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SYNTHESIS, STRUCTURAL, ANTIBACTERIAL AND SPECTRAL STUDIES OF Co(II) COMPLEXES WITH SALICYLALDEHYDE AND *p*-CHLORO-BENZALDEHYDE 4-PHENYLTHIOSEMICARBAZONE

(Sintesis, Penstrukturan, Antibakteria dan Analisis Spektrum Sebatian Kompleks Co(II) dengan Salisilaldehid dan *p*-klorobenzaldehid 4-feniltiosemikarbazon)

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Abstract

The Co(II) complexes derived from salicylaldehyde 4-phenylthiosemicarbazone; SaOHtsc, *p*-chlorobenzaldehyde 4-phenylthiosemicarbazone; ph-HClbtsc is reported and characterized based on elemental analysis, IR, magnetic susceptibility measurement, ^{1}H and ^{13}C NMR spectra. The Co(II) complexes have the molecular formula CoL_2 where the ligand corresponding to SaOHtsc and ph-HClbtsc. The elemental analysis for the ligands and complexes were in a good agreement with the theoretical values. The ligands coordinate to metal ions in different ways which is through mononegative bidentate or dinegative tridentate. The magnetic susceptibility measurements showed that the CoL_2 complexes with ligand SaOHtsc are diamagnetic thus making this complexes suitable for NMR studies. The signals at the 10.04 ppm were assigned to N^2H in the ^{1}H-NMR spectra of the free ligands was absent in the spectra of the complexes due to the deprotonation of the N^2H and coordination to the metal centres. The absence of the band in IR spectrum which is assigned to $\nu(N^2\text{-}H)$ in the spectra of CoL_2 complexes is due to the deprotonation of the ligands upon complexation through azomethine nitrogen and thionic sulphur atom to metal ion. The thiosemicarbazones and its Co(II) complexes showed moderate inhibitory against bacteria *Bacillus Subtilis, Staphylococcus Epidermis, Escherichia Coli* and *Proteus Mirabilis* in 10 µg/disc.

Keywords: Thiosemicarbazone, Co(II), antibacterial

Abstrak

Sebatian kompleks Co(II) diterbitkan daripada salisilaldehid 4-feniltiosemikarbazon, SaOHtsc, dan *p*-klorobenzaldehid 4-feniltiosemikarbazon, ph-HCltsc telah dilaporkan serta dilakukan pencirian melalui analisis unsur, IR, ukuran kebolehrentangan magnet momen, ^{1}H dan ^{13}C RMN. Sebatian kompleks tersebut mempunyai formula molekul CoL_2 di mana ligan mewakili SaOHtsc dan ph-HClbtsc. Analisis unsur bagi sebatian ligan dan kompleks mematuhi nilai teori. Sebatian ligan berkoordinat dengan ion logam melalui pelbagai cara iaitu sama ada mononegatif monodentat atau dinegatif tridentat. Pengukuran kebolehrentangan magnet momen menunjukkan bahawa sebatian kompleks CoL_2 dengan ligan SaOHtsc adalah diamagnet di mana sesuai untuk pencirian RMN. Puncak pada 10.04 ppm mewakili N^2H dalam spektrum ^{1}H-RMN bagi ligan bebas di mana puncak tersebut tidak muncul dalam spketrum sebatian kompleks disebabkan nyahprotonan N^2H dan pengkoordinatan dengan logam pusat. Ketiadaan jalur serapan $\nu(N^2H)$ dalam spektrum IR bagi sebatian kompleks, CoL_2 merujuk kepada nyahprotonan ligan semasa berlaku pengkoordinatan melalui azometina N dan tionik S dengan logam pusat. Sebatian ligan tiosemikarbazon dan kompleks Co(II) menunjukkan sifat sederhana rencatan menentang bakteria *Bacillus Subtilis, Staphylococcus Epidermis, Escherichia Coli* dan *Proteus Mirabilis* dalam 10 µg / cakera.

