HETEROCYCLES, Vol. 91, No. 2, 2015, pp. 275 - 286. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 20th November, 2014, Accepted, 8th January, 2015, Published online, 19th January, 2015
DOI: 10.3987/COM-14-13134

SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF SOME NOVEL GLYCOSYL 1,3,4-THIADIAZOLE DERIVATIVES AS ACETYLCHOLINESTERASE INHIBITORS

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Abstract – The corresponding 4-substituted glycosyl thiosemicarbazide derivatives (**6a-6l**) were obtained by the reaction of glycosyl isothiocyanate **4** with various hydrazides. Further cyclization with the system of *p*-TsCl/TEA led to the formation of *N*-glycosyl-5-substituted 1,3,4-thiadiazole-2-amine (**7a-7l**). Subsequent removal of the acetyl groups were conducted using the system of NaOMe/MeOH. The chemical structures of all new products were confirmed by IR, ^{1}H NMR and ESI-HRMS. The acetylcholinesterase (AChE) inhibitory activities of those compounds were tested by Ellman's method. Among them, the compound **8h** possessed the best acetylcholinesterase-inhibition activity with IC_{50} of 18.38 ± 0.89 μM.

INTRODUCTION

Over the past few years, glycobiology, and carbohydrate chemistry have been paid enormous attention owing to the remarkable role played in many biological events.[1-3] D-Glucosamine, as a natural amino monosaccharide, widely exists in ocean organisms, and is an indispensable substance of our human beings' gristle, ligament, muscle tendon.[4] Furthermore, D-glucosamine and its derivatives present many excellent bioactivities such as antitumor, antibacterial, anti-inflammatory, liver-protecting and improving

immune function.[5-9] Recently, the C1-glycosyl heterocycles have given rise to considerable interest, mainly due to their role in a multitude of biological and pharmaceutical applications like biological inhibitors and probes,[10-13] antitumor,[14,15] antimicrobial,[16] and anti-inflammatory.[17]

On the other hand, the 1,3,4-thiadiazoles are commonly utilized heterocyclic pharmacophores, which display a broad spectrum of biological activities including antimicrobial,[18,19] anticancer,[20,21] antioxidant,[22,23] antidepressant,[24,25] anticonvulsant, [26,27] antihypertensive,[28] and antifungal activity.[29,30] In particular, the most significant 2-amino-1,3,4-thiadiazole derivatives are used in treatment of glaucoma,[31] epileptic seizures,[32] hemiplegic migraine,[33] cystinuria,[34] tuberculosis,[35] etc. Moreover, some 1,3,4-thiadiazoles also exhibit high potential employed as acetylcholinesterase inhibitors for the treatment of Alzheimer's disease (AD).[36,37]

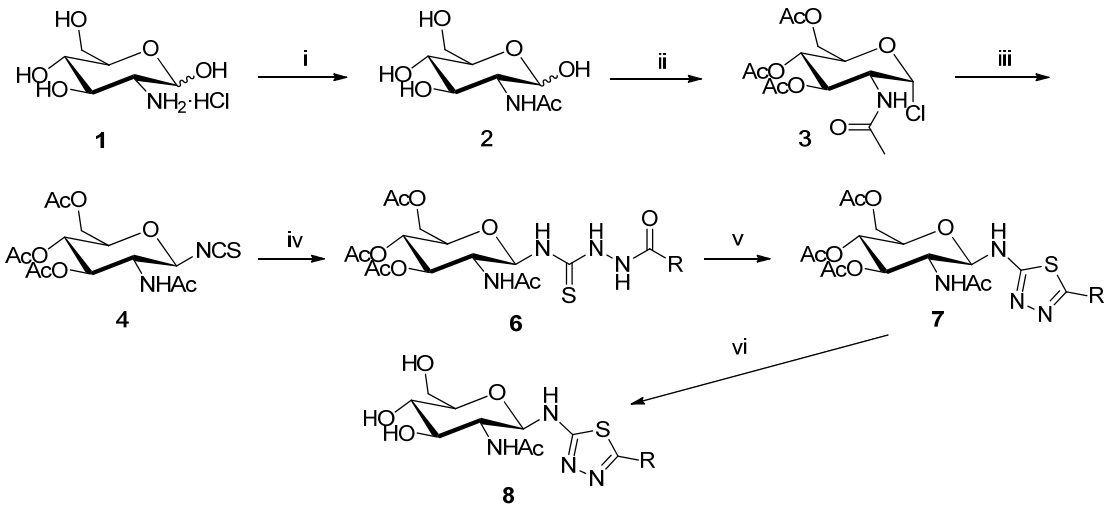
Therefore, it is very interesting for us to report the synthesis of a series of new compounds in which the glycosyl moieties have been used as carriers for the heterocycles possessing thiadiazole ring.

Following our previous studies in searching of novel AChE inhibititors,[38,39] the acetylated GlcNAc of *N*-bit modification didn't show enough inhibitory activity toward AChE. Here, we report a mild and convenient protocol for the synthesis of glycosyl 1,3,4-thiadiazole derivatives via the link of nitrogen glycoside evaluated by Ellman's method in order to screen a novel AChE inhibitor and explore the influence of GlcNAc unit against AChE-inhibition activity.

