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Design, Synthesis and Structure-Activity Relationship Studies of Glycosylated Derivatives of Marine Natural Product Lamellarin D

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ABSTRACT

Lamellarin D, a marine natural product, acts as a potent inhibitor of DNA topoisomerase I (Topo I). To modify its physicochemical property and biological activity, a series of mono- and di-glycosylated derivatives were designed and synthesized through 22–26 multi-steps. Their inhibition of human Topo I was evaluated, and most of the glycosylated derivatives exhibited high potency in inhibiting Topo I activity as well as lamellarin D. All the 15 target compounds were evaluated for their cytotoxic activities against five human cancer cell lines. The typical lamellarin derivative ZL−3 exhibited the best activity with IC_{50} values of 3 nM, 10 nM, and 15 nM against human lung cancer A549 cells, human colon cancer HCT116 cells and human hepatocellular carcinoma HepG2 cells. Compound ZL−1 exhibited anti-cancer activity with IC_{50} of 14 nM and 24 nM against human colon cancer HCT116 cells and human hepatocellular carcinoma HepG2 cells, respectively. Cell cycle analysis in MDA-MB-231 suggested ZL−3 inhibited cell growth through arresting cells at the G2/M phase of the cell cycle. Further tests showed a significant improvement in aqueous solubility of ZL−1 and ZL−7. This study suggested that glycosylation could be utilized as a useful strategy to optimize lamellarin D derivatives as Topo I inhibitors and anticancer agents.

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1. Introduction

Lamellarins were firstly isolated by Faulkner and coworkers from the prosobranch mollusk *Lamellaria* sp. on Ngarol Island, which contain a common 14-phenyl-6*H*-benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinoline scaffold [1]. In the past 30 years, more than 70 lamellarins and their analogues have been found mainly but not exclusively in a variety of marine mollusks, sponges, and ascidians [1–4]. They can be classified into two categories according to their structural features [5–7], including lamellarin *type I*: pentacyclic skeleton with a pyrrolo [2,1-*a*]isoquinoline core, and lamellarin *type II*: monocycle skeleton with an unfused 3,4-diarylpyrrole core. The lamellarins exhibit broad anticancer activity [8,9], and the majority of Structure-Activity Relationship (SAR) studies indicated that they are multi-target anticancer agents, for instance, they have Topo I inhibition activity [10,11], mitochondrial apoptotic activity [12–14], reversal of multidrug resistance activity [15,16], and kinase inhibition activity [17,18]. In addition, some lamellarins even displayed immunomodulating activity [19,20], HIV integrase inhibitory activity [19,21], antioxidant, and antibacterial [22] activities et al.

One of the most notable lamellarins is lamellarin D, which has been proved to be a potent inhibitor of Topo I [10,23,24]. Bailly and coworkers proposed a ternary complex model of DNA-Topo I-lamellarin D by docking simulation studies [25], which demonstrated that the hydroxy groups at C_8 and C_{20} are hydrogen-bonded with Asn722 and Glu356, and the carbonyl of the lactone ring is hydrogen-bonded with Arg364 [25]. Besides, the SAR study indicated the C_5=C_6 double bond ensures the planarity, which is essential for Topo I inhibition of lamellarin D [26–28].

Despite the potent biological activities of lamellarin D *in vitro*, further progression was limited due to its poor aqueous solubility [29], which is caused by its unique pentacyclic skeleton. For improving the solubility, intensive researches have been developed on lamellarin D, for instance, introducing nuclear location signal peptide [25], amino acid residues [30], PEG conjugates [29] or Mannich side chains [31].

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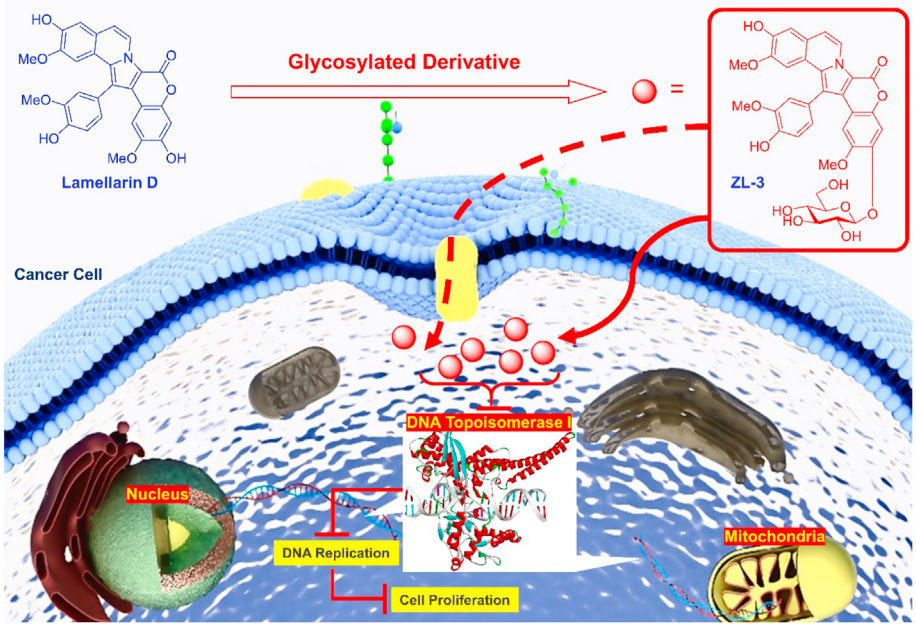


Fig. 1. Novel glycosylated derivative of marine natural product lamellarin D as a cytotoxic agent towards cancer cells.

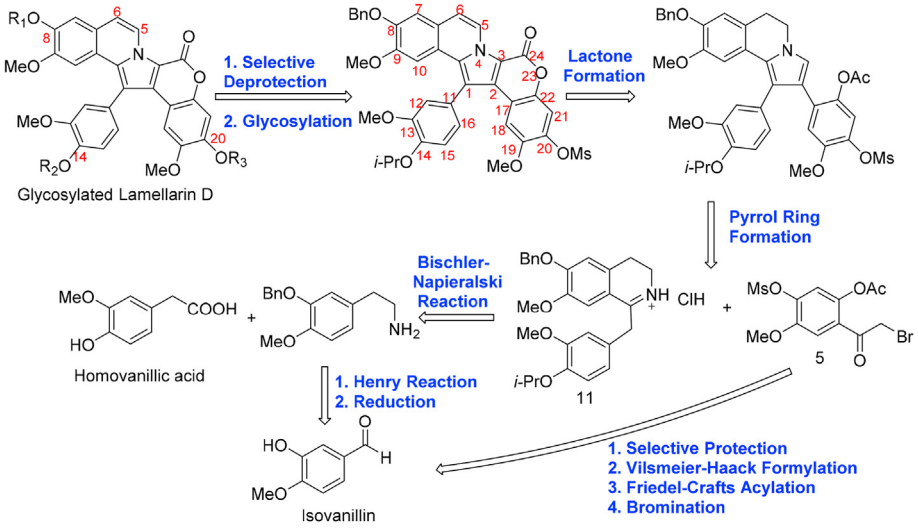
Glycosylation is an effective method to improve the solubility of lead compounds [32–34]. The use of glycosyl moieties generally improves biological activity [35] and targeting effect [36]. Otto Warburg and coworkers demonstrated that cancer cells exhibit an increased dependence on glycolytic pathways even in the presence of sufficient oxygen [37], which indicated that cancer cells would consume more glucose during their high-speed growth than normal cells. Such theory was proved to be related to the over-expression of membrane glucose transporters GLUT in cancer cells [35,38,39]. Further studies showed a variety of anticancer drugs, such as doxorubicin and paclitaxel, could be delivered specifically to cancer cells by covalently binding to carbohydrates [35,40,41]. Hence, novel glycosylated optimization on C-8, 14, or 20-OH of lamellarine D could be an effective strategy for improving the physicochemical bioactive properties which has not been applied in the research of lamellarin D yet.

