Synthesis and Evaluation of Ferrocenyl- Subsituted Compounds as Novel Antimicrobial Agents

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Introduction

The rise and escalating threat of antibiotic resistance has shifted the attention towards alternative antibacterial strategies, specifically natural compounds with antibacterial properties. Curcumin (diferuloylmethane) **(Compound 1)** is a yellow-colored, non-toxic polyphenol of hydrophobic nature which has been identified as the naturally-occurring active compound of turmeric [1].

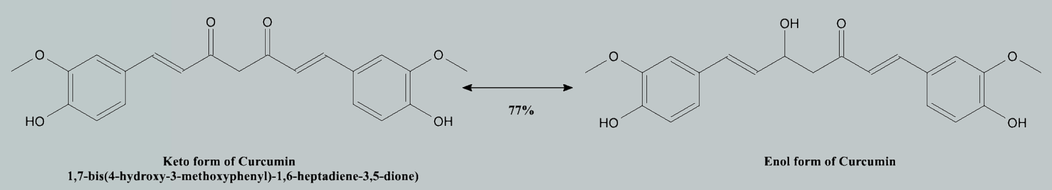


Figure 1: Tautomeric forms of curcumin **(compound 1)** in its commercial form: the **keto** form (predominant in solid state and acidic conditions) and the **enol** form (stabilized in solution and under neutral to basic conditions). These forms contribute to curcumin's chemical reactivity and biological activity.

To date, there have been numerous studies on the application of curcumin in biological systems while reporting its antioxidant, anti-inflammatory, anti-proliferative, antitumor, proapoptotic, neuroprotective and anti-microbial potentials. Curcumin exhibits low bioavailability due to its symmetrical structure and the presence of functional groups—namely, two methoxy (-OCH_3) and two hydroxy (-OH) groups, aromatic rings, and keto-enol tautomerism—which contribute to its rapid metabolism and excretion [1], [2].

Novel asymmetric monocarbonyl analogs of curcumin were synthesized as part of the effort to develop highly potent, more stable and efficient compounds through Claisen-Schmidt aldol condensation and Wittig olefination reaction.

Furthermore, a ferrocenyl-bearing arylbenzamide was designed as an analogue of the brain-penetrant, mutation-selective LRRK2 inhibitor **GSK2578215A (GSK)**, with the aim of investigating its potential antimicrobial activity.

Objectives

- Investigate the potential antimicrobial effects of various **structural analogs of curcumin and GSK** the active compound of turmeric and LRRK2 inhibitor, respectively.
- Synthesize and evaluate **ferrocenyl-substituted compounds** as novel antimicrobial agents on Gram-negative bacterial species (*Escherichia coli, Pseudomonas aeruginosa).*
- Explore the **role of iron** in bacterial growth and how ferrocenyl groups may impact iron acquisition during infection.
- Utilize diverse **bacterial culturing and screening techniques**, including:
  - Quantitative plating
  - High-throughput screening assays
  - **Antibacterial photodynamic therapy (aPDT)** to assess activity of compounds under light irradiation.
  - Compare bacterial **growth inhibition** and **compound efficacy** under non-irradiated and irradiated conditions.

Methodology

Synthesis of Curcumin- and GSK-Derived Ferrocenyl-Substituted Analogues:

To address curcumin's bioavailability, in vivo and in vitro studies have explored monocarbonyl derivatives of curcumin—compounds that lack the central β-diketone moiety—as more stable and bioavailable alternatives. One such derivative, C1, incorporates di-ortho-methoxy substitutions on its aromatic rings, which may enhance its pharmacokinetic properties and interaction with biological targets compared to native curcumin.

Initially, a 'half-C1' intermediate was synthesized, designed to serve as a versatile reagent for the preparation of additional analogues with structural diversity.

