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Synthesis of phenylazonaphthol- β -D-O-glycosides, evaluation as substrates for beta-glycosidase activity and molecular studies

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Abstract

Background: Phenylazonaphthol- β -D-O-glycosides are alternative substrates for the detection of enzymatic activity of β -glycosidases which are involved in various important processes. These azoic compounds are currently exploited as prodrugs for colonic disease due the presence of β -glycosidase activity in the gut flora and therefore allowing the release of the drug at the specific site.

Results: Phenylazonaphthol- β -D-O-glucoside **3a** and galactoside **3b** were prepared via diazonium salt conditions under weak acidic conditions which do not compromise the O-glycosidic bond stability, by coupling reaction between 2-naphthol sodium salt with aminoglycosides **1a** and **1b**. The resulting phenylazonaphthol glycosides **2a** and **2b** were deprotected affording the phenylazonaphthol glycosides **3a** and **3b** in quantitative yield. The galactoside glycoside **3b** was assayed as substrate for *in vitro* β -galactosidase enzymatic activity showing strong absorbance after releasing of the azoic chromophore. Also, docking studies were performed to determine the best pose as well as the interactions between the ligand and the residues located at the active site.

Conclusions: The methodology developed for synthesizing the phenylazonaphthol glycosides described proved to be convenient for generating azoic functionalities in the presence of glycosidic bonds and the glycosides suitable as alternative substrates and potentially useful prodrugs in the treatment of colonic diseases.

Keywords: β -galactosidase; Synthesis; Phenylazonaphthol glycoside; Substrate; Docking

Background

β -glycosidases are hydrolytic enzymes involved in a number of important processes such as defense mechanism, growth regulation [1], gene markers in transgenic plants [2], and prodrugs [3]. The most commonly used substrates for the histochemical localization of β -glycosidases contain the 5-bromo-4-chloro-3-indolyl chromophore attached to most of the known monosaccharides through an O-glycosidic bond [4]. After enzymatic hydrolysis, the water soluble indoxyl intermediate must undergo an oxidative dimerization to produce a blue precipitate. However, in some cases before dimerization occurs to produce the indigo dye, some diffusion takes place, producing false

positives at some regions in cells lacking enzymatic activity [5]. In addition, phenylazonaphthol glucosides have been synthetically prepared and tested in transgenic plants containing the β -glucuronidase activity which is a widely used gene marker. A comparative test between the commercially available glycosidic indoxyl substrates with the alternative phenylazonaphthol glucuronides resulted in partial diffusion for the indigo dye and no detectable diffusion for the phenylazonaphthol chromophore [6,7].

On the other hand, glycosides when attached to pharmacologically active substances can be used as prodrugs for improving availability, stability, and particularly for specific delivery of the active compound at the target organ. For instance, steroids, antitumor, and anti-inflammatory compounds have been attached to glycosides and evaluated as specific delivery prodrugs [8]. Also, azoic β -glycosides provided to be useful as prodrugs particularly against colitis,

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Crohn's disease, and colorectal cancer since the delivery at colon level might be achieved by the action of azoreductase or glycosidase present at the colonic microflora [9].

Due the potential usefulness of phenylazonaphtol glycosides in processes mentioned above, we developed a methodology for preparing phenylazonaphtol glycosides under weak acidic conditions and the resulting azoic glycosides evaluated as β -galactosidase substrates with potential application as prodrugs for colon diseases.

Methods

The preparation of the phenylazonaphtol glycosides **3a** and **3b** was achieved according to Scheme 1 consisting in the coupling reaction between 2-naphtol in the sodium salt form with 4-aminophenyl tetracetyl glucopyranoside [10-12] **1a** and **1b** under modified diazonium salt condition, which is mainly the use of acetic acid instead of stronger acids, with sodium nitrite at 0°C to produce the diazonium salts which was condensed *in situ* with 2-naphtol sodium salt providing the corresponding phenylazonaphtol tetracetyl glucopyranoside **2a** and **2b**. Final removal of the acetate protecting groups under Zemplen conditions provided the target phenylazonaphtol glycosides **3a** and **3b** as a reddish solid in 60% yield.

