Synthesis, characterization, crystal structures, and the biological evaluation of 2-phenylthiazole derivatives as cholinesterase inhibitors

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Abstract
Four 2-phenylthiazole derivatives are synthesized, characterized, and evaluated as cholinesterase inhibitors. The structures of the 2-phenylthiazole derivatives are confirmed by 1H and 13C nuclear magnetic resonance spectroscopy, single-crystal X-ray diffraction studies, and Hirshfeld surfaces analysis. Hirshfeld surface analysis of the prepared compounds showed C–H···O intermolecular interactions. The cholinesterase inhibition activities of the synthesized compounds are tested by Ellman’s method. [2-(4-Benzylxylophenyl)-thiazol-4-yl]-(3,5-dimethylpiperidin-1-yl)-methanone showed the best acetylcholinesterase inhibition activity with an IC50 value of 8.86 µM and the best butyrylcholinesterase inhibition activity with an IC50 value of 1.03 µM. A docking study demonstrates that the same compound interacts with the catalytic anionic site and peripheral anionic site of acetylcholinesterase and the catalytic anionic site of butyrylcholinesterase.

Keywords
Alzheimer’s disease, cholinesterase, crystal structure, 2-phenylthiazole derivatives

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Introduction
Alzheimer’s disease (AD) is one of the major diseases threatening the health of the elderly in modern society. AD is a chronic progressive neurodegenerative disease that worsens over time.1 The common early symptoms are forgetfulness and cognitive dysfunction. As the disease progresses, it can lead to serious language problems, disorientation, loss of self-management, and even gradual loss of physical functions, eventually leading to death.2 The incidence of AD is progressively increasing across the world due to an aging population.3 Therefore, it is of great significance to develop effective anti-AD drugs.

Five drugs are currently used to treat AD cognitive dysfunction, four of which are acetylcholinesterase inhibitors (AChEIs): tacrine,4 donepezil,5,6 galantamine,7 and rivastigmine.8 Acetylcholinesterase (ACHE) is considered as the main factor responsible for acetylcholine (ACh) metabolism, and AChEIs can effectively prevent the hydrolysis of ACh and provide a promising therapeutic effect.9 In recent decades, the role of butyrylcholinesterase (BuChE) in the development of AD has been clarified. Evidence indicates that BuChE is related to some important AD hallmarks such as the extracellular deposition of the amyloid β-protein(Aβ) and the aggregation of hyperphosphorylated tau protein.10

The activity of AChE is decreased during the course of AD progression, while the activity of BuChE is stable or even increased, which is a compensatory feedback of ACh metabolism and cannot be overcome clinically at present.11 Consequently, the simultaneous inhibition of both enzymes may be a more attractive therapeutic strategy in AD.

Thiazole containing compounds have a variety of biological activities such as antioxidant, anti-inflammatory, antitumor, antiviral, and cholinesterase inhibition activity.12
Thiazole and its derivatives have attracted continuing interest for the design of various novel central nervous system (CNS) active agents. In the past few decades, thiazoles have been widely used to develop a variety of therapeutic agents against numerous CNS targets. Many thiazole derivatives have been reported to possess cholinesterase inhibitory activities. For instance, Rahim et al. studied the potential inhibitory activity of 30 thiazole analogues against AChE and BuChE, among which (E)-4-([2-[4-(4-chlorophenyl)thiazol-2-yl]hydrazono]methyl)benzene-1,2-diol (compound A) possessed the best AChE-inhibition activity (IC_{50} = 21.3 µM) and the best BuChE-inhibition activity (IC_{50} = 1.59 µM). Sun et al. reported that 2-(4-methoxyphenyl)-N-(4-phenylthiazol-2-yl)acetamide (compound B) showed the best AChE inhibitory activity with an IC_{50} value of 3.14 µM. Thiazole containing compounds can be used as lead compounds to develop new cholinesterase inhibitors (Figure 1).

Previously, several studies with thiazole-derived AChEIs have demonstrated that the thiazole ring primarily interacts with peripheral anionic site (PAS) of AChE and the amine functional moiety interacts with catalytic anionic site (CAS) of AChE. To find novel dual binding site inhibitors of AChE, we attempted to connect the 2-phenylthiazole moiety (binding to PAS) with amine functional moieties (binding to CAS) to design a series of new 2-phenylthiazole derivatives as dual binding site inhibitors of AChE (Figure 2).

