EurJIC
European Journal of Inorganic Chemistry

Research Article
doi.org/10.1002/ejic.202300447

Chemistry Europe
European Chemical Societies Publishing

www.eurjic.org

Ligand and Linkage Isomers of Bis(ethylthiocarbamato) Copper Complexes with Cyclic C_6H_8 Backbone Substituents: Synthesis, Characterization, and Antiproliferation Activity

Kritika Bajaj,[a] Sarah A. Andres,[b] Dillon T. Hofsommer,[a] Okolocha Chekwube Michael,[a] Mark S. Mashuta,[a] Paula J. Bates,[b] Robert M. Buchanan,*[a] and Craig A. Grapperhaus*[a]

A series of isomeric bis(alkylthiocarbamate) copper complexes have been synthesized, characterized, and evaluated for antiproliferation activity. The complexes were derived from ligand isomers with 3-methylpentyl (H_2L^2) and cyclohexyl (H_2L^3) backbone substituents, which each yield a pair of linkage isomers. The thermodynamic products $CuL^{2a/3a}$ have two imino N and two S donors resulting in three five-member chelate rings (555 isomers). The kinetic isomers $CuL^{2b/3b}$ have one imino and one hydrazino N donor and two S donors resulting in four-, six-, and five-member rings (465 isomers). The 555 isomers have more accessible $Cu^{II/I}$ potentials ($E_{1/2}$ = 811/ 768 mV vs. ferrocenium/ferrocene) and lower energy charge transfer bands than their 465 counterparts ($E_{1/2}$ = 923/-854 mV). Antiproliferation activities were evaluated against the lung adenocarcinoma cell line (A549) and nonmalignant lung fibroblast cell line (IMR-90) using the MTT assay. CuL^{2a} was potent ($^{A549}EC_{50}$ = 0.080 μM) and selective ($^{IMR\text{-}90}EC_{50}/^{A549}EC_{50}$ = 25) for A549. Its linkage isomer CuL^{2b} had equivalent A549 activity, but lower selectivity ($^{IMR\text{-}90}EC_{50}/^{A549}EC_{50}$ = 12.5). The isomers CuL^{3a} and CuL^{3b} were less potent with $^{A549}EC_{50}$ values of 1.9 and 0.19 M and less selective with $^{IMR\text{-}90}EC_{50}/^{A549}EC_{50}$ ratios of 2.3 and 2.65, respectively. There was no correlation between reduction potential and A549 antiproliferation activity/selectivity.

Introduction

The variability of coordination modes and ligand substituents provides for many different types of isomers in metal complexes including different types of stereoisomers and constitutional isomers. Some of these, such as enantiomers and diastereomers, are common to metal free systems while others, such as linkage isomers, are unique to metal complexes. Linkage isomers are complexes with common ligands, but different coordination modes as first reported by Werner to describe the yellow (Co NO_2) and red (Co ONO) forms of pentamminenitritocobalt(III).[1] Linkage isomers have also been observed with other monodentate ligands such as CN^-, SCN^-, and NCO^-,[2] as well as with chelate ligands such as amino acids,[3] Schiff bases,[4] oximes,[5] and bis(thiosemicarbazones) (BTSCs).[6]

Copper BTSC complexes (Figure 1) are neutral, square planar N_2S_2 complexes that have received significant attention as diagnostic[7] and therapeutic[7b,8] agents. To date, all structurally characterized Cu(BTSC) complexes share a common binding motif in which the imino nitrogen atoms of the ligand coordinate to the metal along with two sulfur donors to yield complexes with three five-membered chelate rings. Recently, we developed bis(alkylthiocarbamato) (BATC) and hybrid thiosemicarbazonato-alkylthiocarbamato (TSTC) metal complexes (Figure 1) to introduce additional variability in the physical and electronic structure of N_2S_2 complexes.[8b,9]

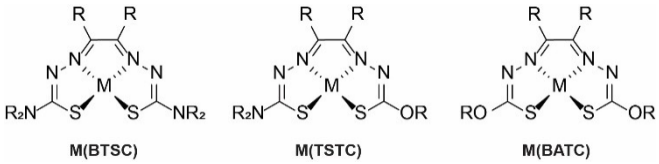


Figure 1. General structures of bis(thiosemicarbazone) (BTSC), thiosemicarbazone-alkylthiocarbamate (TSTC), and bis(alkylthiocarbamate) (BATC) metal complexes with three five-membered chelate rings (555 isomers).

With the TSTC framework, variation of the relative positions of the pendent functional groups (NR_2 and OR) with respect to backbone substituents (R) yielded constitutional isomers with nearly identical electronic structures, but different physical structures.[10] Additionally, the BATC ligand phenylglyoxal bis(ethylthiocarbamate) allowed isloation of the pair of linkage isomers $CuL^{1a/1b}$ (Figure 2).[11] One of the isomers, CuL^{1a}, had the common coordination mode observed for Cu(BTSC) complexes; hereinafter referred to as the 555 isomer. In the other, one imino nitrogen, one hydrazino nitrogen, and two sulfur atoms coordinated to the metal resulting in the formation of four-,

[a] Dr. K. Bajaj, Dr. D. T. Hofsommer, O. Chekwube Michael, Dr. M. S. Mashuta, Prof. R. M. Buchanan, Prof. C. A. Grapperhaus
Department of Chemistry
University of Louisville
40292 Louisville, KY, United States
E-mail: robert.buchanan@louisville.edu
grapperhaus@louisville.edu
Homepage: craiggrapperhaus.wixsite.com/reserachgroup

[b] Dr. S. A. Andres, Prof. P. J. Bates
Department of Medicine and Brown Cancer Center
University of Louisville
40202 Louisville, KY (USA)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/ejic.202300447

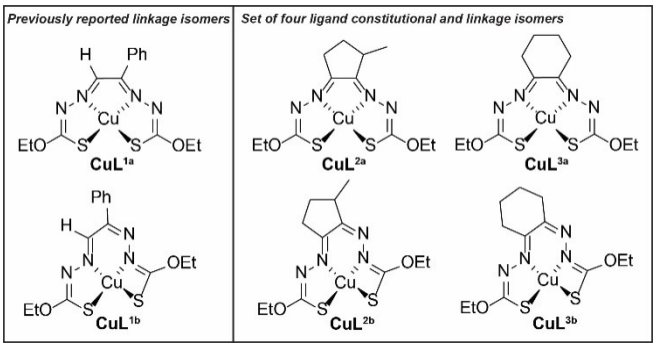
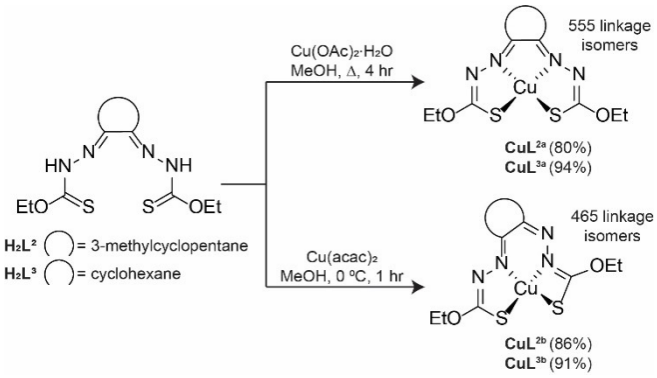


Figure 2. Previously reported linkage isomers $CuL^{1a/b}$ (ref. [11]) and ligand constitutional and linkage isomers $CuL^{2a–3b}$.



