Synthesis, Characterization and the Antibacterial Activity of a New Ni(II) Complex of Thiosemicarbazone Ligand.

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ABSTRACT

Novel complex of Ni(II) of ligand 2-(anilinoacetyl)-N-(3-methylphenyl)hydrazine-1- carbothioamide (H_2L) has been synthesized and characterized by 1HNMR, IR, elemental analyses, molar conductance, UV-visible spectra, magnetic susceptibility measurements ,thermogravimetric analysis (TGA/DTG) and X-ray differaction pattern. The result confirmed that the ligand behaved as neutral bidentate, coordination take place via carbonyl oxygen(C=O) and N(2)H groups. Nickel complex is more thermally stable than free ligand . Nickel(II) complex is mononuclear, adopt square planar geometry . The ligand and complex have been tested for their inhibitory effect on the growth of bacteria against gram-positive (*Streptococcus pyogenes*) and gram-negative (*Escherichia coli*). The result revealed that Ni(II) complex showed a higher antibacterial activity against gram positive (*Streptococcus pyogenes*) and gram negative (*Escherichia coli*) bacteria than the ligand.

Key words: Ni(II)complex, IR, Thermal analysis, X-ray, Antibacterial activity.

1. INTRODUCTION

The chemistry of thiosemicarbazones has received considerable attention because of their variable bonding modes, promising biological implications, structural diversity, and ion-sensing ability (Casas *et al.,* 2000; Mishra *et al.,* 2006 and Kizilicikli *et al*., 2004). They have been used as drugs and are reported to possess a wide variety of biological activities against bacteria, fungi, and certain type of tumors, and they are also a useful model for bioinorganic processes (Singh *et al*., 2001; Offiong and Martelli 1997 and Labisbal *et al*., 2002) . The activity of these compounds is strongly dependent on the nature of the heteroatomic ring and the position of attachment to the ring as well as the form of thiosemicarbazone moiety (Singh *et al*.,2005). These are studied extensively due to their flexibility, selectivity, and sensitivity towards the central metal atom, and structural similarities with natural biological substances, and due to the presence of imine group (−N=CH−) which imparts the biological activity (Chandra *et al* ., 2001 ; Raman *et al* ., 2001 ; Raman *et al* ., 2002 ;Singh *et al*., 2004 and Raman et al .,2005) .

New (Z)-2-(pyrrolidin-2-ylidene) hydrazine carbothioamide (L) was synthesized in a good yield by the reaction of pyrrolidone with thiosemicarbazide. Co(II), Ni(II), and Cu(II) complexes of (L) were prepared and characterized by FT-IR, UV/visible spectra, 1HNMR and CHN analyses. Moreover, charge, bond length, bond angle, twist angle, heat of formation, and steric energy were calculated by using of the Chem Office program, and the DFT calculations for the complexes were done. The free ligand and its metal complexes were tested in vitro against several microorganisms (Staphylococcus aurous, E. coli, Proteus vulgaris, Pseudomonas, and Klebsiella pneumoniae) to assess their antimicrobial properties. Results. The study shows that these complexes have octahedral geometry; in addition, it has high activity against tested bacteria. Conclusion/Recommendations. Based on the reported results, it may be concluded that ligand acts as bidentate, neutral ligand, coordinating through one of the nitrogen and sulfur atoms (Al-Amiery *et al* .,2011) .

A series of metal complexes of Cu(II) and Ni(II) having the general composition [M(L)X2] with benzil bis(thiosemicarbazone) has been prepared and characterized by element chemical analysis,molar conductance, magnetic susceptibility measurements, and spectral (electronic, IR, EPR, mass) studies. The IR spectral data suggest the involvement of

sulphur and azomethane nitrogen in coordination to the central metal ion. On the basis of spectral studies, an octahedral geometry has been assigned for Ni(II) complexes but a tetragonal geometry for Cu(II) complexes. The free ligand and its metal complexes have been tested in vitro against a number of microorganisms in order to assess their antimicrobial properties (Chandra etal ., 2007) .

