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α-Glucosidase Inhibitors from *Brickellia cavanillesii*

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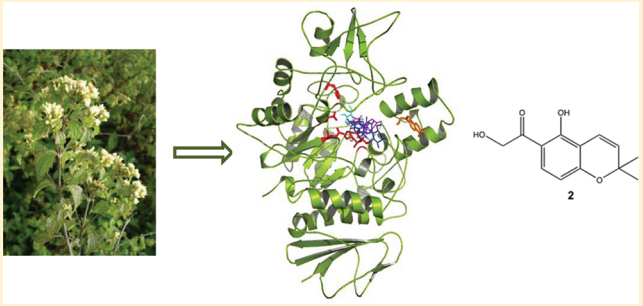
Supporting Information

ABSTRACT: An aqueous extract from the aerial parts of *Brickellia cavanillesii* attenuated postprandial hyperglycemia in diabetic mice during oral glucose and sucrose tolerance tests. Experimental type-II DM was achieved by treating mice with streptozotocin (100 mg/kg) and β-nicotinamide adenine dinucleotide (40 mg/kg). These pharmacological results demonstrated that *B. cavanillesii* is effective for controlling fasting and postprandial blood glucose levels in animal models. The same aqueous extract also showed potent inhibitory activity (IC_{50} = 0.169 vs 1.12 mg/mL for acarbose) against yeast α-glucosidase. Bioassay-guided fractionation of the active extract using the α-glucosidase inhibitory assay led to the isolation of several compounds including two chromenes [6-acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**1**) and 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**2**)], two sesquiterpene lactones [caleins B (**3**) and C (**4**)], several flavonoids [acacetin (**5**), genkwanin (**6**), isorhamnetin (**7**), kaempferol (**8**), and quercetin (**9**)], and 3,5-di-*O*-caffeoylquinic acid (**10**). Chromene **2** is a new chemical entity. Compounds **2**, **4**, **7**, and **9** inhibited the activity of yeast α-glucosidase with IC_{50} 0.42, 0.28, 0.16, and 0.53 mM, respectively, vs 1.7 mM for acarbose. Kinetic analysis revealed that compounds **4** and **7** behaved as mixed-type inhibitors with K_i values of 1.91 and 0.41 mM, respectively, while **2** was noncompetititive, with a K_i of 0.13 mM. Docking analysis predicted that these compounds, except **2**, bind to the enzyme at the catalytic site.



Brickellia cavanillesii (Cass.) A. Gray (Asteraceae), commonly known as "prodigiosa" and "atanasia amarga", is a bitter-tasting shrub widely distributed in Mexico.[1] Previous phytochemical studies of the aerial parts of this species resulted in the isolation and characterization of three flavonoids (brickellin, atanasin, and pendulin)[2−4] and 6-acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**1**).[5] This species, alone or in combination with other herbs, is widely commercialized in Mexico for the treatment of ulcers, dyspepsia, and diabetes.[1−6] According to a recent review, this is one of the 306 species most employed for the treatment of type-II diabetes mellitus (DM),[6] a chronic metabolic disease characterized by major imbalances in glucose metabolism and abnormalities in fat and protein metabolism.[7] About 246 million people suffer from type-II DM worldwide, and its incidence and serious complications continue to grow rapidly. Although there are several classes of antidiabetic drugs, achieving and maintaining long-term glycemia control is often challenging. In addition, many current agents have treatment-limiting side effects.[8] Therefore, there is an urgent need to find novel antidiabetic agents, including herbal remedies.[8] Thus, as part of our continuing efforts[9,10] to discover new therapies for treatment of diabetes derived from Mexican medicinal plants, the goals of the present study were to establish if *B. cavanillesii* had hypoglycemic activity and to identify new α-glucosidase inhibitors that could efficiently control postprandial glucose levels.

RESULTS AND DISCUSSION

The use of *B. cavanillensii* in Mexican folk medicine prompted us to determine its efficacy as an antidiabetic agent using well-known animal models. First, an aqueous extract (56.2 and 316 mg/kg) of the aerial parts was tested using acute hypoglycemic as well as oral glucose and sucrose tolerance tests (OGTT and OSTT, respectively) in two sets of animals: normal and diabetic. Experimental type-II DM was achieved by treating mice with streptozotocin (STZ, 100 mg/kg) 15 min after an injection of β-nicotinamide adenine dinucleotide (NAD, 40 mg/kg).[10,11] This preliminary treatment with NAD provokes partial protection against the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic β-cell mass, creating a diabetic syndrome close to type-II DM. In the acute preliminary experiments, the aqueous extract decreased ($p < 0.05$) blood glucose levels in both normal (Table S1, Supporting Information) and NAD-STZ-diabetic mice (Figure 1) at doses of 100 and 316 mg/kg, an

