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Ni(II) complex of (3,3-dimethyl-7-oxo-6-(2-Phenylacetamido)-4-thia-1-Azabicyclo[3.2.0]heptane-2-carboxylic acid : Synthesis, characterization and antibacterial activities

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Abstract *The ligand (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0-] heptane-2-carboxylic acid (DPTA) is a β-lactamin derivative used in the treatment of infections caused by gram-positive bacterial strains and few gram-negative bacterial strains.[Ni(DPTA)] was synthesized by the reaction of DPTA with $NiCl_2.6H_2O$. Physical properties such as solubility, colour and melting point were determined for the ligand, DPTA and the synthesized complex, [Ni(DPTA)]. The complex is found to be light green in colour. The ligand and the complex are ionic in nature with molar conductivity values of 218.2 and 126.0 Sm^2mol^{-1} respectively. The complex was characterized based on elemental analysis, UV-Visible, infrared, ^1H NMR and ^{13}C NMR spectroscopy. Spectroscopic data suggested that the DPTA coordinated to Ni ion through OH, C=O of amide, C=O of carboxylic acid, C=O of β-lactam and NH functional groups. Also since DPTA was coordinated to nickel centre through five sites it was also proposed that it acted as a pentadentate ligand around the nickel centre. The antibacterial studies of the ligand and its nickel complex were carried out against four-gram negative bacterial strains (Escherichia coli, Enterobacter cloacae, Pneumonia aeruginosa and Campylobacter fetus) and four-gram positive bacterial strains (Staphylococcus aureus, Bacillus substilis, Bacillus cereus and Enterococcus faecalis). The results showed that [Ni(DPTA)] exhibited better antibacterial activity than DPTA. Its study concluded that the process of chelation affected the biological behavior of the compound which in turn increase the inhibitory potential against the bacterial strains.*

Keywords: *Ligand, chelation, spectra, bacteria, inhibition. complexes*

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1.0 Introduction

There is increasing demand for new antimicrobial compounds that can combat the growing threat of widespread antibiotic resistance. Metal complexes are currently in clinical development for the treatment of microbial infections (Angelo *et al*., 2020). Metal complexes have unique mode of action and exist in a wider range of three-dimensional geometries than purely organic compounds (Angelo, 2020). Advances in inorganic chemistry has provided better opportunities of using metal complexes as therapeutic agents (Rafique *et al*., 2010; Thompson and Orgiv, 2004, 2006). Several metal complexes are known to accelerate the drug action and the efficacy of the organic therapeutic agent. The efficacies of the various organic therapeutic agents can be enhanced through coordi-

nation with a suitable metal ion. The pharmacological activity of metal complexes is highly dependent on the nature of the metal ions and the donor sequence of the ligands because different ligands exhibit different biological properties. Generally, drug combinations have proven to be an essential feature of antimicrobial treatment due to a number of important considerations including (i) they increase activity through the use of compounds with synergistic or additive activity; (ii) they thwart drug resistance; (iii) they decrease required doses, reducing both cost and the chances of toxic side effects; (iv) they increase the spectrum of activity. Various biological aspects of the metal-based drugs/ligands entirely depend on the ease of cleaving the bond between the metal ion and the ligand. Pahontu *et al*. (2013) synthesized Ni(II) complexes with salicylidene thiosemicarbazones. These Ni(II) salicylidene thiosemicarbazones complexes showed higher antimicrobial activities than the standard Furacillinum towards *Staphylococcus* and *Streptococcus* bacteria. The minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) were influenced by the nature of thiosemicarbazone and amine of the inner sphere coordination compound. New Ni(II) complex derived from *N*-Isonicotinoyl-N^1-(3-metoxy-2-hydroxybenzaldehyde)-hydrazine were synthesized and screened for *in vitro* antimicrobial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* strains. The quantitative antimicrobial activity test results indicated that both ligand and complex combinations exhibited specific antimicrobial activity that varied with the tested specie of microorganism (Pahontu *et al*., 2017). Islam *et al*. (2007) synthesized new Ni(II) tyrosine complex. The Ni(II) tyrosine complex was screened as potential antimicrobial agent against a number of medically important bacteria (*Bacillus subtillis, Streptococcus β-haemolytica, Escherichia coli and Shigella dysentarae)*. It was observed that the metal chelates exhibited more inhibitory effects than the parent ligands. Observed increase in lipophilic character of these complexes was responsible for their enhanced biological potency. In continuation, Islam *et al*. (2015) synthesized a new Ni(II) pyridine complex and its antibacterial effect was evaluated against human pathogenic bacteria (*Salmonella typhi, Shigella dysentarae, Escherichia coli and Bacillus cereus).* Ni(II) pyridine complex was more effective against *Salmonella typhi, Shigella dysentarae, Escherichia coli and Bacillus cereus* than the free ligand. The aim of this research is to synthesize, characterize and compare the antibacterial activities of DPTA and [Ni(DPTA)] with the intention of enhancing access to pharmacologically interesting [Ni(DPTA)] complex.