Taking all the above-mentioned aspects into consideration, we initiated our work to generate a new class of antineoplastic agents via attaching different glycosyl groups on hydroxy groups at C-8, 14 or 20 position of lamellarin D, to improve the solubility and cytotoxic activity of lamellarin D (Fig. 1). In this study, fifteen mono- and di-glycosylated analogues were designed and synthesized with glucose [35], mannose, galactose [34,42] or ribose [43] as glycosyl donors, and all these glycosylated derivatives were tested for their cytotoxicity towards cancer cells and Topo I inhibition, in parallel with the structure–cytotoxicity analysis.

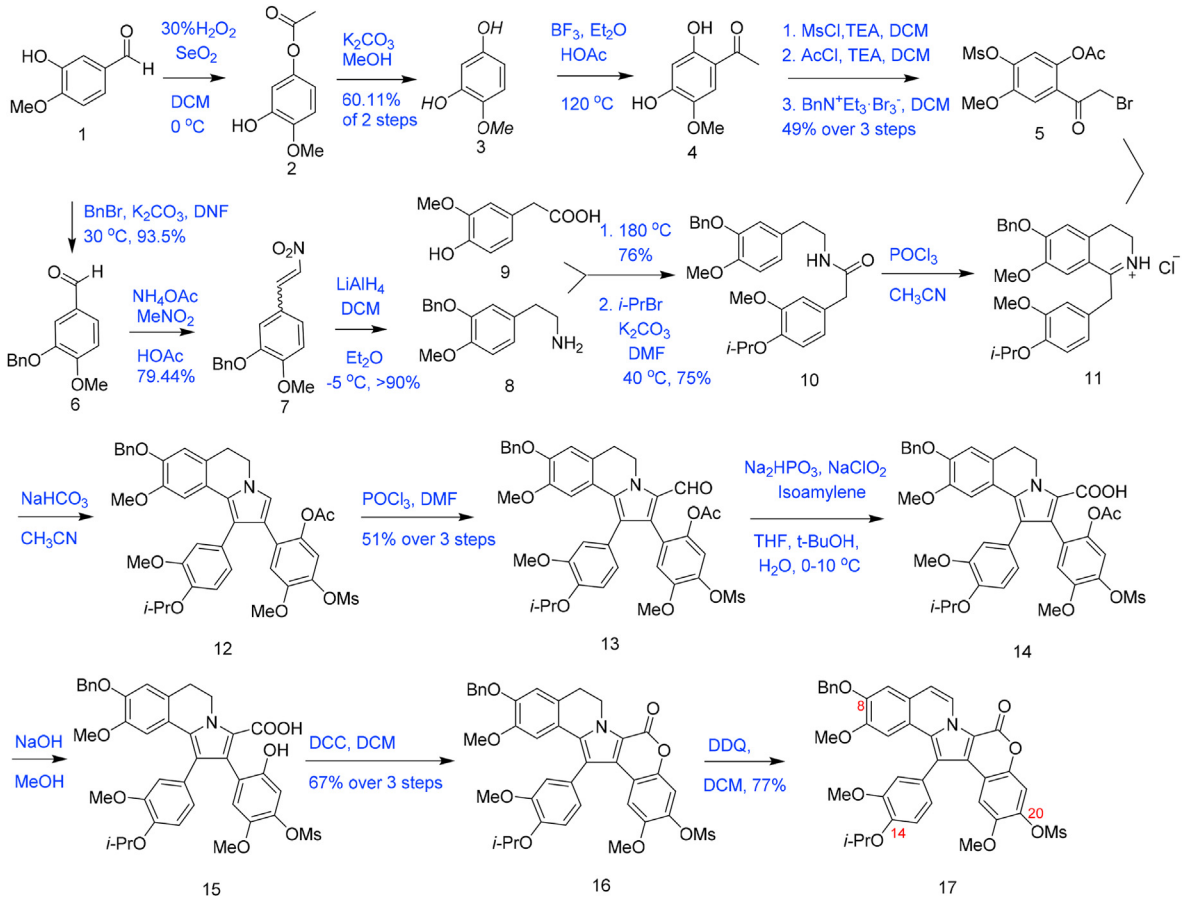
2. Results and discussion

2.1. Chemistry

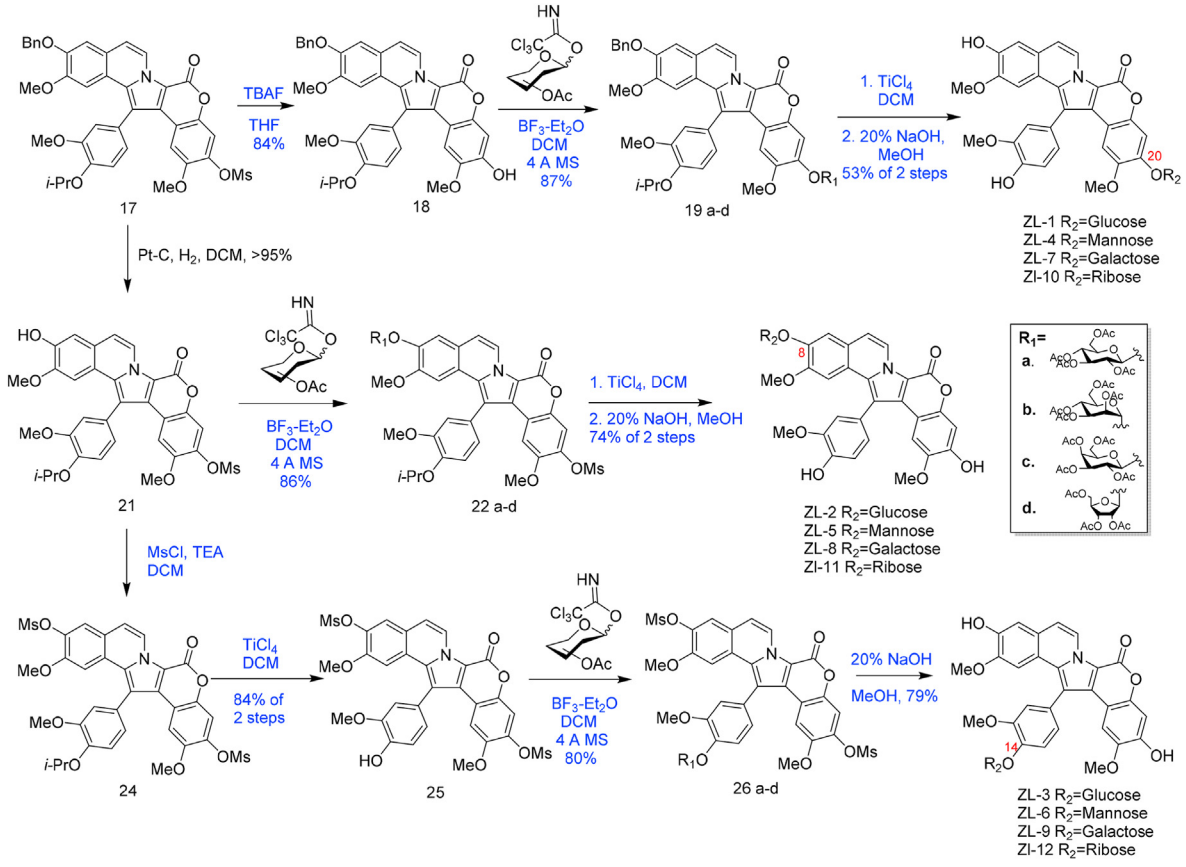
The formation of pyrrole ring through a modified *N*-ylide method [44–46] has been adopted as the key step in our route, which has been shown in Scheme 1 for the retrosynthesis. The glycosylated lamellarin D could be started from the lamellarin D derivative with C-8, 14 and 20-OH selective exposure. The lactone ring D was



