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ANALYSING BIOLOGICAL ACTIVITIES AND STABILITY OF LIGANDS

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ABTRACT

Transition metals have an important place within medicinal inorganic chemistry. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals led to the recent development of drugs which are based on metals and are considered to be potential candidates for pharmacological and therapeutic applications. This review focuses on research undertaken over the past few decades which has sought to possess preclinical pharmacological screenings like anti-microbial, anti-inflammatory and anti-tumor action of synthetic transition metal complexes. It concentrates primarily on a limited number of first row transition metal complexes particularly V (IV), Co (II), Ni (II), Cu (II) and Zn (II) complexes and traces the pharmacological applications of these coordination compounds. In the first part, the nitrogen, oxygen and sulfur donor ligands chelating to transition metals used in metallodrugs are described. The second part describes the pre-clinical screenings viz., anti-microbial, anti-inflammatory and anti-tumor responses of the above coordination compounds incorporating these nitrogen, oxygen and sulfur donor ligands. This survey encourages further research in this field for future applications

A series of novel bidentate anodyne quinoline ligands were synthesized with various p-aromatic amines like p-(OCH_3, CH_3, H, Cl and NO_2). Novel anodyne (HL_n) and complexes [Cu (II)/Ni (II)] of these ligands have been characterized on the basis of elementalanalysis, molarconductance and magnetic measurements, infrared and electronic spectral studies. Suitable structures have been proposed for these complexes. The synthesized ligands and their metal complexes were screened for their antimicrobial activity against four local bacterial species, two Gram positive bacteria (Bacillus cereus and Staphylococcus aureus) and two Gram negative bacteria (Escherichia coli and Klebsiella pneumoniae) as well as against four local fungal species; namely Aspergillus Niger, Alternaria alternata, Penicillium talinum and Fusarium oosporous. The tested compounds have good antibacterial activity against B. cereus, E. coli and K. pneumoniae. Very low effect was detected against S. aureus and F. oosporous. We found that the results of antifungal activity of HL_n revealed that the complexes are more toxic than ligands against fungi due to the transition metal involved in the coordination. Also Cu^{2+} complexes are more active than Ni^{2+} complexes against B. cereus, E. coli and K. pneumoniae. The size of the clear zone was in the following order p-($OCH_3 < CH_3 < H < Cl < NO_2$) as expected from Hammett's constants σ^R.

KEYWORDS:

Complexes,Hydrogen-bonding stability,Spectroscopic studies
Antimicrobial activities

INTRODUCTION:

Transition metal ions are playing an important role in biological processes in the human body. Coordination compounds combine the features of metals, which have a wide range of coordination numbers, geometries, variable oxidation states, and ability to bind a variety of organic ligands or mixed ligands in an attempt to get the optimal stability and the biological in vitro activity, where the action of many drugs depends on the coordination with metal ions or the inhibition on the formation of metallo-enzyme. Researchers have published reviews about complex metals and their contributions to biological activities; it was made clear that a number of antibiotics contain a metal-binding site. Sometimes, transition metal ions are tightly bound forming stable coordination connections, which have an important structural function and/or are responsible for effective antibiotic action. There are a number of antibiotics that require metal ions to function properly and complexes often show better physicochemical properties and are much more effective than parents' drugs. Therefore, bioinorganic chemistry provides a powerful weapon for overcoming numerous challenges encountered in antibiotic chemistry; researchers showed the importance of metal chelation to tetracycline which is an antibiotic used to treat many different bacterial infections, such as urinary tract infections, acne, gonorrhea, chlamydia, and others [6]. Coordination chemistry of mixed-ligands with transition and non-transition metal ions is important in metallo-enzymes and other biological activities. In most cases, metal complexes show higher bioactivities than the free ligands and some side effects and drug-resistance may be reduced upon complexation. Mixed ligand complexes differ from traditional complexes in the sense that they are having at least two different kinds of ligands associated with the same metal ion in a complex. The presence of more than one type of ligand in a complex increases chances of variation in properties expected for the complex. This makes the researchers interested in the synthesis of mixed ligand complexes with varying properties. In recent years, many publications are devoted to synthesis and characterization of mixed ligand complexes. Numerous mixed ligands transition metal complexes have been investigated by various techniques and their biological activities and, exhibit many neurophysiological and neuron pharmacological effects like antimicrobial, antiviral, anticonvulsant, anticancer, anti-mycobacterial, antimalarial, cytocidal, herbicidal and anti-inflammatory activity were extensively studied. Chelating ligands containing O, S and N donor atoms and metal complexes containing nitrogen and Sulphur donors have been proved to show broad biological activity, to be potential antibacterial and fungal agents as well as component of several vitamins and drugs.Nickel complexes with nitrogen and sulfur donor ligands are highly interesting because several hydrogenases and carbon monoxide dehydrogenases contain such nickel complexes as their active site. The role of mixed ligand complexes in biological process has been well recognized. The stabilities of mixed chelates are of great importance in biological systems as many metabolic and toxicological functions are dependent upon this stability. Many attempts have been made to correlate the stability of the metal-ligand complexes with their antimicrobial activity , biological important metal ions with mixed ligands where mixed ligand complexes are used for storage as well as for transport of active material through membrane .Schiff bases were important class of ligands, such ligands and their metal complexes had a variety of applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis [26-28]. Mixed ligand complexes are found to be more active biologically than the