Scheme

The beta galactosidase assay. β -galactosidase (2 mg; 2.1 U/mg) was suspended in 0.8 mL of buffer containing 0.06 M Na_2HPO_4 , 0.04 M NaH_2PO_4 , 0.01 M KCl, 0.001 M MgSO_4 ,

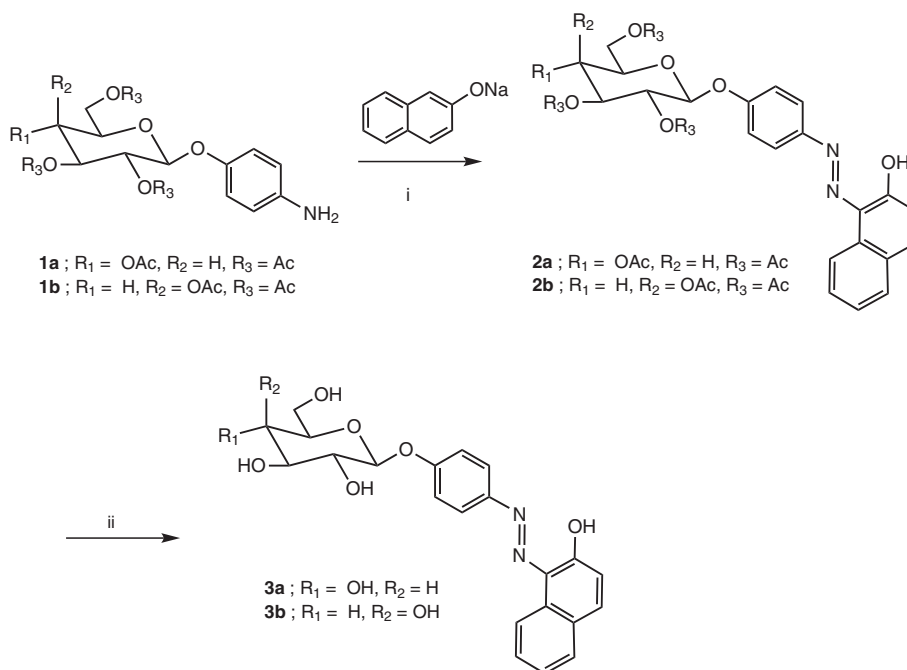
and 1 mM DTT, pH 7.0, and then incubated with glycoside **3b** (4 mg; 9.3×10^{-3} mM) in a mixture of 50 mM acetate buffer with pH 5.0 (0.5 mL) and methanol (0.5 mL) at 37°C for 3 h. The reaction was stopped by adding 0.5 mL 1 M Na_2CO_3 . The amount of the released phenylazonaphtol chromophore was measured spectrophotometrically at 410 and 455 nm [13].

Results and discussion

The deprotected glycoside **3b** was tested as substrate for the detection of glycosidase activity using commercial *Escherichia coli* β -galactosidase. Thus, the hydrolytic activity resulted in the cleavage of the glycosidic linkage, releasing the phenylazonaphtol chromophore which is observed as an intense reddish color with absorption at 410 and 455 nm, as it is observed in the absorption graphic (Figure 1) and as observed in the vials (Figure 2).

Docking studies

The active site architecture showing the interactions between the phenylazonaphtol galactoside **3b** and *E. coli* β -galactosidase pocket was performed using AutoDock 4.2 [14] and Chimera [15] software packages as visualization system. The protein assigned for the docking analysis was *E. coli* β -galactosidase (PDB IDs: 1JYN), and the residues chosen were Asn102, Asp201, Asn460, His418, Glu461, Glu537, His540, Phe 601, and Trp999 which have been described to participate in the interactions with its natural substrate lactose [16]. β -galactosidase is a retaining glycosidase with a double displacement mechanism, and



Scheme 1 The preparation of the phenylazonaphtol glycosides **3a** and **3b**.

