DIFFERENT EFFECTS OF FORSYTHOSIDE *E* ON ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE BY FLUORESCENT SPECTROSCOPY**

Ch. Gao, H. Du*

Institute of Molecular Science at Shanxi University,
Taiyuan, China; e-mail: duhuizhi@sxu.edu.cn.

Forsythoside E is one of the major secondary metabolites in Forsythia suspensa. The interactions between forsythoside E and two types of cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were investigated in PBS buffer (pH 7.40) by using multispectroscopic techniques. Forsythoside E increased the fluorescence intensity of AChE but quenched the fluorescence of BChE. Synchronous fluorescence studies showed that forsythoside E mainly acts on tyrosine residues of AChE and tryptophan residues of BChE. It was also proved that the complex between the compound and cholinesterases formed spontaneously at a stoichiometric ratio of 1:1 via multispectral technology. Finally, forsythoside E inhibited the activities of cholinesterases with similar IC_{50} values of 1.08 mM for AChE and 0.92 mM for BChE. The results illuminate the details of the interaction between forsythoside E and cholinesterases.

Keywords: *forsythoside E, acetylcholinesterase, butyrylcholinesterase, spectroscopy.*

ВЛИЯНИЕ ФОРСИТОЗИДА *E* НА АЦЕТИЛХОЛИНЭСТЕРАЗУ И БУТИРИЛХОЛИНЭСТЕРАЗУ ПО ДАННЫМ ФЛУОРЕСЦЕНТНОЙ СПЕКТРОСКОПИИ

Ch. Gao, H. Du*

УДК 535.37;543.42

Институт молекулярных наук Шаньсийского университета,
Тайюань, Китай; e-mail: duhuizhi@sxu.edu.cn.

(Поступила 24 июня 2021)

Исследовано взаимодействие между одним из основных вторичных метаболитов Forsythia suspensa форситозидом E и двумя типами холинэстераз — ацетилхолинэстеразой (АХЭ) и бутирилхолинэстеразой (БХЭ) — в буфере PBS (pH 7.40) с использованием спектроскопических методов. Показано, что форситозид E увеличивает интенсивность флуоресценции АХЭ, но подавляет флуоресценцию БХЭ, при этом воздействует преимущественно на тирозиновые остатки АХЭ и триптофановые остатки БХЭ. Доказано, что комплекс между форситозидом E и холинэстеразами образуется спонтанно при стехиометрическом соотношении 1:1 и форситозид E ингибирует активность холинэстераз с близкими значениями IC_{50} 1.08 мМ для АХЭ и 0.92 мМ для БХЭ. Описаны детали взаимодействия между форситозидом E и холинэстеразами.

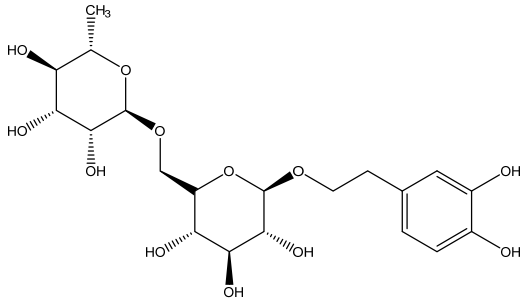
Ключевые слова: *форситозид E, ацетилхолинэстераза, бутирилхолинэстераза, спектроскопия.*

Introduction. Alzheimer's disease (AD) is a common progressive and age-related neurodegenerative disorder. Memory impairment and cognitive decline are the typical pathological features of AD patients. The cholinergic system plays an important role in memory and cognition, and the loss of cholinergic neurons from the nucleus basalis of Meynert that takes place in the AD patient's brain appears to be a very important factor contributing to AD memory deficit [1]. The 'cholinergic hypothesis' that AD is closely associated with an acetylcholine (ACh) deficit and the impairment of the cholinergic transmission was known [2].

**Full text is published in JAS V. 89, No. 3 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 89, No. 3 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

There are two types of cholinesterases – acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) – in the nervous system [3]. Although both cholinesterases are glycoproteins with highly homologous amino acid sequences, they display a big difference regarding substrate specificity. AChE principally hydrolyzes ACh into choline and acetic acid. Butyrylcholine (BCh) is the primary substrate of BChE and ACh is also the secondary substrate. BChE hydrolyzes ACh much more slowly than AChE. The ability of BChE to assist in hydrolyzing Ach recommends it as a substitute for AChE in the absence of AChE or in the later stages of AD. The active site of AChE contains the esteratic subsite for the catalytic machinery pocket and anionic subsite for the choline-binding pocket, along with one or more additional binding sites of ACh [4]. Overexpression of AChE accelerated the aggregation of amyloid β (Aβ) into amyloid fibrils and finally the senile plaques formed in AD. During this progress, the interactions between AChE and Aβ yielded the AChE-Aβ complex which caused neurotoxicity [5]. Currently, the most effective therapeutic treatment for AD symptoms still focuses on the cholinergic system. Many AChE inhibitors have been synthesized or extracted and proved to have good inhibitory activity on AChE, some of which have been used in the clinical trials to treat AD, including natural ingredients such as lignanamide, huperzine A, and fucoidan, etc. [6]. Among these, donepezil and galantamine selectively inhibit AChE, while tacrine and rivastigmine have inhibitory effects on AChE and BChE [7]; however, none of them are disease-modifying drugs. Therapeutic interventions aiming to replenish lost neurons and/or avoid neuronal death have the potential to modify AD progression by rescuing cholinergic neuronal death as well as preventing the loss of cortical and hippocampal neurons, thus preventing AD progression [8]. Since tacrine has been withdrawn because of serious side effects, it is thus urgent to find drugs that inhibit the activity of AChE and BChE at the same time.

