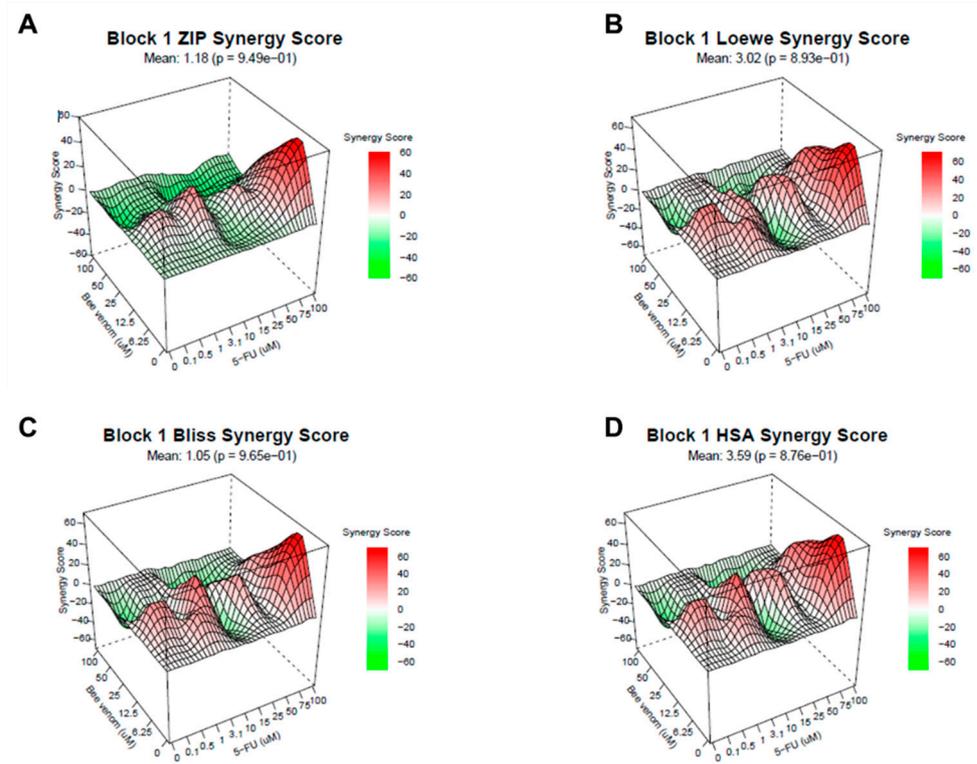
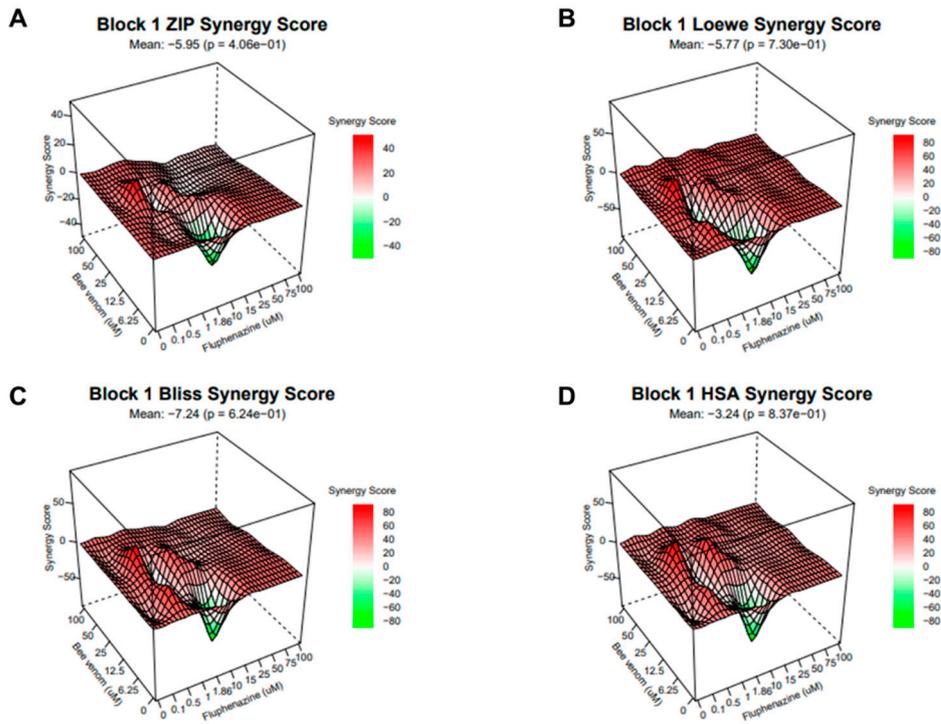


