Synthesis and characterisation of oxomolybdenum(V) and dioxomolybdenum(VI) complexes with Schiff base derived from isonicotinoylhydrazide

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Synthesis of some new oxomolybdenum(V) and dioxomolybdenum(VI) complexes with a Schiff base, 3-methoxysalicylaldehydeisonicotinoylhydrazone derived from 3-methoxysalicylaldehyde and isonicotinoylhydrazide are reported. The complexes have been characterized by elemental analyses, molar conductance, magnetic susceptibility data, IR, UV-vis, EPR, $^1$H NMR and FAB mass spectral studies. The physico-chemical studies and spectral data indicate that the ligand acts as a monovalent tridentate chelating agent. The FAB mass and X-band EPR spectra indicate that the pentavalent Mo in the complex $[\text{MoO(MSINH)Cl}_2]$ is monomeric in nature. The X-ray diffraction studies of $[\text{MoO(MSINH)Cl}_2]$ correspond to orthorhombic crystal lattice with unit cell dimensions: $a = 8.043\text{Å}$, $b = 12.49\text{Å}$ and $c = 14.936\text{Å}$. All the complexes are found to be neutral with distorted octahedral geometry. The thermal properties of the complex $[\text{MoO(MSINH)Cl}_2]$ have been investigated by thermogravimetric techniques. The ligand, $[\text{MoO(MSINH)Cl}_2]$ and $[\text{MoO}_2(\text{MSINH})\text{Cl}]$ have been screened for their in vitro anticancer and antibacterial activity. The complex, $[\text{MoO(MSINH)Cl}_2]$, exhibits much higher activity than the ligand (MSINH) and its dioxocomplex, $[\text{MoO}_2(\text{MSINH})\text{Cl}]$.

Keywords: Coordination chemistry, Molybdenum, Schiff bases, Anticancer activity, Antibacterial activity

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Molybdenum is a versatile transition metal with a large number of stable and accessible oxidation states. Within the second series of transition metals only molybdenum represents a biometal, important for microorganisms, plants and animals. The increasing biological applications, namely antibacterial, antifungal, antitubercular, antitumour activities, etc., of the complexes of transition metals with hydrazones have intensified interest in research and analytical studies on these metallic complexes. Higher biological activity compared to the parental hydrazone has frequently been thought to be due to their ability to chelate trace metals. This enables their application as antitumor, antifungal, antibacterial and antitubercular drugs.

Studies on complexes of oxomolybdenum(V) and dioxomolybdenum(VI) have opened up a new vista of research and analysis of uncharted biochemical significance. We report herein the synthesis and characterization of some new complexes of oxomolybdenum(V) and dioxomolybdenum(VI) with a tridentate Schiff base, 3-methoxysalicylaldehyde isonicotinoylhydrazone (MSINH) derived from 3-methoxysalicylaldehyde and isonicotinoylhydrazide. During our investigation, various spectral (IR, UV-vis, NMR, FAB mass and EPR) methods have been used. The X-ray diffraction patterns, thermal behaviour, anticancer and antibacterial activities of some selected complexes have also been studied.

Materials and Methods
Molybdenum pentachloride (Alfa Aesar, Lancaster) and molybdenum trioxide (Loba Chemie, Mumbai, India) were used. All other chemicals were of AR grade.

Metal and chloride were estimated by standard methods. The elemental analyses (C, H, N and S) were carried out at the Sophisticated Test and Instrumentation Center (STIC), Kochi. The IR spectra of ligand and complexes were recorded in the region 4000-400 cm$^{-1}$ on a Perkin-Elmer 397 spectrophotometer. Room temperature molar conductance of the complexes in DMF was recorded on an Elico direct reading conductivity meter at a concentration of $\sim 10^{-3} M$. Electronic absorption spectral measurements of the complexes in methanol were measured using Jasco-V-550-UV-vis spectrophotometer. $^1$H NMR spectra of the ligand and the complexes were recorded on a 300 MHz FT NMR instrument using TMS as reference. Thermal analysis of one of the complex
[MoO(MSINH)Cl₂] was carried out by heating in air at a rate of 10 °C per minute on a Mettler TG-50 thermo balance. X-ray powder diffraction pattern was recorded using a Philips X-ray PW1710 diffractometer. The FAB mass spectrum of [MoO(MSINH)Cl₂] was recorded on a Jeol JMS600H mass spectrometer. The X-Q band EPR spectrum was recorded at room temperature on a Varian E-112 spectrophotometer with DPPH as the standard, at RSIC, Chennai. The magnetic susceptibilities were recorded at room temperature by Gouy method. Diamagnetic corrections for various atoms and structural units were computed using Pascal’s constants⁹.

