

Researching, Describing, and Synthesizing the Biological Activity and Toxicity of Heterocyclic Molecules Derived From (Triazole)

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Abstract: Novel heterocyclic compounds containing a 1,2,4-triazole moiety were produced by modifying the antibiotic triazole. Physical characteristics, FTIR, ¹H-NMR, and melting point studies were used to ascertain their structures. . To evaluate the new chemicals' toxicity, the LD50 value was calculated utilizing Dixon's up-and-down methodology. The antibacterial activity of these compounds against gram positive and negative bacteria was tested at concentrations of 200–500 mg/mL. , such as *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus*, and *Staphylococcus aureus*. The 1,2,4-triazoles are a common structural motif among heterocyclic compounds containing three nitrogen heteroatoms that have garnered significant interest from the scientific community and medical uses. Despite not occurring in nature, the 1,2,4-triazole moiety finds extensive application in drug development, polymer chemistry, supramolecular chemistry, chemical biology, and organic synthesis. Therefore, it is essential to establish a straightforward and unambiguous process for the synthesis of derivatives of 1,2,4-triazoles. When compared to conventional antibiotics, the results showed that all of these substances exhibit strong antibacterial and antifungal properties. In the end, tests were carried out to ascertain each produced molecule's antioxidant activity, revealing varying levels of activity.

Key words: Triazole, Heterocyclic substances, Schiff bases, The biological process, Properties of Toxicity, Imiadazoldine, β -Lactam, oxazepine

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Introduction

Any significant organic chemical compound family in which all or a portion of the atoms are organized in rings including at least one atom of an element other than carbon is known as a heterocyclic compound, or heterocyclic (C) compound [1]. Our survival depends on heterocyclic chemicals, which include hormones, hemoglobin, alkaloids, antibiotics, and a wide range of specially made drugs and colors.. [2] Any noteworthy family of organic compounds, vitamins, medications, and hallucinogens are examples of heterocyclic compounds. Schiff bases are well-known in the pharmaceutical and medical industries because they are commonly used in forensic and clinical contexts. A wide range of biological efficacy has been demonstrated for schiff bases, which makes them useful as relaxants, light tranquilizers, hypnotics, and muscle relaxants[3].. Schiff bases are the condensation products of energetic carbonyl groups and primary amines. according to their chemical makeup, working method, etc.

There has been a great deal of interest in and research into on the biological properties of 1,2,4-triazole derivatives, including their diuretic, antiprotozoal, cardiogenic, fungicidal, sedative, anesthetic, antimalarial, CNS depressant, hypoglycemic, anti-inflammatory, and antimicrobial activities... Given this, the goal of the current effort was to create new heterocyclic substituted 1,2,4-triazole compounds with less potential side effects, such as antitubercular and antidiuretic qualities. [4-6]. The 1,2,4-triazole unit is a physiologically active molecule with several uses as an anti-cancer agent that helps the pharmaceutical and medical industries tackle the challenges of modern medicine in the wake of the worldwide emergence of drug-resistant diseases.. The well-known heterocyclic compound triazole, on the other hand, has five members and three nitrogen atoms. Triazole compounds are commonly used in agrochemicals as herbicides and in pharmaceuticals as antibacterial, anti-inflammatory, anticonvulsant, and anticancer drugs. [7]. Recently, medicinal chemistry has been more interested in the use of triazoles as a selective analgesic and anti-inflammatory drug. The majority of anticancer drugs work by inhibiting enzymes, interfering with the synthesis of proteins, RNA, or DNA, or interfering with the metabolism of other cell components. Many times, these drugs have little to no specific antitumor impact. It is frequently impossible for conventional anticancer drugs to distinguish between normal and nontumorous cells. [8], particularly with regard to the quickly proliferating cells that comprise malignancies. Prodrugs of anticancer medications, antibody-drug conjugates, anticancer agents customized to specific (cancer) tissues, and micro-drug anticancer agents targeted to tumors via transporter-related mechanisms are only a few examples of cutting-edge technologies.. The fungus *Candida albicans*, Gram-negative (G-) bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, as well as Gram-positive (G+) microorganisms like *Bacillus* and *Staphylococcus aureus*, were recently assessed in connection with antimicrobial drugs. It's been shown that derivative inhibitors that contain heteroatoms, such as "N, O, and S," harm bacterial and fungal cell walls and membranes, changing their permeability. They can also cause growth and cell death, interfere with a cell's metabolic process, and prevent cell proteins from functioning. Both in theory and in fact, these impacts have been noted. [9-10].

