

Synthesis of New Series of Phenyl diazene Based Metal Complexes for Designing Most Active Antibacterial and Antifungal Agents

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Summary: In this study, *In Vitro* antibacterial and antifungal activities of azo series based on transition metal complexes (Cu²⁺, Zn²⁺, Mn²⁺, Co²⁺ and Ni²⁺) with already our reported ligands named as; [(E)-1-(1, 3-dioxolan-2-yl)-2-phenyldiazene] (K-1), [(E)-1-(1, 3-dioxolan-2-yl)-2-(4-methylphenyl)diazene] (K-2), 2-[(E)-phenyl diazenyl]-1H-benzimidazole] (K-3), [(E)-1-(1, 3-dioxolan-2-yl)-2-(4-ethylphenyl)diazene] (K-4), and [(E)-1-(1, 3-dioxolan-2-yl)-2-(2-methylphenyl)diazene] (K-5) were studied. FTIR ¹H-NMR and mass spectrometric techniques were applied for interpretation of synthesized complexes. 4.05-4.07 ppm chemical shift appearance of azo group confirms azo coupling with transition metal complexes. N=N, C-H, C-N and C-O groups are also confirmed by FTIR which exhibited peaks at 1400-1500, 2090-3090, 1100-1180, 1010-1060 and 625-780 cm⁻¹. Furthermore, mass spectroscopic data also gives strong indication for the synthesis of metal complexes.

All the newly synthesized complexes were screened for their antibacterial and antifungal activities. Antibacterial and antifungal activity against *S. aureus*, *E. coli* and *A. niger*, *A. ustus* and *C. albican* at conc. 250 µg/ml showed excellent activity by K-1 complexes (Co²⁺, Cu²⁺, Ni²⁺), K-5 complexes (Zn²⁺, Mn²⁺, Cu²⁺), K-2 complexes (Co²⁺, Cu²⁺, Mn²⁺) and K-3 (Zn²⁺, Mn²⁺, Co²⁺, Cu²⁺) as compared to standard drug (Ampicillin). Hence, based on this study, it was concluded that these azo based complexes may act as a platform for designing more active antibacterial and antifungal agents.

Keywords: Active methylene, Azo based complexes, Therapeutic potential, Anti-fungal agents and Anti-bacterial agents.

Introduction

Gram positive and gram negative families of bacteria are highly contagious and epidemic in nature that cause respiratory infections and thousands of death each year in humans [1-8]. Currently, there are two ways to decrease the effect of bacterial infections *i.e.* in some cases vaccination or anti-bacterial or anti-fungal drugs [9, 10]. There are many limitations for use of these antibacterial and antifungal drugs because these medicines are very limited and if present in markets are becomes resistant to these pathogens [11, 12]. Further it is impossible in case of vaccines that it can be useful and provided to every person to fulfil the requirements against these pathogens [13-20]. Many studies have been established to explore the inhibitory activities of compounds derived from natural sources and synthetic drugs [21, 22]. Among them, plant derived neuraminidase inhibitors showed lesser activity compared to the synthetic drugs. Therefore, it is always a dire need of such novel synthetic antimicrobial agents that are less compromised by

rapid drug resistant viral strains, efficient and have minimal side effects [23].

Azo dyes (-N=N-) based metal complexes are a class of compounds which have fascinated attention over the past few years due to their widespread use as dyeing and pigments and diverse biomedical applications [6] such as antiviral, antibacterial, antifungal, antitumor, hypotensive, anti-inflammatory and antioxidants etc. [24-29]. Specifically against viruses, Naicker *et al.* identified an azo compound as a lead inhibitor of HIV-1 [28], Farghaly *et al.* discovered some of the highly active anti-HCV arylazobenzosuberones compared to ribavirin [30]. Moreover, a series of highly active aminoarylazo compounds was evaluated by Tonelli *et al.* against a panel of RNA viruses [31]. Azo compounds have most important and versatile applications as antibiotic, antifungal, anti-diabetic, antineoplastic, anti-inflammatory, cosmetics, food, paints, plastics, textile dyes, paper printing dyes and color photography [32-36]. The diazotized-p-

