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# REREARCH ARTICLE

## Design, Synthesis, and Molecular Modeling Study of Substituted 1, 3, 4-Thiadiazoles as Antibacterial Agents

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## ABSTRACT

The present study reports the design, synthesis, and molecular modeling of substituted 1,3,4-thiadiazoles as potential antibacterial agents. The heterocyclic 1,3,4-thiadiazole nucleus was selected for its well-documented pharmacological versatility. A series of novel derivatives were synthesized, employing microwave-assisted synthesis to reduce reaction time and improve yields. Structural characterization was performed using IR, ^1H-NMR, ^13C-NMR, and mass spectrometry, confirming the expected functionalities, including thiadiazole core signals and characteristic substituent peaks. Molecular docking studies were conducted to predict the binding affinity of the synthesized compounds toward bacterial target proteins, with compounds 5(a) and 5(d) showing the highest docking scores and favorable interaction profiles. In vitro antibacterial evaluation using the disc diffusion method demonstrated significant activity against both Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) strains, with MIC values ranging between 15–38 µg/mL. Ciprofloxacin served as the reference drug. The results indicate that the presence of a 4-substituted phenyl group linked via thioster/amide to the thiadiazole ring enhances antibacterial activity. This study suggests that the synthesized molecules have promising antibacterial potential, supported by both computational and experimental findings. Further investigations into their mechanism of action and potential structural optimizations are warranted to develop more potent derivatives.

**Keywords:** 1,3,4-Thiadiazole, Antibacterial agents, Molecular docking, Heterocyclic compounds, Microwave-assisted synthesis


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## INTRODUCTION

The relentless rise of antibiotic-resistant bacteria, driven by overuse and misuse of conventional antibiotics, poses a significant global health threat. This scenario has intensified the imperative for discovering novel antibacterial agents with unique mechanisms of action. Heterocyclic compounds, particularly those containing nitrogen and sulfur, have historically been fruitful in drug development due to their structural versatility and bioactivity [1–3]. Among these, 1,3,4-thiadiazole derivatives have garnered considerable attention because of their known pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer effects [4–6].

Structurally, 1,3,4-thiadiazoles feature a five-membered ring with two nitrogen and one sulfur atom, lending them both stability and the capacity to engage in hydrogen bonding and π–π interactions within biological targets [7]. Synthetic modification of this core through introduction of different substituents at the 2- and 5-positions enables fine-tuning of physical properties, such as lipophilicity and electronic distribution, which can enhance biological activity and target selectivity [8,9].

Microwave-assisted organic synthesis (MAOS) has emerged as a powerful tool in medicinal chemistry. The application of microwave irradiation can drastically decrease reaction times, improve yields, and minimize solvent usage compared to conventional heating methods [10]. Employing MAOS in the synthesis of substituted 1,3,4-thiadiazoles aligns well with green chemistry principles and expedites the generation of compound libraries for biological evaluation.

Molecular modeling, particularly docking studies, complements chemical synthesis by predicting ligand-target interactions. Docking allows rapid in silico screening of synthesized compounds against protein targets such as bacterial enzymes or receptors, facilitating rational design and structure-activity relationship (SAR) insights [11]. This approach not only guides compound selection for further evaluation but also elucidates binding mechanisms.

Given this context, the present study focuses on the design, microwave-assisted synthesis, characterization, molecular docking, and antibacterial evaluation of novel substituted 1,3,4-thiadiazoles. The compounds feature diverse substituents on a phenyl ring, connected via a thioster/amide linkage to the heterocyclic core, aimed at optimizing binding to key bacterial targets. We validated structural identity through spectroscopic techniques, explored interaction potential via docking studies, and assessed antibacterial efficacy against both Gram-positive and Gram-negative strains using disc diffusion and MIC assays. Our integrated approach seeks to identify lead molecules with promising multimodal activity and offers direction for future optimization efforts.

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## SYNTHESIS AND CHARACTERIZATION

### 1. Synthesis of substituted 1, 3, 4-thiadiazoles

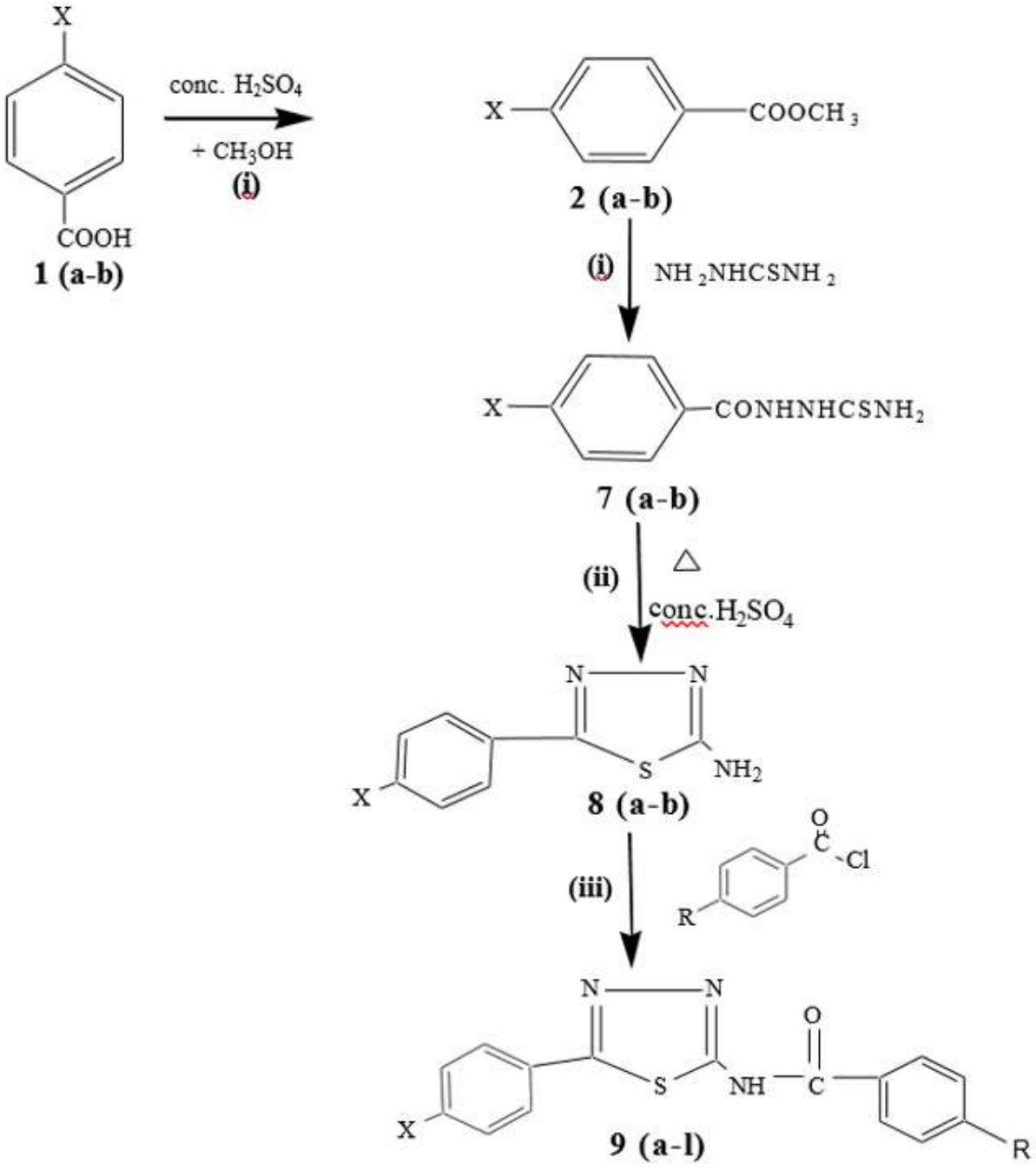


**Figure-1: Scheme for synthesis of substituted 1,3,4-thiadiazoles**

All the chemicals and reagents used of Spectrochem, Rankem Chemicals, Merck (P) Ltd. and Hi-Media Suppliers, India available in pure form.


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**Step (i): Synthesis of methyl 4-halo benzoate, 2(a-b):**

Synthesis of methyl ester of benzoic acid was done using reported method. The substituted benzoic acid [1(a-b); 0.1 mol] (Table-1) was taken in a 250 mL round bottom flask which was fitted with reflux condenser and calcium chloride guard tube. Anhydrous methanol (150 mL) and 5 mL of concentrated sulphuric acid were added and the contents refluxed for 8-9 hours. Excess of methanol was distilled off under reduced pressure. The contents were poured into 150 mL of water contained in a separating funnel. The ester was extracted multiple times with carbon tetrachloride (CCl4). The organic phase was extracted with 10% w/v cold solution of sodium bicarbonate to get rid of unreacted aromatic acid. The solvent was distilled off to get required ester. The melting point or boiling points were determined (Table 2).

**Table-1: Aromatic carboxylic acids used for synthesis of methyl benzoates, 2(a-b)**

| Compound Code | X | Mol. Formula | Mol. Wt. | Quantity taken (g) |
|---|---|---|---|---|
| 1(a) | - F | $C_7H_5FO_2$ | 140.11 | 14.0 |
| 1(b) | - I | $C_7H_5IO_2$ | 248.02 | 24.8 |

**Table-2: Physical data of methyl 4-substituted benzoates, 2(a-b):**

| Compound Code | X | M.P.(B.P.) $^oC$ | Yield (%) | Reaction time |
|---|---|---|---|---|
| 2(a) | -F | -- (202-204) | 80.4 | 9 h |
| 2(b) | - I | 112-14 | 85.2 | 8 h |

**Microwave Technology in the organic synthesis**

**(a) Microwave-Induced Organic Reaction Enhancement (MORE) Chemistry:**

Conventional methods in synthetic chemistry usually need longer heating time which requires high energy consumption and excessive use of solvents. This leads to environmental pollution. Alternative source of energy like microwave radiation holds significant potential for the use.

There are a number of reports where the alternative source i.e. microwave oven has been used with success. The main advantage of this technique, in which reaction rate is drastically improved. The techniques have helped the chemists to carry out syntheses in much lesser time with good yields. Microwave irradiations produces less side products, therefore the

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product is recovered in higher yield and purification step is faster and easier.[48] The ester as reported above was again synthesized in microwave synthesizer following step (i).

**(b) Validation of Power (watt) and reaction time using microwave synthesizer:**

The syntheses were carried out in specially designed reaction vessels of a synthesizer (Catalyst Systems, Scientific Microwave system, Pune, India, CATA-R). The equipment is provided with power (in watt) switch which directly operates the microwave source. The different power sources provided in the equipment are 140, 210, 245, 280, 350, 420 455, 490, 560 and 700 watts. The first attempt to use the maximum power (watt) i.e. 700 watt provided in the equipment failed because of uncontrolled reaction. The vigorous reactions continue till power was lowered to 420 watt.

The whole process was based on trial and error for various steps of research work. As result of trial and error method, finally it was decided to perform all experiments for 140, 210, 245, 280, 350 and 420 W. Out of the several studies conducted 350W was chosen for present synthetic research purpose (Table 3). This table indicates that the optimal time period for microwave assisted reaction is 8 min.