Materials and methods:

1- Chemical study: [11-12]

{X1}: produced by mixing (0.03) moles of 3-mercapto-4-amino-5 methyl-1,2,4-triazole with (25 ml) of pure ethanol, dissolving (0.03) moles of 2-chloro benzaldehyde in the mixture, and aggressively stirring the mixture for 20 minutes on a magnetic stirrer for eight hours at 65–70°C. The mixture was dried and evaporated to prepare it for recrystallization from pure ethanol.

{X2 } : produced by dissolving (0.03) moles of {X1} in 25 milliliters of dry benzene while continuously shaking the mixture to complete it. To complete the reaction in 12 hours at 65 °C, we then progressively added 0.03 mol of phthalic anhydride. The solution was dried for 100% ethanol to recrystallize.

{X3} : prepared by vigorously shaking a mixture consisting of 0.03 mole of {X1} dissolved in 25 milliliters of THF (tetra hydrofuran). The "imiazoldine" derivative was then made by gradually adding

"0.03 mole" of glycine over a period of 15 hours at "50 C0." To recrystallize pure ethanol, the solution must be dry.

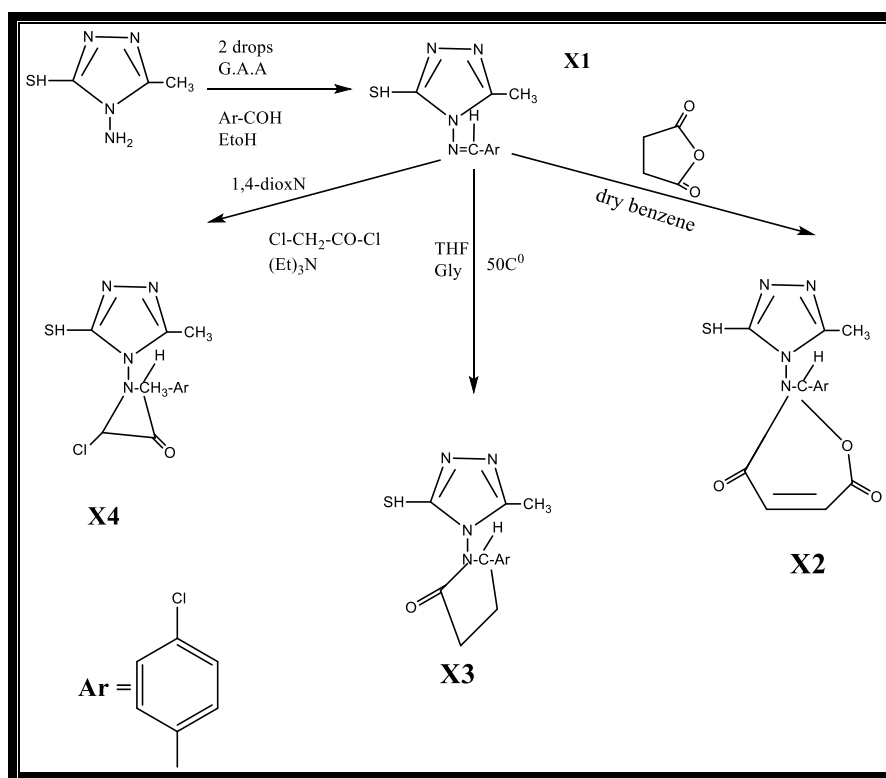
{X4} : Dioxin was used as a solvent to dissolve (0.03) mol of {X1} in (25) ml of triethylamine and chloroacetyl chloride for eight hours at 10 °C to create the β -lactam derivative. The mixture was dried in order to provide the solution for recrystallization from pure ethanol. and evaporated. Table 1 (Scheme 1) displays the physical properties of each target molecule [X1–X4]. [

NOTE : Every product that was produced underwent thin layer chromatography, or TLC.