RESULTS AND DISCUSSION

Chemistry

The starting material, *N*-acetyl-tri-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate **4**, was prepared from the corresponding D-glucosamine hydrochloride **1** via acylation, chlorination and S_N2 reaction.[40,41] Treatment of the isothiocyanate **4** with various hydrazides **5** in ethanol at 78 °C for 2 h afforded the glycosyl acylthiosemicarbazides **6a-6l** with almost quantitative yield. A series of glycosyl 1,3,4-thiadiazoles (**7a-7l**) were synthesized by cyclizing system of *p*-TsCl/TEA in *N*-methylpyrrolidone (NMP) under mild conditions from the compounds **6a-6l** and gave satisfactory isolated yields (65%−89%). The acetyl derivatives **7** were efficiently deprotected to the hydroxyl derivatives **8** in the presence of NaOMe/MeOH. The general synthetic pathway to prepare glycosyl 1,3,4-thiadiazole derivatives was performed in Scheme 1. The chemical structures of all the new compounds were confirmed by IR, ^{1}H NMR and high resolution mass spectroscopy (HRMS), which were in agreement with the proposed structures.



6a, 7a, 8a : R = Me
6b, 7b, 8b: R = C_6H_5
6c, 7c, 8c: R = 4-MeC_6H_4
6d, 7d, 8d: R = 3-$MeOC_6H_4$
6e, 7e, 8e: R = 4-FC_6H_4
6f, 7f, 8f: R = 2-ClC_6H_4
6g, 7g, 8g: R = 4-IC_6H_4
6h, 7h, 8h: R = 4-$NO_2C_6H_4$
6i, 7i, 8i: R = 4-$MeCO_2C_6H_4$
6j, 7j, 8j: R = 4-(*N*, *N*-di-Me)-C_6H_4
6k, 7k, 8k: R = 3-C_6H_4N
6l, 7l, 8l: R = 2-C_5H_3S

Scheme 1: Synthetic pathways of glycosyl 1,3,4-thiadiazoles. Reagents and conditions: (i) Ac_2O, TEA, MeOH, 0 °C; (ii) AcCl, rt; (iii) KSCN, $(n\text{-}C_4H_9)_4NHSO_4$, MeCN, 4 Å molecular sieves; (iv) $RCONHNH_2$ (**5**) EtOH, reflux; (v) *p*-TsCl, TEA, NMP, rt; (vi) NaOMe, MeOH, rt.

In the IR spectra, characteristic absorption bands (the compounds **7**) for fragments: C=O and C-O-C are visible in the ranges of: 1660-1750 cm^{-1} and 1035-1042 cm^{-1}, respectively. There are moderate bands in the region about 3307-3429 cm^{-1} corresponding to *v* (N-H) and band of *v* (C-H) appears in the region 2924-2936 cm^{-1}. In the products **8,** the wide and strong hydroxyl absorption bands in the region 3614-3630 cm^{-1} appeared. Meanwhile, the strong absorption peaks (C=O) about 1743-1750 cm^{-1} disappeared.

In the ^1H NMR spectra, all the compounds **7** showed a characteristic triplet within 5.20–5.35 ppm region having a coupling constant (*J*) in the range of 8.6-9.8 Hz, which indicated sugar ring was *β*-configuration, due to the presence of H_1 proton of the sugar ring coupled by the C_2-H and N-H while the signals of other glycosyl aliphatic protons were shown in the range 3.86-5.20 ppm. In compounds **8**, the presence of only a single peak (CH_3) in the chemical shift of about 2.0 indicated that hydroxy protection groups have been removed. However, the chemical shift of H proton on the sugar ring varied little.

In the mass spectra (ESI), the compounds **7** and **8** all show molecular ion peaks and give $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$ peak stating this kind of compounds easily combine with metal ions. The detailed results of IR, ^1H NMR, and ESI-MS are presented in the experimental part.

Biological activity

The AChE-Inhibition activities of the newly synthesized compounds were evaluated *in vitro* by Ellman's method,[42] in which the AChE extracts from *Electric eel* were used. Their inhibitory potency was described as the inhibition rate and the half of maximal inhibitory concentration, IC_{50}. Tacrine was measured as a standard for the comparative purpose. The results were summarized in Table 1.

Table 1. In vitro inhibitory activities of glycosyl 1,3,4-thiadiazoles against AChE

Compound	R	Inhibition	
		Inhibition (%)[a]	IC_{50} (μM)
7a	Me	< 10	–
7b	C_6H_5	20.34	–
7c	4-MeC_6H_4	24.89	–
7d	3-MeOC_6H_4	19.76	–
7e	4-FC_6H_4	< 10	–
7f	2-ClC_6H_4	43.67	–
7g	4-IC_6H_4	25.60	–
7h	4-$NO_2C_6H_4$	64.52	21.91±1.64
7i	4-$MeCO_2C_6H_4$	< 10	–
7j	4-(*N*,*N*-di-Me)-C_6H_4	53.21	35.14±2.18
7k	3-C_6H_4N	27.53	–
7l	2-C_5H_3S	58.76	28.86±4.24
8c	4-MeC_6H_4	32.64	
8d	3-MeOC_6H_4	23.42	
8h	4-$NO_2C_6H_4$	73.11	18.38±0.89
8j	4-(*N*,*N*-di-Me)-C_6H_4	59.23	30.53±1.32
m[b]		18.12	
n[c]		14.96	
Tacrine			0.26±0.004

[a] The inhibition activities of the compounds at the concentration of 50 μg/mL.
[b] m stands for 5-(4-$NO_2C_6H_4$)-1,3,4-thiadiazole-2-amine.
[c] n stands for 5-[4-(*N*,*N*-di-Me)-C_6H_4]-1,3,4-thiadiazole-2-amine.

