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Synthesis and *in-vitro* antibacterial study of some Schiff base copper(II) complexes derived from 4-phenylsemicarbazone

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Abstract
Six new Schiff base ligands derived from the condensation of 5-substituted 2-hydroxybenzaldehyde with 4-phenylsemicarbazide (HL1-HL6) and their copper(II) complexes were synthesized and characterized using melting point, ^{1}H NMR and Infrared (IR). IR data confirmed that the ligands coordinated to the metal ion in a bidentate manner through the azomethine nitrogen and carbonyl oxygen resulting in metal complexes with a 1:2 metal :ligand ratio. The Schiff bases and copper complexes exhibited good in-vitro antibacterial activity against S.aureus and *E.coli in ethyl acetate with zones of inhibition ranging from 9 -27 mm indicating their potential as good broad spectrum antibacterial agents.*
Keywords: Copper(II) Schiff bases, 4-phenylsemicarbazide, *in-vitro*, antibacterial screening

Introduction
The increase in the number of infections caused by bacteria resistance to antibiotics, macrolides, quinolones and vancomycin is of growing concern in contemporary medicine [1]. Antibacterial reisitance may lead to treatment failures and associated complications thereby making the treatment of bacterial infections a challenging therapeutic problem. These observations have made the search of new classes of potential antibiotic agents highly desirable. Current attention is focused on Schiff base transition metal complexes as these complexes have been reported to show numerous applications as fungicides [2], antibacterial [3-4] antiviral and anti-tubercular [5] agents and also exhibit other biological properties [6-7]. One of the important classes of Schiff base transition metal complexes are those containing semicarbazide and thiosemicarbazide groups. Their Schiff bases are among the most relevant nitrogen, oxygen or sulphur donor ligands that provide potential binding sites for a wide variety of metal ions. These ligands can control the stereochemistry of the complexes and provide numerous examples of unusual geometries about the central metal ion. Hence, thiosemicarbazones, semicarbazones and their transition metal complexes have been extensively studied in recent years [8]. These compounds show increased anticonvulsant[9-10], antimicrobial [11-12], antioxidant [13], anticancer [14-16], antituberculosis [17-18] activities compared to other related compounds.
The varying ligation behaviour and biological activity shown by these Schiff base ligands and their metal complexes motivated our investigation of the substituent effects on the 2-hydroxybenzaldehyde Schiff bases derived from 4-phenylsemicarbazide and their copper complexes.

Materials And Methods
4-phenylsemicarbazide, 2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, 5-chloro-2-hydroxybenzaldehyde, 5-methyl-2-hydroxybenzaldehyde, 5-methoxy-2-hydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde and analytical grade solvents were obtained commercially from Aldrich Chemical Ltd, Germany and used without further purification. Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in deuterated chloroform solutions on a Bruker 600 MHz spectrometer with chemical shifts reported in ppm relative to TMS as internal standard. IR spectra of compounds were obtained on solid samples using a Bruker FTIR model Alpha, Laser Class 1 Table Top spectrophotometer equipped with Attenuated Total Reflectance (ATR) in range 4000-500 cm^{-1}.

Typical Synthesis of Schiff bases (HL1– HL6)
To a solution of 4-phenylsemicarbazide Hydrochloride (0.5 mmol.) in methanol (15 mL) was added a solution of the desired 5-substituted 2-hydroxybenzaldehyde (0.5 mmol.) in methanol (15 mL). The mixture was stirred at room temperature for 1 h. The solid product obtained was collected by filtration, recrystallized from methanol and stored over silica gel in a dessicator.

