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Original article

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

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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

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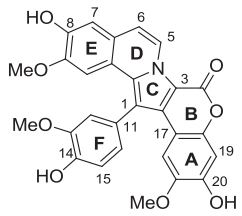


Fig. 1. Lamellarin D.

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

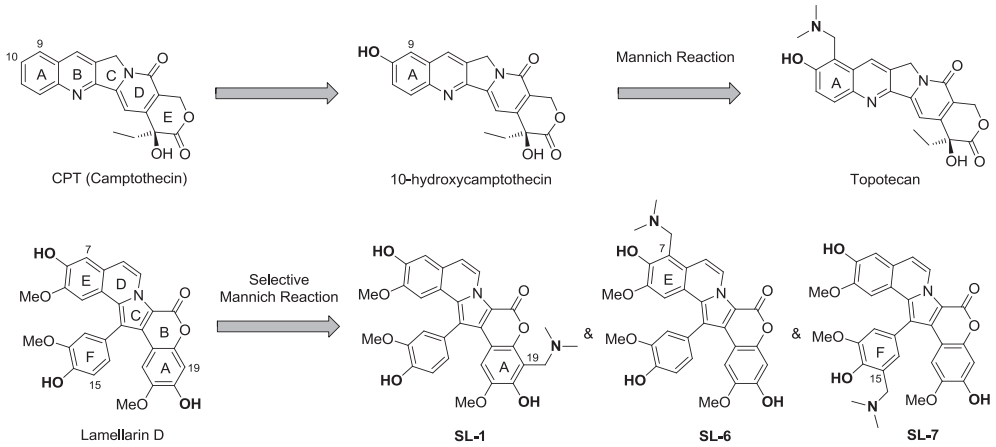
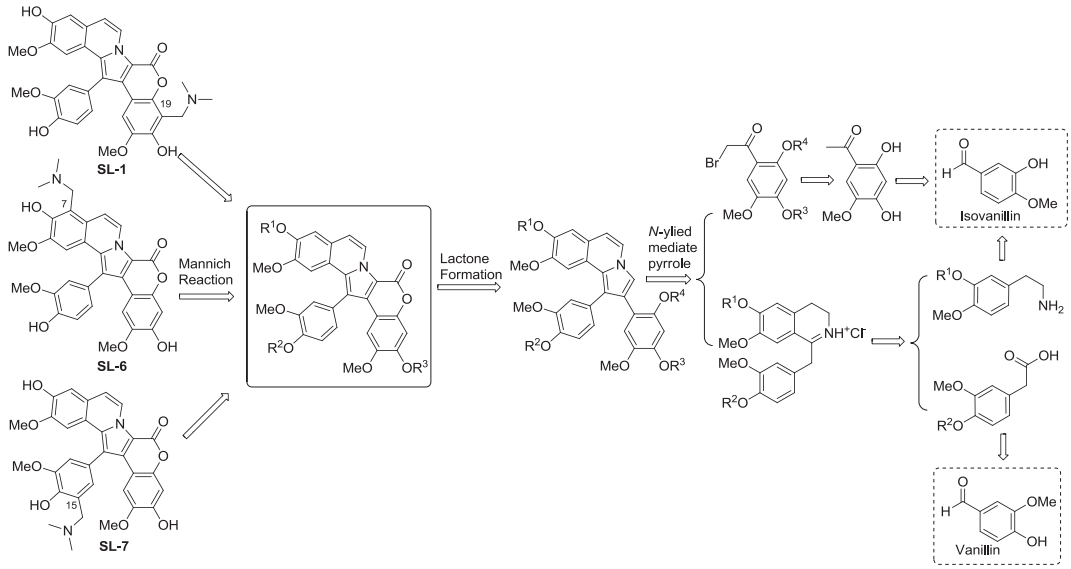


Fig. 2. Mannich derivation design on CPT (camptothecin) and lamellarin D (**SL-1**, **SL-6** and **SL-7** as the model compounds).



Scheme 1. Retrosynthetic analysis and strategy for the synthesis of the Mannich derivatives of lamellarin D, in which **SL-1**, **SL-6** and **SL-7** were chosen as the model compounds.

than that of compounds **SL-7~9** with a Mannich base group at C-15 position. Compounds (**SL-10** and **SL-11**) with two Mannich base groups at C-19 and C-7 position could keep their Topo I inhibition and anti-proliferative activities against HT-29, MDA-MB-231 and K562 cells. Unexpectedly, **SL-1** and **SL-6** lost the ability to inhibit Topo I, but their anti-proliferation activities were quite good. **SL-9** maintain potent Topo I inhibitory activity, but its anti-proliferative activity was not as good as its Topo I inhibition. There might be other targets or mechanisms of the Mannich derivatives for the anti-proliferative activity.

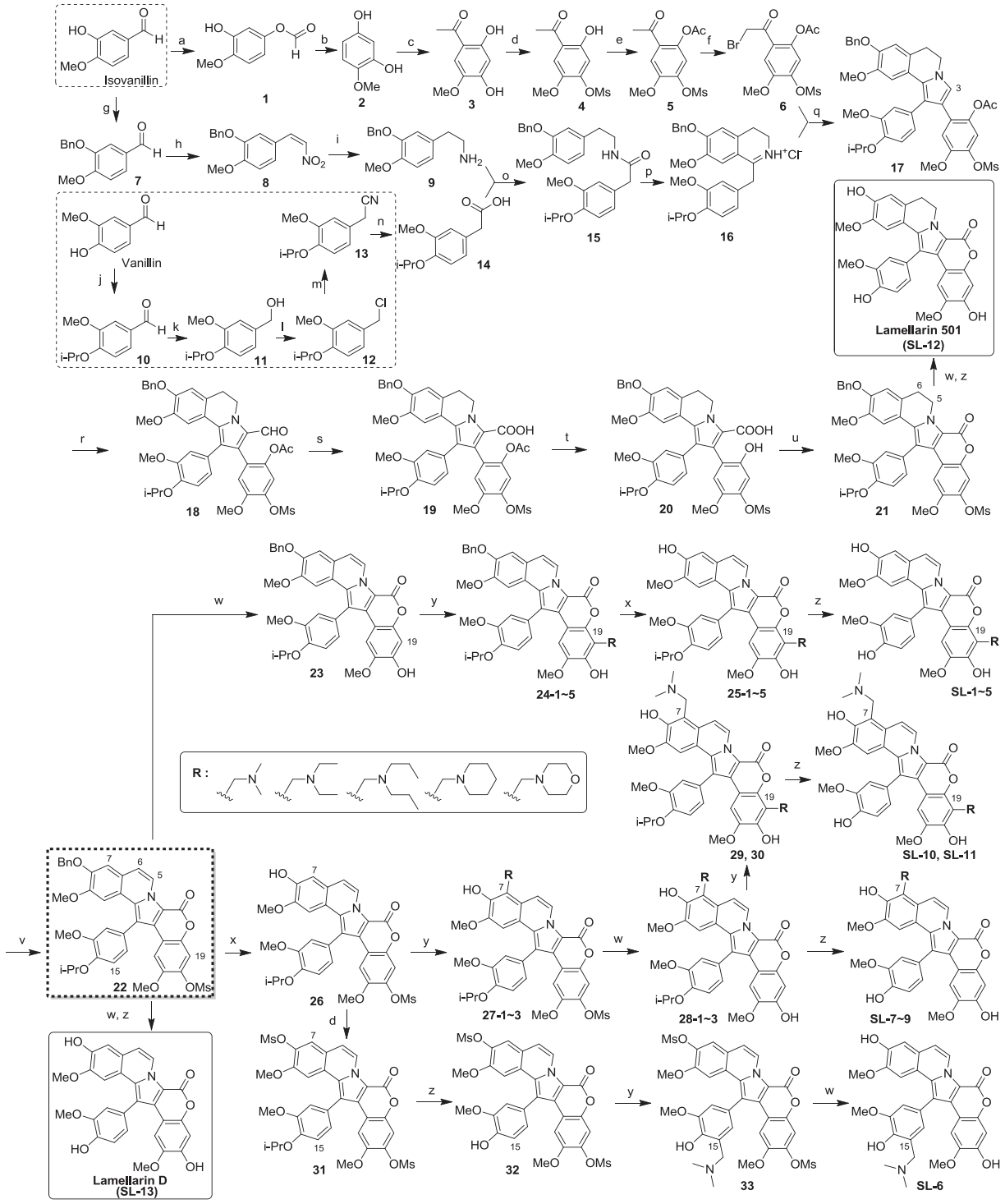
5. Conclusions

Based on the retrosynthetic analysis, we synthesized eleven novel lamellarin D Mannich derivatives were synthesized and evaluated for their Topo I activity and anti-proliferative activity. Most of the Mannich derivation of lamellarin D could keep the Topo I inhibitory activity and partially enhance the anti-proliferation activity against colon carcinoma HT-29, breast carcinoma MDA-MB-231, leukaemia K562 and liver hepatocellular carcinoma HepG2 cancer cell lines *in vitro*. Compounds **SL-2**, **3**, **4**, **5**, **7**, **8**, **10** and **11** exhibited moderate Topo I inhibitory activities. Compounds **SL-2**, **3**, **4**, **5** and **11** exhibited better anti-proliferation activity against HT-29 cells than that of lamellarin D. Our study on lamellarin D-based Mannich derivation may provide new insights for the development of derivatives of lamellarin D as anti-cancer agents. The study on other derivatization and further mechanism research are still in progress.