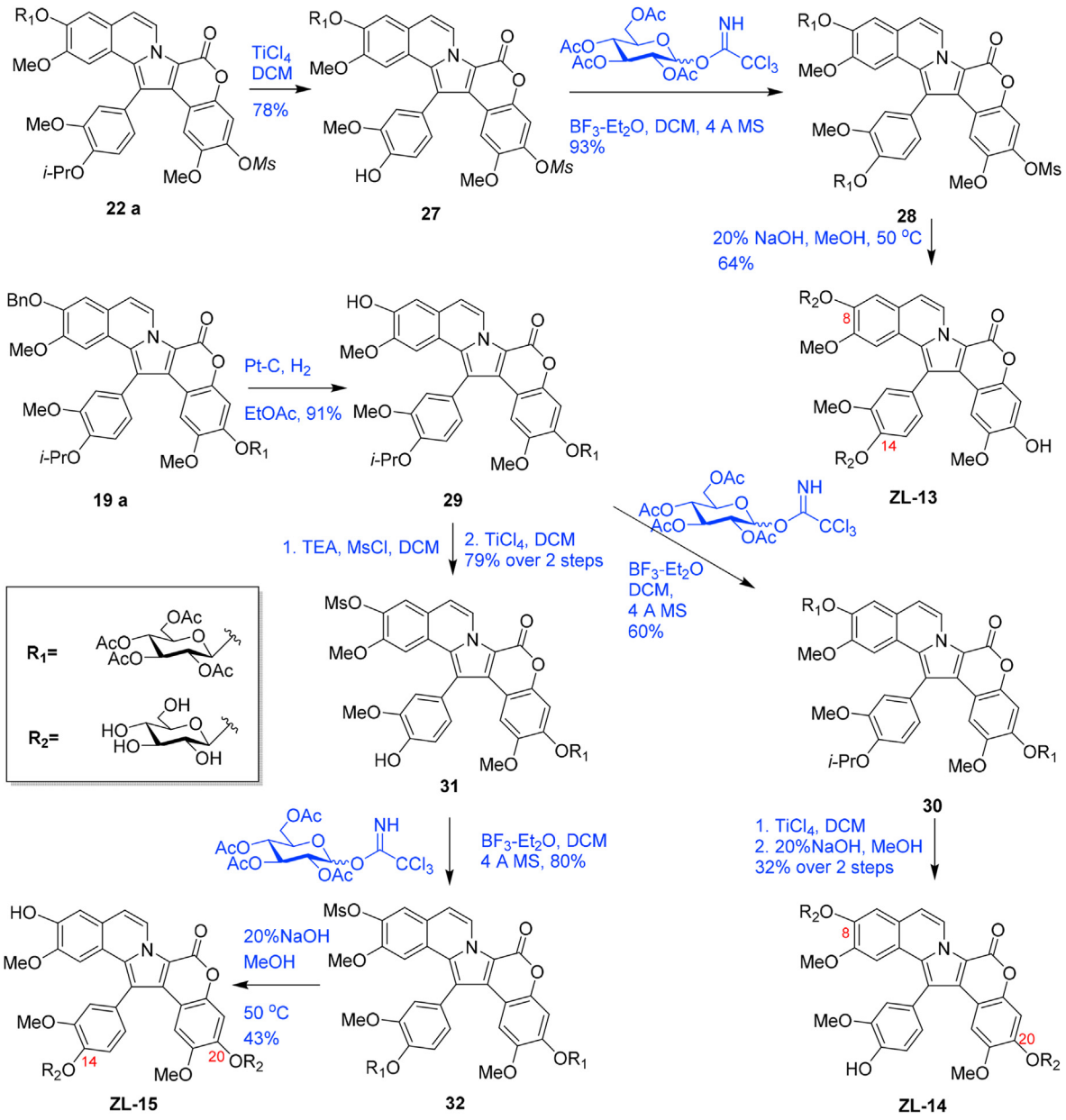
Scheme 1. Retrosynthetic analysis for monoglycosylated lamellarin D.



Scheme 2. Synthesis of hydroxyl selectively protected lamellarin D.



Scheme 3. Synthesis of the monoglycosylated derivatives of lamellarin D.



Scheme 4. Synthesis of the diglycosylated derivatives of lamellarin D.

constructed at a late stage from *N*-ylide mediated pyrrole from the key precursor phenacyl bromide 5 and isoquinoline 11 which would be achieved by multistep reactions from commercially available homovanilic acid and phenylethanamine 8. Both of the phenylethanamine 8 and phenacyl bromide 5 could be synthesized starting from isovanillin.

Based on our previous work [31] (Scheme 2), the preparation of lamellarin D derivative with all phenols protected 17 began with the commercially available isovanillin 1, followed by Baeyer-Villiger oxidation, hydrolysis, Friedel-Crafts acylation [47], methanesulfonation, acetation and α-bromination to obtain phenacyl bromide 5. Meanwhile, isovanillin was readily converted into phenylethanamine 8 via a three-step sequence involving hydroxyl protection, Henry reaction [48,49] and reduction. Amide moiety 10 was obtained by acetylation of phenylethylamine 8 and homovanilic acid 9, and it was subsequently submitted to a Bischler-Napieralski reaction [47], affording isoquinoline 11. Condensation of isoquinoline 11 with phenacyl bromide 5 under basic condition gave the key intermediate 12. Then the carboxylic acid 14 was afforded by a Vilsmeier-Haack formylation 13 [44] and oxidation. Later, the acetyl group of 14 was further hydrolyzed to give the compound 15 which was then transformed to lactone 16 by intramolecular cyclization. Lactone 16 was treated with DDQ [50] to provide the compound 17.

