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Platinated Copper(3-Clip-Phen) Complexes as Effective DNA-Cleaving and Cytotoxic Agents

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Abstract: The synthesis and biological activity of three heteronuclear platinum–copper complexes based on 3-Clip-Phen are reported. These rigid complexes have been designed to alter the intrinsic mechanism of action of both the platinum moiety and the Cu(3-Clip-Phen) unit. The platinum centers of two of these complexes are coordinated to a 3-Clip-Phen moiety, an ammine ligand and two chlorides, which are either *cis* or *trans* to each other. The third complex comprises two 3-Clip-Phen units and two chloride ligands bound in a *trans* fashion to the platinum ion. DNA-cleavage experiments show that the complexes are highly efficient nuclease agents. In addition, a markedly difference in their aptitude to perform direct double-strand cleavage is observed, which appears to be strongly related to the ability of the platinum unit to coordinate to DNA. Indeed, complex **6** is unable to coordinate to DNA, which is reflected by its incapability to carry out double-strand breaks. Nonetheless, this complex exhibits efficient DNA-cleavage activity, and its cytotoxicity is high for several cell lines. Complex **6** shows better antiproliferate activity than both cisplatin and Cu(3-Clip-Phen) toward most cancer cell lines. Furthermore, the cytotoxicity observed for **1** is for most cell lines close to that of cisplatin, or even better. Cu(3-Clip-Phen) induces very low cytotoxic effects, but a marked migratory activity. Complex **6** presents DNA-cleavage properties comparable to the one of Cu(3-Clip-Phen), but it does not show any migratory activity. Interestingly, both Cu(3-Clip-Phen) and **6** induces vacuolisation processes in the cell in contrast to complex **1** and cisplatin. Thus, the four complexes cisplatin tested, Cu(3-Clip-Phen), **1** and **6** stimulate different cellular responses.

Keywords: antitumor agents · cisplatin · DNA cleavage · platinum · primer extension

Introduction

Among the different therapeutic strategies to eradicate cancer cells, the development of DNA-targeting drugs is a rising topic of investigations in bioinorganic chemistry.[1,2] The interaction of such drugs with DNA often gives rise to the formation of coordination, covalent or non-covalent adducts, thereby disrupting the transcription and/or replication. Cisplatin and bleomycin are well-known representatives of this category of highly efficient antitumor agents.[3,4] The spectrum of tumors cured by cisplatin and bleomycin is complementary; in some cases, their combined use is more effective, like for testicular cancer therapy.[5]

After the discovery of the antitumor activities of cisplatin in 1965,[6,7] a great deal of effort has been accomplished to determine its mechanism of action. Nowadays, the main target of cisplatin is generally accepted to be DNA. The main adduct formed is the very stable intrastrand GpG cross-link located in the major groove, which is likely to be

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responsible for the antitumor properties of cisplatin.[8,9] However, cisplatin treatment is still accompanied by severe side effects, and by both intrinsic and acquired resistance to the drug.[10–12]

The bleomycin family was isolated from *Streptomyces verticillus* for the first time in 1966.[13] Associated to iron(II) or copper(I), and in the presence of a reductant, the resulting complexes can catalyze the formation of single-strand and double-strand DNA lesions, which are lethal for the cancer cells.[4] This finding has led to the design and preparation of synthetic bleomycin models, such as Cu(3-Clip-Phen) and derivatives.[14–16] Due to strong interactions with DNA, dominated both by electrostatic interactions and partial intercalation, these Clip-Phen derivatives show very high nuclease activities. However, Cu(3-Clip-Phen) is not capable of performing a direct double-strand break (DSB), and it is also not sequence selective.[17,18]

Several strategies have been developed to overcome cisplatin resistance, such as the use of multinuclear platinum complexes,[19,20] compounds exhibiting a *trans* configuration,[21,22] or derivatives containing a second functionality.[23] In the present study a new approach aimed at reducing, or even annihilating cisplatin resistance, is reported where the platinum compounds are directly linked to the DNA-cleaving complex in Cu(3-Clip-Phen) (Figure 1). As a result, the platinum unit plays two roles: i) as an antitumor drug and ii) as an anchor to DNA, thus allowing the Cu(3-Clip-Phen) moiety to perform cleavages in the close proximity to the Pt-DNA adducts. Thereby the possibilities to achieve double-stranded breaks (DSBs), which are highly cytotoxic, are enhanced. Normally, the Cu(3-Clip-Phen) moiety abstracts protons from the minor groove of DNA.[17] Cisplatin is able to bind in the major groove of DNA. These rigid hybrid complexes are not able to interact with the major groove and the minor groove at the same time. Therefore, either the platinum moiety or the Cu(3-Clip-Phen) part will not interact with its ideal site of interaction, thereby changing its intrinsic mechanism of action. Two asymmetric complexes and one symmetric complex having *cis* or *trans* configurations and containing both active entities have now been synthesized, and their nuclease activity and cytotoxicity have been evaluated.

Results and Discussion

Design and synthesis of the complexes: The synthetic pathway for the preparation of the hybrid platinum/copper complexes is depicted in Scheme 1. 3-Clip-Phen (**8**) has been prepared as previously described.[16] Complex **1** is obtained through a two-step process with the initial synthesis of the intermediate species $(NBu_4)[Pt(NH_3)Cl_3]$ (**7**).[24] The second step then consists of the addition of 1 equivalent 3-Clip-Phen to **7**, giving rise to the precipitation of pure product **1**. The preparation of compound **2** first requires the activation of cisplatin via the removal of one chloride anion using 1 equivalent of $AgNO_3$. Next, 3-Clip-Phen is put to react for 2 days at room temperature with the resulting Pt moiety, producing complex $[Pt(3\text{-Clip-Phen})(NH_3)_2Cl]NO_3$ (**9**) as a precipitate. The final step involves the treatment of **9** with a large excess of HCl at 85 °C for 6 h, as earlier described.[25] The pure complex **2** precipitates after neutralization of the reaction mixture with NaOH. Complex **3** is prepared in three synthetic steps. First, $K_2[PtCl_4]$ is treated with $AgNO_3$ to substitute three chloride ligands by water molecules. Reaction of the resulting complex $[Pt(H_2O)_3Cl](NO_3)$ (**10**) with an excess of 3-Clip-

