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# Five quinolizidine alkaloids with anti-tobacco mosaic virus activities from two species of *Sophora*

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ABSTRACT

Three novel matrine-type alkaloids (**1**–**3**) and two unprecedented aloperine-type alkaloids (**4** and **5**) were isolated from the root of *Sophora tonkinensis* and the seeds of *Sophora alopecuroides* respectively. Notably, compound **1** possessed an unprecedented 6/5/6 tricyclic skeleton, while compounds **2** and **3** characterized by rare 6/6/5/6 tetracyclic system and 6/6/6/6/6 pentacyclic system respectively. Moreover, compound **4** possessed an unprecedented 6/7/6/6 tetracyclic core, and compound **5** characterized by rare 6/6/6/6 tetracyclic skeleton. Their structures were elucidated by comprehensive spectroscopic data analysis and electronic circular dichroism (ECD) calculations. Biological tests indicated that compound **5** displayed significant anti-tobacco mosaic virus (TMV) activity compared with the positive control ningnanmycin.



Quinolizidine alkaloids (QAs) are characteristic natural products of many Fabaceae, especially abundant in the tribes *Genisteae*, *Sophoreae*, and *Thermopsideae* [1–5]. They are biosynthesized from L-lysine through the decarboxylated intermediate cadaverine, which undergoes a series of enzymatic reactions to form different types of QAs, such as matrine-type, aloperine-type, cytisine-type, anagyrine-type, thermopsine-type, and sparteine-type [6–8]. QAs have been reported to have various biological activities including antibacterial, anti-inflammatory, antiviral, anti-allergic, and antitumor activities [9–17]. Previously, we found several new QAs with anti-plant virus activities from the seeds of *T. lanceolata* [18,19]. In our continuing efforts to discover novel, highly effective antiviral agents from QAs, the chemical components of the root of *S. tonkinensis* and the seeds of *S. alopecuroides* were carried out, which led to the isolation of three novel matrine-type alkaloids (**1**–**3**) and two unprecedented aloperine-type alkaloids (**4** and **5**) respectively. Notably, compounds **1**–**3** possessed unprecedented 6/5/6 tricyclic system, 6/6/5/6 tetracyclic skeleton, and 6/6/6/6/6 pentacyclic frame respectively. In addition, compound **4** possessed an unprecedented 6/7/6/6 tetracyclic skeleton, while compound **5** characterized by rare 6/6/6/6 tetracyclic skeleton (Fig. 1). The anti-tobacco mosaic virus (TMV) activities of isolates were screened using the half-leaf method. Herein, the isolation, structural determinations, and bioactivity assays were described.

Sophortonkine A (**1**) with the molecular formula of $C_{11}H_{12}N_2O$ on the basis of the high resolution electrospray ionization mass spectroscopy (HRESIMS) (*m*/*z* 211.0846 $[M+Na]^+$, calcd. for 211.0842). The nuclear magnetic resonance (NMR) data of **1** displayed a pyridine ring [$\delta_C$ 157.9, 149.6 ($\delta_H$ 8.54, d, $J$ = 4.9 Hz), 134.2, 129.7 ($\delta_H$ 7.54, d, $J$ = 7.7 Hz), 122.4 ($\delta_H$ 7.24, dd, $J$ = 7.7, 4.9 Hz)] (Tables S1 and S2 in Supporting information). Moreover, the $^1H$–$^1H$ correlation spectroscopy (COSY) correlations of H-6/$H_2$-7/$H_2$-8/$H_2$-9, combined with the heteronuclear multiple bond correlation spectroscopy (HMBC) correlations from $H_2$-12 to C-5 ($\delta_C$ 134.2)/C-6 ($\delta_C$ 61.9)/C-10 ($\delta_C$ 169.0)/C-13 ($\delta_C$ 157.9), from H-9 to C-10, and from H-6 to C-5 indicated the existence of hexahydro-5(1*H*)-indolizinone unit (rings B and C) (Figs. 1 and 2) [20]. Furthermore, the key HMBC correlations from $H_2$-12 to C-5/C-13, and from H-4 to C-6/C-13 demonstrated that above two units were fused *via* the $C_5$–$C_{13}$ bond (Fig. 2). Therefore, compound **1** with a rare 6/5/6 tricyclic ring system.

