Synthesis, Characterization and Antibacterial study of some 1, 3, 4-thiadiazole ring appended azo derivatives

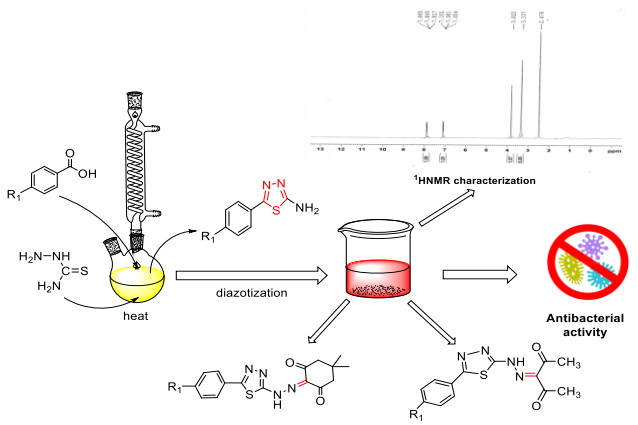
Sanjay Belowar[1*] and Md. Abdur Rashid[2]

Abstract

A series of 1,3,4-thiadiazole ring appended azo derivatives were synthesized in good yields via diazo coupling reaction between 5-(4-substituted)-1,3,4-thiadiazole-2-amine and some active methyline compounds such as dimedone and acetylacetone. All the synthesized compounds were characterized by FT-IR and [1]HNMR spectroscopic methods. Further, all synthesized compounds were screened for the antibacterial activity against gram negative bacteria (Escherichia coli, Pseudomonas aruginosa, Shigella boydii) and gram-positive bacteria (Staphylococcus aureus, Bacillus megaterium). The thiadiazole ring appended azo derivative containing a chloro group showed potent activity against Shigella boydii (67.85%) and Staphylococcus aureus (78.57%) and the compound having a nitro group in its structure exhibited maximum zone of inhibition against Staphylococcus aureus (89.28%) and Shigella boydii (82.14%).

Keywords: *2-amino-1,3,4-thiadiazole, IR and NMR characterization, antibacterial property*

Graphical Abstract



[1] *Instructor, Organic Chemistry Research Laboratory, Department of Natural Science, BGMEA University of Fashion and Technology*

[2]*Professor, Department of Chemistry, Bangladesh University of Engineering and Technology*

[*]***Corresponding author email:*** *sanjay.belowar@buft.edu.bd*

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Introduction

Azo compounds are a class of organic compounds characterized by the presence of an azo (-N=N-) functional group, which imparts them with unique and tunable properties. Over the years, azo compounds have gained considerable attention in various fields, including materials science, medicinal chemistry, and dye chemistry, owing to their intriguing chemical reactivity and vibrant coloration (Keerthi et al., 2013; Benkhaya, et al,2020). Among the diverse range of azo derivatives, those incorporating the 1,3,4-thiadiazole ring have emerged as a distinct and intriguing subset with profound implications across multiple disciplines (Jain, et al., 2013).

The 1,3,4-thiadiazole ring is a structurally significant moiety renowned for its exceptional versatility and biological significance. It serves as a key scaffold in the design and synthesis of compounds with diverse pharmacological, photophysical, and materials-related properties. When integrated into azo compounds, this unique fusion opens up a new dimension of possibilities, leading to a plethora of compounds with unprecedented applications.

This paper presents an exploration of 1,3,4-thiadiazole ring-appended azo derivatives, shedding light on their synthetic strategies, characterization, and their anti-bacterial properties. Drawing upon a broad spectrum of recent research, we delve into the synthetic methodologies employed for the preparation of these intriguing compounds and examine their remarkable chemical, physical, and biological properties.

The intriguing combination of the azo group's color-changing abilities and the 1,3,4-thiadiazole ring's inherent pharmacophoric and photophysical properties makes these derivatives particularly interesting for a range of applications (Ivan, et al, 2014; Kudelko, et al., 2020). In the field of materials science, these compounds have found utility as versatile components in the development of organic semiconductors, liquid crystals, and photo responsive materials. Their unique optoelectronic properties, including photoisomerization and photomechanical effects, have fueled innovations in the design of smart materials and devices.

