

Supporting Information

Identification, rapid screening, docking mechanism and in vitro digestive stability of novel

DPP-4 inhibitory peptides from wheat gluten with ginger protease

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Supporting Information Description

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Fig.S1. Mass spectra of the identified peptides (A, B, and C represented PPFS, APFGL, and PPFW, respectively) in sequence.

Table S1. Enzyme activity for different proteases at their optimal pH and optimal temperature.

Enzyme	Temperature/°C	pH	Enzyme activity
Papain	60	6.0	$(2.3 \pm 0.2) \times 10^4$ U/g
Alcalase	60	8.0	$(5.2 \pm 0.1) \times 10^5$ U/mL
Flavourzyme	55	7.0	$(2.4 \pm 0.1) \times 10^4$ U/mL
Trypsin	37	8.0	$(4.2 \pm 0.1) \times 10^4$ U/g
Ginger protease	60	6.5	$(5.8 \pm 0.3) \times 10^4$ U/g protein

Table S2. Binding energy of the positive inhibitors after molecular docking.

Positive inhibitor	ΔG	$\Delta G_{\min}$
IPI	-6.04 ± 0.53^a	-6.80
Sitagliptin	-7.12 ± 0.54^b	-8.10
Omarigliptin	-7.38 ± 0.41^b	-8.00

Note: ΔG and $\Delta G_{\min}$ represented the average and the lowest binding energy of docking results, respectively (kcal/mol). And the binding energy data was expressed as the average of the 9 docking results $\pm$ standard deviation. Different letters indicate statistical differences ($p < 0.05$).

Table S3. The binding energy, peptide ranker score, toxicity prediction and inhibitory activity of the screened peptides.

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
A1	QFPFWQ	-8.60±0.30	-9.10	801.4	0.94	Non	
A2	PPFW	-8.27±0.31	-8.80	545.3	1.00	Non	ACF93464.1
A3	VPPGVGT	-7.97±0.34	-8.40	675.4	0.38	Non	ACA63873.1
A4	QPPFWQ	-7.74±0.41	-8.50	801.4	0.95	Non	ACF93464.1
A5	PPFS	-7.72±0.55	-8.50	446.2	0.86	Non	XP_044447670.1
A6	QPPFW	-7.42±0.12	-7.60	673.3	0.99	Non	ACF93464.1
A7	QYPQPEPQ	-7.40±0.66	-8.50	1082.5	0.43	Non	BAS02406.1
A8	QYPQPQPQ	-7.40±1.09	-8.20	1081.5	0.49	Non	AGO17673.1
A9	LPYPQP	-7.38±0.35	-8.20	713.4	0.66	Non	BAS02406.1
A10	APFGL	-7.38±0.32	-8.00	503.3	0.95	Non	
A11	MPW	-7.34±0.21	-7.70	432.2	0.99	Non	XP_044433310.1
A12	EPSPYQ	-7.33±0.38	-7.90	719.3	0.32	Non	AJG03094.1
A13	APFGIM	-7.26±0.31	-7.80	634.3	0.94	Non	
A14	LPYPQPQ	-7.23±0.19	-7.50	841.4	0.50	Non	BAS02406.1
A15	DPFS	-7.23±0.19	-7.50	464.2	0.73	Non	XP_044433310.1
A16	QPTPIQPQ	-7.22±0.46	-8.10	907.5	0.28	Non	ACQ83639.1
A17	QPFSQPQ	-7.20±0.30	-7.80	830.4	0.48	Non	ACQ83639.1
A18	LPPFSQ	-7.18±0.17	-7.40	687.4	0.56	Non	ACA63873.1
A19	QPPFSQQ	-7.16±0.35	-7.80	830.4	0.40	Non	AAT36483.1
A20	QFPQPQ	-7.12±0.31	-7.50	840.4	0.74	Non	QXF30551.1
A21	VPPHTPVQ	-7.10±1.50	-8.60	1020.5	0.40	Non	
A22	VADIGGQ	-7.09±0.19	-7.40	658.3	0.16	Non	
A23	SPVAMPQ	-7.08±0.36	-7.60	728.4	0.55	Non	ACA63873.1
A24	QFPWQPQ	-7.00±0.66	-7.70	1026.5	0.83	Non	
A25	QPQFPQ	-6.99±0.54	-7.70	840.4	0.80	Non	ALN96402.1
A26	QPGYYPT	-6.98±0.34	-7.70	824.4	0.68	Non	AEO45112.2
A27	EASPYQ	-6.94±0.35	-7.60	693.3	0.20	Non	
A28	FAL	-6.91±0.13	-7.10	349.2	0.89	Non	NP_001392006.1
A29	LPYPQPH	-6.90±0.48	-7.50	850.4	0.63	Non	AFQ13464.1
A30	APFGI	-6.90±0.25	-7.40	503.3	0.88	Non	BAS02406.1
A31	FAI	-6.90±0.17	-7.20	349.2	0.73	Non	
A32	VAF	-6.90±0.21	-7.30	335.2	0.56	Non	P17314.1
A33	DPFDTR	-6.88±0.33	-7.40	896.4	0.89	Non	KAF7027305.1
A34	HPFILVE	-6.86±0.40	-7.60	853.5	0.38	Non	KAF7048142.1
A35	PAL	-6.86±0.44	-7.70	299.2	0.63	Non	P01084.1
A36	QPLQPQ	-6.80±0.19	-7.00	806.4	0.48	Non	ACX37111.1
A37	QYPQPQP	-6.78±0.68	-7.70	953.5	0.61	Non	BAS02406.1