ligand itself and its binary complexes and it was widely reported that transition metal mixed ligand complexes are used in fighting microbial infections. In his most recent article for the first time, Lobamba also reported some nickel complexes of thoseAmi carbazones with a co-ligand, the biological activities of both above mentioned ligands are attributed to their chelating ability with transition metal ions coordinating to them through either throne or thiolate sulfur, and one of the nitrogen atoms. In addition, various applications transition metal complexes of thiocarbazones have been described such as catalytic activity, imaging and therapy,in sensor, antimicrobial, antiviral, cytotoxic,antibacterial, anticancer, antioxidant activities, antiparasitic,and antitumoractivities,fungicidal and antineoplastic. It is well known that some drugs exhibit increased activity when administered as metal complexes and several metal chelates have been shown to inhibit tumor growth. Among all transition metals, this work is much emphasized on nickel, which is an important transition metal normally stable in the +2 oxidation state and it more attracted by the researchers in recent years because of their numerous importance in biological systems. The role of nickel in bioinorganic chemistry has been rapidly expanded since the discovery that unease is a nickel enzyme in 1975. Since then, the list of nickel-dependent enzymes has been significantly increased , Ni(II) complexes as antibacterial, antifungal, and anticancer agents have been studied and proposed as potent catalysts in homogenous and heterogeneous reactions. The coordination chemistry of nickel ion is significant because of its participation in redox cycles of several metallo-enzymes. Square planar nickel complexes can cause cleavage of plasmid DNA, under special factors. A large number of nickel complexes with capability of acting as vitamins are known. Nickel possesses an important role in physiological processes as a co-factor in absorption of iron from the intestine. It can increase absorption of iron from the diet in iron deficient rats (female) under the condition that dietary iron is in the unavailable ferric form. In this review, the focus is placed on anti-bacterial and anti-fungal activities of various kinds of mixed ligand nickelcomplexesInteractions between nucleic acids and proteins are essential and central to many biochemical processes. Protein–nucleic acid complexes have very diverse structures and the interface may depend on both the shape of the protein and the structure of the nucleic acid. The diversity of DNA and RNA sequences dictates their structures, which in turn control their binding specificity to proteins. The structure of protein–DNA complexes may vary and sometimes even small nuances in the geometrical parameters of the major or minor grooves are fundamental to achieving specificity [and therefore function. An RNA strand can fold into diverse three-dimensional (3D) structures, including double-stranded A-form helices and higher-order tertiary structures that interact specifically with proteins. Stable complexes between proteins and nucleic acids are essential and their disruption can lead to a range of diseases, including several neurodegenerative disorders and cancers. Structures can be formed transiently between proteins and double-stranded DNA (dsDNA) during transcription, replication, recombination, and dsDNA repair. Structures between proteins and single-stranded DNA and RNA are also essential for function, for example, in telomeric overhangs at the end of chromosomes, at double stranded breaks, and at replication forks.