**Table-3: Assessment of reaction time for methyl esters of benzoic acid:**

| S. No. | Compound Code | Power (Watt) | Reaction time (Min) | Yield (%) |
|---|---|---|---|---|
| 1 | 2 (a) | 350 | 4 | 80 |
| 2 | | 350 | 6 | 82.8 |
| 3 | 2 (b) | 350 | 8 | 87.6 |
| 4 | | 350 | 10 | 87.6 |

**Step (ii): Synthesis of substituted benzoyl thiosemicarbazides, 3 (a-b):**

The esters 2 (a-b) as synthesized earlier in step-(i) was used for synthesis of substituted benzoyl thiosemicarbazides Benzoic acid esters (0.01 mol) (Table-4 and Table-5) and thiosemicarbazide (0.015 mol, 1.5 g) were suspended in 50 mL of methanol. The contents were dissolved and reaction mixture was refluxed in a microwave reactor at 350 Watt for 6-12 min (Table-6).

**Table-4: Methyl 4-substituted benzoates used for synthesis of 4-substituted benzoyl thiosemicarbazides, 3(a-b):**

| Compound Code | R | Molecular weight | Quantity taken(g) |
|---|---|---|---|
| **2(a)** | F- | 154.14 | 1.5 |

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| | | | |
|---|---|---|---|
| **2(b)** | I- | 262.04 | 2.6 |

**Table-5: Assessment of reaction time for 4-substituted benzoyl thiosemicarbazides**

| S. No. | Compound Code | Power (Watt) | Reaction time (Min) | Yield (%) |
|---|---|---|---|---|
| 1 | 3(a) | 350 | 6 | 66.5 |
| 2 | | 350 | 8 | 70 |
| 3 | | 350 | 10 | 74.5 |
| 4 | | 350 | 12 | 74.5 |

The percentage yield was remained unchanged look at 10 min & 12 min keeping the source at 350 W and at his power all of the reactions were performed. Hence, the optimum time is 10 min.

**Table-6: Physical data of 4-substituted benzoyl thiosemicarbazides, 3(a-b):**

| Compound Code | R | M.P. $^{o}$C | Yield (%) |
|---|---|---|---|
| 2 (a) | -F | 142-46 | 66.5 |
| 2 (b) | - I | 209-11 | 74.6 |

**Step (iii): Synthesis of 2- amino 5-(4-substituted phenyl)-1, 3, 4-thiadiazole 4 (a-b):**

The 4-substituted benzoyl thiosemicarbazide (0.02 mol, Table-6) (3a-b) was added portion wise to a flask containing contconcentrated sulphuric acid (30-40 mL) with shaking. The mixture was heated at 70-80$^{o}$C for half an hour with occasional stirring. Reaction mixtutre was left overnight at room temperature and poured onto crushed ice. The compound was obtained by precipitation with aqueous ammonia solution. Precipitate, so obtained was filtered and washed with cold water to get free from sulphate impurities. Recystallization of product was done using ethanol (Table-7).

**Table-7: 4-substituted benzoyl thiosemicarbazide used for synthesis of 2- amino 5-(4-substituted phenyl)-1, 3, 4-thiadiazole 4(a-b):**

| Compound Code | R | Molecular weight | Quantity taken(g) |
|---|---|---|---|
| **3 (a)** | F- | 213.23 | 4.26 |

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| | | | |
|---|---|---|---|
| **3 (b)** | I- | 321.14 | 6.42 |

**Table-8: Physical data of 2-amino 5-(4-substituted phenyl)-1, 3, 4-thiadiazoles, 4(a-b):**

| Compound Code | X | M.P. ºC | Yield (%) |
|---|---|---|---|
| 4(a) | -F | 233-35 | 74.8 |
| 4(b) | - I | 282-84 | 87.5 |

**Step (iv): Synthesis of 5-(4-substituted phenyl)-1, 3, 4-thiadiazoles, 5 (a-l):**

Compound 8 (a-b) (0.02 mol; Table-9) was dissolved in 100 mL of methanol and potassium hydroxide (0.02 mol). 4-substituted benzoic acid chlorides (0.022 mole; Table-12) was added dropwise in the contents of flat bottom flask which was stirred using magnetic stirrer for an hour. The contents were poured on crushed ice. The precipitated compound was filtered, washed with ice-cold water and dried. Recystallization was done with rectified spirit.

**Table-9: 5-(4-substituted phenyl)-1,3,4-thiadiazole-2-amines used for synthesis of 5-(4-substituted phenyl)-1, 3, 4-thiadiazoles 5(a-l):**

| Compound Code | X | Molecular weight | Quantity taken (g) |
|---|---|---|---|
| **4 (a)** | F- | 195.22 | 3.90 |
| **4 (b)** | I- | 303.12 | 6.06 |

**Table-10: Physical data of 5-(4-substituted phenyl)-1, 3, 4-thiadiazole-2- benzamides, 5(a-l):**

| S. No. | Compound Code | X | R | Melting Point (ºC) | Yield (%) |
|---|---|---|---|---|---|
| 1 | 5(a) | F | Cl | 188-190 | 57.8 |
| 2 | 5(b) | F | Br | 197-198 | 60.5 |
| 3 | 5(c) | F | I | 208-209 | 66.2 |
| 4 | 5(d) | F | $CH_3$ | 192-194 | 62.6 |
| 5 | 5(e) | F | $OCH_3$ | 184-186 | 65.4 |
| 6 | 5(f) | F | $OC_2H_5$ | 191-193 | 72.7 |
| 7 | 5(g) | I | Cl | 194-195 | 70.3 |

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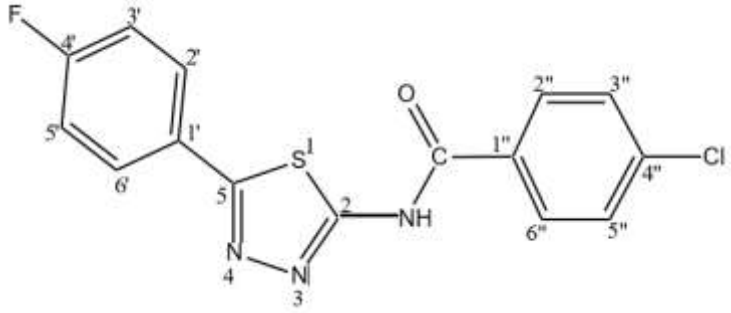
| | | | | | |
|---|---|---|---|---|---|
| 8 | 5(h) | I | Br | 202-204 | 64.8 |
| 9 | 5(i) | I | I | 211-212 | 71.5 |
| 10 | 5(j) | I | CH3 | 190-192 | 61.4 |
| 11 | 5(k) | I | OCH3 | 193-194 | 64.7 |
| 12 | 5(l) | I | OC2H5 | 196-197 | 68.5 |

## 2 Analytical and Spectral Data of Synthesized Compounds:

Melting points of synthesized compounds wherever mentioned were established in open capillaries and are uncorrected. Purity of compounds were ascertained by performing TLC on Silica Gel G using various solvent systems and visualized by iodine vapors. The Rf values were determined for checking the progress of reactions and to assess any side reaction occured. The solubility of the compounds was checked at room temperature in different solvents. IR spectra (in KBr) were acquired on a Shimadzu FTIR spectrophotometer and the values are expressed in terms of $cm^{-1}$. 13C NMR spectra were recorded on a Brucker-400 NMR spectrometer (chemical shift values with expressed in δ ppm) using dimethyl sulphoxide (DMSO) as solvent and tetramethyl silane (TMS) was as reference standard. Mass spectra were recorded on a Shimadzu LC-MS 2010 Spectrometer using methanol as solvent. Elemental analysis was determined using CHNS analyzer (Thermo Finnigan, Italy, Flash EA 1112 series). The alcohol and water mixtures were used to recrystallize the synthesized compounds in various synthetic steps




Compound Code: 5 **(a)**

Chemical Name: 4”-Chloro-N-[5-(4’-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide
TLC ($R_f$ Value): 0.80

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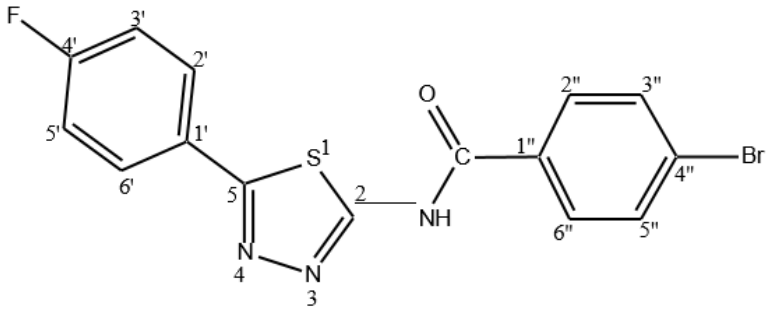
**Elemental Analysis:**

| S. No. | Elements | *Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | ND | 53.98 |
| 2 | Hydrogen | ND | 2.72 |
| 3 | Nitrogen | ND | 12.59 |
| 4 | Sulphur | ND | 9.61 |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3192 | N-H stretching | N-H bond of amide group |
| 2 | 3098 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1652 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1522, 1461 | C=C stretching | C-C bonds of benzene ring |
| 5 | 1131 | C-F stretching | Fluoro group of benzene ring |
| 6 | 1685 | C=O stretching | C=O of amide group |
| 7 | 685 | C-S stretching | C-S-C bond of thiadiazole ring |
| 8 | 782 | C-Cl streching | Chloro group of benzene ring |




Compound Code: **5 (b)**

Chemical Name: 4”-Bromo-N-[5-(4’-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide”

TLC ($R_f$ Value): 0.83

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**Elemental Analysis:**

| S. No. | Elements | Calculated (%) | *Found (%) |
|---|---|---|---|
| 1 | Carbon | 47.63 | ND |
| 2 | Hydrogen | 2.40 | ND |
| 3 | Nitrogen | 11.11 | ND |
| 4 | Sulphur | 8.48 | ND |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3116 | N-H stretching | N-H bond of amide group |
| 2 | 3012 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1630 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1558, 1406 | C=C stretching | C-C bonds of benzene ring |
| 5 | 1124 | C-F stretching | Fluoro group of benzene ring |
| 6 | 1664 | C=O stretching | C=O of amide group |
| 7 | 663 | C-S stretching | C-S-C bond of thiadiazole ring |
| 8 | 638 | C-Br stretching | bromo group of benzene ring |


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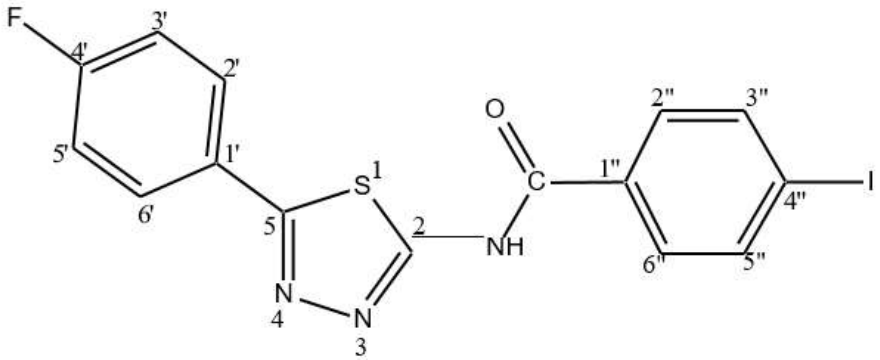
**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 151.9 | C-2 of thiadiazole ring |
| 2 | 165.5 | C-5 of thiadiazole ring |
| 3 | 161.1 | C-4' of benzene ring |
| 4 | 129.1 | C-2' & C-6' of benzene ring |
| 5 | 128.5 | C-1' of benzene ring |
| 6 | 117.1 | C-3'& C-5' of benzene ring |
| 7 | 165.3 | Carbonyl carbon of benzamide |
| 8 | 137.6 | C-4" of benzene ring |
| 9 | 130.2 | C-2" & C-6" of benzene ring |
| 10 | 131.9 | C-1" of benzene ring |
| 11 | 128.6 | C-3"& C-5" of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 378 | M+ | molecular ion peak |