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
A38	SPGA	-6.77±0.40	-7.30	330.2	0.61	Non	XP_044322813.1
A39	IPVDQPT	-6.72±0.45	-7.40	768.4	0.21	Non	D6QZM4.1
A40	QPFQPQ	-6.64±0.21	-7.10	743.4	0.64	Non	
A41	VPFDVGTG	-6.62±0.21	-7.00	790.4	0.47	Non	
A42	QAFPIPQ	-6.62±0.36	-7.20	799.4	0.69	Non	
A43	PAI	-6.60±0.51	-7.20	299.2	0.42	Non	P21292.1
A44	QAFPQPQ	-6.56±0.51	-7.30	814.4	0.57	Non	
A45	QPFVVVV	-6.51±0.21	-6.80	786.5	0.21	Non	ACQ83639.1
A46	APFGLM	-6.44±0.18	-6.90	634.3	0.96	Non	
A47	LPYPQPQPFL	-6.42±1.23	-8.50	1198.6	0.85	Non	BAS02406.1
A48	QPFQPQPFP	-6.40±2.12	-7.90	1084.5	0.91	Non	
A49	QPFPTPQ	-6.40±0.54	-7.70	813.4	0.70	Non	ACQ83639.1
A50	LPYPQPQP	-6.38±0.50	-7.60	938.5	0.51	Non	BAS02406.1
A51	LAGA	-6.38±0.68	-7.30	330.2	0.30	Non	
A52	QPTPQPQ	-6.34±0.43	-7.10	794.4	0.34	Non	XP_044447612.1
A53	IAHASSQVS	-6.34±0.24	-6.70	898.5	0.08	Non	CAY54134.1
A54	QPVPQPH	-6.32±0.45	-7.30	801.4	0.49	Non	
A55	FPIPYPPQ	-6.29±0.74	-7.30	957.5	0.85	Non	AJG03094.1
A56	RPHFPQ	-6.27±0.47	-7.10	877.5	0.82	Non	
A57	IAGA	-6.27±0.36	-6.60	330.2	0.26	Non	XP_044439199.1
A58	LAV	-6.23±0.42	-6.90	301.2	0.10	Non	NP_001392006.1
A59	IPMT	-6.22±0.36	-6.60	460.2	0.63	Non	XP_044366523.1
A60	FAGDDAPR	-6.22±0.42	-7.00	847.4	0.64	Non	XP_044396063.1
A61	APFGIFGTN	-6.21±0.68	-7.50	922.5	0.80	Non	BAS02406.1
A62	LPMT	-6.12±0.15	-6.40	460.2	0.64	Non	
A63	VPI	-6.06±0.35	-6.50	327.2	0.23	Non	P21292.1
A64	IAV	-6.06±0.27	-6.60	301.2	0.08	Non	Q41593.1
A65	AAADDEEMK	-6.03±0.28	-6.50	978.4	0.30	Non	P01544.2
A66	QPYPQPQ	-5.98±0.45	-6.70	856.4	0.57	Non	BAS02406.1
A67	VPFGVGTQ	-5.98±0.28	-6.50	803.4	0.21	Non	AAT36483.1
A68	QPFQPQPFP	-5.97±0.29	-6.30	1181.6	0.90	Non	BAS02406.1
A69	HPEWEFPR	-5.90±0.46	-6.10	1096.5	0.84	Non	KAF7098210.1
A70	PAG	-5.90±0.57	-6.70	243.1	0.68	Non	P93692.1
A71	LPYPQPPPF	-5.88±1.13	-7.40	1054.5	0.92	Non	
A72	VPVPQPQPQ	-5.88±0.61	-6.90	988.5	0.28	Non	
A73	AAIHSVVH	-5.84±0.18	-6.10	832.5	0.13	Non	ACX37111.1
A74	QPTPLQPQ	-5.83±0.39	-6.60	907.5	0.30	Non	
A75	QPLPQQPPF	-5.77±0.98	-7.40	1050.5	0.81	Non	
A76	FPIPYPPQQ	-5.76±1.19	-6.90	1085.6	0.72	Non	AJG03094.1
A77	FPQPQFP	-5.76±0.95	-7.50	956.5	0.95	Non	BAS02406.1
A78	APFASIVA	-5.74±0.38	-6.40	774.4	0.42	Non	AFC98436.1

