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Synthesis And *In Vitro* Biological Evaluation Of Metal Complexes Containing 2-Aminothiazole

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Abstract
In the present study, Schiff bases and metal complexes containing 2-aminothiazole have been synthesized and characterized by physicochemical and spectral means. Broth dilution assay (96 well microtiter plate method) was used to evaluate antimicrobial activity of the synthesized compounds against Gram positive bacteria (*B. subtilis*), Gram negative bacteria (*E. coli*) and fungal species (*A. niger* and *A. terreus*) using cefadroxil (antibacterial) and fluconazole (antifungal) as standard drugs. Among the synthesized derivatives, Schiff base **EVL_1** showed good activity against *A. niger, A. terreus, B. subtilis* ($MIC_{an,at,bs}$ = 3.12 µg/ml). Complex **MV_1** showed good antifungal activity against *A. niger* (MIC_{an} = 1.56 µg/ml) similarly complex **MV_1** showed significant activity against *E. coli, B. subtilis* and *A. terreus* ($MIC_{ec,bs,at}$ = 3.12 µ/ml) and **MV_5** against *A. niger* (MIC_{an} = 1.56 µ/ml). Antioxidant (DPPH assay) 1,1-Diphenyl-2-picrylhydrazyl assay activity of the synthesized derivatives was determined using weight loss method. Schiff base **MV_1** showed excellent antioxidant activity (IC_{50} = 46.56).

Keywords: Aminothiazole, antibacterial, antifungal, antioxidant, Schiff's base, metal complxes

Introduction

Heterocyclic compounds are useful therapeutic agents which play an important role biologically as well as medicinally. Various researchers concluded that heterocyclic compounds play significant role in treatment of numerous life-threatening diseases such as nervous system disorders, tuberculosis, microbial infection and cancer. 2-aminothiazole based transition metals complexes are mainly used in coordination chemistry due to their high stability, low toxicity and compensating ability to different types of resistance with already existing drugs (Martins *et al*., 2015).

The inhibition of microbial growth serves as a crucial parameter for assessing the therapeutic efficacy of antimicrobial agents. However, the emergence and prevalence of microbial resistance have become significant challenges in improving health conditions worldwide. Consequently, there is a growing need to develop new therapeutically active compounds capable of overcoming this resistance problem (Mallikarjunaswamy C, et al., 2012).

In the pursuit of combating microbial resistance, compounds containing the 2-aminothiazole nucleus have shown remarkable effectiveness against a wide range of bacterial and fungal strains, including both Gram-positive and Gram-negative species. The unique structural features and functional groups present in these compounds contribute to their potent antimicrobial properties.

By harnessing the potential of 2-aminothiazole-containing compounds, researchers aim to develop novel therapeutics that can overcome microbial resistance mechanisms. The rational design and synthesis of these compounds allow for the exploration of different structural modifications and chemical functionalities to optimize their antimicrobial activity. Through such efforts, researchers strive to develop compounds that exhibit enhanced efficacy, improved pharmacokinetic profiles, and reduced susceptibility to resistance mechanisms.

Moreover, the versatility of the 2-aminothiazole nucleus allows for the development of targeted therapies. Researchers can design compounds that selectively target specific microbial species or strains, enabling more tailored and effective treatments. This approach helps minimize the indiscriminate use of broad-spectrum antimicrobials, which can contribute to the development of resistance.

The synthesis of new therapeutically active compounds, particularly those containing the 2-aminothiazole nucleus, holds great promise in combating microbial resistance. These compounds demonstrate efficacy against various Gram-positive, Gram-negative bacterial, and fungal strains. Continued research and development in this field are essential to overcome the challenges posed by microbial resistance and improve global health conditions. (Asif *et al*., 2012).

Naturally occurring oxidants possess remarkable potential in protecting cells from oxidative stress through diverse mechanisms and processes. Oxidative stress, known to contribute to various degenerative disorders, arises from an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant defense system. Antioxidants play a crucial role in mitigating oxidative stress by neutralizing ROS and preventing damage to cellular components.

The benefits of antioxidants to human health are substantial, as they help to alleviate the detrimental effects of oxidative stress. These compounds act as scavengers, intercepting and neutralizing harmful ROS, thereby reducing the risk of

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oxidative damage to biomolecules, including proteins, lipids, and DNA. By preventing or minimizing oxidative damage, antioxidants support cellular health and contribute to overall well-being.

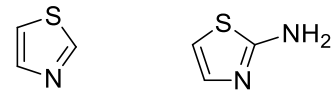
Assessing the antioxidant property of a compound is a significant area of research. Scientists employ various methods and assays to evaluate the effectiveness of compounds in scavenging ROS and inhibiting oxidative stress. These studies enable the identification and characterization of potential antioxidants, aiding in the development of therapeutic strategies to combat oxidative stress-related disorders.

The exploration of natural sources, such as plants, fruits, and vegetables, has revealed a plethora of antioxidant compounds with diverse chemical structures and properties. Researchers investigate these natural antioxidants, analyzing their mechanisms of action and potential benefits to human health. Additionally, synthetic compounds are designed and synthesized to mimic or enhance the antioxidant properties found in nature, expanding the range of potential therapeutic options.

As research in this field progresses, scientists continue to uncover novel antioxidants and elucidate their molecular mechanisms. This knowledge contributes to the development of preventive and therapeutic interventions aimed at reducing oxidative stress and mitigating the risk of degenerative diseases.

Naturally occurring oxidants provide significant protection against oxidative stress by employing various routes and processes within cells. These antioxidants offer valuable benefits to human health by mitigating oxidative damage. The investigation of compounds with antioxidant properties is an essential field of research, facilitating the identification of potential therapeutic agents. Continued advancements in this area hold promise for enhancing our understanding of oxidative stress-related disorders and developing effective strategies for their prevention and treatment. (Belin *et al*., 2003).

A significant number of Schiff bases and their metal complexes have been found to possess biological and catalytic properties (Soliman et al 2013). It is widely recognized that N and S atoms play a crucial role in coordinating metals at the active sites of numerous metallobiomolecules. These compounds are employed as models for biological systems and have shown promise in biomimetic catalytic reactions (Chandra *et al*.,2004).



Thiazole 2-Aminothiazole

Fig. 1: Chemical structure of thiazole and 2-aminothiazole

Table 1. Physico-chemical properties of 2-aminothiazole

Property	**Descriptor**
Chemical Formula	$C_3H_4N_2S$
Molecular Weight	100.14 g/mol
Appearance	Colourless to light yellow crystals/powder
Melting Point	108-110 °C
Boiling Point	315-320 °C
Density	1.353 g/cm³
Solubility	Soluble in water and most organic solvents
pKa	2.28 (acidic)
Odor	Characteristic Odor
Stability	Stable under normal conditions

Heterocycles, particularly thiazoles, are of great importance due to their diverse bioactivities attributed to the presence of multifunctional groups. Among them, 2-aminothiazoles stand out as an incredibly versatile group of compounds, exhibiting numerous biological activities and finding recent applications in drug development. Schiff base and their metal complexes which is derived from the 2-amino thiazole are also showing diverse bioactivities (Das Det al 2016) Thiazoles and their derivatives play a crucial role as components of vitamin B1 and coenzyme carboxylase, making them essential for several biological processes. These compounds belong to a fascinating class of substances renowned for their diverse applications, including antimicrobial, anti-inflammatory, anti-degenerative, and anti-HIV activities (Neelakantan MA et al., 2008)

Due to their wide range of biological properties, thiazoles and their derivatives have attracted considerable attention from researchers, who aim to explore their potential in developing novel therapeutic agents and pharmaceuticals. This versatile group of compounds holds great promise in addressing various health-related challenges and advancing the field of medicine. (Shirodkar *et al*.,2004).

Metal complexes with these ligands are gaining increasing significance in various fields such as biochemical research, analytical chemistry, and antimicrobial applications. They serve as valuable reagents in the design of molecular models and materials in the field of chemistry.

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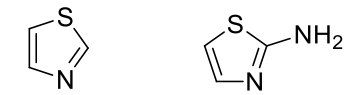
Famotidine is a notable example of a drug available in the market for the treatment of peptic ulcers and gastro-esophageal reflux, and it features the 2-aminothiazole nucleus in its structure. In addition to famotidine, there are several other drugs that incorporate the 2-aminothiazole nucleus (Laine *et al*., 2012) and offer therapeutic benefits in various medical conditions.

For instance, abafungin is a drug employed for the treatment of dermatomycoses (Borelli *et al*., 2008), while cefdinir belongs to the third generation of cephalosporins and exhibits broad-spectrum semi-synthetic antibiotic activity (Guay *et al*., 2002). Another noteworthy example is meloxicam, which belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) (Lugar *et al*., 1996).



