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Discovery and synthesis of new immunosuppressive alkaloids from the stem of *Fissistigma oldhamii* (Hemsl.) Merr.

Yi-Nan Zhang,† Xiang-Gen Zhong,† Zong-Ping Zheng, Xu-Dong Hu, Jian-Ping Zuo* and Li-Hong Hu*

Shanghai Research Center for Modernization of Traditional Chinese Medicine and First Department of Pharmacology, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China

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Abstract—Three new alkaloids (**1**–**3**) and twenty-one known compounds were isolated from the stem of *Fissistigma oldhamii* (Hemsl.) Merr. which was the ruler herb in an approved Traditional Chinese herbal formula used for treatment of rheumatoid arthritis in China and synthesis of one new immunosuppressive alkaloid was achieved. These compounds, including the crude extracts of this herb, exhibited strong activities in the inhibition of T and B cell proliferation.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints. It is a disease that afflicts large population around the world with no effective treatment available so far for its cure. Scientific evidence exists that RA is a type of autoimmune disease[1] and that it has strong relevance with T and B cells.[2–4] The evidences provided prove that T and B lymphocytes play a vital role in the pathogenesis of RA suggesting that blockade of these cells may prove to be an effective therapeutic mechanism in a variety of autoimmune disorders. Thus, many immunosuppressive agents were employed for the therapy of RA.

In search of new anti-RA agents with high efficacy and low toxicity, attention to anti-RA herbs or formulas from Traditional Chinese Medicine was an obvious logical research option resulting in the findings we like to report here. A SFDA (State Food and Drug Administration in China) approved herbal formula, zuanshanfeng syrup,[5] and its ruler herb, the stem of *Fissistigma oldhamii* (Hemsl.) Merr.,[6] were found to have strong inhibitory activities against T and B lymphocyte proliferation (see Table 2). *Fissistigma oldhamii* was previously reported to contain alkaloids,[7] furanones,[8] cyclopentenones,[9] flavonoids, and chalcones.

In order to identify the active constituents responsible for inhibition of T and B lymphocyte proliferation from this herb we systematically examined the ethanolic extract in the present study. Twenty-four compounds, including 9 aporphine alkaloids, 8 aristolactams, 4 oxoaporphines, 2 dioxoaporphines, and 1 morphinandiene, are being reported. The structures of three new compounds **1**–**3** were mainly elucidated by NMR data. The two oxoaporphine alkaloids exhibited stronger activities against T and B cell inhibition than others. Due to low yield of **1** from the plant material, we have accomplished its total synthesis which enabled us to synthesize sufficient compound for further biological studies.

2. Results and discussion

2.1. Structural elucidation of three new compounds (1–3)

2.1.1. Oxodiscoguattine (1). Compound **1**, isolated as red amorphous powder, showed a molecular ion peak at *m/z* 335.0788 in its HREI-MS, indicating a molecular formula of $C_{19}H_{13}NO_5$ (calcd for $[M]^+$, 335.0794). Its UV spectrum (absorption maxima at 230, 265 sh, 330,

* Corresponding authors. Tel./fax: +86 21 50802221 (L.-H.H.); tel./fax: +86 21 50806701 (J.-P.Z.); e-mail addresses: simmhulh@mail.shcnc.ac.cn; jpzuo@mail.shcnc.ac.cn
† The two authors contributed equally to this work.

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and 356 nm) and IR spectrum (a conjugated carbonyl group absorption band at 1660 cm^{-1}) indicated **1** was an oxoaporphine derivative.[10] Its NMR spectra exhibited the presence of two methoxyl groups [δ_H 3.98 and δ_C 55.6; δ_H 3.97 and δ_C 55.8], one methylenedioxy group [δ_H 6.22 (2H, s) and δ_C 101.7], one aromatic protons [δ_H 7.10 (1H, s) and δ_C 101.7], two *meta*-coupled aromatic protons [δ_H 7.65 (1H, d, $J = 2.6$) and δ_C 120.4; δ_H 6.83 (1H, d, $J = 2.6$) and δ_C 105.2], and two *ortho*-coupled aromatic protons [δ_H 7.71 (1H, d, $J = 5.1$) and δ_C 124.2; δ_H 8.82 (1H, d, $J = 5.1$) and δ_C 143.7]. In addition, one carbonyl group [δ_C 182.3] and 10 quaternary carbons were present in the molecule. These data, together with comparison with NMR data of the known aporphine alkaloid, dicentrinone from the literature,[11] established the structure of compound **1**, as a new 9,11-dimethoxy-1,2-methylenedioxyoxoaporphine, named oxodiscoguattine,[12] and its ^1H and ^{13}C NMR spectra (Table 1) were assigned unambiguously by the long-range correlations in its HMBC, HMQC, and NOESY spectra, as shown in Scheme 1. The structure was also confirmed by our synthesis work below (Scheme 2).

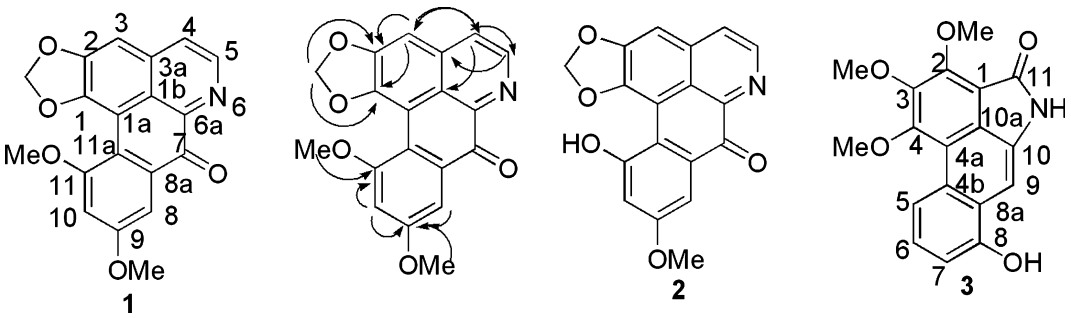
2.1.2. Oxocalycinine (2). Compound **2** was obtained as red amorphous powder. The molecular formula of $C_{18}H_{11}NO_5$ was assigned to **2** on the basis of its HREI-MS (found *m/z* 321.0623, calcd for $[M]^+$ was *m/z* 321.0637). The ^1H and ^{13}C NMR data of **2** were very similar to those of compound **1** (see Table 1), which was only lack of a methoxyl group. Cross peaks between the methoxyl proton to H-8 and H-10 in its NOESY spectrum (see Fig. 1) determined that the hydroxyl group should be located at C_{11} and the residual methoxyl group to C_9. So, compound **2**, named oxocalycinine,[13] was determined as a new 11-hydroxy-9-methoxy-1,2-methyl-enedioxyoxoaporphine.

