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(RESEARCH ARTICLE)

Synthesis and evaluation of some novel heterocyclic compounds containing an oxadiazole moiety

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Abstract

Heterocyclic compounds analog have attracted wide attention due to their useful biological properties. Among them, 1,3,4-oxadiazoles have exhibited a wide range of biological properties including anti-bacterial, anti-viral, anti-fungal, anti-cancer, anti-tumor, anti-inflammatory, anti-hypertensive, anti-convulsant and anti-diabetic properties. The purpose of this study was to collect the literature work reported by researchers on oxadiazole derivatives for their various pharmacological activities and also efforts made on this moiety. This study covers the work reported on various biological activities of oxadiazole derivatives.

Keywords: Heterocyclic compounds; 1, 3, 4-oxadiazoles; Pharmacological activities

1. Introduction

Compounds containing heterocyclic ring systems are of great significance both medicinally and industrially. Oxadiazoles are the heterocyclic compounds comprehend one oxygen and two nitrogen atoms in a five membered ring [1, 2] derived from furan by substitution of two methylene groups (=CH) with two pyridine type nitrogen's (-N=). They possess a diversity of useful biological effects [3]. There are four known isomers of oxadiazole such as 1,2,3-Oxadiazole, 1,2,4-Oxadiazole, 1,3,4-Oxadiazole and 1,2,5-Oxadiazole [4] but the 1,2,3-Oxadiazole is quite unstable and revert in the form of diaziketone tautomer [5]. Oxadiazoles are frequently occurring motifs in drug like molecules, and they are often used with the intention of being bioisosteric replacements for ester and amide functionalities.Some of the recent review proclaimed that 1,3,4-oxadiazoles and its derivatives were reported to acquire antimicrobial [6], tuberculostatic [7], anti-inflammatory [8], antifungal [9], antibacterial [10] anticancer [11], analgesic [12] activities, anti-convulsant [13], anti-hepatitis B [14], antiparasitic [33]. Many drugs containing oxadiazole are in late clinical trials, including zibotentan (15) as an anticancer agent, ataluren (16) for the treatment of cystic fibrosis and raltegravir (17) an antiretroviral drug for the treatment of HIV infection. Some compounds having 1,3,4-oxadiazole unit are currently used as medicines: Fenadiazole is a hypnotic drug [18], MK-0633 p-toluenesulfonate is a 5-lypoxynase inhibitor [19], Nesapidil is an antihypertensive agent [20] and ABT-751-oxadiazole [21] and Furamizole are antibiotics [22, 23]. Several methods have been reported in the literature for the synthesis of 1,3,4-oxadiazoles. The most often used synthetic route for 1,3,4-oxadiazoles includes reactions of acid hydrazides (or hydrazine) with acid chlorides/carboxylic acids and direct cyclization of diacylhydrazines using a variety of dehydrating agents such as phosphorous oxychloride [24], thionyl chloride [25], phosphorous pentaoxide [26], triflic anhydride [27], polyphosphoric acid [28], and direct reaction of acid with (N-isocyanimino-) triphenylphosphorane [29–32]. Various 2,5 substituted 1,3,4-oxadiazoles have been shown to be effective against a wide range of gram-positive and gram-negative bacteria [34-36].

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2. Material and methods

2.1. Material

Reagent and solvents used were analytical grade and purchased from Merck. Analytical thin layer chromatography was carried out on TLC plates of 3 × 15 cm coated with silica gel G for reaction monitoring and for determination of retardation factor. Spots of TLC were located by iodine chamber. Melting points of newly synthesized derivatives were determined on digital melting point apparatus (Flora; Perfit, India) and were found uncorrected. The IR spectra were recorded on FTIR-Shimadzu spectrometer using Nujol method. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER AVANCE II 400 NMR. Chemical shift values were expressed in δ ppm.

2.2. Synthesis of substituted oxadiazolemannich bases

2.2.1. Synthesis of aryloxy acetic acid

A solution of phenolic compounds 2-chlorophenol and 4-chlorophenol (10 g) in NaOH (33%, 35 ml), to this solution freshly prepared chloro acetic acid (50%, 25 ml) was added and the sodium salt separated was dissolved by adding sufficient amount of water. The total mixture is taken in round bottom flask (RBF) and reflux for one hour. A clear solution obtained was cooled and acidified with HCl.

