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

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Abstract: New C2-glycosyl triazole derivatives **6a–l** were synthesized by cyclization of glycosyl acylthiosemicarbazides **5** in refluxing 3 N sodium hydroxide aqueous solution. Substrates **5** were obtained by the reaction of glycosyl isothiocyanate **3** with various hydrazides. The acetylcholinesterase (AChE) inhibitory activities of compounds **6** were tested by Ellman's method. Compounds that exhibited over 85% inhibition were subsequently evaluated for the IC_{50} values. Compound **6f** possesses the best acetylcholinesterase-inhibition activity with IC_{50} of 1.46 ± 0.25 μg/mL.

Keywords: acetylcholinesterase; C2-glycosyl triazole derivatives; synthesis.

Introduction

Carbohydrates play an important role in biological and industrial applications [1–6]. D-Glucosamine is a naturally occurring amino sugar [7, 8] that has been widely used for the prevention and treatment of rheumatoid arthritis and osteoarthritis [9, 10]. It also exhibits a broad variety of bioactivities including anti-inflammatory [11], antioxidant [12], antibacterial [13] and antitumor properties [14]. Modified naturally occurring amino sugars are used for the development of anti-proliferative [15], anti-acetylcholinesterase [16], anticandidal [17] and other active agents [18–21].

In recent years, triazoles and their fused heterocyclic derivatives have received considerable attention owing to their importance in drug discovery [22–24]. 1,2,4-Triazoles and their derivatives are commonly utilized heterocyclic pharmacophores, which are an important class of heterocyclic molecules presenting numerous biological activities such as antimicrobial [25], antiproliferative [26], antiviral [27], anti-inflammatory [28] and anticonvulsant [29] activities. Recent studies have shown that many compounds containing the triazole skeleton act as choline esterase inhibitors for the treatment of Alzheimer's disease [30–32].

Up to now, researchers have been interested in molecular hybrid-based approaches to find some new compounds of potential biological activities [33–35]. Based on these findings and our previous work, in an attempt to discover new potent acetylcholinesterase (AChE) inhibitors, we designed and synthesized a series of novel C2-glycosyl triazole derivatives. The synthesized compounds were screened by Ellman's method to explore the influence of D-glucosamine for AChE-inhibition activity.

Results and discussion

Chemistry

The starting material, 1,3,4,6-tetra-*O*-benzyl-*β*-D-glucosamine hydrochloride (**1**), was synthesized as per the literature [36, 37]. Treatment of compound **1** with triethylamine in acetonitrile followed by addition of carbon disulfide to the mixture and stirring for 2 h furnished dithiocarbamic acid salt **2**. Subsequent reaction of **2** with tosyl chloride (TsCl) yielded the key glycosyl isothiocyanate product **3** (Scheme 1) [38].

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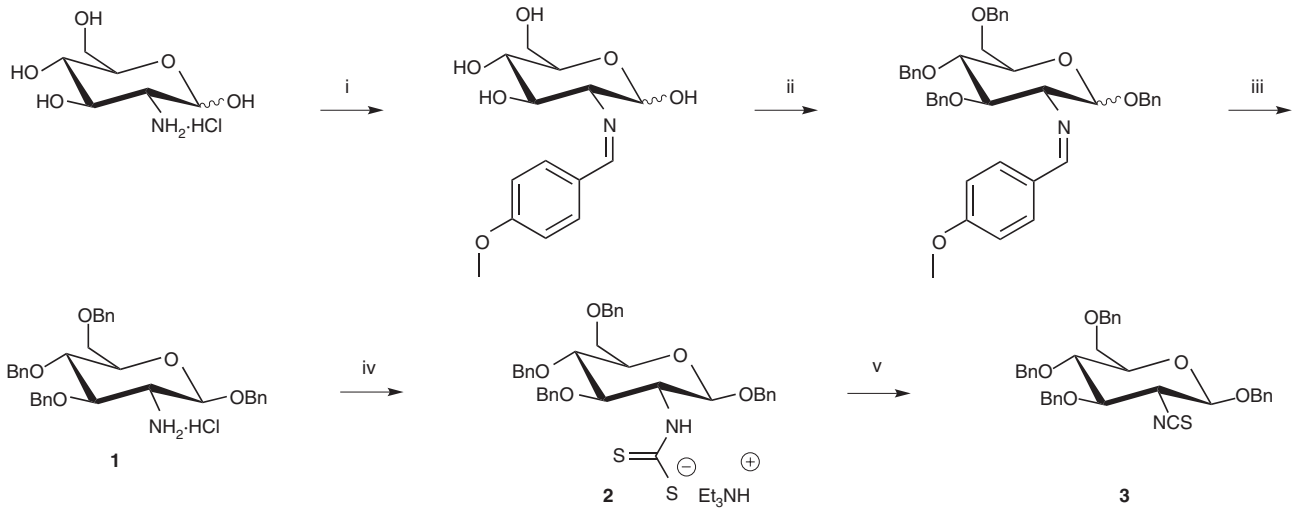


