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Synthesis and Antimicrobial Screening of Some Substituted 1,3,4-Oxadiazole Derivatives

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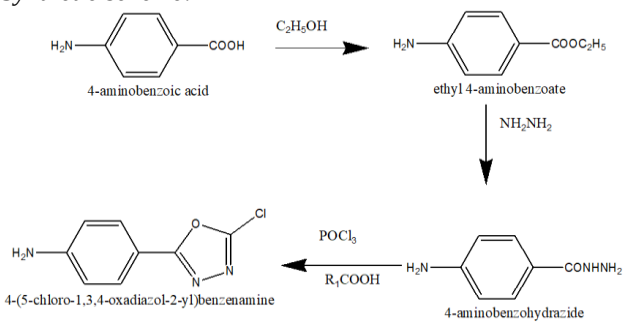
***Abstract*: The 1,3,4-oxadiazole derivatives are aromatic heterocycles with low lipophilicity, which are useful in medication development. By replacing at the 2- and 5-positions, the heterocycle's electrical and hydrogen bond-accepting properties can be altered, allowing it to be used as a carbonyl bioisotere. The 1,3,4-oxadiazole derivative [4-(5chloro-1,3,4-oxadiazole-2-yl) benzenamine is formed when para-aminobenzoic acid is combined with ethyl alcohol to make para-aminoethylbenzoate, which then combines with hydrazine to form para-aminoethylbenzoate]. The structure of freshly synthesized compounds was determined using the IR method. Furthermore, for antibacterial and antifungal activity against all of the selected microbial strains, these compounds were compared to ciprofloxacin and ketoconazole, which served as the gold standard.**

***Keywords*: IR–investigations, antibacterial and antifungal activities, 4-(5-chloro-1,3,4-oxadiazole-2-yl) benzenamine.**

1. Introduction

Oxadiazoles are heterocyclic compounds with one oxygen and two nitrogen atoms in a five-membered ring that have a variety of biological effects [1, 2]. Oxadiazole is thought to be the consequence of replacing two methane (–CH=) groups with two pyridine type nitrogen atoms (–N=) in furan [2]. For the synthesis of 1,3,4-oxadiazoles, several techniques have been documented in the literature. Acid hydrazides (or hydrazine) react with acid chlorides/carboxylic acids in the most popular synthesis pathway for 1,3,4-oxadiazoles. Anticancer, antibacterial, antifungal, analgesic, anti-inflammatory, anticonvulsant, antihypertensive, antiviral, anti-HIV, and antidiabetic effects have been discovered in compounds containing the 1,3,4-oxadiazole moiety. In pharmaceutical chemistry, 1,3,4-oxadiazoles have gained popularity as substitutes for carboxylic acids, esters, and carboxamides [4]. Oxadiazoles have a high biological potential due to their unique structure, which has made them crucial for molecular designing. The number of scientific investigations including these chemicals has risen dramatically in recent years. We have selected to examine the primary synthetic techniques utilized for synthesizing the oxadiazole moiety, as well as the vast spectrum of pharmacological actions documented in the literature, due to the importance of these molecules to both heterocyclic and medicinal chemistry. One of the most pressing issues in modern medicine is antimicrobial resistance (AMR). Some microbes have become resistant to currently used antibiotics due to poor infection management, over-prescription of antibiotics, and inappropriate use by patients. This complicates treatment since previously used antibiotics or antimicrobial medicines are no longer effective, and infections become increasingly difficult to treat [5, 6]. The expense of care for patients with drug-resistant infections rises without appropriate antibiotic therapy, and there is a significant risk during surgery and other medical operations [7, 8]. Researchers all across the world are developing novel chemicals to prevent the spread of resistance. Most newly produced compounds have a heterocyclic moiety, and those with the 1,3,4-oxadiazole ring make up a major category of antibacterial derivatives. The newly synthesized 4-(5chloro-1,3,4-oxadiazole-2-yl) benzenamine was tested against a standard for antibacterial and antifungal activities.

Synthetic scheme:



2. Methods

1. 4-(5 Chloro-1,3,4-oxadiazole-2-yl) benzenamine Synthesis.
2. The produced compound's infrared spectral analysis.
3. The disc diffusion method was used to assess antifungal and antibacterial activity.
4. 4-(5 Chloro-1,3,4-oxadiazole-2-yl)benzenamine synthesis.

