Synthesis and anti-acetylcholinesterase activities of novel glycosyl coumarylthiazole derivatives

You-Xian Wang¹, Shu-Hao Liu¹, Zhong-Bai Shao¹, Lian-Gong Cao¹, Kai-Jun Jiang¹, Xing Lu¹, Lei Wang¹, Wei-Wei Liu¹,²,³,⁴, Da-Hua Shi¹,² and Zhi-Ling Cao¹,²

Abstract
Eleven glycosyl coumarylthiazole derivatives are synthesized by cyclization and condensation of glycosyl thiourea with 3-bromoacetyl coumarins in ethanol. The reaction conditions are optimized and good yields of products (80%–95%) are obtained. The structures of all new products were confirmed by IR, ¹H and ¹³C NMR, and by HRMS (electrospray ionization). The in vitro acetylcholinesterase (AChE) inhibitory activities of these new compounds are tested by Ellman’s method. Among them, N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-(6-nitrocoumarinyl)-1,3-thiazole-2-amine showed the best activity with an in vitro AChE inhibitory rate of 58% and an IC₅₀ value of 12 ± 0.38 μg/mL.

Keywords
acetylcholinesterase inhibitory activity, coumarylthiazole, glucosamine

Date received: 29 May 2020; accepted: 29 June 2020

Introduction
Alzheimer’s disease (AD) is a degenerative disease of the central nervous system, mainly manifested as neuropsychiatric symptoms, such as progressive memory disorders, cognitive dysfunction, personality changes, and language disorders.¹–³ With the aging of the global population, the incidence of AD has increased year on year.⁴ The disease has become the third leading cause of death among elderly people after cancer and cardiovascular disease. The cholinergic hypothesis was the first proposed explanation for the pathogenesis of AD. According to the cholinergic hypothesis,⁵ the decrease of cholinergic activity and acetylcholine (ACh) concentration is caused by the decrease of acetylcholinesterase (AChE) and cholineacetyl transferase (ChAT). Drugs currently approved for the treatment of AD are mainly AChE inhibitors such as tacrine and galantamine.⁶,⁷

In the early stage of our research, a series of glycosyl thiazole derivatives, as AChE inhibitors, was synthesized with glucosamine as the leading compound.⁸ Among them, glycosyl thiazole derivative 1 (the structure of which is shown in Figure 1) showed the best anti-AChE activity with an in vitro AChE inhibitory rate of 43%. This indicated that the presence of a thiazole moiety improved the activity of the GlcNAc unit. On this basis, our research group conducted a more in-depth exploration in order to obtain new compounds with better activity toward AChE. According to the literature, coumarins are an important class of heterocyclic compounds with various biological activities such as anti-arrhythmic, antibacterial, anticancer, anti-HIV, and anti-hypertensive.⁹–¹³ In addition, coumarin derivatives also have an inhibitory effect on AChE, such as 8-hydroxycoumarin (2) and cnidium (3);¹⁴–¹⁶ the structures of which are shown in Figure 2.

The combination of coumarin and a thiazole ring is of great significance in pharmaceutical chemistry. Coumarin–thiazole compounds have many activities such as antibacterial, antioxidant, anticonvulsive, anticancer, and for treating hypertension.¹⁷–²¹ Combining multiple active molecules...
within the same structure is an important means to synthesize new substances, and it is also the main way to find compounds with new biological and physiological activities. Based on the above analysis, we have designed and synthesized a series of novel glycosyl coumarylthiazole derivatives in order to find new and effective AChE inhibitors. The AChE inhibitory activity of the compounds was determined by Ellman’s method, which revealed that glycosyl heterocyclic compounds with good AChE inhibitory activity had been obtained.

**Results and discussion**

**Chemistry**

For the synthesis of the glycosyl coumarylthiazole derivatives, 2-acetylamino-3,4,6-tri-O-acetyl-2-deoxy-β-D-pyranosethiourea (8) and 3-bromoacetylcoumarins 11 are the key intermediates. The acetyl group was selected as the protecting group for glucose to achieve highly selective chemical modification of glucosamine. First, compound 8 was synthesized based on previous work by our group. Next, compound 8 and coumarin 11a were dissolved in ethanol and heated at 60 °C until the reaction was complete. The solvent was evaporated under reduced pressure, and the coumarin derivative 12a was purified by column chromatography (Scheme 1).

In the second stage, taking the reaction of glycosyl thiourea 8 and compound 11a as an example, optimum reaction conditions were found. The reaction molar ratio, solvent, temperature, and time were screened, and the results are summarized in Table 1. The optimum conditions for synthesizing compounds 12 were as follows: ethanol as solvent, a molar ratio of glycosyl thiourea 8 to 3-bromoacetylcoumarin 11 of 1:1.1, and the reaction was carried out at 60°C for 35 minutes.

