Journal of Organometallic Chemistry 893 (2019) 21–31

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

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

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ARTICLE INFO

Article history:
Received 11 March 2019
Received in revised form
3 April 2019
Accepted 4 April 2019
Available online 21 April 2019

Keywords:
Copper
NHC
Nitrogen ligand
Cytotoxicity
Cancer

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.

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1. 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 seems 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 half growth Inhibition Concentrations (IC_{50}) in the 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 [CuX(NHC)] and $[Cu(NHC)_2][X]$ [19–24]. Gautier et al. have firstly selected the [CuX(NHC)] complexes and

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https://doi.org/10.1016/j.jorganchem.2019.04.003
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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 mitotic 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 strong emission 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 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).

2. Material and methods

2.1. 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 (^{1}H) 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 [CuCl(SIPr)] (**9**), [CuCl(IMes)] (**10**) and [CuCl(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 **[CuCl(NHC)]** (**1**, **5**–**8**) where synthesized according to reported procedures [51,52]. Complexes **[CuI(IPr)]** (**2**) [53] **[CuOH(IPr)]** (**3**) [54] were prepared by the reported methods. Complexes **[Cu(NHC)(dpa)][PF$_6$]** (**12**, **18**–**26**) were prepared following our previously reported procedure [38].

Complex **[CuCN(IPr)]** (**4**). In a flame-dried Schlenk tube under argon atmosphere, [CuCl(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 h. 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}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 ***[Cu(IPr)(dpa)][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**.

[Cu(IPr)(dpa)][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}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}C-NMR ($CDCl_3$, 100 MHz):** δ 24.0 ($4xCH_3$), 24.2 ($4xCH_3$), 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}F-NMR ($CDCl_3$, 377 MHz):** δ 151.27 ppm. **^{11}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 $C_{37}H_{45}CuN_5$ $[M-BF_4]^+$: 622.2971; found: 622.2989. **Elemental Anal.** Calcd for $C_{37}H_{45}BCuF_4N_5$: C, 62.58; H, 6.39; N, 9.86; found: C, 62.31; H, 6.52; N, 9.89.

[Cu(IPr)(dpa)][BPh$_4$] 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 $NaBPh_4$ as salt for the final aqueous solution, complex **14** was obtained as a white powder (293 mg, 94% yield). **^{1}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, BPh_4), 6.97 (t, *J* = 7.3 Hz, 8H, BPh_4), 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, BPh_4), 7.56 (t, *J* = 7.8 Hz, 2H) ppm. **^{13}C-NMR ($CDCl_3$, 125 MHz):** δ 24.0 ($4xCH_3$), 24.2 ($4xCH_3$), 28.7 (4xCH), 115.2 (2xCH), 116.3 (2xCH), 123.4 (4xCH, BPh_4), 123.4 (2xCH), 124.7 (4xCH), 125.8 (q, $^2J_{13C\text{-}11B}$ = 2.8 Hz, 8xCH, BPh_4), 130.6 (2xCH), 135.9 (2xC), 136.2 (8xCH, BPh_4), 138.7 (2xCH), 146.0 (4xC), 147.3 (2xCH), 152.0 (2xC), 163.1 (q, $^1J_{13C\text{-}11B}$ = 49.3 Hz, 4xC, BPh_4), 181.9 (C) ppm. **^{11}B-NMR** ($CDCl_3$, 100 MHz): δ −6.40 ppm. **IR** (neat): ν 3339, 2961, 1619, 1578, 1466, 1157, 731, 705 cm^{-1}. **HRMS** (ESI): *m/z* calcd for $C_{37}H_{45}CuN_5$ $[M\text{-}BPh_4]^+$: 622.2971; found: 622.2989. **Elemental Anal.** Calcd for $C_{61}H_{65}BCuN_5$: C, 77.73; H, 6.95; N, 7.43; found: C, 77.53; H, 7.12; N, 7.49.