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sulphanilic acid cardanol, 1-(2-diazo-6-ethoxybenzothiazolyl) and 2-[2-hydroxyphenylazo]-1-naphthol-4-sulphonic acid were evaluated for their antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aureus* and *Staphylococcus aureus* [32]. The antifungal activity of the synthesized compounds; benzimidazole-azophenol, 3-(p-ethylazo) benzaldehyde, 3-(4-Nitrophenylazo) benzaldehyde, 5-((4-amino phenyl) diazenyl)-2-hydroxybenzoic acid and 3-(p-substituted phenyl azo)-6-pyridon have been evaluated against seven strains; *A.nigar*, *A.flowous*, *R.nigaricans*, *A.nidulens*, *C.herbarum*, *T.rosesum* and *A.alternate*. Antipyrine or phenazone derivatives are well known compounds used mainly as analgesic and anti-pyretic drugs [37]. Further it was studied that when these azo based ligands are reacted with transition metal salts then results in the formation of complexes. These complexes can be synthesized at room temperature or at refluxed for minutes to hours. This room temperature and reflux time required on the basis of ligands. These transition metal complexes with azo compounds are successfully proved for many activities like antibacterial, antifungal, antiviral and pesticides. From all transition metals Ni, Co, Mn, Fe and Cu metal complexes are reported as excellent compounds for inhibition of microorganisms. In many studies it is reported that when these azo

ligands are formed into complexes with metals then their activities are increased majestically [38-41]. The azo Schiff's base and their complexes with Mn(II), Zn(II), Ni(II) and Cu(II) showed anti-oxidant, antibacterial and anti-fungal activities [42, 43].

Herein we synthesized Phenyldiazene based azo complexes based on dioxolane because the presence of dioxolane in the compounds as well as their complexes exhibits antibacterial and antifungal activities [43, 44]. To our knowledge, no study has been conducted before to evaluate the bioactive potential of Phenyldiazene based azo complexes against antibacterial and antifungal strains. Therefore, based on such evidences, a series of Phenyldiazene based azo complexes with our reported ligands [45], like as [(E)-1-(1,3-dioxolan-2-yl)-2-phenyldiazene] (K-1), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-methylphenyl)diazene] (K-2), 2-[(E)-phenyldiazanyl]-1H-benzimidazole] (K-3), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-ethyl-phenyl)diazene] (K-4) and [(E)-1-(1,3-dioxolan-2-yl)-2-(2-methylphenyl)diazene] (K-5) were synthesized and structural determination/characterization was done by spectroscopic analysis and the efficacy of synthesized compounds was assayed by *In Vitro* antibacterial and antifungal activities.

