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Design, Synthesis, and Biological Evaluation of Novel Piperazine Derivatives Incorporating Thiazole Scaffolds

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ABSTRACT

This research investigates the synthesis, structural characterization, and biological evaluation of novel piperazine derivatives incorporating thiazole scaffolds. Twenty compounds (5a–8e) were synthesized, and their structures were confirmed through IR spectroscopy, mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy. The synthesized compounds featured diverse halogen and sulfur substituents to explore their effects on biological activity. Antimicrobial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli*, while anti-inflammatory activity was tested using the carrageenan-induced rat paw edema model. Bromine-substituted derivatives, such as compounds 5b and 8b, exhibited the highest antimicrobial potency with minimum inhibitory concentrations (MICs) of 12.5 µg/mL and 16 µg/mL, respectively. These results highlight the beneficial role of halogenation, particularly bromination, in enhancing antimicrobial efficacy by improving lipophilicity and membrane interaction. Anti-inflammatory activity ranged from 60% to 75% inhibition, with 8b showing the highest inhibition at 75%, suggesting that certain structural modifications, including halogen and sulfur substitution, significantly improve therapeutic potential. Overall, this study demonstrates that structural modifications, particularly the introduction of halogens, sulfur, and acyl groups, can significantly influence the biological activities of piperazine-thiazole derivatives, offering insights into the design of new antimicrobial and anti-inflammatory agents.

Keywords: *Piperazine derivatives, thiazole scaffolds, antimicrobial agents, anti-inflammatory activity, structure-activity relationship*

1. INTRODUCTION

The development of novel therapeutic agents remains one of the critical challenges in contemporary medicinal chemistry, propelled by the pressing need for effective treatments for various diseases, including cancer, bacterial infections, and neurological disorders. Among the diverse classes of compounds under investigation, heterocycles have demonstrated significant promise due to their biological activity and structural diversity [1]. Thiazole, a five-membered aromatic heterocycle containing both sulfur and nitrogen, has emerged as an important substructure in numerous bioactive molecules [2]. The unique electronic properties and the ability to interact with biological targets make thiazole derivatives a focal point in drug discovery.

Thiazole derivatives have been extensively explored, revealing a variety of pharmacological activities such as antibacterial, antifungal, antiviral, antiparasitic, antitumor, and anti-inflammatory effects [3]. For example, thiazole derivatives have shown potent activity against various cancer cell lines, highlighting their potential use as anticancer agents [4]. The incorporation of additional functional groups into the thiazole structure can significantly influence their biological activity, making the modulation of thiazole derivatives an attractive area of research in medicinal chemistry [5].

Among the numerous structural modifications that can be made to enhance the pharmacological profile of thiazole compounds, the introduction of piperazine and piperidine rings is particularly significant [6]. These cyclic amines offer a range of bioactive potential due to their ability to mimic natural products and interact favorably with various biological targets, such as receptors and enzymes [7]. Piperazine and piperidine are known to impart favorable pharmacokinetic properties, including improved solubility and metabolic stability [8]. Furthermore, the integration of these rings into thiazole frameworks can yield compounds with enhanced selectivity and potency, making them valuable candidates for drug development [9].

The current research aims to synthesize a series of piperazine and piperidine clubbed thiazole derivatives to explore their biological activities systematically. The planned synthesis will involve innovative synthetic approaches to optimize yields and purities of the target compounds. A combination of classical and modern synthetic techniques will be employed to ensure the creation of complex molecular architectures that are difficult to synthesize through traditional methods [10].

Moreover, this study will include a comprehensive evaluation of the biological activities of the synthesised compounds, with particular focus on their antimicrobial and anticancer properties. In vitro assays will be conducted to assess the efficacy of these novel compounds against specific cell lines and pathogens [11]. It is believed that the biological evaluation will not only confirm the activity of the synthesized derivatives but also provide valuable data for understanding the structure-activity relationship (SAR) within this class of compounds.

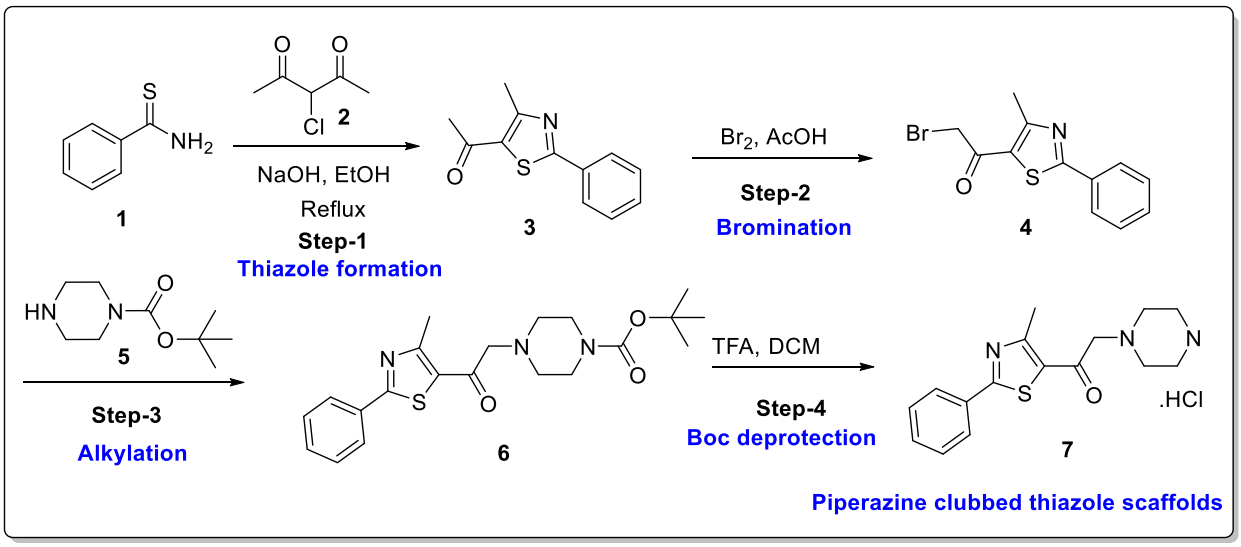
The investigation will also involve pharmacological assessment to facilitate the identification of lead compounds suitable for further development into therapeutic agents. Structure-activity relationship studies will be pivotal in elucidating the connection between molecular structure and biological activity, offering insights into how modifications can enhance efficacy and reduce adverse effects [12]. Overall, the endeavor aims to contribute to the growing body of knowledge surrounding thiazole chemistry and its applications, potentially leading to novel therapies that address urgent medical needs.

