

Supplementary Information

A chemical family-based strategy for uncovering hidden bioactive molecules and multicomponent interactions in herbal medicines

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| | | |
|-----|-----------------------------------------------------------------------------------------------------------------------------------------|----|
| 1 | Supplementary Figures | |
| 1.1 | Fig. S1: The chromatographic profiles of DQP performed by analytical column (a) and semi-preparative column (b) | 4 |
| 1.2 | Fig. S2: The thrombin inhibitory activity of salvianolic acid A increased with the time of dissolving in phosphate buffer solution..... | 4 |
| 1.3 | Fig. S3: The kinetics of thrombin catalyzing substrate to product influenced by the representative compounds in different families..... | 5 |
| 1.4 | Fig. S4: The docking results of dihydrotanshinone I, cryptotanshinone, tanshinone I and tanshinone IIA to thrombin..... | 6 |
| 1.5 | Fig. S5: The docking results of glucose (Glu), protopanaxadiol (PPD) and protopanaxatriol (PPT) to thrombin..... | 7 |
| 1.6 | Fig. S6 The structure-activity comparison of cryptotanshinone (a) and tanshinone IIA (b) | 8 |
| 2 | Supplementary Tables | |
| 2.1 | Table S1: Collection window, collection volume and compound identification of 18 peak fractions..... | 9 |
| 2.2 | Table S2: Comparison of the peak areas in DQP extract and the reconstructed library..... | 10 |
| 2.3 | Table S3: Additive thrombin-inhibitory effects (IC_{50} , μM) of tanshinone IIA and dihydrotanshinone I..... | 11 |
| 2.4 | Table S4: Additive thrombin-inhibitory effects (IC_{50} , μM) of dihydrotanshinone I and cryptotanshinone..... | 11 |

| | |
|------------------------------------------------------------------------------------------------------------------------|----|
| 2.5 Table S5: Additive thrombin-inhibitory effects (IC_{50} , μM) of tanshinone IIA and cryptotanshinone..... | 11 |
| 2.6 Table S6: The interactions between TN family and SA family for thrombin inhibitory activity..... | 12 |

