Study of antimicrobial activity of cyano hydrazone and their metal complexes

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Abstract

Hydrazones are a class of organic compounds with the structure $R^1R^2C{=}N{-}NH_2$.[1] They are related to ketones and aldehydes by the replacement of the oxygen =O with the =N−NH2 functional group. They are formed usually by the action of hydrazine on ketones or aldehydes. Currently, we report the preparation of transition metal complexes Co(II), Ni(II), and Cu(II) of hydrazone Schiff base ligands, which are obtained by the condensation reaction of substituted salicylaldehyde and hydrazines. The synthesized hydrazone ligands and their metal complexes were characterized by spectroscopic methods such as Fourier transform infrared (FT-IR), UV–vis, nuclear magnetic resonance (^1H NMR and C^{13} NMR), and mass spectrometry analyses. All of the quantum chemistry calculations were performed using DFT executed in the Gaussian 09 software package. The geometry was optimized by using the density functional theory (DFT) approximation at the B3LYP level with a basis set of 6-31G (d, p). There was excellent agreement between the FT-IR values obtained experimentally and those obtained theoretically for the test compounds. It is worth noting that none of the optimized geometries for any of the Schiff base and metal complexes had any eigenvalues that were negative, indicating that these geometries represent the true minimum feasible energy surfaces. We also analyzed the electrostatic potential of the molecule and NBO calculation at the same level of theory. Gauss View 6 was utilized for the file organization of the input data. Gauss View 6.0, Avogadro, and Chemcraft were used to determine the data. Additionally, synthesized compounds were screened for antimicrobial activity against Gram-negative bacteria (Salmonella typhi, Escherichia coli) and Gram-positive bacteria (Bacillus halodurans, Micrococcus luteus) and two fungal strains (Aspergillus flavus, Aspergillus niger). These research findings have established the potential of ligands and their metal complexes as antimicrobial agents. Additionally, the compounds demonstrated promising nonlinear optical (NLO) properties, with potential applications across a wide range of contemporary technologies.

Introduction :-

In the field of medicinal chemistry, hydrazide–hydrazones are still in continuous interest due to their diverse and wide spectrum of biological properties. Additionally, hydrazide–hydrazones are versatile compounds for the synthesis of heterocyclic systems, preparing metal complexes and are used as ligands in coordination chemistry.

Among bioactivity profiles of hydrazide–hydrazones, antimicrobial properties are the most common in the scientific literature. This is especially important due to the fact that bacterial and fungal infections became more and more difficult and sometimes impossible to treat as a result of the increase of antibiotic and chemotherapeutic resistant strains. It is worth mentioning that hydrazide–hydrazone moiety is also present in the chemical structure of medicines with antimicrobial activity, such as nitrofurazone, furazolidone, or nitrofurantoin.

This review is an update and continuation of the review, which was previously published in 2017, and focuses on the most recently described (2017–2021) potent hydrazide– hydrazones with applications as antibacterial, antimycobacterial, and antifungal agents.

Taking into account the role of hydrazones and their metal complexes in the biological field and possessing a

comprehensive research background encompassing both synthetic and computational fields, here, we reported the synthesis of hydrazone ligands **L1** 4-chloro-2-((4-isopropylphenyl)hydrazono)methyl)phenol and **L2** 4-(2-(5-chloro-2-hydroxybenzylidene) hydrazinyl)benzonitrile and their Cu(II), Ni(II), and Co(II) complexes and then studied their spectroscopic and nonlinear optical properties. The synthesized compounds were subjected to the determination of antimicrobial activity against two Gram-negative bacteria and Gram-positive bacteria and two fungal strains, wherein the complexes showed a moderate activity against pathogenic bacteria. The computations of density functional theory (DFT) provide a multitude of useful information about the compounds. The correlation between these calculations and the physicochemical properties of the compounds is strong. It is well known that the physicochemical properties of compounds also affect their biological activity. The purpose of the DFT calculations was to examine the relationship between theoretically obtained data and experimental data.

Results and Discussion :-

The synthesized compounds were examined by FT-IR and NMR spectroscopic analysis. In the FT-IR spectrum of the ligands (**L1** and **L2**),absorption bands at 3388 and 3306 cm^{-1} due to the OH group, a strong absorption band at 3306–2936 cm^{-1} for (N–H) stretching frequencies, and an absorption band at 1665–1600 cm^{-1} for the ν(C = N) vibrations were observed. The OH vibrations disappeared from the spectra of the complexes, which indicated the coordination of metal ions to the hydroxyl group after deprotonation. In the complexes' spectra, the azomethine bands ν(C=N) and ν(C–O) (i.e., phenolic oxygen) were shifted to lower frequencies and changes in band frequency indicate that the nitrogen atom of the azomethine group was brought into being involved in coordination with metal ions. So, it can be concluded that ligands phenolic oxygen and azomethine nitrogen were responsible for coordinating with central metal ions. Furthermore, some imperative bands appeared in complexes in ranges between 653 and 558 cm^{-1} assigned to (M–O) and 560–541 cm^{-1} assigned to (M–N), which gives further confirmation of complex formation. The appearance of new spectral bands suggests the presence of chemical linkages that include heteroatoms, such as azomethine nitrogen, deprotonated oxygen, and chlorine atoms. These heteroatoms are coordinated to the core metal ion. This discovery provides strong evidence for the adoption of an octahedral geometry in all of the metal complexes being studied. The main absorption bands of ligands **L1**and **L2** and their complexes **1a**–**3a** and **1b**–**3b** are listed in.

