Structural Modification of Sanguinarine and Chelerythrine and Their *in Vitro* Acaricidal Activity against *Psoroptes cuniculi*

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Sanguinarine (1) and chelerythrine (2) are two quaternary benzo[*c*]phenanthridine alkaloids (QBAs). Eighteen derivatives of 1 and 2 were synthesized by modification of $C=N^+$ bond and evaluated for their *in vitro* acaricidal activity against *Psoroptes cuniculi*, a mange mite. A new method was developed to prepare 6-alkoxy dihydro derivatives of 1 and 2 (1a–e, 2a–e). Among all the compounds, only 6-alkoxy dihydrosanguinarines (1a–e) showed significant acaricidal activity at 5.0 mg/mL and 1a possessed the strongest activity (50% lethal concentrations (LC_{50})=339.70±0.75 mg/L, 50% lethal time (LT_{50})=6.53±0.04 h), comparable with a standard drug ivermectin (LC_{50}=168.19±11.79 mg/L, LT_{50}=16.54±0.11 h). The iminium moiety in 1 and 2 was proven to be the determinant for their acaricidal properties. 6-Alkoxy dihydro derivatives (1a–e, 2a–e) were prodrugs of 1 and 2. Compared with 7,8-dimethoxy groups, 7,8-methylenedioxy group was able to significantly improve the bioactivity. The present results suggested that QBAs are promising candidates or lead compounds for the development of new isoquinoline acaricidal agents.

Key words sanguinarine; chelerythrine; acaricidal activity; *Psoroptes cuniculi*; benzo[*c*]phenanthridine alkaloid

Sanguinarine (**1**) and chelerythrine (**2**) (Fig. 1) belong to quaternary benzo[*c*]phenanthridine alkaloids (QBAs) and widely present in a number of plant species of the Fumariaceae, Papaveraceae, and Rutaceae families.[1)] QBAs have given rise to a lot of attention because of their extensive bioactivities including antitumor,[2–4)] antimicrobial,[5,6)] anti-inflammatory,[7)] antiviral[8)] including anti-human immunodeficiency virus (HIV),[9)] anti-platelet aggregation,[10)] anti-angiogenesis[11)] and anti-acetylcholinesterase.[12)] Recently, QBAs were also found to have antiparasitic actions against *Trichodina* sp.,[13)] *Dactylogyrus intermedius*[14)] and malaria.[15)] However, the acaricidal activity of QBAs was not reported until now.

Nevertheless, QBAs have the common drawback of low compatibility with physiological conditions. The highly polar iminium moiety easily reacts with biological reducing agents such as nicotinamide adenine dinucleotide (NADH), or non-target nucleophiles in biological fluids to become the corresponding neutral nonactive phenanthridine derivatives.[16–20)] Therefore, during the past ten years, a great deal of work had focused on improving the chemical stability of QBAs by structural modification.[16,21)]

Psoroptes cuniculi is an important veterinary ectoparasite in rabbits, goats, horses, sheep and so on.[22)] It can cause intense pruritus, reduction of weight gain or even death of animals.[23,24)] Therapy and control of both human scabies and animal mange are based mainly on the use of effective drugs and chemicals. However, many of the chemical acaracides have limitations such as resistance,[25,26)] toxicity[27,28)] and environmental damage.[27,28)] Ivermectin is increasingly being used to treat human scabies and animal mange but often treatment failures, recrudescence and reinfection can occur.[29)] These problem have lead to research efforts to discover new effective acaricides derived from some active natural products.

Macleaya microcarpa (Maxim.) Fedde (the family Papaveraceae) is a perennial herb and widely distributed in the northwest of China. This plant is rich in compounds **1** and **2** and has been used as Chinese traditional medicine for the treatment of some skin diseases caused by pathogenic fungi or parasite, such as rosacea, scabies, brothers tinea, psoriasis and so on. Our preliminary research revealed that the methanol extract contained 4.75% **1** and 7.38% **2** of the plant possessed significant *in vitro* acaricidal activity against *P. cuniculi*. This result suggested that **1** and **2** as the main compounds of the extract may have acaricidal activity. The purpose of the present study is to examine the acaricidal activity *in vitro* of **1**, **2** and their derivatives against *P. cuniculi* and understand their structure–activity relationship.

