

Synthesis, Characterization and anti-tubercular activity of substituted thiosemicarbazones and their Ni(II) complexes

Qurat Ul Ain[#], Sheikh Insha[#], Rekha Sharma^{#*}

[#]School of Chemical Engineering and Physical Sciences, Lovely Professional University, Phagwara, Punjab, India

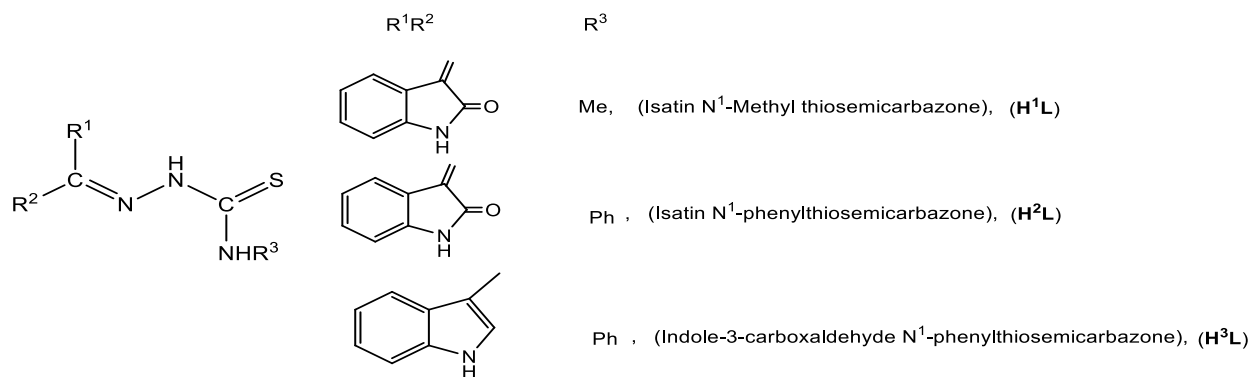
Abstract

Complexes of substituted isatin-3-thiosemicarbazones ($H_2itsc-N^1-Me, H^1L$; $H_2itsc-N^1-Ph, H^2L$) and substituted indole-3-thiosemicarbazone ($HIntsc-N^1-Ph, H^3L$) with Ni (II) and triphenylphosphine of formula, $[NiCl(L)(Ph_3P)]$ (anionic form of $L = {}^1L, 1; {}^2L, 2, {}^3L, 3$) have been synthesized. The ligands and complexes have been characterized using spectroscopic techniques (IR, 1H NMR). 1HNMR of complexes supports binding of thio- ligands in anionic form. All the ligands ($H^1L - H^3L$) and their complexes were evaluated for their antitubercular activities. The enhancement in anti-TB activity of ligands on complexation with Ni(II) has been observed.

Keywords: *isatin-3-thiosemicarbazone, indole, triphenylphosphine, anti-tubercular activity*

Introduction

Chemistry of thiosemicarbazones, a class of N, S- donor ligands has gained a lot of attention due to interesting bonding behavior, structural diversity, analytical and biological applications [1-19]. It has been observed that biological activities of thio- ligands get enhanced on complexation with transition metals by providing binding site to the anionic ligands [20]. Ni-thiosemicarbazone complexes are one of such examples [21-24]. A lot of work has already been done on Ni-thiosemicarbazone complexes, but complexes with additional co-ligands like Ph_3P , pyridine or bi-pyridine are less explored [25-29]. In present research work, synthesis and characterization of Ni(II) complexes of substituted isatin-3-thiosemicarbazones ($H_2itsc-N^1-Me, H^1L$; $H_2itsc-N^1-Ph, H^2L$) and N^1 -phenyl indole-3-thiosemicarbazone ($HIntsc-N^1-Ph, H^3L$) (Scheme 1) in the presence of Ph_3P is reported. Antitubercular activities of ligands and their complexes have been evaluated.



Experimental

Materials and methods

Nickel chloride and triphenyl phosphine were purchased from Loba Pvt.Ltd, where as thiosemicarbazide, N-phenyl thiosemicarbazide, N-methyl thiosemicarbazide, isatin and indole-3-carboxaldehyde were purchased from sigma Aldrich chemicals Ltd and used without further purification. AR Grade solvents used were purchased from sigma-Aldrich chemicals Ltd and Calibochem Ltd. The melting point of synthesized ligands and their complexes were determined with a lab fit electrically heated apparatus. Infrared spectra were recorded from KBr pellets in the range 4000-200 cm^{-1} on a SHIMADZU FTIR 8400s spectrophotometer (Department of Chemistry, Lovely Professional University). ^1H NMR were recorded on a Bruker Advance II spectrometer operating at a frequency of 400 MHz using d^6 -dmsO as a solvent with TMS as the internal standard (NMR LAB SAIF, P.U.CHD). Antitubercular activities of synthesized ligands and their complexes were checked from Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum, Karnataka.