2-(2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL1): white powder. Yield: 32 %; mp: 216 – 218 °C; IR (cm^{-1}): 3255.34, 3173.34, 3034.64, 1645.04, 1606.24, 1552.06, 1498.09, 1585.88, 1443.11, 1356.88, 1310.06, 1270.67, 1216.51, 1155.21, 1135.78, 1111.30, 1059.83, 1031.90, 954.05, 894.14,852.27, 803.53, 750.50, 689.00, 642.51, 604.17, 560.00. ^{1}HNMR (CDCl3, 600MHz): 9.59 (s, 1H), 8.76(s, 1H), 7.59 (s, 1H), 7.54 (d, 2H), 7.40-7.35 (m, 4H), 7.18-7.15 (t, 1H), 7.05 (d, 2H), 7.00-6.97(t,1H).

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2-(5-bromo-2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL2): white solid. Yield: 11.47 %; mp: 209 -211 °C; IR (cm^{-1}): 3306.20, 3195.25, 3106.54. 1593.61. 3306.20, 3195.25, 3106.54, 2965.33, 1654.50, 1593.61, 1561.46, 1526.13, 1474.10, 1449.69, 1371.73, 1346.64, 1327.91, 1309.99, 1258.01, 1239.14, 1206.26, 1177.23, 1155.49, 1126.17, 1076.81, 1030.93, 947.81, 940.41, 920.98, 907.35, 875.15, 825.96, 791.29, 756.76, 719.45, 696.57, 624.54, 579.11, 556.87, 535.78. ^{1}HNMR (CDCl3, 600MHz): 9.55 (s, 1H), 8.56(s, 1H), 7.88 (s, 1H), 7.53 (d, 2H), 7.45-7.39 (m, 4H), 7.32 (d, 2H), 7.19 -7.16 (t, 1H), 6.95-6.93 (t,1H).

2-(5-chloro-2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL3): white powder. Yield: 34.58 %; mp: 212 – 214 °C; IR (cm^{-1}): 3306.39, 3199.79, 1655.72, 1592.88, 1566.85, 1526.48, 1476.94, 1448.24, 1391.68, 1369.76, 1347.35, 1326.19, 1256.81, 1175.88, 1077.05, 1029.48, 940.63, 913.10, 875.09, 827.16, 791.62, 756.72, 748.99, 727.15, 689.30, 643.20, 560.52, 534.29. ^{1}HNMR (CDCl3, 600MHz): 9.53 (s, 1H), 8.83(s, 1H), 7.91 (s, 1H), 7.53 (d, 2H), 7.41-7.33 (m, 3H), 7.29 (t, 1H), 7.19 (d, 1H), 6.99 (d,1H).

2-(2-hydroxy-5-methylbenzylidene)-N-phenylhydrazinecarboxamide (HL4): white powder. Yield: 31.44 %; mp: 207– 209 °C; IR (cm^{-1}): 3269.39, 3191.29, 3035.51, 2916.77, 1643.07, 1608.37, 1552.94, 1491.49,1444.75, 1349.56, 1316.46, 1272.02, 1218.26, 1137.06, 1079.76, 1059.22, 954.02, 815.19, 755.99, 732.49, 689.53, 671.37, 609.57, 564.28. ^{1}HNMR (CDCl3, 600MHz): 2.34 (s,1H), 9.19 (s, 1H), 8.36(s, 1H), 7.94 (s, 1H), 7.52 (d, 2H), 7.40-7.31 (m, 4H), 7.17-7.11 (t, 1H), 6.97 (d, 2H), 6.78 (d,1H).

2-(2-hydroxy-5-methoxybenzylidene)-N-phenylhydrazinecarboxamide (HL5): white powder. Yield: 61.3 %; mp: 194 – 196 °C; IR (cm^{-1}): 3427.79, 3200.14, 1595.00. 3427.79, 3200.14, 3098.66, 2996.18, 2957.14, 1675.19, 1595.00, 1531.67, 1492.65, 1444.95, 1383.92, 1360.55, 1329.69, 1290.47, 1260.64, 1220.76, 1196.88, 1152.23, 1109.19, 1035.81, 941.89, 897.58, 843.36, 804.15, 753.98, 688.86, 617.67, 548.41. ^{1}HNMR (CDCl3, 600MHz): 3.83 (s,1H), 9.35 (s, 1H), 8.39 (s, 1H), 8.00 (s, 1H), 7.54 (d, 2H), 7.40-7.35 (m, 4H), 7.18-7.15 (t, 1H), 7.00-6.97(t,1H).

