

Electronic Supplementary Material

Identification of Potential human Ryanodine Receptor 1 Agonists and Molecular Mechanisms of Natural Small-molecule Phenols as Anxiolytics

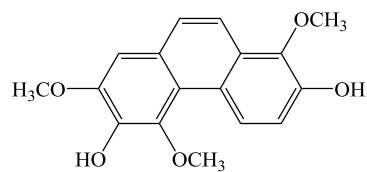
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¹*School of Chinese Materia Medica, Beijing University of Chinese Medicine, Yangguang South Road, Fangshan District, Beijing 102488, China*

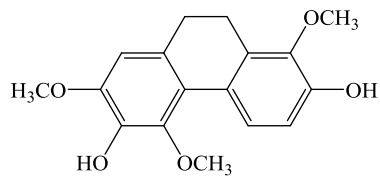
²*National Institute on Drug Dependence, Peking University, 38#, Xueyuan Road, Haidian District, Beijing 100191, China*

Corresponding author.E-mail: Jianmei Huang; huangjm@bucm.edu.cn

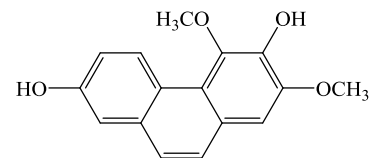
Figure S1. The structural information of 132 NSMPs.



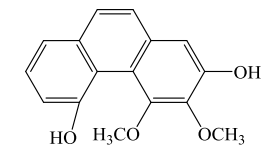
1,5,7-Trimethoxy-2,6-phenanthrenediol



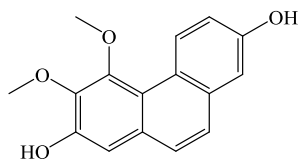
1,5,7-Trimethoxy-9,10-dihydro-2,6-phenanthrenediol



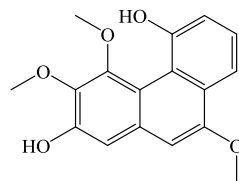
2,4-Dimethoxy-3,7-phenanthrenediol



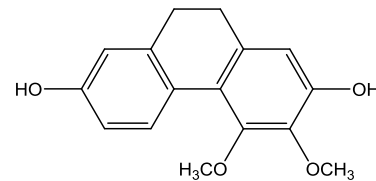
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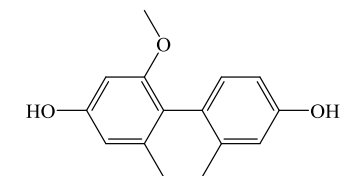
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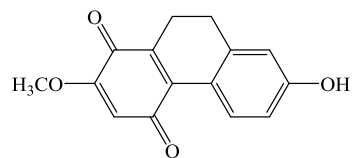
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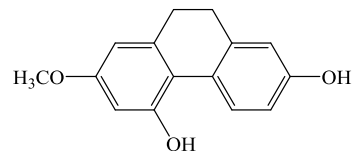
9,10-Dihydro-3,4-dimethoxy-2,7-phenanthrenediol



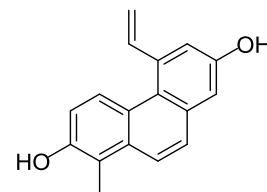
9,10-Dihydro-4-methoxy-2,7-phenanthrenediol



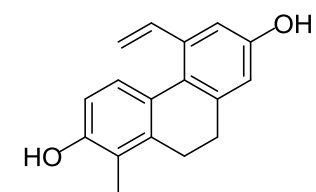
9,10-Dihydro-7-hydroxy-2-methoxy-1,4-phenanthrenedione



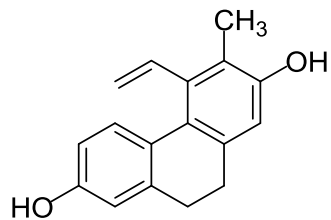
9,10-Dihydro-7-methoxy-2,5-phenanthrenediol



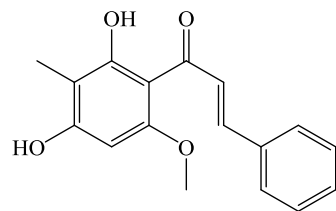
Dehydroeffusol



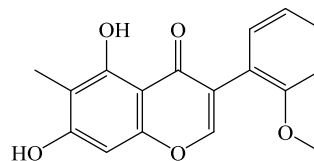
Effusol



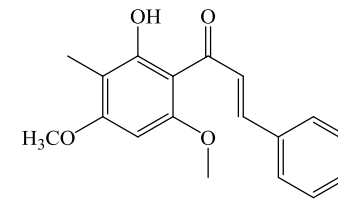
Juncusol



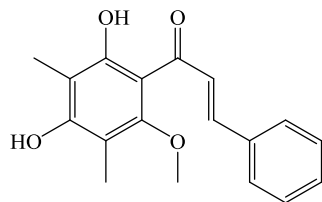
2',4'-Dihydroxy-6'-methoxy-3'-methylchalcone



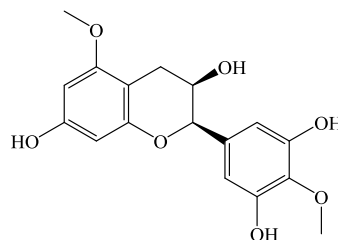
2'-O-methylabronisoflavone



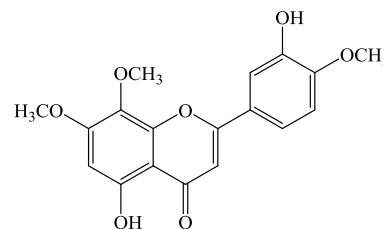
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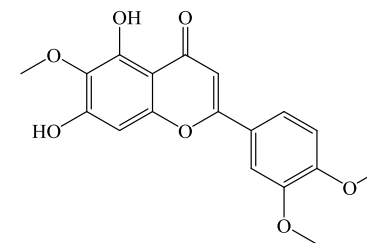
2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone