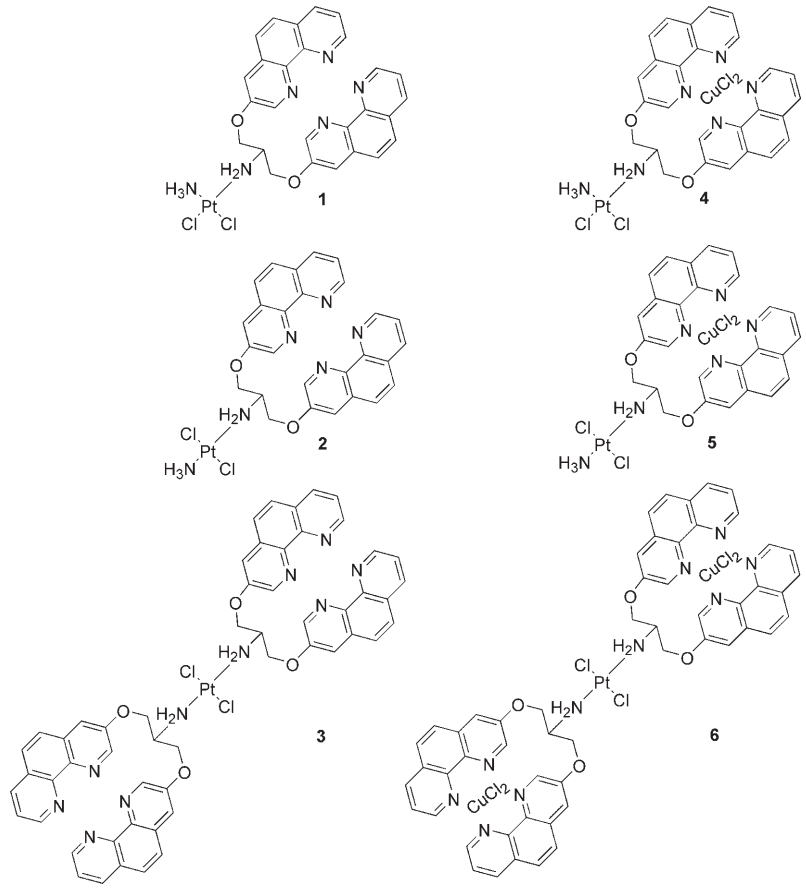
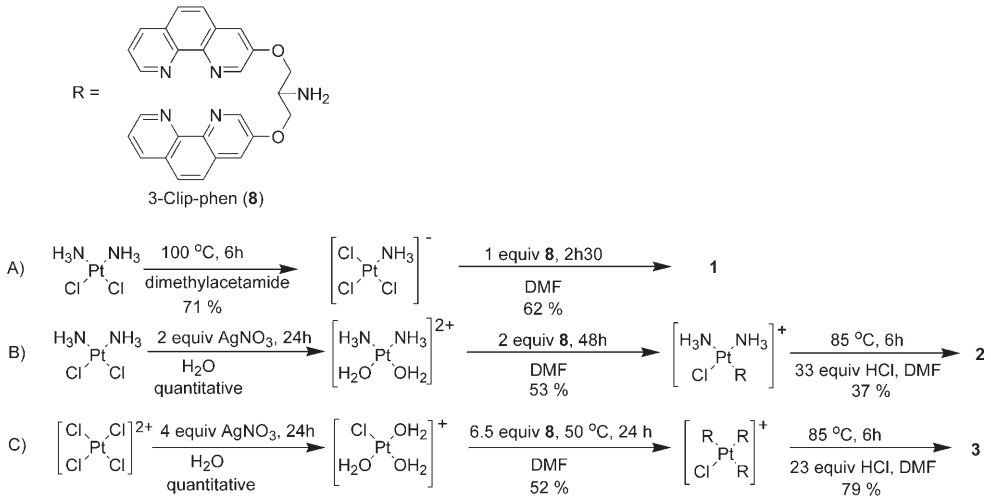


Figure 1. Schematic representations of the platinum complexes **1**–**3** and the heteronuclear platinum-copper complexes **4**–**6**.



Scheme 1. Reaction scheme for the preparation of the platinum complexes **1**–**3**.

Phen at 50 °C for 24 h, yields the compound [Pt(3-Clip-Phen)$_3$Cl](NO$_3$) (**11**) via ligand exchange. **11** is isolated by filtration and washed extensively with water, methanol and diethyl ether, to remove the unreacted products. **11** is subsequently treated with a large excess of HCl at 85 °C for 6 h. Neutralization using NaOH results in the precipitation of the HCl salt of **3**. The coordination of compounds **1**–**3** to copper is achieved in situ with one equivalent of copper(II) chloride per 3-Clip-Phen residue, which produces the heteronuclear complexes **4**–**6**.

DNA-cleavage studies: Relaxation experiments of supercoiled *Φ*X174 DNA (form I) into the circular (form II) and the linear (form III) forms have been performed (agarose gel electrophoresis) to monitor the relative cleavage activities of complexes **4**–**6** in the presence of a reducing agent, in air. For this purpose, the different complexes are pre-incubated for 20 h with supercoiled DNA to allow the binding of the platinum moiety. The cleavage reaction is initiated via the addition of mercaptopropionic acid (MPA) in aerobic conditions (Figure 2).

The absence of nuclease activity for all complexes investigated is observed when no reductant is added (unpublished results). In the presence of MPA, the following order of cleaving ability is observed for 100 nM solutions of the various complexes (Figure 2): **6** (lane 3) ⩾ **4** (lane 6) > Cu(3-Clip-Phen) (lane 12) ⩾ **5** (lane 9). In addition, at a complex concentration of 250 nM, a smear (multi-fragmented DNA) is observed for compounds **6** and in lesser extent for **4** (Figure 2, lanes 4 and 7). In the same experimental conditions, that is, 250 nM, the activity of complex **5** is comparatively reduced (Figure 2, lane 10); however, a 500 nM solution of **5** leads to the cleavage of supercoiled DNA into its circular and linear forms (Figure 3, lane 3). Interestingly, at this concentration, complex **5** generates DNA form III fragments, while the Form I is still present (Figure 3, lane 3). The same feature is observed with complex **4** at a concentration of 100 nM (Figure 3, lane 2).

These important results clearly indicate that these complexes are able to perform direct double-strand cuts.[26] In contrast, Cu(3-Clip-Phen) and complex **6** do not show this form I/form III pattern (Figure 3), suggesting that both complexes are only capable of performing repetitive single-strand cuts. Cleavage experiments with or without pre-incubation time (20 h) have been carried out to further investigate the influence of the platinum–DNA adduct formation

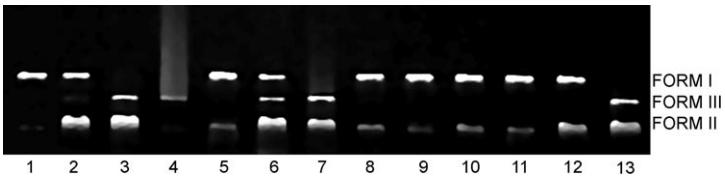


Figure 2. Comparative oxidative cleavage experiments of *Φ*X174 plasmid DNA for complexes **4**–**6** and Cu(3-Clip-Phen) in the presence of 5 mM MPA. Lane 1: control DNA. Lane 2: 50 nM **6**. Lane 3: 100 nM **6**. Lane 4: 250 nM **6**. Lane 5: 50 nM **4**. Lane 6: 100 nM **4**. Lane 7: 250 nM **4**. Lane 8: 50 nM **5**. Lane 9: 100 nM **5**. Lane 10: 250 nM **5**. Lane 11: 50 nM Cu(3-Clip-Phen). Lane 12: 100 nM Cu(3-Clip-Phen). Lane 13: 250 nM Cu(3-Clip-Phen).