Results and Discussion

Chemistry Compounds **1a–i** and **2a–i** in Fig. 2 were synthesized by using **1** and **2** as the starting material, respectively. Compounds **1g–i** and **2g–i** were prepared according to the methods previously reported by us.[6)] Compounds **1a–e** and **2a–e** were respectively synthesized by reaction of **1h** or **2h** with the corresponding alcohol (methanol, ethanol, *n*-propanol, iso-propanol or *n*-butanol) in the presence of 20 mol% of $CuCl_2 \cdot 2H_2O$ under oxygen. Compounds **1** or **2** reacted with potassium cyanide in water solution to provide **1f** or **2f**.

In our previous research, **1a**, **1b**, **2a** and **2b** were respectively synthesized by the reaction of **1** or **2** with sodium methylate or ethylate. In addition, the reaction of **1** or **2** with the corresponding alcohol in the presence of triethylamine might also give **1a–e** or **2a–e**. However, the methods above only gave

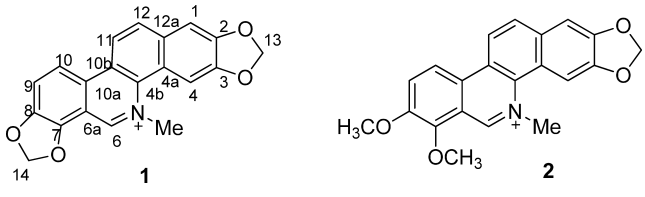

Fig. 1. Structures of Sanguinarine (**1**) and Chelerythrine (**2**)

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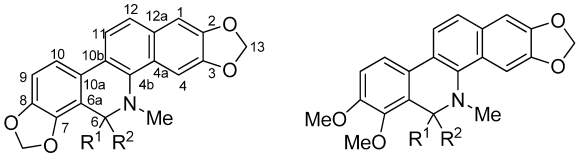

1a-i **2a-i**

a: R^1 = H, R^2 = MeO, **b:** R^1 = H, R^2 = EtO, **c:** R^1 = H, R^2 = PrO, **d:** R^1 = H, R^2 = *iso*-PrO
e: R^1 = H, R^2 = *n*-BuO, **f:** R^1 = H, R^2 = CN, **g:** R^1 = H, R^2 = acetonyl, **h:** R^1 = R^2 = H, **i:** R^1 + R^2 =O

Fig. 2. Derivatives of Sanguinarine (**1**) and Chelerythrine (**2**) (**1a**–**i**, **2a**–**i**)

lower yields and lower purity of the products. In the present research, we report a new method for preparation of **1a**–**e** and **2a**–**e**, *i.e.*, a $CuCl_2$-catalyzed aerobic oxidative coupling reaction of dihydrosanguinarine (**1h**) or dihydrochelerythrine (**2h**) with aliphatic alcohols. Compared with the two methods mentioned above, the present method has some obvious advantages such as nearly quantitative yields, simple work-up operation and easily obtaining high purity products.

The structures of all compounds were elucidated by spectroscopic analyses. Compounds **1** and **2** respectively revealed singlet signals at δ 9.95 and 9.92 ppm in ^{1}H-NMR spectra due to the corresponding HC=N^+ proton (*i.e.* H-6), signals at δ 150.7 and 152.1 ppm in ^{13}C-NMR spectra due to the corresponding C-6 and characteristic ion peaks at *m/z* 332 $[M-Cl]^+$ and 348 $[M-Cl]^+$ in electrospray ionization (ESI)-MS. With respect to **1a**–**e** and **2a**–**e**, each compoumd showed a singlet signal of H-6 in the region of δ 5.45–5.65 ppm in ^{1}H-NMR spectum, one signal of C-6 in the region of δ 82–86 ppm ^{13}C-NMR spectrum and a pseudomolecular ion peak $[M+Na]^+$ and a characteristic fragment ion peak $[M-OR]^+$ in ESI-MS. Compounds **1f** and **2f** respectively displayed singlet signals at δ 5.94 and 5.93 ppm in their ^{1}H-NMR spectra due to the corresponding H-6, and signals at δ 47.3 and 47.5 ppm in their ^{13}C-NMR spectra due to the corresponding C-6, and signals at δ 118.0 and 118.5 ppm due to the corresponding C≡N carbon. In positive ESI-MS spectra, **1f** and **2f** showed a quasimolecular ion peak $[M+H]^+$, a pseudomolecular ion peak $[M+Na]^+$ and a characteristic fragment ion peak $[M-CN]^+$.