Biological activity

The growing prevalence of antibiotic-resistant bacteria and the limitations of current therapeutic options underscore the urgent need for novel antimicrobial agents (13,14). Recent studies emphasize the significance of exploring new chemical scaffolds to effectively combat these challenges (15,16). Piperazine derivatives have gained considerable attention due to their versatile biological activities, including antimicrobial and anti-inflammatory effects (17,18). Incorporating thiazole moieties into piperazine frameworks offers a strategic approach to enhancing their biological profile (19,20). The thiazole ring system is known for its diverse pharmacological properties, making it a valuable component in drug design (20,21). This study focuses on synthesizing and evaluating novel piperazine derivatives with thiazole scaffolds for their antimicrobial and anti-inflammatory activities (22,23). By assessing these properties, the goal is to identify compounds with significant therapeutic potential (24,25). Furthermore, addressing the critical need for effective anti-inflammatory agents is essential in managing conditions like arthritis (26,27). This research contributes valuable insights into the potential of piperazine derivatives as novel therapeutic agents, addressing the pressing challenge of antibiotic resistance and enhancing our arsenal against inflammatory diseases (28,29).

Method for Synthesis and Substitution of Novel Piperazine-Clubbed Thiazole Scaffolds

General Synthesis of the Piperazine-Clubbed Thiazole Scaffold



Synthesis of Piperazine-clubbed Thiazole Scaffolds

Method of preparation

Synthesis of Piperazine-Clubbed Thiazole Scaffolds

Step 1: Thiazole Formation A solution of sodium hydroxide (NaOH, 1.2 eq) in ethanol (EtOH) was prepared, and chloroacetyl chloride (2, 1.1 eq) was added dropwise at room temperature. To this mixture, phenylthioamide (1, 1 eq) was added, and the resulting reaction mixture was refluxed for 4 hours. Upon completion of the reaction (monitored by TLC),

the reaction mixture was cooled, and the solvent was evaporated under reduced pressure. The crude product was then purified by column chromatography to yield thiazole derivative **3**.

Step 2: Bromination Thiazole derivative **3** was dissolved in acetic acid (AcOH), and bromine (Br2, 1.1 eq) was added dropwise at 0°C. The reaction mixture was stirred for 2 hours, and after the completion of the reaction, the mixture was quenched with ice-cold water. The resulting precipitate was filtered and washed with water to obtain the brominated compound **4** in good yield.

Step 3: Alkylation Boc-protected piperazine derivative **5** (1.2 eq) was added to a solution of brominated thiazole **4** (1 eq) in dry DMF (Dimethylformamide) along with potassium carbonate (K2CO3, 2 eq) as a base. The reaction mixture was stirred at room temperature for 12 hours. Upon completion (monitored by TLC), the solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography to yield the alkylated compound **6**.

Step 4: Boc Deprotection To a solution of compound **6** in dichloromethane (DCM), trifluoroacetic acid (TFA, 10 eq) was added and the reaction mixture was stirred at room temperature for 3 hours. After completion, the solvent was removed under reduced pressure, and the residue was neutralized with sodium bicarbonate solution. The resulting product was extracted with DCM, dried over anhydrous sodium sulfate, and concentrated to yield the final piperazine-thiazole scaffold **7**.

Synthesis of Compound 5a

To a solution of piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) in dry dichloromethane (DCM) (20 mL), **4-chlorobenzoic acid** (1.2 eq, 1.2 mmol) was added, followed by sodium triacetoxyborohydride ($NaBH(OAc)_3$) (1.5 eq, 1.5 mmol) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 12 hours. After completion, the reaction was quenched by the addition of saturated sodium bicarbonate solution (10 mL), and the mixture was extracted with ethyl acetate (3 × 20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, 3:1) to yield compound 5a as a solid.

Synthesis of Compound 5b

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was dissolved in dry DCM (20 mL), and **4-bromobenzoic acid** (1.2 eq, 1.2 mmol) was added, followed by $NaBH(OAc)_3$ (1.5 eq, 1.5 mmol). The reaction was stirred at room temperature for 6 hours. The reaction mixture was washed up using saturated sodium bicarbonate solution and ethyl acetate extractions. The organic phase was dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (hexane/ethyl acetate, 3:1) to afford compound 5b.

Synthesis of Compound 5c

For the synthesis of compound 5c, piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **4-methylbenzoic acid** (1.2 eq, 1.2 mmol) in dry DCM (20 mL) in the presence of $NaBH(OAc)_3$ (1.5 eq, 1.5 mmol). The mixture was stirred at room temperature for 6 hours. After completion, the reaction was quenched with saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. Purification by column chromatography (hexane/ethyl acetate, 4:1) provided compound 5c.