As a traditional Chinese medicine, the fruits of *Forsythia suspensa* (Thunb.) Vahl (*F. suspensa*, Lianqiao in Chinese) have long been used in China and other Asian counties [9]. Forsythoside E, one of the major secondary metabolites from *F. suspensa*, has attracted extensive attention in the last few years [10]



Forsythoside E

The interaction of forsythoside E and bovine serum albumin (BSA) was studied by our group [11]. Forsythoside E had completely different effects from that of the other two isomers, e.g., forsythoside A and forsythoside I, from *F. suspensa*. Forsythoside E increased the fluorescence of BSA, while the two isomers quenched it. Forsythoside A inhibited AChE with an IC_{50} of 45.00 μM and suppressed AChE overexpression caused by $A\beta_{25–35}$ in PC12 cells [12, 13]. Forsythoside B proved to be an effective radical scavenger and cholinesterase inhibitor [14]. These phenomena aroused the authors' interest to further study the interactions between forsythoside E and the two cholinesterases (AChE and BChE) and the inhibitory effects of forsythoside E on cholinesterases. The interactions between proteins and various types of drugs are commonly found in pharmacological, biological, and clinical applications. The parameters of the interactions between proteins and drugs, such as the binding sites, binding modes, binding constants, etc., are important information regarding the pharmacodynamics and pharmacokinetics of drugs. Thus, they are very meaningful to help understand the transport and distribution of the drug at the biological, pharmacological, and clinical levels [15]. Because of their advantages over other technologies, spectroscopic techniques are still the most commonly used techniques to explore detailed information on protein–drug interactions. Therefore, in the present work, the interactions of forsythoside E with two cholinesterases (AChE and BChE) and the inhibitory effects of forsythoside E on cholinesterases were studied using multispectroscopic technologies.

Material and methods. Forsythoside E with a purity of 98.6% was purchased from Must (Chengdu, China), whereas AChE from electric eel (200 U/g, EC 3.1.1.7) and BChE from equine serum (4,000 U/g, EC 3.1.1.8; P06276) were bought from Macklin (Shanghai, China) and Aladdin (Shanghai, China), respectively. S-acetylthiocholine iodide (AChI) and S-butyrylthiocholine iodide (BChI) were from Sangon (Shanghai, China) and Sigma, respectively. 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) and sodium dodecyl sul-

fate (SDS) were bought from Sangon and Sigma, respectively. All other reagents used in the work were of analytical grade. Forsythoside E was prepared as a stock solution in purified water and AChE and BChE were prepared as stock solutions in PBS buffer (pH 7.40).

The fluorescence spectra were recorded on a fluorescence spectrophotometer (Fluromax-4, HORIBA Scientific, USA), which was equipped with a temperature controller. A quartz cuvette with 1-cm path length was used in the study. The fluorescence was recorded 5 min after adding the compound to the AChE solution and 10 min after adding the compound to the BChE solution. The fluorescence of cholinesterases was recorded between 290 and 500 nm at a 280-nm excitation wavelength, with a 10-nm excitation and emission slit width. The flow chart for adding forsythoside E to the AChE solution is shown in Fig. S1.

The synchronous fluorescence study was conducted on a fluorescence spectrophotometer (Fluromax-2500, Hitachi, Japan). The initial excitation wavelength was 220 nm and the scan proceeded to 500 nm. The difference ($\Delta\lambda$) between the excitation wavelength and the emission wavelength was set to 15 nm, characteristic of tyrosine (Tyr) residues, or 60 nm, characteristic of tryptophan (Trp) residues. The slit widths for excitation and emission were both 10 nm.

UV absorption experiments were performed at 200–350 nm on a UV-visible spectrophotometer (Cary 50, Varian, USA). The baseline was calibrated by recording the absorption spectra of PBS buffer (pH 7.40). All spectroscopic recordings were performed at room temperature (RT) except where otherwise specified and were repeated at least twice. The concentrations of AChE and BChE were kept at 1 μM in the same volume in the spectroscopic recording experiments.

