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Evaluation of Propolis Activity as Sucrose-Dependent and Sucrose-Independent of *Streptococcus mutans* Inhibitor to Treat Dental Caries Using In Silico Approach

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Supplementary Material

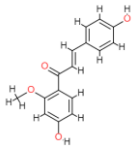
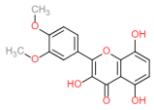
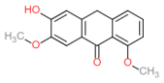
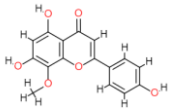
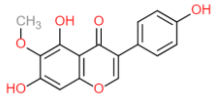
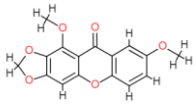
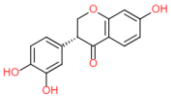
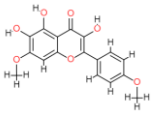
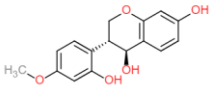
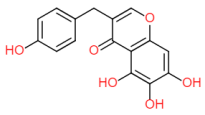
Table S1. Docking validation between acarbose and 3AIC protein

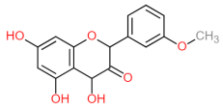
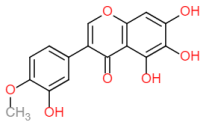
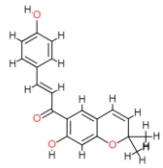
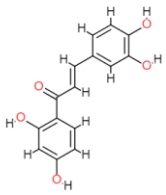
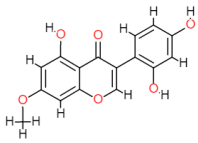
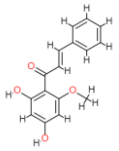
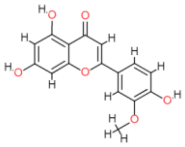
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	3	-7.16	0.00	1.00	RANKING
1	2	10	-7.13	0.50	0.77	RANKING
1	3	4	-7.11	0.47	0.81	RANKING
1	4	6	-7.10	0.54	0.82	RANKING
1	5	9	-7.08	0.49	0.73	RANKING
1	6	2	-7.07	0.55	0.85	RANKING
1	7	7	-7.00	0.41	0.97	RANKING
1	8	1	-6.95	1.93	1.90	RANKING
1	9	8	-6.87	0.30	0.87	RANKING
2	1	5	-6.53	0.00	2.61	RANKING

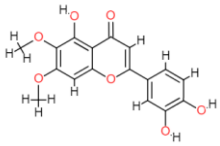
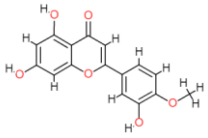
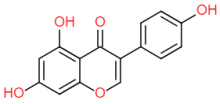
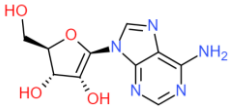
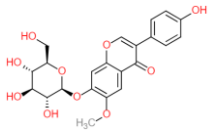
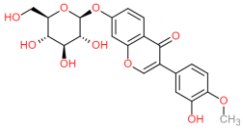
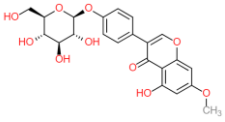
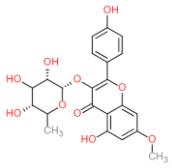
Table S2. Docking validation between Phenylmethanesulfonic Acid (PMS) and 3IPK protein

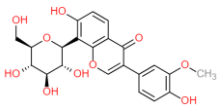
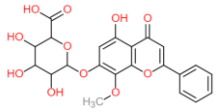
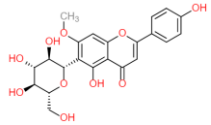
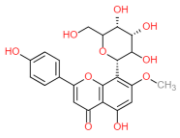
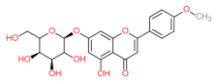
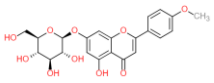
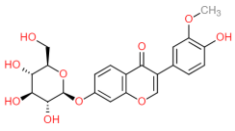
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	3	-4.75	0.00	1.24	RANKING
1	2	10	-4.74	0.54	1.04	RANKING
1	3	6	-4.73	0.21	1.14	RANKING
1	4	5	-4.70	0.56	1.21	RANKING
1	5	7	-4.70	0.52	1.17	RANKING
1	6	4	-4.70	0.33	1.19	RANKING
1	7	8	-4.66	0.68	1.16	RANKING
1	8	1	-4.65	0.52	0.94	RANKING
1	9	2	-4.63	0.92	1.09	RANKING
1	10	9	-4.60	0.90	1.10	RANKING

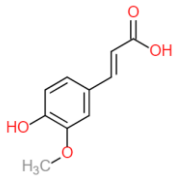
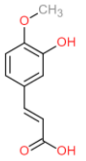
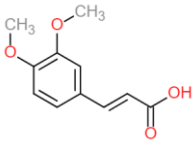
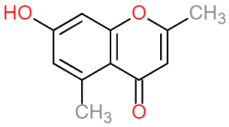
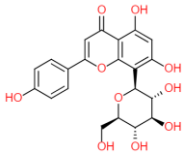
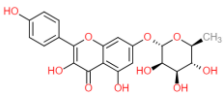
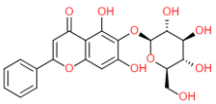
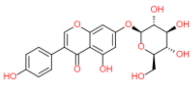
Table S3. Ligands from propolis screening

