


Advances and perspectives in chemical and biological studies of *Baphicacanthus cusia*: a focus on antitumor constituents

Zhi-Hui Feng^{1#}, Shuang Jing^{1#}, Yu-Ping Shen^{2#}, Yu-Qing Tong³, Cheng-Jie Xiao³, Jian-Ping Xue¹, Hong Zhang¹, Qi-Zhang Li^{1*}  and Lei Zhang^{1,3,4*}

¹ Innovative Drug R&D Center, College of Life Sciences, Huaibei Normal University, Huaibei 235000, Anhui, China

² College of Chemistry and Bioengineering, Hunan University of Science and Engineering, Yongzhou 425199, China

³ State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China

⁴ Department of Pharmaceutical Botany, School of Pharmacy, Naval Medical University, Shanghai 200433, China

Authors contributed equally: Zhi-Hui Feng, Shuang Jing, Yu-Ping Shen

* Corresponding authors, E-mail: qizhang-li@hotmail.com; Leizhang100@163.com

Abstract

Baphicacanthus cusia (Nees) Bremek has a rich historical significance in China. Its stems and leaves have been used for traditional Chinese medicine or a dye called Qing-Dai in Chinese, while its roots are referred to as Nan-Ban-Lan-Gen in Chinese after processing. Both have been recognized and documented in the *Chinese Pharmacopoeia*. Modern pharmacological studies have revealed that *B. cusia* has numerous bioactive properties, such as antitumor, antiviral, anti-inflammatory, antioxidant, hepatoprotective, and neuroprotective properties. Moreover, *B. cusia* has been employed in clinical settings for its antitumor activity. The pivotal bioactive compounds in *B. cusia* are indirubin, indigo, and tryptanthrin. These potent substances and their derivatives demonstrate antitumor effects through inducing cell cycle arrest, triggering programmed cell death, inhibiting metastasis, and overcoming multidrug resistance. Nonetheless, the metabolic pathway of these compounds, particularly indole alkaloids, in *B. cusia* remains inadequately investigated. To increase the production of indole alkaloids such as indirubin and indigo, various biosynthetic approaches are employed. Consequently, this review discusses the bioactive compounds present in *B. cusia*, and elucidates their antitumor mechanisms, metabolism, and biosynthesis pathway. Existing studies indicate that *B. cusia* is a crucial medicinal plant harboring numerous biologically active substances, and its exploitation and application hold significant potential for substantial economic gains.

Citation: Feng ZH, Jing S, Shen YP, Tong YQ, Xiao CJ, et al. 2024. Advances and perspectives in chemical and biological studies of *Baphicacanthus cusia*: a focus on antitumor constituents. *Medicinal Plant Biology* 3: e001 <https://doi.org/10.48130/mpb-0024-0001>

Introduction

Baphicacanthus cusia (Nees) Bremek ('Ma-Lan' in Chinese; Fig. 1), a member of the genus *Baphicacanthus* with the family Acanthaceae, has a long history of medicinal use and recorded in some ancient books of traditional Chinese medicine (TCM), such as *Compendium of Materia Medica* (Bencao Gangmu in Chinese). *B. cusia* is widely distributed at 21° to 26° N latitude and 99° to 120° E longitude with an elevation below 1,000 m altitude. In China, the wild *B. cusia* is mainly distributed in Guangxi, Yunnan, Fujian, Guangdong, Guizhou, Hainan, and other places^[1]. In Fujian Province, Xianyou County is the main cultivation area^[2,3]. The Chinese medicine processed using the plant grown in this place is called 'Dao-Di' (in Chinese) medicinal materials, which indicates better quality. In some studies, it is believed that *Baphicacanthus cusia* (Nees) Bremek and *Strobilanthes cusia* (Nees) Kuntze are the same plant^[1,4,5]. Based on abundant evidence, Huang suggested that *Strobilanthes cusia* (Nees) Kuntze should be 'Ban-Lan' in Chinese, and *Baphicacanthus cusia* (Nees) Bremek should be 'Ma-Lan' in Chinese. The same issue involves *isatidis folium* ('Da-Qing-Ye' in Chinese)^[6,7]. Thus, normalizing the name is an issue that needs tackling. In the present paper, the studies of the plant named *B. cusia* are mainly discussed.

Based on the TCM theory, *B. cusia* has the activity to clear heat, detoxify, cool blood, and remove freckles. The whole

plant of *B. cusia* can be used as a source of medicine. The above-ground parts, including stems and leaves, are processed as medicine named indigo naturalis ('Qing-Dai' in Chinese), which is a kind of Daodi herb in Fujian and is known as 'Jian Qing-Dai' in Chinese. The below-ground parts, roots of *B. cusia*, is a source of medicine named 'Nan-Ban-Lan-Gen' in Chinese. Both are officially listed in the *Chinese Pharmacopoeia*. In TCM, indigo naturalis are widely used in traditional Chinese medicine formulae (Table 1). Besides, modern pharmacology has found



Fig. 1 *Baphicacanthus cusia* (Nees) Bremek.

that indigo naturalis effectively treats human leukemia^[8]. And 'Nan-Ban-Lan-Gen' are also widely used in China. Due to the application of indigo naturalis and 'Nan-Ban-Lan-Gen' in the medicinal industry, the growing demand for *B. cusia* has led to an expansion of cultivation efforts. With the rapid development of detection technologies and the utilization of multi-omics approaches, more compounds have been identified, and more detailed metabolic pathways elaborated. Thus, this review focuses on the chemical compositions and the major biological activity—antitumor of *B. cusia*. Moreover, the metabolism and synthesis of the major bioactive compounds, and their related genes are also discussed.

Chemical compositions

Because of a traditional Chinese medicine, research on the chemistry of *B. cusia* began decades ago. Studies showed that the plant and corresponding medicinal materials include indole alkaloids (indirubin, indigo, and tryptanthrin^[9]), sterols (β -sitosterol, γ -sitosterol, stigmasta-5,22-diene-3 β ,7 β -diol, and stigmasta-5,22-diene-3 β ,7 α -diol^[9,10]), triterpenoids (lupeol, lupenone, and botulin^[11]), lignans ((2*R*,3*R*)-(+)-lyoniresinol^[12]) and flavonoids (5,7,4'-trihydroxy-6-methoxyflavone and 5,7,4'-trihydroxy-6-methoxyflavone-7-*O*- β -D-glucopyranoside^[13]) (Fig. 2). With the deepening of research and the development of detection technology, more and more new compounds are constantly being discovered from *B. cusia*. For example, Liu et al. firstly found monoterpenoids (–)-loliolide and (+)-isololiolide^[13]; Feng et al. obtained two new alkaloids, baphicacanthin A and baphicacanthin B, from the roots of *B. cusia*^[14]; Zhu et al. identified four stereoisomeric indole alkaloids, (\pm)-baphicacanthcusines A–D, and one new indole alkaloid, baphicacanthcusine E, together with nine known compounds from the leaves^[15]; Xu et al. identified 11 compounds from leaves of *B. cusia* for the first time, such

as 1,3-dihydro-2*H*-indol-2-one, 2-hydroxy-*N*-(2-hydroxyphenyl) propionamide, (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigma-dien-9-one, 3 β -hydroxy-5 α ,6 α -epoxy-7-megastigmen-9-one, calendin, 3 β -hydroxy- β -ionone, hispidulin, bungein A, bis(2-ethylhexyl) benzene-1,2-dicarboxylate, nonacosane and *p*-hydroxyacetophenone^[5]; and Bai et al. firstly isolated 18 compounds from roots of *B. cusia*, including indole-3-acetic acid, methyl indol-3-ylacetate, acanthiline A, hyptatic acid-A, 2 α -hydroxy oleanolic acid, β -amyrin, nobiletin, tangeretin, wrightiadione, (+)-evofolinB, nitidanin, (7'*S*,8'*S*)-bilagrewin, (+)-lyoniresinol, cerevisterol, 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxypropan-1-one, *O*-phthalic acid bis-(2-ethyl decyl)-ester, 1-linoleoylglycerol, and 10,12-octadecadienoic acid-9-hydroxymethylester^[16]. However, studies on chemical compositions are focused on several secondary metabolites with clearly biological activity, such as flavonoids and indole alkaloids^[4,17]. Further studies of isolation, purification, identification, and characterization of new compounds from *B. cusia* are needed.

Antitumor properties of bioactive compounds

Combination of the TCM theory and modern pharmacological study, some indigo naturalis-containing Chinese medicine, such as the *Danggui Longhui Wan*^[18] and the *Realgar-indigo naturalis formula*^[19], are clinically administrated to treat human leukemia. Indirubin, indigo, and tryptanthrin are believed to be the main bioactive substances in *B. cusia*. Indirubin exhibits inhibitory activity towards cyclin-dependent kinase (CDK) 1-cyclin B with half-maximal inhibitory concentration (IC₅₀) = 10 μ M, and its derivatives inhibit CDK1 efficiently as well, indirubin-3'-monoxime with IC₅₀ = 0.18 μ M, 5-chloro-indirubin with IC₅₀ = 0.4 μ M, and indirubin-5-sulphonic acid with IC₅₀ = 0.055

Table 1. Traditional Chinese Medicine prescriptions containing indigo naturalis (Qing-Dai).