[Cu(IPr)(dpa)][NTf$_2$] 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 $LiNTf_2$ as salt for the final aqueous solution, complex **15** was obtained as a white powder (269 mg, 99% yield). **^{1}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}C-NMR ($CDCl_3$, 100 MHz):** δ 23.9 (s, $4xCH_3$), 24.1 (s, $4xCH_3$), 28.7 (s, 4xCH), 114.9 (s, 2xCH), 116.3 (s, 2xCH), 119.9 (q, *J* = 321 Hz, 2xC, $2xCF_3$ from NTf_2 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),

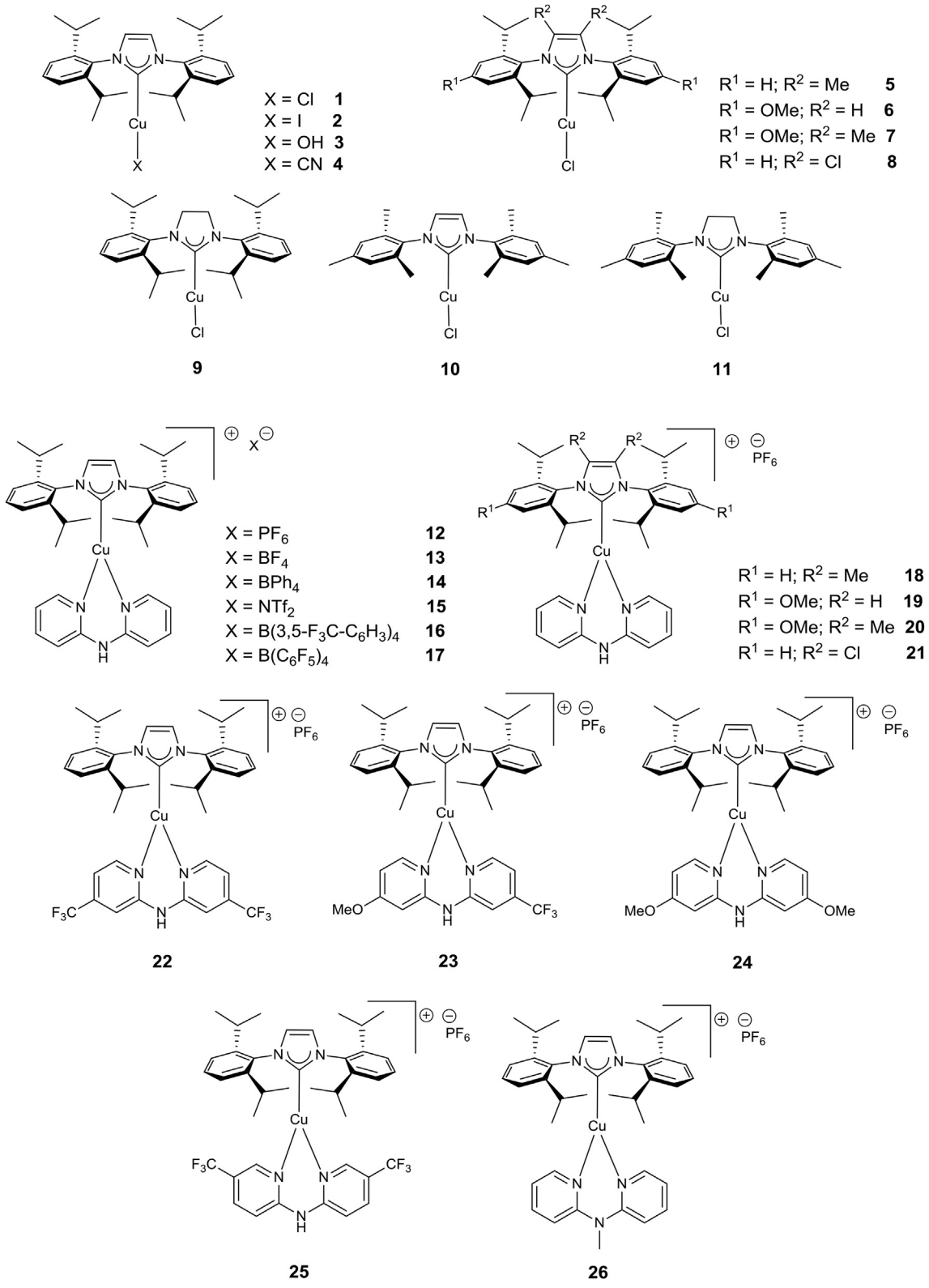


Fig. 1. NHC copper complexes used in this study.

