

Tyrosinase is the key enzyme that controls melanin formation in the human skin. We performed a screening of 96 extracts of mushroom cultures and fruiting bodies for examining their inhibitory activity against mushroom tyrosinase. The ethyl acetate extracts of culture filtrate of *Neolentinus lepideus* exhibited the strongest inhibitory activity. The active compounds **1** and **2** were purified by repeated chromatographic separations from the extract. On the basis of spectroscopic analyses, **1** and **2** were identified to be 1,3-dihydroisobenzofuran-4,5,7-triol and 5-methoxy-1,3-dihydroisobenzofuran-4,7-diol, respectively. Lineweaver-Burk plot of the enzyme reaction in the presence of **1** indicated that **1** was a potent competitive inhibitor. The respective IC<sub>50</sub> values of **1** and **2** were 173 and 263 µg/mL. Compound **1** at 15 µg/mL suppressed melanin accumulation stimulated by α-MSH in the murine melanoma B16 cells, as well as the induced accumulation of both tyrosinase transcript and protein without inhibiting cell proliferation.

We identified new tyrosinase inhibitors in the extract of culture filtrate of *Neolentinus lepideus*.