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Supplementary Information

Stimulation of Insulin Secretion and Inhibition of K_{ATP} Channels by Afzelechin and Coniferaldehyde from *Ensete glaucum* Seeds

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METHODS

Fractionation and isolation: The seeds of *E. glaucum* were dried and ground to afford a fine powdered material. The plant material (13 kg) was extracted with methanol (ratio of 1: 20 *w/v*) by percolation. The solvent was combined and evaporated under reduced pressure using a rotary evaporator to afford the methanol extract. The methanol extract (351 g) was diluted with distilled water and subjected to solvent–solvent extraction with *n*-hexane, ethyl acetate and *n*-butanol (the extraction was repeated until the respective fraction was pale) to afford four fractions,

the *n*-hexane-soluble fraction (55.5 g), the ethyl acetate-soluble fraction (190.49 g), the *n*-butanol-soluble fraction (9.1 g), and the aqueous-soluble fraction. The ethyl acetate fraction (180 g) was subjected to silica gel column chromatography (CC) with gradient elution using CH_2Cl_2/CH_3OH (100:0 ~ 0:100) to afford 15 main fractions (EC1–EC15). A portion of main EC3 was also subjected to CC eluted with *n*-hexane/ethyl acetate (100:1 ~ 0:100) to afford 14 subfractions (EC3.1–EC3.14). Subfraction EC3.8 was continuously subjected to CC eluted with CH_2Cl_2 to afford compound EG2 (257 mg). A portion of main EC12 was subjected to CC with gradient elution using 100% $CHCl_3$, $CHCl_3$/CH_3OH (10:1 ~ 5:1 ~ 3:1 ~ 1:1), and 100% CH_3OH to afford 6 main fractions (EC12.1–EC12.6). Subfraction EC12.2 was continuously subjected to CC eluted with $CHCl_3$/CH_3OH (99:1 ~ 90:1 ~ 80:1 ~ 70:1 ~ ...) to afford compound EG1 (23 mg).

Protein assay: Briefly, 4 µL cell lysate was mixed to 96 µL distilled water and 1 mL Coomassie Brilliant Blue G-250. The reaction mixture was well mixed and absorbance was measured at 595 nm. The content of protein (µg/mL) was calculated by the linear regression equation of the BSA (Thermo Scientific) standard.

Reference: Kielkopf CL, Bauer W, Urbatsch IL. Bradford assay for determining protein concentration. Cold Spring Harb Protoc. 2020;2020:102269. https://doi.org/10.1101/pdb.prot102269

Molecular docking simulation

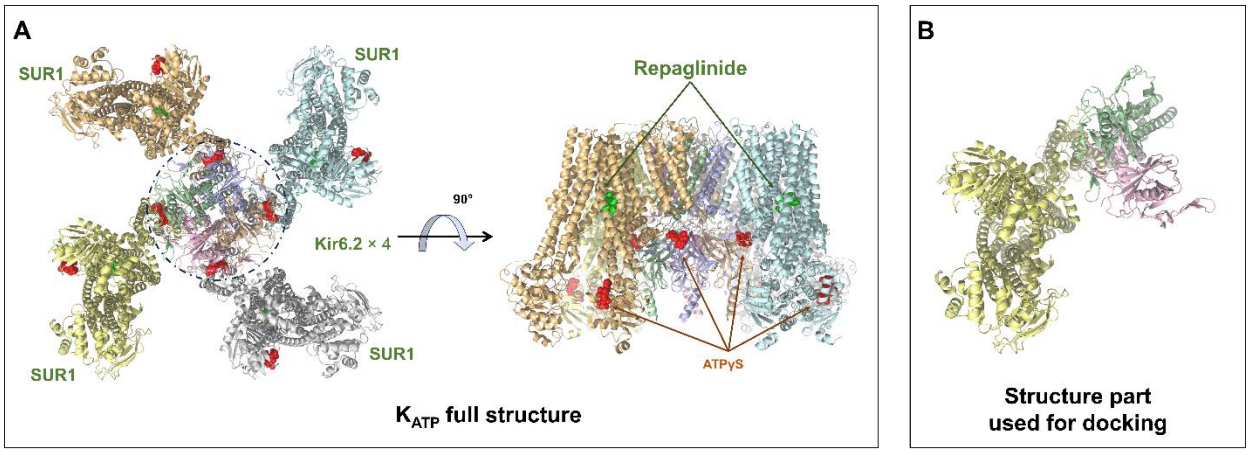

FIGURE S1. The overall structure of the K_{ATP} channel in complex with ATPγS and repaglinide. (A) The eight subunits of the K_{ATP} channel were represented with ribbons of different colors, ATPγS (an analog of ATP) and repaglinide (an inhibitor) were represented by ball molecules of

red and green color, respectively. (B) The structure part of K_{ATP} channel used for docking in this structure, consisted of one SUR1 subunit and two adjacent KIR6 subunits.

