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Note

Study on glycosylated prodrugs of toxoflavins for antibody-directed enzyme tumor therapy

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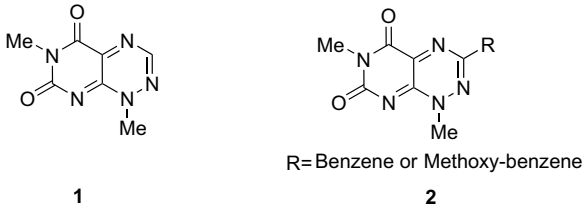
Abstract—Eight novel toxoflavin glycosides, which are potential prodrugs in antibody directed enzyme prodrug therapy (ADEPT), were synthesized. The structures of all toxoflavin glycosides were characterized by ^{13}C NMR spectroscopy, elemental analysis, and MS. Their enzymatic hydrolysis activities were tested against β-glucosidase (EC.3.2.1.21).

Keywords: Toxoflavins; Toxoflavin glycosides; Prodrugs; β-Glucosidase; Anticancer

Toxoflavin, a yellow crystalline solid isolated in 1933 from *Pwsendomoras cacovenenan*, was determined by total synthesis in 1961.[1] In previous studies, toxoflavin has stimulated considerable interest because of its broad-spectrum antibacterial activity.[2,3] Recently, toxoflavin (**1**) and its analogs (**2**) (Scheme 1) were identified as potential novel anticancer agents by their action against Polo-like kinase (PLK1). However, there are still several limitations for their clinical use because of their high toxicity and poor solubility.[4] As glycosylation usually increases the solubility and changes the activities of compounds, it is possible to overcome these drawbacks if we convert toxoflavin into glycosides and use them in ADEPT.[5] Thus, in this paper we report a trial for the synthesis of toxoflavin glycosides and their hydrolysis catalyzed by β-glucosidase.



Scheme 1. Toxoflavin and toxoflavin analogs.

We have synthesized eight toxoflavin glycosides (**10a–d**), (**17a–d**) containing glucose and lactose as the carbohydrate residue. For the toxoflavin glycosides (**10a–d**), glucose and lactose were linked to the toxoflavin by aromatic space-armed and the synthetic route is described in Scheme 2. Here, the corresponding 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide (**3a**) and 2,3,4,6,2′,3′,6′-hepta-*O*-acetyllactosyl bromide (**3b**) were firstly prepared by the method described in the literature and used as glycosyl donor.[6] The commercially available compound **4a** (4-hydroxybenzaldehyde) and **4b** (4-hydroxy-3-methoxy-benzaldehyde, Vanillin) as glycosyl accepters were separately coupled to the glycosyl donors **3a** and **3b** by using TBAB (tetrabutylammonium bromide) as phase-transfer catalyst in satd aq K_2CO_3 and dichloromethane.[7,8] The reactions were completed as indicated by TLC monitoring and subjected to flash chromatographyic purification to afford the four glycosides (**5a–d**) as the white solid in yields of 26–62%. The toxoflavin glycosides (**10a–d**) were synthesized according to the known procedure.[9] Stirring of glycosides (**5a–d**)

