Synthesis Of New Hybrid Molecules Having AzoleMoieties And Evaluation Of Their Antimicrobial Activities

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Abstract
Infectious microbes are attacking our health and spreading enormous types of virus, bacterial, and fungal-associated life-threatening diseases. In this era of medicine, various categories of medicines are available on the market. Each belongs to a different class, and each class has a different mechanism. In the present study, we synthesised a new series of 2-aminobenzothiazole and benzimidazole derivative hybrid molecules to investigate their antimicrobial activities. IR and 1HNMR confirmed the structures of synthesised azole derivative compounds. In the study, some newly synthesised azole compounds were studied on different bacterial strains, and it was found that each azole compound has different efficacy. The synthesised test compounds like T1, T3, T4, B5, and Tt2 showed good activity against gram +ve bacteria, whereas T3, T4, T5, Bb1, and Tt5 showed good antibacterial activity against gram –ve bacteria, while T4 and Bb5 showed good results against fungi. Antimicrobial activity against some bacterial strains was measured by the broth microdilution method.
Keywords: Antimicrobial; Azole; 2-aminobenzothiazole; Benzimidazole; Broth microdilution method

Introduction

Microorganisms are small in size, so they are invisible to our naked eyes [1]. The importance of microorganisms is such that the environment is incomplete without them and is so dangerous that it can become a threat to human existence. These are generally classified as bacteria, fungi, viruses, and yeast [2]. Everybody knows both aspects of microbes, whether they are beneficial orharmful to human life (Figure 1). Microorganisms play crucial roles in various ecological processes. They help decompose organic matter, cycle nutrients, fix nitrogen, and maintain the balance of ecosystems [3]. They also have numerous applications in medicine, industry, and biotechnology, such as producing antibiotics, enzymes, and biofuels [4]. However, some microorganisms can also cause infectious diseases and pose health risks to humans [5], animals,and plants.

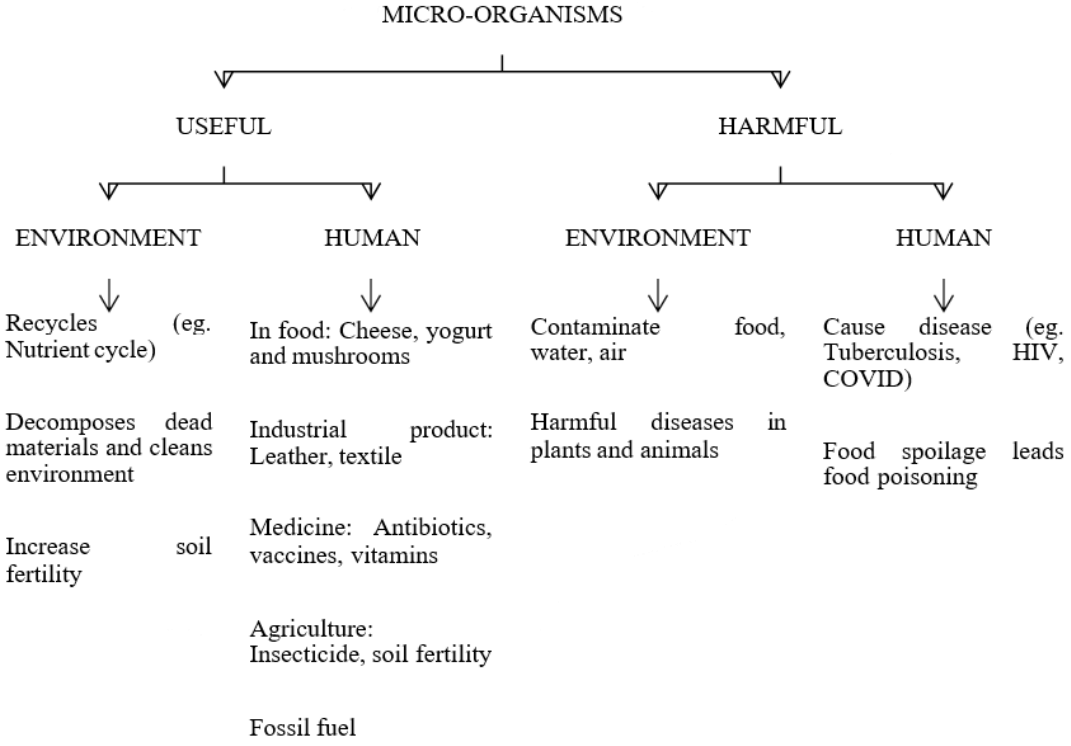


Figure 1: Classification of microorganism

Any microorganism causing disease is called a pathogen. The organism from which a pathogen gets nutrition or shelter to grow is well known as its host. Pathogens infect host organisms in various ways (Figure 2). These organisms have the ability to invade and multiply within the body, disrupting normal bodily functions and causing various diseases.
Pathogens can be transmitted through various means, such as direct contact with an infected individual, inhalation of airborne particles, ingestion of contaminated food or water, or through vectors like mosquitoes or ticks [6]. Once inside the body, pathogens can target specific tissuesor organs, leading to localised or systemic infections.
Antimicrobials are substances that are capable of killing or inhibiting the growth of microorganisms, including bacteria, viruses, fungi, and parasites [7]. They are commonly used in medicine and various industries to prevent or treat infections caused by these microorganisms. Antimicrobials can be classified into several categories based on their specific targets and modes of action.
Azole derivatives are a class of organic compounds that contain a five-membered ring structure with three carbon atoms and two nitrogen atoms [8]. They are known for their antifungal properties and are widely used in the treatment of fungal infections. Azole derivatives work by inhibiting the growth and replication of fungi, thereby helping to control and eliminate the infection.