2.2.2. Synthesis of aryloxy acyl hydrazide

Appropriate quantities of acid (0.1 mole) and ethanol (50 ml) was introduced into a clean and dry RBF and stirred well for 10 min. To the above mixture few drops of concentrated sulphuric acid were added and refluxed for 6 hrs. The reaction mixture was concentrated by distilling the excess ethanol under the reduced pressure and treated with saturated solution of sodium bicarbonate. The ester formed in the reaction was used for the preparation of hydrazides directly. The appropriate ester (0.1 mole) was dissolved in 50ml of ethanol in a clean and dry RBF and to this hydrazine hydrate (0.1 mole) was added. The reaction mixture was refluxed for period of 12 hrs. The excess ethanol was distilled off under reduced pressure. The resultant mixture was then poured into ice cold water and obtained solid was filtered and recrystallized from ethanol.

2.2.3. Synthesis of substituted oxadiazole

A mixture of aryloxy acyl hydrazide, potassium hydroxide (0.01 mole), ethanol (50 ml) and carbon disulphide (3 ml) was refluxed until the evolution of hydrogen sulphide ceased. The reaction mixture was concentrated, dissolved in water and acidified with HCl. The precipitate was filtered and recrystallized from ethanol to give substituted oxadiazole.

2.2.4. Synthesis of oxadiazolemannich bases

A solution of substituted oxadiazole (0.001 mole) in absolute ethanol (10 ml) was treated with formaldehyde (0.5 ml). Later an amine was added with stirring and the reaction mixture was magnetic stirred for 2hrs in an ice bath. The precipitated mannich bases was collected and recrystallized from ethanol.

2.3. Antibacterial and antifungal activity

2.3.1. Preparation of Nutrient agar media and Nutrient broth

Nutrient agar medium (100 ml) was been prepared by adding agar (2 g), beef extract (1 g), peptone(1 g), sodium chloride (0.5 g) in water with heating and the pH was adjusted to 7.0 - 7.5. To prepare Nutrient broth(100 ml) in water beef extract (1 g), peptone (1 g), sodium chloride (0.5 g) were dissolved with heating and the pH was adjusted to 7.0 - 7.5. The nutrient media and Nutrient broth were been autoclaved at 121 °C at 15 lb pressure for 15 min and used for sub-culturing of bacteria

2.3.2. Preparation of Potato dextrose agar media and Potato dextrose broth

To prepare potato dextrose agar (100 ml) peeled and chopped potatoes (20 g) added to enough water and boiled until they become soft. Potato extract was obtained by straining the above mixture with the help of muslin cloth. To it dextrose (2 g) and agar (2 g) was been added and then boiled until they dissolve and made up to the volume with distilled water. The pH was adjusted to 5. For potato dextrose broth (100 ml) peeled and chopped potatoes (20 g) added to enough water and boiled until they become soft. Potato extract was obtained by straining the above mixture with the help of muslin cloth. To it dextrose (2 g) was been added and then boiled until dissolved and made up to the volume

with distilled water. The pH was adjusted to 5. Further the prepared potato dextrose agar media and potato dextrose broth were subjected to sterilization in autoclave at 121 °C, 15 lb pressure for 15 minutes.

2.3.3. Preparation of cultures

Nutrients broth was taken and the organisms i.e. *Bacillus subtillis, Proteus vulgaris, Staphylococcous aureus* were sub-cultured from the stock culture and were incubated for 24 hrs. Potato dextrose broth was taken and the organisms i.e. *Aspergillus niger, Penicillium* marneffei were sub-cultured from the stock culture and were incubated for 24 hrs.

2.3.4. Preparation of test samples

Different concentrations of test samples were prepared by dissolving 100mg of test sample in DMSO 100 ml. Collect 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml separately into different volumetric flask and made up to the volume (10 ml) with DMSO to get 100 µg, 200 µg, 300 µg, 400 µg and 500 µg per ml concentrations respectively.