147.5 (s, 2xCH), 153.0 (s, 2xC), 183.0 (s, C) ppm. **^{19}F-NMR ($CDCl_3$, 400 MHz):** δ −78.80 (s, 2xF, 2xCF_3 from NTf_2 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 $C_{37}H_{45}CuN_5$ $[M\text{-}NTf_2]^+$: 622.2971; found: 622.2973. **Elemental Anal.** Calcd for $C_{39}H_{45}CuF_6N_6O_2S_2$: 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-$(CF_3)_2$Ph)$_4$] complex **16**. Following the

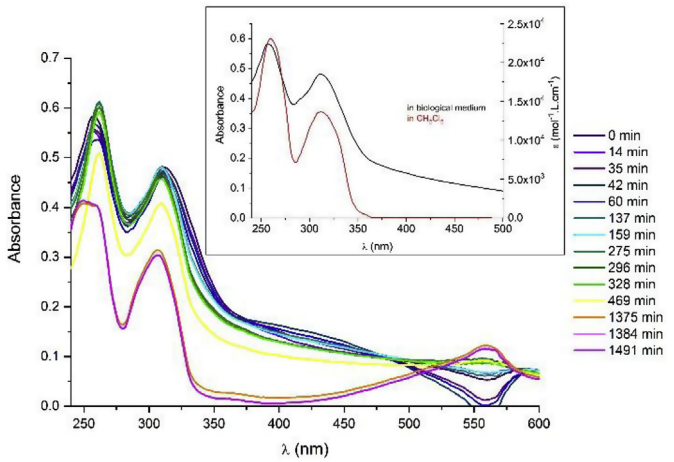


Fig. 2. Stability of **12** followed by UV-vis spectroscopy over 25 h in 1%DMSO/DMEM + GlutaMAX-I™ solution (c = 50 μM) and UV-visible spectra of **12** in dichloromethane solution (c = 100 μM) (red line, inset) and in 1%DMSO/DMEM + GlutaMAX-I™ solution (c = 50 μM) in absence of cell line (dark line, inset). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

general procedure with copper complex **1** (87.8 mg, 0.18 mmol), **dpa** ligand (32.5 mg, 0.19 mmol) and $NaB(3,5\text{-}(CF_3)_2Ph)_4$ as salt for the final aqueous solution, complex **16** was obtained as a white powder (208 mg, 78% yield). **^{1}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, $B(3,5\text{-}(CF_3)_2Ph)_4$), 7.57 (t, J = 7.8 Hz, 2H), 7.70 (*br* s, 8H, $B(3,5\text{-}(CF_3)_2Ph)_4$) ppm. **^{13}C-NMR ($CDCl_3$, 100 MHz):** δ 23.8 ($4xCH_3$), 24.1 ($4xCH_3$), 28.7 (2xCH), 113.4 (2xCH), 117.3 (2xCH), 117.4–117.5 (m, 4xCH, $B(3,5\text{-}(CF_3)_2Ph)_4$), 123.5 (2xCH), 124.5 (q, $^1J_{13C\text{-}19F}$ = 272.5 Hz, $8xCF_3$, $B(3,5\text{-}(CF_3)_2Ph)_4$), 124.7 (4xCH), 128.8 (qq, $^2J_{13C\text{-}19F}$ = 31.4 Hz and $^4J_{13C\text{-}19F}$ = 2.8 Hz, 8xC, $B(3,5\text{-}(CF_3)_2Ph)_4$), 130.7 (2xCH), 134.8 (8xCH, $B(3,5\text{-}(CF_3)_2Ph)_4$), 135.8 (2xC), 139.5 (2xCH), 146.0 (4xC), 148.4 (2xCH), 152.0 (2xC), 161.7 (q, $^1J_{13C\text{-}11B}$ = 49.8 Hz, 4xC, $B(3,5\text{-}(CF_3)_2Ph)_4$), 182.5 (C). ^{19}F NMR ($CDCl_3$, 377 MHz): δ −60.41 ppm. **^{11}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 $C_{37}H_{45}CuN_5$ $[M\text{-}B(3,5\text{-}(CF_3)_2Ph)_4]^+$: 622.2971; found: 622.2959. **Elemental Anal.** Calcd for $C_{69}H_{57}BCuF_{24}N_5$: C, 55.75; H, 3.87; N, 4.71; found: C, 55.62; H, 4.02; N, 4.72.

