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RESEARCH ARTICLE

Structure–activity study of fluorine or chlorine-substituted cinnamic acid derivatives with tertiary amine side chain in acetylcholinesterase and butyrylcholinesterase inhibition

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Abstract

In this study, a series of new fluorine or chlorine-substituted cinnamic acid derivatives that contain tertiary amine side chain were designed, synthesized, and evaluated in acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition. The results show that almost all the derivatives containing tertiary amine side chain (compounds **4a–9d**) exhibit moderate or potent activity in AChE inhibition. By contrast, their parent compounds (compounds **3a–3f**) in the absence of tertiary amine moitery exhibit poor inhibitory activity against AChE. For the compounds containing pyrroline or piperidine side chain, the bioactivity in AChE inhibition is much intense than those containing *N*,*N*-diethylamino side chain. The chlorine or fluorine substituted position produces a significant effect on the bioactivity and selectivity in AChE inhibition. Most of the compounds that contain *para*-substituted fluorine or chlorine exhibit potent activity against AChE and poor activity against BChE, while *ortho*-substituted analogs show the opposite effect. It is worth noticing that the compounds containing *N*,*N*-diethylamino side chain are exceptions to this pattern. Among the newly synthesized compounds, compounds **6d** are the most potent in AChE inhibition (IC_{50} = 1.11 ± 0.08 μmol/L) with high selectivity for AChE over BChE (selectivity ratio: 46.58). An enzyme kinetic study of compounds **6d** suggests it produces a mixed-type inhibitory effect in AChE.

KEYWORDS

cholinesterase inhibitors, cinnamic acid, halogen

1 | INTRODUCTION

Cinnamic acid is a commonly occurring natural compound in *Cinnamomum cassia* and other plants (Jayaprakasha & Rao, 2011). It has been reported that cinnamic acid can be used as condiment and antioxidant (Letizia, Cocchiara, Lapczynski, Lalko, & Api, 2005; Li et al., 2014). Some recently synthesized cinnamic acid derivatives exhibit anti-tyrosinase, hepatoprotective and α-glusidase inhibition effects, etc. (Hu et al., 2016; Yan, Wang, Yen, Lee, & Yin, 2016; Song et al., 2016). It has also drawn great attention of medicine researchers to the treatment of Alzheimer's disease (AD) using cinnamic acid derivatives (Estrada et al., 2016; Zhang et al., 2018). Acetylcholinesterase (AChE) inhibitors, as the major AD therapeutic drugs, exhibit adverse effects such as vomiting, cardiotoxicity in clinical use yet (Hansen et al., 2008; Birks, Grimley Evans, Iakovidou, Tsolaki, & Holt, 2009; Takaya et al., 2009). Therefore, developing new acetylcholinesterase inhibitors has become an area of intense interest in recent years. In our laboratory, after screening more than 100 compounds, flavokawain B Mannich base was found to have an inhibitory effect on AChE (Liu et al., 2014a). As flavokawain B is a naturally occurring chalcone isolated

from the root of *Piper methysticum* (Jeong et al., 2015), a series of chalcone with tertiary amine side chains were synthesized, which were confirmed possessing inhibitory effect on AChE in subsequent experiments. Some of them have better selectivity in inhibiting AChE over butyrylcholinesterase (BChE) in our reports (Liu et al., 2014b; Liu et al., 2016). Most recently, we have discovered cinnamic acid derivatives similar to chalcone scaffold can inhibit AChE. The investigations on the structure–activity relationship reveal that cinnamic acid derivatives with *para*-substituted tertiary amine possess more potent bioactivity in AChE inhibition than *meta*- or *ortho*-substituted ones (Gao et al., 2018b).

Based on the above investigations, in consideration of important effects of halogens in drug discovery and development (Lu et al., 2012; Vulpetti & Dalvit, 2012), in this study a new series of fluorine or chlorine-substituted cinnamic acid derivatives with *para*-substituted tertiary amine side chain are designed, synthesized, and evaluated for AChE and BChE inhibition (Figure 1). Compound **6d**, which is confirmed having the most potent activity in AChE inhibition among new synthesized compounds in subsequent bioactivity evaluation, is selected for conducting an enzymatic kinetics study.

2 | MATERIALS AND METHODS

2.1 | Chemistry

All chemicals or reagents are of analytical reagent-grade and used without further purification. The melting points of compounds were measured on a WRS-IA melting point detector (temperature uncorrected). ^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. Mass spectra were obtained on Waters XEVO G2-XS QTOF by electrospray ionization. Infrared spectrum was obtained by Shimadzu Infinity-1 infrared spectrometer. The purity of compounds was checked by Shimadzu LC-20A high performance liquid chromatography.

2.2 | General method for synthesis of halogen-substituted cinnamamides (3a–3f)

Compounds **3a–3f** were synthesized according to previous reported method (Gao et al., 2018a). Halogen-substituted cinnamic acid (2-,3-, or 4-fluoro or chlorine cinnamic acid, 10 mmol), dichloromethane (30 mL) and a small amount of dimethylformamide (DMF) were added into the flask, followed by the addition of oxalyl chloride (2.55 mL, 30 mmol) drop by drop. The mixture was refluxed, and the reaction process was monitored by thin layer chromatography (TLC) until the reaction is completed. Then the solution was cooled to room temperature, dichloromethane (30 mL × 3) was added into the solution. After the solvent and excess oxalyl chloride was removed by the evaporation under reduced pressure, the halogen-substituted cinnamic acyl chlorides were gained. These compounds were used as the intermediates to synthesize compounds **3a–3f** without further purification.

