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Synthesis and bioactivity of novel C2-glycosyl oxadiazole derivatives as acetylcholinesterase inhibitors

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Abstract: A series of glycosyl-substituted 1,3,4-oxadiazoles were synthesized by cyclization of glycosyl-acylthiosemicarbazides via a base-catalyzed reaction. The starting glycosyl-acylthiosemicarbazide derivatives were obtained by the reaction of glycosyl isothiocyanate with various hydrazides. The acetylcholinesterase (AChE) inhibitory activities of the products were tested by Ellman's method. The most active compounds were subsequently evaluated for the 50% inhibitory concentration (IC_{50}) values. *N*-(1,-3,4,6-tetra-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole-2-amine (**6i**) possesses the best AChE -inhibition activity with an IC_{50} of 1.61 ± 0.34 μM.

Keywords: Acetylcholinesterase inhibitors; glycosyl 1,3,4-oxadiazoles; synthesis.

Introduction

Carbohydrates have long interested chemists and biochemists as a large natural resource [1–4]. As an energy source and an element of many metabolic processes, sugars are present in every part of the human body [5]. D-glucosamine is a naturally occurring amino sugar [6, 7], one of the most abundant monosaccharides, and has been widely used in the prevention and/or treatment of rheumatoid arthritis and osteoarthritis [8, 9]. Furthermore, D-glucosamine exhibits a broad variety of bioactivities such as anti-inflammatory [10], anti-cancer [11] and antibacterial [12] properties and it suppresses tumor growth [13]. Modified carbohydrates provide access to potential mimetics of naturally occurring amino sugars and represent targets for the development of anti-oxidant [14], anti-acetylcholinesterase (AChE) [15], anti-proliferative [16] and other active agents [17–20].

The emergence of heterocyclic compounds with novel structures may promote discovery of new drugs and treatment of stubborn diseases [21–24]. Recently, the oxadiazole chemistry has been developed extensively. 1,3,4-Oxadiazoles structurally resemble amides and esters [25], and some compounds show similar pharmacokinetic properties [26–31]. Moreover, recent studies have shown that a number of compounds containing the 1,3,4-oxadiazole skeleton act as monoamine oxidase inhibitors for the treatment of Alzheimer's disease (AD) [32–34]. AD is a chronic, neurodegenerative disorder that causes an irreversible dementia in elderly people [35].

Researchers have been interested in molecular hybrid-based approaches to find new compounds of potential biological activities [36, 37]. Based on the aforementioned information, herein we report the design and synthesis of novel D-glucosamine/1,3,4-oxadiazole hybrids with the oxadiazole ring offering an important pharmacophore to discover new potent AChE inhibitors. The synthesized derivatives were evaluated by Ellman's method for AChE inhibitors and to explore the influence of D-glucosamine against AChE inhibition.

Results and discussion

Chemistry

In the development of C2-glycosyl oxadiazoles, glycosyl isothiocyanate **3** was the critical intermediate compound.