The aim of this work was to prepare Nickel(II) complex with 2-(anilinoacetyl) -N-(3-methylphenyl) hydrazine-1- carbothioamide (H2L) characterized by physical and spectral data, including microanalysis, IR and UV-visible spectroscopy, conductivity measurements, thermal analyses (TG/ DTG), and the antibacterial activity of ligand and Nickel(II) complex against two pathogenic bacteria (*Streptococcus pyogenes*) as gram-positive bacteria and (*Escherichia coli*) as gram-negative bacteria) were investigated.

2. MATERIAL AND METHODES

2.1 Material

All the chemicals and solvents of Sigma Aldrich/CDH/Rankem/Merck were Analar grade and used without any further purification. All the reactions were carried out under normal atmospheric conditions.

2.2 Synthesis of Ligand

The ligand of 2-(anilinoacetyl)-*N*-(3-methylphenyl) hydrazine-1- carbothioamide (H_2L) was prepared by mixing (0.01 mol) of desired hydrazide with(0.01 mol) of phenyl isothiocyanate in 15 ml of absolute ethanol . The reaction mixture was refluxed for 6 hrs. The reaction mixture was recrystalized several times from ethanol.

2.3. Synthesis of Nickle(II) Complex

Nickle(II) complex of the ligand 2-(anilinoacetyl)-*N*-(3-methylphenyl)hydrazine-1-carbothioamide (H_2L)was prepared by adding stoichiometric amount of the $NiCl_2$ in EtOH to a hot solution of (H_2L) in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at for certain about 6hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10} .

2. 4. Measurements

Elemental analyses (C, H and N) were performed by Microanalytical unit of the Cairo University, Egypt. Ni(II) and chloride were estimated using standard methods **(El-Boraey and El-Gammal 2015).**

IR absorption spectra were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometer.

^{1}HNMR spectra were recorded in d6-DMSO using 300 MHz Varian NMR spectrometer. The electronic spectra were carried out as solution (10^{-3} M) in DMF using a Perkin Elmer Lambada 4B spectrophotometer. The molar conductivity measurements were made in DMF solution (10^{-3}M) using a Tacussel conductometer type CD6N.

Thermal analyses were determined thermal behavior of the ligand and its complex has been studied using TG/DTAG measurements. The TGA/DTA curves of ligand and its complexes were recorded in nitrogen atmosphere from room temperature up to 800c.

X--ray powder diffraction analyses _of solid sample was measured using APD 2000 PROModel GNR-X-ray Diffractometer at The National Research Center, Tanta University, Egypt. X-ray diffractograms gives computer control formally finished by PHILIPS® MPDX´PERT X-ray diffratometer ready with Cu radiation CuKα (λ=1.540 56 Å). The x´pert diffractometer has the Bragg- Brentano geometry. The x-ray tube used was a copper tube operating at 40 KV and 30 mA. The scanning range (2θ) was 5–90 ° with step size of 0.050° and counting time of 2 s/step. Quartz was used as the standard material to accurate for the instrumental expansion. This identification of the complexes was done by a known method From the fit identified Scherrer formula, the average crystallite size, T, is

$$T = (K\lambda / \beta \cos \theta)$$

Where λ is the X- ray wavelength in the manometer, K (constant equal to 0.9) is related

to crystallite shape, and ß is the peak width at half maximum height. The value of ß in the 2θ axis of diffraction shape must be in radians. The θ is the Bragg angle and be able to in radians since the Cos θ compatible with the same number.

