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Vialinin A and thelephantin G, potent inhibitors of tumor necrosis factor-α production, inhibit sentrin/SUMO-specific protease 1 enzymatic activity

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ABSTRACT

Several *p*-terphenyl compounds have been isolated from the edible Chinese mushroom *Thelephora vialis*. Vialinin A, a *p*-terphenyl compound, strongly inhibits tumor necrosis factor-α production and release. Vialinin A inhibits the enzymatic activity of ubiquitin-specific peptidase 5, one of the target molecules in RBL-2H3 cells. Here we examined the inhibitory effect of *p*-terphenyl compounds, including vialinin A, against sentrin/SUMO-specific protease 1 (SENP1) enzymatic activity. The half maximal inhibitory concentration values of vialinin A and thelephantin G against full-length SENP1 were 1.64 ± 0.23 μM and 2.48 ± 0.02 μM, respectively. These findings suggest that *p*-terphenyl compounds are potent SENP1 inhibitors.

SUMO (small ubiquitin-related protein modifier) family proteins share a conserved ubiquitin-like domain, and the covalent modification of proteins by SUMO has a dynamic role in transcription regulation, nuclear transport, chromosome organization, DNA replication and repair, mitotic progression, and ribosome biosynthesis.[1–5] In SUMOylation, SUMO protein is covalently attached to the ε-group of the target lysine residue in specific substrate proteins via an enzymatic cascade that requires sequential action of a set of enzymes, including an activating enzyme (E1), a conjugating enzyme (E2), and a ligase (E3). SUMOylation is a reversible modification regulated by SUMOylating and deSUMOylating enzymes, SENPs (SUMO-specific proteases). SENPs also have endopeptidase (C-terminal hydrolase) activity essential for processing SUMO precursor proteins to expose the C-terminal di-glycine motif and yield mature SUMO. Six SENPs have been identified in humans: SENP 1, 2, 3, 5, 6, and 7. Most SENPs have both isopeptidase and endopeptidase activity, but which of these activities is most essential for each SENP remains unclear.[6] SENP1 is an effector with an emerging role in insulin exocytosis downstream of secretory granule docking in rodents and humans.[7] Recently, SENP inhibitors was summarized successful examples of computational screening that allowed the identification of candidates with therapeutic potential.[8]

Previous studies revealed that vialinin A, isolated from an edible mushroom, *Thelephora vialis*, is a potent inhibitor of tumor necrosis factor alpha (TNF-α) production and release from antigen-stimulated rat basophilic leukemia (RBL-2H3) cells with a half maximal inhibitory concentration (IC_{50}) of 0.09 nM and from murine bone marrow-derived mast cells with an IC_{50} of 0.04 nM.[9–11] Ubiquitin-specific peptidase 5/isopeptidase T (USP5/IsoT, EC3, 1, 2, 15), a deubiquitinating enzyme, was identified as a target molecule of vialinin A in RBL-2H3 cells and USP5 activity is inhibited by vialinin A.[12] Furthermore, we demonstrated in USP5 small interfering RNA-knockdown cells that USP5 plays an essential role in TNF-α production.[13]

In the present study, we investigated the inhibitory effect of *p*-terphenyl compounds, isolated with *T. vialis*, and their derivatives against SENP1 enzymatic activities. IC_{50} and K_i values of *p*-terphenyl compounds on isopeptidase activity of SENP1were determined using a fluorescent substrate, SUMO-1-7-amino-4-

Abbreviations: cSENP1, catalytic human SUMO-specific protease; DUB, deubiquitinating enzyme; IC_{50}, half maximal inhibitory concentration; rhSENP1, recombinant human SUMO-specific protease; SENP, SUMO-specific protease; SUMO, small ubiquitin-related protein modifier; SUMO1-AMC, SUMO1-7-amino-4-methylcoumarin; TNFα, tumor necrosis factor alpha; USP5, ubiquitin-specific peptidase/isopeptidase; NEM, *N*-ethylamalemide.

