Article

In Vitro Antibacterial Activities of Aniline Dithiocarbamate Crystals with its Corresponding Oxovanadium(IV) and Zinc(II) Coordination Compounds

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Abstract: Antibacterial activities can be improved using mixed ligands. Mixed ligands involved in this research are sodium sulfadiazine (Na-sfz) and dithiocarbamate (ai-dtc). One-pot synthesis was used to synthesize ligand of aniline dithiocarbamate (ai-dtc) and the corresponding coordination compounds of [VO(sfz)(ai-dtc)] and [Zn(sfz)(ai-dtc)]. Crystals of ai-dtc, which grew from the solution when refrigerated after five days, were diffracted with technique of single crystal x-ray crystallography to reveal the structure. Other characterization techniques involving physicochemical parameters, FT-IR, UV-Vis and NMR (^{1}H NMR and ^{13}C NMR) were carried out on ligands of ai-dtc, sfz and corresponding coordination compounds. Differences in results of FT-IR, UV-Vis and NMR between ligands and their respective metal ions confirmed the coordination. The *in vitro* antibacterial studies showed that the ligands (not the metal complexes) had modest activity against Gram negative bacteria: *Staphylococcus aureus*, whereas, the coordination compounds had modest activities against the Gram negative bacteria: *Escherichia coli and Pseudomonas aeruginosa*.

Keywords: one-pot synthesis; single crystal x-ray crystallography; oxovanadium(IV); zinc(II); spectroscopic studies; *in vitro* antibacterial studies.

1. Introduction

Outbreak of bacterial infections affected some developed and developing countries [1]. Besides, when bacterial infections reemerge, they become very active giving rise to *Multiple Drug Resistance* (MDR). Factors which contribute to reemergence are wrong diagnosis, prescriptions without doctor's advice and patients' incorrect uses of antibiotics [1]. In order to control MDR, this paper reported the use of chemotherapeutic approach. The reagents involved in the chemotherapy are sulfadiazine (Na-sfz) and dithiocarbamate (ai-dtc) as mixed ligands to litigate aqueous oxovanadium(IV) and zinc(II) ions respectively to form oxovanadium(IV) and zinc(II) coordination compounds. Sulfadiazine (sfz) is an approved antibacterial drug recognized by World Health Organization (WHO) [2]. It is used as a topical antibacterial agent in victims of burns [3], and can act a twofold antibacterial agent against both Gram positive and Gram negative bacteria [4]. On the other hand, dithiocarbamates (dtc) are compounds that consist of carbon, nitrogen and sulfur atoms [5, 6]. Their biological activities were reported to have been widely utilized in medicine, metalloenzymes, fungicides, herbicides and insecticides [5-8]. Recently, dithiocarbamates were combined with heterocyclic compounds to enhance their biological activities [9]. Shifting the focus on the metal ions used for this research, Rehder in one of his papers wrote that, "vanadium compounds possess pharmaceutical activities to treat bacterial and viral infections" [10]. From Arafat et al's paper, they also wrote that zinc(II) compounds can be medically applied to treat parasitic diseases. They further

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said, "when ligands are coordinated to zinc(II) compounds, they give structural and functional models" [11]. The choice of vanadium and zinc metals was got from Rehder and Arafat et al. Other factors of moderate toxicities, essential metals in human bodies and cost effectiveness were considered for choosing of vanadium and zinc metals. In order to apply the synthesized oxovanadium(IV) and zinc(II) coordination compounds for antibacterial studies, this study focused on four bacteria namely; *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* because they are the common bacteria which cause infections. The aim of this research was to assess the prospects of sulfadiazine, aniline dithiocarbamate, as well as, oxovanadium(IV) and zinc(II) coordination compounds as antibacterial agents.

2. Experimental

2.1. Materials and methods

2.1.1. Materials

Aniline was obtained from Merck (Germany), sodium hydroxide was bought from Merck, (Republic of South Africa), Sodium sulfadiazine salt (Na-sfz) and Vanadium(IV) oxide hydrate were purchased from Sigma-Aldrich (USA), Anhydrous zinc(II) chloride ($ZnCl_2$) and carbon(IV) sulfide (CS_2) were supplied by Associated Chemical Enterprises (Pty) Ltd (RSA). Deionised water was produced in house. All the chemicals were of analytical reagent grade and were used as bought, without further purification.

2.1.2. Instrumentations

Open-end capillary tube melting point determination was carried out on a STUART SMP11 melting point apparatus and recorded uncorrected. Molar conductivity of compound was recorded on a CRINSON EC- Meter BASIC 30+ conductivity meter. The UV-Vis spectra were measured using a Perkin-Elmer Lambda 25 UV-Vis Spectrometer. The FT-IR spectra were recorded from a KBr disc in the range of 370-4000 cm^{-1} on a Perkin-Elmer 2000 FT-IR Spectrophotometer. The NMR spectra were recorded on a Varian *Unity Inova* 400 NMR spectrometer operating at frequencies of 400 MHz for ^{1}H NMR and at 150 MHz for ^{13}C NMR frequencies. The ^{1}H NMR and ^{13}C NMR were determined from solutions of the compounds in DMSO-d_6. The chemical shifts were in ppm in relation to internal standard of TMS.

2.1.3. Methods

Compounds of ai-DTC, [VO(sfz)(ai-dtc)] and [Zn(sfz)(ai-dtc)] were synthesized using the method of one pot synthesis [5].

- Sodium sulfadiazine ($C_{10}H_9N_4NaO_2S$); Na-sfz

It was used as bought as the first ligand between the mixed ligands.

White solid. Assay: ≥ 98%. M. P.> 300 °C. Molar Conductivity: 8.16 Ω^{-1} cm^2 mol^{-1}, Selected FT-IR (KBr Pellets), v (cm^{-1}): 3404 $(NH_2)_{as}$ 3268 $(NH_2)_s$; 3237 (SO_2NH); 1577 (C=N); 1229 $(SO_2)_{as}$; 1115 $(SO_2)_s$. Selected λ_{max} in DMSO solvent (nm): 274 (π-π^*, N-C=S); 319 (π-π^*, S-C=S); 322, (n- π^*). ^{1}H NMR (DMSO-d_6, 400Hz, ppm): δ 8.10 (s, 1H, SO_2-NH_2); δ 7.47-7.48 (s, 1H, NH_2) δ 6.87-7.19 (m, 1H, C_6H_5-H) δ 5.40 (s, 1H, C=N). ^{13}C NMR (DMSO-d_6, 100.6 MHz, ppm) δ 164.19 (CSO_2-NH_2), δ 157.19 (^{13}C-NH_2); δ 149.17 (N=^{13}CH); δ 105.10-135.20 (C_6H_5-). The molecular structure of Na-sfz is shown in Figure 1.

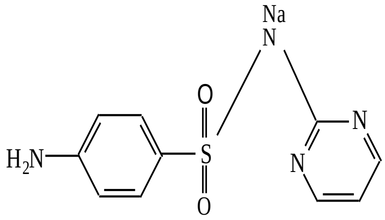
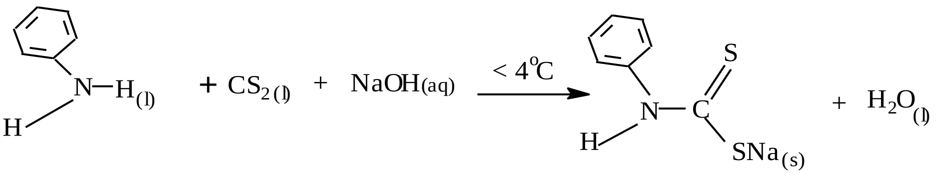


Figure 1: Sodium sulfadiazine (Na-sfz).

