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SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTION OF SOME NOVEL DERIVATIVES OF ISOBUTYLPHENYL-OXADIAZOLE

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ABSTRACT

In this work we synthesized new isobutylphenyl-linked oxadiazole derivatives and evaluated the antimicrobial action of the synthesized molecules. The anti-microbial activity of the compounds was assessed against gram positive and gram negative bacteria and fungus. The zone of inhibition of each compounds was measured it was found that the compounds exhibited poor activity against gram negative bacteria compared to gram positive. The zone of inhibition suggested that compounds 5b and 5d were more potent in preventing microbial growth compared to the other compounds. The results of MIC study were similar to zone of inhibition and it was found that 5b had IC_{50} of 25µg/mL against all the tested microbes and 5d has IC_{50} of 50µg/mL against the tested microbes. The studies led to the conclusion that the synthesized compounds were having medium antimicrobial activity and the presence of electron withdrawing groups in the phenyl ring was beneficial for activity. A *meta* substituted electron withdrawing group was unable to present antimicrobial action (5c) and on the other hand an electron donating on *ortho* position (5e) was having better activity then the meta substituted compound with electron withdrawing group.

Keywords: Isobutylphenyl, oxadiazole, antifungal, antibacterial, electron donating

INTRODUCTION

Oxadiazoles are five membered heterocyclic ring systems bearing two carbons, two nitrogen and an oxygen atom **[1]**. This moiety is well known to demonstrate a wide spectrum of pharmacological activities. This array includes antitubercular **[2]**, analgesic, anti-

inflammatory **[3]**, antimicrobial **[4]**, antimalarial, anti-oxidant **[5]**, anticancer **[6]**, antiviral and many more **[7]**. Oxadiazoles have been known to exert a good pharmacological potential with a significant number of clinically approved molecules **[8-12]**. Several prominent reports on antimicrobial property of oxadiazole based compounds has been found in literature. Isobutylphenyl-propionic acid exerts antimicrobial as well as anti-inflammatory actions, though its anti-inflammatory potential is clinically utilized. Hence in the present work we attempted to conjugate the isobutylphenyl-propionic acid as well as the oxadiazole nucleus in one compound with an objective to achieve potent antimicrobial compound.

MATERIAL AND METHODS

Benzoic acid derivatives were purchased from Avra Chemicals, hydrazine hydrate and 2-(4-isobutylphenyl) propanoic acid was purchased from Loba Chemie. Other chemicals and reagents were purchased from Final and Rankem and used as procured.

The scheme for the synthesis of the oxadiazole derivatives was adapted from the procedures reported by Mishra *et al* **[13]** and the scheme is depicted in **Figure 1**.

