https://dx.doi.org/10.4314/ijs.v26i2.19

ANTIMICROBIAL ACTIVITY AND MOLECULAR REACTIVITY OF PHENYLTHIOSEMICARBAZONES CONTAINING THE THIOPHENYL NUCLEUS AND THEIR Cu(II) COMPLEXES

Sobola, A. O.[1,*], Yusuff, O. K.[2], Raji, A. T.[3], Shaibu, R. O.[4], Osundiya, M.O.[1], Orungbamila, F. O.[1], Ante, D. A.[1,5], Odukaye, F. S.[1] and Alliu, A. A.[1]

[1]Department of Chemistry, Lagos State University, Ojo, Nigeria.
[2]Department of Chemistry, University of Ilorin, Nigeria.
[3]Center for Augmented Intelligence & Data Science (CAIDS), College of Science, Engineering and Technology (CSET), University of South Africa (UNISA), South Africa.
[4]Department of Chemistry, University of Lagos, Akoka, Nigeria.
[5]Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, USA.
[*]Corresponding Author's E-mail: abdullahi.sobola@lasu.edu.ng
(Received: 24th March, 2024; Accepted: 22nd July, 2024)

ABSTRACT

The antimicrobial activity and molecular reactivity of two phenylthiosemicarbazone ligands containing the thiophenyl nuclues (L_1 & L_2) with their Cu(II) complexes have been investigated. The thiosemicarbazone ligands were obtained by condensing 4-phenylthiosemicarbazone with 2-thiophene carboxaldehyde and 3-methyl-2-thiophene carboxaldehyde respectively. The antimicrobial activity of the ligands and the metal complexes were evaluated using *gram*-positive and *gram*-negative bacterial strains. In addition the compounds were further characterised with various global molecular reactivity descriptors using DFT calculations. The compounds exhibited mild inhibition against the bacterial strains, especially *Staphylococus aureus* and *Escherichiia coli*. In addition, the activity of the uncomplexed thiosemicarbazones became enhanced upon chelation with Cu(II) ions. The DFT studies showed that ligand L_1 is more reactive than L_2 and the energy gaps for the complexes were 0.936 eV and 1.103 eV for $[CuL_1]$ and $[CuL_2]$ respectively. Furthermore, the results showed that the copper(II) complexes are soft molecules having higher molecular reactivity, high electro-optic responses and low kinetic stability than their respective ligands.

Keywords: chelation; ligands; disc diffusion; global hardness; chemical reactivity; spectral data.

INTRODUCTION

The emergence of several drug resistance microbial strains such as the methicillin resistance staphylococcus aureus (MRSA) has elicited a sustained focus on the synthesis of new organo-metallic compounds as potential drug agents. Consequently, heterocyclic compounds containing varying pharmacologically significant moieties, such as thiophenyl-derived thiosemicarbazones, could be considered as suitable lead compounds towards the discovery of new drug agents with enhanced potency. Thiosemicarbazones are important organo-sulphur compounds with varying biological applications. They contain the thio-urea group (-HN-C=S-NH-) and serve as potent precursors for the synthesis of various pharmaceuticals and bioactive compounds. The earliest reported medical application of thiosemicarbazones was in their application for the treatment of tuberculosis and leprosy cases (Bernstein *et al*., 1951; Maia *et al*., 2009). Interestingly, the medical application of thiosemicarbazone-based drug agents is largely related to the variation of substitution at the hydrazine-N^1 or the amide-N^4 positions of the thiosemicarbazone moiety. For instance, methisazone (Figure 1a) is a long established anti-viral drug with an N-methyl-isatin substitution at the N^4- position. It is very active against small pox (Bauer *et al*., 1969; Kune, 1964).

Similarly, triapine (Figure 1b) is another clinically significant substituted thiosemicarbazone compound with broad anti-tumour agent arising from the N^4-substituted 3-amino-pyridine-2-carboxaldehye group (Knox *et al*., 2007; Kunos & Sherertz, 2014; Kunos *et al*., 2017).

(a) (b)

Figure 1: Structures of (a) Methisazone and (b) Triapine

Subsequently, various substituted thiosemicarbazone compounds have been investigated for their potency as antiviral (Karaküçük-İyidoğan *et al.*, 2011; Padmanabhan *et al.*, 2017; Arslan *et al.*, 2021), antimicrobial (Ibrahim *et al.*, 2018; Gaber *et al.*, 2021), antimalarial (Duan & Zhang, 2011; Matsa *et al.*, 2019) and anti-tumour (Demoro *et al.*, 2013; Lukmantara *et al.*, 2013; Qi *et al.*, 2020) agents.

In particular, thiosemicarbazone compounds containing the thiophenyl nucleus have been reported to enhance apoptosis in human liver hepatocellular carcinoma HepG2 in a dose-dependent manner (Wang *et al.*, 2015). Consequently, an investigation into the antimicrobial activity of the thiophenyl-derived thiosemicarbazone compounds would suggest their suitability as potential broad-spectrum pharmacological agents. In addition, studies have shown that the incorporation of metal ions into the matrix of thiosemicarbazone ligands enhances the pharmacological efficacy of the non-coordinating ligands (El-Sawaf *et al.*, 2018; Ibrahim *et al.*, 2018; Arslan *et al.*, 2021).

This study seeks to assess the antimicrobial potential and molecular reactivity of two thiophenyl-derived thiosemicarbzones with their copper(II) complexes. The thiosmicarbazone compounds were prepared from 4-phenylthiosemicarbazone, 2-thiophene carboxaldehyde and 3-methyl-2-thiophene carboxaldehyde. The free ligands and their copper(II) complexes were screened for their antimicrobial efficacy against *Bacillus substillis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. In addition, the compounds have been characterized with various global molecular reactivity descriptors using DFT calculations. Global molecular reactivity descriptors are related to the reactivity and site selectivity of molecules and thus helps to predict the toxicity of compounds (Talmaciu *et al.*, 2016; Molski, 2021).