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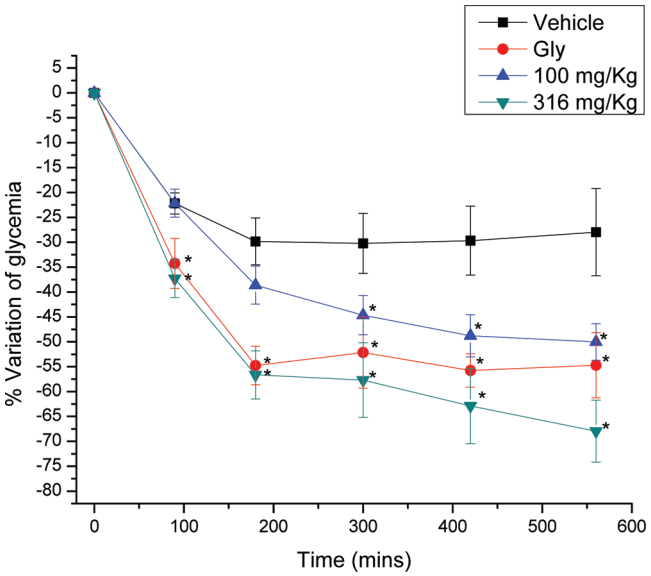

Figure 1. Effect of an infusion of *B. cavanillesii* on blood glucose levels in NAD-STZ-diabetic mice using the acute hypoglycemic test. Each value is the mean ± SEM for six mice in each group. *p < 0.05 significantly different ANOVA followed by Dunnett's t-test for comparison with respect to negative control values at the same time.

effect similar to that produced by glibenclamide (Gly, positive control) at the dose of 10 mg/kg. The effect in all cases was attained after 1 h and was maintained throughout the experiment. In the oral glucose tolerance test, the treatments provoked significant (p < 0.05) decrease of the postprandial peak (Table S2 and Figure S-1 Supporting Information); the glucose-lowering effect was more pronounced in NAD-STZ-diabetic mice (Figure 2) at all doses tested, being noticed after 30 min and persisting throughout the experiment time. Next, an oral sucrose tolerance test, in both normal (Table S3 and Figure S2, Supporting Information) and diabetic (Figure 3) mice was carried out. This test is usually performed to evaluate if the hypoglycemic action of a drug involves inhibition of intestinal α-glucosidase. The enzyme reduces the rate of

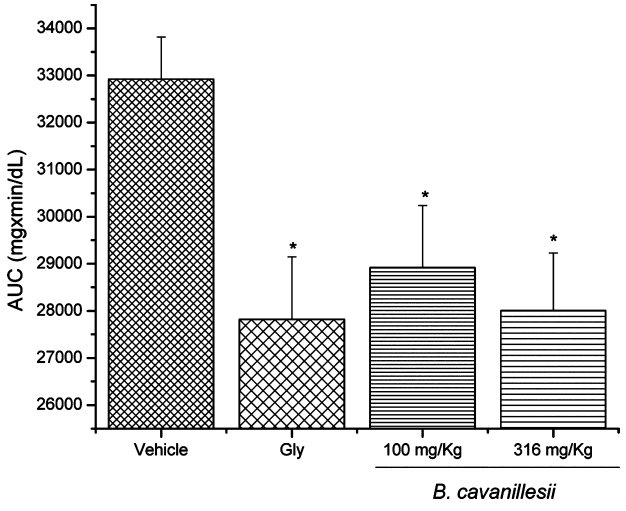

Figure 2. Influence of an infusion of *Brickellia cavanillesii* on postprandial blood glucose levels in NAD-STZ-diabetic mice during an OGTT. *p < 0.05 significantly different ANOVA followed by Dunnett's t-test for comparison with respect to the vehicle.

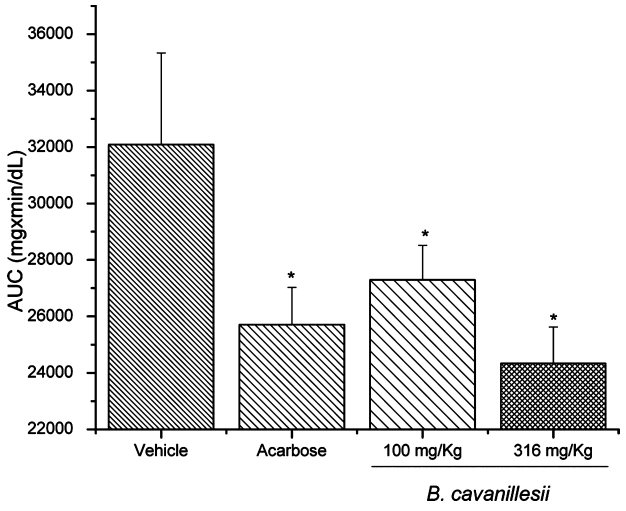

Figure 3. Influence of an infusion of *Brickellia cavanillesii* on postprandial blood glucose levels in NAD-STZ-diabetic mice during an OSTT. *p < 0.05 significantly different ANOVA followed by Dunnett's t test for comparison with respect to negative control.

digestion of carbohydrates by preventing their immediate breakdown into monosaccharides, which then would be absorbed quickly into the bloodstream. Slowing the absorption of carbohydrates gives the β-cells in the pancreas more time to secrete adequate insulin to cover the meal.[7] The infusion lowered glucose levels in both normal (100 and 316 mg/kg) and diabetic mice (56.2, 100, and 316 mg/kg); these results indicated that an inhibition of α-glucosidases at the intestinal level was mediating the observed effect. The activity was compared with that of acarbose (5 mg/kg), a natural α-glucosidase inhibitor currently used to control blood glucose levels. Finally, the in vitro α-glucosidase inhibitory activity of the extract was tested using a spectrophotocolorimetric assay with p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate and α-glucosidase from *Sacharomyces cerevisiae* (yeast).[12,13] The results were also compared with those of acarbose (IC_{50} = 1.12 mg/mL) and revealed that the extract inhibited the activity of yeast α-glucosidase with an IC_{50} of 0.169 mg/mL. Altogether, the above findings showed that the analyzed aqueous extract of *B. cavanillesii* possessed active principles useful to attenuate fast and postprandial hyperglycemia conditions.