The inhibitory activity of forsythoside E on cholinesterases was measured by using the Ellman's method, with slight modifications [13, 16]. Forsythoside E at different concentrations, AChE (or BChE) at a final concentration of 0.07 U/mL, and DTNB at a final concentration of 1 mM were mixed in a PBS buffer, pH 7.40. The mixed solution (100 μL) was added to a 96-well plate, which was then kept in a 37°C incubator for 15 min. Next, AChI (or BChI) was added to each well at a final concentration of 0.19 mM. After 25 min, SDS with the final concentration of 0.19 mM was also added to each well, and OD values (A_{FE}) were recorded at 412 nm on the microplate reader (Spectra Max 190, MD, USA). The percent inhibition was calculated according to the following equation:

$$\text{Inhibition (\%)} = [1 - (A_{FE} - A_{FE\text{-}Ctrl})/(A_{Ctrl\text{-}1} - A_{Ctrl\text{-}2})] \times 100\%,$$

where A_{FE} is the OD value under the same conditions forsythoside E; $A_{FE\text{-}Ctrl}$ is the OD value under the same conditions without cholinesterases; $A_{Ctrl\text{-}1}$ is the OD value under the same conditions without forsythoside E; $A_{Ctrl\text{-}2}$ is the OD value under the same conditions without forsythoside E and cholinesterases; and, the total solution for each group was brought to the same volume of solvent.

Results and discussion. Fluorescence spectroscopy is one of the valuable tools to study ligand-protein interactions. The cholinesterases both have a distinct fluorescent emission peak at approximately 340 nm with a 280-nm excitation wavelength. The interactions of forsythoside E with two cholinesterases were studied by adding the compound into the cholinesterase solutions. The fluorescence of forsythoside E at different concentrations was also recorded (Fig. S2). Forsythoside E shows almost no intrinsic fluorescence emission at 348 nm with λ_{ex} = 280 nm at RT at concentrations of 10, 20, 30, and 40 μM. As shown in Fig. 1a, the fluorescent emission of AChE increases regularly when the concentration of forsythoside E increases to 5, 10, and 15 μM. While the fluorescence intensity is almost unchanged with low concentrations of forsythoside E, it increased significantly at high concentrations. Although forsythoside E may bind with the first binding site at a low concentration, it will bind with the second binding site at an increased concentration. The first binding site should be a strong binding site for forsythoside E and detailed information should be further studied. Meanwhile, the fluorescence peak gradually moves towards a shorter emission wavelength, which is called the blue shift. Forsythoside E may combine with AChE to induce a more hydrophobic amino acid microenvironment that finally causes the blue shift of fluorescence. BChE fluorescence decreases regularly with the addition of forsythoside E at 5, 10, 15 μM (Fig. 1b). In addition, the blue shift of the fluorescent peak is observed, which indicates that BChE exhibited an increasingly hydrophobic environment.

Synchronous fluorescence has been used to study the microenvironment in proteins due to its high sensitivity, narrow spectral bandwidth, simple spectra and minimal interference [17]. The synchronous fluorescence of the interactions of forsythoside E and the cholinesterases was investigated by adding the compound into the protein solution; the spectra are shown in Fig. 2. The synchronous fluorescent spectra with $\Delta\lambda$ = 15 and 60 nm are characteristic of Tyr and Trp residues, respectively [18].

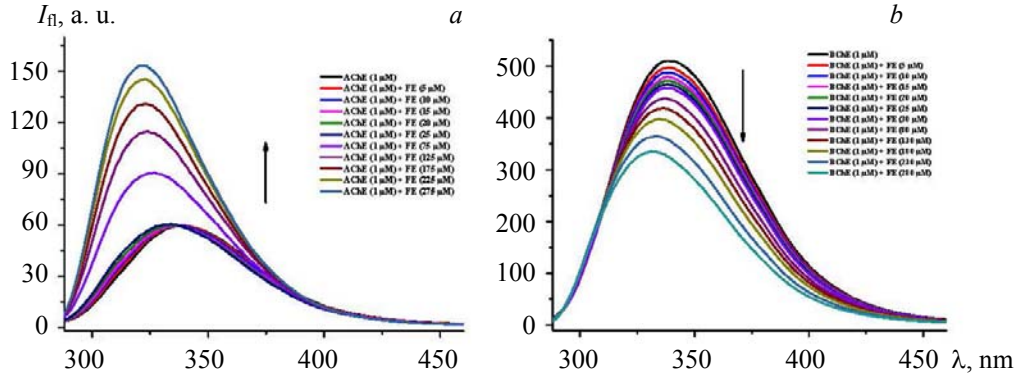


Fig. 1. The fluorescent emission spectra of AChE (a) and BChE (b) at λ_{ex} = 280 nm with the increasing concentration of forsythoside E at 0, 5, 10, 15, ..., 280 μM. The concentrations of AChE and BChE are 1 μM.

