Indigodole E from *Strobilanthes cusia* exhibits anti-IL-17A effect

Chia-Lin Lee, Chien-Ming Wang, Hung-Rong Yen, Ying-Chyi Song and Chao-Jung Chen

ABSTRACT

One new indazole alkaloid, indigodole E (1), was isolated from a traditional Chinese medicine Qing Dai prepared from the aerial parts of *Strobilanthes cusia*. The structure of 1 was elucidated by NMR, MS, UV, and IR spectra as well as optical rotation. Additionally, compound 1 could obviously inhibit not only IL-17A protein production at concentrations from 1.25 to 2.5 μg/mL, but also IL-17 gene expression at concentrations from 5.0 to 10.0 μg/mL without cytotoxicity toward Th17 and Jukat cells, respectively. Overall, indazole analogue 1 could be the anti-IL-17A contributor of Qing Dai in this investigation.

CONTACT Chia-Lin Lee chilee@mail.cmu.edu.tw

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2022.2041633.

© 2022 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

Indole alkaloids are a kind of natural compounds which were biosynthesized from the precursors of tryptophan or tryptamine and then metabolized to produce a large structural variety of indole analogues (Rosales et al. 2020). Numerous pharmacological properties, including anticancer, anti-inflammatory, antibacterial, antifungal, antiviral, antimalarial, antiparasitic, anti-acetylcholinesteras, and anti-butyrylcolinesterase activities have been reported for indole alkaloids (Rosales et al. 2020).

The aerial part of *Strobilanthes cusia* (Acanthaceae) owns indole compounds (Sun et al. 2021) and could be processed to manufacture Qing Dai used as a traditional Chinese medicine (TCM) in Asia (Lee et al. 2019). It was found to ameliorate inflammatory bowel disease and psoriasis in clinical studies (Naganuma 2019; Sun et al. 2021). Additionally, Qing Dai was also proved to reverse the IL-17A gene expression in human psoriatic skin lesions (Cheng et al. 2017), and our previous studies indicated that the indole alkaloids, indigodoles A, C, D, cephalandole B, tryptanthrin, and indirubin could contribute to anti-IL-17A properties of Qing Dai (Lee et al. 2019; 2020). Therefore, the active phytochemicals of aforementioned TCM are interesting for us. In this study, continuing on chromatographic fractionation of Qing Dai provided one new alkaloid (1) and its IL-17A inhibitory effect was also discussed within.

2. Results and discussion

HRESIMS of compound 1 (Figure 1) showed a [M + H]+ ion at m/z 286.0970, indicating a molecular formula of C18H11N3O with 15 degrees of unsaturation. The IR spectrum showed absorptions for carbonyl (1700 cm⁻¹) and aromatic (1598, 1579, 1493, 1467 cm⁻¹) functional groups. Eighteen carbon signals, including one methyl, eight methines, and nine quaternary carbons, were observed in 1D NMR spectra of 1 (Table S1). Among the nine quaternary carbons, one was identified as a carbonyl carbon on the basis of the chemical shift at δC 184.2. Therefore, the 13C NMR data supported the

