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PREPARATION AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITIES OF PYRIDINE-BASED 1,3,4-OXADIAZOLE DERIVATIVES

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Abstract – Fourteen pyridine-based 1,3,4-oxadiazole derivatives were synthesized from pyridine-2-carboxaldehyde via iodine-mediated oxidative cyclisation with substituted hydrazide by using the impregnation method. Their structures were confirmed by melting point, ^{1}H NMR, ^{13}C NMR and HRMS. Preliminary bioassay of these derivatives' activities inhibiting acetylcholinesterase (AChE) was also evaluated *in vitro* at the concentration of 1 μmol/mL. The result showed that compounds **4c**, **4j** and **4k** had moderate inhibitory activities with 52%, 59% and 59%, respectively. The preliminary structure-activity relationships revealed that the introduction of pyridine ring could enhance the activity. Molecular docking study demonstrated that compound **4k** possessed an optimal docking pose with interactions at the middle of the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterised by the destruction of nerve cells, the rapid deterioration of memory and other important cognitive functions, which not only harms the health of patients, but also brings a heavy burden to their families and society.[1,2] AChE, enzymes that terminate cholinergic neurotransmission in the brain, act by catalysing the hydrolysis of ACh, so their inhibition can be used to alleviate memory and cognitive deficits in AD.[3,4] Therefore, the development of highly effective AChE inhibitors has become one of the hot spots in the treatment of AD. Pyridine alkaloid derivatives are biologically very active and versatile in the pharmaceutical industry. They are having antiviral, anticancer, antialzheimer, antichagasic, antioxidant, antibacterial, antidote

activities.[5] The study found that some derivatives of the pyridinium type have potent AChE inhibitory activity.[6] 1,3,4-Oxadiazoles are an important class of N-heterocyclic compounds with a wide range of biological activities such as anticancer, antimicrobial, antiviral, analgesic etc.[7] In light of the above-mentioned interesting results, and in continuation of our program aimed at the discovery and development of novel AChE inhibitor,[8] herein we connected the two active moieties in one, designed with multiple active fragment of derivatives, successfully prepare a series of pyridine-based 1,3,4-oxadiazole derivatives by the iodine-mediated oxidative cyclisation, and determined their anti-AChE activity by the method of Ellman with slight modifications.

RESULTS AND DISCUSSION

As show in **Figure 1**, according to the method reported in some literatures,[9-11] in the presence of HOAc, pyridine-2-carboxaldehyde was first condensed with each different arylhydrazide to give the intermediates (**3a-n**). After the first reaction was complete, the solvent was evaporated under reduced pressure, and the products were not purified, redissolved directly in DMSO, followed by reacted with I_2 to obtain the the target compounds containing 1,3,4-oxadiazole (**4a-n**). All compounds were characterized for structural characterized by mp, ^{1}H NMR, ^{13}C NMR and HRMS.

