IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

THE BIOLOGICAL C HARACTERISTICS OF COMPOUNDS DERIVED FROM TRIAZOLE AND THEIR TRANSITION METAL COMPLEXES

Pallavi Sinha[1*] and Heena Innani[2]
[1]Research Scholar, Lords University, Alwar Rajasthan
[2]Assistant Professor, Lords University, Alwar Rajasthan

Corresponding author – Pallavi Sinha, Research Scholar, Lords University, Alwar Rajasthan

ABSTRACT

Synthetic organic chemists benefit greatly from modern efficient methods for the synthesis of azole derivatives. The main aim of the study is Biological Properties of Triazole Derived Compounds and Their Transition Metal Complexes. We can conclude that from the experiment, the target compounds were effectively obtained and characterized. Based on the results of their screening, chelation or coordination is followed by an increase in antibacterial and antifungal activities.

Keywords: Synthetic, Chelation, Triazole, Metal, Azole, Anti-bacterial, Anti-fungal

1. INTRODUCTION

Azole refers to a family of heterocyclic compounds with a nitrogen atom and at least one additional non-carbon element (other than hydrogen) in the ring's five carbon-sharing positions. Their naming convention may be traced back to the Hantzsch-Widman system. The original compounds are aromatic and include two double bonds, whereas the derivatives (azolines and azolidines) feature progressively fewer double bonds. In an azole, the aromatic bonding involves exactly one lone pair of electrons from each heteroatom in the ring. When azoles are reduced, their names retain their prefixes (such pyrazoline and pyrazolidine). In azoles, the ring atoms are numbered beginning with the heteroatom that is not involved in a double bond. Many pharmaceuticals, bioactive compounds, and natural products include azoles as an essential heterocyclic component. Synthetic organic chemists benefit greatly from modern efficient methods for the synthesis of azole derivatives. The significance of azoles in medicinal chemistry is already reported earlier (Eicher T, HaupVtmann S. 2003).

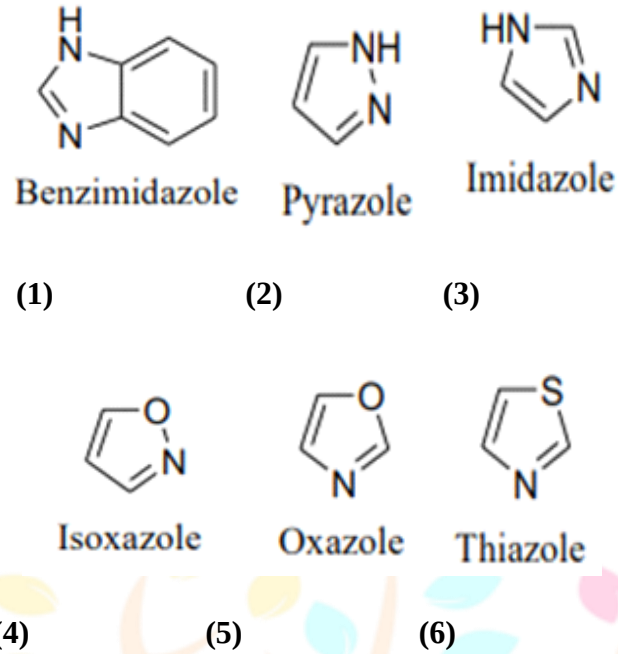


Fig. 1.1: Azoles

Nitrogen heterocyclic ring compounds with at least one additional non-carbon element, such as nitrogen, sulfur, or oxygen, are known as azoles. The original molecules feature two double bonds and are aromatic; from there, one may derive a series of progressively smaller analogues (azolines and azolidines). Each heteroatom in the azole ring contributes exactly one lone pair of electrons to the aromatic bonding. When azoles are reduced, their names keep their prefixes (for example, pyrazoline, pyrazolidine). In azoles, the ring atoms are numbered beginning with the heteroatom that is not involved in a double bond. Many azoles are utilized as antifungal medications because they block the production of ergosterol (a vital component of the fungal plasma membrane) by the enzyme 14-demethylase in the fungus.

1.2 Biological Activity Azole Derivatives

Benzimidazole (1), an azole molecule, was initially reported to have antifungal action in 1944 by Woolley, who was investigating the effects of biotin shortage in animals and microorganisms. He pointed out that biotin and purines had structural similarities to benzimidazole (1), although biotin was unable to counteract the drug's biological effects, but the purines adenine and guanine were. Woolley's original finding was largely dismissed since mycotic infections were of little interest in 1944, but his results were verified in 1949. After 30 years, Vanden Bossche noticed that another azole moiety with antifungal action, phenethylimidazole, interfered with the absorption of purines in yeast from Candida spp. The work of Woolley was resurrected in 1952 when Jerchel et al. revealed the considerable antifungal activity of several substituted benzimidazole (1) compounds. This study prompted other researchers to examine this class of compounds for potential antifungal therapeutic use. Chlormidazole, a chlorobenzyl imidazole, was produced and evaluated in clinical trials for the first time in 1958–1959. In the beginning, there was chlormidazole, the first azole derivative to be produced and commercialized as an antifungal medicine.