Pharmacology. Acaricidal Activity *in Vitro* The *in vitro* acaricidal activity of compounds (**1**, **2**, **1a**–**i** and **2a**–**i**) at the concentration of 5 mg/mL are listed in Table 1. Among all the compounds, only the 6-alkoxy dihydrosanguinarines (**1a**–**e**), also named sanguinarine pseudoalcoholates, displayed significant acaricidal activity. Compared with **1a**–**e**, all **2a**–**e** only gave lower activity. Compound **1a** showed the highest activity with 100% of average mortality, which was the same as the standard acaricide ivermectin. Compared with the parent compounds **1** and **2**, all the pseudoalcoholates (**1a**–**e** or **2a**–**e**) were able to remarkably enhance the activity. Among each group of the pseudoalcoholates (**1a**–**e** or **2a**–**e**), **1a** and **2a** gave the strongest activity while the other pseudoalcoholates (**1b**–**e** or **2b**–**e**) showed the nearly identical lower activity. This result indicated that the activity improvement effect of 6-methoxy group was far beyond that of the other 6-alkoxy group. Furthermore, comparison of the structures and activity of **1a**–**e** with that of **2a**–**e** showed that 7,8-methylenedioxy group was more benifical to the improvement of activity than 7,8-dimethoxy group.

Acaricidal Toxicity Based on the acaricidal screening results above, the most effective compound **1a** was further evaluated for its acaricidal toxicity. Ivermectin was used as a standard acaricide. The corrected mortality rates caused by **1a** and ivermectin at different concentrations at 24 h against *P. cuniculi* are shown in Fig. 3. Toxicity regression equations for concentration effect and the median lethal concentrations (LC_{50}) are given in Table 2. The mortality rates of **1a** and ivermectin increased as the concentration increased. Both **1a** and ivermectin showed significant linear correlation between the probability of mortality rate and log[concentration] value ($R^2 \geq 0.96$). It was worth noting that there was a cross point at the concentration of *ca.* 0.7 mg/mL between the curves of **1a** and ivermectin in Fig. 3, indicating that **1a** was more effective than ivermectin at more than *ca.* 0.7 mg/mL. Because of the presence of the cross point, **1a** showed a bigger LC_{50} value (339.70±0.75 mg/L) and a smaller LC_{90} value (1243.90±36.09 mg/L) than ivermectin (LC_{50}=168.19±11.79, LC_{90}=1693.63±126.18 mg/L) (see Table 2).

Table 1. Acaricidal Activity of Compounds **1**, **2**, **1a**–**i** and **2a**–i against *P. cuniculi* at the Concentration of 5.0 mg/mL at 24 h

Compd. No.	Mortality (%) (mean±S.D.)	Compd. No.	Mortality (%) (mean±S.D.)[a)]
1	3.3±2.9	**2**	3.3±2.9
1a	100.0±0.0	**2a**	16.7±2.9
1b	73.3±2.9	**2b**	8.3±2.9
1c	71.7±2.9	**2c**	8.3±2.9
1d	73.3±2.9	**2d**	6.7±2.9
1e	71.7±2.9	**2e**	6.7±2.9
1f	6.7±2.9	**2f**	3.3±2.9
1g	11.7±2.9	**2g**	3.3±2.9
1h	5.0±5.0	**2h**	3.3±2.9
1i	6.7±2.9	**2i**	3.3±2.9
Ivermectin	100.0±0.0	Control	1.7±2.9

The corrected mortality rates of **1a** and ivermectin at different times at 10 mg/mL are showed in Fig. 4. Toxicity regression equations for post-treatment time effect and the median lethal time (LT_{50}) are listed in Table 3. Similar to the case of the concentration effect, the mortality rates caused by two compounds increased as the post-treatment time increased and showed significant linear correlations with log*T* value ($R^2 \geq 0.99$). From Fig. 4, it might be seen that the acaricidal action of **1a** was much faster than ivermectin. Both the LT_{50} and 90% lethal time (LT_{90}) of **1a** (LT_{50}=6.53±0.04, LT_{90}=7.93±0.11 h) were significantly smaller than ivermectin (LT_{50}=16.54±0.11, LT_{90}=20.26±0.12 h) (see Table 3).

