

Soyasapogenol C from Fermented Soybean (*Glycine Max*) Acting as a Novel AMPK/PPAR α Dual Activator Ameliorates Hepatic Steatosis: A Novel SANDA Methodology

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Supplementary Materials:

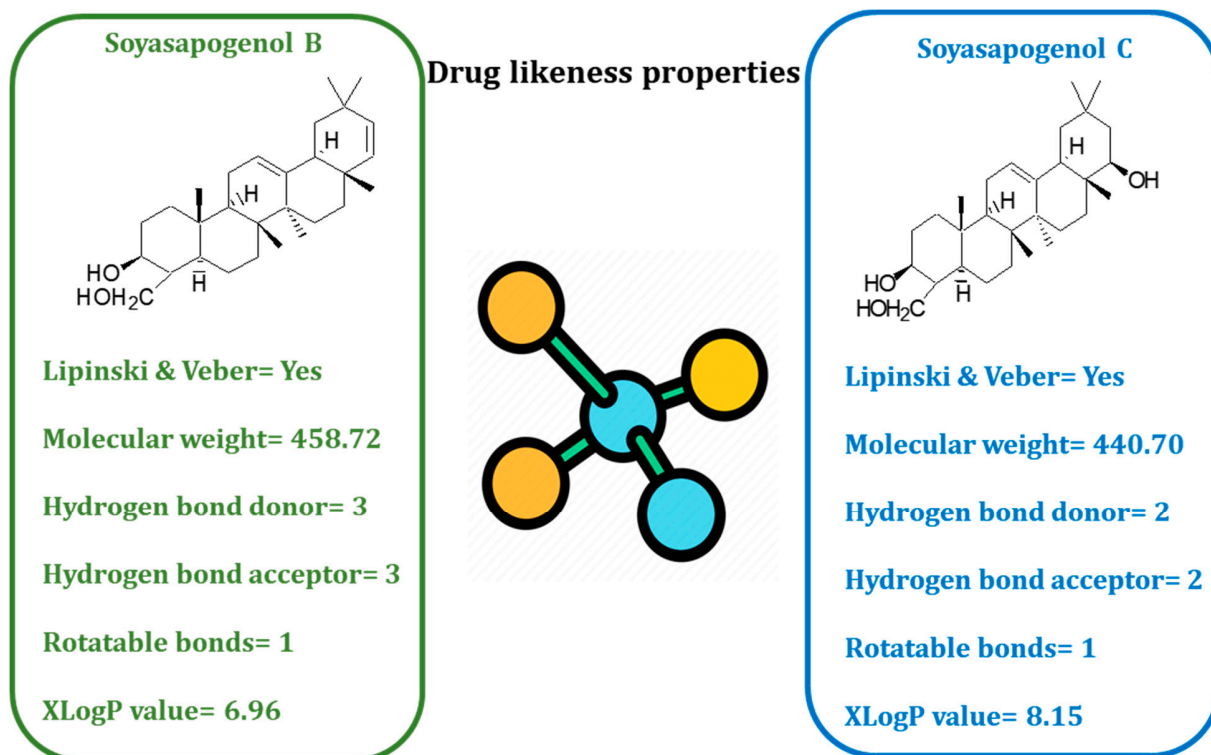


Figure S1. The structural similarity evaluation of soyasapogenol B and soyasapogenol C.