2. Supplementary Figures

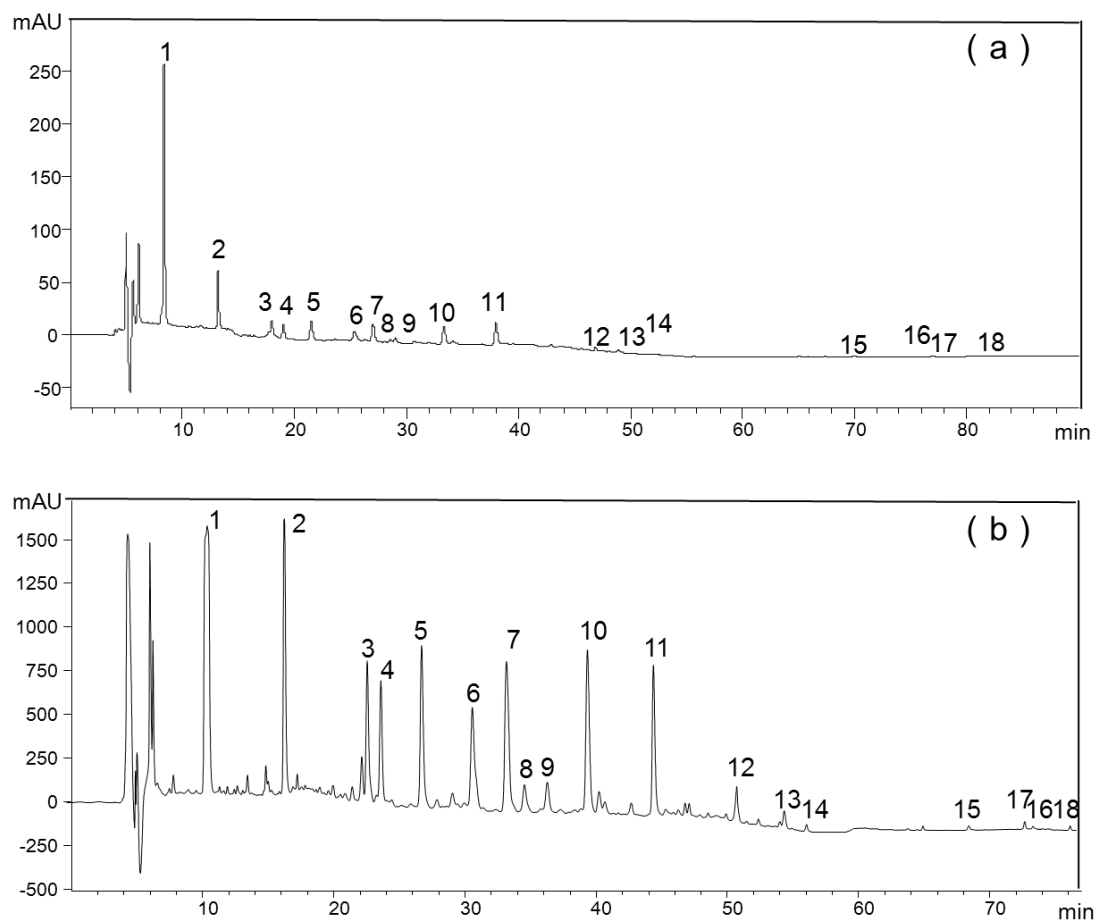


Fig. S1 The chromatographic profiles of DQP performed by analytical column (a) and semi-preparative column (b).

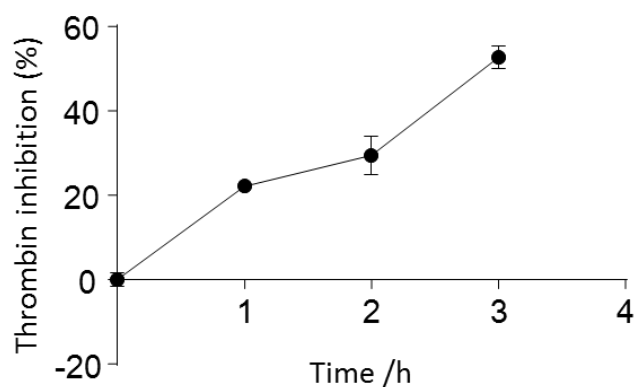


Fig. S2 The thrombin inhibitory activity of salvianolic acid A increased with the time of dissolving in phosphate buffer solution.

