Synthetic and Systems Biotechnology 7 (2022) 750–755

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology

Original Research Article

Carbon-nitrogen bond formation to construct novel polyketide-indole hybrids from the indole-3-carbinol exposed culture of *Daldinia eschscholzii*

Li Ping Lin [a], Min Wu [a], Nan Jiang [c], Wei Wang [d], Ren Xiang Tan [a,b,*]

[a] State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine, Nanjing, 210023, China
[b] State Key Laboratory of Pharmaceutical Biotechnology, Institute of Functional Biomolecules, Nanjing University, Nanjing, 210023, China
[c] Key Laboratory of Cardiovascular & Cerebrovascular Medicine, School of Pharmacy, Nanjing Medical University, Nanjing, 211166, China
[d] Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao, 266003, China

ARTICLE INFO

Keywords:
Polyketide-indole hybrids
Daldinia eschosholzii
Indole-3-carbinol
C–N bond formation
P450
Precursor-directed biosynthesis

ABSTRACT

A plenty of cytochrome P450s have been annotated in the *Daldinia eschosholzii* genome. Inspired by the fact that some P450s have been reported to catalyze the carbon-nitrogen (C–N) bond formation, we were curious about whether hybrids through C–N bond formation could be generated in the indole-3-carbinol (I3C) exposed culture of *D. eschscholzii*. As expected, two skeletally undescribed polyketide-indole hybrids, designated as indolpolyketone A and B (**1** and **2**), were isolated and assigned to be constructed through C–N bond formation. Their structures were elucidated by 1D and 2D NMR spectra. The absolute configurations of **1** and **2** were determined by comparing the recorded and calculated electronic circular dichroism (ECD) spectra. Furthermore, the plausible biosynthetic pathways for **1** and **2** were proposed. Compounds **1** and **2** exhibited significant antiviral activity against H1N1 with IC_{50} values of 45.2 and 31.4 μM, respectively. In brief, compounds **1** and **2** were reported here for the first time and were the first example of polyketide-indole hybrids pieced together through C–N bond formation in the I3C-exposed culture of *D. eschscholzii*. Therefore, this study expands the knowledge about the chemical production of *D. eschscholzii* through precursor-directed biosynthesis (PDB).

1. Introduction

Microbes and plants produce diverse natural products (also called secondary metabolites), which remain an important source of medicines, pesticides and chemical tools [1,2]. In particular, the central significance of natural products as prime starting molecules for the drug discovery is reflected both by the fact that half of all the new chemical entity-based drugs introduced in the last three decades—540 out of 1073—are 'nature-derived' [3]. With the development of society, the demand for new compounds for drug discovery pipelines has become more and more urgent. To address the issue, several strategies have been developed, such as bioactivity-guided isolation [4], engineering strategies [5], and genome-focused approaches [4]. However, we are increasingly disturbed by the fact that more and more known natural products were re-isolation, which is incredibly time consuming [4]. To overcome such frustration, precursor-directed biosynthesis (PDB) approach was thus established to selectively and efficiently produce target new natural or semi-natural skeletons [6,7].

Scrutiny of the structures of marketed drugs, we found that both polyketides [8] and indole alkaloids are among the privileged scaffolds [9]. Polyketides represent a viable source of chemically diverse and biologically active natural compounds, of which many have become important clinical therapeutics [10]. Besides, indole alkaloids are one of the hot topics as an important source of lead compounds that have resulted in diverse clinical medicines [11]. Such information reminds us to ask whether the hybrids of polyketides and indoles could be another valuable source of lead compounds. Screening of our fungal library, we found that *D. eschscholzii*, a fungus residing originally in the *Tenodera aridifolia* gut generated a plenty of polyketides without detecting any alkaloids [12,13]. After supplementation of indole-3-carbinol (I3C) in the culture of *D. eschscholzii,* we obtained an antibacterial and anti-inflammatory polyketide-indole hybrid, dalesindole, which was stereoselectively biosynthesized by *D. eschscholzii* through class II aldolase catalyzed C–C bond formation between 3,3′-diindolylmethane (DIM) and C-3 of fungal chromone [14]. Furthermore, two more skeletal polyketide-indole hybrids, named indolchromins A and B, were

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author. State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine, Nanjing, 210023, China.
E-mail address: rxtan@nju.edu.cn (R.X. Tan).

https://doi.org/10.1016/j.synbio.2022.02.004
Received 15 January 2022; Received in revised form 29 January 2022; Accepted 7 February 2022

identified by piecing polyketide and indole together through C–C bond formation [15]. Notably, C–N bond formation is another key step toward the construction of novel skeletons [16] and many cytochrome P450s have been evidenced to be responsible for C–N bond formation [17,18]. Through the bioinformatics analysis, we found that a total of 119 cytochrome P450s have been annotated in the *D. eschscholzii* genome [19] (Fig. 1), encouraging us to hypothesize that the I3C exposed-culture of *D. eschscholzii* could generate new polyketide-indole hybrids through C–N bond formation. Gratifyingly, the endeavor led to the characterization of two new skeletal polyketide-indole hybrids, named indolpolyketone A and B (**1** and **2**), formed through C–N bond formation between polyketide and DIM, an oxidative dimer of I3C. We herein provide details of the structure identification, plausible biosynthetic pathway and biological activities of **1** and **2**.