RESULTS

Characterization of compound EG1: The ^{1}H-NMR spectrum (δH, 600 MHz, ppm) revealed the presence of two methine protons at 5.71 (H-6) and δ_H 5.89 (H-8) that are characteristic for two protons at the *meta* position in the A ring of a flavane, in the A ring there is also a signal of two methine sp3 groups carrying oxygen δH 4.02 (H-3) and δH 4.80 (H-2), a methylene benzil group δH 2.48 (Ha-4) and δH 2.68 (Hb-4). In addition, two other methine protons at δH 6.71 (H-2', H-6') and δH 7.21 (H-3', H-5') allowed prediction of these two proton signals attached to the B ring of the flavane framework in a symmetrical position. The ^{13}C-NMR spectrum (δC, 150 MHz, ppm) showed resonances with 15 carbon signals including 6 quaternary carbons at δC 98.4 (C-10), δC 129.9 (C-1'), δC 155.7 (C-7), δC 156.2 (C-9), δC 156.5 (C-5), and δC 156.5 (C-4'). The remaining 9 carbon signals included one methylene carbon signal at δC 28.1 (C-4) and six olefin carbon signals (-CH=) at δC 94.1 (C-6), δC 95.1 (C-8), δC 114.4 (C-2', C-6'), δC 128.2 (C-3', C-5') and 2 oxymethine (-O-CH<) carbon signals at δC 64.8 (C-3), δC 78.0 (C-2). The HSQC (Heteronuclear single quantum coherence) spectrum identified the proton attached to the corresponding carbon. Proton at δ_H 5.89 (H-8) attached to carbon δ_C 95.1 (C-8), proton at δ_H 5.71 (H-6) attached to carbon δ_C 94.1 (C-6), proton at δ_H 6.71 (H-2', H-6') attached to carbon δ_C 114.4 (C-2', C-6'), proton at δ_H 7.21 (H-3', H-5') attached to carbon δ_C 128.2 (C-3', C- 5'), proton at δ_H 4.80 (H-2) attached to carbon δ_C 78.0 (C-2), proton at δ_H 4.02 (H-3) attached to carbon δ_C 64.8 (C-3), proton at δ_H 2.48 (Ha-4) and proton δ_H 2.68 (Hb-4) attached to carbon δ_C 28.1 (C-4). HMBC (Heteronuclear multiple bond correlation) spectrum revealed the correlations from δ_H 4.80 (H-2) to δ_C 129.9 (C-1'); from δ_H 4.02 (H-3) to δ_C 98.4 (C-10) and δ_C 129.9 (C-1'); from δ_H 2.48 (Ha-4) and δ_H 2.68 (Hb-4) to δ_C 64.8 (C-3), δ_C 78.0 (C- 2), δ_C 94.1 (C-6), δ_C 95.1 (C-8), δ_C 98.4 (C-10), δ_C 156.2 (C-9), and δ_C 156.5 (C-5); from δ_H 5.71 (H-6) to δ_C 95.1 (C-8), δ_C 98.4 (C-10), δ_C 156.5 (C-5), and δ_C 155.7 (C-7); from δ_H 5.89 (H-8) to δ_C 94.1 (C-6), δ_C 98.4 (C-10), δ_C 156.2 (C-9), and δ_C 155.7 (C-7); from δ_H 6.71 (H-2', H-6') to δ_C 78.0 (C-2), δ_C 156.5 (C-4'), δ_C 128.2 (C-3', C-5'), and δ_C 129.9 (C-1'); from δ_H 7.21 (H-3', H-5') to δ_C 78.0 (C-2), δ_C 156.5 (C-4'), δ_C 114.4 (C-2', C-6').

Characterization of compound EG2: The ^{1}H-NMR (600 MHz, acetone-d6): δ (ppm): 6.91 (1H, *d*, *J*=6.5Hz, H-3), 7.19 (1H, *dd*, *J*=1.5; 7.0 Hz, H-4), 7.35 (1H, *d*, *J*=2.5Hz, H-6), 7.56 (1H, *d*, *J*=13.5 Hz, H-7), 6.65 (1H, *dd*, *J*=1.5; 13.0 Hz, H-8), 9.63 (1H, *d*, *J*=6.5Hz, H-9), 5.37 (H, *s*, H-1-OH), 3.91 (3H, *s*, H-2-OCH_3). ^{13}C-NMR (150MHz, acetone-d6): δ (ppm): 56.3 (OCH_3), 148.9 (C, C-1), 150.9 (C, C-2), 116.2 (CH, C-3), 124.7 (C, C-4), 127.3 (CH, C-5), 111.6 (CH, C-6), 154.2 (CH, C-7), 126.8 (CH, C-8), 194.1 (CH, C-9). The ^{1}H-NMR spectrum in the low-medium magnetic field appeared a signal of three aromatic ring protons interacting ABX type at δH 6.91 (1H, *d*, *J* = 6.5 Hz, H-3), 7.19 (1H, *dd*, *J* = 1.5, 7.0 Hz, H-4), 7.35 (1H, *d*, *J* = 2.5 Hz, H-6), and the signal of the two trans-paired protons at δH 7.56 (1H, *d*, *J* = 13.5 Hz, H-7) and 6.65 (1H, *dd*, *J* = 13.0 Hz, H-8). In addition, a characteristic signal of a methoxy group at δH 3.91 and a –CHO group at δH 9.63 (1H, *d*, *J* = 6.5 Hz, H-9) was also recorded. ^{13}C-NMR spectrum and DEPT (Distortionless enhancement by polarization transfer) spectrum of compound EG2 showed the presence of 10 signals including three quaternary carbons at δC 148.9 (C-1), 116.2 (C-3) and 124.7 (C-4), 6 methyl groups and one methoxy group (–OCH_3) at 56.3. At the low magnetic field, the signal of the aldehyde group appeared at δC 194.1 (C-9).

