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Synthesis, Characterization, and Biological Activity Evaluation of Thiopyrimidone Derivatives from Chalcone Precursors

Shivam Solanki[1*], Hardik Bhatt[2], Bhumika Gajjar[3], Sanjay Bamaniya[1] , Jigar Raval[4]

[1-]Department of Chemistry and Forensic science BKNMU Junagadh Gujarat 362263.
[2] Shri J.N. Bhatu College of Education Junagadh Gujarat 362001
[3] Ananya Institute of Science Kalol Gandhinagar 382721
[4] School of Engineering & Technology National Forensic Science University Gandhinagar

*E-mail: alchemyresearch99@gmail.com

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Abstract

This study explores the synthesis, characterization, and biological activity evaluation of thiopyrimidone derivatives derived from chalcone precursors. Heterocyclic compounds play a crucial role in medicinal chemistry, given their presence in many pharmaceutical agents. Our research focuses on synthesizing various chalcones through Claisen-Schmidt condensation and their subsequent transformation into thiopyrimidone derivatives via condensation with thiourea. Characterization of these compounds was performed using Proton NMR (1H NMR), Carbon-13 NMR (13C NMR), IR spectroscopy, and mass spectrometry, ensuring precise identification and structural integrity. The synthesized compounds were then assessed for their antibacterial activity against four bacterial strains: E. coli, B. subtilis, B. amyloliquefaciens, and B. megaterium, using nutrient agar and broth methods. Comprehensive 2D and 3D plots illustrated the dose-dependent responses of % cell viability across various concentrations, highlighting the IC50 values of each compound. Among the tested derivatives, B4 emerged as the most potent compound with consistent IC50 values around 20 µg/mL, significantly outperforming kanamycin, which exhibited IC50 values near 80 µg/mL. The variations in efficacy across different strains underscore the importance of strain-specific evaluations in antimicrobial research. Our findings suggest that B4 holds significant promise as a therapeutic agent, providing a foundation for future optimization and mechanistic studies. This research contributes to the advancement of heterocyclic chemistry and the development of novel antimicrobial agents, demonstrating the potential of thiopyrimidone derivatives in addressing antimicrobial resistance and expanding the arsenal of effective therapeutic compounds.

Keywords: Thiopyrimidone, Chalcone, E. coli, B. subtilis, B. amyloliquefaciens, and B. megaterium

Introduction

In the exploration of heterocyclic chemistry, both the synthesis and degradation of pyrimidines play pivotal roles, often resulting in mechanisms of ring opening or closing. These processes are catalyzed by a diverse group of enzymes known as amidohydrolases, part of a larger superfamily that primarily facilitates hydrolysis reactions and, to a lesser extent, isomerization processes. These enzymes act on a variety of substrates including nucleic acids, amino acids, and organophosphate esters. Notably, several amidohydrolases have been identified as crucial for either the synthesis or the depletion of pyrimidines, underlining their significance in biochemical pathways [1]. The field of drug discovery heavily relies on heterocyclic chemistry due to the prevalence of heterocycles in pharmaceuticals, which range

from planar heteroaromatics to saturated, fused, or spirocyclic moieties. Functionalized heterocyclic compounds are indispensable in the advancement of medicinal chemistry, and the development of novel synthetic methodologies over the past decades, such as the widely utilized SNAr Substitution, has significantly contributed to this field [2] .

Recent studies have demonstrated the synthesis, characterization, and evaluation of heterocyclic compounds incorporating benzoxazinone, benzimidazole, quinazolinone, and benzofuranone rings, showcasing potent anticancer activities against the HepG2 human hepatocellular carcinoma cell line through sulforhodamine B (SRB) and dimethylthiazol-diphenyltetrazolium bromide (MTT) assays [3]. Additionally, platinum-based compounds like cisplatin have been recognized for their efficacy in cancer treatment, while N-heterocyclic carbenes (NHCs) have emerged as versatile ligands for the development of metal-NHC complexes with promising applications as catalysts, antimicrobial, and anticancer agents [4]

The structural diversity of heterocyclic compounds, including those with nitrogen, oxygen, and sulfur atoms, plays a crucial role in various biological processes and applications, ranging from photosynthesis in plants to the development of antibiotics like penicillin. Their presence in nature and synthetic versatility enables the production of a broad spectrum of compounds with potential applications as dyes, insecticides, and polymers, owing to their ease of modification which can enhance or diminish their reactivity [5]. Heterocyclic compounds form the backbone of the vast and diverse library of organic compounds, crucial for the innovation and discovery of new drugs by providing a plethora of scaffolds and chemical entities through established synthetic strategies.

