

SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIBACTERIAL POTENTIAL OF NEW IBUPROFEN-BASED 1,3,4-OXADIAZOLE- THIOACETAMIDE HYBRIDS

Mahrukh Akram^{*1}, Nagina Ashraf², Ayesha Mehmood³, Aqsa Anwar⁴, Sehrish Saeed⁵, Dr. Abdul Razaq⁶

^{*1,3,4,5}Department of Chemistry, Faculty of Science, Government College University Faisalabad, Pakistan.

²Department of Chemistry, Faculty of Science, Riphah International University, Faisalabad, Pakistan.

⁶Department of Chemistry, Faculty of Science, University of the Punjab Lahore, Pakistan.

¹alislahschool372@gmail.com, ²naginaashraf185@gmail.com, ³ayeshagrewal188@gmail.com,

⁴aqsaanwarshah@gmail.com, ⁵mrsamirshahzad21@gmail.com, ⁶arazaqch@gmail.com

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Corresponding Author: *

Mahrukh Akram

Abstract

A new series of 1,3,4-oxadiazole-thioacetamide hybrids (7a-7j) based on ibuprofen was synthesized (61-78% yields) and tested for antibacterial activity in order to address multidrug resistance (MDR). Ibuprofen was converted into ethyl 2-(4-isobutylphenyl) propanoate (2) in the multi-step synthesis process, which was then hydrazinolized to produce 2-(4-isobutylphenyl) propanohydrazide (3). The primary building block, 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2-thiol (4), was produced via base-catalyzed cyclization of 3 with carbon disulfide. Target hybrids (7a-7j) were successfully produced by nucleophilically substituting 4 with different N-substituted aryl acetamides (6a-j) in the presence of lithium hydride (LiH). Melting point tracking and high-resolution FT-IR spectrum analysis (4000-400 cm⁻¹ range) were used to evaluate structural frameworks. In vitro screening against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) strains using the agar well diffusion method showed that hybridization introduces remarkable antibacterial characteristics in comparison to the fully inactive parent ibuprofen (0.0 mm). Higher susceptibility was shown by Gram-positive bacteria, and the most effective candidate in the entire collection was the 4-nitro-substituted hybrid (7j).

INTRODUCTION:

A novel strategy in contemporary medicinal chemistry is molecular hybridization, which combines two or more different pharmacophores into a single molecular scaffold to produce multi-functional hybrid molecules [1]. Highly personalized modifications that maximize oral

bioavailability and key cell membrane distribution pathways are made possible by the structural engineering and chemical modification of these frameworks [2]. In recent decades, there has been a notable decrease in the global discovery and therapeutic licensing of innovative small-molecule clinical medicines that target common infectious

illnesses. This has created serious health issues for the scientific community [3]. In order to find structurally distinct non-traditional antimicrobial scaffolds with high efficacy and improved receptor selectivity, intensive, financially feasible research is required due to these growing resistance mutations [4].

Because these hybrid frameworks may concurrently bind to several biological target sites or prevent crucial changes, the judicious deployment of bi- and trivalent core ligand combinations provides an incredibly appealing track in this context [5]. Due to their remarkable broad-spectrum antibacterial [6], antifungal [7], and dynamic antioxidant [8] capabilities, heterocyclic templates generated from non-steroidal anti-inflammatory medications (NSAIDs) have become extremely popular. Moreover, improved anti-inflammatory, analgesic, antipyretic, anticonvulsant, and anti-HIV modalities are being investigated in several ongoing clinical studies using synthetic variants based on these frameworks [9]. By selectively inhibiting intracellular pyrophosphatases, phosphodiesterases, and associated neurological receptors, these well-established frameworks operate systemically [10].

Standard non-selective NSAIDs provide remarkable clinical relief for ailments like ankylosing spondylitis, rheumatoid arthritis, and osteoarthritis [11, 12], but their long-term, continuous use is still severely restricted by localized side effects, such as severe internal gastrointestinal bleeding and tissue degradation [13]. The presence of an unmasked free carboxylic acid group, which easily undergoes ionization inside gastric cells and damages the protective mucosal layers, is the main cause of the mechanical induction of localized gastric ulceration and cellular erosion caused by conventional non-selective NSAIDs [14]. By chemically concealing the acidic carboxyl center [15] or structurally substituting it with non-classical heterocyclic bioisosteres such as oxadiazole and tetrazole units [16], this localized tissue damage can be effectively reduced or avoided.

