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Synthesis, Characterization and DFT of ^{64}Cu based complex for Hypoxia Imaging.

Abstract:

An important contributing factor to the growth of aggressive and treatment-resistant tumors is tumor hypoxia. In this investigation, we created a unique tracer, ^{64}Cu-biacetyl-bis(4-methyl-3-semicarbazone) Complex (^{64}Cu-[APSC]$_2$), for hypoxia imaging. Numerous spectral and electrochemical analyses were used to characterize the synthesized ligand and its metal complex. The coordination of the metal in the metal complex was also supported by DFT (B3LYP model) calculations. The radiochemical yields and purities of ^{64}Cu-[APSC]$_2$ were found to be quantitative, as determined by TLC and HPLC analyses.

Stability studies in human plasma demonstrated that ^{64}Cu-[APSC]$_2$ remained steady for at least four hours while being incubated at 37°C. MDA-MB-231 cell-line xenografts were placed in nude mice for biodistribution research. The results revealed significant accumulation of the tracer in tumors after 2 hours post-injection, which correlated with the redox potential and reactive oxygen species. These findings suggest that ^{64}Cu-[APSC]$_2$ shows promising evidence as a theranostic radiopharmaceutical for hypoxic tumors.

Keywords: Tumor hypoxia, Hypoxia imaging, Theranostic radiopharmaceutical, Redox potential

1. Introduction

Bifunctional chelators derived from bis-thiosemicarbazones have shown promise in radiopharmaceutical chemistry, particularly in hypoxia imaging [1-2]. Low tissue oxygenation, or tumor hypoxia, is a harmful state linked to radiotherapy resistance, malignant progression, and a dismal prognosis. Positron emission tomography (PET) employing the tracer [^{64}Cu] [Cu-diacetyl-bis(N(4)-methyl thio semi carbazone)] is one technique for identifying tumor hypoxia. ^{64}Cu-ATSM [3-4].

Fujibayashi and co-workers, [5-6] have created ^{64}Cu-ATSM, which seems to be more suitable for imaging tissues that are hypoxic. When Cu-ATSM interacts with thiol groups or redox-active proteins with NADH acting as an enzymatic cofactor, it displays a distinct behavior. This results in variations in 64Cu accumulation among different cell lines, indicating disparate uptake kinetics, maximum accumulation, and oxygen-dependent responses [5,7-8]. Cu-ATSM demonstrates rapid uptake and a higher hypoxic/normoxic tissue activity ratio, which can be attributed to its enhanced membrane permeability and faster blood clearance compared to other tracers like FMISO [9]. Studies have illustrated that complexes with lower $E_{1/2}$ values, indicative of reduced reducibility, exhibit hypoxia selectivity, unlike easily reducible complexes [6,10].

However, ^{64}Cu-ATSM, like FMISO, is decreased and retained in tissue that is hypoxic or normoxic. Its slow blood clearance and low target-to-background ratio have also restricted its application in clinical settings. It has been demonstrated that ^{64}Cu-ATSM preferentially accumulates in hypoxic cells, rapidly separates from the blood, and quickly washes out of normoxic cells. [11-12].

To address these limitations, we have been investigating methods to control redox potential and lipophilicity independently by substituting alkyl groups at the diimine backbone and terminal nitrogen atoms, respectively. As a result, complexes with and without selectivity for hypoxic cells have been developed.

In this article presents a novel asymmetric compound containing a pyridine group as a potential improvement over the parent Cu-ATSM in terms of hypoxic selectivity. The compound has been radiolabeled with ^{64}Cu, and its physicochemical properties, we evaluate its cellular uptake in MDA-MB-231 cells under hypoxic conditions and further investigate its performance through acute biodistribution studies.