In order to identify the α-glucosidase inhibitors from the active extract, it was subjected to bioassay-guided fractionation. This process led to the isolation of several compounds including two chromenes [6-acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**1**) and 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**2**)], two sesquiterpene lactones [caleins B (**3**) and C (**4**)], several flavonoids [acacetin (**5**), genkwanin (**6**), isorhamnetin (**7**), kaempferol (**8**), and quercetin (**9**)], and 3,5-di-*O*-caffeoylquinic acid (**10**). Compound **2** is a new natural product, and compounds **3**–**10** are new to the species and like chromene **1** were identified by comparison of their spectroscopic and spectrometric data with those described previously.[14–24]

The molecular formula of **2** ($C_{13}H_{14}O_4$) was derived from NMR spectroscopic data (see Experimental Section) and the HREIMS ion at m/z 234.0887 $[M]^+$. Comparison of the NMR, UV, and IR data of **2** with those of **1**[24] suggested a close resemblance between both compounds. In fact, the ^{13}C and ^1H NMR spectra of **2** (see Experimental Section and Figures S3–

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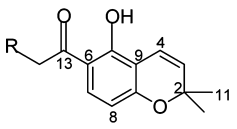
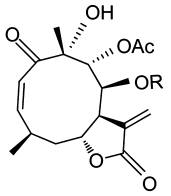
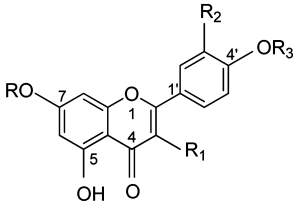


R = H 1
R = OH 2
R= OAc 2a



R = angeloyl 3
R = tigloyl 4



R = R_1 =R_2 = H, R_3 = Me 5
R = Me, R_1 = R_2 = R_3 = H 6
R = R_3 = H, R_1 = OH, R_2 = OMe 7
R = R_2 = R_3 = H, R_1 = OH 8
R= R_3 = H, R_1=R_2 = OH 9

S8 of the Supporting Information) were almost identical to that of **1**, except for the absence of signals for the C-14 methyl group. Instead, signals for a hydroxymethylene moiety were observed at δ_H/δ_C 4.82 (s, H-14)/65 (C-14) and δ_H 4.81 (OH-14). As for compound **1**, the spectra of **2** also showed signals for the C-13 ketone group (δ_C 203.6), a chelated OH group (δ_H 12.55), a *cis* double bond [δ_H/δ_C 6.67 (dd, J = 10, 0.5 Hz, H-4 and 5.68 d, J = 10 Hz, H-3/116.3 (C-4) and 129.7(C-3)], two *ortho*-oriented aromatic protons [δ_H/δ_C 7.55 (d, J = 8.5 Hz, H-7), 6.34 (dd, J = 9.0, 1.0 Hz, H-8/131.1 (C-7), and 109.8 (C-8)], and two geminal methyl groups [δ_H/δ_C 1.42, s, H-11 and H-12)/28.6 (C-11 and C-12)]. The HMBC data (Supporting Information) obtained for compounds **1** and **2** gave almost the same set of correlations, in agreement with the same 6-acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene core. In particular, the strong correlations of C-13 with H-14 and H-7 as well as C-6 with H-8 and H-7 were consistent with attachment of the hydroxymethylene moiety to C-13. The strong NOESY correlation observed between H-7 and both H-14 signals further supported the position of the primary alcohol functionality. Acetylation of **2** with one equivalent of acetic anhydride and pyridine yielded **2a**, whose ^{1}H NMR spectrum showed the hydroxymethylene signal paramagnetically shifted to δ_H 5.34; as expected, the signal at δ_H 12.55 was no longer observed. Thus, compound **2** was established as 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene.

Compounds **1**−**7** and **10** were next evaluated to determine their effect on the enzyme α-glucosidase from yeast; compounds **8** and **9** are well-known mixed-type α-glucosidase[25,26] and α-amylase[27] inhibitors; therefore only **9** was included in our assays as a second positive control. Compounds **2**, **4**, and **7** were the most active, with IC_{50} values of 0.42, 0.28, and 0.16 mM, respectively, similar to those of acarbose and **9** (IC_{50} = 0.34 and 0.53 mM, respectively). Previously, Xu[26] proposed that 7, 3, 3′, and 4′ hydroxy groups in the flavonoid core were important structural features for the inhibitory effect of this type of compound on yeast α-glucosidase. However, our results revealed that the nature of the 3′ substituent is not relevant for better activity against the enzyme since isorhamnetin (**7**), with a 3′ methoxy group, was more active than quercetin (**9**), possessing a 3′ hydroxy moiety. The best activity found for calein C (**4**) in comparison with calein B (**3**) (data not shown) suggested that the nature of the acyl residue at C-8 has an impact on the enzymatic activity.