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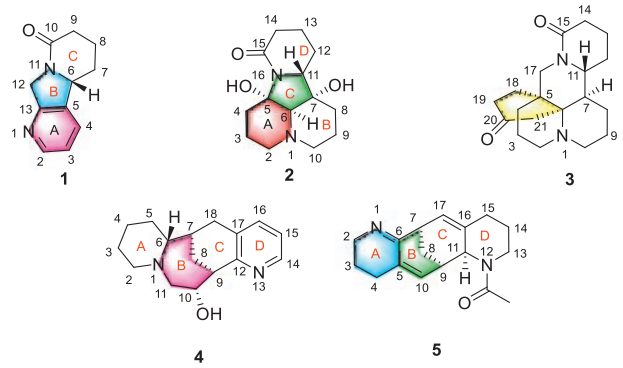


**Fig. 1.** Structures of compounds **1**–**5**.

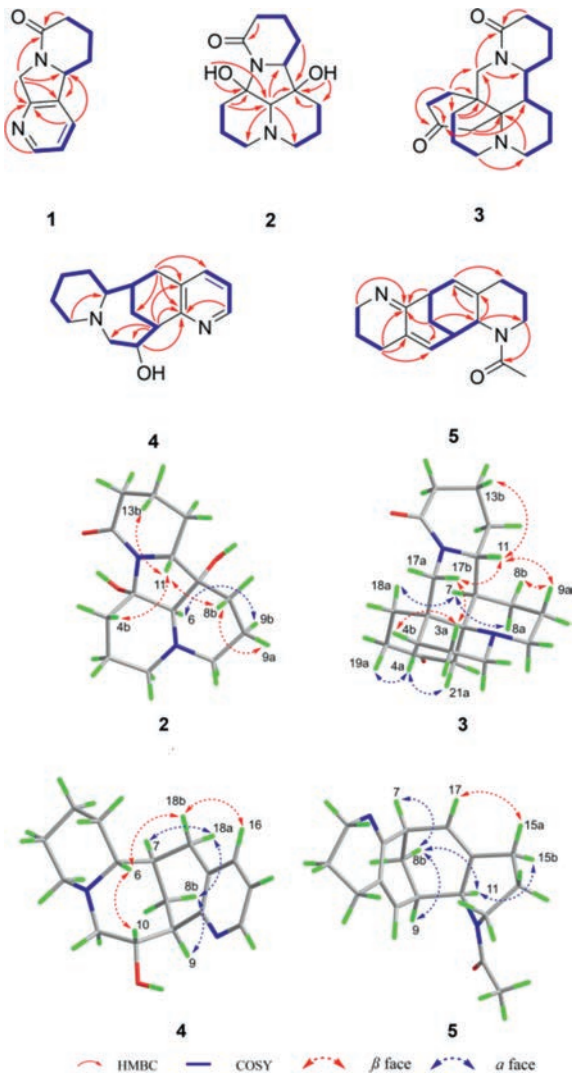



**Fig. 2.** The key HMBC, $^{1}$H–$^{1}$H COSY, and NOESY of **1**–**5**.

The absolute configuration of **1** was further verified through electronic circular dichroism (ECD) calculations [21]. As a result, the calculated ECD spectrum of (6*R*)–**1** matched well with the experimental circular dichroism (CD) spectrum of **1** (Fig. 3). Therefore, the absolute configuration of the stereogenic center of **1** was assigned as 6*R*.

