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Cytotoxicity of cationic NHC copper(I) complexes coordinated to 2,2'-bis-pyridyl ligands

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ABSTRACT

Cytotoxicity of cationic (NHC)Cu(I) complexes bearing 2,2'-dipyridylamine (dpa) type ligands has been evaluated toward 4 cancer cell lines, and compared to the one of neutral (NHC)Cu(I) complexes. The high cytotoxicity of these novel cationic (NHC)Cu(I) complexes, combined with the straightforward synthesis, and versatility of dpa type ligands may offer new prospects in

cancer research, toward the development of novel carrier linked prodrugs.

INTRODUCTION

Cancer therapy research was strongly impacted by the discovery of the cytotoxicity of cis-platin against different cancer cell lines,[1] and the development of its congeners.[2, 3] Transition metal complexes have been then extensively studied for anticancer applications.[4-9] An important attention was focused on gold complexes which were considered as alternative to cis-platin because of their different biological mechanisms. Indeed, many research groups have demonstrated that the cytotoxicity of gold(I) and gold(III) complexes[10-12] is mainly driven by the metal center which binds cysteine or selenocysteine protein fragments, inhibits thioredoxin reductase (TrxR) or leads to mitochondria swelling.[13-18] These effects on cancer cells would allow to overcome some cell-line resistance to cis-platin. The NHC complexes based on isoelectronic less expensive and more abundant transition metals like silver(I) and copper(I) have been also investigated because they might behave similarly from an organometallic point of view.[19-24] Indeed, gold(I) and copper(I) complexes present similar affinities toward selenium and could be considered to have similar biological mechanism.[25] However, regarding the biological mechanisms proposed in the literature for different organometallics congeners of the column 11, they seem to act differently and the simple previous assumption appears to be an incorrect hypothesis. Indeed, as example, the group of Hecht has reported that the cleavage of DNA by bleomycin depends on the presence of copper(I) as metal cofactor.[26] Gautier *et al.* assumed that NHC copper complexes could generate reactive oxygen species (ROS) leading to a DNA strand break, responsible of the observed cytotoxicity.[27] Consequently, the development of new cytotoxic transition metal complexes and more specifically Earth-abundant metal based complexes, might increase the panel of anticancer agents and furnish more drugs to overcome some cancer cell line resistance.

In the literature, several copper(I) complexes have been reported to exhibit IC₅₀ micromolar (μM) range against different cancer cell lines. These copper(I) complexes are generally coordinated to phosphane and/or nitrogen ligands.[28-33] Unlike its group 11 congeners, (NHC) copper(I) complexes are scarcely reported and are based on two families of general formula $[\text{CuX}(\text{NHC})]$ and $[\text{Cu}(\text{NHC})_2][\text{X}]$. [19-24] Gautier *et al.* have firstly selected the $[\text{CuX}(\text{NHC})]$ complexes and compared their activities on different

cancer cell lines such as MCF-7, MCF-7R, LNCaP, HL60 and KB. They demonstrated that the copper complexes stopped the cell cycle progression at the G1 phase.[27] Then, the group of Tacke has synthesized copper complexes bearing benzyl-substituted NHC ligands and studied their cytotoxicity against CAKI-1.[34-36] *In-vivo* studies on nude mice with these aforementioned complexes showed encouraging results as strong inhibition growth of the tumor was noticed.[35, 36]

We have recently reported a series of cationic (NHC)copper(I) complexes bearing 2,2'-dipyridylamine ligands (dpa) and exhibiting highly blue emissive properties.[37-39] We anticipated that such complexes would be of interest as cytotoxic agents. Indeed, their straightforward synthesis[40] allows rapid modulation of the electronic and steric properties of the dpa ligands. Moreover, the functionalization *via* alkylation of the central nitrogen atom of the dpa ligand may open a route to their potential vectorization.[41-43] Adding a recognized biological framework to cytotoxic complexes would also allow their preferential accumulation in the targeted cells, and consequently, reduce the quantity of prodrug absorbed by the patient.[44] Before going further with the development of such sophisticated ligands, the influence of different functionalized ligands (NHC and dpa) and anions on the cytotoxicity against cancer cells had to be evaluated. This study aimed to demonstrate (i) the influence of the substitution on dpa ligand or the nature of the anion on the anticancer activity and (ii) whether a well-functionalized dpa moiety could open a route to vectorization.[43] Moreover, the emissive properties of our cationic copper(I) complexes may also be interesting as potential fluorescent probes to detect the copper accumulation in the cell as their photophysical design is now understood.[45, 46]

Herein, we present a comparative study of the cytotoxicity of various cationic (NHC) (dpa) copper(I) complexes and neutral (NHC) copper complexes having different anionic ligands such as chloride, iodide, hydroxide and cyanide (Fig. 1).

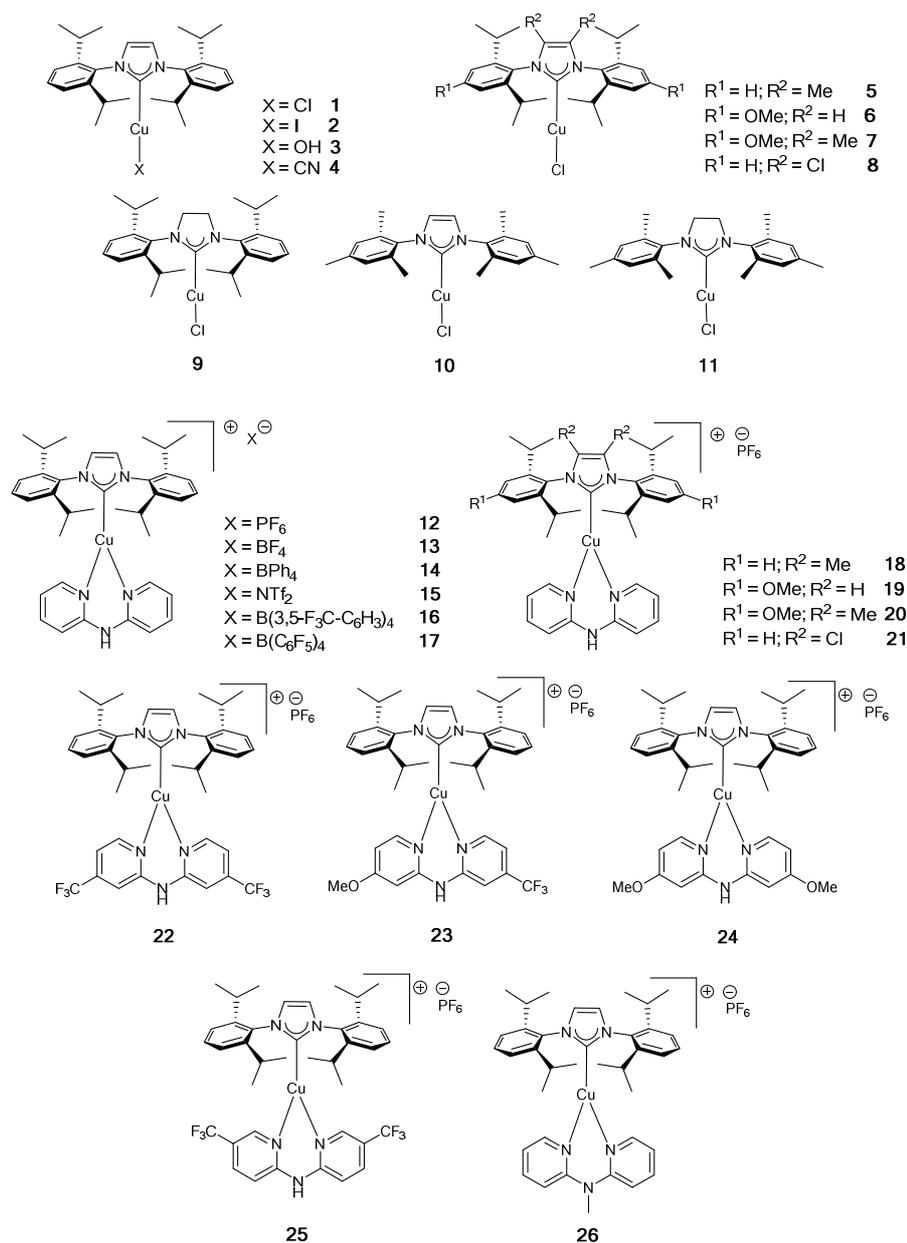


Figure 1. NHC copper complexes used in this study

MATERIAL AND METHODS

Synthesis of the copper(I) complexes.

