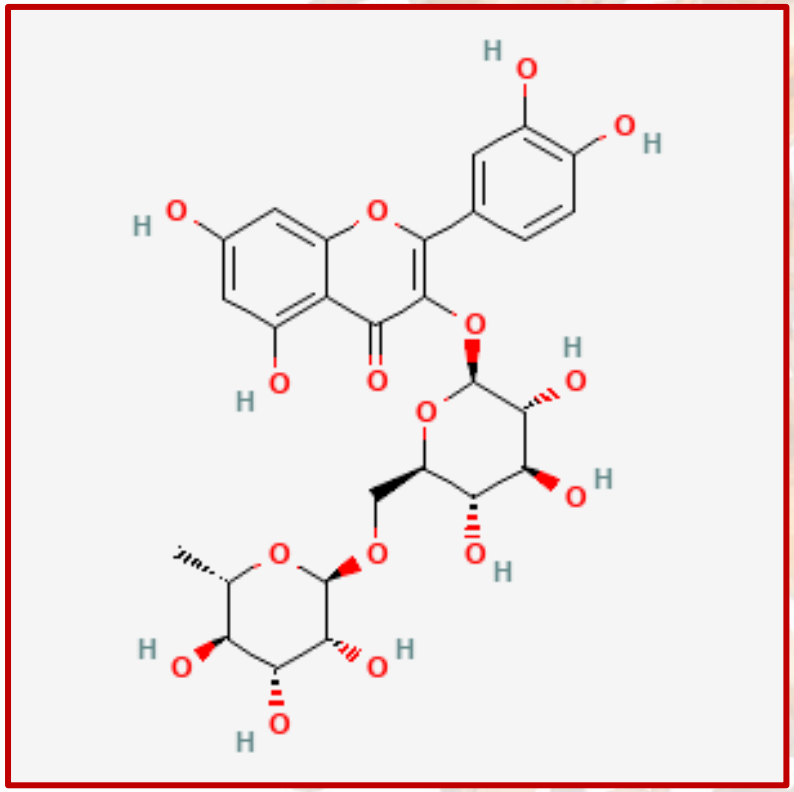
Bioactivity	FC	FRAP	DPPH	HT29	α-Amy	α-Gls	DPP III
Unit	mg GAE/g LF	mg AAE/g LF	IC_{50} (mg/mL) *	EC_{50} (mg/mL)*	IC_{50} (mg/mL) *	IC_{50} (mg/mL) *	IC_{50} (μg/mL) **
plum	5.99 ± 1.59^{g}	2.63 ± 0.31^{gh}	5.71 ± 0.13^{j}	23.27 ± 1.05^{f}	46.69 ± 4.33^{d}	5.72 ± 0.25^{gh}	–
'vlaškača'	10.81 ± 0.38^{d}	6.32 ± 0.66^{e}	2.47 ± 0.06^{f}	15.69 ± 0.55^{d}	45.54 ± 3.33^{d}	1.48 ± 0.07^{e}	101.71 ± 2.71^{e}
damson plum	10.29 ± 0.13^{de}	5.38 ± 0.29^{ef}	3.55 ± 0.02^{g}	21.40 ± 0.96^{ef}	103.45 ± 5.43^{e}	1.39 ± 0.06^{e}	69.44 ± 1.67^{c}
white damson	8.43 ± 1.37^{ef}	3.76 ± 0.62^{fg}	4.04 ± 0.03^{h}	30.88 ± 1.36^{g}	$43.63 \pm 3.88^{d,e}$	4.11 ± 5.98^{fg}	78.49 ± 2.24^{d}
purple-leafcherry plum	2.96 ± 1.52^{hi}	1.35 ± 0.38^{h}	28.82 ± 0.37^{h}	24.03 ± 1.08^{f}	136.23 ± 17.34^{e}	28.44 ± 1.22^{i}	179.60 ± 11.60^{g}
white cherry plum	3.78 ± 0.27^{hi}	2.37 ± 0.47^{gh}	15.75 ± 0.21^{m}	19.80 ± 0.69^{e}	–	6.18 ± 0.27^{gh}	213.67 ± 14.32^{g}
red cherry plum	2.25 ± 0.07^{i}	1.95 ± 0.32^{gh}	29.12 ± 0.50^{n}	11.65 ± 0.50^{b}	40.35 ± 3.07^{d}	10.15 ± 0.46^{hi}	129.90 ± 2.61^{f}
sweet cherry	6.54 ± 0.58^{fg}	4.20 ± 0.59^{fg}	5.19 ± 0.09^{i}	50.27 ± 2.26^{h}	41.80 ± 5.20^{d}	3.06 ± 0.11^{f}	–
sweet cherry, wild type	17.45 ± 0.42^{c}	17.69 ± 0.92^{c}	1.32 ± 0.03^{c}	29.25 ± 1.26^{g}	37.46 ± 4.45^{d}	1.40 ± 0.05^{e}	–
sour cherry	15.81 ± 1.50^{c}	14.99 ± 0.90^{d}	2.31 ± 0.04^{e}	13.12 ± 0.58^{c}	18.44 ± 1.86^{c}	0.62 ± 0.69^{b}	–
steppe cherry	28.78 ± 0.16^{a}	27.23 ± 1.58^{a}	0.83 ± 0.00^{a}	6.68 ± 0.29^{a}	4.61 ± 0.39^{b}	0.41 ± 0.02^{a}	33.76 ± 0.06^{b}
mahaleb cherry	16.72 ± 0.98^{c}	19.67 ± 1.44^{b}	1.66 ± 0.03^{d}	52.70 ± 1.84^{h}	43.95 ± 7.10^{d}	0.96 ± 0.04^{d}	$196.23 \pm 4{,}31^{g}$
blackthorn	26.36 ± 0.82^{b}	20.89 ± 0.67^{b}	1.18 ± 0.02^{b}	6.84 ± 0.30^{a}	1.11 ± 0.04^{a}	0.78 ± 0.03^{c}	27.49 ± 0.04^{a}
peach	4.84 ± 0.45^{gh}	3.30 ± 0.16^{g}	6.53 ± 0.16^{k}	32.56 ± 1.14^{g}	–	3.89 ± 0.18^{fg}	205.93 ± 6.55^{g}
apricot	$3{,}80 \pm 0{,}63^{hi}$	3.06 ± 0.27^{gh}	12.28 ± 0.13^{l}	11.56 ± 0.51^{b}	–	–	–
Reference compound*			Trolox 0.04 ± 0.00		Acarbose 0.11 ± 0.01	Acarbose 3.73 ± 0.08	

Results are given as mean value ± SD ($n = 3$). Values in the same row with different letters are significantly different at $p < 0.01$. * mg of lyophilized fruit (LF)/mL of the added extract. ** μg of lyophilized fruit (LF)/mL of the reaction medium.