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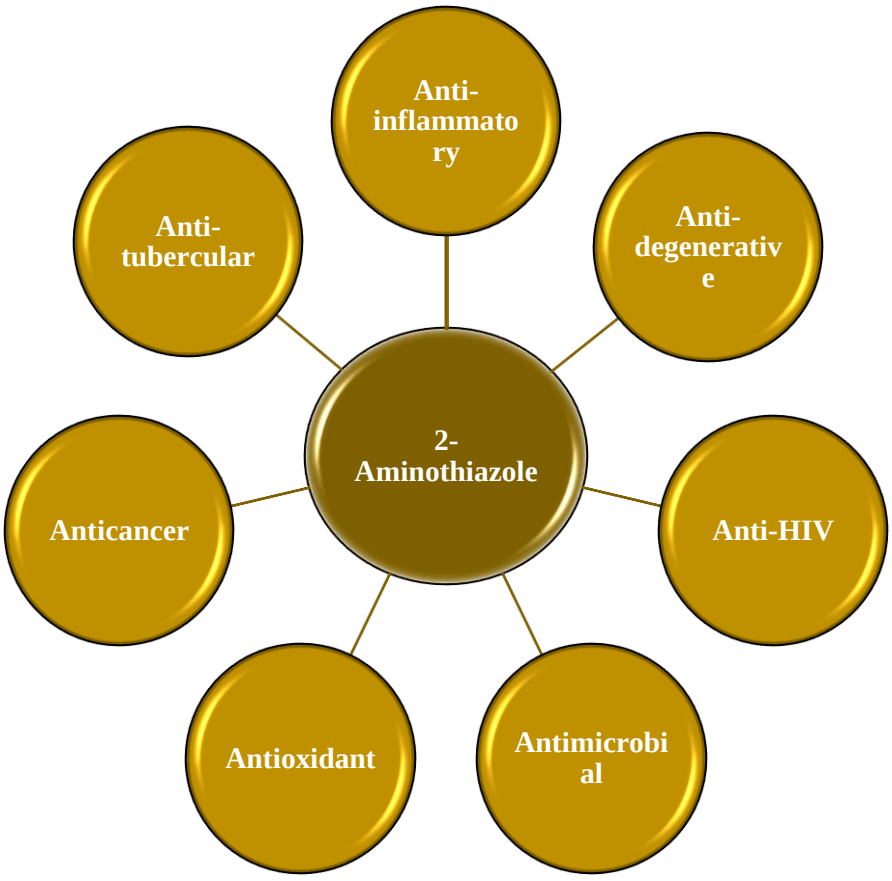

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Thiazole derivatives have gained significant attention due to their potential antitumor and cytotoxic effects. Many of these derivatives have been specifically designed to target and modulate specific pathways involved in cancer. Dabrafenib and dasatinib exemplify thiazole-containing compounds that have progressed to clinical trials and are now utilized in cancer therapy. These compounds possess tyrosine kinase inhibitory activity, making them valuable in combating certain types of cancers.

The utilization of thiazole derivatives in the development of therapeutic agents highlights their importance in the field of medicine. The distinct structural characteristics of thiazole-containing compounds allow for the design and synthesis of molecules with specific pharmacological properties, enabling researchers to explore new avenues for disease treatment and management. Continued research and investigation into the potential of thiazole derivatives are expected to yield further insights and advancements in the field of drug discovery and cancer therapy (Ayati *et al*., 2019). Some marketed formulations of 2-aminothiazole moiety are given in **Fig. 3.**

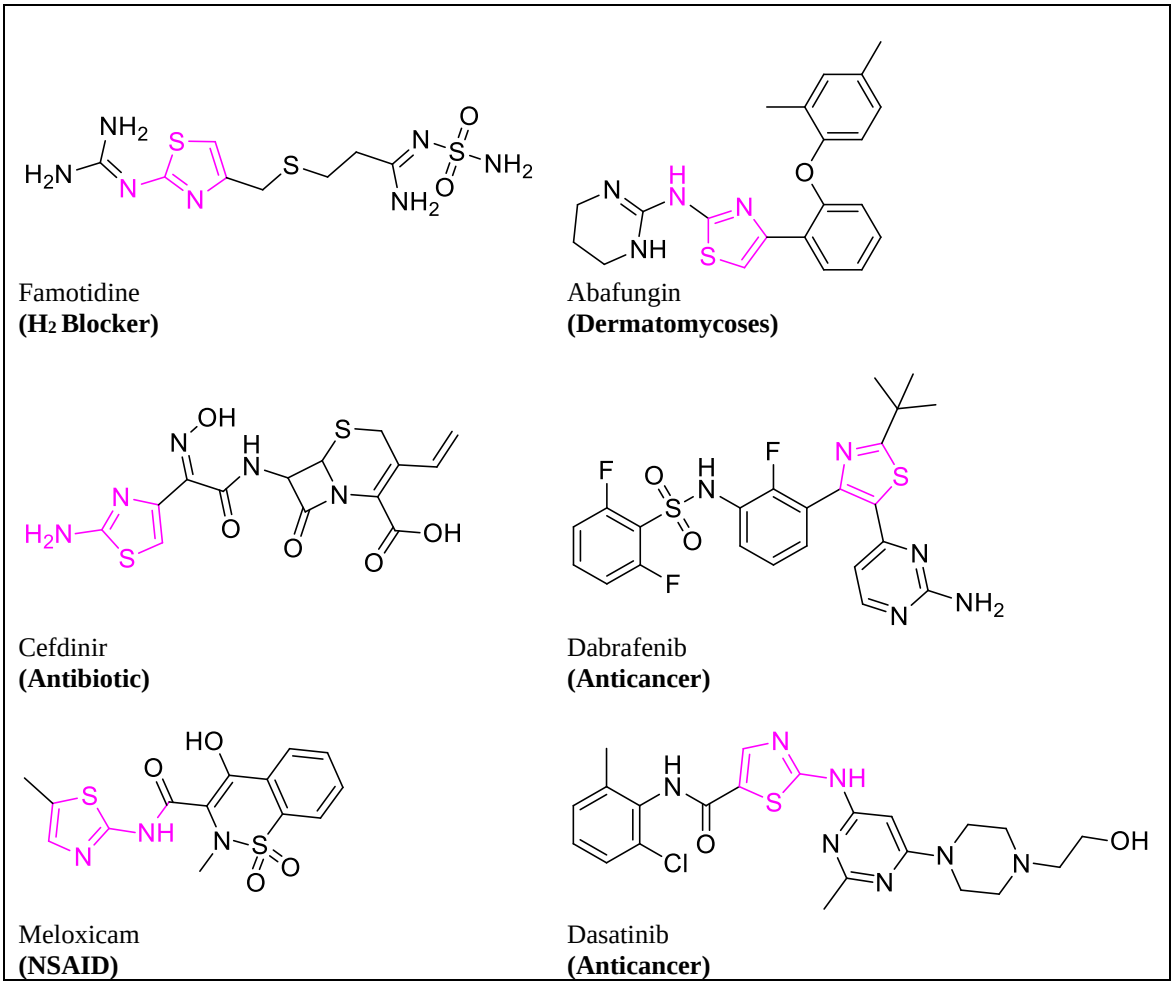

Fig. 3: Marketed formulations containing 2-aminothiazole moiety

1. Experimental

1.1. *Materials and Method*

The starting materials were acquired from different sources (CDH Pvt. Ltd, Loba Chemie Pvt. Ltd, HiMedia Laboratories Pvt. Ltd.). The completion of a chemical reaction was monitored by TLC. Silica gel G was used to prepare TLC plates used as stationary phase and chloroform: toluene, ethyl acetate: n-hexane and benzene as mobile phases for synthesized derivatives. Sonar melting point apparatus (Sunbim, India) was used to determine melting points of the synthesized derivatives. ^{1}H-Nuclear magnetic resonance was recorded at 600 MHz on Bruker Avance III 600 NMR spectrometer by appropriate deuterated solvents (DMSO). Infra-red Bruker 12060280, Software: OPUS 7.2.139.1294 spectrophotometer was used to evaluate IR spectra (4000-400 cm^{-1}). The thermo scientific multiscan FC elisa plate reader and scant software 7.0.2 RE was used to measure the absorbance of 96 well microtiter plate. DPPH assay method was used to determine antioxidant activity of the synthesized derivatives. The NMR data of compounds is specified as multiplicity singlet (s), doublet (d), triplet (t) and multiplet (m) number of protons present in compounds.

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1.2. ***Synthetic procedure for synthesis of 2-aminothiazole derivatives:***

Step a: Synthesis of Schiff base (EVL_1-TPL_2)

A methanolic solution of 2-aminothiazole (0.01 mol) and corresponding substituted benzaldehyde (0.01 mol) (3-ethoxy-4-hydroxybenzaldehyde and 1,4-Benzenedicarboxaldehyde) was refluxed for 4-5h in presence of few drops of glacial acetic acid. Reaction mixture was cooled in ice cold water and the precipitated Schiff base was separated, dried over anhydrous $CaCl_2$ and recrystallized in methanol. The completion of reaction was monitored by using TLC method [18].