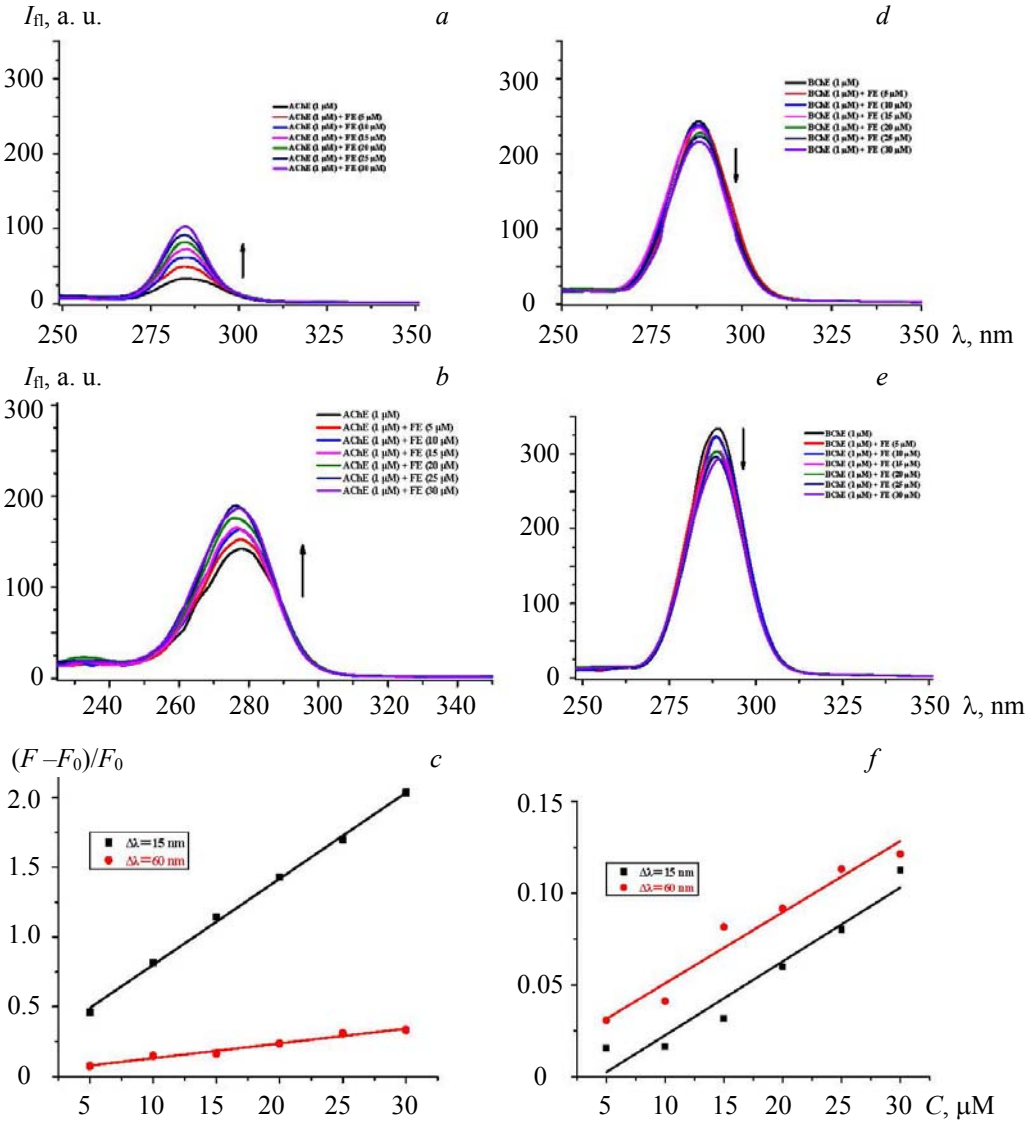


Fig. 2. The effects of forsythoside E on the synchronous fluorescence spectra of AChE (a–c) and BChE (d–f) with forsythoside E concentration at 0, 5, 10, 15, ..., 30 μM, the concentrations of AChE and BChE are 1 μM; Δλ = 15 (a, d) and 60 nm (b, e).

For AChE, consistent with the previous fluorescence phenomenon, the fluorescence intensities at $\Delta\lambda$ = 15 and 60 nm both gradually increase when the compound concentration increases. At the same time, there is a much smaller fluorescence increase at a $\Delta\lambda$ = 60 nm than at a $\Delta\lambda$ = 15 nm, which means that the effect of the compound on Tyr is greater than the effect on Trp in AChE. For BChE, both fluorescent intensities decreased regularly when the compound is gradually added, which further confirms the previous quenching data on fluorescence. The decrease in the fluorescence intensity at a $\Delta\lambda$ = 60 nm is greater than that at a $\Delta\lambda$ = 15 nm, which reveals that the compound mainly affects Trp and that the microenvironment around the Trp residue becomes more hydrophobic.