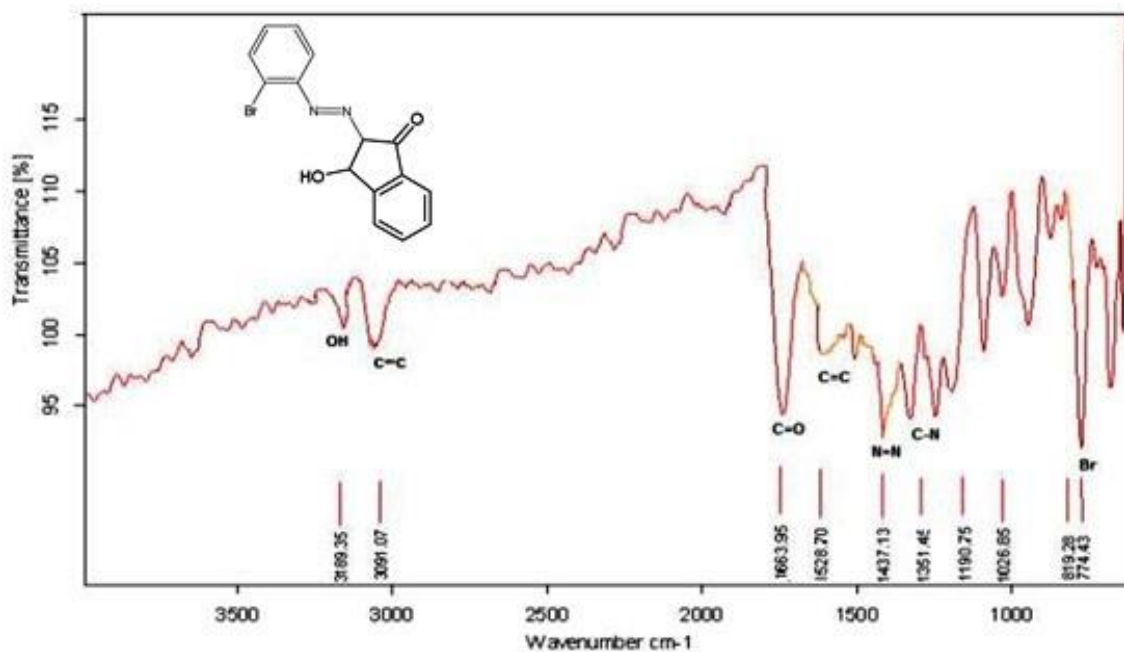


Fig. 1: FTIR spectra of K-1 Ligand.

