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Anticancer potential of metal thiosemicarbazone complexes: A review

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ABSTRACT

Thiosemicarbazones are Schiff based ligands which have gained importance over the decades as potential drug candidates. When coordinated to metals, they have proved as good anticancer, antimicrobial, antioxidant and antiprotozoal agents. Numerous applications of these ligands in the field of Analytical Chemistry have also been reported. The present review aims to summarize some of the recent advances in the design/synthesis of novel substituted metal-ligand thiosemicarbazone complexes with special emphasis on the efficacy of transition metal-ligand complexes as potential anticancer agents as well as to unravel their possible mode of action on in vitro living systems viz. cell lines as well as a few animal models. Transition-metal based complexes hold several advantages over other metal complexes because of their better acceptability and low toxicity in biological systems. Moreover, the role of transition metals as micronutrients as well as co-factors of several metallo-enzymes in living systems further corroborates the rationale behind synthesis and evaluation of novel transition-metal based thiosemicarbazones complexes for their anticancer effects. After a careful review of the literature available, it has been found that the activity enhancement has been achieved by many possible modifications in the ligand moiety and some of the best modifications have been cited in the review. Future use of substituted thiosemicarbazones as effective anticancer agents would depend on such structural modifications as would afford them better potency against a number of tumors/cancers, together with low toxicity and better solubility, when tested in vivo.

Keywords: Thiosemicarbazones, Schiff bases, Substituted metal-ligand complexes, *in vitro*, *in vivo*, Anticancer activity

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INTRODUCTION

Schiff based ligands (Fig. 1) are synthesized by condensation reactions between primary amines and aldehydes or ketones (R''CR'=NR where R and R' and R" represent alkyl and/or aryl substituents). The ligands are of great biological importance as they have shown a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic [1].

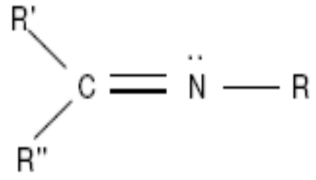

Fig. 1 General structure of Schiff base

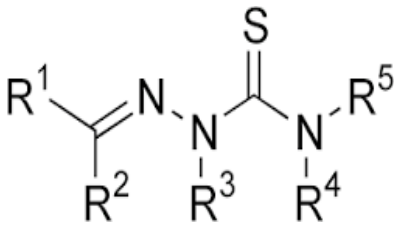

Fig. 2. General Structure of Thiosemicarbazones. R^1, R^2, R^3, R^4, R^5 = H, or any organic substituent

Thiosemicarbazones (Fig.2) represent a versatile class of Schiff based ligands having sulphur and nitrogen as donor atoms. They are usually prepared by the condensation reaction between aldehydes or ketones with thiosemicarbazide [2]. Medical applications of thiosemicarbazones began to appear in the fifties against tuberculosis and leprosy [3-4]. In the sixties, their antiviral properties were discovered and after a considerable amount of research methisazone and Marboran® were commercialized to treat smallpox [5]. Methisazone has direct therapeutic efficiency against variola virus, but it increased morbidity and mortality and is no longer manufactured as a drug [6]. In this period, one of the first antitumor activity results for thiosemicarbazones was published [7]. The anticancer potential of Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone; Fig.3) was discovered and presently, it has reached clinical phase II trials on several types of cancer [8-9]. Its antitumor activity is very broad as well butit is very much dependent on the typology of tumor cells.

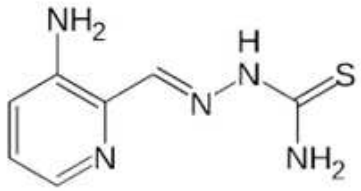

Fig.3. Triapine

It has been seen that the presence of a metal ion increases the activity of overall complex and makes a significant contribution in overcoming the side effects of the organic parent compounds [10]. Thiosemicarbazone is a polar ligand and its interaction with a charged metal ion results in a structure in which hydrophobic part of the molecule gets exposed to outside and this makes the entry of the complex inside a cell membrane feasible (Fig.4).

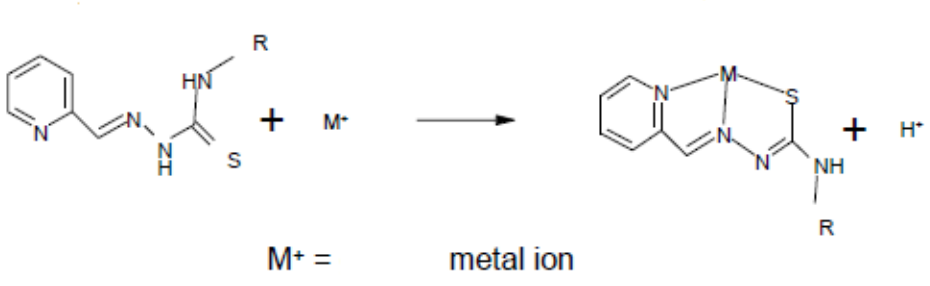

Fig.4. Metal ion coordination to a thiosemicarbazone ligand induces structural changes in the complex

