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Novel sesquiterpene lactone analogues as potent anti-breast cancer agents

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ABSTRACT

Triple-negative breast cancer (TNBC) is associated with high grade, metastatic phenotype, younger patient age, and poor prognosis. The discovery of an effective anti-TNBC agent has been a challenge in oncology. In this study, fifty-eight ester derivatives (DETDs) with a novel sesquiterpene dilactone skeleton were organically synthesized from a bioactive natural product deoxyelephantopin (DET). Among them, DETD-35 showed potent antiproliferative activities against a panel of breast cancer cell lines including TNBC cell line MDA-MB-231, without inhibiting normal mammary cells M10. DETD-35 exhibited a better effect than parental DET on inhibiting migration, invasion, and motility of MDA-MB-231 cells in a concentration-dependent manner. Comparative study of DETD-35, DET and chemotherapeutic drug paclitaxel (PTX) showed that PTX mainly caused a typical time-dependent G2/M cell-cycle arrest, while DETD-35 or DET treatment induced cell apoptosis. In vivo

Abbreviations: DEAD, diethyl azodicarboxylate; DET, deoxyelephantopin; DETD, deoxyelephantopin derivative; DMAP, dimethylaminopyridine; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; ER, estrogen receptor; HER2, human epidermal growth receptor 2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PTX, paclitaxel; PR, progesterone receptor; SAR, structure-activity relationship; TNBC, triple-negative breast cancer.

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Lung metastasis
Paclitaxel

efficacy of DETD-35 was evaluated using a lung metastatic MDA-MB-231 xenograft mouse model. DETD-35 significantly suppressed metastatic pulmonary foci information along with the expression level of VEGF and COX-2 in SCID mice. DETD-35 also showed a synergistic antitumor effect with PTX *in vitro* and *in vivo*. This study suggests that the novel compound DETD-35 may have a potential to be further developed into a therapeutic or adjuvant agent for chemotherapy against metastatic TNBC.

1. Introduction

Breast cancer is a commonly diagnosed cancer among women. Although multiple breast cancer therapy protocols including hormone modulators and antibody preparations have been developed, the mortality rate from breast cancer has not improved over the past decade (Howlader et al., 2012). Eighty percent of breast cancers are invasive and recurrence is problematic. The search for effective therapies for breast cancers is complicated by its heterogeneous pathologies and molecular profiles. It is known that many breast cancers are associated with overexpression of either one or multiple hormone receptors such as estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth receptor 2 (HER2). Hormone therapies and/or molecular targeted therapies are applied as an effective treatment for such types of breast cancer. However, difficulties are associated with the treatment of breast cancer with heterogeneous triple-negative type lacking ER, PR, and HER2 expression, which accounts for 15–20% of breast cancers and usually has poor prognosis, and the discovery of an effective anti-breast cancer agent that targets triple-negative breast cancer (TNBC) has been a challenge in oncology.

Elephantopus scaber, a plant from the Asteraceae family, is widely distributed in Eastern and Southeast Asia, Africa, Australia, as well as the Indian subcontinent. It has been widely used as a traditional medicine for the treatment of various diseases and symptoms, such as hepatitis, fever, cough, asthma, arthritis, leukemia, and rheumatism (Kabiru and Por, 2013). Recent research has also revealed its varied pharmacological activities, such as antimicrobial, antidiarrheal, antidiabetic, analgesic, anti-inflammatory, as well as antitumor activities (Hiradeve and Rangari, 2014). Deoxyelephantopin (DET, **1**), a sesquiterpene dilactone, is a major component of *E. scaber*, and was first isolated in 1970 from a different *Elephantopus* species (Govindac et al., 1970; Kurokawa et al., 1970). Among the diverse sesquiterpene lactones, DET contains a germacranolide skeleton, a ten-membered ring with a *trans*-fused α-methylene-γ-lactone, combined together with an additional γ-lactone and methacrylate at C-8. It is believed that α,β-unsaturated ketones including an α-methylene-γ-lactone group on the skeleton are associated with a variety of biological effects. This often acts as a Michael acceptor for nucleophiles such as the thiol group commonly found in the target protein and enzyme (Kupchan et al., 1971; Lee et al., 1971; Picman, 1986). Biological activities, such as hepatoprotective (Huang et al., 2013), antitrypanosomal (Zahari et al., 2014), and anti-cancer cell activities (Farha et al., 2014; Geetha et al., 2012; Kabeer et al., 2013; Lee et al., 2010; Lee and Shyur, 2012; Su et al., 2011; Zou et al., 2008) have been reported for plant sesquiterpene lactones. Our recent studies have also revealed that DET possesses potent activity against TS/A (ER+) mammary tumor growth and metastasis in syngeneic mice through inhibiting m-calpain activity and induction of centrosomal ubiquitinated protein aggregates, protein carbonylation and ER stress-mediated apoptosis that led to significant attenuation of cancer cell motility and metastasis (Huang et al., 2010; Lee et al., 2010; Lee and Shyur, 2012). In spite of the already observed anti-tumor activities of DET, whether DET or its derivatives is effective for inhibiting TNBC remains unknown.

In this study, we report the design and synthesis of a series of DET derivatives (DETDs), and the structure-activity relationship (SAR) of the parental DET and DETDs were addressed. The most potent DETD derivate, namely DETD-35, as well as DET, along with a reference chemotherapeutic drug paclitaxel against TNBC effects *in vitro* and *in vivo* and the underlying mechanisms were comparatively investigated. This study provides a novel DETD that may constitute a new class of anti-TNBC agent.

2. Material and methods

2.1. Chemicals and reagent

All chemicals and solvents were used as purchased. All melting points were measured on a Fisher-Johns melting point apparatus and reported without correction. ^{1}H and ^{13}C-NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) or Varian Inova (400 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. NMR spectra were referenced to the residual solvent peak, chemical shifts δ in ppm, apparent scalar coupling constants *J* in Hz. Mass spectroscopic data were obtained on a Shimadzu LCMS-IT-TOF instrument and JEOL JMS-700 MStation for FAB. Analytical thin-layer chromatography was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). CombiFlash (Isco Companion systems) were used for flash chromatography. All target compounds were characterized and determined to be at least >95% pure by ^{1}H-NMR, HRMS, and analytical HPLC.

2.2. Deoxyelephantol 2

Aqueous 1 N NaOH (25.0 mL) at 0 °C was added to a solution of DET (**1**, 1.023 g, 2.97 mmol) in dioxane (25.0 mL). The mixture