Three Tyrosinase Inhibitors and Antioxidant Compounds from *Salsola foetida*

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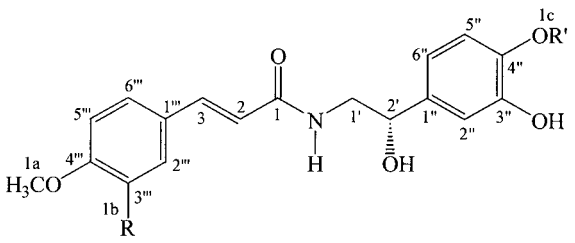
Phytochemical investigation on the whole plant of *Salsola foetida* resulted in the isolation of three new phenolic compounds **1**, **2**, and **3**, which exhibit tyrosinase inhibition with moderate antioxidant activity. Compounds **1**–**3** inhibited tyrosinase with IC_{50} 2.61, 1.85, and 0.40 μM, while exhibiting DPPH radical scavenging activity with IC_{50} 383, 427 and 378 μM, respectively. The structures of **1**, **2**, and **3** were determined by modern spectroscopic techniques.

Introduction. – Tyrosinase (E.C. 1.14.18.1) is a multifunctional Cu-containing enzyme widely distributed in plants and animals. It catalyzes the *o*-hydroxylation of monophenols and also the oxidation of *o*-diphenols to *o*-quinones. It is known to be a key enzyme for melanin biosynthesis in plants and animals. Therefore, tyrosinase inhibitors are clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. Moreover, these are also important in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms [1].

A number of diseases occur as a result of the damaging effects of free radicals. Free radicals can react with biochemicals such as nucleic acids, proteins, and cell membranes to cause structural damages and resulting malfunction of vital molecules. They are also involved in aging, cancer, and heart diseases [2]. To protect from the damaging effect of free radicals, all aerobic organisms possess a natural antioxidant defense system. Several studies have also suggested that balance intake of dietary antioxidants may also help in prevention of diseases [3].

In search of bioactive substances of plant origin [4][5], we have recently studied the chemical constituents of *Salsola foetida* LINN. (Chenopodiaceae). The aqueous and AcOEt extracts of the plant exhibited antioxidant and tyrosinase inhibitiory activities.

Salsola foetida LINN. is widely distributed in Punjab, northern Gujrat, Rajasthan, and Cholistan, where it is used to prepare *sajji,* a crude form of carbonate of soda. The plant is vermifuge, and its ashes are applied to itch [6]. Some alkaloids and phytosterols have earlier been idenified in *Salsola* genus [7][8]. Here, we report the isolation and characterization of three new compounds **1**, **2**, and **3** with tyrosinase inhibitory and antioxidant activities.



1 R = H, R' = H

2 R = MeO, R' = H

3 R = H, R' = Me

Results and Discussion. – The HR-EI-MS of compound **1** showed the M^+ signal at *m/z* 329.1264, corresponding to the molecular formula $C_{18}H_{19}NO_5$ (calc. 329.1263), which indicates ten degrees of unsaturation. The ion at *m/z* 311.1161 ($C_{18}H_{17}NO_4$) was due to the loss of H_2O from the molecular ion. The ions at *m/z* 294.1128 ($C_{18}H_{16}NO_3$) and 277.1110 ($C_{18}H_{15}NO_2$) appeared due to the successive loss of OH groups from the ion at *m/z* 311.1161. Furthermore, the ions at *m/z* 176.0714 ($C_{10}H_{10}NO_2$) and 145.0531 (C_9H_7NO) resulted from the subsequent loss of a phenethyl ($C_8H_7O_2$) moiety from the ion at *m/z* 311.1161 and MeO group from the ion at *m/z* 176.0714, respectively.