Scheme 1. Synthetic routes for the 555 linkage isomers $CuL^{2a/3a}$ and the 465 linkage isomers $CuL^{2b/3b}$.

six-, and five-membered chelate rings; hereinafter referred to as the 465 isomer.

In the current study, we report a series of four new Cu(BATC) complexes that are isomers with the molecular formula $C_{12}H_{18}N_4O_2S_2Cu$ (Figure 2). The complexes are based on a pair of ligands, H_2L^2 and H_2L^3, that are constitutional isomers with cyclic C_6H_8 groups in the ligand backbone. Each ligand can coordinate with Cu^{II} to yield a pair of 555 and 465 linkage isomers. The effects of the ligand isomerism and linkage isomerism on the physical and electronic structures of the four complexes are herein described along with preliminary investigations of their antiproliferation activity.

Results and Discussion

Synthesis and Characterization

A pair of bis(ethylthiocarbamate) ligands (H_2L^2 and H_2L^3) with fused C_6H_8 backbones were prepared via acid catalyzed condensation reactions of hydrazinecarbothioic acid *O*-ethyl ester with the appropriate dione in ethanol at room temperature. The two ligands are constitutional isomers that differ in the size and substitution of the cyclic backbone of the ligand. The ^1H and ^{13}C NMR spectra of H_2L^2 and H_2L^3 (Figures S1–S4) display doubling and broadening of peaks associated with multiple interconverting isomers in solution as previously reported with other bis(alkylthiocarbamate) ligands.[9,11] The dropwise addition of $Cu(OAc)_2 \cdot H_2O$ in methanol to a refluxing solution of H_2L^2 in methanol yielded the thermodynamically favored 555 linkage isomer CuL^{2a} as a deep-red product in 80% (Scheme 1). The complex with the ligand constitutional isomer, CuL^{3a} was prepared in 94% from H_2L^3 using the same methodology. The 465 linkage isomers CuL^{2b} and CuL^{3b} were isolated as kinetic products by addition of $Cu(acac)_2$ to the H_2L^2 and H_2L^3 in methanol at 0 °C yielding yellow-brown solids in 86% and 91% yields, respectively.

The electronic spectra of $CuL^{2a/b}$ and $CuL^{3a/b}$ were recorded in acetonitrile (Figure 3 and Figure S5). Each complex displays three ligand centered absorption bands and one metal-to-ligand charge transfer band. The differences in the coordination environment of the linkage isomers are well pronounced with

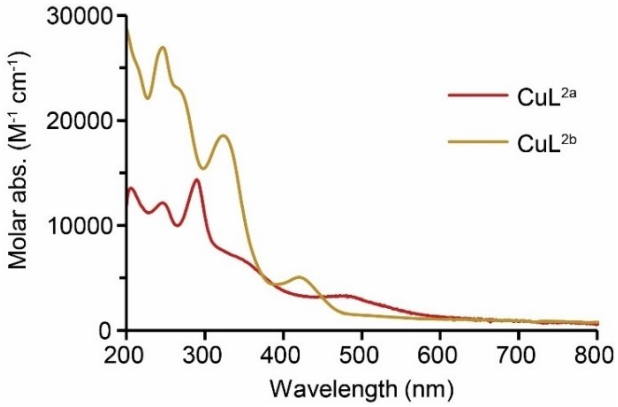


Figure 3. UV-visible spectra for the linkage isomer pair $CuL^{2a/b}$ in acetonitrile.

CuL^{2a} displaying bands at 246, 289 (sh), 368, and 480 nm similar to the previously reported 555 linkage isomer CuL^{1a}, Table 1. For CuL^{2b}, the bands are generally shifted to higher energy resulting in the observed color change from red to yellow-brown, as also observed for the 465 isomer CuL^{1b}. The spectra of linkage isomers $CuL^{3a/b}$ are nearly indistinguishable from their constitutional ligand isomer derivatives $CuL^{2a/2b}$. The FT-IR spectra of the linkage isomers $CuL^{2a/b}$ display common general features (Figures S8 and S9) although there are clear differences in the fingerprint region (Figure S10). The FT-IR spectra of their $CuL^{3a/b}$ derivatives are similar (Figures S11–S13).

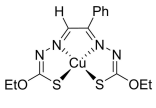
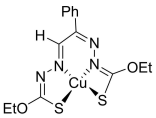
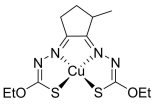
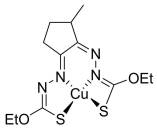
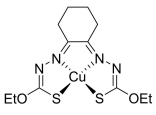
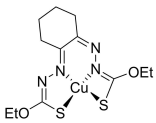
Crystallography

The solid-state structures for CuL^{2a}, CuL^{2b}, and CuL^{3a} were determined using single crystal X-ray diffraction (Figure 4). Crystal structure data and structure refinement details are provided in Table S1. Discussions of disorder within the refinements is provided in the Experimental Section. Selected bond distances and angle are provided in Table 2.

The 555 linkage isomers CuL^{2a} and CuL^{3a} contain Cu^{II} centers coordinated to two imino nitrogen atoms (N1 and N1') and two sulfur atoms (S1 and S1') resulting in three five-member chelate rings, (Figure 4a and 4c). The geometry is best described as

Research Article
doi.org/10.1002/ejic.202300447

Table 1. Summary of spectroscopic, electrochemical, and antiproliferation data for $CuL^{1a–3b}$.

	CuL^{1a}	CuL^{1b}	CuL^{2a}	CuL^{2b}	CuL^{3a}	CuL^{3b}
Structure						
Color[a]	Red	Yellow-Brown	Red	Yellow-Brown	Red	Yellow-Brown
λ_{max} (ε),[b] nm ($M^{-1}cm^{-1}$)	257 (20,800) 293 (30,000) 356 (7,300) 491 (3,500)	268 (25,000) 293 (19,100) 344 (16,100) 441 (3,800)	246 (12,200) 289 (14,300) 368 (5,600) 480 (3,300)	246 (27,000) 264 (23,100) 323 (18,500) 420 (5,100)	246 (12,100) 289 (14,300) 368 (5,600) 480 (3,300)	246 (31,800) 264 (26,300) 323 (20,400) 420 (6,300)
$E_{1/2}$[c] [mV]	603	807	811	923	768	854
$^{A549}EC_{50}$ [μM]	0.11	0.12	0.08	0.08	1.9	0.19
$^{IMR\text{-}90}EC_{50}$ [μM]	1.9	0.77	2.0	1.0	4.5	0.5
Selectivity[d]	17	6.4	25	12.5	2.3	2.6
Reference	Ref. [11]	Ref. [11]	this work	this work	this work	this work

[a] Color of product as a solid powder. [b] Electronic spectroscopy data collected in acetonitrile. [c] $CuI^{I/I}$ reduction potentials in acetonitrile (0.1 M NBu_4PF_6) versus Fc^+/Fc. [d] Selectivity defined as $^{IMR\text{-}90}EC_{50}/^{A549}EC_{50}$.

