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Synthesis and in vitro inhibitory activity of matrine derivatives towards pro-inflammatory cytokines

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ABSTRACT

Matrine, a sophora alkaloid, exhibited good anti-inflammation effects in our previous report. In the present study, a series of matrine derivatives were synthesized via classical Michael addition. Biological studies showed that the synthetic derivatives had good inhibitory effect towards TNF-α production and NFκB transcriptional activity. The introduction of various amino groups to the keto beta position could improve the biochemical profile, resulting in the identification of more potent derivates, such as **1f**, with higher inhibitory activity than both matrine and sophoramine.

Sophora alkaloids, isolated from *Sophora flavescens* Ait. (Kushen), have been found to possess a variety of pharmacological effects, including anti-inflammation and immunity-regulation activity.[1–5] The main active components are matrine, sophocarpine, and oxymatrine, whose structures are shown in Figure 1.[6] Our previous studies[7–9] have found that matrine significantly inhibited the production of pro-inflammatory cytokines (TNF-α, IL-1, or IL-6) in lipopolysaccharide (LPS) stimulated murine peritoneal macrophages and Kupffer cells as well as in several animal inflammation models. Other groups also reported that matrine and oxymatrine exerted anti-inflammation effects through inhibiting nuclear factor-kappa B (NFκB) activation.[10,11]

Considering the good pharmacological effects of matrine, we are interested in developing a general and practical strategy for the preparation of matrine derivates for structure–activity relationship studies and for the exploration of new anti-inflammation drugs. To explore the synthetic strategy, sophocarpine is a suitable starting material because it contains an α,β-unsaturated carbonyl group that is reactive toward a variety of useful nucleophiles. Therefore, in the present study, matrine derivatives **1a–j** and **5** were designed and synthesized by using sophocarpine (Fig. 2), and their influence on TNF-α production and NFκB transcriptional activity in RAW264.7 cells were evaluated to investigate the structure–activity relationship.

The synthesis of **1a–j**, as shown in Scheme 1, started from sophocarpine **2**. First, **2** was transformed into **3** in high yield by treatment with Lawesson's reagent.[12] The product was characterized by ^{1}H NMR, ^{13}C NMR and MS. Then, reaction of **3** with different amines afforded the target compounds **1a–j** in almost quantitative yields.[13] All the derivatives were identified by ^{1}H NMR, ^{13}C NMR, and MS.[14]

Furthermore, to verify the influence of the sulfur atom on biological activity of the derivatives, compound **5** was synthesized in two steps according to Tinarelli's method.[15] As shown in Scheme 2, treating **2** with azidotrimethylsilane, AcOH, and DBU

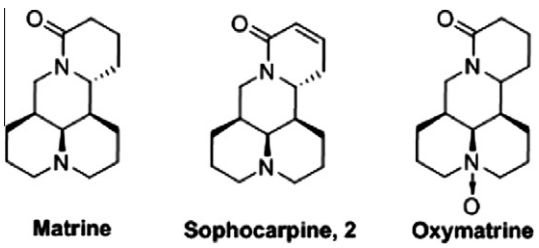

Figure 1. Structures of sophora alkaloids.

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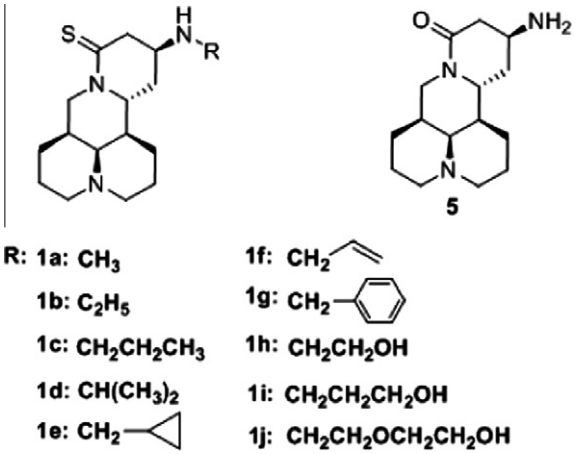
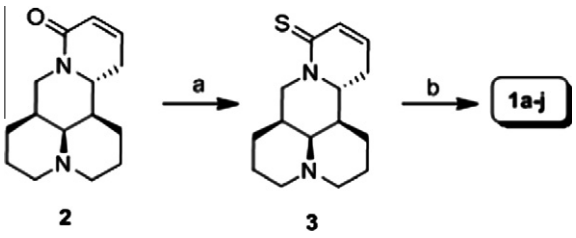