Prescription	Formula	Chinese ancient books
Baidai Powder	Bark of <i>Phellodendron chinense</i> , 2 qian (6 g); Leaves and/or stems of <i>Baphicacanthus cusia</i> , <i>Polygonum tinctorium</i> , <i>Indigofera tinctoria</i> or <i>Isatis indigotica</i> , 2 qian (6 g)	Dongtian Aozhi
Bijing Pill	Calomel, 1.5 qian (4.5 g); Talcum, 1.5 qian (4.5 g); Tubers of <i>Arisaema erubescens</i> , <i>A. heterophyllum</i> or <i>A. amurense</i> , 1 qian (3 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 5 fen (1.5 g)	Puji Fang
Biyu Tongshen Powder	Bark of <i>P. chinense</i> , 5 qian (15 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 fen (0.3 g); Camphor (Branches, stems, leaves and roots of <i>Cinnamomum camphora</i>), a modicum (~ 0.1 g)	Puji Fang
Chaihu Qingdai Decoction	Roots of <i>Bupleurum chinensie</i> or <i>B. scorzoniferolium</i> , 5 fen (1.5 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 5 fen (1.5 g); Rhizomes of <i>Cyperus rotundus</i> , 1 qian (3 g); Rhizomes of <i>Ligusticum chuansiang</i> , 1 qian (3 g); Pericarps of <i>Citrus reticulata</i> or its cultivated varieties, 8 fen (2.4 g); Rhizomes of <i>Coptis chinensis</i> , <i>C. deltoidei</i> or <i>C. teeta</i> , 8 fen (2.4 g); Fruits of <i>Cardenia jasminoides</i> , 8 fen (2.4 g); Roots and rhizomes of <i>Glycyrrhiza uralensis</i> , <i>G. inflata</i> or <i>G. glabra</i> , 8 fen (2.4 g)	Miscellaneous Diseases
Chaya Niu Huang Qingdai Powder	Gallstones of <i>Bos taurus</i> , 5 fen (1.5 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 5 fen (1.5 g); Borax, 2 qian (6 g); Cinnabar, 1 qian (3 g); Naturally deposited solids in healthy human urine, 2 fen (0.6 g); Skeletal fossils of ancient mammals, 2 fen (0.6 g); Borneol, 3 fen (0.9 g)	Yizong Jinjian
Chunbi Biyu Powder	Refined nitrokalite, 1 fen (0.3 g); Camphol, 1 qian (3 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 qian (3 g)	General Records of Saints
Daibai Powder	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 qian (3 g); Bark of <i>P. chinense</i> , 2 qian (6 g); Borneol, 1 qian (3 g)	Integrative Medicine Dermatology
Daiehuang Powder	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 2 qian (6 g); Bark of <i>P. chinense</i> , 2 qian (6 g); Bassanite, 2 liang (60 g); Liuyi Power (Talcum : Roots of <i>G. uralensis</i> = 6 : 1), 24 qian (72 g)	Drug dowry secret

(to be continued)

Table 1. (continued)

Prescription	Formula	Chinese ancient books
Daige Powder	Shell of <i>Meretrix meretrix</i> or <i>Cyclina sinensis</i> , 10 liang (300 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 liang (30 g)	Medical Theory
Danggui Longhui Pill	Roots of <i>Angelica sinensis</i> , 1 liang (30 g); Roots and rhizomes of <i>Gentiana scabra</i> , <i>G. triflora</i> or <i>G. manishurica</i> , 1 liang (30 g); Fruits of <i>Gardenia jasminoides</i> , 1 liang (30 g); Rhizomes of <i>C. chinensis</i> , <i>C. deltoidea</i> or <i>C. teeta</i> , 1 liang (30 g); Bark of <i>Phellodendron chinense</i> , 1 liang (30 g); Roots of <i>Scutellaria baicalensis</i> , 1 liang (30 g); Roots and rhizomes of <i>Rheum palmatum</i> , <i>R. tanguticum</i> or <i>R. officinale</i> , 5 qian (15 g); Concentrated dried juices of <i>Aloe barbadensis</i> or <i>A. ferox</i> , 5 qian (15 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 5 qian (15 g); Roots of <i>Aucklandia lappa</i> , 1 fen (0.3 g); Secretions from mature male sachets of <i>Moschus berezovskii</i> , <i>M. sifanicus</i> or <i>M. Moschiferus</i> , 5 fen (1.5 g)	Danxi Xinfa
Daihong Powder	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> ; Rhizomes of <i>Coptis chinensis</i> , <i>C. deltoidei</i> or <i>C. teeta</i> ; Flowers of <i>Carthamus tinctorius</i> ; with each equal division	Famous selection and sequel
Daihuang Powder	Bark of <i>P. chinense</i> , 1 liang (30 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 2 qian (6 g); Rhizomes of <i>Coptis chinensis</i> , <i>C. deltoidei</i> or <i>C. teeta</i> , 1.5 qian (4.5 g); Roots of <i>A. dahurica</i> or <i>A. dahurica</i> , 1.5 qian (4.5 g); Roots of <i>Paeonia lactiflora</i> or <i>P. veitchii</i> , 1 qian (3 g); Tea, 1 qian (3 g); Secretions from mature male sachets of <i>M. berezovskii</i> , <i>M. sifanicus</i> or <i>M. Moschiferus</i> , 2.5 fen (0.75 g)	Continued name family selection
Gualou Qingdai Pill	Seeds of <i>Trichosanthes kirilowii</i> or <i>T. rosthornii</i> , 1 liang (30 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 3 qian (9 g)	Danxi Xinfa
Hezi Qingdai Pill	Fruits of <i>Terminalia chebula</i> ; Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> ; Seeds of <i>Armeniaca vulgaris</i> , <i>A. vulgaris</i> , <i>A. sibirica</i> or <i>A. mandshurica</i> ; Egg-banding of <i>Notarchus leachii cirrosus</i> ; Rhizomes of <i>Cyperus rotundus</i> ; Seeds of <i>T. skirilowii</i> or <i>T. rosthornii</i> ; Rhizoma <i>Pinelliae</i> Fermentata; with each equal division	Miscellaneous Disease Origin Rhinoceros Candle
Hupo Biyu Powder	Talcum, 6 liang (180 g); Roots and rhizomes of <i>G. uralensis</i> , <i>G. inflata</i> or <i>G. glabra</i> , 1 liang (30 g); Amber, 5 qian (15 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 8 fen (2.4 g)	Yizong Jinjian
Jiujing Pill	Above ground part of <i>Mentha haplocalyx</i> , 3 qian (9 g); Larvae of <i>Bombyx mori</i> infected or artificially inoculated with <i>Beauveria bassiana</i> , 3 qian (9 g); Roots of <i>Saposhnikovia divaricate</i> , 3 qian (9 g); Tubers of <i>Gastrodia elata</i> , 3 qian (9 g); <i>Buthus martensii</i> , 3 qian (9 g); Roots and rhizomes of <i>G. uralensis</i> , <i>G. inflata</i> or <i>G. glabra</i> , 3 qian (9 g); Dried exudate from stems of <i>Bambusa terilis</i> or <i>Schizostachyum chinense</i> , 3 qian (9 g); Stems and branches containing hooks from <i>Uncaria rhynchophylla</i> , <i>U. macrophylla</i> , <i>U. hirsute</i> , <i>U. sinensis</i> or <i>U. sessilifructus</i> , 3 qian (9 g); Mixture of prepared tubers of <i>A. erubescens</i> , <i>A. heterophyllum</i> or <i>A. amurense</i> and bile from <i>Bos</i> spp., <i>Ovis</i> spp. or <i>Sus</i> spp., or fermented mixture of tuber power of <i>A. erubescens</i> , <i>A. heterophyllum</i> or <i>A. amurense</i> and bile from <i>Bos</i> spp., <i>Ovis</i> spp. or <i>Sus</i> spp., 4 qian (12 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> , or <i>I. indigotica</i> , 4 qian (12 g); Tubers of <i>A. erubescens</i> , <i>A. heterophyllum</i> or <i>A. amurense</i> , 4 qian (12 g); Tubers of <i>Typhonium giganteum</i> , 2 qian (6 g); Cinnabar, 2 qian (6 g); Amber, 2 qian (6 g); Secretions from mature male sachets of <i>M. berezovskii</i> , <i>M. sifanicus</i> or <i>M. Moschiferus</i> , 8 fen (2.4 g); Gallstones of <i>Bos taurus</i> , 5 fen (1.5 g); Pearl, 5 fen (1.5 g)	National Traditional Chinese Medicine Prescription Collection
Kexue Recipe	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 2qian (6 g); Seeds of <i>T. skirilowii</i> or <i>T. rosthornii</i> , 3 qian (9 g); Egg-banding of <i>Notarchus leachii cirrosu</i> , 3 qian (9 g); Fruits of <i>G. jasminoides</i> , 3 qian (9 g); Fruits of <i>Terminalia chebula</i> , 2 qian (6 g)	Danxi heart method
Lijing Pill	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 qian (3 g); Calomel, 1 qian (3 g); Power of seeds of <i>Ipomoea nil</i> or <i>I. biflora</i> , 5 qian (15 g); Dried exudate from stems of <i>B. terilis</i> or <i>S. chinense</i> , 2 qian (6 g)	Children's medicine certificate straight recipe
Qingdai Shigao Decoction	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1.5 qian (4.5 g); Fresh roots of <i>Rehmannia glutinosa</i> , 2 liang (60 g); Gypsum, 8 qian (24 g); Rhizome of <i>Cimicifuga heracleifolia</i> , <i>C. dahurica</i> or <i>C. foetida</i> , 6 fen (1.8 g); Roots of <i>S. baicalensis</i> , 2 qian (6 g); Processing fruits of <i>Gardenia jasminoides</i> , 3 qian (9 g); <i>Allium fistulosum</i> , 3 pieces	Redefinition of popular typhoid theory
Yangdu Neixiao Powder	Secretions from mature male sachets of <i>M. berezovskii</i> , <i>M. sifanicus</i> or <i>M. Moschiferus</i> , 2 qian (6 g); Borneol, 2 qian (6 g); Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 2 qian (6 g); Tubers of <i>Bletilla striata</i> , 3 qian (9 g); Tubers of <i>A. erubescens</i> , <i>A. heterophyllum</i> or <i>A. amurense</i> , 3 qian (9 g); Prepared scales of <i>Manis pentadactyla</i> with Rhizomes of <i>Curcuma longa</i> , 3 qian (9 g); Camphor (Branches, stems, leaves and roots of <i>Cinnamomum camphora</i>), 3 qian (9 g); Verdigris, 3 qian (9 g); Chalcantithite, 3 qian (9 g)	Drug dowry secret
Yanhou Biyu Powder	Leaves and/or stems of <i>B. cusia</i> , <i>P. tinctorium</i> , <i>I. tinctoria</i> or <i>I. indigotica</i> , 1 liang (30 g); Mirabilite, 1 liang (30 g); Pollen of <i>Typha angustifolia</i> , <i>T. orientalis</i> or similar plants, 1 liang (30 g); Roots and rhizomes of <i>G. uralensis</i> , <i>G. inflata</i> or <i>G. glabra</i> , 1 liang (30 g)	Yuyao Yuanfang

Note: Li, fen, qian, and liang are units of measurement in ancient China. 1 liang = 10 qian = 100 fen = 1000 li = 30 g.

