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Synthesis and biological evaluation of Schiff bases from substituted 1,3, 4 Thiadiazole as multi-functional agents with antimicrobial and antioxidant activities

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Abstract:
Schiff bases have gradually evolved into powerful biological agents because of their biological and medicinal properties. In this work, an efficient method is developed for the synthesis of Schiff bases from substituted 1,3,4 Thiadiazole. All the synthesized molecules were characterized using various spectral techniques including FTIR, 1HNMR,13CNMR, and Mass spectrometry.All the new synthesized compounds were then screened for their antimicrobial activity against three types of bacteria and three fungal strains, by application of the MIC assays. The antioxidant activities were also estimated and strongly correlated with the potential of BHT or butylated hydroxy toluene. All the synthesised compounds showed good antimicrobial activity at concentrations ranging between at 1–2.25 g/mL. The synthesised compounds also showed promising antioxidant activities, as compared with BHT, with 3b, 3d, and 3h showing appreciable antioxidant efficacy.

Keywords: 1,3,4 Thiadiazols, Synthesis, Biological Efficacy, Antimicrobial activity, Antioxidant activity

Introduction

Numerous research groups have shown interest in investigating aromatic nitrogen heterocycles with five-membered rings due to their intriguing biological activities and therapeutic qualities (Pund et al., 2023; Gur et al., 2020). Schiff bases have garnered significant attention in scientific research owing to their diverse range of biological functions (Aggarwal et al., 2022; Li et al., 2019).

Thiadiazole compounds have anextensive spectrum on pharmacological academics'scope due to the biological activities they possess. 1,3,4-Thiadiazoles are a substantial category of azoles that possess notable biological characteristics. Numerous instances of their antifungal, antibiotic, and antioxidant actions have been documented in the literature (Ali et al., 2022; Dueke-Eze et al., 2020; Ibrahim et al., 2022). Moreover, several studies have shown that the incorporation of heterocyclic units may greatly enhance the antibacterial properties (Gopi et al., 2017; Merugu et al., 2020).

The main objective of this work is Schiff bases'creation and biological assessment from substituted 1,3, 4 Thiadiazole.For 1,3,4-thiadiazole's biological relevance, Novel Schiff base derivatives were devised and subsequently synthesised. Following that, the selected compounds were assessed for their biological action, including their ability to combat oxidative stress and their antibacterial properties.

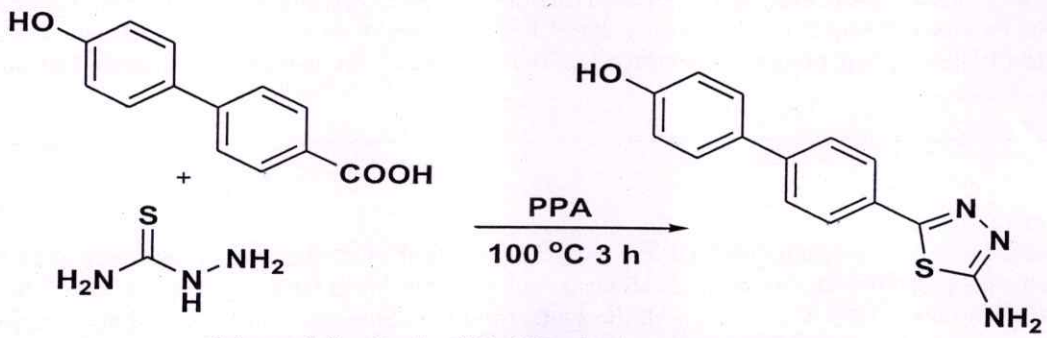
Material and method

Anydrous solvents and reagents were procured from commercial suppliers such as TCI Chemical, Alfa Assar, Sigma Aldrich Chemical Company, and E. Merck India Ltd., all of which are located in the United States of America and India, respectively. TLC was utilized in order to track the reaction's development. Column chromatography with silica gel particles ranging in size from 100 to 200 mesh packed in a glass column was utilized to purify the product. In DMSO-d6, BRUKER-AV300, BRUKER-AV400, and BRUKER-AV500 spectrometers were utilized to acquire ^{1}H and ^{13}C spectra.

2.1 Synthesis

2.1.1 Synthesis of Thaidiazoles

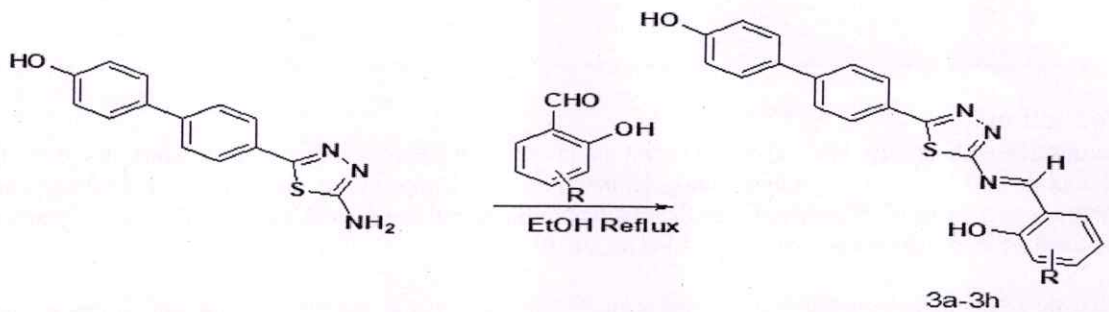
Thiosemicarbazide(0.011 moles) and 4'-hydroxy-[1,1'-biphenyl]-4-carboxylic acid (0.011 moles) were charged into a 100 mL polyphosphoric acid round bottom flask (Scheme 1). 30 minutes were spent stirring the reaction mixture on a hot plate. While monitored by TLC, the reaction mixture refluxed for around three hours. After the reaction, 50% of the solvent was vacuum-collected at 35–40 °C. After 30 minutes of stirring, the reaction mass was filtered, washed, and quenched in 20 g of crushed ice.By recrystallizing the isolated solid from the hexane-ethyl acetate combination, the pure compound was obtained.



