ORIGINAL ARTICLE

Exploring the Combined Efficacy of 3-Hydrazinoquinoxaline-2-thiol and Flucloxacillin Against Methicillin-Resistant *Staphylococcus aureus*

Abdelbagi Alfadil, MD, PhD

Department of Clinical Microbiology and Immunology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
Centre of Research Excellence for Drug Research and Pharmaceutical Industries, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence
Dr. Abdelbagi Alfadil
Department of Clinical Microbiology and Immunology, Faculty of Medicine, King Abdulaziz University
P.O. Box 80205, Jeddah 21589
Kingdom of Saudi Arabia
e-M: aegmusa@kau.edu.sa

Submission: 20 Feb. 2024
Accepted: 07 Mar. 2024

Citation
Alfadil A. Exploring the combined efficacy of 3-hydrazinoquinoxaline-2-thiol and flucloxacillin against methicillin-resistant *Staphylococcus aureus*. JKAU Med Sci 2024; 31(1): 51–57. DOI: 10.4197/Med.31–1.6.

Abstract

The escalating resistance observed in different antimicrobial agents, especially those considered as last-line options, highlights the urgent required for innovative ways and novel agents to combat MRSA infections. Utilizing antibiotic combinations can enhance efficacy, broaden the spectrum against bacteria, and mitigate the risk of resistance development, offering a promising strategy to address the rising challenge of antimicrobial resistance. Resistance has emerged against nearly all antibiotics, making drug discovery difficult and costly. Developing a significant number of effective antibiotics to combat resistance in a short period is nearly impossible. This paper aims to explore the probability of synergistic effects between 3-hydrazinoquinoxaline-2-thiol (3HX) and flucloxacillin (FLX) versus a different range of MRSA clinical isolates, which may provide valuable insights into the combination of the antimicrobial activity. Broth microdilution was conducted on 22 clinical MRSA isolates to assess the Minimum Inhibitory Concentrations (MICs) of both 3HX and FLX. Following by a checkerboard assay was subjected to assess the interaction activity between the two agents, focusing on the Fractional Inhibitory Concentration Index (FICI). The MICs of FLX and 3HX were evaluated for 22 clinical MRSA strains, with FLX displaying MICs ranging from 128 to 512 µg/ml, while 3HX MICs varying from 16 to 64 µg/ml. Surprisingly, the combination of 3HX and FLX demonstrated a synergistic effect, leading to a considerable reduction of MIC up to 64-fold. The potential of combining 3HX with FLX as an effective way versus MRSA appears promising. However, further rigors, testing, and experimentation are imperative to establish its practical utility.

Keywords

Antimicrobial resistance, *Staphylococcus aureus*, MRSA, FLX, Antibiotic combination therapy, 3HX, MIC, FICI

INTRODUCTION

The global rise in antimicrobial resistance presents substantial health and economic obstacles, with its rapid emergence underscoring pressing challenges. This predicament stems from both the improper utilization of antibiotics and the dearth of novel antimicrobial agents in development[1]. AMR infections have resulted in approximately 700,000 fatalities worldwide, and projections indicate that these resistant bacteria could be responsible for up to 10 million deaths by 2050. This trend also signifies a substantial economic toll due to lost benefits[2]. Specifically, there is an immediate worldwide concern regarding multidrug-resistant (MDR) bacteria, such as *Staphylococcus aureus*, due to their ability to resist multiple drugs and their high virulence potential[3,4]. Infections attributed to *S. aureus* are both severe and recurrent, with the situation further compounded by the emergence of antibiotic-resistant variants[5]. Antibiotic resistance has become a pervasive issue, with resistance emerging against nearly all classes of antibiotics currently available. This situation poses significant challenges in the field of drug discovery, which is both difficult and expensive. The process of identifying, developing, and bringing new antibiotics to the market involves extensive research, rigorous testing, and substantial financial investment. Additionally, the timeline for developing a new class of antibiotics that is effective against resistant strains is lengthy, often spanning several years or even decades. As a result, it is nearly impossible to develop a considerable number of effective antibiotics to combat the rising tide of antibiotic resistance within a short period. This underscores the urgency for innovative approaches and alternative strategies in addressing this critical global health threat[1,6].