General Considerations. All reactions were carried out using standard Schlenk technique under an atmosphere of dry argon. Solvents were purchased from Carlo Erba and degassed prior to use by bubbling argon gas directly in the solvent. Solvents for NMR spectroscopy were dried over molecular sieves. NMR spectra were recorded on 400 MHz and 500 MHz Bruker spectrometers. Proton (^1H) NMR information is given in the following format: multiplicity, coupling constant(s) (J) in Hertz (Hz),

number of protons. Carbon (^{13}C) NMR spectra are reported in ppm (δ) relative to residual CHCl_3 (δ 77.0) unless noted otherwise. HRMS were performed by LCMT analytical services. NMR solvent was passed through a pad of basic alumina before uses. 2,2'-dipyridylamine (**dpa**) was purchased from Sigma-Aldrich and used without prior purification. The complexes $[\text{CuCl}(\text{SIPr})]$ (**9**), $[\text{CuCl}(\text{IMes})]$ (**10**) and $[\text{CuCl}(\text{SIMes})]$ (**11**) were purchased from Strem and used without prior purification. The imidazolium salts used for the preparation of the *N*-Heterocyclic Carbenes[47-50] and the 2,2'-dipyridylamine derivatives[38, 40] were synthesized as previously reported. Complexes $[\text{CuCl}(\text{NHC})]$ (**1, 5-8**) were synthesized according to reported procedures.[51, 52] Complexes $[\text{CuI}(\text{IPr})]$ (**2**)[53] $[\text{CuOH}(\text{IPr})]$ (**3**)[54] were prepared by the reported methods. Complexes $[\text{Cu}(\text{NHC})(\text{dpa})][\text{PF}_6]$ **12, 18-26** were prepared following our previously reported procedure.[38]

Complex $[\text{CuCN}(\text{IPr})]$ (**4**). In a flame-dried Schlenk tube under argon atmosphere, $[\text{CuCl}(\text{IPr})]$ (**1**) (0.3 mmol, 150 mg, 1 equiv.) and KCN (0.3 mmol, 19 mg, 1 equiv.) were introduced in degassed MeOH (5 mL) and the reaction mixture was stirred under reflux (50 °C) for 4 hours. After returning to room temperature, the reaction mixture was concentrated to dryness under vacuum. The complex was then dissolved in dichloromethane and filtered through a pad of Celite® and concentrated again under vacuum. A purification by recrystallization by slow diffusion of pentane in a THF solution of the complex led to the pure complex (**4**) as a white powder (143 mg, 97 % yield). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 1.22 (d, $J = 6.9$ Hz, 12H), 1.27 (d, $J = 6.9$ Hz, 12H), 2.50 (sept, $J = 6.9$ Hz, 4H), 7.14 (s, 2H), 7.30 (d, $J = 7.8$ Hz, 4H), 7.50 (t, $J = 7.8$ Hz, 4H) ppm. (spectroscopic data in good agreement with the literature).[54]

*General procedure for the synthesis of complexes having general formula of $[\text{Cu}(\text{IPr})(\text{dpa})][\text{X}]$ **13-17**.*

Following the general procedure described previously for the synthesis of complexes **12**,[38] the KPF_6 salt of the aqueous treatment at the end of the reaction was replaced by the appropriate salt to give complexes **13-17**.

$[\text{Cu}(\text{IPr})(\text{dpa})][\text{BF}_4]$ complex **13**. Following the general procedure with copper complex **1** (162.5 mg, 0.33 mmol), **dpa** ligand (59.9 mg, 0.35 mmol) and NaBF_4 as salt for the final aqueous solution, complex

13 was obtained as a white powder (189 mg, 80% yield). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 1.07 (d, J = 6.9 Hz, 12H), 1.22 (d, J = 6.9 Hz, 12H), 2.65 (sept, J = 6.9 Hz, 4H), 6.13 (dd, J = 5.5 and 1.5 Hz, 2H), 6.28 (t, J = 6.3 Hz, 2H), 7.20-7.23 (m, 4H), 7.33 (d, J = 7.8 Hz, 4H), 7.44 (dt, J = 1.8 and 6.9 Hz, 2H), 7.58 (t, J = 7.8 Hz, 2H), 8.81 (s, 1H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 24.0 (4xCH₃), 24.2 (4xCH₃), 28.3 (4xCH), 115.3 (2xCH), 116.1 (2xCH), 123.4 (2xCH), 124.7 (4xCH), 130.61 (2xCH), 136.0 (2xC), 138.9 (2xCH), 146.0 (4xC), 147.4 (2xCH), 153.2 (2xC), 183.2 (C) ppm. $^{19}\text{F-NMR}$ (CDCl_3 , 377 MHz): δ 151.27 ppm. $^{11}\text{B-NMR}$ (CDCl_3 , 100 MHz): δ -0.76 ppm. **IR** (neat): ν 2964, 1634, 1582, 1478, 1228, 1072, 761 cm^{-1} . **HRMS** (ESI): m/z calcd for $\text{C}_{37}\text{H}_{45}\text{CuN}_5$ $[\text{M-BF}_4]^+$: 622.2971; found: 622.2989. **Elemental Anal.** Calcd for $\text{C}_{37}\text{H}_{45}\text{BCuF}_4\text{N}_5$: C, 62.58; H, 6.39; N, 9.86; found: C, 62.31; H, 6.52; N, 9.89.

[Cu(IPr)(dpa)][BPh₄] complex **14**. Following the general procedure with copper complex **1** (162.5 mg, 0.33 mmol), **dpa** ligand (59.9 mg, 0.35 mmol) and NaBPh₄ as salt for the final aqueous solution, complex **14** was obtained as a white powder (293 mg, 94% yield). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 1.06 (d, J = 6.8 Hz, 12H), 1.23 (d, J = 6.8 Hz, 12H), 2.62 (sept, J = 6.8 Hz, 4H), 5.67 (d, J = 8.4 Hz, 2H), 5.91 (s, 1H), 6.07 (d, J = 4.4 Hz, 2H), 6.22 (t, J = 6.2 Hz, 2H), 6.79 (t, J = 6.8 Hz, 4H, BPh₄), 6.97 (t, J = 7.3 Hz, 8H, BPh₄), 7.13 (s, 2H), 7.16 (dd, J = 11.4 and 4.4 Hz, 2H), 7.32 (d, J = 7.8 Hz, 4H), 7.49 (*br* s, 8H, BPh₄), 7.56 (t, J = 7.8 Hz, 2H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 125MHz): δ 24.0 (4xCH₃), 24.2 (4xCH₃), 28.7 (4xCH), 115.2 (2xCH), 116.3 (2xCH), 123.4 (4xCH, BPh₄), 123.4 (2xCH), 124.7 (4xCH), 125.8 (q, $^2J_{13\text{C}-11\text{B}}$ = 2.8 Hz, 8xCH, BPh₄), 130.6 (2xCH), 135.9 (2xC), 136.2 (8xCH, BPh₄), 138.7 (2xCH), 146.0 (4xC), 147.3 (2xCH), 152.0 (2xC), 163.1 (q, $^1J_{13\text{C}-11\text{B}}$ = 49.3 Hz, 4xC, BPh₄), 181.9 (C) ppm. $^{11}\text{B-NMR}$ (CDCl_3 , 100MHz): δ -6.40 ppm. **IR** (neat): ν 3339, 2961, 1619, 1578, 1466, 1157, 731, 705 cm^{-1} . **HRMS** (ESI): m/z calcd for $\text{C}_{37}\text{H}_{45}\text{CuN}_5$ $[\text{M-BPh}_4]^+$: 622.2971; found: 622.2989. **Elemental Anal.** Calcd for $\text{C}_{61}\text{H}_{65}\text{BCuN}_5$: C, 77.73; H, 6.95; N, 7.43; found: C, 77.53; H, 7.12; N, 7.49.

[Cu(IPr)(dpa)][NTf₂] complex **15**. Following the general procedure with copper complex **1** (146.3 mg, 0.30 mmol), **dpa** ligand (53.6 mg, 0.32 mmol) and LiNTf₂ as salt for the final aqueous solution, complex **15** was obtained as a white powder (269 mg, 99% yield). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 1.08

(d, $J = 6.7$ Hz, 12H), 1.23 (d, $J = 6.7$ Hz, 12H), 2.65 (sept, $J = 6.7$ Hz, 4H), 6.17 (d, $J = 4.4$ Hz, 2H), 6.33 (t, $J = 6.3$ Hz, 2H), 7.10 (d, $J = 8.4$ Hz, 2H), 7.22 (s, 2H), 7.33 (d, $J = 7.8$ Hz, 4H), 7.48 (t, $J = 7.2$ Hz, 2H), 7.58 (t, $J = 7.8$ Hz, 2H), 8.49 (*br s*, 1H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 23.9 (s, 4xCH₃), 24.1 (s, 4xCH₃), 28.7 (s, 4xCH), 114.9 (s, 2xCH), 116.3 (s, 2xCH), 119.9 (q, $J = 321$ Hz, 2xC, 2xCF₃ from NTf₂ anion), 123.5 (s, 2xCH), 124.7 (s, 4xCH), 130.6 (s, 2xCH), 135.9 (s, 2xC), 139.0 (s, 2xCH), 146.0 (s, 4xC), 147.5 (s, 2xCH), 153.0 (s, 2xC), 183.0 (s, C) ppm. $^{19}\text{F-NMR}$ (CDCl_3 , 400 MHz): δ -78.80 (s, 2xF, 2xCF₃ from NTf₂ anion) ppm. **IR** (neat) ν 3357, 1626, 1581, 1469, 1356, 1197, 1139, 1050, 806, 762, 740, 696 cm⁻¹. **HRMS** (ESI): m/z calcd for C₃₇H₄₅CuN₅ [M-NTf₂]⁺: 622.2971; found: 622.2973. **Elemental Anal.** Calcd for C₃₉H₄₅CuF₆N₆O₂S₂: C, 51.85; H, 5.02; N, 9.30; found: C, 51.67; H, 5.21; N, 9.33.

