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Monoamine oxidases inhibitors from *Colvillea racemosa*: Isolation, biological evaluation, and computational study

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Abstract

Bioassay-guided fractionation and chemical investigation of *Colvillea racemosa* stems led to identification of two new α , β -dihydroxydihydrochalcones, colveol A (**1**) and colveol B (**2**) along with fifteen known compounds. The structures were elucidated via interpretation of spectroscopic data. The absolute configurations of the dihydrochalcones **1** and **2** were assigned by a combination of chemical modification and electronic circular dichroism data. The isolated compounds were evaluated for their inhibition activity toward recombinant human monoamine oxidases (rhMAO-A and -B). Compound **1** demonstrated preferential inhibition against hMAO-A isoenzyme (IC₅₀ 0.62 μ M, SI_{A/B} 0.02) while *S*-naringenin (**13**) and isoliquiritigenin (**15**) demonstrated preferential hMAO-B inhibition (IC₅₀ 0.27 and 0.51 μ M, SI_{A/B} 31.77 and 44.69, respectively). Fisetin (**11**) showed inhibition against hMAO-A with IC₅₀ value of 4.62 μ M and no inhibitory activity toward hMAO-B up to 100 μ M. Molecular docking studies for the most active compounds were conducted to demonstrate the putative binding modes. It suggested that **1** interacts with Gln215, Ala111, Phe352, and Phe208 amino acid residues which have a role in the orientation and stabilization of the inhibitor binding to hMAO-A, while *S*-naringenin (**13**) occupies both entrance and substrate cavities and interacts with Tyr326, a critical residue in inhibitor recognition in hMAO-B.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fitote.2017.11.009>.

Keywords

Colvillea; Dihydrochalcones; Colveol; Monoamine oxidase; Docking

1. Introduction

Monoamine oxidases (MAOs) are mitochondrial flavoenzymes that catalyze oxidative deamination of monoaminergic neurotransmitters and dietary monoamines [1]. In mammalian cells, gene coded biosynthesis results in two separate MAO isoforms. Serotonin, adrenaline, and noradrenaline are metabolized by MAO-A isoenzyme, while β -phenylethylamine and benzylamine are metabolized by MAO-B isoenzyme, whereas, dopamine and *p*-tyramine are common nonselective substrates for both isoenzymes. Hence the MAO-A isoform is a pharmacological target for the treatment of depression, anxiety, and major depressive disorders, while the MAO-B isoform is a target for the therapy of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [1]. A plethora of natural products have been reported to inhibit MAOs, such as flavonoids which exhibit desired pharmacological effects on central nervous system due to their potential to pass the blood brain barrier [2].

Colvillea racemosa

Bojer ex Hook (*Colvillea*'s glory tree, whip tree) belongs to a monotypic genus of the family Leguminosae and is native to Madagascar [3]. The plant is cultivated as an ornamental in some tropical countries and is used locally as a decorative shade tree. Its wood is used for construction purposes and its trunks are hollowed out to make canoes [4]. Previous chemical and biological studies on the plant are quite limited. The antibacterial potentials of the acetone extract of *C. racemosa* plant were reported [5], while 6-methoxy-7-hydroxy bis-coumarin has been reported from the plant seeds [6]. Our preliminary pharmacological evaluation revealed that the ethanol extract of *C. racemosa* stems displayed significant human MAO-A and -B (hMAO-A and -B) inhibition (Table 2). Therefore, a series of extraction, separation, and purification procedures were performed on *C. racemosa* stems, which resulted in the isolation and characterization of two new α,β -dihydroxydihydrochalcones (**1** and **2**) and fifteen known compounds, including lantibeside C (**3**) [7], lantibeside (**4**) [7], vicanin2 (**5**) [8], vitexin (**6**) [9], isovitexin (**7**) [10], *R*-liquiritigenin (**8**) [11,12], *R,R*-aromadendrin (**9**) [13,14], 2*S*-7,3',5'-trihydroxyflavanone (**10**) [12,15], fisetin (**11**) [16], genkwanin (**12**) [17], *S*-naringenin (**13**) [12,18], kaempferol (**14**) [19], isoliquiritigenin (**15**) [19–21], lup20(29)-ene (**16**) [22], and lupeol (**17**) [23] (Fig. 1). Herein, we describe the isolation, structural elucidation and hMAOs inhibition activity of the isolated compounds. The study was further extended to molecular docking analysis and determination of the putative mode of binding of the active compounds to hMAO-A and -B isoenzymes.

2. Results and discussion

A bioassay guided fractionation procedure of the EtOH extract of *C. racemosa* stems using various chromatographic techniques (see Experimental Section), afforded compounds **1–17**.