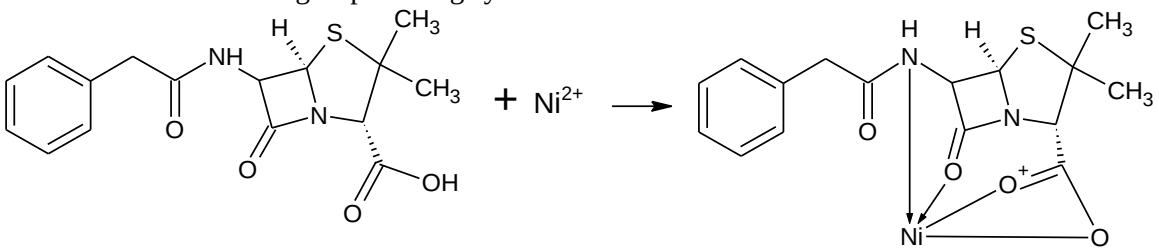
2.0 Materials and Methods

All the chemicals used in this study were of analytical grade. The ligand, (3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid was obtained from Shanxi Federal Pharmaceutical Company limited, Shanxi, China. Melting points of both the ligand and the complex were determined using Gallenkamp melting point apparatus. The solubility of the ligand and the metal complex were tested using various organic solvents at 25^{o}C. Conductivity measurements of the ligand and its nickel (II) complex (10^{-3} M) were recorded at room temperature using Jenway conductivity Meter 4510. DMSO was used as the solvent. Nickel analyses were carried out on AAS spectrophotometer (bulk 210). The elemental analysis for C, N, H and S were obtained using a Perkin-Elmer 240B elemental analyzer. The liquid state UV-Vis spectra of the ligand and its nickel complex were recorded on Uv-1800 series using Dimethylsulfoxide (DMSO) as the solvent in the range 200-800 nm. The solid state FTIR spectra of the ligand and its nickel complex were recorded on a Perkin Elmer Spectrum BX FTIR spectrophotometer (4400-350 cm^{-1}) in KBr pellets. The NMR spectral measurement were recorded on nuclear magnetic resonance Bruker spectrophotometer using tetramethylsilane internal standard and DMSO-d6 as solvent.

2.1 Synthesis of Ni(II) (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-zabicyclo[3.2.0]heptanes-2-carboxylate, [Ni(DPTA)]

The complex was prepared following reported procedure by Anacona and Figueroa, (1999). Ni(II) solution was prepared by dissolving 3.09g (0.013mole) $NiCl_2.6H_2O$ in 10 ml of water. The metal solution was added to a solution of DPTA(0.013 mol). The mixture was stirred for 1 hour and the solid complex which separated was removed by filtration and washed with water, ethanol and ether. The compound was dried under

vacuum at room temperature for 48 hours. The complex was then stored in a neatly labelled container after determining its percentage yield. The general synthesis of the metal complex is proposed in Scheme 1.



3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino] -4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (DPTA)

Ni(II) 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino] -4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [Ni(DPTA)]

Scheme 1: Proposed synthesis of Ni(II) (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxylate, [Ni(DPTA)]

2.2 Antibacterial activity test

The organisms used were Gram-negative *Escherichia coli, Enterobacter cloacae, Pneumonia aeruginosa,* and *Campylobacter fetus*. The Gram-positive bacterial strains were *Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis.* Theywere obtained from Federal Medical Centre, Umuahia, Abia State. Antibacterial activity of sample was determined by using agar well diffusion method and bacterial growth were subcultured on nutrient broth for their *in vitro* testing which were prepared by dissolving (24 g) of nutrient broth. The mixture was autoclaved for 15 minutes at 120 °C. Stock solution for *in vitro* antibacterial activity was prepared by dissolving 5 mg of compound in 9 cm^3 of DMSO to make the stock solution of 100 g/mL. Exactly 1.15 cm^3 of liquid nutrient agar was prepared separately for tested target microorganism cultures and 1 cm^3 of nutrient broth for antibacterial activity. Inoculation was done with the help of micropipette with sterilized tips and 100 µL of activated strain was placed onto the surface of agar plate. It spread over the whole surface and then two wells having diameter of 10 mm were dug in media and incubated at 37°C for 48 hours. Zone of inhibition was measured around the disc and has been expressed in mm.

