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Research Article

SYNTHESIS AND CHARACTERIZATION OF SUBSTITUTED 1,3,4 THIADIAZOLE AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

Background: Heterocyclic chemistry continues to draw the attention of synthetic organic chemists and is of great scientific interest. **Objective**: The present study was undertaken to assess the synthesis and characterization of substituted 1,3,4 thiadiazole as potential antimicrobial agents. **Materials and Methods**: synthesis of three (TDZ1, TDZ2, TDZ3) substituted hetero cyclic compounds and the study its Physio-chemical properties and structural interpretation by IR spectroscopy and NMR spectrum. And *in vitro* antifungal and antibacterial activities performed by nutrient agar medium. **Results**: All the compounds were screened for antibacterial activity. However the compounds TDZ 1 and TDZ 2 have shown promising antibacterial activity, while the remaining compound such as TDZ 3 have also shown moderate antibacterial activity, when compared with the standard drug Ciprofloxacin. Compounds TDZ 1 and TDZ 2 have shown promising antifungal activity, while the remaining compound such as TDZ 3 have also shown moderate antifungal activity, when compared with the standard drug Griseofulvin. **Conclusion**: In this work is concluded that there exists ample scope for the medicinal chemists to further study in this class of compounds and used for the treatment of various types of deadly microbial infections.

Keywords: Thiadiazole, antibacterial, antifungal, Heterocyclic chemistry.

1. INTRODUCTION

Because of the diversity in synthetic procedures, physiological and industrial significance, hetero cyclic chemistry has been and continues to be one of the most active areas of organic chemistry. As a result numerous heterocyclic compounds such as thiazoles, thiadiazoles, indoles, oxadiazoles, benzisoxazoles and pyrroles have been successfully used as antibacterial, anticancer, antipyretic, schistosomicidal, hypoglycemic, antihypertensive, antitubercular, anti-inflammatory and anti-HIV4 agents. In addition, they have also been used in agriculture, plastics, polymers, dyes and textiles. Hence heterocyclic chemistry still continues to draw the attention of synthetic organic chemists and is of great scientific interest. All large number of organo - sulfur compounds occur in living and non-living object. They belong to open chain, alicyclic, aromatic and heterocyclic types of compounds containing sulfur atoms or atoms as a part of chain/ring or both in the structure. Isolation, identification and applications of these organo – sulfur compounds lead to the fact that some of the compounds are useful in scientific, technical and industrial growth. During the last three decades organo-sulfur chemistry developed at a much faster pace than any other branches of organic chemistry.[1,2] The role of organic sulphides in rubber vulcanization, hair curling, muscle contraction, natural aromas, vitamins, hormones, antibiotics, radio-protective agents, dye stuffs, binding materials organic semiconducting materials and organic light emitting diodes etc. may be cited.

Among the sulfur containing heterocyclic compounds, lot of research in the field of 1,3,4-thiadiazoles and imidazo [2,1-b][1,3,4] thiadiazoles has been reported. Some salient features regarding structure, chemical reactivity, spectral studies, synthetic pathways and biological interest of 1,3,4-thiadiazole and condensed imidazo [2,1-b][1,3,4] thiadiazole are discussed briefly as background information.[3,4]

2. MATERIALS AND METHODS

2.1. Analytical Techniques

2.1.1. Physical data

Melting points of the synthesized compounds were determined using Microcontroller based melting point apparatus CL 725/726 were found corrected.

2.1.2. Thin Layer Chromatography (TLC)

Purity of the compounds was checked by thin layer chromatography using alumina as stationary phase and various combinations solvent, as mobile phase. The spots resolved were visualized by using UV chamber and iodine chamber[5,8].