2-(2, 5-dihydroxybenylidene)-N-Phenylhydrazinecarboxamide. (HL6): white solid**.** Yield: 72.18 %; mp: 206 – 208 °C; IR cm^{-1}: 3437.70, 3305.72, 3174.52, 2208.39, 2170.71, 1632.27, 1590.05, 1537.40, 1408.68, 1330.25, 1304.11, 1214.28, 1171.39, 1141.02, 979.13, 856.42, 824.66, 736.00, 595.87, 526.96. ^{1}HNMR (CDCl3, 600MHz): 10.13 (s, 1H), 9.12(s, 1H), 8.14 (s, 1H), 7.54 (d, 2H), 7.46-7.30 (m, 3H), 7.19-7.11 (m, 1H), 6.97(d,2H).

Typical procedure for the synthesis of copper(II) complexes

A solution of $CuCl_2.2H_2O$ (0.4 mmol.) in methanol (5 mL) was added dropwise to a hot solution of the desired ligand (0.4 mmol.) in methanol (10 mL) with constant stirring. The mixture was refluxed for 3 h, allowed to cool to room temperature and the resultant precipitate was collected by filtration, washed with cold methanol and dried in desiccator.

CuHL1: Colour: Brown solid; mp: 232 – 235 °C; IR cm^{-1} 3267.43, 3091.13, 3053.55, 1616.56, 1593.98, 1573.89, 1520.24, 1497.01, 1473.14, 1438.45, 1372.08, 1327.82, 1274.95, 1246.56, 1206.32, 1150.17, 1121.79, 1056.73, 1031.82, 937.96, 907.00, 822.67, 766.08, 742.06, 711.01, 682.71, 606.05 and 543.48.

CuHL2: Colour: Brown solid; mp: 256 –258 °C; IR cm^{-1}: 3263.33, 1596.21, 1596.21, 597.35 544.08. 3263.33, 3205.32, 3159.15, 3100.51, 3011.51, 2942.63, 1620.46, 1596.21, 1578.03, 1520.06, 1498.68, 1469.79, 1448.35, 1364.44, 1329.25, 1270.94, 1246.18, 1205.60, 1192.83, 1154.07, 1134.84, 1060.13, 954.49, 900.99, 852.20, 826.67, 748.63, 708.58, 688.69, 645.63, 597.35 and 544.08.

CuHL3: Colour: Brown solid; mp: 251 – 253 °C; IR cm^{-1} 1577.54, 3263.65, 3206.18, 3160.86, 3102.10, 3013.38, 2941.92, 1619.95, 1498.64, 1470.64, 1448.49, 1365.49, 1328.97, 1269.97, 1245.87, 1204.19, 1191.29, 1153.72, 1134.06, 1060.62, 1029.89, 949.11, 827.61, 748.17, 732.31, 688.25, 661.34, 597.66, 544.48 and 504.81.

CuHL4: Colour: Brown solid; mp: 225 – 227 °C; IR cm^{-1}3266.38, 3205.93, 3163.53, 3101.88, 3016.99, 1621.76, 1579.79, 1482.56, 1448.52, 1371.90, 1328.74, 1283.06, 1251.62, 1225.12, 1177.35, 1139.58, 1061.21, 948.71, 899.39, 855.40, 825.17, 793.31, 746.89, 721.16, 687.86, 600.79 and 547.97.