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
A79	QPFPGPQ	-5.58±0.59	-6.80	769.4	0.85	Non	AGO17673.1
A80	QAIHNVVH	-5.57±0.75	-6.70	916.5	0.14	Non	
A81	QPYPQQPFPQ	-5.55±1.21	-7.20	1228.6	0.69	Non	
A82	NPSDQELQ	-5.54±0.25	-6.00	929.4	0.21	Non	
A83	LPYPQPPPFSPQ	-5.50±0.28	-5.70	1366.7	0.76	Non	
A84	LPYPQPQFPF	-5.50±1.13	-6.30	1279.7	0.78	Non	CAY54134.1
A85	FPQPQFPFPQ	-5.50±0.98	-6.90	1181.6	0.89	Non	BAS02406.1
A86	QPYPQPQPF	-5.43±0.92	-7.00	1100.5	0.87	Non	BAS02406.1
A87	IAHASSQVLQ	-5.43±0.42	-6.00	1052.6	0.14	Non	BAS02406.1
A88	PPFSQQQQPQ	-5.39±1.02	-7.60	1183.6	0.30	Non	ALN96402.1
A89	YPQPQFPFS	-5.36±1.17	-6.90	1059.5	0.73	Non	BAS02406.1
A90	LPYPQPQFPF	-5.27±1.42	-6.90	1182.6	0.77	Non	CAY54134.1
A91	VPYANIDAGIGGQ	-5.20±1.23	-6.10	1273.6	0.32	Non	QCD24996.1
A92	QPQPFRPQ	-5.20±0.65	-6.40	996.5	0.77	Non	AGO17673.1
A93	VPFDVGTGVGA	-5.16±0.73	-6.10	1017.5	0.31	Non	ACA63873.1
A94	IPFVHPSILQQ	-5.15±0.78	-5.70	1277.7	0.38	Non	
A95	IALRTLPM	-5.09±0.49	-5.80	913.5	0.43	Non	
A96	WADPFDTR	-5.00±0.42	-5.30	1153.5	0.94	Non	
A97	RADIYNPR	-4.99±0.44	-5.60	1003.5	0.54	Non	
A98	IPFVHPSIL	-4.96±1.09	-6.80	1021.6	0.66	Non	ACA63873.1
A99	QAQQPFPQPQ	-4.95±1.20	-5.80	1167.6	0.53	Non	ACQ83639.1
A100	FAEIRNLAL	-4.95±1.25	-6.80	1045.6	0.38	Non	BAS02455.1
A101	QPFPTQPQ	-4.94±0.55	-5.90	1038.5	0.57	Non	CAY54134.1
A102	IAHASSQVSQ	-4.94±0.81	-6.00	1026.5	0.08	Non	
A103	LPFPQQPQ	-4.92±0.71	-6.10	953.5	0.49	Non	
A104	IALRILPT	-4.92±0.96	-6.40	895.6	0.30	Non	
A105	HASSQVLQ	-4.92±0.70	-6.10	868.4	0.18	Non	
A106	LPYPQPQPF	-4.90±1.51	-6.30	1085.6	0.81	Non	BAS02406.1
A107	VAMDIPPPR	-4.90±0.98	-6.50	994.5	0.72	Non	CAY54134.1
A108	YPQPQFPFPQ	-4.88±1.16	-6.10	1197.6	0.75	Non	
A109	QPFQPQPQ	-4.86±0.85	-6.30	1065.5	0.63	Non	
A110	YPQPQFPFPQ	-4.85±0.35	-5.10	1256.6	0.72	Non	
A111	QPLQFPQPQ	-4.85±0.64	-5.30	1178.6	0.62	Non	
A112	FAEIRNLALQTL	-4.80±1.27	-5.70	1387.8	0.32	Non	BAS02455.1
A113	EAIDDGRIGPR	-4.78±1.06	-6.50	1197.6	0.42	Non	KAF7045286.1
A114	IPYVQPSILQ	-4.70±0.40	-5.40	1156.6	0.35	Non	AAT36483.1
A115	QAQLPFPQ	-4.67±1.07	-6.60	927.5	0.67	Non	D6QZM4.1
A116	EAIRQQQPQ	-4.60±0.75	-5.80	1096.6	0.09	Non	
A117	QPAQYEVIRS	-4.54±1.16	-6.50	1189.6	0.26	Non	
A118	APFASIVAGIGGQ	-4.46±1.16	-6.10	1186.6	0.51	Non	
A119	EAEGIVEYLK	-4.43±0.90	-5.50	1149.6	0.14	Non	

