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Original Article

Thiazolidinone derivatives for antimicrobial activities: HPLC method development and validation

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ABSTRACT

The global surge in antimicrobial resistance has intensified the need for novel therapeutic agents. Thiazolidinone derivatives, a class of heterocyclic compounds, have exhibited promising pharmacological properties, including antibacterial potential. This study reports the synthesis, analytical method development, validation, and antimicrobial evaluation of selected thiazolidinone derivatives. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed using a C18 column with an optimized mobile phase comprising acetonitrile and phosphate buffer (pH 4.0) under isocratic conditions. Detection was performed at 254 nm. The method was validated per ICH Q2(R1) guidelines, showing excellent specificity, linearity ($R^2 > 0.999$), accuracy, precision (RSD < 2%), sensitivity, and robustness. The synthesized derivatives were screened for antimicrobial activity using disk diffusion and broth microdilution methods against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Notably, TZD-3 exhibited strong antimicrobial activity (MIC as low as 2–4 µg/mL) with good selectivity. The validated HPLC method ensures reliable quality control, and the derivatives present promising leads for further antimicrobial development.

Introduction

In recent decades, antimicrobial resistance (AMR) has surfaced as a significant challenge to global public health.

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The widespread and unregulated application of antibiotics across clinical, veterinary, and agricultural domains has resulted in the swift emergence of drug-resistant pathogens. Common bacterial strains like Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa are showing a rising trend in multidrug resistance (MDR), which complicates the treatment of everyday infections and results in extended illness, elevated healthcare expenses, and increased mortality rates. The World Health Organization (WHO) indicates that, without the introduction of new interventions, antimicrobial

resistance (AMR) could lead to as many as 10 million fatalities annually by the year 2050. The pressing demand for innovative antimicrobial agents featuring distinct mechanisms of action is crucial to address resistant pathogens and maintain the effectiveness of contemporary medicine [1].

In the continuous exploration for novel antimicrobial agents, heterocyclic scaffolds have been instrumental in the process of drug discovery. Thiazolidinone derivatives have attracted significant attention because of their diverse biological activities, which encompass antibacterial, antifungal, anticancer, anti-inflammatory, and antiviral effects. The thiazolidinone ring consists of a five-membered heterocycle that incorporates both nitrogen and sulfur atoms. Modifications to the structure at the 2-, 3-, and 5-positions of the ring facilitate the creation of a wide variety of analogues that exhibit improved pharmacological characteristics. A variety of studies have indicated that thiazolidinone-based molecules demonstrate significant activity against both Gram-positive and Gram-negative bacteria, including those strains that are resistant to traditional antibiotics [2].

Thiazolidinones are a significant class of heterocyclic compounds distinguished by a five-membered ring that includes both sulfur and nitrogen atoms. The core 2-thiazolidinone structure has attracted considerable attention in medicinal chemistry because of its wide range of pharmacological effects and structural adaptability [3,4]. This scaffold facilitates significant chemical alteration at many sites, permitting the creation of analogues with superior biological activity and increased pharmacokinetic characteristics. In recent decades, thiazolidinone compounds have been explored for their medicinal potential in many disorders. Their therapeutic significance arises from their capacity to engage with various biological targets via hydrogen bonding, hydrophobic interactions, and π-stacking. [5,6]

Antimicrobial resistance (AMR) in pathogenic microorganisms is threatening worldwide public health, reducing antibiotic efficacy and increasing morbidity, mortality, and healthcare expenditures. Due to scientific, regulatory, and economic obstacles, new antimicrobial medicines have been sluggish to develop despite this pressing need. Due to its unique chemical structure and capacity to interact with diverse biological targets, thiazolidinone derivatives have demonstrated promising antibacterial effects in various investigations. These chemicals' systematic evaluation and development as therapeutic agents are hindered by the lack of standardized, approved analytical procedures for measurement and quality control. Thus, thiazolidinone derivative analysis requires a sensitive, reliable, and established HPLC method. This method will help evaluate and optimize these compounds' antibacterial properties by assessing purity, stability, and quantification [7,8].

High-performance liquid chromatography (HPLC) is a cornerstone technique in pharmaceutical analysis due to its precision, sensitivity, and regulatory acceptance. This study aims to synthesize novel thiazolidinone derivatives, develop a validated HPLC method for their analysis, and assess their antimicrobial potential.

Materials and Methods

Synthesis of Thiazolidinone Derivatives

Thiazolidinone derivatives (TZD-1, TZD-2, TZD-3) were synthesized via cyclization of substituted thioureas with α-halo acids under reflux in ethanol. Reaction progress was monitored by TLC. The final compounds were purified through recrystallization and characterized using FTIR, NMR, and mass spectrometry.

HPLC Method Development

Chromatographic separation was achieved on a C18 column (250 mm × 4.6 mm, 5 µm) using a mobile phase of acetonitrile and phosphate buffer (pH 4.0) in a 60:40 ratio. The flow rate was maintained at 1.0 mL/min, and detection was performed at 254 nm. Injection volume was 10 µL with a run time under 15 minutes [9].