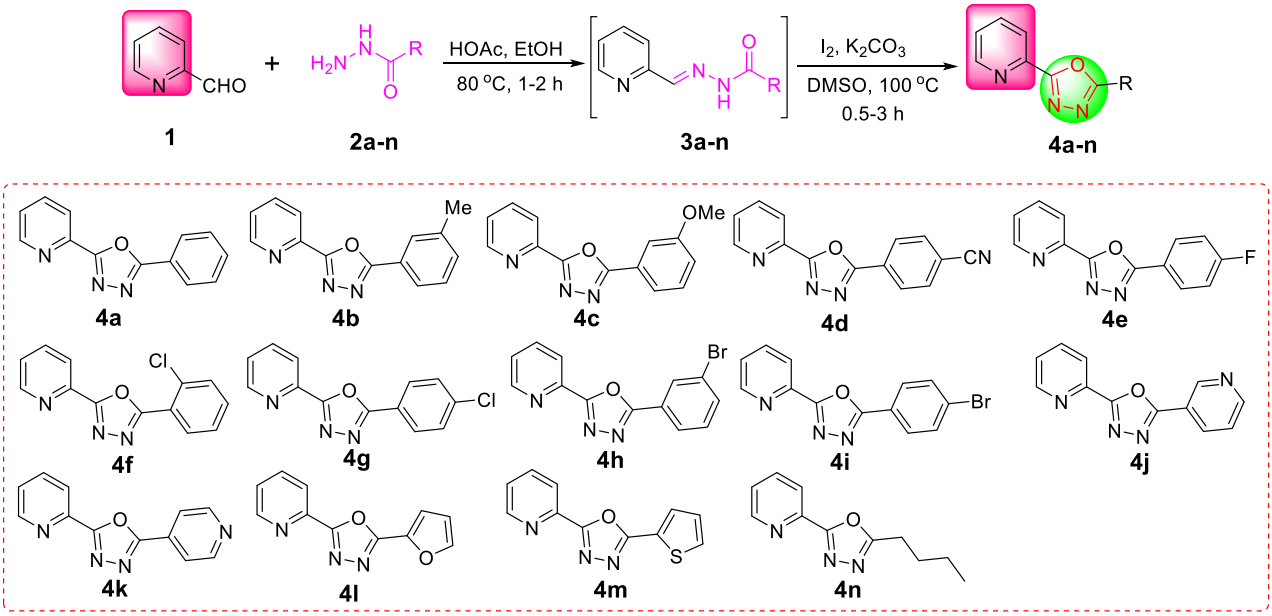

Figure 1. Synthetic route and chemical structure of pyridine-based 1,3,4-oxadiazole derivatives (**4a-n**)

Preliminary bioassay of these derivatives' activities inhibiting AChE was evaluated *in vitro* at the concentration of 1 μmol/mL, and the result was shown in **Figure 2**. Among all compounds, **4c**, **4j** and **4k** showed moderate inhibitory activities against AChE with 52%, 59% and 59%, respectively. Additionally, analysis of the structure-activity relationship shows that introduction of an electron-donating group on the

phenyl ring of **4a** generally increased the inhibition rate (**4a** *vs* **4b**, **4c**). On the contrary, introduction of electron-withdrawing group had little influence on inhibition rates (e.g., **4d**). Obviously, once heterocyclic moiety was introduced into pyridine-based 1,3,4-oxadiazoles, the corresponding compounds **4j-m** all showed the potent inhibitory activities with 59%, 59%, 38% and 46%, respectively.

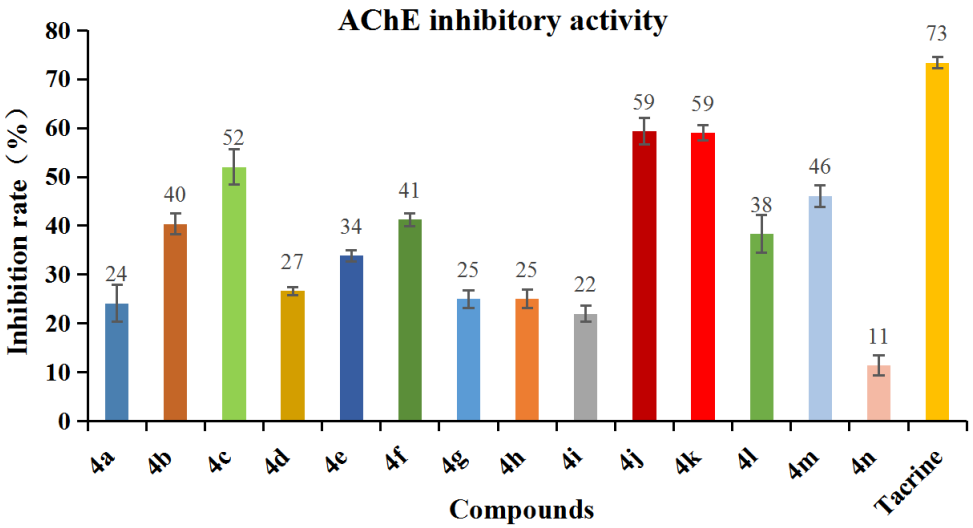

Figure 2. The AChE inhibitory activities of titled compounds (**4a-n**) at 1 μmol/mL; Values represent means ± SD; Tacrine was control and tested in 1 $\times 10^{-3}$ μmol/mL.

