A Review on Recent Developments, Design and Biological Applications of Thiosemicarbazone Derivatives

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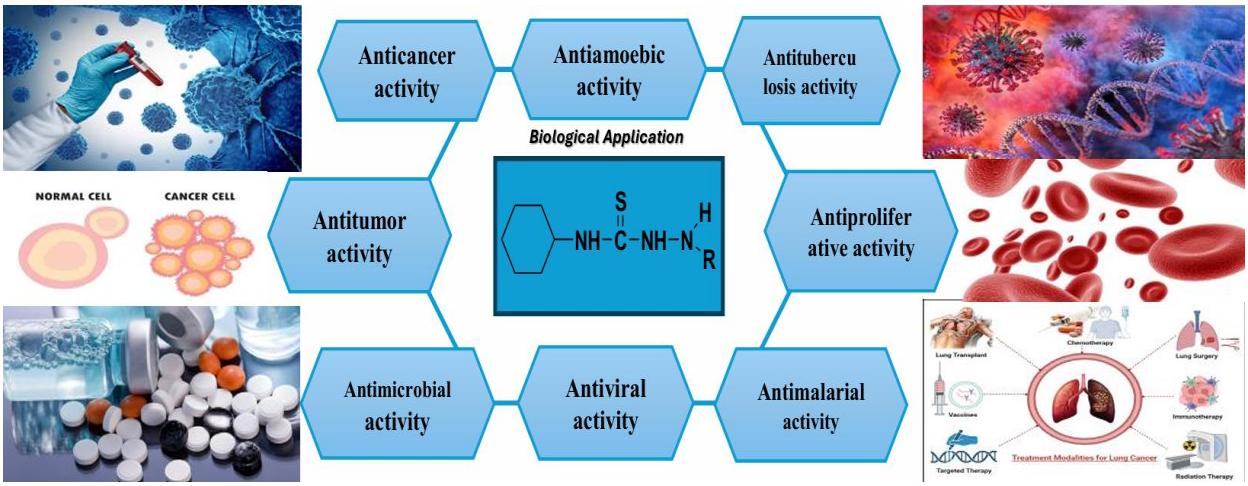
GRAPHICAL ABSTRACT



Abstract—**Objective**

Thiosemicarbazones typically bind through the sulphur and hydrazinic nitrogen atoms to interact as chelating ligands with metal ions. These metal complexes offer a fresh chance to broaden their application in many areas of medicine since they have been linked to the biological activities. Understanding the connections between thiosemicarbazone structures and activity, examining their behavioural mechanism, and maximizing character development are the objectives.

Methods

The several thiosemicarbazone derivatives have been prepared and designed by various scientists and these compounds were tested for their multiple biological activities like anticancer, antibacterial, antifungal, antimalarial, antioxidant, antiproliferative activities. On the basis of literature study of thiosemicarbazones, this review material has been collected.

Results

The results of thiosemicarbazone derivatives shows various biologically active roles such as anticancer, antibacterial, antifungal, antimalarial, antioxidant, antiproliferative activities. The resulted thiosemicarbazone ligands can be utilized to create a further metal based thiosemicarbazone compounds. The main focus of the authors is on the cyclohexyl thiosemicarbazone derivatives and their pharmaceutical applications.

Discussion

Almost all the thiosemicarbazone based compounds shows biological potency but the results of the compounds vary according to the concentration of the substances. The addition of metal salts into the ligand are leading to form metal complexes and obviously shown a more ligating behaviour with the metal ion.

Conclusion

The metal complexes show more potent pharmacological activity than its free ligands. The cyclohexyl group substitution attached on the thiosemicarbazone ligand

gives more enhanced pharmacological activities. The therapeutic properties of the compounds and structural elucidation of the various thiosemicarbazone based compounds.

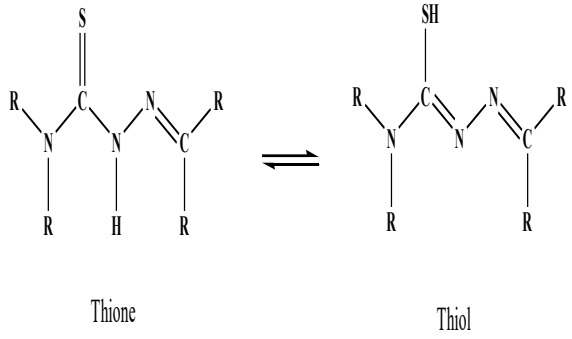
***Index Terms*—Thiosemicarbazone, Biological activity, Human cell lines, Standard drugs, Anticancer activity, Antiproliferative activity, Coordination**

I. INTRODUCTION

Thiosemicarbazones are crucial to metal coordination chemistry because of their remarkable versatility, chelating capacity, and high solubility in the majority of organic solvents [[1]-[3]]. Because of their significant biological activity, thiosemicarbazones and their derivatives have gained significant pharmacological importance [[4],[5]]. Thiosemicarbazones and their metallic complexes possess unique structural and biological characteristics. Thiosemicarbazones and their metal complexes exhibits a broad range of pharmaceutical actions, including anticancer [[6],**Error! Reference source not found.**], antiviral, antituberculosis [[8]], antibacterial [[9],[10]], antimalarial [[11]] antitumor [[12]] and antiamoebic [[13]] qualities, in addition to their structural and chemical characteristics. The biological effects due to their multifunctionality, which includes the hydrophobic domain as an aryl substituent and the NH, C=S groups as coordination-related electron donor sites [[14]]. The many scientists have been very interested in nitrogen-sulfur bearing ligands since they are crucial to the formation of physiologically active substances [[15]].

The biological actions of thiosemicarbazones, which are mixed hard-soft nitrogen–sulphur chelating ligands, have drawn significant attention to their chemistry [[16]]. These actions have been linked to their reductive and metal-chelating capacities. Thiosemicarbazone (R_2C=N-NH-CS-NH_2) is the Schiff base that results from condensing thiosemicarbazide with a carbonyl molecule (aldehyde or ketone). Thiosemicarbazide (H_2N-NH-CS-NH_2) is a nucleophilic reagent that is employed as a precursor [[17]]. Thiosemicarbazone have azomethine (>C=N) which helps to enhances the metal chelation and biological entities of the compounds [[18]]. The adaptability and intriguing behavior of thiourea as building blocks in polydentate ligands for metals have drawn attention in recent years due to the existence of S, N, and O donor atoms. The strong attaching function of thiosemicarbazones containing ONS-coordinated sites with transition metals makes them crucial for coordination chemistry [[19]].

In the fields of medical chemistry and drug development, metals have proven to be quite helpful. The bonding of metal atoms or ions with ligands produces coordination complexes [[20]]. Numerous human illnesses, including cancer, infectious diseases, diabetes, and neurological conditions, have been treated by metal-ligand complexes [[21]]. Thiosemicarbazones occur in thione-thiol tautomeric form, because of intramolecular proton transfer and coordination with metals [[22],[23]]. Depending on the environment's pH, oxidation, and the presence of relative metal ions, they can form anionic, cationic, or neutral chelates.

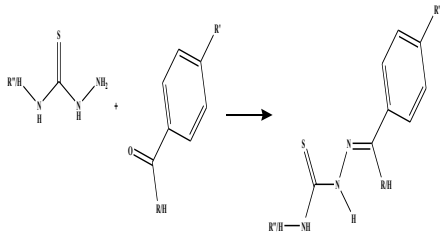


The current study involved the review of significant coordination of cyclohexyl thiosemicarbazone [[24]] and their substitutions with the metals. Additionally, it relates the presence of substituents linked to the parent cyclohexyl thiosemicarbazone and the type of metal bonded to the cyclohexyl thiosemicarbazone moiety to variations in biological activity. The authors examined their potential usage as combination medications and attempted to correlate the synergistic impact displayed by metal TSC complexes.

II. SYNTHESIS OF SUBSTITUTED THIOSEMICARBAZIDES/ THIOSEMICARBAZONES AND THEIR METAL COMPLEXES

Thiosemicarbazones were created when aldehyde or ketone condenses with thiosemicarbazide [[25]]. When the process is carried out in an alcohol-based

solvent like ethanol or methanol, an acidic catalyst that encourages condensation, like glacial acetic acid, is often present [[26]]. The ethanolic solution of the thiosemicarbazide refluxes with carbonyl compounds to get unique substituted thiosemicarbazones. This function as neutral, monoprotonic, or diprotonic ligands. The ability to substitute alkyl, aryl, or heterocyclic derivatives for the substituent groups has led to the development of TSC synthesis. This has produced a wide range of novel multi-dentate ligands. These ligands can coordinate to metallic centres via the imine nitrogen and sulfur atoms [[27]]. Hence, synthesized ligands were further converted into their metal complexes by using a particular metal ion. They are subsequently purified through recrystallization [[28]]. The biological activities of several substituted TSCs have been investigated and synthesized [[29]]. These studies findings show how they are used in the medical industry and bioinorganic chemistry.



