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Synthesis of α-Mannosylated Phenolics as α-Glucosidase Inhibitors*

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BF_3OEt_2-catalysed glycosidation of phenolic compounds 3 and 6 with the mannofuranosyl glycosyl donor 2 separately gave the corresponding α-mannofuranosyl derivatives 4 and 7 in good yield, and the latter on selective deacetonation (hydrolysis) with 2% aqueous HCl afforded 5 and 8 respectively. Compounds 4 and 7 inhibited rat intestinal α-glucosidase more effectively than a standard drug acarbose.

Keywords: α-Glucosidase; glycosidation; mannosylated phenolic; α-glucosidase inhibitor; acarbose; NIDDM

INTRODUCTION

Type-2 noninsulin-dependent diabetes mellitus (NIDDM),[1–7] a multifactorial disease, accounts for 90–95% of all diabetes and affects about 150 million people globally. Although several drugs[8] for NIDDM with the known target exist today, yet they are associated with many drawbacks such as liver toxicity,[9] adverse gastrointestinal symptoms[10] and risk of heart disease. Therapeutic approaches with herbal medicines also exist,[11] but the lack of well organized and rigorous clinical trial evidence to advocate their scientific merit warrants the introduction of new synthetic drugs against diabetes. Glycosidases, involved in the biosynthesis of N-glycoprotein and many other biological processes, are well-known targets in the design and development of antidiabetic,[12–17,32,33] antiviral,[12–16,18–20] antibacterial[12–16,21] and anticancer[22] agents. In NIDDM the delaying of glucose absorption after meal by inhibition of α-glucosidase is beneficial in therapy.[23,24] A pseudosachharide (acarbose) and an azasugar (miglitol) are being clinically used[25–27] for this purpose in the management of diabetes, but these are associated with severe side effects including adverse gastrointestinal effects and abdominal discomfort. Consequently, efforts are being made to develop new α-glucosidase inhibitors for studying and treating metabolic disorders, particularly NIDDM and lysomate storage disease.[18–20] The enzymatic mechanism of α-glucosidase is though to involve a transient oxocarbonium ion with a flattened chair conformation stabilized by an active-site catalytic residue and a complementary charge, identified as carboxylate in most of the glycosidases.[28] Many azapyranoses, furanoses and their derivatives are known as glycosidase inhibitors.[12–16,29–33] Very little attention has been paid to mimic the aglycone part of the glycoside, which plays an important role in the interaction of the inhibitor with the enzyme. Although much work has been done with azafuranoses on inhibition of α-glucosidase, no report is available, to the best of our knowledge, on intestinal α-glucosidase inhibition with mannofuranosyl glycoside. Since phenyl glycosides are often accepted as substrate by the enzyme, we have chosen the phenolic aglycons, compounds **3** and **6**, as such compounds are known to be associated with biological activities.[34–37] Our choice of the furanose form of the sugar is based on the fact that the requirement for the transition state can be met even with such glycosides. Keeping in view the above points,

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we have synthesized a few of the titled compounds and evaluated their effect on α-glucosidase from rat intestinal mucosa.

EXPERIMENTAL

Materials

All glassware were dried over an open flame before use in connection with an inert atmosphere. Concentration was performed under reduced pressure at $<50°C$. Freshly distilled BF_3OEt_2 was used. CH_2Cl_2 was dried and distilled over P_2O_5 before use. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer, and values were within $\pm 0.4\%$ of the calculated values. FABMS was used to determine the molecular mass of the compounds and was recorded on a Jeol (Japan)/SX-102 instrument. Infrared spectra was taken with KBr on a Perkin-Elemer RX-1. Tetramethylsilane (0.0 ppm) was used as an internal standard in ^{1}HNMR. The abbreviations used to indicate the peak multiplicity were; s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; Hz, Hertz. Melting points were determined on a Buchi 535 digital melting point apparatus and are uncorrected.

