Supporting Information

Synthesis of Polyhydroxy Piperidines and their Analogues: A novel approach for selective inhibitors of α-glucosidase.

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X-ray Crystal Structure Analysis for compound 18a and 18b

Crystal Data: Data for both the compounds were collected at T = 293 K, on SMART APEX CCD Single Crystal X-ray diffractometer using Mo-Kα radiation (λ = 0.7107 Å) to a maximum θ range of 25.00°. The structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[1] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. The refinements were carried out using SHELXL-97.

Compound **18a:** Single crystals of the complex were grown by slow evaporation of the solution mixture of ethyl acetate and pet ether. Colorless needle of approximate size 0.34 x 0.14 x 0.05 mm, was used for data collection. Crystal to detector distance 6.05 cm, 512 x 512 pixels / frame, Hemisphere data acquisition. Total scans = 3, total frames = 1283, Oscillation / frame -0.3º, exposure / frame = 20.0 sec / frame, maximum detector swing angle = –30.0º, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration, θ range = 2.16 to 25.0 º, completeness to θ of 25.0 º is 98.8 %. SADABS correction applied, C16 H23 N O4, M = 293.35. Crystals belong to Monoclinic, space group $P2_1$, a = a = 10.872 (1), b = 5.6676 (5), c = 12.916 (1) Å, β = 106.839 (1)º V = 761.75 (12) $Å^3$, Z = 2, D_c = 1.279 mg m^{-3}, μ (Mo–K_α) = 0.091 mm^{-1}, 3761 reflections measured, 2445 unique [I>2σ(I)], R value 0.0482, wR2 = 0.1185. Largest diff. peak and hole 0.212 and -0.165 e. $Å^{-3}$.[1]

[1] G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997

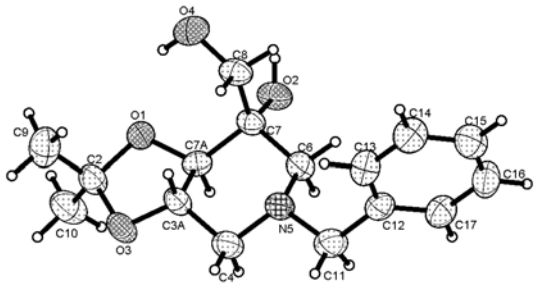

Fig 1. ORTEP diagram of the **18a**. Ellipsoids are drawn at 50% probability.

Compound **18b:** Single crystals of the complex were grown by slow evaporation of the solution mixture of ethyl acetate and pet ether. Colorless needle of approximate size 0.21 x 0.05 x 0.02 mm, was used for data collection. Crystal to detector distance 6.05 cm, 512 x 512 pixels / frame, multiscan data acquisition. Total scans = 5, total frames = 2545, Oscillation / frame -0.3º, exposure / frame = 20.0 sec / frame, maximum detector swing angle = –30.0º, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration, θ range = 1.65 to 23.99 º, completeness to θ of 23.99 º is 92.7 %. SADABS correction applied, C16 H23 N O4, M = 293.35. Crystals belong to monoclinic, space group $P2_1$, a = 10.9255(6), b = 5.6715(3), c = 12.9037(8) Å, beta = 107.143(1) deg, V = 764.04(8) $Å^3$, Z = 2, D_c = 1.275 mg m^{-3}, μ (Mo–K_α) = 0.091 mm^{-1}, 5981 reflections measured, 2186 unique [I>2σ(I)], R value 0.0565, wR2 = 0.1142. Largest diff. peak and hole 0.201 and -0.224 e. $Å^{-3}$.

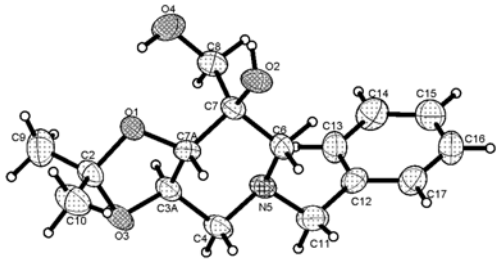

Fig 2. ORTEP diagram of the **18b**. Ellipsoids are drawn at 50% probability.