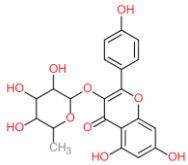
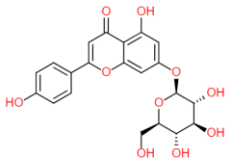
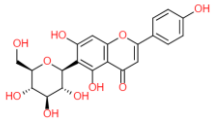
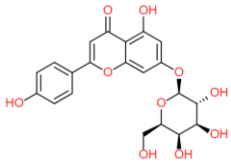
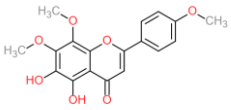
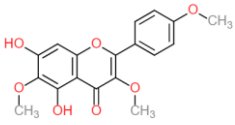
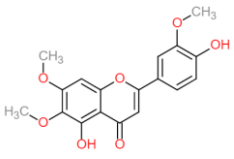
No	Compound	Formula	Structure	PubChem ID	Classification
1	2'-O-Methylisoliquiritigenin	C16H14O4		5319688	chalcones
2	3,5,8-Trihydroxy-3',4'-dimethoxyflavone	C17H14O7			flavonols
3	3-Hydroxy-2,8-dimethoxyxanthone	C15H12O5			simple oxygenated xanthone
4	4'-Hydroxywogonin	C16H12O6		5322078	flavones
5	Tectorigenin	C16H12O6		5281811	isoflavones
6	1,7-Dimethoxy-2,3-methylenedioxyxanthone	C16H12O6		85670503	simple oxygenated xanthone
7	3′,4′,7-Trihydroxy flavanone	C15H10O5		5281611	flavones
8	3,5,6-Trihydroxy-4',7'-dimethoxyflavone	C17H14O7		5322058	flavones
9	4,7,2′-Trihydroxy-4′-methoxyisoflavanol	C16H16O5			flavonols
10	5,6,7-Trihydroxy-3-(4'-hydroxybenzyl) chromone	C16H12O6			Homoisoflavanones

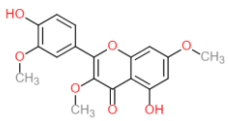
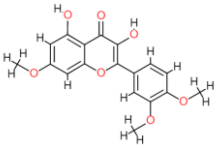
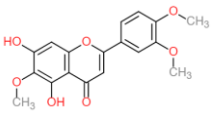
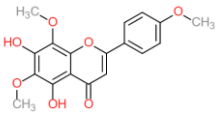
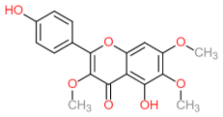
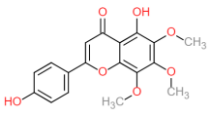
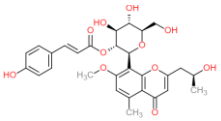
11	5,7,4′-Trihydroxy flavanone	C15H12O5		129847910	flavones
12	Alpinetin	C16H14O4		154279	flavonols
13	Bavachromene	C20H18O4		5321800	chalcones
14	Butein	C15H12O5		5281222	chalcones
15	Cajanin	C16H12O6		5281706	flavones
16	Cardamonin	C16H14O4		641785	chalcones
17	Chrysoeriol	C16H12O6		5280666	flavones

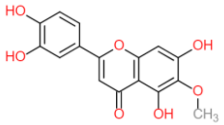
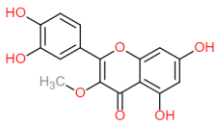
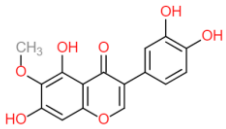
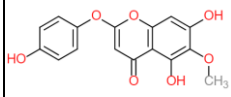
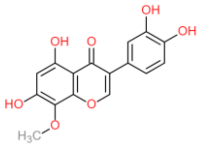
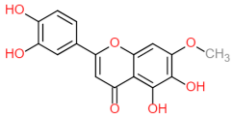
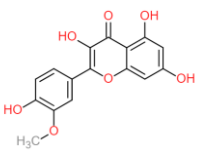
18	Cirsiliol	C17H14O7		160237	flavonols
19	Diosmetin	C16H12O6		5281612	flavonols
20	genistein_1	C15H12O5		25201420	isoflavones
21	Adenosin	C10H13N5O4		60961	Purines base
22	Glycitin	C22H22O10		187808	isoflavones
23	Calycosin-7-O-β-D-glucopyranoside	C22H22O10		5318267	isoflavones
24	Prunetin 4 glucoside	C22H22O10		5918474	isoflavones
25	Rhamnocitrin 3 rhamnoside	C22H22O10		44259556	flavones

