Title

Enzymatic Benzofuranoindoline Formation in the Biosynthesis of the Strained Bridgehead Bicyclic Dipeptide (+)-Azonazine A.

Journal

Angewandte Chemie, 62(40)

Authors

Liu, Mengting
Ohashi, Masao
Zhou, Qingyang
et al.

Publication Date

2023-10-02

DOI

10.1002/anie.202311266

Peer reviewed

HHS Public Access

Author manuscript

Angew Chem Int Ed Engl. Author manuscript; available in PMC 2024 April 02.

Published in final edited form as:

Angew Chem Int Ed Engl. 2023 October 02; 62(40): e202311266. doi:10.1002/anie.202311266.

Enzymatic Benzofuranoindoline Formation in the Biosynthesis of the Strained Bridgehead Bicyclic Dipeptide (+)-Azonazine A

Mengting Liu,
Department of Chemical and Biomolecular Engineering; Department of Chemistry and Biochemistry University of California, Los Angeles, California 90095, USA

Masao Ohashi[*],
Department of Chemical and Biomolecular Engineering; Department of Chemistry and Biochemistry University of California, Los Angeles, California 90095, USA

Qingyang Zhou,
Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, USA

Jacob N. Sanders,
Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, USA

Erin P. McCauley,
Department of Chemistry and Biochemistry, California State University–Dominguez Hills, Carson, California 90747, USA

Phillip Crews,
Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, USA

K. N. Houk,
Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, USA

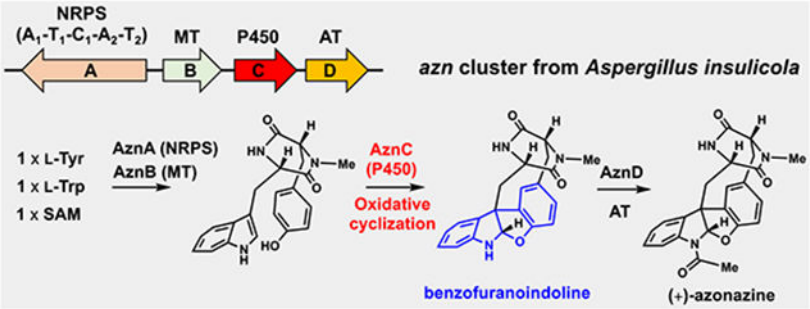
Yi Tang[*]
Department of Chemical and Biomolecular Engineering; Department of Chemistry and Biochemistry University of California, Los Angeles, California 90095, USA; Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, USA

Abstract

We uncovered and reconstituted a concise biosynthetic pathway of the strained dipeptide (+)-azonazine A from marine-derived *Aspergillus insulicola.* Formation of the hexacyclic benzofuranoindoline ring system from cyclo-(L-Trp–*N*-methyl-L-Tyr) is catalysed by a P450 enzyme via an oxidative cyclization. Supplementing the producing strain with various indole-substituted tryptophan derivatives resulted in the generation of a series of azonazine A analogues.

[*] gph422001@ucla.edu; yitang@ucla.edu, Twitter handles: @phdmasao, @yitang_ucla.

Graphical Abstract



We uncovered the biosynthetic pathway of the strained dipeptide (+)-azonazine A from marine-derived *Aspergillus insulicola* that features a rarely observed benzofuranoindoline ring system. Heterologous reconstitution of the fourth-enzyme pathway showed a P450 catalyzes an oxidative cyclization of cyclo-(L-Trp–*N*-methyl-L-Tyr) to form the hexacyclic ring system. Analogues of azonazine A were generated by supplementing the producing strain with different indole-substituted tryptophan analogues.

Keywords

oxidative cyclization; benzofuranoindolines; biosynthesis; indole alkaloids

Amino acids with nitrogen-containing side chains are important building blocks to construct and diversify structures of alkaloid natural products.[1] Many nitrogen-containing heterocycles such as piperidine, pyrazine, pyridine, quinolones, and quinazoline are derived from these amino acid in biosynthetic pathways.[2] A notable heterocycle derived from the coupling of tyrosine and tryptophan is the hemiaminal benzofuranoindoline, which can be found in complex natural products such as diazonamide A,[3] bipleophylline,[4] and (+)-azonazine (**1**)[5] (Figure 1A). Numerous synthetic methods for preparation of these benzofuranoindoline natural products have been developed, although stereoselective synthesis remains challenging.[6] In contrast, biosynthesis of the benzofuranoindoline ring system has not been reported to date, and consequently the chemical logic of tetracyclic heterocycle formation remains unknown.