General procedure for enzyme inhibition assay:

Inhibition assay for the inhibitory potencies of the azasugars were determined by measuring the residual hydrolytic activities of the glycosidases of the corresponding *p*-nitrophenyl glycosides in the presence of azasugars spectrophotometrically. The absorbance of the resulting solution was read at 405 nm.

In the case of β-galactosidase (*Aspergillus oryzaie*) each assay was performed in citrate phosphate buffer (pH 4.5) with *p*-nitrophenyl β-D-galactpyranooside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-galactosidase (Green coffee beans), the assay was performed in a potassium phosphate buffer (pH 6.5) with *p*-nitrophenyl α-D-galactopyranoside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of β-mannosidase (Snail), the assay was performed in a citrate phosphate buffer (pH 4.0) with *p*-nitrophenyl β-D-mannopyranoside as the substrate. Varying concentrations of the substrate (200 μM, 150 μM) were employed. The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-mannosidae (Jack Beans), the assay was performed in citrate phosphate buffer (pH 4.5) with *p*-nitrophenyl α-D-mannpyranoside as the substrate

Varying concentrations of the substrate (50 μM, 30 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 25 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of β-glucosidase (Almond), the assay was performed in a potassium phosphate buffer (pH 5.5) with *p*-nitrophenyl β-D-glucopyranoside as the substrate. Varying concentrations of the substrate (100 μM, 50 μM) were employed. The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 30 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution

In the case of α-glucosidase (Yeast), the assay was performed in a potassium phosphate buffer (pH 6.8) with *p*-nitrophenyl α-D-glucopyranoside as the substrate. Varying concentrations of the substrate (200 μM, 100 μM) were employed (except for compound **23** and **8a** were (200 μM, 150 μM) and (300 μM, 200 μM) was used, respectively). The reaction was initiated by the addition of 50 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 20 min at 37 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

In the case of α-mannosidase (*Aspergillus fischeri*), the assay was performed in a potassium phosphate buffer (pH 6.5) with *p*-nitrophenyl α-D-mannopyranoside as the substrate. Varying concentrations of the substrate (500 μM, 250 μM) were employed. The reaction was initiated by the addition of 25 μL of appropriately diluted enzyme. The reaction mixture (having inhibitor) which had a final volume of 0.5 mL was incubated for 15 min at 50 °C, and then quenched by the addition of 1.0 mL of 1M Na_2CO_3 solution.

Dixon method was employed for the determination of *Ki*. In this method, hydrolytic activity of enzyme was measured in the presence of two different concentrations of substrates and varying concentrations of inhibitors. The reciprocals of substrate

hydrolysis (1/V) were plotted against the inhibitor concentration and the Ki was determined by fitting the data using ORIGIN 6.1.

General experimental methods

Unless mentioned, all reactions were performed under argon atmosphere. All commercially available reagents were used without further purification unless otherwise noted. Enzymes are purchased from commercial sources. Tetrahydrofuran was freshly distilled from benzophenone ketyl radical under argon prior to use. Column chromatography was performed with silica gel (100-200 and 230-400 mesh). The combined organic layers were dried over $NaSO_4$. Solvents were evaporated under reduced pressure. All yields given refer to as isolated yields. Melting points reported are uncorrected. Optical rotations were measured on a precision automated polarimeter. NMR spectra were recorded on a 200, 400, and 500 MHz spectrometer. Chemical shifts are reported in ppm. Coupling constants (*J* values) are reported in Hertz. ^{13}C peak multiplicity assignments were made based on DEPT data. IR spectra were recorded on a FT-IR spectrometer. MS experiments were performed on a low resolution magnetic sector mass spectrometer. GC analysis was performed on a Varian CP 3800 GC using CP-Sil 5CB column. The optical density measurements were carried out on a Varian CARY-50 BIO UV-VIS spectrophotometer.

