Synthesis and in Vitro Evaluation of 1,3,4-Thiadiazol-2-yl Urea Derivatives as Novel AChE Inhibitors

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1,3,4-Thiadiazole and urea group were hybridized to form new molecular skeleton and 11 compounds were synthesized and evaluated as acetylcholinesterase (AChE) inhibitors. Most of them showed comparable effects in inhibition of AChE, especially compound 6b which exhibited activity with IC50 value 1.17 μM, as strong as galanthamine. This information offered by our research would be valuable for further investigation of structure–activity relationship (SAR) and useful in future research of AChE inhibitors.

Key words 1,3,4-thiadiazole; urea group; hybridization; acetylcholinesterase (AChE) inhibitor

Neurodegenerative diseases like Alzheimer’s (AD) and Parkinson’s (PD) are the most enigmatic and problematic issues in biomedicine. AD, the most common form of dementia, is an age-related neurodegenerative disorder characterized by a progressive memory loss, a decline in language skills, and other cognitive impairments. Scientists have proposed several hypotheses for AD development. One of the oldest AD hypotheses, the cholinergic hypothesis, has led to the development of acetylcholinesterase inhibitors (AChEIs) that increase levels of acetylcholine (ACh) through inhibition of acetylcholinesterase (AChE).

Several antiacetylcholinesterase agents such as donepezil, tacrine, galantamine, and ensaculin have shown to induce modest improvement in memory and cognitive functions. Unfortunately, the potential effectiveness offered by these inhibitors is often limited by the appearance of central and peripheral side effects. For example, clinical studies have shown that tacrine has hepatotoxic liability. Recent research aims at new types of compounds as potential AChE inhibitors. Carbamates, which are the most widely studied class of anticholinesterase agents, have attracted a great deal of interest among medicinal chemists and considerable research on them in relation to AD has been accomplished. Rivastigmine, which is the dual AChE and butryrylcholinesterase (BuChE) inhibitor, is one of the most widely used anticholinesterase agents bearing carbamate group.

To discover more potent and metabolically stable cholinomimetic ligands, it points out to the possibility of replacing the ester group with five-membered rings like thiadiazoles, triazoles, tetrazoles as well as oxadiazoles. Some scientists have studied widely 1,3,4-thiadiazole derivatives as potential drugs to treat AD. The thiadiazole ring can act as the “hydrogen binding domain,” “two-electron donor system” as well as it enables to create π−π stacking interactions. At the same time, urea group has widely used as bioisosteric replacement of carbamate, and it is more stable in metabolism. Furthermore, Jean-Louis et al. have successfully introduced urea group in novel design of AChE inhibitor in 1994.

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was equivalent to reference drug galanthamine (IC 50 had the strongest inhibitory activity of anticholinesterase and 1.17.

**Table 1.** AChE Inhibitory Activity of Target Compounds

| Compound | X | R¹ | Y | R² | IC₅₀ (µM) |
|----------|---|----|---|----|-----------|
| 6a       | C | 4-OMe | C | 3,4-Dimethyl | 124.42±7.11  |
| 6b       | C | 4-OMe | N | H   | 1.17±0.06  |
| 6c       | C | 4-OMe | C | 4-OMe | 3.00±0.23  |
| 6d       | C | 3,4-Dimethyl | C | 4-OMe | 206.15±12.57 |
| 6e       | C | 3,4-Dimethyl | N | H   | 111.09±6.34 |
| 6f       | C | 3,4-Dimethyl | C | 3,4-Dimethyl | 293.89±14.78 |
| 6g       | N | H   | C | 4-OMe | 131.31±9.27 |
| 6h       | N | H   | C | 3,4-Dimethyl | 152.76±11.59 |
| 6i       | N | H   | N | H   | 2.05±0.17  |
| 6j       | C | H   | C | H   | 169.97±1.89 |
| 6k       | C | 4-F  | C | 4-F  | 9.87±0.11  |

Galanthamine — — — — 1.07±0.08

**Fig. 2.** Synthesis of Target Compounds 6a-k

Reagents and conditions: a) POCl₃, 80°C, 60–72%; b) Et₃N, DPPA, toluene, 40°C; c) 3a–k, reflux, 36–86%.

**Experimental**

All reagents were purchased from Shanghai Chemical Reagent Company. Column chromatography (CC): silica gel 60 (200–300 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). mp: capillary tube; uncorrected. 1H-NMR spectra: Bruker ACF-300Q apparatus at 300 MHz, in DMSO-d₆ unless
General Procedure for Preparing Compounds 3a–k
A mixture of substituted benzoic acid (40.61 mmol) and thioemcarbazide (48.73 mmol) in POCl₃ (45 mL) was stirring for 2 h at 80°C. After cooling to room temperature, water (60 mL) was added in 30 min. Ice-water was added, and the pH was adjusted to 8 with 1 M sodium hydroxide solution. The mixture was filtered. The obtained solid was crystallized from DMF–H₂O to give 3a–k.

General Procedure for Preparing Compounds 6a–k
A mixture of substituted benzoic acid (3 mmol) and triethylamine (3.3 mmol) in anhydrous toluene 40 mL was stirring for 45 min at room temperature. To the above reaction mixture 6a–k thiazol-2-yl) urea (0.52 mmol) was stirring for 30 min. Ice-water was added, and the pH was adjusted to 50 mL methanol for 0.5 h. After cooling, the mixture was refluxed for 10 h. Cooled to room temperature and concentrat.