Scheme 1:Synthesis of Thaidiazoles in presence of PPA

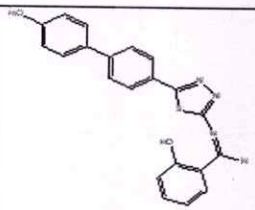
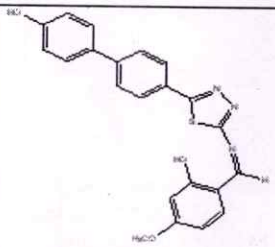
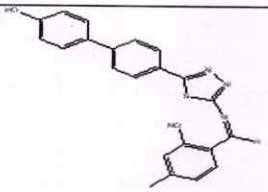
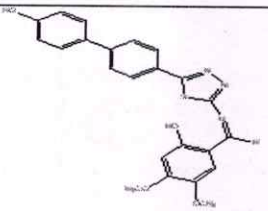
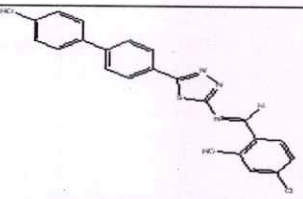
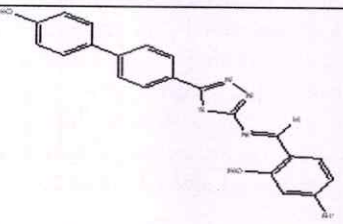
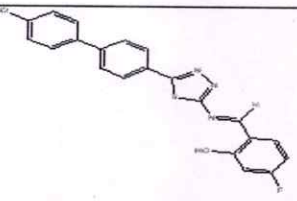
2.1.2 Synthesis of 1,3,4-Thiadiazole Schiff Bases

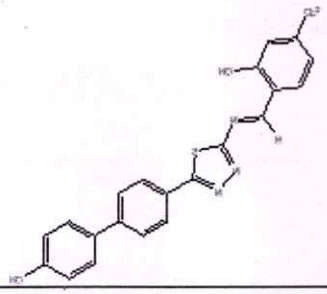
Schiff bases were formed by reacting equimolar quantities of 4'-(5-amino-1,3,4-thiadiazol-2-yl)-[1,1'-biphenyl]-4-ol and substituted salicylaldehyd (Scheme 2). Each component was dissolved in a little ethanol before mixing. Two hours were spent stirring the reaction mixture at room temperature. After placing reaction mass inside cold water, solid product got filtered along with oven-dried at 80 °C. Drying and re-crystallizing the product from ethanol yielded 85% gave the pure product.



Scheme 2: Synthesis of 1,3,4-Thiadiazole Schiff Bases (3a-3h)

Table 1: Physical data of 1,3,4-Thiadiazole Schiff Bases:

Sr. No.	Name of derivative	Structure	Color and State	Yield (in %)	MP (°C)
1	3a		White Solid	82	102
2	3b		White Solid	80	100
3	3c		White Solid	80	106
4	3d		White Solid	81	98
5	3e		White Solid	84	100
6	3f		White Solid	78	102
7	3g		White Solid	80	104

8	3a		Solid Yellow	67	108

2.2. Biological Evaluation

2.2.1. Antimicrobial activity

In vitro antimicrobial activities were determined using micro broth dilution assay. Microbial strains *Pseudomonas aeruginosa* NCIM 5031, *Escherichia coli* NCIM 2065, *Bacillus subtilis* NCIM 2699, along with *Aspergillusniger* NCIM 620, *Aspergillusfumigatus*NCIM 902, along with *Aspergillusflavus*NCIM 549, were acquired from the National Chemical Laboratory, Pune, India.

2.2.1.1 Preparation of inoculums

For Bacteria -The bacterial strains were quantified in terms of CFU utilising the serial plate dilution technique. The bacterial counts were then standardised to a range of 1×105 - 1×106 CFU/ml in order to conduct susceptibility testing.

For Fungus -The fungal inocula were generated by adjusting each fungus's spore density utilising a spectrophotometer at a wavelength of A_{595} nm, aiming to achieve a final conc. of roughly 10^5 spores/ml.

Micro broth dilution assay

The measurement of MIC was conducted utilising the micro broth dilution technique, following the standards established by NCCLS. The compounds were then dissolved directly in DMSO to set up eight dissimilar concentrations viz. 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/ml in the wells utilisingtwo-fold dilution system. Also, negative control - Dimethyl sulphoxidepositive control - Tetracycline along with Amphotericin B.

2.2.2. Antioxidant activity

The reported method produced DPPH radical-scavenging action.EC50 value (g/mL) is calculatedby reducing the concentration of DPPH by 50% BHT, or butylated hydroxy toluene, used as the reference

Result and Discussion

3.1. Characterization of the Synthesized Compounds

The synthesized derivatives' chemical structures were deduced based on spectral data. The ^{1}HNMR spectrum of 3a displayed a multiple singlet because of 2 OH groups and 1 NH group at δ 6.3 ppm, and a multiplet δ 7- 8 ppm for aromatic hydrogens (Fig. 1).Fig 2 shows the 1HNMR of the reactant. It is observed from the spectra thatthe NH_2 singletsharp peak at 5.9 ppm disappears in the Schiff base spectra (fig 1), due toNH2 conversion to imine group (HC=N). The 13C NMR spectra shows in range between 75ppm to 165ppm range, where carbon and oxygen bond shows in between 60-70ppm and imine carbon shows near 155 ppm.3a compound's IR spectrum displayed bands at 1602 cm^{-1} for C=N stretching) along with at 2962 cm^{-1} because of C-H stretching. Mass spectra showed a peak at exact mass of 373.09.