Abbreviations: FC, Folin-Ciocalteu reducing capacity; FRAP, ferric reducing antioxidant power; DPPH, scavenging capacity of DPPH radical; HT29, antiproliferative effect in human colon cancer cells; αAmy, α-amylase inhibition activity; α-Gls, α-glucosidase inhibition activity; DPP III, dipeptidyl peptidase III inhibition activity.

Molecular docking

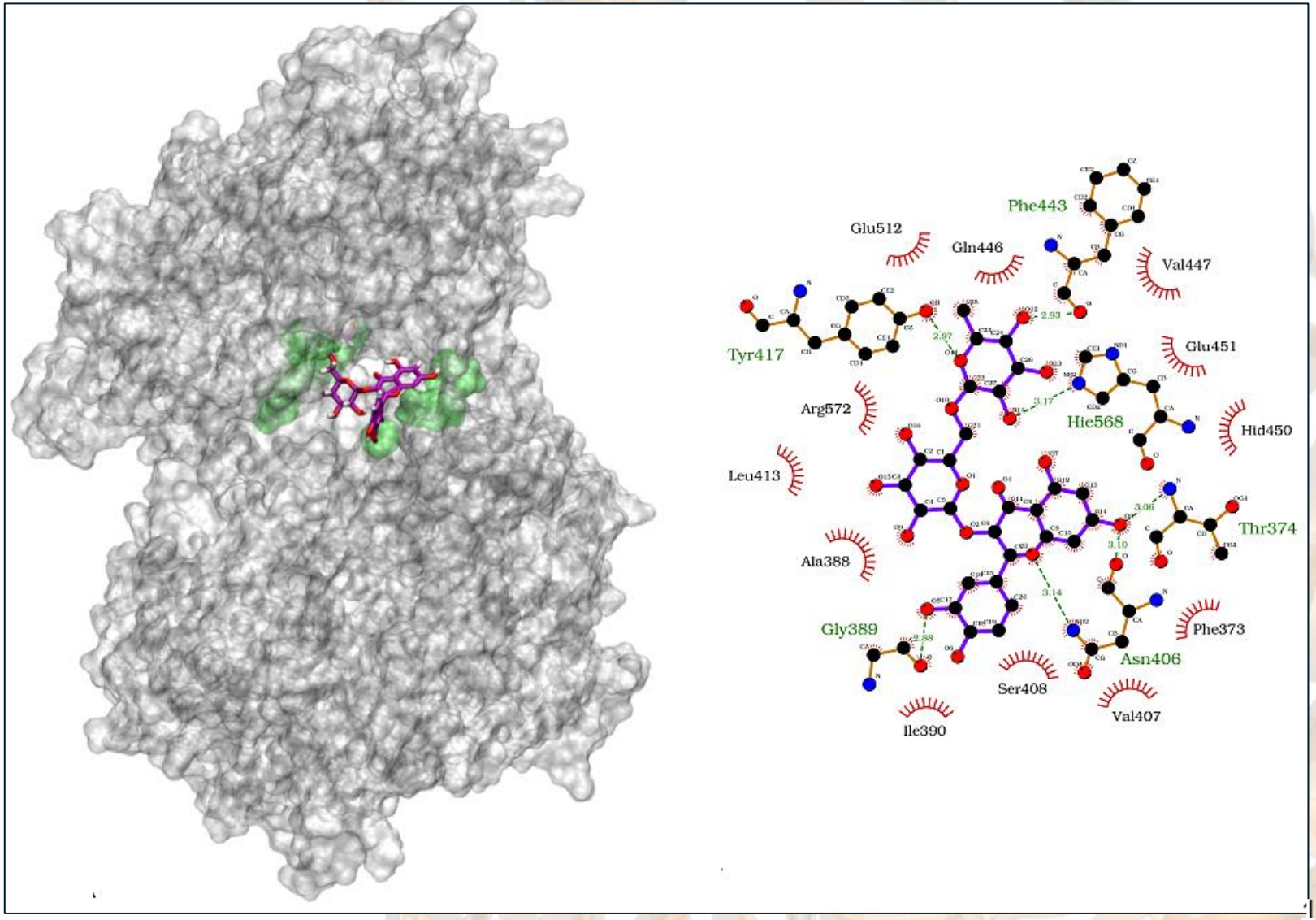
- **- 3D structure of hDPP III (PDB ID: 3FVY, resolution 1.9 Å)**
- **- Compounds (PubChem)**
- **- AutoDock Vina 1.1.2**
- **- VMD 1.9.3 & LigPlot+ (visualization & ligand-receptor interactions)**



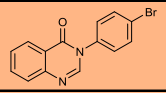
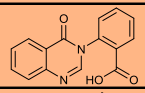
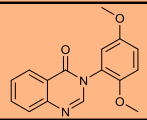
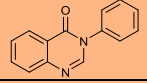
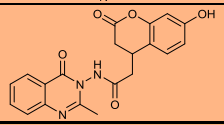
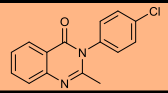
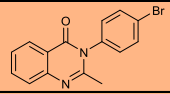
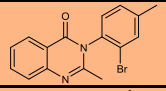
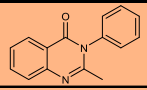
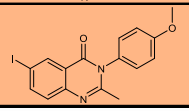
Quercetin 3-*O*-rutinoside

