

## Cholinesterase Inhibitory Activities of Alkaloids from *Corydalis Tuber*<sup>†</sup>

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**Abstract** – Several isoquinoline alkaloids (**1 - 18**), which have basic chemical structures as protoberberine and aporphine skeletons, were evaluated for their inhibitory activities on AChE and BuChE. Among them, compounds **3**, **4**, **6**, **8** and **12** showed the potent AchE activity with the IC<sub>50</sub> values ranging from 10.2 ± 0.5 μM to 24.5 ± 1.6 μM, meanwhile, compound **14 - 17** exhibited strong inhibitory activity with IC<sub>50</sub> values from 2.1 ± 0.2 to 5.5 ± 0.3 μM. Compounds **14 - 17** exhibited selective inhibition for AChE compared with BuChE. The isoquinoline alkaloid possesses aromatic methylenedioxy groups and quaternary nitrogen atoms are crucial for the anti-cholinesterase inhibitory activity.

**Keywords** – *Corydalis turtschaninovii*, Papaveraceae, Isoquinoline alkaloids, Cholinesterase, Alzheimer's disease, Structures and activity relationship

### Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease with many cognitive and neuropsychiatric manifestations that result in progressive disability and eventual incapacitation. A decrease of acetylcholine in the brain of patients with AD appears to be a critical element in producing dementia (Becker *et al.*, 1988). Loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine. One approach is to inactivate acetylcholinesterase, the enzyme that cleaves synaptic acetylcholine and terminates neuronal signaling. Acetylcholinesterase (AChE) inhibitors increase the availability of acetylcholine in central cholinergic synapses and are the most promising currently available drugs for the treatment of AD (Giacobini, 1990, 2000). AChE inhibitors from general chemical classes such as physostigmine, tacrine, donepezil, galanthamine, huperzine A and heptylphysostigmine have been tested for the symptomatic treatment of AD. Although there have been a number of reports on the

designing and development of synthetic AChE inhibitors, that were necessary for those other studies, which have been reported the AChE inhibitors derived from medicinal plants (Oh *et al.*, 2004; Houghton *et al.*, 2006; Adersen *et al.*, 2007). In particular, tacrine, one of the most extensively studied AChE inhibitors, has hepatotoxic side effects because it also inhibits butyrylcholinesterase (BChE), an enzyme found in both plasma and brain (Thomsen *et al.*, 1991). A selective AChE inhibitor with fewer or less severe side effects may be of therapeutic utility in AD treatment.

Our previous study reported that a 70% ethanolic extract of *Corydalis turtschaninovii* Besser forma *yanhusuo* (Papaveraceae) exhibited significant AChE inhibitory activity. Several species of the genera *Corydalis* have been used in the treatment of memory dysfunction in folk medicine (Oh *et al.*, 2004; Houghton *et al.*, 2006). *C. turtschaninovii* have been used in traditional medicine for the treatment of gastric, duodenal ulcer, cardiac arrhythmia disease (Kamigauchi and Iwasa, 1994), rheumatism, dysmenorrheal (Tang and Eisenbrand, 1992), and also memory dysfunction (Oh *et al.*, 2004; Houghton *et al.*, 2006). It contains several pharmacologically important alkaloids (Ito *et al.*, 1990; Lee *et al.*, 2001). In this study,

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several isolated alkaloids from this plant were investigated with the regarding active principals and structural relationship in cholinesterase activity.

## Experimental

**General experimental procedures** – Melting points were measured by using an electrothermal apparatus. Optical rotation was determined on a JASCO DIP-100 KUY polarimeter. UV spectra were obtained with a Beckman Du-650 UV/VIS recording spectrophotometer. IR spectra were recorded on a Jasco Report-100 infrared spectrometer. Mass were carried out with a JEOL JMS-700 Mstation mass spectrometer.  $^1\text{H-NMR}$  (300 and 400 MHz) and  $^{13}\text{C-NMR}$  (75 and 100 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers. Two-dimensional (2D) NMR spectra ( $^1\text{H-}^1\text{H}$  COSY, HMQC, and HMBC) were recorded on a Bruker Avance 500 spectrometer. For column chromatography, silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) was used. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F<sub>254</sub> (0.25 mm, Merck) and RP-18 F254S (0.25 mm, Merck), and spot were detected under an UV light and by spraying with 10% H<sub>2</sub>SO<sub>4</sub>.

