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A New Flavonol Glycoside from the Leaves of *Boscia senegalensis*

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Detailed chemical investigation of *Boscia senegalensis* (Per) Lam. ex Poir. led to the isolation of one new flavonol glycoside, rhamnocitrin-3-*O*-*β*-D-(6"-*O*-*E*-feruloyl)-glucopyranoside named bosenegaloside A (**1**), with seven known compounds, rhamnocitrin-3-*O*-*β*-D-(6"-*O*-*E*-*p*-coumaroyl)-glucopyranoside (**2**), rhamnocitrin-3-*O*-*β*-D-glucopyranoside (**3**), 3,4,5-trimethoxyphenol-*β*-D-glucopyrinoside (**4**), lasianthionoside A (**5**), 3,7-dimethyl-1-octene-3,6,7-triol-6-*O*-*β*-D-glucopyranoside (**6**), syringin (**7**), and austroside B (**8**). The chemical structures of these compounds were elucidated from spectroscopic data and by comparison of these data with previously published results. The inhibitory activity of the isolated compounds on soluble epoxide hydrolase (sEH) was assessed. Compounds **1-3** potently inhibited sEH activity with IC_{50} values of 12.8 ± 0.5, 18.4 ± 0.2, and 11.3 ± 0.9 μM, respectively.

Key Words : *Boscia senegalensis*, Capparaceae, Flavonol glycoside, Soluble epoxide hydrolase

Introduction

Epoxyeicosatrienoic acids (EETs), which are metabolites of arachidonic acid generated by cytochrome P450 epoxygenases, play important roles in the regulation of hypertension, inflammation, and other cardiovascular diseases.[1] Soluble expoxide hydrolase (sEH) has been described as the key enzyme in the metabolism of eicosanoid epoxides, including the metabolism of EETs to their less active metabolites dihydroxyeicosatrienoic acids (DHETs) and of leukotoxin (LTX) to leukotoxin diol (LTX diol). These metabolic events generally reduce the biological activity of compounds.[2]

The genus *Boscia* (Capparaceae) contains more than 37 species distributed mainly in Africa, excluding one species found in southern Arabia.[3] *Boscia senegalensis* (Pers.) Lam. ex Poir. is an evergreen shrub reaching 7 m in height.[4] It is native to the Sahel and Sahara savannas stretching from Mauritania, Senegal, Mali, Niger, and Nigeria to Cameroon and across Africa to Egypt, Sudan, Ethiopia, Somalia, and Kenya.[4] The importance of *B. senegalensis* for the rural agro-economy in Africa has been discussed in several reports, making it a plant of high value for both humans and animals.[5] Previous phytochemical reports on *B. senegalensis*, which were conducted on the leaves and fruits, identified glucosinolate.[6,7] However, to our knowledge there have been no adequate reports of the isolation of secondary metabolites from the leaves of *B. senegalensis*. In our efforts for the isolation of lead compounds for the treatment of cardiovascular diseases, we investigated the chemical constituents of the *B. senegalensis* plant and evaluated their biological activities.

Experimental

General Experimental Procedures. Optical rotations were determined using a JASCO P-2000 automatic digital polarimeter. The FT-IR spectra were measured using a Jasco Report-100 infrared spectrometer. The NMR recorded spectra were using a JEOL ECA 600 spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Gas chromatography was performed on a Shidmazu-2010 Spectrometer; Column, column DB-5 (0.25 mm × 30 m); detector, μ-ECD; column temp, 250 °C; injector temperature, 300 °C; detector temperature, 280 °C; carrier gas, N_2. Column chromatography was performed using silica gel (Kieselgel 60, 70-230, and 230-400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins. Thin layer chromatography (TLC) was performed using pre-coated silica-gel 60 F_{254} and RP-18 F_{254S} plates (both 0.25 mm, Merck, Darmstadt, Germany). Spots were visualized by spraying with 10% aqueous H_2SO_4 solution followed by heating.

Plant Material. Dried leaves of *Boscia senegalensis* were purchased from a folk medicine market in El Obied, North Kurdufan state, southern Sudan in September 2013 and identified by Professor A. M. Hamdoun, Faculty of Agricultural Sciences, University of Gezira, Sudan. A voucher specimen (CNU 13111) was deposited at the Herbarium of College of Pharmacy, Chungnam National University.

