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Catalytic repertoire of bacterial bisindole formation

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Bacterial bisindole alkaloids that derive from the oxidative dimerization of L-tryptophan possess diversified biological activities and unique molecular structures. In recent years, the number of bisindoles and their gene clusters has greatly expanded, revealing a large genetic toolbox for the generation of unique structural modifications. In this review, we will discuss the enzymatic pathways leading to diverse bisindoles structures. We will focus on the discovery of molecules through metagenomic mining, the elucidation of new enzymatic mechanisms, and the identification of new biosynthetic protecting group strategies. We will also highlight the newest work in combinatorial engineering and synthetic biology.

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Introduction

Bacterial bisindole alkaloids that derive from the oxidative dimerization of L-tryptophan are produced by a variety of bacteria from different genera, including *Chromobacterium*, *Streptomyces*, *Lechevalieria*, *Salinispora*, and *Nonomuraea*. Although there is little known about their physiological roles and the regulatory mechanism that governing the biosynthesis, their diversified biological activities and unique molecular scaffolds have attracted great interests. These molecules include violacein (**1**), a purple colored compound with antibacterial activity [1] and staurosporine (**2**), a molecule widely used in research as a polykinase inhibitor [2]. The bisindoles also include several natural products whose synthetic derivatives were brought forward through Phase II/III clinical trials against various cancers [3–5]. Pioneering work in the last decades identified the genes and enzymes responsible for production of such microbial bisindoles, revealing that many microbial bisindoles share common transformations early in their biosynthetic pathways [6,7].

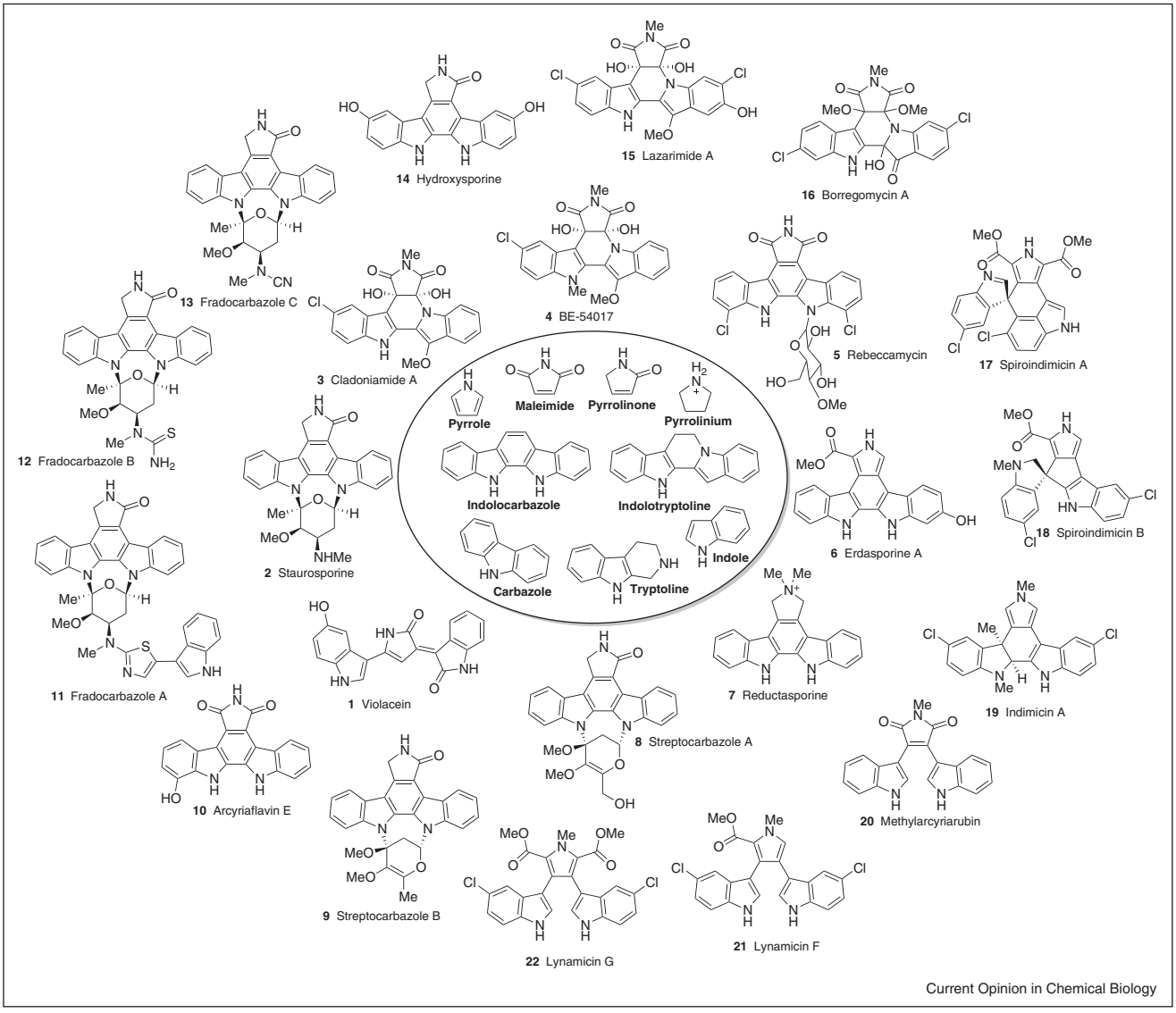
In recent years, the field of bisindole biosynthesis has been reinvigorated with the identification of new bisindole scaffolds and their corresponding biosynthetic pathways. In particular, targeted screening of environmental DNA (eDNA) and microbial libraries to identify new bisindole genes has led to a number of new compounds [8••,9••,10]. Furthermore, the identification of cladoniamide A (**3**) and BE-54017 (**4**) as potent cytotoxic agents that putatively target the vacuolar-type H^+-ATPase in eukaryotic cells [11•] has revived interest in bisindoles as potentially useful chemotherapeutics. In this review, we will highlight the most recent work in the field, focusing on the enzymatic and non-enzymatic transformations that convert shared biosynthetic intermediates into diverse, bisindolic structures.