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
A120	IAHGRSQVLQ	-4.42±0.73	-6.10	1107.6	0.13	Non	SCW25730.1
A121	QPAQLEGIRSLVL	-4.38±0.60	-4.90	1422.8	0.48	Non	QCD24996.1
A122	APFSSVVAGIGGQ	-4.37±0.55	-4.90	1188.6	0.45	Non	
A123	LPYSQPQPF	-4.33±0.88	-5.50	1075.5	0.71	Non	AGO17673.1
A124	QPQQPFPQPQ	-4.25±0.21	-4.40	1193.6	0.62	Non	QCD24996.1
A125	IPVVQPSILQ	-4.23±1.62	-6.10	1092.7	0.25	Non	ACF93464.1
A126	LPVVQPSILQ	-4.20±0.92	-5.60	1092.7	0.29	Non	
A127	IAQDFKTDLRF	-4.20±0.85	-4.80	1352.7	0.60	Non	XP_044417466.1
A128	QPYPQPQFPF	-4.15±1.77	-5.40	1197.6	0.83	Non	BAS02406.1
A129	QPAQLEAIRS	-3.93±1.03	-5.80	1111.6	0.23	Non	
A130	QPHQFPQPQ	-3.90±1.56	-5.00	1202.6	0.66	Non	
A131	QPAQLEVIRSLV	-3.85±1.91	-5.20	1351.8	0.26	Non	ACX37111.1
A132	QPAQLEAIRSL	-3.80±1.12	-5.30	1224.7	0.36	Non	
A133	NPKDETDQHWL	-3.78±1.15	-5.30	1266.6	0.47	Non	XP_044330354.1
A134	RPFDEVSRLL	-3.76±0.94	-5.30	1230.7	0.49	Non	ACJ65515.1
A135	VARQYEQTVVPPK	-3.70±1.70	-4.90	1513.8	0.09	Non	ACD44935.1
A136	LAHGRSQVLQ	-3.69±0.80	-5.40	1107.6	0.15	Non	
A137	QFPQPQPF	-3.68±1.15	-4.70	1084.5	0.93	Non	BAS02406.1
A138	IPFVHPSILQ	-3.66±1.28	-4.90	1149.7	0.50	Non	ACA63873.1
A139	QPAQLEVIRS	-3.54±0.90	-4.70	1139.6	0.21	Non	ACX37111.1
A140	QPAQYEVIRSLVL	-3.50±0.57	-3.90	1514.8	0.40	Non	AFC98436.1
A141	QFPQPQPFPPQ	-3.50±0.28	-3.70	1406.7	0.84	Non	BAS02406.1
A142	QPYPQPQPFPA	-3.28±1.24	-5.00	1268.6	0.81	Non	BAS02455.1
A143	VPWVVVDGKPL	-3.28±0.90	-4.70	1207.7	0.50	Non	XP_044360304.1
A144	RAKDQQDEGFVA	-3.23±1.24	-4.30	1362.7	0.15	Non	ACJ65515.1
A145	QPYPQPQPFPS	-3.20±0.87	-4.20	1284.6	0.75	Non	BAS02406.1
A146	LPYPKPQFPF	-3.10±1.35	-4.50	1182.6	0.81	Non	BAS02432.1
A147	EPQRDFAKVL	-3.07±1.00	-4.10	1201.6	0.50	Non	XP_044435885.1
A148	QFPQPQPFPP	-3.05±0.49	-3.40	1278.6	0.91	Non	BAS02406.1
A149	LPYPQPQSFP	-2.90±0.86	-4.00	1172.6	0.61	Non	ABD85198.1
A150	QPYPQPQPFPSQ	-2.85±0.21	-2.70	1412.7	0.65	Non	BAS02406.1
A151	QPAQLEAIRSLVL	-2.81±1.27	-4.50	1436.8	0.45	Non	
A152	QPYPQPQPFPPQ	-2.80±1.54	-4.10	1422.7	0.73	Non	
A153	LPYPKPQFPF	-2.80±1.56	-4.30	1279.7	0.82	Non	BAS02432.1
A154	PPFSQQQQQFPQPQ	-2.73±1.52	-3.90	1555.7	0.47	Non	
A155	LPYPRQPF	-2.47±1.15	-4.30	1113.6	0.88	Non	
A156	QPAQLEVIRSLVL	-2.45±1.12	-3.90	1464.9	0.41	Non	ACX37111.1
A157	LPYPRQPFLPQ	-2.40±0.14	-2.50	1451.8	0.72	Non	
A158	LPLDIVSVNNRFE	-2.38±1.02	-3.30	1514.8	0.29	Non	KAF7098210.1
A159	QPAQLEVIRSL	-2.38±0.96	-4.00	1252.7	0.34	Non	ACX37111.1
A160	RAPFASIVAGIGGQ	-2.13±1.54	-3.90	1342.7	0.39	Non	