As shown in the Table 1, it was found that 13 of the 16 tested compounds showed the AChE-inhibition activities. Among them, the compounds **7h**, **7l** with 4-nitrophenyl and 2-thienyl substituent exhibited a moderate inhibition on AChE with IC_{50} = 21.91 μM and 28.86 μM, respectively. Deprotected compound **8h** showed the best AChE-inhibition activity with the IC_{50} value of 18.38 μM. Compared with the positive control tacrine (IC_{50} = 0.26 μM) of the first drug used for the treatment of AD,[43] it presented

promising inhibition activity against AChE, potentially useful in the treatment of AD. In addition, the compounds m and n showed weak inhibitory against AChE compared with compound **7** and **8**, indicating that the presence of GlcNAc unit improved activities of 1,3,4-thiadiazole compounds. And compound **8c, d, h, j** all revealed higher potency as compared with **7c, d, h, j**, suggesting that the removal of hydroxyl protection groups also improved activities in a certain extent. This may be due to the removal of acetyl groups leading to better water solubility of the compound **8**. Compound **8h** inhibited AChE with a dose-dependent relationship (Figure. 1).

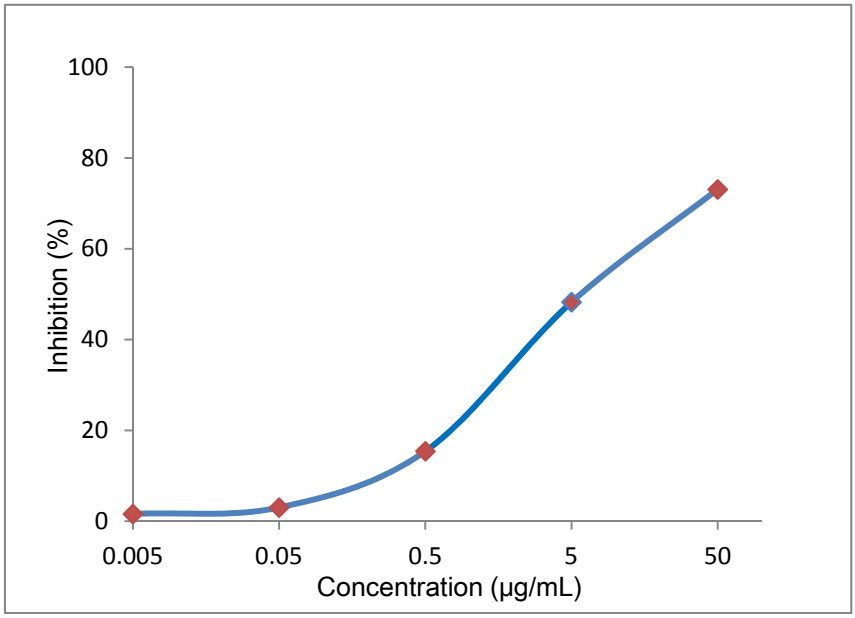


Figure 1. Dose-dependent inhibition of compound **8h** against AChE. Values are means ± SD, n = 3.

EXPERIMENTAL

Chemistry

All chemicals and solvents were purchased from commercial sources and used without further purification unless otherwise stated. Melting points were measured on a Yanaco melting point apparatus and were uncorrected. IR spectra were recorded on a Bruker Tensor 27 spectrometer with KBr pellets. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz instrument using DMSO-d_6 or D_2O as solvents and tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in ppm and coupling constants (*J*) are reported in Hz. HRMS (ESI) analysis was performed on a Agilent 6230 Q-TOF mass spectrometer. The purity of the compounds was checked by TLC on plates precoated with silica gel GF254. Flash column chromatography was performed on silica 200-300 mesh. The compounds **2**, **3** and **4**

were prepared in this laboratory according to the procedure reported in literature.[40,41]

General procedure for the preparation of glycosyl acylthiosemicarbazide (6a−6l)

Glycosyl isothiocyanate **4** (0.388 g, 1 mmol) was added in one portion to a stirred solution of hydrazide **5** (1 mmol) in EtOH (10 mL). The reaction mixture was reflux for 1-2 h, and then the solvent was removed via a rotary evaporator. The residue was recrystallized from aqueous EtOH to obtain the desired product. (The characterization of the representative compound **6a** was only presented in this paper.)