# Supplementary Materials: Honeybee Venom Synergistically Enhances the Cytotoxic Effect of CNS Drugs in HT-29 Colon and MCF-7 Breast Cancer Cell Lines

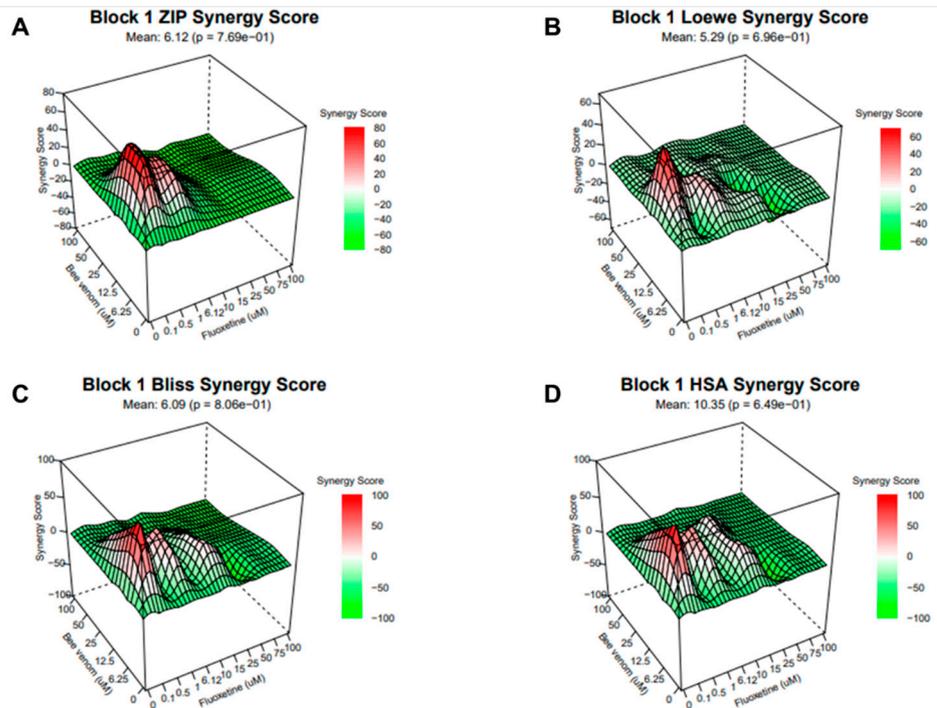
Diana Duarte, Soraia I. Falcão, Iourouine El Mehdi, Miguel Vilas-Boas and Nuno Vale



