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# SYNTHESIS, CHARACTERIZATION AND ANTI-TUBERCULAR ACTIVITY OF SELENOSEMICARBAZONES CONTAINING FUSED AROMATIC RINGS

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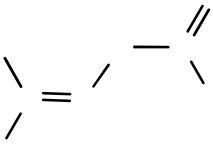
## Abstract

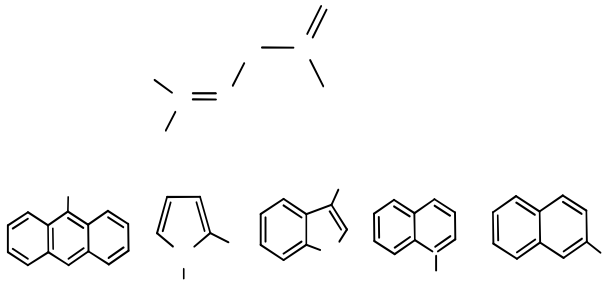
Reaction of cyclohexanone selenosemicarbazone with 9-anthraldehyde, N-methyl-2-pyrrole carbaldehyde, Indole-3-carbaldehyde, 1-naphthaldehyde and 2-naphthaldehyde in 1:1 molar ratio resulted into the formation of 9-anthraldehyde selenosemicarbazone (9-Hansesc, $H^1L$), N-methyl-2-pyrrole carbaldehyde selenosemicarbazone (N-MeHpsesc, $H^2L$), Indole-3-carbaldehyde selenosemicarbazone (HInsesc, $H^3L$), 1-naphthaldehyde selenosemicarbazone (1-Hnapsesc, $H^4L$) and 2-naphthaldehyde selenosemicarbazone (2-Hnapsesc, $H^5L$) respectively. All the synthesized compounds were characterized using elemental analysis, IR and $^1$HNMR. These compounds were tested for anti-tubercular activity and selenosemicarbazone ligands with no heteroatom are found to be more active.
***Keywords*** : cyclohexanone selenosemicarbazone, naphthaldehyde, elemental analysis, anti-tubercular activity.

## Introduction

Ligands containing chalcogen (O, S, Se) or nitrogen as donor atoms are prime focus of research for many researchers. Main reason for that is the number of biological activities exhibited by them for example, 1,2,3-triazole based ligands show antibacterial activity (Singh *et al*., 2018), chromone based thiosemicarbazones exhibit antioxidant properties (Singh *et al*., 2019; Singh *et al*., 2018; Singh *et al*., 2015) and chalcone based ligands exhibit ant-tubercular activities (Jaryal *et al*., 2017; Rawat *et al*., 2017; Talniya *et al*., 2016) Many other such ligands known to be bioactive molecules (Arora *et al*., 2016; Handa *et al*., 2019) and also exhibit number of biological applications (Bashary *et al*., 2019; Bhat *et al*., 2019; Sharma *et al*., 2017; Sharma *et al*., 2017; Sharma *et al*., 2016; Sharma *et al*., 2016; Shiekh *et al*., 2014; Mansoori *et al*., 2018; Niranjan *et al*., 2019; Masta *et al*., 2019; Divya *et al*., 2019; Kumar *et al*., 2018; Mansoori *et al*., 2018; Bedi *et al*., 2018; Datusalia *et al*., 2018; Khatik *et al*., 2018; Kumar *et al*., 2018; Sarma *et al*., 2017; Kumar *et al*., 2017; Sharma *et al*., 2016; Sharma *et al*., 2017). Apart from biological activites, these ligands can be used as sensors (Singh *et al*., 2019; Singh *et al*., 2019; Kaur *et al*., 2018; Kaur *et al*., 2014), in photocells (Kumar *et al*., 2018; Malik *et al*., 2018), as corrosion inhibitors (Ansari *et al*., 2014; Ansari *et al*., 2015; Ansari *et al*., 2015; Ambrish *et al*., 2015; Bashir *et al*., 2019) and sensors (Gupta *et al*., 2012). Ligand of selenium donor are less common as earlier it was considered toxic. Importance of selenium in human body comes into existence after the discovery of selenocystein, the 21$^{st}$ amino acid (Sunde *et al*., 1997). Selenoproteins with enzymatic activity have selenocystein in their active site, where selenium acts as redox centre (Sunde *et al*., 1997; Allan *et al*., 1999; Diplock *et al*., 1994). Thus now a days, selenium compounds like selenosemicarbazones are not treated as toxic, rather they exhibit number of biological activities like antitumor, antimicrobial, antiviral etc. (Liu *et al*., 1992; Turk *et al*., 1986; Al-Eisawi *et al*., 2016; Filipovic *et al*., 2014). But the chemistry of selenosemicarbazone is still not explored much due to: i) elemental selenium get separated out during complexation (Castle *et al*., 2003)], ii) ligands get changed, leaving hydrogen selenide as side product (Todorovic *et al*., 2006), iii) undergo oxidation to form diselenide bridge (Andaloussi *et al.,* 2010). Selenosemicarbazones, $\{R^1C^2H{=}N{-}NH{-}C^1(=S)NHR^2\}$(I) known till date can be categorized into: a) having un-substituted and substituted aromatic ring at $C^2$ carbon (Bippus *et al*., 2010; Pizzo *et al*., 2016; Liu *et al*., 1992; Calcatierra *et al*., 2015; Gingrxs *et al*., 1965); b) with aliphatic chain at $C^2$ carbon (Bhoon *et al*., 1984); c) with heterocyclic ring at $C^2$ carbon (Al-Eisawi *et al*., 2016; Shen *et al*., 2014; Ma. Lourenco *et al*., 2007).