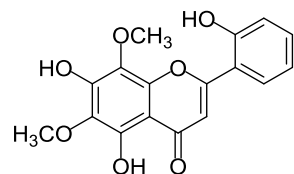
(-)-4'-Methylepigallocatechin



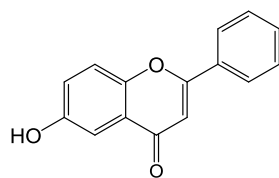
5,3'-Dihydroxy-7,8,4'-trimethoxyflavone



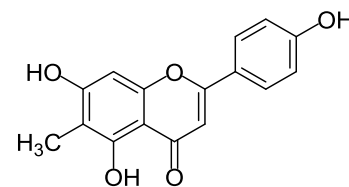
5,7-Dihydroxy-6,3',4'-trimethoxyflavone



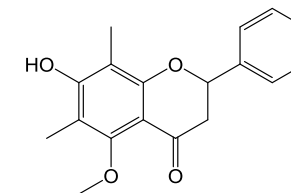
5,7,2'-Trihydroxy-6,8-dimethoxyflavone



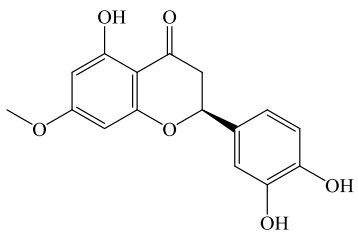
6-Hydroxyflavone



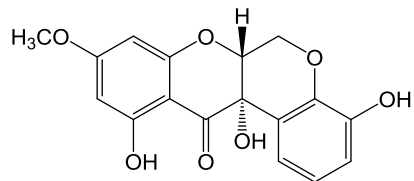
6-Methylapigenin



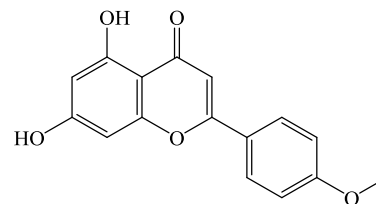
7-hydroxy-5-methoxy-6,8-dimethylflavanone