Scheme 1 Synthesis of 1,3,4,6-tetra-*O*-benzyl-2-deoxy-β-D-glucopyranose-2-isothiocyanate (**3**).
Reagents and conditions: (i) NaOH, *p*-methoxybenzaldehyde, H_2O, rt; (ii) NaH, BnBr, DMF, 0°C – rt; (iii) 5 N HCl, acetone, reflux; (iv) Et_3N, CS_2, 0°C, 1.5 h; (v) TsCl, 0.5 h.

The glycosyl isothiocyanate **3** was treated with various hydrazides **4** to yield the glycosyl acylthiosemicarbazide derivatives **5a–l**. Compounds **5** were cyclized in 3 N sodium hydroxide solution for 5 h to furnish the glycosyl triazoles **6a–l** in high yield (Scheme 2) [39].

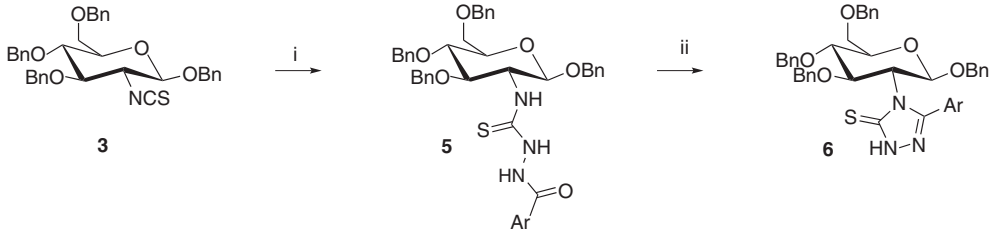
Biological activity

The AChE-inhibition activities of compounds **6** were evaluated *in vitro* by Ellman's method [40], in which the AChE extracts from *Electric eel* were used. Their inhibitory potency is defined as the inhibition rate and the half of maximal inhibitory concentration, IC_{50}. The results are summarized in Table 1.

As shown in Table 1, all compounds are better inhibitors of AChE than D-glucosamine hydrochloride (**m**). Eight of the 12 tested compounds that exhibited over 85% inhibition were subsequently evaluated for the IC_{50} value with tacrine and galantamine used as reference drugs. The best compound **6f** shows the IC_{50} value of 1.461 and inhibit AChE with a dose-dependent relationship (Figure 1). Other compounds are much less active than tacrine and galantamine.

Conclusion

New C2-glycosyl triazole derivatives were designed, synthesized and subjected to biological evaluation. The AChE inhibitor activity data revealed that most of the synthesized



4a,**5a**,**6a**: Ar = Ph
4b,**5b**,**6b**: Ar = 2-thienyl
4c,**5c**,**6c**: Ar = 2-FC_6H_4
4d,**5d**,**6d**: Ar = 2-ClC_6H_4
4e,**5e**,**6e**: Ar = 4-OHC_6H_4
4f,**5f**,**6f**: Ar = 4-$CH_3C_6H_4$
4g,**5g**,**6g**: Ar = 4-$NO_2C_6H_4$
4h,**5h**,**6h**: Ar = 4-$[(CH_3)_2N]C_6H_4$
4i,**5i**,**6i**: Ar = 4-FC_6H_4
4j,**5j**,**6j**: Ar = 4-ClC_6H_4
4k,**5k**,**6k**: Ar = 4-BrC_6H_4
4l,**5l**,**6l**: Ar = 4-IC_6H_4

Scheme 2 Synthesis of products **6**.
Reagents and conditions: (i) $ArCONHNH_2$ (**4a–l**), acetonitrile, reflux; (ii) 3 N NaOH, reflux.