(i). Synthesis of sodium aniline dithiocarbamate (ai -dtc)

Aniline (9.11 mL, 0.10 mol), carbon(IV) sulfide (6.00 mL, 0.10 mol) and sodium hydroxide (4.00 g, 0.10 mol) were used as starting materials. Whitish yellow crystals were obtained. Percentage Yield: 90%. M. P. 72 °C. Molar Conductivity: 1.09 Ω^{-1} cm^2 mol^{-1}. Selected FT-IR (KBr Pellets), v (cm^{-1}): 3409 $(NH_2)_{as}$ 3277 $(NH_2)_s$; 1517 (C-N), 981 (CS_2). Selected λ_{max} in DMSO solvent (nm): 246 (π-π^*, N-C=S); 317 (π-π^*, S-C=S); 381 (n- π^*) 381. ^{1}H NMR (DMSO-d_6, 400Hz, ppm) δ 10.00 (s, 1H, NCS_2); δ 7.19-7.89 (d, 2H NH_2); δ 6.87-7.16; 7.16-7.19 (d, t, m, Ar-H). ^{13}C NMR (DMSO-d_6, 150 MHz, ppm) δ 214.93 (NCS_2), δ 143.76 (s 1H NH_2), δ 137.23 (s 1H HC-N), 125.68-128.85 (C_6H_5-H); The synthesis for ai-dtc is shown in Scheme 1.



(ii). Synthesis of oxovanadium(IV) coordination compound of [VO(sfz)(ai-dtc)]

Vanadium (IV) oxide sulfate hydrate (0.25 g, 1.50 mmol), solution of sodium sulfadiazine, L_1, (0.41 g, 1.50 mmol) in methanol (100.00 mL) and aniline dithiocarbamate $L_{2ai\text{-}dtc}$, (0.29 g, 1.50 mmol) were magnetically stirred for 3 h at room temperature. A light green solid precipitate was formed, filtered, washed with deionized water (3 x 5 mL) and dried over silica gel in a desiccator. Percentage Yield: 75%. M. P. 250 °C. Molar Conductivity (DMSO): 0.74 Ω^{-1} cm^2 mol^{-1}. Selected FT-IR (KBr Pellets), v (cm^{-1}): 3464 $(NH_2)_{as}$ 3227 $(NH_2)_s$; 3150 (SO_2NH), 1615 (C=N), 1528 (C-N), 1223 $(SO_2)_{as}$; 1145 $(SO_2)_s$ 1002(CS_2) ; 934 (V=O), 395 (V-N) 466 (V-S). Selected λ_{max} in DMSO solvent (nm): 319 (π-π^*, N-C=S); 322 (π-π^*, S-C=S); 355 (n- π^*); Band I: 828, 738; Band II: 617; Band III: 396. The synthesis for oxovanadium(IV) coordination compound of [VO(sfz)(ai-dtc)] is shown in Equation 1.

$$\text{Na-sfz}_{(aq)} + \text{ai-dtc}_{(l)} + VOSO_4.xH_2O_{(s)} \rightarrow [VO(sfz)(ai\text{-}dtc)]_{(s)} + Na_2SO_4.\ xH_2O_{(aq)} \text{.... (i)}$$

(iii). Synthesis of zinc(II) coordination compound of [Zn(sfz)(ai-dtc)]

Sodium sulfadiazine (0.4085 g, 1.5 mmol), sodium aniline dithiocarbamate ($L_{2ai\text{-}dtc}$) (0.29 g, 1.50 mmol) and $ZnCl_2$ (0.20 g, 1.5 mmol) were used. A white solid was formed, washed and dried over silica gel. Yield: 72%. M. P. 216-218 °C. Molar Conductivity (DMSO): 0.01 Ω^{-1} cm^2 mol^{-1}. Selected FT-IR (KBr), v(cm^{-1}): 3400 $(NH_2)_{as}$ 3222 $(NH_2)_s$; 3090 (SO_2NH), 1518 (C=N), 1487(C-N), 1246 $(SO_2)_{as}$; 1141 $(SO_2)_s$. 963$(CS_2)_{as}$, 391 (Zn-N), 434 (Zn-S). Selected λ_{max} in DMSO solvent (nm): 235, 256 (π-π^*, N-C=S); 292 (π-π^*, S-C=S); 382, (n- π^*). Selected ^{1}H NMR (DMSO-d_6, 400Hz, ppm): δ 10.08-10.11 (d 1H, H-NCS_2), δ 9.78; 8.47-8.50 (m, 1H, SO_2NH_2); δ 7.92 (-NH_2); δ 6.56-7.92 (d, t, Ar-H); δ 5.99 (s, 1H, C=H). Selected ^{13}C NMR (DMSO-d_6, 150 MHz, ppm); δ 179.43 (NCS_2), δ 158.23(-SO_2N=C), 139.10 (NH_2C); δ112.14-128.44(Ar-C) δ. The synthesis for zinc(II) coordination compound of [Zn(sfz)(ai-dtc)] is shown in Equation (ii).

$$\text{Na-sfz}_{(aq)} + \text{ai-dtc}_{(l)} + ZnCl_{2(s)} \rightarrow [Zn(sfz)(ai\text{-}dtc)]_{(s)} + 2NaCl_{(aq)} \text{.... (ii)}$$

2.2. Crystal growth and crystallographic activities

Crystals of aniline, ($C_7H_6NNaS_2$.$3H_2O$) were grown out of solution when refrigerated after the fifth day. Crystals were washed with diethyl ether and subjected to x-ray diffraction. The crystals were collected and mounted in a four circles diffractometer Gemini of Oxford Diffraction, using a graphite monochromated CuKα radiation (λ = 1.54184 Å). Super flip program was used to solve the crystal structure [12, 13], as well as, refinement done using full matrix least-squares technique with the support of F_2 with Jana 2006 [14]. DIAMOND program was also used to shape the structural graphics [15, 16].

2.3. Antibacterial screening and mechanism of action of antibacterial agents

The ligands of Na-sfz and ai-dtc, as well as, coordination compounds of oxovanadium(IV) and zinc(II) were screened against two Gram positive bacteria (*Staphylococcus aureus* MRSA252 and *Enterococcus faecalis* ATCC 19433) and two Gram negative bacteria (*Escherichia coli* MC4100 and *Pseudomonas aeruginosa* PAO1) species. The antibacterial screening method used was Kirby-Bauer Method [17].

The mechanism of action entails the biological effects of the antibacterial agents (studied compounds) on the four bacterial strains through interaction.

3. Results and Discussion

The two ligands (Na-sfz and ai-dtc) were mixed and coordinated with oxovanadium(IV) and zinc(II) ions to yield metal complexes of [VO(sfz)(ai-dtc)] and [Zn(sfz)(ai-dtc)].They were characterized to obtain their physicochemical, spectroscopic, nuclear magnetic resonance, crystals and antibacterial characteristics.

3.1. Physicochemical properties

The chemical reaction of mixed ligands of white solids of Na-sfz and ai-dtc with hydrated blue oxovanadium(IV) ion yielded a coordination compound of light green solid precipitate of [VO(sfz)(ai-dtc)]. For synthesized zinc(II) of coordination compound of [Zn(sfz)(ai-dtc)], a white solid was formed compared to the mixed ligands of sfz and ai-dtc. Ligands were soluble in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), methanol and deionized water, while the two coordination compounds are stable at room temperature, insoluble in deionized water, but soluble in DMF and DMSO solvents. Ligand of sfz and the two coordination compounds have higher melting points than ai-dtc, which might be due to their higher molecular masses [17]. All compounds have molar conductivities of less than 20 $\Omega^{-1}cm^{2}$ mol^{-1}, which showed they are non electrolytic in nature [17]. Table 1 shows the physicochemical parameters for all the compounds.