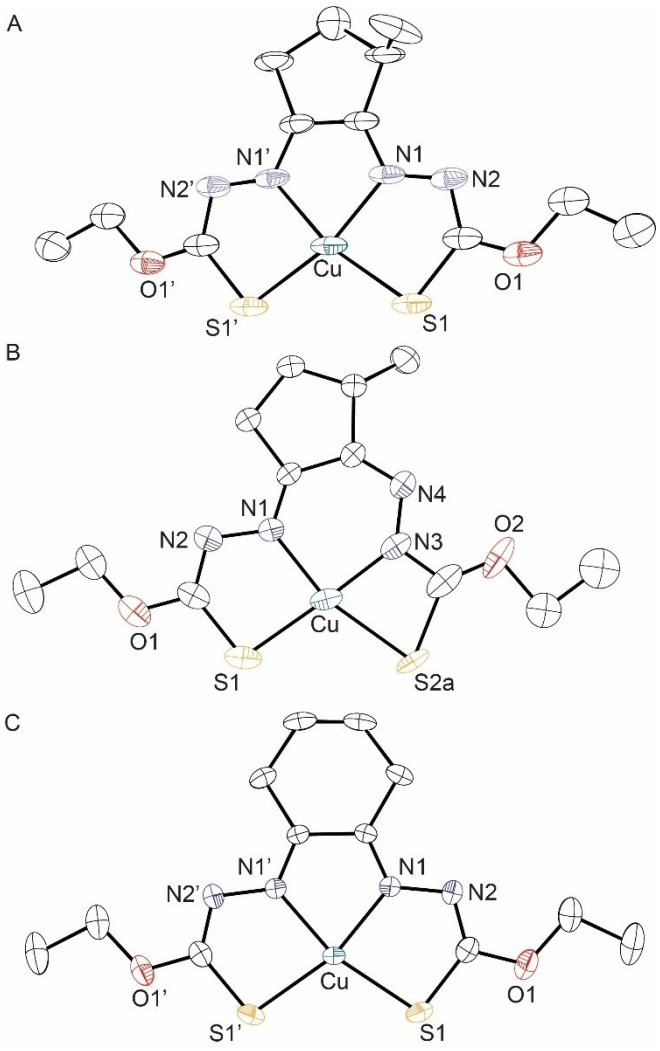


Figure 4. ORTEP representations of A) CuL^{2a}; B) CuL^{2b}; and C) CuL^{3a}.

Table 2. Selected bond distances (Å) and bond angles (°) for CuL^{2a}, CuL^{2b}, and CuL^{3a}.

	CuL^{2a}	CuL^{2b}	CuL^{3a}
Cu N1	1.982(4)	1.945(5)	1.9638(19)
Cu N3		1.922(6)	
Cu S1 Cu	2.2621(14)	2.221(2)	2.2495(7)
S2 N1		2.326(4)[b]	
N2 N1	1.373(6)	1.392(7)	1.379(3)
C1 N3	1.301(5)	1.290(7)	1.289(3)
N4 N3/4		1.359(7)	
C2		1.279(7)	
N1 Cu N3	82.1(2)[a]	91.0(2)	79.83(11)[c]
N1 Cu S1	83.23(12)	86.71(15)	84.57(6)
N3 Cu S2	83.23(12)[a]	72.99(18)[b]	84.57(6)[c]
S1 Cu S2	111.50(7)[a]	109.29(13)[b]	111.18(4)[c]
Sum of four angles	360(3)	360(3)	360(1)

[a] Positions N3 and S2 in CuL^{2b} correspond to symmetry generated positions (2 x, y, 0.5 z) N1′ and S1′ in CuL^{2a}. [b] The position of S2 is disordered. Value reported is for S2a (50% occupancy). [c] Positions N3 and S2 in CuL^{2b} correspond to symmetry generated positions in CuL^{3a} (2 x, y, 2.5 z) N1′ and S1′.

distorted square planar with the sum of the four angles associated with the Cu core equal to 360(3)° and 360(1)° for CuL^{2a} and CuL^{3a}, respectively. The Cu N and Cu S bond distances range are 1.982(4) and 2.2621(14) Å for CuL^{2a}, which are consistent with other Cu(BATC) complexes with three five-member chelates.[9,11] Similar Cu N and Cu S bond distances of 1.9638(19) and 2.2495(7) Å are observed for CuL^{3a}. The N Cu N bond angle of 82.1(2)° associated with the diimine backbone of CuL^{2a} is comparable to the related angle of 80.11(6)° in CuL^{1a} indicating the fused ring in the backbone of CuL^{2a} does not hinder Cu N orbital overlap. A similar N Cu N angle of

Research Article
doi.org/10.1002/ejic.202300447

79.83(11)° is observed for CuL^{3a}. Overall, the inclusion of fused rings in the backbone of the BATC ligand has no significant effect on the coordination sphere.

The Cu^{II} ion of the 465 linkage isomer CuL^{2b} is coordinated to one imino nitrogen (N1), one hydrazino nitrogen (N3) and two sulfurs (S1 and S2a) resulting in a four-, a six-, and a five-atom chelate ring configuration (Figure 4b). The Cu N bond distance to the imine nitrogen, N2, of 1.945(5) Å is slightly longer than the Cu N distance of 1.922(6) Å to the hydrazino nitrogen, N3. The Cu S bond distances are 2.221(2) Å for S1 in the five-membered ring and 2.326(4) Å for S2a in the four-membered ring. The six- and five-member chelate rings have bond angles of 86.71(15)° (S1 Cu N1) and 91.0(2)° (N1 Cu N3), whereas the S2a Cu N3 bond angle associated with the four-member chelate ring is very acute at 72.99(18)°. Overall, the metric parameters are very similar to our previously reported Cu(BATC) 465 linkage isomer.[11]

Electrochemistry

Cyclic voltammetry studies of the four isomeric complexes $CuL^{2a–3b}$ were conducted in acetonitrile and each displays a single reversible reduction assigned to the $Cu^{II/I}$ couple (Figure S18 and Table 1). The $E_{1/2}$ values for 555 linkage isomers CuL^{2a} and CuL^{3a} are observed at 0.811 V and 0.768 V vs. ferrocenium/ferrocene (Fc^+/Fc), respectively. The 465 linkage isomers CuL^{2b} and CuL^{3b} have $Cu^{II/I}$ potentials of 923 and 857 mV, respectively. These values are more cathodic than their 555 counterparts by 112 mV and 89 mV indicating better electron donation from the ligand to the metal for the 465 isomers.