Sophortonkine B (**2**) was obtained as colorless oil. Its molecular formula, $C_{14}H_{22}N_2O_3$, was determined by the HRESIMS (*m/z*: 289.1539 $[M+Na]^+$, calcd. 289.1528). The $^{1}$H NMR spectrum of **2** showed the signals of two nitrogenated methines ($\delta_H$ 2.26, m, H-6; $\delta_H$ 4.20, d, $J$ = 10.6 Hz, H-11) and two nitrogenated methenes ($\delta_H$ 2.72, m, $H_2$-2a; $\delta_H$ 2.28, m, $H_2$-2b; $\delta_H$ 2.74, m, $H_2$-10a; $\delta_H$ 2.25, m, $H_2$-10b). The $^{13}$C and heteronuclear single quantum correlation spectroscopy (HSQC) spectra revealed 14 carbon signals due to one $sp^2$ quaternary carbon, two $sp^3$ quaternary carbons, two $sp^3$ methines, and nine $sp^3$ methylenes (Tables S1 and S2). The $^{1}$H–$^{1}$H COSY correlations of $H_2$-2/$H_2$-3/$H_2$-4 and $H_2$-8/$H_2$-9/$H_2$-10, together with the HMBC correlations of H-6 to C-2 ($\delta_C$ 52.4)/C-7 ($\delta_C$ 76.6)/C-10 ($\delta_C$ 53.8), of $H_2$-4 to C-5 ($\delta_C$ 92.1)/C-6 ($\delta_C$ 72.7), and of $H_2$-8 to C-7 displayed the presence of quinolizidine core (rings A and B) (Figs. 1 and 2) [22]. Meanwhile, the $^{1}$H–$^{1}$H COSY correlations of H-11/$H_2$-12/$H_2$-13/$H_2$-14, and the HMBC correlations of H-14 to C-15 ($\delta_C$ 173.7), of H-6 to C-7/C-11 ($\delta_C$ 64.6), and of $H_2$-12 to C-7 indicated the existence of hexahydro-5(1*H*)-indolizinone moiety (rings C and D) (Figs. 1 and 2) [23]. Moreover, the HMBC correlations of H-6 to C-11/C-7, of H-8 and $H_2$-12 to C-7, and of $H_2$-4 to C-5/C-6 indicated the above two moieties were connected *via* the $C_5$–$C_6$–$C_7$ bond. Thus, the unique 6/6/5/6 tetracyclic core have been formed on the basis of the fragments established above. Furthermore, the key HMBC correlations of 5-OH to C-5/C-6, and of 7-OH to C-7/C-8 demonstrated that two hydroxyl groups were connected to C-5 and C-7 respectively (Fig. 2).

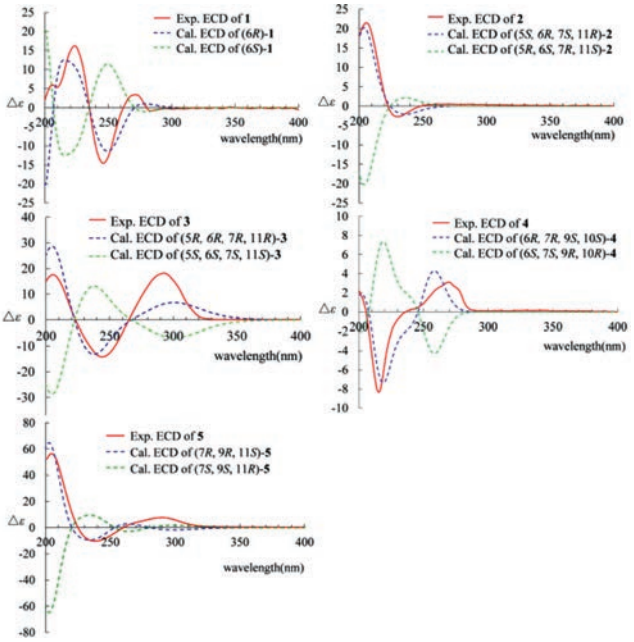



**Fig. 3.** Experimental and calculated ECD spectra of **1**–**5**.

The nuclear overhauser effect spectroscopy (NOESY) correlations of H-11/$H_2$-13b, H-11/$H_2$-8b, $H_2$-8b/$H_2$-9a, and $H_2$-9b/H-6 indicated that H-11 and H-6 were $\beta$- and $\alpha$-oriented respectively. Meanwhile, the correlations of $H_2$-4b/H-11 and $H_2$-8b/H-11 revealed that C-5 and C-7 were all *S**. The absolute configuration of **2** was established as (5*S*, 6*R*, 7*S*, 11*R*) by the aforementioned evidence, the biosynthetic pathway, and X-ray diffraction analysis of biogenic homologue [20,23]. Moreover, the absolute configuration of **2** was further verified through ECD calculations (Fig. 3).