In order to explore the relationship between inhibitory activities of potent compounds and concentration, the inhibitory activity of compounds **4c**, **4j** and **4k** was further evaluated at concentrations of 1 μmol/mL, 0.1 μmol/mL and 0.01 μmol/mL, respectively. As shown in **Figure 3**, the inhibitory activity of the compounds gradually weaken as the concentration decreased. It demonstrated that the inhibitory activity was positively correlated with the concentration of the compounds.

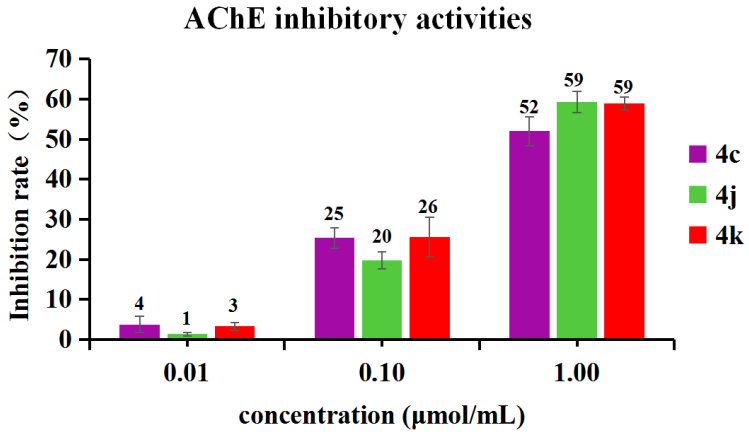

Figure 3. The AChE inhibitory activities of compounds **4c**, **4j**, **4k** in three concentrations; Values represent means ± SD.

Molecular docking studies were performed to investigate the possible binding mode of the most active compounds of pyridine-based 1,3,4-oxadiazole derivatives in AChE active site. The 3D structure of human AChE was selected (PDB code: 3LII) for docking studies.[12] Literature reported that AChE had a catalytic active site which catalyzed the hydrolysis of acetylcholine into acetic acid and choline to stop the excitatory effect of neurotransmitters on the post-synaptic membrane and ensure the normal transmission of biological signals in the body.[13] As shown in **Figure 4**, compound **4k** exhibited four binding interactions with AChE. The two nitrogen atoms of 1,3,4-oxadiazole core interacts with Tyr124 and Tyr337 residues, located in the middle of the catalytic active channel of AChE, via two hydrogen bonds. Also, the pyridine ring of compound **4k** maked π-π interaction with Trp286 to enhance the combination of compound **4k** with the catalytic active site of AChE. In this way, the active channel of AChE was blocked, and acetylcholine could not enter the catalytic active site (CAS) of AChE for hydrolysis, thus showing that the activity of AChE was inhibited.

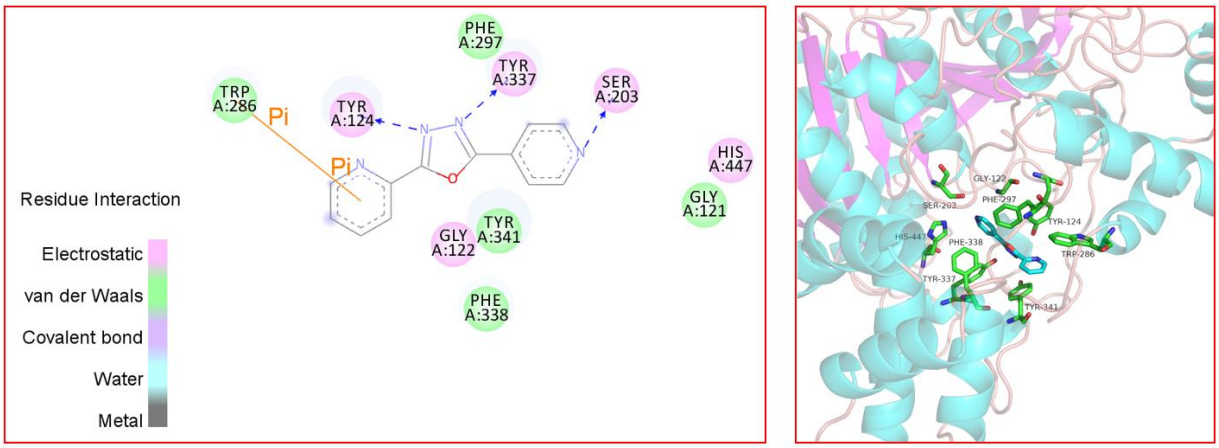

Figure 4. Docking pose of compound **4k** inside AChE. (**a:** The 2D structure of the compound binding model with AChE, the blue dotted lines show the hydrogen bonds, the full lines show the π-π interactions. **b**: The 3D structure of the compound binding model with AChE).

