Natural Product Analogues as Antibacterial Agents: The Case of Cinnamaldehyde and Colupulone

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ABSTRACT

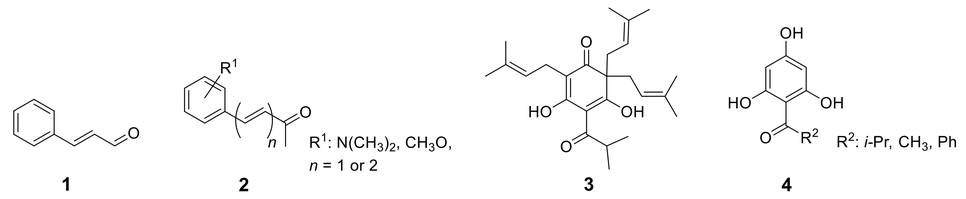
The ever-growing antibiotic resistance to currently prescribed antibiotics constitutes a main reason for investigating natural compounds for antibacterial action. Natural products can be used as leads for new synthetic antibacterial agents or as a source of novel bioactive compounds. Cinnamaldehyde and colupulone were selected as lead compounds for the purposes of this study. Cinnamaldehyde, a byproduct of the stem bark of *Cinnamomum cassia*, was isolated in 1834 by Jean-Baptiste Dumas, with uses ranging from the food and cosmetics to pharmaceutical industries. Colupulone, is a known hop β-acid found in *Humulus lupulus*, a plant also used in the pharmaceutical and food industry. Previous studies have shown that both compounds exhibit antibacterial properties. In order to investigate essential structures responsible for enhanced action, some functionalities on the selected parental compounds, cinnamaldehyde and colupulone, were preserved while others altered. Synthesis of these analogues was based on short synthetic routes, including efficient methods, such as Wittig reaction, Friedel Crafts and C-alkylation of phloroglucinol derivatives. Subsequent testing for their antibacterial action against gram-positive and gram-negative microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* revealed important functionalities required for increased activities. The prospective development of a ligand-based pharmacophore was also investigated, by analyzing the structural-activity relationship of their bacterial growth inhibitory potencies. Enhance inhibitory action was observed for the para-methoxy analogue of *trans*-cinnamaldehyde against *E.coli*. In addition, all tested colupulone analogues exhibited enhanced activities for both *E. coli* and *S.aureus*. These results set the base for designing new compounds to better understand structure-activity relationship and improve activity.

INTRODUCTION

Combating antibacterial resistance has been the focus of research efforts over the past few years [1]. The main factor contributing to the crisis of antibiotic resistance is antibiotic overuse. The development of novel antibiotics from natural product analogues is an attractive strategy [2]. This study focuses on two natural products as lead compounds for studying a first series of their analogues, cinnamaldehyde (**1**) and analogue structures of type **2**, as well as colupulone (**3**) and analogue structures of type **4** (Fig. 1). The antibacterial activity of type **2** compounds may be related to their ability to act as Michael acceptors and serve as covalent drugs by binding to catalytic domains involved in bacterial cell wall biosynthesis, a known antimicrobial target [3], while type **4** compounds activity may result from their reactive oxygen species (ROS) generating ability and closely depend on their lipophilicity [4].

Figure 1

NATURAL COMPOUNDS LEADS & ANALOGUE STRUCTURES



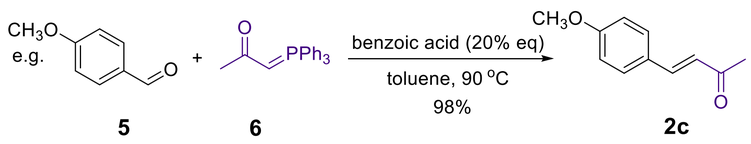
*Natural products trans-cinnamaldehyde (**1**), colupulone (**3**) and a first series of analogues (**2, 4**). Left: Cinnamomum cassia. Right: Humulus lupulus*

Based on the observed bacterial growth inhibition (EC_{50}), Structure-Activity Relationships (SAR) between a first series of known easily prepared multifunctional compounds, **2** and **4**, is employed to determine the most effective functionalities that will, in turn, lead to the development of a series of more active analogues and possibly a ligand–based pharmacophore.