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methylcoumarin (SUMO1-AMC).[14] Moreover, SENP1 enzymatic activity in RBL-2H3 cells was revealed using a suicide substrate, hemagglutinin- SUMO1-vinyl sulfone (HA-SUMO1-VS).[15,16]

First, we examined the inhibitory effects of *p*-terphenyl compounds against SENP1 enzymatic activity. The structures of *p*-terphenyl compounds are shown in Figure 1. Vialinin A, thelephantin G, and atromentin were isolated from *T. vialis*. 5′,6′-Dimethyl-1,1′:4′,1″-terphenyl-2′,3′,4,4″-tetraol (DMT), and biotin-DMT, a biotinylated probe of DMT, were synthesized as bioactive analogs and a probe, respectively, to search for the target molecules of vialinin A. To examine whether these *p*-terphenyl compounds inhibit SENP1 activity, catalytic domain human SENP1 (cSENP1) protein and SUMO1-AMC were purchased from LifeSensors Inc. (Malvern, PA, USA) and Boston Biochem Inc. (Cambridge, MA, USA). SENP1 activity was based on the fluorescence of AMC after adding SENP1. The method for determining SENP1 activity was as follows. *p*-Terphenyl compounds were dissolved in dimethyl sulfoxide. All reagents were diluted with reaction buffer [50 mM Tris–HCl, pH7.8, 100 μg/mL ovalbumin, and 10 mM dithiothreitol]. Then, either concentrations of test samples or solvent as a blank were added to a 96-well microplate, followed by the addition of 20 nM SENP1, and incubated for 15 min at 37 °C. After adding 200 nM or 400 nM SUMO1-AMC as a substrate, the plates were incubated for 1 h at 37 °C and then fluorescence was measured at excitation and emission wavelengths of 380 nm and 460 nm, respectively. We demonstrated that vialinin A (IC_{50}: 1.89 ± 0.04 μM), thelephantin G (IC_{50}: 1.52 ± 0.06 μM), DMT (IC_{50}: 3.76 ± 0.21 μM), atromentin (IC_{50}: 6.10 ± 0.11 μM), and biotin-DMT (IC_{50}: 9.73 ± 0.64 μM) inhibited cSENP1 enzymatic activity (Fig. 2).

SENPs share a conserved C-terminal cysteine protease domain of approximately 250 amino acids containing the catalytic triad His-Asp-Cys. The SENP N-terminal domain is not well conserved and is believed to be responsible for substrate specificity and location.[17] The SENP N-terminal domains vary in primary sequence and size.[18] SENP1 has nuclear localization signals and nuclear export signals that play a role in the regulation of cellular localization, along with other potential regulatory motifs, which vary for each individual SENP.[17] All SENPs share most of the same sequences found in the catalytic domain of human SENPs (Table 1).

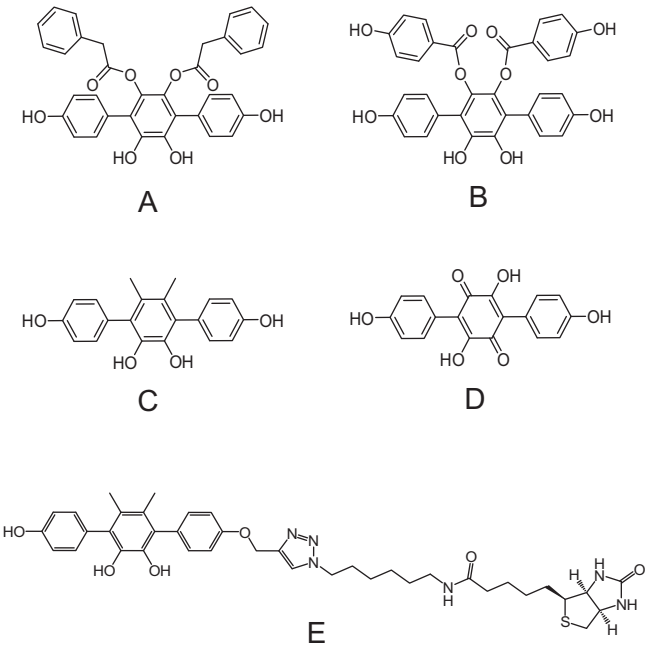

Figure 1. Structures of compounds isolated from *Thelephora vialis* and their derivatives. Chemical structures of vialinin A (A), thelephantin G (B), 5′,6′-dimethyl-1,1′:4′,1″-terphenyl-2′,3′,4,4″-tetraol (DMT) (C), atromentin (D), and biotin-DMT (E).