OBJECTIVES OF PROPOSED WORK:

1) To determine the biological activities of ligands.

2) To study the stabilities and development of ligands with their benefits.

REVIEW OF LITRATURE

Nanoparticle interactions with biological structures

Nanoparticle (NP)-based delivery systems (nanoconstructs) have gained recent attention because of their promise in enhancing delivery efficiency and therapeutic efficacy for cancer treatment. By design, engineered nanomaterials offer advantages over other approaches because their sizes and surface ligand presentations are commensurate with biological markers and active regions on the plasma membrane. However, there is still a lack of detailed information on interactions between the ligands grafted to the NP core and targets in physiological conditions. Since NPs enter most cells via energy-dependent processes that depend on the properties of the nano constructs, an understanding of how physicochemical parameters including NP size, NP shape, ligand density and overall surface charge affect local interactions is needed in order to optimize the cellular uptake pathway. Most reports on nanoconstruct optimization have focused on in vivo applications; however, the type and density of ligands on NP cores have recently emerged as critical factors that dictate how nanoconstructs interact with receptors on the cell membrane, which will ultimately affect their effectiveness in tumors. The next leap in nanomedicine will occur when we understand – at the nano scopes level – how a single, engineered nanoconstruct interacts with an individual cell.Cell stimulation and induced cellular responses are sensitive to local ligand concentrations of cytokines, chemokinas, growth factors and related molecules. For example, membrane–receptor clustering and lipid-raft formation can initiate signal cascades within ligand-induced cellular pathways. Such processes modulate uptake by controlling when endocytic pathways are initiated. Hence, controlled ligand density and presentation on nanoconstructs is emerging as a promising route to modulate cellular responses and as a potential tool to understand the mechanism of NP uptake. Recently, gold NPs (AuNPs) have been exploited to investigate how surface ligand densities affect interactions with cells at the nanoscale. AuNPs offer distinct advantages, including: they are biocompatible and do not produce adverse effects in vitroorin vivo their unique optical (localized surface Plasmon) properties such as absorption and scattering enable their use in bioaffinity sensors, photothermal therapy and bioimaging and unlike soft materials, AuNPs do not deform in physiological conditions, which enables long-term studies of how they impact cellular behavior. These strengths suggest that AuNPs can function as a model system to study ligand–receptor interactions in gene and therapeutic agent delivery processes.

How ligand density & the presentation of ligands on NPs can be controlled

Spherical AuNPs with high ligand loading are believed to derive their unique properties via a multivalent effect that can result in high effective affinities to cell surface receptors. A wide range of ligands (e.g., nucleic acids, peptides and proteins) can be grafted to the Au surface. Because they can be easily synthesized and attached via thiols chemistry to AuNPs, nucleic

acids are the most common system to study the effects of ligand density on cellular behavior. Moreover, their small size (duplexes ˜2 nm in diameter) enables dense packing and oriented presentation for binding targets, in contrast to proteins that can suffer from low loading, site-specific conjugation and low activity on NPs .The packing density of ligand such as DNA is less sensitive to the diameter of NPs compared with the composition of DNA. As spherical NP diameters increase to 60 nm, the surface coverage effects are similar to those of a planar Au surface. Nucleic acid sequences containing poly(thymine) (10-mer) spacers near AuNP surfaces show decreased loading as a function of NP size, while the same sequence with poly(adenine) spacers has nearly the same ligand density for different NP sizes because of the much stronger relative affinity of adenine compared with Au for thymine.

The method most widely used to control the loading of ligand on AuNPs is to tune the molar ratio of ligands and NPs during the conjugation process however such approaches do not allow tight control over the efficiency of ligand packing. To address this issue, recent reports have suggested tailoring the chemical environment around AuNPs by adjusting the concentrations of salt and pH conditions in solution. This approach can improve DNA ligand grafting on AuNPs by reducing repulsion between the particle surface and oligonucleotides. When we have applied salt-aging strategies at low pH to nucleic acids with secondary structures (G-quadraplexes), not only was the necessary excess of ligands reduced and the fictionalization time significantly faster (24 vs. 1 h), but also higher numbers of ligands (over two-times higher) could be attached to the AuNP surface.