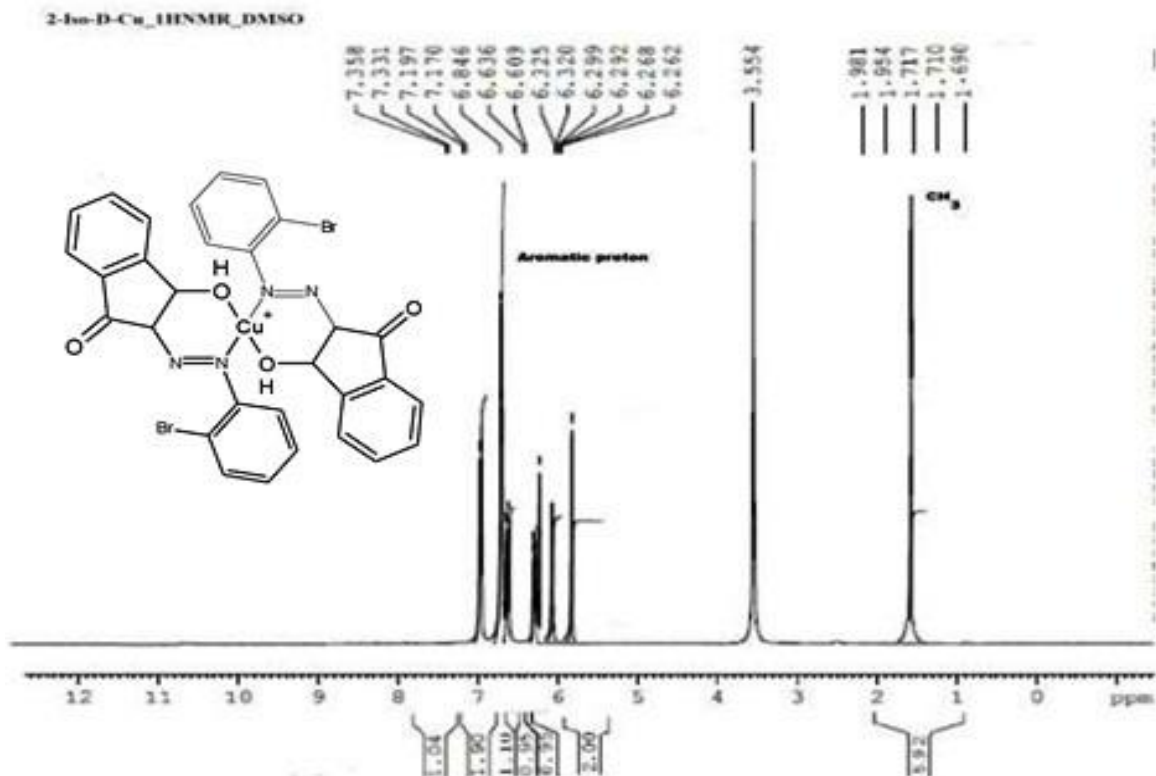
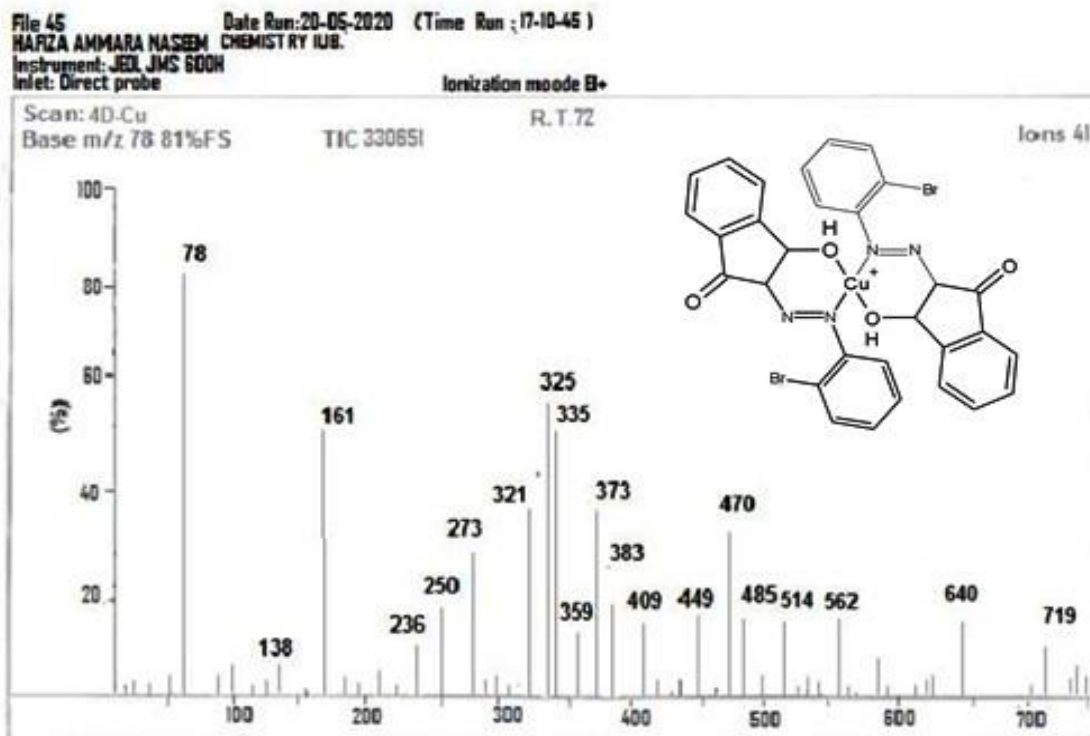
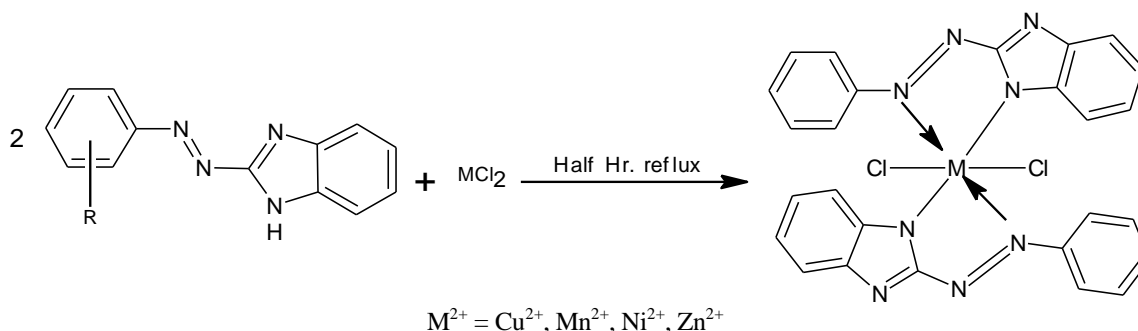
Fig. 2: ^1H NMR spectrum of K-1 with Cu metal.