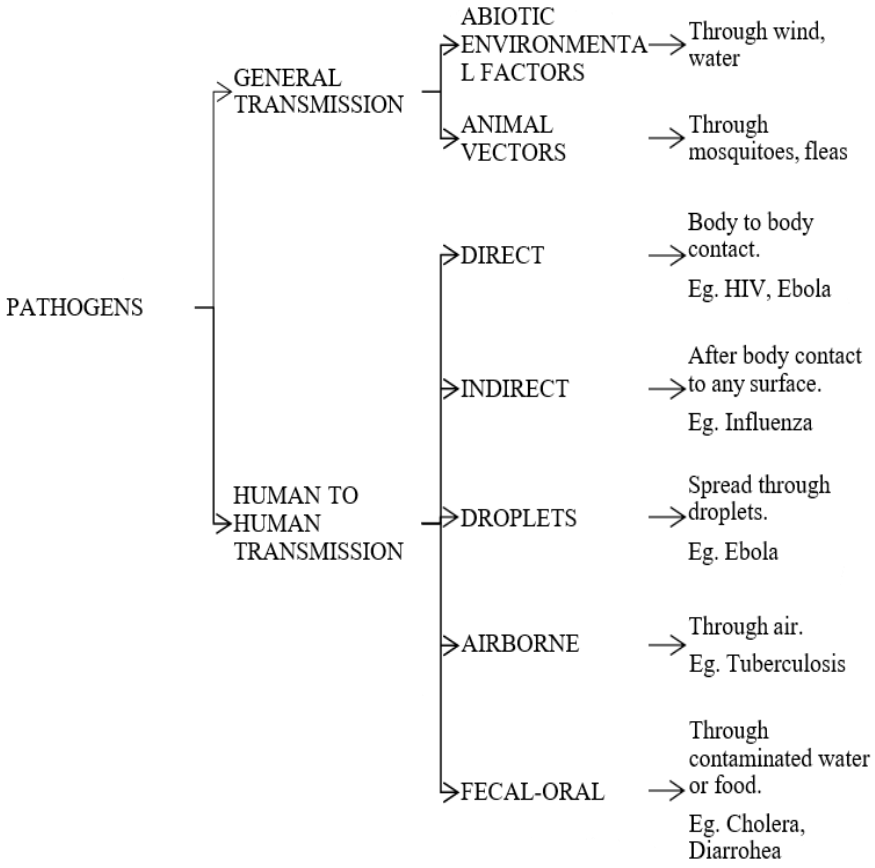


Figure 2: Transmission Of Pathogens

Azole derivatives kill fungi by killing an enzyme called cytochrome P450 14-alpha-demethylase (CYP51), which is needed to make ergosterol, which is an important part of fungi's cell membrane [9]. By inhibiting this enzyme, azole derivatives disrupt the integrity and function of the fungal cell membrane, leading to fungal growth inhibition and ultimately, the death of the microorganism.
These compounds are effective against a broad range of fungal pathogens, including Candida species, Aspergillus species, Cryptococcus neoformans, and dermatophytes, among others.
From time to time, new drug discovery is necessary for pathogens due to various reasons. Firstly,the emergence of new infectious diseases and pathogens is a constant threat. Examples from the recent past include the SARS-CoV-2 virus-driven COVID-19 pandemic. These novel pathogens often lack effective treatments or vaccines, making it essential to develop new drugs to combat them effectively. Secondly, pathogens can develop resistance to existing drugs over time [10] [11]. New drug discovery endeavours seek to develop more effective treatments with better outcomes, such as higher cure rates, shorter treatment durations, and reduced side effects. Such advancements can significantly enhance patient care and quality of life.

Material And Methods
Material
The chemicals were procured from CDH Lab, Qualigens, and HiMedia. All the chemicals are of standard quality and

have a very good packing technique to avoid infection. Chemicals are: Aniline, phenylimide, Phenylhydrazine, 2,4-dinitrophenylhydrazine, 4-aminoantipyrine, 2- chloroacetyl chloride, 4-chlorobutyryl chloride, 2-aminobenzothiazole, Benzimidazole, Glacial acetic acid, Sodium acetate, Potassium Carbonate, and dimethylformamide. By employing the Thin Layer Chromatography (TLC) method on aluminium sheets of silica gel G obtained from Merck and chloroform: methanol (9:1) as the eluent, the purity of the substances was evaluated. A Shimadzu (UV-254) spectrometer and an iodine chamber were used to visualise the TLC spots of synthetic substances. For hoover filtration, ashless Whatman filter paper was employed. Compounds' melting points were determined using melting points apparatus. 1H-NMR spectra were obtained at 400 MHz with BRUKER, while IR spectra (of KBr discs and pellets) were recorded on a SHIMADZU FT-IR 8400 and reported in cm1. Chemical shifts are measured using DMSO/CDCl3 as the solvent, and are represented as parts per million (ppm) δ-values in relation to the internal standard trimethyl silane (TMS).

Synthesis of Compound:

Step 1:

Aniline (0.066 mol) was dissolved in 25 ml of glacial acetic acid. 2-chloroacetyl chloride (0.074 mol) was added dropwise to this solution while cooling in an ice bath. The reaction mixture was stirred in an ice bath for 30 minutes and 1 hour at room temperature. The mixture was poured into a saturated Sodium acetate solution. The precipitate of 2-chloro-N-phenylacetamide (Chloroanilide) was filtered, washed with cold water, and purified by crystallisation.