Table 1. Physical properties of compounds [X1–X4]

Compounds	Color	m.p. (C ⁰)	Rf	Yield(%)	(TLC)
X1	Orange	135-140	0.8	87%,	Ethanol:Hexane
X2	Brown	145-150	0.6	88%,	Ethanol:Hexane
X3	Deep Yellow	188-190	0.75	76%,	Ethanol:Hexane
X4	Pale yellow	186 -196	0.8	75%	Ethanol:Hexane

Scheme 1. [X1–X4]: Pathways for chemical synthesis



2- Research on microbes:

The antibacterial potential of the compounds (X2, X3, X4) was tested in vitro. Gram-negative bacteria The Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, as well as the agar diffusion method, The antibiotic tetracycline was used to calibrate and compare the antibacterial compounds.. Doses of 200, 250, and 500 mg/mL of tetracycline and the medications under study were dissolved in

DMSO. A sterile cotton swab was used to evenly distribute 0.3 mL of bacterial inoculums on a sterile Mueller Hinton Agar Petri plate.. Potato dextrose agar, agar, and fungus were employed for each component. Each well received 200–500 mg/mL of tetracycline and the investigated drugs (agar gel was perforated with holes 20 mm apart and 7 mm in diameter). For twenty-four hours, the plates were incubated in an aerobic environment at "36" C0. Confluent bacterial growth after incubation was seen.. The reduction of bacterial growth was expressed on a millimeter scale. Furthermore, the minimum inhibitory concentrations (MIC) of the medications. The minimal inhibitory concentration (MIC) was found to be the concentration at which no discernible development was seen. [13–14].

Results and Discussion :

Spectral Examination: [15-16]

Melting points (m.p.) and FT-IR spectroscopy were typically employed to investigate [Schiff base] (X1). The absorption peaks of the NH₂ and C=O groups disappeared in the FT-IR spectra, and a new stretching vibration, emerged at 1660 cm⁻¹ and was attributable to the CH=N azomethine group; the C=O group was responsible for the peak at 2470 cm⁻¹; the (C – Cl) group was responsible for another band at 760 cm⁻¹; and the (C–H) aromatic peak at 3064. During the addition reaction (2+5) of azomethine C=N with maleic anhydrides in dry benzene, new bands appeared at 1651 cm⁻¹ due to the cyclic amid group (CO-N) in lactam, which resulted in 1,3-oxazepine (X1). A band at 1720 cm⁻¹ was caused by lactones, and a band at 3044 cm⁻¹ was caused by alkenes (CH=CH). The most characteristic FT-IR absorption band evidence for the chemical (X3) showed another band at (1612) cm⁻¹ due to the (C = O) Amide. bands due to the (N–H) aliphatic group at 3142 cm⁻¹ and the (C–H) aliphatic group at 2965. According to (X4), the band at (1293) was due to (C-N) β-Lactam, and the band at (1776) was due to (N-C=O).

Spectral identification using 1H-NMR: [17-18]

The chemical (X1) (DMSO-d₆) 1H-NMR spectra showed that a single proton of (CH=N) produced a strong signal at δ 8.3 ppm, While several indications at δ 7.83-7.77 ppm are attributed to aromatic protons. .

The compounds (X2) displayed For one proton of (CH-N), there is a doublet signal at 6.06-6.04 ppm and a singlet signal at δ 7.05 ppm . for (2H, CH=CH) in the "1H-NMR" spectra (DMSO-d₆).

In Compound (X3)'s , one proton of the (CH-N) imiadazoldine ring displayed a singlet signal at (6.5) ppm, and twelve protons that are aromatic were seen at δ 7.83-7.54 ppm.

In its 1H-NMR spectra (DMSO-d₆), compound (X4) showed a singlet signal at (6.4) ppm for one proton of the (CH-N) β-Lactam ring. A singlet proton signal of (S-H) at 11.36 ppm was also found.