[Cu(IPr)(dpa)][B(3,5-(CF₃)₂Ph)₄] complex **16**. Following the general procedure with copper complex **1** (87.8 mg, 0.18 mmol), **dpa** ligand (32.5 mg, 0.19 mmol) and NaB(3,5-(CF₃)₂Ph)₄ as salt for the final aqueous solution, complex **16** was obtained as a white powder (208 mg, 78% yield). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ 1.05 (d, $J = 6.9$ Hz, 12H), 1.22 (d, $J = 6.9$ Hz, 12H), 2.63 (sept, $J = 6.9$ Hz, 4H), 6.19 (dd, $J = 5.5$ and 1.3 Hz, 2H), 6.39 (t, $J = 6.9$ Hz, 2H), 6.49 (d, $J = 8.4$ Hz, 2H), 6.59 (s, 1H), 7.24 (s, 2H), 7.33 (d, $J = 7.8$ Hz, 4H), 7.42 (dt, $J = 1.8$ and 6.9 Hz, 2H), 7.49 (*br s*, 4H, B(3,5-(CF₃)₂Ph)₄), 7.57 (t, $J = 7.8$ Hz, 2H), 7.70 (*br s*, 8H, B(3,5-(CF₃)₂Ph)₄) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ 23.8 (4xCH₃), 24.1 (4xCH₃), 28.7 (2xCH), 113.4 (2xCH), 117.3 (2xCH), 117.4-117.5 (m, 4xCH, B(3,5-(CF₃)₂Ph)₄), 123.5 (2xCH), 124.5 (q, $^1J_{13\text{C}-19\text{F}} = 272.5$ Hz, 8xCF₃, B(3,5-(CF₃)₂Ph)₄), 124.7 (4xCH), 128.8 (qq, $^2J_{13\text{C}-19\text{F}} = 31.4$ Hz and $^4J_{13\text{C}-19\text{F}} = 2.8$ Hz, 8xC, B(3,5-(CF₃)₂Ph)₄), 130.7 (2xCH), 134.8 (8xCH, B(3,5-(CF₃)₂Ph)₄), 135.8 (2xC), 139.5 (2xCH), 146.0 (4xC), 148.4 (2xCH), 152.0 (2xC), 161.7 (q, $^1J_{13\text{C}-11\text{B}} = 49.8$ Hz, 4xC, B(3,5-(CF₃)₂Ph)₄), 182.5 (C). $^{19}\text{F-NMR}$ (CDCl_3 , 377 MHz): δ -60.41 ppm. $^{11}\text{B-NMR}$ (CDCl_3 , 100 MHz): δ -6.60 ppm. **IR** (neat): ν 2967, 1625, 1585, 1473, 1352, 1272, 1156, 1124, 887, 681, 668 cm⁻¹. **HRMS** (ESI): m/z calcd for C₃₇H₄₅CuN₅ [M-B(3,5-(CF₃)₂Ph)₄]⁺: 622.2971; found: 622.2959. **Elemental Anal.** Calcd for C₆₉H₅₇BCuF₂₄N₅: C, 55.75; H, 3.87; N, 4.71; found: C, 55.62; H, 4.02; N, 4.72.

[Cu(IPr)(dpa)][B(F₅Ph)₄] complex **17**. Following the general procedure with copper complex **1** (97.5 mg, 0.20 mmol), **dpa** ligand (36 mg, 0.21 mmol) and NaB(F₅Ph)₄ as salt of the final aqueous solution, complex **17** was obtained as a white powder (198 mg, 76% yield). **¹H-NMR (CDCl₃, 400 MHz):** δ 1.07 (d, *J* = 6.7 Hz, 12H), 1.23 (d, *J* = 6.7 Hz, 12H), 2.65 (sept, *J* = 6.7 Hz, 4H), 6.21 (d, *J* = 4.8 Hz, 2H), 6.42 (t, *J* = 6.3 Hz, 2H), 6.61 (d, *J* = 8.3 Hz, 2H), 6.89 (*br s*, 1H), 7.24 (s, 2H), 7.35 (d, *J* = 7.8 Hz, 4H), 7.50 (t, *J* = 7.9 Hz, 2H), 7.59 (t, *J* = 7.8 Hz, 2H) ppm. **¹³C-NMR (CDCl₃, 100 MHz):** δ 23.8 (s, 4xCH₃), 24.1 (s, 4xCH₃), 28.7 (s, 4xCH), 113.62 (s, 2xCH), 117.2 (s, 2xCH), 123.5 (s, 2xCH), 124.8 (s, 4xCH), 130.7 (s, 2xCH), 135.0 (m, 4xC, B(F₅Ph)₄), 135.9 (s, 2xC), 137.5 (m, 8xC, B(F₅Ph)₄), 139.5 (s, 2xCH), 146.0 (s, 4xC), 147.0 (m, 4xC, B(F₅Ph)₄), 148.3 (s, 2xCH), 149.3 (m, 8xC, B(F₅Ph)₄), 152.2 (s, 2xC), 182.7 (s, C) ppm. **¹⁹F-NMR (CDCl₃, 400 MHz):** δ -166.8 (t, *J* = 17.5 Hz, 8xF, B(F₅Ph)₄), -163.0 (t, *J* = 20.5 Hz, 8xF, B(F₅Ph)₄), -132.7 (*br s*, 4xF, B(F₅Ph)₄) ppm. **¹¹B-NMR (CDCl₃, 100 MHz):** δ -16.67 (s, B(F₅Ph)₄) ppm. **IR (neat):** ν 2966, 1638, 1515, 1462, 1088, 979, 773 cm⁻¹. **HRMS (ESI):** *m/z* calcd for C₃₇H₄₅CuN₅ [M-B(F₅Ph)₄]⁺: 622.2971; found: 622.2974. **Elemental Anal.** Calcd for C₆₁H₄₅BCuF₂₀N₅: C, 56.26; H, 3.48; N, 5.38; found: C, 56.02; H, 3.65; N, 5.41.

Biological study details

The growth level of four cancer cell lines was determined using a colorimetric MTT (thiazolyl blue tetrazolium bromide, Sigma) assay. Cancer cell lines and growth medium were obtained from CLS Cell Line Service GmbH, Eppelheim, Germany). Human skin melanoma SK-Mel-28 and human brain glioma HS-683 were grown in DMEM supplemented with 4.5 g/l glucose, L-glutamine and 10% FBS. The human lung carcinoma cell line A549 was grown in DMEM:Ham's F12 (1:1) supplemented with L-glutamine and 5% FBS and human breast adenoma carcinoma MCF-7 in EMEM supplemented with L-glutamine, sodium pyruvate, NEAA and 10 % FBS. MTT assay is based on the reduction of the yellow product thiazolyl blue tetrazolium bromide (MTT) to purple-blue formazan by mitochondrial dehydrogenase of metabolically active cells. The number of living cells after incubation in the presence (or absence, control) of the tested molecule is directly proportional to the blue color which was measured by spectrophotometry. Briefly, cells were seeded (200 μl of a 5.10⁴ cells/ml suspension) in

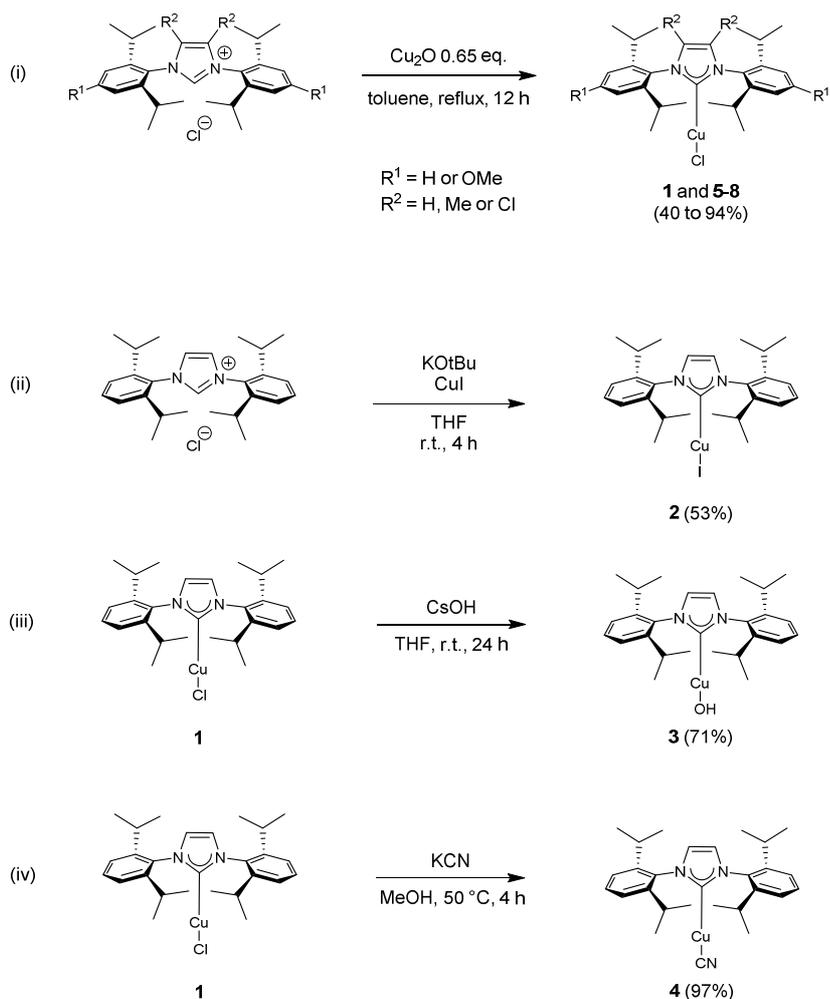
96-well culture plates (TPP, Trasadingen, Switzerland) and incubated for 24h. Each compound (starting from DMSO solutions, stable for months) was assessed in serial dilution (four concentrations in 0.5% DMSO at the highest concentration) in six replicates (n=6) and incubated for 72 h. Thereafter, MTT (5 mg/ml solution in PBS) was added to each well (10% v/v) and cells were further incubated for 4 hours. Then, after removing the culture medium, the blue crystals were dissolved in 100 μ L SDS-acidic-isopropanol solution (0.5% SDS; 80 mM HCl) and absorbance measured at 540 nm using a 620 nm reference. Absorbance of the serial dilution of each cell line treated under the same conditions but without the tested compounds was measured to generate a standard curve allowing IC₅₀ determination (IC₅₀ is defined as the concentration reducing cell growth by 50%).