2.3 *Statistical analysis*

Statistical significance was determined using Duncan Multiple Range Test. Results were considered statistically significant at $P<0.05$ and were expressed as mean ±SD.

3.0 Results and discussion

Some physical and analytical data of DPTA and [Ni(DPTA)] are shown in Table 1. Table 1 reveals that the % yield of [Ni(DPTA)] was 75%. The complex is air and photo stable. The high decomposition temperature from 209 to 212 ^{0}C associated with the change in colour from white (DPTA) to light green [Ni(DPTA)] suggest that coordination occurred since transition metal complexes are known to exhibit colour as one of their characteristics (Najlaa *et al*., 2020). The measured conductivity of DPTA and [Ni(DPTA)] are 236.0 and 218.2 $Sm^2.mol^{-1}$ respectively. This suggest that the ligand and complex are electrolyte (Geary, 1971). Data obtained from % elemental analysis were compared with the calculated data and were found to be in good agreement with the calculated data. This suggested metal ligand ratio of 1:1.

Table 2 shows the solubility of DPTA and [Ni(DPTA)] in various solvents. DPTA was found to be soluble in distilled water, n-hexane, ethanol, methanol, petroleum ether and DMSO. However, the complex was found to be insoluble in n-hexane and petroleum ether. It was slightly soluble in ethanol and methanol but completely soluble in DMSO. The solubility data indicate that the complex is highly polar.

Fig. 1 shows FTIR spectra of DPTA and [Ni(DPTA)]. FTIR spectrum of the complex was compared with that of the free ligand to determine the coordination sites that were involved in the bonding. In the FTIR spectrum of DPTA, the

Table 1: Some physical and analytical data of DPTA and [Ni(DPTA)]

Ligand/complex	Colour	M.P. (°C)	Yield (%)	Conductance ($Sm^2.mol^{-1}$)	C (%) Found Calc.	H (%) Found Calc.	N (%) Found Calc.	S (%) Found Calc.	Ni (%) Found Calc.
DPTA	White	209	-	236.0	57.42 (57.47)	5.41 (5.43)	8.37 (8.38)	9.59 (9.58)	-
[Ni(DPTA)]	Light green	212	75	218.2	48.99 (49.01)	4.35 (4.37)	7.15 (7.14)	8.14 (8.18)	14.95 (14.97)

Table 2: Solubility data of DPTA and [Ni(DPTA)] in some selected solvents

Ligand/Complex	n-Hexane	Distilled water	Petroleum ether	Ethanol	Methanol	DMSO
DPTA	S	S	S	S	S	S
[Ni(DPTA)]	IS	IS	IS	SS	SS	S

Key: S-Soluble, SS-Slightly Soluble, IS-Insoluble

carbonyl of amide stretching frequency was observed at 1697.66 cm^{-1} but in the FTIR spectrum of [Ni(DPTA)], the band was observed at 1642.00 cm^{-1}. This indicates the existent of interaction and that C=O of amide was involved in coordination to nickel. In the FTIR spectrum of DPTA, carbonyl of β-lactam was observed at 1178.04 cm^{-1} but this vibration frequency was absent in the spectrum of the complex indicating this functional group participated in coordination. It is hereby proposed that C=O is converted to C-O during complexation. C-O stretching frequency was observed at 1134.00 cm^{-1} in the spectrum of the metal complex. Also, the vibration frequency of hydroxyl group was observed at 3542.26 cm^{-1} in the spectrum of the ligand but was absent in the spectrum of the metal complex. This suggests deprotonation of OH during complex formation. N-H stretching frequency was observed at 3351.50 cm^{-1} in the spectrum of the ligand. This vibration frequency was shifted to lower wavenumber 3300.00 cm^{-1} in the spectrum of the complex, indicating the participation of N-H functional group in complex formation. Increase in electron density can increases N-H bond length and consequently slows down the vibration frequency as observed in this study.

