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Synthesis, Characterization and Biological Evaluation of Some Novel Azo-Imine Compounds

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The oxidative cyclization of thiosemicarbazone (**III**) synthesized from reaction between substituted aryl aldehydes (**I**) with thiosemicarbazide (**II**) was done using ferric chloride as oxidative agent to get 2-amino-5-phenyl-1,3,4-thiadiazole (**IV**). 2-Amino-5-phenyl-1,3,4-thiadiazole was introduced in condensation reactions with substituted aldehydes to obtain benzylidene imine derivatives (**VI**)$_{1-5}$. Further these were treated with sulphanilic acid to give new 2-{[5-phenyl-1,3,4-thiadiazole-2-imino] substituted benzene}diazenyl benzene sulfonic acid derivatives (**VII**)$_{1-5}$. These derivatives were further characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectral studies and were screened for antibacterial, antifungal and antioxidant activities.

Keywords: 2-Amino-5-phenyl-1,3,4-thiadiazole, Benzylidene imine, Sulphanilic acid, Thiosemicarbazone.

INTRODUCTION

Thiadiazoles are five-membered heterocyclic rings, which are not found in the nature. In such a ring, sulphur, nitrogen, and carbon atoms can be orchestrated more than one manner, leading to a few isomers *viz*. 1,2,4-thiadiazole, 1,2,3-thiadiazole, 1,3,4-thiadiazole and 1,2,5-thiadiazole [1,2]. Of these four isomers, subordinates of 1,3,4-thiadiazole appear to be the most well known among researchers. Many mixtures containing such a framework display an expensive range of biologic communications and have been displayed to have antifungal [3,4], antimicrobial [5], anti-inflammatory [6] and anticancer activities [7]. Just as other modern applications, it is worth focusing on their utilization as thickness stabilizers in elastic preparing [8], added substances for the creation of lithium battery anodes [9], colours [10] and optoelectronic materials [11]. There are likewise reports of the utilizations of 1,3,4-thiadiazole subsidiaries, specifically 2,5-dimercapto-1,3,4-thiadiazoles, as lubricants [12]. Azo colours, described by the presence of azo moiety (N=N) in their construction, are the most fundamental gathering of disperse dyes [13]. They have tracked down a wide application principally in dyestuff industry, yet additionally in food, beauty care products and drugs creation. For the most part azo colours display respond shading properties and proposition striking tones, beginning from yellows, through oranges, reds and winding up in blues. One of the subgroups of this family are formed 1,3,4-thiadiazoles containing an azo gathering (N=N) in their construction. Such heterocyclic azo scatter colours have gotten consideration from mainstream researchers because of their splendor, lucidity and partiality to different strands [14,15]. The most widely recognized strategy to bring this kind of gathering into a last azo compound is a two-venture change through proper diazonium salts [16]. These are are normally created from the response of essential sweet-smelling amines with nitrites within the sight of solid mineral acids at low temperatures. Because of the outrageous unsteadiness of diazonium salts [17], they are utilized following their development in coupling responses with phenols or amines, subbed with electron-giving gatherings. Different techniques used to get ready azo colors incorporate the buildup of nitro compounds with amines [18], decrease of nitro compounds [19,20], oxidation of amines [21,22] or buildup of nitroso compounds with amines [23].

Although thiadiazoles contain a significant thiazole chromophoric centre in their design, regular fragrant amines, diazotization and resulting coupling of 1,3,4-thiadiazole-2-

amine subordinates rarely reported [24-26]. This may occur due to the presence of an additional electronegative nitrogen, which reduces the basicity and reactivity of the outside amino group. To further review the adaptable uses and amalgamation of 1,3,4-thiadiazole subsidiaries [27-29], we endeavoured to portray and blend the primary provisions of the series of 2-phenylazo-1,3,4-thiadiazoles, which is functionalized with aryl substituents on its heterocyclic ring. Such frameworks with a 1,3,4-thiadiazole ring joined to other fragrant mixtures through the azo linkers may offer modern applications in the fields of not only old style engineered colours and shades, laser colours and monomers for OLED creation but also medicine and agriculture due to the presence of the toxophoric N-C-S moiety [30]. Various biological activities and structural aspects of imine salts and azo compounds motivated the synthesis of five novel azo compounds. These compounds were also explored for the antifungal, antibacterial and antioxidant properties.

