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Synthesis, characterization, and biological evaluation of new *N*-glycosides derived from *O*-pivaloylated *β*-D-glucopyranosylamine

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Abstract The novel synthesis of *N*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-glucopyranosyl) benzo[*d*] oxazol-2-amine **4** was described in this study. The new compounds *N*-arylthioureas **3a-c** and **4**, along with a series of glucose-modified imines **5a-g**, were evaluated for their antitumor activity against human myeloid leukemia cell lines (HL-60 cells), gastric carcinoma (BGC-823 cells), liver carcinoma (Bel-7402 cells), and oral carcinomas (KB cells). The antibacterial potency of these compounds was also determined using an inhibition zone diameter test. Although none of the compounds were active against human cancer cells, compound **4** was found to be the most active compound against *Escherichia coli*.

Keywords Glucosyl thioureas · Glucose-modified benzoxazole · Glucose-modified imines · Antitumor activity · Antimicrobial activity

Introduction

Carbohydrates and their derivatives exerted important effects on many complex biological events [1], such as cellular recognition in the processes of immune response [2], cell migration [3], inflammation [4], carbohydrate recognition [5], tumor metastasis, and viral infections [6]. Meanwhile, glycoconjugates play a key role as receptors for proteins and enzymes on the cell surface [7], and they have

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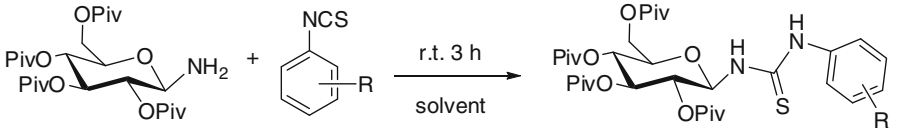
been shown to interact with either RNA or the backbone phosphate of DNA [8]. These derivatives, bearing nitrogen scaffolds in different ring positions on the sugar skeleton, are called *N*-glycosides, and many *N*-glycosides derivatives are of increasing interest because of their diverse biological properties such as antimicrobial [9], anti-inflammatory [10], anti-influenza [11], antiviral [12], and antitumor activities [13]. All this explains that certain series of *N*-glycosides are regarded as major target molecules for many years, owing to their biological and medicinal importance [14, 15]. Therefore, studies of the synthesis and pharmacology of *N*-glycosides derivatives have attracted more interest in recent years [3, 16, 17].

Following our interesting on synthesis of glycoconjugates [18–20], we were eager to extend our approach to the synthesis of other new classes of sugar-based heterocycles, which are of considerable importance in medicinal chemistry [21]. We anticipated that some glucosyl heterocycles could be synthesized in the presence of Pd catalyst through new cyclization model.

Results and discussion

Firstly, the reaction of 2,3,4,6-tetra-*O*-pivaloyl-*β*-D-glucopyranosylamine **1** with 2-hydroxyphenylisothiocyanate affords exclusively the glucosyl thiourea **3a** in 81% yield in isopropanol at room temperature (Table 1, entry 1). Then, the effects of various solvents were studied for the preparation of **3a** and comparative results are presented in Table 1. The reaction of glucopyranosylamine **1** proceeded smoothly in acetonitrile within 3 h at room temperature to afford the desired products in very high yield (Table 1, entry 4). In other cases, the yields varied from 81 to 92%. These results encouraged further studies with various other substituted phenylisothiocyanates and these phenylisothiocyanates readily reacted with glucopyranosylamine

Table 1 Synthesis of glucose-modified thioureas **3a-c**



1 **2a-c** **3a-c**

Entry	R	Product	Solvent	Yield(%)[a]
1	*o*-OH	**3a**	Isopropanol	81
2	*o*-OH	**3a**	Toluene	90
3	*o*-OH	**3a**	CH_2Cl_2	92
4	*o*-OH	**3a**	CH_3CN	95
5	*o*-CH_3	**3b**	CH_3CN	91
6	*p*-OH	**3c**	CH_3CN	93

All reactions were carried out using **1** (1 mmol), **2** (1.1 mmol), in 5 mL solvent

[a] Isolated yield after chromatography

under identical conditions to produce glucosyl thioureas **3b** and **3c** in high yields (Table 1, entries 5 and 6).

