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Year: 2020

Version: Accepted version (Final draft)

CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

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To be cited as: *Chem. Eur. J.* 10.1002/chem.201905325

Link to VoR: http://dx.doi.org/10.1002/chem.201905325

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10.1002/chem.201905325

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Luminescent Pt^{II} and Pt^{IV} Platinacycles with Anticancer Activity Against Multiplatinum-Resistant Metastatic CRC and CRPC Cell Models

Ariadna Lázaro,[+] Cristina Balcells,[+] Josefina Quirante, Josefa Badia, Laura Baldomà, Jas S. Ward, Kari Rissanen, Mercè Font-Bardia, Laura Rodríguez, Margarita Crespo * and Marta Cascante *

Abstract: Platinum-based chemotherapy persists to be the only effective therapeutic option against a wide variety of tumours. Nevertheless, the acquisition of platinum resistance is utterly common, ultimately cornering conventional platinum drugs to only palliative in many patients. Thus, encountering alternatives that are both effective and non-cross-resistant is urgent. In this work, we report the synthesis, reduction studies and luminescent properties of a series of cyclometallated (C,N,N') Pt^{IV} compounds derived from amine-imine ligands, and their remarkable efficacy at the high nanomolar range and complete lack of cross-resistance, as an intrinsic property of the platinacycle, against multiplatinum-resistant colorectal cancer (CRC) and castration-resistant prostate cancer (CRPC) metastatic cell lines generated for this work. We have also determined that the compounds are effective and selective for a broader cancer panel, including breast and lung cancer. Additionally, selected compounds have been further evaluated, finding a shift in their antiproliferative mechanism towards more cytotoxic and less cytostatic than cisplatin against cancer cells, being also able to oxidize cysteine residues and inhibit topoisomerase II, thereby holding great promise as future improved alternatives to conventional platinum drugs.

Platinum-based chemotherapy is often the only effective treatment against a broad spectrum of tumors, even if their success is limited by side-effects and resistance. Octahedral Pt^{IV} compounds have been shown to be a very promising kind of prodrugs since they are kinetically inert compared to Pt^{II} analogues and the two extra coordination positions allow the tuning of their properties.[1],[2],[3] ,[4] In particular, multiple action Pt^{IV} prodrugs derived from cisplatin have attracted much attention in recent years,.[5],[6] [7]

On the other hand, cyclometallated Pt^{II} compounds containing bidentate (C,N) or tridentate (C,N,N') ligands display interesting properties.[8],[9],[10] The presence of a σ(Pt-C) bond increases the stability of these compounds, thus allowing them to reach the cell unaltered. In addition, covalent coordination to DNA is favoured since the strong Pt-C bond increases the lability of the ligand in a *trans* position. Moreover, the presence of aromatic planar groups might favour intercalative binding to DNA through non-covalent π-π stacking interactions. Furthermore, several cyclometallated Pt^{II} anticancer agents exhibit luminescence properties which make them potential luminescent probes for DNA in living cells and also allows easy tracing of their cellular uptake and distribution by fluorescence microscopy.[11],[12] Surprisingly, little attention has been devoted to Pt^{IV} compounds containing a metallacycle in spite of their promising properties.[13]

Within the last years, we have attempted to optimize the ligand design of cyclometallated Pt^{II} and Pt^{IV} compounds to maximize their efficacy and selectivity against cancer cells. Indeed, the present work represents a definitive milestone for the optimization of the general formulae [PtX(C,N,N')] and [PtXYZ(C,N,N')], respectively, in which (C,N,N') is a terdentate ligand and X, Y, Z are different ligands such as halido or C-donor ligands.[14],[15],[16], An additional interesting feature of these (C,N,N')-cycloplatinated compounds relies on their luminescence

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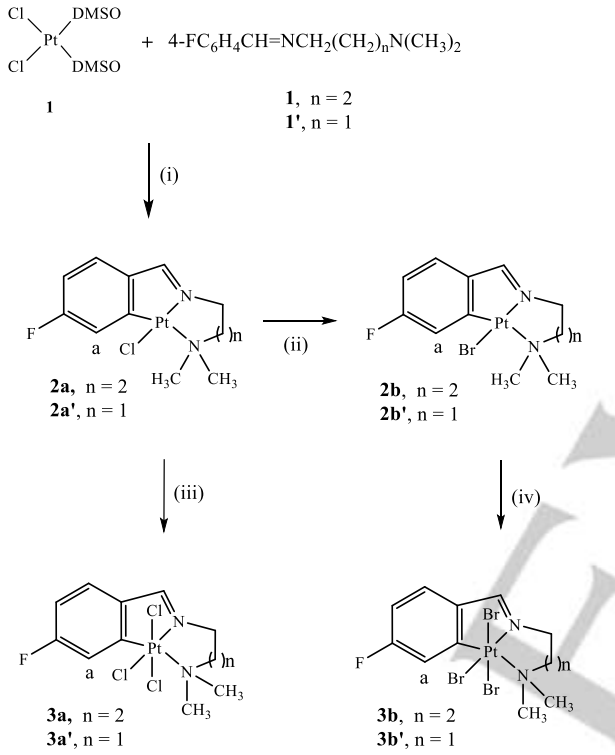
10.1002/chem.201905325

properties since we have unveiled that the intensity and the energy of the emission can be easily modulated by altering the nature of the ligands, the size of the (N,N')-chelate ring and the substituents in the aryl ring.[17],[18] Indeed, this property of the reported compounds can also be of remarkable interest for *in vivo* cell imaging and flow cytometry applications.