The higher activity of the pseudoalcoholates **1a**–**e** and **2a**–**e** might be related with their hydrolyzable property, higher

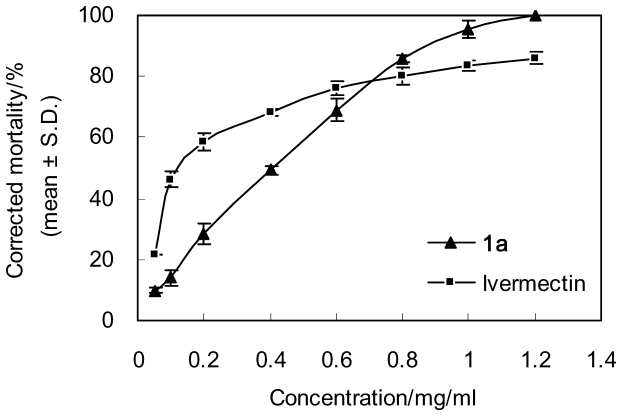

Fig. 3. Average Corrected Mortality of *P. cuniculi* for the Treatment of **1a** and Ivermectin at Different Concentrations

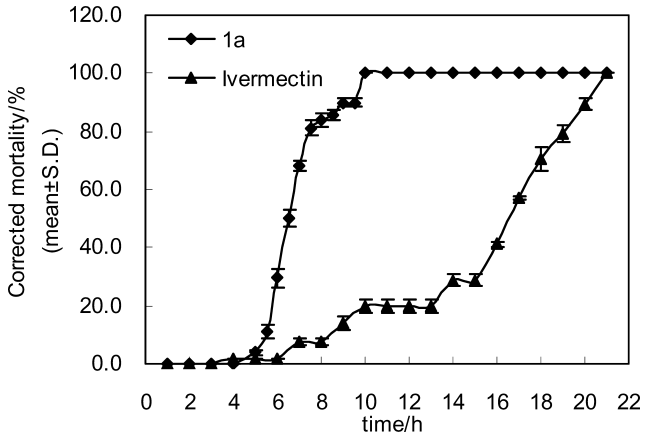

Fig. 4. Average Corrected Mortality of *P. cuniculi* for the Treatment of **1a** and Ivermectin (10 mg/mL) at Various Time

lipophilicity and higher compatibility with physiological conditions. **1a**–**e** and **2a**–**e** are structurally *N*,*O*-acetals and very easily converted to their corresponding iminium ion (**1** or **2**) under an acidic condition[30] even physiological acidic environment, such as the lysosome of cells.[31] Moreover, unlike ionic compounds **1** and **2**, **1a**–**e** and **2a**–**e** are nonionic compounds and have higher lipophilicity. They are able to more easily penetrate target cell membranes than **1** and **2**, and then accumulate to high levels and convert back to the iminium ions in the acidic environment of the lysosome. Unlike the higher chemical activity of **1** and **2**, **1a**–**e** and **2a**–**e** are inactive to biological reducing agents such as NADH and nucleophiles due to the absence of the iminium moiety. Based on the analysis above, we thought that the pseudoalcoholates might be prodrugs of **1** and **2** and the real active components should be **1** and **2**. Although **1f**–**i** and **2f**–**i** have similar molecular structures to the pseudoalcoholates (**1a**–**i**, **2a**–**i**), they cannot convert back to **1** and **2** under physiological conditions. It may be for this reason that **1f**–**i** and **2f**–**i** showed the lower or less activity. Obviously, the C=N$^+$ double bond in **1** and **2** were the determinant for their acaricidal properties. To a certain degree, the above conclusion was also supported by the fact of the different activity of the pseudoalcoholates in the same group (**1a**–**e** or **2a**–**e**). Compared with the other 6-alkoxy groups, 6-methoxy group has the smallest volume and the weakest steric hindrance effect on the hydrolytic reaction. **1a** and **2a** more easily converted back to the corresponding ion forms (**1**, **2**) than the other pseudoalcoholates (**1b**–**e** or **2b**–**e**). Therefore, both **1a** and **2a** gave the best activity among the corresponding pseudoalcoholates (**1a**–**e** or **2a**–**e**).

