Journal of Science and Mathematics Letters, Vol 8, Issue 2, 2020 (15-21)
ISSN 2462-2052, eISSN 2600-8718

RESEARCH PAPER

Mechanochemical Synthesis and Potentiation of the Antimicrobial Activity of 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one by Metal Chelation

Aguzue Onyinye Chinelo*[1], Oviawe Amowie Phillip[2], Adebiyi Adedayo[1]

[1]Sheda Science and Technology Complex, Abuja, Nigeria
[2]Department of Chemistry, University of Benin, Nigeria

*Corresponding author: aguzueonyii@gmail.com

DOI: https://doi.org/10.37134/jsml.vol8.2.3.2020

Received: 29 January 2020; Accepted: 5 March 2020; Published: 9 March 2020

Abstract

A Schiff base ligand, 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one has been synthesized by the condensation of Methoxycinnamaldehyde and 4-aminoantipyrine. Its divalent metal complexes of Co, Ni, and Cu were also synthesized using the mechanochemical solvent-free method. The ligand and the complexes were characterized by FTIR, UV/visible, ^{1}H NMR, ^{13}C NMR, and GC-MS. The ligand behaved as a bidentate donor by using its carbonyl and azomethine N as binding sites for the metals. The ligand showed low activity against some microbes but the complexes were remarkably active against the fungi species.

Keywords: mechanochemical; methoxycinnamaldehyde; 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one; 4-aminioantipyrine

INTRODUCTION

Schiff base is derived by the condensation reaction of aldehydes or ketones and primary amines (Bahl and Bahl, 1993). There are several works on Schiff bases and more researches is ongoing because they have served as synthetic intermediates and have wide applications in natural products, pharmacology, industries, mineralogy and other interdisciplinary sciences (Abdul, 2005). In light of these significances, a variety of synthetic strategies have been developed for the preparation of Schiff base. The solvent-free reactions are green methods in a synthesis that have numerous advantages: reduced pollution, low cost, and simplicity in process and handling (Tanaka and Toda, 2005). In this work, we have synthesized a Schiff base ligand by mechanochemical solvent-free reaction between methoxycinnamaldehyde and 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenylpyrazal-5-one). Methoxycinnamaldehyde is an active constituent of *Agastche rugosa*, a medicinal plant of traditional Chinese medicine and has been reported to have extensive antimicrobial activities and antiviral activity (Kuo et al., 2009; Ooi et al., 2006). Antipyrine is very much used in medicine and it's believed that its amino derivative would equally be of much use in medicine possibly as intermediates in antipyretic and analgesic drugs (Agarwal et al., 2006).

MATERIAL AND METHODS

Chemicals and reagents

All reagents used in this analysis are of analytical grade and obtained from Sigma-Aldrich Chemical Ltd and BDH chemicals. The reagents include Methoxycinnamaldehyde, 4-aminoantipyrine, dimethyl sulphoxide (DMSO), dimethylformamide (DMF), $NiSO_4.6H_2O$, $CuCl_2.2H_2O$, and $CoCl_2.6H_2O$.

Instrumental analysis

The melting point was detected using the melting point apparatus, electronic spectra were determined using Cecil UV C.E7500 7000 series spectrophotometer. IR spectra were also determined using the FTIR-8400S spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were recorded in D_6-DMSO on a Bruker FT-NMR spectrometer. The GC-MS was performed using QP2010 plus Shimadzu. The melting point of the ligands and complexes were recorded using a Gallenkamp Melting Point Apparatus with a thermometer range of 0-360 ºC.

Molar conductance

The molar conductances of each of the metal (II) complexes were determined using 430 Jenway conductivity meter at a concentration of 10^{-3} M in DMSO. This measurement is used to determine whether a given compound is an electrolyte or a non-electrolyte in solution. Molar conductance is the conductivity of an electrolyte solution divided by the molar concentration of the electrolyte and so, measures the efficiency with which a given electrolyte conducts electricity in solution.