RESULTS AND DISCUSSION

Synthesis and characterization

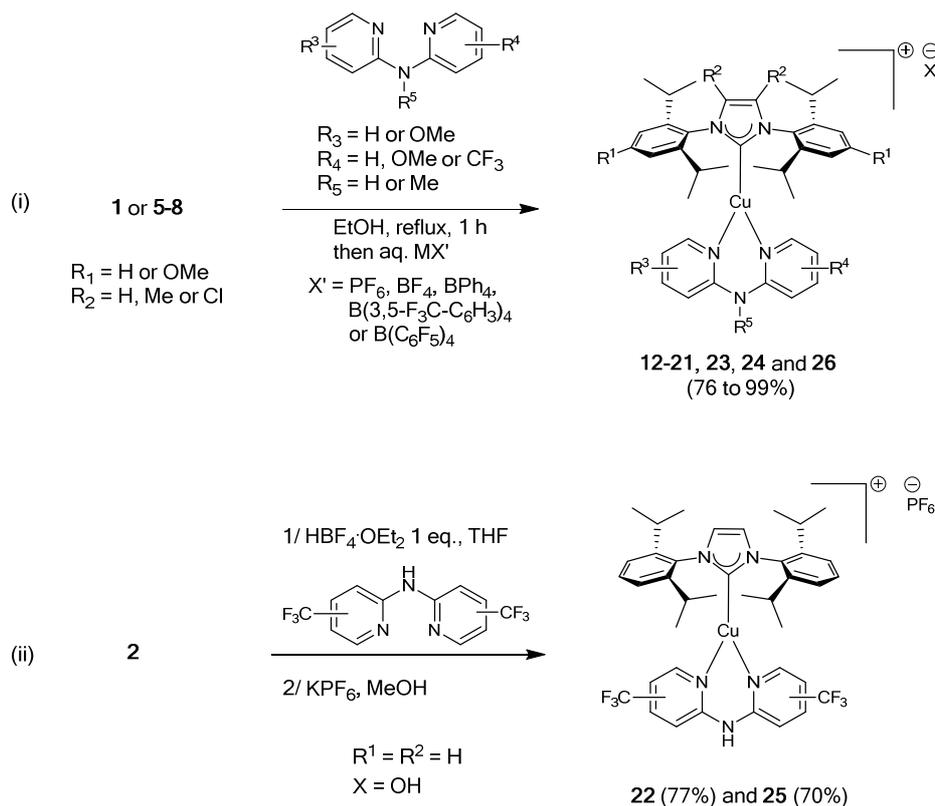
The imidazolium salts[47-50] and 2,2'-dipyridylamine derivatives[38, 40] were prepared according to reported procedures.

The [CuCl(NHC)] precursors **1** and **5-8** were synthesized following the Nolan-Cazin procedure using Cu₂O as metal precursor and the appropriate imidazolium salt and complexes **1**, **5-8** were isolated in 40 to 94% yield (Scheme 1, i).[51, 52] Then, complex **2** was prepared in 53% isolated yield by reacting 1,3-bis(-2,6-di-iso-propylphenyl)-4,5-dichloroimidazolium chloride in the presence of KOtBu and CuI (Scheme 1, ii).[53] The other NHC copper(I) complexes **9-11** were commercially available and were introduced in order to evaluate other *N*-aromatic substituent on the NHC ligand. The (NHC)copper hydroxide complex **3** was synthesized *via* a known procedure[54] and was also used as precursor for the synthesis of some cationic complexes described below (Scheme 1, iii). The exchange of the chlorine ligand by a cyano ligand in degassed methanol furnished the complex **4** in 97% isolated yield (Scheme 1, iv).



Scheme 1. General scheme for the synthesis of neutral complexes **1-8**.

Then, complexes **1**, **3** and **5-8** were used as precursors for the synthesis of cationic copper complexes bearing 2,2'-dipyridylamine (dpa) ligands. Complexes **12-21**, **23**, **24** and **26** were obtained from **1**, or **5-8** *via* the exchange of the chloride atom by a dpa ligand in refluxed methanol, followed by an anion metathesis (Scheme 2, i).[38] The expected cationic (NHC)copper(I) complexes were isolated in 76 to 99% yields. In details, the synthesis of complexes **12** to **17** were carried out with KPF_6 , NaBF_4 , NaBPh_4 , LiNTf_2 , $\text{NaB}((3,5\text{-CF}_3)_2\text{-Ph})_4$, $\text{NaB}(\text{F}_5\text{Ph})_4$ salts, respectively.



Scheme 2. General scheme for the synthesis of cationic complexes **12-26**.

Complexes **18-21**, **23**, **24**, and **26**, having different substituted dpa, were obtained with the PF_6^- anion. As previously noticed,[38] the synthesis of (NHC) copper(I) complexes coordinated to electron-deficient dpa ligands required a different procedure involving $[\text{CuOH}(\text{NHC})]$ **3** as precursor. In details, the cationic copper complexes **22** and **25** were obtained from complex **4** by addition of 1 equivalent of $\text{HBF}_4 \cdot \text{OEt}_2$ in the presence of 1 equivalent of dpa ligand. Complexes **22** and **25** were isolated after an anion metathesis in 77 and 70 % overall yields, respectively (Scheme 1, ii).

Cytotoxicity studies.

Half growth inhibition concentration (IC_{50}) of all the copper(I) complexes were measured on four cancer lines, namely MCF-7 (breast cancer), A-549 (lung carcinoma), SK-Mel-28 (melanoma) and HS-683 (glioma) in order to establish structure/cytotoxicity relationships toward each cancer cell lines independently. The choice for the three first cancer lines was directed by the previous studies with gold complexes.[19-24] In addition, the cytotoxicity of $[(\text{NHC})\text{CuCl}]$ complexes against MCF-7 was also reported by Gautier and this cell line was used as reference for this work.[27]

The growth level of the four cancer cell lines was determined using a colorimetric MTT (thiazolyl blue tetrazolium bromide, Sigma) assay. MTT assay is based on the reduction of the yellow product thiazolyl blue tetrazolium bromide (MTT) to purple-blue formazan by mitochondrial dehydrogenase of metabolically active cells. The number of living cells after incubation in the presence (or absence, control) of the tested molecule is directly proportional to the blue color, which was measured by spectrophotometry. Absorbance spectra were measured to generate a standard curve allowing IC₅₀ determination (IC₅₀ is defined as the concentration reducing cell growth by 50 %). All active complexes were then subjected to their IC₅₀ evaluation on a 10 000 cells population.

Results on the MCF-7 are presented in table 1 and chart 1. Etoposide and 5-fluorouracil, two commercially available generic drugs classically used to treat cancer, were used as positive controls. We were pleased to observe cytotoxicity toward the breast cancer MCF-7 cell lines for all copper complexes with IC₅₀ around 100 to 1000 fold lower than the two reference compounds i.e. Etoposide and 5-fluorouracil (Table 1, Chart 1).

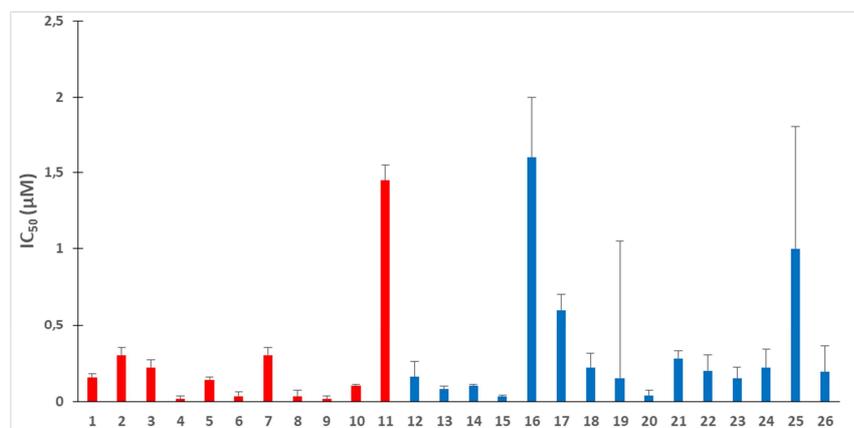


Chart 1. IC₅₀ of neutral (NHC)copper(I) complexes **1-11** (red) and [Cu(NHC)(dpa)][X] **12-26** (blue) on MCF-7.

Concerning the neutral copper complexes **1-11**, the IC₅₀ were found between $0.015 \pm 0.020 \mu\text{M}$ (for complex **4** and **9**, entries 4 and 9, Table 1) and $0.30 \pm 0.05 \mu\text{M}$ (for complexes **2** and **7**, entries 2 and 7, Table 1) except for complex **11** bearing SIMes ligand with a much higher IC₅₀ of 1.45 ± 0.10 than other neutral (NHC)copper(I) complexes. This surprising IC₅₀ for complex **11** is 20 times higher than the IC₅₀ previously reported by Gautier *et al.* ($0.075 \pm 0.002 \mu\text{M}$ on a 3 000 cells population).[27] Nevertheless,

the IC_{50} value of complex **11** is still lower than the IC_{50} of cis-platin in similar conditions.[55] Then, when neutral complexes **1-4** are now compared, the best X ligand appeared to be the cyanide present in complex **4**. Indeed, **4** exhibited a IC_{50} of at least 10 fold lower than the complexes **1-3** (Entries 1-4, Table 1). Gratefully, cationic copper(I) complexes **12-26** also presented a cytotoxic activity against MCF-7 cell-line. To the best of our knowledge, no cytotoxicity against breast cancer MCF-7 cell lines has ever been reported for cationic (NHC)copper(I) complexes. Most of the IC_{50} ranged from 0.03 ± 0.01 to 0.28 ± 0.05 excepted for complexes **16**, **17** and **25** (Entries 20, 21 and 29, Table 1) and were generally slightly higher than those determined for neutral copper(I) complexes **1-10**. These values seem to indicate that large anions (complexes **16** and **17**) and electron deficient substituent at the 5 position of the pyridine ring in dpa ligand (complex **25**) are not suitable for a high cytotoxicity. The best activities were found for complexes **15** and **20**, both having an unsubstituted dpa, small anion and electron rich NHC (complex **20**), with a IC_{50} of 0.03 ± 0.01 and 0.04 ± 0.03 μ M, respectively (Entries 19 and 24, Table 1). The comparison of these IC_{50} gave us some trends on the structure/cytotoxicity property relationships, (i) the presence of electron donor or withdrawing substituent on the pyridine ring of the dpa skeleton in these cationic copper(I) complexes led to higher IC_{50} and unsubstituted dpa should be favoured, and (ii) large fluorinated tetraarylborate anions $B(3,5-F_3C-C_6H_3)_4^-$ and $B(C_6F_5)_4^-$ have a negative effect on the cytotoxicity against MCF-7. The latter might be due to solubility or lipophilicity issues. Very interestingly, the *N*-alkylated dpa complex **26** presents a higher IC_{50} than the most active cationic copper(I) complex but still below 1 μ M (0.19 ± 0.17 μ M, Entry 30, Table 1). Nevertheless, this complex appears more active than Etoposide, 5F-uracil and the cis-platin.