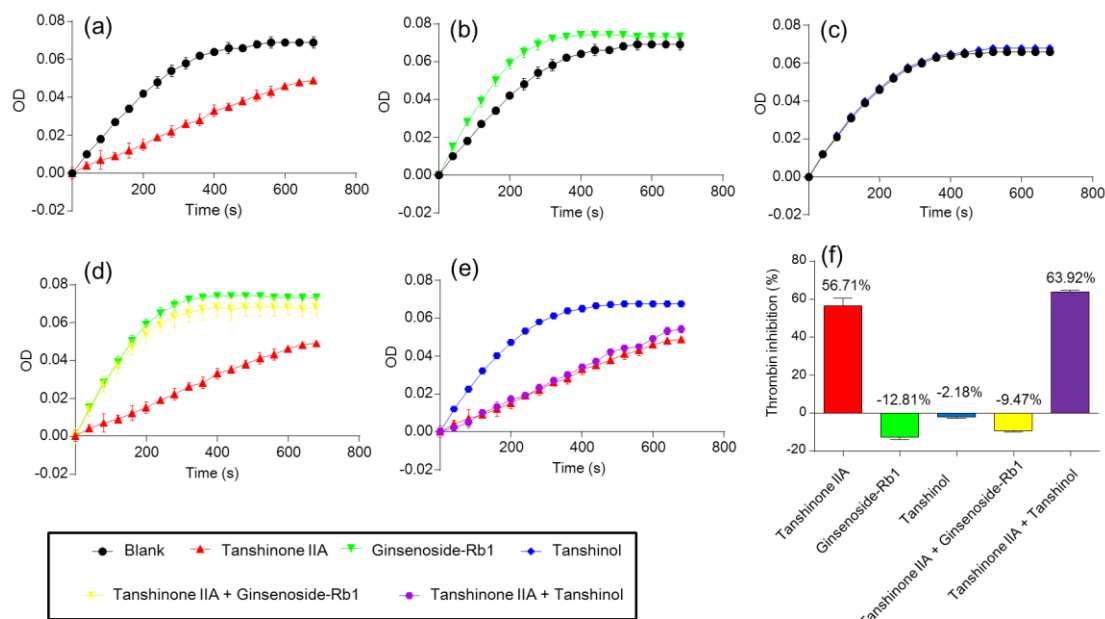


Fig. S3 The kinetics of thrombin catalyzing substrate to product influenced by the representative compounds in different families. (a) The kinetics influenced by tanshinone IIA (TN family, red) and the normal control (black). Tanshinone IIA obviously decreased the reaction rate and delayed the time to reach the plateau. (b) The kinetics influenced by ginsenoside-Rb1 (GS family, green) and the normal control (black). Ginsenoside-Rb1 increased the reaction rate and shortened the time to reach the plateau. (c) The kinetics influenced by tanshinol (SA family, blue) and the normal control (black). Tanshinol had no influence on the kinetics. (d) The kinetics influenced by ginsenoside-Rb1 (green), tanshinone IIA (red), and ginsenoside-Rb1 + tanshinone IIA (yellow). The yellow curve was more similar to the green curve, showing that ginsenoside-Rb1 completely reversed the thrombin inhibition caused by tanshinone IIA. (e) The kinetics influenced by tanshinol (blue), tanshinone IIA (red) and tanshinol + tanshinone IIA (purple). The purple curve was more similar to the red curve, suggesting that tanshinol had no influence on the thrombin inhibition caused by

tanshinone IIA. (f) The quantitative thrombin inhibitory ratios of tanshinone IIA, ginsenoside-Rb1, tanshinol, ginsenoside-Rb1 + tanshinone IIA, and tanshinol + tanshinone IIA.