6. Experimental materials and methods

6.1. Chemistry

Melting points were determined on a Büchi B-540 melting apparatus and uncorrected. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker AM-400 FT and Bruker AVIII500M spectrometers. Chemical shifts values were expressed in δ ppm with TMS as an internal standard. High resolution mass spectra, ESI (positive) were recorded on an Agilent 6224 Accurate-Mass TOF LC/MS spectrometer. Reactions were run in oven-dried glassware and distilled solvents. Common reagents and intermediates were purified by established procedures [42].



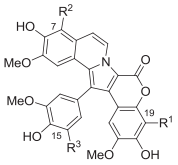
Scheme 2. Reagents: (a) 30% H_2O_2, SeO_2, DCM, 0 °C; (b) 17% KOH a.q., MeOH, 20 °C; (c) BF_3•Et_2O, AcOH, 120 °C; (d) MsCl, TEA, DCM, 0 °C; (e) AcCl, TEA, DCM, 0 °C; (f) $BnN^+Et_3Br^-$, DCM, 0 °C; (g) BnCl, K_2CO_3, DMF, 20 °C; (h) $MeNO_2$, NH_4Ac, HOAc, ultrasonic; (i) $LiAlH_4$, THF, 0–20 °C; (j) i-PrBr, K_2CO_3, DMF, 20 °C; (k) KBH_4, MeOH, 20 °C; (l) $SOCl_2$, DCM, 0 °C; (m) NaCN, CH_3CN, reflux; (n) KOH a.q., EtOH, reflux; (o) heat, 180 °C; (p) $POCl_3$, CH_3CN, reflux; (q) $NaHCO_3$, CH_3CN, reflux; (r) $POCl_3$, DMF, 0–20 °C; (s) 2-methylbut-2-ene, $NaClO_2$, NaH_2PO_4, t-BuOH : THF : H_2O (3:3:1), 0–20 °C; (t) 17% KOH a.q., MeOH, 0–20 °C; (u) DCC, DCM, 0 °C; (v) DDQ, DCM, reflux; (w) TBAF, THF, 0–20 °C; (x) Pd/C, H_2(1 atm), EtOAc, 20 °C; (y) 37% HCHO a.q., HOAc, secondary amine, 70 °C; (z) $TiCl_4$, DCM, 0–20 °C.

*6.1.1. 2-(2-Bromoacetyl)-4-methoxy-5-((methylsulfonyl)oxy)phenyl acetate (**6**)*

This compound was prepared from appropriate acetophenone **5** according to literature procedures [25] in 70.6% yield. Mp 91–92 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (s, 1H, C-6-Ar-*H*), 7.24 (s, 1H, C-3-Ar-*H*), 4.39 (s, 2H, $-CH_2-$Br), 3.95 (s, 3H, C-5-OC*H*$_3$), 3.24 (s, 3H, C-4-OMs), 2.38 (s, 3H, C-2-OAc).

*6.1.1.1. 1-(2,4-Dihydroxy-5-methoxyphenyl)ethanone (**3**).* This compound was prepared from isovanillin according to literature

Table 1
The structures and anti-proliferative activities of lamellarin D Mannich derivatives.



No.	R^1	R^2	R^3	Topo I inhibition	GI_{50} (μM)[a]			
					HT-29	MDA-MB-231	K562	HepG2
SL-1		H	H	–	5.46 ± 1.47	3.30 ± 0.37	0.43 ± 0.03	2.63 ± 0.99
SL-2		H	H	+	2.52 ± 0.51	5.78 ± 0.51	1.04 ± 0.28	5.04 ± 2.98
SL-3		H	H	+	2.92 ± 0.70	9.75 ± 0.71	2.37 ± 0.43	6.83 ± 2.69
SL-4		H	H	+	2.50 ± 0.60	7.25 ± 1.37	1.95 ± 0.36	6.99 ± 2.81
SL-5		H	H	+	2.64 ± 0.88	4.87 ± 1.27	2.25 ± 0.58	3.69 ± 2.07
SL-6	H	H		–	6.41 ± 1.97	3.98 ± 0.20	1.85 ± 0.40	30.14 ± 1.64
SL-7	H		H	+	14.99 ± 1.36	13.05 ± 2.28	1.34 ± 0.06	6.37 ± 3.85
SL-8	H		H	+	18.27 ± 4.08	33.13 ± 3.57	9.94 ± 2.64	21.32 ± 3.41
SL-9	H		H	+	13.00 ± 2.16	19.94 ± 3.71	2.84 ± 1.08	4.47 ± 0.98
SL-10			H	+	5.68 ± 1.78	9.89 ± 0.53	9.62 ± 1.94	>50
SL-11			H	+	2.83 ± 0.89	6.15 ± 0.71	7.16 ± 2.20	15.81 ± 2.62
SL-12	(Lamellarin 501)			– (– [2])	40.78 ± 4.66	17.89 ± 1.84	1.61 ± 0.63	2.14 ± 0.61
SL-13	(Lamellarin D)			+ (+ [2])	4.47 ± 1.69 (5.10 [13])	0.32 ± 0.06 (0.25 [13])	0.08 ± 0.01	0.09 ± 0.01 (0.02 [8])

[a] Data was expressed as mean ± SEM from three independent experiments.

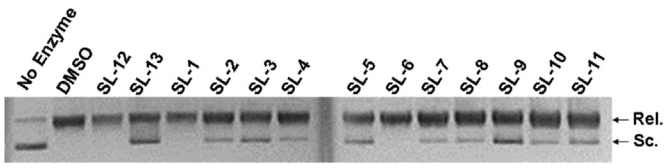


Fig. 3. Inhibition of Topo I-mediated relaxation of DNA by lamellarin 501, Lamellarin D and its Mannich derivatives **SL-1~11**. Native supercoiled pBR322 DNA (0.5 μl) was incubated for 30 min at 37 °C with 1 unit of calf thymus topoisomerase I in the absence or presence of the compound at 100 μM. Reactions were stopped with sodium dodecyl sulphate. The DNA samples were run on an agarose gel followed by gel stain staining and photographed under UV light. (Rel.: relaxed, Sc.: supercoiled).

procedures [24] in 3 steps with 62.4% yield as white solid. Mp 170.5–172.8 °C (Lit. [12]: 171–172 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.58 (s, 1H, C-2-Ar-O*H*), 7.04 (s, 1H, C-6-Ar-*H*), 6.51 (s, 1H, C-3-Ar-*H*), 6.28 (s, 1H, C-4-Ar-O*H*), 3.90 (s, 3H, C-5-OCH_3), 2.55 (s, 3H, –CH_3).