**Plant material** – The tuber of *C. turtschaninovii* was purchased at Yuseong oriental herbarium market in Daejeon, Korea on September 2006. The plant was botanically identified by Professor KiHwan Bae, College of Pharmacy, Chungnam National University where the voucher specimen was deposited (CNU-00124). The tuber (5 kg) were dried and extracted with 70% EtOH. The compounds (purity > 97%) were isolated and structurally identified by our previous report (Hung *et al.*, 2008). Compound **18**, magnoflorine, was selected from *Coptidis chinensis* due to its aporphine chemical structure with the expectation in the structure and activity relationship comparison purpose (Hung *et al.*, 2007).

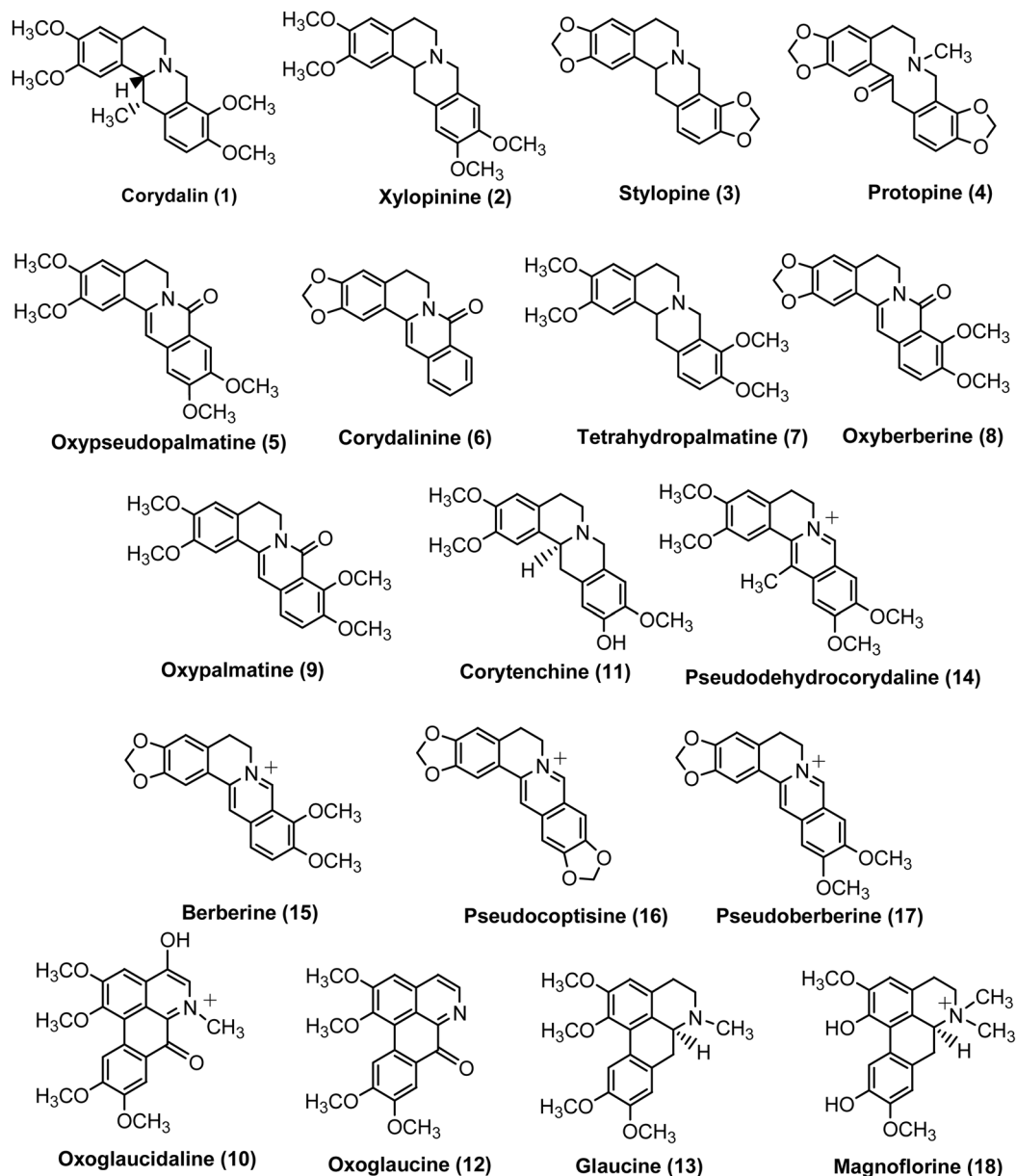
**Determination of cholinesterase activity** – Electric-eel AChE (EC 3.1.1.7) and horse-serum BChE (EC 3.1.1.8) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The inhibitory activities of the AChE and BuChE were determined by the modified method of Ellman 1961 (Ellman *et al.*, 1961). AChE and BuChE were diluted in phosphate buffer (pH 8.0, 100 mM) at 4.3 units/mL and 1.2 units/mL, respectively. The color reagent, DNTB, samples and authentic AChE and BuChE were added to a spectrophotometric cuvette and preincubated at 25 °C. Acetylthiocholine iodide and butylthiocholine iodide were added as substrates and incubation continued