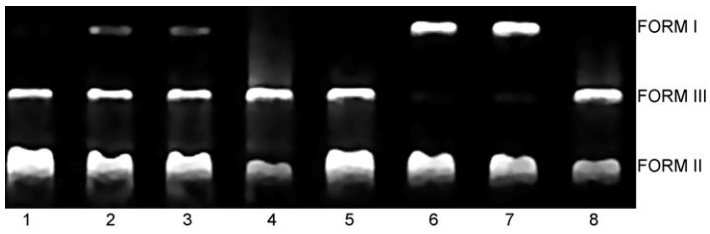


Figure 3. Comparative oxidative cleavage experiments of *Φ*X174 plasmid DNA for complexes **4**–**6** and Cu(3-Clip-Phen) in the presence of 5 mM MPA, with and without incubation time (20 h). Lane 1: 100 nM **6** with 20 h. pre-incubation time. Lane 2: 100 nM **4** with 20 h. pre-incubation time. Lane 3: 500 nM **5** with 20 h. pre-incubation time. Lane 4: 250 nM Cu(3-Clip-Phen) with 20 h. pre-incubation time. Lane 5: 100 nM **6**. Lane 6: 100 nM **4**. Lane 7: 500 nM **5**. Lane 8: 250 nM Cu(3-Clip-Phen).

on the cleaving activities of the corresponding heteronuclear complexes (Figure 3). The coordination of platinum to DNA is a slow process, which typically requires several hours. A pre-incubation time is thus needed to ensure that the platinum moiety of the heteronuclear compound is effectively bound to DNA. Complexes **4** (Figure 3, lanes 2 and 6) and **5** (lanes 3 and 7) show a very strong decrease in activity when no pre-incubation is performed. These remarkable results reveal that the coordination of the platinum moiety to DNA is crucial for their cleaving ability. In contrast, Cu(3-Clip-Phen) (Figure 3, lanes 4 and 8), and complex **6** (Figure 3, lanes 1 and 5) do not show any noticeable differences between the experiments with or without pre-incubation times. The DNA-cleavage behavior exhibited by complex **6** is comparable to the one of Cu(3-Clip-Phen), thus suggesting that the platinum moiety of the bulky complex **6** does not coordinate to DNA. The steric hindrance due to the two coordinated 3-Clip-Phen units and the less reactive *trans*-platinum moiety most likely prohibits the coordination of the platinum ion to the DNA molecule. As a result, the cleaving ability of complex **6** is solely dominated by its two Cu(3-Clip-Phen) entities. This hypothesis is corroborated by the absence of direct double-strand cleavage induced by **6**. Indeed, contrary to complexes **4** and **5**, compound **6** is not able to directly generate form-III fragments of DNA (see Figure 3, lanes 5–7), which shows that, similarly to Cu(3-Clip-Phen), the compound **6** more or less behaves as a "free" cleaving agent.

Analyses with 5′-^{32}P-end-labeled DNA: To further investigate the platinum coordination of the complexes to DNA, high resolution analyses on a 36 bp (base pairs) ODN I–ODN II DNA fragment (Figure 4) have been performed. Sequence of this duplex was chosen to have GG and AG sequences included (which are the two major binding sites of *cis*-Pt^{II} complexes) on the ODN I strand. Complexes were incubated for 20 or 96 h in order to allow the platinum to coordinate to the DNA duplex.

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ODN I   5′-AACTTAATAGTATACATTGGACACAACAACAACAAC-3′
ODN II  3′-TTGAATTATCATATGTTACCTGTGTTGTTGTTGTTG-5′
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Figure 4. 36 bp fragment used for the analysis. The major cisplatin binding sites are identified with bold letters. The sequence of the primer used during these experiments corresponds to the underlined region of ODN II.

Molecules able to irreversibly bind to DNA will retard the rate of migration of the modified ODN, compared to the free ODN, therefore ODN–Pt adducts appear as retarded bands on PAGE. Hence, the amount of formed platinum–ODN I can be quantified. The error of quantifications is between 5 and 10 percent. Complexes **1**, **4**, **2**, **5**, **3** and **6** form 15–20, 15–20, 10–15, 10–15, 5–10 and 5–10% platinum–ODN I adducts, respectively, after an incubation time of 20 h. After 96 h, complexes **1**, **4**, **2**, **5**, and **3** form 50–60, 70–80, 15–20, 5–10, 5–10% platinum–ODN I adducts, respectively, while the quantity of **6** is lower than the detection limit. The amounts of platinum–ODN I adducts developed show that complexes **1** and **4** are the most reactive. Moreover, a significant enhancement of the formation of platinum–ODN I adducts is observed when the incubation time is increased. Comparatively, complexes **2** and **5** are much less reactive, and a significant increase in incubation time does not result in an increase of platinum–ODN I adducts. In the case of complexes **3** and **6**, their platinum parts can be considered as non-binding, since only insignificant quantities (within the experimental error) of platinum–ODN I adducts could be detected. Even after an incubation time of 96 h, the action of complex **6** does not yield any detectable amounts of platinum–ODN I adducts.

Primer extension experiments are performed to investigate the sequence selective binding of platinum complexes **1**, **2** and **3** to DNA (Figure 5).[27–32] TAQ polymerase has proven to effectively stop at platination sites, and is therefore used for these studies. The use of cisplatin results in clear stops of TAQ polymerase, at the expected preferential binding sites (of cisplatin), that is, mainly at the GG, but also at the AG sites (Figure 5, lane 4). The stop is mainly observed at the adenosine preceding the GG site and is distributed over the G base. The binding of complexes **1** and **2** to DNA also results in TAQ polymerase stops at the G and A nucleobases near the GG binding site. The results obtained with complex **3** confirm previous observations that the use of **3** does not lead to TAQ polymerase stops; therefore, the platinum moiety of **3** is evidently not capable of binding to the DNA fragment.

