α-Glucosidase Inhibitory Activity of Triterpenoids from *Cichorium intybus*

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Two new triterpenoids, 18α,19β-20(30)-taraxasten-3β,21α-diol (cichoridiol) (**1**) and 17-*epi*-methyl-6-hydroxyangolensate (intybusoloid) (**2**), were obtained from the methanolic extract of seeds of *Cichorium intybus* along with 11 known compounds, lupeol (**3**), friedelin (**4**), β-sitosterol (**5**), stigmasterol (**6**), betulinic acid (**7**), betulin (**8**), betulinaldehyde (**9**), syringic acid (**10**), vanillic acid (**11**) 6,7-dihydroxycoumarin (**12**), and methyl-α-D-galactopyranoside (**13**). Compounds **1**, **1a**, and **11** showed a good α-glucosidase inhibitory activity.

Plants of the genus *Cichorium* (Asteraceae) are extensively used in indigenous medicines.[1] The genus comprises about 14 species of herbaceous plants, distributed in Europe, the Mediterranean regions, and northern Asia.[2] These plants have been used in folk medicines for the treatment of liver disorders, gallstones, and inflammation of the urinary tract.[3] Cultivated whole plant of *Cichorium intybus* is used in Indian medicine as a tonic and is also reputed to be useful in the treatment of fever, vomiting, diarrhea, and enlargement of the spleen. A decoction of root has been found effective in jaundice, liver enlargement, gout, and rheumatism. The alcoholic aqueous extract of the seeds is also used in the treatment of headache, asthma, and digestive disorders.[5] This genus is known to contain caffeic acid derivatives, flavonoids, polyphenols, glucosides, cichorin, lactucin, intybin, and some bitter substances.[3,4] Pharmacological studies on the root extracts of *C. intybus* have shown anti-inflammatory and hepatoprotective activities.[6,7] An antihepatotoxic effect of the seeds of this species was also reported.[8]

Our investigation on the methanolic extract of this plant afforded two new triterpenoids, namely, cichoridiol (**1**) and intybusoloid (**2**), along with 11 known compounds, lupeol (**3**),[9] friedelin (**4**),[10] β-sitosterol (**5**), stigmasterol (**6**),[11] betulinic acid (**7**), betulin (**8**),[12] betulinaldehyde (**9**),[13] syringic acid (**10**),[14] vanillic acid (**11**),[15] 6,7-dihydroxycoumarin (**12**),[16] and methyl-α-D-galactopyranoside (**13**).[17] Except compounds **8**, **10**, and **11**, all other compounds were obtained for the first time from this species.

The petroleum ether-soluble extract of *C. intybus* seeds was subjected to repeated column chromatography to obtain compound **1** as a colorless crystalline material. The molecular formula of compound **1** ($C_{30}H_{50}O_2$) was deduced by HREIMS *m/z* 442.3829 (calc 442.3811), indicating six degrees of unsaturation. The UV spectrum exhibited absorption at 202 nm, indicating the absence of any chromophore.[18] The IR spectrum of compound **1** showed absorption bands for olefinic (1640 cm^{-1}) and hydroxy (3665 and 3363 cm^{-1}) functionalities.

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of compound **1** exhibited six 3H singlets at δ 0.75, 0.76, 0.79, 0.90, 0.95, and 1.13, which were assigned to the tertiary C-28, C-23, C-25, C-27, C-26, and C-24 methyl protons, respectively. A 3H doublet at δ 1.23 (J = 7.0 Hz) was assigned to the C-29 secondary methyl protons. A characteristic downfield double-doublet at δ 3.17 ($J_{3ax,2ax}$ = 11.1 Hz, $J_{3ax,2eq}$ = 4.3 Hz) was due to a proton geminal to a hydroxy group. The most downfield doublets at δ 4.98 and 4.88 (J_{gem} = 1.4 Hz) were assigned to the *exo*-cyclic C-30 olefinic protons. A 1H double-doublet at δ 4.39 (J = 8.9, 5.3 Hz) was assigned to the C-21 hydroxy proton. The EIMS of compound **1** showed ions at *m/z* 442, 424, 409, 315, 207, 189, 133, 119, and 95, characteristic of a pentacyclic taraxastane skeleton with saturated rings A, B, C, and D.[19,20] Furthermore, the peaks at *m/z* 207 and 234 in the EIMS arose from the cleavage of ring C, indicating two hydroxy groups, one on either rings A and B or rings D and E.