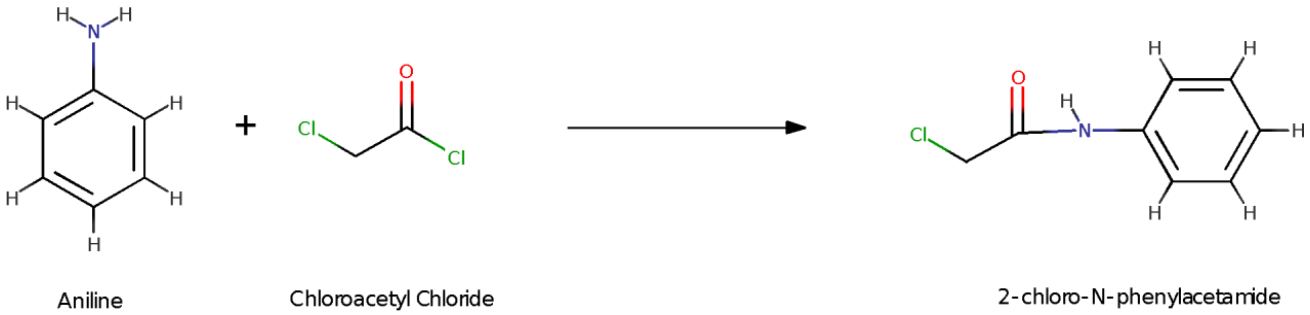


Figure 3: Synthesis of intermediate compound 2-chloro-N-phenylacetamide

Similarly, different combinations of molecules were synthesised by replacing Aniline with Phthalimide, phenylhydrazine, 2, 4-Dinitrophenylhydrazine, 4-Aminoantipyrine. Other compounds were created by substituting 4-Chlorobutyryl chloride for chloroacetyl chloride.

Step 2:

To a solution of 2-aminobenzothiazole (0.002 mol) in a suitable volume of DMF (Dimethylformamide) and then Potassium Carbonate (.006 mol) and the corresponding Chloroanilide (2-chloro-N-phenylacetamide) were added and refluxed for 10 h. Then the content was poured into crushed ice, and the resulting precipitate was filtered, dried, and recrystallized from the methanol or ethanol.

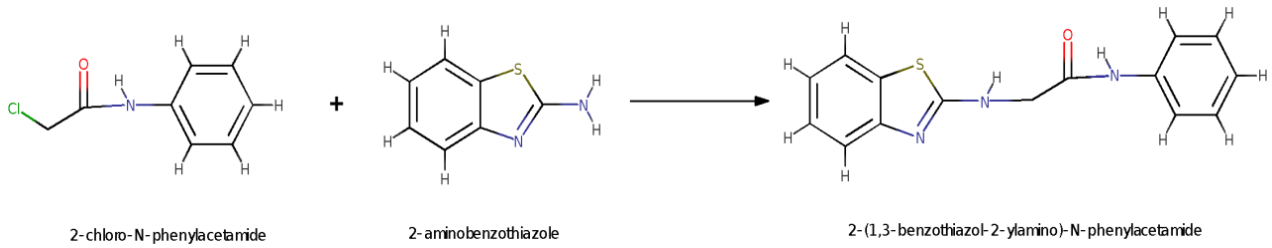
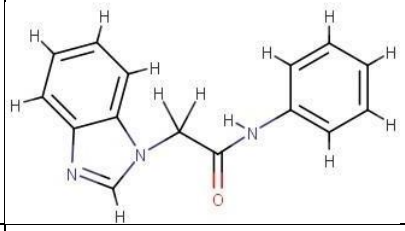
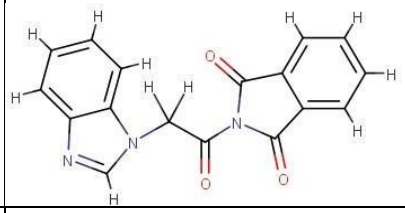
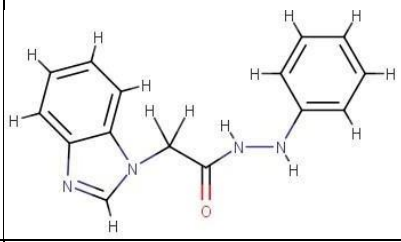
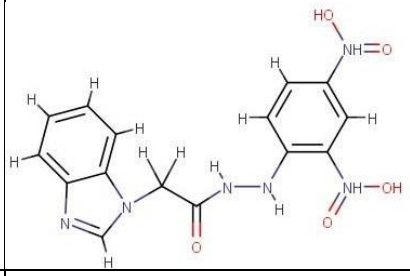
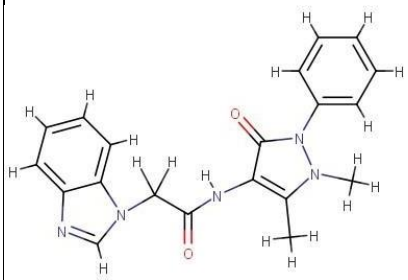
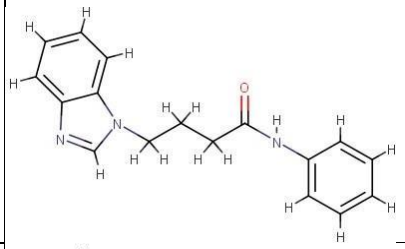
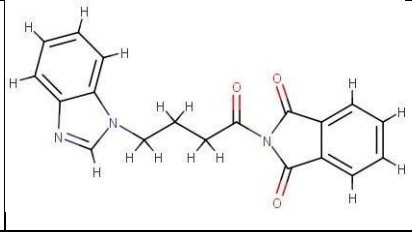


