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Synthesis, characterization and in vitro anti-tumor activities of matrine derivatives

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ABSTRACT

Nineteen previously unreported matrine derivatives were synthesized and characterized using elemental analysis, infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, and mass spectrometry. Target compounds **6a–6l** and **7a–7c** showed stronger inhibitory activities than matrine in the in vitro antitumor tests and inhibited the growth of the Hep7402, B16-F10, A549, and TW03 cell lines. In addition, compound **6i** exhibited a potent antitumor activity similar to that of colchicine.

Matrine (1) is one of the active components isolated from the traditional medicinal herb—*Sophora flavescens*[1] and has attracted considerable attention because of its broad biological activities, such as anti-tumor,[2] anti-inflammation,[3] anti-nociceptive[4] effects. In China, matrine injection is clinically used to treat hepatitis and matrine suppository cures colpitis and chronic cervicitis. However, up to now, matrine has not been become an anticancer drug because of its moderate anti-tumor activities. Some scientists focused on the structure modification of matrine and synthesized a series of derivatives and evaluated their biological activities,[5–8] but they did not find any compound which could become a drug candidate.

According to the moderate anti-tumor activities and many modification points of matrine, we designed, synthesized and characterized 19 matrine derivatives. The synthetic pathway of compounds **3a**, **3b,** and **4** are shown in Scheme 1. Matrine was hydrolyzed with sodium hydroxide to produce acid **2**, which was transformed to acyl chloride using sulfoxide chloride and then reacted with methanol/ethanol to produce **3a** and **3b**. Compound **3a** was aminated with ammonia and calcium chloride to produce compound **4**.

Matrine is a stable, saturated six-membered ring lactam that is not easily hydrolyzed. Two hydrolysis methods, namely, the inorganic acid and base methods, can be used to break the amide bond of matrine.[6,9] The yield of acid hydrolysis is very low, and the product easily forms a hydrochloric salt, thereby increasing the difficulty of the post process. Compared with the acid method, the alkaline hydrolysis method is simple and more convenient for post-processing. Therefore, the alkaline hydrolysis method was used to obtain intermediate **2** from matrine.

In the synthesis of compounds **3a** and **3b**, we tried to use the conventional sulfuric acid catalysis method; however, the yield was very low, possibly due to the tertiary amine structure in the matrinic acid that induced the formation of the sulfate during the reaction and reduced the effect of acid catalysis. In acidic conditions, matrinic acid also very easily redehydrates and reverts back to matrine. After the experimental exploration, thionyl chloride was added to the alcohol to form ethyl chlorosulfate, and the alcohol solution of the matrinic acid was added to produce compounds **3a** and **3b** with relatively high yields and purities.

Two methods were used to synthesize compound **4**. The first is the reaction between **3a** and ammonia at 130 °C in a microwave synthesizer for 15 min, and the other is the reaction between **3a** and an calcium chloride–ammonia system at 80 °C for 18 h.[10,11] A comparison of the two experimental methods shows that in the first approach, a portion of compound **3a** was recycled to produce matrine and several impurities were also produced. On the other hand, the conditions of the second method are milder, and the yield is relatively higher than that of the first method. Therefore, the second method was used to synthesize compound **4**.

Matrine was reduced using lithium aluminum hydride under an anhydrous condition to produce compound **5** (Scheme 2). Sodium

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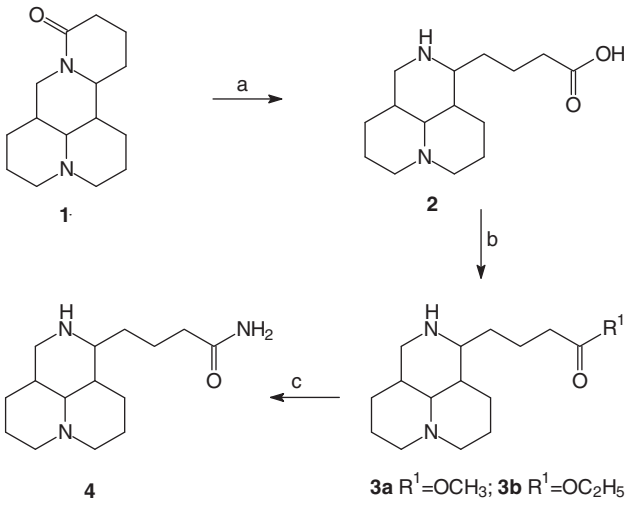
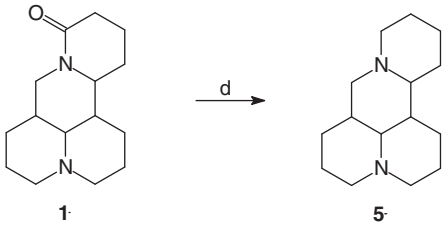
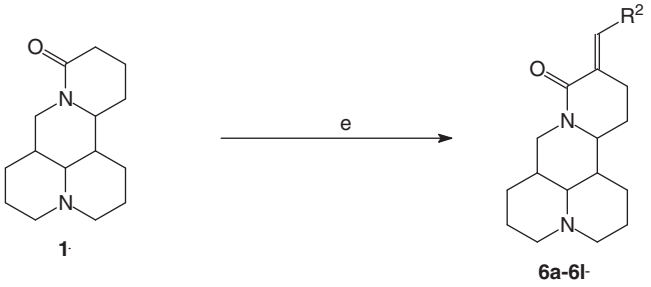