![Figure 1. Structure of indigodole E (1).](image-url)
presence of one carbonyl, eight olefins ($\delta_C$ 118.2, 119.0, 119.5, 121.4, 122.5, 123.9, 124.5, 125.0, 128.5, 134.9, 136.5, 139.9, 140.9, 145.1, 158.7, 161.0), and six rings to fulfill the 15 degrees of unsaturation. 1D NMR and HSQC data also indicated the presence of two sets of $o$-disubstituted benzene rings, one at $\delta_H/\delta_C$ 7.47 (t, $J = 7.5$ Hz)/128.5, 7.80 (t, $J = 7.5$ Hz)/136.5, 7.89 (d, $J = 7.5$ Hz)/125.0, 8.87 (d, $J = 7.5$ Hz)/119.0 and the other at $\delta_H/\delta_C$ 7.28 (t, $J = 7.5$ Hz)/122.5, 7.73 (d, $J = 7.5$ Hz)/119.5, 7.66 (t, $J = 7.5$ Hz)/134.9 and 8.18 (d, $J = 7.5$ Hz)/123.9. In the HMBC spectrum, the methyl protons at $\delta$ 3.05 (3H, s) exhibited $^2J$ interaction with C-11 ($\delta$ 139.9), $^3J$ interaction with C-11a ($\delta$ 118.2), and small $^4J$ interaction with C-12 ($\delta$ 184.2), respectively (Figure S4). Additionally, the HMBC correlation of the methine proton H-1 ($\delta$ 7.89, d) showed $^3J$ interaction with the quaternary carbon at C-12 suggested that the methyl and carbonyl groups were positioned at C-11 and C-12, respectively. The last four rings were connected between the two sets of $o$-disubstituted benzene rings due to the HMBC correlations between H-7 ($\delta$ 8.18, d)/C-6 ($\delta$ 158.7) and H-1/C-12. These and other key HMBC connections are shown in Figure S8. Compound 1 was named as indigodole E (Figure 1).

In anti-IL-17 bioassay, primary mouse CD4+ T lymphocytes were obtained from BALB/c mice and then polarized into Th17 cells in order to investigate the anti-IL-17 effect of compound 1. Consequently, compound 1 could obviously inhibit IL-17A protein production of Th17 cells at concentrations from 1.25 to 2.5 $\mu$g/mL (Figure 9SB) without cytotoxicity (cell viabilities > approximately 80%; Figure 9SA). In the IL-17 luciferase reporter assay, compound 1 could inhibit the IL-17 gene expression at concentrations from 5.0 to 10.0 $\mu$g/mL in Jukat cells (immortalized T lymphocytes) which were transfected with IL-17 luciferase reporters (Figure 10). Our previous studies indicated that the indole alkaloids, indigodoles A, C, D, cephalandole B, tryptanthrin, and indirubin could contribute to anti-IL-17 properties of Qing Dai (Lee et al. 2019, 2020). However, compound 1 is an indazole analogue which has a five-membered pyrazole ring instead of the pyrrole ring in indole alkaloids. Indazole alkaloid 1 obtained in this work could be the anti-IL-17 candidate of Qing Dai as well.

3. Experimental

3.1. Plant material

The Qing Dai products processed from the aerial parts of Strobilanthes cusia were imported, identified, and analyzed (Lot. No. BR0308980) by Sheng Chang Pharmaceutical Co., Ltd., a GMP pharmaceutical factory, in Zhongli District, Taoyuan City, Taiwan, and then was offered for this study in March, 2016. A voucher specimen (IN 201603) was stored at the CMRDC, CMUH, Taiwan (Lee et al. 2019).

3.2. Extraction and isolation

A crude extract (INM, 175.2 g) was obtained from the Qing Dai dry powders (10.0 kg) by MeOH extraction (36 L x 4) at room temperature. The INM was liquid-liquid partitioned and further separated into the hexane- (INH, 74.8 g), 90% MeOH$_{aq}$- (INEA), n-BuOH-, and H$_2$O-soluble crude fractions, respectively. Both INH and INEA fractions
showed anti-IL-17A properties at the concentrations of 20 and 10 μg/mL, respectively (Lee et al. 2019). The active INH fraction was isolated and separated into fourteen sub-fractions (INH-A ~ INH-N) by an open column chromatography on silica gel (63–200 μm, column: 8.0 × 52.5 cm), using gradients of hexane–EtOAc–MeOH (10:1:0 to 0:1:1, v/v/v). Subfraction INH-F (2.2 g) was further isolated by silica chromatography (40–63 μm, column: 3.5 × 42 cm; hexane–CH₂Cl₂–EtOAc, 1:2:0 to 0:0:1, v/v/v) to give eleven subfractions. Subfraction INH-F-9 (108.9 mg) was subjected to silica gel chromatography (column: 3.5 × 39 cm; hexane–EtOAc, 5:1, v/v) to give INH-F-9-3 (5.06 mg) that was further purified by silica gel (column: 2.5 × 29 cm; CH₂Cl₂–MeOH, 1:1, v/v) and RP-18 gel (column: 3.5 × 22 cm; MeOH–H₂O, 80:20, v/v) chromatography to give compound 1 (1.2 mg) (silical TLC, R<sub>f</sub> = 0.57 in hexane–EtOAc, 3:1, v/v; RP-18 TLC, R<sub>f</sub> = 0.14 in MeOH–H₂O, 4:1, v/v; HPLC retention time shown in Figure S7).