Experimental:

Synthesis of isatin- N^1 -methyl thiosemicarbazone ($\text{H}_2\text{itsc-N}^1\text{-Me}$, H^1L): To a solution of N^1 -methyl thiosemicarbazide (1 g, 0.01 mmol) was dissolved in 60 ml methanol. To it was added isatin (1.61 g, 0.010 mmol), and reaction mixture was refluxed for 6-7 hours. A yellow coloured clear solution thus formed was filtered and kept for crystallization. After two days yellow coloured needles were formed. (Yield, 80%, m. pt. 220-222 $^\circ\text{C}$). Main IR peaks (KBr, cm^{-1}) $\nu_{\text{as}}(\text{N-H})$, 3418m; $\nu_{\text{s}}(\text{N-H})$, 3260m; $\nu(-\text{NH}-)$, 3163m; $\nu(\text{C=O})$, 1670s; $\nu(\text{C=N})$, 1589s; $\nu(\text{C=C})$, 1460s; $\nu(\text{C=S})$, 852s. ^1H NMR (δ , ppm, d^6 -dmsO) 12.60s (1H, $-\text{N}^2\text{H}-$), 11.22s (1H, N^4H , Pyrrole), 9.26s (1H, N^1H), 7.64s (1H, C^5H), 7.37s (1H, C^6H), 7.11s (1H, C^7H), 6.94s (1H, C^8H), 3.17m (3H, CH_3).

Synthesis of isatin- N^1 -methyl thiosemicarbazone ($\text{H}_2\text{itsc-N}^1\text{-Ph}$, H^2L): To a solution of N-phenylthiosemicarbazide (1g, 5.97 mmol) in 60 ml of methanol was added isatin (0.87g, 5.97 mmol). The mixture was refluxed for 6-7 hours. Dark yellow coloured solution thus formed was filtered and kept for crystallization. After two days, yellow coloured needles were formed than

filtered and dried in vacuo. (Yield, 75%, m.pt.198-200°C). Main IR peaks (KBr, cm^{-1}) $\nu(\text{N-H})$, 3433m; $\nu(-\text{NH-})$, 3117m; $\nu(\text{C-H})$, 3059m; $\nu(\text{C=O})$, 1687s; $\nu(\text{C=N})$, 1593s; $\nu(\text{C=C})$, 1541s; $\nu(\text{C=S})$, 792s. $^1\text{H NMR}$ (δ , ppm, d^6 -dmsO) 12.81s (1H, $-\text{N}^2\text{H-}$), 10.84s (1H, N^4H , Pyrrole), 7.78s (1H, N^1H), 7.43t (2H, $\text{C}^{5,6}\text{H}$, $J = 6\text{Hz}$), 7.68-7.11(6H, ring proton).

Synthesis of indole-3-carboxaldehyde- N^1 -phenyl thiosemicarbazone (**Hitsc- N^1 -Ph**, **H³L**): To a solution of N-phenylthiosemicarbazide (1g, 5.97 mmol) in 60 ml of methanol was added indole-3-carboxaldehyde (0.868g, 5.97 mmol). The mixture was refluxed for 6-7 hours. Brown coloured solution thus formed was filtered and kept for crystallization. After two days, brown coloured needles were formed. Needles were filtered and dried in vacuo.(Yield, 75%, m.pt.165-167°C). Main IR peaks (KBr, cm^{-1}) $\nu_{\text{as}}(\text{N-H})$, 3404m; $\nu_{\text{s}}(\text{N-H})$, 3313m; $\nu(-\text{NH-})$, 3107m; $\nu(\text{C=N})$, 1657s; $\nu(\text{C=C})$, 1597s; $\nu(\text{C=S})$, 804s. $^1\text{H NMR}$ (δ , ppm, d^6 -dmsO) 11.71s (1H, N^4H); 11.62s (1H, N^2H); 8.42s (N^1H); 9.63s (1H, C^2H); 8.24d (2H, $\text{C}^{6,9}\text{H}$, $J = 8\text{Hz}$); 7.66d (2H, $\text{C}^{7,8}\text{H}$, $J = 8\text{Hz}$); 7.15-7.46m (5H, phenyl ring).