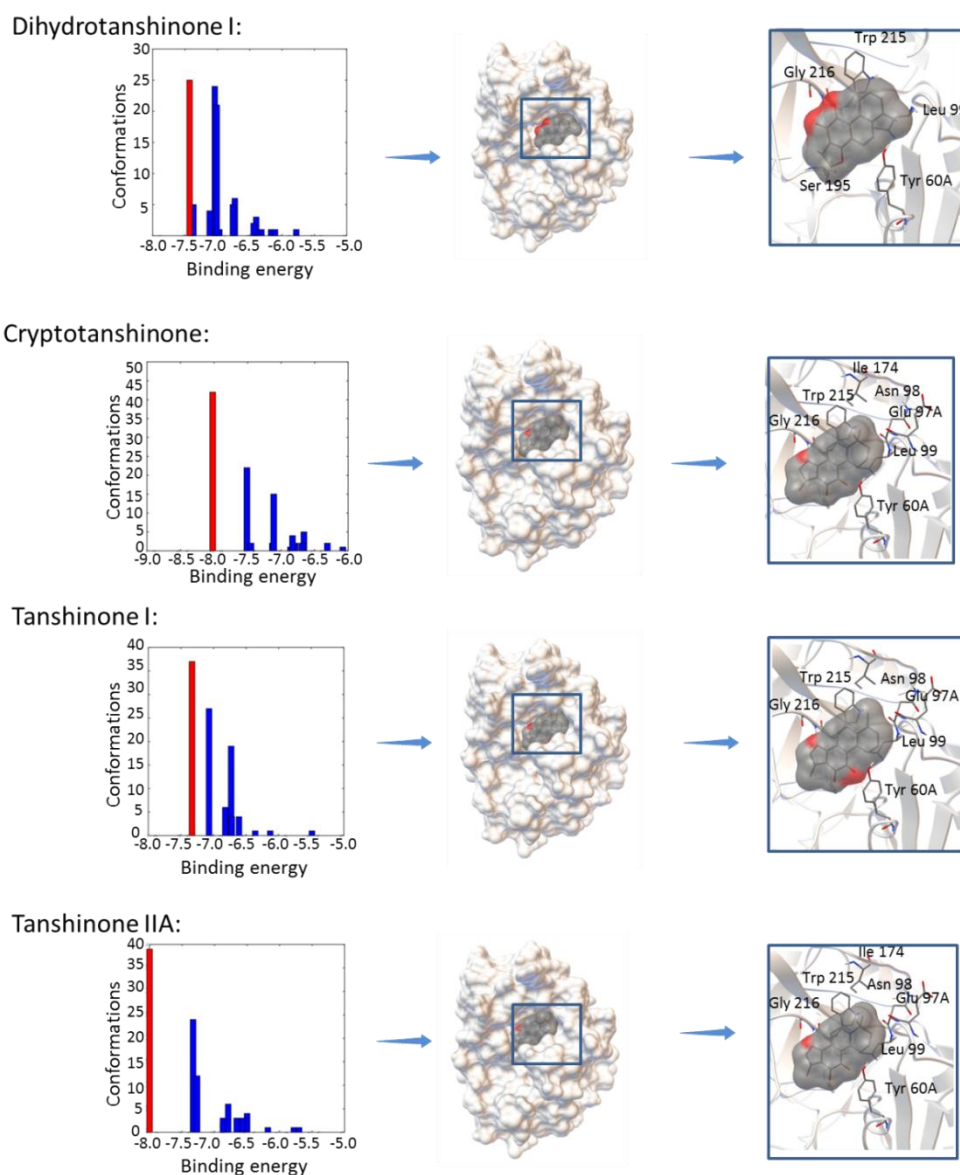
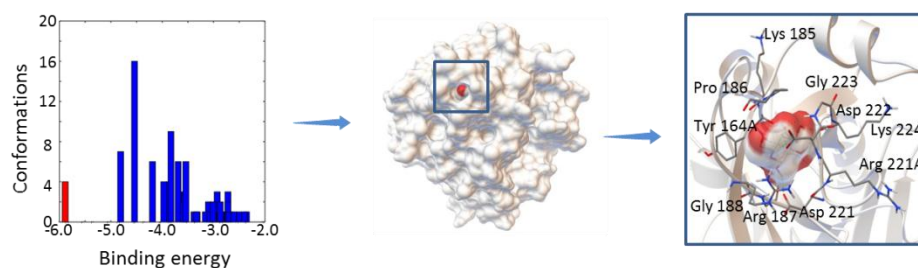
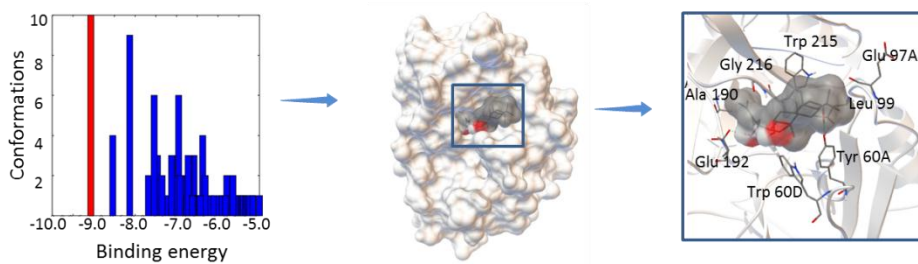


Fig. S4 The docking results of dihydrotanshinone I, cryptotanshinone, tanshinone I and tanshinone IIA to thrombin.

Glucose (Glu):



Protopanaxadiol (PPD):



Protopanaxatriol (PPT):

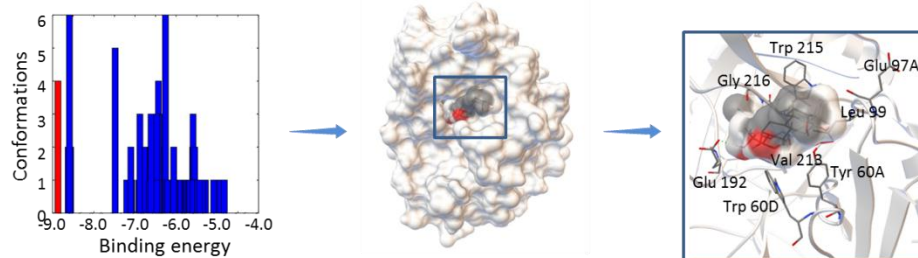


Fig. S5 The docking results of glucose (Glu), protopanaxadiol (PPD) and protopanaxatriol (PPT) to thrombin.