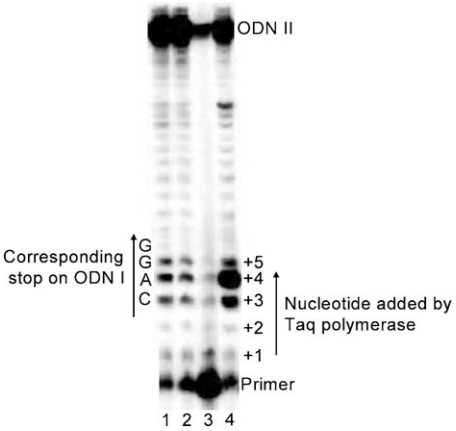

Figure 5. Phosphor image of a DNA-sequencing gel comparing the sequence specificity of cisplatin, **1**, **2** and **3**. All the samples were extended using Taq polymerase, starting from the 5′-end-labelled primer. Lane 1: 10 μM **1**; Lane 2: 10 μM **2**; Lane 3: 10 μM **3**. Lane 4: 3 μM cisplatin. It is noteworthy that the GGAC sites give the sequence of the opposite strand that induced the stop of the primer extension.

Cytotoxicity assays: The cytotoxic activities of complexes **1**–**6**, Cu(3-Clip-Phen), and cisplatin have been determined for breast (MCF7), two glioblastomas (Hs683 and U373), two colorectal (HCT-15 and LoVo) and lung (A549) cancer cell

lines. The results of the activities are summarized in Table 1. A complex with an IC_{50} value higher than 10 μM (>10) is considered as being inactive.

Table 1. In vitro cytotoxicity assays for **1**–**6**, Cu(3-Clip-Phen), and cisplatin, against several cancer cell lines.

	IC_{50} values[a] [μM] Cell lines					
Complexes	Hs683	U373	HCT-15	Lovo	A549	MCF-7
1	7.2	3.3	4.5	4.4	1.2	1.1
2[b]	>10	7.7	>10	>10	>10	5.8
3[b]	>10	5.4	>10	>10	6.2	2.5
4[b]	>10	8.3	>10	>10	>10	1.9
5[b]	>10	>10	>10	>10	>10	0.9
6	2.7	0.6	4.6	0.4	0.9	1.6
Cu(3-Clip-Phen)[b]	>10	>10	>10	>10	>10	>10
cisplatin	0.4	5	10	0.3	1.5	9

[a] IC_{50} = concentration of drug required to eradicate 50% of the cancer cells. [b] An IC_{50} value higher than 10 μM (> 10) is indicative of an inactive compound.

Although unmodified Cu(3-Clip-Phen) has shown antiproliferative activities in a number of cell lines,[26,33] the IC_{50} values herein reported for distinct cell lines are higher than 10 μM (Table 1). Major differences in the cytotoxic activities of the complexes described in the present report are observed. The copper-free complex **1** exhibits IC_{50} values comparable to, or in some cases better than those achieved with cisplatin (Table 1, cell lines U373, HCT-15, A549 and MCF-7). Pt-complex **1** is drastically more cytotoxic than Cu(3-Clip-Phen); however, the corresponding Pt-Cu(3-Clip-Phen) complex **4** shows low cytotoxic activities (except for the MCF-7 cell line), suggesting that its action is mostly governed by its copper component. The IC_{50} values determined for the *trans* complexes **2** and **5** are generally inferior to the ones of cisplatin. However, for the MCF-7 cell line, compound **5** is about ten times more cytotoxic than cisplatin. The *trans*-platinum complex **3** bearing two 3-Clip-Phen ligands shows antiproliferative activities comparable to those of cisplatin toward the U373 and MCF-15 cell lines (Table 1). If copper is coordinated to both 3-Clip-Phen units of complex **3**, the resulting complex **6** becomes a highly efficient cytotoxic agent. Indeed, compound **6** is more cytotoxic than cisplatin toward a number of cell lines (Table 1). Remarkably, the IC_{50} value for the cell line U373 is ten times higher, compared to cisplatin. Complex **6** is the most effective DNA cleaving agent (Figure 2, lanes 3 and 4), which is in total agreement with the cytotoxicity assays. In the same way, the cytotoxicities achieved with complex **5** corroborate its DNA cleaving abilities, as DNA cuts are only observed at very high concentrations (Figure 2, lanes 8–10). The coordination of copper to complexes **1** and **3**, respectively producing complexes **4** and **6**, induces major diversities regarding the corresponding cytotoxic activities. These variations indicate that, either the permeability of the cells, and/or the cellular distribution is affected by the presence of copper ions.

Cellular imaging studies: Cancer cells have an inbuilt urge to survive, so that any genetic change that favors survival against adverse conditions will be selected. As a consequence, some tumors survive exposure to even the most potent therapeutic agents. It should be borne in mind that i) migrating cancer cells (responsible for the formation of metastases) are resistant to pro-apoptotic insults, ii) ≈80% of current drugs used by oncologists to fight cancer are pro-apoptotic agents that display limited efficacy against metastatic cancers, iii) ≈90% of cancer patients die from their metastases, and iv) reducing the level of migration of metastatic cancer cells restores significant sensitivity to pro-apoptotic agents.

In the present study, we report on the fact that platinated Cu(3-Clip-Phen) complexes with weak cytotoxic activity in vitro (as revealed by the MTT colorimetric assay)[34–36] are associated with marked antimigratory effects (as revealed by computer-assisted phase-contrast microscopy),[37–39] and vice versa.

The human A549 non-small-cell-lung cancer (NSCLC) and the human U373 glioblastoma (GBM) models have been used. Both A549 NSCLC[40] and U373 GBM[41] cells are resistant to pro-apoptotic agents. Of the four compounds that have been submitted to these various assays, Cu(3-Clip-Phen) was associated with the weakest cytotoxicity activity (Figure 6 and Table 1), but the highest antimigratory activity (Figure 7). In contrast, the compound that was associated with the highest cytotoxicity activity (Figure 6), that is, **6**, displayed weak antimigratory effects (Figure 7). Cisplatin displayed both moderate cytotoxic (Figure 6) and antimigratory (Figure 7) activity. **1** displayed moderate cytotoxic activity (Figure 6), but no antimigratory activity (Figure 7) may be due to its poor solubility. Indeed, the experiments had to be stopped after 30 h instead of the 72 h-scheduled experiment because the compound markedly precipitated in the culture media (data not shown). Cellular imaging enables specific morphological features to be evidenced in terms of mechanisms of action of antitumor drugs.[34,37,40] The current study clearly indicates that cisplatin and **1** induced no vacuolisation processes in the cytoplasm of A549 (Figure 8) and U373 (data not shown) cancer cells. In contrast, both Cu(3-Clip-Phen) and **6** induced such vacuolisation in A549 (Figure 8) and U373 (data not shown) cancer cells. These vacuolisation processes could relate for example to lysosomal membrane permeabilization-related cell death[40] or to autophagy.[34] When comparing the data from Figures 6–8, it seems that the four compounds investigated exert their antitumor activity through distinct mechanisms of action. Indeed: i) cisplatin displays moderate cytotoxic ac-