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
A161	QPAQYEVIRSL	-2.06±0.93	-3.40	1302.7	0.39	Non	AFC98436.1
A162	LPYSQPQPFRP	-2.00±2.12	-3.50	1328.7	0.72	Non	AGO17673.1
A163	QPYPQPQPFL	-2.00±0.91	-2.80	1213.6	0.88	Non	CAY54134.1
A164	HAVSEGTKAVTKFTSS	-2.00±0.71	-2.50	1648.8	0.09	Non	KAF6991516.1
A165	QPYPQPQPFPQ	-1.85±0.64	-1.40	1254.6	0.85	Non	
A166	QPAQLEAIRSLVLQ	-1.73±1.50	-3.40	1564.9	0.37	Non	
A167	QPHLPYPQPQ	-1.53±1.33	-3.00	1203.6	0.51	Non	AFQ13464.1
A168	LPYPQPQPFRPQ	-1.30±0.14	-1.20	1466.8	0.67	Non	AFQ13464.1
A169	LPYPKPQPFPPQ	-0.75±1.91	-2.10	1407.8	0.71	Non	BAS02432.1
A170	EAIRSLVLQTLPT	-0.70±0.42	-1.00	1439.8	0.12	Non	
A171	LPYPQPQPFRP	-0.35±1.48	-1.40	1338.7	0.83	Non	AFQ13464.1
A172	LPFPQPQPFPPQ	-0.30±0.89	-1.00	1391.7	0.81	Non	XP_044410789.1
A173	SAEPDFLERHIPR	0.20±1.21	-1.10	1565.8	0.57	Non	KAF7067328.1
A174	LPYSQPQPFRPQ	0.35±0.35	0.10	1456.7	0.56	Non	AGO17673.1
A175	QPYPQPQLFPQ	0.43±1.30	-1.40	1341.7	0.77	Non	BAS02455.1
A176	PPFSQQQPFQ	0.80±4.38	3.90	1299.6	0.64	Non	
A177	FAEIRNLALQTLPT	1.85±0.78	1.30	1484.8	0.33	Non	BAS02455.1
A178	IAHASSQVSQQSYQLL	2.65±2.90	0.60	1758.9	0.18	Non	CAY54134.1
A179	LPQFEEIRNLAL	4.80±1.21	4.10	1441.8	0.48	Non	BAS02406.1
A180	LPYPQPQPFPPQ	5.10±0.57	5.50	1407.7	0.69	Non	CAY54134.1
A181	HASSQVSQQSYQLLQ	6.55±0.92	5.90	1702.8	0.17	Non	CAY54134.1
A182	KPFDELVVDSKDFDVS	8.22±1.02	6.60	1838.9	0.25	Non	KAF7048142.1
A183	DAVSVNNRFEKGDELRPQ	13.5±2.12	12.00	2073.0	0.15	Non	XP_044372060.1
A184	QARPPSVMDFIADHPF	14.67±1.47	13.00	1826.9	0.46	Non	KAF7067328.1
A185	QPFPSQQPYPQPQPFPS	14.90±2.29	14.00	1968.9	0.60	Non	
A186	QPPFSQQQPPF	22.95±7.85	28.50	1299.6	0.71	Non	ACA63873.1
A187	QPFPSQLPYQPQPFPPQ	32.75±7.71	38.20	2092.0	0.59	Non	
B1	QQGHYPT	-7.83±0.37	-8.50	829.4	0.36	Non	ACD44935.1
B2	HWYYPT	-7.63±0.29	-8.10	865.4	0.80	Non	
B3	ELPW	-7.57±0.19	-7.90	543.3	0.74	Non	
B4	QTFPPQ	-7.54±0.34	-8.40	716.3	0.52	Non	BAS02455.1
B5	QQPFPQ	-7.50±0.41	-8.20	743.4	0.71	Non	QCF42053.1
B6	QQPFPQ	-7.39±0.27	-7.80	840.4	0.81	Non	AGO17673.1
B7	QGSFRPS	-7.31±0.37	-8.10	777.4	0.51	Non	AGO17673.1
B8	NVYIPPY	-7.29±0.56	-8.20	864.4	0.53	Non	BAS02406.1
B9	TFLQPH	-7.21±0.40	-7.90	741.4	0.40	Non	ACA63873.1
B10	QSGYYPT	-7.13±0.50	-8.00	814.3	0.50	Non	AEO45112.2
B11	TQPFPPQ	-7.11±0.26	-7.70	813.4	0.63	Non	
B12	HQQPFPQ	-7.11±0.28	-7.60	880.4	0.60	Non	
B13	QQFPQPQ	-7.06±0.37	-7.50	871.4	0.54	Non	AFC98436.1
B14	GQVEWPQ	-7.04±0.44	-7.80	842.4	0.28	Non	