EXPERIMENTAL

All the chemicals and reagents were purchased from Sigma-Aldrich, USA. The completion of the reaction was monitored by TLC using various solvent system and iodine vapour. The IR spectra of the compound were recorded in the region of 4000-400 cm^{-1} using KBr pallet on FT-IR Perkin spectrometer. The ^1H (400 MHZ) and ^{13}C (100 MHZ) NMR spectra were recorded on a Bruker instrument in DMSO-d_6 as solvent and TMS as an internal standard. Melting point of all the synthesized compounds were carried out in open capillaries and is uncorrected.

General procedure: 2-Amino-5-aryl-1, 3, 4-thiadiazole was synthesized following two step according to literature procedure [31].

Synthesis of thiosemicarbazones (III): Aromatic aldehyde (**I**, 0.2 mol) dissolved in 300 mL of warm alcohol and thiosemicarbazide (**II**, 0.2 mol) was dissolved in 300 mL of warm water with continuous stirring. The product separated immediately after cooling. The product was filtered through suction, dried, and recrystallized from ethanol to obtain thiosemicarbazone (**III**).

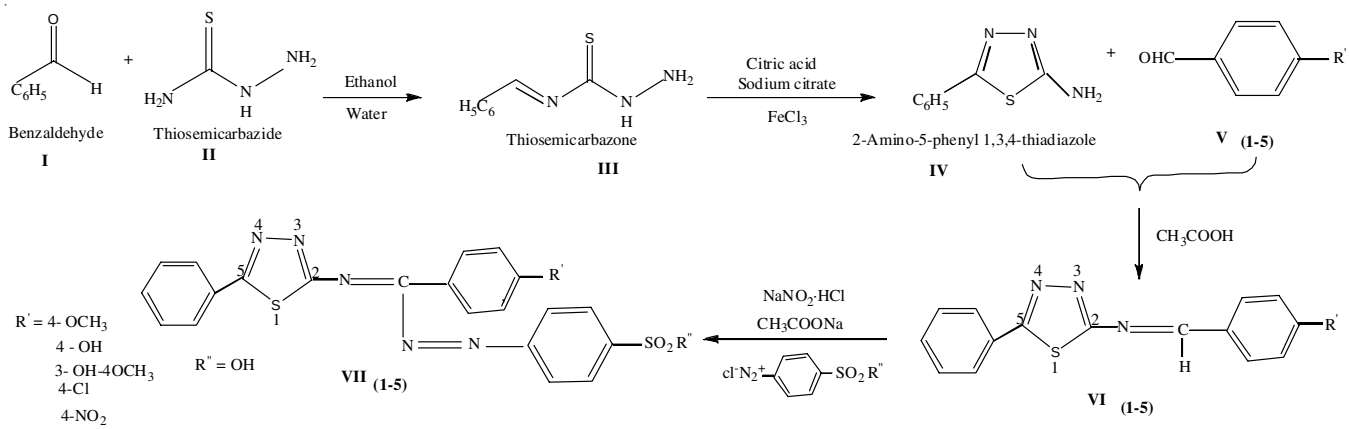
Synthesis of 2-amino-5-aryl-1,3,4-thiadiazole (IV): Compound (**III**, 0.05 mol) was suspended in 300 mL of warm water. To this, 0.15 mol of $FeCl_3$ was added slowly with constant stirring. The contents were heated for 45 min at 80-90 ºC and then filtered. Sodium citrate (0.05 mol) and citric acid (0.11 mol) were added to the filtrate. The resulting mixture was divided into four parts. Each part was neutralized separately with aqueous NH_3 (10%). Thiadiazole (**IV**) was separated out, filtered with suction, dried and recrystallized with an appropriate solvent.

