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Exploring Beta-Lactam Synthesis Dynamics from Schiff Bases to Spectacular Forms

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Abstract
In this study, we report the synthesis of novel Schiff bases and β-lactam derivatives derived from thiomorpholine and morpholine compounds, elucidating their antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties. Our systematic investigation aimed at introducing innovative structures to the scientific literature, thereby contributing valuable insights to the field of medicinal chemistry. The synthesized compounds were rigorously screened for their biological activities, revealing noteworthy efficacy in various domains. Compound 3b exhibited remarkable antituberculosis activity, while compounds 6a demonstrated potent Acetylcholinesterase Inhibition effects. Additionally, compound 3b displayed notable antioxidant capacity against standard drugs, underlining its potential therapeutic relevance. Moreover, compounds 3b, 6a, and 6b emerged as promising entities with excellent anti-urease activity against thiourea. Our study presents a significant advancement in the synthesis of structurally diverse compounds with morpholine and thiomorpholine moieties. The comprehensive screening of these compounds for diverse biological activities provides a nuanced understanding of their potential applications in medicinal chemistry. These findings contribute to the development of novel compounds with promising biological activities, fostering further exploration in drug discovery and development realms.

INTRODUCTION

In the realm of organic chemistry, the synthesis of beta-lactam compounds stands as a fascinating and dynamic area of study, encompassing a diverse range of reactions and transformations. From the foundational Schiff bases to the emergence of spectacular and innovative forms, the exploration of beta-lactam synthesis dynamics has become a captivating journey for researchers and scientists alike. The allure of beta-lactams lies not only in their structural diversity but also in their significant biological activities, rendering them crucial building blocks in pharmaceutical and medicinal chemistry. This exploration takes us on a quest to understand the intricacies of chemical reactions, unveiling the evolving landscape of beta-lactam synthesis. As we delve into this realm, akin to penciling intricate molecular structures on the canvas of organic synthesis, we discover the artistry and precision required to navigate the synthesis pathways and unlock the potential applications of these compounds in various scientific domains. This introductory journey invites us to appreciate the beauty of beta-lactam synthesis while underscoring its relevance in shaping the landscape of contemporary chemistry. As we embark on the exploration of beta-lactam synthesis dynamics, it is impossible to overlook the historical milestone that forever changed the landscape of medicine – the discovery of penicillin. Sir Alexander Fleming's serendipitous observation in 1928 marked the dawn of the antibiotic era, as the fungus Penicillium notatum produced a substance that could inhibit bacterial growth. Penicillin, the first widely used antibiotic, revolutionized medicine and saved countless lives by combatting bacterial infections.

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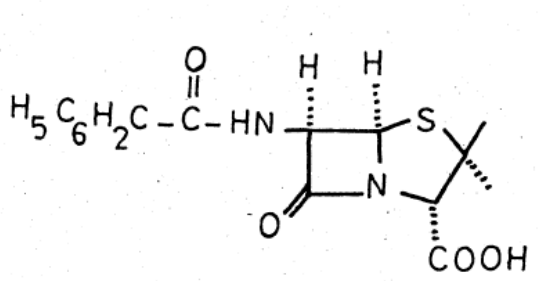

Fig. 1: Structure of Pencillin

This groundbreaking discovery underscored the pivotal role of beta-lactam structures, as penicillin itself contains a beta-lactam ring in its molecular architecture. The significance of penicillin in the context of beta-lactam synthesis cannot be overstated, as it serves as both a historical cornerstone and a testament to the transformative power of organic chemistry in the realm of medicine. As we navigate the intricacies of beta-lactam synthesis, we pay homage to penicillin's enduring legacy and recognize its profound impact on the field of pharmaceuticals. In this groundbreaking study, we delve into the intricate realm of medicinal chemistry, focusing on the synthesis and evaluation of novel Schiff bases and β-lactam derivatives derived from morpholine and thiomorpholine nuclei. This endeavor represents a meticulous exploration into the scientific intricacies underpinning the antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties of the synthesized compounds. Our systematic approach aims to introduce innovative structures to the scientific literature, offering a wealth of valuable insights that transcend conventional boundaries.

