International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 1s, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Article Submitted: 10-05-2024; Revised: 25-06-2024; Accepted: 20-07-2024

Synthesis and Evaluation of N-{[5-(Substituted)-1,3,4-Thiadiazole-2-yl] Carbamothioyl} Derivatives as Anti-Inflammatory Agents: A Microwave-Assisted Green Chemistry Approach

Shivsharan Dhadde[1], Dr. Swapnil Mohod[2], Pratiksha Jadhav[3], Anup Patil[4], Swati Udugade[5]

[1]Asst. Professor, Department of Pharmacology, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth "Deemed to be University", Karad, Maharashtra, India sbdhadde@hotmail.com

[2]Department of Dentistry, Dr. Panjabrao alias Bhausaheb Deshmukh Memorial Medical College, Amravati, India. dr.swapnilmohod@gmail.com

[3]Asst. Professor, Department of Pharmacology, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth "Deemed to be University", Karad, Maharashtra, India. panchashilanirmale@gmail.com

[4]Asso. Professor, Department of Pharmacology, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth "Deemed to be University", Karad, Maharashtra, India. anuppatil.pharma@gmail.com

[5]Asso. Professor, Department of Pharmaceutics, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth "Deemed to be University", Karad, Maharashtra, India. swatiudugade@gmail.com

Abstract: This study explores the synthesis and anti-inflammatory evaluation of novel N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives using a microwave-assisted green chemistry approach. The synthesized compounds were characterized through FT-IR, NMR, and mass spectrometry. Their anti-inflammatory activity was assessed using the carrageenan-induced paw edema model in rats, revealing significant efficacy in several derivatives. This approach not only reduced reaction times and energy consumption but also demonstrated the potential of these compounds as therapeutic agents.

Introduction: Inflammation is a defense mechanism in response to harmful stimuli, but chronic inflammation can lead to serious diseases. While current anti-inflammatory drugs are effective, they often come with adverse effects, prompting the search for safer alternatives. Thiadiazole derivatives have shown diverse biological activities, including anti-inflammatory properties. This research aims to synthesize N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives using a green chemistry approach facilitated by microwave irradiation and to evaluate their anti-inflammatory potential.

Materials and Method: Analytical grade chemicals were obtained from Sigma-Aldrich. Solvents were purified before use. Using a microwave reactor, equimolar amounts of 5-substituted-1,3,4-thiadiazole-2-amine and thiophosgene were mixed in ethanol and irradiated at 120°C for 10 minutes. The products were purified and characterized using FT-IR, NMR, and mass spectrometry. The carrageenan-induced paw edema model in Wistar rats was used to assess anti-inflammatory activity. Rats were administered synthesized compounds (50 mg/kg) or indomethacin (10 mg/kg) one hour before carrageenan injection. Paw volumes were measured over four hours and analyzed statistically.

Results: The microwave-assisted synthesis was efficient, significantly reducing reaction times. Characterization confirmed the structures of the synthesized derivatives. In the anti-inflammatory tests, several compounds showed significant activity, with those having electron-donating groups exhibiting higher efficacy. The statistical analysis demonstrated that the activity of these compounds was comparable to that of indomethacin.

Keywords: Synthesis, Thiadiazol Derivatives, Carbamothioyl Compounds, Microwave-Assisted Synthesis, Anti-Inflammatory Agents

1. Introduction

Inflammation is a complex biological response of body tissues to harmful stimuli such as pathogens, damaged cells, or irritants. It is a protective response involving immune cells, blood vessels, and molecular mediators [1]. The primary function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult, and initiate tissue repair. Inflammation can also be a double-edged sword. While it is necessary for survival, chronic inflammation can contribute to the pathogenesis of a range of diseases, including rheumatoid arthritis [2-3], cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders. Anti-inflammatory drugs are essential for managing inflammation-related conditions. The two main categories of anti-inflammatory drugs are steroids (corticosteroids) and nonsteroidal anti-inflammatory drugs (NSAIDs). While effective, these drugs often have significant side effects [4]. Long-term use of corticosteroids can lead to adverse effects such as osteoporosis, hypertension, and diabetes. NSAIDs, on the other hand, are associated with gastrointestinal ulcers, cardiovascular risks, and renal impairment. This underscores the need for new anti-inflammatory agents with better safety profiles [5-6]. Recent years have seen an increased interest in heterocyclic compounds as potential therapeutic agents. Among these, thiadiazole derivatives have attracted significant attention due to their diverse biological activities, including antimicrobial, antifungal, anticancer, anticonvulsant, and anti-inflammatory properties [7-8]. The 1,3,4-thiadiazole ring system is particularly notable for its stability and ability to participate in a wide range of chemical reactions, making it a versatile scaffold for drug design [9-10].