As shown in Scheme 3, the synthesis of the glycosylated analogues was started with hydroxy groups selectively protected lamellarin D 17. Treatment of 17 with different methods allowed to obtain hydroxy groups selectively exposed lamellarin D (18, 21 or 25), which were then treated with trichloroacetimidate glycosyl donors (glucose, mannose, galactose and ribose) to afford the essential precursors. The remaining hydroxyl protecting groups were removed to provide mono-glycosylated Lamellarin D ZL 1–12 as target moleculars.

After successful synthesis of the mono-glycosylated lamellarin D, we turned our attention towards the synthesis of di-glycosylated

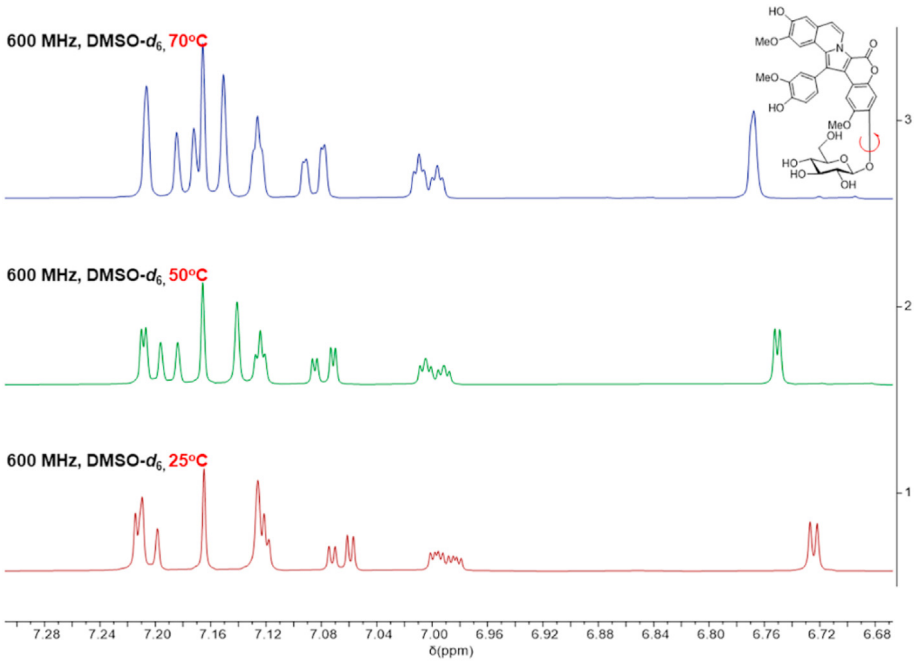


Fig. 2. Variable temperature NMR experiment of compound ZL-3 in DMSO-d_6 from 25 to 70 °C.

lamellarin D (Scheme 4), which began with a hydroxyl selective exposure of 19a or 22a, followed by a similar synthetic sequence (glycosylation and deprotection of hydroxyl group), to furnish the final di-glycosylated lamellarin D ZL 13–15. However, after many attempts, we still failed to obtain tri-glycosylated derivatives.

Finally, fifteen target compounds were prepared using commercially available isovanillin, homovanilic acid, and glycosyl donors (glucose, mannose, galactose, and ribose) as the original material through 22–26 multi-steps, with an overall yield of 0.6–2.8%. All the target compounds were purified by silica gel column chromatography and identified by high resolution mass spectrometry (HRMS), ^{1}H and ^{13}C NMR spectroscopy (Supporting Information).

Interestingly, according to the effect of neighboring group participation, a single favorable steric configuration could be obtained. However, the ^{1}H and ^{13}C NMR spectra of most of the synthesized compounds showed the presence of two sets of NMR signals of comparable intensity, and the *J*-value of hydrogen on anomeric carbon is consistent, for example, the two sets of *J*-values on compound ZL-3 are 6.6 Hz. We assumed that the two sets of NMR signals are caused by a pair of slowly interconverting conformers and our assumption was supported by variable temperature proton spectra for typical compound ZL–3 (Fig. 2). The two rotameric underwent coalescence at 70 °C and these changes in the NMR spectra were reversible upon cooling, which demonstrated the presence of rotational isomers.

2.2. Biological evaluation

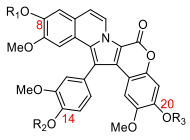
All of the lamellarin D analogues ZL 1–15 were assayed for their anticancer activities against five human cell lines, including human breast cancer cells (MDA-MB-231), human gastric carcinoma cells (HGC27), human lung cancer cells (A549), human colon cancer cells (HCT116) and human hepatoma cells (HepG2). The cytotoxic activities of the target compounds were evaluated by MTT assay and their IC_{50} values are shown in Table 1. Among the glycosylated derivatives, ZL–1, ZL–3, ZL–7, ZL–9 and ZL–14 showed potent anti-proliferation activities against the five cell lines mentioned above. The cytotoxic activities of ZL–1 ($IC_{50HCT116}$ = 14 nM; $IC_{50HepG2}$ = 24 nM) and ZL–3 ($IC_{50HCT116}$ = 10 nM; $IC_{50HepG2}$ = 15 nM) against HCT116 and HepG2 were even better than lamellarin D ($IC_{50HCT116}$ = 25 nM; $IC_{50HepG2}$ = 88 nM). ZL–4, ZL–6, ZL–10, ZL–11 and ZL–12 showed moderate cytotoxic activity against the five cancer cells with IC_{50} < 5 μM. However, cytotoxicities of lamellarin D bearing glycosyl group at C-14 OH (ZL–2, ZL–5, ZL–8, ZL–13 or ZL–15) were decreased in the cultures of all the five cell lines.

According to the IC_{50} values shown in Table 1, the cytotoxic activity was related to the monosaccharide types, the connecting position and amounts on the lamellarin D. In most case, the tested compounds with glycosyl group at C-8 or 20-OH position showed much better anti-proliferation activities than the test compounds with glycosyl group at C-14-OH position. The test compounds with glucose and galactose substitution generally resulted in higher anti-proliferation activities than mannose or ribose substitution. In addition, when introducing the same glycosyl group, mono-glycosylated derivatives exhibited better anticancer activities than the di-glycosylated derivatives.

To investigate the selectivity of glycosylated derivatives toward Topo I, we used *in vitro* Topo I relaxation inhibition assays. As illustrated in Fig. 3, the supercoiled DNA entirely changed to relaxed DNA in the presence of Topo I, and the positive control lamellarin D inhibited the relaxation, as indicated by the increase of the supercoiled DNA, which was because lamellarin D stabilizes Topo I-DNA cleavable complexes to inhibit Topo I activity. Based on the result of Topo I inhibitory assays, most of glycosylated derivatives showed potent Topo I inhibitory activity which were similar to lamellarin D. Unexpectedly, ZL–2, ZL–5, ZL–8, ZL–13, and ZL–15 retained the Topo I inhibitory activity, but their anti-proliferative activities were decreased compared with lamellarin D. There might be other targets or mechanisms of the glycosylated derivatives for the anti-proliferative activities in different levels.