The enzyme inhibition properties of compounds **2**, **4**, **7**, and acarbose were analyzed using Lineweaver−Burk and Dixon plots.[28] Compound **7** (Figure S10, Supporting Information), like other flavonoids,[26] showed a mixed type of inhibition against *S. cerevisiae* α-glucosidase. The same trend was observed for compound **4** (Figure 4). The inhibition constant (K_i) values were 1.91 and 0.41 mM, respectively. As expected, acarbose[29] (Figure S9, Supporting Information) behaved as a competitive inhibitor, with a K_i of 0.26 mM, while compound **2** (Figure 5) acted as noncompetititive,[30] with a K_i of 0.13 mM.

In order to envisage the binding model of inhibitor compounds **2**, **4**, and **7** with α-glucosidase, a molecular docking study was next performed using the program AutoDock4.[31,32] After geometric optimization, the ligands were docked into the entire protein, and the best conformations observed in this preliminary analysis were docked into a smaller area, in order to refine the results. The analysis (Figure 6) predicted that **7** bound in the catalytic region of the α-glucosidase and formed hydrogen bonds with Asp214, Glu304, and Asp349 through the OH groups at C-7, C-5, and C-4′, respectively; two of these

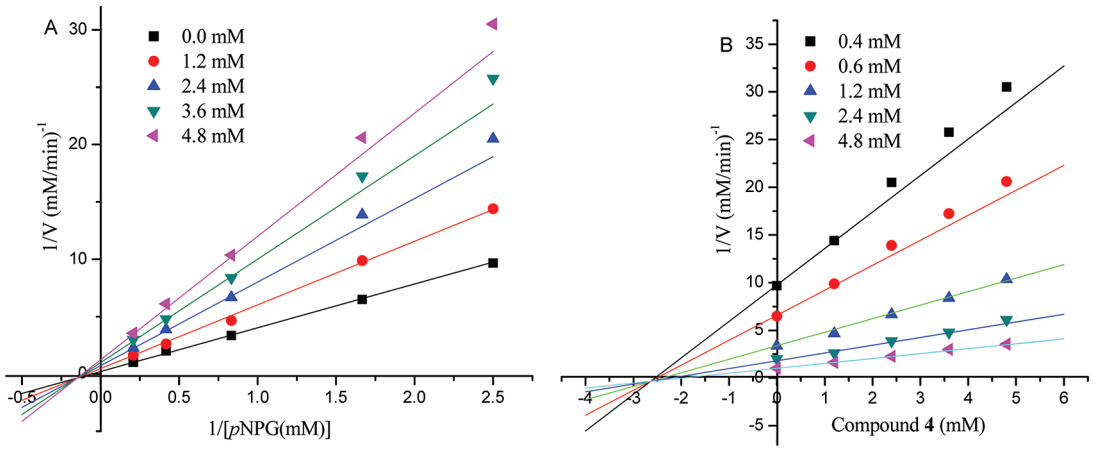


Figure 4. Lineweaver−Burk (A) and Dixon (B) plots of α-glucosidase inhibition at different concentrations of substrate, inhibitors, and compound **4**.

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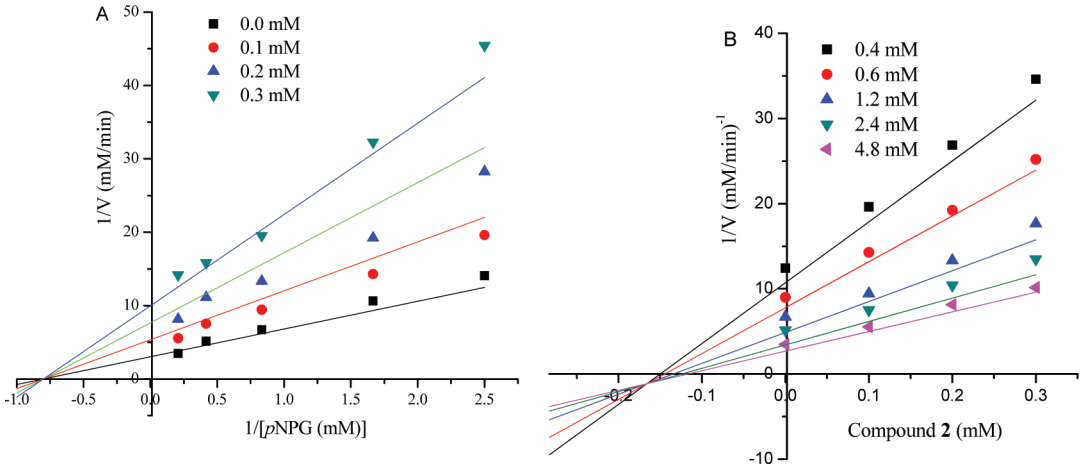

Figure 5. Lineweaver–Burk (A) and Dixon (B) plots of α-glucosidase inhibition at different concentrations of substrate and compound **2**.

amino acids, Asp214 and Asp349, are part of the catalytic residues of the enzyme. The predicted K_i value for **7** was 7.9 μM, while that for acarbose was 0.024 μM. Compound **4** also bound close to the catalytic site of the enzyme, with a K_i value of 0.30 μM. The amino acids involved in the binding of calein (**4**), including those forming hydrogen bonds (Glu304 and Ar349), are also shown in Figure 6. Finally compound **2** binds more probably on a region different from the catalytic area, with a K_i value of 13 μM and consistent with its noncompetitive nature of inhibitor.

