Synthesis and evaluation of 5-benzyl-1,3,4-thiadiazole derivatives as acetylcholinesterase inhibitors

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Three novel 5-benzyl-1,3,4-thiadiazole derivatives were synthesised starting from phenylacetic acid derivatives. These compounds were characterised by NMR, HRMS and single-crystal X-ray diffraction analysis. 2-Pyrrolidyl-5-[2-(4-bromophenyl)methyl]-1,3,4-thiadiazole showed moderate acetylcholinesterase-inhibition activity with a 50% inhibitory concentration value of 33.16 μM. 2-Pyrrolidyl-5-[2-(4-bromophenyl)methyl]-1,3,4-thiadiazole and acetylcholinesterase docking was demonstrated using the Molecular Operating Environment program.

Keywords: 5-benzyl-1,3,4-thiadiazole derivatives, acetylcholinesterase, Alzheimer's disease

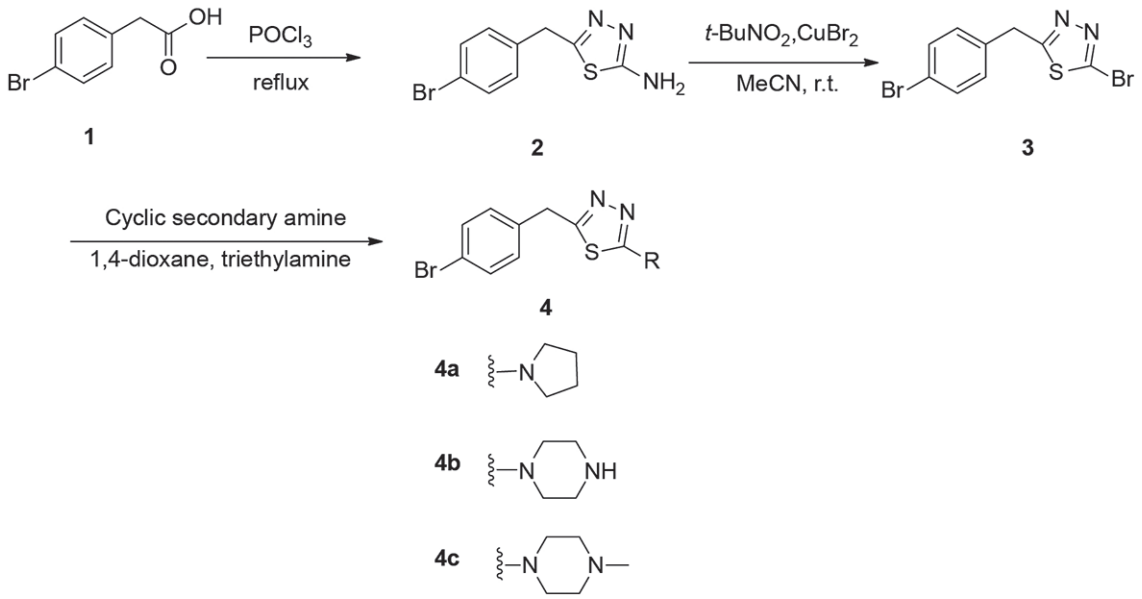
Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder characterised at the molecular level by protein misfolding and aggregation, oxidative stress, mitochondrial abnormalities and neuroinflammatory processes.[1] Acetylcholinesterase (AChE) inhibitors (*e.g.* tacrine, donepezil, rivastigmine and galantamine), developed based on the cholinergic hypothesis of memory dysfunction, the depletion of neurotransmitter acetylcholine and loss of associated synapses during the progression of AD, and *N*-methyl-D-aspartate receptor antagonists (*e.g.* memantine) are used for the treatment of AD.[2] However, AChE inhibitors have some limitations in their use for the treatment of AD, such as non-selectivity, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity.[3,4] Therefore, further studies of novel AChE inhibitors are necessary.