3.3. Indigodole E (1)

Dark purple powders; [α]<sub>D</sub> <sub>22</sub> –127.0 (c 0.1, MeOH); UV (MeOH) λ<sub>max</sub> nm (log ε): 215 (4.11), 238 (3.99), 261 (4.00), 285 (3.90), 339 (3.71), 408 (3.83) (Figure S5); IR (neat) ν<sub>max</sub> cm<sup>−1</sup>: 2956, 2921, 2852, 1700, 1598, 1579, 1493, 1467, 1436, 1294, 1188, 1124, 1089, 982, 859, 804, 757 (Figure S6); For <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table S1; HRESIMS m/z 286.0970 [M + H]<sup>+</sup> (calcd for C₁₈H₁₂N₃O, 286.0975).

4. Conclusions

In this study, one new indazole alkaloid, indigodole E (1), was obtained from Qing Dai and showed inhibition against IL-17A protein production and gene expression. Consequently, compound 1 could be further researched to apply for IL-17A related diseases, such as psoriasis.

Supplementary materials

The detailed experimental procedures and all spectroscopic data of 1 are available online at https://doi.org/10.1080/14786419.2022.2041633.

Acknowledgements

The authors deeply appreciated to Sheng Chang Pharmaceutical Co., Ltd to offer authentic materials for this research. Mass spectrometric analyses were performed by the Proteomics Research Core Laboratory, Office of Research & Development at China Medical University, Taichung, Taiwan, R.O.C.

Disclosure statement

No potential conflict of interest was reported by the authors.
Funding
This work was financially supported by the “Chinese Medicine Research Center, China Medical University” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan (CMRC-CHM-1).

ORCID
Chia-Lin Lee http://orcid.org/0000-0002-5949-1989

References
Cheng HM, Wu YC, Wang Q, Song M, Wu J, Chen D, Li K, Wadman E, Kao ST, Li TC, et al. 2017. Clinical efficacy and IL-17 targeting mechanism of Indigo Naturalis as a topical agent in moderate psoriasis. BMC Complement Altern Med. 17(1):439.
Lee CL, Wang CM, Hu HC, Yen HR, Song YC, Yu SJ, Chen CJ, Li WC, Wu YC. 2019. Indole alkaloids indigodoles A-C from aerial parts of Strobilanthes cusia in the traditional Chinese medicine Qing Dai have anti-IL-17 properties. Phytochemistry. 162:39-46.
Lee CL, Wang CM, Kuo YH, Yen HR, Song YC, Chou YL, Chen CJ. 2020. IL-17A inhibitions of indole alkaloids from traditional Chinese medicine Qing Dai. J Ethnopharmacol. 255:112772.
Naganuma M. 2019. Treatment with indigo naturalis for inflammatory bowel disease and other immune diseases. Immunol Med. 42(1):16-21.
Rosales PF, Bordin GS, Gower AE, Moura S. 2020. Indole alkaloids: 2012 until now, highlighting the new chemical structures and biological activities. Fitoterapia. 143:104558.
Sun Q, Leng J, Tang L, Wang L, Fu C. 2021. A comprehensive review of the chemistry, pharmacokinetics, pharmacology, clinical applications, adverse events, and quality control of Indigo Naturalis. Front Pharmacol. 12:664022.