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
B15	SGPW	-7.01±0.32	-7.50	445.2	0.96	Non	XP_044344245.1
B16	EQPVPH	-7.00±0.33	-7.80	705.3	0.20	Non	XP_044452568.1
B17	QQTFPQ	-7.00±0.37	-7.60	747.4	0.37	Non	ACX37111.1
B18	QGFFQPS	-6.96±0.28	-7.30	809.4	0.69	Non	
B19	GTFLQPH	-6.89±0.24	-7.20	798.4	0.47	Non	ACA63873.1
B20	QQLFPE	-6.88±0.18	-7.10	760.4	0.48	Non	
B21	QQQFPQ	-6.86±0.41	-7.60	774.4	0.44	Non	QCD24996.1
B22	TIPQPH	-6.84±0.47	-7.50	691.4	0.28	Non	ACX37111.1
B23	GQVPWPQ	-6.83±0.45	-7.60	810.4	0.70	Non	
B24	QTFPQPE	-6.83±0.55	-8.10	845.4	0.37	Non	QCD24996.1
B25	RSAWEPQ	-6.83±0.28	-7.10	872.4	0.33	Non	XP_044447612.1
B26	QQPFPQPQ	-6.83±0.31	-7.20	897.4	0.77	Non	
B27	KQFPQPQ	-6.81±0.35	-7.20	871.5	0.47	Non	AWK59777.1
B28	QQPFPW	-6.79±0.31	-7.20	801.4	0.97	Non	
B29	LQQPFPQ	-6.79±0.41	-7.50	856.4	0.55	Non	
B30	QQQQPPF	-6.78±0.32	-7.40	871.4	0.74	Non	AGO17755.1
B31	QQPILPQ	-6.72±0.26	-7.30	822.5	0.45	Non	ACF93464.1
B32	KWQEPG	-6.70±0.23	-7.20	743.4	0.41	Non	ACD44935.1
B33	QLFPQPQ	-6.70±0.49	-7.70	856.4	0.60	Non	BAS02455.1
B34	QQTFFH	-6.66±0.31	-7.20	756.4	0.46	Non	
B35	QQPLLQ	-6.64±0.45	-7.20	822.5	0.50	Non	
B36	QLGYYPT	-6.62±0.45	-7.40	840.4	0.49	Non	CAA43331.1
B37	QQQLPPF	-6.60±0.48	-7.20	856.4	0.82	Non	ACA63873.1
B38	SFPL	-6.58±0.22	-7.00	462.2	0.94	Non	
B39	GQQGHYPT	-6.58±0.32	-7.20	886.4	0.38	Non	ACD44935.1
B40	NMQADPS	-6.56±0.34	-7.20	761.3	0.19	Non	
B41	QGVYQPQ	-6.56±0.38	-7.00	818.4	0.19	Non	
B42	GQGSFRPS	-6.54±0.43	-7.20	834.4	0.56	Non	AGO17673.1
B43	QQVPQPQ	-6.53±0.73	-7.50	823.4	0.20	Non	
B44	AQGPVQPQ	-6.53±0.28	-7.20	823.4	0.25	Non	ABD85198.1
B45	QQQPIQ	-6.53±0.37	-7.10	837.4	0.36	Non	
B46	QTIPQPH	-6.52±0.19	-6.90	819.4	0.27	Non	ACX37111.1
B47	QQPYPH	-6.51±0.44	-7.10	768.4	0.60	Non	
B48	GQQPFPPQ	-6.48±0.20	-6.90	897.4	0.80	Non	
B49	SFPI	-6.47±0.42	-7.40	462.2	0.86	Non	
B50	NMQVDPS	-6.46±0.45	-7.30	789.3	0.14	Non	QCD24996.1
B51	QSGYFPT	-6.46±0.70	-7.90	798.4	0.72	Non	
B52	EIPW	-6.43±0.45	-7.40	543.3	0.70	Non	XP_044331132.1:
B53	FAGDDAPR	-6.40±0.48	-7.20	847.4	0.64	Non	XP_044396063.1
B54	QQGYYP	-6.38±0.62	-7.60	855.4	0.45	Non	XP_044414375.1
B55	GRVPVPQ	-6.33±0.35	-6.80	751.4	0.24	Non	AGO17673.1

