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Original article

Development of novel conformation-constrained cytotoxic derivatives of cheliensisin A by embedment of small heterocycles

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ABSTRACT

Cheliensisin A is a natural styryl-lactone isolated from *Goniothalamus cheliensis* Hu in considerably large quantity with putative anticancer activities. However, its poor water solubility and chemical instability have precluded cheliensisin A as a potential drug candidate. To explore the strategy to overcome these problems, 21 novel derivatives of cheliensisin A with different substitutions at C-7 and C-8 positions were designed and synthesized. Inhibition of proliferation against five tumors cell lines indicates that eight new derivatives with embedment of oxazole or oxazoline exhibit improved cytotoxicity on SK-BR-3 and PANC-1, and compounds **2d** and **2g** show 5–8 folds higher potency than cisplatin. HPLC investigation of representative compounds indicates that oxazole and oxazoline analogs exhibit much improved chemical stability than their natural parent.

1. Introduction

Natural products based drug discovery has become a major strategy in modern pharmaceutical research and development, and roughly half of the currently used drugs are directly or indirectly derived from natural products [1]. The styryl-lactones from *Goniothalamus* (Annonaceae) are an interesting family of natural products, many of which were found to exhibit impressive biological activities [2,3]. In 1998, Li and coworkers isolated cheliensisin A (**1**) as a novel cytotoxic styryl-lactone compound from *Goniothalamus cheliensis* Hu (Fig. 1) [4]. Due to its potent antitumor properties, great attentions have been paid to cheliensisin A. Mechanism studies revealed that cheliensisin A induced leukemia cell apoptosis involving activation of caspase-3 and down-regulation of Bcl-2 mRNA expression [5]. In addition to its significant *in vitro* antitumor activities, cheliensisin A also exhibited potent *in vivo* antitumor effect on murine BALB/c and murine H22 [6,7]. It was also found that no apparent side effect was observed when it was administrated on mice [7]. Because it is relatively abundant in *G. cheliensis*, cheliensisin A was apparently superior, compared to some conventional chemotherapeutic drugs, for further development as a promising candidate of anticancer drug. Unfortunately, further application of cheliensisin A has been strongly impeded by its chemical instability and poor water solubility [6]. Considering the pharmaceutical potential of cheliensisin A and its unsolved problems in further drug development, we decided to explore its structural modification in hope of pursuing more promising anticancer derivatives.

Previous studies indicated that proper modifications of the styryl-lactone compounds at the C-7 and C-8 positions would affect the biological activities [3]. We also envisioned that the relatively reactive epoxy functionality in cheliensisin A might be a major cause of its chemical instability, which led to failure in the *in vivo* studies [6]. On the other hand, the C-7/C-8 epoxide is the most chemically accessible functionality for potential new modifications in cheliensisin A. Accordingly, proper transformations at the C-7 and C-8 positions might result in the change of chemical and physical properties, which would further affect the molecular shapes, bond angles, and partition coefficients [3,8]. In addition, we believe that proper conformation of the major subunits of cheliensisin A, such as the spaciously stretching direction of the phenyl ring and the unsaturated D-lactone, should be very important for

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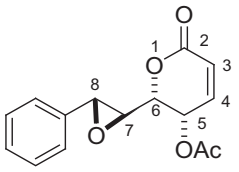


Fig. 1. Structure of cheliensisin A (**1**).

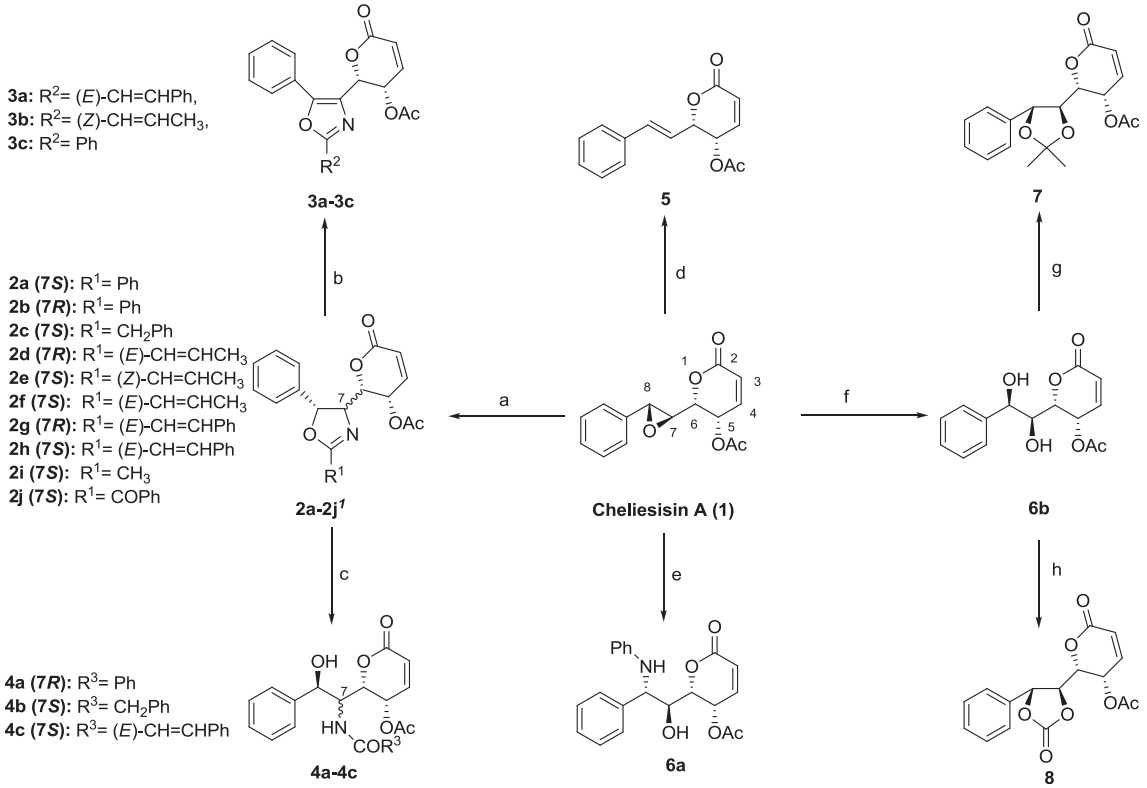
the bioactivities. To achieve the above purposes, conversion of the epoxide moiety of cheliensisin A to the corresponding small heterocycles was thus considered as an option in our synthesis. Herein, we want to report our synthesis of twenty one new derivatives of cheliensisin A and their biological activities.