2.1.3. Oldhamactam (3). Compound **3**, mp 224–226 °C, was obtained as brownish-yellow powder. The HREI-MS, exhibiting a molecular ion peak at *m/z* 325.0934, indicated that the molecular formula was $C_{18}H_{15}NO_5$

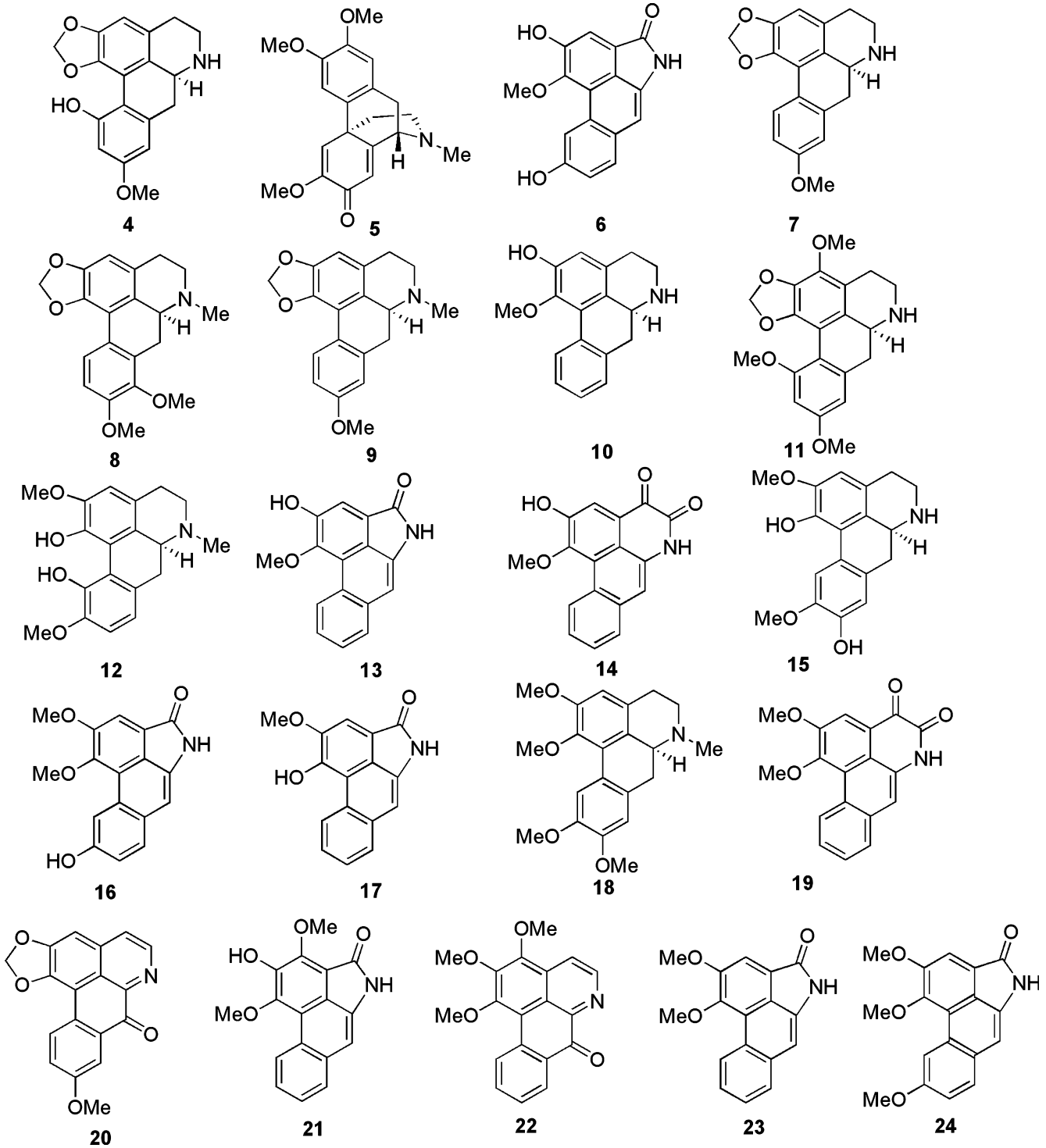
Table 1. NMR spectroscopic data of compounds **1**[a], **2**, and **3**

Position	**1** (in $CDCl_3$)		**2** (in DMSO)		Position	**3** (in DMSO)	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1		147.5		144.0	**1**		115.7
1a		124.1		122.4	**2**		153.5
1b		123.1		124.0	**3**		153.3
2		152.8		156.8	**4**		146.0
3	7.10, s	101.7	7.41, s	107.9	**4a**		125.1
3a		136.1		135.4	**4b**		109.6
4	7.71, d, (5.1)	124.2	7.95, d, (5.1)	124.0	**5**	8.58, d, (8.1)	117.0
5	8.82, d, (5.1)	143.7	8.73, d, (5.1)	144.1	**6**	7.35, dd, (8.1, 7.5)	126.8
6					**7**	7.03, d, (7.5.)	110.8
6a		144.0		146.5	**8**		156.3
7		182.3		181.3	**8a**		125.7
8	7.65, d, (2.6)	102.4	7.34, d, (2.7)	102.7	**9**	7.53, s	99.5
8a		116.2		113.6	**10**		133.2
9		161.0		160.3	**10a**		123.0
10	6.83, d, (2.6)	105.2	6.85, d, (2.7)	101.5	**11**		166.2
11		158.4		152.2	OMe, 2	4.39, s	62.5
11a		134.1		134.2	OMe, 3	3.90, s	61.4
OCH_2O	6.22, s	101.7	6.34, s	102.1	OMe, 4	4.08, s	60.7
OMe, 9	3.98, s	55.6	4.02, s	55.5	OH, 8	10.10, s	
OMe, 11	3.97, s	55.8			NH	10.90, s	