2 Experimental: Materials and Methods:

The detailed materials and methods used in this study are provided in the supplementary information **(SF).**

2.1 Synthesis of (APSC)H: HL Ligand:

The compound acetyl-bis(4-methyl-3-semicarbazone) (APSC)H: HL was synthesized according to a previously reported method [13]. The detailed procedure is as follows: in the presence of sodium acetate (1.36 g, 1.0 mmol), an aqueous solution of semicarbazide hydrochloride (1.11 g, 1.0 mmol) was added to an aqueous solution of 2-acetyl pyridine (1.20 g, 1.0 mmol). For two hours, the reaction mixture was vigorously stirred. After filtering and an ether wash, the crystalline white result produced a 96% yield. M.p 188-192 °C. Elemental analysis showed the following composition: C: 54.0% (calculated: 53.9%), H: 5.8% (calculated: 5.7%), and N: 31.2% (calculated: 31.4%).FTIR (υ_{max}/cm^{-1}): (NH, 3473), (NH_2, 3375), (CH_3, 2280), ($C{=}O_{as}$, 1686), ($C{=}O_{sy}$, 1579), (C=N, 1443), (C–O, 1104), and various peaks for heterocyclic bases. NMR spectroscopy indicated

peaks at 3.39 ppm (3H, CH_3), 7.3 ppm (2H, NH_2), 7.3 ppm (1H, CH=C), 7.7 ppm (1H, CH=C), 8.3 ppm (1H, CH–C), 8.5 ppm (1H, CH–N), and 9.5 ppm (1H, NH). Mass spectroscopy showed a peak at m/z 179 ($[M]^+$).

2.2 Synthesis of Cu-biacetyl-bis(4-methyl-3-semicarbazone) Complex: $Cu(APSC)_2$:

Hot ethanol (EtOH, 10 mL) was used to dissolve the ligand APSC (0.019 g, 0.106 mmol), and $CuCl_2$ was added dropwise to the EtOH solution (1 mL, 0.05 mmol). For three hours, the reaction mixture was refluxed while being constantly stirred. The precipitate was filtered and then cleaned with ether once it had cooled to room temperature. Yield: 89%. decomposed at 220°C. The empirical formula of the complex was determined as $[C_{16}H_{20}N_8O_2]Cu$. Elemental analysis (calculated): C: 49.79 (48.63%), H: 4.89 (4.85 %),N: 27.57 (28.36%). FTIR (υ_{max}/cm^{-1}): (OH, 3387); (NH_2, 3268); (CH_3, 2320); ($C{=}0_{as}$, 1686); ($C{=}O_{sy}$, 1579); (C=N, 1449); (C-O, 1103); (Cu-N, 564); (Cu-O, 482); (heterocyclic bases, 771, 626, 561, 448).UV/Vis spectroscopy in DMSO (wavelength in nm, molar absorptivity in $mol^{-1}dm^3cm^{-1}$): λ_{max} = 230 (1500), 290 (800), 455 (1300).Molar conductivity ($1x10^{-3}$ mol L^{-1} in DMF): 0.54 $mS.cm^{-1}$.HPLC retention time: 7.5 min.

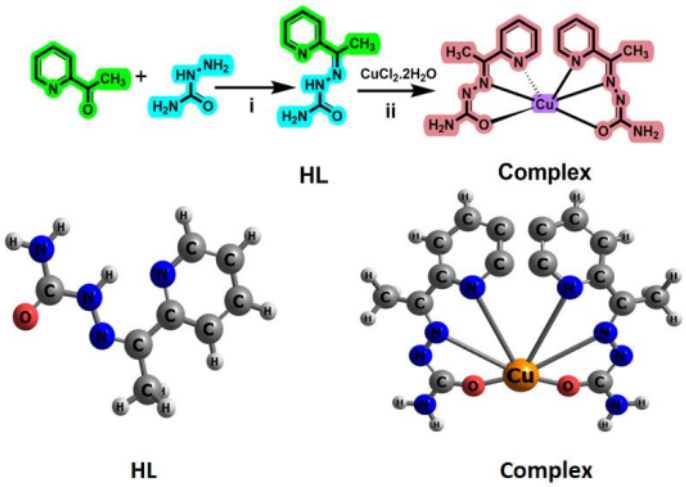
2.3 Synthesis of ^{64}Cu-biacetyl-bis(4-methyl-3-semicarbazone) Complex: $^{64}Cu(APSC)_2$:

$^{64}CuCl_2$ was produced via reported methods explained in the supporting information [14]. To a sealed vial containing 370 MBq of $^{64}CuCl_2$, 200 µg of APSC was added along with 1.2 mL of sodium acetate buffer (5 M, pH ~ 4.6). The reaction, which was the same as the one described in Scheme 2, was conducted for 30 minutes at 95 °C. Following the reaction, the mixtures were run through a C18 Sep-Pak cartridge after being diluted with 3 mL of water (H_2O). After the eluted solution was dried, the dried mixture was extracted from the cartridge using ethanol (EtOH). This produced about 314.5 MBq of activity in 1 mL of ethanol. After that, the ethanol was evaporated

and the residue was mixed back together with regular saline. Next, a 0.22 µm pore membrane filter was used to filter the reconstituted solution so that it could be used in both in vitro and in vivo experiments.