It has been observed that some cell lines are more sensitive than others to a particular metal-ligand complex. Although many effective metal complexes are known as chemotherapeutic agents in numerous human diseases,

there is still a need to improve on already used ones and search for the new and more effective drugs. Apart from thiosemicarbazones, very potent anticancer agents have been found in the group of complexes of semicarbazones also. The results indicate strong antiproliferative activities, even stronger than those of cis-platin. The complexes have been found to inhibit tumor cell proliferation by arresting S phase of the cell cycle [11]. Metal ion complexes have been found to be interesting and attractive compounds in the search and development of novel anticancer drugs because of their chemical reactivity. In addition, metal ions can show diverse coordination geometries that enable the synthesis of compounds with unique stereochemistry as compared to pure organic ligands [12]. The present review is an attempt to summarize some of the recent developments in the field of thiosemicarbazone-metal complexes and attempts to explore their potential for use as anticancer agents. In the current review, significant strategies by which the activity of thiosemicarbazones has been enhanced as well as the corresponding effects on biological systems/cell lines have been discussed. In addition, the possible mechanism(s) by which these thiosemicarbazones based complexes exert their anticancer effects have also been reviewed.

STRUCTURE ALTERATIONS/MODIFICATIONS ATTEMPTED

1. *Enhancement in Anticancer Activity due to Positioning of Substituents*

It has been observed that cytotoxic activity depends not only on the metal ion but also on the position of the substituent group on aromatic ring. Recently complexes of Fe (III) and Ni (II) with **S**-methyl-thiosemicarbazones of 2-hydroxy-R-benzaldehyde have shown maximum cytotoxic potential when methoxy (-OCH_3) group was placed in the side chain aromatic ring (Fig.5). The cytotoxic activity has been tested against chronic myeloid leukemia (K562) and human endothelial (ECV304) cell lines by MTT assay. 4-Methoxy substituted iron chelates have been shown to possess considerable cytotoxic activity against K562 while 3-methoxy chelate with nickel has shown enhanced activity against both ECV304 and K562 [13].

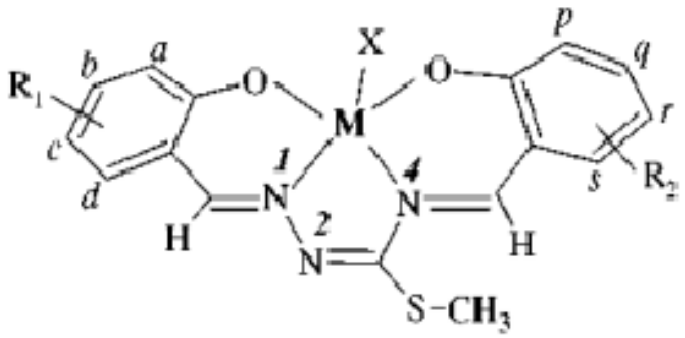

Fig.5. General Structure of iron, nickel chelates (a, where M/X=Fe/Cl) and nickel chelates (b, where M/X=Ni/-) chelates

2. ***Anticancer Activity of N(4)-Substituted Thiosemicarbazones Complexes***

Considerable work has been done on modification of sites such as aldehydes or ketonic carbon, thione group and the N^4 position along with the position of attachment to the pyridine/isoquinoline moiety in α-N heterocyclic thiosemicarbazones [14-15]. N^4 substituted thiosemicarbazones have shown great cytotoxic activity enhancement as reported in literature. N^4-substituted thiosemicarbazones and many of their metal complexes have been shown to possess substantial *in vitro* activity against various human cancer cell lines [16–18]. However, these complexes are usually insoluble in aqueous solutions, and have shown less promising *in vivo* activity and need improvement and more exploration. Cu (II) complexes prepared from N(4) substituted thiosemicarbazones [Cu (pclbhtsc)2]$Cl_2.2H_2O$ [1], [Cu (p-mbhtsc)2]Cl_2. $2H_2O$ [2] and [Cu (p-nbhtsc)2]$Cl_2.2H_2O$ [3], where (pclbhtsc) = para-chloro benzaldehyde thiosemicarbazone, (p-mbhhtsc)=para-methoxy benzaldehyde thiosemicarbazone, (p-nbhtsc)=para-nitro benzaldehyde thiosemicarbazone have been tested for their cytotoxic activity against breast cancer cell lines MCF-7. All three Cu (II) complexes showed modest in vitro cytotoxic properties. IC_{50} values were compared with cisplatin and the results have revealed that complex 1 possesses highest activity. But detailed studies are required to understand the mechanistic action at the cellular level and to better understand the role of the metal [19]. In another example, cobalt (III) complexes containing pyridoxal N(4)-substituted thiosemicarbazone ligands with the composition [Co ($HL^{1\text{-}2}\cdot Cl$)($HL^{1\text{-}2}\cdot H_2O$)] (1-2) have been synthesized from the reaction of [$CoCl_2(PPh_3)2$] and pyridoxal-N-methyl-thiosemicarbazone hydrochloride ($H_3L^1\cdot Cl$)/pyridoxal N-phenyl -thiosemicarbazone