Synthesis of 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one

The Schiff base derived from methoxycinnamaldehyde and 4-aminioantipyrine was prepared by carefully weighing 2.03 g (0.01 mol) of 4-aminoantipyrine and 1.62 g (0.01 mol) methoxy cinnamaldehyde into a clean ceramic mortar and ground at room temperature for 20 mins. The resultant was stored in a desiccator over the $CaCl_2$ vacuum[8]. The yield was recorded.

Synthesis of the complexes

The complexes were prepared by the reaction of the ligand (0.01 mol) with the respective metal (II) salts under mechanochemical solvent-free condition, 0.01 mol $NiSO_4.6H_2O$ (2.63 g); 0.01 mol $CuCl_2.2H_2O$ (1.72 g) and 0.01 mol $CoCl_2.6H_2O$ (2.38 g). The various 0.01 mol of the metal salts were each ground with 0.01 mol of the ligand in mortar for 20 mins. The resultant was collected without further purification and stored in the desiccator (Suresh et al., 2012). The yield was recorded.

Antimicrobial sensitivity test

The sensitivity tests on the samples were carried out using the agar well diffusion method (Sulekh et al., 2009). The nutrient agar was prepared according to the manufacturer's recommendation and was poured into Petri dishes to set. The test organisms; bacteria positive gram: *Pseudomonas aereginosa and Escherichia coli* and negative gram: *Staphylococcus aureus and Bacillus subtillis*; Fungi: *Candida albicans* were cultured. The overnight broth cultures of the test organisms were properly diluted to the turbidity of Macfarland's standards and were inoculated on the surface of the agar. The inoculated agar was left for 20 minutes and holes were bored into it using corn borer. The prepared ligand and complexes were dissolved in DMSO and were then introduced into the

agar using a sterile swab stick. The inoculated plates were then incubated at 37ºC for 18 hours thereafter the resultant zones of inhibition were measured using meter rule and results obtained in centimeters were recorded. Ciprofloxacin and fluconazole which are antibacterial and antifungal agents respectively were used as control drugs.

RESULTS AND DISCUSSION

Synthesis of 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one ligand

The synthesis of 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one ligand is shown in Figure 1. All the complexes were air-stable, colored solids and nonhygroscopic. The physical properties of the compounds are presented in Table 1.

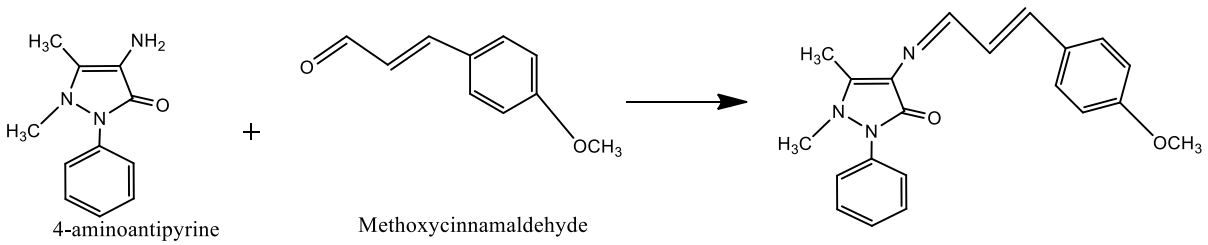


Figure 1. Synthesis of 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one ligand.

Table 1. Physical properties of the ligand and complexes.

Compounds	Colour	MW	MP	Yield (%)	Molar conductance
L*	Yellow	347	166-168ºC	82.2	
CoL	Greenish yellow	576	185-186ºC	62.6	23.7
NiL	Lemon green	608	166-168ºC	62.2	3.86
CuL	Brown	509	178-180ºC	60.0	39.1

*L: 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one; MW: molecular weight; MP: melting point

Infra-red spectra

The infra-red spectra of the ligand and complexes are presented in Table 2. The infra-red spectra of cobalt and nickel complex exhibited a broadband at 3393 and 3414 cm^{-1} respectively. These are due to the presence of water molecules. The sharp bands between 1563-1598 cm^{-1} are due to C=N azomethine vibrations. The free ligand has the C=N vibration at 1592 cm^{-1} so the shifting of the band to higher or lower frequencies in the complexes indicates complexation (Quiroga et al., 1988). The bands that appeared below 650cm^{-1} are assigned to the metal-nitrogen (M-N), metal-oxygen (M-O) and metal chlorine (M-Cl) bonds.