Then *para*-aminophenol (1.1 g, 10 mmol) and acetonitrile (10 mL) was added into the flask to dissolve by heating, followed by the addition of triethylamine (2.0 mL, 15 mmol) and halogen-substituted cinnamic acyl-chloride solution. The mixture solution was refluxed and the reaction process was monitored by TLC until the reaction was completed. Then the reaction solution is cooled to room temperature, followed by the addition of 10% NaOH solution. The solution was filtered, adjusting the pH of filtrate to 4–5 by hydrochloric acid. Then a large number of solid precipitates were produced. The precipitates were collected by filtration, and were washed by water to neutral. Finally solid products were gained with the yield more than 90%.

*(E)-3-(2-fluoro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3a**)*: white solid, mp: 210.6–210.9 °C (Lit.211–213 °C). It is a known compound as commercial block but no biological activity has been reported (Ramalakshmi, Deepa, Sumanth Srinivas, Puratchikody, & Arunkumar, 2009).

*(E)-3-(3-fluoro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3b**):* white solid, mp: 227.7–228.5 °C (Lit.228–230 °C). It is a known compound as commercial block but no biological activity has been reported (Ramalakshmi et al., 2009).

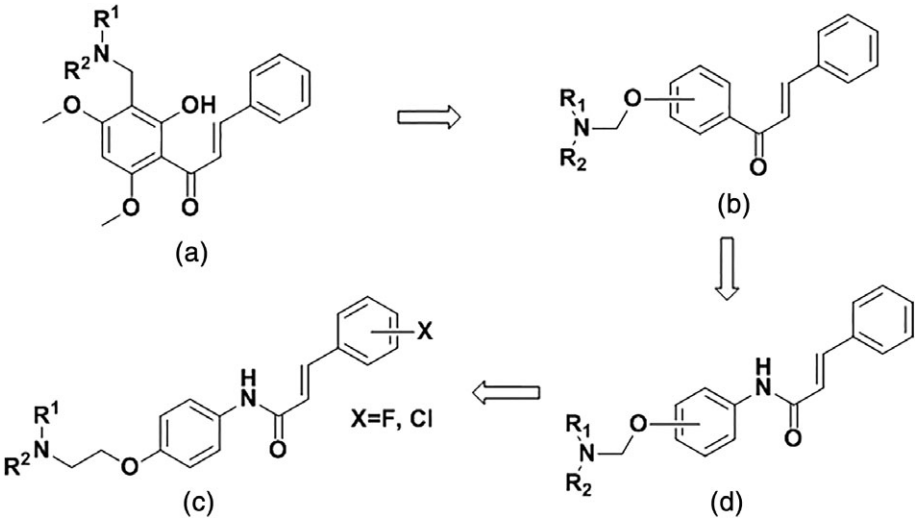


FIGURE 1 Design pathway of halogen-substituted cinnamic acid derivatives. (a) Flavokawain B Mannich base derivatives; (b) chalcone derivatives with tertiary amine moiety; (c) cinnamic acid derivatives with tertiary amine moiety; and (d) halogen-substituted cinnamic acid derivatives with tertiary amine moiety

*(E)-3-(4-fluoro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3c**)*: white solid, mp: 215.5–216.3 °C (Lit.217–218 °C). It is a known compound as commercial block but no biological activity has been reported (Ramalakshmi et al., 2009).

*(E)-3-(2-chloro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3d**)*: white solid, mp: 225.3–226.2 °C (Lit.226–228 °C). It is a known compound in a report possessing antibacterial activity (Ramalakshmi et al., 2009).

*(E)-3-(3-chloro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3e**)*: white solid, mp: 237.5–238.3 °C (Lit.239–240 °C). It is a known compound as commercial block but no biological activity has been reported (Ramalakshmi et al., 2009).

*(E)-3-(4-chloro phenyl)-N-(4-hydroxy phenyl) acrylamide (**3f**)*: white solid, mp: 226.6–227.2 °C (Lit.228–230 °C). It is a known compound with antimicrobial activity (Ramalakshmi et al., 2009).

2.3 | General method for synthesis of compounds 4a–9d

Compounds **4a–9d** were synthesized according to previous reports (Liu et al., 2017; Sang et al., 2017). One of cinnamic amides (**3a–3f**) (1 mmol), anhydrous K_2CO_3 (0.68 g, 5 mmol) were added into a flask with 25 mL acetone as the solvent, stirred and refluxed for 45 min. Subsequently, one of aminoethyl chlorides hydrochloride (3 mmol) and NaI (0.009 g, 0.05 mmol) were added to the flask and continued to refluxed, monitoring the reaction process by TLC. After the reaction was completed, the reaction mixture cooled to room temperature, filtered, removed the solvent by rotary evaporation. Then residue was dissolved by dichloromethane (30 mL), washed by saturated NaCl (aq, 30 mL × 2), followed by drying using anhydrous sodium sulfate. The solution was filtered and the solvent was removed to afford the crude product. The crude product was purified by column chromatography with the eluent (V(MeOH):V(DCM) = 1:60~70).

*(E)-3-(2-fluoro phenyl)-N-(4-(2-[dimethylamino] ethoxy) phenyl) acrylamide (**4a**)*

Grayish white powder, yield:49.3%, mp: 113.1–114.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.34 (6H, s, 2× NCH_3), 2.76 (2H, t, J = 6.0 Hz, OCH_2CH_2), 4.12 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.98–7.02 (3H, m, Ar—H and Ar—CH═CH), 7.36–7.42 (2H, m, Ar—H), 7.52–7.57 (1H, m, Ar—H), 7.67–7.71 (3H, m, Ar—H and Ar—CH═CH), 7.76–7.80 (1H, m, Ar—H), 10.27 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}FN_2O_2$ + ([M]+): 328.2; found M + 1:329.2. IR (KBr) cm^{-1}: 3367, 3,007, 1,654, 1,600, 1,577, 1,350, 1,278, 972, 742.