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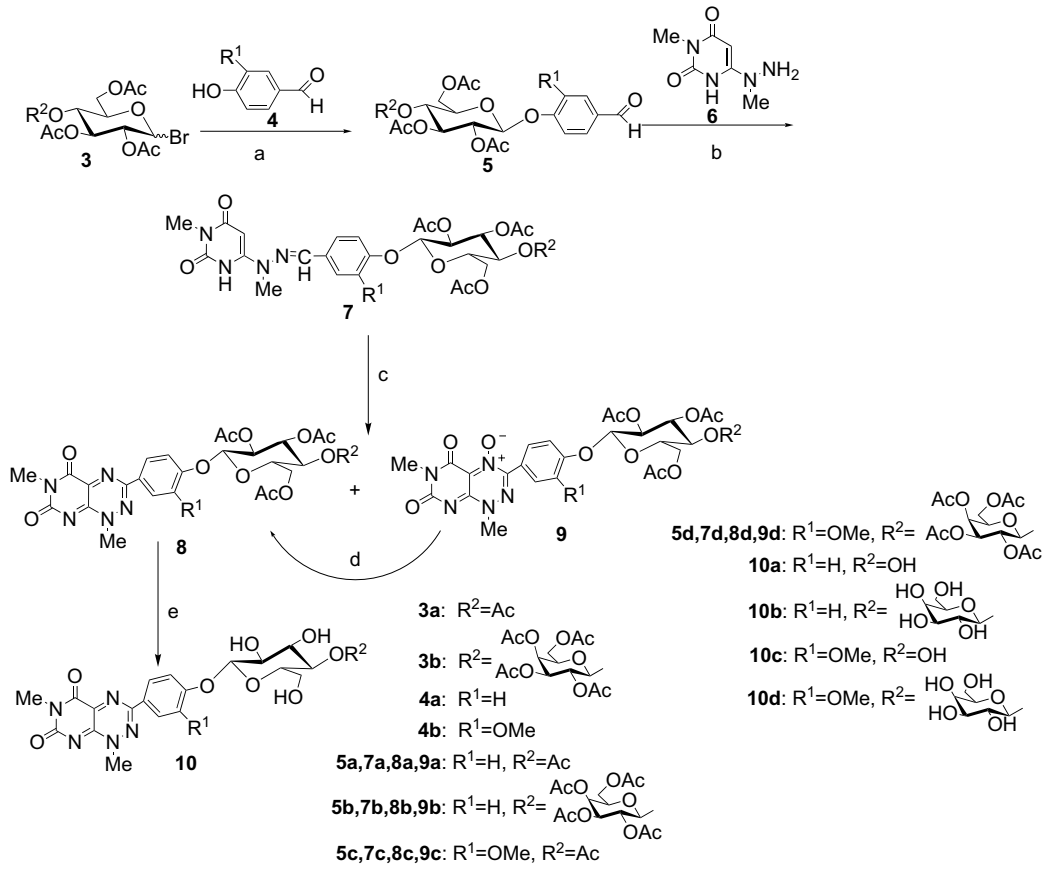
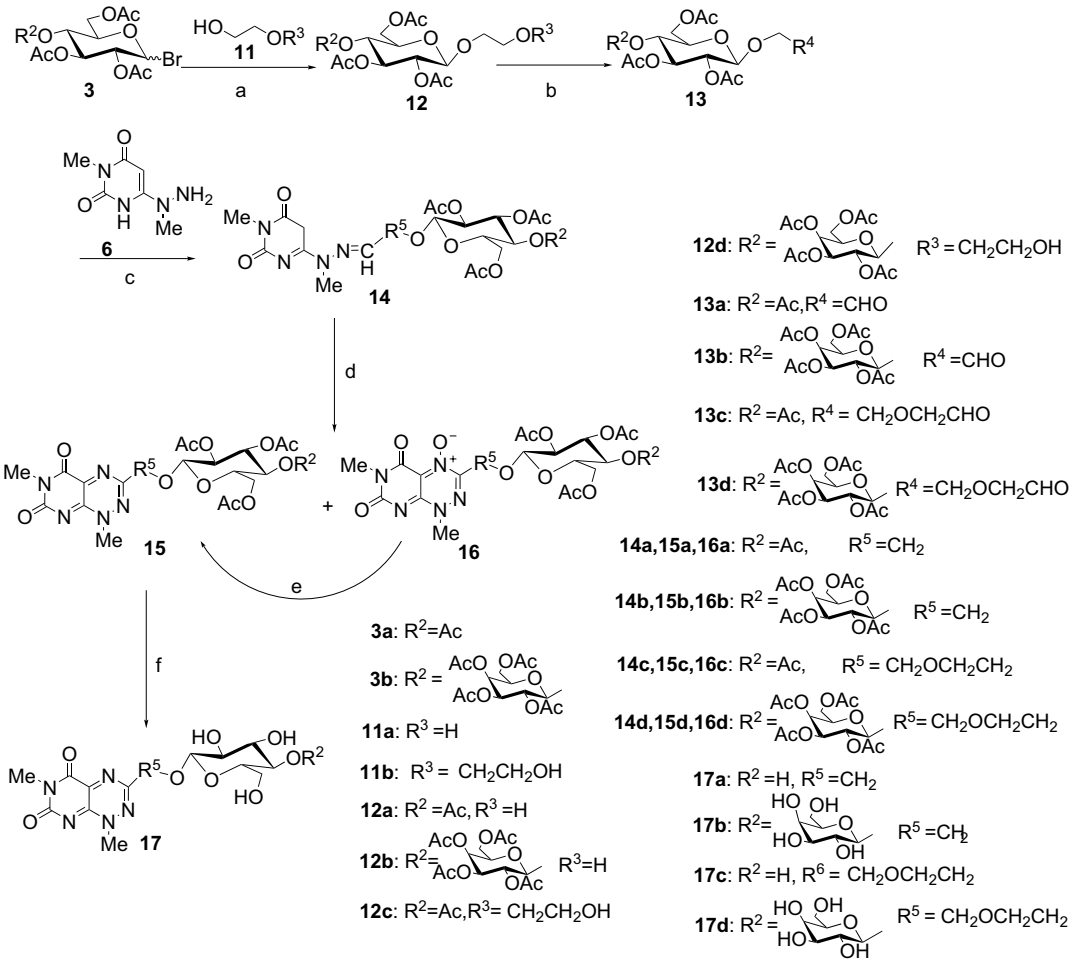


Scheme 2. Reagents and conditions: (a) TBAB (tetrabutylammonium bromide), CH_2Cl_2: satd aq K_2CO_3 = 1:1, 60 °C, 3 h; (b) anhydrous acetic acid, 5 °C, 90 min; (c) $NaNO_2$, rt, 90 min; (d) $Na_2S_2SO_4$, CH_2Cl_2:water = 1:1, rt, overnight; (e) MeONa in MeOH, rt, overnight.