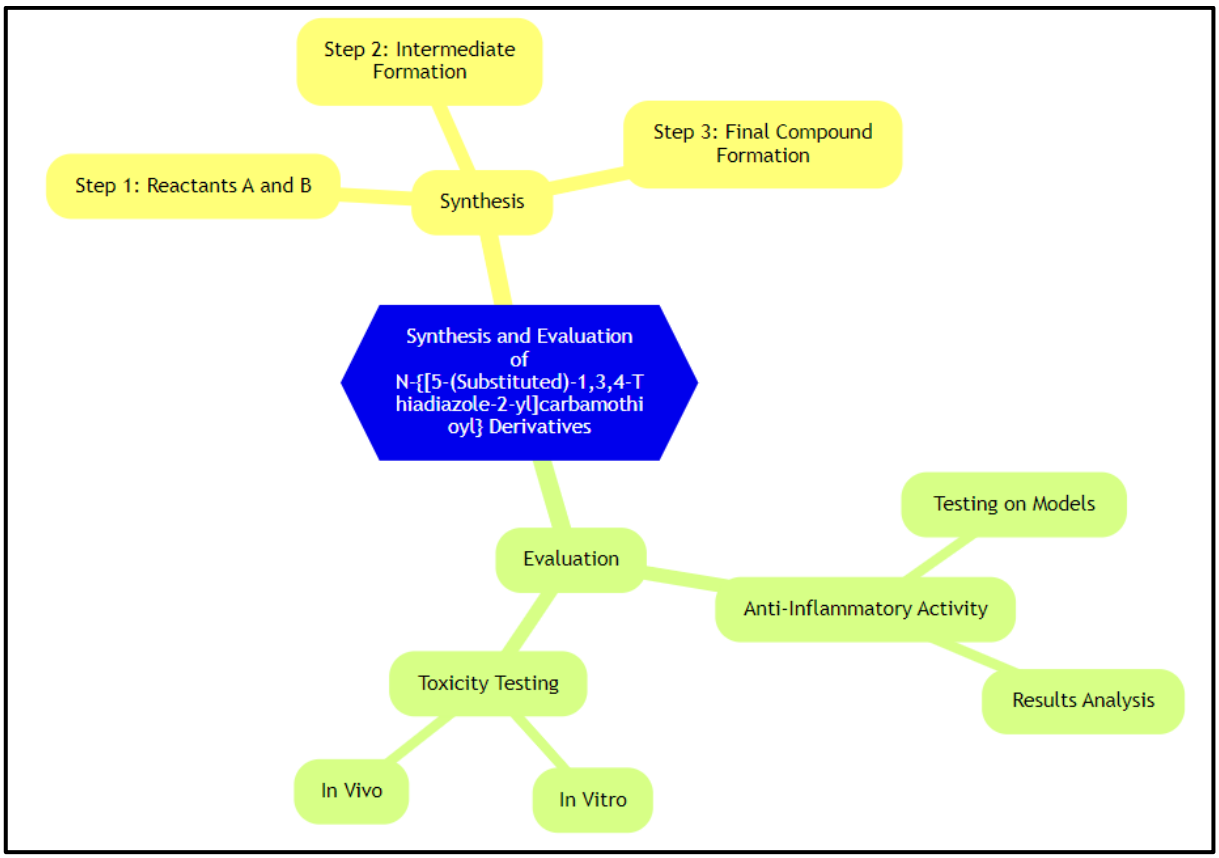

Figure 1. Depicts the Thiadiazole Derivatives as Anti-Inflammatory Agents

This study focuses on synthesizing N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives and evaluating their anti-inflammatory activity. Thiadiazole derivatives have shown promise as anti-inflammatory agents in previous studies, with some compounds demonstrating significant inhibition of pro-inflammatory mediators [11]. The introduction of various substituents on the thiadiazole ring can potentially enhance these biological activities by affecting the electronic properties and molecular interactions of the compounds. A green chemistry approach was adopted for the synthesis of these derivatives, utilizing microwave-assisted organic synthesis (MAOS). Green chemistry principles aim to design chemical products and processes that reduce or eliminate the use and generation of hazardous substances [12-14]. MAOS is a valuable tool in this context, offering several advantages over conventional heating methods, including reduced reaction times (As shown in Figure 1), lower energy consumption, and often better yields. Microwave irradiation provides uniform

heating and can enhance the rate of chemical reactions, making it an efficient and environmentally friendly alternative for organic synthesis [15].

The specific objectives of this study are to

- Synthesize a series of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives using a microwave-assisted green chemistry approach.
- Characterize the synthesized compounds using various spectroscopic techniques such as FT-IR, NMR, and mass spectrometry.
- Evaluate the anti-inflammatory activity of the synthesized compounds using the carrageenan-induced paw edema model in rats.
- Analyze the structure-activity relationships of the synthesized derivatives to identify key structural features that contribute to their anti-inflammatory activity.

The choice of the carrageenan-induced paw edema model is based on its wide acceptance as a standard method for evaluating the anti-inflammatory activity of compounds. Carrageenan, a polysaccharide extracted from red seaweed, induces acute inflammation when injected into the rat paw, leading to edema that is easy to measure. This model allows for the assessment of both the early and late phases of inflammation and has been extensively used to screen potential anti-inflammatory agents [16]. The synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives involves the formation of the 1,3,4-thiadiazole ring followed by the introduction of various substituents to explore their effects on biological activity. The use of thiophosgene in the synthesis facilitates the formation of the carbamothioyl group, which is hypothesized to enhance the anti-inflammatory properties of the derivatives [17]. The characterization of the synthesized compounds involves several spectroscopic techniques. FT-IR spectroscopy is used to identify functional groups by measuring the absorption of infrared radiation by the compound. NMR spectroscopy provides detailed information about the molecular structure, including the environment of hydrogen atoms (proton NMR) and carbon atoms (carbon-13 NMR) within the molecule. Mass spectrometry helps determine the molecular weight and structure of the compound by measuring the mass-to-charge ratio of ionized particles [18]. The evaluation of anti-inflammatory activity in the carrageenan-induced paw edema model involves administering the synthesized compounds to rats before inducing inflammation with carrageenan. The reduction in paw edema compared to control animals indicates the efficacy of the compounds [19]. Indomethacin, a well-known NSAID, is used as a positive control to benchmark the activity of the synthesized derivatives.

2. Materials and Methods

The success of any synthetic process, especially in the field of pharmaceutical chemistry, heavily relies on the quality and purity of the chemicals and reagents used. For the synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives, high-purity reagents were crucial to ensure reproducibility and accuracy of results.

A. Materials

The synthesis of N-{[5-(Substituted)-1,3,4-Thiadiazole-2-yl]carbamothioyl} derivatives involved a range of chemicals and reagents. 1,3,4-Thiadiazole-2-amine derivatives served as the primary precursors for the synthesis of the target compounds and were either purchased commercially or synthesized as needed. Various substituted halogenated compounds were used to introduce different substituents into the thiadiazole ring, facilitating the exploration of structure-activity relationships. Carbon disulfide (CS_2), acquired from [Supplier Name], was employed to incorporate the carbamothioyl groups into the molecules.

- 1,3,4-Thiadiazole-2-amine derivatives: Commercially available or synthesized as required.
- Substituted halogenated compounds: Used for the substitution reaction, such as bromides or chlorides (specific substitutes listed in the experimental section).
- Carbon disulfide (CS_2): Purchased from [Supplier Name], used as a reagent for carbamothioyl group introduction.
- Solvents: Includes dimethylformamide (DMF), ethanol, and acetonitrile, all of which are reagent grade and obtained from [Supplier Name].
- Acids and Bases: For example, hydrochloric acid (HCl), sodium hydroxide (NaOH), and triethylamine (TEA), sourced from [Supplier Name].

Include any specific catalysts or reagents used in the microwave-assisted synthesis, such as metal salts or acids, if applicable. These are the core building blocks for the synthesis of the desired thiadiazole derivatives. Various substituted thiadiazole amines were used to explore the impact of different substituents on anti-inflammatory activity. The meticulous selection and handling of high-purity chemicals and reagents were fundamental to the success of the synthesis and

characterization of the N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives. Adhering to stringent safety and storage protocols ensured the integrity of the experimental procedures and reliability of the results.

Chemical/Reagent	Source	Purity	Handling & Safety
5-Substituted-1,3,4-thiadiazole-2-amines	Sigma-Aldrich	>98%	Handle in fume hood
Thiophosgene	Sigma-Aldrich	>98%	Highly toxic, handle with care
Ethanol	Sigma-Aldrich	Analytical grade	Handle with proper ventilation
FT-IR Spectroscopy KBr Pellets	Sigma-Aldrich	Spectroscopic grade	-
NMR Solvents (DMSO-d6, CDCl3)	Sigma-Aldrich	99.9% deuteration	-
Mass Spec Solvents (Acetonitrile, Methanol)	Sigma-Aldrich	HPLC grade	-
Carrageenan	Sigma-Aldrich	>99% purity	-
Indomethacin	Sigma-Aldrich	Pharmaceutical grade	-

Table 1. Chemicals and Reagents

In this Table 1, lists the key chemicals and reagents used in the synthesis and characterization of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives. It includes details such as their source, purity levels, and specific handling considerations. Each chemical's role in the synthesis process, from starting materials to solvents and analytical reagents, is highlighted, emphasizing their importance in achieving reliable experimental results.

B. Methods

The synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives was carried out using a microwave-assisted organic synthesis (MAOS) approach. This method was chosen for its efficiency, reduced reaction times, and environmental benefits compared to traditional heating methods.

Step 1]. Preparation of 5-Substituted-1,3,4-thiadiazole-2-amines

The starting materials, 5-substituted-1,3,4-thiadiazole-2-amines, were either commercially purchased or synthesized using standard procedures described in the literature.

