Synthesis and spectral characterization of bis(thiosemicarbazones)

2.1. Introduction

Heterocyclic thiosemicarbazones capable of tridentate coordination have been studied extensively [1-5], but heterocyclic bis(thiosemicarbazones), which are capable of higher denticity, have received less attention. This lack of attention is particularly true for those heterocyclic bis(thiosemicarbazones) with substituents other than NH$_2$ in the $N^d$-position of the thiosemicarbazone moiety. The coordinating ability of thiosemicarbazides to both transition and main group metallic cations is attributed to the extended delocalization of electron density over the thiosemicarbazone skeleton, which is enhanced by substitution at the $N^d$ position. Condensation of thiosemicarbazides with aromatic aldehydes or ketones extends the electron delocalization along the azomethine bond [6].

Transition metal complexes of bis(thiosemicarbazone) (btsc) ligands have been investigated as metallotherapeutics for a number of years. It has been reported that $\alpha$-diketone and $\alpha$-ketoaldehyde bis(thiosemicarbazones) and their metal complexes show antitumor activity [7, 8]. Since biological activity has been found to have a significant dependence on the thiosemicarbazone moiety's $N^d$-substituent [9], a series of bis(thiosemicarbazones) and their metal complexes were prepared and studied [10-13]. Spectral and biological studies have been carried out on metal complexes of 2,6-diacetylpyridine bis{$N^d$-substituted thiosemicarbazones} [14, 15]. Recent studies of metal complexes of tetradeutate bis(thiosemicarbazones) have included a number of structural [11-13, 16-17], as well as biological studies [18, 19]. However considerable
interest has been shown in 2,6-diacetylpyridine bis(thiosemicarbazone), and its metal complexes [14, 15].

Coordination chemistry of pentadentate 2,6-diacetylpyridine bis(thiosemicarbazone) Schiff base ligands has been intensively studied due to the versatility of the molecular chain in order to obtain very different geometries [20] as well as their broad therapeutic activity [21]. Metal complexes of thiosemicarbazones with aldehydes and ketones have been widely reported. But there have been fewer reports on potential pentadentate bis(thiosemicarbazones) formed from 2,6-diacetylpyridine [14, 15]. Keeping these in view, we have synthesized four bis(thiosemicarbazone) systems with 2,6-diacetylpyridine. The ligands synthesized are

- 2,6-diacetylpyridine bis (N⁺-phenylthiosemicarbazone) [H₂Ac₄Ph]
- 2,6-diacetylpyridine bis (N⁺-cyclohexylthiosemicarbazone) [H₂Ac₄Cy]
- 2,6-diacetylpyridine bis (N⁺-methylthiosemicarbazone) [H₂Ac₄Me]
- 2,6-diacetylpyridine bis (N⁺-ethylthiosemicarbazone) [H₂Ac₄Et]

Thus the ligands are diprotic and coordinate with metal centre both in keto form as well as in enolate form under neutral conditions. All the four ligands in spite of two protons, sometimes remained in monoanionic form upon coordination. This chapter deals with the synthesis and spectral characterization of ligands. The general structure and the numbering of the two thiosemicarbazones are given in Figure 2.1. This numbering pattern is used throughout the entire work.
Figure 2.1. Structures and numbering schemes of bis(thiosemicarbazones)
2.2. Experimental

2.2.1. Materials

2,6-Diacetylpyridine (Aldrich), hydrazine hydrate, $N^a$-phenyl isothiocyanate, $N^a$-cyclohexyl isothiocyanate, $N^a$-methylthiosemicarbazide, $N^a$-ethylthiosemicarbazide.

Solvents used.

(i) Ethanol: Commercially supplied ethanol, distilled and dried using standard methods and procedures.

(ii) Methanol: Analytical quality sample was used (Merck).

(iii) Dimethylformamide: Analytical quality was used.

(iv) Chloroform: Analytical quality was used.