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-*N′*-methyl-acylthiosemicarbazide (6a)**

Yield 84%, white solid, mp 148-149 °C. IR (KBr) ν (cm^{-1}): 3320 (NH), 2972 (C-H), 1749 (CH_3C=O), 1667 (NHC=O), 1044 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 9.79 (br, 1H, NH), 9.72 (br, 1H, NH), 8.21 (d, J = 8.9 Hz, 1H, NH), 7.84 (d, J = 7.3 Hz, 1H, NH), 5.32 (m, 1H, H_1), 5.15 (d, J = 9.4 Hz, 1H, H_3), 4.82 (t, J = 9.8 Hz, 1H, H_4), 4.19-3.81 (m, 4H, H_2, H_5, H_6, $H_{6'}$), 1.99-1.77 (5s, 15H, $5CH_3$). ESI-HRMS (*m/z*): Calcd for $C_{17}H_{26}N_4O_9SNa$ $(M+Na)^+$: 485.1313; Found: 485.1326.

General procedure for the preparation of glycosyl 1,3,4-thiadiazole (7a−7l)

Tosyl chloride (0.21 g, 1.1 mmol) was added to a stirred solution of acylthiosemicarbazide **6** (1 mmol) and triethylamine (0.29 mL, 2.1 mmol) in NMP (5 mL). The solution was stirred at room temperature for 4-6 h (TLC), and extracted with EtOAc (2×10 mL) and distilled water (2×10 mL). After the aqueous layer removed, the aqueous layer was re-extracted thrice with EtOAc (3×10 mL). The organic phases were combined and dried over anhydrous $MgSO_4$. The solid was filtered out and the solution was evaporate to afford the crude product, which was purified by column chromatography on silica gel (MeOH/DCM) to give the pure product.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-methyl-1,3,4-thiadiazole-2-amine (7a)**: Yield 81%, White solid, mp 209-210 °C. IR (KBr) ν (cm^{-1}): 3328 (NH), 2928 (C-H), 1749 (CH_3C=O), 1662 (NHC=O), 1040 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.33 (d, J = 9.3 Hz, 1H, NH), 8.06 (d, J = 9.0 Hz, 1H, NH), 5.20 (t, J = 8.6 Hz, 1H, H_1), 5.15 (t, J = 8.9 Hz, 1H, H_3), 4.85 (t, J = 9.8 Hz, 1H, H_4), 4.20 (dd, J = 12.4, 4.4 Hz, 1H, H_6), 3.97-3.86 (m, 3H, H_2, H_5, $H_{6'}$), 1.98-1.74 (4s, 12H, $COCH_3$), 1.24 (s, 3H, CH_3). ESI-HRMS (*m/z*): Calcd for $C_{17}H_{24}N_4O_8SNa$ $(M+Na)^+$: 467.1207; Found: 467.1202.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-phenyl-1,3,4-thiadiazole-2-amine (7b):** Yield 84%, White solid, mp 225-226 °C. IR (KBr) ν (cm^{-1}): 3358 (NH), 2928 (C-H), 1744

(CH_3C=O), 1670 (NHC=O), 1035 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.77 (d, *J* = 9.1 Hz, 1H, NH), 8.12 (d, *J* = 9.0 Hz, 1H, NH), 7.79 (dd, *J* = 7.2, 2.3 Hz, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 5.31 (t, *J* = 9.5 Hz, 1H, H_1), 5.19 (t, *J* = 9.9 Hz, 1H, H_3), 4.88 (t, *J* = 9.8 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.1 Hz, 1H, H_6), 3.99-3.91 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{22}H_{26}N_4O_8S$-Na $(M+Na)^+$: 529.1364; Found: 529.1365.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-methylphenyl)-1,3,4-thiadiazole-2-amine (7c):** Yield 89%, White solid, mp 207-208 °C. IR (KBr) *v* (cm^{-1}): 3307 (NH), 2924 (C-H), 1747 (CH_3C=O), 1660 (NHC=O), 1037 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.70 (d, *J* = 9.2 Hz, 1H, NH), 8.11 (d, *J* = 9.0 Hz, 1H, NH), 7.67 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.1 Hz, 2H, Ar-H), 5.29 (t, *J* = 9.4 Hz, 1H, H_1), 5.18 (t, *J* = 9.8 Hz, 1H, H_3), 4.88 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.3 Hz, 1H, H_6), 3.99-3 .90 (m, 3H, H_2, H_5, $H_{6'}$), 2.35 (s, 3H, CH_3), 1.98-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{23}H_{29}N_4O_8S$ $(M+H)^+$: 521.1701; Found: 521.1703.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(3-methoxyphenyl)-1,3,4-thiadiazole-2-amine (7d):** Yield 86%, White solid, mp 208-209 °C. IR (KBr) *v* (cm^{-1}): 3269 (NH), 2936 (C-H), 1747 (CH_3C=O), 1668 (NHC=O), 1042 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.77 (d, *J* = 9.1 Hz, 1H, NH), 8.10 (d, *J* = 9.0 Hz, 1H, NH), 7.41 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.33 (t, *J* = 4.4 Hz, 2H, Ar-H), 7.06 (m, 1H, Ar-H), 5.29 (t, *J* = 9.4 Hz, 1H, H_1), 5.19 (t, *J* = 9.8 Hz, 1H, H_3), 4.88 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 3.99-3.91 (m, 3H, H_2, H_5, $H_{6'}$), 3.82 (s, 3H, CH_3), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{23}H_{28}N_4O_9S$ $(M+H)^+$: 537.1650; Found: 537.1662.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-fluorophenyl)-1,3,4-thiadiazole-2-amine (7e):** Yield 89%, White solid, mp 206-207 °C. IR (KBr) *v* (cm^{-1}): 3348 (NH), 2957 (C-H), 1743 (CH_3C=O), 1662 (NHC=O), 1041 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.74 (d, *J* = 9.1 Hz, 1H, NH), 8.09 (d, *J* = 9.0 Hz, 1H, NH), 7.84 (m, 2H, Ar-H), 7.33 (t, *J* = 8.8 Hz, 2H, Ar-H), 5.30 (t, *J* = 9.4 Hz, 1H, H_1), 5.19 (t, *J* = 9.8 Hz, 1H, H_3), 4.88 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 4.00-3.92 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{22}H_{25}FN_4O_8S$-Na $(M+Na)^+$: 547.1269; Found: 547.1269.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(2-chlorophenyl)-1,3,4-thiadiazole-2-amine (7f):** Yield 78%, White solid, mp 198-199 °C. IR (KBr) *v* (cm^{-1}): 3332 (NH), 2930 (C-H), 1748 (CH_3C=O), 1663 (NHC=O), 1039 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.78 (d, *J* = 9.2 Hz, 1H, NH), 8.10 (d, *J* = 9.0 Hz, 1H, NH), 7.99 (m, 1H, Ar-H), 7.64 (m, 1H, Ar-H), 7.51 (m, 2H, Ar-H), 5.33 (t, *J*

