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Alkaloids from *Oxytropis ochrocephala* and Antiproliferative Activity of Sophoridine Derivatives Against Cancer Cell Lines

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Abstract

Ten alkaloids (**1**−**10**), with sophoridine (**1**) as the most abundant component, were obtained from the whole plants of *Oxytropis ochrocephala* Bunge. Furthermore, eight new sophoridine derivatives (**11**−**16, 20, 21**), with modification on the C-14 position of **1** were synthesized. All compounds (**1**−**16, 20, 21**) were evaluated for antiproliferative activity against five human tumor cell lines. Among them, the newly synthesized derivative **20** exhibited the best inhibitory activity against the tested cell lines. Its activity was increased by more than fourfold as compared with parent compound **1**.

Graphical Abstract

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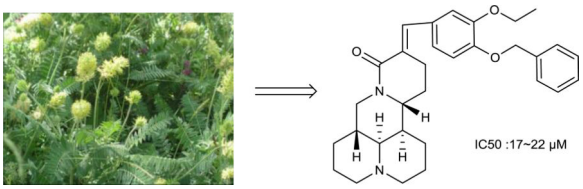
[‖]These authors contributed equally to this work

Supplementary Material

Supplementary data (experimental details and compound characterization for all synthesized compounds) associated with this article can be found, in the online version, at



Keywords

Oxytropis ochrocephala; Poisonous plant; Antiproliferative; Sophoridine derivatives

Locoweed is a common name for specific plants poisonous to livestock and belonging to the genera *Oxytropis* and A*stragalus*, which are widely distributed in Eastern and Central Asia, Western North and South America, and Australia[1–2]. Livestock ingesting locoweed can develop a chronic neurological disease, characterized by a staggering gait, and muscular incoordination, giving rise to the vivid name 'locoweed' for these poisonous plants.[2–3]. Today, locoweed is a main threat to rangelands worldwide[1]. In China, locoweeds cover over 11 m ha, amounting to 3.3% of the total western grassland[2].

The plant *Oxytropis ochrocephala* Bunge is one of the common locoweeds found on the western grassland in China[2, 4]. It spreads 3 m ha across Qinghai, Ningxia, Gansu and Xizang provinces of China. In recent years, due to overgrazing, salinization and damage from drought and rodents, this poisonous plant has grown very so rapidly and even become the dominant species in some places[2]. On the other hand, if the chemical constituents in this plant had a therapeutic use, harm to the grassland could be mitigated.

To date, some efforts have been made to study locoism and poisonous alkaloid constituents of *O. ochrocephala*. As a result, five alkaloids, including one indolizidine and four quinolizidine alkaloids, were isolated previously from this plant[5]. Quinolizidine alkaloids exhibit broad pharmacological effects, such as antibacterial, antipyrotic, antipyretic, antiarrhythmic, antiasthmatic, antiulcerative, antivirus and antineoplastic properties.[6–7] However, the antiproliferative activities of the quinolizidine alkaloids isolated from *O. ochrocephala* remain unclear.

As part of our ongoing research program on the identification of alkaloids from *Oxytropis ochrocephala*, ten known alkaloids (Figure 1), sophoridine (**1**), isosophoridine (**2**), matrine (**3**), sophoramine (**4**), 7,11-dehydromatrine (**5**), sophocarpine (**6**), lupanine (**7**), (+)-9α-hydroxymatrine (**8**), (−)-9α-hydroxysophocarpine (**9**) and swainonine (**10**)[8–15] were obtained from the whole plants of *Oxytropis ochrocephala*. Among them, compounds **1–9** are quinolizidine alkaloids, while compound **10** is an indolizidine alkaloid. Moreover, in our study, sophoridine (**1**, Figure 1), obtained in 5 gram quantity, provided an ideal starting material for further modifications. Compound **1** is one of three main chemical ingredients of Fufang Kushen injection, which was approved by the Chinese FDA (CFDA) in 1995 as an anticancer drug for treating non-small cell lung carcinoma, liver cancer, and gastric cancer in combination with other anticancer drugs.[16–19] While some synthetic derivatives of **1** with an open D ring have exhibited good antiproliferative activity[20–21], no research on the activity as well as structure-activity relationship (SAR) correlations of derivatives of **1** with four intact

rings has been reported. In the present study, eight new derivatives of **1** with various substituents on the C-14 position were synthesized.