Number	Peptide Sequence	ΔG	$\Delta G_{\min}$	Mass	Peptide Ranker Score	Toxicity prediction	Protein Sources
B56	FMGPK	-6.31±0.21	-6.80	578.3	0.91	Non	P16159.1
B57	SSTTIMPF	-6.31±0.24	-6.60	882.4	0.70	Non	
B58	QQPVIPQ	-6.28±0.28	-6.70	808.4	0.28	Non	
B59	YFMGPK	-6.26±0.46	-7.10	741.4	0.87	Non	P16159.1
B60	RTIPQPH	-6.24±0.34	-6.80	847.5	0.23	Non	QCD24996.1
B61	GQGFFQPS	-6.24±0.30	-6.70	866.4	0.68	Non	
B62	TQHLQPQ	-6.20±0.29	-6.70	850.4	0.11	Non	BAS02455.1
B63	QIPVVQPS	-6.19±0.42	-6.90	866.5	0.13	Non	ACF93464.1
B64	QQYPQPQ	-6.19±0.56	-7.20	887.4	0.36	Non	
B65	QQGYYPPI	-6.16±0.36	-7.00	867.4	0.52	Non	1501416A
B66	VRVPVPQ	-6.11±0.29	-6.70	793.5	0.12	Non	BAS02406.1
B67	QTFPQPQ	-6.09±0.68	-7.40	844.4	0.39	Non	P21292.1
B68	QQLPFPQ	-6.09±0.54	-7.20	856.4	0.71	Non	
B69	QGTLFPFQ	-6.07±0.92	-7.60	886.5	0.59	Non	
B70	SQVQWPQ	-6.04±0.26	-6.50	871.4	0.46	Non	
B71	QQPVL PQ	-6.03±0.51	-7.00	808.4	0.33	Non	ALN96402.1
B72	YYPISPQ	-5.99±0.66	-7.10	866.4	0.31	Non	CAA43331.1
B73	QMNFPVI	-5.97±0.33	-6.60	847.4	0.60	Non	XP_044376899.1
B74	NVYVPPE	-5.94±0.62	-7.10	816.4	0.20	Non	
B75	GQQGHYPA	-5.88±0.44	-6.60	856.4	0.40	Non	1501416A
B76	QQSPFPQ	-5.87±0.4	-6.60	830.4	0.66	Non	ACF93464.1
B77	TNGLPFPQ	-5.86±0.35	-6.50	872.4	0.48	Non	
B78	QLPVVQPS	-5.84±0.60	-6.80	866.5	0.13	Non	
B79	GQSGYYPT	-5.82±0.43	-6.40	871.4	0.49	Non	AEO45112.2
B80	MENSHIPG	-5.82±0.38	-6.70	883.4	0.28	Non	ACA63873.1
B81	GEVQQDPT	-5.79±0.41	-6.50	872.4	0.08	Non	KAF7098210.1
B82	STTIMPF	-5.78±0.45	-6.40	795.4	0.73	Non	
B83	TVHNTPY	-5.72±0.28	-6.20	830.4	0.10	Non	P16159.1
B84	RTAWEPH	-5.66±0.42	-6.50	895.4	0.23	Non	XP_044452568.1
B85	TTIMPF	-5.61±0.28	-6.10	708.4	0.63	Non	
B86	YSPYQPQ	-5.60±0.69	-6.80	881.4	0.42	Non	
B87	QQQPLPQ	-5.56±0.15	-5.80	837.4	0.37	Non	
B88	HTFPQPQ	-5.53±0.45	-6.30	853.4	0.39	Non	
B89	QQQPFPQ	-5.44±0.66	-6.30	871.4	0.60	Non	AFC98436.1
B90	QIFPQPQ	-5.40±0.24	-5.80	856.4	0.55	Non	
B91	MVVTFGPT	-5.38±0.45	-6.10	850.4	0.24	Non	KAF7067965.1
B92	NVYIPPH	-5.34±0.91	-6.80	838.4	0.43	Non	BAS02455.1
B93	QSYQPQ	-4.93±0.64	-6.30	846.4	0.40	Non	CAY54134.1