Kata kunci: Tiosemikarbazon, Co(II), antibakteria

Introduction

Thiosemicarbazones also known as Schiff or soft bases are widely applied in analytical determination by using complex formation reactions. Most Schiff bases are chemically unstable and showed tendency to be involved in various equilibria such as tautomerism, hydrolysis or formation of ionized species [3]. Thiosemicarbazones can behave as mononegative or neutral bidentate ligands to coordinate with Co(II) through sulfur azomethine and the hydrazinic nitrogen atoms which is known as N, S- donor ligands. They can exist as thione-thiol tautomer and can bind to a metal centre in the neutral or the anionic forms act as monodentate, bidentate or bridge ligands [8]. The 4-phenylthiosemicarbazone derivatives is an interesting compound because of their ability to form highly stable and intense coloured complexes with metal ion [1]. A wide range of biological activities of heterocyclic thiosemicarbazones species has become a great interesting field including antibacterial, antifungal, antitumor and antiviral [5]. Sometimes, the biological activity of the ligands is enhanced or only takes place when the presence of metal ions. Bingol and Atalay 2006 [1] also said that determination of Co(II) in natural water is of considerable interest because it is important for living species in the forms complexes with vitamin B12.

Experimental

Material and instrumentation

All chemicals and solvents used for synthesis were of reagent grade and used without further purification. Melting points were taken on a melting point apparatus. IR spectra were recorded on a Perkin Elmer GX spectrophotometer between 400-4000 cm^{-1} by using KBr pellet while $\geq$ 400 cm^{-1} using polyethylene. The micro-elemental analysis data was obtained on a Model Fison EA 1108 Analyzer, NMR data also obtained from 600MHz FT-NMR Cryoprobe and the molar magnetic susceptibilities were measured on powdered samples using the Gouy balance.

Synthesis of the ligands

Salicylaldehyde 4-phenylthiosemicarbazone, SaOHtsc, L^1

The synthesis of SaOHtsc ligand involved condensation between ethanolic solution of salicylaldehyde (0.001 mol, 0.122 g) was slowly added dropwise into an ethanolic solution of 4-phenylthiosemicarbazide (0.001 mol, 0.167 g) were stirred for 2 hour at 60-70 °C. After that, the solution of mixture was filtered and cooled at room temperature. After 3 days later, the yellow precipitate was formed and collected by filtration, washed with cold ethanol and dried overnight in a dessicator. Yield: 82 %. Analyses: Calculated: C, 61.97; H, 4.83; N, 15.49; S, 11.82 %. Found: C, 62.60; H, 4.86; N, 16.93; S, 12.07 %. IR ν_{max} cm^{-1}: 1620 (C=N), 1079 (N-N), 1152 (C=S), 3308 (N-H), 1033 (C-N). ^{1}H NMR (DMSO) (δ, ppm): 10.04 (N^2H, 1H, s), 11.70 (N^1H, 1H, s), 6.83-8.04 (aromatic, 9H, m), 8.48 (aliphatic, 1H, s). ^{13}C NMR (DMSO) (δ, ppm): 140.81 (C=N), 176.23 (C=S), 116.53-139.53 (aromatic).

p-chlorobenzaldehyde 4-phenylthiosemicarbazone, ph-HClbtsc, L^2

The synthesis of ph-HClbtsc ligand involved condensation between ethanolic solution of *p*-chlorobenzaldehyde (0.001 mol, 0.141 g) was slowly added dropwise into an ethanolic solution of 4-phenylthiosemicarbazide (0.001 mol, 0.167 g). Yield:79 %. Analyses: Calculated: C, 58.03; H, 4.17; N, 14.50; S, 12.23 %. Found: C, 57.95; H, 4.11; N, 15.87; S, 11.51. IR ν_{max} cm^{-1}: 1597 (C=N), 1086 (N-N), 1199 (C=S), 3311 (N-H), 1013 (C-N). ^{1}H NMR (DMSO) (δ, ppm): 10.16 (N^2H, 1H, s), 11.84 (N^1H, 1H, s), 7.20-7.96 (aromatic, 9H, m), 8.14 (aliphatic, 1H, s). ^{13}C NMR (DMSO) (δ, ppm): 141.94 (C=N), 176.63 (C=S), 125.89-139.51 (aromatic).

Synthesis of complexes

The complexes were prepared by mixing with ratio 2:1 of ligand and metal salt in refluxing ethanolic solution for 3h.