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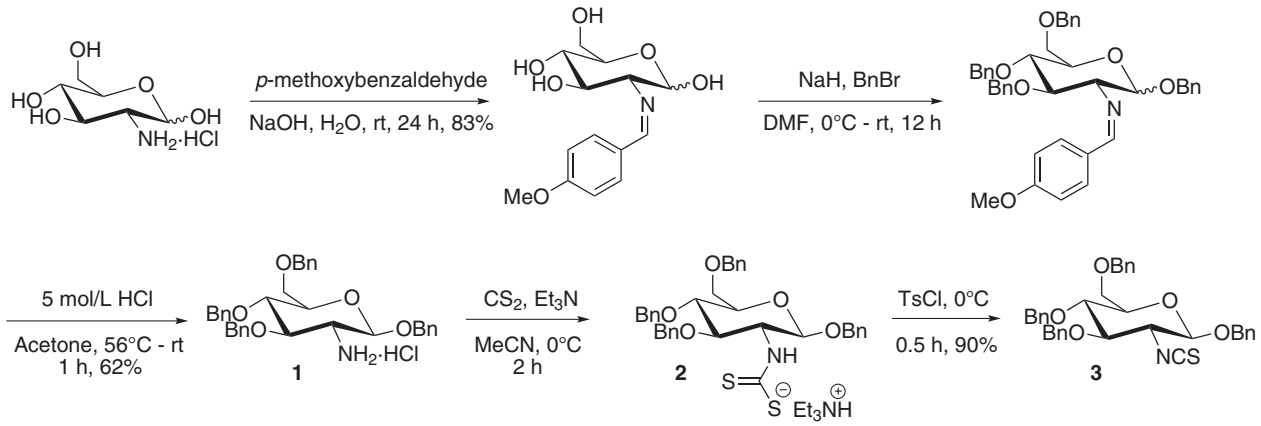
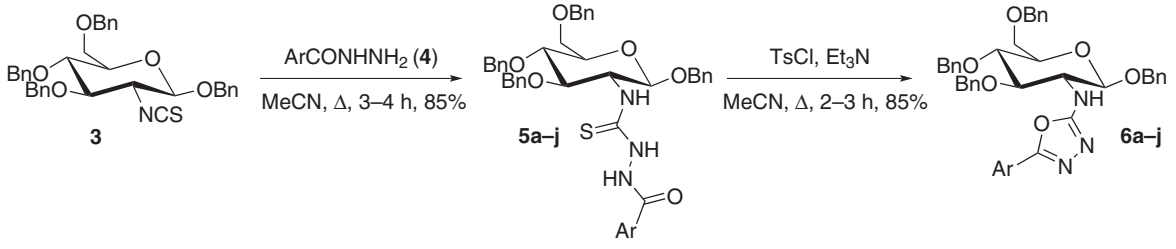


Scheme 1



a: Ar = C_6H_5
b: Ar = 4-CH_3-C_6H_4
c: Ar = 2-thienyl
d: Ar = 4-pyridyl
e: Ar = 3-OCH_3-C_6H_4
f: Ar = 2-Cl-C_6H_4
g: Ar = 4-OH-C_6H_4
h: Ar = 4-I-C_6H_4
i: Ar = 4-F-C_6H_4
j: Ar = 4-(N,N-di-Me)-C_6H_4

Scheme 2

Table 1 *In vitro* inhibitory activities of glycosyl oxadiazoles against AChE.

Compound	Inhibition (%)[a]	IC_{50} (μM)
1	46.2	–
6a	47.7	–
6b	59.4	–
6c	92.1	2.6 ± 0.5
6d	90.2	4.2 ± 0.2
6e	55.1	–
6f	91.3	3.1 ± 0.4
6g	68.3	–
6h	83.3	11.6 ± 0.8
6i	96.8	1.6 ± 0.3
6j	91.1	2.7 ± 0.5
m[b]	17.3	–
n[c]	14.5	–
Tacrine		0.269 ± 0.004
Galantamine		2.67 ± 0.15

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

Our strategy began with an attempted synthesis of this starting material without protection of hydroxyl groups at D-glucosamine hydrochloride but, to our disappointment, this attempt failed. The benzyl group was used to protect the hydroxyl groups. 1,3,4,6-Tetra-*O*-benzyl-*β*-D-glucosamine hydrochloride **1** was synthesized according to the literature [38, 39]. Treatment of compounds **1** with triethylamine in acetonitrile was followed by the addition of carbon disulfide, which furnished dithiocarbamic acid salt **2**. Subsequent reaction of **2** with tosyl chloride (*p*-TsCl) yielded the key glycosyl isothiocyanate immediate **3** (Scheme 1). Compound **3** was treated with various hydrazides **4** to yield the glycosyl acylthiosemicarbazide derivatives **5a–j**. Cyclization of the intermediate products **5** in a base-catalyzed reaction afforded the glycosyl oxadiazole derivatives **6a–j** in high yields (Scheme 2).