In the ^{1}H NMR spectra of hydrazone ligand **L1**, a doublet appears at 1.18 ppm due to the (CH3)2 protons of the isopropyl group. A multiplet signal appears due to the −CH– proton of the isopropyl group at 2.80 ppm. The chemical shift of the azomethine N=CH proton in both ligands (**L1** and **L2**) appeared at 8.05 and 8.20 ppm. The singlet peak of NH proton appeared in both ligands at 10.40 and 10.32 ppm due to the electronegative atom attached to the proton, which in turn increases the deshielding effect of the proton. Furthermore, the singlet peak at 10.55 ppm corresponds to hydroxyl (OH) protons. The aromatic protons were resonated at 6.86–7.59 and 6.88–7.72 ppm. In ^{13}C NMR, the peak for azomethine (C=N) carbon in **L1** and **L2** was observed at 159.2 and 157.3 ppm. In the **L1** spectra, signals appeared at 20.3 and 44.9 ppm due to the presence of methyl and methylene carbon of the isopropyl group. In the **L2** spectra, one signal appeared at 110.2 ppm to recognize the presence of cyano carbon (CN). All of the aromatic carbons in **L1** and **L2** were resonated around 120.3–131.4 and 120.3–131.4 ppm.

Methodology :-

The various works of literature, scienti ic papers,original articles are surveyed and reviewed from dif-ferent search engines viz. Research Gate, GoogleScholar, PubChem, ChemSpider, Scopus etc. forthis write up. The authors have gone through andreviewed many full-text articles with abbreviationsas hydrazone and its metal complexes; imidazolebased heterocyclic compounds, biological poten-cies of hydrazones and its derivatives, QSAR studyof these metal complexes etc. for the successfulreview. The authors drew all the structures inACD/ChemSketch 2017.2.1(Freeware).

Biological Potential of Hydrazonederevatives :-

Antimicrobial activitiesChemicals are used to resist transmittable diseasesagainst different bacteria. As an antibacterial agent,hydrazones containing imidazoles wight againstdifferent bacterial strains. In one research paper,Researchers have evaluated the antibacterial activ-ity of cobalt(II), nickel(II), zinc(II), copper(II) andcadmium(II) complexes of acetophenone-4-aminobenzoyl hydrazone and 4-hydroxy acetophenone-4-amino benzoyl hydrazone against Escherichiacoli and Aspergillus niger. They also reportedthat copper(II) is more active than zinc(II) ofthese hydrazone complexes at every concen- tration. The authors of the previous researchhave reported the antibacterial activity of 2, 3,4-pent aneotrione-3[4-[(5-nitro-2-furyl)methylenehydrazide]carbonyl]phenyl] hydrazone againstStaphylococcus aureus and Mycobacterium tubercu-losis (Savini et al.,2004).Figure 1: Formation of Hydrazones fromketones/aldehydesFigure 2: Versatile Biological Potential ofHydrazone DerivativesSome novel transition metal complexes ofbenzylidene-hydrazo derivatives containing quino-line ring were evaluated against some Gram (+)ve,Gram (-) ve and fungi.Thiosemicarbazone, alongwith hydrazone derivatives, has promising effec-tiveness against Mycobacterium tuberculosis, andethyl- 2-arylhydrazone-3-oxobytyrates has antibac-terial activity against Staphylococcus aureus (Pavanet al.,2010).Fungal infections are generally observed as topic.

DFT Calculations :-

Hydrazone ligands and their metal complexes are optimized using DFT with B3LYP/6- 31G (d, p).

1. Frontier Molecular Analysis

The FMO theory is presently regarded as an effective tool for determining a molecule's reactivity as well as its electrical and optical properties. This is useful for investigating how excited- state lowest unoccupied and highest occupied molecular orbital energies are corelated to one another. The B3LYP/6-311G (d, p) level of theory is used to calculate the frontier molecular orbitals of molecules. The visual representation is illustrated in, and the energy band gap is given in . A large energy gap indicates that the structure is thermodynamically stable. A small energy gap in a structure indicates it is more reactive and more polar. Red indicates the negative part, and blue indicates the positive part.

