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SYNTHESIS, CHARACTERIZATION & ANTI BACTERIAL ACTIVITY OF 1,3,4-OXADIAZOLE

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ABSTRACT

To synthesize, Characterization of 1,3,4-Oxadiazole along with the evaluation of Antibacterial activity. 1,3,4-Oxadiazoles are the five membered ring compound which are commonly used pharmacophores due to their fantastic metabolic profile. They are thermostabile compounds by which they can stay at any temperature during synthesis process. They are reported to have wide range of Biological activities such as antitubercular, antibacterial, antifungal, antipyretic, analgesic activities. Sulfonamide derivatives of 1,3,4-Oxadizoles are reported to have excellent hypoglycemic action. 1,3,4-oxadiazole were synthesized by conventional method in the scheme by using 5-phenyl-1,3,4-oxadiazole-2-thiol. Seven (a-g) biologically active compounds of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl) acetamide were prepared by using incorporation of Chloro-, Nitro-, Amino- & Nitro- moieties to the side benzene rings. Physical & spectral data was evaluated. NMR Spectra (δ ppm) of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl) acetamide 10.4(S, 1H, -CONH), 7.2 – 7.6 (M, 10H, Ar-H) & 4.4 (S, 2H, CH2), Mass Spectra got Molecular ion peak (M+1) at m/z 312. Antibacterial activity of derived compounds was evaluated by using Agar diffusion method, to estimate the inhibitory concentration. Ampicillin (10µg/ml) taken as the standard control. Compound b, c, g was found to have good antibacterial activity & the results were significantly comparable to the standard control.

Key Words:- 1,3,4-Oxadiazole, Antibacterial activity, Agar diffusion method, Ampicillin(10µg/ml), DMSO.

INTRODUCTION

Organic substances possess a great number of advantages such as possibility to adjust their properties for a given purpose by different chemical and physical techniques in the preparation process. One such example is 1, 3, 4-oxadiazole ring.

1, 3, 4-Oxadiazole compounds are thermally stable, hetero aromatic molecules. They are commonly utilized pharmacophores due to their metabolic profile and ability to engage in hydrogen bonding. Other properties include good hydrolytic stability, high glass transition temperature, low dielectric constants and tough mechanical properties.

1, 3, 4-Oxadiazolin-5-ones and 1, 3, 4-oxadiazoline-5-thiones are reported to possess antitubercular (Alireza Foroumadi *et al*., 2002; Suresh Kumar GV *et al*., 2010), antifungal, antibacterial (Padmavathi V *et al*., 2009), antihypertensive, analgesic, antipyretic and antiphlogistic properties. The sulfonamide derivatives of 1,3,4-oxadiazole are established not only as bactericides but also as hypoglycemic agents (Alex S Kiselyov *et al*., 2010). In this study Synthesis,

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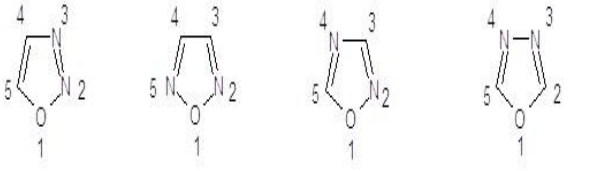
Characterization of 1,3,4-Oxadiazole were carried out to evaluate its Antibacterial activity. (Jumat Salimon *et al*., 2010).

Chemistry of 1,3,4-Oxadiazole:

Compounds having a five membered ring containing one oxygen and two nitrogen atoms are called oxadiazoles.

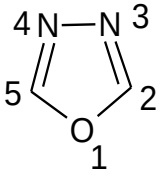
Four types of oxadiazole are known namely 1,2,3-, 1,2,4-, 1,2,5- and 1,3,4-oxadiazoles. Out of these 1,3,4-oxadiazoles are found to be most potent biologically.

Oxadiazoles are considered to be derived from furan by the replacement of two methine (-CH=) groups by two pyridine type of nitrogens (-N=). There are four isomeric types of oxadiazoles depending on the position of nitrogen atoms in the oxadiazole ring and are numbered as:



The replacement of two methane (-CH=) groups by two pyridine type of nitrogens (-N=) reduces aromaticity of the resulting oxadiazole ring to such an extent that the oxadiazole ring exhibits character of a conjugated diene.