Biological activity

The AChE-inhibition activities of the compounds were evaluated *in vitro* by Ellman's method [40] using the AChE extract from *electric eel*. The results are summarized in Table 1. Selected compounds were subsequently evaluated for the maximal inhibitory concentration,

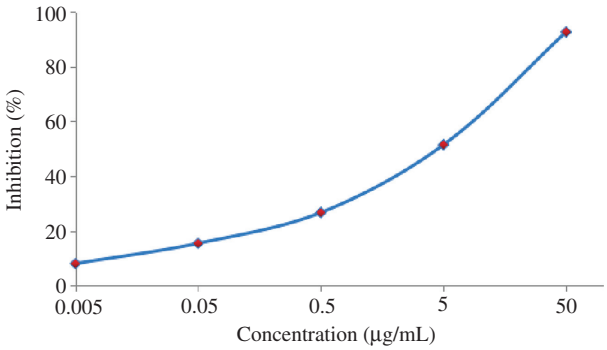


Figure 1 Dose-dependent inhibition of AChE by compound **6i**. Values are presented as mean ± SD, n = 3.

IC_{50}, with tacrine and galantamine as the reference compounds. The results indicate that all compounds show higher inhibitory activities against AChE than the precursor compound **n**. The best compound **6i** shows the highest activity with an IC_{50} value of 1.61 ± 0.34 against AChE and inhibits AChE in a dose-dependent relationship (Figure 1). Compounds **1** and **m** demonstrate weak inhibition of AChE compared with compound **6i**, suggesting that the presence of 1,3,4-oxadiazole unit improves the activity.

Conclusion

A new series of C2-glycosyl oxadiazole derivatives were designed, synthesized and subjected to biological evaluation. These compounds were characterized by NMR, IR and HRMS. Most of the compounds are active against AChE. Compound **6i** shows the best AChE-inhibition activity with an IC_{50} of 1.61 ± 0.34 μM.

Experimental

Chemistry

All chemicals were purchased from commercial sources and used without further purification. Melting points were determined on a Yanaco melting point apparatus and were uncorrected. IR spectra were recorded on a Bruker Tensor 27 spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz at ambient temperature using dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent and tetramethylsilane (TMS) as an internal standard. HRMS (ESI) analysis was performed on an Agilent 6230 mass spectrometer. Flash column chromatography was performed on silica 200–300 mesh.

2-Amino-1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose hydrochloride (1)

A solution of D-glucosamine hydrochloride (10 g, 46.4 mmol) in water (70 mL) at room temperature was stirred and treated with NaOH (1.86 g, 46.5 mmol), and 15 min later dropwise with *p*-methoxybenzaldehyde (5.7 mL, 46.6 mmol). The mixture was stirred at ambient temperature for an additional 24 h, after which time the resulting white solid was filtered and washed with 500 mL of water to afford 2-(4-methoxy benzylidene)-2-deoxy-*β*-D-glucopyranose (11.4 g, 83%). A mixture of this product and BnBr (14 mL, 118 mmol) in dimethylformamide (DMF) (50 mL) at 0°C was treated portion-wise with NaH (60%, 5 g, 125 mmol). The mixture was stirred at room temperature for 12 h, then diluted with a large amount of water and extracted with CH_2Cl_2 (3 × 50 mL). The extract was concentrated under reduced pressure to give a yellow viscous liquid. The solution of the yellow liquid in acetone (100 mL) was treated with hydrochloric acid (7 mL, 5 N) to afford a white solid of **1** after reflux for 1 h. Product **1** was washed with acetone; yield 7.9 g (62%).

2-Isothiocyanato-1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose (3)

A solution of 1,3,4,6-tetra-*O*-benzyl-*β*-D-glucosamine hydrochloride **1** (1 mmol) and triethylamine (3 mmol) in acetonitrile (15 mL) was cooled in an ice bath and treated dropwise with carbon disulfide (1 mmol). The mixture was stirred for 2 h, then treated with *p*-TsCl (1 mmol) and stirred for another 0.5 h on the ice bath. The precipitate of product **3** was crystallized from ethanol; yield 90% of white amorphous powder; mp 55–56°C; IR: 3433, 3030, 2873, 2078, 1454, 1359, 1313, 1068 cm^{-1}; ^{1}H NMR: δ 7.45–7.25 (m, 18H, ArH), 7.24–7.17 (dd, *J* = 7 Hz, 2H, ArH), 4.81 (dd, *J* = 16, 10 Hz, 4H, $PhCH_2$, HGlu), 4.73–4.62 (m, 2H, $PhCH_2$), 4.61–4.48 (m, 3H, $PhCH_2$), 3.95–3.87 (m, 2H, HGlu), 3.67 (ddd, *J* = 14, 12, 7 Hz, 3H, HGlu), 3.54 (dd, *J* = 12, 7 Hz, 1H, HGlu); ESI-HRMS (*m/z*): Calcd for $C_{35}H_{35}NNaO_5S$ $[M+Na]^+$: 604.2128; found: 604.2130.