The 1,3,4-thiadiazole nucleus is a common and integral feature of a variety of natural products and medicinal agents.[5] Recently, some 1,3,4-thiadiazole derivatives with AChE inhibition activity were designed and synthesised.[6–8] We have previously synthesised a series of 1,3,4-thiadiazole derivatives and found that they showed AChE inhibitory activity.[9,10] Following our studies on AChE inhibitors,[11–13] here we report the synthesis of a series of 5-benzyl-1,3,4-thiadiazole derivatives derived from phenylacetic acids. The AChE inhibition activity of these compounds was tested to find inhibitors for the treatment of AD.

Results and discussion

The synthetic route for the 5-benzyl-1,3,4-thiadiazole derivatives is shown in Scheme 1. The commercially available starting material 2-(4-bromophenyl)acetic acid (**1**) was subjected to reaction with thiosemicarbazide to give the 5-(4-bromobenzyl)-1,3,4-thiadiazol-2-amine (**2**).[14] Compound **2** was reacted with $CuBr_2$ and tert-butyl nitrite to give the compound 2-bromo-5-(4-bromobenzyl)-1,3,4-thiadiazole (**3**). The final compounds **4a–c** were obtained by reaction of intermediate **3** with cyclic secondary amines.[15] The structures of the compounds were confirmed by NMR and MS. The detailed physical and analytical data are listed in the Experimental section. Their analytical and spectroscopic data were in accordance with the predicted structures.

The structure of compound **4a** was demonstrated by X-ray diffraction analysis. The crystal data are presented in Table 1 and Fig. 1 and have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1571930. The compound is monoclinic: space group



Scheme 1 Synthesis of 5-benzyl-1,3,4-thiadiazole derivatives.

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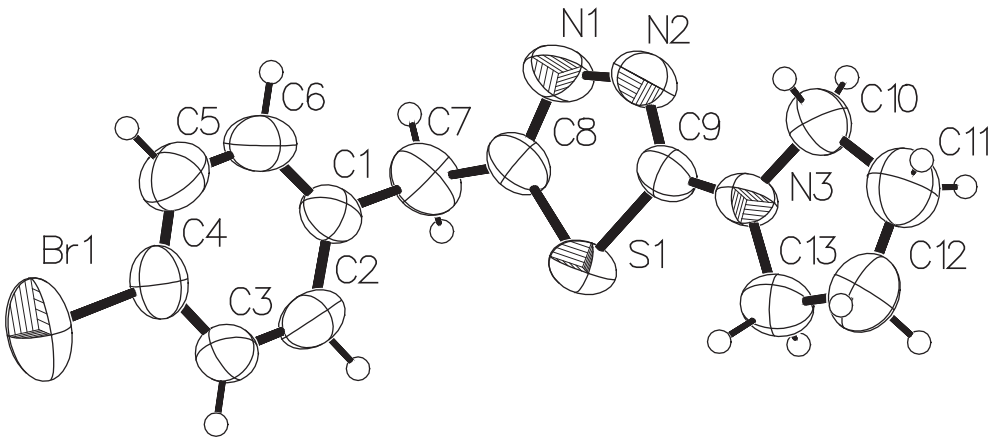

Fig. 1 Molecular structure of compound **4a** at 30% probability ellipsoids.

Table 1 Crystallographic data for the compound **4a**

Formula	$C_{13}H_{14}BrN_3S$
FW	324.24
Crystal shape/colour	Block/white
Crystal size (mm)	0.21 × 0.20 × 0.18
T (K)	296(2)
λ (Mo$K\alpha$) (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2(1)/n$
a (Å)	6.3086(7)
b (Å)	27.282(3)
c (Å)	8.7338(10)
α (°)	90.00
β (°)	109.0690(10)
γ (°)	90.00
V (Å^3)	1420.7(3)
Z	4
μ(Mo$K\alpha$) (mm^{-1})	3.026
T_{min}	0.5690
T_{max}	0.6119
D_c (g cm^{-3})	1.516
Measured reflections	11441
Unique reflections	2742
R_{int}	0.0519
Observed reflections	1359
Data/restraints/parameters	2742/0/163
Goodness of fit on F^2	1.011
R_1 [$I \geq 2\sigma(I)$]	0.0592
wR_2 [$I \geq 2\sigma(I)$]	0.1412
R_1 (all data)	0.1303
wR_2 (all data)	0.1799
Large diff. peak and hole (e Å^{-3})	1.086 and −0.555