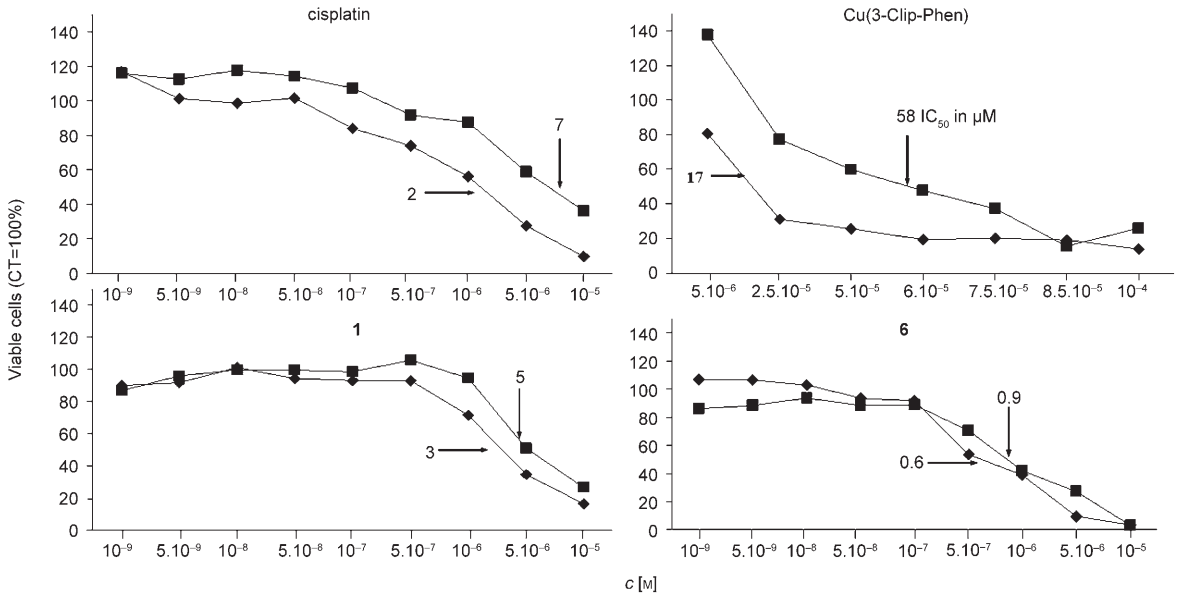

Figure 6. In vitro antiproliferative effects contributed by four tested compounds relative to the two tested human cancer cell lines, that is, A549 (NSCLC cell line, ■) and U373-MG (glioblastoma cell line, ◆). Each sample was assessed in sextuplicate and the data are presented as mean values (SEM lower than 5%). The data are expressed as percentage of viable cells at the end of the treatment with the untreated control condition set at 100%. The IC_{50} values (in µM; representing the concentrations at which each compound reduced the overall growth rate of the cell lines by 50% after three days of treatment) are pointed out by the arrows.

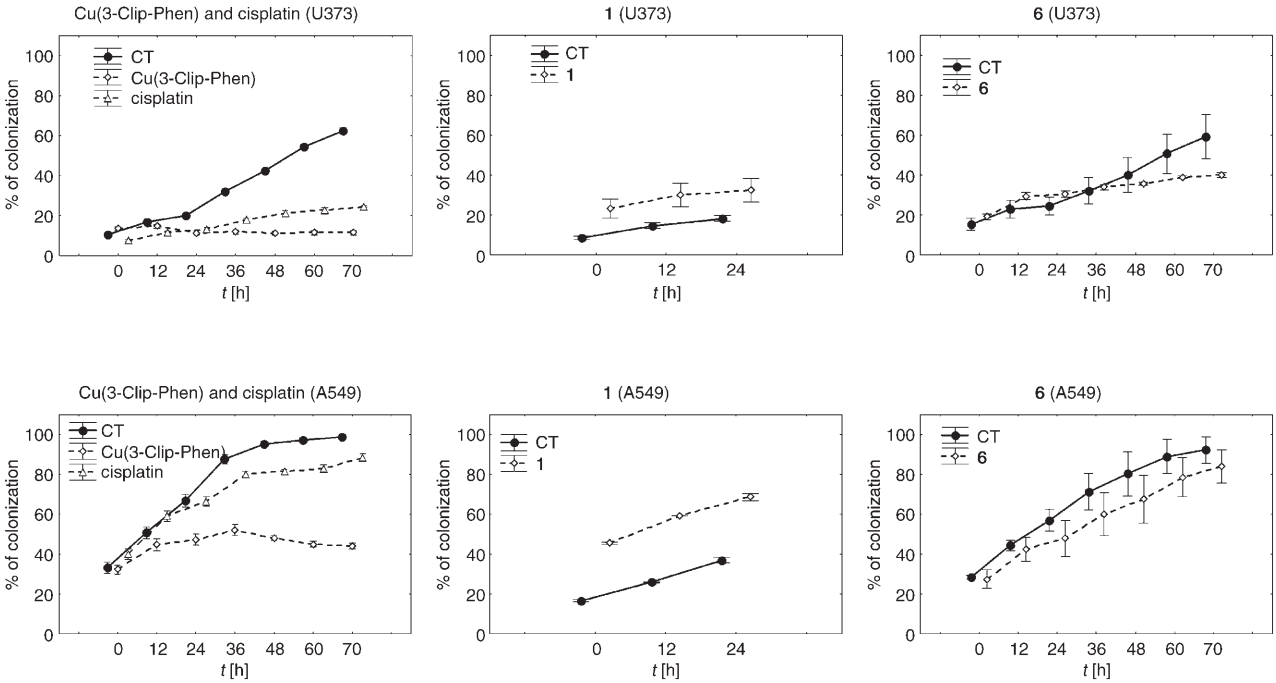

Figure 7. Cellular-imaging-based quantification of the colonization process of a total field under monitoring by treated *versus* untreated cells during the experiment. The software developed in our laboratory enables the quantification, at each observation time, of the percentage of the colonized area by the cells in presence or absence of test compounds. The percentage of colonized area was followed over time, as indicated. Control (untreated) condition is presented by filled black dots and black line while treated conditions are presented by open symbols and dotted lines. The experiment is performed in triplicate and the data are presented as mean + SEM. Complex concentrations used for this experiment are 5 µM of cisplatin, 50 µM of Cu(3-Clip-Phen), 5 µM of **1** and 0.5 µM of **6**.

tivity (Figure 6), moderate antimigratory activity (Figure 7) and no vacuolisation processes in A549 NSCLC and U373 GBM cells (Figure 8), which is compatible with a pro-apoptotic effect. ii) Cu(3-Clip-Phen) displays weak cytotoxic ac-