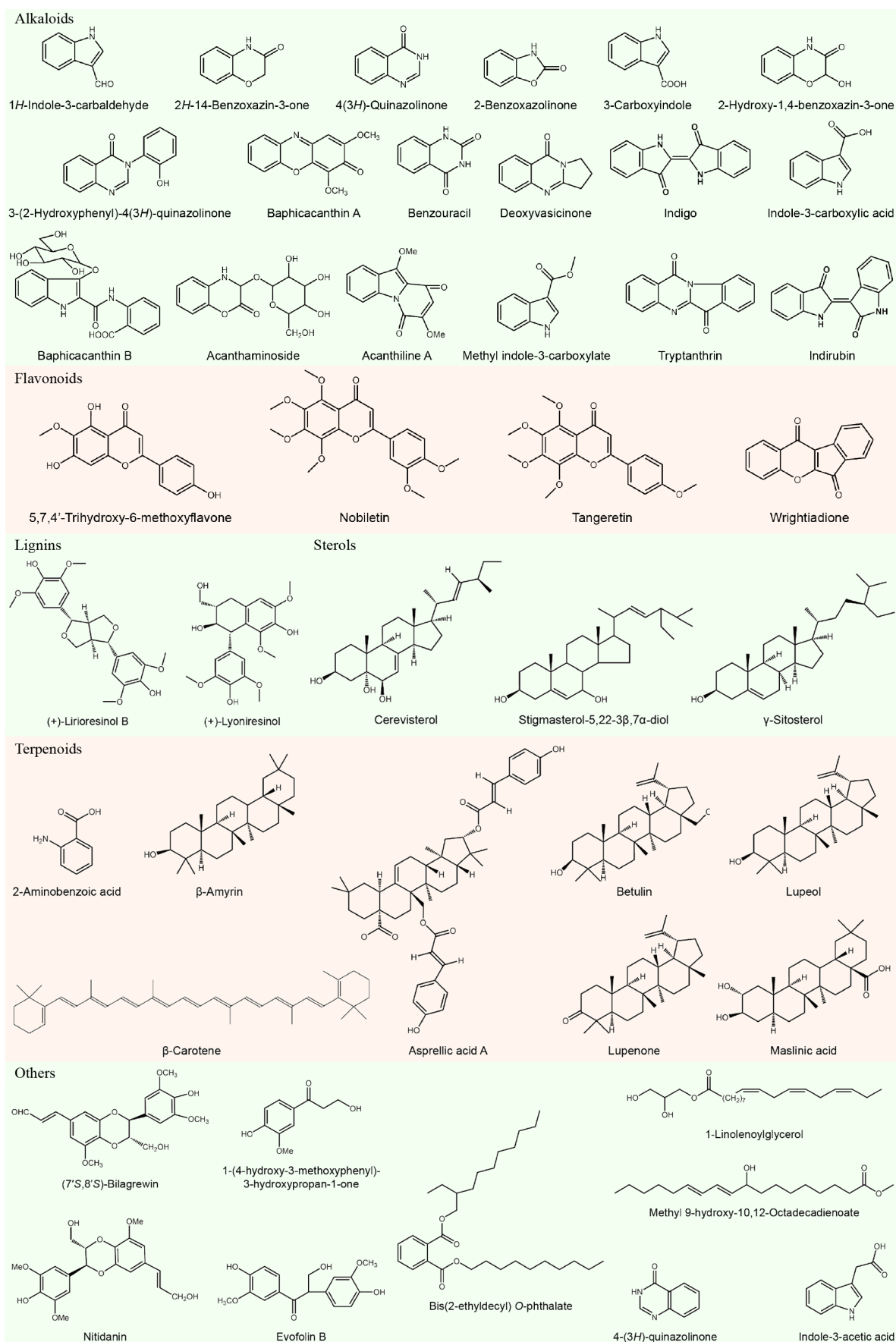


Fig. 2 Chemical compositions of *Baphicacanthus cusia* (Nees) Bremek.

μM ^[18]. Cancer statistics, 2023 depicts that prostate cancer is the most common cancer diagnosed in men and breast cancer in women. The report also shows that lung cancer in men and women will cause the maximum number of deaths^[20]. Fortunately, indirubin, indigo, and their derivatives (Fig. 3) exhibit significant antitumor effects against these cancers *in vitro* and *in vivo* through multiple pathways (Fig. 4).

Induction of cell cycle arrest

In the CDK-dependent mechanism, cyclin D1 (CCND1) and CDK 4/6 form an active CCND1-CDK4/6 complex, and then phosphorylate retinoblastoma protein (Rb), which is dissociated from the bound E2F transcription factor 1 (E2F1) to initiate transcription, resulting in cell cycle progression^[21]. Wei et al. found that indirubin inhibits CCND1 expression and induces

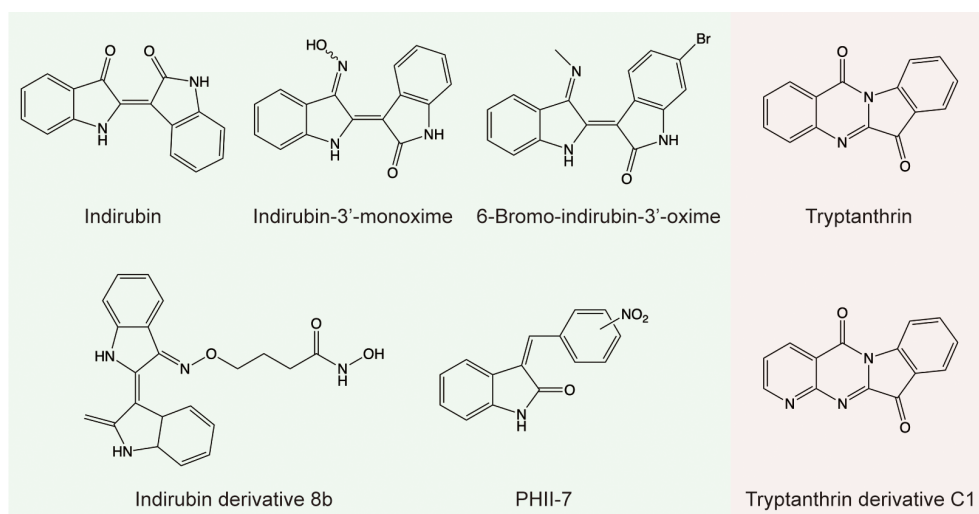


Fig. 3 Structures of indirubin and tryptanthrin and their derivatives.

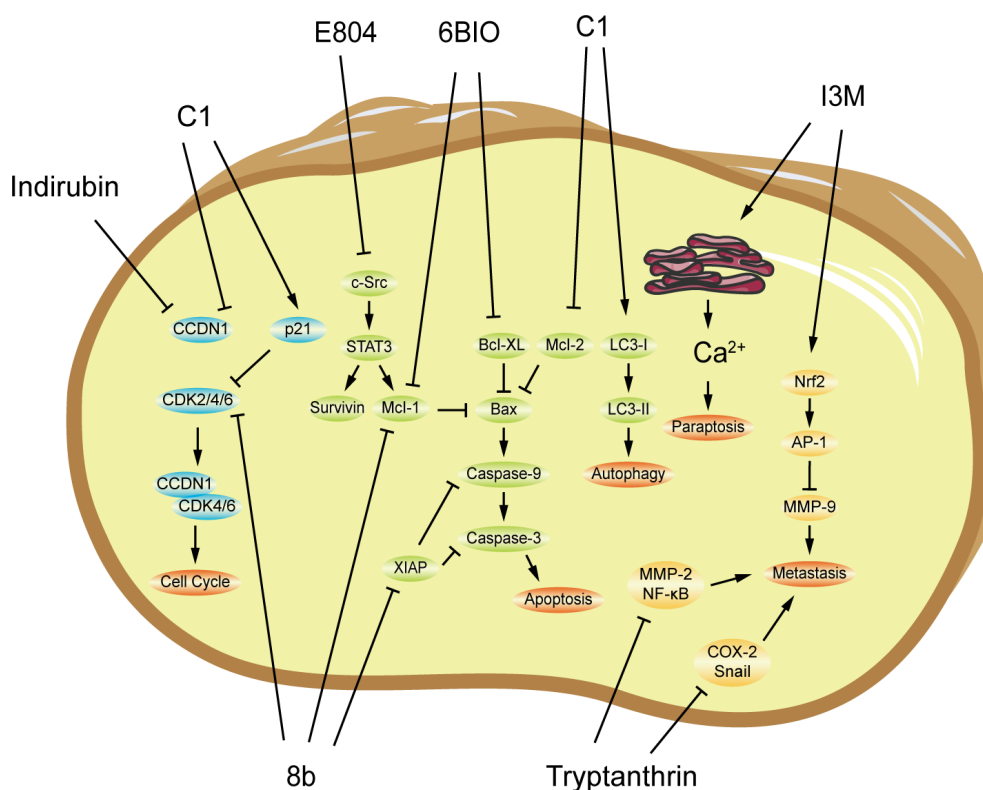


Fig. 4 The possible mechanisms of antitumor activity of indirubin and tryptanthrin and their derivatives. 8b, 6BIO E804, and I3M are indirubin derivatives; and C1 is tryptanthrin derivative. AP-1, activator protein-1; Bax, Bcl-2-associated X protein; Bcl-xL, B-cell lymphoma extra-large; CCND, cyclin D; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase 2; IL, interleukin; LC3, microtubule-associated protein 1 light chain 3; Mcl-1, mantle cell lymphoma 1; MMP, matrix metalloprotein; NF- κ B, nuclear factor κ B; Nrf2, nuclear factor-erythroid 2-related factor 2; Src, proto-oncogene tyrosine-protein kinase; STAT, signal transducer and activator of transcription; TNF- α , tumor necrosis factor- α ; and XIAP, X-linked inhibitor of apoptosis.

cell cycle arrest with an increase in the G0 and G1 phases and a decrease in the S and G2/M phases in human prostate cancer PC-3 cells^[22]. Cao et al. design and synthesize a series of indirubin derivatives as dual inhibitors against CDK and histone deacetylase (HDAC)^[23]. Compound 8b, one of which possesses remarkable CDK2/4/6 and HDAC6 inhibitory activity, induces S-phase arrest in human breast cancer MCF-7 and non-small cell lung cancer A549 cells. Zou et al. synthesized tryptanthrin derivatives, wherein compound C1 significantly downregulates CCND1 level and upregulates the expression of CDK inhibitor p21, resulting in an increase in the percentage of the A549 cells in the G2/M phase^[24].