2.2.2. Synthesis of Ligands

i) 2,6-Diacetylpyridine bis($N^a$-phenylthiosemicarbazone) [H$_2$Ac$_4$Ph]

Step-1:-

Preparation of $N^a$-phenylthiosemicarbazide:

Phenyl isothiocyanate (100 mmol, 11.94 ml) in 25 ml ethanol and hydrazine hydrate (100 mmol, 4.86 ml) in 25 ml methanol were mixed with constant stirring. The resulting solution was kept in a stirred condition for $\frac{1}{2}$ an hour. The white product, $N^a$-phenylthiosemicarbazide formed was filtered, washed with ethanol and ether and dried in vacuo over P$_4$O$_{10}$ (Scheme 2.1). m.p.=135 °C.
Step-2:-

_Synthesis of 2,6-diacetylpyridine bis(N<sup>4</sup>-phenylthiosemicarbazone) [H<sub>2</sub>Ac<sub>4</sub>Ph]_

To a hot solution of 0.836 g (5 mmol) of phenylthiosemicarbazide in 25 ml of ethanol, added 0.407 g (2.5 mmol) of 2,6-diacetylpyridine in 25 ml of ethanol with constant stirring. The above mixture was slowly refluxed for 5 hrs. After cooling, the compound obtained as pale yellow solids, which was filtered, washed with ethanol and dried _in vacuo_ over P<sub>4</sub>O<sub>10</sub> (Scheme 2.2).
ii) 2,6-diacetylpyridine bis(N\textsuperscript{4}-cyclohexylthiosemicarbazone) [H\textsubscript{2}Ac4Cy]

Step-1:-

*Preparation of N\textsuperscript{4}-cyclohexylthiosemicarbazide*

Cyclohexyl isothiocyanate (15 mmol, 2 ml) in 15 ml ethanol and hydrazine hydrate (90 mmol, 4.3 ml) in 15 ml ethanol were mixed with constant stirring. The resulting solution was kept in a stirred condition for \( \frac{1}{2} \) an hour. The white product, N\textsuperscript{4}-cyclohexylthiosemicarbazide formed was filtered, washed with ethanol and dried *in vacuo* over P\textsubscript{2}O\textsubscript{5} (Scheme 2.3). m.p=140 °C.

![Scheme 2.3]

Step-2:-

*Synthesis of 2,6-diacetylpyridine bis(N\textsuperscript{4}-cyclohexylthiosemicarbazone) [H\textsubscript{2}Ac4Cy]*

To a hot solution of 0.886 g (5 mmol) of N\textsuperscript{4}-cyclohexyl thiosemicarbazide in 25 ml of ethanol, added 0.407 g (2.5 mmol) of 2,6-diacetylpyridine in 25 ml of ethanol with constant stirring. Two drops of glacial acetic acid was added to the above solution and the mixture was slowly refluxed for 5 hrs. After cooling, the compound was obtained as yellow
solids, which was filtered, washed with ethanol and ether and dried in vacuo over P₄O₁₀ (Scheme 2.4).

![Chemical structure]

**Scheme 2.4**

iii) 2,6-diacetylpyridine bis(N⁴-methylthiosemicarbazone) [H₂Ac₄Me]

To a hot solution of 0.537 g (5 mmol) of N⁴-methylthiosemicarbazide in 25 ml of ethanol, added 0.407 g (2.5 mmol) of 2,6-diacetylpyridine in 25 ml of ethanol with constant stirring. Two drops of glacial acetic acid was added to the above solution and the mixture was slowly refluxed for 5 hrs. After cooling, the compound was obtained as pale yellow solid, which was filtered, washed with ethanol and ether and dried in vacuo over P₄O₁₀ (Scheme 2.5).
iv) 2,6-diacetylpyridine bis($N^4$-ethylthiosemicarbazone) [H$_2$Ac4Et]