Table 1. *In vitro* IC₅₀ growth inhibitory concentrations (μM) for breast cancer MCF-7.

Entry	Neutral complex	IC50 (μM) ^a	Entry	Cationic complex	IC50 (μM) ^a
1	1	0.15 ± 0.03	16	12	0.16 ± 0.10
2	2	0.30 ± 0.05	17	13	0.08 ± 0.02
3	3	0.22 ± 0.05	18	14	0.10 ± 0.01
4	4	0.015 ± 0.020	19	15	0.03 ± 0.01
5	5	0.14 ± 0.02	20	16	1.6 ± 0.4
6	6	0.03 ± 0.03	21	17	0.6 ± 0.1
7	7	0.30 ± 0.05	22	18	0.22 ± 0.09
8	8	0.03 ± 0.04	23	19	0.15 ± 0.90
9	9	0.015 ± 0.020	24	20	0.04 ± 0.03
10	10	0.10 ± 0.01	25	21	0.28 ± 0.05
11	11	1.45 ± 0.10	26	22	0.20 ± 0.10
12	11 ^b	0.075 ± 0.002	27	23	0.15 ± 0.07
13	Cis-platin[55]	8.8 ± 1.3	28	24	0.22 ± 0.12
14	Etoposide	25.0 ± 0.8	29	25	1.00 ± 0.80
15	5F-Uracil	19.5 ± 2.0	30	26	0.19 ± 0.17

^a ± values, means standard error of at least 6 determinations. ^b IC₅₀ of complex **11** previously reported by Gautier *et al.* on 3000 cells populations.[27]

In the second survey, all the copper complexes were studied toward lung carcinoma A-549. All complexes were found active, the IC₅₀ were determined and reported in table 2 and chart 2. In the neutral complexes series, complexes **1-3**, **5-6** and **8-10** exhibited low IC₅₀ between 0.04 ± 0.01 μM (complex **1**) and 0.15 ± 0.03 μM (complex **10**) (Entries 1-3, 5-6, 8-10, Table 2). Then, complexes **4** and **7** presented a higher IC₅₀ of 0.6 ± 0.2 and 0.30 ± 0.06 μM respectively, but still lower than Etoposide and 5-fluorouracil (Entries 4 and 7 vs. 12 and 13). Noteworthy, because of solubility issues above 3 μM of complex **11** its IC₅₀ was not determined (Entry 11, Table 2). In details, the copper complexes of general formula [CuX(NHC)], **1** and **2** appeared to be more active than other inner sphere anion such as

HO⁻ (complex **3**) and NC⁻ (complex **4**) (Entries 1-4, Table 2). In sharp contrast with the results on the MCF-7 cell line, the complex **4** bearing a cyanide ligand showed the lowest activity with a IC₅₀ of 0.6 ± 0.2 μM (Entry 4, Table 2). Cationic copper(I) complexes presented much higher IC₅₀ than neutral (NHC)copper complexes, except for complexes **13-15** having unsubstituted dpa and IPr ligands (Entries 15-17, Table 2). However, these copper complexes remained more active than the reference drugs (Entries 12-13, Table 2). In this cationic copper(I) complexes series, the non-coordinating anion appeared to be structurally important as complexes **13-15** with BF₄⁻, BPh₄⁻ and NTf₂⁻ anions exhibited IC₅₀ (at maximum 0.06 ± 0.02 μM) similar to the most active neutral copper complexes **1** and **8** (Entries 1, 8, 15-18, Table 2). Then, adding substituents on the IPr motif decreased the IC₅₀ (Complex **12** vs. complexes **18-21**, entries 14 and 20-23, Table 2) and, when the dpa substitution is considered, the *N*-substituted dpa complex presented the lowest IC₅₀ (complex **26** vs. complexes **12**, **22-25**), with a value of 0.78 ± 0.10 μM for complex **26** (Entry 28, Table 2).

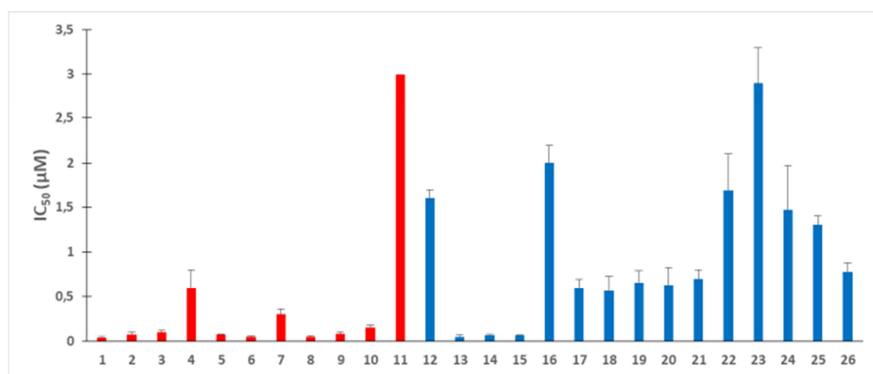


Chart 2. IC₅₀ of neutral (NHC)copper(I) complexes **1-11** (red) and [Cu(NHC)(dpa)][X] **12-26** (blue) on A-549.

Table 2. *In vitro* IC₅₀ growth inhibitory concentrations (μM) for lung carcinoma A-549.

Entry	Neutral complex	IC ₅₀ (μM) ^a	Entry	Cationic complex	IC ₅₀ (μM) ^a
1	1	0.04 ± 0.01	14	12	1.60 ± 0.10
2	2	0.07 ± 0.03	15	13	0.05 ± 0.02
3	3	0.10 ± 0.02	16	14	0.06 ± 0.02
4	4	0.6 ± 0.2	17	15	0.06 ± 0.01
5	5	0.07 ± 0.01	18	16	2.0 ± 0.2
6	6	0.050 ± 0.005	19	17	0.60 ± 0.10
7	7	0.30 ± 0.06	20	18	0.56 ± 0.17
8	8	0.050 ± 0.005	21	19	0.66 ± 0.13
9	9	0.08 ± 0.02	22	20	0.63 ± 0.20
10	10	0.15 ± 0.03	23	21	0.70 ± 0.10
11	11	> 3 ^b	24	22	1.70 ± 0.40
12	Etoposide	3.0 ± 0.2	25	23	2.90 ± 0.40
13	5F-Uracil	3.5 ± 0.3	26	24	1.47 ± 0.50
			27	25	1.30 ± 0.10
			28	26	0.78 ± 0.10

^a ± values, means standard error of at least 6 determinations. ^b higher concentrations were not considered due to solubility issue and IC₅₀ was not determined.

The cytotoxicity of the copper(I) complexes toward the Melanoma SK-Mel-28 cancer cells was then evaluated (Table 3 and Chart 3). For neutral complexes **1-11**, all the IC₅₀ were in a smaller range (0.04 ± 0.002 for **5** to 0.42 ± 0.04 for **11**) compared to the two previous cancer cell lines. In addition, complexes **1-11** have IC₅₀ at least 15 fold lower than etoposide (IC₅₀ of 6.5 ± 0.8), the most active commercially available reference used in this study (Entries 1-13, Table 3).

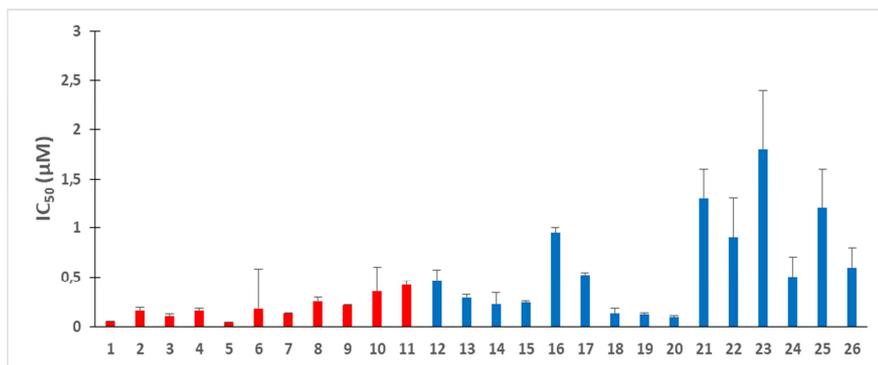


Chart 3. IC₅₀ of neutral (NHC)copper(I) complexes 1-11 (red) and [Cu(NHC)(dpa)][X] 12-26 (blue) on SK-Mel-28.

Concerning the cationic copper complexes, the range of IC₅₀ was found between 0.09 ± 0.02 for complex **20** and 1.80 ± 0.60 µM for complex **23** (Entries 14-28, Table 3). The role of the anion seemed to have a little impact on the activity and its nature was not as important as observed with the MCF-7 and A-549 cell lines (Entries 14-19, Table 3). Then, it appeared that electron donating substituent on the IPr moiety decreased the IC₅₀ (Complex **12** vs complexes **18-20**, Entries 14 and 20-22, Table 3), while, in sharp contrast, the electron withdrawing chloride substituent on the C4 and C5 positions of the IPr ligand led to an increase of the IC₅₀ (Complex **12** vs complex **21**, Entries 14 and 23, Table 3).