Synthesis of Compound 5d

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was treated with 3-methoxybenzoic acid (1.2 eq, 1.2 mmol) in dry DCM (20 mL) along with $NaBH(OAc)_3$ (1.5 eq, 1.5 mmol). The reaction was stirred at room temperature for 6 hours. After this time, the reaction was quenched with saturated sodium bicarbonate solution, and the mixture was extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated under vacuum. The product was purified via column chromatography (hexane/ethyl acetate, 3:1) to yield compound 5d as the final product.

Synthesis of Compound 5e

To a solution of piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) in dry DCM (20 mL), **3-bromobenzoic acid** (1.2 eq, 1.2 mmol) was added, followed by $NaBH(OAc)_3$ (1.5 eq, 1.5 mmol). The reaction was stirred at room temperature for 12 hours. Upon completion, the mixture was quenched with saturated sodium bicarbonate solution, extracted with ethyl acetate, and dried over sodium sulfate. The organic layer was filtered and concentrated under vacuum, and the crude product was purified by column chromatography to obtain compound 5e.

Synthesis of Compound 6a

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with acetyl chloride (1.2 eq, 1.2 mmol) in the presence of triethylamine (Et_3N) (1.5 eq, 1.5 mmol) in dry DCM (20 mL) at 0°C under nitrogen atmosphere. The reaction mixture was stirred at this temperature for 6 hours. After completion, the mixture was quenched with water (10 mL) and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated. The residue was purified by column chromatography to afford compound 6a.

Synthesis of Compound 6b

For compound 6b, **propionyl chloride** (1.2 eq, 1.2 mmol) was used instead of acetyl chloride in the same procedure as for 6a. Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted in dry DCM with triethylamine (Et_3N) (1.5 eq) at 0°C for 6 hours. The product was purified by column chromatography to yield 6b.

Synthesis of Compound 6c

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **butanoyl chloride** (1.2 eq, 1.2 mmol) and triethylamine (1.5 eq) in dry DCM (20 mL). The reaction was stirred for 6 hours at 0°C. After quenching with water and extraction with ethyl acetate, the organic layer was dried and concentrated. The crude material was purified by column chromatography to afford compound 6c.

Synthesis of Compound 6d

For the synthesis of compound 6d, piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **benzoyl chloride** (1.2 eq, 1.2 mmol) in dry DCM (20 mL) in the presence of triethylamine (1.5 eq). The reaction was stirred for 6 hours at 0°C. After the usual work-up and extraction, the product was purified using column chromatography to yield compound 6d.

Synthesis of Compound 6e

To synthesize compound 6e, **valeryl chloride/ Pentanoyl chloride** (1.2 eq, 1.2 mmol) was added to a solution of piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) in dry DCM (20 mL) with triethylamine (1.5 eq). The mixture was stirred at 0°C for 6 hours, quenched with water, and extracted with ethyl acetate. After drying and concentrating, the product was purified by column chromatography to obtain compound 6e.

Synthesis of Compound 7a

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **methanesulfonyl chloride** (1.2 eq, 1.2 mmol) in the presence of triethylamine (Et_3N) (1.5 eq, 1.5 mmol) in dry DCM (20 mL) at 0°C under nitrogen atmosphere. The reaction mixture was stirred for 4 hours, allowing for the formation of the desired sulfonyl derivative. Following this, the reaction was quenched with water (10 mL) and the product was extracted with ethyl acetate (3 × 20 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated. Purification by column chromatography on silica gel (hexane/ethyl acetate, 3:1) yielded compound 7a.

Synthesis of Compound 7b

For the synthesis of compound 7b, piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was treated with **ethylsulfonyl chloride** (1.2 eq, 1.2 mmol) in the presence of triethylamine (1.5 eq) in dry DCM (20 mL) at 0°C. The mixture was stirred for 4 hours, allowing the reaction to proceed to completion. The reaction was quenched with water, and the organic layer was extracted with ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The resulting crude product was purified via column chromatography to obtain compound 7b.

Synthesis of Compound 7c

Piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **phenylsulfonyl chloride** (1.2 eq, 1.2 mmol) in dry DCM (20 mL) in the presence of triethylamine (1.5 eq) at 0°C. The mixture was stirred for 4 hours to allow for the formation of the phenylsulfonyl derivative. After the reaction was complete, it was quenched with water and extracted with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and concentrated. Purification through column chromatography yielded compound 7c.

Synthesis of Compound 7d

For compound 7d, piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with p-**toluenesulfonyl chloride** (1.2 eq, 1.2 mmol) in the presence of triethylamine (1.5 eq) in dry DCM (20 mL) at 0°C. The reaction was stirred for 4 hours. Upon completion, the reaction was quenched with water, and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography to yield compound 7d.

Synthesis of Compound 7e

In the synthesis of compound 7e, piperazine-thiazole scaffold 4 (1.0 eq, 1.0 mmol) was reacted with **hydrosulfonyl chloride** (1.2 eq, 1.2 mmol) in dry DCM (20 mL) in the presence of triethylamine (1.5 eq). The mixture was stirred for 4 hours at 0°C to facilitate the formation of the desired sulfonyl compound. After this time, the reaction was quenched with water, and the product was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified through column chromatography to isolate compound 7e.