The structures of compounds **3–17** were established by comparison of their observed and reported data (S1, Supplementary data). Compound **1**, obtained as yellow amorphous powder, was assigned the molecular formula $C_{15}H_{14}O_6$ based on HRESIMS data m/z 273.0762 $[M + H - H_2O]^+$, calcd for $C_{15}H_{13}O_5$, 273.0763. The 1H and ^{13}C NMR spectroscopic data (Table 1) of **1** showed two doublets for two aliphatic oxygenated methines of AB spin system at δ_H 4.51 and 4.99 ($J_{AB} = 11.8$ Hz) and a carbonyl carbon at δ_C 194.4, suggesting the presence of a $-COCH(OH)CH(OH)-$ subunit [24], which confirmed by HMBC 3J correlations between δ_H 4.99 (H- β) and δ_C 194.4 (CO) and 2J correlations between δ_H 4.51 (H- α) and both δ_C 194.4 (CO) and δ_C 85.4 (C- β) and between δ_H 4.99 (H- β) and δ_C 74.5 (C- α), whereas the ABX spin system of three mutually coupled aromatic protons at δ_H 6.31 (1H, d, $J = 2.0$ Hz, H-3'), δ_H 6.52 (1H, dd, $J = 2.0, 8.7$ Hz, H-5'), and δ_H 7.72 (1H, d, $J = 8.7$ Hz, H-6') and signals of two oxygenated sp^2 -hybridized carbons at δ_C 165.2 (C-2') and δ_C 167.6 (C-4') indicate a dioxygenated A-ring, where the coupling pattern is compatible with a 2',4'-dioxygenation of this ring and this is corroborated by the HMBC cross peak pattern of H-6' at δ_H 7.72 with the carbonyl carbon at δ_C 194.4, and the oxygenated carbons at δ_C 165.2 (C-2') and δ_C 167.6 (C-4') (Table 1). The AA'XX' spin system [δ_H 6.84 (2H, d, $J = 8.5$ Hz, H-3/H-5) and δ_H 7.37 (2H, d, $J = 8.5$ Hz, H-2/H-6)] of the B-ring and oxygenated sp^2 -hybridized carbon at δ_C 159.1 (C-4) indicate 1,4-disubstitution. The HMBC spectrum of **1** showed 3J correlations between the aromatic protons H-2/6 (δ_H 7.37) and C- β (δ_C 85.4) along with a cross peak between H- β (δ_H 4.99) and C-2/6 (δ_C 130.4) confirming the connection of B-ring to C- β . The 1H and ^{13}C NMR spectroscopic data of **1** were similar to those of rhusopolyphenol E reported in the literature [25], but with different chiroptical data where the ECD spectrum of compound **1** exhibited negative Cotton effects at λ (θ) 219 (-2.36×10^3), 252 (-0.89×10^3), 271 (-0.62×10^3) nm. In order to determine the absolute configuration of **1**, it was converted to the corresponding flavanol using a Mitsunobu cyclization (Scheme 1) [26,27] which was identified as 3,7,4'-trihydroxyflavanone (garbanzol) by its NMR spectrum [28]. It has been reported previously that the six-membered flavan-3-ols were the only products of Mitsunobu cyclization and the theoretically possible formation of five-membered rings was not observed under the used conditions [27]. The assignment of the absolute configuration of garbanzol proceeded in two steps. Firstly, identification of the relative configuration of the two stereogenic centers C-2 and C-3 which was assigned as *trans* from NMR vicinal coupling constant $J_{2,3} = 11.8$ Hz [29]. The thermodynamically more stable conformation for the *trans*-isomers is when H-2 and H-3 are diaxial and hence the absolute configuration should be (2*R*, 3*R*) or (2*S*, 3*S*). The next step is using ECD data to determine the absolute configuration at C-2 where it showed sequential positive and negative Cotton effects at 338 and 297 nm for the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions, respectively [30]. These observed Cotton effects are reminiscent of dihydroflavonol exhibiting *P*-helicity of the hetero-ring with a C-2 equatorial B-ring, and, hence, (2*R*, 3*R*) absolute configuration [12,30]. Thus, the absolute configuration of **1** was inferred as $\alpha R, \beta S$ in regard to the absolute configuration of garbanzol since an alteration in the configuration at C-2 occurs during the S_N2 -type Mitsunobu reaction [26,27], therefore the structure of **1** was established as ($\alpha R, \beta S$)- $\alpha, \beta, 4, 2', 4'$ -pentahydroxydihydrochalcone and assigned the name "colveol A".

Compound **2**, obtained as yellow amorphous powder, was assigned the molecular formula $C_{15}H_{14}O_7$ based on HRESIMS data m/z 289.0717 $[M + H - H_2O]^+$, calcd for $C_{15}H_{13}O_6$, 289.0712. 1H and ^{13}C NMR spectroscopic data (Table 1) were similar to those of **1**, except for the different substitution pattern of the B-ring in **2**, which showed ABX system at δ_H 6.80 (1H, d, J = 8.0 Hz, H-5), δ_H 6.85 (1H, dd, J = 1.6, 8.0 Hz, H-6), and δ_H 6.99 (1H, d, J = 1.6 Hz, H-2) attributed to the disubstituted B-ring. The absolute configuration of **2** was the same as that of **1** by comparing its coupling constants, specific rotation, and ECD data λ (θ) 225 (-13.9×10^3), 251 (-7.12×10^3), 277 (-3.2×10^3) nm with those of **1** (θ) 219 (-2.36×10^3), 252 (-0.89×10^3), 271 (-0.62×10^3) nm. Consequently, compound **2** was established as (*αR,βS*)-*α,β*,3,4,2',4'-hexahydroxydihydrochalcone and assigned the name "colveol B".

Inhibitory activity and selectivity toward the A and B isoforms of hMAO were evaluated for the isolated compounds; IC_{50} values are shown in Table 2. Compound **1** emerged as a selective inhibitor of hMAO-A with an IC_{50} value of 0.62 μM , while inhibiting hMAO-B moderately (IC_{50} = 29.90 μM) with a selectivity index ($SI_{A/B}$) value of 0.02 (Table 2), while isoliquiritigenin (**15**) was found to inhibit hMAO-B more selectively with an IC_{50} value of 0.51 μM with moderate inhibition of hMAO-A (IC_{50} = 22.66 μM , $SI_{A/B}$ = 44.69). Similarly, *S*-naringenin (**13**) also inhibited hMAO-B more selectively with IC_{50} value of 0.272 μM ($SI_{A/B}$ = 31.77). Interestingly, *S*-naringenin has been previously reported to inhibit rat MAO-A and -B with IC_{50} values of 955 and 288 μM , respectively [18], which confirms the species-dependent differences of MAOs isozymes either in inhibitor specificity or its selectivity [31], and raises the uncertainty about the capability of the results of rat enzyme assays to be extrapolated for human clinical use. Fisetin (**11**) showed hMAO-A inhibition with IC_{50} value of 4.62 μM and no inhibitory activity toward hMAO-B up to 100 μM . Compounds **6–10** showed inhibitory activity toward hMAO-A with IC_{50} values of 38.65, 25.85, 9.97, 14.58 and 13.64 μM , respectively and hMAO-B with IC_{50} values of 34.15, 23.19, 3.91, 68.08 and 12.13 μM , respectively.