CuHL5: Colour: Brown solid; mp: 240 – 242 °C; IR cm^{-1}: 3265.77, 3207.00, 3161.17, 3097.97, 3005.91, 2945.93, 2830.07, 1622.09, 1579.65, 1521.56, 1486.03, 1450.59, 1376.24, 1329.25, 1283.65, 1233.78, 1198.34, 1177.25, 1149.15, 1061.38, 1035.77, 954.21, 899.63, 848.45, 827.41, 796.45, 746.80, 687.90, 630.24 and 598.92.

CuHL6: Colour: Brown solid; mp: 239 – 241 °C; IR cm^{-1}: 3321.78, 3161.50, 3111.09, 2983.81, 1619.39, 1573.11, 1496.66, 1432.05, 1374.35, 1307.65, 1261.88, 1217.95, 1176.20, 1147.38, 1060.36, 970.80, 940.10, 897.76, 861.91, 828.48, 809.56, 742.86, 685.65 and 590.26.

Biological Activity study.

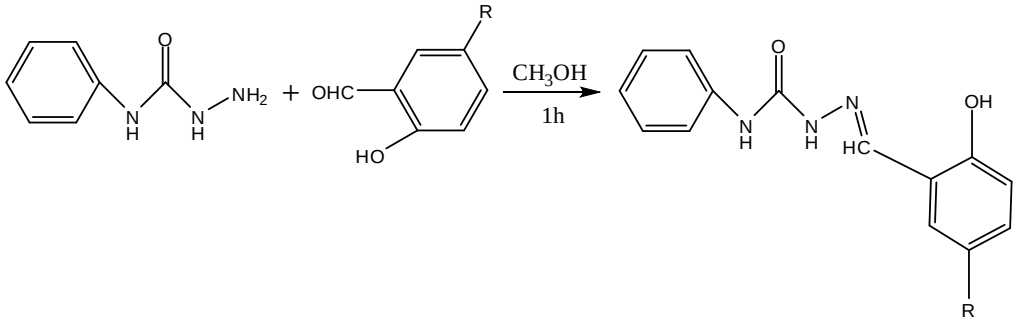
The *in-vitro* antibacterial activities of compounds were assayed using Agar well diffusion technique as described by Ochei and Kolhatkar[19]. The inocula were prepared from the typed cultures, which were maintained in glycerol-peptone water at 4˚C in the pure culture laboratory of Microbiology Department of the

University of Lagos, Akoka, Lagos state, Nigeria and were sub cultured into sterile nutrient broth (Biomark Laboratories, India) using a micropipette fitted with sterile tips. A Muller Hinton agar plate was aseptically prepared. The plate was flooded with standardized (0.5 McFarland) test microorganism and allowed for two minutes to adjust to the environment. A sterilized cork borer (8 mm) was used to make three wells which were filled with the test compounds using a micropipette and incubated at 37°C for 24 h. The diameter of the zone of inhibition surrounding each well was measured and recorded. In order to clarify any participating role of the solvent in the biological screening, control tests were included using the solvent (negative control) and standard broad spectrum Streptomycin disc (positive control).

Results

Synthesis

Condensation reactions of 4-phenylsemicarbazide with the corresponding 5-substituted-2-hydroxybenxaldehyde readily gave rise the corresponding Schiff bases HL1-HL6 respectively (Scheme 1).



Scheme 1: Reaction Scheme for synthesis of Schiff bases HL1-HL6

Treatment of the Schiff base ligands with Cu(II) chloride in methanol afforded the copper complexes. Physical and spectroscopic data of the compounds are presented in Tables 1 and 2.