In present paper, synthesis of new selenosemicarbaze namely, 9-anthraldehyde selenosemicarbazone (9-Hansesc), N-methyl-2-pyrrole carbaldehyde selenosemicarbazone (N-MeHpsesc), Indole-3-carbaldehyde selenosemicarbazone (HInsesc), 1-naphthaldehyde selenosemicarbazone (1-Hnapsesc) and 2-naphthaldehyde selenosemicarbazone (2-Hnapsesc) (Scheme 1) has been done. Characterization of synthesized molecules is done by elemental analysis, IR, $^1$H NMR. All these compounds were also tested for their anti-tubercular activity.



## Materials and Methods

**Chemicals and instrumentation:**

Hydrazine hydrate, Potassium Selenocynate, Cyclohexanone, 9-anthraldehyde, 1-naphthaldehyde, 2-naphthaldehyde, indole-3-carbaldehyde and N-methyl-2-pyrrole carbaldehyde are procured from Sigma-Aldrich Chemicals. SHIMADZU FTIR 8400S, Fourier Transform, Infrared spectrophotometer was used to record IR spectra. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a BRUCKER ADVANCE III NMR Spectrophotometer at 500 MHz in $d^6$-dmso and $CDCl_3$ with TMS as the internal reference. Thermoelectron FLASHEA1112 CHNS analyzer was used for C, H and N analysis of complexes.

**Laboratory procedures:**

**1. Synthesis of Cyclohexanoneselenosemicarbazone:** Cyclohexanoneselenosemicarbazone was synthesized from hydrazine hydrate, KSeCN and cyclohexanone using literature method (Bippus *et al*., 2010). Yield, 70 %, m.p.180-182°C. Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3417s, 3255s; $\upsilon$(–NH–) 3142s; $\upsilon(C–H_{cyclo})$, 2929s, 2355s; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta$ ($NH_2$) 1589, 1512s, 1398s; $\upsilon$(C=Se) 856s (selenoamide moiety). $^{1}H$ NMR ($\delta$, ppm; $CDCl_3$): 8.92 s (1H, $N^2H$), 7.65 s (1H, $N^1H_2$), 6.64 s (1H, $N^1H_2$), 2.35-1-67 m (10H, Cy ring proton). $^{13}C$ NMR ($\delta$, ppm; $CDCl_3$):185.4 ($C^1$), 136.1, ($C^2$) 103.4 ($C^3$), 135.6 ($C^4$), 115.2, ($C^5$), 112.4 ($C^6$), 115.4 ($C^7$), 131.8 ($C^8$), 125.4 ($C^9$), 119.7 ($C^{10}$), 55.9.