= 9.4 Hz, 1H, H_1), 5.19 (t, *J* = 9.8 Hz, 1H, H_3), 4.89 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 4.00-3.93 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{22}H_{25}ClN_4O_8SNa$ $(M+Na)^+$: 563.0974; Found: 563.0970.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-iodophenyl)-1,3,4-thiadiazole-2-amine (7g):** Yield 82%, White solid, mp 232-233 °C. IR (KBr) *v* (cm^{-1}): 3348 (NH), 2927 (C-H), 1743 (CH_3C=O), 1661 (NHC=O), 1041 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.81 (d, *J* = 9.0 Hz, 1H, NH), 8.11 (d, *J* = 9.0 Hz, 1H, NH), 7.86 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.59 (d, *J* = 8.6 Hz, 2H, Ar-H), 5.31 (t, *J* = 9.4 Hz, 1H, H_1), 5.19 (t, *J* = 9.7 Hz, 1H, H_3), 4.89 (t, *J* = 9.9 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 4.01-3.92 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{22}H_{25}IN_4O_8SNa$ $(M+Na)^+$: 655.0330; Found: 655.0316.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-nitrophenyl)-1,3,4-thiadiazole-2-amine (7h):** Yield 83%, Yellow solid, mp 117-118 °C. IR (KBr) *v* (cm^{-1}): 3422 (NH), 2927 (C-H), 1749 (CH_3C=O), 1662 (NHC=O), 1041 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 9.01 (d, *J* = 9.0 Hz, 1H, NH), 8.33 (m, 2H, Ar-H), 8.08 (m, 3H, NH, Ar-H), 5.35 (t, *J* = 9.3 Hz, 1H, H_1), 5.20 (t, *J* = 9.8 Hz, 1H, H_3), 4.90 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 4.02-3.96 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{22}H_{25}N_5O_{10}SNa$ $(M+Na)^+$: 574.1214; Found: 574.1208.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-acetoxyphenyl)-1,3,4-thiadiazole-2-amine (7i):** Yield 76%, White solid, mp 116-117 °C. IR (KBr) *v* (cm^{-1}): 3382 (NH), 2936 (C-H), 1750 (CH_3C=O), 1666 (NHC=O), 1040 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.75 (d, *J* = 9.1 Hz, 1H, NH), 8.09 (d, *J* = 9.1 Hz, 1H, NH), 7.84 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 5.31 (t, *J* = 9.4 Hz, 1H, H_1), 5.19 (t, *J* = 9.8 Hz, 1H, H_3), 4.89 (t, *J* = 9.7 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.4 Hz, 1H, H_6), 3.99-3.91 (m, 3H, H_2, H_5, $H_{6'}$), 2.31 (s, 3H, CH_3), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{24}H_{28}N_4O_{10}SNa$ $(M+Na)^+$: 587.1418; Found: 587.1410.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-*N*,*N*-dimethylphenyl)-1,3,4-thiadiazole-2-amine (7j):** Yield 88%, White solid, mp 197-198 °C. IR (KBr) *v* (cm^{-1}): 3429 (NH), 2927 (C-H),1748 (CH_3C=O), 1662 (NHC=O), 1042 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.50 (d, *J* = 9.2 Hz, 1H, NH), 8.09 (d, *J* = 8.9 Hz, 1H, NH), 7.58 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.76 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.26 (t, *J* = 9.4 Hz, 1H, H_1), 5.16 (m, 1H, H_3), 4.87 (dd, *J* = 11.5, 8.5 Hz, 1H, H_4), 4.21 (m, 1H, H_6), 4.00-3.93 (m, 3H, H_2, H_5, $H_{6'}$), 2.98 (s, 6H, CH_3), 1.99-1.75 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*):