The UV spectrum of **1** exhibited absorptions at 327, 324, and 217 nm, which indicated the presence of a conjugate aromatic system [9][10]. The IR spectrum of **1** showed strong absorptions at 3363 (O–H), 2922 (C–H), 1652 (C=O), 1603, 1517 (aromatic C=C), and 1221 (C–O) cm^{-1}. The ^{1}H-NMR spectrum (*Table 1*) of **1** indicated the presence of two sets of signals for a total of seven aromatic H-atoms, two olefinic H-atoms, two CH_2 H-atoms, one methine H-atom, and H-atoms of one MeO group. This indicated the acrylamide nature of the compound in hand [8][9]. Signals in the aromatic region indicated the presence of two benzene rings, one disubstituted and the other trisubstituted. The aromatic H-atoms, which resonated at δ 7.12 (*d*, $J(2'',6'') = 1.3$, 1 H), 7.01 (*dd*, $J(6'',5'') = 8.5$, $J(6'',2'') = 1.3$, 1 H) and 6.84 (*d*, $J(5'',6'') = 8.5$, 1 H) were assigned to H–C(2″), H–C(6″), and H–C(5″), respectively. Another set of aromatic signals appeared as a pair of *ortho*-coupled doublets at δ 7.25 (H–C(2‴), H–C(6‴) and 6.78 (H–C(3‴), H–C(5‴)). A *singlet* integrating for 3 H at δ 3.87 was assigned to MeO–C(4‴). Two *AB* double doublets at δ 3.50 and 3.42 were assigned to the CH_2(1′) H-atoms, which showed vicinal couplings with H–C(2′) ($J(1'\beta, 2'\beta) = 7.7$, $J(1'\alpha,2'\beta) = 4.9$). The H–C(2′) geminal to the OH group appeared as a double *doublets* at δ 4.71 ($J(2'\beta,1'\beta) = 7.7$, $J(2'\beta,1'\alpha) = 4.9$). A pair of downfield *doublets* at δ 7.46 (H–C(3)) and 6.45 (H–C(2)) with a *J* value of 15.6 Hz each indicated the presence of a C=C bond with (*E*)-configuration [11][12]. The COSY-45° spectrum of **1** exhibited coupling between olefinic H–C(3) (δ 7.46) and H–C(2) (δ 6.45). The CH_2(1′) H-atoms resonating at δ 3.50 and 3.42 displayed a cross-peak with the H–C(2′), which resonated at δ 4.71. Couplings between the aromatic H–C(6″) (δ 7.01), H–C(5″) (δ 6.84), H–C(2‴)/H–C(6‴) (δ 7.25) and H–C(3‴)/H–C(5‴) (δ 6.78) were also observed.

The ^{13}C-NMR, BB, and DEPT spectra of **1** showed resonances for all 18 C-atoms comprising one CH_2, ten CH, one Me and six quaternary C-atoms. The downfield signals at δ 169.5 ((C(1)) indicated the presence of the amide C-atom, while signals at δ 142.2 (C(3)) and 118.7 (C(2)) indicated that one C=C bond is present in the molecule. The downfield signals at δ 158.1 (C(4‴)), 149.8 (C(3″), 149.3 (C(4′), 134.1 (C(1″)), 128.5 (C(2‴)/C(6‴)), 128.2 (C(1‴)), 123.3 (C(6″)), 116.5 (C(5″)), 116.1 (C(3‴)/C(5‴)) and 111.6 (C(2″)) suggested the presence of two benzene rings that comprise seven CH and five quaternary C-atoms of which three were O-bearing centers [10][13].

In the HMQC spectrum, the H-atoms resonating at δ 7.46 (H–C(3)), 7.25 (H–C(2‴)/H–C(6‴)), 7.12 (H–C(2″)), 7.01 (H–C(6″)), 6.84 (H–C(5″)), 6.78 (H–C(3‴)/H–C(5‴)), 6.45 (H–C(2)), 4.71 (H–C(2′)), 3.85 (MeO–C(4‴)), 3.50 (H_α–C(1′)) and 3.42 (H_β–C(1′)) were found to be coupled with the C-atoms at δ 142.2 (C(3)), 128.5 (C(2‴)/C(6‴)), 111.6 (C(2″)), 123.3 (C(6″)), 116.5 (C(5″)), 116.1 (C(3‴)/C(5‴)), 118.7 (C(2)), 73.4 (C(2′)), 56.4 (MeO–C(4‴)), and 48.3 (C(1′)), respectively. The HMBC experiment showed long-range coupling of H–C(1′) (δ 3.50, 3.42) with C(2′) (δ 73.4), C(1″) (δ 134.1), and C(1) (δ 169.5), suggesting that the hydroxyethyl unit is interconnected with the amide C=O group and benzene ring A (C(1″)). The H–C(2) (δ

