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Novel quinoxaline compound against extended-spectrum beta-lactamases producing bacteria

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Abstract. – OBJECTIVE: Extended-spectrum beta-lactamases (ESBLs) targeting beta-lactam antibiotics pose a major healthcare challenge. Carbapenems are known to be less impacted. However, the emergence of carbapenem-resistant strains can add further complexity to this existing challenge. With slow drug discovery and rapid resistance, repurposing existing drugs is crucial. This research study aims to provide insight into the antimicrobial effectiveness of 3-hydrazinoquinoxaline-2-thiol against diverse clinical ESBL-producing isolates.

MATERIALS AND METHODS: The broth microdilution assay was conducted on a total of sixty-nine clinical ESBL-producing isolates to assess the minimum inhibitory concentrations (MICs) of 3-hydrazinoquinoxaline-2-thiol. The assay was conducted in triplicate, and the average MIC values were calculated.

RESULTS: The most repeatedly observed MIC was 64 µg/ml (37.7%), followed by 256 µg/ml (23.2%) and 128 µg/ml (17.4%). Other MICs: 32 µg/ml (11.6%), 16 µg/ml (7.2%), 4-8 µg/ml (1.4%).

CONCLUSIONS: This study demonstrated an effect of 3-hydrazinoquinoxaline-2-thiol on various ESBL-producing strains *in vitro*, indicating its promising therapeutic potential. To comprehensively understand the drug, rigorous testing, including pharmacokinetics, resistance assays, safety assessments, and exploration of potential synergies with other antibiotics against ESBL-producing organisms, is crucial.

Key Words:
ESBL, 3-hydrazinoquinoxaline-2-thiol, Antimicrobial resistance, Repurposing, MIC.

Abbreviations

ESBL, extended-spectrum beta-lactamases; MIC, minimum inhibitory concentration; ROS, reactive oxygen species; UTI, urinary tract infections.

Introduction

Extended-spectrum beta-lactamases (ESBLs) represent a critical challenge in current healthcare settings due to their association with multidrug-resistant organisms[1,2]. These enzymes are primarily produced by the Enterobacteriaceae family of Gram-negative organisms, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*)[3]. They are also produced by nonfermentative Gram-negative organisms, such

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as *Pseudomonas aeruginosa* (*P. aeruginosa*)[4]. The emergence of ESBLs has profound implications for treatment protocols, as these enzymes confer resistance to a broad spectrum of antibiotics, particularly beta-lactam drugs[5].

The enzymes ESBLs confer resistance to a wide array of beta-lactam antibiotics, leading to the loss of their effectiveness[6]. Affected antibiotics include penicillins, cephalosporins, and the monobactam aztreonam[7]. Carbapenems (such as imipenem and meropenem) and beta-lactamase inhibitors (such as clavulanic acid) are less impacted by ESBLs[8]. Carbapenems are frequently regarded as the preferred treatment for infections attributed to ESBL-producing organisms[9]. Nonetheless, the emergence of carbapenem-resistant strains introduces an extra layer of complexity in managing infections caused by ESBL-producing bacteria[10]. The selection of antibiotic therapy is dependent upon the susceptibility patterns of the particular bacterial strain and the prevailing resistance profiles in the local context[11].

Bacteria-producing ESBLs can cause various infections, such as urinary tract infections (UTIs), pneumonia, bloodstream infections, intra-abdominal infections, and wound infections[12]. The reduced or diminished efficacy of the antibiotics used against these infections can lead to limited treatment options[13]. Moreover, the associated bacteria may contribute to the spread of antibiotic resistance through the transmission of ESBL-encoding genes[9]. Consequently, infections associated with ESBL-producing bacteria demand effective preventive infection control strategies as well as specific antibiotics or combination therapies in order to mitigate further dissemination of these resistant strains[14].

