Engineering Microbiology 3 (2023) 100064

Contents lists available at ScienceDirect

Engineering Microbiology

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

Review

Advances on the biosynthesis of pyridine rings

Carolina E. Portero [a,b,c], Yong Han [a,b,d], M. Raquel Marchán-Rivadeneira [b,c,d,e,*]

[a] Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701, USA
[b] Edison Biotechnology Institute, Ohio University, Athens, OH 45701, USA
[c] Center for Research on Health in Latinamerica (CISeAL) - Biological Science Department, Pontificia Universidad Católica del Ecuador (PUCE), Quito 170143, Ecuador
[d] Translational Biomedical Sciences Graduate Program, Ohio University, Athens, OH 45701, USA
[e] Department of Biological Sciences, Ohio University, Athens, OH 45701, USA

ARTICLE INFO

Keywords:
Natural products
Biosynthesis
Pyridine ring

ABSTRACT

Numerous studies have investigated the biosynthesis of pyridine heterocycles derived from nicotinic acid. However, metabolic pathways generating pyridine heterocycles in nature remain uninvestigated. Here, we summarize recent contributions conducted in the last decade on the biosynthetic pathways of non-derivate from nicotinic acid pyridine rings and discuss their implication on the study of natural products with pyridine structures.

1. Introduction

Biomolecules containing nitrogen heterocycles are an important class of biologically active compounds [1], as they are constituents of essential molecules in biological systems, including nitrogenous bases in nucleic acids, energy molecules such as ATP, second messengers, c-di-GMP and its analogs, and various coenzymes [2–4]. In addition, these compounds also comprise secondary metabolites with medical applications [5]. In fact, around 60% of small molecule drugs used in humans present N-based heterocycle moieties [6], including the antitumoral drug camptothecin [7], the widely used analgesic morphine [8], the antimalaria alkaloid quinine [9], and antibiotics such as β-lactams [10]. The reason for their use as drugs is the N-heterocycle's stability and effectiveness in the human body [11].

Nitrogen shows weak acidity in N–H bonds and weak basicity in heterocyclic rings [5], being able to accept or donate protons. In addition, nitrogen heterocycles can establish intermolecular forces like dipole-dipole, hydrophobic, van der Waals, hydrogen bonds, and π-stacking interactions [10]. These features allow N-heterocycles to interact with several molecules in living systems [5]. Furthermore, their presence improves pharmacokinetics and pharmacodynamics in drug candidates [12].

N-heterocycles can be aromatic or aliphatic [13]. Aromatic N-heterocyclics include functional groups such as indole, quinoline, pyrrole, pyridine, pyrimidine, and purine –ubiquitous in natural products and with potent biological activity [5]. Among them, pyridines are the most common aromatic heterocycle in FDA-approved drugs [6] having demonstrated biocompatibility and efficacy as a biological agent [14].

The pyridine moieties in drugs and bioactive molecules can increase their biochemical potency and metabolic stability and membrane permeability, and fix protein-binding issues [15]. For this reason, there is great interest in the synthesis of pyridine and its derivates [16–19]. Indeed, although chemical synthesis arose >200 years ago [20], given its high energy consumption and associated pollution, biosynthesis is receiving increasing attention [21,22].

There is extensive knowledge on the formation of pyridine rings in secondary metabolites from plants, such as alkaloids [23,24]. Particularly, nicotinic acid is the source of the pyridine ring in these compounds and created by some of the same enzymes implicated in nicotinamide adenine dinucleotides biosynthesis [23]. However, little is known about their biosynthesis in other organisms or metabolic pathways. In this mini-review, we summarize recent progress in the biosynthesis of compounds containing pyridine heterocycles not derived from nicotinic acid and describe three instances of pyridine ring production and their potential for abiotic reactions.

2. Biosynthetic pathways of pyridine ring formation

In general, pyridine rings are derived from amino acids [25,26]. Classically, research on pyridine biosynthesis was limited by the use of radioactively-labeled precursors [26,27]. However, advances in molecular biology, genome mining, and gene editing offer a new tool for the synthesis and structure prediction of natural products which allowed discovering new biosynthetic reactions and enzymes involved in pyridine ring formation. Hereafter, we describe three types of mechanisms of pyridine ring formation found in nature.

Abbreviations: NRPS, Non-ribosomal peptide synthase; PKS-NRPS, Polyketide synthase non-ribosomal peptide synthetase; PK, Polyketide; NRP, Non-ribosomal peptide; PK-NRP, Polyketide non-ribosomal peptide; DA, Diels-Alder reaction; DAases, Diels-Alderases; RiPPs, Ribosomally synthesized and post-translationally modified peptides.

* Corresponding author.

https://doi.org/10.1016/j.engmic.2022.100064
Received 31 August 2022; Received in revised form 1 December 2022; Accepted 5 December 2022
Available online 13 December 2022