3 Results and Discussion:

The Cu-[APSC]$_2$ complex was successfully obtained by reacting APSC with $CuCl_2$, resulting in a high yield, as illustrated in **Scheme 1**. The supporting figures SF-1 to SF-5 provide the detailed spectroscopic data for the synthesized compounds.



Scheme 1: Synthesis of acetyl-bis(4-methyl-3-semicarbazone) (APSC) ligand (HL) and Cu-biacetyl-bis(4-methyl-3-semicarbazone) (Cu-[APSC]$_2$) complex (CuL).

The EPR spectra provide valuable information about the electronic and geometric structure of the Cu(II) complex, as well as the nature of its ligand environment. In Figure 1(a), the g-values are reported as: $g_1 = 2.0650$, $g_2 = 2.0762$, $g_3 = 2.285$. The magnetic hyperfine coupling constant, Az, is measured as 169 x 10^{-4} cm^-1 for the I=3/2 nucleus of ($^{63}Cu/^{65}Cu$). Two signals have been identified in the EPR spectrum: one at g =2 and the other at the half-field. These signals are thought

to originate from the ΔMs=±1 and ΔMs=±2 transitions, respectively. These transitions show that a binuclear complex is forming. The unpaired electron on copper appears to have dx2-y2 character, according to the trend in the g-values ($g_{\parallel} > g_{\perp} > 2$), suggesting a square planar structure for the complex [6,15].

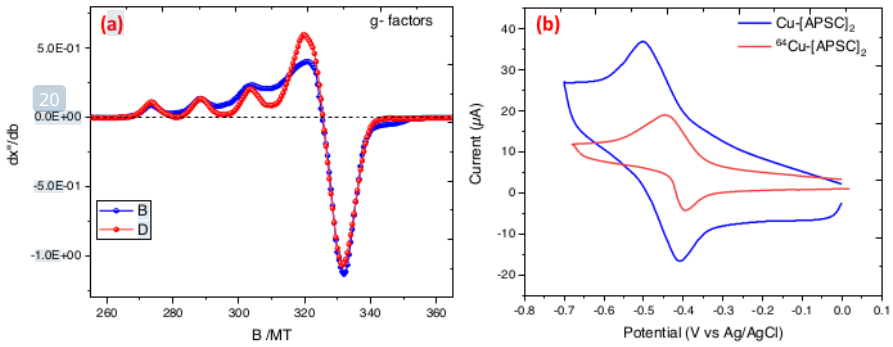


Figure 1. (a) displays the X-band EPR spectrum of the Cu-biacetyl-bis(4-methyl-3-semicarbazone) complex in DMF. (b) cyclic voltametric chart of Cu-Complex.

The modulation of cellular retention and hypoxia selectivity in the copper complex is largely dependent on the reduction potential of the Cu(II) component, as demonstrated in Figure 1(b). Hypoxia selectivity is demonstrated by complexes with an $E_{1/2}$ value less than -0.50 V, whereas easily reducible complexes lack this selectivity. This class of complexes' redox capacity plays a significant role in determining how well they retain cells and how selectively they react to hypoxia. Using $Ag/AgNO_3$ as the reference electrode, cyclic voltammetry was used to examine the redox potentials of the $[Cu^{II}L]0/[Cu^{I}L]-$ couples in dry DMSO at 20°C in order to better understand the biological behavior of these new complexes. [7]. The $E_{1/2}$ value of -0.48 V for the Cu-$[APSC]_2$ complex is in close agreement with the value of -0.54 V reported for Cu-ATSM [6]. The characteristics of the complex are changed when the methyl group at the BTSC chelator's N-

terminus is swapped out for a pyridine ring, more especially, its nitrogen atom [16]. Furthermore, because the oxygen atom withdraws electrons, changing sulfur to oxygen as the donor atom alters the properties of the complex. Because of this interaction, the metal's electronic density is increased, which raises the potential for reduction of -0.48 V for Cu-[APSC]$_2$.

