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Synthesis and fungicidal activities of sanguinarine derivatives

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Sanguinarine is one of the important alkaloids possessing various biological properties such as antitumor, insecticidal activity and fungistatic activity. In the course of discovering agricultural fungicides, sanguinarine and its derivatives were synthesized and evaluated for fungicidal activities. Three groups of 32 sanguinarine analogues were synthesized *via* facile methods and characterized with ^{1}H and ^{13}C nuclear magnetic resonance and high-resolution mass spectrometry. Their fungicidal activities against the fungi *Alternaria solani*, *Gibberella zeae*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Cercospora arachidicola* were evaluated. Thirteen new derivatives displayed a medium effective concentration (EC_{50}) between 1.0 and 4.4 μg/mL against *R. solani*, whereas sanguinarine showed an EC_{50} of 11.6 μg/mL. The 32 sanguinarine synthetic analogues showed low to moderate activities against the other four fungi. Structural modification of sanguinarine has improved its potency for >11 fold against *R. solani*. Two of the chemicals (**II-d** and **III-a**) had approximately one third of the potency of the commercial fungicide tebuconazole against *R. solani in vitro*. They can be considered as a leading structure for further design of agricultural fungicides.

1. Introduction

Natural products have been directly used for crop protection [1], used as lead structures to derive new potent pesticides, and used as probes to illucidate modes of action [2]. Sanguinarine is considered one of the important benzo[*c*]phenanthridine alkaloids possessing a wide range of biological properties such as antitumor [3,4], antiviral [5], anti-inflammatory [6], antimicrobial activities [7,8], trypanocidal activity [9], acaricidal activity [10], and insecticidal activity [11]. Recently, 1% sanguinarine W.P. has been reported as a new botanical termiticide for effective control of *Tetranychus urticae* on apple [12]. Some plant extracts containing sanguinarine were also fungistatic to several phytopathogens [13–15]. In addition to the fungicidal activity, sanguinarine is toxic to insects, mites and vertebrates [16]. However, safety assessments of sanguinarine on rats and pigs in 90 days feeding experiments did not show lymphocyte or hepatocyte genotoxic damage in rats [17], histological, haematological toxicity or DNA adduct formation in pigs despite plasma sanguinarine levels reaching 0.11 μg/mL [18].

Benzo[*c*]phenanthridine alkaloids possess a common phenanthrine core fused with a quatenary nitrogen, which imparts inherent chemical reactivity into alkaloids. Nucleophiles can readily add to the adjacent carbon to create a neutral pseudobase [19,20]. For example, 5,6-dihydrosanguinarine was reported as a metabolite of sanguinarine detoxification by *Verticillium dahliae* [21]. 6-ethoxy-5,6-dihydrosanguinarine have been reported as byproducts formed during the isolation of sanguinarine from plants utilizing ethanol extraction, [6-(5,6-dihydrosanguinarinyl)] ether and bis[6-(5,6-dihydrosanguinarinyl)]amine could be extracted by treatment of sanguinarine chloride with an excess aqueous Na_2CO_3 and NH_3, respectively [20]. The conversion to the alkanolamine of sanguinarine was essentially complete at pH 7.0 and the concentration of iminium ion decreased with increasing pH, while these addition products easily converts to the quaternary cation in the presence of acids [22]. Yang et al. [23] reported the antifungal activity of oxysanguinarine and seven nucleophilic reaction products of sanguinarine against the phytopathogenic fungi *Curvularia lunata*, *Alternaria alternate*, *Fusarium solani*,

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Fusarium oxysporum sp. *vasinfectum*, *Valsa mali*, *Fusarium oxysporum* sp. *niveum* and *Pyricularia oryza*. Some structural relationships among cytotoxic and antitumor of sanguinarine derivatives have also been studied [24]. Conversion from the iminium ion to the alkanolamine improves the lipophilicity of sanguinarine, which may enhance the antimicrobial activity by increasing the cellular availability of the alkaloid [22]. As the alkanolamine, alkyldiamine structures are able to easily convert back to the corresponding parent compound sanguinarine in acidic conditions, the stability of the iminium, alkanolamine, alkyldiamine and alkylamine forms will be critical to any prospective use of sanguinarine.

In our efforts to discover new agricultural fungicides, sanguinarine was structurally modified at three positions to systematically evaluate fungicidal activities *in vitro* against five agriculturally significant fungal species and understand their structure-activity relationships. The results indicated that several derivatives can be fungicide candidates against *R. solani*.