Sophtonkine C (**3**) was obtained as colorless oil. Its molecular formula was deduced to be $C_{19}H_{28}N_2O_2$, by HRESIMS (*m/z* 317.2220 $[M+H]^+$, calcd. 317.2224). The $^{13}$C and HSQC spectroscopic data revealed the presence of total 19 carbon resonances, corresponding to two $sp^2$ quaternary carbons, two $sp^3$ quaternary carbons, two $sp^3$ methines, and thirteen $sp^3$ methylenes (Table S2). The aforementioned evidence suggested that compound **3** was an analogue of matrine [24]. The notable difference was that an

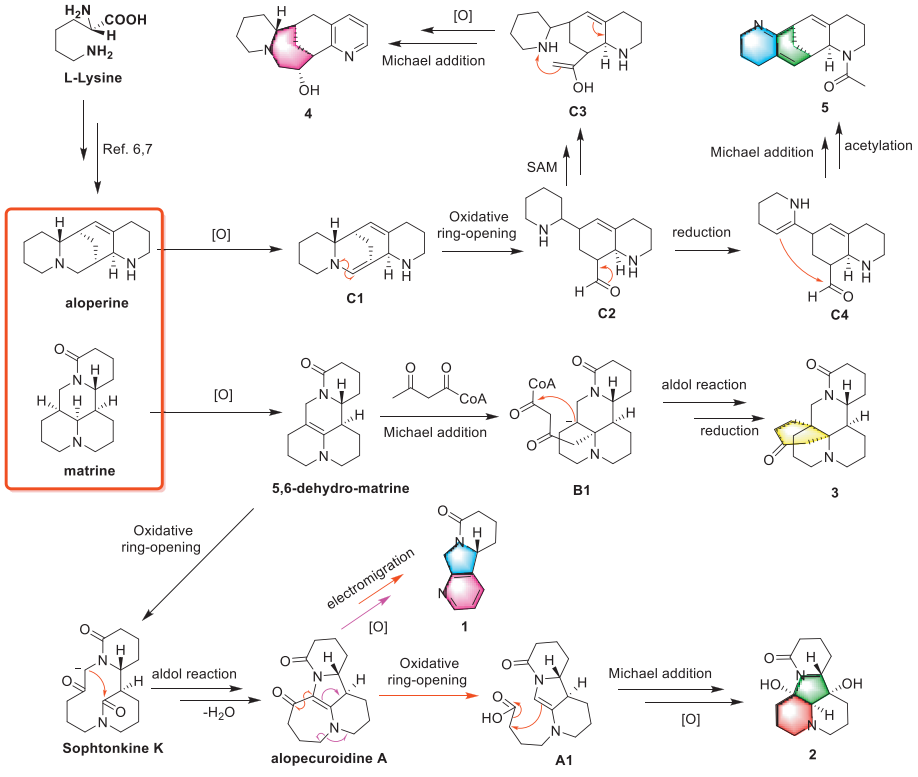
additional butan-2-one group ($\delta_C$ 30.8, 34.7, 37.0, 211.9) was fused between C-5 ($\delta_C$ 36.1) and C-6 ($\delta_C$ 62.6), which was supported by the $^1$H–$^1$H COSY correlation $H_2$-18/$H_2$-19, and the obvious HMBC correlations between $H_2$-18/$H_2$-19/$H_2$-21 and C-20 ($\delta_C$ 211.9), between $H_2$-18 and C-4 ($\delta_C$ 28.7)/C-5/C-17 ($\delta_C$ 45.2), and between $H_2$-21 and C-6/C-7 (Fig. 2). Thereby, compound **3** possessed an undescribed 6/6/6/6/6 pentacyclic skeleton.

The NOESY correlations of H-11/$H_2$-9a/$H_2$-8b, H-11/$H_2$-17b, $H_2$-17b/$H_2$-3a, and $H_2$-3a/$H_2$-4b revealed that these protons were $\beta$-oriented. Accordingly, the key NOESY correlations of $H_2$-19a/$H_2$-4a/$H_2$-21a and $H_2$-8a/H-7/$H_2$-18a revealed that C-5, C-6 and C-7 were 5*R**, 6*R**, 7*R** respectively (Fig. 2). Ultimately, the absolute configuration of **3** was identified as 5*R*, 6*R*, 7*R*, 11*R* by ECD calculations (Fig. 3).