UV absorption spectra are also used to study the structural change of the protein induced by drugs. The characteristic absorption peak of BChE falls at 280 nm, which is mainly caused by Trp residues, while the absorption of the compound at 280 nm may be induced by the phenolic group. Here, the UV absorption spectra of the interaction of forsythoside E with AChE and BChE were recorded by the addition of forsythoside E (5, 10, 15, …, 130 μM) into the protein solution. With increasing concentration of the compound, the absorption intensity accordingly increases (Fig. S3), and the UV absorption spectrum of forsythoside E also increases with increasing concentration (Fig. S4). The difference is observed before and after forsythoside E interacts with cholinesterases at the same concentration of compound, and the results are shown in Fig. S5. It is found that the UV absorption difference is enhanced with increasing forsythoside E concentration. The results further suggest that a complex is formed between forsythoside E and cholinesterases and that the quenching mechanism of BChE may be static quenching [19, 20].

Two protein fluorescence-quenching modes induced by a quencher are the dynamic and static modes. The quenching mode of forsythoside E on BChE fluorescence may be further characterized by the following method. The quenching rate constant (k_q) and the Stern-Volmer quenching constant (K_{SV}) were calculated by using Stern-Volmer plots and the corresponding equations [21]. The binding constant (K) and the number of sites (n) of forsythoside E binding with cholinesterase were obtained from the modified Stern-Volmer plots: $\lg[(F_0–F)/F]$ or $\lg[(F–F_0)/F_0]$ *vs*. lg[Forsythoside E] and the corresponding equations [22]. The thermodynamic parameter-free energy change (ΔG) is the other main evidence confirming the binding modes [23] and may be calculated at different temperatures according to the equation. The equations are shown in the ESM, and the results are summarized in Table 1. The k_q value of BChE is much higher than the maximum value of the quenching constant in the scatter collision (2×10^{10} $M^{-1}s^{-1}$), which suggests that the quenching is not triggered by the dynamical collision but rather by a complex formation between forsythoside E and BChE [21]; thus, static quenching is the main quenching mode in the binding process of forsythoside E with BChE. The K value of AChE-forsythoside E is more than 100 times that of BChE-forsythoside E. The n numbers of the two types of cholinesterases approximately are equal to one, indicating that there is only one site on each cholinesterase applicable for forsythoside E to bind to; however, the details should be investigated in-depth. In addition, the negative signs of ΔG for both cholinesterases demonstrate that there is spontaneous binding between forsythoside E and the cholinesterases [24].

Finally, the effects of forsythoside E on the activity of two cholinesterases were explored by commonly used methods [13, 16] (Fig. 3). Forsythoside E inhibits the activity of AChE and BChE, and the inhibition rate increases with increasing the compound concentration. Fitting and calculating showed that the IC_{50} values of the inhibition of AChE and BChE by forsythoside E are 1.08±0.48 and 0.92±0.33 mM, respectively. Forsythoside E showed similar inhibitory effects on the two types of cholinesterases.

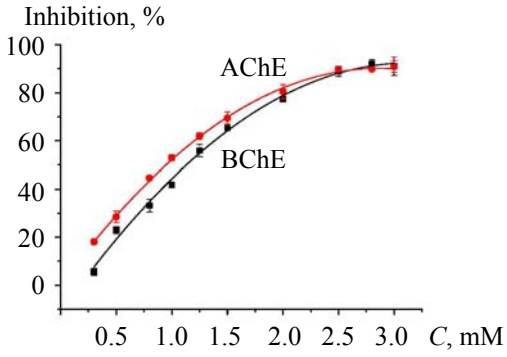


Fig. 3. Forsythoside E inhibits the activity of AChE (●) and BChE (■). Error bars indicate SD, n = 3–5.

TABLE 1. The Binding Parameters for the Interaction between Forsythoside E and Cholinesterases

Cholinesterases	K_{SV}, 10^4 M^{-1}	k_q, 10^{12} $M^{-1}\cdot s^{-1}$	K, M^{-1}	n	ΔG, kJ/mol
AChE	–	–	7.51×10^5	1.12	–28.36
BChE	0.02	0.91	3.39×10^3	1.05	–20.48

Conclusions. The interactions between one of the major secondary metabolites from *F. suspensa,* forsythoside E, and two types of cholinesterases, AChE and BuChE, were studied via multispectroscopic techniques. Forsythoside E inhibits the enzyme activities with similar IC_{50} values of 1.08 μM for AChE and 0.92 mM for BuChE; however, it has the opposite effects on their fluorescence – namely, forsythoside E increases the fluorescence intensity of AChE but quenches the fluorescence of BChE. The fluorescence of AChE is almost unchanged at a low concentration of forsythoside E, but is significantly increased at a high concentration. This interesting result indicates that forsythoside E at any concentration binds with only one binding site of AChE, which is also confirmed by the number of binding sites of 1.12. Synchronous fluorescence studies reveal that forsythoside E affects mainly the Tyr residues of AChE and the Trp residues of BChE. Multispectral technology also proves that forsythoside E spontaneously forms complexes with the cholinesterases. The results help to explore the interaction between forsythoside E and two types of cholinesterases.