2. Molecular Electrostatic Potential

The investigation of the three-dimensional plot of electron density on the entire compound is conducted by the study of molecule electrostatic potential, employing

$$V(r)=\sum(ZARA-r)-\int(p(r\rightarrow)/r\rightarrow-r)dr\rightarrow V(r)=\sum(ZARA-r)-\int(p(r\rightarrow)/r\rightarrow-r)dr\rightarrow$$

The equation shown above introduces the variable $V(r)$ that denotes the molecular electrostatic potential. Additionally, ZA is used to indicate the charge density over the nucleus. The electronic density function is defined as $p(r')$ at a certain location denoted as RA, where r' represents the integration variable. The color observed in MEP (molecular electrostatic potential) analysis serves to delineate the regions inside a molecule that are susceptible to electrophilic and nucleophilic attack. The magnitude of electrostatic potential increases in the following order: red < orange < yellow < green < blue. The red hue signifies the optimal location for electrophilic attack, whereas the blue hue designates the spot most conducive to nucleophilic attack. The MEP analysis was conducted using the B3LYP/6-31G (d, p) basis set. The obtained results are depicted in. The observation reveals that oxygen atoms exhibit a negative potential, as indicated by the red hue, whereas the positive potential is predominantly displayed in the blue area by nitrogen atoms, with hydrogen and carbon atoms partly contributing. The green area represents the mean potential, which refers to the range between the two extremes. The presence of distinct reaction sites in all molecules can be inferred from the observation of red and blue colors.

3. Global Reactivity Parameters

The HOMO and LUMO orbital energies are used in the equations to generate global reactivity descriptors. The stability, selectivity, and reactivity of the species are all heavily dependent on these global reactivity factors. Tabulated reactivity parameters and their corresponding computed values are listed in.

4. NBO Analysis

Gaussian 09W software was used to do NBO analysis on complexes at the B3LYP/6-311G (d, p) level of theory. Natural bond orbital (NBO) studies are useful for understanding the charge relocation between full and unoccupied orbitals. NBO data helps us understand intermolecular delocalization and the charge concentrations that shift in D-π-A structures from donor to acceptor regions.

There were typically three distinct types of electronic transitions detected. Among these, σ-σ* transitions were more prominent. Other transitions include σ-π*, L.P-σ*, and L.P-π*. For calculating the relationships between the donor and acceptor, a second-order perturbation model study of the Fock matrix was performed and stabilization energy was calculated using

$$E2=qi(F2ij)\varepsilon i-\varepsilon jE2=qi(Fij2)\varepsilon i-\varepsilon j$$

All types of vibrations in structures and their stabilization energies are given in the table. The charge transfer characteristics found by NBO analysis of these compounds are significant for their possible NLO features. Natural bond orbital analysis with second-order perturbation study for hydrazone ligands and metal complexes using B3LYP/6-311G (d,p) is presented in.

5. NLO Properties

Optical switches, communication technology, signal processing, and optical memory devices all make substantial use of NLO compounds. The optical response, which is related to both the nonlinear (hyper polarizabilities) and linear (polarizability, etc.) responses is caused by the electrical properties of the entire compound.

6. FT-IR Calculations

Molecular vibrations obtained by modern vibrational spectroscopy have sparked the interest of both the computational and the experimental communities. On the optimized geometries of molecules, we estimated the theoretical vibrational spectra of the synthesized compounds using DFT at the B3LYP/6-31G (d, p) level of theory. The vibrational modes in the investigated compounds were assigned using the animation feature of Gauss-View. The experimental and computed harmonic vibrational frequencies for all compounds are given below.

7. ADMET Properties

Swiss ADMET was used to forecast the results of ADMET investigations of isolated substances (**1a**–**3a**, **1b**–**3b**, **L1**, and **L2**) to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) features to assess the bioavailability of the compounds. The skin's ability to absorb molecules is measured by its permeability (Kp) in cm/s. The skin permeability, Kp, values of all compounds varied from 1.59 to −5.45 cm/s in silico, indicating low skin permeability. Additionally, the blood-brain barrier (BBB) and gastrointestinal (GI) permeability show how medication molecules are absorbed and distributed. The findings of the in silico predictions for the chemicals (**1a**–**3a**, **1b**–**3b**, **L1**, and **L2**) under study's absorption, distribution, metabolism, and excretion (ADME) are shown in. According to the Swiss ADME prediction parameters, the complexes showed low GI absorption, whereas **L1** and **L2** showed high GI absorption. The blood-brain barrier (BBB) permeability of **L1** and **L2** was similarly demonstrated by Swiss ADME prediction, but not those of the complexes. Additionally, a variety of cytochromes (CYPs) control how drugs are metabolized, with CYP1A2, CYP2C9, CYP2C19, CYP3A4, and CYP2D6 being particularly important for the biotransformation of drug compounds.