The glucose-modified benzoxazoles **4** was obtained in 86% yield in the presence of 15% $Pd(OAc)_2$ in dimethyl sulfoxide from **3a**. Noteworthy, the other glucosyl thioureas cannot give the corresponding product in the selected condition. The structures of the products **4** were established and confirmed on the basis of their elemental analysis and spectral data (MS, ^{1}H NMR and ^{13}C NMR). ^{1}H NMR spectroscopy was used to confirm the product and the ^{1}H NMR spectrum of **4** showed the anomeric proton as a doublet at $\delta = 5.34$ ppm with a $J = 9.2$ Hz indicating the β-configuration. The other six protons of the glucopyranosyl ring resonate in the $\delta = 3.95$–5.52 ppm region. In addition, the right NH groups of the glucosyl thioureas **3a** with a singlet at 8.41 ppm disappeared in the product and the left NH groups of glucose-modified benzoxazoles **4** show distinctive chemical shifts at 6.05 ppm. Complementarily, a singlet at 182.39 ppm on the ^{13}C NMR spectrum is assigned to the C=S bond also disappeared and the new C=N bond of benzoxazole ring can also be detected as a carbon resonance at 160.19 ppm in the ^{13}C NMR spectrum, which should be attributed to the formation of compound **4** (Fig. 1) (Scheme 1).

Next, we also prepared another series of glucose-modified imines using aromatic aldehydes instead of substituted phenylisothiocyanates. The reaction of glucopyranosylamine **1** with aromatic aldehydes **5a-g** proceeded smoothly in isopropanol to afford the desired glucose-modified imines **6a-g** in high yields (Scheme 2) [19].

Finally, the newly synthesized glucose-modified benzoxazoles **4**, glucosyl thiourea **3b-c** and glucose-modified imines **6a-g** were screened for their cytostatic activities against human myeloid leukemia cell lines (HL-60 cells) and other three different cell lines derived from various human solid tumors including gastric carcinoma (BGC-823 cells), liver carcinoma (Bel-7402 cells), and oral carcinomas (KB cells). However, the IC_{50} values of selected compounds against four types of

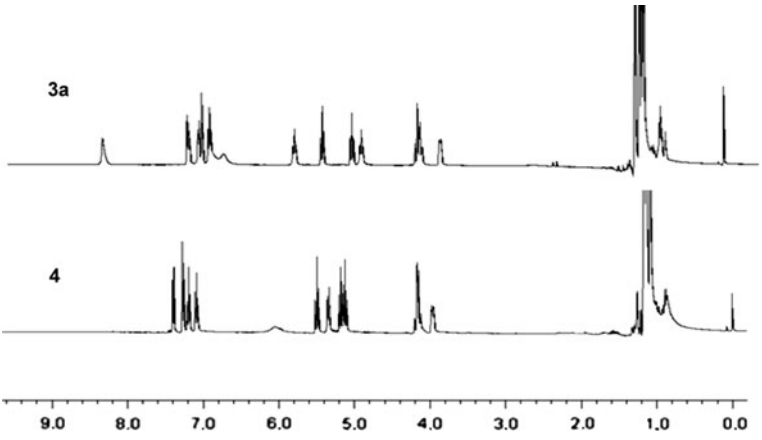
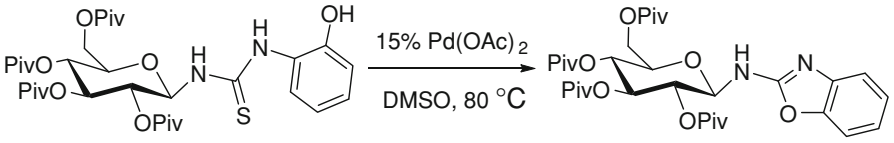
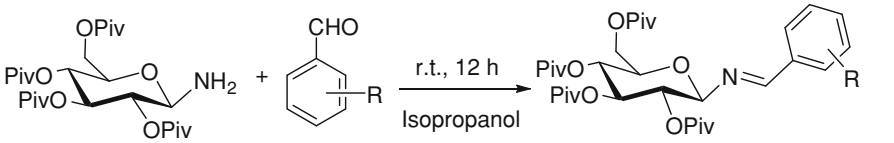


Fig. 1 ^{1}H NMR spectra of compound **3a** and **4** in $CDCl_3$



3a **4**

Scheme 1 Synthesis of glucose-modified benzoxazoles **4**



1 **5a-g**

6a: R = H
b: R = *p*-CH_3
c: R = *p*-OCH_3
d: R = *p*-NO_2
e: R = *p*-Cl
f: R = *m*-CH_3
g: R = *o*-OH

Scheme 2 Synthesis of glucose-modified imines **6a-g**

Table 2 Antimicrobial activity of *N*-glycosides against *Escherichia coli*

Microorganism EC	Compounds							
	3a	**3b**	**3c**	**4**	**6a**	**6c**	**6e**	**6f**
Zone diameter	9	8	6	20	8	11	ND	5

Test microorganism: *EC Escherichia coli*; *ND* not determined

tumor cells in culture were larger than 30, which indicated none of the compounds were active against human myeloid leukemia cell.

