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Research Paper

# Synthesis, Characterization And In- Vitro Antibacterial Activities Of Schiff Base Compounds Derived From 5-Amino-1, 3, 4-Thiadiazole-2-Thiol

Chemistry

**KEYWORDS :** Thiadiazole; schiff base; NMR spectroscopy; antibacterial activity.

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## ABSTRACT

*Nitrogen and sulphur based heterocyclic schiff base compounds were synthesized by simple condensation of 5-amino-1, 3, 4-thiadiazole-2-thiol with carbonyl derivatives in alcoholic medium and characterized with the help of elemental analyses, IR, 1H and 13C-NMR spectroscopic techniques. The in vitro antibacterial activities of the compounds were also screened against different bacteria and were found to exhibit moderate antibacterial activities. The minimum inhibitory concentration of the tested compounds were also determined and compared with standard drugs.*

## INTRODUCTION

Thiadiazole and its derivatives are well known for their potential biological activities. The interesting biological activity of thiazole derivatives is because of their strong aromaticity of the ring system which enhances in vivo stability[1]. Thiadiazole, the heterocyclic analogue of thiosemicarbazide having the deprotonated heterocyclic thioamide (N-C-S-) group are interesting owing to their wide range of coordination modes and possible toxophore in many pesticides[2]. Thiadiazole compounds were also reported to possess antimicrobial[3-5], antitumor[6-7], antituberculosis[4, 8], anticancer[9], anti-HIV[10] and antihypertensive[11] activity. The reactivity of thiadiazoles in polymerization processes, substitution reactions at different moieties[12] have been greatly influenced by the existence of thiol-thione tautomerism in substituted thiadiazoles. There are four isomers of thiadiazoles, out of which more work has been reported on 1, 3, 4-thiadiazole as compare to other isomers. These types of thiadiazoles were found to possess diverse applications as oxidation inhibitors, pharmaceuticals, cyanine dyes and metal complexing agents[13]. In recent years, continuing attention has also been given in ambidentate nitrogen-sulfur heterocyclic thiolato ligands owing to their presence of both endocyclic hard nitrogen donor and an exocyclic soft sulphur donor in large number of compounds[14]. One such example of versatile ligand is the schiff base compound of 5-amino-1, 3, 4-thiadiazole-2-thiol because it can coordinate to a metal ion in several ways. In view of this important class of these thidiazoles and as part of our continuous effort to search effective antimicrobial compounds; in this present work, we have reported synthesis, characterization and *in vitro* antibacterial properties of some schiff base thiazoles derived from 5-amino-1, 3, 4-thiadiazole-2-thiol. One of the thiadiazole schiff base compound resulted from salicylaldehyde and 5-amino- 1, 3, 4- thiadiazole-2-thiol was reported in literature[15]. However, this compound is also included along with other thiadiazoles for their spectral and in vitro antibacterial studies.

## EXPERIMENTAL

Salicylaldehyde (Loba), 2,4dihydroxyaceto phenone (Aldrich), Vaniline, Acetyl acetone and 5-amino-1, 3, 4-thiadiazole-2-thiol (Merck) were used without further purification. The solvents used in the reactions were of AR grade and dried using standard procedures. Elemental analyses were carried out on a Perkin-Elmer 2400 series II elemental analyser. IR spectra in the range of 4000-400 $cm^{-1}$ were obtained on Shimadzu FT-IR-8400S model spectrophotometer using KBr discs. The $^1H$ and $^{13}C$ -NMR spectra were recorded on a Bruker AMX 400 spectrophotometer measured at 400.12 and 100.62 MHz respectively. $^1H$ and $^{13}C$ chemical shifts were referred to $Me_4Si$ set at 0.00 ppm. Reactions were monitored by TLC on silica gel 60 $F_{254}$ (0.25 mm).