By methodically increasing the steric bulk of the parent core pharmacophore, this deliberate alteration optimizes binding orientations and greatly enhances biological receptor selectivity [17]. The 1,3,4-oxadiazole ring is one of the most active and adaptable heterocycles because of its distinct electronic structure and excellent aromatic stability. It interacts with target cell components through extensive π - π stacking networks and specialized hydrogen bonding [18–20]. By serving as stable stand-ins for unstable esters, amides, and carbamates, the transformation of free acids into 1,3,4-oxadiazole compounds maximizes systemic metabolic stability and drug-like behavior [21]. Furthermore, these oxadiazole rings enable target-specific receptor modifications when connected by flexible sulfur bridges like thioether or thioacetamide chains, leading to improved broad-spectrum antibacterial characteristics [22].

These thio-linked targets exhibit exceptional antioxidant characteristics at the same time because of the strong conjugation of the aromatic system and electron-dense sulfur atom networks [23, 24]. Despite being officially recognized in 1969 as a safer substitute for aspirin, ibuprofen (IBU) still faces significant patient compliance issues, such as immediate gastrointestinal pain, nausea, and vomiting [25]. An issue shared by traditional single-action NSAIDs is that prolonged use of unmodified IBU causes irreversible harm to the gastrointestinal tract [26]. Similar-active DIT NSAIDs in the classical sense [20]. Derivatizing the active carboxylic acid group of the IBU core into a hybrid heterocyclic framework with an oxadiazole center is a potent medicinal chemistry strategy to reduce this stomach toxicity profile [27].

One effective method for obtaining synergistic biological activity is the invention of hybrid heterocyclic platforms that combine many pharmacophores. The combination of pyrazole and oxadiazole units with sulfur-containing features such as a thioacetamide bridge is very appealing in this context [28]. By increasing baseline lipophilicity and membrane permeability, sulfur incorporation improves antibacterial and antioxidant screening standards [29, 30].

Additionally, by optimizing steric and electronic binding orientations, an electron-rich, stiff fused pyrano [2,3-*c*]pyrazole scaffold improves cellular target affinity [31]. In order to investigate the therapeutic efficacy of a novel series of Ibuprofen-based 1,3,4-oxadiazole-thioacetamide hybrids against drug-resistant clinical bacterial strains, the current study focuses methodically on the synthesis, structural characterization, and in vitro broad-spectrum antibacterial evaluation [32].

The cyclization of intermediates into physiologically active 1,3,4-oxadiazole ring derivatives with anti-COX, analgesic, and antibacterial potential is depicted in this diagrammatic representation of the molecular hybridization idea.

MATERIALS AND METHODS

2.1 Materials

The entire set of chemical precursors, functional reagents, and analytical-grade processing solvents needed for this multi-step synthetic procedure was purchased from global commercial networks, such as suppliers of Sigma-Aldrich and Merck, and used directly without the need for additional purification or distillation processes. Among the materials utilized are: Core acid of pure ibuprofen ($\text{C}_{13}\text{H}_{18}\text{O}_2$), or absolute ethanol Sulfuric acid concentrated (H_2SO_4) 80% hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$), Carbon disulfide (CS_2) that is volatile, analytical pellets Potassium hydroxide (KOH), ClCH_2COCl , or chloroacetyl chloride Dimethylformamide anhydrous (DMF), Powdered high-purity lithium hydride (LiH), A digital Gallenkamp melting point instrumentation block was used to comprehensively monitor the melting points of all created solid intermediate scaffolds and target hybrids in a variety of structurally variable electron-donating and electron-withdrawing substituted aromatic anilines (5a-j). High-resolution Fourier Transform Infrared (FT-IR) measurements performed on a Bruker FT-IR spectrophotometer were used to validate structural monitoring, functional group shifts, and chemical cyclizations. Pressurized anhydrous KBr pellet frameworks were used to scan the broad 4000- 400 cm^{-1} wavenumber range.

2.2 Methods :

2.2.1 Method for Producing Ethyl 2-(4-isobutylphenyl)propanoate (2)

A round-bottom flask was filled with pure ibuprofen core acid (1, 10.0 g, 48.5 mmol), which was thoroughly dissolved in 50 mL of 100% ethanol while being smoothly stirred. To serve as an acid catalyst, concentrated sulfuric acid (H_2SO_4 , 2.5 mL) was gradually added dropwise to the mixture. For six to eight hours, the reaction flask was refluxed continuously while being constantly stirred over a thermal heating mantle. Thin-Layer Chromatography (TLC) was used to regularly check the starting material's absolute conversion. After finishing, a rotary evaporator was used to remove surplus ethanol under lower pressure. After neutralizing the residual crude bulk with a 5% aqueous sodium bicarbonate (NaHCO_3) solution and extracting it using diethyl ether, intermediate **ester 2** was isolated as a clear, colorless, oily liquid (yield: 81%).