General preparatory route of thiosemicarbazones

Coordination modes of Thiosemicarbazones:

The amount of donor atoms that can concurrently couple with a central metal atom or ions determines how the ligands are categorized. TSCs can interact with metal ions via a variety of donor atoms, mainly N and S. Their binding mechanism dictates whether they function as monodentate, bidentate, or tridentate ligands [[30],[31]]:

a) Monodentate coordination:

In this form, a single donor atom usually the sulfur atom—connects the TSC ligand to the metal core. This leads to a straightforward connection without the formation of a chelate ring. This may happen if an electrical factor or steric impediment precludes synchronous binding through both donor atoms. This leads to a straightforward connection without the formation of a chelate ring. This may happen if an electrical factor or steric impediment precludes synchronous binding through both donor atoms. Cl^-, OH^-, CN^- and water molecule etc are included as the examples.

b) Bidentate coordination:

The TSC ligand forms a five-membered chelate ring by coordinating with the metal via two donor atoms, typically the azomethine nitrogen and the thiocarbonyl sulfur. A typical example is Ethylenediamine (en), which has two nitrogen atoms with a lone pair that can bond to the metal [[32]].

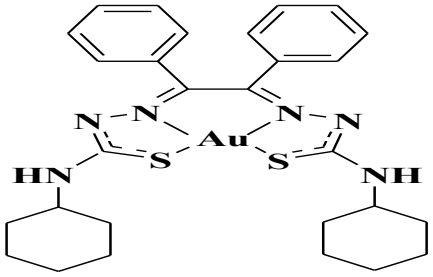
c) Tridentate coordination:

TSC ligands can occasionally function as tridentate ligands, coordinating through three donor atoms for example, two nitrogen atoms and one sulfur atom, to form bigger chelate rings. This results in a chelate ring with six members, which makes the metal complexes more stable. Metals such as Ru (III) and Co (III) can develop tridentate complexes with aromatic or extended thiosemicarbazones that have extra donor atoms. The metal in question and the particular structural characteristics of the ligand determine whether they can act as monodentate, bidentate, or tridentate ligands. Although the bidentate modality is more prevalent, A longer ligand structure may enable tridentate binding resulting in metal complexes that are more stable and useful.

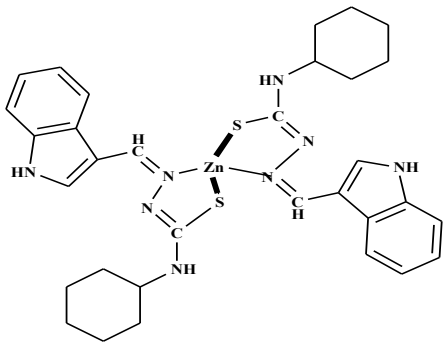
III. BIOLOGICAL APPLICATIONS:

3.1. Anticancer activity

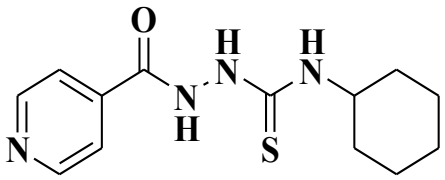
In the study of cancer, Fanjul et.al has focused more on gold(III) compound. Benzil bis(4-cyclohexyl-3-thiosemicarbazonate, which has been synthesized and well characterized. Comparing the cytotoxic activity of the Au(III) complex to cisplatin (CDDP) in three breast cancer cell lines—the triple-negative MDA-MB-231 cell line, MCF10A cells, and HER2 negative MCF7 cell line. MCF10A cells generally exhibit higher levels of Au(III) complex activity. The synthesized compound has more potency, has an activity profile that is comparable to that of CDDP in the three cell lines (the IC_{50} of CDDP is 4.8 M (MCF10A), 7.1 M (MDA-MB-231), and 8.9 M (MCF7)). For MCF7 cells treatment, compound resulted in the largest intracellular concentrations of gold (136.5 ± 11.1 ng Au/10^6 cells) [[33]].



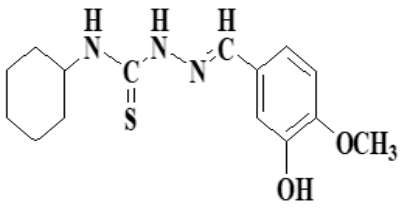
N. Balakrishnan et al. have synthesized and characterized the Zn(II) complex which contains an indole thiosemicarbazone ligand. It was determined that the complex was effective against two human cancer cell lines (A549 and MCF7) using an in vitro cytotoxicity test using cancer cell lines. With an N-terminal cyclohexyl group, the Zn(II) complex exhibited moderate activity [IC_{50}=37.9 (A549) and 60.3 µM (MCF7)], which was similar to that of the well-known anticancer medication cisplatin. Using an MTT assay, it was found that the complex with superior cytotoxicity showed reduced toxicity to two human non-cancerous cell lines (MCF-10A, HEK-293) and one non-cancerous mouse fibroblast cell line (L929). Furthermore, the Zn(II) complex causes apoptosis cell death in both the cancer (A549 and MCF7) cell lines. The experimental findings of cytotoxicity research indicate that complexes containing the bulky cyclohexyl group, such as the N-cyclohexyl, have superior anticancer activity [[34]].

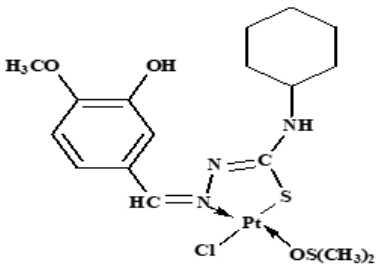


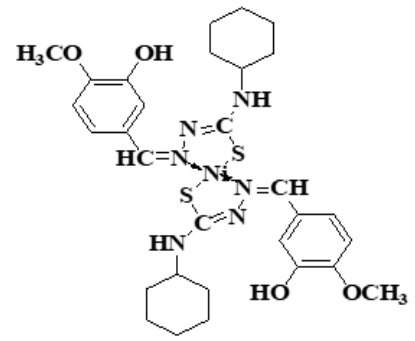
M. A. Bhat et al. synthesized N-(cyclohexyl)-2-(pyridine-4-yl carbonyl) hydrazine carbothioamide derivative from N-(4-cyclohexyl) hydrazine carbothioamide and 2-(pyridine-4-yl carbonyl). This compound was tested in comparison to itraconazole and 10 clinical strains of Candida spp. MIC values of >50 µg/mL were found for compound with a cyclohexyl ring to be the less effective against all the isolates used, while MIC values of 0.04–1.56 µg/mL were found for itraconazole to exhibit inhibitory activity against all tested strains. Hence, cyclohexyl group insertion, which results in a decrease in activity. These unsatisfactory outcomes caused to move to the electron withdrawal groups [[35]].

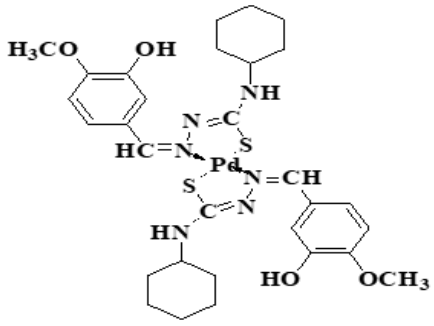


N-cyclohexyl hydrazine carbothioamide and 3-hydroxy-4-methoxybenzaldehyde were combined to create the bidentate N-cyclohexyl-2-(3-hydroxy-4-methoxybenzylidene) hydrazine -1-carbothioamide schiff base ligand, which coordinated to divalent Ni, Pd and Pt ions. All the compounds were evaluated against the human colon cancer cell (HCT-116), human cervical cancer cells (Hela), melanoma (B16F10) cells and human normal endothelial cell lines (Eahy926) to determine their antiproliferative effects. The findings indicated that, in comparison to other compounds, Ni complex had the highest activity. On normal endothelium Eahy926 cell lines, however, the ligand and Ni complex shown more toxicity. Among all the compounds examined, only Ni complex shown considerable cytotoxicity against HCT-116 cells (IC_{50}=7.9 ± 0.2 µM). Only a mild inhibitory effect on cell growth was shown by the ligand, Pd and Pt [[36]].