With **1** as starting material, eight new sophoridine derivatives were synthesized. As shown in Scheme 1, aldol reaction of various aromatic aldehydes with **1** produced compounds **11−16** (Scheme 1). Addition of a benzyl or methoxymethyl group on OH-4 of **17** produced **18** and **19**, respectively (Scheme 2). Compounds **18** and **19** were further reacted with **1** to yield **20** and **21**, respectively.

The anti-proliferative activities of the natural alkaloids (**1–10**) and newly synthesized derivatives (**11−16, 20, 21**) against five tumor cell lines (A549, KB, KB-VIN, MDA-MB-231, MCF7) were evaluated by the sulforhodamine B (SRB) colorimetric assay. Paclitaxel was used as the positive control. The results are summarized in Table 1.

At 40 μM, none of the naturally occurring indolizidine and quinolizidine alkaloids (**1−10**) from *O. ochrocephala* exhibited inhibitory activity against the five tested human tumor cell lines. Compound **1** was inactive even at 80 μM. As compared with **1**, neither a conformation change at C-6 (**2**) or C-5 (**3**) nor the presence of unsaturation in the D or C ring (**4−6**) resulted in significant antiproliferative activity. However, among the eight newly synthesized derivatives with a substituted phenylmethylene group on C-14, some beneficial effects on activity were seen. Regarding the substitution pattern on the phenyl ring, 2-methyl-4-methoxy substituted **13** was less active than 3-methoxy-4-methyl substituted **12**. Notably, when the 3,4-substituted pattern was retained, with an ethoxy group at position-3 and a benzyloxy group at position-4, the resulting compound, **20**, showed significantly increased antiproliferative activity, with IC_{50} values around 20 μM against all five cancer cell lines, fourfold higher than those of **1**. Interestingly, a change from a benzyloxy (**20**) to methoxymethoxy (**21**) group on the same position of the phenyl ring did not lead to increased antiproliferative activity. Thus, in this limited data set, the benzyloxy moiety is crucial for activity. Notably, compound **20** was equipotent against KB and MDR-subline KB-VIN, suggesting that this compounds is not a P-gp substrate. Finally, compound **16** with a dimethylamino group at position-4 of the phenyl ring was not active indicating that this particular basic group is not good for antiproliferative activity.