hydrochloride ($H_3L^2 \cdot Cl$). The ligand has been found to change from neutral ($H_3L \cdot Cl$) (L) to dianionic ($HL \cdot Cl$) (L^{2-}) and monoanionic forms ($HL \cdot H_2O$) (L^-) and coordinated as tridentate dinegative form with cobalt (III) ion and formed a neutral complex. Substitution from Me or Ph groups on terminal N^4-nitrogen of thiosemicarbazone has been found to exhibit significant influence on the potential binding and cleavage ability with DNA, free radical scavenging and cytotoxicity [20]. 2, 6-diacetylpyridine bis (N^4-p-chlorophenylthiosemicarbazone) ligand, H_2L, and its palladium (II) and platinum (II) complexes [PdL] and [PtL] have shown good cytotoxic effect. In complex formation, the ligand acts as dianionic tetradentate donor and forms square planar complex with the metal through the pyridine nitrogen atom, azomethine nitrogen and thione sulfur atoms from one thiosemicarbazone moiety. The fourth coordination site is occupied by the hydrazine nitrogen atom of the other thiosemicarbazone moiety. The free ligand and its metal complexes have been tested for antiproliferative activity in vitro against NCI-H460, T-47D, A2780 and A2780cisR human cancer cell lines. The cytotoxicity data suggests that they possess potent antitumor properties, especially H_2L and [PtL] since they are capable of not only circumventing cisplatin resistance in A2780cisR cells but also exhibit high antiproliferative activity in breast cancer T-47D cells [21]. Molybdenum, a second transition series element has been utilized to form dioxomolybdenum (VI) complexes by reaction of $[MoO_2(acac)_2]$ with thiosemicarbazones derived from 5-allyl-2-hydroxy-3-methoxybenzaldehyde (1), 2-hydroxynaphthaldehyde (2), 2, 3-dihydroxybenzaldehyde (3), or 5-tert-butyl-2-hydroxybenzaldehyde (4). The ligands have been found to coordinate to Mo as tridentate ONS donors. Binding of the ligand and complexes with calf thymus DNA (CT-DNA) has been investigated by UV, fluorescence titrations, and viscosity measurement techniques. Gel electrophoresis studies have revealed that all the complexes cleave pBR322 plasmid DNA. X-ray crystallography studies have shown that the distorted octahedral coordination of molybdenum is completed by methanol in complex 1, 3 and 4 and by H_2O in complex 2. The cytotoxic properties of the complexes against human colorectal (HCT 116) cell line have shown strong antiproliferative activities in the order 4 > 3 > 1 > 2 respectively with IC_{50} values of 1.6, 4.0, 4.8, and 6.7 μM. The complexes have been found to be more active than the standard reference drug, 5-fluorouracil (IC_{50} 7.3 μM) [22].

3. *Anticancer Activity of Macrocyclic Metal Thiosemicarbazones Complexes*

Synthetic macrocyclic chelates of transition metals have attracted much attention in coordination and supra molecular chemistry [23]. Some of the chelates formed by thiosemicarbazones have received considerable attention as anticancer drug compounds. Novel complexes of the type $[MLX_2]$ where L = 2-hydroxy benzylidene carbohydrazone; M = Co (II), Ni (II) and Cu (II); X = Cl^- , NO_3^- , CH_3COO^- ions have been synthesized by template condensation of 2-hydroxy benzylidene carbohydrazone and thiosemicarbazide in the presence of divalent metal salts in methanolic solution. They have exhibited good anticancer activity [24]. Palladium(II) bis-chelate complexes of the type [Pd (TSC1-5)2] (6-10), with 4-phenyl-1-(acetone)-thiosemicarbazone, HTSC1 (1), 4-phenyl-1-(2'-chloro-benzaldehyde)-thiosemicarbazone, HTSC2 (2), 4-phenyl-1-(3'-hydroxy-benzaldehyde)-thiosemicarbazone, HTSC3 (3), 4-phenyl-1-(2'-naphthaldehyde)-thiosemicarbazone, HTSC4 (4), and 4-phenyl-1-(1'-nitro-2'-naphthaldehyde)-thiosemicarbazone, HTSC5 (5), have been synthesized and characterized by elemental analysis and spectroscopic techniques (IR and ^{1}H- and ^{13}C-NMR). The molecular structure of HTSC3, HTSC4, and [Pd (TSC1)2] (6) were determined by single crystal x-ray crystallography. Complex 6 had a square planar geometry with two deprotonated ligands coordinated to Pd (II) through the azomethine nitrogen and thione sulfur atoms arranged in a cis-form. The in vitro cytotoxic activity measurements have indicated that the palladium (II) complexes (IC_{50} = 0.01-9.87 μM) exhibit higher antiproliferative activity than the free ligands (IC_{50} = 23.48-70.86 and >250 μM) against different types of human tumor cell lines. Among all the studied palladium (II) complexes, the [Pd(TSC3)2] (8) complex has been found to exhibit highest antitumor activity on the DU145 prostate carcinoma and K562 chronic myelogenous leukemia cells, with very low values of the inhibitory concentration (0.01 and 0.02 μM, respectively) [25]. Neutral and cationic copper bis (thiosemicarbazone) complexes with Me, Ph, and H as substituents on the diketo-backbone of the ligand have been synthesized and characterized by spectroscopic methods and in three cases by x-ray crystallography. *In vitro* cytotoxicity studies have revealed their cytotoxic potential. Bis Copper chelates Cu (GTSC) and Cu(GTSCHCl) derived from glyoxal bis(4-methyl-4-phenyl-3-thiosemicarbazone) (GTSCH2) have been found to be cytotoxic against various human cancer cell lines, with an efficiency similar to that of the anticancer drug adriamycin and up to 1000 fold higher than that of the corresponding Zn complex. Tritiated thymidine incorporation assay have revealed that Cu (GTSC) and Cu (GTSCHCl) inhibit DNA synthesis substantially. Cell cycle analyses have shown that Cu (GTSC) and Cu (GTSCHCl) induce apoptosis in HCT116 cells. The Cu(GTSCHCl) complex causes distinct DNA cleavage and Topo IIα inhibition unlike that for Cu(GTSC). *In vivo* administration of Cu (GTSC) has been found to significantly inhibit tumor growth in HCT116 xenografts in nude mice [26].

