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Original article

# Design and total synthesis of Mannich derivatives of marine natural product lamellarin D as cytotoxic agents

Li Shen [a], Nan Xie [b], Bo Yang [b], Yongzhou Hu [a, *], Yongmin Zhang [a, c]

[a] ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China
[b] Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China
[c] Institut Parisien de Chimie Moléculaire, CNRS UMR 8232, Université Pierre et Marie Curie-Paris 6, 4 Place Jussieu, 75005 Paris, France

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ABSTRACT

Enlightened by the modification route from Camptothecin (CPT) to Topotecan and based on classical drug design theory, a series of Mannich derivatives of lamellarin D were designed and synthesized in 26–27 steps starting from vanillin and isovanilin. All synthesized compounds were then biologically evaluated for their *in vitro* anti-cancer activities and Topo I inhibitory activities. The results showed that most target compounds exhibited Topo I inhibitory activities in equivalent level with that of lamellarin D. Compound **SL-9** exhibited better Topo I inhibitory activity than that of lamellarin D. Compounds **SL-2**, **SL-3**, **SL-4**, **SL-5** and **SL-11** exhibited better anti-proliferative activity against HT-29 cells than that of lamellarin D.


## 1. Introduction

Lamellarin D (Fig. 1), one of the first isolated lamellarins from marine prosobranch mollusk *Lamellaria sp*. [1] in 1985, is a potent topoisomerase I (Topo I) inhibitor [2] that induces apoptosis through mitochondria mediated pathway [3] and reverses multi-drug resistance caused by P-glycoprotein-mediated drug efflux [4]. Lamellarin D is one of the most extensively studied members in lamellarins because of its potent anti-proliferative activities [5–8]. Increasing efforts have been involved in studying the total synthesis, mechanism and modification of lamellarins, leading to the presence of several instructive reviews in the past years [6–11]. Lamellarin D incorporates a benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*] isoquinolin-6-one scaffold.

SARs research of lamellarin D shows: 20-OH on ring A is essential to maintain cytotoxicity [2,5,6] and participate in hydrogen bond interactions with the side chains of Glu356 [14]. Incorporating amino acid residues [15], nuclear localization signal peptide conjugates [16] or PEG conjugates [17] on 20-OH may increase activity and solubility. Replacing the 20-OH group with a 20-sulphate group, its biological function would change from cytotoxic activity to HIV-1 inhibition [18]. The 21-$OCH_3$ group is not essential [5]. Lactone analogues with opened ring B show decrease of the cytotoxicity [13]. The carbonyl group of the lactone is a bonding side for Arg364 [14]. Replacement of the lactone ring with a lactam [19] ring leads to partial retainment of cytotoxicity. Certain derivatives with a 1-amine moiety rather than a 4-amine moiety showed even more potent cytotoxicity than that of lamellarin D [20]. The $\Delta^{5,6}$ of ring D is an essential element for Topo I inhibition and retaining anti-proliferative activity [2]. The 8-OH of ring E is essential for cytotoxicity [5,6] and participates in hydrogen bond interactions with the side chains of Asn722 [14]. The 14-OH and 13-$OCH_3$ groups of ring F are not essential to maintain the cytotoxicity [5,6]. The amino derivative with a 3-aminoprop-1-en-2-yl group rather than a ring F shows inhibition against Topo I but introduces apoptosis through mitochondria mediated pathway [3]. Topo I inhibition and anti-proliferative activity for the analogues without F ring [21] have not been reported. We now report a novel design and total synthesis of Mannich derivatives of lamellarin D, which is described in details in the following sections.

## 2. Mannich derivation design

Increasing the water-solubility of lamellarin derivatives has been posed as a main challenge for structural modification and

* Corresponding author.
*E-mail address:* huyz@zju.edu.cn (Y. Hu).

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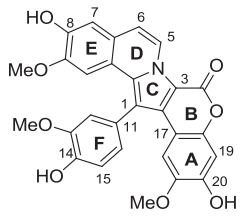


**Fig. 1.** Lamellarin D.

optimization. Incorporating amino acid residues [15], nuclear localization signal peptide conjugates [16] or PEG conjugates [17] on 8, 14, 20-OH of lamellarin D has been reported, however no significant improvement in activity and solubility have been observed yet. Enlightened by the modification route from the typical Topo I inhibitor Camptothecin (CPT) to Topotecan and based on classical drug design theory, a series of Mannich derivatives of lamellarin D were designed by attaching different Mannich base groups onto A-, E- or F-ring of lamellarin D together with the application of several protection strategies on hydroxy groups at 8-, 14-, and 20-position of lamellarin D (Fig. 2).

## 3. Synthetic studies

Based on our previous work in synthesizing lamellarin analogues [22], a modified *N*-ylide-mediate pyrrole formation strategy was adopted, with isovanilin and vanilin as starting materials (Scheme 1). The retrosynthetic analysis on the basis of **SL-1**, **SL-6** and **SL-7** as the model compounds showed that selectively exposing the OH group of C-8, 14 or 20 position could successfully introduce aminomethyl group on C-7, 15 or 19 position through Mannich reaction. And the lamellarin D with selectively protected hydroxyl groups could be built on lactone formation of *N*-ylide-mediate pyrrole which condensed with isoquinoline and phenacyl bromide. The phenacyl bromide could be prepared from isovanilin through Baeyer–Villiger oxidation, hydrolyzation and Friedel–-Crafts acylation, and then selectively protected hydroxyl groups and bromination in turn. The isoquinoline could be prepared through Bischler–Napieralski reaction of ethanamide which condensed with phenylacetic acid from isovanilin and phenylethanamine from vanillin. Furthermore, the strategy of phenol protection should be considered at the beginning of the multistep synthesis.