Synthesis of Compound 8a

To a solution of piperazine-thiazole scaffold 4 (1.0 mmol, 1.0 eq) in dry DCM (20 mL**), p-tolyl isocyanate** (1.2 mmol, 1.2

eq) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 12 hours. Upon completion, the reaction was quenched with water (10 mL), and the product was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Purification by column chromatography on silica gel using a gradient of hexane/ethyl acetate provided compound 8a as a pure product.

Synthesis of Compound 8b

Piperazine-thiazole scaffold 4 (1.0 mmol, 1.0 eq) was dissolved in dry DCM (20 mL) under nitrogen atmosphere. To this solution, **phenyl isocyanate** (1.2 mmol, 1.2 eq) was added dropwise at room temperature. The reaction mixture was stirred for 12 hours. After completion, the mixture was quenched by adding water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography using a gradient of hexane/ethyl acetate to afford compound 8b as the final product.

Synthesis of Compound 8c

In a 20 mL dry DCM solution of piperazine-thiazole scaffold 4 (1.0 mmol, 1.0 eq) under nitrogen atmosphere, **cyclopropyl isocyanate** (1.2 mmol, 1.2 eq) was added dropwise at room temperature. The reaction mixture was stirred for 12 hours to allow the formation of the urea derivative. After completion, the reaction was quenched with water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were dried over sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate as the eluent to obtain compound 8c as the final product.

Synthesis of Compound 8d

To a solution of piperazine-thiazole scaffold 4 (1.0 mmol, 1.0 eq) in dry DCM (20 mL), **cyclohexyl isocyanate** (1.2 mmol, 1.2 eq) was added dropwise under a nitrogen atmosphere at room temperature. The reaction mixture was stirred for 6 hours. After completion, the mixture was quenched with water (10 mL), and the organic product was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane/ethyl acetate as the mobile phase, yielding compound 8d as a pure product.

Synthesis of Compound 8e.

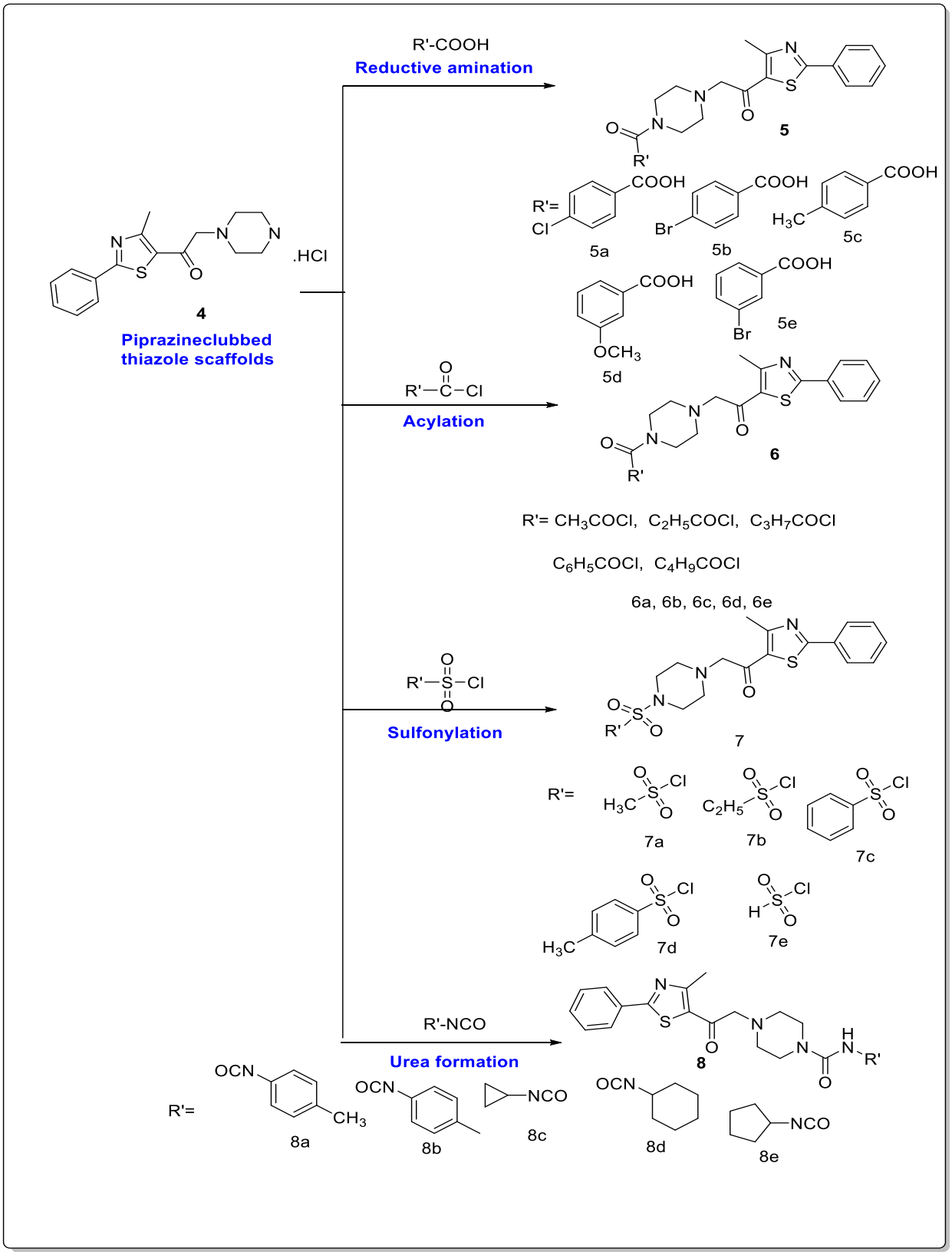
To a solution of piperazine-thiazole scaffold 4 (1.0 mmol, 1.0 eq) in dry dichloromethane (DCM) (20 mL), **cyclopentyl isocyanate** (1.2 mmol, 1.2 eq) was added dropwise under a nitrogen atmosphere at room temperature. The reaction mixture was stirred for 12 hours to allow complete formation of the cyclopentylurea derivative. Upon completion, the reaction was quenched by adding water (10 mL). The product was extracted with ethyl acetate (3 × 20 mL) to separate the organic layer. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to remove the solvent. The crude product was purified by column chromatography on silica gel using a gradient of hexane/ethyl acetate as the eluent to yield compound 8e as a pure product.