4. *Anticancer Activity of Other Thiosemicarbazone Derivatives*

In recent years many different derivatives of thiosemicarbazone have been prepared and tested. Synthesis and characterization of palladium complex of phenanthrene quinone thiosemicarbazone and evaluation of its antiproliferative properties in the breast cancer cells and normal cells have been studied [27]. The study suggests that the complex is a potent anti neoplastic agent that acts selectively against tumor cells and is effective against drug resistant breast cancer cells. Thiosemicarbazones containing benzimidazole moiety have been shown to inhibit growth in numerous human cancer cell lines. 2-{1-(5-chloro-1H-benzimidazole-2-yl) ethylidine]-N-phenylhydrazine carbothiomide have been selected for five dosage screenings and have shown remarkable anticancer activity [28]. In the group of p-isopropyl benzaldehyde thiosemicarbazones, zinc (II) complexes have been shown to exhibit specific cytotoxic activity against Pam-ras cancer cell line, probably due to cell killing by apoptosis. This complex may be considered as potential antitumor agent because it induced apoptosis in the ras-transformed cell line [29]. 2(E)-2-[1-(4-pyridinyl)ethylidene]hydrazinecarbothioammidehydrochloride and two of its copper complexes have been found to be cytotoxic against malignant RT2 glioblastoma cells (expressing p53 protein) with IC_{50} values in the 5.1-13.2 μM range, and against malignant T98 glioblastoma cells (expressing mutant p53 protein) with IC_{50} values in the 4.5-31μM range. Coordination to copper increased the cytotoxic potential considerably when compared to that of free ligand [30].

4-(p-X-phenyl)thiosemicarbazone of naphthaldehyde {where X = Cl^- (HL1) and X = Br^- (HL2)}, thiosemicarbazone of quinoline-2-carbaldehyde (HL3) and 4-(p-fluorophenyl)thiosemicarbazone of salicylaldehyde (H_2L_4) and their complexes with copper(I) i.e. {[Cu(HL1)(PPh_3)2Br]•CH_3CN (1) and [Cu(HL2)(PPh_3)2Cl]•DMSO (2)} and copper(II) i.e. {[(Cu_2L_3 2Cl)2(μ-$Cl)_2$]•$2H_2O$ (3) and [Cu(L_4)(Py)] (4)} have been synthesized and characterized by elemental analysis, cyclic voltammetry, NMR, ESI-MS, IR and UV-visible spectroscopy. Molecular structures of all the Cu (I) and Cu (II) complexes have been determined by x-ray crystallography. All the complexes (1-4) have been tested for their DNA-binding ability and cleavage activity. The complexes have been found to effectively interact with CT-DNA possibly by groove binding mode, with binding constants ranging from 104 to 105 M-1. Complex 3 has shown the highest chemical (60%) as well as photo-induced (80%) DNA cleavage activity against pUC19 DNA. The *in vitro* antiproliferative activity of all the complexes has been assayed against the HeLa cell line. Some of the complexes have proven to be as active as the clinically referred drugs, and the enhanced activity of complex 3 may be attributed due to its aqueous solubility and the presence of the quinonoidal group in the thiosemicarbazone ligand coordinated to the metal [31]. Novel phytochemicals quercetin thiosemicarbozone copper(II)metal complex, quercetin 3-O-glucoside thiosemicarbozone and its rutin derivative have been synthesized and characterized by FT-IR, HNMR, ESR, C^{13}NMR, UV-Vis and Mass spectrometry techniques. The spectral and other data indicate that all the Cu (II) metal complexes possess tetrahedral and octahedral (rutin) structure. The transitional metal complexes of quercetin thiosemicarbazone (QTSC) and quercetin-3-O-glucoside thiosemicarbozone (QOTSC) ligands possess anti-oxidant, anti-tumor, anti-cancer, anti-viral, anti-malarial, anti-fungal, and anti-microbial activities. These activities have been found to increase upon co-ordination, hence making them suitable ligands for the synthesis of potential anti-cancer agents. In an extended study by the same authors, Schiff bases of certain constituents viz. flavanoids/phytochemicals and transition metal complexes with platinum, gold, palladium, ruthenium, cobalt, iron, nickel, zinc and chromium have been synthesized. The precursors, the derivatives/analogs and the transition metal complexes all have been found to be excellent candidates for development of novel anticancer drugs [32]. The reaction of 4-R-benzaldehyde thiosemicarbazones (denoted as H_2L-R, where H_2 stands for the two dissociable protons and R (R = OCH_3, CH_3, H, Cl^- and NO_2^-) for the substituent on the Ph ring) with [Pt ($PPh_3)_2Cl_2$] in the presence of NEt_3 produced a series of organometallics of Pt (II) with general formula [Pt (PPh_3)(L-R)]. Reaction of the same group of ligands with K_2 [$PtCl_4$] in the presence of NEt_3 afforded complexes [Pt (HL-R) $_2$]. The crystal structure of [Pt (PPh_3) (L-CH_3)] and [Pt (HL-$CH_3)_2$] has been determined. In [Pt (PPh_3) (L-R)] complexes, the benzaldehyde thiosemicarbazones are coordinated to Pt as dianionic tridentate CNS donors. In [Pt (HL-$R)_2$] complexes, the benzaldehyde thiosemicarbazones are coordinated to the metal center as bidentate NS donors and form five-membered chelate rings, and with reference to the structure of the uncoordinated thiosemicarbazone ligand, this coordination mode is associated with a change in stereochemistry around the CN bond. All the [Pt (PPh_3) (L-R)] and [Pt (HL-R) $_2$] complexes have been found to exhibit intense absorptions in the visible and UV regions. The cytotoxic effects of these complexes have been examined on the human leukemia cell line HL-60 and human lymphoma cell line U-937, thereby showing that all the [Pt(PPh_3)(L-R)] and [Pt(HL-$R)_2$] complexes are cytotoxic in nature and their IC_{50} values is indicative of their potential use as antitumor agents [33]. Organometallic and inorganic ruthenium and copper complexes bearing thiosemicarbazone ligands have been tested as anti-tumor agents. These compounds have been found to be cytotoxic against breast cancer (MCF-7 and MDA-MB-231) and colon cancer (HT29 and HCT116) cell lines with