General procedure for the preparation of *N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-aryl-1,3,4-oxadiazole-2-amines 6a–j

Glycosyl isothiocyanate **3** (0.58 g, 1 mmol) was added in one portion to a stirred solution of hydrazide **4** (1 mmol) in MeCN (10 mL). The reaction mixture was heated under reflux for 3–4 h, and then the solvent was eliminated under reduced pressure. The residue was crystallized from aqueous ethanol to obtain the desired product **5a–j**. *p*-TsCl (0.21 g, 1.1 mmol) was added to a stirred solution of acylthiosemicarbazide **5** (1 mmol) and triethylamine (0.16 mL, 2.0 mmol) in MeCN (10 mL). The mixture was stirred for about 2–3 h at 81°C, and the reaction progress was monitored by thin-layer chromatography (TLC). The desired compound **6a–j** was purified by column chromatography on silica gel eluting with ethyl acetate/petroleum ether (1:2) to give a white amorphous product.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-phenyl-1,3,4-oxadiazole-2-amine (6a)** Yield 86%; mp 166–168°C; IR (cm^{-1}): 3421, 3226, 3060, 2924, 1630, 1496, 1453, 1397, 1116, 1064; ^1H NMR: δ 8.15 (d, *J* = 9 Hz, 1H, NH), 7.86–7.80 (m, 2H, ArH), 7.56–7.50 (m, 3H, ArH), 7.40–7.27 (m, 8H, ArH), 7.25–7.12 (m, 12H, ArH), 4.82 (d, *J* = 12 Hz, 1H, H-1^{Glu}), 4.80–4.65 (m, 4H, $PhCH_2$), 4.62–4.51 (m, 4H, $PhCH_2$), 3.85–3.69 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.65–3.50 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{42}H_{41}N_3NaO_6$ $[M+Na]^+$: 706.2888; found: 706.2890.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-methylphenyl)-1,3,4-oxadiazole-2-amine (6b)** Yield 88%; mp 159–160°C; IR (cm^{-1}): 3446, 3170, 2923, 1629, 1400, 1071, 1028; ^1H NMR: δ 8.08 (d, *J* = 9 Hz, 1H, NH), 7.71 (d, *J* = 8 Hz, 2H, ArH), 7.42–7.27 (m, 10H, ArH), 7.25–7.13 (m, 12H, ArH), 4.82 (d, *J* = 12 Hz, 1H, H-1^{Glu}), 4.78–4.70 (m, 3H, $PhCH_2$), 4.68–4.52 (m, 5H, $PhCH_2$), 3.83–3.68 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.63–3.52 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}), 2.40–2.32 (s, 3H, CH_3); ESI-HRMS (*m/z*): Calcd for $C_{43}H_{43}N_3NaO_6$ $[M+Na]^+$: 720.3044; found: 720.3046.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(2-thienyl)-1,3,4-oxadiazole-2-amine (6c)** Yield 84%; mp 147–148°C; IR (cm^{-1}): 3420, 3201, 3026, 2923, 1496, 1468, 1400, 1114, 1066; ^1H NMR: δ 8.36 (d, *J* = 9 Hz, 1H, NH), 7.66 (d, *J* = 8 Hz, 1H, ArH), 7.42 (d, *J* = 4 Hz, 1H, ArH), 7.40–7.29 (m, 8H, ArH), 7.27–7.11 (m, 13H, ArH), 4.82 (d, *J* = 12.5 Hz, 1H, H-1^{Glu}), 4.76–4.65 (m, 4H, $PhCH_2$), 4.63–4.52 (m, 4H, $PhCH_2$), 3.86 (t, *J* = 8 Hz, 1H, H-4^{Glu}), 3.78–3.67 (m, 2H, H-6a^{Glu}, H-6b^{Glu}), 3.61–3.50 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{40}H_{39}N_3NaO_6S$ $[M+Na]^+$: 712.2452; found: 712.2456.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-pyridyl)-1,3,4-oxadiazole-2-amine (6d)** Yield 86%; mp 182–183°C; IR (cm^{-1}): 3421, 3230, 3060, 2924, 1627, 1465, 1398, 1061, 1028; ^1H NMR: δ 8.98 (d, *J* = 2 Hz, 1H, ArH), 8.70 (dd, *J* = 5, 2 Hz, 1H, ArH), 8.26 (d, *J* = 9 Hz, 1H, NH), 8.16 (2t, *J* = 2 Hz, 1H, ArH), 7.56 (dd, *J* = 8.5 Hz, 1H, ArH), 7.40–7.27 (m, 8H, ArH), 7.25–7.10 (m, 12H, ArH), 4.82 (d, *J* = 12 Hz, 1H, H-1^{Glu}), 4.79–4.64 (m, 4H, $PhCH_2$), 4.62–4.52 (m, 4H, $PhCH_2$), 3.84–3.69 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.65–3.55 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{41}H_{40}N_4NaO_6$ $[M+Na]^+$: 707.2840; found: 707.2843.