IJPSR (2019), Volume 10, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 December 2018; received in revised form, 09 April 2019; accepted, 19 April 2019; published 01 September 2019

SYNTHESIS AND CHARACTERIZATION OF 2-(PYRIDIN-2-YL)GUANIDINE DERIVATIVES AND THEIR METAL COMPLEXES AS POTENTIAL ANTIBACTERIAL AGENTS USING PHOSPHORYL CHLORIDE

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Keywords:

Guanidine, Guanidine-pyridine, Antibacterial study, Zone inhibition diameter, Broth dilution, Agar dilution

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ABSTRACT: Guanidines were prepared by the condensation reaction of primary amine (-NH_2) with urea derivatives (R_2NCONR_2) in the presence of $POCl_3$. In this research guanidine-pyridine, hybrid derivatives were synthesized by substituted urea derivatives namely 1,1,3,3-tetramethylurea and N, N′-dimethylurea with 2-aminopyridine. The metal complexes were obtained by reaction of metal(II) chloride with guanidine ligands in the molar ratio 1:1 and 1:2 (M:L). The structure of synthesized compounds was assessed by nuclear magnetic resonance (^{1}H-NMR), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet-visible (UV-Visible) mass spectroscopy studies and molar conductance. The measured molar conductance values indicated that the complexes were non-electrolytic. An octahedral geometry is proposed for the cobalt complexes but, square-planar geometry is suggested for both copper and nickel complexes. The synthesized compounds were also subjected to antibacterial study. Evaluation of antibacterial activities indicated that the metal complexes more inhibited the Gram-positive and Gram-negative bacteria strains as compared to the parent ligands. Among all the metal complexes the CuL_2' showed highest antibacterial activity with zone inhibition diameter of 29 mm and MIC value of 15.62 µg/ml based on broth dilution method and 31.25 µg/ml based on agar dilution method.

INTRODUCTION: It is known that resistance to most antibacterial agents is a serious health concern [1, 2]. Further, the growth of multidrug resistance, among both Gram-negative and Gram-positive pathogens, has been identified as a major problem in health care systems in recent decades [3]. Finding antibacterial agents that can offer new modes of actions has always been an important and often difficult task [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(9).4304-14

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(9).4304-14

Developing materials that can limit bacteria colonization on their surface is known as an approach to control infections to prevent the threatening growth of bacteria strains [5].

Chelation and its relationship with different biological processes seem to be a promising research area aiming at designing a novel therapeutic methodology for discovering new antibiotic compounds to control and prevent the growth of drug-resistant bacterial strains worldwide [6-8]. Guanidine functionalities can be seen in a wide range of natural compounds, which are either in cyclic form or terminal groups of pendent substituents [9, 10]. Compounds containing CN_3 unit belong to the group of compounds named guanidine [11-13].

They can be found in important antibiotic drugs including Streptomycin, Trimethoprim, Chlorhexidine, Polyhexamethylene bi-guanidine, *etc*. [14-17] Pyridine ring unit is known as a common structural feature employed in numerous pharmaceutically significant molecules [18]. It has been demonstrated by a large number of literature reports that there can be many medicinal applications of guanidine-pyridine hybrid compounds [19, 20]. Accordingly, both pyridine and guanidine moiety can display considerable biological features [21-23].

Regardless of the biological significance of guanidine derivatives, a large number of methods have been employed in recent years to make these compounds [24-27]. The use of urea derivatives as starting material is one of such methods [28]. The reaction of urea derivatives with different amines can be achieved using $POCl_3$ [29]. In this way, a wide range of substituents can be readily considered for the guanidine moiety, representing the stepwise synthesis. These substituents are likely to have the additional donor atoms; these atoms are likely to play important roles in the coordination of guanidine toward the metal center [30-32].

In this paper, the synthesis, spectroscopic characterization and biological activities of the new Co(II), Ni(II) and Cu(II) complexes of two different guanidine-pyridine hybrid ligands were carried out to evaluate the antibacterial activities of these new series of metal-organic compounds.

MATERIALS AND METHODS: All the reagents and solvents were bought from Aldrich and Merck, and used without further purification. Solvents were distilled from the drying agents before use. Infrared Spectra of ligands and metal complexes were taken as 1% dispersion in KBr pellets using Shimadzu 300 spectrometer. Melting points were obtained on an Electrothermal type 9100 melting point apparatus. UV-Visible spectra were recorded on a Varian Cary 100 UV-Vis spectrophotometer using a 1 cm path length cell. ^{1}H-NMR spectra of ligands were recorded on a Brucker AMX 250 MHz spectrometer at ambient temperature in DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. The splitting of proton resonances in the reported ^{1}H NMR spectra are defined as s= singlet, d= doublet, t= triplet, q= quartet, and m= complex pattern; coupling constants are reported in Hz. The molar conductance of the complexes in DMSO (1×10^{-3} M solution) was recorded at 25 °C using Oakton ECTester 11 dual-range, conductivity tester. The Mass spectra were run at 70 eV at 230 °C with Agilent technologies. The completion of reactions was monitored by thin layer chromatography (TLC) on silica gel polygram SILG/UV 254 nm plates. All the bacteria strains were maintained as stock strains in Microbank cryovials and kept at -80 °C until further used.