*6.1.1.2. 4-Acetyl-5-hydroxy-2-methoxyphenyl methanesulfonate (**4**).* Acetophenone **3** (0.75 g, 4.12 mmol) and TEA (0.57 mL, 4.12 mmol) were dissolved in DCM (25 mL) at 0 °C. MsCl (0.32 mL, 4.12 mmol) in DCM (2 mL) was added dropwise over 30 min. The mixture was stirred at 0 °C–20 °C for 2 h. Then the solvent was removed at reduced pressure. The residue was taken up in DCM (50 mL), washed with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was recrystallized from ether to obtain 1.03 g pale white solid **4** in 96% yield. Mp 139–140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.08 (s, 1H, C-2-Ar-O*H*), 7.25 (s, 1H, C-6-Ar-*H*), 6.97 (s, 1H, C-3-Ar-*H*), 3.90 (s, 3H, C-5-OCH_3), 3.24 (s, 3H, C-4-OMs), 2.63 (s, 3H, –CH_3). HR-MS (ESI, *m*/*z*): calcd for $C_{10}H_{12}O_6S$ $[M+1]^+$ 261.0388, found 261.0433.

*6.1.1.3. 2-Acetyl-4-methoxy-5-((methylsulfonyl)oxy)phenyl acetate (**5**).* Acetophenone **4** (1.0 g, 3.8 mmol) and TEA (0.55 mL, 4.0 mmol) were dissolved in DCM (25 mL) at 0 °C. AcCl (0.28 mL, 4.0 mmol) in DCM (2 mL) was added dropwise over 30 min. The mixture was stirred at 0 °C–20 °C for 1.5 h. Then ice-water was added to quench the reaction. The mixture washed with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was recrystallized from ether to obtain 1.16 g white solid **5** in 97% yield. Mp

103.4–105.8 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H, C-6-Ar-*H*), 7.36 (s, 1H, C-3-Ar-*H*), 3.96 (s, 3H, C-5-OCH_3), 3.24 (s, 3H, C-4-OMs), 3.23 (s, 3H, −CH_3), 2.67 (s, 3H, C-2-OAc).

6.1.2. 2-(3-Benzyloxy-4-methoxyphenyl)ethanamine (**9**)

This compound was prepared according to literature procedures [31] from isovanillin in 3 steps with 60% yield as pale yellow oil. ^{1}H NMR (Lit. [31], 500 MHz, $CDCl_3$) δ 7.43 (d, J = 7.0 Hz, 1H, C-3-Ar-*H*), 7.35 (t, J = 7.0 Hz, 1H, C-3-Ar-*H*), 7.29 (t, J = 7.0 Hz, 1H, C-3-Ar-*H*), 6.83 (d, J = 8.0 Hz, 1H, C-5-*H*), 6.7 (dd, J = 8.0, 2.0 Hz, 1H, C-6-*H*), 6.72 (d, J = 2.0 Hz, 1H, C-5-*H*), 5.14 (s, 2H, C-3-O-CH_2Ar) 3.89 (s, 3H, C-4-OCH_3), 2.86 (t, J = 7.0 Hz, 2H, −$CH_2CH_2NH_2$), 2.62 (t, J = 7.0 Hz, 2H, −$CH_2CH_2NH_2$), 1.78 (s, 2H, −$CH_2CH_2NH_2$).

6.1.3. 2-(4-Isopropoxy-3-methoxyphenyl)acetic acid (**14**)

This compound was prepared according to literature procedures [22,43] from vanillin by etheration, reduction, chlorination, cyanation and hydrolysation 5 steps of 53% yield as pale yellow solid **14**. Mp 72.4–73.1 °C (Lit. [22]: 67–69 °C). ^{1}H NMR (Lit. [22], 400 MHz, $CDCl_3$) δ 8.80 (brs, 1H, −CH_2COOH), 6.84 (d, 1H, J = 8.4 Hz, C-5-Ar-*H*), 6.81 (s, 1H, C-2-Ar-*H*), 6.79 (d, 1H, J = 8.4 Hz, C-6-Ar-*H*), 4.49 (m, 1H, −$CH(CH_3)_2$), 3.86 (s, 3H, C-3-OCH_3), 3.57 (s, 2H, −CH_2COOH), 1.33(d, 6H, J = 6.4 Hz, −OCH(CH_3)$_2$).

6.1.4. N-(3-benzyloxy-4-methoxyphenethyl)-2-(4-isopropoxy-3-methoxyphenyl) acetamide (**15**)

Phenylacetic acid **14** (16.0 g, 71.3 mmol) and phenylethanamine **9** (19.8 g, 76.9 mmol) were stirred at 180 °C for 2 h under nitrogen atmosphere. The mixture was cooled down and dissolved in DCM (150 mL), washed with 1 M HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography (PE:EtOAc = 1:1) to get a white solid **15** in 67% yield. Mp 92.2–92.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 7.5 Hz, 2H, N-3-Ar-3′-Ar-*H*), 7.36 (t, J = 7.5 Hz, 2H, N-3-Ar-3′-Ar-*H*), 7.29 (t, J = 7.5 Hz, 1H, N-3-Ar-3′-Ar-*H*), 6.81 (d, J = 8.0 Hz, 1H, N-3-Ar-5′-*H*), 6.73 (d, J = 8.0 Hz, 1H, C-2-Ar-5′-*H*), 6.66 (s, 1H, C-2-Ar-2′-*H*), 6.64 (s, 1H, N-3-Ar-2′-*H*) 6.63 (d, J = 8.0 Hz, 1H, N-3-Ar-6′-*H*), 6.52 (d, J = 8.0 Hz, 1H, C-2-Ar-6′-*H*), 5.39 (brs, 1H, −N*H*), 5.10 (s, 2H, N-3-Ar-3′-OCH_2Ar), 4.53–4.45 (m, 1H, C-2-Ar-4′-OC*H*(CH_3)$_2$), 3.86 (s, 3H, C-2-Ar-3′-OCH_3), 3.78 (s, 3H, N-3-Ar-4′-OCH_3), 3.44 (s, 2H, C-2-CH_2), 3.37 (q, J = 6.5 Hz, 2H, N-2-CH_2), 2.62 (t, J = 6.5 Hz, 2H, N-3-CH_2), 1.36 (d, J = 6.0 Hz, 6H, C-2-Ar-4′-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{28}H_{33}NO_5$ $[M+1]^+$ 464.2392, found 464.2441.

6.1.5. 6-Benzyloxy-1-(4-isopropoxy-3-methoxybenzyl)-7-methoxy-3,4-dihydroisoquinolin-2-ium chloride (**16**)

$POCl_3$ (4.64 g, 2.82 mL, 30.2 mmol) was added slowly to a solution of ethanamide **15** (7.0 g, 15.1 mmol) in dry MeCN (15 mL) at room temperature. The mixture was heated to reflux and stirred for 4 h, then cooled down and evaporated solvent in vacuo. The remaining crude product was recrystallized from ether to give 5.1 g of white solid in 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (7, J = 7.5 Hz, 1H, C-6-Ar-*H*), 7.35 (t, J = 7.5 Hz, 2H, C-6-Ar-*H*), 7.29 (d, J = 7.5 Hz, 2H, C-6-Ar-*H*), 7.02 (s, 1H, C-8-*H*), 6.84 (d, J = 8.5 Hz, 1H, C-1-Ar-*H*), 6.81(d, J = 8.5 Hz, 1H, C-1-Ar-*H*), 6.67(s, 1H, C-5-*H*), 5.15 (s, 2H, C-6-OCH_2Ar), 4.43(m, 1H, C-1-Ar-OC*H*(CH_3)$_2$), 3.97 (s, 2H, C-1-CH_2), 3.78 (s, 3H, C-7-OCH_3), 3.74 (s, 3H, C-1-Ar-OCH_3), 3.69 (t, 2H, C-3-CH_2), 2.58 (t, 2H, C-4-CH_2), 1.32 (d, J = 6.0 Hz, 6H, C-1-Ar-OCH(CH_3)$_2$).

