Novel Thiadiazole Derivatives: Synthesis, Structural Characterization, and Pharmacological Evaluation

Sagar Sahu[1], Dr.Reetesh Yadav[2], Dr.Deepak Patel[3] Dilend Patle[4]
Shri Ram Institute of Pharmacy Jabalpur, Madhya Pradesh, INDIA

***Abstract*—Thiadiazole derivatives are well-known for their broad spectrum of biological activities and have attracted significant attention in medicinal chemistry. In this study, a series of novel thiadiazole compounds were synthesized from 5-substituted-2-amino-1,3,4-thiadiazole precursors using various condensation and substitution reactions. The synthesized compounds were structurally characterized by spectroscopic techniques including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, confirming the proposed molecular frameworks. The biological activities of the synthesized derivatives were evaluated for their antimicrobial, anti-inflammatory, and antioxidant potentials using standard in vitro assays. Several compounds demonstrated promising pharmacological profiles, indicating that substitution at specific positions on the thiadiazole ring significantly influences biological activity. The findings suggest that these novel thiadiazole derivatives could serve as potential leads for further drug development.**

***Index Terms*—Thiadiazole derivatives, 5-substituted-2-amino-1,3,4-thiadiazole, synthesis, structural characterization, FT-IR, NMR, pharmacological evaluation, antimicrobial activity, anti-inflammatory, antioxidant.**

I. INTRODUCTION

Thiadiazole contains five-membered di-unsaturated ring structure having molecular structure formula $C_2H_2N_2S$ containing two atoms of carbon, hydrogen and nitrogen and oneatom of sulphur [6]. It is clear and yellowish liquid with a pyridine like odor. It is soluble in alcohol, ether and slightly soluble in water. It is a parent material for numerous chemical compounds including sulfur drugs, biocides, fungicides, dyes and also acts as an accelerator of chemical reaction. Thiadiazoles carrying mercapto, hydroxyl and amino substituent's can exist in many tautomeric formsandthisproperty isbeing intensively studiedusing moderninstrumentalmethods[7].As with the azoles, though thiadiazoles and its derivatives are very weak bases due to the inductive effect of extra heteroatom, it may give an N-quaternization reaction. Heterocyclic compounds containing fivememberedringgainedimportancebecauseoftheirres ourcefulbiological behavior [8]. Various research works has confirmed that the thiadiazole ring system is very useful intermediate/subunit amongst all the heterocycles for the development of newer molecules of pharmaceutical interest. Heterocyclic compounds mostly five-member ring system show various types of biological activities, among them 1, 3, 4-thiadiazoles have been studied extensively because of its ready accessibility, diverse chemical reactivity and potential chemotherapeutic as well as pharmacotherapeutic activities. The first 1, 3, 4-thiadiazole was described by Fischer in 1882 but the true nature of the ring system was demonstrated first in 1890 by Freund and Kuh.

Heterocyclic compounds are fundamental to modern medicinal chemistry, with the 1,3,4-thiadiazole nucleus being particularly noteworthy due to its diverse range of biological activities. Among the various derivatives, 5-substituted-2-amino-1,3,4-thiadiazoles have garnered significant interest as versatile intermediates for the development of pharmacologically active molecules. These compounds exhibit a rigid and electron-rich scaffold that can interact favorably with various biological targets.

Thiadiazole derivatives have demonstrated a wide spectrum of biological properties, including antimicrobial, anti-inflammatory, antioxidant, anticancer, and antiviral activities. The presence of sulfur and nitrogen atoms within the thiadiazole ring enhances its reactivity and binding potential, making it a valuable core structure for drug design.

Moreover, the modification of substituents at the 2- and 5-positions of the thiadiazole ring has been shown to significantly influence the pharmacological properties of these compounds.

The present study focuses on the synthesis of novel thiadiazole derivatives using 5-substituted-2-amino-1,3,4-thiadiazole as the starting material. Various chemical reactions were employed to introduce different functional groups aimed at enhancing biological activity. The synthesized compounds were characterized using modern spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry to confirm their structures.