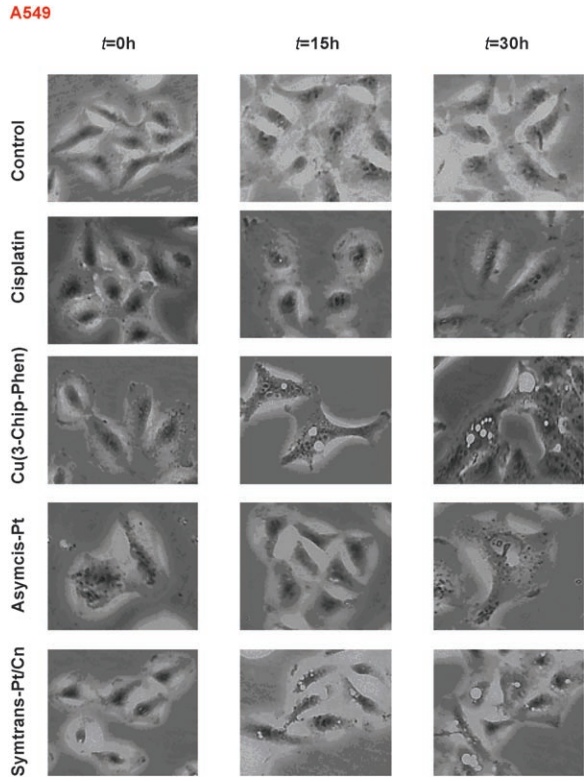

Figure 8. Illustrations of the morphological features observed during the cellular imaging of the A549 cell line. Still pictures were taken from the same scratch wound experiment presented on Figure 7. Complex concentrations used for this experiment are 5 μM of cisplatin, 50 μM of Cu(3-Clip-Phen), 5 μM of **1** and 0.5 μM of **6**.

tivity (Figure 6), marked antimigratory activity (Figure 7) and marked vacuolisation processes in A549 NSCLC and U373 GBM cells (Figure 8), which is compatible with pro-autophagic and/or LMP-related cell death effects.[34,40] iii) **1** displays moderate cytotoxic activity (Figure 6), no antimigratory activity (Figure 7) and no vacuolisation processes in A549 NSCLC and U373 GBM cells (Figure 8). iv) **6** displays potent cytotoxic activity (Figure 6), no antimigratory activity (Figure 7) and marked vacuolisation processes in A549 NSCLC and U373 GBM cells (Figure 8).

In summary, of the four compounds studied, Cu(3-Clip-Phen) merits further investigations, including *in* vivo ones as a single agent or in combination with conventional cytotoxic agents (as for example taxol, irinotecan, temozolomide and gemcitabine) in orthotopic xenografts models of human pancreas,[34] NSCLC,[34] breast[34] and GBM[41] cancers, in order to investigate the actual potential of this compound as a novel anticancer drug.

Conclusion

Three bifunctional Pt–Cu complexes are reported. These complexes contain a(n) (a)symmetric platinum moiety with different configurations (*cis* or *trans*) which can bind to DNA, and one or two Cu(3-Clip-Phen) groups that can cleave the DNA strands. The three complexes show excellent DNA-cleavage activities. Complex **6** is the pre-eminent nuclease active agent, whose activity is significantly superior to the one of Cu(3-Clip-Phen). The platinum moieties of the two latter complexes are able to bind to the DNA strands, whereas complex **3** acts as two single Cu(3-Clip-Phen) units, probably owing to well-known lower activities of *trans* complexes combined with the bulkiness of the two 3-Clip-Phen ligands. Complexes **1** and **2** show binding specificities analogous to the those of cisplatin, thus preferentially binding at GG sites. Complex **6** appears to be the most active cleaving agent. In addition, its cytotoxicity is superior to that all other complexes, including cisplatin. Cu(3-Clip-Phen) has the strongest antimigratory effect followed by cisplatin, **1** and **6** do not show any antimigratory effect. Cu(3-Clip-Phen) and **6** induce vacuolisation processes inside the cytoplasm of the cells in contrast to cisplatin and **1**. Appealingly, cisplatin, Cu(3-Clip-Phen), **1** and **6** exert their antitumor activity through distinct mechanisms of action.

Experimental Section

Synthesis of complex *cis*-[Pt(3-Clip-Phen)(NH_3)Cl_2] (1): The preparation of {(C_4H_9)$_4$N}[Pt(NH_3)Cl_3] (**7**) was carried out as previously described.[24] {(C_4H_9)$_4$N}[Pt(NH_3)Cl_3] (**7**) (0.433 g, 0.77 mmol) and (C_4H_9)$_4$NCl (0.36 g, 1.30 mmol) were dissolved in MeOH (4 mL). One equivalent of 3-Clip-Phen (**8**) (0.346 g, 0.77 mmol) dissolved in dimethylformamide (DMF, 3 mL) was added, resulting in an immediate precipitation of a yellow compound. The suspension was stirred for 1 h 30 at room temperature in the dark. The precipitate was isolated on a glass filter and washed with methanol (3×10 mL) and diethyl ether (2×10 mL) respectively. The yellow product was dried in air. Yellow powder; 0.349 g, yield: 62%; ^{1}H NMR (300 MHz, [D$_7$]DMF): δ = 9.11 (br, 2H), 8.92 (br, 2H), 8.46 (br, 2H), 8.19 (br, 2H), 7.71 (br, 2H), 4.90 (d, 4H), 4.13 ppm (m, 1H); ^{195}Pt NMR ([D$_7$]DMF): δ = −2161 ppm; elemental analysis calcd (%) for $C_{27}H_{24}Cl_2N_6O_2Pt\cdot0.2(C_4H_9)_4NCl$: C 46.14, H 4.00, N 11.05; found: C 46.58, H 4.26, N 11.14.

Synthesis of complex *trans*-[Pt(3-Clip-Phen)(NH_3)Cl_2] (2)

***Step 1*: Synthesis of [Pt(3-Clip-Phen)(NH_3)$_2$Cl]Cl (9)**: Cisplatin (0.400 g, 1.33 mmol) was dissolved in MilliQ water (100 mL) in the dark. Two equivalents of $AgNO_3$ (0.456 g, 2.66 mmol) were added dropwise. The solution was stirred overnight in the dark. The white AgCl precipitate was filtered off. Compound **8** (0.717 g, 1.60 mmol) dissolved in DMF (5 mL) was added dropwise to the filtrate, and the resulting reaction mixture was stirred during 2 d in the dark. The greenish precipitate [Pt(NH_3)$_2$(3-Clip-Phen)Cl]NO_3 was collected and washed with CH_2Cl_2/5% methanol (3×15 mL), ethanol (3×15 mL), and diethyl ether (3×15 mL) to remove unreacted reagents. Greenish powder; 0.527 g, yield: 53%; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.07 (d, 2H, J=8.94 Hz), 8.88 (d, 2H, J=2.60 Hz), 8.44 (d, 2H, J=8.02 Hz), 8.06 (d, 2H, J=2.73 Hz), 7.99–7.92 (m, 4H), 7.70 (dd, 2H, J=7.99, 4.32 Hz), 4.63 (br, 4H), 4.26 ppm (br, 1H); ^{195}Pt NMR ([D$_6$]DMSO): δ = −2403 ppm.