Utilizing meropenem, for example, against ESBL-producing bacteria comes with certain constraints[15]. Overreliance on broad-spectrum antibiotics raises the risk of promoting the emergence of new resistant strains, which can potentially disturb the equilibrium of microbial flora[16]. Moreover, these antibiotics may induce side effects, necessitating vigilant monitoring to prevent adverse reactions. The economic impact also plays a role, as the cost of carbapenems, including meropenem, can be higher than that of alternative antibiotics, posing potential financial challenges in various healthcare settings[17]. Consequently, there is a pressing need to explore alternative treatment options in light of these considerations.

The slow pace of drug discovery, coupled with the rapid emergence of drug-resistant bacteria, necessitates urgent innovative approaches[18,19]. Repurposing, or drug repositioning, is a strategic method in pharmaceutical research that identifies new therapeutic uses for existing drugs[20]. Compared to traditional drug development, the approach of utilizing drugs that are already tested for safety and efficacy in clinical trials is efficient and cost-effective. Researchers aim to expedite drug discovery and address unmet medical needs across various conditions through repurposing established drugs[21,22].

In a previous study, Elfadil et al[23] highlighted the significant effectiveness of quinoxaline derivatives against various clinical MRSA strains[23]. Expanding on this research, we hypothesize that these quinoxaline derivatives might exhibit activity against clinical ESBL-producing strains. To our knowledge, the efficacy of 3-hydrazinoquinoxaline-2-thiol against ESBL has not been previously investigated. In order to explore whether 3-hydrazinoquinoxaline-2-thiol has a novel indication against Gram-negative strains producing ESBL, the herein research study assessed the efficacy of 3-hydrazinoquinoxaline-2-thiol *in vitro* against different ESBL clinical strains.

Materials and Methods

Antibacterial Compounds

The tested compounds of 3-hydrazinoquinoxaline-2-thiol were obtained from Fluorochem Ltd., United Kingdom. It was dissolved in Dimethyl sulfoxide (DMSO) solvent.

Bacterial Isolates

This study examined sixty-nine ESBL isolates of *E. coli* (n=29), *K. pneumoniae* (n=24), *Pseudomonas aeruginosa* (n=9), *Morganella morganii* (n=1), *Enterobacter aerogenes* (n=1), *Enterobacter cloacae* (n=1), *Stenotrophomonas maltophilia* (n=1), *Citrobacter freundii* (n=1) *Proteus mirabilis* (n =1) were evaluated in this study. They are obtained from the hospital of King Abdulaziz University in Jeddah, Saudi Arabia. The isolates were preserved in glycerol and stored at -80°C. Before testing, all isolates underwent thawing and were cultured on either blood agar or MacConkey agar, followed by overnight incubation at 37°C in an aerobic environment. All isolates were identified and tested for susceptibility by the Vitek 2 system (bioMerieux, Marcy-l'Étoile, France) using the Gram-negative strain card type AST-N417 according to the manu-

facturer's recommendations. Isolates were obtained as part of routine procedures conducted by King Abdulaziz University Hospital. The isolates were collected without any associated patient data such as age, sex, or other sensitive information. Our study solely focused on the isolates themselves, and we did not have access to or utilize any patient-related data. As such, we confirm that ethical approval was not required for our study since we did not handle patient data or sensitive information. Our research solely involved the analysis of bacterial isolates obtained through routine hospital procedures. We confirm that informed consent was not applicable to our study as it did not involve human subjects or patient interactions.

Broth Microdilution Assay

A broth microdilution assay was implemented to evaluate the antimicrobial sensitivity. Two-fold serial dilutions of the tested compound were prepared in Mueller Hinton Broth (Sigma-Aldrich, Burlington, MA, USA). Subsequently, 100 µl aliquots of the prepared antimicrobial solutions were dispensed into individual wells of 96-well plates (Corning, Pisa, Italy). The density of the inoculum suspension was adjusted to 0.5 McFarland using a suspension turbidity detector (Biosan Densitometers DEN-1B; Riga, Latvia). Following this calibration, 5 µl aliquots of the prepared inoculum were dispensed into the wells containing various concentrations of the antimicrobial. The plates were then incubated overnight at 37°C to facilitate optimal bacterial growth.