2.2.2 2-(4-isobutylphenyl)propane hydrazide synthesis (3)

Absolute ethanol (40 mL) and the intermediate liquid ester (2, 8.0 g, 34.1 mmol) were combined in a sterile reaction flask. The flask's neck was methodically filled with hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 80%, 5.0 mL). For around five to six hours, the reaction mixture was continuously stirred and allowed to reflux smoothly.

The crude solid chemical freely precipitated out after the reaction vessel was naturally cooled to room temperature following the completion of the reflux run. In order to get pure white crystalline crystals of hydrazide 3 (Melting Point: 74-76 °C, Yield: 79%), the produced precipitate mass was filtered out under suction, repeatedly washed with ice-cold distilled water to eliminate excess hydrazine traces, and recrystallized using hot ethanol.

2.2.3 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2-thiol (4) synthesis

An ethanolic solution comprising analytical potassium hydroxide pellets (KOH, 1.27 g, 22.7 mmol) in 50 mL of pure ethanol was used to

completely dissolve the solid propane hydrazide intermediate (**3**, 5.0 g, 22.7 mmol). Under an effective fume hood, volatile carbon disulfide (CS₂, 3.0 mL) was introduced to the reaction medium very slowly and dropwise while continuous magnetic stirring was maintained. After that, the reaction mixture was put under reflux for 12 hours to stop the poisonous hydrogen sulfide (H₂S) gas from evolving. A rotary evaporator was used to concentrate the solution, which was then chilled in an ice bath before being carefully acidified with a diluted hydrochloric acid (HCl) solution to a pH of 2-3. Filtration was used to collect the ensuing off-white amorphous precipitate of the central 1,3,4-oxadiazole-2-thiol core (**4**), which was then carefully cleaned with cold water and vacuum-dried (Melting Point: 118-120 °C, Yield: 81%).

2.2.4 General Method for N-Substituted Chloroacetamide Synthesis (**6a-j**)

A 10% aqueous sodium carbonate solution (Na₂CO₃, 30 mL) was poured into an ice bath framework under magnetic stirring to suspend several primary substituted aromatic anilines (**5a-j**, 20.0 mmol). Over the course of 30 minutes, chloroacetyl chloride (2.5 mL, 22.0 mmol) was gradually added dropwise while maintaining a temperature that was strictly below 5 °C. Before being used in the last coupling step, the resultant crude solid precipitate of N-substituted chloroacetamides (**6a-j**) was filtered out, dried, and recrystallized from an aqueous ethanol mixture after being thoroughly cleaned of salts with cold distilled water.

2.2.5 Target Hybrid Synthesis General Procedure (**7a-7j**)

To enable conventional thiol deprotonation, the central 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2-thiol core (**4**, 1.0 mmol) was dissolved in anhydrous DMF (15 mL) loaded with lithium hydride powder (LiH, 0.02 g, 2.5 mmol) and left to stir at room temperature for 30 minutes. The corresponding N-substituted chloroacetamide

derivative (**6a-j**, 1.0 mmol) was then gradually added. For four to five hours, the reaction setup was constantly agitated at 60 to 70 °C. Following completion (as determined by TLC), the liquid mass was gradually poured over crushed ice while being continuously stirred. To produce publication-ready crystals (Yield: 61-78%), the separated final solid derivatives (**7a-7j**) were filtered, cleaned with water, dried, and purified by recrystallization from heated methanol/ethanol solutions.

2.2.6 Agar Well Diffusion Technique In Vitro Antibacterial Assay

The conventional agar well diffusion technique was used to examine the biological potential of all freshly synthesized hybrid compounds (**7a-j**). Standard target bacterial strains were uniformly cultivated on sterile nutrient agar plates, comprising Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*). Using a sterile cork borer, functional wells with a precise 6 mm diameter were drilled into the agar plates.

The test hybrids' stock solution frameworks were made in 1 mg/mL of pure DMSO. One hundred microliters of the corresponding chemical solution were accurately added to each well. Standard Ciprofloxacin was used as the positive clinical reference medicine, while the pure parent medication Ibuprofen was used as a negative baseline control.

Results and Discussion:

A multi-step chemical approach was used to methodically produce the targeted 1,3,4-oxadiazole-thioacetamide hybrids derived from the parent ibuprofen pharmacophore core. Physical constants, reaction yields, and high-resolution Fourier Transform Infrared (FT-IR) spectrographic configurations were used to trace, authenticate, and structurally confirm each intermediate conversion and the final molecule's structure.