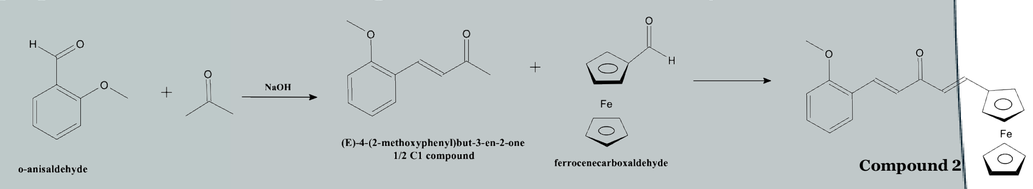


Figure 2: The synthesized compounds were developed through aldol condensation reactions, specifically Claisen-Schmidt condensation from the respective aromatic aldehyde (benzaldehydes) and acetone with sodium hydroxide as the main catalyst which deprotonates an alpha proton from the carbonyl reagent and forms enolate ions.

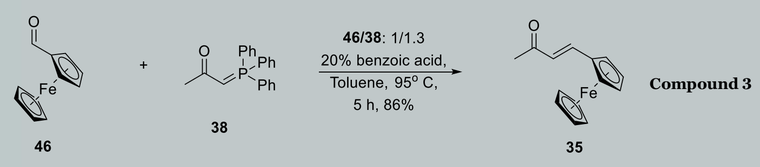


Figure 3: Compound 3 (analogue 35), one of the synthesized curcumin analogues, was prepared through a Wittig-type olefination reaction designed to introduce a ferrocenyl moiety into the molecular framework. The reaction involved the condensation of ferrocenecarboxaldehyde with ylide 38 in 3 mL of toluene, using benzoic acid as a mild acid catalyst.

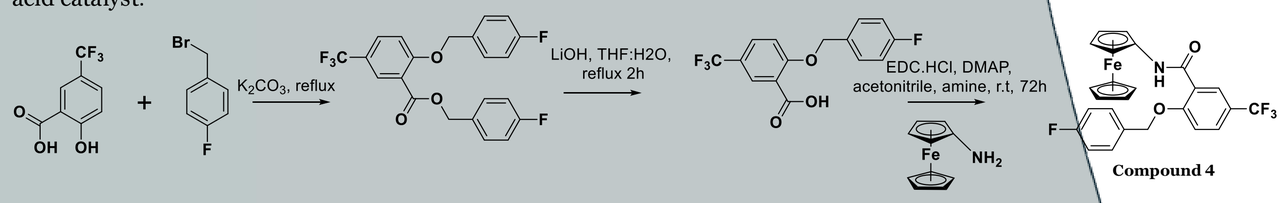


Figure 4: The synthesis reaction for **Compound 4** begins with the etherification of a trifluoromethylated catechol and fluorinated benzyl bromide under basic conditions. The resulting bis-ether intermediate undergoes ester hydrolysis yielding the corresponding carboxylic acid. Lastly, amidation with ferrocenylamine produces the ferrocenyl-arylbenzamide product.

Evaluation of Antibacterial Activity

UV-Vis spectroscopy (250–700 nm) was used to determine the light absorption of the tested compounds. Photodynamic activity was evaluated by irradiating treated samples (ranging from 6.2 mM to 0.01 mM concentration) with a 470 nm blue LED for 3 hours at a 10 cm distance from the light source and light intensity of ~10 mW/cm² [3]. Controls included dark (non-irradiated), light-only, DMSO and Ampicillin antibiotic. Quantitative plating was performed using colony-forming unit (CFU) assays, in which *E. coli* was treated with the compounds (0.0023 mM to 10 mM). Bacterial growth was quantified by measuring OD_{600} following a 16–18-hour incubation at 37°C, and IC_{50} values were determined by fitting data to sigmoidal dose–response curves.

Results

- **Absorption Spectra and Photophysical Characterization**

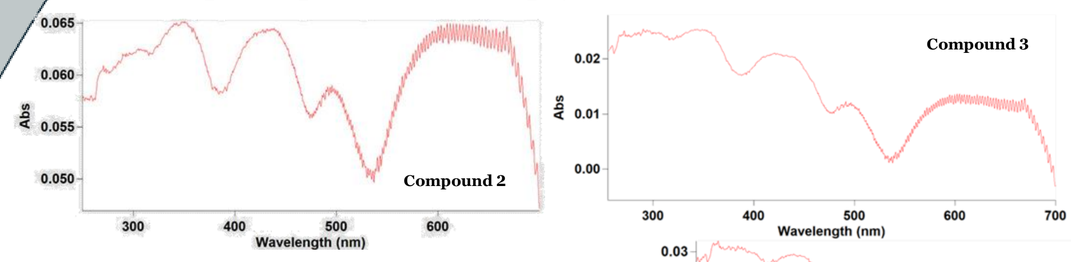


Figure 5: UV-Vis absorption spectra of the ferrocenyl-substituted compounds 2,3 and 4 (10 μM) were recorded in H_2O over the 250–700 nm range. Notable features include absorption peaks around 470 nm and 600 nm, corresponding to the blue and orange regions of the visible spectrum, respectively.