6.1.6. 2-(8-Benzyloxy-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinolin-2-yl)-4-methoxy-5-((methylsulfonyl)oxy)phenyl acetate (**17**)

Phenacyl bromides **6** (3.8 g, 10.0 mmol) and $NaHCO_3$ (3.4 g, 40.0 mmol) was added in portion to a solution of dihydroisoquinolin-2-ium chloride **16** (4.8 g, 10.0 mmol) in anhydrous MeCN (100 mL) at room temperature. The mixture was stirred under refluxing for 20 h. The solvent was removed in vacuo. The residue was dissolved in EtOAc (100 mL), washed with brine (50 mL) and dried over Na_2SO_4. The solvent was evaporated to give a residue, which was purified by silica gel column chromatography (PE:EtOAc = 1:1) to afford 5.1 g of pure compound **17** as pale yellow solid in 70% yield. Mp 95.4–96.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 7.0 Hz, 2H, C-7-Ar-*H*), 7.35 (t, 2H, J = 7.0 Hz, C-7-Ar-*H*), 7.29 (t, J = 7.0 Hz, 1H, C-7-Ar-*H*), 7.04 (s, 1H, C-2-Ar-*H*), 6.90 (s, 2H, C-1-Ar-*H*, C-9-*H*), 6.80–6.82 (d, J = 7.5 Hz, 3H, C-1-Ar-*H*, C-7,10-*H*), 6.73 (s, 1H, C-3-*H*), 6.53 (s, 1H, C-2-Ar-*H*), 5.13 (s, 2H, C-8-OCH_2Ar), 4.47 (m, 1H, C-1-Ar-OC*H*(CH_3)$_2$), 4.06 (t, 2H, C-5-CH_2), 3.64 (s, 3H, C-2-Ar-OCH_3), 3.41 (s, 3H, C-1-Ar-OCH_3), 3.40 (s, 3H, C-9-OCH_3), 3.13 (s, 3H, C-2-Ar-OMs), 2.98 (t, 2H, C-6-CH_2), 2.17 (s, 3H, C-2-Ar-OAc), 1.34 (d, J = 6.0 Hz, 6H, C-1-Ar-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{40}H_{41}NO_{10}S$ $[M+NH_4^+]^+$ 745.2789, found 745.2789.

6.1.7. 2-(8-Benzyloxy-3-formyl-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinolin-2-yl)-4-methoxy-5-((methylsulfonyl)oxy)phenyl acetate (**18**)

$POCl_3$ (295 mg, 0.18 mL, 1.92 mmol) was added slowly to dry DMF (1 mL) at 0 °C and stirred for 0.5 h. Then a solution of **17** (900 mg, 1.24 mmol) in dry DMF (3 mL) was added dropwise. The reaction mixture was warmed to 20 °C and stirred for 6 h, then quenched by ice-water, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography (PE:EtOAc = 1:1) to give **18** (710 mg, 76%) as yellow solid. Mp 181.0–181.8 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H, C-3-C*H*O), 7.43 (d, J = 7.0 Hz, 2H, C-7-Ar-*H*), 7.37 (t, J = 7.0 Hz, 2H, C-7-Ar-*H*), 7.31 (t, J = 7.0 Hz, 1H, C-7-Ar-*H*), 7.11 (s, 1H, C-2-Ar-*H*), 6.86 (t, J = 6.9 Hz, 3H, C-1-Ar-*H*), 6.77 (s, 1H, C-9-*H*), 6.75 (s, 1H, C-7-*H*), 6.55 (s, 1H, C-2-Ar-*H*), 5.20 (s, 1H, C-5-CH_2), 5.16 (s, 2H, C-8-OCH_2Ar), 4.49 (dt, J = 12.0, 6.0 Hz, 1H, C-1-Ar-OC*H*(CH_3)$_2$), 4.19 (s, 1H, C-6-CH_2), 3.60 (s, 3H, C-2-Ar-OCH_3), 3.49 (s, 3H, C-1-Ar-OCH_3), 3.38 (s, 3H, C-9-OCH_3), 3.16 (s, 3H, C-2-Ar-OMs), 3.07(m, 1H, C-6-CH_2), 2.89 (m, 1H, C-6-CH_2), 2.07 (s, 3H, C-2-Ar-OAc), 1.33 (d, J = 6.0 Hz, 6H, C-1-Ar-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{41}H_{41}NO_{11}S$ $[M+1]^+$ 756.2434, found 756.2474.

6.1.8. 2-(2-Acetoxy-5-methoxy-4-((methylsulfonyl)oxy)phenyl)-8-(benzyloxy)-1-(4-isopropoxy-3-methoxyphenyl)-9-methoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline-3-carboxylic acid (**19**)

To a solution of aldehyde **18** (1.25 g, 1.64 mmol) in THF (20 mL) and t-BuOH (20 mL) was added 2-methylbut-2-ene (1.4 mL, 13.2 mmol) at 0 °C. A solution of $NaClO_2$ (447 mg, 4.94 mmol) and NaH_2PO_4 (772 mg, 4.95 mmol) in 7 mL of water was added and the mixture was stirred for 36 h at 20 °C. The mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc twice. The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purificated by column chromatography (PE:EtOAc = 1:1 to 1:20) to give 1.23 g of **19** (96%) as white solid. Mp 132.9–133.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$ filtered by dry K_2CO_3) δ 7.43 (d, J = 7.0 Hz, 1H, C-7-Ar-*H*), 7.37 (t, J = 7.0 Hz, 1H, C-7-Ar-*H*), 7.31 (t, J = 7.0 Hz, 1H, C-7-Ar-*H*), 7.09 (s, 1H, C-2-Ar-*H*), 6.83 (d, J = 8.0 Hz, 1H, C-1-Ar-*H*), 6.78 (d, J = 8.0 Hz, 1H, C-1-Ar-*H*), 6.76 (s, 1H, C-7-*H*), 6.68 (d, J = 2.0 Hz, 1H, C-1-Ar-*H*), 6.55 (s, 1H, C-2-Ar-*H*), 5.15 (s, 2H, C-8-OCH_2Ar), 4.95 (s, 1H, C-5-CH_2), 4.50 (m, 1H, C-1-Ar-OC*H*(CH_3)$_2$), 4.20 (s, 1H, C-5-CH_2), 3.59 (s, 3H, C-2-Ar-OCH_3), 3.51 (s,

3H, C-1-Ar-OCH_3), 3.36 (s, 3H, C-9-OCH_3), 3.14 (s, 3H, C-2-Ar-OMs), 3.08 (m, 1H, C-6-CH_2), 2.91 (m, 1H, C-6-CH_2), 2.08 (s, 1H, C-2-Ar-OAc), 1.32 (d, J = 6.0 Hz, 3H, C-1-Ar-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{41}H_{41}NO_{12}S$ $[M+NH_4^+]^+$ 789.2693, found 789.2697.

*6.1.9. 11-Benzyloxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-8,9-dihydro-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-3-yl methanesulfonate (**21**)*

Acid **19** (100 mg, 0.13 mmol) was dissolved in MeOH (5 mL) at 0 °C and 17% LiOH (0.5 mL) solution was added dropwise. The mixture was stirred at 0 °C–20 °C for 1 h and concentrated. The residue was dissolved in EtOAc (25 mL), washed with brine (10 mL) and dried over Na_2SO_4. The solvent was evaporated to give a residue, which was dissolved in dry DCM (10 mL). Then a solution of DCC (53 mg, 0.26 mmol) in DCM was added dropwise into the reaction mixture with ice-water bath and stirred at 0 °C–20 °C for 6 h. The reaction mixture was concentrated and purified by silica gel column chromatography (DCM:EtOAc = 20:1) to give 53 mg of **21** (2 steps, 58%) as white solid. Mp 214.5–215.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, J = 7.0 Hz, 1H, C-11-O-Ar-H), 7.37 (t, J = 7.0 Hz, 1H, C-11-O-Ar-H), 7.33 (s, 1H, C-4-H), 7.30 (t, J = 7.0 Hz, 1H, C-11-O-Ar-H), 7.11 (d, J = 8.0 Hz, 1H, C-14-Ar-5′-H), 7.06 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-H), 7.02 (d, J = 2.0 Hz, 1H, C-14-Ar-2′-H), 6.81 (s, 1H, C-13-H), 6.78 (s, 1H, C-10-H), 6.74 (s, 1H, C-1-H), 5.16 (s, 2H, C-11-OCH_2Ar), 4.79 (m, 1H, C-8-CH_2), 4.73 (m, 1H, C-8-CH_2), 4.57 (m, 1H, C-14-Ar-OCH(CH_3)$_2$), 3.84 (s, 3H, C-2-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 3.37 (s, 3H, C-12-OCH_3), 3.19 (s, 3H, C-3-OMs), 3.06 (t, J = 8.0 Hz, 2H, C-9-CH_2), 1.40 (t, J = 6.0 Hz, 3H, C-14-Ar- OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{39}H_{37}NO_{10}S$ $[M+1]^+$ 712.2172, found 712.2208.