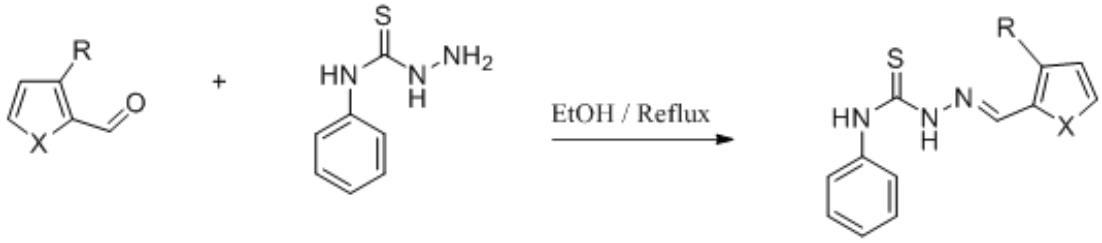
MATERIALS AND METHODS

The chemicals and reagents used for this study were of reagent grade and were used as supplied by Sigma-Aldrich.

The Fourier transform infrared and the electronic spectral data of the thiophene-derived thiosemicarbazone compounds were obtained using a Bruker TENSOR 27 single channel infrared spectrometer and T-80 UV-Visible spectrophotometer respectively. Similarly, Bruker Avance spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) was used to record the proton and carbon-13 NMR spectra of the thiosemicarbazone ligands in $CDCl_3$ or DMSO-d_6 at 25 °C. The elemental analysis was done using Vario EL cube model elemental analyser while Jenway 4510 conductivity meter was used for the measurement of the conductivity of the metal complexes. Griffin melting point apparatus was used for the melting points determantion while the antimicorbial study was conducted using two gram-positive and two gram-negative American Type Culture Collection (ATTC) bacterial strains.

Synthesis of the Thiosemicarbazone ligands

The thiophene-derived thiosemicarbazone compounds were prepared based on the previously reported methods as illustrated in Scheme 1 (Wang *et al.*, 2015; Prabhu & Ramesh, 2016; El-Sawaf *et al.*, 2018) by reacting equimolar mixture of 4-phenylthiosemicarbazide with 2-thiopenecarboxaldehyde and 3-methyl-2-thiophenecarboxaldehyde respectively, under reflux condition with glacial acetic acid as a catalyst.



L_1: R = H; X = S L_2: R = CH_3; X = S

Scheme 1: Synthesis of the Thiophene-derived Phenylthiosemicarbazone Ligands

2-((Thiophen-2-yl)methylene)-*N*-phenylthiosemicarbazone (Ligand L_1)

The ligand L_1 was prepared by reacting 7.5 mmol (1.25 g) of 4-phenylthiosemicarbazide with 2-thiophene carboxaldehyde (7.5 mmol, 2.23 g) in ethanol under reflux condition for 6 h to obtain a cream colour precipitate. The solid precipitate was obtained using suction filteration, washed with ethanol and dried using concentrated H_2SO_4 in a dessicator. Yield: 1.88 g (71.92%). m.p.:178 – 180 °C). CHNS for $C_{12}H_{11}N_3S_2$: Found: %C: 55.44, %H: 4.13, %N: 16.06, %S: 24.98; Calculated: %C: 55.16, %H: 4.24, %N: 16.08, %S: 24.49. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 10.10 (1H, s, N-H), 9.09 (1H, s, N-H); 8.10 (1H, s, HC=N), 7.67 – 7.65 (2H, d, Ar-H); 7.43 – 7.41 (3H, t, Ar-H); 7.31 (1H, d, Ar-H); 7.27 – 7.25 (1H, t, Ar-H), 7.08 – 7.07 (1H, t, Ar-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 175.54 (C=S), 137.83 (C=N), 137.80, 131.23, 128.89, 128.85, 127.89, 126.35, 124.66 (Ar-C).

2-((3-Methyl-thiophen-2-yl)methylene-*N*-phenylthiosemicarbazone (Ligand L_2)

The procedure was the same using 4-phenylthiosemicarbazide (7.5 mmol, 1.25 g) and 3-methyl-2-thiophene carboxaldehyde (7.5 mmol, 0.81 mL). Yield: 1.78 g (95.90%). m.p.: 180 – 182 °C. CHNS for $C_{13}H_{13}N_3S_2$: Found: %C: 56.82, %H: 4.85, %N: 15.17, % S: 23.15. Calculated. %C: 56.69, %H: 4.76, %N: 15.26, %S: 23.24. ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.67 (1H, s, N-H), 9.68 (1H, s, N-H), 8.47 (1H, s, HC=N), 7.59 (3H, t, Ar-H), 7.35 (2H, t, Ar-H), 7.19 (1H, t, Ar-H), 6.97 (1H, d, Ar-H), 2.31 (3H, s, Ar-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 175.69 (C=S), 141.08 (C=N), 139.44, 138.21, 132.32, 131.42, 128.78, 126.60, 125.63, 125.61 (Ar-C) and 14.19 (CH_3).

Synthesis of the thiophene-derived thiosemicarbazone copper(II) complexes

The thiophene-derived thiosemicarbazones (L_1 & L_2) were reacted with copper(II) acetate in 2:1 molar ratio under reflux condition for 6 h in order to obtain their respective copper(II) complexes.

$Cu(L_1)_2$

1.91 mmol (0.5 g) of L_1 in 10 mL dimethylformamide (DMF) and 0.96 mmol (0.19 g) of $Cu(OAc)_2.H_2O$ in 10 mL ethanol were mixed together in 100 mL round bottom flask and refluxed for 6 h to obtain a dark purple precipitate. The solid precipitate was obtained using suction filteration, washed with DMF and dried using concentrated H_2SO_4 as a dessicant. Yield: 0.27 g (46.69%). m.p.: 188 – 190 °C. CHNS for $Cu(C_{24}H_{22}N_6S_4)$: Found: %C: 49.30, %H: 3.34, %N: 14.34, %S: 22.20. Calculated: %C: 49.18, %H: 3.78, %N: 14.33, %S: 21.83. Λ_M = 11.42 $\Omega^{-1}cm^2mol^{-1}$. The spectral data for the copper(II) compounds are presented in Table 1.

