Supplementary material

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].

1. Faculty of Agrobiotechnical Sciences Osijek. Josip Juraj Strossmayer University of Osijek. 31000 Osijek. Croatia; dejan.agic@fazos.hr (D.A.); maja.karnas@fazos.hr (M.K.); domagoj.subaric@fazos.hr (D.Š.); vesna.rastija@fazos.hr (V.R.); drago.beslo@fazos.hr (D.B.); miroslav.lisjak@fazos.hr (M.L.)
2. Faculty of Food Technology Osijek. Josip Juraj Strossmayer University of Osijek. 31000 Osijek. Croatia; melita.loncaric@ptfos.hr (M.L.); maja.molnar@ptfos.hr (M.M.)
3. Divison of Organic Chemistry and Biochemistry. Ruđer Bošković Institute. 10000 Zagreb. Croatia; sanja.tomic@irb.hr (S.T.); zrinka.karacic@irb.hr (Z.K.)
4. Department of Field and Vegetable Crops. Faculty of Agriculture. University of Novi Sad. 21000 Novi Sad. Serbia; boris.popovic@polj.uns.ac.rs (B.P.)

*Correspondence: dejan.agic@fazos.hr (D.A.)

Table S1 The values of the descriptors included in QSAR model equation (2): log % hDPP III inh. = - 4.07 + 1.85 (0.59) *EEig05x* + 1.60 (0.52) *Mor10u* + 0.56 (0.39) *nArOH*

MolID in QSARINS	Compound	EEig05x	Mor10u	nArOH	log(% inh DPP III) exp.	log(% inh DPP III) calc. by Eq 2
1	31	2.864	-0.442	0	0.00	-
2	15	3.015	-0.538	0	0.98	0.65
3	11	3.474	-0.735	0	1.36	1.18
4	24	3.010	-0.387	0	0.00	-
5	34	3.000	-0.434	1	1.37	1.35
6	27	3.010	-0.188	1	1.82	1.76
7	25	3.010	-0.359	0	1.30	0.93
8	9	3.295	-0.450	0	0.00	-
9	37	2.951	-0.522	0	0.00	0.56
10	29	2.962	-0.719	0	0.00	0.26
11	35	3.000	-0.529	0	1.00	0.64
12	30	2.867	-0.543	0	0.00	0.37
13	14	3.211	-0.852	0	0.00	-
14	33	3.001	-0.013	0	1.33	1.46
15	26	3.011	-0.009	0	1.78	1.49
16	7	2.857	-0.127	0	0.89	1.01
17	23	2.868	-0.355	0	0.00	-
18	6	2.857	0.116	1	0.00	-

19	21	2.874	-0.086	1	1.80	1.67
20	4	2.916	-0.117	1	1.21	1.70
21	3	2.929	-0.525	0	0.00	-
22	1	2.966	-0.080	0	1.45	1.29
23	16	3.027	-0.348	0	0.90	0.98
24	2	2.966	-0.041	1	1.11	excluded as outlier
25	18	3.027	-0.174	1	1.65	1.81
26	20	2.984	-0.492	0	0.00	-
27	17	3.027	-0.442	0	1.30	0.83
28	5	2.857	-0.541	0	0.00	0.35
29	22	2.875	-0.676	0	0.00	0.17
30	38	2.863	-0.397	0	0.35	0.59
31	19	3.312	-0.639	0	0.85	1.04
32	36	2.948	-0.277	1	2.00	1.50
33	12	3.239	-0.374	1	2.00	1.89
34	32	3.000	-0.439	0	0.81	0.78
35	8	3.210	-0.540	0	0.64	1.01
36	13	3.239	-0.716	0	1.22	0.78
37	10	3.210	-0.304	1	1.83	1.95
38	28	3.273	-0.102	0	1.47	1.82
39	39	2.344	-0.399	0	0.00	-0.37
40	40	2.410	-0.289	1	0.33	0.49
41		3.340	-0.091	2		3.08
42		3.295	-0.088	2		3.01