Table 1: Physical data of Schiff bases and their Copper complexes

Compound	**Molecular Formula**	**mp(°C)**	**Colour**
HL1	$C_{14}H_{13}N_3O_2$	216 - 218	White
CuHL1	$Cu(C_{14}H_{13}N_3O_2)_2$	241 - 243	Brown
HL2	$C_{14}H_{12}BrN_3O_2$	209 - 211	White
CuHL2	$Cu(C_{14}H_{12}BrN_3O_2)_2$	231-233	Brown
HL3	$C_{14}H_{12}ClN_3O_2$	212 - 214	White
CuHL3	$Cu(C_{14}H_{12}ClN_3O_{2)}$	224-226	Brown
HL4	$C_{15}H_{15}N_3O_2$	207 - 209	White
CuHL4	$Cu(C_{15}H_{15}N_3O_2)_2$	209-211	Brown
HL5	$C_{15}H_{15}N_3O_3$	194 - 196	White
CuHL5	$Cu(C_{15}H_{15}N_3O_3)_2$	187-189	Brown
HL6	$C_{14}H_{13}N_3O_3$	206 - 208	White
CuHL6	$Cu(C_{14}H_{13}N_3O_3)_2$	208-210	Brown

Table 2: Diagnostic IR and ^{1}H NMR bands the Schiff bases and their copper complexes

Compound	IR (ν cm^{-1})				^{1}H NMR (□ ppm)	
	ν(NH))	ν (C=O)	ν(C=N)	ν(M-N)	N=CH	NH
HL1	3255.34	1645.04	1606.24		9.59 7.59	3.76
CuHL1	3267.43	1616.56	1593.98	543.48		
HL2	3306.20	1654.50	1593.61		9.55 7.88	3.56
CuHL2	3263.33	1620.46	1578.03	544.08		
HL3	3306.39	1655.72	1592.88		9.53 7.91	3.83
CuHL3	3263.65	1619.95	1577.54	544.48		
HL4	3269.39	1643.07	1608.37		9.19 7.94	3.36
CuHL4	3205.93	1621.76	1579.79	547.97		
HL5	3427.79	1675.19	1595.00			39
CuHL5	3265.77	1622.09	1579.65	598.92		
HL6	3436.70	1632.27	1590.05		9.12	3.14
CuHL6	3321.78	1619.39	1573.11	590.26		

Results of the in-vitro anti-bacterial assay using 5mgmL-1 of each compound are presented in table 3.

Table 3: *In-vitro* antibacterial activity of HL1-HL6 and their Cu(II) complexes.

Sample	***S.aureus***	***E.Coli***
HL1	19	13
CuHL1	14	18.5
HL2	9	16
CuHL2	11	8
HL3	21	24
CuHL3	13	14
HL4	10	17
CuHL4	18.5	15
HL5	-	14
CuHL5	18	12
HL6	27	26
CuHL6	14	20.5
Streptomycin	18	15
Ethylacetate	0	0

Discussion

Six Schiff bases namely 2-(2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL1), 2-(5-bromo-2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL2), 2-(5-chloro-2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL3), 2-(5-chloro-2-hydroxybenzylidene)-N-phenylhydrazinecarboxamide (HL4), 2-(2-hydroxy-5-methoxybenzylidene)-N-phenylhydrazinecarboxamide (HL5) and 2-(2, 5-dihydroxybenylidene)-N-Phenylhydrazinecarboxamide (HL6) were obtained from the reaction of 4-phenylsemicarbazide with the corresponding 5-substituted-2-hydroxybenxaldehyde.

All the compounds are air stable with sharp melting points indicating the purity of the compounds. The compounds were soluble in common organic solvents namely chloroform, ethyl acetate, N,N'-dimethylformamide, dimethylsulphoxide and acetonitrile.

IR spectra*:* The Diagnostics IR and NMR spectral bands of the compounds are presented in Table 2. The infra-red absorption bands of the Schiff base ligands observed at 1590.05 – 1608.37, 1632.27 – 1675.19 and 3255.34 – 3436.70 cm^{-1} are assigned to C=N, C-O and NH vibrations respectively. The presence of signals in the proton NMR spectra in the region 8 attributed to the imine proton further confirmed formation of the Schiff bases, HL1-HL6.