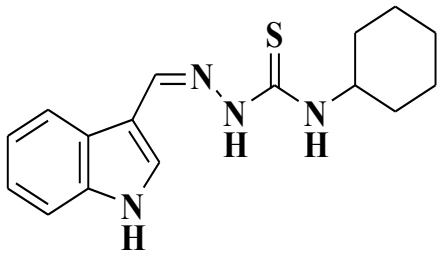


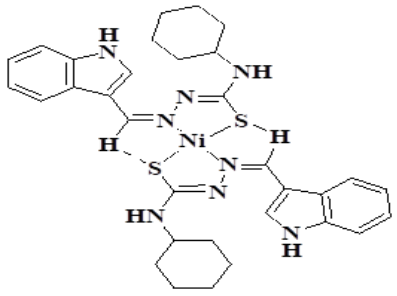




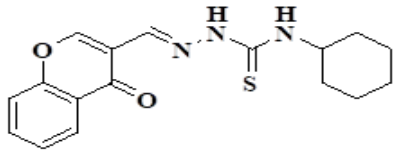


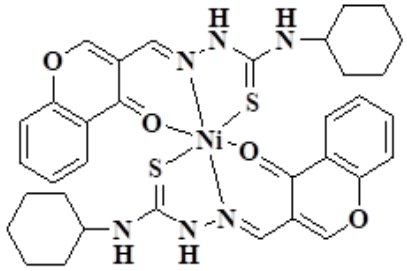
J. Haribabu and his group were created the ligand (E)-2-((1H-indol-3-yl) methylene) -N-cyclohexyl hydrazine carbothioamide and its Ni(II) complex. The investigations were conducted on the in vitro cytotoxicity of Ni(II) complex against mouse embryonic fibroblasts (L929), human breast cancer (MCF7), and lung cancer (A549) cell lines. The presence of bulky substitution (cyclohexyl) at the ligand's terminal nitrogen atom may be the cause of the elevated cytotoxicity. Utilizing Hoechst 33258 staining, the mechanism of cell death caused by the complex has been investigated. Complex demonstrated a greater inhibitory impact, with IC_{50} value that were comparable to cisplatin. Fortunately, the higher IC_{50} values (>750 µM) showed that the complex was less harmful to the normal cell (L929) [[37],[38]].





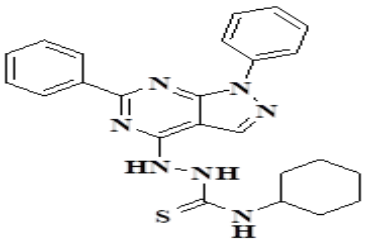
S. Selvamurugan and partners generated the Ni(II) complex from 4-chromone-4-cyclohexylthiosemicarbazone ligand. This prepared complex further investigated for in vitro cytotoxicity against the human breast cancer cell line (MCF-7). Because of the terminal cyclohexyl thiosemicarbazones, the compound exhibits strong anticancer action against the MCF-7 cancer cell line. Significant activity of the nickel(II) complex against MCF-7 cancer cell lines was demonstrated by the obtained IC_{50} value. When complex concentrations were raised from 0.1 µM to 100 µM, the number of cells dropped as the complex concentration increased. The inhibitory activity of the complex against MCF-7 is lower than that of cisplatin when the IC_{50} values of the complex and cisplatin are compared [[38],[39]].



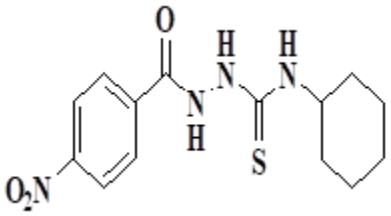


A. Gaber et al. have been formed 1-H -pyrazolo[3,4-d]pyrimidine hybrid thiosemicarbazide derivative. The inhibitory property of newly synthesized drug against three cancer cell lines having $EGFR^{WT}$ (MCF-7, HepG2, A549) were assessed in vitro. In contrast, the molecule exhibited similar inhibitory effects to

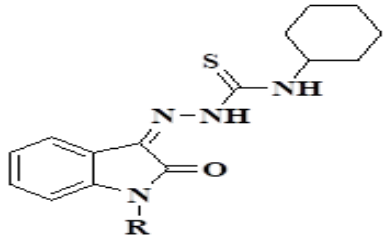
erlotinib, with IC_{50} values between 0.44 ± 0.24 and 0.73 ± 0.31 µM [[40]].



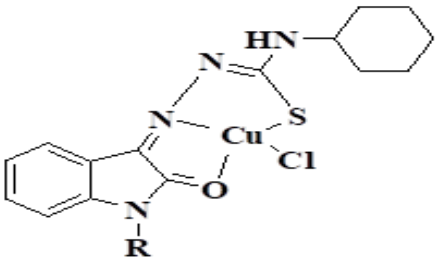
Wos and his teammates discovered the compound 4-cyclohexyl-1-(4 nitrophenyl) carbonyl thiosemicarbazide. The normal human skin fibroblasts (BJ cell line) and dose-dependent antiproliferative activity against cancer cells has been proven by the prepared compound. The concentration that caused a 50% (CC_{50}) reduction of normal fibroblast viability values for the thiosemicarbazide based compound ranged 24.21 ±1.45 µg/mL, indicating a variety of cytotoxic activities. 4-cyclohexyl-1-(4-nitrophenyl) carbonyl thiosemicarbazide demonstrated the most cytotoxic activity among all the substances examined using the MTT assay [[41]].



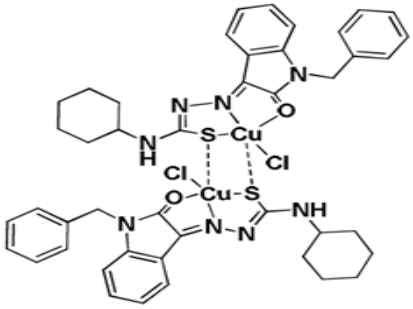
M. Muralisankar and his company employed a new series of N-cyclohexyl isatin thiosemicarbazone ligand and their Copper(II) complexes. The metal complexes were screened for cytotoxic study. The MTT assay has been used to investigate the cytotoxicity of the monometallic and bimetallic complexes targeting the human breast cancer cells MCF7 and the human lung cancer cells A549. The Cu(II) complexes were evaluated under the same circumstances as compared to standard cyclophosphamide [IC_{50}= 6.58 (MCF7) and 22.36 µM (A549)]. With IC_{50} values of 88.59 and 82.56 µM against A549, respectively, both the complexes demonstrated moderate cytotoxicity. Against the MCF7 cell line, the same complexes exhibited 50% inhibition concentrations of 120.68 and 110.34 µM, respectively. Complex 1 exhibited the least amount of activity. The bimetallic structure of complex may be the cause of its increased activity when compared to other complexes [[42]].



R = C_3H_3 (L_1), C_3H_5 (L_2), C_7H_7 (L_3)

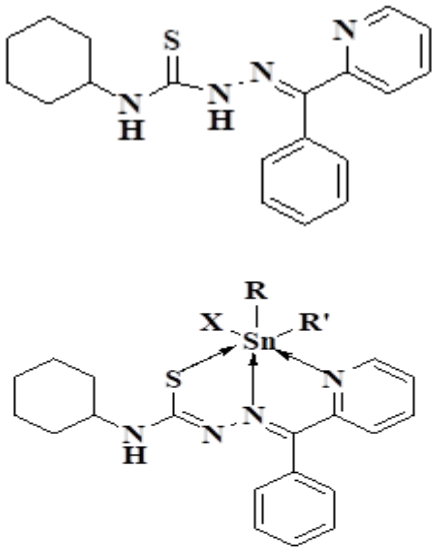


R = C_3H_3 (1) or C_3H_5 (2)



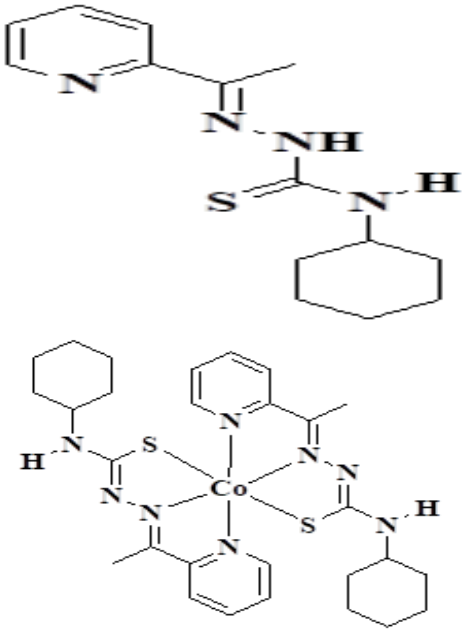
Production of four distinct substituted Sn(IV) metal complexes and the ligand 2-benzoylpyridine-N(4)-cyclohexyl thiosemicarbazone by Salam et al. The cytotoxic potential of ligand and its complexes was evaluated in vitro against the ovarian cancer cell lines A2780 and A2780/Cp8. Cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines were used to compare the effects of complexes. For ligand IC_{50} values observed 0.38 and 0.90 µmolL^{-1}, respectively. Complex 1 is 8 and 7 times more active than a cisplatin against A2780 and A2780/Cp8, with IC_{50} values of 0.30 and 0.94 µmolL^{-1} against A2780 and A2780/Cp8, respectively. Complex 2 outperforms cisplatin by 7 and 11 times greater effective for selected cell lines. The Sn(IV) complexes 3 and 4 shows 10 and 19; 45 and 13 times greater activity than control. Their

actions against two ovarian cancer cell lines demonstrate how effective they are as anticancer drugs. Hence, the addition of metal to the respective ligand enhances the activity of the complexes than the ligand [[43]].