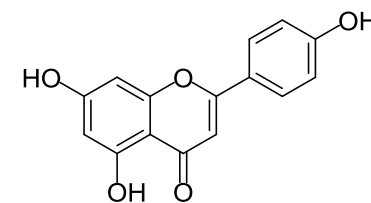
7-O-methyleriodictyol



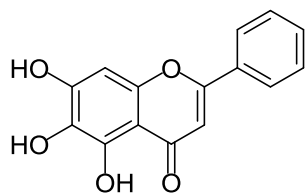
10-Demethylboeravinone C



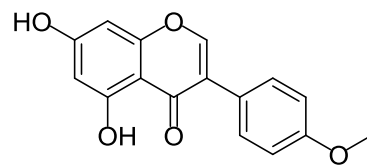
Acacetin



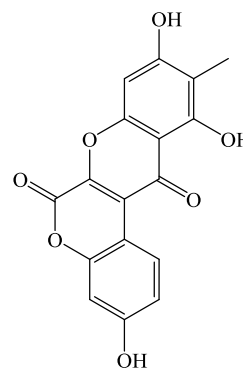
Apigenin



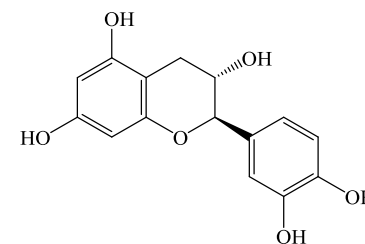
Baicalein



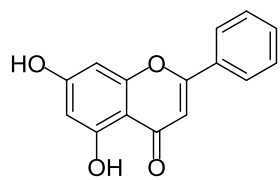
Biochanin A



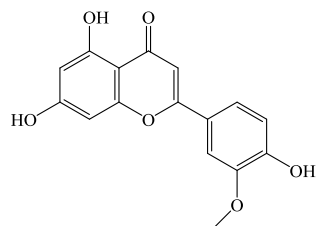
Boeravinone F



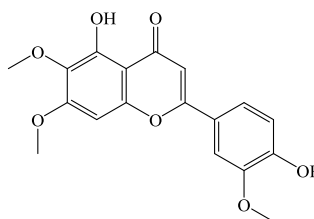
Catechin



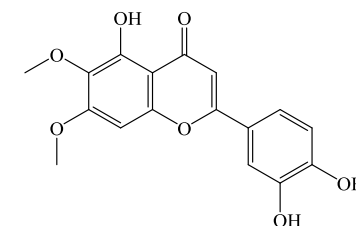
Chrysin



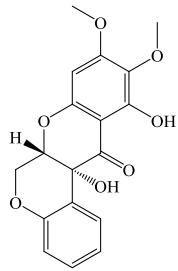
Chrysoeriol



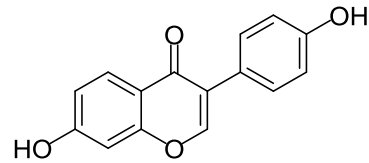
Cirsilineol



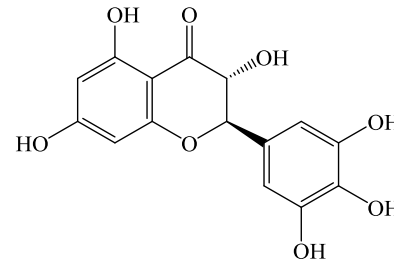
Cirsiliol



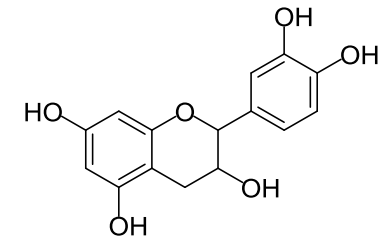
Coccineone E



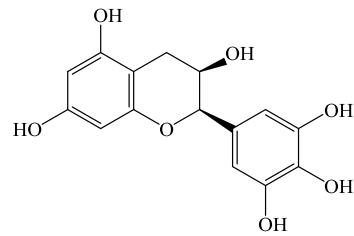
Daidzein



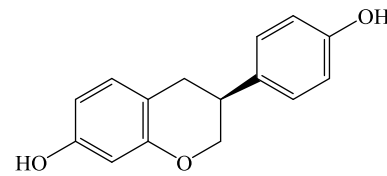
Dihydromyricetin



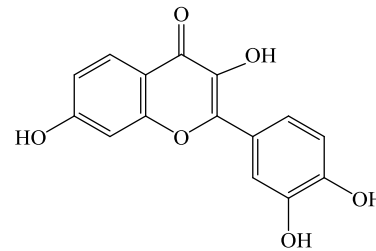
(-) Epicatechin



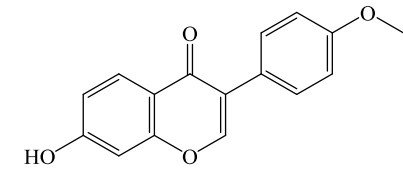
(-) Epigallocatechin



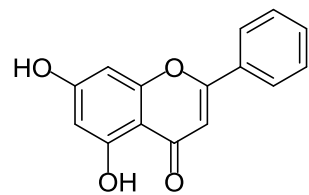
Equol



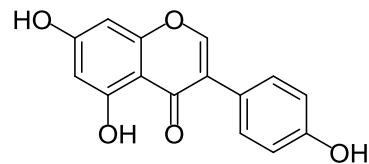
Fisetin



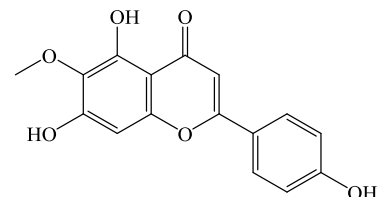
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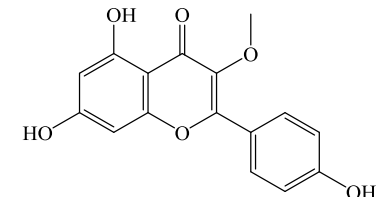
Chrysin



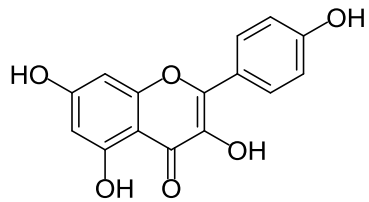
Genistein



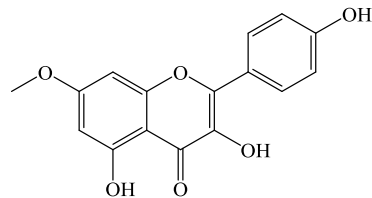
Hispidulin



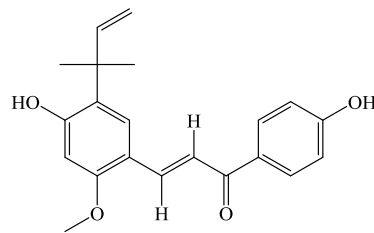
Isokaempferide



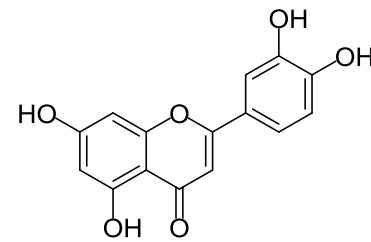
Kaempferol



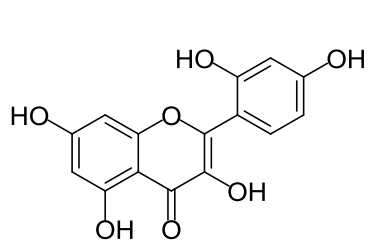
Kaempferol 7-methyl ether



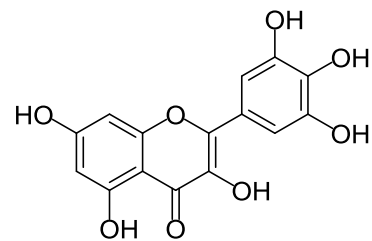
Licochalcone A



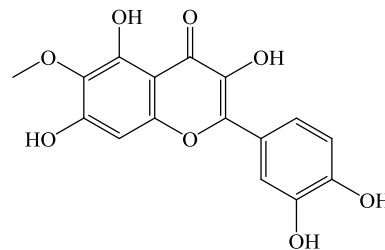
Luteolin (3',4',5,7-tetrahydroxyflavone)



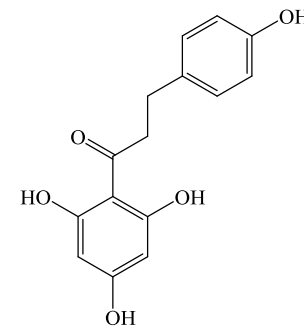
Morin



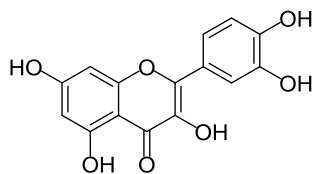
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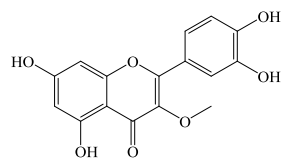
Patuletin



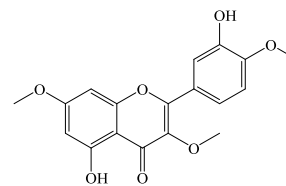
Phloretin



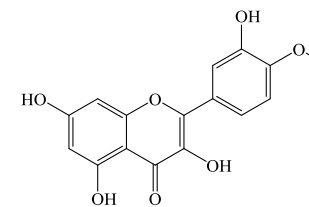
Quercetin



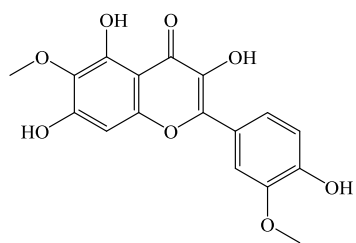
Quercetin 3-methyl



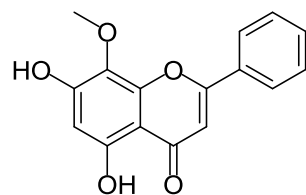
Quercetin 3,4',7-trimethyl ether



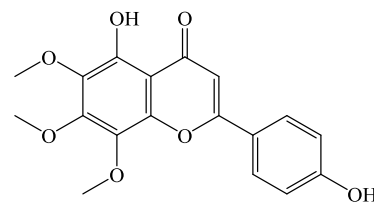
Quercetin 4'-methyl ether



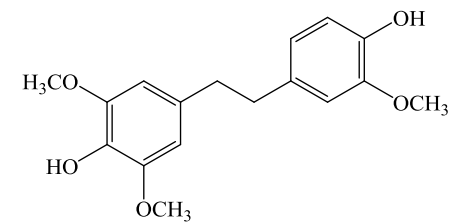
Spinacetin



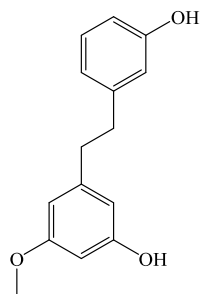
Wogonin



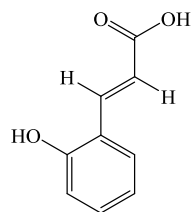
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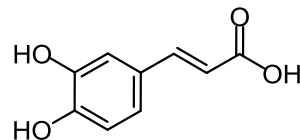
Aloifol II