The ^{13}C NMR spectrum of **1** ($CDCl_3$, 100 MHz) showed resonances for 30 carbon atoms including seven methyl, 10 methylene, seven methine, and six quaternary carbons. The downfield resonances at δ 156.7 and 111.5 were assigned to the C-20 and C-30 olefinic carbons, respectively. Two methine carbons at δ 76.7 and 68.9 were assigned to the OH-bearing C-3 and C-21, respectively. The resonances at δ 27.1, 21.7, 18.3, 16.1, 15.8, 15.6, and 14.6 were ascribed to the methyl carbons (C-23, C-29, C-28, C-25, C-26, C-24, and C-27, respectively). The overall NMR data were in good agreement with a taraxastane-type skeleton[21] (Table 1).

The HMBC spectrum of compound **1** showed correlations between various protons and carbon atoms. The C-3 methine proton (δ 3.17) showed long-range correlations with C-2 (δ 27.2), C-4 (δ 38.4), C-1 (δ 38.3), and C-5 (δ 54.8). The C-5 proton (δ 0.69) showed correlations with C-23 (δ 27.1), C-6 (δ 17.9), and C-25 (δ 16.1). The C-18 methine proton (δ 1.19) showed HMBC couplings with C-17 (δ 33.5) and C-22 (δ 49.6). The C-19 proton (δ 2.05) showed HMBC connectivities with C-20 (δ 156.7) and C-30 (δ 111.5). The C-30 *exo*-methylene protons exhibited correlations with C-19 (δ 37.7) and C-21 (δ 68.9).

The configuration at various stereogenic centers was deduced on the basis of NOE difference measurements (Figure 2). Irradiation of the C-3 methine proton (δ 3.17) led to the enhancement of the C-23 methyl protons (δ 0.76). On irradiation of the C-25 methyl protons (δ 0.79), enhancements of the C-24 and C-26 methyl resonances were observed, which indicated that these groups were all in close proximity and coplanar. The characteristic C-29 methyl group was found to have a β-disposition, since irradiation of the resonances at δ 1.23 resulted in enhancement of H-21β and H_3-28β signals. The other key NOE interactions are shown in Figure 2.

Acetylation of **1** yielded the diacetate derivative **1a**, as deduced from the IR absorptions at 1750 and 1282 cm^{-1} (C−O, ester) and two 3H singlets at δ 2.00 and 2.02 in the ^1H NMR spectrum. Protons geminal to acetoxy groups appeared downfield at δ 4.53 and 5.46. On the basis of the above evidence, compound **1** was deduced to be 18α,19β-20(30)-taraxasten-3β,21α-diol, trivially named cichoridiol.

Compound **2** was obtained as an amorphous powder from the $CHCl_3$ extract. The molecular formula was determined as $C_{27}H_{34}O_8$ (M^+; *m/z* 486.2262) by HREIMS. The FABMS (positive) showed the $[M + H]^+$ ion at *m/z* 487, fragment ions at *m/z* 398 ($M^+ - C_3H_5O_3$), 392 ($M^+ - C_5H_3O_2$), 391 ($M^+ - C_5H_4O_2$), and 363 ($M^+ - C_6H_4O_3$), and the base peak at *m/z* 105 $(C_3H_5O_4)^+$. The UV

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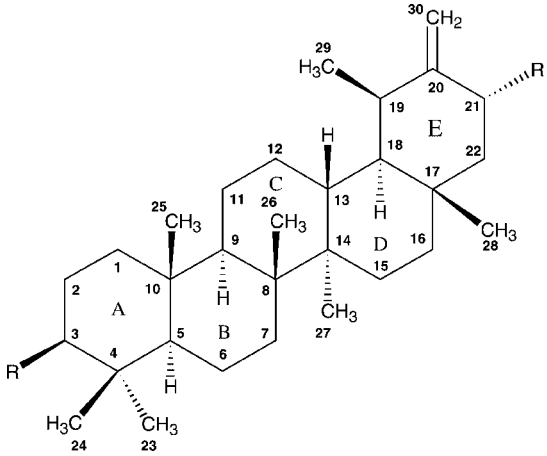
Table 1. NMR Data for Compounds **1** and **2** (100 and 500 MHz)[a]