The following is a summary of the connections between structure, lipophilicity, redox potential, and hypoxia selectivity: Firstly, the redox potential has a significant influence on hypoxia selectivity. Reduced reduction potential complexes (< -0.50 V) show improved hypoxia selectivity. Secondly, lipophilicity is not a sign of hypoxia selectivity, even though it is required for cell membrane penetration. [17-18]. The complexes generally have log P values greater than zero. The log P value for the ^{64}Cu-[APSC]$_2$ complex was found to be 1.34 ± 0.05, indicating it is relatively more hydrophilic compared to ^{64}Cu-ATSM (log P = 1.85 ± 0.05) [7,19].

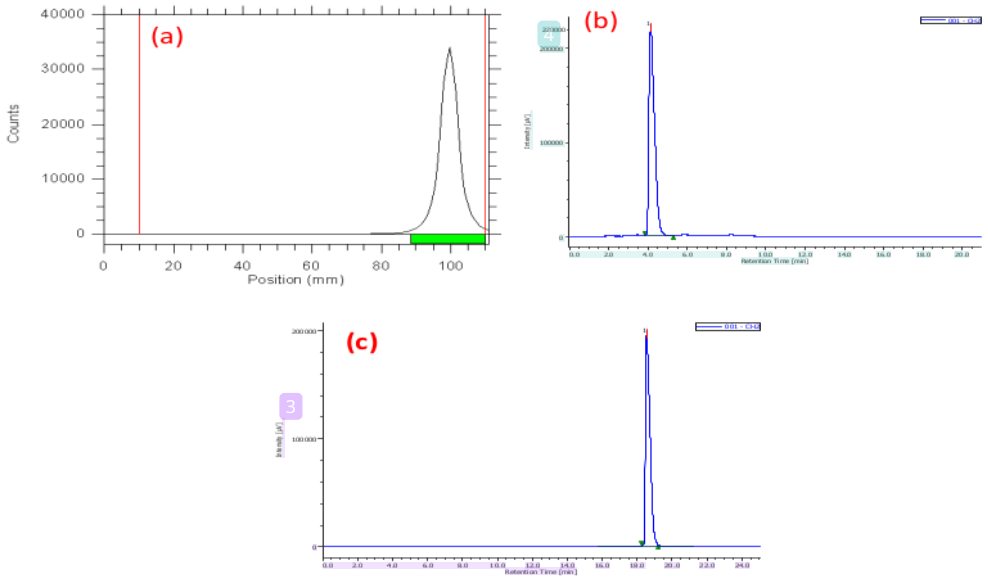


Figure 2. (a) TLC chromatogram of (^{64}Cu-[APSC]$_2$), HPLC chromatograms of (b) ^{64}CuCl$_2$ and (c) (^{64}Cu-[APSC]$_2$) complex.

The TLC showed that the Rf value for $^{64}CuCl_2$ was 0.0, while the Rf value for the ^{64}Cu-$[APSC]_2$ complex was 0.9 (Figure 2a). This significant difference in Rf values indicates a substantial change in the polarity and lipophilicity of the ^{64}Cu-$[APSC]_2$ complex compared to the precursor $^{64}CuCl_2$.

Furthermore, the HPLC analysis revealed distinct differences in the retention times between $^{64}CuCl_2$ and the ^{64}Cu-$[APSC]_2$ complex (Figure 2b, c). These chromatographic results demonstrate the successful formation of the ^{64}Cu-$[APSC]_2$ complex and its distinct separation from the starting material, $^{64}CuCl_2$.

The ^{64}Cu-$[APSC]_2$ complex exhibited good stability in plasma. Proteolytic degradation studies showed that the complex remained over 95% intact during incubation at 37°C and maintained this stability for up to 4 hours.