The antimicrobial susceptibility testing was executed in triplicate, and the mean values were recorded for subsequent analysis and interpretation.

The minimum inhibitory concentration (MIC) that impedes visible bacterial growth was evaluated for the antibacterial agent tested using the exact broth microdilution technique and subsequent interpretation in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI)[24]. This methodological thoroughness ensures the precision and alignment of the study outcomes with established standards in microbiological assessment[25,26].

Results

The Analysis of 3-Hydrazinoquinoxaline-2-Thiol Susceptibility Among ESBL Isolates

Susceptibility testing of the 69 ESBL-producing isolates, using the broth microdilution assay, revealed that the predominant MIC of 3-hydrazinoquinoxaline-2-thiol was 64 µg/mL for 37.7% of the isolates. This was followed by 256 µg/mL (23.2%), 128 µg/mL (17.4%), 32 µg/mL (11.6%), and 16 µg/mL (7.2%). At 4 µg/mL and 8 µg/mL, 1.4% of the isolates were inhibited (Table I).

These findings suggest a notable efficacy of 3-hydrazinoquinoxaline-2-thiol against specific ESBL-producing strains while indicating a comparatively lower activity against other ESBL-producing bacterial strains (Table II).

Discussion

The rise of antibiotic-resistant bacteria poses a critical challenge to global public health, necessitating the exploration of novel compounds to combat bacterial infections[27]. To our knowledge, the efficacy of 3-hydrazinoquinoxaline-2-thiol against a panel of resistant ESBL-producing strains has not been evaluated before in the research literature. The compound under investigation displayed promising antimicrobial activity, yet the most notable observation was the substantial variation in MIC of the 3-hydrazinoquinoxaline-2-thiol across different bacterial strains.

The concept of MIC, representing the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism, is pivotal in assessing the potency of antimicrobial compounds[28]. The observed range of MIC values in our study underscores the diverse responses of different bacterial strains to the tested compound. This variability may be attributed to the inherent genetic differences among bacterial species, including variations in their resistance mechanisms and susceptibility profiles[29].

The compound's ability to elicit an antimicrobial effect against certain strains at lower con-

Table I. Variation in MICs in µg/ml of 3-hydrazinoquinoxaline-2-thiol against ESBL-producing organisms.

No. of ESBL isolates/(%)	MIC values
1 (1.4%)	4
1 (1.4%)	8
5 (7.2%)	16
8 (11.6%)	32
26 (37.7%)	64
12 (17.4%)	128
16 (23.2%)	256

Minimum inhibitory concentrations (MICs), extended-spectrum beta-lactamases (ESBL).

Table II. Minimum inhibitory concentrations (MICs) of 3-hydrazinoquinoxaline-2-thiol in µg/ml against 69 different clinical ESBL-producing strains.