[a] ^1H NMR (300 MHz) was referenced to δ 2.50 (DMSO) and ^{13}C NMR (75 MHz) to δ 39.51 (DMSO); ^1H NMR (300 MHz) was referenced to δ 7.26 ($CDCl_3$) and ^{13}C NMR (75 MHz) to δ 77.23 ($CDCl_3$); chemical shifts are shown in the δ scale with J values (Hz) in parentheses.



Scheme 1. Structure of compound **1** ,**2**, **3** and HMBC correlations of **1**.



Scheme 2.

(calcd for $[M]^+$, 325.0951). The IR spectrum showed absorptions for NH (3378 cm^{-1}), C=O (1667 cm^{-1}) groups, and phenolic hydroxyl group (3439 cm^{-1}), respectively. The UV spectrum also exhibited absorptions at 252, 296, and 397 nm, which corresponded to a phenanthrene chromophore. Comparing its ^1H and ^{13}C NMR data (see Table 1) with that of the known compound aristolactam F II (**21**),[14] **3** had an extra methoxyl group. In the ^1H NMR spectrum of **3**, an AB_2 pattern was observed at δ 8.58 (1H, d, J = 8.1 Hz), δ 7.35 (1H, dd, J = 8.1, 7.5 Hz), and δ 7.03 (1H, d, J = 7.5 Hz). The NOESY correlations observed between δ 4.39/3.90, 3.90/4.08, 4.08/8.58, 8.58/7.35, 7.35/7.03, and 10.10 (–OH)/7.03 (see Fig. 1), and determined that the hydroxyl group was located at C_8 and three methoxyl groups were located at C_2, C_3, and C_4. Thus, **3** was established

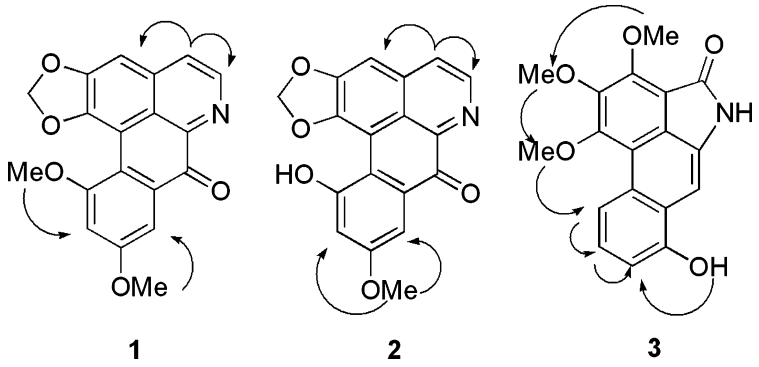


Figure 1. The NOESY correlations of compounds **1**, **2**, and **3**.

as 10-amino-8-hydroxy-2,3,4-trimethoxyphen-anthrene-1-carboxylic acid lactam, which was given a trivial name, oldhamactam.

2.2. Known compound isolated from the bark of *F. oldhamii* (Hemsl.) Merr.

The other known compounds (see Scheme 2) obtained in this study, calycinine **(4)**,[13] *N*-methyl-2,3,6-trimethoxymorphinandien-7-one **(5)**,[7a] aristololactam A IIIa **(6)**,[15] xylopine **(7)**,[16] crebanine **(8)**,[13] isolaureline **(9)**,[13] asimilobine **(10)**,[13] duguevanine **(11)**,[13] corytuberine **(12)**,[17] aristololactam A II **(13)**,[18] 4,5-dioxodehydro asimilobine **(14)**,[19] isoboldine **(15)**,[14] goniothalactam **(16)**,[14] aristolactam F I **(17)**,[20] glaucine **(18)**,[17] norcepharadione B **(19)**,[19] oxoxylopine **(20)**,[16] aristolactam F II **(21)**,[14] *O*-methyl-moschatoline **(22)**,[21] aristololactam B II **(23)**,[18] and aristololactam B III **(24)**,[18] were identified by comparing their spectroscopic data (^{1}H NMR, ^{13}C NMR, DEPT, NOESY, and MS) with published values.

2.3. Biological activity evaluation and discussion

Extract from herbal formula syrup (**sample 1**), 95% ethanol extract of *F. oldhamii*, (**fraction 0**), and all isolated compounds were tested in vitro for their cytotoxicity on murine spleen cells, inhibition activity on concanavalin A (ConA)-induced T cell proliferation, and lipopolysaccharide (LPS)-induced B cell proliferation, with CsA (see Experimental section) as control. The pharmacological results of these compounds are summarized in Table 2. The cytotoxicity activity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (CC_{50}). The immunosuppressive activity of each compound was expressed as the concentration of compound that inhibited T and B cell proliferation to 50% (IC_{50}) of the control value. The selective index (SI) value was used to evaluate the bioactivity of compounds. In the experiments for testing bioactivity of each compound, results were expressed as means ± standard error. Student's *t*-test was used to determine variances between groups where appropriate, although these data are not shown in Table 2.

