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A novel antiproliferative agent, phenylpyridineylbutenol, isolated from *Streptomyces* sp.

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Abstract—A novel compound showing antiproliferative effect was isolated from *Streptomyces* sp. Its structure was determined based on the interpretation of the NMR spectra, and its conformation was elucidated using molecular modeling and 2D NOESY. It was determined to be (*E*)-4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol.

In a normal cell, the cellular proliferation and apoptosis are well balanced, whereas in a cancer cell, the balance is not maintained.[1] Because abnormal proliferation increases in the cancer cell, tumor growth is observed. Therefore, the induction of apoptosis can be correlated with cancer chemotherapy. In order to discover good anticancer drugs, potential candidates for apoptotic inducers have been reviewed and screened.[2] While apoptosis preserves the integrity of a cell membrane, necrosis destroys it, so that these two processes can be distinguished by the morphologies of membranes.[3] In addition, physiological and structural changes associated with apoptosis were analyzed by flow cytometry and antiproliferative effect of cell growth was determined by MTT assay.

In searching for novel anticancer agents, natural products have been recognized as a major focus of interest. *Streptomyces* produces over 70% of known antibiotics, so that in this study over one hundred *Streptomyces* strains were tested. Culture broths fermented from *Streptomyces* strains isolated from soil were screened.[4] Culture broth of *Streptomyces* sp. KACC91010 showed a potent antiproliferative activity. A brief description of this research is as follows. In order to isolate an active compound from the broth, 40 L of the broth (Bennet's medium, 28 °C, 10 days) was collected and added into an equal volume of isopropyl alcohol (IPA). The mixture was concentrated under reduced pressure and fractionated by Diaion HP-20 column chromatography (Mitsubishi Chemical, Tokyo, Japan, IPA/water = 0%, 30%, 50%, 70%, and 100%). The fraction with 70% IPA showed the best activity, and further fractionation was carried out by another column chromatography (LiChroprep® RP-18, 40–63 μm, Merck, Darmstadt, Germany, IPA/water = 0%, 30%, 50%, 70%, and 100%). The fraction with 30% IPA showed the best activity, which was concentrated under reduced pressure and was separated with Prep-HPLC (Waters prep-LC system, C_{18} column, 250×22 mm, photodiode array detector at λ_{max} 210 nm, Oregon, USA, eluent of 35% acetonitrile in water, 20 ml/min flow rate). Because the fraction observed at 12.5 min showed the best activity, it was collected and considered to be a single compound (2.5 mg, compound **1**) based on the chromatogram of two-dimensional photodiode array detector.

The structure of compound **1** was determined using NMR spectroscopy, All NMR experiments were performed on a Bruker Avance 400 spectrometer system (9.4 T, Karlsruhe, Germany) at 298 K.[5] Thirteen ^{13}C

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peaks were observed in the ^{13}C NMR spectrum. Among them, two peaks at 129.5 and 129.6 ppm showed with double intensities, so that it was a suspected compound **1** with a benzene ring which was confirmed by the ^{1}H NMR and COSY spectra. Four ^{13}C peaks at 123.1, 123.7, 138.6, and 149.7 ppm were connected to ^{1}H peaks at 7.47 (d, $J = 8.03$ Hz), 7.23 (m), 7.69 (ddd, $J = 1.8$, 7.7, 7.7 Hz), and 8.47 (m) ppm in HMQC. Because these ^{1}H peaks showed cross peaks in COSY and TOCSY, the existence of pyridine ring was suspected and it was proven by the interpretation of the HMBC spectrum. The ^{13}C peak at 60.3 ppm was triplet in DEPT so that it could be hydroxylated methylene group. In COSY and TOCSY, the proton of this methylene was correlated with the ^{1}H at 6.45 ppm (d, $J = 6.4$ Hz) which was attached directly to the ^{13}C at 133.9 ppm in HMQC and long-range coupled to 140.7 ppm in HMBC. Therefore, two ^{13}C peaks at 133.9 and 140.7 ppm should be neighbored. The ^{1}H peaks contained in a benzene ring were correlated to the 1H peak at 4.03 ppm (s) in COSY and TOCSY. This proton was attached to the ^{13}C peak at 35.2 ppm whose multiplicity was determined to be triplet in DEPT. Among thirteen ^{13}C peaks, two peaks at 140.5 ppm and 160.3 ppm were not determined yet, but they should belong to the benzene ring and the pyridine ring. The data obtained from COSY, TOCSY, and HMBC are shown in Figure 1. According to the interpretation of these data, the structure of compound **1** seems to be 4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol. The NMR data are shown in the Supplementary materials.[6] In order to confirm the structure, mass spectrometry (MS) was carried out on a JMS-700 Mstation (JEOL Ltd., Japan). The molecular ion (M+H) was found at 226.2, so that the molecular mass of compound **1** was 225.1. The structure determined by NMR spectroscopy showed its formula as $C_{15}H_{15}NO$ and this was consistent with the MS result. The complete assignments of ^{1}H and ^{13}C NMR data are listed in the references and notes section.[7]

