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Synthesis and evaluation of biological activities of vibsanin A analogs

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ABSTRACT

Vibsanin A is an 11-membered vibsane diterpenoid and is reported to induce myeloid cell differentiation via activation of protein kinase C (PKC) without tumor-promoting activity. Therefore, vibsanin A is thought to be an attractive compound for acute myeloid leukemia (AML) therapy. In this study, we synthesized vibsanin A analogs and compared the activity of these compounds for PKC activation and myeloid cell differentiation. We found that the hydroxymethyl group in vibsanin A is an important substituent to induce differentiation of AML cells. Collectively, our results showed the biochemical features of vibsanin A and provided new insights into the development of new antileukemic drugs.

Acute myeloid leukemia (AML) is a common type of leukemia characterized by abnormal expansion of clonal precursor myeloid cells and arrested cell differentiation.[1] Chemotherapeutic agents are widely used for AML treatment; however, its severe side effects in patient limit the clinical use of chemotherapy for AML.[2] Actually, the survival for elderly patients who are unable to receive intensive chemotherapy due to unacceptable side effects remains poor.[3] It is known that induction of cell differentiation by retinoids inhibits the proliferation of leukemia cells *in vitro*,[4] and cell differentiation therapy with all-*trans* retinoic acid (ATRA) has been recognized as another approach for the treatment of AML. Although ATRA is clinically effective, this agent can be used only for acute promyelocytic leukemia (APL), the M3 subtype of AML, and most patients usually relapse due to acquired resistance, showing its limited use. Other differentiation inducers, such as PKC activators, were also expected to show therapeutic potentials,[1] but many naturally derived PKC activators, including phorbol 12-myristate 13-acetate (PMA), have tumor-promoting activity with inflammation or skin toxicity.[5] Therefore, the development of differentiation-inducing agents may contribute to construct the next-generation AML treatment.

Vibsanin A (**1**), isolated from *Viburnum odoratissimum* Ker., was recently reported to induce differentiation of myeloid leukemia cells by activating PKC and to prolong survival of mouse xenograft models of AML.[6] Surprisingly, **1** did not induce skin toxicity and also inhibits PMA-induced inflammatory responses.[6] However, the detailed chemical feature of **1** remains unclear. In 2015, we achieved the first total synthesis of **1**.[7] In this study, to know the key partial structure of **1** for its activity, we compared the activity of vibsanin A analogs in the activation of PKC and differentiation of AML cells.

Compounds **2** and **3** were synthetic intermediates in the total synthesis of **1**.[7] Compounds **4** and **5** were obtained by oxidation of **3** and **1**, respectively (Fig. 1 and Supplementary Data). To know whether the analogs functioned as PKC activators similar to **1**, we detected phosphorylation of ERK, which acts downstream of PKC, by western blotting method.[8–11] As a result, only **5** induced ERK phosphorylation in a dose-dependent manner, like PMA and **1**; however, **2**, **3**, and **4** had no effect on ERK phosphorylation in THP-1 cells, derived from peripheral blood of a childhood case of AML (M5 subtype) (Fig. 2A, B). Next, we examined whether **5**-induced ERK phosphorylation was mediated by PKC activation, as well as **1**. Treatment with PMA for the long term down-regulates PKC. As shown in Fig. 2C, 5-induced ERK phosphorylation was suppressed by pre-treatment with PMA for the long term, indicating that **5** activated PKC and thereby ERK phosphorylation. These data suggested that only **5** retained the ability to activate PKC, which was originally observed in **1**.

We next investigated whether **5** induced differentiation of THP-1 cells. Generally, PKC activators trigger the differentiation of non-adherent leukemia cells into adherent monocyte cells, changing cell size and morphology. We observed that **5**-treated THP-1 cells became adherent to the culture plate and showed considerable morphological changes, characterized by loss of cell circularity (Fig. 3A, B).[12] Moreover, several lines of evidence, including

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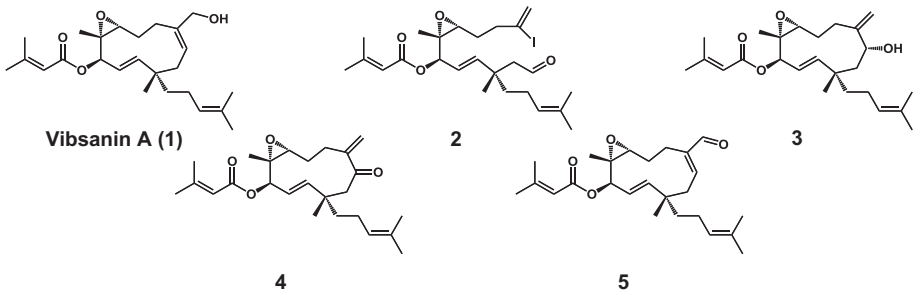

Fig. 1. Structures of **1** and its analogs.