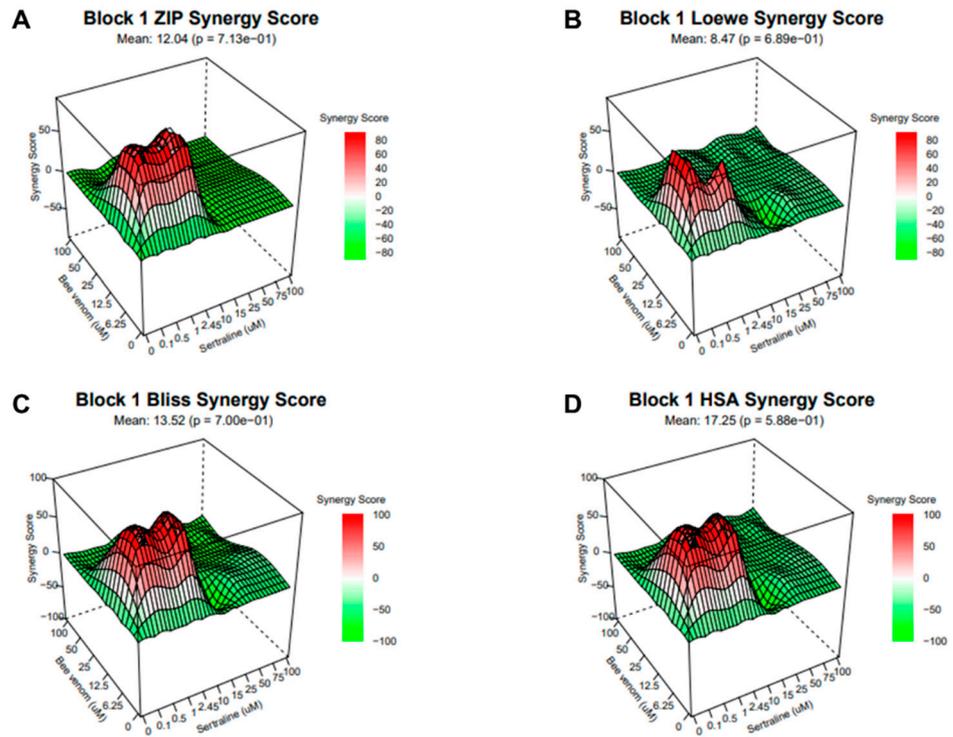
**Figure S1.** Synergy plots of combined treatments of 5-FU combined with honeybee venom on HT-29 colon cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



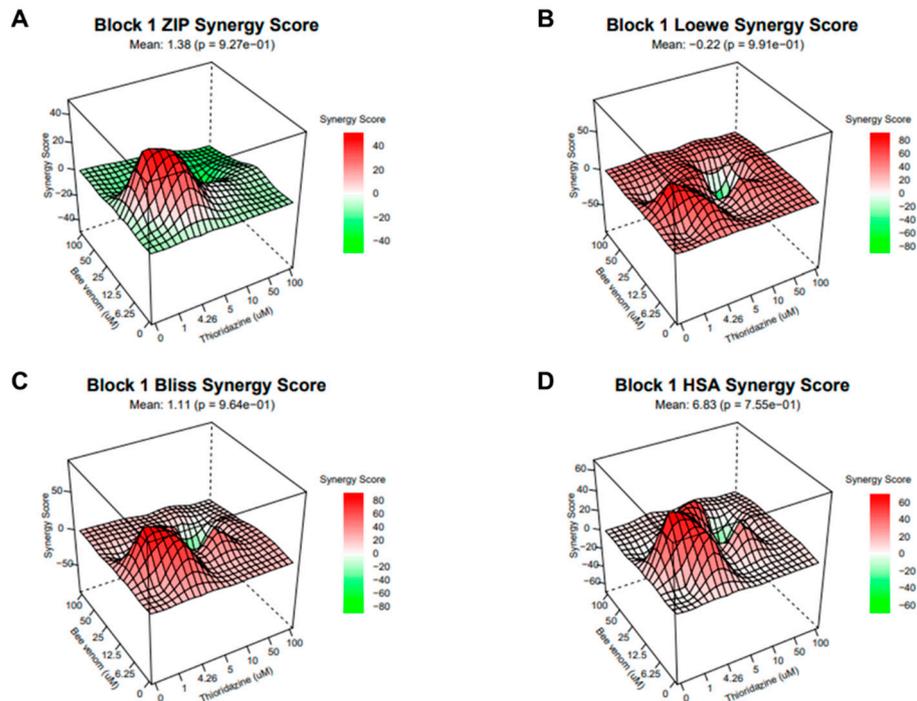
**Figure S2.** Synergy plots of combined treatments of fluphenazine combined with honeybee venom on HT-29 colon cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