In order to determine the binding mode of Schiff bases to metal in the complexes, IR spectra of the free ligand was compared with IR spectra of the metal complexes. The band due to azomethine nitrogen atom of the Schiff bases underwent a negative shift to 1573.11 – 1593.98 cm^{-1} upon complexation with copper indicating involvement of the azomethine nitrogen atom in coordination. The band arising from the carbonyl group (C=O) also underwent a shift from 1632.27 – 1675.19 to 1616.56 – 1622.09 cm^{-1}. These observations suggest that the Schiff bases function as bidentate ligand coordinating *via* the azomethine nitrogen and the carbonyl oxygen atoms. This is further corroborated by the observation of new bands in the region 540 - 590 cm^{-1} assigned to M-N bond. The bands for metal-oxygen bands were not observed due to limitation of the spectrometer used. The absence of bands in region 3500 cm^{-1} reveals that the complexes are anhydrous complexes with no coordinated water molecules present. The complexes are proposed to compose of a four coordinate copper(II) ion having a 1:2 Metal Ligand ratio as shown in figure1.

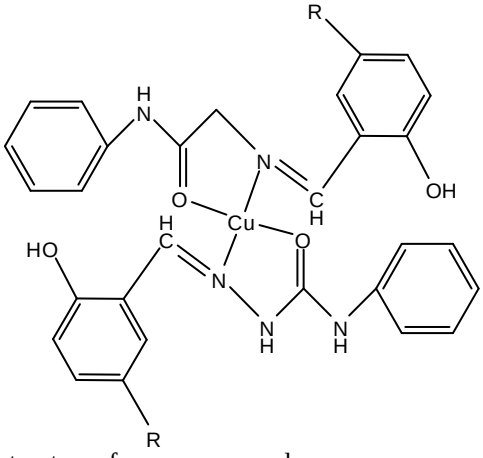


Figure 1: Proposed structures for copper complexes

Biological Activity study

Results of the *in-vitro* antibacterial activity of the investigated compounds against *S.aureus* and *E.Coli* in ethyl acetate solution are summarized in table 3. The Schiff bases were active against the bacterial strains tested with inhibition zones in the range 9-27 mm with exception of HL5 which was inactive against *S. aureus*. All the copper complexes were highly active against the bacterial strains having inhibition zones in the range 11-20.5 mm. The Schiff base bearing the dihydroxy substituents, HL6 exhibited the highest activity of all compounds and was more active compared to the standard drug streptomycin. While the compounds bearing the methoxy, bromo and methyl substituents (HL5, HL2 and HL4) had the low activities towards the gram positive *S.aureus* strain. In these compounds ligation with the copper ion improved the activity observed. Expectedly, the chloro compound (HL3) exhibited high activity against the bacterial strains. This corroborates the reported antiseptic property of chlorine compounds as many antiseptic agents currently in use are chlorine containing compounds [20]. A comparative study of the ligands and their copper complexes indicates that some of the complexes exhibited antimicrobial activity over the free ligands. This is evident in the higher activity of CuHL1 against *E. coli* and CuHL4 and CuHL5 against *S.aureus*. This is probably due to the high electron donating ability of the corresponding Schiff bases HL4 and HL5 which results in formation of more stable metal complexes which would increase the lipophilicity of the compounds thereby favouring diffusion cross the cell membrane. It is generally observed that metal chelates have higher antibacterial activity than the ligands. This is because of increase in cell permeability which increases lipophilicity favouring the passage of lipid soluble material [21].

Conclusion: The synthesis of Schiff base ligands obtained from 5-substituted-2-hydroxybenzaldehyde and 4-phenylsemicarbazide and their corresponding copper complexes is described. Schiff base coordinates in a bidentate fashion giving rise to metal complexes with 1:2 Metal ligand ratio. The antimicrobial results reveal that all compounds were active against bacterial strains tested and activity is dependent on the substituent present. Schiff bases with the 5-chloro and 5-hydroxy substituents are potential candidates for formulation of broad spectrum antimicrobial agents. Further work on the cytotoxicity of the compounds are underway.

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