

Spectral and Biological investigation of 5-Phenyl-1,3,4-oxadiazole-2-thiol

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Abstract

In the current study, a compound 5-phenyl-1,3,4-oxadiazole-2-thiol was synthesized by converting variously benzoic acids successively into the corresponding esters, hydrazides. The structure of the synthesized compound was confirmed based on ¹H-NMR, IR, UV-Vis and mass spectral data. The synthesized compounds were screened for antimicrobial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and fungus *Candida albican*, *Aspergillusniger* and *Candida krusei*.

1. Introduction

Over the last few decades, the rapid emergence of drug resistance in the treatment of infectious diseases emphasizes worldwide need for novel antimicrobial agents so current scenario greatly demands newer, safer and effective antimicrobial agents that will the overcome this problem. A number of researchers have reported antimicrobial activities in 1,3,4-oxadiazoles.

In this direction, various scientists have been engaged to synthesize new compounds related to various chemical groups. Literature survey revealed that 1,3,4-oxadiazole derivatives possessed broad spectrum of biological activities *viz* anthelmintic [1], anticancer [2], antiprotozoal [3], anticonvulsant [4-5], anti-inflammatory [6], analgesic [7], antifungal [8], antibacterial [9] and many more.

The vibrational analysis of the title compound has been studied [10-11]. The complete vibrational analysis of the polyatomic molecules is possible only when both the IR and Raman spectral data are available [12]. The ¹H-NMR analysis [13] and UV-Vis analysis [14] of the title compound also has been studied.

2. Methods and Materials

First ethyl benzoate (2) was prepared by refluxing benzoic acid (1) with ethyl alcohol in the presence of concentrated sulfuric acid for 4 hours. After maximum completion of reaction, conc. solution of base was added to the reaction mixture for neutralization of excess organic acid and sulfuric acid to their respective salts. By using solvent extraction method we get ethyl benzoate in organic layer and both the salts were washed by aqueous layer. Next step is the synthesis of benzohydrazide (3) by allowing ethyl benzoate to react with 80% hydrazine hydrate in methanol at room temperature along with vigorous stirring for 3 hrs. The solid separated out was filtered and washed with *n*-hexane. Further the compound 5-phenyl-1,3,4-Oxadiazol-2-thiol (4) was synthesized by refluxing compound 3, with carbon disulphide and potassium hydroxide in ethanol for 6 hours. On completion of reaction, ice cold distilled water was added to the reaction mixture and it was then acidified to set the pH around 2-3 to put out the synthesized product in the form of precipitates which were filtered and washed with distilled water. The product was finally recrystallized from methanol [15,16] as shown in Fig.1.

All the reagents and solvents were generally received from commercial supplier. Reactions were done in dried glassware. Melting points were taken in open capillaries by thermionic melting point apparatus, (Campbell Electronic Mumbai, India) and are uncorrected. The purity of the newly synthesized compounds was

checked by thin layer chromatography (TLC) on silica gel-G coated plates by using different solvent systems. Infrared (IR) spectra were determined on Bruker IFS-66 FTIR (Bruker Bioscience, USA) using KBr pallets and wave number (ν) was reported in cm^{-1} . The ¹H-NMR spectra were taken on Jeol GSX -300 FT NMR (Jeol, Tokyo, Japan) in CDCl₃ or DMSO-d₆ and chemical shifts (δ) are given in ppm. Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on Spec Finnigan Mat 8230 MS. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found within $\pm 0.4\%$ of the theoretical values. The electronic spectra (UV-Vis) were recorded on a Perkin-Elmer Lambda 15 UV-Vis spectrophotometer, using $10^{-3} \text{ mol} \cdot \text{dm}^{-3}$ solutions in DMF.