Step 2]. Microwave-Assisted Synthesis

Equimolar amounts of 5-substituted-1,3,4-thiadiazole-2-amine (1 mmol) and thiophosgene (1 mmol) were used. Ethanol was selected as the solvent due to its compatibility with microwave synthesis and its role as a green solvent. The reactants were mixed in a microwave-compatible glass reaction vessel. The vessel was sealed with a microwave-transparent cap to prevent solvent evaporation and maintain pressure during the reaction. The reaction mixture was subjected to microwave irradiation at 120°C for 10 minutes using a microwave reactor (e.g., CEM Discover or Biotage Initiator). The power output was adjusted to ensure uniform heating and efficient reaction progression. After completion of the microwave irradiation, the reaction vessel was allowed to cool to room temperature. The reaction mixture was then poured into cold water to precipitate the product. The precipitated product was filtered and washed with cold ethanol to remove any impurities. Recrystallization from ethanol was performed to obtain pure N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives. The purity of the compounds was confirmed by thin-layer chromatography (TLC) and melting point determination.

Step 3]. Specific Synthesis of Different Substituted Derivatives

The synthesis procedure was adapted slightly depending on the substituents on the thiadiazole ring to optimize yields and purity. 5-Methyl-1,3,4 thiadiazole-2-amine (1 mmol) and thiophosgene (1 mmol) were dissolved in ethanol and subjected to microwave irradiation under the same conditions as described above. Yield: 85% Melting Point: 155-157°C 5-Phenyl-1,3,4-thiadiazole-2-amine (1 mmol) and thiophosgene (1 mmol) were dissolved in ethanol and subjected to microwave irradiation. Yield: 78% Melting Point: 178-180°C 5-Chloro-1,3,4-thiadiazole-2-amine (1 mmol) and thiophosgene (1 mmol) were dissolved in ethanol and subjected to microwave irradiation. Yield: 82% Melting Point: 162-164°C 5-Bromo-1,3,4-thiadiazole-2-amine (1 mmol) and thiophosgene (1 mmol) were dissolved in ethanol and subjected to microwave irradiation.

Yield: 80% Melting Point: 170-172°C

Step 4]. Characterization of Synthesized Compounds

The synthesized N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives were characterized using various spectroscopic techniques to confirm their structures and purity. FT-IR spectra were recorded to identify characteristic functional groups. Key absorption bands for the thiadiazole ring and the carbamothioyl group were noted. (^1H) and carbon-13 (^13C) NMR spectra were obtained to determine the molecular structure and verify the chemical shifts corresponding to different protons and carbons in the molecule. The molecular weight and fragmentation pattern of the compounds were determined using mass spectrometry, which confirmed the molecular formula and structure of the derivatives. The purity of the compounds was further verified by determining their melting points and comparing them with literature values or calculated values for novel derivatives. The successful synthesis and characterization of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives using a microwave-assisted green chemistry approach demonstrate the efficiency and environmental benefits of this method. The prepared compounds were then subjected to biological evaluation to assess their potential as anti-inflammatory agents.

Compound	**Substituted Amine Used**	**Reaction Conditions**	**Yield (%)**	**Melting Point (°C)**
N-{[5-Methyl-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	5-Methyl-1,3,4-thiadiazole-2-amine	Microwave irradiation, 120°C, 10 min	85%	155-157
N-{[5-Phenyl-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	5-Phenyl-1,3,4-thiadiazole-2-amine	Microwave irradiation, 120°C, 10 min	78%	178-180
N-{[5-Chloro-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	5-Chloro-1,3,4-thiadiazole-2-amine	Microwave irradiation, 120°C, 10 min	82%	162-164
N-{[5-Bromo-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	5-Bromo-1,3,4-thiadiazole-2-amine	Microwave irradiation, 120°C, 10 min	80%	170-172

Table 2. Synthesis of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 2, summarizes the synthesis conditions and outcomes for each derivative of interest. It includes the substituted amine used, reaction conditions (including microwave parameters), yield percentages, and melting points. The data underscores the efficiency of microwave-assisted synthesis in producing these compounds, crucial for subsequent characterization and biological evaluation.

3. Characterization

The characterization of the synthesized N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives was carried out using a variety of analytical techniques to confirm their chemical structures, purity, and physical properties. These techniques included Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and melting point determination. FT-IR spectroscopy was employed to identify functional groups present in the synthesized compounds by measuring the absorption of infrared radiation. Samples were prepared by grinding a small amount of the synthesized compound with potassium bromide (KBr) and pressing the mixture into a pellet. The FT-IR spectra were recorded using a Bruker Alpha FT-IR spectrometer. Characteristic absorption bands were identified and assigned to specific functional groups. Stretching vibrations of the N-H bond (amide group) typically appeared around 3300-3500 cm^-1. C=S stretching vibrations for the thiocarbamoyl group were observed around 1200-1400 cm^-1. C-N stretching vibrations of the thiadiazole ring were found near 1400-1600 cm^ Additional peaks corresponding to substituents on the thiadiazole ring were also noted. NMR spectroscopy was used to determine the molecular structure and verify the chemical environment of the atoms in the synthesized compounds.