2. Chemistry

Preparation of compounds **2a**–**2j**, **3a**–**3c**, **4a**–**4c 5**, **6a**–**6b**, **7** and **8** was depicted in Scheme 1 starting from the natural sample of cheliensisin A isolated in a large quantity from *G. cheliensis* of Yunnan province, China. Literatures show that many chemically stable compounds containing oxazoline/oxazole moiety exhibit various biological activities [9,10]. Therefore, we decided to embed a small heterocycle, oxazoline/oxazole moiety, at the C-7 and C-8 positions of cheliensisin A through proper chemical transformations.

To our delight, cheliensisin A was found to undergo the desired transformation regio- and stereoselectively by reacting with cyano-group-containing reagents in the presence of $BF_3 \cdot OEt_2$ (**2a**–**2i**) [11]. These oxazoline derivatives were characterized and confirmed by NMR methods. For example, chemical shifts of $\delta_{H\text{-}8}$ at 5.69 and $\delta_{H\text{-}7}$ at 4.06 of compound **2b** suggest that its O/N regio-chemistry as indicated. In addition, the appearance of J_{H7-H8} at 9.6 Hz in ^{1}H NMR mentions the relative stereochemistry of **2b** at C-7/C-8 is *cis* [12]. With the oxazoline derivatives in hand, we further explored the further hydrolysis and aromatization, respectively (Scheme 1). Treatment of the oxazoline derivatives with DDQ in refluxing toluene provided the corresponding aromatic products successfully (**2j**, **3a**–**3c**) [13]. Alternatively, by refluxing the oxazoline derivatives with HCl in ethanol, the corresponding hydrolysis products were successfully furnished (**4a**–**4c**, Scheme 1).

We envisioned that replacement of the epoxide moiety of cheliensisin A with the corresponding bioisostere, the alkenyl group, can maintain its conformation and improve the chemical stability. For such purpose, derivative **5** was designed. After extensive investigations, we found that treatment of cheliensisin A with $Mo(CO)_6$ in toluene yielded compound **5** in an acceptable yield [14]. As mentioned above, the presence of epoxide moiety in cheliensisin A is the major reason of its instability [6]. Two epoxide ring-opening derivatives **6a** and **6b** were then synthesized. Compound **6a** was provided by refluxing cheliensisin A with aniline in toluene. In parallel, compound **6b** was successfully achieved by treatment of cheliensisin A with aqueous citric acid in almost quantitatively yield based on the recovery of starting material. In order to decrease the flexibility of compound **6b** and determine the relative configurations at C-7/C-8 of compound **6b**, compounds **7** and **8** were prepared. Compound **7** was provided by treating compound **6b** with DMP in the presence of catalytic amount of TsOH. It was characterized by the appearance of J_{H7-H8} at 4.8 Hz in the ^{1}H NMR, and the correlations between H-5 and H-7, H-6 and H-8 found in the NOESY experiment (Fig. 2). The relative configurations at C-7/C-8 of compound **6b** were deduced from compound **7**. Compound **6b** could also be converted to the corresponding cyclic carbonate **8** in 70% yield by facile reaction with triphosgene in pyridine (Scheme 1) [15].



Scheme 1. Reagents and conditions: (a) $BF_3.OEt_2$, RCN, −20 °C to RT; (b) DDQ, toluene, reflux; (c) HCl, ethanol, reflux; (d) $Mo(CO)_6$, toluene, reflux; (e) aniline, toluene, reflux; (f) citric acid, CH_3CN/H_2O, RT; (g) DMP, TsOH, RT; (h) triphosgene, pyridine, CH_2Cl_2, RT. ([1]**2j** was prepared under the conditions b).

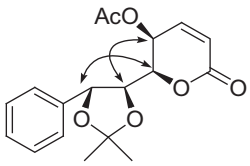


Fig. 2. The key NOESY of compound **7**.

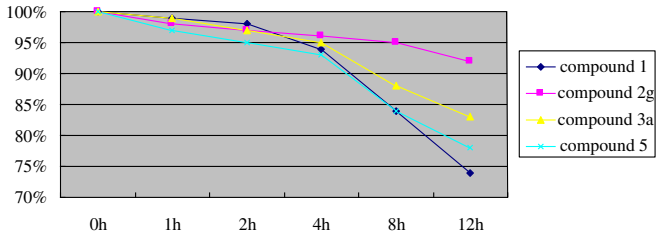


Fig. 3. Chemical stability investigation of compounds **1**, **2g**, **3a** and **5**.

3. Biological results and discussions

To examine the effect of the above C-7/C-8 modifications to biological activities, all synthesized derivatives were evaluated in the cytotoxic assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Five human tumor cell lines were screened, including blood (HL-60), liver (SMMC-7721), lung (A-549), breast (SK-BR-3) and pancreas (PANC-1), and anticancer drug cisplatin (DDP) was applied as the positive control in the screening (Table 1).