Method

(2,3,5,6-bis-O-isopropylidene-1-O-)-trichloroacetamidyl-α-D-mannofuranose (2)

To a magnetically stirred mixture of K_2CO_3 (0.8 g, 5.88 mmol) in anhydrous CH_2Cl_2 (20 ml), (2,3,5,6-*bis*-*O*-isopropylidene-1-*O*)-α-D-mannofuranose (1.53 g, 5.88 mmol) was added slowly, followed by trichloroacetonitrile (1.0 ml, 6.92 mmol) and the mixture was stirred for 24 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a crude product, which on column chromatography (SiO_2), using hexane: ethyl acetate (95:5) as eluant, afforded the required trichloroacetamidyl derivative (**2**) as a white crystalline solid. Yield: 70%, mp = 103–105°C, $[\alpha]_D = +49.01$ (c. 1.0, $CHCl_3$); MS (FAB): 409 $(M+H)^+$; IR (KBr): ν_{max} cm^{-1} 3794 (NH), 3344 and 2988 (CH_3 and CH_2 stretching); ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H, NH), 6.26 (s, 1H, H-1), 4.94 (m, 2H, H-2 and H-3), 4.42 (m, 1H, H-4), 4.04 (m, 3H, H-5 and H-6), 1.59, 1.50, 1.45 and 1.33 [each s, each 3H, 2 × > $C(CH_3)_2$]; Calc. for $C_{14}H_{20}O_6NCl$ (403): C, 41.68; H, 4.96; N, 3.47. Found; C, 41.08; H, 4.81; N, 3.38%.

Ethyl 2-cyano-3-[3′-methoxy-4′-O-(2,3,5,6-bis-O-isopropylidene-1-O-α-D-mannofuranosyl)-phenyl]-propenoate (4)

A mixture of (2,3,5,6-*bis*-*O*-isopropylidene-1-*O*-) trichloroacetamidyl-α-D-mannofuranose **2** (1.2 g, 2.97 mmol), ethyl 2-cyano-3-[3′-methoxy-4′-hydroxy-phenyl)-propenoate **3** (0.78 g, 3.16 mmol) and 4 Å molecular sieve (1.0 g) in anhydrous CH_2Cl_2 (20 ml) was magnetically stirred under N_2 atmosphere at room temperature for 5 min. Freshly distilled anhydrous BF_3OEt_2 (0.4 ml, 2.52 mmol) was added and stirring was continued at the same temperature for a further 3 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a gummy mass which on column chromatography (SiO_2), using chloroform:methanol (98:2) as eluant, afforded the required compound as a light yellow crystalline solid. Yield: 65%, mp = 102–105°C, $[\alpha]_D = +49.68$ (*c*, 0.11, $CHCl_3$); MS (FAB): 490 $(M+H)^+$; IR (KBr): ν_{max} cm^{-1} 2988, 2928 and 2881 (CH_3 and CH_2 stretching); 2222 (–CN); ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.16 (s, 1H, H-3), 7.81 (d, $J = 1.5$ Hz, 1H, H-2′), 7.41 (dd, $J = 1.5$ Hz and 1.2 Hz, 1H, H-6′), 7.16 (d, $J = 1.5$ Hz, 1H, H-5′), 5.71 (s, 1H, H-1″), 4.98 (m, 2H, H-2″ and H-3″), 4.36 (m, 3H, H-5″ and H-6″), 4.09 (q, $J = 7.5$ Hz, 2H, OCH_2CH_3), 3.92 (s, 3H, OCH_3), 1.58, 1.51, 1.43 and 1.39 [each s, each 3H, 2 × > $C(CH_3)_2$], 1.25 (t, $J = 7$ Hz, 3H, OCH_2CH_3); Calc. $C_{25}H_{31}O_9N$ (489):C, 61.34; H, 6.34; N, 2.86. Found; C, 61.52; H, 6.38; N, 2.91%.

Ethyl 2-cyano-3-[3′-methoxy-4′-O-(2,3,-O-isopropylidene-1-O-α-D-mannofuranosyl)-phenyl]-propenoate (5)