2. Materials and methods

2.1. General experimental procedures

Silica gel (200–300 mesh) for column chromatography (CC) and GF_{254} (10–20 mm) for TLC (thin layer chromatography) were produced by the Qingdao Marine Chemical Company, China. The ODS silica gel (50 μm) was purchased from YMC Co. Ltd, Japan, and Sephadex LH-20 from Pharmacia Biotech, Sweden. Reagents and solvents used in the study were of analytical grade. HPLC separations were carried out using Agilent ODS column (5 μm, 250 × 10 mm), a 1260 infinity II preparative binary pump G7161A, and a UV detector G7114A. MS spectra were determined on an Agilent 6546 LC/TOF-MS spectrometer operating in a positive mode with direct infusion. NMR spectra were analyzed on a Bruker DRX400 and Varian Inova-600 NMR spectrometer with TMS as an internal standard. Electronic CD (ECD) spectra were acquired on a JASCO J-810 chirascan. Optical rotations were recorded in MeOH on a Rudolph Research Analytical Autopol IV automatic polarimeter. IR spectra were acquired in KBr disks on a Nexus 870 FT-IR spectrometer.

2.2. Fungal cultivation and extraction

Daldinia eschscholzii IFB-TL01 was cultured on Petri dishes of potato dextrose agar (PDA) at 28 °C for 3 days. The fresh mycelium taken from the grown fungal colony was inoculated into the flasks (always 1-L sized), each containing 400 mL of malt-extract medium (20 g/L malt extract, 20 g/L sucrose, 1 g/L peptone). After a 2-day incubation at 28 °C with an agitation of 200 rpm, 20 mL of culture liquid was transferred as inoculating seed into each flask containing 400 mL of ME medium. I3C at the final concentration of 1.0 mM was added into the flasks at 72 h after inoculation. Cultivations were continued for the ensuing 10 days at 28 °C with agitation (200 rpm). The broth was collected and extracted with EtOAc.

2.3. Polyketide-indole hybrids from the I3C-exposed fungal cultivation

In vacuo evaporation of EtOAc from the above extract gave the residue (25.63 g), which was subjected to CC over silica gel with petroleum ether/acetone mixtures (v/v 100:2, 100:5, 10:1, 5:1, 3:1, 2:1, 1:1) to yield seven fractions (A–G). The CC fraction F (5.14 g) derived from the petroleum ether/acetone (2:1) was verified to be alkaloid-rich by the LC-HR-MS analysis, thereby being subjected to CCs over ODS with a gradient of MeOH/H_2O (30:70 → 100:0, v/v) to give seven subfractions

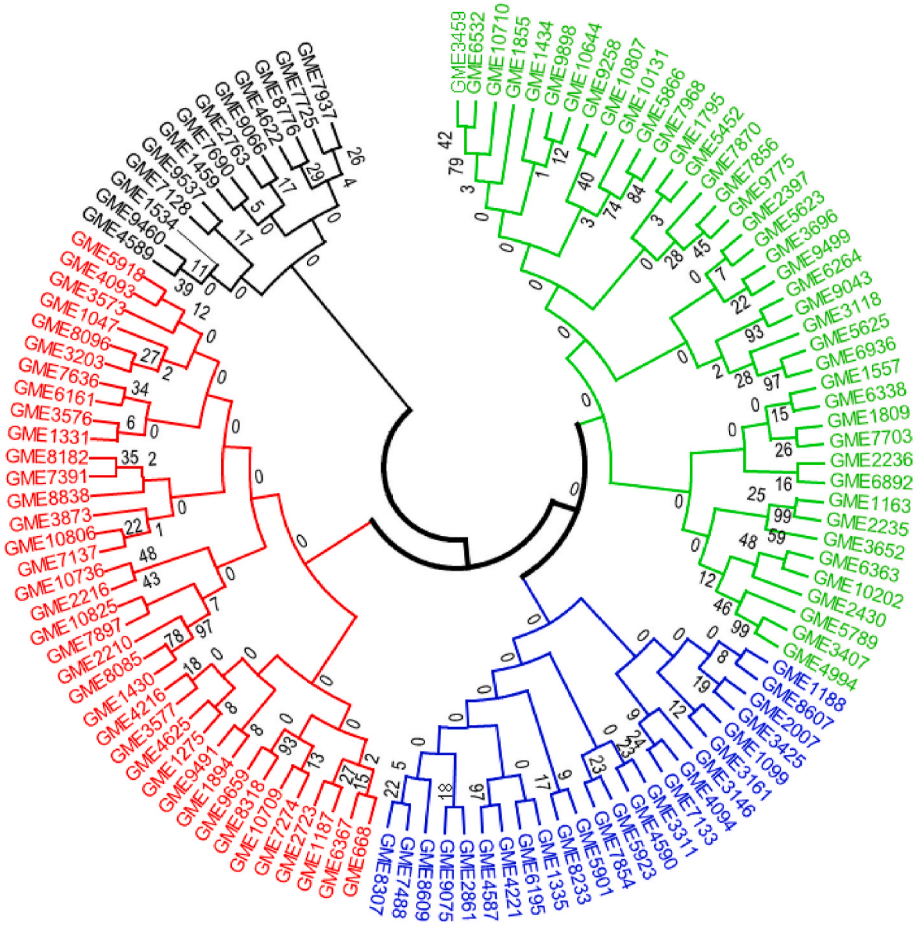

Fig. 1. Phylogenetic analysis-based recognition of cytochrome P450 in the *D. eschscholzii*.