With the biological results in hand, the inherent discipline between structure and cytotoxicity was concluded and discussed. As the results illustrate in Table 1, some new derivatives show impressive cytotoxicities against SK-BR-3 and PANC-1, while cheliensisin A is found more potent to inhibit the cell growth of SMMC-7721 and A-549. Obviously, these newly developed derivatives expand the spectrum of cytotoxicity. On the other hand, epoxide-opening compounds, **4a**, **4b**, **4c**, **6a**, **6b**, show little or modest cytotoxicity, whereas most of the C-7/C-8 ring-containing derivatives (compounds **2b**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **3a**, **3c**) possess much better antitumor activities. These results mention that the C7/C8 conformation of these derivatives tightly links with their potency of cytotoxicity. Compared to cheliensisin A (**1**), the epoxide-opening derivatives **4a**, **4b**, **4c**, **6a** and **6b** are relatively conformationally flexible, and they generally exhibit much less potency than that of natural product **1**. However, over high rigidity is not helpful either. For instance, rigid oxazole analog **3b** shows much less potency against the proliferation of HL60, SMMC-7721 and PANC-1 cells than its corresponding oxazoline precursor **2e**. Only those derivatives with suitable rigidity have more opportunity to exhibit higher potency, such as cheliensisin A, and compounds **2b**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i** and **5**. Based on these evidences, suitable rigidity is thought to be needed to achieve better bioactivities. Additionally, the C-7/C-8 diol **6b** and its cyclic acetal and carbonate derivatives **7** and **8** did not show any activity at all in our test. It means that introduction of a nitrogen atom into these derivatives may be helpful for the improvement of biological activity. To our surprise, compound **5**, a simpler derivative by elimination of epoxide functionality of natural product **1**, is listed into the most potent ones in this study, exhibiting the general cytotoxicities against all five tested cancer cell lines.

Table 1
In Vitro cytotoxicity of cheliensisin A derivatives in HL-60[a], SMMC-7721[b], A-549[c], SK-BR-3[d] and PANC-1[e] cell lines.

Entry	IC_{50} (μM)				
	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
1[g]	1.97	4.70	5.99	20.31	25.65
2a[f]	> 40	> 40	> 40	> 40	> 40
2b[f]	4.30	17.74	18.27	3.65	12.75
2c[f]	17.79	> 40	> 40	35.21	> 40
2d[f]	1.26	14.47	30.66	2.22	3.87
2e[f]	4.59	27.02	> 40	> 40	22.97
2f[f]	6.93	> 40	> 40	18.03	24.43
2g[g]	0.48	20.99	18.92	4.03	4.10
2h[g]	4.05	24.02	21.94	23.12	21.14
2i[g]	2.52	16.26	> 40	7.26	16.57
2j[f]	> 40	> 40	> 40	> 40	> 40
3a[g]	5.89	24.59	24.80	7.36	7.22
3b[g]	> 40	> 40	> 40	> 40	> 40
3c[g]	2.59	15.69	14.71	11.96	13.90
4a[g]	> 40	> 40	> 40	> 40	> 40
4b[g]	5.19	> 40	> 40	38.76	> 40
4c[f]	> 40	> 40	> 40	> 40	> 40
5[g]	2.68	29.04	38.83	4.63	7.95
6a[f]	> 40	> 40	> 40	> 40	> 40
6b[g]	> 40	> 40	> 40	> 40	> 40
7[f]	> 40	> 40	> 40	> 40	> 40
8[g]	> 40	> 40	> 40	> 40	> 40
DDP[f,h]	1.51	16.92	17.82	10.32	24.44
DDP[g,h]	1.09	12.93	17.70	18.20	23.13

[a] HL-60, Human promyelocytic leukemia cell line.
[b] SMMC-7721, human hepatocellular carcinoma cell line.
[c] A-549, Human lung carcinoma cell line.
[d] SK-BR-3, Human breast adenocarcinoma cell line.
[e] PANC-1, Human pancreatic carcinoma, epithelial-like cell line.
[f] the first biological evaluation.
[g] the second biological evaluation.
[h] DDP (cisplatin, which was used as a positive control).

Dramatic change of the cytotoxicity of compounds (7*S*)-**2a** and (7*R*)-**2b** indicates that the stereochemistry is also very important. The *cis* C-7/C-8 configuration is more favorable than the corresponding *trans* isomer. We also noticed that minor differences in the substitutes of oxazoline derivatives (compounds **2a**, **2c**, **2e**, **2f**, **2h**, **2i** and **2j**) could result in subtle fluctuation of the inhibitory activities. Among these, the vinylogous (2-propenyl and 2-styryl) oxazoline analogs are more potent than 2-phenyl and 2-methyl oxazolines, whereas 2-benzyl and 2-benzoyl oxazolines show little or none of cytotoxicity. In addition, compound **2g** exhibits good to excellent cytotoxicities against all five tested cancer cell lines, and its IC_{50} of anti-proliferation against HL-60 cells is 0.48 mM (4 times potent than **1**).

4. Chemical stability investigation

Three representative compounds (compounds **2g**, **3a** and **5**) were selected for the investigation of chemical stability in aqueous phase with comparison of cheliensisin A (**1**). The results indicate that compounds **3a** and **5** exhibit better chemical stability, and compound **2g** is the most stable one under the specific conditions (37 °C, *p*H 7.0) (Fig. 3) (see Supporting Information for the details). Obviously, incorporation of oxazole and oxazoline moiety improved the chemical stability of cheliensisin A. These improvements make them much more drug-like than their natural parent cheliensisin A, and would be promising for the future further development.

5. Conclusion

In summary, twenty one new derivatives of natural styryllactone cheliensisin A were designed, synthesized and evaluated with consideration of improvement of chemical stability and conformational constraints by small heterocycles in this

work. Eight compounds (**2b**, **2d**, **2g**, **2h**, **2i**, **3a**, **3c** and **5**) were found to exhibit potent inhibitory activities against the cell proliferation of SK-BR-3 and PANC-1. In addition, the preference of C7/C8 *cis*-configuration was observed, and the reasonable rigid conformation was found to favor the bioactivities. In addition, our investigation revealed that oxazoline analog **2g** presented much higher chemical stability than cheliensisin A. All these findings will be helpful for the further development of new druggable derivatives of cheliensisin A and other cytotoxic styryl-lactones in this family.

6. Experimental sections

6.1. Material and methods

Reagents and solvents were used as commercial grade, and dichloromethane and tetrahydrofuran were treated as anhydrous solvents prior to use. Chromatographies were performed with 300–400 mesh silica gels. Thin layer chromatographies were carried out on Merck silica plates (0.25 mm layer thickness). ESIMS and HRESIMS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument. Optical rotations were measured with a Horiba SEPA-300 polarimeter. ^{1}H and ^{13}C NMR experiments were performed on a Bruker AM-300, AM-400 and DRX-500 NMR spectrometer at ambient temperature. And chemical shifts were given in δ with TMS as internal reference.