Synthesis of $[\text{NiCl}(\text{Hitsc-}\text{N}^1\text{-Me})\text{Ph}_3\text{P}]$ **1**. Isatin N-Methyl thiosemicarbazone (0.050g, 0.198 mmol) was dissolved in 30 ml of ethanol. To it was added, $\text{NiCl}_2(\text{PPh}_3)_2$ (0.139g, 0.212mmol) and reaction mixture was refluxed for 2-3 hours. Clear solution thus formed was kept for crystallization. Dark red compound was obtained after evaporation. (Yield, 75%, m.pt. 240-244°C). Main IR peaks (KBr, cm^{-1}) $\nu(\text{N-H})$, 3396m; $\nu(\text{C-H})$, 2818m; $\nu(\text{C=N})$, 1682s; $\nu(\text{C=C})$, 1551s; $\nu(\text{C=S})$, 797s. $^1\text{H NMR}$ (δ , ppm, d^6 -dmsO) 11.3s (1H, N^4H , Pyrrole); 9.26s (2H, N^1H_2); 7.65-6.93m (19H, Ring proton); 3.09m (3H, CH_3).

Synthesis of $[\text{NiCl}(\text{Hitsc-}\text{N}^1\text{-Ph})\text{Ph}_3\text{P}]$ **2**. Isatin N-Phenyl thiosemicarbazone (0.050g, 0.159 mmol) was dissolved in 30 ml of ethanol. To it was added, $\text{NiCl}_2(\text{PPh}_3)_2$ (0.12g, 0.183 mmol) and reaction mixture was refluxed for 2-3 hours. Clear solution thus formed was kept for crystallization. Dark red coloured compound formed after evaporation.(Yield, 71%, m.pt.256-258°C) Main IR peaks (KBr, cm^{-1}) $\nu(\text{N-H})$, 3383m; $\nu(\text{C-H})$, 3061m; $\nu(\text{C=N})$, 1664s; $\nu(\text{C=C})$, 1232s; $\nu(\text{C=S})$, 742s. $^1\text{H NMR}$ (δ , ppm, d^6 -dmsO) 9.605s (1H, N^1H), 7.55-7.40 (23H, ring protons)

$[\text{NiCl}(\text{Intsc-}\text{N}^1\text{-Ph})\text{Ph}_3\text{P}]$ **3**. Indole 3 carboxaldehyde N-Phenyl thiosemicarbazone (0.050g, 0.235 mmol) was dissolved in 30 ml of ethanol. To it was added, $\text{NiCl}_2(\text{PPh}_3)_2$ (0.11g, 0.168 mmol) and reaction mixture was refluxed for 2-3 hours. Clear solution thus formed was kept for crystallization. Green coloured compound formed after evaporation. (Yield, 75%, m.pt.222-225°C) Main IR peaks (KBr, cm^{-1}) $\nu(\text{N-H})$, 3228m; $\nu(\text{C-H})$, 2985m; $\nu(\text{C=N})$, 1560s; $\nu(\text{C=C})$, 1508s; $\nu(\text{C=S})$, 752s. $^1\text{H NMR}$ (δ , ppm, d^6 -dmsO) 12.15s (1H, N^4H); 7.91s (1H, C^2H); 7.47-7.04m (18H, ring proton + N^1H_2).

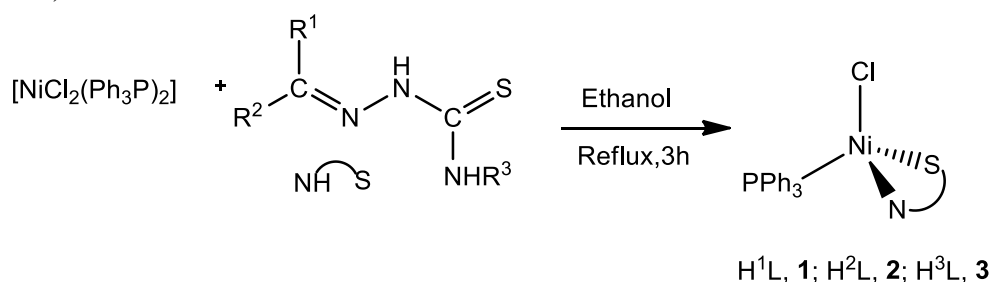
Anti tuberculosis activity:

The anti-mycobacterial activity of the selected (ligands and their complexes) were assessed against M.tuberculosis using microplate Alamar Blue assay (MABA)[24]. This method used is non-toxic, use a thermally stable reagent and shows good correlation with comparative and BACTEC radiometric methods. Briefly, 200 μl of sterile de-ionized water was added to all outer

perimeter wells of a sterile 96 wells plate to minimized evaporation of the medium in the test wells during incubation. The 96 wells plate received 100 μl of the Middlebrooks 7H9 broth and serial dilution of compounds was carried out directly on the plate. The final drug concentrations tested were 100 to 0.2 $\mu\text{g/ml}$. Each test was carried out in triplicate. Plates were covered and sealed with para film and incubated at 37°C for five days in sealed plastic bags with a 5% CO_2 atmosphere. After this time, 25 μl of a freshly prepared 1:1 mixture of Almar Blue reagent and 10% between 80 was added to the plate and incubate for 24 hours. A blue shade in the well was interpreted as no bacterial growth, and a pink color was scord as growth. Pyrazinamide, Ciprofloxin and streptomycin was incorporated as standard drugs. The acceptable range (MIC), of standard drugs are 3.125 $\mu\text{g/ml}$, 3.125 $\mu\text{g/ml}$ and 6.25 $\mu\text{g/ml}$ respectively.

Result and Discussion:

Reaction of N¹-substituted thiosemicarbazones (H^1L - H^3L) with $[\text{NiCl}_2(\text{Ph}_3\text{P})_2]$ in 1 : 1 molar ratio in ethanol yielded complexes of stiochiometry, $[\text{NiCl}(\text{Ph}_3\text{P})(\text{L})]$ ($\text{L} = {}^1\text{L}$, **1**; ${}^2\text{L}$, **2**; ${}^3\text{L}$, **3**) (Scheme 2).



Scheme 2

Discussion on IR

The important IR peaks of ligands (H^1L - H^3L) and their complexes (**1-3**) are given in table 1. The $\nu(\text{N-H})$ and $\nu(-\text{NH}-)$ vibrational modes appeared in the range 3433-3228 cm^{-1} and 3163-3107 cm^{-1} respectively in free ligands. Absence of $\nu(-\text{NH}-)$ band in the range, 3163-3107 cm^{-1} in complexes supports that deprotonation has taken place during complex formation [1]. Characteristic $\nu(\text{C}=\text{S})$ band in free ligands (852 cm^{-1} (H^1L), 792 cm^{-1} (H^2L), 804 cm^{-1} (H^3L)), showed a significance shift to lower energy in complexes, (797 cm^{-1} (**1**); 742 cm^{-1} (**2**); 752 cm^{-1} (**3**)). This low energy shift of $\nu(\text{C}=\text{S})$ band in complexes supports binding of ligand through thiolate sulfur ($\text{C}-\text{S}^-$) [1]. Coordination of triphenylphosphine ligand with the metal is confirmed by the presence of characteristic $\nu(\text{P}=\text{C})$ band at 1091 cm^{-1} (**1**), 1089 cm^{-1} (**2**) and 1099 cm^{-1} (**3**) [1].

Table 1. Important IR peaks of ligands (H^1L - H^3L) and complexes (**1-3**)

IR	$\nu(\text{N-H})$	$\nu(-\text{NH}-)$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{C})$	$\nu(\text{C}=\text{S})$	$\nu(\text{P}-\text{C}_{\text{Ph}})$
H^1L	3418m 3260m	3163s	1589s	1460s	852s	-
Complex 1	3396m	-	1682s	1551s	797s	1091
H^2L	3433m	3117	1593s	1541s	792s	-

Complex 2	3383m	-	1664s	1473s	742s	1089
H ³ L	3404m 3313m	3107m	1657s	1597s	804s	-
Complex 3	3228s	-	1560s	1508s	752s	1099

Discussion on ¹H NMR

The important ¹H NMR signals of ligands (H¹L-H³L) and complexes (1-3) are given in table 2. The N²H signal in ligands appeared in the range, δ 11.62-12.60 ppm. Absence of signal due to N²H in complexes ensured de-protonation of ligand during complexation and its binding as bidentate anionic chelating ligand. The N¹H signal in ligands in the range, δ 8.42-10.84 ppm showed upfield shift in complexes (δ 7.55-9.26 ppm 1-3) The N⁴H signal appeared in the range, δ 10.84-11.71 ppm in ligands and in the range, δ 9.60-12.15 ppm in complexes. The CH₃ signals in H¹L appeared at δ 3.17, In complex 1 appeared at δ 3.09 ppm. The ring protons of ligands and triphenylphosphine appeared in the range, δ 6.93-7.91 ppm respectively, which ensures coordination of triphenylphosphine to metal center.