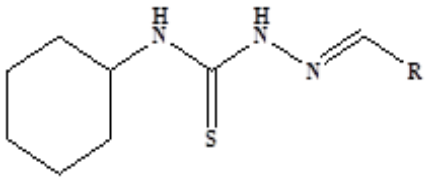


Complex	R	R'	X
1.	Me	Cl	Cl
2.	Bu	Cl	Cl
3.	Cl	Me	Me
4.	Cl	Ph	Ph

The Co(III) complex obtained from 2-acetylpyridine N(4)-cyclohexylthiosemi- carbazone ligand. Initially, VERO epithelial cells (ATCC CCL81) were used for in vitro cytotoxicity tests (IC_{50}). Using the J774A.1 (ATCC TIB-67) murine macrophage cell line, the most selective chemical (greater SI) was investigated. The MIC and IC_{50} (VERO cells) values of the complex found to be 2.41 and 18.31 μmol L^{-1} respectively [[44]].

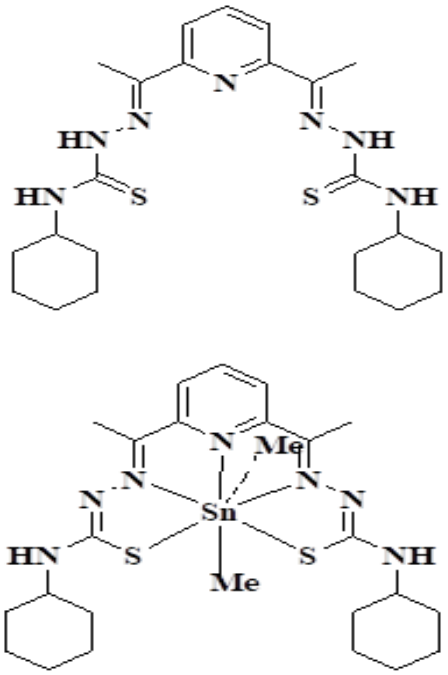


Bhat et al. worked on a series of cyclohexylthiosemicarbazone derivatives (C1–14) were created, described, and assessed on HER-2 overexpressed breast cancer cells. Four breast cancer cell lines: SKBr-3, MCF-7, MDA-MB-468, and MDA-MB-231 were used to screened the synthetic compounds in vitro. With IC_{50} values ranging from 25.6 ± 0.07 to 61.6 ± 0.4 μM, each drug demonstrated efficacy against HER-2 overexpressed SKBr-3 cells. The most potent substances block ALDH $^{+}$ breast cancer stem cells more successfully than salinomycin, an agent that is unique to cancer stem cells. Compound C2 dramatically reduced breast cancer cell lines ability to migrate and adhere to one another. The most effective chemical in this series, compound C2, was discovered to target HER-2 overstated breast cancer cells [[45]].

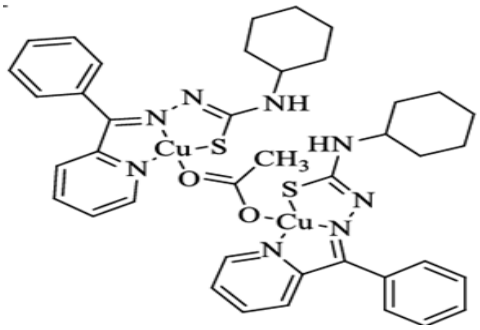


Regarding the cytotoxic effect of thiosemicarbazones, Han Yu and coworkers first assessed ligand and its tin(IV) complex capacity to prevent the proliferation of tumor cells in human liver cancer cell line HepG2 (hepatocellular carcinoma) cells. The effects of these

substances on normal hepatocyte QSG7701 cells are also discussed in order to investigate their toxicity. Compared to ligand (> 100 μM), tin(IV) complex shows much lower IC_{50} values (17.99 μM) against HepG2 cells, suggesting that linking the thiosemicarbazone to tin(IV) increases the cytotoxicity of Schiff base ligand. Additionally, compared to ligand, IC_{50} values of Sn(IV) complex are higher in QSG7701 cells than in HepG2 cells, suggesting that complex is less hazardous to QSG7701 cells [[46]].

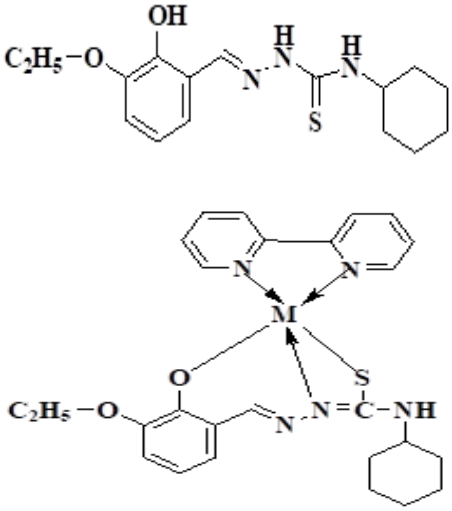


When tested in vitro against the human leukemia K562 cell line, the binuclear copper(II) complex of 2-benzoyl pyridine thiosemicarbazone demonstrated incredible cell death with an IC_{50} of 0.37 ± 0.028 μM, which compared to cisplatin. It also induced apoptosis in HepG2 cells in a dose-dependent manner, increasing intracellular formation of ROS and decreasing mitochondrial membrane potential (MMP) [[47]].

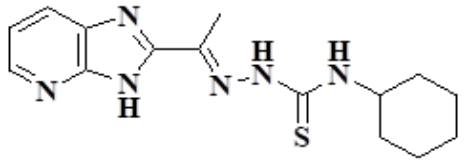


3.2. Antitumor activity

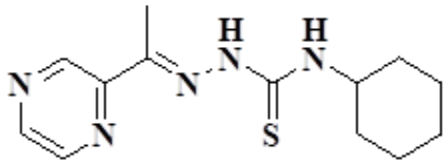
The Cu(II) and Zn(II) complexes were shown to be more cytotoxic than their respective proligands when tested in vitro against Dalton lymphoma ascites cell lines. With the copper ion serving as the core metal, Cu(II) complex demonstrated the maximum activity, and the cytotoxicity recorded was even greater than that of the common chemical cyclophosphamide [[48]].



60 human cell lines from nine clinically identified cancer types will be used to test the compound's in vitro antitumor efficacy (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast) in accordance with a standard methodology. The antitumor action was exhibited by compound. The activity of this molecule is slightly enhanced by the addition of a saturated ring, which demonstrates the sensitivity of various cell lines [[49]].

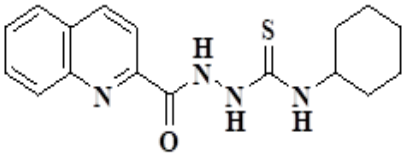


The transition metal Mn^{+2} and Ni^{+2} complexes of 4-cyclohexyl-1-(1-(pyrazin-2-yl)ethylidene) thiosemicarbazide, have been synthesized and characterized. Despite the cytotoxic effect of thiosemicarbazone, they have investigated, the capacity of both the complexes to prevent the proliferation of tumor cells against the K562 leukemia cell line. Ligand exhibits a significantly lower IC_{50} value (9.92 mM) than the previously documented cases of 2-acetylpyrazine-N(4)-methylthiosemicarbazone (25.9 mM) and 2-acetylpyrazine thiosemicarbazone (47.5 mM), indicating that the addition of a bulky (cyclohexyl) group at position N(4) of the thiosemicarbazone moiety significantly improved the antitumor activities. Furthermore, it is well known that complexation with metals has a synergistic effect on these compounds' anticancer activity, and that this effect varies depending on the type of metal. This supports the idea that the connection to metal ions can increase the anticancer actions of thiosemicarbazones. The K562 leukemia cell line was used to study the effects of the free ligand and its two complexes on cell death, mitochondrial membrane potential (MMP). The cytotoxic effect of the investigated drugs may be mediated by the induction of MMP loss [[50]].