[Cu(IPr)(dpa)][$B(F_5Ph)_4$] complex **17**. Following the general procedure with copper complex **1** (97.5 mg, 0.20 mmol), **dpa** ligand (36 mg, 0.21 mmol) and $NaB(F_5Ph)_4$ as salt of the final aqueous solution, complex **17** was obtained as a white powder (198 mg, 76% yield). **^{1}H-NMR ($CDCl_3$, 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. **^{13}C-NMR ($CDCl_3$, 100 MHz):** δ 23.8 (s, $4xCH_3$), 24.1 (s, $4xCH_3$), 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, $B(F_5Ph)_4$), 135.9 (s, 2xC), 137.5 (m, 8xC, $B(F_5Ph)_4$), 139.5 (s, 2xCH), 146.0 (s, 4xC), 147.0 (m, 4xC, $B(F_5Ph)_4$), 148.3 (s, 2xCH), 149.3 (m, 8xC, $B(F_5Ph)_4$), 152.2 (s, 2xC), 182.7 (s, C) ppm. **^{19}F-NMR ($CDCl_3$, 400 MHz):** δ −166.8 (t, J = 17.5 Hz, 8xF, $B(F_5Ph)_4$), −163.0 (t, J = 20.5 Hz, 8xF, $B(F_5Ph)_4$), −132.7 (*br* s, 4xF, $B(F_5Ph)_4$) ppm. **^{11}B-NMR ($CDCl_3$, 100 MHz):** δ −16.67 (s, $B(F_5Ph)_4$) ppm. **IR** (neat): ν 2966, 1638, 1515, 1462, 1088, 979, 773 cm^{-1}. **HRMS** (ESI): *m/z* calcd for $C_{37}H_{45}CuN_5$ $[M\text{-}B(F_5Ph)_4]^+$: 622.2971; found: 622.2974. **Elemental Anal.** Calcd for $C_{61}H_{45}BCuF_{20}N_5$: C, 56.26; H, 3.48; N, 5.38; found: C, 56.02; H, 3.65; N, 5.41.

2.2. 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 5.10^4 cells/ml suspension) in 96-well culture plates (TPP, Trasadingen, Switzerland) and incubated for 24 h. 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 h. Then, after removing the culture medium, the blue crystals were dissolved in 100 μL 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 IC_{50} determination (IC_{50} is defined as the concentration reducing cell growth by 50%).