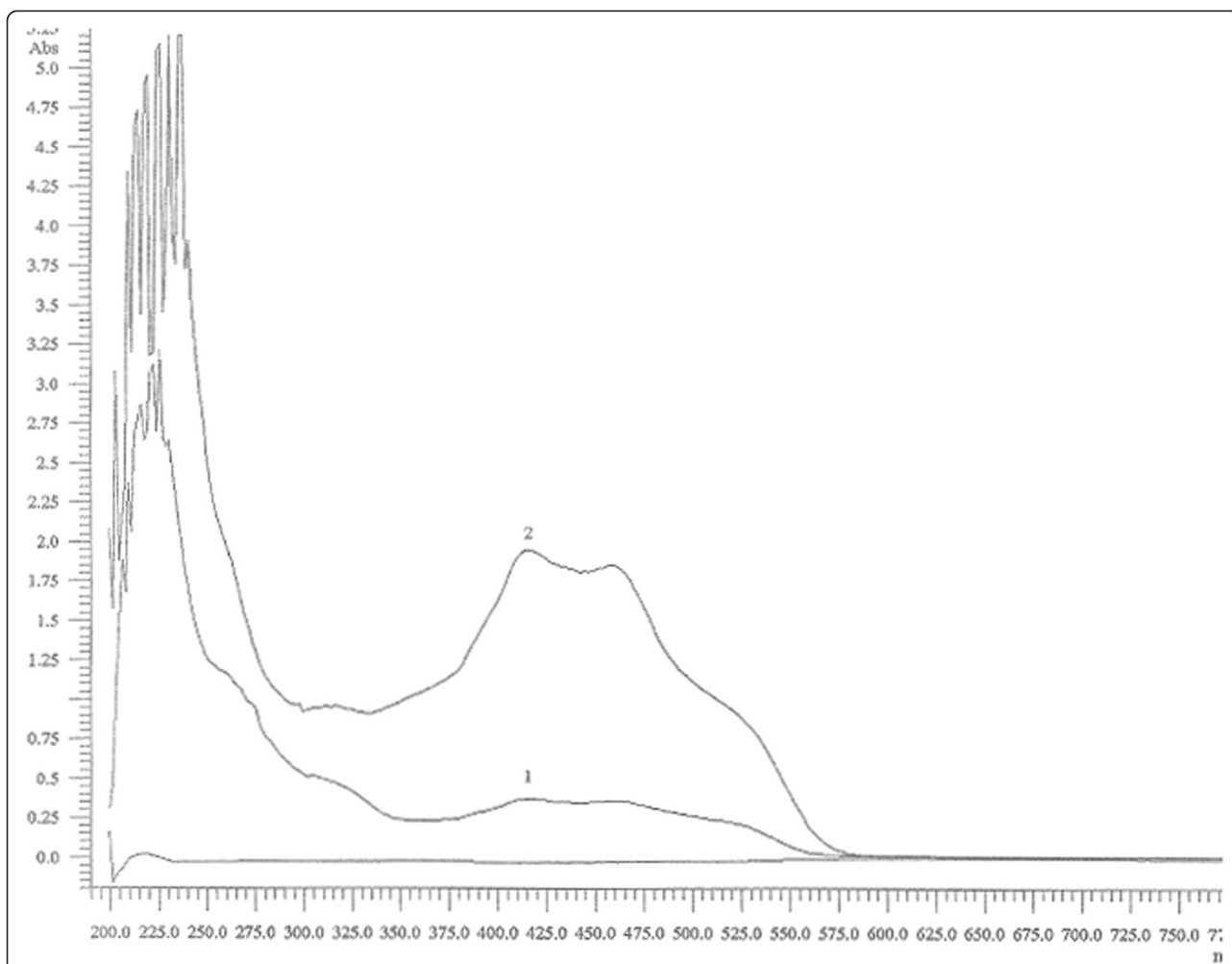


Figure 1 Adsorption of **3b** without β -galactosidase (1) and with β -galactosidase (2).