In conclusion, the information generated in this study indicates that the infusion of *B. cavanillesii* is effective in vivo for controlling fasting and postprandial blood glucose levels in animal models of diabetes mellitus and represents a good phytotherapeutic agent. These effects are due in part to its high content of metabolites with significant inhibitory activity against α-glucosidase, including **2**, **4**, **7**, and **9**. Although, flavonoids are well-known inhibitors of α-glucosidase, reports of sesquiterpene lactones as inhibitors of the enzyme are scarce. So far, only a norcadinanolide,[33] 3-methoxypterolactone, from *Cyclocarya paliurus* (Cyclocaryaceae) has been reported to inhibit α-glucosidase. Finally, no benzopyrane possessing an inhibitory effect on this enzyme has been reported. According to the results of the OGTT test, the aqueous extract of *B. cavanillesii* also contains compounds affecting glucose uptake or utilization and/or insulin secretion or action.[7] Indeed, quercetin (**9**), the major infusion metabolite, potentiates insulin secretion and protected β-cell function and viability against oxidative damage; these effects were correlated with a major increase in ERK1/2 (extracellular signal-regulated kinases 1/2) phosphorylation.[34] Kaempferol, on the other hand, improved insulin secretory function and synthesis in β-cells and human islets.[35]

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks or films on a JASCO FTIR-410 infrared spectrometer. UV spectra were recorded on a Shimadzu 160 UV spectrometer in MeOH solution. NMR spectra including NOE differential, COSY, HMBC, and HMQC experiments were recorded in $CDCl_3$ or CD_3OD on a Varian Unity Plus 500 spectrometer or on a Bruker DMX500 spectrometer operating at 500 or 300 MHz (^{1}H) or 125 or 75 MHz (^{13}C NMR), using tetramethylsilane (TMS) as an internal standard. HREIMS were obtained on a JEOL JMS-AX505HA mass spectrometer. Reversed-phase high-performance liquid chromatography (RP-HPLC-DAD) was carried out with a Waters HPLC instrument equipped with a Waters 996 UV photodiode array detector (900) set at 270–400 nm, using a Purospher RP-18 column (4.6 mm i.d. × 250 mm) and isocratic conditions (CH_3CN–H_2O 48:52; 2.8 mL/min). Control of the equipment, data acquisition, and processing and management of chromatographic information were performed by the Millennium 32 software program (Waters). The absorbances in the enzymatic assay were determined at 405 nm in a BIO-RAD microplate reader model 680. Column chromatography was performed using silica gel 60 (70–230 mesh, Merck) or Sephadex LH-20 (Sigma-Aldrich-Fluka). TLC analyses were performed on silica gel 60 F254 plates (Merck), and visualization of plates was carried out using a ceric sulfate (10%) solution in H_2SO_4.

Chemicals and Reagents. CH_2Cl_2, MeOH, *n*-hexane, CH_3CN (HPLC grade), H_2O (HPLC grade), and MeOH (HPLC grade) were purchased from Merck, Darmstadt, Germany, whereas CD_3OD and $CDCl_3$, streptozotocin 98%, γ-nicotinamide-dinucleotide hydrate 98%, glybenclamide, glucose, sucrose ACS reagent, acarbose, α-glucosidase, type I from *S. cerevisiae*, and *p*-nitrophenyl α-D-glucopyranoside were obtained from Sigma-Aldrich, St. Louis, MO, USA.

Plant Material. Leaves and twigs of *B. cavanillesii* were collected on September 29, 2009, in Yecapixtla Morelos, Mexico. The plant was identified by authors R.B. and E.L. A voucher specimen (R. Bye and E. Linares 36171) has been deposited at the National Herbarium (MEXU), UNAM, Mexico City.

Preparation of Extracts. Dried and ground aerial parts (540 g) of *B. cavanillesii* were extracted with 13.5 L of boiling water (100 °C) during 30 min and then filtered and dried in vacuo to yield 40 g of extract (AE), which was then extracted with CH_2Cl_2 (3 × 13.5 L) to yield an active CH_2Cl_2-soluble fraction (DSF; 4.5 g, at 0.5 mg the activity of the enzyme was inhibited by 100%) and an inactive H_2O-soluble fraction (WSF, 35 g). The process was repeated as needed.

Separation and Isolation. The CFS (4.5 g) was dissolved in MeOH; from this solution 223 mg of **8** spontaneously crystallized. The remainder of the DSF was separated by Sephadex CC (800 mL MeOH) to afford nine secondary fractions (DSF1–DSF9). From the enzymatic assay only fractions DSF3, DSF6, DSF7, and DSF9 were active, all inhibiting the activity of the enzyme with IC_{50}'s near 0.17 mg/mL. Column chromatography on silica gel (120 g) of DSF3 (3 g) eluting with hexane with increasing amounts of EtOAc (7:3 → 1:9) gave **1** (60 mg) and **2** (62 mg) and a mixture of sesquiterpene lactones (0.8 g) **3** and **4**. The latter was further separated by HPLC (CH_3CN–H_2O, 48:52, 3.8 mL/min) to give **4** (335 mg) and **3** (184 mg). DSF6 (128 mg) was subjected to preparative TLC ($CHCl_3$–MeOH, 6:4) to yield compound **10** (22 mg). A yellow powder (20 mg) precipitated from DSF7 (62 mg), which was resolved by HPLC to give **5** (9.6 mg) and **6** (6.4 mg). Preparative TLC (CH_2Cl_2–MeOH, 9:1) of fraction DSF9 (90 mg) yielded 40 mg of **7** and 14 mg of **8**.

