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Synthesis of Novel 1,3-Oxazepine Group and Study its Activity against Pathogenic Bacteria

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ABSTRACT

The present research work involved synthesizing derivatives of the chemical compound 1,3-oxazepine-4,7-dione [F_1-F_2]. The synthesis was achieved using the [2-5] cycloaddition reaction of 1 mole maleic anhydride with azo-imine [D_1-D_2]. Additionally, the azo [C_1-C_2] was synthesized from p-hyroxyaniline by coupling diazonium salt with 4-alkoxy benzaldehyde. The structures of compounds F_1 and F_2 were characterized via spectroscopic technique. In this study this chemical compound was used against four different kinds of pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. In addition to testing the growth inhibition of antibacterial antibiotics such as ampicillin and amoxicillin, the synthesized compounds F_1 and F_2 also showed positive results in terms of growth zone inhibition. In conclusion, a novel 1,3-oxazepine-4,7-dione derivative was created using Schiff's base synthesis. Furthermore, the newly synthesized 1,3-oxazepine-4,7-dione compounds (F_1, F_2) were effective as antibacterial agent for *S. aureus*, *B. subtilis, P. aerugiuosea* and *E. coli*. Synthetic compounds showed similar biological activity to bacterial growth inhibitors, such as commonly used drugs like amoxicillin and penicillin, because the oxazepine-derived compounds contained many active groups that affect bacterial growth.

Key words: Antibiotics, 1,3-oxazepine, biological activity, bacteria

INTRODUCTION

Some bacteria are pathogenic, but most are not (Vouga and Greub, 2016). They are highly distributed in various environments such as soil, water, plants, animals, radioactive waste deep inside the earth's crust, polar ice and glaciers and hot springs. Chemical compounds known as antibiotics work by killing bacteria or slowing down their development (Miethke *et al.*, 2021). They are sometimes useful (Begum *et al.*, 2021). Antibiotics could be useful for bacterial disorders but worthless for viral infections (Gallagher and MacDougall, 2022).
The formula of the seven-ring heterocyclic compound oxazepine has two distinctive oxygen and nitrogen heteroatoms (Asif and Imran, 2020). Biological processes involving oxyazepine molecules include anti-convulsants (Asif and Imran, 2022), antivirals, antifungals, and other uses in a variety of fields (Ahn *et al.*, 2020).
For formulation of this new antibacterial components, some of aspects should be described. The azo group is an essential component of the structure (Ali *et al.*, 2018). Schiff bases are widely applied in medicine and other fields (Abdulameer and Alias, 2022). Pharmacological and biological activity is an essential property of chemical compounds (like azo group), among others, since it suggests potential medical uses for the chemicals. Nevertheless, chemicals may be hard to use in biomedical uses because they can have

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harmful and dangerous side effects (Tanase *et al.*, 2019; Aftan *et al.*, 2021).

The present research was aimed at synthesizing and characterizing a new azo compound bearing oxazepine. These classes of molecules evaluate their activity as antibacterial biomolecules and can be useful for future microbiological works (Alfatlawi *et al.*, 2018; Hassan and Hame, 2019).

MATERIALS AND METHODS

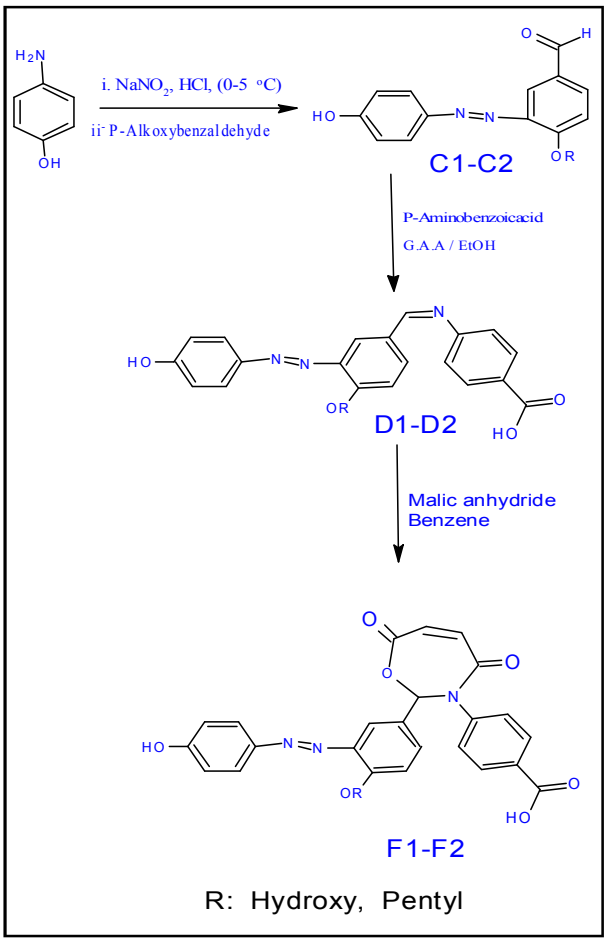
The range of 4000-400/cm was used in technical FT-IR, Model 8300 Shimadzu (Shimadzu Company, Japan). The 1 HNMR spectra were recorded using an ACF 500 spectrometer on Brüker, with solvents such as DMSO and TMS as internal standards. The chemicals used in this research were obtained from BDH® (England) and Merck®(Merck, Germany).