Synthesis of the Hybrid Guanidine Ligands (L, L′): A solution of phosphoryl chloride (0.3 mmol) in dry toluene was added drop-wise under constant stirring to an ice-cooled solution of urea derivative (0.1 mmol). The resulting suspension was stirred at room temperature overnight, then a solution of 2-aminopyridine (0.1 mmol) in dry toluene was added to it in several portions. After 16-20 h at 100ºC under reflux condition, an aqueous solution of 25 wt. % NaOH (20 ml) was added to bring the solution pH to about 14. The solution was then extracted into the toluene phase (3 × 20 ml). The organic layer obtained was dried with $MgSO_4$ and after filtration; the solvent was evaporated under reduced pressure. Finally, the resulting residue was recrystallized from ethanol to prepare the corresponding compounds.

1, 1, 3, 3-tetramethyl-2-(pyridin-2-yl) guanidine (L): Brown solid; m.p. 120-122 ºC; Yield: 87%; FT-IR (KBr, cm^{-1}): 3100, 2927, 1567, 1386, 1012; ^{1}H NMR (250 MHz, DMSO-d_6): δ ppm 8.11 (t, 1H, J = 5.0 Hz, ArH), 7.44 (d, 1H, J = 2.5 Hz), 6.69 (t, 1H, J = 12.5 Hz, Ar-H), 6.57 (d, 1H, J = 7.5 Hz, Ar-H), 2.63 (s, 12H, CH_3); UV/Vis (DMSO): λ_{max} [nm] = 280, 310.

1,3-dimethyl-2-(pyridin-2-yl)guanidine (L′): Pale yellow solid; 85%; m.p. 175-177 ºC; FT-IR (KBr, cm^{-1}): 3483, 3006, 2941, 1641, 1509, 1378, 1063; ^{1}H NMR (250 MHz, DMSO-d6): δ ppm 7.86 (d, 1H, J = 5.0 Hz, Ar-H), 7.32 (m, 1H, Ar-H), 6.45 (t, 2H, J = 15 Hz, Ar-H), 5.84 (s, 1H, N-H), 3.80 (s, 6H, CH_3); UV/Vis (DMSO): λ_{max} [nm] = 320.

General Procedure for Synthesis of Metal Complexes: Reaction of guanidine ligands with metal(II) ions in the molar ratio 1:1 and 2:1

afforded the corresponding stoichiometry transition metal complexes. A solution of metal(II) chloride (1 mmol) in methanol (20 mL) was added to the solution of the corresponding amount of ligand, *i.e*., for mono complexes, 1 metal: 1 ligand and bis complexes 1 metal: 2 ligands. The solutions were stirred for 30 min and heated under reflux for 2-3 h and 5-6 h for mono and bis-complexes, respectively. The resulting precipitate was filtered off, then washed with methanol and finally dried in a vacuum desiccator.

Mono (1, 1, 3, 3- tetramethyl- 2- (pyridin-2-yl) guanidine)copper (II)(CuL): Dark brown solid; 85%; m.p. >300 ºC; FT-IR(KBr, cm^{-1}): 3350, 3100, 2900, 1664, 1634, 1550, 1048, 561; UV-Vis: λ_{max} = 290, 380, 420 nm; Mass: $[m/z]^+$ = 326, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 12.

Mono (1, 1, 3, 3- tetramethyl- 2- (pyridin-2-yl) guanidine)cobalt(II) (CoL): Dark blue solid; 73%; m.p. >300 ºC; FT-IR (KBr, cm^{-1}): 3424, 2924, 1635, 1317, 558; UV-Vis: λ_{max} = 280, 310, 600, 690 nm; Mass: $[m/z]^+$ = 357, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 16.

Mono (1, 1, 3, 3- tetramethyl- 2- (pyridin-2-yl) guanidine)nickel(II) (NiL): Green solid; 56%; m.m. >300 ºC; FT-IR (KBr, cm^{-1}): UV-Vis: λ_{max} = 270, 410 nm; Mass: $[m/z]^+$ = 321, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 20.

Bis (1, 1, 3, 3- tetramethyl- 2- (pyridin- 2-yl) guanidine)copper (II) (CuL_2): Dark green solid; 79%; m.p. >300 ºC; FT-IR (KBr, cm^{-1}): 3448, 2996, 1913, 1658, 1437, 1018, 670, 532; UV-Vis: λ_{max} = 290, 380, 420 nm; Mass: $[m/z]^+$ = 447, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 22.

Bis (1, 1, 3, 3- tetramethyl- 2- (pyridin- 2- yl) guanidine)cobalt(II) (CoL_2): Blue solid; 80%; m.p. >300 ºC; FT-IR(KBr, cm^{-1}): 3407, 2900, 1634, 1423, 1049, 561; UV-Vis: λ_{max} = 260, 290, 605, 690 nm; Mass: $[m/z]^+$ = 514, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 10.

