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Evaluating the Efficacy of 4-Arylidene-2-phenyl-5-(H)-oxazolones compounds as Potential Antimicrobial Agents Against some Antibiotic-Resistant Strains

*Safa alshebanie[1] , Hamed albogdadi[1] , Arabya A.A. Almutaleb[2] , Shamsi saad[1]

[1] Botany Department , Faculty of science , Sebha university , Sebha , Libya
[2] Department of Anesthesia Technology , Faculty of Medical Technology , Sebha university , Sebha , Libya

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ABSTRACT

The emergence of antibiotic-resistant bacteria presents a significant challenge in developing effective antimicrobial agents. This study investigates the antibacterial activity of a series of 4-Arylidene-2-phenyl-5(H)-oxazolones, a class of compounds known for their versatility in medicinal chemistry. The synthesis of these compounds was performed according to established protocols, and their antibacterial efficacy was evaluated using broth microdilution and agar well diffusion assays against five bacterial strains, including Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia Cepacia, E. coli, and Acinetobacter Baumannii. The results demonstrated variable inhibitory effects across the different bacterial strains, with compound A1 showing significant activity against Acinetobacter Baumannii and compound CL1B exhibiting potent inhibition of E. coli and Enterobacter Cloacae. Statistical analysis indicated a trend towards compound specificity, although the differences did not reach conventional statistical significance. The study highlights the potential of 4-Arylidene-2-phenyl-5-(H)-oxazolones as a platform for developing targeted antibacterial agents. The observed variability in antibacterial activity underscores the importance of structural diversity and functional groups in designing effective antimicrobial compounds. Further research is necessary to elucidate these compounds' mechanisms of action and optimize their antibacterial properties for clinical applications.

تقييم فعالية مركبات 4-Arylidene-2-phenyl-5 - (H)-oxazolones كعوامل مضادة للميكروبات واعدة ضد بعض السلالات البكتيرية مقاومة للمضادات الحيوية

* صفاء محفوظ محمد الشيباني[1] و حامد عبدالسلام البغدادي[1] و عربية عبدالسلام عبدالنبي المغربي[2] و شمسي سعد شمسي[1]

[1] قسم علم النبات، كلية العلوم، جامعة سبها، سبها، ليبيا
[2] قسم تقنية التخدير، كلية التقنية الطبية، جامعة سبها، ليبيا

الكلمات المفتاحية:
مركبات الأوكزازولون
سلالات بكتيرية
مضادات حيوية

الملخص

ظهور البكتيريا السلبية الغرام المقاومة للمضادات الحيوية يشكل تحديًا كبيرًا في تطوير عوامل مضادة للميكروبات فعالة. تستكشف هذه الدراسة النشاط المضاد للبكتيريا لسلسلة من مركبات 4-Arylidene-2-phenyl-5(H)-oxazolones، وهي فئة من المركبات المعروفة بتنوعها في الكيمياء الطبية. تم تخليق هذه المركبات وفقًا للبروتوكولات المعتمدة، وتم تقييم فعاليتها المضادة للبكتيريا باستخدام اختبارات التخفيف الميكروبي بالمرق واختبارات الانتشار بالأجار ضد خمس سلالات بكتيرية، بما في ذلكPseudomonas aeruginosa ، Staphylococcus aureus، Burkholderia Cepacia، E. coli، و.Acinetobacter Baumannii أظهرت النتائج تأثيرات مثبطة متغيرة عبر السلالات البكتيرية المختلفة، حيث أظهر المركب A1 نشاطًا ملحوظًا ضد Acinetobacter Baumannii وأظهر المركب CL1B تثبيطًا قويًا لـ E. coli و Enterobacter Cloacae. أشار التحليل الإحصائي إلى اتجاه نحو تخصص المركبات، على الرغم من أن الاختلافات لم تصل إلى الدلالة الإحصائية التقليدية. تسلط الدراسة الضوء على إمكانية مركبات 4-Arylidene-2-phenyl-5(H)-oxazolones كمنصة لتطوير عوامل مضادة للبكتيريا مستهدفة. يؤكد التباين الملحوظ في النشاط المضاد للبكتيريا على أهمية التنوع الهيكلي والمجموعات الوظيفية في تصميم مركبات مضادة للميكروبات