Step d: Synthesis of Metal complexes (MV_1-MV_8)

After that methanolic solution of metal chlorides (0.01 mol) [$CuCl_2$, $CoCl_2$, $ZnCl_2$ and $NiCl_2$] was added in above Schiff's base (0.02 mol), and refluxed for 6-7h. After completion of reaction, the mixture was cooled in ice cold water, filtered and the resultant solid product was dried over anhydrous $CaCl_2$. The completion of reaction was monitored by using TLC method [18].

1.3 In vitro antimicrobial evaluation

An antimicrobial agent refers to a substance that impedes the growth or eliminates microorganisms, including bacteria, fungi, and protozoans. These substances can either halt the growth of microorganisms or result in their death.

1.3.1 Method for antimicrobial evaluation

Pure cultures were employed to assess the in vitro antimicrobial activity of the synthesized compounds (Klemba et al., 2003). The assessment of antimicrobial activity is crucial in understanding the effectiveness of various compounds against microorganisms. In vitro antimicrobial assays provide valuable insights into the potential of antimicrobial agents to inhibit the growth or kill microorganisms, such as bacteria, fungi, and protozoans.

In vitro antimicrobial assays play a crucial role in the development of new antimicrobial agents and the evaluation of their efficacy. These assays aid in identifying potential candidates for further investigation, guiding drug formulation and dosage determination, and assessing the development of antimicrobial resistance. Additionally, in vitro assays serve as preliminary screening tools in research and development, providing a cost-effective and time-efficient means of evaluating numerous compounds.

By determining the MIC, MBC/MFC, and inhibition zones, these assays provide valuable information on the potential of compounds to inhibit microbial growth or cause microbial death. With their widespread application in research, pharmaceutical development, and clinical microbiology, in vitro antimicrobial assays continue to contribute significantly to our understanding of antimicrobial agents and their impact on combating microbial infections. (Andrews *et al*., 2001)

Two different methods were utilized to determine the susceptibility of microorganisms to these compounds in a laboratory setting.

- Diffusion through agar media.
- Tube Dilution method (Broth micro-dilution method)

Synthesized metal complexes of 2-aminothiazole were screened for their *in vitro* antimicrobial activity against bacterial strains: Gram positive bacteria- *B. subtilis* (MTCC 121) and Gram-negative bacteria *E. coli* (MTCC 443) and fungal strains: *A. niger* (MTCC 281) and *A. terreus* using broth microdilution method (96 well microtiter plate technique).

The nutrient broth media and sabouraud dextrose broth were prepared by dissolving 2.5 grams of nutrient broth powder in 100 ml of distilled water. The prepared media was sterilized by autoclaving for 30 minutes at 121°C. Revive bacterial strains (*E. coli* and *B. subtilis*) and fungal strain (*A. niger and A. terreus*) by sub-culturing them in nutrient broth media incubate the cultures in a vortex shaker incubator at 37°C for 24 hours and 25°C for 7 days respectively. A stock solution (1000 µg/ml) of the synthesized compounds and standard drugs (cefadroxil and cefadroxil) using dimethyl sulfoxide (DMSO) as the solvent was prepared.

The wells of a 96-well microtiter plate with 50 µL of the media was filled, leaving the last two wells as positive and negative controls for the entire procedure. The positive control contains 100 µl of the microbial inoculum, while the negative control contains both media and inoculum in equal quantities. (50 µL).

Pipette 100 µl of the prepared sample into the first well of each row. Serial dilution was performed by transferring 50 µL of the sample from the first well of each row to the second well, and subsequently from the second well to the third well, resulting in various concentrations of the solution (i.e., 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml, 0.78 µg/ml, 0.38 µg/ml).

The revived microbial strains were added to each well, excluding the last well of each row, which serves as the positive control. All 96-well plates were covered with parafilm to ensure a sterile environment. All the plates were incubated to allow for proper microbial growth and interaction with the samples. The absorbance was measured of each well using an ELISA plate reader at a wavelength of 620 nm to assess the bacterial response to the samples. (Tang *et al*., 2021)

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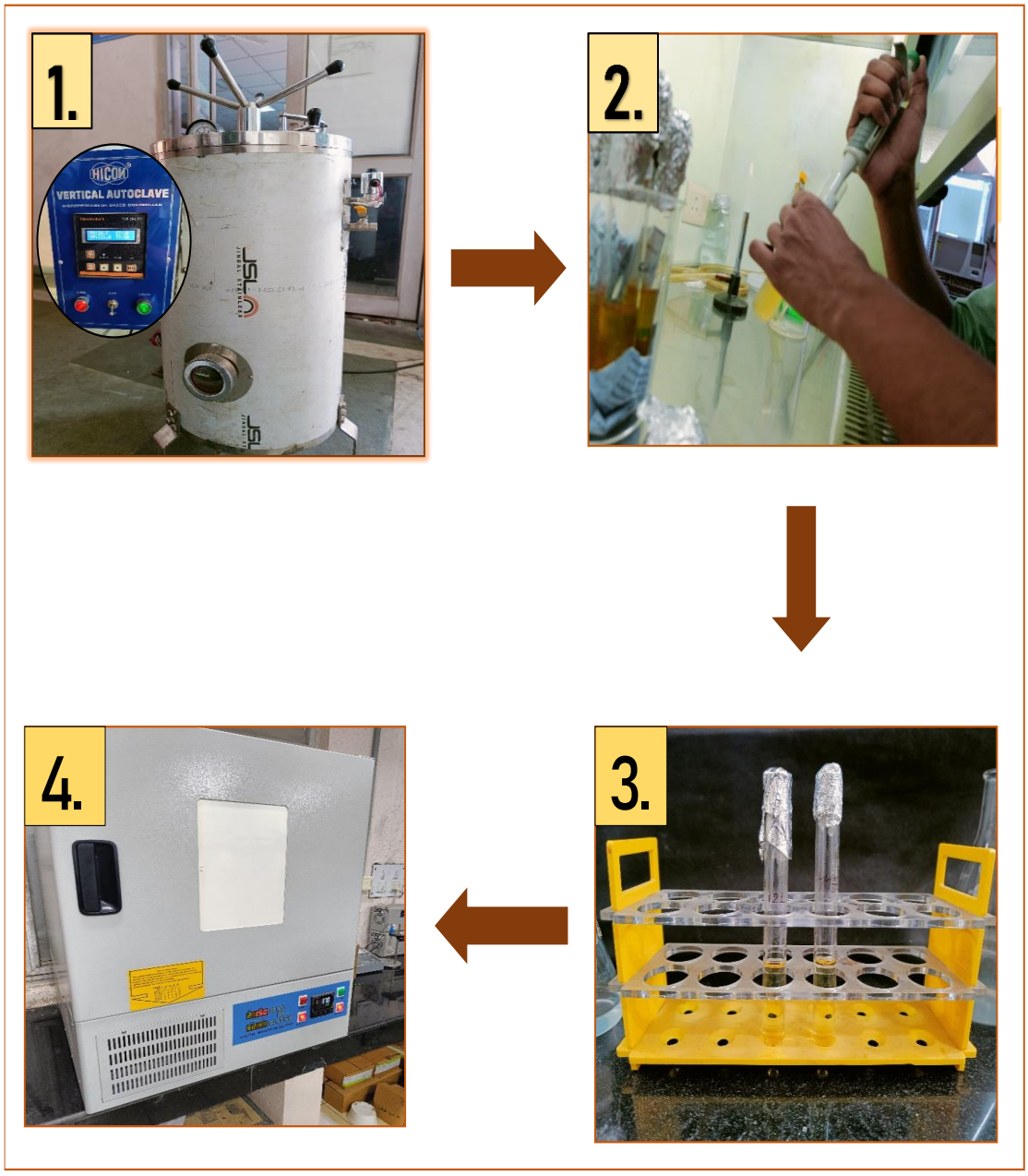
Bacterial strain (*E. coli*)	37±1º C for 24 h
Bacterial strain (*B. subtilis*)	37±1º C for 24 h
Fungal strain (*A. niger*)	25±1º C for 7 days
Fungal strain (*A. terreus*)	25±1º C for 7 days



STEPS INVOLVED IN NUTREINT AND SABOURAUD DEXTROSE BROTH ASSAY
Step 1: Preparation & Sterilization of broth media.
Step 2: Revival of Microbes.
Step 3 & 4: Incubation of test tubes containing nutrient broth or sabouraud dextrose broth media & inactive microbes.