Bis (1, 1, 3, 3- tetramethyl- 2- (pyridin- 2- yl) guanidine)Nickel(II) (NiL_2): Pale green solid; 63%; m.p. >300 ºC; FT-IR(KBr, cm^{-1}): 3437, 2996, 2913, 1656, 1437, 1028, 670, 524; UV-Vis: λ_{max} = 290, 450 nm; Mass: $[m/z]^+$ = 443, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 14.

Mono (1, 3- dimethyl- 2-(pyridin-2-yl)guanidine) copper (II) (CuL′): Green solid; 75%; m.p. 92-94 ºC; FT-IR (KBr, cm^{-1}): 3436, 2997, 2913, 1565, 1312, 669; UV-Vis: λ_{max} = 580 nm; Mass: $[m/z]^+$ = 297, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 14.

Mono (1, 3- dimethyl- 2-(pyridin-2-yl)guanidine) cobalt(II) (CoL′): Blue solid; 83%; m.p. 98-100 ºC; FT-IR (KBr, cm^{-1}): 3431, 2997, 2913, 1663, 1313, 520; UV-Vis: λ_{max} = 490, 570 nm; Mass: $[m/z]^+$ = 329, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 14.

Mono (1, 3- dimethyl- 2-(pyridin-2-yl)guanidine) nickel(II) (NiL′): Pale green solid; 56%; m.p. 85-87 ºC; FT-IR (KBr, cm^{-1}): 3436, 2997, 2913, 1658, 1312, 669; UV-Vis: λ_{max} = 560 nm; Mass: $[m/z]^+$ = 292, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 20.

Bis (1, 3- dimethyl- 2- (pyridin- 2- yl) guanidine) copper (II)) (CuL_2'): Dark green solid, 70%; 102-105 ºC; FT-IR (KBr, cm^{-1}): 3436, 2996, 2912, 1565, 1312, 669; UV-Vis: λ_{max} = 570 nm; Mass: $[m/z]^+$ = 389, Molar conductance($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 20.

Bis (1, 3- dimethyl- 2- (pyridin- 2- yl) guanidine) cobalt(II) (CoL_2'): Dirty green solid; 70%; 108-111 ºC; FT-IR (KBr, cm^{-1}): 3434, 2998, 2913, 1656, 1313, 670; UV-Vis: λ_{max} = 410, 490, 580 nm; Mass: $[m/z]^+$ = 456, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 20.

Bis (1, 3- dimethyl- 2- (pyridin- 2- yl) guanidine) nickel(II) (NiL_2'): Pale green solid; 67%; 95-97 ºC; FT-IR (KBr, cm^{-1}): 3428, 2997, 2913, 1659, 1312, 669; UV-Vis: λ_{max} = 550 nm; Mass: $[m/z]^+$ = 384, Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) in DMSO: 20.

Biological Studies: A variety of laboratory methods can be used to evaluate the *in-vitro* antimicrobial activity of compounds. The most known and basic methods are disc diffusion and broth or agar dilution methods. In this study *in-vitro* antibacterial activity of ligands and their metal complexes were investigated by applying agar dilution and disc diffusion methods. The Minimal Inhibitory Concentration (MIC) values of synthesized compounds were determined by serial

broth dilution and agar dilution tests. The inhabitation zone of each test compound was measured via the disc diffusion method. The tests were performed using the methodology described in the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [33]. In this study, two Gram-positive (*S. aureus* ATCC: 6838, *B. subtilis* ATCC: 6633) and two Gram-negative (*E. coli* ATCC: 25922, *S.marcescens* ATCC: 13880) microorganisms were used. Each of the bacterial strain was cultured onto Muller-Hinton agar (MHA) plate and incubated for 18-24 h at 35 °C to obtain colony counts. Each test was repeated three times.

Disc Diffusion Method: The solution of the synthesized compounds was prepared at 20 mg/mL in DMSO (which contained no antibacterial activity) under sterile conditions. Amikacin was used as a standard drug reference. Suspensions of each bacteria strain were prepared from their 24 h cultures to obtain approximately 1.5×10^8 colony forming units (CFU) per ml. Paper discs of 8 mm diameter were impregnated individually with a constant amount (100 µg/ml) of the compounds and then were allowed to dry. Next, the dried discs were placed on the inoculated agar surface and incubated for 18-24 h at 35 °C. The antibacterial activity was indicated by the presence of clear inhibition zones around the discs.

Broth Micro-Dilution Test: The MIC values of compounds were determined by serially diluted of 1 ml of the antibacterial agent (stock solution 2000 µgml-1) added to 1 ml of Muller Hinton Broth (MHB) medium and 0.1 ml of bacteria suspension (1.5×10^8 CFU/ml). Amikacin used as reference antibacterial drug was dissolved in DMSO. The two-fold dilution of the compounds and amikacin were prepared in the tubes ranging from 1000 to 1.95 μgml^{-1}. The negative control tube did not contain bacterial strain, and the positive control tube was free of an antibacterial compound. The tubes were incubated for 18-24 h at 35 ºC. The MIC is defined as the endpoint where no visible turbidity could be detected concerning controls.

