Phytochemistry 149 (2018) 132–139

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Alkaloids with neuroprotective effects from the leaves of *Isatis indigotica* collected in the Anhui Province, China

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ARTICLE INFO

*Article history:*
Received 27 December 2017
Received in revised form
5 February 2018
Accepted 23 February 2018

*Keywords:*
*Isatis indigotica* fortune
Cruciferae
Alkaloids
Neuroprotective activity
Apoptosis

ABSTRACT

Six undescribed alkaloids, indiforine A-F, together with four known ones, were isolated from the leaves of *Isatis indigotica* Fortune. Their structures were elucidated on the basis of extensive spectroscopic analyses. The absolute configurations of indiforine A and B were determined by comparison of the experimental and calculated electronic circular dichroism spectra, as well as experimental and calculated optical rotations. The isolated alkaloids were evaluated for their neuroprotective activities against $H_2O_2$-induced cell injury in human neuroblastoma SH-SY5Y cells. The results showed that in $H_2O_2$-induced SH-SY5Y cell injury models, indiforine A and B exhibited potent neuroprotective activities. Further investigation of the most potent indiforine A by Hoechst 33258 staining and Annexin V/PI analysis demonstrated that it could protect SH-SY5Y cells from oxidative damage through inhibiting cell apoptosis.


## 1. Introduction

*Isatis indigotica* Fortune (Cruciferae) is a biennial herb widely distributed and cultivated in China (Yang et al., 2014). Its dried roots and leaves are named “Ban lan gen” and “Da qing ye”, respectively (Li et al., 2016). “Da qing ye” is an important raw material of *Indigo naturalis* (Qing dai), a dark blue powder with diverse effects used for thousands of years in China (Chang et al., 2015). It has been widely used in Traditional Chinese Medicine for the treatment of influenza, mumps, viral pneumonia and hepatitis (Liu et al., 2007). Previous phytochemical investigations of *I. indigotica* have led to the isolation of various natural compounds including alkaloids (Chen et al., 2012a, 2012b; Meng et al., 2017a; Wu et al., 1997), lignans (Li et al., 2015; Meng et al., 2017b), flavonoids and phenolic acids (Liu et al., 2006; Wang et al., 2013), among which alkaloids are the main constituents. These chemical constituents in *I. indigotica* have been reported to show diverse biological effects, such as antibacterial (Hu et al., 2010), antitumor (Wu et al., 2011), anti-inflammatory (Li et al., 2016), antiviral (Hsuan et al., 2009; Liu et al., 2015), and antioxidant activities (Zhao et al., 2017).

Oxidative stress results in can be viewed as an imbalance between the prooxidants and antioxidants and thus results in tissue damage in the body, has been implicated in etiology of many diseases including cancer, cardiovascular diseases, as well as neurodegenerative disorders (Bhat et al., 2015; Rahal et al., 2014). It is known that oxidative stress mediates the cell damage in several neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (Hu et al., 2015). Thus, pharmacological approach to resist oxidative stress is considered to be a promising therapeutic strategy. Hydrogen peroxide ($H_2O_2$) can induce apoptosis in many cells especially in neuronal cells, which is commonly used in studying neuronal cell death caused by oxidative stress (Sherer et al., 2002).

In order to search for compounds with potent neuroprotective activities from this plant, the chemical constituents of the leaves of *I. indigotica* were investigated, which resulted in the isolation of six undescribed alkaloids Indiforine A-F (**1**–**6**) together with four known ones (**7**–**10**) (Fig. 1). In this report, the isolation and

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https://doi.org/10.1016/j.phytochem.2018.02.016
0031-9422/