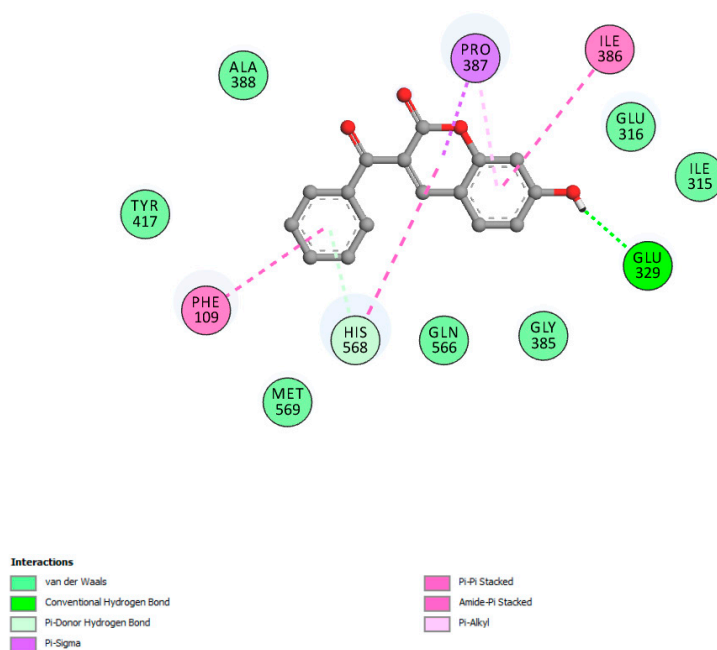- Inactive compounds excluded from QSAR

Figure S1 2D diagram of compound **12** interactions with the hDPP III residues for the best docking pose.

Table S2 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.

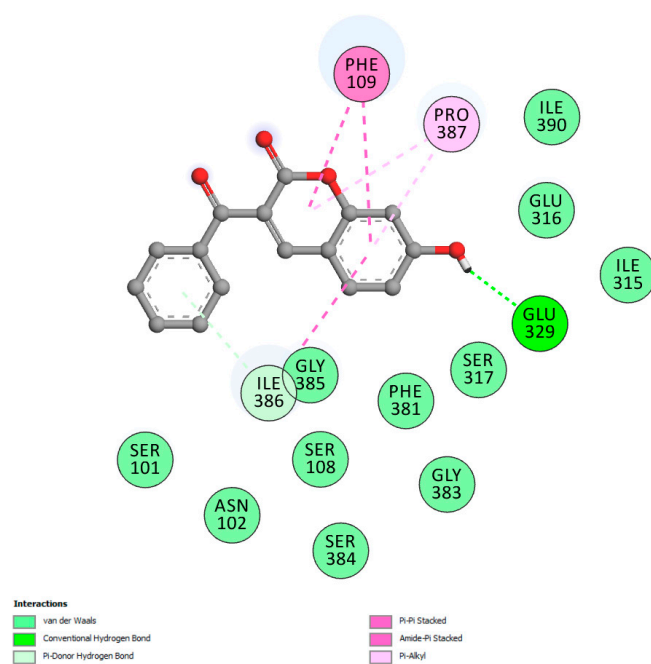| RUN	Acceptor	Donor H	Donor	Frames	Fraction	AvgDist	AvgAngle
1	GLU_329@OE2	LIG@H1	LIG@O4	29794	0.9931	2.6228	165.3042
	LIG@O3	GLN_566@HE22	GLN_566@NE2	6794	0.2265	2.8563	155.2041
	LIG@O2	GLN_566@HE22	GLN_566@NE2	1476	0.0492	2.8484	147.1674
	LIG@O2	TYR_318@HH	TYR_318@OH	1241	0.0414	2.7842	160.8479
2	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

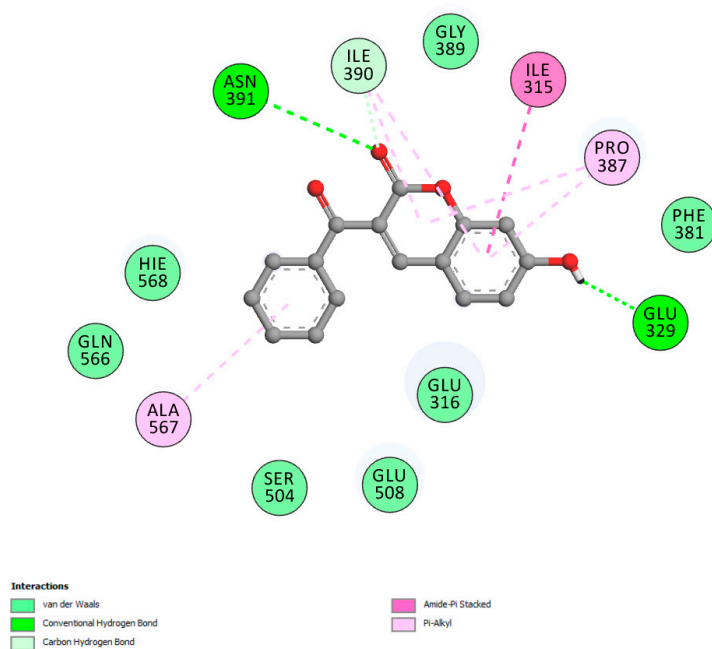**Figure S2** 2D diagram of compound **12** interactions with the hDPP III residues for run 1