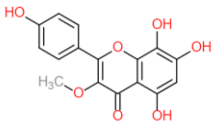
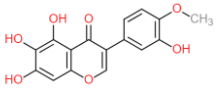
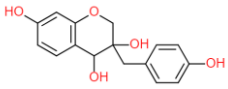
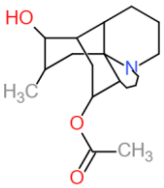
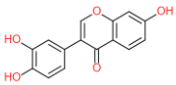
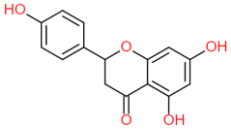
26	3 methoxypuerarin	C22H22O10		5319485	isoflavones
27	Wogonoside	C22H22O10		12004622	flavones
28	Swertisin	C22H22O10		124034	flavones
29	Isoswertisin	C22H22O10		44258317	flavones
30	Acacetin 7 galactoside	C22H22O10		44257885	flavones
31	Tilianin	C22H22O10		5321954	flavones
32	3 methoxydaidzin	C22H22O10		10527347	isoflavones

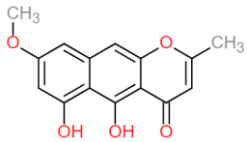
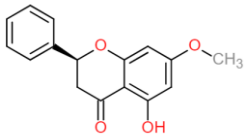
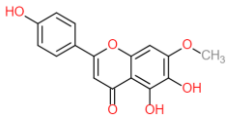
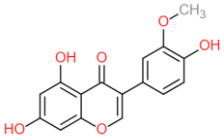
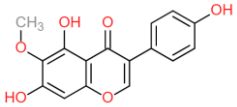
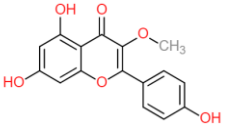
33	Ferulic acid	C10H10O4		445858	Phenolic acids
34	Isoferulic acid	C10H10O4		736186	Phenolic acids
35	3,4-Dimethoxy-cinnamic acid	C10H10O4		717531	Phenolic acids
36	2,5-Dimethyl-7-hydroxychromone	C11H10O3		5316891	isoflavones
37	Vitexin	C21H20O10		5280441	flavones
38	Kaempferol-7-O-α-L-rhamnoside	C21H20O10		25079965	flavonols
39	Baicalein-7-O-β-D glucopyranoside	C21H20O10		5321896	flavones
40	Genistin	C21H20O10		5281377	isoflavones

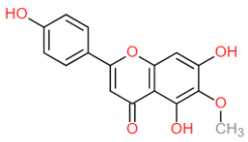
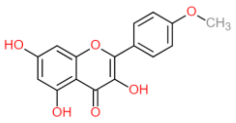
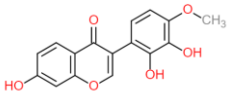
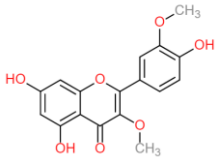
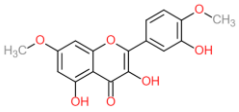
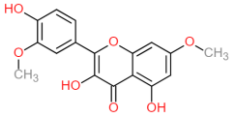
41	Kaempferol 3 rhamnoside	C21H20O10		5835713	flavonols
42	Cosmosiin	C21H20O10		5280704	flavones
43	Isovitexin	C21H20O10		162350	flavones
44	Apigenin-7-O-galactopyranoside	C21H20O10			flavones
45	5,6-Dihydroxy-7,8,4'-trimethoxyflavone	C18H16O7		44258635	flavonols
46	Santin	C18H16O7		5281695	flavonols
47	Cirsilineol	C18H16O7		162464	flavonols

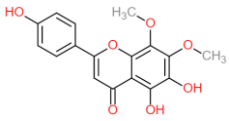
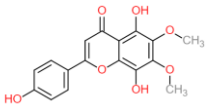
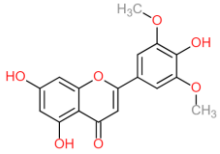
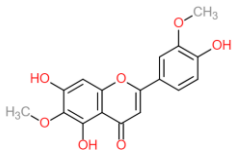
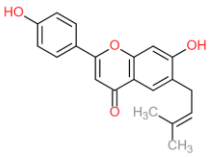
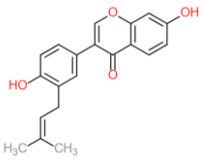
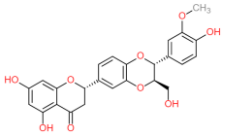
48	Pachypodol	C18H16O7		5281677	flavonols
49	3',5-Dihydroxy-3,4',7-trimethoxy flavone	C18H16O7		319310128	flavones
50	Eupatilin	C18H16O7		5273755	flavonols
51	Nevadensin	C18H16O7		160921	flavonols
52	Penduletin	C18H16O7		5320462	flavonols
53	5,4'-Dihydroxy-6,7,8-trimethoxyflavone	C18H16O7		73207	flavonols
54	Isoaloeresin D	C29H32O11		76332505	Phenolic compounds

55	Eupafolin	C16H12O7		5317284	flavones
56	Quercetin-3-methyl ether	C16H12O7		5280681	flavonols
57	Irilin D	C16H12O7		10495590	isoflavones
58	Capillarisin	C16H12O7		5281342	Phenolic compounds
59	3′,4′,5,7-Tetrahydroxy-8-methoxyisoflavone	C16H12O7		5493449	isoflavones
60	Pedalitin	C16H12O7		31161	isoflavones
61	Isoharmentin	C16H12O7		5281654	flavonols