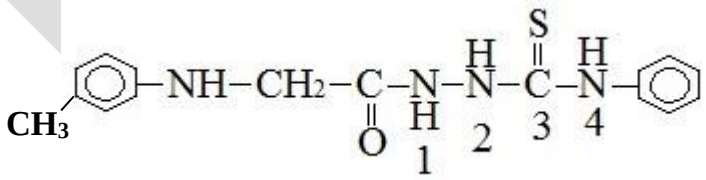
2.5. Antibacterial tests

The in vitro antibacterial activity studies were carried out as described by **(El-Boraey *et al*., 2016)** with some modification, the inhibitory effect of both the synthesized ligand and its complex was tested on the pathogenic gram-positive organism *Streptococcus pyogenes* and the gram-negative bacterium *Escherichia coli*. Biological effect of the ligand and its complex was carried out on Brain Heart Infusion (BHI) was used to grow *S. pyogenes* cells and Nutrient Broth (NB) medium was used to grow *E. coli* cells. Compounds under investigation were dissolved in DMSO which has no inhibition activity on both microbes. Two different concentrations (1 µg/ml and 5 µg/ml) were prepared. Bacterial strains were prepared by activating them on the proper broth media with shaking. The bacteria were then cultured for 24hrs at 37^{o} C in an incubator. With this subcultures, fresh broth media were inoculated by one mille of the standard bacterial culture.

For growth studies, *S. pyogenes* and *E. coli* cultures were inoculated and grown aerobically on BHI broth medium and NB medium respectively. Growth was calculated turbidometrically at 650 nm using conventional spectrophotometer, in which turbidity produced is measured by taking absorbance and compared with turbidity produced by control. After growing bacterial cultures on media that contain the ligand, absorption measurements of complexes and control were measured by spectrophotometer after 24hrs and 48hrs of incubation to determine the number of viable cells count per milliliter of sample and were used to calculate the inhibition percentage.

3. RESULTS and DISCUSSION

The ligand of 2-(anilinoacetyl)-*N*-(3-methylphenyl)hydrazine-1- carbothioamide (H_2L) was confirmed by elemental analysis as shown in table (1), infrared as shown in table (2) and ^{1}H NMR spectroscopy. The reaction of the ligand H_2L with $NiCl_2$ produce complex of the general formulae $Ni(H_2L)\ Cl_2.\ 2H_2O$. These air stable complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities were measured in DMF (10^{-3}M) solution. Table (1) shows that, the complex is non-electrolyte, indicating that coordination of the anion to the ligand. The solid complex is colored, insoluble in water, methanol and ethanol but soluble in DMF at 10^{-3}M **(Al-Shaheen and Al-Mula 2014).**



H_2L

Scheme 1: Chemical structure of ligand (H_2L)

3.1. ^{1}H NMR Spectra

The ^{1}H NMR spectrum of the ligand (H_2L) in DMSO –d^6 revealed a chemical shift (δ/ppm) at 9.1 ppm and at 9.8 ppm attributed to N(4)H and N(1)H. The peaks of N(1)H and N(2)H appeared as signals at 8.9 and 8.3 ppm .The singlet peak appeared at 3.4 ppm due to CH_2 group and the multiple peak at 7.5 ppm appeared due to aromatic protons of phenyl group **(El-Saied *et al.,* 2017).**

Table 1: Analytical and physical data for the ligand (H_2L, $C_{16}H_{18}N_4OS$) and Ni(II) complex

No.	Compound	Color	Found (Cal. %)					
			C	H	N	Cl	Ni	Λ_M
	H_2L	Buff	61 (60.9)	6.2 (6.0)	18.0 (17.8)	-	-	-
1	$Ni(H_2L)\ Cl_2.\ 2H_2O$	Brown	36.7(36.3)	4.7(4.1)	11.4(11.7)	15.0(14.8)	12.3 (12.2)	20

Where, Λ_M = molar conductivity $ohm^{-1}\ cm^2\ mol^{-1}$ in 10^{-3}M in DMF solution.

3.2. The Infrared Spectra of Ligand and Ni(II) Complex

Fundamental IR spectral bands for the ligand and nickel complex are given in table (2). The IR spectrum of the free ligand is characterized mainly by the strong bands at 3384 cm^{-1}, 3262 cm^{-1}, 3150 cm^{-1}, 1671 cm^{-1} and 749 cm^{-1} are attributed to the stretching frequencies of υ(N4-H), υ(N2- H), υ(N1-H), υ(C=O) and υ(C=S) wagging vibrations, respectively. This supports the nature of the ligand H_2L as bidentate one and coordination take place via(C=O) and (N2-H). The bonding mode of the ligand to Ni ion has been judged by a careful comparison of the infrared spectra of the complex with that of the free ligand. In general, the infrared spectra of the Ni(II) complex shows significant changes compared to the spectrum of the free ligand. The IR spectra of complex show strong band at 3385, 3292 , 3173 , 1601 and 728 cm^{-1} which attributed to the stretching frequencies of υ(N4-H), υ(N2-H), υ(N1-H), υ(C=O) and υ(C=S) wagging vibrations, respectively. The new bands appeared at 608 and 489 cm^{-1} assigned to υ(Ni-O) and υ(Ni-N) respectively, **(Meena and Jain 2014)**.