In conclusion, a series of C=N$^+$-modified derivatives of **1** and **2** were synthesized and evaluated for their acaricidal activity *in vitro* against *P. cuniculi*. 6-Alkoxy dihydrosanguinarines (**1a**–**e**) were found to have significant acaricidal activity for the first time. Compared with ivermectin, the most effective compound **1a** possessed a slightly bigger LC_{50} value and much smaller LT_{50} value. 6-Alkoxy dihydro derivatives of **1** and **2** were considered as prodrugs and the real active components were **1** and **2**. The C=N$^+$ double bond in **1** and **2** were the determinant for their acaricidal properties. The present results suggested that quaternary benzo[*c*]phenanthridine alkaloids are very good candidates or lead compounds for the development of new isoquinoline acaricidal agents.

Experimental

Materials Ivermectin (≥91% 22,23-dihydroavermectin B1 consisting of 95% avermectin B_{1a} and 5% avermectin B_{1b}) was purchased from Sigma-Aldrich Trading Co., Ltd., Shanghai, China. Other Chemicals used in the present study were purchased from J&K Chemical Ltd. (China) and used without further purification. Sanguinarine chloride (**1**) and chelerythrine chloride (**2**) were isolated by us from the entire plant of *Macleaya microcarpa* (Maxim) Fedde according to the method reported by us.[6] Compound **1g**–**i**, **2g**–**i** were prepared according to the methods previously reported by us using **1** and **2** as

Table 2. Toxicity Regression Equation for Concentration Effect and LC_{50} Values (mg/L) of **1a** against *P. cuniculi*

Compd. No.	Toxicity regression equation[a)]	R^2	LC_{50}±S.D.	LC_{90}±S.D.
1a	$y=2.2344x-0.6541$	0.9677	339.70±0.75	1243.90±36.09
Ivermectin	$y=1.2794x+2.1510$	0.9862	168.19±11.79	1693.63±126.18

a) y and x express the probability of mortality rate and log[concentration (mg L^{-1})], respectively.

Table 3. Toxicity Regression Equation for Time Effect and LT_{50} Values of **1a** (10 mg/mL) against *P. cuniculi*

Compd. No.	Toxicity regression equation[a)]	R^2	LT_{50}±S.D.	LT_{90}±S.D.
1a	$y=15.566x-7.6942$	0.9970	6.53±0.04	7.93±0.11
Ivermectin	$y=14.7330x-12.942$	0.9943	16.54±0.11	20.26±0.12

a) y: the probability of mortality rate. x: log T (h).

starting materials.[6)]

Apparatus Melting points (mp) were determined on XT-4 micro-melting point apparatus and uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AVANCE III operating at 500 and 125 MHz, respectively and using TMS as an internal standard. ESI-MS was measured on Trace mass spectrometer.

Synthesis of 1a–e and 2a–e. General Procedure To the solution of saguinarine chloride (**1**) or chelerythrine chloride (**2**) (0.33 mmol) in *ca.* 20 mL of different alcohol (methanol for **1a**, **2a**, ethanol for **1b**, **2b**, *n*-propanol for **1c**, **2c**, iso-propanol for **1d**, **2d** or *n*-butanol for **1e**, **2e**) was added 11 mg (0.06 mmol) of $CuCl_2 \cdot 2H_2O$ at room temperature. The resulting solution was stirred at 80°C for 10 h using an oil bath under an oxygen atmosphere. The reaction solution was passed through a pad of celite and washed with *ca.* 40 mL of the same alcohol. The combined filtrate was evaporated to dryness in high vacuum to give **1a**–**e** and **2a**–**e** as solids.

6-Methoxysanguinarine (**1a**): White crystal (MeOH), yield 96%. ^{1}H-, ^{13}C-NMR, ESI-MS and mp data were in agreement with that previously reported by us.[6)]

6-Ethoxysanguinarine (**1b**): White crystal (EtOH), yield 97%. ^{1}H-, ^{13}C-NMR, ESI-MS and mp data were in agreement with that previously reported by us.[6)]