**Biological activity**

Using the classic Ellman colorimetric method, cholinesterase hydrolyzes the substrate (acetylthiocholine iodide or butyrylthiocholine iodide) to form acetic acid or butyric acid and thiocholine. Thiocholine reacts with 5,5-disulfide-bis-2-nitrobenzoic acid (DTNB) turning the solution yellow and showing a characteristic absorption at 412 nm. When the sample under test is different, after the same time, the
absorption value is also different. The inhibition of cholinesterase by the sample under investigation slows down the rate of cholinesterase hydrolysis of ACh, so the absorbance value shows a small difference over the same time. The results are summarized in Table 2. For compounds with an inhibition rate exceeding 50%, the half of maximal inhibitory concentration (IC$_{50}$) is determined, with tacrine and galantamine as the reference compounds.

As shown in Table 2, the AChE inhibitory activity of the synthesized target compounds were higher than that of the precursor compound, and three of the 11 target compounds showed higher values than compound 1. Compound 12f showed the best activity with an IC$_{50}$ value of 12 ± 0.38 μg/mL against AChE and inhibits AChE in a dose-dependent relationship (Figure 3).

**Conclusion**

The synthesis of a new series of glycosyl coumarylthiazole derivatives under mild conditions and simple has been accomplished. All yields were above 80%. The structures of the products were determined by 1H NMR, 13C NMR, infrared spectroscopy (IR), and high-resolution mass spectrometry (HRMS). All the products 12a–k exhibited in vitro AChE inhibitory activity, which were higher than that of glucosamine hydrochloride. Compound 12f showed the best activity with an in vitro AChE inhibitory rate of 58% and an IC$_{50}$ value of 12 ± 0.38 μg/mL. Compared with the previously synthesized AChE inhibitor 1, compound 12f exhibited an increased inhibition of AChE, suggesting that the coumarin group may increase the inhibition of AChE to some extent. This work provides the basis for studying amino sugar derivatives for the treatment of AD.

**Experimental**

**Chemistry**

Unless otherwise stated, all chemicals were purchased from commercial sources and were used without further purification. Melting points were measured on a Yanaco melting point apparatus and are uncorrected. Infrared spectra were recorded on a Bruker Tensor 27 spectrometer as KBr disks. 1H NMR and 13C NMR spectra were recorded at 500 and 126 MHz, respectively, on a Bruker Avance at ambient temperature using DMSO-d$_6$ as the solvent and TMS as the internal standard. Chemical shifts are reported in ppm and coupling constants (J) are given in hertz (Hz). HRMS (electrospray ionization (ESI)) analysis was performed on an Agilent 6230 mass spectrometer. Flash column chromatography was performed on 200–300 mesh silica of Harveybio.

**Synthesis of N-(2-acetylamino-3,4,6-tri-O-acetyl-2-deoxy-β-D-pyranose)-4-(coumarin-3-yl)-1,3-thiazole-2-amins (12a–k); general procedure**

A solution of compound 8 (0.5 mmol) and compound 11a–k (0.6 mmol) in EtOH (30mL) a 50 mL single-necked flask was stirred in an oil bath at 60°C. The reaction was monitored by thin-layer chromatography (TLC) and the solvent was evaporated under reduced pressure. Purification by column chromatography gave pure compound 12a–k.

| Table 1. Optimization of the conditions for the synthesis of 12a. |
|---|---|---|---|---|
| Entry | n(8):n(11a) | Time (min) | Temp (°C) | Solvent | Yield (%) |
| 1 | 1:0.9 | 35 | 60 | EtOH | 69 |
| 2 | 1:1.0 | 35 | 60 | EtOH | 77 |
| 3 | 1:1.1 | 35 | 60 | EtOH | 86 |
| 4 | 1:1.2 | 35 | 60 | EtOH | 86 |
| 5 | 1:1.3 | 35 | 60 | EtOH | 86 |
| 6 | 1:1.1 | 35 | 0 | EtOH | NR |
| 7 | 1:1.1 | 35 | 15 | EtOH | 8 |
| 8 | 1:1.1 | 35 | 30 | EtOH | 30 |
| 9 | 1:1.1 | 35 | 45 | EtOH | 57 |
| 10 | 1:1.1 | 35 | 60 | EtOH | 85 |
| 11 | 1:1.1 | 35 | 79 | EtOH | 85 |
| 12 | 1:1.1 | 35 | 60 | CH$_2$Cl$_2$ | 5 |
| 13 | 1:1.1 | 35 | 60 | EtOAc | 14 |
| 14 | 1:1.1 | 35 | 60 | MeCN | 40 |
| 15 | 1:1.1 | 35 | 60 | MeOH | 82 |
| 16 | 1:1.1 | 35 | 60 | EtOH | 84 |
| 17 | 1:1.1 | 10 | 60 | EtOH | NR |
| 18 | 1:1.1 | 15 | 60 | EtOH | 13 |
| 19 | 1:1.1 | 20 | 60 | EtOH | 24 |
| 20 | 1:1.1 | 25 | 60 | EtOH | 55 |
| 21 | 1:1.1 | 30 | 60 | EtOH | 73 |
| 22 | 1:1.1 | 35 | 60 | EtOH | 84 |
| 23 | 1:1.1 | 40 | 60 | EtOH | 84 |