General procedure for N-debenzylation and acetonide cleavage:

A solution of substrate in ethanol was hydrogenated (1 atm, rt) using $Pd(OH)_2$ on charcoal (20 %) as catalyst (20% by weight of substrate) for 10 h. The reaction mixture was passed through a pad of celite and the solvent was removed under reduced pressure. To a solution of this in methanol was added conc. HCl (2 to 4 drops) and the reaction mixture was stirred at rt for 4h. The solvent was removed under reduced pressure to afford product.

General procedure for conversion of hydroxyl moiety to amine:

To a solution of alcohol (1 eq.) in dry DCM at 0 °C under argon atmosphere was added triethyl amine (1.2 eq.) and methanesulphonyl chloride (1.1 eq.). The reaction mixture was stirred at room temperature for 6 h. Water was added to the reaction mixture and extracted with DCM. The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under *vacuo* and purified by column chromatography (pet. ether/ethyl acetate, 5:2), to get mesylate.

To a solution of mesylate (1 eq.) in DMF was added LiN_3 (10 eq) and heated to 110 °C for 20 h. It was extracted with EtOAc, giving water wash. The organic layer was dried over Na_2SO_4, concentrated and purified (as required) by column chromatography (pet. ether/ethyl acetate, 4:1), to get azide**.**

To a solution of azide (1 eq.) in dry THF was added LAH (2 eq.) and stirred overnight at room temperature. Reaction mixture was quenched by dropwise addition of 2N NaOH solution, dried over Na_2SO_4, filtered, concentrated and chromatographed (DCM / MeOH, 4:1) to get corresponding amine compound.

N-benzyl-1-((4S,5S)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)-N-((trimethylsilyl)methyl)methanamine (16a) A mixture of **15** (8 g, 34.1 mmol), $PhCH_2NHCH_2TMS$ (13.15 g, 68.3 mmol), anhydrous K_2CO_3 (19.15 g, 136.7 mmol) catalytic amount of TBAI (1.0 g, 4.1 mmol) in dry CH_3CN (60 mL) was refluxed for 96 h under an argon atmosphere. Solvent was removed under reduced pressure, water was added and the reaction mixture was extracted with EtOAc (2 X 100 mL). The combined organic extracts were dried over Na_2SO_4, concentrated in *vacuo* and the residue was column chromatographed (petroleum ether-ethyl acetate, 19:1) to afford pure **16a** (7.96 g, 80 %) as a colorless liquid. $[\alpha]^{27}_D$ -0.73, (c 0.5, $CHCl_3$); Lit[14b] $[\alpha]^{20}_D$ -0.7, (c 11.0, $CHCl_3$); Rest of the data is as for its enantiomer ie **16b.**