2.1.3 Instrumentation

The techniques employed for the characterization of the synthesized compounds were IR spectra (Fourier transform IR spectrometer model Shimadzu using KBr pellets, Bangalore), Mass spectra (GC-MS spectrophotometer, University Science Instruments Centre, Karnataka University, Dharawad).[6,7]

2.2. Chemicals and Reagents

Diethyl ether, Dimethyl sulphoxide, N,N-dimethyl formamide, 1,4-dioxon, Ethyl acetate, Thiosemicarbazide. The chemicals and reagents used in the present project were of LR grade, purchased from S.D. Fine Chem. Ltd., Mumbai, India.

2.2.1. 4-Hydroxyphenyl-1-(4-pyridine carboxyamido)-thiosemicarbazide (INTC 1)

Isoniazid 2.74g (0.02 mol) and 4-hydroxyphenyl isothiocyanates 2.35g (0.02 mol) was dissolved in 15 ml of ethanol. The resulting mixture was refluxed on a boiling water bath for 6 hr with occasional stirring. The reaction completion was monitored by TLC and the reaction mixture was concentrated and kept overnight at room temperature.[9,10] The needle shaped crystals of substituted thiosemicarbazides obtained was filtered & dried. Recrystallized from methanol. The yield is 2.38 g, m.p was found to be 142-145.

2.2.2. 4-aminoyphenyl-1-(4-pyridine carboxy amido)-thiosemicarbazide (INTC 2)

Isoniazid 2.74g (0.02 mol) and 4-aminophenyl isothiocyanates 2.35g (0.02 mol) was dissolved in 15 ml of ethanol. The resulting mixture was refluxed on a boiling water bath for 6 hr with occasional stirring. The reaction completion was monitored by TLC and the reaction mixture was concentrated and kept overnight at room temperature. The needle shaped crystals of substituted thiosemicarbazides obtained was filtered & dried. Recrystallized from methanol. The yield is 2.38 g, m.p was found to be 157-159.

2.2.3. 4-Bromophenyl-1-(4-pyridinecarboxy amido)-thiosemicarbazide (INTC 3)

Isoniazid 2.74g (0.02 mol) and 4-bromophenyl isothiocyanates 2.35g (0.02 mol) was dissolved in 15 ml of ethanol. The resulting mixture was refluxed on a boiling water bath for 6 hr with occasional stirring. The reaction completion was monitored by TLC and the reaction mixture was concentrated and kept overnight at room temperature.[11,12] The needle shaped crystals of substituted thiosemicarbazides obtained was filtered & dried and Recrystallized from methanol. The yield is 2.38 g, m.p was found to be 134-136.

2.2.4. Synthesis of 4-(5-(pyridin-4-yl)-1, 3, 4-thiadiazol-2-ylamino) phenol (TDZ 1)

4-hydroxyphenyl thiosemicarbazides (INTC 1) 2.10 g (0. 02 mol) was added with sulphuric acid slowly and the mixture was cooled at 0-5^{o}C. The reaction mixture was powered in to ice cold water, filtered and washed with water and recrystallized from methanol. The yield is 1.83 g, m.p was found to be 116-118.

2.2.5. Synthesis of N-(5-(pyridin-4-yl)-1, 3, 4-thiadiazol-2-yl) benzene-1, 4-diamine (TDZ 2)

4-aminophenyl thiosemicarbazides (INTC 2) 2.10 g (0. 02 mol) was added with sulphuric acid slowly and the mixture was cooled at 0-5oC. The reaction mixture was powered in to ice cold water, filtered and washed with water and recrystallized from methanol. The yield is 1.65 g, m.p was found to be 125-128.

2.2.6. Synthesis of N-(4-bromophenyl)-5-(pyridin-4-yl)-1, 3, 4-thiadiazol-2-amine (TDZ 3)

4-bromophenyl thiosemicarbazides (INTC 3) 2.10 g (0. 02 mol) was added with sulphuric acid slowly and the mixture was cooled at 0-5oC. The reaction mixture was powered in to ice cold water, filtered and washed with water and

recrystallized from methanol. The yield is 1.48 g, m.p was found to be 112-115.