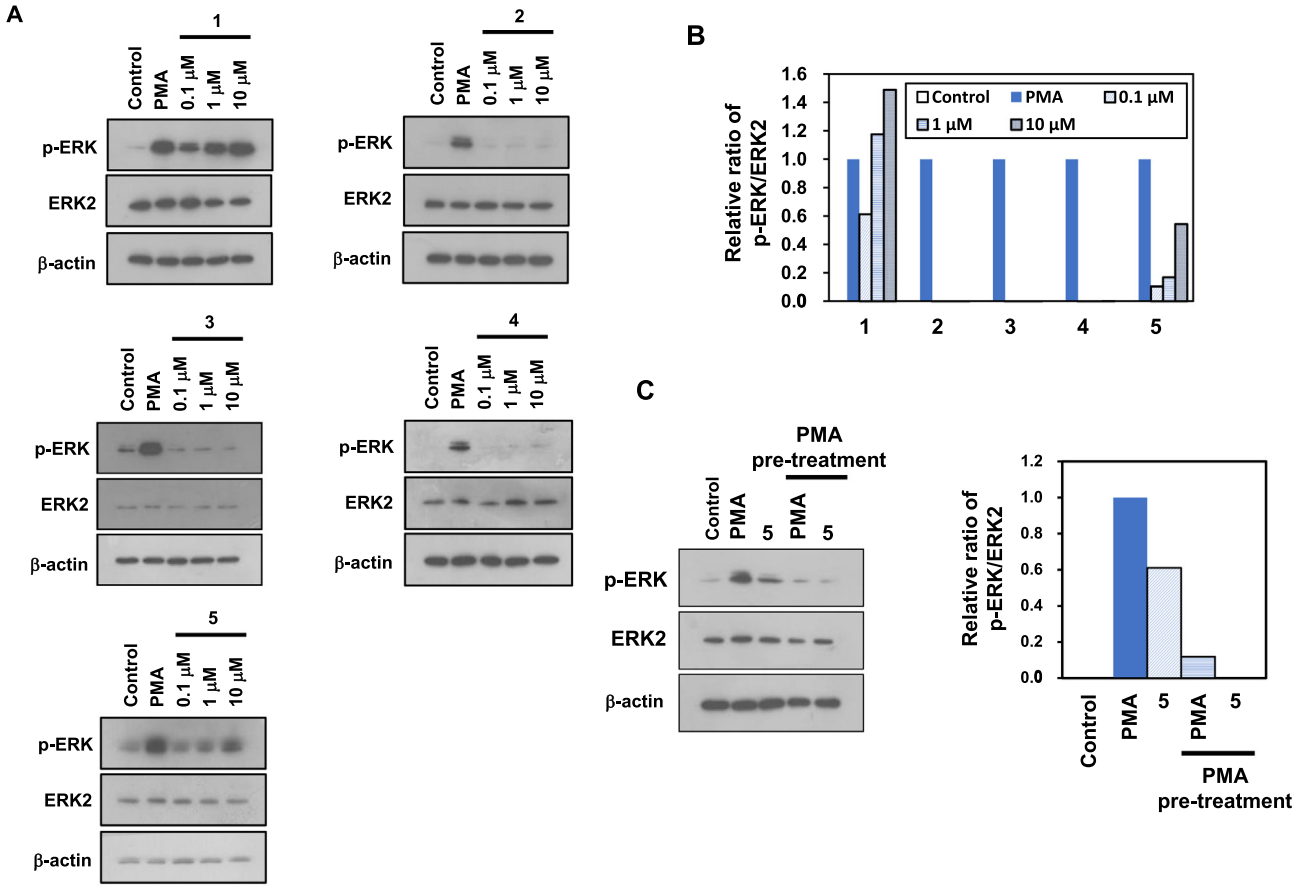

Fig. 2. Compound **5** induced ERK phosphorylation by activating PKC. (A) THP-1 cells were treated with 100 nM PMA or indicated concentrations of **1** or its analogs for 15 min. Cell lysates were immunoblotted with anti-phospho-ERK1/2 (Thr202/Tyr204), anti-ERK2, and anti-β-actin antibodies. β-actin was used as a loading control. (B) Signal intensities of p-ERK were quantified, normalized to total ERK expression, and compared to the PMA-treated condition using ImageJ software. The degrees of PMA-induced ERK phosphorylation were defined as 1.0. (C) THP-1 cells were pre-treated with or without 100 nM PMA for 24 h and treated with 100 nM PMA or 10 μM **5** for 15 min. Cell lysates were immunoblotted with anti-phospho-ERK1/2 (Thr202/Tyr204), anti-ERK2, and anti-β-actin antibodies (*left*). Signal intensities of p-ERK were quantified, normalized to total ERK expression, and compared to the PMA-treated condition using ImageJ software. The degrees of PMA-induced ERK phosphorylation were defined as 1.0 (*right*).

increased cell size, lower nuclear-to-cytoplasmic ratio, and highly vacuolated cytoplasm characterized by Giemsa's staining,[13] suggested that **5** triggered monocytic differentiation, similar to **1** (Fig. 3C). The quantitative analysis showed that differentiation-inducing activity of **5** was significantly weaker than that of **1** (Fig. 3D) as well as PKC activating activity. In addition, **1** and **5** also activate ERK thereby cell differentiation in HL-60 cells derived from APL patient (data not shown).

A previous report showed that the induction of myeloid differentiation by **1** was associated with an inhibition of cell growth and induction of G1 cell cycle arrest.[6] Thus, we detected cytotoxicity of these analogs to THP-1 cells. Trypan blue dye exclusion assay[14–16] demonstrated that **5**, which induced cell differentiation, showed modest cytotoxic effects to THP-1 cells, as well as **1**; however, **4**, which had no effect on cell differentiation, exhibited cytotoxicity (data not shown). We found the positive correlation between differentiation induction and cytotoxicity compared to active compounds (**1** and **5**) and inactive analogs (**2** and **3**), excluding **4**. Thus, we thought **4** might display the cytotoxic effects independently of PKC.