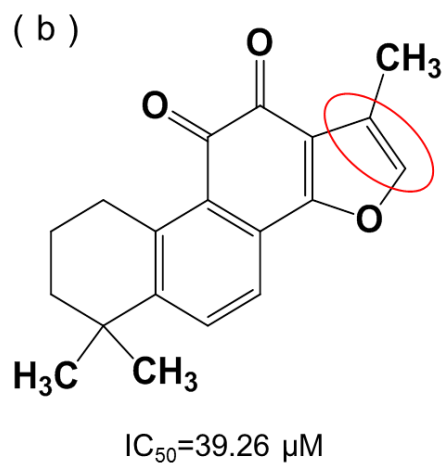
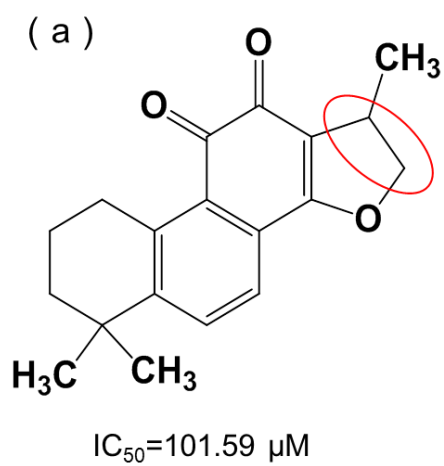


Fig. S6 The structure-activity comparison of cryptotanshinone (a) and tanshinone IIA

(b).

3. Supplementary Tables

Table S1 Collection window, collection volume and compound identification of 18 peak fractions.

| Fraction No. | Starting Time (min) | Ending Time (min) | Time Window (min) | Collection Volume (mL) | Identification |
|---------------------|----------------------------|--------------------------|--------------------------|--------------------------------|-------------------------|
| 1 | 10.21 | 11.30 | 1.09 | 2.18 | Tanshinol |
| 2 | 16.36 | 17.08 | 0.72 | 1.44 | Protocatechuic aldehyde |
| 3 | 22.71 | 23.43 | 0.72 | 1.44 | Isolithospermic acid A |
| 4 | 23.72 | 24.36 | 0.64 | 1.28 | Isolithospermic acid B |
| 5 | 26.73 | 27.87 | 1.14 | 2.28 | Salvianolic acid D |
| 6 | 30.57 | 31.62 | 1.05 | 2.10 | Salvianolic acid G |
| 7 | 33.08 | 34.43 | 1.35 | 2.70 | Rosmarinic acid |
| 8 | 34.43 | 35.61 | 1.18 | 2.36 | Lithospermic acid |
| 9 | 36.31 | 37.31 | 1.00 | 2.00 | Ginsenoside-Rg1 |
| 10 | 39.35 | 40.32 | 0.97 | 1.94 | Salvianolic acid B |
| 11 | 44.07 | 45.43 | 1.36 | 2.72 | Salvianolic acid A |
| 12 | 50.63 | 51.53 | 0.90 | 1.80 | Ginsenoside-Rb1 |
| 13 | 54.53 | 55.13 | 0.60 | 1.20 | Ginsenoside-Rh1 |
| 14 | 56.18 | 56.94 | 0.76 | 1.52 | Ginsenoside-Rd |
| 15 | 68.49 | 69.01 | 0.52 | 1.04 | Dihydrotanshinone I |
| 17 | 72.60 | 73.38 | 0.78 | 1.56 | Tanshinone I |
| 16 | 73.38 | 74.16 | 0.78 | 1.56 | Cryptotanshinone |
| 18 | 76.19 | 76.79 | 0.60 | 1.20 | Tanshinone IIA |

Table S2 Comparison of the peak areas in DQP extract and the reconstructed library.

