Supporting Information

Ruptured organosilica nanocapsules immobilized acetylcholinesterase coupled with MnO_2 nanozyme for screening inhibitor from *Inula macrophylla*

Jia Liu [a, c], Wei Ha [a], Eshbakova Komila Alibekovna [b], Rui Ma [a], Yan-Ping Shi [a*]

[a] CAS Key Laboratory of Chemistry of Northwestern Plant Resources, Key Laboratory for Natural Medicines of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Lanzhou 730000, P. R. China

[b] S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan

[c] University of Chinese Academy of Sciences, Beijing 100049, P. R. China

[*] Corresponding author. E-mail address: shiyp@licp.cas.cn (Y.-P. Shi).

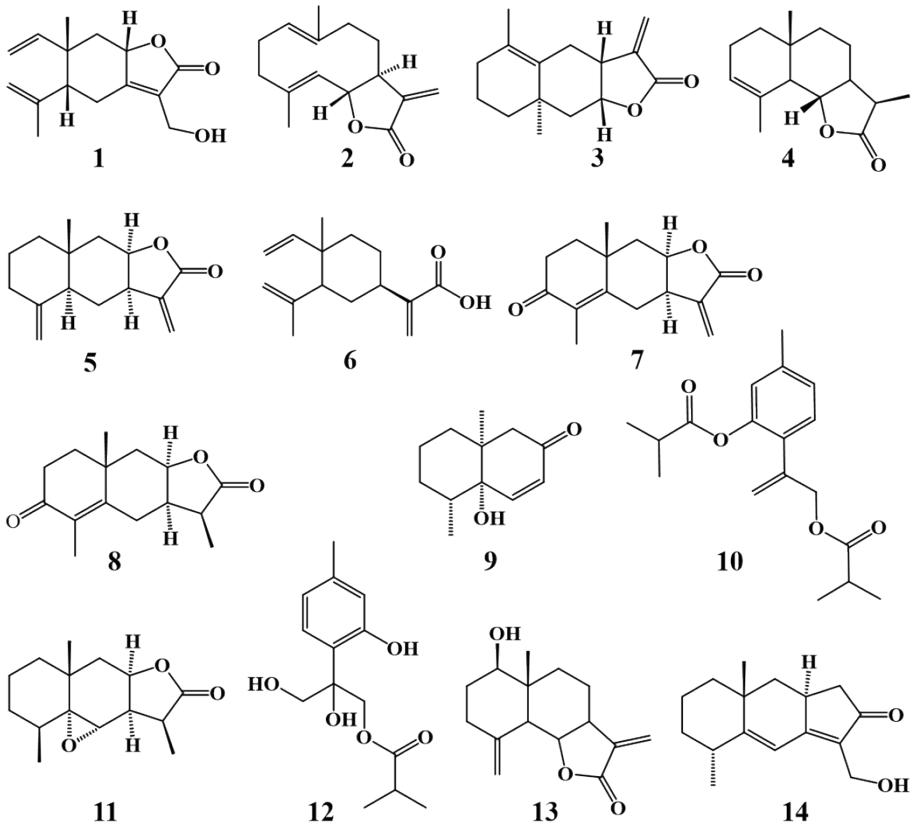

Fig. S1. The structures of 14 compounds isolated from *Inula macrophylla*.

Optimization of RONs immobilized AChE

The catalytic activity of RONs immobilized AChE was associated with several conditions, including immobilization pH (3, 4, 5, 6, 7), immobilization time (1, 2, 3, 4, 5 h), immobilization AChE concentration (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/mL), enzymatic reaction temperature (25, 30, 35, 40, 45°C) and enzymatic reaction time (5, 10, 15, 20, 25, 30 min). The relative activity of RONs immobilized AChE was optimized by ΔP ($\Delta P=P_0-P$), where P_0 and P are the peak areas of the product (yellow-colored ox OPD) in the absence and presence of RONs immobilized AChE, respectively. More information was given in the Fig. S2. Regarding above experimental conditions, immobilization pH of 5, immobilization time of 3 h, immobilization AChE concentration of 0.5 mg/mL, reaction temperature of 35°C and reaction time of 15 min were optimized as the best parameters.

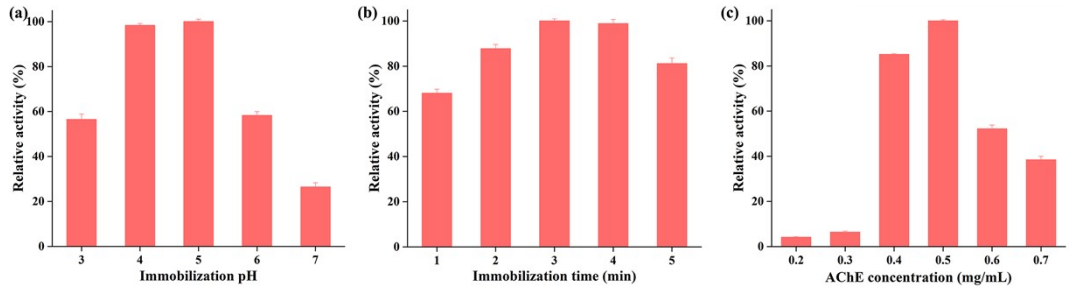

Fig. S2. Influence of (a) immobilization pH, (b) immobilization time and (c) immobilization enzyme concentration on the relative activity of the RONs immobilized AChE.