for 5 min. AChE and BuChE activities were terminated by the addition of neostigmine or TPPA. Finally, the absorbance at 410 nm was measured.

## Results and Discussion

The isolated compounds were identified as corydalin (**1**), xylopinine (**2**), stylopinine (**3**), protopine (**4**), oxypseudopalmatine (**5**), corydaliline (**6**), tetrahydropalmatine (**7**), oxyberberine (**8**), oxypalmatine (**9**), corytenchine (**11**), pseudodehydrocorydalin (**14**), berberine (**15**), pseudocoptisine (**16**), and pseudoberberine (**17**), which have basic skeleton as protoberberine, meanwhile oxoglucodalin (**10**), oxoglucine (**12**), glaucine (**13**), and magnoflorine (**18**) have aporphine skeleton (Fig. 1) (Kim *et al.*, 1999; Halbsguth *et al.*, 2003; Kim *et al.*, 2004; Hung *et al.*, 2008). The inhibitory effects of isolated compounds in AChE (Type V-S) and BuChE (human serum) are presented in Table 1. In the AChE inhibitory effect, compounds **1**, **2**, **5**, **7**, **9** - **11**, **13** and **18** exhibits from weak to moderate inhibitory activity with the IC<sub>50</sub> values over than 25.1 ± 2.3 μM. Compounds **3**, **4**, **6**, **8** and **12** showed the potent activity for the inhibition of AChE with the IC<sub>50</sub> values ranging from 10.2 ± 0.3 μM to 24.5 ± 0.6 μM. Compound **14** - **17** exhibited strong inhibitory activity with IC<sub>50</sub> values from 2.1 ± 1.1 to 5.5 ± 1.7 μM, compared with tacrine and galanthamine, which were used as positive controls with IC<sub>50</sub> = 0.04 ± 0.001 and 1.8 ± 0.2 μM, respectively. To determine the selectivity of strong active compounds for AChE, compounds **1** - **4**, **6**, **8**, **12**, and **14** - **17** showed inhibitory activity on BuChE was assayed (Table 1). Compounds **14** - **17** exhibited selective inhibition for AChE compared with BuChE, with selectivities of 0.11, 0.04, 0.07, and 0.04, respectively. In this assay, tacrine, a well-known anti-cholinesterase agent, had low selectivity (5.0) with a stronger inhibitory activity on BuChE (IC<sub>50</sub> = 0.008 μM) than AChE in this assay system.

Based on the results, there were significant differences in the relationship between activity and chemical structures. Previously, the protoberberine alkaloids from *Corydalis* Tuber as corydaline, tetrahydropalmatine (THP), and protopine were assessed for their analgesic, hypnotic action (Kiriyakov *et al.*, 1982), and alleviate pain (Xu *et al.*, 2004; Halbsguth *et al.*, 2003). In those published studies, the introduction of same substitution patterns in the structures was demonstrated conclusively. The mechanisms by which those of alkaloids exert the effects are in somewhat understandable, even not so much clearly on case by case. Relate to our experiment, AChE