3.1 Synthesis and FT-IR Spectral Interpretation of Intermediates

Table 3.1: Physical characteristics data of intermediate compounds (2-4)

Compound No	Compound Name	Physical state	Melting Point °C	Yield (%)	Molecular Formula
2	Ethyl 2-(4-isobutylphenyl) propanoate	Colorless liquid	Liquid (N/A)	81	C ₁₅ H ₂₂ O ₂
3	Ethyl 2-(isobutylphenyl)propane hydrazide	White Crystals	74-76 °C	79	C ₁₃ H ₂₀ N ₂ O
4	5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole -2-thiol	Off-White solid	118-120 °C	81	C ₁₄ H ₁₈ N ₂ OS

3.1.1 Ethyl 2-(4-isobutylphenyl) propanoate (2)

The complete transformation of the dimeric carboxylic acid group of the parent ibuprofen was used to track the structural assignment of the production of the first liquid ester intermediate (2). The formation of a very strong, abrupt carbonyl stretching vibration (C=O stretching) recorded at 1734 cm⁻¹ provided strategic validation of effective esterification in the isolated spectrographic tracking configuration. Simultaneously, substantial overlapping peaks centered at 2955 cm⁻¹ were produced by the

symmetric and asymmetric vibrations of the saturated aliphatic backbone (C-H stretching) for the methyl and isobutyl configurations. **While** the dynamic single-bond ester skeletal movement (C-O asymmetric stretch) was identified by a deep peak at 1245 cm⁻¹, confirming the assignment of structure 2, the aromatic core stretching bands associated with the central benzene skeleton (C=C skeletal stretching) were clearly recorded at 1605 cm⁻¹.

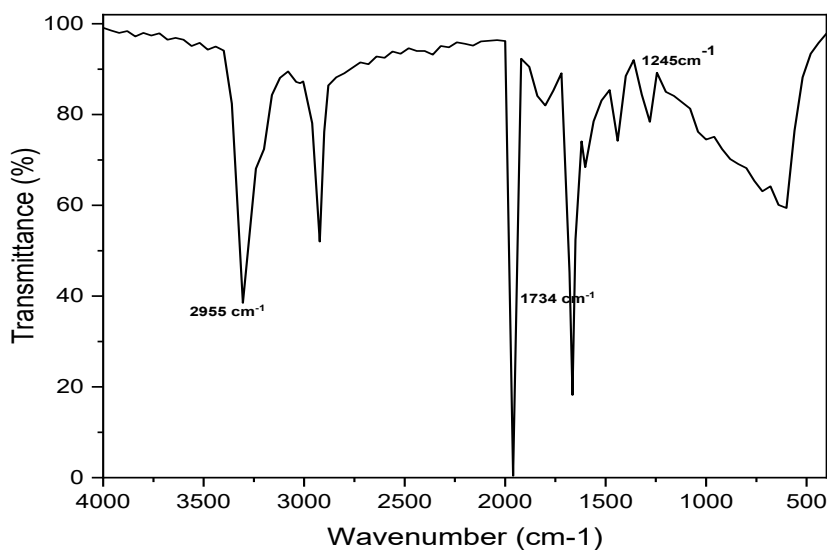


Figure 3.1: FT-IR spectrum of Ethyl 2-(4-isobutylphenyl)propanoate (2)

3.1.2 2-(4-isobutylphenyl)propane hydrazide (3)

Significant downfield spectrum shifts verified the nucleophilic conversion of liquid ester

intermediate 2 into the white crystalline 2-(4-isobutylphenyl) propane hydrazide intermediate (3). The ester single-bond stretching line

completely vanished at 1245 cm^{-1} , as seen by high-resolution infrared scanning. The creation of a very unique, wide multi-band absorption system peaking at 3305 cm^{-1} , which corresponds to the secondary amine and primary amide functions (N-H stretching vibrations), replaced this functionality. The powerful ester carbonyl band dramatically downshifted to 1665 cm^{-1} due to

localized hydrogen bonding, indicating a secondary amide carbonyl environment ($\text{C}=\text{O}$ amide stretching). The active amide bending and C-N stretching bands were evident at 1540 cm^{-1} , whereas the framework vibrations for the aliphatic and aromatic system segments were seen around 2922 cm^{-1} (C-H stretching) and 1602 cm^{-1} (C=C stretching).

