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 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 dihydro-sanguinarines (1a–e) showed significant acaricidal activity at 5.0 mg/mL and 1a possessed the strongest activity (50% lethal concentrations (LC₅₀)=339.70±0.75 mg/L, 50% lethal time (LT₅₀)=6.53±0.04 h), comparable with a standard drug ivermectin (LC₅₀=168.19±11.79 mg/L, LT₅₀=16.54±0.11 h). The iminium moiety in compound 1 and 2 was proven to be the determinant for their acaricidal properties. 6-Alkox 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 study 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.5 QBAs have been given rise to a lot of attention because of their extensive bioactivities including antitumor,6–9 antimicrobial,6,10 anti-inflammatory,7 antiviral8 including anti-human immunodeficiency virus (HIV),9 anti-platelet aggregation,10 anti-angiogenesis11 and anti-acetylcholinesterase.12 Recently, QBAs were also found to have antiparasitic actions against Trichodina sp.,13 Dactylogyrus intermedius14 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.6–20 Therefore, during the past ten years, a great deal of work has 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.23 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 acaricides 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 problems have lead to research efforts to discover new effective acaricides derived from some active natural products.

Macleaya microcarpa (MAXIM.) FDDE (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.5 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 CuCl₂·2H₂O 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 methyate 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

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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 CuCl2-catalyzed aerobic oxidative coupling reaction of dihydroxyanisamine (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 1H-NMR spectra due to the corresponding H=C=N proton (i.e. H-6), signals at δ 150.7 and 152.1 ppm in 13C-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 compound showed a singlet signal of H-6 in the region of δ 5.45–5.65 ppm in 1H-NMR spectrum, one signal of C-6 in the region of δ 82–86 ppm 13C-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 1H-NMR spectra due to the corresponding H-6, and signals at δ 47.3 and 47.5 ppm in their 13C-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 dihydroxyanisamines (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 beneficial 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 (LC50) 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 (R²=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 LC50 value (339.70±0.96 mg/L) than ivermectin (LC50=168.19±11.79, LC90=1693.63±126.18 mg/L) (see Table 2).

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 (LT50) 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 (R²=0.99). From Fig. 4, it might be seen that the acaricidal action of 1a was much faster than ivermectin. Both the LT50 and 90% lethal time (LT90) of 1a (LT50=6.53±0.04, LT90=7.93±0.11 h) were significantly smaller than ivermectin (LT50=16.54±0.11, LT90=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

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**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**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.3±2.9</td>
<td>2</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>1a</td>
<td>100.0±0.0</td>
<td>2a</td>
<td>16.7±2.9</td>
</tr>
<tr>
<td>1b</td>
<td>73.3±2.9</td>
<td>2b</td>
<td>8.3±2.9</td>
</tr>
<tr>
<td>1c</td>
<td>71.7±2.9</td>
<td>2c</td>
<td>8.3±2.9</td>
</tr>
<tr>
<td>1d</td>
<td>73.3±2.9</td>
<td>2d</td>
<td>6.7±2.9</td>
</tr>
<tr>
<td>1e</td>
<td>71.7±2.9</td>
<td>2e</td>
<td>6.7±2.9</td>
</tr>
<tr>
<td>1f</td>
<td>6.7±2.9</td>
<td>2f</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>1g</td>
<td>11.7±2.9</td>
<td>2g</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>1h</td>
<td>50.0±5.0</td>
<td>2h</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>1i</td>
<td>6.7±2.9</td>
<td>2i</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>100.0±0.0</td>
<td>Control</td>
<td>1.7±2.9</td>
</tr>
</tbody>
</table>
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 even physiological acidic environment, such as the lysosome of cells. 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\textsuperscript{+} 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\textsuperscript{+}-modified derivatives of 1 and 2 were synthesized and evaluated for their acaricidal activity in vitro against P. cuniculi. 6-Alkoxy dihydroxyammoniums (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\textsubscript{50} value and much smaller LT\textsubscript{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\textsuperscript{+} double bond in 1 and 2 were the determinant for their acaricidal properties. The present results suggested that quaternary benzylphenanthridine 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\textsubscript{1a} and 5% avermectin B\textsubscript{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.\textsuperscript{30} Compound 1g–i, 2g–i were prepared according to the methods previously reported by us using 1 and 2 as