Co(II) salicylaldehyde 4-phenylthiosemicarbazone, CoSaOHtsc (1)

$C_{28}H_{24}N_6O_2S_2Co$. Yield:65 %. Analyses: Calculated: C, 56.17; H, 4.04; N, 14.04; S, 10.72 %. Found: C, 55.95; H, 3.69; N, 14.07; S, 9.06. IR ν_{max} cm^{-1}: 1596 (C=N), 1081 (N-N), 650 (C-S), 3357 (N1H), 1250 (C-N), 500 (Co-N), 352 (Co-S).

Co(II) p-chlorobenzaldehyde 4-phenylthiosemicarbazone, Coph-HClbtsc (2)

$C_{28}H_{22}N_6S_2Cl_2Co$. Yield:68 %. Analyses: Calculated: C, 52.83; H, 3.48; N, 13.21; S, 10.08 %. Found: C, 51.63; H, 3.43; N, 13.13; S, 9.46 %. IR ν_{max} cm^{-1}: 1594 (C=N), 1093 (N-N), 661 (C-S), 3386 (N^1-H), 1251 (C-N), 536 (Co-N), 353 (Co-S). 1H NMR (DMSO) (δ, ppm): 9.51(N^1H, 1H, s), 6.65-7.71 (aromatic, 9H, m), 8.74 (aliphatic, 1H, s). ^{13}C NMR (DMSO) (δ, ppm): 171.21 (C=N), 158.12 (C-S), 116.34-141.47 (aromatic)

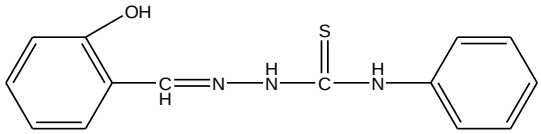

Figure 1. Salicylaldehyde 4-phenylthiosemicarbazone, SaOHtsc, L^1

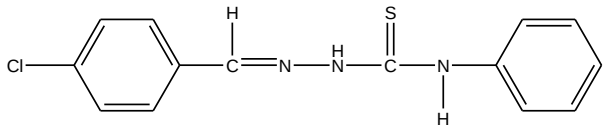

Figure 2. *p*-chlorobenzaldehyde 4-phenylthiosemicarbazone, ph-HClbtsc, L^2

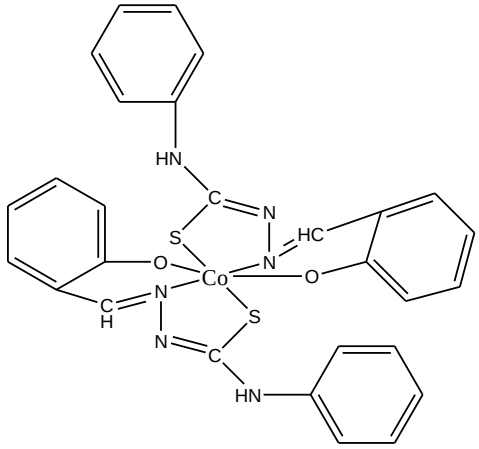

Figure 3. Co(II) salicylaldehyde 4-phenylthiosemicarbazone, CoSaOHtsc (**1**)

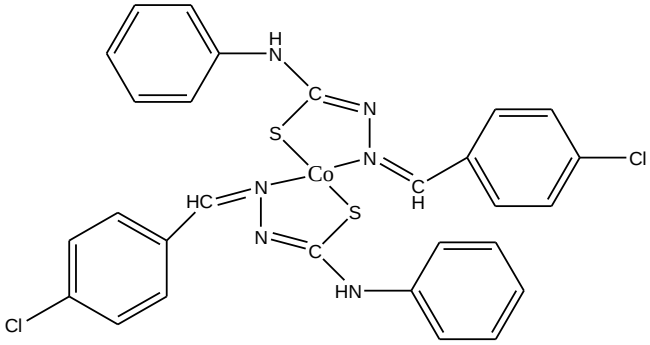

Figure 4. Co(II) *p*-chlorobenzaldehyde 4-phenylthiosemicarbazone, Coph-HClbtsc (**2**)

Antibacterial and antifungi test

The ligands and the complexes were tested for antibacterial and antifungi activities. The test organisms chosen for antibacterial activity were two Gram positive and two Gram negative while for antifungi activity were only one fungi species. The screening property was done by the disc diffusion method. The bacterial cultures were maintained in nutrient agar slants and fungi in potato dextrose. The diameter of inhibition zone resulting with DMSO for both antibacterial and antifungi activities was measured and compared with standard values.