Synthesis of 3-methoxysalicylaldehyde isonicotinoylhydrazone (MSINH)

The Schiff base, MSINH (C₆H₄N₂O₃) (I) was prepared by mixing equimolar solutions of 3-methoxysalicylaldehyde and isonicotinoylhydrazone in methanol and refluxing the mixture for ~30 min. The pale yellow solid separated was filtered, washed with methanol and dried. Purity of the ligand was monitored by TLC. It was characterized by elemental analysis, IR, UV and NMR spectra.

![Schiff base structure](image)

(I)

Synthesis of oxomolybdenum (V) complexes

The chloride complex was prepared by adding a methanolic solution of MoCl₃ (2 mmol) in small quantities with stirring to a hot methanolic solution of the ligand (2 mmol) in methanol. The pH of the mixture was adjusted to ~ 4 with NaOAc/HOAc buffer and stirring was continued for ~10-15 min. The solid complex which separated out was suction filtered, washed first with aqueous methanol and finally with ether and dried over P₂O₅ in vacuo.

The thiocyanate complex was prepared⁷ by adding a methanolic solution of MoCl₃ (2 mmol) containing ~ 0.5 g of NH₄CNS to a hot methanolic solution of the ligand (2 mmol). The pH of the mixture was adjusted to ~4 with NaOAc/HOAc buffer. The complex was precipitated on stirring the solution using a magnetic stirrer at 40 °C for ~ 2 h. The precipitated complex was suction filtered, washed with aqueous methanol (1:1) followed by dry ether and dried over P₂O₅ in vacuo.

Synthesis of dioxomolybdenum(VI) complexes

MoO₃ (1 mmol) was dissolved in minimum amount of hot conc HCl (2 ml). It was then added to the ligand (1 mmol) solution in methanol with constant stirring. The solid which separated out immediately was suction filtered, washed with aqueous methanol, then with ether and dried over P₂O₅ in vacuo. The method adopted for the preparation of the thiocyanate complex was the same as that adopted for the preparation of oxomolybdenum(V) complexes.

Biological activity of the compounds

Anticancer screening was done at the Regional Cancer Center, Thiruvananthapuram by MTT assay and antibacterial screening by disc diffusion method. Cytotoxicity was measured on human cervical cancer cell lines (HeLa) using the assay MTT.

The ligand (MSINH) and the complexes [MoO(MSINH)Cl₂] and [Mo₂(MSINH)Cl₄] were screened in vitro for their possible antibacterial activities using the disc diffusion diffusion method¹⁰ (Kirby Bauer method) For the pathogenic bacteria, viz., Salmonella typhi MTCC 734, Pseudomonas aeruginosa MTCC 2642, Escherichia coli 585, Proteus vulgaris 177, Bacillus subtilis 2248 and Streptococcus thermophilus 1938.

Results and Discussion

The chemical analyses data and physical properties of the ligand and their complexes are given in (Table I). All the complexes are coloured, non-hygroscopic solids and stable in air. They are sparingly soluble in common organic solvents like acetone, chloroform, acetonitrile and completely soluble in ethanol, methanol, DMF and DMSO.

The molar conductance values of 10⁻³ M solutions of complexes lie in the range 45-56 Ω⁻¹ cm² mol⁻¹ in dry DMF indicate their non electrolyte behaviour¹¹. All the oxocomplexes show magnetic moment values in the range of 1.66-1.78 BM, which corresponds to spin-only value (1.73 BM) expected for oxomolybdenum(V) complexes showing the absence of Mo-Mo interaction¹². All the dioxomolybdenum(VI) complexes are found to be diamagnetic as expected for a d⁰ system. The results show that the ligand coordinates to the metal ion in 1:1 ratio and suggest that the proposed formulas are [MoO(MSINH)ClX] and [MoO₂(MSINH)X], where X = Cl, NCS.