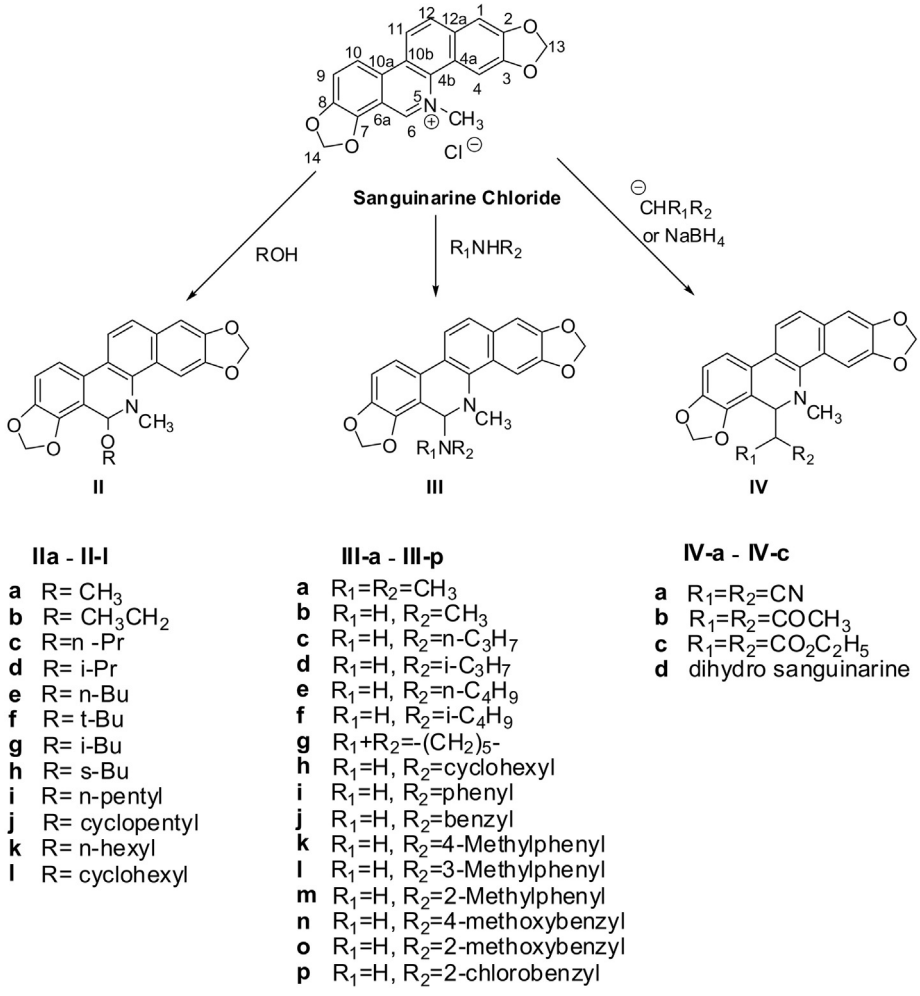
2. Materials and methods

2.1. Chemicals, solvents and instruments

NMR spectra were recorded at 400 MHz with a Bruker AV 400 spectrometer or at 600 MHz with an Agilent DD2 600 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solution with tetramethylsilane as the internal standard. Chemical shift values (δ) were given in ppm. High-resolution mass spectrometry (HRMS) data were obtained on a Varian QFT-ESI instrument, the source voltage was 4 kV. (Most of the reported addition products eliminate to form sanguinarine, it indicates the structural characteristics of the chemicals the bond between 6C and the substituents is rather weak.). Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. Synthetic yields were not optimized. Reagents were all analytically or chemically pure. All solvents and liquid reagents were dried by standard methods in advance and distilled prior to use. Tebuconazole (98%) were purchased from J&K Chemical Ltd. (Shanghai, China). Tebuconazole is a commercial fungicide and was used as a positive control.

2.2. Chemistry

Sanguinarine was synthesized [25] and then used as the starting material to synthesize **II-a–II-l**, **III-a–III-p** and **IV-a–IV-c**. Structure modifications of sanguinarine included introduction of alkoxyl, amine, anion of malononitrile, acetylacetone and diethyl malonate by nucleophilic addition.



Scheme 1. Synthetic routes for compounds **II-a–II-l**, **III-a–III-p** and **IV-a–IV-c**.

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