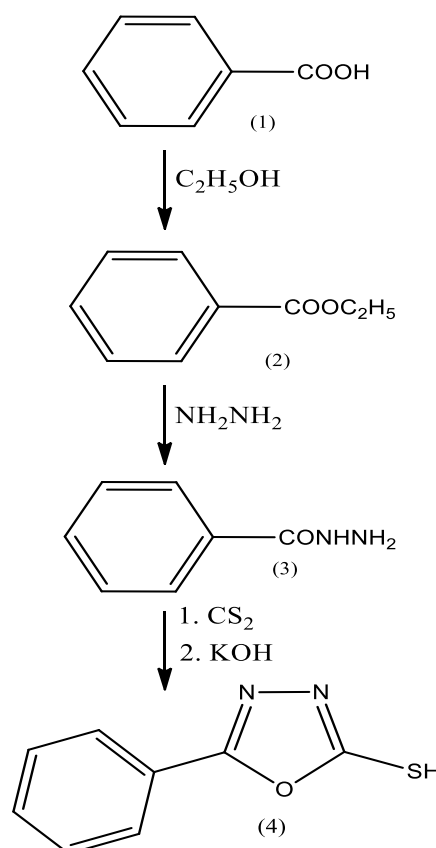


Fig.2 5-Phenyl-1,3,4-oxadiazole-2-thiol

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3. Antimicrobial activity

The antimicrobial activity was assayed *in vitro* by the twofold broth dilution [17] against bacteria *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and fungus *Candida albicans*, *Aspergillus niger* and *Candida krusei*. The minimal inhibitory concentrations (MIC, $\mu\text{g/ml}$) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds, dissolved in dimethylsulfoxide, were added to culture media. Mueller Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi to obtain final concentrations ranging from 125 $\mu\text{g/ml}$ to 1.592 $\mu\text{g/ml}$. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.0×10^4 bacteria/ml and 1.0×10^3 fungi/ml. The MICs were read after incubation at 37 °C for 24 h (bacteria) and at 30°C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were employed as growth controls. Chloroamphenicol and fluconazole were used as reference antibacterial and antifungal drugs, respectively.

To detect the type of antimicrobial activity, subcultures were performed by transferring 100 μl of each mixture remaining clear in 1 ml of fresh medium. The minimal bactericidal concentrations (MBC, $\mu\text{g/ml}$) and the minimal fungicidal concentrations (MFC, $\mu\text{g/ml}$) were read after incubation at 37 °C for 24 h and at 30 °C for 48 h, respectively.

4. Result and Discussion

4.1 Spectral analysis

Heteroaromatic structure shows the presence of C-H stretching, in-plane bending vibrations in the regions 3200-3000 cm^{-1} and 900-1200 cm^{-1} respectively. In this region the bands are not affected appreciably by the nature of the substituent. The FTIR bands at 3111, 2935, 2742 and 2548 cm^{-1} and FT-Raman bands at 3068, 2634 and 2563 cm^{-1} in 5-phenyl-1,3,4-oxadiazole-2-thiol is assigned to C-H stretching modes. The bands at 1271, 1265, 1173 cm^{-1} have been assigned to C-H in-plane bending vibrational modes.

The IR and Raman bands identified at 2438 and 2398 cm^{-1} are assigned to S-H stretching mode.

The C=N stretching frequencies in the Raman spectrum of crystalline 5-phenyl-1,3,4-oxadiazole-2-thiol occur in the range 1600-1450 cm^{-1} . In the present investigation, the Raman bands observed at 1467 cm^{-1} have been assigned to C=N stretching vibrations. The very strong IR peak and the strong Raman peak observed at 1471 cm^{-1} is assigned to C-N stretching mode.

The carbon-carbon stretching vibrations of the title compound have been observed at 1400 to 1320 cm^{-1} . The medium Raman bands identified at 912 and 825 cm^{-1} have been assigned to C-C in-plane bending.

Carbon-sulphur stretching vibrations of the title compound have been observed at 704 cm^{-1} . The medium Raman bands identified at 680 cm^{-1} have been assigned to C-S in-plane bending. These assignments are in good agreement with the literature. (table1).

In the $^1\text{H-NMR}$ spectra, the singlet signal at $\delta 13.77$ ppm is assigned to SH based on the position of this peak in the spectrum of the parent 5-phenyl-1,3,4-oxadiazole-2-thiol molecule. The assignment of the peak at $\delta 7.62$ -7.49 ppm of five proton of phenyl ring in the title compound. (table2).