1.3 Overview of Heterocyclic Compounds

All or most of the atoms in the molecules of heterocyclic compounds, also known as heterocycles, are connected in rings that include at least one atom of an element other than carbon (C). The prefix hetero-

designates any atoms in the ring that are not carbon, while the cyclic component of heterocycles denotes the existence of at least one ring structure. While carbon remains the most common ring atom in heterocyclic compounds, there has been a steady expansion of this field to encompass compounds with a wider variety of heteroatoms in their rings.

To the untrained eye, heterocyclic compounds like cyclopropane (with three-carbon atoms in the ring) and benzene (with six-carbon atoms in the ring) are virtually indistinguishable from their all-carbon ring analogues. However, the presence of the heteroatoms gives heterocyclic compounds physical and chemical properties which might be regularly pretty exceptional from those of their all-carbon ring analogues. Many of the biological building blocks necessary for life are found in heterocyclic molecules. Nucleic acids, which transmit hereditary information, are composed of lengthy chains of heterocyclic devices bound together by a variety of chemical and physical forces. Heterocyclic compounds include the majority of hallucinogens and many naturally occurring colors, vitamins, and medicines.

A significant amount of interest in medicinal chemistry has focused on the discovery and development of novel triazole medicines with extended biological activity using the bioisosteric replacement approach. There are several drugs on the market that contain the triazole moiety, including: Myclobutanil, tebuconazole, posaconazole, itraconazole, fluconazole, paclobutrazole, and paclobutrazole are antifungal drugs. Anastrozole, litrozole, and vorozole are anticancer and antimigraine drugs. Rizatriptan with the antiviral ribavirin. The issue of antibiotic resistance among various bacteria strains is now plaguing our medical field.

2. LITERATURE REVIEW

S.Jubie,*et al.* **[2011]** developed several new ciprofloxacin analogues as antibiotics by chemical synthesis. Mannich reaction has been used to integrate ciprofloxacin into a novel family of 1,2,4-triazole Schiff bases. Antimicrobial activity of the novel compounds was tested in vitro at a 10 g/ml concentration against B. subtilis, K. pneumoniae, and P. aeruginosa. The in vitro gram-positive and gram-negative activity of all of the compounds was either on par with or even somewhat higher than that of the standard ciprofloxacin. Twenty-eight 4-amino-5-substituted aryl-3-mercapto-1,2,4-triazole derivatives were synthesized by Bijul Lakshman, and their in vitro efficacy against Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum, Pythium aphanidermatum, Puccinia recondite, and Bipolarissorokiana was evaluated.

Tomasz Plech *et al.* **[2011],** Some 1,4-disubstituted thiosemicarbazide compounds were synthesized rapidly and effectively, as disclosed. Thiosemicarbazide derivatives were produced in high yields from the reaction of 3-chlorobenzoic acid hydrazide with a variety of aryl isothiocyanates. Compounds with a 1,2,4-triazole ring were produced by cyclization in the presence of 2% NaOH. In addition, a group of novel Mannich bases with 1,2,4-triazole-like structures has been synthesized. The effect of substituent type and location on the antibacterial activity of the compounds reported was explored. New s-triazoles and Mannich bases were also synthesized. Especially against Gram-positive bacteria, certain compounds shown encouraging antibacterial efficacy.

Aniket Kshirsagar*et al*. **[2012]** synthesized Schiff's bases of 5-mercapto-3-(3-pyridyl)-4H-1,2,4-triazole-4-yl-thiosemicarbazidebymicrowaveassistedmethod.Thesynthesizedcompoundshavebeenevaluated*invitro*fortheir antibacterial,antifungalandanticonvulsantactivities.

R.K.Mali*etal*.**[2019]**Using Fluconazole as a standard, they synthesized 5-(N-substituted carboxamidomethylthio)-3-(3'-pyridyl)-1,2,4-triazole and tested its antifungal and antitubercular activity at 50 and 100 g/mL against Candida albicans and Aspergillus Niger, respectively. A series of new coumarin based 1,2,4-triazoles were synthesized and evaluated for antimicrobial activity in vitro against Gram-positive bacteria (Staphylococcus aureus, MRSA, Bacillus substilis and micrococcus luteus), and Gram-negative bacteria (Escheichia coli, Proteus vulgaris, Salmonella typi and Shigella dysenteriae) as well as fungi (Candida albicans, Sacchoromyces cerevisiae and Aspergillus fumigatus) by two-fold serial dilution techniques.