Since compound **1** contains a double bond between C-2 and C-3, its stereochemistry should be determined. Molecular modeling was performed on a Silicon Graphics workstation O2 R12,000 with InsightII software (Accelrys, San Diego, CA, USA).[8] The force field used for molecular dynamics calculation was consistent valence force field. The molecule built up using InsightII

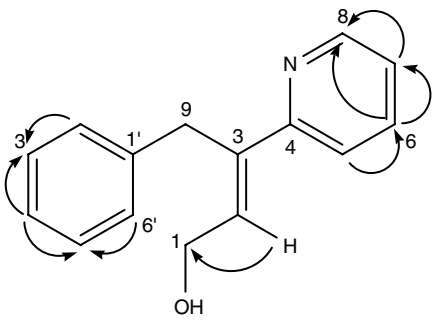

Figure 1. The structure and nomenclature of compound **1**, (*E*)-4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol, and the connectivities obtained from the interpretations of the two-dimensional NMR experiments.

was subjected to energy minimization. After energy minimization, molecular dynamics was performed at 300 K, 1 atm for 100 ps with 1 fs each step. The conformer with the lowest energy was chosen. In the case of *E*-form, the distances between H-2 and H-5, and H-2 and H-9 are 2.93 and 3.89, respectively, but in *Z*-form, they are 4.21 and 2.50, respectively (Fig. 2).

From the NOESY experiment, while the NOE cross peak between H-2 and H-5 was observed, the peak between H-2 and H-9 was not. In *Z*-form, if the NOE between H-2 and H-5 was observed, the peak between H-2 and H-9 should be observed, because the distance between H-2 and H-9 is shorter than that between H-2 and H-5. However, the NOESY data showed only the NOE peak between H-2 and H-5 (Fig. 3). As a result, the conformation of compound **1** should be *E*-form, and the final structure should be (*E*)-4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol.

For the bioassay of compound **1**, the antiproliferative effect was determined by the MTT assay (Sigma Chemical, St. Louis, MO, USA). This MTT assay was carried out to assess cell viability according to the manufacturer's instructions. The human hepatocellular carcinoma cell line HepG2, human cervical adenocarcinoma cell line

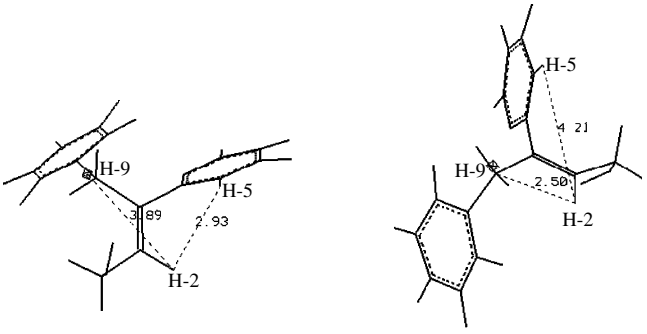

Figure 2. The *E*-conformer (left) and the *Z*-conformer (right) obtained from molecular modeling calculation.

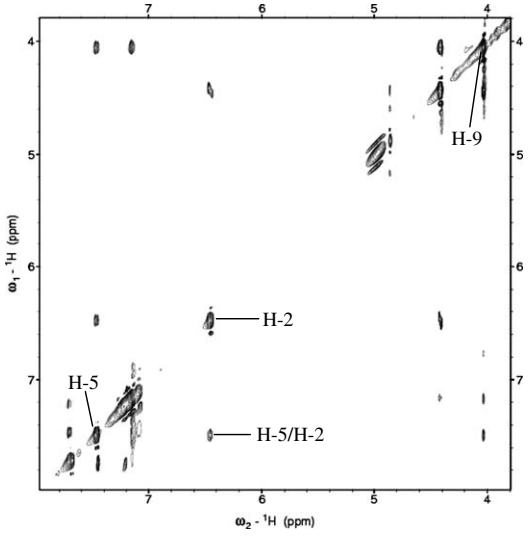

Figure 3. The partial spectrum of NOESY corresponding to the cross peaks between H-2 and H-5, H-2 and H-9, respectively.