*(E)-3-(2-fluoro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**4b**)*

Light yellow powder, yield:55.6%, mp: 112.6–113.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.98–1.04 (6H, m, 2× NCH_2CH_3), 2.50 (2H, br, OCH_2CH_2), 2.71–2.92 (4H, m, 2× NCH_2CH_3), 4.06 (2H, br, OCH_2CH_2), 6.90–6.94 (3H, m, Ar—H and Ar—CH═CH), 7.28–7.34 (2H, m, Ar—H), 7.44–7.49 (1H, m, Ar—H), 7.59–7.64 (3H, m, Ar—H and Ar—CH═CH), 7.68–7.72 (1H, m, Ar—H), 10.19 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{25}FN_2O_2$ + ([M]+): 356.2; found M + 1:357.2. IR (KBr) cm^{-1}: 3311, 2,970, 1,660, 1,625, 1,600, 1,336, 1,253, 968, 752.

*(E)-3-(2-fluoro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**4c**)*

White powder, yield: 48.5%, mp: 108.5–108.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.38–1.39 (2H, m, piperidine-H), 1.47–1.51 (4H, m, piperidine-H), 2.44–2.51 (6H, m, 3× NCH_2), 4.04 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.90–6.93 (3H, m, Ar—H and Ar—CH═CH), 7.28–7.34 (2H, m, Ar—H), 7.44–7.49 (1H, m, Ar—H), 7.59–7.62 (3H, m, Ar—H and Ar—CH═CH), 7.68–7.72 (1H, m, Ar—H), 10.18 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{22}H_{25}FN_2O_2$ + ([M]+): 368.2; found M + 1:369.2. IR (KBr) cm^{-1}: 3317, 3,314, 1,666, 1,627, 1,558, 1,355, 1,232, 985, 750.

*(E)-3-(2-fluoro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**4d**)*

Light green powder, yield:57.8%, mp: 180.9–182.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.84 (4H, br, pyrrolidine-H), 2.99 (4H, br, pyrrolidine-H), 3.23 (2H, br, OCH_2CH_2), 4.17 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.91–6.99 (3H, m, Ar—H and Ar—CH═CH), 7.28–7.34 (2H, m, Ar—H), 7.44–7.50 (1H, m, Ar—H), 7.60–7.66 (3H, m, Ar—H and Ar—CH═CH), 7.69–7.72 (1H, m, Ar—H), 10.21 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{23}FN_2O_2$ + ([M]+): 354.2; found M + 1:355.2. IR (KBr) cm^{-1}: 3259, 3,028, 1,668, 1,600, 1,558, 1,344, 1,294, 989, 754.

*(E)-3-(3-fluoro phenyl)-N-(4-(2-[dimethylamino] ethoxy) phenyl) acrylamide (**5a**)*

White powder, yield: 48.6%, mp: 119.1–119.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.22 (6H, s, 2× NCH_3), 2.61 (2H, t, J = 6.0 Hz, OCH_2CH_2), 4.02 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.84 (H, d, J = 16.0 Hz, Ar—CH═CH), 6.91–6.93 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.50 (3H, m, Ar—H and Ar—CH═CH), 7.52–7.51 (H, m, Ar—H), 7.58–7.62 (2H, m, Ar—H), 10.27 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}FN_2O_2$ + ([M]+): 328.2; found M + 1:329.2. IR (KBr) cm^{-1}: 3288, 2,981, 1,664, 1,608, 1,581, 1,328, 1,276, 993, 775.

*(E)-3-(3-fluoro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**5b**)*

Light yellow powder, yield: 56.8%, mp: 139.2–140.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (6H, t, J = 8.0 Hz, 2× NCH_2CH_3), 2.51 (2H, t, J = 4.0 Hz, OCH_2CH_2), 2.61–2.83 (4H, m, 2× NCH_2CH_3), 4.02 (2H, br, OCH_2CH_2), 6.84 (H, d, J = 16.0 Hz, Ar—CH═CH), 6.91–6.93 (2H, m, Ar—H), 7.22–7.26 (1H, m, Ar—H), 7.44–7.50 (3H, m, Ar—H and Ar—CH═CH), 7.52–7.54 (1H, m, Ar—H), 7.58–7.62 (2H, m, Ar—H), 10.12 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{25}FN_2O_2$ + ([M]+): 356.2; found M + 1:357.2. IR (KBr) cm^{-1}: 3288, 2,981, 1,662, 1,602, 1,583, 1,357, 1,294, 979, 783.

*(E)-3-(3-fluoro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**5c**)*

Grayish white powder, yield: 47.8%, mp: 131.3–131.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.75 (4H, br, piperidine-H), 2.49–2.51 (2H, m, piperidine-H), 2.72–2.97 (6H, m, 3× NCH_2), 4.10 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.84 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.93–6.95 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.50 (3H, m, Ar—H and Ar—CH═CH), 7.52–7.58 (1H, m, Ar—H), 7.61–7.63 (2H, m, Ar—H), 10.13 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{22}H_{25}FN_2O_2$ + ([M]+): 368.2; found M + 1:369.2. IR (KBr) cm^{-1}: 3079, 2,933, 1,664, 1,602, 1,583, 1,357, 1,292, 987, 781.

*(E)-3-(3-fluoro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**5d**)*

Grayish white powder, yield: 59.1%, mp: 123.0–123.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.41–1.54 (4H, m, pyrrolidine-H), 2.50–2.51 (4H, m, pyrrolidine-H), 2.68 (2H, br, OCH_2CH_2), 4.08 (2H, br, OCH_2CH_2), 6.84 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.93–6.96 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.53 (3H, m, Ar—H and Ar—CH=CH), 7.53–7.58 (1H, m, Ar—H), 7.60–7.63 (2H, m, Ar—H), 10.12 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{23}FN_2O_2$ + ([M]+): 354.2 found M + 1:355.2. IR (KBr) cm^{-1}: 3049, 2,931, 1,668, 1,604, 1,583, 1,357, 1,292, 987, 779.