*6.1.10. 11-(Benzyloxy)-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-3-yl methanesulfonate (**22**)*

To a solution of **21** (80 mg, 0.13 mmol) in DCM (10 mL) was added DDQ (45 mg, 0.20 mmol), stirred and refluxed for 12 h. Then the reaction mixture was diluted by another portion of DCM (10 mL), washed with saturated aqueous $NaHCO_3$ (50 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography (DCM:EtOAc = 20:1) to give **22** (74 mg, 80%) as white solid. Mp 236–237 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (d, J = 7.4 Hz, 1H, C-8-H), 7.46 (d, J = 7.3 Hz, 2H, C-11-O-Ar-H), 7.39 (s, 1H, C-4-H), 7.40 (t, J = 7.3 Hz, 2H, C-11-O-Ar-H), 7.33 (t, J = 7.3 Hz, 1H, C-11-O-Ar-H), 7.19 (d, J = 8.0 Hz, 2H, C-14-Ar-5′-H), 7.19 (s, 1H, C-13-H), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-H), 7.13 (s, 1H, C-10-H), 7.12 (d, J = 2.0 Hz, 1H, C-14-Ar-2′-H), 7.04 (d, J = 7.4 Hz, 1H, C-9-H), 6.91 (s, 1H, C-1-H), 5.26 (s, 2H, C-11-OCH_2Ar), 4.65 (m, 1H, C-14-Ar-OCH(CH_3)$_2$), 3.86 (s, 3H, C-2-OCH_3), 3.48 (s, 2H, C-14-Ar-OCH_3), 3.47 (s, 3H, C-12-OCH_3), 3.21 (s, 3H, C-3-OMs), 1.44 (d, J = 6.0 Hz, 6H, C-14-Ar- OCH(CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.75, 151.56, 149.84, 149.40, 147.83, 147.43, 145.40, 137.55, 136.20, 134.41, 128.72, 128.18, 127.80, 127.25, 124.58, 123.75, 123.00, 119.35, 117.42, 116.90, 114.81, 113.59, 113.16, 111.91, 109.57, 108.36, 106.89, 105.52, 71.84, 70.80, 56.26, 55.64, 55.19, 49.17, 38.62, 33.93, 25.61, 24.93, 21.91. HR-MS (ESI, m/z): calcd for $C_{39}H_{35}NO_{10}S$ $[M+1]^+$ 710.2015, found 710.2048.

*6.1.10.1. 11-(Benzyloxy)-3-hydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**23**).* To a solution of **22** (49.5 mg, 69.7 μmol) in dry THF (25 mL), a solution of TBAF (20 mg, 76.7 μmol) in THF (2 mL) was added in dropwise at 0 °C. The mixture was stirred at 0 °C–20 °C for 12 h and concentrated. The residue was dissolved in EtOAc (25 mL), washed with brine (10 mL), dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:EtOAc = 20:1) to give **23** (38 mg, 86%) as white solid. Mp 230–231 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (d, J = 7.3 Hz, 1H, C-8-H), 7.46 (d, J = 7.5 Hz, 2H, C-11-O-Ar-H), 7.39 (t, J = 7.5 Hz, 2H, C-11-O-Ar-H), 7.32 (t, J = 7.5 Hz, 1H, C-11-O-Ar-H), 7.20 (s, 1H, C-13-H), 7.18 (d, J = 8.0 Hz, 1H, C-14-Ar-H), 7.17 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-H), 7.13 (d, J = 2.0 Hz, 1H, C-14-Ar-H), 7.11 (s, 1H, C-10-H), 7.02 (s, 1H, C-4-H), 6.98 (d, J = 7.3 Hz, 1H, C-9-H), 6.73 (s, 1H, C-1-H), 5.77 (s, 1H, C-3-OH), 5.26 (s, 2H, C-11-OCH_2Ar), 4.66 (dt, J = 12.0, 6.0 Hz, 1H, C-14-Ar-OCH(CH_3)$_2$), 3.85 (s, 3H, C-2-OCH_3), 3.51 (s, 3H, C-14-Ar-OCH_3), 3.47 (s, 3H, C-12-OCH_3), 1.44 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{38}H_{33}NO_8$ $[M+1]^+$ 632.2240, found 632.2276.

*6.1.10.2. 11-Hydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-3-yl methanesulfonate (**26**).* Compound **22** (40.0 mg, 56.4 μmol) was hydrogenated (1 atm H_2) over 5% Pd-C (4.0 mg) in EtOAc (10 mL) at 25 °C for 12 h. Then the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM:MeOH = 20:1) to afford **28** as white solid (36 mg, 96%). Mp 249–250 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, J = 7.4 Hz, 1H, C-8-H), 7.38 (s, 1H, C-4-H), 7.21 (s, 1H, C-10-H), 7.18 (d, J = 8.0 Hz, 1H, C-14-Ar-H), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-H), 7.14 (s, 1H, C-13-H), 7.12 (d, J = 2.0 Hz, 1H, C-14-Ar-H), 7.06 (d, J = 7.4 Hz, 1H, C-9-H), 6.91 (s, 1H, C-1-H), 4.66 (dt, J = 12.0, 6.0 Hz, 1H, C-14-Ar-OCH(CH_3)$_2$), 3.86 (s, 3H, C-2-OCH_3), 3.51 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 3.21 (s, 3H, C-3-OMs), 1.44 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{32}H_{29}NO_{10}S$ $[M+1]^+$ 620.1546, found 620.1588.

*6.1.10.3. 11-(Benzyloxy)-4-((dimethylamino)methyl)-3-hydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**24-1**).* To a solution of **23** (49.5 mg, 15.8 μmol) in HOAc (1 mL) was added a solution of 37% HCHO (9.3 mg, 25 μL, 309 μmol) and 33% aqueous Me_2NH (8.3 mg, 25 μL, 201 μmol). The mixture was stirred at 70 °C for 12 h and concentrated. The residue was dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ to pH = 10, dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:MeOH = 20:1) to give **24** as brown solid (8.5 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (d, J = 7.3 Hz, 1H, C-8-H), 7.45 (d, J = 7.3 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.33 (d, J = 7.3 Hz, 1H, C-11-O-Ar-H), 7.18 (s, 1H, C-13-H), 7.17 (d, J = 8.0 Hz, 1H, C-14-Ar-H), 7.14 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-H), 7.10 (d, J = 2.0 Hz, 1H, C-14-Ar-H), 7.09 (s, 1H, C-10-H), 6.97 (d, J = 7.4 Hz, 1H, C-9-H), 6.75 (s, 1H, C-1-H), 5.25 (s, 2H, C-11-OCH_2Ar), 4.67–4.60 (m, 1H, C-14-Ar-OCH(CH_3)$_2$), 4.34 (s, 2H, C-4-CH_2N(CH_3)$_2$), 3.84 (s, 3H, C-2-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 3.46 (s, 3H, C-12-OCH_3), 2.68 (s, 6H, C-4-CH$_2$N(CH_3)$_2$), 1.43 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(CH_3)$_2$).

*6.1.10.4. 10-((Dimethylamino)methyl)-11-hydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-3-yl methanesulfonate (**27-1**).* To a solution of **26** (23.0 mg, 37 μmol) in HOAc (2 mL) was added a solution of 37% HCHO (9.3 mg, 25 μL, 309 μmol) and 33% aqueous Me_2NH (8.3 mg, 25 μL, 201 μmol). The mixture was stirred at 70 °C for 12 h and concentrated. The residue was dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ to pH = 10, dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:MeOH = 20:1) to afford **19-1** as brown solid (18.0 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (d, J = 7.5 Hz, 1H, C-8-H), 7.38 (s, 1H, C-4-H), 7.23 (s, 1H, C-13-H), 7.20 (d, J = 7.5 Hz, 1H, C-9-H), 7.18 (d, J = 8.0 Hz, 1H, C-14-Ar-H), 7.16 (dd,

J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.11 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 6.89 (s, 1H, C-1-*H*), 4.67–4.62 (m, 1H, C-14-Ar-OC*H*$(CH_3)_2$), 4.16 (s, 2H, C-10-CH_2N$(CH_3)_2$), 3.86 (s, 3H, C-2-OCH_3), 3.48 (s, 3H, C-12-OCH_3), 3.45 (s, 3H, C-14-Ar-OCH_3), 3.21 (s, 3H, C-3-OMs), 2.50 (s, 6H, C-10-CH_2N(C$H_3)_2$), 1.44 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(C$H_3)_2$). HR-MS (ESI, *m*/*z*): calcd for $C_{35}H_{36}N_2O_{10}S$ $[M+1]^+$ 677.2124, found 677.2165.