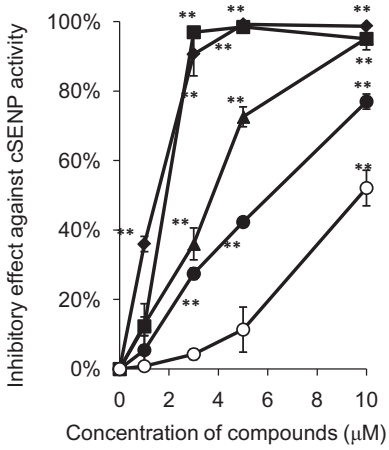

Figure 2. Inhibitory effects of *p*-terphenyl compounds against cSENP1 enzymatic activity. *p*-Terphenyl compounds: vialinin A (■), thelephantin G (◆), DMT, (▲) atromentin (●), and biotin-DMT (○). Each value represents mean ± standard deviation of triplicate determinations. Significance of differences from control values (without inhibitor) was estimated using Student's *t*-test ($^{*}p$ <0.05, $^{**}p$ <0.01).

We then expressed full-length recombinant human SENP1 (rhSENP1) protein in *Escherichia coli* BL21 (DE3) to examine whether the inhibitory effects of SENP1 enzymatic activity were due to differences between the catalytic domain and the full-length protein. Complementary DNA of recombinant human full-length SENP1 (rfSENP1) was synthesized from total RNA, which was extracted from human basophilic leukemia KU812 cells by reverse transcription-polymerase chain reaction (PCR) using PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara Bio, Inc.; Shiga, Japan). The primer sequences were 5′-TAAGGCCTCTGTCGACATG-GATGATATTGCTGATAG-3′ (sense) and 5′-CAGAATTCGCAAGCTT-CAAGAGTTTTCGGTGGAG-3′ (antisense). The underlined regions of the primers indicate the in-fusion region. The PCR conditions were as follows: 98 °C, 30 s (denaturation); 54 °C, 30 s (annealing); and 68 °C, 2 min (extension) for 40 cycles. The PCR product was purified with Wizard® SV Gel and the PCR Clean-Up System (Promega Corp., Madison WI), and ligated with pET-6xHN-N (Clontech Laboratories, Mountain View, CA) using an In-Fusion® HD Cloning Kit (Clontech), and the plasmid was transformed into *ECOS*™ Competent *E. coli* DH5α (Nippon Gene Corp., Tokyo, Japan). The plasmid was extracted with NucleoSpin® Plasmid QuickPure (Takara Bio, Inc., Japan) from *E. coli* incubated in Luria Bertani medium and transformed into *ECOS*™ Competent *E. coli* BL21 (DE3; Nippon Gene). Bacterial cultures were grown overnight in Luria Bertani medium with ampicillin at 30 °C with constant shaking, diluted 1:100 into the same medium, and grown at 37 °C. After 2 h, the incubation temperature was changed to 15 °C. After 1 h, cultures were induced by adding 100 μM

Table 1
IC_{50} value (μM) of *p*-terphenyl compounds against SENP1 enzymatic activity

Compounds	IC_{50} value (μM)	
	cSENP1	rfSENP1
Vialinin A	1.89 ± 0.04	1.64 ± 0.23
Thelephantin G	1.52 ± 0.06	2.48 ± 0.02
DMT	3.76 ± 0.21	2.71 ± 0.12
Atromentin	6.10 ± 0.11	3.79 ± 0.24
Biotin-DMT	9.73 ± 0.64	>50