BioTOF™Q mass spectrometer (Bruker); in 

HR-TOF-MS

1-(3,4-Dimethylphenyl)-3-(5-(3-pyridine-3-yl)-1,3,4-thiadiazol-2-yl)urea (6f): Yield 81.9%; mp 341.2–344.6°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 2.17–2.20 (t, 6H), 2.27–2.29 (d, 6H), 7.06–7.08 (d, J=6 Hz, 1H), 7.20–7.29 (m, 3H), 7.60–7.62 (d, J=6 Hz, 1H), 7.68 (s, 1H), 8.86 (s, 1H), 10.92 (s, 1H). HR-TOF-MS m/z 353.1436 ([M+H]+, C₁₁H₁₀N₂O₅S; Calcd 353.1431).

1-(4-Methoxyphenyl)-3-(5-(3-pyridine-3-yl)-1,3,4-thiadiazol-2-yl)urea (6g): Yield 50.8%; mp 321.6–324.4°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 3.75 (s, 3H), 6.92–7.94 (d, J=6 Hz, 2H), 7.42–7.44 (d, J=6 Hz, 2H), 7.56–7.59 (m, 1H), 8.29–8.31 (d, J=6 Hz, 1H), 8.70–8.71 (d, J=3 Hz, 1H), 8.92 (s, 1H), 9.11 (s, 1H), 11.13 (s, 1H). HR-TOF-MS m/z 328.0868 ([M+H]+, C₁₁H₁₀N₂O₅S; Calcd 328.0863).

1-(3,4-Dimethylphenyl)-3-(5-(3-pyridine-3-yl)-1,3,4-thiadiazol-2-yl)urea (6i): Yield 58.9%; mp 318.2–321.6°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 2.05–2.31 (m, 6H), 7.08–7.29 (m, 3H), 7.56 (s, 1H), 8.29–8.30 (m, 10H), 8.70 (s, 1H), 8.90 (s, 1H), 9.10 (s, 1H), 11.16 (s, 1H). HR-TOF-MS m/z 326.1076 ([M+H]+, C₁₁H₁₀N₂O₅S; Calcd 326.1070).

1-(Pyridine-3-yl)-3-(5-(3-pyridine-3-yl)-1,3,4-thiadiazol-2-yl)urea (6j): Yield 36.0%; mp 319.4–324.2°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 7.34 (m, 1H), 7.57–7.58 (m, 1H), 7.99–8.00 (d, J=3 Hz, 1H), 8.29–8.31 (d, J=6 Hz, 2H), 8.70 (s, 2H), 9.10 (s, 1H), 9.34 (s, 1H), 11.78 (s, 1H). HR-TOF-MS m/z 299.0714 ([M+H]+, C₁₁H₁₀N₂O₅S; Calcd 299.0710).

1-(Phenyl-1,3,4-thiadiazol-2-yl)-3-phenyl urea (6j): Yield 75.8%; mp 309.5–311.9°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 7.04–7.09 (m, 1H), 7.31–7.36 (m, 2H), 7.89–7.91 (m, 2H), 7.50–7.52 (m, 2H), 9.05 (s, 1H), 11.04 (s, 1H). HR-TOF-MS m/z 295.0660 ([M+H]+, C₁₁H₁₀N₂O₅S; Calcd 295.0654).

1-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)-3-(4-fluorophenyl)urea (6k): Yield 81.2%; mp 335.7–336.9°C. ¹H-NMR (300 MHz, DMSO-d₆) δ: 7.14–7.20 (t, J=8.7 Hz, 2H), 7.34–7.40 (t, J=8.7 Hz, 2H), 7.51–7.55 (t, J=8.1 Hz, 2H), 7.94–7.99 (t, J=8.4 Hz, 2H), 9.13 (s, 1H), 11.14 (s, 1H). HR-TOF-MS m/z 313.0472 ([M+H]+, C₁₁H₁₀N₂O₂F₂S; Calcd 313.0465).

In Vitro AChE Inhibition Assay
AChE inhibitory activity was detected by a microtitre plate assay based on Ellman’s method (Rhee et al., 2001), using the Amplite™ Fluorimetric Acetylcholinesterase Assay Kit. In 96-well plates, 25 µL Acetylcholine, 2 µL Thioioltie™ Green, 62.2 µL of buffer and 10 µL of the test compound at concentration of 0.01, 0.1, 1, 10, 100, 1000 µM, 10 mM were added and the fluorescence intensity was measured at 485/528 nm every 13 s for five times. After adding 25 µL of 0.125 % AChE, the fluorescence intensity was read again every 13 s for five times. The fluorescence intensity was measured using a Multi-Plate Reader (Synergy HT, BioTek Ltd.). Percentage of inhibition was calculated by comparing the rates for the sample to the blank (DMSO), control contained all components except the tested extract. Galanthamine was used as positive control. The mean of four measurements for each concentration was determined (n=4). The 50% inhibitory concentration (IC₅₀) was calculated from a doseeresponse curve obtained by plotting the percentage of inhibition versus the log concentration with the use of GraphPad Prism 5.0 software.

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