2.3.5. Antibacterial and antifungal assay

In the present study, antimicrobial activities of the synthesized compounds were studied by the paper disc diffusion method. To evaluate the anti-bacterial activity standard drug ampicillin and anti-fungal activity standard drug ketoconazole was used. The strains of *Bacillus subtillis, Proteus vulgaris, Staphylococcous aureus, Aspergillus niger* and *Penicillium marneffei* were used from Pulla Reddy institute of pharmacy, Medak. One loop full of each strain of microorganism was transferred into a suitable agar slant by using a sterile Pasteur loop. These slants were incubated for 24 hrs at 30 °C for bacteria and 37 °C for fungi. Prepared plates were collected and divided into 4 equal portions. In every part of the plate one sterilized filter discs were placed which were previously dipped in different concentrations of test samples and incubated for 24 hrs. After incubation the diameter of the circular inhibition zones were measured and from these values minimum inhibitory concentrations and biological activities were calculated [37-38].

3. Results and discussion

Compounds (K_1, K_2 and K_3) were synthesized as shown in figure 1. Compounds (K1, K2 and K3) were characterized by infra-red spectroscopy and ^{1}H NMR spectra. The IR and NMR spectral details are given in table 1 and table 2 respectively. The details of synthesized compounds (K_1, K_2 and K_3)like molecular structure, nature of compound, yield, molecular formula and molecular weight is given are table 3.

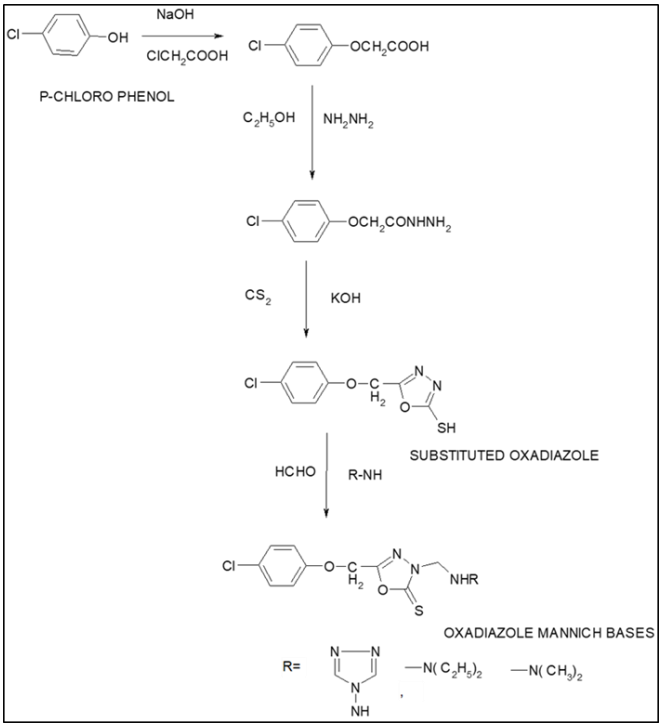


Figure 1 Scheme for synthesis of substituted oxadiazolemannich bases

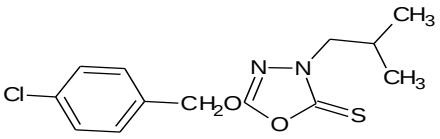
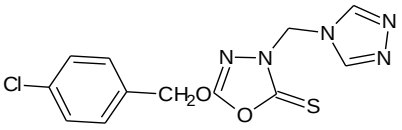
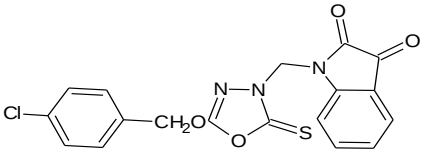
Table 1 Infra -red spectral study of the synthesized compounds

Compound	Spectral peaks (cm^{-1})	Molecular nature
K_1	2918.53	C-H(aromatic)
	2849.91	C-H(aliphatic)
	823.09	C=S
	659.55	Cl-Cl
K_2	2921.68	C-H
	1633.83	C=O
	823.04	C=S
	659.94	C-Cl
K_3	2922.75	C-H
	822.16	C=S
	659.71	C-Cl

Table 2 ^1H NMR Spectral data of synthesized compounds

Compound	Chemical shift value	Proton nature
K_1	7.093-7.409	4Ar-H
	5.292-5.336	2CH2
	1.333-1.380	2CH3
K_2	7.093-7.407	6Ar-H
	5.315-5.332	2CH2
K_3	7.079-7.407	Ar-H
	5.290-5.331	$-2CH_2$