Table 1: Biodistribution of (^{64}Cu-$[APSC]_2$) in tumor bearing nude mice.

organ	1 h	2 h	6 h
Blood	7.75 ± 0.78	4.35 ± 0.03	1.70 ± 0.13
Liver	5.09 ± 0.09	4.01 ± 0.13	1.59 ± 0.10
Lung	7.42 ± 0.17	2.84 ± 0.18	1.83 ± 0.17
Kidney	6.02 ± 0.05	3.66 ± 0.23	2.67 ± 0.03
Intestine	5.03 ± 0.04	4.71 ± 0.06	3.17 ± 0.79
Heart	6.15 ± 0.11	2.27 ± 0.38	1.0 ± 0.09
Muscle	2.24 ± 0.59	1.56 ± 0.16	0.42 ± 0.25
Spleen	5.26 ± 0.24	2.5 ± 0.18	2.25 ± 0.11
Tumor	2.80 ± 0.14	4.74 ± 0.19	2.51 ± 0.14
Tumor/muscle	1.25	3.03	5.97

For $n = 3$, the values are the average of the percent injected dose/gram ± SD. A radiotracer called ^{64}Cu-[APSC]$_2$ was injected into the animals.

The ^{64}Cu-[APSC]$_2$ complex's biodistribution was assessed in naked mice that were given xenografts of the human MDA-MB-231 cell line (Table 1). High blood uptake of the complex was observed one hour after injection. (7.75% ID/g), which gradually decreased to 4.35% ID/g by 2 hours. This contrasts with the more lipophilic ^{64}Cu-ATSM, which is rapidly and efficiently cleared from the blood (3.65% ID/g at 1 min, and 2.22% ID/g over 4 hours) [4,20]. The liver uptake of ^{64}Cu-[APSC]$_2$ was 5% ID/g at 1 hour, significantly lower than the 20.84% ID/g observed for ^{64}Cu-ATSM at the same time point [20,18]. Other major organs, including the lungs, heart, spleen, and kidneys, also showed high initial uptake, but the radioactivity began to clear by 2 hours post-injection. The tumor uptake of ^{64}Cu-[APSC]$_2$ reached an optimal peak of 4.76 ± 0.19% ID/g at 2 hours post-injection, comparable to to the highest tumor uptake recorded for ^{64}Cu-ATSM (4.78 ± 1.00% ID at 10 minutes) [20,18, 4]. At two hours, the tumor-to-muscle ratio was 3.1, indicating that the radiotracer had accumulated favorably in the tumor tissue.

4 Computational Results:

Molecular modeling using density functional theory (DFT) at the B3LYP level was conducted to examine the structures of the ligand and the Cu-[APSC]$_2$ complex. The optimized structures, shown in Scheme 1, indicate that the Cu(II) center has a hexacoordinate bonding mode in the complex. The DFT-computed bond lengths and angles are reported in Supporting Figure SF-6.

In the complex, the Cu(II) center is coordinated to the oxygen atoms of the two ligand (L1 and L2) molecules. The bond lengths are 1.898 Å for both the Cu-O(40) and Cu-O(26) bonds. The bond angles in the complex show some variation: For the L2 ligand, the C(24)-O(26)-Cu(41) angle is

158.5°. For the L1 ligand, the C(36)-O(40)-Cu(41) angle is 158.7°. The O(26)-Cu(41)-O(40) angle between the two ligands is 149.5°. The bond angles in the complex range from 158.7° to 90°, and the bond lengths range from 1.898 Å to 1.006 Å. The longest bond length is observed for the O(26)-Cu(41) bond.

In the free ligand, the bond length range is 1.863 Å to 1.006 Å, with the maximum value observed for the N(6)-C(18) bond. The bond angle range is 139.3° to 101.5°, with the maximum value observed for the C(1)-N(6)-C(18) angle. The DFT-calculated geometric parameters for both the ligand and the complex are in good agreement, providing a solid starting point for further property calculations and understanding the complex's structural characteristics.

Table 2: The HOMO-LUMO, band gap (eV) calculated.

Molecules	HOMO (eV)	LUMO (eV)	Energy gap (eV)	HOMO-1	LUMO+1	Energy gap (eV)
HL	-5.846	-1.541	4.305	-6.668	-0.945	5.724
Complex	-4.694	-1.949	2.745	-5.405	-1.432	3.973

Frontier molecular orbitals have two crucial quantum chemical properties: the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO). These properties can be used to assess a compound's reactivity, where the HOMO and LUMO represent the electron donor and acceptor behavior, respectively. The energy difference between HOMO and LUMO, known as the energy gap (ΔE), is a crucial factor in predicting the molecule's electrical transport properties. A smaller ΔE value indicates a more reactive system. The data in **Table 2** shows that

the free ligand (HL) has the largest energy gap of 4.305 eV, while the complex species have a smaller energy gap of 2.745 eV, suggesting the latter are more stable.