Extraction and Isolation. Dried leaves of *B. senegalensis* (3.0 kg) were extracted with 100% hot MeOH three times. After removing the solvent under reduced pressure, the MeOH extract (580 g) was dissolved in 1.0 L of H_2O to form a suspension that was successively partitioned with dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) to yield CH_2Cl_2 (85 g), EtOAc (20 g), and aqueous extract (475 g), respectively. Aqueous extract was chromatographed on a column of highly porous polymer (Diaion HP-20) using a stepwise gradient of H_2O and MeOH (0%, 25%, 50%, 75%

and 100% MeOH in H_2O) successively to yield five fractions (1a-1e). Fraction 1b was repeatedly separated by column chromatography over silica gel (70–230 mesh) eluted with CH_2Cl_2/MeOH/H_2O (20:1:0.1 – 1:1:0.1 v/v/v) and $CHCl_3$/MeOH/H_2O (6:1:0.1 v/v/v) to obtain **4** (30 mg), **5** (50 mg), **6** (10 mg) and **7** (15 mg). Fraction 1c was subjected to chromatography on silica gel (70–230 mesh) eluted with CH_2Cl_2/MeOH/H_2O (15:1:0.1 – 1:1:0.1 v/v/v) and further purified using YMC reverse-phase column chromatography (150 g, 2.0 · 90.0 cm) and eluted with MeOH/Me_2CO/H_2O (0.3:0.3:1 v/v/v) to afford **8** (10 mg). Compounds **1** (70 mg), **2** (50 mg) and **3** (90 mg) were obtained from fraction 1d after chromatography on silica gel (70–230 mesh) eluted with CH_2Cl_2/MeOH/H_2O (12:1:0.1 – 1:1:0.1 v/v/v) and then purified using YMC reverse-phase column chromatography (150 g, 2.0 × 90.0 cm) eluted with MeOH/Me_2CO/H_2O (0.25:0.25:1 v/v/v).

Bosenegaloside A (1): Yellow amorphous powder, $[\alpha]_D^{25}$ = –24.58 (c 0.1, MeOH); UV λ_{max} (MeOH) nm: 269.0, 326.0; FT-IR (KBr): ν_{max} 3421.4, 1654.4, 1253.4, 1213.2, 1164.8, cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR data (DMSO-d_6, 150 MHz), see Table 1; HR-ESI-Q-TOF MS *m/z* 661.1538 $[M + Na]^+$ (calcd for $C_{32}H_{30}O_{14}Na$, 661.1533).

Acid Hydrolysis and Sugar Identification. Compound **1** (3 mg) was heated in 3 mL of 10% HCl-dioxane (1:1) at 80 °C for 3 h. After the solvent was removed *in vacuo*, the residue was partitioned between EtOAc and H_2O to yield the aglycone and the sugar, respectively. The sugar component in the aqueous layer was analyzed using silica gel TLC by comparison with standard sugars. The solvent system was CH_2Cl_2-MeOH-H_2O (2:1:0.2) and spots were visualized by spraying with 95% EtOH-H_2SO_4-anisaldehyde (9:0.5:0.5, v/v) and heating to 180 °C for 5 min. For sugars, the R_f of glucose was 0.30. The results were confirmed based on GC analysis, as follows. The aqueous layer was evaporated to dryness to yield a residue and was dissolved in anhydrous pyridine (100 μL) and then mixed with a pyridine solution of 0.1 M L-cysteine methyl ester hydrochloride (100 μL). After warming at 60 °C for 2 h, trimethylsilylimidazole solution was added and warmed to 60 °C for 2 h. The mixture was then evaporated *in vacuo* to yield a dried product, which was partitioned between *n*-hexane and H_2O. The *n*-hexane layer was filtered and analyzed using GC.[8] The retention times of D-glucose (t_R, 14.85 min) were confirmed by comparison with an authentic standard.