62	3-Methoxyherbacetin	C16H12O7		51642537	flavonols
63	3',5,6,7-Tetrahydroxy-4'-methoxyisoflavone	C16H12O7		10543410	isoflavones
64	3'-Deoxysappanol	C16H16O5		13846660	isoflavones
65	Fawcettiine	C18H29NO3		50841427	alkaloid
66	3',4',7-Trihydroxyisoflavanone	C15H12O5		22065265	isoflavones
67	Naringenin	C15H12O5		932	flavonols

68	Rubrofusarin	C15H12O5		72537	simple oxygenated xanthone
69	Pinostrobin	C16H14O4		73201	flavonols
70	Sorbifolin	C16H12O6		3084390	flavonols
71	3'-O-Methylorobol	C16H12O6		5319744	flavones
72	Hydroxygenkwanin	C16H12O6		5318214	flavones
73	Isokaempferide	C16H12O6		5280862	flavonols

74	Hispidulin	C16H12O6		5281628	flavonols
75	Kaempferide	C16H12O6		5281666	flavonols
76	Koparin	C16H12O6		5318834	flavones
77	Quercetin-3,3'-dimethyl-ether	C17H14O7		5316900	flavonols
78	Ombuine	C17H14O7		5320287	flavonols
79	Rhamnazin	C17H14O7		5320945	flavonols

80	Thymusin	C17H14O7		628895	flavonols
81	Isothymusin	C17H14O7		630253	flavonols
82	Tricin	C17H14O7		5281702	flavonols
83	Jaceosidin	C17H14O7		5379096	flavonols
84	Licoflavone A	C20H18O4		5319000	flavonols
85	Neobavaisoflavone	C20H18O4		5320053	isoflavones
86	Silandrin	C25H22O9		441663	flavonols

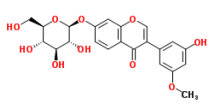
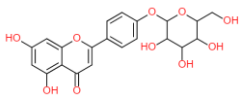
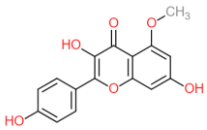
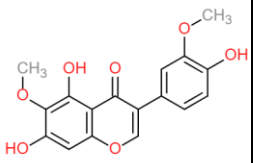
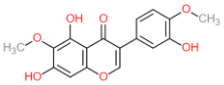
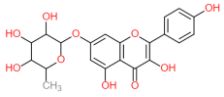
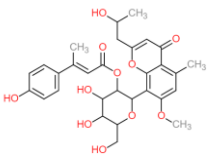
87	3'-methoxy-5'-hydroxyisoflavone-7-O-β-D-glucoside	C22H22O10			isoflavones
88	Flavone,5,7-dihydroxy-4′-O-α-D-glucoside	C21H20O10			isoflavones
89	5-Methyl kaempferol	C16H12O6		5319694	flavonols
90	Iristectorigenin A	C17H14O7		5491637	isoflavones
91	Iristectorigenin B	C17H14O7		5488781	isoflavones
92	7-O-α-L-Rhamnopyranosyl-kaempferol	C21H20O10			flavonols
93	Coumaroyl	C29H32O11			Phenolic acids

Table S4. Interaction and residue involving diosmetin and cosmosiin to 3AIC protein. Bold-black word as the key residue among potential and native ligands, while bold-purple word as the similar binding residue between diosmetin and genistin.

Protein		Interaction		Ligand
Name	**Residue**	**Category**	**Type**	
3AIC	**ASP 477** **TRP 517** **GLU 515**	Hydrogen Bond	Conventional Hydrogen Bond	**Diosmetin**
	ASP 909 **ASN 481**		Carbon-Hydrogen Bond	
	TYR 916	Hydrophobic	Pi-Pi T Shaped	
	HIS 587 **ALA 478** **LEU 433**		Pi-Alkyl	
	ASN 914 **ARG 475** ASN 862 **ASP 480**	Van Der Waals		
	ASP 588	Electrostatic	Pi-Anion	
	ASP 480 **ASP 588** **GLU 515** **ASP 477** GLN 960	Hydrogen Bond	Conventional Hydrogen Bond	**Cosmosiin**
	ASP 909 **ASN 481**		Carbon-Hydrogen Bond	
	TRP 517	Hydrophobic	Pi-Pi T Shaped	
	LEU 433 LEU 382		Pi-Sigma	
	ALA 478		Pi-Alkyl	
	TYR 430 PHE 907 GLN 592 LEU 434 TYR 916 **ARG 475** **HIS 587**	Van Der Waals		

Table S5. Interaction and residue involving genistin and 3′-methoxy-5′-hydroxyisoflavone-7-O-β-D glucoside to 3IPK protein. Bold-black word as the key residue among potential and native ligands, while bold-purple word as the similar binding residue between genistin and 3′-methoxy-5′-hydroxyisoflavone-7-O-β-D glucoside.