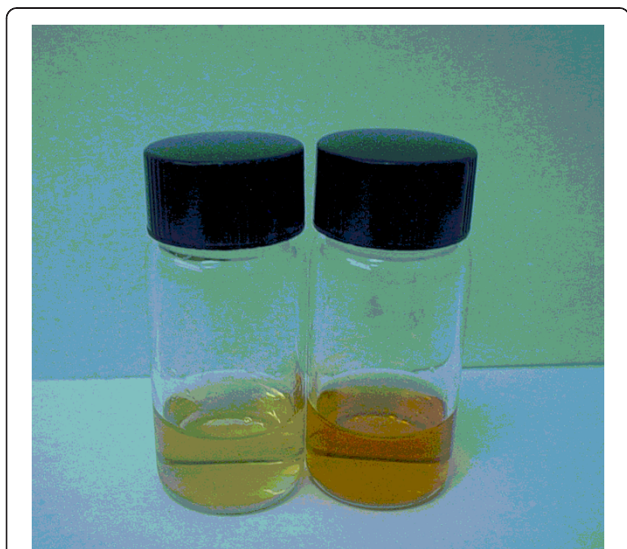


Figure 2 Vials containing **3b** without β -galactosidase (less colored) and **3b** incubated with β -galactosidase.

its interactions with the ligand proceed with participation of water and the ions Mg^{2+} and Na^{1+} within the catalytic cavity [17]. The docking analysis was performed to find the minimized conformations according to the docking protocols which use the Lamarckian Genetic Algorithm for searching the most favorable ligand-protein complex interactions. Thus, as a result of the docking analysis, we found the minimized conformer with the galactose moiety positioned toward the active site having a binding energy of -11.62 , showing hydrogen bond interactions of C-3 and C-4 hydroxyl groups of galactoside moiety with Glu461 residue, in agreement with those reported for the natural substrate lactose [16] (Figure 3).

The ribbon representation was also analyzed by using the chimera visualizing system to show the region of the cavity and the residues involved in the interaction with the substrate **3b** (Figure 4).

In addition, the surface representation shows that the galactopyranoside moiety is embedded into the pocket interacting with the residues while the azoic chromophore

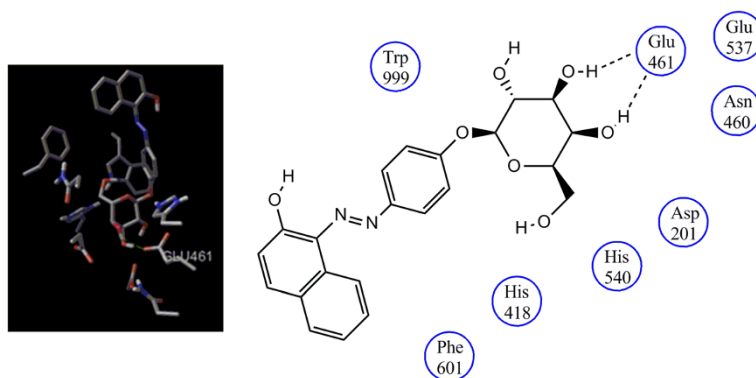


Figure 3 The first scored conformation assumed by 3b presenting hydrogen bond interactions with Glu461 residue.

is pointed out toward the pocket exit as shown in Figure 5.

Experimental

All the reagents were purchased from Aldrich Chemical Co. (St. Louis, MO, USA) except HBr/acetic acid which was purchased from Fluka Chemical Co. (Buchs, Switzerland). Column chromatography was performed on silica gel with a 70-230 mesh, and thin-layer chromatography on Kieselgel, both from Merck Co. (Darmstadt, Germany), which was used as a detection system. A cerium sulfate solution followed by heating on hot plate. Infrared (IR) spectra were obtained on Perkin-Elmer spectrometer (Waltham, MA, USA); nuclear magnetic resonance (NMR) spectra were recorded on Varian 300-MHz spectrometer (Palo Alto, CA, USA) and Bruker 500 MHz (Karlsruhe, Germany). Mass spectrum (MS) spectra were obtained using a Hewlett-Packard 5989A (Palo Alto, CA, USA). Optical rotations were measured

with a Perkin-Elmer 341 polarimeter at 23°C. Enzyme galactosidase was purchased from Sigma Chemical Company (St. Louis, MO, USA).