Sophoralopeine A (**4**) was obtained as colorless oil. Its molecular formula, $C_{16}H_{22}N_2O$, was determined by the HRESIMS (*m/z*: 259.1809 $[M + H]^+$, calcd. 259.1805). The $^1$H NMR spectrum of **4** showed a set of AMX spin system of aromatic H atoms ($\delta_H$ 8.40, d, $J$ = 5.0 Hz, H-14; $\delta_H$ 7.39, d, $J$ = 7.8 Hz, H-16; $\delta_H$ 7.09, dd, $J$ = 7.8, 5.0 Hz, H-15), a nitrogenated methine ($\delta_H$ 1.79, m, H-6), and two nitrogenated methenes ($\delta_H$ 3.91, dd, $J$ = 10.9, 5.6 Hz, $H_2$-11a; $\delta_H$ 3.79, dd, $J$ = 10.9, 2.0 Hz, $H_2$-11b; $\delta_H$ 3.15, d, $J$ = 11.8 Hz, $H_2$-2a; $\delta_H$ 1.72, m, $H_2$-2b). The $^{13}$C and HSQC spectra displayed resonances for two sp$^2$ quaternary carbons, three sp$^2$ methines, four sp$^3$ methines, and seven sp$^3$ methylenes (Tables S1 and S2). The $^1$H–$^1$H COSY correlations of $H_2$-2/$H_2$-3/$H_2$-4/$H_2$-5/H-6/H-7/$H_2$-8/H-9/H-10/$H_2$-11, the HMBC correlations of H-2 to C-6 ($\delta_C$ 62.3), together with the chemical shift of C-2 ($\delta_C$ 53.5), C-6 ($\delta_C$ 62.3), and C-11 ($\delta_C$ 64.8) indicated the existence of decahydropyrido[1,2-$\alpha$]azepine skeleton (rings A and B) (Figs. 1 and 2) [25]. Moreover, the $^1$H–$^1$H COSY correlations of H-14/H-15/H-16 and $H_2$-18/H-7/$H_2$-8/H-9, together with the HMBC correlations of H-9 to C-12 ($\delta_C$ 162.3)/C-17 ($\delta_C$ 130.4), of $H_2$-18a to C-16 ($\delta_C$ 138.1)/C-17/C-12, and of H-14 to C-12 indicated the existence of 5,6,7,8-tetrahydroquinoline moiety (rings C and D) (Figs. 1 and 2) [26]. Furthermore, the $^1$H–$^1$H COSY of H-7/$H_2$-8/H-9, and the HMBC correlation of H-10 to C-12 indicated the above two moieties were connected *via* the $C_7$–$C_8$–$C_9$ bond. Thus, the unique 6/7/6/6 tetracyclic core have been formed on the basis of the fragments established above.

The NOESY correlations of $H_2$-18b/H-6 and H-6/H-10 indicated that these protons were cofacial and were arbitrarily assigned as $\beta$-oriented. Meanwhile, the correlations of H-7/$H_2$-18a, $H_2$-18a/$H_2$-8b, $H_2$-8b/H-9 revealed that H-7 and H-9 were all $\alpha$-oriented (Fig. 2). Finally, the absolute configuration of **4** (6*R*, 7*R*, 9*S*, 10*S*) was determined by ECD calculations (Fig. 3).