*6.1.10.5. 14-(4-Isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinoline-3,11-diyl dimethanesulfonate (**31**).* To a mixture of **23** (10.0 mg, 16 μmol) in DCM (2.0 mL) and 0.1 M TEA (0.5 mL) in DCM was added dropwise over 30 min at 0 °C 0.1 M MsCl (0.3 mL) in DCM. The reaction mixture was stirred at 0 °C–20 °C for 1 h. Then the solvent was removed under reduced pressure. The residue was taken up in DCM (20 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), brine (10 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was purified by silica gel column chromatography (DCM:EtOAc = 30:1) to provide 11.0 mg of **31** (99%) as yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.66 (s, 1H, C-10-*H*), 7.38 (s, 1H, C-4-*H*), 7.31 (s, 1H, C-13-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-5′-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-*H*), 7.13 (d, J = 2.0 Hz, 1H, C-14-Ar-2′-*H*), 7.09 (d, J = 7.4 Hz, 1H, C-9-*H*), 6.88 (s, 1H, C-1-*H*), 4.69–4.63 (m, 1H, C-14-Ar-OC*H*$(CH_3)_2$), 3.88 (s, 3H, C-2-OCH_3), 3.48 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 3.23 (s, 3H, C-11-OMs), 3.21 (s, 3H, C-3-OMs), 1.45 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(C$H_3)_2$).

*6.1.10.6. 4-((Dimethylamino)methyl)-3,11-dihydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**25-1**).* Compound **24-1** (8.5 mg, 12 μmol) was hydrogenated (1 atm H_2) over 5% Pd-C (2 mg) in EtOAc (80 mL) at 25 °C for 12 h. Then the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM:MeOH = 10:1) to give 5.0 mg of **25-1** (68%) as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.16 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.15 (s, 1H, C-10-*H*), 7.14 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.12 (s, 1H, C-13-*H*), 7.10 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 6.98 (d, J = 7.4 Hz, 1H, C-9-*H*), 6.69 (s, 1H, C-1-*H*), 4.63 (dt, J = 12.0, 6.0 Hz, 1H, C-14-Ar-OC*H*$(CH_3)_2$), 4.16 (s, 2H, C-4-CH_2N$(CH_3)_2$), 3.83 (s, 3H, C-2-OCH_3), 3.47 (s, 3H, C-12-OCH_3), 3.44 (s, 3H, C-14-Ar-OCH_3), 2.47 (s, 6H, C-4-CH_2N(C$H_3)_2$) 1.42 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(C$H_3)_2$).

*6.1.10.7. 10-((Dimethylamino)methyl)-3,11-dihydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**28-1**).* To a solution of **27-1** (6.0 mg, 8.9 μmol) in dry THF (5 mL) was added dropwise at 0 °C a solution of TBAF (20.0 mg, 76.7 μmol) in THF (1 mL). The mixture was stirred at 0 °C–20 °C for 12 h and concentrated. The residue was dissolved in EtOAc (25 mL), washed with brine (10 mL), dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:EtOAc = 20:1) to give **30-1** (4.0 mg, 80%) as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (d, J = 7.7 Hz, 1H, C-8-*H*), 7.25 (s, 1H, C-4-*H*), 7.23 (d, 1H, J = 7.7 Hz, 1H, C-9-*H*), 7.18 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.15 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (s, 1H, C-13-*H*), 7.11 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 6.70 (s, 1H, C-1-*H*), 4.67–4.60 (m, 1H, C-14-Ar-OC*H*$(CH_3)_2$), 4.23 (s, 2H, C-10-CH_2N$(CH_3)_2$), 3.84 (s, 3H, C-2-OCH_3), 3.49 (s, 3H, C-12-OCH_3), 3.45 (s, 3H, C-14-Ar-OCH_3), 2.54 (s, 6H, C-10-CH_2N(C$H_3)_2$), 1.44 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(C$H_3)_2$).

*6.1.10.8. 10-((Dimethylamino)methyl)-3,11-dihydroxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**29**).* To a solution of **28-1** (5.0 mg, 8.4 μmol) in HOAc (1 mL) was added a solution of 37% HCHO (1.85 mg, 5 μL, 62 μmol) and 33% aqueous Me_2NH (1.65 mg, 5 μL, 40 mmol). The mixture was stirred at 70 °C for 12 h and concentrated. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ to pH = 10, dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:MeOH = 20:1) to provide **29** (4.1 mg, 75%) as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, J = 7.7 Hz, 1H, C-8-*H*), 7.23 (s, 1H, C-13-*H*), 7.18 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.16 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.13 (d, J = 7.7 Hz, 1H, C-9-*H*), 7.11 (s, 1H, C-14-Ar-*H*), 6.71 (s, 1H, C-1-*H*), 4.67–4.61 (m, 1H, C-14-Ar-OC*H*$(CH_3)_2$), 4.21 (s, 2H, C-10-CH_2N$(CH_3)_2$), 4.12 (s, 2H, C-4-CH_2N$(CH_3)_2$), 3.84 (s, 3H, C-2-OCH_3), 3.46 (s, 3H, C-12-OCH_3), 3.45 (s, 3H, C-14-Ar-OCH_3), 2.52 (s, 6H, C-10-CH_2N(C$H_3)_2$), 2.47 (s, 6H, C-4-CH_2N(C$H_3)_2$), 1.43 (d, J = 6.0 Hz, 6H, C-14-Ar-OCH(C$H_3)_2$).

*6.1.10.9. 14-(4-Hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6-oxo-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinoline-3,11-diyl dimethanesulfonate (**32**).* To a solution of **31** (11.0 mg, 16.0 μmol) in dry DCM (2 mL) was added dropwise a solution of 0.01 M $TiCl_4$ in DCM (0.5 mL) at 0 °C. The mixture was gradually warmed to 20 °C and stirred for 12 h. The mixture was extracted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography (DCM:EtOAc = 10:1) to give **32** (8.0 mg, 76%) as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (d, J = 7.5 Hz, 1H, C-8-*H*), 7.37 (s, 1H, C-4-*H*), 7.29 (s, 1H, C-13-*H*), 7.23 (d, J = 8.0 Hz, 1H, C-14-Ar-5′-*H*), 7.17 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-*H*), 7.08 (d, J = 7.5 Hz, 2H, C-14-Ar-2′-*H*, C-9-*H*), 6.87 (s, 1H, C-1-*H*), 5.89 (s, 1H, C-14-Ar-4′-O*H*), 3.93 (s, 3H, C-2-OCH_3), 3.50 (s, 3H, C-12-OCH_3), 3.49 (s, 3H, C-14-Ar-OCH_3), 3.24 (s, 3H, C-11-OMs), 3.22 (s, 3H, C-3-OMs).

*6.1.10.10. 4-((Dimethylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-1**).* To a solution of **25-1** (5.0 mg, 8.4 μmol) in dry DCM (5 mL) was added dropwise a solution of 0.01 M $TiCl_4$ in DCM (3.4 mL) at 0 °C. The mixture was gradually warmed to 20 °C and stirred for 12 h. The mixture was extracted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to give the crude product, which was purified by column chromatography (DCM:EtOAc = 10:1) to provide **SL-1** (3.4 mg, 73%) as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, J = 7.0 Hz, 1H, C-8-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.19 (s, 1H, C-10-*H*), 7.15 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.12 (s, 1H, C-13-*H*), 7.06 (s, 1H, C-14-Ar-*H*), 7.01 (d, J = 7.0 Hz, 1H, C-9-*H*), 6.71 (s, 1H, C-1-*H*), 4.21 (s, 2H, C-4-CH_2N$(CH_3)_2$), 3.89 (s, 3H, C-2-OCH_3), 3.52 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 2.51 (s, 6H, C-4-CH_2N(C$H_3)_2$). HR-MS (ESI, *m*/*z*): calcd for $C_{31}H_{28}N_2O_8$ $[M+1]^+$ 557.1879, found 557.1927.