	1		2	
position	δ_C	δ_H	δ_C	δ_H
1	38.3	1.08, 2.05 (m)	78.2	3.64 (5.17, 2.25, dd)
2	27.2	1.45, 1.12 (m)	39.1	2.31 (14.1, 2.7, dd); 3.07 (14.1, 2.6, dd)
3	76.7	3.17 (11.1, 4.31, dd)	211.9	
4	38.4		48.7	
5	54.8	0.69 (m)	47.5	2.79 (s)
6	17.9	1.35, 1.51 (m)	72.3	4.50 (s)
7	33.6	1.38 (m)	176.6	
8	40.7		145.9	
9	49.7	1.29 (m)	50.7	2.37 (14.2, 2.8, dd)
10	37.8		44.7	
11	20.8	1.57 (m)	24.1	2.10 (m)
12	26.3	1.12, 1.6 (m)	28.6	1.0 (s, H_β-12); 1.07 (m, H_α-12)
13	38.7	1.58 (m)	41.3	
14	41.7		80.4	
15	25.9	1.61, 0.90 (m)	33.7	2.62 (18.1, d, H_α-15); 3.13 (18.1, d, H_β-15)
16	37.5	1.03, 1.23 (m)	169.9	
17	33.5		79.4	5.59 (s)
18	49.4	1.19 (13.1, 6.9, dd)	13.7	0.89 (s)
19	37.7	2.05 (7.1, d)	23.7	1.39 (s)
20	156.7		120.9	
21	68.9	4.39 (8.9, 5.3, dd)	140.7	7.50 (s)
22	49.6	1.24, 1.75 (m)	109.9	6.42 (s)
23	27.1	0.76 (s)	142.7	7.48 (s)
24	15.6	1.13 (s)		
25	16.1	0.79 (s)		
26	15.8	0.95 (s)		
27	14.5	0.90 (s)		
28	18.3	0.75 (s)	23.4	1.48 (s)
29	21.6	1.23 (7.0, d)	24.7	1.02 (s)
30	111.5	4.88 (br s), 4.98 (1.4, d)	111.5	5.01, 5.22 (s, 2H)
OCH_3			53.3	3.74 (s, 3H)

[a] Assignments confirmed by decoupling, $^1H/^1H$ COSY, NOESY, HMQC, and HMBC spectra. *J* values are given in Hz.



	R
1	OH
1a	OAc

Figure 1

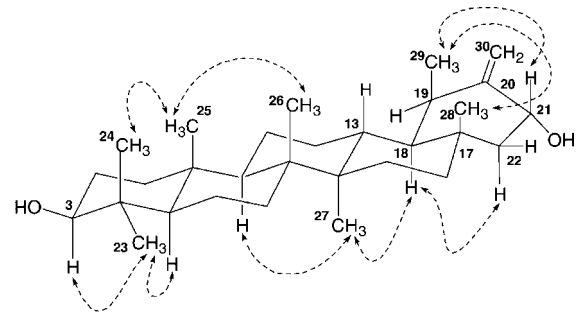


Figure 2. Key NOE interactions in compound **1**.

spectrum exhibited an absorption maximum at 206 nm (log ϵ 4.16), while IR bands appeared at 3663, 3698 (−OH), 2938 (−CH), 1729 (ester carbonyl), 1720 (δ lactone), 1590 (C=C), 1385 (geminal methyls), 1030 (ether linkage), and 875 cm^{-1} (furan ring).[22] Out of 11 double-bond equivalents, four accounted for ester, acetyl, ketone, and *exo*-methylene, three for the furan ring, and the remaining four for the four rings of the limonoid nucleus.