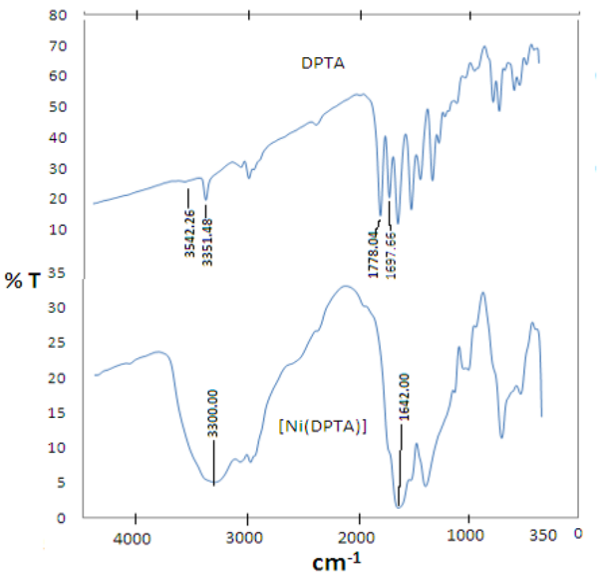


Fig. 1: FTIR spectra of DPTA and [Ni(DPTA)]

Fig. 2 shows UV-Vis spectra of DPTA and [Ni(DPTA)]. The ligand and its nickel (II) complex absorbed maximally at λ_{max} = 97.50, 203.50,209.50, 215.50, 226.50, 238.50, 255.50, 259.50, 270.50, 278.50, 284.50 and 317.50 nm. These absorption bands are assigned to π–π* and n–π* transitions. These bands are known as Intra-Ligand Charge

Transfer (ILCT). Similarly, the UV-Vis spectra of [Ni(DPTA)] showed an absorption band at λ_{max}= 389.00 nm. This absorption band is due to ligand to metal charge transfer (LMCT).

Figs.3 and 4 show the ^{1}H NMR spectra of DPTA and [Ni(DPTA)]. The ^{1}H NMR spectrum of DPTA shows a singlet at 11.00 ppm. This chemical shift was assigned to OH proton of carboxylic acid. The shift was absent in the spectrum of [Ni(DPTA)]. The absent of OH chemical shift in the complex indicated deprotonation of OH during coordination. A doublet observed at 8.72 ppm in ^{1}H NMR spectrum of DPTA was assigned to NH proton.