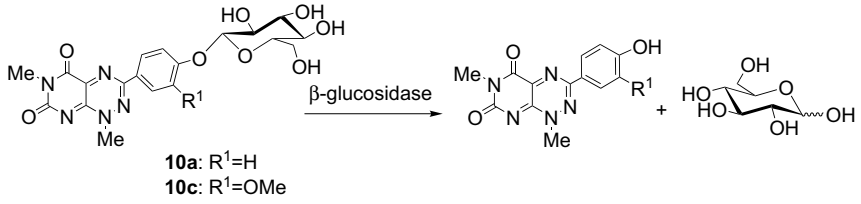
and compound **6** (3-methyl-6-(1-methylhydrazino)-uracil) in anhydrous acetic acid at 5 °C for 90 min gave the Schiff bases (**7a**–**d**). In the acid condition, after sodium nitrite was added and stirring for 90 min at room temperature, obtained compounds (**8a**–**d**) and its 4-oxide analog compounds (**9a**–**d**). Then compounds (**9a**–**d**) were reduced to compounds (**8a**–**d**) by the mild reducer hyposulphite in dichloromethane and water.[10] Finally compounds (**8a**–**d**) were deprotected in NaOMe/MeOH.[11] The water soluble toxoflavin glycosides (**10a**–**d**) were synthesized as the yellow solid in yields of 56–70%.

For the other toxoflavin glycosides (**17a**–**d**), glucose and lactose were linked to the toxoflavin by an aliphatic spacer-arm. The synthetic route is described in Scheme 3. The glycosyl donors **3a**, **3b** were reacted, respectively, with glycol **11a** and diethylene glycol **11b** in the presence of $HgBr_2$ as catalyst in dry dichloromethane (under Koenigs–Knorr conditions), by flash chromatographic purification to give the four glycosides (**12a**–**d**) as the white solids in yields 22–30%.[12] Then, the free hydroxyl group of glycosides (**12a**–**d**) was converted into aldehyde by using a highly efficient oxidant Dess–Martin periodinane in anhydrous dichloromethane at room temperature,[13] the glycosides (**13a**–**d**) were obtained as the white solid in yields 89–94% without purification. The synthesis of toxoflavin glycosides (**17a**–**d**) were carried out according to the synthetic method of (**10a**–**d**). Stirring of glycosides (**13a**–**d**) and compound **6** in anhydrous acetic acid at 5 °C for 90 min, the Schiff bases (**14a**–**d**) were formed then, by nitrative cyclization, afforded the compounds (**15a**–**d**) and its 4-oxide analogs (**16a**–**d**). Followed via reduction and deprotection, we obtained the water soluble toxoflavin glycosides (**17a**–**d**) as yellow solid in yields 60–80%.

In the ADEPT approach, a key step is that the anticancer drug must be released from inactive prodrug by enzyme. The β-glucosidase (EC.3.2.1.21) was used to hydrolyze the eight toxoflavin glycosides (**10a**–**d**), (**17a**–**d**) in this study. The kinetics were measured via analysis of generating of reduced sugar by 3,5-dinitrosalicyclic acid assay (Scheme 4).[14] the result showed only **10a** and **10c** can be hydrolyzed at pH 7.4 (0.002 M phosphate buffer) and 37 °C in vitro, the other six toxoflavin glycosides **10b**, **10d** and (**17a**–**d**) would not be hydrolyzed in the same condition. Furthermore, for the same glycosides concentration (0.01 mmol/mL) and enzyme concentration (0.69 μm/mL), the speed of enzymatic hydrolysis indicated that glycoside **10a** was more quicker than **10c** (Fig. 1). From the result, it clearly



Scheme 3. Reagents and conditions: (a) $HgBr_2$, CH_2Cl_2, 4 Å molecular sieves, rt, overnight; (b) Dess–Martin Periodinane, CH_2Cl_2, rt, 90 min; (c) anhydrous acetic acid, 5 °C, 90 min; (d) $NaNO_2$, rt, 90 min; (e) $Na_2S_2SO_4$, CH_2Cl_2:water = 1:1, rt, overnight; (f) MeONa in MeOH, rt, overnight.



Scheme 4. Enzymatic hydrolysis of toxoflavin glucosides **10a** and **10c**.

appears that toxoflavin glucosides **10a** and **10c** are the suitable substrates for β-glucosidase (EC.3.2.1.21) and they can be used as the potential prodrugs for ADEPT in vitro studies and consequently in vivo studies.

1. Experimental

1.1. General methods

NMR spectra were recorded with the Varian INOVA 500 MHz and Bruke 600 MHz. ^1H chemical shifts are expressed in parts per million (ppm) based on internal TMS (0.00 ppm) in $CDCl_3$. ^{13}C chemical shifts are expressed in parts per million (ppm) based on solvent signal of D_2O or DMSO. Mass spectra were obtained at 70 eV with a Finnigan MAT spectrometer. IR spectra were registered on a Specord IR 75 spectrometer in KBr pellets. Optical rotations were measured on a Perkin Elmer Model 341LC polarimeter in a 1-dm cell on solvent H_2O at 20 °C. Elemental analyses were performed on a VarioEL analyzer. All reactions were monitored by thin layer chromatography (TLC) on Silica Gel60 F_{254} (E. Merck), with detection by UV light or by visualizing

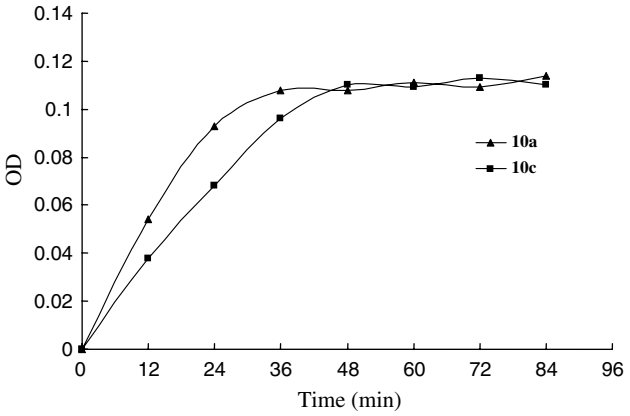


Figure 1. Kinetic result of enzymatic hydrolysis toxoflavin glucosides **10a** and **10c**.

with 10% H_2SO_4 in ethanol and heating. β-glucosidase (EC.3.2.1.21) purchased from Sigma–Aldrich Co. All solvents were dried and freshly distilled prior to use.

