Secondary metabolites from *Eupenicillium parvum*, and their in vitro binding affinity for human opioid and cannabinoid receptors

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X-ray Crystallographic Study of compound (6).

A single crystal X-ray diffraction study was conducted on (E)-6-(4-acetoxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-y1)-4-methylhex-4-enoic acid. Colorless plates were obtained with the slow evaporation of a solution in methanol. A single crystal, approximate dimensions, 0.05 × 0.13 × 0.23 mm, was used for data collection on a Bruker Smart Apex II system, using CuKα radiation with a graphite monochromator, fine-focus sealed tube. The crystal was kept at 100 K under a stream of cooled nitrogen gas from a KRYO-FLEX low temperature device. Compound 6, C₁₉H₂₂O₇, MW = 362.37, crystallizes in the triclinic space group P-1 with two molecules in one asymmetric unit (Z = 2). Cell dimensions are: a = 7.54570(10) Å, b = 10.10260(10) Å, c = 11.8352(2) Å, α = 76.2670(10)°, β = 83.8830(10)°, γ = 87.3210(10), V = 871.21(2)Å³. Data collection, indexing and initial cell refinements were all carried out using APEX II software [1]. All non-hydrogen atoms were located in the difference electron density maps. Anisotropic displacement parameters were included in the refinement for all non-hydrogen atoms. The final cell parameters were determined from least-squares refinement on 2982 reflections, with R_int = 0.0396, and wR(F²) = 0.1243, and Goof = 1.089. The crystal was stabilized by two major interactions: carboxylic acid of two adjacent molecules formed an intermolecular H-bonded network (O7–H7…O6: 2.645(2)Å) which formed a six-membered ring; isobenzofuran ring is a planer structure and they packed in layers in the crystal. Face to face π-π stacking interactions was found between these layers.

The structure was solved and refined using the Bruker SHELXTL Software Package [2]. The largest peak in the final difference electron density synthesis was 0.454 e/Å³ and the largest hole was -0.401 e/Å³ with an RMS deviation of 0.104 e/Å³. Hydrogen on hydroxyl group was located on difference map and refined as riding with U_iso(H) = 1.5 U_eq. All other hydrogen atoms
were placed in idealized positions using the HFIX command and were included in the final cycles of least squares refinement, with isotropic Uij's related to the atom’s ridden upon. The crystallographic data have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 927196), and this data can be obtained free of charge from CCDC via http://www.ccdc.cam.ac.uk/deposit.


Fig. 1S \(^1\)H NMR of compound 1.
Fig. 2S $^{13}$C NMR of compound 1.
Fig. 3S HSQC experiment of compound 1.
Fig. 4S HMBC experiment of compound 1.
Fig. 5S $^1$H NMR of compound 2.
Fig. 6S $^{13}$C NMR of compound 2.
Fig. 6S COSY Experiment of compound 2.
Fig. 6S HSQC Experiment of compound 2.
Fig. 6S HMBC Experiment of compound 2.
Fig. 7S NOESY Experiment of compound 2.
Fig. 8S HPLC-UV chromatograms at 210 (A) and 254 (B) of the EtOAc extract of *E. parvum*. **HPLC conditions**: Analytical Alliance (2695 model Waters) equipped with a 996 photodiode array detector, and a 4.6 x 250 mm, 100 Å, 5 μm, Atlantis T3 C18 column (Waters). The system used was water/acetonitrile started at 15% acetonitrile, which was increased to 100% over 20 min and then held at 100% for 5 min, at rate flow of 0.4 mL/min. Sample volumes of 10 μL were injected onto the column.
Fig. 9S HPLC-UV chromatograms at 210 for compound 1 (A) and compound 2 (B). Same conditions explained above.