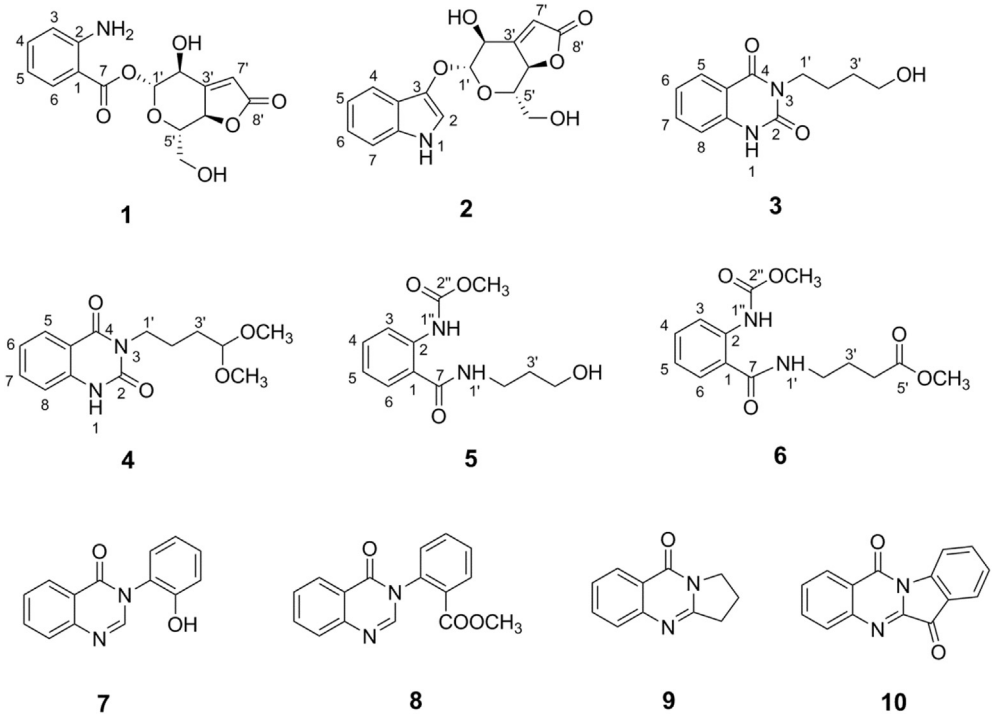

**Fig. 1.** The structures of compounds **1**–**10**.

structural characterization of all compounds and their neuroprotective effects on the human neuroblastoma SH-SY5Y cell injuries induced by $H_2O_2$ were described. The findings provide a preliminary indication of the neuroprotective activities of alkaloids in *I. indigotica* for the first time, laying a foundation for the application of this plant for treatment of neurodegenerative diseases to a certain degree.

## 2. Results and discussion

Compound **1** was obtained as a yellow oil. Its molecular formula was established as $C_{15}H_{15}NO_7$ by HRESIMS at *m/z* 344.0744 $[M + Na]^+$ (calcd for 344.0741), demonstrating nine degrees of unsaturation. The $^1$H-NMR spectrum of **1** (Table 1) indicated the presence of an *ortho*-disubstituted benzene ring at $\delta_H$ 7.83 (1H, d, $J = 7.5$ Hz, H-6), 7.31 (1H, t, $J = 7.5$ Hz, H-4), 6.81 (1H, d, $J = 7.5$ Hz, H-3) and 6.57 (1H, t, $J = 7.5$ Hz, H-5), two nitrogen-bearing protons at $\delta_H$ 6.74 (2H, s, H-2), one extra olefinic proton at $\delta_H$ 6.10 (1H, s, H-7′), four oxygenated methine groups at $\delta_H$ 5.54 (1H, d, $J = 7.4$ Hz, H-1′), 5.00 (1H, d, $J = 9.3$ Hz, H-4′), 4.67 (1H, d, $J = 7.4$ Hz, H-2′), 3.42 (1H, m, H-5′), an oxygenated methylene at $\delta_H$ 3.71 and 3.60 (each 1H, m), and two hydroxyl protons at $\delta_H$ 6.51 (1H, br s) and $\delta_H$ 5.13 (1H, br s). The $^{13}$C-NMR (Table 1) and HSQC spectra displayed 15 carbon signals, corresponding to the above described protons, as well as two additional carbonyl signals and one $sp^2$ quaternary carbon. The HMBC correlation from H-6 to C-7 ($\delta_C$ 165.7) indicated the linkage of the ester carbonyl to C-1 ($\delta_C$ 107.3), suggesting the presence of an *ortho*-aminobenzoyl in **1**, and C-1′ was linked to C-7 through an oxygen atom confirmed by correlation peak of H-1′ to C-7 ($\delta_C$ 165.7). The vicinal coupling correlations of H-1′/H-2′ and H-4′/H-5′/H-6′ in the $^1$H–$^1$H COSY and HMBC correlations from H-2′ to C-3′ ($\delta_C$ 171.5), H-7′ to C-4′ ($\delta_C$ 76.1), H-4′ to C-3′ ($\delta_C$ 171.5), C-5′ ($\delta_C$ 79.1) and C-6′ ($\delta_C$ 60.4) determined the rest of fragment of **1**, as shown in Fig. 2.