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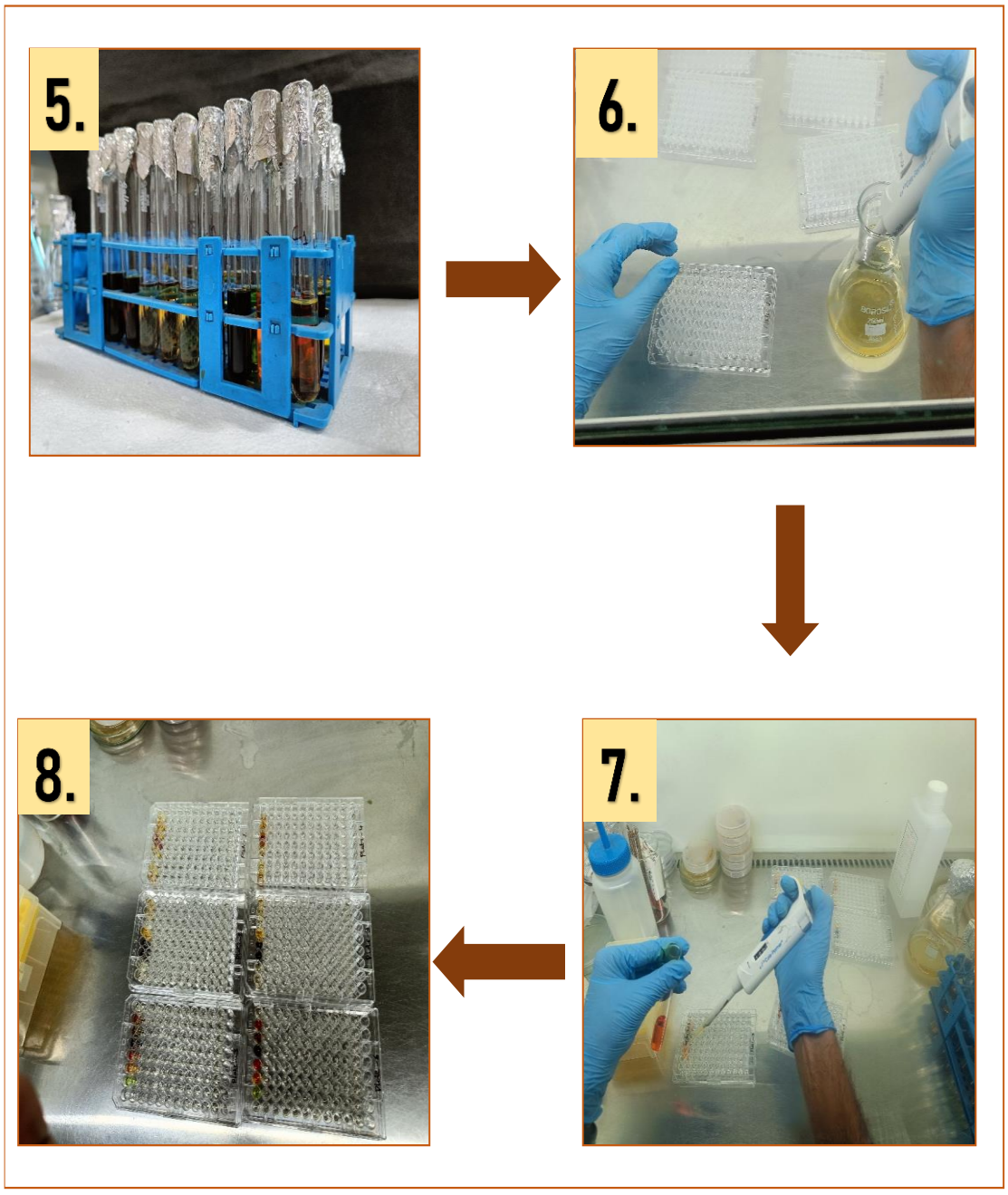

Step 5: Preparation of sample using DMSO as the solvent.
Step 6: Preparation of microplate wells using sterilized broth media and positive and negative controls.
Step 7: Pouring of sample into 96-well plate.
Step 8: Plate containing sample and sterilized broth media.

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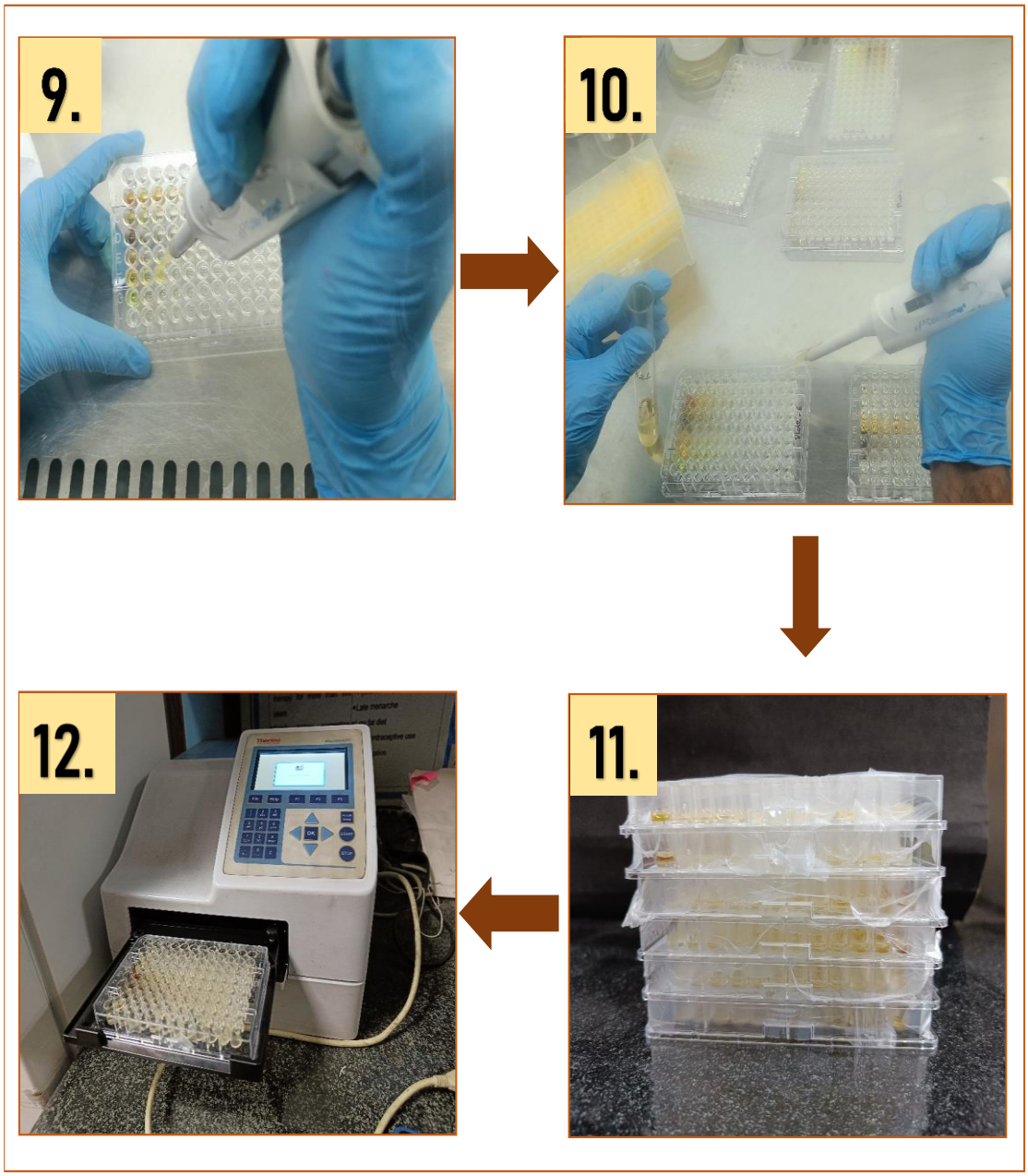

Step 9: Serial Dilution process to create concentration gradient.
Step 10: Inoculation of Revived microbes Strains into Microplate Wells (Excluding Positive Control).
Step 11: Covering of 96-well plate with parafilm for sterility protection for the process of incubation.
Step 12: Measurement of absorbance using ELISA plate reader after incubation.

1.4. Antioxidant activity

Free radicals (O, N, H) species are universally acknowledged for performing a double function in human bodies, both detrimental and beneficial. It has a metabolic mechanism for its production. Oxidative stress develops in human bodies due to a high surge in free radical generation and a reduction in antioxidant levels; nonetheless, at minimum concentrations, these free radical species fulfill essential biological tasks in the human body. According to scientific research, antioxidants decrease the risk of lethal chronic illness or diseases like cardiovascular disease and epithelial cancer. Atoms, molecules, or ions containing unpaired electrons in an outer shell are referred to as free radicals. At a time, free radicals may have a zero, negative, or positive charge.