sEH Inhibitory Activity Assay. The sEH inhibitory activity was determined using a hydrolysis reaction of PHOME in the presence or absence of the Inhibitor. The final reaction volume was 200 μL, and contained 25 mM bis-Tris HCl buffer (including 0.1% BSA (bovine serum albumin), pH 7.0), 20 μL of various concentrations of samples, 50 μL sEH enzyme, and 50 μL of 40 μM PHOME, AUDA (IC_{50}: 7.2 ± 2.7 nM) was used as the positive control. Reaction systems were incubated at 37 °C for 1 h, and fluorescence intensity was then monitored every 2 min (during 1 h) using a Genios microplate reader (Tecan, Mannedorf, Switzerland) at excitation and emission wavelengths of 330 and 465 nm, respectively. sEH inhibitory activity for each sample was calculated as follows:

$$\text{Enzyme activity (\%)} = [(S - S_0)/(C - C_0)] \times 100$$

where C is the fluorescence of the control (enzyme, buffer, MeOH, and substrate) after 60 min of incubation, C_0 is the fluorescence of the control at zero time, S is the fluorescence of the tested samples (enzyme, buffer, sample solution, and substrate) after incubation, and S_0 is the fluorescence of the tested samples at zero time.[9,10]

sEH Kinetic Assay. To study the kinetics of sEH inhibition by isolated compounds **1–3**, various concentrations of compounds **1–3** were added in 96 well plate containing 80 μL of 25 mM bis-Tr-is HCl buffer (including 0.1% BSA (bovine serum albumin), pH 7.0) with a series of substrate concentrations range of 10-80 μM, and fluorescence intensity was monitored at 37 °C every 2 mins for 20 mins. The *Ki* value was also derived by plotting slopes obtained from Lineweaver–Burk plots and Dixon plots using SigmaPlot (SPSs Inc., Chicago, IL).[10,11]

Results and Discussion

The present study reported the isolation and structural identification of one new flavonol glycoside, rhamnocitrin-3-*O*-*β*-D-(6"-*O*-*E*-feruloyl)-glucopyranoside, named bosenegaloside A (**1**),[12,13] together with seven known compounds: rhamnocitrin-3-*O*-*β*-D-(6"-*O*-*E*-*p*-coumaroyl)-glucopyranoside (**2**),[14] rhamnocitrin-3-*O*-*β*-D-glucopyranoside (**3**),[15] 3,4,5-trimethoxyphenol-*β*-D-glucopyrinoside (**4**),[16] lasianthionoside A (**5**),[17] 3,7-dimethyl-1-octene-3,6,7-triol-6-*O*-*β*-D-glucopyranoside (**6**),[18] syringin (**7**),[19] and austroside B (**8**),[20] which were isolated for the first time from *B. senegalensis*. Their chemical structures (Fig. 1) were elucidated based on 1D and 2D NMR spectra, MS data, and by comparisons with previously published data acquired from similar compounds (see Supplementary Information). The inhibitory activity of the isolated compounds (**1-8**) on soluble epoxide hydrolase (sEH) was assessed.

Compound **1** was obtained from water fractions as a yellow amorphous powder, mp (183-185 ºC), $[\alpha]_D^{25}$ = –24.58 (c 0.1, MeOH), UV λ_{max} (MeOH) nm: 269.0, 326.0, with a molecular formula of $C_{32}H_{30}O_{14}$ determined based on a peak in the MicroQ-TOF III mass spectrum (ESI-Q-TOF MS) at *m/z* 661.1538 $[M + Na]^+$ (calcd for $C_{32}H_{30}O_{14}Na$, 661.1533). FT-IR (KBr) absorbance peaks were observed at ν_{max} 3421.4, 1654.4, 1253.4, 1213.2, and 1164.8 cm^{-1}. The ^1H-NMR spectrum confirmed many of the above features and revealed a set of rhamnocitrin signals; namely, a feruloyl group and a glucopyranose moiety. The presence of rhamnocitrin was suggested based on the following signals: two doublets at δ_H 6.20 (d, J = 2.0 Hz, H-6) and 6.53 (d, J = 2.0 Hz, H-8); A_2B_2-type aromatic proton signals were observed at δ_H 7.97 (2H, d, J = 8.8 Hz, H-2',6') and 6.84 (2H, d, J = 8.8 Hz, H-3',5'); a methoxyl group singlet at δ_H 3.70 (3H, s, 7-OMe) and its HMBC correlation with δ_C 166.02 (C-7). ABX-type aromatic proton signals at δ_H 6.77 (d, J = 8.2 Hz, H-5‴),

