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Antibacterial Activity of Some Newly Synthesized 2, 5-Substituted Oxadiazoles and Thiadiziole and Their Transition Metal Complexes

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ABSTRACT

The 1, 3, 4-oxadiazole and thiadiazole derivatives were synthesized by oxidative cyclization of semicarbazones obtained from the reaction of semicarbazides with corresponding aromatic aldehydes. The ligands were characterized by CHNS analyses, IR, LCMS, ^{1}H NMR and ^{13}CMR spectral analysis. The antibacterial activity of synthesized compounds and their transition metal complexes with Cu (II), VO (IV), Ni (II), Zn (II) and Cd (II) ions were screened in vitro. The activities were screened against Gram negative strains (*Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*) and Gram positive strain (*Staphylococcus aureus*). The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of selected compounds were determined.
Keywords: Antibacterial activity, 1, 3, 4-oxa- /-thiadiazole derivatives, metal complexes, MIC, MBC

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INTRODUCTION

There are numbers of five member heterocycles containing nitrogen and sulphur atom, to be potential chemotherapeutics and parmacotherapeutics agents. The biological profile of oxadiazoles and thiadiazoles is very extensive. Among the five-membered nitrogen heterocycles, the 1, 3, 4-oxadiazoles are known to be associated with a broad spectrum of biological activities [1-3]. Their derivatives have been known to possess antibacterial [4], antimicrobial [5], insecticidal [6], herbicidal, fungicidal [7], anti-inflammatory [8], hypoglycaemic [9], and hypotension [10] characteristics, as well as antiviral [11] and anti-tumour activities [12]. Also the thiadiazoles are known to have pesticidal, antifungal [13], antibacterial [14].

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. In addition, primary and opportunistic microbial infections continue to increase the number of immunocompromised patients, those suffering from such as AIDS or cancer or who have undergone organ transplantation. We designed and prepared a series of oxadiazoles and thiadiazole derivatives and their metal complexes in an effort to investigate their antimicrobial activities. These above observations promoted us to synthesis of the title compounds and their metal complexes for antimicrobial activity (Figure 1).

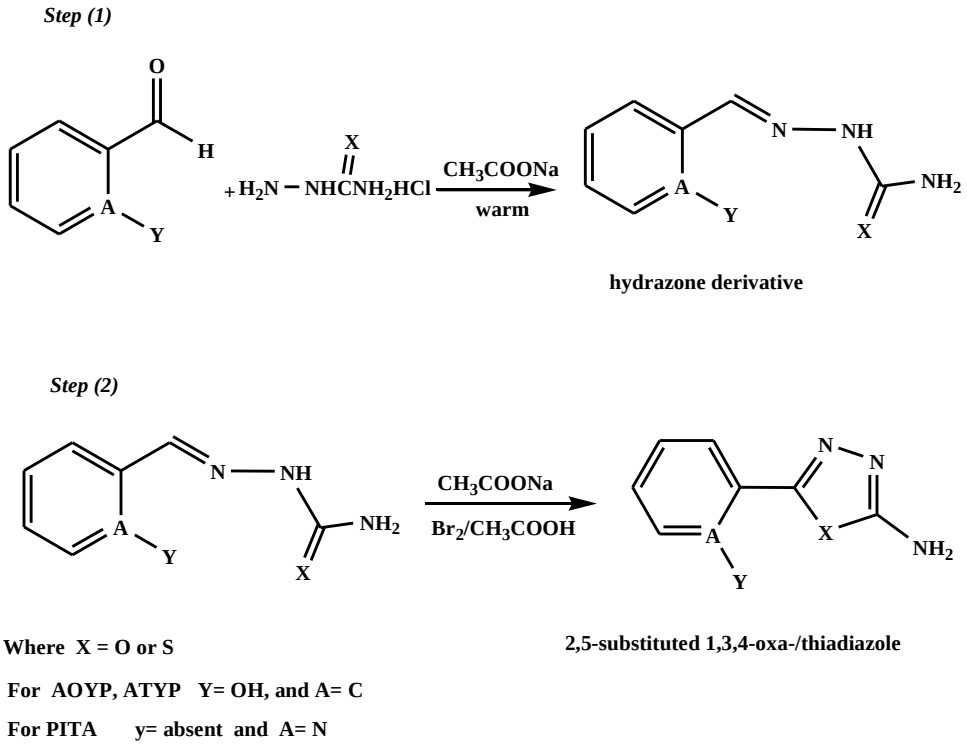

Figure 1: Synthesis of 2, 5-substituted 1, 3, 4-oxa/thiadiazole derivatives

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MATERIALS AND METHODS

The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapors as detecting agent. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were done using CHNS Analyzer at I.R.M.R.A. Pune. Infra-red spectra were recorded on Shimadzu 8000-FTIR Spectrophotometer in KBr Phase. Proton NMR spectra were recorded in DMSO at SAIF Punjab University, Chandigarh, using tetramethyl silane as internal standard. The synthesised complexes were also characterized by elemental, magnetic, spectral, thermal, XRD and ESR studies.