1-[(4-tetra-O-acetyl-β-D-glucopyranosyloxy phenyl)azo]-2-naphtol (2a)

Compound 1a (0.543 g, 1.23 mmol) was dissolved in 8 mL of THF, and the flask was cooled to 0°C. After the addition of sodium nitrite (85 mg, 1.23 mmol) and 0.5 mL of acetic acid/H₂O (1:1, v/v), the reaction was kept under ice-bath temperature with stirring for 15 min. 2-naphtol sodium salt dissolved in 5 mL of THF/H₂O (1:1, v/v) was added dropwise in another flask, and the reaction was kept at 0°C with stirring for 30 min, allowing the reaction to reach room temperature and stirred for another 60 min. The solvent was removed under *vacuo*, diluted with CH₂Cl₂ (30 mL) and washed with cold 2 N NaOH solution (3 × 15 mL) and water (20 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated on

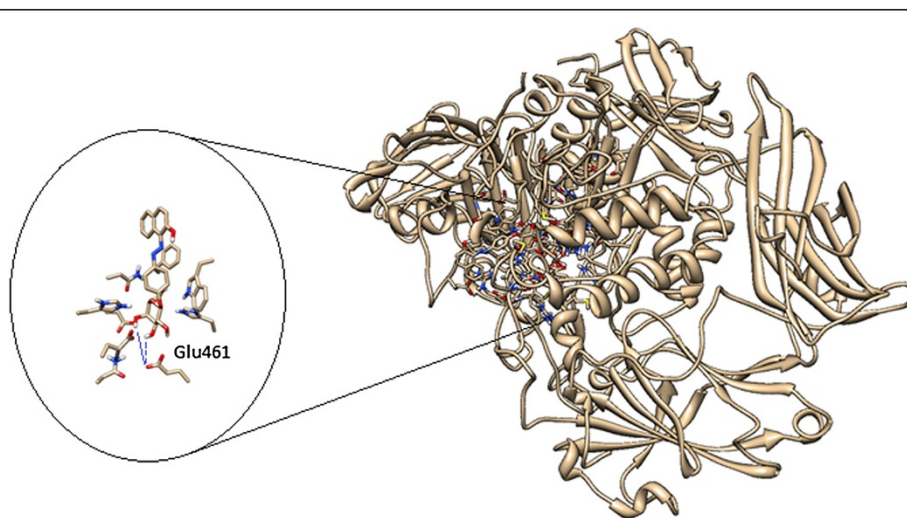


Figure 4 Ribbon representation. This shows the region of the active site among the protein and residues involved in the interaction with the ligand.