The advent of modern synthetic techniques has facilitated rapid access to functionalized heterocyclic compounds, significantly impacting drug discovery by offering drug-like properties and improving ADMET profiles. This has been particularly pivotal in addressing challenges such as antimicrobial resistance, which poses a significant threat to healthcare due to the overuse of antibacterial and antifungal agents [6]. The rise in immunocompromised populations has also highlighted the clinical importance of targeting fungal infections, with research indicating a dire need for novel antimicrobial drugs [7]. Moreover, heterocyclic

compounds have shown promising antimalarial activity against chloroquine-sensitive and resistant strains of Plasmodium falciparum [8].

The synthesis of chalcones through acetic and perchloric acid under acidic conditions opens new avenues for producing potent tyrosinase inhibitors, acting as antioxidants and depigmenting agents [9]. Chalcones, as 1,3-diphenyl-2-propen-1-ones, play a crucial role as natural products in plants, serving as precursors for flavonoids and isoflavonoids, with specific derivatives displaying significant fluorescence [10]. The preparation of chalcones via the Claisen-Schmidt condensation highlights their foundational role in flavonoid and isoflavonoid biosynthesis [11]. This study focuses on synthesizing various chalcones and their subsequent condensation with thiourea to produce thiopyrimidine derivatives, showcasing the ongoing innovation and significance of heterocyclic compounds in medicinal chemistry and drug discovery.

1. Methods and Materials

1.1 Chemicals and Reagents

The study utilized chemicals of reagent-grade quality to maintain consistency and accuracy in experimental results. Sourced from Merck, Mumbai, India, the research involved various aldehydes, 3 nitro p-cresol, 1-(3-chlorophenyl) ethenone, thiourea, sodium hydroxide (NaOH), and ethanol. These chemicals were used as received, without further purification, to synthesize the desired heterocyclic compounds.

1.2 Experimental

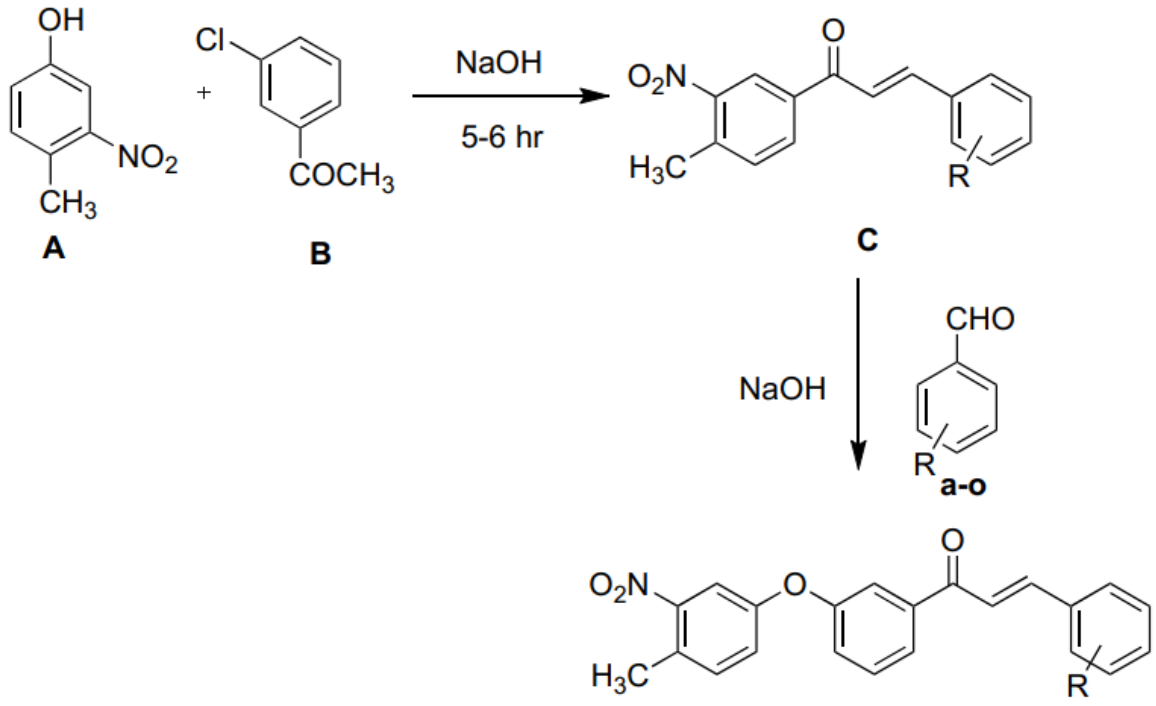
For characterizing the synthesized compounds, a comprehensive analytical approach was employed: Proton NMR (^{1}H NMR) and Carbon-13 NMR (^{13}C NMR) analyses were performed using a Bruker Avance-400 instrument and a 100 MHz instrument respectively, to elucidate the molecular structures, with chemical shifts reported in parts per million (ppm). Infrared (IR) spectroscopy, conducted on an FT-IR 3000 Spectrophotometer from ABB Bomem Inc., provided insights into functional groups and molecular bonding, with data presented in cm^-1. Mass spectrometry analysis, utilizing a Shimadzu LCMS-2010, determined the molecular weights and fragmentation patterns, while elemental composition was verified through a Perkin Elmer-2400 Series II CHNS/O Elemental Analyzer. This rigorous analytical regimen ensured precise and