6.2. Synthesis

6.2.1. Typical procedure for preparation of oxazoline compounds **2a**–**2i** (Preparation of compounds **2a** and **2b**) [11]

To a solution of cheliensisin A (548 mg, 2 mmol) in PhCN (10 mL) at −20 °C, a solution of $BF_3{\cdot}OEt_2$ (668 μL, 2 mmol) in PhCN (5 mL) was added over 15 min. The reaction completed in 1 h with TLC judgment. A saturated aqueous $NaHCO_3$ solution (12 mL) was then added to quench the reaction. The mixture was extracted with dichloromethane (100 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:3) to give compounds **2a** and **2b** as white foams in 34% (257 mg) and 53% (403 mg), respectively.

*6.2.1.1. (4S,5R)-2-phenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′ -yl]-5-phenyl-(5H,6H)-oxazoline (**2a**).* White foam, 34% yield; $[\alpha]_D^{20}$ + 121.9 (*c* 0.19, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 8.01–8.03 (d, *J* = 7.2 Hz, 2H), 7.31–7.55 (m, 8H), 7.09 (dd, *J* = 9.6, 6.3 Hz, 1H), 6.28 (d, *J* = 9.6 Hz, 1H), 5.51 (d, *J* = 4.8 Hz, 1H), 5.48 (dd, *J* = 6.0, 3.0 Hz, 1H), 4.80 (dd, *J* = 9.3, 4.8 Hz, 1H), 4.56 (dd, *J* = 9.3, 2.4 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.7, 163.2, 161.2 , 142.1, 141.1, 140.5, 132.0, 128.8, 128.7, 128.6, 128.5, 128.0, 127.8, 126.7, 125.5, 81.6, 78.0, 72.8, 61.1, 20.6; HRMS (ESI, *m/z*): calcd. for $C_{22}H_{20}NO_5$ $[M + H]^+$, 378.1341, found 378.1352.

*6.2.1.2. (4R,5R)-2-phenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′ -yl]-5-phenyl-(5H,6H)-oxazoline (**2b**).* White foam, 53% yield; $[\alpha]_D^{27}$ + 256.5 (c 0.33, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.99–8.02 (d, *J* = 7.5 Hz, 2H), 7.45–7.56 (m, 3H), 7.27–7.36 (m, 5H), 6.93 (dd, *J* = 9.6, 6.3 Hz, 1H), 6.05 (d, *J* = 9.9 Hz, 1H), 5.69 (d, *J* = 9.3 Hz, 1H), 5.42 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.05 (dd, *J* = 9.6, 9.3 Hz, 1H), 4.06 (dd, *J* = 9.6, 2.7 Hz, 1H), 2.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.7, 163.8, 160.6 , 139.8, 135.6, 132.1, 129.7, 128.6, 128.4, 128.3, 128.3, 128.2, 126.4, 126.8, 124.8, 78.1, 75.5, 71.2, 61.5, 20.6; HRMS (ESI, *m/z*): calcd. for $C_{22}H_{20}NO_5$ $[M + H]^+$, 378.1341, found 378.1353.

*6.2.1.3. (4S,5R)-2-benzyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′ -yl]-5-phenyl-(5H,6H)-oxazoline (**2c**).* White foam, 31% yield; $[\alpha]_D^{24}$ + 42.8 (c 0.78, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.21–7.31 (m, 10H), 6.93 (dd, *J* = 9.6, 6.0 Hz, 1H), 6.13 (d, *J* = 9.6 Hz, 1H), 5.21 (d, *J* = 4.5 Hz, 1H), 5.00 (dd, *J* = 5.7, 2.7 Hz, 1H), 4.54 (dd, *J* = 9.0, 5.1 Hz, 1H), 4.25 (dd, *J* = 9.0, 2.7 Hz, 1H), 3.65 (d, *J* = 3.0 Hz, 2H), 1.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.9, 162.5, 161.8, 141.0, 140.1, 134.6, 131.1, 130.1, 129.1, 128.9, 128.7, 127.2, 126.7, 126.5, 125.4, 125.0, 81.7, 72.3, 70.7, 61.1, 34.6, 20.4; HRMS (ESI, *m/z*): calcd. for $C_{23}H_{22}NO_5$ $[M + H]^+$, 392.1497, found 392.1491.

*6.2.1.4. (4R,5R)-2(E)-propenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′- one-6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2d**).* White foam, 22% yield; $[\alpha]_D^{26}$ + 29.8 (c 0.38, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.14–7.32 (m, 5H), 6.86 (dd, *J* = 9.6, 6.3 Hz, 1H), 6.60 (td, *J* = 22.5,6.9,6.9 Hz, 1H), 6.09 (d, *J* = 15.6 Hz, 1H), 5.97 (d, *J* = 9.6 Hz, 1H), 5.47 (d, *J* = 9.3 Hz, 1H), 5.25 (dd, *J* = 6.0, 2.7 Hz, 1H), 4.65 (t, *J* = 9.6, 9.6 Hz, 1H), 3.92 (dd, *J* = 9.6, 2.7 Hz, 1H), 2.10(s, 3H), 1.89 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.6, 163.0, 160.6, 141.0, 139.8, 135.1, 128.2, 128.1, 124.7, 124.7, 118.1, 77.4, 75.5, 70.9, 61.5, 20.5, 18.5; HRMS (ESI, *m/z*): calcd. for $C_{19}H_{20}NO_5$ $[M + H]^+$, 342.1341, found 342.1351.