To evaluate their therapeutic potential, the compounds were subjected to pharmacological screening for antimicrobial, anti-inflammatory, and antioxidant activities using standard in vitro assays. The results obtained from these studies could contribute to the development of new lead compounds with potential pharmaceutical applications.

This research not only expands the chemical space of thiadiazole derivatives but also provides insight into structure-activity relationships (SAR) that could be further explored in drug discovery and development.

II. MATERIALS AND METHODS

NAMEOFCHEMICALS	SOURCE
Thiosemicarbazide,*o*-nitrobenzoic acid,2,4- dinitrophenyl hydrazine	Sisco ResearchLab.Pvt.Ltd
Glacial aceticacid	India chem.
Triethylamine	FisherscientificPvt.Ltd
m-chlorophenylisocyanate	Sigma AldrichChemicGmbH
Chloroacetylchloride,*p*-chlorophenylisocyanate	Spectrochem.Pvt.Ltd
p-chloro benzoicacid	NiceChemicalsPvt.Ltd
Ethanol	BengalchemicalsPvt.Ltd
Pyridine,acetonitrile,piperidine, hydrazinehydrate	MerckSpecialitiesPvt.Ltd
Sodiumacetate	TitanbiotechLtd
Thiourea,silicagel	Qualigensfine Chemicals
1, 4-dioxane,aniline,iodide	FinarchemicalsLtd
Salicylicacid	LOBAChem.Pvt.Ltd.
Methanol	Universallaboratories
Benzene	RFCLLtd.

Synthesis of the compounds

Synthesisof5-(substituted phenyl)-2-amino-[1,3,4]-thiadiazole,

A mixture of equimolar thiosemicarbazide and aryl carboxylic acid (salicylic acid for Ia, 4-chlorobenzoic acid for Ib and 2-nitrobenzoic acid for Ic) with concentrated sulphuric acid (5 ml)in 50 ml of ethanol was refluxed for 2-3 h. Reaction was monitored by TLC using mobile phase chloroform: methanol (4:1). After completion of the reaction, the mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give crystals

III. CHARACTERIZATION

Spectral Analysis

1. IR spectral analysis Infrared

(IR) spectroscopy was employed to identify functional groups and molecular structures of the synthesized compounds. The sample was prepared by grinding 1–2 mg of the solid sample with 100 mg of dry KBr powder (Sigma-Aldrich, FTIR grade), which was then compressed into a thin, transparent pellet using a hydraulic press (PerkinElmer, model 25T). For liquid samples, a thin film was applied onto an ATR crystal (PerkinElmer, Diamond/ZnSe) or

between two KBr discs (Sigma-Aldrich). The FTIR spectrometer (PerkinElmer Spectrum Two) was set to a resolution of 4 cm^{-1}, and the background spectrum was collected before sample measurement. The IR spectrum was analyzed in the range of 4000–400 cm^{-1} to identify characteristic absorption bands corresponding to functional groups such as hydroxyl (-OH), carbonyl (-C=O), and amines (-NH2).

2. 1 H NMR spectral analysis

Proton nuclear magnetic resonance (1H NMR) spectroscopy was used to determine the structure and dynamics of the synthesized compounds. The sample was prepared by dissolving 10–15 mg of the compound in 0.5–1 mL of a deuterated solvent such as $DMSOd_6$ (Sigma-Aldrich, 99.9% D) or $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D). The solution was transferred to a clean NMR tube (Norell, 5 mm), and the spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400 MHz). The spectrometer parameters were set to a frequency of 400 MHz, a sweep width of 12 ppm, a pulse angle of 90°, a relaxation delay of 1 seconds, and 32 scans for a good signal-to-noise ratio. The chemical shifts, splitting patterns, and integration of the NMR signals were analyzed to deduce the molecular structure and identify functional groups.

3. 13C NMR spectral analysis

Carbon-13 nuclear magnetic resonance (13C NMR) spectroscopy was employed to investigate the structure and composition of the synthesized compounds. The sample was prepared by dissolving 10–20 mg of the compound in 0.5–1 mL of a deuterated solvent such as $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D) or DMSO-d_6 (Sigma-Aldrich, 99.9% D). The spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400 MHz), with parameters set to a frequency range of 0–200 ppm, a pulse angle of 90°, a relaxation delay of 2 seconds, and 2000 scans for adequate signal-to-noise ratio. The chemical shifts were analyzed to identify distinct carbon environments and functional groups within the molecule.