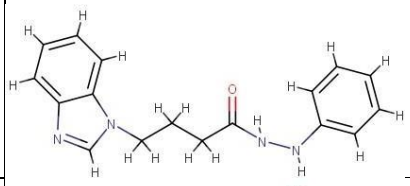
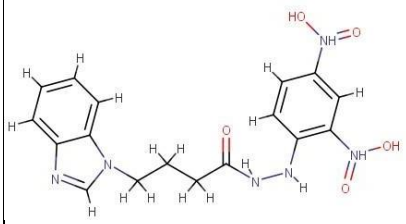
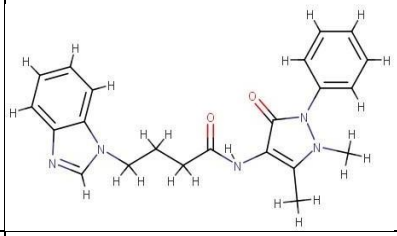
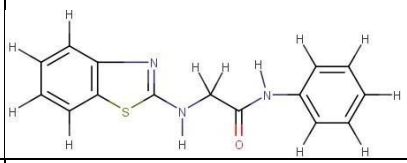
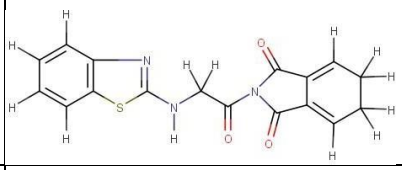
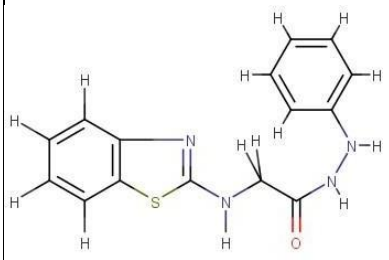
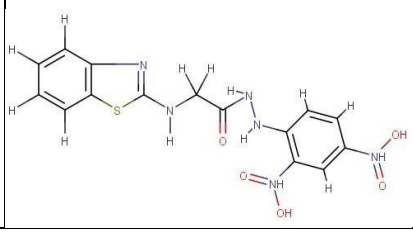
Figure 4: Synthesis of Azole Compound

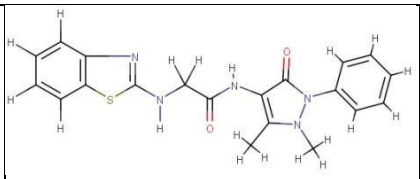
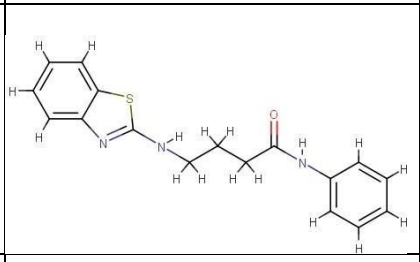
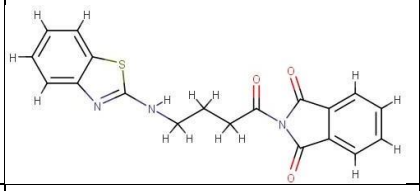
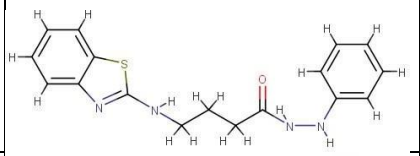
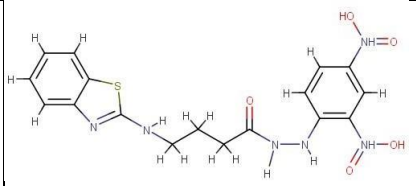
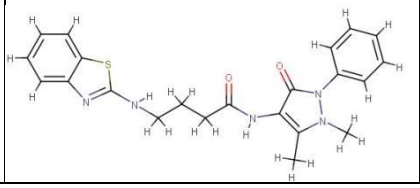
After synthesising 10 compounds with 2-aminobenzothiazole and Chloroanilide (prepared as the final compound in step 1), synthesise 10 more compounds by replacing 2-aminobenzothiazole with Benzimidazole.

Like this, synthesized up to 20 compounds in the lab. At each step to check the completion of thereaction, a TLC study was performed.

Table 1: List of ligand compound sketched on Marvin Tool (MarvinSketch).

S. No.	Abbreviation	Chemical Structure	IUPAC Name
1	B1		2-(1H-1,3-benzodiazol-1-yl)-N-phenylacetamide
2	B2		2-[2-(1H-1,3-benzodiazol-1-yl)acetyl]-2,3-dihydro-1H-isoindole-1,3-dione
3	B3		2-(1H-1,3-benzodiazol-1-yl)-N'-phenylacetohydrazide
4	B4		{2-[2-(1H-1,3-benzodiazol-1-yl)acetohydrazido]-5-(hydroxynitroso)phenyl}azinic acid
5	B5		2-(1H-1,3-benzodiazol-1-yl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide
6	Bb1		4-(1H-1,3-benzodiazol-1-yl)-N-phenylbutanamide
7	Bb2		2-[4-(1H-1,3-benzodiazol-1-yl)butanoyl]-2,3-dihydro-1H-isoindole-1,3-dione

8	Bb3		4-(1H-1,3-benzodiazol-1-yl)-N'-phenylbutanehydrazide
9	Bb4		{2-[4-(1H-1,3-benzodiazol-1-yl)butanehydrazido]-5-(hydroxynitroso)phenyl}azinic acid
10	Bb5		4-(1H-1,3-benzodiazol-1-yl)-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)butanamide
11	T1		2-[(1,3-benzothiazol-2yl)amino]-N-phenylacetamide
12	T2		2-{2-[(1,3-benzothiazol-2-yl)amino]acetyl}-2,3,5,6-tetrahydro-1H-isoindole-1,3-dione
13	T3		2-[(1,3-benzothiazol-2-yl)amino]-N'-phenylacetohydrazide
14	T4		(2-{2-[(1,3-benzothiazol-2-yl)amino]acetohydrazido}-5-(hydroxynitroso)phenyl)azinic acid

15	T5		2-[(1,3-benzothiazol-2-yl)amino]-N-(1, 5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide
16	Tt1		4-[(1,3-benzothiazol-2-yl)amino]-N-phenylbutanamide
17	Tt2		2-{4-[(1,3-benzothiazol-2-yl)amino]butanoyl}-2,3- dihydro-1H-isoindole-1,3-dione
18	Tt3		4-[(1,3-benzothiazol-2-yl)amino]-N'-phenylbutanehydrazide
19	Tt4		(2-{4-[(1,3-benzothiazol-2- yl)amino]butanehydrazido}-5-(hydroxynitroso)phenyl)azinic acid
20	Tt5		4-[(1,3-benzothiazol-2-yl)amino]-N-(1, 5-dimethyl-3- oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)butanamide

IR, and 1HNMR spectra of all synthesized compound were recorded.