An alternative approach to modulate the ligand density is to control the spatial presentation of the ligands, which can easily be accomplished by changing the shape of the AuNP core. Different NP shapes with average sizes similar to spherical ones can affect ligand loading due to differences in surface area, in that anisotropic NPs support higher surface-to-volume ratios. In addition, the protruding structures of anisotropic AuNPs can result in more accessible binding sites for molecules at the surface of NPs, which can influence their reactivity. Therefore, tuning the shape of NPs can provide an effective way to achieve quantitative loading as well as an understanding of ligand surface coverage on NPs as a function of curvature.

How ligand density affects cellular uptake & therapeutic activity in vitro

Recent work has demonstrated that ligand density and the presentation of ligands on NPs can affect cell targeting efficacy as well as cellular uptake. Linear nucleic acids with six-times higher loading on spherical AuNPs enhanced cellular uptake by over threefold in representative cell types that were selected to compare different species (mouse and human) and the inherent differences between cell and tissue types (yolk sac, cervix and lung) . In our own work, we have shown that oligonucleotides with secondary structures grafted to AuNPs also show enhanced uptake in cancer cells depending on ligand density. We found that a 2.5-times increased loading of the DNA aptamers AS1411 (G-quadruplex) on gold nanostars (AuNSs) showed two-times higher uptake in different cancer cell lines that overexpress the surface marker nucleoli. Moreover, the highly loaded nanoconstructs were taken up by cancer cells at faster rates compared with constructs with lower densities of AS1411.

So, the question remains: why and how does ligand loading density affect cellular uptake? One hypothesis is that high ligand loading can increase extracellular protein absorption on the NPs. ζ-potential results of spherical AuNPs with DNA (28-mer) indicated that nanoconstructs become more positively charged in phosphate-buffered saline as the packing density of DNA increased from approximately ten to 80 strands/NP. This reduction in overall charge (DNA–AuNPs were still negatively charged) was due to the higher adsorption of extracellular proteins (over two-times higher), which then facilitated higher cellular uptake. Although the identification of key proteins that contribute to the internalization of NPs is still unanswered, interactions between nanoconstructs and proteins are involved in the endocytosis process. A second hypothesis is that high ligand densities affect the binding capability of ligands to receptors, in that multiple ligand on AuNPs can interact with numerous target receptors simultaneously, resulting in increased affinity.

The densities of both nontargeting and targeting ligand on AuNPs appear to affect uptake however, downstream results – the therapeutic effects – have been largely unexplored. In our recent work, we showed that the therapeutic efficacy of AS1411 in vitro was improved through high loading on AuNS in pancreatic cancer and fibrosarcoma cells. AS1411–AuNSs with high loading densities (126 ± 6 dimers/AuNS) showed an average 42% increase in cancer cell death compared with AS1411–AuNSs with a lower loading density (55 ± 3 dimers/AuNS). These results strongly suggest that AuNS nanoconstructs with increased multi valences from higher local concentrations of aptamer drug can improve therapeutic effects.

MATERIAL AND METHODOLOGY

Synthesis of Salicylaldehyde-Hexamine Schiff base ligands

1) Conventional method: Salicylaldehyde (3mmol) dissolved in ethanol (25cm3) is mixed with Hexamine (3mmol) dissolved in ethanol (25cm3). To this a few drops of acetic acid is added and the mixture is refluxed for (1-1 1/2) hour. Then it is cooled, filtered off, washed with water and dried under vacuum. The crude product thus obtained is recrystallized from ethanol.
2) Grinding method: Salicylaldehyde (3mmol) dissolved in 10mL ethanol (25cm3) is mixed with Hexamine(3mmol) dissolved in 10mL of ethanol (25 cm3). To this a few drops of citric acid is added and the mixture is grinded for 20minutes.Pestle and Mortar are used for grinding to attain the powdered form. And then cooled water is added. The Precipitate was obtained. International Journal of Advanced Scientific Research and Management, Special Issue 4, ICAMA-18, Apr 2019
3) Stirring method: Hexamine (3mmol) in 10mL of water is mixed with Salicylaldehyde (3mmol) and then stirred for 10minutes.The precipitate was obtained. The yields of the above said three methods were compared and the conventional method was found to be a better method with maximum yield. Since the yields of the other two methods are meager, they were not used for the further studies.