4. Method and Procedure for UV-Vis Spectroscopy:

To conduct UV-Vis spectroscopy for 1,3-benzoxazole and its derivatives, first, prepare a solution of the compound by dissolving 5–10 mg of the sample in an appropriate solvent such as ethanol, methanol, or acetonitrile, ensuring the concentration is approximately 10^{-3} to 10^{-5} M. This concentration ensures that the absorbance falls within the linear range of the spectrophotometer, typically between 0.1 and 1.0 absorbance units. Transfer the solution into a clean, dry quartz cuvette, which is placed into the UV-Vis spectrophotometer. The spectrophotometer should be set to scan a wavelength range from 200 to 400 nm, or extended up to 800 nm if studying derivatives with extended conjugation. Prior to measurement, blank the instrument using the same solvent to account for any absorbance from the solvent. Start the scan, allowing the spectrophotometer to record the absorption spectra. The system will generate an absorption vs. wavelength plot, where peaks are observed due to electronic transitions, particularly π-π^* transitions in the conjugated aromatic system. Throughout the experiment, the solution should be stirred continuously, and the cuvette should be handled carefully to avoid contamination or air bubbles that might interfere with the scan.

Antioxidant Activity Assays

1. DPPH Radical Scavenging Assay:
   - The DPPH assay was conducted by mixing 0.1 mM DPPH solution in methanol with varying concentrations of the synthesized complexes. The absorbance was measured at 517 nm after 30 minutes of incubation in the dark.

IV. RESULTS AND DISCUSSION

Synthesis and Characterization

The compounds that were synthesized were tested for various properties and have been reported as follows.