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(3-methoxyphenyl)-1,3,4-oxadiazole-2-amine (6e)** Yield 90%; mp 147–148°C; IR (cm^{-1}): 3426, 3174, 3028, 2926, 1598, 1497, 1453, 1400, 1216, 1125, 1069, 1043; ^1H NMR: (δ 8.37 (d, *J* = 9 Hz, 1H, NH), 7.43–7.27 (m, 12H, ArH), 7.25–7.15 (m, 11H, ArH), 7.05–7.01 (m, 1H, ArH), 4.83 (d, *J* = 12.5 Hz, 1H, H-1^{Glu}), 4.78–4.67 (m, 4H, $PhCH_2$), 4.64–4.51 (m, 4H, $PhCH_2$), 3.87 (t, *J* = 8 Hz, 1H, H-4^{Glu}), 3.82 (s, 3H, OCH_3), 3.77–3.68 (m, 2H, H-6a^{Glu}, H-6b^{Glu}), 3.61–3.51 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{43}H_{43}N_3NaO_7$ $[M+Na]^+$: 736.2993; found: 736.2998.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole-2-amine (6f)** Yield 83%; mp 121–122°C; IR (cm^{-1}): 3421, 3231, 3062, 2924, 1627, 1508, 1454, 1397, 1362, 1149, 1076, 1028; ^1H NMR: δ 8.25 (d, *J* = 9 Hz, 1H, NH), 7.78 (dd, *J* = 8, 1.8 Hz, 1H, ArH), 7.65 (dd, *J* = 8, 1.1 Hz, 1H, ArH), 7.58–7.47 (m, 2H, ArH), 7.40–7.27 (m, 8H, ArH), 7.26–7.13 (m, 12H, ArH), 4.83 (d, *J* = 12.5 Hz, 1H, H-1^{Glu}), 4.79–4.63 (m, 4H, $PhCH_2$), 4.62–4.51 (m, 4H, $PhCH_2$), 3.84–3.68 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.63–3.50 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{42}H_{40}ClN_3NaO_6$ $[M+Na]^+$: 740.2498; found: 740.2501.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-hydroxylphenyl)-1,3,4-oxadiazole-2-amine (6g)** Yield 86%; mp 181–182°C; IR (cm^{-1}): 3421, 3142, 3030, 2956, 1650, 1611, 1497, 1398, 1279, 1173, 1072, 1027; ^1H NMR: δ 10.10 (s, 1H, OH), 7.98 (d, *J* = 9 Hz, 1H, NH), 7.66 (t, *J* = 9 Hz, 2H, ArH), 7.41–7.12 (m, 20H, ArH), 6.89 (d, *J* = 9 Hz, 2H, ArH), 4.81 (d, *J* = 12 Hz, 1H, H-1^{Glu}), 4.78–4.50 (m, 8H, $PhCH_2$), 3.83–3.68 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.69–3.50 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{42}H_{41}N_3NaO_7$ $[M+Na]^+$: 722.2837; found: 722.2838.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-iodinylphenyl)-1,3,4-oxadiazole-2-amine (6h)** Yield 82%; mp 176–177°C; IR (cm^{-1}): 3446, 3229, 3059, 2922, 1627, 1478, 1453, 1397, 1362, 1203, 1055, 1005; ^1H NMR: δ 8.19 (d, *J* = 9 Hz, 1H, NH), 7.90 (d, *J* = 8.5 Hz, 2H, ArH), 7.58 (d, *J* = 8.5 Hz, 2H, ArH), 7.40–7.27 (m, 8H, ArH), 7.25–7.10 (m, 12H, ArH), 4.81 (d, *J* = 12 Hz, 1H, H-1^{Glu}), 4.77–4.61 (m, 4H, $PhCH_2$), 4.60–4.50 (m, 4H, $PhCH_2$), 3.83–3.67 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.64–3.51 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{42}H_{40}IN_3NaO_6$ $[M+Na]^+$: 832.1854; found: 832.1860.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole-2-amine (6i)** Yield 88%; mp 156–157°C; IR (cm^{-1}): 3425, 3179, 3029, 2923, 1601, 1518, 1498, 1400, 1219, 1124, 1070; ^1H NMR: δ 8.34 (d, *J* = 9 Hz, 1H, NH), 7.84–7.77 (m, 2H, ArH), 7.41–7.26 (m, 11H, ArH), 7.25–7.13 (m, 11H, ArH), 4.82 (d, *J* = 12.5 Hz, 1H, H-1^{Glu}), 4.76–4.66 (m, 4H, $PhCH_2$), 4.63–4.51 (m, 4H, $PhCH_2$), 3.86 (t, *J* = 8 Hz, 1H, H-4^{Glu}), 3.78–3.67 (m, 2H, H-6a^{Glu}, H-6b^{Glu}), 3.62–3.52 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}); ESI-HRMS (*m/z*): Calcd for $C_{42}H_{40}FN_3NaO_6$ $[M+Na]^+$: 724.2793; found: 724.2796.