Protein		Interaction		Ligand
Name	Residue	Category	Type	
3IPK	**ASP 512** **ARG 824** ASP 760 **TRP 816** **THR 586**	Hydrogen Bond	Conventional Hydrogen Bond	**Genistin**
	SER 697		Carbon-Hydrogen Bond	
	LYS 822	Hydrophobic	Pi-Alkyl	
	TRP 816		Pi-Pi Stacked	
	LEU 653 **ASN 820** **SER 762** **VAL 587**	Van Der Waals		
	THR 652 **ASN 820** **ARG 824** **TRP 816** **THR 586**	Hydrogen Bond	Conventional Hydrogen Bond	**3′-Methoxy-5′-Hydroxyisoflavone-7-O-β-D Glucoside**
	ASP 909 ASN 481		Carbon-Hydrogen Bond	
	TRP 816	Hydrophobic	Pi-Pi stacked	
	LEU 653		Alkyl	
	LYS 822		Pi-Alkyl	
	ASP 512 **SER 762** **VAL 587**	Van Der Waals		

Description	Scientific Name	Max Score	Query Cover	E value	Per. ident
LPXTG cell wall anchor domain-containing protein	Limosilactobacillus reuteri	79.3	83%	9.00E-15	27.88
LPXTG cell wall anchor domain-containing protein	Limosilactobacillus reuteri	70.5	35%	5.00E-12	30.96
hypothetical protein	Limosilactobacillus reuteri	68.2	87%	1.00E-11	24.9
KxYKxGKxW signal peptide domain-containing protein	Limosilactobacillus reuteri	68.6	34%	1.00E-11	32.29
hypothetical protein	Limosilactobacillus reuteri	67	51%	2.00E-11	28
hypothetical protein	Limosilactobacillus reuteri	66.6	50%	3.00E-11	27.31
LPXTG cell wall anchor domain-containing protein	Limosilactobacillus reuteri	67.8	35%	4.00E-11	31.5
LPXTG cell wall anchor domain-containing protein	Limosilactobacillus reuteri	67.8	35%	4.00E-11	31.5
hypothetical protein	Limosilactobacillus reuteri	66.2	35%	4.00E-11	30.96

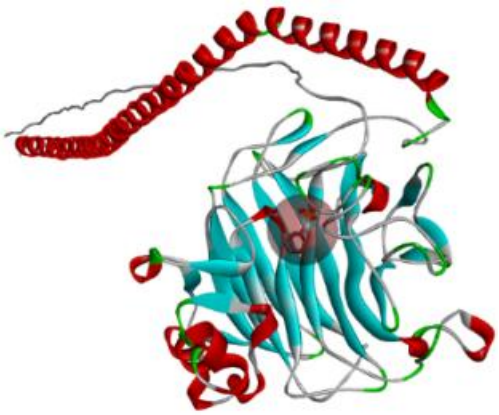
Figure S1. BLAST-P result between 3IPK protein and *Lactobacillus reuteri* protein

Description	Scientific Name	Max Score	Query Cover	E value	Per. ident
KxYKxGKxW signal peptide domain-containing protein	Limosilactobacillus reuteri	82	40%	4.00E-16	28.67
glycosyl hydrolase 53 family protein	Limosilactobacillus reuteri	82.8	40%	2.00E-15	28.57
glycosyl hydrolase 53 family protein	Limosilactobacillus reuteri	82	40%	3.00E-15	27.36
glycosyl hydrolase 53 family protein	Limosilactobacillus reuteri	81.6	40%	3.00E-15	27.36
KxYKxGKxW signal peptide domain-containing protein	Limosilactobacillus reuteri	79.7	50%	4.00E-15	26.33
KxYKxGKxW signal peptide domain-containing protein	Limosilactobacillus reuteri	80.1	50%	4.00E-15	25.61
KxYKxGKxW signal peptide domain-containing protein	Limosilactobacillus reuteri	79.3	40%	5.00E-15	28.47
Arabinogalactan endo-1,4-beta-galactosidase precursor	Limosilactobacillus reuteri subsp. porcinus	80.9	40%	7.00E-15	28.38
glycosyl hydrolase 53 family protein	Limosilactobacillus reuteri	80.5	40%	9.00E-15	28.38

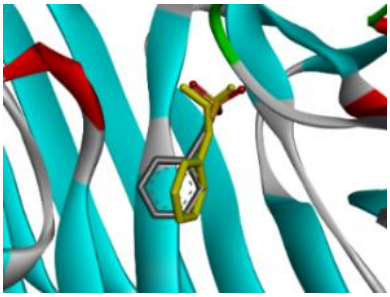
Figure S2. BLAST-P result between 3AIC protein and *Lactobacillus reuteri* protein

Description	Scientific Name	Max Score	Query Cover	E value	Per. ident
zonadhesin isoform 3 precursor	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin splice variant 3	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin variant 3	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin splice variant 6	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin isoform 6 precursor	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin variant 6	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin splice variant 5	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin variant 5	Homo sapiens	43.9	16%	0.003	36.26
zonadhesin variant 1	Homo sapiens	43.9	16%	0.003	36.26