$Cu(L_2)_2$

A mixture of 10 mL methanolic solution of 1.82 mmol (0.50 g) of L_2 and 10 mL ethanolic solution of 0.181 g (0.91 mmol) of $Cu(OAc)_2.H_2O$ was similarly refluxed for 6 h to obtain $Cu(L_2)_2$.Yield: 0.33 g (58.07%). m.p.: 190 – 192 °C. CHNS for $Cu(C_{26}H_{26}N_6S_4)$. Found: %C: 49.89, %H: 4.02, %N: 13.57, %S: 19.47. Calculated: %C: 49.38, %H: 4.46, %N: 13.29, %S: 20.24. Λ_M = 12.00 $\Omega^{-1}cm^2mol^{-1}$.

Computational Studies

The molecular reactivity parameters for the thiophene-derived thiosemicarbazones and their Cu(II) complexes were obtained by carrying out Density functional theory (DFT) calculations. The

geometry of the structures was optimized using the B3LYP functional (Lee *et al.*, 1988) with the 6–31G(d) basis set (Nascimento *et al.*, 2021). The study was done under polarizable continum solvation model (PCM) using ethanol as solvent. Furthermore, the vibrational frequency calculations and the characterization of the electronic transitions of the compounds were performed using the time-dependent density functional theory (TD-DFT) (Nakatsukasa *et al.*, 2016) at B3LYP/631-G(d) level of theory.

The spectroscopic and the global molecular reactivity descriptors such as energies of the frontier molecular orbitals, (E_{LUMO}) and (E_{HOMO}), frontier orbitals energy gap (ΔE), electron affinity (A), electronegativity (χ), chemical potential (μ), global hardness (η), electrophilicity index (ω), and global softness (S) were evaluated from the DFT calculations in order to better characterize the thiophene-derived thiosemicarbzone ligands and their Cu(II) complexes.

Antimicrobial Study of Thiophene-Derived Thiosemicarbazone Compounds

The thiophene-based thiosemicarbazones and their corresponding copper(II) complexes were studied for their inhibitory activity on four bacterial strains using Kirby–Bauer disc diffusion method (Bauer, 1996; NCCLS, 2015). The bacterial strains were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, and *Staphlylococcus aureus* ATCC 25923 while streptomycin was used as positive control at 10 μg/mL. The test compounds were prepared at a final concentration of 1000 μg/mL in dimethylsulphoxide (DMSO) and were loaded unto sterile discs of 5 mm. In addition, petri dishes containing nutrient agar were inoculated with 0.5 McFarland turbid control isolates of the bacterial strains. The sterile discs were applied on the nutrient agar at a distance of 30 mm and then incubated at 37 °C for 24 h. The antimicrobial susceptibility of the compounds was determined by measuring the diameter of zone of inhibition around each disc in millimetre.

The minimum inhibitory concentrations (MIC) for the compounds were obtained using standard micro-dilution method (NCCLS, 2015). The two-fold serial dilution was carried out in a 96-well microtitre plate and the incubation was once again carried out at 37 °C in ambient air for 24 h. The MIC for each of the compounds corresponds to the minimum concentration without any noticeable turbidity (NCCLS, 2015). In addition, the antimicrobial study of the compounds involved the determination of the minimum bactericidal concentration (MBC) by sub-culturing from two to three-fold dilutions of each well showing the MIC onto nutrient agar plates. The plates were equally incubated at 37 °C in ambient air for 24 h and the MBC corresponds to the lowest concentration of the sample that kills the bacterial isolates.

RESULTS AND DISCUSSION

CHNS analysis and conductivity of the compounds

The microanalysis data of the prepared thiophene-derived thiosemicarbazone compounds agree with the expected values indicating the purity of the compounds. The copper complexes are of the form $[CuL_2]$ as indicated by the CHNS results. In addition, the low conductivity values of the complexes indicated their non-electrolytic nature and by extension their existence as neutral compounds (Geary, 1971). Furthermore, the non-electrolytic nature of the complexes could be related to the coordination of the thiophene-derived thiosemicarbazone ligands as anionic species through the deprotonated thiolate-S atom (Lobana *et al.*, 2009).

NMR spectral study

The proton and carbon-13 NMR spectra for ligands L_1 were recorded in $CDCl_3$, while ligand L_2 spectra were recorded in dimethylsulfoxide, d_6. The thiosemicarbazone ligands exhibit signals at 11.67 – 10.10 ppm and 9.09 ppm corresponding to the hydrazinic N^3- H and the thiourea N^4- H amino groups respectively (Al-Amiery *et al.*, 2011; Sampath *et al.*, 2013; Haribabu *et al.*, 2017). In addition, the azomethine proton, HC=N, resonates as one proton singlet at 8.47 – 8.10 ppm (Khalaji *et al.*, 2010; Sampath *et al.*, 2013). The aromatic and the thiophene rings signals of the thiosemicarbazone ligands were observed at 7.67 – 6.97 ppm. The strong signal at 2.31 ppm characterizes the methyl protons of the 3-methyl-thiophene-derived thiosemicarbazone ligand (L_2).

Furthermore, the ^{13}C-NMR spectra of the ligands exhibit signal at about 176 ppm due to the thiocarbamide functional group (C=S) of the thiosemicarbazone ligands. This observation indicates the existence of the thiophene-derived thiosemicarbazones as thione tautomers. The azomethine carbon, C=N, resonated at 141.08 ppm while the aromatic signals were observed at 144.99 – 123.89 ppm. The ^{13}C-NMR spectra of ligand L_2 exhibit an addition signal at 14.19 ppm corresponding to the methyl, CH_3. The NMR spectroscopic data for the compounds have been presented above under experimental section.