Table 2: IR and 1H NMR Spectral Interpretation of Test compound

S. No.	**Compound**	**IR (KBr, cm^{-1})**	**1H NMR**
1	T1	3318.34 (N-H stretching), 1700 (C=O stretching), 686.25 (C-S stretching), 1620.25 (C=N stretching)	δ 3.76 (2H, s), 7.04-7.16 (2H, 7.12 (ddd, *J* = 7.8, 7.4, 1.3 Hz), 7.07 (tt, *J* = 7.8, 1.2 Hz)), 7.22-7.34 (3H, 7.30 (ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.27 (dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz)), 7.38 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.47 (2H, dddd, *J* = 8.2, 1.5, 1.2, 0.5 Hz), 7.78 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz).
2	T2	3326.32 (N-H stretching),	δ 2.64 (4H, dddd, *J* = 13.0, 9.7, 6.6, 4.3 Hz), 3.86 (2H, s), 7.09 (2H, dd, *J* = 6.6, 4.0 Hz),
		1698 (C=O stretching), 688.20 (C-S stretching), 1619.25 (C=N stretching)	7.22-7.34 (2H, 7.30 (ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.26 (ddd, *J* = 7.8, 7.4, 1.3 Hz)), 7.38 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.78 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
3	T3	3280.24 (N-H stretching), 1695.25 (C=O stretching), 691.25 (C-S stretching), 1630 (C=N	δ 3.75 (2H, s), 6.91 (1H, tt, *J* = 8.1, 1.1 Hz), 7.01-7.16 (3H, 7.05 (dddd, *J* = 8.2, 1.2, 1.1, 0.5 Hz), 7.12 (ddd, *J* = 7.8, 7.4, 1.3 Hz)), 7.17-7.34 (3H, 7.22 (dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.30

		stretching)	(ddd, *J* = 8.1, 7.4, 1.6 Hz)), 7.38 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.78 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
4	T4	3295.25 (N-H stretching), 1696.45 (C=O stretching), 689.35 (C-S stretching), 1628.65 (C=N stretching)	δ 3.75 (2H, s), 7.12 (1H, ddd, J = 7.8, 7.4, 1.3 Hz), 7.22-7.44 (2H, 7.30 (ddd, J = 8.1, 7.4, 1.6 Hz), 7.38 (ddd, J = 8.1, 1.3, 0.5 Hz)), 7.78 (1H, ddd, J = 7.8, 1.6, 0.5 Hz), 7.92 (1H, dd, J = 7.8, 0.5 Hz), 8.42 (1H, dd, J = 7.8, 1.9 Hz), 8.75(1H, dd, J = 1.9, 0.5 Hz)
5	T5	3290.45 (N-H stretching), 1690 (C=O stretching), 692.25 (C-S stretching), 1622.15 (C=N stretching)	δ 2.41 (3H, s), 3.49 (3H, s), 3.77 (2H, s), 7.12 (1H, ddd, *J* = 7.8, 7.4, 1.3 Hz), 7.25-7.43 (5H, 7.38 (ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.33 (tt, *J* = 7.6, 1.3 Hz), 7.30 (ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.40 (dddd, *J* = 8.2, 1.5, 1.3, 0.5 Hz)), 7.60 (2H, dddd, *J* = 8.2, 7.6, 1.5, 0.5 Hz), 7.78 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
6	Tt1	3312.65 (N-H stretching), 1691.25 (C=O stretching), 689 (C-S stretching), 1623.20 (C=N stretching)	δ 1.87 (2H, quint, *J* = 7.3 Hz), 2.37 (2H, t, *J* = 7.4 Hz), 3.35 (2H, t, *J* = 7.3 Hz), 7.04-7.16 (2H, 7.11 (ddd, *J* = 7.8, 7.4, 1.3 Hz), 7.07 (tt, *J* = 7.8, 1.2 Hz)), 7.21-7.32 (3H, 7.26 (ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.27 (dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz)), 7.36 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.47 (2H, dddd, *J* = 8.2, 1.5, 1.2, 0.5 Hz), 7.77 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
7	Tt2	3302.25 (N-H stretching), 1693.20 (C=O stretching), 687.45 (C-S stretching), 1621.25 (C=N stretching)	δ 1.94 (2H, tt, *J* = 7.4, 7.1 Hz), 2.62 (2H, t, *J* = 7.4 Hz), 3.34 (2H, t, *J* = 7.1 Hz), 7.11 (1H, ddd, *J* = 7.8, 7.4, 1.3 Hz), 7.26 (1H, ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.36 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.77 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz), 7.89 (2H, ddd, *J* = 7.8, 7.6, 1.3 Hz), 8.10 (2H, ddd, *J* = 7.8, 1.3, 0.5 Hz)
8	Tt3	3312.75 (N-H stretching), 1696 (C=O stretching), 689.65 (C-S stretching), 1622.75 (C=N stretching)	δ 1.87 (2H, quint, *J* = 7.3 Hz), 2.27 (2H, t, *J* = 7.4 Hz), 3.34 (2H, t, *J* = 7.3 Hz), 6.91 (1H, tt, *J* = 8.1, 1.1 Hz), 7.01-7.16 (3H, 7.05 (dddd, *J* = 8.2, 1.2, 1.1, 0.5 Hz), 7.11 (ddd, *J* = 7.8, 7.4, 1.3 Hz)), 7.17-7.31 (3H, 7.22 (dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.26 (ddd, *J* = 8.1, 7.4, 1.6 Hz)), 7.36 (1H, ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.77 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
9	Tt4	3293.30 (N-H stretching),	δ 1.87 (2H, tt, J = 7.5, 7.4 Hz), 2.31 (2H, t, J =
		1700 (C=O stretching), 687.25 (C-S stretching), 1627 (C=N stretching)	7.4 Hz), 3.39 (2H, t, J = 7.5 Hz), 7.11 (1H, ddd, J = 7.8, 7.4, 1.3 Hz), 7.19-7.43 (2H, 7.26 (ddd, J = 8.1, 7.4, 1.6 Hz), 7.36 (ddd, J = 8.1, 1.3, 0.5 Hz)), 7.77 (1H, ddd, J = 7.8, 1.6, 0.5 Hz), 7.92 (1H, dd, J = 7.8, 0.5 Hz), 8.42 (1H, dd, J = 7.8, 1.9 Hz), 8.75 (1H, dd, J = 1.9, 0.5 Hz)
10	Tt5	3297.78 (N-H stretching), 1690.25 (C=O stretching), 688.15 (C-S stretching), 1619.25 (C=N stretching)	δ 1.88 (2H, quint, *J* = 7.3 Hz), 2.38 (2H, t, *J* = 7.4 Hz), 2.41 (3H, s), 3.34 (2H, t, *J* = 7.3 Hz), 3.49 (3H, s), 7.11 (1H, ddd, *J* = 7.8, 7.4, 1.3 Hz), 7.21-7.43 (5H, 7.36 (ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.33 (tt, *J* = 7.6, 1.3 Hz), 7.26 (ddd, *J* = 8.1, 7.4, 1.6 Hz), 7.40 (dddd, *J* = 8.2, 1.5, 1.3, 0.5 Hz)), 7.60 (2H, dddd, *J* = 8.2, 7.6, 1.5, 0.5 Hz), 7.77 (1H, ddd, *J* = 7.8, 1.6, 0.5 Hz)
11	B1	3265.59 (N-H stretching), 1666.55 (C=O stretching), 1630.25 (C=N stretching)	δ 4.78 (2H, s), 6.91-7.10 (3H, 7.02 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 7.07 (tt, *J* = 7.8, 1.2 Hz), 6.96 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.27 (2H, dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz), 7.47 (2H, dddd, *J* = 8.2, 1.5, 1.2, 0.5 Hz), 7.64-7.75 (2H, 7.72 (dddd, *J* = 7.7, 1.3, 0.5, 0.5 Hz), 7.67 (ddt, *J* = 7.9, 1.2, 0.5Hz)), 8.04 (1H, t, *J* = 0.5 Hz)