**Fig. 1.** Chemical structures of compounds 1 - 18.

is a substrate specific enzyme degrading neurotransmitter acetylcholine in the nerve synapses. As the active components, the protoberberine skeleton compounds from other *Corydalis* sp. plants also showed the effects on AChE in many previous studies. Corydaline, one of the tetrahydroberberine skeletal type, was the most active compound that inhibited AChE activity in a dose-dependent manner with  $IC_{50}$  value of  $15 \pm 3 \mu\text{M}$  (Miyazawa *et al.*, 1998) using a crude extract from the heads of *Drosophila melanogaster* as enzyme source. Corynoxidine, the same type, has been isolated from *C. speciosa*, was shown to display much weaker inhibition with an  $IC_{50}$

value of  $89.0 \mu\text{M}$  but it was considered inactive against BuChE due to  $IC_{50} > 100 \mu\text{M}$ . The protopine from *C. ternata* inhibited AChE activity with  $IC_{50}$  value as  $50 \mu\text{M}$  (Kim *et al.*, 1999). Quaternary protoberberines as palmatine and berberine isolated from *C. speciosa* showed  $IC_{50}$  values of  $5.8 \mu\text{M}$  and  $3.3 \mu\text{M}$ , respectively (Kim *et al.*, 2004), also berberine from *C. ternata* was expressed with an  $IC_{50}$  value of  $2.5 \mu\text{M}$  (Hwang *et al.*, 1996; Ulrichova *et al.*, 1983). Corynoline, another benzophenanthridine alkaloid isolated from *C. incise* inhibited AChE with an  $IC_{50}$  value of  $30.6 \mu\text{M}$  (Kim, 2002).

Studies concerning cholinesterase-inhibiting activity of

**Table 1.** Inhibition of compounds (**1 - 18**) on AChE (Type V-S) and BuChE (human serum)

Compounds	Inhibitory activity, IC <sub>50</sub> μM <sup>a)</sup>		Selectivity <sup>b)</sup>
	AChE	BuChE	
<b>1</b>	25.1 ± 2.3	108.7 ± 3.2	0.23
<b>2</b>	26.5 ± 2.4	92.0 ± 2.1	0.29
<b>3</b>	20.8 ± 0.6	95.6 ± 2.8	0.21
<b>4</b>	10.2 ± 0.3	26.8 ± 3.2	0.38
<b>5</b>	76.1 ± 0.7	-	-
<b>6</b>	20.3 ± 1.2	85.5 ± 2.7	-
<b>7</b>	52.4 ± 3.4	-	-
<b>8</b>	10.5 ± 1.0	117.5 ± 6.4	0.89
<b>9</b>	82.1 ± 3.5	-	-
<b>10</b>	48.5 ± 2.8	-	-
<b>11</b>	85.7 ± 1.5	-	-
<b>12</b>	24.5 ± 0.6	-	-
<b>13</b>	89.0 ± 0.8	-	-
<b>14</b>	5.5 ± 1.7	47.5 ± 2.1	0.11
<b>15</b>	2.7 ± 0.6	61.9 ± 3.1	0.04
<b>16</b>	2.1 ± 1.1	29.2 ± 4.1	0.07
<b>17</b>	2.5 ± 0.4	58.2 ± 5.5	0.04
<b>18</b>	48.6 ± 2.1	55.7 ± 1.4	-
Tacrine <sup>c</sup>	0.04 ± 0.001	0.008 ± 0.0001	5.0
Galanthamine <sup>c</sup>	1.8 ± 0.2	5.3 ± 0.4	3.39

a) Mean ± S.D.

b) AChE/BuChE

c) Reference controls

(-) not determined.

benzylisoquinoline alkaloids have shown that the most active alkaloids are found within compounds with a quaternary nitrogen atom that produced the strongest inhibitory activity on both enzymes (Ulrichova *et al.*, 1983). It was unequivocally demonstrated that the action of some compounds on BuChE was weaker than the action on AChE. In accordance with our results, some selected quaternary alkaloids as compounds **14** (pseudo-dehydrocorydaline), **15** (berberine), **16** (pseudocoptisine), and **17** (pseudoberberine) can manifest the strong ability to inhibit AChE. As the chemical structures viewed on Figure 1, although compounds **1**, **2**, **5**, **7**, **9 - 11** are belonging to protoberberine skeleton with tertiary nitrogen atom and have the structures of aromatic methoxyl and/or aromatic hydroxy, they are less active with IC<sub>50</sub> over than 30 μM. In those structures, there were no components appearing on C-2, C-3 and C-9, C-10, and C-11 as aromatic methylenedioxy groups. Thus, it is possible to note that no aromatic methylenedioxy group and no

