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Organic and bioinorganic chemistry is the branch which has much more remained for exploring medicinal and analytical techniques. Various methods for the synthesis of organic molecules have now been reported which have opened a new window to look and work on [1-6]. The nature of bonding in organic molecules can be evaluated by various theories like Ligand field theory, Molecular orbital theory, which greatly helped in understanding the factors affecting their stability. Recent advancement in drug discovery is based on the structure, stability and effectiveness of organic molecules. Ever since the discovery of organic molecules as therapeutic agents, many changes have occurred in the field of Medicinal Chemistry. From finding a therapeutic use for the available organic molecules, there has been a paradigm shift towards designing molecules to treat specific diseases [7-15].

Organic molecules acts as ligand which can bind with more than one donor atom hence called chilators, the stability increases with the increase in number of covalent and coordinate bonding with neighbor atoms. The solubility and effectiveness of drug molecules are thus predicted by this index and is a key feature. The physicochemical properties of an organic molecule to develop drug, are done by evaluating various parameters [16, 17]. This approach has been facilitated by Quantitative Structure Activity Relationship (QSAR) studies. The techniques used for evaluating several drug properties are performed by in silico techniques using various softwares, which help in improvement of activities. The modern approach of tailoring a drug to meet a requirement has been facilitated because of identification and characterization of the three
dimensional aspect of several protein targets which acts as signaling agents in various disease states [18].

The structural theory of organic chemistry was developed in the last half of the nineteenth century. It led to the concept that biochemical properties must be a function of change in their structure. Examples are the directional effect of substituents on the benzene ring with respect to electrophilic aromatic substitution and orientation in transition metal complexes acts as catalyst in many industrial processes like Wacker process, Oxo process, Monsanto processes etc. Various enzymes having prosthetic group enzymes contain usually metal ion e.g. Heamoglobin, Myoglobin, Chlorophyll and Cytochrome [19-23].

Ligand can bind to the metal ion by covalent and coordinate bonds to form complexes known as organometallic complexes. Critical parameters for the design of a new chelating agent are complex strength, solubility of the ligand and its complexes selectivity for particular metal or class of metals and the thermal stability of the chelate complexes. This can be obtained by the mixing exact molar ratio of ligand and metal ions [24].

Modern coordination chemistry occupies a special interdisciplinary position in chemical sciences, joining inorganic, organic, physical, analytical and biological branches of chemistry [25]. The spectacular development of modern coordination chemistry relies upon programmed ligands, which are organic molecules designed to generate complexes with pre-established properties. Acyclic and cyclic Schiff bases are special class of modern coordination chemistry due to the possibility of creation on their basis novel polyfunctional materials: magnetic, luminophores, bioactive chelates, chemosensors and liquid crystals. Schiff base metal complexes play an important role in supra- and nanochemistry [26-30].
A recent advancement of bioorganic chemistry, medicinal, inorganic chemistry is at the junction between medicine and inorganic chemistry which includes metal-based drugs, metal sequestering or mobilizing agents, metal-containing diagnostic aids and the medicinal recruitment of endogenous metal ions. Ligands can be modified for oral/systemic bioavailability of metal ions, can assist in targeting specific tissues or enzymes, can deliver, protect or sequester a particular metal ion, depending on the requirements for therapy or diagnosis [31-37].

The hybridization of metal complexes determines the geometry of the molecules, which may be affected by the reaction conditions used. Coordination compounds brought revolution in inorganic chemistry leading to generation of novel products, applicable in analytical chemistry, fungicides, paints, pigments, polymers, pharmaceuticals, catalysis, and photoconductors [38-40].

Complexation reactions are used in qualitative as well as quantitative analysis. Some extremely sensitive and selective organic reagents have been reported for the determination of metal ions [41]. Various techniques have been developed by the scientists for the detection and determination of metal ion complexes. While the older techniques like conductometry, pH metry, elemental analysis are helpful in determining the physical properties [42], spectroscopic techniques like FTNMR, MASS, X-RAY, FTIR and UV-Vis are proven to be of great value for evaluating their physicochemical properties. Metal complexes have been used for a wide variety of applications, e.g. C.Maria et al synthesized cobalt and nickel complexes of versatile imidazole- and pyrrole-2-carbaldehyde thiosemicarbazones and studied their antimicrobial activity. Schiff base complexes have been amongst the most widely studied coordination compounds in the past few years, since they are becoming increasingly important as biochemical, analytical and antimicrobial reagents [43-46].
Schiff base derivatives, amidines, are well known pharmacophores in drug discovery. These privileged structures have not been studied and represented in chemical compound libraries. In the framework of this doctoral thesis, five different scaffolds which are not frequently used or are totally absent in commercial compound libraries have been synthesized [47-50]. The discovery of metal based medicinal product have opened up new scientific area and posed questions which are still at an early stage of exploration. This area is of major interest to the immediate and long-range goals. It is better to focus on the rapidly expanding research areas associated with peptide chemistry and its potential application to drug abuse research and therapeutic treatment. Major classes of pharmaceutical agents containing metal compounds have current clinical use thus new areas of application are rapidly emerging [51].