| Peak No. | t _R | Compound | Chemical family | Peak area | |
|----------|----------------|-------------------------|-----------------|----------------|-----------------------|
| | | | | Herbal extract | Reconstructed library |
| 1 | 8.32 | Tanshinol | SA | 2531 | 2184 |
| 2 | 13.16 | Protocatechuic aldehyde | SA | 605 | 2044 |
| 3 | 18.01 | Isolithospermic acid A | SA | 281 | 2593 |
| 4 | 19.00 | Isolithospermic acid B | SA | 199 | 2301 |
| 5 | 21.48 | Salvianolic acid D | SA | 310 | 2282 |
| 6 | 25.37 | Salvianolic acid G | SA | 197 | 2601 |
| 7 | 27.00 | Rosmarinic acid | SA | 313 | 2377 |
| 8 | 28.60 | Lithospermic acid | SA | 48 | 2408 |
| 9 | 29.06 | Ginsenoside-Rg1 | GS | 80 | 218 |
| 10 | 33.40 | Salvianolic acid B | SA | 308 | 2284 |
| 11 | 37.98 | Salvianolic acid A | SA | 371 | 2534 |
| 12 | 46.89 | Ginsenoside-Rb1 | GS | 44 | 224 |
| 13 | 50.31 | Ginsenoside-Rh1 | GS | 25 | 284 |
| 14 | 52.36 | Ginsenoside-Rd | GS | 6 | 247 |
| 15 | 69.95 | Dihydrotanshinone I | TN | 8 | 746 |
| 16 | 76.25 | Cryptotanshinone | TN | <5 | 721 |
| 17 | 77.03 | Tanshinone I | TN | 9 | 783 |
| 18 | 82.12 | Tanshinone IIA | TN | 5 | 754 |

Table S3 Additive thrombin-inhibitory effects (IC₅₀, μM) of tanshinone IIA and dihydrotanshinone I.

| Tanshinone IIA | Dihydrotanshinone I | C _{IIA} :C _{Dih} | Mixture | CI | Interaction |
|----------------|---------------------|------------------------------------|--------------|------|-------------|
| 39.26 ± 0.96 | 92.46 ± 4.89 | 1:10 | 94.77 ± 4.19 | 1.09 | Additive |
| | | 1:5 | 75.68 ± 0.79 | 1.07 | Additive |
| | | 1:2 | 67.70 ± 2.21 | 1.01 | Additive |
| | | 1:1 | 56.82 ± 2.56 | 1.08 | Additive |
| | | 2:1 | 50.07 ± 1.86 | 1.02 | Additive |
| | | 5:1 | 41.55 ± 2.84 | 1.02 | Additive |
| | | 10:1 | 39.17 ± 4.43 | 1.05 | Additive |

Table S4 Additive thrombin-inhibitory effects (IC₅₀, μM) of dihydrotanshinone I and cryptotanshinone.

| Dihydrotanshinone I | Cryptotanshinone | C _{Dih} :C _{Cry} | Mixture | CI | Interaction |
|---------------------|------------------|------------------------------------|---------------|------|-------------|
| 92.46 ± 4.89 | 101.59 ± 3.84 | 1:10 | 100.08 ± 4.35 | 1.01 | Additive |
| | | 1:5 | 99.72 ± 2.65 | 1.01 | Additive |
| | | 1:2 | 93.67 ± 3.41 | 0.97 | Additive |
| | | 1:1 | 88.59 ± 4.72 | 0.93 | Additive |
| | | 2:1 | 92.24 ± 1.55 | 0.99 | Additive |
| | | 5:1 | 91.57 ± 0.96 | 1.00 | Additive |
| | | 10:1 | 84.97 ± 1.16 | 0.93 | Additive |

Table S5 Additive thrombin-inhibitory effects (IC₅₀, μM) of tanshinone IIA and cryptotanshinone.

| Tanshinone IIA | Cryptotanshinone | C _{IIA} :C _{Cry} | Mixture | CI | Interaction |
|----------------|------------------|------------------------------------|--------------|------|-------------------|
| 39.26 ± 0.96 | 101.59 ± 3.84 | 1:10 | 96.04 ± 1.3 | 0.94 | Additive |
| | | 1:5 | 80.76 ± 3.29 | 0.95 | Additive |
| | | 1:2 | 66.94 ± 4.12 | 1.03 | Additive |
| | | 1:1 | 61.35 ± 4.70 | 1.03 | Additive |
| | | 2:1 | 50.47 ± 3.53 | 1.07 | Additive |
| | | 5:1 | 44.77 ± 2.15 | 0.93 | Additive |
| | | 10:1 | 44.19 ± 3.85 | 1.17 | Slight Antagonism |