Table 1: Physicochemical parameters for studied compounds.

Compounds	**Molecular formulae**	**Colour and state of matter**	**Assay/Yield (%)**	**Melting point (0°C)**	**Molar conductivity (Ω^{-1} cm^{2} mol^{-1})**
Na-sfz	$C_{10}H_9N_4NaO_2S$	white solid	≥ 98	> 300	8.16
ai-dtc	$C_7H_6NS_2Na$	whitish yellow crystals	90	72	1.09
[VO(sfz)(ai-dtc)]	$C_{17}H_{15}N_5S_3O_3Na_2V$	green solid	75	250	0.74
[Zn(sfz)(ai-dtc)]	$C_{17}H_{15}N_5S_3O_2Na_2Zn$	white solid	72	216-218	0.01

3.2. Infra-red spectroscopy

- Coordination compound of [VO(sfz)(ai-dtc)]

Bellú et al reported that the bands which appeared near 3500 and 3400 cm^{-1} were due to asymmetric amino group $(NH_2)_{asy}$ and symmetric amino group $(NH_2)_{sy}$ [18]. The FT-IR of asymmetric amino group in Na-sfz at 3404 $cm^{-1}{}_{(m)}$ shifted slightly to lower frequency in the coordination compound of [VO(sfz)(ai-dtc)] appearing with a sharp peak at 3401$cm^{-1}{}_{(s)}$. Similarly, the FT-IR of symmetric amino group has a frequency of 3268 cm$^{-1}_{(m)}$ in Na-sfz and also shifted to lower frequency in coordination compound of [VO(sfz)(ai-dtc)] at 3227 cm$^{-1}_{(sh)}$. The FT-IR wavelengths are within 3500 and 3400 cm^{-1} as reported by Bellú et al [18]. The slight differences between the ligand and the coordination compound indicate no involvement of the two amino groups in coordination [3, 18].

For azomethine group (C=N), a medium band at 1584 cm$^{-1}_{(m)}$ appeared in Na-sfz, but shifted to higher and sharp frequency at 1615 cm$^{-1}_{(s)}$ in [VO(sfz)(ai-dtc)]. This indicates a difference of 31 cm^{-1} compared with Na-sfz. The shift to higher frequency is in support of Athar et al study and confirms the presence of the azomethine band in the coordination [19]. Medium bands at 1229 cm$^{-1}_{(m)}$ and 1115 cm$^{-1}_{(m)}$ can be assigned to the assymmetric and symmetric v(SO$_2$) respectively in Na-sfz but in [VO(sfz)(ai-dtc)] there are slight changes of 1223 cm$^{-1}_{(m)}$ and 1145 cm$^{-1}_{(m)}$ which could be assigned to the assymmetric and symmetric v(SO$_2$) respectively [18, 19]. The results due to slight difference agree with Bellú et al and Athar et al that the sulfonamide oxygen (SO$_2$NH) did not participate in the coordination to metal ion [18, 19]. The stretching vibration of the terminal V=O bond in [VO(sfz)(ai-dtc)] appeared with a sharp signal at 937 cm$^{-1}_{(shp)}$ [20], while the V-N band has a frequency of 395 cm$^{-1}_{(m)}$ [20].

According to Ajibade et al, infrared spectra of coordination compounds have three distinct regions, which are region of 1580-1450 cm^{-1} for the thioureide band ν(C-N)) band, region of 1060-940 cm^{-1} for the ν(C=S) and region of 430-250 cm^{-1} for the ν(M-S) [3]. Ligand of ai-dtc stretching vibration for ν(C-N) appeared sharply at a frequency of 1453 cm^{-1}, while it shifted to higher frequency in [VO(sfz)(ai-dtc)] with a small value of 1528 cm^{-1}. The presence of the thioureide bond between double bond of ν(C=N) and single bond of ν(C-N) indicates partial delocalization of the π electron density of the thioureide bond [21]. Similarly, the ν(C=S) for ai-dtc appeared with a stretching vibration at 985 cm^{-1} and also shifted to higher frequency in [VO(sfz)(ai-dtc)] with a medium value of 1002 cm^{-1}.The ν(V-S) wavenumber is 466 cm^{-1}.

- Coordination compound of [VO(sfz)(ai-dtc)]

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For azomethine group (C=N), a medium band at 1584 cm$^{-1}_{(m)}$ appeared in Na-sfz, but shifted to higher and sharp frequency at 1615 cm$^{-1}_{(s)}$ in [VO(sfz)(ai-dtc)]. This indicates a difference of 31 cm^{-1} compared with Na-sfz. The shift to higher frequency is in support of Athar et al study and confirms the presence of the azomethine band in the coordination [19]. Medium bands at 1229 cm$^{-1}_{(m)}$ and 1115 cm$^{-1}_{(m)}$ can be assigned to the assymmetric and symmetric v(SO$_2$) respectively in Na-sfz but in [VO(sfz)(ai-dtc)] there are slight changes of 1223 cm$^{-1}_{(m)}$ and 1145 cm$^{-1}_{(m)}$ which could be assigned to the assymmetric and symmetric v(SO$_2$) respectively [18, 19]. The results due to slight difference agree with Bellú et al and Athar et al that the sulfonamide oxygen (SO$_2$NH) did not participate in the coordination to metal ion [18, 19]. The stretching vibration of the terminal V=O bond in [VO(sfz)(ai-dtc)] appeared with a sharp signal at 937 cm$^{-1}_{(shp)}$ [20], while the V-N band has a frequency of 395 cm$^{-1}_{(m)}$ [20].

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- Coordination compound of [Zn(sfz)(ai-dtc)]

The FT-IR of asymmetric amino group in Na-sfz still at 3404 cm$^{-1}_{(m)}$ shifted slightly to lower frequency in the coordination compound of [Zn(sfz)(ai-dtc)] appearing with a medium peak at 3401cm$^{-1}_{(s)}$. I n the same way, the FT-IR of symmetric amino group has a frequency of 3277 cm$^{-1}_{(m)}$ in Na-sfz and also shifted to lower frequency in coordination compound of [Zn(sfz)(ai-dtc)] at 3266 cm$^{-1}_{(sh).}$ The FT-IR wavelengths are within 3500 and 3400 cm^{-1} as reported by Bellú et al [18]. The slight differences between the ligand and the coordination compound indicate no involvement of the two amino groups in coordination [3, 18].

For azomethine group (C=N), a medium band at 1583 cm$^{-1}_{(m)}$ appeared in Na-sfz, but shifted to lower and medium frequency at 1578 cm$^{-1}_{(s)}$ in [Zn(sfz)(ai-dtc)]. This indicates a difference of 5 cm^{-1} compared with Na-sfz. The shift to higher frequency is in support of Athar et al study and confirms the presence of the azomethine band in the coordination [19]. Medium bands at 1225 cm$^{-1}_{(m)}$ and 1112 cm$^{-1}_{(m)}$ can be assigned to the assymmetric and symmetric v(SO$_2$) respectively in Na-sfz but in [Zn(sfz)(ai-dtc)] there are slight changes of 1246 cm$^{-1}_{(m)}$ and 1141 cm$^{-1}_{(m)}$ which could be assigned to the assymmetric and symmetric v(SO$_2$) respectively [18, 19]. The results due to slight difference agree with Bellú et al and Athar et al that the sulfonamide oxygen (SO$_2$NH) did not participate in the coordination to metal ion [18, 19]. The Zn-N band has a frequency of 391 cm$^{-1}_{(shd)}$ [20].