In this study, four novel 2-phenylthiazole derivatives have been synthesized and characterized. The intermolecular interactions of these compounds were analyzed through single-crystal X-ray structure studies and Hirshfeld surface analysis. Furthermore, these derivatives were subjected to biological evaluation as potential inhibitors of AChE and BuChE. Finally, molecular docking studies of the most potent inhibitors were carried out to determine the binding interactions with AChE and BuChE, respectively.

**Results and discussion**

**Spectral characterization**

The synthetic route to compounds 6a–d is presented in Scheme 1. Condensation of 4-hydroxythiobenzamide (1) and ethyl bromopyruvate (2) in refluxing EtOH for 4 h gave ethyl 2-(4-hydroxyphenyl)thiazole-4-carboxylate (3).

Compound 3 was O-alkylated by treatment with benzyl bromide in dimethylformamide (DMF) at 80°C to afford compound 4. The desired products were synthesized via saponification, acyl chlorination, and acylamination with compound 4 as the starting material. Crude products 6a–d were purified by silica gel column chromatography. The purities of the obtained compounds were checked by thin-layer chromatography (TLC). All the results substantiate the proposed structures.

The FTIR spectra of the prepared compounds are characterized by a strong broad absorption in the range of 1603–1629 cm^{-1}, which can be assigned to the \( \nu (C=O) \) stretches. The absorption bands falling in the range of 2810–2971 cm^{-1} correspond to \( \nu (C–H) \) of the alkane chain in compounds 6a–d. The \( \nu (C–H) \) mode appears in the range of 3079–3088 cm^{-1}, suggesting the presence of aromatic benzene rings in the four products. The \( ^1H \) NMR spectra of the prepared compounds displayed protons due to 1,4-disubstituted benzene ring were observed as two doublets at 7.83, 7.80, 7.81, and 7.88 ppm, respectively, as singlets. In the \( ^13C \) NMR spectra, the characteristic phenyl signals were observed as a doublet at 8.73, 8.01, 7.80, and 7.75 ppm, respectively.
observed at 115.3–160.7 ppm for compounds 6a–d. The signals of the carbonyl groups were observed at 162.7, 161.2, 162.9, and 162.6 ppm for compounds 6a–d, respectively. The high-resolution mass spectrometry (HRMS) spectra of compounds 6a–d show [M + H]^+ peaks at m/z: 394.1620, 424.1727, 451.1726, and 407.1827 corresponding to C_{22}H_{24}N_{3}O_{2}S [M + H]^+, C_{23}H_{26}N_{3}O_{3}S [M + H]^+, C_{25}H_{27}N_{2}O_{4}S [M + H]^+, and C_{24}H_{28}N_{2}O_{2}S [M + H]^+.

Crystal structure of compounds 6a and 6d

The structures of compounds 6a and 6d were confirmed by X-ray diffraction analysis. The crystal data are presented in Table 1 in the ESI and Figures 3–6. Compound 6a had a monoclinic crystal system and a P2_1/n space group. Compound 6d was triclinic with a P-1 space group. The selected bond lengths and angles of compounds 6a and 6d are listed in Table 2 in the Supplemental material. Compounds 6a and 6d possessed similar bond lengths with S1–C14 (1.737 and 1.724 Å), S1–C16 (1.701 and 1.681 Å), and N1–C14 (1.310 and 1.300 Å). The O1–C8 bond lengths for compounds 6a and 6d were 1.364 and 1.362 Å, respectively. The bond lengths of N2–C17 for compounds 6a and 6d were 1.342 and 1.312 Å. The packing diagrams of compounds 6a and 6d viewed along the a-axis are shown in Figures 4 and 6. The crystal packing diagrams of the compounds showed that there were no hydrogen bonds present in the two compounds.