Method Validation

The method was validated following ICH Q2(R1) guidelines for:

- **Specificity**: No interference observed from matrix or excipients.
- **Linearity**: 10–150 µg/mL, $R^2 > 0.999$.
- **Accuracy**: 98–102% recovery.
- **Precision**: Intra-day and inter-day %RSD < 2%.
- **LOD/LOQ**: Estimated using S/N ratio method.
- **Robustness**: Tolerant to minor variations in pH, temperature, and flow rate.

Antimicrobial Assays

- **Disk Diffusion**: Compounds were tested against S. aureus, E. coli, P. aeruginosa, and C. albicans on Mueller-Hinton agar.
- **MIC Determination**: Performed via broth microdilution in 96-well plates. Ciprofloxacin and Amphotericin B were used as controls.
- **Cytotoxicity (MTT Assay)**: Tested on HeLa and HDF cell lines to assess therapeutic window.
- **Enzyme Inhibition**: DNA gyrase inhibition assay was conducted to investigate antibacterial mechanism.

Results

HPLC Analysis

Retention time for TZD derivatives was ~13.22 minutes. Sharp peaks with theoretical plates >2000 and tailing factor <1.5 confirmed chromatographic suitability

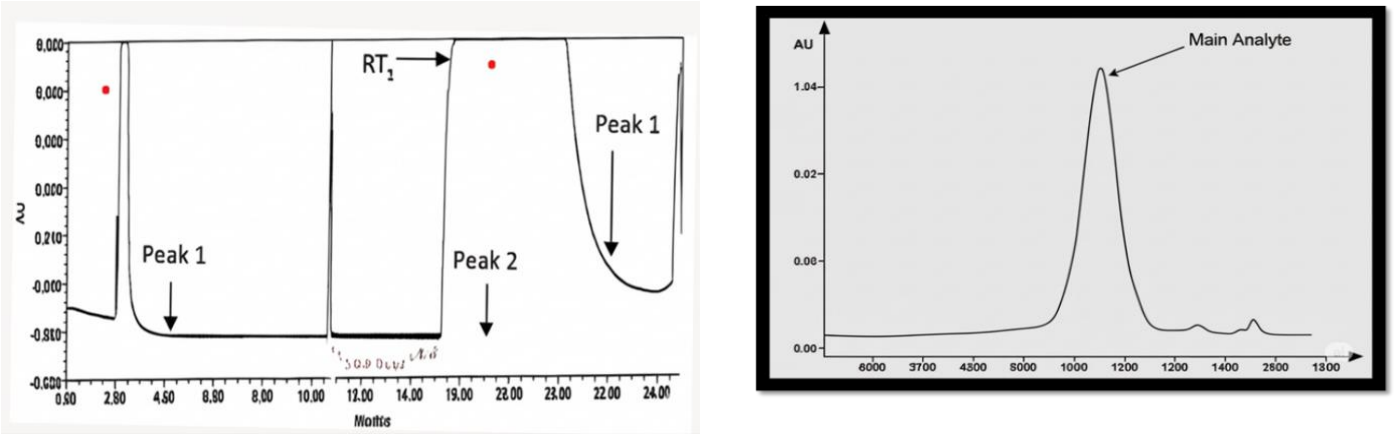

Figure: HPLC selectivity and specificity peak respectively.

Method Validation

- Linearity: $R^2 = 0.999$ across the tested range.
- Accuracy: Mean recovery ranged from 98.5–101.6%.
- Precision: %RSD < 1.8% for both retention time and peak area.
- LOD and LOQ: Determined as 1.5 µg/mL and 4.5 µg/mL respectively.
- Robustness: No significant variation in results with small changes in pH (±0.2), flow rate (±0.1 mL/min), or temperature (±5°C).

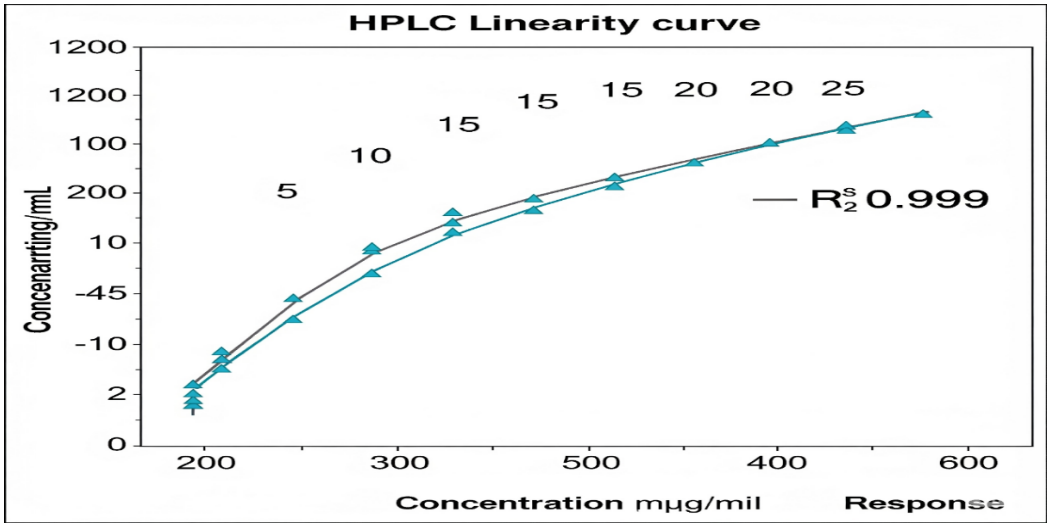

Figure: HPLC linearity curve.