Agar Dilution Test: Dilution of each compound was freshly prepared in DMSO for each experiment. Different dilutions of the antibacterial stock solutions were incorporated into Muller Hinton agar medium. An agar plate without antibacterial compound was established as positive control plate. After solidification of the agar, the plates were inoculated with a bacterial suspension of 1.5×10^8 CFU/mL by a sterile inoculation loop. After the plates were incubated for 18-24 h, the MIC values were recorded as the lowest concentration of tested compound that inhibited the growth of microorganism.

RESULTS AND DISCUSSION:

Characterization: The guanidine ligands were prepared by the condensation of 2-aminopyridine and urea derivatives in the molar ratio 1:1 in the presence of $POCl_3$ **Fig. 1**. To optimize the reaction conditions to obtain the highest yield, the reaction of N, N′-dimethylurea, and 2-aminopyridine was carried out using various reaction parameters such as solvent type and amount of phosphoryl chloride as shown in **Table 1**. According to the results given in **Table 1**, when $POCl_3$ was not added to the reaction mixture, no compound was produced. By increasing the $POCl_3$ level in the reaction mixture using toluene compared to benzene resulted in the higher percent yield.

TABLE 1: THE REACTION OF N,N′-DIMETHYLUREA (0.1 mmol) AND 2-AMINOPYRIDINE (0.1 mmol) IN THE PRESENCE OF $POCl_3$ IN DIFFERENT CONDITIONS

Entry	$POCl_3$ (mmol)	Solvent	Time (h)	Yield (%)
1	-	toluene	30	-
2	0.1	toluene	20	46
3	0.2	toluene	18	57
4	0.3	toluene	16	85
5	0.4	toluene	18	63
6	0.5	toluene	24	53
7	0.1	benzene	24	42
8	0.2	benzene	23	50
9	0.3	benzene	20	57
10	0.4	benzene	25	38
11	0.5	benzene	28	35

The optimum value was obtained when the amount of $POCl_3$ reached 0.3 mmol which was found to be 85%. However, by increasing $POCl_3$>0.3 mmol the percent yield of the reaction decreased to 53% and 35% using toluene and benzene, respectively.

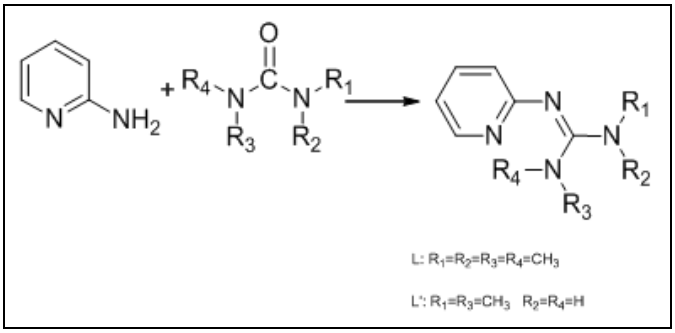


FIG. 1: PREPARATION OF GUANIDINE LIGANDS

The molar conductivity of metal complexes was calculated using Equation (1):

$$\Lambda_m = K / C \text{.....(1)}$$

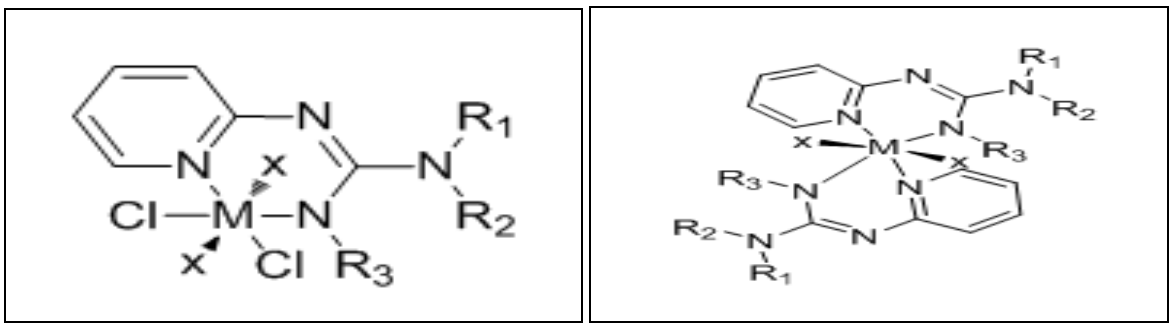
Where κ is the measured conductivity and C is the concentration of the solutions. The molar conductance values of all the complexes were in the range 8-22 ($\Omega^{-1}cm^2mol^{-1}$**)** which indicated these complexes were non-electrolytes. Therefore, in all of the metal complexes, the chlorine atoms are present in the inner sphere of these complexes.