An Anees Siddiqui*etal*.**[2011]** they have produced some 4-[1-(aryl) methylidene-amino] Starting with isonicotinic acid hydrazide, potassium hydroxide, and carbon disulfide, we synthesized 3-(4- pyridyl)-5-mercapto-4H-1,2,4-triazole and tested it for analgesic and antipyretic effects. Rats given 25 mg/kg were tested for analgesic effects using the tail-flick technique, and antipyretic effects were tested using Brewer's yeast-induced pyrexia. The rectal temperature was taken using a clinical thermometer after a 20 ml/kg dose of a 20% aqueous solution of Brewer's yeast in normal saline was injected subcutaneously below the nape to produce fever. The antipyretic effect of several substances was compared to that of aspirin (300 mg/kg).

Due to its wide variety of biological applications, triazole has earned a special place among heterocyclic molecules in the field of chemistry. Analgesic, antiseptic, antibacterial, antioxidant, antiurease, anti-inflammatory, diuretic, anticancer, anticonvulsant, antidiabetic, and antimigraine properties are only some of the therapeutically intriguing drug possibilities in the 1,2,4-triazole class.

3. METHODOLOGY

IR, ^{1}H and ^{13}C NMR, mass spectrometry, magnetic susceptibility and conductivity tests, and CHN analytical data were used to analyze triazole derived Schiff bases and their metal complexes (cobalt(II), copper(II), nickel(II), and zinc(II)). X-ray diffraction was also used to identify the structure of L^2, also known as N-[(5-methylthiophen-2-yl)methylidene]-^{1}H-1,2,4-triazol-3-amine. In vitro tests for antibacterial, antifungal, and cytotoxic activities have been conducted on the triazole ligands and their metal complexes.

3.1 DESIGN, SYNTHESIS, AND BIOLOGICAL PROPERTIES OF TRIAZOLE DERIVED COMPOUNDS AND THEIR TRANSITION METAL COMPLEXES

The Analar grade reagents and solvents were utilized in all experiments. Chloride salts of all metals were used. A Fisher Johns melting point instrument was used to record the melting points. Shimadzu FT-IR spectra were acquired using an infrared (IR) spectrometer. We used a PerkinElmer model for our C, H, and N research. Using a Bruker Spectrospin Avance DPX-500 spectrometer, ^{1}H and in vitro studies on the antibacterial, antifungal, and cytotoxic effects of several compounds.

3.1.1 Synthesis of ligands

N-(Thiophen-2-ylmethylidene)-1H-1,2,4-triazol-3-amine (L1)

For 5 hours, 1.12 grams of thiophene-2-carboxaldehyde and 0.84 grams of 3-amino-1,2,4-triazole (10 mmol) were combined mixed in 40 milliliters of methanol while being monitored by thin layer chromatography (TLC). A fine off-white solid product separated from the clear solution after just 1 hour after the reaction mixture was cooled to room temperature and filtered. Recrystallization from hot ethanol

followed filtration, washing with methanol, drying, and repackaging. All additional ligands were synthesized using the same method.

3.1.2 Physical, analytical, and spectral data of the ligands (L1–L5)

N-(Thiophen-2-ylmethylidene)-1H-1,2,4-triazol-3-amine (L1)

Yield (1.26g, 71%); m.p. 172°C; IR (KBr, cm−1): 3175 (NH), 1628 (HC=N), 1611 (C=N, triazole), 1570, 1540 (C=C), 1020 (N-N), 960 (C-S); 1 H NMR (DMSO-d6): δ 7.24 (dd, 1H, J = 4.6, 4.0 Hz, C4 -H), 7.76 (d, 1H, J = 4.0 Hz, C3 -H), 7.83 (d, 1H, J = 4.6 Hz, C5 -H), 8.25 (s, 1H, C6 -H), 9.30 (s, 1H, triazole), 13.98 (s, 1H, triazole, NH); 13C NMR (DMSO-d6): δ 126.7 (C5), 129.5 (C4), 132.6 (C3), 143.6 (C2), 152.7 (C8), 156.2 (C6), 157.9 (C7); EIMS (70 eV) m/z (%): 178 ([M]+ , 77), 177 (100), 151 (6), 145 (11), 137 (15), 122 (12), 110 (20), 96 (11), 69 (16); Anal. Calcd. for C7 H6 N4 S (178.21): C, 47.18; H, 3.39; N, 31.44. Found: C, 47.30; H, 3.41; N, 31.35%.