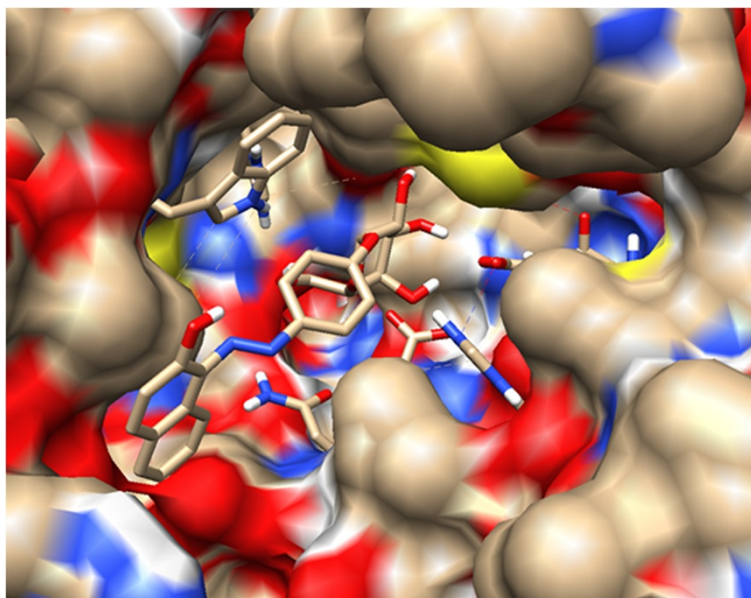


Figure 5 Surface representation showing the pose of ligand 3b at the active.

rotavapor. The crude was purified by column chromatography with AcOEt/hexane (1:3) to give an orange-red solid (325 mg, 60%). m.p. 81°C to 83°C, $[\alpha]_D -13.1$ (c 0.6, CHCl₃), IR (thin film) 1738 cm⁻¹, ¹H NMR (CDCl₃) δ 2.05 to 2.14 (4 s, 12 H), 3.92 (m, H-5), 4.20 (dd, H-6, $J = 12.3$ Hz, 2.1 Hz), 4.33 (dd, H-6', $J = 12.3$ Hz, 5.1 Hz), 5.17 (d, H-1, $J = 7.5$ Hz), 5.20 (t, H-4, $J = 9.6$ Hz), 5.31 (t, H-2, $J = 9.6$ Hz), 5.34 (t, H-3, $J = 9.6$ Hz), 6.97 (d, H-13, $J = 9.3$), 7.15 (d, H-8, 8', $J = 5.1$ Hz), 7.41 (t, H-16, $J = 7.5$ Hz, 7.2 Hz), 7.58 (t, H-17, $J = 7.5$ Hz, 7.2 Hz), 7.66 (d, H-15,

$J = 8.1$ Hz), 7.75 (d, H-14, $J = 9.6$ Hz), 7.76 (d, H-9,9', $J = 9.0$ Hz), 8.62 (d, H-18, $J = 7.8$ Hz). ¹³C NMR (CDCl₃) δ 20.5, 20.6 (4 CH₃-), 61.8 (C-6), 68.1 (C-4), 71.1 (C-3), 72.1 (C-2), 72.6 (C-5), 98.9 (C-1), 117.8 (C-8), 121.0 (C-9), 121.5 (C-18), 123.0 (C-13), 125.2 (C-16), 127.7 (C-14a), 128.1 (C-11), 128.4 (C-17), 129.7 (C-15), 133.3 (C-18a), 138.2 (C-10), 142.6 (C-14), 156.8 (C-7), 165.1 (C-12), 169.2, 169.3, 170.1, 170.4 (4 C = O). HRMS calculated for C₃₀H₃₀N₂O₁₁ (M⁺) 594.1850, found 594.1845 (Figure 6).