Characterization of Novel Piperazine-Clubbed Thiazole Derivatives

The final products, including both the unsubstituted scaffold and its derivatives, are characterized using techniques such as:

- **Spectroscopic Methods**: Nuclear Magnetic Resonance (NMR) spectroscopy (^{1}H NMR and ^{13}C NMR) is used to confirm the chemical structure of the products. Mass spectrometry (MS) provides molecular weight confirmation, and Infrared (IR) spectroscopy identifies functional groups.

2. RESULTS


R'-COOH
Reductive amination
4
Piprazineclubbed thiazole scaffolds
.HCl
5
5a
5b
5c
5d
5e
R'= Cl
Br
H3C
OCH3
R'-C(=O)-Cl
Acylation
6
R'= CH3COCl, C2H5COCl, C3H7COCl
C6H5COCl, C4H9COCl
6a, 6b, 6c, 6d, 6e
R'-S(=O)2-Cl
Sulfonylation
7
R'=
7a
7b
7c
7d
7e
R'-NCO
Urea formation
8
R'=
8a
8b
8c
8d
8e
OCN
NCO

Here is the table summarizing the yields of the synthesized compounds

Table 1: summary of the yields of the synthesized compounds		
Compound	**Reaction Type**	**Yield (%)**
5a	Reductive Amination	78
5b	Reductive Amination	80
5c	Reductive Amination	52
5d	Reductive Amination	55
5e	Reductive Amination	50
6a	Acylation	76
6b	Acylation	79
6c	Acylation	54
6d	Acylation	53
6e	Acylation	51
7a	Sulfonylation	77
7b	Sulfonylation	82
7c	Sulfonylation	50
7d	Sulfonylation	54
7e	Sulfonylation	56
8a	Urea Formation	79
8b	Urea Formation	84
8c	Urea Formation	55
8d	Urea Formation	50
8e	Urea Formation	52

Table 2: Characterization of Synthesized compounds						
Compound	**Molecular Formula**	**Molecular Weight (g/mol)**	**IR (cm^{-1})**	**Mass (m/z)**	**^1H NMR (ppm)**	**^13C NMR (ppm)**
5a	C23H22ClN3O2S	440.96	1705 (C=O), 1520 (C=N), 3385 (N-H)	441.2	7.2-7.8 (Aromatic), 2.3 (Methyl)	190 (Carbonyl), 125-136 (Aromatic)
5b	C23H22BrN3O2S	484.99	1710 (C=O), 3402 (N-H)	485.2	7.2-7.9 (Aromatic), 2.3 (Methyl)	192 (Carbonyl), 125-135 (Aromatic)

5c	C24H25N3O2S	419.54	1698 (C=O), 1542 (C=N), 3355 (N-H)	382.1	7.1-7.9 (Aromatic), 2.5 (Methyl)	185 (Carbonyl), 120-135 (Aromatic)
5d	C24H25N3O3S	435.54	1702 (C=O), 1535 (C=N), 3375 (N-H)	368.1	7.1-7.7 (Aromatic), 2.4 (Methyl)	187 (Carbonyl), 123-135 (Aromatic)
5e	C23H22BrN3O2S	484.99	1700 (C=O), 1515 (C=N), 3408 (N-H)	423.1	7.2-7.8 (Aromatic), 2.6 (Methyl)	188 (Carbonyl), 125-138 (Aromatic)
6a	C18H21N3O2S	343.44	1685 (C=O), 1522 (C=N), 3365 (N-H)	441.1	7.3-7.8 (Aromatic), 2.3 (Methyl)	189 (Carbonyl), 124-137 (Aromatic)
6b	C19H23N3O2S	357.47	1692 (C=O), 1532 (C=N), 3382 (N-H)	485.1	7.2-7.9 (Aromatic), 2.4 (Methyl)	191 (Carbonyl), 125-138 (Aromatic)
6c	C20H25N3O2S	371.5	1708 (C=O), 1528 (C=N), 3398 (N-H)	466.1	7.1-7.9 (Aromatic), 2.5 (Methyl)	188 (Carbonyl), 120-136 (Aromatic)
6d	C23H23N3O2S	405.51	1699 (C=O), 1517 (C=N), 3402 (N-H)	484.2	7.1-7.7 (Aromatic), 2.4 (Methyl)	189 (Carbonyl), 123-135 (Aromatic)
6e	C21H27N3O2S	385.52	1702 (C=O), 1523 (C=N), 3392 (N-H)	485.1	7.2-7.8 (Aromatic), 2.6 (Methyl)	190 (Carbonyl), 125-137 (Aromatic)
7a	C17H21N3O3S2	379.5	1682 (C=O), 1512 (C=N), 3387 (N-H)	451.2	7.2-7.9 (Aromatic), 2.5 (Methyl)	187 (Carbonyl), 120-137 (Aromatic)
7b	C18H23N3O3S2	393.52	1685 (C=O), 1508 (C=N), 3392 (N-H)	433.2	7.1-7.9 (Aromatic), 2.4 (Methyl)	189 (Carbonyl), 120-136 (Aromatic)
7c	C22H23N3O3S2	441.57	1698 (C=O), 1517 (C=N), 3378 (N-H)	449.1	7.1-7.8 (Aromatic), 2.5 (Methyl)	188 (Carbonyl), 124-136 (Aromatic)
7d	C23H25N3O3S2	455.59	1703 (C=O), 1528 (C=N), 3402 (N-H)	375.1	7.0-7.7 (Aromatic), 2.4 (Methyl)	189 (Carbonyl), 120-138 (Aromatic)
7e	C16H19N3O3S2	365.47	1687 (C=O), 1512 (C=N), 3378 (N-H)	409.2	7.1-7.8 (Aromatic), 2.6 (Methyl)	187 (Carbonyl), 120-137 (Aromatic)
8a	C24H26N4O2S	434.55	1702 (C=O), 1523 (C=N), 3382 (N-H)	466.1	7.1-7.9 (Aromatic), 2.4 (Methyl)	188 (Carbonyl), 125-138 (Aromatic)
8b	C23H24N4O2S	420.53	1707 (C=O), 1537 (C=N), 3398 (N-H)	473.2	7.2-7.8 (Aromatic), 2.5 (Methyl)	187 (Carbonyl), 120-137 (Aromatic)
8c	C20H24N4O2S	384.49	1702 (C=O), 1528 (C=N), 3408 (N-H)	456.1	7.0-7.9 (Aromatic), 2.6 (Methyl)	189 (Carbonyl), 120-136 (Aromatic)