UV-Vis absorption spectra of 5-phenyl-1,3,4-oxadiazole-2-thiol after the continuous prolonged irradiation (0, 5, 15, 30, 45 and 60 min) with UV-A light. Both the absorption maxima ($\lambda_{\text{max}} = 203$ nm and $\lambda_{\text{max}} = 215$ nm) decrease, and a slight bathochromic shift have been detected, at the end of any particular UV-irradiating period. The log values of the absorbance maxima plotted against irradiation time yielded a linear plot, suggesting the involved kinetics to be probably of pseudo-first order, depending on the 5-phenyl-1,3,4-oxadiazole-2-thiol concentration only. (table3).

Table:1 Vibrational assignments of fundamental frequencies (cm^{-1}) of 5-phenyl-1,3,4-oxadiazole-2-thiol.

Species	Observed Frequencies (cm^{-1})		Calculate Frequencies (cm^{-1})	Assignment
	FTIR	Raman		
4	3111(s)	-	3115	C-H stretching
4	-	3068(s)	-	C-H stretching
4	2935(s)	-	-	C-H stretching
4	2742(s)	2634(w)	-	C-H stretching
4	2548(s)	-	-	C-H stretching
4	2438(s)	-	2450	S-H stretching
4	-	2398(w)	-	S-H stretching
4	1597(s)	-	1585	C=N stretching
4	1489(w)	-	-	C=N stretching
4	-	1467(w)	-	C=N stretching
4	1389(w)	-	-	C=C stretching
4	-	1375(w)	-	C=C stretching
4	1336(w)	-	-	C=C stretching
4	1271(s)	-	1265	C-H in plane bending
4	1176(w)	-	-	C-H in plane bending
4	1060(s)	-	1078	N=N stretching
4	954(s)	-	-	N=N stretching
4	-	912(m)	-	C-C in plane bending
4	-	825(w)	-	C-C in plane bending
4	704(s)	-	-	C-S-C in plane bending
4	-	680(m)	-	C-S-C in plane bending
4	623(s)	-	-	C-O-C in plane bending
4	569(s)	-	-	C-C-H in plane bending
4	491(s)	-	-	C-C-H in plane bending

Table 2: ¹H-NMR data of 5-phenyl-1,3,4-oxadiazole-2-thiol.

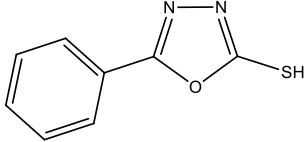
Compound	δ/ ppm	Assignments
	7.62-7.49	m, 5H of CH of phenyl
	13.77	s, 1H of SH of thiol

Table 3: Electronic spectral data in 95% ethanol and DMF, λ_{max}(nm) / ε_{max}(10³ mol¹.dm³.cm)

Solvent	5-phenyl-1,3,4-oxadiazole-2-thiol			
	I	II	III	IV
Ethanol	202.5/0.82	222.5/0.34	306.5/0.47	250.00/0.42
DMF	-	225.6/0.61	-	315.2/0.52