A comparison of the $Cu^{II/I}$ reduction potentials of $CuL^{2a–3b}$ with $CuL^{1a/b}$ is provided in Figure 5. For the 555 isomers, CuL^{1a} with a single phenyl substituent in the backbone is the easiest to reduce ($E_{1/2}$ = 603 mV) whereas CuL^{2a} is most difficult ($E_{1/2}$ = 811 mV). The cyclohexyl derivative CuL^{3a} is 43 mV easier to reduce than CuL^{2a} For the 465 linkage isomers, the potentials are shifted cathodically by 112 and 89 mV for the cyclic C_6H_8 backbone derivatives CuL^{2b} and CuL^{3b} and by 204 mV for CuL^{1b}. Overall, a ranking of the complexes based on the ease of reduction shows CuL^{1a} @ CuL^{3a} > CuL^{1b} □ CuL^{2a} > CuL^{3b} > CuL^{2b}. Interestingly, the potentials of CuL^{1b} and CuL^{2a} are similar (807 vs. 811 mV) indicating that both the identity of the backbone substituents and the donor atom (N_{imino} vs. $N_{hydrazino}$) are useful tools to tune the reduction potential of Cu(BATC) and related complexes.

Antiproliferation activity

The four isomeric complexes CuL^{2a-3b} were screened against lung adenocarcinoma (A549) and nonmalignant lung fibroblast (IMR-90) cell lines to evaluate antiproliferation activity and selectivity for cancer vs. non-cancer cells (Table 1 and Figure S19). The complexes are sparingly soluble in aqueous solutions (2.0 μg/mL for $CuL^{2a/3a}$ and 1.3 μg/mL for $CuL^{2b/3b}$). For

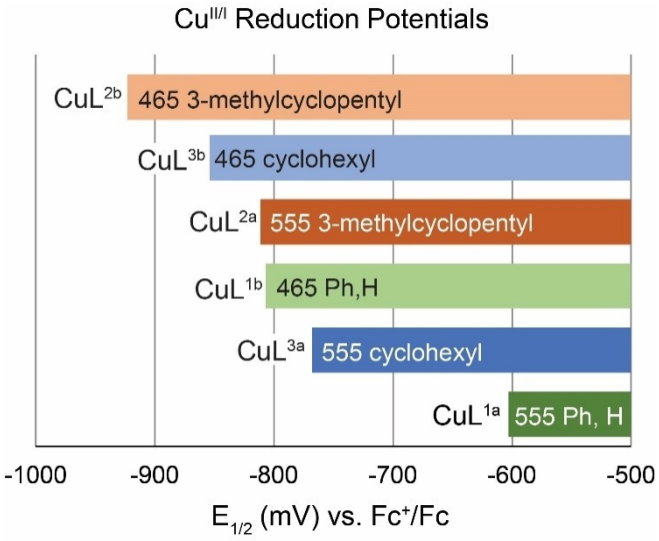


Figure 5. Comparison of $Cu^{II/I}$ reduction potentials versus ferrocenium/ferrocene for CuL^{1a-3b}. The end of the bar represents the $E_{1/2}$ value for each complex. The bars are color coded by ligand with the 555 isomer in a darker shade with white lettering and the 465 isomer in the lighter shade with black lettering. Cyclic voltammograms were recorded in acetonitrile with 0.1 M NBu_4PF_6 as supporting electrolyte at a scan rate of 200 mV s^{-1}.

the antiproliferatoin studies, the complexes were first dissolved in 100% DMSO and then diluted in aqueous medium. The complexes displayed overall good stability in DMSO/water and DMSO/culture medium over 12 hours (Figure S20). The 555 isomer CuL^{2a} retained 82% and 95% of its initial absorbance intensity in DMSO/H_2O and DMSO/culture medium, respectively (Table S2). Complex CuL^{2b} showed good stability in DMSO/H_2O (98%), but its limited solubility in DMSO/culture medium resulted precipitation and decreased relative intensity (63%). Each of the 465 isomers ($CuL^{2b/3b}$) were very stable with no loss of absorbance intensity in either solution over 12 hours.

The linkage isomer pair CuL^{2a} and CuL^{2b} possess equivalent $^{A549}EC_{50}$ values of 0.08 μM for A549. However, CuL^{2a} is slightly less active ($^{IMR\text{-}90}EC_{50}$ = 2.0 μM) against IMR-90 than CuL^{2b} ($^{IMR\text{-}90}EC_{50}$ = 1.0 μM) indicating a modestly higher degree of selectivity of the 555 isomer. These results are consistent with previously reported observations for $CuL^{1a/1b}$ (Table 1) in which the 555 isomer was more selective despite both isomers having similar A549 potencies. Interestingly, for the $CuL^{3a/3b}$ linkage isomer pair the 555 isomer is an order of magnitude *less active* ($^{A549}EC_{50}$ = 1.9 μM) than the 465 isomer ($^{A549}EC_{50}$ = 0.19 μM) and both isomers display similar selectivity. Although inclusion of peripheral cyclohexyl containing pendents as mimics of sugars has generally shown to improve cellular uptake,[12] for complex 3a the cyclohexyl group appears to decrease the solubility of the complex in the DMSO/culture medium potentially affecting the antiproliferation activity.

Overall, the activity and selectivity are similar to our previously reported hybrid thiosemicarbanto-alkylthiocarbamate complexes, which show better selectivity than the BTSC complex Cu(GSTM).[10] The A549 antiproliferation activity is also generally better than recently reported transition metal carba-

Research Article
doi.org/10.1002/ejic.202300447

mates, which demonstrated $^{A549}EC_{50}$ values as low for 5.4 μM and 0.4 μM for $^{A2780}EC_{50}$.[13] Although the mechanism of action for $CuL^{2a\text{-}3b}$ has not been established, the presence and redox cycling of copper can be toxic to cells by multiple mechanisms. Released Cu(I) can activate hydrogen peroxide (H_2O_2) to generate hydroxide (OH) and hydroxyl radical (OH·) via Fenton reaction to produce reactive oxygen species (ROS).[14] Our studies on similar complexes showed higher Cu loading in cancer cells compared to non-cancerous cell lines, but no significant ROS activity at concentrations of 1–10 μM.[10] Other possible mechanisms include inactivation of proteasomal activity, topoisomerase inhibition, protein transmetalation, and damage to proteins, membranes, mitochondria, or DNA.[7b,15]

Conclusions

A series of four isomeric Cu(BATC) complexes with cyclic C_6H_8 backbone substituents have been synthesized, characterized, and evaluated for their antiproliferation activity. The isomers are based on the constitutional isomer ligands H_2L^2 and H_2L^3, which each yield a pair of linkage isomers upon coordination to Cu^{II}. The 555 linkage isomers, $CuL^{2a/3a}$, are thermodynamic products that are readily prepared at higher temperature, whereas the 465 isomers $CuL^{2b/3b}$ are kinetic products that are isolated when metal complexation is performed at low temperatures. The electronic structures of the complexes are largely determined by the physical structure of the complexes with the 555 isomers having lower energy bands in the electronic spectra and being easier to reduce than their 465 counterparts. Notably, there is no correlation between the $Cu^{II/I}$ reduction potential and A549 antiproliferation activity. Four of the complexes ($CuL^{1a–2b}$) display similar $^{A549}EC_{50}$ values between 0.08 and 0.12 μM despite having reduction potentials that span 320 mV from 603 to 923 mV. Additionally, the reduction potentials of the ligand constitutional isomer complexes CuL^{2a} (811 mV) and CuL^{3a} (768) are similar despite CuL^{2a} being substantially more active ($^{A549}EC_{50}$ = 0.08 vs. 1.9 μM) and selective (25 vs. 2.3) for A549 antiproliferation. These results reinforce our recent observations that the $Cu^{II/I}$ reduction potentials are important, but do not play a dominate role in antiproliferation activity of these (N_2S_2)Cu complexes against A549.[10,11] The activity and selectivity is more dependent on the physical structure of the complexes with the more planar 3-methylcyclopentyl derivatives having better performance than the cyclohexyl derivatives.