reliable identification of the synthesized heterocyclic compounds, underpinning their potential applications in medicinal chemistry.

1.3 Method of Synthesis

1.3.1 Synthesis of (E)-1-(3-(4-methyl-3-nitrophenoxy)phenyl)-3-phenylprop-2-en-1-one A1-A15.

In the synthesis of (E)-1-(3-(4-methyl-3-nitrophenoxy)phenyl)-3-phenylprop-2-en-1-one derivatives A1-A15, the process begins with the combination of 3 nitro p-cresol (0.1 mol) and 1-(3-chlorophenyl)ethanone in a 250 mL round-bottom flask, to which sodium hydroxide (30 mL) is added while maintaining constant shaking to keep the temperature below 25°C, ensuring the reactants dissolve completely. Following dissolution, the mixture is refluxed for 3-4 hours, then cooled and poured into crushed ice to facilitate the precipitation of the intermediate product, which is isolated by filtration and recrystallized from ethanol for purification. The next stage involves the addition of an aromatic aldehyde (0.01 mol) dropwise at 0°C to a vigorously stirred solution of the purified intermediate (0.01 mol) in ethanol (40 mL) and 40% sodium hydroxide (40 mL), aimed at controlling the reaction temperature and pace, thereby preventing the formation of by-products. After completing the addition, the mixture is stirred for an additional 2-3 hours and left to stand overnight, allowing the reaction to go to completion. The resulting mixture is then poured into ice water and the chalcone derivatives A1-A5 are precipitated, filtered, and crystallized from ethanol to achieve purified final products. This carefully orchestrated synthesis procedure underscores the importance of controlled reaction conditions, precise reagent additions, and meticulous purification techniques in the successful production of chalcone derivatives with potential applications in medicinal chemistry.

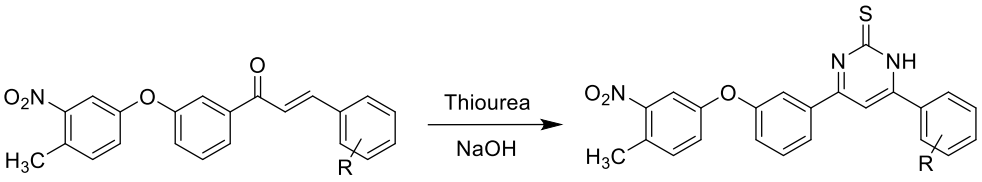


Scheme 1: Synthesis of Chalcones A1-A5

1.3.2 Synthesis of Thiopyrimidones B1-B5

To synthesize Primidone, a pharmacologically relevant compound, from chalcone derivatives, a methodical approach is employed utilizing basic reagents and solvent systems. Begin by introducing 0.01 mol of the chosen chalcone derivative into a 250 mL round-bottom flask, which serves as the reaction vessel for this synthesis. To this flask, add an equimolar amount (0.01 mol) of thiourea, a key reagent in the formation of Primidone's characteristic heterocyclic structure. Following the addition of thiourea, incorporate 40 mL of ethanol, which acts both as a solvent and a medium facilitating the dissolution of reactants, thereby ensuring a homogenous reaction mixture. Additionally, integrate 40 mL of 40% sodium hydroxide (NaOH) solution into the mixture; this strong base is crucial for initiating the reaction by providing the necessary alkaline environment for the ensuing chemical transformation. Once all reagents are combined, subject the mixture to reflux conditions for a duration of 30-50 minutes. Refluxing is a critical step in this synthesis, as it allows the reaction to proceed at an elevated temperature without the loss of solvent, thus driving the reaction towards completion. The process of refluxing not only accelerates the