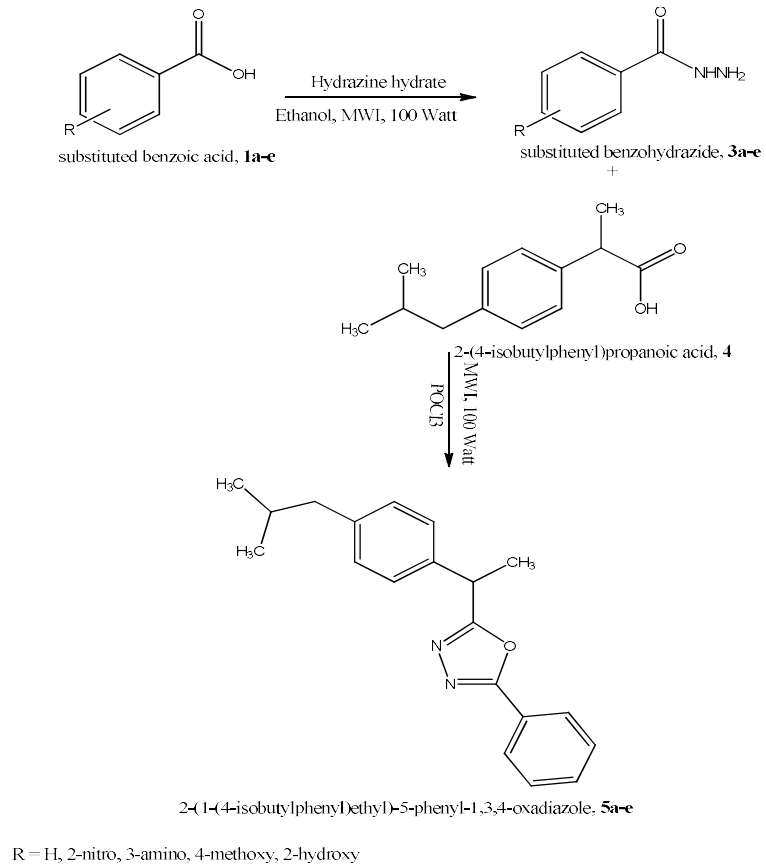


Figure 1: Synthetic pathway for desired oxadiazole derivatives

General Method for synthesis of substituted benzohydrazide

0.1 moles of subsituted benzoic acid, **1a-e** was dissolved in 25 ml ethanol and and 5 drops of concentrated H_2SO_4 was added to the mixture. This mixture was irradiated using 100 Watt power in microwave under reflux for 3 minutes. On cooling, a solid separated which was filtered and recrystallised using ethanol to give the carboethyoxy derivative of the benzoic acid, **2a-e**. Completion of the reaction was monitored by TLC.

The hydrazide derivative of the substituted benzoic acids was synthesized by the reaction of **2a-e** by hydrazine hydrate, **2** in presence of ethanol with catalytic amount of concentrated sulfuric acid. To 0.001 mole of product **2a-e** dissolved in 20 ml ethanol, 0.0015 mole of hydrazine hydrate was added. To the mixture, catalytic amount of concentrated sulfuric acid was added. The mixture was irradiated using 100 Watt power in microwave under reflux. On cooling, a solid separated, which was recrystallized from ethanol to give the product **3a-e**.

General method for synthesis of 2-(4-isobutylphenyl)propanoic acid linked oxadiazole derivative

Product **3a-e** (0.001 mol) and the 2-(4-isobutylphenyl)propanoic acid (0.001 mol) were dissolved in phosphorus oxychloride and irradiated using 100 Watt power in microwave under reflux for 15 minutes. The reaction mixture was slowly poured over crushed ice and allowed to stand overnight. The solid that precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **5a-e.**

Characterization of synthesized compounds

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR).

Evaluation of Antimicrobial Activity

Escherichia coli, *Staphylococcus aureus*, *Aspergillus niger* were used for the present investigation and were purchased as MTCC lyophilized strains. The lyophilized cultures were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. Revival of the fungal culture was done using 0.3 mL of water.

Nutrient agar plates or potato dextrose agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labeled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening.

Determination of zone of inhibition

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method **[14]**. All the synthesized compounds were dissolved in DMSO to obtain 1mg/mL, 1.5 mg/mL and 2mg/mL solutions of each. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the compounds were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters and the average diameter of the zone of inhibitions was calculated. The activity index was calculated by subtracting the diameter of the well from the diameter of the zone of inhibition and dividing the result by the diameter of the well.

The IC_{50} of the synthesized compounds was established by broth dilution method, measuring the optical density (absorbance) of each bacterial broth dilution at 640 nm using UV-Visible spectrophotometer **[15]**.

Results and Discussion

A total of five compounds were synthesized using the synthetic pathway and the compounds were coded as **5a-e** according to the synthetic pathway in **Figure 1**. The retention factor (R_f) of each compound on silica gel TLC plates was calculated, solubility in various solvents observed, yield (%) and melting point (°C) of the compounds was determined. The physicochemical properties of **5a-e** are presented in **Table 1**.

The ^{1}H-NMR, mass and FT-IR spectra of the synthesized compounds 5a-e were obtained from NIPER-Hyderabad and the spectra were observed for chemical shifts of protons, molecular ion peak or fragment peaks as well as the stretching and bending vibrations that occur due to the presence of various functional group in the compounds **(Table 2)**.

Antimicrobial study

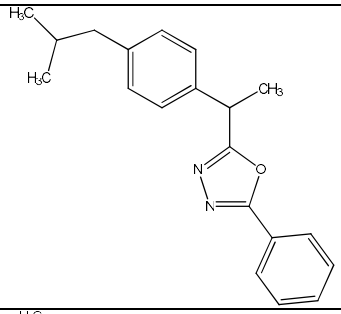
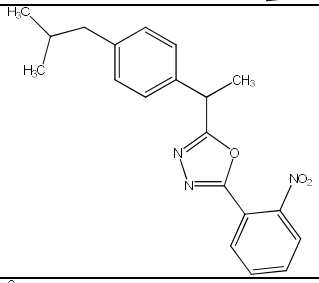
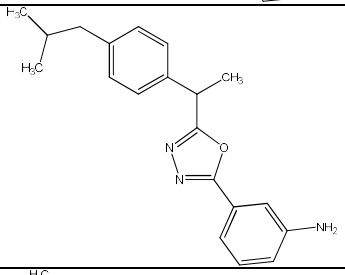
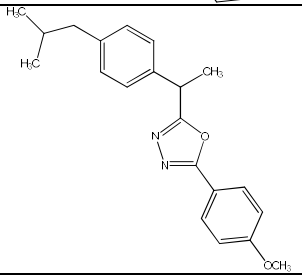
The zone of inhibition against growth of microbe was taken as the primary indicator to assess the anti-microbial activity of the synthesized derivatives. Four concentrations of the conjugates were tested for antibacterial action **(Figure 2)**.