NR: no reaction.

Yield of isolated product after purification.

| Table 2. In vitro inhibitory activities of compounds 12a–k against AChE. |
|---|---|---|---|
| Compound | R | Inhibition (%) | IC$_{50}$ (μg/mL) |
| 12a | H | 5.96 ± 0.02 | – |
| 12b | 6-Cl | 11.77 ± 0.04 | – |
| 12c | 6-F | 45.61 ± 0.09 | – |
| 12d | 6-Br | 10.58 ± 0.03 | – |
| 12e | 6, 8-Br | 9.94 ± 0.02 | – |
| 12f | 6-NO$_2$ | 58.88 ± 0.07 | 12 ± 0.38 |
| 12g | 6-CH$_3$ | 6.94 ± 0.05 | – |
| 12h | 6-OCH$_3$ | 9.81 ± 0.02 | – |
| 12i | 7-OCH$_3$ | 25.64 ± 0.09 | – |
| 12j | 8-OCH$_3$ | 55.17 ± 0.08 | 17 ± 0.44 |
| 12k | 8-OC$_6$H$_5$ | 22.74 ± 0.02 | – |
| 4 | 0.38 ± 0.13 | 0.0533 ± 0.0008 |
| Tacrine | 92.17 ± 0.17 | 0.767 ± 0.043 |

*Inhibition activities at a concentration of 50 μg/mL.
N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-(6-alkoxycoumarinyl)-1,3-thiazole-2-amine (12b): Dichloromethane/methanol (25:1) as eluent, Rf = 0.46; yellow solid; yield 0.26 g (88%); m.p. 137.7–138.7 °C; IR (cm⁻¹): 3348, 2928, 1746, 1735, 1666, 1556, 1537, 1527, 1483, 143.0, 137.7, 121.4, 120.5, 119.0, 118.8, 113.7, 111.0, 83.5, 73.6, 72.3, 68.9, 62.0, 52.6, 22.9, 20.7, 20.6, 20.6. ESI-HRMS: m/z [M+Na⁺] cale for C_{26}H_{24}F_{2}Na_{2}O_{10}S: 751.9520; found: 751.9517.

N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-(6-bromocoumarinyl)-1,3-thiazole-2-amine (12e): Dichloromethane/methanol (25:1) as eluent, Rf = 0.35; yellow solid; yield 0.31 g (86%); m.p. 205–206 °C; IR (cm⁻¹): 3345, 2985, 1742, 1637, 1565, 1404, 1043, 903; 1H NMR (500 MHz, DMSO-d6): δ = 8.59-8.56 ppm (1H, H-4 GlcN), 7.73 (s, 1H, thiazole), 7.67 (m, 2H, NH, ArH), 8.14 (d, J = 9.0 Hz, 1H, ArH), 7.87 (dd, J = 9.0, 2.5 Hz, 1H, ArH), 7.71 (s, 1H, thiazole), 7.44 (d, J = 9.0 Hz, 1H, ArH), 5.35 (t, J = 9.5 Hz, 1H, H-1(GlcN)), 5.22 (t, J = 9.0 Hz, 1H, H-3(GlcN)), 4.91 (t, J = 10.0 Hz, 1H, H-4(GlcN)), 4.22 (dd, J = 12.5, 5.0 Hz, 1H, H-6(GlcN)), 3.96-3.92 (m, 3H, H-2 GlcN, H-5 GlcN, H-6b GlcN), 2.01 (s, 3H, CH₃), 1.97 (s, 3H, CH₃), 1.95 (s, 3H, CH₃); 13C NMR (126 MHz, DMSO-d6): δ = 170.2, 169.8, 169.5, 166.5, 156.0, 153.7, 152.6, 151.0, 140.4, 137.5, 132.6, 124.6, 122.2, 120.0, 117.6, 111.5, 83.4, 73.7, 72.3, 68.8, 62.0, 52.6, 22.9, 20.7, 20.6, 20.6. ESI-HRMS: m/z [M+Na⁺] cale for C_{26}H_{24}Br_{2}Na_{2}O_{10}S: 674.0414; found: 674.0418.
N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-(6-nitrocoumarinyl)-1,3-thiazole-2-amine (12g): Dichloromethane/methanol (25:1) as eluent, R<sub>f</sub> = 0.40; pale yellow solid; yield 0.24 g (81%); m.p. 238–239 °C; IR (cm<sup>−1</sup>): 3318, 2927, 1742, 1746, 1652, 1550, 1437, 1317, 1261.6, 1140.2, 1138.7, 1120.6, 1118.5, 1073.7, 1038.9, 1024.5, 1004.9, 917.4, 834.8, 735.7, 68.8, 24.2, 20.9, 20.6, 20.3. ESI-HRMS: m/z [M + Na]<sup>+</sup> calcd for C<sub>27</sub>H<sub>33</sub>N<sub>5</sub>O<sub>14</sub>S<sub>3</sub>: 641.1460; found: 641.1456.