Experimental Section

Materials and Methods. All the reagents were commercially available and used as received unless otherwise noted. Hydrazinecarbothioic acid *O*-ethyl ester[16] was prepared by literature methods.[16] Solvents were dried and purified using an MBraun solvent purification system. The complexes in this study are air and water stable as solids and were handled on the benchtop with no required protection from the atmosphere. All reactions were performed open to air and under ambient conditions unless otherwise indicated.

H_2L^2: 3-methylcyclopentanedione (0.50 g, 4.5 mmol) was first dissolved in ethanol (25 mL) and a catalytic amount of concentrated hydrochloric acid (5 drops) was added to the solution. To this, hydrazinecarbothioic acid *O*-ethyl ester (1.07 g, 8.92 mmol) was added and the reaction was stirred overnight at room temperature. A white precipitate was isolated by filtration and washed with ethanol. Yield = 0.88 g (61%). ^{1}H NMR, (DMSO-d_6, 700 MHz) δ 1.11 (m, 3H), 1.33 (m, 6H), 2.11 (s, 1H), 2.75 (m, 2H), 4.58 (q, 4H), 11.94 (s, 1H), 12.14 (s, 1H), 12.30 (s, 1H), 13.46 (br, 1H), 13.56 (br, 1H). ^{13}C NMR, (DMSO-d_6, 700 MHz) δ 13.7, 14.4, 14.7, 18.0, 25.0, 28.1, 29.3, 29.4, 37.9, 66.7, 68.0, 130.5, 146.6, 151.7, 154.2, 155.9, 163.0, 185.5, 186.8, 189.5. FT-IR, cm ^{1}: 3198 and 1499 (NH), 2981 and 1307 (CH), 1217 (CN), 1138 (CO), 1051 (CS). Elemental analysis calculated for $C_{12}H_{20}N_4O_2S_2$: C, 45.55; H, 6.37; N, 17.71. Found: C,45.66; H, 6.22; N, 17.69.

H_2L^3: cyclohexanedione (1.0 g, 8.9 mmol) was first dissolved in ethanol (20 mL) and a catalytic amount of concentrated hydrochloric acid (5 drops) was added to the solution. To this, hydrazinecarbothioic acid *O*-ethyl ester (2.14 g, 17.8 mmol) was added and the reaction was stirred overnight at room temperature. A white precipitate was isolated by filtration and washed with ethanol. Yield = 1.44 g (51%). ^{1}H NMR, (DMSO-d_6, 700 MHz) δ 1.32 (m, 6H), 1.66 (m, 3H), 1.80 (m, 2H), 2.14 (m, 2H), 2.65 (m, 2H), 4.55 (m, 4H), 5.52 (t, 1H), 7.38 (s, 1H), 10.25 (s, 1H), 11.33 (s, 1H), 12.32 (s, 1H), 12.69 (s, 1H), 13.27 (s, 1H). ^{13}C NMR, (DMSO-d_6, 700 MHz) δ 14.1, 20.9, 22.1, 26.8, 33.5, 65.7, 67.0, 67.4, 151.0, 173.7, 183.0, 186.6, FT-IR, cm ^{1}: 3178 and 1498 (NH), 2952 and 1280 (CH), 1211 (CN), 1139 (CO), 1045 (CS). Elemental analysis calculated for $C_{12}H_{20}N_4O_2S_2$: C, 45.55; H, 6.37; N, 17.71. Found: C,45.59; H, 6.61; N, 17.80.

CuL^{2a}: In a two neck round bottom flask, H_2L^2 (0.20 g, 0.63 mmol) was suspended in methanol (15 mL) and reflux for 30 mins. To this solution, copper acetate monohydrate (0.13 g, 0.63 mmol) was added dropwise using a dropwise addition funnel leading to the formation of a red solution. Upon complete addition of the Cu^{II} salt, the resultant red solution was refluxed for four hours. The red solid was isolated by filtration and washed with methanol. Yield = 0.19 g (80%). FT-IR, cm ^{1}: 2973 and 1415 (CH), 1384 and 1231 (CN), 1013 (CO), 801 (CuS), 632 (CuN). UV-vis spectrum in acetonitrile, nm (M ^{1} cm ^{1}): 246 (12,200), 289 (14,300), 368 (5,600), 480 (3,300). Elemental analysis calculated for $C_{12}H_{18}CuN_4O_2S_2$: C, 38.13; H, 4.80; N, 14.82. Found: C, 38.21; H, 4.80; N, 14.70. Mass spectrum m/z calculated for {$CuL^{2a} + H^+$}: 378.0240. Found: 378.0243.

CuL^{2b}: In a round bottom flask, H_2L^2 (0.10 g, 0.32 mmol) was suspended in methanol (5 mL). In another round bottom flask, copper acetylacetonate (0.83 g, 0.32 mmol) was suspended in methanol (8 mL). Both the solutions were held at 0 °C for 10 mins prior to mixing. The cold Cu^{II} salt solution was then transferred the cold ligand solution resulting in the formation of yellow-brown solution. The resultant yellow-brown solution was reacted for one hour at 0 °C. After one hour, cold pentane (at 20 °C) was added to the solution to precipitate the product. The yellow-brown solid was isolated by filtration and washed with cold pentane. Yield = 0.10 g (86%). FT-IR, cm ^{1}: 2974 and 1445 (CH), 1384 and 1260 (CN), 1073 (CO), 762 (CuS), 654 (CuN). UV-vis spectrum in acetonitrile, nm (M ^{1} cm ^{1}): 246 (27,000), 264 (23,100), 323 (18,500), 420 (5,100). Elemental analysis calculated for $C_{12}H_{18}CuN_4O_2S_2$: C, 38.13; H, 4.80; N, 14.82. Found: C, 38.80; H, 3.74; N, 14.34. Mass spectrum m/z calculated for {$CuL^{2b} + H^+$}: 378.0240. Found: 378.0242.