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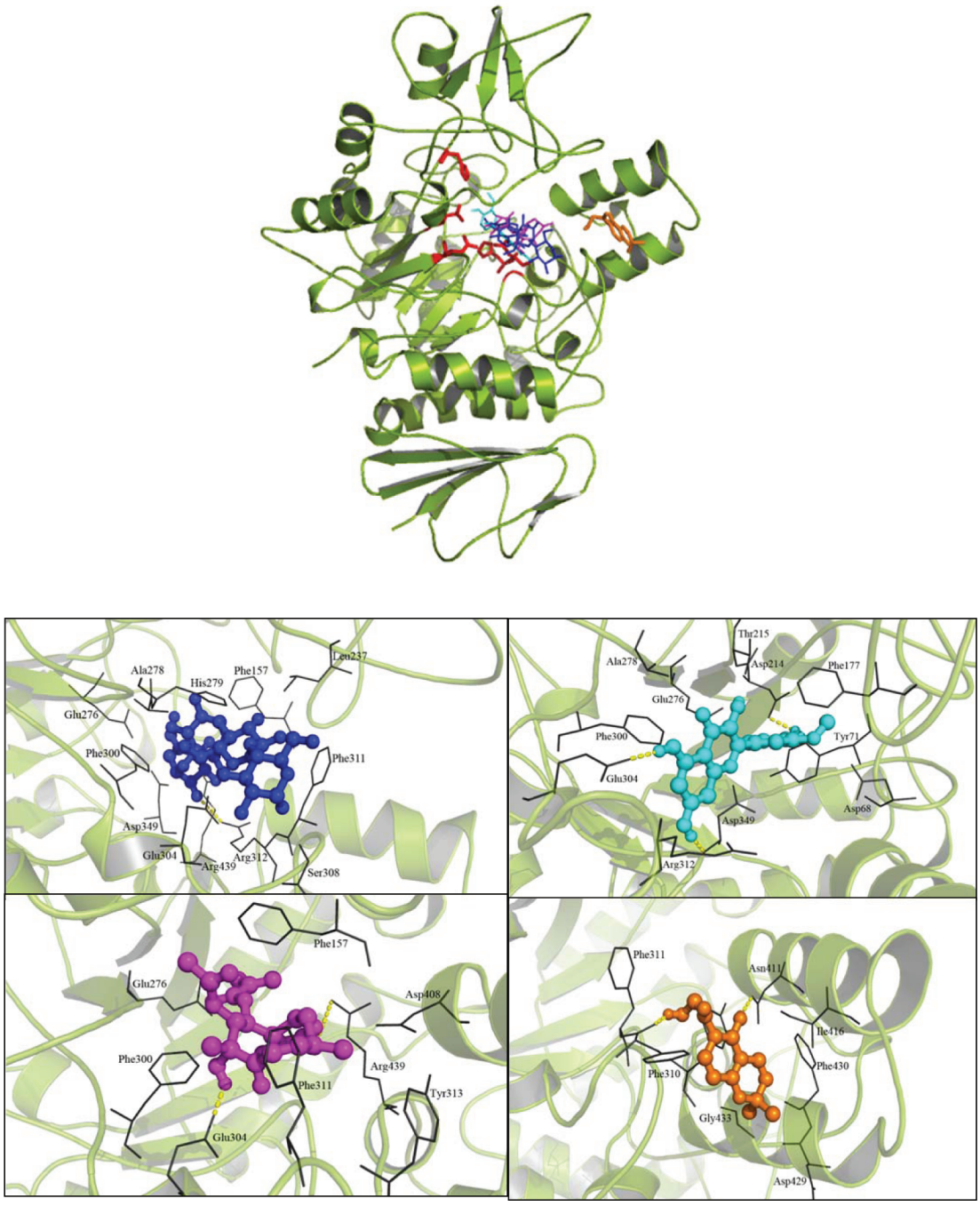

Figure 6. Docking results using the structural model of the α-glucosidase: (A) site of binding of acarbose (blue), which comprises the catalytic site of the enzyme; (B) binding conformation of compounds **7** (cyan), **4** (magenta), and **2** (orange).