**2. Synthesis of 9-anthraldehyde selenosemicarbazone (9-Hansesc, $H^1L$):**

Cyclohexanone selenosemicarbazone, (0.5g, 2.29mmol) was dissolved in 20 ml of ethanol with heating. To it was added 9-anthraldehyde (0.473g, 2.29mmol) and the mixture was refluxed for 2 hours. 1ml of glacial acetic acid was added during refluxing. Reddish brown ppt. obtained after evaporation. Yield, 60%, m.p.210-213°C. Analysis found: C, 58.83; H, 3.97; N, 12.67 %. Calculated for $C_{16}H_{13}N_3Se$: C, 58.89; H, 3.98; N, 12.88 %; Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3416m, 3254m; $\upsilon$(–NH–) 3144w; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta(NH_2)$ 1668s, 1589m, 1516s; $\upsilon$(C=Se) 839s (selenoamidemoiety). $^{1}H$ NMR ($\delta$, ppm; $d^6$-dmso and $CDCl_3$): 11.57s (1H, $N^2H$), 10.17s (1H, $C^2H$), 9.23d ( 2H, $C^{4,12}H$, 8.2Hz), 9.02d ( 2H, $C^{7,9}H$, 8.2Hz), 8.71m (4H, $C^{5,6,10,11}H$), 8.63s (1H, $C^8H$), 7.66s (1H, $N^1H_2$), 6.65s (1H, $N^1H_2$).

**3. Synthesis of N-methyl-2-pyrrole carbaldehyde selenosemicarbazone (N-MeHpsesc, $H^2L$):** Cyclohexanone selenosemicarbazone, (0.50g, 2.29mmol) was dissolved in 20 ml of ethanol with heating. To it was added N-methyl-2-pyrrole carbaldehyde( (0.25g, 2.29mmol) and the mixture was refluxed for 2 hours. 1ml of glacial acetic acid was added during refluxing. Yellow ppt. obtained after evaporation. Yield, 50%, m.p.150-152°C. Analysis found: C, 36.63; H, 4.34; N, 24.44%. Calculated for $C_7H_{10}N_4Se$: C, 36.68; H, 4.36; N, 24.45%; Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3356m, 3246m; $\upsilon$(–NH–) 3153w; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta(NH_2)$ 1656s, 1591m, 1487s; $\upsilon$(C=Se) 854s. $^{1}H$ NMR ($\delta$, ppm; $d^6$-dmso and $CDCl_3$): 9.01s (1H, $N^2H$), 8.01s (1H, $C^2H$), 7.79s ( 1H, $C^4H$), 7.27s (1H, $C^5H$), 7.29s (1H, $C^6H$), 6.92s (1H, $N^1H_2$), 6.46s (1H, $N^1H_2$).$^{13}C$ NMR ($\delta$, ppm;$CDCl_3$): 152.4.0 ($C^2$), 139.6 ($C^3$), 135.78 ($C^4$), 122.2 ($C^5$), 31.54 (N-$C^6$).

**4. Synthesis of Indole-3-carbaldehyde selenosemicarbazone (HInsesc, $H^3L$):** Cyclohexanone selenosemicarbazone, (0.5g, 2.29mmol) was dissolved in 20 ml of ethanol with heating. To it was added Indole-3-carbaldehyde (0.33g, 2.29mmol) and the mixture was refluxed for 2 hours. 1ml of glacial acetic acid was added during refluxing. Orange ppt. formed after evaporation. Yield, 70%, m.p.210-213°C. Analysis found: C, 46.95; H, 3.96; N, 21.99%. Calculated for $C_{10}H_{10}N_4Se$: C, 47.05; H, 3.92; N, 21.96%; Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3252m; $\upsilon$(–NH–) 3138w; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta(NH_2)$ 1654s, 1572m, 1496s; $\upsilon$(C=Se) 854s (selenoamidemoiety). $^{1}H$ NMR ($\delta$, ppm; $d^6$-dmso and $CDCl_3$): 9.04s (1H, $N^2H$), 8.80 s (1H, $C^2H$), 8.50 s (1H, $C^5H$), 8.10 s (1H, $C^8H$), 7.68 s (1H, $C^6H$), 7.67 s (1H, $C^7H$), 7.61s (1H, $N^1H_2$), 6.65s (1H,$^1N_2$).