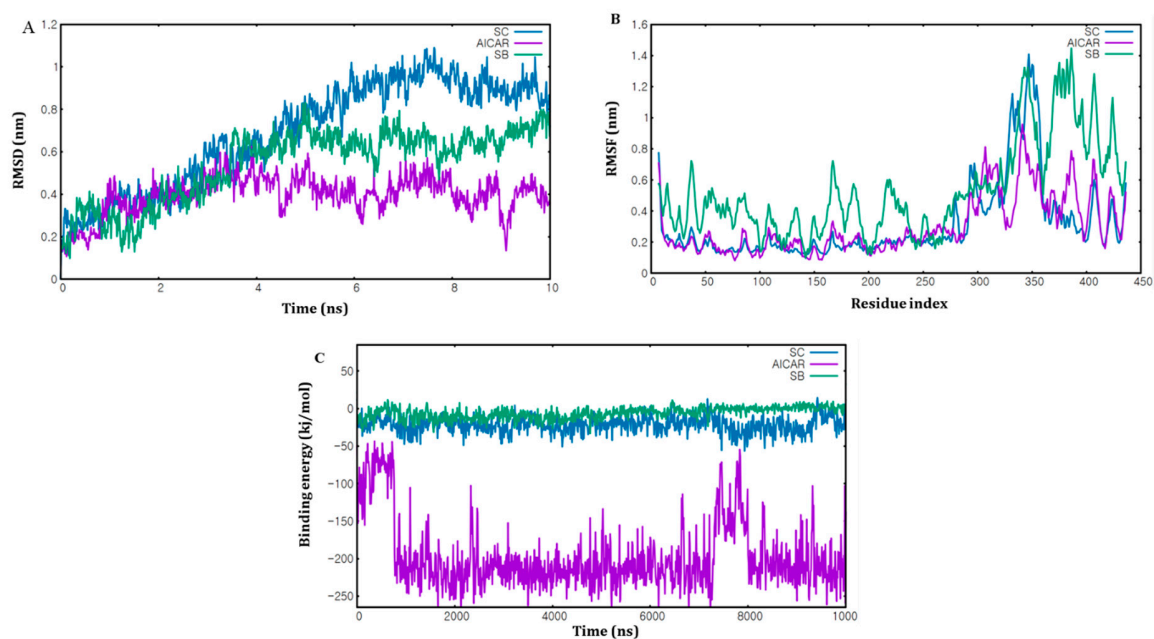


Figure S2. Molecular dynamic simulation: (A) RMSD plots between soyasapogenol C (blue), soyasapogenol B (green), and AICAR (purple) with AMPK; (B) RMSF plots between soyasapogenol C (blue), soyasapogenol B (green), and AICAR (purple) with AMPK; (C) Binding interaction plots between soyasapogenol C (blue), soyasapogenol B (green), and AICAR (purple) with AMPK at 10ns. *SC—soyasapogenol C; SB—soyasapogenol B; AICAR—5-Aminoimidazole-4-carboxamide ribonucleotide.