Synthesis of Schiff base transition metal complexes:

The following general procedure was carried out for the preparation of Schiff base complexes with transition metals Cu (ll) and Ni (ll).

Synthesis of Schiff base Nickel (ll) complex: To the 10 ml ethanolic solution add 1 gm of NiCl2 (H2O) 6 complex. Take 2 gm of Schiff base ligand in 10 ml ethanol. Heat the solution. Add solution of Schiff base ligand in solution of NiCl2 (H2O) 6. Few drops of ammonium solution were added until pH 6-8 was obtained. And then the reaction mixture is stirred at room temperature for 1 hour. The obtained product washed were filtered and washed with ethanol. Dried well and the Schiff base metal complex were formed. Greenish yellow color complex was obtained and the yield is 66.26%.

Synthesis of Schiff base Copper (ll) complex: To the 10 ml ethanolic solution add 1 gm of CuCl2 (H2O) 6 complex. Take 2 gm of Schiff base ligand in 10 ml ethanol. Heat the solution. Add solution of Schiff base ligand in solution of CuCl2 (H2O) 6. Few drops of ammonium solution were added until pH 6-8 was obtained. And then the reaction mixture is stirred at room temperature for 1 hour. The obtained product washed were filtered and washed with ethanol. Dried well and the Schiff base metal complex were formed. Greenish yellow color complex was obtained and the yield is 66.26%.

Antimicrobial activity

2.3.1 Preparation of test microorganisms: A loopful of the test organism was transferred to already sterilized 10 ml Nutrient agar and incubated overnight at 370C for bacteria and 300C for fungi. Aspergallonages was cultured as a slant culture in an acidified PDA (Potato Dextrose Agar) media 25 ml of sterilized Muller-Hinton Agar (MHA) (Hi Media, Mumbai, India) was poured in Petri plates and allowed to solidify at room temperature on which the test organisms were inoculated.

Antimicrobial assay

The antimicrobial activity was measured by Disc Diffusion method. The sterile discs were impregnated with the known concentration of the various extracts (15 µl) and standard drug the discs were then placed on the already inoculated Petri dishes containing the inoculums of test microbes in such a way that there is no overlapping of the zones of inhibition. The seeded plates were then incubated at 370C for 24 hours and 48 hours for bacteria and fungi respectively. The antimicrobial activity of the animal extracts was recorded as the mean diameter of the resulting inhibition zone of growth measured in milli meters. From the results, the Active Index (AI) and Proportion Index (PI) were calculated using the

Following formulae,

$$\text{Active Index (AI)} = \frac{\text{Inhibition zone of the test sample}}{\text{Inhibition zone of the standard}}$$

$$\text{Proportion Index (PI)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

Antioxidant Activity DPPRadicals scavenging activity:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H [5]. The free radical scavenging activity of all the extracts was evaluated by1,1- diphenyl-2- picryl-hydroxyl (DPPH) according to the previously reported method.Briefly,an0.1mM solution of DPPH in methanol was prepared and1ml of this solution was added to 3ml of the solution of all various solvent extracts at different concentration(50,100,200,400&800µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30min. Then the absorbance was measured at 517 nm using a UVVIS spectrophotometer (Genesys10s UV, Thermo Electron Corporation).Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula. DPPH scavenging effect (%inhibition) = {(A0– A1)/A0) *100} Where, A0 is the absorbance of the control reaction and A1 is the absorbance in presence of all of the extract samples and reference.All the tests were performed in triplicates and the results were averaged.

RESULT:

MIC values (µg/ml) for antimicrobial activity of ligands and their Co(II) complexes.