N-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-(6-methoxycoumarinyl)-1,3-thiazole-2-amine (12i): Dichloromethane/methanol (25:1) as eluent, R<sub>f</sub> = 0.33; white solid; yield 0.29 g (95%); m.p. 200–201 °C; IR (cm<sup>−1</sup>): 3434, 2945, 1748, 1730, 1669, 1570, 1501, 1495, 1437, 1317.7, 1261.6, 1140.2, 1138.7, 1120.6, 1118.5, 1073.7, 1038.9, 1024.5, 1004.9, 917.4, 834.8, 735.7, 68.8, 24.2, 20.9, 20.6, 20.3. ESI-HRMS: m/z [M + Na]<sup>+</sup> calcd for C<sub>27</sub>H<sub>33</sub>N<sub>5</sub>O<sub>14</sub>S<sub>3</sub>: 641.1460; found: 641.1456.
Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Postgraduate Research and Practice Innovation Program of Jiangsu Province (KYCX20-2894, KYCX20-2297 and SJCX20-1212), a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Project 521 Funded by Lianyungang (LYG52105-2018023), and the Student Innovation and Entrepreneurship Program (SY201911641319002).

ORCID iD
You-Xian Wang https://orcid.org/0000-0002-5840-488X

References
1. Wenk GL. J Clin Psych 2003; 64: 7.
2. Selkoe DJ. Science 2002; 298: 789.
3. Bookheimer SY, Strojwas MH, Cohen MS, et al. New Engl J Med 2000; 343: 450.
4. Macke L. Nature 2009; 461: 895.
5. Craig LA, Hong NS and McDonald RJ. Neurosci Biobehav R 2011; 35: 1397.
6. Eagger SA, Levy R and Sahakian BJ. Lancet 1991; 337: 989.
7. Lamb HM and Goa KL. Pharmacoeconomics 2001; 19: 303.
8. Yin L, Cheng FC, Li QX, et al. J Chem Res 2016; 40: 545.
9. Abysev AZ, Nguen CB, Ivkin DY, et al. Butlerov Commun 2018; 53: 121.
10. Singh I, Kaur H, Kumar S, et al. Int J Chemtech Res 2010; 2: 1745.
11. Thakur A, Singla R and Jaitak V. Eur J Med Chem 2015; 101: 476.
12. Kumar V, Pandey IP, Jain J, et al. Indian J Pharm Educ 2019; 53: 624.
13. Razavi BM, Arasteh E, Imenshahidi M, et al. Iran J Basic Med Sci 2015; 18: 153.
14. Anand P, Singh B and Singh N. Bioorg Med Chem 2012; 20: 1175.
15. Ali MY, Jannat S, Jung HA, et al. Asian Pac J Trop Med 2016; 9: 103.
16. Chimenti F, Carradori S, Secci D, et al. J Heterocycl Chem 2009; 46: 575.
17. Wang G, He D, Li X, et al. Bioorg Chem 2016; 65: 167.
18. Gouda MA, Berghot MA, Buz EA, et al. Med Chem Res 2012; 21: 1062.
19. Arshad MF, Siddiqui N, Elkerdasy A, et al. Am J Pharmacol Toxicol 2014; 9: 132.
20. Abdellatif KRA, Abdelgawad MA, Elshemy HAH, et al. Lett Drug Des Discov 2017; 14: 773.
21. Dingova D, Leroy J, Check A, et al. Anal Biochem 2014; 462: 67.
22. Ellman GL, Courtney KD, Andres V, et al. Biochem Pharmacol 1961; 7: 88.
23. Shetab-Boushehri SV. EXCLI J 2018; 17: 798.