Ethyl 2-cyano-3-[3′-methoxy-4′-*O*-(2,3,5,6,-*bis*-*O*-isopropylidene-1-*O*-α-D-mannofuranosyl)-phenyl]-propenoate (**4**, 0.5 g, 1.12 mmol) in ethanol (2 ml) was stirred with aqueous HCl (2%, 10 ml, pH 1–2) at room temperature for 3 h. The reaction mixture was cooled and neutralized with solid sodium bicarbonate, filtered and the filtrate evaporated azeotropically with ethanol: toluene (50:50). The residue obtained, was extracted with chloroform (3 × 50 ml), dried (Na_2SO_4), and the chloroform evaporated under reduced pressure to give a crude product, which on column chromatography (SiO_2), using chloroform: methanol (95:5) as eluant, gave compound **5** as a colourless oil. Yield: 70%; $[\alpha]_D = +48.52$ (c, 0.09, $CHCl_3$); MS (FAB): 450 $(M+H)^+$; IR (KBr): ν_{max} cm^{-1} 3020, 2986 and 2881 (CH_3 and CH_2 stretching), 2222 (–CN); ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H, H-3), 7.80 (d, $J = 1.5$ Hz, 1H, H-2′), 7.42 (dd, $J = 1.5$ Hz and 1.2 Hz, 1H, H-6′), 7.14 (d, $J = 1.5$ Hz, 1H, H-5′), 5.70 (s, 1H, H-1″), 4.96 (m, 2H, H-2″ and H-3″),

4.35 (m, 3H, H-5″ and H-6″), 4.08 (q, $J = 7.5$ Hz, 2H, OCH_2CH_3), 3.88 (s, 3H, OCH_3), 1.48 and 1.37 [each s, each 3H, $2 \times > C(CH_3)_2$]; 1.25 (t, $J = 7.5$ Hz, 3H, OCH_2CH_3); Calc. $C_{22}H_{27}O_9N$ (449): C, 58.79; H, 8.24; N, 3.11. Found; C, 58.85; H, 8.28; N, 3.01%.

7-O-α-(2,3,5,6-bis-O-isopropylidene-1-O-α-D-mannofuranosyl)-4-propyl-coumarin (7)

A mixture of 2,3,5,6-*bis*-*O*-isopropylidene-1-*O*-trichloroacetamidyl-α-D-mannofuranose (2, 2.56 g, 6.35 mmol), 7-hydroxy-4-propyl coumarin (**6**, 1.3 g, 6.37 mmol) and 4 Å molecular sieve (2.2 g) in anhydrous CH_2Cl_2 (80 ml) was stirred magnetically for 5 min at room temperature. Freshly distilled anhydrous BF_3OEt_2 (1.18 ml, 7.42 mmol) was added under N_2 atmosphere and the stirring continued for further 3 h at the same temperature. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a crude product which was chromatographed (SiO_2) using methanol: chloroform (2:98) as eluant to afford the required compound **7** as a white crystalline solid. Yield: 60%, mp = 118–122°C, $[\alpha]_D = +48.24$ (c, 0.11, $CHCl_3$); MS (FAB): 447 $(M + H)^+$; IR (KBr): ν_{max} cm^{-1} 3282 and 2974 (CH_3 and CH_2 stretching), 1730 (>C═O); ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (d, $J = 1.5$ Hz, 1H, H-5), 7.00 (d, $J = 1.2$ Hz, 1H, H-8), 6.95 and 6.90 (dd, $J = 1.5$ Hz and 1.2 Hz, 1H, H-6), 6.17 (s, 1H, H-3), 5.68 (s, 1H, H-1′), 4.92 (m, 2H, H-2′ and H-3′), 4.41 (m, 1H, H-4′), 4.14–3.97 (m, 3H, H-5′ and H-6′), 2.71 (t, $J = 7$ Hz, 2H, $CH_2CH_2CH_3$), 1.74 (m, 2H, $-CH_2CH_2CH_3$), 1.53, 1.42, 1.38 and 1.37 [each s, each 3H, $2 \times > C(CH_3)_2$], 1.05 (t, $J = 7.5$ Hz, 3H, $CH_2CH_2CH_3$); Calc. $C_{24}H_{30}O_8$ (446): C, 64.58; H, 6.72. Found; C, 64.62; H, 6.68%.

7-O-α-(2,3-O-isopropylidene-1-O-α-D-mannofuranos-1-yl)-4-propyl-coumarin (8)