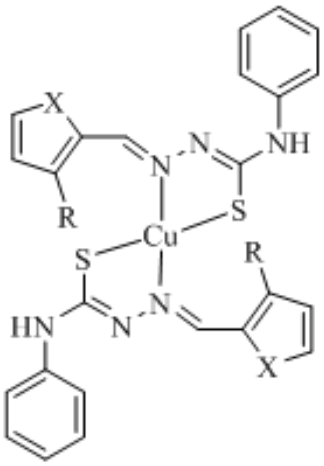
Infrared spectral study

The infrared spectral data for the thiopene-derived phenylthiosemicarbazones are presented in Table 1 while the spectra are presented as supplementary data. The spectra of the uncoordinated ligands exhibit two distinct N-H symmetric absorption bands between 3300 cm^{-1} and 3100 cm^{-1} region. The thiourea N-H band appeared at 3297 – 3280 cm^{-1} while the broad band at 3144 – 3127 cm^{-1} is associated with the stretching vibration of the hydrazinic N-H group (Afrasiabi *et al.*, 2004; Lobana *et al*; 2009; Tan *et al.*, 2012). The presence of the hydrazinic N-H and the non-appearance of any band within 2700 - 2500 cm^{-1} due to the S-H band imply that the thiosemicarbazone ligands did not exhibit thione – thiol tautomerism (Afrasiabi *et al.*, 2004; Li *et al.*, 2010; Tan *et al.*, 2012; Savir *et al.*, 2020). On the contrary, the hydrazinic N-H band was not observed in the spectra of the copper(II) complexes, suggesting the bonding of the free thiosemicarbazones in the anionic form via the thiolate sulphur as N=C-S$^-$ (Lobana *et al.*, 2009).

Table 1: Infrared and Electronic Spectral Data for the Thiophene-derived Compounds

S/N	Compounds	ν_{N-H} (cm^{-1})	ν_{C-H} (cm^{-1})	$\nu_{C=N}$ (cm^{-1})	$\nu_{C=S}$(cm^{-1})	λmax / nm
1.	Ligand L_1	3297, 3136	3103, 3067	1589	1270	245, 345
2.	$Cu(L_1)_2$	3347	3089	1528, 1593	1244	290, 345, 395
3.	Ligand L_2	3280, 3144	3047, 3027	1589	1275	245, 350
4.	$Cu(L_2)_2$	3406	3043	1525, 1600	1243	280, 340, 390

Furthermore, the strong band at 1589 cm^{-1} corresponds to the stretching vibration of the azomethine group, C=N, (Haribabu *et al.*, 2017; Savir *et al.*, 2020) of the thiophene-derived thiosemicarbazone ligands. The band, however, underwent a negative shift (1528 – 1525 cm^{-1}) for the copper(II) complexes, suggesting bonding to the Cu(II) ions through the imine-N. Similarly, the thioamide, $\nu_{C=S}$, band was observed at 1275 – 1269 cm^{-1} in the non-complexed ligand and this underwent a negative shift of ~30 cm^{-1} in the Cu(II) complexes. This is suggestive of the coordination of the thiosemicarbazone ligands via the S –atom as illustrated in Figure 2.



$Cu(L_1)_2$]: X = S, R = H ;
[$Cu(L_2)_2$]: X = S, R = CH_3

Figure 2: Proposed Structures for the Thiosemicarbazone Copper(II) Complexes

The Cu(II) complexes are neutral species as indicated by the conductivity data and this suggests that the thiosemicarbazone ligands coordinated in an anionic form through the deprotonated thiolate sulphur. Therefore, the lowering of the thioamide (C=S) frequency corresponds to a decrease in the double bond character of the C=S bond due to the transfer of electrons to the Cu(II) ions. Consequently, this results in the formation of a double bond between the C^3 – and N^2 – atoms (Figure 2) and thus the appearance of an additional imine band at 1600 – 1590 cm^{-1} in the infrared spectra of the complexes (Prathima *et al.*, 2010). The proposed structure for the thiophene-derived thiosemicarbazone copper(II) complexes is presented as Figure 2.

Electronic spectral study

The UV/visible study of the compounds were carried out in dimethylformamide and the spectral data are presented in Table I. The electronic spectral data for the free thiophene-derived thiosemicarbazone ligands show two distinct absorption bands at 280 – 295 nm and 340 – 395 nm. The lower energy band corresponds to $\pi \rightarrow \pi^*$ transition arising from the delocalization of pi electrons of the aromatic ring and the $n \rightarrow \pi^*$ transition of the imine, C=N (West *et al.*, 1993; García-Tojal *et al.*, 1999; Rahman *et al.*, 2017;). In the metal complexes, however, the bands underwent redshift and were observed at 280 – 295 nm and 340 – 395 nm respectively. The ligand field transition of the Cu(II) complexes were, however of very low intensity and thus not observed.

Molecular reactivity of the thiophene-derived thiosemicarbazone compounds

The values of the various global molecular reactivity descriptors for the thiophene-derived thiosemicarbazone compounds are presented in Table 2.

It is evident that the addition of $–CH_3$ nucleophile to the thiophene ring resulted in increments in the values of the ionization energy, frontier orbital energy gap, electron affinity, electrophilicity index, electronegativity and global hardness. On the other hand, there was a corresponding reduction in frontier orbitals energies, chemical potential, and global softness. Similarly, the frontier orbital energy difference, ionization energy and the global hardness of the Cu(II) complexes were lower than that of the free ligands. This indicates that the metal complexes are soft molecules with high chemical reactivity, low kinetic stabilities and high electro-optic responses. The Cu(II) complexes are, therefore, considered to be more polarizable and magnetizable than the free uncoordinated thiophene-derived thiosemicarbazone ligands (Nakatsukasa *et al.*, 2016; Nascimento et al., 2021). Furthermore, the Cu(II) complexes have higher electron affinity, global softness and electrophilicity index. Comparatively, the order of reactivity for the ligands is $L_1 > L_2$ while CuL_1 is similarly more reactive than CuL_2. The lower the value of LUMO–HOMO energy gap (ΔE); the less kinetically stable and more reactive is the molecule.