Calcd for $C_{24}H_{31}N_5O_8SNa$ $(M+Na)^+$: 572.1786; Found: 572.1776.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(3-pyridyl)-1,3,4-thiadiazole-2-amine (7k):** Yield 71%, Yellow solid, mp 113-114 °C. IR (KBr) *v* (cm^{-1}): 3426 (NH), 2930 (C-H), 1749 (CH_3C=O), 1663 (NHC=O), 1040 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.98 (d, *J* = 2.1 Hz, 1H, Pyridine), 8.89 (d, *J* = 9.0 Hz, 1H, NH), 8.65 (dd, *J* = 4.8, 1.5 Hz, 1H, Pyridine), 8.18 (m, 1H, Pyridine), 8.13 (d, *J* = 9.0 Hz, 1H, NH), 7.53 (dd, *J* = 7.9, 4.8 Hz, 1H, Pyridine), 5.33 (t, *J* = 9.3 Hz, 1H, H_1), 5.19 (t, *J* = 9.9 Hz, 1H, H_3), 4.89 (t, *J* = 9.8 Hz, 1H, H_4), 4.23 (dd, *J* = 12.4, 4.3 Hz, 1H, H_6), 4.00-3.93 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{21}H_{25}N_5O_8SNa$ $(M+Na)^+$: 530.1316; Found: 530.1320.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-5-(2-thienyl)-1,3,4-thiadiazole-2-amine (7l):** Yield 65%, White solid, mp 201-202 °C. IR (KBr) *v* (cm^{-1}): 3418 (NH), 2926 (C-H), 1745 (CH_3C=O), 1668 (NHC=O), 1037 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ: 8.76 (d, *J* = 9.1 Hz, 1H, NH), 8.10 (d, *J* = 9.0 Hz, 1H, NH), 7.68 (dd, *J* = 5.1, 1.1 Hz, 1H, Thiophene), 7.50 (dd, *J* = 3.7, 1.1 Hz, 1H, Thiophene), 7.15 (dd, *J* = 5.1, 3.7 Hz, 1H, Thiophene), 5.27 (t, *J* = 9.4 Hz, 1H, H_1), 5.18 (t, *J* = 9.8 Hz, 1H, H_3), 4.88 (t, *J* = 9.8 Hz, 1H, H_4), 4.22 (dd, *J* = 12.4, 4.5 Hz, 1H, H_6), 4.00-3.91 (m, 3H, H_2, H_5, $H_{6'}$), 1.99-1.76 (4s, 12H, $COCH_3$). ESI-HRMS (*m/z*): Calcd for $C_{20}H_{24}N_4O_8S_2Na$ $(M+Na)^+$: 535.0928; Found: 535.0930.

General procedure for deprotection of *O*-acetyl-substituted compounds 7 to hydroxy derivatives 8

A solution of compound **7** (0.5 mmol) in MeOH (5 mL) was treated with sodium methoxide (1 M in MeOH, 0.2 mL). The mixture was stirred at ambient temperature for 2 h and then neutralized with Amberlite IR 120 H+ resin and filtered. The filtrate was concentrated to afford the deprotected product **8**. Selected products **8c, d, h, j** are characterized below.

***N*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-5-(4-methylphenyl)-1,3,4-thiadiazole-2-amine (8c):** Yield 90%, White solid, mp 156-157 °C. IR (KBr) *v* (cm^{-1}): 3414 (OH), 2928 (C-H), 1626 (NHC=O), 1074 (C-O-C). ^{1}H NMR (400 MHz, D_2O) δ: 7.66 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.81 (t, *J* = 8.7 Hz, 1H, H_1), 3.66-3.15 (m, 6H, H_2, H_3, H_4, H_5, H_6), 2.34 (s, 3H, CH_3), 1.81 (s, 3H, CH_3). ESI-HRMS (*m/z*): Calcd for $C_{17}H_{22}N_4O_5SNa$ $(M+Na)^+$: 417.1203; Found: 417.1202.

***N*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-5-(3-methoxyphenyl)-1,3,4-thiadiazole-2-amine (8d):** Yield 88%, White solid, mp 101-102 °C. IR (KBr) *v* (cm^{-1}): 3416 (NH), 2934 (C-H), 1629 (NHC=O), 1044 (C-O-C). ^{1}H NMR (400 MHz, D_2O) δ: 7.39 (m, 1H, Ar-H), 7.32 (m, 2H, Ar-H), 7.03 (m, 1H, Ar-H),

4.85 (t, *J* = 9.0 Hz, 1H, H_1), 3.76-3.21 (m, 6H, H_2, H_3, H_4, H_5, H_6), 3.80 (s, 3H, CH_3), 1.82 (s, 3H, CH_3). ESI-HRMS (*m/z*): Calcd for $C_{17}H_{22}N_4O_6SNa$ $(M+Na)^+$: 433.1152 ; Found: 433.1150.

***N*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-5-(4-nitrophenyl)-1,3,4-thiadiazole-2-amine (8h):** Yield 81%, Yellow solid, mp 175-176 °C. IR (KBr) *v* (cm^{-1}): 3430 (OH), 2926 (C-H), 1634 (NHC=O), 1048 (C-O-C). ^1H NMR (400 MHz, D_2O) δ: 8.30 (m, 2H, Ar-H), 8.10 (m, 2H, Ar-H), 4.92 (t, *J* = 8.9 Hz, 1H, H_1), 3.81-3.26 (m, 6H, H_2, H_3, H_4, H_5, H_6), 1.84 (s, 3H, CH_3). ESI-HRMS (*m/z*): Calcd for $C_{16}H_{19}N_5O_7SNa$ $(M+Na)^+$: 448.0897 ; Found: 448.0895.