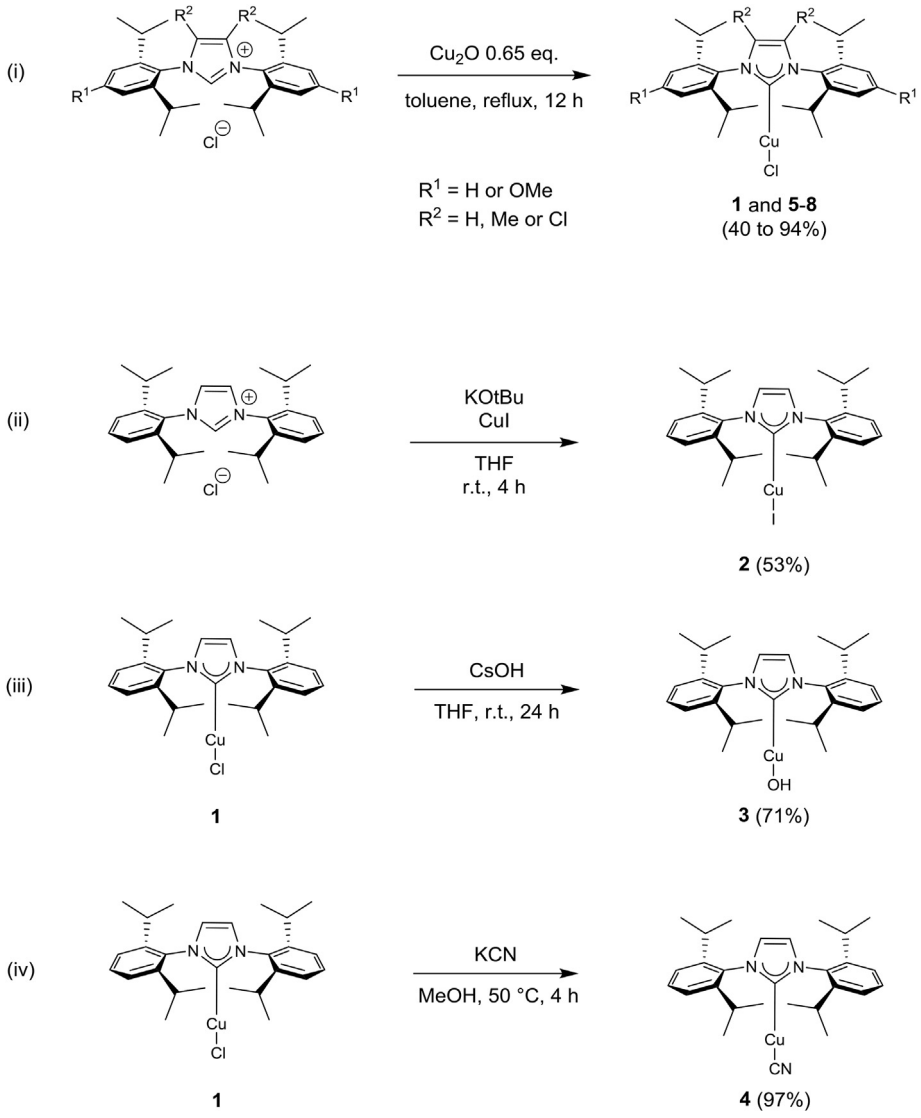
3. Results and discussion

3.1. 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 Cu_2O as metal precursor and the appropriate imidazolium salt and complexes **1**, **5**–**8** were isolated in 40–94% yield (Scheme 1, i) [51,52]. Then, complex **2** was prepared in 53% isolated yield by reacting 1,3-bis(-2,6-di-isopropylphenyl)-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 and Scheme 2, ii). The exchange of the chlorine ligand by a cyano ligand in degassed methanol furnished the complex **4** in 97% isolated yield (Scheme 1, iv).

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 refluxing methanol, followed by an anion metathesis (Scheme 2, i) [38]. The expected cationic (NHC)copper(I) complexes were



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

isolated in 76–99% yields. In details, the synthesis of complexes **12** to **17** were carried out with KPF_6, $NaBF_4$, $NaBPh_4$, $LiNTf_2$, $NaB((3,5\text{-}CF_3)_2\text{-}Ph)_4$, $NaB(F_5Ph)_4$ salts, respectively.

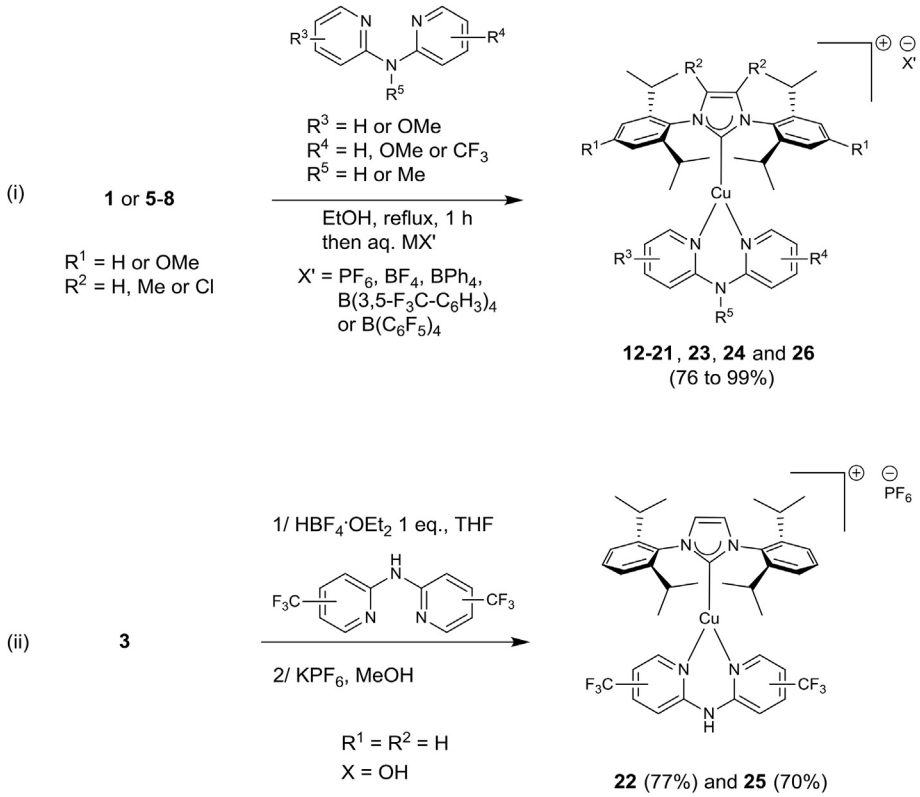
Complexes **18**–**21**, **23**, **24**, and **26**, having different substituted dpa, were obtained with the PF_6^- anion. As previously noticed [38], the synthesis of (NHC) copper(I) complexes coordinated to electron-deficient dpa ligands required a different procedure involving [CuOH(NHC)] **3** as precursor. In details, the cationic copper complexes **22** and **25** were obtained from complex **3** by addition of 1 equivalent of HBF_4OEt_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 2, ii).