Table 1. *^{1}H- and ^{13}C-NMR, and HMBC Data for Compound* **1** (CD_3OD)

Position	δ(C)[a])	δ(H)[b])	HMBC[c]) (H → C)
1	169.5 (C)		
2	118.7 (CH)	6.45 (*d*, *J* = 15.6)	1, 3, 1‴
3	142.2 (CH)	7.46 (*d*, *J* = 15.6)	1, 2, 1‴, 2‴, 6‴
1′	48.3 (CH_2)	3.42 (*dd*, *J* = 13.5, 7.7), 3.5 (*dd*, *J* = 13.5, 4.9)	1, 2′, 1″ 2’
73.4 (CH)	4.71 (*dd*, *J* = 7.7, 4.9)	1′, 1″, 2″, 6″	
1″	134.1 C		
2″	111.6 (CH)	7.12 (*d*, *J* = 1.3)	2′, 1″, 3″, 4″
3″	149.8 (C)		
4″	149.3 (C)		
5″	116.5 (CH)	6.84 (*d*, *J* = 8.5)	1″, 3″, 4″
6″	123.3 (CH)	7.01 (*dd*, *J* = 8.5, 1.3)	1″, 5″
1‴	128.2 (C)		
2‴, 6‴	128.5 (CH)	7.24 (*d*, *J* = 8.5)	3, 3‴, 4‴, 5‴
3‴, 5‴	116.1 (CH)	6.78 (*d*, *J* = 8.5)	1‴, 4‴
4‴	158.1 (C)		
1a	56.4 (MeO)	3.87 (*s*)	4‴

[a]) Recorded at 100 MHz. [b]) Recorded at 400 MHz. [c]) Recorded at 400 MHz

6.45) was found to be coupled with C(1‴) (δ 128.2), C(3) (δ 142.2), and C(1) (δ 169.5). Similarly, H–C(3) (δ 7.25) showed couplings with C(2) (δ 118.7), C(1‴) (δ 128.2), C(2‴)/C(6‴) (δ 128.5), and C(1) (δ 169.5), thereby indicating that there is a C=C bond that interconnects the amide carbonyl C-atom (C(1)) with benzene ring B (C(1‴)). The couplings of C(2″) protons at δ 7.12 with C(4″) (δ 149.3), C(1″) (δ 134.1), C(3″) (δ 149.8) and C(2′) (δ 73.4) while δ 6.84 (H–C(5″)) with C(3″) (δ 149.8), C(4″) (δ 149.3) and C(1″) (δ 134.1), indicated that two hydroxyl groups are substituted on benzene ring A. Furthermore, the downfield H–C(2‴)/H–C(6‴) (δ 7.25) displayed correlations with C(4‴) (δ 158.1), C(3) (δ 142.2), C(1‴) (δ 128.2) and C(5‴) (δ 116.1), which indicated that the MeO group is substituted on benzene ring B.

The optical rotation of **1** was found to be -16° for the (–)-(*S*)-configuration at C(2′) [11–13]. The absolute configuration at C(2′) was also established by *Horeau*'s procedure [14] (see the *Exper. Part*). On the basis of these spectroscopic studies, it was concluded that the compound **1** is *N*-[2-(3,4-dihydroxyphenyl)-2-hydroxyethyl]-3-(4-methoxyphenyl)prop-2-enamide.