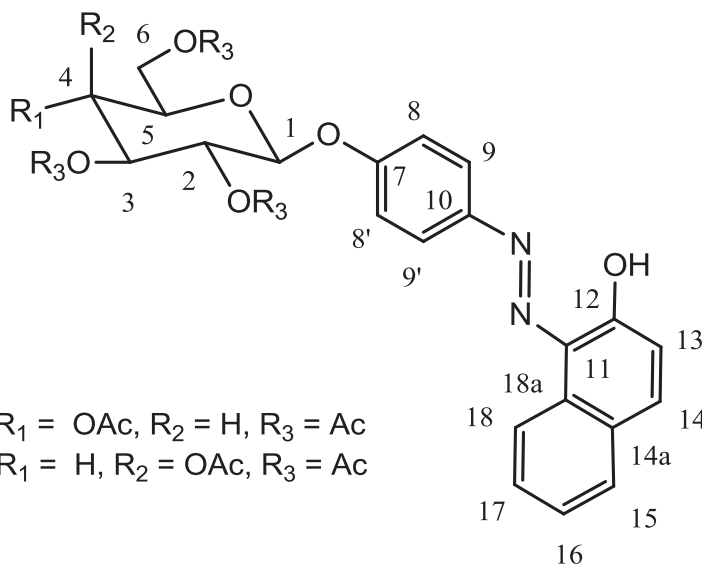
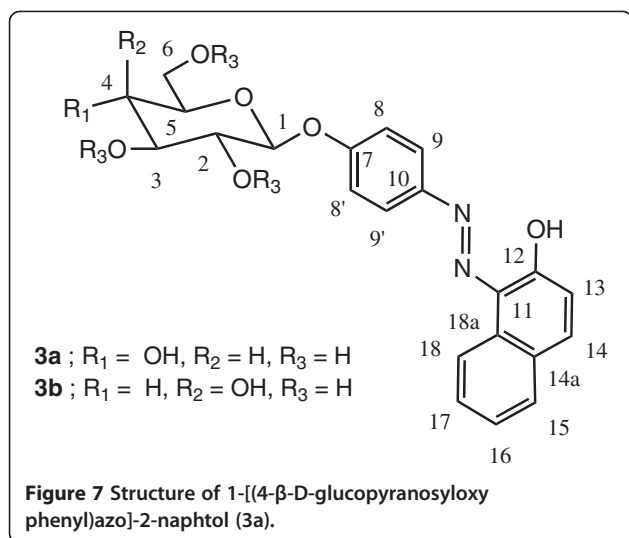


Figure 6 Structure of 1-[(4-tetra-O-acetyl- β -D-glucopyranosyloxy phenyl)azo]-2-naphthol (2a).



1-[(4-tetra-O-acetyl-β-D-galactopyranosyloxy phenyl)azo]-2-naphthol (2b)

Same reaction conditions as for **2a** except that compound **1b** is used instead of **1a**.

¹H NMR (CDCl₃) δ 2.09 to 2.12 (4 s, 12 H), 4.12 (1H, m, H-5), 4.22 (1H, dd, H-6), 4.26 (1-H, dd, H6'), 5.11 (1H, d, H-1), 5.13 (1H, dd, H-4), 5.48 (1H, t, H-2), 5.54 (1H, dd, H-3), 6.97 (d, H-13, *J* = 9.3), 7.15 (d, H-8, 8', *J* = 5.1 Hz), 7.41 (t, H-16, *J* = 7.5 Hz, 7.2 Hz), 7.58 (t, H-17, *J* = 7.5 Hz, 7.2 Hz), 7.66 (d, H-15, *J* = 8.1 Hz), 7.75 (d, H-14, *J* = 9.6 Hz), 7.76 (d, H-9,9', *J* = 9.0 Hz), 8.62 (d, H-18, *J* = 7.8 Hz). ¹³C NMR (CDCl₃) δ 20.8, 20.9 (4 CH₃⁻), 61.5 (C-6), 67.0 (C-4), 68.7 (C-2), 71.2 (C-3), 71.4 (C-5), 99.7 (C-1), 117.8 (C-8), 121.0 (C-9), 121.5 (C-18), 123.0 (C-13), 125.2 (C-16), 127.7 (C-14a), 128.1 (C-11), 128.4 (C-17), 129.7 (C-15), 133.3 (C-18a), 138.2 (C-10), 142.6 (C-14), 156.8 (C-7), 165.1 (C-12), 169.2, 169.3, 170.1, 170.4 (4 C = O). MS (EI) *m/z* 594.18 [M⁺], 169 (100), 264 (62).