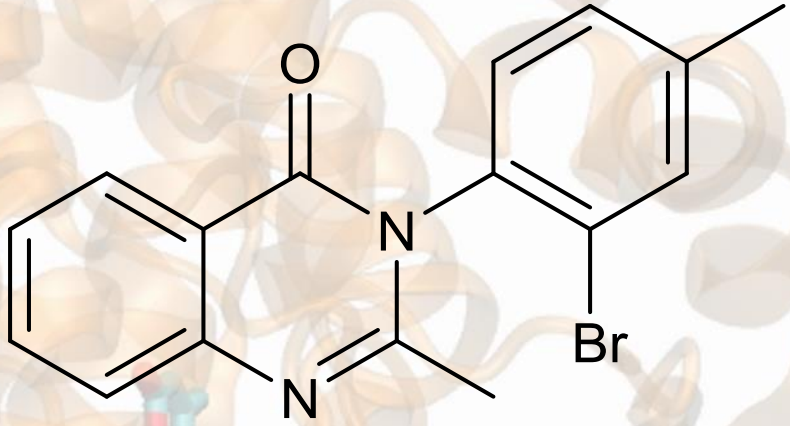
No.	Compound	Binding energy kcal/mol
1.	3-*O*-Caffeoylquinic acid	-7.9
2.	5-*O*-Caffeoylquinic acid	-7.5
3.	Methyl 3-*O*-caffeoylquinate	-7.8
4.	Methyl 4-*O*-caffeoylquinate	-8.5
5.	Ethyl 4-*O*-caffeoylquinate	-7.6
6.	Methyl 5-*O*-caffeoylquinate	-7.5
7.	Methyl 3-*O*-*p*-coumaroylquinate	-7.2
8.	Methyl 4-*O*-*p*-coumaroylquinate	-7.8
9.	Methyl 5-*O*-*p*-coumaroylquinate	-7.2
10.	3-*p*-Coumaroylquinic acid*	-7.6
11.	4-*p*-Coumaroylquinic acid*	-8.6
12.	5-*p*-Coumaroylquinic acid*	-8.0
13.	Caffeic acid*	-6.2
14.	*p*-Coumaric acid*	-5.9
15.	*o*-Coumaric acid*	-6.4
16.	*m*-Coumaric acid*	-6.1
17.	Ferulic acid*	-5.9
18.	**Quercetin 3-*O*-rutinoside**	**-9.4**
19.	Quercetin 3-*O*-glucoside	-7.4
20.	Quercetin 3-*O*- rhamnoside	-7.9
21.	Quercetin	-8.2
22.	Kaempferol 3-*O*-rutinoside	-9.3
23.	Isorhamnetin 3-*O*-rutinoside	-9.1
24.	Cyanidin 3-*O*-glucoside	-7.4
25.	Cyanidin 3-*O*-rutinoside	-8.9
26.	Cyanidin 3-*O*-sophoroside	-8.5
27.	Cyanidin 3-*O*-(2′-glucosyl)rutinoside	-8.0
28.	Cyanidin 3-*O*-(xylosyl)rutinoside	-9.1
29.	Peonidin 3-*O*-glucoside	-7.2
30.	Peonidin 3-*O*-rutinoside	-8.5
31.	Pelargonidin 3-*O*-rutinoside	-9.1

Best docking pose for quercetin - 3-O-rutinoside at the hDPP III binding site



Quinazolinone derivatives as hDPP III inhibitors

Compound	Structure	Inhibition at 100 μM concentration (%)
QD1		48.90
QD2		76.26
QD3		75.54
QD4		64.75
QD5		34.35
QD6		74.78
QD7		80.00
QD8		81.90
QD9		77.78
QD10		77.37



Compound QD8 (3-(4-bromophenyl)-2-methylquinazolin-4(3H)-one)

INACTIVATION OF HUMAN DIPEPTIDYL PEPTIDASE III BY QUINAZOLINONE DERIVATIVES

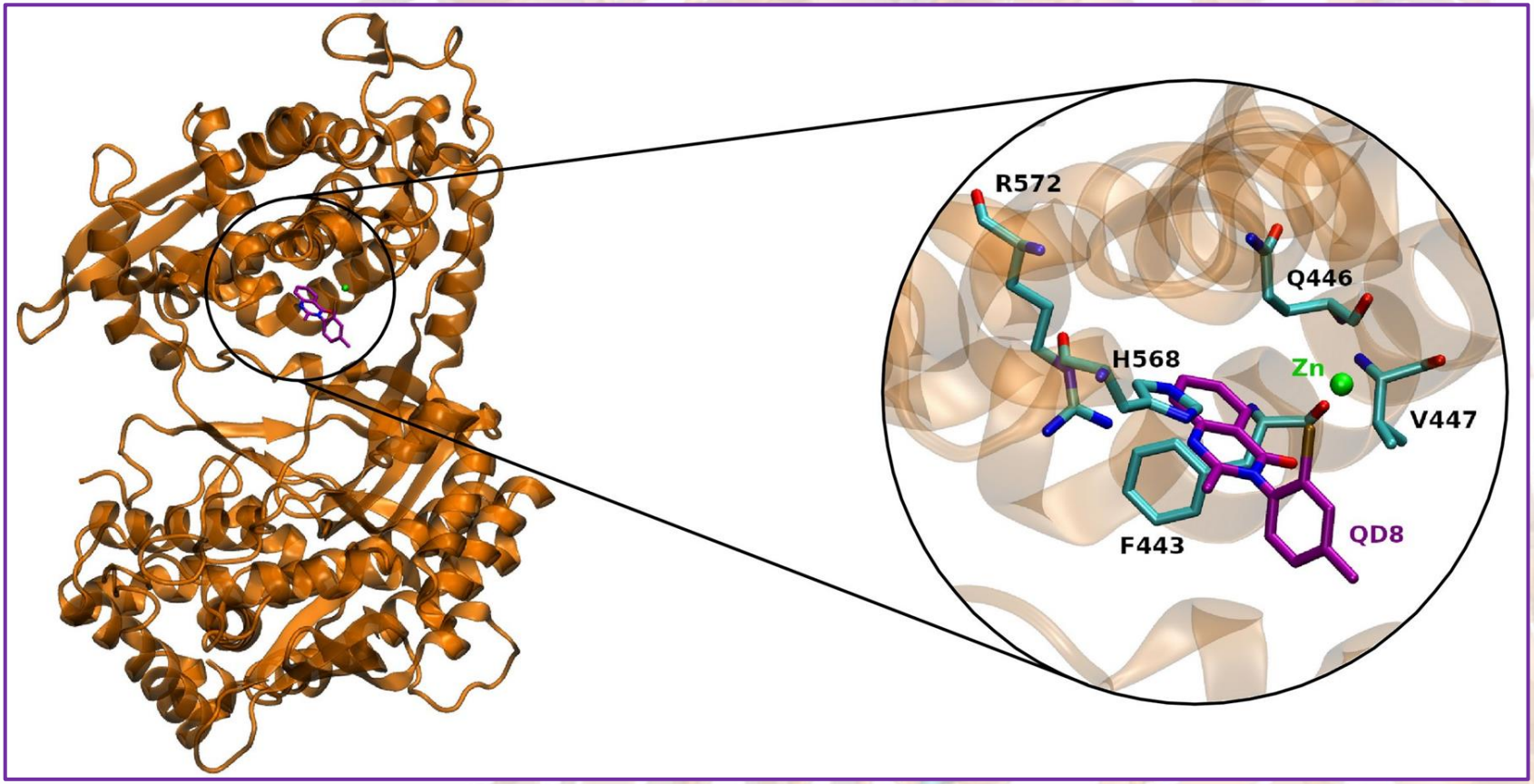
Dejan Agić, Maja Karnaš, Domagoj Šubarić, Mario Komar, Zrinka Karačić, Sanja Tomić, Maja Molnar

International conference 18th Ružička Days "Today Science – Tomorrow Industry", 2020,124.