Initially, azo compounds (C_1 – C_2) were synthesized according to Benkhaya *et al.* (2020). Five ml of 10% HCl solution was added to the 0.01 mol P-hydroxy aniline in the cooling bath. Then three drops of concentrated HCl were added. 0.01 mol $NaNO_2$ was prepared in 5 ml distilled water. It was inserted in the previous solution at 0 to 5 °C. Now, 0.01 mol 4-hydroxybenzaldehyde and 4-pentyl benzaldehyde were prepared in 10% sodium hydroxide in separate tubes. Previous solution was added to these tubes. Each solution was filtered, and the precipitate was collected. Physical properties of these C_1-C_2 compounds are tabulated in Table 1 and procedure is shown in Scheme 1.

Table 1. Physical properties of compounds C_1-C_2

Compounds number	R	Yield (%)	Colour	Melting point (°C)
C_1	Hydroxyl	71	Dark brown	225-230
C_2	Pentyl	73	Dark brown	217-221

Now, Shiff's bases D_1-D_2 were synthesized according to Marwani *et al.* (2012). 0.01 mol of the produced molecule (C_1-C_2) was reacted with 0.01 mol of 4-aminobenzoic acid in 100% ethanol as the solvent. The reaction mixture was refluxed for four hours using glacial acetic acid as the catalyst. The resulting crystals were collected by filtration and dried. Table 2 displays the physicochemical properties of these synthesized D_1 and D_2 compounds.



Scheme 1. Synthesis procedure for 1,3-oxazepine (F_1-F_2).

Table 2. Physico-chemical properties Schiff's base D_1-D_2

Compounds number	R	Yield (%)	Colour	Melting point (°C)
D_1	Hydroxyl	71	Dark brown	225-230
D_2	Pentyl	73	Dark brown	217-221

A mixture of each 0.01 mole Schiff D_1-D_2 and 0.01 mole maleic anhydride in 15 ml dry benzene was reflexed for 3 h. The final steps were filtering the solution, collecting the precipitate and recrystallizing the resulting coloured crystalline solid utilizing 1,4-dioxane dry. Table 3 shows the physico-chemical properties of the synthesized 1,3-oxazepine derivatives F_1-F_2. This synthesis procedure for

Table 3. Physico-chemical properties of 1,3-oxazepine compounds F_1-F_2

Compounds number	R	Yield (%)	Colour	
Q_1		69	Dark orange	281-285
Q_2		67	Orange	262-266

1,3-Oxazepine (F_1-F_2) has been shown in scheme 1.

The four commonly isolated and diagnosed bacteria were prepared by culturing them overnight at 37 °C, followed by isolation into *E. coli, Klebsiella, Streptococcus* and *Staphylococcus*.

RESULTS AND DISCUSSION

Various techniques have been described for testing the synthesized azo dye (Shah, 2019). Infrared spectroscopy (FTIR) was used in the present research to identify the presence of functional groups. The stretching vibration band of an azo group (υ N=N) was shown at 1509/cm (Fig. 1). The υ C–H aromatic was detected at 3073/cm, while the υ C–H aliphatic was detected at 2878/cm. The υ carbonyl group was shown at 1722/cm.

