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Highly Cytotoxic Copper(II) Complexes with Modified Paullone Ligands

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The reaction of copper(II) chloride or copper(II) acetate with 6-*N*-(2-*N*′,*N*′-dimethylaminoethylamino)-7,12-dihydroindolo-[3,2-*d*][1]benzazepine (HL^1), 9-bromo-6-*N*-(2-*N*′,*N*′-dimethylaminoethylamino)-7,12-dihydroindolo[3,2-*d*][1]-benzazepine (HL^2), *N*-(9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-yliden-*N*′-(1-pyridin-2-yl-methylidene)azine (HL^3), or *N*-(9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-yliden-*N*′-(1-pyridin-2-yl-ethylidene)azine (HL^4) in methanol affords the novel copper(II) complexes $[Cu(HL^1)Cl_2]$ (**1**), $[Cu(HL^2)Cl_2]$ (**2**), $[Cu(HL^3)Cl_2]$ (**3**), $[Cu(HL^4)Cl_2]$ (**4**), and $[Cu(L^4)(CH_3COO)(CH_3OH)]$ (**5**). The new ligands (HL^2 and HL^3) and the complexes **1**−**5** were characterized by ^1H and ^{13}C NMR, IR and electronic absorption spectroscopy, ESI mass spectrometry, and X-ray crystallography. Two ligands, HL^1 and HL^2, and complexes **1**−**4** were tested for cytotoxicity in three human cancer cell lines, namely, CH1 (ovarian carcinoma), A549 (non-small cell lung cancer), and SW480 (colon carcinoma). Additionally, complexes **1**, **2**, and **4** were assayed in an isogenic pair of ovarian cancer cell lines, one being sensitive to cisplatin (A2780) and the other having acquired cisplatin resistance (A2780cisR). All of the compounds evaluated are cytotoxic, with complexes **3** and **4** exhibiting IC_{50} values in the nanomolar range.

Introduction

Many anticancer drugs used today are either classic non-targeted coordination complexes or organic alkylating agents, but they are increasingly used in combination with targeted organic compounds that inhibit specific enzymes. In recent years, efforts to construct hybrid drug systems in which a targeted organic drug is attached to a non-targeted metal center have emerged.[1] Indeed, metal complexes with biologically active ligands display many favorable properties that could overcome the limitations encountered in combination therapies.[2−13] In this regard, recent efforts by us have focused on the synthesis of metal complexes with indolo-[3,2-*d*]benzazepines (Paullones), which inhibit the activity of cyclin-dependent kinases (CDKs) and show high cytotoxicity in vitro.[14,15] Structure−activity relationship studies on metal-free compounds have revealed certain features required for CDK-inhibitory properties that do not necessarily parallel their antiproliferative activity profile.[16] As a result, other intracellular targets for Paullones have also been suggested.[15,17] Our primary aim has been to investigate the effect of metal

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coordination of Paullone compounds on both CDK-inhibition and cytotoxicity. Since the original Paullone derivatives do not contain binding sites to accommodate metal ions, a number of novel indolo[3,2-*d*]benzazepines have been designed, synthesized and subsequently coordinated to gallium(III), ruthenium(II), and osmium(II) centers.[18−21] The effect of metal coordination on the aqueous solubility, antiproliferative activity, and impact on the cell cycle has been reported.[18−21] As a natural extension to this work, we turned our attention to ligands that are able to bind copper(II), a more biologically compatible metal ion.

Copper is an essential element for most aerobic organisms, employed as a structural and catalytic cofactor, and consequently it is involved in many biological pathways.[22−25] Taking this into account, much attention has been given to research on the mechanisms of absorption,[26−28] distribution,[29−31] metabolism, and excretion of copper,[32−34] as well as on its role in the development of cancer and other diseases.[35,36] In the case of cancer, numerous studies have shown that the concentration of copper is elevated in the serum, as well as in the tumor,[24,37−51] as copper is an endogenous angiogenesis stimulator.[52−54] Nevertheless, several bis(thiosemicarbazones) and their copper(II) complexes show encouraging antitumor activity, which has been demonstrated in vitro and in vivo.[55,56] Moreover, a number of copper(II) complexes with purine-based, benzohydroxamic acid, imidazole, and polycarboxylate ligands have shown good cytotoxicities.[57]

Herein, we report on the synthesis of the first Paullone-based copper(II) complexes of the general formulas $[Cu(HL^{1-4})Cl_2]$ and $[Cu(L^4)(CH_3COO)(CH_3OH)]$, where HL^1 and HL^2 are bidentate ligands and HL^3 and HL^4 tridentate ligands (Scheme 1). Their spectroscopic properties, solid state structures, and cytotoxicity in human cancer cell lines are also reported.

Experimental Section

Starting Materials. 7,12-Dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-thione (**A**),[58] 9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-thione (**B**),[17] 9-bromo-7,12-dihydroindolo[3,2-*d*]-[1]benzazepin-6-ylhydrazine (**C**),[20] 6-*N*-(2-*N*′,*N*′-dimethylaminoethylamino)-7,12-dihydroindolo[3,2-*d*][1]benzazepine (HL^1),[59] and *N*-(9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-yliden-*N*′-(1-pyridin-2-yl-ethylidene)azine (HL^4)[60] were prepared according to published protocols. Tetrahydrofuran and ethanol were dried using standard procedures. Methanol (HPLC-grade) was purchased from Fisher Scientific. Hydrazine hydrate, *N*,*N*-dimethylethylenediamine, 2-pyridinecarbaldehyde, 2-acetylpyridine, and copper(II) chloride were purchased from Sigma-Aldrich and used without further purification.

9-Bromo-6-*N*-(2-*N*′,*N*′-dimethylaminoethylamino)-7,12-dihydroindolo[3,2-*d*][1]benzazepine (HL^2). To 9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5H)-thione (**B**) (1.01 g, 3.0 mmol) in dry THF (60 mL) was added *N*,*N*-dimethylethylendiamine (0.38 mL, 3.5 mmol), and the reaction mixture was heated to reflux under argon for 45 h. Afterwards, the solvent was evaporated and the solid residue extracted with diethyl ether (120 mL). The filtered extract was evaporated to ca. 50% of the initial volume and allowed to stand at −20 °C overnight. The colorless crystals formed were removed by filtration, washed with diethyl ether, and dried in vacuo. Yield: 0.78 g, 68%. Anal. Calcd for $C_{20}H_{21}BrN_4$ (M_r = 397.31 g/mol) (%): C, 60.46; H, 5.33; N, 14.10. Found: C, 60.52; H, 5.63; N, 13.78. ^{1}H NMR (DMSO-d_6, δ): 11.61 (s, 1H, N12), 7.84 (s, 1H, C8), 7.68 (d, 1H, $^3J(H_{C2})$ = 7.5 Hz, C1), 7.36 (d, 1H, $^3J(H_{C10})$ = 8.5 Hz, C11), 7.25 (dd, 1H, $^3J(H_{C2})$ = 8.5 Hz, $^3J(H_{C4})$ = 8.0 Hz, C3), 7.23 (d, 1H, $^3J(H_{C11})$ = 8.5 Hz, C10),