IC_{50} values ranging from 2.7 to 40 mM. Their biophysical reactivity with DNA and human serum albumin (HSA) has been studied. These compounds have been shown to interact with DNA*in vitro* which might be the target for their biological activity. The binding constants for the organometallic complexes with DNA are of the order of 103 M^{-1} but the inorganic ruthenium and copper compounds have binding constants that are of higher magnitude. The interactions of Cu (II), Zn (II) and Fe (II) with Triapine (3-aminopyridine-2-carbaldehyde thiosemicarbazone), which is currently undergoing phase II clinical trials as a chemotherapeutic antitumor agent have been investigated in a water/DMSO mixture. The proton-dissociation constants of the ligands, the stability constants and the coordination modes of the metal complexes formed have been determined by pH-potentiometric, UV/Vis spectrophotometric, EPR, ^{1}HNMR spectroscopic and ESI-MS methods. Two terminally-N-dimethylated derivatives of Triapine have also been studied. Mono- and bis-ligand complexes in different protonation states have been identified. Furthermore, the formation of the dinuclear species with copper i.e. $[Cu_2L_3]^+$ has been confirmed for all ligands by EPR spectroscopy and ESI-MS measurements. The results have indicated that the N-terminally dimethylated ligands act as much more potent chelating agents than Triapine for the divalent metal ions. The ligands form the least stable complexes with Zn (II), whereas the Fe (II) complexes are somewhat more stable than the corresponding Cu (II) species [34]. A new ligand, 6-hydroxy chromone-3-carbaldehyde thiosemicarbazone (L), and its Ni (II) complex have been synthesized and characterized. The crystal structure of Ni (II) complex has been determined by single crystal X-ray diffraction. Cytotoxic activity of Ni (II) complex and ligand L has been investigated *in vitro* using THP-1, Raji and Hela cancer cell lines. Compared with the ligand, Ni (II) complex has been shown to exhibit significant cytotoxic activity against these cancer cell lines. The interactions of Ni (II) complex and ligand with CT-DNA have also been investigated by spectrometric titrations, ethidium bromide displacement experiments and viscosity measurement methods. Results have indicated indicated that Ni (II) complex binds to DNA by intercalative mode via the ligand. The intrinsic binding constants of Ni(II) complex and ligand L with DNA have been found to be in the range of $(1.10 \pm 0.65) \times 106\ M^{-1}$ and $(1.48 \pm 0.57) \times 105\ M^{-1}$, respectively [35]. Four $[Cu(HL)Cl_2]$ complexes of chalcone-derived thiosemicarbazones have also been synthesized and characterized. Complexes 1-3 have been found to interact with CT-DNA. The cytotoxic activities of the thiosemicarbazones and complexes (1-4) have been tested against wild type human promyelocytic leukemia (HL60), human immortalized line of T lymphocyte (Jurkat), human breast carcinoma (MDA-MB 231) and human colorectal carcinoma (HCT-116) tumor cell lineages. Upon co-ordination to Cu(II) cytotoxicity significantly increased in Jurkat, MDA-MB 231 and HCT-116 cells. Unlike the free thiosemicarbazones, 1-4 induced DNA fragmentation in solid tumor cells indicating their pro-apoptotic potential [36].