In an attempt to rationalize the previous results and get insight into possible binding modes of the most active compounds within hMAO-A and -B active sites, molecular docking study was accomplished. Both hMAOs consist of a flavin adenine dinucleotide (FAD) binding domain conserved among other flavoprotein oxidases, a substrate-binding domain, and a membrane-binding domain. The substrate binding sites of hMAO-A and -B are both mostly hydrophobic, enclosed by aromatic and aliphatic residues [32]. Tyr407 and Tyr444 in hMAO-A (Tyr398 and Tyr435 in hMAO-B) are situated on opposite directions of the covalently bound inhibitors, these tyrosines have been defined as aromatic cage and suggested to activate the inhibitor by enhancing its nucleophilicity [32]. Molecular docking simulation of compound **1** into hMAO-A active site revealed several interactions considered to be responsible for the activity (Fig. 2A, Fig. S90, Supplementary data); four hydrogen bond interactions were observed, one of them between the carbonyl oxygen of the ligand and amino group of Gln215, which has been reported to have a critical role in the orientation and stabilization of the inhibitor binding [33], two hydrogen bonds between C=O of Ala111 and both the hydroxyl at C-4' and hydrogen at C-3' in the ligand, in addition to a hydrogen bond interaction between C-3 of the ligand and C₄=O of FAD. A π - π stacking is established between A-ring and Phe352 and between B-ring and Phe208. Lack of Hydrogen bonding

with Gln215, and π - π stacking with Phe352 may contribute to the reduced inhibition activity of fisetin (**11**) ($IC_{50} = 4.62 \mu M$) than compound **1** ($IC_{50} = 0.62 \mu M$) (Fig. 2B, Fig. S90, Supplementary data). The active site of hMAO-B consists of two separate but jointly operating subdomains, entrance cavity ($\sim 290 \text{ \AA}^3$) and substrate cavity ($\sim 420 \text{ \AA}^3$), the residues Phe168, Leu171, Ile199, and Tyr326 act as “internal gate” between the two cavities [33,34]. Small inhibitors induce Ile199 to rotate into a closed conformation, leading to a dual cavity active site, while bulky inhibitors force Ile199 to the open conformation and fusing the two cavities into one ($\sim 700 \text{ \AA}^3$) [34]. *S*-Naringenin (**13**) and isoliquiritigenin (**15**) appeared to properly occupy both entrance and substrate pockets (Fig. 2C and D, Fig. S90, Supplementary data) which influence the selectivity to hMAO-B than hMAO-A [35], in addition, *S*-naringenin showed a π - π stacking between A-ring and Tyr326; a critical residue in inhibitor recognition [36] and one of the residues comprising the gate between the two cavities, the opening of this gate enables the inhibitor to fill both cavities and exert higher selectivity toward hMAO-B [34]. In contrast, docking simulation of compound **1** into active site of hMAO-B revealed intramolecular hydrogen bonds between carbonyl oxygen and both H-2 and OH- β of the ligand, which appear to be unfavorable for the activity as it keeps the ligand in the entrance cavity without reaching the recognition area. On the other hand no hydrogen bonding or π - π interactions were observed between fisetin (**11**) and hMAO-B residues.

3. Experimental section

3.1. General experimental procedures

Optical rotations were recorded at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV/vis spectrometer, and ECD data were collected using an Olis Cary-17 spectrophotometer (1 cm path length cell). NMR spectra were acquired on a Bruker 400 MHz NMR spectrometer at 400 (1H) and 100 MHz (^{13}C) and a Bruker 500 MHz NMR spectrometer at 500 (1H) and 125 MHz (^{13}C), using the residual solvent as an internal standard. Multiplicity determinations (DEPT) and 2D-NMR spectra (HMQC, HMBC, NOESY) were obtained using standard Bruker pulse programs. HRESIMS data were acquired by direct injection using a Bruker Bioapex-FTMS with electrospray ionization (ESI). TLC was conducted on precoated silica gel 60F254, 0.25 mm and reversed phase (RP) C₁₈ F254 silica gel (EMD Chemicals Inc., Darmstadt, Germany). Column chromatography was carried out on silica gel G60 (60–120 mesh, Merck, Darmstadt, Germany) and sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). Solid phase extraction (SPE) cartridges C₁₈ (Supelco Inc., Bellefonte, PA, USA) were used in the fractionation experiments. The compounds were visualized by spraying the TLC plates with 1% vanillin–H₂SO₄ spray reagent.