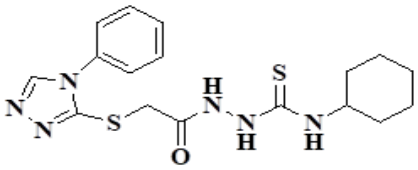


3.3. Antimicrobial activity

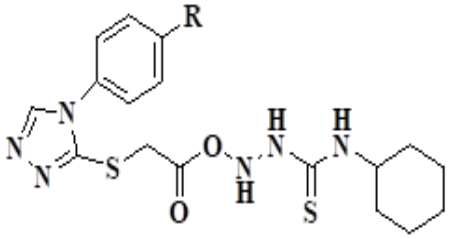
The 2-quinolone-4-cyclohexyl-3-thiosemicar bazide was created by Keshk and colleagues using quinoline 2-carboxylic acid, cyclohexyl isothiocyanate and hydrazine hydrate. Its in-vitro antibacterial activity was further examined. The compound was tested for one gram positive: Staphylococcus aureus and one gram negative: E. coli bacterial strains. The substance shown excellent effectiveness against both the strains, S. aureus and E. coli [[51]].



Popiolek and coworkers discovered that the 1,2,4-triazole hybrid 4-cyclohexyl-3-thiosemicarbazide derivative. The agar well diffusion method was used to screen the tested chemical for in vitro antibacterial activity. The gram-positive bacterial species: S. aureus, B. subtilis, B. cereus, S. epidermidis etc. were used to detect the antibacterial activity. From the results, it was seen that the tested compound does not show much effect against above bacterial strains used [[52]].

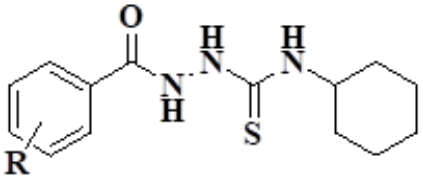


In their study, Trotsko and co-workers showed how to synthesize a 1,2,4-triazol N-cyclohexyl-3-thiosemicarbazide derivative from ethyl 2-((4-phenyl-1,2,4-triazol-3-yl)thio)acetate and assessed its antimicrobial efficacy in vitro. Against gram-positive bacteria, these derivative does not show much good efficacy [[53]].



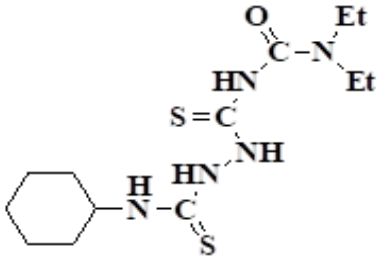
Plech and his team developed a three various thiosemicarbazide derivatives namely, 4-Cyclohexyl-1-(2-hydroxybenzoyl) thiosemi carbazide, 4-Cyclohexyl-1-(3-hydroxybenzoyl) thiosemicarbazide and 4-Cyclohexyl-1-(4-hydroxybenzoyl) thiosemicarbazide. These molecules were tested for multiple antibacterial pathogens. The gram-positive pathogens have been chosen such as S. aureus, S.

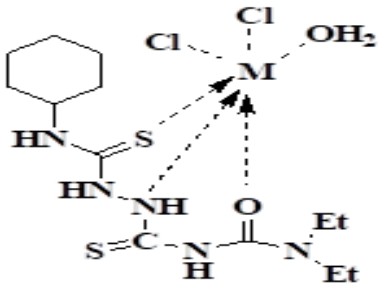
epidermidis, B. subtilis, B. cereus and M. luteus. E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, these gram-negative strains were taken for evaluation of the compounds. Vancomycin, cefuroxime, and ampicillin were employed as control antibacterials.2-hydrxy derivative shows 7.82-31.25 µg/mL of MIC for all the selected strains. It shows highest MIC for the strain B. cereus i.e. 1000 µg/mL. Remaining both the 3-OH and 4-OH derivatives does not exhibit any active potency against any pathogen used [[54]].



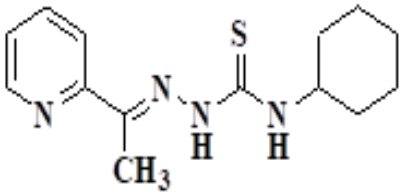
R= 2-OH, 3-OH, 4-OH

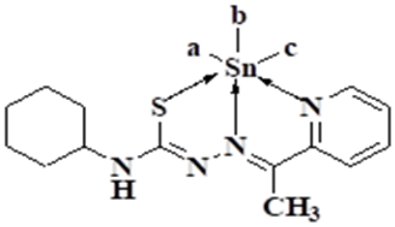
The ligand has been manufactured by T. Khalaf and N. Hussien using a mixture of N-cyclohexyl hydrazinecarbothioamide and diethyl carbamoyl chloride and utilized to make three novel Mn^{+2}, Co^{+2} and Ni^{+2} metal complexes. The ligand and complexes were tested for antibacterial activity against both gram-positive (S. aureus) and gram-negative microorganisms (E. coli). The Penicillin was used as the negative control, whereas Cefotaxime was the positive control. Complexation can enhance antibacterial action when compared to free ligands, according to research. The Mn^{+2} complex does not show any activity but Co^{+2} and Ni^{+2} complexes show greatest activity among selected bacteria. The tested substances show antibacterial activity in the order of $Ni^{+2} > Co^{+2} > (Mn^{+2} = Ligand)$ [[55]].





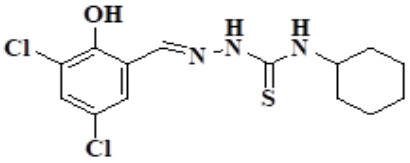
The ligand 2-acetylpyridine-N(4)-cyclohexylthiosemicarbazone and five distinct substituted Sn(II) metal complexes were synthesized and tested for their antibacterial activity versus S. aureus, E. coli, E. aerogenes and S. typhi bacterial strains. The results discovered that the free ligand had minimal effect against microorganisms. The overall results indicated that the metal complexes showed high antibacterial potency than the respective ligand and low potency than the reference drug used doxycycline. The largest inhibitory zones for the development of organisms were found in complex 5, which had inhibition zones of 27.6, 27.5, 25.9, and 24.8 mm against S. aureus, E. coli, E. aerogenes, and S. typhi respectively. Complex 4 was the second most anti poisonous to all of the bacteria. The complex 2 and 3 showed more enhanced activity than the complex 1. Increased in activity has been reported as a result of ligand-metal coordination and effective metal complex diffusion into bacterial cells [[56]].



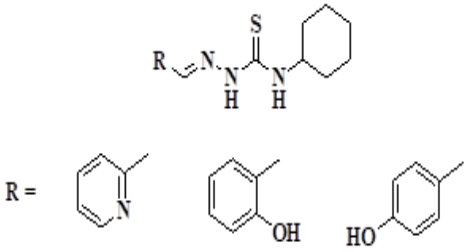


Complex	a	b	c
1.	Cl	Me	Cl
2.	Cl	Bu	Cl
3.	Cl	Ph	Cl
4.	Me	Cl	Me
5.	Ph	Cl	Ph

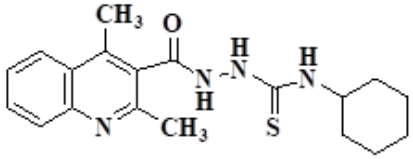
Mathews et Al. studied on the antibacterial performance of the synthesized ligand 3,5-dichlorosalicylaldehyde-N4-cyclohexyl thiosemicarbazone and its Cu and Zn metal complexes by using bacterial microbes: E. coli, S. typhi, E. aerogenes, S. dysentriae, B. cereus and S. aureus. The ligand shown activity against E. coli, E. aerogenes, S. typhi, and S. dysentriae, while the complexes demonstrated activity against E. Coli and S. typhi. In the case of ligand, the zone of inhibition produced 0.6, 0.9 and 0.4 mg/ml of E. aerogenes, S. dysentriae and E. coli respectively. For complexes, E. Coli provides 0.3, 0.4, and 0.6 mg/ml while S. typhi provides 0.4, 0.5, and 0.8 mg/ml [[57]].



