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Radical Scavenging Hydroxyphenyl Ethanoic Acid Derivatives from a Marine-Derived Fungus

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Abstract Bioassay-guided fractionation of an organic extract of the culture broth from an unidentified marine-derived fungus led to the isolation of a new metabolite, *N*-[2-(4-hydroxyphenyl) acetyl]formamide (**1**), along with four known polyketides, 4-hydroxyphenyl acetamide (**2**), 4-hydroxyphenyl acetic acid (**3**), 3,4-dihydroxyphenyl acetic acid (**4**), and *N*-[2-(4-hydroxyphenyl)ethenyl]formamide (**5**). The structures of **1**–**5** were elucidated by spectral data analyses. Among them, compounds **1**, **4**, and **5** exhibited significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 8.4, 11.9, and 0.2 μM, respectively.

Key words: Marine-derived fungus, radical scavenging activity, *N*-[2-(4-hydroxyphenyl)acetyl]formamide, 4-hydroxyphenyl acetamide, 4-hydroxyphenyl acetic acid, 3,4-dihydroxyphenyl acetic acid, *N*-[2-(4-hydroxyphenyl)ethenyl]formamide

Marine-derived fungi, which are emerging as a significant new chemical resource for drug discovery, have proven to be a rich source of structurally novel and biologically active secondary metabolites [1, 3].

In our search for bioactive compounds from the marine microorganisms [5], one new hydroxyphenyl acetic acid derivative, *N*-[2-(4-hydroxyphenyl)acetyl]formamide (**1**), and the known polyketides, 4-hydroxyphenyl acetamide (**2**) [2, 4], 4-hydroxyphenyl acetic acid (**3**) [2, 7], 3,4-dihydroxyphenyl acetic acid (**4**) [2, 8], and *N*-[2-(4-hydroxyphenyl)ethenyl]formamide (**5**) [9], have been isolated from the broth of an unidentified fungus, which was separated from the surface of the marine brown alga *Ishige okamurae* collected at Uljin, Gyeongbuk Province, Korea in December 2002.

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The fungus was cultured (10 l) for four weeks (static) at 29°C in SWS medium of soytone (0.1%), soluble starch (1.0%), and seawater (100%). The resulting broth and mycelium were extracted separately with EtOAc and CH_2Cl_2-MeOH (1:1) to afford the broth extract (430 mg) and the mycelium extract (1.1 g), respectively. The broth extract showed radical (DPPH) scavenging activity with an IC_{50} value of 1.1 μg/ml, however, the mycelium extract was inactive. Therefore, the broth extract was subjected to column chromatography on silica gel (*n*-hexane/EtOAc), and then octadesyl silica (ODS) gel (H_2O/MeOH) to furnish five fractions containing compounds **1**–**5**. Further purification of each fraction by recycling HPLC (JAI ODS, MeOH), followed by HPLC (C_{18} Apollo, MeOH-H_2O=3:2), yielded compounds **1** (4.4 mg), **2** (2.5 mg), **3** (2.9 mg), **4** (3.3 mg), and **5** (5.6 mg).

The physicochemical properties of the new compound (**1**) and the known compounds (**4, 5**) are as follows. Compound (**1**): colorless oil; UV (MeOH) λ_{max} (log ε) 203 (3.98), 227 (3.94), 278 (3.37) nm; IR (KBr) ν_{max} 3,395, 3,222, 1,663, 1,609, 1,595, 1,517, 1,416, 1,232, 1,026 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 3.53 (2H, s, H-2), 7.06 (1H, dd, J= 8.5, 1.9 Hz, H-2'/-6'), 6.70 (1H, dd, J=8.5, 1.9 Hz, H-3'/-5'), 11.25 (1H, br.s, 4'-OH), 9.33 (1H, br.s, H-1"), 8.98 (1H, s, H-2"); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.8 (s, C-1), 41.4 (t, C-2), 124.0 (s, C-1'), 130.3 (d, C-2'/-6'), 115.1 (d, C-3'/-5'), 156.3 (s, C-4'), 163.4 (d, C-2"); HMBC correlations: H_2-2/C-1, -1', -2'/-6'; H-2'/C-2, -3', -4'; H-3'/C-1', -2', -4'; LREIMS *m/z* 179 $[M]^+$ (28), 151 $[M\text{-}CO]^+$ (67), 135 $[M\text{-}NHCHO]^+$ (39), 134 $[M\text{-}NHCHO\text{-}H]^+$ (94), 107 $[M\text{-}CONHCHO]^+$ (100), 90 $[M\text{-}CONHCHO\text{-}OH]^+$ (19); HREIMS *m/z* 179.0621 $[M]^+$ (calcd for $C_9H_9NO_3$, 179.0582). Compound (**4**): colorless oil; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.53 (2H, s, H_2-2), 6.64 (1H, s, H-2'), 6.47 (1H, d, J=7.5 Hz, H-5'), 6.63 (1H, d, J=7.5 Hz, H-6'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.1 (s, C-1), 40.2 (t, C-2), 125.6 (s, C-1'), 115.3 (d, C-2'), 144.0 (s, C-3'), 145.0 (s, C-

