Antileishmanial activity of $sp^2$-minosugar derivatives

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List of Contents

1. General Procedure for the Glycosidase Inhibition Assay S2
2. Lineweaver-Burk and Double Reciprocal Analysis Plots of $2\alpha$, 5, 7, 8 S3-S4
3. Spectroscopic Data of $13\beta$ and $2\beta$ S5
4. Copies of $1\text{H}$ and $^{13}\text{C}$ NMR Spectra of 2-8 and 13-19 S6-S21

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1. General Procedure for the Glycosidase Inhibition Assay

Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective o- (for β-glucosidase/β-galactosidase from bovine liver and β-galactosidase from E. coli) or p-nitrophenyl α- or β-D-glycopyranoside, in the presence of the corresponding inhibitor. Each assay was performed in phosphate buffer at the optimal pH for each enzyme. The $K_m$ values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: α-glucosidase (yeast), $K_m = 0.35$ mM (pH 6.8); isomaltase (yeast) $K_m = 1.0$ mM (pH 6.8), β-glucosidase (almonds), $K_m = 3.5$ mM (pH 7.3); β-glucosidase/β-galactosidase (bovine liver), $K_m = 2.0$ mM (pH 7.3); β-galactosidase (E. coli), $K_m = 0.12$ mM (pH 7.3); α-galactosidase (coffee beans), $K_m = 2.0$ mM (pH 6.8); trehalase (pig kidney), $K_m = 4.0$ mM (pH 6.2); amyloglucosidase (Aspergillus niger), $K_m = 3.0$ mM (pH 5.5); β-mannosidase (Helix pomatia), $K_m = 0.6$ mM (pH 5.5); α-mannosidase (jack bean), $K_m = 2.0$ mM (pH 5.5); naringinase (Penicillium decumbens, β-glucosidase/β-rhamnosidase activity). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10-30 min at 37 °C or 55 °C the reaction was quenched by addition of 1 M Na₂CO₃. The absorbance of the resulting mixture was determined at 405 nm or 505 nm. Each experiment was performed in duplicate using $[I] = 2, 0.4, 0.08, 0.04$ y 0.02 μM and $[S]$ nearly $K_m$ value. In those cases were $K_i$ values lower that 10 μM were obtained by this procedure (2α, 5, 7 and 8 against yeast α-glucosidase), refined $K_i$ values and the enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis (Figures S1-S4).
2. Lineweaver-Burk and Double Reciprocal Analysis Plots

**Figure S1.** Lineweaver-Burk Plot for $K_i$ determination (1.3 \( \mu \)M) of 2\( \alpha \) against \( \alpha \)-glucosidase (baker yeast) (pH 6.8).

**Figure S2.** Lineweaver-Burk Plot for $K_i$ determination (14.3 \( \mu \)M) of 5 against \( \alpha \)-glucosidase (baker yeast) (pH 6.8).
**Figure S3.** Lineweaver-Burk Plot for $K_i$ determination (11.8 μM) of 7 against $\alpha$-glucosidase (baker yeast) (pH 6.8).

**Figure S4.** Lineweaver-Burk Plot for $K_i$ determination (6.4 μM) of 8 against $\alpha$-glucosidase (baker yeast) (pH 6.8).
3. Spectroscopic Data of 13β and 2β