(F1–F7). The subfraction F6 (0.32 g) was then subjected to the semi-preparative RP-HPLC equipped with a xbridge C_{18} PN FWXB 12S05-2510 (10 × 250 mm) using 75% MeOH in water for 30 min (flow rate: 2 mL/min) to yield **1** (5.7 mg, t_R = 17.6 min) and **2** (4.2 mg, t_R = 21.2 min).

2.4. Chiral HPLC separation of 1 and 2

The commercialized chiral column (CHIRALPAK® IA, Lot No. IA00CG-RE001, 10 × 250 mm) was used for the Chiral HPLC resolution of racemates **1** and **2** with *n*-hexane/ethanol (85:15, v/v) as the mobile phase.

2.5. Computational details

The corresponding excited-state calculations were performed at the ground-state optimized geometries. Time-dependent density functional theory (DFT) in combination with polarizable continuum model (PCM, dielectric constant ε = 32.64 for MeOH) (TD-DFT/PCM) with the same basis set was carried out to calculate the spin-allowed excitation energy and rotatory strength of the lowest 100 excited states. The ECD spectra were generated using the program SpecDis [20] by applying a Gaussian band shape with the width of 0.20 eV, from oscillator strengths and dipole-velocity rotational strengths, respectively.

2.6. Cells and viruses [21]

Vero cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in 5% CO_2. Madin-Darby canine kidney (MDCK) cells were grown in DMEM medium supplemented with 10% FBS, 100 U/mL of penicillin and 100 μg/mL of streptomycin. Influenza A virus H1N1 (A/Puerto Rico/8/34), and H1N1 (A/Virginia/ATCC1/2009) were purchased from ATCC (USA), and propagated in 10-day-old embryonated eggs for three days at 36.5 °C.

2.7. Cytopathic effect (CPE) inhibition assay

The antiviral activity was evaluated by the CPE inhibition assay [21]. Briefly, MDCK or Vero cells in 96-well plates were infected with influenza A virus at a multiplicity of infection (MOI) of 0.1, respectively, and then treated with indicated concentrations of compounds in triplicate after removal of virus inoculum. After 24 h incubation, the cells were fixed with 4% formaldehyde for 20 min at room temperature (RT). After removal of the formaldehyde, the cells were stained with 0.1% (w/v) crystal violet for 30 min at 37 °C. The plates were washed and dried, and the intensity of crystal violet staining for each well was measured at 570 nm. The concentration required for a test compound to reduce the CPE of virus by 50% (IC_{50}) was determined.

3. Results

3.1. Phylogenetic analysis-based recognition of cytochrome P450 in D. eschscholzii

The genome of *D. eschscholzii* was deposited at https://www.ncbi.nlm.nih.gov. GenBank: GCA_000751375.2, GenBank: GCA_001951055.1, and GenBank: GCA_000261445.1, respectively. A total of 119 cytochrome P450s have been annotated in the *D. eschscholzii* genome [19] (Fig. 1).

3.2. Structure elucidation

Indolpolyketone A (**1**) was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{27}H_{24}N_2O_2$ on the basis of high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) ion at *m/z* 431.1731 (431.1730 calcd for $C_{27}H_{24}N_2O_2Na$), implying 17° of unsaturation. The ^{1}H NMR spectrum of **1** showed characteristic signals for the presence of a 2-methylchroman-5-ol moiety and a DIM motif by comparing with those of indolchromins A [15] and DIM [22], respectively (Table S1 and Fig. S4-9). However, in the case of **1**, the 2-methylchroman-5-ol motif was found to anchor on the N-1 atom of DIM by the absence of NH-1 signal and the HMBC correlations of the H-4 double doublet at δ 5.88 with C-2', C-10, C-5 and C-2 (Fig. 2). Chiral HPLC of **1** (Fig. S1) afforded (+)-(2*R*,4*R*)-**1** (2.1 mg, t_R = 10.556 min) and (−)-(2*S*,4*S*)-**1** (1.8 mg, t_R = 12.076 min), whose absolute configurations were demonstrated by comparing the recorded and calculated ECD spectra (Fig. 3). In theory, **1** should have four enantiomers, but only two more stable *trans*-oriented enantiomers were obtained owing to be interchangeable via retro-Michael reaction.

Indolpolyketone B (**2**) was obtained as a yellow amorphous powder, which was evidenced to have a molecular formula of $C_{36}H_{31}N_3O_2$ (23° of unsaturation) from the Na^+-liganded molecular ion at *m/z* 560.2307 (560.2308 requires $C_{36}H_{31}N_3O_2Na$) in its HR-ESI-MS. Its ^{1}H and ^{13}C NMR spectral data (Table S2 and Fig. S10-16) were well comparable to those of **1** except for a set of signals ascribable for an additional (3-indolyl)methyl motif, that was shown to attach to C-2″ resonating downfield at δ_C 134.9. Such an indole trimer was demonstrated to anchor on C-4 via a C–N bond with the pentaketide moiety by the absence of NH-1 and 4-carbonyl resonances, but the presence of H-4 signal at δ_H 5.84 (Fig. 2). This assumption was further determined by the HMBC correlations of H-4 with C-2′ and C-7′a (Fig. 2).