8. In Silico Pharmacokinetics (Drug-likeness) and Toxicity Analysis

The SwissADME tool was used to predict in silico pharmacokinetic characteristics (drug-likeness qualities) based on Lipinski's rule of five using the structures of isolated compounds (**1a**–**3a**, **1b**– **3b**, **L1**, and **L2**) converted to their canonical simplified molecular-input line-entry system (SMILE). Lipinski's rule of five suggests that the drugs and/or candidates should abide by the five- parameter rule, which specifies that the hydrogen-bond donors (HBDs) should be less than 5, the hydrogen-bond acceptors (HBAs) should be less than 10, the molecular mass should be less than 500 Da, log *P* should not be less than 5, and the total polar surface area (TPSA) should not be greater than 140. Drug-likeness is a test that determines whether a specific organic molecule possesses characteristics that are typical of an orally active medication. The SwissADME tool confirmed that all complexes followed Lipinski's rule of five and are likely to be orally active The bioavailability of the compounds and their hydrogen bonding potentials are closely connected to the TPSA value. As a result, the examined compounds' TPSA values were found to be between 44.62 and 114.14, which is significantly less than the limit of 140. The complexes and ligands' computed number of rotatable bonds (NRB) values are fewer than 10, indicating that the compounds are conformationally stable.

9. Antimicrobial Activities

The synthesized hydrazone ligands (**L1** and **L2**) and their metal complexes were tested on two Gram-negative strains (*Escherichia coli* and *Salmonella*), Gram-positive strains (*Bacillus halodurans* and *Micrococcus luteus*), and fungal strains (*Aspergillus flavus* and *Aspergillus niger*) by a disc diffusion method. The test compounds (ligand/complex) were dissolved in DMSO. Azithromycin was used as a standard drug in pathogenic bacteria, and terbinafine was used as a standard drug in fungal strains. The antibacterial and antifungal activity results show that ligands were active toward these bacterial strains, but the complexes found moderate activity against pathogenic bacteria. The hydrazone ligand **L1** exhibits maximum inhibition of 11 mm against *E. coli* and *B. halodurans* bacterial strains and shows less activity against other bacterial strains. The hydrazone ligand **L2** shows a 15 mm zone of inhibition against the *E. coli* bacterial strain. Among all the complexes, complex **2b** shows the highest activity with 13, 18, and 14 mm inhibition zones against *E. coli*, *B. halodurans*, and *M. luteus*, respectively. Furthermore, the hydrazone ligand **L1** shows 6 mm zone of inhibition against *A. flavus*, while **L2** shows a 9 mm inhibition zone against *A. niger*. Complex **1b** shows the highest activity with a 14 mm inhibition zone against *A. niger*, and complexes **3a and 3b** show moderate activity with a 9 mm inhibition zone against *A. niger* as compared to other metal complexes but less activity as compared to the standard drug. The zones of inhibition against the tested bacterial and fungal strains are presented in Table and. The activity of the hydrazone metal complexes is enhanced due to complexation with central metal atoms. Co(II) and Ni(II) complexes show better activity against bacteria as compared to the other compounds. Cu(II) and Ni(II) complexes show greater activity against bacterial strains, but other complexes showed better activity against fungal strains but less activity as compared to the standard drug. Partial sharing between the azomethine nitrogen and phenolic oxygen with a positively charged metal atom can cause the metal to be less polar during complexation. Chelation can increase the lipophilicity of metal ions that enhances the permeability in lipid membranes and consequently supports the inhibition of bacterial growth by

Conclusions :-

Novel hydrazone ligands and their transition metal complexes Co(II), Ni(II), and Cu(II) were synthesized and characterized by spectroscopic data (FT-IR, UV–vis, ^{1}H NMR, C^{13} NMR, and HRMS). FT-IR and HRMS analysis results show that the formation of complexes was successful and also that the complexes formed octahedral geometry. Additionally, we present the results of DFT analysis of the synthesized compounds. Resolution of the chemical structure was attained by means of NLO and IR analyses as well as NBO, FMO, and global reactivity parameters. The DFT and experimental calculations were in good agreement. FT-IR calculations were reproduced with DFT calculations. The newly prepared hydrazone ligands and their metal complexes were screened for their antimicrobial activities, and some compounds were active against Gram-negative bacteria *Salmonella* and *E. coli* and Gram-positive bacteria *B. halodurans* and *M. luteus* and show activity against *A. flavus* and *A. niger*. The results indicated that while the ligands displayed activity against the bacterial strains, the metal complexes exhibited varying degrees of effectiveness. Complex **2b**, for instance, showed the highest inhibition zones against multiple bacterial strains. The enhanced activity of the hydrazone metal complexes can be attributed to chelation with central metal

atoms, leading to improved permeability and the inhibition of bacterial growth.

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