Table 3 Characterization of synthesized compounds

Sr. No	Molecular structure	Nature of compound	Yield (%)	Molecular formula	Molecular weight (gm)
K_1		White	65	$C_{14}H_{18}O_2N_3SCl$	327.5
K_2		White	54	$C_{12}H_{10}O_2N_5SCl$	325.5
K_3		White	45	$C_{18}H_{12}O_4N_3SCl$	389.5

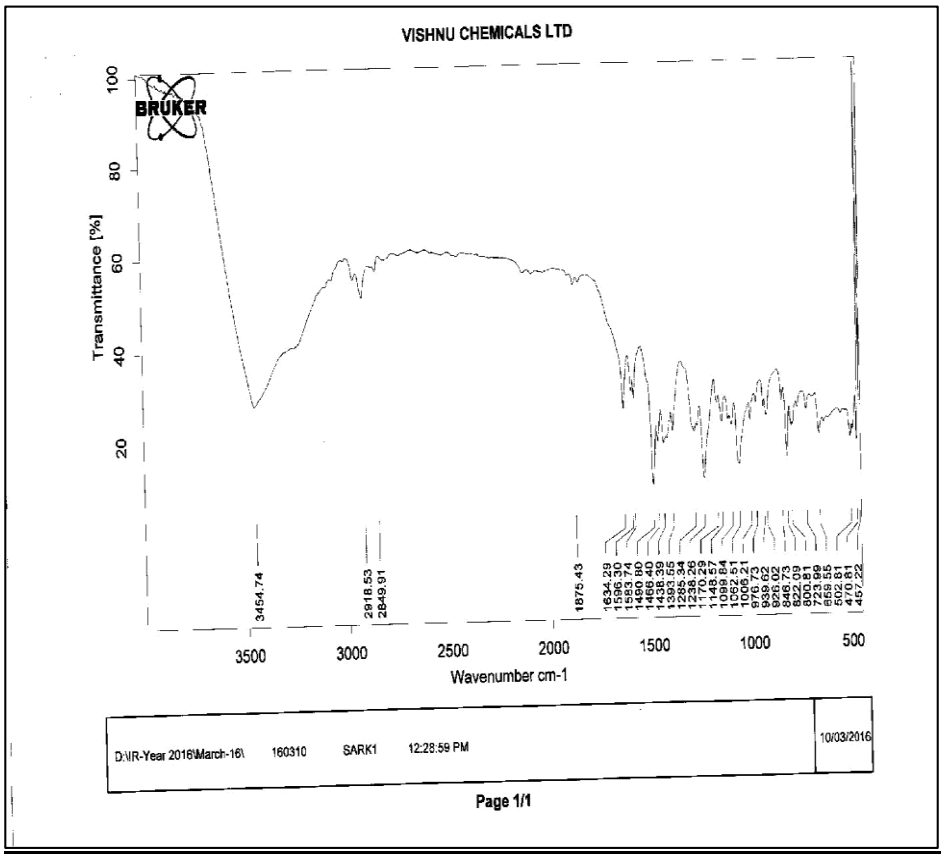


Figure 2 Infra-red spectroscopy of K_1

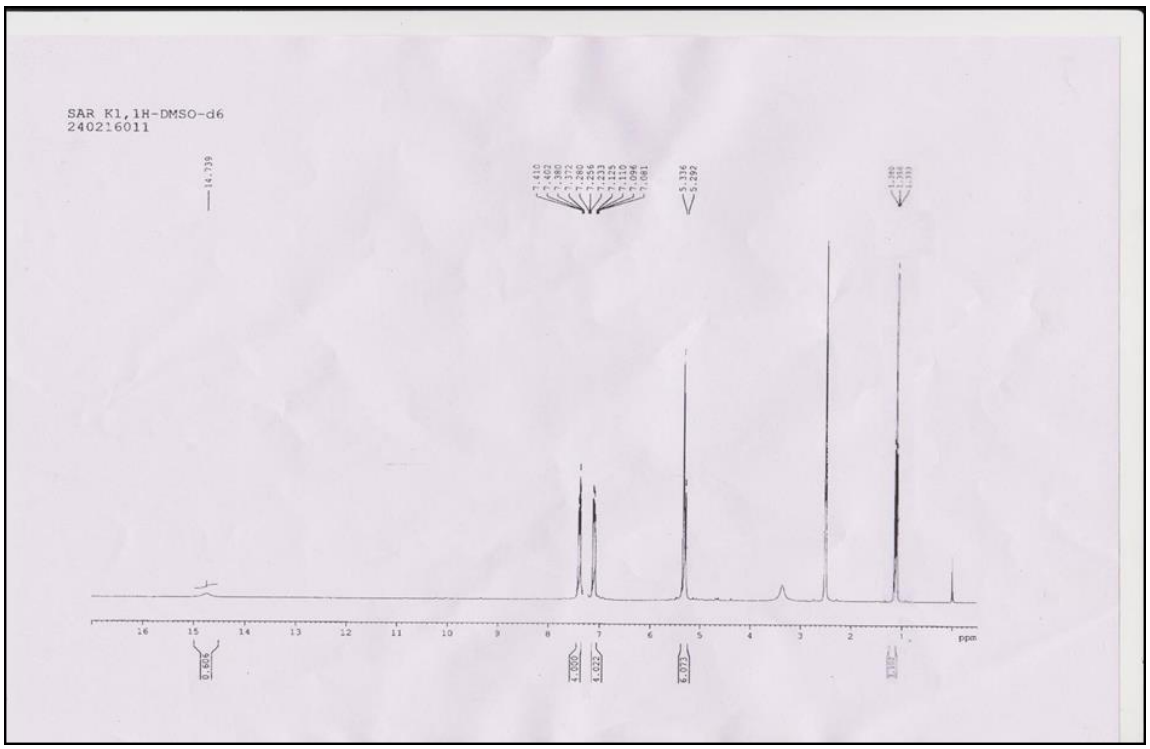


Figure 3 ^1H NMR of K_1

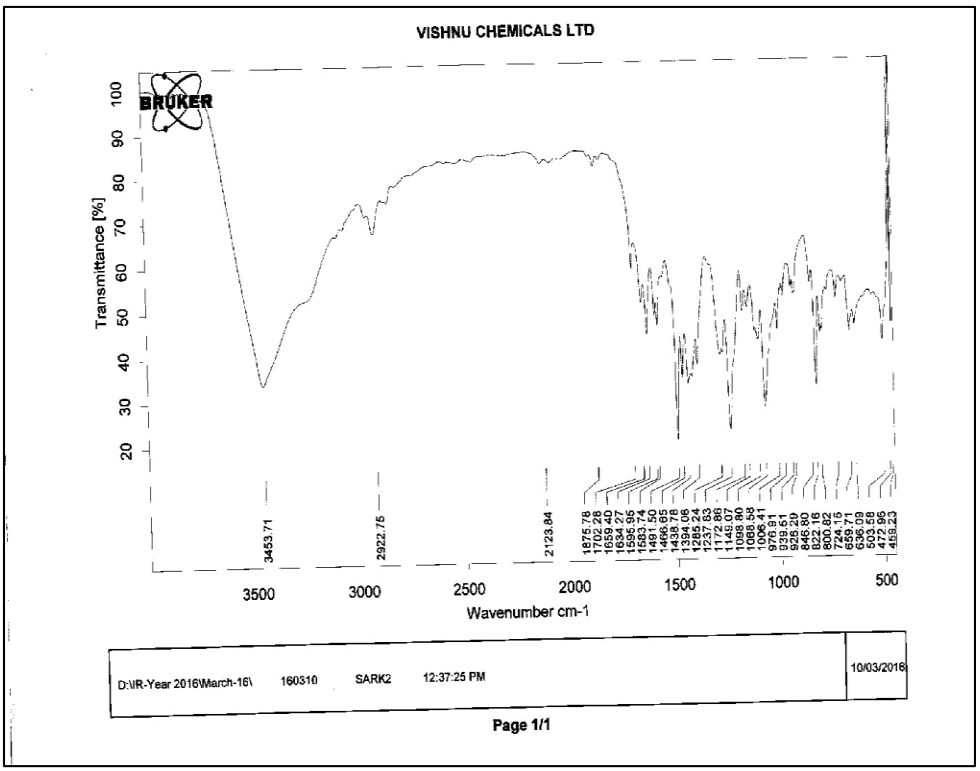


Figure 4 Infra-red spectroscopy of K_2