Ten natural alkaloids from *O. ochrocephala* and eight synthetic sophoridine analogues were evaluated for antiproliferative activity. Among them, synthetic derivative **20** exhibited the best inhibitory activity against five human tumor cell lines. The activity results and SAR correlations of this compound series were reported. Our research produced fundamental information for further study and exploitation of the poisonous plant *O. ochrocephala*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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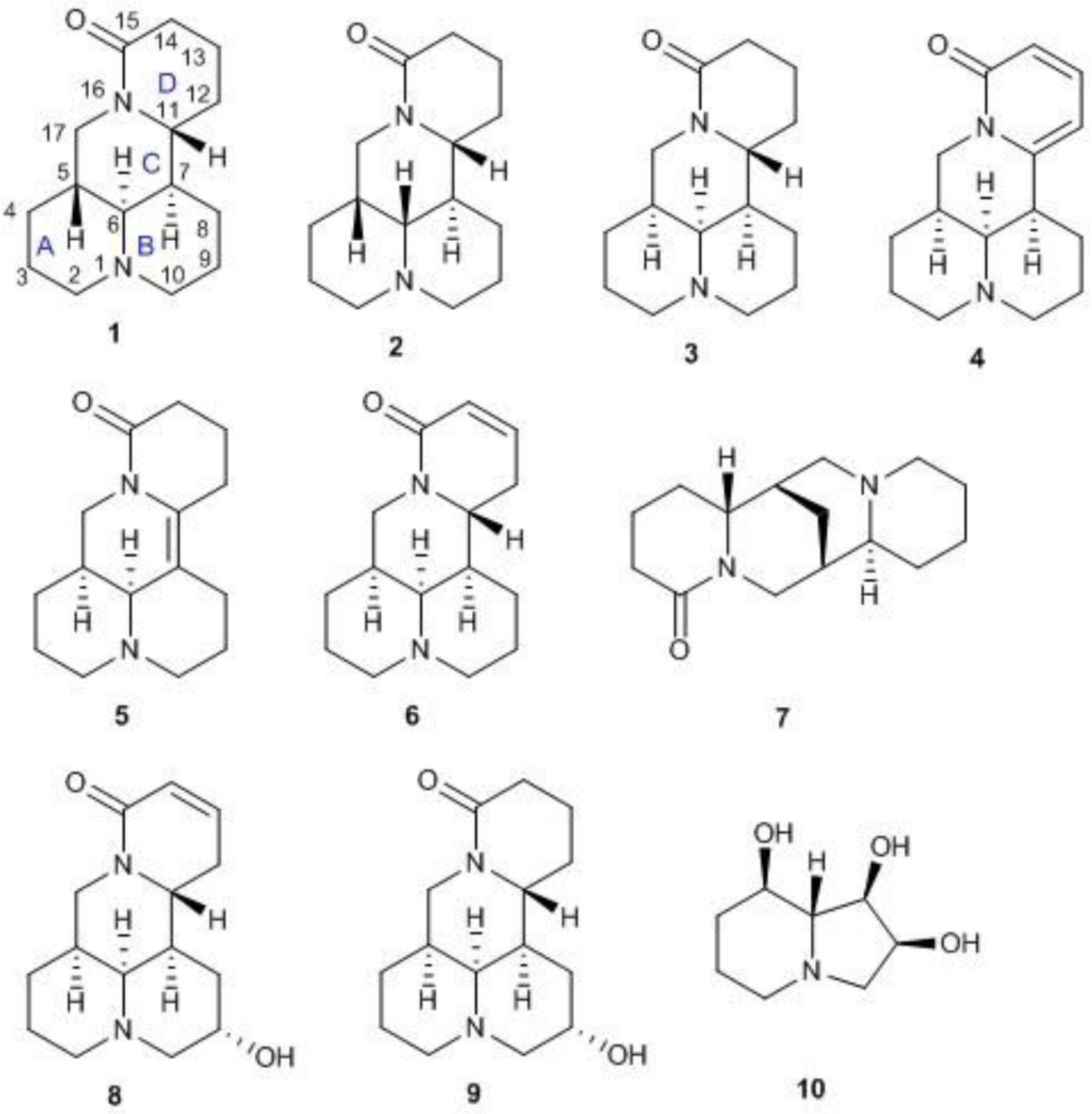
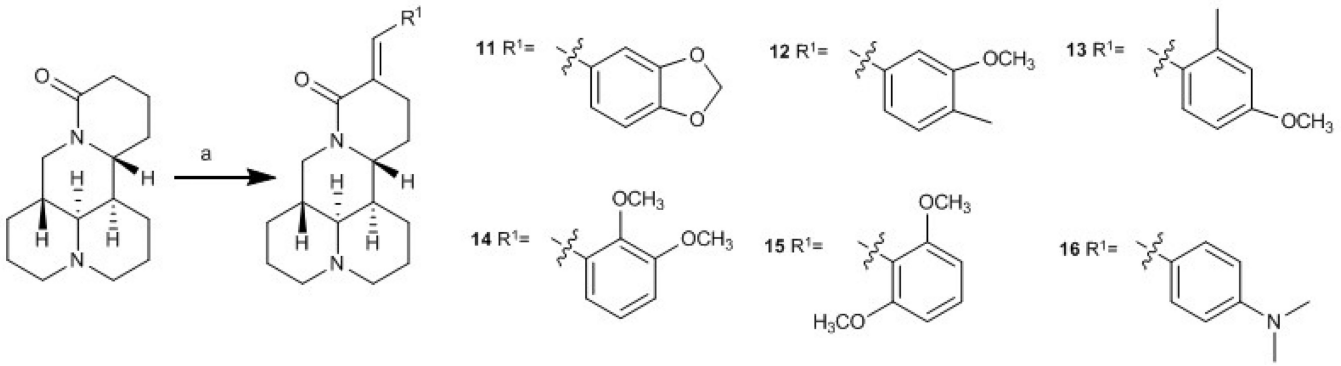
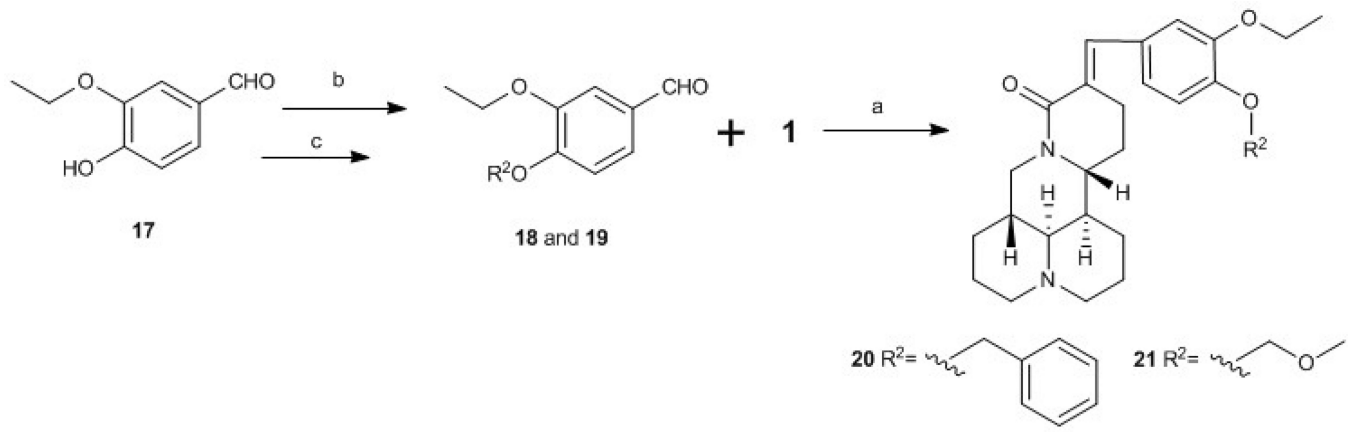