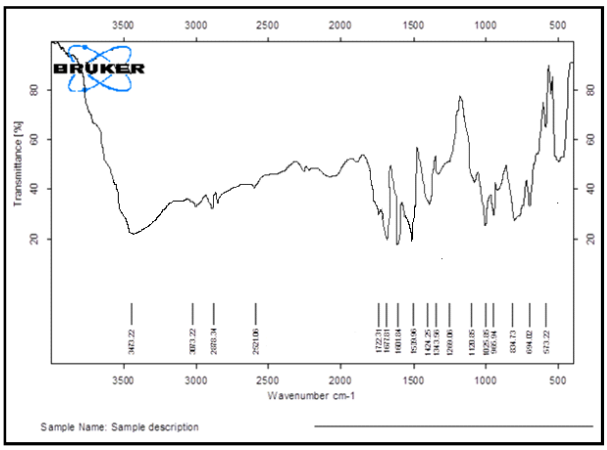


Fig. 1. FTIR spectrum of 3- [(4-hydroxy phenyl) diazenyl]-4-pentoxybenzaldehyde C_2.

Absorption bands in FTIR spectroscopy are shown in Fig. 2. The synthesized compound D_1 showed bands υ O–H stretching 3307/cm. The aromatic stretching of υ C–H was determined in 3024/cm. The value of the υ C–H aliphatic stretching was 2961/cm. The azo groups appeared at 1508 and 1594/cm due to υ CH=N stretching. The band at 1724/cm corresponded to the υ carbonyl of the carboxylic acid.

The ^{1}H-NMR spectrum of molecule [D_2] showed that the phenolic hydroxyl proton (2H) generated a signal at 10.10 ppm and the imine group in its structure appeared at 8.37 ppm (Fig. 3). The protons of the aromatic group appeared at 6.52-8.17 ppm (22H). The signals at 1.04-1.06 ppm were attributed to –CH_3, and the signals at 3.32-4.06 ppm were attributed to –OCH_2.

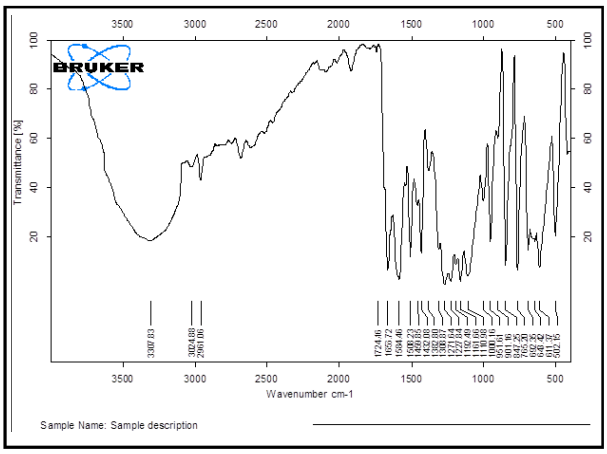


Fig. 2. FTIR spectrum of 4-[(Z)-{4-hydroxy-3-[(4-hydroxyphenyl)-diazenyl] benzylidene} amino] benzoic acid D_1.

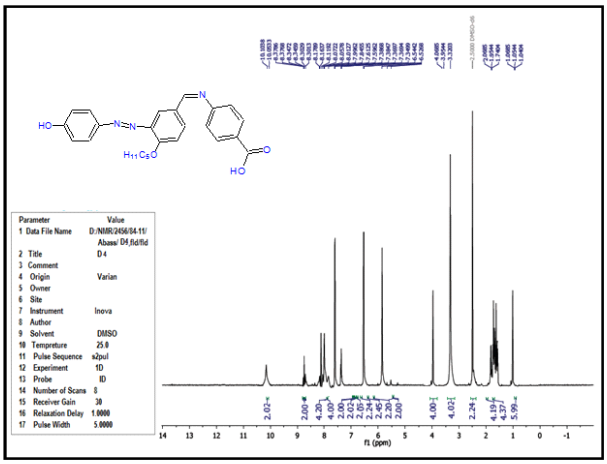


Fig. 3. HNMR spectrum of 4-[(Z)-{4-hydroxy-3-[(4-pentoxyphenyl)-diazenyl] benzylidene} amino] benzoic acid D_2.

The FTIR spectra of derivative F_1 showed (Fig. 4) that the synthesized compounds had bands in the range of 1725/cm for the lactone υ C=O and 1685/cm for the lactam υ C=O. The bands at 3036/cm were assigned to υ Ar-H, while the bands at 2976/cm were assigned to υ C-H aliphatic. The band at 3427/cm was assigned to υ O-H.