Determination of the antimicrobial activities of the new compounds was carried out in vitro by the agar well diffusion method [22]. The antimicrobial activity of these compounds was evaluated by measuring the inhibition zone diameter observed and were found to be significant at 5 mM concentration. It is obvious that our synthesized compounds showed significant activity against the tested microorganisms with inhibition zones ranging from 5 to 20 mm. Among the *N*-glycosides derivatives studied, the most active compound was **4** that showed good antimicrobial activity (Table 2).

Conclusions

In summary, the novel *N*-glycosides containing benzoxazole ring were synthesized in good yield. A series of glucosyl thioureas and imines were also obtained from the same starting materials. All of the target compounds were screened for their

antitumor activities against HL-60, BGC-823, Bel-7402, and KB cells. Among the *N*-glycosides derivatives studied, glucose-modified benzoxazoles **4** possess better antibacterial activity than other compounds. This study forms part of our research program to evolve new methodologies for the construction of novel glycoconjugates and to discover new molecules with antitumor activities. Further synthesis and biological investigation of new glycoconjugates for antitumor activity is continuing within our group.

Experimental section

2,3,4,6-Tetra-*O*-pivaloyl-*β*-D-glucopyranosylamine **1** and glucose-modified imines **6a-g** were prepared according our previous reports [18–20]. Other chemicals and solvents were either purchased or purified by standard techniques. Analytical TLC was performed on a Merck precoated TLC (silica gel 60 F254) plate. Melting points were recorded on an X_4-Data microscopic melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet 380 FT-IR spectrophotometer using KBr discs. ESI–MS were acquired on a Bruker Esquire 3000 plus spectrometer. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ using TMS as internal standard. Elemental analysis were performed on Carlo-Erba 1106.

General procedure for the synthesis of glucosyl thiourea **3a–b**

To a glass vial charged with acetonitrile (5 mL) was added glucosyl glucopyranosylamine (1.00 mmol) and substituted isothiocyanate (1.10 mmol). The resulting mixture was stirred at room temperature and monitored by TLC. Upon completion of the reaction ($\sim$3 h), the mixture was poured into water (10 mL), and extracted three times with dichloromethane (10 × 3 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and filtered. The solvents were removed via rotary evaporation and the product was purified by silica gel column chromatography using hexane–AcOEt (5:1) as an eluent to yield the desired products.

N-(2,3,4,6-Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)-*N*′-*o*-hydroxyphenyl thiourea **3a**

White solid; yield: 95%; mp 181–183 °C. ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.41 (s, 1H, NH), 7.25 (t, J = 7.6 Hz, 1H, ArH), 7.11 (d, J = 7.6 Hz, 1H, ArH), 7.06 (d, J = 8.4 Hz, 1H, ArH), 6.97 (t, J = 7.6 Hz, 1H, ArH), 6.77 (s, 1H, NH), 5.82 (t, J = 8.8 Hz, 1H, G_1H), 5.44 (t, J = 9.2 Hz, 1H, G_2H), 5.04 (t, J = 9.6 Hz, 1H, G_4H), 4.91 (t, J = 9.6 Hz, 1H, G_3H), 4.18-4.11 (m, 2H, G_6H), 3.85 (m, 1H, G_5H), 1.21–1.09 (m, 36H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.39, 178.72, 178.52, 176.81, 176.52, 150.95, 129.60, 126.94, 121.30, 118.33, 83.07, 74.10, 72.01, 70.50, 67.68, 61.60, 38.94, 38.89, 38.73, 38.65, 27.11, 27.05, 26.98, 26.90; *m*/*z* (EI): 667.23 $[M + H]^+$; Anal. Calcd. For : $C_{33}H_{50}N_2O_{10}S$: C, 59.42; H, 7.59; N, 4.11; Found: C, 59.40; H, 7.61; N, 4.16.