3.1.3 X-ray structure of N-[(5-methylthiophen-2-yl) methylidene]-1H-1,2,4-triazol-3-amine (L2)

Here we authenticate our previous work by presenting Figures 3.1 and 3.2 showing the X-ray structure of one of the ligands, N-[(5- methylthiophen-2-yl)methylidene]-^{1}H-1,2,4-triazol3-amine(L^{2}). One of the ligands, N-[(5-methylthiophen-2-yl)methylidene]-^{1}H-1,2,4-triazol-3-amine (L2), has its X-ray structure identified and reported by us before.

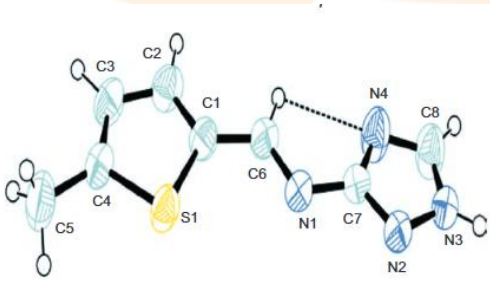


Figure 3.1 ORTEP diagram of a single molecule in asymmetric unit of L2.

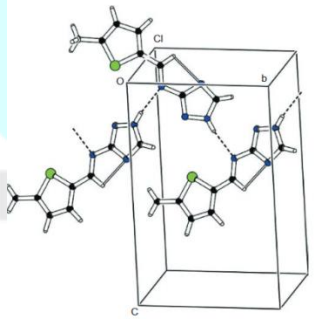


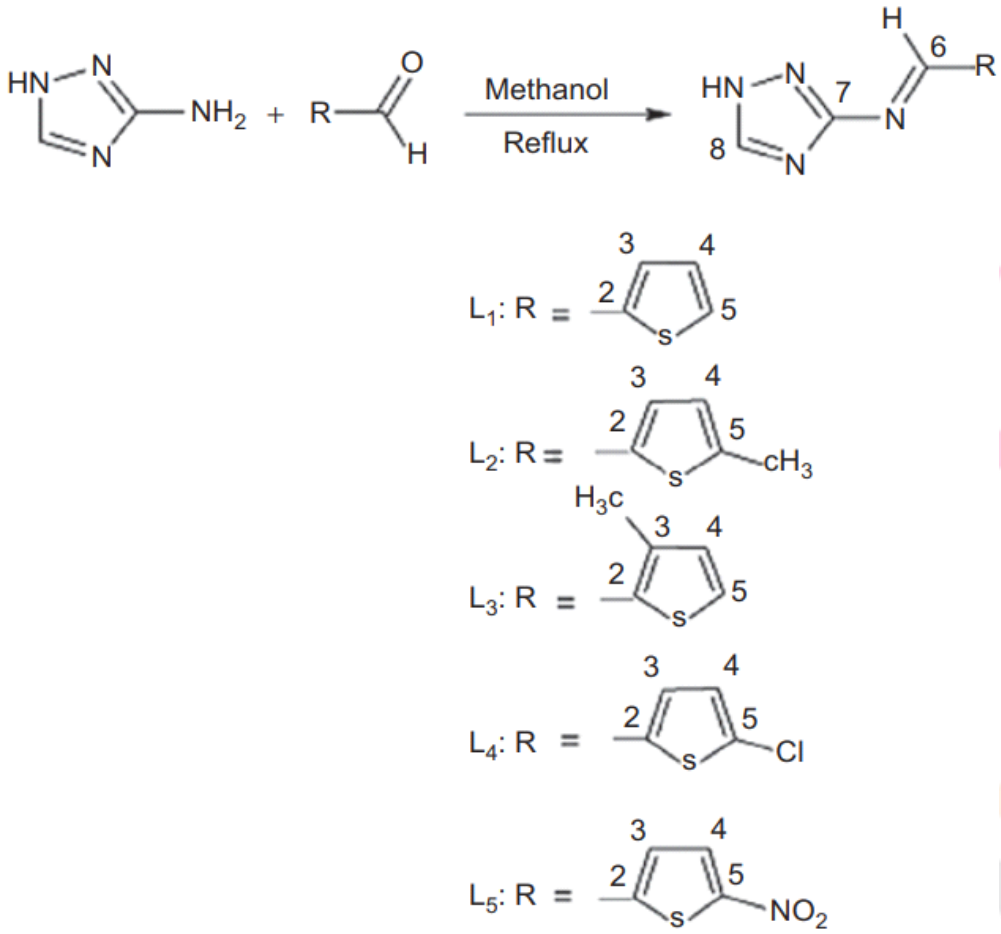
Figure 3.2 The unit cell packing in L2.

