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Letter

Comparative cytotoxicity of triphenylstibane and fluorine-substituted triarylpnictogens in cultured vascular endothelial cells

Masaki Murakami[1,*], Tomoya Fujie[1,*], Mio Matsumura[2], Eiko Yoshida[1], Chika Yamamoto[3,4], Yasuyuki Fujiwara[3,5], Shuji Yasuike[2,3] and Toshiyuki Kaji[1,3]

[1]Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510, Japan
[2]School of Pharmacy, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan
[3]Division of Bio-organometallics, Research Institute for Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510, Japan
[4]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi 274-8510, Japan
[5]School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji 192-0392, Japan

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ABSTRACT — The toxicity of organic-inorganic hybrid molecules appears to depend on the toxicity of the organic structure, the metals, and their interaction. However, very little is known about the structure-activity relationship of these molecules. In the present study, we investigated the cytotoxicity of triphenylstibane (Sb25) and its fluorine-substituted derivatives the triarylstibanes, using a culture system of bovine aortic endothelial cells. The results showed that the cytotoxicity of tris(4-fluorophenyl)stibane (Sb33) and tris(3,4,5-trifluorophenyl)stibane (Sb49) was higher than that of Sb25, suggesting that introduction of fluorine atoms into the benzene rings may potentiate the cytotoxicity of Sb25 in vascular endothelial cells. However, interestingly, tris(pentafluorophenyl)stibane (Sb35) was nontoxic. The pnictogen analogues tris(pentafluorophenyl)arsane (As35) and tris(pentafluorophenyl)phosphane (P35) showed a higher cytotoxicity than that of Sb35. In addition, the potentiation was much stronger with P35 than it was with As35. The intracellular accumulation of Sb35 was very low while the accumulation of As35 was higher than that of Sb25. These results collectively suggest that the hydrophobicity and metal of the organometallic compounds do not necessarily predict their cytotoxicity and intracellular accumulation in vascular endothelial cells.

Key words: Triphenylstibane, Bio-organometallics, Cytotoxicity, Endothelial cell

INTRODUCTION

Organic-inorganic hybrid molecules are composed of an organic structure and metal(s). Although the molecules are widely used in synthetic chemical reactions, very little is known about their biological activities. Recently, we found that organobismuth compounds with certain organic structures exhibit cytotoxicity, which can be diminished by replacing the bismuth atom with an antimony atom (Kohri *et al.*, 2015). This suggests that the toxicological theory of organic and inorganic compounds cannot be applied to predict the cytotoxicity of organometallic compounds, in the more complex cases. A new toxicological approach to the biology of organic-inorganic hybrid molecules (bio-organometallics) may be required to enhance our understanding.

Antimony is a pnictogen element that is no longer used as an ingredient of pigments because of its irritant effects (Iavicoli *et al.*, 2006). After that, inorganic antimonial compounds such as sodium stibogluconate and meglumine antimoniate were medically used to treat parasitic infections such as leishmaniasis (Wyllie *et al.*, 2004). Antimony and arsenic belong to the same group of elements in the periodic table; however, the cytotoxicity of inorganic arsenic has been investigated more than that of inorganic antimony. Little is known about the cytotoxicity

Correspondence: Toshiyuki Kaji (E-mail: t-kaji@rs.noda.tus.ac.jp)
*These authors equally contributed to this work.

of organoantimony compounds, despite the fact that their biological activities have been elucidated (Jiang *et al.*, 2013; Chen *et al.*, 2014; Islam *et al.*, 2014).

In the present study, we investigated the cytotoxicity and intracellular accumulation of triphenylstibane (Sb25) and its fluorine-substituted triarylstibanes in vascular endothelial cells to determine their structure-activity relationships. In addition, the cytotoxicity and intracellular accumulation of tris(pentafluorophenyl)stibane (Sb35) were also compared with those of tris(pentafluorophenyl)arsane (As35) and tris(pentafluorophenyl)phosphane (P35). The results indicate that the hydrophobicity and metal of these compounds do not necessarily predict their cytotoxicity and intracellular accumulation in vascular endothelial cells.