Bacteria Gram(+)	Bacteria Gram(-)	Fungi
i. B. Subtilis	i. *E. Coli*	i. *Candida Albican*
ii S. Epidermis	ii. *P. Mirabilis*	

Results and Discussion

Infrared spectra

Selected vibration bands of ligands and its metal complexes are given in Table 1 and Table 2.

Table 1. IR spectral assignments for ligands

Molecular formula	**wavenumber (cm^{-1})**				
	C=N	N-N	C=S	N-H	C-N
SaOHtsc, L^1	1620	1079	1152	3308	1033
ph-HClbtsc, L^2	1597	1086	1199	3311	1013

Table 2. IR spectral assignments for cobalt(II) complexes

Compounds	wavenumber (cm^{-1})						
	C=N	N-N	C-S	N^1-H	C-N	M-N	M-S
CoSaOHtsc (**1**)	1596	1081	650	3357	1250	500	352
Coph-HClbtsc (**2**)	1594	1093	661	3386	1251	536	353

In principle, Qing et al. 2006 [6] said that the ligands can exhibit thione-thiol tautomerism, since it contains a thioamide –NH-C=S functional group. The vibration of ν(S-H) band at 2600-2800 cm^{-1} is absence from the IR spectral of the ligands but the vibrations of ν(N^2H) band at 3308 and 3311 cm^{-1} where corresponding ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ are present indicated that in the solid state the ligands remain as the thione tautomer. In the complexes spectral, the vibration of ν(N^2H) band in the ligands spectral disappears which indicates the deprotonation of the –N^2H proton and coordination of azomethine nitrogen atom to the central metal ion, Co(II). The bands appearing at 1152 and 1199 cm^{-1} which assigned to the ν(C=S) band for the ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ are absent in the spectral of the complexes. While the vibration band of ν(C-S) are appeared at 650 and 661 cm^{-1} in the spectral of the complexes which confirmed the coordination occur through the sulfur atom to the metal ion. For the ligand $\mathbf{L^1}$, the broad band of ν(OH) overlapping with ν(N^2H) at 3308 cm^{-1}. The vibration band of ν(OH) deprotonated and coordinate to the central metal ion through oxygen atom. The coordination between azomethine nitrogen, thiolate sulfur and oxygen atom from the ligand $\mathbf{L^1}$ to the Co(II) ion can be approved by the presence of bands at 500, 352 and 466 cm^{-1} which the ligand act as dinegative tridentate. Whereas for the ligand $\mathbf{L^2}$, the coordination between azomethine N and thiolate S atoms to the Co(II) ion can be approved by the presence of bands at 536 and 353 cm^{-1} where the ligand act as mononegative bidentate.

^{1}H and ^{13}C NMR

Table 3. Selected chemical shifts of ligands and its metal complexes for ^{1}H NMR.

Compounds	Chemical shift, δ (ppm)			
	N(2)H	**N(1)H**	**Aromatic**	**Aliphatic (C-H)**
SaOHtsc, $\mathbf{L^1}$	10.04, 1H (s)	11.70, 1H (s)	6.83-8.04, 9H (m)	8.48, 1H (s)
ph-HClbtsc, $\mathbf{L^2}$	10.16, 1H (s)	11.84, 1H (s)	7.20-7.96, 9H (m)	8.14, 1H (s)
Coph-HClbtsc (**1**)	-	9.51, 1H (s)	6.65-7.71, 9H (m)	8.74, 1H (s)

Table 4. Selected chemical shifts of ligands and its metal complexes for ^{13}C NMR.