Table 3. *In vitro* IC₅₀ growth inhibitory concentrations (μM) for melanoma SK-Mel-28.

Entry	Neutral complex	IC ₅₀ (μM) ^a	Entry	Cationic complex	IC ₅₀ (μM) ^a
1	1	0.045 ± 0.005	14	12	0.47 ± 0.10
2	2	0.16 ± 0.03	15	13	0.29 ± 0.03
3	3	0.100 ± 0.025	16	14	0.22 ± 0.12
4	4	0.16 ± 0.02	17	15	0.24 ± 0.02
5	5	0.040 ± 0.002	18	16	0.95 ± 0.05
6	6	0.18 ± 0.40	19	17	0.52 ± 0.03
7	7	0.13 ± 0.01	20	18	0.13 ± 0.05
8	8	0.25 ± 0.04	21	19	0.12 ± 0.02
9	9	0.21 ± 0.01	22	20	0.09 ± 0.02
10	10	0.35 ± 0.25	23	21	1.30 ± 0.30
11	11	0.42 ± 0.04	24	22	0.90 ± 0.40
12	Etoposide	6.5 ± 0.8	25	23	1.80 ± 0.60
13	5F-Uracil	13.4 ± 2.0	26	24	0.50 ± 0.20
			27	25	1.20 ± 0.40
			28	26	0.60 ± 0.20

^a ± values, means standard error of at least 6 determinations.

The fourth cancer cell line we studied in this work was Glioma HS-683. The IC₅₀ were presented in table 4 and chart 4. Fortunately, all the copper(I) complexes presented a higher cytotoxicity toward Glioma HS-683 than the commercially available drugs etoposide and 5-fluorouracil, which exhibited IC₅₀ of 4.00 ± 0.52 and 16.7 ± 12.2, respectively (Entries 12 and 13, Table 4). And even more interesting was the micromolar range of the IC₅₀ of some (NHC) copper(I) complexes (Entries 1-3, 5-6, 8-11, 16-17, 20, 22 and 24, Table 4). As example, in the neutral copper complex family, the most active (NHC) copper(I) complexes were **5** and **10** with IC₅₀ of 0.21 ± 0.01 and 0.20 ± 0.05, respectively (Entries 5 and 10, Table 4). Cationic copper(I) complexes exhibited a lower cytotoxicity than neutral copper(I)

complexes **1-11** except complex **14**, for which an IC_{50} of $0.22 \pm 0.01 \mu\text{M}$ was determined, similarly to complexes **5** and **10** (Entry 16 vs entries 5 and 10, Table 4).

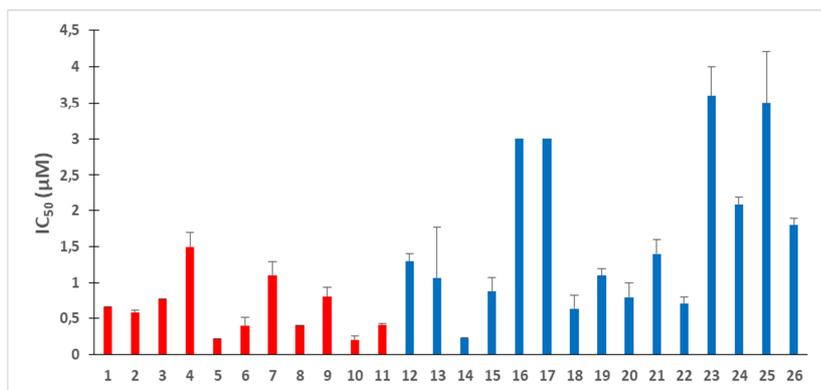


Chart 4. IC_{50} of neutral (NHC)copper(I) complexes **1-11** (red) and $[\text{Cu}(\text{NHC})(\text{dpa})][\text{X}]$ **12-26** (blue) on HS-683.

In the cationic complex series, IC_{50} were not determined for **16** and **17** due to solubility issues above $3 \mu\text{M}$ (Entries 16 and 17, Table 4). Complexes **23** and **25** presented the lowest cytotoxicity with IC_{50} of 3.60 ± 0.40 and 3.50 ± 0.70 , respectively (Entries 25 and 27, Table 4). Concerning complex **26**, having the *N*-alkylated dpa ligand, its IC_{50} was found at $1.8 \pm 0.1 \mu\text{M}$ representing a cytotoxicity 10 fold lower than the one of **14** (Entries 16 and 28, Table 4). Even if BPh_4^- anion is very hydrophobic, this anion appeared to bring the highest cytotoxicity to cationic NHC copper complexes as **14** presented the lowest IC_{50} compared to complexes **12-17** (Entry 16, Table 4). Therefore, an eventual anion metathesis on complex **26** might lead to more active complexes and balance the negative effect of the *N*-alkyl substitution on the dpa ligand.

Table 4. *In vitro* IC₅₀ growth inhibitory concentrations (μM) for Glioma HS-683.

Entry	Neutral complex	IC ₅₀ (μM)	Entry	Cationic complex	IC ₅₀ (μM)
1	1	0.65 ± 0.01	14	12	1.30 ± 0.10
2	2	0.58 ± 0.03	15	13	1.07 ± 0.70
3	3	0.77 ± 0.01	16	14	0.22 ± 0.01
4	4	1.5 ± 0.2	17	15	0.89 ± 0.18
5	5	0.21 ± 0.01	18	16	> 3 ^b
6	6	0.39 ± 0.12	19	17	> 3 ^b
7	7	1.1 ± 0.2	20	18	0.63 ± 0.20
8	8	0.39 ± 0.01	21	19	1.10 ± 0.10
9	9	0.81 ± 0.13	22	20	0.80 ± 0.20
10	10	0.20 ± 0.05	23	21	1.40 ± 0.20
11	11	0.41 ± 0.02	24	22	0.70 ± 0.10
12	Etoposide	4.00 ± 0.52	25	23	3.60 ± 0.40
13	5F-Uracil	16.7 ± 12.2	26	24	2.10 ± 0.10
			27	25	3.50 ± 0.70
			28	26	1.80 ± 0.10

^a ± values, means standard error of at least 6 determinations. ^b higher concentrations was not considered due to solubility issue and IC₅₀ was not determined.

Finally, the evolution and stability of complexes **1** and **12** in DMSO-d₆ solution was evaluated by ¹H NMR spectroscopy (Figure S42 and S43 in supplementary information). After 14 h, no evolution of both complexes was noticed. Then, the stability study was assessed for complex **12** in biological medium (1%DMSO/DMEM+GlutaMAX-ITM solution) in absence of cells and was monitored by UV-visible absorption spectroscopy for 25 hours (Figure 2). As control, the same experiment was performed in the biological medium. Surprisingly, the absorption spectra of the medium presented important changes in both UV

(274 nm) and visible region (560 nm) (See Supporting Information, Figure S1). As the ligands (dpa and NHC) and complex **12** have only their maximal absorption wavelength in the UV region, our analysis of the evolution, in function of time, of the absorption spectra of complex **12**, in 1% DMSO/DMEM+GlutaMAX-ITM solution, was only focused in the 250 to 380 nm region. The maxima of the absorption wavelength in a dichloromethane solution were previously measured at 260 and 315 nm, assigned to π - π^* and d- π^* transitions, respectively (Figure 2, inset).[37]

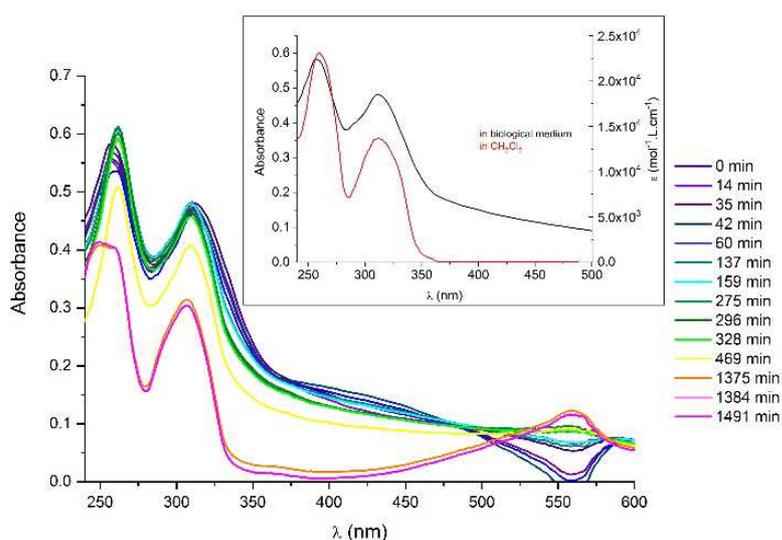


Figure 2. Stability of **12** followed by UV-vis spectroscopy over 25 h in 1% DMSO/DMEM+GlutaMAX-ITM solution ($c = 50 \mu\text{M}$) and UV-visible spectra of **12** in dichloromethane solution ($c = 100 \mu\text{M}$) (red line, inset) and in 1% DMSO/DMEM+GlutaMAX-ITM solution ($c = 50 \mu\text{M}$) in absence of cell line (dark line, inset).

Encouragingly, the UV-visible spectra in the 1% DMSO/DMEM+GlutaMAX-ITM solution did not dramatically change in the 260 and 315 nm region within 4 to 5 h. After this period of time, a slow decrease of the absorbance was observed: 13 and 26% at 260 nm, 17 and 39% at 312 nm after 8 and 25 h, respectively. In a few words, the integrity of the cationic complex **12** seems to be maintained during the first 4 to 5 h in the biological medium and such stability might open a door to vectorizable copper complexes bearing a dpa ligand.