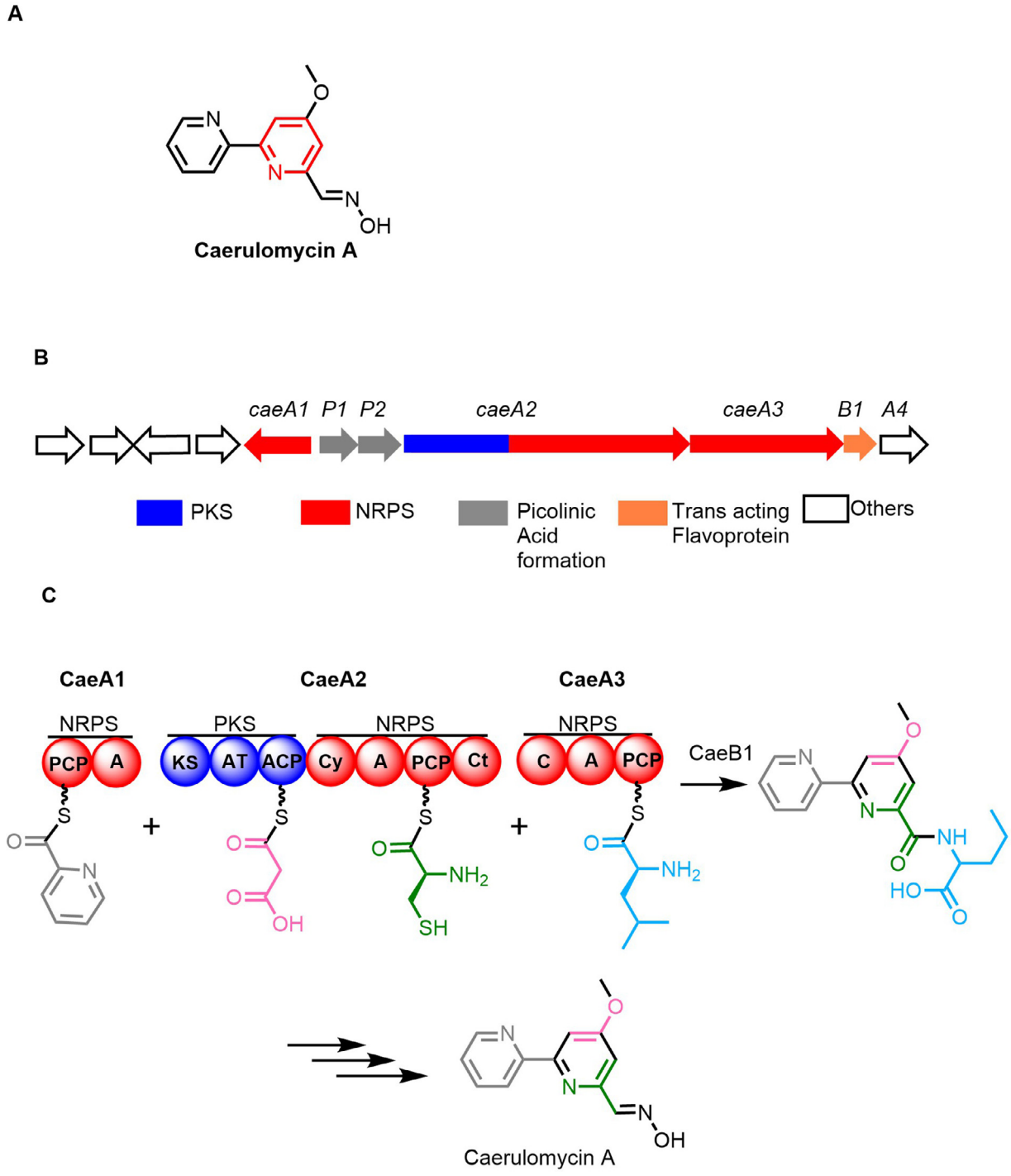

Fig. 1. Polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) complex for 2,2′-bipyridine biosynthesis. A. Caerulomycin A structure. B. Diagram of genes responsible for caerulomycin A synthesis C. Abbreviated PKS-NRPS assembly line. Protein complex formations include their functional domains (KS: ketosynthase, AT: acyltransferase, ACP: acyl carrier protein, Cy: condensation/cyclization, A: adenylation, PCP: peptide carrier protein, Ct: terminal C: condensation). The molecule's identity is indicated by colors: gray for picolinyl, pink for malonyl, green for *L*-cysteinyl, and light blue for *L*-leucinyl. Adapted from [29].

2.1. Bipyridine biosynthesis by the polyketides synthetase (PKS)–non-ribosomal peptide synthetase (NRPS) assembly line

Polyketides (PKs) and non-ribosomal peptide (NRPs) hybrids are natural products derived from large multimodular enzymes [28]. Pyridine formation using this chemical assembly line has been described in several polyketide synthase–non-ribosomal peptide synthetase (PKS-NRPS) hybrids [29]. Below, we provide a few examples of symmetrical bipyridines.

2,2′-Bipyridine is a unique molecular scaffold of bioactive natural products represented by caerulomycin A (Fig. 1A), which has multiple antifungal and antitumor activities and is a dual-target antitumor agent [29,30]. In this compound, the PKS-NRPS assembly line consists of three modular proteins (CaeA1, CaeA2, and CaeA3) (Fig. 1B, C). CaeA1 has been proposed to incorporate picolinic acid, which provides an unmodified pyridine unit. CaeA2, an atypical PKS-NRPS hybrid complex, integrates malonyl-CoA and *L*-cysteinyl to picolinic acid, using a unique peptidyl elongation reaction through C–C linkage. Finally,