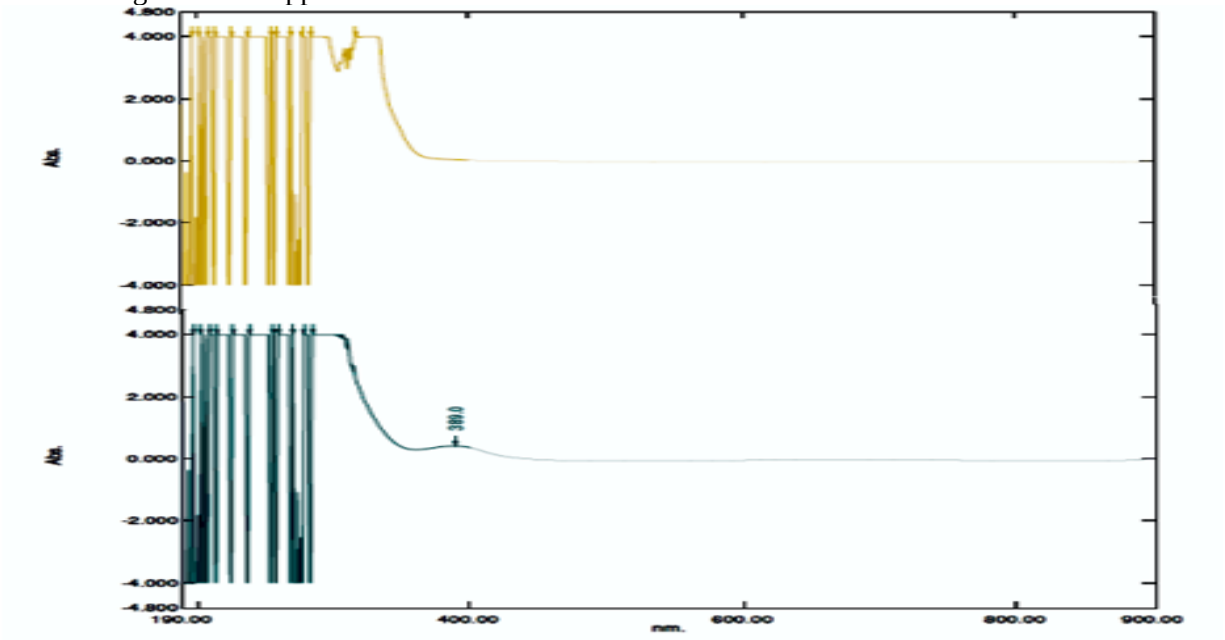


Fig. 2: Uv-Visible spectra of DPTA and [Ni(DPTA)]

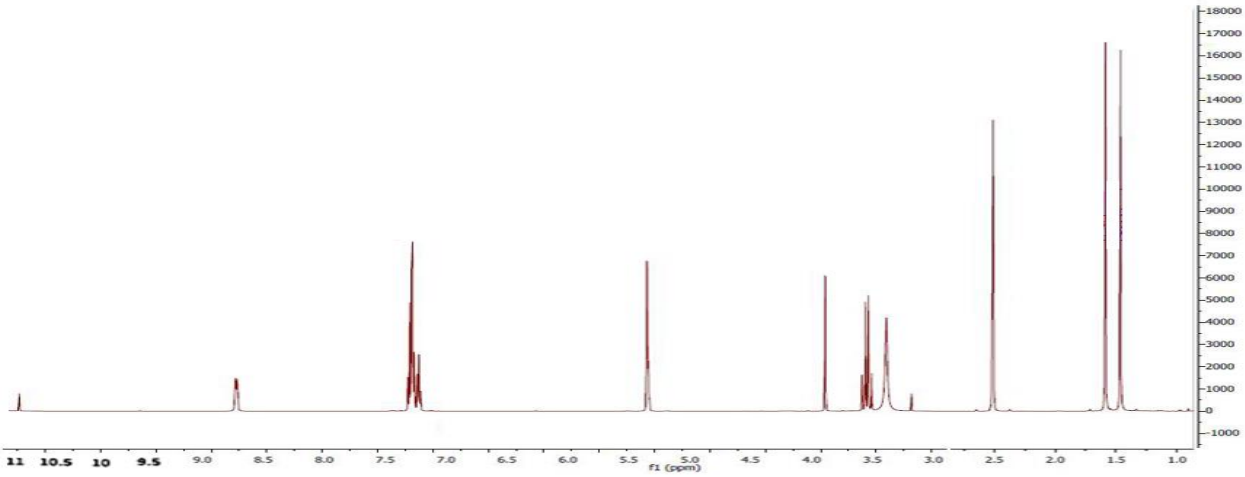


Fig. 3: ^{1}H NMR Spectrum of DPTA

Multiplets observed in the ^{1}H NMR spectrum of the ligand at 7.12-7.32 ppm were assigned to aromatic protons and it appeared at 7.25 ppm in the ^{1}H NMR spectrum of the nickel (II) complex. Multiplets observed at 3.39 ppm in the ^{1}H NMR spectrum of the ligand were attributed to CO-CH_2 of thiazolidine ring and was observed at 3.52 ppm in the spectrum of the nickel complex. A singlet observed at 1.46 and 1.59 ppm was assigned to protons of methyl group of the thiazolidine ring.

However, in the ^{1}H NMR spectrum of [Ni(DPTA)], these were observed at 1.55 b 1.15 ppm respectively. Fig. 5 and 6 show the ^{13}C NMR spectra of DPTA and [Ni(DPTA)]. A comparison of the ^{13}C NMR (DEPT 135) spectrum of DPTA and [Ni(DPTA)] were made. In the ^{13}C NMR (DEPT 135) spectra of DPTA, the chemical shift of C=O amide was observed at173.73 ppm.