CONCLUSION

We reported the synthesis of several new neutral and cationic (NHC)copper(I) complexes. The cytotoxicity of all these copper(I) complexes was studied on three cancer lines, breast cancer MCF-7, lung carcinoma A-549 and Melanoma SK-Mel-28 which were previously reported to be sensitive to (NHC)gold(I) complexes. In addition, we also reported cytotoxicity of these (NHC)copper(I) complexes toward Glioma HS-683. This work further demonstrates that copper complexes can act as anticancer agents like their group 11 congeners and are more active than commercially available anti-cancer agents. Some of the cationic (NHC)copper(I) complexes **12-26**, bearing NHC and dpa ligands exhibit similar sub-micromolar IC_{50} than neutral $[CuX(NHC)]$ complexes **1-11** and, in all cases, lower than the commercial Etoposide and 5-fluorouracil drugs.

Chart 5 depicts the whole IC_{50} , on the four cell lines determined in this study. This overview highlights that neutral (NHC) copper(I) complexes are in general more active than the cationic (NHC) copper(I) analogs bearing dpa ligands. Nevertheless, in few cases, the cytotoxicity of some cationic copper(I) complexes is comparable to the one observed with the neutral copper(I) complexes and depends on the nature of the anion, the electronic property or the steric hindrance of their ligands. Complexes **13-15** having a small anion, present similar cytotoxicity to neutral copper(I) complexes toward MCF-7 and lung carcinoma A-549. Complexes **18-20**, having electron rich substituent on the NHC moiety, present also IC_{50} in the range of the most active neutral (NHC)copper(I) complexes toward MCF-7 and SK-Mel-28. Finally, complexes bearing substituted dpa present higher IC_{50} , whatever the electron donating or electron withdrawing properties of the substituent. Such results might be due to steric hindrance issues. Finally, the complex **26**, which could be seen as a model of a future vectorizable complex if the dpa is substituted by a biologically recognized framework, has higher IC_{50} than its non-substituted congeners. However, its cytotoxicity against the four cell

lines is higher than commercially available anticancer agents. All these observations might give some guidelines for the future design of more efficient cationic copper(I) complexes.

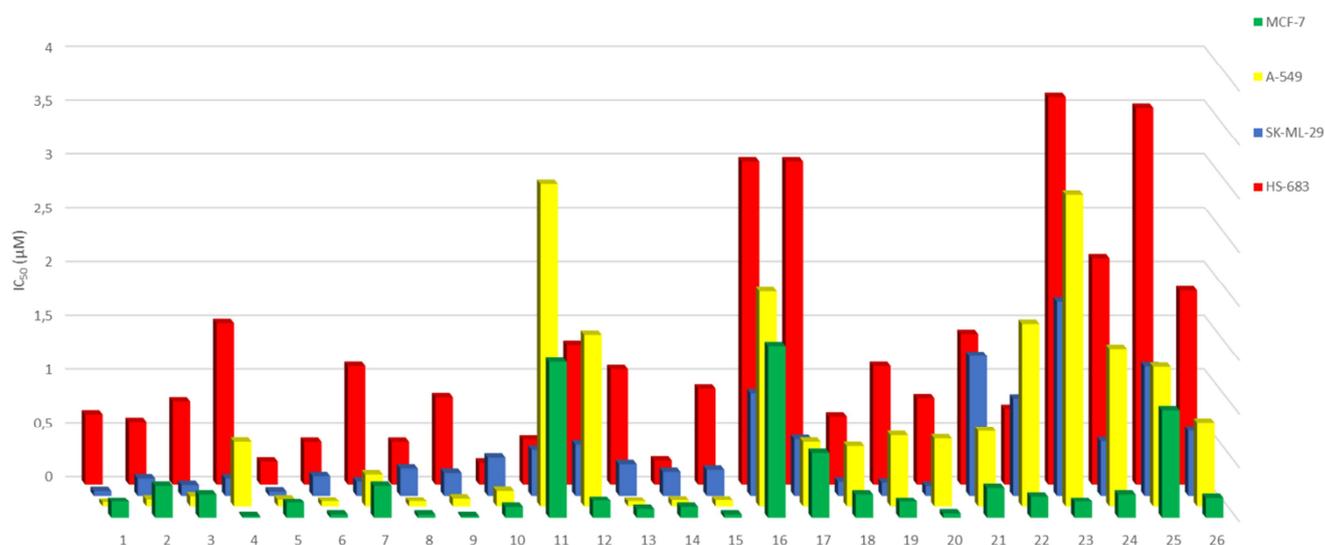


Chart 5. IC₅₀ of complexes **1-26** on the four cancer cell lines (MCF-7, A-549, SK-Mel-28 and HS-68)

Table of Abbreviation

dpa 2,2'-dipyridylamine derivative.

IC₅₀ half growth inhibitory concentration

NHC *N*-Heterocyclic Carbene

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Supporting Information.

Supporting information available: experimental procedures for the synthesis of the iridium (III) complexes, their characterization, and the biological evaluation of the inhibition. See DOI: 10.1039/c000000x/

References

- [1] B. Rosenberg, L. Vancamp, J.E. Trosko, V.H. Mansour, Platinum Compounds: a New Class of Potent Antitumour Agents, *Nature* 222 (1969) 385-386.
- [2] S. Dasari, P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, *Eur. J. Pharmacol.* 740 (2014) 364-378.
- [3] T.C. Johnstone, K. Suntharalingam, S.J. Lippard, The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs, *Chem. Rev.* 116 (2016) 3436-3486.
- [4] F. Michael, H. Sarmad, C. Di, D. Andrew, S. Sara, T. Dajena, Q.P. Dou, Novel Metals and Metal Complexes as Platforms for Cancer Therapy, *Current Pharmaceutical Design* 16 (2010) 1813-1825.
- [5] J. Zhang, F. Zhang, H. Li, C. Liu, J. Xia, L. Ma, W. Chu, Z. Zhang, C. Chen, S. Li, S. Wang, Recent Progress and Future Potential for Metal Complexes as Anticancer Drugs Targeting G-quadruplex DNA, *Current Medicinal Chemistry* 19 (2012) 2957-2975.
- [6] I. Romero-Canelon, P.J. Sadler, Next-generation metal anticancer complexes: multitargeting via redox modulation, *Inorg. Chem.* 52 (2013) 12276-12291.
- [7] M.B. Baile, N.S. Kolhe, P.P. Deotarse, A.S. Jain, A.A. Kulkarni, Metal Ion Complex - Potential Anticancer Drug- A Review, *International Journal of Pharma Research & Review* 4 (2015) 59-66.
- [8] A. Bergamo, G. Sava, Linking the future of anticancer metal-complexes to the therapy of tumour metastases, *Chem. Soc. Rev.* 44 (2015) 8818-8835.

- [9] For an overview on metal anticancer compounds, see also the special issue 48 of Dalton Trans. 2009.
- [10] F. Cisnetti, A. Gautier, Metal/N-heterocyclic carbene complexes: opportunities for the development of anticancer metallodrugs, *Angew. Chem. Int. Ed. Engl.* 52 (2013) 11976-11978.
- [11] B. Bertrand, A. Casini, A golden future in medicinal inorganic chemistry: the promise of anticancer gold organometallic compounds, *Dalton Trans* 43 (2014) 4209-4219.
- [12] T. Zou, C.T. Lum, C.N. Lok, J.J. Zhang, C.M. Che, Chemical biology of anticancer gold(III) and gold(I) complexes, *Chem. Soc. Rev.* 44 (2015) 8786-8801.
- [13] J.L. Hickey, R.A. Ruhayel, P.J. Barnard, M.V. Baker, S.J. Berners-Price, A. Filipovska, Mitochondria-Targeted Chemotherapeutics: The Rational Design of Gold(I) N-Heterocyclic Carbene Complexes That Are Selectively Toxic to Cancer Cells and Target Protein Selenols in Preference to Thiols, *Journal of the American Chemical Society* 130 (2008) 12570-12571.
- [14] K.M. Hindi, M.J. Panzner, C.A. Tessier, C.L. Cannon, W.J. Youngs, The Medicinal Applications of Imidazolium Carbene-Metal Complexes, *Chem. Rev.* 109 (2009) 3859-3884.
- [15] R. Rubbiani, I. Kitanovic, H. Alborzinia, S. Can, A. Kitanovic, L.A. Onambele, M. Stefanopoulou, Y. Geldmacher, W.S. Sheldrick, G. Wolber, A. Prokop, S. Wolfl, I. Ott, Benzimidazol-2-ylidene gold(I) complexes are thioredoxin reductase inhibitors with multiple antitumor properties, *J. Med. Chem.* 53 (2010) 8608-8618.
- [16] L. Messori, F. Scaletti, L. Massai, M.A. Cinellu, C. Gabbiani, A. Vergara, A. Merlino, The mode of action of anticancer gold-based drugs: a structural perspective, *Chem. Commun.* 49 (2013) 10100-10102.
- [17] B.K. Rana, A. Nandy, V. Bertolasi, C.W. Bielawski, K. Das Saha, J. Dinda, Novel Gold(I)- and Gold(III)-N-Heterocyclic Carbene Complexes: Synthesis and Evaluation of Their Anticancer Properties, *Organometallics* 33 (2014) 2544-2548.