The hexacyclic dipeptide (+)-azonazine A (**1**), isolated from *Aspergillus insulicola* (strain no. 088708a), has anti-inflammatory activity through the inhibition of NF-κB activity and NO production.[5] **1** contains a benzofuranoindoline ring system that results in a highly strained diketopiperazine (DKP). While the absolute stereochemistry of **1** was originally proposed based on electronic circular dichroism (ECD) spectroscopy,[5] the total synthesis of *ent*-**1** by Yao et al.,[6g-h] later revised the absolute stereochemistry of **1** as 2*S*, 10*R*, 11*S*, 19*S*. Therefore, **1** is derived from L-tryptophan and L-tyrosine instead of their D-enantiomers as initially proposed. The first total synthesis of *ent*-**1** featured biomimetic oxidative cyclization[7] of cyclo-(D-Trp–*N*-Me-D-Tyr) *ent*-**2** using hypervalent-iodine (III) as the oxidation reagent to form the benzofuranoindoline core, which gave an equal ratio of *ent*-**1** and the diastereomer.[6g-h] The relatively low yield of the cyclized products indicated

that it is challenging to construct the congested hexacyclic core of **1**. This implies that Nature must use dedicated enzymes to stereoselectively construct the benzofuranoindoline core from a DKP precursor. We now report the concise biosynthetic pathway of **1**, which involves a key P450-catalyzed oxidative phenol-indole coupling reaction.

To identify the biosynthetic gene cluster (BGC) of **1**, we first sequenced the **1**-producing strain *A. insulicola*. Based on the retro-biosynthetic analysis (Figure 1B), the BGC of **1** should encode at least i) a two-module nonribosomal peptide synthetase (NRPS), homologous to other DKP synthetases such as NotE from the notoamide pathway;[8] ii) an *N*-methyltransferase (*N*-MT) to account for the *N*-Me-L-Tyr; iii) an acetyltransferase (AT) for *N*-acetylation of the indole nitrogen; and iv) a redox enzyme for oxidative cyclization of the benzofuranoindoline. Guided by this proposal, we located the putative *azn* BGC (Figure 2A), which encodes a two-module NRPS (AznA) with the domain architecture of ATCAT, a predicted *N*-MT (AznB), a P450 (AznC), and a predicted AT (AznD). An orthologous BGC was found in the genome of *Aspergillus flocculosus*, which shares 99% identity to the *azn* cluster (Figure S3).

To determine the functions of enzymes in the *azn* cluster, we performed heterologous expression of *aznA-D* in *Aspergillus nidulans* A1145 ΔSTΔEM strain.[9] After three days of culture on CD-ST agar, the fungal strain produced a new compound with the same retention time and molecular weight as **1** with an isolated titer of ~3 mg/L (Figure 2B, trace iv, and Figure S4). Upon purification from scaled-up culture, the compound was assigned to be **1** by NMR, HRMS as well as optical rotation (Table S4 and Figures S16-S18). We therefore confirmed that the *azn* cluster is the BGC of **1**.

To understand the chemical logic in biosynthesis of **1**, we next performed a gene-by-gene reconstitution of the *azn* cluster in *A. nidulans*. When AznA was the sole enzyme expressed, no new products were observed compared to the vector-only control (Figure 2B, trace i). This is consistent with the bioinformatics prediction that AznA does not contain a releasing domain; such a terminal condensation (C_T) domain is found in other DKP-forming NRPSs.[10] However, coexpression of AznA and the methyltransferase (AznB) led to the formation of a new product **2** with molecular weight of 363 (Figure 2B, trace ii). Large-scale cultivation of the transformant allowed us to isolate **2** (~8 mg/L), which was structurally determined to be cyclo-(L-Trp–*N*-Me-L-Tyr). This suggests that the installation of α-*N*-Me group on L-Tyr is essential for the formation and release of the DKP from AznA. To examine the timing of *N*-methylation, AznB was expressed and purified from *Escherichia coli* BL21 (DE3) (Figure S5), and assayed with L-Tyr or cyclo-(L-Trp–L-Tyr) as substrate. While no methylation was detected using cyclo-(L-Trp–L-Tyr), AznB readily catalyzed the *N*-methylation of L-Tyr with SAM to form *N*-Me-L-Tyr (Figure 2C and Figure S7). This suggested that one of the A domains in AznA activates and incorporates *N*-Me-L-Tyr to form **2**. To test this, we supplemented *N*-Me-L-Tyr to *A. nidulans* expressing only AznA. As expected, the accumulation of **2** was only observed upon *N*-Me-L-Tyr feeding (Figure S6).