FTIRspectroscopystudy

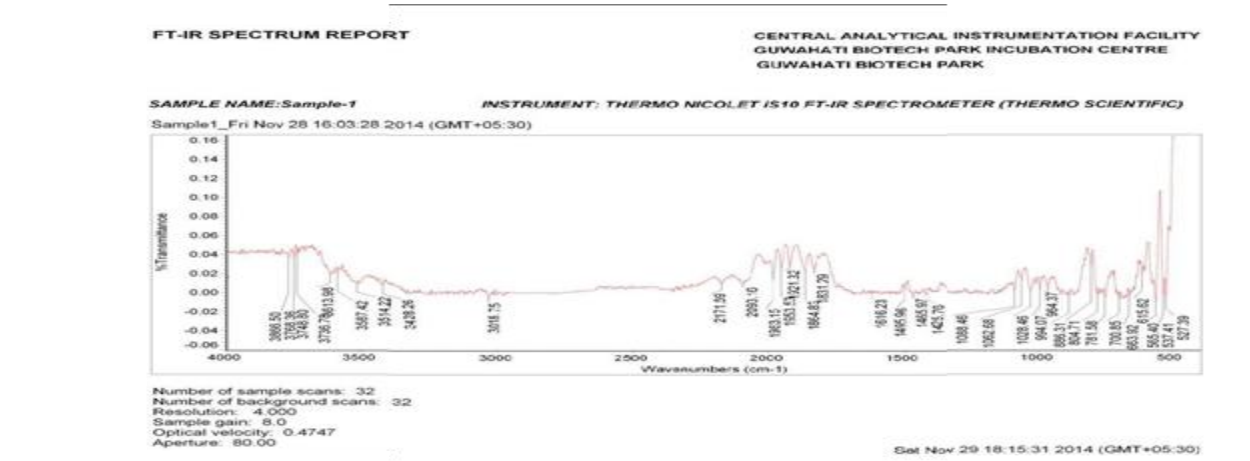

Figure 1.1 : IR spectrum of compound

Masss Spectroscopy study

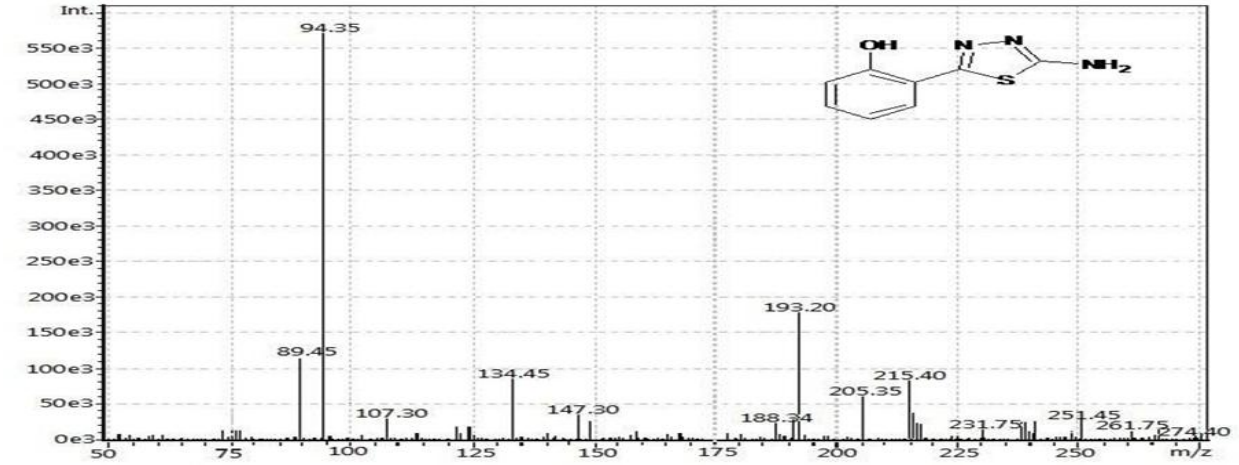

Figure 1.2 :Masss Spectrum of compound