## SYNTHESIS OF SCHIFF BASE COMPOUNDS

Thiadiazole schiff base compounds, **1**-**4** were synthesized by the equimolar condensation reaction of 5-amino-1, 3, 4-thiadiazole-2-thiol with carbonyl derivatives *viz*. Salicylaldehyde (**1**), 2, 4-dihydroxy acetophenone (**2**), Vaniline (**3**) and Acetylacetone (**4**) in methanol at ambient temperature . The reaction mixture was refluxed for 3 h and evaporated to give yellow crystalline compounds. The compounds were dried in vacuum and its purity was confirmed by TLC technique.

**Compound 1:***5[(2Hydroxyphenyl)methylene amino]-1,3,4-thiadiazole-2(3H)-thione* M.P °C: 220, Calculated (%) for $C_9H_7N_3OS_2$ (237.30) : C = 45.55; H = 2.97; N = 17.71; S = 27.02; Found (%): C = 45.30; H = 2.50; N = 17.10 ; S = 26.85. FT-IR (KBr disc, $cm^{-1}$): $\upsilon(OH)_{str}$ 3074, υ(S-H) 2650, υ(C=N) 1604, υ(S-C=N) 1527, υ(C-S)979. $^1H$ NMR (DMSO-$d_6$), δ: 11.2 (s, 1H, OH), 8.86 (s, 1H, CH=N), 7.84-6.95 (m, 4H, Ar-H), 14.5 (s, 1H, S-H) ($D_2O$ exchange). $^{13}C$ NMR (DMSO-$d_6$) δ: 192.61, 181.66 (thiadiazole carbons), 162.25(C-OH), 161.01(C=N), 137.36, 130.15, 122.43, 120.19, 117.71(Ar-C).

**Compound2:***4(1(5mercapto1,3,4thiadiazol-2-ylimino)ethyl) benzene-1,3-diol* M.P°C: 180, Calculated (%) for $C_{10}H_9N_3O_2S_2$ (267.33): C = 44.93 ; H = 3.39; N = 15.72; S = 23.99; Found (%): C = 44.70; H = 3.15; N = 15.25; S = 23.60. FT-IR (KBr disc, $cm^{-1}$): $\upsilon(OH)_{str}$ 3142, υ(S-H) 2769, υ(C=N) 1610, υ(S-C=N) 1554, υ(C-S) 982. . $^1H$ NMR (DMSO-$d_6$) δ: 12.69 (s, 1H, OH), 7.27-6.38 (m, 3H, Ar-H), 2.57 (s, 3H, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$)δ: 192.15, 180.56(thiadiazole carbons), 162.20(C-OH, *ortho*),161.35(C=N), 152.50(C-OH, *para*), 136.36, 132.86, 115.15, 108.25(Ar-C), 25.75($CH_3$).

**Compound3:***4-((5-mercapto1,3,4thiadiazol-2-ylimino)methyl)-2-methoxyphenol* M.P °C: 193, Calculated (%) for $C_{10}H_9N_3O_2S_2$ (267.01) : C = 44.93; H = 3.39; N = 15.72; S = 23.99; Found (%): C = 44.40; H = 3.20; N = 15.30; S = 23.55. FT-IR (KBr disc, $cm^{-1}$): $\upsilon(OH)_{str}$ 3144, υ(S-H) 2720, υ(C=N) 1602, υ(S-C=N) 1516, υ(C-S) 990. $^1H$ NMR (DMSO-$d_6$) δ: 13.17 (s, 1H, OH), 8.51 (s, 1H, CH=N), 7.51-6.91 (m, 3H, Ar-H), 3.82 (s, 3H, $OCH_3$). $^{13}C$ NMR (DMSO-$d_6$) δ: 191.22, 180.77 (thiadiazole carbons), 161.46(C=N), 152.91(C-OH), 148.05, 128.60, 126.07, 115.28, 110.47(Ar-C), 55.46($OCH_3$).

