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Current Opinion in Chemical Biology

Indolocarbazole antitumour compounds by combinatorial biosynthesis

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The indolocarbazoles constitute a family of natural products with potential therapeutic applications in the treatment of cancer and neurodegenerative disorders. Members of this family are either potent stabilizers of topoisomerase I-DNA covalent complex or potent inhibitors of protein kinases. Rebeccamycin, staurosporine, AT2433 and K252a are members of this family, which are produced by different actinomycete strains, and whose biosynthesis gene clusters have been isolated and characterized. A number of indolocarbazole derivatives have been generated by applying combinatorial biosynthesis technologies to these clusters either in the producer strain or in the heterologous hosts after expression of part or the entire gene cluster. Combinatorial biosynthesis is therefore providing a new approach for increasing structural diversity in this family of natural products.

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Current Opinion in Chemical Biology 2009, **13**:152–160

This review comes from a themed issue on
Molecular Diversity
Edited by Christian Hertweck and Hiroyuki Osada

Available online 27th February 2009

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2009.02.003

Introduction

The indolocarbazole alkaloids constitute an important class of natural products, which have been isolated from actinomycetes, cyanobacteria, fungi, slime moulds and marine invertebrates [1•]. After the isolation of the first indolocarbazole in 1977, this family of compounds has attracted the attention of many researchers from different disciplines because of the variety of chemical structures and the interesting biological activities showed by this family of compounds. Structurally (Figure 1a), the members of this family are characterized by a core consisting of either an 'open' bisindolylmaleimide (e.g. arcyriarubin A), or a 'closed' indolo[2,3-α]carbazole (e.g. rebeccamycin, staurosporine). Most of the latter compounds are, in fact, derivatives of the indolo[2,3-α]pyrrolo[3,4-c]carbazole ring system, to which a sugar residue is often attached, but they differ in the way the sugar is attached to the indolocarbazole moiety: through one (as in rebeccamycin and AT2433) or two (as in staurosporine and K-252a) N-glycosidic bonds.

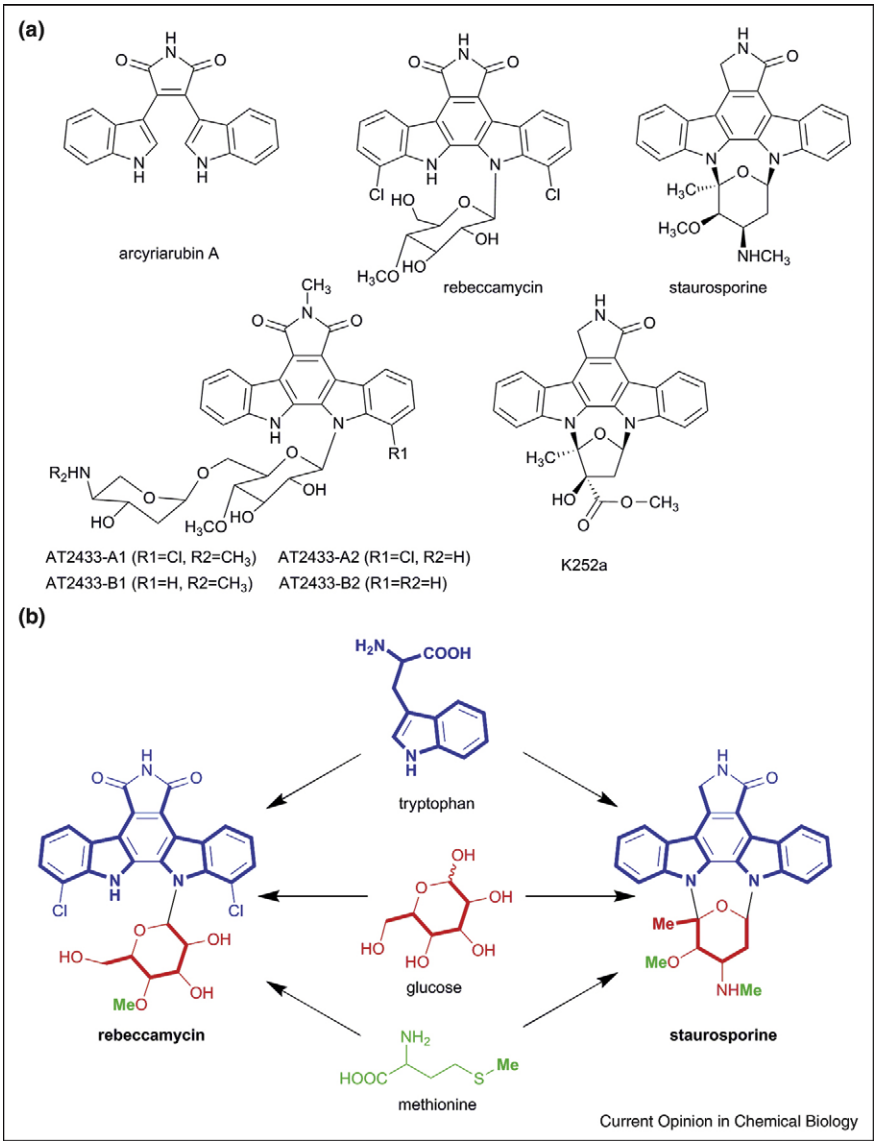
Indolocarbazoles display a wide range of biological activities, including antibacterial, antifungal, antiviral, hypotensive, antitumour or neuroprotective properties, but their greatest interest is based on their antitumour properties [2,3], and great efforts are made to generate indolocarbazole derivatives with improved properties for the treatment of cancer. Some indolocarbazole derivatives have entered into clinical trials for cancer and also for the treatment of neurodegenerative disorders, and diabetes-associated pathologies [4•]. When considering the mode of action, indolocarbazoles can be subdivided into two different subgroups. The first subgroup is represented by rebeccamycin that is a potent stabilizer of topoisomerase I-DNA covalent complex. The second one, represented by staurosporine, includes potent inhibitors of protein kinases A, C and K. This suggests that the structural differences between these two indolocarbazoles are essential for target selectivity.