Compound Code: 5 **(c)**

Chemical Name: 4"-Iodo-N-[5-(4'-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]-benzamide

TLC ($R_f$ Value): 0.84

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**Elemental Analysis:**

| S. No. | Elements | *Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | ND | 42.37 |
| 2 | Hydrogen | ND | 2.13 |
| 3 | Nitrogen | ND | 9.88 |
| 4 | Sulphur | ND | 7.54 |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3176 | N-H stretching | N-H bond of amide group |
| 2 | 2956 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1646 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1544, 1470 | C=C stretching | C-C bonds of benzene ring |
| 5 | 1140 | C-F streching | Fluoro group of benzene ring |
| 6 | 658 | C-I stretching | Iodo group of benzene ring |
| 7 | 1674 | C=O stretching | C=O of amide group |
| 8 | 678 | C-S stretching | C-S-C bond of thiadiazole ring |


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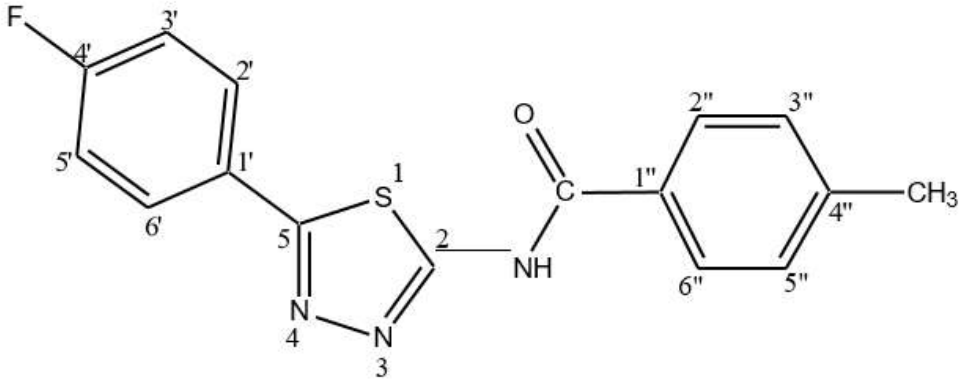
**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.3 | C-2 of thiadiazole ring |
| 2 | 165.6 | C-5 of thiadiazole ring |
| 3 | 161.2 | C-4' of benzene ring |
| 4 | 129.2 | C-2' & C-6' of benzene ring |
| 5 | 128.3 | C-1' of benzene ring |
| 6 | 116.9 | C-3'& C-5' of benzene ring |
| 7 | 165.5 | Carbonyl carbon of benzamide |
| 8 | 105.0 | C-4" of benzene ring |
| 9 | 128.8 | C-2" & C-6" of benzene ring |
| 10 | 131.8 | C-1" of benzene ring |
| 11 | 136.5 | C-3"& C-5" of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 425 | M+ | molecular ion peak |




Compound Code: 5 **(d)**

Chemical Name: 4"-Methyl-N-[5-(4'-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

 https://doi.org/10.5281/zenodo.16940719

TLC ($R_f$ Value): 0.81

**Elemental Analysis:**

| S. No. | Elements | *Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | ND | 61.33 |
| 2 | Hydrogen | ND | 3.86 |
| 3 | Nitrogen | ND | 13.41 |
| 4 | Sulphur | ND | 10.23 |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3180 | N-H stretching | N-H bond of amide group |
| 2 | 3092 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2897 | C-H stretching | C-H bonds of $CH_3$ group (Aliphatic) |
| 4 | 1644 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1550, 1475 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1445 | C-H bending | C-H bonds of $CH_3$ group (Aliphatic) |
| 7 | 1131 | C-F stretching | Fluoro group of benzene ring |
| 8 | 1675 | C=O stretching | C=O of amide group |
| 9 | 680 | C-S stretching | C-S-C bond of thiadiazole |

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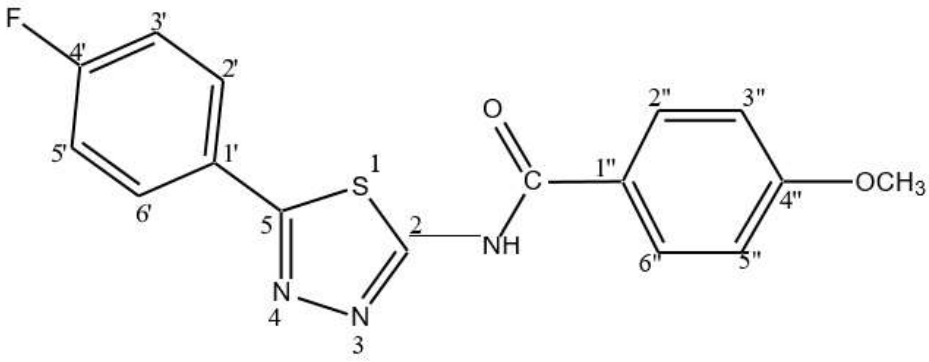
**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.1 | C-2 of thiadiazole ring |
| 2 | 165.5 | C-5 of thiadiazole ring |
| 3 | 161.1 | C-4' of benzene ring |
| 4 | 130.1 | C-2' & C-6' of benzene ring |
| 5 | 129.7 | C-1' of benzene ring |
| 6 | 117.0 | C-3'& C-5' of benzene ring |
| 7 | 165.3 | Carbonyl carbon of benzamide |
| 8 | 141.6 | C-4" of benzene ring |
| 9 | 127.5 | C-2" & C-6" of benzene ring |
| 10 | 131.8 | C-1" of benzene ring |
| 11 | 129.1 | C-3"& C-5" of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 313 | M+ | molecular ion peak |




Compound Code: 5 **(e)**

Chemical Name: 4"-Methoxy-N-[5-(4'-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.86

 https://doi.org/10.5281/zenodo.16940719

**Elemental Analysis:**

| S. No. | Elements | *Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | ND | 58.35 |
| 2 | Hydrogen | ND | 3.67 |
| 3 | Nitrogen | ND | 12.76 |
| 4 | Sulphur | ND | 9.74 |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3184 | N-H stretching | N-H bond of amide group |
| 2 | 2958 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2850 | C-H stretching | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 4 | 1656 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1565, 1459 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1422 | C-H bending | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 7 | 1104 | C-F stretching | Fluoro group of benzene ring |
| 8 | 1672 | C=O stretching | C=O of amide group |
| 9 | 684 | C-S stretching | C-S-C bond of thiadiazole |
| 10 | 1022 | C-O stretching | C-O-C bond of aryl alkyl ether |

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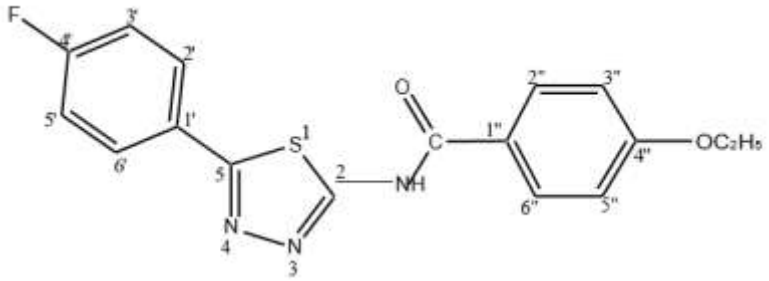
**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.7 | C-2 of thiadiazole ring |
| 2 | 167.2 | C-5 of thiadiazole ring |
| 3 | 165.2 | C-4' of benzene ring |
| 4 | 131.9 | C-2' & C-6' of benzene ring |
| 5 | 129.2 | C-1' of benzene ring |
| 6 | 117.1 | C-3'& C-5' of benzene ring |
| 7 | 165.7 | Carbonyl carbon of benzamide |
| 8 | 142.8 | C-4" of benzene ring |
| 9 | 138.1 | C-2" & C-6" of benzene ring |
| 10 | 137.2 | C-1" of benzene ring |
| 11 | 114.5 | C-3"& C-5" of benzene ring |
| 12 | 55.7 | $OCH_3$ carbon at C-4" of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 329 | M+ | molecular ion peak |




Compound Code: **5 (f)**

Chemical Name: 4"-Ethoxy-N-[5-(4'-fluoro-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.85

 https://doi.org/10.5281/zenodo.16940719

**Elemental Analysis:**

| S. No. | Elements | *Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | ND | 45.24 |
| 2 | Hydrogen | ND | 3.13 |
| 3 | Nitrogen | ND | 9.31 |
| 4 | Sulphur | ND | 7.11 |

*Elemental analysis of compounds containing Fluorine would not be determined by Research Laboratories in India.

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3160 | N-H stretching | N-H bond of amide group |
| 2 | 3092 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2885 | C-H stretching | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 4 | 1646 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1570, 1472 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1442 | C-H bending | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 7 | 1126 | C-F stretching | Fluoro group of benzene ring |
| 8 | 1668 | C=O stretching | C=O of amide group |
| 9 | 1085 | C-O stretching | C-O-C bond of aryl alkyl ether |
| 10 | 682 | C-S stretching | C-S-C bond of thiadiazole |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

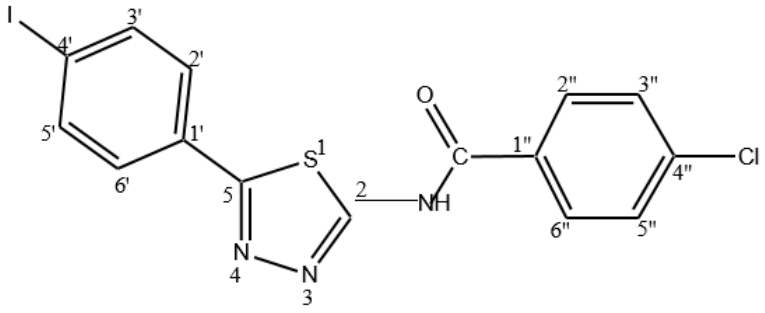
| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 151.8 | C-2 of thiadiazole ring |
| 2 | 166.1 | C-5 of thiadiazole ring |
| 3 | 160.7 | C-4’ of benzene ring |

 https://doi.org/10.5281/zenodo.16940719

| | | |
|---|---|---|
| 4 | 130.0 | C-2’ & C-6’ of benzene ring |
| 5 | 128.7 | C-1’ of benzene ring |
| 6 | 115.4 | C-3’& C-5’ of benzene ring |
| 7 | 154.3 | Carbonyl carbon of benzamide |
| 8 | 138.6 | C-4” of benzene ring |
| 9 | 129.3 | C-2” & C-6” of benzene ring |
| 10 | 125.6 | C-1” of benzene ring |
| 11 | 114.5 | C-3”& C-5” of benzene ring |
| 12 | 64.7 | $O\underline{C}H_2CH_3$ carbon at C-4” of benzene ring |
| 13 | 14.9 | $OCH_2\underline{C}H_3$ carbon at C-4” of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 451 | M+ | molecular ion peak |




Compound Code: **5 (g)**

Chemical Name: 4”-Chloro-N-[5-(4’-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.76

**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 40.47 | 40.79 |
| 2 | Hydrogen | 1.94 | 2.05 |

 https://doi.org/10.5281/zenodo.16940719

| | | | |
|---|---|---|---|
| 3 | Nitrogen | 9.26 | 9.51 |
| 4 | Sulphur | 7.05 | 7.26 |