3.2. Plant material

The stems of *Colvillea racemosa* were collected in Alexandria, Egypt in October 2010. The sample was identified by Dr. Abdel Halim Mohamed, Flora and Phytotaxonomy Department, Agricultural Research Center, Cairo, Egypt and a voucher specimen (#

BUPD-58) was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

3.3. Extraction and isolation

Air-dried and pulverized stems (1.2 Kg) were exhaustively extracted using 70% EtOH (3×5 L) by maceration at room temperature. The combined extract was filtered and evaporated under reduced pressure (40 g). A portion of the crude extract (30 g) was subjected to VLC fractionation (RP-C₁₈) starting with 100% H₂O with an increasing gradient of MeOH in H₂O as a mobile phase to afford 12 fractions. Fraction 3 (2.5 g, eluted with 20% MeOH in H₂O) was chromatographed on Sephadex LH-20 CC using MeOH-H₂O (1:1) as eluent. Sub fraction 3-A (180 mg) was further chromatographed on silica gel CC using EtOAc-CH₂Cl₂-MeOH-H₂O mixture (15:8:4:1) as eluent to yield two compounds **3** (10 mg) and **4** (20 mg). Fraction 4 (700 mg, eluted with 20% MeOH in H₂O) was chromatographed on Sephadex LH-20 CC using 10% MeOH in H₂O as eluent to afford compound **5** (10 mg). Fraction **5** (760 mg, eluted with 30% MeOH in H₂O) was subjected to sephadex LH-20 CC using 10% MeOH in H₂O as mobile phase, sub fraction 5E (91 mg) was purified on silica gel CC using mixtures of CH₂Cl₂-MeOH as eluent in the order of increasing polarities to afford compound **1** (3 mg), sub fraction 5F (27 mg) was subjected to silica gel CC eluted with mixtures of CH₂Cl₂-MeOH in the order of increasing polarities to afford compound **2** (0.8 mg). Fraction 6 (1.2 g, eluted with 40% MeOH in H₂O) was passed through sephadex LH-20 CC and eluted with 10% MeOH in H₂O, sub fraction 6B (57 mg) was chromatographed on silica gel CC using EtOAc-CH₂Cl₂-MeOH-H₂O mixture (15:8:2:0.5) as mobile phase to afford two compounds **6** (3 mg) and **7** (12 mg), sub fraction 6C (45 mg) was subjected to silica gel CC, eluted using gradient of CH₂Cl₂-MeOH to afford compound **8** (3 mg), sub fraction 6D (62 mg) was purified on silica gel CC using gradient of CH₂Cl₂-MeOH as eluent in the order of increasing polarities to yield two compounds **9** (15 mg) and **10** (8 mg). Sub fraction 6E (25 mg) was subjected to sephadex LH-20 CC and eluted with 30% acetone in MeOH to afford compound **11** (6 mg). Fraction 7 (700 mg, eluted with 60% MeOH in H₂O) was fractionated by applying on sephadex LH-20 CC using MeOH-CH₂Cl₂ (1:1) as mobile phase, sub fraction 7B (142 mg) was passed through solid phase extraction (SPE) cartridge (RP-C₁₈, 10 g) using a gradient elution of H₂O-MeOH (0–100%), sub fraction 7B1 (100 mg, eluted with 20% MeOH in H₂O) was chromatographed on silica gel CC using a gradient elution of CH₂Cl₂-MeOH to afford compound **12** (2 mg), sub fraction 7C (40 mg) was chromatographed on silica gel CC using mixtures of CH₂Cl₂-MeOH as eluent in the order of increasing polarities to afford compound **13** (2 mg), and sub fraction 7D (30 mg) was purified on silica gel CC using gradient of CH₂Cl₂-MeOH as eluent to yield compound **14** (1.5 mg). Fraction 8 (450 mg, eluted with 60% MeOH in H₂O) was chromatographed on silica gel CC using a gradient elution of *n*-hexane-acetone (10–100%), sub fraction 8A (18.5 mg, eluted with 30% acetone in *n*-hexane) was purified on silica gel CC using CH₂Cl₂-MeOH, 9:1 as a mobile phase to afford compound **15** (1.5 mg). Fraction 12 (160 mg, eluted with 100% MeOH) was subjected to silica gel CC eluting with a gradient of *n*-hexane-CH₂Cl₂ (30–100%) to afford two compounds **16** (1 mg) and **17** (15 mg).

Colveol A (**1**): yellow amorphous powder; $[\alpha]_D^{25}$: +10.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log *e*) 311 (2.7), 370 (1.9) nm; ECD (MeOH) λ (θ) 219 (-2.36×10^3), 252 (-0.89×10^3),

271 (-0.62×10^3) nm; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 273.0762 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_{15}\text{H}_{13}\text{O}_5$, 273.0763).

Colveol B (**2**): yellow amorphous powder; $[\alpha]_{\text{D}}$: +12.5 (c 0.1, MeOH), ECD (MeOH) λ (θ) 225 (-13.9×10^3), 251 (-7.12×10^3), 277 (-3.2×10^3); ^1H and ^{13}C NMR data, Table 1; HRESIMS m/z 289.0717 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_{15}\text{H}_{13}\text{O}_6$, 289.0712).

3.4. General procedure of Mitsunobu reaction

A solution of compound **1** (0.005 mmol) in anhydrous THF (0.05 mL) was added to Ph_3P (2 mg, 0.008 mmol). The mixture was stirred for 15 min, then, diethyl azodicarboxylate (DEAD) (1.5 μL , 0.008 mmol) was added drop wise to the mixture. After stirring for 3 h at room temperature and monitoring of the reaction by TLC, the reaction mixture was diluted with EtOAc (5 mL) and washed with H_2O (5 mL) and brine (4 mL). The organic phase was then dried over MgSO_4 and the solvent was then removed in vacuo. The residue was chromatographed by silica gel column using the mobile phase (*n*-hexane–EtOAc, 3:1) to give the cyclized product as amorphous solid.