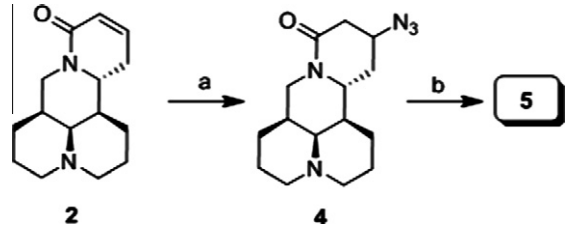
Figure 2. Target matrine derivatives.



Scheme 1. Synthesis of **1a–j**. Reagents and conditions: (a) Lawesson's reagent, toluene, reflux, 2 h, in 85% yield; (b) RNH_2, Et_3N, CH_2Cl_2–MeOH (1:1), rt, overnight.

in anhydrous toluene gave **4** in good yield, which was followed by reduction of the azide group by catalytic hydrogenation to afford **5** in excellent yield (95%). Both **4** and **5** were characterized by ^1H NMR, ^{13}C NMR, and MS.[16]

The comparison of inhibitory effects of sophora alkaloids and compounds **1a–j** on TNF-α production by LPS-stimulated macrophages were determined by the method described in our previous report.[9] RAW264.7 cells (5×10^5 cells/ml) were incubated with LPS (Sigma, St. Louis, MO, USA) (1 μg/ml) for 6 h in the presence or absence of matrine, sophocarpine, **5**, and **1a–j**. The culture supernatants were collected, and the concentrations of TNF-α were measured by a commercially available ELISA kit (eBioscience, California, CA, USA). Data was analyzed by logarithm fit to generate a dose–response curve using CurveExpert 1.3 Software (Daniel J. Hyams, Hixson, TN). The calculated IC_{50} value is the concentration of the test compound that caused a 50% decrease in the maximal TNF-α production. The results are summarized in Table 1. From the results, TNF-α production by LPS-stimulated RAW264.7 cells were significantly inhibited by matrine derivatives in a concentration-dependent manner. These inhibitory effects were not attributed to their unspecific cytotoxic effects (data not shown).



Scheme 2. Synthesis of **5**. Reagents and conditions: (a) Me_3SiN_3, PhMe, AcOH, DBU, 0 °C to rt, 24 h, in 95% yield; (b) H_2, 10% Pd/C, MeOH, rt, 2 h, in 98% yield.

Table 1
Effects of sophora alkaloids and derivatives on TNF-α production by LPS-stimulated RAW264.7 cells

Treatment	TNF activity	
	Inhibition (%, 10 μM)	IC_{50} (μM)
Matrine	—	>200
Sophoramine	—	>200
5	—	>200
1a	9.1	93.2
1b	41.5	23.5
1c	9.6	93.5
1d	23.99	52.3
1e	16.17	119.4
1f	52.5	9.4
1g	33.9	62.0
1h	29.3	39.2
1i	22.8	53.8
1j	8.4	57.5

Compound **1f** showed the strongest inhibitory effect with an IC_{50} value of 9.4 μM. It was clear that introduction of small substituents to the amine group could improve the biochemical profile. Moreover, the sulfur atom had good contribution to the inhibitory effect.