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3145 | N-H stretching | N-H bond of amide group |
| 2 | 3088 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1645 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1540, 1469 | C=C stretching | C-C bonds of benzene ring |
| 5 | 658 | C-I stretching | Iodo group of benzene ring |
| 7 | 1676 | C=O stretching | C=O of amide group |
| 8 | 683 | C-S stretching | C-S-C bond of thiadiazole |
| 9 | 774 | C-Cl streching | Chloro group of benzene ring |

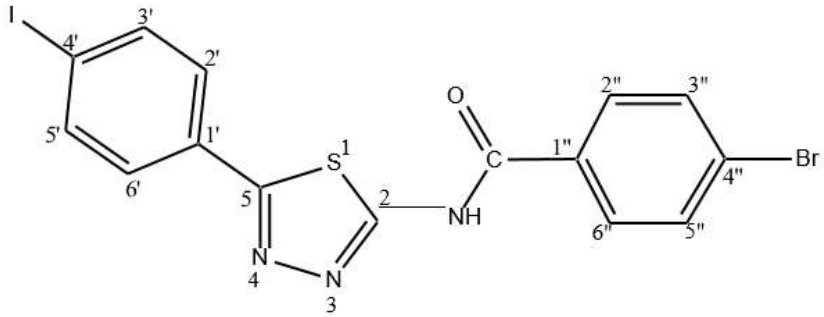
**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.4 | C-2 of thiadiazole ring |
| 2 | 167.2 | C-5 of thiadiazole ring |
| 3 | 93.7 | C-4' of benzene ring |
| 4 | 129.6 | C-2' & C-6' of benzene ring |
| 5 | 132.4 | C-1' of benzene ring |
| 6 | 138.3 | C-3'& C-5' of benzene ring |
| 7 | 165.5 | Carbonyl carbon of benzamide |
| 8 | 137.4 | C-4" of benzene ring |
| 9 | 130.5 | C-2" & C-6" of benzene ring |
| 10 | 131.3 | C-1" of benzene ring |
| 11 | 129.1 | C-3"& C-5" of benzene ring |


https://doi.org/10.5281/zenodo.16940719

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 441 | M+ | molecular ion peak |




Compound Code: **5 (h)**

Chemical Name: 4”-Bromo-N-[5-(4’-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.81

**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 36.84 | 37.06 |
| 2 | Hydrogen | 1.73 | 1.87 |
| 3 | Nitrogen | 8.49 | 8.64 |
| 4 | Sulphur | 6.44 | 6.60 |

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3138 | N-H stretching | N-H bond of amide group |
| 2 | 2972 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1642 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1508, 1454 | C=C stretching | C-C bonds of benzene ring |
| 5 | 646 | C-I stretching | fluoro group of benzene ring |
| 7 | 1677 | C=O stretching | C=O of amide group |

 https://doi.org/10.5281/zenodo.16940719

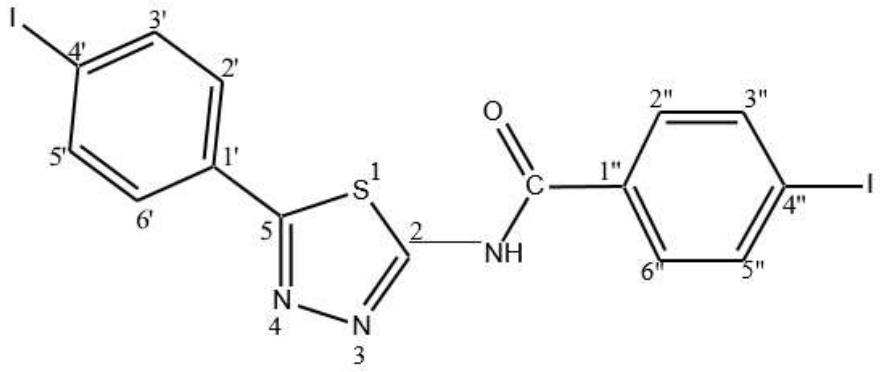
| | | | |
|---|---|---|---|
| 8 | 681 | C-S stretching | C-S-C bond of thiadiazole |
| 9 | 710 | C-Br streching | bromo group of benzene ring |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.2 | C-2 of thiadiazole ring |
| 2 | 168.5 | C-5 of thiadiazole ring |
| 3 | 93.9 | C-4' of benzene ring |
| 4 | 129.4 | C-2' & C-6' of benzene ring |
| 5 | 131.9 | C-1' of benzene ring |
| 6 | 138.2 | C-3'& C-5' of benzene ring |
| 7 | 165.3 | Carbonyl carbon of benzamide |
| 8 | 133.4 | C-4" of benzene ring |
| 9 | 129.8 | C-2" & C-6" of benzene ring |
| 10 | 131.8 | C-1" of benzene ring |
| 11 | 131.5 | C-3"& C-5" of benzene ring |

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 486 | M+ | molecular ion peak |




Compound Code: **5 (i)**

Chemical Name: 4"-Iodo-N-[5-(4'-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]-benzamide

Molecular Weight: 533.13 TLC ($R_f$ Value): 0.84

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**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 33.57 | 33.79 |
| 2 | Hydrogen | 1.58 | 1.70 |
| 3 | Nitrogen | 7.72 | 7.88 |
| 4 | Sulphur | 5.84 | 6.01 |

**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3158 | N-H stretching | N-H bond of amide group |
| 2 | 3086 | C-H stretching | C-H bonds of benzene ring |
| 3 | 1641 | C=N stretching | C-N bond of thiadiazole ring |
| 4 | 1542, 1481 | C=C stretching | C-C bonds of benzene ring |
| 5 | 654 | C-I stretching | Iodo group of benzene ring |
| 7 | 1692 | C=O stretching | C=O of amide group |
| 8 | 671 | C-S stretching | C-S-C bond of thiadiazole |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 152.1 | C-2 of thiadiazole ring |
| 2 | 169.1 | C-5 of thiadiazole ring |
| 3 | 94.0 | C-4’ of benzene ring |
| 4 | 129.2 | C-2’ & C-6’ of benzene ring |
| 5 | 130.4 | C-1’ of benzene ring |
| 6 | 138.0 | C-3’& C-5’ of benzene ring |
| 7 | 165.1 | Carbonyl carbon of benzamide |
| 8 | 97.8 | C-4” of benzene ring |
| 9 | 129.3 | C-2” & C-6” of benzene ring |
| 10 | 133.0 | C-1” of benzene ring |
| 11 | 134.7 | C-3”& C-5” of benzene ring |


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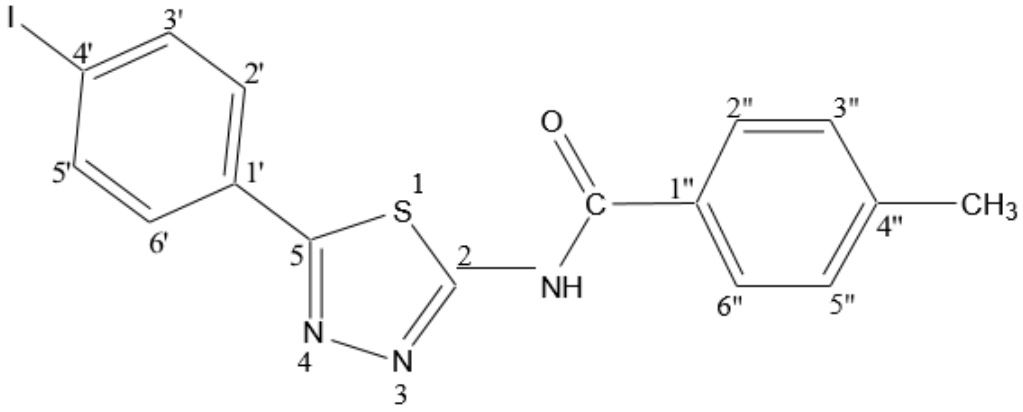
**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 533 | M+ | molecular ion peak |




Compound Code: **5 (j)**

Chemical Name: 4”-Methyl-N-[5-(4’-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.83

**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 45.24 | 45.62 |
| 2 | Hydrogen | 2.56 | 2.87 |
| 3 | Nitrogen | 9.69 | 9.97 |
| 4 | Sulphur | 7.43 | 7.61 |

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**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3151 | N-H stretching | N-H bond of amide group |
| 2 | 3082 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2895 | C-H stretching | C-H bonds of $CH_3$ group (Aliphatic) |
| 4 | 1640 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1564, 1482 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1446 | C-H bending | C-H bonds of $CH_3$ group (Aliphatic) |
| 7 | 648 | C-I stretching | Iodo group of benzene ring |
| 8 | 1682 | C=O stretching | C=O of amide group |
| 9 | 674 | C-S stretching | C-S-C bond of thiadiazole acid |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 151.9 | C-2 of thiadiazole ring |
| 2 | 168.7 | C-5 of thiadiazole ring |
| 3 | 94.1 | C-4’ of benzene ring |
| 4 | 128.8 | C-2’ & C-6’ of benzene ring |
| 5 | 132.0 | C-1’ of benzene ring |
| 6 | 135.6 | C-3’& C-5’ of benzene ring |
| 7 | 165.4 | Carbonyl carbon of benzamide |
| 8 | 141.7 | C-4” of benzene ring |
| 9 | 127.5 | C-2” & C-6” of benzene ring |
| 10 | 131.2 | C-1” of benzene ring |
| 11 | 129.3 | C-3”& C-5” of benzene ring |
| 12 | 21.2 | $\underline{C}H_3$ carbon at C-4” of benzene ring |


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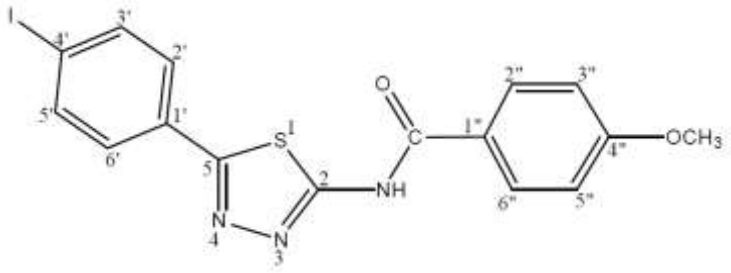
**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 421 | M+ | molecular ion peak |



Compound Code: **5 (k)**
Chemical Name: 4”-Methoxy-N-[5-(4’-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide
TLC ($R_f$ Value): 0.78

**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 43.68 | 43.95 |
| 2 | Hydrogen | 2.55 | 2.77 |
| 3 | Nitrogen | 9.46 | 9.61 |
| 4 | Sulphur | 7.20 | 7.33 |

 https://doi.org/10.5281/zenodo.16940719

**IR (KBr ν, $cm^{-1}$):**

| S.No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3152 | N-H stretching | N-H bond of amide group |
| 2 | 3088 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2892 | C-H stretching | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 4 | 1643 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1565, 1479 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1443 | C-H bending | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 7 | 650 | C-I stretching | Iodo group of benzene ring |
| 8 | 1679 | C=O stretching | C=O of amide group |
| 9 | 667 | C-S stretching | C-S-C bond of thiadiazole |
| 10 | 1082 | C-O stretching | C-O-C bond of aryl alkyl ether |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 151.7 | C-2 of thiadiazole ring |
| 2 | 169.4 | C-5 of thiadiazole ring |
| 3 | 93.6 | C-4' of benzene ring |
| 4 | 128.7 | C-2' & C-6' of benzene ring |
| 5 | 130.2 | C-1' of benzene ring |
| 6 | 134.8 | C-3'& C-5' of benzene ring |
| 7 | 165.5 | Carbonyl carbon of benzamide |
| 8 | 160.3 | C-4" of benzene ring |
| 9 | 128.5 | C-2" & C-6" of benzene ring |
| 10 | 126.4 | C-1" of benzene ring |
| 11 | 114.5 | C-3"& C-5" of benzene ring |
| 12 | 55.9 | O$\underline{C}H_3$ carbon at C-4" of benzene ring |