*6.1.10.11. 4-((Diethylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-2**).* The title compound was synthesized from **23** by the similar procedure as that for **SL-1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.19 (s, 1H, C-10-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.12 (s, 1H, C-13-*H*), 7.07 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.00 (d, J = 7.4 Hz, 1H, C-9-*H*), 6.69 (s, 1H, C-1-*H*), 4.27 (s, 2H, C-4-CH_2N), 3.89 (s, 3H, C-2-OCH_3), 3.52 (s, 3H, C-12-OCH_3), 3.47 (s, 3H, C-14-Ar-OCH_3), 2.79 (q, J = 7.0 Hz, 4H, C-4- CH_2N(CH_2C$H_3)_2$), 1.22 (t, J = 7.0 Hz, 6H, C-4-CH_2N(CH_2C$H_3)_2$). HR-MS (ESI, *m*/*z*): calcd for $C_{33}H_{32}N_2O_8$ $[M+1]^+$ 585.2192, found 585.2228.

*6.1.10.12. 4-((Dipropylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-3**).* The title compound was synthesized from **23** by the similar procedure as that for **SL-1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.21 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.20 (s, 1H, C-10-*H*), 7.17 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (s, 1H, C-13-*H*), 7.08 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.01 (d, J = 7.4 Hz, 1H, C-9-*H*), 4.28 (s, 2H, C-4-CH_2N), 3.90 (s, 3H, C-2-OCH_3), 3.53 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 2.65 (m, 4H, C-4-CH$_2$N(CH_2CH$_2$CH$_3$)$_2$), 1.68 (m, 4H, C-4-CH$_2$N(CH$_2$CH_2CH$_3$)$_2$), 0.92 (t, J = 7.2 Hz, 6H, C-4-CH$_2$N(CH$_2$CH$_2$CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{35}H_{36}N_2O_8$ $[M+1]^+$ 613.2505, found 613.2553.

*6.1.10.13. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-4-(piperidin-1-ylmethyl)-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-4**).* The title compound was synthesized from **23** by the similar procedure as that for **SL-1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.19 (s, 1H, C-10-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (s, 1H, C-13-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.00 (d, J = 7.4 Hz, 1H, C-9-*H*), 6.69 (s, 1H, C-1-*H*), 4.18 (s, 2H, C-4-CH_2N), 3.89 (s, 3H, C-2-OCH_3), 3.52 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 2.71 (m, 4H, piperidin-*H*), 1.70 (m, 4H, piperidin-*H*), 1.54 (m, 2H, piperidin-*H*). HR-MS (ESI, m/z): calcd for $C_{34}H_{32}N_2O_8$ $[M+1]^+$ 597.2192, found 597.2243.

*6.1.10.14. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-4-(morpholinomethyl)-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-5**).* The title compound was synthesized from **23** by the similar procedure as that for **SL-1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.21 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.19 (s, 1H, C-10-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.12 (s, 1H, C-13-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.02 (d, J = 7.4 Hz, 1H, C-9-*H*), 6.71 (s, 1H, C-1-*H*), 4.23 (s, 2H, C-4-CH_2N), 3.89 (s, 3H, C-2-OCH_3), 3.81 (m, 4H, morpholin-*H*), 3.52 (s, 3H, C-12-OCH_3), 3.48 (s, 3H, C-14-Ar-OCH_3), 2.76 (m, 4H, morpholin-*H*). HR-MS (ESI, m/z): calcd for $C_{33}H_{30}N_2O_9$ $[M+1]^+$ 599.1985, found 599.2037.

*6.1.10.15. 14-(3-((Dimethylamino)methyl)-4-hydroxy-5-methoxyphenyl)-3,11-dihydroxy-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-6**).* To a solution of **32** (4.0 mg, 6.1 μmol) in HOAc (2 mL) was added a solution of 37% HCHO (1.0 mg, 3 μL, 33 μmol) and 33% aqueous Me_2NH (1.3 mg, 4 μL, 30 μmol). The mixture was stirred at 70 °C for 12 h and concentrated. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ to pH = 10, dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:MeOH = 20:1) to give a brown solid, which was dissolved in dry THF (5 mL). A solution of TBAF (5.0 mg, 19 μmol) in THF (1 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C–20 °C for 12 h and concentrated. The residue was dissolved in EtOAc (25 mL), washed with brine (10 mL), dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:EtOAc = 20:1) to give 1.5 mg of **SL-6** as pale white solid (2 steps, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (d, J = 7.4 Hz, 1H, C-8-*H*), 7.47 (s, 1H, C-14-Ar-2′-*H*), 7.23 (s, 1H, C-13-*H*), 7.21 (s, 1H, C-14-Ar-6′-*H*), 7.04 (s, 1H, C-10-*H*), 7.02 (d, J = 7.4 Hz, 2H, C-4, 9-*H*), 6.60 (s, 1H, C-1-*H*), 3.91 (s, 3H, C-2-OCH_3), 3.67 (s, 2H, C-14-Ar-3′-CH_2N(CH$_3$)$_2$), 3.50 (s, 6H, C-14-Ar-5′-OCH_3, C-12-OCH_3), 2.37–2.31 (m, 6H, C-14-Ar-3′-CH$_2$N(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{31}H_{28}N_2O_8$ $[M+1]^+$ 557.1879, found 557.4447.

*6.1.10.16. 10-((Dimethylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-7**).* To a solution of **28-1** (3.0 mg, 5.0 μmol) in dry DCM (5 mL) was added dropwise a solution of 0.01 M $TiCl_4$ in DCM (1.0 mL) at 0 °C. The mixture was gradually warmed to 20 °C and stirred for 12 h. The mixture was extracted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to give the crude product, which was purified by column chromatography (DCM:EtOAc = 10:1) to provide **SL-7** (1.9 mg, 70%) as pale white solid. ^{1}H NMR (500 MHz, MeOD) δ 9.15 (brs, 1H, C-8-*H*), 7.44 (brs, 1H, C-9-*H*), 7.42 (s, 1H, C-4-*H*), 7.19 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.04 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 6.78 (s, 1H, C-13-*H*), 6.75 (s, 1H, C-1-*H*), 4.78 (s, 2H, C-10-CH_2N(CH$_3$)$_2$), 3.89 (s, 3H, C-2-OCH_3), 3.53 (s, 3H, C-12-OCH_3), 3.50 (s, 3H, C-14-Ar-OCH_3), 2.98 (s, 6H, C-10-CH$_2$N(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{31}H_{28}N_2O_8$ $[M+1]^+$ 557.1879, found 557.1926.

*6.1.10.17. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-10-(piperidin-1-ylmethyl)-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-8**).* The title compound was synthesized from **26** by the similar procedure as that for **SL-7**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, J = 7.6 Hz, 1H, C-8-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.19 (s, 1H, C-4-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.11 (d, J = 7.6 Hz, 1H, C-9-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.01 (s, 1H, C-13-*H*), 6.68 (s, 1H, C-1-*H*), 4.14 (s, 2H, C-10-CH_2N), 3.89 (s, 3H, C-2-OCH_3), 3.52 (s, 3H, C-12-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 2.67 (m, 4H, piperidin-*H*), 1.71 (m, 4H, piperidin-*H*), 1.54 (m, 2H, piperidin-*H*). HR-MS (ESI, m/z): calcd for $C_{34}H_{32}N_2O_8$ $[M+1]^+$ 597.2192, found 597.2242.

*6.1.10.18. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-10-(morpholinomethyl)-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-9**).* The title compound was synthesized from **26** by the similar procedure as that for **SL-7**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (d, J = 7.7 Hz, 1H, C-8-*H*), 7.22 (s, 1H, C-13-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.16 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (d, J = 7.7 Hz, 1H, C-9-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 7.02 (s, 1H, C-4-*H*), 6.68 (s, 1H, C-1-*H*), 4.15 (s, 2H, C-10-CH_2N), 3.89 (s, 3H, C-2-OCH_3), 3.80 (m, 4H, morpholin-*H*), 3.51 (s, 3H, C-12-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 2.71 (m, 4H, morpholin-*H*). HR-MS (ESI, m/z): calcd for $C_{33}H_{30}N_2O_9$ $[M+1]^+$ 599.1985, found 599.2030.