4. RESULTS AND DISCUSSION

4.1 DESIGN, SYNTHESIS, AND BIOLOGICAL PROPERTIES OF TRIAZOLE DERIVED COMPOUNDS AND THEIR TRANSITION METAL COMPLEXES

4.1.1 Chemistry

As illustrated in Scheme 1, the Schiff base derivatives of triazole (L1–L5) were produced by refluxing an adequate quantity of 3-amino-1,2,4-triazole with a succession of methyl-, chloro-, and nitro-substituted thiophene-2-carboxaldehydes. The only solvents that were capable of dissolving triazole derivatives were methanol, ethanol, dioxane, dimethylformamide, and dimethylsulfoxide. The findings that they obtained from microanalysis and mass spectrometry were in agreement with the compositions. Stoichiometric molar ratios of metal to ligand were used throughout the preparation of the metal(II) complexes (1–20).



Scheme 2 Preparation of ligands

Chlorides of cobalt, copper, nickel, and zinc were used in this process. The tables 4.1 and 4.2 provide the analytical data and physical measurements of complexes 1 through 20 respectively.

Table 4.1 Antibacterial bioassay (concentration used 1mg/mL of DMSO) of ligands and metal (II) complexes

Compound			Zone of inhibition (mm)							
	Gram-negative						Gram-positive			
	(a)	(b)		(c)	(d)		(e)		(f)	SA
L1	12	11		17	18		16		07	3.50
L2	18	12		18	09		14		17	3.33

L3	08	16		15	18		12		14	2.56
L4	12	08		15	17		16		17	2.67
L5	13	06		17	14		18		16	3.00
1	12	12		19	21		17		12	3.50
2	13	17		18	19		13		14	2.33
3	17	16		23	20		17		13	2.56
4	18	17		17	22		16		11	2.22
5	20	16		19	12		17		13	2.50
6	21	12		18	18		18		15	2.33
7	19	16		25	17		16		21	2.67
8	21	12		18	13		17		24	3.50
9	13	19		14	20		13		18	2.83
10	12	20		13	23		16		20	3.67
11	12	18		15	21		14		19	2.83
12	13	17		18	20		19		20	1.89
13	17	12		19	19		17		21	2.17
14	18	11		19	20		19		19	2.22
15	16	16		18	22		20		21	2.17
16	20	16		15	17		18		24	2.44
17	16	12		19	18		19		20	2.22
18	16	16		15	21		23		17	2.67
19	19	11		22	17		21		19	2.78
20	17	16		23	18		20		15	2.22
SD	**26**	**24**		**32**	**28**		**27**		**29**	**2.00**

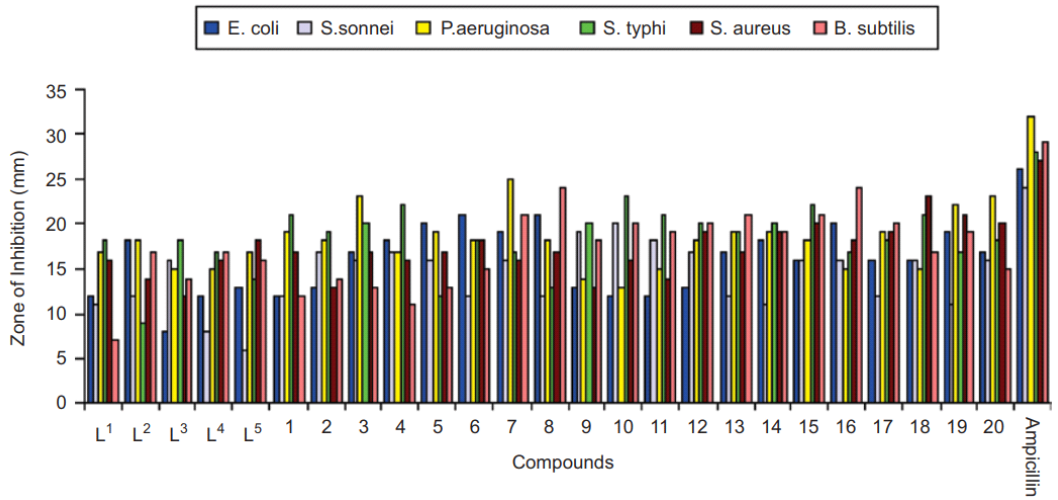


Figure 4.1 Comparison of antibacterial activity

Table 4.2 Antifungal bioassay (concentration used 200 µg/mL) of ligands and metal(II) complexes