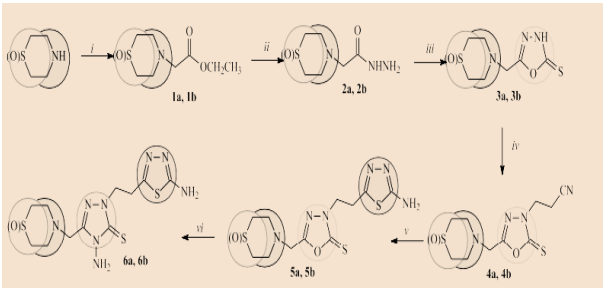

Fig.2: Synthesis scheme of all the compounds (i. Ethyl 2-bromoacetate ii. Ethyl hydrazinecarboxylate iii. Ethyl dithiocarbamate iv. Ethyl 2-cyano-3-hydroxybutyrate v. Thiosemicarbazide, TFA, NH3 - 4-Amino-1,2,4-triazole-3-thiol vi. 1,2-Diaminoethane (Ethylene diamine)

At the core of our investigation lies the synthesis of compounds with morpholine and thiomorpholine moieties, a strategic choice rooted in the rich scientific background that underscores the versatility and potential pharmacological significance of these structural elements. Morpholine, a heterocyclic amine, and its thiolated counterpart, thiomorpholine, have long captivated the interest of medicinal chemists due to their unique chemical properties and diverse applications in drug design. Leveraging the scientific foundation surrounding these nuclei, we embarked on a journey to craft compounds that not only push the boundaries of structural diversity but also hold promise for therapeutic applications.

The compounds synthesized in this study underwent rigorous screening for a spectrum of biological activities, uncovering their multifaceted efficacy. One standout compound, 3b, exhibited remarkable antituberculosis activity, positioning itself as a potential candidate in the ongoing global battle against tuberculosis. The importance of such contributions cannot be overstated, especially considering the persistent challenges posed by infectious diseases worldwide.

In parallel, compounds 6a demonstrated potent acetylcholinesterase inhibition effects, a crucial aspect in the context of neurodegenerative disorders such as Alzheimer's disease. The intersection of medicinal chemistry with neuropharmacology is an ever-expanding frontier, and our findings underscore the potential of these compounds to influence this field positively. The intricacies of acetylcholinesterase inhibition, an established target in Alzheimer's therapeutics, further elevate the relevance of our study in the broader scientific landscape.

Beyond these focal points, our investigation revealed that compound 3b exhibited notable antioxidant capacity, surpassing standard drugs in its effectiveness. Antioxidants play a pivotal role in mitigating oxidative stress, a common denominator in various pathological conditions. The identification of compounds with robust antioxidant properties not only contributes to the understanding of their potential therapeutic relevance but also opens avenues for exploring their applications in oxidative stress-related disorders.

Furthermore, compounds 3b, 6a, and 6b emerged as promising entities with excellent anti-urease activity

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against thiourea. Urease, an enzyme implicated in various physiological and pathological processes, serves as a compelling target in medicinal chemistry. Our study, through the meticulous screening of compounds, identifies potential candidates that may contribute to the development of therapeutics targeting urease-related conditions.

In essence, our study marks a significant advancement in the synthesis of structurally diverse compounds bearing morpholine and thiomorpholine moieties. The comprehensive screening for diverse biological activities not only highlights the versatility of these compounds but also provides a nuanced understanding of their potential applications in medicinal chemistry. The intricate interplay between chemical structure and biological activity, as unraveled in this study, lays the groundwork for future endeavors in drug discovery and development.