Induction of programmed cell death

Apoptosis is a kind of cell death processes. Signal transducer and activator of transcription (STAT) 3 is a transcription factor and is involved in numerous biological processes, including cell survival^[25]. Various factors can activate STAT3, which then regulates downstream genes, such as apoptosis-related genes B cell lymphoma 2 (Bcl-2), B-cell lymphoma extra-large (Bcl-xL), Survivin, and mantle cell lymphoma 1 (Mcl-1). Subsequently, the Bcl-2 family regulates caspases, leading to trigger apoptosis. Targeting STAT3 is an attractive therapeutic strategy for cancer management^[26]. In human breast cells, indirubin derivative E804 blocks tyrosyl phosphorylation and DNA-binding activity of STAT3 by directly inhibiting c-Src kinase activity with $IC_{50} = 0.43 \mu M$, and then the expression levels of Mcl-1 and Survivin are reduced, leading to apoptosis^[27]. A bromo-substituted indirubin 6-bromo-indirubin-3'-oxime (6BIO), as a strong inhibitor of GSK-3 β with $IC_{50} = 0.005 \mu M$, CDK1 with $IC_{50} = 0.320 \mu M$, and CDK5 with $IC_{50} = 0.083 \mu M$, induces apoptosis in various cancer cell lines^[28]. In breast cancer cells, 6BIO downregulates Mcl-1 and Bcl-xL expression levels and activates the intrinsic caspase-9 and caspase-3 mediated apoptotic pathway^[29,30]. Indirubin derivative 8b downregulates Mcl-1 and X-linked inhibitor of apoptosis (XIAP) in A549 cells^[23]. Tryptanthrin derivative C1 treatment increases Bax/Bcl-2 ratio, with reduced Bcl-2 expression and increased Bax expression^[24]. Additionally, the C1 can also induce autophagy in NSCLC with the increased transformation from LC3-I to LC3-II^[24]. An indirubin derivative, indirubin-3'-monoxime (I3M), triggers proteasome dysfunction, induces ER stress, and promotes Ca^{2+} release with transmission to the mitochondria regulated by MCU, resulting in paraptosis^[31].

Metastasis inhibition

The tumor microenvironment (TME), including surrounding cells, signaling molecules, and extracellular matrix (ECM), is critical to cancer progression. Active transforming growth factor (TGF)- β stored in the ECM complex is released into the TME^[32]. The TGF- β signaling pathway has been proven to be a significant inducer of Epithelial-to-mesenchymal transition (EMT) and promotes tumor metastasis in human cancers^[33]. ECM is degraded by matrix metalloproteinase (MMP), which results in tumor cell invasion and migration^[34]. Indirubin derivative I3M slightly decreases viability of human prostate cancer LNCaP cells, but inhibits phorbol 12-myristate 13-acetate (PMA)-induced invasion of LNCaP cells^[35]. I3M activates nuclear factor-erythroid 2-related factor 2 (Nrf2), subsequently stimulates its movement to the nucleus, and consequently reduces activator protein-1 (AP-1) activity, thereby downregulating MMP-9 expression. This results in the reduction of prostate cancer

invasion. In MCF-7 cells, tryptanthrin inhibits cell proliferation, migration and invasion, induces poor adherence, and suppresses the TGF- β 1-induced transition *in vitro*^[36]. Moreover, tryptanthrin enhances the E-cadherin expression level and depresses expressions of MMP-2 and Snail stimulated by TGF- β 1, suggesting that tryptanthrin suppresses the TME-associated EMT induced by TGF- β 1. In 4T1 mouse breast cancer models, tryptanthrin also has considerable inhibitory activity with much lower toxic and side effects on organisms compared with the positive drug cyclophosphamide. Tryptanthrin suppresses the expression levels of NOS1, COX-2, and NF- κ B in mouse tumor tissues, and upregulates serum expression levels of IL-2, IL-10, and TNF- α in tumor-bearing mice. It is showed that tryptanthrin exerts anti-breast cancer activities by modulating the inflammatory TME *in vitro* and *in vivo*.

Multidrug resistance overcoming

Most tumors develop resistance during therapy, and multidrug resistance often leads to failure of cancer chemotherapy. New therapeutic strategies are urgently needed to overcome chemotherapy resistance. Indirubin derivative PHIL-7 significantly inhibits cell growth ($IC_{50} = 6.07 \mu M$ against MCF-7 cells; $IC_{50} = 5.51 \mu M$ against adriamycin-selected MCF-7 cells), potentiates adriamycin cytotoxicity, and restores chemotherapy sensitivity in MCF-7 cells with adriamycin resistance^[37]. It is suggested that the downregulation of the P-glycoprotein level could reverse multidrug resistance. The indirubin derivative 6BIO overcomes tumor necrosis factor α -related apoptosis inducing ligand (TRAIL) resistance and synergizes with TRAIL to abrogate cell proliferation and induce caspase-dependent apoptosis^[30]. 6BIO in combination with TRAIL augments caspase-dependent apoptosis. Tryptanthrin and its derivative benzo[b]-tryptanthrin reverse multidrug resistance through the downregulation of multidrug resistance protein 1 (MDR1) level in MCF-7 cells^[38,39].

Omics study

Hu et al. applied flow cytometry to estimate the *B. cusia* genome size^[40]. They used the genome size of *Oryza Sativa* L. spp. japonica as a reference, and found that the *B. cusia* genome size is 0.99 ± 0.01 Gb. For the first time, this team performed *de novo* transcriptome sequencing of *B. cusia*^[41,42]. They found 51,381 unique sequences based on 137,216,248, 122,837,394, and 140,240,688 clean reads from leaves, stems, and roots, respectively, and annotated 33,317 unigenes using the databases of Nr, Swiss-Prot, KEGG, and KOG. Within the 51,381 examined unigenes, 6,782 unigenes consist of 8,471 SSR markers, and 1,350 unigenes contain more than one SSR. Trinucleotide is the most abundant SSR, and penta-nucleotide is the least frequent SSR. In order to analyze the content of indigo and indirubin in the leaf and root tissues of *B. cusia*, the team performed *de novo* RNA-seq of transcriptional profiles of *B. cusia* leaf and root treated by methyl jasmonate (MeJA)^[43]. In response to MeJA treatment, 33,317 unigenes are annotated and contain 8,355 DEGs with 5,999 DEGs in MeJA-treated roots and 2,356 DEGs in the treated leaves. Huang also performed transcriptome sequencing and annotation of *B. cusia* using the Illumina Hiseq platform^[3]. They established a transcriptome database containing 293,666 unigenes.

For the species determination of Nan-Ban-Lan-Gen, Chen et al. analyzed and characterized the complete chloroplast (cp)

genome sequence of *S. cusia*^[44]. The genome is 144,133 bp in length and encodes 113 unique genes consisting of 79 protein-coding, 30 transfer RNA, and four ribosomal RNA genes. Combination of single-molecule sequencing and high-throughput chromosome conformation capture technologies, Xu et al. analyzed the chromosome scale genome of *S. cusia*^[45]. The genome size is approximately 865 Mb, containing about 79% of repetitive sequences and 32,148 annotated protein-coding genes.

Metabolism biology

Indirubin and indigo are isomers and are believed to be the most important bioactive components in *B. cusia*. Thus, exploration and analysis of their biosynthesis are vital. In the past 30 years, indole alkaloids biosynthesis has been believed to be one of the most investigated secondary metabolic pathways in blue-genera plants. This biosynthesis pathway is originally studied in microorganisms^[46]. It comprises the shikimate pathway and tryptophan metabolism pathway. The shikimate

pathway acts like a bridge for connecting primary and secondary metabolism^[47]. The secondary metabolism pathway of indoles in plants begins the tryptophan metabolism pathway (Fig. 5). This pathway of indole alkaloids in plants is still unclear, and the work is still in progress.

Analysis of the synthesis of indole alkaloids in *B. cusia* is just beginning. Some genes are predicted, identified, and cloned based on mining omics data. In *S. cusia*, Hu et al. confirmed 18 indole alkaloids-related coding genes^[48]. It was found that three UGT and two CYP450 genes are mainly expressed in the stems and leaves, and two UGT, one ASA, one TSB, one BGL, one CS, and one EPSPS genes are significantly expressed in roots. Plant hormones are necessary for plant growth and development^[49] and secondary metabolism^[50]. Using MeJA-treated *B. cusia* or *S. cusia*, comparative transcriptome analyses reveal several genes upregulated by MeJA and involved in the synthesis of indole alkaloids^[43,48].