Garbanzaol: white amorphous powder, $[\alpha]_{\text{D}}$: +32.0 (c 0.1, MeOH); ECD (MeOH) λ (θ) 261 ($+1.63 \times 10^3$), 297 (-1.36×10^3), 343 ($+0.64 \times 10^3$) nm; ^1H NMR (500 MHz, Methanol- d_4) δ 7.80 (1H, d, J = 8.8 Hz, H-5), 7.40 (2H, d, J = 8.4 Hz, H-2' and H-6'), 6.86 (2H, d, J = 8.5 Hz, H-3' and H-5'), 6.68 (1H, dd, J = 8.8, 2.4 Hz, H-6), 6.52 (1H, d, J = 2.3 Hz, H-8), 5.06 (1H, d, J = 11.9 Hz, H-2), 4.58 (1H, d, J = 11.9 Hz, H-3).

3.5. Determination of absolute configuration of sugar moieties of compounds 3 and 4

Compound **3** (1 mg) and **4** (1 mg) were separately hydrolyzed with 2 M HCl (200 μL) at 90 °C for 2 h, then each reaction mixture was neutralized with 9 M NH_4OH and dried under N_2 gas. Each residue was stirred with L-cysteine methyl ester solution (0.3 mmol/mL) in pyridine and incubated at 90 °C for 1 h, followed by addition of phenyl isothiocyanate solution in pyridine (0.69 mmol/mL; 160 μL) and heating for another hour., the final mixture was analyzed by UPLC-MS using a gradient elution of 0.05% formic acid in H_2O (A) and acetonitrile/methanol/isopropanol (50:25:25, v/v) - 0.05% formic acid (B), PDA detection wavelength of 254 nm and ESI-MS in the positive mode according to the protocol described by Wang et al. [37]. Acid hydrolysis of compounds **3** and **4**, followed by chiral derivatization, permitted the identification of D-(+)-glucose and D-(+)-xylose as the two sugar units of each compound based on comparisons with authentic samples' retention times and mass spectra.

3.6. MAO inhibition assay

In order to investigate the inhibition effect of the extract, fractions and isolated compounds from *C. racemosa* on recombinant human MAO-A and MAO-B, the kynuramine deamination assay was performed in 384-well plates as previously reported [38]. Human monoamine oxidase-A and -B enzymes were obtained from BD Biosciences (Bedford, MA, USA). Kynuramine, harmine, clorgyline, deprenyl, and DMSO were purchased from Sigma Chemical (St. Louis, MO, USA). A fixed concentration of kynuramine (80 μM for MAO-A and 50 μM for MAO-B) and different concentrations of the test compounds were used to

determine the IC₅₀ values. The concentrations run for extract/fractions were from 0.001 to 100 µg/mL and for isolated pure compounds were from 0.001 to 100 µM. The inhibitors and compounds were dissolved in DMSO, diluted in the buffer solution, and pre-incubated at 37 °C for 10 min (1.0% of DMSO final). Reactions were initiated by the addition of 18.75 µL of MAO-A (to 5 µg/mL) and -B (to 12.5 µg/mL), incubated for 20 min at 37 °C. The enzyme reactions were terminated by the addition of 28 µL of 2 N NaOH. The enzyme product formation (4-hydroxyquinoline) was recorded fluorometrically using a Spectra-Max M5 fluorescence plate reader (Molecular Devices, Sunnyvale, CA, USA) with an excitation (320 nm) and emission (380 nm) wavelength, using the Soft Max Pro program. The assays were calculated as percent of product formation compared to the corresponding control (enzyme–substrate reaction) without inhibitors. Control assays including samples, where the enzyme or the substrate was added after stopping the reaction were setup simultaneously to determine the interference with the fluorescence measurements and the backgrounds. The determination of IC₅₀ values for MAO-A and -B inhibition by the selected samples was performed using varying concentrations of the inhibitor and fixed concentration of the substrate. The IC₅₀ values were calculated from the concentration-dependent inhibition curves using XLFit software.