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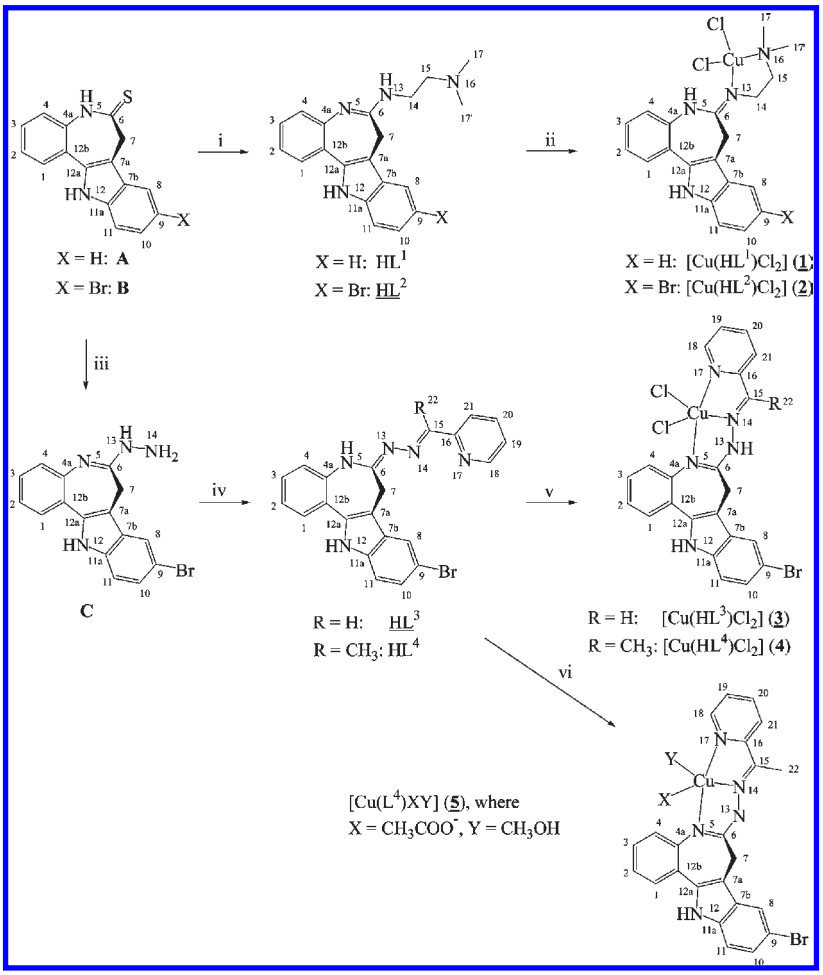
Scheme 1. Synthesis of **1**−**5** and Relevant Numbering Scheme[a]



[a] Underlined compounds have been characterized by X-ray crystallography. Reagents and conditions: (i) N,N-dimethylethylenediamine, THF, Ar, reflux, 24 h (HL^1, 60%) or 45 h (HL^2, 68%); (ii) $CuCl_2 \cdot 2H_2O$, CH_3OH, reflux, 30 min [68% (**1**), 69% (**2**)]; (iii) $N_2H_4 \cdot H_2O$, C_2H_5OH, reflux, 1.5 h (85%); (iv) 2-pyridinecarbaldehyde, CH_3OH, reflux, 45 min (HL^3 68%) or 2-acetylpyridine, CH_3OH, reflux, 3.5 h (HL^4 73%); (v) $CuCl_2 \cdot 2H_2O$, CH_3OH, reflux, 30 min [95% (**3**), 91% (**4**)]; (vi) $Cu(CH_3COO)_2 \cdot H_2O$, CH_3OH, reflux, 30 min (83%) (**5**).

7.14 (t, 1H, $^3J(H_{C14})$ = 5.0 Hz, N13), 7.13 (d, 1H, $^3J(H_{C3})$ = 8.0 Hz, C4), 7.03 (dd, 1H, $^3J(H_{C1})$ = 7.5 Hz, $^3J(H_{C3})$ = 8.5 Hz, C2), 3.32 (s, 2H, C7), 3.28 (dt, 2H, $^3J(H_{N13})$ = 5.0 Hz, $^3J(H_{C15})$ = 6.5 Hz, C14), 2.15 (s, 6H, C17+C18). $^{13}C\{^1H\}$ NMR (DMSO-d_6, δ): 155.52 (C6), 147.25 (C4a), 136.78 (C11a), 136.24 (C12a), 129.09 (C7b), 128.36 (C4), 128.23 (C3), 127.42 (C1), 124.49 (C10), 122.59 (C12b), 121.07 (C2), 121.00 (C8), 113.96 (C11), 112.01 (C9), 109.31 (C7a), 58.61 (C15), 46.12 (C17+C18), 39.90 (C14), 28.66 (C7). UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 263 (45 000), 280 (44 000), 319 (41 000). Single crystals of $HL^2 \cdot C_2H_5OH$ suitable for an X-ray diffraction study were obtained by slow evaporation of an ethanolic solution of HL^2 at room temperature.

***N*-(9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-yliden-*N*′-(1-pyridin-2-yl-methylidene)azine (HL^3).** To a refluxing suspension of **C** (0.25 g, 0.73 mmol) in methanol (62 mL) was added 2-pyridinecarbaldehyde (0.08 g, 0.74 mmol). As soon as all starting material had dissolved, the reaction mixture was filtered hot, cooled to room temperature, and allowed to stand at −20 °C overnight. The yellow precipitate formed was removed by filtration, washed with diethyl ether, and dried in vacuo. Yield: 0.21 g, 68%. Anal. Calcd for $C_{22}H_{16}BrN_5 \cdot H_2O$ (M_r = 448.32 g/mol) (%): C, 58.94; H, 4.05; N, 15.62. Found: C, 59.23; H, 4.17; N, 15.62. ^1H NMR (DMSO-d_6, δ): 11.82 (s, 1H, N12), 9.41 (s, 1H, N5), 8.59 (d, 1H, $^3J(H_{C19})$ = 4.7 Hz, C18), 8.40 (d, 1H, $^3J(H_{C20})$ = 8.2 Hz, C21), 8.34 (s, 1H, C15), 7.92 (s, 1H, C8), 7.84 (dd, 1H, $^3J(H_{C19})$ = 7.3 Hz, $^3J(H_{C21})$ = 7.9 Hz, C20), 7.74 (d, 1H, $^3J(H_{C2})$ = 7.9 Hz, C1), 7.54 (d, 1H, $^3J(H_{C3})$ = 7.6 Hz, C4), 7.41 (dd, 1H, $^3J(H_{C2})$ = 7.5−8.5 Hz, $^3J(H_{C4})$ = 8.0 Hz, C3), 7.39 (d, 1H, $^3J(H_{C10})$ = 7.7 Hz, C11), 7.38 (dd, 1H, $^3J(H_{C18})$ = 4.0 Hz, $^3J(H_{C20})$ = 7.5 Hz, C19), 7.28 (dd, 1H, $^3J(H_{C1})$ = 7.9 Hz, $^3J(H_{C3})$ = 7.5−8.5 Hz, C2), 7.27 (d, 1H, $^3J(H_{C11})$ = 8.5 Hz, C10), 3.7 (s, 2H, C7). $^{13}C\{^1H\}$ NMR (DMSO-d_6, δ): 160.31 (C6), 154.77 (C15), 154.25 (C16), 149.77 (C18), 136.88 (C4a), 136.85 (C20), 136.35 (C11a), 134.74 (C12a), 128.73 (C3), 128.63 (C7b), 127.68 (C1), 124.98 (C10), 124.81 (C19), 124.03 (C2), 124.00 (C4), 122.95 (C12b), 121.85 (C21), 120.87 (C8), 113.90 (C11), 112.22 (C9), 109.48 (C7a), 27.66 (C7). UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 268 (15 300), 317 (25 400), 339 (22 200), 462 (760). X-ray diffraction quality single crystals of $HL^3 \cdot 2CH_3OH$ were picked directly from the reaction vessel.