Tests for toxic qualities and antibacterial activity:

The overabundance of microorganisms that are resistant to the most commonly utilized antibiotics in recent years has come to the attention of the general public and healthcare organizations. Because of this, researchers and experts have been looking for safer and more potent antibiotic substitutes.. as shown in Table 2. Persistent compounds [X2-X4] showed an inhibitory impact against the bacterial and fungal strains that were the subject of this study (Figure 1).. Gram-positive bacteria were more susceptible to all of the study's examined derivatives than Gram-negative ones, whereas compound [X4] exhibited better antibacterial activity against fungal and Gram-positive/negative organisms than other compounds [X2, X3]... The antibacterial activity varies according to the type of derivative chemical [19–20]. This activity may be caused by triazole isons, which have been demonstrated to block the bacterial efflux pump. The compound [X4] is composed of (N, Cl, and O) atoms and possesses a (β-Lactam) ring.. The antibacterial activity of derivative compounds may stem from their ability to alter and disrupt the permeability of bacterial membranes and cell walls. or impede a cell's ability to grow and perish by interfering with its metabolic process, interfering with the creation of proteins, and decreasing its activity. The chemical properties that give these compounds their antimicrobial action dictate their antibacterial activity.

Amoxicillin and fluconazole were used as references for the antibacterial and antifungal properties, respectively. The compounds [X2-X4] seem to have strong antibacterial and weak antifungal properties [21]. The antibacterial and fungal activity of all derivatives [X2-X4] against the bacteria and fungi under investigation increased with compound concentration.. Furthermore, by intraperitoneally injecting dosages with similar spacing [concentrations] between doses, ranging from 500 to 700 mg/kg, the fatal dose (LD50) of the examined compounds [X2-X4] in-vivo was determined in mice. Our findings show that the LD50 values of Z3C and Z3C' are 658.45 and 718.6 mg/kg, respectively [22–23]. The results may provide light on the drugs' comparatively harmful effects as well as the clinical alterations the mice had after receiving different dosages. Injected mice may exhibit a range of behaviors, including tremors, The overabundance of microorganisms that are resistant to the most commonly utilized , convulsions, capillary swelling, and the tortuous reflex under specific circumstances. Finally, fatalities may occur from especially lethal doses (Table 3).

Table 2. At different concentrations (200, 250, and 500 mg/ml-1), the compounds X2-X4 show inhibitory zone diameters (mm) against the growth of bacteria and fungi.