2.3. BIOLOGICAL ACTIVITY

The inhibition of microbial growth under standardized conditions may be utilized for demonstrating the therapeutic efficacy of antibiotics. Any subtle change in the antibiotics molecule which may not be detected by chemical methods will be revealed by a change in the antimicrobial activity and hence microbiological assays are very useful for resolving doubts regarding possible change in potency of drugs and their preparation. The microbiological assay is based upon a comparison of the inhibition of growth of microorganisms by measured concentrations of the antibiotics to be examined with that produced by known concentrations of a standard preparation of the antibiotic having a known activity. [13]

The cylinder-plate method depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a Petri dish or plate to an extent such that growth of the added micro-organisms is prevented entirely in a zone around the cylinder containing a solution of the antibiotic. The newly synthesized compounds were tested *in vitro* for their antibacterial activity against four microorganisms viz., *Staphylococci* for gram + ve and *E. coli, Entireo cocci* for gram – ve and at 100, 200 and 300 µg/ml concentration using Ciprofloxin as reference standard and antifungal activity against two microorganisms Penicillium and A. niger at 100, 200 and 300 µg/ml concentration, using control as reference.

2.3.1. MATERIALS USED

- ✓ Nutrient Agar.
- ✓ 18 - 24 hr growth culture in nutrient agar.
- ✓ Sterile test tubes.
- ✓ Sterile micropipettes.
- ✓ Sterile cotton swabs.
- ✓ Sterile inoculating needle.
- ✓ Sterile glass rod.

2.3.2. Culture

The media required for the preparation of test organism inoculation are made from the ingredients like agar, peptone, yeast extract, beef extract, dextrose and pancreatic digest of casein i.e., nutrient agar medium, etc. For antibacterial activity and use sabouraud dextrose agar for antifungal activity. [14] Where agar is specified in a formula, use agar that has a moisture content of not more than 15%. Purified water is used. Unless otherwise indicated, the media should be sterilized by heating in an autoclave at 115 °C for 30 min. Dissolve the soluble solids in the water, using heat if necessary, to effect complete solution and add solutions of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the required pH in the medium when it is ready for use.

2.3.3. Nutrient broth

For the preparation of nutrient agar broth for bacteria 5 g of nutrient agar is dissolved in the 100 ml of distilled water and adjust the pH at 7.2±0.2. Pour in test tubes as per requirement and then sterilized by autoclave at 121 °C, 15 lb pressure. For antifungal activity: 3 g Sabouraud dextrose in 100 ml of distilled water.

2.3.4. Preparation of test solution

Each test compound (5 mg) was dissolved in dimethyl sulphoxide (5 ml) to give stock solution of concentration 1000 µg/ml. Then 0.10, 0.20 and 0.30 ml of this solution were used for testing.

2.3.5. Preparation of standard solution

Standard drug, Ciprofloxin was used at the concentration of 100 µg/ml for antibacterial activity.

2.3.6. Protocol for antibacterial activity

The sterilized (autoclaved at 120 °C for 30 min) nutrient agar medium (40-50 °C) was inoculated (1 to 100 ml) with the suspension of microorganisms and mixture was transferred to sterile petri dishes and allowed to solidify.[16] Specified concentration solution of synthesized compounds and standard were placed on surface of the agar plates. DMSO was used as control. The plates were left for 1 hr at room temperature as period of pre incubation diffusion to minimize effects of variations in time between applications of different solutions. The plates were incubated at 37±2 °C for 18 hr and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of standard, the values were tabulated.

2.3.7. Protocol for antifungal activity

The sterilized (autoclaved at 120 °C for 30 min) nutrient agar medium (40-50 °C) was inoculated (1 to 100 ml) with the suspension of different fungi were transferred to sterile petri dishes and allowed to solidify. Specified concentration solution of synthesized compounds and standard were placed on surface of the agar plates. DMSO was used as control. The plates were left for 1 hr at room temperature as period

of pre incubation diffusion to minimize effects of variations in time between applications of different solutions. The plates were incubated at 37±2 °C for 18 hr and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of standard, the values were tabulated.