AznA lacks the C_T domain that catalyzes intramolecular cyclization and product release found in other DKP synthetases such as NotE[8] or GliP[11] (Figure S1). Therefore, release of **2** from AznA is likely nonenzymatic. *N*-methylation of L-Tyr by AznB is essential to facilitate

this step. Mechanistically, the formation of cyclodipeptide must require a *cis*-configured peptide bond between the two amino acids in order to position the nucleophilic *N*-terminal amine (N_a-amine) in a conformation to attack the electrophilic thioester carbonyl.[12] It was shown that *N*-alkylation of the second amino acid from *N*-terminus in peptides changes the conformation of amide bond configuration from *trans* to *cis*.[13] We therefore reason that while A_1 domain of AznA, which has moderate identity (37%) with the L-Trp activating A_1 domain of NotE,[8] activates L-Trp; while *N*-Me-L-Tyr is activated by A_2 domain of AznA. C_1 domain of AznA then catalyzes the condensation of T_2-tethered *N*-Me-L-Tyr with T_1-tethered L-Trp to form the T_2-tethered linear L-Trp–*N*-Me-L-Tyr as shown in Figure 2E. The *cis*-configured amide bond then facilitates the nonenzymatic nucleophilic attack of N_a-amine of L-Trp to the T_2-tethered thioester to release **2** from AznA.

To determine the subsequent biosynthetic steps, including the formation of benzofuranoindoline core, additional *azn* genes were then coexpressed with *aznAB*. Coexpression of the acyltransferase *aznD* with *aznAB* in *A. nidulans* did not give any new compounds (Figure S4). However, when the P450 *aznC* was coexpressed with *aznAB* in *A. nidulans*, two new metabolites **3** and **3'** with MW=361 were produced (Figure 2B, trace iii). We performed a large-scale cultivation of the *A. nidulans* transformant and isolated **3** (8 mg/L). NMR and HRMS characterization revealed the structure of **3**, which contains the strained benzofuranoindoline ring system as in **1** (Table S6, Figures S24-S29). **3** and **3'** can interconvert during purification and characterization, with **3** being the dominant form in NMR solvent (DMSO-d_6). Given the interchangeable nature of **3** and **3'**, the structure of **3'** is proposed to be the imine form of **3** that results from the C11-O7 bond cleavage of the hemiaminal. The biosynthesis of **3** suggests that AznC alone is sufficient to catalyze an oxidative cyclization of **2** into benzofuranoindoline **3** with the formation of C8—C10 and C11-O7 bonds. To confirm the substrate and the function of AznC, we supplemented **2** to the *A. nidulans* transformant expressing only AznC, which led to the direct formation of **3** and **3'** (Figure 2D, trace ii). When directly assayed with **2,** the microsomal fraction of the *A. nidulans* transformant converted **2** to **3,** albeit with low efficiency (Figure S8). This evidence strongly supports the proposal that AznC is solely responsible for the oxidative cyclization of **2** to **3**. Further expression of AznD led to conversion of of **3** to **1**, thereby completing the biosynthetic pathway (Figure 2B, trace iv). The putative imine form of **1** analogous to **3'** was not observed, which is expected following acylation of the indole nitrogen that leads to decreased electron density on N_1. Hence, the logic of the *N*-acylation as the final step is to prevent unraveling of the newly formed benzofuranoindoline ring system.

Biosynthetic pathways of DKP natural products such as (−)-ditryptophenaline often involve a P450 that catalyzes intermolecular or intramolecular couplings to generate structural complexity.[14-17] AznC is most similar to AneD[18] from the aculene BGC and share ~43% sequence identity. However, AznC facilitates the unprecedented intramolecular oxidative cyclization that forms two new bonds (one C-C, one C-O) stereoselectively. Based on proposed mechanisms of other intramolecular coupling P450s, such as CYP121,[16] DtpC,[12] and HqlC,[17] abstraction of hydrogen atom from OH or NH by a P450 must be the first step to generate a reactive radical species for oxidative coupling reactions. Therefore, the abstraction of the phenolic (O7) and/or the indole *N*1-hydrogen in **2** is likely catalyzed by

AznC. To probe the mechanism of this reaction, we first prepared cyclo (L-Trp–*N*-Me-L-Phe) (**7**) as the analogue of **2** lacking the phenolic OH and cyclo (1-Me-L-Trp–N-Me-L-Tyr) (**8**) as the analogue of **2** lacking the indole NH (Figure 2E). Feeding *N*-Me-L-Phe or 1-Me-L-Trp to the *A. nidulans* transformant expressing AznA or AznA with AznB led to the production of **7** or **8**, respectively. Both **7** (Table S7, and Figures S30-S31) and **8** (Table S8 and Figures S32-S36) were purified and structurally verified. With **7** and **8** in hand, we next supplied each compound to the *A. nidulans* transformant expressing AznC. However, feeding neither **7** nor **8** led to the formation of any new product. This suggests that both phenolic hydrogen and indole hydrogen in **2** are required for the AznC-catalysis.