MECHANISMS OF ACTION RESPONSIBLE FOR THE ANTICANCER/CYTOTOXIC ACTIVITY OF THIOSEMICARBAZONE COMPLEXES

Thiosemicarbazone complexes may exhibit their antitumor/antiproliferative effect by any of the following main mechanisms:

1. Ribonucleoside Diphosphate Reductase(RR) Inhibition

Thiosemicarbazones in their neutral or deprotonated form, act as a N,N,S-thiodentate ligands while forming chelates with essential metal ions. They display antiproliferative activity on different tumor cell lines. A strong co-relation has been established between tumor growth rate and the enzyme Ribonucleotide Reductase (RR), a necessary enzyme for DNA synthesis [37]. Thiosemicarbazone compounds have been found to inhibit RR [38]. It is an iron-dependent enzyme that promotes the reduction of ribose to deoxyribose through a free radical mechanism. The activity is initiated by a tyrosyl radical. Inhibition of this enzyme leads to blockage in the synthesis phase of cell cycle and eventually to cell death by apoptosis. Pyrazine carboxaldehyde thiosemicarbazone and 1-formylisoquinoline thiosemicarbazone which are α (N)-heterocyclic thiosemicarbazones have shown high activity owing to their tridentate nature making them better chelators [39]. Certain formyl-pyridyl thiosemicarbazones have been found to act as powerful inhibitors of RR [40, 41]. One of the earliest thiosemicarbazone RR inhibitors tested was 5-HP (5-hydroxy-2-formylpyridine) which despite positive results in animals showed relatively low potency and rapid excretion in man. The mechanism of action has revealed that by exposing RR to these molecules, tyrosyl free radical of the enzyme is targeted by the drug and that the thiosemicarbazone complex exhibits its inhibitory effect on the enzyme by destroying the tyrosyl radical [42]. The mechanism requires oxygen and adds a new dimension to the role of thiosemicarbazones as simple iron chelators. It is also reported that the reaction is reversible, and this is in accordance with the experimental observations. The greater inhibitory activity of 1-formylisoquinoline thiosemicarbazone than 2-formylpyridine thiosemicarbazone has pointed towards the presence ofa hydrophobic pocket in enzyme with which the aromatic system interacts, which could justify the fact that upon

methylation of the aromatic ring in 2-formylpyridine thiosemicarbazone leads to a activity significant increase in activity[43].

2. Inhibition of Topoisomerase II and DNA interactions

Topoisomerase II (Topo-II) is a eukaryotic cell nuclear enzyme that decatenates DNA coils, passing one helix through another to prevent supercoiling during DNA replication [44-45]. Topo-II is necessary for DNA synthesis and cellular division; rapidly proliferating cells such as those in tumors generally contain high level of this enzyme and this renders it an interesting target in cancer cells. Thiosemicarbazones are potent antitumor agents that inhibit Topo-II. Relationship between *in vitro* and *in vivo* behavior of ^{64}Cu-labelled thiosemicarbazide complexes and the expression of Topo-II activity has been investigated [46]. Four ^{4}N-azobicyclo [3.2.2] nonane thiosemicarbazide ligands were prepared and radiolabelled with ^{64}Cu to form lipophilic cations (Fig.6).