β-lactam antibiotics are frequently prescribed to combat bacterial infections due to their favorable efficacy, minimal toxicity, and wide-ranging effectiveness against various bacterial strains[7]. The yearly spending on β-lactam antibiotics is estimated at around $15 billion USD, constituting approximately 65% of the entire antibiotics market[8]. In MRSA, resistance mechanisms are associated with the presence of the *mecA* gene, which codes for penicillin-binding protein2A (PBP2A). PBP2A is notable for its decreased affinity to most β-lactam antibiotics, except 5th-generation cephalosporins (ceftaroline), thereby reducing their efficacy against MRSA strains[9–11]. New approaches are essential to address the challenge of antibiotic resistance and rejuvenate the efficacy of existing antibiotics[6]. One potential strategy involves identifying novel targets essential for bacterial resistance mechanisms[6]. The discovery of a small molecule inhibitor targeting such a new target is anticipated to exhibit synergistic effects when combined with an antibiotic. Another viable approach is the repurposing of older antibiotics through the utilization of synergistic combinations with current antibiotics[6].

Considering the use of antibiotic combinations provides numerous advantages, such as improving treatment effectiveness, broadening the spectrum of targeted pathogens, lowering the risk of adverse effects by reducing dosage and toxicity, and diminishing the likelihood of resistance emergence[12,13]. Augmentin stands out as a successful example of combining β-lactam antibiotics, illustrating the revival of β-lactam effectiveness. This combination includes amoxicillin, a β-lactam antibiotic, paired with clavulanic acid, a β-lactamase inhibitor. Clavulanic acid exhibits strong binding affinity to diverse bacterial β-lactamases, shielding amoxicillin from degradation by these enzymes, thus ensuring its efficacy. Consequently, this combination enables the utilization of amoxicillin in treating bacterial infections that demonstrate resistance to β-lactam antibiotics[14]. This spurs us to seek out a substance exhibiting antimicrobial properties, with the aim of revitalizing the effectiveness of β-lactam antibiotics.

FLX, a novel isoxazole penicillin, effectively combats penicillinase-producing strains of *S. aureus* and demonstrates high absorption rates in humans when administered orally or intramuscularly. In comparison to other isoxazole penicillins like oxacillin, cloxacillin, and dicloxacillin currently employed in clinical settings, FLX exhibits superior activity against Gram-positive cocci, including strains resistant to penicillin. As, this antimicrobial agent is resistant to penicillinase, an enzyme pivotal for breaking the beta-lactam ring in penicillins, thereby rendering them inactive. FLX, formulated for both oral and injectable delivery, exerts bactericidal effects. It is the preferred pharmaceutical form for administration[15,16].

Among the benzodiazine family, quinoxaline stands out as a significant six-membered ring template. These compounds including 3HX are of great significance in pharmacology due to their diverse and intriguing biological activities, such as antibacterial, antitubercular, antimalarial, antiviral, and anti-HIV

properties[17]. In their earlier work, Elfadil et al. showed the significant efficacy of quinoxaline derivatives, particularly 3HX, in enhancing the action of penicillin versus various clinical MRSA strains[18]. Expanding on this discovery, we are intrigued by whether 3HX possesses the potential to augment the effectiveness of FLX against diverse MRSA clinical strains. Put differently, we seek to explore if 3HX synergistic effects are exclusive to penicillin or if they extend to FLX in combating MRSA.

This study aims to assess the *in vitro* antimicrobial effectiveness of combining 3HX with FLX against various clinical MRSA strains.

MATERIAL AND METHODS

BACTERIAL ISOLATES AND GROWTH CONDITIONS

In this study, we examined 22 MRSA isolates obtained from King Abdulaziz University Hospital in Jeddah, Saudi Arabia. These isolates were stored in glycerol at -80°C. Prior to testing, they were thawed and cultured on blood agar from HiMedia, India, and then incubated overnight at 37°C in an aerobic environment. Colony identification was performed using standard procedures, including catalase and tube coagulase tests. Sample collection was performed in agreement with the ethics and research committee of the Faculty of Applied Medical Sciences at King Abdulaziz University (No. 38-712-456) and complied with the Declaration of Helsinki.