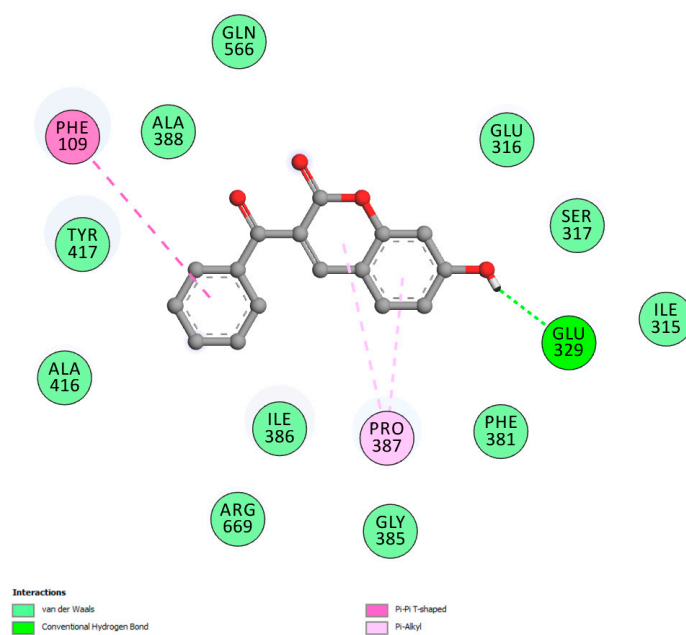**Figure S3** 2D diagram of compound **12** interactions with the hDPP III residues for run 2

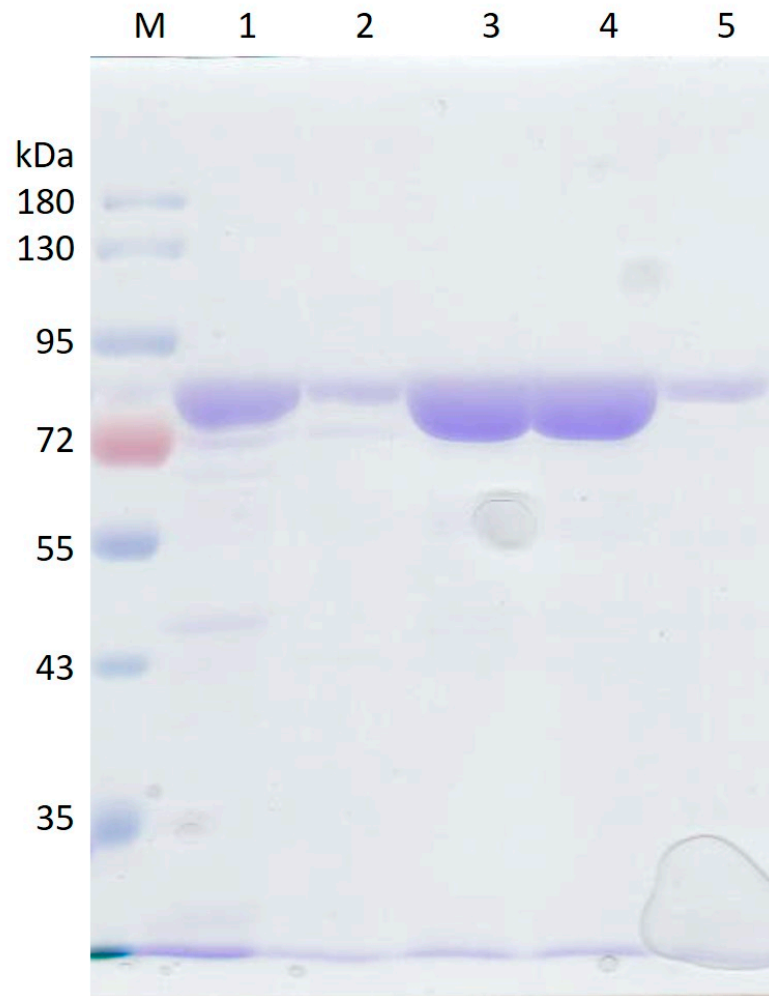**Figure S4** 2D diagram of compound **12** interactions with the hDPP III residues for run 3

Figure S5 SDS-PAGE demostrating purity of hDPP III sample on a 10% gel: M. PageRuler Prestained protein marker. with 72 kDa band in red; lane 1. hDPP III sample after affinity chromatography; lanes 2-5. fractions of the main hDPP III peak after gel-filtration.