General procedure of Synthesis of 2, 5-substituted oxa-/thiadiazoles:

An ethanolic solution (25 ml) of aldehyde (10 mmol) was added with stirring to an aqueous solution (40 ml) of a mixture of semicarbazide hydrochloride (10.2 mmol) and sodium acetate (10.3 mmol). The mixture was warmed on a water bath for 5-10 min and allowed to cool in ice water. The semicarbazone crystals were filtered off, washed with cold water, dried and recrystallised with ethanol. The obtained semicabazone (0.01M) and sodium acetate (0.02 M) were dissolved in 30-40 ml of glacial acetic acid taken in a round bottom flask equipped with separating funnel for addition of bromine. Bromine (7 ml in 5 ml glacial acetic acid) was added slowly to it, while stirring magnetically. After half an hour stirring, the solution was poured on crushed ice. The resulting solid was separated, dried and recrystallised from ethonal. The physical and analytical data of the synthesized title compounds (Figure 2) are given as follows.

2-(5-amino-1,3,4-oxadiazol-2-yl) phenol (AOYP): m. p. 137°C, molecular formula ($C_8H_7N_3O_2$), molecular weight 177, recrystallization solvent ethanol, yield 92%, elemental analysis (%)calculated H(3.98), C(54.24), N(23.72), found H(4.02), C(55.23), N(23.80). IR cm^{-1}: 3382(OH), 3273-3251(NH_2), 1597(C=N). 1255 (Phenolic C-O), 1013(C-O-C). ^{1}H NMR (DMSO-d_6) ppm: 12.14 (2H, s, Ar-NH_2), 7.35-7.75 (4H, m, Ar-H), 5.21 (1H, s, OH).

2-(5-amino-1,3,4-thiadiazol-2-yl) phenol (ATYP): m. p. 140°C, molecular formula ($C_8H_7N_3OS$), molecular weight 193, recrystallization solvent ethanol, yield 85%, elemental analysis: (%)calculated H(3.65), C(49.73), N(21.75), S(16.59) found H(3.54), C(50.03), N(21.59), S(17.05). IR cm^{-1}: 3382(OH), 3273-3251(NH_2), 1597(C=N), 1255(Phenolic C-O), 705(C-S-C). ^{1}H NMR (DMSO-d_6) ppm: 10.85 (2H, s, Ar-NH_2), 6.95-7.75 (4H, m, Ar-H), 5.25 (1H, s, OH).

2,2'-(1,3,4-oxadiazole-2,5-diyl) diphenol (ODDP): m. p. 133°C, molecular formula ($C_{14}H_{10}N_2O_3$), molecular weight 254, recrystallization solvent ethanol, yield 78%, elemental analysis: (%)calculated H(3.96), C(66.14), N(11.02) found H(4.12), C(66.56), N(11.57). IR cm^{-1}: 3203(OH), 3273-3251(NH_2), 1605(C=N), 1265(Phenolic C-O), 1012(C-O-C). ^{1}H NMR (DMSO-d_6) ppm: 6.98-7.69 (8H, m, Ar-H), 5.31(2H, s, OH).

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5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-amine (PITA): m. p. 125°C, molecular formula ($C_7H_6N_4S$), molecular weight 178, recrystallization solvent ethanol, yield 91%, elemental analysis: (%)calculated H(3.39), C(47.18), N(31.44), S(17.99) found H(3.56), C(48.09), N(32.12), S(18.24). IR cm^{-1}: 3262-3193(NH_2), 1596(C=N), 689(C-S-C). ^{1}H NMR (DMSO-d_6) ppm: 10.55 (2H, s, Ar-NH_2), 7.35-8.64 (4H, m, Ar-H).

N-(5-Pyridin-2-yl)-1,3,4-oxadiazol-2-yl) acetamide (PIOA): m. p. 136°C, molecular formula ($C_9H_8N_4O_2$), molecular weight 204, recrystallization solvent ethanol, yield 80%, elemental analysis: (%)calculated H(3.95), C(52.94), N(27.44) found H(4.23), C(52.60), N(27.89). IR cm^{-1}: 3260(NH), 1690(C=O), 1596(C=N), 1044(C-O-C). ^{1}H NMR (DMSO-d_6) ppm: 9.92 (1H, s, NH), 7.34-8.72(4H, m, Ar-H), 2.38(3H, s, CH3).