Transition metal complexes have been studied broadly due to their significance as biomimetic models for metalloproteins, as effective catalysts for oxygenation processes and utilization in asymmetric catalysis, as building blocks for metal-containing liquid crystals and the functional self-assembled supramolecular architectures with interesting structures and properties. Much effort has been devoted in recent years to the design with the aim to develop novel therapeutic agents [52-55].

![Schiff base and amidine molecules](image)

Figure 1: Schiff base and amidine molecules
Schiff bases have effect for substrate chirality, leading the metal centered electronic factor, enhancing the solubility and stability of homogeneous or heterogeneous catalysts. Reports are available on the chemistry of biocidal activities of transition metal complexes containing O, N and S donor atoms, possess unusual configuration, structural lability and are sensitive to molecular environment. The geometrical orientation of transition metal complexes is the key factor for metalloproteins to carry out specific physiological functions [56-58].

Number of amidines and their metal complexes using different reagents have been synthesized and characterized. Some drugs like Nimesulide, have also been modified and used as ligands which have been found to have better antimicrobial and bacterial activities as compared to the original drug [59-63]. A. Golcu et al have synthesized various schiff base ligand and prepared coordinate compounds of Cd(II) and Cu(II). Their antimicrobial activities were also studied and reported [48]. B. Clement et al studied the new prodrug principle with their pharmacokinetic properties to improve their bioavailability [64, 65].

![Fig.2. Anthranilic amidines, H.Nakamura et al.](image)

Hiroyuki Nakamura et al have studied (Fig.2) many benzamidines and evaluated their VEGFR tyrosine kinase activity [35]. Mohabey et al have developed methods for extraction and
determination of Mo, Fe, Cu, etc. using hydroxyamidine hydrochloride [34, 44] (Fig.3). These were done in the presence of thiocyanate ion forming orange red color in strong acidic medium. K.S. Patel et al proposed a method (Fig.4) for the detection and determination of Cr(II) in acidic medium in coal dust cement dust mainly based on chloroform of Cr(II) complex [32, 33].

Various researchers have performed experiments on the synthesis and characterization of N-hydroxyamidines. Previously their applications were mainly focused on the heavy metal extractions and determination of metals like Mo, Co, V and Cr etc. Several researches were also based on the biological application of amidines, but we have found only a few number of research work which implicate their biological activities. N-hydroxyamidines have not much been explored for their biological activity [66-69].
The application of computational methods to study the formation of intermolecular complexes has been the subject of intensive research during the last decade. It is widely accepted that drug activity is obtained through the molecular binding of one molecule (the ligand) to the pocket of another, usually larger, molecule (the receptor), which is commonly a protein [69]. A complex of a protein with a therapeutical drug. In their binding conformations, the molecules exhibit geometric and chemical complementarity, both of which are essential for successful drug activity. The computational process of searching for a ligand that is able to fit both geometrically and energetically the binding site of a protein is called molecular docking [74].

The rapid generation of quality lead compounds is a major hurdle in the design of therapeutics, so that accurate automated procedures would be of tremendous value to pharmaceutical and other biotechnology companies. However, designing a drug based on the knowledge of the target receptor structure as determined by current experimental techniques is a process prone to error [53]. The two major reasons responsible for failures are inaccuracies in the energy models used to score potential ligand/receptor complexes, and the inability of current methods to account for conformational changes that occur during the binding process not only for the ligand, but also for the receptor [75].

