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Communication

Bis(4-hydroxybenzyl)sulfide: a Sulfur Compound Inhibitor of Histone Deacetylase Isolated from Root Extract of *Pleuropterus ciliinervis*

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Abstract: A sulfur compound, bis(4-hydroxybenzyl)sulfide (**1**) was isolated from the root extract of *Pleuropterus ciliinervis*. Its structure was elucidated using NMR spectroscopic techniques and mass spectrometric analysis. Compound **1** showed potent inhibitory activity in a histone deacetylase (HDAC) enzyme assay. It also exhibited growth inhibitory activity on five human tumor cell lines and more sensitive inhibitory activity on the MDA-MB-231 breast tumor cell line.

Keywords: Sulfur compound; HDAC; *Pleuropterus ciliinervis*; tumor cell lines.

Introduction

The turnover of histone acetylation is regulated by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) [1]. The inhibition of HDACs causes an accumulation of acetylated histones in the nucleus and subsequent activation of transcription of target genes. HDAC catalyzes deacetylation of ε-amino group in lysines located near the N-terminal of core histone proteins. Abnormal recruitment of HDAC is related to carcinogenesis. Thus, the identification of potent HDAC inhibitors has been considered as a very intriguing approach for the development of

cancer chemotherapy [2]. Recently, HDAC inhibitors are emerging as an exciting new class of potential anticancer agents for the treatment of solid and hematological malignancies [3]. A number of natural and synthetic HDAC inhibitors have shown an anti-proliferative activity on tumor cells. Among them, trichostatin A (TSA) [4], apicidin [5], trapoxin B (TPX) [6] and FK-228 [7] were classified as natural substances, while suberoylanilide hydroxamic acid (SAHA) [8] and other TSA or SAHA-like analogues were reported as synthetic HDAC inhibitors [9].

The root of *Pleuropterus ciliinervis* Nakai (Polygonaceae) has been used in the traditional Chinese folk medicine ‘‘Hasuo’’, which is used to treat inflammation and bacterial infections [10]. Stilbenes, anthraquinones and flavonoids have been isolated from the genus *Pleuropterus* [11,12]. These compounds have attracted much attention for their biological activity, which include antioxidant [13], or tyrosinase-inhibitory effects [14].

In the context of our natural product chemistry program dealing with the development of new potent anticancer agents, we have examined the isolation of sulfur compounds as leads for novel HDAC inhibitors. Herein, we describe our results on the isolation, enzyme inhibition activity and cancer cell growth inhibition of one such sulfur-based HDAC inhibitor.

Results and Discussion

Silica gel and Sephadex LH-20 column chromatography and preparative HPLC of the EtOAc-soluble fraction of the MeOH extract of *Pleuropterus ciliinervis* roots led to the isolation of the sulfur compound **1** (Figure 1). Compound **1** was identified as bis(4-hydroxybenzyl)sulfide by comparing its spectral data with those previously reported. [15]. We have evaluated the HDAC inhibitory activities of the newly isolated sulfur compound on partially purified HDAC enzyme obtained from HeLa cell lysate and their anti-proliferative effects using PC-3 cells as well. Compound **1** was active in the HDAC enzyme assay. The compound showed potential growth inhibitory activities on the PC-3 cell line (Table 1).

Figure 1. Structure of **1.**

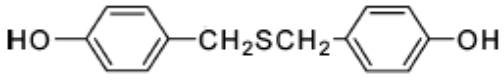

Table 1. HDAC enzyme and growth inhibition by sulfur compound **1** and SAHA.

Compound	IC_{50} (μM) Enzyme	GI_{50} (μM) PC-3
1	1.43 ± 0.03	7.65 ± 0.05
SAHA	0.13 ± 0.01	0.74 ± 0.01

* Values are means of a minimum of three experiments.

Growth inhibitory activities of **1** and SAHA were evaluated in six human tumor cell lines. Growth inhibition (GI_{50}) measured by the MTT assay of these HDAC inhibitors and the tumor cell line types are listed in Table 2. With a similar pattern to the enzyme inhibition, compound **1** exhibited growth inhibitory activity on five human tumor cell lines. Among them, **1** showed the most potent inhibitory activity on the MDA-MB-231 breast tumor cell line.

Table 2. GI_{50} and origin type for cells treated with sulfur compound and SAHA.

Cell line	Origin	Growth inhibition (μM)	
		1	**SAHA**
ACHN	Kidney	6.23 ± 0.07	0.67 ± 0.02
NCI-H23	Lung	>10	1.32 ± 0.02
PC-3	Prostate	7.86 ± 0.06	0.75 ± 0.02
MDA-MB-231	Breast	1.45 ± 0.03	0.89 ± 0.02
LOX-IMVI	Melanoma	6.14 ± 0.08	1.67 ± 0.02
HCT-15	Colon	7.89 ± 0.12	0.93 ± 0.06

* Values are means of a minimum of three experiments.