Based on these data and the proposed mechanism of P450-catalyzed reactions reported by others,[14-20] several mechanisms for the AznC-catalyzed oxidative [3+2]-cyclization such as a diradical recombination or a single radical mechanism can be proposed. To investigate the plausible mechanism of the AznC-catalyzed reaction, we performed DFT calculations on the transformation of **2** to **3**. As shown in Figure 3, the reaction can be initiated by a H-abstraction of the indole N—H bond (**TS1**) or the phenol O-H bond (**TS2**) by the ferryl-oxo Compound I of AznC, which can generate an indolyl (**Int1**) or a phenolyl radical (**Int2**). While **TS1** has a lower barrier, **TS2** leads to a thermodynamically more stable product (**Int2**). Given the comparable low energy barriers of both pathways, both routes are plausible. The resultant compound II of AznC can readily abstract the indole N-H or the phenolic O-H, generating the diradical intermediate **Int3**. Additionally, we explored the potential direct attack of radical **Int1**/**Int2** on the phenol/indole aromatic ring, but observed higher energy barriers that disfavor such reactions (Figure S12). **Int3** then undergoes radical recombination via **TS5** with a low coupling barrier (11.1 kcal/mol) to form the ketone intermediate **Int4**. The resulting C7-ketone in **Int4** readily tautomerizes to form the phenolic alcohol (**Int5**), which subsequently attacks the C11-indole imine from the *si*-face leading to the thermodynamically stable product **3** (ΔG_{rxn} = −19.7 kcal/mol). Since the newly formed stereocenter at C11 could be strictly controlled by the stereochemistry of C10 and the strained structure of **Int5**, the stereoselective formation of C11-O7 bond may not require enzymatic control. The spontaneous hemiaminal formation in the active site of AznC supports the observation that **3'** (the putative imine form) and **3** are interconvertible.

A similar P450-mediated tandem one-electron C-C coupling followed by two-electron intramolecular cyclization was proposed in the biosynthesis of duclauxins.[21] Another notable example for such tandem reaction is proposed for the biosynthesis of communesin.[22] The P450 CnsC catalyzes the hetero radical-radical coupling reaction between the indole moieties of tryptamine and aurantioclavin, and forms a heterodimer connected by vicinal quaternary stereocenters. The resulting heterodimer is subjected to intramolecular nucleophilic attack from the two amine groups on the electrophilic indole imines to form the heptacyclic communesin.[22] AznC, DuxL, and CnsC are all proposed to generate an electrophilic imine or ketone next to a quaternary carbon resulted from one-electron C-C coupling. The quaternary carbon prevents tautomerization of the imine (or ketone) to enamine (or enol), and leads to subsequent nucleophilic intramolecular cyclization. Therefore, this efficient strategy using both one and two electron chemistries employed by those P450s is a general strategy to form complex heterocyclic scaffolds. We expect that

this chemical logic will be found in biosynthesis of other multiheterocyclic natural products such as diazonamide A and bipleiophylline.