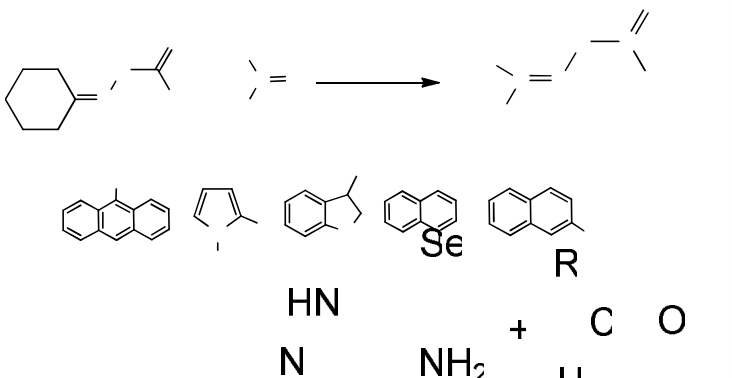
**5. Synthesis of 1-Naphthaldehyde selenosemicarbazone (1-Hnapsesc, $H^4L$):** Cyclohexanone selenosemicarbazone, (0.5g, 2.29mmol) was dissolved in 20 ml of ethanol with heating. To it was added 1-naphthaldehyde (0.35g, 2.29mmol) and the mixture was refluxed for 2 hours. 1ml of glacial acetic acid was added during refluxing. Yellowish ppt. obtained after evaporation. Yield, 50%, m.p.175-179°C. Analysis found: C, 46.01; H, 3.86; N, 16.04%. Calculated for $C_{12}H_{10}N_3Se$: C, 45.97; H, 3.83; N, 16.09%; Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3400m, $\upsilon$(–NH–) 3147w; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta(NH_2)$ 1599s, 1516m, 1452s; $\upsilon$(C=Se) 871s (selenoamidemoiety). $^{1}H$ NMR ($\delta$, ppm; $d^6$-dmso and $CDCl_3$): 9.51s (1H, $N^2H$), 9.15 s (1H, $C^2H$), 8.50 s (1H, $C^3H$), 8.45 s (1H, $C^4H$), 8.32 s (1H, $C^5H$), 8.22s (1H, $C^6H$), 8.20s (1H, $C^7H$), 8.17 s (1H, $C^8H$), 8.10 s (1H, $C^9H$), 7.61s (1H, $N^1H_2$).

**6. Synthesis of 2-Naphthaldehyde selenosemicarbazone( 2-Hnapsesc, $H^5L$):** Cyclohexanone selenosemicarbazone, (0.5g, 2.29mmol) was dissolved in 20 ml of ethanol with heating. To it was added 2-naphthaldehyde (0.358g, 2.29mmol) and the mixture was refluxed for 2 hours. 1ml of glacial acetic acid was added during refluxing. Yellow ppt. formed after evaporation. Yield, 50%, m.p., 178-180°C. Analysis found: C, 45.99; H, 3.80; N, 16.07%. Calculated for $C_{12}H_{10}N_3Se$: C, 45.97; H, 3.83; N, 16.09%; Main IR peaks (KBr, $cm^{-1}$): $\upsilon(NH_2)$ 3352m; $\upsilon$(–NH–) 3124w; $\upsilon$(C=N) + $\upsilon$(C=C) + $\delta(NH_2)$ 1683s, 1597m, 1533s; $\upsilon$(C=Se) 855s (selenoamidemoiety). $^{1}H$ NMR ($\delta$, ppm; $d^6$-dmso and $CDCl_3$): 8.91s (1H, $C^2H$), 8.50 s (1H, $C^3H$), 8.45 s (1H, $C^4H$), 8.32 s (1H, $C^5H$), 8.22s (1H, $C^6H$), 8.20s (1H, $C^7H$), 8.17 s (1H, $C^8H$), 8.10 s (1H, $C^9H$), 7.65s (1H, $N^1H_2$).