Immobilization pH is a crucial factor for the activity of RONs immobilized AChE. Fig. S2a presented the effect of pH (from 3.0 to 7.0) on the relative activity. Increasing pH value from 3.0 to 5.0 resulted in an enhancement of ΔP. But, continuing increasing pH leaded to the decrease of ΔP. Thus, the optimum immobilization pH is 5.

Immobilization time was directly related to immobilization efficiency. Immobilization time was investigated ranging from 1 h to 5 h. As shown in Fig. S2b,

ΔP increased as the immobilization time was prolonged. When the immobilization time was up to 3 h and the relative activity was gradually decreased. Therefore, 3 h is utilized as the optimal immobilization time.

Immobilization AChE concentration directly affects the amount of AChE on the RONs, further affects the enzymatic reaction rate. The effect of immobilization AChE concentration on the relative activity was investigated ranging from 0.2 to 0.7 mg/mL (Fig. S2c). The optimal immobilization AChE concentration on the RONs is 0.5 mg/mL and was employed in the following experiment.

Enzymatic reaction temperature and time were studied and the result was presented in Fig. S3. According to the above-mentioned procedures of RONs immobilized AChE activity assay, 35°C and 15 min were selected as the most appropriate reaction temperature and reaction time, respectively.

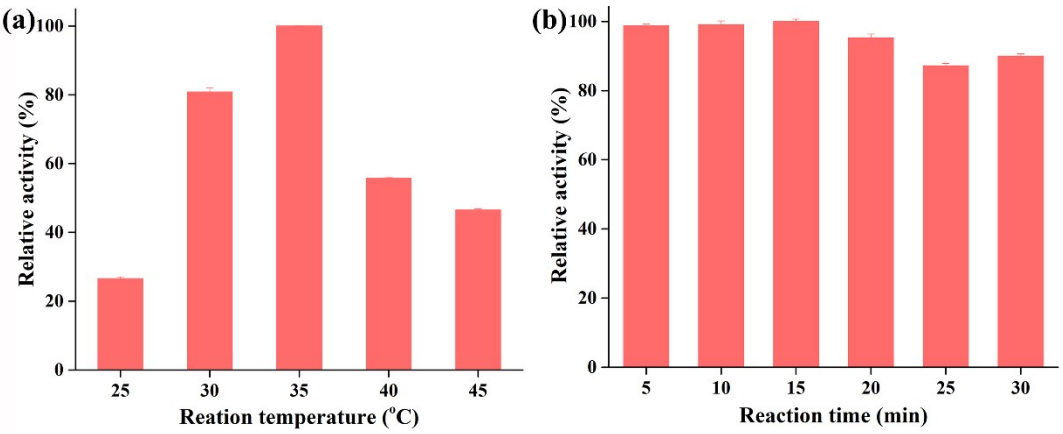

Fig. S3. Influence of (a) reaction temperature and (b) reaction time on the relative activity of the RONs immobilized AChE.

Optimization of chromogenic reaction

After the RONs immobilized AChE activity assay, the produced TCh could trigger the decomposition of the MnO_2 nanosheets, impair the oxidase-like activity of MnO_2 in the chromogenic reaction with OPD. To obtain optimum chromogenic reaction parameters, the concentration of OPD, coloration pH and coloration temperature should be optimized for monitoring RONs immobilized AChE activity. In this process, the relative activity of RONs immobilized AChE was optimized by P. Regarding the optimization results (Fig. S4), we chose 4 mM as the proper OPD concentration, pH 4 as the optimum coloration pH and 30°C as the suitable coloration temperature.

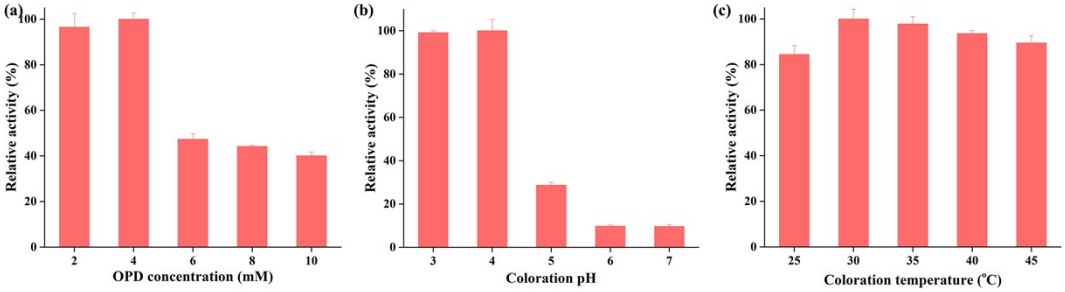

Fig. S4. Influence of (a) OPD concentration, (b) coloration pH, (c) coloration temperature on the relative activity of the RONs immobilized AChE.