Synthesis of 2-[5-phenyl-1,3,4-thiadiazole-2-imino] substituted benzene (VI)$_{1-5}$: 2-Amino-5-aryl-1,3,4-thiadiazole (IV) compound (0.01mol) was dissolved in 30 mL of glacial acetic acid. To this equimolar (0.01mol) quantity of aromatic aldehyde (**V**) in 20-30 mL of ethanol was added and refluxed for 6 h. The reaction mixture was allowed to stand for cool. After cooling, the resulting reaction mass was poured into crushed ice and left overnight. The solid benzylidene imine (**VI**) were separated out, filtred, washed thoroughly with petroleum ether, dried and recrystallized from hot ethanol.

Synthesis of 2-{[5-phenyl-1,3,4-thiadiazole-2-imino] substituted benzene}diazenyl benzene sulfonic acid (VII)$_{1-5}$: Benzylidene imines (VI) (0.02 mol), were dissolved in an ethanolic solution of sodium acetate (5 g) with constant stirring. Sodium nitrate (0.02 mol) was dissolved in H_2O and added to a well cooled solution of sulphanilic acid (0.02mole) initially dissolved in 3N HCl (25 mL). The content were left at room temperature for 2 h, compound (**VII**) was precipitated and then filtered, washed with water, dried and recrystallized. The results obtained were analyzed by TLC. All other compounds of this series were synthesized using the same method (**Scheme-I**).

2-{[5-Phenyl-1,3,4-thiadiazole-2-ylimino]-4-methoxybenzene}diazenyl benzene sulfonic acid (VII)$_1$: Yield: 81%; m.p.: 244-246 ºC; IR (KBr, ν_{max}, cm^{-1}): 3358 (OH), 3051 (C-H arom.), 2844 (-OCH_3), 1667 (C=N), 1527 (N=N), 1326 (S=O), 1287 (C-N); ^1H NMR (400 MHz, DMSO-d_6): δ 3.86(s, 3H, -OCH_3), 3.44 (s, 3H, Ar-SO_3H), 7.88 (d, 2H, J = 7.6 Hz), 7.68 (d, 4H, J = 7.2 Hz), 7.25 (d, 4H, J = 6.8 Hz), 7.14 (d, 2H, J = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.54 (C-N), 115.00-132.31 (Ar C-H), 164.70 (C=N), 56.17 (-OCH_3). Elemental analysis of $C_{22}H_{17}N_5O_4S_2$ calcd. (found) %: C, 55.10 (55.06); H, 3.57 (3.50); N, 14.60 (14.52).

2-{[5-Phenyl-1,3,4-thiadiazole-2-ylimino]-4-hydroxybenzene}diazenyl benzene sulfonic acid (VII)$_2$: Yield: 80%;



Scheme-I: Synthesis of azo dye

m.p.: 249-250 ºC; IR (KBr, ν_{max}, cm^{-1}): 3202 (OH), 3084 (C-H arom.), 1650 (C=N), 1544 (N=N), 1376 (S=O), 1281 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.364 (s, 3H, Ar-SO_3H), 4.526 (s, 1H, OH), 6.619 (s, 4H, Ar-H), 7.140 (s, 4H, Ar-H), 7.566 (s, 4H, Ar-H), 7.833 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6):δ 40.52 (C-N), 122.18-131.58 (Ar-CH), 156.56 (C=N), 169.38 (C-OH). Elemental analysis of $C_{21}H_{15}N_5O_4S_2$ calcd. (found) %: C, 54.18 (54.12); H, 3.25 (3.25); N, 15.04 (15.01).