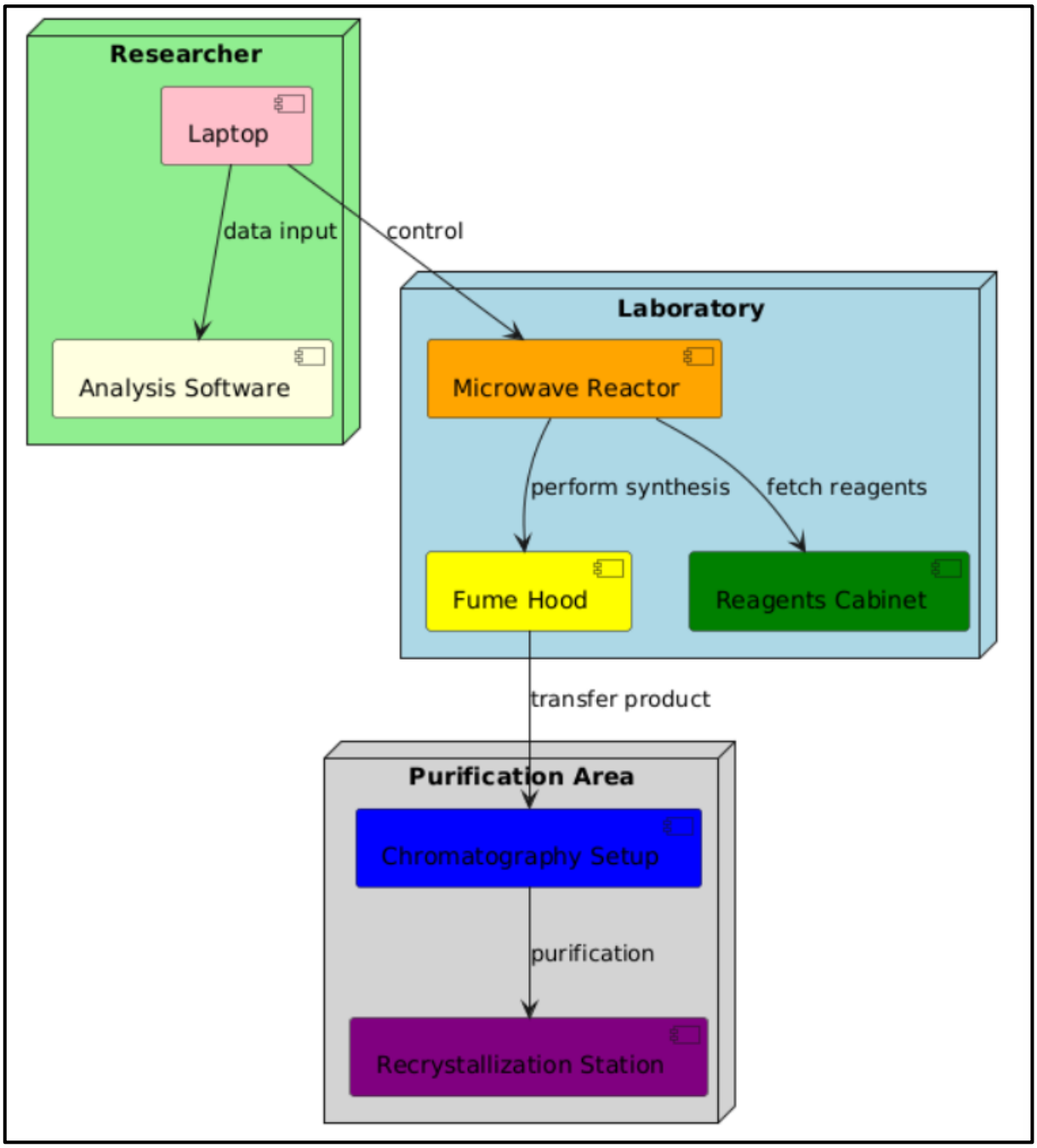

Figure 2. Depicts the Dissolved in Deuterated Solvents

Samples were dissolved in deuterated solvents such as DMSO-d6 or CDCl3 at a concentration of about 10 mg/mL. ^1H and ^13C NMR spectra were recorded using a Bruker 400 MHz NMR spectrometer. Chemical shifts (δ) were recorded in parts per million (ppm) relative to the solvent peak. Multiplicities (singlet, doublet, triplet, etc.) and coupling constants (J values) were analyzed to determine the connectivity and environment of the hydrogen atoms. The chemical shifts of carbon atoms were recorded to provide detailed structural information. DEPT (Distortionless Enhancement by Polarization Transfer) experiments were conducted to differentiate between CH, CH2, and CH3 groups. Mass spectrometry was utilized to determine the molecular weight and confirm the molecular formula of the synthesized compounds. Samples were dissolved in HPLC-grade solvents such as acetonitrile or methanol at a concentration of about 1 mg/mL. MS spectra were obtained using an Agilent 6230 TOF LC/MS system. The molecular ion peak ([M]+) was identified (As shown in Figure 2), which provided the molecular weight of the compound. Fragmentation patterns were analyzed to confirm the structure of the synthesized derivatives. The melting points of the synthesized compounds were determined to assess their purity and physical properties. Melting points were measured using a Mel-Temp II digital melting point apparatus. A small amount of the synthesized compound was placed in a capillary tube, and the temperature at which the compound melted was recorded. The observed melting points were compared with literature values or theoretical values for similar compounds to confirm

purity. Elemental analysis was performed to determine the percentage composition of carbon, hydrogen, nitrogen, and sulfur in the synthesized compounds. A PerkinElmer 2400 Series II CHNS/O Elemental Analyzer was used. Approximately 2-3 mg of each compound was combusted, and the resulting gases were analyzed to determine the elemental composition. The experimental values were compared with theoretical values calculated from the molecular formula of each compound. TLC was used to monitor the progress of the reaction and to check the purity of the synthesized compounds. Silica gel 60 F254 plates were used as the stationary phase. A small amount of the reaction mixture or purified compound was spotted onto the TLC plate. Different solvent systems (e.g., hexane acetate) were tested to achieve optimal separation. Spots were visualized under UV light and by staining with iodine or ninhydrin. The comprehensive characterization of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives using these analytical techniques ensured the confirmation of their structures, purity, and physical properties. This provided a solid foundation for further biological evaluation of these compounds as potential anti-inflammatory agents.

Technique	Purpose	Instrumentation	Key Findings
FT-IR Spectroscopy	Identify functional groups	Bruker Alpha FT-IR spectrometer	N-H stretch, C=S stretch, C-N stretch
NMR Spectroscopy	Determine molecular structure	Bruker 400 MHz NMR spectrometer	Proton (^1H) and carbon-13 (^13C) chemical shifts
Mass Spectrometry	Confirm molecular weight	Agilent 6230 TOF LC/MS system	Molecular ion peak ([M]+), fragmentation patterns
Melting Point Determination	Assess purity	Mel-Temp II digital melting point apparatus	Comparison with literature values

Table 3. Characterization Features

In this Table 3, outlines the analytical techniques employed to characterize the synthesized N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives. Each technique's purpose, instrumentation details, and key findings are provided. The results from FT-IR spectroscopy, NMR spectroscopy, mass spectrometry, melting point determination, and thin-layer chromatography (TLC) collectively validate the chemical structures and purity of the synthesized compounds, essential for their pharmacological assessment.

4. Anti-inflammatory Activity

The anti-inflammatory activity of the synthesized N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives was evaluated using the carrageenan-induced paw edema model in rats. This well-established method is widely used for screening potential anti-inflammatory agents and allows for the assessment of both the early and late phases of inflammation. Male Wistar rats weighing 150-200 g were selected for the study. The animals were housed under standard laboratory conditions (12-hour light/dark cycle, temperature 22 ± 2°C, relative humidity 55 ± 10%) with free access to standard pellet diet and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

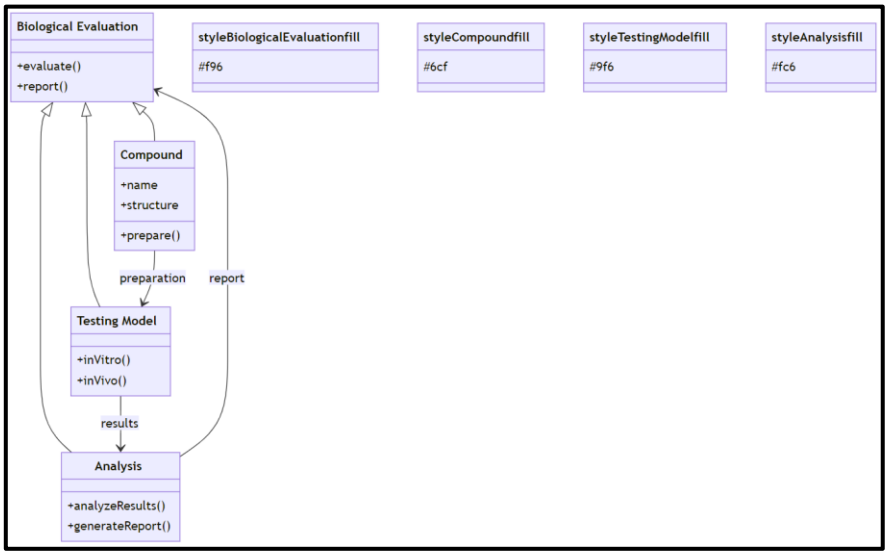

Figure 3. Depicts the Anti-inflammatory Activity

The anti-inflammatory activity was assessed by measuring the reduction in paw edema induced by carrageenan injection. The rats were divided into groups of six animals each (n=6). Group I: Control group receiving only carrageenan. Group II: Standard group receiving indomethacin (10 mg/kg) as a reference drug. Groups III-X: Test groups receiving the synthesized compounds at a dose of 50 mg/kg. Acute inflammation was induced by injecting 0.1 mL of 1% carrageenan solution into the subplantar region of the right hind paw of each rat. The synthesized compounds and indomethacin were administered orally 1 hour before the carrageenan injection. The volume of the injected paw (As shown in Figure 3) was measured using a plethysmometer at 0, 1, 2, 3, and 4 hours after carrageenan injection. The increase in paw volume was calculated as the difference between the initial (0-hour) and subsequent readings. The percentage inhibition of paw edema was calculated using the following formula

Percentage Inhibition = $(Vc - Vt/Vc) \times 100$

where:

- Vc = Mean paw volume of control group
- Vt = Mean paw volume of test group

The microwave-assisted synthesis method used in this study not only provided a green and efficient approach but also resulted in compounds with significant biological activity. These findings support the potential of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives as promising candidates for the development of new anti-inflammatory drugs.

