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Synthesis and antimicrobial evaluation of novel oxadiazolyl-benzenamine derivatives

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ABSTRACT

The pursuit of development of newer treatments for existing diseases has been the doorstep for the present research work. In the present work newer antibacterial agents based on oxadiazole-benzenamine scaffold were synthesized using microwave irradiation and evaluated. All the compounds were yellowish to brown in colour and were obtained in 73-81% yields using the optimized reaction conditions. The compounds were insoluble in water, methanol, soluble in chloroform and DMSO. The confirmation of the structure of the synthesized compounds was done by IR, ^{1}HNMR and mass spectral studies. All the compounds exhibited the absorption bands of C=O, C=N, C-H, C=C stretching in the IR spectra. The compounds were evaluated for anti-bacterial potential using disc diffusion method (*in vitro*). The results obtained led to the conclusion that the antibacterial activity of the oxadiazole-benzenamine derivatives as depends on position of the hydroxyl substitution present in the scaffold.

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Introduction

1.1 Oxadiazole

Oxadiazoles are five membered heterocyclic ring systems containing two carbons, two nitrogen and one oxygen atom. They are known to exist in different isomeric forms viz., 1,2,4-Oxadiazole, 1,3,4-oxadizole, 1,2,5-oxadiazole and 1,2,3-oxadiazole [1]. The isomer 1,2,3-oxadiazole is a slightly unstable and gets converted into a diazoketone tautomer. Amongst all the isomers, 1,3,4-oxadiazole has high significance in the area of research. Several pharmacological actions of various synthesized oxadiazole derivatives have been reported in literature. Some recent reported pharmacological potential of oxadiazole based molecules include anti alzheimers [6], anticholinesterase action [8], analgesic and anti-inflammatory [9, 14], antiproliferative [15], anticancer [17], and anticonvulsant [20].

Oxadiazoles have been known to exert a good anti microbial property [25,26] and have been recenlty widely researched for their vivid biological potentials. The objective of the current work is to synthesize some derivatives bearing oxadiazole-benzenamine conjugates using microwave assisted synthesis method and to assess their anti microbial potential.

Material and Methods

The scheme for the synthesis of the oxadiazole derivatives was adapted from the procedures reported by Amir et al [41] and Mishra et al [42] and the scheme is depicted in Figure 1. The scheme was modified for microwave assisted synthesis and validated for the reaction conditions.

General method for synthesis of substituted benzohydrazides

0.1 moles of substituted benzoic acid **(1a-e)** was dissolved in 25 ml ethanol and the mixture was irradiated using microwave for 7 min at 100 Watt in presence of 5 drops of concentrated H_2SO_4. On cooling, a solid separated which was filtered to give an intermediate. The intermediate was reacted by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid. Briefly, 0.1 mole of the intermediate in 20 ml ethanol, 0.1 mole of hydrazine hydrate was added. To the mixture, catalytic amount of concentrated sulfuric acid was added. The mixture was irradiated at 100 Watt using microwave until the completion of the reaction (approximately 3 min). On cooling, a solid separated, which was recrystallized from ethanol to give the products **2a-e**.

Synthesis of N-(2,3-dimethylphenyl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl) benzenamine

Compound **2a-e** (0.001 mol) and mefenamic acid, **3** (0.001 mol) were dissolved in phosphorus oxychloride and irradiated at 100 Watt using microwave for 25 min. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus precipitated was filtered, washed with water, dried and recrystallized from ethanol to obtain compounds **4a-e.**

Chemical Characterization

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).

Melting point was determined using open capillary employing melting point apparatus, the solubility was qualitatively assessed in solvents of varying polarity and the TLC was performed on precoated silica gel plates.

Antibacterial acivity

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganisms viz. *Bacillus subtilis, Streptococcus aureus, Escherichia coli and Salmonella*, which are the representative types of gram positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion.