***Step 2*: Synthesis of *trans*-[Pt(3-Clip-Phen)(NH_3)Cl_2] (2)**: Compound **9** (0.1231 g, 0.16 mmol) was dissolved in DMF (3 mL). 33 equiv HCl (0.437 mL, 5.28 mmol) were added to this solution in the dark. A precipitate occurred instantly. The suspension was refluxed at 85 °C for 6 h in the dark. After cooling the reaction, NaOH was added in excess to neutralize the HCl. The orange precipitate was filtered off and washed MilliQ H_2O (3×5 mL), methanol (5 mL), and diethyl ether (5 mL). Orange powder; 0.043 g, yield: 37%; ^{1}H NMR (300 MHz, [D$_7$]DMF): δ = 9.09 (br, 2H), 8.94 (br, 2H), 8.48 (br, 3H), 8.19 (br, 1H), 7.72 (br, 2H), 5.17–4.92 (br, 4H), 4.46 ppm (br, 1H); ^{195}Pt NMR ([D$_7$]DMF): δ =

−2169 ppm; ESI-MS: *m/z*: calcd for $C_{27}H_{24}N_6O_2Pt^+$: 735.0, 736.0, 737.0, 738.0, 739.0; found: 734.0, 735.0, 736.0, 737.0, 738.0 $[M-Cl+H_2O+Na]^+$; elemental analysis calcd (%) for $C_{27}H_{24}Cl_2N_6O_2Pt\cdot 4HCl$: C 37.00, H 3.22, N 9.59; found: C 37.09, H 3.45, N 9.09.

Synthesis of complex *trans*-[Pt(3-Clip-Phen)$_2$Cl$_2$] (3)

Step 1: **Synthesis of [Pt(3-Clip-Phen)$_3$Cl]NO$_3$ (11)**: K_2PtCl_4 (500 mg, 1.20 mmol) was dissolved in MilliQ water (3 mL). A solution of $AgNO_3$ (818 mg, 4.80 mmol, 4 equiv) in MilliQ H_2O (7 mL) was added. The resulting solution was stirred in the dark overnight. The precipitate of AgCl was removed and the clear yellowish filtrate was evaporated until the yellow product $[Pt(H_2O)_3Cl](NO_3)$ (**10**) precipitated. The complex was filtered and washed with cold MilliQ water (5 mL), ethanol (3× 5 mL), and diethyl ether (3×5 mL). 6.5 equiv **8** (864 mg, 1.93 mmol) were dissolved in DMF (10 mL). A solution of $[Pt(H_2O)_3Cl](NO_3)$ (**10**) (111 mg, 0.32 mmol) dissolved in MilliQ H_2O (20 mL) was added dropwise to the 3-Clip-Phen solution in the dark. A yellow precipitate immediately appeared. The mixture was stirred at room temperature for about 3.5 h, before being heated to 50 °C with an oil bath. The reaction mixture was stirred for 21 h at this temperature, in the dark. The dense yellow precipitate was filtered and washed with MilliQ H_2O (3×10 mL), methanol (3×10 mL), and diethyl ether (3×10 mL). The compound turned brown during the drying under reduced pressure. Brown powder; 1.02 g, yield: 52 %; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.02 (d, 2H, J= 3.06 Hz), 8.81 (br, 2H), 8.48 (d, 2H, J=7.86 Hz), 8.06 (br, 2H), 7.98–7.89 (m, 4H), 7.74 (dd, 2H, J=7.91, 4.42 Hz), 4.40 (br, 4H), 3.74 ppm (br, 1H); ^{195}Pt NMR ([D$_6$]DMSO): δ = −3064 ppm (1 solvent DMSO coordinated); important IR absorptions (neat): $\tilde{\nu}$=3368 (br), 1590 (s), 1428 (s), 1326 (br), 1238 (s), 1039 (s), 1014 cm^{-1} (s).

Step 2: **Synthesis of complex *trans*-[Pt(3-Clip-Phen)$_2$Cl$_2$] (3)**: [Pt(3-Clip-Phen)$_3$Cl](NO$_3$) (**11**) (148.5 mg, 0.09 mmol) was suspended in DMF (3 mL), 23 equiv HCl (200 μL, 1.53 mmol) were added to this solution. The remaining solution was heated at 85 °C for 6 h in the dark. The HCl was neutralized with NaOH. The consequent brown precipitate was filtered and washed with MilliQ H_2O (3×10 mL), methanol (3×10 mL), and diethyl ether (3×10 mL), and dried in air. Brown powder; 0.083 g, yield: 79 %; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.03 (d, 2H, J= 3.03 Hz), 8.81 (d, 2H, J=2.55 Hz), 8.46 (d, 2H, J=7.96 Hz), 8.09 (br, 2H), 7.95 (m, 4H), 7.72 (dd, 2H, J=8.00, 4.33 Hz), 4.55 (br, 4H), 4.06 ppm (br, 1H); ^{195}Pt NMR ([D$_6$]DMSO): δ = −3488 ppm (2 DMSO molecules coordinated); important IR absorptions (neat): $\tilde{\nu}$=3370 (br), 1590 (s), 1428 (s), 1359 (s), 1328 (s), 1240 (s), 1040 (s), 1021 cm^{-1} (s); elemental analysis calcd (%) for $C_{54}H_{42}Cl_2N_{10}O_4Pt\cdot 8HCl$: C 44.65, H 3.47, N 9.64; found: C 43.45, H 3.44, N 9.88.

Cleavage studies: The solutions of the complexes were prepared as 1 mM solutions in DMSO, and diluted to the appropriate concentration with water, typically corresponding to a concentration four times higher than the final concentration of the cleavage experiment. 5 μL of complex solution were added to 10 μL of supercoiled *Φ*X174 DNA (Invitrogen, 7 nM, 40 μM base pairs) in 6 mM NaCl, 20 mM sodium phosphate buffer (pH 7.2), and incubated for 20 h at 37 °C. To initiate the cleavage, 5 μL of a 20 mM mercaptopropionic acid solution in water were added, and the resulting reaction mixture was incubated at 37 °C for 1 h. The reaction was quenched at 4 °C, followed by the addition of 4 μL of loading buffer (bromophenol blue) prior to its loading on a 0.8 % agarose gel containing 1 μg mL^{-1} of ethidium bromide. The gels were run at a constant voltage of 70 V for 90 min in TBE buffer containing 1 μg mL^{-1} of ethidium bromide. The gels were visualized under a UV transilluminator, and the bands were quantified using a BioRad Gel Doc 1000 apparatus interfaced with a computer.