1,3,4-oxadiazole ring is an aromatic molecule with resonance energy 167.4 KJ/mole. The ring is symmetrical and planar with the following structural parameters:



1,3,4-Oxadiazole contains pyridine type nitrogen at position 3 and 4 which cause electron withdrawal from the carbons at positions 2 and 5. Therefore these have low electron density on the nitrogen atoms. Because of very low π-electron density on the carbon atoms the attack of electrophiles preferentially occurs at nitrogen whereas the nucleophiles attack at 2 and 5 carbon atoms (Asif Husain *et al*., 2012; Azza T Taher *et al*., 2012).

MATERIALS AND METHODS

Step I: Synthesis of benzoic acid hydrazide (II):

A mixture of hydrazine hydrate (12ml, 0.24mol) and ethylbezoate/ethyl-p-aminobenzoate (27.33gm, 0.2mol) was taken into a round bottomed flask and heated under reflux for 15 min. Ethanol was added through the condensor to produce a clear solution, refluxed for another 4-5hrs. The reaction was monitored by TLC. After completion of the reaction, the excess of solvent was distilled off and the contents were cooled to room temperature. The crystals of acid hydrazide formed were filtered, dried and further purified by recrystallization from ethanol.

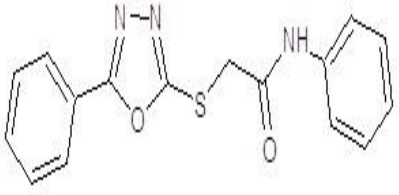
Step II: Synthesis of 5-phenyl-1,3,4-oxadiazole-2-thiol (III):

A mixture of benzoicacid hydrazide (II, 0.1mol) in ethanol (30ml), KOH (0.1mol) in absolute ethanol (50ml) and carbon disulfide (CS_2) was refluxed for about 16hrs, till evolution of hydrogen sulfide was ceased. The reaction mixture was cooled at room temperature and poured over crushed ice. On acidification with dil. HCl, the required oxadiazole was precipitated. The solid mass that separated out was filtered, dried and recrystallized from ethanol to get desired product as a solid. The yield of the compound was found to be 58%. The purity of the compound was checked by TLC.

Step III: Synthesis of n-phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide(IV):

A mixture of 5-Phenyl-1,3,4-oxadiazole-2-thiol (III,0.01mol) and 2-chloro-N-phenyl acetamide (0.01mol) were refluxed in dry pyridine(20ml) for 24hrs. The reaction mixture was then poured into a beaker containing ice cold water, the solid obtained was filtered, washed with water and recrystalized from alcohol to yield white coloured crystals of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl) acetamide. (Nida N Farshori *et al*., 2010; Joanna Matysiak *et al*., 2006; Rune Severinsen *et al*., 2005; John Kallikat Augustine *et al*., 2009; Vivek Polshettiwar *et al*., 2008).

Spectral data of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide(IVa-h



N-phenyl-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide

(IVa, R,R^1 = H)

TLC: Rf : (n-hexane:ethylacetate-3:2)
Solubility : methanol
IR (KBr) Cm^{-1} : 3328 (NH), 1674(C=O), 1616(C=N) & 1544(C=C).
NMR Spectra(δ ppm) : 10.4(S, 1H, -CONH), 7.2 – 7.6(M, 10H, Ar-H) & 4.4 (S, 2H, CH2)
Mass Spectra : Molecular ion peak (M+1) at m/z 312.

Biological Activity

In view of varied biological and pharmacological importance of different 1,3,4-Oxadiazoles, it has been prompted us to evaluate the new series of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide (IVa-IVg) for antimicrobial (Bhat KI *et al.*, 2011; Shakya AK *et al*., 1992; Joanna Matysiak *et al*., 2006).