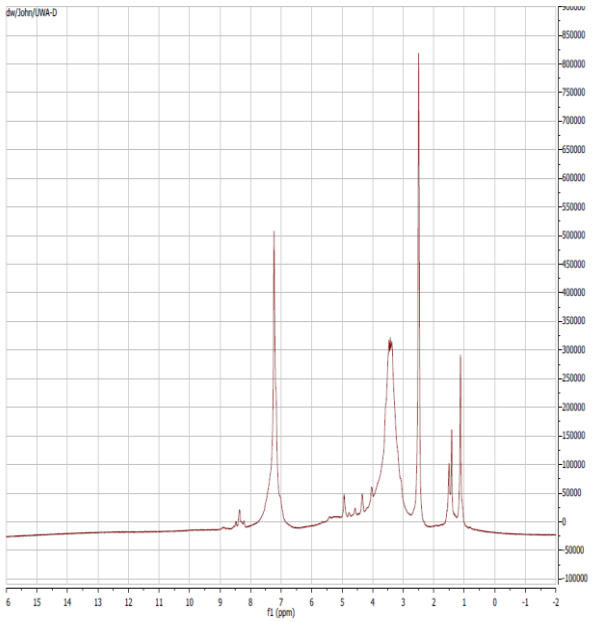


Fig. 4: ^{1}H NMR Spectrum of [Ni(DPTA)]

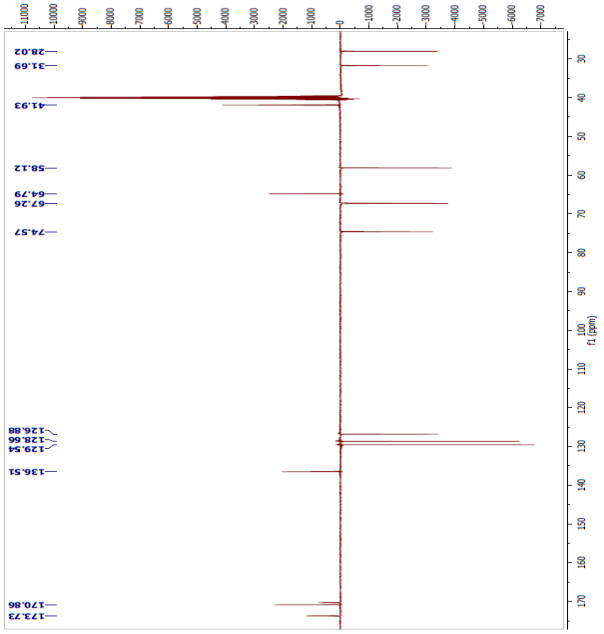


Fig. 5 : ^{13}C NMR (DEPT 135) spectrum of DPTA

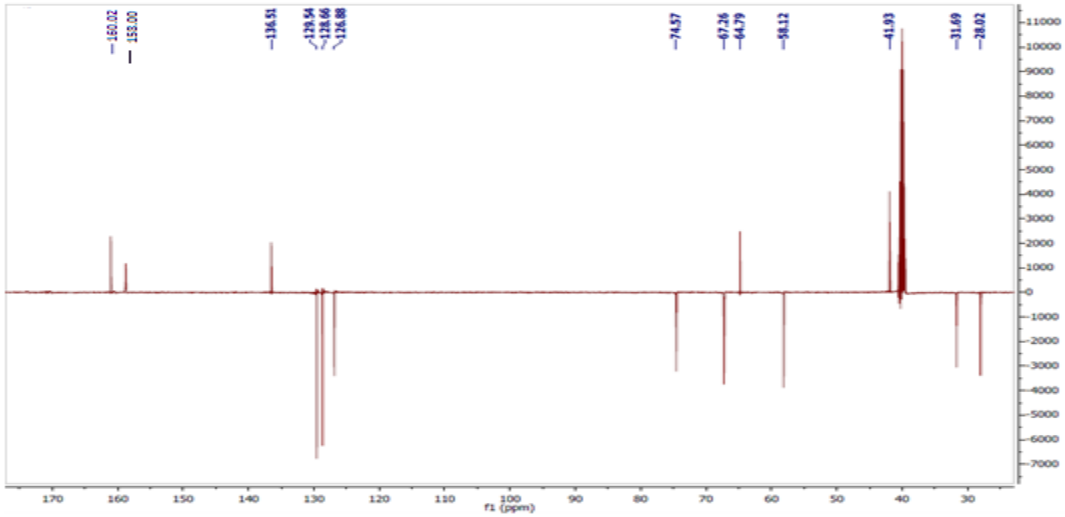


Fig. 6: ^{13}C NMR (DEPT 135) spectrum of [Ni(DPTA)]