Strain number	ESBL producing organism	MIC of 3-hydrazinoquinoxaline-2-thiol
1	*E. coli*	64
2	*E. coli*	64
3	*E. coli*	32
4	*E. coli*	128
5	*E. coli*	64
6	*E. coli*	64
7	*E. coli*	32
8	*E. coli*	128
9	*E. coli*	64
10	*E. coli*	64
11	*E. coli*	64
12	*E. coli*	32
13	*E. coli*	64
14	*E. coli*	64
15	*E. coli*	64
16	*E. coli*	128
17	*E. coli*	32
18	*E. coli*	64
19	*K. pneumoniae*	128
20	*K. pneumoniae*	128
21	*K. pneumoniae*	128
22	*K. pneumoniae*	64
23	*K. pneumoniae*	64
24	*K. pneumoniae*	64
25	*K. pneumoniae*	128
26	*K. pneumoniae*	64
27	*K. pneumoniae*	128
28	*K. pneumoniae*	32
29	*K. pneumoniae*	128
30	*K. pneumoniae*	64
31	*K. pneumoniae*	128
32	*P. aeruginosa*	256
33	*P. aeruginosa*	256
34	*P. aeruginosa*	256
35	*P. aeruginosa*	256
36	*P. aeruginosa*	256
37	*P. aeruginosa*	256
38	*Enterobacter aerogenes*	64
39	*Morganella morganii*	64
40	*Acinetobacter baumannii*	64
41	*Stenotrophomonas maltophilia*	256
42	*Enterobacter cloacae*	256
43	*P. aeruginosa*	256
44	*Proteus mirabilis*	128
45	*K. pneumoniae*	64
46	*K. pneumoniae*	64
47	*K. pneumoniae*	64
48	*K. pneumoniae*	64
49	*K. pneumoniae*	64
50	*E. coli*	16
51	*E. coli*	32
52	*E. coli*	16
53	*E. coli*	8
54	*K. pneumoniae*	256
55	*K. pneumoniae*	256
56	*K. pneumoniae*	256
57	*K. pneumoniae*	256

Continued

Table II *(Continued)*. Minimum inhibitory concentrations (MICs) of 3-hydrazinoquinoxaline-2-thiol in μg/ml against 69 different clinical ESBL-producing strains.

Strain number	ESBL producing organism	MIC of 3-hydrazinoquinoxaline-2-thiol
58	*Citrobacter freundii*	64
59	E. coli	32
60	*E. coli*	32
61	*E. coli*	16
62	*E. coli*	64
63	*E. coli*	16
64	*E. coli*	16
65	*E. coli*	4
66	*K. pneumoniae*	256
67	*P. aeruginosa*	256
68	*P. aeruginosa*	256
69	*K. pneumoniae*	128

Escherichia coli (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), extended-spectrum beta-lactamases (ESBL).

centrations suggests a promising avenue for therapeutic intervention. Conversely, the higher MIC values observed in some bacterial strains indicate potential challenges in achieving optimal efficacy against specific resistant phenotypes. Understanding the mechanisms behind these variations in MIC is crucial for the targeted application of the compound.

The observed MIC variations may also reflect the adaptability of bacteria and the dynamic nature of resistance mechanisms. Bacterial populations can exhibit heterogeneous responses to antimicrobial agents, with subpopulations harboring distinct resistance profiles. Further investigations, including genotypic and phenotypic analyses, are required to elucidate the underlying factors contributing to the observed MIC variability[30].

Variations in the 3-hydrazinoquinoxaline-2-thiol efficacy, with a lower MIC against *E. coli* than *Pseudomonas aeruginosa*, may be associated with differences in bacterial responses, resistance mechanisms, target affinity, and membrane permeability. *P. aeruginosa* is known to have a number of antibiotic resistance mechanisms, including efflux pumps, enzymatic inactivation of antibiotics, and alterations in target sites[31]. Higher MIC values observed against *P. aeruginosa* may indicate the presence of resistance mechanisms, which 3-hydrazinoquinoxaline-2-thiol is less effective in overcoming than *E. coli*. Potential variations in target susceptibility and membrane penetration may also contribute to the higher MIC. It has been previously reported[32] that *Pseudomonas aeruginosa* exhibits significantly lower outer membrane permeability, ranging from 12 to 100 times lower compared to *Escherichia coli*. It has been shown in this study that the susceptibility of *Escherichia coli* to 3-hydrazinoquinoxaline-2-thiol is notable, with a MIC of 16 μg/ml, showing a 16-fold reduction compared to *Pseudomonas aeruginosa*, which has a MIC of 256 μg/ml. Further studies, including molecular investigations, are crucial to understanding these differences and exploring resistance mechanisms that may result in the observed MIC variation of 3-hydrazinoquinoxaline-2-thiol.