3. RESULTS AND DISCUSSION

The synthesized compounds were subjected to various antibacterial and antifungal activities by the standard methods. All the compounds were screened for antibacterial activity. However the compounds TDZ1 and TDZ2 have shown promising antibacterial activity, while the remaining compound such as TDZ 3 have also shown moderate antibacterial activity, when compared with the standard drug Ciprofloxacin. All the compounds were also screened for antifungal activity. However compounds TDZ 1 and TDZ 2 have shown promising antifungal activity, while the remaining compound such as TDZ 3 have also shown moderate antifungal activity, when compared with the standard drug Griseofulvin.

Table 1: Physicochemical properties of the synthesized compounds

Compound Name	Molecular Formula	Molecular Weight	Percentage yield	M.P°C	Elemental Analysis Calcd (Found)		
					C	H	N
INTC1	$C_{13}H_{12}N_4O_2S$	288.32	82.14	142-145	54.09 (54.15)	4.16 (4.20)	19.38 (19.43)
INTC2	$C_{13}H_{13}N_5OS$	287.34	87.23	157-159	54.30 (54.34)	4.51 (4.56)	24.30 (24.37)
INTC3	$C_{13}H_{11}BrN_4S$	351.22	55.87	134-136	44.41 (44.46)	3.10 (3.16)	15.89 (15.95)
TDZ1	$C_{13}H_{10}N_4OS$	270.31	62.35	116-118	57.70 (57.76)	3.68 (3.73)	20.68 (20.73)
TDZ2	$C_{13}H_{11}N_5S$	269.32	74.56	125-128	57.91 (57.97)	4.07 (4.12)	25.96 (26.00)
TDZ3	$C_{13}H_9BrN_4S$	333.21	51.27	112-115	46.80 (46.86)	2.67 (2.72)	16.75 (16.81)

Table 2: Interpretation of IR and NMR spectral values

S. No	Compound Name	IR Spectra values cm-1	1HNMR Spectra values (ppm)
1	INTC 1	3521, OH-str; 3327 NH-str, broad; 3086 Ar-CH-str; 1715 C=O-str; 1300, C-N str; 625, C-S str;	9.06 2H, Ar-CH; 8.13 1H, NH; 7.96 2H, Ar-CH; 6.48 2H, Ar-CH; 6.29 2H, Ar-CH; 4.42 1H, NH; 2.23 1H, NH; 5.07 1H, OH.
2	INTC 2	3455, NH_2 str; 3335, NH-str; 3086, Ar-CH-str; 1715, C=O-str; 1300, C- N str; 625, C=S-str.	9.12 2H, Ar-CH; 8.09 1H, NH; 7.96 2H, Ar-CH; 6.21 4H, Ar-CH; 4.24 1H, NH; 2.56 1H, NH; 4.06 2H, NH_2.
3	INTC 3	3288, NH-str; 3086, Ar-CH-str; 1710, C=O-str; 1300, C-Nstr; 651, C=S-str.	9.20 2H, Ar-CH; 8.26 1H, NH; 7.96 2H, Ar-CH; 7.18 2H, Ar-CH; 6.35 2H, Ar-CH; 4.32 1H, NH; 2.30 1H, NH.
4	TDZ 1	3530, OH-str; 3327, NH-str; 3086, Ar-CH-str; 1300, C-N str; 625, C=S-str.	8.56 2H, Ar-CH-str; 7.60 2H, Ar-CH- str; 6.48 2H, Ar-CH-str; 6.29 2H, Ar- CH-str; 5.07 1H, OH; 4.12 1H, NH. m/e : 270.31.
5	TDZ 2	3485, NH_2- str; 3385, NH-str; 3086, Ar-CH-str; 1300, C-Nstr; 729, C=S- str.	8.61 2H, Ar-CH-str; 7.63 2H, Ar-CH- str; 6.21 4H, Ar-CH-str; 5.07 1H, OH; 4.12 1H, NH; 4.06 2H, NH_2. m/e : 269.36.
6	TDZ 3	3251, NH-str; 3086, Ar-CH-str; 1300, C-Nstr; 651, C=S-str.	8.65 2H, Ar-CH-str; 7.60 2H, Ar-CH- str; 7.18 2H, Ar-CH-str; 6.35 2H, Ar- CH-str; 4.15 1H, NH. m/e: 330.21.