*6.2.1.5. (4S,5R)-2(Z)-propenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′- one-6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2e**).* White foam, 13% yield; $[\alpha]_D^{26}$ + 351.1 (c 0.47, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.27–7.39 (m, 5H), 7.09 (dd, *J* = 9.6, 6.0 Hz, 1H), 6.26 (td, *J* = 18.9, 7.5, 7.5 Hz, 1H), 6.25 (d, *J* = 9.9 Hz, 1H), 5.98 (dd, *J* = 12.0, 1.5 Hz, 1H), 5.38(d, *J* = 5.7 Hz, 1H), 5.35 (dd, *J* = 6.0, 2.7 Hz, 1H), 4.65 (dd, *J* = 9.3, 4.8 Hz, 1H), 4.50 (dd, *J* = 9.3, 2.7 Hz, 1H), 2.10 (d, *J* = 6.9 Hz, 3H), 2.04(s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.6, 163.0, 160.6, 140.9, 139.7, 135.7, 128.2, 128.1, 128.1, 126.9, 124.8, 118.2, 77.4, 75.5, 70.9, 61.6, 20.5, 18.4; HRMS (ESI, *m/z*): calcd. for $C_{19}H_{20}NO_5$ $[M + H]^+$, 342.1341, found 342.1341.

*6.2.1.6. (4S,5R)-2(E)-propenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′- one-6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2f**).* White foam, 40% yield; $[\alpha]_D^{27}$ + 409.8 (c 0.59, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.27–7.39 (m, 5H), 7.06 (dd, *J* = 9.6, 6.3 Hz, 1H), 6.69 (td, *J* = 22.5, 6.9, 6.9 Hz, 1H), 6.24 (d, *J* = 9.6 Hz, 1H), 6.11 (dd, *J* = 15.9, 1.5 Hz, 1H), 5.36 (dd, *J* = 6.0, 2.7 Hz, 1H), 5.34(d, *J* = 4.8 Hz, 1H), 4.6 (t, *J* = 9.3, 4.8 1H), 4.48 (dd, *J* = 9.3, 2.7 Hz, 1H), 2.04 (s, 3H), 1.91 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.6, 162.4, 161.2 , 141.2, 141.0, 140.1, 128.7, 128.5, 127.6, 126.6, 118.1, 81.0, 78.0, 72.5, 61.0, 20.5, 18.4; HRMS (ESI, *m/z*): calcd. for $C_{19}H_{20}NO_5$ $[M + H]^+$, 342.1341, found 342.1346.

*6.2.1.7. (4R,5R)-2(E)-styryl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one -6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2g**).* White foam, 63% yield; $[\alpha]_D^{26}$ + 78.8 (c 0.55, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.23–7.45 (m, 10H), 6.99 (dd, *J* = 6.6, 0.9 Hz, 1H), 6.92 (dd, *J* = 9.6, 6.0 Hz, 1H), 6.80 (d, *J* = 16.5 Hz, 1H), 6.02 (d, *J* = 9.9 Hz, 1H), 5.60 (d, *J* = 9.3 Hz, 1H), 5.38 (dd, *J* = 9.0, 2.7 Hz, 1H), 4.97 (t, *J* = 9.6, 9.6 Hz, 1H), 4.02 (dd, *J* = 9.6, 2.7 Hz, 1H), 2.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 163.7, 160.7, 141.5, 139.8, 135.5, 134.7, 129.9, 129.8, 128.9, 128.3, 128.2, 127.7, 124.8, 113.9, 77.7, 75.5, 71.1, 61.6, 20.6; HRMS (ESI, *m/z*): calcd. for $C_{24}H_{22}NO_5$ $[M + H]^+$, 404.1497, found 404.1497.

*6.2.1.8. (4S,5R)-2(E)-styryl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one -6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2h**).* White foam, 11% yield; $[\alpha]_D^{26}$ + 276.2 (c 0.55, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.33–7.55 (m, 11H), 7.12(dd, *J* = 9.6, 5.7 Hz, 1H), 6.74 (d, *J* = 16.2 Hz, 1H), 6.28 (d, *J* = 9.6 Hz, 1H), 5.45 (dd, *J* = 6.0, 2.7 Hz, 1H), 5.45 (d, *J* = 5.4 Hz, 1H), 4.74 (dd, *J* = 9.0, 4.8 Hz, 1H), 4.54 (dd, *J* = 9.3,

2.7 Hz, 2H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 162.9, 161.2, 141.4, 141.1, 140.2, 134.7, 129.9, 128.9, 128.8, 127.8, 127.6, 126.6, 125.2, 113.9, 81.2, 78.0, 72.7, 61.1, 20.5; HRMS (ESI, *m/z*): calcd. for $C_{24}H_{22}NO_5$ $[M + H]^+$, 404.1497, found 404.1487.

*6.2.1.9. (4S,5R)-2-methyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′ -yl]-5-phenyl-(5H,6H)-oxazoline (**2i**).* White foam, 68% yield; $[\alpha]_D^{26}$ + 308.1 (c 0.69, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.30–7.39 (m, 5H), 7.11(dd, *J* = 9.6, 5.7 Hz, 1H), 6.26 (d, *J* = 9.9 Hz, 1H), 5.30(d, *J* = 2.1 Hz, 1H), 5.28 (d, *J* = 3.0 Hz, 1H), 4.62 (dd, *J* = 9.0, 5.1 Hz, 1H), 4.48 (dd, *J* = 9.0, 2.1 Hz, 1H), 2.12 (s, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.7, 164.2, 161.2 , 141.2, 140.8, 130.1, 128.7, 128.6, 127.6, 126.5, 125.2, 123.2, 81.5, 78.1, 69.7, 61.2, 20.4, 13.8; HRMS (ESI, *m/z*): calcd. for $C_{17}H_{17}NO_5$ $[M + H]^+$, 316.1184, found 316.1177.

*6.2.2. Typical procedure for preparation of oxazole derivatives, compounds **2j** and **3a–3c** (Preparation of compound **3a**) [13]*

The mixture of **2h** (121 mg, 0.3 mmol) and 2, 3-dichloro-5, 6-dicyanobenzoquinon (DDQ, 69 mg, 0.3 mmol) in dry toluene (10 mL) was heated to reflux at 110 °C for 3.5 h until no starting material was detected by TLC. The solvent was removed and the residue was treated with dichloromethane. The solid was removed by filtration. The solution was concentrated under reduced pressure. The crude product was subjected to flash chromatography on silica gel (ethyl acetate/petrol ether = 1:2.5) to afford **3a** as a white foam (102 mg, 85%).