Compound	Chemical shift, δ (ppm)			
	C=N	**C=S**	**C-S**	**Aromatic**
SaOHtsc, $\mathbf{L^1}$	140.81	176.23	-	116.53-139.53
ph-HClbtsc, $\mathbf{L^2}$	141.94	176.63	-	125.89-139.51
Coph-HClbtsc, (**2**)	171.21	-	158.12	116.34-141.47

The ^{1}H and ^{13}C NMR spectra of all the ligands and complexes have been taken in DMSO-d_6. The ligands does not show any peak attributable to S-H proton in the ^{1}H NMR spectra but the spectra show the signal of ν(N^2H) at 10.04-10.16 ppm which indicates that the ligand exist as thione tautomer. Aromatic protons appear as a multiplet at 6.83-8.04 ppm and 7.20-7.96 ppm which is assigned for the ligands $\mathbf{L^1}$ and $\mathbf{L^2}$. The diamagnetic Co(II) complex (**2**) is soluble in DMSO-d_6, thus when the spectral data are compared with the free ligands, several differences can be observed. The peak corresponding to the proton N^2H is absent in the spectrum of complex which is evidence of the deprotonation of the ligand and coordination between azomethine N with metal centre. The chemical shift for aliphatic hydrogen, C-H in spectrum complex (**1**) shifted downfield to be compared with its ligand where the signal appeared to be at 8.74 ppm. This happens due to the influenced of coordination between azomethine N with Co(II) [7]. The proton OH in the spectrum of ligand $\mathbf{L^1}$ at 10.01 ppm is absent in spectrum of complex (**1**) which is the evidence of deprotonation of OH and coordination between O phenolic with metal centre [4]. The chemical shift for C=S in the spectral of ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ appeared at the lowest downfield which is at the range 176.23 and 176.63 ppm. The phenomenon happens due to the influence of electronegativity of N and S atoms that attached it. The peak of C=S is absent in the spectrum of ligand $\mathbf{L^1}$ but the peak of C-S appear at the range 158.12 ppm in spectrum of complex (**1**) which is the evidence that coordination occur between thiolate S with Co(II).

Antibacterial properties

The antibacterial properties of the ligands and complexes were determined by the standard "disc diffusion' method. The bacteria were growth in nutrient agar slants. The compounds to be tested were dissolved in DMSO and soaked in filter paper disc No.3. The disc incubated at 28°C for 28 h. The diameter clearing zone around the disc was measured which the diameter indicated the inhibitory activity of the compound on the bacteria. The results of antibacterial studies were given in Table 5 and 6.

Table 5. Diameter clearing zone around the disc for bacteria G(+).

Compound	*B. Subtilis* (cm)	*S. Epidermis* (cm)
Standard (*Tobramycin)*	1.7	1.7
SaOHtsc, $\mathbf{L^1}$	0.6	1.0
ph-HClbtsc, $\mathbf{L^2}$	0.7	0.6
CoSaOHtsc , (**1**)	1.0	0.7
Coph-HClbtsc, (**2**)	0.7	1.8

Table 6. Diameter clearing zone around the disc for bacteria G(-)

Compound	*E. Coli* (cm)	*P. Mirabilis* (cm)
Standard (*Tobramycin)*	1.2	1.5
SaOHtsc, $\mathbf{L^1}$	0.7	0.7
ph-HClbtsc, $\mathbf{L^2}$	0.7	0.7
CoSaOHtsc, (**1**)	0.6	0.6
Coph-HClbtsc, (**2**)	0.7	0.6

From the Table 5 and 6, the ligands and Co(II) complexes are found to be moderate active against bacteria. While the compound (**1**) was very active against *S. Epidermis sp*. The compound have electron withdrawing group which will increase the activity of antibacterial. The velocity of compound to diffuse into membrane cell was depends on the density of electron in the compound. The faster the compound to diffuse into membrane cell, more active antibacterial activity of the compound [2].

Conclusion

The coordination ability of the thiosemicarbazone derivatives as mononegative and dinegative bidentate through thiolate sulfur, azomethinic nitrogen and phenolic oxygen has been proved in complexation reaction with Co(II) and characterization with elemental analysis, NMR and IR spectroscopy. The biological behaviour revealed that most of the ligands are moderate active as antibacterial activity. While the complex (**2**) are very active as antibacterial against *S. Epidermis* sp due to presence of withdrawing group in the complex structure.

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