Important infrared spectral bands of the ligand and complexes and their tentative assignments are given in Table 2. IR spectra of the ligand and their complexes are given in (Table I). All the complexes are coloured, non-hygroscopic solids and stable in air. They are sparingly soluble in common organic solvents like acetone, chloroform, acetonitrile and completely soluble in ethanol, methanol, DMF and DMSO.

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Important infrared spectral bands of the ligand and complexes and their tentative assignments are given in Table 2. IR spectra of the ligand and its complexes are quite complex due to the presence of various ring
The metal complexes have been assigned to repulsive forces in the adjacent nitrogen atoms. The spectra of the complexes confirming coordination shift of azomethine nitrogen atom is further supported by the ligand to higher frequency by ~30 cm\(^{-1}\). The coordination through the carbonyl oxygen of the intense ligand band in all the complexes. In the complexes this band remains unaltered. This suggests non-participation of the NH group in bonding. The infrared spectrum of the free ligand exhibits bands at 3438 cm\(^{-1}\) due to phenolic OH. These bands are absent in the spectra of the complexes, indicating the deprotonation of phenolic OH. The Mo–O stretch of the complexes displays two Mo=O stretches at ~940-960 cm\(^{-1}\) and ~898-920 cm\(^{-1}\) due to symmetric and antisymmetric stretching of cis-MoO\(^{2+}\) core. The MoO\(_2\) prefers to form a cis configuration due to maximum utilization of the \(d\alpha\) groups. A very strong band observed at ~940 cm\(^{-1}\) in the spectra of oxomolybdenum(V) complexes corresponds to Mo=O stretching frequency. The N-coordinated \(\mu\)-sulfido Mo=O core and \(\sigma\)-thiocyanate group is characteristic of phenolic OH and \(\delta\)-thiocyanate (~490 cm\(^{-1}\)) bands.

The \(^1\)H NMR spectra of (MSINH) and [MoO\(_2\)(MSINH)Cl] were recorded in DMSO-\(d_6\). \(^1\)H NMR spectrum of the free ligand shows signal due to OH at \(\delta\) (12.27) characteristic of phenolic OH proton which is absent in the complex, suggesting coordination through deprotonated phenolic oxygen. Signal due to NH at \(\delta\) (10.74) in the free ligand appears at 10.5 in the complex. Presence of NH proton in the complex indicates that the ligand exists in the keto form. A singlet at \(\delta\) (8.7) observed in the spectrum for the free ligand shows downfield shift to \(\delta\) (9.05) indicating coordination of azomethine nitrogen in the complexes, due to the reduction of electron density at the azomethine C-H. The methoxy protons of the ligand and the complex appear at \(\delta\) (3.97). The seven aromatic protons appear as vibrations and C-H absorptions. A broad band centered at 3201 cm\(^{-1}\) in the free ligand can be assigned to the \(\nu_{\text{NH}}\) stretching. In the complexes this band remains unaltered. This suggests non-participation of the NH group in bonding. The infrared spectrum of the free ligand exhibits bands at 3438 cm\(^{-1}\) and 1353 cm\(^{-1}\) due to phenolic OH. These bands are absent in the spectra of the complexes, indicating the deprotonation of phenolic OH on coordination with metal ions. This is further supported by the shifting of the intense ligand band at 1317 cm\(^{-1}\) due to phenolic C–O to ~1340 cm\(^{-1}\). The \(\nu_{\text{C=O}}\) observed at 1670 cm\(^{-1}\) in the spectrum of the ligand shows a downward shift by ~30 cm\(^{-1}\) in all the complexes indicating coordination through the carbonyl oxygen. The vibrational band at 1626 cm\(^{-1}\) assigned to the azomethine nitrogen atom is further supported by the shifting of \(\nu_{\text{NH}}\) vibration observed at 998 cm\(^{-1}\) in the ligand to higher frequency by ~20 cm\(^{-1}\) in the complexes. It is due to the reduction of lone pair repulsive forces in the adjacent nitrogen atoms.