Compound	Bacillus subtilis	Staphylococcus aureus	Proteus vulgaris	Klebsiella pneumoniae	Candida albicans
HL_1	–	–	–	–	–
HL_2	80	80	80	80	80
HL_3	–	–	–	–	–
HL_4	–	–	–	–	–
$[Co(L_1)_2]\cdot H_2O$	80	80	80	80	80
$[Co(L_2)_2]\cdot 3H_2O$	30	20	30	20	30
$[Co(L_3)_2]\cdot 2H_2O$	50	50	50	50	55
$[Co(L_4)_2]\cdot 2H_2O$	80	80	–	80	–
Kanamycin	04	10	08	11	–
Clotrimazole	–	–	–	–	10

From the results, none of the compounds are effective against the tested microorganisms when compared with the standard antibiotics like kanamycin and clotrimazole.

Nematocidal activity

Plant parasitic nematodes are the main pathogens on most fiber crops, horticultural, food and vegetable crops and without adequate control; they cause loss of yield and quality. Nematode Meloidogyne species is known to attack almost all types of plants and cause considerable damage (Adekunle and Akinlua, 2007). M. incognita produces galls on the roots of many host plants and is also responsible for 44.87% of yield loss in brinjal (Kapoor et al., 2012). The past literature works concerning nematode problems have indicated that there is a need to check this pest by control practices, using various chemicals.
The nematocidal activity of all the ligand and their Co (II) complexes were evaluated against M. incognita with different concentrations after 24 and 48 h and the details are given in Table. The results revealed that, all the ligand except HL_2 and HL_3, and their Co (II) complexes showed very less activity. The ligand HL_2 and HL_3 showed more than 70% mortality in 250 µg/ml concentration after 48 h. The highest activity was observed at higher concentrations and activity also increased with time.

Table. Nematocidal activity (% mortality) values of ligand and their Co (II) complexes.

Compound	After 24 h			After 48 h		
	250 (µg/ml)	150 (µg/ml)	50 (µg/ml)	250 (µg/ml)	150 (µg/ml)	50 (µg/ml)
HL_2	51	35	18	70	51	28
HL_3	55	38	20	77	57	33
$[Co(L_1)_2]\cdot H_2O$	7	–	–	10	–	–
$[Co(L_2)_2]\cdot 3H_2O$	–	–	–	–	–	–
$[Co(L_3)_2]\cdot 2H_2O$	5	–	–	7	–	–
$[Co(L_4)_2]\cdot 2H_2O$	–	–	–	–	–	–

DPPH radical scavenging activity

In DPPH free radical scavenging activity, antioxidants are reacting with the stable free radical 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) producing a colorless 1, 1-diphenyl-2-picryl-hydrazine. When DPPH receives an electron or hydrogen radical to become more stable, its absorption decreases (Konzen et al., 2006). The DPPH scavenging activity was expressed as IC_{50}, whose concentration is sufficient to obtain 50% of maximum scavenging activity. The IC_{50} values of ligands and their Co(II) complexes are depicted in Table. BHT was used as standard. From the results, ligands HL_3 (IC_{50} = 1.27 µg) and HL_4 (IC_{50} = 0.4 µg) showed

good activity, whereas remaining ligands HL_1 and HL_2 did not show any antioxidant activity. The HL_4 (IC_{50} = 0.40 µg) ligand showed effective activity when compared to standard drug BHT (IC_{50} = 0.67 µg). All the Co(II) complexes did not show prominent activity except $[Co(L_2)_2]$ $\cdot 3H_2O$ (IC_{50} = 2.32 µg) complex. The free radical scavenging activity of the compounds depends on the structural factors such as the phenolic hydroxyl, carboxylic groups and other structural features. The order of antioxidant activity of ligands and their Co(II) complexes according to their IC_{50} values is as followsHL4>HL3>[Co(L2)2]·3H2O

Table. IC_{50} values of DPPH radical scavenging activity of ligands and their Co (II) complexes.