ANTIMICROBIAL AGENTS

This study evaluated medications designed to combat MRSA, including a 3HX compound obtained from Fluorochem Ltd in the United Kingdom and FLX powder acquired from Sigma.

BROTH MICRODILUTION ASSAY

Both drugs were initially prepared as 10 mg/ml stock solutions. To assess antibiotic and antimicrobial sensitivity, a broth microdilution test was carried out. This entailed diluting the drugs in Mueller Hinton Broth from Sigma-Aldrich in the United States in a two-fold manner (starting with 1024 µg/ml). Then, 100 µl of each drug solution was dispensed into the wells of 96-well plates from Corning, USA.

The density of the inoculum suspension was carefully set to 0.5 McFarland using a Biosan Densitometers DEN-1B turbidity detector. Subsequently, 5 µl of the inoculum was added to each well containing varying concentrations of antibiotics. The plates were left to incubate overnight at 37°C. Antibiotic susceptibility testing was conducted in triplicate, and the resulting mean values were recorded for analysis.

The Minimum Inhibitory Concentration (MIC) signifies the lowest concentration of a drug that prevents the visible growth of a microorganism. MIC results for both antibacterial agents were determined through the broth microdilution method and interpreted in accordance with guidelines from the Clinical and Laboratory Standards Institute (CLSI)[18,19].

CHECKERBOARD ASSAY

In order to assess the interaction of 3HX and FLX checkerboard assay was used. For this, we prepared a two-fold serial dilution of each antibiotic in Muller-Hinton broth (MHB) and dispensed 50 µl of each dilution into 96-well plates from Italy Inc. The density of the inoculum suspension was precisely adjusted to 0.5 McFarland using a Biosan Densitometers DEN-1B turbidity detector. Then, 5 µl of the diluted bacteria were added to each well of the 96-well plates. To assess interactions, we calculated the fractional inhibitory concentration index (FICI) using the formula: (MIC of drug A in combination / MIC of drug A alone) + (MIC of drug B in combination / MIC of drug B alone). A FIC index of ≤ 0.5 indicated a synergistic effect[20].

RESULTS

MICS OF FLX AND 3HX AGAINST MRSA CLINICAL STRAINS

In preparation for the checkerboard assay experiment, it is necessary to determine the MICs of both FLX and 3HX. The MICs for FLX vary between 128 and 512 µg/ml (Table 1), with higher MIC values expected when dealing with MRSA strains. Conversely, the MICs for 3HX range from 16 to 64 µg/ml (Table 1). The MIC was defined as the lowest concentration that inhibits bacterial growth[6].

Table 1. Minimum Inhibitory Concentrations (MICs) in µg/ml of FLX and 3HX against MRSA strains

Number of Strain	MRSA	MIC 3HX	MIC FLX
1	70	32	512
2	72	32	512
3	91	32	512
4	80	16	512
5	90	32	512
6	75	32	512
7	73	32	512
8	93	64	512
9	95	32	512
10	96	32	512
11	97	32	512
12	106	64	128
13	104	32	256
14	102	32	512
15	100	32	512
16	105	32	256
17	107	32	256
18	98	32	128
19	92	32	128
20	101	32	256
21	1	32	128
22	2	32	128

3HX SYNERGIZES FLX AGAINST DIFFERENT MRSA CLINICAL STRAINS

To investigate the potential synergy between 3HX and different beta-lactam antibiotics, such as FLX, against MRSA strains, we employed a checkerboard assay. Interestingly, when FLX was administered alone, it failed to inhibit MRSA growth. However, when combined with 3HX, a significant decrease in the MICs of FLX was observed, with reductions of up to 64-fold in some cases. Similarly, the MICs of 3HX were notably lower, up to 16-fold, when used in combination with FLX (Figure 1).