1.2. 4-(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyloxy)benzaldehyde (5a)

2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl bromide **3a** (3 g, 7.69 mmol) was dissolved in CH_2Cl_2 (50 mL) in which TBAB (2.49 g, 7.69 mmol), 4-hydroxybenzaldehyde **4a** (1.2 g, 10.6 mmol) and satd aq K_2CO_3 (50 mL) were added. The emulsion was stirred at 60 °C for 3 h after addition of H_2O (50 mL), the aqueous layer was separated and extracted with CH_2Cl_2 (50 mL × 2). Combined organic layers were washed with H_2O (100 mL × 2), then dried (anhydrous Na_2SO_4) and concentrated. Purification by chromatography (petroleum ether:ethyl acetate = 2:1), afforded compound **5a** (1.6 g, 3.59 mmol) as a white solid in yield of 46%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 3.91–3.94 (m, 1H, H-5Glc), 4.15–4.18 (d, 1H, H-6aGlc), 4.27–4.30 (dd, 1H, H-6bGlc), 5.16–5.22 (m, 2H, H-3Glc, H-4Glc), 5.29–5.34 (m, 2H, H-1Glc, H-2Glc), 7.09 (d, 2H, H-arom2, H-arom6, $J_{2,3} = J_{5,6} = 8$), 7.84 (d, 2H, H-arom3, H-arom5, $J_{2,3} = J_{5,6} = 8$), 9.92 (s, 1H, CHO). Anal. Calcd for $C_{21}H_{24}O_{11}$: C, 55.75; H, 5.35. Found: C, 55.82; H, 5.31.

1.3. 4-(2,3,4,6,2′,3′,6′-Hepta-*O*-acetyl-β-lactosyloxy)benzaldehyde (5b)

2,3,4,6,2′,3′,6′-Hepta-*O*-acetyl-β-lactosyl bromide **3b** (5 g, 7 mmol) was dissolved in CH_2Cl_2 (50 mL) in which TBAB (2.2 g, 7 mmol), 4-hydroxybenzaldehyde **4a** (1.2 g, 10.5 mmol) and satd aq K_2CO_3 (50 mL) were added. Stirring at 60 °C for 3 h after addition of H_2O (50 mL), separated the aqueous layer and extracted with CH_2Cl_2 (50 mL × 2). The combined organic phase was washed with H_2O (100 mL × 2), dried (anhydrous Na_2SO_4) and concentrated. Purification by chromatography (petroleum:ethyl acetate = 1:1) gave compound **5b** (1.4 g, 1.9 mmol, 27%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.98–2.17 (m, 24H, OAc), 3.90 (d, 1H, H-5Gal), 3.91 (d, 1H, H-5Glc), 4.07–4.25 (m, 4H, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.51–4.65 (m, 2H, H-4Gal, H-3Gal), 4.99–5.01 (m, 2H, H-3Glc, H-2Gal), 5.20–5.22 (m, 2H, H-2Glc, H-4Glc), 5.30 (d, 1H, H-1Gal), 5.36 (d, 1H, H-1Glc), 7.08 (d, 2H, H-arom6, H-arom2 $J_{2,3} = J_{5,6} = 5$), 7.84 (d, 2H, H-arom3, H-arom5 $J_{2,3} = J_{5,6} = 5$), 9.92 (s, 1H, CHO). Anal. Calcd for $C_{33}H_{40}O_{19}$: C, 53.51; H, 5.44. Found: C, 53.58; H, 5.36.

1.4. 3-Methoxy-4-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyloxy)benzaldehyde (5c)

Compound **3a** (3 g, 7.69 mmol) was reacted with compound **4b** (1.6 g, 10.8 mmol) as described for compound **5a** to give **5c** (2.3 g, 4.77 mmol) as a white solid in yield of 62%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 3.86–3.88 (m, 3H, OCH_3), 3.83–3.87 (m, 1H, H-5Glc), 4.17–4.20 (dd, 1H, H-6aGlc, $J_{5,6a} = J_{6a,6b} = 5$), 4.25–4.29 (dd, 1H, H-6bGlc, $J_{5,6b} = J_{6a,6b} = 5$), 5.09–5.10 (d, 1H, H-4Glc), 5.16–5.20 (m, 1H, H-3Glc), 5.29–5.35 (m, 2H, H-2Glc, H-1Glc), 7.04 (d, 1H, H-arom6, $J_{5,6} = 5$), 7.20 (d, 1H, H-arom5, $J_{5,6} = 5$), 7.42 (s, 1H, H-arom2), 9.89 (s, 1H, CHO). Anal. Calcd for $C_{22}H_{26}O_{12}$: C, 54.77; H, 5.43. Found: C, 54.82; H, 5.24.