Figure 1.
The structures of alkaloids from *Oxytropis ochrocephala*



Scheme 1.

Synthesis of sophoridine derivations **11−16**. (a) Aromatic aldehyde, NaH, THF, reflux, 6 h

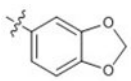
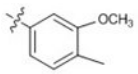
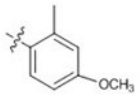
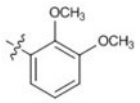
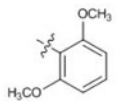
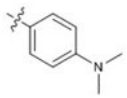
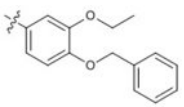


Scheme 2.

Synthesis of sophoridine derivations **20** and **21**. (b) BnBr, 10% K_2CO_3, rt; (c) MomCl, DIPEA, 0 °C to rt

Table 1

Antiproliferative Activity of Natural (**1–10**) and Synthetic (**11–16, 20, 21**) Alkaloids

Compd	IC_{50} (μM)					
	R_1	A549	KB	KB-VIN	MDA-MB-231	MCF7
1		>80	>80	>80	>80	>80
2–10		>40	>40	>40	>40	N/D[a)]
11		>40	>40	>40	>40	N/D
12		42 ± 0.94	58 ± 2.1	72 ± 1.6	45 ± 7.8	54 ± 21
13		74 ± 1.9	74 ± 4.0	>80	67 ± 1.7	>80
14		>80	71 ± 0.61	>80	68 ± 4.7	>80
15		>40	>40	>40	>40	N/D
16		>40	>40	>40	>40	N/D
20		17.6 ± 0.31	20.7 ± 0.56	21.6 ± 2.011	21.9 ± 0.54	20.5 ± 1.49

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Compd	R_1	IC_{50} (μM)				
		A549	KB	KB-VIN	MDA-MB-231	MCF7
21		>40	>40	>40	>40	N/D
PXL (nM)[b]		0.03 ± 0.03	0.04 ± 0.03	2600 ± 160	18 ± 3.2	3.9 ± 1.3

[a] N/D: not determined.

[b]: IC_{50} of paclitaxel (PXL) is represented in nM.