The mis-regulation of the nuclear factor-kappa B (NFκB) signal pathway is involved in a variety of inflammatory diseases that leads to the production of inflammatory mediators.[17] To further determine the possible molecular mechanisms involved in the action of sophora alkaloids on TNF-α production, we assessed the effects of sophora alkaloids on NFκB transcriptional activity in LPS-stimulated RAW264.7 cells using a reporter gene assay.[18] RAW264.7 cells (2×10^5) co-transfected for 24 h with the mixture of pGL3.5XκB-luciferase, and pRL-TK-Renilla-luciferase were pretreated with sophora alkaloids (100 μM) for 30 min and then stimulated with LPS (1 μg/ml) for 6 h. NFκB luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Data are normalized for transfection efficiency by dividing firefly luciferase activity with that of Renilla luciferase. As shown in Table 2, sophora alkaloids significantly inhibited NFκB transcriptional activity. The inhibitory effects of the derivatives with liner substituents on amine group were better than those with branched and ring substituents. Compound **1f** which contained an allylamino group was identified as the most potent inhibitor.

In summary, a highly efficient and versatile synthetic method was developed for the synthesis of matrine derivatives. Biological studies of the synthetic compounds suggest that introducing small

Table 2
Effects of sophora alkaloids on NFκB transcriptional activity in LPS-stimulated RAW264.7 cells

Treatment	Relative NFκB activity (NFκB/TK, fold)
Control	1.18 ± 0.37
LPS	11.26 ± 2.52**
Matrine	5.11 ± 1.24##
Sophoramine	3.47 ± 0.47##
5	6.54 ± 0.06##
1a	3.69 ± 1.07##
1b	1.96 ± 0.53##
1c	3.40 ± 0.16##
1d	6.56 ± 0.47##
1e	7.83 ± 1.38##
1f	1.47 ± 0.33##
1g	8.39 ± 3.89##
1h	4.85 ± 1.97##
1i	3.14 ± 1.90##
1j	4.75 ± 1.23##

$N = 3$.
** $P < 0.01$ versus control.
$P < 0.01$ versus LPS.

substituents at the 13-position of matrine had a significant impact on its anti-inflammatory activity. This discovery, as well as further studies on the structures and activities of matrine derivatives which should be significantly facilitated by the method presented here, can be generally useful for understanding the functions of substituent replacements in matrine and for the development of new anti-inflammation agents.

Acknowledgment

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14. *Representative analytical data for compound* **1a**: yield 92.3%, ^{1}H NMR (600 MHz, $CDCl_3$, TMS): δ 5.46 (dd, 1H, J = 12.0 Hz, 3.6 Hz), 4.29 (m, 1H), 3.55 (t, 1H, J = 12.6 Hz), 3.26 (m, 1H), 3.00 (m, 1H), 2.89–2.78 (m, 3H), 2.46 (s, 3H), 2.18 (m, 1H), 2.08–1.86 (m, 7H), 1.80–1.68 (m, 2H), 1.67–1.52 (m, 3H), 1.51–1.39 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.42, 63.44, 56.86, 56.84, 55.42, 50.74, 49.30, 47.79, 42.62, 35.52, 33.52, 30.31, 27.54, 26.44, 21.02, 20.49. HR-Q-TOF-MS, calcd for $C_{16}H_{28}N_3S^+$, M+H$^+$, 294.1998; found, 294.2003.
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16. *Compound* **5**: ^{1}H NMR (600 MHz, DMSO-d_6, TMS): δ 8.50–7.80 (m, 2H), 4.11 (dd, 1H, J = 12.0 Hz, 4.2H), 3.96–3.89 (m, 1H), 3.53–3.45 (m, 1H), 3.01 (t, 1H, J = 12.0 Hz), 2.80–2.70 (m, 2H), 2.62–2.57 (m, 1H), 2.40–2.35 (m, 1H), 2.15–1.96 (m, 3H), 1.95–1.82 (m, 3H), 1.66–1.52 (m, 2H), 1.50–1.26 (m, 5H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.40, 63.23, 56.58, 56.48, 49.62, 42.19, 41.46, 40.77, 36.03, 35.09, 27.71, 27.31, 26.06, 20.66, 19.98. HR-Q-TOF-MS, calcd for $C_{15}H_{26}N_3O^+$, M+H$^+$, 264.2070; found, 264.2079.
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