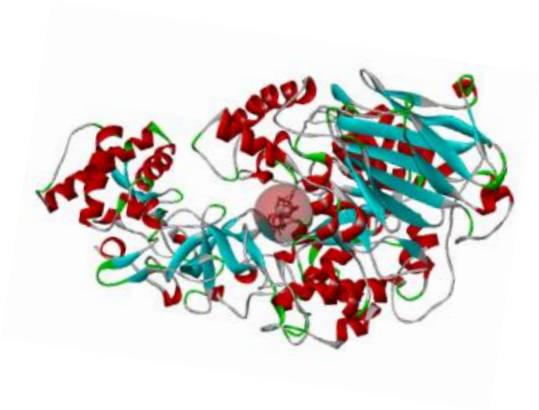
Figure S3. BLAST-P result between 3AIC protein and *Homo sapiens* protein



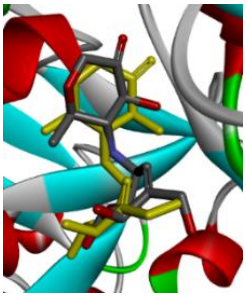
(A)



(B)



(C)



(D)

Figure S4. Protein-ligand binding site. 3AIC binding site (A), acarbose before and after (yellow) validation (B). 3IPK binding site (C), PMS before and after (yellow) validation (D)

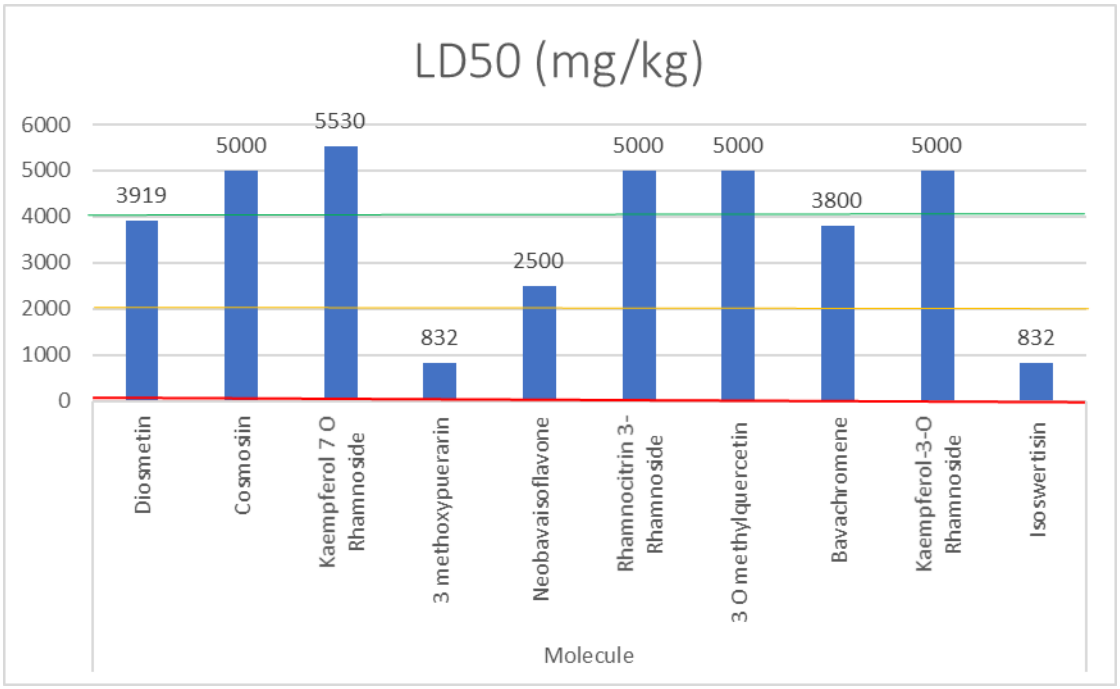


Figure S5. Lethal dose top ten ligands of 3AIC protein. Class 1 fatal if swallowed (red line), class IV harmful if swallowed (yellow line), and Class V may be harmful if swallowed (green line)