The concentration of drug required to inhibit the growth of 50% microbes was determined using serial dilution method. The optical density of each broth was measured at 640 nm to determine the MIC value of the compounds **(Table 3)**.

The IR spectra were analyzed to identify the unique peaks associated with the presence of specific functional groups. All of the compounds displayed peaks corresponding to

the stretching of aromatic C=C bonds, C-H bonds, C=N bonds, and C-O bonds. The compounds 5e and 5c exhibited absorption bands corresponding to O-H and N-H, respectively. The ^{1}HNMR spectra of all the substances displayed chemical shifts of aromatic and aliphatic hydrogen. Additionally, they displayed any prominent peak that could occur as a result of protons from functional groups such as -OH and NH_2. The compounds' mass spectra were analyzed to determine the presence of a molecular ion peak or isotopic peaks, which would validate the production of the compounds.

Table 1: Physicochemical properties of 5a-e

Compound code	Structure	% Yield	Rf value	Melting Range (°C)	Solubility
5a		**71**	**0.63**	**138-140**	**Soluble in methanol and chloroform**
5b		**75**	**0.58**	**159-162**	
5c		**74**	**0.47**	**164-168**	
5d		**67**	**0.49**	**145-147**	

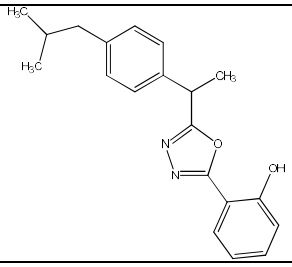
5e		**72**	**0.38**	**128-130**	

Table 2: Spectral features of 5a-e

Compound	^{1}HNMR chemical shift (δ, ppm)	FT-IR (cm^{-1})
5a	**6.99-8.35 (CH, ar); 4.33 (oxadiazole CH); 4.16 (NH); 2.47 (methylene); 1.85 (CH isobutyl); 1.72 (CH_3 adj oxadiazole); 0.87 (CH_3, isobutyl)**	**1639.00 (C=N); 3228.13 (C-H); 1289.63 (C-O); 1456.90 (C=C)**
5b	**6.99-8.31 (CH, ar); 4.33 (oxadiazole CH); 2.47 (methylene); 1.85 (CH isobutyl); 1.72 (CH_3 adj oxadiazole); 0.87 (CH_3, isobutyl)**	**1639.54 (C=N); 3100.40 (C-H); 1289.17 (C-O); 1456.90 (C=C)**
5c	**6.99-8.35 (CH, ar); 4.33 (oxadiazole CH); 2.47 (methylene); 1.85 (CH isobutyl); 1.72 (CH_3 adj oxadiazole); 0.87 (CH_3, isobutyl)**	**1653.56 (C=N); 3107.54 (C-H); 1288.54 (C-O); 1477.79 (C=C); 3554.99 (N-H)**
5d	**6.99-8.35 (CH, ar); 4.33 (oxadiazole CH); 3.80 (methoxy); 2.47 (methylene); 1.85 (CH isobutyl); 1.72 (CH_3 adj oxadiazole); 0.87 (CH_3, isobutyl)**	**1610.83 (C=N); 3042.73 (C-H); 1267.37 (C-O); 1510.55 (C=C)**
5e	**6.99-8.35 (CH, ar); 4.33 (oxadiazole CH); 2.47 (methylene); 1.85 (CH isobutyl); 1.72 (CH_3 adj oxadiazole); 0.87 (CH_3, isobutyl)**	**1652.12 (C=N); 3112.55 (C-H); 1287.91 (C-O); 1481.33 (C=C); 3733.71 (O-H)**