(3aS,7aS)-5-benzyl-2,2-dimethyl-7-methylenehexahydro-[1,3]dioxolo[4,5-c]pyridine (17) A solution containing the substrate **16a** (1.0 g, 3.02 mmol) and 1,4-dicyanonaphthalene (DCN) (0.18 g, 1.01 mmol) in 2-propanol (80 mL per mmol of substrate), in an open vessel, was irradiated using a 450-W Hanovia medium pressure mercury vapor lamp as the light source. The lamp was housed in a Pyrex water-jacketed immersion well so as to allow only the wavelengths greater than 280 nm to pass through. The reaction was monitored by TLC or GC till consumption of the starting material (>90% ~ 1 h). The solvent was then removed under reduced pressure and the crude product was purified by column chromatography (petroleum ether-ethyl acetate, 92.5:7.5) to get the pure compound **17** (0.47g) 60% as white solid. . mp 96-98 °C (from EtOAc / pet. ether) Lit.[14b] 94-96 °C; $[\alpha]^{22}_D$ +53 (c 0.75, $CHCl_3$); *ent* $[\alpha]^{28}_D$ = -52, (c 1.0, $CHCl_3$) lit[14b] $[\alpha]^{20}_D$+49.2 (c 0.7, $CHCl_3$); $[\alpha]^{20}_D$ -50.9, (c 1.9, $CHCl_3$); respectively; Anal. Calcd for $C_{16}H_{21}NO_2$: C, 74.10; H, 8.16; N, 5.40. Found: C, 74.23; H, 8.56 N, 5.46; IR υ_{max} cm^{-1} in $CHCl_3$, 3020, 1216, 757; ^1H NMR (200 MHz, $CDCl_3$), δ 1.39 (s, 6H), 2.24-2.34 (m, 1H), 2.70 (d, 1H, *J* = 12.63 Hz), 3.19 (s, 1H), 3.26 (s, 1H), 3.40-3.55 (m, 1H), 3.60 (s, 1H), 3.61 (s, 1H), 3.73 (td, 1H, *J* = 9.22, 1.77 Hz), 4.81 (d, 1H, *J* = 1.26 Hz), 4.94 (s, 1H), 7.19-7.29 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$), δ 26.7 (CH_3), 26.9 (CH_3), 54.5 (CH_2), 57.2 (CH_2), 61.6 (CH_2), 77.4 (CH), 81.7 (CH), 105.3 (CH_2), 111.0 (C), 127.2 (CH), 128.3

(CH), 128.9 (CH), 137.7 (C), 140.3 (C); Mass: *m/z* (%)282 (M^++Na, 63), 260 (M^++H, 100), 250 (30);

(3aS,7S,7aR)-5-benzyl-7-(hydroxymethyl)-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (18a)

To a suspension of **17** (1.0 g, 3.8 mmol) in *t*-BuOH (10 mL) was added N-methylmorpholine-N-oxide (50% aq. solution) (0.634 mL, 7.69 mmol) and osmium tetroxide (0.5 mL, 1% solution of OsO_4 in *t*-BuOH). The reaction mixture was stirred for 24 h at room temperature. Solvent was removed under reduced pressure, and the residue was column chromatographed (pet. ether / ethyl acetate, 7:3) to afford pure **18a** (1.02 g, 90 %) as a white solid. mp 152-154 °C (from EtOAc / pet. ether); Lit.[14e] 150-152 °C. $[\alpha]^{29}_D$ +24.2 (c 0.6, MeOH), *ent* $[\alpha]^{29}_D$ -23.6, (c 1.2, MeOH); Lit.[14d,14e] $[\alpha]^{25}_D$ +23.1, (c 0.46, MeOH); *ent* $[\alpha]^{25}_D$ -21.6, (c 1.4, MeOH); Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77. Found: C, 65.43; H, 7.81 N, 4.97; IR υ_{max} cm^{-1} in $CHCl_3$ 3491, 1216; ^1H NMR (400 MHz, $CDCl_3$, D_2O exchange), δ 1.35 (s, 6H), 2.02 (d, 1H, *J* = 11.80 Hz), 2.12 (dd, 1H, *J* = 10.04, 9.79 Hz), 2.78 (d, 1H, *J* = 11.80 Hz), 3.11 (dd, 1H, *J* = 9.54, 4.01 Hz), 3.42 (d, 1H, *J* = 9.54 Hz), 3.55 (s, 2H), 3.62 (d, 1H, *J* = 11.55 Hz), 3.64 (dt, 1H, *J* = 9.78, 4.01 Hz), 4.02 (d, 1H, *J* = 11.30 Hz), 7.16-7.26 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$), 26.5 (CH_3), 26.6 (CH_3), 54.4 (CH_2), 59.1 (CH_2), 61.4 (CH_2), 64.7 (CH_2), 71.4 (C), 73.5 (CH), 86.0 (CH), 110.8 (C), 127.4 (CH), 128.4 (CH), 128.7 (CH), 137.7 (C); Mass: *m/z* (%), 316 (M^++Na, 77), 294 (M^++H, 100).