Preparation of test solution

20 mg of the synthesized compounds were dissolved separately in 20 ml chloroform. 1 ml of this solution was diluted to 10 ml with chloroform. 0.5 ml (50 µg) and 1 ml (100 µg) of this solution was further diluted upto 2 ml by addition of chloroform to obtain a solution of 25 and 50 µg/ml strength. These sample solutions were sterilized test tubes. These test compounds (25, 50 and 100 µg/ml) were soaked on small circular disc of 5 mm.

Procedure of antibacterial testing

Hardness test

The sterilized media (nutrient agar) was cooled to 45°C with gentle shaking for uniform cooling and then inoculated with 18-24 h old bacterial subculture under aseptic conditions in a laminar air flow bench and mixed well by gentle shaking. This was poured in to sterile Petri dishes and allowed to set. After solidification all the Petri dishes were transferred to laminar flow unit and the test sample discs were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept in the laminar air flow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The extent diameter of inhibition after 24 h was measured as the zone of inhibition in millimeters (mm).

Results and Discussion

Chemical characterization

The physicochemical properties are shown in Table 1. All the compounds were found to be soluble in chloroform and insoluble in water and methanol.

The structure elucidation of the synthesized compounds was confirmed by interpretation of the IR, ^{1}HNMR and Mass spectra of the compounds. The IR spectra were observed for the characteristic peaks obtained due to the presence of the functional groups. All the compounds exhibited the peaks of aromatic C=C stretching, C-H stretching, C-N and C=N stretching and C-O stretching. The occurrence of absorption bands for C-O and C=N may occur at the same frequency and Fermi resonance peaks may be the diagnostic of a carbonyl group in the compounds. The ^{1}HNMR spectra of all the compounds exhibited chemical shifts of aromatic hydrogen. They also exhibited any peak that may arise due to certain functional groups like –OH and NH protons. The mass spectra of the compounds were examined for the presence of molecular ion peak or the isotopic peaks to confirm the formation of the compounds.

Antibacterial Action

The antibacterial activity of the synthesized oxadiazole-benzenamine conjugates was determined measuring the zone of inhibition in the agar plate. Three concentrations of the synthesized compounds were tested for antibacterial action against ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table 2.

The results revealed that the antibacterial action of the synthesized compounds was dose dependent. The compounds were mild to moderately antibacterial. The presence of hydroxy group at para position in the phenyl ring attached to oxadiazole in the compounds favored antibacterial activity against gram negative bacteria (**4a, & 4c**) whereas hydroxyl group on meta or ortho position of the phenyl ring attached to oxadiazole favored activity against gram positive bacteria (**4d, 4e**). Compound 4b did not exhibit significant antibacterial action as compared to the control suggesting the importance of the hydroxyl substitution in the compounds for antibacterial potency.

Conclusion

The objective of the present investigation was to synthesize anti-microbial molecules based on oxadiazole-benzenamine scaffold using microwave irradiation method. It was accomplished by converting the carboxyl group of substituted aromatic acids mefenamic acid to oxadiazole nucleus while conjugating it with me-

fenamic acid. The synthesized compounds presented good yield and antibacterial activity comparable to that of the standard drug.

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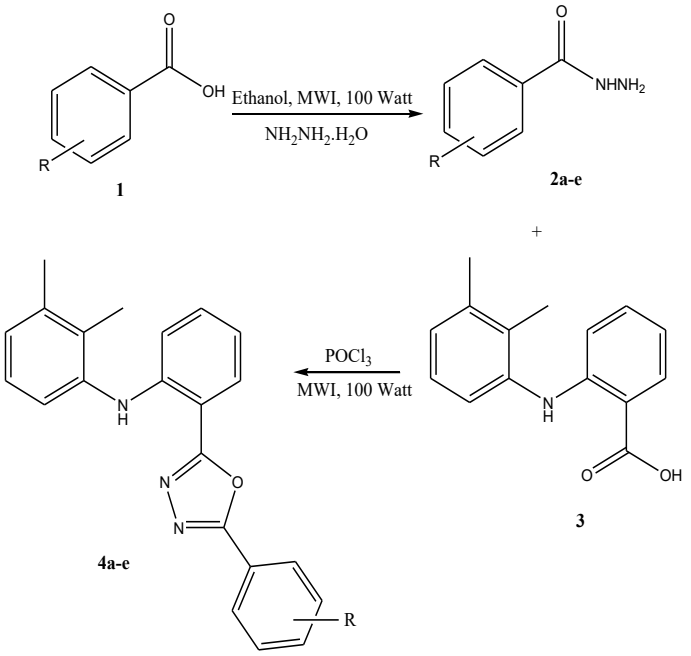