A number of indolocarbazole derivatives have been produced by chemical synthesis or semi-synthesis [5,6]. However, in the past few years the isolation and characterization of biosynthesis gene clusters for several indolocarbazoles produced by actinomycetes offers a promising alternative for increasing structural diversity in this family of compounds through the use of combinatorial biosynthesis technology. This article revises the gene clusters characterized, the enzymatic characterization of different steps in indolocarbazole biosynthesis and how combinatorial biosynthesis is being used as a tool for diversifying and expanding the chemical space in this family of natural products.

Biosynthetic origin of indolocarbazoles

Early studies on the biosynthetic origin of indolocarbazoles shed light on the biosynthetic pathways through the identification of metabolic precursors and some biosynthesis intermediates. Furthermore, the flexibility of the biosynthetic machinery was tested for accepting alternative precursors, and several new analogues were generated. The biosyntheses of staurosporine and rebeccamycin were studied by feeding isotope-labeled precursors to their producer organisms: *Lentzea albida* (formerly *Streptomyces staurosporeus*) staurosporine producer and *Lechevalieria aerocolonigenes* (formerly *Saccharothrix aerocolonigenes*) rebeccamycin producer [7–10]. It was established that

Figure 1



Indolocarbazoles: structures and biosynthetic origin. **(a)** Chemical structures of the indolocarbazoles whose gene clusters have been characterized. **(b)** Biosynthetic origin of indolocarbazoles. Feeding isotope-labeled precursors and analysis of the incorporation pattern showed that L-tryptophan (blue), D-glucose (red) and methionine (green) are precursors in the biosynthesis.

the indolocarbazole core was derived from two tryptophan units with the carbon skeleton incorporated intact, while the sugar moiety was derived from glucose and methionine (Figure 1b). The identity of early tryptophan-derived intermediates was studied by feeding tryptamine, indolepyruvate, indoleacetaldehyde, indoleacetamide and indoleacetate, and showing that indolepyruvate was an intermediate in rebeccamycin biosynthesis, while tryptamine was not incorporated [8,9]. On the contrary, feeding tryptamine to the staurosoporine producer resulted in efficient incorporation into the molecule, suggesting that tryptamine was a precursor in staurosporine biosynthesis. The apparently conflicting results obtained with the two strains were perhaps due to the different experimental conditions employed, in addition to putative metabolic differences existing between the two organisms such as transaminases and other enzymes not directly involved in indolocarbazole biosynthesis.

Novel indolocarbazoles accumulated by mutants or produced by precursor feeding

A number of indolocarbazole derivatives or biosynthesis intermediates have been isolated from mutants generated by classical mutagenesis, by changing the fermentation conditions or by feeding precursors. After UV irradiation of *Streptomyces longisporoflavus* (staurosporine producer)