Using the transcriptome data, some genes have been cloned, such as BcASA^[51], BcASB^[52], BcIGPS^[53], BcEPSPS^[54], and BcTSA^[55]. Transcription factors control expressions of

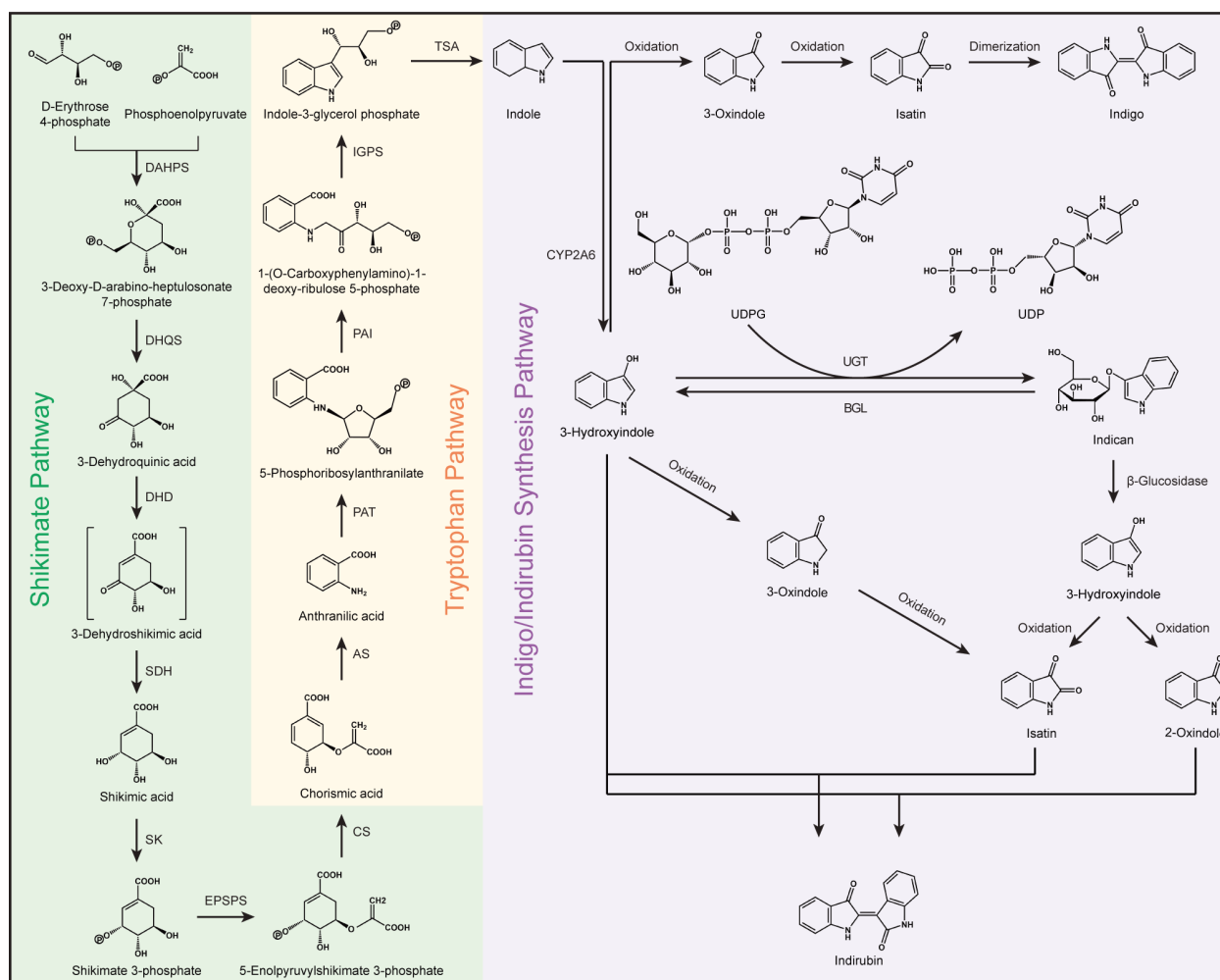


Fig. 5 Possible metabolic pathways of indigo and indirubin in *B. cusia*. AS, anthranilate synthase; BGL, β -glucosidase; CS, chorismate acid; CYP2A6, cytochrome P450 monooxygenase; DAHPS, 3-deoxy-D-arabinoheptulosonate 7-Phosphate synthase; DHD, 3-dehydroquinic acid dehydratase; DHQS, 3-dehydroquinic acid synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; IGPS, indole-3-glycerol phosphate synthase; PAI, phosphoribosyl anthranilate isomerase; PAT, phosphoribosyl anthranilate transferase; SDH, shikimate dehydrogenase; SK, shikimate kinase; TSA, tryptophan synthase α -subunit; UDPG, uridine diphosphate glucose; UDP, uridine diphosphate; and UGT, uridine diphosphate glucuronide transferase.

biosynthetic genes, thereby participating in the production of indole alkaloids. Zeng et al. cloned a WRKY transcription factor BcWRKY1, which is 916 bp in length containing three exons and two introns, and has 534 bp of open reading frame ORF encoding 177 amino acids residues^[56].

Both *in vitro* and *in vivo* experimental approaches are employed to verify the functions of these pathway-related genes. The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) catalyzes the reaction from enolpyruvyl moiety of phosphoenol pyruvate (PEP) to 5-hydroxyl shikimate-3-phosphate (S3P), thereby producing shikimic acid and chorismite. Yu et al. expressed and identified the recombinant BcEPSPS protein in *E. coli* strain BL21^[54]. After purification, the enzymatic activity assays are performed. Model organisms are an essential intermediate tool to confirm gene functions *in vivo* experiments. The functions of BcTSA^[55] and BcWRKY1^[56] are investigated in *E. coli* and *Arabidopsis thaliana*, respectively. Besides model organisms, *Isatis tinctoria* containing indole alkaloids pathway is also used to study the functions of the pathway related genes, such as BcTSA^[55] and BcEPSPS^[54].

Synthetic biology

Generally, there are three ways to acquire natural products including indigo and indirubin, such as extraction, chemical synthesis, and biosynthesis. The typical extraction method mainly consists of microbial fermentation and organic solvent extraction. Nevertheless, this method is cumbersome in operation and low in extraction purity, with high cost and toxicity^[57]. After the chemical structure of indigo is determined, the chemical synthesis method replaces the plant extraction and dramatically increases the yield of indigo pigments^[58]. To this day, the majority of indigo is chemically synthesized. Although the chemical synthesis method is simple, efficient, high yield, and high purity^[59], the used primary raw materials, catalysts, and a significant amount of produced toxic by-products bring a severe potential threat to human beings and the environment^[60].

Since the microbial synthesis of indigo is first reported, the green synthesis of indigo has paid much attention. The first indigo-reducing alkaliphile, *Bacillus alkaliphiles*, is isolated in the 1960s, and then multiple microorganisms involved in the reduction of indigo during natural fermentation are reported, such as alkaliphilic bacterium IDR2-2^T^[61], *Amphibacillus* spp. strain C40^[62], and *Oceanobacillus* spp. strain A21^[62]. *Escherichia coli* is capable of producing indirubin from indican, a primary metabolite of indole, as well^[63]. Indole can be catalyzed from indole 3-glycerol phosphate, forming L-tryptophan; tryptophan can also reversibly convert to indole. Thus, the metabolic engineering strategies mainly include enhancement of the production of biomass-derived substrates, L-tryptophan, and indicant^[64]. Besides, the indole is oxidized to indoxyl, followed by being oxidized to indigo. Thus, microbial synthesis of indigo and indirubin also depends on the indole oxidative enzymes (Fig. 6).

Baeyer-Villiger monooxygenase (BVMO) can catalyze chemo-, regio-, and enantio-selective Baeyer-Villiger oxidations^[65]. Phenylacetone monooxygenase (PAMO) is a new variant of the BVMO family^[66]. Núñez-Navarro et al. developed two PAMOs, PAMO_{HPCD} and PAMO_{HPED}, by iterative saturation mutagenesis^[67]. The *E. coli* with PAMO_{HPCD} expression and

L-tryptophan supplement produces approximately 3,000 mg/L of indigo and 130.0 mg/L of indirubin. Flavin-containing monooxygenases (FMOs) belong to a family of molecular oxygen-dependent enzymes and are involved in oxidative processes. Recombinant *E. coli* DH5 α cells harboring an FMO gene cloned from *Methylophaga aminisulfidivorans* MPT produce about 5 mg/L of indirubin and 920 mg/L of indigo in tryptophan medium^[68,69]. Then, in optimal tryptophan medium by adding cysteine, the cells produce 223.6 mg/L of indirubin^[70]. Toluene 4-monooxygenase (T4MO) is a multicomponent hydroxylating enzyme involved in the conversion of toluene to hydroxytoluene^[71]. T4MO can also catalyze the production of indigo and indirubin^[72]. The *tmoABCDEF* operon encoding T4MO components is cloned from *Pseudomonas* sp. M4 and expressed in *E. coli*. When the developed two-phase (dioctyl phthalate/aqueous medium) culture system combines with the optimized culture conditions, the strain yields 102.4 mg/L of indirubin^[73]. Styrene monooxygenase (SMO) catalyzes the conversion of styrene into (S)-styrene oxide in a highly enantioselective manner^[74]. As the key rate-limiting enzyme in the indigo biosynthesis pathway, SMO can catalyze the oxidation of indole efficiently and consequently promote indigo biosynthesis^[75]. Recently, an engineering *E. coli* is constructed with co-expressed styrene monooxygenase StyAB from *Pseudomonas putida* and the chaperone groES-groEL^[76]. The yield of indigo is increased to 550 mg/L. When the StyAB gene is co-expressed with the malate dehydrogenase (mdh) gene selected for NADH regeneration, the yield of indigo is up to 787.25 mg/L after 24 h of fermentation^[77]. Rieske-type oxygenases are a class of non-heme iron enzymes involved in the degradation of aromatic compounds^[78]. In *E. coli*, tryptophanase oxidizes tryptophan into indole, and then heterologous oxygenases such as naphthalene dioxygenase (NDO), a Rieske non-heme iron dioxygenase, convert indole into precursors of indigo and indirubin^[79]. Zhang et al. cloned an NDO gene from *Comamonas* sp. MQ and induced it into *E. coli*, which can produce 57.98 mg/L of indirubin^[80].

Besides monooxygenases and dioxygenases, cyclases such as flavin-dependent terpenoid cyclases Xiamycins (Xias) also involve in the production of indigo and indirubin^[81]. Yin et al. found that Xial can produce 1.7 mg/L of indigoids in *E. coli* BL21 (DE3)^[82]. Through introducing other vital enzymes such as flavin-reducing enzyme Fre, tryptophan-lysing and -importing enzymes TnaA and TnaB, and H₂O₂-degrading enzyme KatE, and optimizing the fermentation parameters, the yield of indigoids reached 101.9 mg/L. Moreover, the productivity of indigo and indirubin in 1 L of the flask is improved to 26.0 and 250.7 mg/L, respectively.