***N*-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-5-(4-*N*,*N*-dimehylphenyl)-1,3,4-thiadiazole-2-amine (8j):** Yield 84%, White solid, mp 184-185 °C. IR (KBr) *v* (cm^{-1}): 3412 (OH), 2925 (C-H), 1627 (NHC=O), 1074 (C-O-C). ^1H NMR (400 MHz, D_2O) δ: 7.63 (d, *J* = 7.3 Hz, 2H, Ar-H), 6.83 (d, *J* = 7.2 Hz, 2H, Ar-H), 4.91 (d, *J* = 9.4 Hz, 1H, H_1), 3.73-3.24 (m, 6H, H_2, H_3, H_4, H_5, H_6), 3.02 (s, 6H, CH_3), 1.90 (s, 3H, CH_3). ESI-HRMS (*m/z*): Calcd for $C_{18}H_{25}N_5O_5SNa$ $(M+Na)^+$: 446.1469; Found: 446.1468.

Biological tests

Acetylcholinesterase (AChE, from *Electric eel*), acetylthiocholine iodide (ATCI), 5,5-dithiobis-(2-nitro-benzoic acid) (DTNB) and tacrine were purchased from Sigma-Aldrich (America). AChE activities were measured by Ellman's colorimetric method with a slight modification,[42] using tacrine as the reference compounds. In assays, 10 μL of rat cortex homogenate was incubated with 10 μL of tested compounds and 140 μL of 0.1 M PBS (pH 8.0) for 10 min in 96-well microplates before addition of 20 μL of 3.33 mM DTNB solution and 20 μL of 5.30 mM ATCI solution. After the addition of DTNB and ATCI, the 96-well microplates were read at 412 nm with a microplate reader (SPECTRAFLUOR, TECAN, Sunrise, Austria) for 15 min. One triplicate sample without inhibitors was always present to yield 100% of AChE activity. One triplicate sample with 20 μM tacrine was always present to yield 100% AChE inhibition (to obviate the ChE-independent substrate hydrolysis). The reaction rates were compared and the percent inhibition due to the presence of tested compounds was calculated. Each concentration was assayed in triplicate. The 50% inhibitory concentration (IC_{50}) was calculated from a dose-response curve obtained by plotting the percentage of inhibition versus the log concentration with the use of Origin 8.0 software. The results were described as the mean ± standard deviation (SD).

ACKNOWLEDGEMENTS

We are grateful to the Major Project of Natural Science Research in Colleges and Universities in Jiangsu

Education Committee (No.10KJA170003), the Industrialization Project of Scientific Research in Colleges and Universities of Jiangsu Province (No.JHB2012-60), the Science and the Technology Critical Project Foundation of Lianyungang (No.CG1105), Collegiate Natural Science Fund of Jiangsu Province (12KJB350001) and Science and Technology Project of Lianyungang (CG1202).

REFERENCES

1. Y. E. Tsvetkov, M. Burg-Roderfeld, and G. Loers, *J. Am. Chem. Soc*., 2011, **134**, 426.
2. M. Kimura, Y. Masui, and Y. Shirai, *Carbohydr. Res*., 2013, **374**, 49.
3. R. M. Schmaltz, S. R. Hanson, and C. H. Wong, *Chem. Rev*., 2011, **111**, 4259.
4. A. Varki, D. C. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, and M. E. Etzler, *Essentials of Glycobiology, 2nd ed.* Cold Spring Habor Laboratory Press: New York, 2009.
5. Y. Guan, Y. Tian, Y. Li, Z. F. Yang, Y. Y. Jia, and T. J. Hang, *J. Pharm. Biomed*., 2011, **55**, 181.
6. B. Liberek, A. Melcer, A. Osuch, and R. Wakiecb, *Carbohydr. Res*., 2005, **340**, 1876.
7. S. S. Shivatare, S. H. Chang, T. I. Tsai, C. T. Ren, H. Y. Chuang, C. W. Lin, S. T. Li, C. Y. Wu, and C. H. Wong, *J. Am. Chem. Soc*., 2013, **135**, 153821.
8. V. Pozsgay, J. Kubler-Kielb, P. Santacroce, J. B. Robbins, and R. Schneerson, *J. Org. Chem.*, 2012, **77**, 5922.
9. A. Fekete, D. Eszenyi, M. Herczeg, and V. Pozsgay, *Carbohydr. Res*., 2014, **386**, 33.
10. S. Kun, G. Z. Nagy, M. Tóth, L. Czecze, T. Docsa, P. Gergely, M. D. Charavgi, T. Patonay, and L. Somsák, *Carbohydr. Res*., 2011, **346**, 1427.
11. S. Bera and R. J. Linhardt, *J. Org. Chem*., 2011, **76**, 3181.
12. A. Chibba, S. Dasgupta, N. Yakandawala, S. Madhyastha, and M. Nitz, *J. Carbohydr. Chem*., 2011, **30**, 549.
13. M. E. Jung, T. A. Dong, and X. Cai, *Tetrahedron Lett*., 2011, **52**, 2533.
14. A. Drozdová, P. Bojarová, K. Křenek, L. Weignerova, B. Hensen, L. Elling, H. Christensen, H. H. Jensen, H. Pelantova, and M. Kuzma, *Carbohydr. Res*., 2011, **346**, 1599.
15. K. Du, X. Cao, P. Zhang, and H. Zheng, *Bioorg. Med. Chem. Lett*., 2014, DOI: 10.1016/ j.bmcl. 2014.09.042.
16. K. A. Dilmaghani, F. N. Pur, N. H. Jazani, A. Alavi, Z. Niknam, and F. Mirfakhraee, *Phosphorus, Sulfur Silicon Relat. Elem*., 2014, **189**, 81.
17. A. R. El-Gazzar, H. N. Hafez, and H. A. S. Abbas, *Eur. J. Med. Chem*., 2009, **44**, 4249.
18. B. Chandrakantha, A. M. Isloor, P. Shetty, H. K. Fun, and G. Hegde, *Eur. J. Med. Chem*., 2014, **44**, 4249.
19. K. R. Alagawadi and S. G. Alegaon, *Arabian J. Chem*., 2011, **4**, 465.
20. M. Juszczak, J. Matysiak, M. Szeliga, P. Pozarowski, and W. Rzeski, *Bioorg. Med. Chem. Lett*., 2012,