Note: A and B represented the peptides with P₂' as Pro and P₂ as Pro, respectively. ΔG , $\Delta G_{\min}$ represented the average and minimum docking binding energy (kcal/mol), respectively. Peptide

Ranker score represented the predicted biological activity of peptides. 'Non' represented that toxicity was not detected by ToxIBTL.

Table S4. The interactions and action regions between peptides and DPP-4 (1WCY).

Inhibitor	Interactions and action regions between peptides and DPP-4	
	Hydrogen bond interactions	Hydrophobic interactions
PPFS	Glu206, Glu205 (S2); Ser209, Phe357 (S3); His740, Ser630 (Triad); Arg669.	Tyr662, Tyr666 (S1); Arg125 (S2); Arg358 (S3); Tyr547.
APFGL	His740 (Triad); Gln553, Tyr547.	Glu206 (S2); Phe357 (S3); Ser630 (Triad).
PPFW	Glu206, Arg125 (S2).	Tyr662 (S1); Glu205 (S2); Ser209, Phe357 (S3); His740, Ser630 (Triad); Ser552, Lys554, Trp629, Tyr547.
PPF	Ser630, His740 (Triad).	Tyr662, Tyr666, Val711, Tyr631, Val656 (S1); Arg125 (S2); Tyr547.
IPI	Tyr662 (S1); Ser630, Asn710 (Triad); Tyr547.	Val656, Val711, Tyr666, Trp659 (S1); Glu205, Glu206, Arg125 (S2); Ser209, Phe357 (S3); Arg669.
Sitagliptin	-	Tyr662, Tyr666 (S1); Glu205, Glu206, Arg125 (S2); Phe357 (S3); Ser630 (Triad); Tyr547.

Note: () represented the active pocket where the amino acid in parentheses is located.

Table S5. K_m and V_{max} of PPFS, APFGL, PPFW, and Sitagliptin

Rate	PPFS(μ M)		
Constants	0	67.5	125
K_m (mM)	0.76	1.01	1.43
V_{max} (Abs $\cdot$ min $^{-1}$)	0.0055	0.0055	0.0053

Rate	APFGL (μ M)		
Constants	0	67.5	125
K_m (mM)	0.75	1.37	2.14
V_{max} (Abs $\cdot$ min $^{-1}$)	0.0049	0.0044	0.0032

Rate	PPFW (μ M)		
Constants	0	125	250
K_m (mM)	0.76	1.75	2.57
V_{max} (Abs $\cdot$ min $^{-1}$)	0.0050	0.0047	0.0040

Rate	Sitagliptin (μ M)		
Constants	0	0.38	0.76
K_m (mM)	0.82	0.99	1.24
V_{max} (Abs $\cdot$ min $^{-1}$)	0.0051	0.0048	0.0048

Fig.S1. MS/MS spectra of identified peptides (A, B, and C represented PPFS, APFGL, and PPFW, respectively).