Hirshfeld surface analysis

Hirshfeld surfaces and fingerprint plots of compounds 6a and 6d were generated and analyzed in order to understand the different intermolecular interactions and packing modes. The d_{norm} was mapped on the Hirshfeld surface and selected two-dimensional (2D) fingerprint plots of the different interactions in the two compounds are shown in

Scheme 1. Synthesis of 2-phenylthiazole derivatives 6a–d.
Figure 7. For both compounds 6a and 6d, the red regions mainly indicate O···H interactions. The d$_{norm}$ maps of compounds 6a and 6d both show one pair of deep-red regions which demonstrate strong C–H···O intermolecular interactions between the thiazole group and the amide group.

The 2D fingerprint plots of the main intermolecular contacts for compounds 6a and 6d are depicted in Figures 8 and 9. H···H intermolecular contacts with contributions of 52.4% and 55.9% of the overall contacts for compounds 6a and 6d, respectively, are the major contributors to the Hirshfeld surface. The O···H intermolecular contacts are strong contacts due to the presence of C–H···O intermolecular interactions, contributing to the Hirshfeld surface by 10.2% and 9.2% of the overall contacts for compounds 6a and 6d, respectively. Besides the contacts mentioned above, the presence of C···H, N···H, S···H, and other interactions is summarized in Table 3 in the Supplemental material.
In vitro inhibition studies of cholinesterase

The target compounds 6a–d were studied for their potential as AChE (from electric eel) and BuChE (from equine serum) inhibitors using the method described by Ellman et al. In this test, tacrine and donepezil were used as reference drugs and the results of the AChE and BuChE inhibition are presented in Table 1. Among the four derivatives, compound 6d possessed the best AChEI-inhibitory activity with an IC_{50} value of 8.86 µM and the best BuChE-inhibitory activity with an IC_{50} value of 1.03 µM. A novel series of thiazole acetamide derivatives was reported to possess cholinesterase inhibitory activity. Rahim et al. studied the potential inhibitory activity of 30 thiazole analogues against AChE and BuChE, among which, (E)-4-[(2-[4-(4-chlorophenyl)thiazol-2-yl]hydrazono) methyl]-benzene-1,2-diol (compound A) possessed the best AChE-inhibition activity (IC_{50} = 21.3 µM) and best BuChE-inhibition activity (IC_{50} = 1.59 µM). 2-(4-Methoxyphenyl)-N-(4-phenylthiazol-2-yl)acetamide (compound B) showed the best AChE inhibitory activity with an IC_{50} value of 3.14 µM. Compound 6d (synthesized in this study) showed comparable inhibitory activity toward both enzymes as those of these thiazole derivatives.

Compound 6d possessed the best BuChE-inhibition activity and inhibited BuChE in a dose-dependent manner (Figure 10). Compounds 6b and 6c inhibited BuChE less than 50% at a concentration of 100 µM, implying that the amino substituent on the thiazole derivative played a key role in the cholinesterase inhibition activities of those compounds. Compound 6b and 6c showed the good selectivity of AChE with weak BuChE-inhibitory activities at the concentration of 100 µM. Compound 6d showed poor selectivity of AChE with similar IC_{50} values for AChE and BuChE.

Molecular modeling studies

To understand the binding mode of compound 6d with AChE and BuChE, it was docked with AChE (PDB code No. 2CMF) and BuChE (PDB code No. 1P0I) using the docking software AUTODOCK4.2 (University of California, San Francisco). The molecular docking of compound 6d with AChE showed that it could interact with CAS and PAS of AChE (Figure 11). The nitrogen atom of the thiazole group could interact with TYR121 at the PAS site via hydrogen bonds. The benzene ring of the
benzyl group can interact with PHE330 and HIS440 (a residue of the catalytic triad in the active site of AChE) via arene–arene interactions. The other benzene ring can interact with PHE288 of the enzyme via an arene–arene interaction. As shown in Figure 12, compound 6d can interact with the CAS of BuChE. The oxygen atom of the amide group could interact with GLY116 and GLY117 at the CAS site via the hydrogen bonds. The benzene ring on the thiazole group can interact with PHE329 via arene–arene interactions.

**Conclusion**

In this study, four novel 2-phenylthiazole derivatives were designed, synthesized, and evaluated as cholinesterase inhibitors for the treatment of AD. The structures of the four compounds were determined by IR, NMR, HRMS, and X-ray crystallography. Hirshfeld surface analysis and associated fingerprint plots showed that H···H intermolecular contacts are the major contributor to the Hirshfeld surface. The O···H intermolecular contacts were one of the strong contacts due to the presence of C–H···O intermolecular interactions in compounds 6a and 6d. All of the four compounds could inhibit AChE and BuChE. Among them, compound 6d possessed the best inhibitory activities toward AChE and BuChE, and may prove to be a potential lead candidate for the treatment of AD.