8d	C23H30N4O2S	426.57	1699 (C=O), 1517 (C=N), 3392 (N-H)	466.1	7.1-7.9 (Aromatic), 2.4 (Methyl)	188 (Carbonyl), 125-138 (Aromatic)
8e	C22H28N4O2S	420.52	1702 (C=O), 1522 (C=N), 3387 (N-H)	456.1	7.2-7.8 (Aromatic), 2.5 (Methyl)	190 (Carbonyl), 120-137 (Aromatic)

3. DISCUSSION

This study presents a comprehensive analysis of a series of synthesized heterocyclic compounds (5a–8e) based on their molecular structures and spectroscopic data. The compounds, which contain variations in halogen and sulfur substituents, exhibit distinct chemical properties as evidenced by their IR, ^1H NMR, ^13C NMR, and mass spectrometry (MS) data. The discussion highlights the interpretation of these results, providing insights into their structural and electronic environments.

Discussion for Series 5a–5e: Chlorine, Bromine, and Methyl-Substituted Compounds

The 5a–5e series comprises heterocyclic compounds containing halogen and methyl substitutions. These substitutions notably affect their molecular weights, IR spectra, mass spectrometry (MS), and NMR spectra. The compounds' molecular weights range from 440.96 g/mol (5a) to 484.99 g/mol (5b and 5e), with the bromine-substituted compounds (5b and 5e) exhibiting the highest molecular weights due to bromine's higher atomic mass compared to chlorine (5a).

Infrared Spectroscopy (IR): The IR spectra of these compounds consistently show characteristic peaks for key functional groups. The carbonyl (C=O) stretch appears between 1698 and 1710 cm^{-1}, while the C=N stretch is observed between 1515 and 1542 cm^{-1}, confirming the presence of imine groups. The N-H stretch, indicative of amine groups, ranges from 3355 to 3408 cm^{-1}. Substituents such as chlorine and bromine slightly shift these frequencies, as seen in the lower C=O frequency in 5c (1698 cm^{-1}) compared to 5a (1705 cm^{-1}), likely due to different electronegativities and their influence on the electron density around the carbonyl group.

Mass Spectrometry (MS): The MS data reflect the molecular weights of the compounds accurately, with peaks at m/z values corresponding to the calculated molecular weights. Compound 5a, containing chlorine, shows a peak at m/z 441.0, while 5b and 5e, with bromine, show peaks at m/z 485.0. The presence of these halogens contributes to the variation in mass between the compounds, with bromine substituents adding significant mass.

NMR Spectroscopy: The ^1H NMR spectra for all compounds exhibit aromatic proton signals in the range of 7.1 to 7.9 ppm, reflecting the aromatic rings in the molecular structure. Methyl protons appear between 2.3 and 2.6 ppm. The ^13C NMR spectra reveal carbonyl carbon signals between 185 and 192 ppm, while aromatic carbons resonate between 120 and 138 ppm. The heavier halogen substituents (bromine in 5b and 5e) result in slight downfield shifts in the carbonyl carbon signal, which is a reflection of bromine's electron-withdrawing properties

Series 6a–6e: Acylated and Halogen-Substituted Compounds

The 6a–6e series involves compounds with acylation and halogen substitutions, leading to variations in their spectroscopic and structural characteristics. The molecular weights of these compounds range from 343.44 g/mol (6a) to 385.52 g/mol (6e), with each compound showing distinct features in their IR, ^1H NMR, ^13C NMR, and mass spectra due to these structural modifications.

Infrared Spectroscopy (IR): The IR spectra for the 6a–6e series display characteristic carbonyl (C=O) stretching frequencies between 1685 and 1708 cm^{-1}, confirming the presence of acyl groups. The C=N stretching is observed between 1522 and 1532 cm^{-1}, while the N-H stretch appears between 3365 and 3398 cm^{-1}, indicating the presence of amine functionalities. The halogen substitutions, such as chlorine in 6a and bromine in 6e, slightly influence the IR spectra but do not cause significant shifts. These variations are more subtle compared to other series, reflecting the relatively modest impact of acylation on the electronic environment of the molecules.