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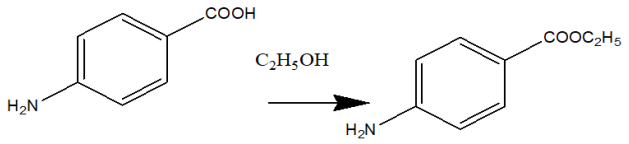
Step 1: Aromatic acid esterification:

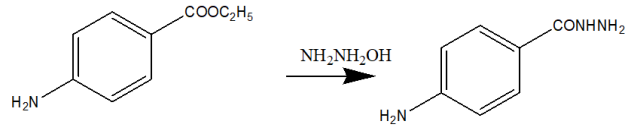
In a round bottom flask, 4-aminobenzoic acid (0.01mol), ethyl alcohol (60ml), and concentrated sulfuric acid (1.5ml) were combined and refluxed for 4 hours. The contents were then cooled and put into a beaker containing crushed ice. The product had been precipitated. From ethanol, it was filtered, dried, and recrystallized. Thin layer chromatography and a chemical test were used to monitor the reaction's completion.



4-aminobenzoic acid ethyl 4-aminobenzoate

Step 2: Acid Hydrazide Preparation:

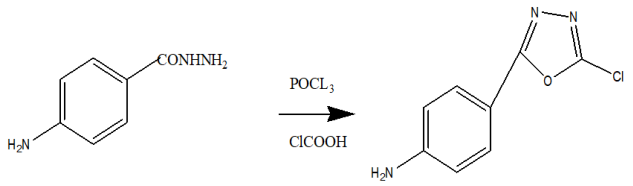
The 4-amino ethyl benzoate (1) (0.01 mol), hydrazine hydrate (0.15 mol), and 30ml of ethanol were refluxed for 4 hours, with the excess ethanol drained off and the contents placed into a beaker and cooled in an ice bath, where the hydrazides separated. From ethanol, the resultant solid was filtered, dried, and recrystallized [9,10].



ethyl 4-aminobenzoate 4-aminobenzohydrazide

Step 3: 2,5-disubstituted-1,3,4-oxadiazoles preparation:

The 4-amino benzo hydrazide (0.01 mol) and a suitable aromatic acid (0.01 mol) were refluxed for 8 hours in POCl3 (5 ml), cooled, and poured over crushed ice before being neutralised with sodium bicarbonate solution. Filtered, dried, and recrystallized from ethanol, the precipitate was obtained. Thin layer chromatography was used to monitor the reaction's conclusion. [11]



4-aminobenzohydrazide 4-(5-chloro-1,3,4-oxadiazole-yl)benzenamine

Synthesized Compound Infrared Spectral Analysis:

Infrared is a useful instrument in chemistry and allied fields (inorganic and organic chemistry, as well as pharmaceuticals) [12, 13]. Studies revealing the lack and presence of functional groups in ligands, as well as evidence of coordination in the proposed compounds [14], have reported on its relevance. Infrared can be used to distinguish between hydroxyl and amine groups from spectra [15], to determine the presence of water in the absence of a thermal analyzer [16], and to determine purity [17,18] in the absence of an elemental analyzer.

The disc diffusion method was used to evaluate antifungal and antibacterial activities.

Antibacterial activity of synthesized compounds:

The antibacterial activity of synthesized compounds was screened using the disc diffusion method against gramme positive Bacillus cereus and gramme negative Pseudomonas aeruginosa in Muller Hinton agar medium at concentrations of 50, 100, and 150 g/ml in dimethyl formamide.

Beef extract 10.0g Muller Hinton Agar composition preparation.

17.5 g casein acid hydrolysis
1.5 g starch
20.0 g agar
1000 mL distilled water

All of the materials are placed in a conical flask with 1000 mL of distilled water and heated in a steam bath to dissolve. The pH was adjusted to 7.0 0.2 and sterilized for 15 minutes in an autoclave at 15 lb at 1200C. The sterile medium was placed into Petridishes and allowed to set before being used.