METHODOLOGY

Figure 2

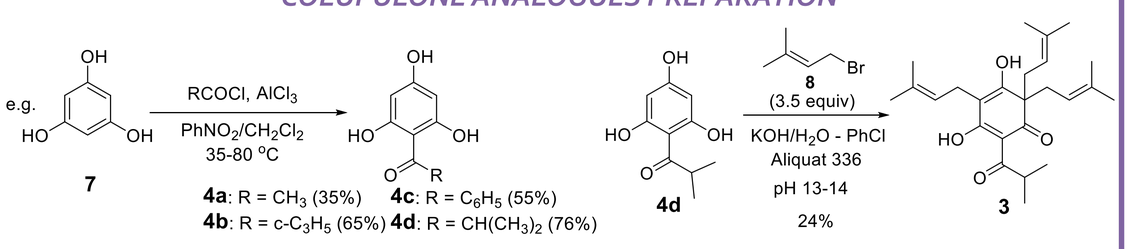
CINNAMALDEHYDE ANALOGUES PREPARATION



A first series of cinnamaldehyde analogues was prepared exploiting a Wittig reaction for the introduction of an α,β-unsaturated ketone system in a commercially available aromatic aldehyde (Fig. 2).

Figure 3

COLUPULONE ANALOGUES PREPARATION



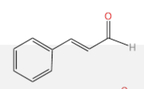
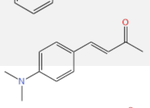
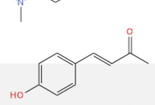
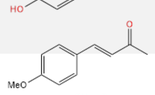
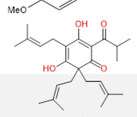
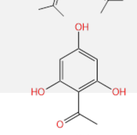
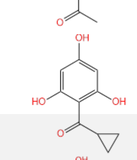
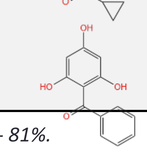
A first series of colupulone analogues **4** was prepared by introducing an acyl group by Friedel Crafts on the phloroglucinol core and colupulone (**3**) was prepared by incorporating prenyl side chains on the aromatic core by C-alkylation in alkaline conditions [5].

All analogues were monitored for antibacterial activity (Fig. 4) at dilutions raging from 9.8μM to 5.0mM, incubated at 37°C and 70 rpm, and MICs were determined by measuring UV/Vis absorbance at 600nm. Compounds **2a, 2c, 4a** and **4c** were also evaluated by quantitative plating of *P. aeruginosa* (0.07 - 20mM).