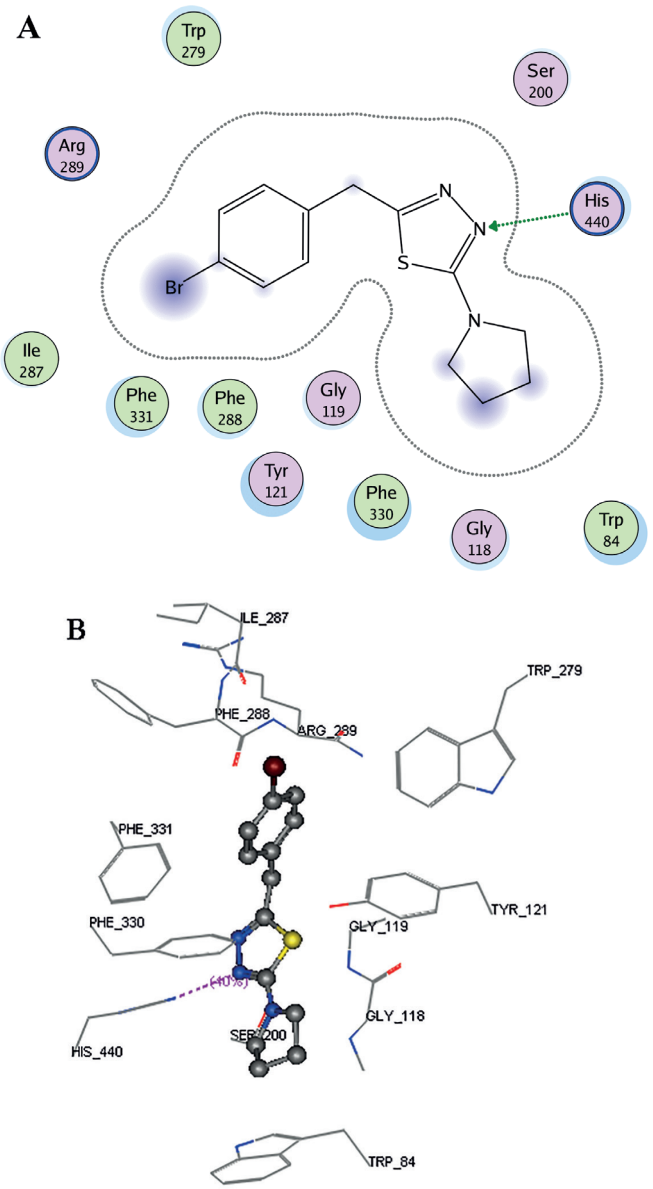

Fig. 2 The AChE active site cavity (A) and interaction map (B) displaying the binding and interactions of compound **4a** with AChE.

$P2(1)/n$, a = 6.3086(7), b = 27.282(3), c = 8.7338(10) Å, β = 109.0690(10)°, V = 1420.7(3) Å^3, Z = 4.

The target compounds **4a**–**c** were tested for AChE (from electric eel) inhibitory activity using the method published by Ellman *et al.*[16] In this test, tacrine was used as the reference drug and the AChE inhibition results are presented in Table 2. Only compound **4a** showed moderate AChE inhibitory activity with a 50% inhibitory concentration (IC_{50}) value of 33.16 **μM.**

Table 2 *In vitro* inhibitory activities of compounds **4a–c** against AChE

Compound	IC_{50} (μM)[a]
4a	33.16 ± 4.32
4b	–
4c	–
Tacrine	0.25 ± 0.05

[a]50% inhibitory concentration (mean ± SD of three experiments) of AChE from electric eel.