**Anti-tuberculosis activity:** The anti-microbial activity of compounds was assessed using literature method (Ma. Lourenco *et al*., 2007)

## Results and Discussion

Reaction of cyclohexanone selenosemicarbazone with 9-anthraldehyde, N-methyl-2-pyrrole carbaldehyde, Indole-3-carbaldehyde, 1-naphthaldehyde and 2-naphthaldehyde in 1:1 molar ratio resulted into the formation of 9-anthraldehyde selenosemicarbazone (9-Hansesc, $H^1L$), N-methyl-2-pyrrole carbaldehyde selenosemicarbazone (N-MeHpsesc, $H^2L$), Indole-3-carbaldehyde selenosemicarbazone (HInsesc, $H^3L$), 1-naphthaldehyde selenosemicarbazone (1-Hnapsesc, $H^4L$) and 2-naphthaldehyde selenosemicarbazone (2-Hnapsesc, $H^5L$) respectively (Scheme 2).




Scheme 2

Characteristic IR peaks of selenosemicarbazones are given in Table-1. The $\upsilon(NH_2)$ band of cyclohexanone selenosemicarbazone appears in the range of 3417-3255 $cm^{-1}$ in IR spectra (Table-1). This band shows low energy shift in ligands $H^1L$ - $H^5L$ as compare to cyclohexanone selenosemicarbazone. The υ(-NH-) band obtained in ligands 3120-3153 $cm^{-1}$ which is at lower wave number in $H^3L$ (3138 $cm^{-1}$) and $H^5L$ (3124 $cm^{-1}$) and higher in $H^1L$ (3144 $cm^{-1}$) , $H^2L$ (3153 $cm^{-1}$) and $H^4L$ 3147 $cm^{-1}$). The characteristic ν(C=Se) band of cyclohexanone selenosemicarbazone appeared at 879 $cm^{-1}$ this band shows low energy shift in ligands $H^1L$ – $H^5L$ {839($H^1L$), 854($H^2L$), 854 ($H^3L$), 871($H^4L$), 855($H^5L$)}. The shift in υ(C=Se) band indicates formation of the ligand, $H^1L$ – $H^5L$.

**Table1.** IR peaks of cyclohexanone selenosemicarbazone and selenosemicarbazone ligands $H^1L$ – $H^5L$

| Compound and ligands | ν($NH_2$) | υ(–NH-) | υ(C=N) + υ(C=C) + δ ($NH_2$) | υ(C=Se) |
|---|---|---|---|---|
| Cyclohexanone selenosemicarbazone | 3417<br>3255 | 3142 | 1589,1512,1398 | 879 |
| $H^1L$ | 3417<br>3254 | 3144 | 1668,1589,1516 | 839 |
| $H^2L$ | 3356<br>3246 | 3153 | 1656,1591,1487 | 854 |
| $H^3L$ | 3252 | 3138 | 1654,1572,1496 | 854 |
| $H^4L$ | 3400 | 3147 | 1599,1516,1452 | 871 |
| $H^5L$ | 3352 | 3124 | 1683,1597,1533 | 855 |

Formation of cyclohexanone selenosemicarbazone and selenosemicarbaones derived from it was confirmed by NMR spectroscopy. In $^1H$ NMR, $N^2H$ proton of this compound appeared at δ 8.92 ppm. Two broad singlets appeared at δ 7.65 ppm and δ 6.64 ppm due to non equivalent $N^1H_2$ proton. Proton of cyclic ring appeared in the range δ 2.34 ppm - δ 1.67 ppm.