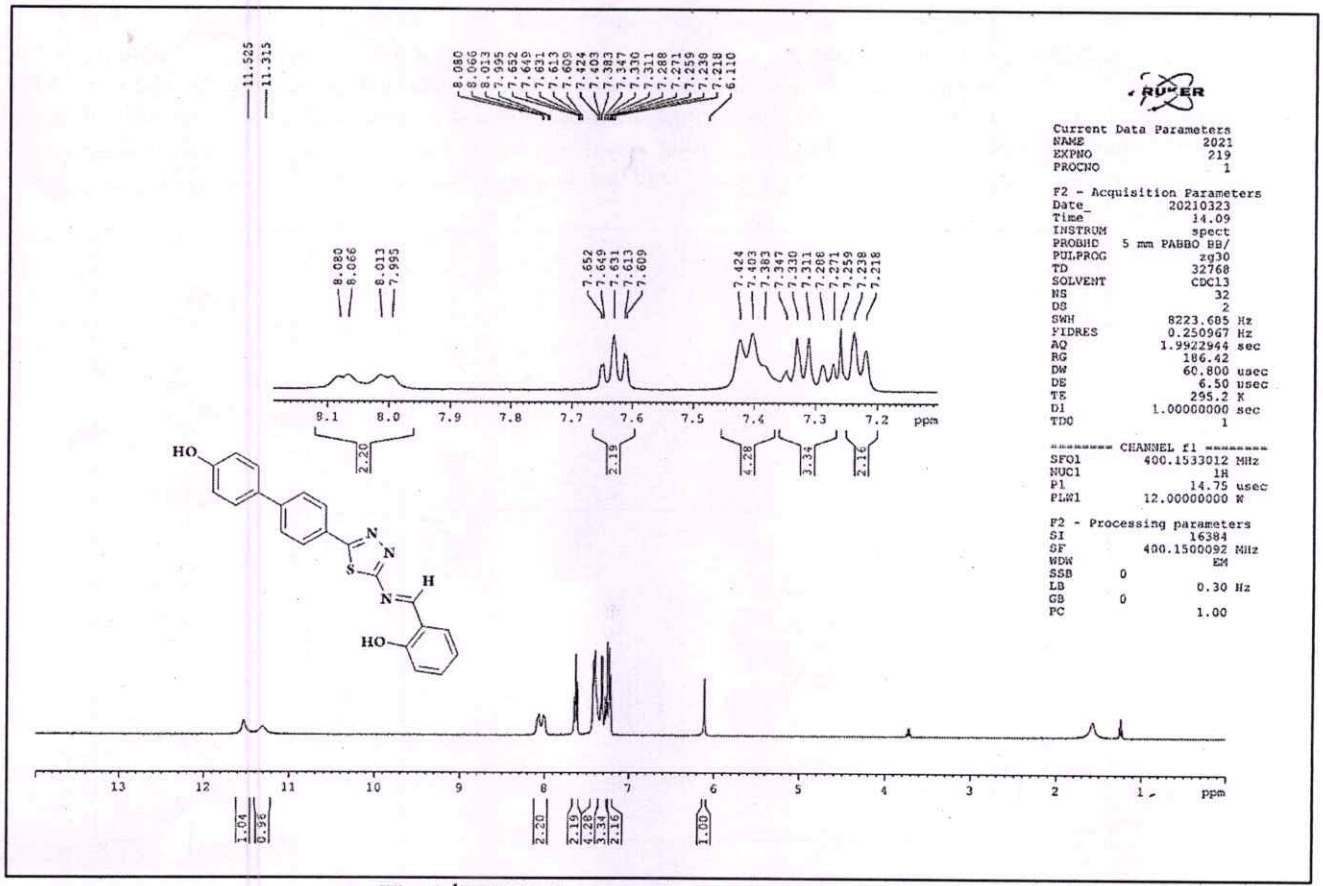


Fig. 1 [1]HNMR Spectra of compound 3a

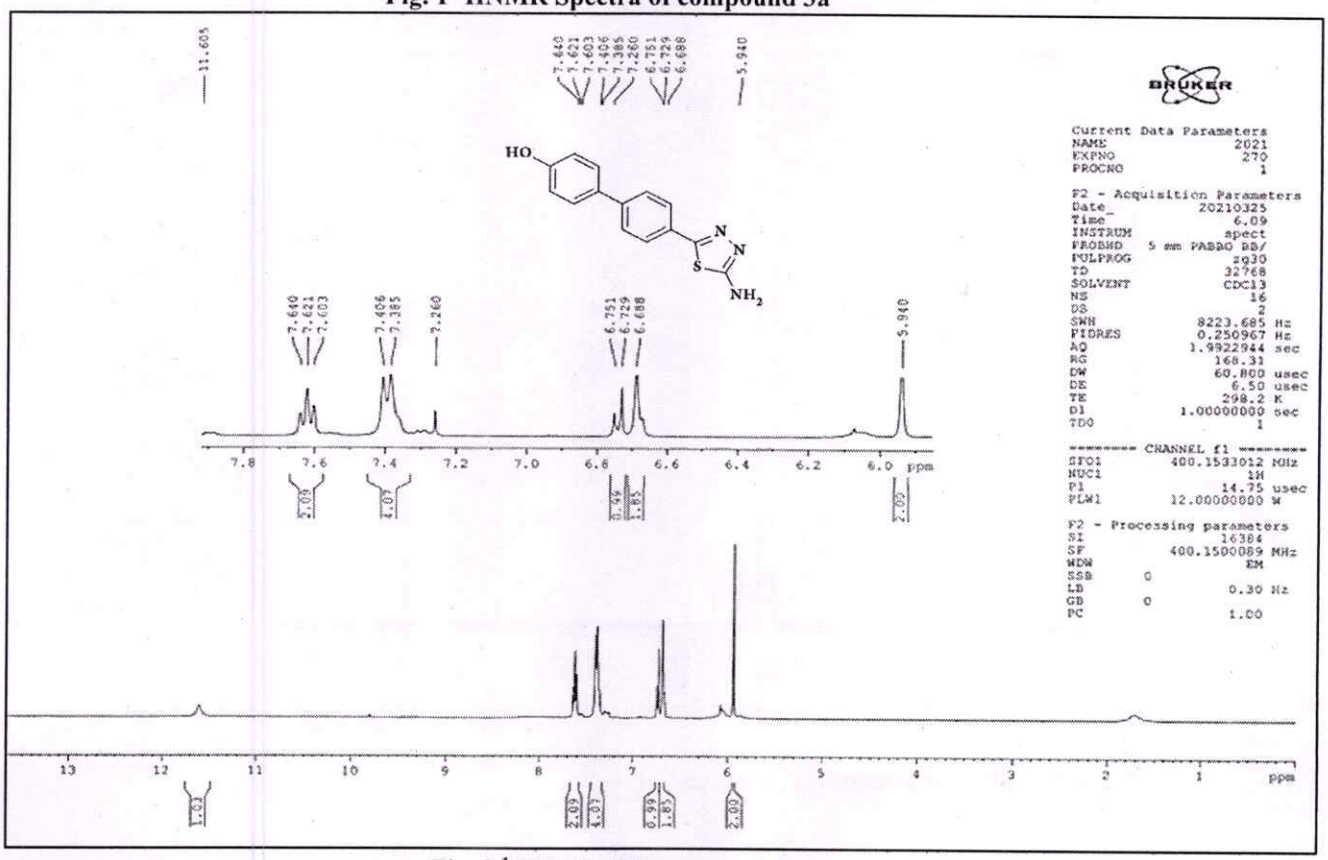


Fig. 2 [1]HNMR of the reactant.

In the 1HNMR spectra of compound 3b, strong singlets were observed at 3.7 ppm (OCH3), 6.1 ppm (CH), two singlets for2 (OH) group as well as a multiplet at 7–8 ppm for aromatic hydrogens (Fig 3). The C 13 NMR spectra shows in range between 35 - 135ppm range, where carbon and oxygen bond show in between 60-70ppm and imine shows near 155 ppm (Fig. 4). Strong absorption bands were observed in the infrared spectra of compound 3b at 1640 cm^1 due to C=N stretching, and the compound had a mass of 403.10 (Fig 5).