*(E)-3-(4-fluoro phenyl)-N-(4-(2-[dimethylamino] ethoxy) phenyl) acrylamide (**6a**)*

White powder, yield: 49.6%, mp: 161.5–162.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.30 (6H, s, 2× NCH_3), 2.70 (2H, t, J = 4.0 Hz, OCH_2CH_2), 4.10 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.83 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.99–7.01 (2H, m, Ar—H), 7.34–7.39 (2H, m, Ar—H), 7.62 (1H, d, J = 16.0 Hz, Ar—CH=CH), 7.66–7.70 (2H, m, Ar—H), 7.74–7.78 (2H, m, Ar—H), 10.15 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}FN_2O_2$ + ([M]+): 328.2; found M + 1:329.2. IR (KBr) cm^{-1}: 3296, 2,935, 1,660, 1,624, 1,597, 1,334, 1,294, 985, 833.

*(E)-3-(4-fluoro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**6b**)*

Light yellow powder, yield: 58.2%, mp: 155.2–156.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.13–1.27 (6H, m, 2× NCH_2CH_3), 2.97 (2H, br, OCH_2CH_2), 2.75–2.97 (4H, m, 2× NCH_2CH_3), 4.71 (2H, br, OCH_2CH_2), 6.76 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.95–6.97 (2H,m, Ar—H), 7.27–7.31 (2H, m, Ar—H), 7.56 (1H, d, J = 16.0 Hz, Ar—CH=CH), 7.63–7.65 (2H, m, Ar—H), 7.66–7.70 (2H, m, Ar—H), 10.11 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{25}FN_2O_2$ + ([M]+): 356.2; found M + 1:357.2. IR (KBr) cm^{-1}: 3294, 2,935, 1,683, 1,641, 1,600, 1,354, 1,294, 1,168, 829.

*(E)-3-(4-fluoro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**6c**)*

Grayish white powder, yield: 46.4%, mp: 165.3–166.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.39–1.52 (4H, m, piperdine-H), 2.50–2.51 (6H, m, 3× NCH_2), 4.06 (2H, br, OCH_2CH_2), 6.74 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.91–6.93 (2H, m, Ar—H), 7.26–7.31 (2H, m, Ar—H), 7.55 (1H, d, J = 16.0 Hz, Ar—CH=CH), 7.57–7.61 (2H, m, Ar—H), 7.66–7.70 (2H, m, Ar—H), 10.07 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{22}H_{25}FN_2O_2$ + ([M]+): 368.2; found M + 1:369.2. IR (KBr) cm^{-1}: 3321, 2,954, 1,662, 1,625, 1,595, 1,332, 1,293, 983, 825.

*(E)-3-(4-fluoro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**6d**)*

Grayish white powder, yield: 61.2%. mp: 189.7–190.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.24 (4H, br, pyrrolidine-H), 2.50–2.51 (4H, m, pyrrolidine-H), 2.64 (2H, t, J = 4.0 Hz, OCH_2CH_2), 4.02 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.80 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.91–6.93 (2H, m, Ar—H), 7.50–7.53 (2H, m, Ar—H), 7.57 (1H, d, J = 16.0 Hz, Ar—CH=CH), 7.59–7.66 (4H, m, Ar—H), 10.09 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{23}FN_2O_2$ + ([M]+): 354.2; found M + 1:355.2. IR (KBr) cm^{-1}: 3284, 2,945, 1,675, 1,635, 1,600, 1,355, 1,244, 970, 825.

*(E)-3-(2-chloro phenyl)-N-(4-(2-[dimethylamino]ethoxy) phenyl) acrylamide (**7a**)*

White powder, yield: 47.9%. mp: 106.4–106.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.46 (6H, s, 2× NCH_3), 2.67 (2H, br, OCH_2CH_2), 4.05 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.87 (1H, d, J = 16.0 Hz, Ar—CH=CH), 6.92–6.94 (2H, m, Ar—H), 7.42–7.46 (2H, m, Ar—H), 7.55–7.57 (1H, m, Ar—H), 7.60–7.63 (2H, m, Ar—H), 7.75–7.77 (1H, m, Ar—H), 7.85 (1H, d, J = 16.0 Hz, Ar—CH=CH), 10.21 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}ClN_2O_2$ + ([M]+): 344.1; found M + 1:355.1. IR (KBr) cm^{-1}: 3296, 2,926, 1,670, 1,631, 1,606, 1,359, 1,298, 981, 823.

*(E)-3-(2-chloro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**7b**)*

Light yellow powder, yield: 56.6%, mp: 113.7–114.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.91 (6H, br, 2× NCH_2CH_3), 3.04 (4H, br, 2× NCH_2CH_3), 3.28 (2H, br, OCH_2CH_2), 4.25 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.96 (1H, d, J = 16.0 Hz, Ar—CH=CH), 7.04–7.07 (2H, m, Ar—H), 7.50–7.55 (2H, m, Ar—H), 7.62–7.65 (1H, m, Ar—H), 7.72–7.74 (2H, m, Ar—H), 7.83–7.86 (1H, m, Ar—H), 7.93 (1H, d, J = 16.0 Hz, Ar—CH=CH) 10.32 (1H, s, NH). MS (ESI) m/z calculated for $C_{21}H_{25}ClN_2O_2$ + ([M]+): 372.2 found M + 1:373.2. IR (KBr) cm^{-1}: 3298, 2,970, 1,658, 1,622, 1,601, 1,336, 1,294, 966, 819.