Ligand of ai-dtc stretching vibration for ν(C-N) appeared sharply at a frequency of 1517 cm^{-1}, while it shifted to lower frequency in [Zn(sfz)(ai-dtc)] with a medium value of 1487 cm^{-1}. Similarly, the ν(C=S) for ai-dtc appeared with a stretching vibration at 981 cm^{-1} and also shifted to lower frequency in [Zn(sfz)(ai-dtc)] with a small value of 963 cm^{-1}.The ν(Zn-S) wavenumber is 551 cm^{-1}. Table 2 shows the FTIR results for all compounds.

Thus, the FT-IR results showed that the coordination modes of Na-sfz and ai-dtc litigated to oxidovanadium(IV) and zinc(II) ions respectively.

Table 2: The FT-IR results for all studied compounds.

	ν(N-H)$_{asy}$	**ν(N-H)$_{sy}$**	**ν(C=N)**	**ν(SO$_2$)$_{asy}$**	**ν(SO$_2$)$_{sy}$**	**ν(C-N)**	**ν(CS$_2$)**	**ν(V=O)**	**ν(M-N)**	**ν(M-S)**
Na-sfz	3404$_{(m)}$	3268 (m)	1577$_{(m)}$	1227$_{(m)}$	1115$_{(m)}$	1453$_{(shp)}$	985 (sm)			
ai-dtc	3409$_{(m)}$	3277$_{(m)}$		1225$_{(m)}$	1112$_{(m)}$	1517$_{(sm)}$	981$_{(sm)}$			
[VO(sfz)(ai-dtc)]	3401$_{(shp)}$	3227$_{(shd)}$	1615$_{(m)}$	1223$_{(m)}$	1145$_{(m)}$	1528$_{(sm)}$	1002$_{(sm)}$	937$_{(shp)}$	395$_{(m)}$	466$_{(m)}$
[Zn(sfz)(ai-dtc)]	3400$_{(m)}$	3266$_{(sm)}$	1578$_{(m)}$	1246$_{(sm)}$	1141$_{(shp)}$	1487$_{(m)}$	961$_{(sm)}$		391$_{(shd)}$	551$_{(m)}$

Key: Na-sfz: sodium sulfadiazine, **ai-dtc**: sodium salt of aniline dithiocarbamate, **[VO(sfz)(ai-dtc)]:** coordination compound of oxidovanadium(IV) ion, **[Zn(sfz)(ai-dtc)]:** coordination compound of oxidovanadium(IV) ion, **m**: medium, **shd**: shoulder, **shp**: sharp, **sm**: small.

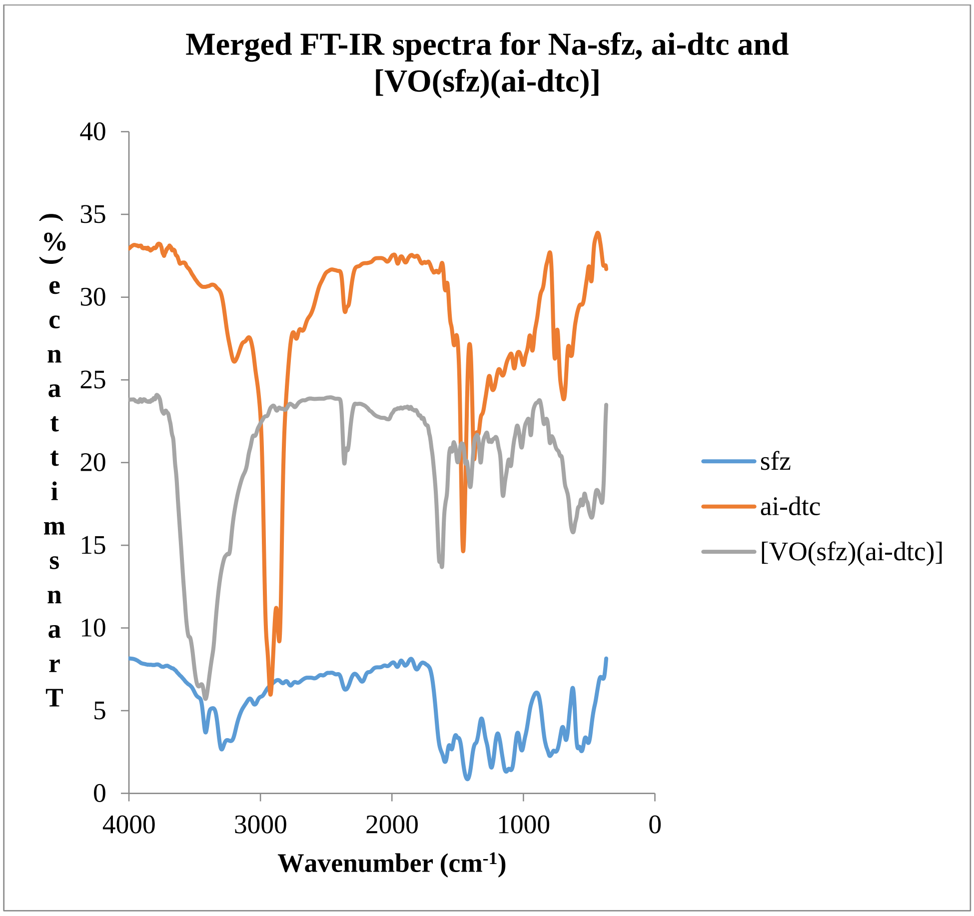

Figure 2: Merged spectra of Na-sfz, ai-dtc and [VO(sfz)(ai-dtc)].

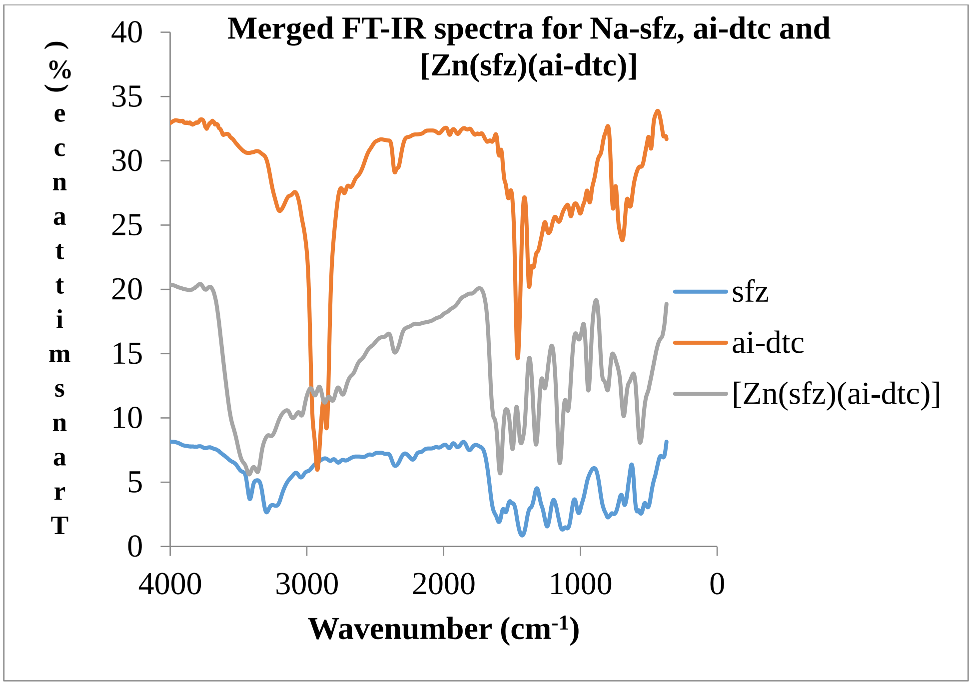

Figure 3: Merged spectra of Na-sfz, ai-dtc and [Zn(sfz)(ai-dtc)].