*Corresponding author.
E-mail addresses: safaalsebhani@gmail.com, (H. Albogdadi)Bogdadi99@gmail.com, (A. Almutaleb)Ara.Almagraby@sebhau.edu.ly, (S. Saad)Sha.saad@sebhau.edu.ly

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فعالة. من الضروري إجراء المزيد من الأبحاث لتوضيح آليات عمل هذه المركبات وتحسين خصائصها المضادة للبكتيريا للتطبيقات السريرية.

Introduction

As is well established, certain types of bacteria can be harmful, causing various human diseases. These diseases can sometimes be severe, posing significant threats to individuals with weakened immune systems[1, 2]. The challenge of bacterial infections has been compounded by the emergence and spread of antibiotic-resistant strains[3], particularly Gram-negative bacteria[3]. Numerous studies [4, 5]have demonstrated the resilience of these bacteria against multiple drugs, leading to increased mortality rates and extended hospital stays in healthcare settings.

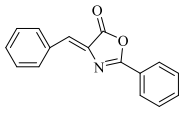
In light of this growing threat, discovering and developing new antimicrobial agents has become critical. Medicinal chemistry[6], a field at the forefront of drug discovery, is pivotal in addressing this challenge. It involves designing and synthesizing compounds that can serve as effective treatments against various pathogens.

One of the significant advancements in medicinal chemistry has been the synthesis of 4-Arylidene-2-phenyl-5(H)-oxazolones[7]. These compounds have earned attention for their role as intermediates in synthesizing a wide array of biologically active molecules. Their versatility is evident in their application in creating antibiotics and antifungals and a range of other therapeutic agents such as sedatives, analgesics, antilipemics, antimalarials, and anticancer agents[8].

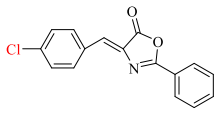
The importance of 4-Arylidene-2-phenyl-5(H)-oxazolones extends beyond their immediate applications[9]. They serve as a foundation for developing new treatments to combat the growing issue of antibiotic resistance[8]. By exploring these compounds' synthesis and potential applications, researchers aim to contribute to the broader field of medicinal chemistry and, ultimately, to develop novel therapeutic agents that can address some of our most pressing health challenges.

Synthesis of 4-Arylidene-2-phenyl-5-(H)-oxazolones derivatives:

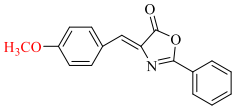
The 4-Arylidene-2-phenyl-5(H)-oxazolones derivatives were synthesized according to the established protocol[10] and [11]. The derivatives were prepared by dissolving the synthesized compound in an appropriate solvent to achieve the desired concentration for testing.



(*Z*)-4-benzylidene-2-phenyloxazol-5(4*H*)-one



(*Z*)-4-(4-chlorobenzylidene)-2-phenyloxazol-5(4*H*)-one



(*Z*)-4-(4-methoxybenzylidene)-2-phenyloxazol-5(4*H*)-one

The differences between the physical (melting points, solubility and colour), and chemical properties (IR, ^{1}H, $^{13}CNMR$ and mass spectra) were the evidence for all the deficient biological effects in this study.

Bacterial Strains and Culture Conditions:

Five bacterial strains were selected for this study, including Gram-negativea and Gram-Positive species (Pseudomonas aeruginosa, Staphylococcus aureus , Burkholderia Cepacia , E. coli , Enterobacter Cloacae , and Acinetobacter Baumannii .Bacterial cultures were grown in Luria-Bertani (LB) broth at 37°C with constant shaking at 200 rpm until they reached the mid-logarithmic phase.