Moreover, this combination exhibited a synergistic interaction across 22 distinct clinical MRSA strains, as evidenced by FICI values consistently below 0.5 with 100% synergy (Table 2). The results strongly imply that 3HX can effectively enhance the effectiveness of FLX in fighting against MRSA strains, indicating the

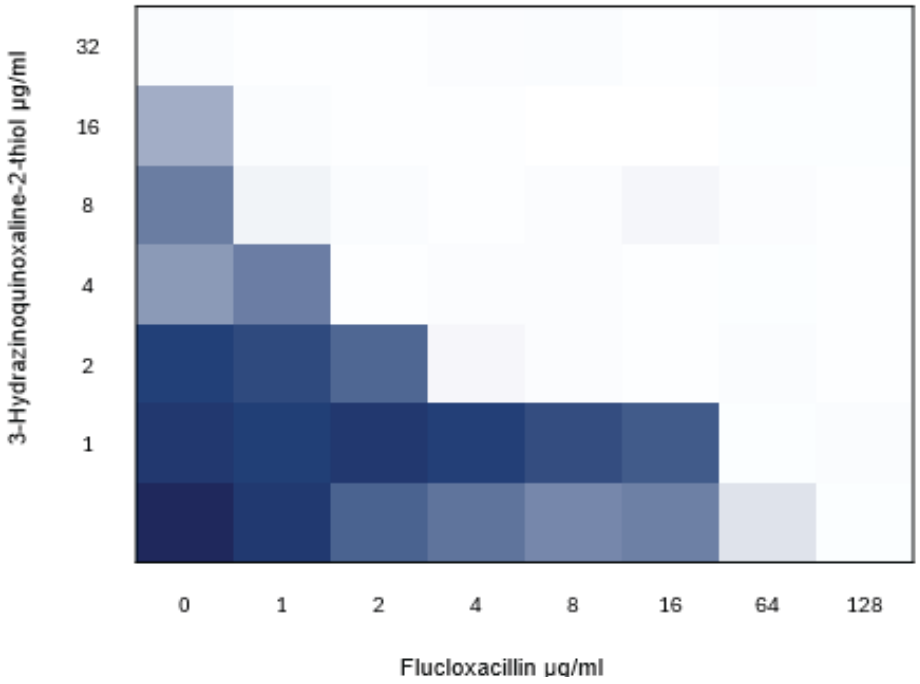

Figure 1. Checkerboard test showing the synergistic effect of flucloxacillin with 3-hydrazinoquinoxaline-2-thiol against MRSA 2. The white color represents 0 % growth, and the dark blue color represents 100% growth.

Table 2. Synergistic Interaction of 3HX and FLX Across 22 Clinical MRSA Strains. Fraction inhibitory concentration (FIC), Fraction inhibitory concentration index (FICI).

Number of Strain	MRSA	FIC 3HX	FIC FLX	FICI
1	70	0.250	0.014	0.264
2	72	0.146	0.032	0.178
3	91	0.25	0.066	0.316
4	80	0.25	0.032	0.282
5	90	0.094	0.047	0.141
6	75	0.167	0.043	0.21
7	73	0.146	0.034	0.18
8	93	0.146	0.073	0.219
9	95	0.104	0.057	0.161
10	96	0.188	0.094	0.282
11	97	0.175	0.131	0.306
12	106	0.208	0.062	0.27
13	104	0.135	0.109	0.244
14	102	0.089	0.057	0.146
15	100	0.146	0.13	0.276
16	105	0.208	0.086	0.294
17	107	0.104	0.045	0.149
18	98	0.135	0.115	0.25
19	92	0.104	0.042	0.146
20	101	0.146	0.043	0.189
21	1	0.156	0.071	0.227
22	2	0.109	0.074	0.035

possibility for enhanced treatment options. Moreover, this implies that the synergistic effects of 3HX are not exclusive to penicillin but also apply to other beta-lactam antibiotics.

DISCUSSION

Combining antibiotics in bacterial infection therapy has demonstrated its effectiveness in tackling the obstacles presented by multidrug-resistant pathogens. The successful application of combination therapy can be seen in the treatment of Mycobacterium tuberculosis[21]. The advantages of combination therapy include ensuring adequate drug delivery to infection sites, enhancing bacterial clearance, preventing resistance emergence, and inhibiting toxin synthesis, which diminishes bacterial virulence[13, 22]. Our prior research demonstrated promising outcomes with the combination of penicillin and 3HX against MRSA[18]. This prompts us to investigate whether hydrazinoquinoxaline-2-thiol can synergize ant staphylococci beta-lactam, such as FLX, against clinical MRSA strains or if this synergy was specific to penicillin.