On the basis of the inhibition of ConA-induced T lymphocyte proliferation, **sample 1** and **fraction 0** both exhibited a satisfactory SI value although their activity was far less than CsA. Compounds **1**, **2**, **4**, **14**, **17**, **19**, **20**, **24**, and **1k** had relatively good activities (IC_{50} < 5 μM), among which compound **4** had the best SI value. The SI value of inhibition on LPS-induced B cell proliferation indicated the **fraction 0** (SI > 93) had more potent value than CsA (SI = 8), although it also showed relatively low activity. Particularly, compounds **1**, **2**, **4**, **5**, **14**, **19**, **21**, **24**, and **1k** had much higher activities (IC_{50} < 5 μM) than others, among which compound **4** (SI = 108) showed the best SI value.

From the preliminary immunosuppressive evaluation in vitro, we could find the following. (i) This syrup and the ruler herb both showed strong activities in inhibiting the T or B cell proliferation, and their action mechanism in treatment of the RA might be attributed to inhibition of T and B cell functions. (ii) The major effective components of this herb were alkaloids, such as aporphines, oxoaporphines, and aristolactams. Furthermore, the aporphines (**4**, **7**, **10**, **11**, **15**, and **1k**) and oxoaporphines (**1**, **2**, **20**, and **22**) were more potent than aristolactams (**3**, **6**, **13**, **16**, **21**, and **23**) except **17** and **24**. For the toxicity of dione groups,[24] dioxoaporphines (**14**, **19**), which were not suitable for further research, displayed similar cytotoxicities to their immunosuppressive activities. (iii) Interestingly in aporphines, secondary amine (**4**, **7**, **10**, **11**, **15**, and **1k**) exhibited a much more potent inhibitory effect than the methyl substituted ternary amine (**8**, **9**, **12**, **18**), and 1,2 methylene group (**4**, **7**, **11**, **1k**) was more active than the methoxy groups (**10**, **15**) at C_1 and C_2 position.

2.4. Synthesis of oxodiscoguattine

As described above, compounds **1**, **2**, and **4** showed better characteristics both in activity and in SI value, however, the isolated yield of **1** (0.014%) and **2** (0.012%) from the title plant was relatively low. Considering that these three compounds' only difference in synthetic work was the different starting substituted benzonate ester, we chose the new compound oxodiscoguattine as our target to probe the synthesis of this kind compound. And in the next further biological study, this synthetic work would give us a chance of looking for the proper targets in vitro and for further structure–activity relationship study. The approach of synthesis is outlined in Scheme 3, starting with methylation of methyl 3,5-

Table 2. Inhibitory effects of CsA (positive control), syrup, crude extract, and compounds **1**–**24**, **1k** on spleen lymphocyte proliferation induced by mitogens in vitro

Compound[a]	Cytotoxicity CC_{50} (M)	IC_{50} (M) [SI[b]]	
		Inhibition of ConA-induced T cell proliferation	Inhibition of LPS-induced B cell proliferation
1	3.23×10^{-5}	3.58×10^{-6} [9]	2.15×10^{-6} [15]
2	4.39×10^{-6}	8.10×10^{-7} [5]	5.60×10^{-7} [8]
3	2.01×10^{-4}	2.28×10^{-5} [9]	4.17×10^{-5} [5]
4	3.16×10^{-4}	4.86×10^{-6} [65]	2.93×10^{-6} [108]
5	5.89×10^{-5}	3.93×10^{-5} [1]	3.97×10^{-6} [15]
6	$>1 \times 10^{-4}$	1.85×10^{-5} [>5]	1.92×10^{-5} [>5]
7	6.46×10^{-5}	5.69×10^{-6} [11]	8.10×10^{-6} [8]
8	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
9	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
10	$>1 \times 10^{-4}$	4.48×10^{-5} [>2]	7.96×10^{-5} [>1]
11	$>1 \times 10^{-4}$	6.86×10^{-6} [>14]	5.54×10^{-6} [>18]
12	$>1 \times 10^{-4}$	2.32×10^{-5} [>4]	2.13×10^{-5} [>4]
13	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
14	5.15×10^{-6}	4.93×10^{-6} [1]	3.12×10^{-7} [17]
15	$>1 \times 10^{-4}$	2.40×10^{-5} [>4]	2.96×10^{-5} [>3]
16	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
17	8.12×10^{-6}	7.98×10^{-7} [10]	1.30×10^{-6} [6]
18	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
19	3.91×10^{-6}	7.95×10^{-7} [5]	6.72×10^{-6} [0.5]
20	1.62×10^{-5}	4.16×10^{-6} [3]	7.29×10^{-6} [2]
21	2.47×10^{-5}	1.00×10^{-5} [2]	3.96×10^{-6} [6]
22	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
23	1.11×10^{-5}	6.96×10^{-6} [1]	7.11×10^{-6} [1]
24	9.41×10^{-6}	2.29×10^{-6} [4]	1.98×10^{-6} [4]
1k	1.56×10^{-5}	2.43×10^{-6} [6]	1.69×10^{-6} [9]
Sample 1	>500 μg/ml	21.5 μg/ml [>23]	9.8 μg/ml [>51]
Fraction 0	>500 μg/ml	6.0 μg/ml [>83]	5.4 μg/ml [>93]
CsA	1.35×10^{-6}	1.37×10^{-9} [985]	1.59×10^{-7} [8]

[a] The compounds tested for immunosuppressive activity were consistent with the description in the Experimental Section.
[b] Selectivity index [SI] is determined as the ratio of the concentration of the compound that reduced cell viability to 50% (CC_{50}) to the concentration of the compound needed to inhibit the proliferation to 50% (IC_{50}) of the control value.

dihydroxybenzonate to form methyl 3,5-dimethoxybenzonate, followed by reduction of the benzonate group and substitution by *N*-bromosuccinimide. After the general method[22] to get the 2-bromo-3,5-dimethoxy phenylacetic acid (**1f**) through the hydrolyzation of corresponding nitrile, **1f** was coupled with 3,4-methylenedioxy-phenethylamine catalyzed by *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt). The amide **1g** was subject to a Bischler-Napieralski cyclization upon treatment with phosphorous oxychloride, then sodium borohydride reduction and di-*tert*-butyl dicarbonate (Boc_2O) protection to give the amine **1j**. Oxodiscoguattine was finally accomplished with 6.7% total yield through intramolecular phenol *ortho*-arylation[23] using Pd-mediated catalysis, which followed oxidation by I_2 in refluxing ethanol.