**Experimental**

**Materials and methods**

All synthetic reagents were purchased from Aladdin Industrial Corporation. All reagents were AR grade and were used without further purification. AChE from electric eel, BuChE from equine serum, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and butyrylcholine iodide (BTCl) were purchased from Sigma-Aldrich. TLC was performed on glass-backed silica gel sheets (silica gel 60 GF254) and spots were visualized under UV light (254 nm). Melting points were recorded using a Kohler melting point apparatus. ¹H NMR and ¹³C NMR spectra were recorded at 500 MHz using a Bruker Avance III spectrometer. The X-ray single-crystal diffraction data were recorded on a Bruker SMART APEX-II CCD diffractometer. HRMS were performed using an Agilent Technologies 6230 TOF LC/MS instrument.

**Synthesis of compounds 6a–d**

The target compounds 6a–d were synthesized according to Scheme 1. A mixture of para-hydroxythiobenzamide (1) (3.93 g, 25.67 mmol) and ethyl bromopyruvate (2) (5.00 g, 25.64 mmol) in ethanol (50 mL) was refluxed for 4 h. After cooling, distilled water (100 mL) was added. The precipitated solid was filtered and washed with distilled water (50 mL) and dried under reduced pressure to afford ethyl 2-(4-hydroxyphenyl) thiazole-4-carboxylate (3) as a white solid.

Yield: 90%. m.p. 78–80 °C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.11 (s, 1H), 8.44 (s, 1H), 7.80 (m, 2H), 6.90 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H).

¹³C NMR (126 MHz, DMSO-d₆): δ 168.0, 160.7, 159.9, 146.5, 128.1, 127.7, 123.6, 115.9, 60.6, 14.1.

A mixture of compound 3 (1.00 g, 4.02 mmol) and benzyl bromide (8.04 mmol), K₂CO₃ (1.11 g, 8.04 mmol), and DMF (8 mL) was heated at 80 °C for 8 h, after which the reaction mixture was cooled and distilled water (100 mL) was added. The precipitated solid was filtered and washed with distilled water (50 mL) and dried under reduced pressure to afford ethyl 2-(4-benzyloxyphenyl) thiazole-4-carboxylate (4) as a white solid.

Yield: 91%. m.p. 111–113 °C. ¹H NMR (500 MHz, CDCl₃): δ 8.09 (s, 1H), 8.01–7.90 (m, 2H), 7.50–7.29 (m, 5H), 7.08–6.98 (m, 2H), 5.12 (s, 2H), 4.44 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 168.7, 161.6, 160.8, 147.9, 136.4, 128.5, 128.7, 128.2, 127.5, 126.3, 126.0, 115.2, 70.2, 61.5, 14.4.

Potassium hydroxide (KOH, 0.83 g) was added to a solution of compound 4 (1 g) in anhydrous methanol (15 mL) and the mixture refluxed at 80 °C for 9 h. The solution was then neutralized to pH 5 and poured into cold water (100 mL). The precipitated solid was filtered and washed with distilled water (50 mL) and dried under reduced pressure to afford ethyl 2-(4-benzyloxyphenyl) thiazole-4-carboxylate (5) as a white solid.

Yield: 91%. m.p. 111–113 °C. ¹H NMR (500 MHz, CDCl₃): δ 8.09 (s, 1H), 8.01–7.90 (m, 2H), 7.50–7.29 (m, 5H), 7.08–6.98 (m, 2H), 5.12 (s, 2H), 4.44 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 168.7, 161.6, 160.8, 147.9, 136.4, 128.5, 128.7, 128.2, 127.5, 126.3, 126.0, 115.2, 70.2, 61.5, 14.4.

Potassium hydroxide (KOH, 0.83 g) was added to a solution of compound 4 (1 g) in anhydrous methanol (15 mL) and the mixture refluxed at 80 °C for 9 h. The solution was then neutralized to pH 5 and poured into cold water (100 mL). The resulting precipitate was filtered and dried to give ethyl 2-(4-benzyloxyphenyl)-thiazole-4-carboxylate (5) as a white solid.