Core structures of microbial bisindoles

The most prevalent group of such bacterial bisindoles is the 'indolocarbazoles,' with the commonly observed arrangement of an indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole. Traditionally, indolocarbazoles were simply divided into those with a maleimide ring, like rebeccamycin (**5**), and those with a pyrrolinone ring, like staurosporine (**2**) (Figure 1). More recently, however, this classification was expanded with the identification of indolocarbazoles with an upper, pyrrole-carboxylic acid ring, like erdasporine (**6**) [12], and those with a pyrrolinium ring, like reductasporine (**7**) [13]. Among these four types of indolocarbazoles, the core indolocarbazole scaffold is fully planar. Indeed, for rebeccamycin and staurosporine, the planarity is thought to be key for both intercalation into DNA and targeting the nucleotide binding site in topoisomerase and kinase targets, respectively [14,15]. Recent examples of bacterial indolocarbazole isolation in the literature include the streptocarbazoles (**8** and **9**) [16], arcyriaflavin E (**10**) [17], fradocarbazoles (**11**–**13**) [18], and hydroxysporine (**14**) [13].

However, not all bacterial bisindoles have a planar core. In the past years, molecules with a so-called 'indolotryptoline' scaffold have been isolated. In all four published examples — cladoniamide (**3**) [19•], BE-54017 (**4**) [8••,20], lazarimide (**15**) [21], and borregomycin A (**16**) [22] — the introduction of two hydroxyl groups across a double bond in the central part of the structure, and rearrangement of one indole, eliminates the extended aromaticity, and the succinimide ring flairs upward. Interestingly, the putative molecular target of this molecule, the vacuolar-type H^+-ATPase, is also thought to be unique from any indolocarbazole [11•]. Whether and how the loss of planarity is key to interaction with the putative target remains to be investigated. Another group of fascinating, non-planar bisindole

Figure 1



Heterocycles (in center) that compose key bisindolic molecules.

structures are the spiroindimicins A-B (**17** and **18**), where the atoms that make up the lower ring have been manipulated into [5,6] or [5,5] spiro-rings, eliminating any planarity [9••]. The spiroindimicin producer also makes the indimicins (**19**), another group of non-planar molecules. However, in this case, the loss of planarity comes from a reductive methylation across of the C2–C3 bond of the indole [23]. Other non-planar bacterial bisindoles include the bisindolylmaleimides, also known as arcyriarubins [24]. These molecules lack a C-C bond linking the C2 carbons of the indoles, and thus the indoles are untethered and flare apart from one another to avoid steric clash. Recent examples of bacterial bisindolylmaleimides include methyarcyriaflavin (**20**), isolated from eDNA [24], and two related compounds, the lynamicins F-G (**21** and **22**), which have an upper ring structure resembling spiroindimicins A-B (**17** and **18**), respectively [23]. Finally, one of the oldest known bisindoles, violacein (**1**), has the most unusual scaffold, with three-linked nitrogen heterocycles, each of which occupies its own plane in energy-minimized calculations.

Oxidative dimerization of L-tryptophan

Despite the large structural diversity of all such bacterial tryptophan dimer bisindoles, the initial biosynthetic steps are shared. These steps are: (1) oxidation of L-tryptophan (or a chlorinated derivative) to the corresponding indolepyruvate (IPA) imine (**23**) by a flavoprotein oxidase [25], and (2) coupling of two molecules of IPA enamine (**24**) by