Best docking pose for compound QD8 at the hDPP III binding site

- 3D structure of semi-closed form of hDPP III
- - Compound QD8 (Avogadro 1.2.0.)
- - AutoDock Vina 1.1.2

VMD 1.9.3 (visualization & ligand-receptor interactions)



Pharmaceuticals **2021**, *14*, 540

MDPI

Article

Coumarin Derivatives Act as Novel Inhibitors of Human Dipeptidyl Peptidase III: Combined In Vitro and In Silico Study

Dejan Agić [1,*], Maja Karnaš [1], Domagoj Šubarić [1], Melita Lončarić [2], Sanja Tomić [3], Zrinka Karačić [3], Drago Bešlo [1], Vesna Rastija [1], Maja Molnar [2], Boris M. Popović [4] and Miroslav Lisjak [1]

In vitro

- **- inhibitory potential of 40 compounds toward hDPP III**

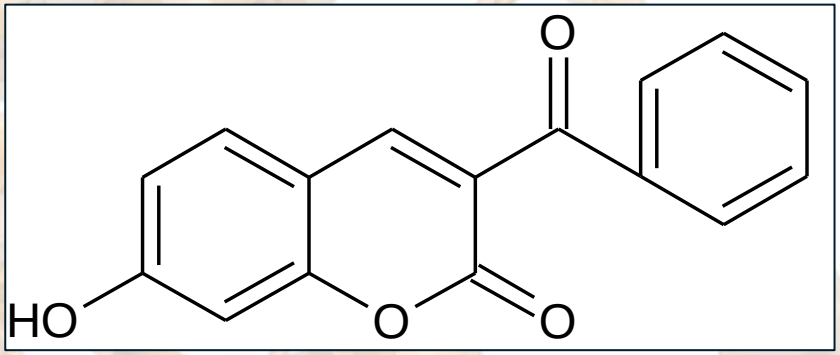
  (% inh, IC_{50} for selected compounds)

In silico

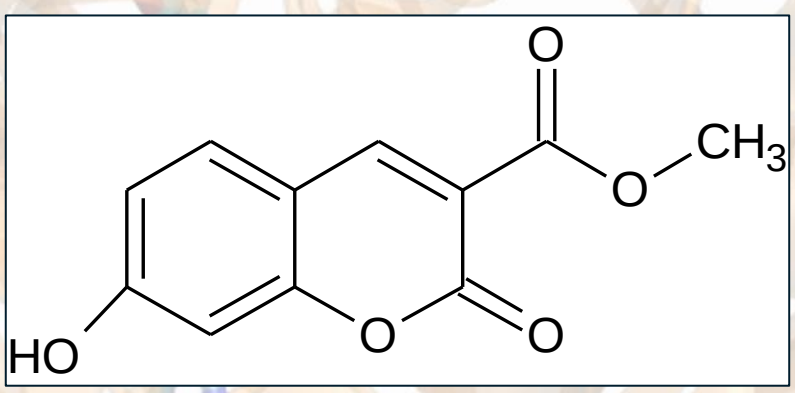
- **- QSAR**
- **- molecular docking**
- **- MD simulations**

DPP III inhibitory activity

Compound No.	Substituents	DPP III inh. (%)*
1	3-acetyl; 6-bromo	28.51
2	3-acetyl; 6-hydroxy	12.79
3	3-acetyl; 7-diethylamino	NA
4	3-acetyl; 7-hydroxy	16.17
5	3-acetyl; 8-ethoxy	NA
6	3-acetyl; 8-hydroxy	NA
7	3-acetyl	7.77
8	3-benzoyl; 6-chloro	4.41
9	3-benzoyl; 6,8-dibromo	NA
10	3-benzoyl; 6-hydroxy	67.53
11	3-benzoyl; 7-benzoyl	22,79
12	3-benzoyl; 7-hydroxy	100 (1.10 µM)
13	3-benzoyl; 7-methoxy	16.50
14	3-benzoyl; 8-ethoxy	NA
15	3-benzoyl	9.61
16	3-cyano; 6-bromo	7.93
17	3-cyano; 6-methoxy	19.82
18	3-cyano; 6-hydroxy	44.55
19	3- cyano; 7-benzoyl	7.05
20	3-cyano; 7-methoxy	NA
21	3- cyano; 8-hydroxy	62.56
22	3-cyano; 8-ethoxy	NA
23	3-cyano	NA
24	3-ethoxycarbonyl; 6-bromo	NA
25	3-ethoxycarbonyl; 6-chloro	20.08
26	3-ethoxycarbonyl; 6-dihydroxyamino	59.71
27	3-ethoxycarbonyl; 6-hydroxy	66.02
28	3- ethoxycarbonyl; 6,8-dibromo	29.41
29	3-ethoxycarbonyl; 7-methoxy	NA
30	3-ethoxycarbonyl; 8-ethoxy	NA
31	3- ethoxycarbonyl	NA
32	3- methoxycarbonyl; 6-bromo	6.48
33	3-methoxycarbonyl; 6-dihydroxyamino	21.19
34	3-methoxycarbonyl; 6-hydroxy	23.50
35	3-methoxycarbonyl; 6-methoxy	9.95
36	3-methoxycarbonyl; 7-hydroxy	100 (2.14 µM)
37	3-methoxycarbonyl; 7-methoxy	NA
38	3-methoxycarbonyl	2.26
39	coumarin	NA
40	7-hydroxy coumarin	2.14