**N-hydroxyamidines**

Major research effort was focused towards finding cheap, effective and simple ways for the synthesis of N-hydroxyamidines and their metal ion complexes. Present work aims to synthesis and characterization of drug candidates for certain kind of human diseases like tuberculosis, malaria etc. Models for the quantitative description of the structural effects of substituents are described in this work. Also described are substituent effects of NO, −C=NOH,
C(O)NHOH and NHOH substituents N-hydroxyamidines have adjacent nitrogen and oxygen atoms, as part of their characteristic N–OH group. Since both these atoms commonly have significant lone pairs, a major determinant of conformations is the need to minimize the repulsion between these lone pairs. This will be illustrated by the example of hydroxylamine, H₂N–OH. We have found two energy minima for H₂N–OH, corresponding to two stable conformers [70-73]. In the less stable, the nitrogen and oxygen lone pairs are in close proximity; the distance between their effective centers, the respective \( V_{\min} \), is only 2.40 Å. (The two oxygen lone pairs give rise to only one \( V_{\min} \) and one \( V_{S\min} \).) When the hydroxyl group is rotated to give, this distance increases to 3.21 Å. The energy simultaneously decreases by 3.9 kcal mol⁻¹.

It is shown in the figure that N-hydroxyamidines are found in two stable tautomeric forms. Rearrangement occurs, resulting in pi bond shifting [74]. It might be thought that may also be stabilized by attractive interactions between the hydroxyl hydrogen and the nitrogen lone pair and/or between the amine hydrogens and the oxygen lone pair(s). To investigate these possibilities, we look at the distances between these hydrogens and the \( V_{S\min} \) of the lone pairs. Here it is appropriate to use \( V_{S\min} \), as the hydrogens are external to the nitrogen and oxygen surfaces [75]. The respective H- - - \( V_{S\min} \) separations are rather large: H(hydroxyl)-- - \( V_{S\min} \) (N) = 2.75 Å, H(amine)-- - \( V_{S\min} \) (O) = 3.05 Å. They are indeed greater than the sums of the H- - - N and H-- -O van der Waals radii 40, 2.72 Å and 2.69 Å. (The sum of the N- - - O van der Waals radii is 3.07 Å) Thus the effect of any such hydrogen bonding interactions in stabilizing should
be quite minor (or none) compared to that of separating the lone pairs. It has been observed that the hydroxyl group to be rotated so as to achieve this separation.

Basic molecular formula of N-hydroxyamidines are written as (R1R2C=NOH) where R represents H, alkyl, cycloalkyl or aryl ring. Amidines are oxygen-containing core that is linked directly to electronegative nitrogen with an unshared electron pair. In general, –NR–OH containing molecules appear to be amenable to biotransformations such as reduction, oxidation, hydrolysis and conjugations with organic and inorganic molecules [76].

In addition, the ability of amidines to form strong complexes with transition metals determines its biological and medicinal behavior [77-80]. These properties have been exploited by few investigators to design and develop novel therapeutic agents that can display acyl group transfer capabilities and/or serve as specific inhibitors of a variety of metalloenzymes. Biological and therapeutic properties in plants, marine sponges, microorganisms and mammals gave considerable impetus to the synthesis and evaluation of novel candidate drugs for the treatment of various diseases, e.g. amidine were proposed as part of a treatment regimen for cyanide toxicity in humans [81].

N–OH containing compounds were further reported as antimalarial, antimicrobial and anticancer drug, possess antioxidant and anti-inflammatory activity, to serve as efficient drugs in nonabsorbed iron chelating therapy and to be potential agents for treatment of osteoarthritis [82]. Heteroaromatic and aliphatic amidines are being used as antidotes to treat organophosphate intoxication caused by nerve agents and commercial pesticides, and some amidine ligands have been shown to possess antimicrobial activity, potential digitalis-like properties, antitumor activity, ability to relax blood vessels and the capacity to initiate oxidative DNA cleavage. Thus,
both the increasing synthetic effort and the growing body of scientific information with respect to N=OH compounds hold great promise for the development of novel therapeutic drugs in various fields of medicine [83,84].

**Structure and properties of N-hydroxyamidines**

Amidines have the general formula of –C(=NH)NH₂. These are much more basic than amides and are among the strongest neutral bases. These are very much active against some bacteria and fungi. The basic nature of their functional group is responsible for the bioactivity. It binds with different enzyme to inhibit their biological function and hence acts as the growth inhibitors against certain microbes. The basic principal behind the development of a new drug candidate is the pharmacophore modeling by using QSAR [85]. A structure of molecule is the key feature for their biological functions. Some parameters are set by the activities of the molecule can be assessed.