1.5. 3-Methoxy-4-(2,3,4,6,2′,3′,6′-hepta-*O*-acetyl-β-lactosyloxy)benzaldehyde (5d)

Compound **3b** (5 g, 7 mmol) was reacted with compound **4b** (1.6 g, 10.5 mmol) as described for compound **5b** to give **5d** (1.9 g, 2.48 mmol) as a white solid in yield of 35%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.97–2.16 (m, 24H, OAc), 3.76–3.88 (m, 1H, H-5Gal), 3.88 (s, 3H, OCH_3), 3.91–3.97 (m, 1H, H-5Glc), 4.06–4.17 (m, 4H, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.50–4.54 (m, 2H, H-4Gal, H-3Gal), 4.95–4.98 (m, 1H, H-3Glc), 5.07 (d, 1H, H-2Gal), 5.11–5.21 (m, 2H, H-2Glc, H-4Glc), 5.29 (d, 1H, H-1Gal), 5.35 (s, 1H, H-1Glc), 7.16 (d, 1H, H-arom6, $J_{5,6} = 5$), 7.40 (s, 1H, H-arom5), 7.42 (s, 1H, H-arom2), 9.88 (s, 1H, CHO). Anal. Calcd for $C_{34}H_{42}O_{20}$: C, 52.99; H, 5.49. Found: C, 52.92; H, 5.43.

1.6. 3-(4′-(β-D-Glucopyranosyloxy)phenyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (10a)

Compound **6** (3-methyl-6-(1′-methylhydrazino)-uracil) (85 mg, 0.5 mmol) and compound **5a** (485 mg,

0.75 mmol) were stirred in 6 mL acetic acid under cooling at 5 °C for 90 min. The Schiff base **7a** was obtained and was stirred for 90 min after the sodium nitrite (138 mg, 2 mmol) was added. The mixture of the compound **8a** and compound **9a** were obtained. Then the solvent were neutralized with aq satd K_2CO_3 and extracted with CH_2Cl_2 (30 mL × 2). The dried (anhydrous Na_2SO_4) extracts were evaporated in vacuum and purification by chromatography. The mixture of **8a** and **9a** was a yellow solid. They were dissolved in 10 mL CH_2Cl_2 which 10 mL $Na_2S_2O_4$ (200 mg, 1.2 mmol) solvent was added. After stirring continuously overnight at room temperature, compound **9a** was all reduced to compound **8a**. The aqueous layer was separated and extracted with CH_2Cl_2 (10 mL × 2). Combined organic layers were washed with H_2O (10 mL × 2), dried and concentrated, afforded **8a** (240 mg, 0.39 mmol) as yellow solid. To dissolve **8a** in 10 mL NaOMe/MeOH (4 mg/mL) and was stirred overnight at room temperature. The solution was neutralized by acetic acid and concentrated. After purified by chromatography, a yellow solid **10a** (152 mg, 0.35 mmol) was give in yield of 70%. $[\alpha]_D$ −160; ^{13}C NMR (600 MHz, DMSO) δ: 20.2, 28.1, 61.5, 67.8, 70.5, 70.9, 71.8, 96.3, 116.4, 131.3, 137.6, 148.3, 150.4, 160.9, 159.57–169.93 (multi peaks, C ring C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3413, 1728, 1381, 1280, 1073 cm^{-1}. ESIMS (*m*/*z*): 448.3 ($M+H^+$). Anal. Calcd for $C_{19}H_{21}N_5O_8$: C, 51.01; H, 4.73; N, 15.65. Found: C, 52.17; H, 4.41; N, 15.62.

The compounds (**10b**–**d**) were synthesized as described for compound **10a**.

1.7. 3-(4′-(β-D-Lactosyloxy)phenyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (10b)

Compound **10b** (180 mg, 0.3 mmol) as a yellow solid in yield of 60%. $[\alpha]_D$ −120; ^{13}C NMR (600 MHz, D_2O) δ: 19.7, 22.6, 59.2, 60.6, 68.1, 70.5, 70.9, 73.0, 73.6, 74.4, 74.9, 77.4, 98.9, 102.8, 116.1, 126.1, 128.2, 130.5, 149.7–173.3 (multi peaks C ring C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3415, 1609, 1559, 1240 cm^{-1}. ESIMS (*m*/*z*): 609.9 ($M+H^+$). Anal. Calcd for $C_{25}H_{31}N_5O_{13}$: C, 49.26; H, 5.13; N, 11.49. Found: C, 49.44; H, 5.03; N, 11.45.

1.8. 3-(3′-Methoxy-4′-(β-D-glucopyranosyloxy)phenyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (10c)

Compound **10c** (180 mg, 0.38 mmol) as a yellow solid in yield of 76%. $[\alpha]_D$ −48; ^{13}C NMR (600 MHz, DMSO) δ: 22.7, 28.3, 55.5, 60.6, 69.6, 73.2, 76.8, 77.2, 99.7, 109.9, 115.1, 119.2, 129.5, 130.6, 141.5–174.0 (multi peaks, C ring, C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3425, 1605, 1557, 1075 cm^{-1}. ESIMS (*m*/*z*): 477.4 ($M+H^+$). Anal. Calcd for $C_{20}H_{23}N_5O_9$: C, 50.31; H, 4.86; N, 14.67. Found: C, 50.27; H, 4.78; N, 14.53.