- **High-Throughput Screening Assays for Antibacterial Activity**

Compounds were dissolved in DMSO (50 mM stock) and tested across a concentration range under both irradiated and non-irradiated conditions.

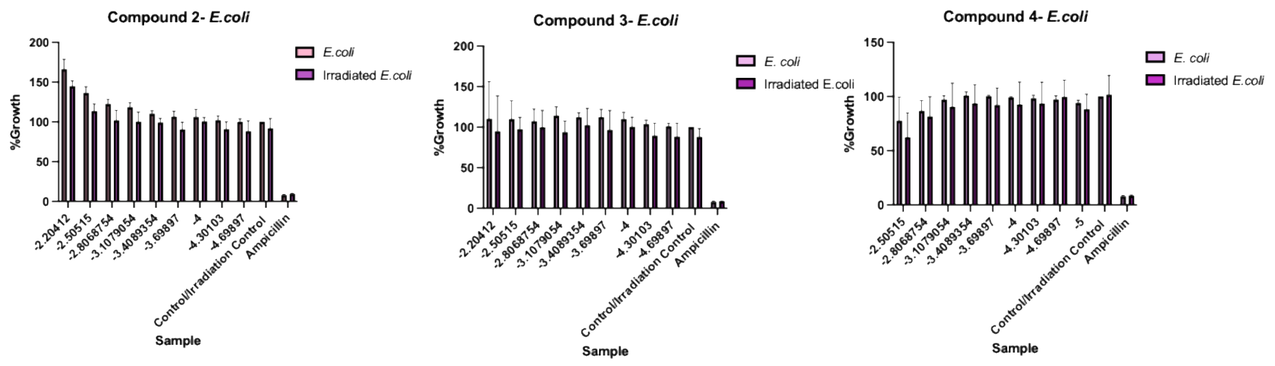


Figure 6: High-throughput growth inhibition assay of *E. coli* exposed to ferrocenyl-bearing compounds under blue light irradiation and dark conditions. *E. coli* cultures were treated with serial dilutions of Compounds 2 and 3 (6.25–0.02 mM) and Compound 4 (3.2–0.01 mM), plotted on a logarithmic scale. Cultures were incubated either in the dark (pink bars) or under 470 nm blue light (purple bars). Percent growth was normalized to untreated controls. DMSO and light-only controls showed no significant effects, while ampicillin (+ control) almost completely inhibited growth. Data represent mean ± SD from three independent experiments, each performed in technical duplicates. Statistical significance was assessed via paired *t*-tests comparing treatment to control at each concentration.