The efficacy of 3-hydrazinoquinoxaline-2-thiol against Gram-positive bacteria, specifically methicillin-resistant *Staphylococcus aureus*, has been previously demonstrated[23]. In this current study, we have demonstrated the activity of 3-hydrazinoquinoxaline-2-thiol against some Gram-negative ESBL-producing bacteria. The findings present a potentially new therapeutic option with distinct advantages. The ability of 3-hydrazinoquinoxaline-2-thiol to target various Gram-positive and Gram-negative organisms suggests broad coverage within a range of bacterial species. Its ability to inhibit bacterial growth may involve mechanisms such as preventing DNA synthesis and inducing reactive oxygen species (ROS)[33-35]. However, comprehensive examinations, including resistance assays to evaluate potential drug resistance development, as well as pharmacokinetic and pharmacodynamic studies to understand drug metabolism, distribution, excretion, and potential drug interactions, are imperative. Additionally, an *in vivo* model is crucial to assess 3-hydrazinoquinoxaline-2-thiol efficacy *in vivo* and ascertain its safety profile.

The findings of this study emphasize the importance of developing antimicrobial agents that take into consideration the diversity of bacterial resistance mechanisms. The tested compound appears to be effective against a range of ESBL-producing strains and has the potential as a broad-spectrum antimicrobial drug. The repurposing approach holds the potential to introduce a novel drug with a new therapeutic indication[36]. 3-hydrazinoquinoxaline-2-thiol demonstrated good efficacy against MRSA in a previous study[23], and it has now exhibited notable effectiveness against some ESBL-producing organisms as well. Despite demonstrating a weaker effect against other ESBL-producing bacteria, 3-hydrazinoquinoxaline-2-thiol presents an opportunity for exploration in combination therapies and advancing treatment modalities. Investigating possible synergistic effects with established antibiotics can enhance the overall efficacy and assist in mitigating the risk of potential bacterial resistance against the new compound and the challenges associated with it.

Conclusions

This study marks the first instance in which the activity of 3-hydrazinoquinoxaline-2-thiol against some ESBL-producing bacteria has been successfully demonstrated *in vitro*. The preliminary findings are promising, revealing the potential therapeutic effect of 3-hydrazinoquinoxaline-2-thiol against Gram-negative ESBL-producing bacteria. To comprehensively characterize this drug and pave the way for its potential market development, further rigorous testing and investigations are imperative. These subsequent tests should encompass a spectrum of analyses, including but not limited to in-depth pharmacokinetic studies, resistance assays, and thorough safety assessments. Potential successful combination with other antibiotics against ESBL-producing organisms also needs to be assessed. Undertaking these essential steps would provide valuable insights into the efficacy of 3-hydrazinoquinoxaline-2-thiol and its potential usage as a market-ready solution.

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Conflict of Interest
The authors declare no conflicts of interest.

Informed Consent and Ethics Approval
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Authors' Contributions
The inception of this study was led by Khalil Alkuwaity, Karem Ibrahem, and Jawahir A. Mokhtar, Mohammed W. Al-Rabia, and Abdelbagi Elfadi. Mohammed Alsieni, Mohammed A. Bazuhair, and Karem Ibrahem conducted the literature review and introduction. Data collection was performed by Hani Abdullah and Dalya Attallah, while article composition was undertaken by Khalil Alkuwaity, Jawahir A. Mokhtar, and Karem Ibrahem. Critical review contributions were provided by Turki Abujamel, Tarfa A. Altorki, Noof R. Helmi, Hind AbdulMajed, Noha A. Juma, Mohammed W. Al-Rabia, and Abdelbagi Elfadi. Khalil Alkuwaity was responsible for editing, formatting, and conducting the final check. Approval for publication was granted by all authors upon review of the final version of the article.

Availability of Data and Materials
The data generated from this study has already been incorporated into the manuscript.

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