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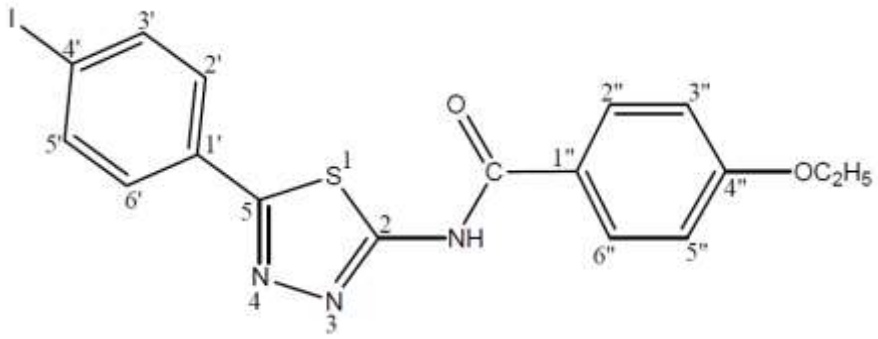
**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 437 | M+ | molecular ion peak |




Compound Code: **5 (l)**

Chemical Name: 4”-Ethoxy-N-[5-(4’-Iodo-phenyl)-[1,3,4]thiadiazole-2-yl]- benzamide

TLC ($R_f$ Value): 0.86

**Elemental Analysis:**

| S. No. | Elements | Found (%) | Calculated (%) |
|---|---|---|---|
| 1 | Carbon | 44.94 | 45.24 |
| 2 | Hydrogen | 3.02 | 3.13 |
| 3 | Nitrogen | 9.18 | 9.31 |
| 4 | Sulphur | 6.95 | 7.11 |

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**IR (KBr ν, $cm^{-1}$):**

| S. No. | Wave Number ($cm^{-1}$) | Mode of vibration | due to |
|---|---|---|---|
| 1 | 3128 | N-H stretching | N-H bond of amide group |
| 2 | 3085 | C-H stretching | C-H bonds of benzene ring |
| 3 | 2890 | C-H stretching | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 4 | 1632 | C=N stretching | C-N bond of thiadiazole ring |
| 5 | 1572, 1484 | C=C stretching | C-C bonds of benzene ring |
| 6 | 1440 | C-H bending | C-H bonds of $CH_3$ & $CH_2$ group (Aliphatic) |
| 7 | 1084 | C-O stretching | C-O-C bond of aryl alkyl ether |
| 8 | 658 | C-I stretching | Iodo group of benzene ring |
| 9 | 1674 | C=O stretching | C=O of amide group |
| 10 | 665 | C-S stretching | C-S-C bond of thiadiazole |

**$^{13}$C NMR (DMSO-$d_6$, TMS):**

| S. No. | chemical shifts, δ ppm | due to |
|---|---|---|
| 1 | 151.8 | C-2 of thiadiazole ring |
| 2 | 170.1 | C-5 of thiadiazole ring |
| 3 | 93.8 | C-4' of benzene ring |
| 4 | 128.7 | C-2' & C-6' of benzene ring |
| 5 | 130.6 | C-1' of benzene ring |
| 6 | 135.1 | C-3'& C-5' of benzene ring |
| 7 | 164.8 | Carbonyl carbon of benzamide |
| 8 | 161.2 | C-4" of benzene ring |
| 9 | 128.4 | C-2" & C-6" of benzene ring |
| 10 | 125.8 | C-1" of benzene ring |
| 11 | 115.1 | C-3"& C-5" of benzene ring |
| 12 | 64.3 | $O\underline{C}H_2CH_3$ carbon at C-4" of benzene ring |
| 13 | 14.4 | $OCH_2\underline{C}H_3$ carbon at C-4" of benzene ring |


https://doi.org/10.5281/zenodo.16940719

**LCMS (m/z):**

| S. No. | m/z value | Peak type | appeared as |
|---|---|---|---|
| 1 | 451 | M+ | molecular ion peak |

## RESULTS AND DISCUSSION

We synthesized and studied a series of 1, 3, 4-thiadiazoles with various substituents. Benzoic acid was first converted to hydrazides and thiosemicarbazides; these, in turn, were cyclized with the right reagents to give 1, 3, 4-thiadiazoles. Whenever it was determined that it would be beneficial to decrease the reaction time, microwave irradiation was employed. At the end of each reaction, the product was recrystallized to remove impurities. At each stage, the melting points were ascertained. Using thin layer chromatography, we confirmed that the produced chemicals were pure. Infrared, 13C-NMR, mass spectral, and elemental analyses were used to characterize the produced chemicals.

One, three, four-thiadiazole nucleus (5a-l) exhibited distinctive absorption bands at 1660–1695 $cm^{-1}$, 1405–1570 $cm^{-1}$, and 1630–1660 $cm^{-1}$, all of which are caused by stretching of the C=O and C=C bonds, respectively. A possible explanation for the occurrence of C-S-C in 1, 3, 4-thiadiazole is the existence of an absorption band between 665-690 $cm^{-1}$.

No matter whether they were substituted thioesters or amides, all of the produced compounds exhibited the characteristic absorption band at C=O of 1, 3, 4-thiadiazoles. Because of their aromatic C-H bands, all of the compounds exhibited a range of 3010-3098 cm-1. The compounds also exhibit bands corresponding to the aryl C-F, C-Cl, C-Br, and C-I stretching, which may be observed between 1040-1150, 755-795, 690-750, and 620-670 Hz, respectively.
Since the substitution process rendered the heterocyclic rings hydrogen-free, 13C NMR spectroscopy was employed to examine them. The establishment of these heterocyclic nuclei has been validated by $^{13}C$ NMR and IR spectrum data, which are in complete concordance with the compounds' predicted structures.

Chemical shift values for C-2 and C-5, respectively, were from 165 to 175 ppm and 151 to 153 ppm for compounds containing 1, 3, 4-thiadiazoles. Typical values were found around 166 ppm for compounds with a carbonyl group (>C=O), such as thiadiazole. Compounds' molecular ion (m/z) peaks (M+) validated their molecular weights in mass spectroscopy using the LC-MS technique.

### DOCKING OF THE SYNTHESIZED COMPOUNDS

Molecular docking describes this process of fitting molecules onto receptor sites. By analyzing the interactions between the ligand and the target, molecular docking aims to accurately predict the binding affinity and discover a scoring function for estimating the binding strength.in the 53rd Docking consists of two interconnected steps. These motions include the ligand searching for a certain conformation at the protein's active site. A computational docking score was used to rank the conformations.

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Molecular docking on the COX-2 site (PBD code-1PXX) was performed using the Glide XP docking tool of Molegro Virtual Docker ver. 6.0. Only five compounds, one from each heterocycle, 5(a), 5(d), 5(e), 5(g), and 9(k), were used for this purpose. The reference standard used in in vivo research, Indomethacin, was included in this docking study for comparison.

Docking scores are based on the binding energies of bonding and other non-bonding interactions between ligands and proteins; the higher the number of binding interactions, the higher the docking value will be for the compounds in question. In table 11, you can see all of the parameters that were calculated by the program.

**Table-44: Glide docking score of five potent anti-inflammatory compounds on COX-2 active site and other binding parameters**

| SN | Comp Code | Docking Score* | Lipo[a] | H bond[b] | Electro[c] | Site Map[d] | Low MW[e] | Rot Pf | Phob En[g] | Penalties[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5(a) | -8.9 | -4.83 | -0.59 | 0.04 | -1.38 | -0.34 | 0.27 | -2.07 | 0 |
| 2 | 5(d) | -8.75 | -5.45 | 0 | 0.17 | -0.64 | -0.45 | 0.32 | -2.7 | 0 |
| 3 | 5(e) | -7.8 | -6.25 | -1.4 | -0.09 | -0.4 | -0.04 | 0.18 | -2.65 | 2.86 |
| 4 | 5(g) | -6.29 | -3.62 | 0 | 0 | -0.4 | -0.5 | 0.18 | -1.95 | 0 |
| 5 | 8(k) | -5.97 | -3.77 | 0 | 0.06 | -0.27 | -0.5 | 0.18 | -1.67 | 0 |
| 6 | Indo[i] | -4.55 | -5.24 | -0.93 | -0.19 | -0.78 | -0.31 | 0.19 | -1.79 | 4.5 |

[a] Lipophilic term, [b] Hydrogen-bonding term, [c] Electrostatic interactions incorporates metal and Coulomb terms, [d]SiteMap-non-Hbonding polar-hydrophobic terms for ligand-receptor, [e]Reward for ligands having low molecular mass [f] Rotatable bond penalty, [g]Hydrophobic interactions, [h] Polar burial & desolvation penalties and penalty used other ligand contacts, [i]Indomethacin.

*The contribution of PhobEnHB (Reward for hydrophobically enclosed H-bond), PhobEnPairHB (Reward for hydrophobic and H-bonds correlation), PiCat (Reward for pi-cation bonding), ClBr (Reward for Cl or Br in a hydrophobic pocket that enclose against Asp or Glu), HBPenal (Penalty for ligands having large hydrophobic and low H-bond contacts), ExposPenal (Penalty for solvated ligand; cancels vander Waals terms) were found to be zero in Glide XP module.

## RESULTS AND DISCUSSION

A perusal of the table-5.1 shows that the reference drug, Indomethacin has low docking score than all the synthesized compounds, though the activity shown was the best. The compound 5(a) show best docking score followed by 5(d), 5(e), 5(g) and 5(k) on COX-2 protein. Using docking score as the parameter, the binding order of the compounds may be shown as-5(a)>5(d)>5(e)>5(g)>5(k)>Indomethacin.