6-*n*-Propoxy Dihydrosanguinarine (**1c**): White granular solid (*n*-PrOH), yield 99%, mp 194–195°C, *Rf* 0.36 (petroleum ether–ethyl acetate, v/v=2 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.78 (d, J=8.5 Hz, 1H), 7.70 (1H, s, H-4), 7.49 (d, J=8.5 Hz, 1H), 7.42 (d, J=8.2 Hz, 1H), 7.14 (s,1H), 6.95 (d, J=8.25 Hz, 1H), 6.14 (s, 2H), 6.08 (s, 2H), 5.49 (s, 1H), 3.83 (m, 1H), 3.63 (1H, m), 2.79 (s, 3H), 1.53 (m, 2H), 0.79 (t, J=7.5 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 148.0, 147.4, 147.3, 145.2, 138.6, 131.0, 126.9, 125.9, 123.6, 123.0, 120.3, 116.4, 113.5, 108.7, 104.6, 101.7, 101.0, 100.7, 84.4, 68.0, 40.9, 22.6, 10.6. ESI-MS *m/z*: 413.96 $[M+Na]^+$, 332.27 $[M-O(CH_2)_2CH_3]^+$.

6-*iso*-Propoxy Dihydrosanguinarine (**1d**): White granular crystal (*iso*-PrOH), yield 98%, mp 228–230°C, *Rf* 0.53 (petroleum ether–ethyl acetate, v/v=5 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.75 (d, J=7.0 Hz, 1H), 7.64 (s, 1H), 7.46 (d, J=7.0 Hz, 1H), 7.42 (d, J=6.5 Hz, 1H), 7.11 (s, 1H), 6.95 (d, J=6.5 Hz, 1H), 6.09 (s, 2H), 6.04 (s, 2H), 5.53 (s, 1H), 4.33 (br s, 1H), 2.72 (s, 3H), 1.27 (s, 3H), 0.91 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ: 148.0, 147.3, 147.3, 145.2, 138.7, 131.0, 127.0, 125.9, 123.6, 123.1, 120.4, 116.4, 113.6, 108.6, 104.6, 101.7, 101.0, 100.6, 82.3, 66.9, 40.8, 23.5, 21.3. ESI-MS *m/z*: 413.92 $[M+Na]^+$, 332.29 $[M-OCH(CH_3)_2]^+$.

6-*n*-Butoxy Dihydrosanguinarine (**1e**): White solid (*n*-BuOH), yield 99%, mp 178–179°C, *Rf* 0.52 (petroleum ether–ethyl acetate, v/v=5 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.75 (d, J=8.5 Hz, 1H), 7.66 (s, 1H), 7.46 (d, J=8.5 Hz, 1H), 7.39 (d, J=8.0 Hz, 1H), 7.11 (s, 1H), 6.92 (d, J=8.0 Hz, 1H), 6.11 (s, 2H), 6.06 (s, 2H), 5.45 (s, 1H), 3.85 (m, 1H), 3.62 (m, 1H), 2.75 (s, 3H), 1.46 (m, 2H), 1.20 (m, 2H), 0.78 (t, J=7.0 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 148.0, 147.4, 147.3, 145.2, 138.5, 131.0, 126.9, 125.9, 123.6, 123.0, 120.3, 116.4, 113.5, 108.7, 104.6, 101.7, 101.0, 100.7, 84.4, 66.2, 40.9, 31.5, 19.3, 13.9. ESI-MS *m/z*: 406.08 $[M+H]^+$, 427.96 $[M+Na]^+$, 332.28 $[M-O(CH_2)_3CH_3]^+$.

6-Methoxychelerythrine (**2a**): Yellow–white prism (MeOH), yield 95%, ^{1}H-, ^{13}C-NMR, ESI-MS and mp data were agreement with that previously reported by us.[6)]

6-Ethoxychelerythrine (**2b**): Yellow–white prism (EtOH), yield 96%, ^{1}H-, ^{13}C-NMR, ESI-MS and mp data were agreement with that previously reported by us.[6)]

6-*n*-Propoxy Dihydrochelerythrine (**2c**): White granular crystal (*n*-PrOH), yield 97%, mp 204–206°C, *Rf* 0.53 (petroleum ether–ethyl acetate, v/v=1 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.77 (d, J=8.5 Hz, 1H), 7.67 (s, 1H), 7.62 (d, J=8.6 Hz, 1H), 7.46 (d, J=8.5 Hz, 1H), 7.12 (s, 1H), 7.03 (d, J=8.6 Hz, 1H), 6.05 (s, 2H), 5.65 (s, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.80–3.85 (m, 1H), 3.57–3.62 (m, 1H), 2.73 (s, 3H), 1.42–1.61 (m, 2H), 0.76 (t, J=7.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 152.1, 147.8, 147.3, 146.6, 138.7, 131.0, 126.8, 126.0, 125.0, 123.3, 122.7, 120.1, 119.0, 112.8, 104.6, 101.0, 100.7, 84.7, 68.1, 55.9, 55.6, 40.6, 22.7, 10.7. ESI-MS *m/z*: 429.75 $[M+Na]^+$, 348.27 $[M-O(CH_2)_2CH_3]^+$.