Compound	**IC_{50}(µg/ml)**
HL_3	1.27
HL_4	0.40
$[Cu(L_2)_2]\cdot 3H_2O$	2.32
BHT	0.67

Activity studies

The activity of the ligands and their Co (II) complexes was determined by MTT assay. The IC_{50} values of ligands and their Co (II) complexes are presented in Table. The pharmacological testing has proved that the cytotoxic effect of the ligands and their Co (II) complexes was considerably moderate to less pronounce compared to the standard drug cisplatin, since calculated IC_{50} values were in the range of 15–400 µg/ml. Among all ligands and their Co(II) complexes evaluated, the Co(II) complex of HL_2 showed the highest anticancer activity against MCF-7 (IC_{50} = 15.4 µg/ml; IC_{50} = 1.7 µg/ml for Cisplatin). Co (II) complex of HL_1 showed the lowest IC_{50} value among all the ligands and their Co (II) complexes against raw cell lines. Furthermore, HL_3 ligand exhibited cytotoxicity with low concentration (IC_{50} = 15.2 µg/ml; IC_{50}: 5.6 µg/ml for Cisplatin) compared to all ligands and complexes against COLO 205 cell lines. However, the ligands have a higher inhibitory effect than their corresponding Co (II) complexes. Several compounds in particular HL_3 and Co(II) complex of HL_2 were endowed with significant cytotoxic potency and can be viewed as new lead compounds for further modifications.

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Table IC values (µg/ml) of anticancer activity of ligands and their Co(II)complexes.

Compound	Raw	MCF-7	COLO 205
HL1	46.8	24.5	20.1
HL2	56.1	34.2	39.1
HL3	52.8	29.2	15.2
HL4	46.9	30.4	68.1
$[Co(L_1)_2]\cdot H_2O$	42.2	41.9	51.6
$[Co(L_2)_2]\cdot 3H_2O$	52.1	15.4	27.1
$[Co(L_3)_2]\cdot 2H_2O$	199.8	231.7	63.9
$[Co(L_4)_2]\cdot 2H_2O$	246.5	405.5	56.7
Cisplatin	1.5	1.7	5.6

DNA cleavage studies

The interaction of plasmid pUC19 DNA with Co(II) complexes were studied using gelelectrophoresis in the presence and absence of oxidizing agent H_2O_2. DNA cleavage was achieved by monitoring the gel electrophoresis for naturally occurring, covalently closed circular form (Form I) transition to the nicked circular (Form II) and linear forms (Form III). When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the super coil form (Form I), slower migration will be observed for nicked circular form (Form II) and linear form occurred between the super coiled and nicked circular forms (Li et al., 2011, Kashanian et al., 2012).. In the absence of H_2O_2, control DNA does not show any activity. $FeSO_4$ was used as standard, disappearance of bands was observed in its lane, indicating the DNA cleavage. All the ligands exhibited significant activity in the presence of H_2O_2 (Lanes 1–4), in the absence of H_2O_2, ligands do not show cleavage activity(Lanes 1–4) (Kavitha et al.,2013). All the Co (II) complexes showed a decrease in the concentration of the super coiled form and increase in the concentration of the nicked circular form. It indicates the DNA cleavage activity of the complexes without any external reagents i.e., H_2O_2. Interestingly, the complexes cleaved DNA hydrolytically in the absence of any reducing agents and light. In the presence of an oxidizing agent H_2O_2, the DNA cleavage activity occurred as evidenced by the total disappearance of DNA [Lanes 5–8]. On the basis of these results, it is concluded that prominent DNA cleavage activity was observed in the presence of an oxidizing agent H_2O_2.

DISCUSSION

The binding, dissociation, and internalization of FLPEP at its receptor on human neutrophils are all processes which occur rapidly at 37 "C. Cellular responses to these ligandreceptor interactions are initiated within seconds following the addition of ligand. In this report we