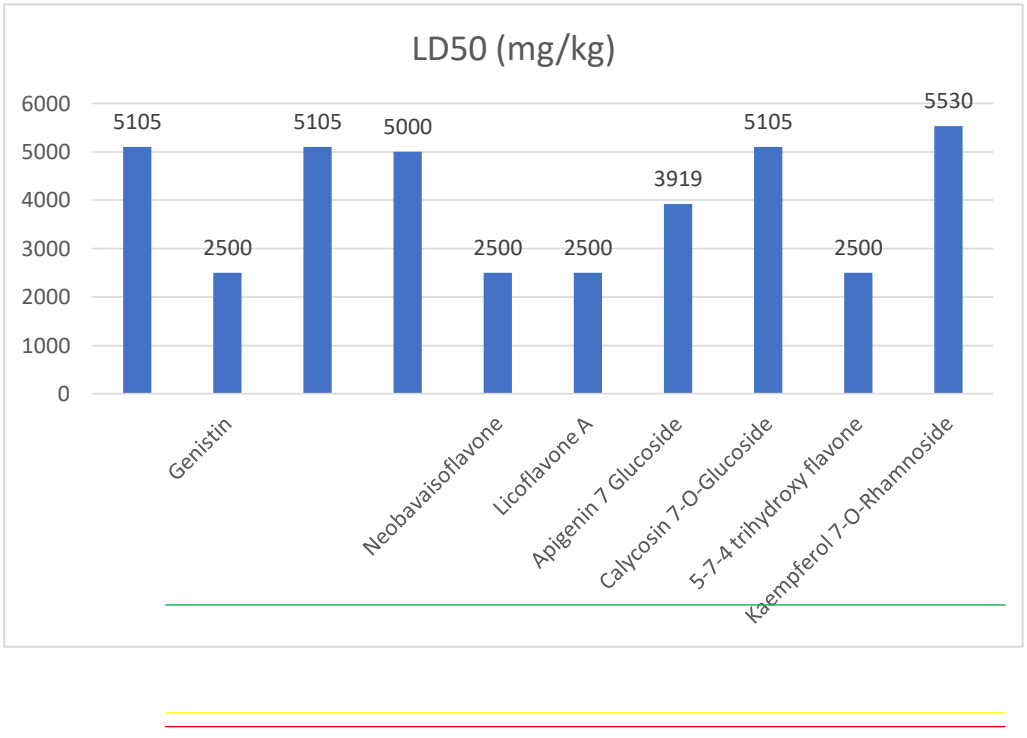
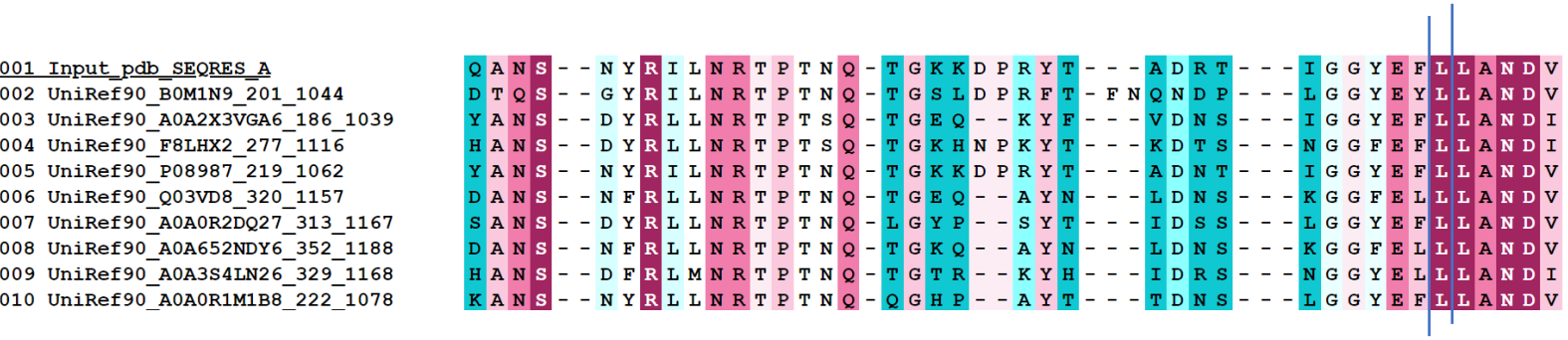
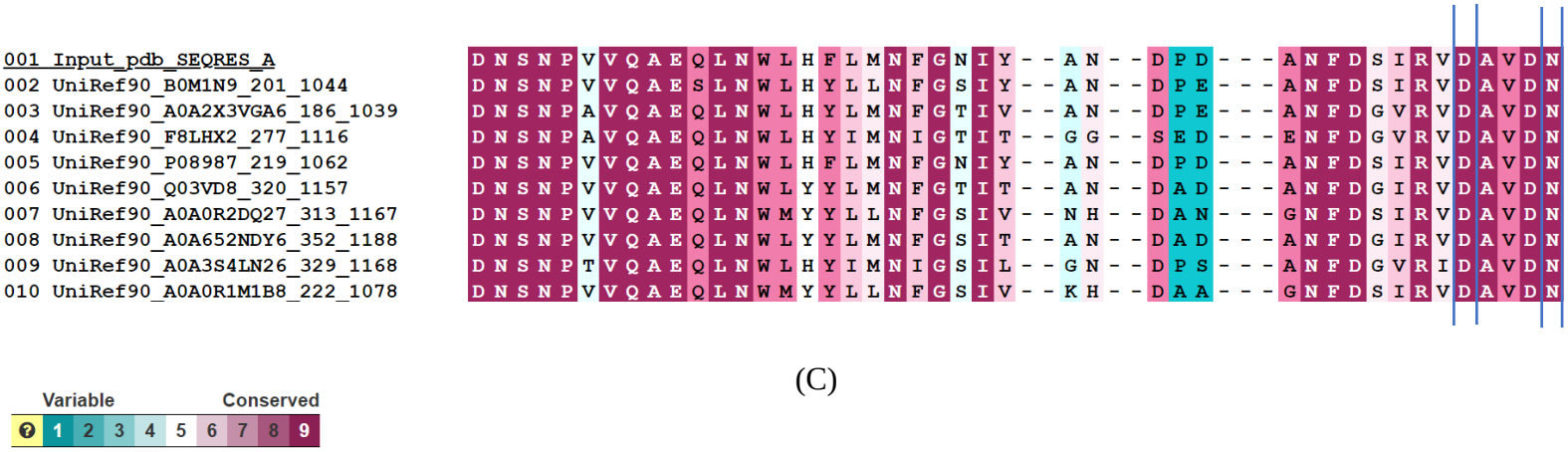