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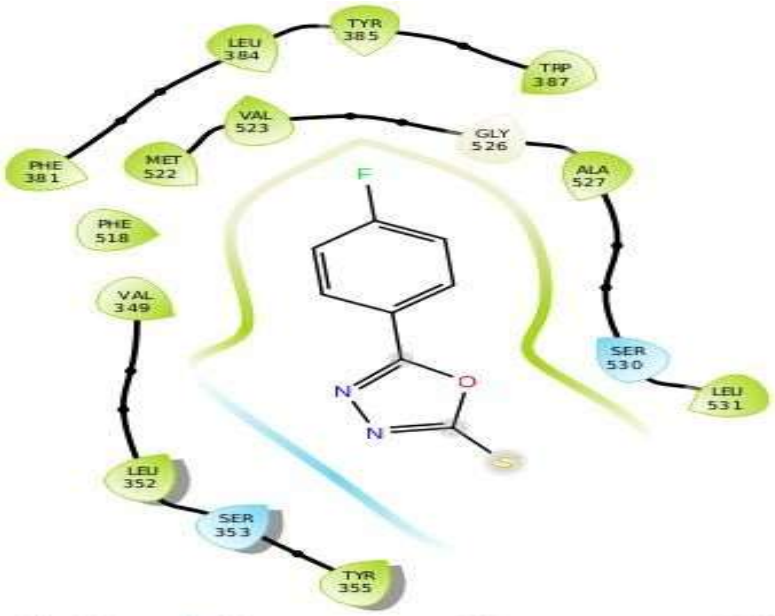
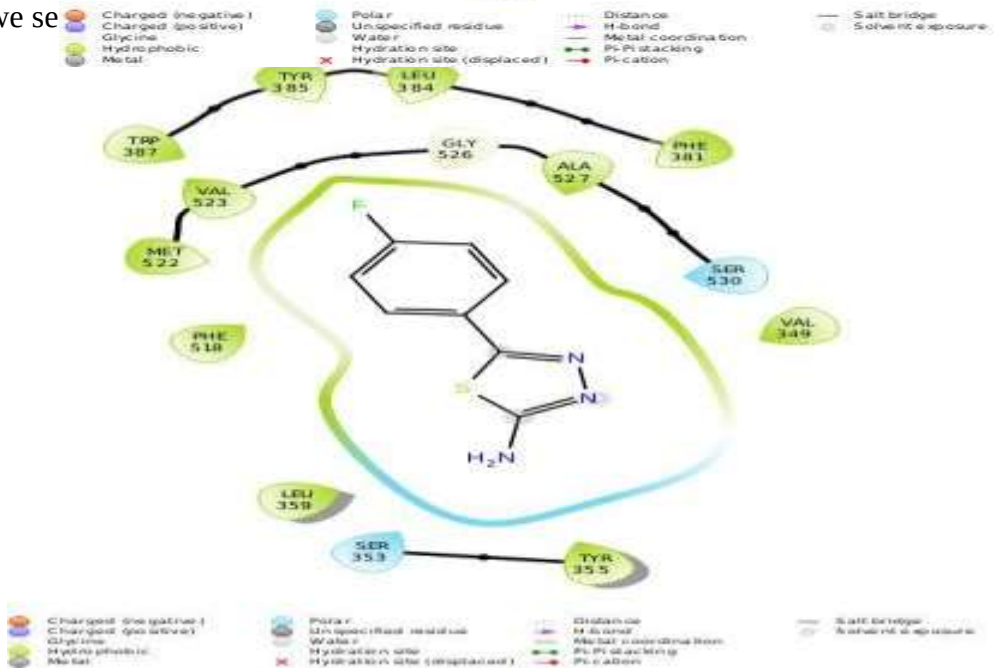
In figure-5.1; we see that there are non-bonding interactions in compound 5(g) but its docking score is good. This may be thought of non-bonding interactions which include vander Waals forces and hydrophobic interactions.




When we se




nd 5(k).

**Figure-5.2: Docking of compound 5(k) on COX-2 active site.**

In figure-5.3 which is representation of docking of Compound 5(e) on COX-2 enzyme it is seen that there are π-π stacking interaction between aromatic ring of compound and that of Tyr385 and Typ387 amino acids present on the protein. There is again presence of hydrogen bonding between oxygen of 4-methoxy and water.

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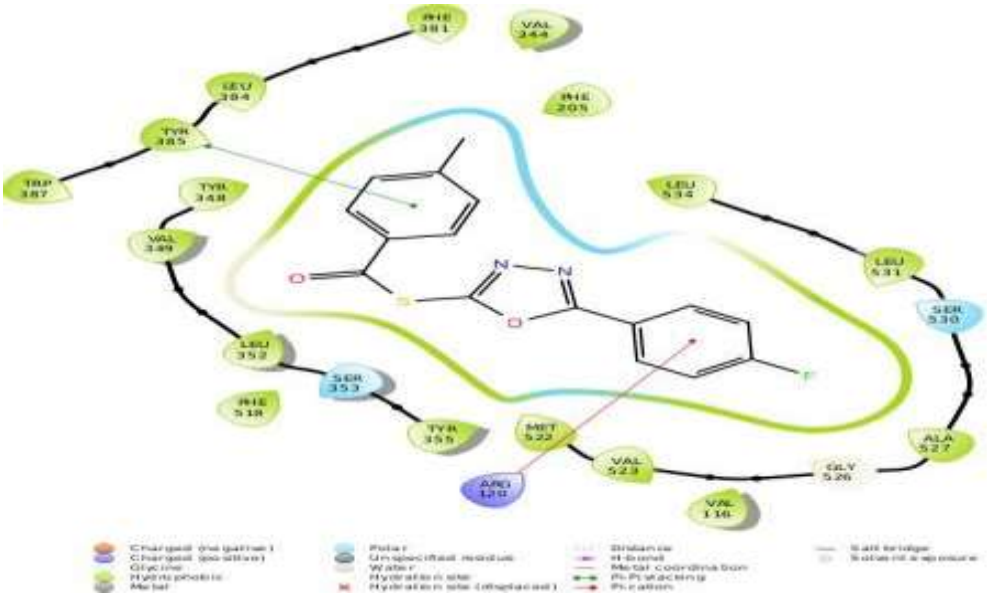


**Figure-5.3: Docking of compound 5(e) on COX-2 active site.**

Compound 5(d) in figure-5.4 shows π-π stacking interaction between the π electrons of aromatic ring of compound with that of Tyr385 of the protein. There is presence of π -cation interaction between the π electrons of the aromatic ring of the compound and cationic head of Arg120 of protein (Figure-5.5). This has been established as an important parameter for a COX-2 anti-inflammatory agent.

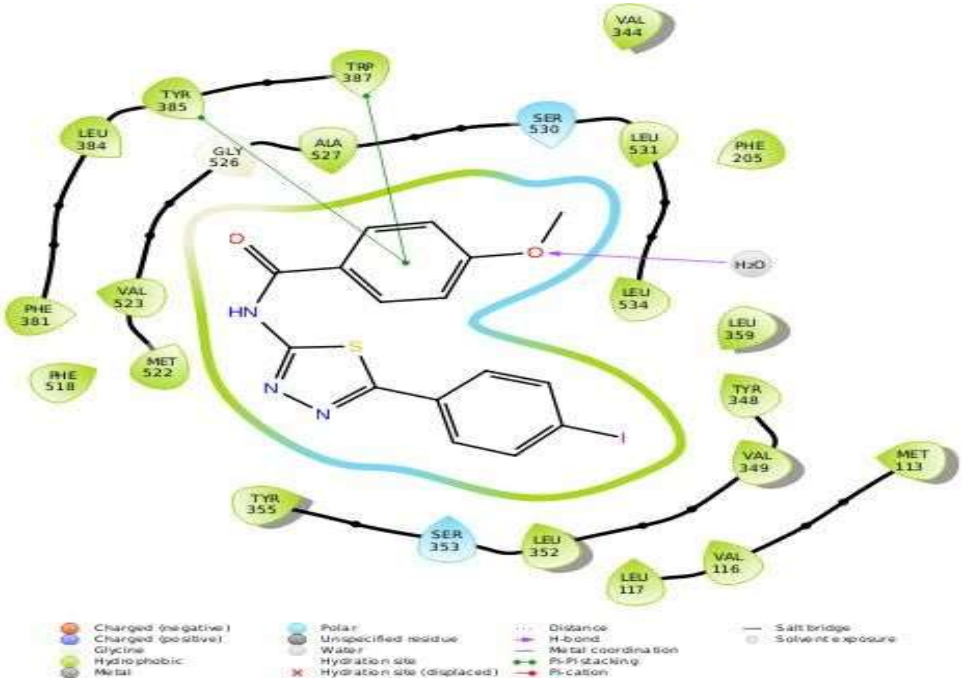


**Figure-5.4: Docking of compound 5(d) on COX-2 active site.**


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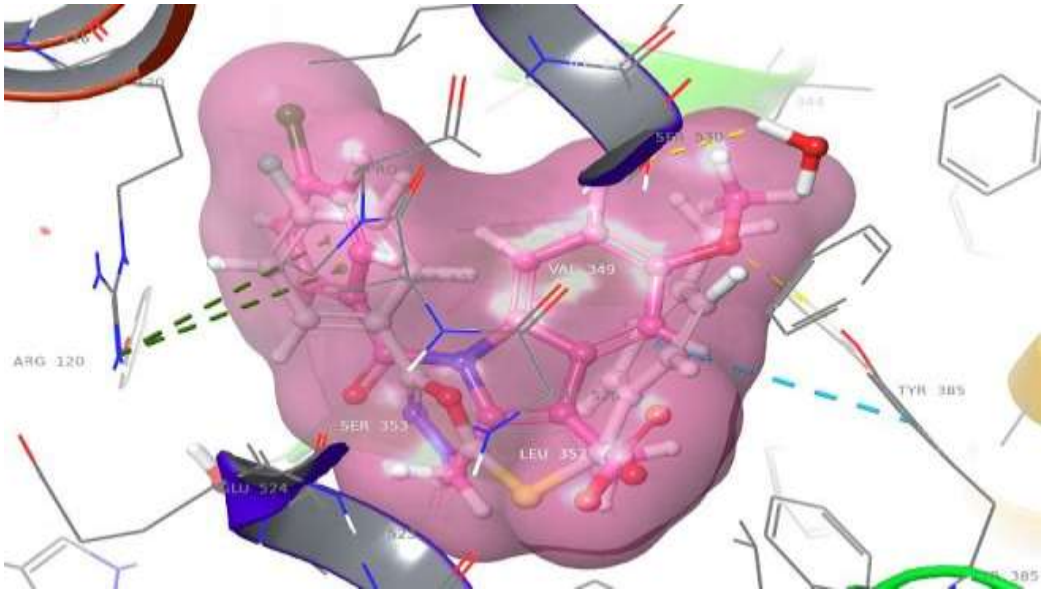


**Figure-5.5: Superimposition of compound 5(d) on Indomethcin in COX-2 pocket (Ball and stick model)**

In compound 5(a) there is π-π stacking interaction of the aromatic rings of the compound with that of Tyr385 on the protein at one place and π -cation interaction between the π electrons of the aromatic ring of the compound and cationic head of Arg120 of protein at other end (figure-5.6). Hydrogen bonding is observed between amino hydrogen and carbonyl oxygen of Val523 shown in figure-5.7.