Bis(4-hydroxybenzyl)sulfide thus has good enzyme inhibitory and cell growth inhibitory activities and showed the most potent growth inhibitory activity to MDA-MB-231 among the six human tumor cell lines tested. Further structure–activity relationships of hydroxyl group from aromatic ring will be reported in due course.

Conclusions

Bis(4-hydroxybenzyl)sulfide (**1**) was isolated from the root extract of *Pleuropterus ciliinervis* as a potential histone deacetylase inhibitor. Its structure was determined by spectral and chemical methods. It inhibited histone deacetylase derived from PC-3 cell lines with GI_{50} values ranging from 7.65 μM.

Experimental

General

UV: UV-2200 UV–VIS recording spectrophotometer (Shimadzu, Japan); IR: Jasco Report-100 spectrophotometer; NMR: Bruker AMX 400 spectrometer (Bruker, USA), the chemical shifts being represented as ppm with tetramethylsilane as a internal standard; GC-MS (HP 5890 series II plus GC, HP 5972 series Mass Selective Detector, Column: HP-1MS); column chromatography: silica gel 60 (70~230 and 230~400 mesh, Merck), Sephadex LH-20 (Pharmacia, Sweden) and YMC-GEL ODS-A (12 nm, S-75 mm, YMC); TLC: pre-coated silica gel 60 F_{254}. Suberoylanilide hydroxamic acid was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Plant material collection, extraction and purification

Pleuropterus ciliinervis was collected in August 2004, at Odae Mt., Kangwondo, Korea. Voucher specimens are deposited at the herbarium of Kon Kuk University (Seoul, South Korea). The botanical identification was made by Dr. Kim Tae-Jin, KRIBB (Dae-Jeon, South Korea). The collected roots of *Pleuropterus ciliinervis* (1 kg) were freeze-dried for 7 days and then extracted twice at room temperature for 2 weeks with EtOAc (5 L each time). The resulting residue of the EtOAC extract after solvent removal (32 g) was suspended in water (500 mL) and partitioned successively with two portions of EtOAC (500 mL each), to give EtOAC (26 g) and water (5 g) soluble fractions. The EtOAC extract fraction was applied to a Si gel column and eluted with hexane-EtOAc mixtures of increasing polarity [10:1~1:10 (v/v)] to give five subfractions, the fourth of which (2.3 g, eluted with 2:3 hexane-EtOAc) was chromatographed with Si gel eluting with $CHCl_3$-EtOAc (13:1), followed by hexane-CH_2Cl_2-EtOAc (1:1:1) to give five fractions A-E. Fraction C (1.6 g) afforded bis(4-hydroxybenzyl)sulfide (**1**, 26 mg) after chromatography on Lobar A (MeOH-H_2O, 8:2). Compound **1**: pale yellow needles ; mp 135-137 °C (from EtOH); IR (KBr) λ_{max} 3285 (OH), 1604, 1600, 1509 (C=C), 1214, 1089 (OH) cm-1; EIMS *m/z* (%) 246 (M+, 35), 200 (15), 107 (100); ^{1}H- and ^{13}C-NMR data were in good agreement with the published data [15]

Cell Culture

Cell lines purchased from ATCC (Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10 % horse serum and 5 % fetal bovine serum and incubated in a CO_2 incubator (5 %) at 37 °C.

MTT Assay

Cells were serum-deprived by three washes of PBS and resuspended in DMEM. The suspended cells were plated on 96-well plates (1 x 10^4 cells/well) and treated with the indicated reagent(s). After treatment for 21 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the medium (0.5 mg/mL), and the mixture was incubated at 37 °C for another 3 h. After discarding the medium, DMSO (100 mL) was then applied to the well to dissolve the formazan crystals, and the absorbances at 570 and 630 nm in each well were measured on a micro-ELISA reader.

Histone deacetylase assay

HDAC fluorescent activity assays using a Fluror de Lys™ Substrate (Biomol, Plymouth Meeting, PA, USA), which contains an acetylated lysine side chain, were performed according to manufacturer's instructions. In brief, HeLa nuclear extracts, which were used as an HDAC enzyme source, were incubated at 25 C with 250 mM of Fluror de Lys™ Substrate and various concentrations of each sample. Reactions were stopped after 20min with Fluror de Lys™ Developer and fluorescence was measured using a microplate spectrofluorometer (BioRad) with excitation at 360 nm and emission at 460 nm.

Statistical analysis

The 50% inhibitory concentration (IC_{50}) values for 1 and SAHA were obtained from the dose–response curves, using non-linear dose response curve fitting analysis with Sigma pro software. Statistical significance was determined using the Student t-tests. Results are presented by means ± standard error of mean (SEM). All p values quoted are two-tailed and were accepted as significantly different when p was ≤ 0.05.

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Sample Availability: Samples of the compounds are available from authors.