3. Conclusion

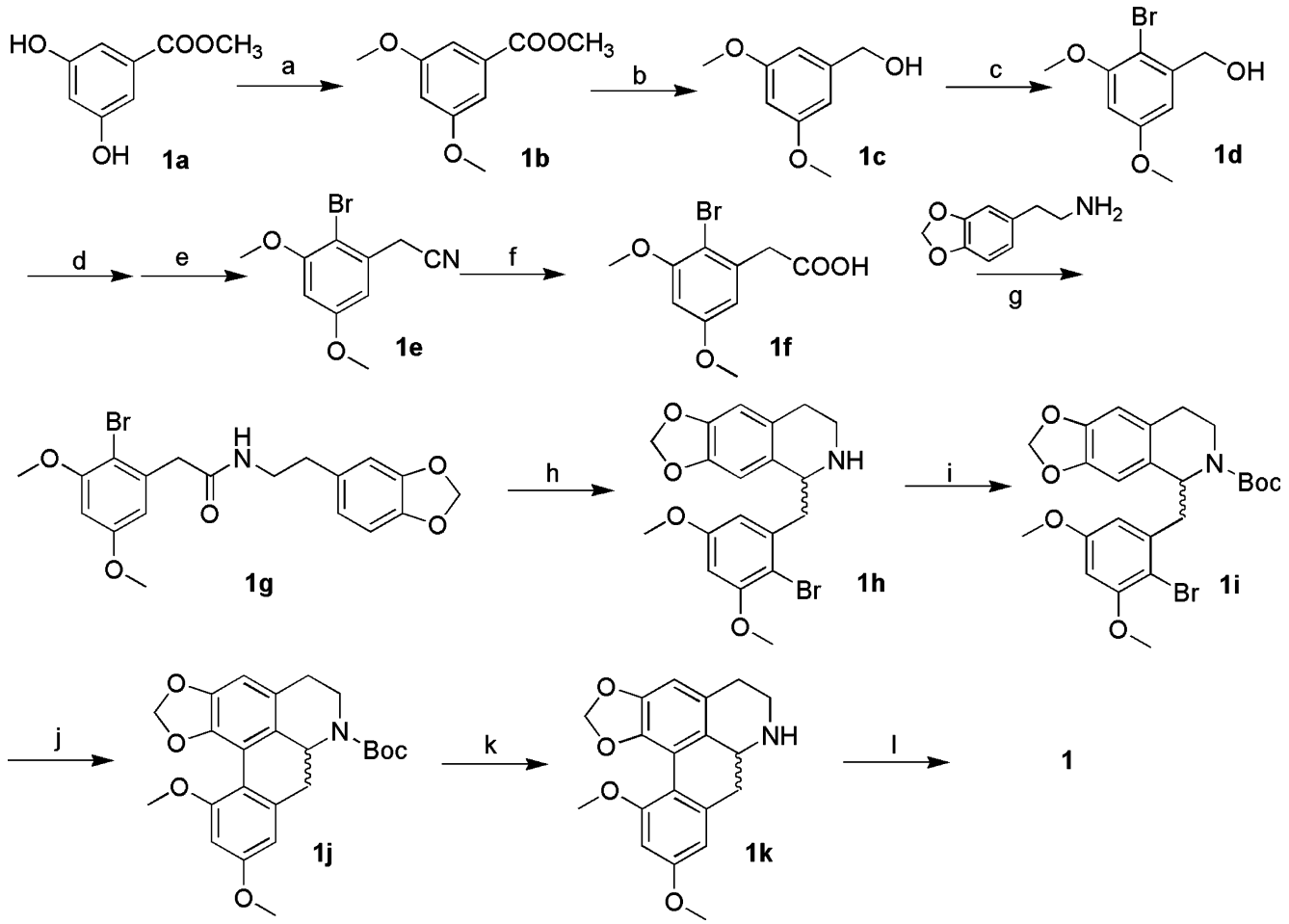
In summary, the ruler herb of a certificated syrup treated for RA has been studied. Twenty-four compounds including three new alkaloids were isolated and identified, among which one new compound was synthesized. All of them as well as the crude extract were assessed for their cytotoxicity of lymphocyte and activities on the mitogen-induced T and B cell proliferation in comparison of CsA in vitro. It is worth noting that compounds **1** and **4** had the most outstanding character after considering the comprehensive evaluation between SI value and activity.

According to observations and analyses above, we presumed that this TCM syrup is a very useful resource to find good anti-RA leads. Isolation on the other six auxiliary herbs in this syrup,[5] further modification of compounds **1** and **4** and evaluation in more vitro or vivo models are now in progress. The qualitative structure–activity relationship was expected to be useful in guiding the design of new immunosuppressive agents with high efficacy and low toxicity.

4. Experimental

4.1. General experimental procedure

^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Mercury-VX300 Fourier transform spectrometer. The chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^1H NMR) and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as internal standards. Low resolution mass spectra were obtained on a Shimadzu GCMS-QP5050A and high resolution mass spectra on a Finnigan MAT-95 spectrometer. The IR and UV spectra were run on a Nicolet Impact 410 spectrophotometer and a Shimadzu UV2501PC spectrophotometer, respectively.



Scheme 3. Reagents and conditions: (a) 2 equiv CH_3I K_2CO_3, acetone, reflux, 6 h; (b) $LiAlH_4$ THF, rt, 4 h; (c) NBS, $CHCl_3$ rt, overnight; (d) MsCl, CH_2Cl_2, 0 °C, 2 h; (e) NaCN, DMF, rt, overnight; (f) 5 N NaOH, MeOH, reflux, overnight; (g) EDC, HOBt, CH_2Cl_2, 4 A molecular sieves, rt, overnight; (h) 8 equiv $POCl_3$, CH_3CN, reflux, 3 h; then, $NaBH_4$, MeOH; (i) Boc_2O, 2 N NaOH (aq)/1,4-Dioxane (v/v, 1/2), rt, 2 h; (j) $Pd(OAc)_2$, Cy_3P, Cs_2CO_3, 4 A molecular sieves, Argon, 100 °C, overnight; (k) TFA, CH_2Cl_2 rt, 1.5 h; (l) I_2, NaOAc, EtOH, reflux, 6 h.