1.9. 3-(3′-Methoxy-4′-(β-D-lactosyloxy)phenyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (10d)

Compound **10d** (296 mg, 0.32 mmol) as a yellow solid in yield of 56%. $[\alpha]_D$ −24; ^{13}C NMR (600 MHz, DMSO) δ: 20.3, 28.1, 55.9, 60.8, 61.9, 67.0, 68.8, 69.6, 70.2, 70.7, 72.0, 72.1, 76.0. 97.1, 99.9, 111.3, 116.0, 124.6, 131.7, 137.6, 149.7–170.1 (multi peaks C ring C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3422, 1554, 1139, 1077 cm^{-1}. ESIMS (*m*/*z*): 640.2 ($M+H^+$). Anal. Calcd for $C_{26}H_{33}N_5O_{14}$: C, 48.83; H, 5.20; N, 10.95. Found: C, 48.86; H, 5.03; N, 10.89.

1.10. 2-Hydroxy-ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (12a)

A mixture of **3a** (4.5 g, 10 mmol) and glycol **11a** (10 mL) in dry CH_2Cl_2 (50 mL), containing actived powdered 4 Å molecular sieves (5 g), was stirred under N_2 for 15 min. Then $HgBr_2$ (3.6 g, 10 mmol) was added and stirring was continue overnight at room temperature. The mixture was diluted with CH_2Cl_2 (50 mL), filtered through Celite and the filtrate was washed with aq 5% KI (30 mL × 3) and water (30 mL × 3), dried (anhydrous Na_2SO_4) and concentrated, purification by chromatography to yield **12a** (1.02 g, 2.60 mmol, 26%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 2.01 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.72–3.76 (m, 3H, H-5Glc, H-6aGlc, H-6bGlc), 3.85 (d, 2H, CH_2), 4.19 (d, 2H, CH_2), 4.55 (d, 1H, OH), 5.00–5.08 (m, 3H, H-2Glc, H-3Glc), 5.22 (t, 1H, H-1Glc1, $J_{1,2} = 10$). Anal. Calcd for $C_{16}H_{24}O_{11}$: C, 48.98; H, 6.17. Found: C, 49.04; H, 6.09.

1.11. 2-Hydroxy-ethyl 2,3,4,6,2′,3′,6′-hepta-*O*-acetyl-β-lactoside (12b)

A mixture of **3b** (5 g, 7.37 mmol), **11a** (10 mL) in dry CH_2Cl_2 (50 mL), containing actived powdered 4 Å molecular sieves (5 g), was stirred under N_2 for 15 min. After adding $HgBr_2$ (1.7 g, 7 mmol) and stirring overnight at room temperature, the mixture was diluted with CH_2Cl_2 (50 mL), filtered through Celite and the filtrate was washed with aq 5% KI (30 mL × 3) and water (30 mL × 3), dried (anhydrous Na_2SO_4) and concentrated, purification by chromatography, afforded **12b** (1.17 g, 1.72 mmol, 23%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.97–2.16 (m, 21H, OAc), 3.66–3.74 (m, 4H, CH_2CH_2), 3.76–3.89 (m, 2H, H-5Gal, H-5Glc), 4.04–4.14 (m, 4H, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.48–4.55 (m, 3H, H-4Gal, H-3Gal, H-

Glc3), 4.92–4.97 (m, 2H, H-2Gal, H-2Glc), 5.10 (d, 1H, H-4Glc), 5.19–5.23 (m, 1H, H-1Gal), 5.35 (d, 1H, H-1Glc). Anal. Calcd for $C_{28}H_{40}O_{19}$: C, 49.41; H, 5.92. Found: C, 49.45; H, 5.89.

1.12. 5-Hydroxy-3-oxa-pentyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (12c)

Compound **3a** (4.5 g, 10 mmol) was reacted with glycol **11b** (10 mL) as described for compound **12a** to give **12c** (1.29 g, 2.96 mmol) as a white solid. Yield: 30%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), 3.56–3.75 (m, 8H, $CH_2CH_2OCH_2CH_2$), 3.95–3.98 (m, 1H, H-5Glc), 4.13–4.28 (m, 2H, H-6aGlc, H-6bGlc), 4.60 (d, 1H, OH), 4.99–5.11 (m, 2H, H-4Glc, H-3Glc), 5.12–5.23 (m, 1H, H-2Glc, H-1Glc). Anal. Calcd for $C_{18}H_{28}O_{12}$: C, 49.54; H, 6.47. Found: C, 49.62; H, 6.46.

1.13. 5-Hydroxy-3-oxa-pentyl 2,3,4,6,2′,3′,6′-hepta-*O*-acetyl-β-lactoside (12d)

Compound **3b** (5 g, 7.37 mmol) was reacted with glycol **11b** (10 mL) as described for compound **12b** to give **12d** (1.48 g, 1.59 mmol) as a white solid. Yield: 22%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.95–2.47 (m, 21H, OAc), 3.56–3.69 (m, 8H, $CH_2CH_2OCH_2CH_2$), 3.71–3.91 (m, 2H, H-5Gal, H-5Glc), 4.04–4.13 (m, 4H, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.49–4.56 (m, 3H, H-4Gal, H-3Gal, H-Glc3), 4.87–4.95 (m, 2H, H-2Gal, H-2Glc), 5.08 (d, 1H, H-4Glc), 5.16–5.20 (m, 1H, H-1Gal), 5.32 (d, 1H, H-1Glc). Anal. Calcd for $C_{30}H_{44}O_{20}$: C, 49.72; H, 6.12. Found: C, 49.87; H, 6.08.