Compound 12

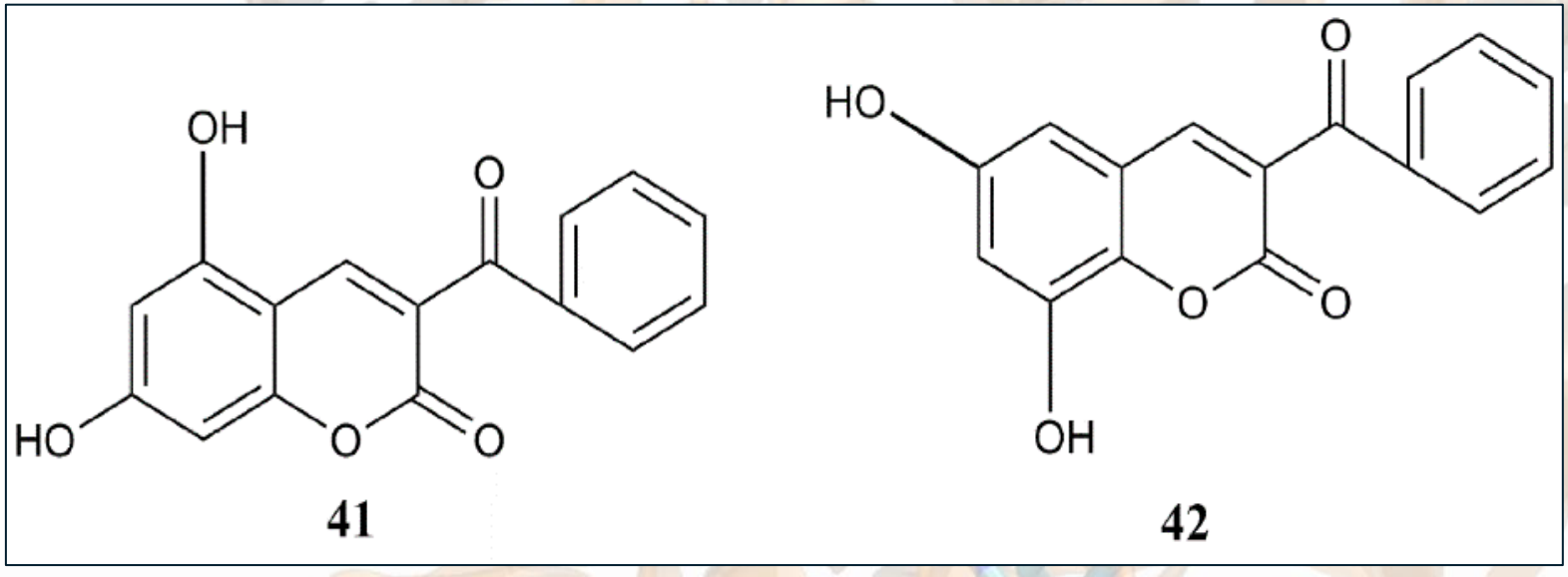


Compound 36

* at the 10 µmol L^{-1} concentration of the compound

QSAR analysis

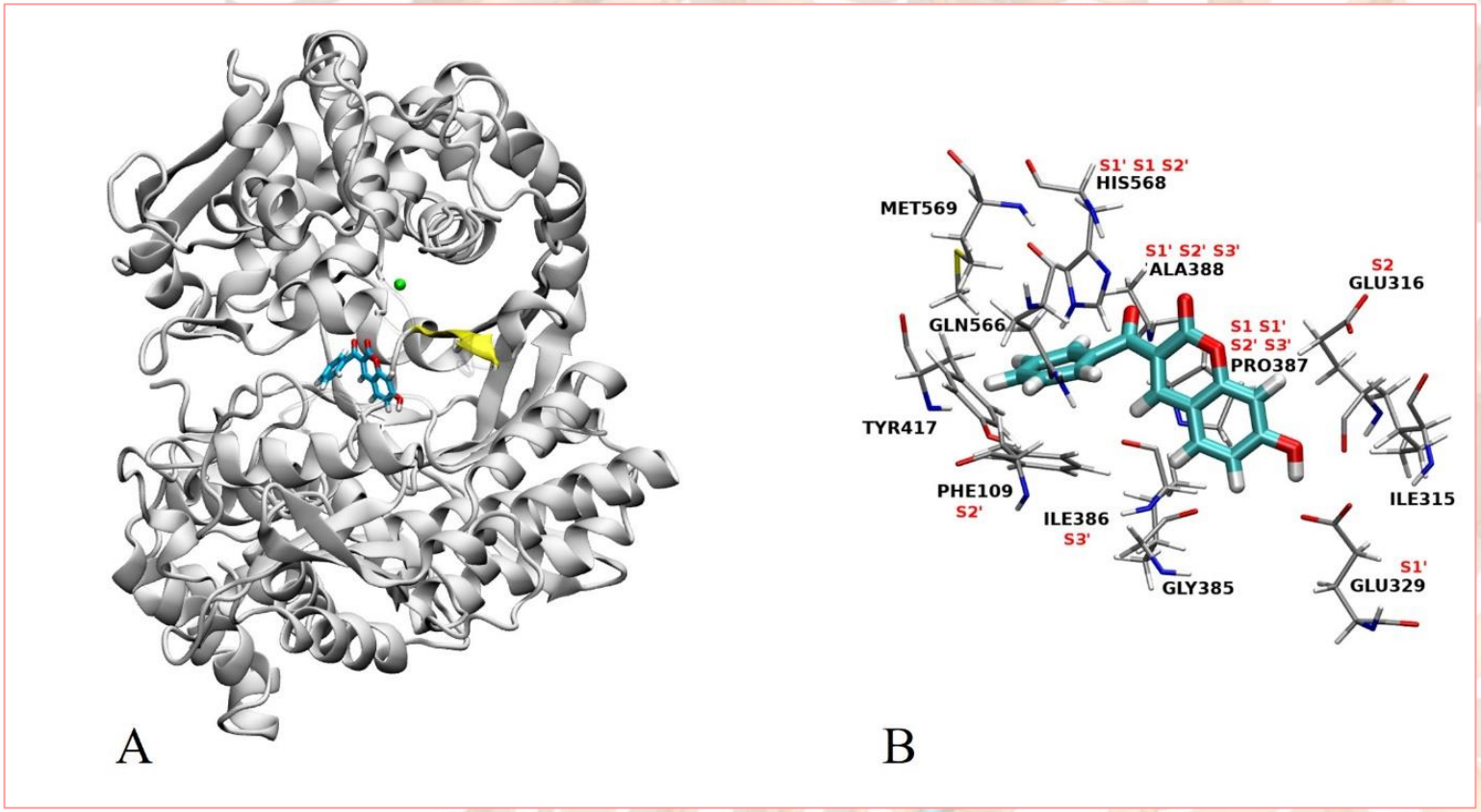
Based on the QSAR analysis, structures of two modified compounds (42 and 43) with possible improved activity are proposed, log (% inh. hDPP III) 3.08 and 3.01, respectively.