Chiral HPLC of **2** (Fig. S2) gave (+)-(2*R*,4*R*)-**2** (1.5 mg, t_R = 7.374 min) and (−)-2*S*,4*S*)-**2** (1.6 mg, t_R = 7.975 min), which were stereochemically assigned by comparing their ECD spectra with those of **1** (Fig. 4). These two enantiomers formed dominantly upon the addition reaction owing to the *trans*-orientation of the 2,4-substituents.

3.3. The putative biosynthetic pathways of 1 and 2

The isolation of **1** and **2** highlights the possibility of expanding the chemical skeleton through hybridizing indole vestiges and polyketides. Therefore, we were encouraged to address the fungal biosynthesis of **1** and **2**. Previously, we identified DIM from the same I3C-exposed fungal culture [22,23], and we have addressed the formation of DIM through three pathways [24]. Due to the slight acidity in the I3C-exposed culture of *D. eschscholzii* (Fig. S3), at least two (CO_2 –liberating and formaldehyde-releasing) pathways may explain the biosynthesis of DIM [24] (Fig. 5). In addition, 1-(2,6-dihydroxyphenyl)but-2-en-1-one (PBEO) was recognized as a reactive polyketide intermediate and results from the orchestration of the polyketide synthase (ChrA) ketoreductase (KR) domain with that of the KR partner (ChrB) in *D. eschscholzii* [19]. Therefore, compound **1** was hypothesized to be produced through the Michael addition of DIM with PBEO, which may be catalyzed by an uncertain P450 enzyme. Compound **2** was generated through another round of Michael addition of **1** with 3-methyleneindolium (3MI, an acidolysis product of I3C) [24] and followed by a Wagner–Meerwein rearrangement (WMR) (Fig. 5).

3.4. Bioactivity tests

The obtained compounds **1** and **2** were assayed for their antiviral activity against H1N1. As a result, **1** and **2** exhibited significant antiviral activity against H1N1 with IC_{50} values of 45.2 and 31.4 μM, respectively.

4. Discussion and conclusion

Natural products are the major source of lead compounds for drug development and represent the majority of small-molecule drugs that were already on the market [25,26]. However, due to the repeated

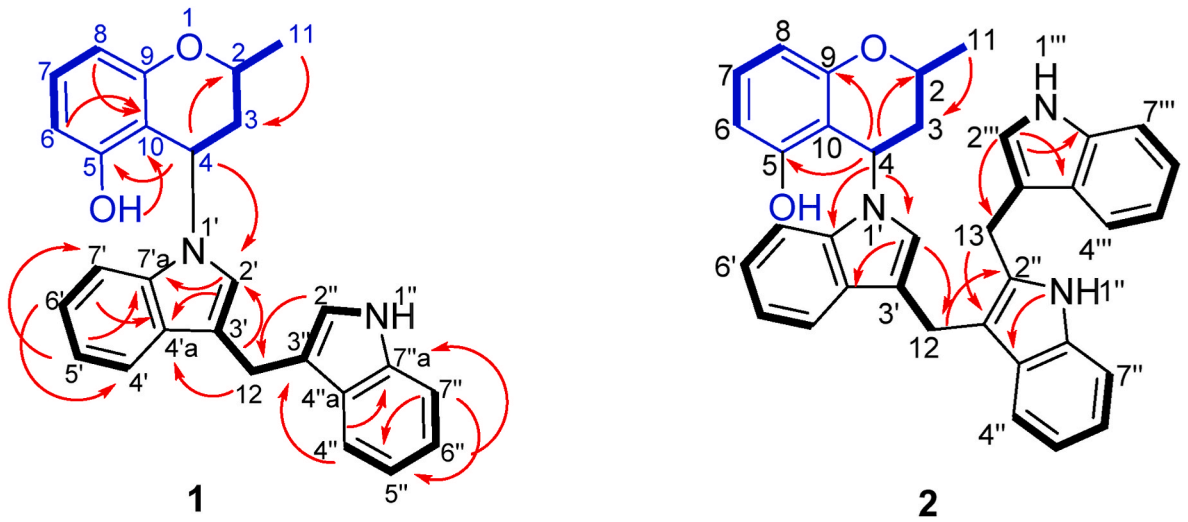

Fig. 2. The structures and key ^{1}H– ^{1}H COSY and HMBC correlations of **1** and **2** (HMBC, red arrows; ^{1}H–^{1}H COSY, bold lines).