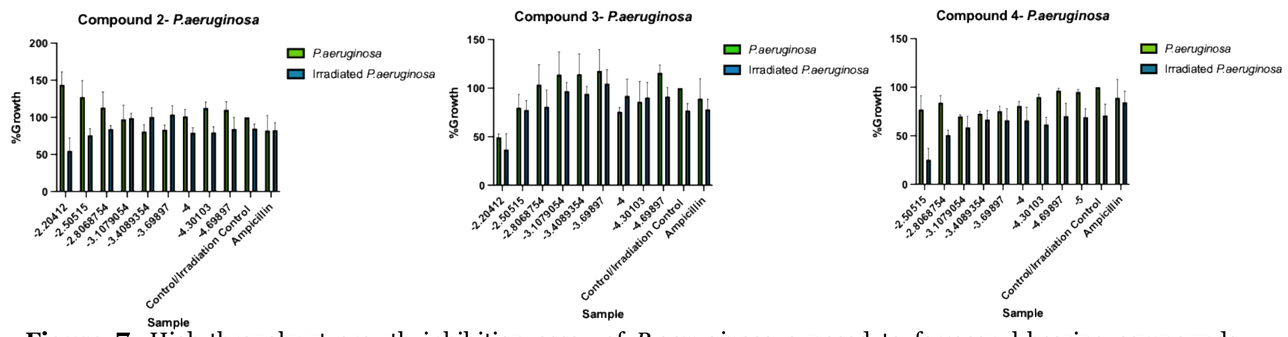


Figure 7: High-throughput growth inhibition assay of *P.aeruginosa* exposed to ferrocenyl-bearing compounds under blue light irradiation and dark conditions. Antibacterial activity of Compounds 2, 3, and 4 against *Pseudomonas aeruginosa* (non-irradiated and irradiated). Treatment conditions for *P.aeruginosa* were similar to those applied for *E.coli*. Cultures were incubated either in the dark (green bars) or under 470 nm blue light (blue bars). No statistical significance were confirmed among conditions; however, all three compounds exhibit effect with irradiation and at higher concentrations.

- **Dose-Response Curves**

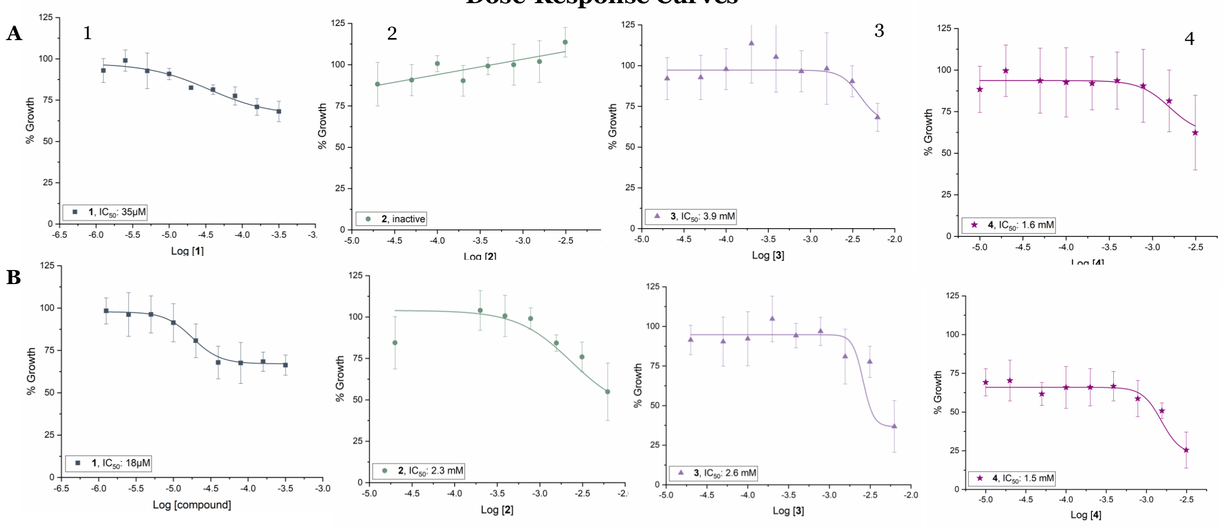


Figure 8: Dose-response curves illustrating antibacterial activity of ferrocenyl- bearing compounds compared to curcumin(compound 1) as a control (curve 1). **A.** *E.coli* % growth after treatment with activated Compound 1, 3 and 4 (active), while inactive for Compound 2. **B.** *P. aeruginosa* % growth after treatment with activated compounds. IC_{50} values present higher than those of curcumin for the respective species **(1A, 1B)** indicating moderate antibacterial activity and potency.

Conclusion

- Light-dependent antimicrobial effect was observed among all tested compounds for both Gram-negative bacterial species.
- Compound 4 (GSK analog) exhibited the lowest IC_{50}value while followed by Compound 3 it was observed a drop in % growth indicating moderate activity.
- In contrast to ampicillin, the tested compounds demonstrated greater efficacy against *P.aeruginosa*; however, a slight decrease in bacterial growth was observed under blue light exposure, suggesting an additional inhibitory effect of the irradiation.
- Quantitative platting using CFU assays indicated non-active compounds at higher concentrations, possibly due to solubility and aggregation effects.
- Ferrocenyl-bearing compounds, even if less potent could offer advantages in terms of improved stability, selectivity or in synergism with compounds/antibiotics.

Acknowledgments

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