All commercially available reagents were used without further purification. The solvents used were all AR grade and were redistilled under positive pressure of dry nitrogen atmosphere in the presence of proper desiccant when necessary. The progress of the reactions was monitored by analytical thin-layer chromatography (TLC) on $HSGF_{254}$ precoated silica gel plates.

4.2. Plant material

The stem of *F. oldhamii* (Hemsl.) Merr. was collected in Jiangxi Province, People's Republic of China, in March 2004, and identified by Xinhua Long. A voucher specimen of the plant (No. 2004134) was deposited at the herbarium of Shanghai Institute of Materia Medica, Shanghai, P.R. China.

4.3. Biological assay

4.3.1. Materials. Stock solutions of compounds were prepared with 100% dimethylsulfoxide (DMSO, Sigma) and diluted with RPMI 1640 medium containing 10% fetal bovine serum (FBS). MTT (3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), Concanavalin A (ConA), and lipopolysaccharide (LPS) were purchased from Sigma. [^{3}H]Thymidine (1 mCi/mL) was purchased from Shanghai Institute of Atomic Energy (SIAE). The related in vitro experimental procedure was performed according to our previous work.[25]

4.3.2. Animals. BALB/c mice, used at 6–8 weeks of age, were purchased from Shanghai Experimental Animal Center and were housed in a controlled environment (12 h of light/12 h of dark photoperiod, 22 ± 1 °C, 55% ± 5% relative humidity). All husbandry and experimental contacts made with the mice were conducted under specific pathogen-free conditions. All mice were allowed to acclimatize in our facility for 1 week before any experiment started. All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica.

4.3.3. Preparation of spleen cell from mice. BALB/c mice were sacrificed, and spleens were removed aseptically. A single cell suspension was prepared after cell debris, and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed three times with PBS containing 2% FBS and

were resuspended in RPMI 1640 medium at the indicated concentration.

4.3.4. Cytotoxicity assay. Fresh spleen cells were obtained from BALB/c mice (male, 7–9 weeks old). Spleen cells (5×10^5 cells) were cultured in 96-well flat plates with 200 μL RPMI 1640 media containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified, 37 °C, 5% CO_2-containing incubator for 48 h in the presence or absence of various concentrations of compounds. An amount of 18 μL MTT (5 mg/mL) was added to each well at the final 5 h of culture. Then 90 μL of lysis buffer (10% SDS, 50% DMF, pH 7.2) was added to each well for 6–7 h and the absorbance value at 570 nm was collected by microplate reader. The percentage of cell death was determined using the following formula:

$$\begin{aligned}&\text{cytotoxicity}(\%)\\&\quad = [\text{compounds}(OD_{570})\\&\quad - \text{background}(OD_{570})]/[\text{control}(OD_{570})\\&\quad - \text{background}(OD_{570})] \times 100\end{aligned}$$

4.3.5. T Cell and B cell function assay. Fresh spleen cells were obtained from BALB/c mice (male, 7–9 weeks old). The 5×10^5 spleen cells were cultured at the same conditions as those mentioned above. The cultures were unstimulated or stimulated with 5 μg/mL ConA or 10 μg/mL LPS to induce T cell or B cell proliferative responses, respectively. The compounds were added to cultures with indicated concentrations to test their bioactivities. Proliferation was assessed in terms of uptake of [^{3}H]thymidine during 8 h of pulsing with 20 kBq [^{3}H]thymidine for each well, and then cells will be harvested onto glass fiber filters by a Basic 96 harvester. The incorporated radioactivity was counted by a liquid scintillation counter.

4.4. Extraction and isolation

The commercial syrup (produced by Jiangxi Jinding Pharmceutical Co. Ltd., China) 100 mL was primarily purified by D-101 porous resin using H_2O as eluant to wash the superfluous sugar and other additives away. The active component of syrup (**sample 1**, 1.4 g) got from the concentration of 95% EtOH eluant was initially tested in the inhibition of T and B lymphocytes. (see Table 2)

The dried and milled stem of *F. oldhamii* (Hemsl.) Merr (4.0 kg) was extracted using 95% ethanol (3×10 L) at reflux temperature. After filteration and evaporation of the solvent under reduced pressure, the combined extracts (86.0 g) were subjected to D-101 porous resin, eluted with H_2O, then 95% EtOH. After that, only the 95% ethanol parts (**fraction 0**) exhibited the activities in T and B lymphocyte inhibition, which was collected, concentrated (19.0 g), and primarily separated by column chromatography on silica gel 200–300 mesh eluted with $CHCl_3$/MeOH ($CHCl_3$, 50:1, 40:1, 20:1, 10:1, and 5:1) to afford six parts (Fr. 1–Fr. 6).

The $CHCl_3$ fraction (Fr. 1) was chromatographed over a silica gel column and eluted with petroleum ether/$CHCl_3$ (3:2–1:1), to give two subfractions (Fr. 1.1, Fr. 1.2). Subfraction Fr. 1.1 was purified using petroleum ether/$CHCl_3$ (2:1) as solvents, to give **1** (12 mg) and **22** (25 mg). The other subfraction Fr. 1.2 was subjected to chromatography (petroleum ether/$CHCl_3$ 1:1) on Silica gel to yield **23** (9 mg) and **24** (8 mg).