Table 3: Antibacterial activity of substituted thiadiazole derivatives against Gram positive bacteria ((TDZ 1-3))

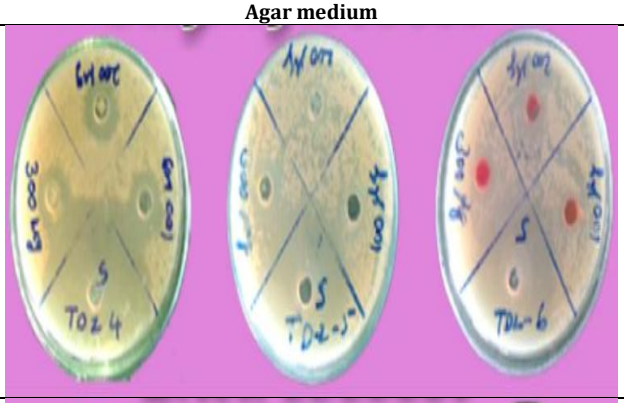
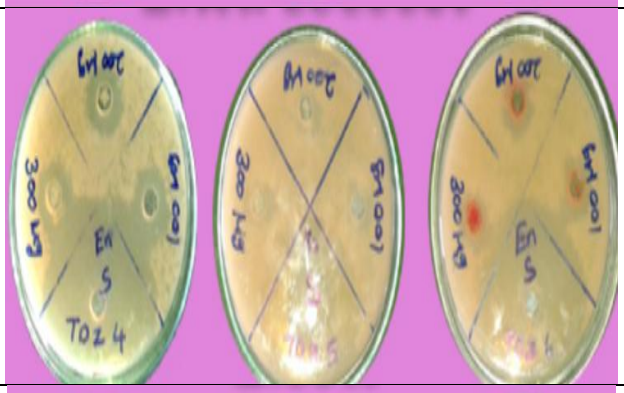
S. No	Name of the compounds	Mean zone of inhibition (in mm)		
		Staphylococcus aureus		
		100 µg	200 µg	300 µg
1	Ciprofloxacin	9	11	12
2	TDZ-1	4	5	6
3	TDZ-2	2	4	5
4	TDZ-3	2	4	5

Table 4: Antibacterial activity of substituted thiadiazole derivatives against gram negative bacteria (TDZ 1-3)

S. No	Name of the compounds	Mean zone of inhibition (in mm)					
		Entireo coli			E. coli		
		100 µg	200 µg	300 µg	100 µg	200 µg	300µg
1	Ciprofloxacin	11	09	10	12	11	09
2	TDZ-1	4	6	8	3	5	7
3	TDZ-2	3	5	6	2	4	5
4	TDZ-3	3	5	6	3	4	5

Table 5: Antifungal activity of substituted thiadiazole derivatives (TDZ 1-3)

S. No	Name of the compounds	Mean zone of inhibition (in mm)					
		Penicillium			A.niger		
		100 µg	200 µg	300 µg	100 µg	200 µg	300µg
1	Griseofulvin	9	11	12	8	9	11
2	TDZ-1	4	5	6	3	2	4
3	TDZ-2	2	3	5	2	3	4
4	TDZ-3	2	3	4	1	3	4