The effects on the cell cycle distribution of compound ZL–3 in MDA-MB-231 cells were examined by flow cytometry after staining of the cells with propidium iodide (Fig. 4A). MDA-MB-231 cells

Table 1
Structures and *in vitro* cytotoxicity of glycosylated lamellarin D.



Comp.	R1	R2	R3	IC_{50} (μM)				
				MDA-MB-231	HGC27	A549	HCT116	HepG2
ZL−1	Glu	H	H	0.032	0.114	0.047	0.014	0.024
ZL−2	H	Glu	H	>50	>50	>50	26.236	42.922
ZL−3	H	H	Glu	0.034	0.162	0.003	0.010	0.015
ZL−4	Man	H	H	1.991	0.219	3.239	1.424	3.881
ZL−5	H	Man	H	>50	31.347	>50	>50	>50
ZL−6	H	H	Man	0.316	0.137	0.374	0.206	0.570
ZL−7	Gal	H	H	0.018	0.114	0.013	0.037	0.048
ZL−8	H	Gal	H	>50	16.594	>50	13.735	>50
ZL−9	H	H	Gal	0.025	0.090	0.020	0.018	0.058
ZL−10	Rib	H	H	5.081	1.238	4.121	1.283	0.937
ZL−11	H	Rib	H	2.113	0.224	0.659	0.614	0.332
ZL−12	H	H	Rib	2.693	0.862	2.856	1.156	0.627
ZL−13	Glu	Glu	H	13.257	27.080	13.707	5.283	40.101
ZL−14	Glu	H	Glu	0.036	0.074	0.026	0.040	0.118
ZL−15	H	Glu	Glu	7.373	7.218	16.363	6.771	28.771
Lam-D	H	H	H	0.031	0.236	0.004	0.025	0.088

(IC_{50} : the half-maximal inhibitory concentration; Lam-D: lamellarin D; MDA-MB-231: human breast cancer cells; HGC27: human gastric carcinoma cells; A549: human lung cancer cells; HCT116: human colon cancer cells; HepG2: human hepatocellular carcinoma cells.).

were exposed to ZL−3 (10 nM, 20 nM and 50 nM) for 24 h and lamellarin D (20 nM and 50 nM) as the positive control. As shown in Fig. 4A and B, both ZL−3 and lamellarin D induced an increase of cells in the G2/M phase arrests in a concentration-dependent manner. Notably, ZL−3 caused more accumulation of cells in G2/M phase, compared to lamellarin D as the positive control. And apoptosis significantly occurred in MDA-MB-231 cells after treatment with a higher concentration of ZL−3 (Fig. 4A and C).

To evaluate the binding mode of glycosylated derivatives, molecular docking studies were performed in the active sites of Topo I (PDB code: 1k4t) by AutoDock 4.0. To confirm that our top docking pose was reasonable, lamellarin D was docking into the binding site of the crystal structure contain ligand camptothecin (CPT), and the fact confirmed that the binding poses of the lamellarin D were successfully docked in the correct binding site in the DNA-Topo I complex.

Top binding poses of lamellarin D (Fig. 5A) showed that the hydroxy groups at C_8, C_{14}, C_{20} and the carbonyl oxygen of the lactone ring were hydrogen-bonded with Glu356, Asn352, Asn722 and Arg364. ZL−1 (Fig. 5B, −10.1 kcal/mol), ZL−3 (Fig. 5D, −10.27 kcal/mol) displayed a better docking energy for DNA-Topo I complex than ZL−2 (Fig. 5C, −9.46 kcal/mol), which was in consistent with the experimental *in vitro* anti-cancer activity observed. The hydroxy groups at C_{14} and C_{20} of ZL−1 established hydrogen bonds with Asn352, Asn722, and the 6-OH of glycosyl formed hydrogen bonds with Glu 356. As for ZL−2, the absence of the C-14 hydroxyl reduced the stability of the ternary complex, only the carbonyl oxygen of the lactone ring bound to Arg364, and the hydroxy group of glycosyl bound to Asn352, Tyr426. However, due to the change of glycosyl configuration, the hydrogen bonds between glycosyl and enzyme were not fixed. In the binding of the ZL−3-DNA-Topo I complex, the hydroxy groups at C_8 and C_{14} and the carbonyl oxygen of the lactone ring were engaged in hydrogen bonds with Asn722, Asn352, and Arg364, and the 6-OH of glycosyl was hydrogen-bonded with Glu356.

As shown in Fig. 6, all three di-glycosylated lamellarin D and tri-glycosylated lamellarin D displayed lower binding affinities for DNA-Topo I complex. The key interactions of the core of lamellarin D with DNA-Topo I complex were significantly disturbed when more glycosyl groups were placed on lamellarin D, such as in Fig. 6A, only the oxygen atom of the glycosyl group interacted with Asp533. In Fig. 6B, the carbonyl oxygen and oxygen atom of the lactone ring established hydrogen bonds with Arg364, the oxygen atoms of the glycosyl groups interacted with Asp533, Tyr426, and Thr718 residues through H-bonds. For ZL−15, the hydroxy group at C_8 was interacting with Met428 and Asn352. As for the tri-glycosylated lamellarin D, it engaged the glycosyl groups with hydrogen bonds to the Arg364, Asn722, Met428, and Tyr426.

The solubilities of lamellarin D, mono- and di-glycosylated lamellarin D derivatives were tested in distilled water with HPLC-HRMS method. As shown in Table 2, the solubility of lamellarin D was 0.563 nM, and some of the glycosylated derivatives possessed significantly improved water solubility than that of lamellarin D. It should be noted that ZL−1, ZL−7 and ZL−15 had the best water solubility of 257.78 nM, 235.82 nM and 257.55 nM, which were almost up to 500-fold higher than that of lamellarin D. However, the water solubilities of ZL−2, ZL−4, ZL−5, ZL−8 and ZL−9 were even not as good as that of lamellarin D. The water solubilities of ZL−10, ZL−11 and ZL−12 were lower than the detection limits.