The FTIR spectra for F_2 showed (Fig. 5) that the synthesized compounds had bands in the range of 1722/cm for the lactone υ C=O and 1694/cm for the lactam υ C=O. The bands at 3087/cm were assigned to υ Ar-H, while the bands at 2964/cm were assigned to υ C-H aliphatic. The band at 3476/cm was assigned to υ O-H.

The ^{1}HNMR spectrum for compound F_2 is shown in Fig. 6. The phenolic hydroxyl proton (2H) produced a signal at 10.10-10.13 ppm, while

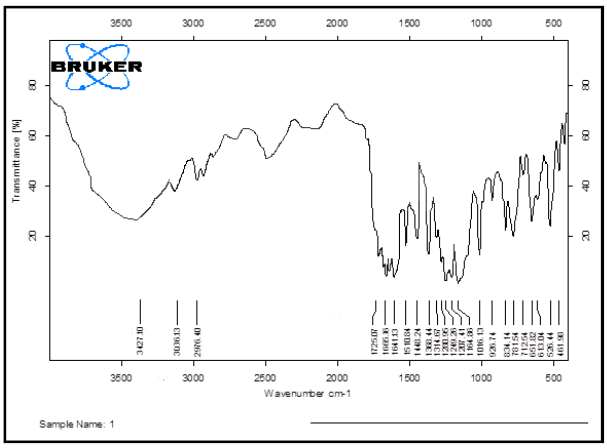


Fig. 4. FTIR spectrum of compound F_1.

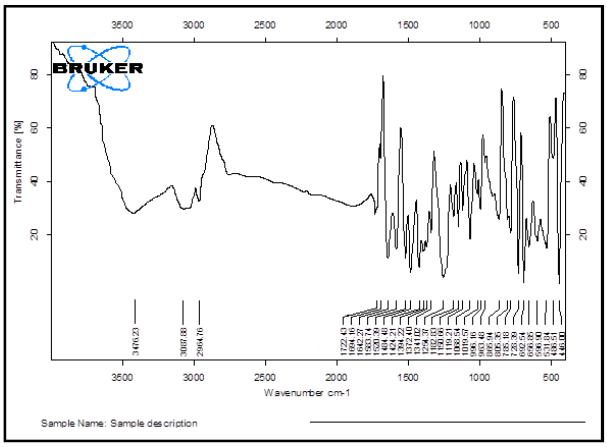


Fig. 5. FTIR spectrum of compound F_2.

aromatic protons had signals at 7.17-8.11 ppm (30H). At 0.97 ppm, three protons showed as triplets, which could be ascribed to $-CH_3$. At 2.77 ppm, the $-OCH_2-$ groups of the pentyl substituent appeared as triplets (2H) (Shah, 2019).

Gram-positive and gram-negative bacteria, including *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* were examined for resistance to the produced oxazepine molecules. The microorganisms were tested by preparing bacterial cultures and utilizing the Agar well diffusion method at doses of 50 and 100 g/ml (24). The inhibitory diameter of each well was

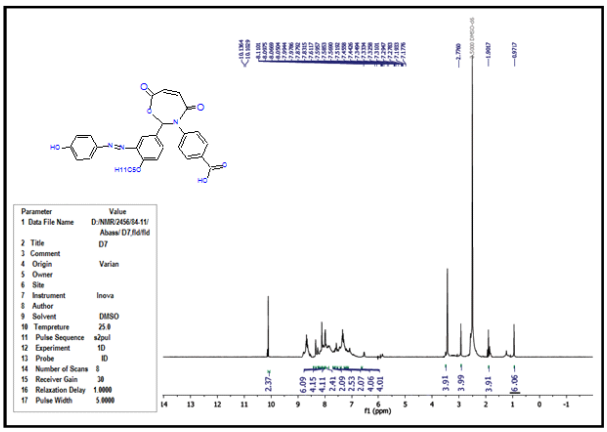


Fig. 6. ^{1}HNMR spectrum of compound F_2.

calculated using a ruler. Antibacterial activity of produced oxazepine is described in Table 4 and Fig. 7. Both the compounds F_1 and F_2 gave high activity against *S. aureus* and very high activity of compound F_1, especially in concentrations (25, 50 mg/ml). Both compounds F_1 and F_2 gave high activity against *B. subtilis* and moderate activity of compound F_2, especially in concentrations (25 mg/ml). In addition, compounds F_1 and F_2 had high activity against

