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Synthesis and AChE inhibitory activity of *N*-glycosyl benzofuran derivatives

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Abstract: Six *N*-glycosyl benzofuran derivatives were synthesized by the catalysis of organic bases and condensation agents. The benzofuran derivatives were obtained by the reaction of various salicylaldehydes in acetone, and then hydrolyzed to the corresponding carboxylic acids. Finally, the target compounds were synthesized by acylation and the reaction conditions were optimized. The acetylcholinesterase (AChE) inhibitory activity of the desired compounds was tested using Ellman's method. Most of the compounds showed acetylcholinesterase-inhibition activity; *N*-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carbxamide (**5a**) showed the best acetylcholinesterase inhibition, with an inhibitory rate of 84%.

Keywords: D-glucosamine; amide; anti-acetylcholinesterase.

Introduction

Benzofurans are an important family of oxygen-containing heterocycles. Natural benzofuran compounds have anti-tumor, anti-fungal, anti-senile dementia and anti-inflammatory activity and thus provide important heterocyclic active molecule for the development of new drugs [1-4]. The main clinical drugs containing a benzofuran skeleton are the anti-arrhythmic amiodarone (I), the anti-hypertensive bufuralol (II) and the anti-fungal griseofulvin (III) [5,6], whose structures are shown in Figure 1. Some studies have shown that many compounds containing a benzofuran skeleton can be used as monoamine oxidase inhibitors, potent acetylcholinesterase (AChE) inhibitors or potent Multitarget-Directed Ligands in the treatment of Alzheimer's disease [7-10].

D-glucosamine is an important natural monosaccharide with many kinds of biological activity, such as anti-inflammatory, anti-cancer and anti-bacterial [11-13]. The synthesis of glucosamine derivatives has recently become of interest with respect to increasing their biological activity, as many studies have shown that glucosamine derivatives have strong biological properties, including anti-oxidant and anti-AChE [14-17].

Due to the excessive lipid-water partition coefficient and poor water solubility of benzofuran, it cannot easily reach the anticipated site of action of the drug. Glucosamine is an active molecule with multi-hydroxyl groups and has strong water solubility. Therefore, unprotected glucosamine was linked to benzofuran via an amide bond in order to increase the water solubility of the coumarin molecule and improve its bioavailability and activity. The synthesized derivatives were tested for AChE inhibitory activity by Ellman's method, and glycosylated heterocyclic compounds with better AChE-inhibitory activity were identified.

Results and Discussion

Chemistry

In our experiment, ethyl benzofuran-2carboxylate **1** was synthesized from substituted salicylaldehyde in acetone

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solution catalyzed by potassium carbonate, and hydrolyzed to benzofuran-2-carboxylic acid **3**. Then, under the catalysis of *N*,*N*-diisopropylethylamine and HATU, **3** and glucosamine **4** were reacted in acetonitrile solution to produce *N*-glycosyl benzofuran derivatives **5a–5f** in high yield (Scheme 1).

In the second stage, coumarilic acid **3a** and the condensation agent HATU were used as representatives to select the best reaction conditions. Table 1 summarizes the molar ratio, solvent, temperature and duration of the various reactions.

Biological activity

The AChE inhibition activity of the newly synthesized compounds was evaluated in vitro by Ellman's method, using AChE extracts from *Electric eel* [18,19]. Table 2 summarizes the compounds' inhibitory potency ('inhibition rate').

As shown in Table 2, the inhibitory activity of all the compounds to AChE is higher than that of the precursor compound, D-glucosamine hydrochloride **m**, and the inhibitory rate of the optimum compound **5a** is 84%, indicating that the presence of benzofurans enhances the inhibitory activity.

Conclusion

Six *N*-glycosyl benzofuran derivatives were designed and synthesized by a green, efficient and convenient method. Optimum reaction conditions were identified and all yields were above 60%. The compounds were determined by NMR, IR and HRMS. Most of the compounds demonstrated acetylcholinesterase inhibition activity and compound **5a** showed the best acetylcholinesterase inhibition with an inhibition rate of 84%.