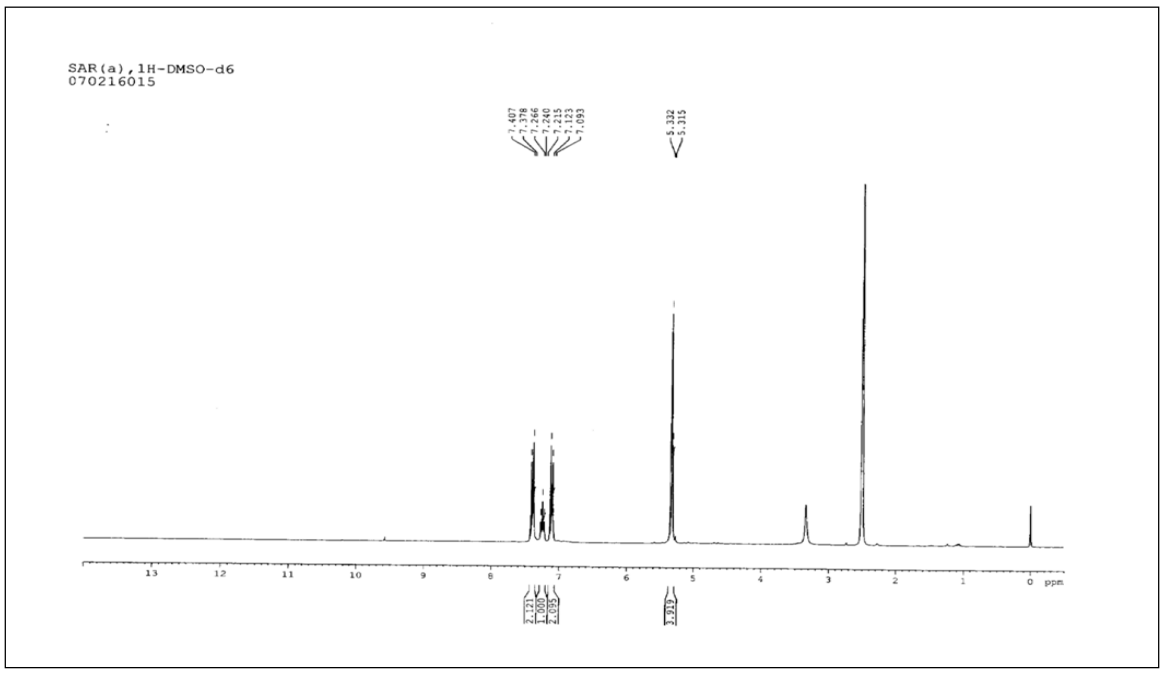


Figure 5 ^1H NMR of K2

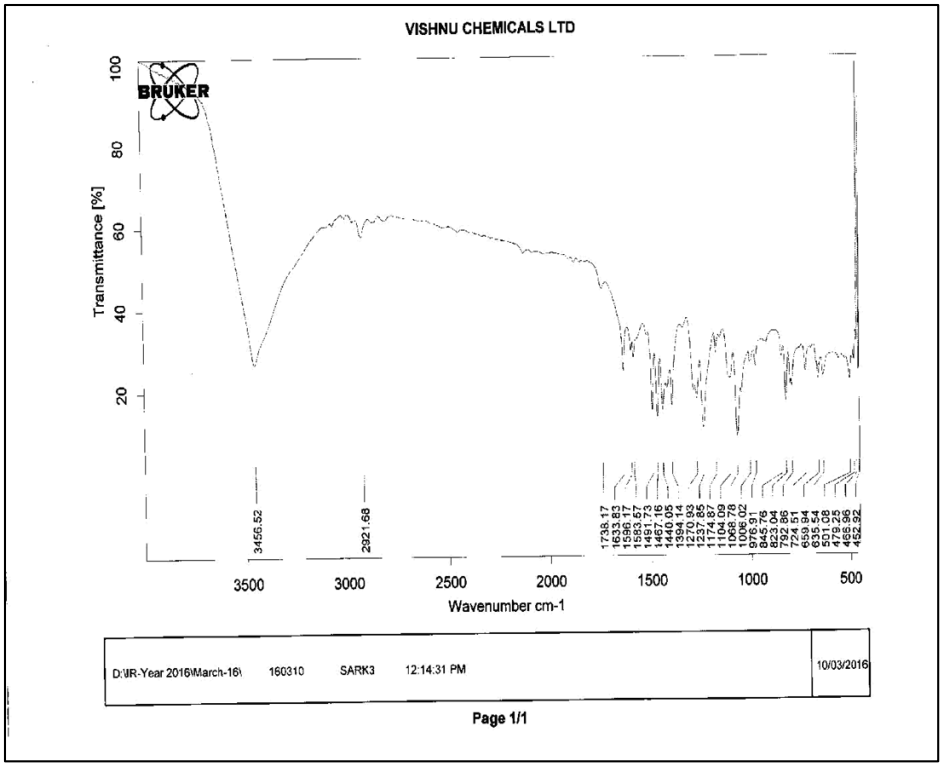


Figure 6 Infra-red spectroscopy of K_3

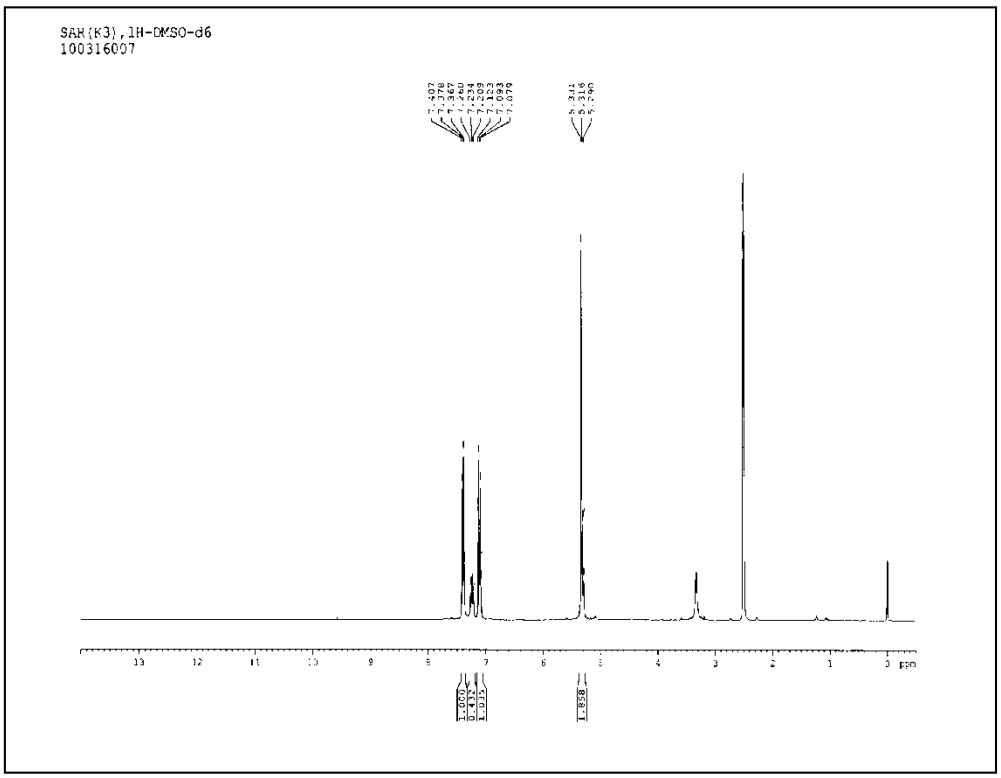


Figure 7 ^{1}H NMR of K_3

All the synthesized compounds of oxadiazoles in the present study showed significant activity against bacteria employed at the concentration of 100µg/ml when compared with that of ampicillin as standard. The zone of inhibition of various concentrations of synthesized compounds are been summarized in the following table 4.