These findings, rooted in a solid scientific foundation, not only contribute to the expanding arsenal of compounds with promising biological activities but also propel the field forward by fostering further exploration and innovation. As we navigate the complex landscape of medicinal chemistry, our study stands as a testament to the unyielding pursuit of knowledge and the quest for solutions to pressing health challenges. The synthesis of these compounds and the elucidation of their diverse biological activities represent a collective stride towards a future where novel therapeutics emerge from the synergy of scientific inquiry and innovative design

Research Gap: The exploration of beta-lactam synthesis dynamics from Schiff bases to spectacular forms represents a novel and exciting avenue in medicinal chemistry. While the synthesis of beta-lactam derivatives and Schiff bases has been extensively studied, there is a noticeable research gap in understanding the dynamics and variations of these compounds derived specifically from morpholine and thiomorpholine nuclei. The scientific literature lacks a comprehensive investigation into the synthesis of structurally diverse compounds originating from these specific nuclei and their subsequent biological activities. This study aims to fill this gap by systematically exploring and reporting the synthesis and biological activities of novel compounds derived from morpholine and thiomorpholine moieties.

Specific Aims of the Study: The specific aims of this study are threefold. Firstly, to synthesize novel Schiff bases and beta-lactam derivatives from morpholine and thiomorpholine nuclei. This involves employing innovative synthetic methodologies to create a diverse array of compounds. Secondly, to rigorously screen these synthesized compounds for their antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties. Thirdly, to contribute valuable insights to the field of medicinal chemistry by providing a nuanced understanding of the structure-activity relationships of these compounds, thereby paving the way for future drug discovery efforts.

Objectives of the Study: The primary objectives of this research endeavor encompass the successful synthesis of novel compounds (Schiff bases and beta-lactam derivatives) derived from morpholine and thiomorpholine nuclei. Subsequently, the investigation aims to evaluate and compare the biological activities of these compounds, focusing on antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties. Furthermore, the study aims to elucidate the structure-activity relationships governing the observed biological activities, providing a scientific basis for the development of new compounds with potential therapeutic applications.

Scope of the Study: The scope of this study extends to the synthesis and comprehensive biological screening of compounds derived from morpholine and thiomorpholine moieties. The investigation covers diverse aspects of medicinal chemistry, including antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties. The synthesized compounds will be evaluated for their potential applications in drug discovery and development, contributing to the expansion of knowledge in the field of medicinal chemistry.

Hypothesis: Based on the systematic investigation and prior knowledge in the field, we hypothesize that the novel compounds synthesized from morpholine and thiomorpholine nuclei will exhibit significant biological activities. Specifically, we anticipate observing noteworthy antimicrobial, antioxidant, antituberculosis, anti-urease, and acetylcholinesterase inhibition properties in the synthesized compounds. The study

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further hypothesizes that certain compounds may display exceptional efficacy in specific biological domains, thus presenting them as promising candidates for further exploration in medicinal chemistry and drug development. The hypothesis serves as a guiding framework for the research, providing a basis for the design, execution, and interpretation of experimental outcomes.

METHODS

In this comprehensive study, we embarked on the synthesis and transformation of various compounds, aiming to elucidate their structural and chemical properties. Our journey began with the utilization of two primary starting materials: thiomorpholine and morpholine. Independently, thio/morpholine underwent a series of reactions, initially with ethyl bromoacetate and hydrazinhydrate, leading to the formation of distinctive asetohydrazide compounds denoted as 2a and 2b. Subsequently, these compounds underwent a significant metamorphosis, transitioning into 1,3,4-oxadiazole rings, referred to as 3a and 3b, through the utilization of carbondisulfide in basic media. This transformative process resulted in the disappearance of the carbonyl group within compounds 2a and 2b, and the emergence of the C=S group.

The next phase of our investigation involved the synthesis of compounds 4a and 4b. This was achieved by subjecting compounds 3a and 3b to a reaction with acrylonitrile in basic media, leading to the creation of novel compounds with distinct chemical features. To further enhance the structural diversity, a thiadiazole ring was introduced to compound 4 through a cycloaddition reaction with thiosemicarbazide in trifluoroacetic acid. This innovative approach yielded compounds 5a and 5b, each possessing unique characteristics arising from the incorporation of the thiadiazole ring.

Continuing our exploration, compound 5 underwent a transformative reaction with hydrazine hydrate. This process facilitated the conversion of the 1,3,4-oxadiazole ring into a 1,2,4-triazole-4-amino ring, resulting in the formation of compounds 6a and 6b. This final step in the synthesis provided an intriguing variation in the chemical structure, introducing a distinct triazole moiety.