Acknowledgements. The study was supported by Natural Science Foundation of Shanxi Province (No. 201801D121290) and the Open Fund from the Key Laboratory of Cellular Physiology (Shanxi Medical University; No. KLMEC/SXMU-201911). We thank Rongrong Gao (College of Chemistry, Nankai University), Yu Li (South China Academy of Advanced Optoelectronics, South China Normal University) and Qin Guo (College of Chemistry and Chemical Engineering, Southwest University) for their advice, technical support and preliminary experiments.

REFERENCES

1. T. H. Ferreira-Vieira, I. M. Guimaraes, F. R. Silva, F. M. Ribeiro, *Curr. Neuropharmacol.,* **14**, 101 (2016).
2. H. Hampel, M. M. Mesulam, A. C. Cuello, A. S. Khachaturian, A. Vergallo, M. R. Farlow, P. J. Snyder, E. Giacobini, Z. S. Khachaturian, *J. Prev. Alzheimers Dis.*, **6**, 2 (2019).
3. S. S. Xing, Q. Li, B. C. Xiong, Y. Chen, F. Feng, W. Y. Liu, H. P. Sun, *Med. Res. Rev.*, **41**, 858 (2020).
4. H. Dvir, I. Silman, M. Harel, T.L. Rosenberry, J. L. Sussman, *Chem. Biol. Interact.*, **187**, 10 (2010).
5. N. C. Inestrosa, A. Alvarez, C. A. Pérez, R. D. Moreno, M. Vicente, C. Linker, O. I. Casanueva, C. Soto, J. Garrido, *Neuron*, **16**, 881 (1996).
6. M. Mehta, A. Adem, M. Sabbagh, *Int. J. Alzheimers Dis.*, **2012**, 728983 (2012).
7. B. Adalat, F. Rahim, M. Taha, F. J. Alshamrani, E. H. Anouar, N. Uddin, S. A. A. Shah, Z. Ali, Z. A. Zakaria, *Molecules*, **25**, 4828 (2020).
8. P. Anand, B. Singh, *Arch. Pharm. Res*., **36**, 375 (2013).
9. [CPH] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China. Part Five, China Medical Science Press, 170 (2020).
10. F. N. Wang, Z. Q. Ma, Y. Liu, Y. Z. Guo, Z. W. Gu, *Molecules*, **14**, 1324 (2009).
11.Y. Li, Q. Guo, Y. Yan, T. Chen, C. Du, H. Du, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, **214**, 309 (2019).
12. X. Yan, T. Chen, L. Zhang, H. Du, *Eur. J. Pharmacol.*, **810**, 141 (2017).
13. X. Yan, T. Chen, L. Zhang, H. Du, *Int. J. Biol. Macromol.*, **119**, 1344 (2018).
14. M. Georgiev, K. Alipieva, I. Orhan, R. Abrashev, P. Denev, M. Angelova, *Food Chem.*, **128**, 100 (2011).
15. B. Ahmad, S. Parveen, R. H. Khan, *Biomacromolecules*, **7**, 1350 (2006).
16. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol.*, **7**, 88 (1961).
17. R. Li, D. Dhankhar, J. Chen, T. C. Cesario, P. M. Rentzepis, *Proc. Natl. Acad. Sci. USA*, **116**, 18822 (2019).
18. J. N. Miller, *Proc. Anal. Div. Chem. Soc.*, **16**, 203 (1979).
19. P. D. Ross, S. Subramanian, *Biochemistry*, **20**, 3096 (1981).
20. U. Anand, C. Jash, S. Mukherjee, *J. Phys. Chem. B*, **114**, 15839 (2010).
21. J. R. Lackowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Plenum Press, New York, 603–606 (2006).
22. S. Nusrat, A. Masroor, M. Zaman, M. K. Siddiqi, M. R. Ajmal, N. Zaidi, A. S. Abdelhameed, R. H. Khan, *Int. J. Biol. Macromol.*, **109**, 1132 (2018).
23. I. M. Klotz, *Ann. N. Y. Acad. Sci.*, **226**, 18 (1973).
24. D. Leckband, *Annu. Rev. Biophys. Biomol. Struct.*, **29**, 1 (2000).

Supplementary.