Activity	Agar medium	Description
Antibacterial activity against *Staphylococci.*		**Compound**: TDZ-1, TDZ-2, TDZ-3. **Concentration** : 100 µg/ml , 200 µg/ml, 300 µg/ml. **Control** : Ciprofloxacin, 100 µg/ml
Antibacterial activity against *Entireococci*		**Compound**: TDZ-1, TDZ-2, TDZ-3. **Concentration**: 100 µg/ml , 200 µg/ml, 300 µg/ml. **Control** : Ciprofloxacin, 100 µg/ml.
Antibacterial activity against *E.coli*		**Compound**: TDZ-1, TDZ-2, TDZ-3. **Concentration**: 100 µg/ml , 200 µg/ml, 300 µg/ml. **Control** : Ciprofloxacin, 100 µg/ml.

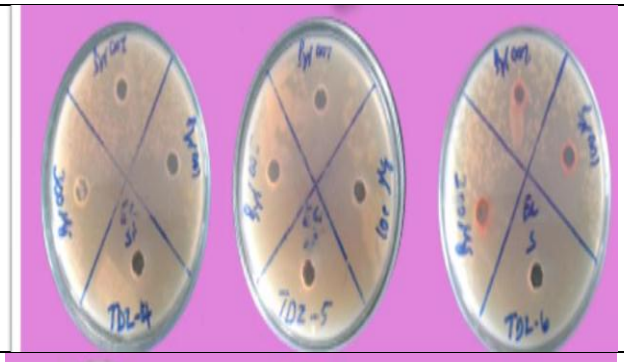
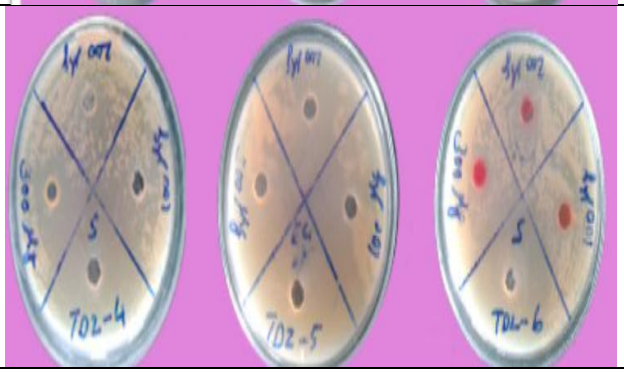
Antifungal activity against *A. niger*		**Compound**: TDZ-1, TDZ-2, TDZ-3. **Concentration** : 100, 200 and 300 µg/ml **Control:** Griseofulvin, 100 µg/ml
Antifungal activity against *Penicillium.*		**Compound:** TDZ-1, TDZ-2, TDZ-3. **Concentration** : 100, 200 and 300 µg/ml **Control:** Griseofulvin, 100 µg/ml

Fig. 1-5: Antibacterial activity of synthesized thiadiazole derivatives

4. CONCLUSION

There are millions of patients worldwide who are sufferings from microbial infections and most of them die because of this so there must an antimicrobial therapy to effectively control such fatal infections. In this connection, a series of novel 1, 3, 4-thiadiazole derivatives were synthesized and the structures of the entire compounds were confirmed by recording by their IR, 1H NMR and Mass spectra. In conclusion, we feel that the preliminary *in vitro* activity results of this class of compounds may possess potential for design of future molecules with modifications on the aryl substituent's as well as pyridine moiety. All the synthesized compounds showed moderate activity against bacteria and fungi. In particular, the compound TDZ1 and TDZ 2 showed good anti-bacterial as well as antifungal activities, while other compound such as TDZ 3 shows moderate activity. Therefore, the detailed literature survey and screening studies have demonstrated that the newly synthesized compounds exhibit promising antibacterial and antifungal properties. Hence, it is concluded that there exists ample scope for the medicinal chemists to further study in this class of compounds and used for the treatment of various types of deadly microbial infections.

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