Our reconstitution data showed that only three enzymes AznA-C are sufficient to biosynthesize the strained benzofuranoindoline **3**, compared to the reported chemical synthesis which requires over 10 synthetic steps. The heterologous expression system in *A. nidulans* can be leveraged to generate azonazine analogues. To test this, we supplemented twelve L-Trp analogues (4-F-L-Trp, 4-Br-L-Trp, 5-F-L-Trp, 5-Cl-L-Trp, 5-Br-L-Trp, 6-F-L-Trp, 6-Cl-L-Trp, 6-Br-L-Trp, 7-Br-L-Trp, 7-Me-L-Trp, 5-OH-L-Trp, and 5-NO_2-L-Trp) to *A. nidulans* transformant expressing *aznA-C*. All of the examined L-Trp analogues except for 5-OH-L-Trp and 5-NO_2-L-Trp supported the biosynthesis of the corresponding analogues of **2,** as detected by selected ion chromatography (Figure S13). The 5-Br- and 6-Br-L-Trp analogues (**16** and **22**) of **2** were isolated from large-scale cultivation of the *A. nidulans* transformant, and structurally characterized by NMR (Tables S9, S11, and Figures S37-S41, S47-S51). This suggests that A and C domains of AznA have relaxed substrate scope towards indole-substituted L-tryptophans. Furthermore, analogues of **3** can be detected in the feeding studies, except when 4-Br-L-Trp, 5-OH-L-Trp, 5-NO_2-L-Trp, and 7-Br-L-Trp were used (Table 1 and Figure S13). The 5-Br- and 6-Br-L-Trp analogues of **3** (**17** and **23**) were isolated from the large-scale cultivations at titers of ~3 mg/L (Table 1), and structurally confirmed by NMR (Tables S10, S12, and Figures S42-S46, S52-S56). Lastly, analogues of **1** were also detected when we supplemented the 5- and 6-substituted L-tryptophan analogues to the *A. nidulans* co-expressing *aznA-D* (Table 1 and Figure S14). 5-F-L-Trp and 5-Cl-L-Trp derived analogues of **1** (**27** and **28**) were also isolated with titers of ~8 mg/L and 4 mg/L (Table 1), and were structurally determined to be 5-F-L-Trp-azonazine and 5-Cl-L-Trp-azonazine (Tables S13-S14, and Figures S57-S66), respectively.

In conclusion, we reconstituted the biosynthesis of **1** using four enzymes from the *azn* BGC, and uncovered the first biosynthetic pathway to the benzofuranoindoline core through the oxidative phenol-indole dihydrofuran-forming coupling reaction catalyzed by the P450 AznC. Our finding enriches the catalytic repertoire of P450s and facilitates the discovery of new benzofuranoindoline-containing natural products by genome mining.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIGMS R35GM118056 to YT and NSF CHE-2153972 to KNH.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

References

[1]. (a)Almhjell PJ, Boville CE, Arnold FH, Chem. Soc. Rev, 2018, 47, 8980–8997. [PubMed: 30280154] (b)Blaskovich MA, J. Med. Chem 2016, 59, 10807–10836. [PubMed: 27589349] (c)Walsh CT, The Chemical Versatility of Natural-Product Assembly Lines. Acc. Chem. Res 2008, 41, 4–10. [PubMed: 17506516]

[2]. (a)Hedges JB, Ryan KS, Chem. Rev 2020, 120, 3161–3209. [PubMed: 31869221] (b)Joule JA, Chapter Four - Natural Products Containing Nitrogen Heterocycles—Some Highlights 1990–2015. In Heterocyclic Chemistry in the 21st Century.(c)Scriven E, Ramsden CA, Heterocyclic Chemistry in the 21st Century; Academic Press. Advances in Heterocyclic Chemistry 2016, 119, 81–106.

[3]. Lindquist N, Fenical W, J. Am. Chem. Soc 1991, 113, 2303–2304.

[4]. Kam TS, Tan SJ, Ng SW, and Komiyama K, Org. Lett 2008, 10, 3749–3752. [PubMed: 18683934]

[5]. Wu QX, Crews MS, Draskovic M, Sohn J, Johnson TA, Tenney K, Valeriote FA, Yao XJ, Bjeldanes LF, and Crews P, Org. lett 2010, 12, 4458–4461. [PubMed: 20866076]

[6]. (a)Li J, Burgett AWG, Esser L, Amezcua C, Harran PG, Angew. Chem. Int. Ed 2001, 113, 4906–4909.(b)Nicolaou KC, Hao JL, Reddy MV, Rao PB, Rassias G, Snyder SA, Huang XH, Chen DY-K, Brenzovich WE, Giuseppone N, Giannakakou P, and O'Brate A, J. Am. Chem. Soc 2004, 126, 12897–12906. [PubMed: 15469287] (c)Nicolaou KC, Bheema Rao P, Hao J, Reddy MV, Rassias G, Huang X, Chen DYK, Snyder SA, Angew. Chem. Int. Ed 2003, 115, 1795–1800. (d)Cheung CM, Goldberg FW, Magnus P, Russell CJ, Turnbull R, and Lynch V, J. Am. Chem. Soc 2007, 129, 12320–12327. [PubMed: 17880079] (e)Burgett AWG, Li QY, Wei Q, Harran PG, Angew. Chem. Int. Ed 2003, 42, 4961–4966.(f)Lachkar D, Denizot N, Bernadat G, Ahamada K, Beniddir MA, Dumontet V, Gallard JF, Guillot R, Leblanc K, N'Nang EO, Turpin V, Kouklovsky C, Poupon E, Evanno L, Vincent G, Nat. Chem 2017, 9, 793–798. [PubMed: 28754932] (g)Zhao JC, Yu SM, Liu Y, and Yao ZJ, Org. Lett 2013, 15, 4300–4303. [PubMed: 23931155] (h)Zhao JC, Yu SM, Qiu HB, Yao ZJ, Tetrahedron 2014, 70, 3197–3210.