MATERIALS AND METHODS

Materials

Bovine aortic endothelial cells were purchased from Toyobo (Osaka, Japan). The following materials were purchased from the specified vendors: Dulbecco's modified Eagle's medium (DMEM) and calcium (Ca^{2+})-and magnesium (Mg^{2+})-free phosphate-buffered saline (PBS, Nissui Pharmaceutical, Tokyo, Japan); fetal bovine serum (FBS, Thermo Scientific, Waltham, MA, USA); penicillin-streptomycin and perchloric acid (Nacalai Tesque, Kyoto, Japan); Cytotox 96 Non-Radioactive Cytotoxicity Assay, a lactate dehydrogenase (LDH) kit (Promega, Madison, WI, USA); May-Grunwald and Giemsa stain solution (Merck KGaA, Darmstadt, Germany); nitric oxide and hydrogen peroxide (Wako Pure Chemical Industries, Osaka, Japan); and 3,5-diaminobenzoic acid and triphenylstibane (Tokyo Chemical Industry, Tokyo, Japan).

Synthesis of fluorine-substituted triarylpnictogens

Tris(4-fluorophenyl)stibane (Sb33), tris(3,4,5-trifluorophenyl)stibane (Sb49), Sb35, As35, and P35 were synthesized according to previously reported procedures (Fild *et al*., 1964; Kant *et al*., 2008; Schäfer *et al*., 2011; Jiang *et al.*, 2013). The structures of the triarylpnictogen compounds are shown in Fig. 1.

Cytotoxicity assay

The bovine aortic endothelial cells were cultured in DMEM supplemented with 10% FBS in 24-well culture plates at 37°C in a humidified atmosphere of 5% CO_2 until they attained confluence. The medium was discarded and the cell layer was washed twice with serum-free DMEM and then incubated at 37°C for 24 hr in 0.25 mL of serum-free DMEM in the presence of increasing concentrations of Sb25, Sb33, Sb49, Sb35, As35, or P35 (5, 10, 30, 50, or 100 μM each). After incubation, the conditioned medium was harvested, and an aliquot was used for the determination of LDH activity as an indicator of