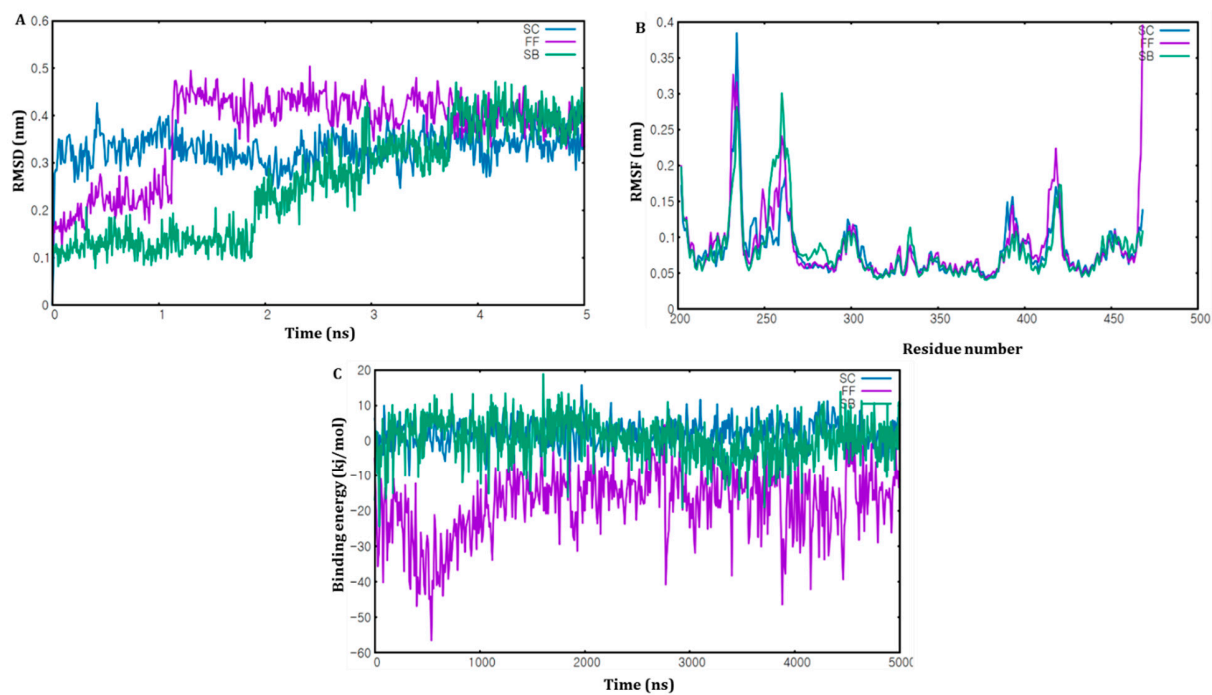


Figure S3. Molecular dynamic simulation: (A) RMSD plots between soyasapogenol C (blue), soyasapogenol B (green), and fenofibrate (purple) with PPAR α . (B) RMSF plots between soyasapogenol C (blue), soyasapogenol B (green), and fenofibrate (purple) with PPAR α at 5 ns. (C) Binding interaction plots between soyasapogenol C (blue), soyasapogenol B (green), and fenofibrate (purple) with PPAR α at 5ns. * SC—soyasapogenol C; SB—soyasapogenol B; FF—fenofibrate.

Table S1. Group B soyasaponin, soyasaponin, DDMP saponin, and soyasaponin aglycones present in fermented soybean Cheonggukjang, and their activities.

Serial. No	Types	Compounds	Effects
1	Group B soyasaponin	Soyasaponin I	Anti-inflammatory, anti-carcinogenic, hepatoprotective, cardiovascular protective, antimicrobial, anti-oxidative, adjuvant kidney protective
2		Soyasaponin II	Hepatoprotective, cardiovascular protective, anti-viral
3		Soyasaponin III	Anti-inflammatory, anti-carcinogenic, hepatoprotective
4		Soyasaponin IV	Anti-carcinogenic, cardiovascular protective
5		Soyasaponin V	
6		Soyasaponin VI	
7	Soyasaponin	Dehydrosoyasaponin I	Potent and reversible calcium-activated potassium (maxi-K) channels activator
8	Soyasaponin aglycone	Soyasapogenol A	Anti-carcinogenic, hepatoprotective, anti-viral
9		Soyasapogenol B	Anti-inflammatory, anti-mutagenic, anti-carcinogenic, hepatoprotective, anti-viral
10		Soyasapogenol C	Anti-Inflammatory, anti-viral
11		Soyasapogenol D	
12	DDMP group of soyasaponin	Soyasaponin α g	Antioxidant, anti-obesity, anti-tumorigenic, anti-hypertension, anti-arcopenia, skin
13		Soyasaponin β a	whitening, immune modulation, sympathetic
14		Soyasaponin γ a	nerve activity, anti-diabetic activity
15		Soyasaponin γ g	

Table S2. Docking score of Soyasaponin B group, soyasaponin, DDMP saponin, and soyasaponin aglycones present in fermented soybean Cheonggukjang using Auto Dock Vina and AutoDock4.