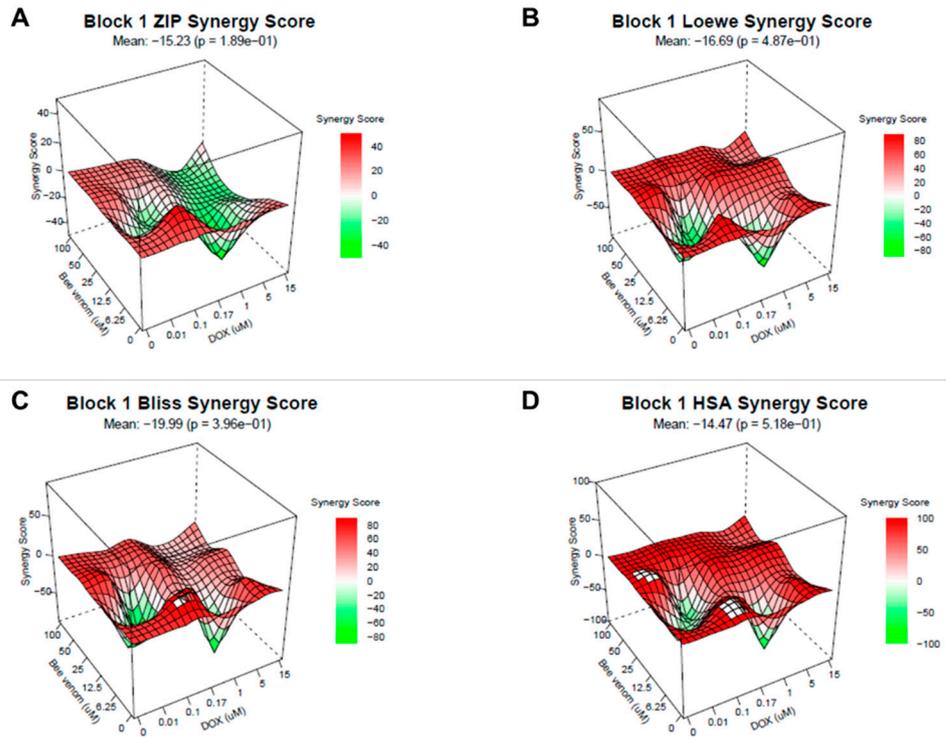
**Figure S3.** Synergy plots of combined treatments of fluoxetine combined with honeybee venom on HT-29 colon cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



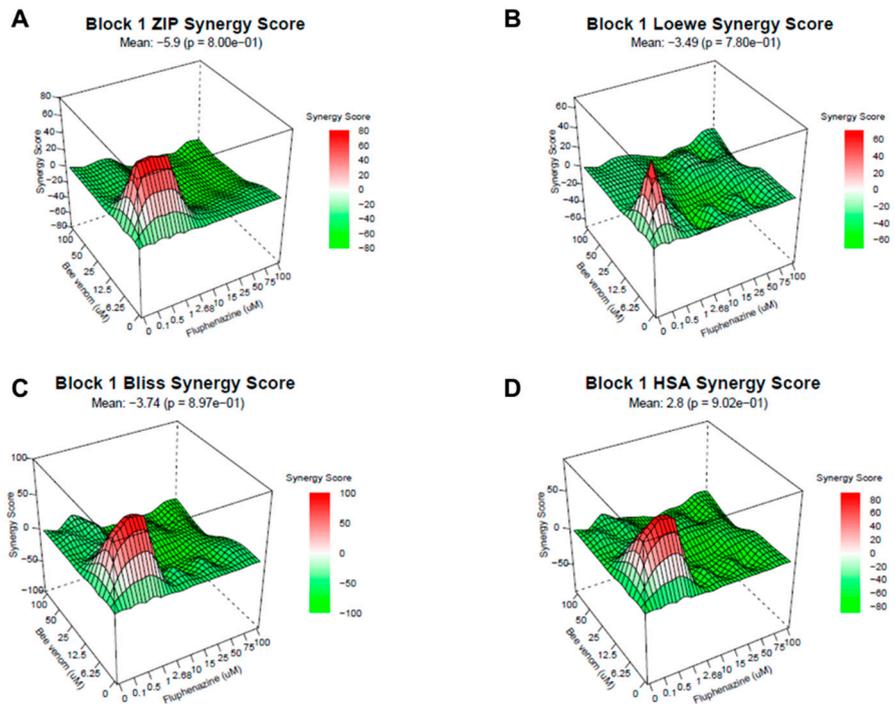
**Figure S4.** Synergy plots of combined treatments of sertraline combined with honeybee venom on HT-29 colon cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores < -10, from -10 to 10 and > 10 indicates antagonism, additivity and synergism, respectively.