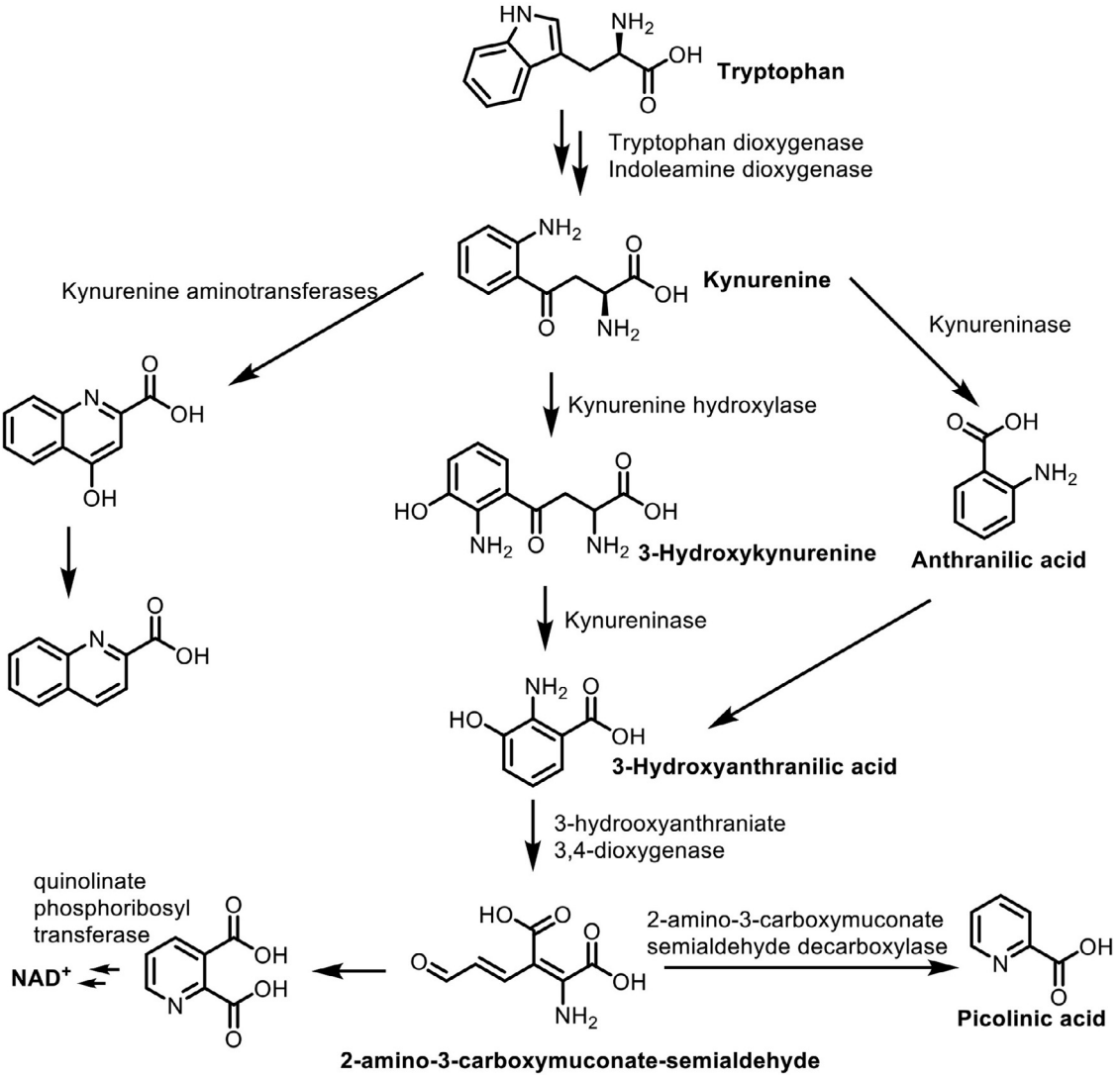

Fig. 2. Kynurenine pathway, the main route of tryptophan metabolism. Adapted from [31].

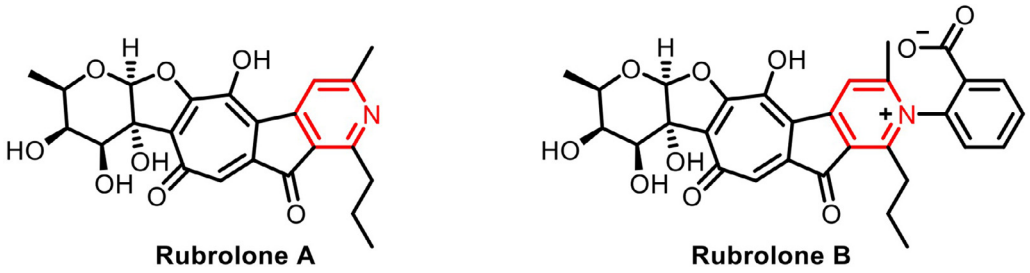

Fig. 3. Chemical structure of rubrolone A and B. Pyridine rings are shown in red.

CaeA3 produces the mature 2,2′-bipyridine through reactions including *L*-leucinyl removal, carboxyl reduction, and transamination (Fig. 1C) [29]. Interestingly, the flavoprotein CaeB1 is needed to form a functional 2,2′-bipyridine. In the CaeA2 protein complex, the Ct domain activates CaeB1, which catalyzes in trans the α,β-dehydrogenation of *L*-cysteinyl [29]. Similarly, collismycin antibiotics, another type of 2,2′-bipyridine, share an assembly line showing an unusual PK-peptide assembly hybrid [29].

Another important precursor in the biosynthetic pathway of caerulomycin and other natural products containing pyridine structures is picolinic acid, a catabolite of tryptophan through the kynurenine pathway (Fig. 2) [31]. Since the metabolism of tryptophan is a well-known primary metabolic pathway, it is not described in detail in this review.

2.2. Non-enzymatic pyridine ring formation in type II polyketide synthases (PKS)

Non-enzymatic pyridine ring synthesis has been reported on some tropolone alkaloids natural products [26,32,33]. In particular, the metabolic pathways of a *Streptomyces*-derived compounds, rubrolones, have been recently described [26]. Specifically, rubrolone A and B possess a tetra-substituted pyridine moiety that differ by an additional ben-