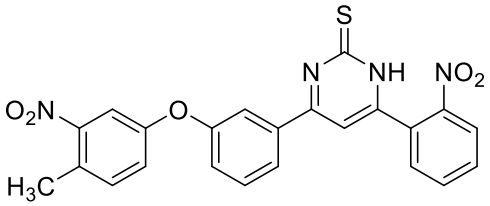
reaction rate but also ensures the thorough interaction of chalcones with thiourea under optimal conditions.To monitor the progress and completion of the Primidone synthesis, employ Thin Layer Chromatography (TLC), a versatile analytical technique. TLC provides a rapid and efficient means of assessing the reaction's advancement by comparing the mobility of the reaction mixture's components against known standards under a controlled solvent system.



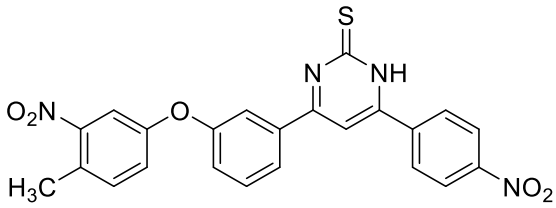
A1-A6 **B1-B6**

1.4 Characterization

B1 & B6 compounds of the series is taken as the representative compound. Inthe ^{1}HNMR spectrum the characteristic signals due to each proton and functionalgroups with protons are well described on the basis of shielding and deshelling effect The signal due to aromatic proton of compound was observed in more downfield region at chemical shift valuearound 6 to 8ppm. ^{1}HNMR, ^{13}CNMR, IR, spectroscopic data of **B1 & B6** compounds shown below. From the supplementary data.

Compound Code: B4	
Molecular Formula $C_{23}H_{16}N_4O_5S$	
M.P. (°C):242	

^{1}H NMR (400 MHz) **δ ppm:**	δ 2.32 (3H, s), 6.88-7.09 (5H, including 6.90 ddd, J = 8.2, 1.8, 0.6 Hz; 6.98 dt, J = 8.2, 1.4 Hz; 7.03 ddd, J = 8.2, 1.0, 0.6 Hz), 7.17-7.59 (6H, including 7.40 ddd, J = 8.2, 7.9, 0.5 Hz; 7.48 dddd, J = 7.8), and 8.21-8.23 (3H, m, including 8.23 dd, J = 8.2, 1.4 Hz; 8.22 dd, J = 8.2, 1.4 Hz; 8.21 dd, J = 8.2, 1.4 Hz).
^{13}C NMR (100 MHz) **δ ppm:**	33.4, 127.2, 129.4, 131.3, 138.6, 132.2, 143.6, 151.8, 153.6, 155.1, 161.8, 165.1.
IR cm^{-1}(KBr) :	3345, 3021, 2978, 1640, 1592, 1564, 742.
Elemental analysis:	**Calculated (%):**C: 59.99, H: 3.5; N:12.17 ,O:17.37,S:6.37 **Found (%):** C: 58.79, H: 4.4; N:12.45 ,O:17.23,S:6.43

Compound Code : B5	
Molecular Formula $C_{23}H_{16}N_4O_5S$	
M.P. (°C):242	
^{1}H NMR (400 MHz) **δ ppm:**	δ 2.23 (3H, s), 6.57 (1H, s), 6.84-7.09 (5H, including 6.90 ddd, J = 8.2, 1.8, 0.6 Hz; 6.98 dt, J = 8.2, 1.4 Hz; 7.03 ddd, J = 8.2, 1.0, 0.6 Hz), and 7.33-7.59 (6H, including 7.40 ddd, J = 8.2, 7.9, 0.5 Hz; 7.48 dddd, J = 7.8).
^{13}C NMR (100 MHz) **δ ppm:**	176.20, 170.29, 158.90, 158.22, 154.83, 154.27, 133.95, 130.44, 130.02, 129.99, 129.33, 129.05, 121.91, 121.40, 118.46, 97.61, 20.63
IR cm^{-1}(KBr) :	3345, 3021, 2978, 1640, 1592, 1564, 742.