Broth Microdilution Inhibition Assay:

The broth microdilution inhibition assay[12] evaluated the antibacterial activity of 4-Arylidene-2-phenyl-5-(H)-oxazolones and their derivatives. Bacterial cultures were diluted to a final concentration of approximately 1×10^6 colony-forming units (CFU)/mL in LB broth. The test compound and organic compounds were added to the bacterial suspension at 500 mg/ml concentration , and the mixture was incubated at 37°C for 24 hours. Meropenem was used as a positive control, and LB broth without the test compound was negative. All experiments were performed in triplicate**.**

Measurement of Optical Density (OD):

After incubation, each culture's optical density (OD) was measured at 600 nm using a spectrophotometer to assess bacterial growth. The OD values were used to calculate the percentage of growth inhibition compared to the negative control**.**

Agar Well Diffusion Assay:

Agar well Diffusion method by [12] was used . Müller-Hinton agar plates were prepared by pouring approximately 20 mL of the molten agar into sterile Petri dishes and allowing them to solidify. Once solidified, the agar surface was marked into sections to accommodate the wells for each test compound and control.

The same five bacterial strains used in the broth microdilution inhibition assay were cultured overnight in Luria-Bertani (LB) broth at 37°C with constant shaking. The bacterial cultures were adjusted to a turbidity equivalent to 0.5 McFarland standard, approximately 1 × 10^8 CFU/mL, using sterile saline.

A stock solution of 4-Arylidene-2-phenyl-5-(H)-oxazolones and their derivatives was prepared at a 500 mg/mL concentration in an appropriate solvent. The solution was then filter-sterilized using a 0.22 µm syringe filter.

The surface of each Müller-Hinton agar plate was evenly inoculated with 100 µL of the bacterial inoculum using a sterile cotton swab, ensuring complete coverage of the agar surface.

Wells with a diameter of approximately 5 mm were punched into the agar using a sterile borer. Then, 50 µL of the 500 mg/mL test compound solution was added to the respective wells. Meropenem was used as a positive control, and the solvent used for dissolving the compounds was a negative control.The inoculated agar plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones around each well were measured in millimetres using a ruler. The diameter of the zone of inhibition was recorded as an indicator of the antibacterial activity of the test compounds.

The results were tabulated, and the average zone of inhibition for each tested compound and control was calculated from triplicate experiments. The antibacterial efficacy of the test compounds was compared to that of the positive control and the solvent control.

Statistical Analysis:

Statistical analysis was performed using one-way ANOVA and post-hoc tests to compare the differences between the treated groups and controls. A p-value of <0.05 was considered statistically significant.

Results

In our investigation of the antibacterial activity of five different4-Arylidene-2-phenyl-5(H)-oxazolones compounds, significant variability in effectiveness was observed across various bacterial strains.

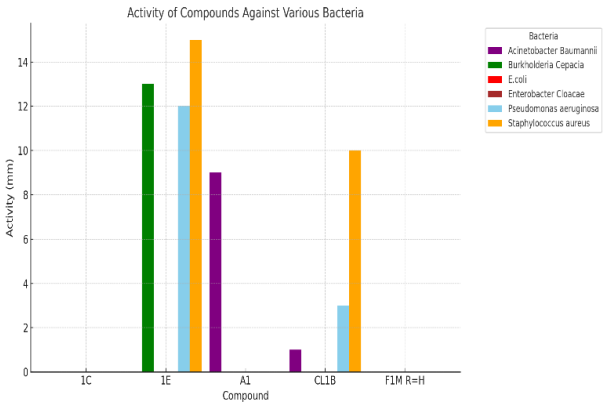


Figure (1) Activity of various 4-Arylidene-2-phenyl-5(H)-oxazolones compounds against different bacterial strains.

The bars represent the mean inhibition zone diameter in millimeters (mm) for each compound tested against Pseudomonas aeruginosa (blue), Staphylococcus aureus (orange), Burkholderia Cepacia (green), E. coli (red), Enterobacter Cloacae (purple), and Acinetobacter Baumannii (brown).

The compound 1E demonstrated the highest mean activity against Staphylococcus aureus and Burkholderia Cepacia, whereas A1 showed notable activity against Acinetobacter Baumannii. The other compounds exhibited minimal to no inhibitory effects. Statistical analysis using one-way ANOVA revealed a bordering significance in the differences between the compounds' mean activities (F-statistic: 2.7113, p-value: 0.0529), suggesting a tendency towards certain compounds being more effective against specific bacteria, although these findings did not reach the conventional level of statistical significance.