Scheme 1. Synthesis of matrine derivatives **3a**, **3b** and **4**. Reagents and conditions: (a) 3 equiv $NaOH/H_2O$, 90 °C, 12 h; (b) $SOCl_2$, CH_3OH, or C_2H_5OH, reflux, 5 h; and (c) $NH_4OH/CaCl_2$, 80 °C, 18 h.



Scheme 2. Synthesis of matrine derivative **5**. Reagents and conditions: (d) $LiAlH_4$, ether, reflux, 10 h.

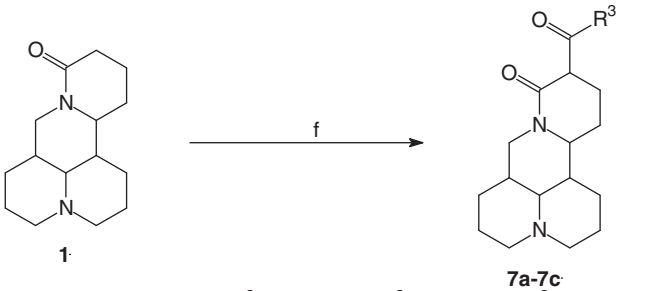


6a: R^2=phenyl; **6b:** R^2=4-nitrophenyl; **6c:** R^2=3-nitrophenyl; **6d:** R^2=4-flourophenyl; **6e:** R^2=4-chlorophenyl; **6f:** R^2=4-bromophenyl; **6g:** R^2=3-flourophenyl; **6h:** R^2=3,4-dichlorophenyl; **6i:** R^2=4-methoxyphenyl; **6j:** R^2=3-methoxyphenyl; **6k:** R^2=3,4,5-trimethoxyphenyl; **6l:** R^2=4-pyridyl

Scheme 3. Synthesis of matrine derivatives **6a**–**6l**. Reagents and conditions: (e) 5 equiv aromatic aldehyde, NaH, THF, reflux, 5 h, 25–35%.

borohydride was chosen as the reducing agent, but the yield was extremely low. Lithium aluminum hydride[12,13] was then used to replace sodium borohydride in the reduction reaction to obtain a good yield of compound **5**.

Matrine reacted with sodium hydride and then with aromatic aldehyde under an anhydrous condition to produce compounds **6a**–**6l**[14] (Scheme 3). Previous studies[15,16] reported that lithium diisopropylamide is usually used in the alkylation of amides at α position; however, the reaction must occur under an anhydrous



Scheme 4. Synthesis of matrine derivatives **7a**–**7c**. Reagents and conditions: (f) 5 equiv aromatic ester, NaH, THF, reflux, 5 h, 19–24%.

Table 1
Inhibition of Hep7402, B16-F10, A549, and TW03 cell proliferation by the target compounds

Compounds	IC_{50} ± SD (μg/mL)			
	Hep7402	B16-F10	A549	TW03
Colchicine	0.4 ± 0.1	0.8 ± 0.04	0.2 ± 0.03	1.3 ± 0.4
Matrine	15.3 ± 0.3	17.0 ± 0.5	9.7 ± 1.1	16.3 ± 0.2
2	NA	NA	NA	NA
3a	NA	NA	31.4 ± 5.2	NA
3b	NA	NA	32.3 ± 3.6	NA
4	11.4 ± 4.3	12.9 ± 6.8	18.6 ± 1.3	21.7 ± 0.8
5	NA	NA	NA	NA
6a	10.0 ± 3.9	5.7 ± 2.4	4.6 ± 5.2	2.4 ± 1.6
6b	8.7 ± 3.1	2.8 ± 2.6	6.2 ± 4.2	3.7 ± 2.3
6c	5.3 ± 4.5	8.5 ± 3.7	7.6 ± 3.3	2.4 ± 1.8
6d	1.8 ± 0.5	2.5 ± 1.2	3.0 ± 1.7	1.9 ± 0.9
6e	1.6 ± 4.5	3.9 ± 0.2	2.6 ± 0.7	2.8 ± 7.1
6f	0.7 ± 1.4	2.6 ± 0.5	1.9 ± 1.1	3.2 ± 1.3
6g	1.5 ± 8.6	2.7 ± 4.8	3.4 ± 6.6	2.2 ± 4.5
6h	11.3 ± 2.7	6.5 ± 3.7	4.7 ± 0.3	6.3 ± 0.6
6i	0.5 ± 3.1	0.8 ± 2.2	0.6 ± 3.7	1.2 ± 5.9
6j	3.4 ± 5.5	8.7 ± 5.7	2.3 ± 0.4	6.4 ± 5.2
6k	7.8 ± 7.6	9.8 ± 8.5	3.8 ± 2.2	5.5 ± 3.4
6l	8.6 ± 2.7	6.2 ± 5.4	5.7 ± 4.8	4.4 ± 2.5
7a	4.3 ± 1.2	10.6 ± 2.1	3.6 ± 2.5	2.4 ± 1.8
7b	11.8 ± 3.8	12.9 ± 7.3	6.6 ± 6.5	4.9 ± 2.4
7c	7.5 ± 4.2	4.5 ± 7.3	2.3 ± 3.8	8.1 ± 4.5

