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Total synthesis of BE-43547A$_2$

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ABSTRACT

An asymmetric total synthesis of BE-43547A$_2$ has been achieved in 16 steps (longest linear) on a 3.2 gram scale. The details of our efforts that led to the total synthesis of BE-43547A$_2$ were reported, the final 6 steps were optimized, and the overall yield was improved from 4.5% to 9.7%. The procedure utilized in the final 2 steps may serve as a better method for separating unstable APD compounds.

1. Introduction

Hypoxia, a common feature of many types of solid tumors [1], is speculated to cause therapeutic resistance [2–5] and tumor metastasis [6]. When O_2 levels were measured in patients with various solid tumors using pO_2 histography [7], pancreatic ductal adenocarcinoma (PDAC) was found to be the most hypoxic tumor [8,9]. As the most severely hypoxic solid tumor, PDAC, the third leading cause of cancer deaths in the United States, is considered one of the deadliest human cancers, with an 8% 5-year survival rate for patients with PDAC and 3% for advanced-stage patients [10–12]. Early metastasis, therapy resistance, and tumor recurrence, which result in poor prognosis for patients with PDAC, are still challenging obstacles to overcome [13].

One of the key factor correlated to poor prognosis for patients with PDAC is the extent of the hypoxic areas [14] within the tumor tissue. The adaptation of pancreatic cancer cells to limited oxygen delivery promotes the induction of an invasive [15,16] and treatment-resistant phenotype [17], triggering metastases [18,19] at an early stage of tumor development as well as inducing the cancer stem cells [20] (CSC, with high tumorigenicity and upregulated migratory potential). *In vitro* experiments also indicated that hypoxic conditions dramatically decreased the cytotoxicity of most tested anticancer drugs to various cancer cells [21]. Therefore, targeting the hypoxic microenvironment of pancreatic cancer should significantly impact the clinical outcome [22]. However, compounds that are able to selectively inhibit the growth of pancreatic cancer cells are rare, and the types of hypoxia-selective compounds reported to date are also very limited.

In 2007, Ikeda and coworkers screened 20000 cultured broths of microorganisms, among which only rakicidin A (Fig. 1) showed hypoxia-selective cytotoxicity [21]. Structurally similar to rakicidin A [23], BE-43547 family members are cyclic depsipeptides [24] that were isolated from *Streptomyces* sp. in 1998. Structurally, the BE-43547s consist of a 4-amido-2,4-pentadienoate (APD) moiety and a polyketide moiety with a lipid chain. Biological assays indicated that these molecules exhibit potent growth inhibitory activities [24] (e.g., for BE-43547A$_2$, $IC_{50} = 67$ nM for colon 26) towards various cancer cell lines. The mechanism of action of the BE-43547s is unknown. The structural novelty of these molecules, together with their cytotoxic activities against tumor cell lines and unresolved mechanistic questions, have prompted the total synthesis studies by numerous chemistry researchers, including us. Recently,

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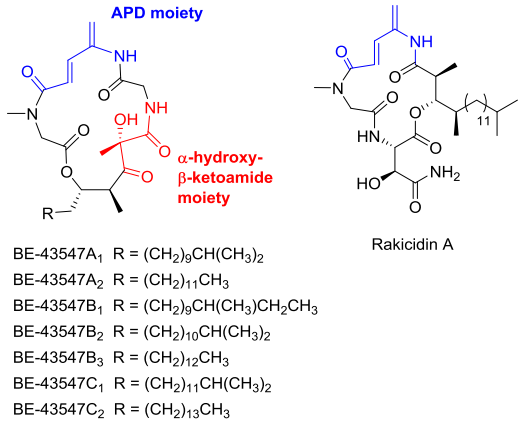


BE-43547A$_1$ R = $(CH_2)_9CH(CH_3)_2$
BE-43547A$_2$ R = $(CH_2)_{11}CH_3$
BE-43547B$_1$ R = $(CH_2)_9CH(CH_3)CH_2CH_3$
BE-43547B$_2$ R = $(CH_2)_{10}CH(CH_3)_2$
BE-43547B$_3$ R = $(CH_2)_{12}CH_3$
BE-43547C$_1$ R = $(CH_2)_{11}CH(CH_3)_2$
BE-43547C$_2$ R = $(CH_2)_{13}CH_3$

Fig. 1. Molecular structures of BE-43547s and rakicidin A.

Poulsen and coworkers made a break-through in designing the first synthetic route to the BE-43547 macrocyclic scaffold [25]. With their chemical synthesis of *ent*-BE-43547A$_1$ and the biosynthesis of the BE-43547 family, they determined the absolute configuration of BE-43547A$_1$-A$_2$ and proposed that the originally reported ^{1}H NMR data for BE-43547A$_1$ and A$_2$ were incorrect [25]. Meanwhile, the researchers demonstrated that BE-43457A$_1$ and A$_2$ possess significant hypoxia-selective growth inhibitory activities against human pancreatic cancer Panc-1 cells [25].