Summary and prospects

B. cusia has been a traditional medicinal plant for thousands of years. In modern medicine, *B. cusia* still possesses great potential for exploration. For example, the *Compound Huangdai Tablet* and the *Compound Nan-Ban-Lan-Gen Tablets* are medicines approved for sale in China. Indigo naturalis is a significant component of the *Compound Huangdai Tablet* used to treat leukaemia. Indigo naturalis also excels in the treatment of psoriasis. Zhang et al. reviewed the functions and mechanisms of indigo naturalis in the treatment of psoriasis through immune cells, signal pathways, and disease-related

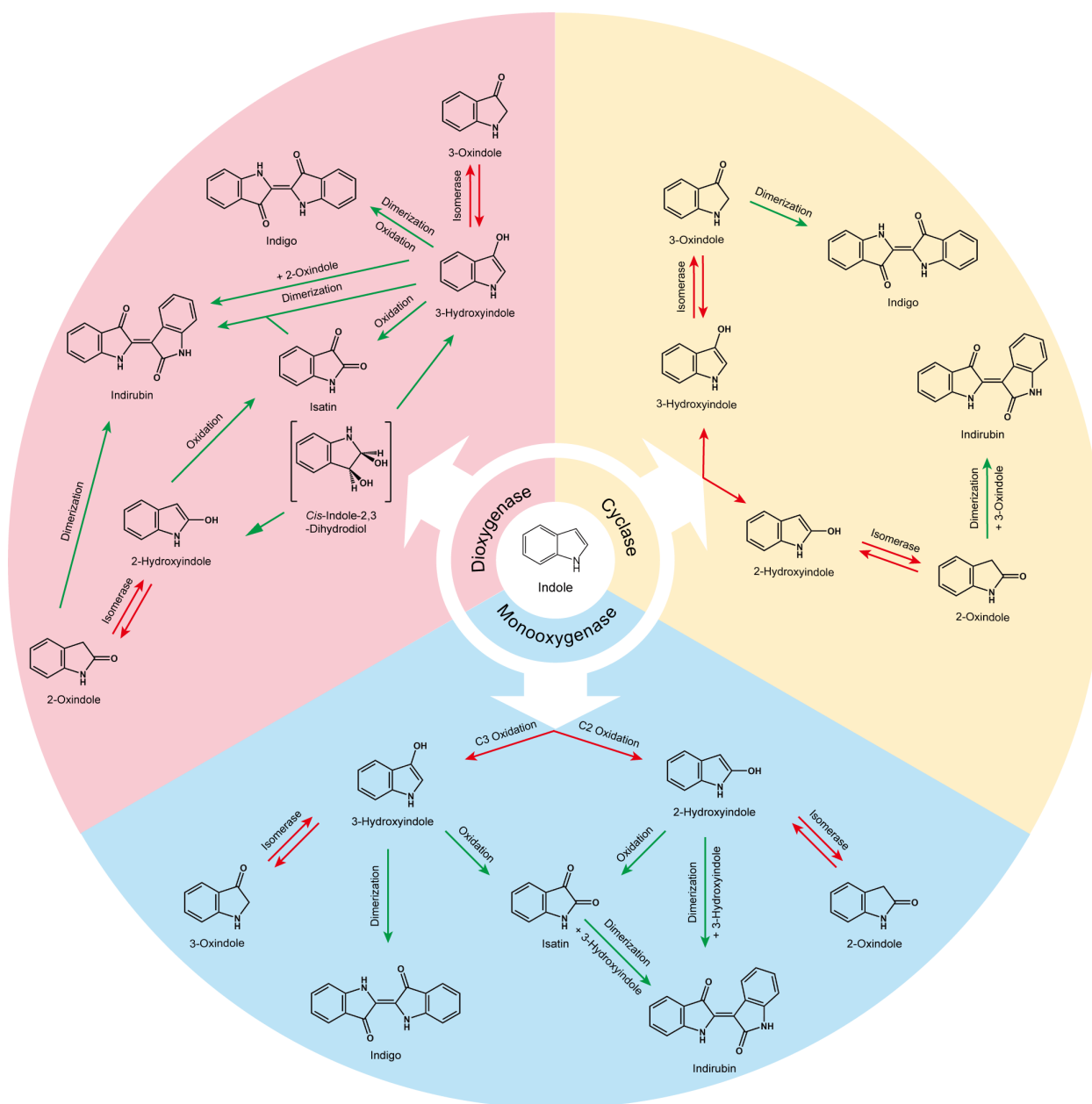


Fig. 6 The pathways of biosynthetic production of indigo and indirubin from indole. Three strategies can be applied for biosynthesis. Monooxygenase oxidizes indole into indole oxide, eventually producing indigo and indirubin; dioxxygenase oxidizes indole into cis-indole-2,3-dihydrodiol, then generating indigo and indirubin; and cyclase catalyzes the hydroxylation of indole to produce indigo or indirubin. The red arrow indicates enzymatic reaction; the green arrow indicates spontaneous process.

mediators^[83]. The *Compound Nan-Ban-Lan-Gen Tablet* contains Nan-Ban-Lan-Gen as the main ingredient and possesses anti-inflammatory and detoxifying properties. Regrettably, the primary source of this medicinal herb is controversial.

The rich and diverse chemical compositions of *B. cusia* confers many bioactive properties, such as antitumor, anti-inflammation, and neuroprotection. Among the numerous chemical components contained in *B. cusia*, indirubin, indigo, and tryptanthrin are mainly bioactive compounds. They exhibit inhibitory effects against a variety of human cancers such as glioma^[84] and skin cancer^[85], besides cancers discussed in the text. Additionally, these bioactive components can also suppress fibroblast^[86], inhibit SARS-CoV-2 infection^[87], and prevent and treat atherosclerosis^[88]. The functions of other

compounds are less well-studied. Thus, there is still a significant scope to explore their effects and application. Moreover, considering the practical biological functions of these bioactive compounds, a series of their derivatives are designed and synthesized to improve the effects and reduce toxicity^[89]. In addition to these mainly bioactive compounds, the biological functions of other compounds need further investigation. Identification of compound targets using the tools of modern biotechnology such as target fishing hook^[90] is of great importance in terms of exploring mechanisms involved.

Although the biological functions of these natural ingredients are very impressive, their metabolic processes, especially the indole alkaloids pathway, in *B. cusia* have been less studied. Only a few of these pathway-related enzyme genes are cloned

from *B. cusia*, and the studies of their functions are even fewer. However, the decipherment of a whole-genome map^[48] and analysis of transcriptome^[43] will provide an essential reference for the analysis of the metabolic pathway of indole alkaloids and other secondary metabolites. Multi-omics and comparative omics analysis is also a maturation-researching strategy for elaborating metabolic pathways. Genetic transformation is a technological tool to study plant gene structure and function. The genetic transformation system is relatively immature for *B. cusia*, leading to difficulty in validating gene function. In addition to model plants, functions of several genes cloned from *B. cusia* have to be studied in *I. indigotica* and other plants. *Catharanthus roseus* also contains a synthesis pathway of indole alkaloids and could be used for genetic transformation^[91]. Thus, the establishment of a genetic transformation system for *B. cusia* is in urgent demand. Based on the refinement of metabolic pathways and genetic transformation system, genetic engineering approaches are employed to enhance the contents of active ingredients. Gene editing has been proved to be an effective strategy. But the efficiency of gene editing varies widely across species^[92]. It is quite necessary to develop species-specific gene-editing technology. Moreover, synthetic biology is an effective way to address the scarcity of resource components. The reactions of oxidation and cyclization contribute to the production of bioactive compounds, especially indigo and indirubin, from indole. Exploration and discovery of new indole oxidases and cyclases with high catalytic efficiency are the keys to promoting the large-scale bioproduction and application of indigo and indirubin in the future^[82].

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Zhang L, Li QZ; data collection: Feng ZH, Jing S, Shen YP; analysis and interpretation of results: Feng ZH, Jing S, Shen YP, Tong YQ, Xiao CJ; draft manuscript preparation: Feng ZH, Jing S, Shen YP, Tong YQ, Xiao CJ, Xue JP, Zhang H, Li QZ, Zhang L. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant numbers 82225047 and 82170274), the National Key Research and Development Program of China (grant number 2022YFC3501703), and the Excellent Scientific Research and Innovation Team of University in Anhui Province (grant number 2022AH010029).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 29 September 2023; Revised 15 December 2023; Accepted 27 December 2023; Published online 23 January 2024