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of compound **2** displayed resonances at δ 1.48, 1.39, 1.02, and 0.89 due to the C-28, C-19, C-29, and C-18 methyl protons, respectively. It also exhibited resonances due to the protons of a β-substituted furan ring at δ 7.50 (H-21, s), 6.42 (H-22, s), and 7.48 (H-23, s). A sharp singlet at δ 5.59 was assigned to the C-17 proton. A downfield double-doublet at δ 3.64 ($J_{1eq,2ax}$ = 5.2 Hz, $J_{1eq,2eq}$ = 2.2 Hz) was assigned to the C-1 proton. Two C-15 methylene protons appeared as AB doublets at δ 2.62 and 3.13 (J_{gem} = 18.0 Hz), while a singlet at δ 2.79 was assigned to the C-5 methine proton. A hydroxy-bearing C-6 methine proton appeared as a sharp singlet at δ 4.50, while the *O*-methyl protons appeared as a sharp singlet at δ 3.74. Both H-5 and H-6 appeared as sharp singlets due to their small dihedral angle.[23] The C-30 *exo*-methylene olefinic protons appeared as singlets at δ 5.22 and 5.01, characteristic of a C-30/C-8 double bond in limonoids.[23] The ^1H NMR chemical shift assignments are presented in Table 1.

The broad band decoupled ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of compound **2** showed 27 carbon resonances, including five methyl, eight methine, five methylene, and nine quaternary carbons. The downfield resonances at δ 211.9, 176.6, and 169.9 were assigned to the carbonyl carbons of ketone, ester, and lactone groups, respectively. Two *exo*-cyclic double-bond carbons (C-8 and C-30) appeared at δ 145.9 and 111.5, respectively. Downfield

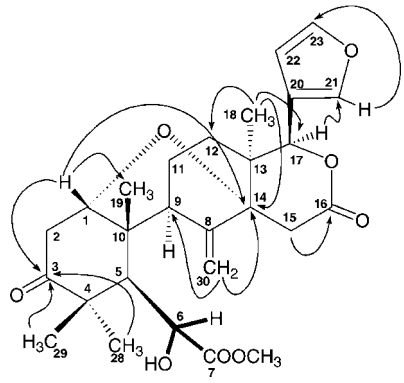


Figure 3. Key HMBC interactions in compound **2**.

Table 2. *In Vitro* Inhibition of α-Glucosidase by Compounds **1–13**

compound	conc (μM)	$IC_{50} \pm$ SEM (μM)
1	51.9	51.9 ± 1.1
1a	10	10 ± 0.62
4	321	321 ± 8.04
11	69	69 ± 0.008
deoxynojirimycin	425	425 ± 8.14

SEM = standard error of the mean.

carbon resonances at δ 72.3, 78.2, and 79.4 were attributed to the oxygen-bearing C-6, C-1, and C-17, respectively. The C-20, C-21, C-22, and C-23 aromatic carbons appeared downfield at δ 120.9, 140.7, 109.9, and 142.7, respectively.

The HMBC spectrum of **2** was used to place various functionalities, based on long-range C−H correlations. The C-1 methine proton (δ 3.64) showed $^3J_{CH}$ correlations with C-3 (δ 211.9), C-19 (δ 23.7), and C-14 (δ 80.4). Similarly, the C-17 methine proton (δ 5.59) exhibited HMBC couplings with C-18 (δ 13.7), C-21 (δ 140.7), C-20 (δ 120.9), and C-22 (δ 109.9) (Figure 3).

The relative configuration of **2** was deduced by NOESY analysis. The observed NOEs between H-17, CH_3-18, and the α-CH-15 suggested that all the groups are α-cofacial. The NOE observed between H-5/CH_3-28 and H-9/H-5 indicated that these groups are α-oriented. Similarly, irradiation of H-1 resulted in the enhancement of H-19 and CH_3-29, showing their β-orientation. On the basis of the above spectroscopic studies, the structure of compound **2** was identified as a new tetranortriterpenoid, 17-e*pi*-methyl-6-hydroxyangolensate.