7-*O*-(2,3,5,6-*bis*-*O*-isopropylidene-1-α-D-mannofuranos-1-yl)-4-propyl-coumarin (**7**, 0.7 g, 1.56 mmol) in ethanol (2 ml) was stirred with aqueous HCl (2%, 10 ml, pH 1–2) at room temperature for 4 h. The mixture was neutralized with solid $NaHCO_3$. Work up of the reaction mixture as above and column chromatography of the crude product in a similar manner to compound **5** afforded compound **8** as colourless oil. Yield: 70%, $[\alpha]_D = +46.18$ (c, 0.08, $CHCl_3$); MS (FAB): 407 $(M + H)^+$; IR (KBr): ν_{max} cm^{-1} 3282 and 2974 (CH_3 and CH_2 stretching), 1728 (>C═O); ^{1}HNMR (300 MHz, $CDCl_3$): δ 7.55 (d, $J = 1.6$Hz, 1H, H-5), 6.99 (d, $J = 1.2$ Hz, 1H, H-8), 6.95 and 6.91 (dd, $J = 1.5$ Hz and 1.2 Hz, 1H, H-6), 6.16 (s, 1H, H-3), 5.74 (s, 1H, H-1′), 4.95 and 4.88 (m, 2H, H-2′ and H-3′), 4.42 (m, 1H, H-4′), 4.17–4.04 (m, 3H, H-5′ and H-6′), 2.70 (t, $J = 7$ Hz, 2H, $CH_2CH_2CH_3$), 1.71 (m, 2H, $-CH_2CH_2CH_3$), 1.45 and 1.33 [each s, each 3H, $2 \times > C(CH_3)_2$], 1.05 (t, $J = 7.5$ Hz, 3H, $CH_2CH_2CH_3$); Calc. $C_{21}H_{26}O_8$ (406): C, 62.06; H, 6.40. Found; C, 62.12; H, 6.38%.

Preparation of α-Glucosidase from Rat Intestinal Mucosa

This was done according to a slight modification of the procedure reported by Cogli *et al.*[43] The intestines of male albino rats (CF strain, average body weight 200 ± 20 g) were excised, opened and the mucosa was collected and pooled. A 100% homogenate was prepared in 150 mM KCl using Potter Elvejhem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1,000 × g for 15 min and the supernatant was decanted and stored at 4°C, The supernatant was dialyzed at 4°C against 50 mM Tris–Hcl buffer pH 7.0 with two or three changes of buffer. The dialyzed supernatant was saturated with ammonium sulphate to a final concentration of 30%. The sample was kept at 4°C overnight and then centrifuged to collect the precipitate and the supernatant separately. The 30% ammonium sulphate saturated supernatant was further saturated to 60% with ammonium sulphate. Again the precipitate and supernatant were separated by centrifugation. Finally the 60% ammonium sulphate saturated supernatant was further saturated to 100% with further addition of ammonium sulphate. The precipitate and supernatant was once again separated and all the samples were analysed for α-glucosidase activity using *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as substrate. The enzyme activity was found maximum in the 60–100% ammonium sulphate precipitate (Table I) and this fraction was stored at 4°C and used as a source of enzyme for the study.

Determination of α-Glucosidase Inhibitory Activity

Fifty μg of partially purified α-glucosidase from rat intestinal mucosa and 100 μg of glutathione were added to 0.67 mM phosphate buffer (pH 6.8). The reaction mixture was incubated at room temperature for 10 min and after the addition of (0.01 M) (PNPG) 0.1 ml, the change in optical density was followed at 400 nm for a further period of 5 min in the presence of 50 μg of test compound in the 1.0 ml assay system. Activity was expressed as nmol *p*-nitrophenol formed per min using a molar extinction coefficient of 9.6×10^3.