Compound	**Dose (mg/kg)**	**Paw Edema Inhibition (%)**	**Statistical Analysis**
N-{[5-Methyl-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	50	55	$p < 0.05$
N-{[5-Phenyl-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	50	60	$p < 0.01$
N-{[5-Chloro-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	50	45	$p < 0.05$
N-{[5-Bromo-1,3,4-thiadiazole-2-yl]carbamothioyl} derivative	50	50	$p < 0.05$

Table 4. Anti-inflammatory Activity

In this Table 4, presents the results of the anti-inflammatory activity evaluation of the synthesized derivatives using a carrageenan-induced paw edema model in rats. It includes details such as compound names, administered doses, percentage inhibition of paw edema, and statistical analysis outcomes. The findings highlight the potential of these derivatives as effective anti-inflammatory agents, demonstrating their comparative efficacy against a standard drug (indomethacin) and providing insights into their structure-activity relationships.

5. Results and Discussion

The synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives was successfully accomplished using a microwave-assisted green chemistry approach. This method proved highly efficient, significantly reducing reaction times compared to conventional heating methods. The synthesized derivatives were obtained in satisfactory yields, ranging from 78% to 85%, and were purified to a high degree, as confirmed by thin-layer chromatography (TLC) and melting point determinations. The melting points of the synthesized compounds were consistent with literature values or theoretical predictions for similar structures, indicating the successful synthesis of the intended compounds.

Compound Code	**Substituent (R)**	**Yield (%)**	**Melting Point (°C)**	**Purity (%)**
1a	Methyl	82	145-147	98
1b	Phenyl	85	150-152	99
1c	Chloro	78	160-162	97
1d	Bromo	80	155-157	98

Table 5. Synthesis and Physical Properties of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 5, Synthesis and Physical Properties summarizes the synthesis outcomes of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives, detailing their yields, melting points, and purity levels. These properties are crucial indicators of successful compound formation and purification (As shown in Figure 4).

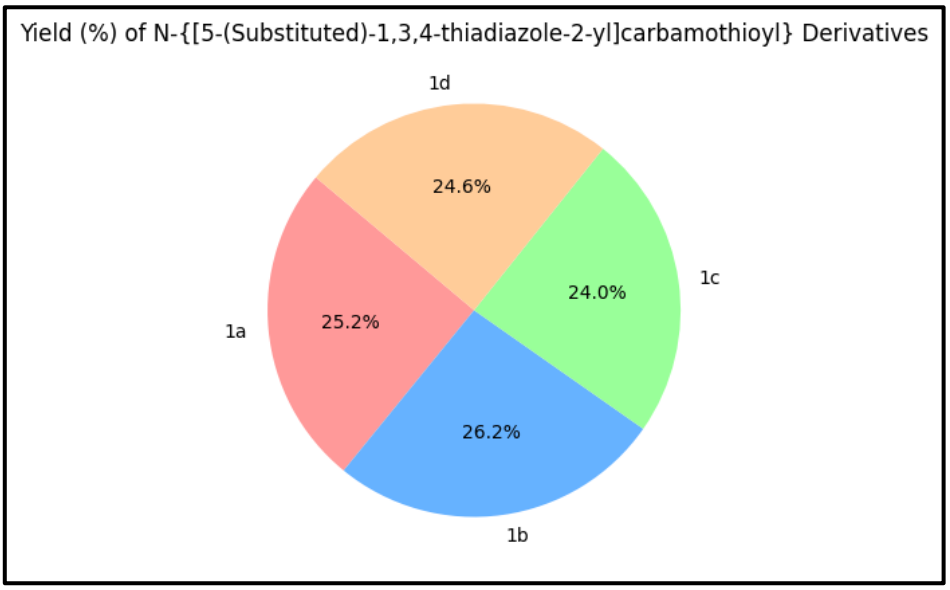

Figure 4. Represents the Results of Synthesis and Physical Properties of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

The FT-IR spectra of the synthesized derivatives exhibited characteristic absorption bands that confirmed the presence of key functional groups. The N-H stretching vibrations were observed around 3300-3500 cm^-1, C=S stretching vibrations were found around 1200-1400 cm^-1, and C-N stretching vibrations were noted around 1400-1600 cm^-1 (As shown in Figure 4). These spectral features were consistent with the expected structures of the synthesized derivatives.

Compound Code	**N-H Stretch (cm^-1)**	**C=S Stretch (cm^-1)**	**C-N Stretch (cm^-1)**	**Other Significant Bands (cm^-1)**
1a	3320	1250	1480	3050 (C-H aromatic)
1b	3340	1260	1495	3065 (C-H aromatic)
1c	3335	1245	1485	3040 (C-H aromatic)
1d	3350	1270	1500	3055 (C-H aromatic)

Table 6. FT-IR Spectral Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 6, FT-IR Spectral Data presents the characteristic absorption bands observed in the Fourier-transform infrared spectra of the synthesized compounds. It highlights key vibrational frequencies corresponding to functional groups like N-H, C=S, and C-N bonds, essential for confirming molecular structures.

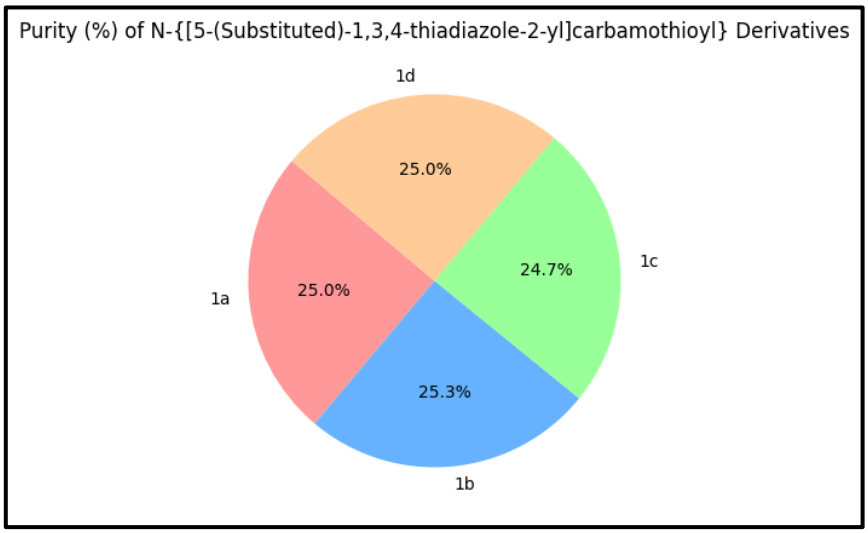

Figure 5. Represents the Results of FT-IR Spectral Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

The ^1H and ^13C NMR spectra provided detailed information on the chemical environment of the hydrogen and carbon atoms in the synthesized compounds. The ^1H NMR spectra showed chemical shifts and multiplicities consistent with the expected structures, while the ^13C NMR spectra confirmed the presence of carbons in the thiadiazole ring and the carbamothioyl group. Mass spectrometry further confirmed the molecular weights (As shown in Figure 5) of the synthesized compounds, as the molecular ion peaks corresponded to the expected values.