3.3. Electronic Spectroscopy

The electronic spectra for Na-sfz, ai-dtc, [VO(sfz)(ai-dtc)] [Zn(sfz)(ai-dtc)]were recorded in the ultraviolet-visible range between 200 and 900 nm in 10^{-3} solution of DMSO solvent. The coordination processes of mixed ligands of Na-sfz and ai-dtc were assessed from their electronic spectra. In the ultraviolet-visible region, dithiocarbamates generally show three bands related to intramolecular charge transfer [22]. These bands are π-π^* which corresponds to N-C=S, π-π^* which corresponds to S-C=S and n-π^* [22]. Another transition known as d-d transition was observed in the coordination compound of [VO (sfz)(ai-dtc)], which might be due to excitation of the metal ions [22]. The chromophores of N-C=S and S-C=S were present in both ligands. Coordination compound of oxovanadium(IV) was red shifted with respect to Na-sfz and ai-DTC. The weak d-d transitions consist of Band I (828, 738 nm), Band II (617 nm) and Band III (396 nm). In the case of [Zn(sfz)(ai-dtc)], it was blue shifted when compared with Na-sfz and ai-dtc. The paramagnetic character of [VO(sfz)(ai-dtc)] made it to be assumed to have square pyramidal geometry [20]. The diamagnetic character of [Zn(sfz)(ai-dtc)] made it to be assumed to have distorted tetrahedral geometry [23].

3.4. Nuclear magnetic resonance spectroscopy (NMR)

This study used both proton NMR (^{1}H NMR) and carbon 13 NMR (^{13}C NMR) for structural determination of Na-sfz, ai-dtc and [Zn(sfz)(ai-dtc)]. The paramagnetic nature of [VO(sfz)(ai-dtc)] did not allow the use of ^{13}C NMR .

- Proton NMR (^{1}H NMR)

The ^{1}H NMR spectra for Na-sfz, ai-dtc, and [Zn(sfz)(ai-dtc)] were recorded at room temperature. Deuterated dimethyl sulfoxide (dmso-d_6) as the internal reference. The results were in agreement with to literature [22]. The proton in sulfonamide nitrogen (SO_2-NH_2) of Na-sfz appeared as singlet and has a signal at δ 8.10 ppm (Figure 4), which moved slightly downfield to δ 9.78, 8.50 ppm with doublet in [Zn(sfz)(ai-dtc)] (Figure 6). This indicates it's not involvement in the coordination. The amino group of the Na-sfz, which also appeared singlet at δ 7.47-7.48 ppm also deshielded slightly to δ 7.91 ppm as singlet in [Zn(sfz)(ai-dtc)]. The amino groups are as well not involved in the coordination. The aromatic protons appeared as doublet in Na-sfz because of the presence of the two aromatic rings at δ 6.50 ppm and shifted downfield slightly to δ 6.60 ppm in [Zn(sfz)(ai-dtc)]. The azomethine proton signaled as a singlet in Na-sfz at δ 5.40 ppm and shifted downfield remaining singlet at δ 5.99 ppm in [Zn(sfz)(ai-dtc)]. In the ai-dtc (Figure 5), the dithiocarbamato proton (-$HNCS_2$) as singlet at δ 10.00 ppm shifted downfield slightly to δ 10.11 ppm. The amino group of ai-dtc, appeared doublet at δ 7.19-7.89 ppm also deshielded slightly to δ 7.91 ppm (overlapping) also as doublet in [Zn(sfz)(ai-dtc)].The aromatic protons resonated as doublet and triplet at δ 6.87-7.16 and δ 7.16-7.19 and δ 7.19-7.89 ppm and became deshielded with multiple singlets at δ 6.56- 7.62 ppm in [Zn(sfz)(ai-dtc)].

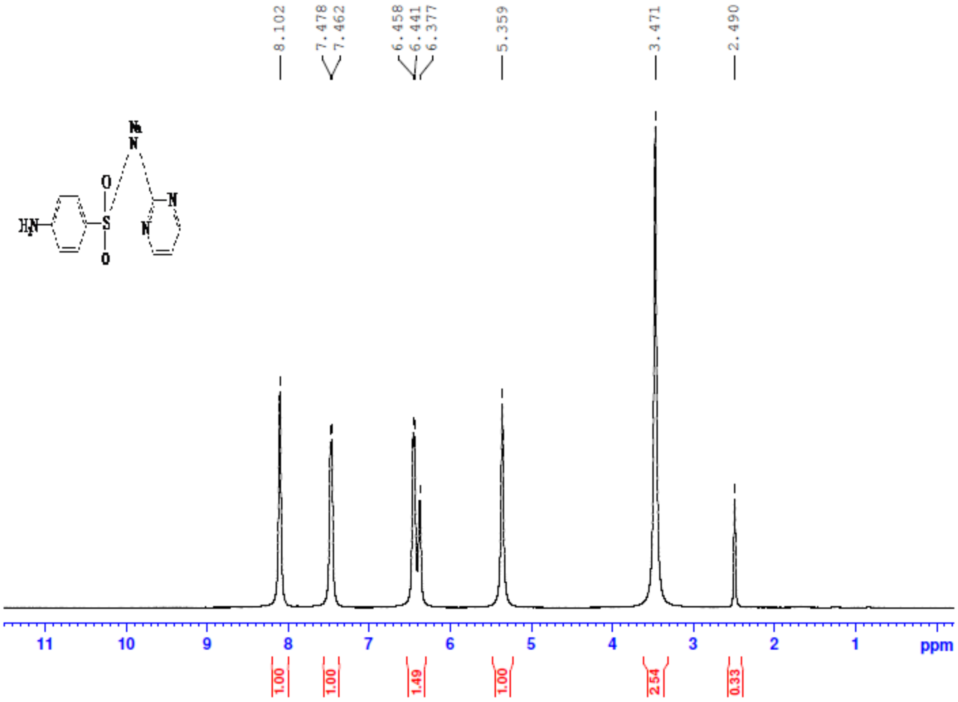

Figure 4: The ^{1}H NMR of Na-sfz.