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1.4.1 DPPH Assay for antioxidant evaluation

The antioxidant evaluation (*in vitro*) of newly synthesized compounds will be performed by U.V spectrophotometry by DPPH assay through the free radical (N, O, H species) scavenging method. After the reaction of DPPH with hydrogen donating species then, it forms hydrazine, and the absorbance is then decreased at 517 nm. Antioxidant agents take the hydrogen of DPPH and reduce it with the result dark violet colour turning into yellow colour. Methanol was used to prepare (3µg/ml) DPPH solution. For blank reference, DPPH and methanol (1:1) solutions will be used. Different dilutions will be prepared of variable concentrations (100 µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml) of the standard drug (ascorbic acid) with each synthesized compound using methanol and in the tube of each concentration one milli litre of DPPH solution will be poured. The initial reaction mixture will be thoroughly mixed, followed by keeping in the dark surrounding in the room for more than 32 min at room temperature (25 °C), and absorbance will be recorded using UV at 517 nm (Mukherjee et; al., 2012). As lower as the inhibitory of free radical of DPPH calculated using equation A concentration exhibit higher free radical scavenging potential.

$$\%\ Inhibition = \frac{A_{Blank} - A_{Sample}}{A_{Sample}}$$

Where,
A_{Blank} = Denotes absorbance of the blank control
A_{Sample} = Denotes absorbance of the test/standard compound

2. Results and discussion

2.1. Chemistry

The metal complexes containing 2-aminothiazole were synthesized using
Scheme 1. Initially, 2-aminothiazole was refluxed with substituted aldehydes (3-Ethoxy-4-hydroxybenzaldehyde and 1.4-Benzenedicarboxaldehyde) to synthesize Schiff base (**EVL_1-TPL_2**) which were treated with different metal chlorides to form metal complexes (**MV_1-MV_8**). Synthesized derivatives were characterized for their physicochemical properties (Table 1).

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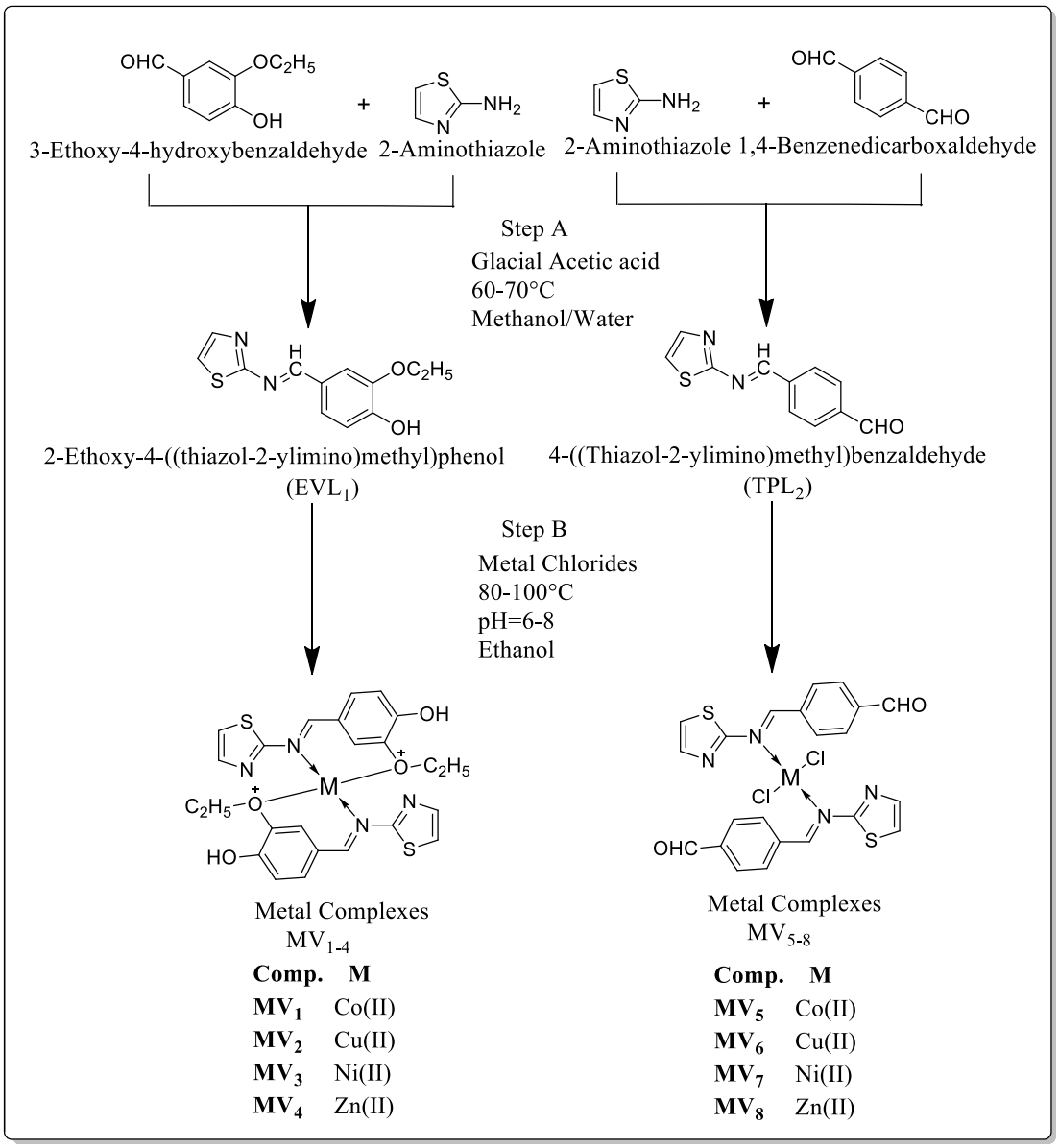

Scheme 1: Synthesis of Schiff bases and metal complexes of 2-aminothiazole

Chemical structures of the synthesized 2-aminothiazole Schiff bases and their metal complexes (**EVL_1, TPL_2, MV_1-MV_8**) were determined by spectral analysis (ATR, ^{1}H-NMR) (Table 3). The tridentate Schiff base have one phenolic ring, one thiazole ring and azomethine linkage, respectively. The IR (cm^{-1}) data of Schiff base showed the characteristic band at 1685.59 (**EVL_1**) and 1649.85 (**TPL_2**) which represented the presence of ν(C=N) azomethine linkage. Nitrogen atoms in the azomethine linkage combine with metal ions and reduce electron density and decrease the ν(C=N) absorption frequency i.e., indicated by the lowering of stretching band due to ν(C=N) shifted towards the lower frequency at 1520-1667 cm^{-1} and characteristic bands at 1511-1591 (C=C), 3104-3395 (C-H), 1630-1750 (C=O) and 3393-3741 (O-H) which confirmed the synthesis of Schiff base. The IR data of metal complexes showed characteristic bands at 700-600 cm^{-1} which represent the linkage of metal ions with N and O atoms present in the formed complexes.

The ^{1}H-NMR spectra of the Schiff base have been recorded in DMSO-d_6 solvent that confirmed the binding of the Schiff base to the metal atoms. The spectra showed the multiplet signals of aromatic protons in the Schiff base in the range of 6.40-8.00 δ ppm while peaks appeared in the region of 6.39-8.33 δ ppm were allotted to chemical shift of protons present in 2-aminothiazole ring. The up field shifting of the substituted aromatic ring showed hydrogen peaks at 6.80-8.0 δ ppm that indicated its coordination with metal complexes.

The NMR spectra of the Schiff base, the proton present in the hydroxyl group of phenolic rings appeared at 6.55 δ ppm, but the metal complexes did not show phenolic proton, showing deprotonation of the OH group. The singlet at 7.23-8.48 δ ppm indicative of the azomethine proton of Schiff base. Likewise, the azomethine proton of metal complexes remain same on complexation.

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Table 3: Physicochemical properties of the synthesized derivatives

Comp.	Mol. Formula	Mol. wt.	Colour	R*f*	Yield (%)	M. pt. (°C)
EVL$_1$	$C_{12}H_{22}N_2O_2S$	248.30	Yellow	0.62[a]	62.84	116-119
TPL$_2$	$C_{11}H_8N_2OS$	216.26	Orange	0.72[a]	77.16	110-112
MV$_1$	$C_{24}H_{24}$ Co $N_4O_4S_2$	555.53	Olive green	0.74[a]	60.64	208-210
MV$_2$	$C_{24}H_{24}$ Cu $N_4O_4S_2$	560.15	Brown	0.63[a]	64.75	207-210
MV$_3$	$C_{24}H_{24}$ Ni $N_4O_4S_2$	555.29	Mustard yellow	0.70[b]	67.68	210-212
MV$_4$	$C_{24}H_{24}$ Zn $N_4O_4S_2$	561.98	Brick red	0.66[b]	61.28	215-217
MV$_5$	$C_{22}H_{16}Cl_2CoN_4O_2S_2$	562.36	Green	0.60[a]	59.18	208-210
MV$_6$	$C_{22}H_{16}Cl_2CuN_4O_2S_2$	566.97	Brown	0.72[a]	68.86	212-215
MV$_7$	$C_{22}H_{16}Cl_2NiN_4O_2S_2$	562.12	Dark orange	0.73[b]	74.80	217-220
MV$_8$	$C_{22}H_{16}Cl_2ZnN_4O_2S_2$	568.80	Light yellow	0.69[b]	45.76	216-218