Best docking pose of compound 12 at the hDPP III binding site

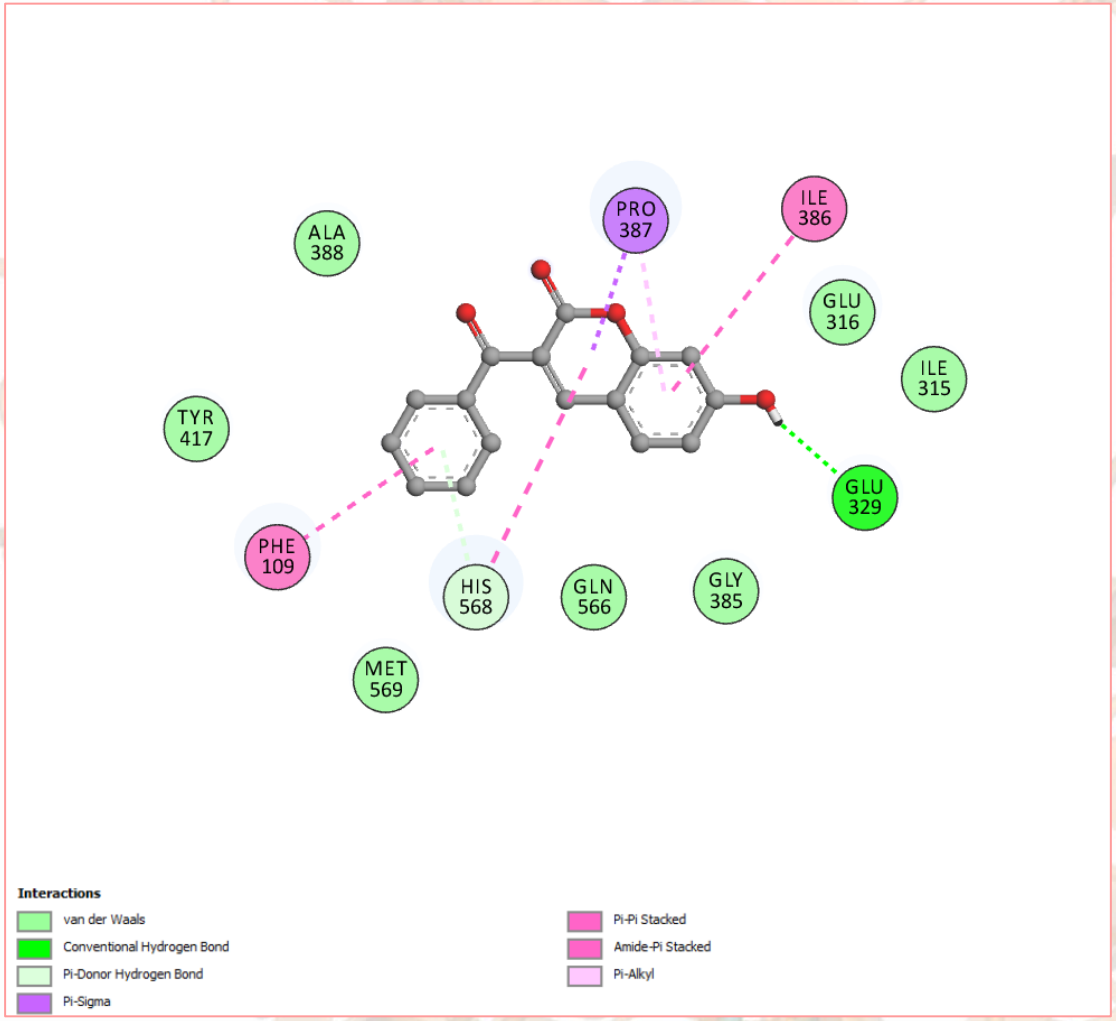
- **- 3D structure of semi-closed form of hDPP III**
- **- Compound 12 (Avogadro 1.2.0.)**
- **- AutoDock Vina 1.1.2**

VMD 1.9.3 & Discovery Studio Visualizer, version 20.1.0.19295 (visualization & ligand-receptor interactions)



(A) Best docking pose for compound 12 in the inter-domain cleft of hDPP III (-8.6 kcal/mol); (B) Potential interactions of compound 12 with amino acid residues of hDPP III. Substrate binding subsites S1, S1', S2, S2' and S3' are indicated.

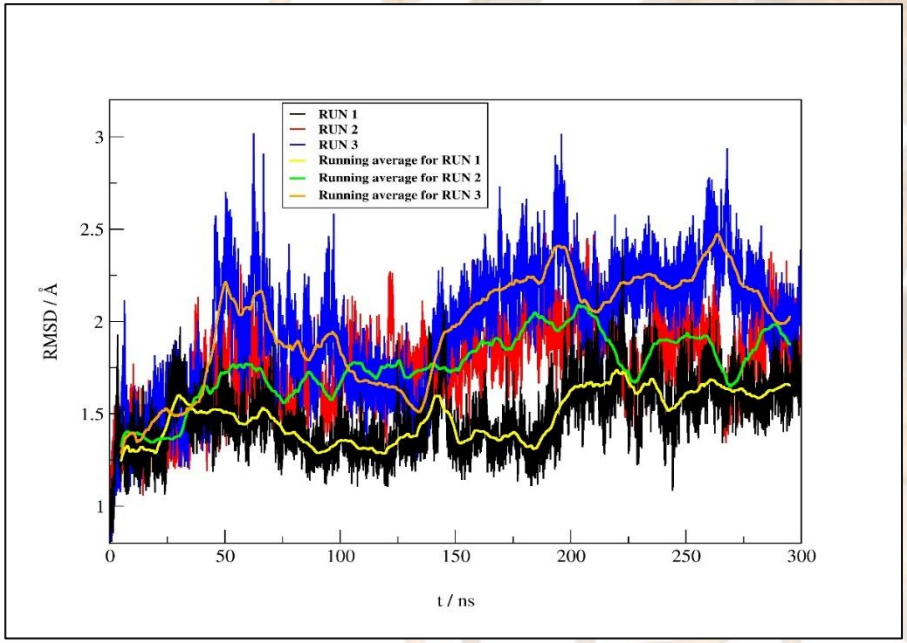
Interactions of compound 12 at the hDPP III binding site

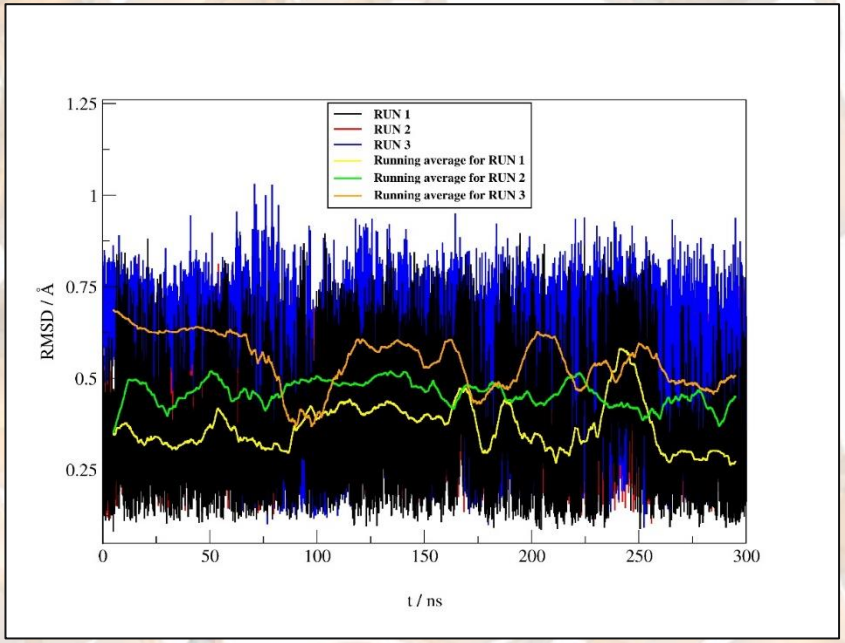


2D diagram of compound 12 interactions with the hDPP III residues for the best docking pose.