Furthermore, the biologically active 1,3,4-thiadiazole ring when integrated with azo moieties can give rise to compounds with notable pharmacological activities. Some of these derivatives exhibit promising anticancer, anti-inflammatory, antifungal, and antimicrobial properties, making them valuable candidates for drug discovery and development (Li, et al.,2015; Zhibing, et al., 2021; Ahmed, et al, 2022).

As the research in this field continues to flourish, this paper aims to provide a convenient root to synthesis some1,3,4-thiadiazole ring appended azo derivatives, their characterization, and their antibacterial properties. By consolidating the latest findings and insights, we hope to inspire further exploration and innovation in the development of these intriguing compounds, both in academia and industry.

1. Experimental

1.1 Materials and physical measurements

All the starting materials and solvents were purchased from Sigma Aldrich and used without further purification. Melting points were determined on electrothermal capillary apparatus and are uncorrected. The FT-IR spectra were obtained using SHIMADZU FTIR prestige 21 spectrophotometer and the NMR spectra were recorded on a BRUKER AVANCE III HD (400 MHz) instrument. All chemical shifts were recorded in δ units and are referenced to the residual protons of the deuterated NMR solvent (^{1}H). All spectral analysis were performed in Wazed Miah Science Research Centre, Jahangirnagar University, Bangladesh.

2.2 General procedure for preparation of 5-(4-substituted)-1, 3, 4-thiadiazol-2-amine *1(a-d)*

The starting material 5-(4-substituted)-1, 3, 4-thiadiazol-2-amine derivatives was prepared by following the reported article of Chinnagiri T. Keerthi Kumar et al. (2013). The experiment was performed with a mixture of 4-substiuted benzoic acid (0.01 mol), thiosemicarbazide (0.01 mol) and $POCl_3$ (10 mL) was refluxed at 85°C for 2 hrs. The reaction mixture was then cooled to room temperature and 50 mL ice cold water was added. The mixture was again refluxed for 4 hrs. After cooling, the mixture was then alkalized to pH 8 by the dropwise addition of 10% KOH solution. The crude solid precipitate was filtered, washed and dried over desiccator and purified by recrystallization from ethanol.

IR (KBr, cm^{-1}, ***1a***) : 3302,3108(w, NH_2), 2765,2709(C-H, Ar), 1633(C=N), 1503, 1392, 1317 (C=C, Ar), 500-600(C-S-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, ***1a***): 7.55(s, 2H, NH_2), 8.05, 8.07 (d, 2H, J=8.0 Hz, Ar-H), 8.17, 8.19 (d, 2H, J=8.0 Hz, Ar-H).

IR (KBr, cm^{-1}, ***1b***) : 3284 (w, NH_2), 3127(w, C-H, Ar), 1634(C=N), 1600, 1517, 1465(C=C, Ar), 560(C-S-C).

IR (KBr, cm^{-1}, ***1c***) 3303(NH_2), 3098 (w, C-H, Ar), 2838, 2777 (C-H, -OCH3), 1609(C=N), 1578, 1524, 1465(C=C, Ar), 560(C-S-C). ^{1}H NMR(400 MHz, $CDCl_3$, δ

ppm, ***1c***) : 1.820-2.024(m, 2H, NH_2), 3.897(s, 3H, OCH_3), 7.0(d, 2H, J= 8.0 Hz, Ar-H), 2.84(d, 2H, J=8.0 Hz, Ar-H).

2.3 General procedure for synthesis of Substituted azo appended thiadiazoles *3(a-f)*

A diazonium salt of thiadiazol ***1a-1c*** was prepared by dissolving 5-(4-substituted)-1,3,4-thiadiazol-2-amine (0.01 mol) in minimum amount of glacial acetic acid then the mixture was diluted with concentrated hydrochloric acid and was kept in an ice bath with stirring. To this mixture, an ice-cold aqueous solution of $NaNO_2$ (0.02 mol) was added drop wise and the the stirring was continued for five minutes. In another flask sodium salt of acetyl acetone was prepared by stirring a mixture of aqueous solution of sodium acetate (0.05 mol) and ethanolic solution of acetyl acetone/dimedone/ ethylacetoacetate (0.01 mol) for 2 hrs at 0^{o}C. Then the ice cold diazonium salt was added dropwise to the salt solution of acetyl acetone/dimedone/ ethylacetoacetate and the stirring was continued for further two hours at the same temperature. The resultant reaction mixture was then neutralized with 10% sodium carbonate solution. The coloured precipitate was filtered, washed, dried and recrystallized from ethanol. The final product was obtained with 50 -65% yield. The general scheme for the synthesis is given in scheme 1.