In our previous paper [26], we presented a concise strategy for the construction of BE-43547A$_2$ featuring highly diastereoselective α-hydroxylation at C15. The concise synthetic sequence resulted in 350 mg of BE-43547A$_2$ as well as several derivatives that enabled a preliminary structure-activity relationship (SAR) study to be conducted. Moreover, we discovered that BE-43547A$_2$ could significantly reduce the percentage of pancreatic cancer stem cells in Panc-1 cells, and dramatically ablate the tumorsphere forming capability of Panc-1 cells [26] (see Fig. 2). Although an amount of BE-43547A$_2$ sufficient for preliminary biological evaluation could be synthesized in one batch, the amount was not sufficient for *in vivo* toxicological and pharmacodynamics studies. Meanwhile, obstacles still hinder the gram-scale synthesis, e.g., the instability of the natural product and the low yields of several steps. In this full paper, we describe the details of our efforts that led to the total synthesis of BE-43547A$_2$ as well as the optimization of some reaction conditions and modification of some procedures.

Retrosynthetically, the APD moiety was introduced via elimination of **2** in the final step, as this sensitive functionality was too challenging to handle over several synthetic steps (Scheme 1). A macrolactamization reaction between C11 and N10 was chosen as the ring-closing step. Further cleavage of the C1–O35 bond dissected **3** into two major pieces, an acid fragment and an alcohol fragment. The alcohol fragment contained all 3 stereocenters in BE-43547A$_2$ with unknown configuration at the onset of our studies. Without enough coupling constants in the ^{1}H NMR for BE-43547A$_2$, computational prediction of the absolute configuration of stereocenters like those in rakicidin A is difficult [27]. Thus, the preparation of at most 5 stereoisomers was necessary to complete the synthetic work.

2. Results and discussion

As illustrated in Scheme 2, the C15–OH unit in the alcohol fragment could be installed via α-hydroxylation [28–30]. We anticipated that with the assistance of a chiral auxiliary, **8** could be assembled via a $TiCl_4$-mediated cyclic TS aldol reaction [31] (syn aldol) or an open TS Mukaiyama aldol reaction [32] (anti aldol) to manipulate the stereochemistry at C19 and C21.

Scheme 3 illustrates our first attempt to construct the alcohol fragment. To explore the synthetic feasibility of this bond formation sequence, we initially chose Bn instead of a chiral auxiliary to mask the acid group. Enolization of **13** with Cs_2O_3 followed by oxidation with O_2 afforded alcohol **14**, which was converted to the silyl ether in the presence of TESOTf and 2,6-lutidine to give **15** in 95% yield. **15** was then subjected to a $TiCl_4$-mediated aldol reaction with tetradecyl aldehyde **9** to give syn-aldol product **16** as a mixture. To avoid lactonization via attack of C14 by O35, the secondary OH group was also protected with TES. Direct amidation between *tert*-butyl glycinate **7** and **17** using DBU as the base was unsuccessful, presumably because of the inability of OBn ester to react with nucleophiles. Thus, we tried unmasking the acid using hydrogenation followed by a coupling reaction with *tert*-butyl glycinate to construct the alcohol fragment. Treatment of **17** with Pd/C and H_2 led to the rapid formation of **19**. This phenomenon could be explained by the tendency of β-keto-acid **18** to decarboxylate. To avoid this side reaction, the C18 carbonyl should be replaced by a hydroxyl group.

With the previous knowledge that the C14–N13 amide bond should be introduced in an early stage, we next aimed to develop an asymmetric strategy utilizing Oppolzer's camphorsultam as the chiral auxiliary. As illustrated in Scheme 4, L-camphorsultam derivative **20** reacted with propionaldehyde in the presence of $TiCl_4$

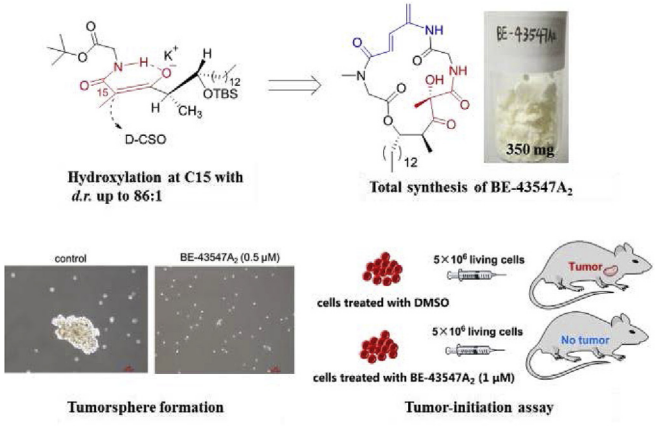
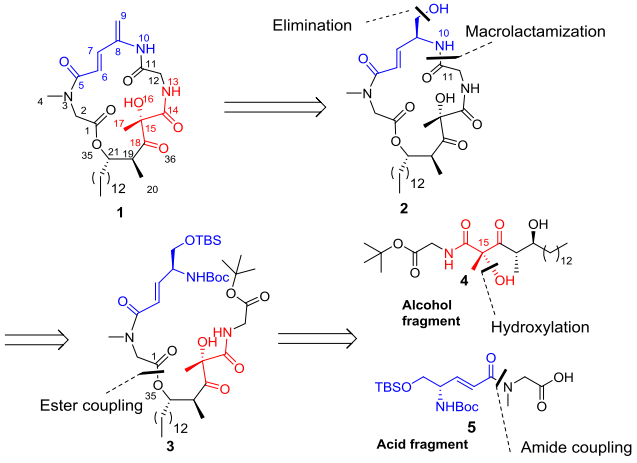


Fig. 2. Total synthesis and anti-pancreatic cancer stem cell activity of BE-43547A$_2$. (Previous work).



Scheme 1. Retrosynthetic analysis of BE-43547A$_2$. (Previous work).