22, 5466.
21. M. N. Noolvi, H. M. Patel, N. Singh, A. K. Gadad, and A. Badiger, *Eur. J. Med. Chem*., 2011, **46**, 4411.
22. D. Sunil, A. M. Isloor, P. Shetty, K. Satyamoorthy, and A. S. B. Prasad, *Arabian J. Chem*., 2010, **3**, 211.
23. I. Khan, S. Ali, S. Hameed, N. H. Rama, M. T. Hussain, A. Wadood, R. Uddin, Z. Ul-Haq, A. Khan, S. Ali, and M. I. Choudhary, *Eur. J. Med. Chem*., 2010, **45**, 5200.
24. F. Clerici, D. Pocar, M. Guido, A. Loche, V. Perlini, and M. Brufani, *J. Med. Chem*., 2001, **44**, 931.
25. S. Jubie, P. N. Ramesh, P. Dhanabal, R. Kalirajana, N. Murugananthamb, and A. S. Antonyc, *Eur. J. Med. Chem*., 2012, **54**, 931.
26. V. Jatav, P. Mishra, S. Kashaw, and J. P. Stablesb, *Eur. J. Med. Chem*., 2008, **43**, 1945.
27. K. P. Harish, K. N. Mohana, and L. S. Mallesha, *Drug Invention Today*, 2013, **5**, 92.
28. T. Hasui, N. Matsunaga, T. Ora, N. Ohyabu, N. Nishigaki, Y. Imura, Y. Igata, H. Matsui, T. Motoyaji, N. Habuka, S. Sogabe, and M. Ono, *J. Med. Chem*., 2011, **54**, 8616.
29. P. Zoumpoulakis, C. Camoutsis, G. Pairas, M. Sokovic, J. Glamoclija, C. Potamitis, and A. Pitsas, *Bioorg. Med. Chem*., 2012, **20**, 1569.
30. W. M. Xu, S. Yang, P. Bhadury, J. He, M. He, L. L. Gao, and B. A. Song, *Pestic. Biochem. Physiol*., 2011, **101**, 6.
31. I. P. Kaur, R. Smitha, D. Aggarwal, and M. Kapil, *Int. J. Pharm*., 2002, **248**, 1.
32. P. Wolf, *CNS Neurosci. Ther*., 2011, **17**, 442.
33. M. B. Russell and A. Ducros, *Lancet Neurol*., 2011, **10**, 457.
34. H. G. Tiselius, *Curr. Opin. Urol*., 2010, **20**, 169.
35. S. Talath and A. K. Gadad, *Eur. J. Med. Chem*., 2006, **41**, 918.
36. A. Skrzypek, J. Matysiak, M. M. Karpinska, and A. Niewiadomy, *Enzyme Inhib. Med. Chem*., 2013, **28**, 816.
37. A. Skrzypek, J. Matysiak, A. Niewiadomy, M. Bajda, and P. Szymanski, *Eur. J. Med. Chem*., 2013, **62**, 311.
38. W. W. Liu, D. H. Shi, C. Z. Tao, Y. Q. Zhao, and L. J. Tang, Proceedings of 2009 International Conference of Natural Products and Traditional Medicine, Xi'An, China, October 16-18, 2009 [C]; Berlin: Springer.
39. D. H. Shi, W. Huang, C. Li, L.T. Wang, and S. F. Wang, *Bioorg. Med. Chem*., 2013, **21**, 1064.
40. V. R. Bouvet and R. N. Ben, *J. Org. Chem*., 2006, **71**, 3619.
41. M. J. Amarasa, P. Fernandez-Resa, and M. T. Garcia-Lopez, *Synthesis*, 1984, 509.
42. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Feather-Stone, *Biochem. Pharmacol*., 1961, **7**, 88.
43. J. L. Cummings, *Am. J. Psychiatry*, 2000, **157**, 4.