*(E)-3-(2-chloro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**7c**)*

White powder, yield: 63.9%. mp:127.2–128.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.38–1.39 (2H, m, piperidine-H), 1.47–1.51 (4H, m, piperidine-H), 2.44–2.51 (6H, m, 3× NCH_2), 4.03 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.90–6.93 (3H, m, Ar—H and Ar—CH=CH), 7.28–7.34 (2H, m, Ar—H), 7.44–7.49 (1H, m, Ar—H), 7.59–7.62 (3H, m, Ar—H and Ar—CH=CH), 7.68–7.72 (1H, m, Ar—H), 10.18 (1H, s, NH). MS (ESI) m/z calculated for $C_{22}H_{25}ClN_2O_2$ + ([M]+): 384.2 found M + 1:385.2. IR (KBr) cm^{-1}: 3294, 2,954, 1,664, 1,627, 1,610, 1,356, 1,305, 981, 825.

*(E)-3-(2-chloro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**7d**)*

Light green powder, yield: 65.2%, mp: 157.7–158.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.84 (4H, br, pyrrolidine-H), 2.92 (4H, br, pyrrolidine-H), 3.23 (2H, br, OCH_2CH_2), 4.18 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.91–6.99 (3H, m, Ar—H and Ar—CH=CH), 7.28–7.34 (2H, m, Ar—H), 7.44–7.50 (1H, m, Ar—H), 7.60–7.66 (3H, m, Ar—H and Ar—CH=CH), 7.69–7.72 (1H, m, Ar—H), 10.21 (1H, s, NH). MS (ESI) m/z calculated for $C_{21}H_{23}ClN_2O_2$ + ([M]+): 370.1; found M + 1:371.1. IR (KBr) cm^{-1}: 3292, 2,968, 1,654, 1,622, 1,600, 1,348, 1,240, 970, 827.

*(E)-3-(3-chloro phenyl)-N-(4-(2-[dimethylamino]ethoxy) phenyl) acrylamide (**8a**)*

White powder, yield: 48.1%. mp: 145.6–146.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.22 (6H, s, 2× NCH_3), 2.61 (2H, t, J = 6.0 Hz, OCH_2CH_2), 4.02 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.84 (H, d, J = 16.0 Hz, Ar—CH=CH), 6.91–6.93 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.50 (3H, m, Ar—H and Ar—CH=CH), 7.52–7.54 (1H, m, Ar—H), 7.58–7.62 (2H, m, Ar—H), 10.27 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}ClN_2O_2$ + ([M]+): 344.1; found M + 1:345.1. IR (KBr) cm^{-1}: 3126, 2,926, 1,670, 1,631, 1,560, 1,359, 1,246, 981, 823.

*(E)-3-(3-chloro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**8b**)*

Light yellow powder, yield: 57.7%, mp: 155.6–156.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (6H, t, J = 8.0 Hz, 2× NCH_2CH_3), 2.61–2.83 (6H, m, 3× NCH_2CH_3), 4.02 (2H, br, OCH_2CH_2), 6.84 (H, d, J = 16.0 Hz,

Ar—CH═CH), 6.91–6.93 (2H, m, Ar—H), 7.22–7.26 (H, m, Ar—H), 7.44–7.50 (3H, m, Ar—H and Ar—CH═CH), 7.52–7.54 (1H, m, Ar—H), 7.58–7.62 (2H, m, Ar—H), 10.12 (1H, s, NH). MS (ESI) m/z calculated for $C_{21}H_{25}ClN_2O_2$ + ([M]+): 372.2 found M + 1:373.2. IR (KBr) cm^{-1}: 3261, 2,929, 1,678, 1,654, 1,598, 1,296, 1,236, 970, 823.

*(E)-3-(3-chloro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**8c**)*

Grayish white powder, yield: 52.8%, mp: 173.2–174.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.75 (4H, br, piperidine-H), 2.49–2.51 (2H, m, piperidine-H), 2.72–2.97 (6H, m, 3× NCH_2), 4.10 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.84 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.93–6.95 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.49 (3H, m, Ar—H and Ar—CH═CH), 7.52–7.58 (1H, m, Ar—H), 7.61–7.63 (2H, m, Ar—H), 10.13 (1H, s, NH). MS (ESI) m/z calculated for $C_{22}H_{25}ClN_2O_2$ + ([M]+): 384.2 found M + 1:385.2. IR (KBr) cm^{-1}: 3284, 2,933, 1,668, 1,622, 1,533, 1,294, 1,236, 970, 823.

*(E)-3-(3-chloro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**8d**)*

Grayish white powder, yield: 54.3%. mp: 166.3–167.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.41–1.54 (4H, m, pyrrolidine-H), 2.50–2.51 (4H, m, pyrrolidine-H), 2.68 (2H, br, OCH_2CH_2), 4.08 (2H, br, OCH_2CH_2), 6.84 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.93–6.95 (2H, m, Ar—H), 7.22–7.27 (1H, m, Ar—H), 7.44–7.51 (3H, m, Ar—H and Ar—CH═CH), 7.53–7.58 (1H, m, Ar—H), 7.60–7.63 (2H, m, Ar—H), 10.12 (1H, s, NH). MS (ESI) m/z calculated for $C_{21}H_{23}ClN_2O_2$ + ([M]+): 370.1; found M + 1:371.1. IR (KBr) cm^{-1}: 3280, 2,824, 1,653, 1,620, 1,598, 1,296, 1,236, 970, 819.