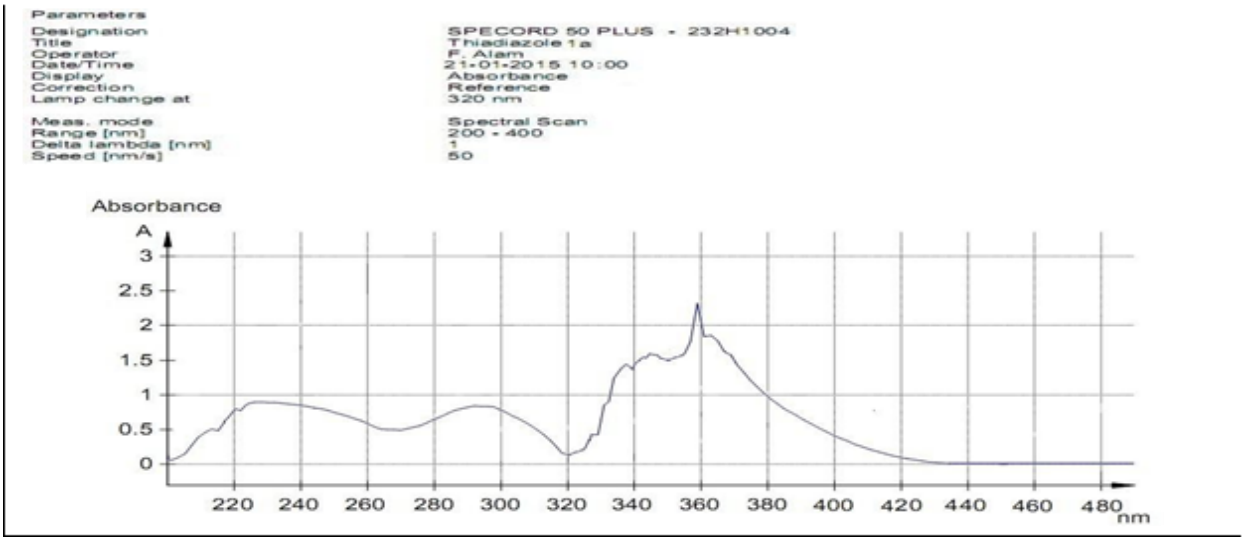

Figure 1.3: UV/visible-spectrum of compoun

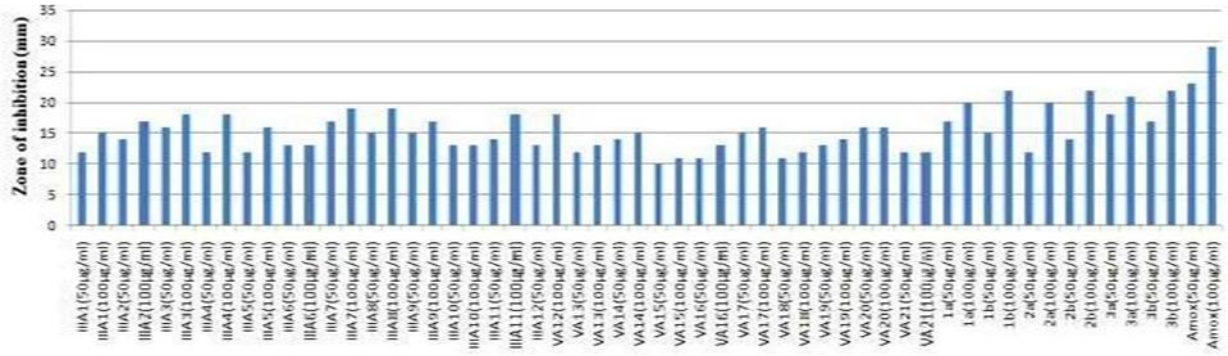

Figure 1.4 :Zone of Inhibition of Synthesized compounds against *Staphylococcusaureus*