6-*iso*-Propoxy Dihydrochelerythrine (**2d**): White needle crystal (*iso*-PrOH), yield 96%, mp 176–178°C, *Rf* 0.49 (petroleum ether–ethyl acetate, v/v=5 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.77 (d, J=8.5 Hz, 1H), 7.65 (s, 1H), 7.61 (d, J=8.6 Hz, 1H), 7.46 (d, J=8.5 Hz, 1H), 7.12 (s, 1H), 7.02 (d, J=8.6 Hz, 1H), 6.06 (s, 2H), 5.76 (s, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 4.37–4.39 (m, 1H), 2.70 (s, 3H), 1.30 (d, J=6.0 Hz, 1H), 0.87 (d, J=7.5 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 152.2, 147.9, 147.3, 146.5, 138.8, 131.0, 126.8, 126.1, 125.1, 123.3, 122.8, 120.2, 119.1, 112.8, 104.6, 101.0, 100.7, 82.6, 66.9, 61.6, 56.0, 40.5, 23.6, 21.5. ESI-MS *m/z*: 408.05 $[M+H]^+$, 429.61 $[M+Na]^+$, 348.14 $[M-OCH(CH_3)_2]^+$.

6-*n*-Butoxy Dihydrochelerythrine (**1e**): White needle crystal (*n*-BuOH), yield 98%, mp 144–146°C, *Rf* 0.53 (petroleum ether–ethyl acetate, v/v=5 : 1). ^{1}H-NMR ($CDCl_3$) δ: 7.77 (d, J=8.5 Hz, 1H), 7.67 (s, 1H), 7.62 (d, J=8.5 Hz, 1H), 7.46 (d, J=8.5 Hz, 1H), 7.12 (s, 1H), 7.03 (d, J=8.5 Hz, 1H), 6.06 (s, 2H), 5.65 (s, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.85–3.90 (m, 1H), 3.63–3.65 (m, 1H), 2.73 (s, 3H), 1.40–1.48 (m, 2H), 1.19–1.2 (m, 2H), 0.79 (t, J=7.5 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ: 152.2, 147.9, 147.3, 146.6, 138.7, 131.0, 126.8, 126.0, 125.0, 123.3, 122.7, 120.1, 119.0, 112.8, 104.6, 101.0, 100.7, 84.7, 66.2, 61.7, 56.0, 40.6, 31.7, 19.4, 13.9. ESI-MS *m/z*: 443.88 $[M+Na]^+$, 348.26 $[M-O(CH_2)_3CH_3]^+$.

Synthesis of 1f and 2f. General Procedure To the solution of potassium cyanide (60 mg, 0.92 mmol) in *ca.* 20 mL of water was added **1** or **2** (0.22 mmol). The resulting solution was heated at 60°C for 30 min using a water bath under stirring. White precipitate was filtered off, washed with a small volume of water for several times and recrystallized in a mixed solution of chloroform and methanol to provide the desired compound **1f** or **2f**.

6-Cyano Dihydrosaguinarine (**1f**): White prism crystal, yield 83%, mp 242–243°C. ^{1}H-NMR (DMSO-d_6) δ: 7.86 (d, J=8.5 Hz, 1H), 7.67 (d, J=8.5 Hz, 1H), 7.54 (d, J=8.2 Hz, 1H), 7.52 (s, 1H), 7.38 (s, 1H), 7.12 (d, J=8.2 Hz, 1H), 6.26 (d, J=0.75 Hz, 1H), 6.20 (d, J=0.75 Hz, 1H), 6.17 (d, J=3.0 Hz, 2H), 5.94 (s, 1H), 2.60 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 148.3, 147.6, 147.6, 144.6, 138.1, 130.7, 125.7, 125.0, 124.8, 122.5, 120.0, 118.0, 117.1, 109.3, 107.2, 104.3, 102.2, 101.4, 99.3, 47.3, 40.8. ESI-MS *m/z*: 332.27 $[M-CN]^+$, 359.25 $[M+H]^+$, 380.82 $[M+Na]^+$;