To a hot solution of 0.595 g (5 mmol) of $N^4$-ethylthiosemicarbazide in 25 ml of ethanol, added 0.407 g (2.5 mmol) of 2,6-diacetylpyridine in 25 ml of ethanol with constant stirring. Two drops of glacial acetic acid was added to the above solution and the mixture was slowly refluxed for 4 hrs. After cooling, the compound was obtained as yellow solid, which was filtered, washed with ethanol and ether and dried in vacuo over P$_4$O$_{10}$ (Scheme 2.6).
2.3. Physical measurements

Elemental analyses were carried out using a Vario EL III CHNS analyzer at SAIF, Kochi, India. Infrared spectral analyses were done using KBr pellets on Thermo Nicolet AVATAR 370 DTGS FT-IR spectrophotometer. Electronic spectra were recorded on a UVD-3500, UV-vis Double Beam Spectrophotometer from a solution in DMF. $^1$H NMR spectra were recorded using Brucker AMX 400 FT-NMR Spectrometer using TMS as the internal standard at the Sophisticated Instrumentation Facility, Indian Institute of Science, Bangalore.

2.4. Results and discussion

Condensation reaction of 2,6-diacetylpyridine and $N^4$-substituted thiosemicarbazides in a molar ratio of 1:2 resulted in ligand systems 2,6-diacetylpyridine bis($N^4$-substitutedthiosemicarbazones). The stoichiometries, colors and elemental analyses data are presented in Table 2.1.

Table 2.1. Stoichiometries and partial elemental analyses of bis(thiosemicarbazones)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stoichiometry</th>
<th>Color</th>
<th>Composition (Found/Calcd) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>H$_2$Ac4Ph</td>
<td>C$<em>{33}$H$</em>{24}$N$_7$S$_2$</td>
<td>yellow</td>
<td>59.44 (59.84)</td>
</tr>
<tr>
<td>H$_2$Ac4Cy</td>
<td>C$<em>{33}$H$</em>{24}$N$_7$S$_2$</td>
<td>yellow</td>
<td>58.70 (58.32)</td>
</tr>
<tr>
<td>H$_2$Ac4Me</td>
<td>C$<em>{13}$H$</em>{10}$N$_7$S$_2$.H$_2$O</td>
<td>yellow</td>
<td>44.16 (43.92)</td>
</tr>
<tr>
<td>H$_2$Ac4Et</td>
<td>C$<em>{15}$H$</em>{22}$N$_7$S$_2$</td>
<td>yellow</td>
<td>49.05 (49.29)</td>
</tr>
</tbody>
</table>
2.4.1. IR spectral studies

The characteristic IR bands for the ligands (H₂Ac₄Ph, H₂Ac₄Cy, H₂Ac₄Me and H₂Ac₄Et) recorded as KBr discs are listed in Table 2.2.

H₂Ac₄Ph: IR spectral analysis confirms the presence of characteristic groups present in the compound. Bands at 3306 and 3220 cm⁻¹ are assigned to ν(4N-H) and ν(2N-H). The thiocarbonyl groups show stretching and bending vibrations at 1322 and 808 cm⁻¹, while additional bands in the broad region of 1500–700 cm⁻¹ are due to vibrations involving interactions between C=S stretching and C–N stretching of the C=S groups attached to a nitrogen atom [22]. Absence of any bands in the range 2500–2800 cm⁻¹ points towards the lack of –SH stretching absorptions in the molecule. It reveals the presence of only the thione tautomer in the solid state [23]. The azomethine stretching vibrations, C=Nₐzo, characteristic of a Schiff base is observed at 1597 cm⁻¹ [24-26]. Medium band observed at 1028 cm⁻¹, assigned for hydrazinic (N–N) bonds. Aromatic and heteroaromatic compounds display strong out-of-plane C–H bending and ring bending absorption bands in the 900-650 cm⁻¹ region (Figure 2.2).

H₂Ac₄Cy: The bands at 3296 and 3259 cm⁻¹ are assigned to N–H stretching vibrations at N(4) and N(2) respectively. Thiocarbonyl vibrations are observed at 1248 and 809 cm⁻¹ and azomethine vibrations are at 1596 cm⁻¹. The band at 1102 is assigned to (N–N) bonds. The –CH stretching vibrations of the cyclohexyl moiety are observed as two sharp bands at 2930 and 2849 cm⁻¹. These bands confirm the existence of cyclohexyl ring in the molecular structure (Figure 2.3).