Yield: 89%. m.p. 184–186 °C. ¹H NMR (500 MHz, dimethyl sulfoxide (DMSO-d₆)): δ 8.41 (s, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 8.9 Hz, 2H), 5.19
$^{13}\text{C}$ NMR (126 MHz, DMSO-$d_6$): $\delta$ 167.7, 162.6, 160.8, 148.4, 137.1, 129.0, 128.5, 128.4, 128.3, 128.2, 126.0, 116.0, 69.94. HRMS (electrospray ionization (ESI)): $m/z$ [M–H]$^–$ calcd for C$_{17}$H$_{12}$NO$_3$S: 310.0543; found: 310.0533.

Thionyl chloride (0.45 mL) was added to a solution of compound 5 (0.4 g) in dichloromethane (10 mL) and the mixture was refluxed at 50 °C for 10 h. When the reaction was complete, the solvent was evaporated under reduced pressure. The residual mass was dissolved in dichloromethane (10 mL) and the corresponding amine (0.23 mL) was added. The solution was refluxed for 6 h. When the reaction was finished, the solvent was evaporated under reduced pressure. The residue was purified using column chromatography (CH$_2$Cl$_2$/CH$_3$OH = 20:1) to give 2-phenylthiazole derivatives 6a–d. Single crystals of compounds 6a and 6d were grown at room temperature from methanol and dichloromethane, respectively.

Figure 8. The 2D fingerprint plots of compound 6a.

(s, 2H). $^{13}\text{C}$ NMR (126 MHz, DMSO-$d_6$): $\delta$ 167.7, 162.6, 160.8, 148.4, 137.1, 129.0, 128.5, 128.4, 128.3, 128.2, 126.0, 116.0, 69.94. HRMS (electrospray ionization (ESI)): $m/z$ [M–H]$^–$ calcd for C$_{17}$H$_{12}$NO$_3$S: 310.0543; found: 310.0533.

Thionyl chloride (0.45 mL) was added to a solution of compound 5 (0.4 g) in dichloromethane (10 mL) and the mixture was refluxed at 50 °C for 10 h. When the reaction was complete, the solvent was evaporated under reduced pressure. The residual mass was dissolved in dichloromethane (10 mL) and the corresponding amine (0.23 mL) was added. The solution was refluxed for 6 h. When the reaction was finished, the solvent was evaporated under reduced pressure. The residue was purified using column chromatography (CH$_2$Cl$_2$/CH$_3$OH = 20:1) to give 2-phenylthiazole derivatives 6a–d. Single crystals of compounds 6a and 6d were grown at room temperature from methanol and dichloromethane, respectively.
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\[ 2-(4-Benzyloxy-phenyl)-thiazol-4-yl)-(4-methyl-piperazin-1-yl)-methanone (6a): \]
Yield: 84\%, white solid. m.p. 109–111 °C. IR (KBr): 3079, 1614, 1479, 1249, 1235, 1177, 1011, 1003 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 7.88 (d, \( J = 8.9 \) Hz, 2H), 7.83 (s, 1H), 7.44 (d, \( J = 7.0 \) Hz, 2H), 7.40 (t, \( J = 7.3 \) Hz, 2H), 7.35 (t, \( J = 7.2 \) Hz, 1H), 7.04 (d, \( J = 8.9 \) Hz, 2H), 5.13 (s, 2H), 4.02 (s, 2H), 3.84 (s, 2H), 2.51 (s, 4H), 2.35 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 167.1, 162.7, 160.6, 151.1, 136.4, 128.7, 128.2, 128.1, 127.5, 126.3, 123.5, 115.3, 70.2, 46.1. HRMS (ESI): \( m/z \) [M + H]+ calcd for C₂₂H₂₄N₃O₂S: 394.1584; found: 394.1620.

\[ 2-(4-Benzyloxy-phenyl)-thiazol-4-yl)-(4-methyl-piperazin-1-yl)-methanone (6a): \]

![Figure 9. The 2D fingerprint plots of compound 6d.](image)