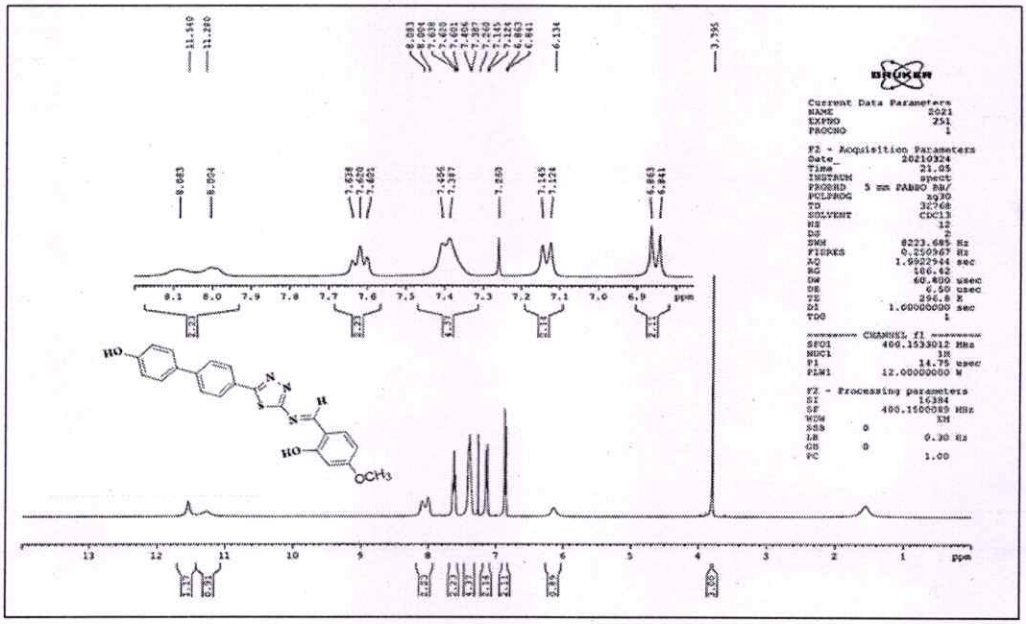


Fig. 3 1HNMR of compound 3b

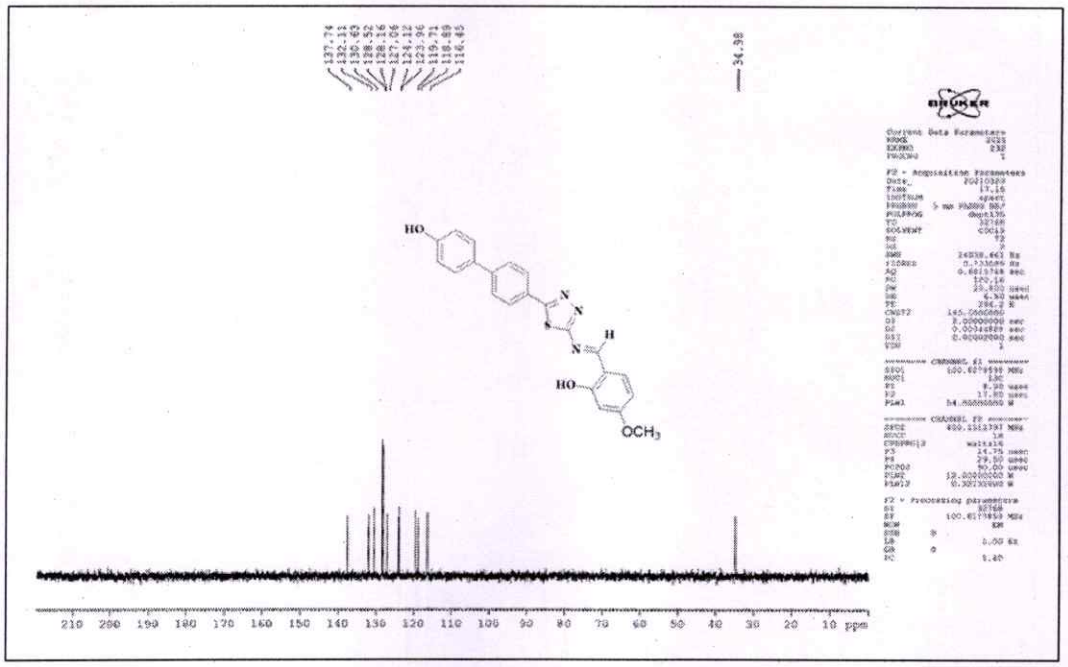


Fig. 4 13C NMR spectra of the compound 3b

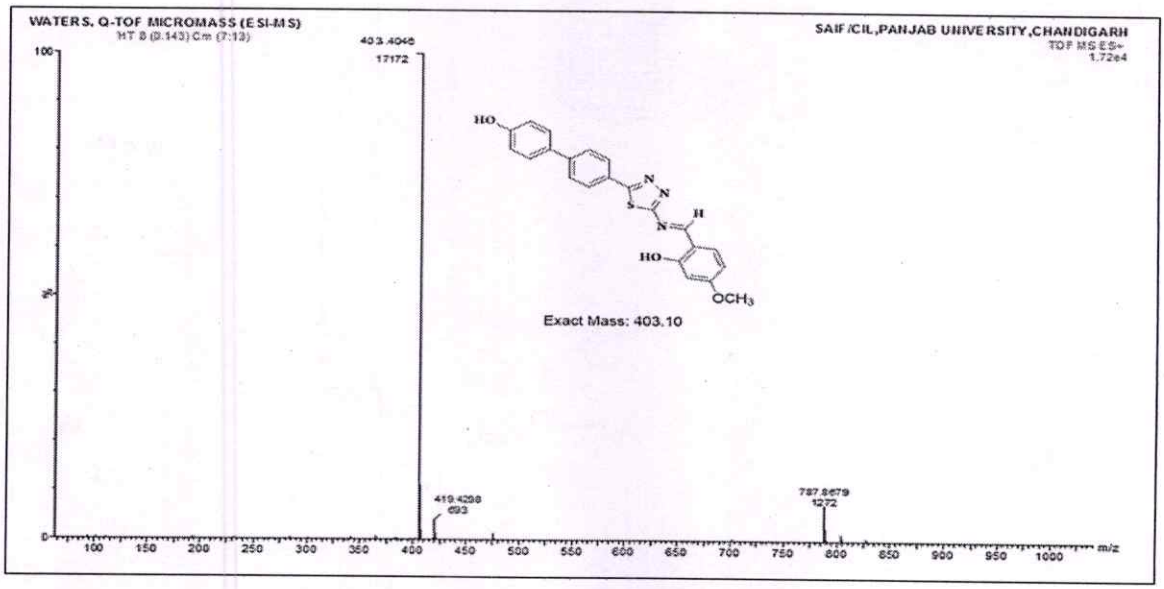


Fig. 5 MASS Spectra of the compound 3b.

1HNMR spectra of compound 3c had substantial multiple singlets at 3.01 ppm, 6 ppm (H-C=N proton), as well as multiple peaks at 7-8 ppm for aromatic hydrogens. The 13C NMR spectra shows peaks in range between 20 - ppm 170 range, where carbon and methyl bond show in between 20-30 ppm and imine show near 155 ppm. The infrared spectra of compound 3c had prominent absorption bands at 1629 cm-1 due to C=N stretching, and the compound's mass obtained via mass spectra is 387.10.

The NMR spectra of compound 3d displayed several singlet peak at 3.17 ppm (OCH3), 3.12 ppm (OCH3), 6.2 ppm (HC=N), and multiplet from 7 to 8 ppm for aromatic hydrogens (Fig 6). The 13C ^{1}NMR spectra shows in range between 55 –210 ppm range, where carbon and oxygen bond show in between 60-70ppm and imine shows near 155 ppm (Fig 7). In a similar fashion, the infrared spectra displayed bands at 1580 cm1 (C=N stretching) with a mass of 433.11 (Fig 8).