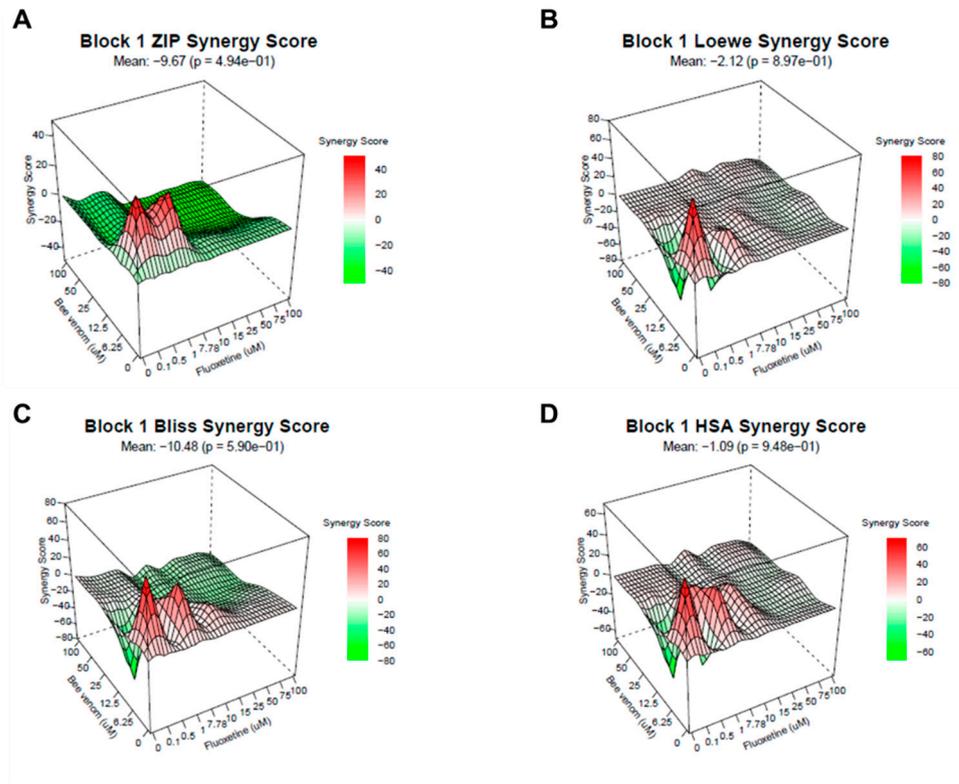
**Figure S5.** Synergy plots of combined treatments of thioridazine combined with honeybee venom on HT-29 colon cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores < -10, from -10 to 10 and > 10 indicates antagonism, additivity and synergism, respectively.



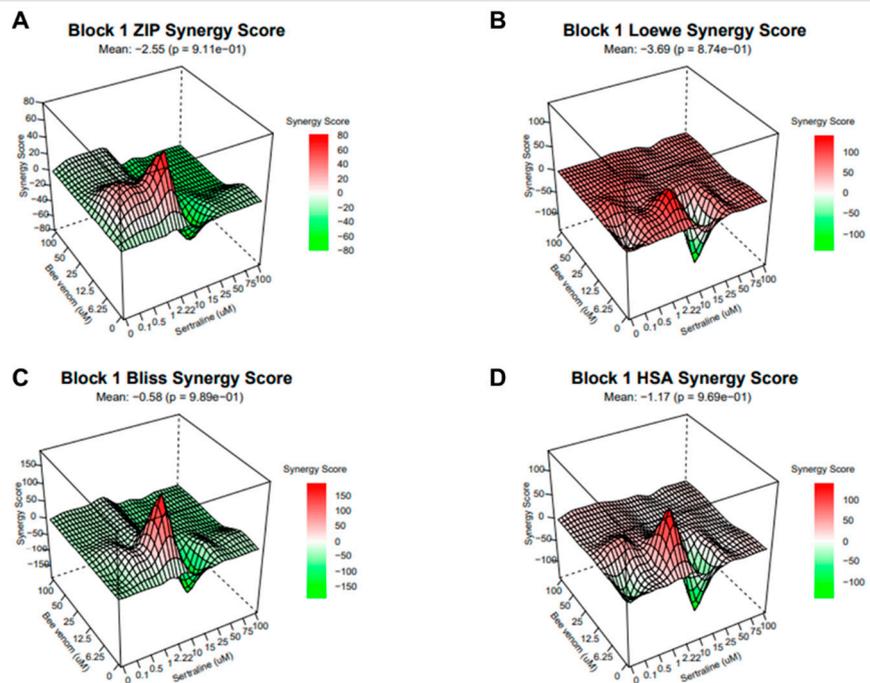
**Figure S6.** Synergy plots of combined treatments of DOX combined with honeybee venom on MCF-7 breast cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