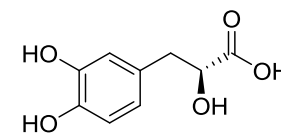
Batatasin III



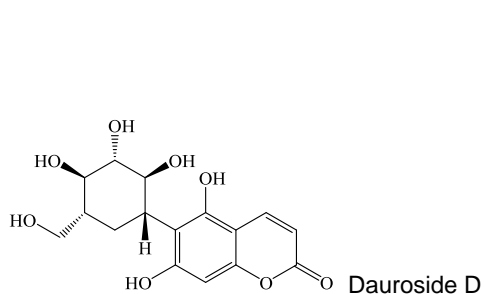
2-Hydroxycinnamic acid



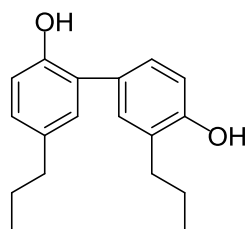
Caffeic acid



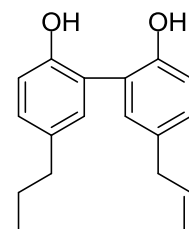
Danshensu [(3-(3,4-dihydroxyphenyl)-(2R)-lactic acid)]



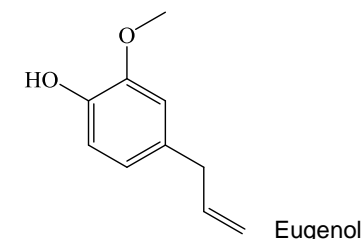
Daurosides



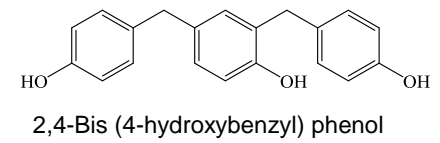
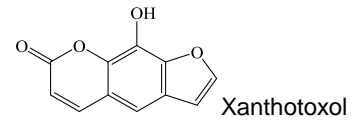
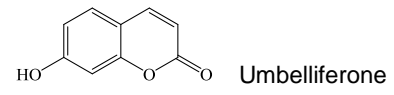
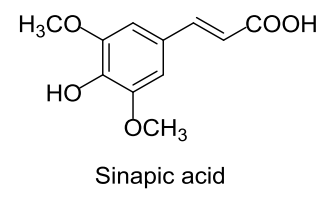
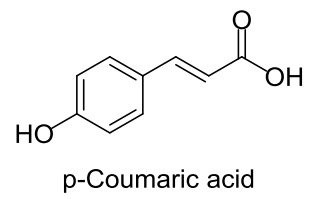
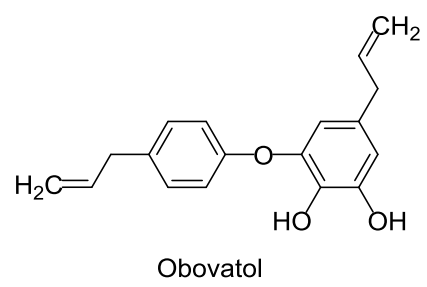
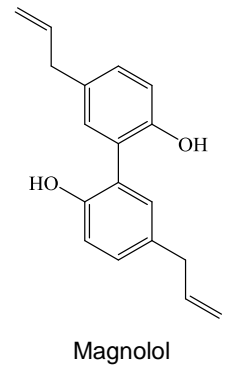
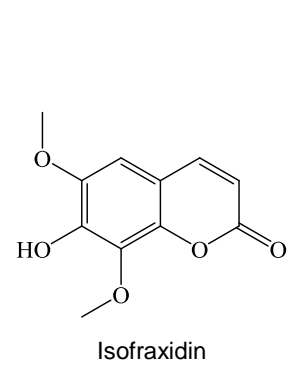
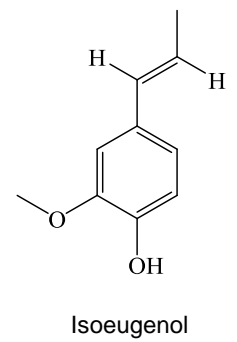
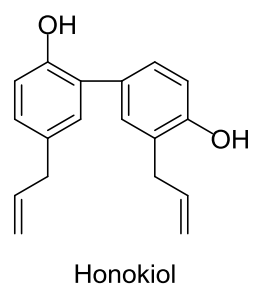
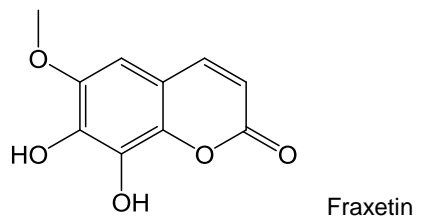
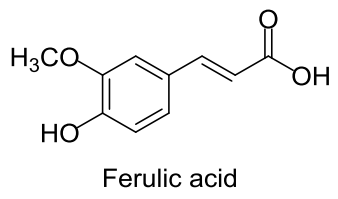
Dihydrohonokiol

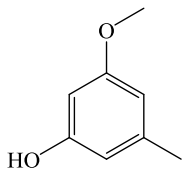


Dihydromagnolol

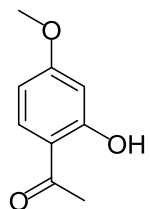


Eugenol

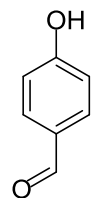




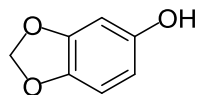
3-Methoxy-5-methylphenol



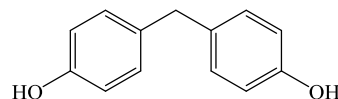
Paeonol



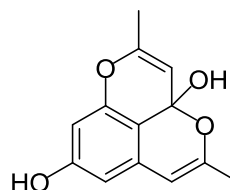
4-Hydroxybenzaldehyde



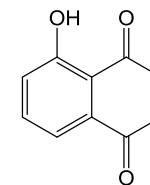
Sesamol



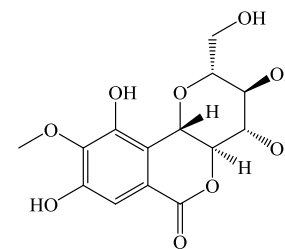
4,4'-Methylenebis (Phenol)