Table (2) : Infrared spectral bands (cm^{-1}) for ligand (H_2L) and Ni(II) complex

No.	Compound	ν(N4-H)/ ν(OH)	ν(N2-H)	ν(N1-H)	ν(C=O)	ν(C=S)	ν(Ni-O)	ν(Ni-N)
	H_2L	3384	3263	3150	1671	749	-	-
1	$Ni(H_2L)Cl_2\ .\ 2H_2O$	3385	3292	3173	1601	749	608	489

3.3. The electronic spectra of the ligand and Ni(II) complex

The electronic spectra of ligand and Ni(II) complex were recorded in DMF solution (10^{-3}M). In UV spectra of ligand shows λ_{max} at 272 nm with a shoulder band. It indicates that in DMF solution the ligand exists in thiol form. Ni(I) complex was found tobe paramagnetic which exclude the possibility of square planar configuration . The calculated magnetic moment value for nickel(II) complex is 2.4 B.M. corresponding to two unpaird electrons and tetrahedral structure **(Yauhang *et al*., 1995).**

3.4. Thermal Studies (TG/DTG)

The thermal properties of ligand and its Nickel complexes were investigated by thermo gravimetric analysis (TG/DTG), under nitrogen

atmosphere from room temperature in the range 25-800°C as shown in table (3) **(Donia *et al*., 2003).**

3.4. 1. Ligand

The TG curve of the ligand shows that the ligand is thermally stable up to 127°C after this point melting point at 128°C. Also the TG curve shows decomposition step in the temperature range 128.5–799 °C, with total mass loss of 100% (found 97.9%).

3.4. 2. Ni(II) Complex

The TG curves of Ni(II) complex show mass loss in the temperature range 29.3 - 38.5°C (Calc./Found % 1.1/ (1.0)), associated with DTG peak at 43°C is assigned to release of two molecule of water of crystallization.

Table (3): Thermal data of ligand and Ni(II) complex.

No.	Compound	TG/°C	Mass loss% Cal. (F.)	Reaction	Leaving species
	H_2L	128	-	-	Melting decomposition
		128.5-799	100(97.9)	C	Decompostion
1	$Ni(H_2L)Cl_2 . 2H_2O$	29.3 - 138.5	1.1(1.0)	d	$-2H_2O$
		138.5 -248.5	28.0(28.1)	c	Decomp.
		248.5 -511	37.5(37.7)	c	Complete Decomp.
		511-799.7	37.5(37.7)	f	-2NiO

Where, d =Dehydration, c= Decomposition and f =Final product percent

3.5. X-ray diffraction

The X-ray powder diffraction is revealed in table 4. and Figure 1. Nickle complex XRD pattern was attained in the range of 5-90° and in steps of 0.050°. The full width at the half- maximum (FWHM) of diffraction peaks observed from the refinement was used to evaluate the particle size. The average of crystallite size(T) is known by Scherer's equation: $T = (K\lambda/\beta Cos\theta)$ (Uarikumaran *et al*., 2012). The diffraction peaks indicate that the synthesized of nickel complex is in the nanometer range . Also the crystaline size of Ni(II) complex is 3.27nm.