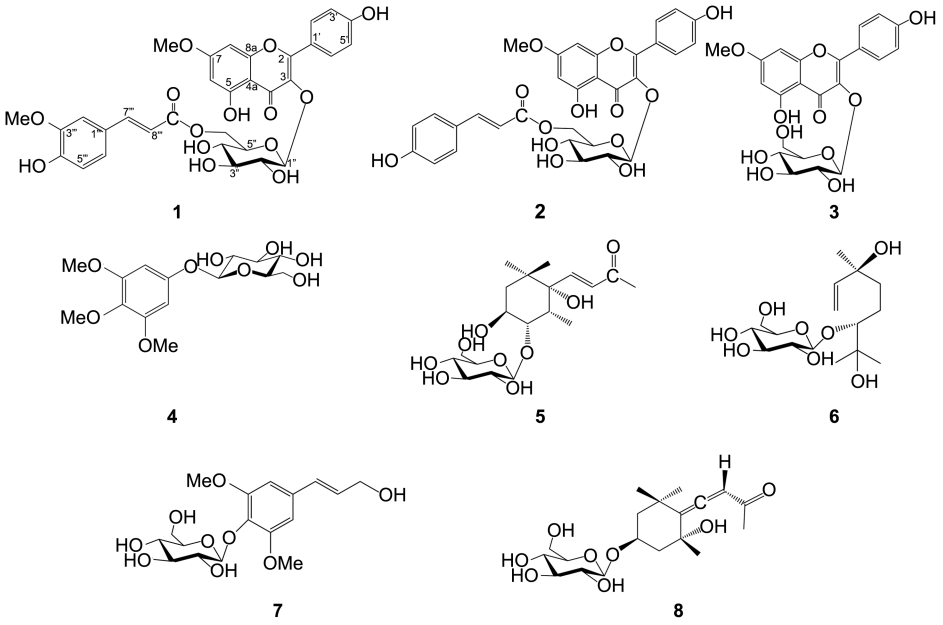

Figure 1. Chemical structures of isolated compounds **1-8**.

Table 1. The ^{1}H and ^{13}C NMR data for **1** in DMSO-d_6 (δ in ppm)

Position	δ_H[a] (H, mult, J in Hz)	δ_C[b]
2		157.83
3		134.21
4		178.56
4a		105.84
5		161.79
6	6.20 (1H, d, J = 2.0 Hz)	98.77
7		166.02
8	6.53 (1H, d, J = 2.0 Hz)	93.21
8a		157.24
1'		121.74
2', 6'	7.97 (2H, d, J = 8.8 Hz)	131.92
3', 5'	6.84 (2H, d, J = 8.8 Hz)	116.13
4'		161.10
1"	5.43 (H, d, J = 8.0 Hz)	101.80
2"	3.19 (m)	75.14
3"	3.25 (m)	77.27
4"	3.13 (m)	71.19
5"	3.36 (m)	75.30
6"	4.18 (H, dd, J = 11.9, 2.0 Hz) 4.11 (H, dd, J = 11.9, 6.9 Hz)	63.92
1‴		126.48
2‴	7.15 (H, d, J = 1.8 Hz)	111.89
3‴		148.84
4‴		150.30
5‴	6.77 (H, d, J = 8.2 Hz)	116.65
6‴	6.90 (H, dd, J = 8.2, 1.8 Hz)	124.07
7‴	7.27 (H, d, J = 16.0 Hz)	145.89
8‴	6.17 (H, d, J = 16.0 Hz)	114.82
9‴		167.18
OMe (7)	3.70 (3H, s)	56.90
OMe (3‴)	3.76 (3H, s)	56.63

Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments. [a]Measured at 600 MHz. [b]Measured at 150 MHz.