CuL^{3a}: In a two neck round bottom flask, H_2L^3 (0.30 g, 0.95 mmol) was suspended in methanol (15 mL) and reflux for 30 mins. To this solution, copper acetate monohydrate (0.19 g, 0.95 mmol) was

added dropwise using a dropwise addition funnel leading to the formation of a red solution. Upon complete addition of the Cu^{II} salt, the resultant red solution was refluxed for four hours. The red solid was isolated by filtration and washed with methanol. Yield = 0.34 g (94%). FT-IR, cm^{-1}: 2938 and 1419 (CH), 1384 and 1231 (CN), 1013 (CO), 814 (CuS), 632 (CuN). UV-vis spectrum in acetonitrile, nm (M^{-1} cm^{-1}): 246 (12,100), 289 (14,300), 368 (5,600), 480 (3,300). Elemental analysis calculated for $C_{12}H_{18}CuN_4O_2S_2$: C, 38.13; H, 4.80; N, 14.82. Found: C, 38.27; H, 4.81; N, 14.85. Mass spectrum m/z calculated for $\{CuL^{3a} + H^+\}$: 378.0240. Found: 378.0242.

CuL^{3b}: In a round bottom flask, H_2L^3 (0.10 g, 0.32 mmol) was suspended in methanol (5 mL). In another round bottom flask, copper acetylacetonate (0.83 g, 0.32 mmol) was suspended in methanol (8 mL). Both the solutions were held at 0 °C for 10 mins prior to mixing. The cold Cu^{II} salt solution was then transferred the cold ligand solution resulting in the formation of yellow-brown solution. The resultant yellow-brown solution was reacted for one hour at 0 °C. After one hour, cold pentane (at 20 °C) was added to the solution to precipitate the product. The yellow-brown solid was isolated by filtration and washed with cold pentane. Yield = 0.11 g (91%). FT-IR, cm^{-1}: 2942 and 1436 (CH), 1383 and 1256 (CN), 1036 (CO), 744 (CuS), 611 (CuN). UV-vis spectrum in acetonitrile (nm) (M^{-1} cm^{-1}): 246 (31,800), 264 (26,300), 323 (20,400), 420 (6,300). Elemental analysis calculated for $C_{12}H_{18}CuN_4O_2S_2$: C, 38.13; H, 4.80; N, 14.82. Found: C, 37.91; H, 4.62; N, 14.88. Mass spectrum m/z calculated for $\{CuL^{3b} + H^+\}$: 378.0240. Found: 378.0242.

Physical Methods. Elemental analyses were performed by Microanalysis, Inc. (Wilmington, DE). The electronic spectra were collected as solutions in acetonitrile in 1 mm quartz cuvettes using an Agilent 8453 diode array spectrometer. The vibrational spectra were obtained as powders on a Nicolet 360 FT-IR with a Smart iTR attachment. The NMR spectra were recorded on a Varian 700 MHz spectrometer at room temperature. Mass spectrometry was performed by the Mass Spectrometry Facility at Indiana University (+ESI-MS).

Electrochemical data were recorded using a Gamry 1000 Potentiostat/Galvanostat/ZRA. The complexes were dissolved in acetonitrile (0.3 mM) under argon in a Gamry three electrode cell. Tetrabutylammonium hexafluorophosphate (0.1 M) was used as a supporting electrolyte. Glassy carbon was used as a working electrode, platinum as a counter electrode, and a silver wire immersed in a glass tube containing 0.1 M tetrabutylammonium hexafluorophosphate and connected to the solution by a Teflon frit as quasi reference electrode. A small amount of ferrocene was added as an internal standard and all potentials are referenced versus ferrocenium/ferrocene (Fc^+/Fc^0).

Crystallographic Studies. The CrysAlisPro[17] CCD software package was used to acquire a total of 544 seventy-five second frame ω-scan exposures of data for a 0.43×0.05×0.03 mm^3 red-orange needle crystal of CuL^{2a} at 103 K to a 2θ max = 54.24° using monochromated MoKα radiation (0.71073 Å) from a sealed tube. Frame data were processed using CrysAlisPro[17] RED to determine final unit cell parameters: a = 18.750(4) Å, b = 9.3696(12) Å, c = 9.1550(14) Å, = 90°, β = 99.633(19)°, γ = 90°, V = 1585.7(5)Å^3, D_{calc} = 1.562 Mg/m^3, Z = 4 to produce raw hkl data that were then corrected for absorption (transmission min./max. = 0.814/1.000; μ = 1.648 mm^{-1}) using SCALE3 ABSPACK.[18] The structure was solved using Patterson methods in the space group C2/c using SHELXS[19] and refined by least squares methods on F^2 using SHELXL.[19] Non-hydrogen atoms were refined with anisotropic atomic displacement parameters with the exception of the half-occupancy C3 atom (isotropic). Hydrogen atoms were calculated and placed in their geometrically generated positions and refined as a riding model on the attached C atom. For data $I > 2\sigma(I)$ (R(int) = 0.056) the final anisotropic full matrix least-squares refinement on F^2 for 102 variables converged at R1 = 0.053 and wR2 = 0.113 with a GOF of 1.08.

Crystals of CuL^{2b} suitable for x-ray analysis were grown from acetonitrile solution in the freezer. X-ray structural analysis was performed on a yellow-brown thin square plate 0.25×0.25×0.01 mm^3 using an identical data acquisition strategy described above for **CuL^{2a}** at 103(1) K to a $2\theta_{max}$ = 56.28°. CuL^{2b} crystallizes in the monoclinic space group $P2_1/m$ with unit cell parameters: a = 10.3530(5) Å, b = 6.8788(3) Å, c = 11.9807(7) Å, β = 107.902(6)°, V = 811.92(7) Å^3, Z = 2 and D_{calc} = 1.521 Mg/m^3. 2,146 raw independent data were corrected for absorption (transmission min./max. = 0.809/1.000; μ = 1.609 mm^{-1}) using SCALE3 ABSPACK.[18] The structure was solved by Direct methods using SHELXTL. Non-hydrogen atoms were refined with anisotropic atomic displacement parameters with the exception of the half-occupancy S2b atom (isotropic) and the disordered C11a, C11b, C12a and C12b atoms (isotropic). The disordered S2 was refined with a wobbling model comprised of two sulfur atoms (S2a and S2b) both at half occupancy using 2 restraints. The disordered OEt group was modeled with half occupancy C11a, C11b, C12a and C12b atoms using 3 restraints. Hydrogen atoms were calculated and placed in their geometrically generated positions and refined as a riding model on the attached C atom. For data $I > 2\sigma$ (I) 1,851 unique reflections (R(int) 0.048) the final anisotropic full matrix least-squares refinement on F^2 for 133 variables converged at R1 = 0.068 and wR2 = 0.134 with a GOF of 1.05.