The $N^2H$ proton in selenosemicarbazone ligands ($H^1L$ – $H^5L$) appeared in the range δ 9.51 ppm- δ11.57 ppm. The signal due to $C^2H$ proton appeared in the range δ 9.51 ppm - δ 10.17 ppm in these ligands indicated the replacement of cyclic ring by aromatic ring. Disappearance of cyclic ring protons and presence of aromatic ring in the range of δ 6.65 ppm - δ 9.23 ppm in $H^1L$-$H^5L$ confirm the replacement of cyclic ring by aromatic or heterocyclic rings (Table-2).

**Table 2.** $^1H$ NMR signals for $H^1L$ – $H^5L$ (δ, ppm).

| Compound and ligands | (1H, $N^2H$) | (1H, $C^2H$) | (1H, $N^1H_2$) | (Ring protons), |
|---|---|---|---|---|
| Cyclohexanone selenosemicarbazone | 8.92 | _ | 7.65(s)<br>6.64(s) | Cy 2.34-1.67 |
| $H^1L$ | 11.57 | 10.17 | 7.66<br>6.65 | 9.23-6.65 |
| $H^2L$ | 9.01 | 8.01 | | 7.85-6.23 |
| $H^3L$ | 9.04 | 8.80 | 7.61 | 8.50-7.67 |
| $H^4L$ | 9.51 | 9.15 | 7.61 | 8.50-8.10 |
| $H^5L$ | - | 8.91 | 7.65 | 8.50- 8.10 |

## Anti-tubercular activity

The anti-tubercular activity of cyclohexanone selenosemicarbazone and $H^1L$-$H^5L$ ligands was evaluated against M. tuberculosis at various concentrations. Minimum Inhibitory Concentration of cyclohexanone selenosemicarbazone and its related ligands complex ($H^1L$-$H^5L$) against *M. Tuberculosis* H37RV (Table-3). Cyclohexanone selenosemicarbazone shows anti-TB activity at MIC = 3.12 μg/ml. This activity is same as that of second line standard drugs Pyrazinamide, Ciprofloxacin and Streptomycin (MIC = 3.125 μg/ml, 3.125 μg/ml and 6.25 μg/ml respectively). The antitubercular activity further gets enhanced when cyclohexanone ring gets replaced by fused aromatic rings in $H^1L$, $H^4L$ and $H^5L$ (MIC = 1.6μg/ml), however activity get reduced on replacement of cyclohexanone ring with rings having hetero-atom in $H^2L$ and $H^3L$ (MIC=25μg/ml and 25μg/ml).

**Table 3.** Anti-tubercular activity of cyclohexanone selenosemicarbazone and selenosemicarbazone ligands ($H^1L$ – $H^5L$)

| Sr. No. | Compound and ligands | MIC (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.6 | 0.8 |
| | Cyclohexanone selenosemicarbazone | S | S | S | S | S | S | R | R |
| 1. | 9-anthracene carbaldehyde selenosemicarbazone ($H^1L$) | S | S | S | S | S | S | S | R |
| 2. | N-methyl-2-pyrrole carbaldehyde selenosemicarbazone ($H^2L$) | S | S | S | R | R | R | R | R |
| 3. | Indole-3-carbaldehyde selenosemicarbazone ($H^3L$) | S | S | S | R | R | R | R | R |
| 4. | 1-naphthaldehyde selenosemicarbazone ($H^4L$) | S | S | S | S | S | S | S | R |
| 5. | 2-naphthaldehyde selenosemicarbazone ($H^5L$) | S | S | S | S | S | S | S | R |

## Conclusion

9-anthraldehyde selenosemicarbazone (9-Hansesc, $H^1L$), N-methyl-2-pyrrole carbaldehyde selenosemicarbazone (N-MeHpsesc, $H^2L$), Indole-3-carbaldehyde selenosemicarbazone (HInsesc, $H^3L$), 1-naphthaldehyde selenosemicarbazone (1-Hnapsesc, $H^4L$) and 2-naphthaldehyde selenosemicarbazone (2-Hnapsesc, $H^5L$) has been formed by reaction of cyclohexanone selenosemicarbazone with respective aldehydes. CHN analysis, IR and $^1$HNMR studies support formation of these compounds. Compounds with fused aromatic rings show very good anti-tubercular activity (MIC = 1.6μg/ml)

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