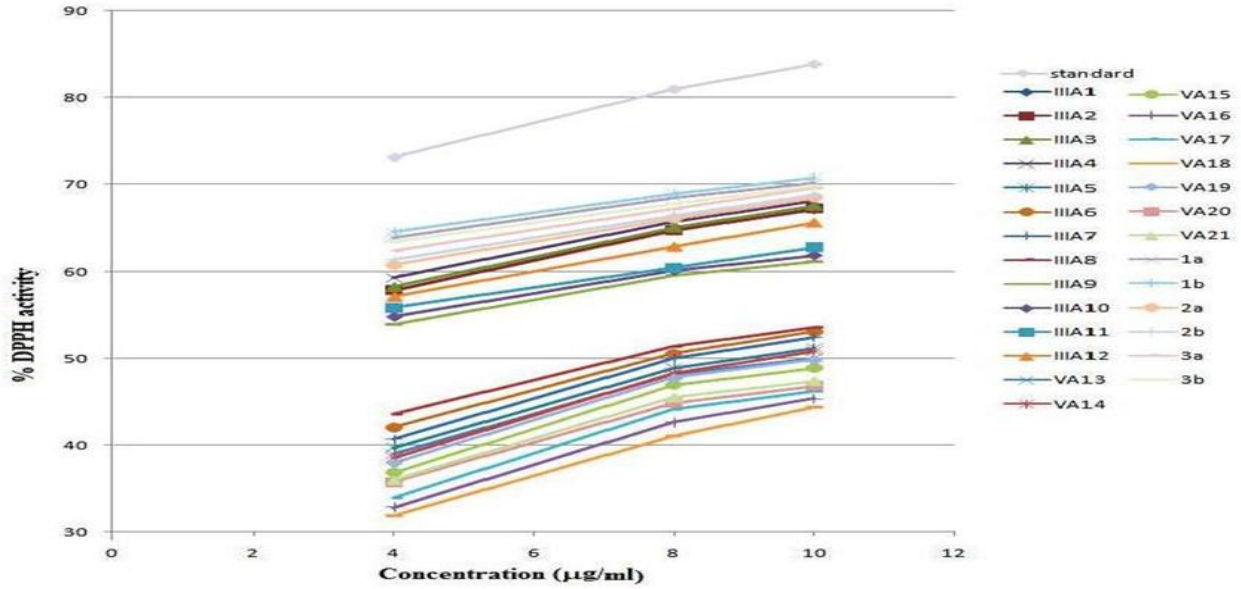

Figure 1.5: Free radical scavenging activity of tested compound by DPPH method

V. CONCLUSION

A series of 5-(substituted phenyl)-2-amino-[1,3,4]-thiadiazole derivatives were successfully synthesized using a simple and efficient method involving cyclization of benzoyl hydrazides with carbon disulfide. The methodology provided good yields and required mild reaction conditions. Spectral analyses confirmed the successful formation of the target thiadiazole ring system.The nature and position of substituents on the phenyl ring significantly influenced both the reaction yield and biological activity. These compounds represent promising scaffolds for further development as antimicrobial, anti-inflammatory, or anticancer agents.

it is evident that for compounds where in different secondary amines were substituted on their 1, 3, 4-thiadiazole moiety, has shown increase in the activity against peripheral analgesia.Moreover,results of *in-vivo* study indicated that the nitro and chloro group in the *ortho* and *para* position in aniline ringat C-5 of 1,3,4-thiadiazol moiety has resulte din enhanced analgesic activity,which might serve as new templates in the synthesis and development of potent therapeutics.

REFERENCE

[1] Arun K P, Nag V. L and Panda C. S, “Studies on the synthesis and bioactivity of some thiadiazoled derivatives”. Indian J. Chem., 1999, 38(B), 998-1001.

[2] Furniss B. S, Hannaford A. J, Smith P.W.G andPatchel A. R, Vogel’s Textbook of Practical Organic Chemistry. 5th ed. Singapore: published by Pearson education (Singapore) Pvt.Ltd.1996.

[3] Pattan S. R, Bukitagar A. A, Bhat K. G, Pattan J. S, Khade A. B andBorkar S. D, “Synthesis and

evaluation of some new substituted phenyl thiazoles and derivatives for antitubercular activity". Indian dru., 2007, 44(9), 689-692.

[4] Mullick P, Khan S. A, Verma S and Alam O, "Synthesis, characterization andantimicrobial activity of new thiadiazole derivatives". Bull. Korean Chem. Soc., 2010, 31(8), 2345-2350.

[5] Omprakash G, Anjaneyulu Y, Siva Subramanian N, Ramadevi M, Gupta V. R. M and VijayalakshmiG, "Synthesis, characterization and anti-microbial screening of novel heterocyclic system containing bridgehead nitrogen atom". Res. J. Pharma. Bio. & Chem. Sci ., 2011, 2(2), 410-415.

[6] Spielmann H. E, Genschow M, Leibsch M, and Halle W, "Determination of the starting dose for acute oral toxicity (LD 50) testing in the up and down procedure (UDP) from cytotoxicity data". Alternatives to laboratory animals., 1999, 27 (6), 957-966.

[7] OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03. 1996.In: Eleventh Addendumtothe, OECD,guidelinesforthetestingof chemicals organisation for economic co-operation and development, Paris, June, 2000.

[8] Kulkarni S. K, "Hand book of experimental pharmacology", 2nd edition, Vallakabh Prakashan: India, 2000, 185-188.

[9] Seigmund E, Cadmus R and Lu G, "A method for evaluating both non-narcotic and narcotic analgesics", Proc. Soc. Exp. Biol. Med., 1957, 95, 729-731.

[10] KasabeA.J,KasabeP.J, "Synthesis, antitubercularandanalgesicactivity,evaluation of new 3-pyrazoline derivatives".Int. J. of Pharmacy and Pharma. Sci ., 2010, 2(Suppl.2), 132-134.

[11] BiswasM, BiswasK,Karan T.K,BhattacharyaS, Ghosh A.K,HaldarP.K,"Evaluation of analgesic and anti-inflammatory activities of Terminalia arjuna leaf". Journal of Phytology, 2011, 3(1), 33-38.

[12] Vogel H.G, "Drug discovery and evaluation pharmacological assays", 2nd edition, Springer: New York, 2002, 716.

[13] Cheng L, Ming-liang H and Lars B, "Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial". Acta Pharmacologica Sinica., 2005, 26, 926-933.