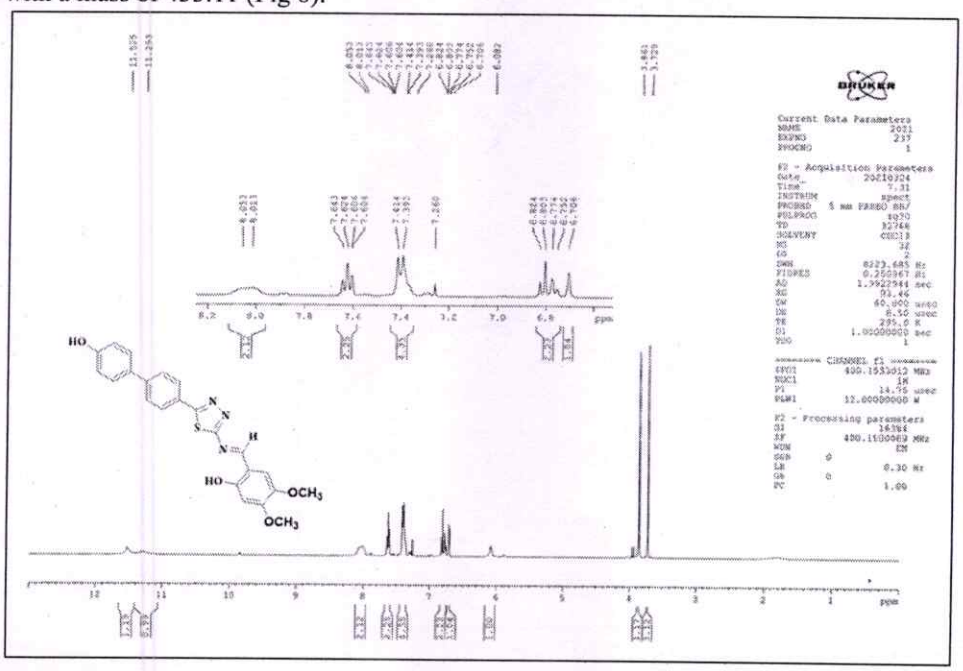


Fig 6 1HNMR of compound 3d

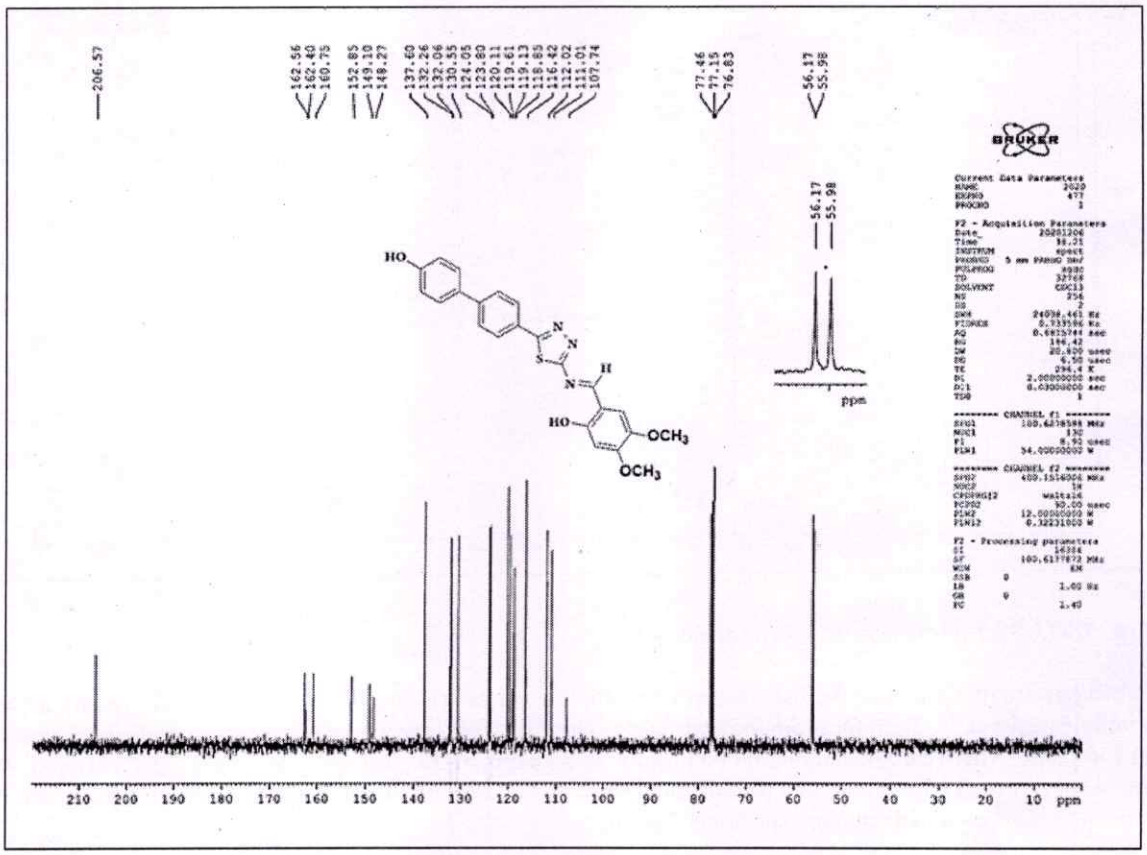


Fig 7 13C NMR of compound 3d

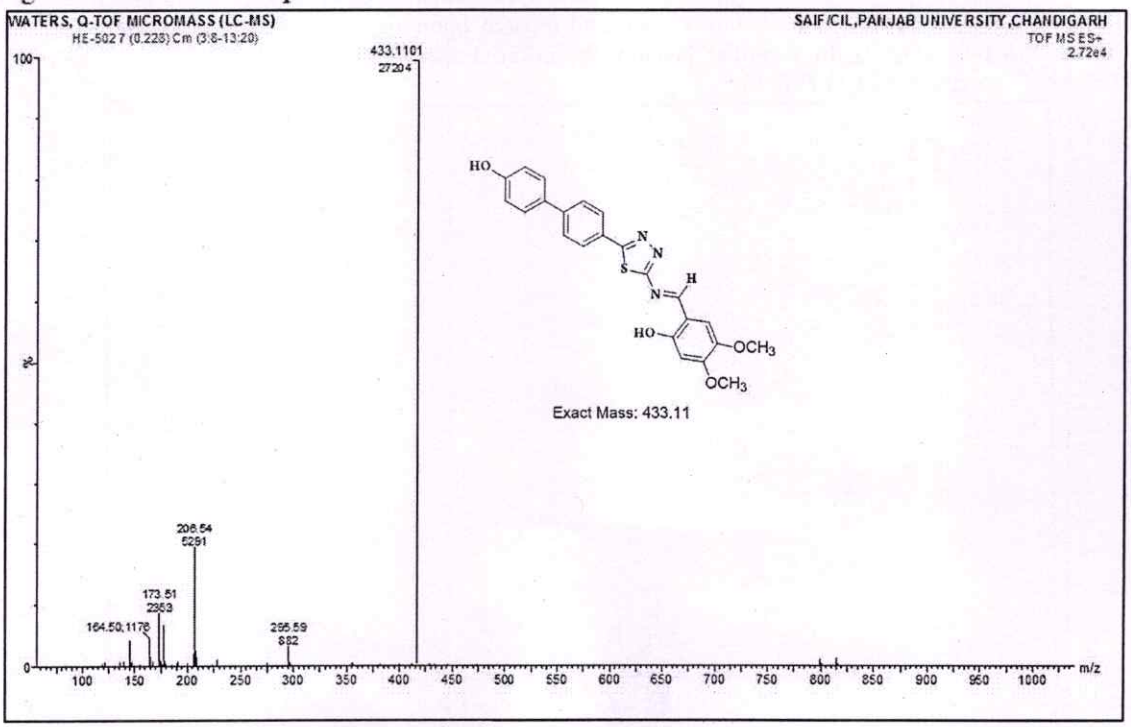


Fig 8 MASS spectra of the compound 3d

The NMR spectra 3e displayed a singlet peak and 6.2 ppm because of (HC=N) group, and peaks at 7-8 ppm for aromatic hydrogen. The 13C NMR spectra shows in range between 65 ppm to 210 ppm range, C-Cl bond show

peak near 128 ppm. Additionally, the IR spectrum displayed bands at 671 cm-1 (C-Cl stretching meta-substituted).

The NMR spectra of the 3f compound displayed singlet signals at 6.2 ppm (HC=N), and several peaks with doublet peaks at 7-8 ppm for aromatic hydrogen. The 13C NMR spectra shows in range between 65 ppm to 210 ppm range. Infrared spectroscopy demonstrated absorption at 528 cm-1 due to C–Br stretching.

The NMR spectrum of compound 3g revealed singlets signals at 6.2 ppm HC=N It also displayed multiplets signals at 7.5 - 8.2 ppm, which are caused by aromatic hydrogens. The 13C NMR spectra shows in range between 65p- 210 ppm range and aromatic carbon-carbon show peak between 150-120 ppm.Additionally, the IR spectrum showed 3467cm-1 (OH stretching) and 500 cm-1 (C-F stretching). The precise mass that was measured was 391.08.

Compound 3h 1HNMR spectra displayed the singlet signal at at 6.2 ppm HC=N, and multiplets signals at 7.5 - 8.2 ppm for aromatic hydrogens. The 13C NMR spectra shows in range between 65 -210ppm range, Aromatic carbons show peak between 150-120 ppm. Additionally, the compound's IR spectrum displayed 3467cm^{-1} (OH stretching) with an exact mass of 441.08.