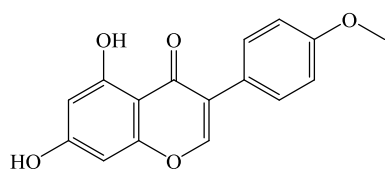
Barakol



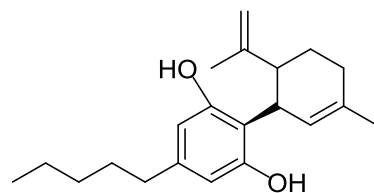
5-Hydroxy-1,4-naphthalenedione



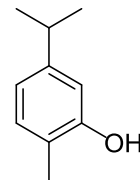
Bergenin



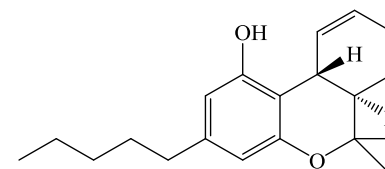
Biochanin A



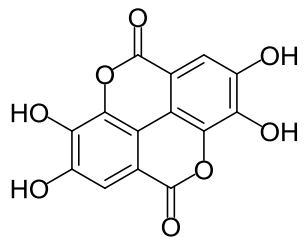
Cannabidiol



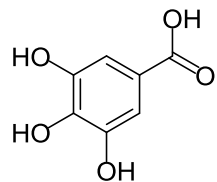
Carvacrol



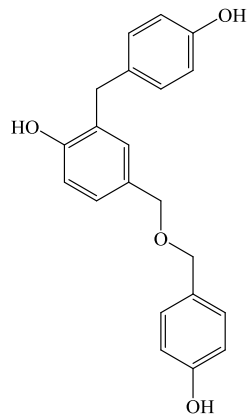
Dronabinol



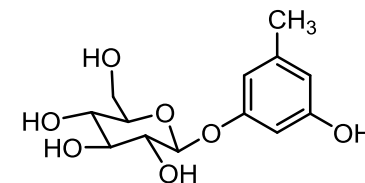
Ellagic acid



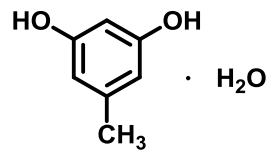
Gallic acid



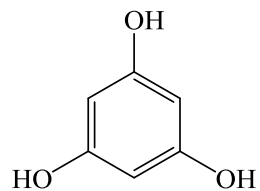
Gestrol



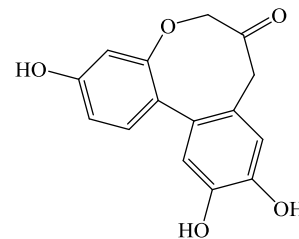
Orcinol glucoside



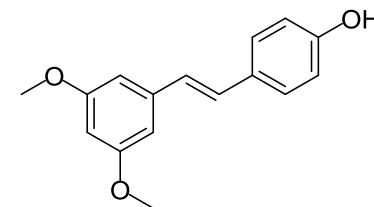
Orcinol monohydrate



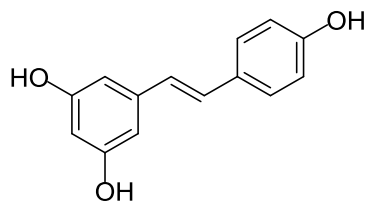
Phloroglucinol



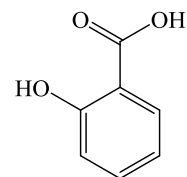
Protosappanin A



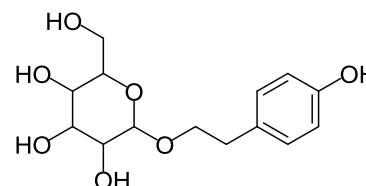
Pterostilbene



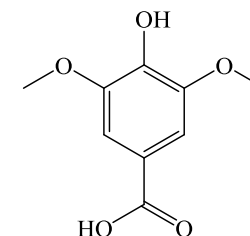
Resveratrol



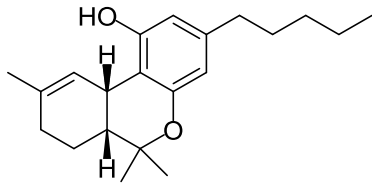
Salicylic acid



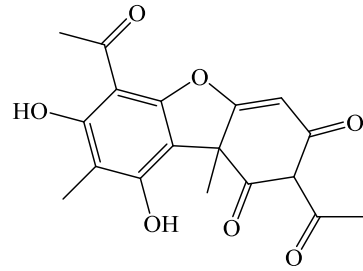
Salidroside



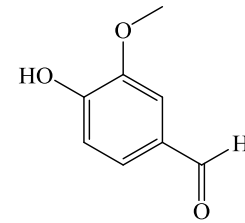
Syringic acid



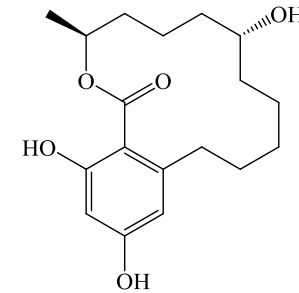
9-Tetrahydrocannabinol



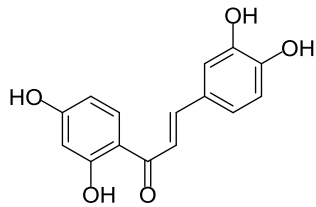
Usnic acid



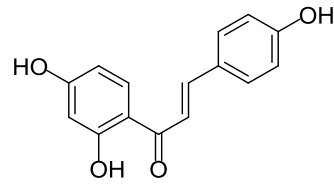
Vanillin



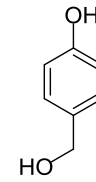
Zeranol



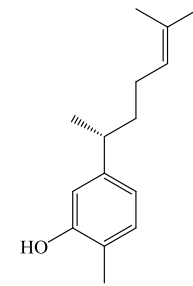
2,4,3,4-Trihydroxychalcone Isoliquiritigenin.BUT