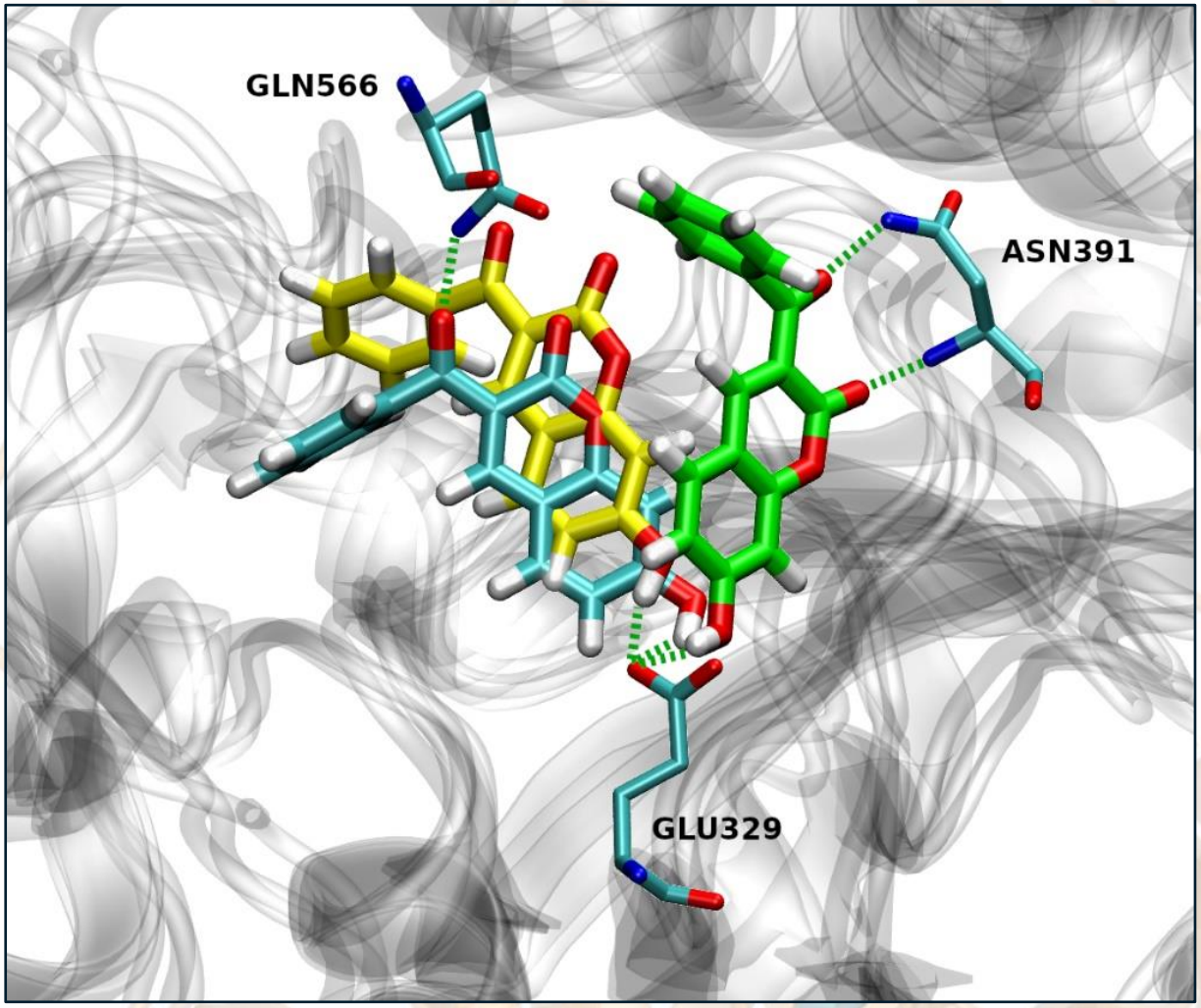
MD simulations

- - 3D structure of semi-closed form of hDPP III in complex with compound 12
- - parameterization: AMBERTools16 (GAFF and FF 14SB force fields for ligand and protein, respectively)
- - zinc cation: new hybrid bonded-nonbonded parameters
- - MD simulations (300 ns x 3): pmemd module of AMBER16 software package