Antibacterial Activity

Four bacterial test organisms such as *Bacillus subtilis* (MTCC441), *Staphylococcus aureus* (MTCC 96) *Escherichia coli* (MTCC 722), and *Proteus vulgaris* (MTCC 109) were selected and obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in Petri dishes prior to testing. The media used was nutrient agar, nutrient procured from HiMedia Laboratories, Mumbai. Stock solutions of the synthesized compounds were prepared in the different concentrations, viz., 100µg/ml, 500µg/ml, 300µg/ml, 150µg/ml, 50µg/ml using dimethyl sulfoxide (DMSO) as solvent for antimicrobial activity. (Padmavathi V *et al.*, 2010).

The antibacterial activity of title compounds was assayed against four different strains of bacteria by *agar diffusion method*.

Two Gram-Positive Bacteria: *Bacillus subtilis* and *Staphylococcus aureus*.

Two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris.*

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods: one is *serial dilution method* and the other is *diffusion method*. The *serial dilution method* is very useful for the determination of antimicrobial activity. It is not much useful for the quantitative detection tests and also for the evaluation of large number of compounds.

The method adopted in this investigation was cup-plate method (one of the type of agar diffusion method). In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of zone of inhibition was measured.

Cultured Medium

Nutrient broth was used for the preparation of inoculums of the bacteria and the nutrient agar used for the screening method.

Preparation of Bacterial culture

The test organism was sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain & incubated at 37 ± 1ºC for 24 hours. The stock cultures were maintained.

Preparation of Test compound solutions

Solution of test compounds was prepared by dissolving 10 mg each in Dimethyl sulfoxide (DMSO, 10ml). A reference standard for Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantities of Ampicillin in DMSO (10µg/ml).

Table 1. Characterization of 1,3,4-Oxadiazole

Compound	Obtained Form	Mol. Formula	Mol. Wt. (gm)	% yield	M.P	TLC:Rf
II	white crystalline compound	$C_7H_8N_2O$	136.15	83.2	112-114^{0}C	(n-hexane:ethylacetate-3:2)
III	light yellow crystalline compound	$C_8H_6N_2OS$	178.21	58	237^{0}C	(n-hexane:ethylacetate-3:2)
IV	light yellow crystalline compound	$C_{16}H_{13}N_3O_2S$	311.35	45	242-244^{0}C	(n-hexane:ethylacetate-3:2)

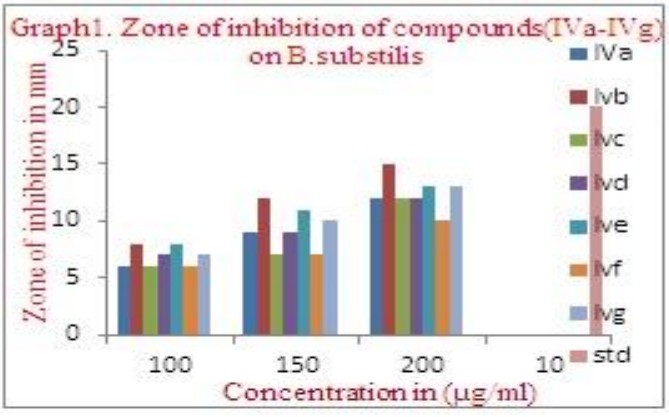
Table 2. Physical data of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide (Compound - IV)

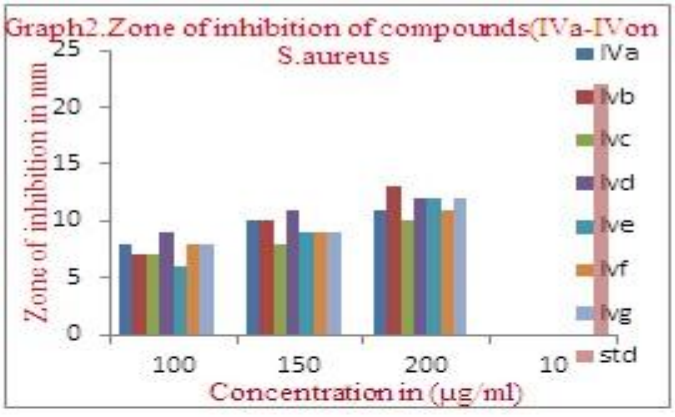
Compound	Substituents		Mol. Formula	IUPAC Name	Mol. Wt.	M.P (^{0}C)	Yield (%)
	R	R^1					
IVa	H	H	$C_{16}H_{13}N_3O_2S$	*N*-phenyl-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide	311	243-245	45
IVb	H	Cl	$C_{16}H_{12}ClN_3O_2S$	*N*-(4-chlorophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide	345	235-237	51
IVc	H	NO_2	$C_{16}H_{12}N_4O_2S$	*N*-(4-nitrophenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide	356	241-243	41
IVd	H	CH_3	$C_{17}H_{15}N_4O_2S$	*N*-(4-methylphenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide	325	248-251	45
IVe	NH_2	Cl	$C_{16}H_{13}ClN_4O_2S$	2-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-chlorophenyl)acetamide	360	233-235	49
IVf	NH_2	NO_2	$C_{16}H_{13}N_5O_2S$	2-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-nitrophenyl)acetamide	371	221-223	53
IVg	NH_2	CH_3	$C_{17}H_{16}N_4O_2S$	2-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-*N*-(4-methylphenyl)acetamide	340	229-231	50