12	B2	3276.25 (N-H stretching), 1656.42 (C=O stretching), 1635 (C=N stretching)	δ 5.10 (2H, s), 6.91-7.06 (2H, 7.02 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.96 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.64-7.75 (2H, 7.72 (dddd, *J* = 7.7, 1.3, 0.5, 0.5 Hz), 7.67 (ddt, *J* = 7.9, 1.2, 0.5 Hz)), 7.92 (2H, ddd, *J* = 7.8, 7.6, 1.3 Hz), 8.07-8.13 (3H, 8.10 (ddd, *J* = 7.8, 1.3, 0.5 Hz), 8.09 (t, *J* = 0.5 Hz)).
13	B3	3261.74 (N-H stretching), 1668.48 (C=O stretching), 1623.45 (C=N stretching)	δ 4.90 (2H, s), 6.91 (1H, tt, *J* = 8.1, 1.1 Hz), 6.91-7.08 (4H, 7.05 (dddd, *J* = 8.2, 1.2, 1.1, 0.5 Hz), 7.02 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.96 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.22 (2H, dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.64-7.75 (2H, 7.72 (dddd, *J* = 7.7, 1.3, 0.5, 0.5 Hz), 7.67 (ddt, *J* = 7.9, 1.2, 0.5 Hz)), 8.04 (1H, t, *J* = 0.5 Hz).
14	B4	3258.57 (N-H stretching), 1686.21 (C=O stretching), 1628.65 (C=N stretching)	δ 4.94 (2H, s), 6.90-7.09 (2H, 6.96 (td, J = 7.7, 1.2 Hz), 7.02 (ddd, J = 7.9, 7.6, 1.3 Hz)), 7.61-7.78 (2H, 7.67 (ddt, J = 7.9, 1.2, 0.5 Hz), 7.72 (ddt, J = 7.7, 1.3, 0.5 Hz)), 7.92 (1H, dd, J = 7.8, 0.5 Hz), 8.04 (1H, t, J = 0.5 Hz), 8.42 (1H, dd, J = 7.8, 1.9 Hz), 8.75 (1H, dd, J = 1.9, 0.5Hz).
15	B5	3319.60 (N-H stretching), 1691.63 (C=O stretching), 1639 (C=N stretching)	^{1}H NMR: δ 2.42 (3H, s), 3.49 (3H, s), 4.80 (2H, s), 6.91-7.06 (2H, 7.02 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.96 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.33 (1H, tt, *J* = 7.6, 1.3 Hz), 7.40 (2H, dddd, *J* = 8.2, 1.5, 1.3, 0.5 Hz), 7.55-7.75 (4H, 7.72 (dddd, *J* = 7.7,
			1.3, 0.5, 0.5 Hz), 7.67 (ddt, *J* = 7.9, 1.2, 0.5 Hz), 7.60 (dddd, *J* = 8.2, 7.6, 1.5, 0.5 Hz)), 8.04 (1H, t, *J* = 0.5 Hz).
16	Bb1	3311.47 (N-H stretching), 1654.72 (C=O stretching), 1642.45 (C=N stretching)	δ 2.11 (2H, quint, *J* = 7.4 Hz), 2.39 (2H, t, *J* = 7.4 Hz), 4.14 (2H, t, *J* = 7.4 Hz), 6.90-7.01 (2H, 6.96 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.95 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.06 (1H, tt, *J* = 7.8, 1.2 Hz), 7.27 (2H, dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz), 7.47 (2H, dddd, *J* = 8.2, 1.5, 1.2, 0.5 Hz), 7.62-7.73 (2H, 7.70 (ddt, *J* = 7.7, 1.3, 0.5 Hz), 7.65 (ddt, *J* = 7.9, 1.2, 0.5 Hz)), 8.01 (1H, t, *J* = 0.5Hz).
17	Bb2	3323.64 (N-H stretching), 1662.14 (C=O stretching), 1633.25 (C=N stretching)	δ 2.14 (2H, tt, *J* = 7.4, 6.9 Hz), 2.68 (2H, t, *J* = 7.4 Hz), 4.14 (2H, t, *J* = 6.9 Hz), 6.90-7.01 (2H, 6.96 (td, *J* = 7.9, 1.3 Hz), 6.95 (ddd, *J* = 7.9, 7.7, 1.2 Hz)), 7.62-7.73 (2H, 7.70 (ddt, *J* = 7.7, 1.3, 0.5 Hz), 7.65 (ddt, *J* = 7.9, 1.2, 0.5 Hz)), 7.89 (2H, ddd, *J* = 7.8, 7.6, 1.3 Hz), 8.01 (1H, t, *J* = 0.5 Hz), 8.10 (2H, ddd, *J* = 7.8, 1.3, 0.5Hz).
18	Bb3	3302.58 (N-H stretching), 1634.18 (C=O stretching), 1642.20 (C=N stretching)	δ 2.11 (2H, quint, *J* = 7.4 Hz), 2.27 (2H, t, *J* = 7.4 Hz), 4.14 (2H, t, *J* = 7.4 Hz), 6.88-7.01 (3H, 6.96 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.91 (tt, *J* = 8.1, 1.1 Hz), 6.95 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.05 (2H, dddd, *J* = 8.2, 1.2, 1.1, 0.5 Hz), 7.22 (2H, dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.62-7.73 (2H, 7.70 (ddt, *J* = 7.7, 1.3, 0.5 Hz), 7.65 (dddd, *J* = 7.9, 1.2, 0.5, 0.5 Hz)), 8.01 (1H, t, *J* = 0.5 Hz).
19	Bb4	3324.28 (N-H stretching), 1646.26 (C=O stretching), 1639.28 (C=N stretching)	δ 2.11 (2H, quint, J = 7.4 Hz), 2.34 (2H, t, J = 7.4 Hz), 4.14 (2H, t, J = 7.4 Hz), 6.88-7.04 (2H, 6.95 (td, J = 7.7, 1.2 Hz), 6.96 (ddd, J = 7.9, 7.6, 1.3 Hz)), 7.59-7.76 (2H, 7.65 (ddt, J = 7.9, 1.2, 0.5 Hz), 7.70 (ddt, J = 7.7, 1.3, 0.5 Hz)), 7.86-8.06 (2H, 7.92 (dd, J = 7.8, 0.5 Hz), 8.01 (t, J = 0.5 Hz)), 8.42 (1H, dd, J = 7.8, 1.9 Hz), 8.75(1H, dd, J = 1.9, 0.5 Hz)