quaternary atom of nitrogen were delayed the inhibitory activity. Interestingly, the active compounds were observed with aromatic methylenedioxy groups such as compound **3**, which possessed two methylenedioxy groups at C2, C-3 and C-9, C-10; compound **4**, which possessed two methylenedioxy groups at the same positions even though they do not content quaternary atom of nitrogen. The compounds having both of aromatic methylenedioxy groups and quaternary atom of nitrogen such as **15**, **16**, and **17** inhibited AChE potentially in the dose dependent way entirely. Specially, compound **14**, which possesses no aromatic methylenedioxy groups but containing quaternary nitrogen atom, was somewhat less active than other quaternary nitrogen atoms compounds (IC<sub>50</sub> = 5.5 μM vs IC<sub>50</sub> = 2.7, 2.1, and 2.5 μM). These results indicated that aromatic methylenedioxy groups and quaternary nitrogen atoms are important for the AChE inhibitory activity, however, the position of methylenedioxy group, whether it is at C-2 and C-3 (A ring), C-9 and C-10, C-10 and C-11 (D ring) seems not to be critical for the activity of protoberberine skeleton. Also by the same assay systems, some other compounds as **12**, **13** and **18** which have aporphine skeleton, can manifest inhibitory activity on AChE but not so much effective due to their IC<sub>50</sub> values (24.5, 89.0, and 48.6 μM). Thus, it can be insisted that aporphine skeleton was less effective than protoberberine skeleton in the inhibition of AChE. In addition, no components appearing on C-8 of C ring does not play an important role in AChE activity.

Together with previous results, our selected compounds can show inhibitory effects on AChE and BuChE, although they are less effective than tacrine and galanthamine using two other sources of enzyme. The difference sources of cholinesterase enzyme led to the difference of inhibitions due to the change in IC<sub>50</sub> values, this can be somewhat understood. And, this may suggest that these compounds might be acted with the enzymes in similar mechanisms but different interactions. Also the inhibitory are less than reference compounds, however, those compounds were purified from a natural medicinal plant, which have been used as a folk medicine for a long time. In addition, the precise active compounds for the AChE inhibitory are still interesting. It is likely that not only the major constituents as berberine, corydaline, protopine, glaucine, but also the other compounds as pseudoberberine, pseudocoptisine, oxoglucine, pseudo-dehydrocorydaline and others can be the active components in the plant. Thus, the investigation of further principles and the biochemical mechanisms of active components remain should be more elucidated.