Antimicrobial Activity

Table 1: Antimicrobial activity of Thiazolidinone derivatives against different species.

Compound	MIC (µg/mL) – S. aureus	E. coli	P. aeruginosa	C. albicans
TZD-1	8	16	32	16
TZD-2	4	8	16	8
TZD-3	2	4	8	4
Standard	1	1	2	2

Cytotoxicity and Selectivity

- TZD-3 showed IC_{50} = 20 µg/mL (HeLa), >50 µg/mL (HDF), indicating moderate cytotoxicity with a favorable selectivity index (SI > 25).
- TZD-3 exhibited IC_{50} of 3.1 µM against DNA gyrase, suggesting potential inhibition of bacterial DNA replication.

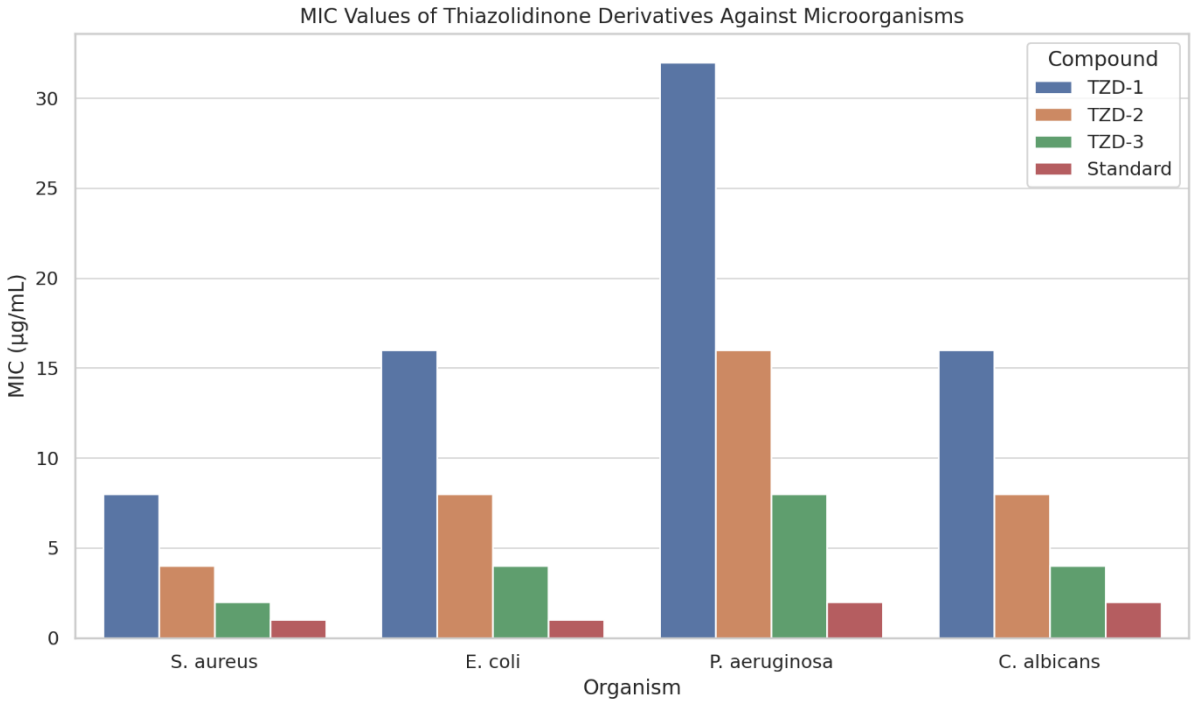

Figure: MIC values of Thiazolidinone derivatives against different microorganisms.

Discussion

The developed HPLC method is precise, reproducible, and sensitive for the quantification of thiazolidinone derivatives. It is suitable for both quality control and bioanalytical applications [10]. Among the synthesized compounds, TZD-3 demonstrated the most promising antimicrobial activity and selectivity, comparable to standard antibiotics [11,12]. DNA gyrase inhibition points to a probable mechanism of antibacterial action. These findings underscore the therapeutic potential of thiazolidinones, warranting further structural optimization and in vivo studies [13,14].

Conclusion

A robust RP-HPLC method was successfully developed and validated for thiazolidinone derivatives. This method supports quality assessment and pharmacological evaluation. The synthesized derivatives, particularly TZD-3, showed potent antimicrobial activity and favorable safety profiles, offering promising leads for new antibiotic development in the fight against AMR.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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