In the OD600 broth inhibition assay, the compounds demonstrated varied activity levels against different bacteria, as indicated by the optical density (OD) measurements taken after 24 hours of incubation. Higher OD readings suggest lower antibacterial activity, as the OD measures bacterial growth; therefore, a reading closer to the control OD (2.5) indicates less inhibition. Contrarily, a lower OD reading implies more significant antibacterial activity.

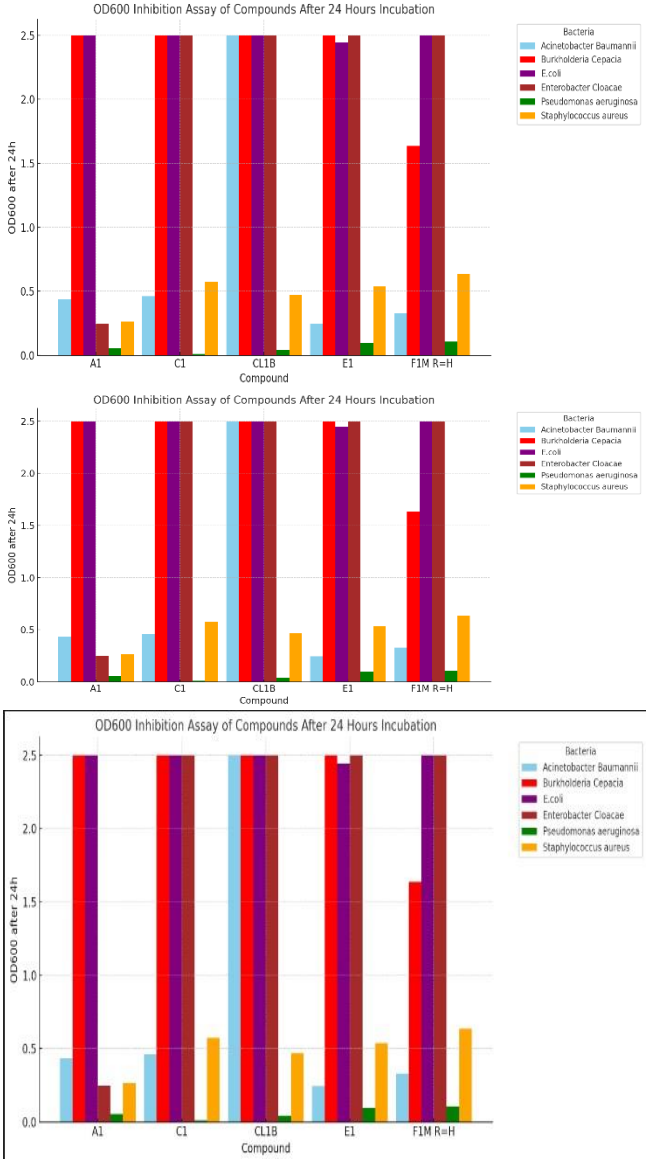


Figure (2) Comparative Analysis of Antibacterial Activity Measured by OD600 Inhibition Assays against selected bacteria

In evaluating antibacterial efficacy through OD600 inhibition assays, percent inhibition serves as the metric for compound activity—higher percentages indicate a greater antibacterial effect. The compounds exhibited a range of activities against the tested bacterial strains, with the following observations:

Compound A1 displayed moderate inhibitory activity, particularly notable against Acinetobacter Baumannii with around 82% inhibition, but its activity varied considerably across other bacterial strains.

Compound CL1B showed high antibacterial activity, achieving up to 100% inhibition in Enterobacter Cloacae and E.coli, suggesting its potential as a potent antibacterial agent against these particular strains.

Compound C1 similarly reached 100% inhibition for Enterobacter Cloacae and E.coli, displaying a consistent inhibitory profile akin to CL1B.

Compound E1 had varying effectiveness, with remarkable activity against Burkholderia Cepacia and lower activity against Pseudomonas aeruginosa and Staphylococcus aureus, indicating a selective antibacterial potential.