The syntheses of the (C,N,N') cyclometallated Pt compounds studied in this work are summarised in scheme 1. These compounds differ in the oxidation state of Pt (II or IV), the length of the hydrocarbyl chain connecting both nitrogen donor atoms (ethylene or propylene) and the halido ligands (Cl or Br).



Scheme 1. Synthesis of cyclometallated Pt^{II} and Pt^{IV} compounds. (i): + *cis*-$[PtCl_2(DMSO)_2]$ and $Na(CH_3COO)$, methanol, reflux, 72 h; (ii): + KBr, methanol, reflux, 48 h; (iii): + $PhICl_2$, acetone, r.t., 1 h; (iv): + Br_2, acetone, r.t., 1 h.

The new compounds **2b'**, **3a'** and **3b'** were characterised by elemental analyses, mass spectra and ^1H and ^{19}F NMR spectra and the molecular structures of these compounds and the previously reported compound **2a'** were determined by X- ray diffraction analysis of suitable crystals (see figures 1, S1-S7). In compounds **2a'** and **2b'** the Pt^{II} central atom adopts a square-planar coordination completed with a tridentate (C,N,N') ligand and an halido (Cl for **2a** or Br for **2a'**) ligand *trans* to the imine. Both the platinacycle and the chelate rings are nearly coplanar with the coordination plane leading to more rigid structures than those previously reported for **2a**[17] and **2b**[18] containing a propylene chain. For compounds **3a'** and **3b'**, Pt^{IV} central atom displays an octahedral coordination with a meridional tridentate (C,N,N') ligand and three chlorido (**3a'**) or bromido (**3b'**) ligands. The axial ligands form a Cl-Pt-Cl angle of 175.86(8)°(**3a'**) or a Br-Pt-Br of 173.85(8)°(**3b'**).

Absorption and emission spectra for all compounds were recorded in $5{\cdot}10^{-5}$ M dichloromethane solutions at 298 K. The results are summarized in Table 1.

Pt^{II} cyclometallated compounds show several absorption bands in the UV-Visible range (Figure S8). The lowest energy bands in the 357-386 nm range can be attributed to Pt(5d) → π* metal-to-ligand charge transfer (MLCT) mixed with intraligand transitions. An additional high energy absorption band, observed in the range 275-301 nm with higher ε values, can be attributed to π → π* intraligand transitions, as it matches the absorption recorded for the free ligand (Figure S9). [18],[19],[20] Pt^{IV} compounds only show absorption bands in the high energy range, except for compound **3b'** which also displays lower energy absorption bands.

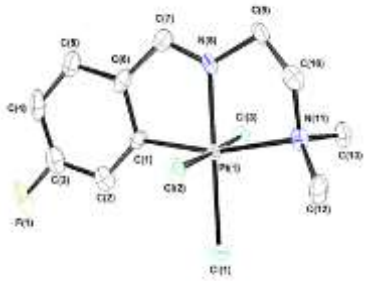


Figure 1.- Molecular structure of compound **3a'**. Selected bond lengths (Å) and angles (deg.) with estimated standard deviations: Pt(1)–N(8): 1.984(8); Pt(1)–N(11): 2.253(8); Pt(1)–C(1): 2.019(9); Pt(1)–Cl(1): 2.320(2); Pt(1)–Cl(2): 2.313(2); Pt(1)–Cl(3): 2.322(2); C(1)–Pt–N(8): 81.6(3); N(8)–Pt–N(11): 82.2(3); N(11)–Pt–Cl(1): 97.8(2); Cl(1)–Pt–C(1): 98.4(3); Cl(2)–Pt–Cl(1): 90.02(8); Cl(2)–Pt–N(8): 90.6(2); Cl(2)–Pt–N(11): 90.0(2); Cl(2)–Pt–C(1): 87.0(3); Cl(3)–Pt–Cl(1): 90.78(8); Cl(3)–Pt–N(8): 88.6(2); Cl(3)–Pt–N(11): 93.9(2); Cl(3)–Pt–C(1): 88.8(3).

When excited at their high energy absorption band, all compounds display a broad emission in the 344-351 nm range assigned as intraligand (^{1}IL) transitions, perturbed by the presence of Pt in the case of the metal complexes (Figure S10). Trends observed for Pt^{II} compounds, indicate that higher luminescent quantum yields are recorded for the more rigid compounds, containing a two carbon hydrocarbyl moiety (**2a'** > **2a** and **2b'** > **2b**) and for chlorido derivatives (**2a** > **2b** and **2a'** > **2b'**). Although no clear trends are observed for Pt^{IV} compounds, the higher quantum yield is obtained for compound **3a'** containing chlorido ligands and the more rigid ethylene fragment. Pt^{II} complexes and compound **3b'** also display a vibronically structured emission band in the 576-630 nm range when excited at their lower energy absorption band (Figure 2). It can be attributed to phosphorescence ^{3}IL emission due to the observation of a large Stokes' shift and the quenching of the emission in the presence of oxygen (Figure S11).[17],[18].