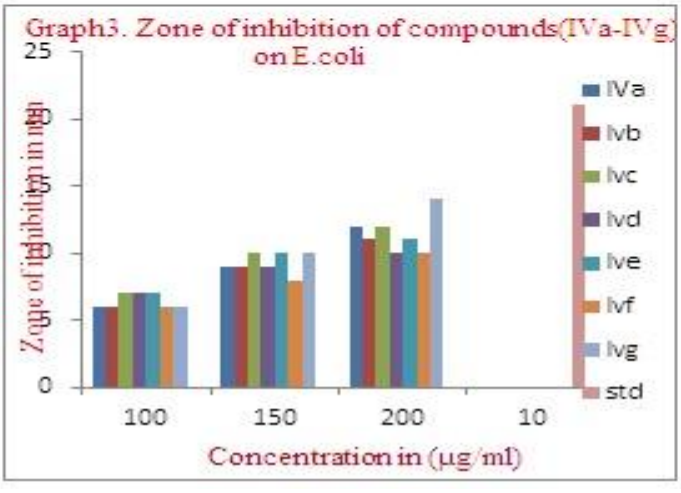
Table 3. Antibacterial activity of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl) sulfanyl) acetamide (IV)

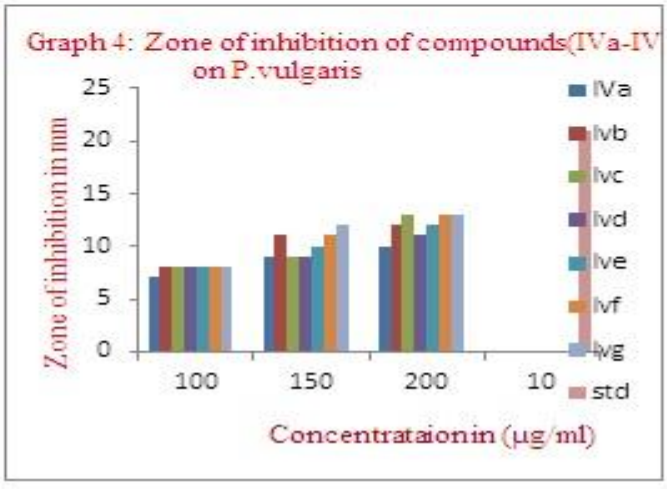
S. No	Substituents			Conc.	Zone of inhibition (in mm)			
	Compound No	R	R^1	Conc. (µg/ml)	B.subtilis	S.aureus	E.coli	P.vulgaris
1	**IVa**	H	H	100	6	8	6	7
				150	9	10	9	9
				200	12	11	12	10
2	**IVb**	H	Cl	100	8	7	6	8
				150	12	10	9	11
				200	15	13	13	12
3	**IVc**	H	NO_2	100	6	7	7	8
				150	7	8	10	9
				200	12	11	12	13
4	**IVd**	H	CH_3	100	7	9	7	8
				150	9	11	9	9
				200	12	12	10	11
5	**IVe**	NH_2	Cl	100	8	6	7	8
				150	11	9	10	10
				200	13	12	11	12
6	**IVf**	NH_2	NO_2	100	6	8	6	8
				150	7	9	8	11
				200	10	11	10	13