Table 2: Global molecular descriptors for the thiophene-derived thiosemicarbazone compounds.

Parameters (eV)	L_1	$Cu(L_1)_2$	L_2	$Cu(L_2)_2$
E_{HOMO}	-5.453	-3.007	-5.860	-3.021
E_{LUMO}	-1.668	-2.071	-1.821	-1.918
ΔE (E_{LUMO} - E_{HOMO})	3.785	0.936	4.039	1.103
I	5.453	3.007	5.860	3.021
A	1.668	2.071	1.821	1.918
η	1.893	0.468	2.020	0.552
S	0.528	2.137	0.495	1.813
χ	3.561	2.539	3.841	2.470
μ	-3.561	-2.539	-3.841	-2.470
ω	3.349	6.887	3.652	5.529

Furthermore, the electron densities of the HOMO and LUMO of both the free thiophene-derived ligands (Figure 3) and their copper complexes (Figure 4) are well distributed around the thiophene ring and the atoms of the thiosemicarbazide moiety but not on the aromatic ring of the ligands. The most favourable electronic transitions for the ligands are the $\pi \rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ transitions.

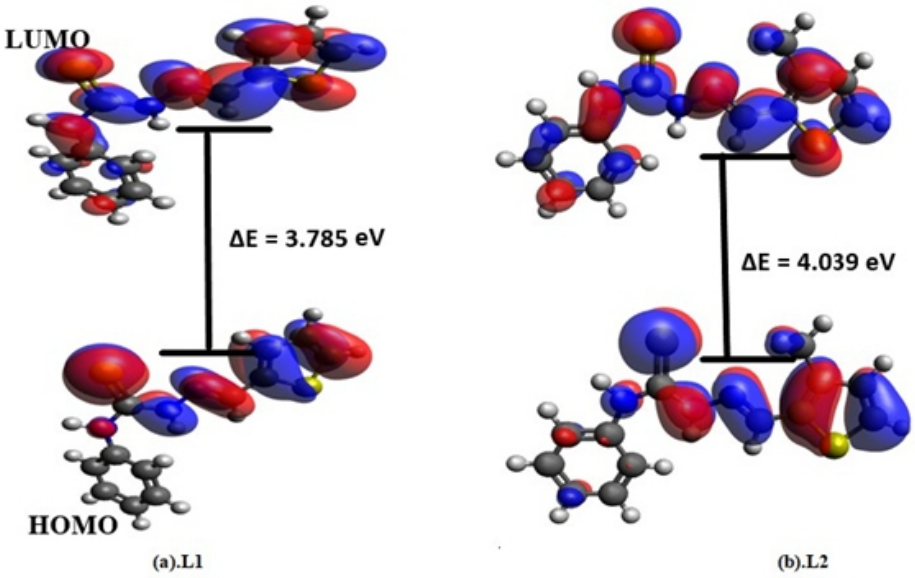


Figure 3: Frontier Molecular orbitals for the LUMO–HOMO of the Ligands

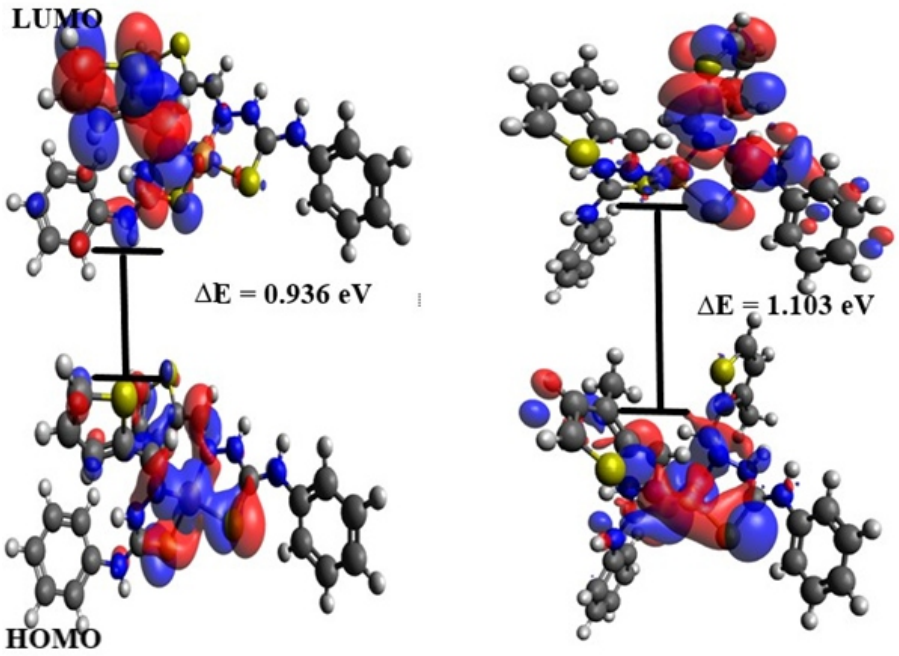


Figure 4: Frontier Molecular orbitals for the LUMO–HOMO of the complexes

Antimicrobial study

The inhibitory activity of the thiophene-derived thiosemicarbazone compounds against the tested microorganisms is presented as zone of inhibition in Figure 5 while the MIC and the MBC results are presented in Table 3.

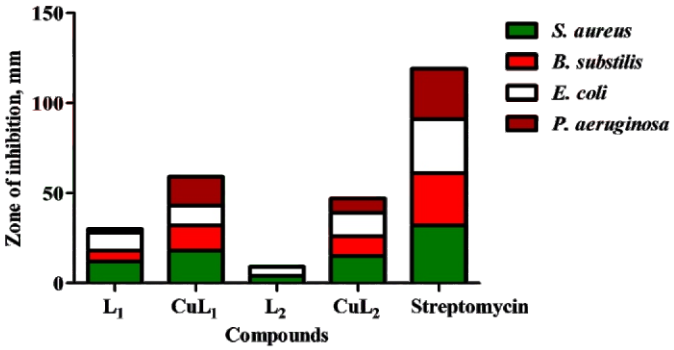


Figure 5: Antimicrobial activity of the thiophene-derived compounds.