Compound **2** showed the molecular ion peak at *m/z* 359.1367 ($C_{19}H_{21}NO_6$; calc. 359.1369). It differs from compound **1** only by the presence of a MeO unit at C(3‴) instead of an aromatic H-atom at C(3‴). The fragmentation pattern in EI-MS as well as the IR spectrum (KBr) of **2** was identical to **1**. The ^{1}H-NMR spectrum displayed a 3-H signal at δ 3.85 (*s*) assigned to MeO–C(3‴) (*Table 2*). The aromatic H-atoms of ring B resonated at δ 6.99 (*J*(2‴, 6‴) = 1.5, 1 H), 6.82 (*dd*, *J*(6‴, 2‴) = 1.5, *J*(6‴, 5‴) = 8.1, 1 H), 6.76 (*d*, *J*(5‴, 6‴) = 8.1, 1 H) were assigned to H–C(2‴), H–C(6‴), and H–C(5‴), respectively. Its ^{13}C-NMR spectrum showed the downfield signals at 147.1 (C(3‴)), 119.9 (C(6‴)), 115.9 (C(5‴)), 110.9 (C(2‴)), 156.5 (C(4‴)) and 128.2 (C(1‴)), which suggested the presence of trisubstituted benzene ring B with two O-bearing centers, whereas signal at δ 55.8 was assigned to MeO–C(3‴). On the basis of these data, compound **2** was identified as *N*-[2-(3,4-dihydroxyphenyl)-2-hydroxyethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide.

Compound **3** showed the molecular ion *m/z* 343.1421 ($C_{19}H_{21}NO_5$; calc. 343.1419). It differs from compound **1** only by the presence of a MeO group at C(4″) instead of a OH group at C(4″). The structure was further supported by MS, IR, and ^{1}H- and ^{13}C-NMR experiments (*Table 3*). The ^{1}H-NMR spectrum of **3** displayed a signal resonating at δ 3.82 (*s*, 3 H), which was assigned to MeO–C(4″). Its ^{13}C-NMR spectrum showed one signal at δ 55.9, which was due to MeO–C(4″). The HMBC spectrum showed that Me–C(4″) (δ 3.82) was coupled with C(4″) (δ 148.8), supporting the presence of a MeO group at ring A. On the basis of these data, compound **3** was identified as *N*-[2-(3-hydroxy-4-methoxyphenyl)-2-hydroxyethyl]3-(4-methoxyphenyl)-prop-2-enamide.

Table 2. *^{1}H- and ^{13}C-NMR, and HMBC Data for Compound* **2** (CD_3OD)

Postion	δ (C)[a])	δ (H)[b])	HMBC[c]) (H → C)
1	169.5 (C)		
2	118.6 (CH)	6.46 (*d*, *J* = 15.68)	1, 3, 1‴
3	142.2 (CH)	7.44 (*d*, *J* = 15.68)	1, 2, 1‴, 2‴, 6‴
1′ (CH_2)	48.3 (CH_2)	3.44 (*dd*, *J* = 13.5, 7.8), 3.53 (*dd*, *J* = 13.5, 4.9)	1, 2′, 1″
2′	73.6 (CH)	4.72 (*dd*, *J* = 7.8, 4.9)	1′, 1″, 2″, 6″
1″	133.5 (C)		
2″	111.5 (CH)	7.12 (*d*, *J* = 1.5)	2′, 3″, 4″
3″	149.9 (C)		
4″	149.3 (C)		
5″	116.4 (CH)	6.78 (*d*, *J* = 8.17)	1″, 3″
6″	123.2 (CH)	7.01 (*dd*, *J* = 8.17, 1.5)	2′, 4″, 5″
1‴	128.2 (C)		
2‴	110.9 (CH)	6.99 (*d*, *J* = 1.5)	3, 1‴, 3‴, 6‴
3‴	147.1 (C)		
4‴	156.5 (C)		
5‴	115.9 (CH)	6.76 (*d*, *J* = 8.17)	1‴, 3‴
6‴	119.9 (CH)	6.82 (*dd*, *J* = 8.17, 1.5)	4‴
1a	56.1 (MeO)	3.90 (*s*)	4‴
1b	55.8 (MeO)	3.85 (*s*)	3‴

[a]) Recorded at 125 MHz. [b]) Recorded at 500 MHz. [c]) Recorded at 400 MHz.