A red-orange needle 0.39×0.05×0.02 mm^3 crystal of CuL^{3a} was mounted on a CryoLoop for collection of x-ray data on an Agilent Technologies/Oxford Diffraction Gemini CCD diffractometer. The CrysAlisPro[17] CCD software package (v 1.171.40.67a) was used to acquire a total of 813 fifty second frame ω-scan exposures of data at 200 K to a 2θ max = 58.26° using monochromated MoKα radiation (0.71073 Å) from a sealed tube. Frame data were processed using CrysAlisPro[17] RED to determine final unit cell parameters: a = 17.4927(7) Å, b = 10.0148(3) Å, c = 9.2019(3) Å, α = 90°, β = 100.980(4)°, γ = 90°, V = 1582.53 Å^3, D_{calc} = 1.586 Mg/m^3, Z = 4 to produce raw hkl data that were then corrected for absorption (transmission min./max. = 0.692/1.00; μ = 1.652 mm^{-1}) using SCALE3 ABSPACK.[18] The structure was solved by Direct methods in the monoclinic space group C2/c using SHELXS[19] and refined by least squares methods on F^2 using SHELXL.[19] Non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Hydrogen atoms were located by difference maps and refined isotropically. For all 2,132 unique reflections (R(int) 0.061) the final anisotropic full matrix least-squares refinement on F^2 for 126 variables converged at R1 = 0.057 and wR2 = 0.098 and a GOF of 1.04.

Stability Studies. The stabilities of $CuL^{2a–3b}$ were evaluated in DMSO/H_2O and DMSO/culture medium by UV-visible spectroscopy (Figure S20). A 3 mM solution of each complex was prepared in DMSO and a 3 mL aliquot was diluted with 1 mL of DI H_2O or 1 mL of Dulbecco's modified Eagle's medium (DMEM, Life Technologies) culture medium supplemented with 10% fetal bovine serum (FBS, Life Technologies, Grand Island, NY), 62.5 μg/mL penicillin and 100 μg/mL streptomycin (Life Technologies). The stability of each complex was quantified by comparison of the initial absorbance intensity with the intensity after 12 hours at room temperature under ambient conditions. Results are summarized in Table S2.

Antiproliferation Studies. A549 and IMR-90 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in the appropriate medium supplemented with 10% fetal bovine serum (FBS, Life Technologies, Grand Island, NY), 62.5 μg/mL penicillin and 100 μg/mL streptomycin (Life Technologies) in a humidified incubator at 37 °C with 5% CO_2. The growth

media were as follows: Dulbecco's modified Eagle's medium (DMEM, Life Technologies) for A549 cells, and Eagle's minimal essential medium (EMEM) with Eagle's balanced salt solution (EBSS), 2 mM L-glutamine, 1500 mg/L sodium bicarbonate (Lonza, Walkersville, MD), supplemented with 1 mM sodium pyruvate (Life Technologies) and non-essential amino acids (Life Technologies) for IMR-90 cells. For treatment, metal complexes were dissolved first in 100% DMSO and then diluted in aqueous medium. The final concentration of DMSO used in the assays was always less than 0.5% v/v.

Antiproliferative activity of the metal complexes was evaluated using a previously published 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay protocol.[8b,20] Cells were seeded in quadruplicate wells in 96-well plates and allowed to adhere overnight. To account for intrinsic differences in growth rates, cells were plated at the following densities to achieve comparable MTT absorbance values (OD570 between 0.5 and 1) for untreated cells: A549, 1000 cells/well; IMR-90, 5000 cells/well. After 72 h of treatment with the various complexes or vehicle control, MTT (Sigma/Millipore Sigma, St. Louis, MO) was added for 4 h prior to cell lysis. All readings were normalized to the vehicle treatment.

Supporting Information

The following files are available free of charge: NMR, UV-vis, FT-IR, UV-vis, high resolution mass spectrometry, cyclic voltamme-try, cell proliferation assays, and crystallographic data and refinement parameters (PDF). Deposition Numbers 2282182 (for CuL^{2a}), 2282183 (for CuL^{2b}), 2282184 (for CuL^{3a}) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures services .

Acknowledgements

This research was supported by the National Science Foundation CHE-1955268 (C. A. G.) and CHE-1800245 (R. M. B.) and the National Institutes of Health U01 HL127518 REACH program, which was funded by NIMHD, NHGRI, NHLBI, NIA, NIAAA, NIBIB, NICHD, NIDA, NINDS, NINR, and NLM. The Department of Energy (DEFG02-08CH11538) and the Kentucky Research Challenge Trust Fund are acknowledged for upgrade of the X-ray facility (MSM).

Conflict of Interests

The authors declare the following competing financial interest-(s): Complexes described in this manuscript are included in an issued US patent (US-11208379-B2) entitled "Compounds, Compositions, Methods for Treating Diseases, and Methods for Preparing Compounds." A portion of the results described in this manuscript are included in the dissertation *Alkylthiocarbamate metal complexes with antiproliferation activity* by Kritika Bajaj, University of Louisville.[21]

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords: copper · isomers · N_2S_2 ligands · antiproliferation activity