*6.2.2.1. (4S,5R)-2-benzoyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one- 6′-yl]-5-phenyl-(5H,6H)-oxazoline (**2j**).* Compound **2j** was prepared as a white foam from **2c** following the above procedure in the yield of 64%. $[\alpha]_D^{19}$ + 285.3 (c 0.07, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 8.34–8.36 (d, *J* = 8.4 Hz, 2H), 7.48–7.69 (m, 8H), 7.19 (dd, *J* = 9.3, 6.0 Hz, 1H), 6.27 (d, *J* = 9.6 Hz, 1H), 5.69 (d, *J* = 5.4 Hz, 1H), 5.32 (dd, *J* = 6.0, 3.3 Hz, 1H), 4.93 (t, *J* = 7.5, 6.0 Hz, 1H), 4.66 (d, *J* = 7.8 Hz, 1H), 1.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 160.7, 158.7, 152.9, 140.2, 136.1, 133.1, 131.8, 129.3, 128.9, 128.7, 128.4, 127.3, 124.9, 78.3, 75.4, 60.3, 59.4, 29.6, 20.4; HRMS (ESI, *m/z*): calcd. for $C_{23}H_{20}NO_6$ $[M + H]^+$ 406.1290, found 406.1284.

*6.2.2.2. 2(E)-styryl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′-yl]- 5-phenyl-oxazole (**3a**).* Compound **3a** was prepared as a white foam from **2 h** following the above procedure in the yield of 85%. $[\alpha]_D^{26}$ + 152.4 (c 0.45, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.63 (d, *J* = 8.1 Hz, 2H), 7.55 (d, *J* = 6.9 Hz, 2H), 7.36–7.48 (m, 7H), 7.00 (dd, *J* = 9.9, 4.8 Hz, 1H), 6.96 (d, *J* = 16.2 Hz, 1H), 6.33 (d, *J* = 9.6 Hz, 1H), 5.88 (d, *J* = 3.9 Hz, 1H), 5.70 (t, *J* = 3.9, 3.9 Hz, 1H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 161.7, 161.6, 142.3, 141.2, 139.1, 131.0, 130.5, 128.9, 128.0, 126.6, 123.9, 128.9, 124.7, 128.8, 126.7, 71.7, 63.4, 20.4; HRMS (ESI, *m/z*): calcd. for $C_{24}H_{20}NO_5$ $[M + H]^+$ 402.1341, found 402.1579.

*6.2.2.3. 2(Z)-propenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′- yl]-5-phenyl-oxazole (**3b**).* Compound **3b** was prepared as a white foam from **2e** following the above procedure in the yield of 42%. $[\alpha]_D^{25}$ + 107.6 (c 0.14, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.5 (d, *J* = 6.9 Hz, 2H), 7.39–7.47 (m, 3H), 6.97 (dd, *J* = 9.9, 4.8 Hz, 1H), 6.81 (td, *J* = 22.8, 6.6 Hz, 1H), 6.33 (dd, *J* = 12.9, 1.5 Hz, 1H), 6.31 (d, *J* = 9.9 Hz, 1H), 5.83 (d, *J* = 3.9 Hz, 1H), 5.69 (t, *J* = 4.2 Hz, 1H), 2.01 (s, 3H), 1.97 (dd, *J* = 7.2, 1.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6, 161.7, 161.1, 141.0, 138.1, 137.0, 130.5, 128.8, 127.9, 123.9, 117.4, 71.7, 63.3, 20.4, 18.5; HRMS (ESI, *m/z*): calcd. for $C_{19}H_{17}NO_5Na$ $[M + Na]^+$ 362.1004, found 362.1002.

*6.2.2.4. 2-Phenyl-4-[(5′S,6′R)-5′-acetoxyl-5′,6′-dihydro-2′H-pyran-2′-one-6′-yl]-5-phenyl-oxazole (**3c**).* Compound **3c** was prepared as a white foam from **2b** following the above procedure in the yield of 53%. $[\alpha]_D^{19}$ + 108.2 (c 0.17, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 500 MHz): δ 8.09–8.11 (m, 2H), 7.68–7.69 (m, 2H), 7.43–7.51 (m, 6H), 7.02 (dd, *J* = 9.5, 5.5 Hz, 1H), 6.35 (d, *J* = 10.0 Hz, 1H), 5.94 (d, *J* = 4.0 Hz, 1H), 5.75 (t, *J* = 4.0, 4.0 Hz, 1H), 2.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.7, 161.7, 161.6, 142.3, 141.2, 139.1, 131.0, 130.5, 128.9, 128.9, 128.8, 128.0, 126.7, 126.6, 124.7, 123.9, 71.7, 63.4, 20.4; HRMS (ESI, *m/z*): calcd. for $C_{22}NO_5Na$ $[M + Na]^+$ 398.1004, found 398.1014.

*6.2.3. Typical procedure for compounds **4a–4c** (Preparation of compound **4a**)*

A solution of **2b** (38 mg, 0.1 mmol) in ethanol (0.8 mL) was treated with 0.5 M aqueous HCl solution (0.2 mL, 0.1 mmol) and refluxed at 80 °C for 2.5 h until no starting material was detected. The mixture was cooled to the room temperature and a saturated aqueous $NaHCO_3$ solution was added to *p*H 7.0. The mixture was concentrated under reduced pressure. The residue was extracted with dichloromethane (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (ethyl acetate/petrol ether = 1:1.5) to afford **4a** as a colorless film (18 mg, 46%).