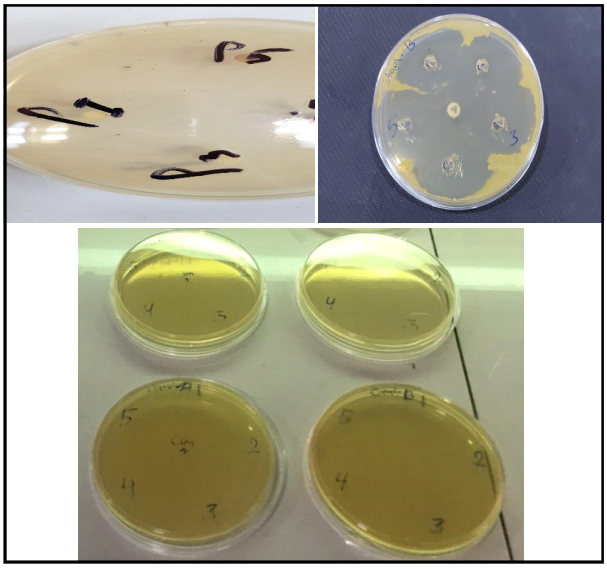


Fig. 7. The biological activity produced oxazepine.

Table 4. Antibacterial activities of compounds F_1-F_2

Bacteria name	Zone of inhibition (mm)			
	Compound F_1		Compound F_2	
	(Con. 25 mg/ml)	(Con. 50 mg/ml)	(Con. 25 mg/ml)	(Con. 50 mg/ml)
Staphylococcus aureus	44	49	41	43
Bacillus subtilis	41	41	39	42
Pseudomonas aeruginosa	46	48	47	47
Escherichia coli	43	46	38	41

Table 5. Antibacterial activities of ampicillin and amoxicillin

Bacteria name	Zone of inhibition (mm)			
	Ampicillin		Amoxicillin	
	(Con. 25 mg/ml)	(Con. 50 mg/ml)	(Con. 25 mg/ml)	(Con. 50 mg/ml)
Staphylococcus aureus	37	39	43	44
Bacillus subtilis	41	41	40	41
Pseudomonas aeruginosa	42	41	44	46
Escherichia coli	40	42	38	39

P. aeruginosa and very high activity of compound F_1, especially in concentrations (50 mg/ml). Similarly, compounds F_1 and F_2 exhibited good activity against *E. coli*. Compound F_2 showed moderate activity, especially at a concentration of 25 mg/ml.

Antibiotics such as ampicillin and amoxicillin are commonly used to treat bacterial infections. These drugs are tested against the same types of bacteria to determine their effectiveness (Table 5). Amoxicillin contains the beta-lactam family of antimicrobials (De Rosa *et al.*, 2021). Beta-lactams block transpeptidation (the cross-linking process in cell wall formation) by binding to penicillin-binding proteins, activating autolytic enzymes in the cell wall of bacteria. Through the breakdown of the bacterial cell wall, this process kills the bacterial cell. For instance, they include sulbactam and clavulanic acid. These beta-lactamase inhibitors work by attaching stably to the catalytic site of an organism's beta-lactamase enzyme, developing resistance to the amoxicillin's initial beta-lactam ring. These medications do not have inherent bactericidal action, but when coupled with amoxicillin, they could increase their effectiveness against bacteria that make the beta-lactamase enzyme (Salvador and Tan, 2017; Breijyeh *et al.*, 2020). Compounds F_1 and F_2 have a considerable inhibitory effect on the activities of DNA gyrase and topoisomerase IV due to the carboxylic acid and carbonyl groups in the seven rings of the oxazepine structure. The hydroxyl group prevents t-RNA from attaching to the ribosome's A site, which contributes to the inhibition of protein synthesis (Kapoor *et al.*, 2017). Other groups have an impact on the region that inhibits bacterial growth. In present study new bioactive chemical- components have been examined and reported for using in antibacterial products for specific bacteria.

CONCLUSION

Using Schiff's base synthesis, a novel 1,3-oxazepine-4,7-dione derivation of 1,3-oxazepine-4,7-dione was created and introduce in present study. Furthermore, the newly synthesized F1 and F2 showed effectiveness as bacterial inhibitors against various strains, including *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*, and *Escherichia coli*. Synthetic compounds are almost similar in biological activity as bacterial growth inhibitors with some commonly used drugs such as amoxicillin and penicillin because the synthesized compounds of Oxazepine contain many several groups that affect the growth of bacteria. Moreover, these compounds can be prepared using simple and inexpensive methods.

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