The $CHCl_3$–MeOH (50:1) fraction (Fr. 2) was crude separated through Sephadex LH-20 column (25–100 μm, Merck, Darmstadt, Germany; $CHCl_3$/MeOH 1:1) to get two subfractions (Fr. 2.1, Fr. 2.2). The simple subfraction (Fr. 2.1) was further purified on silica gel ($CHCl_3$/MeOH 50:1) to obtain **5** (28 mg) and **11** (13 mg). The relative complex subfraction (Fr. 2.2) was chromatographed over MCI gel CHP 20P (75–150 μm, Mitsubishi Kasei Industry Co., Ltd., Tokyo, Japan, MeOH–water, 50%, 60%, and 80%) to give three further subfractions (Fr. 2.2.1–Fr. 2.2.3). The Fr. 2.2.1 and Fr. 2.2.3, both advanced on purified Sephadex LH-20 column (MeOH), gave **16** (6 mg) and **19** (6 mg), respectively. The residue subfraction (Fr. 2.2.2) was subjected to silica gel ($CHCl_3$/MeOH 100:1–50:1) to obtain **17** (61 mg), **2** (10 mg) and a subfraction (Fr. 2.2.2.1). Interestingly, the unsolvable solid in MeOH, when we prepared to concentrated Fr. 2.2.2.1 in vacuum, was filtered and washed to obtain **3** (20 mg).

The other four fractions ($CHCl_3$/MeOH, 40:1, 20:1, 10:1, and 5:1), separated through repeated Silica gel, MCI, and Sephadex LH-20 following the procedure described as above, were got as **15** (25 mg), **18** (7 mg), **20** (6 mg), and **21** (9 mg) in Fr. 3; **8** (12 mg), **9** (5 mg), and **13** (18 mg) in Fr. 4; **4** (85 mg), **7** (12 mg), and **10** (15 mg) in Fr. 5 and **6** (32 mg), **12** (12 mg), and **14** (33 mg), in Fr. 6, respectively.

4.5. Oxodiscoguattine (1)

Red amorphous powder; ^{1}H and ^{13}C NMR spectrum, see Table 1; HREI-MS *m*/*z* 335.0788 (calcd for $[M]^+$, *m*/*z* 335.0794); EI-MS *m*/*z* 335 (100), 334 (58), 319 (9), 305 (13), 292 (15), 264 (23), 249 (20), 221 (18), 44 (30); UV (MeOH) λ_{max} (log ε) 230 (4.62), 265 (4.45), 330 (4.06), 356 (4.03) nm; IR υ_{max} (KBr) 3047, 2958, 1660, 1601, 1457, 1413, 1282, 1043, 939 cm^{-1}.

4.6. Preparation of compound 1b–1

4.6.1. 2-(2-bromo-3,5-dimethoxyphenyl)acetonitrile (1e). To the dry CH_2Cl_2 (30 mL) solution of **1d** (2.50 g, 10 mmol) in the presence of triethyl amine (2.1 mL, 15.0 mmol), the solution of methanesulfonyl chloride (0.85 mL, 11.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise in 1 h under ice bath. Finishing the addition, the reaction mixture was still stirred for further 2 h at room temperature. The mixture was then separated by CH_2Cl_2 (2×100 mL) and saturated $NaHCO_3$ solution (50 mL). The collected organic layers were washed by water (50 mL), and brine, dried over Na_2SO_4, and concentrated. Without further purification, the crude product was directly added to the solution of DMF (30 mL)

with sodium cyanide (740 mg, 15.0 mmol) followed. The mixture was stirred overnight and then extracted by ether (3 × 50 mL) after diluted with water (50 mL). The combined organic layer were evaporated in vacuum, and the residue was subjected to column chromatography on silica gel using 5% ethyl acetate in petroleum ether as eluant to give 2.08 g **1e**, yield 81.2%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.53 (d, J = 2.8 Hz, 1H), 6.48 (d, J = 2.8, 1H), 3.91 (s, 2H), 3.86 (s, 3H), 3.78 (s, 3H). EI-MS m/z 255.

4.6.2. *N*-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)-2-(2-bromo-3,5-dimethoxyphenyl)acetamide (1g). A solution of **1f** (1.95 g, 7.1 mmol), HOBt (1.08 g, 8.0 mmol), 3,4-methylenedioxyphenethylamine (1.40 g, 8.5 mmol), and 4 Å molecular sieves (1.00 g) in dry CH_2Cl_2 (35 mL) was treated with EDC (1.50 g, 8.0 mmol) and the resulting reaction mixture was stirred at room temperature overnight. The mixture was partitioned by CH_2Cl_2 (2 × 100 mL) and water (50 mL), washed by 1 N HCl (25 mL), saturated $NaHCO_3$ (50 mL), and brine, dried over (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel using petroleum ether/acetone (1:1) as eluant to give 2.00 g product **1g** (66.9%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.64 (d, J = 7.5 Hz, 1H), 6.55 (d, J = 1.8 Hz, 1H), 6.48 (dd, J = 7.5, 1.8 Hz, 1H), 6.45 (d, J = 2.8 Hz, 1H), 6.42 (d, J = 2.8 Hz, 1H), 5.91 (s, 2H), 5.44 (br s, 1H), 3.88 (s, 3H), 3.78 (s, 2H), 3.68 (s, 3H), 3.40 (q, J = 6.6 Hz), 2.66 (t, J = 6.6 Hz); EI-MS m/z 421.

4.6.3. (±) 5-(2-Bromo-3,5-dimethoxybenzyl)-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g] isoquinoline (1h). A solution of **1g** (2.00 g, 4.8 mmol) and $POCl_3$ (3.5 mL, 38.0 mmol) in dry acetonitrile was refluxed for 3 h, and its color turned to dark red. After working up, the reaction mixture was concentrated in vacuum and then dissolved in MeOH (20 mL). Excess amount of sodium borohydride was added to the mixture by portion under ice bath until the color turned to pale yellow. The mixture was separated by ethyl acetate (2 × 50 mL) and saturated $NaHCO_3$ (30 mL), washed by water (50 mL) and brine, then dried by Na_2SO_4 and concentrated in vacuum. The residue was chromatographed over silica gel using $CHCl_3$/MeOH (100:1) to get 800 mg **1h** (41.6%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.87 (s, 1H), 6.57 (s, 1H), 6.46 (d, J = 2.8 Hz, 1H), 6.42 (d, J = 2.8 Hz, 1H), 5.91 (s, 2H), 4.25 (dd, J = 10.5 Hz, 2.7 Hz, 1H), 3.89 (s, 3H), 3.81 (s, 3H), 3.38 (dd, J = 13.5 Hz, 2.9 Hz, 1H), 3.22 (m, 1H), 2.91 (m, 2H), 2.72 (m, 2H); EI-MS m/z 405.