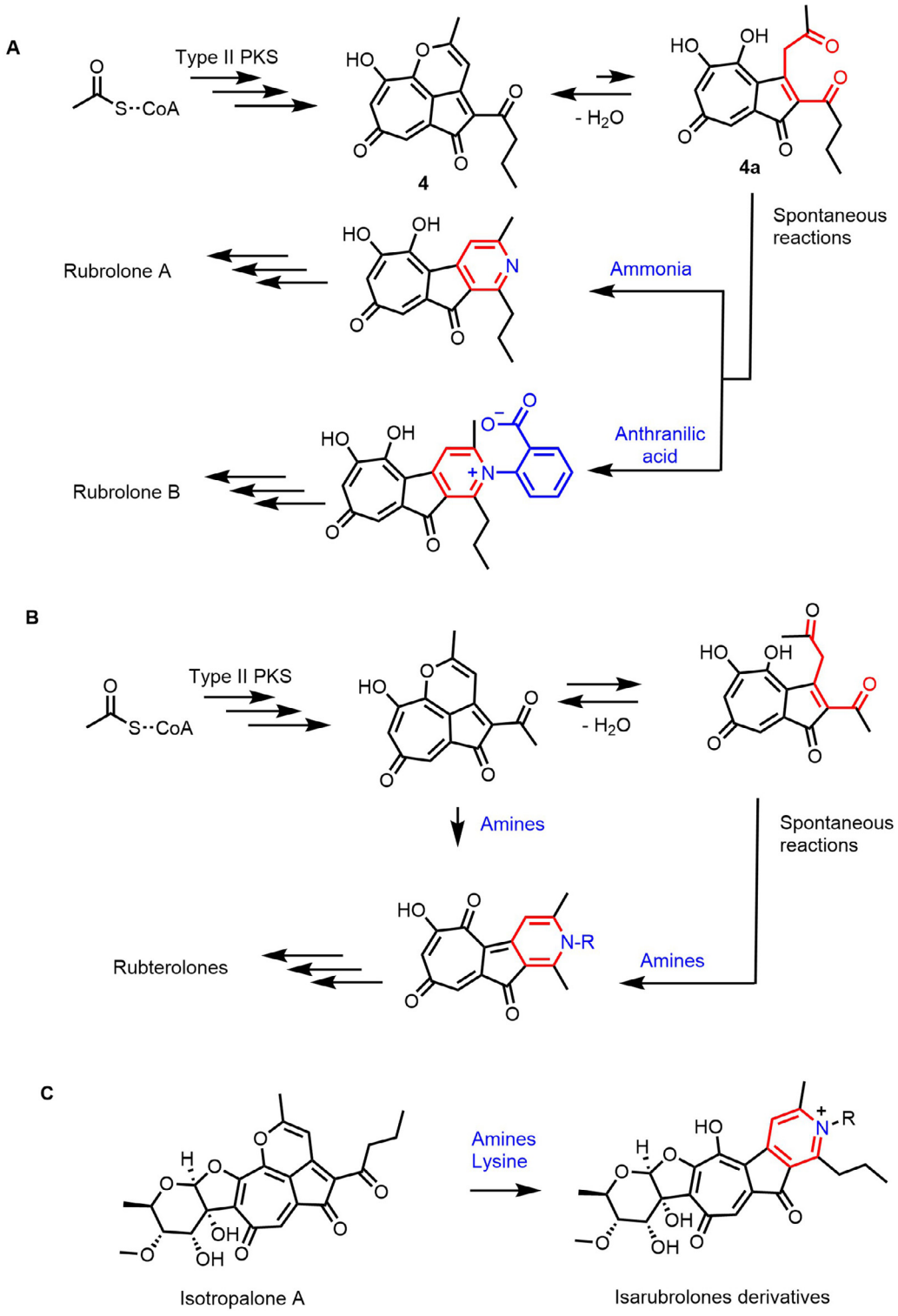

Fig. 4. Non-enzymatic pathways for pyridine ring biosynthesis in tropolone alkaloids. A. Rubrolone biosynthesis. Adapted from [26]. B. Biosynthesis of rubterolones. Adapted from [33] C. Reaction of isotropalone A with amines and amino acids. Adapted from [32]. The 1,5-dicarbonyl moiety and their derived pyridine ring are shown in red.

zoic acid on the pyridine ring in B (Fig. 3), which shows cardioprotective activity [26,34]. This unique pyridyl moiety structure makes the pyridine ring formation in these compounds highly interesting.

Indeed, the tropolone ring construction in rubrolone A and B biosynthesis derives from a type II polyketide synthases (PKS) pathway that includes the synthesis of a long PK chain, enzymatic oxidative rearrangements, and cyclization [26] (Fig. 4A). As a key intermediate, a rubrulone precursor is formed (Compound **4**), the divergent point in rubrulone A and B synthesis. Compound **4** has a reactive 1,5-dione moiety that reacts with ammonia or o-aminobenzoic acid in the non-enzymatic pathway to form the pyridine rings (Fig. 4A) [26]. Interestingly, these non-enzymatic pathways require hydrolysis of intermediate **4** to form