In conclusion, a series of pyridine-based 1,3,4-oxadiazole derivatives were designed, synthesised, and evaluated for their AChE inhibitory activities *in vitro*. Compouds **4c**, **4j** and **4k** demonstrated moderate inhibitory activities and the inhibitory activity was positively correlated with the concentration. SAR analysis revealed that the introduction of pyridine ring could enhance the activity. Molecular docking study revealed that compound **4k** possessed an optimal docking pose with interactions at the middle of the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE.

EXPERIMENTAL

General

All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., China). Melting points (mp) were determined on a XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance 400 MHz instrument, and tetramethylsilane (TMS) was used as the internal standard. High-resoiution mass spectrometry (HRMS) was carried out with a Thermo DSQ GC/MS instrument.

Syntheses

General procedure for the preparation of intermediates (3a-n)

A mixture of pyridine-2-carboxaldehyde (**1**, 1.0 mmol, 107.11 mg), the corresponding hydrazides (1.0 mmol) and two drops of HOAc in absolute EtOH (10 mL) was refluxed until the reaction was complete according to TLC analysis (1-2 h). Then the solvent was removed under reduced pressure to give the intermediates (**3a-n**). The product was not purified and went straight to the next step.

General procedure for the preparation of 1,3,4-oxadiazole derivatives (4a-n)

The intermediates (**3a-n**) were redissolved in DMSO (5 mL), followed by addition of K_2CO_3 (3.0 mmol, 414.6 mg) and I_2 (1.1 mmol, 279.4 mg) in sequence. The reaction mixture was stirred at 100 ^{o}C until the reaction was complete according to TLC analysis (0.5-3 h). After being cooled to room temperature, the mixture was treated with saturated $Na_2S_2O_3$ (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layer was washed with brine (3×20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated. The given residue was purified by PTLC to give the target products **4a-n** in 42-91% yield. All compounds were characterized by ^{1}H NMR (400 MHz), HRMS and mp.

2-Phenyl-5-(pyridin-2-yl)-1,3,4-oxadiazole (4a): Yield: 50%, white solid, mp 127-129 ^{o}C (124-125 ^{o}C, lit.[14]); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.34 (dt, J = 8.0, 1.2 Hz, 1H), 8.24 (dd, J = 8.0, 1.6 Hz, 2H), 7.93 (td, J = 7.8, 1.6 Hz, 1H), 7.51-7.59 (m, 3H), 7.47-7.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.6, 163.8, 150.3, 143.6, 137.2, 132.0, 129.0, 127.3, 125.8, 123.6, 123.2; HRMS (ESI) calcd for $C_{13}H_{10}N_3O_2^+$ $[M+H]^+$: 224.0824, found 224.0825.

2-(Pyridin-2-yl)-5-(*m*-tolyl)-1,3,4-oxadiazole (4b): Yield: 62%, white solid, mp 98-100 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.82-8.84 (m, 1H), 8.34 (dt, J = 8.0, 1.2 Hz, 1H), 8.05 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.93 (td, J = 8.0, 1.6 Hz, 1H), 7.48-7.50 (m, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.7, 163.7, 150.3, 143.7, 138.9, 137.2, 132.8, 128.9,

127.7, 125.7, 124.5, 123.4, 123.2, 21.2; HRMS (ESI) calcd for $C_{14}H_{12}N_3O^+$ $[M+H]^+$: 238.0980, found 238.0981.