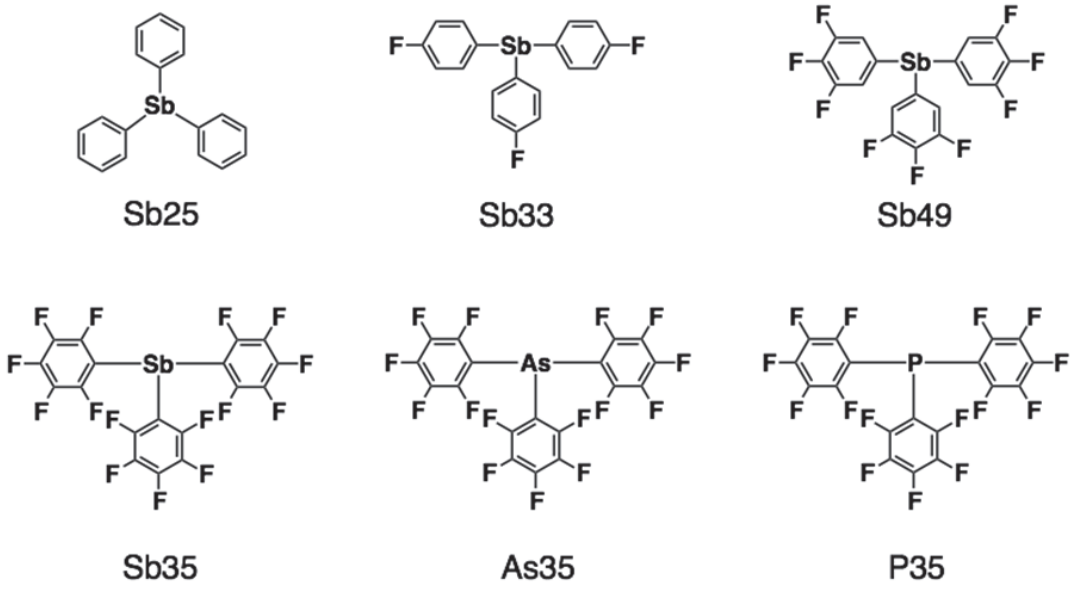

Fig. 1. Structures of the triphenylstibane and fluorine-substituted triarylpnictogens used in this study. Sb25, triphenylstibane; Sb33, tris(4-fluorophenyl)stibane; Sb49, tris(3,4,5-trifluorophenyl)stibane; Sb35, tris(pentafluorophenyl)stibane; As35, tris(pentafluorophenyl)arsane; P35, tris(pentafluorophenyl)phosphane.

cytotoxicity. The cell layer was washed twice with ice-cold Ca^{2+}- and Mg^{2+}-free PBS, fixed with methanol, and stained with Giemsa for morphological observation.

Intracellular accumulation of Sb25, Sb33, Sb49, Sb35, As35, and P35

Confluent cultures of the bovine aortic endothelial cells were incubated at 37°C in 6-well plates for 24 hr in 1 mL of serum-free DMEM in the presence of increasing concentrations of Sb25, Sb33, Sb49, Sb35, As35, or P35 (5, 10, or 30 μM each). After incubation, the medium was discarded and the cell layer was washed twice with ice-cold Ca^{2+}- and Mg^{2+}-free PBS. The cells were sonicated in 1 mL of 50 mM Tris-HCl buffer solution (pH 8.0) and a portion of the homogenate was treated with a mixture of 9.5 M nitric acid and 7% hydrogen peroxide at 130°C for 1 day and then diluted to 4 mL with 0.1 M nitric acid. This mixture was used for the determination of antimony, arsenic, or phosphorus by inductively coupled plasma mass spectrometry (Nexion 300S, PerkinElmer, Waltham, MA, USA) as a marker of the intracellular accumulation of triarylstibanes (Sb25, Sb33, Sb49, and Sb35), As35, and P35, respectively. Another portion of the cell homogenate was analyzed for DNA content using the fluorometric method (Kissane and Robins, 1958) to express the content of antimony, arsenic (pmol/g DNA), and phosphorus (nmol/μg DNA).

Statistical analysis

The data were statistically analyzed using the one way analysis of variance (ANOVA) and Bonferroni's multiple *t*-test where applicable. *P* values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Figs. 2 and 3 show the morphological appearance of and LDH leakage, respectively, in vascular endothelial cells after treatment with Sb25, Sb33, Sb49, Sb35, As35, or P35 for 24 hr. The cell damage evident from the mor-