Table 3: The chemical reactivity **parameters** (in eV) calculated at the B3LYP/6-31G(d, p) level of theory

Molecules	IP	EA	η	ω	χ	μ	σ
HL	5.846	1.541	2.152	3.169	3.693	-3.693	0.232
Complex	4.694	1.949	1.372	4.019	3.321	-3.321	0.364

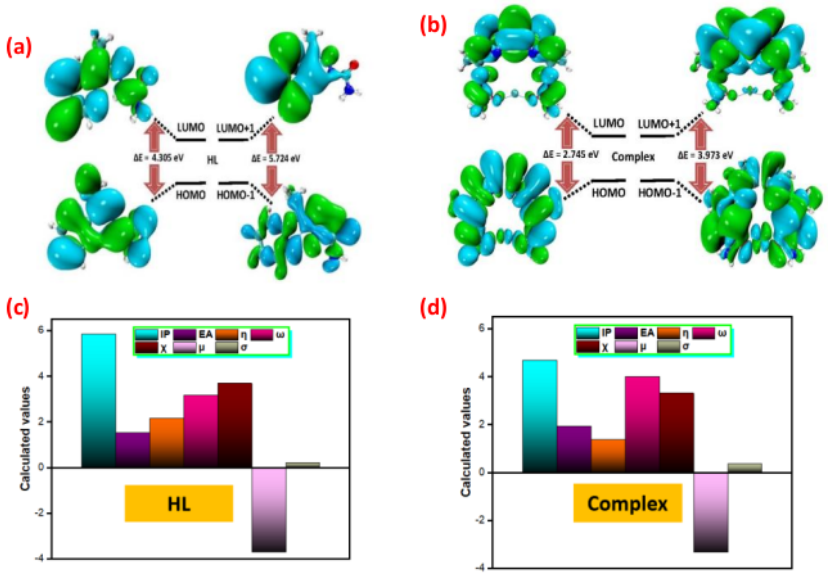


Figure 3. The Contour plots representing HOMO and LUMOs of (a,c) HL and (b,d) complex

Using the equations {1-7}, the values of electronegativity (χ), chemical potential (μ), global hardness (η), and global softness (σ) were determined [25], and these values were reported in Table 3 and Figure 3. Based on these characteristics, the HL ligand and the complex have the best

electron-accepting capacity, the best stabilization energy, and the lowest chemical hardness, making them potentially suitable for use in the medical field.

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$\sigma = \frac{1}{2\eta} \quad (3)$$

$$\eta = \frac{[IP-EA]}{2} = -\frac{[E_{LUMO}-E_{HOMO}]}{2} \quad (4)$$

$$\omega = \frac{(IP+EA)^2}{4(IP-EA)} \quad (5)$$

$$X = \frac{[IP+EA]}{2} = -\frac{[E_{LUMO}+E_{HOMO}]}{2} \quad (6)$$

$$\mu = \frac{E_{HOMO}+E_{LUMO}}{2} \quad (7)$$

5 Conclusion:

The synthesis of ^{64}Cu-[APSC]$_2$ resulted in excellent radiochemical yield and purity within a short time frame of less than 30 minutes. At two hours post-injection (p.i.), biodistribution studies using human MDAMB-231 cell line xenografts in a model of naked mice demonstrated a significant accumulation of ^{64}Cu-[APSC]$_2$ in tumor tissue, indicating substantial tumor uptake. Furthermore, the pharmacokinetic profile of ^{64}Cu-[APSC]$_2$ was favorable compared to previously investigated radiotracers of its kind.

The theoretical stability of the compound was determined using advanced quantum chemistry simulations, including DFT and TD-DFT methods. The complex under investigation exhibits promising anticancer activity, according to analyses of its frontier molecular orbitals (FMO), natural bond orbitals (NBO), geometric parameters, electron localization function (ELF), non-covalent interactions (NCI), localized orbital locator (LOL), molecular electrostatic potential

(MEP), and Mullikan charge. This implies possible uses in the creation of innovative medications. Significantly, it was discovered that the low end of the range's redox potential was necessary for hypoxia selectivity, a desirable quality for the compound's use in tumor detection and staging.

Overall, the findings from this study suggest that the ^{64}Cu-[APSC]$_2$ radiotracer holds promise as a PET probe for the detection and staging of hypoxic tumors. However, further evaluation and investigation are necessary to fully validate its potential clinical utility.