	% Inhibition						
Compound	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	**SA**
L1	29	08	53	42	54	21	18.07
L2	46	22	29	10	39	54	13.00
L3	33	46	00	50	57	48	15.00
L4	24	42	08	62	39	47	14.00
L5	32	24	48	59	66	18	16.50
1	38	12	59	34	59	29	13.67
2	41	22	38	48	61	36	09.00
3	24	26	57	51	44	39	10.50
4	35	16	56	44	55	33	11.83
5	39	38	43	16	49	67	11.00
6	54	40	58	12	43	46	10.78
7	44	27	37	24	45	56	09.50
8	53	35	39	14	53	45	10.50
9	20	42	39	57	46	50	08.67
10	14	46	44	49	25	67	14.22
11	41	54	10	54	23	54	15.22
12	24	49	25	50	52	53	11.78
13	33	43	20	72	45	43	10.78
14	38	57	09	63	41	29	14.17
15	29	34	14	56	43	52	10.67
16	39	43	28	59	38	53	08.44
17	35	29	53	59	74	20	17.17
18	39	21	37	65	59	31	13.33
19	41	29	44	67	69	14	16.00
20	34	15	36	70	68	23	18.67
SD	A	B	C	D	E	F	

4.1.9 In vitro antifungal bioassay

According to the technique outlined in the relevant literature, an antifungal screening of all the compounds was performed using fungal strains including T. longifusus, C. albicans, A. flavus, M. canis, F. solani, and C. glabrata (Table 4). Some of the Schiff base derivatives of triazole exhibited either a low or no inhibitory impact, while others showed either a moderate to large degree of inhibitory effect on the development of the tested strains. On the other hand, the Schiff base L1 demonstrated significant activity (53–54%) against both (c) and (e), while L2 demonstrated significant activity (54%) against (f), L3 demonstrated significant

activity (57%) against (e), L4 demonstrated significant activity (62%) against (d), and L5 demonstrated significant activity (59–66%) against both (d) and (e). The results given in Table 4 show that compounds 6 and 8 possessed significant activity (53–54%) against (a), 11 and 14 showed significant activity (54–57%) against (b), 1, 3, 4, 6, and 17 had significant activity (54–59%) against (c), 9, 11, and 13–20 possessed significant activity (54–72%) against (d), 1, 2, 4, 8, and 17-20 had significant activity (53–74%) against (e), and 5, 7, 10, 11, 12, and 13 showed significant activity (53–67%) against (f). Most of the other compounds had moderate activity, while just a few of them displayed lesser activity against the fungal strains T. longifusus, C. albicans, A. flavus, M. canis, F. solani, and C. glabrata. Miconazole and amphotericin B44 were used as benchmarks, and the findings of the inhibition test were compared to those of the standard medicines (Figures 4.2 and 4.3).

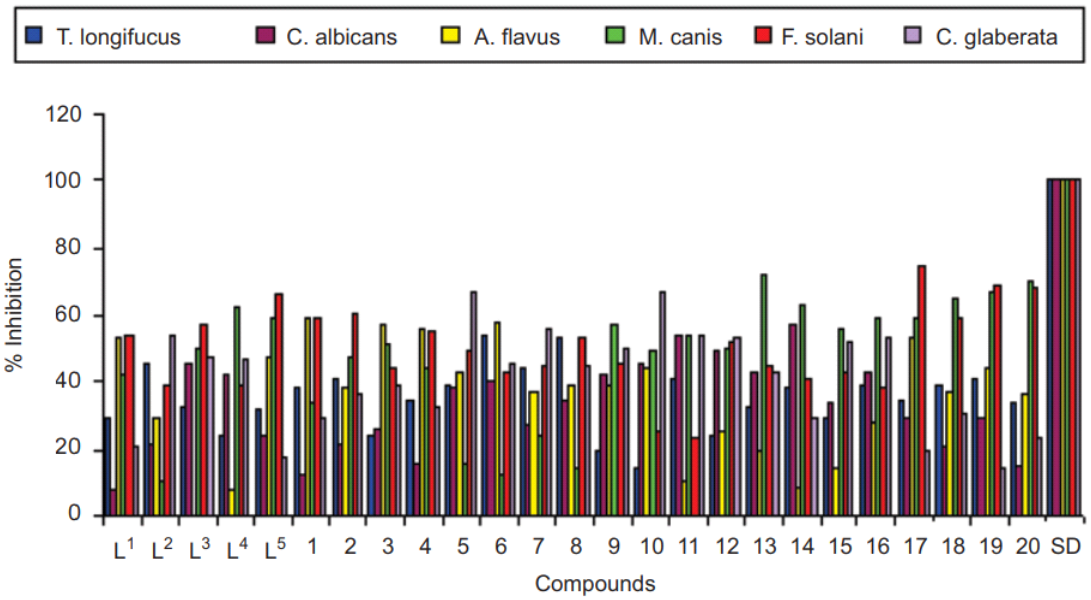


Figure 4.2 Comparison of antifungal activity.

