European Journal of Medicinal Chemistry 156 (2018) 692–710

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and biological evaluation of novel larotaxel analogues

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ARTICLE INFO

Article history:
Received 5 February 2018
Received in revised form
7 July 2018
Accepted 10 July 2018

Keywords:
Larotaxel
Toxoids
Structure-activity relationship
Semi-synthesis
Drug-resistant
Cytotoxicity

ABSTRACT

Taxoids are a class of successful drugs and have been successfully used in chemotherapy for a variety of cancer types. However, despite the hope and promises that these taxoids have engendered, their utility is hampered by some clinic limitations. Extensive structure-activity relationship (SAR) studies of toxoids have been performed in many different laboratories. Whereas, SAR studies that based on the new-generation toxoid, larotaxel, have not been reported yet. In view of the advantages in preclinical and clinical data of larotaxel over former toxoids, new taxoids that strategicly modified at the C3'/C3′-*N* and C2 positions of larotaxel were designed, semi-synthesized, and examined for their potency and efficacy *in vitro*. As a result, it has been shown that the majority of these larotaxel analogues are exceptionally potent against both drug-sensitive tumor cells and tumor cells with drug resistance arising from P-glycoprotein over expression. Further *in vivo* antitumor efficacies investigations revealed **A2** might be a potent antitumor drug candidate for further preclinical evaluation.

1. Introduction

Taxoids have contributed to the mainstay treatment in a number of tumors, particularly breast, head and neck, and lung cancers [1]. These taxoids anticancer drugs bind to the β-tubulin subunit, accelerate the polymerization of tubulin, and stabilize the resultant microtubules, thereby inhibiting their depolymerization [2,3]. This results in the arrest of the cell division cycle mainly at the G2/M stage, leading to apoptosis through the cell-signaling cascade [2,3].

Paclitaxel and docetaxel (Fig. 1) are two epoch-making anticancer drugs and have been successfully used in chemotherapy for a variety of cancer types. However, despite the hope and promises that these taxoids have engendered, their utility is hampered by clinic limitations such as acquired or intrinsic drug resistance of tumors, poor CNS activity, allergic reactions and unfavorable toxicity profiles [4,5]. Therefore, it is important to develop new taxoid anticancer agents with fewer side effects, superior pharmacological properties, and improved activity against various classes of tumors, especially against drug-resistant human cancer.

Larotaxel (XRP9881, Fig. 1) [5,6] is a new-generation semi-synthetic taxoid that has a similar mode of action to docetaxel and paclitaxel, but stands for advantages in preclinical and clinical data over former taxanes. Structurally, larotaxel is a taxane derivative similar in origin to docetaxel but is featured in the presence of a 3-membered ring on C-7/C-8 positions of the baccatin III moiety. The 3-membered ring in larotaxel are believed to minimize the recognition by P-glycoprotein [6], which therefore makes larotaxel potentially overcome the multi-drug resistance (MDR) mechanism and cross the blood-brain barrier (BBB) [7]. In addition, based on the pharmacokinetics results from clinical studies, larotaxel may have a more predictable toxicity and efficacy [4].

Extensive structure-activity relationship (SAR) studies of taxoids have been performed in many different laboratories. However, SAR studies that based on the new-generation taxoid, larotaxel, have not been reported yet for discovery and development of better taxane anticancer agents. In view of the advantages in preclinical and clinical data of larotaxel over former toxoids, here we described

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http://dx.doi.org/10.1016/j.ejmech.2018.07.029
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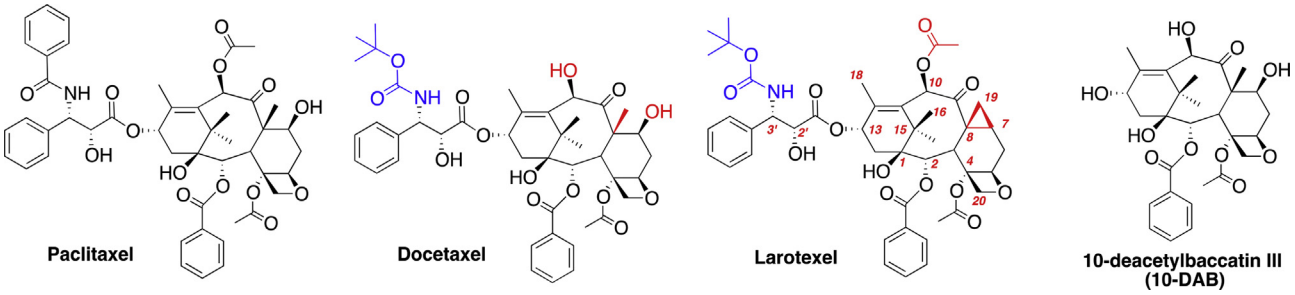


Fig. 1. The structures of paclitaxel, docetaxel, larotaxel and 10-deacetylbaccatin III.

our work on the semi-synthesis of larotaxel and a series of its analogues bearing various substituents at the C3'/C3′-*N* and C2 positions, as well as their effects on cytotoxicities toward both drug-sensitive tumor cells and tumor cells with drug resistance arising from P-glycoprotein over expression. Two of the potent compounds were selected for further *in vivo* antitumor efficacies investigations.