Table 3. *^{1}H- and ^{13}C-NMR, and HMBC Data for Compound* **3** (CD_3OD)

Position	δ (C)[a])	δ (H)[b])	HMBC[c]) (H → C)
1	169.4 (C)		
2	119.2 (CH)	6.43 (*d*, *J* = 16.1)	1, 3, 1‴
3	141.1 (CH)	7.47 (*d*, *J* = 16.1)	1, 2, 1‴, 2‴, 6‴
1′	49.0 (CH_2)	3.42 (*dd*, *J* = 14.2, 8.3), 3.5 (*dd*, *J* = 14.2, 4.8)	1, 2′, 1″
2′	73.6 (CH)	4.75 (*dd*, *J* = 8.3, 4.8)	1′, 1″, 2″, 6″
1″	134.5 (C)		
2″	111.8 (CH)	7.14 (*d*, *J* = 1.5)	2′, 3″, 4″
3″	150.6 (C)		
4″	148.8 (C)		
5″	116.8 (CH)	6.82 (*d*, *J* = 8.4)	3″, 4″, 6″
6″	124.2 (CH)	7.01 (*dd*, *J* = 8.4, 1.5)	1″, 5″
1‴	129.1 (C)		
2‴, 6‴	128.7 (CH)	7.27 (*d*, *J* = 8.4)	3, 3‴, 4‴, 5‴
3‴, 5‴	116.3 (CH)	6.81 (*d*, *J* = 8.4)	1‴, 4‴
4‴	159.1 (C)		
1a	56.8 (MeO)	3.91 (*s*)	4‴
1c	55.9 (MeO)	3.82 (*s*)	4″

[a]) Recorded at 100 MHz. [b]) Recorded at 400 MHz. [c]) Recorded at 400 MHz.

The inhibitory activity of the three new isolated compounds **1**–**3** on mushroom tyrosinase inhibition was studied (*Fig. 1*). In these experiments, compounds **1** (IC_{50} = 2.61 μM), **2** (IC_{50} = 1.85 μM), and **3** (IC_{50} = 0.40 μM), exhibited pronounced activities when compared with standard tyrosinase inhibitors like kojic acid (IC_{50} = 16.67 μM) and L-mimosine (IC_{50} = 3.68 μM).

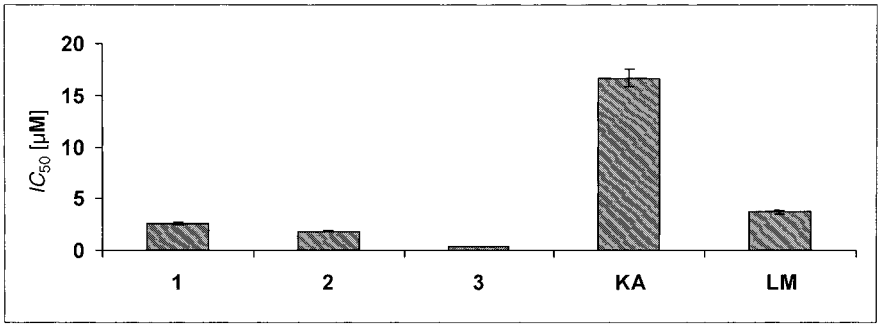


Fig. 1. *Graphical presentation of the comparative* IC_{50} *values of the compounds* **1**, **2**, **3**, *and KA* (kojic acid) *and LM* (L-mimosine) *against mushroom tyrosinase*