As shown in Fig. 7, ZL−1 and ZL−7 bearing glucosyl or galactosyl group at C-8-OH position exhibited remarkable water solubility. However, introducing a mannosyl or ribosyl at C-8-OH position of lamellarin D led to ZL−4 and ZL−10, which showed poor water solubility. The water solubility decreased after introducing glycosyl into the C-14 or 20-OH position of lamellarin D. Compared with the mono-glycosylation derivatives, there was no significant improvement in the solubility of di-glycosylation derivatives. These results suggested that the aqueous solubility of glycosylated lamelllarin D was related to the types of monosaccharide and the connecting position on lamellarin D. The water solubility and cytotoxic activity has been compared, it was found that the compounds with good

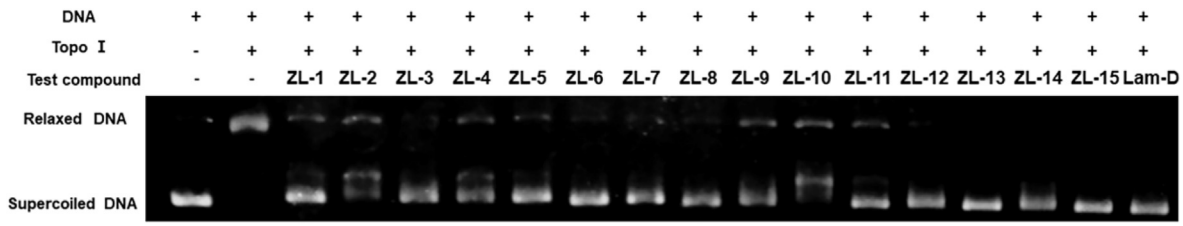


Fig. 3. Topo I inhibitory activity was evaluated by agarose gel electrophoresis. Inhibition of Topo I relaxation activity at 10 μM. Lamellarin D was a positive control. Reaction samples were incubated at 37 °C for 30 min and separated by electrophoresis on a 1% agarose gel, then stained with 4s green, and photographed under the irradiation of UV light. Lane 1 is pBR322 DNA only; Lane 2 is the mixture of pBR322 DNA and Topo I; Lines 3–17 are the mixture of pBR322 DNA, Topo I and test compounds. Line 18 is the mixture of pBR322 DNA, Topo I and positive control lamellarin D.

water solubility often showed potent antiproliferative activity such as ZL–1, ZL–7 and ZL–14. These data indicated that the water solubility of glycosylated derivatives could affect their antiproliferative potencies. Interestingly, ZL–3 and ZL–9 exhibited remarkable antiproliferative potencies, albeit their poor water soluble which meant that their antiproliferative activity did not entirely depend on the water solubility.

3. Conclusion

In this study, a new class of glycosylated derivatives were designed and synthesized based on the skeleton of lamellarin D, the corresponding cytotoxic activities, and Topo I inhibitions were investigated. The result demonstrated that most of the glycosylated derivatives kept the potent Topo I inhibitory activity which were similar to lamellarin D. The cytotoxicity was improved after glycosylation with glucose or galactose at the C-8 and 20-OH positions of lamellarin D. In contrast, the presence of a glycosyl residue at the C-14-OH position lowered the cytotoxicity, which was not consistent with their Topo I inhibitory activity, so there might be other targets of the glycosylated derivatives for anticancer activity. The docking study confirmed that C-8 or 20-OH glycosylated lamellarin D-Topo I-DNA ternary complex was stable than C-14-OH glycosylated lamellarin D-Topo I-DNA ternary complex, and with the increase of glycosyl groups, the compounds led to a lower binding affinity to the Topo I-DNA complex. In addition, when the MDA-MB-231 cells were exposed to ZL–3, the number of cells in G0/G1 phase decreased and those in G2/M phase increased compared with the control, and the population of cells in G2/M phase increased in a dose-dependent manner. The investigation suggested that ZL–3 induced cell-cycle arrest in the G2/M phase and increased cell apoptosis. The water-soluble study indicated that the introduction of glucosyl or galactosyl to the C-8-OH position of lamellarin D increased their aqueous solubility and their anticancer activity. The synthesis and study on tri-glycosylated derivatives or other derivatives are currently in progress.

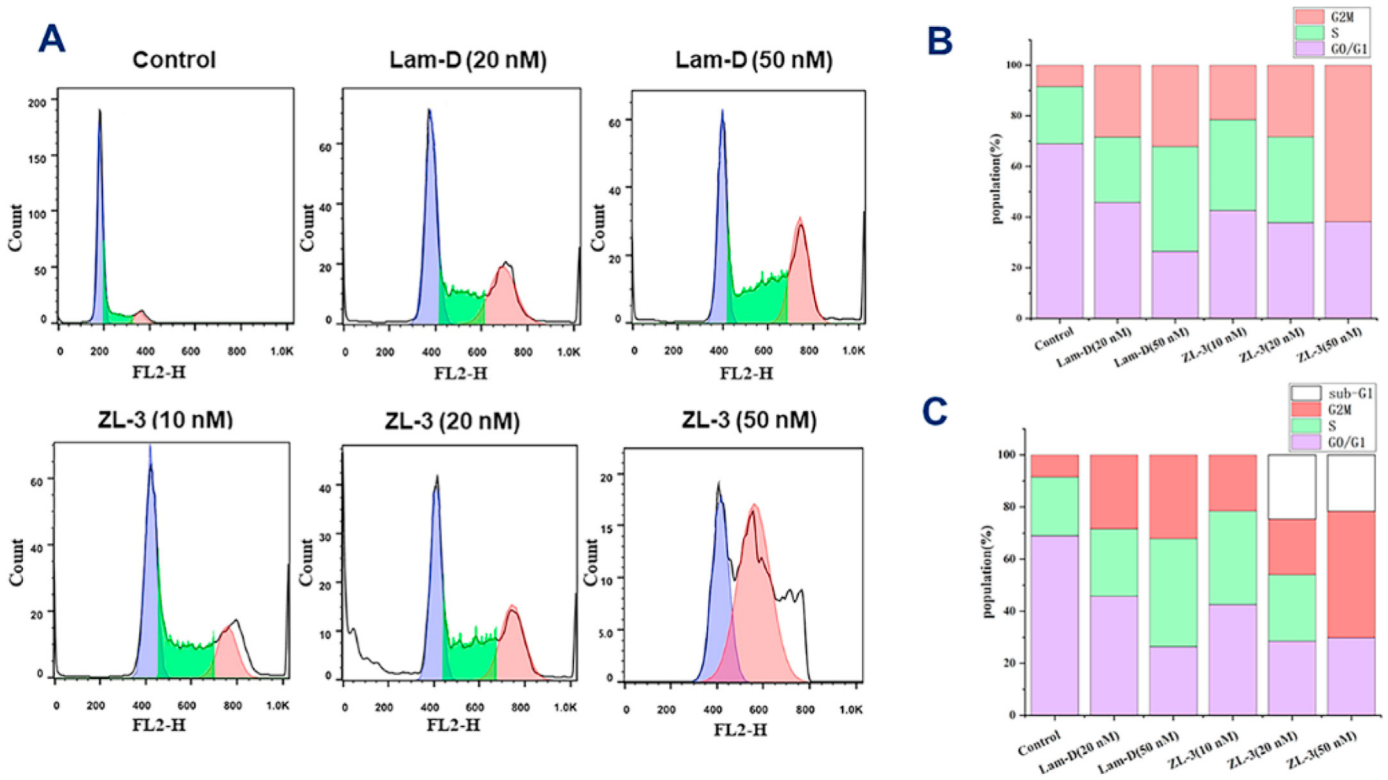


Fig. 4. (A) Cell cycle analysis: MDA-MB-231 cells were treated with lamellarin D (20 nM and 50 nM) or ZL–3 (10 nM, 20 nM and 50 nM) for 24 h and PI staining was used to analyze the cell cycle distribution. (B) The percentages of MDA-MB-231 cells in the different phases of cell cycle were presented. (C) The percentages of MDA-MB-231 cells in the different phases of cell cycle and sub-G1 were presented.