*(E)-3-(4-chloro phenyl)-N-(4-(2-[dimethylamino]ethoxy) phenyl) acrylamide (**9a**)*

White powder, yield: 54.5%, mp: 180.3–181.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.24 (6H, s, 2× NCH_3), 2.64 (2H, t, J = 6.0 Hz, OCH_2CH_2), 4.02 (2H, t, J = 6.0 Hz, OCH_2CH_2), 6.80 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.91–6.93 (2H, m, Ar—H), 7.50–7.57 (3H, m, Ar—CH═CH and Ar—H), 7.59–7.65 (4H, m, Ar—H), 10.09 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{19}H_{21}ClN_2O_2$ + ([M]+): 344.1; found M + 1:345.1. IR (KBr) cm^{-1}: 3271, 2,980, 1,658, 1,622, 1,597, 1,294, 1,231, 981, 821.

*(E)-3-(4-chloro phenyl)-N-(4-(2-[diethylamino] ethoxy) phenyl) acrylamide (**9b**)*

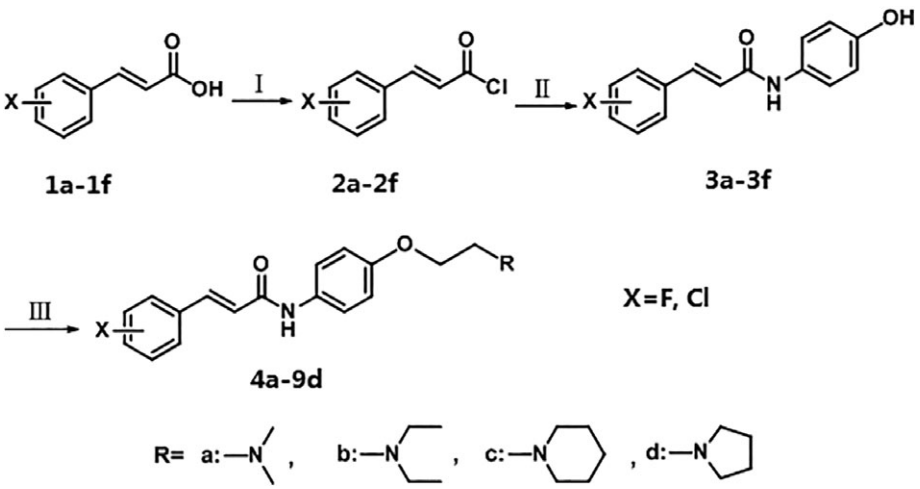
Light yellow powder, yield: 58.4%, mp: 183.8–185.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.32–1.68 (6H, m, J = 4.0 Hz, 2× NCH_2CH_3), 2.58–2.59 (6H, m, 2× NCH_2CH_3 and OCH_2CH_2), 4.23 (2H, br, OCH_2CH_2), 6.89 (1H, d, J = 16.0 Hz, Ar—CH═CH), 7.03–7.04 (2H, m, Ar—H), 7.58–7.65 (3H, Ar—CH═CH and Ar—H), 7.69–7.74 (3H, m, Ar—H), 10.20 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{25}ClN_2O_2$ + ([M]+): 372.2 found M + 1:373.2. IR (KBr) cm^{-1}: 3271, 2,970, 1,658, 1,624, 1,597, 1,294, 1,232, 977, 823.

*(E)-3-(4-chloro phenyl)-N-(4-(2-[piperidin-1-yl] ethoxy) phenyl) acrylamide (**9c**)*

Grayish white powder, yield: 57.6%, mp: 179.7–181.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.74 (4H, br, piperdine-H), 2.50–2.51 (2H, m, piperdine-H), 2.69 (4H, br, piperdine-H), 2.94 (2H, br, OCH_2CH_2), 4.08 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.80 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.92–6.95 (2H, m, Ar—H), 7.50–7.57 (3H, m, Ar—CH═CH and Ar—H), 7.60–7.65 (3H, m, Ar—H), 10.10 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{22}H_{25}ClN_2O_2$ + ([M]+): 384.2 found M + 1:385.2. IR (KBr) cm^{-1}: 3288, 2,933, 1,660, 1,624, 1,597, 1,292, 1,232, 985, 823.

*(E)-3-(4-chloro phenyl)-N-(4-(2-[pyrrolidin-1-yl] ethoxy) phenyl) acrylamide (**9d**)*

Grayish white powder, yield: 55.1%. mp: 184.3–185.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (6H, br, pyrrolidine-H), 2.50–2.51 (2H, m, pyrrolidine-H), 2.68 (2H, t, J = 4.0 Hz, OCH_2CH_2), 4.04 (2H, t, J = 4.0 Hz, OCH_2CH_2), 6.87 (1H, d, J = 16.0 Hz, Ar—CH═CH), 6.92–6.94 (2H, m, Ar—H), 7.43–7.47 (2H, m, Ar—H), 7.55–7.57 (1H, m, Ar—H), 7.61–7.63 (2H, m, Ar—H), 7.75–7.77 (1H, m, Ar—H), 7.85 (1H, d, J = 16.0 Hz, Ar—CH═CH), 10.20 (1H, s, NH). MS (ESI-Q-TOFMS) m/z calculated for $C_{21}H_{23}ClN_2O_2$ + ([M]+): 370.1; found M + 1:371.1. IR (KBr) cm^{-1}: 3292, 2,924, 1,660, 1,622, 1,595, 1,294, 1,232, 987, 825.



SCHEME 1 Reagents and conditions: (I). $(COCl)_2$, DMF, DCM; (II). Aminophenol, TEA, acetonitrile, 60 °C; (III) $Cl(CH_2)_2NR$, K_2CO_3, NaI, acetone reflux

TABLE 1 Effect of compound **3a–3f** on inhibitory activity against AChE and BChE

Compound	X	IC_{50} [a](μmol/L) AChE	IC_{50} [a](μmol/L) BChE	Selectivity for AChE[b]
3a	2-F	>500	>500	–
3b	3-F	>500	>500	–
3c	4-F	>500	>500	–
3d	2-Cl	>500	>500	–
3e	3-Cl	>500	>500	–
3f	4-Cl	>500	>500	–
Rivastigmine[c]	–	10.54 ± 0.86	0.26 ± 0.08	0.02

[a] IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss.
[b] The selectivity is defined as the ratio of IC_{50} of compounds inhibiting BChE/AChE.
[c] Used for positive control for IC_{50}.