Table 2. Infrared data of the ligand and complexes (cm^{-1}).

Compounds	OH, H_2O	C-H Aromatic	C=O	C=N	C=C Aromatic	M-N	M-Cl
L*	-	3029	1624	1592	1462	-	-
CoL	3393	2924	1639	1575	1458	503	398
NiL	3414	2985	1650	1598	1421	567	456
CuL	-	3029	1640	1563	1490	589	424

*L: 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one

UV/Visible electronic spectra

The electronic spectra of the ligand and its complexes were recorded and shown in Table 3. The ligand's spectra data displayed three bands at 422 nm, 347 nm, and 317 nm which resulted from Intra ligand charge transfer due to the π→π* transition of the phenyl bonds and n→π* transition of the C=N bonds (El-Hassy, 2004). Co (II) complex showed two absorption frequencies at 674 nm and 420 nm. The transitions have been assigned to metal to ligand charge transfer (MLCT) or ligand to metal charge transfer (LMCT) and d→d transitions. In the case of Ni (II) complex (L_2Ni), three absorption bands were observed at 420 nm, 343 nm, and 330 nm. Cu (II) complex exhibited bands at 428nm and 348nm. The intense absorption was due to MLCT or LMCT and the weak absorption has been assigned to d→d transition.

Table 3. UV/Visible electronic spectra data and possible assignments

Compounds	λ nm	λ nm	λ nm
L*	422	347	317
CoL	674	42	-
NiL	420	343	330
CuL	428	348	-

*L: 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one

^{1}H-NMR spectral data

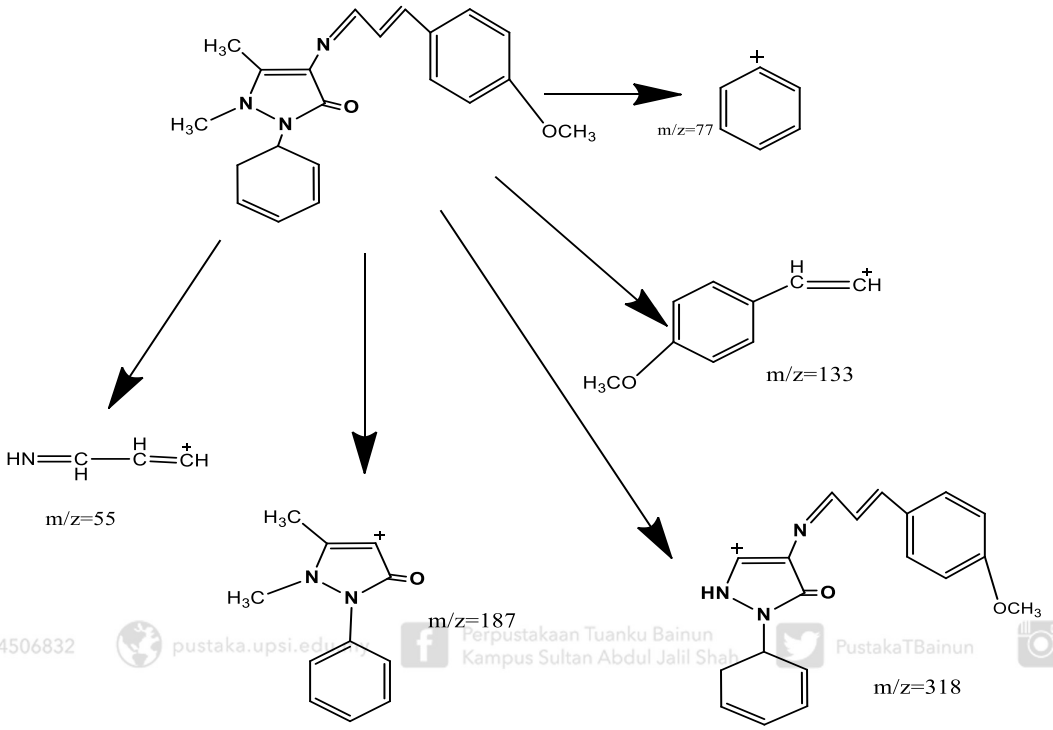
The ^{1}H-NMR signal at 2.41ppm (3H, s) is assigned to the antipyrine methyl proton, H_3C-C. The signal at 3.89ppm (3H, s) is assigned to methyl proton, H_3C-O, while the signal at 3.10ppm (3H, s) is assigned to the methyl proton, H_3C-N. The signals at 7.02 ppm (1H, d) and 6.85m (1H, d) are assigned to ethylene protons, HC=C(H)C. The signal at 9.52 ppm is assigned to azomethine proton, C(H)=N. The azomethine proton (HC=N) appeared at 9.52 ppm for the ligand but shifted upfield (9.39ppm) for the complex showing complexation through the azomethine linkage (El-Hassy, 2004).