Table 2. Important NMR signals of ligands (H¹L-H³L) and complexes (1-3)

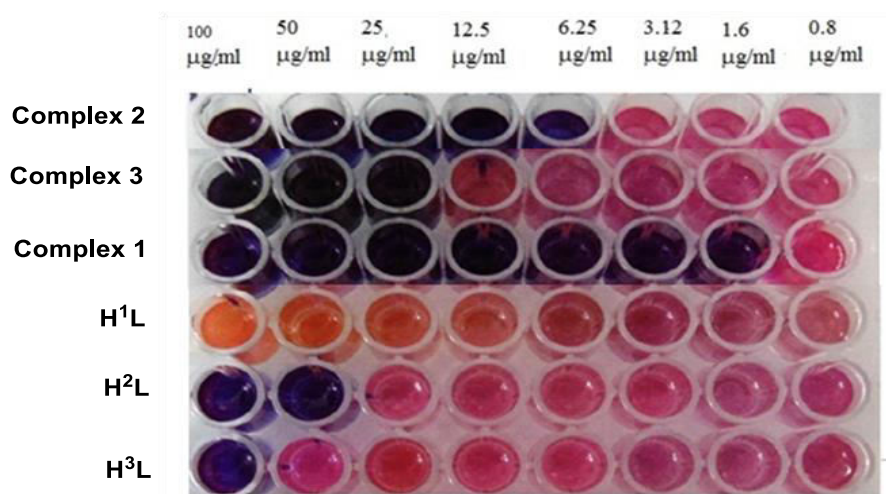
NMR	N ⁴ H	N ² H	N ¹ H	Ring protons	CH ₃
H ¹ L	11.22s	12.60s	9.26s	6.94-7.37	3.17m
Complex 1	11.30s	-	9.26s	7.65-6.93	3.09m
H ² L	10.84s	12.81s	7.78s	7.68-7.11	-
Complex 2	-	-	9.605s	7.55 -7.40	-
H ³ L	11.71s	11.62s	8.42s	7.46-7.15	-
Complex 3	12.15s	-	8.05s	7.47-7.04	-

Antituberculosis activity of ligands (H¹L-H³L) and Complexes (1-3):

Anti M.Tuberculosis activity of ligands and their complexes were evaluated against the M.Tuberculosis H37RV strain The Minimum Inhibitory Concentration (MIC) values of all the ligands and their complexes has given in table 4 and changes observed after activity were given in Figure 1. Ligands H¹L-H³L either show no activity (H²L) against M. Tuberculosis H37RV strain or show activity at high concentration (H¹L, H³L). However, the anti-TB activity of ligands get enhanced significantly on complexation with Ni(II). Complex 1 showed highest anti-TB activity (1.6 μ g/ml) amongst all the complexes. It is even more active than standard drugs Pyrazinamide (MIC, 3.125 μ g/ml), Ciprofloxacin (MIC, 3.125 μ g/ml) and streptomycin (MIC, 6.25 μ g/ml). Complexes 2 and 3 showed anti-TB activity at 12.5 μ g/ml 6.25 μ g/ml and 25 μ g/ml respectively.

Table 4. MIC values of ligands (H^1L - H^3L) and complexes (**1-3**)

Mycobacterium tuberculosis H37RV strain									
S. No	Compound	MIC ($\mu\text{g/mL}$)							
		100	50	25	12.5	6.25	3.12	1.6	0.8
1.	H^1L	S	R	R	R	R	R	R	R
2.	1	S	S	S	S	S	S	S	R
3.	H^2L	R	R	R	R	R	R	R	R
4.	2	S	S	S	S	S	R	R	R
5.	H^3L	S	R	R	R	R	R	R	R
6.	3	S	S	S	R	R	R	R	R

Figure 1. Well plates showing anti-TB activity of ligands (H^1L - H^3L) and complexes (**1-3**)

Conclusion

Absence of $\nu(-NH-)$ band in IR spectra of complexes supports deprotonation of ligand on complexation, which was further ensured by absence of N^2H signal in 1HNMR of complexes. The shift of characteristic $\nu(C=S)$ band to low energy in complexes **1-3** ($797-752\text{ cm}^{-1}$) vis-a-vis free ligands ($852-804\text{ cm}^{-1}$) indicates binding of ligand to metal center in thiolate ($C-S^-$) form. Presence and coordination of triphenylphosphine to metal centre in complexes has been supported by both IR and 1HNMR spectra of complexes. Complexes has shown significantly enhanced anti-TB activity vis-à-vis free ligands.

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