3.7. Molecular modeling study

The crystal structures of human MAO-A (PDB ID: 2Z5Y) [39] and MAO-B (PDB ID: 2V61) [40] were downloaded from the protein databank repository [41]. Protein structures were prepared by Protein Preparation Wizard (PrepWizard) [42,43] of the Schrödinger suite for adding missing hydrogen atoms, assigning hydrogen bonds, adjusting bond orders, and relaxing the protein – ligand complex. Filling the missing loops and amino acid side chains were accomplished using Prime [44–46]. The center of the docking receptor grid was determined as the coordinate of cognate ligand. The potential of nonpolar parts of the receptor was softened by scaling the van der Waals radius by a factor of 1.0 and partial charge cutoff of 0.25. Rotation of the receptor thiol and hydroxyl groups was allowed for optimal hydrogen bond formation with predicted docked poses. Ligand preparation was performed using LigPrep [47] of Schrödinger by using the OPLS3 force field [48]. Possible ligand states at pH 7.4 were generated by Epik [49], and ligand stereogenic centers were retained during ligand preparation. Then, the compounds were docked using Glide [50] SP (standard precision) mode, and the best five poses were kept for analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Carradori S, Gidaro MC, Petzer A, Costa G, Guglielmi P, Chimenti P, Alcaro S, Petzer JP, Inhibition of human monoamine oxidase: biological and molecular modeling studies on selected natural flavonoids, *J. Agric. Food Chem* 64 (2016) 9004–9011. [PubMed: 27933876]
- [2]. Orhan IE, Potential of natural products of herbal origin as monoamine oxidase inhibitors, *Curr. Pharm. Des* 22 (2016) 268–276. [PubMed: 26561069]
- [3]. Tropical legumes, Resources for the Future, The National Academies Press, Washington, DC, 1979, p. 345.
- [4]. Du Puy D, K. Royal Botanic Gardens, The Leguminosae of Madagascar, Royal Botanic Gardens, Kew, 2002.
- [5]. Ravikumar A, Rathinam KMS, Antibacterial activity of hexane and acetone extracts of *Peltophorum pterocarpum*, *Calvillea racemosa* and *Bauhinia purpurea*, *Int. J. Chem. Sci* 7 (2009) 1751–1756.
- [6]. Sreenath D, Rao JT, A new bis-coumarin from the seeds of *Colvillea racemosa*, *Asian J. Chem* 12 (2000) 917–918.
- [7]. Li T, Hao XJ, Gu QQ, Zhu WM, Minor furofurano lignans from the Tibetan herb, *Lancea tibetica*, *Planta Med.* 74 (2008) 1391–1396. [PubMed: 18666045]
- [8]. Sato S, Akiya T, Nishizawa H, Suzuki T, Total synthesis of three naturally occurring 6,8-di-*C*-glycosylflavonoids: phloretin, naringenin, and apigenin bis-*C*- β -D-glucosides, *Carbohydr. Res* 341 (2006) 964–970. [PubMed: 16545348]
- [9]. Kim JH, Lee BC, Kim JH, Sim GS, Lee DH, Lee KE, Yun YP, Pyo HB, The isolation and antioxidative effects of vitexin from *Acer palmatum*, *Arch. Pharm. Res* 28 (2005) 195–202. [PubMed: 15789751]
- [10]. Peng J, Fan G, Hong Z, Chai Y, Wu Y, Preparative separation of isovitexin and isoorientin from *Patrinia villosa* Juss by high-speed counter-current chromatography, *J. Chromatogr. A* 1074 (2005) 111–115. [PubMed: 15941046]
- [11]. Kulesh NI, Vasilevskaya NA, Veselova MV, Denisenko VA, Fedoreev SA, Minor polyphenols from *Maackia amurensis* wood, *Chem. Nat. Compd* 44 (2008) 712–714.
- [12]. Zaki MA, Nanayakkara NP, Hetta MH, Jacob MR, Khan SI, Mohammed R, Ibrahim MA, Samoylenko V, Coleman C, Fronczek FR, Ferreira D, Muhammad I, Bioactive formylated flavonoids from *Eugenia rigida*: isolation, synthesis, and X-ray crystallography, *J. Nat. Prod* 79 (2016) 2341–2349. [PubMed: 27618204]
- [13]. Jeon SH, Chun W, Choi YJ, Kwon YS, Cytotoxic constituents from the bark of *Salix hulteni*, *Arch. Pharm. Res* 31 (2008) 978–982. [PubMed: 18787784]
- [14]. Takahashi H, Li S, Harigaya Y, Onda M, Heterocycles. Xxii.: stereoselective synthesis of (+)-aromadendrin trimethyl ether and its enantiomer, and their reduction, *Chem. Pharm. Bull* 36 (1988) 1877–1881.
- [15]. Shi J, Chen B, Sun ZH, Hu CQ, Studies on flavonoid constituents of *Caragana intermediia*, *Acta Pharmacol. Sin* 38 (2003) 599–602.
- [16]. Lee S, Park Y, Moon B-H, Lee E, Hong S, Lim Y, Substitution effect of hydroxyl groups on the ^1H and ^{13}C chemical shifts in hydroxyflavonols, *Bull. Kor. Chem. Soc* 29 (2008) 1597–1600.
- [17]. Gomes RA, Ramirez RRA, Maciel J.K.d.S., Agra M.d.F., Souza M.d.F.V.d., Falcão-Silva VS, Siqueira-Junior JP, Phenolic compounds from *Sidastrum micranthum* (A. St.-Hil.) fryxell and evaluation of acacetin and 7,4'-Di-*O*-methylisoscuteallarein as motulator of bacterial drug resistance, *Quím. Nova* 34 (2011) 1385–1388.
- [18]. Olsen HT, Stafford GI, van Staden J, Christensen SB, Jager AK, Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L, *J. Ethnopharmacol* 117 (2008) 500–502. [PubMed: 18372132]
- [19]. Itoh T, Ninomiya M, Yasuda M, Koshikawa K, Deyashiki Y, Nozawa Y, Akao Y, Koketsu M, Inhibitory effects of flavonoids isolated from *Fragaria ananassa* Duch on IgE-mediated degranulation in rat basophilic leukemia RBL-2H3, *Bioorg. Med. Chem* 17 (2009) 5374–5379. [PubMed: 19596200]