6-Hydroxyacetyl-5-hydroxy-2,2-dimethyl-2H-chromene (**2**)*:* yellow oil; UV (MeOH) (log ε) λ_{max} nm 265 (4.48) and 310 (3.82); IR (neat) ν_{max} cm^{-1} 3451 (OH), 2974, 2931, 1640 (C═O), 1618, 1576, 1487, 1428, 1362, 1280, 1164, 1109, 1076, 1022; ^{1}H NMR (CD_3OD, 500 MHz) δ 12.55 (1H, s, OH-5), 7.55 (1H, d, J_{7-8} = 8.5 Hz, H-7), 6.67 (1H, dd, J_{4-3} = 10 Hz, J_{4-8} = 0.5 Hz, H-4), 6.34 (1H, dd, J_{8-7} = 9.0 Hz, J_{8-4} = 1.0 Hz, H-8), 5.67 (1H, d, J_{3-4} = 10 Hz, H-3), 4.82 (2H, s, H-14), 1.42 (6H, s, H-11 and H-12); ^{13}C NMR (CD_3OD, 125 MHz) δ 203.6 (C-13), 161.1 (C-5), 160.0 (C-9), 131.1 (C-7), 129.8 (C-3), 116.3 (C-4), 112.3 (C-6), 110.3 (C-10), 109.7 (C-8), 78.9 (C-2), 65.12 (C-14) 28.6 (C-11 and C-12); EIMS (rel int) m/z 234 [M]$^+$ (44), 219 [M − 15]$^+$ (90), 201 (100), 173 (43), 161 (31), 77 (59), 51 (43); HREIMS m/z 234.0887 (calcd for $C_{13}H_{14}O_4$ 234.0892).

Acetylation of 6-Hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (2). Compound **2** (5 mg) was treated with acetic anhydride (1 mL) and pyridine (1 mL). The mixture was left at room temperature for 24 h; then, 2 mL of water was added to the reaction mixture and extracted with CH_2Cl_2 (5 mL × 3). The organic phase was successively washed with 1 N HCl (5 mL × 3) and H_2O (5 mL × 3), dried over Na_2SO_4, and concentrated in vacuo to yield 5.5 mg of **2a** as an oil.

6-Acetoxyacetyl-5-hydroxy-2,2-dimethyl-2H-chromene (**2**)*:* yellow oil; IR (neat) ν_{max} cm^{-1} 3450 (−OH, weak), 2973, 2929, 1720, 1640, 1620, 1570, 1280; ^{1}H NMR (CD_3OD, 500 MHz) δ 12.61 (OH-5), 7.60 (1H, d, J_{7-8} = 8.5 Hz, H-7), 6.66 (1H, dd, J_{4-3} = 10 Hz, J_{4-8} = 0.5 Hz, H-4), 6.36 (1H, dd, J_{8-7} = 9.0 Hz, J_{8-4} = 1.0 Hz, H-8), 5.67 (1H, d, J_{3-4} = 10 Hz, H-3), 5.34 (2H, s, H-14), 2.17 (3H, s, CH_3-CO),

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1.42 (6H, s, H-11 and H-12); EIMS (rel int) m/z 276 $[M]^+$ (5), 234 (58), 217 (25), 43 (100); HREIMS m/z 276.0995 (calcd for $C_{15}H_{16}O_5$ 276.0998).

Experimental Animals. Acute hypoglycemic assays were conducted in both (Supporting Information) normoglycemic and diabetic mice.[9,10] Male ICR normoglycemic mice, weighing 20−25 g, 20−25 days old, were obtained from Centro UNAM Harlan (Harlan México, SA de CV). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with international rules on care and use of laboratory animals. The animals were housed in groups of eight under standard laboratory conditions (12 h light−dark cycle under controlled temperature, 22 ± 1 °C) and maintained on a standard pellet diet and water ad libitum.

Preparation of the Test Samples. The infusion was dissolved in saline solution. Gly (10 mg/kg) was used as hypoglycemic control drug. Acarbose (5 mg/kg) was used as an antihyperglycemic drug. Glucose (1.5 g/kg) and sucrose (3 g/kg) were used for the carbohydrate tolerance tests. The control mice group received only saline solution. The administration was oral in all cases.[9,10]

Induction of Experimental Type-II DM. Type-II DM was induced by ip administration of STZ (100 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5, and maintained on ice prior to use, 15 min after an ip administration of NAD (40 mg/kg) dissolved in distilled water. One week later the blood glucose levels in each mouse were measured by the enzymatic glucose oxidase method using a commercial glucometer. Blood samples were collected from the tail vein by means of a small incision at the end of the tail. Only those mice with blood glucose levels higher than 140 mg/dL were included in the study.[9,10]

Acute Hypoglycaemic Assay. Normal and diabetic animals were placed in acrylic boxes in groups of six deprived of food but free access to water 12 h before experimentation. The infusion was administrated orally at doses of 56.2, 100, and 316 mg/kg of body weight. Gly as positive control (10 mg/kg) and saline solution as vehicle control were administrated by the same route. Gly and the infusion were suspended in the vehicle. Blood samples were collected at 0, 1.5, 3, 4, 5, 7, and 9 h after respective administrations.[9,10]

Oral Glucose Tolerance Test. Normal and diabetic mice were placed in acrylic boxes, forming groups of six animals (I to VI). Group I was administrated with the vehicle; group II received Gly; groups III to V received different amounts of aqueous extract (56.2, 100, and 316 mg/kg of body weight). Time 0 min was set before treatment with the extract or control; 30 min later a glucose load (1.5 g/kg) was given to the animals. Blood samples were obtained 30, 60, 90, 120, and 180 min after the carbohydrate load.[9,10] Area under the curve (AUC) was calculated applying the following formula: AUC (mg/dL × min) = $\sum(VG_{T1} + VG_{T2})/2) \times (T2 - T1)$, where VG_{T1} and VG_{T2} represent blood glucose levels at 0, 30, 60, 90, 120, and 180 min after oral glucose feeding to mice.