Preparation of 3-(2-(5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-yl) hydrazineylidene) pentane-2, 4-dione, *3a*: The red colored azo derivative was synthesized from 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine and acetyl acetone was yielded 55% with melting point 210-212^{o}C. IR (KBr, cm^{-1}, ***3a***) : 3061 (N-H), 2925(C-H, aliphatic), 1693(C=O), 1601(C=N), 1510, 1398 (C=C, Ar), 841(C-S-C); ^{1}H NMR (400 MHz, DMSO-d6, δ ppm, ***3a***): 3.417(s, 3H, OCH_3), 8.21 (d, 2H, J=8.0 Hz, Ar-H), 8.36(d, 2H, J=8.0 Hz, Ar-H).

Preparation of 3-(2-(5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl) hydrazineylidene) pentane-2,4-dione, *3b*: The azo derivative obtained from 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine and acetyl acetone was appered as yellow colored powder with 58% yield and the recorded melting point is between 114-116^{o}C . IR (KBr, cm^{-1}, ***3b***) : 3082 (N-H), 3021 (C-H, Ar), 2934, 2851 (C-H, aliphatic), 1599 (=N-CO, conjugated), 1511 (C=N), 1443 (C=C, Ar), 688 (C-S-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm,***3b***): 1.604(s, 6H, 2 $COCH_3$), 7.50(d, 2H, J=8.4 Hz, Ar-H), 7.86(d, 2H, J=8.4 Hz, Ar-H), 13.0(s, 1H, N-H).

Preparation of 3-(2-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)hydrazineylidene) pentane-2, 4-dione, *3c*: The dye was obtained from 5-(4-methoxyphenyl)-1,3,4-

thiadiazol-2-amine and acetyl acetone appered as brown colored powder with 65% yield and recorded melting point is between 108-110^{o}C.IR (KBr, cm^{-1}, ***3c***) : 3280-3360 (N-H), 2924 (C-H, Ar), 2792(C-H, aliphatic), 1603 (C=N), 1441, 1411 (C=C, Ar), 562(C-S-C).^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm,***3c***): 3.337(s, 6H, 2 $COCH_3$), 3.822(s, 3H, OCH_3), 7.09 (d, 2H, J=8.0 Hz, Ar-H), 7.87(d, 2H, J=8.0 Hz, Ar-H).

Preparation of 3-(2-(5-phenyl-1,3,4-thiadiazol-2-yl)hydrazineylidene) pentane-2, 4-dione, *3d*:

The dye was synthesized from 5-phenyl-1,3,4-thiadiazol-2-amine and acetyl acetone was appered as pale yellow colored powder with 55% yield and the melting point was recorded between 115-117^{o}C.IR (KBr, cm^{-1},***3d***) : 3300-3440 (w, N-H), 3065 (C-H, Ar), 2840(C-H, aliphatic), 1674=N-CO, conjugted), 1599(C=N), 1544, 1460, 1428(C=C, Ar). ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm, ***3d***): 3.473(s, 6H, 2 $COCH_3$), 8.210(t, 3H, J=8.0 Hz, Ar-H), 8.34(d, 2H, J=8.0 Hz, Ar-H), 13.2(s, 1H, N-H).

Preparation of 5, 5-dimethyl-2-(2-(5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-yl) hydrazineylidene) cyclohexane-1, 3-dione, *3e*:

The dye was obtained from 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine and dimedone was appered as yellow colored powder with 60% yield and melting point 200-202^{o}C. IR (KBr, cm^{-1},***3e***) : 3110(N-H), 2931(w, C-H, Ar), 2852(C-H, aliphatic), 1694(=N-CO, conjugated), 1599(C=N), 1521, 1407(C=C, Ar), 689(C-S-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, ***3e***): 1.633(s, 6H, 2 CH_3), 7.50(d, 2H, J=8.0 Hz, Ar-H), 7.86(d, 2H, J=8.0 Hz, Ar-H).