2-{[5-Phenyl-1,3,4-thiadiazole-2-ylimino]-3-hydroxy-4-methoxybenzene}diazenyl benzene sulfonic acid (VII)$_3$: Yield: 76%; m.p.: 232-234 ºC; IR (KBr, ν_{max}, cm^{-1}): 3431(OH), 3069 (C-H arom.), 2851 (-OCH_3), 1664 (C=N), 1572 (N=N); 1383 (S=O), 1289 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.870 (s, 3H, -OCH_3), 3.566 (s, 3H, Ar-SO_3H), 7.140 (d, 2H, *J* = 8.4 Hz),7.312 (t, 2H, *J* = 8.0 Hz), 7.428 (d, 2H, *J* = 8.0 Hz), 7.715 (d, 2H, *J* = 8.0 Hz), 7.503 (s, 2H, Ar-H), 7.781 (s, 1H, Ar-H), 9.774 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6): 40.50 (C-N), 112.08-131.25 (Ar-CH), 153.80 (C=N), 56.28 (-OCH_3). Elemental analysis of $C_{22}H_{17}N_5O_5S_2$ calcd. (found) %: C, 53.32 (53.30); H, 3.46 (3.38); N, 14.13 (14.01).

2-{[5-Phenyl-1,3,4-thiadiazole-2-ylimino]-4-chloro-benzene}diazenyl benzene sulfonic acid (VII)$_4$: Yield: 80%; m.p.: 240-242 ºC; IR (KBr, ν_{max}, cm^{-1}): 3421 (OH), 3089 (C-H arom.), 1632 (C=N), 1543 (N=N), 1383 (S=O), 1244 (C-N), 763 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.775 (s, 3H, Ar-SO_3H), 7.542 (s, 16H, Ar-H), 7.126 (d, 4H, Ar-H, *J* = 5.6 Hz), 7.814 (s, 2H, Ar-H), 8.288 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 39.22 (C-N), 40.48 (C-Cl), 127.15- 131.89 (Ar-CH), 156.47 (C=N). Elemental analysis of $C_{21}H_{14}N_5O_3S_2Cl$ calcd. (found) %: C, 52.11 (52.99); H, 2.92 (2.82); N, 14.47 (14.38).

2-{[5-Phenyl-1,3,4-thiadiazole-2-ylimino]-4-nitro-benzene}diazenyl benzene sulfonic acid (VII)$_5$: Yield: 78%; m.p.: 228-230 ºC; IR (KBr, ν_{max}, cm^{-1}): 3431(OH), 3103 (C-H arom.), 1632 (C=N), 1542 (N=N), 1383 (S=O), 1345 (C-NO_2); 1245 (C-N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.776 (s, 3H, Ar-SO_3H), 7.312 (s, 4H, Ar-H), 7.545 (s, 6H, Ar-H), 7.815 (s, 2H, Ar-H), 8.061 (s, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 39.68 (C-N), 127.10-131.61 (Ar-CH), 156.55 (C=N); Elemental analysis of $C_{21}H_{14}N_6O_5S_2$ calcd. (found) %: C, 51.00 (51.08); H, 2.85 (2.76); N, 16.99 (16.90).

Biological studies

Antibacterial activity: The screening of all the novel synthesised compounds (**VII**)$_{1-5}$ was conducted for study their antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *E. coli* and *Klebsilla pneumonia* by using the diffusion method with streptomycin as the standard drug at concentration of 1000 μg mL^{-1} for antibacterial activity [32,33]. The bacterial strains were stored in the nutrient agar (NA) medium. The dissolved medium was autoclaved at 121 ºC at 15 Lbs pressure for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured into petri dishes (25 mL/plate). The plates were swabbed using the pathogenic bacteria culture. Finally, the sample-loaded disc was placed on the Mueller-Hinton Agar medium surface. The plates were incubated at 37 ºC for 24 h. The inhibition zone size (including disc) was estimated in millimetres.