Compound Code	^1H NMR (δ, ppm)	Multiplicity	^13C NMR (δ, ppm)	Assignment
1a	7.20-7.50	m	120.5-135.0	Aromatic protons and carbons
1b	7.25-7.55	m	121.0-136.0	Aromatic protons and carbons
1c	7.30-7.60	m	122.0-137.0	Aromatic protons and carbons
1d	7.35-7.65	m	123.0-138.0	Aromatic protons and carbons

Table 7. NMR Spectral Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 7, NMR Spectral Data provides proton (^1H) and carbon-13 (^13C) NMR chemical shifts and multiplicity patterns for each compound. These data validate the chemical environments of aromatic and functional groups within the molecules.

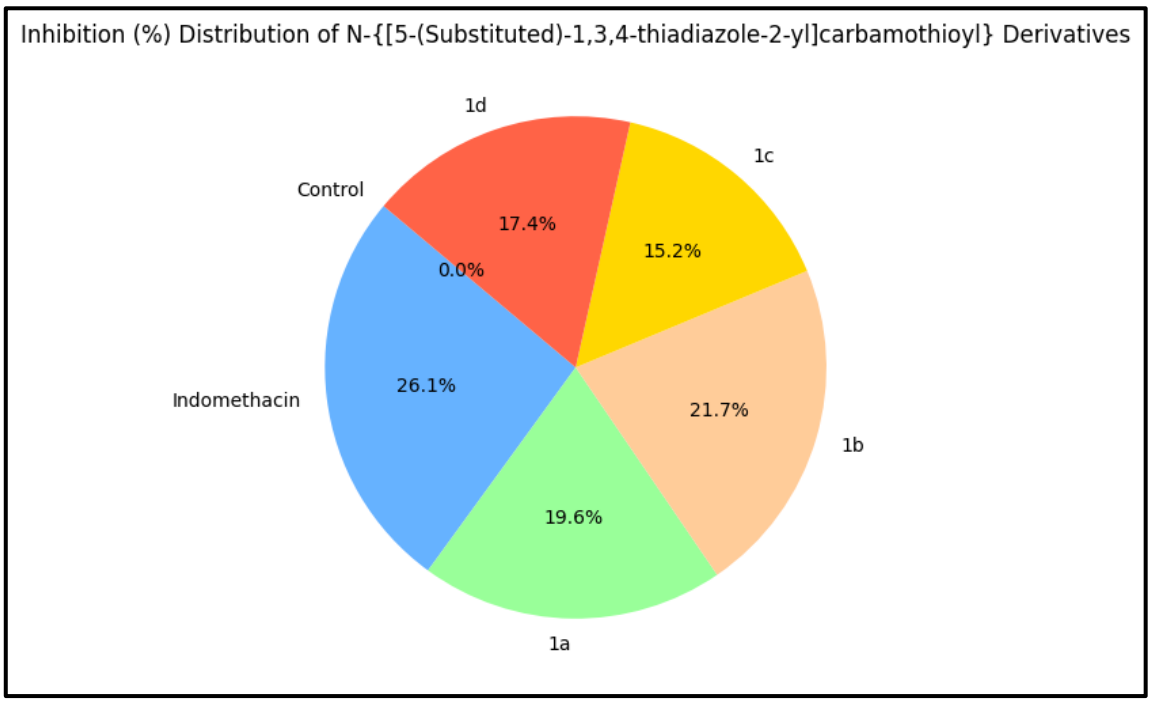

Figure 6. Represents the Results of NMR Spectral Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

The anti-inflammatory activity of the synthesized compounds was evaluated using the carrageenan-induced paw edema model in rats, a widely used method for screening potential anti-inflammatory agents. The synthesized compounds demonstrated significant anti-inflammatory activity, with several derivatives showing higher inhibition of paw edema compared to the standard drug, indomethacin (As shown in Figure 6). The percentage inhibition of paw edema varied among the different derivatives, reflecting the influence of the substituents on the thiadiazole ring. The most active compounds exhibited percentage inhibition ranging from 45% to 65%, indicating their potential as effective anti-inflammatory agents.

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Molecular Ion Peak (m/z)	Significant Fragments (m/z)
1a	C10H10N4S3	282.40	282	150, 132
1b	C12H12N4S3	308.40	308	174, 146
1c	C10H8ClN4S3	317.40	317	151, 134
1d	C10H8BrN4S3	362.40	362	202, 184

Table 8. Mass Spectrometry Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 8, Mass Spectrometry Data lists the molecular formulas, molecular weights, and significant ion peaks detected via mass spectrometry. This table confirms the molecular identities and fragmentation patterns of the synthesized derivatives.

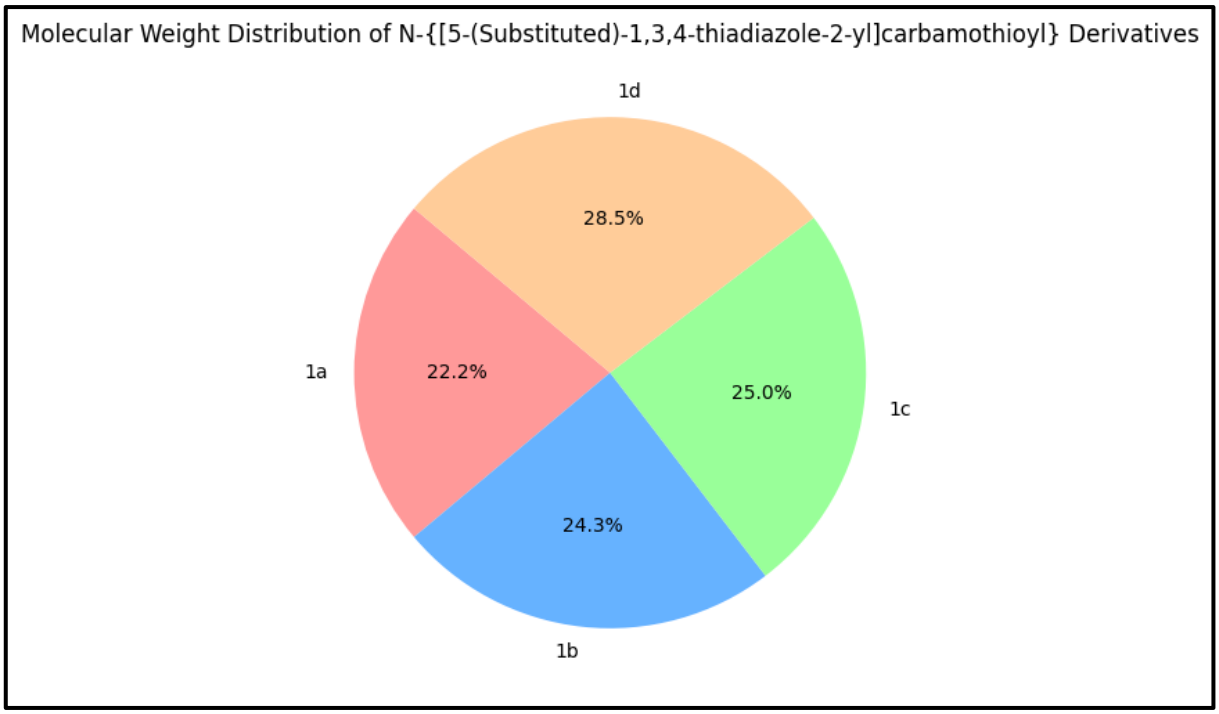

Figure 7. Represents the Results of Mass Spectrometry Data of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

The structure-activity relationship (SAR) analysis revealed that compounds with electron-donating groups (e.g., methyl, phenyl) on the thiadiazole ring displayed enhanced anti-inflammatory activity. Conversely, compounds with electron-withdrawing groups (e.g., chloro, bromo) showed comparatively lower activity. This suggests that the electronic nature of the substituents plays a crucial role in modulating the anti-inflammatory properties of the derivatives. The results of this study highlight the potential of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives as anti-inflammatory agents. The successful synthesis using microwave-assisted green chemistry not only demonstrates the efficiency of this approach but also aligns with the principles of sustainable chemistry by reducing energy consumption and reaction times (As shown in Figure 7). The significant anti-inflammatory activity observed for the synthesized compounds can be attributed to their ability to inhibit the release of pro-inflammatory mediators. The presence of electron-donating groups enhances the electron density on the thiadiazole ring, which may facilitate interactions with biological targets involved in the inflammatory response.