NA: no activity.

condition and at −78 °C. By employing some methods, sodium hydride was used as the strong base in the reaction, and good results were obtained.

Matrine was acylated with aromatic esters in the presence of sodium hydride to obtain compounds **7a**–**7c** (Scheme 4). First, aromatic acids were transformed into methyl esters, which reacted with matrine under the same conditions used in the synthesis of compound **6a** to obtain the target products.

The anti-proliferative activities of all synthesized compounds against human hepatoma Hep7402 cells, melanoma B16-F10 cells, human non-small cell lung cancer A549 cells, and human nasopharyngeal carcinoma TW03 cells were evaluated; matrine and colchicine were used as the positive control groups.[17] The results are summarized in Table 1.

The anti-proliferative activities of compounds **6a**–**6l** and **7a**–**7c** against HepG2, B16-F10, and A549 cells are generally much stronger than that of matrine, possibly because the substituted groups in the matrine derivatives increased the oil-solubility of the derivatives and enhanced their interaction with the tumor cells. Of these compounds, the halophenyl derivatives **6d**–**6g** showed almost similar moderate anti-proliferative activities, with IC_{50} values 2- to 10-fold higher than that of matrine. In addition, the halogen groups

affected the anti-proliferative activities of compounds **6d**–**6g**. The methoxyphenyl analog **6i**[18,19] exhibited the most potent inhibitory activity (IC_{50} = 0.5 μg/mL for Hep7402, 0.8 μg/mL for B16-F10, 0.6 μg/mL for A549, and 1.2 μg/mL for TW03) compared with the positive control colchicine. The structure–activity relationships of the target compounds demonstrate that the amido bond is necessary for the anti-tumor activities of matrine. When this bond is broken, the anti-proliferative activities are lost. For example, compounds **2**, **3a**, and **3b** showed no activities. Compound **6i** was significantly more active than compounds **6b** and **6c**, in which the electron-withdrawing nitro group of the benzene ring changed the electron cloud density distribution of **6b** and **6c**, resulting in a weak interaction with the tumor cells. Compound **6j** was less active than compound **6i**; the only difference between them is the position of the methoxy group, which is in the *m*-position for **6j** and in the *p*-position for **6i**.

In conclusion, a series of matrine derivatives has been successfully synthesized and characterized via infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, mass spectrometry, and elemental analysis. The results of the in vitro antitumor tests show that 15 of the 19 matrine derivatives exhibited good activities against cancer cells, suggesting that one of the synthesized matrine derivatives, such as compound **6i**, can be used in the treatment of certain drug-resistant cancers. The results of the present study provide useful information for further structural modifications of these compounds and for the synthesis of new, potent antitumor agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.069.

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18. *Preparation for compound **6i**:* Anhydrous tetrahydrofuran (50 mL) was added into a round-bottomed flask (100 ml) containing matrine (0.005 mol) and sodium hydride (0.1 mol). The solution was stirred and 4-methoxybenzaldehyde (0.025 mol) was added at room temperature. The solution was then refluxed for 8 h. After cooling to room temperature, the mixture was treated with hydrochloric acid (5%, 20 mL) to hydrolyze the excess sodium hydride and then extracted with chloroform (20 mL × 3). The combined organic layer was concentrated, and the residue was purified in a reverse-phase silica gel column (petroleum ether/ethyl acetate = 1:5, v/v) to give the compound.
19. *Analytical data for key compound **6i**:* Yield 27%, yellow liquid, IR(KBr, νcm^{-1}): 3058.16, 2930.25, 2812.32, 2751.42, 1608.61, 1510.66, 1478.00, 1453.13; ^1H NMR (DMSO, δ ppm): 7.66 (s, 1H), 7.41 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 4.49 (dd, *J* = 4.2 Hz, 4.2 Hz, 1H), 3.94 (s,1H), 3.80 (s, 3H), 3.20 (m, 1H), 2.84 (m, 3H), 2.28 (m, 1H), 2.06 (m, 1H), 1.80 (m, 2H), 1.76–1.64 (m, 3H), 1.53–1.20 (m, 9H); MS (EI, *m*/*z*): 366(M^+), 323, 245, 177, 150, 135, 96, 41. Elemental anal. Calcd: C, 75.37; H, 8.25; N, 7.64. Found: C, 75.53; H, 8.36; N, 7.83.