3.2. Antimicrobial Screening

Table 1: Antimicrobial screening of the synthesized compounds (3a-3h)

Compound codes	*P. aeruginosa*	*E. coli*	*B. subtilis*	*A. niger*	*A. fumigatus*	*A. flavus*
3a	1.25	1.25	2.5	1.25	1.25	1.25
3b	2.5	1.25	--	1.25	1.25	--
3c	--	1.25	1.25	1.25	--	2.5
3d	2.5	2.5	1.25	1.00	2.5	--
3e	2.5	--	1.25	--	1.25	1.25
3f	1.25	1.25	1.25	2.5	--	1.25
3g	--	1.25	1.25	2.5	1.25	--
3h	2.5	--	--	2.5	1.25	1.25
Tetracycline	0.00125	0.01	0.00125	-	-	-
Amphotericin B	-	-	-	0.00125	0.000156	0.000156

The in vitro growth inhibitory effects of all the recently synthesised compounds were assessed versus a panel containing pathogenic microorganisms' conventional strains, which included three bacteria along with three strains of fungus. The MIC refers to the most diluted form of a substance that exhibits a transparent fluid ~~without any formation of turbidity (Husain et al., 2018).~~

The results of the antibacterial along with antifungal detection demonstrated that some examined compounds, including compounds 3a-3h, had significant efficacy at doses ranging from 1 to 2.25 g/mL. In a study conducted by Cinar et al. (2023), it was shown that the thiadiazoles exhibited notable antibacterial efficacy against a wide range of bacterial and fungal species.

It is noteworthy that the inclusion of an azomethine linkage in hydrazones has been shown to be crucial for the significant antibacterial along with antifungal properties exhibited by these compounds.