- [18] M. Porchia, M. Pellei, M. Marinelli, F. Tisato, F. Del Bello, C. Santini, New insights in Au-NHCs complexes as anticancer agents, *Eur. J. Med. Chem.* 146 (2018) 709-746.
- [19] M.L. Teyssot, A.S. Jarrousse, M. Manin, A. Chevy, S. Roche, F. Norre, C. Beaudoin, L. Morel, D. Boyer, R. Mahiou, A. Gautier, Metal-NHC complexes: a survey of anti-cancer properties, *Dalton Trans* (2009) 6894-6902.
- [20] L. Mercks, M. Albrecht, Beyond catalysis: N-heterocyclic carbene complexes as components for medicinal, luminescent, and functional materials applications, *Chem. Soc. Rev.* 39 (2010) 1903-1912.
- [21] A. Gautier, F. Cisnetti, Advances in metal-carbene complexes as potent anti-cancer agents, *Metallomics* 4 (2012) 23-32.
- [22] W. Liu, R. Gust, Metal N-heterocyclic carbene complexes as potential antitumor metallodrugs, *Chem. Soc. Rev.* 42 (2013) 755-773.
- [23] L. Oehninger, R. Rubbiani, I. Ott, N-Heterocyclic carbene metal complexes in medicinal chemistry, *Dalton Trans* 42 (2013) 3269-3284.
- [24] S.B. Aher, P.N. Muskawar, K. Thenmozhi, P.R. Bhagat, Recent developments of metal N-heterocyclic carbenes as anticancer agents, *Eur. J. Med. Chem.* 81 (2014) 408-419.
- [25] H.C. Wang, M. Riahi, J. Pothen, C.A. Bayse, P. Riggs-Gelasco, J.L. Brumaghim, Interactions of Cu(I) with selenium-containing amino acids determined by NMR, XAS, and DFT studies, *Inorg. Chem.* 50 (2011) 10893-10900.
- [26] G.M. Ehrenfeld, J.B. Shipley, D.C. Heimbrook, H. Sugiyama, E.C. Long, J.H. van Boom, G.A. van der Marel, N.J. Oppenheimer, S.M. Hecht, Copper-Dependent Cleavage of DNA by Bleomycin, *Biochemistry* 26 (1987) 931-942.
- [27] F. Lazreg, D.B. Cordes, A.M.Z. Slawin, C.S.J. Cazin, Synthesis of Homoleptic and Heteroleptic Bis-N-heterocyclic Carbene Group 11 Complexes, *Organometallics* 34 (2015) 419-425.

- [28] C. Marzano, M. Pellei, T. F., C. Santini, Copper Complexes as Anticancer Agents, *Anti-Cancer Agents in Med. Chem.* 9 (2009) 185-211.
- [29] V. Gandin, M. Porchia, F. Tisato, A. Zanella, E. Severin, A. Dolmella, C. Marzano, Novel mixed-ligand copper(I) complexes: role of diimine ligands on cytotoxicity and genotoxicity, *J. Med. Chem.* 56 (2013) 7416-7430.
- [30] C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, Advances in copper complexes as anticancer agents, *Chem. Rev.* 114 (2014) 815-862.
- [31] U.K. Komarnicka, R. Starosta, M. Plotek, R.F. de Almeida, M. Jezowska-Bojczuk, A. Kyziol, Copper(I) complexes with phosphine derived from sparfloxacin. Part II: a first insight into the cytotoxic action mode, *Dalton Trans* 45 (2016) 5052-5063.
- [32] J. Lopes, D. Alves, T.S. Morais, P.J. Costa, M.F. Piedade, F. Marques, M.J. Villa de Brito, M. Helena Garcia, New copper(I) and heteronuclear copper(I)-ruthenium(II) complexes: Synthesis, structural characterization and cytotoxicity, *J. Inorg. Biochem.* 169 (2017) 68-78.
- [33] T.S. Morais, Y. Jousseume, M.P. MF, C. Roma-Rodrigues, A.R. Fernandes, F. Marques, M.J. Villa de Brito, M.H. Garcia, Important cytotoxic and cytostatic effects of new copper(i)-phosphane compounds with N,N, N,O and N,S bidentate ligands, *Dalton Trans* 47 (2018) 7819-7829.
- [34] W. Streciwilk, F. Hackenberg, H. Müller-Bunz, M. Tacke, Synthesis and cytotoxicity studies of p-benzyl substituted NHC–copper(I) bromide derivatives, *Polyhedron* 80 (2014) 3-9.
- [35] W. Walther, I. Fichtner, F. Hackenberg, W. Streciwilk, M. Tacke, In Vitro and In Vivo Investigations into The Carbene Copper Bromide Anticancer Drug Candidate WBC4, *Lett. in Drug Design and Discovery* 11 (2014) 825-832.

- [36] M. Tacke, Benzyl-substituted carbene–metal complexes: Potential for novel antibiotics and anticancer drugs, *Journal of Organometallic Chemistry* 782 (2015) 17-21.
- [37] R. Marion, F. Sguerra, F. Di Meo, E. Sauvageot, J.F. Lohier, R. Daniellou, J.L. Renaud, M. Linares, M. Hamel, S. Gaillard, NHC copper(I) complexes bearing dipyridylamine ligands: synthesis, structural, and photoluminescent studies, *Inorg. Chem.* 53 (2014) 9181-9191.
- [38] M. Elie, F. Sguerra, F. Di Meo, M.D. Weber, R. Marion, A. Grimault, J.F. Lohier, A. Stallivieri, A. Brosseau, R.B. Pansu, J.L. Renaud, M. Linares, M. Hamel, R.D. Costa, S. Gaillard, Designing NHC-Copper(I) Dipyridylamine Complexes for Blue Light-Emitting Electrochemical Cells, *ACS Appl. Mater. Interfaces* 8(23) (2016) 14678-14691.
- [39] M. Elie, M.D. Weber, F. Di Meo, F. Sguerra, J.F. Lohier, R.B. Pansu, J.L. Renaud, M. Hamel, M. Linares, R.D. Costa, S. Gaillard, Role of the Bridging Group in Bis-Pyridyl Ligands: Enhancing Both the Photo- and Electroluminescent Features of Cationic (IPr)Cu(I) Complexes, *Chem. Eur. J.* 23 (2017) 16328-16337.
- [40] S. Gaillard, M.K. Elmkaddem, C. Fischmeister, C.M. Thomas, J.-L. Renaud, Highly efficient and economic synthesis of new substituted amino-bispyridyl derivatives via copper and palladium catalysis, *Tetrahedron Letters* 49 (2008) 3471-3474.
- [41] M.J. Rauterkus, S. Fakhri, C. Mock, I. Puscasu, B. Krebs, Cisplatin analogues with 2,2'-dipyridylamine ligands and their reactions with DNA model nucleobases, *Inorganica Chimica Acta* 350 (2003) 355-365.
- [42] S. Wang, H. Huang, V. Dorcet, T. Roisnel, C. Bruneau, C. Fischmeister, Efficient Iridium Catalysts for Base-Free Hydrogenation of Levulinic Acid, *Organometallics* 36 (2017) 3152-3162.

- [43] E. Sauvageot, M. Elie, S. Gaillard, R. Daniellou, P. Fechter, I.J. Schalk, V. Gasser, J.L. Renaud, G.L.A. Mislin, Antipseudomonal activity enhancement of luminescent iridium(III) dipyrindylamine complexes under visible blue light, *Metallomics* 9 (2017) 1820-1827.
- [44] W.X. Ren, J. Han, S. Uhm, Y.J. Jang, C. Kang, J.H. Kim, J.S. Kim, Recent development of biotin conjugation in biological imaging, sensing, and target delivery, *Chem. Commun. (Camb)* 51 (2015) 10403-10418.
- [45] D.L. Ma, H.Z. He, K.H. Leung, D.S. Chan, C.H. Leung, Bioactive luminescent transition-metal complexes for biomedical applications, *Angew. Chem. Int. Ed. Engl.* 52 (2013) 7666-7682.
- [46] C. Hemmert, H. Gornitzka, Luminescent bioactive NHC-metal complexes to bring light into cells, *Dalton Trans* 45 (2016) 440-447.
- [47] A.J.I. Arduengo, R. Krafczyk, R. Schmutzler, Imidazolylidenes, Imidazolinyliidenes and Imidazolidines, *Tetrahedron* 55 (1999) 14523-14534.
- [48] S. P. Nolan, Synthesis of 1,3 disubstituted Imidazolium Salts., US-Patent, No WO2008/036084, A1, 2006.
- [49] A.J. Arduengo, 3rd, L.I. Iconaru, Fused polycyclic nucleophilic carbenes - synthesis, structure, and function, *Dalton Trans* (35) (2009) 6903-6914.
- [50] T.K. Lane, B.R. D'Souza, J. Louie, Iron-catalyzed formation of 2-aminopyridines from diynes and cyanamides, *J. Org. Chem.* 77 (2012) 7555-7563.
- [51] C.A. Citadelle, E. Le Nouy, F. Bisaro, A.M. Slawin, C.S. Cazin, Simple and versatile synthesis of copper and silver N-heterocyclic carbene complexes in water or organic solvents, *Dalton Trans* 39 (2010) 4489-4491.
- [52] O. Santoro, A. Collado, A.M. Slawin, S.P. Nolan, C.S. Cazin, A general synthetic route to [Cu(X)(NHC)] (NHC = N-heterocyclic carbene, X = Cl, Br, I) complexes, *Chem. Commun. (Camb)* 49(89) (2013) 10483-10485.

[53] S. Zheng, F. Li, J. Liu, C. Xia, A novel and efficient (NHC)CuI (NHC=N-heterocyclic carbene) catalyst for the oxidative carbonylation of amino compounds, *Tetrahedron Letters* 48 (2007) 5883-5886.

[54] G.C. Fortman, A.M.Z. Slawin, S.P. Nolan, A Versatile Cuprous Synthon: [Cu(IPr)(OH)] (IPr = 1,3 bis(diisopropylphenyl)imidazol-2-ylidene), *Organometallics* 29 (2010) 3966-3972.

[55] N.A. Johnson, M.R. Southerland, W.J. Youngs, Recent Developments in the Medicinal Applications of Silver-NHC Complexes and Imidazolium Salts, *Molecules* 22 (2017) 1263.

Highlights

We evaluated cytotoxicity of cationic NHC copper(I) complexes bearing dipyridylamine ligands

We compared cytotoxicity of cationic NHC copper(I) complexes bearing dipyridylamine ligands with their neutral NHC copper(I) complexes congeners