Table 4 Antibacterial activity against different bacterial strains

Compound	Concentration (µg/ml)	Zone of inhibition (cm)*		
		Bacillus subtilis	***Staphylococcus aureus***	***Proteus vulgaris***
K_1	100	0.3	0.7	0.8
	200	0.5	0.8	1.0
	300	0.8	1.0	1.2
	400	1.0	1.1	1.4
	500	1.2	1.4	1.9
K_2	100	0.4	0.3	0.7
	200	0.5	0.6	0.9
	300	0.85	1.1	1.0
	400	1.0	1.2	1.3
	500	1.3	1.5	1.5
K_3	100	0.6	1.0	0.7
	200	0.7	1.1	1.0
	300	0.75	1.3	1.5
	400	0.9	1.3	1.8
	500	1.1	1.5	2.0
Ampicillin	100	0.5	0.5	0.9
	200	0.9	0.9	1.2
	300	1.3	1.3	1.5
	400	1.5	1.5	1.8
	500	1.6	1.6	2.0

* Zone of inhibition (cm) without diameter of the disc

All the synthesized compounds of oxadiazole in the present study showed significant activity against the fungi employed at the concentrations of 100µg/ml when compared with that of ketoconazole as standard. The zone of inhibition results was summarized below in table 5.

Table 5 Antifungal activity against different strains

Compound	Concentration (µg/ml)	Zone of inhibition (cm)	
		Aspergillus niger	***Penicillium marneffei***
K_1	100	0.9	0.8
	200	1.0	0.9
	300	1.1	1.1
	400	1.2	1.1
	500	1.3	1.3
K_2	100	0.7	0.8
	200	0.9	0.9
	300	1.2	1.1
	400	1.3	1.2
	500	1.5	1.4
K_3	100	0.9	0.9
	200	1.1	1.1
	300	1.2	1.2
	400	1.3	1.3
	500	1.4	1.4
Ketokonazole	100	0.9	0.9
	200	1.0	1.0
	300	1.2	1.2
	400	1.2	1.2
	500	1.4	1.4

The paper disc diffusion method is one of the methods that may be used for determining the relative effectiveness of the antimicrobial activity. The results obtained by this method depend not only on the toxicity of the antimicrobial

agents but also on its ability to diffuse through the medium. Comparative study of minimum inhibitory concentration (MIC =100µg/ml) of synthesized compounds against microbial strains reveals K_3 compound as significant antibacterial activity against *Staphylococcus aureus*. K_3 and K_2 are more significant against fungal strains than bacterial strains used in the present investigation (figure 7).

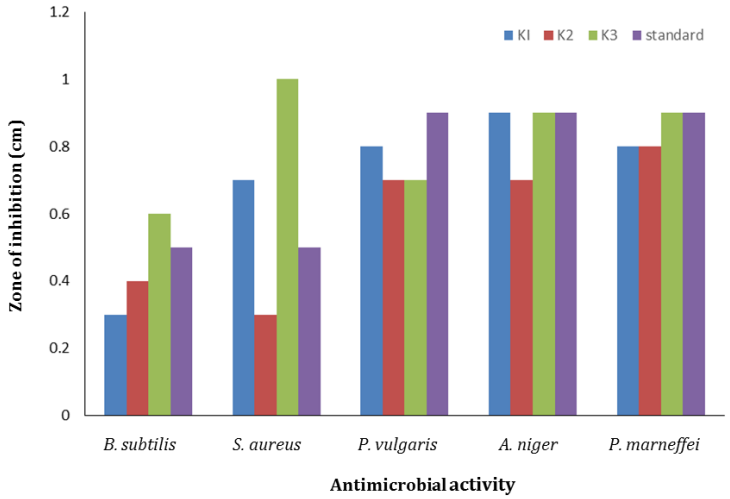


Figure 7 Comparative study of MIC (100 µg/ml) of synthesized compounds against microbial strains

4. Conclusion

In the present study, three compounds (K_1, K_2, K_3) containing oxadiazole moiety were synthesized as potential antimicrobial agents. They were evaluated for their biological activity. All the three compounds were found to possess antibacterial and antifungal activity. Compound K_3 showed the significant antibacterial and antifungal activity when compared with the standards. The result of this investigation encourages us to synthesize similar other related compounds and evaluate them for a wide range of biological activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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