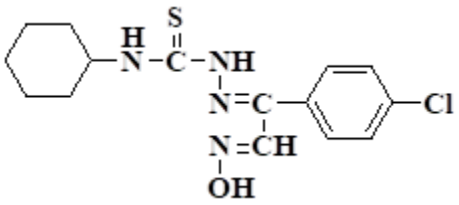
The synergistic antibacterial properties of thiosemicarbazones were examined by measuring the minimum inhibitory concentration (MIC). According to in vivo antibacterial activity tests, thiosemicarbazone and meropenem can work together to combat the clinical problem of carbapenem-resistant microorganisms carrying NDM-1. Meropenem and thiosemicarbazones have a MIC of 16 µg/mL against E. coli-NDM-1 gives 16, 64 and 128 µg/mL of the following compounds respectively [[58]].



Patel D. B. et al discovered a quinoline based thiosemicarbazone derivative, N-(Cyclohexyl carbamothioyl) 2,4-dimethylquinoline-3-carboxamide were tested for its antibacterial activity. The compound shows very good antibacterial activity because of the cyclohexyl group having MIC 25µg/mL. The various bacterial strains have been used, out of those E. coli shows very prominent effect compared to reference drug [[59]].

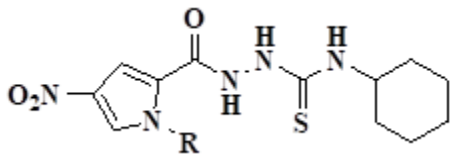


S. V. Kadam and R. M. Patil discovered thiosemicarbazone based ligand namely,4-chloroisinitrosoacetophenonecyclohexylthiosemicarbazone and its various metal complexes. All the compounds were tested for its antimicrobial properties against bacterial and fungal strains. The results were compared with the standard dugs ciprofloxacin and fluconazole. The overall observation seen that the metal complexes show more intense antimicrobial activities than that of free ligand [[60]].

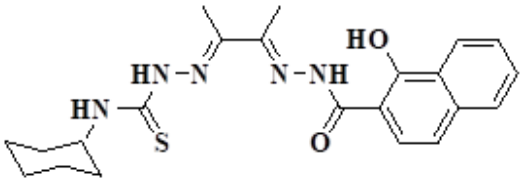


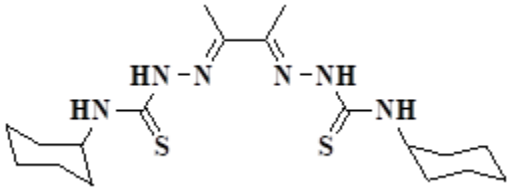
R. Rane and associates produced 4-nitropyrrole-4-cyclohexylthiosemicarbazide derivative. The synthesized derivative examined. The derivative demonstrated that the pyrrole core's N-methylation improved its antifungal effectiveness against C. albicans. In comparison to standard Amphotericin-B (MIC =3.125 µg/mL), the synthesized hybrid showed exceptional antifungal activity (MIC = 3.125 µg/mL) against Candida albicans. In thiosemicarbazides, this combination showed noticeably better antifungal activity that was four fold stronger. Thus, N-methylation on the pyrrole core and the thiosemicarbazides domain are more preferred

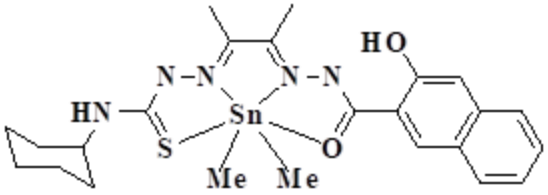
characteristics for antifungal action against C. albicans [[61]].

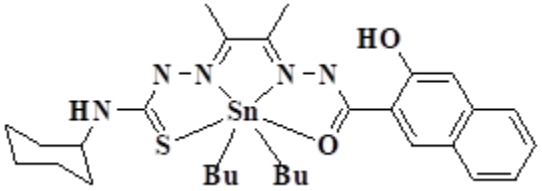


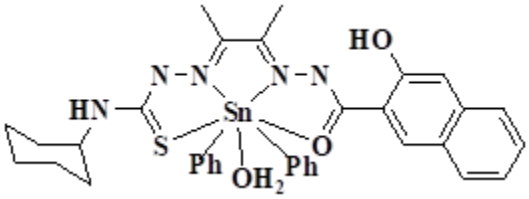
The antifungal activity of the ligands and their corresponding diorganotin (IV) complexes was investigated by C. Gonzalez-Garcia and co-workers. All the compounds were tested for different yeasts and mould such as, Candida tropicalis (ATCC1369), Saccharomyces cerevisiae (ATCC9763) as a yeast and Aspergillus niger (ATCC6275) as mould. Only some Sn(IV) complexes were found to be active in terms of the novel compounds' antifungal characteristics. Both the new ligands and their complexes demonstrated antifungal activity that was lower than that of the antifungal positive control, miconazole. A. niger growth was suppressed by complexes at concentrations of 200 and 100 μgmL^{-1}, respectively. Compared to the ligand, the antifungal spectrum of metal complexes shows more active potency. Minimum fungicidal concentrations (MFCs) were higher than the equivalent minimum inhibitory concentrations (MICs) in all the compounds, indicating fungistatic behaviour of the compounds [[62]].

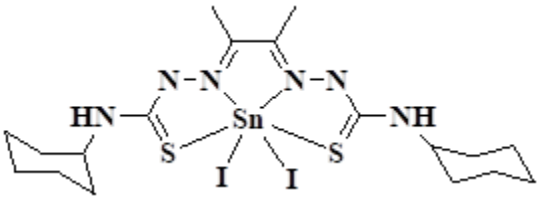






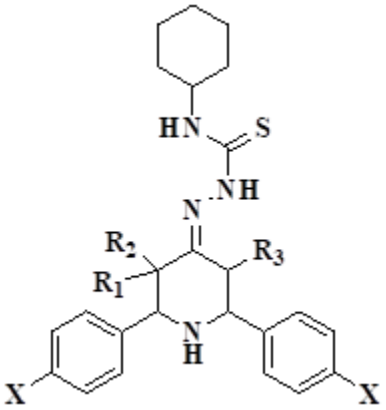






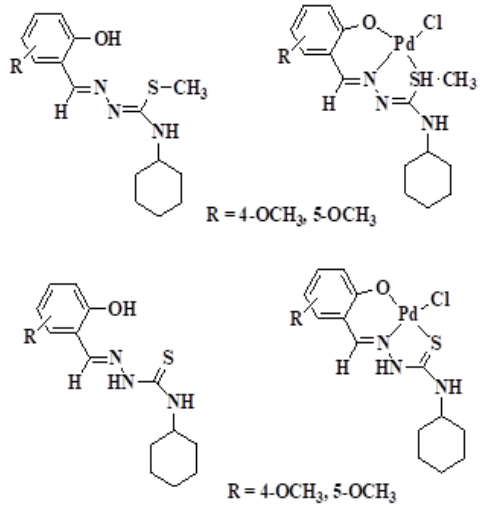
By reacting 2,6-diarylpiperidin-4-ones with cyclohexylthiosemicarbazide, a new series of 2,6-diarylpiperidin-4-one-N(4)-cyclohexyl thiosemicarbazones was produced and explained by A. Sethukumar et al. All the synthetic molecules in vitro antifungal activity was tested against five different fungal strains: Aspergillus flavus, Aspergillus niger, Penicillium chryogenum, Trioderma veride, and Fusarium oxysporum. With the methyl group in heterocyclic ring, demonstrated equal potency to the standard medicine drug amphotericin-B against all fungal strains, with the exception of A. niger. Fluorine and methoxy groups were introduced at the para position of phenyl group, which led to a minor to slight increase in inhibitory potency versus all tested fungal species. A compound which replaces the methyl group with an isopropyl group at the C-3 position, has lower activity against the tested strains. In addition to fluorine, it did not show any improvement in potency. The molecule which has two methyl groups, exhibited fine potency against A. niger, T. veride, and F.

oxysporum, but it was one-fold less active against A. flavus and P. chryogenum than the control. The remaining substances show reduced activeness over all studied strains [[63]].