6-*N*-(2-*N*′,*N*′-dimethylaminoethylenamino)-7,12-dihydroindolo-[3,2-*d*][1]-benzazepino-dichlorido-copper(II) [Cu(HL^1)Cl_2] (1). To a solution of HL^1 (0.26 g, 0.81 mmol) in methanol (23.5 mL) under reflux was added $CuCl_2 \cdot 2H_2O$ (0.14 g, 0.84 mmol) in methanol (1.5 mL). The greenish-brown solution formed was refluxed for 5 min, filtered hot, and allowed to cool to room temperature. The next day, the crystals formed were removed by filtration, washed with methanol, and dried in vacuo. Yield: 0.25 g, 69%. Anal. Calcd for $C_{20}H_{22}Cl_2CuN_4$ (M_r = 452.88 g/mol) (%): C, 53.04; H, 4.90; N, 12.37. Found: C, 53.05; H, 4.90; N, 12.28. ESI-MS in methanol (positive): 274 $[HL^1 - N(CH_3)_2]^+$, 319 $[H_2L^1]^+$, 380 $[Cu(L^1)]^+$, 416 $[Cu(HL^1)Cl]^+$, 440 [unidentified], 454 $[Cu(L^1)ClK]^+$, 470 $[Cu(HL^1)Cl(CH_3O)Na]^+$. IR spectrum in KBr, selected bands,

cm^{-1}: 3318, 3213, 3048, 2093, 1628, 1407, 768, 741. UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 263 (26 700), 323 (17 300). Single crystals of **1** suitable for an X-ray diffraction study were picked directly from the reaction vessel.

9-Bromo-6-*N*-(2-*N*′,*N*′-dimethylaminoethylenamino)-7,12-dihydroindolo[3,2-*d*][1]benzazepino-dichlorido-copper(II) [Cu(HL^2)Cl$_2$] (2). To a refluxing solution of HL^2 (0.20 g, 0.51 mmol) in methanol (10 mL) was added a solution of $CuCl_2 \cdot 2H_2O$ (0.09 g, 0.52 mmol) in methanol (1 mL). The dark-green solution formed was refluxed for 5 min, filtered hot, and allowed to cool to room temperature. The next day, the crystals formed were removed by filtration, washed with methanol, and dried in vacuo. Yield: 0.21 g, 78%. Anal. Calcd for $C_{20}H_{21}BrCl_2CuN_4$ (M_r = 531.76 g/mol) (%): C, 45.17; H, 3,98; N, 10.54. Found: C, 44.82; H, 4.04; N, 10.21. ESI-MS in methanol (positive): 352 $[HL^2 - N(CH_3)_2]^+$, 397 $[H_2L^2]^+$, 458 $[Cu(L^2)]^+$, 494 $[Cu(HL^2)Cl]^+$, 518 [unidentified], 532 $[Cu(L^2)ClK]^+$, 548 $[Cu(HL^2)Cl(CH_3O)Na]^+$. IR spectrum in KBr, selected bands, cm^{-1}: 3272, 1627, 1398, 807, 761, 748, 626. UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 274 (31 000), 317 (21 000). Single crystals of **2** suitable for X-ray diffraction study were picked directly from the reaction vessel.

***N*-(9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-yliden)-*N*′-(1-pyridin-2-yl-methylidene)-azin-5-ide-dichlorido-copper(II) [Cu(L^3)Cl$_2$]·1.5CH$_3$OH (3·1.5CH$_3$OH).** To a solution of HL^3 (0.04 g, 0.10 mmol) in methanol (7 mL) under reflux was added $CuCl_2 \cdot 2H_2O$ (0.019 g, 0.11 mmol) in methanol (1 mL). The yellowish-brown solution formed was filtered hot and allowed to cool to room temperature. The next day, the crystals formed were removed by filtration, washed with methanol, and dried in air. Yield: 0.06 g, 95%. Anal. Calcd for $C_{22}H_{16}BrCl_2CuN_5 \cdot 1.5CH_3OH$ (M_r = 612.81 g/mol) (%): C, 46.06; H, 3.62; N, 11.43. Found: C, 46.06; H, 3.33; N, 11.38. ESI-MS in methanol (positive): 430 $[H_2L^3]^+$, 491 $[Cu(L^3)]^+$, 549 $[Cu(L^3)ClNa]^+$, 573 $[(L^3)Cu(CH_3OH)_2(H_2O)]^+$. IR spectrum in KBr, selected bands, cm^{-1}: 3015, 1618, 1546, 1400, 1303, 1021, 758, 738. UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 311 (20 300), 338 (16 000), 463 (9700). Single crystals of $3 \cdot 0.75H_2O$ suitable for an X-ray diffraction study were obtained by recrystallization of $3 \cdot 1.5CH_3OH$ from methanol in air.

***N*-(9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-yliden)-*N*′-(1-pyridin-2-yl-ethylidene)-azin-5-ide-dichlorido-copper(II) [Cu(HL^4)Cl$_2$]·1.5H$_2$O (4·1.5H$_2$O).** To a suspension of HL^4 (0.11 g, 0.25 mmol) in refluxing methanol (19 mL) a solution of $CuCl_2 \cdot 2H_2O$ (0.045 g, 0.26 mmol) in methanol (1 mL) was added. The reaction mixture was refluxed for 10 min, filtered hot and allowed to cool to room temperature. The next day the product formed was removed by filtration, washed with methanol, and dried in vacuo. Yield: 0.13 g, 91%. Anal. Calcd for $C_{23}H_{18}BrCl_2CuN_5 \cdot 1.5H_2O$ (M_r = 605.80 g/mol) (%): C, 45.60; H, 3.49; N, 11.56. Found: C, 45.52; H, 3.30; N, 11.19. ESI-MS in methanol (positive): 505 $[Cu(L^4)]^+$, 587 $[(HL^4)Cu(CH_3O)CH_3OH(H_2O)]^+$. IR spectrum in KBr, selected bands, cm^{-1}: 3343, 3095, 3065, 1594, 1520, 1466, 1183, 770. UV−vis (DMSO), λ_{max} (ε, M^{-1} cm^{-1}): 310 (23 000), 314 (20 000), 454 (12 800).

***N*-(9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-yliden)-*N*′-(1-pyridin-2-yl-ethylidene)-azin-5-ide-acetato-methanolo-copper(II) [Cu(L^4)(CH$_3$COO)(CH$_3$OH)]·3CH$_3$OH (5·3CH$_3$OH).** To a suspension of HL^4 (0.445 g, 1.0 mmol) in refluxing methanol (9 mL) was added a solution of $Cu(CH_3COO)_2 \cdot H_2O$ (0.100 g, 0.5 mmol) in methanol (7 mL). The dark-brown hot solution was filtered and saturated slowly with diethyl ether. After 3 days, the crystals formed were removed by filtration, washed with methanol, and dried in air. Yield: 0.30 g, 84%. Anal. Calcd for $C_{25}H_{20}BrCuN_5O_2$ (M_r = 565.91 g/mol) (%): C, 53.06; H, 3.56; N, 12.38. Found: C, 52.83; H, 3.91; N, 12.76. ESI-MS in methanol (positive): 444 $[H_2L^4]^+$, 505 $[Cu(L^4)]^+$, 527 $[(L^4\text{-}H)CuNa]^+$, 593 $[L^4CuK(CH_3O)H_2O]^+$. IR spectrum in KBr, selected bands, cm^{-1}: 1634, 1491, 1461, 1387, 1152, 898, 762. UV−vis (methanol), λ_{max} (ε, M^{-1} cm^{-1}): 233 (52 600), 261 (29 600), 316 (30 200), 436 (8500). Single crystals of $5 \cdot 3CH_3OH$ suitable for X-ray diffraction study were picked directly from the reaction vessel.