***N*-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-5-(4-dimethylaminophenyl)-1,3,4-oxadiazole-2-amine (6j)** Yield 83%; mp 123–124°C; IR (cm^{-1}): 3422, 3234, 3060, 2906, 1633, 1614, 1519, 1397, 1197, 1068, 1028; ^1H NMR: δ 7.89 (d, *J* = 9 Hz, 1H, NH), 7.62 (d, *J* = 9 Hz, 2H, ArH), 7.41–7.25 (m, 8H, ArH), 7.24–7.15 (m, 12H, ArH), 6.80 (d, *J* = 9 Hz, 2H, ArH), 4.81 (d, *J* = 12.5 Hz, 1H, H-1^{Glu}), 4.78–4.62 (m, 4H, $PhCH_2$), 4.60–4.51 (m, 4H, $PhCH_2$), 3.83–3.66 (m, 3H, H-4^{Glu}, H-6a^{Glu}, H-6b^{Glu}), 3.63–3.49 (m, 3H, H-5^{Glu}, H-3^{Glu}, H-2^{Glu}), 2.98 (s, 6H, CH_3); ESI-HRMS (*m/z*): Calcd for $C_{44}H_{46}N_4NaO_6$ $[M+Na]^+$: 749.3310; found: 749.3312.

In vitro cholinesterase activity assay

AChE, acetylthiocholine iodide (ATCI), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), galantamine and tacrine were purchased from Sigma-Aldrich. AChE activities were measured using Ellman's colorimetric method with a slight modification [39]; galantamine and tacrine were the reference compounds. For the determination, a 96-well plate was used as the carrier. First, 130 μL of buffer solution, 20 μL of AChE solution, 20 μL of color developer and 10 μL of methanol were added to the first column of the 96-well plate as a control blank system. Then, 130 μL of buffer solution, 20 μL of AChE solution, 20 μL of developer and 10 μL of the analyte solution were added. After all samples were added, the plate was treated with 20 μL of substrate and shaken evenly. The plate was quickly placed in the microplate reader and the temperature was maintained at 20–25°C. The reaction rates were compared and the percent inhibition due to the presence of tested compounds was calculated. All samples were 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 the Origin 8.0 software. The results were described as mean ± standard deviation (SD).

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