(1S)-2,3,4-Tri-O-acetyl-1-dodecylthio-5N,6O-oxomethylidenenojirimycin (13β):
Column chromatography (1:5 → 1:2 EtOAc:cyclohexane). Yield: 33 mg (6%). White solid. Rf 0.67 (1:1 EtOAc-cyclohexane). [α]D +4.9 (c 1.0 in DCM). 1H NMR (500 MHz, CDCl3) δ 5.19 (dd, 1 H, J4,5 = 10.5 Hz, J3,4 = 7.0 Hz, H-4), 5.13 (t, 1 H, J1,2 = J2,3 = 4.0 Hz, H-2), 4.62 (d, 1 H, H-1), 4.33 (dd, 1 H, J6a,6b = 8.8 Hz, J5,6a = 7.7 Hz, H-6a), 4.08 (t, 1 H, J5,6b = 8.8 Hz, H-6b), 3.90 (ddd, 1 H, H-5), 2.87-2.74 (m, 2 H, SCH2), 2.08-1.98 (3 s, 9 H, MeCO), 1.65-1.10 (m, 20 H, CH2), 0.81 (t, 3 H, JH,H = 7.0 Hz, CH3). 13C NMR (125.7 MHz, CDCl3) δ 169.8-168.7 (MeCO), 156.0 (CO), 73.6 (C-3), 73.3 (C-2), 72.7 (C-4), 67.1 (C-6), 59.2 (C-1), 53.9 (C-5), 34.3 (SCH2), 31.9-22.7 (CH2), 20.8-20.6 (MeCO), 14.1 (CH3). ESIMS: m/z 538.4 [M + Na]+. Anal. Calcd for C25H41NO8S: C 58.23, H 8.01, N 2.72, S 6.22. Found: C 57.86, H 7.73, N 2.63, S 6.47.

(1S)-1-Dodecylthio-5N,6O-oxomethylidenenojirimycin (2β): Yield: 18 mg (91%). Rf 0.80 (1:5 MeOH-EtOAc). [α]D -9.0 (c 1.3 in DMSO). 1H NMR (400 MHz, DMSO-d6) δ 4.29 (dd, 1 H, J6a,6b = 8.6 Hz, J5,6a = 7.0 Hz, H-6a), 4.21 (d, 1 H, J1,2 = 8.0 Hz, H-1), 4.05 (dd, 1 H, J5,6b = 4.6 Hz, H-6b), 3.60 (dd, 1 H, J4,5 = 10.0 Hz, H-5), 2.73-2.62 (m, 2 H, SCH2), 1.52 (quint., 1 H, JH,H = 7.0 Hz, SCH2CH2), 1.40-1.20 (m, 18 H, CH2), 0.86 (t, 3 H, JH,H = 7.0 Hz, CH3). 13C NMR (75.5 MHz, DMSO-d6) δ 156.0 (CO), 77.6-72.6 (C-3, C-4), 74.7 (C-2), 65.8 (C-6), 62.5 (C-1), 57.9 (C-5), 33.3 (SCH2), 31.3-22.2 (CH2), 14.0 (CH3). ESIMS: m/z 412.3 [M + Na]+. Anal. Calcd for C19H35NO5S: C 58.58, H 9.06, N 3.60, S 8.23. Found: C 58.32, H 8.88, N 3.39, S 7.85.
4. Copies of \(^1\)H and \(^{13}\)C NMR Spectra

![NMR Spectra Diagram]

**Figure S5.** \(^1\)H and \(^{13}\)C NMR spectra (500 MHz and 125.7 MHz, CDCl₃) of \(13\alpha\).
Figure S6. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 13β
Figure S7. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CD$_3$OD) of 2α
Figure S8. $^1$H and $^{13}$C NMR spectra (400 MHz and 75.5 MHz, DMSO-d$_6$) of 2β.
Figure S9. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 14
Figure S10. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 15
Figure S11. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CD$_3$OD) of 3
Figure S12. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CD$_3$OD) of 5
Figure S13. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 16
Figure S14. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 17
Figure S15. $^1\text{H}$ and $^{13}\text{C}$ NMR spectra (500 MHz and 75.5 MHz, CD$_3$OD) of 4
Figure S16. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, DMSO-d$_6$) of 6
Figure S17. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 18
Figure S18. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CDCl$_3$) of 19
Figure S19. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, CD$_3$OD) of 7
Figure S20. $^1$H and $^{13}$C NMR spectra (500 MHz and 125.7 MHz, DMSO-d$_6$) of 8