3.2. 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 carcimona), 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 [(NHC)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 IC_{50} determination (IC_{50} is defined as the concentration reducing cell growth by 50%). All active complexes were then subjected to their IC_{50} 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 IC_{50} around 100–1000 fold lower than the two reference compounds i.e.



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

Table 1
In vitro IC_{50} 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] IC_{50} of complex **11** previously reported by Gautier et al. on 3000 cells populations [27].

Etoposide and 5-fluorouracil (Table 1, Chart 1).

Concerning the neutral copper complexes **1**–**11**, the IC_{50} were found between 0.015 ± 0.020 μM (for complex **4** and **9**, entries 4 and 9, Table 1) and 0.30 ± 0.05 μM (for complexes **2** and **7**, entries 2 and 7, Table 1) except for complex **11** bearing SIMes ligand with a much higher IC_{50} of 1.45 ± 0.10 than other neutral (NHC)copper(I) complexes. This surprising IC_{50} for complex **11** is 20 times higher than the IC_{50} previously reported by Gautier et al. (0.075 ± 0.002 μM 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. Indeed, complex **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 μM, 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 $B(3,5\text{-}F_3C\text{-}C_6H_3)_4^-$ 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 μM (0.19 ± 0.17 μM, Entry 30, Table 1). Nevertheless, this complex appears more active than Etoposide, 5F-uracil and the cis-platin.

In the second survey, all the copper complexes were studied toward lung carcimona A-549. All complexes were found active, the IC_{50} 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 IC_{50} 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**

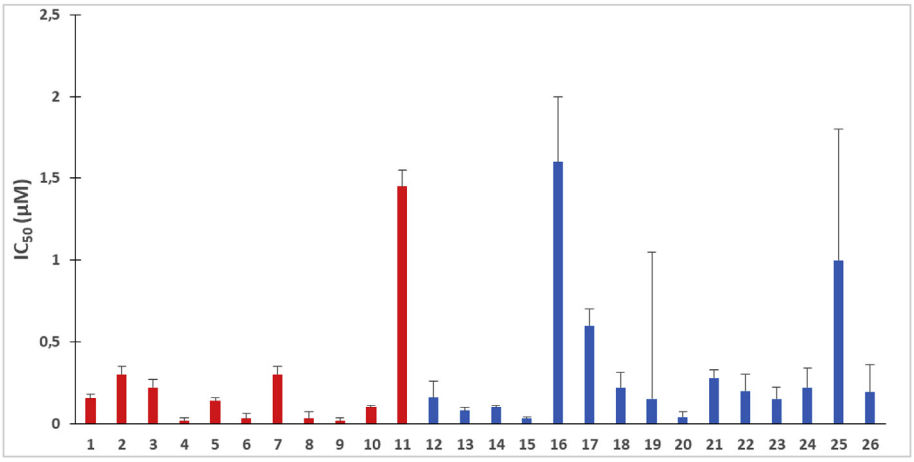


Chart 1. IC_{50} of neutral (NHC)copper(I) complexes **1**–**11** (red) and [Cu(NHC)(dpa)][X] **12**–**26** (blue) on MCF-7.

Table 2
In vitro IC_{50} 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 IC_{50} was not determined.

and **7** presented a higher IC_{50} 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 IC_{50} 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 IC_{50} of 0.6 ± 0.2 μM (Entry 4, Table 2). Cationic copper(I) complexes presented much higher IC_{50} 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 BF_4^-, BPh_4^- and NTf_2^- anions exhibited IC_{50} (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 IC_{50} (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 IC_{50} (complex **26** *vs.* complexes **12**, **22**–**25**), with a value of 0.78 ± 0.10 μM for complex **26** (Entry 28, Table 2).