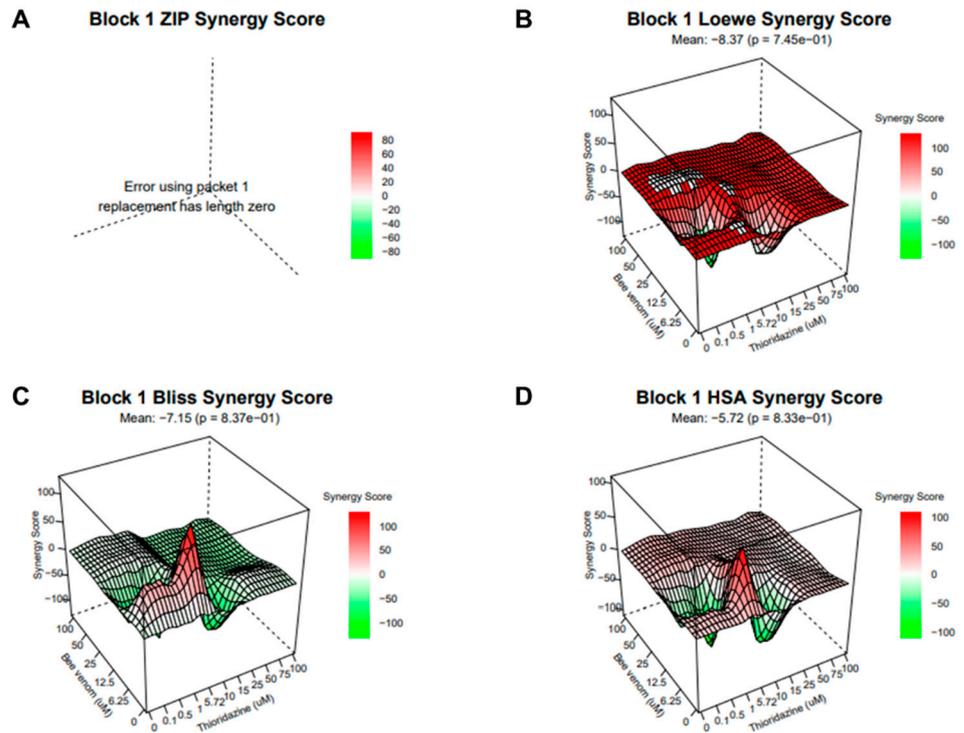
**Figure S7.** Synergy plots of combined treatments of fluphenazine combined with honeybee venom on MCF-7 breast cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