[7]. (a)Denizot N, Pouilhes A, Cucca M, Beaud R, Guillot R, Kouklovsky C, Vincent G, Org. Lett 2014, 16, 5752–5755. [PubMed: 25347388] (b)Liu K, Tang S, Huang P, Lei A, Nat. Commun 2017, 8, 775. [PubMed: 28974679] (c)Blum TR, Zhu Y, Nordeen SA, Yoon TP, Angew. Chem. Int. Ed 2014, 53, 11056–11059.

[8]. Li SY, Srinivasan K, Tran H, Yu F, Finefield JM, Sunderhaus JD, McAfoos TJ, Tsukamoto S, Williams RM, Sherman DH, Medchemcomm 2012, 3, 987–996. [PubMed: 23213353]

[9]. Liu N, Hung YS, Gao SS, Hang L, Zou Y, Chooi Y-H, Tang Y, Org. Lett 2017, 19, 3560–3563. [PubMed: 28605916]

[10]. (a)Gao X, Haynes SW, Ames BD, Wang P, Vien LP, Walsh CT, Tang Y, Nat. Chem. Biol 2012, 8, 823–30. [PubMed: 22902615] (b)Haynes SW, Ames BD, Gao X, Tang Y, Walsh CT, Biochemistry 2011, 50, 5668–79. [PubMed: 21591693]

[11]. Baccile JA, Le HH, Pfannenstiel BT, Bok JW, Gomez C, Brandenburger E, Hoffmeister D, Keller NP, Schroeder FC, Angew. Chem. Int. Ed 2019, 58, 14589–14593.

[12]. Fischer PM, J. Pept. Sci 2003, 9, 9–35. [PubMed: 12587880]

[13]. (a)Misicka A, Verheyden PMF, Binst GV, Letters in Peptide Science 1998, 5, 375–377.(b)Kessler H, Angew. Chem. Int. Ed 1970, 9, 219–235.(c)Berry D, Mace W, Grage K, Wesche F, Gore S, Schardl CL, Young CA, Dijkwel PP, Leuchtmann A, Bode HB, Scott B, Proc. Natl. Acad. Sci. U.S.A 2019, 116, 25614–25623. [PubMed: 31801877]

[14]. Saruwatari T, Yagishita F, Mino T, Noguchi H, Hotta K, Watanabe K, Chembiochem 2014, 15, 656–659. [PubMed: 24677498]

[15]. Liu J, Liu A, Hu Y, Nat. Prod. Rep 2021, 38, 1469–1505. [PubMed: 33404031]

[16]. Shende VV, Khatri Y, Newmister SA, Sanders JN, Lindovska P, Yu F, Doyon TJ, Kim J, Houk KN, Movassaghi M, Sherman DH, J. Am. Chem. Soc 2020, 142, 17413–17424. [PubMed: 32786740]

[17]. Mazzaferro LS, Huttel W, Fries A, Muller M, J. Am. Chem. Soc 2015, 137, 12289–12295. [PubMed: 26389790]

[18]. Gao SS, Zhang T, Garcia-Borras M, Hung YS, Billingsley JM, Houk KN, Hu Y, Tang Y, J. Am. Chem. Soc 2018, 140, 6991–6997. [PubMed: 29741874]

[19]. Belin P, Du MHL, Fielding A, Lequin O, Jacquet M, Charbonnier JB, Lecoq A, Thai R, Courçona M, Masson C, Dugave C, Genet R, Pernodet JL, and Gondry M, PNAS 2009, 106, 7426–7431. [PubMed: 19416919]

[20]. Yu X, Liu F, Zou Y, Tang MC, Hang L, Houk KN, Tang Y, J. Am. Chem. Soc 2016, 138, 13529–13532. [PubMed: 27690412]

[21]. Lee CF, Chen LX, Chiang CY, Lai CY, Lin HC, Angew. Chem. Int. Ed 2019, 58, 18414–18418.