Elemental analysis:	**Calculated (%):**C: 59.99, H: 3.5; N:12.17 ,O:17.37,S:6.37 **Found (%):** C: 58.79, H: 4.4; N:12.45 ,O:17.23,S:6.43

1.5 Antimicrobial activity

To assess bacterial activity, nutrient agar was utilized, prepared by mixing 5 grams of peptone, 3 grams of meat extract, 5 grams of NaCl, and 15 grams of agar-agar in one liter of distilled water. This mixture was heated until all components were dissolved. The medium was then sterilized in an autoclave at 15 pounds pressure and 125°C for 20 minutes. After autoclaving, the medium was allowed to cool to 45°C and 20 ml of it was poured into sterilized Petri dishes. The pH of the medium was adjusted to fall between 7.0 and 7.5. The bacterial culture was prepared in nutrient broth, which consisted of 10 grams of beef extract, 10 grams of peptone, and 5 grams of sodium chloride dissolved in distilled water. This nutrient broth was sterilized and then used for culturing the bacteria. The bacterial culture was grown at 37°C in an incubator. A sterile swab was used to spread the bacterial culture evenly over the surface of the agar plates. For the application of the test compound, 5 mm diameter paper discs were prepared and sterilized in an autoclave. The test compound was applied to these paper discs using a micropipette, and the discs were then dried to remove any solvent. The sterile, compound-coated discs were placed on the agar plates containing the bacterial culture media. The discs were gently pressed onto the media to ensure proper contact. The Petri dishes were incubated for 24 hours at 37°C. After the incubation period, the zones of inhibition around each disc were measured to assess the antibacterial activity of the test compound. The size of the inhibition zone indicates the effectiveness of the compound against the bacteria.

1.6 Result and Discussion

Table 1 Data showing synthesis of ThioPyrimidone B1-B15.

Sr. No.	Compounds Code	R	Reaction Time (hr)	% Yield
1	B1	2-Cl	2.5	74
2	B2	4-Cl	2.3	80
3	B3	2-NO_2	2	83
4	B4	4-NO_2	2	83

5	B5	3-NO_2	2	82
6	B6	3-Br	3	78

In our recent study, we synthesized a series of compounds with varying substituents and analyzed their reaction times and yields. The compound B1, which contains a 2-chlorine substituent, exhibited a reaction time of 2.5 hours and achieved a yield of 74%. On the other hand, B2, with a 4-chlorine substituent, demonstrated a slightly shorter reaction time of 2.3 hours and a higher yield of 80%. Compounds B3, B4, and B5, all containing nitro substituents at different positions (2-NO_2, 4-NO_2, and 3-NO_2, respectively), showed reaction times of 2.0 hours. Interestingly, both B3 and B4 achieved the highest yields of 83%, while B5 exhibited a slightly lower yield of 82%. Lastly, the compound B6, which features a 3-bromine substituent, had the longest reaction time of 3.0 hours and a yield of 78%. These results highlight the influence of different substituents on the reaction times and yields, providing valuable insights for future synthesis optimization. Compound B5, identified by its molecular formula $C_{23}H_{16}N_4O_5S$, was thoroughly characterized using various analytical techniques. The melting point was determined to be 242°C, suggesting good thermal stability and purity. The 1H NMR spectrum provided detailed insights into the compound's hydrogen environment. The spectrum showed a singlet at δ 2.23 ppm corresponding to three equivalent hydrogen atoms, likely from a methyl group. Another singlet at δ 6.57 ppm indicated the presence of a single hydrogen atom, possibly from an isolated hydrogen on an aromatic ring. Additionally, the 1H NMR spectrum displayed a multiplet between δ 6.84-7.09 ppm, corresponding to five hydrogen atoms. This multiplet included signals at δ 6.90 ppm (ddd, J = 8.2, 1.8, 0.6 Hz), δ 6.98 ppm (dt, J = 8.2, 1.4 Hz), and δ 7.03 ppm (ddd, J = 8.2, 1.0, 0.6 Hz). Another multiplet between δ 7.33-7.59 ppm accounted for six hydrogen atoms, with notable peaks at δ 7.40 ppm (ddd, J = 8.2, 7.9, 0.5 Hz) and δ 7.48 ppm (dddd, J = 7.8 Hz). The 13C NMR spectrum revealed the presence of multiple carbon environments, with chemical shifts at δ 176.20, 170.29, 158.90, 158.22, 154.83, 154.27, 133.95, 130.44, 130.02, 129.99, 129.33, 129.05, 121.91, 121.40, 118.46, 97.61, and 20.63 ppm. The IR spectrum (KBr) showed characteristic absorption bands at 3345, 3021, 2978, 1640, 1592, 1564, and 742 cm-1, indicating the presence of various functional groups. Elemental analysis calculated for $C_{23}H_{16}N_4O_5S$ gave C: 59.99%, H: 3.5%, N: 12.17%, O: 17.37%, S: 6.37%, while the found values were C: 58.79%, H: 4.4%, N: 12.45%, O: 17.23%, S: 6.43%. These analytical data confirm the structure and composition of Compound B5.