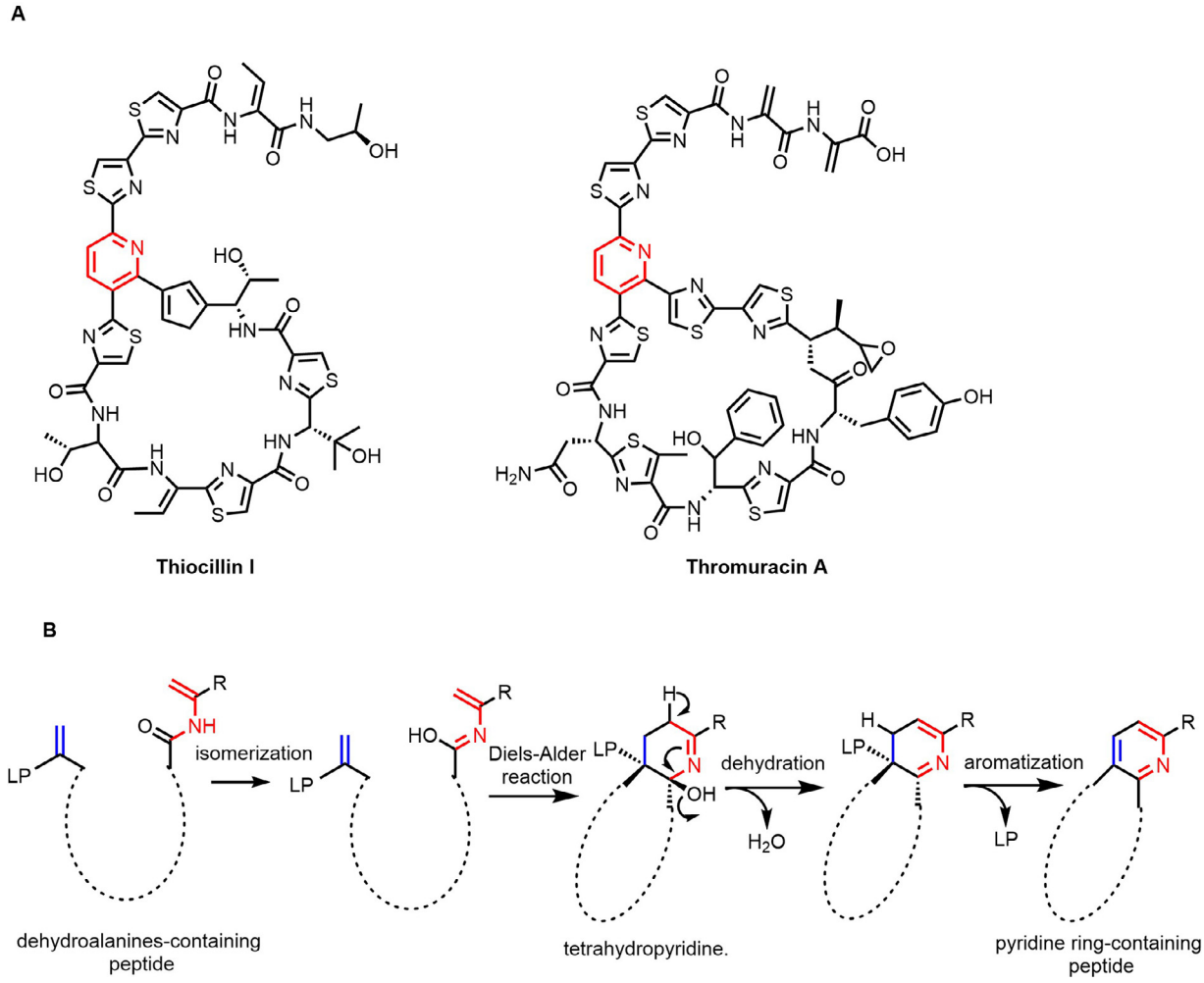

Fig. 5. Pyridine ring formation in thiocillins and thiomuracins. A. Structure of thiocillins and thiomuracins. The pyrimidine ring is shown in red B. Potential mechanism of the Diels-Alder reaction. Adapted from [44].

an amino-acceptor compound (**4a**) with a 1,5-dicarbonyl structural unit (Fig. 4A) [26].

The chemical structure and reactivity of the biosynthetic intermediate **4**, the presence of excess anthranilic acid in the fermentation culture of the wild type *Streptomyces* strain, and the absence of suitable gene candidates in the rub cluster, together suggest that amination may occur non-enzymatically [26].

To confirm the non-enzymatic origin of the pyridine ring, Yan et al. [26] restored rubrolone B production in an anthranilic acid deficient strain after feeding the mutant with [^{15}N]-labeled o-aminobenzoic acid. Additionally, *in vitro* reactions to mimic the fermentation media between the biosynthetic intermediate **4** and either ammonia or anthranilic acid analogs in phosphate buffer at pH 8.0 yielded new HPLC peaks with a retention time corresponding to that of the intermediates having a pyridine ring. These results demonstrate intermediate **4** can react with ammonia or anthranilic acid to generate non-enzymatic pyridyl moieties [26].

The 1,5-dicarbonyl structural unit may represent a widespread mechanism of pyridine ring formation in nature [26]. For instance, biosynthesis of rubterolones A–D, natural compounds derived from the gut of a fungus-growing termite, includes an intermediate with a 1,5-dione moiety which can react with amines, triggering non-enzymatic pyridine ring formation (Fig. 4B) [33,35]. Furthermore, the *Streptomyces* compounds isatropolones have a 1,5-diketone moiety, similar to compound **4** in rubrolone biosynthesis, which can produce pyridine rings when reacting with ammonia, amines, and lysine (Fig. 4C) [32].

2.3. Hetero-Diels-Alderases catalyzing pyridine ring formation

The Diels-Alder (DA) reaction has a wide range of applications in organic synthesis [36], particularly in the total synthesis of natural products [37]. This reaction is highly stereoselective and yields a six-membered ring with four chiral centers [37]. Moreover, it can form a pyridine ring from a 1,3-butadiene moiety (diene) and an olefin/double bond structure (dienophile) using a [4 + 2] cycloaddition reaction [38]. Five different families of enzymes, called Diels-Alderases (DAases) catalyze DA reactions in biosynthetic pathways [39]. Near 10 DAases have been described, with different catalytical mechanisms. Among them, ribosomal peptides that contain thiazole are the only ones capable of producing pyridine rings [40].

Thiazole peptides are a class of ribosomally synthesized and post translationally modified peptide natural products (RiPPs) considered interesting because of their strong biological activity [41–43]. The biosynthesis of the central pyridine ring at the junction of the macrocyclic system is particular to this class of compounds [44]. Recently, genome sequencing and mining has shown that the synthesis of the pyridine ring of thiazole peptides is catalyzed by DAases [44]. *In vitro* synthesis of thiopeptides (thiomuracins and thiocillins; Fig. 5A) has shown that

the enzymes TclM and Tbt can produce pyridine rings without intermediates [45,46]. First, a variable-size peptide chain with two dehydroalanines with synergistic cyclization via isomerization of the amide carbonyl group forms a tetrahydropyridine. Later, spontaneous dehydration, excision of the N terminal region (leader peptide), and aromatization result in a mature pyridine ring (Fig. 5B) [44].

Interestingly, dehydration is an enzymatic process in other macrocyclic RiPPs. For example, in pyritidine A, dehydratase MroBC acts after [4 + 2] cycloaddition of the DA enzyme MroD. Remarkably, both enzymes recognize specific parts in the peptide; mutations in these sections can produce analogs with various sequences and sizes (14−68 atoms) [47]. Moreover, these heteroDA enzymes are unexpectedly homologous to the elimination enzymes which provide dehydroalanine/dehydrobutyrine, the precursors for DA reactions in lantibiotic biosynthesis [48].

3. Discussion

Nitrogen containing natural products have received extensive attention from pharmaceutical scientists due to their complex structures and wide range of biological activities [10]. The biosynthesis processes of common nitrogenous substances, such as non-ribosomal polypeptides and RiPPs has been investigated in depth [49,50]. However, the mechanism of pyridine ring formation in some pyridine alkaloids is not so well-known [26]. In this review, we list three types of compounds containing pyridine structures, which have very different mechanisms of pyridine ring formation.

The discovery and identification of novel biosynthetic pathways and related enzymes catalyzing the synthesis of pyridine heterocycles is meaningful research, as not only expands the understanding on the origin of pyridine rings in natural products but also allows synthetic preparation of compounds containing pyridine rings.

In particular, the biosynthesis of rubrolones and rubterolones includes enzymatic generation of a highly reactive 1,5-dione intermediate that forms a pyridine ring under non-catalytic conditions [26,35]. This finding provides a good reference for the future development of new structures of pyridine alkaloids, expands the chemical diversity of natural products, enhances their bioactivity, and avoids the use of extreme temperatures and pH required for *in vitro* pyridine synthesis [26,32,51].