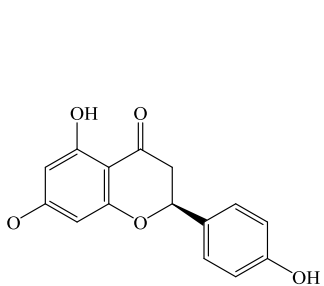
2,4,4-Trihydroxychalcone Isoliquiritigenin.ISL



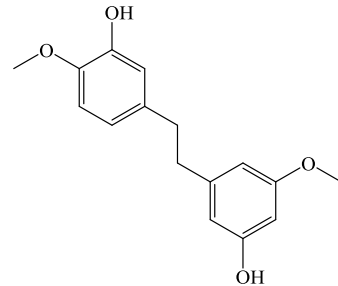
4-Hydroxybenzyl alcohol



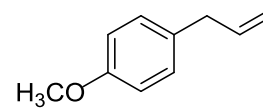
Xanthorrhizol



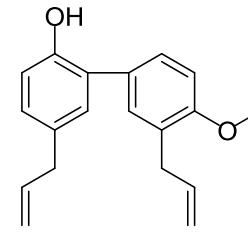
Sakuranetin



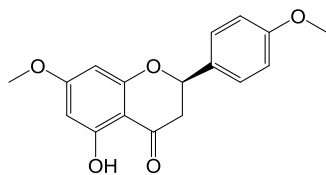
Gigantol



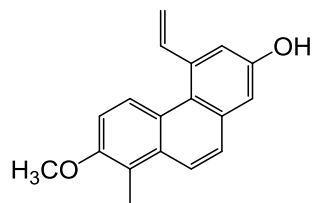
Estragole



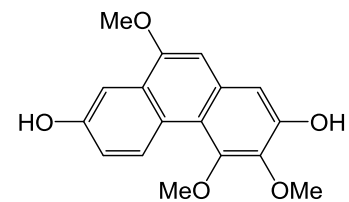
4-O-methylhonokiol



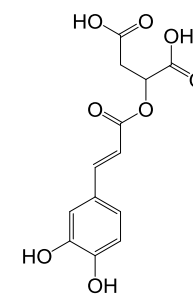
naringenin-4',7-dimethyl



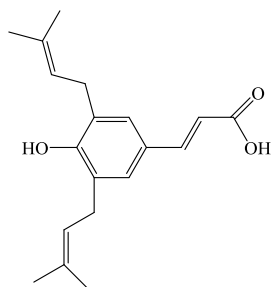
2-Methoxy-7-hydroxy-1-methyl-5-vinyl Phenanthrene



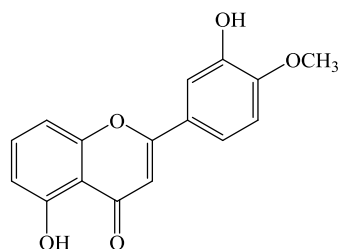
2,3,4-Trimethoxy-7,9-phenanthrenediol



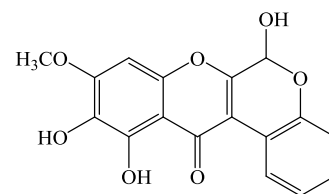
(+)-Caffeoylmalic acid



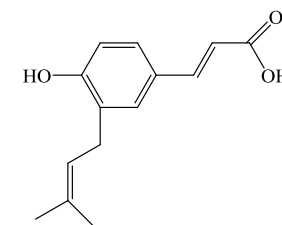
3,5-Diprenyl-4-hydroxycinnamic acid



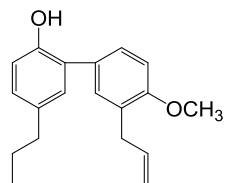
5,3'-Dihydroxyl-4'-methoxyl-flavone



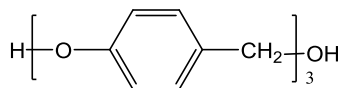
9-O-methyl-10-hydroxycoccineone B



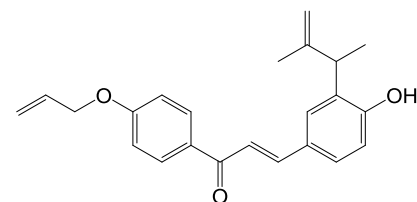
3-Prenyl-4-hydroxycinnamic acid



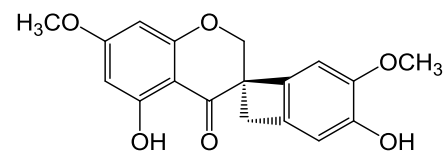
4-O-methoxydihydroonokiol



4-[[4-[(4-Hydroxyphenyl)methoxy]phenyl]methoxy]-benzenemethanol



3-[3-(1,2-dimethyl-2-propen-1-yl)-4-hydroxyphenyl]-1-[4-(2-propen-1-yloxy)phenyl]- (2E)-2-Propen-1-one



(R)-3,5-dihydroxy-4,7-dimethoxyspiro [2H-1-benzopyran-3(4H),7-bicyclo[4.2.0]-octa[1,3,5]-trien]-4-one

Figure S2. Docking modes between potential agonists of hRyR1 and hRyR1.