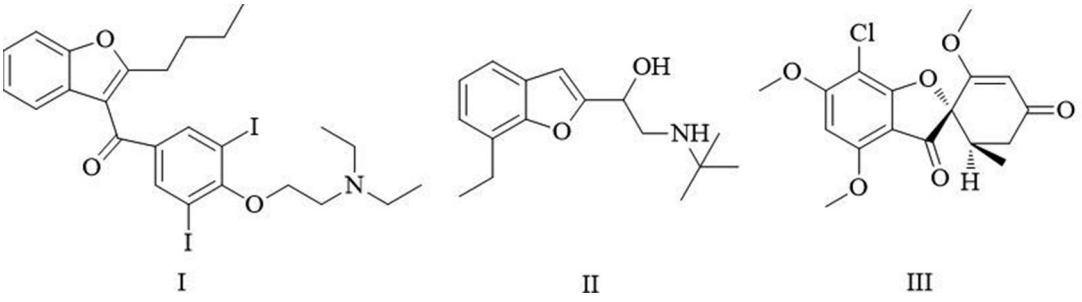
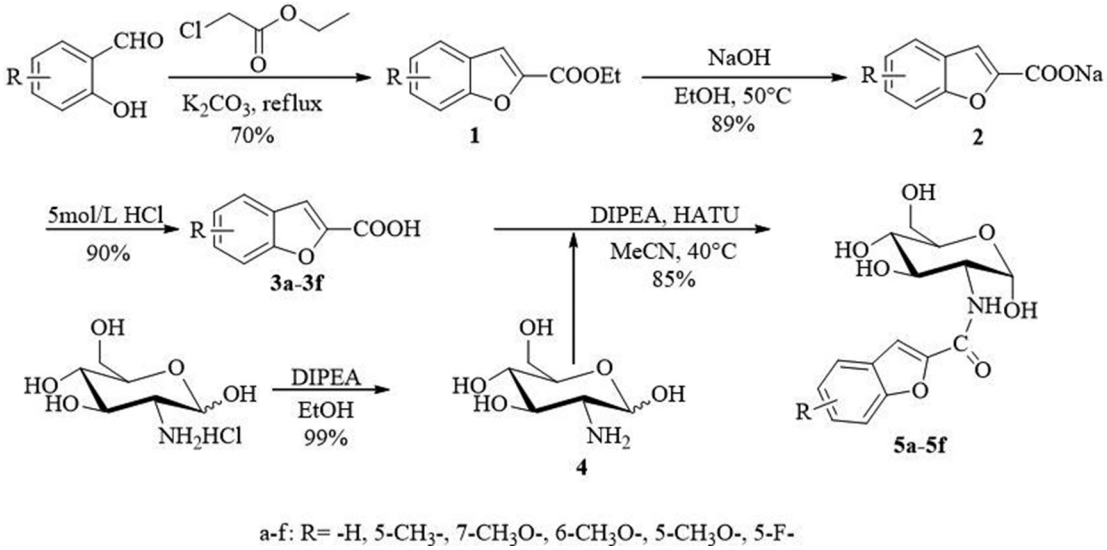


Figure 1 Drugs containing a benzofuran skeleton.



Scheme 1 Synthetic pathways of the *N*-glycosyl benzofuran derivatives.

Table 1 Optimizing the conditions for the synthesis of **5a**

Entry	n(3a) : HATU	Time (min)	Temp. (°C)	Solvent	Yield (%)
1	1:1.0	50	30	ACN	56
2	1:1.2	50	30	ACN	77
3	1:1.3	50	30	ACN	85
4	1:1.4	50	30	ACN	85
5	1:1.3	50	20	ACN	66
6	1:1.3	50	30	ACN	85
7	1:1.3	50	40	ACN	85
8	1:1.3	50	30	DCM	NR
9	1:1.3	50	30	MeOH	38
10	1:1.3	50	30	EtOH	46
11	1:1.3	50	30	ACN	85
12	1:1.3	20	30	ACN	32
13	1:1.3	40	30	ACN	73
14	1:1.3	50	30	ACN	85
15	1:1.3	60	30	ACN	85

Table 2 In vitro inhibitory activity of target compounds against AChE.

Compound	Inhibition rate (%)[a]
5a	84
5b	53
5c	65
5d	77
5e	58
5f	64
m[b]	1.79

[a] Inhibitory activity at a concentration of 1mg/mL.
[b] **m** stands for D-glucosamine hydrochloride.

Experimental

Chemistry

All chemicals were purchased from commercial sources and used without further purification unless otherwise stated. Melting points were determined 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 500 MHz at ambient temperature using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts were reported in ppm. HRMS (ESI) analysis was performed on an Agilent 6230 mass spectrometer.