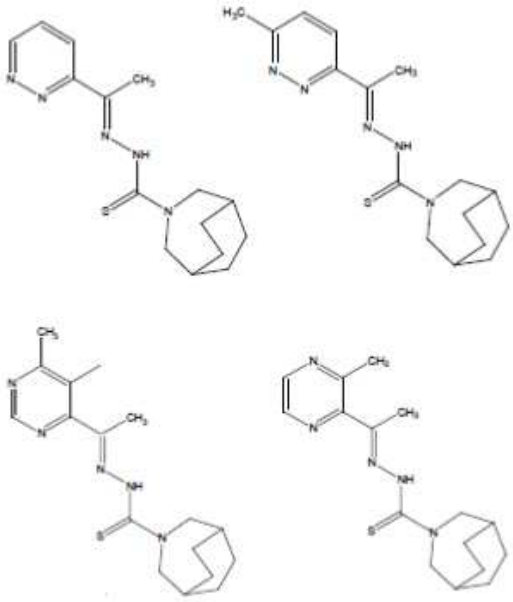

Fig.6. ^{4}N-azobicyclo [3.2.2] nonane Thiosemicarbazone ligands

Of the four ligands(1-4) examined in the study (Fig.6.) ligand three has been found to possess significantly higher growth inhibitory activity when compared with non-radioactive copper with IC_{50} value of 0.004µmol/l in HT29 cells . A wide range of tridentate TSCs with various nitrogen based heterocycles has been examined. A ligand bearing a quinoline group has been identified to have particularly high cytotoxicity and also to inhibit Topo-II. The mechanism of inhibition has suggested that the free ligand bound to the ATPase domain of the enzyme, blocked enzyme mediated ATP hydrolysis [47]. A subsequent paper has examined the Topo-II inhibition by Cu complexes of same ligand types and has shown them to be effective inhibitors of Topo-II in cells [48]. Cu-thiosemicarbazone complexes have significantly higher growth inhibitory activity than the uncomplexed ligand and have lower IC_{50} values against tumor cells than the reported Topo-II inhibitors [47]. Antitumor activity of 1,2 naphthoquinone-2-thiosemicarbazone and its metal complexes of Cu (II), Pd (II) and Ni (II) has been investigated against breast cancer (MCF-7) cell line establishing them as effective antitumor agents. The Ni complex has been most effective based on IC_{50} values [49]. Further investigation of the ligands and complexes has shown that they can only stabilize the single-strand DNA, but not double-strand breakage intermediates. The metal complexes of these ligands have been found to exert an antagonizing effect on Topo-II activity, as compared to the free ligands. In another study, Cu (II) complexes of 4-hydroxy-3-methyl-1,2 naphthoquinone-1-thiosemicarbazone have shown highest cytotoxicity compared to those of Fe(II), Ni(II), Pd(II) and Pt(II) metal complexes with the same ligand. This has been explained by the generation of Cu (I) species during intracellular enzymatic reduction or greater binding affinity of Cu (I) to an estrogen receptor protein complex [50]. The binding is thought to prevent the protein complex from functioning properly during its interaction with DNA. Further studies on mechanism of action have shown that metal complexes could stabilize the cleavable complex formed by DNA and Topo-II. Studies have shown that iron and copper

complexes are more active in cell destruction as well as in the inhibition of DNA synthesis than the uncomplexed thiosemicarbazone [51]. 5-hydroxy-2-formyl thiosemicarbazone has been shown to cause lesions in DNA [52]. It has been proven [53] that a tridentate nature and a high formation constant is a prerequisite for enhanced activity by comparing the activity of pyrazine thiosemicarbazone derivatives and an analog derived from acetophenone. It has also been reported that these compounds prevent iron uptake from the serum transferrin thereby altering the iron homeostasis. In a very recent study, Topo-IIα inhibition and antiproliferative activity of α-heterocyclic thiosemicarbazones and their corresponding copper (II) complexes has been investigated. Cu (II) (thiosemicarbazonato)Cl complexes have been shown to catalytically inhibit Topo-IIα at concentrations (0.3-7.2 μM) over an order of magnitude lower than their corresponding thiosemicarbazone ligands alone. The copper complexes have also shown inhibitory action towards the proliferation of breast cancer cells (SK-BR-3) expressing high levels of Topo-IIα at lower concentrations than breast cancer cells (MCF-7) expressing lower levels of the enzyme [54]. The copper complex of acetylpyridine methylthiosemicarbazone has also been shown to inhibit Topo-IIα enzyme. To test the hypothesis that the palladium(II) and platinum(II) complexes of the same ligand would also inhibit the enzyme due to the same structural geometry (square planar around the metal), a series of acetylpyridine thiosemicarbazone ligands, and their Cu(II) and Pd(II) metal complexes have been synthesized and characterized by NMR. The results have demonstrated that the Pd (II) complexes have much the same high anti-proliferative activity as the Cu (II) complexes [55].

3. Generation of Reactive Oxygen Species

Redox metal complexes can act as ROS generators. Since most thiosemicarbazone complexes contain redox metal ions, they potentially can activate O_2 and generate OH° radicals. $[Cu\ (L)_2(pz)](ClO_4)$ and $\{[Cu(L)_2\ (dca)](ClO_4)\}$ complexes where L=2-formylpyridine TSC, pz=pyrazine and dca=dicyanamide have been tested for their biological activity on DNA. The oxidative cleavage of DNA has been assayed in the presence of 3-mercaptopropionic acid as reducing agent by gel electrophoresis using supercoiled pUC18. Both complexes have been found to produce single and double strand breaks in DNA [56]. It is well-known that copper is an essential micronutrient and has important biological functions, such as cellular trafficking, redox regulation [57-58] and angiogenesis modulation etc. [59-60]. An abnormal level of intracellular copper will induce cellular apoptosis. The related investigations on Cu (II) complex-mediated cytotoxicity are on the rise [61-62]. Four novel thiosemicarbazone metal complexes, $[Cu(Am_4M)(OAc)]\cdot H_2O$ (1),$[Zn(HAm_4M)Cl_2]$ (2),$[Zn_2(Am_4M)_2Br_2]$ (3) and $[Zn_2(Am_4M)_2(OAc)_2]\cdot 2MeOH$ (4) [HAm_4M = (Z)-2 (amino(pyridin-2-yl) methylene)-N-methylhydrazinecarbothioamide], have been synthesized and tested against HepG-2 cell. IC_{50} value (11.2±0.9 μM) of complex 1 against HepG-2 cells has been found to be nearly 0.5 fold of that against human hepatic cell lines LO2, showing a lower toxicity to human liver cells. Additionally, it has been found to display a stronger inhibition on the viability of HepG-2 cells than cis-platin (IC_{50}=25±3.1 μM), suggesting complex-1 might be a potential and ahighly efficient antitumor agent. It is well-known that copper (II) is very sensitive to electron transfer, and zinc (II) is difficult to participate in redox reaction owing to lack of a variable valence. Fluorescence microscopy observation and flow cytometry analysis has revealed that complex-1 cannot significantly suppress HepG-2 cell viability and induce apoptosis. Several indexes, such as DNA cleavage, reactive oxygen species (ROS) generation, comet assay and cell cycle analysis have indicated that the antitumor mechanism of complex 1 on HepG-2 cells might be *via* ROS-triggered apoptosis pathway [63]. Complexes 1–4 have been found todisplay different coordination geometries even in similar synthetic conditions, which is attributed to the nature of metal ion and its ionic radius, coordination numbers, different counter anions (Cl^-, Br^- and OAc^-) etc.