3.3 Antioxidant Efficacy

Table 2: Antioxidant activity of synthesized compounds 3a-3h

Sr. No.	Compound Code	Antioxidant Test EC_{50} in µg/ml
1.	3a	34.12 ± 0.123
2.	3b	0.3608 ± 0.154
3.	3c	32.54± 0.224
4.	3d	0.3683 ± 0.210
5.	3e	18.02 ± 0.245
6.	3f	26.08 ± 0.145
7.	3g	18.25± 0.115
8.	3h	0.4036± 0.117
9.	BHT(STD)	0.1801± 0.245

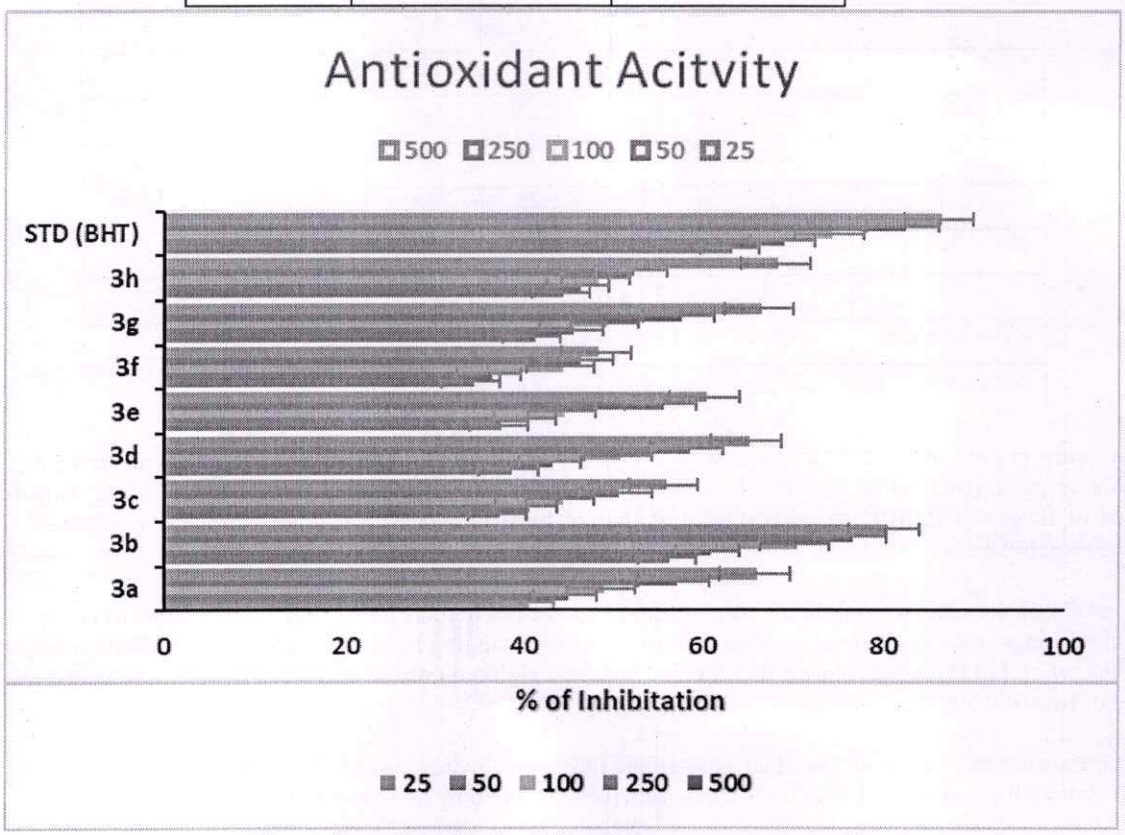


Figure 9: Antioxidant activity of the compounds 3a-3h

In recent studies, antioxidant activity was observed in 1,3,4 Thiadiazole Schiff base derivatives (Pund et al., 2023; Kumar et al., 2023; Cinar et al., 2021). 3b, 3d and 3h showed higher antioxidant activity with EC_{50} 0.3608±0.154, 0.3683±0.210, 0.4036±0.117 µg/ml. Figure 9 showed the antioxidant activity of the synthesised compounds against the % Inhibition. It is known that the antioxidantactionrelies on the nature of the substituent existing on the aldehyde ring (Tople et al., 2023; Dueke-Eze et al., 2020).

Conclusion

In conclusion, we synthesised new biologically significant 1,3,4-thiadiazole-tagged Schiff bases and investigated their antimicrobial and antioxidant properties. The simplicity, widely accessible starting materials, brief reaction durations, catalyst-free and moderate conditions, and high yields of up to 84% of the products are the primary advantages of this reaction. 1,3,4-thiadiazole and its derivatives continue to play an important role in the design and development of new drugs for the treatment of a wide range of complex diseases, as well as in the enhancement of their traditional use as antibacterial and antifungal agents. All synthesised compounds possess a wide variety of biological activities with pharmaceutical significance. Antimicrobial assays revealed that the majority of synthesised thiadiazole derivatives exhibited good to outstanding antimicrobial activity. Of all the synthesised compounds, only 3a exhibited antimicrobial activity against all of the bacterial and fungal isolates. 3d compounds demonstrated potent antibacterial activity and 3h compounds demonstrated potent antifungal activity. Antioxidant agents capable of scavenging DPPH were discovered among the synthesised compounds. 3a-3h have EC_{50} values between 0.3608 and 34.12 µg/mL for their antioxidant activity. 3b exhibited the highest antioxidant activity especially among all of the synthesised compounds. Recently, as multi-target techniques in drug discovery began to receive much-deserved attention, so did the use of 1,3,4 thiadiazole derivatives products as multi-target agents.

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