^{13}C-NMR spectral data

The ^{13}C-NMR spectrum of the ligand gave a satisfactory result. The peak at 160.21 m is assigned to the azomethine C=N carbon. The peaks at 10.18 ppm, 35.89 ppm, 55.35 ppm are assigned to the methyl carbons. The peaks at 114.24 ppm and 124.38pp m are assigned to the HC=CH. The peaks at 128.79 ppm and 129.17 ppm are assigned to the benzene carbon attached to the antipyrine. The peak at 16.21 ppm is assigned to the benzene carbon attached to O-CH_3. (Obasi L. N. et al., 216; Shipman et al., 1986).

GC-MS spectral data

In the GC-MS analysis of ligand, there were many peaks. The molecular ion peak of the ligand had a mass/charge (*m/z*) ratio of 347 which corresponded to the molecular mass of the compound. The base peak had *m/z* ratio at 318. Other fragments occurred at 33, 45, 55, 77, 89, 104, 117, 133, 161, 187, 234, 29 and 318. The major fragmentations are represented in Scheme 1.



Scheme 1. Mass fragmentation of ligand.

Antimicrobial activity

The results in Table 4 are zones of inhibition. From the results, we can see that the complexes proved potent against some bacteria and especially the fungus more than the ligand. Complexation improved the antimicrobial activities of the ligand. CoL inhibited the growth of all the organisms used in this work more than other complexes except for *Bacillus subtillis* which NiL inhibited most. But NiL, showed no activity against *Escherichia coli* and *Staphylococcus aureus*. CuL was only active against *Staphylococcus aureus* and *Candida albicans*, with the lowest activity when compared to the other complex (Aguzue, 2019). The control drugs were ciprofloxacin and fluconazole for the bacteria and fungi respectively.

Table 4. Antimicrobial test results

Compounds	*Escherichia coli*	*Pseudomonas aeureginosa*	*Bacillus subtillis*	*Staphylococcous aureus*	*Candida albicans*
L*	-	-	10 cm	15 cm	9 cm
CoL	15 cm	21 cm	22 cm	20 cm	15 cm
NiL	-	16 cm	23 cm	-	12 cm
CuL	-	-	-	17 cm	13 cm
Control	14 cm	10 cm	15 cm	-	13 cm

*L: 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one

CONCLUSION

The mechanochemical synthesis and potentiation of the antimicrobial activity of 4-[3-(4-methoxyphenyl)-allylidenea mino]-1,5-dimethyl-2-phenylpyrazol-3-one by metal chelation have been studied. The Schiff base ligand, 4-[3-(4-methoxyphenyl)-allylideneamino]-1,5-dimethyl-2-phenylpyrazol-3-one have been synthesized by the condensation of methoxycinnamaldehyde and 4-aminoantipyrine. Its divalent metal complexes of Co, Ni, and Cu were also synthesized. The ligand and the complexes have been characterized by FTIR, UV/visible, ^{1}HNMR, ^{13}C-NMR, and GCMS. The ligand behaved as a bidentate donor by using its carbonyl and azomethine N as binding sites for the metals. The ligand showed low activity against some microbes but the complexes were remarkably active against some of the bacteria and especially the fungi species. We hereby suggest that this ligand and its metal complexes (especially the Cobalt) be considered as metal-based drugs.

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