Table 1 *In vitro* inhibitory activities of glycosyl triazoles against AchE.

Compound	Ar	Inhibition (%)[a]	IC_{50} (μg/mL)
6a	C_6H_5	94.07 ± 1.36	5.64 ± 0.86
6b	2-C_5H_3S	92.43 ± 1.05	4.51 ± 0.49
6c	2-FC_6H_4	92.11 ± 0.99	2.16 ± 0.20
6d	2-ClC_6H_4	85.51 ± 0.48	9.14 ± 0.41
6e	4-OHC_6H_4	79.01 ± 0.83	–
6f	4-$CH_3C_6H_4$	94.43 ± 1.60	1.46 ± 0.25
6g	4-$NO_2C_6H_4$	64.41 ± 3.41	–
6h	4-4-(*N*,*N*-di-Me)-C_6H_4	69.39 ± 2.42	–
6i	4-FC_6H_4	98.38 ± 1.05	3.41 ± 0.54
6j	4-ClC_6H_4	93.96 ± 0.86	1.99 ± 0.13
6k	4-BrC_6H_5	87.23 ± 0.89	9.68 ± 0.89
6l	4-IC_6H_5	74.42 ± 1.55	–
m[b]	–	14.46 ± 1.49	–
n[c]	–	19.80 ± 1.84	–
Tacrine		98.46 ± 0.13	0.0533 ± 0.0008
Galantamine		92.17 ± 0.17	0.767 ± 0.043

[a]The inhibition activities at the concentration of 50 μg/mL.
[b]m stands for D-glucosamine hydrochloride.
[c]n stands for 5-(4-methylphenyl)-1,2,4-triazole-3-thione.

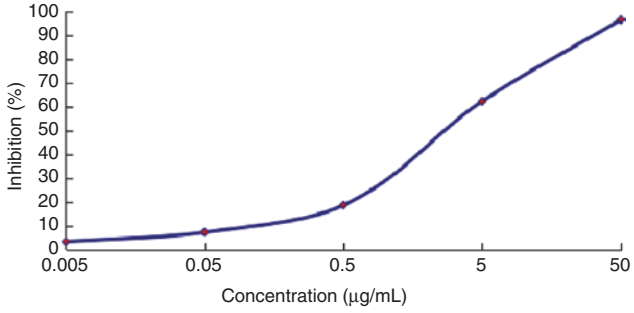


Figure 1 Dose-dependent inhibition of AChE by compound **6f**. Values are means ± SD, n = 3.

compounds are active against acetyl cholinesterase enzymes. The present study finds that it is ineffective to remove the benzyl group by catalytic hydrogenation using Pd/C or $Pd(OH)_2$/C due to the poisoning of the catalyst by the sulfur atom in the molecule. In the following work, we will be searching for other methods to solve this problem.

Experimental

Chemistry

All chemicals were purchased from commercial sources and used without further purification. All reactions were monitored by thin layer chromatography (TLC) using plates and visualized with 254 nm ultra violet (UV) light. Melting points were determined on a Yanaco melting point apparatus and are uncorrected. Infra red (IR) spectra were recorded on a Bruker Tensor 27 spectrometer with KBr pellets. ^{1}H NMR spectra were recorded with a Bruker Avance spectrometer at 500 Hz using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as a solvent. ESI-HRMS analysis was performed on an Agilent 6500 mass spectrometer. Flash column chromatography was performed using a silica gel 200–300 mesh.