5-(1H-pyrrol-2-yl)-1,3,4-oxadiazol-2-amine (PYOA): m.p. 130°C, molecular formula ($C_6H_6N_4O$), molecular weight 150, recrystallization solvent ethanol, yield 81%, elemental analysis: (%)calculated H(4.03), C(48.00), N(37.32) found H(4.80), C(48.37), N(37.03). IR cm^{-1}: 3232-3188(NH_2), 3134(NH-pyrole), 1613(C=N), 1035(C-O-C). ^{1}H NMR (DMSO-d_6) ppm: 10.92 (2H, s, Ar-NH_2), 8.63(1H, s, NH-pyrole), 6.28-6.96 (3H, m, Ar-H).

General procedure of Synthesis of metal complexes of oxa-/thiadiazoles:

0.02 moles of ligand (in slight excess) was taken in round bottom flask containing 30ml of ethanol. 0.01 moles of metal (II) chloride dissolved in 20ml of ethanol and was gradually added into the solution of ligand. Ten percent alcoholic ammonia solution was added drop wise till precipitation of complex was obtained. The precipitated complex was digested for one hour. Any change in pH if observed was readjusted and digested for one more hour. The coloured precipitate of complex was filtered in hot condition. It was washed with alcohol followed by petroleum ether (40–60^{0}C) and dried in vacuum desiccators over calcium chloride.

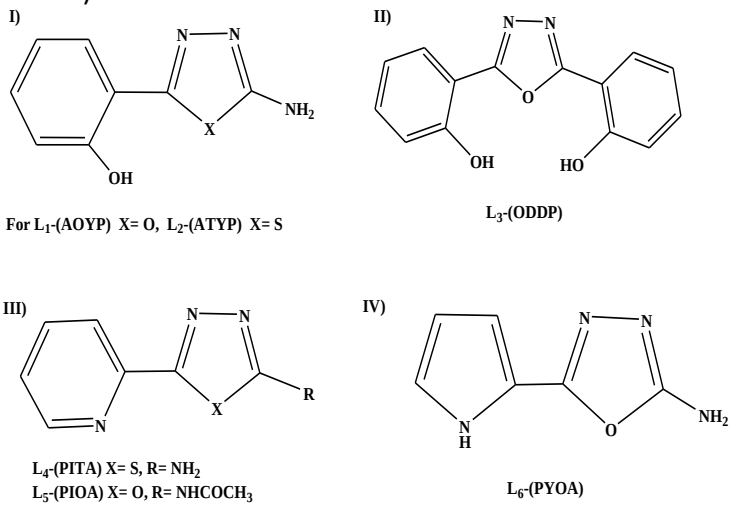

Figure 2: Structures of synthesized compounds

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RESULTS AND DISCUSSION

Antimicrobial Activity

Bacterial strains used in the study were clinical isolates of Staphylococcus aureus, Escherechia coli, Proteus mirabilis, and Pseudomonas aeroginosa, all of them were collected from Pharmacy College, Nanded, Maharashtra.

All the 1, 3, 4-oxa-/thiadiazole derivatives and its complexes were screened for their in vitro antimicrobial activity by using the well diffusion method reported in the literature by Perez et al [15]. Three colonies of bacteria were transferred to sterile tubes each containing 5 ml of Tryptic Soy Broth. Turbidity of the bacterial suspensions was adjusted to reach an optical density equivalent to a 0.5 McFarland standard to give a bacterial suspension of 10^8 cfu/ml. Mueller-Hinton agar plates were inoculated by streaking bacterial swabs over the entire surface of the plates. Plates were allowed to dry at room temperature. Six millimeter wells were punched in the plates. Ciprofloxacin was used as a standard drug. All the compounds were dissolved in dimethyl sulfoxide. Fifty microliters of 4 mg/ml solutions of each of the 1, 3, 4-oxa-/thiadiazole derivatives and their complexes were added into duplicate wells. Plates were allowed to stand at room temperature to let the tested derivative diffuse into the agar, and afterwards, they were incubated at 37^o C for 18 to 24 hours. Plates were examined for bacterial growth inhibition and zones of inhibition were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) [16]

MIC was determined by Broth dilution method. Drugs with inhibitory zones against the above mentioned bacterial strains were used in this part. Two-fold serial dilutions were prepared from the drugs in Tryptic Soy Broth. Duplicate tubes of each dilution were inoculated with $5x10^5$ of the bacterial strains. All tubes were incubated at 37^o C for 18 to 24 hours. The highest dilution of the drug that resulted in inhibition of bacterial growth was considered as the MIC.