Compound F1M R=H was less effective than the others, with the highest activity observed against Acinetobacter Baumannii at approximately 34% inhibition and its lowest against Burkholderia Cepacia.

The differential antibacterial effects underscore the importance of compound specificity and the necessity to effectively tailor antibacterial agents to target specific bacterial pathogens. The variation in percent inhibition across different bacterial strains for each compound warrants further investigation into their mechanisms of action and potential applications in combating bacterial infections.

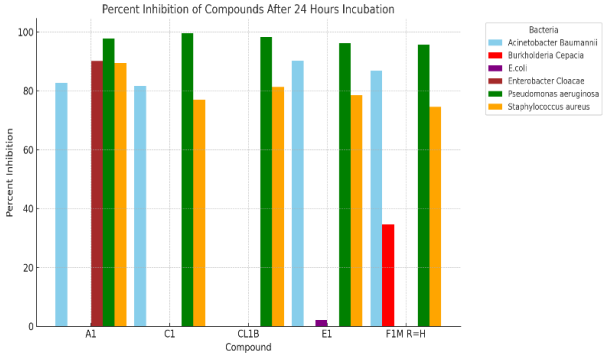


Figure (3) Percent Inhibition of Bacterial Growth by Compounds A1, CL2, CL1B, and C1 after 24 hours, showcasing varied efficacy across Acinetobacter Baumannii, Enterobacter Cloacae, E. coli, and Staphylococcus aureus

Discussion

Our research outlines the interplay between the antibacterial activities of 4-Arylidene-2-phenyl-5-(H)-oxazolones and the methodological frameworks employed to evaluate them. Key findings from agar well diffusion and OD600 broth inhibition assays underscore the distinct responses of bacterial strains to the compounds, highlighting the complexity of antibiotic interactions. Variability in the zones of inhibition and OD600 values points to the importance of considering both the physical properties of the compounds, such as solubility and diffusion, and the kinetic aspects of their bacterial interactions[13].

Functional groups within the chemical structures of 4-Arylidene-2-phenyl-5-(H)-oxazolones are critical in determining their bacteriostatic and bactericidal properties[14]. These findings align with previous research[15] emphasizing the role of molecular functionality in the permeability and binding affinities of antibacterial agents. Notably, the varying efficacy of these compounds against different strains, such as the reduced activity of F1M R=H, may correlate with the presence or modification of functional groups[15] that mediate the interaction with bacterial targets.

Positioned within the broader context of antimicrobial research, this study advances the collective understanding of structure-activity relationships in drug design. It narrows the existing gap by providing empirical data on the antibiotic potential of 4-Arylidene-2-phenyl-5-(H)-oxazolones[16], thus pushing the boundaries of current knowledge. Our results potentially modify the perspective on the strategic incorporation of functional groups in developing antibacterial agents against resistant strains.

In the study of future research trajectories, it is proposed that investigations search deeper into the mechanical aspects of these compounds, mainly through molecular modeling and in vivo studies. Examining the synergy between these compounds and existing antibiotics could unveil new avenues for combination therapies, addressing the escalating concern of antimicrobial resistance. Additionally, expanding the scope of research to include more

diverse bacterial models could further validate the applicability of 4-Arylidene-2-phenyl-5-(H)-oxazolones in clinical settings, ultimately leading to more targeted and effective antimicrobial strategies.

Conclusion:

The varying efficacious of the 4-Arylidene-2-phenyl-5-(H)-oxazolones emphasize the importance of structural diversity when developing antibacterial agents. Functional groups are crucial in determining the spectrum and potency of antibacterial activity. This insight can guide the design of future compounds and the optimization of existing ones. Different bacterial strains have distinct susceptibilities, so it is vital to consider the specific target bacterial pathogens when choosing the appropriate antibacterial compounds for further development.

The difference in results between the agar well diffusion and OD broth inhibition assays serves as a reminder of the complexity of interpreting antibacterial activity and the necessity to utilize multiple methods to understand a compound's potential comprehensively. The findings also underscore the significance of chemical diversity in the quest for new and effective antibacterial therapies.

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