RESULTS AND DISCUSSION

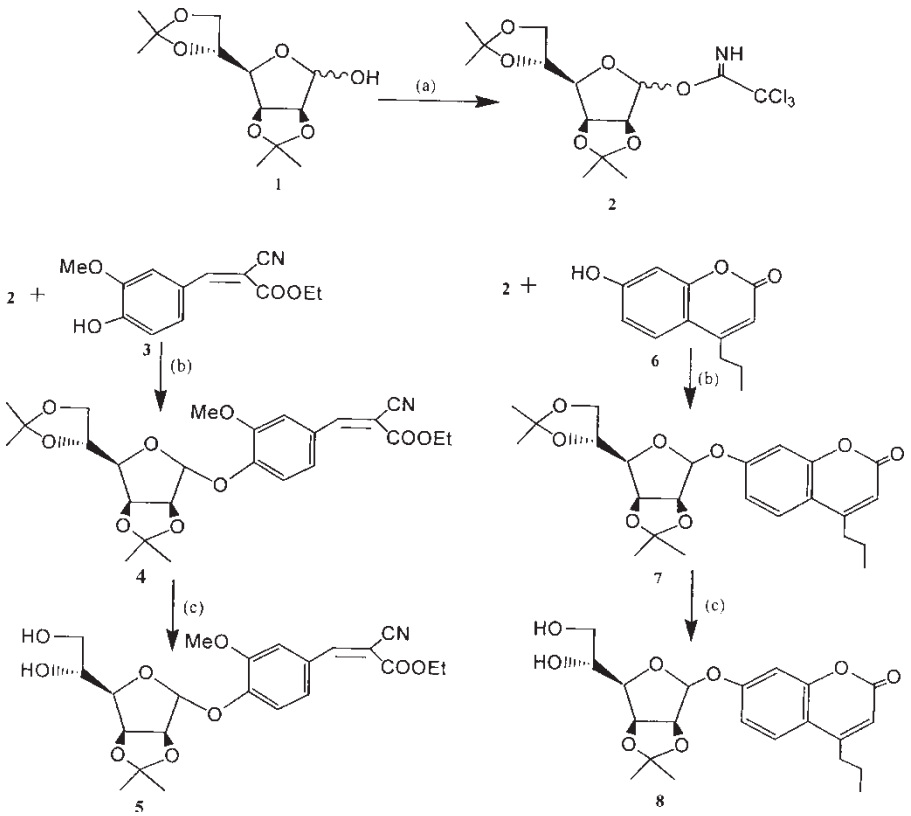
The required glycosyl donor 2,3,5,6-bis-*O*-isopropylidene-1-*O* trichloroacetamidyl-α-D-mannofuranose (**2**)

TABLE I Purification steps of α-glucosidase from rat intestinal mucosa

	Total protein mg/ml	Total activity (nmol/min)	Specific activity (nmol/min/mg protein)	Fold purification
Crude extract	1.50 ± 0.03	433.4 ± 0.30	288.94 ± 10.1	1.0
1000 × g supernatant	0.74 ± 0.09	358.02 ± 8.07	483.81 ± 89.7	1.68
0–30% ammonium sulphate precipitate	0.18 ± 0.02	99.48 ± 0.78	552.69 ± 38.9	1.91
30–60% ammonium sulphate precipitate	0.31 ± 0.03	224.24 ± 2.83	723.36 ± 94.2	2.50
60–100% ammonium sulphate precipitate	0.60 ± 0.06	822.55 ± 3.23	1370.91 ± 53.8	4.74
100% saturated ammonium sulphate supernatant	0.12 ± 0.02	Nil	Nil	

Maximum activity was observed in 60–100% saturated dialyzed precipitate.

was obtained by the reaction of (2,3,5,6-bis-*O*-isopropylidene-1-*O*-)-α-D-mannofuranose (**1**) with trichloroacetonitrile as reported in the literature.[38] Aglycones, ethyl 2-cyano-3- (3′-methoxy-4′-hydroxyphenyl)-propenoate (**3**) and 7-hydroxy-4-propyl coumarin (**6**), were prepared as reported earlier and structures were in agreement with their spectral data and analysis. BF_3OEt_2 catalysed condensation[39–41] of phenol 3 with glycosyl donor 2 in CH_2Cl_2 in the presence of 4 Å molecular sieve under a N_2 atmosphere gave the corresponding α-D-mannoside **4** in good yield (Scheme 1). The structure was assigned on the basis of spectral data and analysis. FAB (MS) showed $[M + H]^+$ (490), and in the IR spectrum an absorption band at 2222 cm^{-1} indicated the presence of the cyano (CN) group. In the ^{1}HNMR spectrum of compound **4** the glycosidic H-1 appeared at δ 5.1 (s), besides the other usual signals. It has been reported that mannosylation of phenols with trichloroacetamidyl mannose donor generally leads to α-glycosides. Selective deketalisation of the 5,6-*O*-isopropylidine moiety in compound **4** with dilute HCl, at ambient temperature gave compound **5** in 70% yield. The structure was confirmed on the basis of spectral data and analysis. Similar glycosidation of coumarin **6** with mannofuranosyl donor **2** afforded the corresponding glycoside **7** in 65% yield. The IR spectrum exhibited an absorption band at 1730 cm^{-1}, which indicates the lactone of the pyrone. MS indicated a molecular ion peak at 448 and in the ^{1}HNMR spectrum



SCHEME 1 (a) CCl_3CN, K_2CO_3, CH_2Cl_2, RT, 18–20 h; (b) $BF_3.OEt_2$, Mol.Sieve, 4 Å, CH_2Cl_2, RT, 3–4 h; (c) 2% Aq. HCl (pH 1–2), rt, 2–3 h.