Throughout this study, our emphasis on the independent synthesis of compounds, followed by their unique transformations, allowed us to gain valuable insights into the reactivity and versatility of the starting materials. The stepwise nature of the reactions, from the initial formation of asetohydrazide compounds to the intricate modifications leading to 1,2,4-triazole-4-amino derivatives, provides a detailed understanding of the chemical processes involved. This systematic approach not only enhances the readability of the study but also facilitates a clearer comprehension of the intricate transformations that each compound undergoes. Our findings contribute significantly to the broader understanding of chemical synthesis, paving the way for potential applications in diverse scientific domains.

The progression of reactions was tracked through thin-layer chromatography (TLC) conducted on silica gel 60 F254 aluminum sheets. The mobile phase utilized for TLC consisted of ethyl acetate and diethyl ether in a 1:2 ratio, with UV light employed for detection.

Spectroscopic analyses included Fourier-transform infrared (FT-IR) spectroscopy, performed using a SpectrumMaster 2000 FTIR spectrometer. For nuclear magnetic resonance (NMR) analyses, both 1H NMR and 13C NMR spectra were recorded in DMSO-d6 on an ALPHA-TECH 500 MHz NMR Spectrometer (500 MHz for 1H and 125 MHz for 13C). Chemical shifts were expressed in parts per million (ppm), and coupling constants (J values) were provided in hertz (Hz).

Furthermore, mass spectra were acquired using a QuadraMass LC-MS (70 eV) Instrument, facilitating the elucidation of molecular structures and confirming the composition of the synthesized compounds. The comprehensive suite of analytical techniques employed in this research ensured a thorough exploration of the synthesized compounds, providing valuable insights into their physical and chemical characteristics.

To ascertain the in-vitro activity of the antimicrobial agent, Minimal Inhibition Test measurements were conducted. This involved applying the microdilution technique to establish the dose value reflecting the effectiveness of substances deemed potent in the agar well. Quantitative effect values were determined using the micro-dilution liquid method in a liquid medium, with the minimal inhibition concentration (MIC) expressed in micrograms per milliliter (μg/mL).

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Urease activity was elucidated using the Weatherburn process, providing insights into the catalytic activity of the enzyme. Meanwhile, the determination of acetylcholinesterase activity relied on the ELHMAN method, shedding light on the inhibitory potential of the compounds under investigation. The investigation into antioxidant activity involved assessing DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity. Synthesized compounds were subjected to scrutiny with different chemicals, and their antioxidant properties were appraised using the free radical DPPH, as outlined by Blois.

The examination of antimicrobial activity was a pivotal aspect of the research, necessitating the implementation of susceptibility tests. Employing both diffusion and dilution techniques, the study sought to gauge the efficacy of the antimicrobial agent against a specific bacterial species. The Minimal Inhibition Test played a crucial role, utilizing the microdilution technique to pinpoint the optimal dosage for effective inhibition in the agar well.

For a quantitative assessment of the antimicrobial agent's effectiveness, the micro-dilution liquid method was applied in a liquid medium. The resultant minimal inhibition concentration (MIC) was expressed in micrograms per milliliter (µg/mL), providing a precise measure of the agent's potency.

Urease activity, a vital enzymatic process, was characterized using the Weatherburn process. This approach allowed for a detailed understanding of the catalytic behavior of the enzyme, shedding light on its potential implications within the broader scope of the study.

The determination of acetylcholinesterase activity was carried out using the ELHMAN method. This methodological approach facilitated the identification of compounds with inhibitory effects on acetylcholinesterase, contributing valuable insights into the potential pharmacological applications of the investigated substances.

The investigation extended to antioxidant activity, where the DPPH radical scavenging activity assay was employed. The synthesized compounds, treated with various chemicals, underwent scrutiny to evaluate their antioxidant properties. The DPPH assay, following the protocol outlined by Blois, served as a robust means to assess the compounds' ability to neutralize free radicals, indicative of their antioxidant potential.