Fig. 3: Mass Spectrum and Fragmentation pattern of ligand K-1 with Cu metal.



Scheme-1: General Reaction for the Synthesis of Phenyl diazene based Azo Complexes.

Experimental

Chemicals

All chemicals were obtained from Merck (Darmstadt, Germany) and BDH (Country British part of Merck Chemicals Limited 1977- 1990s) and used as such. Solvents were used methanol, ethanol, chloroform, n-hexane, carbon tetrachloride, acetone and distilled water.

General method for the synthesis of complex

Ligand (2mmol) were mixed in methanol taken in a 50 ml round bottom flask and 1 mmol solution of metal salt in methanol was added in it drop wise with continuous stirring. After 5-10 minutes the color was changed and the product was washed with distilled water and dried [20, 29,46]. The general reaction for the synthesis of complexes is given as follows:

Synthesis of metal complexes

General procedure for synthesis of metal complexes

All the ligands (Phenyl diazene based azo compounds) were synthesized according to our reported method. For the synthesis of complexes 3 mmol of Phenyl diazene based azo ligands was added in beaker and then added 2 mmol metallic solution of transition metals in methanolic solution slowly and refluxed for 60 minutes. after refluxing the ppt were formed and filtered, dried and used for further purposes [29].

Synthesis of [(E)-1-(1, 3-dioxolan-2-yl)-2-phenyl diazene] (K-1) metal complexes

Cu^{2+} , Zn^{2+} , Mn^{2+} and Ni^{2+} complexes respectively. Yield: 86 %, m.p: $>300^{\circ}C$, Color: black, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H

sp^2 : 3077; CO (carbonyl): 1658, N=N: 1474; C-N: 1233, O-Cu: 787. Yield: 55 %, m.p: $279-281^{\circ}C$, Color: Orange, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Zn: 712. Yield: 75 %, m.p: $220^{\circ}C$, Color: white, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3060; CO (carbonyl): 1650, N=N: 1480; C-N: 1231, O-Mn: 660. Yield: 61 %, m.p: $279-281^{\circ}C$, Color: Orange, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Ni: 650.

Synthesis of [(E)-1-(1, 3-dioxolan-2-yl)-2-(4-methylphenyl) diazene] (K-2) metal complexes

Cu^{2+} , Zn^{2+} , Mn^{2+} and Ni^{2+} complexes respectively. Yield: 72 %, m.p: $>300^{\circ}C$, Color: white, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3077; CO (carbonyl): 1658, N=N: 1474; C-N: 1233, O-Cu: 787. Yield: 55 %, m.p: $279-281^{\circ}C$, Color: Orange, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Zn: 712. Yield: 75 %, m.p: $220^{\circ}C$, Color: white, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3060; CO (carbonyl): 1650, N=N: 1480; C-N: 1231, O-Mn: 660. Yield: 61 %, m.p: $279-281^{\circ}C$, Color: Orange, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Ni: 650.

Synthesis of complexes of [2-[(E)-phenyl diazenyl]-1H-benzimidazole] K-3 ligand.

Cu^{2+} , Zn^{2+} , Mn^{2+} and Ni^{2+} complexes respectively. Yield: 81 %, m.p: $>300^{\circ}C$, Color: purple, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3077; CO (carbonyl): 1658, N=N: 1474; C-N: 1233, O-Cu: 787. Yield: 55 %, m.p: $279-281^{\circ}C$, Color: Orange, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}): C-H sp^2 : 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Zn: 712. Yield: 75 %, m.p: $220^{\circ}C$, Color: white, Solubility: C_6H_6 , $CHCl_3$ and DMSO. FTIR (cm^{-1}):

¹): C-H sp²: 3060; CO (carbonyl): 1650, N=N: 1480; C-N: 1231, O-Mn: 660. Yield: 61 %, m.p: 279-281°C, Color: Orange, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Ni: 650.