As an important part of our research work on selective protection hydroxy groups of lamellarin D, protection strategies of phenol groups on C-8, 14 and 20 positions were considered at the beginning of the synthetic work according to the reagents and reaction conditions. Based on the experience of trials, we found that MOM, TMS and TBDMS were unstable under bromination and Henry reaction, so that they did not meet our requirements in phenacyl bromide or ethanamine part on $R^1$, $R^3$ and $R^4$. The Ac and Ms were unstable under base condition, so these two were not suitable to choose on $R^2$ of phenylacetic acid part. The PMB was not suitable to choose on $R^1$, $R^2$ or $R^3$, because it was easy to be deprotected on $R^1$, $R^2$ or $R^3$ at the presence of DDQ, which was the key reagent for the oxidation of $\Delta^{5,6}$. After repeated tests, we finally selected Ms for $R^1$ protection, Bn for $R^2$ protection, i-Pr for $R^3$ protection and Ac for $R^4$ protection.

On the basis of retrosynthetic analysis and selective protection strategy, we took the synthetic route starting from vanillin and isovanilin (Scheme 2).

Firstly, one part of isovanilin underwent Baeyer–Villiger oxidation, hydrolysis, Friedel–Crafts acylation [23,24], methanesulfonation, acetation and α-bromination [25] to obtain **6**. Another part of isovanilin underwent hydroxyl protection, Henry reaction [26–28] on aldehyde and reduction [22,28–31] to obtain **9**. Secondly, the vanillin was treated with protection of hydroxyl group, reduction, nucleophilic substitution [32] and hydrolysis [22] to give **14**. Then **14** and **9** were directly acylation [33] to yield **15**, which was transferred to isoquinoline **16** by Bischler–Napieralski reaction [22,33]. Condensation of isoquinoline **16** with phenacyl bromide (**6**) under basic condition [22,25,34] gave the 1,2-diphenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**17**). Through Vilsmeier–Haack formylation [25], a formyl group was introduced at C-3 position of **17** to provide **18**. Then the formyl group of **18** was oxidized [35] to carboxyl group to give **19**. Hydrolysis of 19 with 17% NaOH aqueous solution afforded the phenol derivative (**20**), which was transformed into lactone (**21**) by intramolecular cyclization reactions. Then reaction of **21** with DDQ [36,37] afforded the lamellarin D with the 8, 14 and 20-OH groups selectively protected (**22**). Finally, the target compounds **SL-1**~**11** was obtained through selective exposure of hydroxyl groups [5,38,39], Mannich reaction [40] and deprotection of residual hydroxyl groups from **22** in turn. Furthermore, lamellarin 501 could be obtained through deprotection of the benzyl, iso-propyl and mesyl groups of **21**, similarly lamellarin D could be obtained through the same method from **22**.

As a result, eleven novel mono- and bis-Mannich derivatives of lamellarin D **SL-1**~**11** (Table 1) were synthesized in 26–27 steps starting from vanillin and isovanilin. Lamellarin D and lamellarin 501 were synthesized and served as the reference compounds for biological studies.

## 4. Biological studies and results discussion

The synthesized compounds (**SL-1~11**) were then measured for their Topo I inhibitory activities by using a Topo I enzyme-reagent kit (Takara, D2240A). And their anti-proliferation activities were measured *in vitro* against four human cancer cell lines, including colon carcinoma HT-29, breast carcinoma MDA-MB-231, leukaemia K562 and liver hepatocellular carcinoma HepG2. Lamellarin 501 (**SL-12**) and lamellarin D (**SL-13**) were used as the negative control and positive control respectively. The results are summarized in Fig. 3 and Table 1.

As illustrated in Fig. 3, in the absence of Topo I (**No Enzyme**), plasmid DNA mostly maintained the supercoiled status in the gel. Meanwhile supercoiled DNA was entirely relaxed after adding Topo I. As expected, lamellarin 501 (**SL-12**), the negative control, didn't show significant inhibition against Topo I. In contrast, after administrating lamellarin D (**SL-13**), the amount of supercoiled DNA was significantly increased, which in line with previous reports that lamellarin D inhibited the catalytic activity of Topo I. These results were consistent with previous reports [2,6,41]. Nine Mannich derivatives **SL-2**, **3**, **4**, **5**, **7**, **8**, **9**, **10** and **11**, rather than **SL-1** and **6**, exhibited Topo I inhibitory activities (summarized in Table 1) in comparison with the parent compound lamellarin D. The Topo I inhibition of **SL-9** was stronger than that of lamellarin D.

The results of *in vitro* anti-proliferative activity evaluation revealed that eleven Mannich derivatives of lamellarin D exhibited moderate to potent anti-proliferative activities against tested four human cancer cell lines. The anti-proliferative activities of **SL-1~5** and **SL-11** against HT-29 were even better than that of lamellarin D. **SL-1** and **SL-5** showed better anti-proliferative activities than other derivatives against all the four cancer cells.

The type and position of Mannich base groups on target compounds could influence their biological activities. Generally speaking, compounds (**SL-1~6**) with a Mannich base group at C-19 or C-7 position showed more potent anti-proliferative activities