6-Cyano Dihydrochelerythrine (**2f**): White prism crystal, yield 89%, mp 244–246°C. ^{1}H-NMR (DMSO-d_6) δ: 7.88 (d, J=8.6 Hz, 1H), 7.76 (d, J=8.7 Hz, 1H), 7.67 (d, J=8.6 Hz, 1H), 7.52 (s, 1H), 7.38 (s, 1H), 7.27 (d, J=8.7 Hz, 1H), 6.18 (s, 1H), 6.17 (s, 1H), 5.93 (s, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 2.58 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ: 152.0, 148.1, 147.5, 145.3, 138.0,

130.6, 125.5, 124.7, 123.7, 122.3, 119.7, 119.7, 119.4, 118.5, 113.9, 104.2, 99.2, 101.2, 60.7, 55.7, 47.5, 40.6. ESI-MS *m/z*: 375.29 $[M+H]^+$, 396.78 $[M+Na]^+$.

Pharmacology. Screening of Acaricidal Activity *in Vitro* *In vitro* acaricidal activity of **1**, **2** and their derivatives (**1a**–**2i**) were performed according to the literature method with slight modification.[32] *Psoroptes cuniculi* adult mites of both sexes isolated from naturally infected rabbits were used as tested mites. The scabs and the cerumen, collected from the infected ears, were observed by means of a stereoscopic microscope to isolate adult mites of both sexes. Mites were placed in 24-well flat-bottomed cell culture plates (20 adult mites per each well). All tested compounds and the standard drug ivermectin were tested at the concentration of 5 mg/mL in 10% dimethyl sulfoxide (DMSO) and 10% Tween-80 in normal saline. Half milliliter of each tested solution was directly added to each well. Three replicates were made for each concentration. As untreated control, the same solution except for the tested compound was used while ivermectin in the same solvent represented the treated control.

All the plates were placed in separate humidity chambers in saturated humidity conditions at 22°C. After 24 h each plate was observed under a stereomicroscope for 5 min. When the persistent immobile mites were stimulated with a needle, lack of reaction was considered as the indication of death. Mortality rates were calculated as the following formula and expressed as means±S.D.

$$\text{mortality (\%)} = \frac{\text{number of death mites}}{\text{number of the tested mites}} \times 100$$

Acaricidal Toxicity Assay 6-Methoxy dihydrosanguinarine (**1a**) with the strongest acaricidal activity was used for acaricidal toxicity determination including toxicity regression equation for concentration effect and time effect, LC_{50} and LT_{50}. Ivermectin was used as the treated control.

The tested compound was completely dissolved in 0.2 mL DMSO, and then 0.2 mL of Tween-80 and 1.6 mL of normal saline were added and completely mixed to prepare a series of concentrations of 1.0, 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL of **1a** and a series of concentrations of 1.5, 0.5, 0.2, 0.1, 0.05, 0.02 mg/mL of ivermectin. Acaricidal activity of each solution was assayed at 24 h according to the method described in "Screening of Acaricidal Activity *in Vitro*." Untreated control mites were treated with the same solution except for the tested compound. Each of the tested concentration was performed in three test groups and each test group consisted of triplicate. The corrected mortality of each group was calculated as the following formula and expressed as means±S.D.

$$\text{corrected mortality (\%)} = \frac{\text{\% test mortality} - \text{\% control mortality}}{1.00\% - \text{\% control mortality}} \times 100$$

Probit value of the corrected mortality at the test concentrations and the corresponding log[concentration (mg/L)] value were used to establish toxicity regression equation for concentration effect by the linear least-square fitting method. LC_{50} value was calculated from the equation.

The test solution containing 10 mg/mL **1a** or ivermectin in the same solvent was prepared and used to determine LT_{50} value, respectively. The determination of acaricidal activity of each solution was carried out according to the method described above. The mites in each well were observed under a stereomicroscope every 0.5 h or 1.0 h and the percentage mortality and corrected percentage mortality for each well in each set time was calculated. The tested compound was performed in three test groups and each group consisted of triplicate. The corrected percentage mortality of each group at each set time was expressed as means±S.D. Probit value of the corrected percentage mortality for each post-treatment time and the corresponding log[post-treatment time (h)] value were used to establish toxicity regression equation for time effect. LT_{50} was calculated from it.

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