Furthermore, caerulomycin A biosynthesis expanded our knowledge on the compounds synthesized by hybrid PKS-NRPS systems. Given the broad spectrum and powerful biological activities of 2,2′-bipyridine analogs, this study highlights the need to further expand PK-NRPs product diversity through synthetic biology. From this perspective, improving the bioactivity or bioavailability of this family of traditionally used chemicals in clinical settings is particularly important [52].

In addition, the construction of pyridine structures in thiazole peptide-like compounds indicated the role of DAases. The discovery of new DAases can provide more environmentally friendly routes to synthesize bioactive compounds [53,54]. Furthermore, knowledge on the functional features of DAases may allow enhancing their natural properties. In addition, protein-based artificial enzymes for DA reactions are a promising tool for abiotic catalysis under eco-friendly conditions [55].

In summary, nature has evolved mechanisms and highly efficient molecules that, by imitating their process, we can improve chemical synthesis [56]. However, a major challenge is enhancing efficiency and production. Therefore, the rapid development of technologies (*e.g.*, omics, synthetic and semisynthetic methods) will open a window for the discovery of more new nature biosynthetic pathways of non-derivate from nicotinic acid pyridine rings.

4. Conclusion

Pyridine moieties are often used in drugs because of their biological importance, however, its biosynthesis in some organisms is poorly understood. In this review, the metabolic pathways analyzed exemplify the significance of pyrimidine biosynthesis and their possible applications.

Recent knowledge on pyridine ring formation has provided the basis to produce structurally varied pyridine compounds using synthetic biology methods and combinatorial biosynthesis, offering in the future a way to develop new pharmacological drugs. With the development of bioinformatics and gene editing technologies, a large number of unknown biosynthetic gene clusters have been discovered. We expect that in the following years, the use of new tools will accelerate the discovery of novel natural biosynthetic pathways, and associated pyridine ring-forming enzymes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the Edison Biotechnology Institute (EBI) and the Baker Fund at Ohio University. Thanks to Javier Serrano for helping to design the graphical abstract.

References

[1] B. Zhang, A. Studer, Recent advances in the synthesis of nitrogen heterocycles via radical cascade reactions using isonitriles as radical acceptors, Chem. Soc. Rev. 44 (2015) 3505–3521, doi:10.1039/C5CS00083A.
[2] L.E. Rodriguez, C.H. House, K.E. Smith, M.R. Roberts, M.P. Callahan, Nitrogen heterocycles form peptide nucleic acid precursors in complex prebiotic mixtures, Sci. Rep. 9 (2019) 9281, doi:10.1038/s41598-019-45310-z.
[3] C.T. Walsh, Nature loves nitrogen heterocycles, Tetrahedron Lett. 56 (2015) 3075–3081, doi:10.1016/j.tetlet.2014.11.046.
[4] R.S. Hosmane, Chapter 2: ring-expanded ('fat') purines and their nucleoside/nucleotide analogues as broad-spectrum therapeutics, in: Prog. Heterocycl. Chem., Elsevier (2009) 35–68, doi:10.1016/S0959-6380(09)70029-7.
[5] B. Gao, B. Yang, X. Feng, C. Li, Recent advances in the biosynthesis strategies of nitrogen heterocyclic natural products, Nat. Prod. Rep. 39 (2022) 139–162, doi:10.1039/D1NP00017A.
[6] E. Vitaku, D.T. Smith, J.T. Njardarson, Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among U.S. FDA approved pharmaceuticals: miniperspective, J. Med. Chem. 57 (2014) 10257–10274, doi:10.1021/jm501100b.
[7] M. Ljungman, P.C. Hanawalt, The anti-cancer drug camptothecin inhibits elongation but stimulates initiation of RNA polymerase II transcription, Carcinogenesis 17 (1996) 31–36, doi:10.1093/carcin/17.1.31.
[8] C. Hartley, F. Moultrie, A. Hoskin, G. Green, V. Monk, J.L. Bell, A.R. King, M. Buckle, M. van der Vaart, D. Gursul, S. Goksan, E. Juszczak, J.E. Norman, R. Rogers, C. Patel, E. Adams, R. Slater, Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomised placebo-controlled trial, Lancet 392 (2018) 2595–2605, doi:10.1016/S0140-6736(18)31813-0.
[9] P.N. Kalaria, S.C. Karad, D.K. Raval, A review on diverse heterocyclic compounds as the privileged scaffolds in antimalarial drug discovery, Eur. J. Med. Chem. 158 (2018) 917–936, doi:10.1016/j.ejmech.2018.08.040.
[10] N. Kerru, L. Gummidi, S. Maddila, K.K. Gangu, S.B. Jonnalagadda, A review on recent advances in nitrogen-containing molecules and their biological applications, Molecules 25 (2020) 1909, doi:10.3390/molecules25081909.
[11] M.M. Heravi, V. Zadsirjan, Prescribed drugs containing nitrogen heterocycles: an overview, RSC Adv 10 (2020) 44247–44311, doi:10.1039/D0RA09198G.
[12] P. Martins, J. Jesus, S. Santos, L. Raposo, C. Roma-Rodrigues, P. Baptista, A. Fernandes, Heterocyclic anticancer compounds: recent advances and the paradigm shift towards the use of nanomedicine's tool box, Molecules 20 (2015) 16852–16891, doi:10.3390/molecules200916852.
[13] J.K. Annor-Gyamfi, Ring-fused Nitrogen Heterocycles By Efficient Synthetic strategies, Doctor of Philosophy, Oklahoma State University, 2020.
[14] E. Khan, Pyridine derivatives as biologically active precursors; organics and selected coordination complexes, ChemistrySelect 6 (2021) 3041–3064, doi:10.1002/slct.202100332.
[15] Y. Ling, Z.-Y. Hao, D. Liang, C.-L. Zhang, Y.-F. Liu, Y. Wang, The expanding role of pyridine and dihydropyridine scaffolds in drug design, Drug Des. Devel. Ther. Volume 15 (2021) 4289–4338, doi:10.2147/DDDT.S329547.
[16] K.M. Elattar, B.D. Mert, Recent developments in the chemistry of bicyclic 6-6 systems: chemistry of pyrido[4,3-d]pyrimidines, RSC Adv. 6 (2016) 71827–71851, doi:10.1039/C6RA12364C.
[17] M. Monier, D. Abdel-Latif, A. El-Mekabaty, B.D. Mert, K.M. Elattar, Advances in the chemistry of 6-6 bicyclic systems: chemistry of Pyrido[3,4- d]pyrimidines, Curr. Org. Synth. 16 (2019) 812–854, doi:10.2174/1570179416666190704113647.