7	**Ivg**	NH_2	CH_3	100	7	8	6	8
				150	10	9	10	12
				200	13	12	14	13
Standard drug: Ampicillin (10µg/ml) shows 20mm Zone of inhibition								

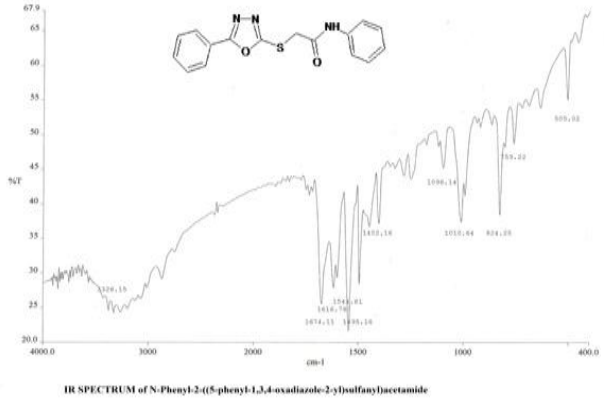


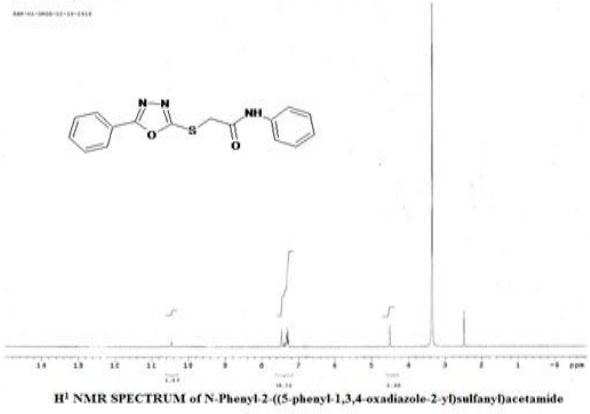


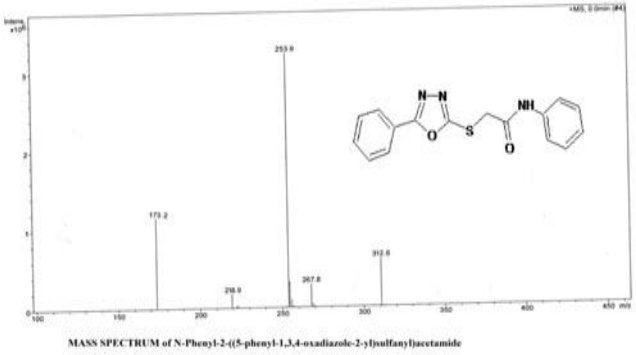




Spectral Pictures of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide: (Fig 1-3)