Since all these compounds have the same basic skeleton, it is, therefore, possible to study their structure-enzyme inhibitory activity relationships. From previous reports [15][16], it is clear that OH group(s) at aromatic rings are important for the tyrosinase inhibitory activity. In compound **1**, two OH groups are present at C(3″) and C(4″). However, it exhibited the least activity compared to the other two compounds. In compound **2**, two OH groups are present at the same positions, but a new MeO group is attached at C(3‴) of the second aromatic ring. This compound showed more-potent inhibitory activity than **1**. On the other hand, compound **3**, which contains one MeO and one OH group at C(4″) and C(3″), respectively, exhibited potent tyrosinase inhibition activity. From these observations, it is also clear that MeO group(s) at the aromatic ring are very important for the tyrosinase inhibitory activities. The tyrosinase inhibitory activities of these compounds were tested in reactions with L-DOPA as substrate and are shown in *Table 4*. Hyperpigmentation is associated with increased plasma melanocytes-stimulating hormone activity with insufficient production of glucocorticoids (*Addison*'s disease). Recently, a number of inhibitors of natural origins have been used in cosmetics [15].

Table 4. *Tyrosinase Inhibitory Activities of Compounds* **1**, **2** *and* **3** *as Compared with the Standard Inhibitors*

Compounds	IC_{50} ± S.E.M.[a]) [μM]
1	2.61 ± 0.01497
2	1.85 ± 0.00315
3	0.40 ± 0.00247
Kojic acid[b])	16.67 ± 0.5190
L-mimosine[b])	3.68 ± 0.02234

[a]) Standard error of the mean. [b]) Standard inhibitors of the enzyme tyrosinase.

Compounds **1**, **2**, and **3** were also subjected to a DPPH radical scavenging assay and showed a varying degree of activity. The results of scavenging activities are depicted in *Table 5*. Compounds **1** and **3** exhibited IC_{50} values of 383 and 378 μM, respectively, suggesting a moderate free radical-scavenging activity, whereas compound **2** also

showed some activity with an IC_{50} value of 427 μM. The antioxidant potentials of test samples were compared with 3-(*tert*-butyl)-4-hydroxyanisol and propylgallate used as a positive control (*Fig. 2*) [17][18].

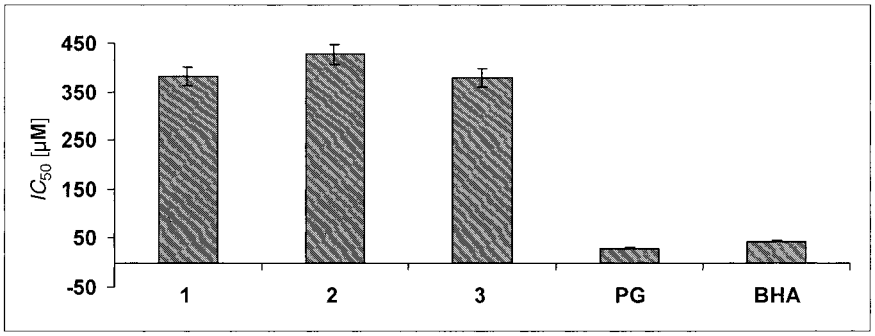


Fig. 2. *Graphical presentation of the comparative* IC_{50} *values of the compounds* **1**, **2**, **3**, *and PG* (propyl gallate) *and BHA* (3-(*tert*-butyl-4-hydroxyanisol) *on DPPH-induced antioxidant assay*

Table 5. *Antioxidant Activities of the Compounds* **1**, **2**, *and* **3** *as Compared with the Standards*

Compounds	IC_{50} ± S.E.M.[a]) [μM]
1	383 ± 0.0471
2	427 ± 7.40
3	378 ± 0.60
Propyl gallate[b])	30 ± 0.5190
3-(*tert*-Butyl)-4-hydroxyanisol[b])	44 ± 0.02234

[a]) Standard error of the mean. [b]) Standard antioxidants.

Conclusions. – From this study, it can be concluded that the new compounds from the plant *Salsola foetida* have potential to be used for the treatment of diseases like hyperpigmentation associated with the over production of melanocytes. There is need of further study for their *in vitro* as well as *in vivo* activities on melanocytes and skin pigmentation.

