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Cinnamaldehyde Analogues as antibacterial agents: A Study of Structure-Activity Relationship

Maria Veisllari [1], Veroniki P. Vidali [2], Georgia Kythreoti [1]

[1] Department of Science and Mathematics, The American College of Greece, 6 Gravias Street, 15342 Athens, Greece
[2] Natural Products Synthesis and Bioorganic Chemistry Laboratory, Institute of Nanoscience & Nanotechnology, National Centre of Scientific Research "Demokritos", Terma Patr. Grigoriou & Neapoleos 27, Ag. Paraskevi, 15341, Greece

INTRODUCTION & AIM

Antimicrobial resistance (AMR) is a major public health threat, projected to cause up to 10 million deaths annually by 2050 [1]. Driven by excessive antibiotic use in both humans and animals, resistant bacteria are undermining decades of medical progress — from infection treatment to procedures like surgery and chemotherapy [2]. To address this crisis, research is turning to natural antimicrobial compounds. Cinnamaldehyde, the primary component of cinnamon bark oil (62–90%), exhibits broad-spectrum antibacterial, antioxidant, and anti-inflammatory properties, as well as some documented synergy with aminoglycosides. However, high volatility, low stability, low solubility, and sensitivity to oxygen, light, and heat limit its clinical use [3]. The antibacterial activity of cinnamaldehyde alone is modest, with MICs (Minimum Inhibitory Concentrations) much higher than conventional antibiotics. This study investigates structurally modified cinnamaldehyde analogues (aldehydes and ketones) against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Antibacterial activity was accessed while structure–activity relationships (SAR) were explored followed by the evaluation of synergistic effects with ampicillin and neomycin using checkerboard assays.

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METHOD

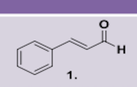
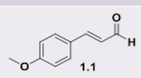
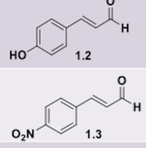
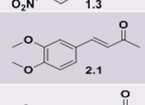
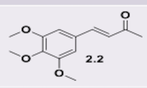
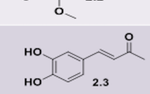
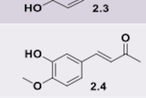
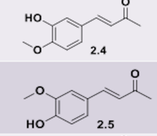
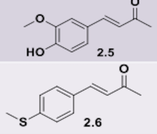
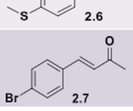
Ten cinnamaldehyde analogues, including aldehydes and unsaturated ketones, were tested against *E. coli, S. aureus,* and *P. aeruginosa.* Antibacterial activity was assessed using a 96-well broth microdilution assay ranging from 5 mM to 0.0098 mM. Bacterial inoculums were standardized to OD_{600} = 0.004, and plates were incubated for 18–24 hours at 37 °C. Controls included solvent (methanol), growth and ampicillin. Bacterial growth was assessed via absorbance at 600 nm.

Additive or synergistic effects for *E.coli* were determined by combining compounds with antibiotics across dilution grids. Each experiment was repeated at least three times in duplicate. IC_{50} values were calculated via non-linear regression. Interactions were classified based on the sum of fractional inhibitory concentrations (ΣFIC): synergistic as ΣFIC < 0.5, additive as 0.5 ≤ ΣFIC ≤ 1.0, and indifferent as ΣFIC > 1.0.