TLC Mobile phase: choloform: toluene (7:3)[a] ethyl acetate: n-hexane (2:3)[b]

Fig. 4: Synthesized Schiff's base (EVL$_1$) and their metal complexes (MV$_1$-MV$_4$)
Fig. 4a: Synthesized Schiff's base (TPL$_2$) and their metal complexes (MV$_5$-MV$_8$)

2.2. Antimicrobial screening results

Micro broth dilution assay (96 well microtiter plate method) was used to evaluate antimicrobial activity (MIC = µg/ml) of the synthesized compounds against Gram positive (*B. subtilis*), Gram negative (*E. coli*) bacterial strain and fungal strains (*A. niger*, *A. terreus*) using cefadroxil and fluconazole as standard drugs (Table 4, Fig. 3a and 3b). Among synthesized derivatives, most of the compounds showed antimicrobial activity. Schiff base **EVL$_1$** showed good activity against *A. niger*, *A. terreus*, *B. subtilis* ($MIC_{an,at,bs}$ = 3.12 µg/ml). Complex **MV$_1$** showed good antifungal activity against *A. niger* (MIC_{an} = 1.56 µg/ml) similarly complex **MV$_1$** showed significant activity against *E. coli*, *B. subtilis* and *A. terreus* ($MIC_{ec,bs,at}$ = 3.12 µ/ml) and **MV$_5$** against *A. niger* (MIC_{an} = 1.56 µ/ml). The increase in the antimicrobial activity may be due to the presence of an additional azomethine (C=N) linkage in metal complexes which may be involved in the binding of antimicrobial target.

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Table 4: Spectral characteristic of synthesized derivatives:

Comp.	IR (ATR, cm^{-1})	^{1}H-NMR (DMSO), δ ppm
EVL_1	[1516.84 (C=C str.), 3393.70 (C-H str.) of aromatic ring], 3610.90 (O-H str.), 3393.70 (N-H str.), 1043.31 (C-O), 2857.10 (CH_3 str.), 1685.59 (C=N str.)	5.28-7.27 (6H, m of Ar-H), 8.48 (1H, s of CH=N), 3.76 (3H, s of CH_3), 6.55 (1H, s of OH), 6.56-8.24 (H, s, thiazole ring)
TPL_2	[1515.93 (C=C str.), 3118.93 (C-H str.) of aromatic ring], 3182.49 (N-H str.), 1694.07 (C=N str.), 1918.85 (C=O str.)	7.53-7.89 (8H, m Ar-H), 8.41 (2H, s of CH=N), 8.09 [(2H, s of CHO], 6.58-8.10 (4H, s of thiazole ring)
MV_1	[1504.30 (C=C str.), 3171.79 (C-H str.) 3612.66 (O-H), of aromatic ring], 3274.78 (N-H str.), 1667.06 (C=N str.), 640.48 (M-N str.), 720.61 (M-O str.)	5.28-7.27 (6H, m Ar-H), 8.48 [(2H, s of $(CH=N)_2$], 1.40 [4H, s of CH_2], 7.60-7.77 (4H, s of thiazole ring), 0.90 [6H, s of $(CH_3)_2$], 5.35 [2H, s of OH]
MV_2	[1439.85 (C=C str.), 3083.81 (C-H str.) 3610.79 (O-H) of aromatic ring], 3139.98 (N-H str.), 1691.97 (C=N str.), 648 (M-N str.), 740 (M-O str.)	6.93-7.55 (6H, m Ar-H), 8.48 [(2H, s of $(CH=N)_2$], 1.40 [4H, s of CH_2], 7.60-7.77 (4H, s of thiazole ring), 0.90 [6H, s of $(CH_3)_2$], 5.35 [2H, s of OH]
MV_3	[1517.47 (C=C str.), 3073.13 (C-H str.) 3604.51 (O-H) of aromatic ring], 3183.44 (N-H str.), 1643.14 (C=N str.), 651 (M-N str.), 716.48 (M-O str.)	6.85-7.28 (6H, m Ar-H), 8.48 [(2H, s of $(CH=N)_2$], 1.40 [4H, s of CH_2], 7.60-7.77 (4H, s of thiazole ring), 0.90 [6H, s of $(CH_3)_2$], 5.35 [2H, s of OH]
MV_4	[1516.12 (C=C str.), 3012.47 (C-H str.) 3610.79 (O-H), of aromatic ring], 3392.87 (N-H str.), 1693.42 (C=N str.), 697.66 (M-N str.), 792.86 (M-O str.)	5.58-7.17 (6H, m Ar-H), 8.48 [(2H, s of $(CH=N)_2$], 1.40 [4H, s of CH_2], 7.60-7.77 (4H, s of thiazole ring), 0.90 [6H, s of $(CH_3)_2$], 5.35 [2H, s of OH]
MV_5	[1518.66 (C=C str.), 3112.36 (C-H str.) 3611.08 (O-H) of aromatic ring], 3254.59 (N-H str.), 1695.36 (C=N str.), 642.39 (M-N str.), 707.51 (M-O str.)	7.53-7.89 (8H, m Ar-H), 8.41 (2H, s of CH=N), 8.09 [(2H, s of CHO], 6.58-8.10 (4H, s of thiazole ring)
MV_6	[1518.65 (C=C str.), 3110.54 (C-H str.) 3609.10 (O-H) of aromatic ring], 3329 (N-H str.), 1747 (C=O), 1652 (C=N str.), 637.05 (M-N str.), 699.26 (M-O str.)	7.53-7.89 (8H, m Ar-H), 8.41 (2H, s of CH=N), 8.09 [(2H, s of CHO], 6.58-8.10 (4H, s of thiazole ring)
MV_7	[1514.69 (C=C str.), 3111.77 (C-H str.) 3610.77 (O-H) of aromatic ring], 3385.10 (N-H str.), 1645.51 (C=N str.), 697 (M-N str.), 802.08 (M-O str.)	7.53-7.89 (8H, m Ar-H), 8.41 (2H, s of CH=N), 8.09 [(2H, s of CHO], 6.58-8.10 (4H, s of thiazole ring)
MV_8	[1515.82 (C=C str.), 3225.60 (C-H str.) 3611.66 (O-H) of aromatic ring], 3109.89 (N-H str.), 1697.17 (C=N str.), 701.65 (M-N str.), 806.84 (M-O str.)	7.53-7.89 (8H, m Ar-H), 8.41 (2H, s of CH=N), 8.09 [(2H, s of CHO], 6.58-8.10 (4H, s of thiazole ring)

Among the synthesized derivatives **MV_1** showed significant antimicrobial activity which may be used as a prime complex to develop better antimicrobial agents.

Table 5: Antimicrobial screening results of the synthesized derivatives (MIC=µg/ml)

Compounds	Bacterial strains		Fungal strains	
	Gram positive	Gram negative		
	B. subtilis	***E. coli***	***A. niger***	***A. terreus***
EVL_1	**3.12**	12.5	**3.12**	**3.12**
TPL_2	12.5	25	12.5	12.5
MV_1	**3.12**	**3.12**	**1.56**	**3.12**
MV_2	12.5	25	12.5	25
MV_3	25	50	25	50
MV_4	25	12.5	6.25	12.5
MV_5	50	25	**1.56**	**3.12**
MV_6	12.5	50	12.5	25
MV_7	12.5	50	25	50
MV_8	6.25	12.5	25	25
Standard	**1.56**[a]	**1.56**[a]	**3.12**[b]	**3.12**[b]

Std. drug =Cefadroxil [a], Fluconazole[b], **MIC**=Minimum Inhibitory Concentration

2.3. Antioxidant evaluation of synthesized 2-aminothiazole derivatives using (DPPH assay) 1,1-diphenyl-2-picrylhydrazyl Assay

The modified Brand-Williams procedure was utilized to assess the free radical scavenging action of the designed and synthesized analogs. Initially, the synthesized derivatives were first mixed within DMSO (Dimethyl sulfoxide) to prepare the solution of varying amounts of the drug (100µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml) of test and reference drug (ascorbic

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acid). These dilutions of test compounds were added separately into an equal amount of methanolic solution of DPPH (3 µg/ml).
After 30-minute of incubation time at 25°c (RT) in the dark surrounding, the absorbance of the final mixture was recorded on UV/vis spectrophotometer at 517nm wavelength (Mukherjee *et al*.,2012). The reaction mixture's coloration shifted from deep violet to yellow, resulting in fall in absorbance of the reaction mixture. The coloration of the reaction mixture shifted from purple to yellow, which led to a decrease in the absorbance of the reaction. The following equation was used to calculate the free radical scavenging activity of designed derivatives.

$$\%\ Inhibition = \frac{A_{Blank} - A_{Sample}}{A_{Sample}}$$

Where,
A_{Blank} = Denotes absorbance of the blank control
A_{Sample} = Denotes absorbance of the test/standard compound
From the graph of % Inhibition vs Concentration was used for the calculation of IC_{50} value of the designed and synthesized 2-aminothiazole derivatives. The outcomes antioxidant activity result revealed that the few only Schiff base ligand **EVL_1** exhibit significant antioxidant activity, and other had medium to modest antioxidant action compared with the reference drug, which is ascorbic acid.