*6.2.3.1. (7S,8R)-7-benzoylamino-8-hydroxyl cheliensisin A (**4a**).* Compound **4a** was prepared as a colorless film from **2b** following the above procedure in the yield of 46%. $[\alpha]_D^{17}$ + 126.5 (*c* 0.12, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.85 (d, *J* = 7.2 Hz, 2H), 7.30–7.54 (m, 8H), 7.05 (dd, *J* = 9.6, 6.0 Hz, 1H), 6.22 (d, *J* = 9.6 Hz, 1H), 5.61 (d, *J* = 7.5 Hz, 1H), 5.45 (dd, *J* = 6.3, 2.1 Hz, 1H), 4.42 (dd, *J* = 9.6, 2.1 Hz, 1H), 4.23 (d, *J* = 6.9 Hz, 1H), 4.10 (d, *J* = 5.7 Hz, 1H) , 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 167.4, 161.6 , 140.4, 138.7, 133.5, 132.0, 128.8, 128.7, 127.8, 127.1, 127.0, 125.2, 77.9, 70.6, 61.4, 54.6, 20.7; HRMS (ESI, *m/z*): calcd. for $C_{22}H_{22}NO_6$ $[M + H]^+$ 396.1447, found 396.1459.

*6.2.3.2. (7S,8R)-7-phenylacetylamino-8-hydroxyl cheliensisin A (**4b**).* Compound **4b** was prepared as a colorless film from **2c** following the above procedure in the yield of 37%. $[\alpha]_D^{27}$ + 137.0 (c 0.15, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.22–7.39 (m, 10H), 7.02 (dd, *J* = 9.3, 6.0 Hz, 1H), 6.21 (d, *J* = 9.6 Hz, 1H), 5.31 (dd, *J* = 7.2, 2.1 Hz, 1H), 5.07 (dd, *J* = 6.0, 2.7 Hz, 1H), 4.62 (dd, *J* = 9.0, 4.8 Hz, 1H), 4.33 (dd, *J* = 9.0, 2.7 Hz, 1H), 3.73 (d, *J* = 3.0 Hz, 2H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.7, 170.5, 161.6, 140.3, 138.7, 134.7, 129.5, 129.2, 129.1, 128.7, 128.7, 127.6, 127.4, 126.8, 126.3, 125.1, 122.0, 77.6, 70.7, 61.3, 54.2, 43.9, 20.6; HRMS (ESI, *m/z*): calcd. for $C_{23}H_{23}NO_6Na$ $[M + Na]^+$ 432.1423, found 432.1410.

*6.2.3.3. (7S,8R)-7-cinnamoylamino-8-hydroxyl cheliensisin A (**4c**).* Compound **4c** was prepared as a white foam from **2h** following the above procedure in the yield of 63%. $[\alpha]_D^{19}$ + 115.4 (c 0.48, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.30–7.47 (m, 10H), 7.06 (dd, *J* = 8.4, 6.0 Hz, 1H), 6.64 (d, *J* = 14.4 Hz, 1H), 6.18 (d, *J* = 16.5 Hz, 1H), 5.46 (d, *J* = 6.4 Hz, 2H), 4.48 (d, *J* = 8.8 Hz, 1H), 4.22 (dd, *J* = 8.8, 5.7 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 166.7, 162.1, 142.6, 140.7, 138.8, 134.3, 130.0, 129.3, 128.8, 128.7, 128.6, 128.2, 128.0, 127.7, 127.1, 126.7, 119.6, 77.9, 70.7, 61.4, 54.9, 20.6; HRMS (ESI, *m/z*): calcd. for $C_{24}H_{23}NO_6Na$ $[M + Na]^+$ 444.1423, found 444.1414.

*6.2.4. 7,8-En-cheliensisin A (**5**)*

A mixture of cheliensisin A (55 mg, 0.2 mmol) and $Mo(CO)_6$ (53 mg, 0.2 mmol) in dry toluene (3 mL) was heated to reflux at 110 °C for 5.5 h until no starting material was indicated by TLC.

Then the mixture was cooled to the room temperature and the solvent was removed under reduced pressure. The residue was washed with ethyl acetate through a short bed of silica gel. Further purification by flash chromatography on silica gel using ethyl acetate/petrol ether (1:2.5) afforded compound **5** as a white foam (31 mg, 60%). $[\alpha]_D^{22}$ + 278.5 (c 0.11, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 500 MHz): δ 7.27–7.40 (m, 5H), 7.00 (dd, J = 9.6, 5.6 Hz, 1H), 6.82 (d, J = 16.0 Hz, 1H), 6.26 (d, J = 10.0 Hz, 1H), 6.19 (dd, J = 16.0, 6.4 Hz, 1H), 5.37 (dd, J = 5.2, 2.4 Hz 1H), 5.19 (ddd, J = 6.4, 2.4, 1.2 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.0, 162.4, 142.1, 140.6, 135.6, 134.8, 129.8, 128.7, 128.5, 126.7, 124.8, 121.1, 79.0, 63.8, 20.5; HRMS (ESI, *m/z*): calcd. for $C_{15}H_{14}O_4Na$ $[M + Na]^+$ 281.0789, found 281.0795.

*6.2.5. (7S,8S)-7-Hydroxyl-8-phenylamino cheliensisin A (**6a**)*

A solution of cheliensisin A (55 mg, 0.2 mmol) and aniline (37 mg, 0.2 mmol) in dry toluene (1.5 mL) was heated to reflux for 4 h until no starting material was detected by TLC. The mixture was cooled to room temperature. An aqueous hydrochloride acid solution (1N) was added to quench the reaction. The aqueous layer was extracted with ethyl acetate (25 mL × 3). The combined organic phase was washed with brine, dried over $NaSO_4$ and concentrated. The residue was subjected to silica gel (petrol ether/ethyl acetate = 2.5:1) to obtain compound **6a** as a white foam (54 mg, 75%). $[\alpha]_D^{18}$ + 24.5 (c 0.39, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 400 MHz): δ 7.37–7.39 (m, 2H), 7.19–7.27 (m, 4H), 7.01–7.05 (m, 2H), 6.85 (dd, J = 9.6, 6.4 Hz, 1H), 6.55–6.66 (m, 2H), 6.15 (d, J = 9.6 Hz, 1H), 5.27 (d, J = 6.4, 2.4 Hz, 1H), 4.89 (d, J = 2.8 Hz, 1H), 4.15 (d, J = 6.0 Hz, 1H), 3.79 (dd, J = 10.0, 2.4 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.5, 161.6, 146.2, 139.8, 137.1, 129.1, 128.7, 128.6, 128.4, 127.9, 125.4, 117.8, 113.6, 78.0, 69.6, 56.6, 53.7, 20.5; HRMS (ESI, *m/z*): calcd. for $C_{21}H_{22}NO_5$ $[M + H]^+$ 368.1497, found 368.1496.