Experimental Part

General. Column chromatography (CC): Sephadex *LH-20* (*Pharmacia,* Uppsala, Sweden); prepacked reverse-phase *C18* column (*Water Sep-Pak Vac 20 cc*). Recycling prep. HPLC: *LC-908* (*Japan Analytical Industry Co., Ltd.*). M. p.: open capillaries, *Büchi*-535 apparatus; uncorrected. UV: *Perkin-Elmer Lambda-5 UV/VIS* spectrometer; MeOH. IR: *Shimadzu IR-460* spectrophotometer; KBr. α_D: *JASCO DIP-370* digital polarimeter; MeOH. NMR: *Bruker* apparatus; at 400 and 500 MHz; δ in ppm rel. to Me_4Si (= 0 ppm) as internal standard. EI-MS, FD-MS, FAB-MS, and HR-EI-MS: *Varian MAT-711* and *MAT-112S* apparatus; *m/z* (rel. %).

Plant Material. S. foetida (30 kg) was collected from the Lal Sohanra National Forest Park of Bahawalpur in August 2001. A voucher specimen has deposited in the Cholistan Institute of Desert Studies, Islamia University Bahawalpur, Pakistan.

Extraction and Isolation. Air-dried whole plants of *S. foetida* (18 kg) were percolated with MeOH at r.t. for two weeks. The residue obtained after removal of the solvent (500 g) was extracted with hexane, Et_2O, and AcOEt (3 × 3 l). The AcOEt extract (40 g) was further extracted with distilled H_2O (3 × 150 ml). The H_2O

extract, on concentration, left a pale yellow syrup (3 g), which was chromatographed over *Sephadex LH-20* column with MeOH/H_2O as a solvent with decreasing polarity. The active fractions were further purified by prepacked reverse-phase *C18* column chromatography with MeOH/H_2O 3:8. The final purification was carried out by prep. recycling HPLC (YMC, *ODS-H80* column) with MeOH/H_2O 1:1 isocratic solvent system to yield pure compounds **1** (11 mg, 2.2×10^{-5} %), **2** (9 mg, 1.8×10^{-5} %), and **3** (8 mg, 1.6×10^{-5} %).

N-*[2-(3,4-Dihydroxyphenyl)-2-hydroxyethyl]-3-(4-methoxyphenyl)prop-2-enamide* (**1**). Yellow amorphous powder. M.p. 218–220°. $[\alpha]_D^{25} = -16$ ($c = 0.1$, MeOH). UV (MeOH): λ_{max} 217 (4.85), 324 (4.51), 327 (4.75). IR (KBr): 3363, 2922, 1652, 1600, 1517, 1271. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 329.1264 ($C_{18}H_{19}NO_5$; calc. 329.1263), 311.1161 ($C_{18}H_{17}NO_4$; calc. 311.1157), 294.1128 ($C_{18}H_{16}NO_3$; calc. 294.1130), 277.1110 ($C_{18}H_{15}NO_2$; calc. 277.1103), 176.0714 ($C_{10}H_{10}NO_2$; calc. 176.0711), 145.0531 (C_9H_7NO; calc. 145.0528), 103.0545 (C_8H_7; calc. 103.0548).

N-*[2-(3,4-Dihydroxyphenyl)-2-hydroxyethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide* (**2**). Yellow amorphous powder. M.p. 225–227° $[\alpha]_D^{25} = -24$ ($c = 0.058$, MeOH). UV (MeOH): λ_{max} 219 (4.60), 324 (4.29), 327 (4.35). IR (KBr): 3360, 2910, 1648, 1605, 1510, 1270. ^{1}H and ^{13}C-NMR: *see Table 2*. HR-EI-MS: 359.1367 ($C_{19}H_{21}NO_6$; calc. 359.1369), 341.1267 ($C_{19}H_{19}NO_5$; calc. 341.1263), 324.1233 ($C_{19}H_{18}NO_4$; calc. 324.1236), 310.1088 ($C_{18}H_{16}NO_4$; calc. 310.1079), 307.1209 ($C_{19}H_{17}NO_3$; calc. 307.1208), 279.0890 ($C_{17}H_{13}NO_3$; calc. 279.0895), 206.0822 ($C_{11}H_{12}NO_3$; calc. 206.0817), 146.0607 (C_9H_8NO; calc. 146.0606), 103.0552 (C_8H_7; calc. 103.0548).