[18] M. Monier, A. El-Mekabaty, D. Abdel-Latif, K.M. Elattar, Chemistry of bicyclic 5-6 systems: synthesis of oxazolo[3,2- *a*]pyridines and their salts with a ring-junction nitrogen atom, Synth. Commun. 49 (2019) 2591–2629, doi:10.1080/00397911.2019.1643889.
[19] M. Monier, D. Abdel-Latif, A. El-Mekabaty, K.M. Elattar, Reactivity and stereoselectivity of oxazolopyridines with a ring-junction nitrogen atom, J. Heterocycl. Chem. 56 (2019) 3172–3196, doi:10.1002/jhet.3727.
[20] A.A. Khalaf, Synthesis and properties of pyridine containing drugs and heterocycles, Thesis (Doctoral), 2017. http://sro.sussex.ac.uk/id/eprint/70414.
[21] M.J. Mulvihill, E.S. Beach, J.B. Zimmerman, P.T. Anastas, Green chemistry and green engineering: a framework for sustainable technology development, Annu. Rev. Environ. Resour. 36 (2011) 271–293, doi:10.1146/annurev-environ-032009-095500.
[22] O.V. Kharissova, B.I. Kharisov, C.M. Oliva González, Y.P. Méndez, I. López, Greener synthesis of chemical compounds and materials, R. Soc. Open Sci. 6 (2019) 191378, doi:10.1098/rsos.191378.
[23] F.F. Zenkner, M. Margis-Pinheiro, A. Cagliari, Nicotine biosynthesis in *nicotiana* : a metabolic overview, Tob. Sci. 56 (2019) 1–9, doi:10.3381/18-063.
[24] A. Katoh, Molecular biology of pyridine nucleotide and nicotine biosynthesis, Front. Biosci. 9 (2004) 1577, doi:10.2741/1350.
[25] X. Tan, A.J. Chen, B. Wu, G.-S. Zhang, G. Ding, Advance of swainsonine biosynthesis, Chin. Chem. Lett. 29 (2018) 417–422, doi:10.1016/j.cclet.2017.08.040.
[26] Y. Yan, J. Yang, Z. Yu, M. Yu, Y.-T. Ma, L. Wang, C. Su, J. Luo, G.P. Horsman, S.-X. Huang, Non-enzymatic pyridine ring formation in the biosynthesis of the rubrolone tropolone alkaloids, Nat. Commun. 7 (2016) 13083, doi:10.1038/ncomms13083.
[27] G.A. Cordell, Fifty years of alkaloid biosynthesis in Phytochemistry, Phytochemistry 91 (2013) 29–51, doi:10.1016/j.phytochem.2012.05.012.
[28] C. Beck, J.F.G. Garzón, T. Weber, Recent advances in re-engineering modular PKS and NRPS assembly lines, Biotechnol. Bioprocess Eng. 25 (2020) 886–894, doi:10.1007/s12257-020-0265-5.
[29] B. Pang, R. Liao, Z. Tang, S. Guo, Z. Wu, W. Liu, Caerulomycin and collismycin antibiotics share a trans-acting flavoprotein-dependent assembly line for 2,2'-bipyridine formation, Nat. Commun. 12 (2021) 3124, doi:10.1038/s41467-021-23475-4.
[30] L. Tong, W. Sun, S. Wu, Y. Han, Characterization of Caerulomycin A as a dual-targeting anticancer agent, Eur. J. Pharmacol. 922 (2022) 174914, doi:10.1016/j.ejphar.2022.174914.
[31] M. Dehhaghi, H. Kazemi Shariat Panahi, G.J. Guillemin, Microorganisms, tryptophan metabolism, and kynurenine pathway: a complex interconnected loop influencing human health status, Int. J. Tryptophan Res. 12 (2019) 117864691985299, doi:10.1177/1178646919852996.
[32] X. Cai, Y.-M. Shi, N. Pöhlmann, O. Revermann, I. Bahner, S.J. Pidot, F. Wesche, H. Lackner, C. Büchel, M. Kaiser, C. Richter, H. Schwalbe, T.P. Stinear, A. Zeeck, H.B. Bode, Structure and biosynthesis of isatropolones, bioactive amine-scavenging fluorescent natural products from *Streptomyces* Gö66, Angew. Chem. Int. Ed. 56 (2017) 4945–4949, doi:10.1002/anie.201701223.
[33] H. Guo, R. Benndorf, S. König, D. Leichnitz, C. Weigel, G. Peschel, P. Berthel, M. Kaiser, C. Steinbeck, O. Werz, M. Poulsen, C. Beemelmanns, Expanding the Rubterolone Family: intrinsic reactivity and directed diversification of PKS-derived pyrans, Chem. - Eur. J. 24 (2018) 11319–11324, doi:10.1002/chem.201802066.
[34] Y. Yan, Y.-T. Ma, J. Yang, G.P. Horsman, D. Luo, X. Ji, S.-X. Huang, Tropolone ring construction in the biosynthesis of Rubrolone B, a Cationic Tropolone Alkaloid from Endophytic *Streptomyces*, Org. Lett. 18 (2016) 1254–1257, doi:10.1021/acs.orglett.6b00074.
[35] H. Guo, R. Benndorf, D. Leichnitz, J.L. Klassen, J. Vollmers, H. Görls, M. Steinacker, C. Weigel, H.-M. Dahse, A.-K. Kaster, Z.W. de Beer, M. Poulsen, C. Beemelmanns, Isolation, Biosynthesis and Chemical Modifications of Rubterolones A-F: rare Tropolone Alkaloids from *Actinomadura* sp. 5-2, Chem. - Eur. J. 23 (2017) 9338–9345, doi:10.1002/chem.201701005.
[36] A. Gandini, The furan/maleimide Diels–Alder reaction: a versatile click–unclick tool in macromolecular synthesis, Prog. Polym. Sci. 38 (2013) 1–29, doi:10.1016/j.progpolymsci.2012.04.002.