(3aS,7S,7aS)-5-benzyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol (21)

To a solution of **18a** (0.50 g, 1.7 mmol) in ethanol-water (10 mL, 4:1) mixture was added sodium periodate (0.44 g, 2.04 mmol) gradually. The white suspension was stirred for 0.5 h at room temperature and filtered. The filtrate was concentrated and the white pasty mass was extracted with ethyl acetate (2 X 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. Sodium borohydride (0.381 g, 2.04 mmol) was added to a solution of ketone in methanol (5 mL). The resulting mixture was stirred for 24 h at room temperature and then quenched with brine. The white suspension was stirred overnight and extracted with ethyl

acetate (2 X 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was column chromatographed (pet ether / ethyl acetate, 3:2) to afford **21** (0.086 g, 85 %) as a colorless liquid. $[\alpha]^{29}_D$ +38.38 (c 1.45, $CHCl_3$), Lit.[14d] $[\alpha]^{25}_D$ +35.0 (c 0.2, $CHCl_3$); Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.82; H, 7.90; N, 5.66; IR (in $CHCl_3$) υ_{max} cm^{-1} 3018, 1216; ^1H NMR (400 MHz, $CDCl_3$, D_2O exchange), δ 1.34 (s, 3H), 1.38 (s, 3H), 2.15 (t, 1H, *J* = 10.04 Hz), 2.23 (d, 1H, *J* = 12.80 Hz), 2.95 (d, 1H, *J* = 12.55 Hz), 3.18-3.22 (m, 2H), 3.61 (s, 2H), 3.92 (dt, 1H, *J* = 10.04, 4.02 Hz), 4.14 (s, 1H), 7.16-7.26 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 26.5 (CH_3), 26.8 (CH_3), 54.8 (CH_2), 56.6 (CH_2), 61.7 (CH_2), 65.6 (CH), 70.8 (CH), 81.6 (CH), 110.3 (C), 127.3 (CH), 128.7 (CH), 129.0 (CH), 137.2 (C); Mass: *m/z* (%), 264 (M^++H, 33), 263 (M^+, 100), 223 (27).

(R)-1'-((4R,5R)-5-ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1',2'-diol (**38**)

To a stirred mixture of **37** (20.0 g, 65.78 mmol) and anhydrous K_2CO_3 (11.97 g, 85.5 mmol) in dry MeOH (250 mL) at 65°C was added the solution of Bestmann-Ohira reagent (16.42 g, 85.5 mmol) in dry MeOH (100 mL) drop wise over a period of 6 h under argon atmosphere. Reaction mixture was neutralized with acetic acid and solvent was removed. Water was added to the residue and it was extracted by ethyl acetate (2 X 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (pet. ether/ethyl acetate, 4:1), furnished **38** (6.73 g, 55%) as white solid. $[\alpha]^{30}_D$ +7.27 (c 0.55, $CHCl_3$); Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.15; H, 7.45; IR υ_{max} cm^{-1} in $CHCl_3$, 3306, 3019, 1215; ^1H NMR (400 MHz, $CDCl_3$), δ 1.40 (s, 3H), 1.48 (s, 3H), 2.56 (d, 1H, *J* = 2.01 Hz), 2.87 (bs, 1H), 3.26 (bs, 1H), 3.66 (dd, 1H, *J* = 11.54, 6.53 Hz), 3.76 (dd, 1H, *J* = 11.54, 3.26 Hz), 3.83-3.86 (m, 1H), 4.11 (t, 1H, *J* = 6.52 Hz), 4.68 (dd, 1H, *J* = 6.78, 2.01 Hz); ^{13}C NMR (100 MHz, $CDCl_3$), δ 25.8 (CH_3), 26.8 (CH_3), 63.1 (CH_2), 66.6 (CH), 71.6 (CH), 74.7 (CH), 81.6 (C), 81.8 (CH), 110.9 (C).