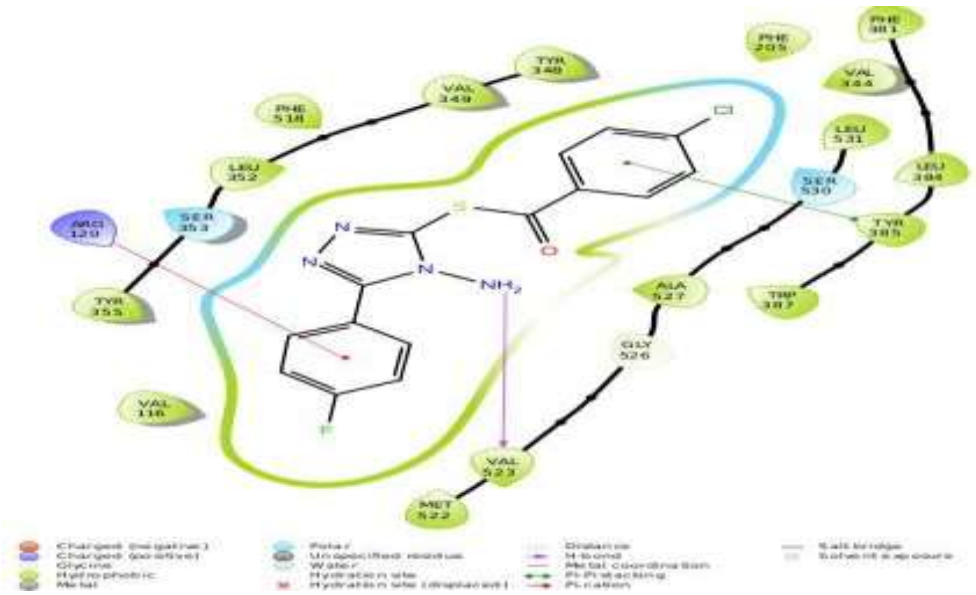


**Figure-5.6: Docking of compound 5(a) on COX-2 active site.**


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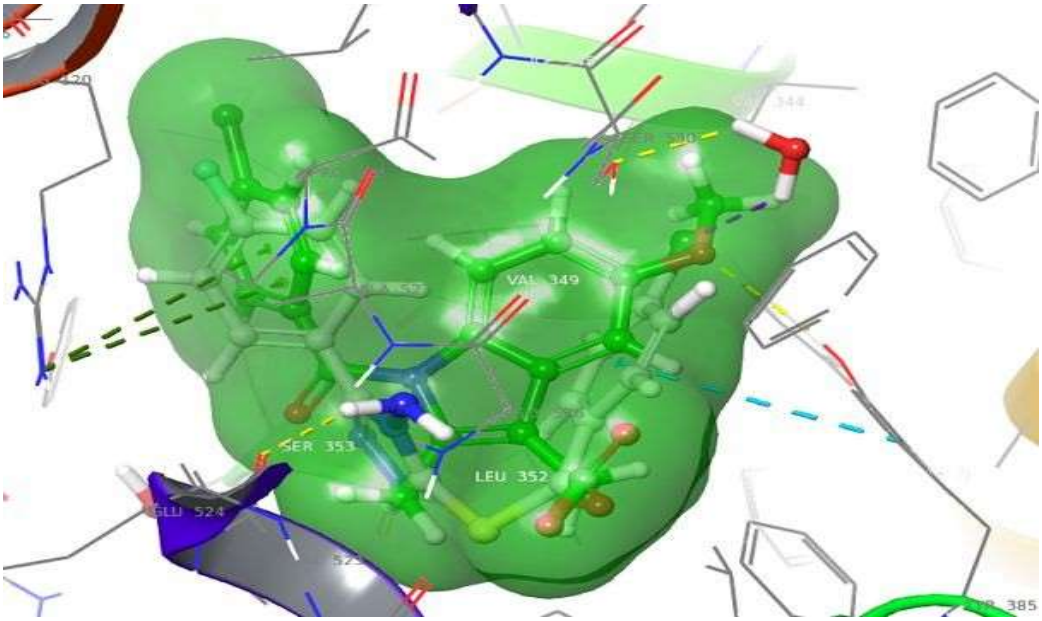


**Figure-5.7: Superimposition of compound 5(a) on Indomethcin in COX-2 pocket ( Ball and stick model)**

Docking studies of Indomethacin with the macromolecule show hydrogen bonding between oxygen of methoxy group and amino hydrogen of Tyr385 (figure- 5.8). The ball-stick model representation of further projects the interaction between π cloud of aromatic nucleus and cationic head of Arg120 (figure-5.9).

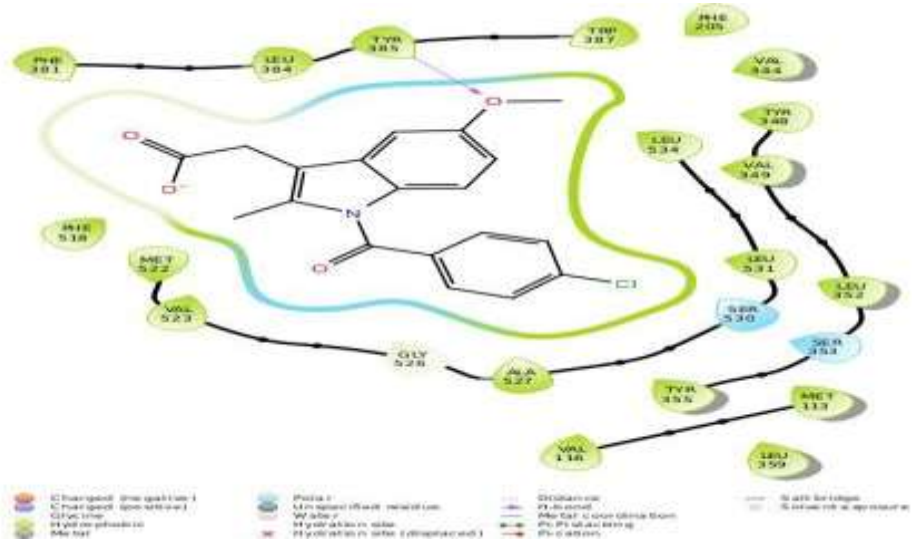

**Figure-5.8: Docking of Indomethacin on COX-2 active site.**


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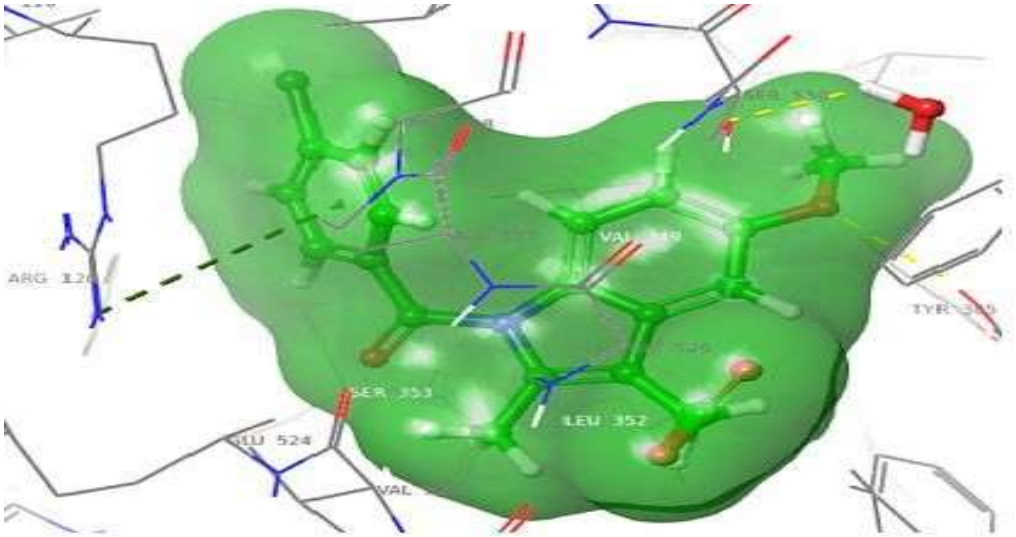


**Figure-5.9: Docking of Indomethacin on binding site of COX-2 pocket (Ball-stick docking model)**

High docking scores for compounds 5(a) and 5(d) supported by enhanced occupancy in the active site (high sitemap value) and high hydrophobic enclosure with COX-2 residues (Table-5.1) prompted us to apply the superimposition technique for these two compounds over Indomethacin on the COX-2 protein in terms of affinity on the protein.

The best fit of compound 5(a) and 5(d) in COX-2 pocket have been observed as given in figure-5.5 and figure-5.7. These best fit compounds were further checked after superimposing on Indomethacin in COX-2 pocket. These observation and comparisons made a conclusion that compound 5(d) showed the interactions on Arg120 amino acid with aromatic rings and on Tyr385 by oxygen of carbonyl group of said compounds/methoxy group of Indomethacin via same type of binding interactions.

Thus, the superimposition studies of the best compound 5(d) on the Indomethacin bound to cyclo-oxygenase-2 (COX-2) enzyme show that these two compounds bind to the said protein in the same way as the Indomethacin.

After all, seeing the superimposition of compound 5(a) and 5(d) in the COX-2 pockets, it has been concluded that out of the five selected potent compounds, compounds 5(a) and 5(d) showed good binding abilities on COX-2 protein.

## ANTIMICROBIAL RESISTANCE

### Methods used in antimicrobial screening:

Various bacteria strains have demonstrated varying degrees of susceptibility to various antimicrobial treatments. Time and pharmacological therapy can change the susceptibility. The efficacy of an antimicrobial agent against a particular pathogen can be determined by a number of tests. Antimicrobial agent evaluations should be based on dependable and repeatable procedures. The different available methods to test antimicrobial susceptibility are-

1. Agar dilution method

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2. Serial dilution method (broth dilution method)
3. Agar diffusion method
    a. Cup-plate method
    b. Paper disc method
4. Turbiditimetric method

Minimum Inhibitory Concentration (MIC) findings can be obtained by a variety of dilution procedures used in antimicrobial susceptibility testing. Soup dilution, agar dilution, and disc diffusion are the three most used approaches. The disc diffusion method yields findings measured in terms of the diameter of the zone of inhibition.

**ANTIBACTERIAL SCREENING**

**Bacterial strains and standard drug selected for Antibacterial Activity:**

For the present study the following bacterial strains were used.

1. *Staphylococcus aureus (Gram +ve), (*MTCC 3160*)*
2. *Bacillus subtilis (Gram +ve),(* MTCC 121)
3. *Escherichia coli (Gram –ve,* (MTCC 443)
4. *Pseudomonas aeruginosa (Gram –ve), (*MTCC 424)

These srains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh.

**Standard drug: Ciprofloxacin**

For this investigation, the standard antibiotic was ciprofloxacin because of its remarkable antibacterial effect against germs. It works similarly to other quinolones in that it inhibits bacterial DNA gyrase and topoisomerase IV enzymes. Interference with DNA gyrase and topoisomerase-IV renders DNA inaccessible to a cell, leading to cell death, and thus changes the DNA twisting level and releases torsional stress in the particle. Different quinolones inhibit these enzymes to different extents.

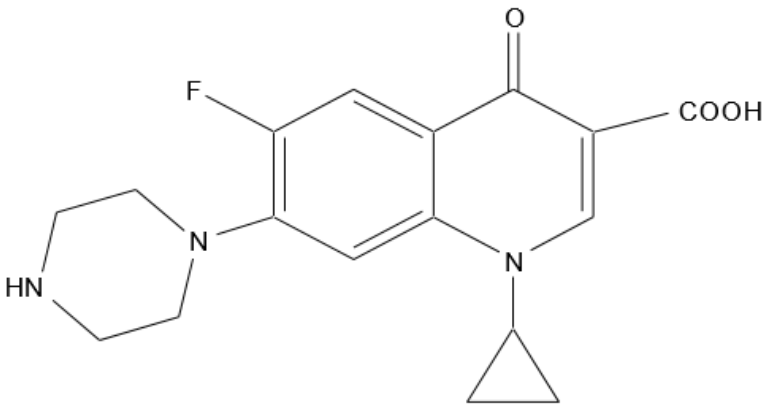


**Figure 6.1: Structure of ciprofloxacin**

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### 6.3.2 Experimental:

**Preparation of Solution of standard Drug:**

N, N-dimethylformamide (DMF) was used to generate a ciprofloxacin stock solution (1 mg/mL). Moreover, concentrations of 20 µg/mL were achieved by diluting the original substance.

**Preparation of Solution of synthesized compounds:**

A solution of the synthesized compounds was prepared in DMF at a concentration of 1 mg/mL and then diluted to a final concentration of 100µg/mL. There was a complete and utter lack of cross-contamination among the antimicrobial screening tools.