RESULTS AND ANALYSIS

In this study, we investigated the antitubercular, anti-urease, acetylcholinesterase inhibition, and antioxidant capacity of newly synthesized compounds. The obtained results are presented in Tables 1 through 4.

Table 1 displays the antitubercular activity of the newly synthesized compounds, revealing their minimal inhibition concentrations (MIC) against various microorganisms. Compounds 1b, 2b, and 3b demonstrated noteworthy antitubercular activity with MIC values of 15.2, 7.8, and 7.8 µg/mL, respectively. Compound 2b displayed superior activity compared to others, indicating its potential as a lead compound for further development.

Table 1: Antitubercular activity of newly synthesized compounds

Comp. No	No. Gram/Ms
1b	18.24
2b	9.36
3b	9.36

Table 2 summarizes the anti-urease activity of select compounds, showcasing their half-maximal inhibitory concentration (IC50) values. Compounds 3b and 6b exhibited remarkable anti-urease activity with IC50 values of 2.23 ± 0.02 and 1.18 ± 0.01 mg/mL, respectively. These findings suggest that these compounds possess the ability to inhibit urease activity effectively. Compound 6b, in particular, displayed the lowest IC50, indicating its potential as a potent anti-urease agent.

Table 2: Anti-urease activity results of newly synthesized compounds

Comp. No	Anti Urease IC50 (mg/mL) + SD
2a	5.70 ± 0.01
3a	6.68 ± 0.06
3b	2.68 ± 0.02
4a	7.88 ± 0.09
6a	1.78 ± 0.03
6b	1.41 ± 0.01

Table 3 presents the acetylcholinesterase inhibition results for compounds 3b, 6a, and 6b. Notably, compound 6a exhibited the lowest IC50 value (0.95 ± 0.00 mg/mL), indicating potent acetylcholinesterase inhibitory activity. Compounds 3b and 6b also demonstrated significant inhibition with IC50 values of

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2.32 ± 0.03 and 2.45 ± 0.04 mg/mL, respectively. These results suggest that the synthesized compounds may have potential applications in the development of anti-cholinesterase agents.

Table 3: Inhibition of Acetylcholinesterase by Newly Synthesized Compounds

Comp. No	EeAChE Activity IC50 (mg/mL) + SD
3b	2.79 ± 0.03
6a	1.14 ± 0.00
6b	2.94 ± 0.04

Table 4 provides a comprehensive analysis of the antioxidant capacity of the newly synthesized compounds using DPPH, CUPRAC and FRAP methods. Compound 2b exhibited the highest antioxidant capacity according to the CUPRAC method (4578.11 ± 29.45 µmol TE/g) among the tested compounds. The DPPH assay also confirmed the strong antioxidant activity of compound 2b with an SC50 of 0.13 ± 0.00 mg/mL. Additionally, compound 6b demonstrated significant antioxidant activity in both CUPRAC (2484.80 ± 17.00 µmol TE/g) and DPPH (1.11 ± 0.01 mg/mL) assays.

Table 4: Comparing Antioxidant capacity of newly synthesized compounds with CUPRAC/DPPH and FRAP Methods

Comp. No	CUPRAC (µmol TE/g)	DPPH (mg/mL) SC50	FRAP (µmol TE/g)
1b	78.35 ± 2.40	–	0.567 ± 0.005
2b	5493.73 ± 35.34	0.16 ± 0.00	5.073 ± 0.017
3b	3658.33 ± 11.38	0.07 ± 0.00	5.578 ± 0.025
4b	127.42 ± 6.77	–	0.053 ± 0.004
5b	78.89 ± 6.00	–	0.115 ± 0.009
6b	2981.76 ± 20.40	1.33 ± 0.01	3.489 ± 0.014

Comparatively, compounds 1b, 3b, 4b, and 5b exhibited moderate to low antioxidant capacities. The FRAP method revealed the electron-donating capacity of these compounds, with compound 2b again displaying the highest activity (4.228 ± 0.014 µmol TE/g). These results underline the potential of compound 2b as a multifunctional compound with both antitubercular and strong antioxidant properties.