Preparation of 2-(2-(5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl) hydrazineylidene)-5, 5-dimethylcyclohexane-1, 3-dione, *3f*:

The yellow colored azo dye was obotained from 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine and dimedone with 60% yield and recorded melting point is 118-120^{o}C. IR (KBr, cm^{-1}, ***3f***) : 3281 (N-H), 2932 (C-H, Ar), 2862 (C-H, aliphatic), 1603 (C=N), 1465 (C=C, Ar), 569 (C-S-C). ^{1}H NMR (400 MHz, $CDCl_3$, ***3f***): 1.596(s, 6H, 2CH_3), 3.902(s, 4H, 2CH_2), 7.01(d, 2H, J=8.8 Hz, Ar), 7.85(d, 2H, J= 8.8 Hz, Ar), 13.1(s, 1H, N-H).

2. Antibacterial Evaluation

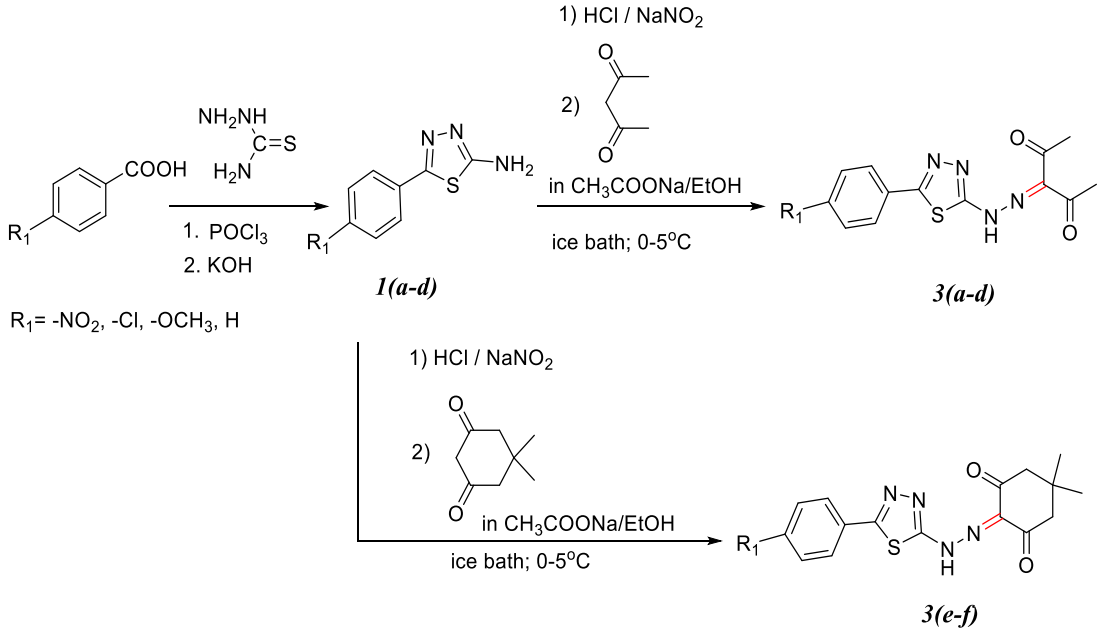
The following bacteria and fungi were used for the experiment. Bacteria: *Staphylococcus aureus*, *Bacillus megaterium*,*Escherichia coli*, *Shigella boydii*,and *Pseudomonas aeruginosa*. All bacterial strains were maintained on nutrient agar medium at 37◦C. These cultures are obtained from the Department of Pharmacy, State

University of Bangladesh. All fungi strains were maintained on potato dextrose agar (PDA) at 25°C.