RESULTS

Figure 4

EXAMINED PARENT COMPOUNDS & ANALOGUES MIC VALUES

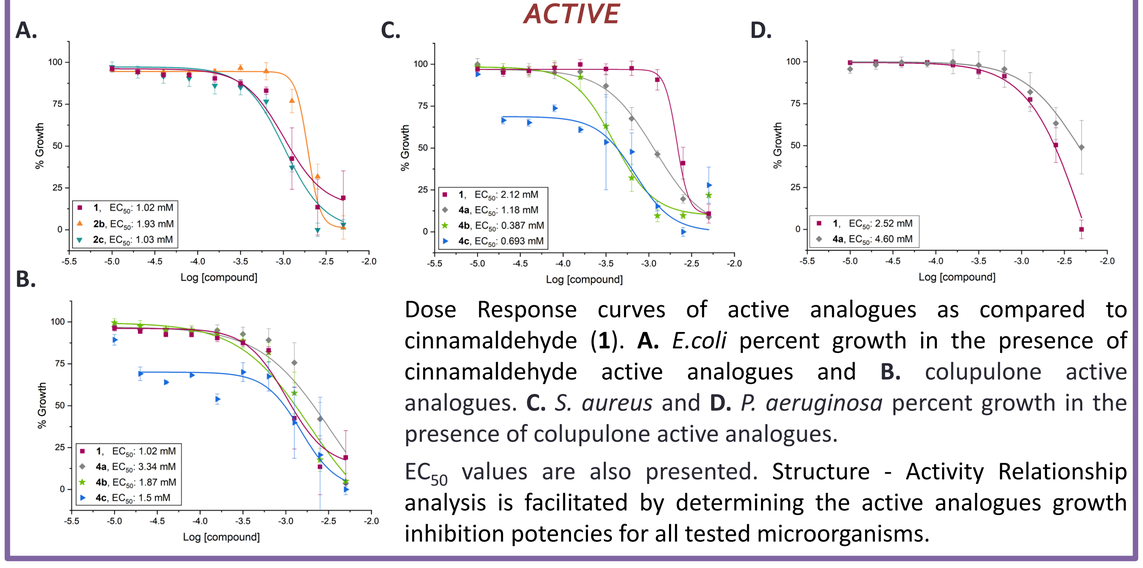
No	Structure	Minimum Inhibitory Concentrations (MIC)		
		E. coli	*S. aureus*	*P. aeruginosa*
1		>0.66mg/ml*	0.66mg/ml	0.66mg/ml
2a		Not active	>0.95mg/ml*	Not active
2b		0.81mg/ml	Not active	>0.81mg/ml*
2c		0.44mg/ml	Not active	>0.88mg.ml*
3		37.5mg/ml [4]	37.5mg/ml [4]	N/A
4a		0.84mg/ml	0.84mg/ml	>0.84mg/ml
4b		0.97mg/ml	0.24mg/ml	Not active
4c		1.15mg/ml	0.58mg/ml	Not active

**Inhibition ranging from 50% - 81%.*

Table of MIC values of tested cinnamaldehyde analogues (**2a, 2b, 2c**) and colupulone analogues (**4a, 4b, 4c**). Parent bacterial growth inhibition effects of *trans*-cinnamaldehyde (**1**) and colupulone (**3**) are also presented.

Figure 5

DOSE RESPONSE CURVES OF SELECTED ANALOGUES ACTIVE



Dose Response curves of active analogues as compared to cinnamaldehyde (**1**). **A.** *E.coli* percent growth in the presence of cinnamaldehyde active analogues and **B.** colupulone active analogues. **C.** *S. aureus* and **D.** *P. aeruginosa* percent growth in the presence of colupulone active analogues.

EC_{50} values are also presented. Structure - Activity Relationship analysis is facilitated by determining the active analogues growth inhibition potencies for all tested microorganisms.

CONCLUSIONS

- Antibacterial resistance has recently been an issue of great concern. Natural products can hold the solution to this problem, especially the creation of natural product analogues (Fig. 1).
- Cinnamaldehyde natural product analogues were synthesized and tested for antibacterial action. The most effective analogue against *E.coli* was **2c** and against *S. aureus* **2a**, while the first series of analogues tested did not improve cinnamaldehyde's active against *P. aeruginosa* (Fig. 4).
- Colupulone natural product analogues were also synthesized and tested for antibacterial action. The most effective analogue against *E.coli* was **4a** and against *S. aureus* **4b**. Colupulone analogues tested were not found active against *P. aeruginosa*, with a 50% inhibition of growth only observed in the presence of 0.84mg/ml of analogue **4a** (Fig. 4).
- Increased *E.coli* growth inhibition was observed by the addition of a methoxy functionality at the para position of the cinnamaldehyde analogue, as well as by simplifying the structure of colupulone. The addition of a cyclopropyl functionality on the simplified colupulone structure yielded increased activity against *S. aureus*, whereas no significant improvement was observed from the first series of analogues when tested for inhibiting *P. aeruginosa* growth (Fig. 5).
- Bacterial growth inhibition studies of additional type **2** cinnamaldehyde analogues and type **4** colupulone analogues are currently underway to further elucidate their structure-activity relationships.

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