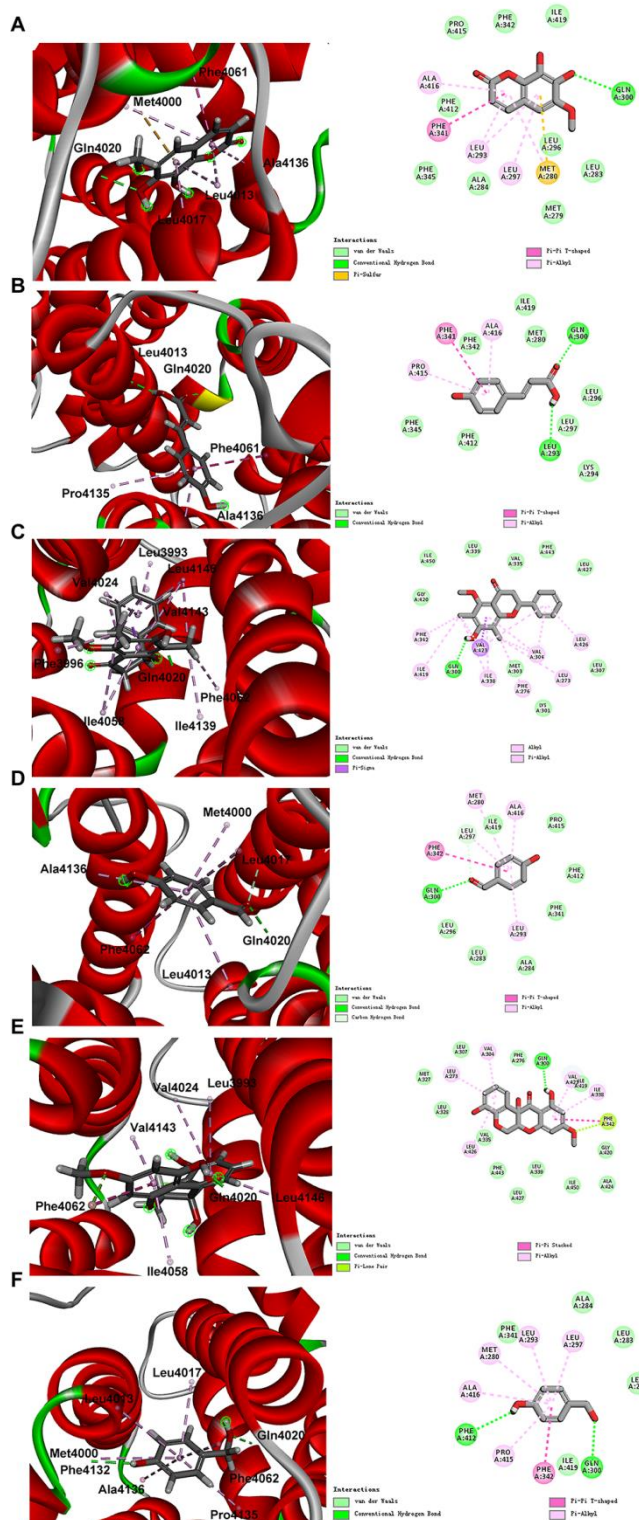


Figure S2. Docking modes between potential agonists of hRyR1 and hRyR1. Note: (A) Fraxetin penetrated into the active pocket, and mainly formed one hydrogen bond with the Gln4020 residue on the protein, a total of 5 residues were bound by hydrophobic interaction on the hRyR1. (B)The *p*-coumaric acid small molecule

completely penetrated into the active pocket, forming 2 hydrogen bonds with the Gln4020, Leu4013 residues on the protein. The hydrophobic interaction residues of the *p*-coumaric acid molecule were 3. (C) The 7-hydroxy-5-methoxy-6,8-dimethyl-flavanone molecule completely entered the active pocket, Gln4020 residues formed one hydrogen bond, a total of 8 hydrophobic residues. (D) 4-hydroxybenzaldehyde small molecule deeped into the active pocket, forming 2 hydrogen bonds with Gln4020, Leu4017 and 5 residues formed a hydrophobic effect. (E)10-Demethylboeravinone C completely entered the active pocket and formed a hydrophobic interaction with 6 residues. (F) The 4-hydroxybenzylalcohol molecule completely penetrated into the active pocket, forming 4 hydrogen bonds with Gln4020 and a total of 6 hydrophobic residues.

Figure S3. Effects of the positive small-molecule phenol 4-CmC on the viability of RTSMCs.

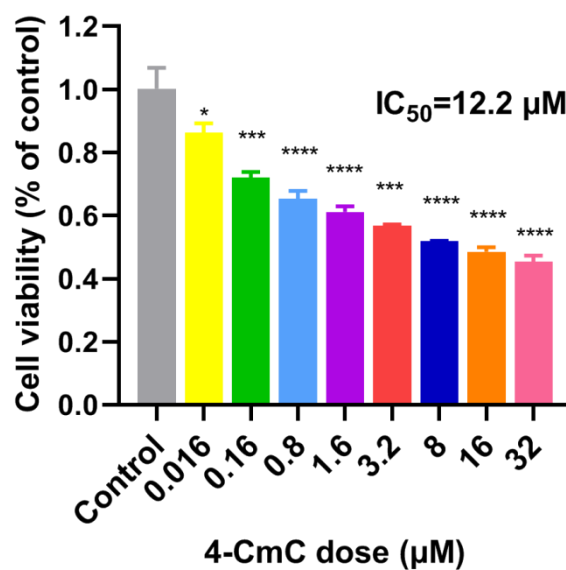


Figure S3. Effects of the positive small-molecule phenol 4-CmC on the viability of RTSMCs. Note:

Significant differences from control group are denoted by * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. Differences were assessed for significance by Dunnett's T3 multiple comparisons test subsequent to significant one-way ANOVA.