Physical Measurements. ^1H and ^{13}C NMR; nuclear Overhauser effect difference, and two-dimensional $^1H-^1H$ correlation spectroscopy, $^1H-^1H$ total correlation spectroscopy, $^1H-^{13}C$ heteronuclear single quantum coherence, and $^1H-^{13}C$ heteronuclear multiple-bond correlation NMR spectra were recorded on a Bruker Avance III spectrometer (Ultrashield Magnet) in DMSO-d_6 at 25 °C using standard pulse programs at 500.10 (^1H) and 125.76 (^{13}C) MHz and a Bruker Avance DPX 400 spectrometer (HL^2) at 400.13 (^1H) and 100.63 (^{13}C) MHz. ^1H and ^{13}C shifts are quoted relative to the residual solvent signals. Elemental analyses were carried out at the Microanalytical Service of the Institute of Physical Chemistry of the University of Vienna. Electrospray ionization mass spectrometry was carried out using a Bruker Esquire 3000 instrument (Bruker Daltonic, Bremen, Germany) on samples dissolved in methanol. Infrared spectra were measured on a Bruker Vertex 70 FT-IR spectrometer. UV−vis spectra were recorded on a Perkin-Elmer Lambda 650 spectrophotometer, using samples dissolved in DMSO (HL^1-HL^4 and **1**−**4**) and methanol (**5**). The aqueous solution behavior of **1**−**4** with respect to hydrolysis was studied at 25 °C over 24 h by UV−vis spectroscopy in a 1.25% (v/v) DMSO−water mixture for **1** and **2** and a 10% (v/v) DMSO−water mixture for **3** and **4**. Magnetic susceptibility measurements were conducted in solution on a Bruker Avance III spectrometer (Ultrashield Magnet) in DMSO-d_6 at 298 K using the Evans method. The μ_{eff} calculated for a 0.018 M $Cu(acac)_2$ solution in DMSO-d_6 was 1.75 μ_B.

Crystallographic Structure Determination. X-ray diffraction measurements were performed with Nonius Kappa CCD (HL^2) and Bruker X8 APEX II CCD diffractometers at 100 or 120 K (HL^2). Single crystals were positioned at 35, 35, 35, 30, 30, and 40 mm from the detector, and 525, 2619, 1643, 2017, 1374, and 3188 frames were measured, each for 50, 20, 60, 60, 60, and 20 s over 1.5, 1, 1, 1, 1, and 1° scan widths for $HL^2 \cdot C_2H_5OH$, $HL^3 \cdot 2CH_3OH$, **1**, **2**, $3 \cdot 0.75H_2O$, and $5 \cdot 3CH_3OH$, respectively. The data were processed using the Denzo-SMN[61] or SAINT software package.[62] Crystal data, data collection parameters, and structure refinement details for the measured compounds are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the non-hydrogen atoms to which hydrogen atoms were bonded. SHELXS-97[63] was used for structure solution and SHELXL-97[64] for refinement. Molecular diagrams were produced with ORTEP,[65] and appropriate scattering factors were used.[66]

Cell Lines and Culture Conditions. CH1 (ovarian carcinoma, human) cells were generously provided by Lloyd R. Kelland

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Table 1. Crystal Data and Details of Data Collection for $HL^2 \cdot C_2H_5OH$, $HL^3 \cdot 2CH_3OH$, **1**, **2**, **3**·0.75H_2O and **5**·3CH_3OH

compound	$HL^2 \cdot C_2H_5OH$	$HL^3 \cdot 2CH_3OH$	**1**	**2**	**3**·0.75H_2O	**5**·3CH_3OH
empirical formula	$C_{22}H_{27}BrN_4O$	$C_{24}H_{24}BrN_5O_2$	$C_{20}H_{22}Cl_2CuN_4$	$C_{20}H_{21}BrCl_2CuN_4$	$C_{22}H_{17.5}BrCl_2CuN_5O_{0.75}$	$C_{29}H_{36}$ $BrCuN_5O_6$
fw	443.39	486.38	452.86	531.76	578.26	694.08
space group	$P2_1/c$	$P2_1/c$	$P2_1/n$	$P\bar{1}$	$P2_1/c$	$P\bar{1}$
a, Å	10.978(2)	12.3094(5)	15.1349(9)	10.1511(5)	12.7662(8)	9.2218(3)
b, Å	9.193(2)	11.1450(4)	7.6083(5)	10.8098(6)	13.5586(8)	12.6553(5)
c, Å	21.116(4)	17.0199(7)	17.3917(11)	11.7944(9)	13.1869(8)	13.7218(6)
α,deg				113.613(4)		107.719(3)
β, deg	94.93(3)	97.995(2)	101.579(4)	93.568(4)	105.691(4)	90.188(2)
γ,deg				115.547(3)		98.000(2)
V, Å^3	2123.2(7)	2312.24(16)	1508.81(10)	1025.78(11)	2197.5(2)	1508.81(10)
Z	4	4	4	2	4	2
λ, Å	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
ρ_{calcd}, g cm^{-3}	1.387	1.420	1.533	1.722	1.748	1.528
cryst size, mm	0.57 × 0.29 × 0.23	0.20 × 0.18 × 0.10	0.16 × 0.11 × 0.02	0.15 × 0.08 × 0.03	0.10 × 0.10 × 0.07	0.40 × 0.15 × 0.08
T, K	120	120	100	100	100	100
μ, cm^{-1}	19.56	18.08	13.98	32.87	30.80	20.98
R1[a]	0.0308	0.0650	0.0423	0.0337	0.0396	0.0355
wR2[b]	0.0792	0.1932	0.0984	0.0800	0.0927	0.1008
GOF[c]	1.021	1.060	1.005	1.019	1.007	1.013

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $wR2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$. [c] $GOF = \{\sum[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

(CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). SW480 (colon carcinoma, human) and A549 (non-small cell lung cancer, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). Cells were grown without antibiotics in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass) as adherent monolayer cultures in Minimal Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, and 2 mM L-glutamine (all purchased from Sigma-Aldrich). Human A2780 and A2780cisR cells were obtained from the European Collection of Cell Cultures (ECACC, Porton Down, Salisbury, U. K.), and cell culture reagents were obtained from Gibco-BRL (Basel, Switzerland). These cells were grown in an RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and antibiotics. All cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cytotoxicity Determination. Cytotoxicity was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma-Aldrich). For this purpose, cells were harvested from culture flasks by trypsinization and seeded in 100 μL aliquots in the appropriate medium into 96-well microculture plates. The following cell densities were chosen to ensure exponential growth of untreated controls throughout the experiment: 1.5×10^3 (CH1), 2.5×10^3 (SW480), 4.0×10^3 (A549), and 2.0×10^4 (A2780, A2780cisR) viable cells per well. For 24 h, cells were allowed to settle and resume exponential growth, followed by the addition of dilutions of the test compounds in aliquots of 100 μL/well in the same medium. For this purpose, the compounds (with the exception of copper chloride) were predissolved in DMSO and then diluted with medium to a maximum DMSO content of 0.5% v/v. After continuous exposure for 96 or 72 h, the medium was replaced by 100 μL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μL/well MTT solution in phosphate-buffered saline (5 mg/mL) (all purchased from Sigma-Aldrich). After incubation for 4 h, medium/MTT mixtures were removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed as a percentage of the untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by interpolation. The evaluation is based on at least three independent experiments, each comprising three replicates per concentration level.

Results and Discussion

The bidentate ligands HL^1 and HL^2 were prepared in good yield by reacting the thiones **A** and **B** (see Scheme 1) with N, N-dimethylethylenediamine in dry THF under reflux for 24 and 45 h, respectively. The Paullone−hydrazine **C** was obtained in 85% yield by the condensation of hydrazine hydrate with thione **B** in dry ethanol at reflux (Scheme 1).[20] Further reaction of **C** with 2-pyridinecarbaldehyde or 2-acetylpyridine, respectively, affords the tridentate ligands HL^3 and HL^4.[60] ^{1}H and ^{13}C NMR spectra of HL^3 and HL^4 in DMSO-d_6 revealed that both ligands adopt a configuration with an exocyclic $C^6{=}N^{13}$ bond (confirmed by the chemical shifts of C^6 and the presence of a proton at N^5), in contrast to HL^1 and HL^2 with an endocyclic $C^6{=}N^5$ bond (see the Experimental Section).[59]

Complexes **1**−**4** were obtained in good to excellent yield (68−95%), as shown in Scheme 1, by reacting the corresponding ligand with $CuCl_2 \cdot 2H_2O$ in refluxing methanol. Complex **5** was obtained in 84% yield from the reaction of HL^4 with $Cu(CH_3COO)_2 \cdot H_2O$ in methanol under reflux. Magnetic susceptibility measurements were carried out in DMSO at 298 K for complexes **2** and **4** using the Evans method.[67,68] The calculated effective magnetic moments of 1.75 and 1.74 μ_B, respectively, are in accord with the d^9 electronic configuration of copper(II) with $S = 1/2$.