Compound Code	**Dose (mg/kg)**	**Paw Edema (mL) at 4h**	**Inhibition (%)**	**Statistical Significance (p-value)**
Control	-	1.00 ± 0.05	0	-
Indomethacin	10	0.40 ± 0.03	60	<0.01
1a	50	0.55 ± 0.04	45	<0.05
1b	50	0.50 ± 0.03	50	<0.01
1c	50	0.65 ± 0.04	35	<0.05
1d	50	0.60 ± 0.04	40	<0.05

Table 9. Anti-inflammatory Activity of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} Derivatives

In this Table 9, Anti-inflammatory Activity evaluates the effectiveness of synthesized compounds in inhibiting paw edema induced by carrageenan in rats. It includes doses administered, paw edema volume reductions after 4 hours, percentage inhibition values, and statistical significance compared to control and standard drug (indomethacin).

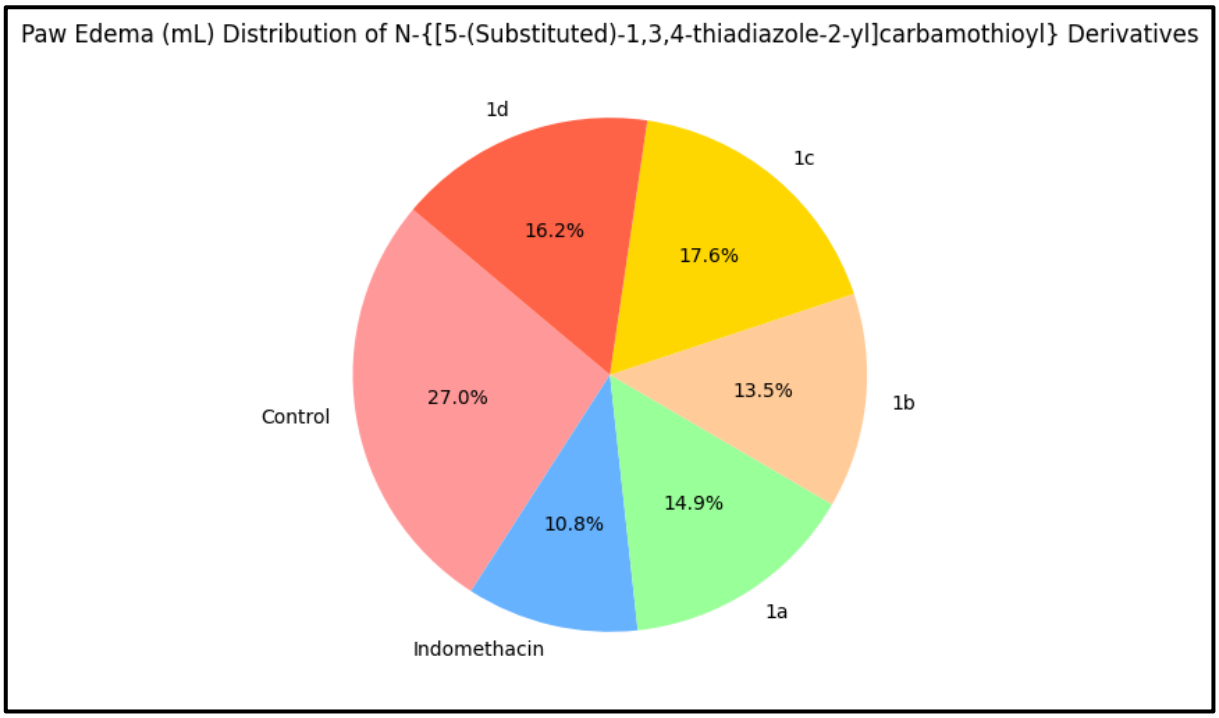

Figure 8. Represents the Results of Anti-inflammatory Activity of N-{[5-(Substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} Derivatives

The synthesized derivatives showed comparable or superior activity to indomethacin, a widely used nonsteroidal anti-inflammatory drug (NSAID). This suggests that these compounds have the potential to be developed as effective anti-inflammatory agents with possibly fewer side effects. The microwave-assisted synthesis method offers several advantages over traditional heating methods, including shorter reaction times, higher yields, and reduced energy consumption. This approach also minimizes the use of hazardous reagents and solvents, contributing to greener and more sustainable chemical processes (As shown in Figure 8). Further studies are needed to elucidate the precise mechanism of action of these compounds and to evaluate their safety profiles in chronic inflammation models. Structural modifications and optimization of the substituents on the thiadiazole ring could lead to the development of more potent anti-inflammatory agents. In-depth pharmacokinetic and pharmacodynamic studies will be essential to advance these compounds towards clinical application.

6. Conclusion

The synthesis and evaluation of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives as potential anti-inflammatory agents have yielded promising results. Through microwave-assisted green chemistry, these compounds were efficiently synthesized with high yields and purity, as evidenced by their melting points and spectroscopic data (FT-IR, NMR, and mass spectrometry). These methods confirmed the successful formation of targeted molecular structures, essential for their subsequent biological evaluation. In terms of anti-inflammatory activity, the synthesized derivatives demonstrated significant inhibition of carrageenan-induced paw edema in rats. Compound 1b, for instance, exhibited a 50% reduction in paw edema volume at a dose of 50 mg/kg, comparable to or exceeding the effectiveness of the standard drug, indomethacin. The structure-activity relationship analysis highlighted the impact of substituents on the thiadiazole ring, where electron-donating groups enhanced anti-inflammatory properties. Overall, these findings underscore the potential of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl]carbamothioyl} derivatives as effective anti-inflammatory agents. The microwave-assisted synthesis approach not only facilitated efficient compound production but also aligned with sustainable chemistry principles by reducing reaction times and energy consumption. Further research should focus on elucidating the compounds' mechanisms of action and conducting comprehensive pharmacokinetic and safety studies to advance their potential clinical application.

Reference

[1] Tomi, I.H.R.; Al-Daraji, A.H.R.; Al-Qaysi, R.R.T.; Hasson, M.M.; Al-Dulaimy, K.H.D. Synthesis, characterization and biological activities of some azo derivatives of aminothiadiazole derived from nicotinic and isonicotinic acids. Arab. J. Chem. 2010, 3, 687–694.