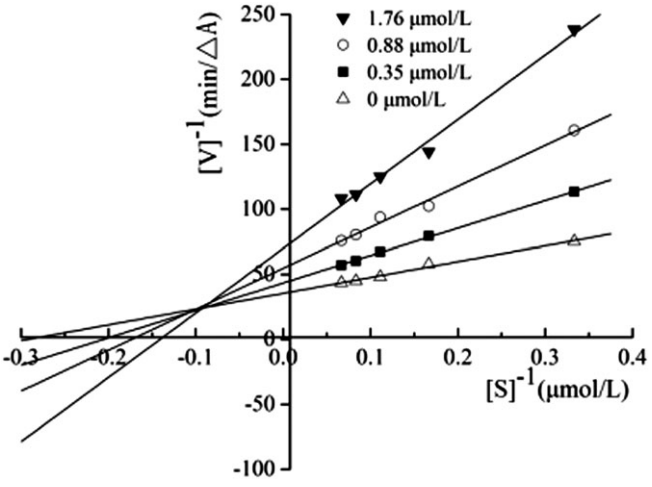


FIGURE 2 Lineweaver–Burk plots for AChE kinetic profile in the presence of compound **6d**

2.4 | AChE and BChE inhibition assay

AChE or BChE activity assays were performed with *Ellman* method (Ellman, Courtney, Andres, & Featherstone, 1961). The brain and serum of Sprague–Dawley rat was used as the resource of AChE and BChE, respectively. Every compound was dissolved in a little Tween 80 and diluted by water to various concentrations immediately before use. The assay mixture consists of 50 μL AChE(BChE), 100 μL S-acetylthiocholine iodide (S-butyrylthiocholine iodide), 2.76 mL Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0, 0.1 mol/L), and 100 μL various concentrations of test compounds, it was incubated at 30 °C for 25 min. Then 100 μL 20% sodium dodecylsulfate (SDS) and 100 μL 10 mmol/L 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was added into the assay mixture to terminate the reaction

TABLE 2 Effect of compound **4a–9d** on inhibitory activity against AChE and BChE

Compound	X	R	IC_{50}[a] (μmol/L) AChE	IC_{50}[a] (μmol/L) BChE	Selective for AChE[b]
4a	2-F	$N(CH_3)_2$	2.80 ± 0.25	39.97 ± 1.82	14.28
4b	2-F	$N(CH_2CH_3)_2$	71.35 ± 4.16	42.58 ± 3.77	0.60
4c	2-F	Piperidine	3.72 ± 0.20	224.65 ± 9.78	60.39
4d	2-F	Pyrrolidine	1.46 ± 0.13	27.69 ± 0.78	18.97
5a	3-F	$N(CH_3)_2$	2.14 ± 0.12	27.85 ± 0.93	13.01
5b	3-F	$N(CH_2CH_3)_2$	26.96 ± 2.89	>500	>18.55
5c	3-F	Piperidine	3.12 ± 0.14	19.33 ± 0.96	6.20
5d	3-F	Pyrrolidine	1.30 ± 0.05	27.21 ± 1.92	20.93
6a	4-F	$N(CH_3)_2$	1.72 ± 0.04	243.03 ± 8.64	141.30
6b	4-F	$N(CH_2CH_3)_2$	131.02 ± 13.33	47.15 ± 1.42	0.36
6c	4-F	Piperidine	1.75 ± 0.23	62.99 ± 2.66	35.99
6d	4-F	Pyrrolidine	1.11 ± 0.08	51.7 ± 3.26	46.58
7a	2-Cl	$N(CH_3)_2$	2.65 ± 0.17	35.37 ± 2.48	13.35
7b	2-Cl	$N(CH_2CH_3)_2$	22.26 ± 1.26	34.71 ± 2.81	1.56
7c	2-Cl	Piperidine	4.25 ± 0.18	98.64 ± 7.45	22.17
7d	2-Cl	Pyrrolidine	1.91 ± 0.09	13.34 ± 1.02	6.98
8a	3-Cl	$N(CH_3)_2$	1.98 ± 0.13	27.56 ± 2.45	13.92
8b	3-Cl	$N(CH_2CH_3)_2$	20.91 ± 1.46	30.12 ± 2.31	1.44
8c	3-Cl	Piperidine	3.44 ± 0.19	76.90 ± 4.37	22.35
8d	3-Cl	Pyrrolidine	1.73 ± 0.13	27.16 ± 2.44	15.70
9a	4-Cl	$N(CH_3)_2$	1.45 ± 0.14	19.22 ± 0.98	13.26
9b	4-Cl	$N(CH_2CH_3)_2$	18.54 ± 2.46	29.41 ± 1.77	1.59
9c	4-Cl	Piperidine	2.52 ± 0.05	44.13 ± 3.12	17.51
9d	4-Cl	Pyrrolidine	1.40 ± 0.11	45.6 ± 3.10	32.57
Rivastigmine[c]	–	–	10.54 ± 0.86	0.26 ± 0.081	0.02

[a] IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss.
[b] The selectivity is defined as the ratio of IC_{50} of compounds inhibiting BChE/AChE.
[c] Used for positive control for IC_{50}.