6.90 (dd, J = 8.2, 1.8 Hz, H-6‴) and 7.15 (d, J = 1.8 Hz, H-2‴) were observed, together with an additional HMBC crosspeak between δ_H 3.76 (3H, s, 3‴-OMe) and δ_C 148.84 (C-3‴). Two olefinic protons with a *trans* coupling constant (J = 16.0 Hz) at δ_H 6.17 (d, H-8‴) and 7.27 (d, H-7‴) confirmed the presence of an *α,β*-unsaturated carbonyl ester group. The feruloyl structure was then deduced from the HMBC correlation from δ_H 6.17 (H-8‴) to δ_C 126.48 (C-1‴) and the crosspeaks between H-2‴/C-7‴ and H-6‴/C-7‴. Detailed analyses of the ^{1}H- (δ_H 5.43, d, J = 8.0 Hz, H-1″) and ^{13}C-NMR (δ_C 101.80, 77.27, 75.30, 75.14, 71.19, and 63.92) suggested that glucopyranose was the sugar moiety (see Table 1). The coupling constant of the anomeric proton and its doublet multiplicity confirmed the β–configuration of the glucose moiety. The β-glucopyranosyl unit was positioned at C-3, which was in agreement with correlations observed between the anomeric proton H-1" (δ_H 5.43) and C-3 (δ_C 134.21) in the HMBC spectra (Fig. 2). According to previous reports, downfield shift of C-6″ was from δ_C 61.8 to 63.92 and an upfield shift of C-5″ was from δ_C 76.8 to 75.30, which were in accordance with acylation of the C-6″ of the glucose moiety.[12] Moreover, the downfield shift of H_2-6″ to 4.11 (dd, J = 6.9, 11.9 Hz) and 4.18 (dd, J = 2.0, 11.9 Hz) further confirmed the presence of a C-6″ feruloyl in compound **1**, which was in agreement with correlations observed between the proton H_2-6″ (δ_H 4.11, 4.18) and C-9‴ (δ_C 167.18) in the HMBC spectra (Fig. 2).[12] ^{1}H-^{1}H COSY spectra of **1** showed correlations between H-2′ (δ_H 7.97) and H-3′ (δ_H 6.84), H-5′ (δ_H 6.84) and H-6′ (δ_H 7.97), H-7‴ (δ_H 7.27) and H-8‴ (δ_H 6.17), and between H-5‴ (δ_H 6.77) and H-6‴ (δ_H 6.90) (Fig. 2). The presence of a D-glucose unit was further confirmed based on acid hydrolysis and gas chromatographic (GC) analyses followed by comparisons of retention times with authentic samples.[8] Based on the above data, compound **1** was established as rhamnocitrin-3-*O*-*β*-D-

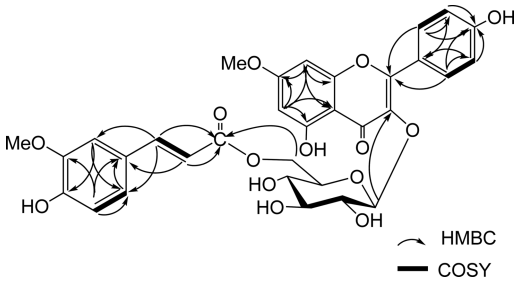

Figure 2. Selected HMBC and COSY correlation for **1**.

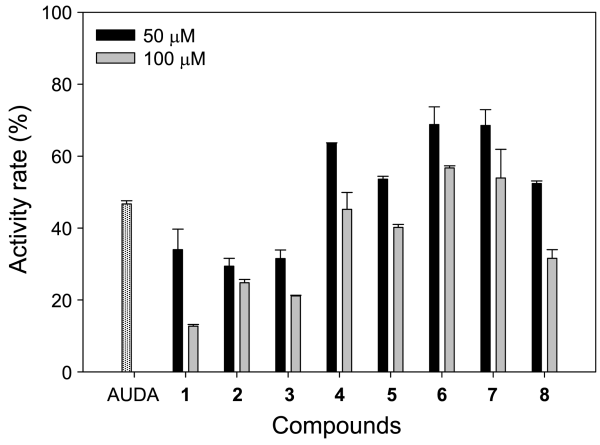

Figure 3. Inhibitory effect of the isolated compounds **1-8** on sEH activity determined using the fluorometric method. The sEH activity is expressed as the percentage of control activity. Values represent means ± SD from triplicate experiments (n = 3). (AUDA (12.5 nM): positive control).