Antifungal activity: The antifungal activity was against *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* was recorded. The antifungal activity was determined using the agar disc diffusion method by employing fluconazole as the standard drug [34]. Up to 40 μL of extract of each concentration was introduced into the sterile discs by using sterile pipettes. The discs were then placed on the SDA medium surface. The compound was allowed to diffuse for 5 min and the plates were incubated at 22 ºC for 48 h. At the end of incubation, the inhibition zones were analyzed near the discs and measured with a transparent ruler in millimetres.

Antioxidant activity: The activity of free radical scavenging of fractions was estimated using 2,2′-diphenyl-1-picryl-hydrazyl (DPPH) assay *in vitro* by following a standard method [35]. A stock solution was produced by dissolving 24 mg of DPPH in 100 mL of ethanol and it was stored at 20 ºC until further use. The functional arrangement was achieved by diluting DPPH with ethanol and 3 mL of this aliquot was mixed with 1 mL of test solution at different concentrations (100, 200 and 300 μg/mL). Then, this reaction mixture was shaken well and incubated at room temperature in dark for 15 min. Subsequently, the absorbance was measured at 517 nm. The control was synthesized without a sample. The scavenging activity was evaluated depending on the amount of DPPH radical scavenged with the following equation:

$$\text{Inhibition } (\%) = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

RESULTS AND DISCUSSION

A novel series of 2-{[5-phenyl-1,3,4-thiadiazole-2-imino] substituted benzene}diazenyl benzene sulfonic acid (**VII**)$_{1-5}$ derivatives were achieved by sequence of reaction as shown in **Scheme-I**. Infrared spectrum of compound (**VII**)$_1$ showed characteristic vibrations at 1650 cm^{-1} for the imine group, at 2844 cm^{-1} for the -OCH_3 group and 3358 cm^{-1} for the H- bonded -OH group. The occurrence of the azo group is exhibited at 1527 cm^{-1}. The peak at 1281 cm^{-1} represent the presence of the C-N group. All the other peaks in the spectra represent other functionalities of the synthesized azo dyes. The ^{1}H NMR spectrum of (**VII**)$_1$ showed a singlet at δ 3.86 ppm for -OCH_3 and δ 7.88 for the doublet, *J* = 7.6 Hz and another doublet at δ 7.68, *J* = 7.2 Hz for Ar-H, which confirmed the formation of this derivative. The ^{1}H NMR data of all compounds show the aromatic protons in the range of 7.14-7.81 ppm. In the ^{13}C NMR spectra, the resonances in the region 164.70 and δ 132.31 ppm were assigned the imine carbon and aromatic carbon located in the region δ 115.00-130.10 ppm. The carbon of the methoxy carbon located at the region δ 56.17 ppm. Substituents in the synthesized compounds (**VII**)$_{1-5}$ were showed the characteristic vibration in the respective regions.

Antibacterial activity: Among the synthesized compouds, compounds (**VII**)$_2$, (**VII**)$_4$ and (**VII**)$_5$ showed good activity with the zone of inhibition 13 mm, 12 mm and 14 mm, respec-

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF THE SYNTHESIZED COMPOUNDS **(VII)$_{1-5}$**

Compd. No.	R	Inhibition zone of diameter (mm)						
		Antibacterial				Antifungal		
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsilla pneumoniae*	*E. coli* (Gram-negative)	*Aspergillus flavus*	*Aspergillus niger*	*Rhizopus stolonifer*
(VII)$_1$	4-OCH_3	10	9	12	8	8	–	–
(VII)$_2$	4-OH	14	15	12	13	8	7	8
(VII)$_3$	3-OH-4-OCH_3	10	11	–	8	10	16	12
(VII)$_4$	4-Cl	–	11	9	12	8	–	–
(VII)$_5$	4-NO_2	8	13	–	14	–	–	–
Positive control	–	17	16	15	15	20	19	21
Negative control	–	–	–	–	–	–	–	–

tively against the bacteria *E. coli*. Compounds **(VII)$_2$**, **(VII)$_3$**, **(VII)$_4$** and **(VII)$_5$** were active against *Bacillus stubtilis*, while compound **(VII)$_2$** showed good activity with *Staphylococcus aureus* and *Klebsilla pneumonia* as compared to the standard drug streptomycin (Table-1). The remaining compounds of the series possessed moderate antibacterial activity.