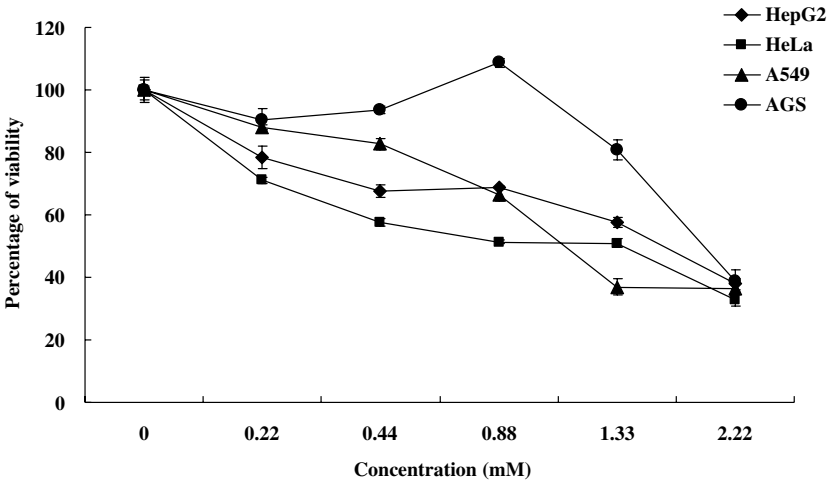

Figure 4. Antiproliferative effect of cell growth of compound 1 in HepG2, HeLa, A549, and AGS cells was determined by MTT assay. The IC_{50} values of HepG2, HeLa, A549, and AGS cells are 1.6, 1.38, 1.13, and 1.98 mM, respectively. Data points represent means ± SD of three experiments.

HeLa, human stomach cancer cell line AGS, and human lung cancer cell line A549 (1.4×10^5 cells per well) were seeded in a 96-well plate containing 100 μl of the cell culture medium and incubated overnight. Then the cells were treated with different concentrations of compound **1** (0.22–2.22 mM) or its vehicle, DMSO (0.1 %), for 24 h. Subsequently, 15 μl of the MTT reagent was added to each well, and the plates incubated again in a CO_2 incubator at 37 °C for 3 h. Thereafter, the supernatant was removed from each well. Then 100 μl DMSO was added to dissolve the colored formazan crystals produced by the MTT. Subsequently, the optical density was measured at 570 nm using an ELISA Reader (Molecular Devices Corp., Sunnyvale, USA).[9] As shown in Figure 4, the growth of all of the four cancer cells tested was inhibited in a concentration-dependent manner by compound **1**. The estimated IC_{50} values determined for compound **1** in these cancer cell lines ranged from 1.13 to 1.98 mM.

In conclusion, even though the antiproliferative effect of compound **1** is lower than that of a control, it is the novel compound isolated from culture broth of *Streptomyces* sp. The results of this study demonstrate that compounds having phenylpyridineylbutenol moiety like compound **1** show activities as anticancer or antitumor agents. To the best of our knowledge, it is the first time to report these findings. Therefore, rational design of derivatives based on the skeleton of compound **1** will provide new approaches for searching novel anticancer agents. Moreover, further study is needed to focus on the improvement of their selectivity as anticancer agents toward caner cells.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.015

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6. Data proving the identity of the compound are provided as Supplementary materials; spectra of ^{1}H NMR, ^{13}C NMR, DEPT, COSY, TOCSY, NOESY, HMQC, and HMBC, and chromatogram of HPLC with photodiode array detector, and MS spectrum.
7. ^{1}H NMR (DMSO-d_6) δ 4.03 (2H, s, H-9), 4.41 (2H, d, J = 6.4, H-1), 6.45 (1H, d, J = 6.4, H-2), 7.09 (1H, m, H-4′), 7.14 (2H, m, H-2′/6), 7.18 (1H, m, H-3′/5), 7.23 (1H, m, H-7), 7.47 (1H, d, J = 8.03, H-5), 7.69 (1H, ddd, J = 1.8, 7.7, 7.7, H-6), 8.47 (1H, m, H-8); ^{13}C NMR (DMSO-d_6) δ 35.2 (t, C-9), 60.3 (t, C-1), 123.1 (d, C-5), 123.7 (d, C-7), 127.2 (d, C-4′), 129.6 (d, C-2′/6′), 129.6 (d, C-3′/5′), 133.9 (d,C-2), 138.6 (d, C-6), 140.5 (s, C-1′), 140.7 (s, C-3), 149.7 (d, C-8), 160.3 (s, C-4).
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