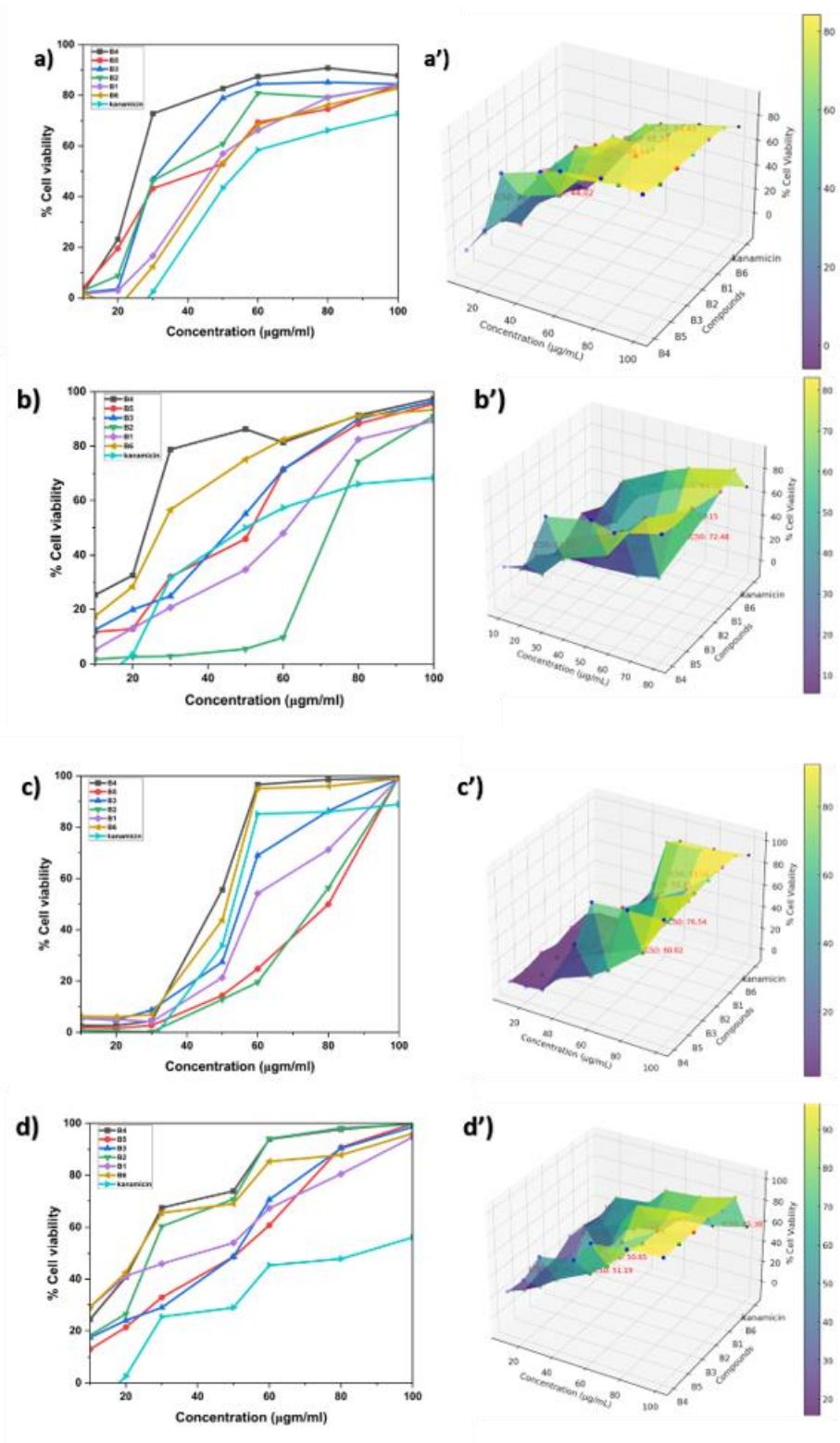


Figure 1: Comparative analysis of % cell viability for compounds (B4, B5, B3, B2, B1, B6) and kanamycin across concentrations for E. coli (a/a'), B. subtilis (b/b'), B.