Sophoralopeine B (**5**) was obtained as colorless oil. Its molecular formula, $C_{17}H_{22}N_2O$, was determined by the HRESIMS (*m/z*: 271.1802 $[M + H]^+$, calcd. 271.1805). The $^1$H NMR spectrum of **5** displayed two olefin protons ($\delta_H$ 5.81, d, $J$ = 6.1 Hz, H-10; $\delta_H$ 5.67, d, $J$ = 6.1 Hz, H-17), a nitrogenated methine ($\delta_H$ 4.94, d, $J$ = 3.1 Hz, H-11), and two nitrogenated methenes ($\delta_H$ 3.69, ddd, $J$ = 17.1, 5.7, 4.3 Hz, $H_2$-2a; $\delta_H$ 3.57, ddd, $J$ = 17.1, 7.8, 4.3 Hz, $H_2$-2b; $\delta_H$ 3.43, dd, $J$ = 14.1, 6.9 Hz, $H_2$-13a; $\delta_H$ 2.90, m, $H_2$-13b). The $^{13}$C and HSQC spectra displayed resonances for one methyl, seven sp$^3$ methylenes, two sp$^2$ methines, three sp$^3$ methines, and four sp$^2$ quaternary carbons (Tables S1 and S2). The $^1$H–$^1$H COSY correlations of $H_2$-2/$H_2$-3/$H_2$-4, and H-7/$H_2$-8/H-9/H-10, combined with the HMBC correlations of H-2, H-7, and $H_2$-8 to C-6 ($\delta_C$ 167.1), and of $H_2$-4 to C-5 ($\delta_C$ 128.8)/C-6/C-10 ($\delta_C$ 132.5) indicated the existence of 2,3,4,6,7,8-hexahydroquinoline skeleton (rings A and B) (Figs. 1 and 2) [27]. Meanwhile, the $^1$H–$^1$H COSY correlations of H-17/H-7/$H_2$-8/H-9/H-11 and $H_2$-13/$H_2$-14/$H_2$-15, together with the HMBC correlations of H-11 to C-13 ($\delta_C$ 42.9)/C-16 ($\delta_C$ 134.4)/C-17 ($\delta_C$ 125.7), of H-17 to C-15 ($\delta_C$ 27.3) indicated the existence of octahydroquinoline moiety (rings C and D) (Figs. 1 and 2) [28]. Furthermore, the $^1$H–$^1$H COSY H-17/H-7/$H_2$-8/H-9/H-10 indicated the above two moieties were connected *via* the $C_7$–$C_8$–$C_9$ bond. Thus, the unique 6/6/6/6 tetracyclic core have been formed on the basis of the fragments established above. In addition, the key HMBC cor-




**Scheme 1.** Hypothetical biosynthetic pathways for **1**–**5**.

relations of $H_2$-13 to the acetyl group ($\delta_C$ 169.8) demonstrated that the acetoxyl group was connected to N-12 (Fig. 2).

The NOESY correlations of $H_2$-15b/H-11, H-11/$H_2$-8b, and H-9/$H_2$-8b/H-7 indicated that H-7, H-9, and H-11 were $\alpha$-oriented (Fig. 2). Finally, the absolute configuration of **5** (7*R*, 9*R*, 11*S*) was determined by ECD calculations (Fig. 3).

The biosynthetic pathway for **1**–**5** are illustrated in Scheme 1. L-Lysine undergoes a series of reactions to yield matrine and aloperine [6,7]. Matrine could be converted to 5,6-dehydro-matrine through oxidation and dehydration, followed by oxidative ring-opening and intramolecular nucleophilic addition to generate the key intermediate alopecuroidine A [20]. On the one hand, alopecuroidine A undergoes ring-opening, C–N bond cleavage, electromigration, and oxidative reaction to generate **1** [29]. On the other hand, alopecuroidine A goes through ring-opening, Michael addition reaction, decarboxylation, and oxidation to yield **2** [30]. Moreover, 5,6-dehydro-matrine undergoes the Michael addition with acetoacetyl-CoA to obtained **B1**. After cyclizition and reduction, **B1** could afford **3** [30]. The key intermediate **C2** was derived from aloperine through reduction and oxidative ring-opening [30]. Subsequently, **C2** could further generate **4** *via* *S*-adenosyl methionine (SAM) catalysed methylation, Michael addition, and reduction [31–33]. In addition, **C2** undergoes a cascade of reduction, Michael addition, dehydration, and acetylation to obtain **5** [34].

The anti-TMV activities of **1**–**5** were evaluated using the half-leaf method (100 mg/L) (Table S3 in Supporting information) [2]. Notably, **5** displayed a significant protective effect and curative effect with concentration for 50% of maximal effect ($EC_{50}$) values of 19.6 μg/mL (curative), which was superior to that of positive control ningnanmycin ($EC_{50}$: 54.8 μg/mL) (Table S4 in Supporting information). Moreover, the transcription levels of the TMV *Cp* and *Rdrp* genes were detected by quantitative real time polymerase chain reaction (qRT-PCR) in the inoculated and systematic leaves for the *in vivo* curative and protective assays. Biological tests indicated that compound **5** strongly inhibited the expression of the TMV *Cp* and *Rdrp* genes (Fig. 4).