4.6.4. (±) Discoguattine (1k). To a mixture of aqueous 2 N NaOH/1,4-dioxane (5 mL/5 mL) with **1h** (800 mg, 2.0 mmol), Boc_2O (475 mg, 2.2 mmol) in 5 mL 1,4-dioxane was added and stirred for 2 h at room temperature. The reaction mixture was extracted by ethyl acetate (2 × 50 mL), washed by water (2 × 30 mL) and brine, dried (Na_2SO_4), and concentrated in vacuum to obtain the crude product **1i**.

A mixture of **1i**, tricyclohexylphosphine (230 mg, 0.8 mmol), anhydrous cesium carbonate (1.95 g, 6.0 mmol), and palladium acetate (93 mg, 0.4 mmol) in dry DMF (40 mL) under an argon atmosphere was heated at 100 °C overnight. The reaction mixture was allowed to cool and then carefully diluted with 1 N HCl (20 mL), then extracted with ethyl acetate (2 × 50 mL). The organic extracts were combined, washed with brine, dried over by Na_2SO_4, and concentrated. The residue was subject to column chromatography on silica gel using petroleum ether/ethyl acetate (10/1) to give **1j** (600 mg, 1.4 mmol) with moderate yield 71.7% from **1h**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.56 (s, 1H), 6.47 (m, 1H), 6.46 (m, 1H), 6.03 (d, J = 1.5 Hz, 1H), 5.86 (d, J = 1.5 Hz, 1H), 4.63 (m, 1H), 4.38 (m, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 2.57–2.93 (m, 6H).

A mixture of TFA/CH_2Cl_2 (2 mL/10 mL) was added to 2 h, and the reaction mixture was stirred for 1.5 h at room temperature. The volatiles were removed in vacuum and the residue was diluted with 5% $NaHCO_3$ (5 mL). The aqueous mixture was extracted with ethyl acetate (2 × 25 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to afford **1k** (400 mg, 1.2 mmol, 87.2%) as white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.55 (s, 1H), 6.47 (d, J = 2.0 Hz, 1H), 6.44 (d, J = 2.0 Hz, 1H), 6.00 (d, J = 1.5 Hz, 1H), 5.84 (d, J = 1.5 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.76 (dd, J = 13.0 Hz, 4.2 Hz, 1H), 3.31 (m, 2H), 2.94 (m, 2H), 2.59–2.79 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 29.45 (CH_2), 38.94 (CH_2), 43.34 (CH_2), 54.02 (CH), 55.59 (CH_3), 55.94 (CH_3), 97.84 (CH), 100.25 (CH_2), 105.15 (CH), 107.05 (CH), 113.91 (C), 126.17 (C), 126.18 (C), 129.51 (C), 139.61 (C), 139.62 (C), 146.93 (C), 157.79 (C), 160.46 (C); EI-MS m/z 325.

4.6.5. Oxodiscoguattine (1). To a refluxing solution of **1k** (100 mg, 0.3 mmol) and sodium acetate (100 mg, 1.2 mmol) in 10 mL ethanol, a solution of iodine (198 mg, 0.8 mmol) in 10 mL ethanol was added dropwise during 15 min. After refluxing for 6 h, the solution was dissolved in $CHCl_3$ and washed by aqueous 5% Na_2SO_3 (30 mL), saturated $NaHCO_3$ (30 mL), and brine. Removing the volatiles under vacuum, the dark green solid was subject to column chromatography on silica gel using $CHCl_3$/MeOH (100:1) as eluant to get 67 mg final product (64.7%) as red powder. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.83 (d, J = 5.1 Hz, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.68 (d, J = 2.5 Hz, 1H), 7.11 (s, 3H), 6.84 (d, J = 2.5 Hz, 1H), 6.24 (s, 2H), 4.00 (s, 3H), 3.98 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 55.59 (CH_3), 55.84 (CH_3), 101.70 (CH), 101.73 (CH_2), 102.53 (CH), 105.20 (CH), 116.15 (C), 123.09 (C), 124.16 (C), 124.20 (CH), 134.06 (C), 136.15 (C), 143.64 (CH), 143.97 (C), 147.4 (C), 152.82 (C), 158.39 (C), 161.02 (C), 182.29 (C); EI-MS m/z 335.

4.6.6. Oxocalycinine (2). Red amorphous powder; ^{1}H and ^{13}C NMR spectrum, see Table 1; HREI-MS m/z 321.0623 (calcd for $[M]^+$, m/z 321.0637); EI-MS m/z 321 (100), 320 (55), 295 (38), 292 (20), 277 (11), 265 (50), 250 (42), 234 (13), 222 (23), 164 (27), 84 (52), 66 (56); UV (MeOH) λ_{max} (log ε) 225 (4.50), 267 (4.28), 356 (4.01) nm, IR υ_{max} (KBr) 3425, 2951, 1660, 1601, 1450, 1283 cm^{-1}.

4.6.7. Oldhamactam (3). Brownish-yellow amorphous powder; ^{1}H and ^{13}C NMR spectrum, see Table 1; HREI-MS *m/z* 325.0951 (calcd for $[M]^{+}$, *m/z* 325.0934); EI-MS *m/z* 325 (100), 310 (42), 296 (25), 252 (22), 196 (10); UV ($CHCl_3$) λ_{max} (log ε) 252 (4.24), 296 (4.82), 397 (4.92) nm; IR υ_{max} (KBr) 3439, 3378, 1667, 1461, 1407, 1245 cm^{-1}.

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