![Diagram showing the pharmacophore modeling of 3-methyl-N-hydroxybenzamidine. First one in pink and green color is showing the hydrogen donor and acceptor and second picture is showing the hydrophobicity.](image)

The amidine moiety of N-hydroxyamidine acts both as hydrogen acceptor and hydrogen donor, Fig.7 showing the hydrogen bond donor and hydrogen bond acceptor activity of 3-
methyl-N-hydroxybenzamidine, illustrated as by the green color as hydrogen acceptor and pink color as hydrogen donor. The hydrophobicity is shown as blue color frame around the same molecule, other parameters are steric, polar surface area and ionizing surface etc. this are referred as the descriptors. This property of molecule finally determines the interaction with the biomolecules. The activity increases when group like \(-\text{NH}_2, -\text{NO}_2\) and \(-\text{CH}_3\) are attached to the phenyl ring it increases the hydrogen bond interaction of these molecules to the biomolecules.

Higher number of alkyl group attached to the phenyl ring increases the hydrogen bond done acceptor property and hence the membrane penetration property increases which is finally responsible for the growth inhibition property of microbes. The present work describes the synthesis, characterization and biological evaluation of some N-hydroxybenzamidines, which are not used frequently or unknown compound. Among the reported methods for synthesis of amidines we have followed the simplest method. Characterization of these molecules was performed using NMR, IR, UV-vis, MASS and Elemental analysis etc. The purification was performed by thin layer technique using two solvent at different ratio.

The thermal stability was examined using thermal gravimetric analysis techniques of some compounds. The biological properties were evaluated using various bacterial and fungal strain cultured in nutrient medium. Some molecules were studied for their in vitro inhibitor property against some enzymes of microbes, e.g. Purine nucleoside phosphorylase of \textit{M.tuberculosis} and \textit{P.falciparum} etc. The antitumor activities of these compounds against breast cancer cell were studied using reported procedure. In the present research work molecular docking simulation studies were also performed to evaluate the enzyme inhibition properties in silico using various softwares, like. Molegro virtual docker, Osiris, Molinspiration, Discovery
Studio, Chem Sketch, Sparton 10 and Chem Bio Draw Ultra etc. Gel electrophoresis was used to study the DNA cleavage analysis. Antioxidant activity was also studied using DPPH reagent.

**Objective and scope of the research work**

In the present work N-hydroxyamidines with Co(II), Cr(II) and Zn(II) complexes were synthesized and characterized are not in use or unknown. Biological evaluations were performed on different microbes. Docking and simulation studies were also performed using various software. The application of our research work is widespread and may be helpful in understanding the biological properties of hydroxyamidines, applicable in certain diseases.

The possible outcome of our work is better understanding of drug interaction with biomolecules and their activities, The N-hydroxyamidine molecules were studied by various spectral techniques and they were evaluated as biologically active compounds which may lead to development of new drug agents against various disease.

**Methodology used in the present work**

In the present work we have used FTIR, NMR, UV-vis, Mass, Elemental analysis, TGA etc. for the characterization of the synthesized compounds. For the synthesis we used water bath. Thin layer chromatography was used to ensure the formation of ligand and complexes. Gel electrophoresis has used to study the DNA cleavage analysis. For the biological activities we have used incubator to maintain the optimal temperature for the growth of bacterial and fungal species. For the molecular docking studies we have used many software like Sparton 10, Chembiodraw Ultra, Argus lab, Chem Draw, Actelion Software, PyMol, Molinspiration software.
Summary of the research work

The thesis has been divided in eight chapters as under:

Chapter 1. Introduction

Recent advances in the field of medicinal chemistry, various techniques used to synthesize ligand and their metal complexes, their characterization, purification processes etc. are introduced in this section. We have contained the discussion of the nature and importance of organic chelating agents and the detailed introduction about the metal ion complexes and their coordination with the ligand were also described. Various Schiff base derived ligands and their metal ion complexes have been discussed, their biological application such as antibacterial, antifungal and anticancer etc. are discussed in detail. The work conducted by various researchers along with their application on certain kind of amidines and their metal complexes are also discussed. The basic structure of amidine and their derivative N-hydroxyamidine are focused in this part. The key structure which is responsible for their biological activity and their chelating effect are discussed in this section. Nature of our work and how its difference from reported are, which mainly emphasized on bioavailability and metal ion extraction is explained.