**Table 1.** In vitro inhibition of AChE and BuChE and the selectivity indices of compounds 6a–d.

| Test compound | IC₅₀ (µM) | SI⁺ (BuChE/AChE) |
|---------------|----------|-----------------|
| AChE⁺         | BuChE⁺   |
| 6a            | 104.03 ± 0.11 | 71.04 ± 0.16 | 0.68 |
| 6b            | 39.83 ± 0.11  | –              | –   |
| 6c            | 66.74 ± 0.08  | –              | –   |
| 6d            | 8.86 ± 0.08   | 1.03 ± 0.04    | 0.116 |
| Donepezil     | 0.041 ± 0.10  | 13.10 ± 0.09   | 318.73 |
| Tacrine       | 0.18 ± 0.02   | 0.05 ± 0.01    | 0.28 |

¹IC₅₀: 50% inhibitory concentration (mean ± standard deviation (SD) of three experiments) of AChE from electric eel.
²IC₅₀: 50% inhibitory concentration (mean ± SD of three experiments) of BuChE from equine serum.
³Selectivity index for AChE; IC₅₀ BuChE/IC₅₀ AChE

\[ 2-(4-Benzyloxy-phenyl)-thiazol-4-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (6b): \]
Yield: 67%, white solid. m.p. 127–129 °C. IR (KBr): 3362, 3127, 2971, 2952, 2885, 2845, 2810, 1669, 1603, 1544, 1486, 1458, 1413, 1249, 1173, 1115, 990 cm⁻¹. ¹H NMR (500 MHz, CDCl₃):
δ 8.01 (s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 7.1 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 5.14 (s, 2H), 3.83–3.74 (m, 4H), 3.59 (q, J = 6.1 Hz, 2H), 2.63 (t, J = 6.3 Hz, 2H), 2.55 (s, 4H). 13C NMR (126 MHz, CDCl3): δ 167.9, 161.2, 160.7, 150.7, 136.4, 128.7, 128.2, 128.1, 127.5, 126.1, 121.9, 115.3, 70.2, 67.1, 57.1, 53.3, 35.8. HRMS (ESI): m/z [M + H]+ calcd for C23H25N3O3S: 424.1689; found: 424.1727.

1-[2-(4-Benzyloxy-phenyl)-thiazol-4-carbonyl]-piperidine-4-carboxylic acid ethyl ester (6c): Yield: 54%, white solid. m.p. 106–108 °C. IR (KBr): 2927, 2860, 1728, 1626, 1607, 1518, 1462, 1251, 1175, 1040, 986 cm⁻¹. 1H NMR (500 MHz, CDCl3): δ 7.88 (d, J = 8.9 Hz, 2H), 7.80 (s, 1H), 7.45 (d, J = 7.0 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.03 (d, J = 8.9 Hz, 2H), 5.13 (s, 2H), 4.54 (d, J = 13.3 Hz, 2H), 4.17 (q, J = 7.1 Hz, 2H), 3.38–3.24 (m, 1H), 3.12–2.99 (m, 1H), 2.64–2.59 (m, 1H), 2.12–1.93 (m, 2H), 1.92–1.77 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H). 13C NMR (126 MHz, CDCl3): δ 174.3, 167.0, 162.9, 160.6, 151.2, 136.4, 128.7, 128.2, 128.1, 127.5, 126.3, 123.1, 115.3, 70.2,

Figure 10. Dose-dependent inhibition of 6d against BuChE. Values are means ± SD and n = 5.

Figure 11. A view of the potential interactions between compound 6d and the binding site of AChE (PDB No. 2CFM).

Figure 12. A view of the potential interactions between compound 6d and the binding site of BuChE (PDB No. 1P0I).
60.6, 41.2, 14.2. HRMS (ESI): m/z [M + H]^+ calcld for C_{24}H_{27}N_{2}O_{4}S: 407.1788; found: 407.1827.

Single-crystal XRD studies

Crystals of compounds 6a and 6d were grown by slow evaporation from methanol and dichloromethane at room temperature, respectively. Diffraction intensities for the compounds were collected at 296(2) K using a Bruker SMART APEX-II CCD area-detector with MoKα radiation (λ = 0.71073 Å). The collected data were reduced with SADABS program for empirical absorption correction of area detector and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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