Determination of Minimal Bactericidal Concentration (MBC)

Subcultures from the above dilutions were done on Muller-Hinton plates and incubated at 37 ^{o}C for 18 to 24 hours. The highest dilution that resulted in total inhibition of bacterial growth was considered the MBC.

Antimicrobial assessment

It can be seen from the screening results (Table1) that amongst the ligands the ATYP and PITA showed better activity than others. ODDP showed the least activity.

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The results also reveal that amongst the metal (II) complexes, the Cd (II) and Cu (II) complexes showed highest comparative activities especially against P. mirabilis and E. coli. The reflection of high values for Zn (II), Cd (II) and VO (IV) complexes of PIOA may be originating from its ionic nature. The best activity showed was by PITA and its complexes against P. mirabilis. All compounds showed intermediate to susceptible activities, according to interpretive criteria defined by the National Committee for Clinical Laboratory Standards that divide the activity of compounds to bacteria as susceptible, intermediate and resistant. The zones of inhibition varied from 07 mm to 30 mm for the analytes. Cd-PITA showed the highest zone of inhibition, it was about 30 mm, and its effect, on an average is at least two fold more than that of the other compounds.

Table 1: The antimicrobial activity of 1, 3, 4-oxa-/thiadiazole derivatives and their complexes against various types of bacteria.

Screening results (Zone of inhibition in mm)

Compound (4 mg/ml)	Zone of inhibition in mm			
	S. aureus	E. coli	P. mirabilis	P. aeroginosa
AOYP	08	12	11	07
Cu-AOYP	15	20	18	19
VO-AOYP	10	15	14	09
Ni-AOYP	12	18	17	13
Zn-AOYP	08	13	13	09
Cd-AOYP	15	22	17	20
ATYP	15	17	18	16
Cu-ATYP	18	26	25	20
VO-ATYP	17	18	20	19
Ni-ATYP	18	24	21	20
Zn-ATYP	17	18	19	16
Cd-ATYP	20	25	21	27
ODDP	07	11	12	09
Cu-ODDP	14	15	15	12
VO-ODDP	09	11	13	09
Ni-ODDP	11	14	15	13
Zn-ODDP	07	12	12	10
Cd-ODDP	12	15	16	08
PITA	16	20	19	21
Cu-PITA	19	27	28	24
VO-PITA	20	20	22	25
Ni-PITA	18	26	27	22
Zn-PITA	19	20	20	22
Cd-PITA	19	30	29	23
PIOA	09	14	11	13
Cu-PIOA	15	20	21	18
VO-PIOA	19	21	23	24
Ni-PIOA	13	17	15	16
Zn-PIOA	18	21	23	23
Cd-PIOA	20	29	30	25
PYOA	09	13	15	12
Cu-PYOA	13	16	17	18
VO-PYOA	10	14	15	15

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Ni-PYOA	12	17	17	16
Zn-PYOA	09	14	15	13
Cd-PYOA	14	16	19	15
Ciprofloxacin	27	36	32	29

MIC and MBC

Owing to the better activity of ATYP, PITA and their complexes against P. mirabilis, the MIC and MBC tests of them were selectively screened for P. mirabilis. The results obtained from it are summarized in Table 2. As shown in Table 2, MIC of ATYP was 500 mg/mL, which is at least two fold higher than the MIC of PITA. The comparative results may indicate that Zn (II) and VO (IV) complexes have slight activity against the bacteria used in this study. MIC for the selected title compounds and their complexes was determined by dilution of the different derivatives to various concentrations and compared with a standard (Ciprofloxacin, Cipla Ltd. Mumbai, India).