20	Bb5	3381.33 (N-H stretching), 1608.69 (C=O stretching), 1627.35 (C=N stretching)	δ 2.11 (2H, quint, *J* = 7.4 Hz), 2.38-2.42 (5H, 2.40 (t, *J* = 7.4 Hz), 2.39 (s)), 3.49 (3H, s), 4.14 (2H, t, *J* = 7.4 Hz), 6.90-7.01 (2H, 6.96 (ddd, *J* = 7.9, 7.6, 1.3 Hz), 6.95 (ddd, *J* = 7.7, 7.6, 1.2 Hz)), 7.33 (1H, tt, *J* = 7.6, 1.3 Hz), 7.40 (2H, dddd, *J* = 8.2, 1.5, 1.3, 0.5 Hz), 7.55-7.73 (4H, 7.70 (ddt, *J* = 7.7, 1.3, 0.5 Hz), 7.65 (ddt, *J* = 7.9, 1.2, 0.5 Hz), 7.60 (dddd, *J* = 8.2, 7.6, 1.5, 0.5 Hz)), 8.01 (1H, t, *J* = 0.5 Hz).

Antimicrobial Activity:

The most common method for determining and estimating the effectiveness of diverse synthetic chemicals against multiple pathogenic and nonpathogenic bacteria was to utilise minimum inhibitory concentrations (MICs) [12] [13]. By using the two-fold serial dilution technique, the antibacterial activity of all compounds from 1 to 20 was assessed against different Gram-positivebacteria (B. subtilis, S. aureus) and Gram-negative bacteria (K. pneumonia, E. coli, P. aeruginosa, S. typhi). Standard fluoroquinolone antibiotics with good bacteriostatic action include ciprofloxacin and norfloxacin. Fluconazole served as the standard drug for the evaluation of the antifungal activity of all the synthesised compounds (1–20) against Candida albicans and Aspergillus niger.

Table 3: Antimicrobial activity details

Strain	**Gram-positive bacteria:***Bacillus subtilis* (MTCC121); *Staphylococcus aureus* (NCIM 2122) **Gram-negative bacteria:***Klebsiellla pneumonia*; *Escherichia coli* (MTCC118); *Pseudomonas aeruginosa* (MTCC647); *Salmonella typhi* (NCIM2501) **Fungi:** *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 1056)
Medium	Double strength nutrient broth
Method	Two fold serial dilution
Culture used	10^8 -10^7 CFU/mL
Test compounds	1-20
Standard	Ciprofloxacin, Norfloxacin, Fluconazole
Incubation Condition	35 - 37 ° C for 24 h
Growth assessment	Visual Observation
MIC	Lowest concentration tested that completely inhibited growth

Only a few of the test substances show antimicrobial activity comparable to that of standard drugs.

In Mueller-Hinton broth, a culture of distinct and unique microorganisms was produced [14]. Through a sterile diluent (often Mueller-Hinton broth), the antibacterial chemicals were diluted numerous times in a 1:1 ratio. To create the stock solution of 2000 µg/ml, all test chemicals weredissolved in DMSO at a concentration of around 10%. To create other concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml, these solutions were serially diluted [15] [16]. Bacteria were grown on Mueller-Hinton Broth nutrient medium, whereas fungi were grown on Sabouraud Dextrose Broth media. In sterile water, the cellular density of microorganisms was corrected to the 0.5 McFarland standard. For bacteria, the final concentration was ~10^7 CFU/mL, while for fungus, it was ~10^6 CFU/mL. The samples were diluted twice and introduced to the microbial inoculums. For bacteria, the diluted test tubes were cultured for 18–24 hours at 37°C ±1°C, and for fungi, for 2–5 days at 25°C ±1°C [17]. The value of the test compounds was given in µg/ml, and the MIC (Minimum Inhibitory Concentration) was defined as the maximum dilution of the test compounds that can totally block the growth of bacteria.