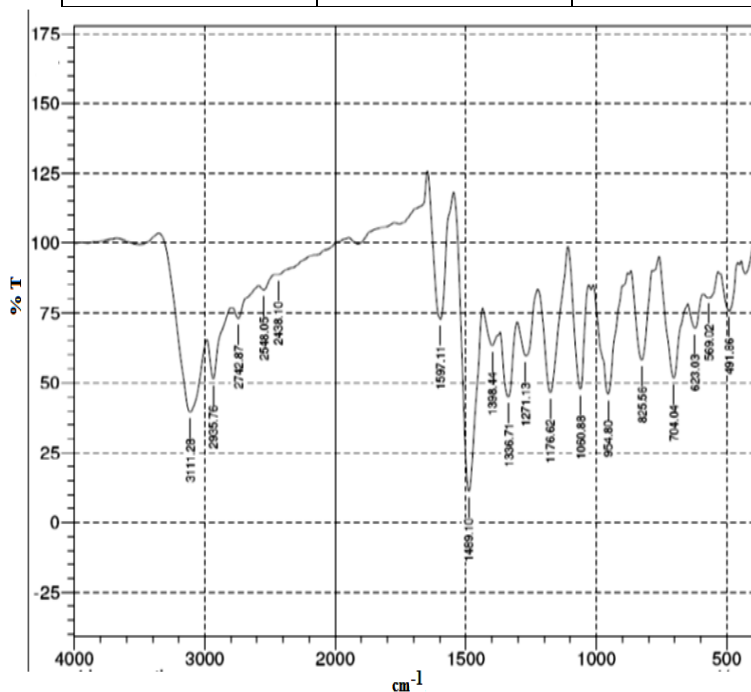


Fig. 2 FTIR-Raman Spectroscopy of 5-phenyl-1,3,4-oxadiazole-2-thiol

Table 4: Minimal inhibitory concentration (MIC) μg/ml of 5-phenyl-1,3,4-oxadiazole-2-thiol against tested bacterial and fungal strains

Compound No.	Minimal Inhibitory Concentration (MIC) μg/ml					
	E. coli	B. subtilis	S. aureus	C. albicans	A. niger	C. krusei
4	1.592	3.125	6.25	1.592	3.125	1.592
Chloroamphenicol	12.5	6.25	12.5	-	-	-
Fluconazole	-	-	-	12.5	12.5	6.25

4.2 Antimicrobial activity (Minimal inhibitory concentration)

Antibacterial activity of 5-phenyl-1,3,4-oxadiazole-2-thiol (4) and standard drug, chloroamphenicol, was carried out at a concentration 250 μg/ml against *E. coli* ATCC 25922, *B. subtilis* ATCC 1633 and *S. aureus* ATCC 25923. Results show the varying degree of antibacterial activity of all the compounds tested as given in table 4.

From the results obtained, it is clear that 5-phenyl-1,3,4-oxadiazole-2-thiol exhibited less activity against *E. coli* ATCC 25922, *B. subtilis* ATCC 1633 than chloroamphenicol but *S. aureus* ATCC 25923 displayed antibacterial property moderate to the reference drug.

The compound 5-phenyl-1,3,4-oxadiazole-2-thiol (4) along with reference drug, fluconazole, were also tested for antifungal activity at a concentration of 250 μg/ml against *C. albicans* ATCC 2091, *A. niger* ATCC 9029 and *C. krusei* ATCC 6518, and it is found that synthesized is showed very weak or moderate active as compared to standard drug.

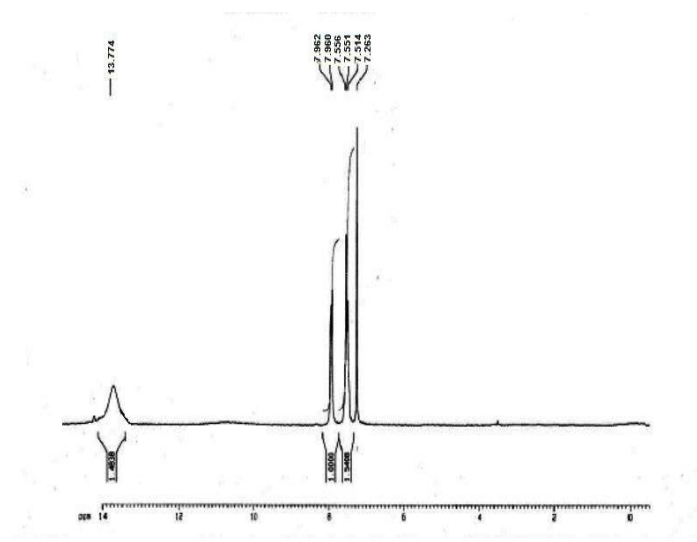


Fig. 2 H-NMR Spectroscopy of 5-phenyl-1,3,4-oxadiazole-2-thiol

5. Conclusions

5-phenyl-1,3,4-oxadiazole-2-thiol established using FTIR, UV-Vis and ¹H-NMR spectroscopic method. Vibrational and electronic spectra confirmed the synthesized compound, 5-phenyl-1,3,4-oxadiazole-2-thiol. The compound was tested for its *in vitro* antimicrobial activity and its activity against bacteria *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and fungus *Candida albicans*, *Aspergillus niger* and *Candida krusei* compared to chloroamphenicol and fluconazole, respectively.

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