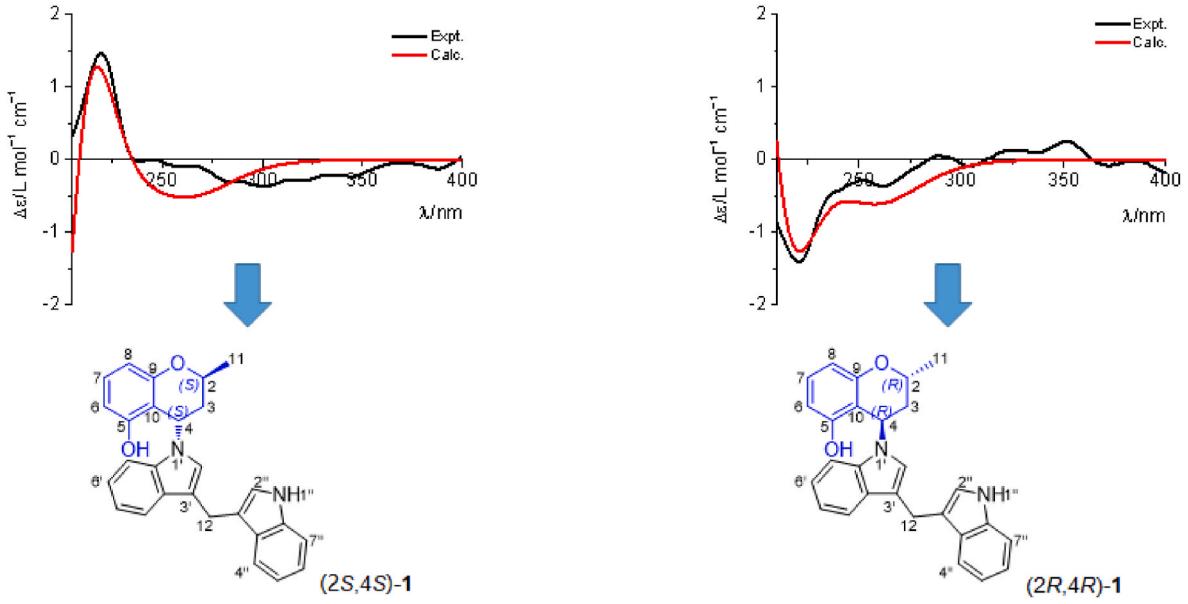

Fig. 3. Comparison of the experimental ECD spectrum (in black) of the compound **1** with those calculated for the optional enantiomers (2*S*,4*S*)- and (2*R*,4*R*)-isomers (in red).

discovery of known compounds, the hit rate of new skeletal compounds come down every year since the study of streptomyces metabolites reached its summit in the 1970s [27]. To further expand the chemical diversity, development of new strategies for the discovery of novel compounds is of great significance.

Recently, precursor-directed biosynthesis (PDB) has become an important approach for the biosynthesis of a variety of semi-natural chemicals. PDB exploits the native biosynthetic machinery of a producing organism in culture medium supplemented with non-native substrates that compete against native substrates *in situ*, thus greatly expands the pharmaceutical library of lead compounds with promising or even enhanced biological performance [28,29]. Although the production of metabolites constructed by PDB are usually in low yield and need to be further purified from a complex mixture, however, through rational design of PDB, such access to target compounds remains economical, practical and environmental benign.

In fact, the key to the successful application of PDB is the selection of the proper platform (such as a fungus) and precursors. *D. eschscholzii* was rich in polyketides that was pieced together through promiscuous couplings of radicals derived from 1,3,6,8-tetrahydroxynaphthalene, 1,3,8-trihydroxynaphthalene and 1,8-dihydroxynaphthalene. Based on the genome of *D. eschscholzii*, *pks*TL gene was found to participate in the biosynthesis of these naphthol-derived polyketides [30,31]. In addition, the combined application of the functional dimorphism of the polyketide synthase ChrA KR with ChrB allows the fungal generation of PBEO, which is inclined to cyclization spontaneously [19]. PBEO is an active intermediate, which facilitates the fungal production of expanded polyketide diversity [19]. Therefore, choosing *D. eschscholzii* as the platform of PDB seems reasonable. A body of work showed that cytochrome P450s involved in the formation of C–N bonds. For example, StaN in *Streptomyces* sp. TP-A0274 is responsible for the C–N bond formation between the nitrogen at N-12 of aglycone and the carbon at C-5' of deoxysugar [32]. TleB from *Streptomyces blastmyceticus* and its homolog HinD from *Streptoalloteichus hindustanus*, were characterized to catalyze unusual intramolecular C–N bond formation to generate indolactam V from the dipeptide *N*-methylvalyl-tryptophanol [33]. A total of 119