(6"-*O*-*E*-feruloyl)-glucopyranoside, named bosenegaloside A (Fig. 1). The known compounds (**2-8**) corresponded to rhamnocitrin-3-*O*-*β*-D-(6"-*O*-*E*-*p*-coumaroyl)-glucopyranoside (**2**), rhamnocitrin-3-*O*-*β*-D-glucopyranoside (**3**), 3,4,5-trimethoxyphenol-*β*-D-glucopyrinoside (**4**), lasianthionoside A (**5**), 3,7-dimethyl-1-octene-3,6,7-triol-6-*O*-*β*-D-glucopyranoside (**6**), syringin (**7**), and austroside B (**8**) (Fig. 1). The structures of these compounds were identified based on comparison of the spectral data (^{1}H-NMR, ^{13}C-NMR) with those reported previously (see supporting information).

sEH inhibitory activity of isolated compounds **1-8** was evaluated *in vitro* using a fluorescent method based on hydrolysis of the specific substrate PHOME in the presence of sEH. AUDA [12-(3-adamantan-1-yl-ureido) dodecanoic acid] one of the most effective sEH inhibitors, was used as a positive control (IC_{50}: 7.2 ± 2.7 nM). The sEH inhibitory activity assessed at doses of 50 and 100 μM. At a concentration of 50 μM, isolated compounds showed sEH activity values ranging from 29.4% (**2**) to 68.8% (**6**) (Fig. 3). Compounds **1-3** inhibited sEH more than 50% and were assessed for further investigation. Effects of those compounds were examined at concentrations from 3.1 to 50 μM, and the 50% inhibitory concentration (IC_{50}) was calculated using a dose-dependent response curve (see Table 2 and Fig. 4).

Table 2. *In vitro* sEH inhibitory activity and kinetic study of compounds **1-3**

Compound	IC_{50}[a] (μM)	Binding mode (Ki[b], μM)
1	12.8 ± 0.5	Non-competitive (7.6 ± 3.2)
2	18.4 ± 0.2	Non-competitive (23.7 ± 1.5)
3	11.3 ± 0.9	Mixed (7.4 ± 2.5)
AUDA[c]	7.2 ± 2.7 nM	

[a]All compounds were examined in a set of experiments three times. [b]Inhibition constant. [c]Positive control

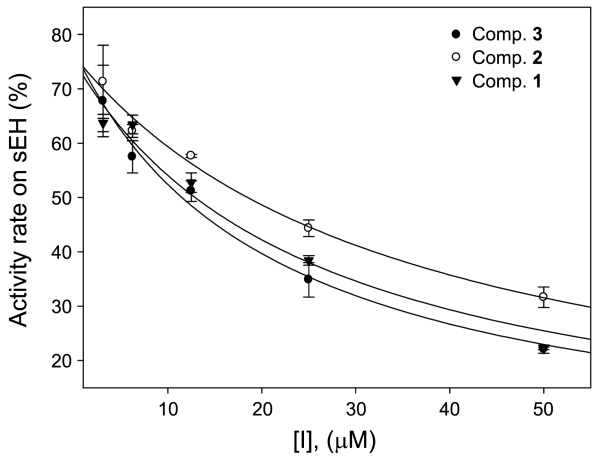

Figure 4. Effects of compounds **1-3** on the activity of sEH for hydroysis of substrate.

Among the 8 isolated compounds, flavonoid compounds **1-3** exhibited high sEH inhibitory activity; their IC_{50} values were 12.8 ± 0.5, 18.4 ± 0.2, and 11.3 ± 0.9 μM, respectively. This data agrees with the conclusion of previous work.[21]

We also characterized the inhibitory mechanism of the compounds **1-3** against sEH activity. The enzymatic activity was measured with a series of substrate concentrations and various inhibitor concentrations. The enzyme inhibition mechanisms of **1-3** were modeled using double-reciprocal plots (Lineweaver-Burk and Dixon plots). The Lineweaver–Burk plot (1/V *vs* 1/[S]) resulted in a series of straight lines passing through the same point on the *x*-axis, as illustrated for representative compounds **1** and **2** (Fig. 5(a) and (b), respectively), while compound **3** (Fig. 5(c)) resulted in a series of straight lines with the same *y*-axis intercept for the sEH inhibitors. In these kinetic plots, the abscissa 1/[S]) is the reciprocal of the concentrations of compounds, whereas the ordinate 1/V is the reciprocal of the change in intensity over time, thus representing a reciprocal of sEH activity. These findings indicate that compounds **1** and **2** exhibit non-competitive inhibition characteristics, whilst compound **3** showed mixed inhibition. The *Ki* values were calculated from the Dixon plots (Fig. 5(d)-(f)), which are useful for determining the *Ki* value of the inhibitor. From these kinetic plots, we calculated that the sEH inhibitors **1-3**, had inhibition constants (*Ki*) of 7.6 ± 3.2, 23.7 ± 1.5, and 7.4 ± 2.5 μM, respectively (see Table 2).