6 Acknowledgements:

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7. References:

[1] Y. Arano, A. Yokoyama, T. Furukawa, K. Horiuchi, T. Yahata, H. Saji, H. Sakahara, T. Nakashima, M. Koizumi, K. Endo, Technetium-99m-labeled monoclonal antibody with preserved immunoreactivity and high in vivo stability, Journal of nuclear medicine 28(6) (1987) 1027-1033.

[2] D. McPherson, G. Umbricht, F. Knapp Jr, Radiolabeling of proteins with radioisotopes of copper using p-carboxyalkylphenylglyoxal bis-(4N-methylthiosemicarbazone)(TSC) bifunctional chelates, Journal of Labelled Compounds and Radiopharmaceuticals 28(8) (1990) 877-899.

[3] A.R. Cowley, J.R. Dilworth, P.S. Donnelly, J.M. Heslop, S.J. Ratcliffe, Bifunctional chelators for copper radiopharmaceuticals: the synthesis of [Cu(ATSM)–amino acid] and [Cu(ATSM)–octreotide] conjugates, Dalton Transactions (2) (2007) 209-217.

[4] P.D. Bonnitcha, A.L. Vāvere, J.S. Lewis, J.R. Dilworth, In vitro and in vivo evaluation of bifunctional bisthiosemicarbazone 64Cu-complexes for the positron emission tomography imaging of hypoxia, Journal of medicinal chemistry 51(10) (2008) 2985-2991.

[5] Y. Fujibayashi, H. Taniuchi, Y. Yonekura, H. Ohtani, Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential, The Journal of Nuclear Medicine 38(7) (1997) 1155.

[6] J.L. Dearling, J.S. Lewis, G.E. Mullen, M.J. Welch, P.J. Blower, Copper bis(thiosemicarbazone) complexes as hypoxia imaging agents: structure-activity relationships, JBIC Journal of Biological Inorganic Chemistry 7(3) (2002) 249-259.

[7] J.L. Dearling, P.J. Blower, Redox-active metal complexes for imaging hypoxic tissues: structure–activity relationships in copper (II) bis (thiosemicarbazone) complexes, Chemical Communications (22) (1998) 2531-2532.

[8] J.P. Holland, F.I. Aigbirhio, H.M. Betts, P.D. Bonnitcha, P. Burke, M. Christlieb, G.C. Churchill, A.R. Cowley, J.R. Dilworth, P.S. Donnelly, Functionalized bis (thiosemicarbazonato) complexes of zinc and copper: synthetic platforms toward site-specific radiopharmaceuticals, Inorganic chemistry 46(2) (2007) 465-485.

[9] T. Liu, M. Karlsen, A.M. Karlberg, K.R. Redalen, Hypoxia imaging and theranostic potential of [64Cu][Cu (ATSM)] and ionic Cu (II) salts: a review of current evidence and discussion of the retention mechanisms, EJNMMI research 10(1) (2020) 1-14.

[10] R.I. Maurer, P.J. Blower, J.R. Dilworth, C.A. Reynolds, Y. Zheng, G.E.D. Mullen, Studies on the Mechanism of Hypoxic Selectivity in Copper Bis(Thiosemicarbazone) Radiopharmaceuticals, Journal of Medicinal Chemistry 45(7) (2002) 1420-1431.

[11] J.S. Lewis, D.W. McCarthy, T.J. McCarthy, Y. Fujibayashi, M.J. Welch, Evaluation of 64Cu-ATSM in vitro and in vivo in a hypoxic tumor model, Journal of Nuclear Medicine 40(1) (1999) 177-183.

[12] F. Dehdashti , Perry. W Grigsby, M. A Mintun, J.S Lewis, B.A Siegel, M. J Welch., Assessing tumor hypoxia in cervical cancer by positron emission tomography with 60Cu-ATSM: relationship to therapeutic response-a preliminary report; Int J Radiat Oncol Biol Phys. 2003 Apr 1;55(5):1233-8. doi: 10.1016/s0360-3016(02)04477-2.

[13] N. S. Al-Hokbany, Basim Alotaibi, Suad Bin Amer, Subhani M. Okarvi, and Ibrahim Al-Jammaz., Synthesis and *In Vitro* and *In Vivo* Evaluation of a New 68Ga-Semicarbazone Complex: Potential PET Radiopharmaceutical for Tumor Imaging., Journal of Chemistry., 2014, Article ID 616459, 6 pages http://dx.doi.org/10.1155/2014/616459.

