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ANTIFUNGAL 5,6-DIHYDROTETRAZOLO[1,5-*c*]QUINAZOLINES SHOW PROMISE AGAINST MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE*

Abstract. *This study evaluated nineteen tetrazolo[1,5-c]quinazoline derivatives for antibacterial activity against a multidrug-resistant Klebsiella pneumoniae strain (resistant to 14 standard antibiotics) isolated from patient blood samples. Only two compounds demonstrated measurable activity in 0.125 – 64 mg/L range: compound* ***63*** *(carboxylic acid derivative) and compound* ***102*** *(2-chlorobenzyl acetamide), both achieving MIC values of 64 mg/L. These results contrast with previously reported potent antifungal activity (0.125 mg/L) of spiroderivatives, indicating organism-specific structural requirements. While the antibacterial activity is modest, this proof-of-concept study establishes 5,6-dihydrotetrazolo[1,5-c]quinazolines as potential scaffolds for developing novel antibacterial agents against resistant bacterial pathogens.*

Introduction. Antimicrobial resistance represents one of the most pressing challenges in contemporary medicine, with multidrug-resistant (MDR) bacteria causing approximately 700,000 deaths annually worldwide [1]. *Klebsiella pneumoniae*, a gram-negative opportunistic pathogen, has emerged as a particularly

concerning organism due to its rapid acquisition of resistance mechanisms, including β-lactamase production (ESBLs, carbapenemases like KPC-2), efflux pump overexpression (AcrAB and OqxAB systems), and target site modifications [2, 3]. The emergence of extensively drug-resistant (XDR) and pandrug-resistant (PDR) *K. pneumoniae* strains has necessitated urgent development of novel antimicrobial agents with alternative mechanisms of action [4, 5].

Tetrazole-containing compounds have demonstrated significant potential as antimicrobial agents due to their ability to mimic carboxylate groups and interact with various biological targets [6]. Previous investigations have established, that 5,6-dihydrotetrazolo[1,5-*c*]quinazoline derivatives exhibit potent antifungal activity against *Nakaseomyces glabrata*, with compounds **c1** and **c5** demonstrating minimum inhibitory concentrations of 0.37 μM and 0.47 μM, respectively [7]. The structural diversity and proven antimicrobial activity of this scaffold prompted investigation of its antibacterial potential against resistant bacterial pathogens.

This study aimed to evaluate the antibacterial activity of structurally diverse tetrazolo[1,5-*c*]quinazoline derivatives against resistant *K. pneumoniae* isolated from patient blood samples and to establish preliminary structure-activity relationships for future optimization.

Materials and methods. A multidrug-resistant *Klebsiella pneumoniae* strain was isolated from patient blood samples at the bacteriological laboratory of MNPE Zaporizhzhia Regional Clinical Hospital of Zaporizhzhia Regional Council. Bacterial identification was confirmed using standard microbiological techniques and the VITEK-2 Compact automated system (bioMérieux, France). The *K. pneumoniae* isolate demonstrated resistance to multiple standard reference antibiotics, with minimum inhibitory concentrations (MIC) determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (version 15.0, 2025). The observed MIC values (≥mg/L) were as follows: amoxicillin/clavulanic acid (32), piperacillin/tazobactam (128), cefuroxime (64), cefotaxime (64), ceftazidime (64), cefepime (32), imipenem (16), meropenem (16), amikacin (64), gentamicin (16), tobramycin (16), ciprofloxacin (4), colistin (16), and trimethoprim/sulfamethoxazole (320).

Antimicrobial susceptibility testing was performed using the standard broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines, employing previously established protocols [7, 8]. Nineteen tetrazolo[1,5-*c*]quinazoline derivatives (Fig. 1) were evaluated for antibacterial activity, including thioether (**60**), ester (**56**), carboxylic acids (**63**, **65**), and various *N*-substituted acetamide derivatives (**96-112**).

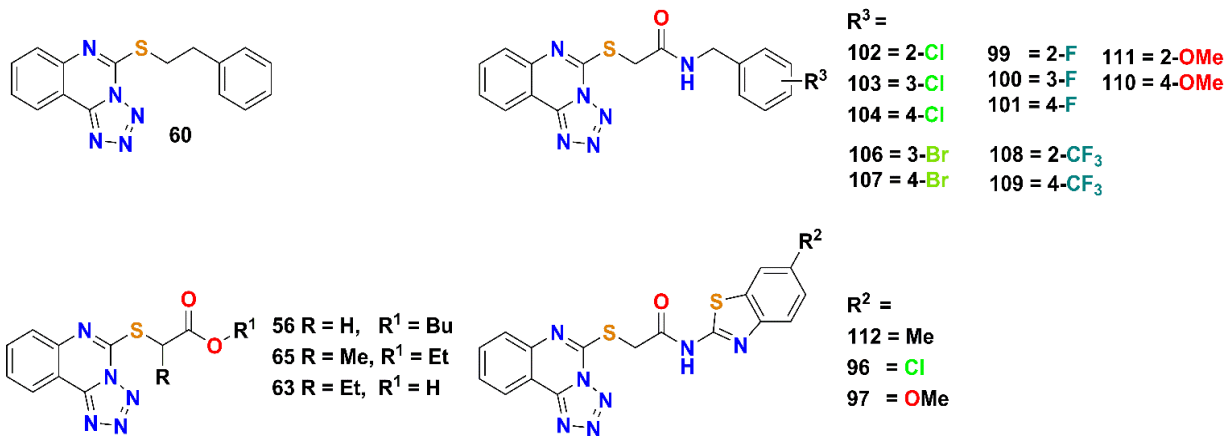

Fig. 1. **Chemical structures of tested tetrazolo[1,5-*c*]quinazoline derivatives against multidrug-resistant *Klebsiella pneumoniae***