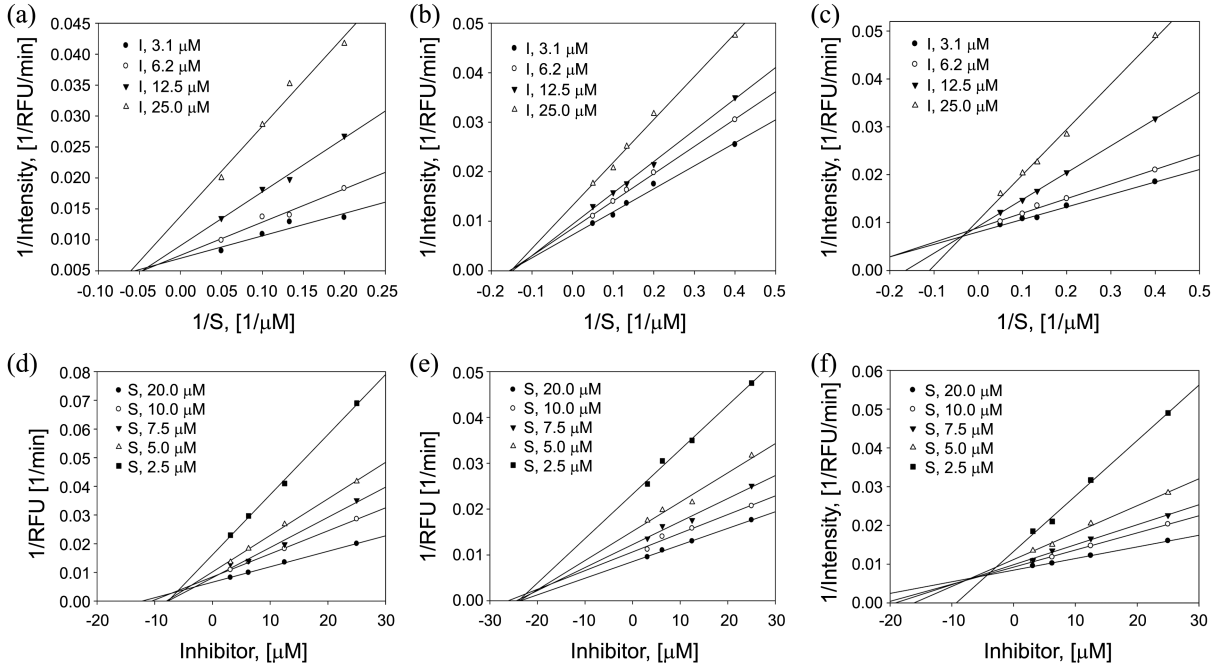

Figure 5. Graphical determination of the inhibition type for isolated compounds **1-3**. (**A-C**) Lineweaver-Burk plots for the inhibtion of compounds **1**, **2**, and **3** on sEH for the hydrolysis of substrate. (**D-F**) Dixon plots for the inhibition of compounds **1**, **2**, and **3** to determined *ki* value.

Conclusion

A phytochemical study of *Boscia senegalensis* leaves resulted in the isolation of 8 compounds, including one new flavonol glycoside (**1**), with seven known compounds. All of the aforementioned compounds were isolated for the first time from *B. senegalensis*. The inhibitory activity of the isolated compounds (**1-8**) on soluble epoxide hydrolase (sEH) was assessed. Among the 8 isolated compounds, flavonoid compounds **1-3** exhibited the most potent sEH inhibitory activity with IC_{50} values of 12.8 ± 0.5, 18.4 ± 0.2, and 11.3 ± 0.9 μM, respectively. In a study of inhibitory mechanism of compounds **1-3** against sEH activity, findings indicate that compounds **1** and **2** exhibit non-competitive inhibition characteristics, while compound **3** showed competitive inhibition, with inhibition constants (*Ki*) of 7.6 ± 3.2, 23.7 ± 1.5, and 7.4 ± 2.5 μM, respectively .

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