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Synthesis and biological activity of novel mono-indole and mono-benzofuran inhibitors of bacterial transcription initiation complex formation

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ABSTRACT

Our ongoing research focused on targeting transcription initiation in bacteria has resulted in synthesis of several classes of mono-indole and mono-benzofuran inhibitors that targeted the essential protein–protein interaction between RNA polymerase core and σ^{70}/σ^{A} factors in bacteria. In this study, the reaction of indole-2-, indole-3-, indole-7- and benzofuran-2-glyoxyloyl chlorides with amines and hydrazines afforded a variety of glyoxyloylamides and glyoxyloylhydrazides. Similarly, condensation of 2- and 7-trichloroacetylindoles with amines and hydrazines delivered amides and hydrazides. The novel molecules were found to inhibit the RNA polymerase–σ^{70}/σ^{A} interaction as measured by ELISA, and also inhibited the growth of both Gram-positive and Gram-negative bacteria in culture. Structure–activity relationship (SAR) studies of the mono-indole and mono-benzofuran inhibitors suggested that the hydrophilic–hydrophobic balance is an important determinant of biological activity.

1. Introduction

Antibiotics have been of fundamental importance in contemporary medicine and pharmacy since the discovery of penicillin, an antibacterial agent from the fungus *Penicillium notatum* by Alexander Fleming in 1928.[1,2] However, as a result of their excessive and sometimes unjustified use, bacterial strains resistant to these drugs have rapidly emerged.[3–25] The loss of potency of existing antibiotics against resistant strains has been exacerbated by the continually decreasing numbers of new antimicrobial compounds being brought to the market.[26–28]

Current antibacterial drug discovery is largely focused on the derivatization of existing classes of antibiotics. However, such compounds may be prone to inactivation by existing bacterial resistance mechanisms. Therefore, in order to combat the rise and spread of antibiotic resistant strains, the discovery and development of novel classes of antibiotics and the elucidation of their molecular targets have to be urgently pursued.[29]

Bacterial RNA polymerase (RNAP) is an attractive target for novel antibacterial agents. Several classes of antibiotics have been discovered that inhibit the activity of RNAP via different molecular mechanisms, and some of these molecules are important components of antibacterial therapies.[30–39] However, only a few classes of antibacterial agents targeting bacterial transcription initiation complex formation, and in particular the β'-CH-$\sigma^{70}/\sigma^{A}_{2.2}$ protein–protein interaction, have been discovered.[2,32,34,35,40] Synthetic molecules **1**, **2** and **3** have been found to efficiently inhibit transcription initiation in bacteria (Fig. 1).[34,35]

Bacterial transcription is a sequential process comprising three main stages: initiation, elongation and termination.[41] There are two forms of bacterial RNAP: core and holoenzyme.[41] Both enzyme forms are catalytically active, but only the holoenzyme formed by the association of the core enzyme with σ factors is able to recognize DNA promoter sequences and initiate transcription with appropriate specificity and efficiency.[41–48] The RNAP core enzyme consists of five subunits: α_2 dimer, β, β' and ω.[32,41] σ factors are

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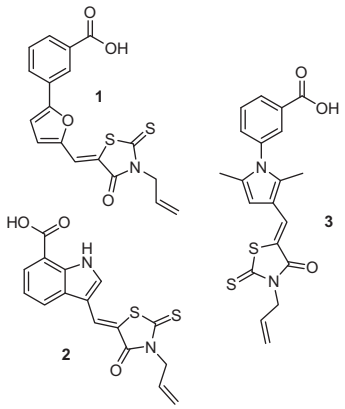


Figure 1. Synthetic molecules inhibiting transcription initiation.

unique to bacteria and the closely related σ^{70} factors in Gram-negative bacteria, and σ^{A} factors in Gram-positive bacteria are essential and required for the transcription of the majority of expressed genes.[2] The RNAP holoenzyme is formed via protein–protein interaction between two highly conserved motifs: the β'-CH region of RNAP core and region 2.2 of the σ^{70}/σ^{A} factor.[49] Since the regions of the σ^{70}/σ^{A} factor family that interact with the RNAP core enzyme are highly conserved across different bacteria species,[32,42,44,48,50,51] molecules capable of inhibiting the β'-CH-$\sigma^{70}/\sigma^{A}_{2.2}$ interaction would be expected to exhibit broad spectrum antibacterial activity. Therefore, the design and development of such novel drug candidates, potentially unique both in terms of their mechanism of antibacterial activity as well as in the structure of their chemical scaffolds, could help to combat bacterial infections that are resistant to currently used antibiotics.