The NOESY correlations of H-1′ with H-5′, H-4′ with H-2′, H-6′, together with the coupling constant ($J_{H\text{-}4', H\text{-}5'}$) value of 9.3 Hz indicated that H-4′ and H-5′ were *trans*-orientated. H-1′ and H-2′ were also determined to be *trans*-orientated according to the coupling constant ($J_{H\text{-}1', H\text{-}2'}$) value of 7.4 Hz in combination with NOSEY correlation (Fig. 3). The absolute configuration was proposed by comparing the experimental circular dichroism spectrum with the ECD spectrum predicted from quantum mechanical time-dependent density functional theory (TDDFT) calculations (Li et al., 2010). The experimental CD spectrum of **1** was in agreement with the calculated ECD spectrum of 1′*R*, 2′*S*, 4′*R*, 5′*S*-isomer (Fig. 4). Moreover, the optical rotation of compound **1** was calculated at

**Table 1**
$^1$H NMR data (400 MHz) and $^{13}$C NMR data (100 MHz) for compounds **1** and **2** in DMSO-d6.

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_H$ (multi, $J$ in Hz) | $\delta_C$ | $\delta_H$ (multi, $J$ in Hz) | $\delta_C$ |
| 1 | | 107.3 | 10.67 s | |
| 2 | | 152.1 | 7.14 d (2.1) | 111.5 |
| 3 | 6.81 d (7.5) | 116.6 | | 136.7 |
| 3a | | | | 119.5 |
| 4 | 7.31 t (7.5) | 134.9 | 7.61 d (7.5) | 117.3 |
| 5 | 6.57 t (7.5) | 114.7 | 6.98 t (7.5) | 118.3 |
| 6 | 7.83 d (7.5) | 131.0 | 7.08 t (7.5) | 121.6 |
| 7 | | 165.7 | 7.31 d (7.5) | 111.6 |
| 7a | | | | 133.3 |
| 1′ | 5.54 d (7.4) | 96.4 | 4.69 d (7.1) | 106.3 |
| 2′ | 4.67 d (7.4) | 69.3 | 4.60 d (7.1) | 70.3 |
| 3′ | | 171.5 | | 172.2 |
| 4′ | 5.00 d (9.3) | 76.1 | 4.98 d (8.2) | 76.5 |
| 5′ | 3.42 m | 79.1 | 3.35 m | 78.5 |
| 6′ | 3.71 overlap | 60.4 | 3.76 m | 60.8 |
| | 3.60 m | | 3.63 m | |
| 7′ | 6.10 s | 112.8 | 6.07 s | 112.5 |
| 8′ | | 172.0 | | 172.2 |
| 2-NH | 6.74 s | | | |
| 2′–OH | 6.51 br s | | 6.60 br s | |
| 6′–OH | 5.13 br s | | 5.14 br s | |