Table S6a The interactions between TN family and SA family for thrombin inhibitory activity.

| SA family TN family | 1.Tanshinol | | | 2.Protocatechuic aldehyde | | | 3.Rosmarinic acid | | |
|----------------------------|---------------------------------------------------------|--------------|---------------|---------------------------------------------------------|--------------|--------------|---------------------------------------------------------|---------------|--------------|
| | Thrombin inhibition (%) at different combination ratios | | | Thrombin inhibition (%) at different combination ratios | | | Thrombin inhibition (%) at different combination ratios | | |
| | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN |
| Dihydrotanshinone I | 45.20 ± 1.78 | 46.97 ± 3.71 | 45.05 ± 1.81 | 40.93 ± 4.63 | 46.83 ± 2.78 | 42.23 ± 1.85 | 56.57 ± 4.15 | 57.85 ± 0.77 | 47.99 ± 3.87 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Tanshinone I | 65.81 ± 2.19 | 70.16 ± 1.62 | 57.93 ± 12.54 | 70.79 ± 0.73 | 62.64 ± 8.04 | 66.35 ± 2.44 | 59.43 ± 1.77 | 68.81 ± 10.26 | 60.08 ± 3.70 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Cryptotanshinone | 42.88 ± 3.28 | 40.36 ± 1.66 | 46.06 ± 3.39 | 36.83 ± 2.15 | 47.76 ± 1.20 | 46.94 ± 6.49 | 58.23 ± 3.83 | 59.74 ± 2.61 | 49.95 ± 1.26 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Tanshinone IIA | 61.99 ± 2.77 | 56.18 ± 2.36 | 63.92 ± 1.16 | 59.17 ± 1.78 | 67.50 ± 1.18 | 65.00 ± 4.71 | 61.24 ± 2.60 | 62.45 ± 0.30 | 44.92 ± 2.96 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |

Table S6b The interactions between TN family and SA family for thrombin inhibitory activity (continued).

| SA family TN family | 4.Lithospermic acid | | | 5.Salvianolic acid B | | | 6.Salvianolic acid A | | |
|----------------------------|---------------------------------------------------------|--------------|--------------|---------------------------------------------------------|--------------|--------------|---------------------------------------------------------|--------------|--------------|
| | Thrombin inhibition (%) at different combination ratios | | | Thrombin inhibition (%) at different combination ratios | | | Thrombin inhibition (%) at different combination ratios | | |
| | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN | 1/4SA+1TN | 1/2SA+1TN | 1SA+1TN |
| Dihydrotanshinone I | 56.80 ± 0.91 | 60.57 ± 1.81 | 53.34 ± 2.01 | 43.90 ± 3.45 | 56.10 ± 3.45 | 50.61 ± 0.86 | 46.95 ± 2.62 | 47.32 ± 1.58 | 45.76 ± 1.90 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Tanshinone I | 71.42 ± 9.86 | 71.78 ± 2.93 | 55.28 ± 6.99 | 75.24 ± 1.01 | 82.93 ± 3.05 | 68.76 ± 2.72 | 70.54 ± 1.26 | 51.61 ± 1.26 | 56.78 ± 0.00 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Cryptotanshinone | 47.50 ± 2.78 | 63.28 ± 2.78 | 60.94 ± 3.91 | 36.22 ± 1.01 | 43.83 ± 3.62 | 39.58 ± 0.82 | 50.52 ± 1.40 | 47.18 ± 1.34 | 64.26 ± 0.46 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |
| Tanshinone IIA | 40.46 ± 0.89 | 48.54 ± 3.44 | 45.08 ± 2.54 | 74.99 ± 0.87 | 71.69 ± 2.86 | 76.96 ± 0.95 | 70.58 ± 2.28 | 51.71 ± 2.23 | 62.32 ± 2.23 |
| | No dose-dependent interaction | | | No dose-dependent interaction | | | No dose-dependent interaction | | |