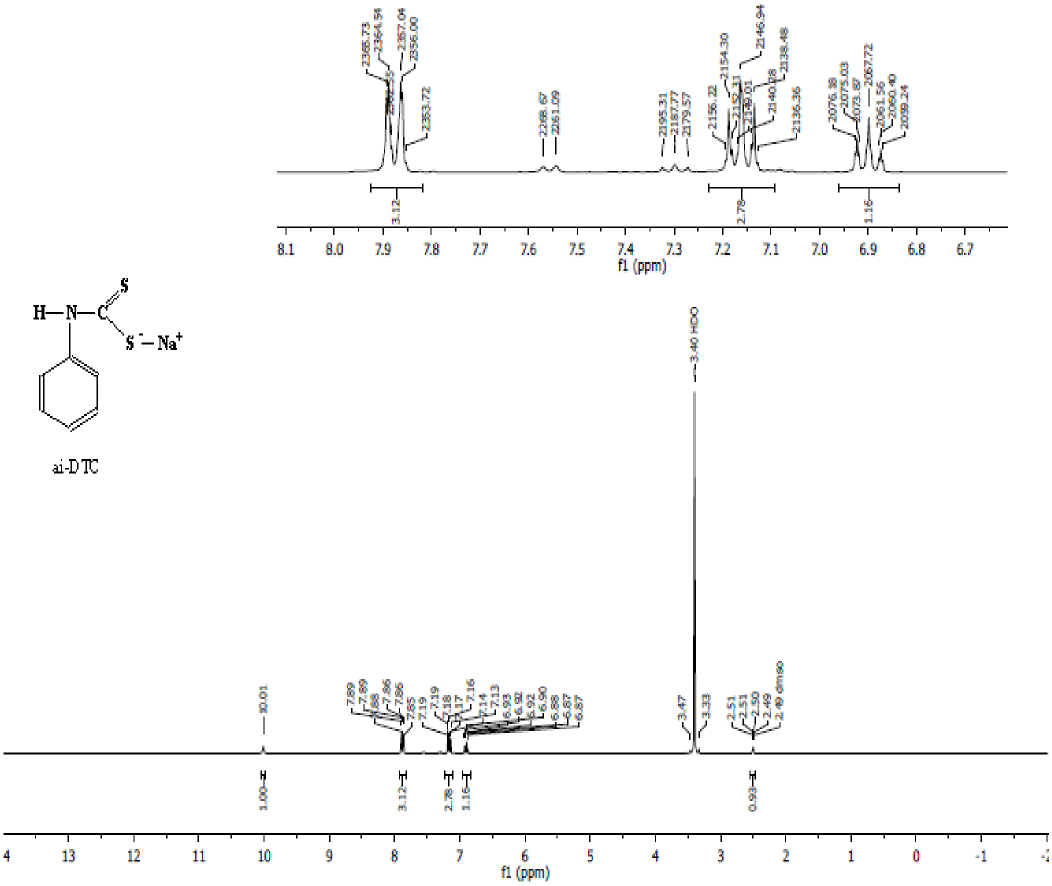

Figure 5: The ^{1}H NMR of ai-dtc.

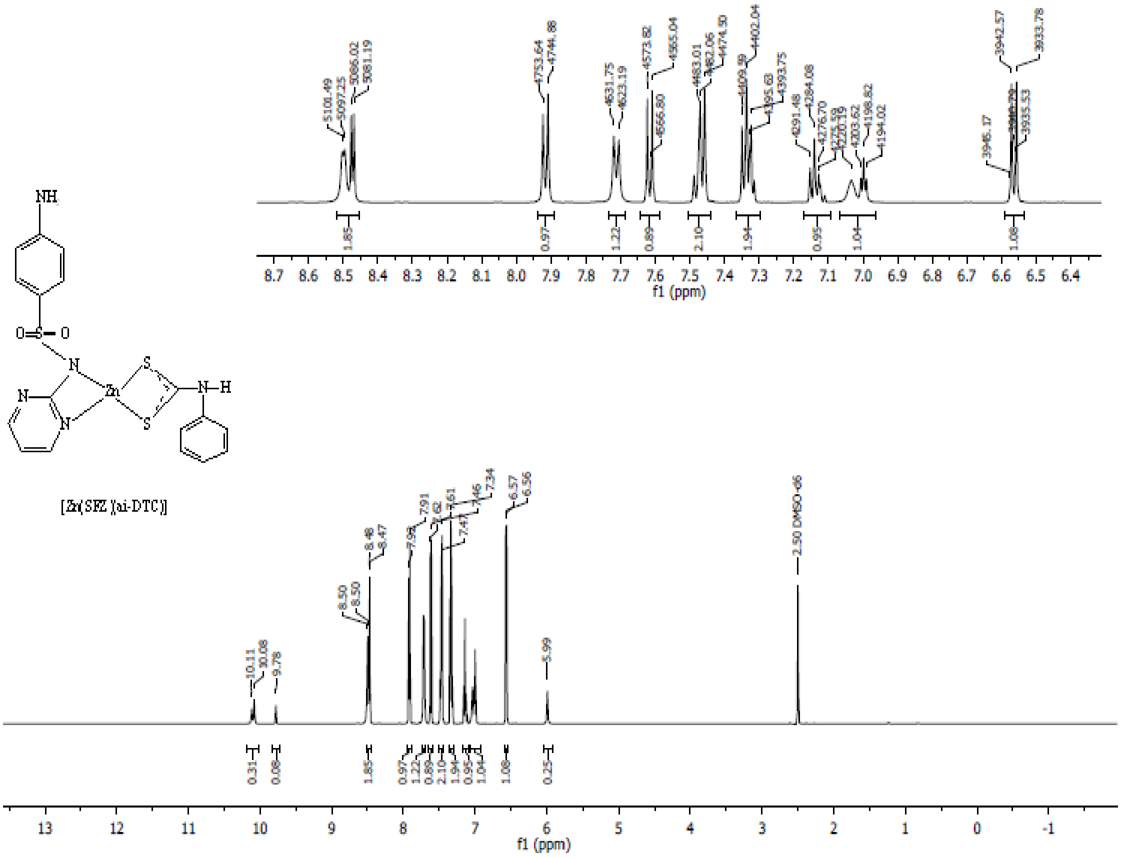

Figure 6: The ^{1}H NMR of [Zn(sfz)(ai-dtc)].

- Carbon 13 NMR (^{13}C NMR) and hybridization

For the hybridization of the carbon atoms, sp^3 hybridized carbon atoms resonate from 0 to 90 ppm, while sp^2 hybridized carbon atoms resonate from 110 to 220 ppm. All the studied compounds, they possess both sp^3 and sp^2 hybridized carbon atoms, therefore, resonance occurred from 0 to 220 ppm.

Carbon of the of aromatic rings resonated in Na-sfz (Figure 7) at δ 105.10, δ 110.91, δ 120.25, δ 135.20 ppm and coincided with the carbons in the aromatic rings of ai-dtc (Figure 8) δ (125.68, 125. 79, 127.94, 128. 85 and 137.23 ppm) for both to have signals which appeared at δ (112.14, δ 121.87, δ 123.74, δ 128.55, δ 128. 44 and δ 139.10 ppm) in [Zn(SFZ)(ai-DTC)] (Figure 9). Carbons of the azomethine group and amino group resonated at δ 149.17 and δ 157.19 ppm respectively, but shifted upfield to δ 158.23 ppm by overlapping in [Zn(sfz)(ai-dtc)], while the carbon of dithiocarbamato moiety resonated at δ 164. 19 ppm and became downshielded to 179.43 ppm in [Zn(sfz)(ai-dtc)]. On the other hand, the thioureide carbon in ai-dtc signaled at 143.76 ppm and resonated downfield to 158. 23 ppm in [Zn(sfz)(ai-dtc)]. Dithiocarbamato moiety, (N^{13}CS$_2$) in ai-dtc resonated at δ 214.93 ppm and had a upfield shifting at δ 179.43 ppm in [Zn(SFZ)(ai-DTC)].