References

1. Yang C, Liu X, Fan S. 2012. Investigation of the current status of resources of *Strobilanthes cusia*. *Modern Chinese Medicine* 14:33–35,38(in Chinese)
2. Chen RF. 2013. *Research on tissue culture technology and vitrification prevention measure for Strobilanthes cusia (Nees) Ktze*. Master's Thesis. Fujian Agriculture and Forestry University, China. pp. 7–8(in Chinese).
3. Huang YX. 2017. *Exploration and functional research of the key genes related to pharmacodynamics substances in Baphicacanthus cusia*. Doctor's Thesis. Huaqiao University, China. pp. 1–2(in Chinese).
4. Liu B, Chen J, Li X, Zhang W, Huang Y, et al. 2021. Production overview, morphological observation and biochemical composition evaluation of *Strobilanthes cusia*. *Chinese Journal of Tropical Agriculture* 41:61–68(in Chinese)
5. Xu Z, Cai Y, Chen Y, Zhao Z, Ma Q, et al. 2021. Chemical constituents from the leaves of *Baphicacanthus cusia*. *Journal of Chinese Medicinal Materials* 44:1875–79(in Chinese)
6. Shao MH, Shang JY. 2018. Pharmacognosy analysis of leaves of *Baphicacanthus cusia* (Nees) Bremek. and its adulterants. *Journal of Clinical Medical* 5:171,76(in Chinese)
7. Zhong WG, Xu QH, Meng FD. 2011. Advances in study of *Isatis indigotica*. *Ginseng Research* 2011(3):38–41(in Chinese)
8. Zhu HH, Hu J, Lo-Coco F, Jin J. 2019. The simpler, the better: oral arsenic for acute promyelocytic leukemia. *Blood* 134:597–605
9. Yang XX, Lv SH, Wu SJ. 1995. Studies of chemical constituents from the leaves of *Baphicacanthus cusia*. *Chinese Traditional and Herbal Drugs* 1995(12):622(in Chinese)
10. Wu YQ, Qian B, Zhang RP, Zou D, Liu G. 2005. Studies of chemical constituents from the roots of *Baphicacanthus cusia*. *Chinese Traditional and Herbal Drugs* 36:982–83(in Chinese)
11. Chen R, Lu Z, Guan D, Chen G, Li S. 1987. Studies of chemical constituents from the roots of *Baphicacanthus cusia*. *Chinese Traditional and Herbal Drugs* 18:488–90(in Chinese)
12. Tanaka T, Ikeda T, Kaku M, Zhu XH, Okawa M, et al. 2004. A new lignan glycoside and phenylethanoid glycosides from *Strobilanthes cusia* BREMEK. *Chemical and Pharmaceutical Bulletin* 52:1242–45
13. Liu Y, Ouyang F, Yu HY, Li L, Wang NL, et al. 2009. Chemical constituents in the leaves of *Baphicacanthus cusia* (Nees) Bremek. *Chinese Journal of Medicinal Chemistry* 19(4):273–275,283(in Chinese)
14. Feng QT, Zhu GY, Gao WN, Yang Z, Zhong N, et al. 2016. Two new alkaloids from the roots of *Baphicacanthus cusia*. *Chemical and Pharmaceutical Bulletin* 64:1505–8
15. Zhu LJ, Cao F, Su XX, Li CY, Lin B, et al. 2020. *Baphicacanthus* A–E, Bisindole Alkaloids from the Leaves of *Baphicacanthus cusia* (Nees) Bremek. *Journal of Organic Chemistry* 85:8580–87
16. Bai B, Zhang Y, Xu Z, Huang Y, Ye S, et al. 2021. Chemical constituents and in vitro antitumor activities of *Baphicacanthus cusia*. *Chinese Pharmaceutical Journal* 56:1299–305(in Chinese)
17. Xu J, Hu Y, Wu J, Wu G, Zhao J. 2022. Extraction and optimization of flavonoids from *Strobilanthes cusia* stems and leaves and their antioxidant activity. *Chemical Engineering & Equipment* 2022(12):10–13+42(in Chinese)(in Chinese)
18. Hoessel R, Leclerc S, Endicott JA, Nobel ME, Lawrie A, et al. 1999. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nature Cell Biology* 1:60–67
19. Wang L, Zhou GB, Liu P, Song JH, Liang Y, et al. 2008. Dissection of mechanisms of Chinese medicinal formula *realgar-indigo naturalis* as an effective treatment for promyelocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America* 105:4826–31
20. Siegel RL, Miller KD, Wagle NS, Jemal A. 2023. Cancer statistics, 2023. *CA: A Cancer Journal for Clinicians* 73:17–48

21. Wang J, Su W, Zhang T, Zhang S, Lei H, et al. 2023. Aberrant cyclin D1 splicing in cancer: from molecular mechanism to therapeutic modulation. *Cell Death & Disease* 14:244
22. Wei YF, Su J, Deng ZL, Zhu C, Yuan L, et al. 2015. Iridubin inhibits the proliferation of prostate cancer PC-3 cells. *National Journal of Andrology* 21:788–91(in Chinese)
23. Cao Z, Yang F, Wang J, Gu Z, Lin S, et al. 2021. Iridubin derivatives as dual inhibitors targeting cyclin-dependent kinase and histone deacetylase for treating cancer. *Journal of Medicinal Chemistry* 64:15280–96
24. Zou Y, Zhang G, Li C, Long H, Chen D, et al. 2023. Discovery of tryptanthrin and its derivatives and its activities against NSCLC in vitro via both apoptosis and autophagy pathways. *International Journal of Molecular Sciences* 24:1450
25. La Sala G, Michiels C, Kükenshöner T, Brandstoetter T, Maurer B, et al. 2020. Selective inhibition of STAT3 signaling using monoclonal antibodies targeting the coiled-coil and N-terminal domains. *Nature Communications* 11:4115
26. Zou S, Tong Q, Liu B, Huang W, Tian Y, et al. 2020. Targeting STAT3 in cancer immunotherapy. *Molecular Cancer* 19:145
27. Nam S, Buettner R, Turkson J, Kim D, Cheng JQ, et al. 2005. Iridubin derivatives inhibit Stat3 signaling and induce apoptosis in human cancer cells. *Proceedings of the National Academy of Sciences of the United States of America* 102:5998–6003
28. Ferandin Y, Bettayeb K, Kritsanida M, Lozach O, Polychronopoulos P, et al. 2006. 3'-Substituted 7-halogenoiridubins, a new class of cell death inducing agents. *Journal of Medicinal Chemistry* 49:4638–49
29. Nicolaou KA, Liapis V, Evdokiou A, Constantinou C, Magiatis P, et al. 2012. Induction of discrete apoptotic pathways by bromo-substituted iridubin derivatives in invasive breast cancer cells. *Biochemical and Biophysical Research Communications* 425:76–82
30. Braig S, Bischoff F, Abhari BA, Meijer L, Fulda S, et al. 2014. The pleiotropic profile of the iridubin derivative 6BIO overcomes TRAIL resistance in cancer. *Biochemical Pharmacology* 91:157–67
31. Dilshara MG, Neelaka Molagoda IM, Prasad Tharanga Jayasooriya RG, Choi YH, Park C, et al. 2021. Iridubin-3'-monoxime induces paraptosis in MDA-MB-231 breast cancer cells by transmitting Ca²⁺ from endoplasmic reticulum to mitochondria. *Archives of Biochemistry and Biophysics* 698:108723
32. Pei L, Liu Y, Liu L, Gao S, Gao X, et al. 2023. Roles of cancer-associated fibroblasts (CAFs) in anti-PD-1/PD-L1 immunotherapy for solid cancers. *Molecular Cancer* 22:29
33. Yang J, Weinberg RA. 2008. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Developmental Cell* 14:818–29
34. Baram D, Vaday GG, Salamon P, Drucker I, HersHKoviz R, et al. 2001. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF- α . *Journal of Immunology* 167:4008–16
35. Dilshara MG, Molagoda IMN, Jayasooriya RGPT, Choi YH, Park C, et al. 2020. Iridubin-3'-monoxime inhibits AP-1-mediated matrix metalloproteinase-9 in LNCaP prostate cancer cells by activating the Nrf2/HO-1 signaling pathway. *Latin American Journal of Pharmacy* 39:269–78
36. Zeng Q, Luo C, Cho J, Lai D, Shen X, et al. 2021. Tryptanthrin exerts anti-breast cancer effects both in vitro and in vivo through modulating the inflammatory tumor microenvironment. *Acta Pharmaceutica (Zagreb, Croatia)* 71:245–66
37. Shi R, Li W, Zhang X, Zhang Y, Peng H, et al. 2011. A novel iridubin derivative PHII-7 potentiates adriamycin cytotoxicity via inhibiting P-glycoprotein expression in human breast cancer MCF-7/ADR cells. *European Journal of Pharmacology* 669:38–44
38. Yu ST, Chen TM, Tseng SY, Chen YH. 2007. Tryptanthrin inhibits MDR1 and reverses doxorubicin resistance in breast cancer cells. *Biochemical and Biophysical Research Communications* 358:79–84
39. Jun KY, Park SE, Liang JL, Jahng Y, et al. 2015. Benzo[b]tryptanthrin inhibits MDR1, topoisomerase activity, and reverses adriamycin resistance in breast cancer cells. *ChemMedChem* 10:827–35
40. Hu YL, Ning SJ, Ye Q, Ma XM, Cai GQ, et al. 2023. Genome size determination of *Baphicacanthus cusia* (Nees) Bremek. based on flow cytometry. *Chinese Traditional Patent Medicine* 45:634–36(in Chinese)
41. Lin WZ. 2015. *Accumulation of effective components and its molecular basis of Baphicacanthus cusia* (Nees) Bremek in Fujian province. Doctor's Thesis. Fujian Agriculture and Forestry University, China. pp. 63–65(in Chinese).
42. Lin W, Huang W, Ning S, Wang X, Ye Q, et al. 2018. De novo characterization of the *Baphicacanthus cusia* (Nees) Bremek transcriptome and analysis of candidate genes involved in indican biosynthesis and metabolism. *PLoS One* 13:e0199788
43. Lin W, Huang W, Ning S, Gong X, Ye Q, et al. 2019. Comparative transcriptome analyses revealed differential strategies of roots and leaves from methyl jasmonate treatment *Baphicacanthus cusia* (Nees) Bremek and differentially expressed genes involved in tryptophan biosynthesis. *PLoS ONE* 14:e0212863
44. Chen H, Shao J, Zhang H, Jiang M, Huang L, et al. 2018. Sequencing and analysis of *Strobilanthes cusia* (Nees) Kuntze chloroplast genome revealed the rare simultaneous contraction and expansion of the inverted repeat region in angiosperm. *Frontiers in Plant Science* 9:324
45. Xu W, Zhang L, Cunningham AB, Li S, Zhuang H, et al. 2020. Blue genome: Chromosome-scale genome reveals the evolutionary and molecular basis of indigo biosynthesis in *Strobilanthes cusia*. *Plant Journal* 104:864–79
46. Lee JH, Lee J. 2010. Indole as an intercellular signal in microbial communities. *FEMS Microbiology Reviews* 34:426–44
47. Herrmann KM, Weaver LM. 1999. The Shikimate Pathway. *Annual Review of Plant Physiology and Plant Molecular Biology* 50:473–503
48. Hu Y, Ma D, Ning S, Ye Q, Zhao X, et al. 2021. High-quality genome of the medicinal plant *Strobilanthes cusia* provides insights into the biosynthesis of indole alkaloids. *Frontiers in Plant Science* 12:742420
49. Huang H, Liu B, Liu L, Song S. 2017. Jasmonate action in plant growth and development. *Journal of Experimental Botany* 68:1349–59
50. Jeon BJ, Yang HM, Lyu YS, Pae HO, Ju SM, et al. 2015. Apigenin inhibits indoxyl sulfate-induced endoplasmic reticulum stress and anti-proliferative pathways, CHOP and IL-6/p21, in human renal proximal tubular cells. *European Review for Medical and Pharmacological Sciences* 19:2303–10
51. Shen J, Ma X, Wei D. 2020. Cloning and bioinformatics analysis of anthranilate synthase gene from *Baphicacanthus cusia*. *Genomics and Applied Biology* 29:5773–80(in Chinese)
52. Ma X, Ning S, Ye Q, Hu Y, Cai G, Wei D. 2021. Cloning, bioinformatic analysis and expression analysis of BcASB gene from *Baphicacanthus cusia*. *Chinese Traditional and Herbal Drugs* 52:5697–706(in Chinese)
53. Cai G, Ning S, Ye Q, Hu Y, Ma X, Wei D. 2021. Cloning, expression analysis and prokaryotic expression of IGPS from *Baphicacanthus cusia*. *Chinese Journal of Tropical Crops* 42:2167–74(in Chinese)
54. Yu J, Zhang Y, Ning S, Ye Q, Tan H, et al. 2019. Molecular cloning and metabolomic characterization of the 5-enolpyruvylshikimate-3-phosphate synthase gene from *Baphicacanthus cusia*. *BMC Plant Biology* 19:485
55. Guo Z, Chen J, Lv Z, Huang Y, Tan H, et al. 2023. Molecular cloning and functional characterization of BcTSA in the biosynthesis of indole alkaloids in *Baphicacanthus cusia*. *Frontiers in Plant Science* 14:1174582
56. Zeng M, Zhong Y, Guo Z, Yang H, Zhu H, et al. 2022. Expression and functional study of BcWRKY1 in *Baphicacanthus cusia* (Nees) Bremek. *Frontiers in Plant Science* 13:919071