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

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Toxicity regression equation\textsuperscript{a})</th>
<th>( R^2 )</th>
<th>LC\textsubscript{50}± S.D.</th>
<th>LT\textsubscript{90}± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>( y = 2.2344x – 0.6541 )</td>
<td>0.9677</td>
<td>339.70± 0.75</td>
<td>1243.90± 36.09</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>( y = 1.2794x + 2.1510 )</td>
<td>0.9862</td>
<td>168.19± 11.79</td>
<td>1693.63± 126.18</td>
</tr>
</tbody>
</table>

\textsuperscript{a}) \( y \) and \( x \) express the probability of mortality rate and log[concentration (mgL\textsuperscript{-1})], respectively.

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

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Toxicity regression equation\textsuperscript{a})</th>
<th>( R^2 )</th>
<th>LT\textsubscript{50}± S.D.</th>
<th>LT\textsubscript{90}± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>( y = 15.566x – 7.6942 )</td>
<td>0.9970</td>
<td>6.53± 0.04</td>
<td>7.93± 0.11</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>( y = 14.730x – 12.942 )</td>
<td>0.9943</td>
<td>16.54± 0.11</td>
<td>20.26± 0.12</td>
</tr>
</tbody>
</table>

\textsuperscript{a}) \( y \): the probability of mortality rate. \( x: \log T \) (h).
starting materials. 

**Apparatus** Melting points (mp) were determined on XT-4 micro-melting point apparatus and uncorrected. ¹H- and ¹³C-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 sanguinarine 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 CuCl₂·2H₂O at room temperature. The reaction 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%. ¹H-, ¹³C-NMR, ESI-MS and mp data were in agreement with that previously reported by us. 6) 

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

6-n-Propano Dihydrogsanguinarine (1c): White granular solid (n-ProOH), yield 99%, mp 194–195°C, Rf 0.36 (petroleum ether–ethyl acetate, v/v=2:1). ¹H-NMR (CDCl₃) 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, m), 2.79 (s, 3H), 1.53 (m, 2H), 0.80 (2H), (7.5 Hz, 3H); ¹³C-NMR (CDCl₃) δ: 148.0, 147.4, 147.3, 145.2, 143.5, 131.0, 126.8, 126.0, 125.0, 123.3, 122.8, 120.2, 119.1, 112.8, 104.6, 101.0, 10.0, 82.6, 65.8, 56.0, 40.5, 23.6, 21.5. ESI-MS m/z: 439.43 [M+Na]⁺, 344.18 [M+O(CH₃)₂CH₃]⁺.

6-iso-Propano Dihydrogsanguinarine (1d): White granular crystal (iso-ProOH), yield 98%, mp 144–146°C, Rf 0.53 (petroleum ether–ethyl acetate, v/v=5:1). ¹H-NMR (CDCl₃) δ: 7.77 (d, J=7.9 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 (brs, 1H), 2.72 (s, 3H), 1.27 (s, 3H), 0.91 (s, 3H); ¹³C-NMR (CDCl₃) δ: 148.0, 147.3, 147.3, 145.2, 143.5, 131.0, 126.8, 126.0, 125.0, 123.3, 122.8, 120.2, 119.1, 112.8, 104.6, 101.0, 10.0, 82.6, 65.8, 56.0, 40.5, 31.7, 19.4, 13.9. ESI-MS m/z: 443.88 [M+Na]⁺, 348.26 [M+O(CH₃)₂CH₃]⁺.

**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 Dihydrogsanguinarine (1f): White prism crystal, yield 83%, mp 242–243°C. ¹H-NMR (DMSO-d₆) 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=3.01 Hz, 2H), 5.94 (s, 1H, 2.60 (s, 3H); ¹³C-NMR (DMSO-d₆) 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, 97.3, 40.8. ESI-MS m/z: 332.27 [M–CN]⁺, 359.25 [M+H]⁺, 380.82 [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. 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 24h 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, LC50 and LT50. Ivermectin was used as the treated control.

The test solution containing 10 mg/mL 1a or ivermectin in the same solvent was prepared and used to determine LT50 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. LT50 was calculated from it.

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References

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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]+.