The formation of the complexes was confirmed by electrospray ionization mass spectra (ESI-MS, positive ion mode) in methanol, in which peaks that may be assigned to the ions $[Cu(L^{1-4})]^+$ with m/z values of 380 (**1**), 458 (**2**), 491 (**3**), and 505 (**4** and **5**) were observed. The formation of the $[H_2L^{1-4}]^+$ ion was observed for all complexes, with the exception of **4**. The presence of intense peaks assigned to $[H_2L^{1-4}]^+$ ions for **1** and **2** indicates their lower stability compared to complexes **3** and **4** toward dissociation. In the spectra of **4**, the peak

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attributed to the ion $[CuL^4]^+$ has the highest relative intensity.

Crystal Structures. The molecular structure of HL^2 established by single-crystal X-ray diffraction is shown in Figure 1, and selected bond lengths and bond angles are given in the caption.

Complexes **1** and **2** (Figure 2) crystallized in the monoclinic space group $P2_1/n$ and triclinic centrosymmetric space group $P\bar{1}$, respectively.

The copper(II) center in **1** and **2** is four-coordinate, and as expected, the ligands HL^1 and HL^2 are bound to the metal via two nitrogen atoms N3 and N4 of the ethylenediamine moiety. The remaining two binding sites are occupied by two chlorido ligands. The Cu−N and Cu−Cl distances are typical of those of other ethylenediamine derivatives of copper(II) chloride (e.g., [Cu(ethylenediamine)Cl_2][69] Cu−N, 2.010(5) and 2.017(5) Å; Cu−Cl, 2.286(2) and 2.301(2) Å; [Cu(*trans-N, N, N′,N′*-tetramethylcyclohexane-1,2-diamine)Cl_2][70] Cu−N, 2.052(7); Cu−Cl, 2.247(2) Å; [Cu(*N*-4-hydroxybenzylmethyl-*N, N′,N′*-trimethylethylenediamine)Cl_2][71] Cu−N, 2.086(3) and 2.045(3) Å; Cu−Cl, 2.263(1) and 2.269(1) Å). The coordination geometry can be described as a distorted tetrahedron. The distortion from the square-planar geometry in **1** and **2** may be estimated by the dihedral angle between the planes through CuN3N4 and CuCl1Cl2, which deviates tremendously from 0° (55.8 and 41.7° for **1** and **2**, respectively).

The conformation of the coordinated ligand HL^2 is very close to that adopted by the metal-free ligand (Figure 1). This is evident when comparing the torsion angle $\Theta_{N1-C5-C6-C7}$ in metal-free HL^2 at −36.84(18) and in coordinated HL^2 in **2** at −35.3(4)°. The ligand backbone mainly consists of sp^2-hybridized carbon and nitrogen atoms. The only exception is the methylene group carbon atom in the seven-membered azepine ring, which is sp^3-hybridized. This atom disrupts the conjugation of the whole π system, and as a result the ligand as a whole is non-planar. Binding to copper(II) is accompanied by a change of the torsion angle $\Theta_{N3-C13-C14-N4}$ from −48.20(15) to −32.4(3)° and migration of the proton from N3 to N2 (Figures 1 and 2). Thereby the distribution of the electron density over the fragment N2−C12−N3 in **2** with an exocyclic C=N bond is opposite to that in the metal-free ligand HL^2 with an endocyclic C=N bond.

The crystal structure of **2** consists of molecules that are involved in two intermolecular hydrogen-bonding interactions, namely, N1−H···Cl1^{i} [N1−H, 0.88; H···Cl1^{i}, 2.371; N1···Cl1^{i}, 3.234 Å; N1−H···Cl1^{i}, 166.97°] and N2−H···Cl^{ii} [N2−H, 0.88; H···Cl1^{ii}, 2.625; N2···Cl1^{ii}, 3.416 Å; N2−H···Cl1^{ii}, 150.06°], (Figure S1, Supporting Information).

The molecular structures of HL^3, **3**, and **5** are shown in Figures 3−5, respectively. Selected bond distances (Å) and bond angles (deg) are given in the legends to the figures. The tridentate ligand HL^3 crystallized in the monoclinic space group $P2_1/c$ with two molecules of methanol. Intermolecular H-bonding interactions of the type O−H···N are evident between co-crystallized methanol molecules and HL^3, namely, O2(x, $y + 3/2$, $z + 1/2$)···H−N12 (O2···H, 1.920; O6···N12, 2.780; H−N12, 0.88 Å; O6···H−N12, 165.24°), O2−H···N13 (O2−H, 0.84; O2−N13, 2.734; N13···H, 1.911 Å; O2−H···N13, 166.00°), O1···H−N5 (O1···H, 2.144; O1···N5, 3.010; H−N12, 0.88 Å; O1···H−N12, 168.17°), O1−H···N14 (O1−H, 0.84; O1···N14, 3.026; H···N14, 2.497 Å; O1−H···N14, 121.81°), O1−H···N17 (O1−H, 0.84; O1···N17, 2.904; H···N17, 2.101 Å; O1−H···N17, 159.92°). The distribution of electron density over the fragment N5−C6−N13 [C6−N5, 1.358(5); C6−N13, 1.309(5) Å] indicates the presence of an exocyclic double bond at the amidrazone moiety, which is in accordance with ^{1}H and ^{13}C NMR data for HL^3.

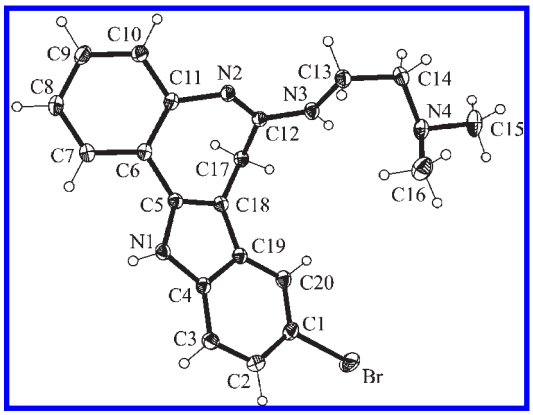

Figure 1. ORTEP plot of HL^2 with thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): C11−N2, 1.4141(16); C12−N2, 1.3029(16); C12−N3, 1.3460(16); C12−C17, 1.5132(17); C17−C18, 1.4954(17); C5−C18, 1.3750(17); C5−C6, 1.4592(17); C6−C11, 1.4221(18) Å; $\Theta_{N3-C13-C14-N4}$, −48.20(15)°.

Complex **3** crystallized in the monoclinic space group $P2_1/c$ with 0.75 molecules of co-crystallized water. As expected, HL^3 acts as a neutral tridentate ligand in **3**. Copper(II) is coordinated via the azepine ring nitrogen atom N5, the hydrazine group nitrogen atom N14, and the pyridine ring nitrogen atom N17.

Complex **5** crystallized in the triclinic centrosymmetric space group $P\bar{1}$ with three molecules of methanol in the asymmetric unit. Strong intermolecular H-bonding interactions of the type O−H···O between co-crystallized methanol molecules [O6···O5($x - 1$, y, z), 2.772; O4···O5($-x + 2$, $-y + 2$, $-z + 1$), 2.892 Å], as well as O−H···N and O−H···O between co-crystallized methanol molecules and molecules of **5**, namely, O6···H−N12 (O6···H, 1.913; O6···N12, 2.760; H−N12, 0.88 Å; O6···H−N12, 160.89°), O5−H···O2($x + 1$, y, z) with O5−H, 0.84; H···O2, 1.844; O5···O2, 2.651 Å; and O5−H···O2, 160.53° are evident in the crystal structure of **5**. Of note is also the H−bond between the molecules of **5** of the type O3−H···N13($-x + 1$, $-y + 1$, $-z + 1$) with O3−H, 0.833; H···N13, 1.970; O3···N13, 2.795 Å; and O3−H···N13, 170.16° (Figure S2, Supporting Information).