**Compound 4:** *(E)-4-(5-mercapto-1,3,4-thiadiazol-2-ylimino) pentan-2-one* M.P °C: 200, Calculated (%) for $C_7H_9N_3OS_2$ (215.30) : C = 39.05; H = 4.21; N = 19.52; S = 29.79; Found (%): C = 38.80; H = 4.05; N = 19.25; S = 29.40. FT-IR (KBr disc, $cm^{-1}$): υ(S-H) 2750, υ(C=N) 1608, υ(S-C=N) 1554, υ(C-S) 997. $^1H$ NMR (DMSO-$d_6$) δ: 13.35 (s, 1H,OH of the *enol* form), 3.76 (s, 1H, -CH=C, *enol* form), 3.72(s, 2H, -$CH_2$ *keto* form), 2.76 -2.49(m, 6H, two $CH_3$ of *keto* and *enol* form). $^{13}C$ NMR (DMSO-$d_6$) δ: 181.54, 180.78(C=O, *keto* form), 165.74, 162.35(C=N), 161.87 and 150.71 (2C,C=C-OH, *enol* form), 118.26($CH_2$, *keto* form), 23.77 and 19.05(2C, $CH_3$).

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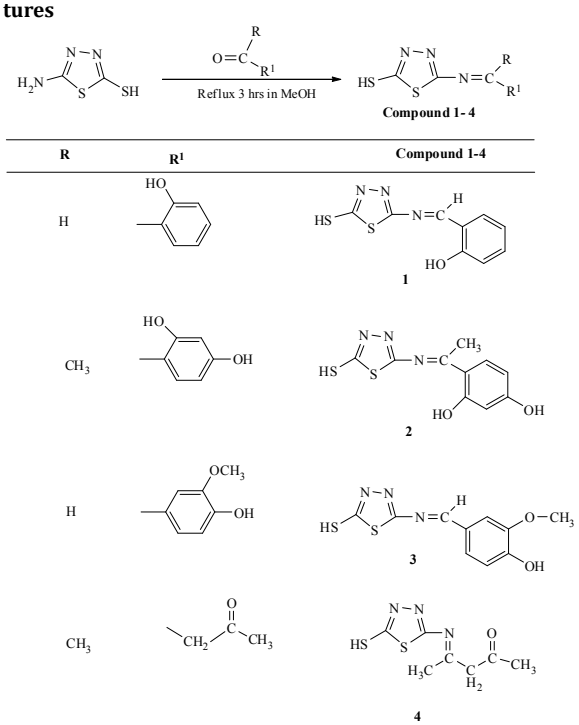
**ANTIBACTERIAL TEST**

The synthesized schiff base compounds **1** and **2** were screened *for their in vitro* antibacterial activities against *Proteus mirabilis*, *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa* strains. The activity was assessed by agar well diffusion method[16] using 20 ml of sterile Nutrient Agar (Hi-Media). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Sterile 6mm diameter cork borer were pierced in the agar at equidistant. The dilutions of the compounds concentration (5mg/mL$^{-1}$ in DMSO) of the test sample were deposited in 20µl on the inoculated well and left for 10 min at room temperature for the compound diffusion. Ciprofloxacin (Hi-Media) was served as positive control while negative control was prepared using DMSO. The plates were inoculated with bacteria were incubated immediately at 37ºC for 24 hr. All the experiment was performed in triplicate and the average results were recorded. The activity was determined by measuring the diameter of the inhibition zone (mm) around the well.

The susceptibility of bacteria was determined by minimum inhibitory concentration determination method[17]. The minimum inhibitory concentrations (MICs) of the compounds were determined by serial dilution against the bacterial strains. The minimum concentrations at which no visible growth were observed is defined as the MICs, which were expressed in µg/ml.