The combination of FLX and 3HX demonstrates synergistic interaction against 22 MRSA clinical strains, leading to a remarkable reduction in MICs of FLX by up to 64-fold. Similarly, when paired with FLX, 3HX derivatives also exhibit a substantial decrease in MICs. This potent cooperative effect was consistently observed across different clinical MRSA strains in our experiments. These results strongly suggest that the combined therapy of FLX and 3HX produces a more robust response against MRSA strains compared to the efficacy of each drug alone. Also, these results suggest that 3HX synergise other beta-lactam drugs (FLX) not only limited to penicillin.

Considering the potential for toxic effects related to elevated concentrations of 3HX, FLX, and other antibiotics, employing reduced doses of each drug in a synergistic approach presents a promising strategy to mitigate potential toxicity[23]. Our research findings support this concept: while FLX alone required 512 µg/ml to impede MRSA growth, an intriguing discovery emerged when combined with 3HX. In this combined regimen, only 16 µg/ml of FLX was sufficient to inhibit the growth of the same MRSA strain. This finding suggests the feasibility of achieving a comparable therapeutic effect with lower drug doses, potentially reducing the risk of adverse effects. However, further investigations are warranted to validate this promising outcome.

Our study has demonstrated that FLX alone was ineffective in inhibiting MRSA growth. However, when combined with 3HX, it gained the ability to suppress MRSA growth. This observed effect can be attributed to the synergistic interaction between FLX and 3HX, highlighting the potential of combination therapy to address the inherent challenges associated with MRSA infections. Furthermore, it has been suggested that the introduction of a second antibiotic in the treatment regimen can compensate for the limitations of the first antibiotic[24]. This may explain why the two antimicrobial drugs in combination exhibit greater efficacy than each drug alone.

The synergy observed between 3HX and FLX seems to stem from their action on distinct pathways. While 3HX interferes with DNA synthesis[25], FLX

inhibits penicillin-binding proteins (PBPs). Inhibiting of PBPs results in abnormalities in bacterial cell wall structure, including elongation, lesions, compromised permeability, and eventual cell lysis[26]. Additionally, FLX exhibits activity against various β-lactamases, including penicillinases and cephalosporinases[27]. Consequently, FLX may inhibit the activity of penicillinases in degrading itself, thereby enhancing the effectiveness of FLX in the combination therapy.

An alternative explanation for the enhanced efficacy of combining FLX and 3HX versus different MRSA strains could involve the increased creation of reactive oxygen species (ROS). These ROS disrupt target-specific cellular processes, ultimately leading to cell death[28]. It is widely recognized that bactericidal antibiotics, particularly β-lactams, can stimulate ROS generation, which is pivotal in bacterial cell eradication[29]. Intriguingly, 3HX is also known to possess the ability to generate reactive oxygen species[30]. Therefore, we assume that the observed synergy among FLX and 3HX may be associated with the heightened production of ROS. Another possible reason for the increased effectiveness of combining FLX and 3HX against different MRSA strains could be the intercalation impact of 3HX on MRSA DNA[31, 32]. The synergism may have resulted from the cumulative impact of FLX inhibiting peptidoglycan production, so enhancing the absorption of 3HX, which disrupts DNA synthesis. Nevertheless, further experimentation is required to substantiate this conjecture.

Time-kill analysis will provide more information about the bactericidal activity of the combined drug[6]. Moreover, resistance assay can reveal whether this combination inhibits the development of new resistance[23]. *In vivo,* the model will analyze the pharmacokinetic and pharmacokinetic of the combined drug as well as the efficacy *in vivo*[33].

CONCLUSION

Our study presents groundbreaking evidence of the synergistic efficacy of combining 3HX with FLX against a wide range of clinical MRSA strains. These findings suggest a promising avenue for the clinical application of this synergy. However, significant steps are needed to translate the combined antibiotics into clinical practice. Further research and clinical trials are essential to fully evaluate their therapeutic potential, refine dosing strategies, and ensure their safety and effectiveness in real-world medical contexts.