Table 6: Antioxidant estimation of synthesized derivatives

Compounds	**(%) Percentage Inhibition**				***IC_{50}***
	25 µg/ml	***50 µg/ml***	***75 µg/ml***	***100µg/ml***	
EVL_1	36.62	52.11	67.82	82.63	**46.56**
TPL_2	15.63	32.63	44.65	66.65	73.66
MV_1	15.86	31.56	50.14	72.35	60.44
MV_2	24.25	40.31	58.67	71.68	64.48
MV_3	33.21	48.62	64.65	81.62	51.61
MV_4	15.62	31.11	46.69	59.63	82.38
MV_5	23.02	39.65	56.32	70.23	66.77
MV_6	24.25	43.89	58.67	71.68	63.09
MV_7	21.67	38.9	56.73	70.72	67.1
MV_8	24.61	35.98	50.33	68.27	71.45
Standard	37.61	53.98	68.74	85.62	**44.41**

Std. drug =Ascorbic acid

Fig. 5: Test tubes showing antioxidant activity

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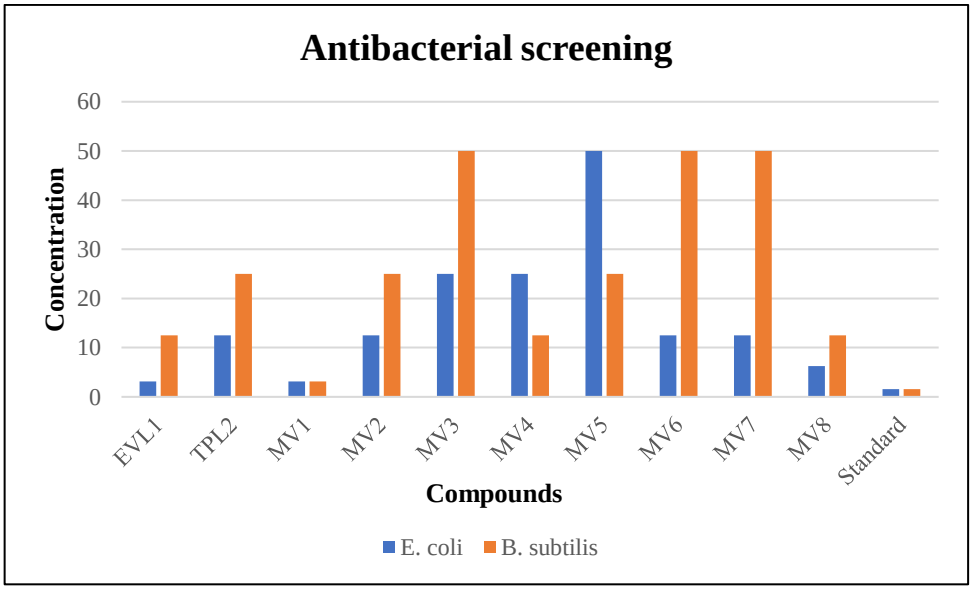

Fig. 6: Graphical representation of antibacterial activity

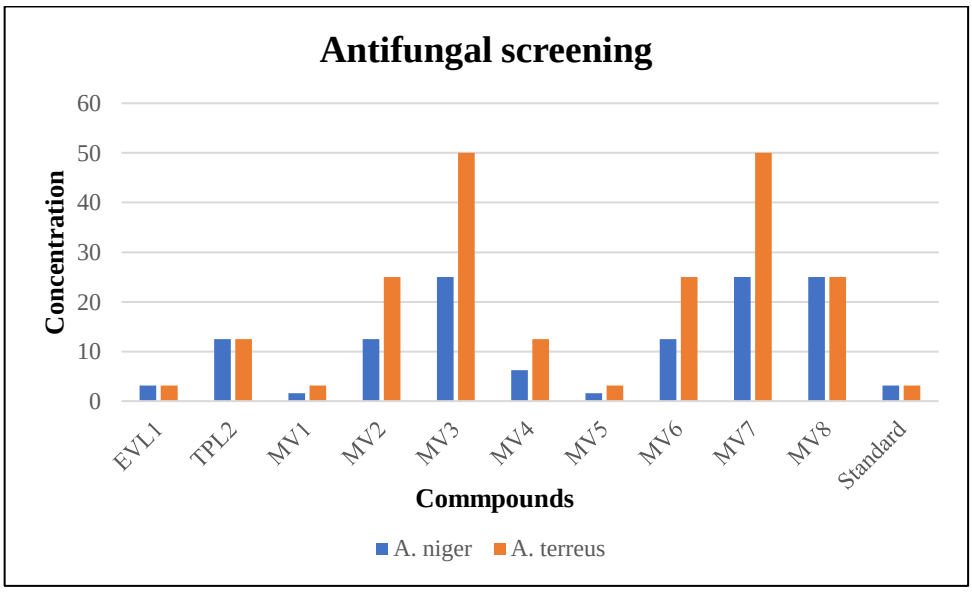

Fig. 6a: Graphical representation of antifungal activity

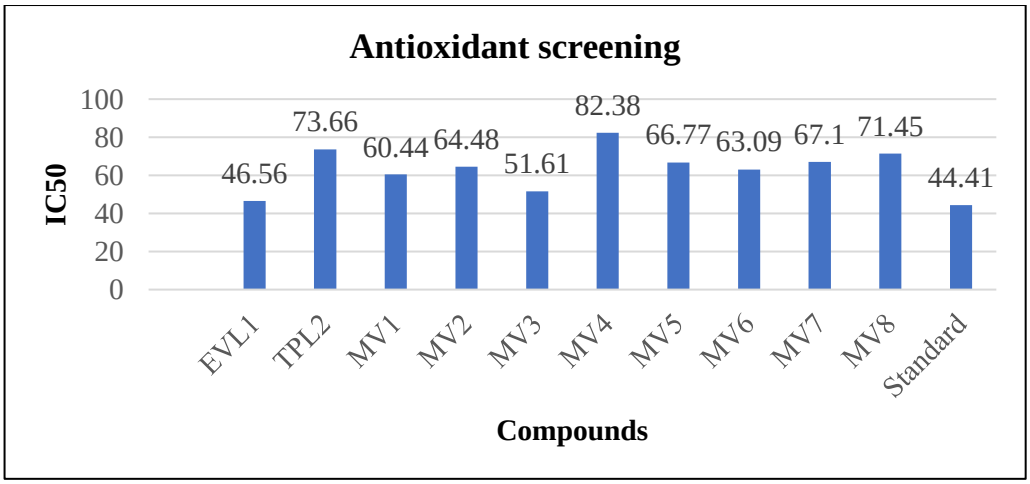

Fig. 7: Graphical representation of antioxidant activity showing IC_{50}

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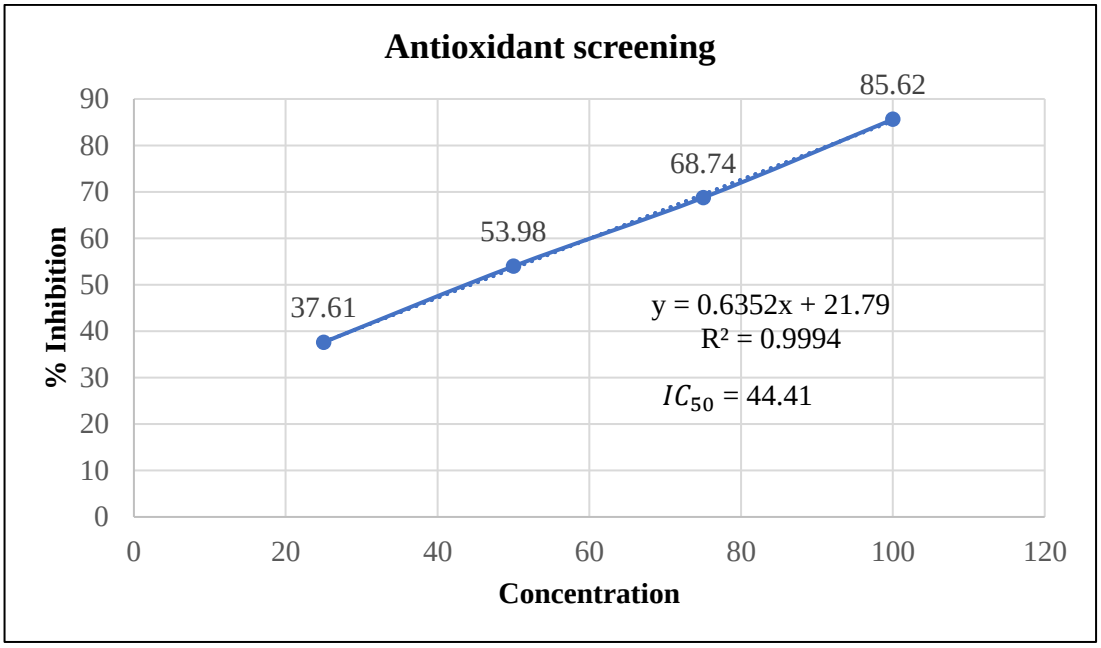