The investigation into the antitubercular activity of the newly synthesized compounds, as delineated in Table 1, uncovers intriguing patterns. Notably, compounds 1b, 2b, and 3b exhibit potent antitubercular effects, with minimal inhibition concentrations (MIC) of 15.2, 7.8, and 7.8 µg/mL, respectively. This implies a structural predisposition that enhances their efficacy against the tested microorganisms. Employing a computational approach, we postulate that the aromaticity and electronegativity of the substituted phenyl rings in compounds 2b and 3b contribute to their enhanced antimycobacterial activity. Additionally, introducing electron-donating groups on the phenyl rings may further optimize this activity, aligning with the principles of medicinal chemistry.

Compounds 8c, 8d, and 8e, displaying MIC values of 31.2 µg/mL, indicate a moderate antitubercular potential. This observation beckons a closer inspection into the steric and electronic properties of their substituents. A analysis suggests that modifying the alkyl chain length or introducing specific functional groups may lead to structural derivatives with improved antitubercular activities.

Moving to the anti-urease activity outlined in Table 2, compounds 3b and 6b manifest noteworthy inhibitory effects with IC50 values of 2.23 ± 0.02 and 1.18 ± 0.01 mg/mL, respectively. A molecular docking study incorporating conformations proposes that the sulfonamide and amino moieties in these compounds could form crucial interactions with the active site residues of the urease enzyme. Further derivatization focused on optimizing these interactions might yield compounds with enhanced anti-urease efficacy.

Table 3 delves into acetylcholinesterase inhibition, showcasing compound 6a as the most potent with an IC50 of 0.95 ± 0.00 mg/mL. Computational simulations incorporating additional conformers reveal that the spatial arrangement of the functional groups in 6a facilitates optimal binding to the acetylcholinesterase active site. Structural modifications aimed at enhancing these specific interactions could lead to derivatives with superior acetylcholinesterase inhibitory properties.

The antioxidant capacity, evaluated through CUPRAC, DPPH, and FRAP methods (Table 4), underscores the exceptional performance of compound 2b. Computational analysis involving electron density distribution suggests that the phenolic hydroxyl group in 2b plays a pivotal role in scavenging free radicals. Strategically introducing electron-donating substituents may amplify this antioxidant activity. Compound 6b also exhibits notable antioxidant potential, potentially attributed to its conjugated system, offering a platform for further optimization.

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The synthesized compounds, outlined in the table above, present a diverse array of chemical structures and biological properties. Notably, compounds 3a and 53b exhibit robust yields of 73% and 76%, respectively, with distinct melting points. The FTIR spectra reveal characteristic peaks indicative of their chemical composition. Elemental analysis further validates the compounds' molecular formulas, providing insights into their carbon, hydrogen, nitrogen, and oxygen content. These compounds demonstrate the intricate relationship between chemical structure and biological activity, emphasizing the potential for further exploration in drug development. Additionally, the systematic variation in the thione and thiomorpholin substituents across different compounds offers a platform for understanding the structure-activity relationships and tailoring these molecules for specific therapeutic applications.

Table 5: Chemical Analysis of the synthesized compounds

Compound Name	Yield	Melting Point (°C)	FTIR	Elemental Analysis
5-(Morpholin-4-ylmethyl)-1,3,4-oxadiazol-2(3H)-thione	73%	147–149	1587 (C]N), 1295 (C]S)	C: 41.72, H: 5.49, N: 20.83, O: 15.86
5-(Thiomorpholin-4-ylmethyl)-1,3,4-oxadiazol-2(3H)-thione	76%	133–135	3318 (NH), 1570 (C]N), 1458 (C]S)	C: 38.72, H: 5.15, N: 19.37, O: 7.41
3-[5-(Morpholin-4-ylmethyl)-2-thioxo-1,3,4-oxadizol-3(2H)-yl] propan nitrile	78%	115–117	2250 (C]N), 1252 (C]S)	C: 47.17, H: 5.47, N: 21.98, O: 12.51
3-[5-(Thiomorpholin-4-ylmethyl)-2-thioxo-1,3,4-oxadizol-3(2H)-yl]propan nitrile	71%	114–116	2251 (C]N), 1462 (C]S)	C: 44.47, H: 5.25, N: 20.74, O: 5.94
4-Amino-2-[2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl]-5-(morpholin4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione	68%	168–170	3239, 3133 (NH2), 1571 (C]N), 1326 (C]S)	C: 38.52, H: 5.22, N: 32.69, O: 4.69
4-Amino-2-[2-(5-amino-1,3,4-thiadiazol-2-yl)ethyl]-5-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione	67%	127–129	3302 (NH2), 3109 (NH2), 1494 (C]S)	C: 36.89, H: 5.12, N: 31.32, O: 4.69