Results And Discussion

All the synthesised compounds (1-20) were evaluated for in vitro antibacterial activity against two strains of Gram-positive bacteria (B. subtilis, S. aureus) and Gram-negative bacteria (K. pneumonia, E. coli, P. aeruginosa, S. typhi) and two strains of the fungus (C. albicans, A. niger) by using the twofold serial dilution technique, and the results are summarised in Tables 4 and 5. For antibacterial action, Ciprofloxacin and Norfloxacin were utilised as the benchmark, and Fluconazole was used as the benchmark for antifungal activity.

The antimicrobial medications that are now on the market have many disadvantages, including toxicity and a limited range of activity. Some antibiotics also show drug-drug interactions. The desire for some good and efficient antibacterial medicines with a broad range of activity and acceptable pharmacokinetic characteristics has also grown due to the increasing risk of infection in immune-compromised individuals. Against diverse Gram-positive and Gram-negative bacterial strains, the majority of the compounds showed weak to moderate antibacterial activity. The

antimicrobial results showed that, albeit lower than that of standard compound, all compounds, ranging from 1 to 20, displayed substantial antibacterial activity against diverse strains. The synthesised test compounds like T1, T3, T4, B5, and Tt2 showed good activity against gram +ve bacteria, whereas T3, T4, T5, Bb1, and Tt5 showed good antibacterial activity against gram –ve bacteria. Table 4 summarises the antibacterial activity of substances (1–20) (MIC in µg/ml).

Table 4: Minimum Inhibitory Concentration (MIC) of Test Compounds 1 to 20 against *Bacillussubtilis*, *Staphylococcus aureus, Klebsiellla pneumonia*; *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*

S. No.	Test compounds	MIC(µg/ml)					
		Gram +ve bacteria		*Gram -ve bacteria*			
		B. subtilis (MTCC121)	*S. aureus* (NCIM 2122)	*K. pneumonia* (MTCC3384)	*E. coli* (MTCC118)	*P. aeruginosa* (MTCC647)	*S. typhi* (NCIM2501)
1	T1	3.125	6.25	6.25	6.25	12.5	6.25
2	T2	12.5	12.5	12.5	12.5	6.25	12.5
3	T3	6.25	3.125	6.25	12.5	12.5	25
4	T4	3.125	3.125	12.5	3.125	6.25	12.5
5	T5	6.25	6.25	12.5	6.25	6.25	3.125
6	Tt1	6.25	6.25	25	12.5	12.5	25
7	Tt2	6.25	3.125	12.5	12.5	12.5	12.5
8	Tt3	12.5	12.5	6.25	12.5	6.25	6.25
9	Tt4	6.25	6.25	12.5	12.5	12.5	12.5
10	Tt5	6.25	6.25	6.25	3.125	6.25	3.125
11	B1	12.5	6.25	6.25	6.25	6.25	6.25
12	B2	6.25	12.5	12.5	6.25	12.5	6.25
13	B3	12.5	12.5	25	12.5	25	25
14	B4	12.5	12.5	12.5	12.5	25	12.5
15	B5	3.125	6.25	6.25	6.25	6.25	6.25
16	Bb1	6.25	6.25	6.25	3.125	3.125	6.25
17	Bb2	6.25	6.25	25	12.5	12.5	6.25
18	Bb3	6.25	6.25	12.5	12.5	12.5	12.5
19	Bb4	6.25	6.25	6.25	6.25	6.25	6.25
20	Bb5	6.25	6.25	25	12.5	12.5	12.5
Ciprofloxacin (Standard)		≤1	≤1	≤1	≤1	≤1	≤1
Norfloxacin (Standard)		≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1

The antifungal activity of each of the synthesised compounds (1–20) was also tested against C. albicans and A. niger. Against C. albicans and A. niger, the majority of the compounds displayed extremely significant antifungal activity. When tested against C. albicans and A. niger, compounds T4, and Bb5 shown significant antifungal activity which was similar to that of regular fluconazole. Table 5 displays the compounds (1-20)'s antifungal activity (MIC in µg/ml).

Table 5: Minimum Inhibitory Concentration (MIC) of Test Compounds 1 to 20 against *Candidaalbicans* and *Asperigillus niger*

S. No.	Test compounds	MIC(µg/ml)	
		CandidaAlbicans (MTCC 227)	*AsperigillusNiger (NCIM 1056)*
1	T1	12.5	6.25
2	T2	12.5	12.5
3	T3	6.25	12.5
4	T4	6.25	3.125
5	T5	6.25	6.25
6	Tt1	6.25	6.25
7	Tt2	6.25	6.25
8	Tt3	12.5	12.5
9	Tt4	6.25	6.25
10	Tt5	6.25	6.25
11	B1	12.5	25
12	B2	6.25	12.5
13	B3	25	12.5
14	B4	12.5	12.5
15	B5	6.25	6.25
16	Bb1	12.5	12.5
17	Bb2	6.25	6.25
18	Bb3	12.5	25

19	Bb4	25	12.5
20	Bb5	3.125	6.25
Fluconazole (Standard)		3.125	3.125

Conclusion

The chemical structures of a group of 20 synthesised azole derivatives (1-20) were determined by IR and NMR. The two-fold serial dilution approach was used to assess synthesised compounds (1–20) for their antimicrobial (antibacterial and antifungal) properties. The results of the current investigations lead us to believe that this is a more recent molecular structure that might serve as a viable scaffold for the creation of broad-spectrum antibacterial medicines.

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