1.14. 2-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)acetaldehyde (13a)

To a stirred solution of **12a** (300 mg, 0.77 mmol) in dry CH_2Cl_2 (10 mL) under N_2 at temperature, a solution of Dess–Martin periodinane (326 mg, 0.77 mmol) in dry CH_2Cl_2 (5 mL) was added. After 90 min the solution was added to a saturated aqueous solution of $NaHCO_3$ (10 mL) which contained 10% $Na_2S_2O_3$. The mixture was stirred vigorously for 5 min and then extracted with CH_2Cl_2 (10 mL × 2), the dried (anhydrous Na_2SO_4) extracts were evaporated in vacuum and gave **13a** (280 mg, 0.7 mmol) as a white solid in yield of 91%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.02–2.10 (m, 12H, OAc), 3.72–4.10 (m, 2H, CH_2), 4.12–4.33 (m, 3H, H-5Glc, H-6aGlc, H-6bGlc), 4.59 (d, 1H, H-4Glc), 5.00–5.09 (m, 2H, H-3Glc, H-2Glc), 5.21 (d, 1H, H-1Glc), 9.67 (s, 1H, CHO). Anal. Calcd for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.68. Found: C, 49.32; H, 5.64.

The compounds (**13b–d**) were synthesized as described for compound **13a**.

1.15. 2-(2,3,4,6,2′,3′,6′-Hepta-*O*-acetyl-β-D-lactosyloxy)acetaldehyde (13b)

Compound **13b** (422 mg, 0.62 mmol) as a white solid. Yield: 89%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.97–2.15 (m, 21H, OAc), 3.70–3.82 (m, 2H, CH_2), 3.87–4.25 (m, 6H, H-5Gal, H-5Glc, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.45–4.55 (m, 3H, H-4Gal, H-3Gal, H-Glc3), 4.94–5.00 (m, 2H, H-2Gal, H-2Glc), 5.09–5.13 (m, 1H, H-4Glc), 5.20–5.23 (m, 21H, H-1Gal, H-1Glc), 9.65 (s, 1H, CHO). Anal. Calcd for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.68; H, 5.60.

1.16. 2-((2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)ethoxy)acetaldehyde (13c)

Compound **13c** (290 mg, 0.66 mmol) as a white solid. Yield: 94%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.00–2.08 (m, 12H, OAc), 3.69–3.80 (m, 6H, $CH_2CH_2OCH_2$), 3.98–4.01 (m, 1H, H-5Glc), 4.12–4.27 (m, 2H, H-6aGlc, H-6bGlc), 4.58 (d, 1H, H-4Glc), 5.00 (t, 1H, H-3Glc, $J_{2,3} = J_{3,4} = 5$), 5.08 (t, 1H, H-2Glc, $J_{1,2} = J_{2,3} = 10$), 5.19 (d, 1H H-1Glc), 9.69 (s, 1H, CHO). Anal. Calcd for $C_{18}H_{26}O_{12}$: C, 49.77; H, 6.03. Found: C, 49.80; H, 6.01.

1.17. 2-((2,3,4,6,2′,3′,6′-Hepta-*O*-acetyl-β-lactosyloxy)ethoxy)acetaldehyde (13d)

Compound **13d** (464 mg, 0.64 mmol) as a white solid. Yield: 91%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.96–2.18 (m, 21H, OAc), 3.72–3.85 (m, 6H, $CH_2CH_2OCH_2$), 3.87–4.16 (m, 6H, H-5Gal, H-5Glc, H-6aGal, H-6bGal, H-6aGlc, H-6bGlc), 4.47–4.55 (m, H-4Gal, H-3Gal, H-Glc3), 4.88–4.96 (m, 2H, H-2Gal, H-2Glc), 5.09–5.12 (1H, H-4Glc), 5.18–5.30 (m, 2H, H-1Gal, H-1Glc), 9.69 (s, 1H, CHO). Anal. Calcd for $C_{30}H_{42}O_{20}$: C, 49.86; H, 5.86. Found: C, 49.93; H, 5.86.

The compounds (**17a–d**) were synthesized as described for compound **10a**.

1.18. 3-((β-D-Glucopyranosyloxy)methyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (17a)

Compound **17a** (141 mg, 0.37 mmol) as a yellow solid. Yield: 74%. $[\alpha]_D$ −12; ^{13}C NMR (600 MHz, D_2O) δ: 19.6, 22.6, 56.9, 60.1, 69.8, 70.1, 72.7, 75.1, 101.9, 133.0–172.6 (multi peaks, C ring, C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3411, 1642, 1564, 1076 cm^{-1}. ESIMS (*m*/*z*): 386.1 (M+H$^+$). Anal. Calcd for $C_{14}H_{19}N_5O_8$: C, 43.64; H, 4.97; N, 18.17. Found: C, 43.75; H, 4.94; N, 18.08.