The new weak bands at ~500 cm\(^{-1}\) and ~430 cm\(^{-1}\) in the metal complexes have been assigned to \(\nu_{\text{Mo–N}}\) and \(\nu_{\text{Mo=O}}\) modes respectively. Dioxomolybdenum(VI)
multiplets within the range $\delta$ (8.8 – 8.96) (isonicotinic 4H) and $\delta$ (7.04 – 6.83) (phenyl 3H) for the ligand and dioxomolybdenum complex. The sharp signal found as a singlet at $\delta$ (2.49) may be due to the water present in DMSO-$d_6$ sample used.

Electronic spectra of tridentate ONO donor hydrazone ligand and the oxomolybdenum(V) complexes were recorded in methanol. The spectra of the complexes with tridentate ligand are similar to each other, thereby suggesting a uniform structure for all. A moderately intense band observed in the region ~360 – 377 nm is attributed to O($\pi$) $\rightarrow$ d (Mo).

The band due to the transition $^2$B$_2$ $\rightarrow$ $^2$A$_1$ ($d_{xy} \rightarrow d_z^2$) is probably masked by the above bands. The complexes exhibit two more bands, a medium intensity band ~ 234 – 280 nm and ~ 668 – 678 nm assigned to $^2$B$_2$ $\rightarrow$ $^2$B$_1$ ($d_{xy} \rightarrow d_{z^2}$) and $^2$B$_2$ $\rightarrow$ $^2$E$_1$ ($d_{xy} \rightarrow d_{xz}, d_{yz}$) transitions respectively (the unpaired electron is in the $d_{xy}$ orbital). The electronic spectra of these complexes indicate octahedral geometry with a strong tetragonal distortion resulting from Mo=O bond. The bands appearing below 360 nm are due to $\pi \rightarrow \pi^*$, n $\rightarrow$ $\pi^*$ and intra ligand transition.

The X-band EPR spectrum (Fig. 1) of [MoO(MSINH)Cl$_2$] recorded in the polycrystalline form at room temperature is characterized by only a single line with unresolved parallel and perpendicular components. The EPR parameters were found to be $g_\| = 1.9355$, $g_\perp = 1.9049$ and $g_{av} = 1.9151$. The calculated $g_{av}$ value indicates that the complex is monomeric with molybdenum in the pentavalent state.

The complex [MoO(MSINH)Cl$_2$] was found to be orthorhombic by X-ray powder diffraction method and was indexed (Fig. 2) using Hesse and Lipson’s procedure. The lattice constants were found to be as follows: $a = 8.043$ Å, $b = 12.49$ Å and $c = 14.936$ Å

Thermal behaviour of the complex [MoO(MSINH)Cl$_2$] was studied by TGA in air at a heating rate of 10 °C per min in conjunction with DTG. (Fig. 3). Thermal decomposition data and kinetic parameters are given in Table 3. The complex is stable till 190 °C with total decomposition of the complex at 550 °C in three stages as denoted by the DTG peaks at 237 °C, 302 °C and 465 °C. First mass loss of 7.72 % corresponds to the elimination of one chlorine atom. Monodentate ligand trans to oxo oxygen is weakly bound to molybdenum and are known to undergo cleavage on heating. The second mass loss of 29.4 % corresponds to the loss of a part of the ligand, C$_6$H$_5$N$_2$O. Finally, a mass loss of 51% has been ascribed to the oxidative decomposition of the remaining part of the complex to give MoO$_3$ as the ultimate residue. The kinetic and thermodynamic parameters were calculated using Coats-Redfern equation.

The FAB mass spectrum of [MoO(MSINH)Cl$_2$] shows a molecular ion peak (M$^+$) at $m/z$ 452.91, suggesting the complex to be monomeric. Fragmentation of the complex occurred giving a series of peaks at $m/z$ 418.29, 382.91, 331.91, 303.97, 270.09,
especially at lower concentrations. The results show IC
concentration dependant as the dioxocomplex,

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and 382.9 [M-Cl-Cl] suggest the presence of two
chlorine atom in the complex.