The results of the qualitative antimicrobial susceptibility of the compounds as shown in Figure 5, indicates that all the thiophene-based thiosemicarbazone compounds, were not as active as streptomycin. Ligand, L_1, exhibited mild broad antimicrobial activity upon the tested organisms, especially, *Staphylococcus aureus* and *Escherichia coli*. However, the 3-methyl-2-thiophene analogue (L_2), was only slightly active against the two organisms but exhibit no activity against *Bacillus substilis* and *Pseudomonas aeruginosa*. Thus, the presence of the methyl substituent impacts negatively on the potency of the thiophene-based thiosemicarbazone. Furthermore, the inhibitory activity of the free un-complexed thiophene-derived ligands (L_1 and L_2) on the tested organisms was enhanced with the introduction of copper(II) ions in the metal complexes as indicated in Figure 5. Similarly, metal complexes have been reported to exhibit higher biological activity than the corresponding uncoordinated organic moiety (El-Sawaf *et al.*, 2018; Arslan *et al.*, 2021; Gaber *et al.*, 2021).

Contrarily, the minimum concentration (MIC) of the free uncoordinated ligands (L_1 and L_2) required to inhibit the growth of *E. coli* was much lesser than that for the metal complexes. The inhibition of the *S. Aureus* strain, however, required lesser concentration of the metal complex [CuL_2] than the uncoordinated 3-methyl-thiophene-derived ligand, L_2, with MIC values of 31.25 and 62.5 μg/mL respectively. The highest bactericidal effect of the thiosemicarbazone compounds was observed against the *S. aureus* strain with MBC value of 500 μgmL^{-1} while the free ligands exhibit no bactericidal effect against *P. Aeruginosa*.

Table 3: MIC and MBC for the thiophene-derived Compounds, μgmL^{-1}

S/N	Compounds	*S. aureus*		*B. substilis*		*E. coli*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1.	L_1	62.5	500	125	1000	62.5	500	250	----
2.	CuL_1	31.25	500	125	1000	125	1000	62.5	500
3.	L_2	31.25	500	125	1000	62.5	1000	250	----
4.	CuL_2	62.5	250	250	----	500	----	250	1000

CONCLUSION

The copper(II) complexes of the thiophene-based thiosemicarbazone and its 3-methyl analogue have been synthesized and screened as possible antimicrobial agents. The compounds exhibited mild inhibitory activity on the tested organisms, especially *Staphylococcus aureus* and *Escherichia coli*. Furthermore, the thiophene-based thiosemicarbazone was more reactive than the 3-methyl analogue while the copper(II) complexes exhibited greater antimicrobial potency than the free ligands. The DFT studies showed that ligand L_1 was more reactive than L_2 and that the copper(II) complexes are soft molecules having higher chemical reactivity, high electro-optic responses and low kinetic stability than their respective ligands.

ACKNOWLEDGEMENTS

The authors are grateful to Lagos State University, Ojo, especially, chemistry department, for providing facilities for the conduct of this

research.

CONFLICT OF INTERESTS

There is no conflict of interest in the submission of this manuscript for publication.

AUTHORS CONTRIBUTIONS

S.A.O: Administration, Conceptualization, Investigation, Data curation, Resources, Methodology, Formal analysis, Writing – review & editing, Writing – original draft; **Y.O.K:** Formal analysis, Software, Data curation, Writing – original draft; **R.A.T:** Formal analysis, Software, Data curation; **S.O.R**: Conceptualization, Methodology, Formal analysis, Writing- review & editing; **O.M.O:** Resources, Writing – review & editing; **O.F.O**; Visualization, Resources, Investigation; **A.D.A:** Visualization, Resources, Investigation; **O.F.S:** Visualization, Resources, Investigation; **A.T.A:** Visualization, Resources, Investigation.

REFERENCES

Afrasiabi, Z., Sinn, E., Chen, J., Ma, Y., Rheingold, A. L., Zakharov, L. N., Rath, N., and Padhye, S. (2004). Appended 1, 2-naphthoquinones as anticancer agents 1: synthesis, structural, spectral and antitumor activities of ortho-naphthaquinone thiosemicarbazone and its transition metal complexes. *Inorganica Chimica Acta,* 357(1), 271-278. doi: 10.1016/S0020-1693(03)00484-5

Al-Amiery, A. A., Al-Majedy, Y. K., Abdulreazak, H., and Abood, H. (2011). Synthesis, characterization, theoretical crystal structure, and antibacterial activities of some transition metal complexes of the thiosemicarbazone (Z)-2-(pyrrolidin-2-ylidene) hydrazinecarbothioamide. *Bioinorganic Chemistry and Applications, 2011*. doi: 10.1155/2011/483101

Arslan, B. A., Kaya, B., Şahin, O., Baday, S., Saylan, C. C., and Ülküseven, B. (2021). The iron (III) and nickel (II) complexes with tetradentate thiosemicarbazones. Synthesis, experimental, theoretical characterization, and antiviral effect against SARS-CoV-2. *Journal of Molecular Structure,* 1246, 131166. doi: 10.1016/j.molstruc.2021.131166

Bauer, A. (1996). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology,* 45, 149-158.