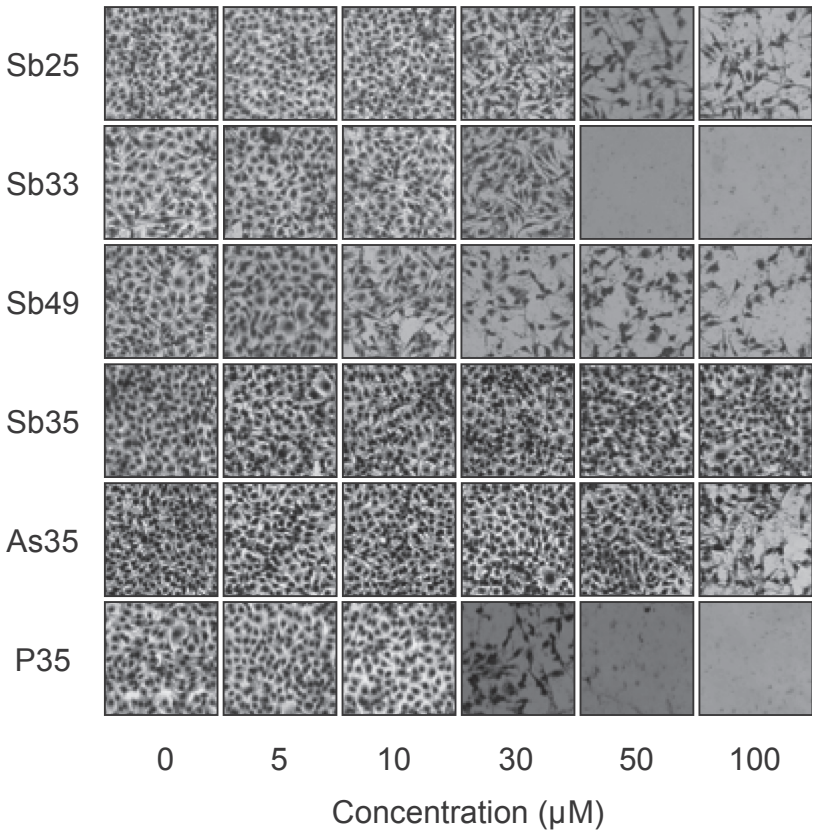

Fig. 2. Morphological appearance of vascular endothelial cells after treatment with triphenylstibane and fluorine-substituted triarylpnictogens. Bovine aortic endothelial cells were treated with Sb25, Sb33, Sb49, Sb35, As35, or P35 (5, 10, 30, 50, and 100 μM each) for 24 hr.