The quenching rate constant (k_q) and the Stern-Volmer quenching constant (K_{SV}) were calculated by using Stern-Volmer plots and the equations:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q],\ k_q = K_{SV}/\tau_0,$$

where F_0 and F are the fluorescence intensities of cholinesterases in the absence and presence of the compound, respectively; k_q is the quenching rate constant of the quenching reaction; τ_0 is the average integral fluorescence lifetime of the protein; K_{SV} is the Stern-Volmer quenching constant and $[Q]$ is concentration of the compound.

The binding constant (K) and the number of the binding sites (n) of forsythoside E binding with AChE and BChE were obtained from the modified Stern-Volmer plots, respectively:

$$\text{with AChE} \quad \lg[(F - F_0)/F_0] = \lg K + n\lg[Q],$$

$$\text{with BChE} \quad \lg[(F_0 - F)/F] = \lg K + n\lg[Q].$$

The thermodynamic parameters free energy change (ΔG) may be calculated according to the equation:

$$\Delta G = -RT \ln K,$$

where R is the gas constant and T is the temperature.

After fitting the plots, IC_{50} values of forsythoside E inhibiting AChE and BChE were calculated:

$$y = 38.78 \ln x + 47.08, \quad R^2 = 0.9833,$$

$$y = 35.99 \ln x + 53.46, \quad R^2 = 0.9910.$$

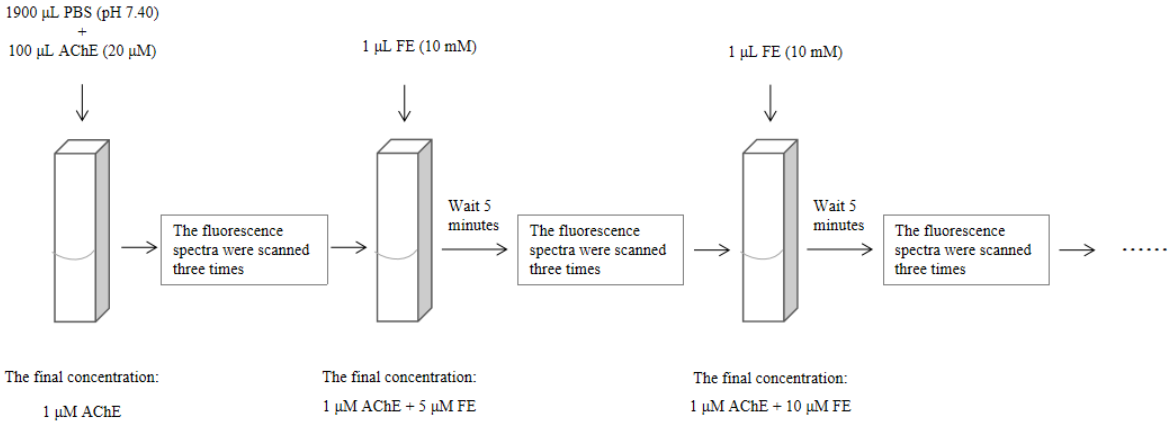


Fig. S1. The flow chart of titrating forsythoside E to AChE solution. The final concentration of AChE is 1 µM.

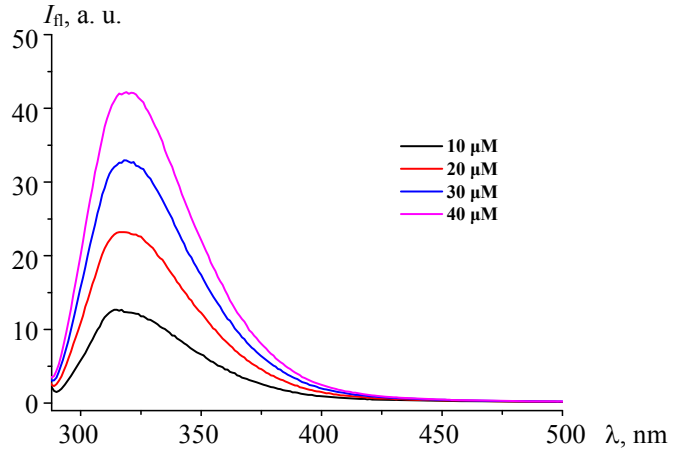


Fig. S2. Forsythoside E shows almost no intrinsic fluorescence emission at 348 nm with λ_{ex} 280 nm at room temperature at concentrations of 10, 20, 30 and 40 µM.