N-(2,3,4,6-Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)-*N*′-*o*methoxyphenyl thiourea **3b**

White solid; yield 91%; mp 166–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.83 (s, 1H, NH), 7.27–7.24 (m, 2H, ArH), 7.02–6.94 (m, 2H, ArH), 6.68 (s, 1H, NH), 5.88 (t, J = 9.6 Hz, 1H, G_1H), 5.44 (t, J = 10.0 Hz, 1H, G_2H), 5.09 (t, J = 10.0 Hz, 1H, G_4H), 4.96 (t, J = 9.6 Hz, 1H, G_3H), 4.14–4.13 (m, 2H, G_6H), 3.89 (m, 1H, G_5H), 3.82 (s, 3H, OCH_3), 1.20–1.07 (m, 36H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.05, 181.79, 178.84, 178.07, 176.64, 175.88, 152.51, 128.37, 124.98, 120.99, 111.87, 83.32, 74.04, 72.07, 70.45, 67.66, 61.61, 55.55, 38.88, 38.74, 38.63, 27.12, 27.07, 26.95, 26.85; *m*/*z* (EI): 681.33 $[M + H]^+$; Anal. Calcd. For $C_{34}H_{52}N_2O_{10}S$: C, 59.98; H, 7.71; N, 4.11. Found: C, 59.99; H, 7.72; N, 4.12.

N-(2,3,4,6-Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)-*N*′-*p*-hydroxyphenylthiourea **3c**

White solid; yield: 93%; mp 193–195 °C. ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.71 (s, 1H, NH), 7.69(d, J = 9.2 Hz, 2H, ArH), 7.44 (d, J = 8.4 Hz, 2H, ArH), 6.90 (s, 1H, NH), 5.82 (t, J = 8.8 Hz, 1H, G_1H), 5.44 (t, J = 9.2 Hz, 1H, G_2H), 5.04 (t, J = 9.6 Hz, 1H, G_4H), 4.91 (t, J = 9.6 Hz, 1H, G_3H), 4.18–4.11 (m, 2H, G_6H), 3.85 (m, 1H, G_5H), 1.21–1.09 (m, 36H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.60, 178.98, 178.18, 17679, 176.48, 140.66, 133.60, 124.16, 118.21, 82.99, 74.47, 70.73, 67.64, 61.77, 39.03, 38.86, 38.77, 38.71, 27.13, 27.09, 26.97; *m*/*z* (EI): 667.50 $[M + H]^+$; Anal. Calcd. For : $C_{33}H_{50}N_2O_{10}S$: C, 59.39; H, 7.51; N, 4.22; Found: C, 59.51; H, 7.69; N, 4.12.

General procedure for the synthesis of glucose-modified benzoxazoles **4**

A mixture of **3a** (0.5 mmol), and $Pd(OAc)_2$ (16.73 mg, 0.075 mmol) in DMSO (5 mL) was stirred at 80 °C under air and the reaction progress was monitored by TLC. Upon completion of the reaction (ca. 12 h), the mixture was cooled to room temperature and poured into 10 mL of water. The mixture was extracted with AcOEt (5 mL × 3) and the combined organic layer was washed with brine (10 mL), then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using hexane–AcOEt (10:1) as an eluent to yield the desired product glucose-modified benzothiazoles **4**.

N-(2,3,4,6-Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl) benzo[*d*] oxazol-2-amine **4**

White solid; yield: 86%; mp 191–193 °C. ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.39 (d, J = 7.6 Hz, 1H, ArH), 7.26 (d, J = 8.0 Hz, 1H, ArH), 7.19 (d, J = 7.6 Hz, 1H, ArH), 7.06 (t, J = 7.6 Hz, 1H, ArH), 6.05 (s, 1H, NH), 5.49 (t, J = 9.2 Hz, 1H, G_1H), 5.34 (d, J = 9.2 Hz, 1H, G_2H), 5.18 (t, J = 9.6 Hz, 1H, G_4H), 5.12 (t, J = 9.2 Hz, 1H, G_3H), 4.18–4.15 (m, 2H, G_6H), 3.98–3.95 (m, 1H, G_5H), 1.18–1.09 (m, 36H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.56, 178.24, 177.15, 176.70, 160.19,

148.80, 142.22, 124.47, 122.11, 117.49, 109.44, 83.04, 74.26, 72.38, 70.83, 68.01, 61.93, 39.14, 39.06, 39.02, 38.97, 27.37, 27.27, 27.13; *m/z* (EI): 633.12 $[M + H]^+$; Anal. Calcd. For $C_{33}H_{48}N_2O_{10}$: C, 62.60; H, 7.52; N, 4.41; 4. Found: C, 62.55; H, 7.42; N, 4.50.