TABLE II Effect of synthetic glycosides (**4**, **5**, **7** and **8**) and acarbose on partially purified α-glucosidase from rat intestinal mucosa

Addition	Concentration (μg/ml)	Residual activity (nmol/min/mg)
Vehicle	–	265.2 ± 18.5
4	50	47.7 ± 3.64
5	50	215.6 ± 3.16
7	50	58.3 ± 4.15
8	50	188.3 ± 2.01
Acarbose	50	84.8 ± 6.12

the glycosidic proton appeared at δ 5.68 (s) and another (s) at 6.17 for the H-3 of the benzopyrone moiety in addition to the other usual signals. Selective deketalisation in compound **7** with aqueous HCl at room temperature gave the required compound **8** in 70% yield. The structure was assigned on the basis of spectral data and analysis.

One of the most direct and beneficial types of therapy for NIDDM is achieved by control of the blood glucose level after a meal by delaying glucose absorption.[23,42,43] To date certain synthetic inhibitors of α-glucosidase (an exo-type α-D-glucosidase O-linkage hydrolase[44]) have been developed and used for the therapeutic treatment of NIDDM.[45] In our studies concerning with prophylaxis of NIDDM, we synthesized and evaluated the α-glucosidase inhibitory potential of mannosylated phenolic compounds.

The prepared glycosides (**4**, **5**, **7** and **8**) were tested for their effect on α-glucosidase from rat intestinal mucosa and compared with the standard drug acarbose. Table II represents the residual activity profiles 10 min after adding the test substance. Figure 1 represent the % inhibition of α-glucosidase by compounds **4**, **7** and acarbose at 50 μg/ml. Compounds **4** and **7** were also studied for their dose-dependent effect. Nearly 82, 61 and 31% inhibition was observed at 50, 25 and 10 μg/ml concentration of compound **4** in the assay system, respectively, which was followed by compound **7** where around 78, 63 and 33% inhibition, respectively, was observed at these

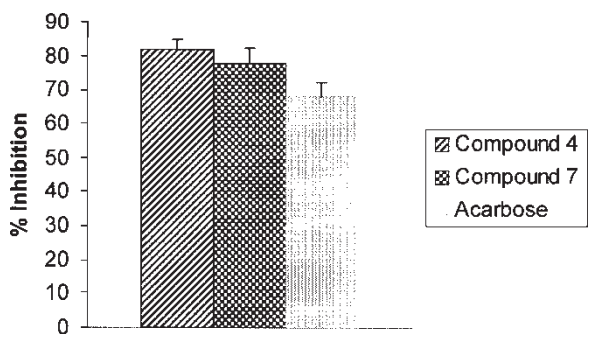


FIGURE 1 Inhibition of α-glucosidase.

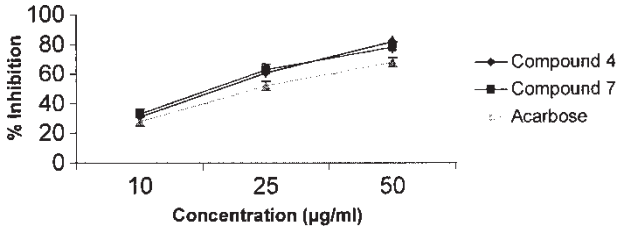


FIGURE 2 Dose-dependency of compound, **4**, **7** and acarbose.

concentrations. The standard drug acarbose showed around 68, 52 and 28% inhibition, respectively, at 50, 25 and 10 μg/ml concentration in the assay system (Figure 2).

The only α-glucosidase inhibitor available commercially is acarbose, a pseudotetrasaccharide of microbial origin, which is registered in many countries for use in patients with NIDDM.[8,46,47] Several non-comparative multicentric and placebo-controlled studies have shown that acarbose indices of blood glucose stability in NIDDM patients treated with diet, oral hypoglycaemic agents or insulin.[48,49] The two mannosylated phenolics described here have strong α-glucosidase inhibitory potential *in vitro* which warrant further *in vivo* studies.

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