2-(3-Methoxyphenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4c): Yield: 59%, white solid, mp 89-91 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.83 (d, J = 6.0 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.93 (td, J = 7.8, 1.8 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.74 (s, 1H), 7.42-7.50 (m, 2H), 7.12 (dd, J = 8.4, 3.6 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.5, 163.8, 159.9, 150.3, 143.5, 137.2, 130.2, 125.8, 124.6, 123.2, 119.7, 118.7, 111.6, 55.5; HRMS (ESI) calcd for $C_{14}H_{12}N_3O_2^+$ $[M+H]^+$: 254.0930, found 254.0918.

2-(4-Cyanophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4d): Yield: 42%, white solid, mp 230-232 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.82-8.84 (m, 1H), 8.28-8.32 (m, 3H), 8.08-8.13 (m, 3H), 7.67-7.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.5, 164.0, 150.4, 143.1, 137.3, 132.8, 127.7, 127.5, 126.2, 123.5, 117.8, 115.5; HRMS (ESI) calcd for $C_{14}H_9N_4O^+$ $[M+H]^+$: 249.077, found 249.0777.

2-(4-Fluorophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4e): Yield: 89%, white solid, mp 170-172 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.33 (dt, J = 8.0, 1.2 Hz, 1H), 8.21-8.24 (m, 2H), 7.94 (td, J = 7.6, 1.6 Hz, 1H), 7.47-7.50 (m, 1H), 7.21-7.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.2, 164.7, 163.8, 150.3, 143.5, 137.2, 129.6, 129.5, 125.8, 123.2, 119.9, 116.5, 116.3; HRMS (ESI) calcd for $C_{13}H_9N_3OF^+$ $[M+H]^+$: 242.0730, found 242.0728.

2-(2-Chlorophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4f): Yield: 49%, white solid, mp 93-95 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.33 (dt, J = 8.0, 1.2 Hz, 1H), 8.12 (dd, J = 7.6, 1.6 Hz, 1H), 7.94 (td, J = 7.6, 1.6 Hz, 1H), 7.60 (dd, J = 8.0, 1.2 Hz, 1H), 7.47-7.52 (m, 2H), 7.45 (td, J = 7.6, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.2, 163.8, 150.4, 143.5, 137.2, 133.4, 132.6, 131.4, 131.2, 127.0, 125.9, 123.3, 123.0; HRMS (ESI) calcd for $C_{13}H_9N_3OCl^+$ $[M+H]^+$: 258.0434, found 258.0435.

2-(4-Chlorophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4g): Yield: 66%, white solid, mp 162-164 ºC (162-164 ºC, lit.[15]); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.33 (dt, J = 8.0, 1.2 Hz, 1H), 8.17 (d, J = 8.8 Hz, 2H), 7.94 (td, J = 7.6, 1.6 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.47-7.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.7, 163.9, 150.3, 143.4, 138.3, 137.2, 129.4, 128.5, 125.9, 123.3, 122.0; HRMS (ESI) calcd for $C_{13}H_9N_3OCl^+$ $[M+H]^+$: 258.0434, found 258.0433.

2-(3-Bromophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4h): Yield: 64%, white solid, mp 157-159 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.82-8.84 (m, 1H), 8.38 (t, J = 1.6 Hz, 1H), 8.34 (dt, J = 8.0, 1.2 Hz, 1H), 8.16-8.18 (m, 1H), 7.94 (td, J = 7.6, 1.6 Hz, 1H), 7.68-7.71 (m, 1H), 7.48-7.52 (m, 1H), 7.44 (t, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.2, 164.1, 150.3, 143.3, 137.3, 134.9, 130.6, 130.0, 126.0, 125.8, 125.4, 123.3, 123.1; HRMS (ESI) calcd for $C_{13}H_9N_3OBr^+$ $[M+H]^+$: 301.9929, found 301.9931.

2-(4-Bromophenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4i): Yield: 59%, white solid, mp 151-153 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.34 (dt, J = 8.0, 1.2 Hz, 1H), 8.10 (d, J = 8.8 Hz, 2H), 7.94 (td, J = 7.6, 1.6 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.48-7.51 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:

164.8, 163.9, 150.3, 143.4, 137.2, 132.4, 128.6, 126.8, 125.9, 123.3, 122.5; HRMS (ESI) calcd for $C_{13}H_9N_3OBr^+$ $[M+H]^+$: 301.9929, found 301.9928.