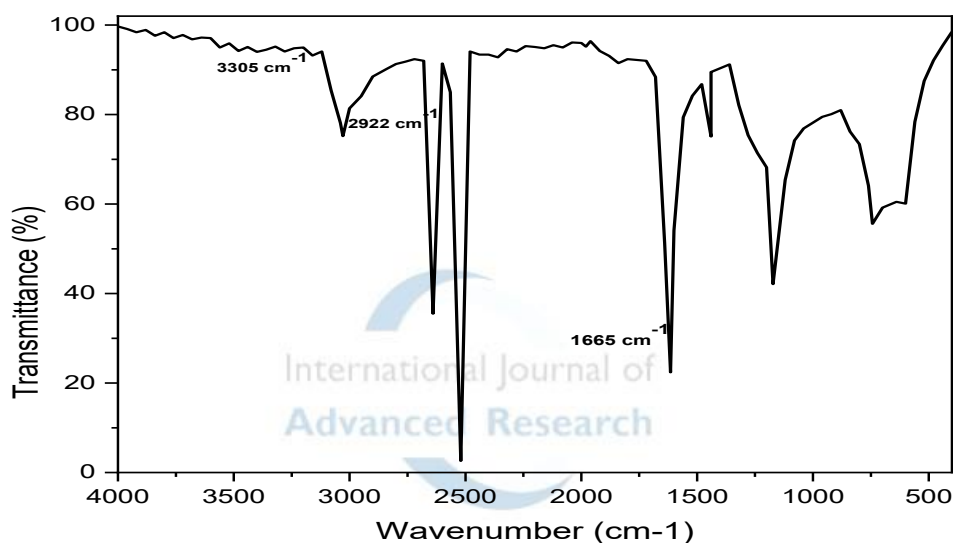


Figure 3.2: FT-IR spectrum of 2-(4-isobutylphenyl) propane hydrazide (3)

3.1.3 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2-thiol (4)

The full, successful base-catalyzed ring cyclization of hydrazide intermediate 3 into the essential 1,3,4-oxadiazole-2-thiol heterocyclic core was verified by the elimination of the potent amide carbonyl stretching band at 1665 cm^{-1} (4). A very clear, characteristic ring absorption band at 1615 cm^{-1} replaced this, confirming the installation of the heterocyclic ring arrangement (C=N

heterocyclic ring stretching). The introduction of the thiol system was verified by a weak but distinct stretching frequency centered at 2565 cm^{-1} (S-H thiol stretching), whereas the cyclic ether core structure and endocyclic thio-link produced deep absorption bands at 1172 cm^{-1} (C-O-C stretching) and 743 cm^{-1} (C-S stretching), respectively. Aromatic out-of-plane bending structures were seen lower in the spectrum, at around 640 cm^{-1} .

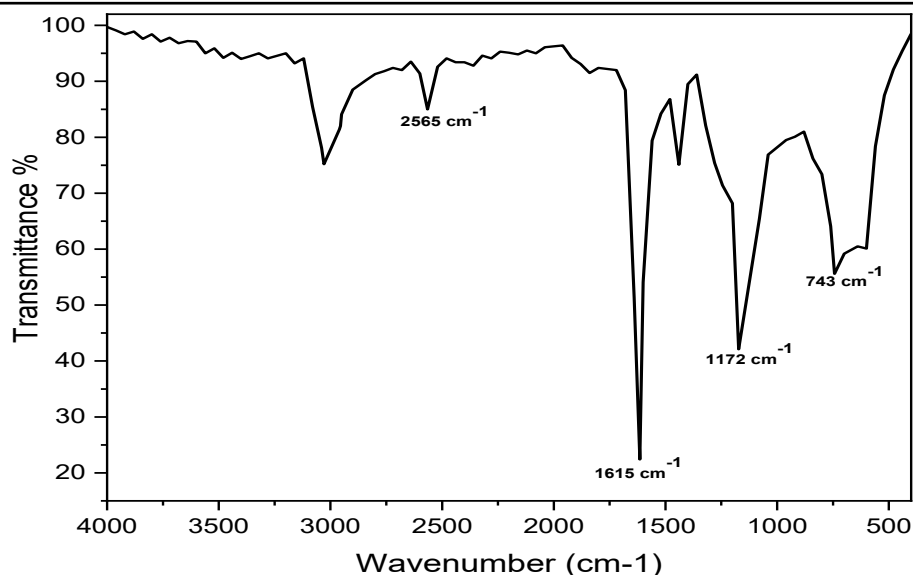


Figure 3.3: FT-IR Spectrum of 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2-thiol (4)

3.2 Target Hybrid Characterization Grids and Physical Constants

Target hybrids (7a-7j) were produced by nucleophilic replacements with various secondary chloroacetamides (6a-j) after the heterocyclic core

building block (4) was generated. The master structural analysis table that follows methodically compiles the physical attribute data of these target hybrids.