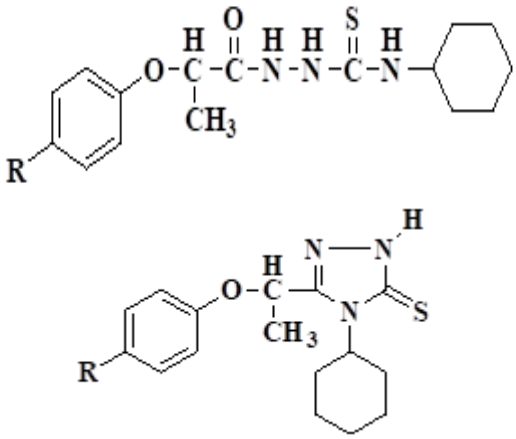


Sr. No.	R_1	R_2	R_3	X
1	CH_3	H	H	H
2	CH_3	H	H	F
3	CH_3	H	H	OCH_3
4	$CH(CH_3)_2$	H	H	H
5	$CH_3(CH_3)_2$	H	H	F
6	CH_3	CH_3	H	H
7	CH_3	CH_3	H	F
8	CH_3	CH_3	H	OCH_3
9	CH_3	H	CH_3	H
10	CH_3	H	CH_3	F
11	CH_3	H	CH_3	OCH_3

Thiosemicarbazone derivatives and their complexes were tested for antifungal activity against three fungi: C. albicans (ATCC10231), C. parapsilosis (ATCC22019) and C. tropicalalis (ATCC750) and MIC values compared with respect to a standard agent. All the thiosemicarbazone ligands showed negligible inhibitory effect against fungi, with the exception of C. tropicalis. The palladium metal complexes shown superior antifungal efficacy against Candida spp. Of MIC values 19.53 and 3.17µg/mL. Complex (4-OCH_3) showed excellent antifungal action towards C. albicans and C. parapsilosis having MIC of 3.17µg/mL [[64]].

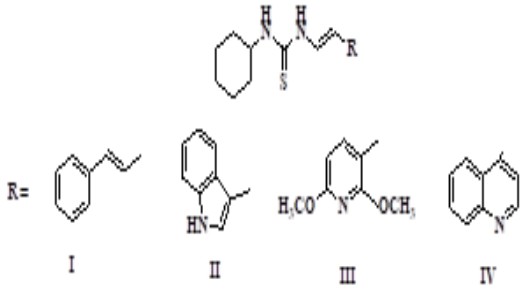


The results of all the synthesized compounds were derived from the screening of antifungal activity were the most significant against C. albicans and C. glabrata was successfully combatted by the majority of the compounds, in contrast with the ketoconazole. The molecule shows strong activity against C. albicans and C. glabrata. The MIC values were expressed in the range between 8-16 µg ml^{-1} [[65]].



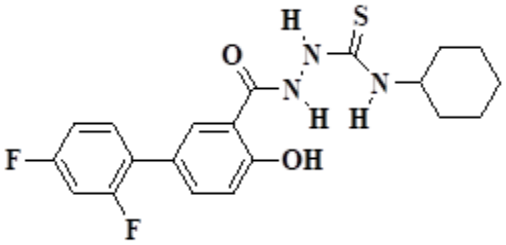
Reis et.al prepared four novel 4-cyclohexyl-3-thiosemicarbazide derivatives from substituted aldehydes. All thiosemicarbazones were tested for their initial in vitro antifungal efficacy against Aspergillus parasiticus and Candida albicans. All thiosemicarbazone derivatives were tested for their initial in vitro antifungal activity against yeasts C. albicans and A. parasiticus. When contrasted with the common fungicide itraconazole, the only thiosemicarbazone derivative II exhibited highest

antifungal character with the MIC of 500 µg/mL and other synthesized derivatives do not show antifungal properties against both the yeasts [[66]].



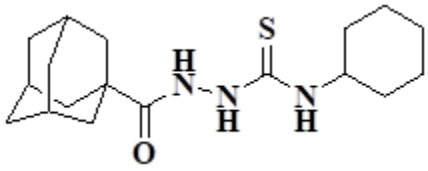
3.4. Antiviral activity

A compound 1-(2′,4′-Difluoro-4-hydroxybiphenyl-3-carbonyl)-4-cyclohexyl thiosemicarbazide have been discovered by Kucukguzel G. et al. The present diflunisal thiosemicarbazide based compound were tested for antiviral activity using various virus. As a result, the obtained compound shows more excellent antiviral potency than diflunisal 4-thiazolidinone [[67]].

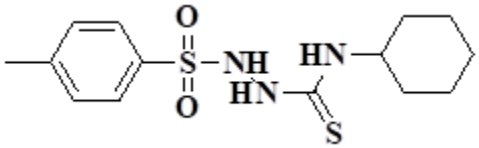


3.5. Antiproliferative activity

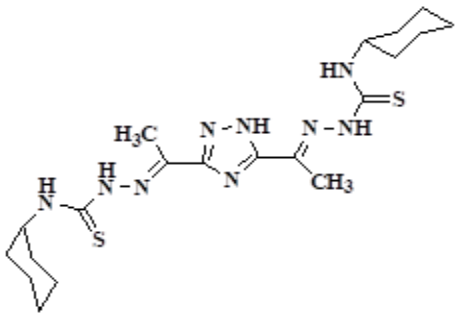
The 2-(admantane-1-carbonyl)-N-cyclohexyl hydrazine-1-carbothioamide have been studied by L. H. Al-Wahaibi and his team. Using the 3-[4,5-dimethylthiazoyl-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay, the in vitro antiproliferative activity of compound was evaluated against five human tumor cell lines: PC-3, HCT-116, HepG-2, HeLa, and MCF-7. According to the results obtained, the compound showed strong antiproliferative activity (IC_{50}<10µM) against HeLa and MCF-7 cell lines. They were marginally active against PC-3 cells and moderately active against HCT-116 and HepG-2 cells. The MIC values were found to be 52.68±4.3, 34.90±2.8, 29.48±1.4, 9.88±1.0 and 16.98±0.6 µM of PC-3, HCT-116, HepG-2, HeLa, and MCF-7 respectively [[68]].



The compound N-(cyclohexylmethyl)-2-[(4-methylphenyl) sulfonyl] hydrazinecarbothio amide were used for its antiproliferative activities on PC3 (androgen-independent) human prostate and MCF7 (oestrogen and progesterone receptors) breast cancer cell lines using an MTT assay. Cisplatin, an anti-cancer drug, served as a positive control. It shows good cytotoxic activity results against both the cells [[69]].

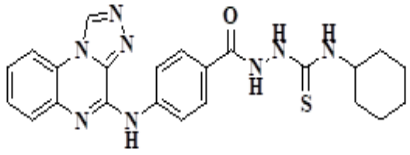


In this article, Matesanz and teammates reported bis(thiosemicarbazone) based compound 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexyl thiosemicarbazone). The prepared compound exhibits low-micromolar antiproliferative activity against lung (NCI-H460) and ovarian (A2780, cisplatin sensitive, and A2780cisR, cisplatin resistant) cancer cells. Additionally, the compound also has antiproliferative effect against breast cancer cells (MCF-7). At the maximal dose of 100 µM, the compound shows very little cellular growth inhibition (<50%), and as a result, there is no detectable cytotoxicity (IC_{50} > 100 µM) [[70]].



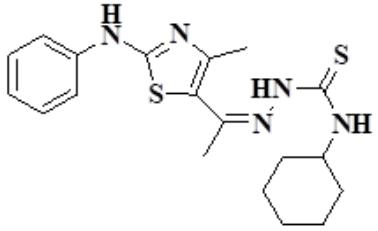
Specifically, this study was discovered that prepared [1,2,4]triazolo[4,3-a]quinoxaline cyclohexylthiosemicarbazone were screened for

antiproliferative activity the most effective compound against the three cancer cell lines, HepG2, HCT116, and MCF-7 having IC_{50} 6.18 ±0.8, 6.49 ±0.5 and 8.08 ±0.8 µM respectively. The prepared compound exhibits higher potency than the standard cytotoxic drug doxorubicin [[71]].

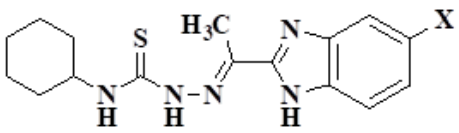


3.6. Antimalarial activity

In this work, a series of novel 2-(1-(4-methyl-2-(substituted-phenylamino) thiazol-5-yl) ethylidene) hydrazine carbothioamide compounds have been prepared and were evaluated for their antimalarial activity against Plasmodium falciparum 3D7 (P. falciparum) strain. The standard drug was used as Chloroquine and Quinine. Compared to the usual control medication, none of the compounds had demonstrated adequate efficacy against P. falciparum [[72]].