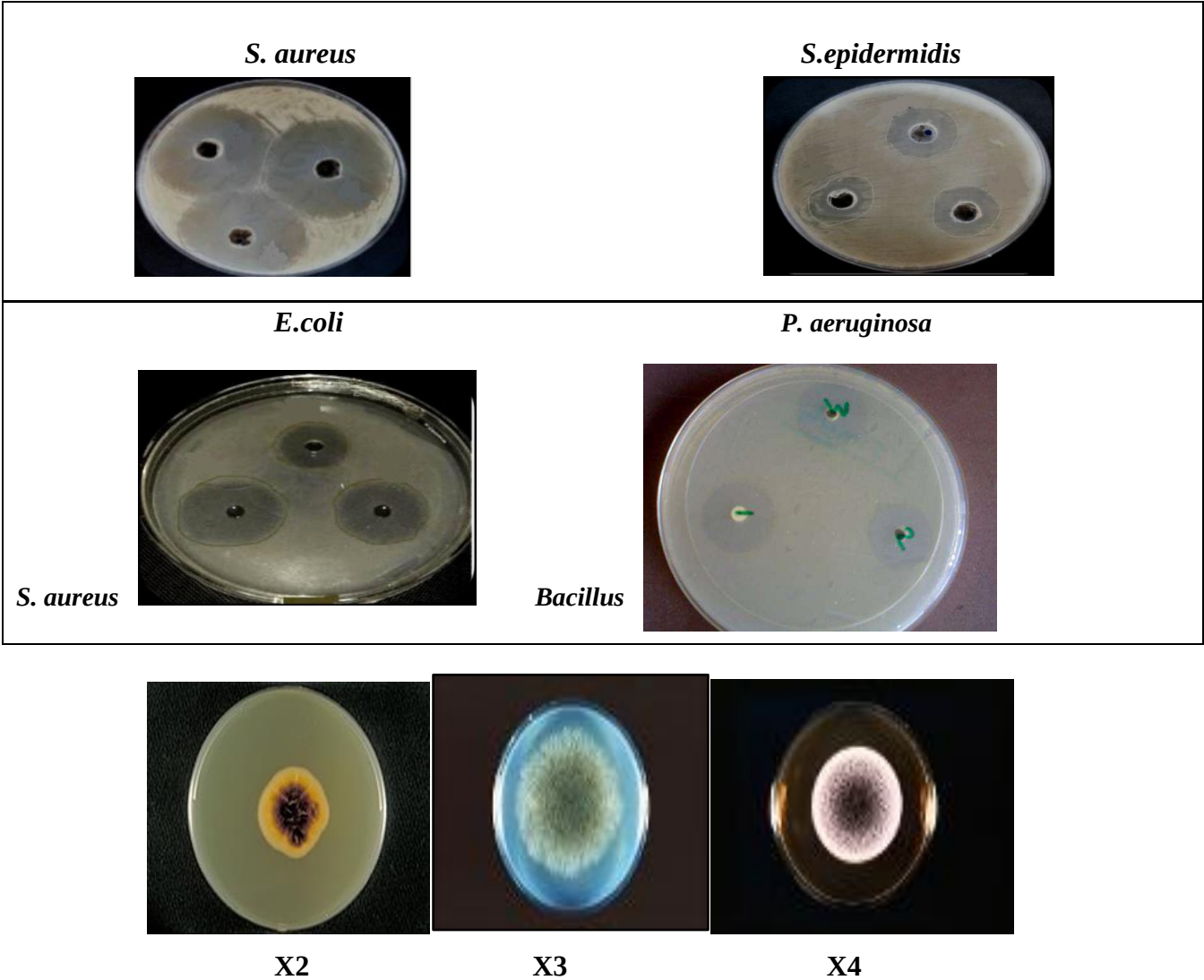
Compounds	Concentrations (mg/ml-1)	Gram positive (G ⁺)		Gram negative (G ⁻)		Fungi
		<i>S. aureus</i>	<i>Bacillus</i>	<i>E.coli</i>	<i>P. aeruginosa</i>	
X2	200	22	14	20	15	14
	250	22	16	21	18	16
	500	26	20	25	20	20
X3	200	23	20	22	18	20
	250	26	22	23	20	22
	500	28	26	26	23	26
X4	200	28	16	27	20	16
	250	30	20	30	24	20
	500	34	22	32	30	22
Amoxicillin		21	20	18	12	-----
Fluconazole	----	-----	-----	-----	-----	22

Table 3: Mice's toxic symptoms and toxicity results (LD50).

Test characterization	Results		
	X2	X3	X4
Doses vary	550–650 = 100 mg/kg	600–700 = 150 mg/kg	750–850 = 100 mg/kg
Initial dosage	500 mg/kg	500 "mg/kg"	500 mg/kg
Final dosage	650 "mg/kg	700 ;mg/kg	750 mg/kg
Dosage fluctuations	50 "mg/kg	50(mg/kg)	50 mg/kg
Lethal dosage median (LD50) in mg/kg	657.48 "mg/kg	728.5 mg/kg	815.15 mg/kg
Effective dose (LD50 /10) mg/kg	69.735 (mg/kg)	70.86 mg/kg	78.81 mg/kg
Onset of toxic signs	(5-15)minutes	5-20 min	5-25 (minutes)
No. of mice	8 (XOXXOXOX)	8 (XXOOXOXX)	8 (OXOXOOXX)

Toxic signs	Death, tremors, excitement, salivation, choreoathetosis, and rolling convulsions	Death, tremors, muscle fasciculation, excitement, convulsions, salivation, and dyspnea	trembling, excitement, salivation, muscle fasciculation, and death
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Figure 1: Antibacterial activity was measured in this study using the agar well diffusion method.



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