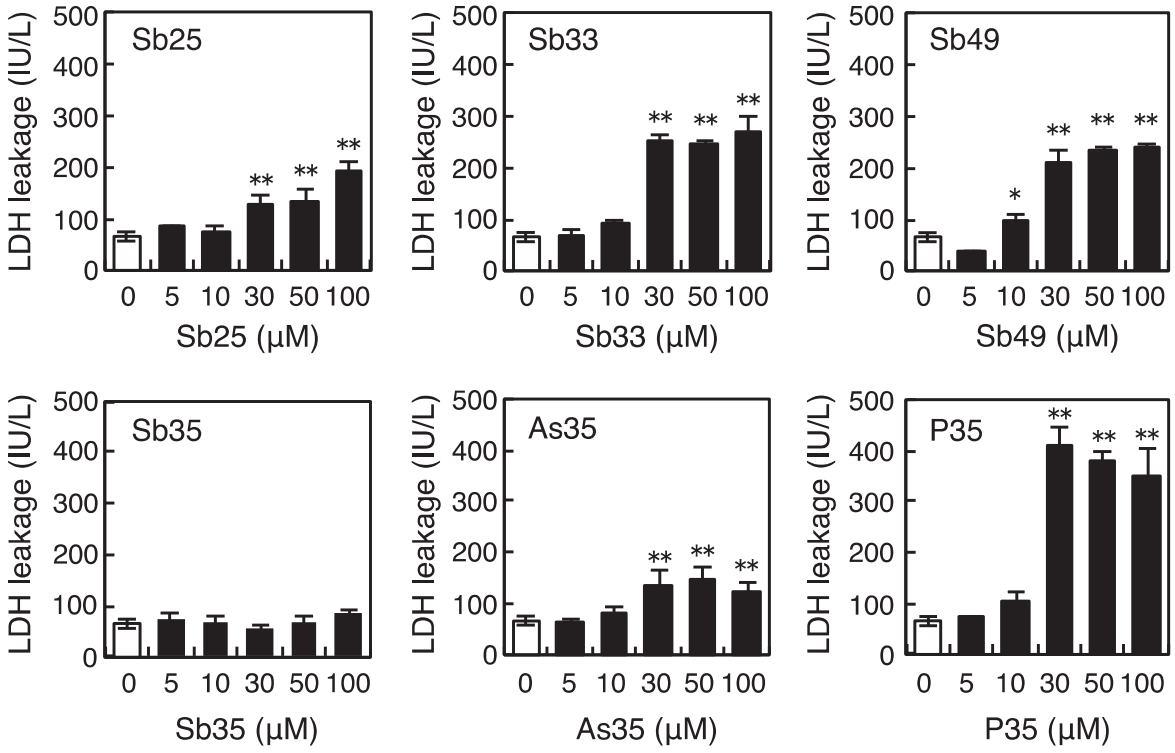

Fig. 3. The leakage of lactate dehydrogenase (LDH) from vascular endothelial cells after treatment with triphenylstibane and fluorine-substituted triarylpnictogens. Bovine aortic endothelial cells were treated with Sb25, Sb33, Sb49, Sb35, As35, or P35 (5, 10, 30, 50, and 100 μM each) for 24 hr. Values are means ± S.E. of four samples. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. Sb25, triphenylstibane; Sb33, tris(4-fluorophenyl)stibane; Sb49, tris(3,4,5-trifluorophenyl)stibane; Sb35, tris(pentafluorophenyl)stibane; As35, tris(pentafluorophenyl)arsane; P35, tris(pentafluorophenyl)phosphane.

phological appearance was almost consistent with the leakage of LDH from the cells. The concentration of Sb49 that induced cell damage was 10 μM, which was lower than that of Sb25 and Sb33 (30 μM each). In addition, the cell damage caused by Sb33 was more severe than that caused by Sb25. These results indicate that the cytotoxicity of triarylstibanes might depend on the degree of fluorination of the benzene rings. However, Sb35, which has the most highly fluorinated benzene rings, did not exhibit cytotoxicity at 100 μM and less. Therefore, it is suggested that the cytotoxicity of triphenylstibane in vascular endothelial cells is influenced by fluorination of the benzene rings, but this does not necessarily correlate to the fluorination degree. When either an arsenic or a phosphorus atom replaced the antimony atom of Sb35, the cytotoxicity was intensified in the vascular endothelial cells, and the cytotoxicity of P35 was stronger than that of As35. Phosphorus is an essential element whereas inorganic arsenic is toxic and not beneficial. The cytotoxicity of an organometallic compound cannot be easily predicted from the cytotoxicity of the inorganic intramolecular metal, particularly in vascular endothelial cells.

The intracellular accumulation of Sb25, Sb33, Sb49, Sb35, As35, and P35 are shown in Fig. 4. Although triarylstibanes (Sb25, Sb33, Sb49, and Sb35) did not accumulate at concentrations of 10 μM and less, a higher accumulation of Sb25, Sb33, and Sb49 but not Sb35 was observed at 30 μM. The lower accumulation of Sb35 was consistent with the lower cytotoxicity of this triarylstibane. However, the degree of cytotoxicity of Sb25, Sb33, and Sb49 (Figs. 2 and 3) did not depend on the intracellular accumulation of these compounds (Fig. 4). The degree of fluorination of the benzene rings may influence the binding of these compounds to their molecular targets rather than their uptake in vascular endothelial cells.

Hydrophobicity is one of the important characteristics that determine the toxicity of organic compounds. The cellular uptake of compounds with higher hydrophobicity is easier because of passive transport, and this results in higher intracellular accumulation and consequently higher