**Scheme 1: Reaction of Compounds 1 to 4 with their structures**




**Scheme 2:** ***Keto-enol*** **tautomer of compound 4**

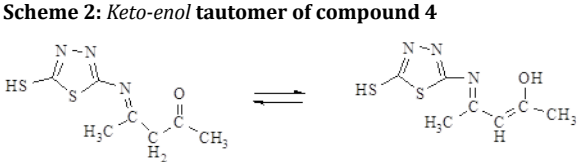


**Table 1: Antibacterial activity of thiadiazole compounds[a] (zone of inhibition in mm) and their Minimum inhibitory concentrations (MICs).**

| Bacrerial strains | Compounds 1 | 2 |
|---|---|---|
| *P. mirabilis* | 10 | 12 |
| *K. pneumoniae* | 10 | 10 |
| *E. coli* | - | 8 |
| *S. paratyphi* | 9 | 10 |
| *P. aeruginosa* | - | - |

**MIC for tested bacteria (Concentration in µg mL$^{-1}$ )**

| Bacrerial strains | Compounds 1 | 2 |
|---|---|---|
| *P. mirabilis* | 625 | 312.5 |
| *K. pneumoniae* | >625 | 625 |
| *E. coli* | >5000 | >625 |
| *S. paratyphi* | >625 | <625 |
| *P. aeruginosa* | >5000 | >5000 |

[a]In vitro agar well- diffusion method, concentrations: 3mg mL$^{-1}$ in DMSO, reference drug, Ciprofloxacin : 16µg mL$^{-1}$ in DMSO, dash(-) indicated inactivity.

**RESULTS AND DISCUSSION**

Thiadiazole shciff base compounds, **1**- **4** were synthesized by the reaction of carbonyl derivatives with 5-amino-1, 3, 4-thiadiazole-2-thiol in methanol as shown in reaction (scheme 1). The compounds have been characterized by elemental analysis, IR and

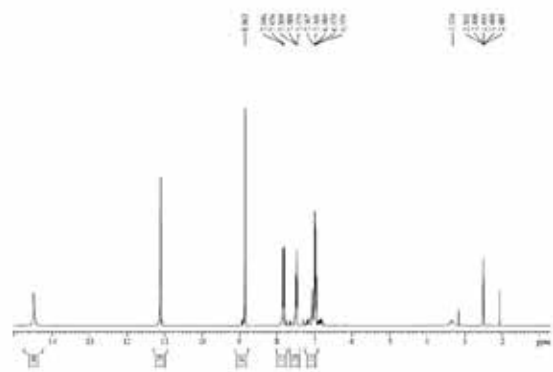


**Fig.1 $^{1}$H NMR spectrum of compound 1**

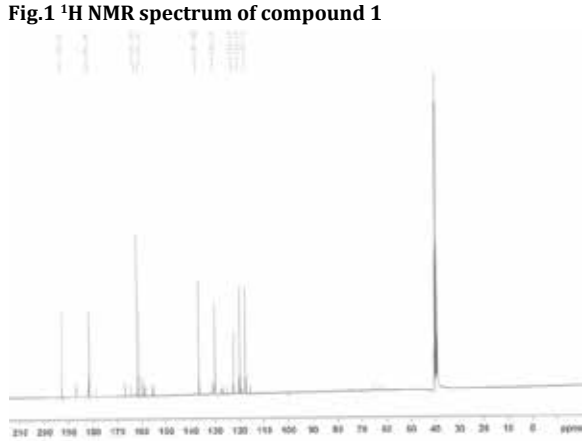


**Fig.2 $^{13}$C NMR spectrum of compound 1**

NMR spectroscopy. The structure of thione form of **1** i.e., 5-[(2-Hydroxy-phenyl) methylene amino]-1, 3, 4-thiadiazole-2(*3H*)-thione was reported earlier[15], however, antibacterial property of the compound have not been reported. Moreover, we have completely characterized the compound by $^{1}$H- and $^{13}$C-NMR spectroscopy. Therefore, compound **1** is also included in this study and antibacterial activity of compound **1** has been studied along with **2**. The IR spectra of the compounds **1**-**4** exhibited IR absorption bands at 2650-2769 cm$^{-1}$, 1602-1610 cm$^{-1}$,1516-1554 cm$^{-1}$ and 979- 997 cm$^{-1}$ which may be attributed to υ(SH), υ(C=N), υ(S-C=N) and υ(C-S) stretching frequencies[18]. $^{1}$H-and $^{13}$C-NMR of the thiadiazole schiff base compounds, **1**-**4** were recorded in DMSO-$d_6$. The $^{1}$H and $^{13}$C NMR spectra of compound **1** are given in Fig.1 and Fig. 2. The singlet peak appeared at δ 8.86 and 8.51 ppm in compound **1** and **3** may be assigned to azomethine proton (-CH=N). The proton NMR spectra of the

