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Antimicrobial Potential of Some Novel N-aryldithiocarbamate Based 1,3,4-Oxadiazoles

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Abstract: *The need of newer antimicrobial agents due to rapid emergence of antimicrobial resistance and importance of 1,3,4-oxadiazoles as promising antimicrobial agents prompted us to synthesize a novel series of 1,3,4-oxadiazole analogues for their antimicrobial activity. The structures of the compounds were confirmed by elemental analysis, IR and ^{1}H-NMR spectral data. The disk diffusion method was employed for antimicrobial evaluation. The synthesized compounds were tested for their in-vitro antimicrobial activity against the Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, the Gram negative bacteria Proteus mirabilis and Pseudomonas aeruginosa, the fungal strain Aspergillus niger and the yeast like pathogenic fungus Candida albicans. Some of the compounds demonstrated marked antibacterial and antifungal activities. Structure activity relationship among the synthesized oxadiazoles was also established.*

Keywords: *1,3,4-oxadiazole, Antimicrobial, Antifungal, Antibacterial, Synthesis.*

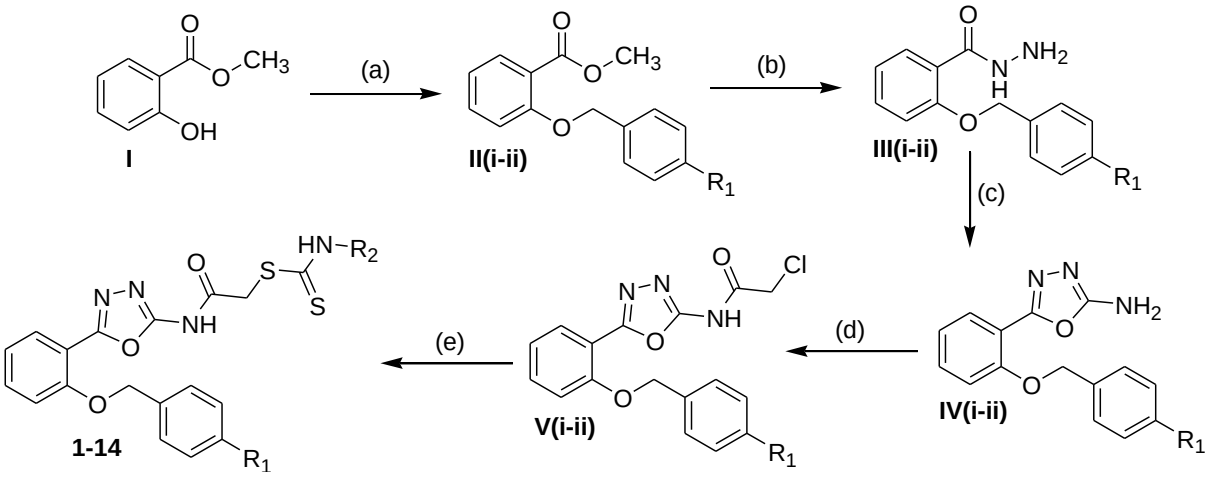
1. INTRODUCTION

Antimicrobial resistance threatens the effectual prevention and cure of a constantly increasing range of infections generally caused by bacteria, viruses and fungi. It is an increasingly serious challenge to global public health requiring urgent action across all government sectors and society. The appearance of resistant strains is a natural incident that takes place when microorganisms replicate themselves inaccurately or when resistant traits are exchanged between them. The use and misuse of antimicrobial drugs has resulted in the speed up of emergence of drug-resistant strains [1]. A number of researchers have reported antimicrobial activities in 1,3,4-oxadiazoles [2-5]. In view of these findings, it was therefore, considered worthwhile to synthesize novel 1,3,4-oxadiazole analogues for their antimicrobial activity.

2. MATERIALS AND METHODS

2.1 Synthesis

All the chemicals were of E-Merck, Aldrich and Himedia. Melting points were determined by open capillary method and are uncorrected. Melting points were determined by open capillary method and are uncorrected. Elemental analysis was done using an elemental analyzer Heraeus Carlo Erba-1108, IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr disc), ^{1}H-NMR spectra on a Bruker DRX-300 NMR spectrometer (DMSO-d_6, TMS) and the electrospray mass spectra on a Micromass Quattro II triple-quadrupole mass spectrometer (Methanol).