As can be seen in **Table 2**, the percent yields of metal complexes were in the range of 56-85%. Among these metal complexes, the highest yield was achieved for CuL metal complex (85%) with molar conductance of 20 ($\Omega^{-1}cm^2mol^{-1}$) whereas NiL and NiL″ metal complexes showed the lowest percent yield of 56% with molar conductance of 16 and 20 ($\Omega^{-1}cm^2mol^{-1}$), respectively. The proposed structures of metal complexes are shown in **Fig. 2**.

As presented in **Table 2**, in all of the complexes derived from L′ ligand, there is a significant decrease in the melting point compared to the free ligand. This is due to the presence of N-H groups is forming hydrogen bonds in this ligand. However, these hydrogen bonds are eliminated by attaching to the ligand thereby resulting in the reduction of the melting point in these metal complexes.

TABLE 2: PHYSICAL PROPERTIES OF LIGANDS AND THEIR METAL COMPLEXES

Compounds	M.W. (g/mol)	M.P. (°C)	Color	Yield (%)	Molar conductance ($\Omega^{-1}cm^2mol^{-1}$)
L	192	120-122	Brown	87	-
L′	164	175-177	Pale yellow	85	-
CuL	326	>300	Dark brown	85	20
CoL	357	>300	Dark blue	73	12
NiL	321	>300	Green	56	16
CuL_2	447	>300	Dark green	79	22
CoL_2	514	>300	Blue	80	10
NiL_2	443	>300	Pale green	63	14
CuL′	297	92-94	Dark brown	75	14
CoL′	329	98-100	Dark blue	83	14
NiL′	292	85-87	Green	56	20
CuL'_2	389	102-105	Dark green	70	22
CoL'_2	456	108-111	Dirty green	70	10
NiL'_2	384	95-97	Pale green	67	8



M=Cu(II), Ni(II) When only M=Co(II), X=H2O **M=Cu(II), Ni(II) When only M=Co(II), X= Cl**

FIG. 2: THE PROPOSED STRUCTURE OF METAL COMPLEXES

The ligands, L, L′, and their metal complexes were characterized by FT-IR, ^{1}H NMR, and UV-Vis and mass spectroscopies. The important FT-IR data and assignments for free ligands and their metal complexes are reported in **Table 3**. In the FT-IR spectra of ligands, two important bands were observed. The first one was associated with the disappearance of the stretching frequency of NH_2 band in the region 3285 and 3267 cm^{-1} of 2-aminopyridine, as well as stretching frequency of carbonyl groups, υ(C=O) of 1,1,3,3-tetramethylurea or N, N′-dimethylurea in 1723 cm^{-1}. The second one was assigned to the appearance of intensive bands at 1632 cm^{-1} and 1641 cm^{-1}, and at 1012 cm^{-1} and 1063 cm^{-1} corresponding to azomethine groups (C=N), and C-N groups for L and L′, respectively.

In the ^{1}H-NMR spectra of ligands the absence of NH_2 group confirmed the condensation of the amine and carbonyl groups. The signals due to the methyl groups (CH_3) and pyridine ring observed at 2.95 ppm and 8.11-6.54 ppm and at 3.80 ppm and 7.84-6.39 ppm for L and L′, respectively. The spectra of L′, display signal due to the N-H protons at 5.84 ppm.

TABLE 3: IMPORTANT FT-IR DATA OF LIGANDS AND THEIR METAL COMPLEXES

Compounds	C=N	C-N	H_2O	M-N
L	1632	1012	-	-
L′	1641	1063	-	-
CuL	1664	1041	3350	561
CoL	1635	1056	3424	558
NiL	1659	1018	3495	530
CuL_2	1659	1018	3488	532
CoL_2	1634	1049	3407	561
NiL_2	1656	1028	3437	524
CuL′	1656	1031	3436	669
CoL′	1663	1028	3431	520
NiL′	1658	1030	3436	669
CuL'_2	1656	1050	3436	669
CoL'_2	1656	1027	3434	670
NiL'_2	1659	1028	3428	669

In the electronic spectra of L-ligand, band at 280 nm can be assigned to π→π* transition of the aromatic ring, while the more intensive band at 310 nm can be assigned to –C=N group. In the electronic absorption of L′- ligand, one intensive band at 320 nm is observed which is due to the π→π* transition. As can be observed in **Table 3**, in the FT-IR spectra of metal complexes the broadband in the range 3495-3359 cm^{-1} and 3100-2996 cm^{-1} are assigned to the υ (H_2O) and υ (ArCH), respectively. The CH_3 groups stretching are observed in the range of 2900-2912 cm^{-1}. The bands at 1664-1620 cm^{-1} and 1048-1026 cm^{-1} are attributed to υ (C=N) and υ (C-N) resonance of guanidine unit, respectively.

The position of the (C=N) and (C-N) bands shifted to the lower frequencies when compared to the free ligands, indicating that the coordination occurs through the nitrogen atoms to the metal ions. The bonding of the nitrogen atoms with metal ions observed in the range 520-670 cm^{-1} is assigned to the υ (M-N) mode of bonding.