As expected, HL^4 acts as a neutral tridentate ligand in **4** and as a monodeprotonated ligand in **5**, coordinating to copper(II) via the azepine ring nitrogen atom N5, the

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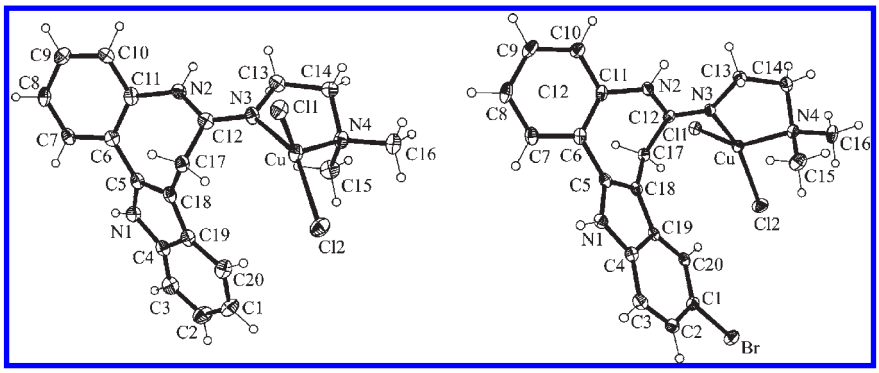

Figure 2. ORTEP plots for $[Cu(HL^1)Cl_2]$ (**1**) (left) and $[Cu(HL^2)Cl_2]$ (**2**) (right) with thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg) for **1**: Cu−Cl1, 2.2715(9); Cu−Cl2, 2.1965(10); Cu−N3, 1.948(3); Cu−N4, 2.021(3); C12−N2, 1.347(4); C12−N3, 1.301(4) Å; Cl1−Cu−N3, 96.70(9); Cl1−Cu−N4, 132.32(9); Cl2−Cu−N3, 148.97(9); Cl2−Cu−N4, 103.92(9); N3−Cu−N4, 82.62(11)°; $\Theta_{N1-C5-C6-C7}$, 33.08(5); $\Theta_{N3-C13-C14-N4}$, 33.8(4). For **2**: Cu−Cl1, 2.2736(7); Cu−Cl2, 2.2085(7); Cu−N3, 1.975(2); Cu−N4, 2.034(2); C12−N2, 1.359(3); C12−N3, 1.302(3) Å; Cl1−Cu−N3, 96.96(6); Cl1−Cu−N4, 147.45(6); Cl2−Cu−N3, 151.99(6); Cl2−Cu−N4, 96.72(6); N3−Cu−N4, 81.77(9) °; $\Theta_{N1-C5-C6-C7}$, −35.3(4); $\Theta_{N3-C13-C14-N4}$, −32.4(3)°.

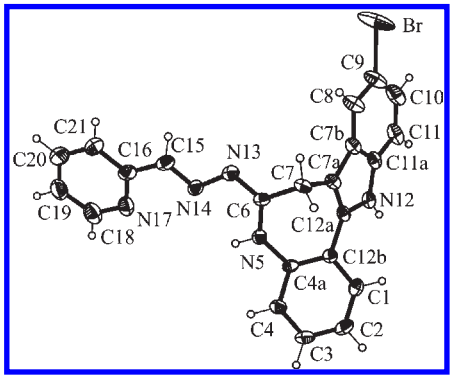

Figure 3. ORTEP plot of HL^3 with thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): C4a−N5, 1.410(5); C6−N5, 1.358(5); C6−N13, 1.309(5); N13−N14, 1.399(5); N14−C15, 1.274(6) Å; $\Theta_{N5-C6-N13-N14}$, −1.5(5); $\Theta_{C4a-N5-C6-C7}$, −2.6(7); $\Theta_{N12-C12a-C12b-C1}$, 34.0(6)°.

hydrazine group nitrogen atom N14, and the pyridine ring nitrogen atom N17. The copper(II) center is five-coordinate, the remaining two binding sites being occupied by two chloride ligands in **3** or by two different ligands, an acetate ion (atom O1) and a methanol (atom O3) in **5**.

The τ descriptor for five-coordinate complexes, expressed as the difference between the angles N5−Cu−N17, 155.53(10)°, and N14−Cu−Cl1, 149.67(8)°, divided by 60°, gives a value of 0.10 for **3**, which is very close to the ideal one for a square pyramid (0).[72] Note that this value in the five-coordinate gallium(III) complex $[Ga(L^4)Cl_2]$[60] is between that for a trigonal bipyramid (1) and that for a square pyramid (0) at 0.52. The angles between the mean plane of the indole moiety and the benzene ring annelated to the seven-membered azepine ring in **3** and **5** are 39.6 and 36.7°, respectively. These values are close to those in the metal-free ligands HL^3 (35.0°) and HL^4 (34.0°)[60] and in $Ga(L^4)Cl_2$ (33.6°).[60] In contrast, the angle between the indole and the pyridine ring is 78.6° in **3** and 83.6° in **5**, differing by ca. 54 and 49°, respectively, from that in the metal-free ligand HL^4 (132.56°) and by ca. 38 and 33°, respectively, from that

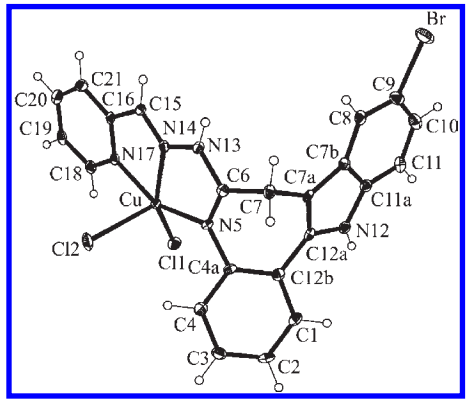

Figure 4. ORTEP plot of $[Cu(HL^3)Cl_2]$ (**3**) with thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): Cu−N5, 2.038(3); Cu−N14, 1.982(3); Cu−N17, 2.062(3); Cu−Cl1, 2.2411(8); Cu−Cl2, 2.4306(8); C6−N5, 1.296(4); C6−N13, 1.366(4); N13−N14, 1.353(3); N5−Cu−N17, 155.53(10); N14−Cu−Cl2, 103.39(7); N14−Cu−Cl1, 149.67(8); Cl1−Cu−Cl2, 106.63(3); N5−Cu−Cl2, 100.75(7); N5−Cu−Cl1, 100.01(7); N14−Cu−N17, 77.75(10); N17−Cu−Cl2, 89.52(7); N17−Cu−Cl1, 98.18(7).

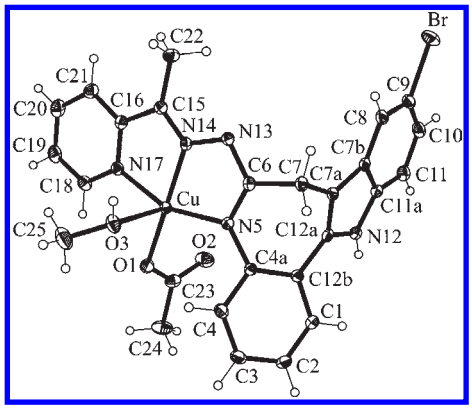

Figure 5. ORTEP plot of $[Cu(L^4)(CH_3COO)(CH_3OH)]$ (**5**) with thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): Cu−N5, 1.9994(17); Cu−N14, 1.9482(18); Cu−N17, 2.0363(18); Cu−O1, 1.9392(15); Cu−O3, 2.2794(15); C6−N5, 1.324(3); C6−N13, 1.351(3); N13−N14, 1.372(2); N5−Cu−N17, 158.21(7); N14−Cu−O1, 173.26(7); N14−Cu−O3, 96.86(6); O1−Cu−O3, 88.73(6); N5−Cu−O1, 103.89(7); N5−Cu−O3, 96.38(6); N14−Cu−N17, 79.92(7); N17−Cu−O1, 96.12(7); N17−Cu−O3, 92.55(6).