Compound Code	R^1	R^2	Compound Code	R^1	R^2
1	H	2-CH_3-C_6H_4	8	Cl	2-CH_3-C_6H_4
2	H	3-CH_3-C_6H_4	9	Cl	3-CH_3-C_6H_4
3	H	4-CH_3-C_6H_4-	10	Cl	4-CH_3-C_6H_4
4	H	4-OCH_3-C_6H_4	11	Cl	4-OCH_3-C_6H_4
5	H	4-OC_2H_5-C_6H_4	12	Cl	4-OC_2H_5-C_6H_4
6	H	4-Cl-C_6H_4	13	Cl	4-Cl-C_6H_4
7	H	2,3-(CH_3)-C_6H_4	14	Cl	2,3-(CH_3)-C_6H_4

Reaction conditions: (i) Benzyl chloride, KOH 10%; CH_3OH, rt, 6-7 h; (ii) $NH_2NH_2.H_2O$; CH_3OH, rt, 5-6 h; (iii) BrCN, $NaHCO_3$; CH_3OH, rt, 3-4 h; (iv) $ClCH_2COCl$, dry benzene, reflux 3-5 h; (v) $NH_4SCSNHR$, acetone, rt, stirring, reflux 2-4 h.

Scheme 1: Synthesis of 1,3,4-oxadiazole analogues

The designed compounds were synthesized according to scheme 1. The methylsalicylate **(I)** was reacted with appropriate benzyl chloride in the presence of alkaline hydromethanolic solution yielded corresponding benzoic acid methyl ester [**II(i-iii)]**. The benzoic acid hydrazides [**III(i-iii)]** were synthesized by reaction of **II** with hydrazine hydrate in methanol. The hydrazides **III** were reacted with cyanogens bromide in methanol to yield 2-amino-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles [**IV(i-iii)]** [6]. Chlroacetamido-1,3,4-oxadiazole derivatives [**V(i-iii)]** were prepared by reaction of [**IV(i-iii)]** with chloroacetyl chloride in the present of benzene under reflux for 3 to 5 h. In the last step, equimolar quantity of **[V(i-iii)]** and ammonium aryldithiocarbamate in dry acetone was stirred for half an h followed by reflux for 2 to 4 h. The excess of solvent was removed under reduced pressure and crude product was recrystallized with ethanol to get the title compounds **1-14.**

2.2 Antimicrobial Evaluation

2.2.1 Experimental Conditions

The synthesized compounds were tested for their *in-vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC-619), the Gram negative bacteria *Proteus mirabilis* (MTCC-425) and *Pseudomonas aeruginosa* (MTCC-424), the fungal strain *Aspergillus niger* (MTCC-1344) and the yeast like pathogenic fungus *Candida albicans* (MTCC-227) using disk diffusion method.[7-8] Norfloxacin and Clotrimazole were used as standard drug for antibacterial and antifungal studies, respectively. Nutrient Agar[9], (beef extract 1 g, Yeast extract 2 g, peptone 5 g, sodium chloride 5 g, Agar 15 g and distilled water q. s. to 1,000 ml) was employed as culture media for antibacterial studies. For antimycotic evaluation against *Aspergillus niger*, czapek yeast extract agar[9], (czapek concentrate 10 ml, K_2HPO_4 1 g, yeast extract 5 g, sucrose 30 g, Agar 15 g and distilled water q. s. to 1,000 ml, where czapek concentrate comprises of $NaNO_3$ 30 g, KCl 5 g, $MgSO_4.7H_2O$ 5 g, Fe SO_4 $.7H_2O$ 0.1 g and distilled water q. s. to 1,000 ml) was employed. Malt yeast Agar[9] (Malt extract 3 g, yeast extract 3 g, Peptone 5 g, glucose 10 g, Agar 20 g and distilled water q. s. to 1,000 ml) with pH 7.0 was employed as culture media in antimicrobial studies against *Candida albicans*. The sterilization of the culture medias, Petri dishes and other

glassware's was done by autoclaving at 15 1b/sq inch pressure for 30 min. For antibacterial studies, incubation was carried out at 37±1ºC for 48 h except for *Bacillus subtilis* where incubation was carried out at 26±1ºC for similar time period. Incubation conditions for *Aspergillus niger* and *Candida albicans* was 25±1ºC for 72 h. All the tests were performed in triplicate (Table 1).