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

Sodium hydroxide (1.86 g, 46.5 mmol) was added to a solution of D-glucosamine hydrochloride (10 g, 46.4 mmol) in water (70 mL) at room temperature with stirring followed by dropwise addition of *p*-methoxybezaldehyde (5.7 mL, 46.6 mmol) 15 min later. The mixture was stirred at ambient temperature for an additional 24 h, after which time the resulting white solid was filtrated and washed with 500 mL water to afford 2-(4-methoxy benzylidene)-2-deoxy-*β*-D-glucopyranose (11.4 g, 83%). A mixture of this product (6.6 g, 22.2 mmol) and BnBr (14 mL, 117.9 mmol) in DMF (50 mL) was treated portion-wise at 0°C with NaH (60%, 5 g, 125 mmol) and then stirred at room temperature for 12 h. After addition of a large amount of water, the mixture was extracted with dichloromethane (3 × 50 mL). The extract was concentrated under reduced pressure and the resultant yellow viscous liquid was dissolved in acetone (100 mL). Treatment of this solution with hydrochloric acid (7 mL, 5 N) and heating under reflux for 1 h afforded a white solid of **1**. The product was filtered off and washed with acetone: yield 7.9 g, (62%).

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

To a solution of 1,3,4,6-tetra-*O*-benzyl-*β*-D-glucosamine hydrochloride (**1**,1 mmol) in acetonitrile (15 mL) was added triethylamine (3 mmol). The mixture was cooled in an ice bath, treated dropwise via a syringe pump with carbon disulfide (1 mmol) and stirred for 2 h. Then tosyl chloride (TsCl) (1 mmol) was added and the mixture was stirred for an additional 0.5 h. Product **3** was crystallized from ethanol; yield 90%, white amorphous powder; mp 55–56°C; IR: *ν* 3433, 3030, 2873, 2078, 1454, 1359, 1313, 1068 cm^{-1}; ^{1}H NMR: δ 7.48–7.19 (m, 20H), 4.81 (dd, *J* = 10.0 Hz and 5.0 Hz, 4H), 4.74–4.63 (m, 2H), 4.61–4.48 (m, 3H), 3.94–3.86 (m, 2H), 3.68–3.47 (m, 3H), 3.54 (t, *J* = 9.0 Hz, 1H). ESI-HRMS. Calcd for $C_{35}H_{35}NNaO_5S$, $[M+Na]^+$: *m/z* 604.2128. Found: *m/z* 604.2130.

General procedure for the preparation of compounds 6a–l

Glycosyl isothiocyanate **3** (0.581 g, 1 mmol) was added in one portion to a stirred solution of hydrazide **4a–l** (1 mmol) in MeCN (10 mL). The mixture was heated under reflux for 3–4 h and then concentrated under reduced pressure to give **5a–l**. Without purification, **5a–l** was added to 3 N sodium hydroxide aqueous solution (20 mL). The

mixture was heated under reflux for 5–6 h and then extracted with dichloromethane (3×10 mL). The extract was washed with water, dried over anhydrous sodium sulfate and concentrated. Compound **6a–l** was purified by silica gel column chromatography eluting with AcOEt/petroleum ether.

5-Phenyl-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6a) Yield 88%, white solid; mp 139–140°C; IR: v 3431 (NH), 3088 (C-H, Ph), 2927 (CH_2-Ph), 1557 (C=N), 1359 (C=S), 1048 cm^{-1} (C-O-C); ^1H NMR: δ 14.08 (s, 1H), 7.57–7.44 (m, 5H, Ar-H), 7.36–7.24 (m, 14H, Ar-H), 7.23–7.19 (m, 2H, Ar-H), 7.18–7.12 (m, 2H, Ar-H), 7.07–7.03 (m, 2H, Ar-H), 6.21 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.65 (dd, *J* = 10.0 Hz and 8.0 Hz, 1H, H^{Glu}-3), 4.82 (d, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.71 (dd, *J* = 11.0 Hz and 4.0 Hz, 2H, $PhCH_2$), 4.59–4.48 (m, 4H, $PhCH_2$), 4.41 (d, *J* = 11.0 Hz, 1H, $PhCH_2$), 3.93 (t, *J* = 9.0 Hz, 1H, H^{Glu}-4), 3.75–3.63 (m, 3H, H^{Glu}-5,6,6), 3.50 (t, *J* = 8.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{42}N_3O_5S$, $[M+H]^+$: *m/z* 700.2840. Found: *m/z* 700.2832.