H₂Ac₄Me: In the IR spectrum of the ligand H₂Ac₄Me, it is seen that the two N–H bands are very close to each other and give absorptions at 3335
and 3228 cm$^{-1}$. Band assignable to $v$(C=Nazo), is observed at 1549 cm$^{-1}$. Medium peak observed at 1045 cm$^{-1}$ corresponds to $v$(N–N) band. The thiocarbonyl group shows stretching and bending vibrations at 1355 and 869 cm$^{-1}$. Absence of any bands in the 2800–2550 cm$^{-1}$ region reveals the presence of only the thione tautomer in the solid state, as they imply the lack of –SH stretching absorptions in the molecule (Figure 2.4).

$H_2Ac4Et$: IR spectrum of the ligand $H_2Ac4Et$ shows bands at 3373 and 3328 cm$^{-1}$ corresponds to $v(^4N–H)$ and $v(^2N–H)$ respectively. The $v$(CS) and $\delta$(CS) vibrations are observed at 1307 and 857 cm$^{-1}$ and $v$(C=N) vibration is observed at 1541 cm$^{-1}$. A medium intensity band at 1050 cm$^{-1}$ assigned to $v$(N–N) vibration. The absence of $v$(S–H) band around 2600 cm$^{-1}$ suggests the existence of the bis(thiosemicarbazone) in the thione form (Figure 2.5). The 1600-1400 cm$^{-1}$ region of the spectra is complicated by the presence of thioamide bands and ring breathing vibrations of the pyridyl and phenyl rings. The band at 639 cm$^{-1}$ in the spectrum of ligand can be assigned as due to the in-plane ring deformation band of the pyridine ring [27].

Table 2.2. Infrared spectral assignments (cm$^{-1}$) of bis(thiosemicarbazones)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$v(^4N-H)$</th>
<th>$v(^2N-H)$</th>
<th>$v$(C=N)</th>
<th>$v$(N–N)</th>
<th>$v$($\delta$(C-S))</th>
<th>py(ip)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2Ac4Ph$</td>
<td>3306</td>
<td>3220</td>
<td>1597</td>
<td>1028</td>
<td>1322,808</td>
<td>628</td>
</tr>
<tr>
<td>$H_2Ac4Cy$</td>
<td>3296</td>
<td>3259</td>
<td>1596</td>
<td>1013</td>
<td>1307,857</td>
<td>601</td>
</tr>
<tr>
<td>$H_2Ac4Me$</td>
<td>3335</td>
<td>3325</td>
<td>1549</td>
<td>1043</td>
<td>1353,869</td>
<td>607</td>
</tr>
<tr>
<td>$H_2Ac4Et$</td>
<td>3373</td>
<td>3328</td>
<td>1541</td>
<td>1050</td>
<td>1307,857</td>
<td>639</td>
</tr>
</tbody>
</table>
Figure 2.2. IR spectrum of H₂Ac₄Ph

Figure 2.3. IR spectrum of H₂Ac₄Cy
Figure 2.4. IR spectrum of H$_2$Ac$_4$Me

Figure 2.5. IR spectrum of H$_2$Ac$_4$Et
2.4.2. Electronic spectral studies

The UV-visible spectra of organic compounds are associated with the electronic transitions between energy levels, and at wavelengths above 200 nm, excitation of electrons from the \( \pi \)-orbitals usually occurs giving rise to informative spectra \cite{28, 29}. The tentative assignments of the significant electronic spectral bands of ligands are presented in Table 2.3.