Table1. *Zone of inhibition[#] of synthesized compounds by disk-diffusion method*

Compo-und No.	Zone of inhibition in mm using disk-diffusion method (100 μg/ 8mm disk)					
	Antibacterial activity				Antifungal activity	
	S. aureus **(MTCC-96)**	***B. subtilis*** **(MTCC-619)**	***P. mirabilis*** **(MTCC-425)**	***P. aeruginosa*** **(MTCC-424)**	***A. niger*** **(MTCC-1344)**	***C. albicans (MTCC-227)***
1	14	12	13	12	12	13
2	13	13	12	12	14	14
3	14	12	14	13	13	13
4	15	14	13	13	14	14
5	14	13	12	13	13	14
6	15	14	13	14	14	14
7	14	12	12	12	13	13
8	16	14	14	14	14	15
9	15	14	14	14	13	14
1	16	15	14	15	14	15
1	18	16	15	16	15	16
1	17	16	15	15	15	16
1	18	17	16	16	15	17
1	15	13	13	15	13	14
Norflo-Xacin	24	21	22	20	NT	NT
Clotri-Mazole	NT	NT	NT	NT	21	23

[#]Microbial strains were procured from Institute of Microbial Technology (IMTECH) Chandigarh, India.

NT = Not tested.

2.2.2 Disk Diffusion Method

The cell density of each inoculum was adjusted with hemocytometer in order to procure a final concentration of approximately 10^5 CFU ml^{-1}. During antimicrobial evaluation the medium after sterilization was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plate solidified, 0.5 ml of (10^5 CFU ml^{-1}) culture of test organism was inoculated and uniformly spread over the agar surface using a sterile L-shaped glass rod. Solutions of the test compound (100 μg/ml) were prepared by dissolving the test compound in dimethyl formamide (DMF). The sterile filter paper disc (8 mm diameter) were moistened with the test compounds solution in DMF of specific concentration (100 μg/disc) placed on the agar culture plates that had been previously inoculated with specific microorganisms. Controls were maintained with DMF and standard drug chosen in the respective case. Inhibition zones were measured and the diameter was calculated in millimeters.

3. RESULT AND DISCUSSION

The results of elemental analysis were in conformity with the structure of the compounds. IR data also confirms the presence of specific functional groups present in the synthesized compounds. The ^{1}H-NMR spectra and mass spectra of the compounds were in conformity with the assigned structure.

The antimicrobial potential of the synthesized compounds was evaluated by disk diffusion method. All the compounds exhibited significant antibacterial and moderate antifungal activities. Out of all the fourteen compounds evaluated for antimicrobial studies, compound **13** showed significant antibacterial activity against all six microbial strains used (zone of inhibition in disk diffusion method: 18 mm against *Staphylococcus aureus*, 17 mm against *Bacillus subtilis*, 16 mm against *Proteus mirabilis*, 16 mm against *Pseudomonas aeroginosa*, 15 mm against *Aspergillus niger* and 17 mm against *Candida albicans*). The compound **7** showed weakest antimicrobial activity among all the synthesized compounds. Other compounds with moderate activity were **4, 6, 8, 9, 10, 11, 12** and **14**.

The most active compound **13** possess R_1 = Cl and R_2 = 4-chlorophenyl substitution. On comparing the structure of title compounds with their biological activity, it was found that chloro substituted compounds (R_1 = Cl) were more active as compared to unsubstituted compounds (R_1 = H). While on considering the effect of R_2 substitution, it was found that biological activity varies considerably on changing the substitution in the following manner: 4-chlorophenyl > 4-methoxyphenyl > 4-ethoxyphenyl > 4-methylphenyl > 2-methylphenyl > 3-methylphenyl > 2,3-dimethylphenyl.

4. CONCLUSION

A novel series of 1,3,4-oxadiazole analogues were synthesized and their antimicrobial activities were evaluated using disk diffusion method. All the compounds were found to possess a broad spectrum of antimicrobial activities, especially compound **13** have shown significant activities against all the pathogenic microorganisms tested including *Pseudomonas aeruginosa* and *Candida albicans* responsible for nosocomial infection. Synthesized oxadiazole analogues exhibited more pronounced inhibitory activity against Gram-positive bacteria than Gram-negative bacteria. Our research work confirms the potential of 1,3,4-oxadiazole as lead for development of novel and better antimicrobial agent.

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