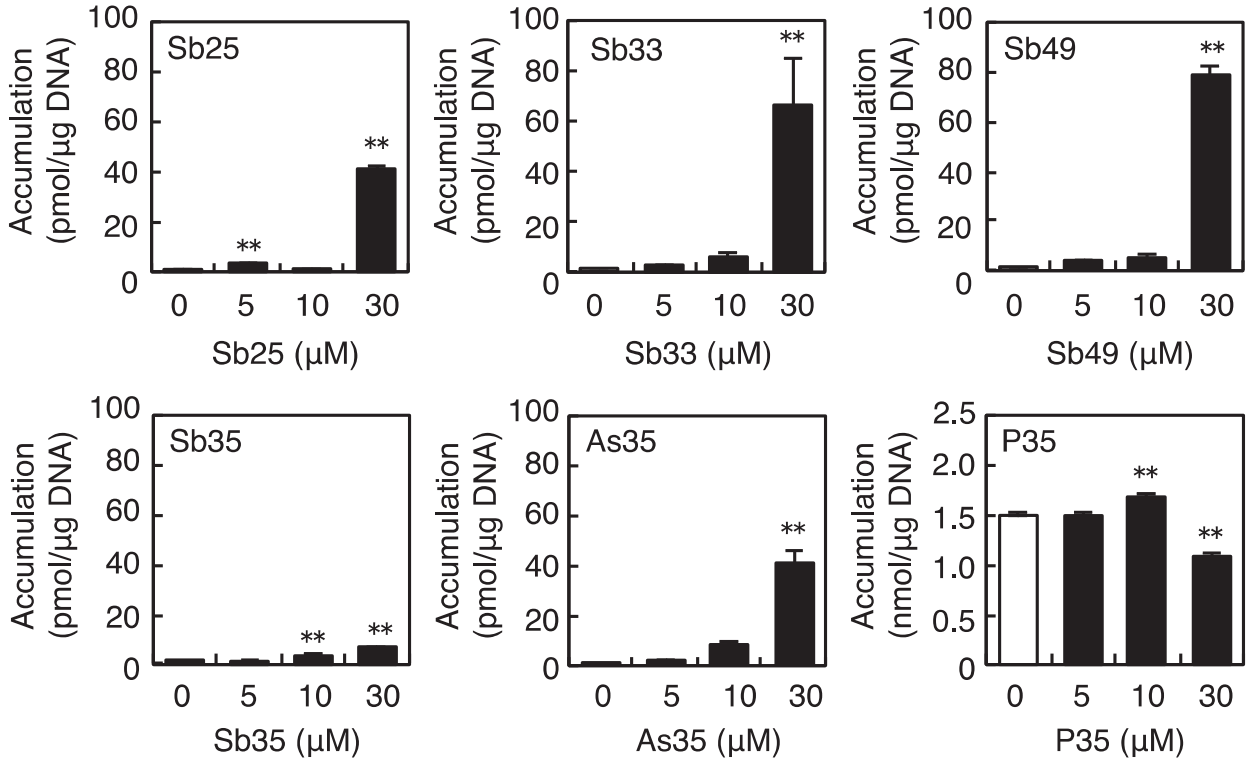

Fig. 4. The intracellular accumulation of triphenylstibane and fluorine-substituted triarylpnictogens in vascular endothelial cells. Bovine aortic endothelial cells were treated with Sb25, Sb33, Sb49, Sb35, As35, or P35 (5, 10, 30, 50, and 100 μM each) for 24 hr. Values are means ± S.E. of three samples. $^{**}P < 0.01$ compared with control. Sb25, triphenylstibane; Sb33, tris(4-fluorophenyl)stibane; Sb49, tris(3,4,5-trifluorophenyl)stibane; Sb35, tris(pentafluorophenyl)stibane; As35, tris(pentafluorophenyl)arsane; P35, tris(pentafluorophenyl)phosphane.

toxicity. However, the principle that higher hydrophobicity results in higher intracellular accumulation and cytotoxicity did not appear to apply to Sb25, Sb33, Sb49, and Sb35. Since the intracellular accumulation of the organobismuth compounds can be much higher than that of the organoantimony compounds with the same organic structure (Kohri *et al.*, 2015), it is most likely that the intracellular accumulation of triphenylstibane and its related compounds is not necessarily predictable by the molecular characteristics including hydrophobicity.

Therefore, the present data shows that the hydrophobicity and the intramolecular metal of the molecules do not necessarily predict the cytotoxicity and intracellular accumulation of organometallic compounds in vascular endothelial cells. Further studies are required for a complete elucidation of the cytotoxicity of organometallic compounds.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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