Table 3.2: Target Hybrids' Physical and Analytical Characteristics Information (7a-7j)

Entry	Compound code	Substitute group	Physical state	Melting point °C	Yield (%)	Molecular Formula	M.W. (g/mol)
1	7a	4-F	Pink solid	142-144 °C	78 %	C ₂₂ H ₂₄ FN ₃ O ₂ S	413.53
2	7b	3-Br	White solid	155-157 °C	68 %	C ₂₂ H ₂₄ BrN ₃ O ₂ S	474.42
3	7c	4-CH ₃	Yellow Crystals	138-140 °C	70 %	C ₂₃ H ₂₇ BrN ₃ O ₂ S	409.55
4	7d	Unsubstituted	Off-white solid	130-132 °C	72 %	C ₂₂ H ₂₆ N ₃ O ₂ S	395.52
5	7e	3,5-di-CH ₃	White powder	162-164 °C	65 %	C ₂₄ H ₂₉ N ₃ O ₂ S	423.58
6	7f	2-NH ₂	Brown solid	148-150 °C	61 %	C ₂₂ H ₂₉ N ₄ O ₂ S	410.54
7	7g	2-CH ₃ ,6-C ₂ H ₅	Off-White solid	168-170 °C	63 %	C ₂₅ H ₃₁ N ₃ O ₂ S	437.60
8	7h	4-C ₂ H ₅	Dull White powder	141-143 °C	69 %	C ₂₄ H ₂₉ N ₃ O ₂ S	423.58
9	7i	2,4-di-CH ₃	White solid	151-153 °C	67 %	C ₂₄ H ₂₉ N ₃ O ₂ S	423.58
10	7j	4-NO ₂	Yellow solid	175-177 °C	75 %	C ₂₂ H ₂₄ N ₄ O ₄ S	440.52

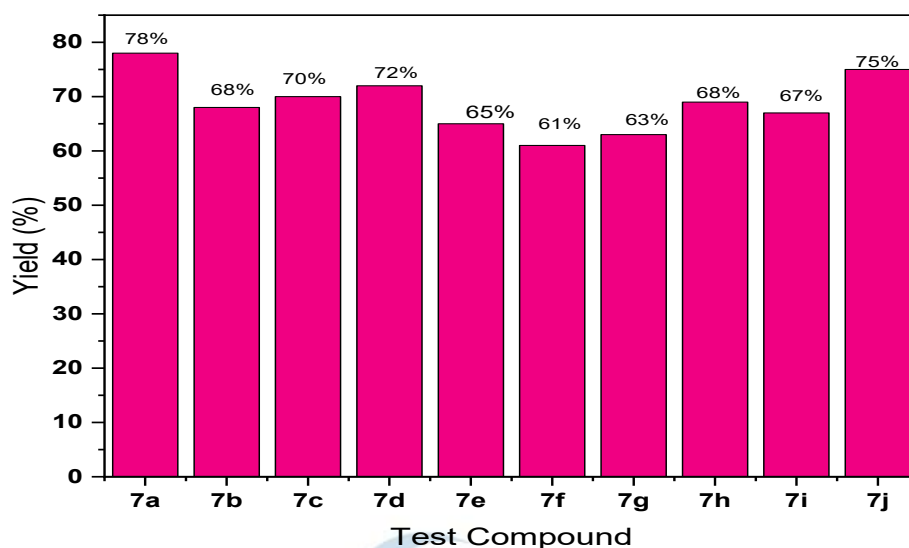


Figure 3.4: Bar Graph representing the reaction yield (%) of Synthesized target hybrids (7a-7j)

3.3 Target Hybrids' Detailed FT-IR Spectral Analysis (7a-7j)

7a: (Unsubstituted Hybrid) Melting point: 142-144 °C; yield: 78%; pink solid; FT-IR (cm^{-1}): 3285 (N-H secondary amide stretching), 3014 (aromatic C-H), 1680 (C=O intense amide stretch), 1584 (C=C skeletal aromatic mapping), 1395 (C-F halogen interaction), 1298 (C-N stretching vibration), 1142 (C-O symmetric link), and 720 (C-S secondary thioether stretching).