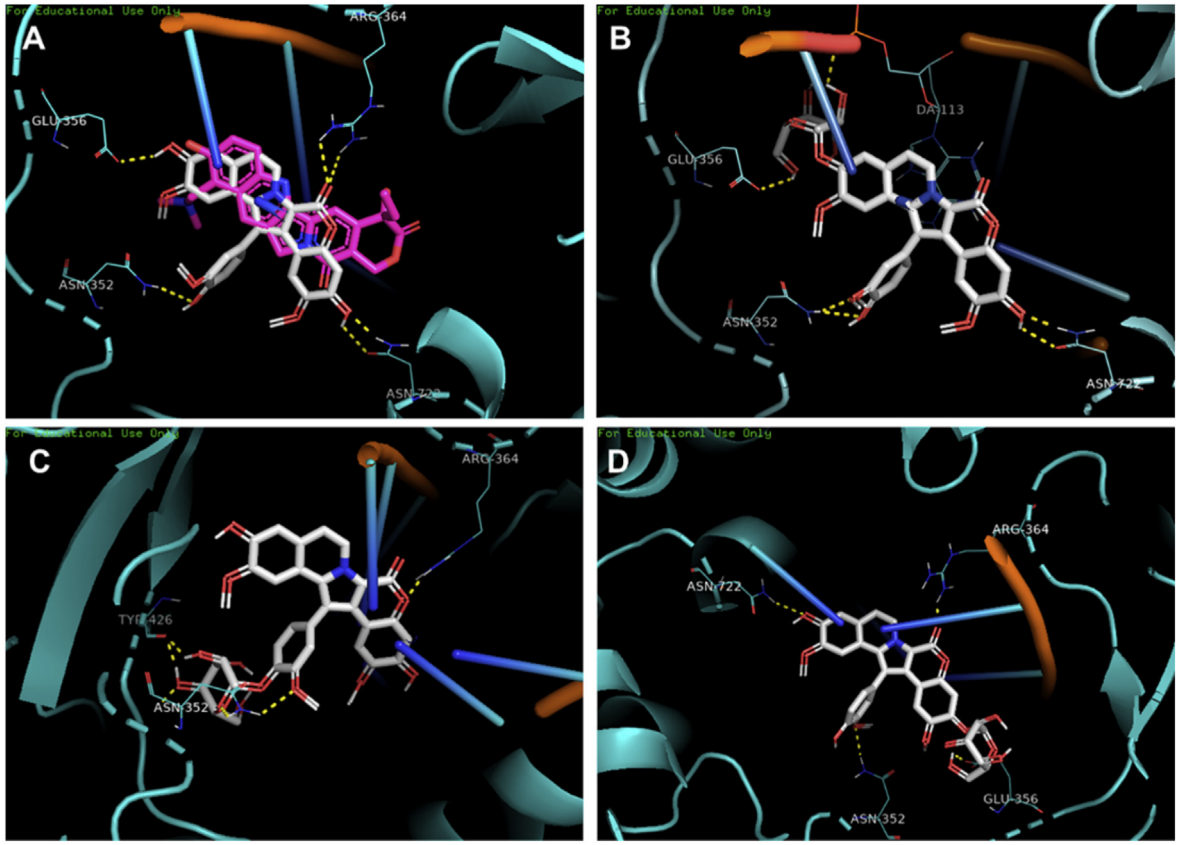


Fig. 5. Calculated binding poses of ligand to the DNA-Topo I complex (PDB ID: 1k4t (http://www.rcsb.org/structure/1K4T); cyan), key hydrogen-bonding interactions are indicated with yellow dashes. (A) Lam D (white)/CPT (magenta)-DNA-Topo I complex, the docking energy of lamellarin D-DNA-Topo I complex was estimated to be −10.87 kcal/mol (B) ZL−1 (white)-DNA-Topo I complex, the docking energy was estimated to be −10.10 kcal/mol (C) ZL−2 (white)-DNA-Topo I complex, the docking energy was estimated to be −9.46 kcal/mol; (D) ZL−3 (white)-DNA-Topo I complex, the docking energy was estimated to be −10.27 kcal/mol.

3.1. Experimental section

3.1.1. General procedures

Proton NMR and carbon NMR were performed on JNM-ECZ600R/S1 (^{1}H, 600 MHz; ^{13}C, 150 MHz). Waterless operation was performed on Unilab Pro Sp (1500/780). Low temperature reaction was performed on PSL2000. The organic solvents were dried and purified by MB-SPS-5. Water Solubility was performed on AB Sciex Triple TOF 5600+. Flash column chromatography was performed using silica gel (200−300 mesh) and sephadex LH-20. Visualization was achieved under a UV lamp (254 nm). Flow cytometry assay was performed on FACS Calibur. The organic solvents were dried and purified before use. Substrates 2−18, 21, 24 and 25 were prepared according to our previous work [31]. Experimental procedures and copies of ^{1}H, ^{13}C NMR spectra and HRMS spectra can be found in the Supporting Information.

3.1.2. Antiproliferative assay

Cells were collected and resuspended in DMEM growth medium +10% fetal bovine serum (FBS). The cells were seeded on 96-well plates at the concentration of 5 000 cells/well and incubated at 37 °C in 5% CO_2 overnight. The following day, 100 μL of compounds with serial dilutions were added into the corresponding wells, and the plates were further incubated at 37 °C for 72 h. The cell viability was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The medium was changed with a fresh medium containing 0.5 mg/mL of MTT, and the cells were then incubated for 4 h. The medium was removed and 100 μL of DMSO was added into the wells to dissolve the formazan crystals and the absorption at 540 nm was recorded by a microplate reader (labsystem multiscan). Cell proliferation rates for each well were calculated as follows: $A540_{treated}/A540_{control} \times 100\%$. IC_{50} values were determined as the *in vitro* concentration of compound required to inhibit cell proliferation by 50%.

3.1.3. Topo I inhibition assay

The reaction mixture (20 μL) contained 10X DNA Topoisomerase I Buffer (2 μL), supercoiled DNA (pBR322, 250 ng, TaKaRa Bio), Topo I (2 U), and indicated drug concentrations were incubated at 37 °C for 30 min. Reactions were terminated by the addition of 2 μL 10% SDS, digested with 2 μL proteinase K for 30 min. The reaction product was subjected to 1% agarose gel electrophoresis in the absence of 4s Green. After electrophoresis, the 4s Green-free gel were stained with 0.5 μg/mL 4s Green for 50 min prior to photodocumentation.