Table 4. XRD data for Nickel complex

Nickel complex							
Angle 2θ	d- value A◦	Full width β	Grain size nm	Angle 2θ	d - value A◦	Full width B	Grain size nm
9.38	9.4197	0.4223	3.271	19.01	4.667	0.3000	4.55
17.11	5.1774	0.8716	1.572	24.58	3.6194	0.3951	3.428
20.74	4.2801	0.6381	2.137	15.16	5.8406	0.4521	3.039
10.92	8.0979	0.2863	4.819	18.26	4.8538	0.3000	4.561
25.16	3.5367	0.7557	1.77	29.10	3.0657	0.8927	1.503
23.94	3.7143	0.7395	1.833				

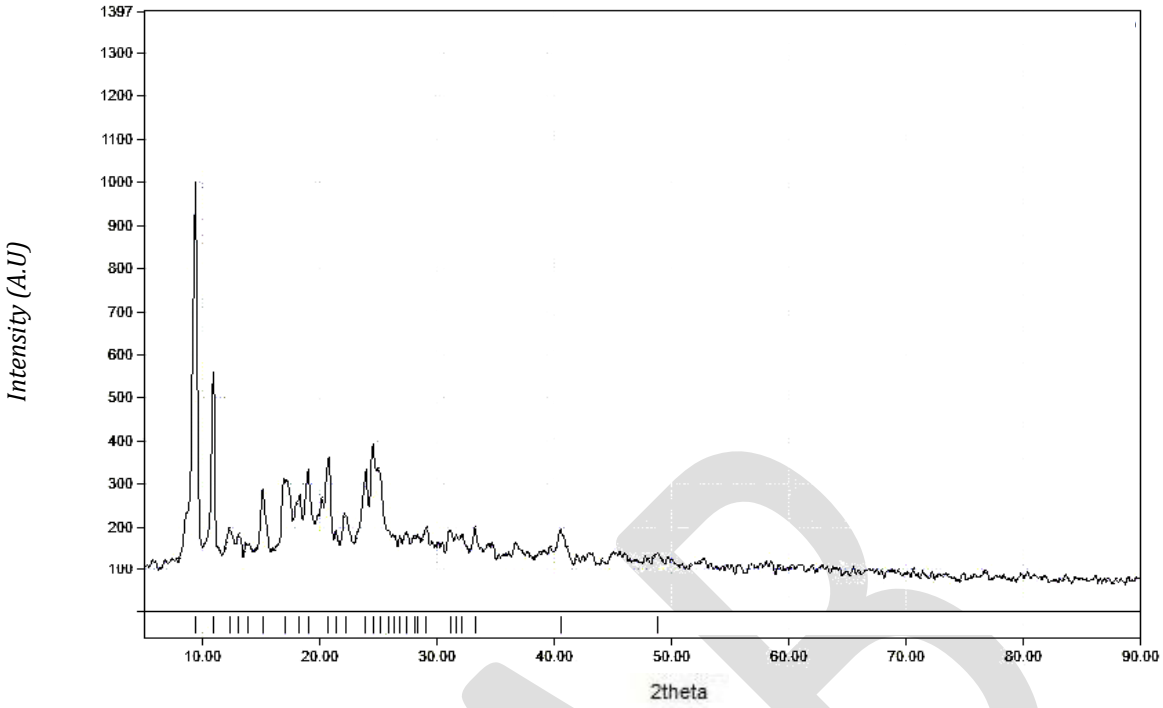


Figure 1: X- ray diffraction patterns of Ni(II) complex

3.6. Antibacterial Activity

The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases in table (5) showed antibacterial activity against the tested microbes. Generally, it was found that the antibacterial activity of both the synthetic ligand and Ni(II) complex was proportionally increased with increased concentration. The tested compounds are found to have remarkable biological activity. For 1 µg/ml concentration of both synthetic ligand and Ni(II) complex, the antibacterial activity of the tested compounds was found to follow the order : Ni(II) complex < ligand in case of *E. coli* as shown in figure (2). On the other hand, a higher antibacterial activity was recorded when using the ligand with *S. pyogenes* as shown in figure (3) with the same concentration. (**El-Boraey *et al*., 2016**).

Antibacterial activity of 5 µg/ml concentration for both the free acyclic ligand and its complex followed the order ligand complex when compounds were used with both *S. pyogenes* and *E. coli* **(Sönmez *et al*., 2010).** Results in Figure (2) suggested that in case of 1 µg/ml Ni(II) complex, the chelation could facilitate the ability to cross the cell membrane of *E. coli* and can be explained by Tweedy's chelation theory. Chelation complication could enhance the lipophilic nature of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane **(Tweedy, 1964).** Complex when used with both concentrations (1 µg/ml and 5 µg/ml) in case of the gram-positive *S. pyogenes* bacterium. It also has been observed that some moieties such as N(2)H linkage introduced into such compounds exhibits extensive biological activity **(Singh *et al*., 2013)** . The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases.