General procedure for synthesis of ethyl benzofuran-2carboxylate (1)

Potassium carbonate, anhydrous (10 mmol) was added to an acetone solution of various salicylaldehydes (8 mmol) and stirred sufficiently. Ethyl chloroacetate (20 mmol) was added to the solution and the reaction mixture was stirred under reflux for 8 h. After removing the solvent, adding H_2O and extracting with ethyl acetate, product **1** was obtained, yield 70%.

General procedure for synthesis of benzofuran-2-carboxylic acid (3)

A sodium hydroxide (4 mmol) water (10 mL) solution was added to 20 mL ethanol containing **1** (3 mmol). After dropping, the reaction liquid was heated to 40°C and stirred for 1 h. After the reaction, the pH was adjusted to 1 by 5 mol/L HCl and the precipitate was filtered, washed with H_2O and ice ethanol, and dried. Product **3** was obtained, yield 90%.

Synthesis of Glucosamine (4)

Glucosamine hydrochloride (2 mmol) and *N*,*N*-diisopropylethylamine (2 mmol) were added to a 50 ml flask and stirred for 3 h to obtain compound **4**, yield 99%.

General procedure for Synthesis of 5a–5f

3 (2 mmol) was added to 15 ml Na_2SO_4 dried acetonitrile, and *N*,*N*-diisopropylethylamine (4 mmol) was added after stirring and dissolving. HATU (2.6 mmol) was dissolved in 5 ml acetonitrile and slowly dripped. When the solution turned yellow, **4** (2.6 mmol) was added. The reaction was complete after 50 minutes' continuous stirring at 30°C. The mixture was filtered and washed with acetonitrile to give **5a–5f**.

N-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5a)

Yield 85%; mp 212–214°C; IR (cm^{-1}): 3401, 3065, 2360, 1593, 1447, 1215, 795; ^{1}H NMR: 7.97 (d, *J* = 8.5 Hz, 1H, NH), 7.79 (d, *J* = 7.5 Hz, 1H, ArH), 7.71 (d, *J* = 8.5 Hz, 1H, ArH), 7.64 (s, *J* = 8.5 Hz, 1H, ArH), 7.51 (m, 1H, ArH), 7.36 (t, *J* = 10.0 Hz, 1H, ArH), 6.61 (d, *J* = 4.0 Hz, 1H, H^{Glu}), 5.10 (t, *J* = 4.0 Hz, 1H, H^{Glu}), 5.02 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.85 (d, *J* = 6.0 Hz, 1H, H^{Glu}), 3.84–3.80 (m, 1H, H^{Glu}), 3.75–3.71 (m, 1H, OH), 3.67–3.64 (m, 2H, OH), 3.55–3.50 (m, 1H, OH), 3.23–3.17 (m, 1H, H^{Glu}); ESI-HRMS (*m/z*): calcd for $C_{15}H_{17}NO_8Na^+$ [M+Na]$^+$: 346.0896; Found: 346.0907.

5-Methyl-*N*-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5b)

Yield 77%; mp 189–190°C; IR (cm^{-1}): 3403, 3060, 2345, 1588, 1437, 1201, 792; ^1H NMR: 7.92 (d, *J* = 8.0 Hz, 1H, NH), 7.56 (m, 2H, ArH), 7.30 (d, *J* = 8.5 Hz, 1H, ArH), 7.17 (d, *J* = 4.0 Hz, 1H, ArH), 6.61 (d, *J* = 4.0 Hz, 1H, H^{Glu}), 5.09 (t, *J* = 3.5 Hz, 1H, H^{Glu}), 5.02 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.85 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 3.84–3.80 (m, 1H, H^{Glu}), 3.75–3.71 (m, 1H, OH) 3.67–3.64 (m, 2H, OH), 3.53–3.49 (m, 1H, OH), 3.22–3.16 (m, 1H, H^{Glu}), 2.42 (s, 3H, C-H); ESI-HRMS (*m/z*): calcd for $C_{16}H_{20}NO_7^+$ $[M + H]^+$: 338.1234; Found: 338.1244.