5-(2-Thienyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6b) Yield 93%, pale yellow solid; mp 145–146°C; IR: v 3447 (NH), 3073 (C-H, Ph), 2929 (CH_2-Ph), 1582 (C=N), 1359 (C=S), 1076 cm^{-1} (C-O-C); ^1H NMR: δ 14.13 (s, 1H, -NH), 7.86 (d, *J* = 5.0 Hz, 1H, thiophene), 7.46 (d, *J* = 5.0 Hz, 1H, thiophene), 7.39–7.31 (m, 7H, Ar-H), 7.30–7.20 (m, 10 H, Ar-H), 7.15–7.10 (m, 2H, Ar-H, thiophene), 7.02 (s, 2H, Ar-H), 6.18 (d, *J* = 8.5 Hz, 1H, H^{Glu}-1), 5.59 (t, *J* = 9.0 Hz, 1H, H^{Glu}-3), 4.82 (d, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.74–4.68 (m, 2H, $PhCH_2$), 4.60–4.51 (m, 4H, $PhCH_2$), 4.39 (d, *J* = 11.5 Hz, 1H, $PhCH_2$), 4.15 (t, *J* = 9.0 Hz, 1H, H^{Glu}-4), 3.77–3.64 (m, 4H, H^{Glu}-5,6,6′), 3.58 (t, *J* = 9.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{40}H_{40}N_3O_5S_2$, $[M+H]^+$: *m/z* 706.2406. Found: *m/z* 706.2405.

5-(2-Fluorophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6c) Yield 91%, white solid; mp 112–113°C; IR: v 3433 (NH), 3089 (C-H, Ph), 2925 (CH_2-Ph), 1560 (C=N), 1359 (C=S), 1059 cm^{-1} (C-O-C); ^1H NMR: δ 14.25 (s, 1H, -NH), 7.68–7.60 (m, 1H, Ar-H), 7.42 (t, *J* = 9.0 Hz, 1H, Ar-H), 7.38–7.25 (m, 16H, Ar-H), 7.20 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.15 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.10 (d, *J* = 4.5 Hz, 2H, Ar-H), 6.12 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.61 (t, *J* = 9.0 Hz, 1H, H^{Glu}-3), 4.80 (d, *J* = 12.5 Hz, 1H, $PhCH_2$), 4.73–4.65 (m, 2H, $PhCH_2$), 4.58–4.43 (m, 5H, $PhCH_2$), 3.73–3.58 (m, 4H, H^{Glu}-4,5,6,6′), 3.47 (t, *J* = 9.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{41}FN_3O_5S$, $[M+H]^+$: *m/z* 718.2745. Found: *m/z* 718.2744.

5-(2-Chlorophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6d) Yield 84%, white solid; mp 106–107°C; IR: v 3421 (NH), 3063 (C-H, Ph), 2926 (CH_2-Ph), 1604 (C=N), 1361 (C=S), 1061 cm^{-1} (C-O-C); ^1H NMR: δ 14.18 (s, 1H, -NH), 7.62–7.34 (m, 3H, Ar-H), 7.33–7.24 (m, 15H, Ar-H), 7.23–7.16 (m, 4H, Ar-H), 7.15–7.13 (m, 2H, Ar-H), 6.11 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.63 (t, *J* = 9.0 Hz, 1H, H^{Glu}-3), 4.88–4.73 (m, 2H, $PhCH_2$), 4.71–4.61 (m, 1H, $PhCH_2$), 4.56–4.45 (m, 5H, $PhCH_2$), 3.69 (t, *J* = 9.0 Hz, 1H, H^{Glu}-4), 3.68–3.47 (m, 3H, H^{Glu}-5,6,6′), 3.45 (t, *J* = 9.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{40}ClN_3NaO_5S$, $[M+Na]^+$: *m/z* 756.2269. Found: *m/z* 756.2265.