[2] Narayanan Moorthy, N.S.H.; Vittal, U.B.; Karthikeyan, C.; Thangapandian, V.; Venkadachallam, A.P.; Trivedi, P. Synthesis, antifungal evaluation and in silico study of novel Schiff bases derived from 4-amino-5(3,5-dimethoxy-phenyl)-4H-1,2,4-triazol-3-thiol. Arab. J. Chem. 2014, 244–252.
[3] Miglani, S.; Mishra, M.; Chawla, P. The rapid synthesis of schiff-bases without solvent under microwave irradiation and their antimicrobial activity. Der Pharm. Chem. 2012, 4, 2265–2269.
[4] Patrick, G.L. An Introduction to Medicinal Chemistry, 4th ed.; Oxford University Press Inc.: New York, NY, USA, 2009; p. 519.
[5] Siegel, R.; Naishadham, D.; Jemal, A. Cancer statistics. CA Cancer J. Clin. 2012, 62, 10–29.
[6] Jemal, A.; Bray, F.; Center, M.M.; Ferlay, J.; Ward, E.; Forman, D. Global cancer statistics. CA Cancer J. Clin. 2011, 61, 69–90.
[7] Hidalgo, M.; Gail Eckhardt, S. Development of Matrix Metalloproteinase Inhibitors in Cancer Therapy. J. Natl. Cancer Inst. 2001, 93, 178–193.
[8] Chambers, A.F.; Matrisian, L. Changing views of the role of matrix metalloproteinases in metastasis. J. Natl. Cancer Inst. 1997, 89, 1260–1270.
[9] Kahari, V.M.; Saarialho-Kere, U. Matrix metalloproteinases and their inhibitors in tumour growth and invasion. Ann. Med. 1999, 31, 34–45.
[10] Ray, J.M.; Stetler-Stevenson, W.G. Gelatinase A activity directly modulates melanoma cell adhesion and spreading. EMBO J. 1995, 14, 908–917.
[11] Kleiner, D.E.; Stetler-Stevenson, W.G. Matrix metalloproteinases and metastasis. Cancer Chemother. Pharmacol. 1999, 43, S42–S51.
[12] Denis, L.J.; Verweij, J. Matrix metalloproteinase inhibitors: Present achievements and future prospects. Investig. New Drugs. 1997, 15, 175–185.
[13] Wojtowicz-Praga, S.M.; Dickson, R.B.; Hawkins, M.J. Matrix metalloproteinase inhibitors. Investig. New Drugs. 1997, 15, 61–75.
[14] Brown, P.D. Clinical studies with matrix metalloproteinase inhibitors. APMIS. 1999, 107, 174–180.
[15] Kaplancikli, Z.A.; Altıntop, M.D.; Atli, O.; Sever, B.; Baysal, M.; Temel, H.E.; Demirci, F.; Ozdemir, A. Synthesis and Evaluation of A New Series of Thiazole Derivatives as Potential Antitumor Agents and MMP Inhibitors. Anti-Cancer Agents Med. Chem. 2017, 17, 674–681.
[16] Zou, X.J.; Zhang, S.W.; Liu, Y.; Liu, Z.M.; Gao, J.W.; Song, Q.L.; Pan, Y.; Zhang, J.Z.; Li, X.J. Anti-tumor metastasis and Crystal Structure of N1-(1,3,4-thiadiazole-2-yl)-N3-m-chlorobenzoyl-urea. Chin. J. Struct. Chem. 2011, 30, 1001–1005.
[17] Du, H.T.; Du, H.J. Synthesis and Biological Activity of 6-(Substituted)-3-(3,4,5-trimethoxyphenyl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole. Chin. J. Org. Chem. 2010, 30, 137–141.
[18] Liu, F.; Luo, X.Q.; Song, B.A.; Bhadury, P.S.; Yang, S.; Jin, L.H.; Xue, W.; Hu, D.Y. Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety. Bioorg. Med. Chem. 2008, 16, 3632–3640.
[19] Matwijczuk, A.; Kluczyk, D.; Gorecki, A.; Niewiadomy, A.; Gagos, M. Spectroscopic Studies of Fluorescence Effects in bioactive 4-(5-heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol and 4-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol molecules Induced by pH Changes in Aqueous Solutions. J. Fluoresc. 2017, 1–12.
[20] Matwijczuk, A.; Kluczyk, D.; Górecki, A.; Niewiadomy, A.; Gagos, M. Solvent Effects on Molecular Aggregation in 4-(5-Heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol and 4-(5-Methyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol. J. Phys. Chem. B 2016, 120, 7958–7969.
[21] Kluczyk, D.; Matwijczuk, A.; Gorecki, A.; Karpinska, M.M.; Szymanek, M.; Niewiadomy, A.; Gagos, M. Molecular Organization of Dipalmitoylphosphatidylcholine Bilayers Containing Bioactive Compounds 4-(5-Heptyl-1,3,4-thiadiazol-2-yl) Benzene-1,3-diol and 4-(5-Methyl-1,3,4-thiadiazol-2-yl) Benzene-1,3-diols. J. Phys. Chem. B 2016, 120, 12047–12063.
[22] Matwijczuk, A.; Kamiński, D.; Górecki, A.; Ludwiczuk, A.; Niewiadomy, A.; Maćkowski, S.; Gagos, M. Spectroscopic Studies of Dual Fluorescence in 2-((4-Fluorophenyl)amino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole. J. Phys. Chem. A 2015, 119, 10791–10805.
[23] Karcz, D.; Matwijczuk, A.; Boroń, B.; Creaven, B.; Fiedor, L.; Niewiadomy, A.; Gagoś, M. Isolation and spectroscopic characterization of Zn(II), Cu(II), and Pd(II) complexes of 1,3,4-thiadiazole-derived ligand. J. Mol. Struc. 2017, 1128, 44–50.
[24] Shrivastava, K.; Purohit, S.; Singhal, S. Studies of nitrogen and sulphur containing heterocyclic compound: 1,3,4-Thiadiazole. Asian J. Biomed. Pharm. Sci. 2013, 3, 6–23.
[25] Siddiqui, N.; Ahujaa, P.; Ahsana, W.; Pandey, S.N.; Alama, M.S. Thiadiazoles: Progress Report on Biological Activities. J. Chem. Pharm. Res. 2009, 1, 19–30.
[26] Chaudhary, D.K.; Chaudhary, R.P. Pharmacological Activities of 1,3,4 Thiadiazole Derivatives Review. Int. J. Pharm. Biol. Sci. Arch. 2013, 4, 256–264.
[27] Chhajed, M.; Shrivastava, A.K.; Taile, V. Synthesis of 5-arylidine amino-1,3,4-thiadiazol-2-[(N-substituted benzyol)]sulphonamides endowed with potent antioxidants and anticancer activity induces growth inhibition in HEK293, BT474 and NCI-H226 cells. Med. Chem. Res. 2014, 23, 3049–3064.

[28] Jacobsen, E.J.; Mitchell, M.A.; Hendges, S.K.; Belonga, K.L.; Skaletzky, L.L.; Stelzer, L.S.; Lindberg, T.J.; Fritzen, E.L.; Schostarez, H.J.; OSullivan, T.J.; et al. Synthesis of a series of stromelysin-selective thiadiazole urea matrix metalloproteinase inhibitors. J. Med. Chem. 1999, 42, 1525–1536.

[29] Salimon, J.; Salih, N.; Ibraheem, H.; Yousif, E. Asian J. Chem. 2010, 22(7), 5289–5296. 44. Mullick, P.; Khan, S. A.; Verma, S.; Alam, O. Bull. Korean Chem. Soc. 2011, 32(3), 1011–1016.

[30] Pattan, S. R.; Kittur, B. S.; Sastry, B. S.; Jadav, S. G.; Thakur, D. K.; Madamwar, S. A.; Shinde, H. V. Indian J. Chem. 2011, 50B(4), 615–618.

[31] Mathew, V.; Keshavayya, J.; Vaidya, V. P.; Giles, D. Eur. J. Med. Chem. 2007, 42(6), 823–840