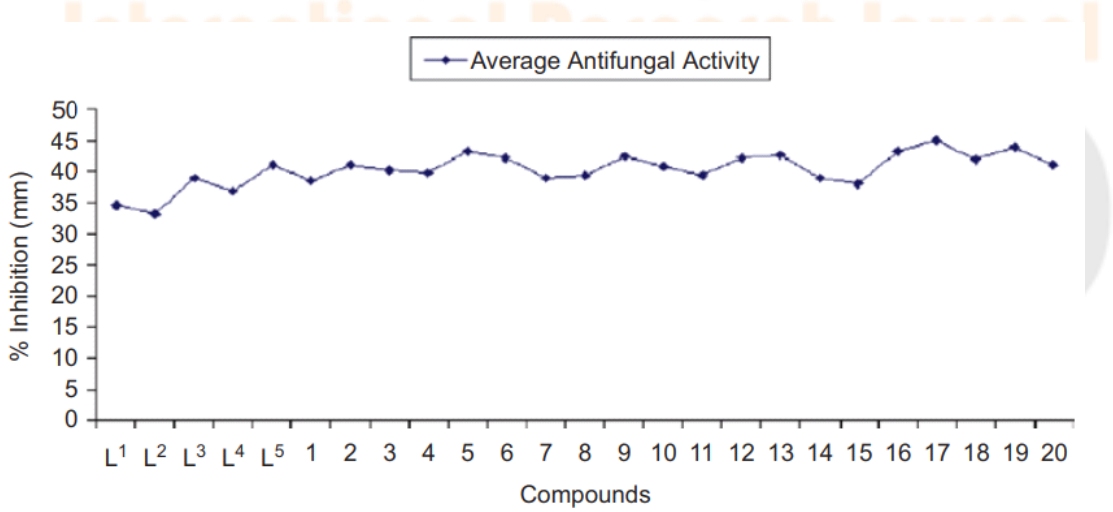


Figure 4.3 Average antifungal activity.

Table 4.3 Minimum inhibitory concentration (µg/mL) of selected compounds 6, 8, 10, 16, and 18 against selected bacteria.

	6	8	10	16	18

Gram-negative					
E.coli	60.66	44.37	—	—	—
S.sonnei	—	—	50.12	—	—
S.typhi	—	—	59.63	—	—
Gram-positive					
S.aureus	—	—	—	—	35.48
B.subtilis	—	56.76	—	70.66	—

4.1.10 Minimum inhibitory concentration (MIC)

Compounds 6, 8, 10, 16, and 18 were found to have antibacterial activity levels of more than 80 percent when compared to the other manufactured compounds that were tested. These findings were achieved following an initial screening. As a result, we decided to conduct research on these five drugs to determine the minimum inhibitory concentration (MIC) (Table 5). The minimum inhibitory concentration (MIC) of these compounds was found to be in the range of 35.48–70.66 g/mL. According to the findings of the MIC test, which are shown in Table 5, compound 18 was the most active. At a concentration of 35.48 g/mL, it was able to suppress the development of S. aureus.

4.1.11 In vitro cytotoxic bioassay

The ligands that were produced (L1–L5) and the metal(II) complexes of those ligands (1–20) were tested for their cytotoxicity using a bioassay using brine shrimp following the procedure developed by Meyer et al.45. Only six of the compounds, 3, 4, 7, 14, 15, and 20, demonstrated substantial cytotoxic action, with LD50 values ranging from 4.47 x 10-5 to 2.52 x 10-4 M, against Artenia salina. The other compounds were nearly completely inert in this test. The cytotoxic results were documented in Table 6. When compared to the ligands, it was intriguing to see that the metal complexes had much higher levels of cytotoxicity. According to the results that can be seen in Table 6, the cytotoxic activity of the copper complexes is much higher than that of the other metal complexes. This can be seen by looking at the values of LD50 46. This activity association may assist to serve as a foundation for future orientation toward the development of cytotoxic drugs for clinical use. This would be a step in the right direction.

Table 4.4 Brine shrimp bioassay data of the ligands (L1 –L5) and their metal(II) complexes (1–20).

Compound	LD_{50} (M/mL)
L^1	$>8.15\times10^{-4}$
L^2	$>8.66\times10^{-4}$
L^3	$>3.64\times10^{-4}$
L^4	$>4.51\times10^{-4}$
L^5	$>4.46\times10^{-4}$
1	$>7.54\times10^{-4}$
2	$>9.39\times10^{-4}$
3	4.47×10^{-5}
4	5.80×10^{-5}
5	5.48×10^{-4}
6	$>4.48\times10^{-4}$
7	1.92×10^{-4}
8	9.44×10^{-4}
9	$>6.32\times10^{-4}$
10	$>4.86\times10^{-4}$
11	$>8.16\times10^{-4}$
12	$>5.78\times10^{-4}$
13	$>9.08\times10^{-4}$
14	2.52×10^{-4}
15	4.61×10^{-5}
16	$>4.51\times10^{-4}$
17	$>4.94\times10^{-4}$
18	$>3.72\times10^{-4}$
19	$>4.59\times10^{-4}$
20	9.60×10^{-5}