[37] M. Juhl, D. Tanner, Recent applications of intramolecular Diels–Alder reactions to natural product synthesis, Chem. Soc. Rev. 38 (2009) 2983, doi:10.1039/b816703f.
[38] J. Sauer, Diels-Alder reactions II: the reaction mechanism, Angew. Chem. Int. Ed. Engl. 6 (1967) 16–33, doi:10.1002/anie.196700161.
[39] L. Gao, J. Yang, X. Lei, Enzymatic intermolecular Diels-Alder reactions in synthesis: from nature to design, Tetrahedron Chem. 2 (2022) 100013, doi:10.1016/j.tchem.2022.100013.
[40] B. Jeon, S.-A. Wang, M.W. Ruszczycky, H. Liu, Natural [4 + 2]-Cyclases, Chem. Rev. 117 (2017) 5367–5388, doi:10.1021/acs.chemrev.6b00578.
[41] M.J. Pucci, J.J. Bronson, J.F. Barrett, K.L. DenBleyker, L.F. Discotto, J.C. Fung-Tomc, Y. Ueda, Antimicrobial evaluation of nocathiacins, a thiazole peptide class of antibiotics, Antimicrob. Agents Chemother. 48 (2004) 3697–3701, doi:10.1128/AAC.48.10.3697-3701.2004.
[42] R. Dahiya, S. Dahiya, N.K. Fuloria, S. Kumar, R. Mourya, S.V. Chennupati, S. Jankie, H. Gautam, S. Singh, S.K. Karan, S. Maharaj, S. Fuloria, J. Shrivastava, A. Agarwal, S. Singh, A. Kishor, G. Jadon, A. Sharma, Natural bioactive thiazole-based peptides from marine resources: structural and pharmacological aspects, Mar. Drugs. 18 (2020) 329, doi:10.3390/md18060329.
[43] C.L. Cox, J.R. Doroghazi, D.A. Mitchell, Data from: the genomic landscape of ribosomal peptides containing thiazole and oxazole heterocycles, (2016) 12095533 bytes. https://doi.org/10.5061/DRYAD.7Q830.
[44] A. Minami, H. Oikawa, Recent advances of Diels–Alderases involved in natural product biosynthesis, J. Antibiot. (Tokyo). 69 (2016) 500–506, doi:10.1038/ja.2016.67.
[45] W.J. Wever, J.W. Bogart, J.A. Baccile, A.N. Chan, F.C. Schroeder, A.A. Bowers, Chemoenzymatic synthesis of thiazolyl peptide natural products featuring an enzyme-catalyzed formal [4 + 2] cycloaddition, J. Am. Chem. Soc. 137 (2015) 3494–3497, doi:10.1021/jacs.5b00940.
[46] G.A. Hudson, Z. Zhang, J.I. Tietz, D.A. Mitchell, W.A. van der Donk, In vitro biosynthesis of the core scaffold of the thiopeptide thiomuracin, J. Am. Chem. Soc. 137 (2015) 16012–16015, doi:10.1021/jacs.5b10194.
[47] D.T. Nguyen, T.T. Le, A.J. Rice, G.A. Hudson, W.A. van der Donk, D.A. Mitchell, Accessing diverse pyridine-based macrocyclic peptides by a two-site recognition pathway, J. Am. Chem. Soc. 144 (2022) 11263–11269, doi:10.1021/jacs.2c02824.
[48] M.A. Ortega, Y. Hao, Q. Zhang, M.C. Walker, W.A. van der Donk, S.K. Nair, Structure and mechanism of the tRNA-dependent lantibiotic dehydratase NisB, Nature 517 (2015) 509–512, doi:10.1038/nature13888.
[49] G.M. Rubin, Y. Ding, Recent advances in the biosynthesis of RiPPs from multicore-containing precursor peptides, J. Ind. Microbiol. Biotechnol. 47 (2020) 659–674, doi:10.1007/s10295-020-02289-1.
[50] S. Hwang, N. Lee, S. Cho, B. Palsson, B.-K. Cho, Repurposing modular polyketide synthases and non-ribosomal peptide synthetases for novel chemical biosynthesis, Front. Mol. Biosci. 7 (2020) 87, doi:10.3389/fmolb.2020.00087.
[51] G.D. Henry, De novo synthesis of substituted pyridines, Tetrahedron 60 (2004) 6043–6061, doi:10.1016/j.tet.2004.04.043.
[52] A. Nivina, K.P. Yuet, J. Hsu, C. Khosla, Evolution and diversity of assembly-line polyketide synthases: focus review, Chem. Rev. 119 (2019) 12524–12547, doi:10.1021/acs.chemrev.9b00525.
[53] A.A. Strauss, Assembly and characterisation of Diels-Alderase polymer conjugates for industrial biocatalysis, Master of Science, University of Bristol, 2019. https://research-information.bris.ac.uk/en/studentTheses/assembly-and-characterisation-of-diels-alderase-polymer-conjugate.
[54] C.O. Marsh, N.R. Lees, L. Han, M.J. Byrne, S.Z. Mbatha, L. Maschio, S. Pagden-Ratcliffe, P.W. Duke, J.E.M. Stach, P. Curnow, C.L. Willis, P.R. Race, A natural Diels-Alder biocatalyst enables efficient [4+2] cycloaddition under harsh reaction conditions, ChemCatChem 11 (2019) 5027–5031, doi:10.1002/cctc.201901285.
[55] W. Ghattas, J. Mahy, M. Réglier, A.J. Simaan, Artificial enzymes for Diels-Alder reactions, ChemBioChem 22 (2021) 443–459, doi:10.1002/cbic.202000316.
[56] R. Bao, H. Zhang, Y. Tang, Biomimetic synthesis of natural products: a journey to learn, to mimic, and to be Better, Acc. Chem. Res. 54 (2021) 3720–3733, doi:10.1021/acs.accounts.1c00459.