amyloliquefaciens (c/c'), and B. megaterium (d/d') with 2D line plots showing dose-dependent responses and 3D surface plots highlighting IC50 values.

The presented data illustrate the % cell viability of various compounds (B4, B5, B3, B2, B1, B6) and kanamycin across different concentrations for four bacterial strains: E. coli, B. subtilis, B. amyloliquefaciens, and B. megaterium. Each bacterial strain is represented by a pair of 2D line and 3D surface plots, labeled as a/a', b/b', c/c', and d/d', respectively. For *E. coli* (Fig.1 a and a'), the 2D line plot (a) shows the dose-dependent response of the compounds, with % cell viability increasing as concentration rises. B4 achieves nearly 100% cell viability at 100 µg/mL, while kanamycin shows significantly lower viability at the same concentration. The corresponding 3D surface plot (a') provides a spatial visualization of the data, with IC50 values marked in red. B4 exhibits an IC50 of approximately 20 µg/mL, indicating higher potency compared to kanamycin, which has an IC50 around 80 µg/mL. For *B. subtilis* (Fig.1 b and b'), the 2D line plot (b) demonstrates variability in compound efficacy under different conditions compared to E. coli. For instance, B2 shows a significant drop in cell viability, indicating a reduced efficacy. The 3D surface plot (b') complements this by illustrating the IC50 values, highlighting that B2's potency decreases under these conditions. For *B. amyloliquefaciens* (Fig.1 c and c'), the 2D line plot (c) indicates trends similar to those observed in E. coli but with some differences in compound efficacy. The 3D surface plot (c') provides a comprehensive view of % cell viability against concentration for each compound, with IC50 values showing differences in potency compared to E. coli and B. subtilis. For *B. megaterium* (Fig.1 d and d'), the 2D line plot (d) reveals a dose-response relationship where some compounds display higher efficacy at lower concentrations compared to the other strains. The corresponding 3D surface plot (d') visually highlights the IC50 values, demonstrating that B4 consistently exhibits high potency across all strains, reinforcing its potential as a therapeutic agent. These comprehensive plots reveal that B4 has the highest potency across all tested bacterial strains, with consistent IC50 values around 20 µg/mL, significantly lower than kanamycin's IC50 values. The variations in compound efficacy across different strains underscore the importance of strain-specific studies in evaluating antimicrobial agents. These findings suggest that B4 holds significant promise for further development as a potent therapeutic agent.

1.7 Conclusion

In this study, we synthesized and characterized a series of thiopyrimidone derivatives

from chalcone precursors, and evaluated their biological activity against various bacterial strains. The synthesis process involved precise control of reaction conditions and rigorous analytical techniques to ensure the structural integrity and purity of the compounds. The antibacterial activity was assessed using nutrient agar and nutrient broth methods, revealing significant variations in the efficacy of the compounds across different bacterial strains. Among the synthesized compounds, B4 consistently demonstrated the highest potency with IC50 values around 20 μg/mL, significantly lower than those of kanamycin, which had IC50 values around 80 μg/mL. This was evident across all tested strains, including E. coli, B. subtilis, B. amyloliquefaciens, and B. megaterium. The detailed 2D line and 3D surface plots provided comprehensive insights into the dose-dependent responses and spatial representation of cell viability, further highlighting the superior efficacy of B4. The results underscore the importance of strain-specific studies in the evaluation of antimicrobial agents and suggest that B4 holds significant promise as a potent therapeutic agent. These findings contribute to the field of medicinal chemistry by offering a novel series of thiopyrimidone derivatives with potential applications in antimicrobial therapy. Future studies will focus on optimizing the synthesis process, expanding the range of bacterial strains tested, and exploring the mechanistic pathways of these compounds to further establish their therapeutic potential.

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