Analysis of platinum adducts by high resolution polyacrylamide gel electrophoresis: The ODNs I, II and the primer were purchased from Eurogentec, and purified on a 15 % polyacrylamide gel. The concentrations of single-stranded ODNs were determined by UV titration at 260 nm.[42] The ODNs were end-labeled with ^{32}P using standard procedures with T_4 polynucleotide kinase (New England BioLabs) and [γ-^{32}P]ATP for the 5′-end, before being purified on a MicroSpin G25 column (Pharmacia).[43]

Analysis of the platinum–DNA adducts: 5′-end labeled ODN I (2 μM) was annealed to 1 equiv of its complementary strand ODN II in 1100 μL of Tris-HCl (20 mM, pH 7.2) by heating to 90 °C for 5 min, followed by slow cooling to room temperature. Then 60 μL of this solution was incubated with 60 μL of complex solution (6 μM cisplatin or 20 μM of complexes **1**–**3**) for 20 or 96 h at 37 °C followed by precipitation with 100 μL of sodium acetate buffer (3 M, pH 5.2) and 1300 μL of cold ethanol. Pellets were rinsed with ethanol and lyophilized. Platinum–DNA adducts were analyzed by denaturing 20 % polyacrylamide gel electrophoresis followed by phosphor imagery.

Primer extension experiments: ODN I (2 μM) was annealed to ODN II (2 μM) in 1100 μL of Tris-HCl (20 mM, pH 7.2) by heating to 90 °C for 5 min, followed by slow cooling to room temperature. Then 60 μL of this solution was incubated with 60 μL of complex solution (6 μM cisplatin or 20 μM of complexes **1**–**3**) for 20 h at 37 °C followed by precipitation with 100 μL of sodium acetate buffer (3 M, pH 5.2) and 1300 μL of cold ethanol. Pellets were rinsed with ethanol and lyophilized. For primer extension, an aliquot of this solution (0.25 μM) was annealed with 5′end labeled primer (0.25 μM) and 1 equiv of ODN I (0.25 μM) in the enzyme buffer before the addition of 250 μM dGTP, dCTP, dATP and dTTP and 2.5 units TAQ polymerase (final concentrations are given, the total volume was 10 μL). The samples were reacted at 37 °C for 120 min followed by the addition of 1 μL of Na_2H_2edta (0.2 M). Then, 5 μL samples were analyzed by denaturing 20 % polyacrylamide gel electrophoresis and subsequent phosphorimagery. Maxam and Gilbert sequencing scale, including a final scale of T4 polynucleotidekinase digestion to remove 3′end-phosphates, was used to analyze DNA fragments.[44]

Cytotoxicity tests: The experimental procedure described below has been used for the following cell lines: Hs683, U373MG, HCT-15, LoVo, MCF-7 and A549.

Hs683 and U-373MG: glioblastomas

HCT-15 and LoVo: colorectal cancers

A549: lung cancer

MCF-7: breast cancer

The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco) supplemented with 5 % fetal calf serum (FCS). All the media were supplemented with a mixture of 0.6 mg mL^{-1} glutamine (Gibco), 200 IU mL^{-1} penicillin (Gibco), 200 IU mL^{-1} streptomycin (Gibco), and 0.1 mg mL^{-1} gentamycin (Gibco). The FCS was heat-inactivated for 1 h at 56 °C.

The MTT test is an indirect technique, which allows the rapid measurement (5 d) of the effect of a given product on the global growth of a cell line.[39] This test is based on the measurement of the number of metabolically active living cells able to transform the yellowish MTT product (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) into the blue product, formazan, via mitochondrial reduction performed by living cells.[39] The amount of formazan obtained at the end of the experiment is measured with a spectrophotometer, and is therefore directly proportional to the number of living cells at that moment. The measurement of the OD (Optical density) provides a quantitative measurement of the effect of the product investigated as compared to control (untreated cells), and enables it to be compared to other reference products.[39]

The cells are put to grow in flat-bottomed 96-well micro-wells with 100 μL of cell suspension per well and between 1,000 and 5,000 cells/well depending on cell type. Each cell line is seeded in its own cell culture medium. After a 24 h period of incubation at 37 °C, the culture medium is replaced by 100 μL of fresh medium in which the substance to be tested has been dissolved at the different concentrations required. In our experiments, the 6 compounds (complexes **1**–**6**, Cu-3-Clip-Phen and cisplatin) were tested at 10^{-5} M to 10^{-9} M concentrations with $^1/_2$ log steps. Each experimental condition is carried out in six different wells. After 72 h of incubation at 37 °C with the drug (experimental conditions) or without the drug (control), the medium is replaced by 100 μL MTT at the concentration of 1 mg mL^{-1} dissolved in RPMI. The micro-wells are then incubated for 3 h at 37 °C and centrifuged at 400 G for 10 min. The MTT is removed, and the formazan crystals formed are dissolved in 100 μL of DMSO. The micro-wells are then shaken for 5 min and read on a spectrophotometer at 2 wavelengths (570 nm: the maximum formazan absorb-

ance wavelength; 630 nm: the background noise wavelength).[39] The IC_{50} value of the 3-Clip-Phen ligand is for: Hs683; 8.4 μM, U373MG; 1 μM, HCT-15 > 10 μM, LoVo; 8.6 μM, A549; 5 μM and MCF-7; 4.5 μM.

Quantitative videomicroscopy for cellular imaging: The human NSCLC A549 (ATCC code CCL-185) and glioblastoma (GBM) U373-MG cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The trypsin-EDTA, fetal bovine serum, cell culture media and supplements were obtained from GibcoBRL (Invitrogen SA, Merelbeke, Belgium). The cells were maintained as previously described in the literature.[40,41]

The tumor cells were incubated for 72 h in the presence (or absence–control) of the various drugs. The drug concentrations ranged between 10^{-9} and 10^{-5} M (with half-log concentration increases). The experiments were carried out in sextuplicate.

Human A549 NSCLC and U373-MG glioblastoma cell migration and proliferation with and without treatments were characterized in vitro by quantitative videomicroscopy, as previously described.[34,37,40] Cellular imaging is a technique of cell observation in time, which allows to evaluate the effect of a given product on the morphology, the motility, the death and proliferation of given cell lines. The cells are maintained alive in closed flasks containing buffered medium at a controlled temperature of 37 ± 0.1 °C during the time required for the experiment. They are observed with the help of a phase-contrast microscope on which a CCD camera is placed. A control monitor placed between the camera and the computer makes it possible to visualize at all times what is happening in the flask. A picture is taken every 4 min. Different parameters can be evaluated such as the proliferation, the motility, the cell death or the morphology of cells in the absence or presence of test compounds. Colonization assay is based on the quantification of the colonization process of a total field under monitoring by treated versus untreated cells during the experiment. The software developed in our laboratory enables us to quantify, at each observation time, the percentage of the colonized area by the cells in presence or absence of test compounds.

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