N-*[2-(3-hydroxy-4-methoxyphenyl)-2-hydroxyethyl]-3-(4-methoxyphenyl)prop-2-enamide* (**3**): Yellow amorphous powder. M.p. 255–257°. $[\alpha]_D^{25} = -20$ ($c = 0.04$, MeOH). UV (MeOH): λ_{max} 204 (4.56), 319 (4.48), 327 (4.53). IR (KBr): 3380, 1650, 1600, 1518, 1278. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-EI-MS 343.1421 ($C_{19}H_{21}NO_5$; calc. 343.1419), 325.1317 ($C_{19}H_{19}NO_4$; calc. 325.1314), 308.1281 ($C_{19}H_{18}NO_3$; calc. 308.1287), 277.1109 ($C_{18}H_{15}NO_2$; calc. 277.1103), 246.0917 ($C_{17}H_{12}NO$; calc. 246.0919), 176.0721 ($C_{10}H_{10}NO_2$; calc. 176.0711), 146.0609 (C_9H_8NO; calc. 146.0606), 103.0550 (C_8H_7; calc. 103.0548).

Horeau's *Procedure.* The sample compound (3 mg, *ca.* 0.0091 mmol) was added to a soln. of racemic 2-phenylbutanoic anhydride (0.08 ml) in 0.4 ml of pyridine. The resulting mixture was stirred overnight at r.t. Distilled H_2O (0.3 ml) was added, and the mixture was allowed to stand for 30 min. NaOH (0.1M) was then added dropwise until the pH became 9, and the soln. was then extracted with CH_2Cl_2. The aq. layer was acidified to pH 3 with 1M HCl, and the acidic layer was extracted with benzene (6 ml). The benzene extract was evaporated to adjust the volume to 2.5 ml. The optical rotation of the resulting 2-phenylbutanoic acid in the aq. soln. was found to be (+)-(*R*), thereby establishing the (−)-(*S*) configuration at C(2′) in compound **1**, **2**, and **3**.

Tyrosinase Inhibition Assays. Tyrosinase inhibition assay was performed with kojic acid and L-mimosin as standard inhibitors for the tyrosinase in *SpectraMax 340* microplate reader (*Molecular Devices*, USA). The compounds were screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate [17]. IC_{50} Studies were carried out with 3.3% soln. in MeOH. 30 units mushroom tyrosinase (28 nM) was preincubated with the compounds in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25°. Then, the L-DOPA (0.5 mM) was added to the mixture, and the enzyme reaction was monitored for the formation of the DOPAchrome by measuring the change in absorbance at 475 nm (at 37°) for 10 min. The percent inhibition of the enzyme was calculated as follows,

$$\% \text{ inhibition} = [ABS_{Blank} - ABS_{Sample}/ ABS_{Blank}] \times 100$$

where the ABS_{Blank} is absorbance for the blank, and ABS_{Sample} is absorbance for the samples.

DPPH Free Radical Scavenging Activity. The reaction mixture containing 5 µl of test samples was dissolved in DMSO and 95 µl of DPPH in EtOH. Different concentrations of test sample were taken in mixture, while the concentration of DPPH was kept as 300 µM. These mixtures were taken in 96-well plate microlitre plates (*Molecular Devices*, USA) and incubated at 37° for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity by samples was determined in comparison with a DMSO-treated control group. IC_{50} Values represent the concentration of sample required to scavenge 50% DPPH free radicals. 3-(*tert*-Butyl)-4-hydroxyanisole (BHA) and propyl gallate were used as positive control [18][19].

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