7b: (4-Substituted Hybrid) Melting point: 155-157 °C; yield: 68%; white solid; FT-IR (cm^{-1}): 3290 (N-H stretch), 3022 (aromatic C-H), 1678 (C=O stretch), 1610 (C=N ring oxadiazole configuration), 1165 (C-O-C link stretching), 730 (C-S thioether bridge), and 615 (C-Br halogen frequency).

7c: (4-CH₃ Substituted Hybrid) 138-140 °C melting point; yellow crystals; yield: 70%. FT-IR (cm^{-1}): 1685 (C=O secondary amide), 1598 (C=C stretch), 1290 (C-N stretching), 725 (C-S bridge stretching), 3275 (N-H stretch), and 2950 (aliphatic C-H vibrations).

7d: (Unsubstituted Amide Hybrid) Off-white solid; 72% yield; 130-132 °C melting point. FT-IR (cm^{-1}): 735 (C-S stretching frequency), 1682 (C=O stretch), 1612 (C=N heterocyclic core), 3045

(aromatic C-H system), and 3310 (N-H amide vibration).

7e: (3,5-di-CH₃ Substituted Hybrid) White powder; 65% yield; 162-164 °C melting point. FT-IR (cm^{-1}): 3268 (N-H stretching), 2920 (C-H stretching system), 1672 (C=O amide), 1590 (C=C aromatic structure), 1170 (C-O-C core ring tracking), and 722 (C-S thioether link).

7f: (2-NH₂ Substituted Hybrid) Brown solid; yield: 61%; melting point: 148-150 °C; FT-IR (cm^{-1}): 3410 (N-H primary amine stretch), 3280 (N-H amide configuration), 1675 (C=O primary stretch), 1618 (C=N skeletal stretching), 1295 (C-N linkage), and 740 (C-S stretch).

7g: (2-CH₃, 6-C₂H₅ Substituted Hybrid) Off-white solid; 63% yield; 168-170 °C melting point. FT-IR (cm^{-1}): 3260 (N-H stretch), 2962 (C-H alkyl system), 1690 (C=O secondary stretch), 1580 (C=C stretching), and 718 (C-S aromatic stretch).

7h: (4-C₂H₅ Substituted Hybrid) 141-143 °C; yield: 69%; dull white powder. FT-IR (cm^{-1}): N-H stretch (3295), C-H alkyl vibrations (2930), C=O mapping stretch (1676), C=C ring stretching (1605), C-O-C system (1175), and C-S stretch (728).

7i:(2,4-di-CH₃ Substituted Hybrid): White solid; yield: 67%; melting point: 151-153 °C; FT-IR (cm⁻¹): 3272 (N-H stretch), 2915 (C-H alkyl backbone), 1680 (C=O stretch), 1595 (C=C stretch), 1285 (C-N core stretch), and 724 (C-S bridge).

7j: (4NO₂ Substituted Hybrid): yellow solid; yield: 75%; melting point: 175-177 °C; FT-IR (cm⁻¹): 3315 (N-H secondary amide stretch); 3080 (aromatic C-H vibrations); 1695 (C=O secondary amide intense stretching frequency); 1535 (N-O asymmetric stretching vibration associated with the recently added nitro group); 1340 (N-O symmetric nitro stretching framework); 1292 (C-N stretching bond); and 732 (C-S secondary bridge link).

3.4 Structure-Activity Relationship (SAR) and In Vitro Antibacterial Efficacy

The biological potential of the newly designed hybrid scaffolds (**7a-7j**) was assessed through quantitative in vitro broad-spectrum antibacterial trials that targeted both Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) clinical pathogens.

The experimental zones of inhibition (ZOI) are summarized within the biological matrix in Table 3.3.

The dynamic screening arrays demonstrated that structural hybridization, which introduces a heterocyclic 1,3,4-oxadiazole ring and a substituted thioacetamide link, transforms ibuprofen, a parent drug framework that is completely inactive (exhibiting 0.0 mm activity against all isolates), into remarkable antimicrobial targets. Compared to isolates of Gram-negative bacteria, Gram-positive pathogenic bacteria showed noticeably greater sensitivity throughout the chemical selection. The varied cross-linking complexity of bacterial outer envelopes is directly responsible for this noticeable variance in biological screening parameters. While the rich peptidoglycan layer of Gram-positive organisms permits smooth cell penetration, the lipopolysaccharide outer wall architecture of Gram-negative organisms creates a highly selective permeability layer that limits candidate dispersion. Compound **7j**, which has a strongly electronegative and highly electron-withdrawing 4-nitro (-NO₂) substitution on the acetamide arm, was found to be the most effective candidate among all tested lines in the structurally diverse library (*S. aureus*: 23.1 mm, *B. subtilis*: 21.5 mm, *E. coli*: 16.4 mm, *P. aeruginosa*: 13.5 mm). The 4-fluoro (-F) functionalized hybrid **7a** closely trailed this remarkable tracking performance, suggesting that strong electron-withdrawing capabilities and electronic characteristics improve binding orientations inside bacterial cellular targets.