**Culture media:**

**Preparation of nutrient broth:**

| | |
|---|---|
| Beef extract | 1.0 g |
| Yeast extract | 2.0 g |
| Peptone | 5.0 g |
| Sodium chloride | 5.0 g |
| Distllied water *q.s.* | 1000 mL |

A nutritional broth was made by dissolving peptone, yeast extract, beef extract, and sodium chloride in the necessary amount of distilled water. Distilled water was used to make up the final volume, and 0.1 M sodium hydroxide was used to correct the pH to 7.2-7.3. We autoclaved the items at 121°C for 15 minutes under 15 pounds of pressure to ensure they were completely sterilized. All organisms obtained from the lab's stock were subcultured onto nutrient broth that was as clean as possible the day before the experiment. Depending on the specifics, incubation was carried out at 37°C for 18–24 hours. Inoculums were prepared from the resulting culture growth and employed in the antibacterial tests.[62]

**Preparation of nutrient agar media:**

The nutrient agar media was prepared by addition of 2% w/v agar to above nutrient broth and sterilized accordingly.

**Sterilization:**

Sterilization of media, petridishes and other materials was done by autoclaving them at 121$^{o}$C (15 psi) for 20 minutes.

**Incubation:**

The incubation was carried out in an electrically heated incubator at 37$^{o}$C ± 1$^{o}$C for 24 hours.

**Stock culture:**

The bacterial strains that had been frozen were brought from IMTECH, Chandigarh and placed in culture tubes with sterile nutritional broth. After 24 hours, the tubes were placed in an incubator that was heated electrically at 37$^{o}$C ± 1$^{o}$C. This was regarded as generic culture.

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To achieve a concentration of Colony Forming Units (CFU) ranging from 105 to 107 per milliliter, the bacteria were cultured in nutritional broth multiple times. To plant the seeds on the agar plates, this broth was utilized.

**Method of testing:**

Each 10 cm petri dish was aseptically filled with 20 mL of sterile molten agar medium. Aseptically, in a laminar air flow, a standard inoculum was added to agar plates that had been properly distributed. Carefully placed on agar plates for antibacterial evaluation were the sterile discs, which were roughly 6 mm in diameter and had previously been saturated with the test medication solution. The incubation period for the agar plates was 24 hours at a temperature of 37±1°C. We used DMF to finish the controls. The usual medication, ciprofloxacin, was taken. Triplicate runs of each experiment allowed us to calculate the average zones of inhibition. The inhibition zones, measured in millimeters, were determined using the Mean±SD. Table 12 shows the percentage of inhibition when compared to Ciprofloxacin, the reference medication. An unpaired t-test was used in the statistical analysis to determine the degree of significance, with a significance threshold of $P < 0.05$.

**Table- 12: Details of all the synthesized compounds with their codes**

| S. No. | Compound Code | X | M | Y | Z |
|---|---|---|---|---|---|
| 1. | 5(a) | -F | S | NH | -$COC_6H_4$(4-Cl) |
| 2. | 5(b) | -F | S | NH | -$COC_6H_4$(4-Br) |
| 3. | 5(c ) | -F | S | NH | -$COC_6H_4$(4-I) |
| 4. | 5(d) | -F | S | NH | -$COC_6H_4$(4-$CH_3$) |
| 5. | 5(e) | -F | S | NH | -$COC_6H_4$(4-$OCH_3$) |
| 6. | 5(f) | -F | S | NH | -$COC_6H_4$(4-$OC_2H_5$) |
| 7. | 5(g) | -I | S | NH | -$COC_6H_4$(4-Cl) |
| 8. | 5(h) | -I | S | NH | -$COC_6H_4$(4-Br) |
| 9. | 5(i) | -I | S | NH | -$COC_6H_4$(4-I) |
| 10. | 5(j) | -I | S | NH | -$COC_6H_4$(4-$CH_3$) |
| 11. | 5(k) | -I | S | NH | -$COC_6H_4$(4-$OCH_3$) |
| 12. | 5(l) | -I | S | NH | -$COC_6H_4$(4-$OC_2H_5$) |

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**Table- 13: Antibacterial activity of synthesized compounds (Zone of inhibition, ZOI and % inhibition)**

| S. No | Comp Code | *S. aureus* | | | *B. substilis* | | | *E. coli* | | | *P. aureginosa* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ZOI ± SD | % Inhib | t-value # | ZOI ± SD | % Inhib | t-value # | ZOI ± SD | % Inhib | t-value# | ZOI ± SD | % Inhib | t-value# |
| 1 | 5(a) | 19.03±0.78 | 94.3 | 2.373 | 20.21±0.91 | 93.3 | 2.680 | 20.17±0.66 | 90.3 | 2.418 | 19.13±0.81 | 94.1 | 2.434 |
| 2 | 5(b) | 18.70±0.87 | 92.7 | 2.776 | 20.17±0.91 | 93.1 | 2.753 | 19.93±1.53 | 89.2 | 2.669 | 19.17±0.81 | 94.3 | 2.353 |
| 3 | 5(c) | 15.02±0.35 | 74.5 | 19.62* | 15.70±0.27 | 72.5 | 28.10* | 16.23±0.29 | 72.7 | 25.76* | 14.77±0.66 | 72.7 | 13.50* |
| 4 | 5(d) | 11.73±0.41 | 58.2 | 29.11* | 11.77±0.42 | 54.3 | 35.80* | 15.50±0.70 | 69.4 | 15.61* | 11.70±0.42 | 57.6 | 29.94* |
| 5 | 5(e) | 12.10±0.40 | 60.0 | 28.29* | 12.43±0.35 | 57.4 | 37.21* | 14.17±0.49 | 63.5 | 24.82* | 11.60±0.40 | 57.1 | 31.33* |
| 6 | 5(f) | 11.77±0.25 | 58.4 | 38.00* | 13.13±0.25 | 60.6 | 41.81* | 11.10±0.33 | 49.7 | 44.28* | 10.67±0.81 | 52.5 | 19.60* |
| 7 | 5(g) | 19.37±0.49 | 96.0 | 2.434 | 19.87±1.15 | 91.6 | 2.649 | 20.25±1.50 | 90.7 | 2.358 | 18.94±0.92 | 93.2 | 2.511 |
| 8 | 5(h) | 13.73±0.35 | 68.1 | 24.54* | 14.63±0.45 | 67.5 | 23.69* | 13.70±0.70 | 61.4 | 19.73* | 12.57±0.41 | 61.8 | 27.38* |
| 9 | 5(i) | 13.70±0.27 | 67.9 | 28.28* | 14.30±0.36 | 66.0 | 29.12* | 11.67±0.81 | 52.3 | 21.46* | 10.43±0.77 | 51.3 | 21.01* |
| 10 | 5(j) | 10.53±0.35 | 52.2 | 36.73* | 12.10±0.27 | 55.8 | 45.05* | 13.63±0.33 | 61.0 | 34.30* | 11.27±0.45 | 55.4 | 29.90* |
| 11 | 5(k) | 11.60±0.40 | 57.5 | 30.04* | 12.33±0.31 | 56.9 | 40.62* | 12.33±0.66 | 55.2 | 24.03* | 11.23±0.35 | 55.2 | 35.66* |
| 12 | 5(l) | 10.43±0.45 | 51.7 | 31.51* | 12.23±0.49 | 56.4 | 29.72* | 11.47±0.49 | 51.4 | 33.04* | 10.47±0.29 | 51.5 | 43.10* |
| 13 | Cipro | 20.17±0.2 | 100 | 0.000 | 21.67±0.2 | 100 | 0.000 | 22.33±0.2 | 100 | 0.000 | 20.33±0.2 | 100 | 0.000 |


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| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 9 | | | 5 | | | 9 | | | 7 | | |
| 14 | DMF | - | - | - | - | - | | - | - | | - | - | |

**Minimum Inhibitory Concentration MIC**

An antimicrobial agent's minimum inhibitory concentration (MIC) is the lowest concentration at which it can halt the growth of a certain microbe. Research groups utilize MICs to rationally determine the efficacy of novel antimicrobials. In many drug development processes, screening potential library drugs for minimum inhibitory concentrations (MICs) against target bacteria is the initial step. Preclinical studies of these antimicrobials often begin with MICs because of this.

**6.4.1 Experimental: Disc Diffusion Method:**

The method developed for Shafi was modified somewhat and applied to the current inquiry. For more powerful chemicals, we simply calculated minimum inhibitory concentrations (MICs). The minimum inhibitory concentration (MIC) was defined as the quantity below which visual inspection of the sample did not reveal any signs of bacterial growth. The following concentrations of synthesized compounds in DMF were prepared: 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/mL. To test for inhibition, we ran the experiment again with varying doses. We prepared more dilutions and tested them after selecting the concentration that showed inhibition and the one that did not. The testing was carried out in this manner until the concentration was determined (Table-14). We display the MIC values as Mean±SD. A level of significance was determined using statistical analysis utilizing an unpaired t-test with a significance level of $P < 0.05$.

**Table-14: Minimum Inhibitory Concentrations (MICs, µg/mL) of title compounds against bacterial strains:**

| S. No. | Comp Code | *S. aureus* | | *B. substilis* | | *E. coli* | | *P. aeruginosa* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ±SD | t-value[#] | Mean ±SD | t-value | Mean ±SD | t-value | Mean ±SD | t-value[#] |
| 1 | 5(a) | 18.00 ±1.00 | 1.225 | 20.33 ±0.58 | 1.345 | 24.33 ±1.53 | 2.715 | 23.33 ±1.53 | 2.648 |
| 2 | 5(b) | 21.33 ±1.53 | 2.208 | 20.67 ±1.15 | 1.427 | 25.17± 2.00 | 2.883 | 23.00± 1.00 | 2.449 |
| 3 | 5(g) | 20.67 ±1.15 | 1.898 | 21.50 ±1.00 | 2.466 | 24.33 ±1.53 | 2.715 | 22.97 ±1.15 | 2.239 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | Cipro | 19.00 ±1.00 | 0.000 | 19.33 ±1.15 | 0.000 | 21.33 ±1.15 | 0.000 | 21.00 ±1.00 | 0.000 |

## RESULTS AND DISCUSSION

Three types of bacteria were utilized in the antibacterial investigations: two Gram+ bacteria, Bacillus subtilis and Staphylococcus aureus, and two Gram-negative bacteria, Escherichia coli and Pseudomonas aureginosa. We employed substances with concentrations of 100 µg/mL. The standard medication for antibacterial purposes was ciprofloxacin. The normal medication concentration of 20 µg/mL was utilized. The inhibitory effects of all the substances ranged from moderate to good. Some of the compounds that made it through the screening showed stronger antibacterial properties than the gold standard medicine (Table 6.2).

Chemicals 5(a), 5(b), and 5(g) were determined to have an active compound. The powerful drugs' minimum inhibitory concentrations (MICs) were found by employing the paper disc diffusion method. The compounds 5(a) (R=4-Cl), 5(b) (R=4-Br) and 5(g) (R=4-Cl), showed significant antibacterial actions. Therefore, the electron withdrawing groups (4-Cl, 4-Br and 4-I) on benzene ring at C-2 position of thiadiazole nucleus determine antibacterial activity.

## SUMMARY & CONCLUSION

The study focused on designing, synthesizing, and evaluating substituted 1,3,4-thiadiazoles for potential anti-inflammatory and antibacterial activity. Microwave irradiation shortened reaction times, and recrystallization ensured purity. Spectral analyses confirmed structures, with key IR and NMR signals supporting the presence of the thiadiazole nucleus. Docking studies revealed compounds 5(a) and 5(d) had strong COX-2 binding affinity, comparable to Indomethacin. Antibacterial tests via disc diffusion showed activity against Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, P. aeruginosa) strains, with notable MIC values. Findings suggest these heterocycles are promising therapeutic candidates, warranting further investigation into their mechanism and broader pharmacological potential.

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