Table 2: MIC Determination of 1, 3, 4-oxa-/thiadiazole derivatives and their complexes (for P. mirabilis)

Conc. (µg/mL)	ATYP	Cu-ATYP	VO-ATYP	Ni-ATYP	Zn-ATYP	Cd-ATYP
4×10^3	-ve	-ve	-ve	-ve	-ve	-ve
2×10^3	-ve	-ve	-ve	-ve	-ve	-ve
$1x\ 10^3$	-ve	-ve	-ve	-ve	-ve	-ve
500	-ve	-ve	-ve	-ve	-ve	-ve
250	**+ve**	-ve	-ve	-ve	**+ve**	-ve
125	+ve	-ve	**+ve**	-ve	+ve	-ve
62.5	+ve	-ve	+ve	**+ve**	+ve	-ve
31.25	+ve	**+ve**	+ve	+ve	+ve	**+ve**
15.62	+ve	+ve	+ve	+ve	+ve	+ve
7.81	+ve	+ve	+ve	+ve	+ve	+ve
3.90	+ve	+ve	+ve	+ve	+ve	+ve
1.95	+ve	+ve	+ve	+ve	+ve	+ve
0.97	+ve	+ve	+ve	+ve	+ve	+ve
P.C.	+ve	+ve	+ve	+ve	+ve	+ve
S.C.	-ve	-ve	-ve	-ve	-ve	-ve
	PITA	Cu-PITA	VO-PITA	Ni-PITA	Zn-PITA	Cd-PITA
4×10^3	-ve	-ve	-ve	-ve	-ve	-ve
2×10^3	-ve	-ve	-ve	-ve	-ve	-ve
$1x\ 10^3$	-ve	-ve	-ve	-ve	-ve	-ve
500	-ve	-ve	-ve	-ve	-ve	-ve
250	-ve	-ve	-ve	-ve	-ve	-ve
125	-ve	-ve	-ve	-ve	-ve	-ve
62.5	**+ve**	-ve	-ve	-ve	**+ve**	-ve
31.25	+ve	-ve	**+ve**	-ve	+ve	-ve
15.62	+ve	-ve	+ve	-ve	+ve	-ve
7.81	+ve	**+ve**	+ve	**+ve**	+ve	-ve
3.90	+ve	+ve	+ve	+ve	+ve	**+ve**
1.95	+ve	+ve	+ve	+ve	+ve	+ve

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0.97	+ve	+ve	+ve	+ve	+ve	+ve
P.C.*	+ve	+ve	+ve	+ve	+ve	+ve
S.C.**	-ve	-ve	-ve	-ve	-ve	-ve

*Positive Control, **Sterility Control, Bacterial growth (+ve or –ve)

The concentration of the compounds that results in a total inhibition of bacterial growth was considered the MBC. Table 3 summarizes MIC and MBC of ATYP, PITA and their complexes.

Table 3: MBC results for P. mirabilis bacteria

Compound	MIC(mg/mL)	MBC(mg/mL)
ATYP	0.5	1
Cu-ATYP	0.0625	0.25
VO-ATYP	0.25	1
NI-ATYP	0.125	0.5
Zn-ATYP	0.5	1
Cd-ATYP	0.0625	0.25
PITA	0.125	0.5
Cu-PITA	0.0156	0.0625
VO-PITA	0.0625	0.5
Ni-PITA	0.0156	0.0625
Zn-PITA	0.125	0.5
Cd-PITA	0.0078	0.0315

The MIC value of 0.5 mg/mL for ATYP shows comparatively lower activity than PITA whose MIC value is 0.125 mg/mL.

The highest MIC result was for Cd-PITA which was about 7.8 µg /mL. For Cu (II), VO (IV), Ni (II), Zn (II) and Cd (II)-ATYP the MIC was equal to 0.0625, 0.25, 0.125, 0.5 and 0.0625 mg/mL respectively. Similarly for Cu (II), VO (IV), Ni (II) and Zn (II)-PITA the MIC was equal to 0.0156, 0.0625, 0.0156, and 0.125 mg/mL respectively. These results indicate that Cu (II), Ni (II) and Cd (II) complexes have higher activities than VO (IV) and Zn (II) complexes.

However, the MBC results for most of the compounds was about 1 and 0.5 mg/ml, except for Cu(II), Ni(II) and Cd(II)-PITA complexes which was 0.0625, 0.0625 and 0.0315 mg/mL respectively.

CONCLUSIONS

In conclusion, all the ligands and their complexes showed antimicrobial activity against all the strains of bacteria. Screening results against Proteus mirabilis and Escherechia coli were better than screening of Staphylococcus aureus, that means the prepared 1, 3, 4-oxa-/thiadiazole derivatives and their complexes are more efficient against gram-negative bacteria than gram-positive. In ligands the thiadiazoles proved better antibacterial activity than the oxadiazoles derivatives. The complex Cd-PITA showed the highest efficiency against P. mirabilis

bacteria. The increase in antibacterial activity is due to faster diffusion of metal complexes as a whole through cell membrane or due to the combined activity effect of the metal and ligand [17-18].

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