Our previous research aimed at the identification of potential inhibitors of the β'-CH-$\sigma^{70}/\sigma^{A}_{2.2}$ protein–protein interaction resulted in the synthesis of a library of novel bis-indole compounds capable of efficiently disrupting this interaction in a cell-free assay.[2] Modelling studies based on a *Bacillus subtilis* RNAP homology model[52] and isothermal titration calorimetry experiments[40] proved that the bis-indoles inhibited transcription initiation by binding to the β'-CH region of the RNAP core enzyme. Interestingly, many of the synthesized molecules were found to exclusively inhibit the growth of *Escherichia coli*,[2] allowing them to potentially be used as non-conventional antibacterial agents against Gram-negative bacteria.[53–55] Compounds **4** and **5** were the most potent inhibitors of *E. coli* and *B. subtilis* growth, respectively (Fig. 2).[2]

Since the bis-indoles synthesized in our previous studies were relatively large, issues related to solubility as well as limited

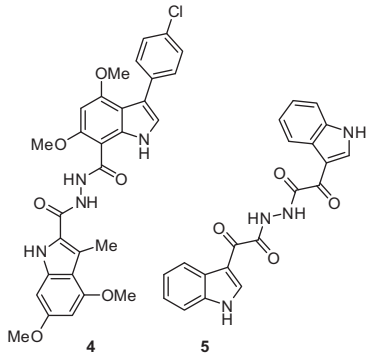


Figure 2. Bis-indole inhibitors of the interaction between RNAP core and σ^{70}/σ^{A} in bacteria.

penetration through the outer membrane in Gram-negative bacteria or the cell wall in Gram-positive bacteria were encountered.[2] Given that the indole nucleus has been discovered to be a bioactive moiety against bacterial transcription initiation complex formation, we were interested in developing smaller compounds based on indole or the related benzofuran scaffolds that could circumvent the problems associated with the larger bis-indoles, while retaining potent antibacterial activity.

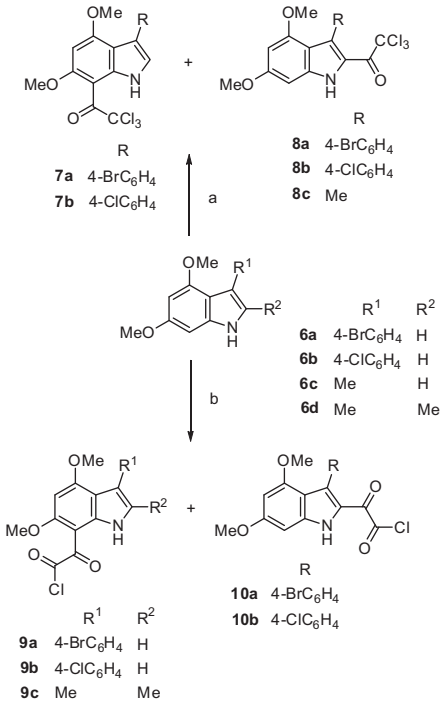
In this paper, we report the synthesis and evaluation of the antibacterial activity of a library of low molecular weight molecules active against both Gram-positive and Gram-negative bacteria, and present our structure–activity relationship (SAR) studies on these novel inhibitors of the essential β'-CH-$\sigma^{70}/\sigma^{A}_{2.2}$ protein–protein interaction.

2. Results and discussion

2.1. Synthesis of starting materials

In order to produce a library of mono-indole- and mono-benzofuran-based amides, hydrazides, glyoxyloylamides and glyoxyloylhydrazides, we employed well-established procedures that had previously been developed in our group for the synthesis of the biologically active bis-indoles **4** and **5** (Fig. 2).[2] 3-Aryl-4,6-dimethoxyindoles **6a–b**,[56] 4,6-dimethoxy-3-methylindole **6c**[56] and 4,6-dimethoxy-2,3-dimethylindole **6d**[57] were synthesized according to previously reported methods. These indoles were subsequently reacted with trichloroacetyl chloride and oxalyl chloride to yield a variety of derivatives, including 7-trichloroacetylindoles **7a–b**, 2-trichloroacetylindoles **8a–c**, indole-7-glyoxyloyl chlorides **9a–b** and indole-2-glyoxyloyl chlorides **10a–b** (Scheme 1).[2]

Following another well-established method developed by our group, 4,6-dimethoxy-3-phenylbenzofuran **11**[58] was synthesized and subsequently reacted with oxalyl chloride to afford benzofuran-2-glyoxyloyl chloride **12**. Additionally, indole **13** was treated



Scheme 1. Reagents and conditions: (a) CCl_3COCl (3 equiv), anhydrous 1,2-dichloroethane, 80 °C, 3.5 h, 20–37% (**7a–b**), 10–23% (**8a–c**); (b) oxalyl chloride (3 equiv), anhydrous diethyl ether, 0 °C → rt, 3 h, 30–33% (**9a–b**), 35–43% (**10a–b**).