This functional group was observed at 158.00 ppm in the ^{13}C NMR (DEPT 135) spectrum of [Ni(DPTA)]. This suggests that C=O of amide was involved in complexation to nickel ion. The chemical shift of C=O carboxylic acid and β-lactam was observed at chemical shift at 170.86 and 170.05ppm in the spectrum of DPTA. In the spectrum of [Ni(DPTA)], the chemical shift corresponding to C=O of carboxylic acid was observed at 160.02 ppm .This shift suggests that C=O of carboxylic acid was involved in coordination to the nickel ion. The absence of C=O

of β-lactam in the ^{13}C NMR(DEPT) spectrum of [Ni(DPTA)] suggests that C=O was transformed to C-O during complexation. The aromatic carbons were observed at 126.88 and 29.54 ppm in the spectra of the ligand and complex respectively.
Based on elemental analysis, FTIR, UV/Visible, ^{1}H NMR and ^{13}C NMR(DEPT) spectroscopic analysis, the structure in Figure 7 was proposed for [Ni(DPTA)]. Table 3 shows the antibacterial activity of DPTA and [Ni(DPTA)] complex against four-gram negative bacterial strains (*Escherichia coli, Enterobacter cloacae, Pneumonia aeruginosa,* and *Campylobacter fetus*) and four gram-positive bacterial strains (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis*). The zone of inhibition of the metal complex was significantly higher ($P<0.05$) compared to the free ligand against the bacterial strains used.

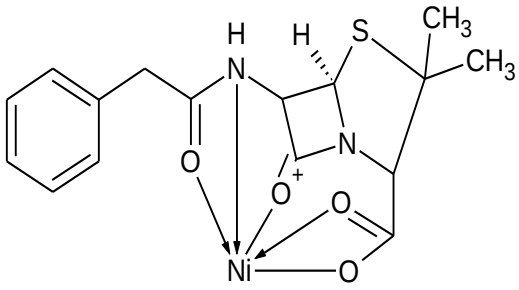


Fig. 7: Proposed structure of [Ni(DPTA)] complex

Metal complexes coordinatively saturate the bacterial strains and operate by physical interactions. From the above, it was concluded that the process of chelation affected the biological behavior of the compound which in turn increased the inhibitory potential against the bacterial strains.

Table 3: Percentage zone of inhibition (mm) of DPTA and [Ni(DPTA)]on the bacterial population

Ligands/ Complexes	Bacteria							
	Gram Positive				Gram Negative			
	Staphylococcus aureus	*Bacillus subtilis*	*Bacillus cereus*	*Enterococcus faecalis*	*Escherichia coli*	*Enterobacter cloacae*	*Pneumonia aeruginosa*	*Campylobacter fetus*
DPTA	2.52±0.03^{a}	6.12±0.03^{a}	4.96±0.01^{a}	2.12±0.03^{a}	10.43±0.03^{a}	1.32±0.03^{a}	6.82±0.03^{a}	7.32±0.02^{a}
[Ni(DPTA)]	6.94±0.02^{b}	12.83±0.04^{b}	7.70±0.01^{b}	8.12±0.03^{b}	11.94±0.06^{b}	6.52±0.06^{b}	13.11±0.01^{b}	12.63±0.04^{b}

****Values are mean ±SD of 3 replicates. Values carrying superscript different from their parent antibiotics for each micro-organism are significantly different ($P<0.05$)**

4.0 Conclusion

The nickel (II) complex of (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate (DPTA) was synthesized and characterized using techniques such as elemental analysis, conductivity measurement, FTIR, UV-Vis, ^{1}HNMR, ^{13}CNMR (DEPT 135). The ligand coordinated to the nickel ion through OH, NH, C=O of amide, C=O of β-lactam and C=O of carboxylic acid. Hence, DPTA behaved as a pentadentate ligand towards nickel ion. The antibacterial activity of DPTA and [Ni(DPTA)] against four-gram negative bacterial strains (*Escherichia coli,Enterobacter cloacae, Pneumonia aeruginosa,* and *Campylobacter fetus*) and four gram-positive bacterial strains (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis*) was studied. It was observed from the result that [Ni(DPTA)] has more inhibitory potential than the uncomplexed DPTA. From the above, it was concluded that the process of chelation affected the biological behavior of the compound which in turn increased the inhibitory potential against the bacterial strains.

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