Table 4 represents all peaks derived from UV-Visible spectra of ligands and Cu(II), Co(II) and Ni(II) complexes, as well as the geometry surround the metal ions. The UV-Vis absorption spectra of metal complexes were recorded in DMSO solution at ambient temperature. The UV-Visible spectra of metal complexes were compared to the spectra of the free ligands. By observing the spectra of metal complexes, the intra-ligand bands (π→π*and n→π*) have shifted to the lower frequencies upon the coordination of ligand to the metal ions.

According to the d-d transition for both Cu(II) and Ni(II) complexes, a square-planner geometry is proposed, but octahedral geometry is suggested for Co(II) metal complexes using L and L′ ligands.

The mass spectrum of the synthesized metal complexes was recorded, and the results are in good agreement with the proposed structures. The mass spectrum of NiL'_2 complex is shown in **Fig. 3**. In the mass spectrum of this complex a molecular ion peak at m/z = 384.3 (calc. M. Wt. =

385) with an intensity of 36.35% which is equal to its molecular weight. The other peaks in the mass spectrum are attributed to the fragmentation of the molecule obtained from the rapture of different bond in the molecule. In addition, in the mass spectrum of other complexes molecular ion peak observed at m/z = 326, 357, 321, 447, 514, 443, 297, 329, 292, 389 and 456 are attributed to CuL, CoL, NiL, CuL_2, CoL_2, NiL_2, CuL′, CoL′, NiL′, CuL'_2 and CoL'_2, respectively.

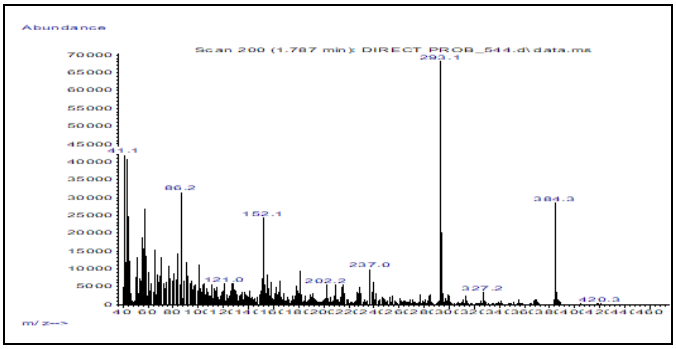


FIG. 3: THE MASS SPECTRUM OF NiL'_2 COMPLEX

TABLE 4: UV-VISIBLE DATA OF LIGANDS AND THEIR METAL COMPLEXES

Compounds	Band position (nm)	Assignment	Geometry
L	280	π→π*	-
L′	310	π→π*	-
CuL	290	π→π,	Square planner
	380	n→π*	
	420	$^2B_1g \rightarrow {}^2B_2g$	
CoL	280	π→π	Octahedral
	310	n→π*	
	600	$^4T_1g \rightarrow {}^4T_1g(p)$	
	690	$^4T_1g \rightarrow {}^4A_2g$	
NiL	270	π→π	Square planner
	410	$^3A_2g \rightarrow {}^3Eg$	
CuL_2	290	π→π	Square planner
	380	n→π*	
	420	$^2B_1g \rightarrow {}^2B_2g$	
CoL_2	260	π→π	Octahedral
	290	n→π*	
	605	$^4T_1g \rightarrow {}^4T_1g(p)$	
	690	$^4T_1g \rightarrow {}^4A_2g$	
NiL_2	290, 450	π→π	Square planner
		$^3A_2g \rightarrow {}^3Eg$	
CuL	580	$^2B_1g \rightarrow {}^2B_2g$	Square planner
CoL	490	$^4T_1g \rightarrow {}^4T_1g(p)$	Octahedral
	570	$^4T_1g \rightarrow {}^4A_2g$	
NiL	560	$^3A_2g \rightarrow {}^3Eg$	Square planner
CuL_2	570	$^2B_1g \rightarrow {}^2B_2g$	Square planner
CoL_2	410	$^4T_1g \rightarrow {}^4T_1g(p)$	Octahedral
	490	$^4T_1g \rightarrow {}^4A_2g$	
	580	$^4T_1g \rightarrow {}^4T_2g$	
NiL_2	550	$^3A_2g \rightarrow {}^3Eg$	Square planner

Biological Study: The antibacterial activities of synthesized compounds were carried out with the following microorganisms under the disc diffusion, broth dilution, and agar dilution methods:

- *Staphylococcus aureus*
- *Bacillus subtilis*
- *Escherichia coli*
- *Serratia marcescens*

Both the broth dilution and the agar dilution tests are known as standard *in-vitro* susceptibility tests which are recommended by the National Committee for Clinical Laboratory Standards (NCCLS). However, it should be noted that these two tests have advantages and disadvantages. The first one, the broth dilution test, is simple, easily manageable, and easy to handle, as well as yielding more reproducible results. Also, this test can be performed in both test tubes and microplates. On the other hand, the agar dilution test takes a long time to carry out; it is also very laborious, and many factors may influence the obtained results. The advantage of agar dilution test is that several bacterial strains on the same plate can be examined, and the growth conditions of the bacteria can be monitored.