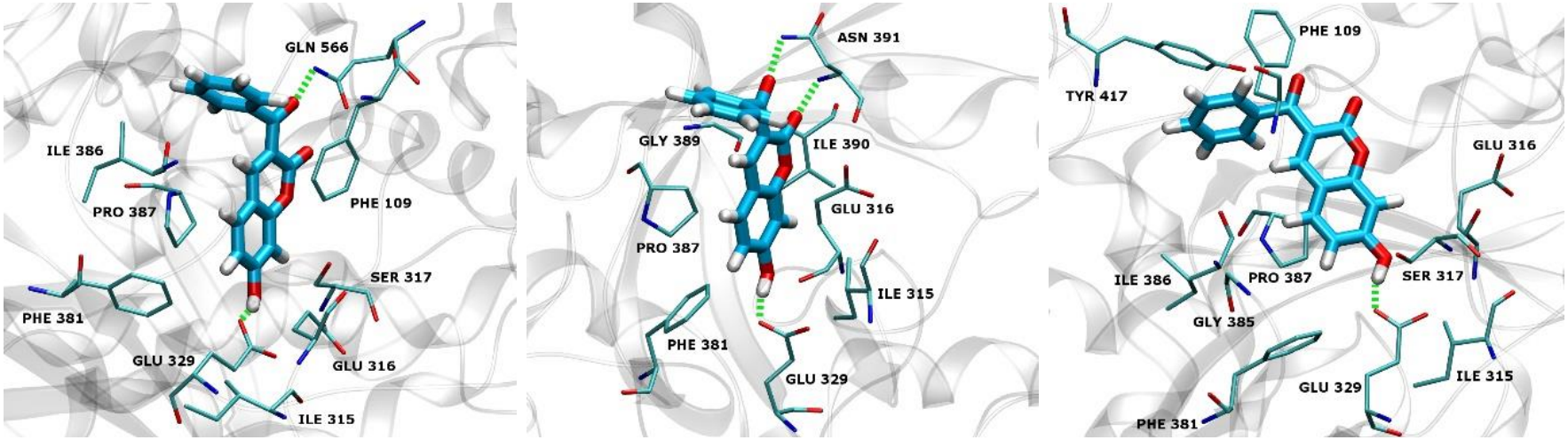




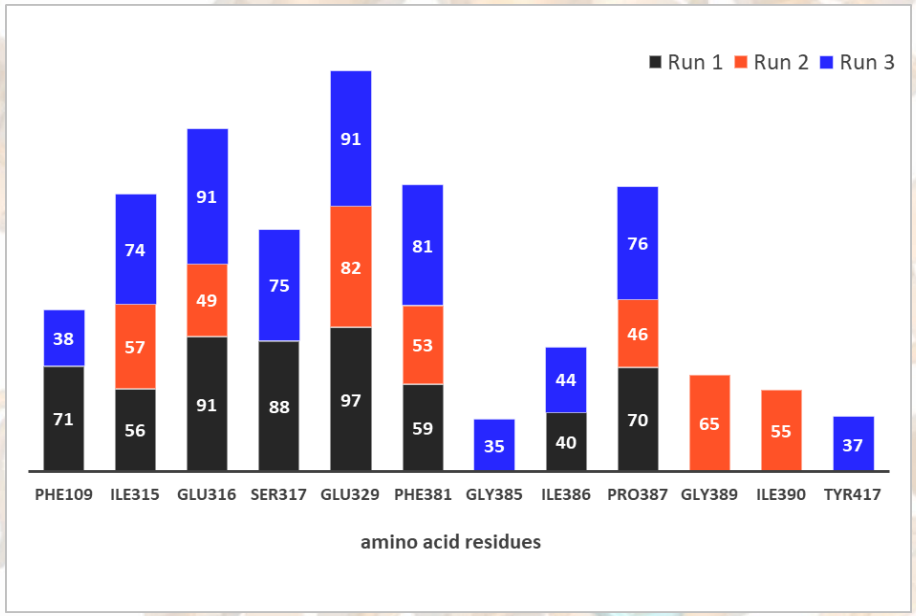
RMSD profile of the protein backbone atoms (left) and ligand havy atoms (right) obtained during 300 ns of MD simulations.



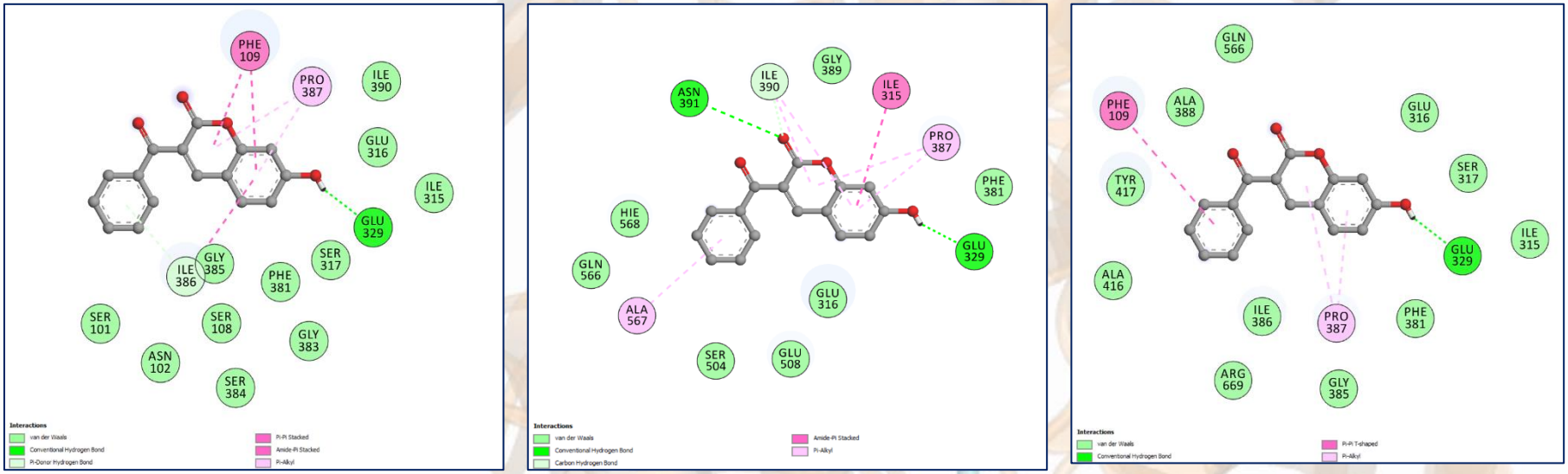
Overlay of the hDPP III with compound 12 in its preferable binding mode after 300 ns of MD simulation for run1 (cyan), run2 (green) and run3 (yellow). Hydrogen bonds are depicted as green dashed line.



hDPP III residues involved in native contacts and H-bonds formation with compound 12 for run 1 (left), run 2 (middle) and run 3 (right). H-bonds are depicted as green dashed line, and compound 12 as light blue sticks.



Native contacts between hDPP III residues and compound 12 with relative occupancy of more than 30% during 300 ns MD simulations.



2D diagrams of compound 12 interactions with the hDPP III residues for run 1, 2, and 3

Detailed analysis of hydrogen bonds between compound 12 (LIG) and hDPP III residues during MD simulations for run 1. 2 and 3 obtained by *hbond* command in CPPTRAJ module.

RUN	Acceptor	Donor H	Donor	Frames	Fraction	AvgDist	AvgAngle
1	GLU_329@OE2	LIG@H1	LIG@O4	29794	0.9931	2.6228	165.3042
	LIG@O3	GLN_566@HE22	GLN_566@NE2	6794	0.2265	2.8563	155.2041
	LIG@O2	GLN_566@HE22	GLN_566@NE2	1476	0.0492	2.8484	147.1674
	LIG@O2	TYR_318@HH	TYR_318@OH	1241	0.0414	2.7842	160.8479
2	GLU_329@OE1	LIG@H1	LIG@O4	25524	0.8508	2.6332	164.773
	LIG@O2	ASN_391@H	ASN_391@N	5150	0.1717	2.8898	156.9507
	LIG@O3	ASN_391@HD22	ASN_391@ND2	4028	0.1343	2.8492	157.513
	LIG@O2	ASN_391@HD22	ASN_391@ND2	1788	0.0596	2.8756	155.3083
3	GLU_329@OE2	LIG@H1	LIG@O4	29832	0.9944	2.6313	164.7486
	LIG@O3	TYR_417@HH	TYR_417@OH	1180	0.0393	2.7920	157.2633
	LIG@O3	HIE_568@HE2	HIE_568@NE2	1078	0.0359	2.8720	151.3918
	LIG@O2	HIE_568@HE2	HIE_568@NE2	911	0.0304	2.8777	153.9514

Binding free energy (kcal mol^{-1}) of the complexes obtained during the last 5 ns of MD simulations for all three replicates.

Energy component	RUN 1	RUN 2	RUN 3
E_{vdw}	-28.33	-27.44	-26.16
E_{ele}	-30.55	-30.76	-23.34
E_{GB}	35.94	41.26	34.69
E_{SA}	-3.44	-4.06	-3.41
ΔG_{gas}	-58.88	-58.20	-49.50
ΔG_{solv}	32.50	37.20	31.28
ΔG_{bind}	**-26.36**	-20.98	-18.22

Thank you for your attention