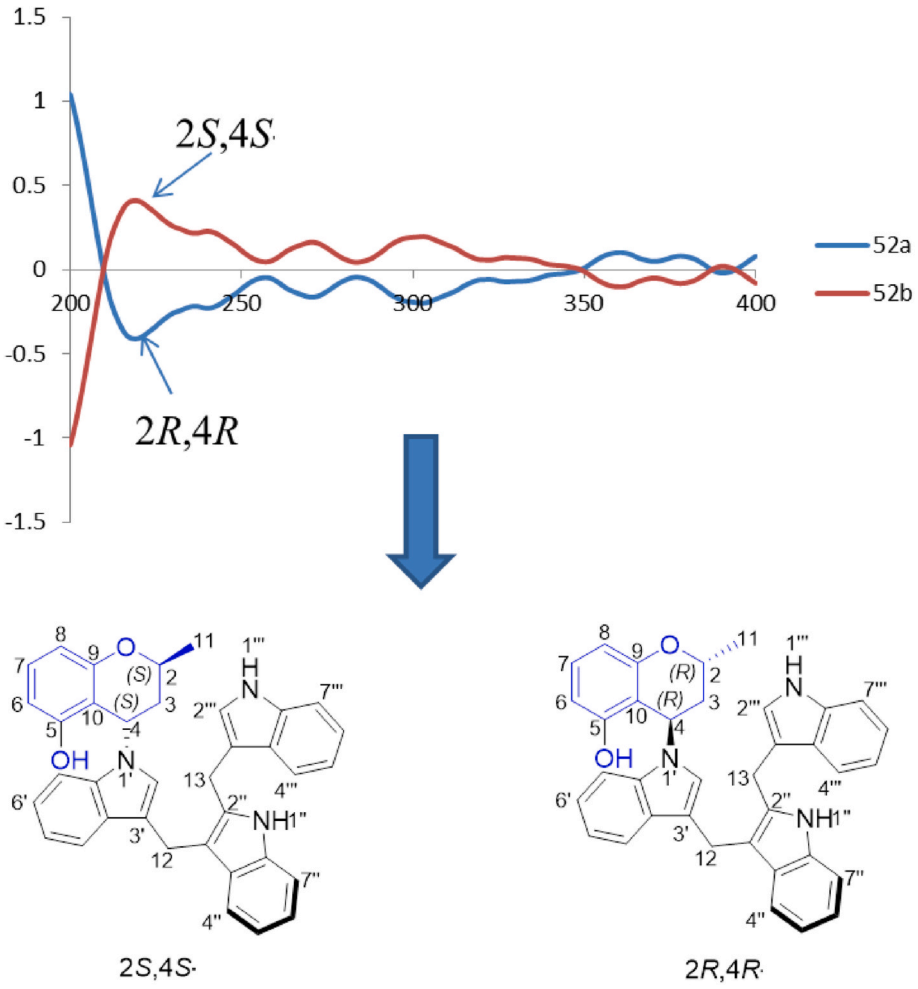

Fig. 4. Absolute configurations assignment of **2** by comparing their ECD spectra with those of **1**.

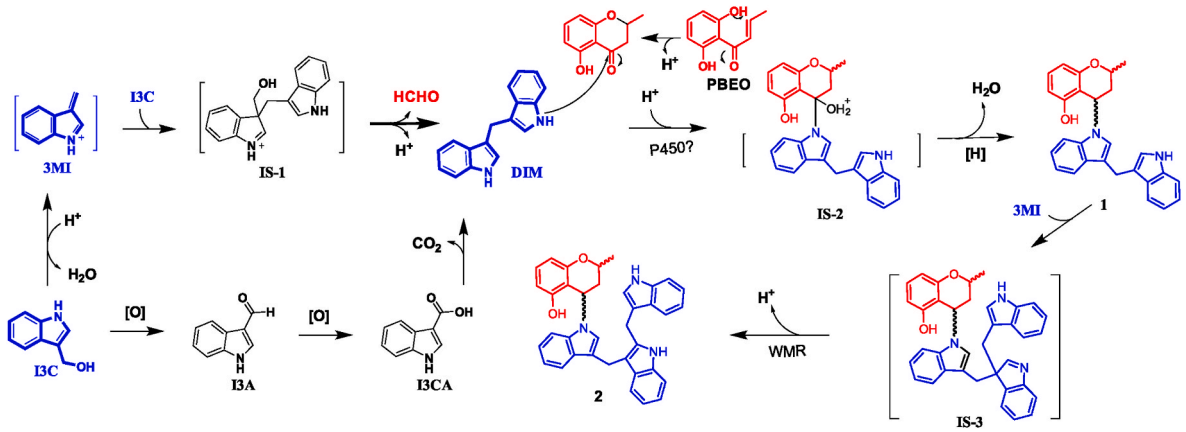

Fig. 5. The putative biosynthetic pathways of **1** and **2** in the I3C-exposed culture of *Daldinia eschscholzii*. I3C, indole-3-carbinol; I3A, indole-3-carbaldehyde; I3CA, indole-3-carboxylic acid; DIM, 3,3′-diindolylmethane; PBEO, 1-(2,6-dihydrox-yphenyl)but-2-en-1-one; 3MI, 3-methyleneindolium; IS, intermediate state; WMR, Wagner–Meerwein rearrangement [24].

cytochrome P450s have been annotated in the *D. eschscholzii* genome [19] (Fig. 1), which endows a great opportunity to construct new compounds by forming C–N bonds. However, further experimental verification is desired to ascertain the exact role and mechanism of the P450 enzyme involved in the C–N bond formation in **1** and **2**.

Additionally, indole-3-carbinol is abundant in cruciferous vegetable and shows cancer-preventive potency in diverse models [34,35]. Under acid environment, I3C dehydrate to form an active intermediate 3MI, which could further polymerize to form oligomeric products, such as DIM and 2-(indol-3-ylmethyl)-3,3′-diindolylmethane (LTr1) [24,36]. The I3C-exposed culture of *D. eschscholzii* was slightly acidic (Fig. S3), thus facilitating the transformation of I3C into 3MI, an acceptable precursor for the PDB-based generation of new chemicals.