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 IC_{50} 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 IC_{50} at least 15 fold lower than etoposide (IC_{50} of 6.5 ± 0.8), the most active commercially available reference used in this study (Entries 1–13,

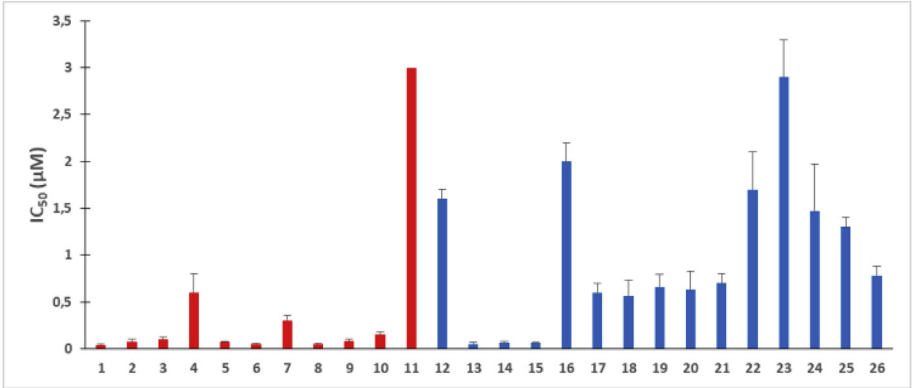


Chart 2. IC_{50} of neutral (NHC)copper(I) complexes **1**–**11** (red) and [Cu(NHC)(dpa)][X] **12**–**26** (blue) on A-549. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
In vitro IC_{50} 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.

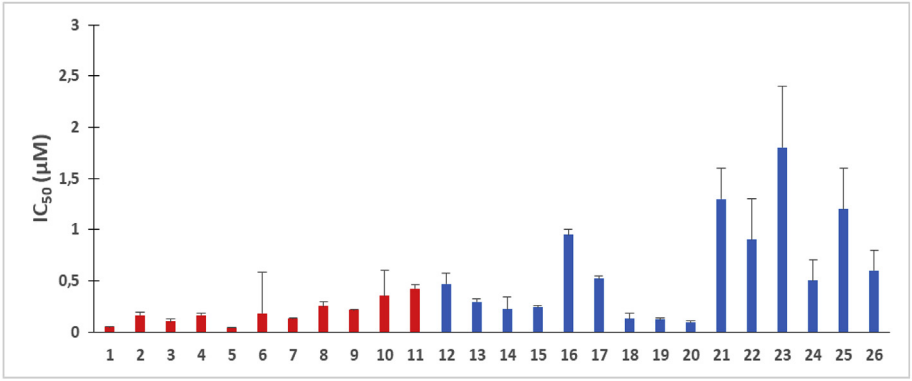


Chart 3. IC_{50} of neutral (NHC)copper(I) complexes 1–11 (red) and [Cu(NHC)(dpa)][X] 12–26 (blue) on SK-Mel-28. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3).

Concerning the cationic copper complexes, the range of IC_{50} 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 IC_{50} (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 IC_{50} (Complex **12** *vs* complex **21**, Entries 14 and 23, Table 3).

The fourth cancer cell line we studied in this work was Glioma HS-683. The IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 ± 0.01 μM was determined, similarly to complexes **5** and **10** (Entry 16 *vs* entries 5 and 10, Table 4).

In the cationic complex series, IC_{50} were not determined for **16** and **17** due to solubility issues above 3 μM (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 ± 0,1 μM 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**

Table 4
In vitro IC_{50} 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 IC_{50} was not determined.

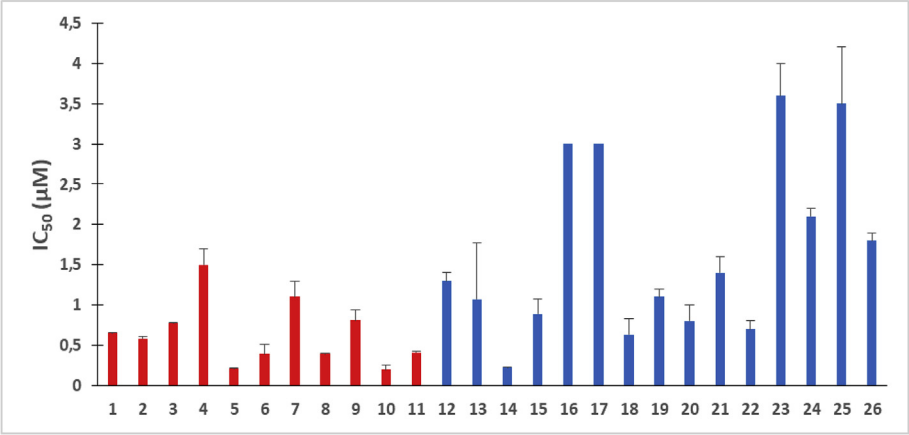


Chart 4. IC_{50} of neutral (NHC)copper(I) complexes **1**–**11** (red) and [Cu(NHC)(dpa)][X] **12**–**26** (blue) on HS-683. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