A docking study of the most potent anti-AChE compound **4a** was conducted to identify the possible interactions between the compound and the enzyme active site. The docking study was performed using the Molecular Operating Environment (MOE) program.[17] The molecular docking of compound **4a** can interact with the catalytic active site (CAS) of AChE (Fig. 2).[18] When **4a** is docked with AChE, one N atom of the 1,3,4-thiadiazole group can interact with His440 *via* the hydrogen bond.

Conclusion

In summary, three 5-benzyl-1,3,4-thiadiazole derivatives **4a–c** were synthesised and their AChE inhibition ability *in vitro* was tested. The data showed that compound **4a** was the most potent anti-AChE derivative, and it could interact with CAS of AChE. All these results suggest that compound **4a** could be a promising multi-target lead candidate against AD.

Experimental

Analytical reagent grade 2-(4-bromophenyl)acetic acid was purchased from Aladdin Industrial Corporation. AChE from electric eel, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (ATCI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were purchased from Sinopharm Chemical Regent Co., Ltd. and were used as received. TLC was performed on glass-backed silica gel sheets (Silica Gel 60 GF254). Melting points were determined on a Yanaco melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet iS10 spectrometer in KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz instrument or 500 MHz instrument using MeOD or $CDCl_3$, with tetra methyl silane (TMS) as an internal standard. The MS (ESI) spectra were recorded using an Agilent Technologies 6224 TOF LC/MS.

5-(4-Bromobenzyl)-1,3,4-thiadiazol-2-amine (**2**)

$POCl_3$ (0.28 mol) was added dropwise to a mixture of 2-(4-bromophenyl)acetic acid (**1**) (0.07 mol) and thiosemicarbazide (0.105 mol) in an ice bath. Then the reaction mixture was refluxed over an oil bath for 6 h. The reaction was monitored by TLC. When the reaction was complete and the mixture had cooled to room temperature, water (100 mL) was added and the reaction mixture was refluxed for 1 h. After cooling, the mixture was basified to pH 8–9 by the dropwise addition of 50% NaOH solution in an ice bath. The precipitate was filtered and recrystallised from ethanol to obtain compound **2** as white crystals; yield 85%; m.p. 203–205 °C; IR (KBr) (cm^{-1}): 3396, 3273, 3122, 1578, 1488, 1420; ^{1}H NMR (400 MHz, CD_3OD): δ 7.48 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 4.16 (s, 2H). HRMS (ESI) *m/z* calcd for $C_9H_9BrN_3S$ [M + H]$^+$: 269.9695; found: 269.9700.

2-Bromo-5-(4-bromobenzyl)-1,3,4-thiadiazole (**3**)

5-(4-Bromobenzyl)-1,3,4-thiadiazol-2-amine (20 mol) was added slowly to a mixture of $CuBr_2$ (24 mmol) and *t*-$BuNO_2$ (30 mmol) in MeCN (100 mL) and the reaction mixture was stirred at room temperature overnight. The reaction was monitored by TLC. When the reaction was completed, saturated NH_4Cl (120 mL) was added to the reaction mixture and the organic layer was extracted with EtOAC, dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain the crude product, which was triturated in MeOH. The precipitate was filtered and recrystallised from ethanol to obtain compound **3** a white solid; yield 96.4%; m.p. 84.2–86.3 °C; IR (KBr) (cm^{-1}): 3435, 3002, 2971, 2928, 1576, 1488, 1428, 1397; ^{1}H NMR (400 MHz, CD_3OD): δ 4.43 (s, 2H, CH2), 7.29–7.22 (m, 2H, Ar–H), 7.53–7.47 (m, 2H, Ar–H); ^{13}C NMR (100 MHz, CD_3OD): δ 174.3, 139.9, 135.8, 131.8, 130.5, 121.2, 34.9. HRMS (ESI) *m/z* calcd for $C_9H_7Br_2N_2S$ [M + H]$^+$: 332.8691; found: 332.8691.