*6.1.10.19. 4,10-Bis((dimethylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4s′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-10**).* To a solution of **29** (4.1 mg, 6.3 μmol) in dry DCM (2 mL) was added dropwise a solution of 0.01 M $TiCl_4$ in DCM (1.5 mL) at 0 °C. The mixture was gradually warmed to 20 °C and stirred for 12 h. The mixture was extracted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to give the crude product, which was purified by column chromatography (DCM:EtOAc = 10:1) to provide **SL-10** (2.5 mg, 65%). as pale white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, J = 7.7 Hz, 1H, C-8-*H*), 7.20 (s, 1H, C-13-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.15 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.11 (d, J = 7.7 Hz, 1H, C-9-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 6.67 (s, 1H, C-1-*H*), 4.14 (s, 2H, C-10-CH_2N(CH$_3$)$_2$), 4.08 (s, 2H, C-4-CH_2N(CH$_3$)$_2$), 3.88 (s, 3H, C-2-OCH_3), 3.47 (s, 3H, C-12-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 2.44 (s, 6H, C-10- CH$_2$N(CH_3)$_2$), 2.43 (s, 6H, C-4-CH$_2$N(CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{34}H_{35}N_3O_8$ $[M+1]^+$ 614.2458, found 614.2504.

*6.1.10.20. 10-((Dimethylamino)methyl)-4-((dipropylamino)methyl)-3,11-dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (**SL-11**).* The title compound was synthesized from **30** (synthesized from **28-1** by the similar procedure as that for **29**) by the similar procedure as that for **SL-10**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (d, J = 7.7 Hz, 1H, C-8-*H*), 7.21 (s, 1H, C-13-*H*), 7.20 (d, J = 8.0 Hz, 1H, C-14-Ar-*H*), 7.17–7.14 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-*H*), 7.13 (d, J = 7.7 Hz, 1H, C-9-*H*), 7.06 (d, J = 2.0 Hz, 1H, C-14-Ar-*H*), 6.67 (s, 1H, C-1-*H*), 4.26 (s, 2H, C-10-CH_2N(CH_3)$_2$), 4.14 (s, 2H, C-4-CH_2N($CH_2CH_2CH_3$)$_2$), 3.89 (s, 3H, C-2-OCH_3), 3.47 (s, 3H, C-12-OCH_3), 3.46 (s, 3H, C-14-Ar-OCH_3), 2.67–2.62 (m, 4H, C-4-CH$_2$N(CH_2CH$_2$CH$_3$)$_2$), 2.49 (s, 6H, C-10-CH$_2$N(CH_3)$_2$), 1.71–1.64 (m, 4H, C-4-CH$_2$N(CH$_2$CH_2CH$_3$)$_2$), 0.91 (t, J = 7.4 Hz, 6H, C-4-CH$_2$N(CH$_2$CH$_2$CH_3)$_2$). HR-MS (ESI, m/z): calcd for $C_{38}H_{43}N_3O_8$ $[M+1]^+$ 670.3084, found 670.3125.

*6.1.10.21. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-8,9-dihydro-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (lamellarin 501, **SL-12**).* To a solution of **21** (15.0 mg, 21.1 μmol) in dry THF (5 mL) was added dropwise at 0 °C a solution of TBAF (20.0 mg, 76.7 μmol) in THF (1 mL). The mixture was stirred at 0 °C–20 °C for 12 h and concentrated. The residue was dissolved in EtOAc (25 mL), washed with brine (10 mL), dried over Na_2SO_4. The solvent was removed in vacuo and purified by silica gel column chromatography (DCM:EtOAc = 20:1) to give a pale white solid, which was dissolved in dry DCM (2 mL). A solution of 0.01 M $TiCl_4$ in DCM (5 mL) was added dropwise at 0 °C. The mixture was gradually warmed to 20 °C and stirred for 12 h. The mixture was extracted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to provide the crude product, which was purified by column chromatography (DCM:EtOAc = 10:1) to afford 6.5 mg of **SL-12** (2 steps, 61%) as white solid. ^{1}H NMR (Lit. [15,44,45], 500 MHz, $CDCl_3$) δ 7.13 (d, J = 8.0 Hz, 1H, C-14-Ar-5′-*H*), 7.08 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-*H*), 6.97 (d, J = 2.0 Hz, 1H, C-14-Ar-6′-*H*), 6.96 (s, 1H, C-4-*H*), 6.81 (s, 1H, C-10-*H*), 6.67 (s, 1H, C-10-*H*), 6.63 (s, 1H, C-1-*H*), 5.75 (s, 1H, Ar-O*H*), 5.70 (s, 1H, Ar-O*H*), 5.63 (s, 1H, Ar-O*H*), 4.96–4.89 (m, 1H, C-8-CH_2), 4.65–4.58 (m, 1H, C-8-CH_2), 3.87 (s, 3H, C-2-OCH_3), 3.50 (s, 3H, C-12-OCH_3), 3.41 (s, 3H, C-14-Ar-4′-OCH_3), 3.10–3.05 (m, 2H, C-9-CH_2); HR-MS (ESI, m/z): calcd for $C_{28}H_{23}NO_8$ $[M+1]^+$ 502.1457, found 502.1499.

*6.1.10.22. 3,11-Dihydroxy-14-(4-hydroxy-3-methoxyphenyl)-2,12-dimethoxy-6H-chromeno[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (lamellarin D, **SL-13**).* The title compound was synthesized from **22** by the same procedure as that for **SL-12** in 81% yield. ^{1}H NMR (Lit. [1,15,35,45], 500 MHz, MeOD) δ 9.02 (d, J = 7.3 Hz, 1H, C-8-*H*), 7.17 (s, 1H, C-4-*H*), 7.16 (d, J = 2.0 Hz, 1H, C-14-Ar-2′-*H*), 7.12 (d, J = 8.0 Hz, 1H, C-14-Ar-5′-*H*), 7.06 (dd, J = 8.0, 2.0 Hz, 1H, C-14-Ar-6′-*H*), 7.06 (s, 1H, C-13-*H*), 7.03 (d, J = 7.3 Hz, 1H, C-9-*H*), 6.78 (s, 1H, C-10-*H*), 6.76 (s, 1H, C-1-*H*), 3.87 (s, 3H, C-2-OCH_3), 3.46 (s, 6H, C-14-Ar-4′-OCH_3, C-12-OCH_3); HR-MS (ESI, m/z): calcd for $C_{28}H_{21}NO_8$ $[M+1]^+$ 500.1301, found 500.1336.

6.2. Biological procedures

6.2.1. Topo I inhibition

Each reaction mixture (20.0 μl) contained 2.0 μl 10 × DNA Topo I buffer, 2.0 μl 0.1% BSA, 0.5 μl pBR322 DNA (0.5 μg), 1.0 μl Topo I (1U), and 2.0 μl of compound solution (100 μM) at desired lane. After incubation for 30 min at 37 °C, the reaction was terminated by addition of 2.0 μl 10% sodium dodecyl sulphate solution. DNA was electrophoresed [46] on 1% agarose gels and soaked with 3.0 μl DNA gel stain for 15 min before photographed under UV by Bio-Rad Universal Hood (Bio-Rad laboratories, Milan, Italy). Purified Topo I (ID: D2240A) and pBR322 DNA (ID: D3050) were purchased from TaKaRa (Dalian, China P.R.). DNA gel stain (ID: S33102) was purchased from Yuyang corporation (Hangzhou, China).

6.2.2. Cell viability analysis

The human cancer cell lines (HT-29, MDA-MB-231, K562 and HepG2) were obtained from ATCC. To investigate the cell growth inhibition activity of the compounds, 4000 cells were seeded into each well from a 96-well plate. After 12 h incubation, the culture medium was replaced with fresh medium containing graded concentrations of the test compound. After 72 h incubation, MTT assay was performed to determine the cell viability. After the treatment, 10 μl of MTT solution (5 mg/mL) was added to each well. After 3 h incubation, the medium was removed and formazan crystals were dissolved with 150 μl of DMSO for 10 min on a shaker. The absorbance of each well was measured by a Multiskan GO UV/Vis microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 540 nm. The concentration induced 50% cell growth inhibition (GI_{50}) was calculated from the dose-dependent curve. This study was repeated for three times, and data were expressed as mean ± SEM of three independent experiments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.038. These data include MOL files and InChiKeys of the most important compounds described in this article.

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