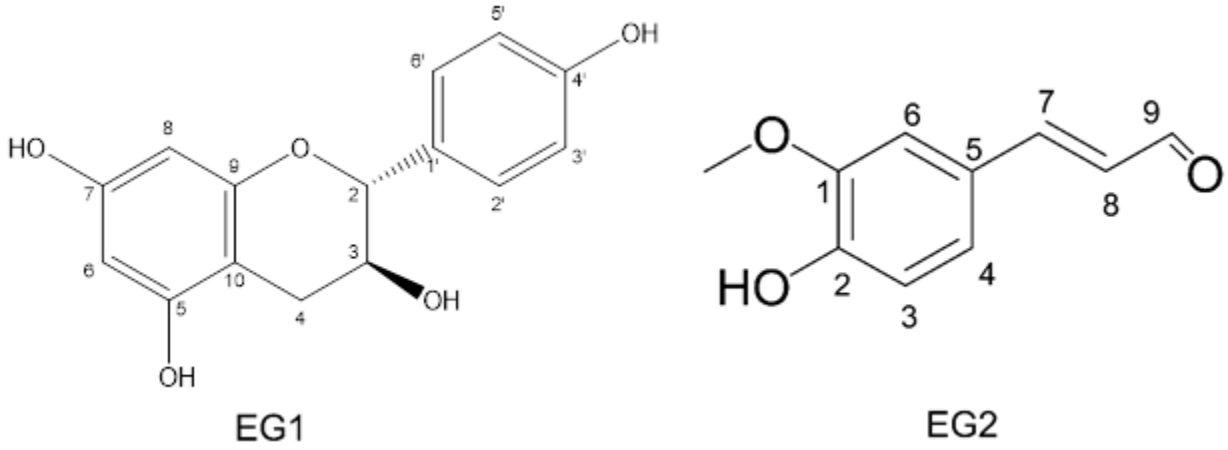

FIGURE S2. Structure of the compound **EG1** and **EG2** isolated from the *E. glaucum* seeds.

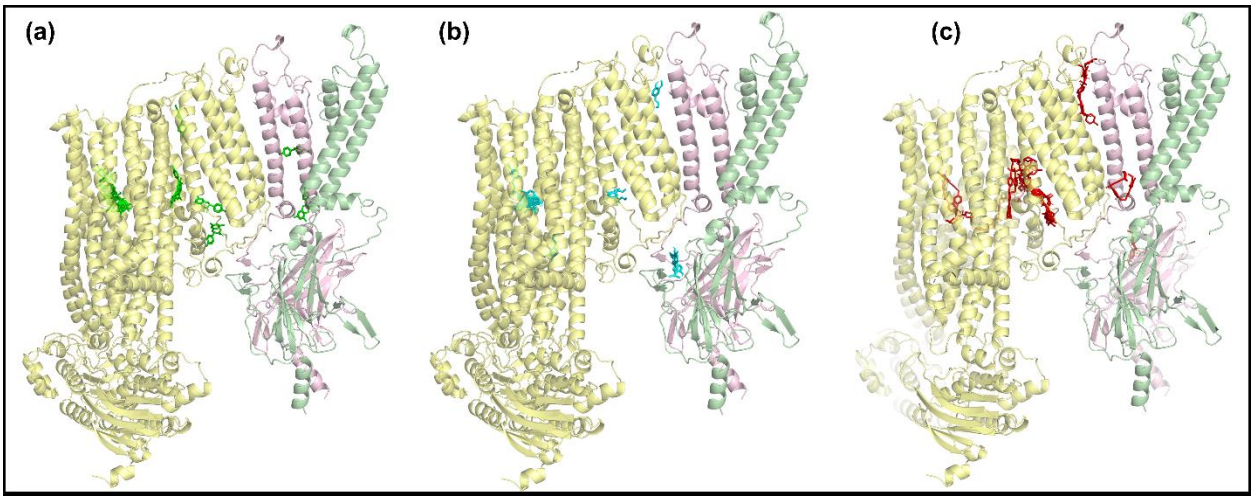

FIGURE S3. All 15 docking poses of (a) afzelechin, (b) coniferaldehyde, and (c) glimepiride to the K_{ATP} channel, generated from the docking analysis.