Synthesis of 2-amino-5-(substituted-benzyl)-1,3,4-thiadiazoles (**4a–c**)*; general procedure*

Triethylamine (2.4 mmol) was added to a mixture of 2-bromo-5-(4-bromophenethyl)-1,3,4-thiadiazole (2 mmol) with amine (4 mmol) in 1,4-dioxane at 70 °C with stirring. The reaction was monitored by TLC. When the reaction was completed and the mixture had cooled to room temperature, the organic layer was extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain the crude product. The precipitate was filtered and recrystallised from methanol or purified by column chromatography using CH_2Cl_2 and MeOH to obtain compounds 4a–c.

2-(4-Bromobenzyl)-5-(pyrrolidin-1-yl)-1,3,4-thiadiazole (**4a**): White solid; yield 80.8%; m.p. 98.7–99.2 °C; IR (KBr) (cm^{-1}): 3446, 2970, 2870, 1637, 1524, 1415; ^{1}H NMR (400 MHz, MEOD): δ 2.02–2.05 (m, 4H, $2CH_2$), 3.41 (s, 4H, $2CH_2$), 4.18 (s, 2H, CH_2), 7.20–7.23 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.46–7.48 (m, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO): δ 169.2, 157.7, 136.8, 131.6, 130.3, 120.7, 50.3, 35.0, 25.3. HRMS (ESI) *m/z* calcd for $C_{13}H_{15}BrN_3S$ [M + H]$^+$: 324.0165; found: 324.0159.

2-(4-Bromobenzyl)-5-(piperazin-1-yl)-1,3,4-thiadiazole (**4b**): White solid; yield 81.7%; m.p. 76.3–79.2 °C; IR (KBr) (cm^{-1}): 3432, 3282, 2979, 2846, 1546, 1487, 1446; ^{1}H NMR (400 MHz, DMSO): δ 2.75–2.78 (m, 4H, $2CH_2$), 3.28–3.31 (m, 5H, $2CH_2$, NH), 4.21 (s, 2H, CH_2), 7.25–7.27 (d, 2H, *J* = 8.4 Hz, Ar–H), 7.52–7.54 (m, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO) δ: 173.0, 158.3, 137.8, 132.1, 131.4, 120.6, 50.9, 45.1, 35.3. HRMS (ESI) *m/z* calcd for $C_{13}H_{16}BrN_4S$ [M + H]$^+$: 339.0274; found: 339.0268.

2-(4-Bromobenzyl)-5-(4-methylpiperazin-1-yl)-1,3,4-thiadiazole (**4c**): White solid; yield 73.6%; m.p. 78.8–81.8 °C; IR (KBr) (cm^{-1}): 3434, 2937, 2848, 2803, 1640, 1536, 1497; ^{1}H NMR (400 MHz, DMSO): δ 2.20 (s, 3H, CH_3), 2.37–2.39 (m, 4H, $2CH_2$), 3.35–3.38 (t, 4H, $2CH_2$), 4.22 (s, 2H, CH_2), 7.25–7.27 (m, 2H, Ar–H), 7.52–7.54 (m, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO) δ: 172.6, 158.7, 137.8, 132.1, 131.4, 120.6, 54.0, 49.7, 46.1, 35.2. HRMS (ESI) *m/z* calcd for $C_{14}H_{18}BrN_4S$ [M + H]$^+$: 353.0430; found: 353.0424.

X-ray crystallography

Diffraction intensities for the complexes were collected at 296(2) K using a Bruker SMART APEX-II CCD area-detector with Mo*K*α radiation (λ = 0.71073 Å). The collected data were reduced with the SAINT program,[19,20] and multi-scan absorption corrections were performed using the SADABS program.[21] Both structures were solved by direct methods. The complexes were refined against F^2 by full-matrix least-squares methods using the Olex2-1.2 package.[22] All of the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions and constrained to their parent atoms. The crystallographic data for the complexes are summarised in Table 1. Crystallographic data for the complexes have been deposited with the Cambridge Crystallographic Data Center (CCDC 1571930).

Biological tests

The methods for biological tests are provided in the ESI.

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Electronic Supplementary Information

The ESI containing the methods for the biological tests is available through:
http://ingentaconnect.com/content/stl/jcr/2017/00000041/00000011/art00012

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