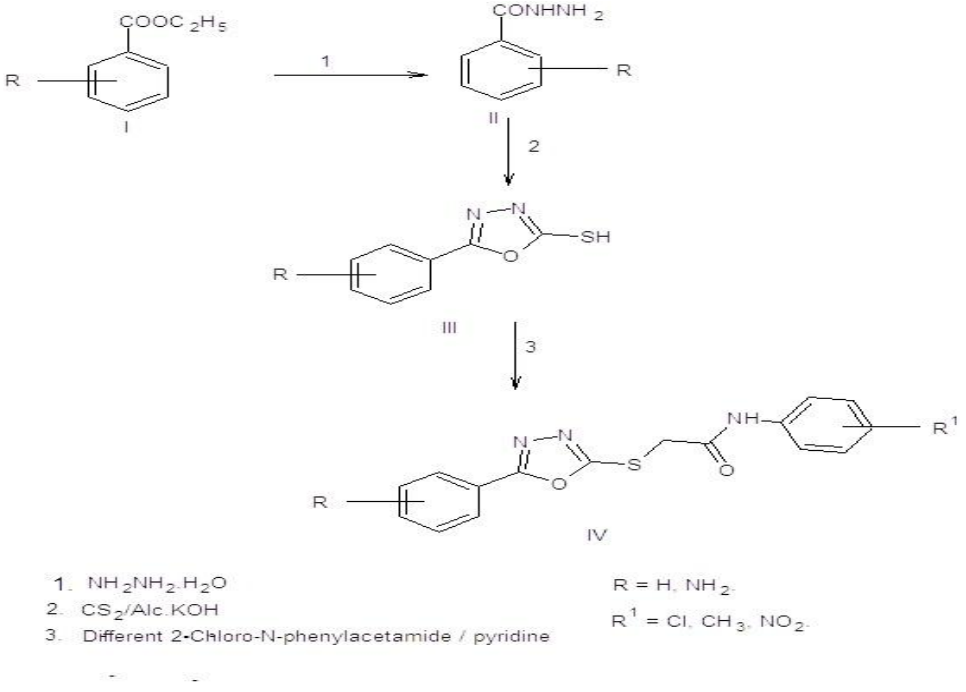

IR SPECTRUM of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide


H^1 NMR SPECTRUM of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide



MASS SPECTRUM of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide

Scheme of Synthesis



Compound I: Ethylbezoate/Ethyl-p-aminobenzoate
Compound II: Benzoicacidhydrazide
Compound III: 5-Phenyl-1,3,4-oxadiazole-2-thiol
Compound IV: N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl)acetamide

The nutrient agar medium was sterilized by autoclaving at 121ºC (15lb/sq.inch) for 15 minutes. Petri-plates, tubes and flasks plugged in cotton were sterilized in hot-air oven at 160ºC for an hour. Into each sterilized Petri-plate (10cm diameter), about 27ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of inoculum into each plate) was transferred aseptically. In each plate, three discs of 6mm diameter were made with a sterile borer after solidification of the plates. These solutions at concentrations (200µg/ml, 150µg/ml, and 100µg/ml) was added to respective disc aseptically and labeled accordingly. The plates were kept undisturbed for 1 hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37 ± 1ºC for 24 hours, the diameter of zone inhibition surrounding each of

discs was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1ml of DMSO to observe the solvent effects and the results were represented in Table 3.

RESULTS & DISCUSSION

The anti-bacterial activity of N-Phenyl-2-((5-phenyl-1,3,4-oxadiazole-2-yl)sulfanyl) acetamide (IV) was screened against four different strains of bacteria by *agar diffusion method* (Table 3).

Two Gram-Positive Bacteria: *Bacillus subtilis* and *Staphylococcus aureus* (Graph 1,2).

Two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris* (Graph 3,4).

Using benzoic acid hydrazide as starting material, 5-phenyl-1,3,4-oxadiazole -2-thiol was synthesized. The method involved reacting the acid hydrazide with carbon disulphide employing methanol as solvent. Acid hydrazide which is the principle starting material for the method employed, was relatively easy to prepare requiring fewer steps compared to other methods for synthesizing 5-phenyl-1,3,4-oxadiazole-2-thiol.

The newly synthesized oxadiazole derivatives were characterized by physical and spectral data. These compounds were evaluated for the Antibacterial activity. (Table 2, Fig 1-3).

It could be evidenced from the results of present investigation that irrespective of their nature, none of the test compounds are comparable with the standard i.e., Ampicillin in their antibacterial activity.

Antibacterial activity among the test compounds is presented in Table 3. The antibacterial activity of test compounds shows that the newly synthesized oxadiazole derivatives (IV_{a-g}) exhibited mild to moderate antibacterial activity against the test organisms employed in the present investigation. However, the degree of inhibition varied with the test compounds and the test bacterium.

Among the treated compounds employed IV_b was relatively more active against all the test organisms. All the compounds were equipotent against *S.aureus*, but IV_b was relatively more potent (Table 3).

The compounds IV_g was relatively more active against *E.coli*, whereas compounds V_b and IV_g were relatively more active against *P.vulgaris*. (Graph 3,4)

CONCLUSION

In the present study new 1,3,4-oxadiazole were synthesized by conventional method in the scheme by using 5-phenyl-1,3,4-oxadiazole-2-thiol and evaluated for their antimicrobial activity. It could be evidenced that none of the test compounds are comparable with the standard. Among the compounds synthesized, IVb, IVc, IVg demonstrated good antibacterial in comparison to the control and other test compounds.

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