Compound	AMPK		LKB1		PPAR α	
	AutoDock Vina	AutoDock4	AutoDock Vina	AutoDock4	AutoDock Vina	AutoDock4
Control	-5.7	-10.81	-7.4	-7.22	-6.7	-7.81
Soyasaponin I	2.6	-3.69	0.6	-10.24	12.8	52.74
Soyasaponin II	0.6	0.66	-3.4	-3.95	19.1	68.81
Soyasaponin III	19.2	416.51	35.6	376.24	122.8	2.37E+03
Soyasaponin IV	-3.3	-10.31	1	-10.85	6.8	19.82
Soyasaponin V	25.6	337.57	26.7	218.58	88.8	6.39E+03
Soyasaponin VI	6.4	86.93	1.2	31.8	25	236.48
Soyasapogenol A	-3.3	-8.22	-8.2	-10.35	3.5	-1.84
Soyasapogenol B	-3.7	-7.68	-8.8	-9.3	2	-5.82
Soyasapogenol C	-7.2	-10.24	-9.4	-8.97	4.2	-8.95
Soyasapogenol D	-6.4	-10.98	-8.2	-10.16	4.2	-2.12
Soyasaponin α g	17.6	362.34	8.4	216.29	26.2	271.11
Soyasaponin β a	9.3	102.16	0.2	138.88	21.5	385.91
Soyasaponin γ a	-4.1	6.67	-6.2	-4.14	3.8	20.45
Soyasaponin γ g	-0.9	1.62	-0.8	-7.13	-1	29.62
Dehydrosoyasaponin I	4.7	-4.62	2	-13.05	14	27.21

Table S3. In silico docking score of active compounds soyasapogenol C, soyasapogenol B, and control compounds AICAR (AMPK) and fenofibrate (PPAR α) with targets AMPK and PPAR α , using four different docking tools.

Compounds	AMPK (PDB ID: 2Y94)			
	Auto Dock Vina	AutoDock4	Swiss Dock	LeDock
AICAR*(Control)	-6	-7.54	-9.01	-6.01
Soyasapogenol B	-3.7	-7.68	-7.39	-4.67
Soyasapogenol C	-7.2	-10.24	-6.96	-4.64

*AICAR- 5-Aminoimidazole-4-carboxamide ribonucleotide

Compounds	PPAR α (PDB ID: 1K7L)			
	Auto Dock Vina	AutoDock4	Swiss Dock	LeDock
Fenofibrate (Control)	-7.4	-9.67	-8.59	-3.67
Soyasapogenol B	2	-5.82	-5.47	-2.31
Soyasapogenol C	4.2	-8.95	-7.91	-2.17

Table S4. The binding energy and interacting residues obtained by calculating the molecular docking data of AMPK and PPAR α with controls and soyasapogenol C, using Auto Dock 4.2.

Compounds	Binding energy (kcal/mol)	Intermolecular energy (kcal/mol)	Common residues
AMPK			
Soyasapogenol C	-10.24	-11.16	VAL24, GLY25, VAL30, LYS45, MET93, GLU100, GLU143, ASN144, LEU146, ALA156, ASP157.
AICAR* (control)	-7.54	-6.20	
PPAR α			
Soyasapogenol C	-8.95	-9.73	CYS276, THR279, LEU321, MET330, VAL332
Fenofibrate (control)	-9.67	-11.41	
<ul style="list-style-type: none">AICAR—5-Aminoimidazole-4-carboxamide ribonucleotide			

Table S5. Human primer sets for real-time PCR.

Gene	Primer	
	Forward (5'→3')	Reverse (3'→5')
<i>ACOX-1</i>	AGGTCACAGCTGTCCAACCA	TTACCCAGCCCTGGCTTAAT
<i>CPT-1α</i>	GGTCCAGGTAGAGCTCAGGC	GTGCTCTGAGGCCTTTGTCA
<i>ACC</i>	GCTGCTCGGATCACTAGTGAA	TTCTGCTATCAGTCTGTCCAG
<i>FASN</i>	GACATCGTCCATTCGTTTGTG	CGGATCACCTTCTTGAGCTCC
<i>GAPDH</i>	CATGTTCGTCATGGGGTGAACCA	AGTGATGGCATGGACTGTGGTCAT