7-Methoxy-*N*-(2,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5c)

Yield 64%; mp 265–266°C; IR (cm^{-1}): 3412, 3050, 2344, 1583, 1424, 1254, 780; ^1H NMR: 8.07 (d, *J* = 6.5 Hz, 1H, NH), 7.20 (s, 1H, ArH), 7.12 (m, 2H, ArH), 6.94 (d, *J* = 8.5 Hz, 1H, ArH), 6.56 (d, *J* = 4.0 Hz, 1H, H^{Glu}), 5.09 (t, *J* = 3.5 Hz, 1H, H^{Glu}), 5.02 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.85 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 3.94 (s, 3H, $-OCH_3$) 3.84–3.80 (m, 1H, H^{Glu}), 3.75–3.71 (m, 1H, OH) 3.67–3.64 (m, 2H, OH), 3.53–3.49 (m, 1H, OH), 3.22–3.16 (m, 1H, H^{Glu}); ESI-HRMS (*m/z*): calcd for $C_{16}H_{19}NO_8Na^+$ $[M+Na]^+$: 376.1003; Found: 376.1009.

6-Methoxy-*N*-(2,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5d)

Yield 68%; mp 168–169°C; IR (cm^{-1}): 3377, 3010, 2340, 1606, 1437, 1197, 812; ^1H NMR: 8.30 (d, *J* = 9.0 Hz, 1H, NH), 7.73 (d, *J* = 8.5 Hz, 1H, ArH), 7.56 (s, 1H, ArH), 7.27 (d, *J* = 1.5 Hz, 1H, ArH), 6.98 (m, 1H, ArH), 6.61 (d, *J* = 6.0 Hz, 1H, H^{Glu}), 5.09 (t, *J* = 4.0 Hz, 1H, H^{Glu}), 5.07 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.85 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 3.84 (s, 3H, $-OCH_3$) 3.83–3.80 (m, 1H, H^{Glu}), 3.75–3.71 (m, 1H, OH) 3.67–3.64 (m, 2H, OH), 3.53–3.49 (m, 1H, OH), 3.22–3.16 (m, 1H, H^{Glu}); ESI-HRMS (*m/z*): calcd for $C_{16}H_{19}NO_8Na^+$ $[M+Na]^+$: 376.1003; Found: 376.1015.

5-Methoxy-*N*-(2,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5e)

Yield 72%; mp 181–182°C; IR (cm^{-1}): 3420, 3017, 2360, 1579, 1436, 1208, 780; ^1H NMR: 7.90 (d, *J* = 3.5 Hz, 1H, NH), 7.60 (d, *J* = 9.0 Hz, 1H, ArH), 7.55 (s, 1H, ArH), 7.27 (d, *J* = 3.0 Hz, 1H, ArH), 7.07 (m, 1H, ArH), 6.60 (d, *J* = 4.5 Hz, 1H, H^{Glu}), 5.09 (t, *J* = 3.5 Hz, 1H, H^{Glu}), 5.01 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.84 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 3.81 (s, 3H, $-OCH_3$) 3.73–3.70 (m, 1H, H^{Glu}), 3.68–3.63 (m, 2H, OH) 3.54–3.50 (m, 1H, OH), 3.46–3.42 (m, 1H, OH), 3.22–3.18 (m, 1H, H^{Glu}); ESI-HRMS (*m/z*): calcd for $C_{16}H_{19}NO_8Na^+$ $[M+Na]^+$: 376.1003; Found: 376.1015.

5-Fluoro-*N*-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)benzofuran-2-carboxamide (5f)

Yield 76%; mp 132–133°C; IR (cm^{-1}): 3421, 3009, 2401, 1573, 1438, 1213, 791; ^1H NMR : 8.06 (d, *J* = 8.0 Hz, 1H, NH), 7.63 (s, 1H, ArH), 7.61 (d, *J* = 8.0 Hz, 1H, ArH), 7.21 (s, 1H, ArH), 7.10 (m, 1H, ArH), 6.61 (d, *J* = 4.0 Hz, 1H, H^{Glu}), 5.09 (t, *J* = 4.0 Hz, 1H, H^{Glu}), 5.02 (d, *J* = 5.5 Hz, 1H, H^{Glu}), 4.85 (d, *J* = 6.0 Hz, 1H, H^{Glu}), 3.85–3.81 (m, 1H, H^{Glu}), 3.74–3.70 (m, 1H, OH), 3.65–3.62 (m, 2H, OH), 3.53–3.46 (m, 1H, OH), 3.24–3.18 (m, 1H, H^{Glu}); ESI-HRMS (*m/z*): calcd for $C_{15}H_{16}FNO_8Na^+$ $[M+Na]^+$: 364.0803; Found: 364.0811.

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