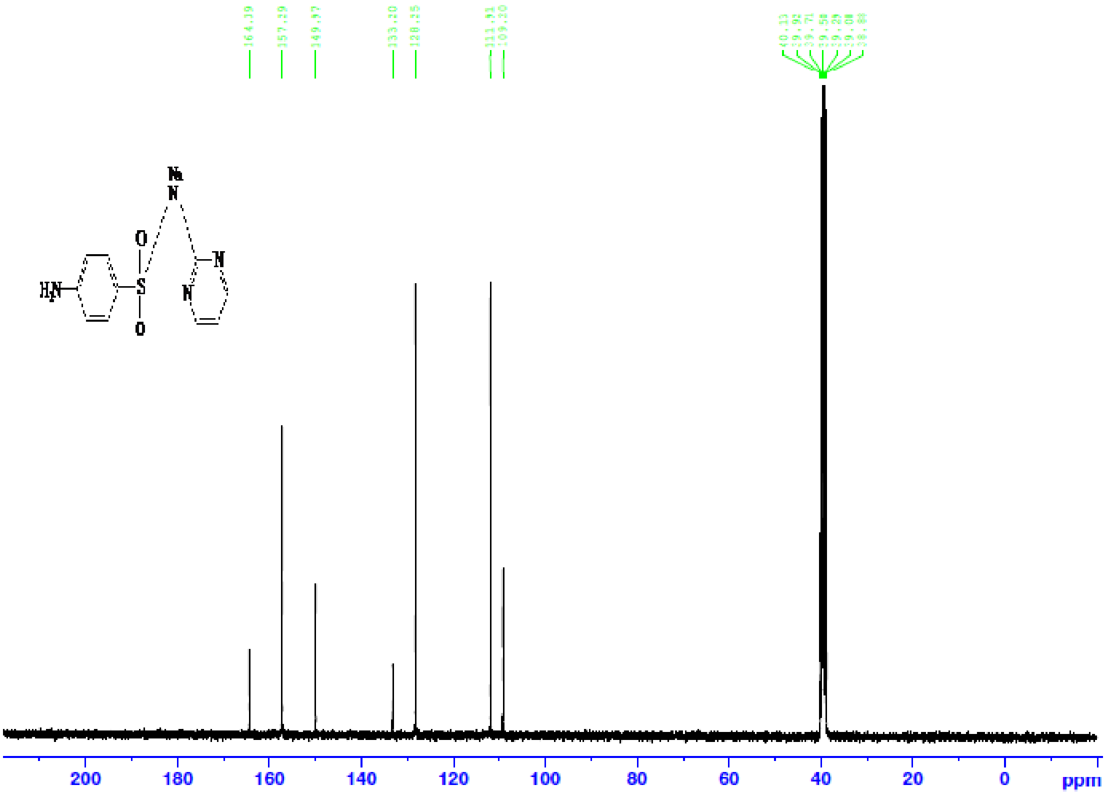

Figure 7: The ^{13}C NMR of Na-sfz.

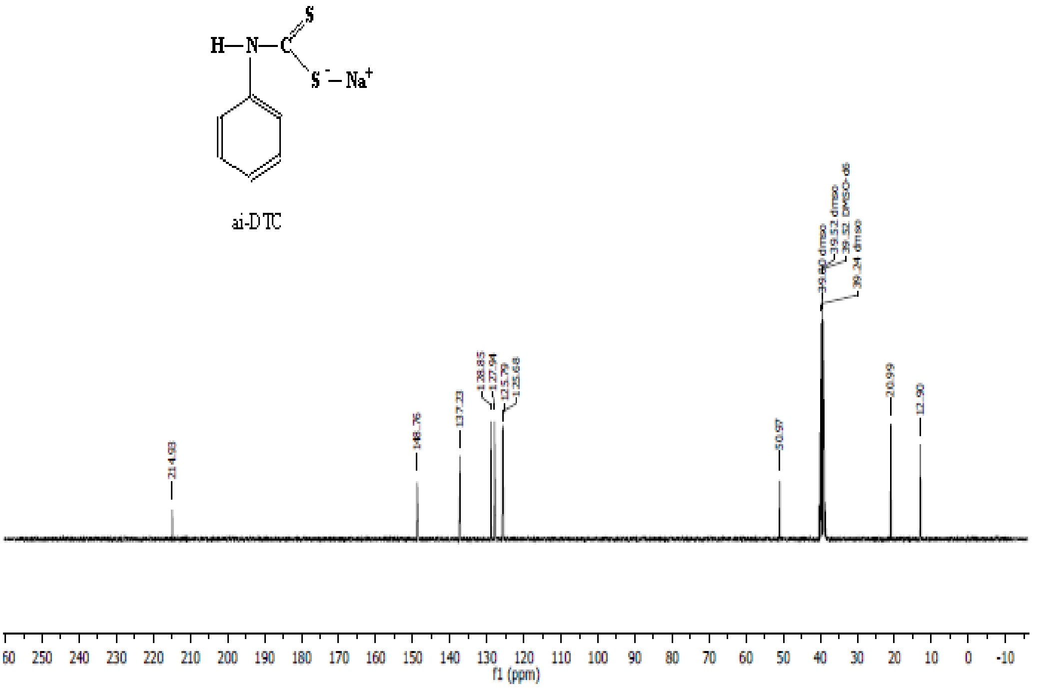

Figure 8: The ^{13}C NMR of ai-dtc.

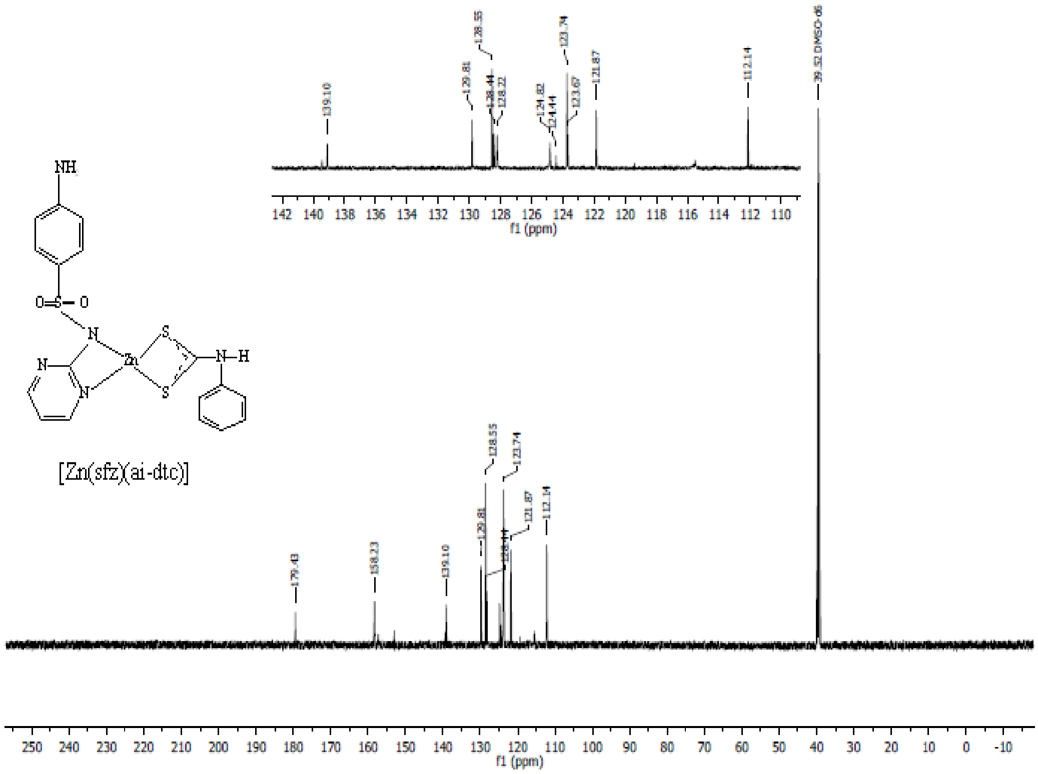

Figure 9: The ^{13}C NMR of [Zn(sfz)(ai-dtc)].

3.5. Crystal data, data collection and refinements of ai-dtc

Computing details

Table 3: Crystal data, data collection special details and refinements.