From a theoretical point of view, both tests can be applied. However, in the case of the agar dilution test, the antibacterial powder is gradually deposited at the bottom of the plate in the course of preparation, due to insolubility. The resulting heterogeneity in the medium can influence the results to a great extent. For regarding the broth dilution test, while antibacterial solutions could be converted to homogeneous suspensions using shaking, avoiding sediments could be very problematic when the solution stopped shaking after inoculation. Consequently, antibacterial agents may not be able to react fully with the bacteria. To tackle this problem, a stirrer could be employed to prevent the sedimentation of antibacterial agents; however, the effect of stirring on the growth of bacteria should be considered.

The evaluation of the antibacterial effect of ligands and their metal complexes against pathogenic strains based on the disc diffusion test was conducted by measuring the diameter of growth inhibition are shown in **Table 5**, and the minimal inhibitory concentration (MIC) was examined based on the broth microdilution method and agar dilution method are listed in **Table 6** and **7**, respectively.

The antibacterial activities of complexes were compared to standard drug amikacin, as well as to free ligands. **Table 6** revealed that the guanidine ligands had poorer antibacterial activity against all the examined bacteria strains compared to standard drug amikacin and metal complexes with zone inhibition in the range 8-12 mm. The other compounds displayed medium to excellent inhibition against both Gram-positive and Gram-negative bacteria. Among all the synthesized compounds, the highest antibacterial activity was performed by CuL'_2-complex against Gram-positive microorganism *(S. aureus*) with zone inhabitation of 29 mm, followed by CuL_2, CuL′ and CuL compound with the same zone inhibition of 20 mm against *B. subtilis*, *S. aureus*, and *S. marcescens*, respectively. It should be noted that antibacterial activity was not observed for DMSO. As can be seen from **Table 6** and **7** the results of the broth dilution test are significantly lower than those of the agar dilution test for both ligands and metal complexes. This implied that antibacterial powders deposited in the former test could still react with the bacteria by releasing some effective ingredients, whereas, in the later test the releasing ingredients into the agar medium may have been reduced, hence resulting in lower values of MIC in agar dilution test. Also, for the same antibacterial agent, no correlation between the results from these two methods was found. These findings further suggest that the distribution of antibacterial powders in the agar dilution test was probably uneven compared to the broth dilution test. Thus, the broth dilution test appears to be more suitable for testing insoluble inorganic antibacterial agents.

TABLE 5: INHIBITION ZONE (mm) OF LIGANDS AND THEIR METAL COMPLEXES AGAINST PATHOGENIC STRAINS BASED ON DISC DIFFUSION TEST

	G(+)		G(-)	
Compounds	***B. subtilis***	***S. aureus***	***E. coli***	***S. marcescens***
L	10	10	10	12
L′	8	8	10	10
CuL	17	10	18	20
CoL	14	15	13	10
NiL	14	10	12	10
CuL_2	20	12	15	15
CoL_2	18	17	12	12
NiL_2	N.A*	12	10	12
CuL′	12	20	10	15
CoL′	13	15	15	16
NiL′	10	16	10	10
CuL'_2	10	29	12	13
CoL'_2	12	15	17	12
NiL'_2	10	11	10	10
Amikacin	14	15	15	17
DMSO	0	0	0	0

* Not active

The data listed in **Table 6** and **7** showed that metal complexes had better antibacterial activities when compared to parent ligands. This can be explained on the basis of Overton's concept and chelation theory [34-36]. The chelation reduces the polarity of metal to some extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion. The chelation increases the delocalization of p-electrons over the whole chelate ring and enhances the lipophilicity of the complexes which in turn, increases the penetration of the complexes into lipid membranes, and results in blockage of metal sites in the enzymes of the microorganisms. In addition, metal complexes hinder the respiration process of the cell and, block the synthesis of proteins and prevent further growth of the organism. The results given in **Table 6** suggested that the MIC values of compounds are in the order $CuL_2 = CoL_2 < CuL = NiL = CoL' < CoL = CuL'_2 = CoL'_2 < NiL_2 = CuL' = NiL'$ against *B. subtilis*, $CuL'_2 < CuL' < CoL' = NiL' = CoL'_2 < CoL = NiL = CuL_2 = CoL_2 < CuL = NiL = NiL'_2$ against *S. aureus*, $CuL = CoL' < CoL'_2 < NiL = CuL_2 = CuL_2 = NiL_2 = CuL'_2 < CoL = CuL' = NiL'$ against *E. coli* and $CuL < CuL_2 = NiL_2 = CoL' = CuL'_2 = CoL'_2 < NiL = CoL_2 = CuL' < CoL = NiL = NiL'_2$ against *S. marcescens* bacteria. Whereas, the data given in **Table 7** showed that the MIC values of compounds are in the order $CoL < CuL = NiL = CuL_2 = CoL' < CuL'_2 = CoL'_2 < CoL = CuL' < NiL_2 = NiL'$ against *B. subtilis*, $CuL'_2 < CuL' < CuL = NiL_2 < CoL' = < CoL'_2 < CoL = NiL = CoL_2 = CuL_2 = NiL'_2$ against *S. aureus*, $CoL' < CoL'_2 < NiL = CuL_2 = CoL_2 = CuL'_2 < CuL = CoL = NiL_2 = CuL' < NiL'$ against *E. coli* and $CoL' < CuL = NiL = CuL'_2 = CoL'_2 < NiL = CuL_2 = NiL_2 = CoL'_2 < CuL' = CuL'_2 < CoL_2 = NiL' < CoL = NiL'_2$ against *S. marcescens* bacteria. From the results obtained in **Table 5-7** it may be deduced that CuL'_2 complex had the highest inhibition activity against *S. aureus* bacteria with zone inhibition diameter of 29 mm and MIC value of 15.62 µg/ml (based on broth dilution test) and 31.25 µg/ml (based on agar dilution test). Hence, the MIC value for CuL'_2 complex suggested that this compound had much stronger antibacterial activity compared to other synthesized compounds and therefore it may be used as antibacterial drug.