Table 3.3: Target Hybrids' Antibacterial Activity (Zone of Inhibition in mm) (**7a-7j**)

Test Compound	<i>S.aureus</i>	<i>B.subtilis</i>	<i>E.coli</i>	<i>Aeruginosa</i>
7a	21.4	19.2	14.1	11.2
7b	18.5	17.1	12.3	10.1
7c	15.2	14.8	11.5	09.4
7d	14.1	13.5	10.2	08.3
7e	16.3	15.1	11.8	09.1
7f	17.8	16.4	13.2	10.5
7g	13.5	12.9	09.5	07.2
7h	15.8	14.2	11.0	08.9
7i	16.1	15.0	11.4	09.0
7j	23.1	21.5	16.4	13.5

<i>Ibuprofen</i>	0.0	0.0	0.0	0.0
<i>Ciprofloxacin</i>	26.5	25.2	24.8	22.1

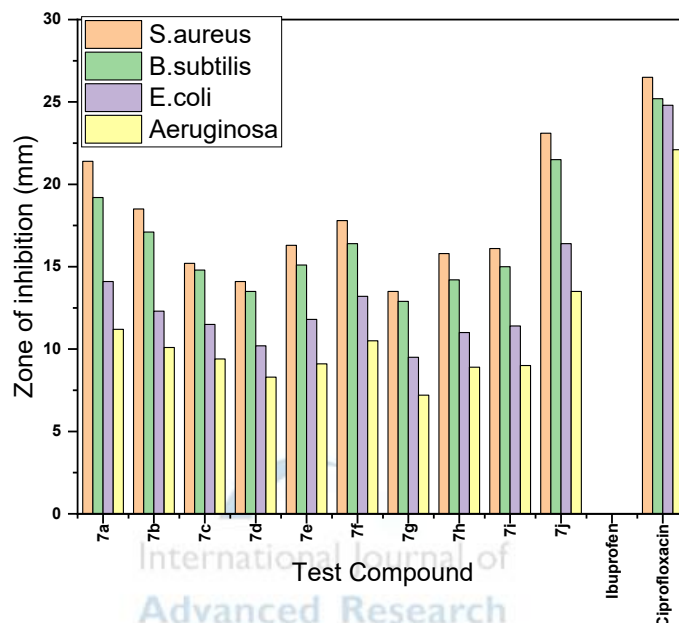


Figure 3.5: Bar graph comparing the in vitro antibacterial activity (Zone of inhibition in mm) of synthesized hybrids (7a-7j), parent Ibuprofen, and standard Ciprofloxacin against clinical pathogens

Conclusion:

In conclusion, a new series of 1,3,4-oxadiazole-thioacetamide hybrids (7a-7j) based on ibuprofen was successfully synthesized with immaculate analytical purity and high product yields ranging from 61% to 78%. Diagnostic spectral alterations, namely the absolute conversion of the hydrazide carbonyl band into a strong heterocyclic core peak (C=N stretching) at 1615 cm and a corresponding thiol signature (S-H stretching) at 2565 cm⁻¹, were used to confirm the multi-step chemical sequence physically.

Molecular hybridization effectively converts the absolutely inactive parent ibuprofen core (0.0 mm inhibition) into extremely powerful bactericidal agents, as shown by the quantitative in vitro broad-spectrum antibacterial experiments. In comparison to Gram-negative envelopes (Escherichia coli and Pseudomonas aeruginosa), Gram-positive isolates (Staphylococcus aureus and Bacillus subtilis) demonstrated noticeably better susceptibility metrics across the whole chemical

selection. The most remarkable and effective antibacterial candidate in the structural library was compound 7j, which was functionalized with a significantly electron-withdrawing 4-nitro (-NO₂) substituent on the acetamide aromatic ring. It was closely followed by the 4-fluoro (-F) derivative 7a. These results demonstrate that, in contemporary medicinal chemistry paradigms, combining NSAID structures with a 1,3,4-oxadiazole core via a thioacetamide bridge provides a potent, scientifically useful molecular blueprint for fighting resistant pathogenic bacterial strains.

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