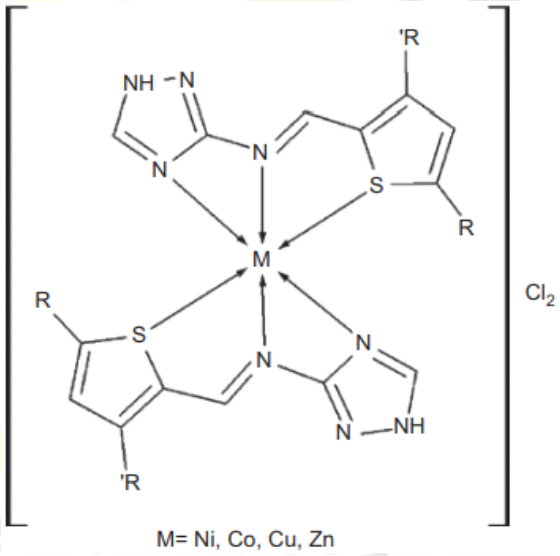


Figure 4.4 Proposed structure of the metal complexes.

5. CONCLUSION

The target compounds were successfully synthesized and characterized after the experiment. According to the findings of their screening, the antibacterial and antifungal activity rises after chelation or coordination has been performed. Chelation decreases the polarity of the metal ion, which ultimately results in an increase in the metal's lipophilic properties. This lipophilic quality that the metal ions encounter further boosts the effective penetration through the lipid layer of the cell membrane of the microbe, resulting in a

more efficient death of the bacteria. In addition, it has been hypothesized that the presence of heteroatoms or certain functional groups in the compounds, such as azomethine (HC=N), may play a significant part in the enhancement of the biological activity of the compounds that have been produced. Antimigraine, antioxidant, anti-urease, antimicrobial, anti-inflammatory, anticonvulsant, anticancer, antiviral, and antiparasitic properties are just a few of the many biological actions of 1,2,4-triazole that have piqued the interest of scientists and make it different from other works.

REFERENCE

1. S. Jubie, P. Sikdar, R. Kalirajan, B. Gowramma, S. Gomathy, S. Sankar, K. Elango, J. Pharm. Res., 2011, 3, 511.

2. Aniket. Kshirsagar, M. P. Toraskar, V. M. Kulkarni, S. Dhanashire, V. Kadam, Inter. J. ChemTech. Research, 2012, 1, 696.

3. R. K. Mali, R. R. Somani, M. P. Toraskar, K. K. Mali, P. P. Naik, P. Y. Shirodkar, Inter. J. ChemTech Research, 2019, 1, 168.

4. Anees, Siddiqui, R. Mishra, R. Kumar, M. Rashid, S. Khaidem, J Pharm. Bio. Sci., 2011, 2, 109.

5. J. A. Joule, K. Mills, G. F. Smith, Heterocyclic chemistry, 3rd Ed, 2015, page 452.

6. L. Harry, Yale, Kathryn, Losee, The Squibb Institute for Medical Research, New Jersey, 2015, p 478.

7. M. Amir, S. A. Javed, Kumar, Harish, Indian J. Chem., 2017, 46B, 1014.

8. H. Asif, A. Mohammed, Acta Pharma., 2019, 59, 223.

9. H. Kumar, A. Javed, Sadique, K. A. Suroor, Eur. J. Med. Chem., 2018, 43, 2688.

10. Woolley, D. W. 1944. Some biological effects produced by benzimidazole and their reversal by purines. J. Biol. Chem. 152:225-232.

11. Vanden Bossche, H., D. Bellens, W. Cools, J. Gorrens, P. Marichal, H. Verhoeven, G. Willemsens, R. De Coster, D. Beerens, C. Haelterman, M.-C. Coene, W. Lauwers, and L. Le Jeune. 1986. Cytochrome P-450: target for itraconazole. Drug Dev. Res. 8:287-298.

12. Jerchel, D., H. Fischer, and M. Fracht. 1952. Zur Darstellung der Benzimidazole. Liebigs Ann. Chem. 575:162-173.

13. Herrling, S., H. Sous, W. Kruppe, G. Osterloh, and H. Muckter. 1959. ExperimentelleUntersuchungenubereineneuegegenPilzewirksameVerbindung. Arneim. Forsch. 9:489-494

14. Seeliger, H. P. R. 1958. PilzemmendeWirkungeinesneuenBenzimidazol Derivatives. Mykosen 1:162-171.

15. Eicher T, Hauptmann S. The Chemistry of Heterocycles: Structure, Reactions, Synthesis, and Applications (2nd ed). John Wiley & Sons. June 2003, ISBN 3527307206.