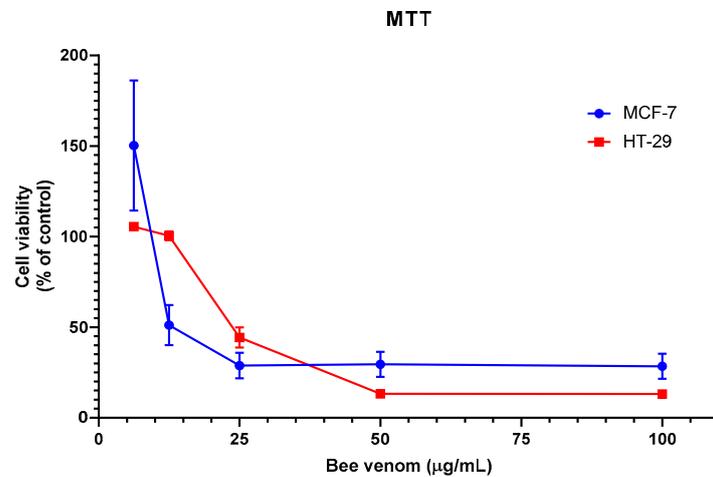
**Figure S8.** Synergy plots of combined treatments of fluoxetine combined with honeybee venom on MCF-7 breast cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



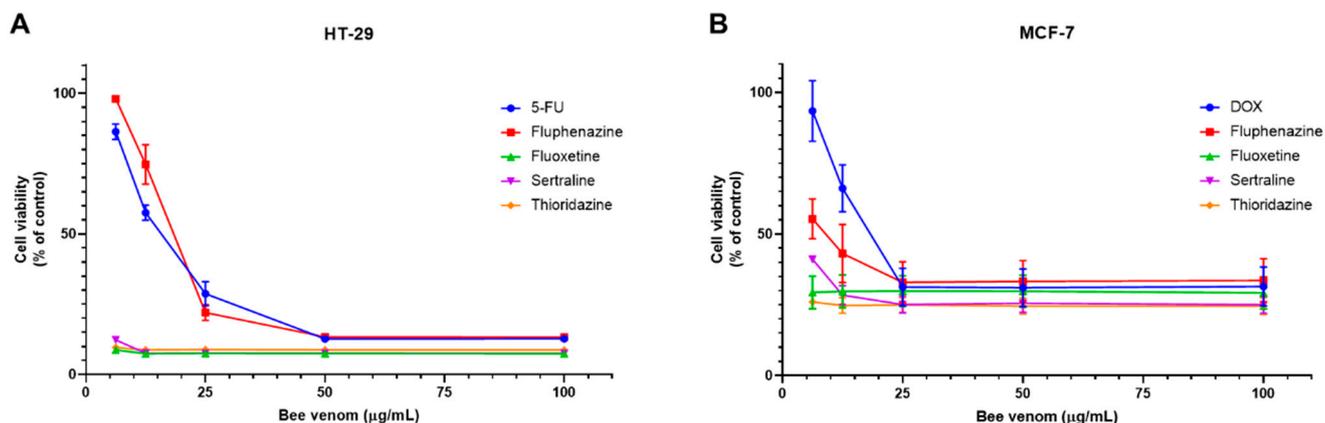
**Figure S9.** Synergy plots of combined treatments of sertraline combined with honeybee venom on MCF-7 breast cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores  $< -10$ , from  $-10$  to  $10$  and  $> 10$  indicates antagonism, additivity and synergism, respectively.