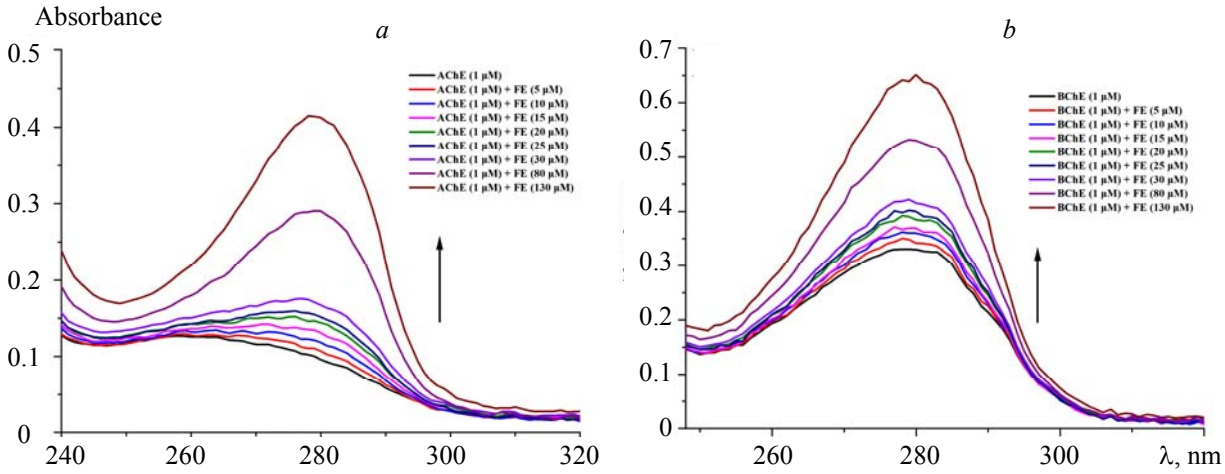


Fig. S3. UV absorption of AChE (a) and BChE (b) increases with increasing the concentration of forsythoside E (5, 10, 15, …... 130 μM). The concentrations of AChE and BChE are 1 μM.

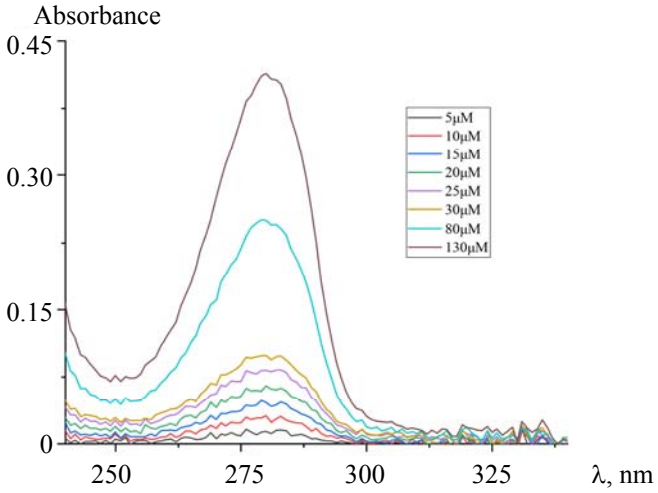


Fig. S4. UV absorption of forsythoside E increases with increasing the concentration (5, 10, 15, …, 130 μM).

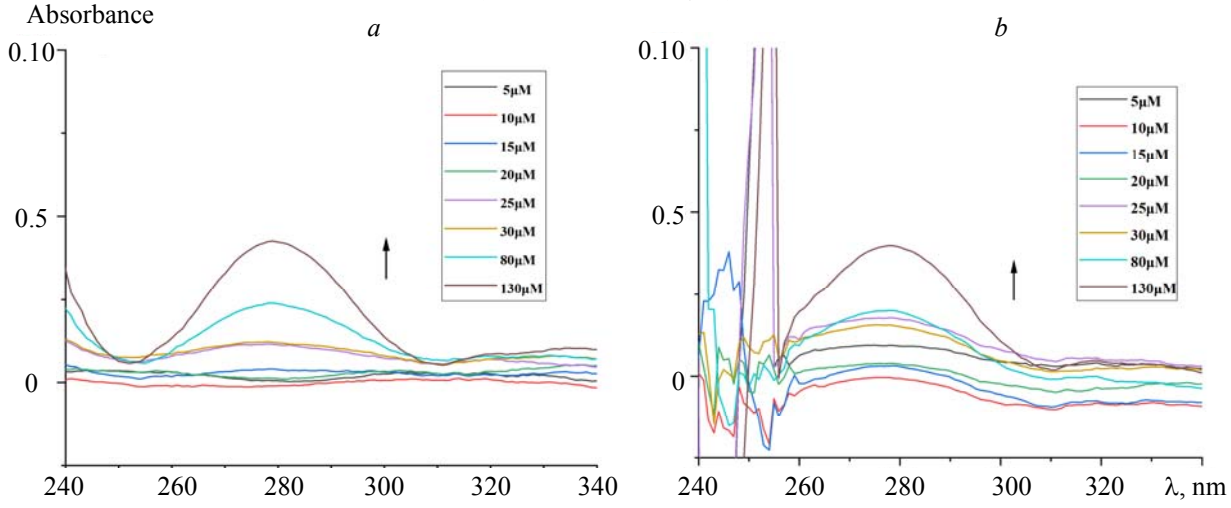


Fig. S5. The difference was calculated before and after forsythoside E interacted with AChE (a) and BChE (b) according to the same concentration of compound.