5-(4-Hydroxyphenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6e) Yield 82%, white solid; mp 96–97°C; IR: v 3418 (NH), 3201 (O-H), 3062 (C-H, Ph), 2924 (CH_2-Ph), 1513 (C=N), 1386 (C=S), 1053 cm^{-1} (C-O-C); ^1H NMR: δ 13.92 (s, 1H, -NH), 10.07 (s, 1H, OH), 7.38–7.24 (m, 16H, Ar-H), 7.21 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.14 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.03 (d, *J* = 7.0 Hz, 2H, Ar-H), 6.83 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.20 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.62 (t, *J* = 9.0 Hz, 1H, H^{Glu}-3), 4.81 (d, *J* = 12.5 Hz, 1H, $PhCH_2$), 4.68 (dd, *J* = 11.0, 7.5 Hz, 2H, $PhCH_2$), 4.59–4.49 (m, 4H, $PhCH_2$), 4.38 (d, *J* = 11.0 Hz, 1H, $PhCH_2$), 3.95 (t, *J* = 9.0 Hz, 1H, H^{Glu}-4), 3.75–3.62 (m, 3H, H^{Glu}-5,6,6′), 3.50 (t, *J* = 9.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{42}N_3O_6S$, $[M+H]^+$: *m/z* 716.2789. Found: *m/z* 716.2785.

5-(4-Methylphenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6f) Yield 88%, white solid; mp 136–137°C; IR: v 3439 (NH), 3089 (C-H, Ph), 2948 (CH_2-Ph), 1516 (C=N), 1360 (C=S), 1057 cm^{-1} (C-O-C); ^1H NMR: *δ* 14.02 (s, 1H, NH), 7.39–7.32 (m, 7H, Ar-H), 7.31–7.25 (m, 11H, Ar-H), 7.24–7.20 (m, 2H, Ar-H), 7.17–7.12 (m, 2H, Ar-H), 7.07–7.02 (m, 2H, Ar-H), 6.20 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.63 (t, *J* = 8.5 Hz, 1H, H^{Glu}-3), 4.82 (d, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.71 (d, *J* = 11.0 Hz, 2H, $PhCH_2$), 4.58–4.48 (m, 4H, $PhCH_2$), 4.40 (d, *J* = 11.0 Hz, 1H, $PhCH_2$), 3.92 (t, *J* = 8.5 Hz, 1H, H^{Glu}-4), 3.75–3.63 (m, 3H, H^{Glu}-5,6,6′), 3.49 (t, *J* = 8.0 Hz, 1H, H^{Glu}-2), 3.30 (s, 3H, CH_3). ESI-HRMS. Calcd for $C_{43}H_{44}N_3O_5S$, $[M+H]^+$: *m/z* 714.2996. Found: *m/z* 714.2999.

5-(4-Nitrophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6g) Yield 79%, yellow solid; mp 103–104°C; IR: v 3424 (NH), 3090 (C-H, Ph), 2924 (CH_2-Ph), 1559 (C=N), 1347 (C=S), 1057 cm^{-1} (C-O-C); ^1H NMR: *δ* 14.28 (s, 1H, -NH), 8.41–8.31 (m, 2H, Ar-H), 7.82–7.69 (m, 2H, Ar-H), 7.44–6.99 (m, 20H, Ar-H), 6.19 (t, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.63 (t, *J* = 8.5 Hz, 1H, H^{Glu}-3), 4.81 (t, *J* = 11.0 Hz, 1H, $PhCH_2$), 4.77–4.67 (m, 2H, $PhCH_2$), 4.60–4.48 (m, 4H, $PhCH_2$), 4.41 (t, *J* = 10.0 Hz, 1H, $PhCH_2$), 3.89 (t, *J* = 8.5 Hz, 1H, H^{Glu}-4), 3.75–3.63 (m, 3H, H^{Glu}-5,6,6), 3.56 (t, *J* = 8.5 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{41}N_4O_7S$, $[M+H]^+$: *m/z* 745.2690. Found: *m/z* 745.2694.