Figure 4.1 Scheme for synthesis of oxadiazole-benzenamine derivatives

Compound code	Aromatic acid Used	Yield (%)	Color	R_f Value	Melting point (°C)
4a	Coumaric acid	81	Yellow	0.67	249-251
4b	Cinnamic acid	75	Yellow	0.63	238-240
4c	Gallic acid	73	Yellow	0.69	222-224
4d	2,4-dihydroxy benzoic acid	77	Yellow	0.64	261-263
4e	2,5-dihydroxy benzoic acid	74	Brown	0.66	265-267

Table 1 Physicochemical characters of synthesized compounds

Compound Code	Zone of Inhibition (mm)*											
	B. subtilis			*S. auerus*			*E.coli*			*Salmonella*		
	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg
4a	7	9	14	6	8	15	11	16	**23**	10	16	**24**
4b	5	5	7	5	7	9	5	8	11	6	11	13
4c	6	9	13	6	8	13	9	15	**24**	8	16	**25**
4d	10	16	**25**	9	16	**24**	6	9	13	5	9	14
4e	11	14	**23**	10	15	**23**	6	8	12	6	10	13
Ciproflox-acin	21	**26**	**31**	20	**24**	**28**	18	**25**	**36**	20	**27**	**36**

Table 2 Zone of Inhibition of synthesized compounds

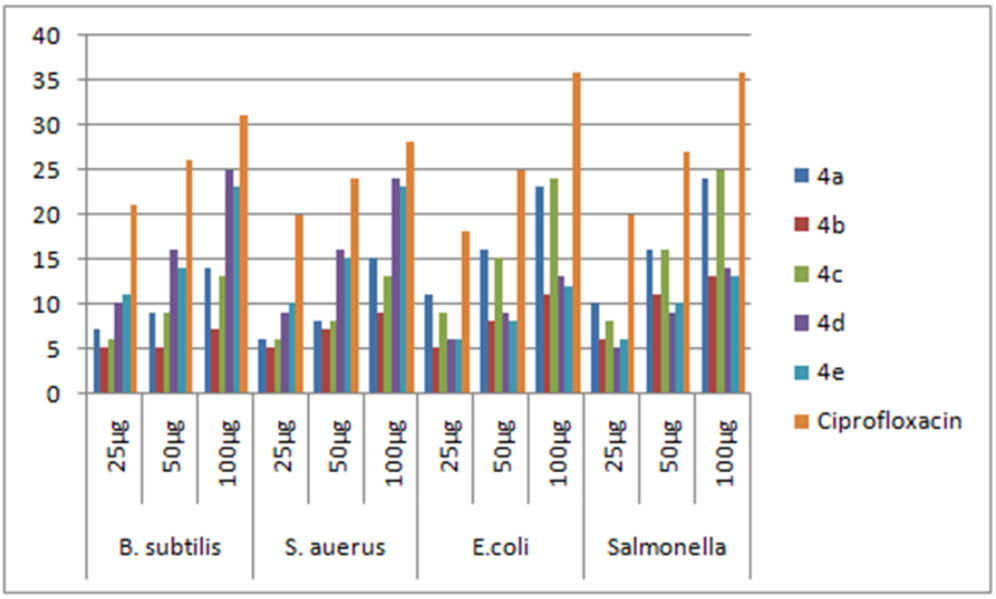

Figure 2 Zone of Inhibition of the tested compounds

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