2-(Pyridin-2-yl)-5-(pyridin-3-yl)-1,3,4-oxadiazole (4j): Yield: 54%, white solid, mp 161-163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.45 (dd, J = 2.4, 0.8 Hz, 1H), 8.83-8.85 (m, 1H), 8.82 (dd, J = 4.8, 1.6 Hz, 1H), 8.52 (dt, J = 8.0, 2.0 Hz, 1H, 1H), 8.35 (dt, J = 8.0, 1.2 Hz, 1H), 7.96 (td, J = 8.0, 1.6 Hz, 1H), 7.48-7.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.3, 163.5, 152.6, 150.4, 148.2, 143.3, 137.4, 128.6, 126.1, 123.7, 123.4, 120.2; HRMS (ESI) calcd for $C_{12}H_9N_4O^+$ $[M+H]^+$: 225.0776, found 225.0777.

2-(Pyridin-2-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazole (4k): Yield: 92%, white solid, mp 158-160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.83-8.86 (m, 3H), 8.37 (dt, J = 7.6, 1.2 Hz, 1H), 8.11-8.06 (m, 2H), 7.95 (td, J = 7.6, 1.6 Hz, 1H), 7.51-7.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.6, 163.7, 150.8, 150.4, 143.1, 137.3, 130.7, 126.2, 123.5, 120.6; HRMS (ESI) calcd for $C_{12}H_9N_4O^+$ $[M+H]^+$: 225.0776, found 225.0778.

2-(Furan-2-yl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (4l): Yield: 60%, white solid, mp 129-131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.79-8.83 (m, 1H), 8.33 (dt, J = 8.0, 1.2 Hz, 1H), 7.93 (td, J = 7.6, 1.6 Hz, 1H), 7.70 (dd, J = 1.8, 0.8 Hz, 1H), 87.47-7.50 (m, 1H), 7.34 (dd, J = 3.6, 0.8 Hz, 1H), 6.65 (dd, J = 3.6, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.1, 158.3, 150.3, 146.0, 143.2, 139.1, 137.2, 125.9, 123.3, 114.9, 112.3; HRMS (ESI) calcd for $C_{11}H_8N_3O_2^+$ $[M+H]^+$: 214.0617, found 214.0613.

2-(Pyridin-2-yl)-5-(thiophen-2-yl)-1,3,4-oxadiazole (4m): Yield: 91%, white solid, mp 54-56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81-8.83 (m, 1H), 8.32 (dt, J = 8.0, 1.2 Hz, 1H), 7.95 (dd, J = 3.6, 1.2 Hz, 1H), 7.93 (td, J = 7.6, 1.6 Hz, 1H), 7.61 (dd, J = 5.2, 1.2 Hz, 1H), 7.46-7.50 (m, 1H), 7.19-7.22 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.2, 161.8, 150.3, 143.4, 137.2, 130.7, 130.5, 128.2, 125.8, 124.8, 123.3; HRMS (ESI) calcd for $C_{11}H_8N_3OS^+$ $[M+H]^+$: 230.0388, found 230.0389.

2-Butyl-5-(pyridin-2-yl)-1,3,4-oxadiazole (4n): Yield: 47%, colourless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78 (d, J = 4.8 Hz, 1H), 8.26 (d, J = 7.6 Hz, 1H), 7.90 (td, J = 7.6, 1.6 Hz, 1H), 7.43-7.46 (m, 1H), 2.99 (t, J = 7.6 Hz, 2H), 1.91 (p, J = 7.6 Hz, 2H), 1.42-1.51 (m, 2H), 0.99 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 163.9, 150.1, 143.6, 137.2, 125.6, 122.9, 28.5, 25.2, 22.1, 13.5; HRMS (ESI) calcd for $C_{11}H_{14}N_3O^+$ $[M+H]^+$: 204.1137, found 204.1137.