The in vitro anticancer study was undertaken to
ascertain the effects of (MSINH), [MoO(MSINH)Cl
human cervix carcinoma cell lines (HeLa). The results indicate
that all the samples exhibit cytotoxic activity. The most
outstanding results were obtained from compound
[MoO(MSINH)Cl], which shows higher cytotoxic
activity against the cell line in a dose-dependent manner.
The anticancer activity of MSINH is not as much
concentration dependant as the dioxocomplex,
especially at lower concentrations. The results show IC₅₀
values of 258 for MSINH, 87 for [MoO(MSINH)Cl] and
295 for [MoO₂(MSINH)Cl]. Low IC₅₀ value indicates that oxomolybdenum complex has high
anticancer activity against the tumor cell lines evaluated.

The cell growth inhibition assays represent the
standard criterion for screening of the antitumor
compounds. However, this approach does not give direct
information on the mechanism of action of the individual
compound, but it may be reasonably concluded that these substances behave differently on
the cells under study probably because they act with
different mechanisms. It may be due to inhibition of
DNA replication and transcription due to distortion of
helical structure resulting from the binding of complex
to DNA. Mo(V) complexes are far more reactive than
Mo(VI) and possibly the anticancer activity of Mo(VI)
involves initial reduction to Mo(V) at the tumour site,
promoted by the altered physicochemical environment
in tumor cells.

Antitumour activity of complexes may also be due to
their ability to produce elevated amounts of damaging
reactive oxygen species (ROS) which can disturb the
redox balance of a system leading to an increase in
DNA damage, DNA-protein crosslink formation, lipid
peroxidation, cellular toxicity, and/or the inappropriate
activation of cellular signalling pathways. It may also
be due to factors like induction of apoptosis or
lipophilicity. Therefore, it is likely that the molecular mechanism of oxomolybdenum mediated
cytotoxicity is different from others, may be due to
difference in metal centers as well as their
coordination sphere surroundings. More experiments
are required to resolve the question of mechanism of
action of these complexes on cancerous and normal
cells and to establish the path ways of cell death,
which are in progress now.

The antibacterial studies reveal that
[MoO(MSINH)Cl] shows moderate activity against
gram positive pathogenic bacteria Strep-tococcus thermophilus (17 mm), Bacillus subtilis
(15 mm). Proteus vulgaris (17 mm), Escherichia coli
(12 mm) and Salmonella typhi (11 mm). The complex
[MoO₂(MSINH)Cl] shows moderate activity against
Proteus vulgaris (14 mm). It also shows trace activity
against Escherichia coli and no activity against all
other bacteria. All the tested compounds show no
activity towards Pseudomonas aeruginosa. The
results reveal that the complexes show higher activity
whereas the ligand (MSINH) is inactive against the
tested pathogenic bacteria. Antibacterial activity of
Mo(V) complexes varies in the following order:
Streptococcus thermophilus/Proteus vulgaris >
Bacillus subtilis> Escherichia coli > Salmonella typhi.

The increased lipophilic character of these
complexes seems to be responsible for their enhanced
potent antibacterial activity. It can be considered
that these compounds deactivate various cellular
enzymes, which plays a vital role in various metabolic
pathways of these microorganisms. It can also be
inferred that the ultimate action of the complex is
denaturation of one or more proteins of the cell,
which as a result, impairs the normal cellular
processes.

On the basis of all the above spectral and
distorted octahedral geometry (II & III) has been tentatively proposed for
three stages of thermal decomposition of [MoO(MSINH)Cl]

<table>
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<th>Stage</th>
<th>Decomp. temp. range (K)</th>
<th>Mass loss (%)</th>
<th>E (kJ mol⁻¹)</th>
<th>A (S⁻¹)</th>
<th>∆S (JK⁻¹mol⁻¹)</th>
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<td>51</td>
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214.89, 121.1, 149, 111, etc., corresponding to various
fragments. Presence of peaks at m/z = 418.29 [M-Cl]⁺ and 382.9 [M-Cl-Cl]⁺ suggests the presence of two
chlorine atom in the complex.

On the basis of all the above spectral and
physicochemical studies, a distorted octahedral geometry (II & III) has been tentatively proposed for
all the complexes. The computer models of the
proposed configurations of above complexes have
been constructed using the software Hyperchem7.5.
The geometrical optimizations of the structures obtained are presented in Figs 4 & 5.

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References