Chapter 2. Synthesis, spectroscopic/structural characterization

This section contains the step by step method for the synthesis of various N-hydroxyamidines ligand and their complexation with various metal ion complexes. The purification of ligand and the metal complexes is also discussed. The percentage yield of the compounds were between 75-93%. This section is divided into various subsections. The spectral characterization of the synthesized compounds using FTIR, NMR, UV-vis and MASS were performed and discussed in details to confirm their structure. Thermo gravimetric, elemental analysis and magnetic behavior was done and included in this chapter to support the structural composition. The molecular ion peak of the ligands and complexes confirms the purity. Vibrational peak at its corresponding position supports the structural activity. $^1$H NMR and $^{13}$C NMR spectral studies of the compounds have been discussed to propose the structural confirmation. The electronic spectra give the idea about the functional group and their transition in solution.
Chapter 3. Antimicrobial activity

In this chapter we have discussed the antimicrobial activity of the N-hydroxyamidines and their metal ion complexes. Antibacterial activity of this compounds on some selected bacterial strains like *S.aureus*, *B.cereus*, *E.coli* and *M.tuberculosis* etc. were studied by the filter paper disc method at various concentrations using nutrient agar as medium, in vitro and only few of them were selected for animal testing. The Inhibition zone and minimum inhibitory concentration (in µM/mL) of the compounds were studied and evaluated using reported methods. Likewise antifungal activity of the compounds on some fungal species like, *T.longifusus*, *C.albican*, *A.flavus* and *P.aerugenosa* etc. were evaluated and the minimum inhibitory concentration and zone of inhibition determined.

The inhibition zones were recorded in percentage. It can be we concluded that some of the compounds are moderate to good inhibitors of both the bacterial and fungal species. It can also be studied further for the better results to develop as the antibacterial and antifungal drugs. The studies and their results of HIV-1 cell inhibition study and *P.falciparum* growth inhibition were also discussed in this chapter.

Chapter 4. DNA cleavage study

This chapter reports the CT DNA cleavage activity studies of N-hydroxyamidine and their metal ion complexes. The gel electrophoresis was performed using CT-DNAThe compounds were taken to an appropriate amount to study the nucleolytic behavior of these compounds. DNA was uncoiled interacting with the different amount of the sample. The results shows that are able to convert supercoiled to nicked circular thus revealed that metal complexes and ligand behave as efficient chemical nuclease for double strand cleavage of DNA.

Chapter 5. Antioxidant activity

In this chapter we have discussed about the antioxidant activity of the N-hydroxyamidines and their metal ion complexes. All the complexes were dissolved in ethanol and added to DPPH reagent having free radical in solution and the absorbance was measured by increasing time [36]. The ability to give hydrogen ion to free radicle hence showing their percentage antioxidant activity. We have concluded that all the compounds have moderate to good antioxidant activity to become a good drug candidate.
Chapter 6. Enzyme inhibition and biological activity

In this chapter the method for the ligand to protein interaction techniques and their results are discussed. We have performed some experiment using reported techniques to inhibit the growth of *M.tuberculosis*, *HIV-1* and *P.falciparum* by the inhibition of their active enzyme like purine nucleoside phosphorylase, reverse transcriptase and thymidylate kinase etc. The results for their inhibition activity was confirmed my in vitro analysis. The results were shown as IC$_{50}$ values of compounds in µM and nM range that is a good range to develop as the drug agent. The results included in this chapter were good to moderate for all the compounds.

Chapter 7. Antitumoral activity of some ligand and their metal ion complexes

This chapter reports the ant antitumoral activity of some compounds including N-hydroxyamidine and their metal complexes. i.e., N-4-(dihydroxy)benzamidine and their Co(II), Zn(II) and Cr(II) complexes were synthesized and evaluated as on breast cancer MCF-7 cell lines preliminary. The results include in this chapter to support their good to moderate nature to so as to propose as the anticancer cell proliferation inhibitor agent.

Chapter 8. Molecular docking and SAR studies of some compounds

The use of computer aided drug design has become a most important tool to predict the structural activity of the compounds. In this chapter we have concluded the SAR study of some N-hydroxyamidine and their metal complexes to validate the experimental results. We have focused on the in silico inhibition of protein assay techniques, their molecular docking simulation studies, ADME, toxicity behavior to achieve low cost, high absorbing and nontoxic medicines. Molecular descriptor like, LogP, LogD, LogS were selected for prediction of their chemical and physical behavior, which were also confirmed by using in silico descriptor calculations.
Reference