1.19. 3-((β-D-Lactosyloxy)methyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (17b)

Compound **17b** (162 mg, 0.3 mmol) as a yellow solid. Yield: 60%. $[\alpha]_D$ −6; ^{13}C NMR (600 MHz, D_2O) δ:

20.1, 22.8, 59.0, 60.5, 68.1, 69.9, 70.4, 72.0, 72.2, 73.6, 73.7, 74.8, 77.7, 99.4, 102.4, 133.1–173.1 (multi peaks C ring C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3411, 1561, 1146, 1115 cm^{-1}. ESIMS (*m*/*z*): 546.2 ($M+H^+$). Anal. Calcd for $C_{20}H_{29}N_5O_{13}$: C, 43.88; H, 5.34; N, 12.79. Found: C, 43.74; H, 5.31; N, 12.62.

1.20. 3-(((β-D-Glucopyranosyloxy)ethoxy)methyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (17c)

Compound **17c** (160 mg, 0.4 mmol) as a yellow solid. Yield: 80%. $[\alpha]_D$ −56; ^{13}C NMR (600 MHz, D_2O) δ: 20.1, 22.8, 59.8, 60.2, 68.2, 69.1, 70.9, 72.6, 75.1, 75.4, 101.8, 133.2–170.6 (multi peaks, C ring, C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3402, 1563, 1110, 1076 cm^{-1}. ESIMS (*m*/*z*): 430.0 ($M+H^+$). Anal. Calcd for $C_{16}H_{23}N_5O_9$: C, 44.76; H, 5.40; N, 16.31. Found: C, 44.72; H, 5.39; N, 16.22.

1.21. 3-(((β-D-Lactosyloxy)ethoxy)methyl)-1,6-dimethylpyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-dione (17d)

Compound **17d** (193 mg, 0.33 mmol) as a yellow solid. Yield: 66%. $[\alpha]_D$ −7.2; ^{13}C NMR (600 MHz, D_2O) δ: 20.2, 22.6, 59.6, 59.9, 60.6, 68.3, 69.1, 70.8, 71.2, 72.1, 72.3, 73.7, 74.3, 74.9, 101.6, 102.7, 133.1–170.6 (multi peaks C ring C-3, C-4a, C-5, C-7, C-8a). IR (KBr): 3415, 1561, 1145, 1116 cm^{-1}. ESIMS (*m*/*z*): 592.4 ($M+H^+$). Anal. Calcd for $C_{22}H_{33}N_5O_{14}$: C, 44. 67; H, 5.62; N, 11.84. Found: C, 44.77; H, 5.48; N, 11.76.

1.22. Enzymatic hydrolysis by β-glucosidase (EC.3.2.1.21)

The toxoflavin glycosides **10a** and **10b** (8 mg, 0.02 mmol) were dissolved in 2 mL (0.02 M) phosphate buffer (pH 7.4). The solvent **10a** and **10b** were, respectively, equally distributed to 8 test tube, a test tube was 0.25 mL, then 0.25 mL β-glycosidase (0.69 μm/mL) was added. Enzymatic hydrolysis was investigated by incubating at 37 °C. Analysis of reduced sugar was performed by 3,5-dinitrosalicyclic acid assay. It is about 12 min that a test tube was heated at 100 °C for 10 min, after rapid cooling to room temperature. The first test tube as contrast, determine the absorbance at 570 nm and the result gave 8 point.

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References

1. Doyle, D. G.; Roland, K. R.; Cheng, C. C. *J. Am. Chem. Soc.* **1961**, *83*, 3904–3905.
2. Edward, C. T.; Robert, W. M. J. *J. Am. Chem. Soc.* **1965**, *87*, 1976–1979.
3. Sadao, N.; Misuzu, I.; Keitaro, S. *J. Org. Chem.* **1983**, *48*, 1628–1631.
4. Kee, C. G.; Haishan, W.; Niefang, Y.; Yifa, Z. *Drug. Dev. Res.* **2004**, *62*, 349–361.
5. Emmanuel, B.; Sylvie, T.; Frederic, S.; Claude, M. *Bioorg. Med. Chem.* **2004**, *12*, 969–977.
6. Garegg, P. J.; Goldstein, I. J.; Iversen, T. *Acta Chem. Scand.* **1976**, *30*, 876–878.
7. Dess, D.; Kleine, H. P.; Weinberg, D. V.; Kaufman, R. J.; Sidhu, R. S. *Synthesis* **1981**, *11*, 883–885.
8. Chunyan, B.; Ran, L.; Ming, J.; Pengchong, X.; Changhui, T.; Yingying, Z.; Guofa, L. *Carbohydr. Res.* **2004**, *339*, 1311–1316.
9. Fumio, Y.; Kazuko, S.; Sadao, N. *Tetrahedron Lett.* **1971**, *13*, 851–854.
10. Shinichi, N.; Kohtaro, S.; Makoto, N. *Heterocycles* **2004**, *63*, 1393–1408.
11. Alena, P.; Karel, K.; Martina, H.; Jitka, M. *Carbohydr. Res.* **2005**, *340*, 161–166.
12. Janos, K.; Zoltan, N.; Zoltan, S. *Carbohydr. Res.* **1997**, *297*, 107–115.
13. Russell, J. C.; William, A. S.; Lister, K. P. L.; John, C. V. *J. Am. Chem. Soc.* **1996**, *118*, 7449–7460.
14. Chaplin, M. F.; Kennedy, J. F. In *Carbohydrate Analysis*, 2nd ed.; Chaplin, M. F., Kennedy, J. F., Eds.; Oxford University Press: Oxford, 1994; p 3.