TABLE 3 Kinetics parameters assay of compound **6d** inhibiting AChE

C (μM)	Michaelis–Menten equation	K_m (mM)	V_{max} ($\Delta Amin^{-1}$)	K_i (μM)	K_i' (μM)
0	1/v = 121.36/[S] + 35.49	3.42	0.028		
0.35	1/v = 210.81/[S] + 43.40	4.86	0.023	0.61	1.86
0.88	1/v = 314.84/[S] + 54.71	5.75	0.018		
1.76	1/v = 495.98/[S] + 69.96	7.09	0.014		

and generate yellow product. The absorbance of each assay mixture was measured at 412 nm by UV spectroscopy. Then the IC_{50} values were calculated by Bliss method and expressed as mean ± *SD*.

2.5 | Kinetic studies

Kinetic study was conducted according to a reported method with some modifications (Skrzypek, Matysiak, Niewiadomy, Bajda, & Szymanski, 2013). The assay solution consists of 100 μL compound **6d**, 100 μL DTNB, 2.76 mL 0.1 mol/L Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0). It was preincubated with AChE at 30 °C, followed by the addition of 100 μL acetylthiocholine iodide with five various concentrations in parallel. Then the absorbance of assay solution was measured spectrometrically at 412 nm, which is proportional to the activity of AChE to catalyze acetylthiocholine iodide. The linear Lineweaver–Burk plot based on Michaelis–Menten equation was used to evaluate the inhibition profile and kinetic parameters. Additionally, the parallel control experiment was conducted in the absence of compound **6d** in the assay solution.

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

The route of synthesis of compounds **4a–9d** is shown in Scheme 1. Firstly, the chloride compounds were prepared using condensation reaction of halogen-substituted cinnamic acids and oxalyl chloride with DMF as the catalyst. Then, cinnamic acid chlorides reacted with *p*-aminophenol to synthesize amide derivatives respectively, which subsequently reacted with four commercially available aminoethyl chlorides (chloroethyldimethylamine hydrochloride, chloroethyldiethylamine hydrochloride, chloroethylpiperidine hydrochloride, and chloroethylpyrrolidine hydrochloride) in the presence of K_2CO_3 and NaI for gaining desired compounds.

Compounds **4a–9d** were gained by Williamson reaction between aminoethyl chlorides and hydroxy cinnamamide. For various tertiary amine substituents, the overall reaction times were arrayed in descending order as: diethylamine > dimethylamine > pyrrolidine > piperidine. However, there is no significant difference in the yield of final products.

New synthetic compounds were characterized by proton nuclear magnetic resonance spectroscopy (H^1NMR), infrared spectrum (IR) and mass spectrometry (MS). The compounds were measured by HPLC, and the purity is over 98.0%. In this study, according to H^1NMR spectrum, the *cis*- or *trans*-conformation of cinnamic acid derivatives can be distinguished. Hydrogen spectrum shows that the ^{3}JHH couplings of *trans*-cinnamic amide are larger than that of *cis*-cinnamic amide. The former is about 16–17 Hz, while the latter is about 12–13 Hz according to previous report (Hanai, Kuwae, Takai, Senda, & Kunimoto, 2001). So cinnamic acids derivatives in the present study can be confirmed as *trans*-conformation (^{3}JHH = 16 Hz). In addition, there are some broad peaks in ^{1}H NMR spectra of some compounds, such as compounds **4b**, **5c**, **6c**. The hydrogen atoms that are adjacent to oxygen or nitrogen atoms with larger electronegativity exhibit broad peaks. It is speculated that proton exchange among these hydrogen atoms leads to the decoupling phenomenon, showing a broad peak in the spectra.

3.2 | Bioactivity evaluation

In this study newly synthesized compounds were evaluated in AChE and BChE inhibition with the results shown in Tables 1 and 2. Cinnamic amides without tertiary amine side chain (compounds **3a–3f**) exhibit poor activity in AChE and BChE inhibition (IC_{50} > 500 μmol/L). By contrast, compounds containing tertiary amine side chain (compounds **4a–9c**) exhibit moderate or potent activity against AChE. All compounds containing dimethylamine, piperidine, diethylamine or pyrrolidine side chain exhibit potent activity in AChE inhibition (IC_{50} < 5.0 μmol/L) except the compounds with diethylamine side chain showing weak bioactivity (IC_{50} > 18.0 μmol/L). In addition, almost all the cinnamic derivatives with *para*-fluoro or chloro substituent are more potent in AChE inhibition than *meta*- or *ortho*-substituted compounds. For those compounds with piperidine or pyrrolidine side chain, fluoro-substituted compounds are more potent in AChE inhibition than chloro-substituted compounds. The results above indicate tertiary side chain may be important in AChE or BChE inhibition. Also, the substitution of fluorine or chlorine in cinnamic acid poses significant influence on the inhibitory effect against AChE and BChE.

Among those newly synthesized compounds, compounds **6d** possess inhibitory potency 9.5 times greater than Rivastigmine (IC_{50} = 10.54 μmol/L) with IC_{50} values of 1.11 μmol/L. Due to the high selectivity for AChE inhibition (Ratio: 46.58), it was selected for further kinetic studies. The analysis result of compounds **6d** in linear Lineweaver–Burk plot is shown in Figure 2. It exhibits a mixed-type inhibitory manner according to the plot. The competitive inhibition constant (K_i) and the noncompetitive constant (K_i') are 0.61 μmol/L and 1.86 μmol/L, respectively (Table 3).

4 | CONCLUSION

In the present study, a series of chlorine or fluorine cinnamic amide derivatives were synthesized with their bioactivity in AChE and BChE inhibition evaluated. The results show the tertiary amine side chain exerts a significant effect on the bioactivity. Moreover, for the compounds containing tertiary amine side chain, the chlorine or fluorine substituted position also produces marked influences on the bioactivity and selectivity in AChE inhibition. Most of the compounds that contain

para-substituted fluorine or chlorine are shown to have potent activity against AChE and poor activity against BChE, while the opposite effect exhibit in the *ortho*-substituted analogs. This result may provide valuable information for future research on new cholinesterase inhibitors.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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