Bauer, D., Vincent, L. S., Kempe, C. H., Young, P., and Downie, A. (1969). Prophylaxis of smallpox with methisazone. *American journal of epidemiology,* 90(2), 130-145. doi: 10.1093/oxfordjournals.aje.a121057

Bernstein, J., Yale, H. L., Losee, K., Holsing, M., Martins, J., and Lott, W. (1951). The chemotherapy of experimental tuberculosis. III. The synthesis of thiosemicarbazones and related compounds1, 2. *Journal of the American Chemical Society,* 73(3), 906-912. doi: 10.1021/ja01147a007

Demoro, B., De Almeida, R. F., Marques, F., Matos, C. P., Otero, L., Pessoa, J. C., Santos, I.; Rodríguez, A., Moreno, V., and Lorenzo, J. (2013). Screening organometallic binuclear thiosemicarbazone ruthenium complexes as potential anti-tumour agents: cytotoxic activity and human serum albumin binding mechanism. *Dalton Transactions,* 42(19), 7131-7146. doi: 10.1039/C3DT00028A

Duan, L., and Zhang, H. (2011). Novel thiosemicarbazones derivatives bearing aromatic iodine moiety: Design, synthesis and anti-malarial activity. *Arabian Journal of Chemistry,* 4(2), 231-234. doi: 10.1016/j.arabjc.2010.06.042

El-Sawaf, A. K., El-Essawy, F., Nassar, A. A., and El-Samanody, E.-S. A. (2018). Synthesis, spectral, thermal and antimicrobial studies on cobalt (II), nickel (II), copper (II), zinc (II) and palladium (II) complexes containing thiosemicarbazone ligand. *Journal of Molecular Structure,* 1157, 381-394. doi: 10.1016/j.molstruc.2017.12.075

Gaber, A., Refat, M. S., Belal, A. A., El-Deen, I. M., Hassan, N., Zakaria, R., Alhomrani, M., Alamri, A. S., Alsanie, W. F., and Saied, E. M. (2021). New mononuclear and binuclear Cu (II), Co (II), Ni (II), and Zn (II) thiosemicarbazone complexes with potential biological activity: antimicrobial and molecular docking study. *Molecules,* 26(8), 2288. doi: 10.3390/molecules26082288

García-Tojal, J., García-Orad, A., Serra, J. L., Pizarro, J. L., Lezama, L., Arriortua, M. I., and Rojo, T. (1999). Synthesis and spectroscopic properties of copper (II) complexes derived from thiophene-2-carbaldehyde thiosemicarbazone. Structure and biological activity of $[Cu(C_6H_6N_3S_2)_2]$. *Journal of Inorganic Biochemistry, 75*(1), 45-54. doi: 10.1016/S0162-0134(99)00031-8

Geary, W. J. (1971). Use of conductivity measurements in organic solvents for the characterization of coordination compounds. *Coordination Chemistry Review,* 7, 81-122. doi: 10.1016/S0010-8545(00)80009-0

Haribabu, J., Jeyalakshmi, K., Arun, Y., Bhuvanesh, N. S., Perumal, P. T., and Karvembu, R. (2017). Synthesis of Ni (II) complexes bearing indole-based thiosemicarbazone ligands for interaction with biomolecules and some biological applications. *Journal of Biological Inorganic Chemistry,* 22(4), 461-480. doi: 10.1007/s00775-016-1424-1

Ibrahim, A. B., Farh, M. K., and Mayer, P. (2018). Copper complexes of new thiosemicarbazone ligands: synthesis, structural studies and antimicrobial activity. *Inorganic Chemistry Communications,* 94, 127-132. doi: 10.1016/j.inoche.2018.06.019

Karaküçük-İyidoğan, A., Taşdemir, D., Oruç-Emre, E. E., and Balzarini, J. (2011). Novel platinum (II) and palladium (II) complexes of thiosemicarbazones derived from 5-substitutedthiophene-2-carboxaldehydes and their antiviral and cytotoxic activities. *European Journal of Medicinal Chemistry,* 46(11), 5616-5624. doi: 10.1016/j.ejmech.2011.09.031

Khalaji, A. D., Grivani, G., Akerdi, S. J., Gotoh, K., Ishida, H., and Mighani, H. (2010). Synthesis, spectroscopic characterization, crystal structures, and theoretical studies of (E)-2-(2, 4-dimethoxybenzylidene) thiosemicarbazone and (E)-2-(2, 5-dimethoxybenzylidene) thiosemicarbazone. *Structural Chemistry,* 21(5), 995-1003. doi: 10.1007/s11224-010-9637-3

Knox, J. J., Hotte, S. J., Kollmannsberger, C., Winquist, E., Fisher, B., and Eisenhauer, E. A. (2007). Phase II study of Triapine® in patients with metastatic renal cell carcinoma: a trial of the National Cancer Institute of Canada Clinical Trials Group (NCIC IND. 161). *Investigational New Drugs,* 25(5), 471-477. doi: 10.1007/s10637-007-9044-9

Kune, G.A. (1964). To-Day's Drugs: Methisazone. *British Medical Journal,* 2(5409), 621-621. doi: 10.1136/bmj.2.5409.621

Kunos, C. A., Chu, E., Beumer, J. H., Sznol, M., and Ivy, S. P. (2017). Phase I trial of daily triapine in combination with cisplatin chemotherapy for advanced-stage malignancies. *Cancer Chemotherapy and Pharmacology,* 79(1), 201-207. doi: 10.1007/s00280-016-3200-x

Kunos, C. A., and Sherertz, T. M. (2014). Long-term disease control with triapine-based radiochemotherapy for patients with stage IB2–IIIB cervical cancer. *Frontiers in Oncology,* 4, 184. doi: 10.3389/fonc.2014.00184

Lee, C., Yang, W., and Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B, 37*(2), 785. doi: 10.1103/PhysRevB.37.785

Li, M. X., Chen, C. L., Zhang, D., Niu, J. Y., and Ji, B. S. (2010). Mn (II), Co (II) and Zn (II) complexes with heterocyclic substituted thiosemicarbazones: Synthesis, characterization, X-ray crystal structures and antitumor comparison. *European Journal of Medicinal Chemistry,* 45(7), 3169-3177. doi: 10.1016/j.ejmech.2010.04.009

Lobana, T. S., Sharma, R., Bawa, G., and Khanna, S. (2009). Bonding and structure trends of thiosemicarbazone derivatives of metals—an overview. *Coordination Chemistry Reviews,* 253(7-8), 977-1055. doi: 10.1016/j.ccr.2008.07.004