ABBREVIATIONS

MHB, Muller-Hinton broth, MRSA, Methicillin-resistant Staphylococcus aureus, MIC, minimum inhibitory concentration, FIC, Fractional inhibitory concentration, FICI, Fractional inhibitory concentration index, ROS, Reactive oxygen species, PBP2A, penicillin-binding protein 2A, PBP, Penicillin-binding proteins

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

This study received approval from the Ethics and Research Committee of the Faculty of Applied Medical Sciences at King Abdulaziz University (Approval No. 38-712-456).

REFERENCES CITED

[1] Ventola CL. The Antibiotic Resistance Crisis Part 1 : Causes and Threats," Pharm. Ther., vol. 40, no. 4, pp. 277–283, 2015.

[2] Nagvekar V, Sawant S, and Amey S. Prevalence of multidrug-resistant Gram-negative bacteria cases at admission in a multispeciality hospital, J. Glob. Antimicrob. Resist., vol. 22, no. 9, pp. 457–461, 2020.

[3] Ramsamy Y, Essack SY, Sartorius B, Patel M, and Mlisana KP, Antibiotic resistance trends of ESKAPE pathogens in Kwazulu-Natal, South Africa: A five-year retrospective analysis, Afr. J. Lab. Med., vol. 7, no. 2, pp. 1–8, 2018.

[4] Zhen X, Lundborg CS, Sun X, Hu X, and Dong H. Economic burden of antibiotic resistance in ESKAPE organisms : a systematic review, Antimicrob. Resist. Infect. Control, vol. 8, no. 1, p. 137, 2019.

[5] Hussain FM, Boyle-vavra S, Bethel CD, Daum RS. Current trends in community-acquired methicillin-resistant Staphylococcus aureus at a tertiary care pediatric facility UCCH in 1998 and 1999 to see whether this trend, Pediatr. Infect. Dis. J., vol. 19, no. 12, pp. 1163–1166, 2000.

[6] Ba X, et al., Old drugs to treat resistant bugs: Methicillin-resistant Staphylococcus aureus isolates with mecC are susceptible to a combination of penicillin and clavulanic

acid, Antimicrob. Agents Chemother., vol. 59, no. 12, pp. 7396–7404, 2015.

[7] Bush K, and Bradford PA. β-Lactams and β-lactamase inhibitors: an overview,"Cold Spring Harb. Perspect. Med., vol. 6, no. 8, p. p.a025247., 2016.

[8] Pandey N, and Cascella M. "eta Lactam Antibiotics,"vol. 1, no. 1, pp. 1–7, 2019.

[9] Stapleton PD, and Taylor PW. Methicillin resistance in Staphylococcus aureus : mechanisms and modulation, Sci. Prog., vol. 85, no. 1, pp. 57–72, 2002.

[10] Stapleton PD, and Taylor PW. Methicillin resistance in Staphylococcus aureus : mechanisms and modulation, Science progress, vol. 85, no. 1. pp. 1–14, 2007.

[11] Lee AS, et al., "Methicillin-resistant Staphylococcus aureus," Nat. Rev. Dis. Prim., vol. 4, no. 1, pp. 1–23, 2018.

[12] Davis JS, Van Hal S, and Tong SYC. Combination antibiotic treatment of serious methicillin-resistant Staphylococcus aureus infections., Semin. Respir. Crit. Care Med., vol. 36, no. 1, pp. 3–16, 2015.

[13] Nguyen HM, and Graber CJ. Limitations of antibiotic options for invasive infections caused by methicillin-resistant Staphylococcus aureus : is combination therapy the answer?, J. Antimicrob. Chemother., vol. 65, no. 1, pp. 24–36, 2009.

[14] Miller LA, Ratnam K, and Payne DJ, β - Lactamase-inhibitor combinations in the 21st century : current agents and new developments, Curr. Opin. Pharmacol., vol. 1, no. 5, pp. 451–458, 2001.

[15] Astwood EB et al. Human Pituitary Disease-Fraser Flucloxacillin, a New Isoxazolyl Penicillin, Compared with Oxacillin, Cloxacillin, and Dicloxacillin, Br. Med. J., no. November, pp. 1962–1967, 1970.

[16] de Menezes MN, de Marco BA, Fiorentino FAM, Zimmermann A, Kogawa AC, and Salgado HRN. Flucloxacillin: A Review of Characteristics, Properties and Analytical Methods, Crit. Rev. Anal. Chem., vol. 49, no. 1, pp. 67–77, 2019.