In conclusion, **1** and **2** were characterized as skeletally undescribed

polyketide-indole hybrids with the two substructures pieced together through C–N bond formation in I3C-exposed culture of *D. eschscholzii*. This study expands the knowledge about the chemical production of *D. eschscholzii*, thereby increasing the possibility to afford new bioactive molecules that may invigorate the drug discovery pipelines.

CRediT authorship contribution statement

Li Ping Lin: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Min Wu:** Methodology, Investigation. **Nan Jiang:** Software, Validation, Data curation. **Wei Wang:** Methodology, Validation, Data curation. **Ren Xiang Tan:** Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was co-financed by grants from the National Natural Science Foundation of China (NSFC) (82073721 and 81991524) and National Key R&D Program of China (2018YFC1706205).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2022.02.004.

References

[1] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 2015;14:111–29. https://doi.org/10.1038/nrd4510.
[2] Oguri H, Schreiber SL. Skeletal diversity via a folding pathway: synthesis of indole alkaloid-like skeletons. Org Lett 2005;7:47–50. https://doi.org/10.1021/ol047945w.
[3] Crane EA, Gademann K. Capturing biological activity in natural product fragments by chemical synthesis. Angew Chem Int Ed Engl 2016;55:3882–902. https://doi.org/10.1002/anie.201505863.
[4] Bauman KD, Butler KS, Moore BS, Chekan JR. Genome mining methods to discover bioactive natural products. Nat Prod Rep 2021;38:2100–29. https://doi.org/10.1039/d1np00032b.
[5] Klaus M, Grininger M. Engineering strategies for rational polyketide synthase design. Nat Prod Rep 2018;35:1070–81. https://doi.org/10.1039/d1np90026a.
[6] Tian Y, Jiang N, Zhang AH, Chen CJ, Deng XZ, Zhang WJ, et al. Mutamycosynthesis of naphthalene analogs. Org Lett 2015;17:1457–60. https://doi.org/10.1021/acs.orglett.5b00335.
[7] Yan W, Ge HM, Wang G, Jiang N, Mei YN, Jiang R, et al. Pictet-Spengler reaction-based biosynthetic machinery in fungi. Proc Natl Acad Sci USA 2014;111:18138–43. https://doi.org/10.1073/pnas.1417304111.
[8] Khabthani S, Rolain JM, Merhej V. *In silico/in vitro* strategies leading to the discovery of new nonribosomal peptide and polyketide antibiotics active against human pathogens. Microorganisms 2021;9:2297. https://doi.org/10.3390/microorganisms9112297.
[9] Davison EK, Brimble MA. Natural product derived privileged scaffolds in drug discovery. Curr Opin Chem Biol 2019;52:1–8. https://doi.org/10.1016/j.cbpa.2018.12.007.
[10] Hertweck C. The biosynthetic logic of polyketide diversity. Angew Chem Int Ed Engl 2009;48:4688–716. https://doi.org/10.1002/anie.200806121.
[11] Hu Y, Chen S, Yang F, Dong S. Marine indole alkaloids-isolation, structure and bioactivities. Mar Drugs 2021;19:658. https://doi.org/10.3390/md19120658.
[12] Zhang YL, Ge HM, Zhao W, Dong H, Xu Q, Li SH, et al. Unprecedented immunosuppressive polyketides from *Daldinia eschscholzii*, a mantis-associated fungus. Angew Chem Int Ed Engl 2008;47:5823–6. https://doi.org/10.1002/anie.200801284.
[13] Zhang YL, Zhang J, Jiang N, Lu YH, Wang L, Xu SH, et al. Immunosuppressive polyketides from mantis-associated *Daldinia eschscholzii*. J Am Chem Soc 2011;133:5931–40. https://doi.org/10.1021/ja110932p.
[14] Lin LP, Yuan P, Jiang N, Mei YN, Zhang WJ, Wu HM, et al. Gene-inspired mycosynthesis of skeletally new indole alkaloids. Org Lett 2015;17:2610–3. https://doi.org/10.1021/acs.orglett.5b00882.
[15] Lin L, Jiang N, Wu H, Mei Y, Yang J, Tan R. Cytotoxic and antibacterial polyketide-indole hybrids synthesized from indole-3-carbinol by *Daldinia eschscholzii*. Acta Pharm Sin B 2019;9:369–80. https://doi.org/10.1016/j.apsb.2018.09.011.
[16] Majeed K, Wang L, Liu B, Guo Z, Zhou F, Zhang Q. Metal-free tandem approach for triazole-fused diazepinone ccaffolds via [3 + 2]cycloaddition/C-N coupling reaction. J Org Chem 2021;86:207–22. https://doi.org/10.1021/acs.joc.0c02022.
[17] Wei J, Liu Y. Mechanistic insights into the P450 TleB-catalyzed unusual intramolecular C-N bond formation involved in the biosynthesis of Indolactam V. J Chem Inf Model 2021;61:3638–48. https://doi.org/10.1021/acs.jcim.1c00542.