Antifungal activity: The antifungal activities of the synthesized compounds **(VII)$_1$**, **(VII)$_2$** and **(VII)$_3$** were very active with the zone of inhibition 11, 16 and 14 mm, respectively. Compound **(VII)$_2$** recorded with the maximum zone of inhibition, whereas compounds **(VII)$_4$** and **(VII)$_5$** showed no activity against *Aspergillus flavus*. Compound **(VII)$_2$** showed good activity zone of inhibition 16 mm and compound **(VII)$_5$** recorded with zone of 8 mm against *Aspergillus niger*. Compound **(VII)$_2$** recorded with maximum zone of inhibition 12 mm and compound **(VII)$_5$** recorded with zone of 8 mm against *Rhizopus stolonifer* (Table-1). Whereas the remaining compounds **(VII)$_1$**, **(VII)$_3$** and **(VII)$_4$** showed no activity against *Aspergillus niger* and *Rhizopus stolonifer*.

Antioxidant activity: The structure-activity relationship (SAR) of synthesized compounds revealed that the compounds having electron donating group [**(VII)$_4$** and **(VII)$_5$**] showed higher radical scavenging activity as compared other compounds [**(VII)$_1$**, **(VII)$_2$** and **(VII)$_3$**]. It was also observed that compounds **(VII)$_1$** and **(VII)$_3$** exhibited good antioxidant activity, whereas compound **(VII)$_2$** displayed low activity when compared to standard ascorbic acid. Moreover, the radical scavenging activity increases with increase in concentration in this method (Table-2).

TABLE-2
ANTIOXIDANT ACTIVITY OF THE SYNTHESIZED COMPOUNDS **(VII)$_{1-5}$**

Compd. No.	Inhibition (%)		
	100 µg/mL	200 µg/mL	300 µg/mL
(VII)$_1$	35.049 ± 0.101	42.622 ± 0.028	48.224 ± 0.036
(VII)$_2$	19.120 ± 0.033	27.471 ± 0.010	27.611 ± 0.015
(VII)$_3$	29.790 ± 0.020	39.195 ± 0.012	40.189 ± 0.039
(VII)$_4$	22.028 ± 0.030	45.649 ± 0.237	90.488 ± 0.002
(VII)$_5$	29.804 ± 0.002	47.006 ± 0.032	91.052 ± 0.010
Ascorbic acid	95.993 ± 0.040	96.135 ± 0.002	96.393 ± 0.005

Conclusion

A series of azo-imine dye *viz*. 2-{[5-phenyl-1,3,4-thiadiazole-2-imino] substituted benzene}diazenyl benzene sulfonic acid [**(VII)$_{1-5}$**] derivatives were synthesized and characterized. The constitution of these compounds assigned on the basis of IR, ^{1}H and ^{13}C NMR spectra and elemental analyses were found to be in correlation with the desired structure. The antibacterial activity screening revealed that compound **(VII)$_2$**, hydroxy substituted azo-imine dye has good activity as compared to the standard drug streptomycin. The antifungal activity showed that the compound **(VII)$_3$**, 3-hydroxy-4-methoxy substituted azo-imine dye has good activity as compared to the standard drug fluconazole. Antioxidant activity of chloro and nitro substituted azoimine compounds [**(VII)$_4$** and **(VII)$_5$**] exhibited higher radical scavenging activity when compared with standard compound ascorbic acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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