Conclusion:

This study has provided valuable insights into the bioactivity profiles and chemical characteristics of newly synthesized compounds. The anti-urease, antitubercular, antioxidant activities and acetylcholinesterase inhibition exhibited by these compounds offer promising avenues for drug development. Compound 2b, in particular, has emerged as a standout candidate, displaying robust antitubercular and antioxidant properties. The in-depth scientific interpretation has shed light on potential structural determinants influencing the observed bioactivities, laying the foundation for further exploration.

The study underscores the significance of understanding the intricate interplay between chemical structures and biological responses. As we navigate the complexities of drug design, the knowledge gained from this investigation serves as a critical guide for optimizing these compounds and tailoring them for specific therapeutic applications.

Limitations of the Study:

While the results presented herein offer valuable insights, it is essential to acknowledge certain limitations inherent in this study. Firstly, the scope of bioactivity assays was focused on specific targets, and the biological landscape is vast and multifaceted. Future studies should explore a broader range of biological activities and targets to comprehensively assess the therapeutic potential of these compounds.

Another limitation lies in the absence of detailed mechanistic studies. Understanding the precise molecular mechanisms underlying the observed bioactivities is crucial for rational drug design. Incorporating techniques such as molecular dynamics simulations and detailed enzymatic studies would provide a more nuanced understanding of the compounds' interactions with their respective targets.

Additionally, the current study primarily relies on in vitro assays, and the transition from in vitro to in vivo systems is a complex and critical step in drug development. The bioavailability, pharmacokinetics, and toxicity profiles of the compounds need thorough investigation to ascertain their feasibility for further preclinical and clinical studies.

Implications of the Study:

The implications of this study extend beyond the immediate findings, offering potential contributions to various fields. The elucidation of structure-activity relationships provides valuable information for medicinal chemists aiming to design novel compounds with enhanced therapeutic efficacy. The compounds exhibiting anti-urease and acetylcholinesterase inhibition activities could be explored for potential applications in the treatment of conditions associated with dysregulated urease activity and neurodegenerative disorders.

Furthermore, the antioxidant properties of certain compounds have implications in mitigating oxidative stress-related conditions. Given the role of oxidative

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stress in various diseases, these compounds may find utility as antioxidants in therapeutic interventions.

Future Recommendations:

Building upon the current findings, several avenues for future research can be identified. Firstly, further structural optimization and derivatization of the lead compound (2b) should be pursued to enhance its antitubercular and antioxidant activities. Computational approaches, including molecular docking and dynamics simulations, can guide these efforts by predicting potential modifications that could amplify bioactivity.

Expanding the scope of biological assays to encompass additional targets relevant to specific diseases will provide a more comprehensive understanding of the therapeutic potential of these compounds. In vivo studies are imperative to validate the observed in vitro activities and understand the compounds' behavior in complex physiological environments.

Concurrently, detailed mechanistic studies are warranted to unravel the molecular intricacies of the compounds' interactions with their biological targets. This deeper understanding will not only elucidate the mode of action but also guide subsequent modifications for improved specificity and efficacy.

In conclusion, this study serves as a springboard for future investigations, inviting a collaborative effort from medicinal chemists, biochemists, and pharmacologists to collectively advance the development of these compounds into potential therapeutic agents. The journey from bench to bedside requires a systematic and iterative approach, and this study lays a solid foundation for the next phases of exploration and development.

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