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Table 2. Cytotoxicity of Paullone-Based Ligands, Their Copper(II) Complexes and $CuCl_2$ in Human Cancer Cell Lines

	IC_{50} (μM), 96 h[a]			IC_{50} (μM), 72 h[a]	
compound	A549	SW480	CH1	A2780	A2780cisR
HL^1	44 ± 1	25 ± 1	21 ± 3		
HL^2	8.2 ± 1.0	4.5 ± 0.4	3.0 ± 0.4		
1	39 ± 5	12 ± 2	3.2 ± 0.2	3.6 ± 0.4	10 ± 1
2	9.9 ± 1.3	4.0 ± 0.4	1.6 ± 0.04	4.0 ± 0.2	5.2 ± 0.5
3·$1.5CH_3OH$	1.2 ± 0.2	0.33 ± 0.06	0.34 ± 0.06		
4·$1.5H_2O$	0.35 ± 0.04	0.12 ± 0.004	0.050 ± 0.003	0.19 ± 0.04	0.49 ± 0.05
$CuCl_2 \cdot 2H_2O$	153 ± 8	> 160	43 ± 3		

[a] 50% inhibitory concentrations (means ± standard deviations from at least three independent experiments), as obtained by the MTT assay using the indicated exposure times.

in metal-free ligand HL^3 (116.39°). In contrast to HL^4, the bond lengths C6−N5 [1.324(3) Å] and C6−N13 [1.351(3) Å] differ from one another to a lesser extent and indicate a delocalized π system, which also extends over the N13−N14 [1.372(2) Å] bond in **5**. The distribution of electron density over the fragment N5−C6−N13 [C6−N5, 1.3621(16); C6−N13, 1.3088(16) Å] indicates the presence of an exocyclic double bond at the amidrazone moiety. The lack of a proton at N13 is corroborated by the role of the amidrazone moiety as a proton acceptor from the coordinated methanol of the neighboring molecule with formation of the hydrogen bond N13···H−O3 in **5**. The τ descriptor for **5**, expressed as the difference between the angles N14−Cu−O1, 173.26°, and N5−Cu−N17, 158.21°, divided by 60°, gives a value of 0.25, which is close to the ideal value for a square pyramid (0).[72]

Stability Studies. The kinetic stability of the complexes in aqueous solution with a DMSO content of 1.25% v/v (**1** and **2**) or 10% v/v (**3** and **4**) was studied by UV−vis spectroscopy over 24 h. For **1** and **2**, the resulting spectra were compared with those of the ligands HL^1 and HL^2 (Figures S3 and S4, Supporting Information). A weak charge-transfer band at 397 nm (Figures S5 and S6, Supporting Information) with a molar extinction coefficient of ca. 570 M^{-1} cm^{-1}, resulting from the coordination of an ethylenediamine moiety to the copper(II) ion, seems to be responsible for the yellow color of the methanol solutions of **1** and **2**. As the spectra of **1** and **2** are very similar to those of the corresponding ligands, no clear statement can be made whether **1** and **2** dissociate or hydrolyze during the measurements in an aqueous DMSO solution. However, ESI mass spectra of **1** and **2** in a methanolic solution with 10% water measured 24 h after dissolution showed the presence of peaks with m/z values of 319 and 397, attributable to $[H_2L^1]^+$ and $[H_2L^2]^+$ correspondingly, indicating their dissociation or hydrolysis. In contrast, coordination of ligands HL^3 and HL^4 to copper(II) via the pyridine nitrogen atom, the hydrazine nitrogen and the azepinone ring nitrogen atom alters significantly the conjugated π systems of the yellow-colored ligands HL^3 and HL^4, resulting in significantly different electronic absorption spectra. As a result, solutions of **3** and **4** show significant absorbances in the visible range, namely, a broad charge-transfer band at 430 and 420 nm, respectively (Figure S7, Supporting Information). These bands are blue-shifted by 29 and 27 nm for **3** and **4**, respectively, when DMSO as a solvent is replaced by water with a content of 10% DMSO (v/v). A small decrease in absorption (ca. 4%) was observed for **3** and **4** over the first 4 h, with no further changes over the next 20 h, indicating that the coordination sphere of copper(II) remained intact.

Biological Studies. The cytotoxicity of **1**, HL^1, **2**, HL^2, **3**·$1.5CH_3OH$, and **4**·$1.5H_2O$ was assessed by means of a colorimetric microculture assay (MTT assay) in three human cancer cell lines (A549, SW480, and CH1). Additionally, complexes **1**, **2**, and **4**·$1.5H_2O$ were tested in an isogenic pair of cell lines, one being sensitive to cisplatin (A2780) and the other having acquired cisplatin resistance (A2780cisR). The compounds showed the strongest effects on cell viability in the more chemosensitive ovarian carcinoma cell line CH1, whereas the more chemoresistant non-small cell lung cancer cell line A549 is the least sensitive for this series of compounds. The IC_{50} values are listed in Table 2, and the concentration−effect curves in three cell lines are depicted in Figures 6 and 7, from which certain structure−activity relationships can be discerned (see below).

The Paullone-derived ligands HL^1 and HL^2 show notable cytotoxicities with IC_{50} values in the 10^{-6} or 10^{-5} M range. Substitution by bromine at position 9 of the Paullone scaffold has a favorable effect on cytotoxicity, as reflected by the significantly lower IC_{50} values of the HL^2 ligand. Previously, this substitution was reported to increase CDK-inhibitory potency, but apparently without being reflected in an enhanced cytotoxicity.[14] Complexation of the Paullones HL^1 and HL^2 with copper(II) results in only a modest increase in cytotoxicity. A clear-cut advantage of complexation could only be observed in the case of the HL^1 ligand, with a significant improvement in cytotoxicity in CH1 cells. Nevertheless, activity is maintained in the complexes, and other advantages relevant for in vivo applications arise from their higher solubility and altered pharmacokinetic behavior. Comparison of the complexes **1** and **2** shows that bromination of the ligand at C9 results in a higher cytotoxicity, depending on the cell line (with the exception of A2780), roughly paralleling the effects in the uncomplexed Paullone derivatives (Figure 6).

While the compounds mentioned above show IC_{50} values in micromolar concentrations, complexes **3** and **4** exhibit cytotoxicities mainly in the nanomolar range. Thus, the structural modifications of the chelating side chain result in 4.7−12 and 11−33 times higher cytotoxicity, respectively, compared to that of complex **2**. As the cytotoxicities of HL^3 and HL^4 could not be determined because of insufficient solubility, it cannot be assessed