Crystal data	
$C_7H_{12}NNaO_3S_2$	F(000)=1032.0
M_r = 247.3	D_x= 1.460 Mgm^{-3}
Orthorhombic, Pbcn	Cu Kα radiation, λ=1.54184 Å
Hall symbol: -P2n2ab	Cell psrameters from 10352 reflections
a=286663(14)Å	Θ= 3.9-53.0°
b=6.9386 (3) Å	μ=4.58 mm^{-1}
c=11.3127(3) Å	T=120 K
β=90	Colourless
V=2250.14(16) Å^3	0.52x 0.32x 0.14 mm
Z=8	
Data collection	
Xcalibur, Atlas, Gemini ultra diffractometer	1997 independent reflections
Radiation source: X-ray tube	1773 reflections with I> 3α(I)
Mirror monochromator	R_{int} = 0.033
Detector resolutions:103745 pixels mm^{-1}	$\Theta_{max = 53.1}$ °, $\Theta_{min = 3.9}$ °
ω scans	h= -34→33
Absorption correction: Gaussian Jana2006	k= -8→8
T_{min} = 0.386, T_{max} = 0.536	l= -13→13

doi:10.20944/preprints201802.0021.v1

24029 measured reflections	
Refinement	
Refinement on F^2	98 constraints
$R[F^2 > 3\sigma(F^2)] = 0.063$	H atoms treated by a mixture of independent and constrained refinement
$wR(F^2) = 0.170$	Weighting scheme based on measured s.u.'s $w = 1/(\sigma^2(I) + 0.0016I^2)$
$S = 1.810$	$(\Delta/\sigma)_{max} = 0.025$
1997 reflections	$\Delta)_{max} = 1.19$ e Å^{-3}
149 parameters	$\Delta)_{min} = -1.05$ e Å^{-3}
2 restraints	

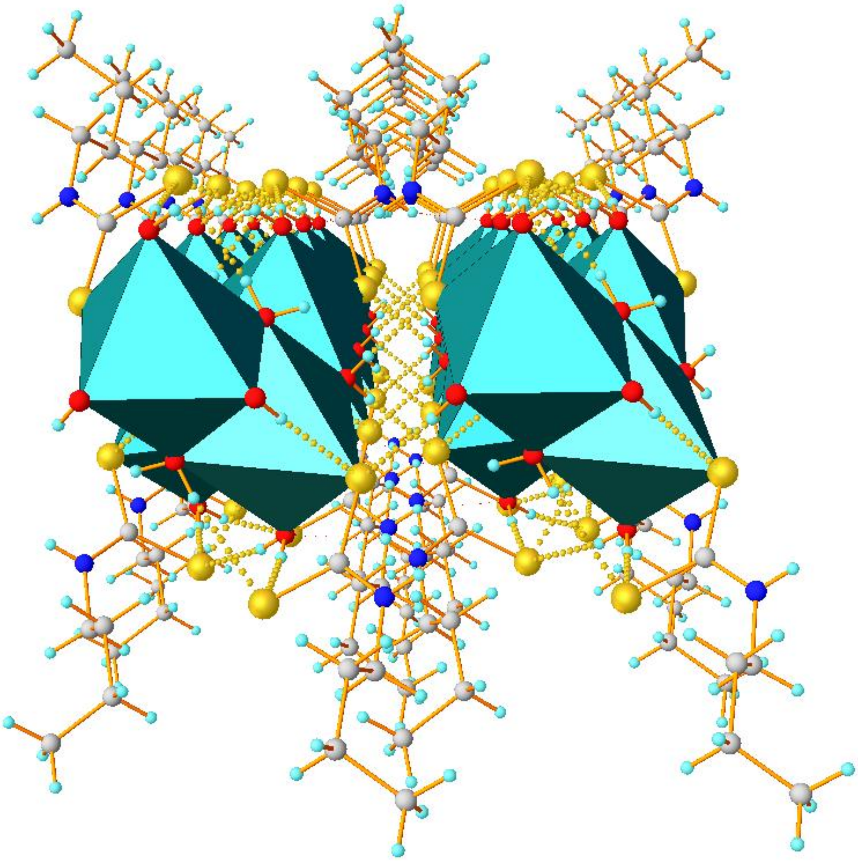

Figure 10: Crystal refinements of ai-dtc**.** (C-Red; N-Blue; O-Oxygen ;S-Sulfur).

3.6. Antibacterial studies and mechanism of action

- Staphylococcus aureus MRSA252

Both ligands (Na-sfz and ai-dtc) were active against *S. aureus* with zone of inhibition of 17 mm and 8 mm respectively, but the corresponding coordination compounds of [VO(sfz)(ai-dtc)]and [Zn(sfz)(ai-dtc)] were inactive.

- Enterococcus faecalis ATCC 19433

None of the ligands and coordination compounds was active against *E. faecalis*.

- Escherichia coli MC4100

Ligand of Na-sfz was active against *E. coli* with zone of inhibition of 16.3 mm and ai-dtc with no ZOI, but the corresponding coordination compounds were less active, where [VO(sfz)(ai-dtc)] has 8 mm and [Zn(sfz)(ai-dtc)] has 10 mm.

- Pseudomonas aeruginosa PAO1

Ligands of Na-sfz and ai-dtc, as well as, coordination compound of [VO(sfz)(ai-dtc)] were not active against *P. aeruginosa*, but the biological activity of [Zn(sfz)(ai-dtc)] was enhanced when compared with individual ligands of Na-sfz and ai-dtc. Both ligands and coordination compounds are potentials had lower antibacterial activities as compared to the positive control; meropenem.

In general, the metallic compounds of vanadium(IV) sulfate. hydrate showed no activity against all the bacterial strains, while zinc(II) chloride showed towards the two Gram-negative bacterial strains of *S.aureus* MRSA252 and *E. coli* MC4100 with ZOI of 14 mm and 9 mm respectively.

Table 4: The zone of inhibition for studied compounds in mm.

Compound	*S.aureus* MRSA252	*E.faecalis* ATCC 19433	*E. coli* MC4100	*P.aeruginosa* PAO1
$VOSO_4$. H_20	0	0	0	**0**
$ZnCl_2$	14	0	9	0
Na-sfz	17	ND	16.3	ND
ai-DTC	8	ND	0	ND
[VO(sfz)(ai-dtc)]	0	NA	8	NA
[Zn(sfz)(ai-dtc)]	0	NA	10	10
Meropenem	NA	NA	NA	30
Tetracycline	30	NA	28	NA
Vancomycin	NA	22	NA	NA
DMSO	0	0	0	0

Na-sfz = Sodium sulfadiazine; ai-dtc = Sodium salt of aniline dithiocarbamate; [VO(sfz)(ai-dtc)]= Coordination compound of oxovanadium(IV) ion; [Zn(sfz)(ai-dtc)] = Coordination compound of zinc(II) ion; ND=Non Detectable; NA= Not Applicable; Negative control: DMSO; Positive controls: Meropenem, Tetracycline and Vancomycin.

4. Conclusion and Future Perspective

All compounds were successfully synthesized and crystals of ai-dtc were diffracted and confirmed the structure contains carbon, nitrogen, oxygen and sulfur. All compounds were non electrolytes. Spectroscopic studies of FT-IR, UV-Vis and NMR revealed differences between ligands and metal complexes which proved the coordination. Both [VO(sfz)(ai-dtc)] and [Zn(sfz)(ai-dtc)] were active against Gram negative bacteria, but not against Gram positive bacteria possibly due to the mechanism of action. Future perspective will entail using the synthesized complexes to form adducts with 1, 10-phenanthroline so as to enhance the antibacterial potentials.

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Appendix A. Supplementary material: CCDC 1018265 contains the supplementary crystallographic data for $C_7H_6NS_2Na$. The data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving html, or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

Conflict of interest: The authors declare no conflict of interest.

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