Supplementary Information

for

Inhibition of human thrombin by the constituents of licorice: inhibition kinetics and mechanistic insights through in *vitro* and in *silico* studies

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This file contains four supplementary figures and one table.

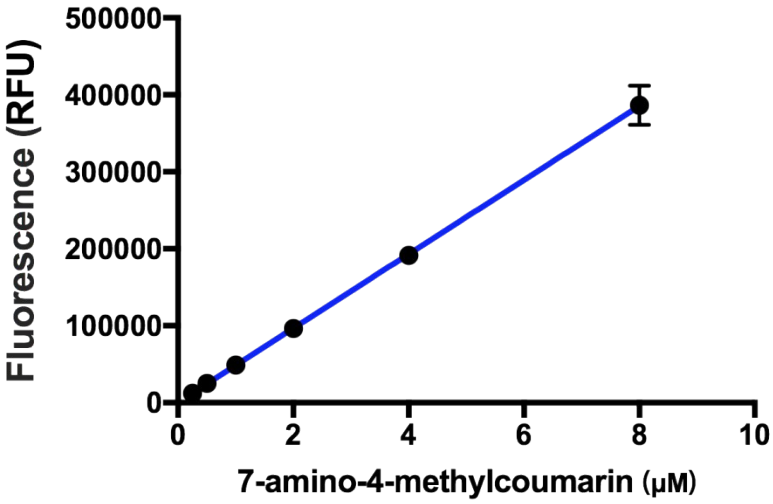

Figure S1. The standard curve of the fluorescent product (7-amino-4-methylcoumarin) after reaction of thrombin with the substrate (Z-Gly-Gly-Arg-AMC acetate), the equation was Y = 48114*X + 570.3; X means concentrations of 7-amino-4-methylcoumarin; Y means fluorescence.

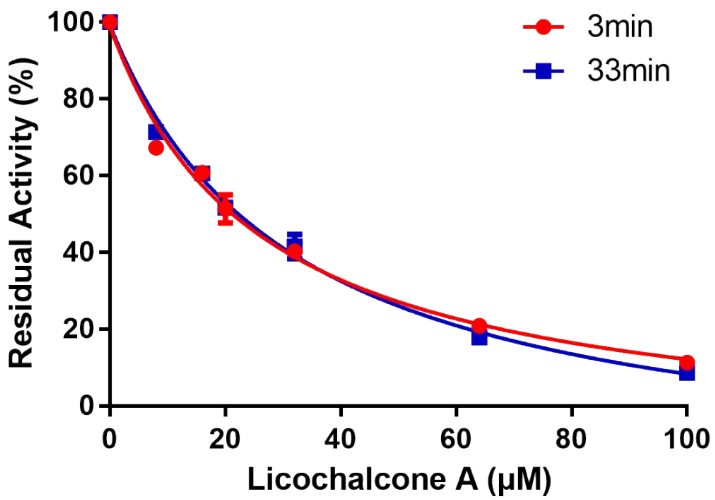

Figure S2. The time-dependent inhibition curves of licochalcone A against thrombin (3 min and 33 min). All inhibition experiments were performed in triplicate (n=3) and the values are expressed as mean ± SD.

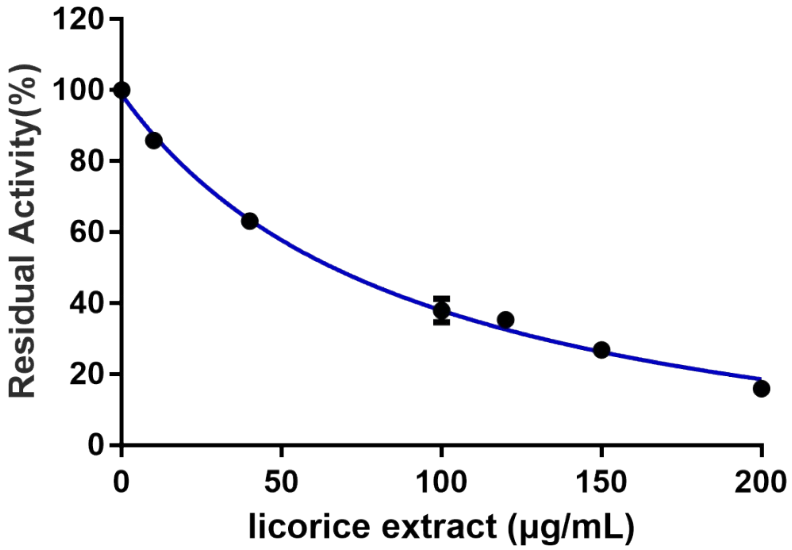

Figure S3. The dose-response curves of licorice extract on human thrombin. All inhibition experiments were performed in triplicate (n=3) and the values are expressed as mean ± SD.

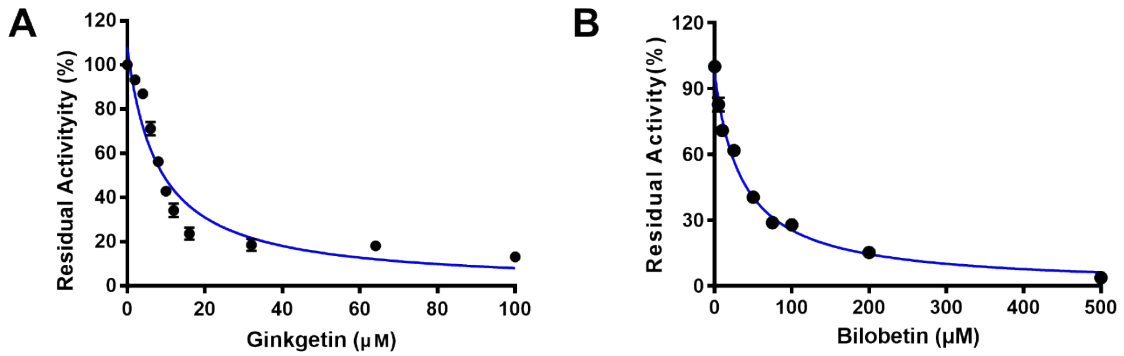

Figure S4. The dose-response curves of ginkgetin and baicalein on human thrombin. All inhibition experiments were performed in triplicate (n=3) and the values are expressed as mean ± SD.

Table S1. The labeling ratios of different lysine residues in human thrombin (The amino acid numbers indicate human thrombin numbering**).**

Sequences	**Residues**	**Labeling ratios**	
		Thrombin without licochalcone A	**Thrombin with licochalcone A**
RKSPQELL	Lys-36	93%	100%

PPWDKNFTENDLL	Lys-60F	Not labeled	Not labeled
ERNIEKISML	Lys-81	98%	100%
MKLKKPVAFSDY	Lys-107, Lys-109, Lys-110	80%	0%
KGRVTGW	Lys-135	100%	100%
GNLKETW	Lys-145	100%	33%
TANVGKGQPSVL	Lys-149E	93%	50%
QVVNLPIVERPVCKDSTRIRITDNM	Lys-169	Not labeled	Not labeled
KPDEGKRGDACEGDSGGPF	Lys-185, Lys-186D	100%	96%
GEGCDRDGKYGF	Lys-224	38%	0%
IQKVIDQFGE	Lys-240	95%	70%