[14] N. AlHokbany, I. AlJammaz, B. AlOtaibi, Y. AlMalki, B. AlJammaz, S.M. Okarvi, Development of new copper-64 labeled rhodamine: a potential PET myocardial perfusion imaging agent, EJNMMI Radiopharmacy and Chemistry 7(1) (2022) 19.

[15] D.X. West, H. Gebremedhin, T.J. Romack, A.E. Liberta, Spectral and biological studies of copper(II) complexes of 2-acetyl-pyridine thiosemicarbazones with bulky 4N-substituents, Transition Metal Chemistry 19(4) (1994) 426-431.

[16] E. Palma, F. Mendes, G.R. Morais, I. Rodrigues, I.C. Santos, M.P.C. Campello, P. Raposinho, I. Correia, S. Gama, D. Belo, Biophysical characterization and antineoplastic activity of new bis (thiosemicarbazonato) Cu (II) complexes, Journal of Inorganic Biochemistry 167 (2017) 68-79.

[17] T.C. Castle, R.I. Maurer, F.E. Sowrey, M.J. Went, C.A. Reynolds, E.J.L. McInnes, P.J. Blower, Hypoxia-Targeting Copper Bis(selenosemicarbazone) Complexes: Comparison with Their Sulfur Analogues, Journal of the American Chemical Society 125(33) (2003) 10040-10049.

[18] P. McQuade, K.E. Martin, T.C. Castle, M.J. Went, P.J. Blower, M.J. Welch, J.S. Lewis, Investigation into 64Cu-labeled Bis (selenosemicarbazone) and Bis (thiosemicarbazone) complexes as hypoxia imaging agents, Nuclear medicine and biology 32(2) (2005) 147-156.

[19] M.A. Green, D.L. Klippenstein, J.R. Tennison, Copper (II) bis (thiosemicarbazone) complexes as potential tracers for evaluation of cerebral and myocardial blood flow with PET, Journal of nuclear medicine 29(9) (1988) 1549-1557.

[20] J. S. Lewis, D. W. McCarthy, T. J. McCarthy, Y. Fujibayashi and M.J. Welch., Evaluation of 64Cu-ATSM In Vitro and In Vivo in a Hypoxie Tumor Model., J.Nucl.Med,. 40.No. 1 . 1999,177-183.

[21] I.A. Jammaz, B. Al-Otaibi, N. Al-Hokbani, S.M. Okarvi, Synthesis of novel gallium-68 labeled rhodamine: A potential PET myocardial perfusion agent, Applied Radiation and Isotopes 144 (2019) 29-33.

[22] M.e. Frisch, G. Trucks, H.B. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, G. Petersson, H. Nakatsuji, Gaussian 16, Gaussian, Inc. Wallingford, CT, 2016.

[23] A.D. Becke., Density-functional thermochemistry. I. The effect of the exchange-only gradient correction., *J. Chem. Phys.* 96, 2155–2160 (1992). https://doi.org/10.1063/1.462066.

[24] T. Lu, F. Chen., Multiwfn: A multifunctional wavefunction analyzer., 2011., https://doi.org/10.1002/jcc.22885.

[25] W. Humphrey, A. Dalke, K. Schulten.., VMD: visual molecular dynamics., Journal of Molecular Graphics 14, 1, 1996, 33-38. https://doi.org/10.1016/0263-7855(96)00018-5.

[26] R. Dennington, T. Keith, J. Millam ., GaussView, Version 5.0.8.: Semichem Inc., Shawnee Mission KS; 2009.

[27] R.A. May, K.J. Stevenson, Software review of Origin 8, ACS Publications, 2009.

[28] O'Boyle, N.M., Tenderholt, A.L. and Langner, K.M. (2008) Cclib: A Library for Package-Independent Computational Chemistry Algorithms. Journal of Computational Chemistry, 29, 839-845., http://dx.doi.org/10.1002/jcc.20823.

[29] Zhurko, G. and Zhurko, D. (2015) Chemcraft Graphical Program for Visualization of Computed Results.., http://www.chemcraftprog.com/.

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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
EMERGING	The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.