- [20]. Namikoshi M, Nakata H, Nuno M, Ozawa T, Saitoh T, Homoisoflavonoids and related compounds. III. Phenolic constituents of *Caesalpinia japonica* sieb. Et zucc, Chem. Pharm. Bull 35 (1987) 3568–3575.
- [21]. Sugamoto K, Matsusita Y.-i., Matsui K, Kurogi C, Matsui T, Synthesis and antibacterial activity of chalcones bearing prenyl or geranyl groups from *Angelica keiskei*, Tetrahedron 67 (2011) 5346–5359.
- [22]. Wenkert E, Baddeley GV, Burfitt IR, Moreno LN, Carbon-13 nuclear magnetic resonance spectroscopy of naturally-occurring substances. LVII. Triterpenes related to lupane and hopane, Org. Magn. Reson 11 (1978) 337–343.
- [23]. Fotie J, Bohle DS, Leimanis ML, Georges E, Rukunga G, Nkengfack AE, Lupeol long-chain fatty acid esters with antimalarial activity from *Holarrhena floribunda*, J. Nat. Prod 69 (2006) 62–67. [PubMed: 16441070]
- [24]. Woldemichael GM, Gutierrez-Lugo MT, Franzblau SG, Wang Y, Suarez E, Timmermann BN, *Mycobacterium tuberculosis* growth inhibition by constituents of *Sapium haematospermum*, J. Nat. Prod 67 (2004) 598–603. [PubMed: 15104489]
- [25]. Kim KH, Moon E, Choi SU, Kim SY, Lee KR, Polyphenols from the bark of *Rhus verniciflua* and their biological evaluation on antitumor and anti-inflammatory activities, Phytochemistry 92 (2013) 113–121. [PubMed: 23752101]
- [26]. Jew S.-s., Kim H.-a., Bae S.-y., Kim J.-h., Park H.-g., Enantioselective synthetic method for 3-hydroxyflavanones: an approach to (2*R*,3*R*)-3',4'-O-dimethyltaxifolin, Tetrahedron Lett. 41 (2000) 7925–7928.
- [27]. Krohn K, Ahmed I, John M, Enantioselective synthesis of flavan-3-ols using a Mitsunobu cyclization, Synthesis 2009 (2009) 779–786.
- [28]. Lee T-H, Chiou J-L, Lee C-K, Kuo Y-H, Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. *roxburghiana*, J. Chin. Chem. Soc 52 (2005) 833–841.
- [29]. van Rensburg H, van Heerden PS, Bezuidenhout BCB, Ferreira D, Stereoselective synthesis of flavonoids. Part 4. *Trans*- and *cis*-dihydroflavonols, Tetrahedron 53 (1997) 14141–14152.
- [30]. Slade D, Ferreira D, Marais JP, Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids, Phytochemistry 66 (2005) 2177–2215. [PubMed: 16153414]
- [31]. Novaroli L, Daina A, Favre E, Bravo J, Carotti A, Leonetti F, Catto M, Carrupt PA, Reist M, Impact of species-dependent differences on screening, design, and development of MAO B inhibitors, J. Med. Chem 49 (2006) 6264–6272. [PubMed: 17034132]
- [32]. Gaweska H, Fitzpatrick PF, Structures and mechanism of the monoamine oxidase family, Biomol. Concepts 2 (2011) 365–377. [PubMed: 22022344]
- [33]. Fierro A, Montecinos A, Gomez-Molina C, Nunez G, Aldeco M, Edmondson DE, Vilches-Herrera M, Luhr S, Iturriaga-Vasquez P, Reyes-Parada M, An Integrated View of the Molecular Recognition and Toxinology - From Analytical Procedures to Biomedical Applications, In Tech, Rijeka, 2013, pp. 405–431 Chapter 16.
- [34]. Mladenovi M, Patsilinakos A, Pirolli A, Sabatino M, Ragno R, J. Chem. Inf. Model (2017), 10.1021/acs.jcim.6b00608.
- [35]. Binda C, Newton-Vinson P, Hubalek F, Edmondson DE, Mattevi A, Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders, Nat. Struct. Biol 9 (2002) 22–26. [PubMed: 11753429]
- [36]. Lee J, Lee Y, Park SJ, Lee J, Kim YS, Suh Y-G, Lee J, Discovery of highly selective and potent monoamine oxidase B inhibitors: contribution of additional phenyl rings introduced into 2-aryl-1,3,4-oxadiazin-5(6H)-one, Eur. J. Med. Chem 130 (2017) 365–378. [PubMed: 28273563]
- [37]. Wang YH, Avula B, Fu X, Wang M, Khan IA, Simultaneous determination of the absolute configuration of twelve monosaccharide enantiomers from natural products in a single injection by a UPLC-UV/MS method, Planta Med. 78 (2012) 834–837. [PubMed: 22532020]
- [38]. Samoylenko V, Rahman MM, Tekwani BL, Tripathi LM, Wang YH, Khan SI, Khan IA, Miller LS, Joshi VC, Muhammad I, Banisteriopsis caapi, a unique combination of MAO inhibitory and antioxidative constituents for the activities relevant to neurodegenerative disorders and Parkinson's disease, J. Ethnopharmacol 127 (2010) 357–367. [PubMed: 19879939]

- [39]. Son SY, Ma J, Kondou Y, Yoshimura M, Yamashita E, Tsukihara T, Structure of human monoamine oxidase A at 2.2 Å resolution: the control of opening the entry for substrates/inhibitors, *Proc. Natl. Acad. Sci. U. S. A* 105 (2008) 5739–5744. [PubMed: 18391214]
- [40]. Binda C, Wang J, Pisani L, Caccia C, Carotti A, Salvati P, Edmondson DE, Mattevi A, Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs, *J. Med. Chem* 50 (2007) 5848–5852. [PubMed: 17915852]
- [41]. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE, The protein data bank, *Nucleic Acids Res.* 28 (2000) 235–242. [PubMed: 10592235]
- [42]. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W, Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments, *J. Comput. Aided Mol. Des* 27 (2013) 221–234. [PubMed: 23579614]
- [43]. Schrödinger SS, Protein Preparation Wizard, Epik, Schrödinger, LLC, New York, NY, Impact, Schrödinger, LLC, New York; Prime; Schrödinger, LLC, New York, NY, 2015, p. 2017.
- [44]. Jacobson MP, Pincus DL, Rapp CS, Day TJF, Honig B, Shaw DE, Friesner RA, A hierarchical approach to all-atom protein loop prediction, *proteins: Struct., Funct, Bioinformatics* 55 (2004) 351–367.
- [45]. Jacobson MP, Friesner RA, Xiang Z, Honig B, On the role of the crystal environment in determining protein side-chain conformations, *J. Mol. Biol* 320 (2002) 597–608. [PubMed: 12096912]
- [46]. Prime, Schrödinger, LLC: New York, NY, 2017.
- [47]. LigPrep, Schrödinger, LLC: New York, NY, 2017.
- [48]. Harder E, Damm W, Maple J, Wu C, Reboul M, Xiang JY, Wang L, Lupyan D, Dahlgren MK, Knight JL, Kaus JW, Cerutti DS, Krilov G, Jorgensen WL, Abel R, Friesner RA, OPLS3: a force field providing broad coverage of drug-like small molecules and proteins, *J. Chem. Theory Comput* 12 (2016) 281–296. [PubMed: 26584231]
- [49]. Epik, Schrödinger, LLC, New York, NY, 2017.
- [50]. Glide, Schrödinger, LLC, New York, NY, 2017.