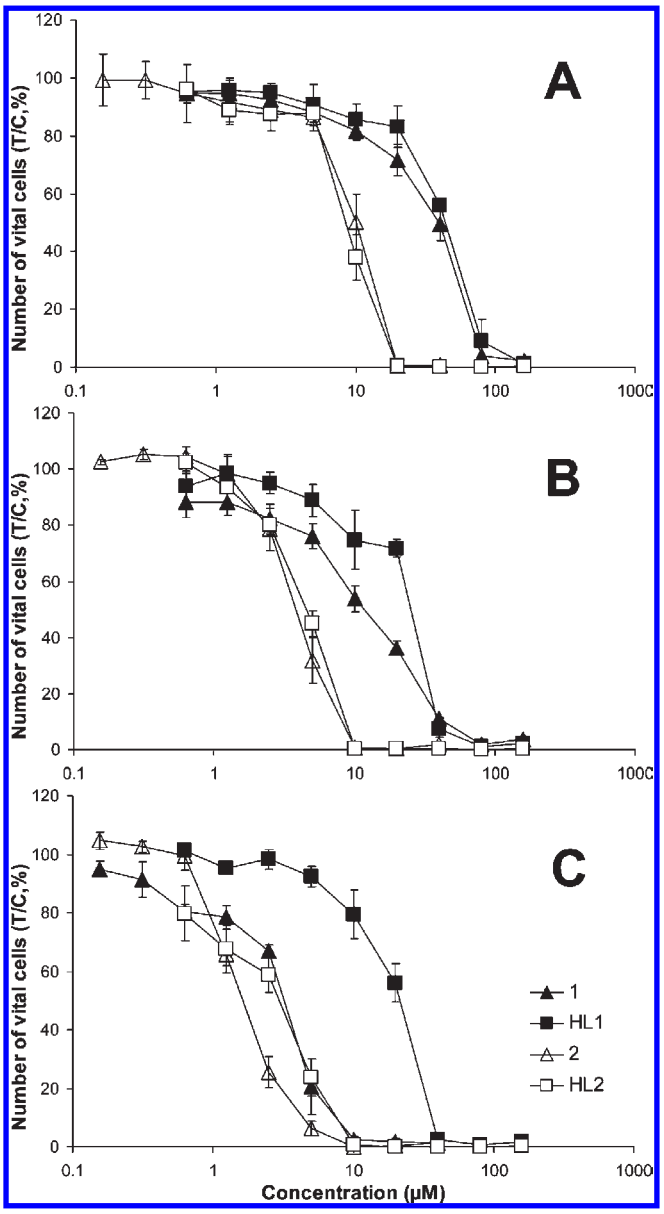

Figure 6. Concentration-effect curves of complexes **1** and **2** in comparison with the corresponding uncomplexed Paullone derivatives HL^1 and HL^2 in the human cancer cell lines A549 (A), SW480 (B), and CH1 (C), obtained by the MTT assay following continuous exposure for 96 h.

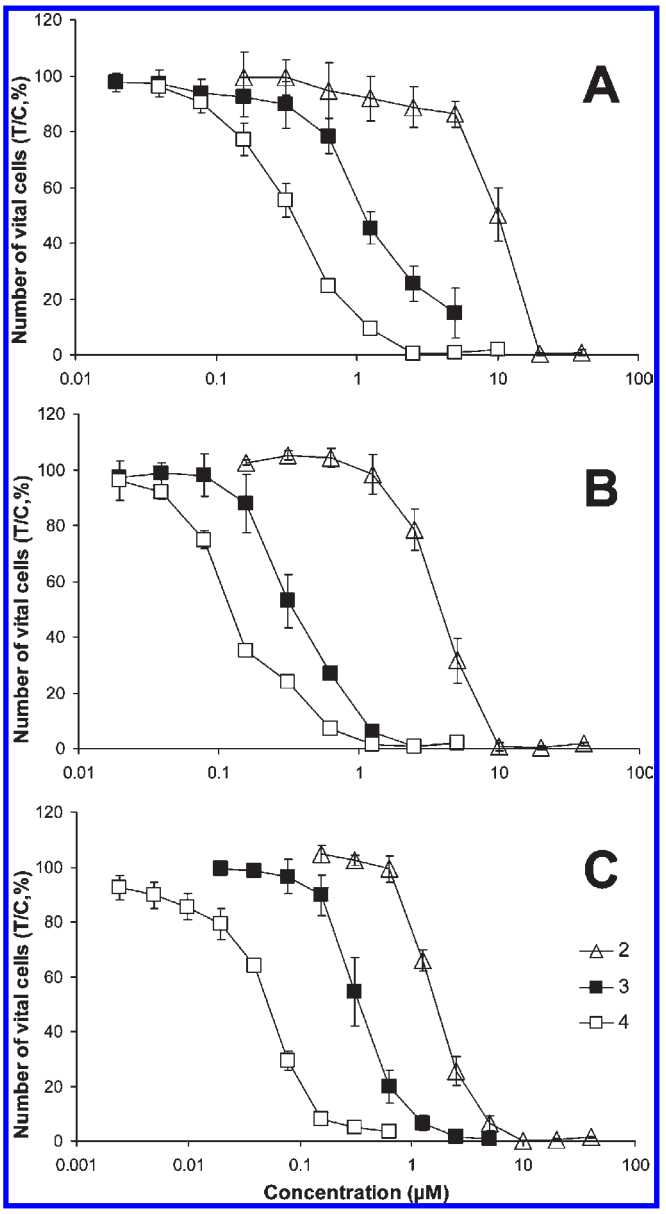

Figure 7. Concentration-effect curves of complexes **2**, **3**, and **4** in the human cancer cell lines A549 (A), SW480 (B), and CH1 (C), obtained by the MTT assay following continuous exposure for 96 h.

whether the modification of the side chain itself or the consequences for binding to copper, that is, a tridentate coordination involving N5 of the azepine ring, accounts for the increase in activity. Thus, it remains unclear whether the effects of complexes **3** and **4** are governed by those of the ligands HL^3 and HL^4 to the same extent as the effects of **2** are by those of HL^2 or whether coordination to copper adds a more substantial component to overall activity in these cases. However, it is not unreasonable to assume that HL^1, HL^2, **1**, and **2** exhibit similar cytotoxicities because the copper ion is released from the bidentate ligand, whereas in **3** and **4** stronger coordination leads to an enhancement in activity. It is worth noting that the IC_{50} values of $CuCl_2$ range between 43 and >160 μM and are thus 1 order of magnitude higher than those of the least cytotoxic complex (Table 2). If copper ions were partially released from the complexes at any stage of exposure, they are unlikely to contribute appreciably to the overall effects.

At least for the copper(II) complexes, the methyl substitution in position 15 of the Paullone ligand is favorable in terms of biological activity, as indicated by the lower IC_{50} values of complex **4** compared to those of **3**, yielding the most cytotoxic compound within the series (Figure 7).

The acquired cisplatin resistance of the ovarian cancer cell line A2780cisR affects sensitivity to complexes **1**, **2**, and **4** to only a moderate extent, as indicated by slightly higher IC_{50} values than in the parent A2780 cell line (Table 2). As resistance in A2780cisR cells does not exclusively pertain to DNA cross-linking agents and can only partially be attributed to increased DNA repair capacity,[73] DNA-damaging effects cannot be

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inferred unequivocally from these data but should be considered in future mechanistic studies of the copper(II) complexes.

Final Remarks. Complexation of copper(II) salts with bidentate and tridentate Paullone-based ligands $HL^{1,2}$ and $HL^{3,4}$ modified at the lactone function resulted in four-coordinate (**1** and **2**) and five-coordinate (**3**−**5**) copper(II) complexes, which show markedly different, but remarkably high, antiproliferative activities in human cancer cell lines with IC_{50} values in the micromolar (**1**, **2**) or the nanomolar (**3**, **4**) concentration range. The high cytotoxicity of the metal-free Paullones $HL^{1,2}$ is at least preserved upon binding to copper(II) or even enhanced, as in the case of HL^1 and **1** in CH1 cells. It should also be stressed that the biological activity of metal-free Paullones $HL^{3,4}$ could not be assayed because of their very low solubility in biocompatible media. Coordination to copper(II) improved the solubility of the resulted species, enabling them to be used as anticancer agents. It is highly probable that for **3** and **4** the copper(II) center remains attached to the Paullone ligands until entry into the cells, although it remains to be clarified whether the metal ion is released once inside the cell. Further experiments are in progress to delineate the mode of action of these compounds. The fact that **3** and **4** are highly cytotoxic, at nanomolar concentrations, could allow their application at very low doses, which could offer a significant advantage over platinum-based chemotherapies.

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Supporting Information Available: Additional figures and a crystallographic information file. This material is available free of charge via the Internet at http://pubs.acs.org.