compounds **1**-**3** show multiplet peaks at around δ 6.38 to 7.84 ppm due to aromatic protons and chemical shift values shown around δ 11.20 to 13.17 ppm may be assigned to -OH protons. A singlet peak appeared at δ 2.57ppm in compound **2** and δ 3.82 ppm in 3 is due to - $CH_3$ and -$OCH_3$ protons respectively. NMR spectroscopy also provides the fruitful method to investigate *keto-enol* tautomers[19]. Compound **4** exhibits *keto-enol* tautomerism (scheme 2) in which one proton from methylene carbon atom transfers to oxygen atom. Thus, NMR spectrum of compound **4** shows a separate and distinct proton and carbon signals for both the tautomer as observed in the case of acetyl acetone in which such type of *keto-enol* tautomer was studied by NMR spectroscopy[19]. The singlet peak appeared at δ 3.72, 3.76 ppm and δ 2.49 to 2.76 ppm in compound **4** are assigned to -$CH_2$, -CH= and two -$CH_3$ protons for the *keto-enol* tautomer whereas the weak and broad singlet peak observed at δ 13.35 ppm may be due to -OH proton of the *enol* form . Compound **1** also shows a singlet peak at δ 14.50 ppm due to S-H proton ($D_2O$ exchangeable)[20]. In other compounds **2**-**4**, the S-H protons ($D_2O$ exchangeable) could not be detected. The $^{13}C$ NMR signals observed at δ 191.02 and 181.66 ppm in compound **1** and **3** may be assigned to thiadiazole carbons and the signals at δ 161 ppm are due to (C=N) carbons. In compound **2** and **3**, the $^{13}C$ signals for $CH_3$ and $OCH_3$ were observed at δ 25.75 and 55.46 ppm. The $^{13}C$ NMR spectrum of compound **4** gives nine $^{13}C$ signals corresponding to *keto* and *enol* tautomer (Scheme 2). The $^{13}C$-NMR signals observed at δ 180.78 and 162.35 ppm are due to (C=O) of the *keto* form and (-C=N) carbon atoms while signals at δ 161.87 and 150.71 ppm may be assigned to the two carbons (-C=C-OH) of the *enol* form. The $^{13}C$-NMR signal appeared at δ 23.77 and 19.05 ppm is due to the carbons of the two -$CH_3$ groups. The numbers of $^{13}C$ signals observed in the $^{13}C$-NMR of the compounds are consistent with the proton NMR data of the compounds and are in agreement with the formation of the products. Antibacterial activities of compounds **1** and **2** were screened against various microbes taking as positive control and DMSO as negative control. The zone of inhibitions shown by the compounds against the bacteria and their minimum inhibitory concentrations (MICs) are shown in Table 1. From the table it is clear that, compound **2** exhibited antibacterial activities against all the tested bacterial strain except against *P. aeruginosa* while compound **1** was found to be ineffective against *E. coli* and *P. aeruginosa*. Both the compounds exhibited almost similar antibacterial activity and the results are comparable with that of the similar type of the reported thiadiazole compound[21].

## CONCLUSIONS

Schiff base thiadiazole compounds were synthesized and characterized by IR and NMR spectroscopy in combination with elemental analysis. NMR spectroscopy study of the compounds indicated the existance of *keto-enol* tautomer in compound **4**. The antibacterial activities of compounds **1** and **2** were screened against various bacteria and were found to exhibit moderate activity against the tested bacteria strain. It has been also observed that compound **1** was found to be inactive against *E. coli* and *P. aeruginosa* whereas except *P. aeruginosa* compound **2** exhibited antibacterial activities against all the tested bacteria.

## ACKNOWLEDGEMENTS

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