Figure S6. Lethal dose top ten ligands of 3IPK protein. Class 1 fatal if swallowed (red line), class IV harmful if swallowed (yellow line), and Class V may be harmful if swallowed (green line)

(A)

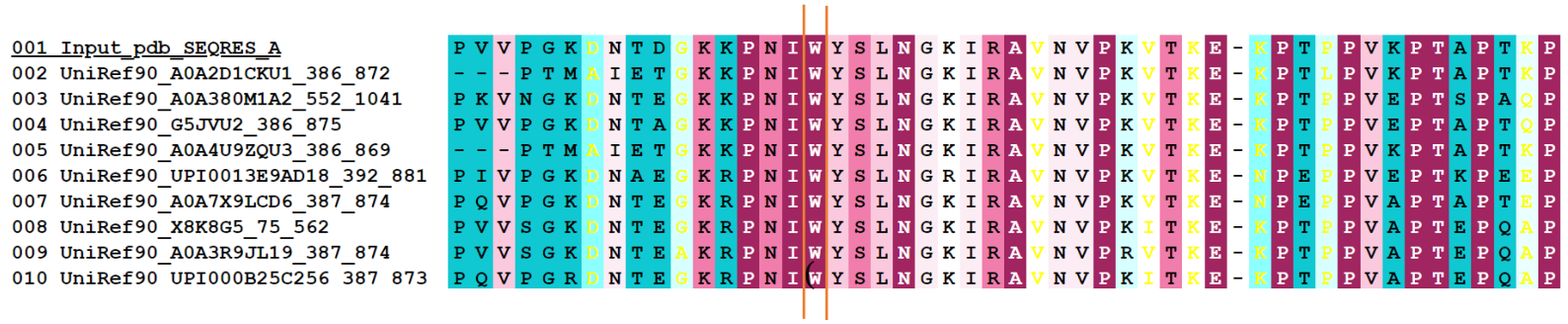


(B)

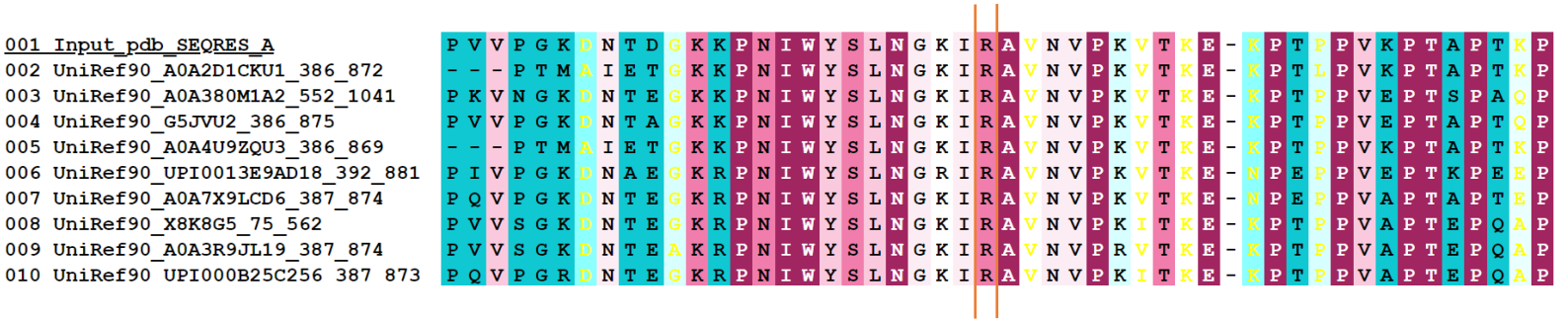


(C)

Figure S7. Partial 3AIC protein sequence. Conserved amino acid shows within the blue line. Amino acid of glutamic acid 515 (A), leucine 433 (B), aspartic acid 477 and asparagine 481 (C).



(A)



(B)

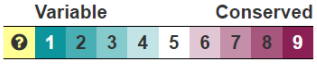


Figure S8. Partial 3IPK protein sequence. Conserved amino acid shows within the orange line. The amino acid of tryptophan 816 (A), and arginine 824 (B).

(A) (B)

Figure S9. 3AIC and 3IPK crystal protein. The fluctuating residue is shown by yellow color. The fluctuating residue of 173-182 (left) and 276-283 (right) from diosmetin and cosmosiin in 3AIC protein (A). The fluctuating residue of 1-23 (left) and 482-489 (right) from genistin and 3′-Methoxy-5′-hydroxyisoflavone-7-O-β-D glucoside in 3IPK protein (B)

(A) (B)

Figure S10. Protein and native ligand interaction between 3AIC-acarbose (A) and 3IPK-Phenylmethanesulfonic Acid (PMS) (B)