3.1.4. Flow cytometry assay

MDA-MB-231 (5 × 10^5) cells were seeded into six-well plates, cultured overnight at 37 °C, and treated with test compounds at indicated concentrations (10 nM, 20 nM, 50 nM) for 24 h. After drug treatments, the cells were collected by trypsinization, washed twice with phosphate-buffered saline (PBS), and fixed with 70% ethanol overnight at −20 °C. Before staining, cells were washed with PBS to remove ethanol. Fixed cells were stained with

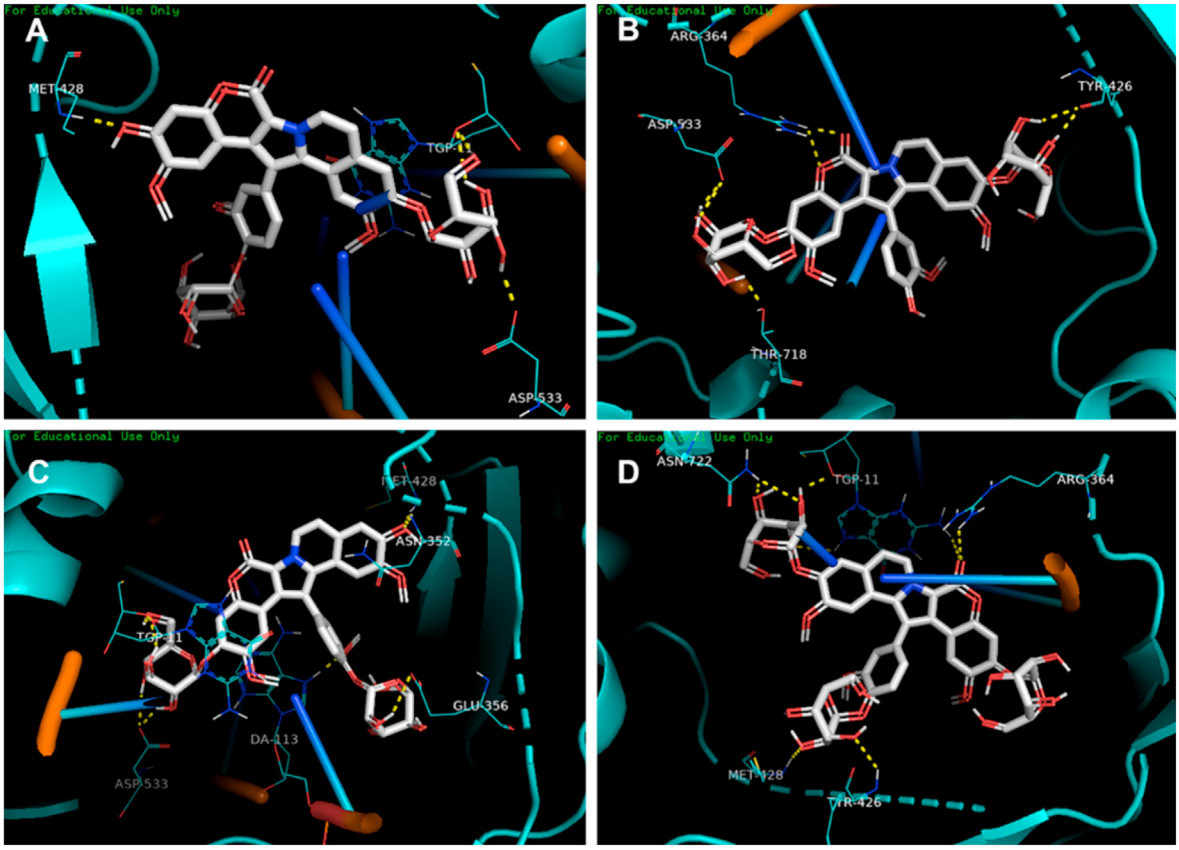
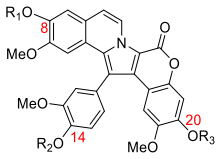


Fig. 6. Calculated binding poses of ligand to the DNA-Topo I complex (cyan), key hydrogen-bonding interactions are indicated with yellow dashes. (A) ZL−13 (white)-DNA-Topo I complex, the docking score was estimated to be −8.23 kcal/mol (B) ZL−14 (white)-DNA-Topo I complex, the docking score was estimated to be −10.17 kcal/mol (C) ZL−15 (white)-DNA-Topo I complex, the docking score was estimated to be −9.27 kcal/mol. (D) Triglycosylated lamellarin D (white)-DNA-Topo I complex, the docking energy was estimated to be −6.87 kcal/mol.

Table 2
Structures and aqueous solubility of glycosylated lamellarin D.



No.	R1	R2	R3	Aqueous solubility (nM)
ZL−1	Glu	H	H	257.78 ± 7.903
ZL−2	H	Glu	H	0.41 ± 0.090
ZL−3	H	H	Glu	2.00 ± 0.288
ZL−4	Man	H	H	0.23 ± 0.000
ZL−5	H	Man	H	0.14 ± 0.006
ZL−6	H	H	Man	0.37 ± 0.085
ZL−7	Gal	H	H	235.82 ± 5.451
ZL−8	H	Gal	H	0.49 ± 0.033
ZL−9	H	H	Gal	0.12 ± 0.001
ZL−10	Rib	H	H	<0.05
ZL−11	H	Rib	H	<0.05
ZL−12	H	H	Rib	<0.05
ZL−13	Glu	Glu	H	96.75 ± 8.796
ZL−14	Glu	H	Glu	182.31 ± 9.772
ZL−15	H	Glu	Glu	257.55 ± 10.369
Lam-D	H	H	H	0.56 ± 0.119

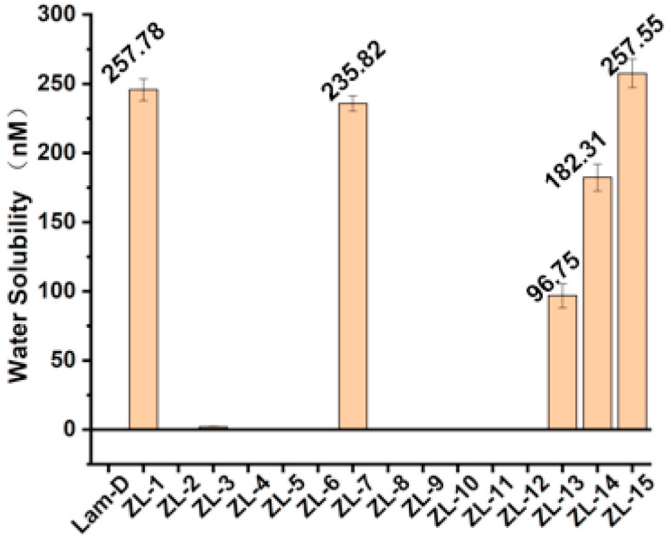


Fig. 7. The water solubility of lamellarin D and glycosylated derivatives. The aqueous solubility was tested by HPLC-HRMS method. The standard curves were shown in Supporting Information Figure. S1.

propidium iodide (20 μg/mL) in PBS containing RNase A (0.2 mg/mL) for 0.5 h at room temperature. Cell cycle distribution was analyzed using flow cytometer.

3.1.5. Water solubility study

The standard curves were made prior with HPLC-HRMS method. The test compounds were dispensed in 400 μL deionized water. The suspension was sonicated for 5 s, stranded for 30 min and centrifuged at the speed of 30,000 r/min, filtered on 0.45 μm cellulose membrane. Compound amount in saturated solution was evaluated by HPLC-HRMS analysis [51].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113226.

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