*6.2.6. (7S,8R)-7,8-dihydroxyl cheliensisin A (**6b**)*

To a solution of cheliensisin A (1.1 g, 4 mmol) in MeCN (15 mL) was added dropwise an aqueous solution of citric acid (10 mL, 0.1 mol/L). The mixture was stirred at room temperature until most of the starting material was transformed. A saturated aqueous $NaHCO_3$ solution (8 mL) was added to quench the reaction. The mixture was extracted by ethyl acetate (150 mL × 3). The organic layers were combined and washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was subjected to flash chromatography on silica gel using ethyl acetate/petrol ether (1:1.5) to give compound **6b** as a white foam (425 mg , 98% based on 66% of the starting material recovered). $[\alpha]_D^{27}$ + 276.4 (c 0.92, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.32–7.45 (m, 5H), 7.11 (dd, J = 9.6, 5.7 Hz, 1H), 6.23 (d, J = 9.6 Hz, 1H), 5.44 (dd, J = 6.0, 2.4 Hz, 1H), 5.23 (d, J = 5.1 Hz, 1H), 4.69 (dd, J = 9.3, 2.4 Hz, 1H), 3.96 (t, J = 8.4 Hz, 1H), 2.55 (d, J = 7.5 Hz, 1H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.2, 162.4, 140.8, 128.6, 128.4, 127.9, 126.0, 124.8, 78.2, 71.8, 70.3, 61.5, 20.5; HRMS (ESI, *m/z*): calcd. for $C_{15}H_{16}O_6Na$ $[M + Na]^+$ 315.0844, found 315.0837.

*6.2.7. (7S,8R)-7,8-acetonyl-dioxa-cheliensisin A (**7**)*

To a solution of compound **6b** (54 mg, 0.2 mmol) in dimethoxypropane (DMP, 2 mL) was added a catalytic amount of tosic acid. The mixture was stirred at room temperature for 14 h until all the starting material was transformed. A saturated aqueous $NaHCO_3$ solution was added to quench the reaction. Water (10 mL) was added and the mixture was extracted by dichloromethane (25 mL × 3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel using ethyl acetate/petrol ether (1:1.5) to give compound **7** as a colorless film (45 mg, 68%). $[\alpha]_D^{22}$ + 230.6 (c 0.83, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.33–7.54 (m, 5H),7.09 (dd, J = 9.9, 6.0 Hz, 1H), 6.19 (d, J = 9.9 Hz, 1H), 5.32 (dd, J = 5.7, 2.7 Hz, 1H), 5.19 (d, J = 6.3 Hz, 1H), 4.58 (dd, J = 9.3, 2.7 Hz, 1H), 4.29 (dd, J = 9.3, 6.3 Hz, 1H), 2.00 (s, 3H), 1.51 (s, 3H), 1.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.8, 161.2, 140.6, 138.7, 128.4, 128.1, 126.8, 124.8, 110.5, 82.0, 80.0, 78.7, 61.6, 27.1, 20.4; HRMS (ESI, *m/z*): calcd. for $C_{18}H_{20}O_6Na$ $[M + Na]^+$ 355.1157, found 355.1157.

*6.2.8. (7S,8R)-7,8-carbonate-dioxa-cheliensisin A (**8**)*

To a solution of compound **6b** (146 mg, 0.5 mmol) in dry dichloromethane (2 mL) and pyridine (0.5 mL) was added dropwise a solution of triphosgene (74 mg, 0.25 mmol) in dry dichloromethane (0.5 mL) at 0 °C. The reaction was completed by TLC in 2 h. A saturated aqueous NH_4Cl solution was added to quench the reaction. Then the mixture was extracted by dichloromethane (50 mL × 3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was chromatographed on silica gel using ethyl acetate/etrol ether (1:1.5) to afford compound **8** as a white foam (110 mg, 70%). $[\alpha]_D^{26}$ + 315.1 (c 0.31, $CHCl_3$); ^{1}H–NMR ($CDCl_3$, 300 MHz): δ 7.43–7.45 (m, 5H), 7.14 (dd, J = 9.6, 6.0 Hz, 1H), 6.25 (d, J = 9.6 Hz, 1H), 5.80 (d, J = 4.2 Hz, 1H), 5.33 (dd, J = 6.0, 2.4 Hz, 1H), 4.87 (d, J = 3.9 Hz, 1H), 4.29 (s, 1H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.2, 160.3, 153.1, 140.2, 135.9, 129.7, 129.3, 125.7, 125.7, 124.9, 79.7, 78.4, 77.3, 60.9, 20.3; HRMS (ESI, *m/z*): calcd. for $C_{16}H_{14}O_7Na$ $[M + Na]^+$ 341.0637, found 341.0640.

6.3. Biological assay

The used cell lines were human promyelocytic leukemia cell line (HL-60), human hepatocellular carcinoma cell line (SK-BR-3), human lung carcinoma cell line (A-549), human breast adenocarcinoma cell line (SMMC-7721), human pancreatic carcinoma cell line (PANC-1). An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in 96-well plates. HL-60 cells at the log phase of their growth cycle (1.25×10^5 cell/mL) were added to each well (90 mL/well), then treated in four replicates at various concentrations of the samples (1–100 μg/mL), and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 10 μL of MTT solution (5 mg/mL) per well was added to each cultured medium, which were incubated for further 4 h. Then, a three-system solution of 10% SDS–5% isobutanole – 0.012 mol/L hydrochloric acid was added to each well (100 μL/well). After 12 h at room temperature, the OD of each well was measured on a Microplate Reader (BIO-TEK instruments Inc EL311S) at a wavelength of 570 nm. In these experiments, the negative reference agents was 0.1% DMSO, and cisplatin (DDP) was used as the positive reference substance with concentration of 1–80 μg/mL. The same method was used in cytotoxic testing against SK-BR-3, A549, SMMC-7721 and PANC-1 cell lines.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.06.028.

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