[22]. Lin HC, McMahon TC, Patel A, Corsello M, Simon A, Xu W, Zhao M, Houk KN, Garg NK, Tang Y, J. Am. Chem. Soc 2016, 138, 4002–4005. [PubMed: 26963294]

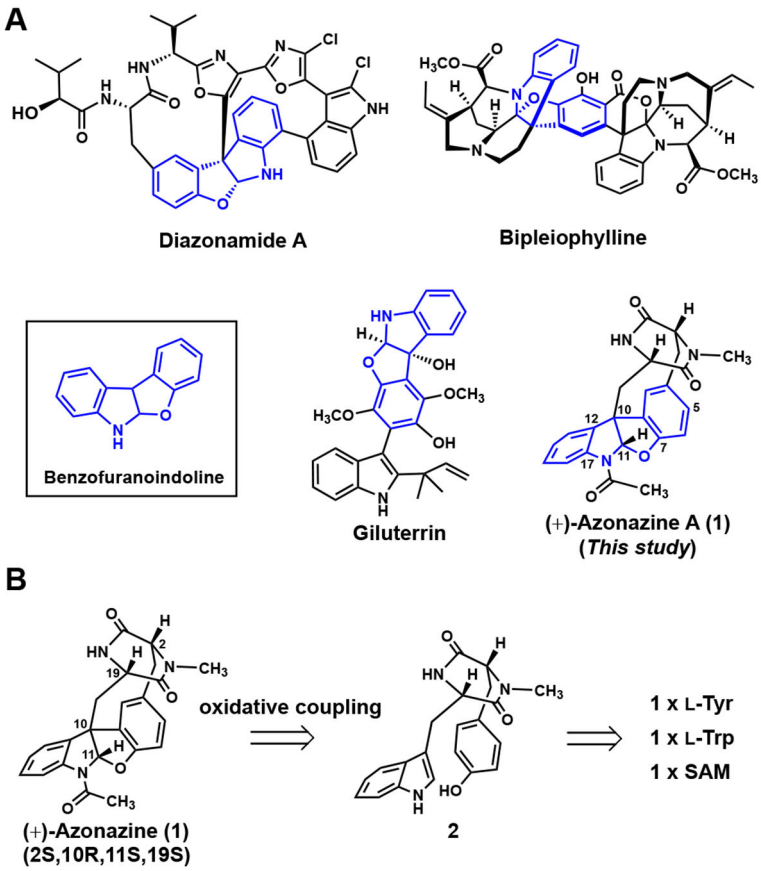


Figure 1.

(**A**) Representative benzofuranoindoline-containing natural products. (**B**) Retrobiosynthetic analysis of **1**.

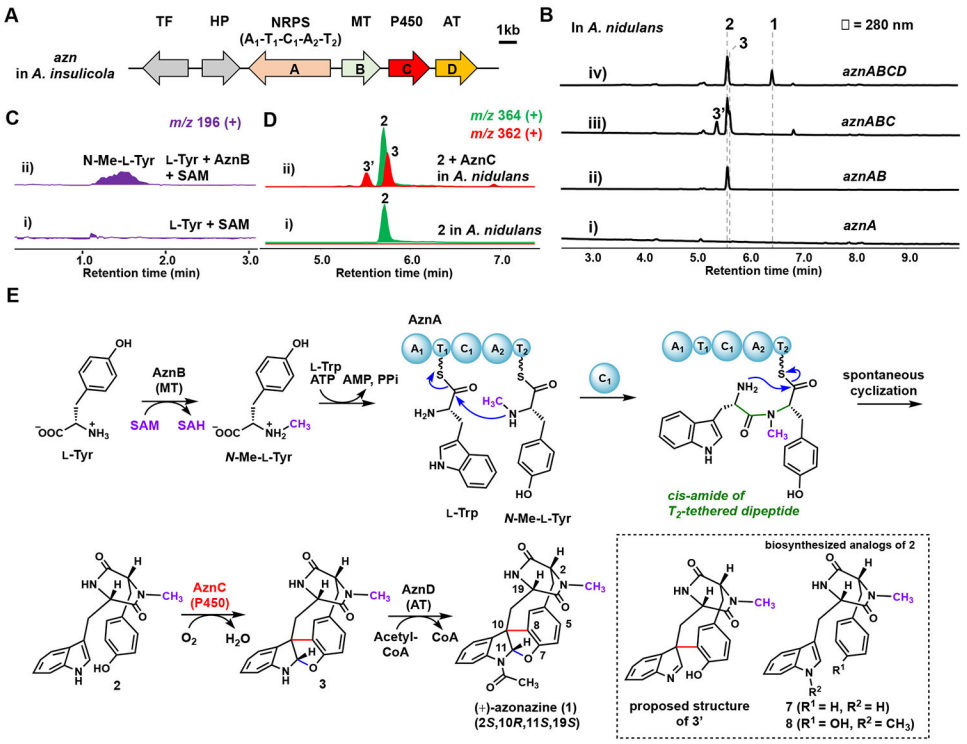


Figure 2.