All the synthesized compounds have been screened for antibacterial activity using disk diffusion method by measuring the inhibition zone in mm. Standard antibiotic (Ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4° C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media. The plates are then inverted and incubated at 37° C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter. In the present study the synthesized compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required. The antimicrobial potency of the test agents was measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

3. Results and Discussion

The compounds 5-(4-substituted)-1, 3, 4-thiadiazol-2-amine ***1(a-d)*** were synthesized by reacting 4-substituted benzoic acid and thiosemicarbazide in presence of phosphorous oxychloride and the azo derivatives (***3a-3f***) were synthesized via diazotization reaction of 5-(4-substituted)-1, 3, 4-thiadiazol-2-amine and coupled with different active methylene group containing compounds such as dimedone, acetylacetone and ethylacetoacetate. The reaction procedures are shown in **scheme 1**. The synthesized compounds were characterized by FT-IR, ^{1}H-NMR. The infrared spectra for all the azo coupled dyes were recorded in the region of 4000 cm^{-1} to 400 cm^{-1}. The regions of expected functional groups such as NH, C-H, CO, C=N, C=C, C-S-C etc. are placed in Table-I. It has been reported that the secondary amine group (-NH-) has a stretching at 3477–3061 cm^{-1}. In addition, the stretching vibrations of C=N, aromatic C–H, aliphatic C–H and aromatic C=C are respectively reported in the following ranges: 1603-1599, 3112-2924, 2934-2792 and 1521-1407 cm^{-1}. Moreover, the peaks belonging to the conjugated C=C and C–S–C are found at around 1694-1519 and 569-689 cm^{-1}.



Scheme1: Preparation of 5-(4-substituted)-1,3,4-thiadiazole-2-amine precursors ***1 (a-d)*** and synthesis of 3-(2-(5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl) hydrazineylidene) pentane-2,4-dione ***3(a-d)*** and 5,5-dimethyl-2-(2-(5-(4-substituted)-1,3,4-thiadiazol-2 yl)hydrazineylidene)cyclohex ane-1,3-dione ***3(e-f)***

Table-I: IR data of synthesized compounds

SI no.	Compound no.	−NH − cm^{-1}	C − H (aliphatic) cm^{-1}	C − H (aromatic) cm^{-1}	C = C (aromatic) cm^{-1}	C = N cm^{-1}	C = O cm^{-1}	C − S cm^{-1}
01	***3a***	3061	2925	-	1398	1601	1693	841
02	***3b***	3082	2934,2851	3021	1443	1511	1599	688
03	***3c***	3280	2792	2924	1441	1603	1519	562
04	***3d***	3440	2840	3065	1460,1428	1599	1674	
05	***3e***	3110	2852	2931	1521,1407	1599	1694	689
06	***3f***	3281	2862	2932	1465	1603	1682	569

[1]H NMR analysis. The [1]H NMR spectra of compound *3a,3c,3d* showed a sharp singlet for the six aliphatic protons at between δ 3.3-3.4 ppm. The doublet with coupling constant J=8.0-19.2 Hz was designated at δ 7.5-8.36 was attributed for aromatic protons. The [1]H NMR spectra of the compound *3b,3e* and *3f* showed a sharp singlet for the six aliphatic protons at between δ1.5- 1.604. Compound 3f showed a singlet peak at δ 3.902 for four aliphatic CH_2 protons. All the H-NMR spectra indicates that the hydrazinylidine (-NH-N=C<) bond is formed via diazotization reaction. The dyes were in moderate to good yield and these are soluble in ethanol, acetone, chloroform and DMSO.

Antibacterial effect. The synthesized compounds were screened for antibacterial activity against gram negative bacteria (*Escherichia coli*, *Pseudomonas aruginosa*, *Shigella boydii*) and gram-positive bacteria (*Staphylococcus aureus*, *Bacillus megaterium*). The results are presented in table -I and table-II. The thiadiazole ring appended azo derivative ***3b*** having a chloro group showed potent activity against *Shigella boydii* (67.85%) and *Staphylococcus aureus* (78.57%) whereas compound ***3a*** having a nitro group in its structure exhibited maximum zone of inhibition against *Staphylococcus aureus* (89.28%) and *Shigella boydii* (82.14%) in compared with standard antibiotic (Ciprofloxacin) discs. Therefore all the synthesized exhibited strong, moderate and weak activities against all mentioned gram positive and gram negative bacteria.