Table S1. Effects of potential NSMPs on RTSMCs viability

Group	Concentration (μM)	Absorbance (A)	Cell viability rate (%)	IC50 (μM)
Xanthotoxol	0	1.02 \pm 0.06	100.00 \pm 0.00	28.29
	0.016	0.95 \pm 0.01	92.94 \pm 0.01	
	0.16	0.80 \pm 0.02	78.72 \pm 0.02 ^{***}	
	0.80	0.71 \pm 0.02	69.89 \pm 0.02 ^{****}	
	1.6	0.67 \pm 0.01 [*]	65.95 \pm 0.01 ^{****}	
	3.2	0.64 \pm 0.02 ^{**}	62.64 \pm 0.02 ^{****}	
	8.0	0.58 \pm 0.01 ^{**}	56.87 \pm 0.01 ^{****}	
	16	0.55 \pm 0.02 ^{**}	53.55 \pm 0.02 ^{****}	
	32	0.50 \pm 0.01 ^{**}	49.55 \pm 0.01 ^{****}	
Danshensu	0	1.02 \pm 0.06	100.00 \pm 0.00	2.57
	0.016	0.86 \pm 0.03 [*]	84.70 \pm 0.02 ^{**}	
	0.16	0.71 \pm 0.02 ^{**}	69.55 \pm 0.02 ^{****}	
	0.80	0.60 \pm 0.02 ^{**}	58.55 \pm 0.02 ^{****}	
	1.6	0.54 \pm 0.03 ^{**}	53.53 \pm 0.03 ^{****}	
	3.2	0.50 \pm 0.02 ^{**}	48.95 \pm 0.02 ^{****}	
	8.0	0.42 \pm 0.01 ^{**}	41.08 \pm 0.01 ^{****}	
	16	0.37 \pm 0.01 ^{**}	36.21 \pm 0.01 ^{****}	
	32	0.34 \pm 0.01 ^{**}	33.57 \pm 0.01 ^{****}	
Juglone	0	1.02 \pm 0.06	100.00 \pm 0.00	0.38
	0.016	0.66 \pm 0.01	64.70 \pm 0.01	
	0.16	0.54 \pm 0.02	52.96 \pm 0.02	
	0.80	0.47 \pm 0.02	46.23 \pm 0.02	
	1.6	0.44 \pm 0.02 [*]	43.22 \pm 0.02	
	3.2	0.40 \pm 0.02 ^{**}	39.76 \pm 0.02 [*]	
	8.0	0.39 \pm 0.01 ^{**}	38.20 \pm 0.01 [*]	
	16	0.34 \pm 0.01 ^{**}	33.81 \pm 0.01 ^{**}	
	32	0.31 \pm 0.02 ^{**}	30.73 \pm 0.02 ^{***}	
Vanillin	0	1.02 \pm 0.06	100.00 \pm 0.00	0.98
	0.016	0.75 \pm 0.01 ^{****}	73.51 \pm 0.01 ^{****}	
	0.16	0.61 \pm 0.02 ^{****}	59.81 \pm 0.02 ^{****}	
	0.80	0.53 \pm 0.03 ^{****}	51.97 \pm 0.03 ^{****}	
	1.6	0.50 \pm 0.01 ^{****}	48.67 \pm 0.01 ^{****}	
	3.2	0.43 \pm 0.02 ^{****}	42.53 \pm 0.02 ^{****}	
	8.0	0.39 \pm 0.01 ^{****}	37.87 \pm 0.01 ^{****}	
	16	0.35 \pm 0.02 ^{****}	34.78 \pm 0.02 ^{****}	
	32	0.29 \pm 0.01 ^{****}	28.36 \pm 0.01 ^{****}	

Table S1 continued

Group	Concentration (μM)	Absorbance (A)	Cell viability rate (%)	IC50 (μM)
Paeonol	0	1.02±0.06	100.00±0.00	29.46
	0.016	0.75±0.02	73.90±0.02 ^{****}	
	0.16	0.69±0.02	67.64±0.02 ^{****}	
	0.80	0.63±0.03	61.56±0.03 ^{****}	
	1.6	0.59±0.01	58.45±0.01 ^{****}	
	3.2	0.59±0.03	57.66±0.03 ^{****}	
	8.0	0.56±0.01 [*]	55.50±0.01 ^{****}	
	16	0.53±0.01 ^{**}	51.97±0.01 ^{****}	
	32	0.50±0.01 ^{***}	48.80±0.01 ^{****}	
Caffeic acid	0	1.02±0.06	100.00±0.00	6.15
	0.016	0.84±0.01 ^{**}	82.76±0.01 ^{**}	
	0.16	0.71±0.01 ^{****}	69.52±0.01 ^{****}	
	0.80	0.61±0.01 ^{****}	59.54±0.01 ^{****}	
	1.6	0.58±0.01 ^{****}	56.79±0.01 ^{****}	
	3.2	0.54±0.01 ^{****}	52.73±0.01 ^{****}	
	8.0	0.52±0.003 ^{****}	51.00±0.003 ^{****}	
	16	0.45±0.03 ^{****}	44.61±0.03 ^{****}	
	32	0.42±0.02 ^{****}	40.99±0.02 ^{****}	
Fraxetin	0	1.02±0.06	100.00±0.00	1.78
	0.016	0.87±0.01 ^{**}	85.81±0.01 ^{**}	
	0.16	0.71±0.02 ^{****}	69.46±0.02 ^{****}	
	0.80	0.56±0.01 ^{****}	55.38±0.01 ^{****}	
	1.6	0.53±0.02 ^{****}	52.07±0.02 ^{****}	
	3.2	0.45±0.02 ^{****}	44.48±0.02 ^{****}	
	8.0	0.39±0.02 ^{****}	38.37±0.02 ^{****}	
	16	0.35±0.02 ^{****}	34.60±0.02 ^{****}	
	32	0.27±0.02 ^{****}	26.52±0.02 ^{****}	
<i>p</i> -Coumaric acid	0	1.02±0.06	100.00±0.00	3.90
	0.016	0.97±0.02	95.63±0.02	
	0.16	0.79±0.002	77.27±0.002	
	0.80	0.65±0.02	64.19±0.02	
	1.6	0.59±0.01	58.02±0.01	
	3.2	0.52±0.02	50.77±0.02	
	8.0	0.44±0.02 [*]	43.30±0.02 [*]	
	16	0.40±0.02 ^{**}	39.73±0.02 ^{**}	
	32	0.32±0.01 ^{***}	31.13±0.01 ^{***}	

Table S1 continued

Group	Concentration (μM)	Absorbance (A)	Cell viability rate (%)	IC50 (μM)
4-hydroxybenzaldehyde	0	1.02±0.06	100.00±0.00	14.48
	0.016	0.75±0.02****	73.23±0.02****	
	0.16	0.68±0.02****	67.04±0.02****	
	0.80	0.60±0.02****	58.84±0.02****	
	1.6	0.59±0.02****	58.34±0.02****	
	3.2	0.56±0.02****	54.95±0.02****	
	8.0	0.53±0.002****	52.10±0.002****	
	16	0.50±0.03****	49.62±0.03****	
4-hydroxybenzylalcohol	0	1.02±0.06	100.00±0.00	9.44
	0.016	0.78±0.005	77.04±0.01	
	0.16	0.67±0.03	66.31±0.03	
	0.80	0.61±0.01	59.86±0.01	
	1.6	0.59±0.01	58.22±0.01	
	3.2	0.54±0.001*	53.10±0.001*	
	8.0	0.53±0.02*	51.61±0.02*	
	16	0.48±0.02***	46.95±0.02***	
	32	0.47±0.02***	45.78±0.02***	

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ compared with the control group, n=3