[1] A. Werner, *Ber. Dtsch. Chem. Ges.* **1907**, *40*, 765–788.
[2] R. J. Balahura, N. A. Lewis, *Coord. Chem. Rev.* **1976**, *20*, 109–153.
[3] T. G. Appleton, *Coord. Chem. Rev.* **1997**, *166*, 313–359.
[4] A. Hazari, A. Ghosh, *J. Indian Chem. Soc.* **2018**, *95*, 1597–1606.
[5] a) M. M. Aly, J. J. Stephanos, *Spectrochim. Acta Part A* **1994**, *50*, 835–840; b) M. J. Lacey, C. Macdonald, J. Shannon, *Aust. J. Chem.* **1973**, *26*, 263–268.
[6] a) E. Bermejo, A. Castiñeiras, L. Ackerman, M. Owens, D. West, *Z. Anorg. Allg. Chem.* **2001**, *627*, 1966–1971; b) A. Castiñeiras, E. Bermejo, D. West, A. K. El-Sawaf, J. Swearingen, *Polyhedron* **1998**, *17*, 2751–2757; c) R. L. Arrowsmith, P. A. Waghorn, M. W. Jones, A. Bauman, S. K. Brayshaw, Z. Hu, G. Kociok-Koehn, T. L. Mindt, R. M. Tyrrell, S. W. Botchway, J. R. Dilworth, S. I. Pascu, *Dalton Trans.* **2011**, *40*, 6238–6252; d) J. K. Bilyj, M. J. Riley, P. V. Bernhardt, *Dalton Trans.* **2018**, *47*, 2018–2030; e) C. F. Wise, D. Liu, K. J. Mayer, P. M. Crossland, C. L. Hartley, W. R. McNamara, *Dalton Trans.* **2015**, *44*, 14265–14271; f) S. I. Pascu, P. A. Waghorn, T. D. Conry, B. Lin, H. M. Betts, J. R. Dilworth, R. B. Sim, G. C. Churchill, F. I. Aigbirhio, J. E. Warren, *Dalton Trans.* **2008**, 2107–2110.
[7] a) D. Palanimuthu, S. V. Shinde, K. Somasundaram, A. G. Samuelson, *J. Med. Chem.* **2013**, *56*, 722–734; b) B. M. Paterson, P. S. Donnelly, *Chem. Soc. Rev.* **2011**, *40*, 3005–3018; c) B. M. Paterson, C. Cullinane, P. J. Crouch, A. R. White, K. J. Barnham, P. D. Rose, W. Noonan, D. Binns, R. J. Hicks, P. S. Donnelly, *Inorg. Chem.* **2019**, *58*, 4540–4552; d) L. W. Hung, V. L. Villemagne, L. Cheng, N. A. Sherratt, S. Ayton, A. R. White, P. J. Crouch, S. Lim, S. L. Leong, S. Wilkins, J. George, B. R. Roberts, C. L. Pham, X. Liu, F. C. Chiu, D. M. Shackleford, A. K. Powell, C. L. Masters, A. I. Bush, G. O'Keefe, J. G. Culvenor, R. Cappai, R. A. Cherny, P. S. Donnelly, A. F. Hill, D. I. Finkelstein, K. J. Barnham, *J. Exp. Med.* **2012**, *209*, 837–854; e) O. C. Brown, J. Baguña Torres, K. B. Holt, P. J. Blower, M. J. Went, *Dalton Trans.* **2017**, *46*, 14612–14630.
[8] a) R. Anjum, D. Palanimuthu, D. S. Kalinowski, W. Lewis, K. C. Park, Z. Kovacevic, I. U. Khan, D. R. Richardson, *Inorg. Chem.* **2019**, *58*, 13709–13723; b) S. A. Andres, K. Bajaj, N. S. Vishnosky, M. A. Peterson, M. S. Mashuta, R. M. Buchanan, P. J. Bates, C. A. Grapperhaus, *Inorg. Chem.* **2020**, *59*, 4924–4935; c) H. G. Petering, H. H. Buskirk, G. E. Underwood, *Cancer Res.* **1964**, *24*, 367–372; d) S. Sandhaus, R. Taylor, T. Edwards, A. Huddleston, Y. Wooten, R. Venkatraman, R. T. Weber, A. González-Sarrías, P. M. Martin, P. Cagle, Y.-C. Tse-Dinh, S. J. Beebe, N. Seeram, A. A. Holder, *Inorg. Chem. Commun.* **2016**, *64*, 45–49.
[9] N. S. Vishnosky, M. S. Mashuta, R. M. Buchanan, C. A. Grapperhaus, *Inorg. Chim. Acta* **2017**, *461*, 45–51.
[10] K. Bajaj, S. A. Andres, D. T. Hofsommer, A. F. Greene, O. Hietsoi, M. S. Mashuta, T. Weis, L. J. Beverly, P. J. Bates, R. M. Buchanan, C. A. Grapperhaus, *J. Inorg. Biochem.* **2023**, *246*, 112288.
[11] K. Bajaj, S. A. Andres, D. T. Hofsommer, M. Galib, M. S. Mashuta, B. Bennett, B. Narayanan, R. M. Buchanan, P. J. Bates, C. A. Grapperhaus, *Inorg. Chem.* **2022**, *61*, 7715–7719.
[12] a) S. Benetti, M. Dalla Pozza, L. Biancalana, S. Zacchini, G. Gasser, F. Marchetti, *Dalton Trans.* **2023**, *52*, 5724–5741; b) F. Marchetti, C. Di Nicola, R. Pettinari, C. Pettinari, I. Aiello, M. La Deda, A. Candreva, S. Morelli, L. De Bartolo, A. Crispini, *Eur. J. Inorg. Chem.* **2020**, *2020*, 1027–1039; c) V. R. Akhmetova, N. S. Akhmadiev, M. F. Abdullin, L. U. Dzhemileva, V. A. D'yakonov, *RSC Adv.* **2020**, *10*, 15116–15123.
[13] G. Bresciani, N. Busto, V. Ceccherini, M. Bortoluzzi, G. Pampaloni, B. Garcia, F. Marchetti, *J. Inorg. Biochem.* **2022**, *227*, 111667.
[14] P. Gupta, A. Lakes, T. Dziubla, in *Oxidative stress and biomaterials*, Elsevier, Amsterdam, **2016**, pp. 1–33.
[15] a) A. G. Dalecki, C. L. Crawford, F. Wolschendorf, *Adv. Microb. Physiol.* **2017**, 193–260; b) D. Denoyer, S. A. Clatworthy, M. A. Cater, *Met. Ions Life Sci.* **2018**, *18*, 469–506; c) C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, *Chem. Rev.* **2014**, *114*, 815–862; d) A. Santoro, B. Vileno, Ò. Palacios, M. D. Peris-Díaz, G. Riegel, C. Gaiddon, A. Krężel, P.

Faller, *Metallomics* **2019**, *11*, 994–1004; e) M. Wehbe, A. W. Y. Leung, M. J. Abrams, C. Orvig, M. B. Bally, *Dalton Trans.* **2017**, *46*, 10758–10773; f) M. F. Zaltariov, M. Hammerstad, H. J. Arabshahi, K. Jovanovic, K. W. Richter, M. Cazacu, S. Shova, M. Balan, N. H. Andersen, S. a. Radulović, *Inorg. Chem.* **2017**, *56*, 3532–3549.

[16] K. Reüfenacht, *Helv. Chim. Acta* **1972**, *55*, 1178–1187.

[17] CrysAlis PRO (CCD and RED), V 1.171.40.67a, (Rigaku OD 2019).

[18] SCALE3 ABSPACK included in CrysAlis PRO RED, V. 1.171.40.67a, (Rigaku OD,2019).

[19] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2008**, *A64*, 112–122.

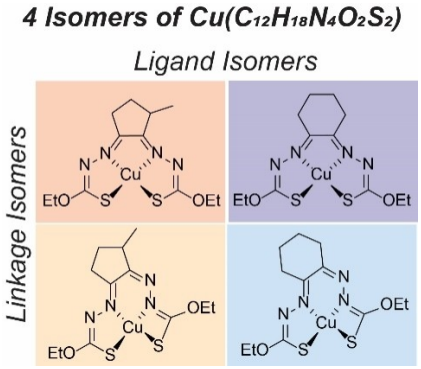
[20] D. M. Morgan, *Methods Mol. Biol.* **1998**, *79*, 179–183.

[21] K. Bajaj, "Alkylthiocarbamate metal complexes with antiproliferation activity." (2022). *Electronic Theses and Dissertations.* Paper 3939. 10.18297/etd/3939.

Manuscript received: July 18, 2023
Revised manuscript received: September 10, 2023
Accepted manuscript online: September 14, 2023
Version of record online: ■■, ■■

RESEARCH ARTICLE

Four isomeric copper(II) complexes based on a pair of ligand isomers that each yield a pair of linkage isomers have been synthesized and characterized. The effect of isomerism on the electronic structure and antiproliferation activity of the complexes is described.



Dr. K. Bajaj, Dr. S. A. Andres, Dr. D. T. Hofsommer, O. Chekwube Michael, Dr. M. S. Mashuta, Prof. P. J. Bates, Prof. R. M. Buchanan, Prof. C. A. Grapperhaus**

1 – 9

Ligand and Linkage Isomers of Bis(ethylthiocarbamato) Copper Complexes with Cyclic C_6H_8 Backbone Substituents: Synthesis, Characterization, and Antiproliferation Activity