5-(4-*N*,*N*-Dimethylphenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6h) Yield 81%, pale yellow solid; mp 131–132°C; IR: v 3423 (NH), 3087 (C-H, Ph), 2868 (CH_2-Ph), 1614 (C=N), 1362 (C=S), 1058 cm^{-1} (C-O-C); ^1H NMR: δ 13.87 (s, 1H), 7.37–7.25 (m, 16H, Ar-H), 7.25–7.21 (m, 2H, Ar-H), 7.17–7.13 (m, 2H, Ar-H), 7.06–7.02 (m, 2H, Ar-H), 6.75 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.22 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.64 (dd, *J* = 10.5 Hz and 8.0 Hz, 1H, H^{Glu}-3), 4.83 (d, *J* = 12.5 Hz, 1H, $PhCH_2$), 4.69 (t, *J* = 10.0 Hz, 2H, $PhCH_2$), 4.59–4.49 (m, 4H, $PhCH_2$), 4.40 (d, *J* = 11.0 Hz, 1H, $PhCH_2$), 4.03 (dd, *J* = 10.5, 8.5 Hz, 1H, H^{Glu}-4), 3.75–3.63 (m, 3H, H^{Glu}-5,6,6′), 3.49 (t, *J* = 8.0 Hz, 1H, H^{Glu}-2), 2.95 (s, 6H, $-CH_3$). ESI-HRMS. Calcd for $C_{44}H_{45}N_4NaO_5S$, $[M+Na]^+$: *m/z* 765.3081. Found: *m/z* 765.3072.

5-(4-Fluorophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6i) Yield 87%, white solid; mp 127–128°C; IR: v 3429 (NH), 3063 (C-H, Ph), 2869 (CH_2-Ph), 1611 (C=N), 1361 (C=S), 1058 cm^{-1} (C-O-C); ^1H NMR: *δ* 14.25 (s, 1H, -NH), 7.68–7.60 (m, 1H, Ar-H), 7.42–7.25 (m, 17H, Ar-H), 7.20–7.10 (m, 6H, Ar-H), 6.12 (d, *J* = 8.0 Hz, 1H, H^{Glu}-1), 5.61 (dd, *J* = 10.0, 8.5 Hz, 1H, H^{Glu}-3), 4.80 (d, *J* = 12.0 Hz, 1H, $PhCH_2$), 4.73–4.65 (m, 2H, $PhCH_2$), 4.58–4.43 (m, 5H, $PhCH_2$), 3.75–3.58 (m, 4H, H^{Glu}-4,5,6,6′), 3.47 (t, *J* = 8.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{41}FN_3O_5S$, $[M+H]^+$: *m/z* 718.2745. Found: *m/z* 718.2748.

5-(4-Chlorophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6j) Yield 85%, white solid; mp 123–124°C. IR: v 3446 (NH), 3088 (C-H, Ph), 2941 (CH_2-Ph), 1606 (C=N), 1361 (C=S), 1057 cm^{-1} (C-O-C); ^1H NMR: *δ* 14.12 (s, 1H, -NH),

7.53–7.48 (m, 2H, Ar-H), 7.38–7.26 (m, 16H, Ar-H), 7.25–7.21 (m, 2H, Ar-H), 7.17–7.12 (m, 2H, Ar-H), 7.07–7.03 (m, 2H, Ar-H), 6.19 (d, J = 8.0 Hz, 1H, H^{Glu}-1), 5.63 (dd, J = 10.0, 8.0 Hz, 1H, H^{Glu}-3), 4.82 (d, J = 12.5 Hz, 1H, $PhCH_2$), 4.71 (dd, J = 11.0 Hz and 5.0 Hz, 2H, $PhCH_2$), 4.60–4.48 (m, 4H, $PhCH_2$), 4.40 (d, 1H, J = 11.0 Hz, $PhCH_2$), 3.87 (dd, J = 10.5, 8.0 Hz, 1H, H^{Glu}-4), 3.75–3.63 (m, 3H, H^{Glu}-5,6,6), 3.52 (t, J = 8.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{41}ClN_3O_5S$, $[M+H]^+$: *m/z* 734.2450. Found: *m/z* 734.2447.