TABLE 6: MINIMAL INHIBITORY CONCENTRATIONS (µg/ml) OF LIGANDS AND THEIR METAL COMPLEXES AGAINST PATHOGENIC STRAINS BASED ON BROTH MICRO-DILUTION METHOD

	G(+)		G(-)	
Compounds	***B. subtilis***	***S. aureus***	***E. coli***	***S. marcescens***
L	500	500	500	500
L′	1000	1000	500	500
CuL	62.5	500	31.25	31.25
CoL	125	125	500	500
NiL	62.5	125	125	125
CuL_2	31.25	125	125	62.5
CoL_2	31.25	125	125	125
NiL_2	500	500	125	62.5
CuL′	500	31.25	500	125
CoL′	62.5	62.5	31.25	62.5
NiL′	500	62.5	500	500
CuL'_2	125	15.62	125	62.5
CoL'_2	125	62.5	62.5	62.5
NiL'_2	N.A	500	N.A	500
DMSO	N.A	N.A	N.A	N.A

TABLE 7: MINIMAL INHIBITORY CONCENTRATIONS (µg/ml) OF LIGANDS AND THEIR METAL COMPLEXES AGAINST PATHOGENIC STRAINS BASED ON AGAR DILUTION METHOD

	G(+)		G(-)	
Compounds	***B. subtilis***	***S. aureus***	**Compounds**	***B. subtilis***
L	1000	1000	1000	1000
L′	1000	1000	1000	500
CuL	125	100	500	125
CoL	500	250	500	1000
NiL	125	250	250	125
CuL_2	125	250	250	125
CoL_2	62.5	250	250	500
NiL_2	1000	100	500	125

CuL′	500	62.5	500	250
CoL′	125	125	62.5	62.5
NiL′	1000	250	1000	500
CuL'_2	250	31.25	250	250
CoL'_2	250	125	125	125
NiL'_2	N.A	1000	N.A	1000
DMSO	N.A	N.A	N.A	N.A

CONCLUSION: With the growing public health concerns of the pathogenic effect by microorganisms, there is an increasing need for new antibacterial agents in many applications such as medical and dental equipment and services, water purifications, food packing and storage, and hospitals. In this study, two new guanidine ligands were synthesized by the reaction of 2-aminopyridine and urea derivatives in optimum conditions. The metal complexes were derived from the reaction of metal ions with different ligands in a molar ratio 1:1 and 2:1. The ligands were coordinated to the metals through nitrogen atoms as bidentate ligands, forming a six-membered ring. The coordination number of Ni and Cu complexes is four with square-planner geometry while the Co complexes have octahedral with a coordination number of six.

The presence of water in the outer sphere of complexes was confirmed by the results of FTIR spectrum in all of the complexes. The antibacterial activities of the ligands and their metal complexes were carried out against gram-positive and gram-negative bacteria such as *B. subtilis*, *S. aureus*, *E. coli* and *S. marcescens* by disc diffusion, agar dilution, and broth dilution methods.

The synthesized compounds were prepared in DMSO which had no antibacterial activity. In general, metal complexes had better biological activities when compared to the parent ligands. In comparison, CuL_2' complex showed the highest antibacterial activity than other metal complexes with zone inhibition diameter of 29 mm and MIC value of 15.62 µg/ml against *S. aureus*.

ACKNOWLEDGEMENT: The authors are grateful to the Chemistry Department, Tehran North Branch, Islamic Azad University for the financial support given in this project.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this article.

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How to cite this article:
Pasdar H, Saghavaz BH, Khadivi R, Davallo M and Foroughifar N: Synthesis and characterization of 2-(pyridin-2-yl)guanidine derivatives and their metal complexes as potential antibacterial agents using phosphoryl chloride. Int J Pharm Sci & Res 2019; 10(9): 4304-14. doi: 10.13040/IJPSR.0975-8232.10(9).4304-14.