4. Other Possible Mechanisms of Action

Other than these common mechanisms it has also been observed that substitution on terminal nitrogen increases the overall activity of the complex. 2-formyl- and 2-acetylthiosemicarbazone and their metal complexes with zinc have been tested for activity against MCF7, T24 and L-929 [64]. Later acetyl derivative was modified by adding an ethyl group on terminal nitrogen and its Pt and Pd complexes were synthesized $[ML_2]$, M=Pd, Pt. The complexes were found to be active towards cis-platin resistant tumour cell lines and role of metal was not found to be significant [65]. In another similar study 8-hydroxy quinolone-2-carboxaldehyde thiosemicarbazone and its 4, 4-dimethyl derivative along with their Cu (II) complexes. The terminal amino substituted complex showed stronger anticancer activity than that of the unsubstituted complex. The activity was tested on SK-N-DZ (a cisplatin resistant neuroblastoma cell lines). Enhanced expression of p53 protein was detected in the SK-N-DZ cells treated with the non- methylated complex suggesting that apoptosis was caused by DNA damage [66]. Quinoline-2-carboxaldehyde thiosemicarbazone derivatives and their Cu (II) complexes have been found to trigger apoptosis by inhibiting proteasome-ubiquitin pathway and not through oxidative stress [67]. This shows an additional pathway of action.

DISCUSSION

Thiosemicarbazones have emerged as ligands of great biological activity. The ability of thiosemicarbazones to chelate metal ions has now been recognized as a major factor in their antiproliferative effects [68]. Interest in metal complexes with thiosemicarbazones and semicarbazones has been stimulated because biological activity is often enhanced on complexation [69-70]. The more reasonable explanation for the higher activity by the metal-thiosemicarbazone complexes when compared to free ligands passes through the prevalence of the diffusive mechanism over the active transport mechanism across the membranes. The chelation of the metal ion by the most polar regions of the ligands (the donor atoms) allows an easier uptake by the cell. Only a limited number of *in vivo* studies have been done which indicate that some thiosemicarbazones show potential as chemotherapeutic agents. However, future studies are warranted.

Many attempts have been made in the last decade to improve the hydrophilicity and reduce the toxic effects by modifying the thiosemicarbazones frameworks [71]. A number of N (4)-substituted thiosemicarbazones and many of their metal complexes have been shown to possess substantial *in vitro* activity against various human tumor lines [16-18]. However, due to their lack of solubility in aqueous solutions, these thiosemicarbazones and their metal complexes show less promising *in vivo* activity. But still detailed studies are required to explore new mechanistic actions and the specific role of metal and ligand inside body. Hence *in vivo* studies should be undertaken. Ligands which have poor or moderate activity should be studied in combination with ligands of good biological significance for activity enhancement. As a futuristic lead, amide thiosemicarbazones have not been tested to date and can be evaluated for their prospective *in vitro* and more importantly, *in vivo* activity.

Anticancer activity of thiosemicarbazone complexes is mainly attributed to inhibition of RR activity, Topo- II activity and generation of ROS, but there are other possible targets as well which need to be explored. In many cases, *in vitro* ribonucleotide inhibitors have been found to be poor proliferation inhibitors on whole cells. Another area which needs attention is metal/ion sequestering since thiosemicarbazones are versatile chelators, they sometimes deprive the cell of essential metal ions by forming stable chelates with them. On the other hand, the fact cannot be overruled that metal-ligand complexes are more active than pure ligands. The redox capability of transition metals like copper play an important role in activity enhancement but it can also trigger off Fenton's reactions producing significant amount of OH° radicals that can create hindrance in normal cell functions. It has also been observed that some of the ligands are more active while others are inactive for the same cell lines, hence questioning the simple diffusion hypothesis. Likewise the interaction of one metal with another can also be explored taking synergestic effect into consideration. Not only this, whether the complex acts in unison or metal and ligand act independently inside the body needs a greater depth of understanding by bridging the gap between chemistry and molecular biology.

CONCLUSION

Thiosemicarbazide is an important structural motif that has the potential to display chemical functionality in biologically active molecules. Optimization of this structure can result in groundbreaking discovery of new class of anticancer agents. In future, the success of thiosemicarbazone based complexes as anticancer agents would largely depend on the possibility of enhancement in activity as can be obtained by such modifications in their structures as have never been attempted till date and as a consequence, their better acceptability and solubility *in vivo*.

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