5-(4-Bromophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6k) Yield 90%, white solid; mp 132–133°C. IR: v 3427 (NH), 3086 (C-H, Ph), 2941 (CH_2-Ph), 1602 (C=N), 1357 (C=S), 1058 cm^{-1} (C-O-C); ^1H NMR: δ 14.17 (s, 1H, NH), 7.75 (d, J = 7.0 Hz, 2H, Ar-H), 7.40–7.24 (m, 18H, Ar-H), 7.16–6.95 (m, 4H, Ar-H), 6.19 (d, J = 7.0 Hz, 1H, H^{Glu}-1), 5.62 (t, J = 8.5 Hz, 1H, H^{Glu}-3), 4.82 (d, J = 11.5 Hz, 1H, $PhCH_2$), 4.76–4.64 (m, 2H, $PhCH_2$), 4.57–4.47 (m, 4H, $PhCH_2$), 4.40 (d, J = 10.5 Hz, 1H, $PhCH_2$), 3.88 (t, J = 8.0 Hz, 1H, H^{Glu}-4), 3.71–3.65 (m, 3H, H^{Glu}-5,6,6′), 3.54 (t, J = 8.0 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_{41}BrN_3O_5S$, $[M+H]^+$: *m/z* 778.1945. Found: *m/z* 778.1942.

5-(4-Iodophenyl)-4-(1,3,4,6-tetra-*O*-benzyl-2-deoxy-*β*-D-glucopyranose-2-yl)-1,2,4-triazole-3-thione (6l) Yield 77%; pale yellow solid; mp 127–128°C; IR: v 3428 (NH), 3087 (C-H, Ph), 2928 (CH_2-Ph), 1600 (C=N), 1360 (C=S), 1027 cm^{-1} cm^{-1} (C-O-C); ^1H NMR: δ 14.12 (s, 1H, NH), 7.90 (d, J = 7.5 Hz, 2H, Ar-H), 7.40–7.21 (m, 18H, Ar-H), 7.14 (d, J = 7.5 Hz, 2H, Ar-H), 7.04 (d, J = 7.0 Hz, 2H, Ar-H), 6.19 (d, J = 8.0 Hz, 1H, H^{Glu}-1), 5.61 (t, J = 8.5 Hz, 1H, H^{Glu}-3), 4.81 (d, J = 12.5 Hz, 1H, $PhCH_2$), 4.70 (d, J = 11.0 Hz, 2H, $PhCH_2$), 4.60–4.47 (m, 4H, $PhCH_2$), 4.39 (d, J = 11.0 Hz, 1H, $PhCH_2$), 3.88 (t, J = 9.0 Hz, 1H, H^{Glu}-4), 3.75–3.60 (m, 3H, H^{Glu}-5,6,6), 3.54 (t, J = 8.5 Hz, 1H, H^{Glu}-2). ESI-HRMS. Calcd for $C_{42}H_4IN_3NaO_5S$, $[M+Na]^+$: *m/z* 848.1626. Found: *m/z* 848.1621.

In vitro cholinesterase activity assay

Acetylcholinesterase (AChE), acetylthiocholine iodide (ATCI), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), galantamine and tacrine were purchased from Sigma-Aldrich (USA). AChE activities were measured using Ellman's colorimetric method with a slight modification [38] with galantamine and tacrine as the reference compounds. An electric eel AChE was dissolved in 0.1 M phosphate-buffered saline (PBS, pH 8.0) to obtain a solution of 0.35 U/mL. In assays, 20 μL of AChE was incubated with 10 μL of tested compounds and 130 μL of 0.1 M PBS (pH 8.0) for 10 min in 96-well microplates before the 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, Austria) for 15 min. One triplicate sample without inhibitors was always present to yield 100% of AChE activity. The reaction rates were compared and the percentage inhibition due to the presence of tested compounds was calculated. Galantamine and tacrine were applied as positive controls. 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 vs. the log concentration with the use of Origin 8.0 software. The results were described as the mean ± standard deviation.

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