[17] Meka G, and Chintakunta R. Analgesic and anti-inflammatory activity of quinoxaline derivatives: Design synthesis and characterization, Results Chem., vol. 5, no. October 2022, p. 100783, 2023.

[18] Elfadil A, Ibrahem K, Abdullah H, Mokhtar JA, Al-Rabia MW, and Mohammed HM. Synergistic Activity of 3-Hydrazinoquinoxaline-2-Thiol in Combination with Penicillin Against MRSA," Infect. Drug Resist., vol. 17, no. January, pp. 355–364, 2024.

[19] Clinical and Laboratory Standards Institute (CLSI). M07-A9: methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—Ninth edition. Available from: https://clsi.org/media/1928/m07ed11_sample.pdf. Access, 2002.

[20] Hu A, Liu Y, and Coates Y. Azidothymidine produces synergistic activity in combination with colistin against antibiotic-resistant Enterobacteriaceae, Antimicrob. Agents Chemother., vol. 63, no. 1, pp. 1–11, 2019.

[21] Kerantzas CA, and Jacobs WR Jr. "Origins of combination therapy for tuberculosis: lessons for future antimicrobial development and application," MBio, vol. 8, no. 2, pp. 1–10, 2017.

[22] Davis J, Hal S, and Tong S. Combination Antibiotic Treatment of Serious Methicillin-Resistant Staphylococcus aureus Infections, Semin. Respir. Crit. Care Med., vol. 36, no. 01, pp. 003–016, Feb. 2015.

[23] Gonzales PR, et al. Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA, Nat. Chem. Biol., vol. 11, no. 11, pp. 855–861, 2015.

[24] Mulani MS, Kamble EE, Kumkar SN, Tawre MS, and Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review, Front. Microbiol., vol. 10, no. 4, 2019.

[25] Cheng G, et al., Quinoxaline 1,4-di-N-oxides: Biological activities and mechanisms of actions, Front. Pharmacol., vol. 7, no. MAR, pp. 1–21, 2016.

[26] Kulanthaivel L, Jeyaraman J, Biswas A, Subbaraj GK, and Santhoshkumar S. Identification of potential inhibitors for Penicillin binding protein (PBP) from Staphylococcus aureus, Bioinformation, vol. 14, no. 9, pp. 471–476, 2018.

[27] Reddy GS, and Reddy CB. Spectrophotometric estimation of flucloaxacillin in pure drug and pharmaceutical dosage formulation, IOSR J. Pharm. Bio. Sci, vol. 3, no. 3, pp. 46–48, 2011.

[28] Léger N, Budin-Verneuil L, Cacaci A, Benachour M, Hartke A, and Verneuil A. β-lactam exposure triggers reactive oxygen species formation in enterococcus faecalis via the respiratory chain component DMK, Cell Rep., vol. 29, no. 8, pp. 2184–2191, 2019.

[29] Dwyer DY, Belenky DJ, Yang PA, MacDonald JH, Martell IC, Takahashi JD, Chan N, Lobritz CT, Braff MA, Schwarz D, and Jonathan EG. Antibiotics induce redox-related physiological alterations as part of their lethality, Proc. Natl. Acad. Sci., vol. 111, no. 20, pp. E2100–E2109, 2014.

[30] Chacón-Vargas KF, et al. Isopropyl quinoxaline-7-carboxylate 1,4-di-N-oxide derivatives induce regulated necrosis-like cell death on Leishmania (Leishmania) mexicana, Parasitol. Res., vol. 117, no. 1, pp. 45–58, 2018.

[31] Bolhuis J, Hand A, Marshall L, Richards JE, Rodger AD, and Aldrich-Wright A. Bacteriophage Therapy: Advances in Formulation Strategies and Human Clinical Trials, Eur. J. Pharm., vol. 42, no. 4, pp. 313–317, 2011.

[32] Bolhuis J, Hand A, Marshall L, Richards JE, Rodger AD, and Aldrich-Wright A. Antimicrobial activity of ruthenium-based intercalators, Eur. J. Pharm., vol. 42, no. 4, pp. 313–317, 2011.

[33] Daina A, Michielin O, and Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep., vol. 7, no. January, pp. 1–13, 2017.