Serial two-fold dilutions were prepared in Mueller-Hinton broth ranging from 0.125 to 64 mg/L. Test compounds were initially dissolved in DMSO (2.5%) to ensure complete dissolution. The tested microorganism strains did not exhibit sensitivity to the chosen DMSO concentration. Bacterial inoculum was adjusted to McFarland standard 0.5 (1.5×10^8 CFU/mL) and further diluted to achieve final concentration of 5×10^5 CFU/mL in each well. Plates were incubated at 35°C for 18-24 hours, and MIC was determined as the lowest concentration showing no visible bacterial growth. All experiments were performed in duplicate with appropriate controls, including DMSO solvent controls and sterile broth controls.

Results and discussion. Among the nineteen evaluated tetrazolo[1,5-*c*]quinazoline derivatives (Fig. 1), only two compounds demonstrated measurable antibacterial activity against resistant *K. pneumoniae* at the tested concentration range: compound **63** (2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)butanoic acid) and **102** (*N*-(2-chlorobenzyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamide). They both exhibited MIC values of 64 mg/L, representing the highest concentration tested, still at the reported level of reference antibiotics cefuroxime, cefotaxime, ceftazidime, cefepime, and amikacin in case they are found active against the strain, and exceeding piperacillin/tazobactam, and trimethoprim/sulfamethoxazole.

Figure 1 illustrates the chemical diversity within the tested tetrazolo[1,5-*c*]quinazoline series, and the structural comparison reveals distinct features, that may contribute to the observed antibacterial properties against resistant *K. pneumoniae*.

Compound **63** represents the carboxylic acid class within the series, featuring a butanoic acid chain with a substituted acetic acid core bearing an ethyl substituent linked through a sulfur atom to the tetrazole ring. The thioether linkage provides conformational flexibility, allowing the ethyl group to extend away from the core

structure. Tetrazoles are widely recognized as metabolism-resistant isosteric replacements for carboxylic acids, with similar pKa values and the ability to form comparable hydrogen bonding interactions [6]. The carboxylic acid functional group serves as an important pharmacophore constituent, functioning as both hydrogen bond donor and acceptor [9]. This structural motif combines a polar carboxylate group for ionic interactions with a flexible alkyl chain, that may facilitate membrane penetration.

Compound **102** belongs to the *N*-substituted acetamide series, incorporating a 2-chlorobenzyl group attached *via* an acetamide linker. The incorporation of tetrazole rings with halogenated substituents has already been shown to enhance biological effects in antimicrobial applications [10, 11]. The ortho-chlorine substitution pattern distinguishes this compound from other halogenated derivatives in the series (**99**-**101**, **103**-**104**), that showed no activity. This positional specificity suggests, that steric and electronic effects of the chlorine atom in the ortho position may be crucial for antibacterial activity.

The remaining seventeen compounds, despite structural diversity including various electron-donating and electron-withdrawing groups, failed to demonstrate measurable activity at the tested concentrations. This selectivity pattern indicates highly specific structural requirements for antibacterial efficacy against gram-negative bacteria, contrasting with the broader structure-activity relationships observed for antifungal activity earlier [7].

The observed antibacterial MIC values (64 mg/L, corresponding to 221.2 μM for compound **63** and 166.4 μM for compound **102**) are substantially higher, than the antifungal MIC values earlier reported for the most potent spiroderivatives against *N. glabrata* (0.125 mg/L ≈ 0.37-0.47 μM) [7]. The structural features optimized for antifungal activity may not be suitable for penetrating gram-negative bacterial cell walls or interacting with bacterial-specific targets.

While the observed antibacterial activity is modest, it provides proof-of-concept that tetrazolo[1,5-*c*]quinazoline scaffold can be modified to target bacterial pathogens. The MIC values of 64 mg/L, while not immediately clinically relevant, represent starting points for medicinal chemistry optimization.

Conclusions. This preliminary investigation demonstrates, that some tetrazolo[1,5-*c*]quinazoline derivatives possess measurable antibacterial activity against multidrug-resistant *K. pneumoniae*. The identification of compounds **63** and **102** as active derivatives provides initial structure-activity insights for future optimization efforts. The carboxylic acid functionality in compound **63** and the 2-chlorobenzyl acetamide substitution in compound **102** represent promising structural features for enhancement.

Future research should focus on increasing the tested concentration to 512 mg/L to establish deeper structure-activity correlation, to modify the lead structures and to improve potency with selectivity. Optimization strategies should include: carboxylic acid and acetamide substituents to enhance bacterial cell penetration; exploration of alternative halogen substitutions and positioning; investigation of hybrid molecules combining tetrazole and other antibacterial pharmacophores; and mechanistic studies to identify specific bacterial targets.

The substantial difference in antifungal *versus* antibacterial potency underscores the importance of target-specific optimization in antimicrobial drug design. While immediate clinical application is not warranted, these findings establish a foundation for developing novel antibacterial agents based on the tetrazolo[1,5-*c*]quinazoline scaffold.

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