1-[(4-β-D-glucopyranosyloxy phenyl)azo]-2-naphthol (3a)

To protected phenylazonaphthol glycoside **2a** (0.3 g, 0.504 mmol), 5 mL of 10% NaOMe in MeOH was added and stirred in a room temperature for 2 h. Ion exchange resin Dowex 50WX2-100 was added to neutralize and the resin filtered. The solvent was removed under *vacuo* to give 0.193 g, 90% as a red solid (Figure 7).

m.p. 177°C to 178°C, [α]_D -23.4 (c 0.9, MeOH), ¹H NMR (DMSO *d*-6) δ 3.13 (H-2), 3.30 (H-4), 3.35 (H-5), 3.37 (H-3), 3.60 (H-6), 3.75 (H-6'), 4.99 (H-1, *J* = 7.8), 6.97 (d, H-13, *J* = 9.3), 7.15 (d, H-8, 8', *J* = 5.1 Hz), 7.41 (t, H-16, *J* = 7.5 Hz, 7.2 Hz), 7.58 (t, H-17, *J* = 7.5 Hz, 7.2 Hz), 7.66 (d, H-15, *J* = 8.1 Hz), 7.75 (d, H-14, *J* = 9.6 Hz), 7.76 (d, H-9,9', *J* = 9.0 Hz), 8.62 (d, H-18, *J* = 7.8 Hz). ¹³C NMR (DMSO *d*-6) δ 61.7 (C-6), 76.8 (C-5), 71.6 (C-4), 74.1 (C-2), 76.7 (C-3), 96.7 (C-1), 117.8 (C-8),

121.0 (C-9), 121.5 (C-18), 123.0 (C-13), 125.2 (C-16), 127.7 (C-14a), 128.1 (C-11), 128.4 (C-17), 129.7 (C-15), 133.3 (C-18a), 138.2 (C-10), 142.6 (C-14), 156.8 (C-7), 165.1 (C-12), 169.2, 169.3, 170.1, 170.4 (4 C = O). HRMS calculated for C₂₂H₂₂N₂O₇ (M + H) 427.1505, found 427.1509.

1-[(4-β-D-galactopyranosyloxy phenyl)azo]-2-naphthol (3b)

Same reaction conditions as for **3a** except that compound **2b** is used instead of **2a**. ¹H NMR (DMSO *d*-6) δ 3.41 (t, H-2), 3.56 (dd, H-3), 3.61 (m, H-5), 3.62 (H-6), 3.70 (H-6'), 3.84 (dd, H-4), 4.98 (d, H-1, *J* = 8.0), 6.97 (d, H-13, *J* = 9.3), 7.15 (d, H-8, 8', *J* = 5.1 Hz), 7.41 (t, H-16, *J* = 7.5 Hz, 7.2 Hz), 7.58 (t, H-17, *J* = 7.5 Hz, 7.2 Hz), 7.66 (d, H-15, *J* = 8.1 Hz), 7.75 (d, H-14, *J* = 9.6 Hz), 7.76 (d, H-9,9', *J* = 9.0 Hz), 8.62 (d, H-18, *J* = 7.8 Hz). ¹³C NMR (DMSO *d*-6) δ 62.0 (C-6), 69.7 (C-4), 72.9 (C-2), 73.8 (C-3), 76.0 (C-5), 97.3 (C-1), 117.8 (C-8), 121.0 (C-9), 121.5 (C-18), 123.0 (C-13), 125.2 (C-16), 127.7 (C-14a), 128.1 (C-11), 128.4 (C-17), 129.7 (C-15), 133.3 (C-18a), 138.2 (C-10), 142.6 (C-14), 156.8 (C-7), 165.1 (C-12), 169.2, 169.3, 170.1, 170.4 (4 C = O). FABMS (M + 1) 427.1.

The Additional file 1 (as word document) shows the ¹H, ¹³C NMR spectra for the synthesized compounds **2a-b** and **3a-b**, and high-resolution mass spectrometry and elemental analysis are also included.

Conclusions

The methodology developed for synthesizing the phenylazonaphthol glycosides described proved to be convenient for generating azoic functionalities in the presence of glycosidic bonds, and the glycosides are suitable as alternative substrates and potentially useful prodrugs in the treatment of colonic diseases.

Additional file

Additional file 1: Bidimensional NMR spectroscopy for compound 2a. Homonuclear through-bond correlation bidimensional spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments are included to provide further evidence for compound **2a**.

Competing interests

The authors declare that they have no competing interests.

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