4'), 116.6 (d, C-5'), 120.0 (d, C-6'); LREIMS *m/z* 168 $[M]^+$ (50), 151 $[M\text{-}OH]^+$ (29), 123 $[M\text{-}COOH]^+$ (100), 107 $[M\text{-}COOH\text{-}OH+H]^+$ (42), 94 (26), 77 (42). Compound (**5**): colorless oil; ^1H NMR (DMSO-d_6, 400 MHz) δ 6.64 (1H, d, *J*=10.0, H-1), 5.60 (1H, d, *J*=10.0, H-2), 7.19 (2H, dd, *J*= 8.5, 1.6 Hz, H-2'/-6'), 6.76 (2H, dd, *J*=8.5, 1.8 Hz, H-3'/-5'), 8.10 (1H, br.s, 4'-OH), 9.81 (1H, br.s, N-1"), 9.50 (1H, br.s, C-2"); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 117.7 (d, C-1), 110.8 (d, C-2), 126.0 (s, C-1'), 129.5 (d, C-2'/-6'), 115.3 (d, C-3'/-5'), 156.2 (s, C-4'), 159.9 (d, C-2").

The structures of the known compounds (**2–5**) were identified by comparison of their ^1H and ^{13}C NMR data with those found in the literature [2, 4, 7–9]. 3,4-Dihydroxyphenyl acetic acid (**4**), commercialized as a chemical, was found to exhibit binding affinity as a substrate for uptake by the dopamine transporter [2]. *N*-[2-(4-Hydroxyphenyl)ethenyl] formamide (**5**) has been isolated from the fungus *Aspergillus fumigatus*, and reported to have inhibitory activity against rabbit platelet aggregation [9].

The molecular formula of the new compound (**1**) was determined by HRFABMS and ^{13}C NMR as $C_9H_9NO_3$.

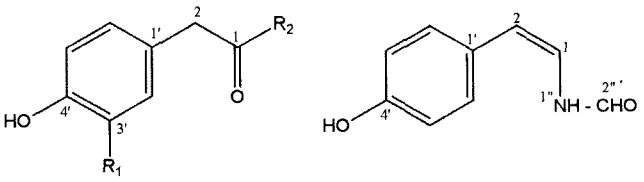
Since **1** showed six unsaturations in HRFABMS, it implied that **1** contained two carbonyls, three double bonds, and one ring. The IR spectrum of **1** showed absorptions for hydroxyl (3,395 cm^{-1}) and a formamide and a secondary amide (3,395, 1,663, 1,232 cm^{-1}) functionalities.

Detailed analyses of the ^1H and ^{13}C NMR data of **1** revealed the presence of three sp^2 quaternary carbons, five sp^2 methines, and one methylene. Extensive analysis of 2D NMR spectra, correlated spectroscopy (COSY), ^1H-detected heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and nuclear Overhauser enhanced and exchange spectroscopy (NOESY) revealed signals ascribable to a 1,4-disubstituted benzene [δ 7.06 (1H, dd, *J*=8.5, 1.9 Hz, H-2'/-6'), 6.70 (1H, dd, *J*=8.5, 1.9 Hz, H-3'/-5'), 124.0 (C-1'), 130.3 (C-2'/-6'), 115.1 (C-3'/-5'), 156.3 (C-4')], a formamide [δ 9.33 (1H, br.s, H-1"), 8.98 (1H, s, H-2"), 163.4 (C-2")], and a secondary acetamide [δ 3.53 (2H, s, H_2-2), 9.33 (1H, br.s, H-1"), 172.8 (C-1), 41.4 (C-2)].

The connection of the functional groups in **1** was achieved on the basis of HMQC, HMBC, and MS data. Key HMBC correlations from H_2-2 to C-1, -1', -2'/-6', and from H-2'/-6' to C-2, as well as the characteristic mass fragments of *m/z* 135 $[M\text{-}NHCHO]^+$ and 107 $[M\text{-}CONHCHO]^+$, were critical in establishing the structure of **1** as shown.

On the basis of all the foregoing evidence, the structure of **1** was proposed as the *N*-[2-(4-hydroxyphenyl)acetyl] formamide.

The antioxidant activity was assessed on the basis of the radical scavenging effect of the DPPH free radical [6]. Compounds **1, 4,** and **5** exhibited significant radical scavenging activity with IC_{50} values of 8.4, 11.9, and 0.2 μM, respectively, which were more potent than the positive control, ascorbic acid (IC_{50}, 20 μM).



1: R_1 = H, R_2 = NHCHO
2: R_1 = H, R_2 = NH_2
3: R_1 = H, R_2 = OH
4: R_1 = R_2 = OH

5

Fig. 1. Structures of hydroxyphenyl ethanoic acid derivatives (1–4) and hydroxyphenyl ethenylformamide (5).

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