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## References

- Adersen, A., Kjøbye, A., Dall, O., and Jøger A.K., Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from *Corydalis cava* Schweigg. & Kort. *J. Ethnopharmacol.* **113**, 179-182 (2007).
- Becker, R., Giacobini, E., Elble, R., McIlhany, M., and Sherman, K., Potential pharmacotherapy of Alzheimer disease. A comparison of various forms of physostigmine administration. *Acta Neurologica Scandinavica. Supplementum* **116**, 19-32 (1988).
- Ellman, G.L., Courtney, K.D., Andres Jr, V., and Featherstone, R.M., A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**, 88-90 (1961).
- Giacobini, E., The cholinergic system in Alzheimer disease. *Prog. Brain Res.* **84**, 321-332 (1990).
- Giacobini, E., Cholinesterase inhibitors: from the calabar bean to Alzheimer therapy. eds. by Giacobini, E., Dunitz, M., Thonex-Geneva, pp. 181-226 (2000).
- Halbgsuth, C., Meissner, O., and Haberlein H., Positive cooperation of protoberberine type 2 alkaloids from *Corydalis cava* on the GABA(A) binding site. *Planta Med.* **69**, 305-309 (2003).
- Houghton, P.J., Ren, Y., and Howes, M.J., Acetylcholinesterase inhibitors from plants and fungi. *Nat. Prod. Rep.* **23**, 181-199 (2006).
- Hung, T.M., Na, M., Dat, N.T., Ngoc, T.M., Youn, U., Kim, H.J., Min, B.S., Lee, J., and Bae, K., Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *J. Ethnopharmacol.* **119**, 74-80 (2008).
- Hung, T.M., Na, M., Min, B.S., Zhang, X., Lee, I., Ngoc, T.M., Thuong, P.T., Sok, D.E., and Bae, K., Protective effect of magnoflorine isolated from *Coptidis rhizoma* on Cu<sup>2+</sup>-induced oxidation of human low density lipoprotein. *Planta Med.* **73**, 1281-1284 (2007).
- Hwang, S.Y., Chang, Y.P., Byun, S.J., Jeon, M.H., and Kim, Y.C., An acetylcholinesterase inhibitor isolated from *Corydalis* tuber and its mode of action. *Kor. J. Pharm.* **27**, 91-95 (1996).
- Ito, C., Mizuno, T., Wu, T.S., and Furukawa, H., Alkaloids from *Corydalis*. *Phytochemistry* **29**, 2044-2045 (1990).
- Kamigauchi, M. and Iwasa, K., *Corydalis* spp. VI: In vitro culture and the biotransformation of protoberberines. Vol. 26., eds. by Bajaj, Y.P.S., Springer-Verlag, Berlin Heidelberg, pp. 93-105 (1994).
- Kim, D.K., Inhibitory effect of corynoline isolated from the aerial parts of *Corydalis incisa* on the acetylcholinesterase. *Arch. Pharm. Res.* **25**, 817-819 (2002).
- Kim, S.R., Hwang, S.Y., Jang, Y.P., Park, M.J., Markelonis, G.J., and Oh, T.H., Protopine from *Corydalis ternata* has anticholinesterase and anti-amnesic activities. *Planta Med.* **65**, 218-221 (1999).
- Kim, D.K., Lee, K.T., Baek, N.I., Kim, S.H., Park, H.W., Lim, J.P., Shin, T.Y., Eom, D.O., Yang, J.H., and Eun, J.S., Acetylcholinesterase inhibitors from the aerial parts of *Corydalis speciosa*. *Arch. Pharm. Res.* **27**, 1127-1131 (2004).
- Kiryakov, H.G., Iskrenova, E., Daskalova, E., Kuzmanov, B., and Evstatieva, L., Alkaloids of *Corydalis slivenensis*. *Planta Med.* **44**, 168-170 (1982).
- Lee, Y.L., Sagare, A.P., Lee, C.Y., Feng, H.T., Ko, Y.C., Shaw, J.F., and Tsay, H.S., Formation of protoberberine-type alkaloids by the tubers of somatic embryo-derived plants of *Corydalis yanhusuo*. *Planta Med.* **67**, 839-842 (2001).
- Miyazawa, M., Yoshio, K., Ishikawa, Y., and Kameoka, H., Insecticidal alkaloids from *Corydalis bulbosa* against *Drosophila melanogaster*. *J. Agric. Food Chem.* **46**, 1914-1919 (1998).
- Oh, M.H., Houghton, P.J., Whang, W.K., and Cho, J.H., Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. *Phytomedicine* **11**, 544-548 (2004).
- Tang, W. and Eisenbrand, G., *Corydalis turtschaninovii* Bess. f. *yanhusuo* Y.H. Chou et C.C. Hsu, Chinese drugs of plant origin, chemistry, pharmacology, and use in traditional and modern medicine, eds. by Tang, W., Eisenbrand, G., Springer-Verlag, Berlin Heidelberg, pp. 377-393 (1992).
- Thomsen, T., Zendeher, B., Fischer, J.P., and Kewitz, H. In vitro effects of various cholinesterase inhibitors on acetyl- and butyrylcholinesterase of healthy volunteers. *Biochem. Pharmacol.* **41**, 139-141 (1991).
- Ulrichová, J., Walterová, D., Preininger, V., Slavk, J., Lenfeld, J., and Cushman M., Inhibition of acetylcholinesterase activity by some isoquinoline alkaloids. *Planta Med.* **48**, 111-115 (1983).
- Xu, X.H., Yu, G.D., and Wang, Z.T., Resource investigation and quality evaluation on wild *Corydalis yanhusuo*. *Zhongguo Zhong Yao Za Zhi* **29**, 399-401 (2004).

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