RESULTS & DISCUSSION

Figure 1

Examined Parent Compound & Analogues

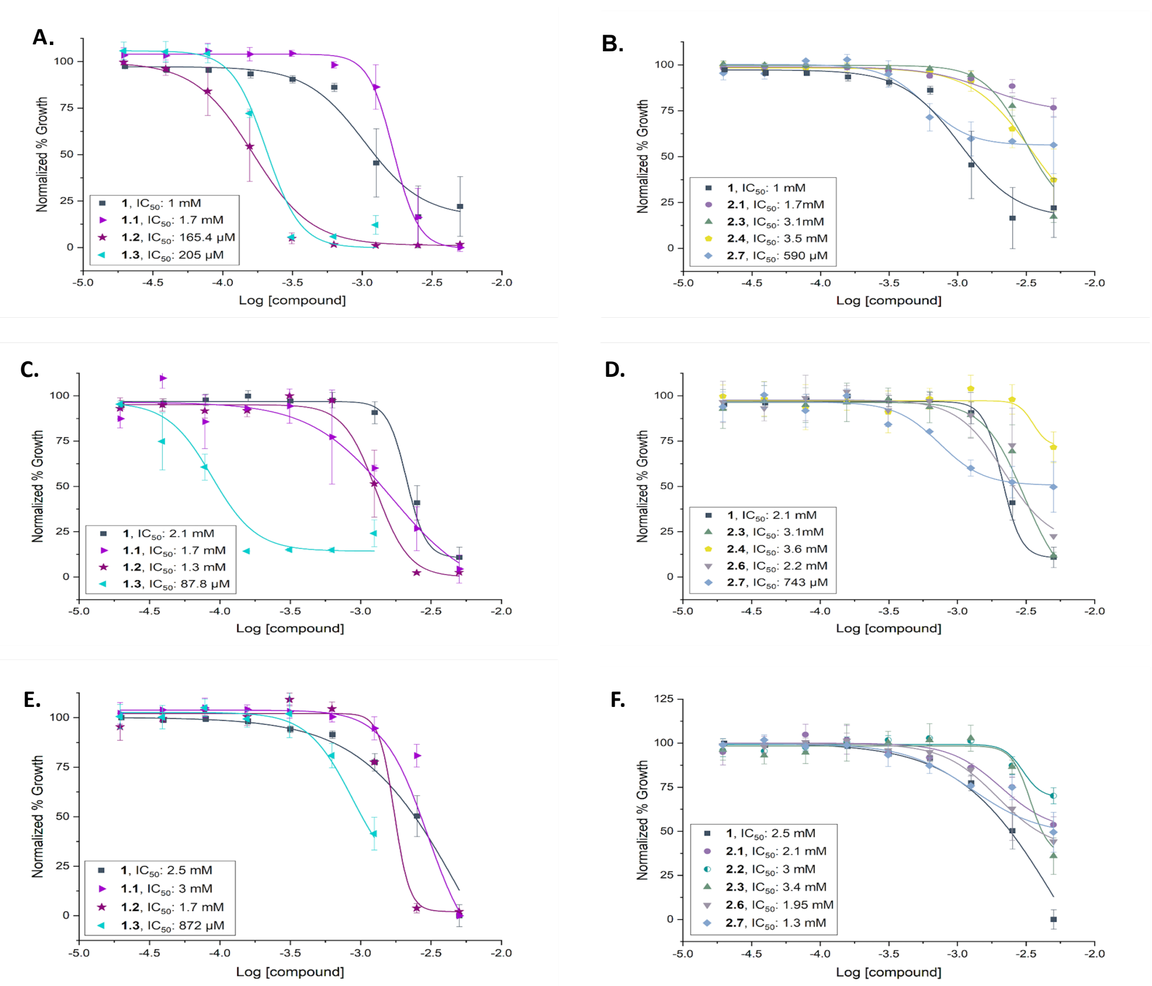
Compound No.	Structure
1 (Cinnamaldehyde)	
1.1	
1.2	
1.3	
2.1	
2.2	
2.3	
2.4	
2.5	
2.6	
2.7	

Cinnamaldehyde (1) and its analogues were grouped into two series: substituted aldehydes (1.1–1.3) and unsaturated ketones (2.1–2.7). Structural modifications included para-positioned methoxy, hydroxy, nitro, and bromo groups, as well as multi-site substitutions with potential to influence antibacterial potency and selectivity.

Figure 2

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 analogues 1, 1.1, 1.2, 1.3, and 2.3, and **B.** analogues 2.1, 2.3, 2.4, and 2.7. **C.** *S. aureus* growth in the presence of analogues 1, 1.1, 1.2, and 1.3, and **D.** analogues 2.1, 2.3, 2.4, 2.6, and 2.7. **E.** *P. aeruginosa* growth with analogues 1, 1.1, 1.2, and 1.3, and **F.** analogues 2.1, 2.2, 2.3, 2.6, 2.7.

IC_{50} values are also presented. These data support structure–activity relationship analysis by comparing the growth inhibition potencies of active analogues across bacterial species.

Figure 3

IC_{50}-Based Synergy Analysis of Cinnamaldehyde Analogues in Combination with Neomycin Against *E. coli*

Combination	FIC (compound in µM)	FIC (neomycin in µM)	ΣFIC	Interaction
2.1 + Neomycin	36.9 / 1700 = 0.0217	3.89 / 9.57 = 0.406	0.428	**Synergistic**
1.2 + Neomycin	31.3 / 165.4 = 0.189	3.11 / 9.57 = 0.325	0.514	**Additive**
1.3 + Neomycin	82.9 / 205 = 0.404	3.89 / 9.57 = 0.406	0.81	**Additive**

Only neomycin combinations are shown; all ampicillin results were classified as indifferent and are not depicted.

Cinnamaldehyde analogues with para-position substitutions, particularly 1.2, 1.3 and 2.7, demonstrated improved antibacterial potency across species, supporting a structure-activity relationship driven by electronic effects. Synergistic or additive effects with neomycin were observed in *E.coli* , but not with ampicillin, suggesting specificity in interaction. These effects may be linked to increased membrane permeability caused by cinnamaldehyde, which has been previously reported to disrupt membrane integrity and facilitate antibiotic uptake [4].

These results highlight the therapeutic potential of natural product–based hybrid scaffolds and offer design insights for future analogue development.

CONCLUSION

- Cinnamaldehyde analogues exhibited strain-specific antibacterial activity, with efficacy influenced by structural modifications. Analogues 1.2 and 1.3 showed the most potent inhibition, particularly against *E. coli* and *S. aureus*. Among the ketone analogues, only the para-bromo substitution (analogue 2.7) improved potency.
- The synergy assay revealed a potential connection between the mode of action of cinnamaldehyde derivatives and neomycin. All combinations with ampicillin were classified as indifferent.
- Two novel hybrid aldehyde analogues are proposed: one with para-bromo and meta-nitro substitutions, and another with meta-bromo and para-nitro. These designs aim to combine the beneficial properties of both functional groups to further enhance antibacterial efficacy.

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