Table-II: antibacterial activity of 1,3,4-thiadiazole ring appended azo derivatives against gram negative bacteria

Sl No.	Sample	Conc. In mg/mL	*Escherichia coli*		*Pseudomonas aruginosa*		*Shigella boydii*	
1	Control	-	0.0		0.0		0.0	
2	Standard Ciprofloxacin	50	Zone of inhibition in mm	% inhibition	Zone of inhibition in mm	% inhibition	Zone of inhibition in mm	% inhibition
			16.5	100%	22	100	14	100%
3	***3a***	50	12	72.72%	18	81.81%	11.5	82.14%
4	***3b***	50	10.5	63.63%	15.5	70.45%	9.5	67.85%
5	***3f***	50	09	54.54%	-	-	8.5	60.71%

Table-III: antibacterial activity of 1,3,4-thiadiazole ring appended azo derivatives against gram positive bacteria

SL No.	Sample	Conc. In mg/mL	*Staphylococcus aureus*		*Bacillus megaterium*	
1	Control	-	0.0		0.0	
2	Standard Ciprofloxacin	50	Zone of inhibition in mm	% inhibition	Zone of inhibition in mm	% inhibition
			14	100%	16	100%
3	***3a***	50	12.5	89.28%	12.5	78.12%
4	***3b***	50	11.0	78.57%	11.0	68.75%
5	***3f***	50	08	57.14%	-	-

4. Conclusions

In this work new hydrazinylidene bond is formed with some new synthesized thiadiazole ring appended azo dyes. This invesitigation proposes convenient, cheaper and useful method for synthesis of thiadiazole ring appended azo dyes which are showing moderate to good antibacterial properties. The aim of this work was to enrich the synthetic data with proper antibacterial properties. The synthesized thiadiazole ring appended azo dyes demonstrating good solubility in some polar solvents—may serve as potential candidates for the dye industry and will be tested in the near future.

References

Ahmed H. M. Elwahy, Elshimaa M. Eid, Samir A. Abdel-Latif, Huwaida M. E. Hassaneen, Ismail A. Abdelhamid. Design, Synthesis, DFT, TD-DFT/PCM Calculations, and Molecular Docking Studies on the Anti-COVID-19, and Anti-SARS Activities of Some New Bis-Thiazoles and Bis-Thiadiazole. Polycyclic Aromatic Compounds 2022, 11 , 1-30.

Bala M, Piplani P, Ankalgi A, Jain A, Chandel L. 1,3,4-Thiadiazole: A Versatile Pharmacophore of Medicinal Significance. Med Chem. 2023;19(8):730-756.

Benkhaya S, M'rabet S, El Harfi A. Classifications, properties, recent synthesis and applications of azo dyes. Heliyon. 2020 Jan 31;6(1)

Ivan Hameed R. Tomi, Ali Hussein R. Al-Daraji, Raya Raad T. Al-Qaysi, Mohammed Mujbel Hasson, Khlood Hamed D. Al-Dulaimy,Synthesis, characterization and biological activities of some azo derivatives of aminothiadiazole derived from nicotinic and isonicotinic acids,Arabian Journal of Chemistry,Volume 7, Issue 5,2014.

Jain AK, Sharma S, Vaidya A, Ravichandran V, Agrawal RK. 1,3,4-thiadiazole and its derivatives: a review on recent progress in biological activities. Chem Biol Drug Des. 2013 May;81(5):557-76.

Kumar, C. T. Keerthi et al. "Synthesis, Characterization, and Biological Activity of 5-Phenyl-1,3,4-thiadiazole-2-amine Incorporated Azo Dye Derivatives." Organic Chemistry International 2013 (2013): 1-7.

Kudelko, A.; Olesiejuk, M.; Luczynski, M.; Swiatkowski, M.; Sieranski, T.; Kruszynski, R. 1,3,4-Thiadiazole-Containing Azo Dyes: Synthesis, Spectroscopic Properties and Molecular Structure. *Molecules* **2020**, *25*, 2822.

Li S., Jing F., Fu X., Zhao J., Wang X., Li B., Chen B. Synthesis and antitumor activities of disulfide derivatives containing 1, 3, 4-thiadiazole moiety. Chin. J. Org. Chem. 2015;35:2624–2628.

Singh, A., et al. (2017). Azo compounds: Synthesis and applications in contemporary chemistry. RSC Advances, 7(49), 30797-30819.

Zhibing W., Jin S., Jixiang C., Deyu H., Baoan S. Design, Synthesis, Antibacterial Activity, and Mechanisms of Novel 1,3,4-Thiadiazole Derivatives Containing an Amide Moiety. Journal of Agricultural and Food Chemistry 2021, 69 (31) , 8660-8670.