The known compounds **3**−**13** were identified through comparison of their physical data (NMR and EIMS) with published information.[9–17]

Compounds **1**−**13** were tested for α-glucosidase inhibitory activity. α-Glucosidase is a membrane-bound enzyme in the small intestine. It catalyzes the final step in the digestive process of carbohydrates. Its inhibitors retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia.[24] Glucosidases are also involved in several important biological processes such as the biosynthesis of glycoproteins and the lysosomal catabolism of glycoconjugates. Glucosidase inhibitors are therefore potentially useful as antiviral, antimetastatic, and immunomodulatory agents. They also have a potential to be useful against the HIV-1 infection.[25] Some triterpenoids were reported to have α-glucosidase inhibitory activity.[26,27] Compound **1a**, containing two acetyl groups, showed more potent α-glucosidase inhibitory activity ($IC_{50} = 10 \pm 0.62$ μM) than that of compound **1** ($IC_{50} = 51.9$ μM), without the acetyl groups. Vanillic acid (**11**) was also active, with an IC_{50} value of 69 ± 0.008 μM. Compounds **2**, **3**, **5**−**10**, **12**, and **13** were found to be inactive. Deoxynojirimycin ($IC_{50} = 425 \pm 8.14$ μM) was used as a standard inhibitor (positive control) in the assay.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco melting point apparatus. Optical rotations were measured on a Schmidt + Haensch Polartronic D instrument. UV and IR spectra were recorded on Hitachi UV 3200 and JASCO 302-A spectrophotometers. EI- and HREIMS were measured on Varian MAT 311A and JEOL HX 110 mass spectrometers (*m/z*, rel int). The NMR (broadband decoupled ^{13}C NMR, COSY, NOESY, NOE, HMQC, HMBC) were recorded on Bruker AMX 400 and AMX 500 MHz NMR spectrometers. The chemical shifts are given in ppm (δ), relative to $SiMe_4$ as internal standard, and coupling constants are in Hz. Column chromatography (CC) was performed on Si gel (70–230 mesh). Thin-layer chromatography (TLC) was performed on precoated Si gel plates (DC-Alufolien 60 F_{254}, E. Merck), and spots were located by using ceric sulfate spraying reagent.

α-Glucosidase (E.C.3.2.1.20) from *Saccharomyces* sp. was purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). Other reagents were purchased from various commercial sources.

Plant Material. The seeds of *Cichorium intybus* Linn. were purchased from the local herbal market of Karachi (Pakistan). The plant was identified by Mr. Sher Wali, Botany Department, University of Karachi, and a voucher specimen (KUH-68260) was deposited in the herbarium of the same University.

Extraction and Isolation. The air-dried seeds (10 kg) of *C. intybus* were ground and soaked in a mixture of 30 L of 80% MeOH/H_2O (4:1) at room temperature for 72 h. The organic extract was filtered, and the solvent was evaporated under reduced pressures. The concentrated aqueous MeOH extract (660.3 g) was suspended in distilled H_2O (1 L) and defatted with petroleum ether (2.5 L). The aqueous layer was further extracted with $CHCl_3$, EtOAc, and BuOH. Each extract was concentrated *in vacuo* to obtain petroleum ether- (55.2 g), $CHCl_3$- (17.3 g), EtOAc- (8.11 g), and BuOH-soluble (52.5 g) fractions. Five compounds were isolated from the petroleum ether-soluble part, four compounds from the $CHCl_3$-soluble portion, three compounds from the EtOAc-soluble portion, and one from the BuOH-soluble portion, by using repeated column and thin-layer chromatographic techniques.

The petroleum ether extract (55.2 g) was repeatedly chromatographed on a Si gel column by using various polarities of petroleum ether, acetone, and MeOH mixtures. From this extract, five main fractions were collected. Fraction Fr-2, which was eluted with petroleum ether/acetone (9:1), yielded compound **1** (14.4 mg) by flash column chromatography. Fr-4, eluted with petroleum ether/acetone (7.5:2.5), was rechromatographed over a Si gel column with gradient mixtures of petroleum ether/acetone to obtain subfractions A (9.5:0.5), B (9:1), and C (8:2). These subfractions were further purified by flash chromatography over Si gel to obtain compounds **3** (11.79 mg), **4** (1.27 g), **5** (26.27 mg), and **6** (9.2 mg).