H\textsubscript{2}Ac4Ph: The electronic spectrum of the ligand (Figure 2.6) in DMF solution (10\textsuperscript{-4} M) shows a band at 28,900 cm\textsuperscript{-1}, which corresponds to \( n \rightarrow \pi^* \) transition of the pyridyl ring and imine function of the thiosemicarbazone moiety. The \( \pi \rightarrow \pi^* \) transition observed as a band at 32,250 cm\textsuperscript{-1} is assigned for the aromatic rings.

H\textsubscript{2}Ac4Cy: The \( n \rightarrow \pi^* \) and \( \pi \rightarrow \pi^* \) transitions are observed as a band and as a shoulder at 29,210 and 32,450 cm\textsuperscript{-1} in the electronic spectrum of the ligand H\textsubscript{2}Ac4Cy (Figure 2.7) in DMF (10\textsuperscript{-4} M).

<table>
<thead>
<tr>
<th>Compound</th>
<th>( n \rightarrow \pi^* )</th>
<th>( \pi \rightarrow \pi^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H\textsubscript{2}Ac4Ph</td>
<td>28,900</td>
<td>32,250</td>
</tr>
<tr>
<td>H\textsubscript{2}Ac4Cy</td>
<td>29,210</td>
<td>32,450</td>
</tr>
<tr>
<td>H\textsubscript{2}Ac4Me</td>
<td>29,620</td>
<td>32,050</td>
</tr>
<tr>
<td>H\textsubscript{2}Ac4Et</td>
<td>29,390</td>
<td>32,240</td>
</tr>
</tbody>
</table>

Table 2.3. Electronic spectral assignments (cm\textsuperscript{-1}) of ligands
H₂Ac₄Me: The spectrum recorded for a 10⁻⁴ M DMF solution of the ligand (Figure 2.8) consists of a shoulder at 29,620 cm⁻¹, which corresponds to the $n \rightarrow \pi^*$ transition. The $\pi \rightarrow \pi^*$ transition was observed at 32,050 cm⁻¹.

H₂Ac₄Et: The band corresponding to $n \rightarrow \pi^*$ transition is observed at 29,390 cm⁻¹ and the $\pi \rightarrow \pi^*$ transition is observed at 32,240 cm⁻¹ (Figure 2.9).

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Figure 2.6. Electronic spectrum of H₂Ac₄Ph

Figure 2.7. Electronic spectrum of H₂Ac₄Cy

Figure 2.8. Electronic spectrum of H₂Ac₄Me

Figure 2.9. Electronic spectrum of H₂Ac₄Et
2.4.3. \(^1\)H NMR spectral studies

Proton magnetic resonance spectroscopy is a helpful tool for the identification of organic compounds in conjunction with other spectrometric informations.

\(\text{H}_2\text{Ac4Ph}:\) The \(^1\)H NMR spectrum of \(\text{H}_2\text{Ac4Ph}\) and the spectral assignments are given in Figure 2.10. Solution of the double-armed \(\text{H}_2\text{Ac4Ph}\) ligand in DMSO showed that N(3)H and N(6)H resonance, occur at \(\delta=10.21.\) The low field position of \(-^4\text{NH}\) and \(-^5\text{NH}\) (\(\delta=10.78\) ppm) could be attributable to the deshielding caused by the phenyl group. The presence of \(-^3\text{NH}\) and \(-^6\text{NH}\) proton signal suggests enolization of \(-^3\text{NH}–\text{C}=\) and \(-^6\text{NH}–\text{C}=\) groups to \(-^3\text{N}=\text{C}–\text{SH}\) and \(-^6\text{N}=\text{C}–\text{SH},\) but \(\text{H}_2\text{Ac4Ph}\) does not show any peak attributable to \(-\text{SH}\) proton. A sharp singlet, which integrates as six hydrogen at \(\delta=2.53\) ppm is assigned to the methyl protons attached to C(7) and C(11) which are chemically and magnetically equivalent. Aromatic protons appear as a multiplet at 7.20-8.62 ppm. A doublet at \(\delta=8.56\) is assigned to pyridyl ring protons at C(2) and C(4) and is more down field compared to the other proton C(3)H in the same ring, \(\delta=7.82,\) which appears as a triplet. Phenyl ring protons at C(13,17)H and C(19,23)H appear as a doublet at \(\delta=7.51.\) Two triplets (\(\delta=7.36\) and \(\delta=7.22\)) are assigned to C(14,16)H, C(20,22)H and C(15,21)H respectively.
H$_2$Ac$_4$Cy: The $^1$H NMR spectrum of H$_2$Ac$_4$Cy is represented in Figure 2.11. In this spectrum, the three protons of the pyridine ring appear at $\delta$ values in the range 7.84 -8.20 ppm and signals for all the aliphatic protons of...
methyl group and the cyclohexyl moiety have appeared at 1.24-3.37 ppm. N(4) and N(7) protons appear at δ= 8.05 ppm and a doublet, which integrates as two hydrogens at δ=8.09 ppm is assigned to the protons attached to the pyridyl ring at C(2) and C(4). A one proton triplet at δ=7.26 ppm is attributed to the C(3)H of the pyridyl ring. The absence of peaks corresponding to the S–H proton in the spectrum supports the fact that in solution, the predominant tautomer is in the thione form.