Synthesis of complexes of [(E)-1-(1, 3-dioxolan-2-yl)-2-(4-ethylphenyl) diazene] K-4 ligand

Cu²⁺, Zn²⁺, Mn²⁺ and Ni²⁺ complexes respectively. Yield: 69 %, m.p: >300°C, Color: brown, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3077; CO (carbonyl): 1658, N=N: 1474; C-N: 1233, O-Cu: 787. Yield: 55 %, m.p: 279-281°C, Color: Orange, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Zn: 712. Yield: 75 %, m.p: 220°C, Color: white, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3060; CO (carbonyl): 1650, N=N: 1480; C-N: 1231, O-Mn: 660. Yield: 61 %, m.p: 279-281°C, Color: Orange, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Ni: 650.

Synthesis of complexes of [(E)-1-(1, 3-dioxolan-2-yl)-2-(2-methylphenyl) diazene] K-5 ligand

Cu²⁺, Zn²⁺, Mn²⁺ and Ni²⁺ complexes respectively. Yield: 69 %, m.p: >300°C, Color: brown, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3077; CO (carbonyl): 1658, N=N: 1474; C-N: 1233, O-Cu: 787. Yield: 55 %, m.p: 279-281°C, Color: Orange, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Zn: 712. Yield: 75 %, m.p: 220°C, Color: white, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3060; CO (carbonyl): 1650, N=N: 1480; C-N: 1231, O-Mn: 660. Yield: 61 %, m.p: 279-281°C, Color: Orange, Solubility: C₆H₆, CHCl₃ and DMSO. FTIR (cm⁻¹): C-H sp²: 3067; CO (carbonyl): 1688, N=N: 1463; C-N: 1267, O-Ni: 650.

FTIR data, physical properties and FTIR spectrum of metal complexes recorded successfully.

Biological Study

Antibacterial activity

For screening of biological activities disc diffusion method was applied according to reported procedure [5, 6, 20].

Sampling of microbes

Sampling of microbes were also done according to our reported method [20].

Results and Discussions

Phenyldiazene based azo-complexes with different ligands; [(E)-1-(1,3-dioxolan-2-yl)-2-phenyldiazene] (K-1), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-methylphenyl)diazene] (K-2), 2-[(E)-phenyldiazanyl]-1H-benzimidazole] (K-3), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-ethylphenyl)diazene] (K-4), and [(E)-1-(1,3-dioxolan-2-yl)-2-(2-methylphenyl)diazene] (K-5) were synthesized by coupling reaction of diazonium salt solutions with active methylene 1,3-dioxalane and benzimidazole in the presence of Na₂CO₃ at 0-5 °C. The transition metal (Mn²⁺, Cu²⁺, Ni²⁺ and Zn²⁺) complexes of all the ligands were also synthesized. All the ligands and complexes were characterized by FTIR. Further ¹H-NMR and Mass spectrometry was also used for the characterization of ligands. Purification of the ligands and complexes was identified by TLC.

In FTIR Study the interpretation of IR spectra of the ligands indicates the formation of ligands as the absence of NH₂ peak at 3600-3400 cm⁻¹, appearance of N=N peak at 1530-1440 cm⁻¹, C-O peak at 1100-1050 cm⁻¹, C-N peak at 1200-1100 cm⁻¹ and C-H peak at 3100-3000 cm⁻¹.

In ¹H-NMR Study all the synthesized ligands exhibited concerned proton peaks that were resolved in their proper range which confirmed the synthesis of compounds. The absence of NH₂ peak at 4.07-4.05 ppm confirmed the synthesis of K-1. The other peaks which observed were: a peak at 4.8 ppm for CH proton, a peak at 3.3 ppm for CH₂, and a multiplet at 7.0-7.5 ppm for phenyl protons. The ¹H-NMR spectrum of all complexes.

In Mass Spectroscopic Study the synthesized compounds showed prominent molecular ion peaks and base peaks. The complexes had distinct molecular ion peaks at m/z 178, 192, 222, 206 and 192 respectively [47]. The mass spectrums of the ligands showed that they were easily patronized into fragments. Major fragmentations were observed at m/z 133, 119, 105, 77, 73 and 51. The fragmentation pattern also revealed that the fragmentation ended into C₄H₃⁺ ion at m/z 51.