57. Liu H, Huang R, Qu C, Liu S. 2022. Aqueous enzymatic method to extract indigo pigment from *Baphicacanthus cusia* leaves. *Agricultural Science & Technology* 23:33–39(in Chinese)
58. Ren J, Wang W, Li H. 1997. Recent development on the study of indigo production process. *Dyestuffs and Coloration* 1997(4):22–24(in Chinese)
59. You SN. 2018. *Biosynthesis of indigo by Cupriavidus sp. IDO and its functional genes*. Master's Thesis. Dalian University of Technology, China. pp. 5–7. (in Chinese)
60. Hsu TM, Welner DH, Russ ZN, Cervantes B, Prathuri RL, et al. 2018. Employing a biochemical protecting group for a sustainable indigo dyeing strategy. *Nature Chemical Biology* 14:256–61
61. Yumoto I, Hirota K, Nodasaka Y, Yokota Y, Hoshino T, et al. 2004. *Alkalibacterium psychrotolerans* sp. nov., a psychrotolerant obligate alkaliphile that reduces an indigo dye. *International Journal of Systematic and Evolutionary Microbiology* 54:2379–83
62. Aino K, Narihiro T, Minamida K, Kamagata Y, Yoshimune K, et al. 2010. Bacterial community characterization and dynamics of indigo fermentation. *FEMS Microbiology Ecology* 74:174–83
63. Lee JY, Shin YS, Shin HJ, Kim GJ. 2011. Production of natural indirubin from indican using non-recombinant *Escherichia coli*. *Biore-source Technology* 102:9193–8
64. Kayastha S, Sagwan-Barkdoll L, Anterola A, Jayakody LN. 2021. Developing synthetic microbes to produce indirubin-derivatives. *Biocatalysis and Agricultural Biotechnology* 37:102162
65. Mansouri HR, Gracia Carmona O, Jodlbauer J, Schweiger L, Fink MJ, et al. 2022. Mutations increasing cofactor affinity, improve stability and activity of a Baeyer-Villiger monooxygenase. *ACS Catalysis* 12:11761–66
66. Fraaije MW, Wu J, Heuts DPHM, van Hellemond EW, Spelberg JHL, et al. 2005. Discovery of a thermostable Baeyer-Villiger monooxygenase by genome mining. *Applied Microbiology and Biotechnology* 66:393–400
67. Núñez-Navarro N, Salazar Muñoz J, Castillo F, Ramírez-Sarmiento CA, Poblete-Castro I, et al. 2022. Discovery of new phenylacetone monooxygenase variants for the development of substituted indigoids through biocatalysis. *International Journal of Molecular Sciences* 23:12544
68. Han GH, Shin HJ, Kim SW. 2008. Optimization of bio-indigo production by recombinant *E. coli* harboring *fmo* gene. *Enzyme and Microbial Technology* 42:617–23
69. Han GH, Bang SE, Babu BK, Chang M, Shin HJ, et al. 2011. Bio-indigo production in two different fermentation systems using recombinant *Escherichia coli* cells harboring a flavin-containing monooxygenase gene (*fmo*). *Process Biochemistry* 46:788–91
70. Han GH, Gim GH, Kim W, Seo SI, Kim SW. 2013. Enhanced indirubin production in recombinant *Escherichia coli* harboring a flavin-containing monooxygenase gene by cysteine supplementation. *Journal of Biotechnology* 164:179–87
71. Bouhajja E, McGuire M, Liles MR, Bataille G, Agathos SN, George IF. 2017. Identification of novel toluene monooxygenase genes in a hydrocarbon-polluted sediment using sequence- and function-based screening of metagenomic libraries. *Applied Microbiology and Biotechnology* 101:797–808
72. McClay K, Boss C, Keresztes I, Steffan RJ. 2005. Mutations of toluene-4-monooxygenase that alter regiospecificity of indole oxidation and lead to production of novel indigoid pigments. *Applied and Environmental Microbiology* 71:5476–83
73. Wongsaroj L, Sallabhan R, Dubbs JM, Mongkolsuk S, Loprasert S. 2015. Cloning of toluene 4-monooxygenase genes and application of two-phase system to the production of the anticancer agent, indirubin. *Molecular Biotechnology* 57:720–26
74. Tischler D, Kermer R, Gröning JAD, Kaschabek SR, van Berkel WJH, et al. 2010. StyA1 and StyA2B from *Rhodococcus opacus* 1CP: a multifunctional styrene monooxygenase system. *Journal of Bacteriology* 192:5220–7
75. Cheng L, Yin S, Chen M, Sun B, Hao S, et al. 2016. Enhancing indigo production by over-expression of the styrene monooxygenase in *Pseudomonas putida*. *Current Microbiology* 73:248–54
76. Du L, Yue J, Zhu Y, Yin S. 2022. Production of indigo by recombinant *Escherichia coli* with expression of monooxygenase, tryptophanase, and molecular chaperone. *Foods* 11:2117
77. Pan Z, Tao D, Ren M, Cheng L. 2023. A combinational optimization method for efficient production of indigo by the recombinant *Escherichia coli* with expression of monooxygenase and malate dehydrogenase. *Foods* 12:502
78. Hu WY, Li K, Weitz A, Wen A, Kim H, et al. 2022. Light-driven oxidative demethylation reaction catalyzed by a Rieske-type non-heme iron enzyme Stc2. *ACS Catalysis* 12:14559–70
79. Pathak H, Madamwar D. 2010. Biosynthesis of indigo dye by newly isolated naphthalene-degrading strain *Pseudomonas* sp. HOB1 and its application in dyeing cotton fabric. *Applied Biochemistry and Biotechnology* 160:1616–26
80. Zhang X, Qu Y, Ma Q, Kong C, Zhou H, et al. 2014. Production of indirubin from tryptophan by recombinant *Escherichia coli* containing naphthalene dioxygenase genes from *Comamonas* sp. MQ. *Applied Biochemistry and Biotechnology* 172:3194–206
81. Kugel S, Baunach M, Baer P, Ishida-Ito M, Sundaram S, et al. 2017. Cryptic indole hydroxylation by a non-canonical terpenoid cyclase parallels bacterial xenobiotic detoxification. *Nature Communications* 8:15804
82. Yin H, Chen H, Yan M, Li Z, Yang R, et al. 2021. Efficient bioproduction of indigo and indirubin by optimizing a novel terpenoid cyclase Xial in *Escherichia coli*. *ACS Omega* 6:20569–76
83. Zhang Q, Xie J, Li G, Wang F, Lin J, et al. 2022. Psoriasis treatment using indigo naturalis: Progress and strategy. *Journal of Ethnopharmacology* 297:115522
84. Li Z, Wang H, Wei J, Han L, Guo Z. 2020. Indirubin exerts anti-cancer effects on human glioma cells by inducing apoptosis and autophagy. *AMB Express* 10:171
85. Shankar GM, Alex VV, Nisthul AA, Bava SV, Sundaram S, et al. 2020. Pre-clinical evidences for the efficacy of tryptanthrin as a potent suppressor of skin cancer. *Cell Proliferation* 53:e12710
86. Wang Q, Yu J, Hu Y, Chen X, Zhang L, et al. 2020. Indirubin alleviates bleomycin-induced pulmonary fibrosis in mice by suppressing fibroblast to myofibroblast differentiation. *Biomedicine & Pharmacotherapy* 131:110715
87. Hagiyaama M, Takeuchi F, Sugano A, Yoneshige A, Inoue T, et al. 2022. Indigo plant leaf extract inhibits the binding of SARS-CoV-2 spike protein to angiotensin-converting enzyme 2. *Experimental and Therapeutic Medicine* 23:274
88. Shi X, Zhang Y, Han J, Peng W, Fang Z, et al. 2021. Tryptanthrin regulates vascular smooth muscle cell phenotypic switching in atherosclerosis by AMP-activated protein kinase/acetyl-CoA carboxylase signaling pathway. *Journal of Cardiovascular Pharmacology* 77:642–49
89. Yang L, Li X, Huang W, Rao X, Lai Y. 2022. Pharmacological properties of indirubin and its derivatives. *Biomedicine and Pharmacotherapy* 151:113112
90. Zhou X, Zhao S, Liu T, Yao L, Zhao M, et al. 2022. Schisandrol A protects AGEs-induced neuronal cells death by allosterically targeting ATP6V0d1 subunit of V-ATPase. *Acta Pharmaceutica Sinica B* 12:3843–60
91. Das A, Sarkar S, Bhattacharyya S, Gantait S. 2020. Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. *Applied Microbiology and Biotechnology* 104:4811–35
92. Zhang Y, Zhou L, Xia J, Dong C, Luo X. 2021. Human microbiome and its medical applications. *Frontiers in Molecular Biosciences* 8:703585



Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.