The $CHCl_3$ extract (17.3 g) was chromatographed on a Si gel column and eluted with increasing polarities of $CHCl_3$ in petroleum ether, $CHCl_3$, and $CHCl_3$ in MeOH. This extract was subjected to column chromatography by using petroleum ether/$CHCl_3$ and $CHCl_3$/MeOH for elution to afford five fractions. By elution of the column with $CHCl_3$/MeOH (90:10), compound **2** (15.7 mg) was obtained. Further elution of the column with $CHCl_3$ and MeOH (1–25% MeOH/$CHCl_3$) yielded compounds **7** (5.1 mg), **8** (6.6 mg), and **9** (2.5 mg).

Compounds **10** (8.9 mg), **11** (4.3 mg), and **12** (12.4 mg) were isolated from the EtOAc-soluble extract. Compound **13** was obtained as dark brown crystals (13.3 mg) from the BuOH-soluble portion obtained by partitioning the MeOH extract of the plant with BuOH.

18α,19*β*-20(30)-Taraxasten-3*β*,21α-diol (1): colorless, crystalline material (14.4 mg, 0.5% acetone/petroleum ether); mp 259–260 °C; $[\alpha]_D^{29}$ +54 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 nm; IR ($CDCl_3$) ν_{max} 3665, 3363 (hydroxy groups) and 1640, 898 (terminal double bond) cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1: EIMS *m/z* (rel int %) 442 (59) $[M]^+$, 424 (46), 409 (11), 372 (19), 342 (9), 273 (5), 207 (76), 189 (78), 135 (77), 119 (73), 95 (86), 55 (100); CIMS *m/z* 442, 425, 407, 273, 245, 217, 79, 57; HREIMS *m/z* 442.3829 (calc 442.3811 for $C_{30}H_{50}O_2$).

Acetylation of 1. To a solution of **1** (5 mg) in pyridine (0.5 mL), was added Ac_2O (0.5 mL), and the reaction mixture was kept at room temperature overnight. On usual workup, the acetylated product **1a** was obtained as a colorless, amorphous powder (2.73 mg); $[\alpha]_D^{27}$ +26.6 (*c* 0.01, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1750, 1282 cm^{-1} (C−O, ester linkage); HREIMS *m/z* 526.4022, (calc 526.4014 for $C_{34}H_{54}O_4$); ^{1}H NMR data

($CDCl_3$, 500 MHz) δ 0.78, 0.82, 0.83, 0.93, 1.00 (each 3H, s), 2.00 and 2.02 (each 3H, s, OAc), 4.53 (dd, $J_{3ax,2ax} = 13.5$ Hz, $J_{3ax,2eq} = 6.2$ Hz, H-3α), 5.46 (dd, $J = 9.6$, 5.2 Hz, H-21β), 1.13 (3H, d, $J = 6.9$ Hz, C-29), 4.95 and 5.06 (br s, 1H each).

17-*Epi*-Methyl-6-hydroxyangolensate (2): amorphous powder (15.7 mg); $[\alpha]_D^{25}$ −88 (*c* 0.21, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 206 (4.1) nm; IR ($CDCl_3$) ν_{max} 3663, 3698 (−OH), 2938 (−CH), 1729 (ester carbonyl), 1720 (a lactone), 1590 (C═C), 1385 (geminal methyls), 1030 (ether linkage), 875 (furan ring) cm^1; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int %) 486 (49) $[M]^+$, 375 (6), 348 (4), 164 (19), 148 (28), 105 (100), 95 (89), 94 (12), 81 (28); HREIMS *m/z* 486.2262, $C_{27}H_{34}O_8$ (calc 486.2254).

Assay for α-Glucosidase Inhibition. The α-glucosidase (E.C.3.2.1.20) enzyme inhibition assay was performed according to the slightly modified method of Oki et al.[28] The inhibition was measured spectrophotometrically at pH 6.9 and at 37 °C using 0.5 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 250 m units/mL enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) and acarbose (0.78 mM) were used as positive controls. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α-glucosidase, was monitored continuously with the spectrophotometer (Spectra Max, Molecular Devices, CA).

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