The Disk's Preparation:

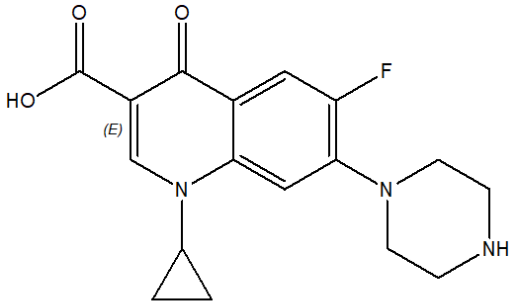
Autoclaving at 1210C for 15 minutes sterilised a paper disc with a diameter of 5 mm and a thickness of 2 mm. For the comparison of antibacterial activity of the produced compounds, ciprofloxacin (10 g/ml) was utilised as a reference antibiotic.

Organism that was used:

Bacillus cereus ATCC 11778 Bacillus cereus ATCC 11778 Bacillus cereus ATCC 11778 Bacillus cereus ATCC

Pseudomonus aeruginosa ATCC 9027 Pseudomonus aeruginosa ATCC 9027 Pseudomonus aeruginosa ATCC 9027.

Standard drug:



Ciprofloxacin

Antibacterial activity procedure:

At 45 degrees Celsius, a suspension of the organism was put to sterile muller hinton agar medium. The liquid was poured onto sterile petridishes and allowed to set. A sterile disc with a diameter of 5mm was dipped in a solution containing various concentrations of test compounds, as well as a standard and control substance, and then placed on the surface of agar plates.

To reduce the impact of variations in time between the applications of the different solutions, the plates were pre-incubated for 1 hour at room temperature. The plates were then incubated for 24 hours at 37°C 1°C to test for antibacterial activity. The size of the inhibitory zone was measured and documented.

Synthesized compound antifungal activity:

Organism that was used:

ATCC 46645 Aspergillus fumigates

The antifungal activity of produced compounds was tested against Aspergillus fumigates in dimethyl formamide at concentrations of 50, 100, and 150 g/ml. The antifungal activity was determined by measuring the zone of inhibition in millimetres; more information on the process may be found below.

Sabouraud's Agar Media Composition Preparation

20 g dextrose

10 g peptone

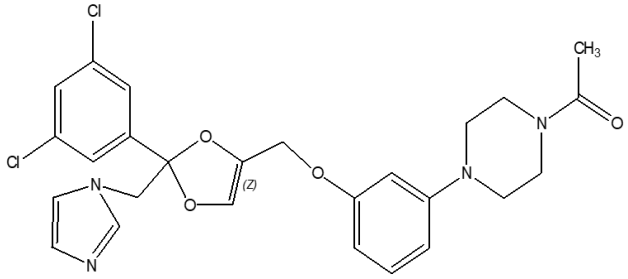
1000 mL purified water

pH = 5.4 +/- 0.2

15 g agar

The media was made by dissolving the necessary amounts of dried materials (Hi-media) in purified water and then distributing it to a thickness of 3-4 mm in petridishes. The plates were autoclaved at 121°C for 15 minutes to disinfect them. The sterile medium was put into petridishes and allowed to set before being used.

Standard drug:



Ketaconazole

Antifungal activity procedure

At 45 degrees Celsius, a suspension of the organism was put to sterile Sabouraud's agar medium. The liquid was poured into sterilised petri plates and let to set. Sterile discs with a diameter of 5 mm were dipped in a solution containing various concentrations of test compounds, as well as standard and control compounds, and then deposited on the surface of agar plates.

To minimise the impacts of variations in time between the applications of the different solutions, the plates were left at room temperature for 1 hour as a period of pre-incubation diffusion. The plates were then incubated for 48 hours at 37°C minus 1°C to test for antifungal activity. The size of the inhibitory zone was measured and documented.

3. IR Spectral Analysis Result and Discussion

IR spectroscopy was used to characterize the produced molecules.

Using a JASCO FT-IR spectrophotometer, the IR spectrum was captured. The important IR values are calculated in cm^{-1}, and the results are presented in the table.