2. Chemical synthesis

2.1. Optimized semi-synthesis of larotaxel

The isolation of 10-deacetylbaccatin III (10-DAB) from the needles (1kg/3000 kg) and leaves (1kg/1000 kg) of European yew, *T. baccata*, which possesses the exact tetracyclic diterpene skeleton of paclitaxel, is a major breakthrough for securing the supply of taxoids through practical semi-synthesis [5,8]. To date, most of taxoids are obtained by semi-synthesis while coupling of a C13 side chain precursor with a pre-modified 10-DAB moiety in general [5].

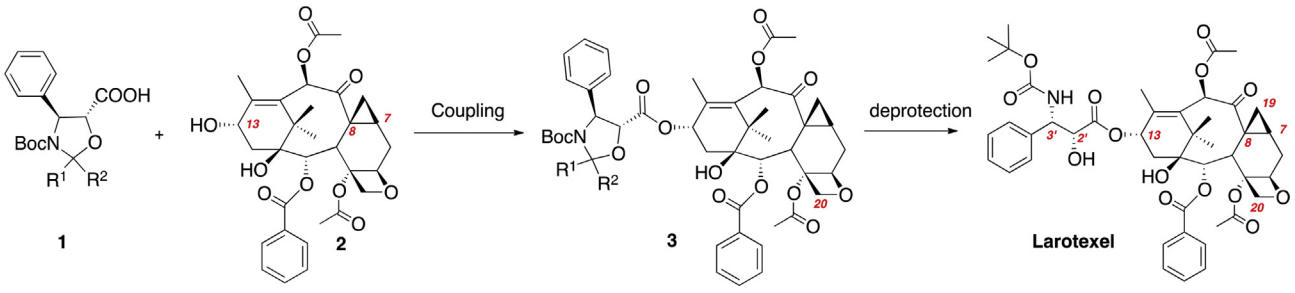
The originally reported semi-synthesis of larotaxel [9–12] adopted this general protocol (Scheme 1), which employed the coupling reaction of 7-deoxy-7β,8β-methanobaccatin III (**2**) with oxazolidine-protected phenylisoserine side chain precursor (**1**) in the presence of condensing reagents to afford the larotaxel scaffold (**3**). This coupling reaction, however, suffered from only modest conversion and substantial epimerization at C2′ as evidenced in the synthesis of many toxoids [5]. Accordingly, more efficient coupling methods were extensively investigated for the practical synthesis and manufacturing of taxoids, but none of them has been applied to larotaxel. Among the coupling methods developed, the β-Lactam Synthon Method (β-LSM, also called "Ojima–Holton coupling"), using the β-lactam as one of the coupling precursor, has been successfully applied for the actual industrial production of some toxoids [5,13]. As the starting point of our efforts on the SAR studies based on larotaxel, we became interested in applying this β-LSM to exploit an efficient and practical semi-synthetic strategy for larotaxel and its congeners.

2.1.1. Synthesis of the 7-deoxy-7β,8β-methanobaccatin III (2)

The originally reported method on the synthesis of 7-deoxy-7β,8β-methanobaccatin III (**2**) from 10-DAB contains a sequence of reactions [9–12], namely 7-OH selective triflation, 10-OH acetylation and 7,8-cyclopropane formation (Route A in Scheme 2). However, there are several drawbacks associated with this method. For example, in this method each of the intermediates (**4**, **5** and **2**) needs to be purified by silica gel chromatography. And the 7-triflate intermediate **4** was found unstable during workup and toward silica gel in our work, which reduced the yield and brought difficulties for purification. In addition, the solubility of both intermediates **4** and **5** are quite low in a pedal of organic solvent systems, further hampered the purification process.

To solve these problems, a reversed sequence of reactions was proposed for the synthesis of intermediate **5** (Route B in Scheme 2). The first step is the regioselective 10-OH acylation on 10-DAB to provide intermediate **6**, which was realized efficiently by reacting of 10-DAB with acetic anhydride in the presence of catalytic amount of $CeCl_3 \cdot 7H_2O$ [14]. Intermediate **6** is shelf-stable, and is conveniently crystalline. With intermediate **6** in hand, the subsequent selective 7-OH triflation [15] becomes straightforward. The reaction of **6** with triflic anhydride in DCM/pyridine led to **5** in 85% isolated yield. Alternatively, after the 7-OH triflation, crude **5** can be subjected to the subsequent 7,8-cyclopropane formation step without further purification, leading to more efficient synthesis of 7-deoxy-7β,8β-methanobaccatin III (**2**).

The conditions for 7,8-cyclopropane formation were screened (Table 1). Johnson [15] reported that simply stirring a solution of **5** with silica gel (w/w = 1/60) in 1,2-dichloroethane at 60 °C produced **2** in good yield. This result was reproduced in our work (Table 1, entry 1). Further experiments revealed that adding more (entry 2) or less silica gel (entry 3) to the reaction mixture led to a drop in yield of **2**. Bouchard et al. disclosed another method in the patents [9–12] that heating the solution of a 7-triflate taxoid precursor in the presence of sodium azide afforded the corresponding 7,8-cyclopropane product in good yield, probably taking advantage of the kinetic salt effect. Inspired by this work and in view of the



Scheme 1. Originally reported semi-synthesis of larotaxel.