Lukmantara, A. Y., Kalinowski, D. S., Kumar, N., and Richardson, D. R. (2013). Structure–activity studies of 4-phenyl-substituted 2'-benzoylpyridine thiosemicarbazones with potent and selective anti-tumour activity. *Organic & Biomolecular Chemistry,* 11(37), 6414-6425. doi: 10.1039/C3OB41109E

Maia, P. I. d. S., Pavan, F. R., Leite, C. Q., Lemos, S. S., de Sousa, G. F., Batista, A. A., Nascimento, O.R., Ellena, J., Castellano, E.E., and Niquet, E. (2009). Vanadium complexes with thiosemicarbazones: synthesis, characterization, crystal structures and anti-Mycobacterium tuberculosis activity. *Polyhedron,* 28(2), 398-406. doi: 10.1016/j.poly.2008.11.017

Matsa, R., Makam, P., Kaushik, M., Hoti, S., and Kannan, T. (2019). Thiosemicarbazone derivatives: Design, synthesis and in vitro antimalarial activity studies. *European Journal of Pharmaceutical Sciences,* 137, 104986. doi: 10.1016/j.ejps.2019.104986

Molski, M. (2021). Theoretical modeling of structure-toxicity relationship of cyanides. *Toxicology Letters,* 349, 30-39. doi: 10.1016/j.toxlet.2021.05.011

Nakatsukasa, T., Matsuyanagi, K., Matsuo, M., and Yabana, K. (2016). Time-dependent density-functional description of nuclear dynamics. *Reviews of Modern Physics,* 88(4), 045004. doi: 10.1103/RevModPhys.88.045004

Nascimento, D. R., Biasin, E., Poulter, B. I., Khalil, M., Sokaras, D., and Govind, N. (2021). Resonant inelastic x-ray scattering calculations of transition metal complexes within a simplified time-dependent density functional theory framework. *Journal of Chemical Theory and Computation,* 17(5), 3031-3038. doi: 10.1021/acs.jctc.1c00144

NCCLS. (2015). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard *CLSI document M02-A12* (12 th ed.). Wayne, PA: Clinical and Laboratory Standards Institute.

Padmanabhan, P., Khaleefathullah, S., Kaveri, K., Palani, G., Ramanathan, G., Thennarasu, S., and Tirichurapalli Sivagnanam, U. (2017). Antiviral activity of Thiosemicarbazones derived from α-amino acids against Dengue virus. *Journal of Medical Virology*, 89(3), 546-552. doi: 10.1002/jmv.24655

Prabhu, R. N., and Ramesh, R. (2016). Square-planar Ni (II) thiosemicarbazonato complex as an easily accessible and convenient catalyst for Sonogashira cross-coupling reaction. *Tetrahedron Letters,* 57(44), 4893-4897. doi: 10.1016/j.tetlet.2016.09.049

Prathima, B., Rao, Y. S., Reddy, S. A., Reddy, Y., and Reddy, A. V. (2010). Copper (II) and nickel (II) complexes of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone: Synthesis, characterization and biological activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 77(1), 248-252. doi: 10.1016/j.saa.2010.05.016

Qi, J., Zhao, W., Zheng, Y., Wang, R., Chen, Q., Wang, F.-A., Fan, W., Gao, H., and Xia, X. (2020). Single-crystal structure and intracellular localization of Zn (II)-thiosemicarbazone complex targeting mitochondrial apoptosis pathways. *Bioorganic & Medicinal Chemistry Letters,* 30(16), 127340. doi: 10.1016/j.bmcl.2020.127340

Rahman, K. A., Haribabu, J., Balachandran, C., Bhuvanesh, N. S., Karvembu, R., and Sreekanth, A. (2017). Copper, nickel and zinc complexes of 3-acetyl coumarin thiosemicarbazone: Synthesis, characterization and in vitro evaluation of cytotoxicity and DNA/protein binding properties. *Polyhedron,* 135, 26-35. doi: 10.1016/j.poly.2017.06.044

Sampath, K., Sathiyaraj, S., and Jayabalakrishnan, C. (2013). Evaluation of DNA-binding, DNA cleavage, antioxidant and cytotoxic activity of mononuclear ruthenium (II) carbonyl complexes of benzaldehyde 4-phenyl-3-thiosemicarbazones. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 115, 287-296. doi: 10.1016/j.saa.2013.06.030

Savir, S., Wei, Z. J., Liew, J. W. K., Vythilingam, I., Lim, Y. A. L., Saad, H. M., Sim, K.S., and Tan, K. W. (2020). Synthesis, cytotoxicity and antimalarial activities of thiosemicarbazones and their nickel (II) complexes. *Journal of Molecular Structure,* 1211, 128090. doi: 10.1016/j.molstruc.2020.128090

Talmaciu, M. M., Bodoki, E., and Oprean, R. (2016). Global chemical reactivity parameters for several chiral beta-blockers from the Density Functional Theory viewpoint. *Clujul Medical,* 89(4), 513. doi: 10.15386/cjmed-610

Tan, K. W., Seng, H. L., Lim, F. S., Cheah, S.-C., Ng, C. H., Koo, K. S., Mustafa, M.R., Ng, S.W., and Maah, M. J. (2012). Towards a selective cytotoxic agent for prostate cancer: Interaction of zinc complexes of polyhydroxybenzaldehyde thiosemicarbazones with topoisomerase I. *Polyhedron,* 38(1), 275-284. doi: 10.1016/j.poly.2012.03.014

Wang, Z., Wu, Y., Fu, Y., Li, M., Tai, Y., and Li, Y. (2015). Synthesis, structure investigation and biological evaluation of 2-thiophene N (4)-phenylthiosemicarbazone and its three metal derivatives. *Journal of Molecular Structure,* 1100, 376-383. doi: 10.1016/j.molstruc.2015.07.031

West, D. X., Liberta, A. E., Padhye, S. B., Chikate, R. C., Sonawane, P. B., Kumbhar, A. S., and Yerande, R. G. (1993). Thiosemicarbazone complexes of copper (II): structural and biological studies. *Coordination Chemistry Reviews,* 123(1-2), 49-71. doi: 10.1016/0010-8545(93)85052-6