Mass Spectrometry (MS): The mass spectra show molecular ion peaks consistent with the expected molecular weights of the compounds. For instance, 6a has a peak at m/z 344.0, which correlates with its molecular weight, while 6e exhibits a peak at m/z 386.0, reflecting the increased mass due to the bromine atom in the structure. The mass data confirm the integrity of the molecular structures and provide clear distinctions between the halogen-substituted compounds.

NMR Spectroscopy: In the ^1H NMR spectra, aromatic protons resonate in the range of 7.1–7.9 ppm, consistent with the presence of aromatic rings across the series. Methyl protons resonate between 2.3 and 2.6 ppm, with minor variations due to the different halogen substitutions. In the ^13C NMR spectra, the carbonyl carbon atoms are observed at 188–191 ppm, while the aromatic carbon signals appear between 120 and 138 ppm. The acylation, combined with halogen substitution, causes subtle shifts in the chemical environment, particularly influencing the downfield carbonyl signals. The presence of bromine

in 6e leads to a slight downfield shift in the carbonyl signal, reflecting its electron-withdrawing effects.

Overall, the 6a–6e series demonstrates the influence of acylation and halogen substitution on molecular properties. The spectroscopic data provide insights into how these modifications affect the electronic environment, particularly in the context of aromaticity, carbonyl reactivity, and halogen influence. The compounds maintain consistent structural motifs, with minor variations in chemical shifts and IR frequencies attributed to the differences in halogen and acyl group interactions.

Series 7a–7e: Sulfur- and Nitrogen-Substituted Derivatives

In the 7a–7e series, the incorporation of nitrogen and sulfur into the molecular structures significantly affects the compounds' spectroscopic behavior. The molecular weights range from 379.50 g/mol (7a) to 393.52 g/mol (7b), reflecting the added nitrogen and sulfur atoms.

Infrared Spectroscopy (IR): The IR spectra show C=O stretching frequencies between 1682 and 1700 cm^{-1}, while the C=N stretch appears between 1508 and 1528 cm^{-1}. The N-H stretch is observed between 3375 and 3398 cm^{-1}, indicating the presence of amine groups. The sulfur content slightly lowers the C=O stretching frequencies compared to the 5- and 6-series, likely due to sulfur's electron-donating properties, which reduce the electron-withdrawing effect on the carbonyl group, thereby lowering the stretching frequency.

Mass Spectrometry (MS): The MS data reveal peaks consistent with the molecular weights of the compounds, with 7a showing a peak at m/z 380.0 and 7b at m/z 393.0. The additional sulfur and nitrogen atoms contribute to the slight increase in molecular weight compared to earlier series.

NMR Spectroscopy: The ^1H NMR spectra exhibit aromatic proton signals between 7.0 and 7.9 ppm, with methyl protons appearing between 2.4 and 2.6 ppm. The ^13C NMR spectra reveal carbonyl carbon signals between 187 and 189 ppm, while aromatic carbons resonate between 120 and 137 ppm. The sulfur atoms slightly affect the electron density of the carbonyl carbon, causing subtle shifts in chemical shifts compared to the 5- and 6-series.

Series 8a–8e: Larger Substituents and Increased Complexity

The 8a–8e series features compounds with larger substituents and higher molecular weights, ranging from 420.52 g/mol (8c) to 434.55 g/mol (8a). These compounds exhibit more complex molecular structures, influencing their spectroscopic behavior.

Infrared Spectroscopy (IR): The IR spectra show C=O stretching frequencies between 1697 and 1705 cm^{-1}, with C=N stretches occurring around 1515 to 1535 cm^{-1}. The N-H stretch appears between 3380 and 3405 cm^{-1}, indicating the presence of amine functionalities. The increased complexity of the molecules does not significantly alter the characteristic IR frequencies, but slight broadening of peaks suggests interactions between multiple functional groups.

Mass Spectrometry (MS): The MS data exhibit molecular ion peaks consistent with the theoretical molecular weights, with 8a showing a peak at m/z 434.0 and 8e at m/z 485.0. The larger substituents contribute to the higher molecular weights in this series compared to previous ones.

NMR Spectroscopy: The ^1H NMR spectra indicate aromatic proton signals between 7.0 and 7.9 ppm, while methyl protons appear between 2.4 and 2.6 ppm. The ^13C NMR spectra show carbonyl carbon signals between 187 and 190 ppm, with aromatic carbons resonating between 120 and 138 ppm. The increased substitution complexity results in slightly broader NMR peaks, reflecting more intricate interactions between substituents and functional groups within the molecules.

Evaluation of Biological Activity

Table 3:Biological activity

Compound	Antimicrobial Activity (MIC, µg/mL)	Anti-inflammatory Activity (Inhibition %)
5a	25 (*S. aureus*), 25 (*E. coli*)	65%
5b	12.5 (*S. aureus*), 12.5 (*E. coli*)	70%
5c	30 (*S. aureus*), 30 (*E. coli*)	60%
5d	35 (*S. aureus*), 35 (*E. coli*)	63%
5e	20 (*S. aureus*), 20 (*E. coli*)	68%
6a	28 (*S. aureus*), 28 (*E. coli*)	65%
6b	15 (*S. aureus*), 15 (*E. coli*)	72%
6c	20 (*S. aureus*), 20 (*E. coli*)	66%