Table 1

S. No.	Wave Number (cm^{-1})	Functional Group
1	3435.20	N-H Stretching
2	2922	C-H Stretching in Aromatic ring
3	1552.35	C=N Stretching (Aromatic nitro group)
4	1563.61	C=C Stretching
5	1102.19	C-O-C Stretching
6	1072.21	C-O Stretching
7	742.59	C-Cl Stretching

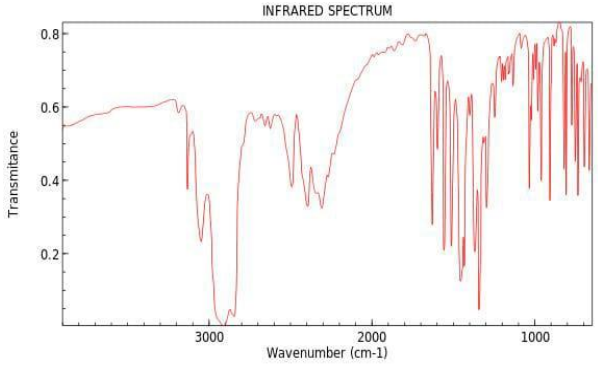


Fig. 1. Infrared spectrum

Antimicrobial Activity Screening:

I Antibacterial Properties:

The antibacterial activity of the produced compounds was tested using the disc diffusion method using ciprofloxacin as a reference against both gramme positive Bacillus cereus and gramme negative Pseudomonas aeruginosa. The outcomes are listed in table 2.

Table 2

Compounds	Zone of Inhibition (in mm)					
	Bacillus cereus			*Pseudomonas aeruginosa*		
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)
4-(5-chloro-1,3,4-oxadiazole-yl)benzenamine	15	18	20	13	19	22
Ciprofloxacin (10µg/ml)	39			38		

Table 3

Compounds	Zone of Inhibition (in mm)		
	Aspergillus fumigates		
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)
4(5chloro-1,3,4-oxadiazol-yl)benzenamine	16	22	24
Ketoconazole (10µg/ml)	39		

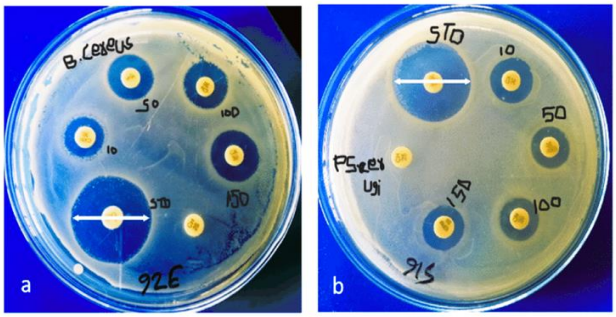


Fig. 2. a) Bacillus cereus antibacterial activity of produced chemicals, b) Pseudomonas aeruginosa antibacterial activity of produced chemicals

Antifungal Activity (ii):

The disc diffusion method was used to screen the antifungal activity of synthesized compounds against Aspergillus fumigates, with ketoconazole as the reference medication.

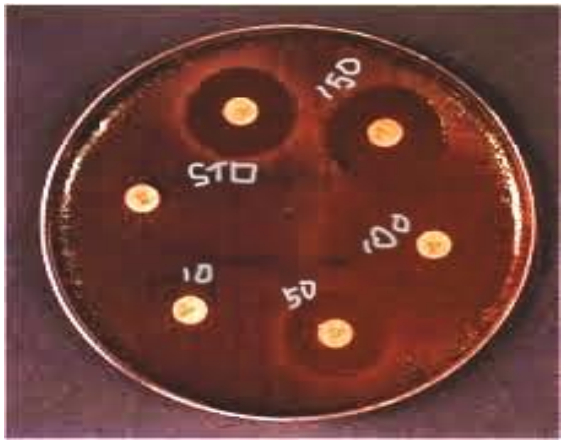


Fig. 3. Synthetic substances have antifungal action against Aspergillus fumigates

4. Conclusion

The antibacterial activity of the synthesized compounds was screened using the disc diffusion method at concentrations of 50, 100, and 150 g/ml against both gramme positive Bacillus cereus and gramme negative Pseudomonas aeruginosa using Muller Hinton agar media, as well as the antifungal activity against Aspergillus fumigates using Sabourand's agar media. The diameter of the inhibitory zone was measured in millimetres and documented. At three different doses, all of the produced compounds demonstrate modest activity.

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