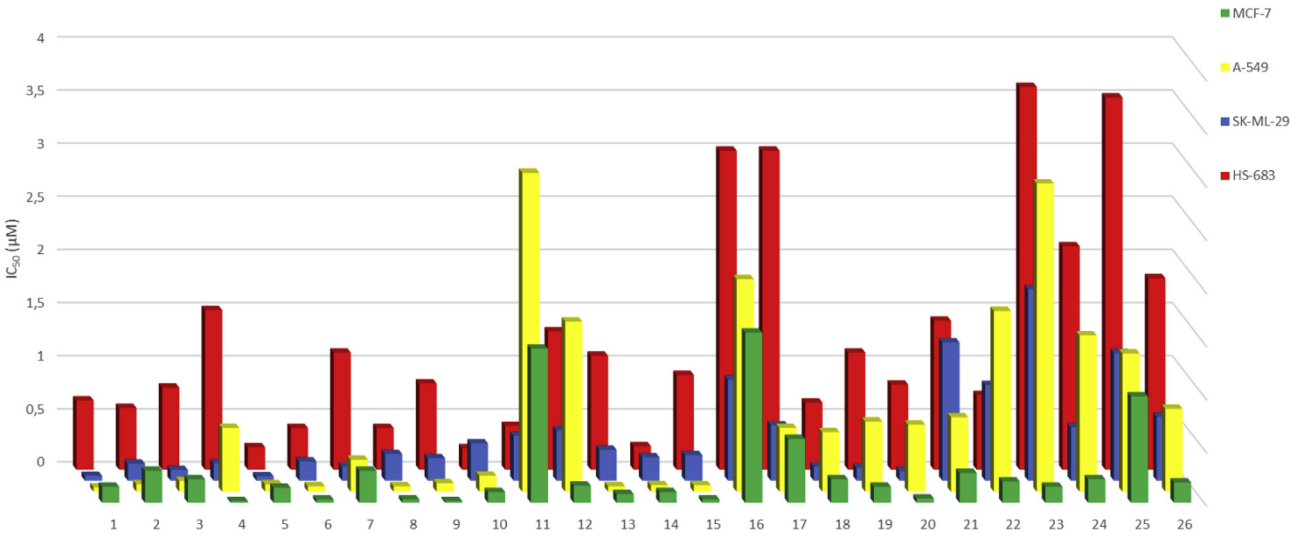


Chart 5. IC_{50} of complexes **1**–**26** on the four cancer cell lines (MCF-7, A-549, SK-Mel-28 and HS-68).

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.

Finally, the evolution and stability of complexes **1** and **12** in DMSO-d_6 solution was evaluated by ^{1}H NMR spectroscopy (Figs. 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 h (Fig. 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, Fig. 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-I™ solution, was only focused in the 250–380 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 (Fig. 2, inset) [37].

Encouragingly, the UV-visible spectra in the 1%DMSO/DMEM + GlutaMAX-I™ solution did not dramatically change in the 260 and 315 nm region within 4–5 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 4–5 h in the biological medium and such stability might open a door to vectorizable copper complexes bearing a dpa ligand.

4. 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 carcimona 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 carcimona 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.

Author contributions

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

Acknowledgements

This work was supported by the "Ministère de la Recherche et des Nouvelles Technologies", CNRS (Centre National de la Recherche Scientifique) and the LABEX SynOrg (ANR-11-LABX-0029). We thank the “Agence Nationale de la Recherche”, within the CSOSG program (ANR-12-SECU-0002-02), the ANR program (ANR-15-CE39-0006), the “Région Basse-Normandie” (M.E.) and Normandie University (G.U.M.) for funding. SG thanks Johnson Matthey for the gift of metals.

Table of Abbreviation

dpa	2,2′-dipyridylamine derivative.
IC_{50}	half growth inhibitory concentration
NHC	*N*-Heterocyclic Carbene

Appendix A. Supplementary data

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jorganchem.2019.04.003.

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