Figure 2.11. $^1$H NMR spectrum of H$_2$Ac4Cy
H$_2$Ac$_4$Me: In the spectrum of H$_2$Ac$_4$Me (Figure 2.12), N(3)H and N(6)H protons at $\delta=10.372$ ppm which are more downfield, are easily distinguished from N(4)H and N(5)H protons ($\delta=8.66$), because the N(4,5)H appear as a doublet due to interaction with the methyl hydrogens of N(4,5)Me group. Absence of any coupling interactions by N(3)H and N(6)H protons due to the lack of availability of protons on neighbouring atoms render singlet peaks for the imine protons. A doublet, which integrates as two hydrogens at $\delta=8.41$ ppm is assigned to the hydrogens attached to the pyridyl ring at C(2) and C(4) positions which are chemically and magnetically equivalent.

![Figure 2.12. $^1$H NMR spectrum of H$_2$Ac$_4$Me](image)

A one proton triplet at $\delta=7.84$ ppm is attributed to the C(3)H of the pyridyl ring, which is unique in nature. It is observed that the protons of methyl groups attached to N(4) and N(5) are more deshielded than the protons attached to C(7) and C(11) ($\delta=2.44$).
H$_2$Ac4Et: In the spectrum of H$_2$Ac4Et (Figure 2.13), N(3) and N(6) protons appear at $\delta = 8.83$ ppm, which are shifted downfield because they are attached to heteroatoms and so are easily subjected to hydrogen bonding and are decoupled by the electrical quadrupole effects. These protons appear as a singlet as expected since the NH protons are decoupled from the nitrogen atoms and the protons from the adjacent atoms.

Figure 2.13. $^1$H NMR spectrum of H$_2$Ac4Et
The N(4)H and N(5)H protons (δ=8.03 ppm) appear as a triplet due to the interaction with the methylene hydrogens of the ethyl group. The three protons of the pyridine ring appear at δ values 7.26 ppm for C(3)H and 7.96 ppm for C(2,4)H. A triplet at δ=1.34 ppm is assigned to the signal corresponding to the six protons of the methyl group attached to –CH₂. The remaining aliphatic protons of two –CH₃ groups and two –CH₂ groups are observed as a multiplet at about 2.41-3.13 ppm where the chemical shift values are very close and hence it is very difficult to be resolved. NMR assignments are in agreement with values already reported [30-33].

**Concluding remarks**

This chapter presents the details regarding the syntheses of four (H₂Ac₄Ph, H₂Ac₄Cy, H₂Ac₄Me, H₂Ac₄Et), SNNNS donor ligands. The double armed bis(thiosemicarbazones) are pentadentate in nature with two thiosemicarbazone moieties in each system. Elemental analysis data is consistent with the empirical formulae of ligands. The compounds are further characterized by IR, ¹H NMR and electronic spectral studies.

**References**


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