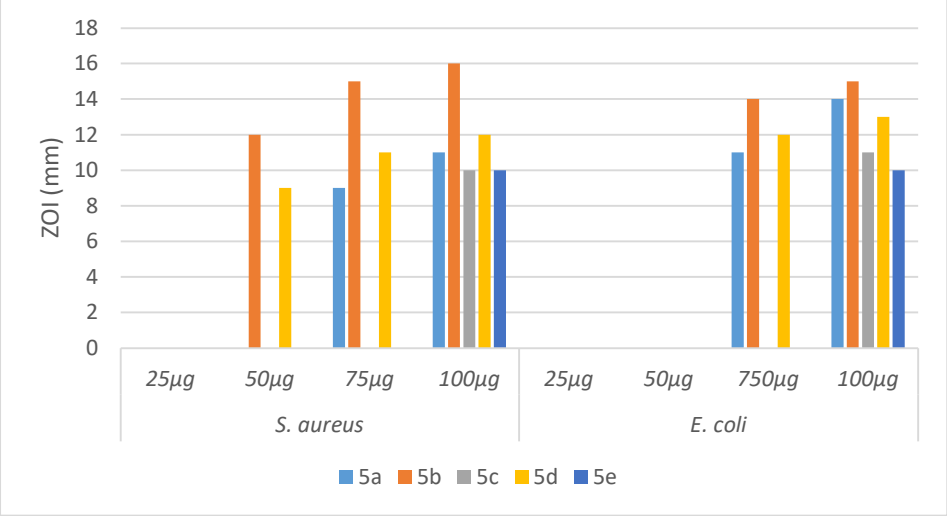


Figure 2: Graphical representation of inhibition zones

Table 3: Optical density of 5a-e against tested microbes

Concentration (µg/ml)	Optical Density at 640 nm														
	E. coli					*S. aureus*					*A. niger*				
	5a	5b	5c	5d	5e	5a	5b	5c	5d	5e	5a	5b	5c	5d	5e
100	**0.621**	**0.463**	**0.711**	**0.471**	**0.701**	**0.623**	**0.353**	**0.713**	**0.36**	**0.703**	**0.609**	**0.532**	**0.699**	**0.538**	**0.689**
50	**0.659**	**0.496**	**0.749**	**0.506**	**0.739**	**0.655**	**0.387**	**0.745**	**0.394**	**0.735**	**0.647**	**0.581**	**0.737**	**0.587**	**0.727**
25	**0.692**	**0.546**	**0.781**	**0.558**	**0.771**	**0.687**	**0.455**	**0.782**	**0.462**	**0.772**	**0.678**	**0.636**	**0.768**	**0.642**	**0.758**
12.5	**0.721**	**0.613**	**0.811**	**0.627**	**0.801**	**0.701**	**0.493**	**0.815**	**0.5**	**0.805**	**0.703**	**0.678**	**0.793**	**0.684**	**0.783**
6.25	**0.778**	**0.649**	**0.868**	**0.659**	**0.858**	**0.743**	**0.525**	**0.855**	**0.532**	**0.845**	**0.756**	**0.708**	**0.841**	**0.716**	**0.831**
3.125	**0.835**	**0.697**	**0.916**	**0.707**	**0.906**	**0.811**	**0.587**	**0.894**	**0.594**	**0.884**	**0.801**	**0.764**	**0.898**	**0.77**	**0.888**
1.5625	**0.896**	**0.748**	**0.975**	**0.755**	**0.965**	**0.867**	**0.634**	**0.929**	**0.641**	**0.919**	**0.844**	**0.801**	**0.937**	**0.807**	**0.927**
Control	**1.025**	**1.025**	**1.025**	**1.025**	**1.025**	**0.964**	**0.964**	**0.964**	**0.964**	**0.964**	**1.091**	**1.091**	**1.091**	**1.091**	**1.091**

The anti-microbial activity of the compounds was assessed against gram positive and gram negative bacteria and fungus. The zone of inhibition of each compounds was measured it was found that the compounds exhibited poor activity against gram negative bacteria compared to gram positive. The zone of inhibition suggested that compounds **5b** and **5d** were more potent in preventing microbial growth compared to the other compounds. The results of MIC study were similar to zone of inhibition and it was found that **5b** had IC_{50} of 25µg/mL against all the tested microbes and **5d** has IC_{50} of 50µg/mL against the tested microbes.

The studies led to the conclusion that the synthesized compounds were having medium antimicrobial activity and the presence of electron withdrawing groups in the phenyl ring was beneficial for activity. On the other hand the presence of electron donating groups (5c and 5e) resulted in poor activity. The position of substitution was also found to be affecting the activity of compounds. A *meta* substituted electron withdrawing group was unable to present antimicrobial action (**5c**) and on the other hand an electron donating on *ortho* position (**5e**) was having better activity then the meta substituted compound with electron withdrawing group.

CONCLUSION

Oxadiazole nuclues has been widely associated with antimicrobial action while the isobutylphenyl propionic acid has been linked to antimicrobial as well as anti-inflammatory actions. The objective of this work was to synthesize new isobutylphenyl-linked oxadiazole derivatives and evaluate the antimicrobial action of the synthesized molecules. The results of the study led to the conclusion that novel isobutylphenyl-linked oxadiazole compounds were having moderated antimicrobial activity and could be optimized to obtain new lead molecules using computer aided drug design techniques.

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