Biosynthesis of (+)-azonazine A (**1**). (**A**) The *azn* cluster from *Aspergillus insulicola* encodes *aznA*: NRPS (A: adenylation; T: peptidyl-carrier protein; C: condensation); *aznB*: MT: methyltransferase; *aznC*: P450; *aznD*: acetyltransferase; TF: transcriptional factor. (**B**) LC-MS analysis of product profiles from each *A. nidulans* transformant expressing *azn* genes. The structure of **3'** is likely the imine form of **3** that results from the C11-O7 bond cleavage of the hemiaminal. (**C**) LC-MS analysis of in vitro assay of AznB with L-Tyr. (**D**) LC-MS analysis of metabolites from the *A. nidulans* transformant expressing AznC supplemented with **2**. (**E**) Proposed biosynthetic pathway of **1**.

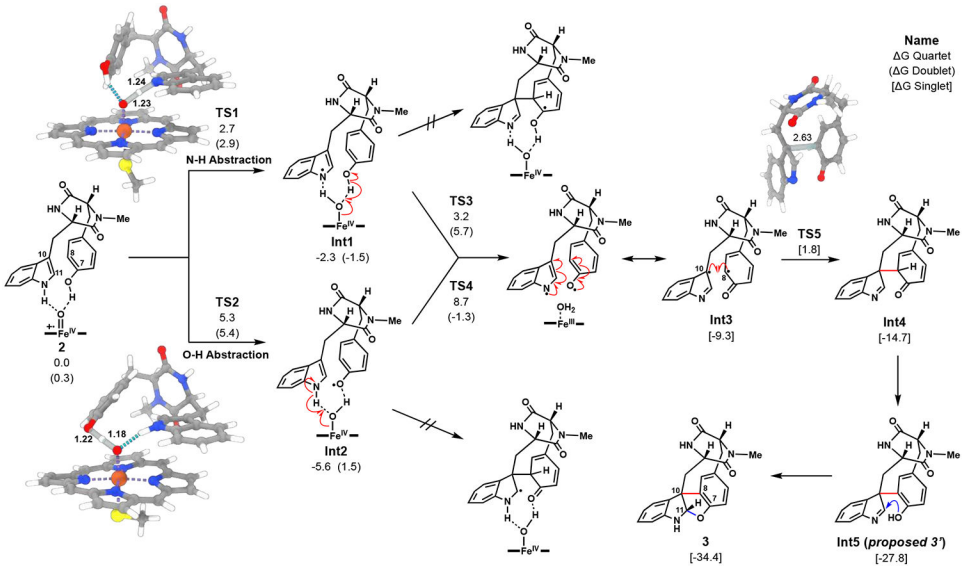


Figure 3.

Proposed mechanism for the AznC-catalyzed oxidative coupling reaction of **2** to **3**. Computed pathways for the formation of **3.** The energies were calculated by B3LYP-D3(BJ) /def2-TZVP/SMD(Et2O)// B3LYP-D3(BJ) /def2-SVP/IEEPCM(Et2O) and are given in kcal/mol. For all iron-containing species, possible spin states with free energy were reported. The spin state with the lowest free energy was reported for the species without iron.

Table 1.

A. nidulans biosynthesis of indole-substituted compound by supplementation of different L-Trp analogues [a].

Entry	Substrate	AznABC	AznABCD
		Analogues of 3	Analogues of 1
1	4-F-L-Trp	+	−
2	4-Br-L-Trp	−	−
3	5-F-L-Trp	++	++ (**27**) (Isolated yield ~8 mg/L)
4	5-Cl-L-Trp	++	++ (**28**) (Isolated yield ~4 mg/L)
5	5-Br-L-Trp	+++ (**17**) (Isolated yield ~3 mg/L)	+
6	6-F-L-Trp	+++	++
7	6-Cl-L-Trp	++	+
8	6-Br-L-Trp	+ (**23**) (Isolated yield ~3 mg/L)	+
9	7-Br-L-Trp	−	−
10	7-Me-L-Trp	+	−
11	5-OH-L-Trp	−	−
12	5-NO_2-L-Trp	−	−

[a]All substrates were fed at ~ 300 μM. Product analogues were detected by selected ion chromatography. − : not detectable. +++: analogues produced at levels > 50% of **3** or **1**; ++: analogues produced at levels > 20% of **3** or **1**; +: analogues produced at levels <20% of **3** or **1**. Isolated yield from large-scale cultivation is shown for **17**, **23**, **27**, and **28**.