All the synthesized complexes were studied for their antibacterial, antifungal activities. Among these, complexes, complexes with K-1 and K-5 revealed outstanding antibacterial activity against *S. aureus* at conc. 250 µg/ml (as shown in Fig 4), while against *E.coli* all the complexes showed no activity. Antifungal activity was studied out on *A.niger*, *A.ustus* and *C.albican*. Antifungal activity of complexes showed that all the complexes except K-5 complexes inhibit maximum activity against *A.niger*. Complexes with

ligands K-1 and K-3 also revealed maximum inhibition activity towards *A.ustus*. All the complexes activity against *C.albican* is less than standard medicine (Fluconazole). Complexes with ligand K-5 was inactive against all the fungal strains. Ligand K-5 was inactive but the complexes of ligand K-5 showed remarkable antifungal activities which revealed that after complexation with cobalt and nickel metal the antifungal inhibition activity of K-5 ligand is increased. Among the complexes, the complexes of nickel and cobalt metal showed excellent inhibition activity at 250 µg/ml concentration (as shown in Fig 5) because these

metal have vacant d-orbitals which can be interacted with microbial DNA and RNA more easily than other metals [48,49]. It can also be inferred from the results of antifungal and antibacterial activity and computational studies that the azo compound (K-5) can be further analyzed against other influenza viral strains especially COVID-19 and can be served as a structural template in designing of novel antibacterial and antifungal agents since there are less synthetic drugs available commercially for bacteria, fungus and especially for viruses.

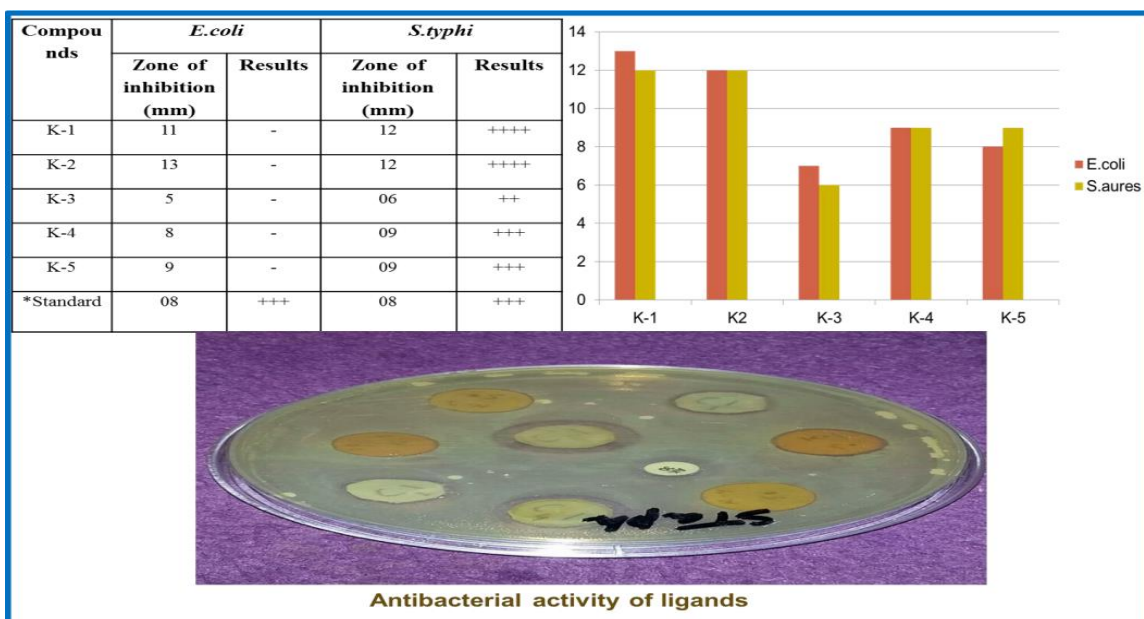


Fig. 4: Antibacterial activity of Ligands.