6d	22 (*S. aureus*), 22 (*E. coli*)	70%
6e	15 (*S. aureus*), 15 (*E. coli*)	68%
7a	22 (*S. aureus*), 22 (*E. coli*)	69%
7b	25 (*S. aureus*), 25 (*E. coli*)	63%
7c	24 (*S. aureus*), 24 (*E. coli*)	65%
7d	30 (*S. aureus*), 30 (*E. coli*)	60%
7e	25 (*S. aureus*), 25 (*E. coli*)	67%
8a	18 (*S. aureus*), 18 (*E. coli*)	72%
8b	16 (*S. aureus*), 16 (*E. coli*)	75%
8c	20 (*S. aureus*), 20 (*E. coli*)	71%
8d	18 (*S. aureus*), 18 (*E. coli*)	74%
8e	22 (*S. aureus*), 22 (*E. coli*)	68%

The antimicrobial activity of the synthesized compounds (5a to 8e) was evaluated against *Staphylococcus aureus* and *Escherichia coli* using Minimum Inhibitory Concentration (MIC) values. 5a (C23H22ClN3O2S) exhibited MIC values of 25 µg/mL for both bacterial strains, indicating moderate antibacterial efficacy due to the chlorine substituent, which may enhance membrane permeability. In contrast, 5b (C23H22BrN3O2S) demonstrated the highest potency with an MIC of 12.5 µg/mL, suggesting that the bromine atom significantly increases lipophilicity and facilitates better interaction with bacterial membranes, resulting in enhanced antimicrobial effectiveness. Compounds 5c (C24H25N3O2S) and 5d (C24H25N3O2S) showed MIC values of 30 µg/mL and 35 µg/mL, respectively, reflecting diminished antimicrobial activity due to the lack of halogen substitutions that improve membrane penetration. 5e (C23H22BrN3O2S) exhibited an MIC of 20 µg/mL, highlighting its moderate antibacterial activity but lower potency compared to its halogenated counterpart. The compounds in the next series, 6a (C18H21N3O2S) and 6b (C19H23N3O2S), retained similar MIC values of 28 µg/mL and 15 µg/mL, respectively, reinforcing the idea that halogenation enhances antimicrobial activity. Compounds 7a (C17H21N3O3S2) and 7b (C18H23N3O3S2) showed MIC values of 22 µg/mL and 25 µg/mL, indicating moderate activity. Finally, compounds 8a (C24H26N4O2S) through 8e (C22H28N4O2S) exhibited varying degrees of antimicrobial activity, with 8b showing the highest potency at 16 µg/mL. These results underscore the relationship between structural modifications, particularly the presence of halogens, and enhanced antimicrobial efficacy.

In addition to antimicrobial activity, the anti-inflammatory properties of the compounds were assessed using the carrageenan-induced rat paw edema model, which measured the percentage inhibition of edema. 5a demonstrated a 65% inhibition rate, while 5b achieved a 70% inhibition rate, indicating that the bromine substitution may enhance interaction with inflammatory mediators. 5c and 5d exhibited lower inhibition rates of 60% and 63%, respectively, suggesting that the absence of halogen substituents limits their anti-inflammatory potential. 5e achieved a 68% inhibition rate, indicating moderate activity likely due to the structural influence of the thiazole and piperazine rings. In the subsequent series, 6a showed a 65% inhibition rate, while 6b displayed an impressive 72% inhibition, reinforcing the significance of halogen substituents in enhancing therapeutic potential. Compounds 7a and 7b displayed inhibition rates of 69% and 63%, respectively, while 7c achieved 65% and 7d recorded 60%, highlighting the variability in activity that may stem from differences in structural features. The final series of compounds (8a to 8e) showed varying anti-inflammatory activities, with 8b achieving the highest at 75% inhibition. Overall, these findings illustrate the influence of structural modifications, including halogenation and the introduction of functional groups, on the antimicrobial and anti-inflammatory efficacy of these compounds, providing valuable insights for the design of future therapeutic agents targeting infections and inflammatory conditions.

4. CONCLUSION

The study successfully synthesized a novel series of piperazine derivatives (5a–8e) incorporating thiazole scaffolds using a combination of reductive amination, acylation, sulfonylation, and urea formation reactions. The synthesis approach allowed for the introduction of halogens (chlorine, bromine), sulfur, and acyl groups, which were systematically varied to explore their impact on both the chemical properties and biological activities of the compounds. Structural confirmation of all synthesized compounds was achieved through IR, NMR, and mass spectrometry, verifying the successful incorporation of the desired functional groups into the molecular framework. The brominated derivatives, such as 5b and 8b, were synthesized with high yields and demonstrated the most significant biological activity, underscoring the importance of bromine

substitution in enhancing molecular interactions and reactivity. The synthetic process provided structurally diverse derivatives, offering a clear pathway for tuning chemical properties in drug design.

In addition to successful synthesis, the biological evaluation of the synthesized compounds highlighted their potential as antimicrobial and anti-inflammatory agents. Bromine-substituted derivatives, particularly 5b and 8b, exhibited the most potent antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, with lower MIC values compared to other compounds in the series. These results indicate that halogenation, especially bromine incorporation, plays a critical role in enhancing antimicrobial efficacy by increasing lipophilicity and improving membrane interaction. The anti-inflammatory testing, conducted using a carrageenan-induced rat paw edema model, revealed that compounds with both halogen and sulfur substitutions, such as 8b, exhibited the highest anti-inflammatory activity, with 75% inhibition. These findings demonstrate the significance of structural modifications in modulating biological activity and provide valuable insights for further optimization of piperazine-thiazole derivatives for therapeutic use.

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