Biological activity assays

Cell culture

Four different human carcinoma cell lines: HL-60, Bel-7402, BGC-823 and KB were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/mL of penicillin and 100 μg/mL of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Solutions

The selected complexes were then dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1.0, 10, 100, and 500 μM as working-solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (v/v) in all experiments.

Cytotoxicity analysis

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the complexes were added to the wells to achieve final concentrations. Control wells were prepared by the addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were performed in quintuplicate. The MTT assay was performed as described by Mosmann for HL-60 [23]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg/mL) was added to each well. After 4 h incubation, 2-propanol (100 mL) was added to solubilize the MTT formazan. The OD of each well was measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described for Bel-7402, BGC-823, and KB. Upon completion of the incubation for 44 h, the cells were fixed in 10% trichloroacetic acid (100 mL) for 30 min at 4 °C, washed five times and stained with 0.1% SRB in 1% acetic acid (100 mL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mM unbuffered Tris base (100 mL) and OD was measured at 540 nm as above. The IC_{50} value was determined from plot of % viability against dose of compounds added.

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References

1. P. Stallforth, B. Lepenies, A. Adibekian, P.H. Seeberger, J. Med. Chem. **52**, 5561–5577 (2009)
2. P.M. Rudd, T. Elliott, P. Cresswell, I.A. Wilson, R.A. Dwek, Science. **291**, 2370–2376 (2001)
3. H.E. Murrey, L.C. Hsieh-Wilson, Chem. Rev. **108**, 1708–1731 (2008)
4. M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, J.C. Paulson, Science. **250**, 1130–1132 (1990)
5. A.P. Davis, Nature. **464**, 169170 (2010)
6. T. Feizi, Curr. Opin. Struct. Biol. **3**, 701–710 (1993)
7. M. Mieszala, G. Kogan, H.J. Jennings, Carbohydr. Res. **338**, 167–175 (2003)
8. B.G. Davis, Chem. Rev. **102**, 579–602 (2002)
9. N.H. Metwally, M.A. Abdalla, M.A.N. Mosselhi, E.A. El-Desoky, Carbohydr. Res. **345**, 1135–1141 (2010)
10. X.B. Meng, D. Han, S.N. Zhang, W. Guo, J.R. Cui, Z.J. Li, Carbohydr. Res. **342**, 1167–1174 (2007)
11. F. Feng, N. Miura, N. Isoda, Y. Sakoda, M. Okamatsu, H. Kida, S.I. Nishimura, Bioorg. Med. Chem. Lett. **20**, 3772–3776 (2010)
12. K. Barral, J. Balzarini, J. Neyts, E.D. Clercq, R.C. Hider, M. Camplo, J. Med. Chem. **49**, 43–50 (2006)
13. R.D. Goff, J.S. Thorson, J. Med. Chem. **53**, 8129–8139 (2010)
14. R.R. Schmidt, Y.D. Vankar, Acc. Chem. Res. **41**, 1059–1073 (2008)
15. N. Yenil, E. Ay, K. Ay, M. Oskay, J. Maddaluno, Carbohydr. Res. **345**, 1617–1621 (2010)
16. J.J. Zhang, C.W.T. Chang, J. Org. Chem. **74**, 685–695 (2009)
17. K. Godula, C.R. Bertozzi, J. Am. Chem. Soc. **132**, 9963–9965 (2010)
18. Q. Zhao, C. Shen, H. Zheng, J.C. Zhang, P.F. Zhang, Carbohydr. Res. **345**, 437–441 (2010)
19. C. Shen, H. Zheng, P.F. Zhang, X.Z. Chen, J. Carbohydr. Chem. **29**, 155–163 (2010)
20. G.B. Zhou, Y.Q. Guan, C. Shen, Q. Wang, X.M. Liu, P.F. Zhang, L.L. Li, Synthesis. **13**, 1994–1996 (2008)
21. J. Neyts, E.D. Clercq, R. Singha, Y.H. Chang, A.R. Das, S.K. Chakraborty, S.C. Hong, S.C. Tsay, M.H. Hsu, J.R. Hwu, J. Med. Chem. **52**, 1486–1490 (2009)
22. C. Perez, M. Pauli, P. Bazerque, Acta. Biol. Med. Exp. **15**, 113–115 (1990)
23. T. Mosmann, J. Immunol. Methods. **65**, 55–63 (1983)