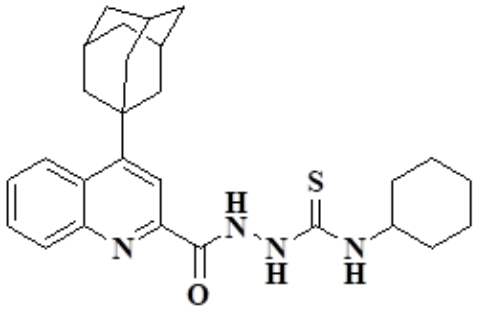
By calculating the lowest inhibitory concentration (µg/mL), the synthetic compounds 7a–7t and 8a–8t were evaluated for their in vitro antimalarial efficacy against P. falciparum. Compounds 7e (0.047 µg/mL) and 8e (0.053 µg/mL) displays moderate activity against Chloroquine. The groups such as cyclohexyl, appear to be very crucial potency for the antimalarial activity [[73]].



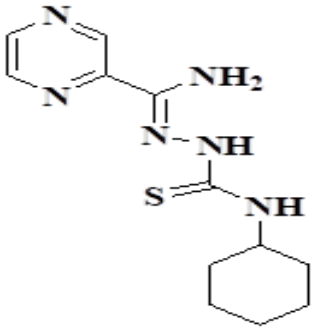
X=Cl, H

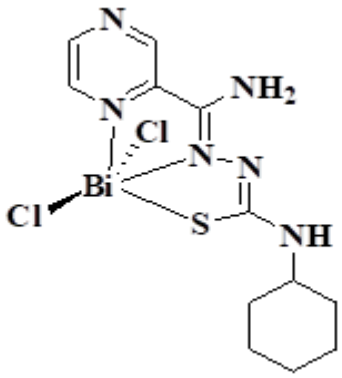
3.7. Anti-tuberculosis activity

The 4-(adamantan-1-yl) quinolone hybrid cyclohexyl thiosemicarbazide derivative was prepared using an effective methodology by Patel et al., who also examined its anti-tuberculosis properties and screened it in vitro against drug-sensitive strains. The first step was employing the Microplate Alamar Blue Assay (MABA) at a concentration of 6.25 mg/mL to test the synthetic analogues' in vitro efficacy against the M. tuberculosis H37Rv strain, which is susceptible to both isoniazid and rifampicin treatments. The compound shown 0% inhibition at MIC value of 6.25 µg/mL [[74]].



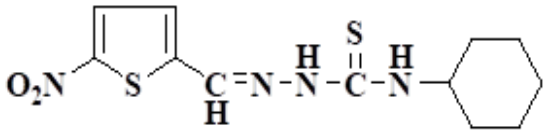
C. L. Almeida and coworkers synthesized a thiosemicarbazone based ligand and its Bi complex. These compounds were checked for their in vitro activity against M. tuberculosis H37Rv strain and results were compared with the standard drugs. Under the tested conditions, thiosemicarbazones ligand are not very effective at slowing the growth of M. tuberculosis. Surprisingly, when the ligand was coupled to Bi, enhancements in the antitubercular action were noted. The MIC values >359.2 and 163.6 $\mu molL^{-1}$ were obtained for ligand and its Bi complex respectively. The ligand has the greatest volume and lipophilicity (log P = 0.96 and V = 252.94 Å3) [[75]].



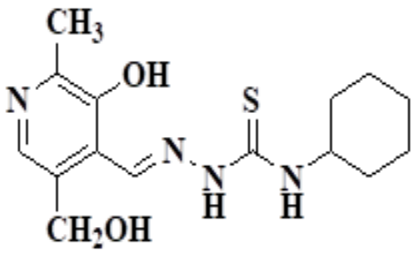


3.8. Antiamoebic activity

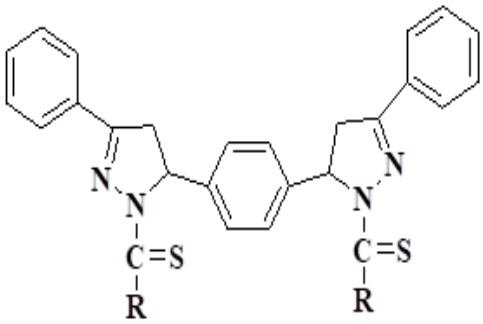
The compound 5-nitrothiophene 2-carbaldehyde-N(4)-cyclohexyl thiosemicarbazone were synthesized by Bharti et.al. The in vitro activity of synthesized drug was evaluated against protozoal strain HK-9 and demonstrated remarkable efficacy against E. histolytica. The IC_{50} for antiamoebic activity of the compound found to be 2.41±0.51 µM and compared with the standard drug Metronidazole [[76]].



The mixture of cyclohexylthiosemicarbazide and pyridoxalhydrochloride prepared a compound. Using the microdilution method, the compound was evaluated in vitro for antiamoeobic activity against the HM1:1MSS strain of E. histolytica. It noted that, thiosemicarbazone with a larger substitution at position N4, such as ligand with a cyclohexyl group. The IC_{50} value of the compound shows higher antiamoebic property than the standard drug metronidazole [[77]].

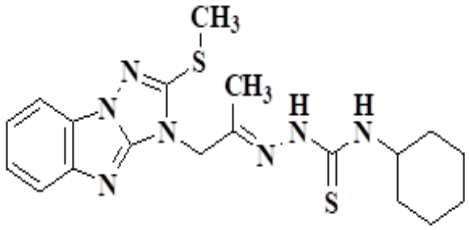


Bhat et. Al. designed and analysed the thiosemicrbazone and bis-pyrazoline based derivative (Cyclohexylamino) [5-(4-{1-[(cyclohexylamino)thioxomethyl]-3-phenyl(2-pyrazoline-5-yl-phenyl)}-3-phenyl(2-pyrazolinyl)]methane-1-thione. This compound was screened for antiamoeobic activity using microdilution method by HM1: IMSS strain of E. histolytica. The IC_{50} of the compound 1.16 µM shown action that was moderate compared to metronidazole. This compound having cyclohexyl ring shows maximum activity than the cyclopentyl ring [[78]].



3.9. Anti-inflammatory activity

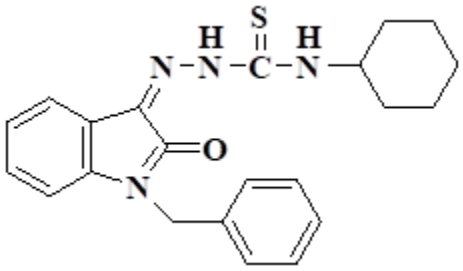
Using a carrageenan-induced paw edema bioassay in rats, Mahmoud and coworkers constructed, manufactured, and analyzed novel thiosemicarbazone of 2,3-disubstituted-[1,2,4]triazolo[1,5-a] benzimidazole as anti-inflammatory drug. The main precursor, thiosemicarbazone, demonstrated substantial anti-inflammatory efficacy, according to the findings [[79],[80]].



3.10. Anti-oxidant and anti-haemolytic activity

The prepared thiosemicarbazone based compound shows superior scavenging ability versus DPPH free radical assay was demonstrated by their antioxidant potential having IC_{50} value 75 µg/ml. This compound also tested for anti-haemolytic activity; the toxicity of the synthesized compound was evaluated using human red blood cells. The outcomes were contrasted with the water-treated control cells, which resulted in 100%

lysis. The findings made it abundantly evident that the test compound had a very low haemolysis percentage. Additionally, since the substituted thiosemicarbazone do not exhibit any toxicity to red blood cells, they may be utilized for pharmacological effects [[81]].



IV. CONCLUSION

The reviewed research demonstrates the enormous potential of thiosemicarbazones and their metal complexes, as well as chances for further study to overcome current obstacles and broaden their uses. The metal complexes of thiosemicarbazones provide a broad range of molecules with significant applications in environmental research and the medical field. Their therapeutic potential is demonstrated by the research, which highlights their many biological activities, such as antibacterial, anticancer, antiviral, antimalarial, and enzyme inhibitory qualities. The complexes greater lipophilicity in comparison to the ligand alone may be the cause of the improved impact. The activity of the complexes is increased when coordination sites are present. The complexes have an inhibiting influence on the cell lines' ability to proliferate and differentiate. Improving specificity and selectivity of the compounds for their biological domains through structure-activity relationship (SAR) investigations should be a major priority. Although, in vitro and in silico methods are the main focus of current research, thorough in vivo studies are necessary to assess their pharmacokinetics, toxicity, and therapeutic efficacy, opening the door for clinical translation. Thiosemicarbazones have the potential to promote medical and industrial improvements while addressing global health issues like cancer, antiviral resistance, and antibiotic resistance. In the future, the creation of analytical reagents for a variety of applications may be greatly aided by the inexpensive cost of thiosemicarbazones and their simple preparation techniques.

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