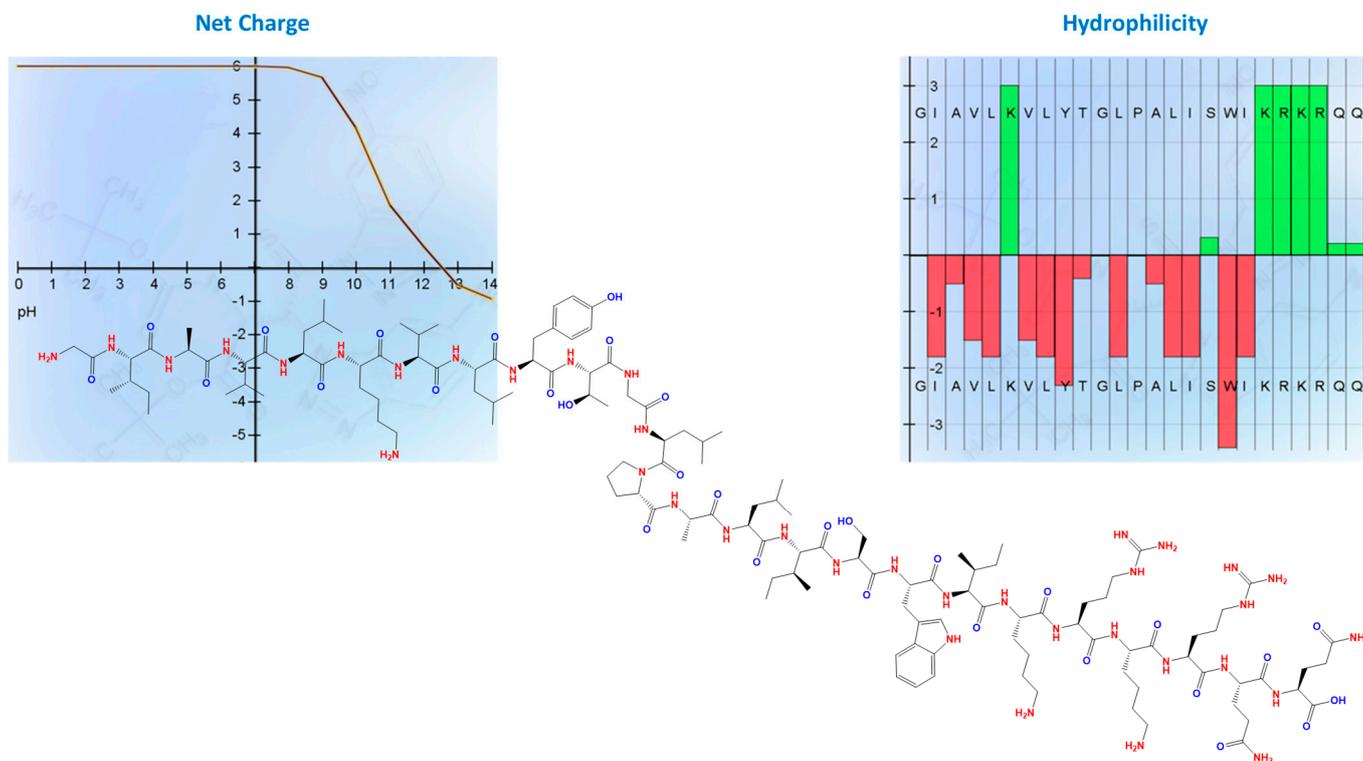
**Figure S10.** Synergy plots of combined treatments of thioridazine combined with honeybee venom on MCF-7 breast cancer cells calculated with (A) ZIP; (B) Loewe; (C) Bliss and (D) HAS reference models. These data were obtained using SynergyFinder software. Synergy scores < -10, from -10 to 10 and > 10 indicates antagonism, additivity and synergism, respectively.



**Figure S11.** Comparison of the cytotoxic effect of increasing concentrations of honeybee venom in MCF-7 and HT-29 cancer cells. Cells were cultured in 96-well plates and treated with increasing concentrations (6.25-100 µg/mL) of honeybee venom for 48 h. Each point represents the mean ± SEM relative to the control cells (0.1% DMSO) of three independent experiences.



**Figure S12.** Comparison of the cytotoxic effect of honeybee venom combined with antineoplastic drugs and CNS drugs in (A) HT-29 colon and (B) MCF-7 breast cancer cells. 5-FU and DOX were used as reference drugs for HT-29 and MCF-7 cancer cells, respectively. Cell viabilities were determined after the final treatment with MTT assay. Each point represents the mean  $\pm$  SEM relative to the control cells (0.1% DMSO) of three independent experiences.



**Figure S13.** Structure of peptide MEL, melittin, with the sequence in 3-letter code of Gly-Ile-Ala-Val-Leu-Lys-Val-Leu-Tyr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln, and N-terminus with H- and C-terminus with  $-NH_2$ . At the top of the image, the peptide properties are calculated with sequence-based functions of Peptide Calculator from BACHEM (<https://www.bachem.com/knowledge-center/peptide-calculator/>) for a net charge of pH = 7 and hydrophilicity with the Eisenberg scale. The molecular weight of MEL is 2851.52 g/mol, isoelectric point is 12.5, net charge at pH 7.0 is 6.0, average hydrophilicity is  $-0.3$  and the ratio of hydrophilicity residues/total number of residues is 32%.