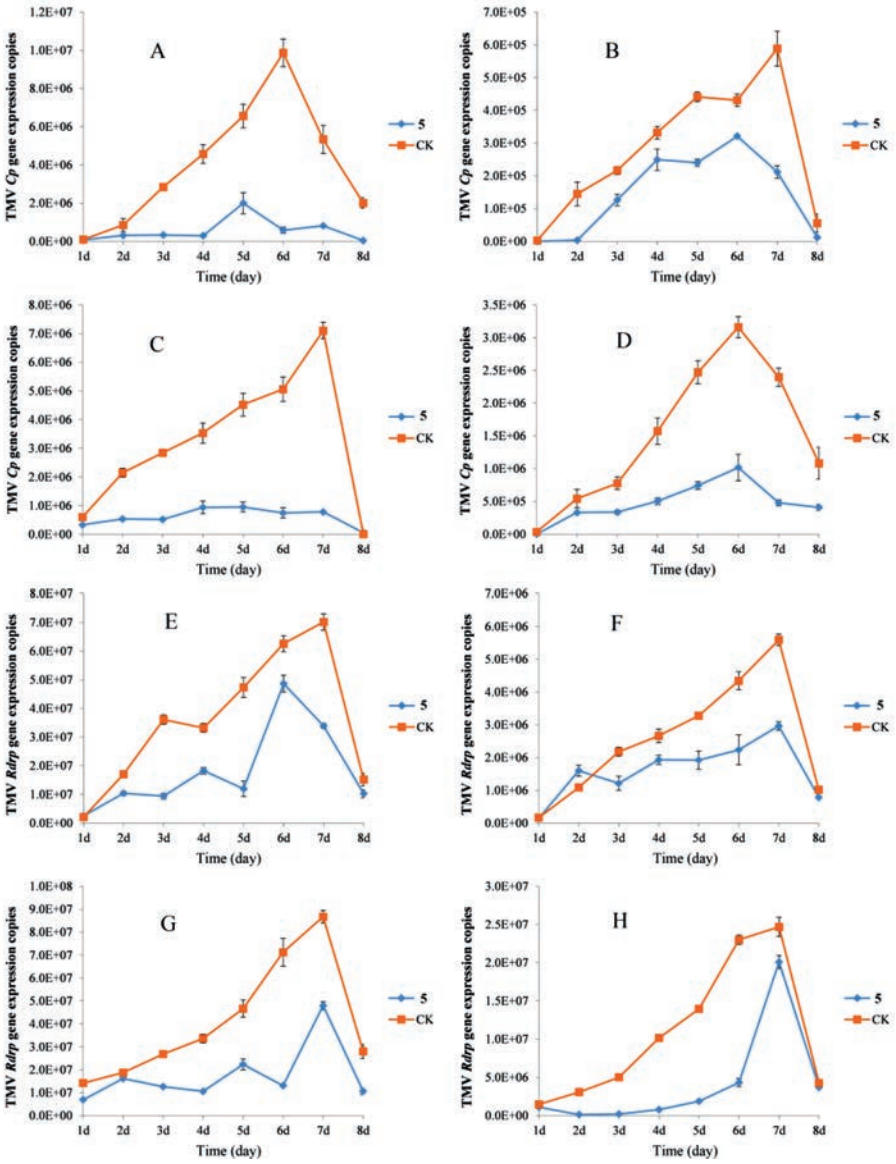



**Fig. 4.** The expression levels of TMV *Cp* and *Rdrp* genes in inoculated and systematic $K_{326}$ leaves following treatment with 5 in protective and curative effects. (A) The level of TMV *Cp* gene expression in inoculated leaves (protective). (B) The level of TMV *Cp* gene expression in systematic leaves (protective). (C) The level of TMV *Cp* gene expression in inoculated leaves (curative). (D) The level of TMV *Cp* gene expression in systematic leaves (curative). (E) The level of TMV *Rdrp* gene expression in inoculated leaves (protective). (F) The level of TMV *Rdrp* gene expression in systematic leaves (protective). (G) The level of TMV *Rdrp* gene expression in inoculated leaves (curative). (H) The level of TMV *Rdrp* gene expression in systematic leaves (curative). Solution of equal DMSO was used as a negative control agent (CK).

In summary, this study established the structures of five new QAs covering five novel skeletons. Bioactivity evaluations revealed that **5** displayed significant anti-TMV activity than the positive control ningnanmycin. Furthermore, compound **5** could not only inhibit the accumulation of TMV *Cp* and *Rdrp* genes but also enhance the host plant's resistance to TMV infection. Therefore, compound **5** can serve as a lead compound for the discovery of new antiviral agents for the management of TMV.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108927.

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