have applied cytometric and Spectrofluorometric methods which are suited to the analysis of ligand-receptor dynamics on this time scale. By a combination of three independent methods, we have analyzed the number of receptors on resting cells (at 4 or 15 "C). These methods involve the use of: 1) particles with known numbers of fluorescein's in a cytometric assay; 2) antibody to fluorescein to discriminate free and bound FLPEP in a Spectrofluorometric assay; and 3) a cytometric assay described previously which examines the variation ofreceptor occupancy as a function of cell concentration. Taken together, these methods yield a value of 53,000 +- 13,000 receptors on a resting ne rophil.These numbers are comparable to those obtained in a variety of studies using radioligands of these three methods, the first is the most convenient, being rapid and requiring the smallest number of cells. Due to the differences in the "brightness" of the two commercial standards, there remains some uncertainty in the absolute instrumental calibration. Of primary significance, however, is the utility of the calibration standard as a tool to standardize the instrumental sensitivity of the cytometer so that cells from the same or different donors may be compared on different days. The affinity of FLPEP for its receptors has been analyzed at equilibrium by the Spectrofluorometric and cytometric methods. Both of these methods assume that the intensity of fluorescence of FLPEP is not influenced by the binding event. Reasonably well described by a single dissociation constant Kd = 0.6 k 0.2 nm. Our present results concerning the kinetics of N-formyl ' We have also now found a few donors whose neutrophils possess in excess of 100,000 receptors/cell. Peptide receptor interaction extends and confirms the earlier observations of Zigmond and Sullivan and Niedel and co-workers. Specifically, we have now quantitatively evaluated the temperature dependence of association and the temperature dependence of the initial rate of internalization. We have begun to dissect the elements which influence ligand dissociation. The kinetics of FLPEP association has been analyzed as a function of FLPEP concentration and temperature. The association rate constant varies roughly 3-fold over a temperature range from 4 to 37 "C. k, at 37 "C equals io9 M" min", a value roughly 1 to 2% of the diffusion limit predicted for a molecule this size. When we consider the fact that the solid angle for entry of the ligand into the receptor binding pocket is likely to be diminished to a few per cent of 4s radians, k, is of comparable magnitude to the diffusion limit.

CONCLUSION:

The analysis of recent literature data shows that catalysis by polymers has become an independent and thriving branch of chemistry. Extensive development of this field is attributed to the success achieved in synthesis and investigation of so-called functional polymers as well as to success attained in homogeneous, metal complex catalysis. The fruitful cooperation of these two directions, namely the fixation of homogeneous catalysts or transition metal compounds on organic polymers, has led to the novel idea of heterogenization of homogeneous metal complex catalysts. Such catalysts obtained by the heterogenization of various polymeric supports by homogeneous complexes of transition metals, retain the advantages of both homogeneous (high selectivity) and heterogeneous (convenient manufacture) catalysts While the former are helpful for elucidating the kinetics and mechanisms of catalytic reactions, the latter are more promising for the production of stable catalytic systems. Although combining the advantages and simultaneously avoiding the drawbacks of each catalyst type would be ideal for heterogenized homogeneous catalysts,

their catalytic sites, too, eventually become deactivated. Two aspects of catalysis involving polymers should be discussed: the catalytic effect of functional groups of polymers andthe use of polymers as supports for homogeneous metal complexes. Such an approach is useful because it enables one to establish a relationship between enzyme-like, homogeneous and heterogeneous catalysis. Enzymes and synthetic polymers are very similar in many respects. However, the main limitation of applying polymers for enzyme catalysis is their insufficient variety of functional groups; polymers cannot, for example, yet perform complex enzymatic functions. Nevertheless, the following functions of both polymers and enzymes are analogous: considerable reaction acceleration realized under normal conditions in neutral media and aqueous solutions; high operational effectivity and selectivity for reagents and reaction products; as well as reaction control. Polymeric catalysts, like enzymes, operate at stereospecific sites. Reactive selectivity is provided by hydrophobic "traps", coordination and hydrogen bonds, and electrostatic interactions. Thus, by applying some general concepts of enzyme catalysis, effective polymeric catalysts can be synthesized. The reactivity of a chain molecule is determined

Chemists and their compounds have contributed tremendously,without any doubt, to the impressive progress of medicine. The exploration of the chemistry of coordination offers real possibilities for a new understanding of intractable diseases and for the design of novel therapeutic and diagnostic agents. The rational design of the chelating agents requiresan understanding of link kinetics, catalysis mechanisms and donor interactions. Ligands can be introduced into a biological system tolimit the adverse effect of the accumulation of a metal ion, to inhibit selected metalloenzymes, or facilitate the redistribution of a metal ion. Some of the mentioned effects involve the modification of reactivity and lipophilicity, stabilization of specific oxidation states or contributions to substitution kinetics. The rational design of efficient chelating agents requires[58] a good knowledge of the electronic and molecular structure of the complexes formed.

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