Table (5) : Antibacterial activity of ligand and its metal complex

No.	Compound	Inhibition %			
		E. coli		*S. pyogenes*	
		1µg/ml	5µg/ml	1µg/ml	5µg/ml
	H_2L	73.41	94.56	94.23	95.76
1	$Ni(H_2L)Cl_2 . 2H_2O$	11.17	3.62	35.41	41.35

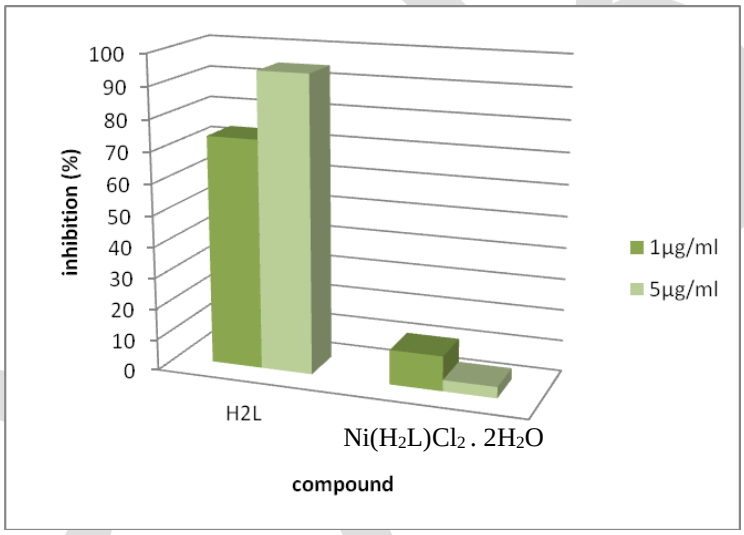


Figure (2) : antibacterial activity of ligand and Ni(II) aganist *E. coli*.

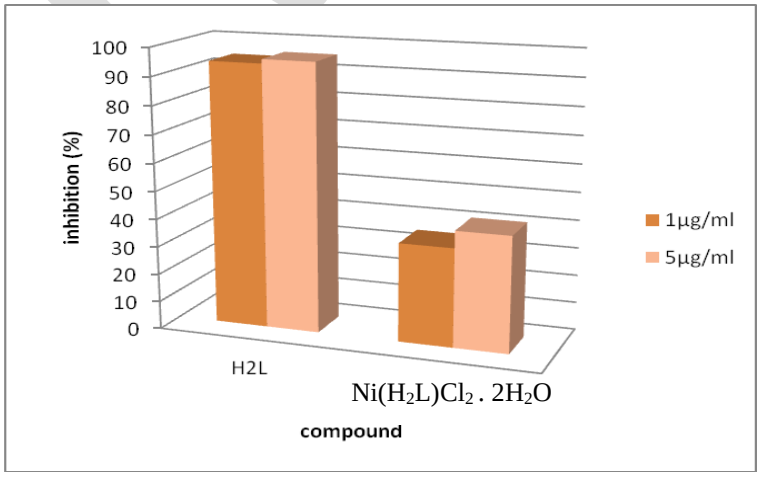


Figure (3) : antibacterial activity of ligand and Ni(II) aganist S. pyogenes.

4. CONCLUSION

In this summary, synthesis and characterization of ligand and its Ni(II) complex is reported. The analytical and physicochemical analysis confirmed the composition and the structure of the newly obtained compound.

1-The spectral analysis of infrared of the Ni(II) complex is square planar in geometry

2- The results obtained can be summarized as follows:

a- The result confirmed that the ligand behaved as neutral bidentate ,coordination take place via carbonyl oxygen(C=O)and N(2)H groups. Nickel Complex is more thermally stable than free ligand .

b- The antibacterial activity of the Ni(II) complex screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit the antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compound exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases.

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