In vitro anticholinesterase assay

The anticholinesterase activity of the pyridine-based 1,3,4-oxadiazole derivatives was determined by the method of Ellman with slight modifications. The *in vitro* inhibition assays of AChE from electric eel run in phosphate buffer 0.1 M, at pH 7.4. Acetylthiocholine iodide was used as substrate. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was used as the chromophoric reagent. To a 96-well plate, 40 μL of phosphate buffer solution (0.1 M, pH = 7.4, PBS), 20 μL of DTNB (2.5 mM in 0.1 M PBS, pH = 8.0) were added sequentially, 10 μL AChE solution (0.2 U/mL in 0.1 M PBS, pH = 7.4), 10 μL of

the sample solution, shaken well, and incubated at 37 °C for 10 min. Then, 20 μL of acetylthiocholine iodide (10 mM in 0.1 M PBS, pH = 7.4) were added, shaken well, and incubated at 37 °C for 10 min. The absorbance at 405 nm of the samples was measured using a spectrophotometer, the sample solution was set to 1 μmol/mL and the experiment was repeated three times. Tacrine, commercial acetylcholinesterase inhibitors containing pyridine structures, was used as a positive control. Inhibitory effect (%) $=[OD_0-(OD_1-OD_2)]/OD_0\times100\%$, where OD_0 is the absorbance of blank group; OD_1 is the absorbance of sample group; OD_2 is the absorbance of sample blank group.

Molecular modelling

The 3D-structure of human AChE was downloaded from the RCSB database (PDB ID: 3LII), a chain A of the structure was used for docking study. Then, Autodock Tool was used to determine the atom types and calculate the partial charges of the protein, and the pdbqt file was generated for docking. The 2D structure of the ligand was drawn by ChemDraw15.0, and saved as cdx file. Then, MM2 force field in Chem3D 15.0 was used to optimize the 3D structure of the ligand. Again, Autodock Tool was used to determine the atom types and calculate the partial charges of the ligand, and pdbqt file was generated for docking. Docking of the ligand was carried out using the Autodock vina 1.1.2 program. A sphere of 20 Å around the carbonyl group of Gly122 was defined as the binding site for the ligand docking and 250 confirmations was allowed. Pymol was used to analyze the docking solutions, and the conformation of lowest affinity value was chosen for further analysis in ligplot.

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REFERENCES

1. J. L. Cummings, *N. Engl. J. Med.*, 2004, **351**, 56.
2. L. Rizzi, I. Rosset, and M. Roriz-Cruz, *Biomed. Res. Int.*, 2014, **2014**, 1.
3. M. Citron, *Nat. Rev. Drug Discov.*, 2010, **9**, 387.
4. M. M. Ibrahim and M. T. Gabr, *Neural Regen. Res.*, 2019, **14**, 437.
5. A. Chaubey and S. N. Padeya, *Asian J. Pharm. Clin. Res.*, 2011, **4**, 5.
6. T. T. Cao, J. T. Chen, C. L. Yang, Y. F. Tian, L. T. Feng, and Z. Y. Ma, *Acta Pharm. Sin.*, 2016, **51**, 1436.

7. H. J. Khalilullah, M. Ahsan, M. Hedaitullah, S. Khan, and B. Ahmed, *Mini-Rev. Med. Chem.*, 2012, **12**, 789.
8. X. Yu, Y. F. Chen, Y. F. Zhao, and G. J. Huang, *Chemistry (Huaxue Tongbao)*, 2020, **83**, 92.
9. W. Q. Yu, G. Huang, Y. T. Zhang, H. X. Liu, L H. Liu, X. J. Yu, and J. B. Chang, *J. Org. Chem.*, 2013, **78**, 10337.
10. Y. Guo, L. L. Qu, X. G. Wang, M. X. Huang, L. Jia, and Y. B Zhang, *RSC Adv.*, 2016, **6**, 93505.
11. Y. Guo, X. G. Wang, J. P. Fan, Q. Zhang, Y. Wang, Y. Zhao, M. X. Huang, M. Ding, and Y. B. Zhang, *R. Soc. Open Sci.*, 2017, **4**, 171053.
12. O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455.
13. I. Silman and J. L. Sussman, *J. Neurochem.*, 2017, **142**, 19.
14. S. Guin, T. Ghosh, S. K. Rout, A. Banerjee, and B. K. Patel, *Org. Lett.*, 2011, **13**, 5976.
15. D. M. Pore, S. M. Mahadik, and U. V. Desai, *Synth. Commun.*, 2008, **38**, 3121.