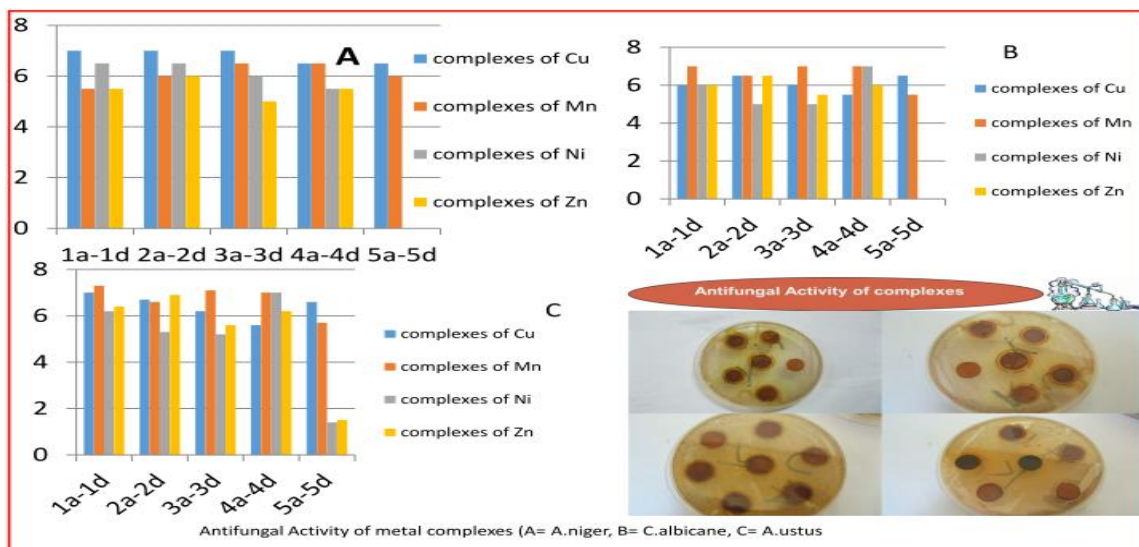


Fig. 5: Antifungal activities of Metal complexes.

Conclusion

The Phenyldiazene based azo complexes were successfully synthesized under reproducible conditions with excellent yields. Azo-derivatives i.e. [(E)-1-(1,3-dioxolan-2-yl)-2-phenyldiazene] (K-1), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-methylphenyl)diazene] (K-2), 2-[(E)-phenyl diazenyl]-1H-benzimidazole (K-3), [(E)-1-(1,3-dioxolan-2-yl)-2-(4-ethylphenyl)diazene] (K-4), [(E)-1-(1,3-dioxolan-2-yl)-2-(2-methyl-phenyl)diazene] (K-5) and transition metal complexes of **K-1 to K-5** and confirmed by spectroscopic techniques like FTIR, ¹H-NMR and Mass spectrometry. In FTIR study of the ligands N=N, C-H, C-N and C-O peaks have appeared around 1400-1500, 2090-3090, 1100-1180 and 1010-1060 cm⁻¹ respectively and in transition metal complexes shifting of C-O/ C-N peak to lower frequency confirmed their synthesis. Another evidence about the formation of ligands and complexes was collected from ¹H-NMR and mass spectroscopy which gives distinct molecular ion peaks at m/z 178, 192, 222, 206 and 192 respectively. The geometry of the bi-dentate coordinated complexes was supposed to be octahedral except K-3 complexes having square planer by spectroscopic data [50-53]. All the newly synthesized compounds were screened for their antibacterial, antifungal activities and their structure activity relationship is also studied. All the complexes specially K-1, K-2 and K-5 ligands complexes showed excellent antibacterial activity against *S. aureus* at conc. 250 µg/ml, while all the complexes were inactive against *E.coli*. Antifungal screening was also carried out against *A.niger*, *A.ustus* and *C.albican* fungal strains. Among the tested complexes Nickel and Cobalt showed highest inhibition at 250 µg/ml concentration. It can also be inferred from the results that the azo complexes of (K-5) ligands can be further analyzed against other influenza viral strains and can be served as a structural template in designing of novel antiviral as well as antibacterial and antifungal agents since there are less synthetic drugs available commercially especially for viruses, bacteria and fungus.

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