[18] Morita I, Mori T, Abe I. Enzymatic formation of indolactam scaffold by C-N bond-forming cytochrome P450 oxidases in teleocidin biosynthesis. Chemistry 2021;27:2963–72. https://doi.org/10.1002/chem.202003899.
[19] Zhou ZZ, Zhu HJ, Lin LP, Zhang X, Ge HM, Jiao RH, et al. Dalmanol biosyntheses require coupling of two separate polyketide gene clusters. Chem Sci 2018;10:73–82. https://doi.org/10.1039/c8sc03697g.
[20] Bruhn T, Schaumlöffel A, Hemberger Y, Bringmann G. SpecDis: quantifying the comparison of calculated and experimental electronic circular dichroism spectra. Chirality 2013;25:243–9. https://doi.org/10.1002/chir.22138.
[21] Wang W, Yin RJ, Zhang M, Yu RL, Hao C, Zhang LJ, et al. Boronic acid modifications enhance the anti-influenza A virus activities of novel quindoline derivatives. J Med Chem 2017;60:2840–52. https://doi.org/10.1021/acs.jmedchem.6b00326.
[22] Lin LP, Yuan P, Jiang N, Mei YN, Zhang WJ, Wu HM, et al. Gene-inspired mycosynthesis of skeletally new indole alkaloids. Org Lett 2015;17:2610–3. https://doi.org/10.1021/acs.orglett.5b00882.
[23] Lin LP, Tan RX. Bioactive alkaloids from indole-3-carbinol exposed culture of *Daldinia Eschscholzii*. Chin J Chem 2018;36. https://doi.org/10.1002/cjoc.201880085. 749-53.
[24] Lin LP, Liu D, Qian JC, Wu L, Zhao Q, Tan RX. Post-ingestion conversion of dietary indoles into anticancer agents. Natl Sci Rev 2021. https://doi.org/10.1093/nsr/nwab144. nwab144.
[25] Kunyavskaya O, Tagirdzhanov AM, Caraballo-Rodríguez AM, Nothias LF, Dorrestein PC, Korobeynikov A, et al. Nerpa: a tool for discovering biosynthetic gene clusters of bacterial nonribosomal peptides. Metabolites 2021;11:693. https://doi.org/10.3390/metabo11100693.
[26] Caesar LK, Kellogg JJ, Kvalheim OM, Cech NB. Opportunities and limitations for untargeted mass spectrometry metabolomics to identify biologically active constituents in complex natural product mixtures. J Nat Prod 2019;82:469–84. https://doi.org/10.1021/acs.jnatprod.9b00176.
[27] Cheng J, Zhang XL, Zhao JY, Wen ML, Ding ZG, Li MG. The recent progress of study on secondary metabolites of streptomyces, vol. 40; 2015. p. 791–800. https://doi.org/10.13461/j.cnki.cja.005629.
[28] Adhikari K, Lo IW, Chen CL, Wang YL, Lin KH, Zadeh SM, et al. Chemoenzymatic synthesis and biological evaluation for bioactive molecules derived from bacterial benzoyl coenzyme A ligase and plant type III polyketide synthase. Biomolecules 2020;10:738. https://doi.org/10.3390/biom10050738.
[29] Reitz ZL, Butler A. Precursor-directed biosynthesis of catechol compounds in *Acinetobacter bouvetii* DSM 14964. Chem Commun (Camb) 2020;56:12222–5. https://doi.org/10.1039/d0cc04171h.
[30] Chan CL, Yew SM, Ngeow YF, Na SL, Lee KW, Hoh CC, et al. Genome analysis of *Daldinia eschscholtzii* strains UM 1400 and UM 1020, wood-decaying fungi isolated from human hosts. BMC Genom 2015;16:966. https://doi.org/10.1186/s12864-015-2200-2.
[31] Fang W, Ji S, Jiang N, Wang W, Zhao GY, Zhang S, et al. Naphthol radical couplings determine structural features and enantiomeric excess of dalesconols in *Daldinia eschscholzii*. Nat Commun 2012;3:1039. https://doi.org/10.1038/ncomms2031.
[32] Onaka H, Asamizu S, Igarashi Y, Yoshida R, Furumai T. Cytochrome P450 homolog is responsible for C-N bond formation between aglycone and deoxysugar in the staurosporine biosynthesis of *Streptomyces* sp. TP-A0274. Biosci Biotechnol Biochem 2005;69. https://doi.org/10.1271/bbb.69.1753. 1753-9.
[33] He F, Mori T, Morita I, Nakamura H, Alblova M, Hoshino S, et al. Molecular basis for the P450-catalyzed C-N bond formation in indolactam biosynthesis. Nat Chem Biol 2019;15:1206–13. https://doi.org/10.1038/s41589-019-0380-9.
[34] Weng JR, Tsai CH, Kulp SK, Chen CS. Indole-3-carbinol as a chemopreventive and anti-cancer agent. Cancer Lett 2008;262:153–63. https://doi.org/10.1016/j.canlet.2008.01.033.
[35] Fujioka N, Fritz V, Upadhyaya P, Kassie F, Hecht SS. Research on cruciferous vegetables, indole-3-carbinol, and cancer prevention: a tribute to Lee W. Wattenberg. Mol Nutr Food Res 2016;60:1228–38. https://doi.org/10.1002/mnfr.201500889.
[36] De Kruif CA, Marsman JW, Venekamp JC, Falke HE, Noordhoek J, Blaauboer BJ, et al. Structure elucidation of acid reaction products of indole-3-carbinol: detection *in vivo* and enzyme induction *in vitro*. Chem Biol Interact 1991;80:303–15. https://doi.org/10.1016/0009-2797(91)90090-t.