Oral Sucrose Tolerance Test. Oral sucrose tolerance test was executed in the same way as for the OGTT, but in this case sucrose (3 g/kg of body weight) was used as carbohydrate load and acarbose (5 mg/kg) as positive control. The percentage variation of glycemia of each group was measured as previously described.[10] Area under the curve was calculated applying the following formula: AUC (mg/dL × min) = $\sum(VG_{T1} + VG_{T2})/2) \times (T2 - T1)$, where VG_{T1} and VG_{T2} represent % variation of glycemia at 0, 30, 60, 90, and 120 min after an oral sucrose feeding to the mice.

Statistical Analysis. Results are expressed as the mean ± SEM of six animals in each group. Analysis of variance (ANOVA, one way) was used to analyze changes in blood glucose level followed by Dunnett's test; $p < 0.05$ was considered statistically significant. Sigma Stat software was used for the data analysis.

In Vitro α-Glucosidase Assay. AE, DSF, WSF, DSF1−DSF9, and compounds **1**−**9** were dissolved in MeOH or DMSO. Aliquots of 10 μL of acarbose or test compounds or fraction solutions (triplicated) were incubated during 5 min with 20 μL of enzyme stock solution (0.9 units/mL of yeast α-glucosidase in 100 mM solution of sodium phosphate buffer). After incubation, 10 μL of substrate [*p*NPG, 5 mM] was added and incubated for 35 min at 30 °C; then, the absorbances were determined. The concentration required to inhibit activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis, using the following equation:

$$v = \frac{A_{100}}{1 + (I/IC_{50})^s}$$

where v is the percentage of inhibition, A_{100} is the maximum inhibition, I is the inhibitor concentration, IC_{50} is the concentration required to inhibit activity of the enzyme by 50%, and s is the cooperative degree.[10]

Enzyme Kinetics. The mode of inhibition of α-glucosidase was determined by the Lineweaver−Burk plots. Dixon plots were used to determine the inhibitory constants. The linear mixed inhibition is described by the following equation:

$$v = \frac{v_{max}S}{(k_m(1 + I/K_i) + S(1 + I/K'_i)}$$

where v is the initial velocity in the absence and presence of the inhibitor, s and I respectively are the concentration of substrate and inhibitor, V_{max} is the maximum velocity, k_m is the Michaelis−Menten constant, K_i is the competitive inhibition constant, and K'_i is the uncompetitive inhibition constant.

The noncompetitive type of inhibition is described with the following equation:[36]

$$v = \frac{v_{max}S^{nh}}{(1 + I/K_i) \times (K \times 10^5)^{nh} + (1 + I/K_i) \times S^{nh}}$$

where K_i is the inhibition constant and nh is the Hill number.

The kinetic data were analyzed using a computer program for nonlinear regressions (Origin 8.0).[10]

Docking of α-Glucosidase Inhibitors. First homology modeling of yeast α-glucosidase was performed as previously described.[10] Blind docking was carried out with AutoDock4 software (http://autodock.scripps.edu/)[31,32] using the default parameters, except for the number of GA runs (100) and the Lamarkian genetic algorithm with local search and 25 million energy evaluations (long. evals.) per run, on a parallel supercomputer of distributed memory; it contains 1368 AMD Opteron processors, around 3 terabytes of memory, and 160 terabytes of storage (http://www.super.unam.mx/). The target in each docking run was obtained from homology modeling of α-glucosidase. The starting conformation of the ligand was an energy-minimized form with geometric optimization using the program HyperChem 8 release. Acarbose and compound **2**, **4**, or **7** molecules were first prepared by AutoDockTools 1.5.4 (http://mgltools.scripps.edu/), adding polar hydrogen atoms and Kollman charges to α-glucosidase and computing Gasteiger charges for acarbose and **2**, **4**, or **7**. The protein was held rigid during the docking process while the ligands were allowed to be flexible. The grid box size was 126 Å × 126 Å × 126 Å in the x, y, and z dimensions, with the center of the grid corresponding to the protein.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Effect of an infusion of *B. cavanillesii* on blood glucose levels of normal and STZ-diabetic mice. Effect of an infusion of *B. cavanillesii* on blood glucose levels of normal and STZ-diabetic mice after an oral load of glucose (1.5 g/kg). Effect of an infusion of *B. cavanillesii* on blood glucose levels of normal and STZ-diabetic mice after a load of sucrose (3 g/kg). Effect of an infusion of *B. cavanillesii* on blood glucose levels in NAD-STZ-diabetic mice after an oral load of glucose (1.5 g/kg). Effect of an infusion of *B. cavanillesii* on blood glucose levels in NAD-STZ-diabetic mice after an oral load of sucrose (3 g/kg). ^{13}C and ^{1}HNMR, DEPT, NOESY, HMBC, and HSQC spectra for compound **2**. Lineweaver−Burk and Dixon plots of α-glucosidase inhibition at different concentrations of substrate

and inhibitors for acarbose. Lineweaver−Burk and Dixon plots of α-glucosidase inhibition at different concentrations of substrate and inhibitors for **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.
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