Fig. 7a: Graphical representation of standard (Ascorbic acid) showing IC_{50}

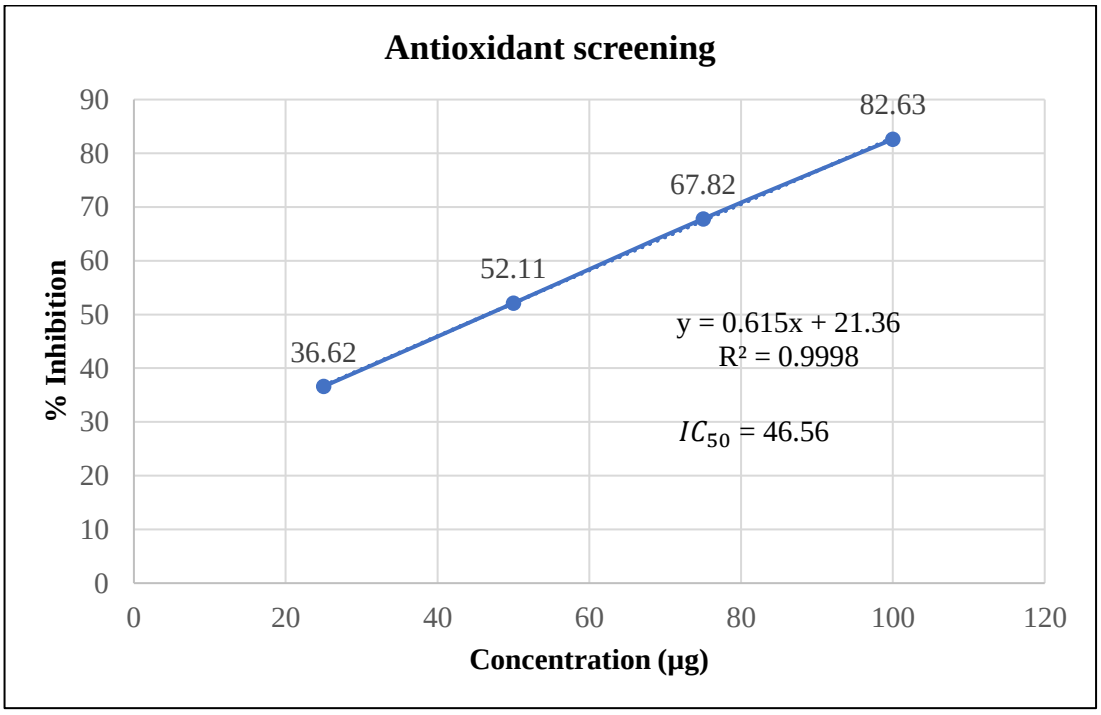

Fig. 7b: Graphical representation of EVL_1 showing IC_{50}

2.4. Structure activity relationship (SAR) study

From the antimicrobial and antioxidant results, the following structure activity relationship (Fig. 8) may be deduced:

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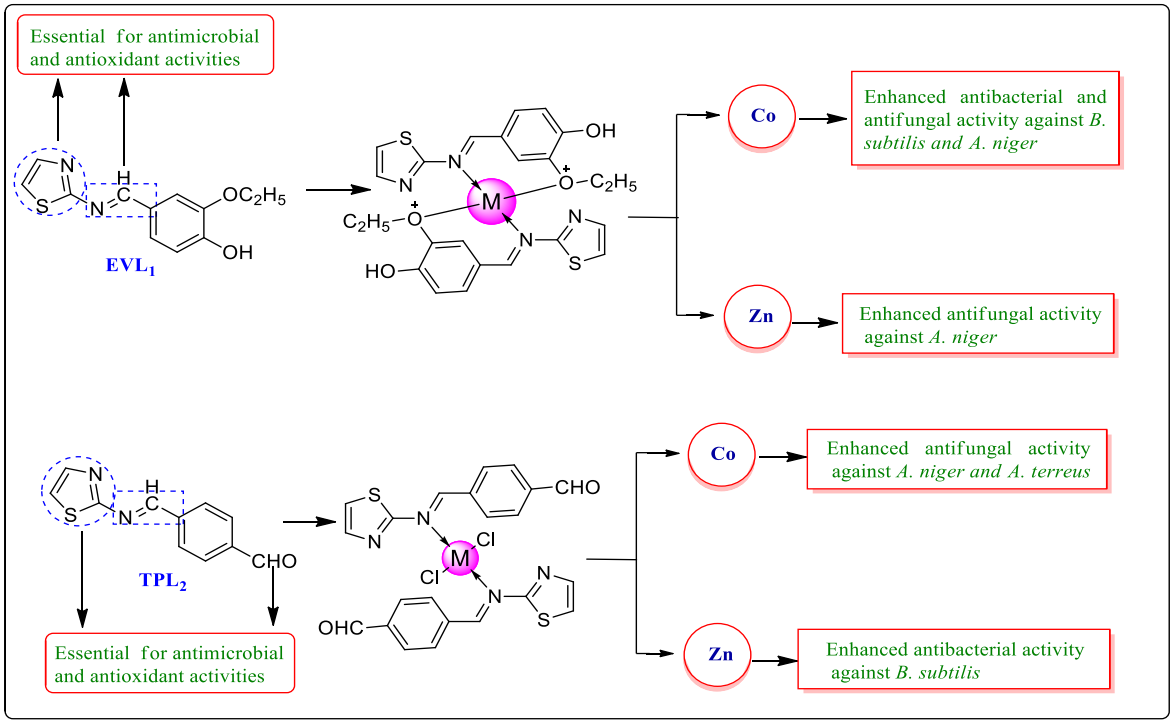

Fig. 8: Structure activity relationship of 2-aminothiazole derivatives

a) Thiazole ring and azomethine group are important for antimicrobial and antioxidant activities, respectively.
b) Presence of Co(II) as transition metal improved antifungal activity against *A. niger*, and *A. terreus*.
c) Presence of Co(II) contributed to antibacterial activity against *B. subtilis* and *E. coli* also enhanced antioxidant activity.
d) Presence of Zn(II) in metal complexes enhanced the antifungal potential against *A. niger*.

Summary and conclusion:

Metal complexes containing 2-aminothiazole were synthesized and characterized by physicochemical and spectral means. The synthesized derivatives showed promising antibacterial and antifungal activities. The Schiff base **EVL₁** and the complex **MV₁** and **MV₅** exhibited promising antimicrobial activity. Antioxidant activity screening by DPPH assay method indicated that complex **EVL₁** had excellent antioxidant efficiency. It can be concluded that Schiff base and metal complexe**, EVL₁, MV₁** and **MV₅** (Fig. 9) may be used as lead molecules for the development of novel antimicrobial and antioxidant agents, respectively.

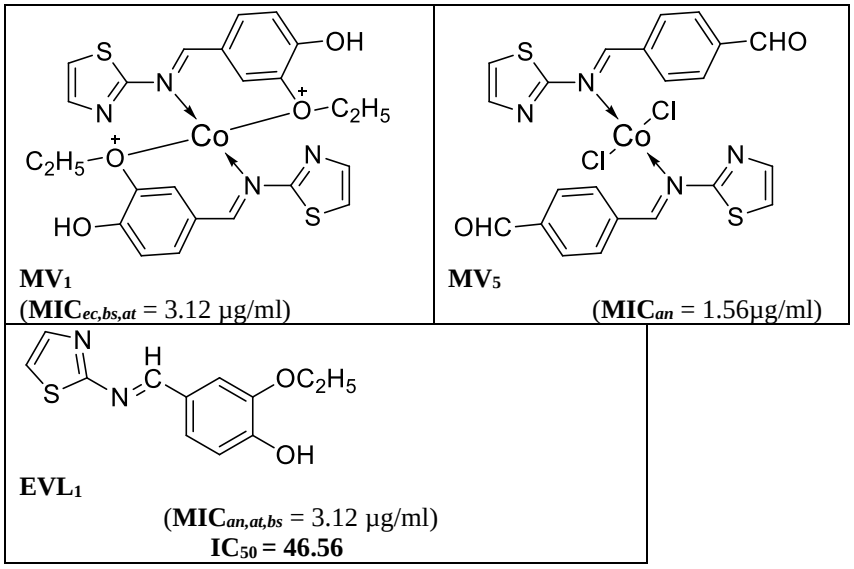

Fig.9: Most active antimicrobial and antioxidant complexes

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