Fig. 1.
Chemical structures of isolated compounds (**1–17**) from *Colvillea racemosa* stems.

Fig. 2. Binding modes of compound **1** (A) and fisetin (**11**) (B) into MAO-A, and *S*-naringenin (**13**) (C) and isoliquiritigenin (**15**) (D) into MAO-B binding cavities. The Connolly channel surfaces of the cavities are displayed in gray. Ligands are depicted in ball and stick model. The residues potentially interacting with the ligands are shown in labelled tube models, FAD cofactor is represented as red tube model and water molecules are represented as wire model (red and white). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Scheme 1.

Cyclization of compound 1 to flavanone via Mitsunobu reaction.^a

^aReagents and conditions: DEAD, Ph₃P, THF, 3 h.

Table 1

^1H and ^{13}C NMR data for (*aR*,*βS*)-*α,β*,4,2',4'-pentahydroxydihydrochalcone (1) and (*aR*,*βS*)-*α,β*,3,4,2',4'-hexaahydroxydihydrochalcone (2).^a

No.	1			2	
	δ_{C} , type	δ_{H} (J in Hz)	HMBC ^b	δ_{C} , type	δ_{H} (J in Hz)
1	129.5, C	-	-	130.4, C	-
2	130.4, CH	7.37, d (8.5)	4,6, β	115.8, CH	6.99, d (1.6)
3	116.1, CH	6.84, d (8.5)	1, 4, 5	146.3, C	-
4	159.1, C	-	-	147.0, C	-
5	116.1, CH	6.84, d (8.5)	1, 3, 4	116.0, CH	6.80, d (8.0)
6	130.4, CH	7.37, d (8.5)	2,4, β	120.9, CH	6.85, dd (1.6,8.0)
α	74.5, CH	4.51, d (11.8)	1, β , CO	74.4, CH	4.42, d (11.6)
β	85.4, CH	4.99, d (11.8)	2, 6, α , CO	85.4, CH	4.94, d (11.9)
1'	113.1, C	-	-	114.8, C	-
2'	165.2, C	-	-	165.6, C	-
3'	103.8, CH	6.31, d (2.0)	1', 2', 4'	104.5, CH	6.16, d (2.1)
4'	167.6, C	-	-	165.6, C	-
5'	112.4, CH	6.52, dd(2.0,8.7)	1', 3'	110.9, CH	6.41, dd (2.2,8.9)
6'	130.0, CH	7.72, d (8.7)	2', 4', CO	129.8, CH	7.64, d (8.7)
C = O	194.4	-	-	193.8	-

^aSpectra run at 400 MHz, (^1H) and 100 MHz (^{13}C) in CD_3OD .

^bHMBC correlations are from proton (s) stated to the indicated carbon.

Table 2

Inhibition of recombinant human monoamine oxidase-A and -B by EtOH extract and compounds isolated from *Colvillea racemosa*.

Compound	Monoamine oxidase A	Monoamine oxidase B	SI _{A/B} index ^b
	IC ₅₀ ^a (μM)	IC ₅₀ ^a (μM)	
<i>Colvillea racemosa</i> (EtOH extract)	11.37 ± 0.13 ^c	8.77 ± 2.13 ^c	1.30
1	0.62 ± 0.03	29.90 ± 6.93	0.02
3	^d	^d	NA
4	^d	^d	NA
5	50.18 ± 0.80	43.29 ± 2.13	1.16
6	38.65 ± 0.87	34.15 ± 3.77	1.13
7	25.85 ± 0.19	23.19 ± 3.28	1.11
8	9.97 ± 2.63	3.91 ± 0.21	2.55
9	14.58 ± 0.01	68.08 ± 3.57	0.21
10	13.64 ± 0.50	12.13 ± 2.10	1.12
11	4.62 ± 0.15	^d	NA
13	8.6411 ± 0.582	0.272 ± 0.022	31.77
14	0.222 ± 0.020	6.718 ± 0.863	0.033
15	22.66 ± 1.84	0.51 ± 0.01	44.69
16	^d	^d	NA
Harmine	0.0039 ± 0.0001	37.500 ± 1.412	0.0001
Clorgyline	0.0053 ± 0.0005	2.1500 ± 0.212	0.0025
Deprenyl	33.500 ± 1.411	0.0447 ± 0.0023	749.44

^aThe IC₅₀ values computed from the dose-response inhibition curves are mean ± SD of triplicate observations.

^bSI_{A/B} selectivity index = IC₅₀ (hMAO-A) / IC₅₀ (hMAO-B).

^cμg/mL.

^dNot active at the highest concentration tested of 100 μM. NA Non applicable.