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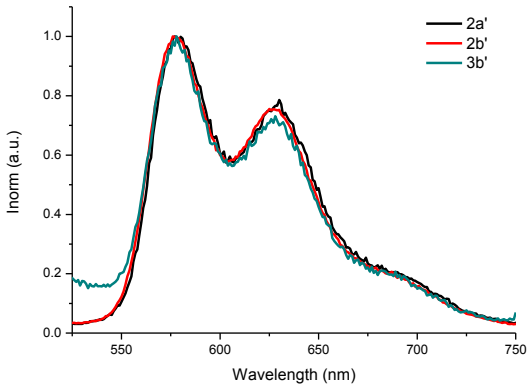

Figure 2. Normalized emission spectra of the Pt compounds in dichloromethane solution at 298 K. λexc (nm) = 385 (**2a'**), 385 (**2b'**), 380 (**3b'**).

Table 1. Absorption and emission data for compounds **1**, **2** and **3** in dichloromethane solution at 298 K.

Compound	Absorption λ_{max}/nm (ε/M^{-1} cm^{-1})	Emission λ_{max}/nm	ϕ
1[18]	276 (1506), 286 (1060)	350	0.074[a]
1'	275 (1994), 286 (1295)	346	0.059[a]
2a[18]	285 (3443), 311 (2563),	347	0.056[a]
	357 (2387), 374 (2618)	577, 620	0.003[b]
2b[18]	288 (4524), 318 (2713),	349	0.044[a]
	357 (2140), 378 (2618)	576, 622	0.004[b]
2a'[17]	277 (7524), 288 (5960),	344	0.069[a]
	324 (4843), 383 (4203)	577, 630	0.005[b]
2b'	280 (7645), 291 (6901),	350	0.065[a]
	326 (5898), 386 (5396)	576, 625	0.006[b]
3a[18]	287 (3831)	349	0.051[a]
3b[18]	286 (6353)	350	0.056[a]
3a'	270 (22702), 336 (2655),	352	0.123[a]
3b'	280 (12290), 301 (9805)	351	0.046[a]
	340 (4097), 380 (2228)	578, 628	0.002[b]

[a]Quantum yields for emission in solution referred to naphthalene in cyclohexane. [b]Quantum yields for emission in solution referred to $[Ru(bipy)_3]Cl_3$ in H_2O.

The stability of the Pt^{IV} compound **3a** in the aqueous biological medium was evaluated by ^1H NMR in D_2O and two drops of d^6-DMSO. The obtained spectra (Figure S12) suggests the formation of a mixture of solvato complexes, which is in line with recent observations of aquation of equatorial ligands of Pt^{IV} compounds.[21]

First, we assessed the effect of the Pt^{II} and Pt^{IV} compounds on the proliferation of a cancer panel including SW620 (lymph node metastasis of colorectal cancer), PC-3 (bone metastasis of prostate cancer), A549 (lung adenocarcinoma) and MCF-7 (breast adenocarcinoma). Our results, summarized in Table 2, indicate that the IC_{50} of all the compounds except **2a'** and **2b'** were significantly lower than both cisplatin and oxaliplatin for the breast cancer cell line MCF-7. Also, some of the Pt^{IV} compounds displayed IC_{50} values in the high nanomolar range for SW620 and PC-3. Compound **3a'** was the most effective against SW620 (0.41 µM), whereas **3a** exhibited the highest efficacy against PC-3 (0.9 µM), A549 (1.4 µM) and MCF-7 (3.3 µM).

Table 2. Antiproliferative activity (IC_{50}, µM) on A549 lung, SW620 colorectal, MCF-7 breast and PC-3 prostate cancer cell lines for the studied compounds, cisplatin and oxaliplatin.

Compound[a]	A549	SW620	MCF-7	PC-3
2a	5 ± 2	5.7 ± 1.1	6 ± 2	1.1 ± 0.6
2b	5 ± 2	5.5 ± 0.4	7 ± 2	2.1 ± 1.3
2a'	57 ± 3	4.6 ± 0.8	>100	19 ± 5
2b'	48 ± 5	3.1 ± 1.1	>100	66 ± 13
3a	1.4 ± 0.5	0.9 ± 0.3	3.3 ± 0.5	0.9 ± 0.2
3b	3.39 ± 0.12	1.8 ± 0.7	6.6 ± 0.8	1.46 ± 0.13
3a'	4.1 ± 0.3	0.41 ± 0.04	5.4 ± 1.0	1.2 ± 0.5
3b'	4 ± 2	0.7 ± 0.4	8.0 ± 0.8	1.46 ± 0.11
Cisplatin[b]	5.5 ± 0.2	1.4 ± 0.5	25.6 ± 0.7	1.5 ± 0.4
Oxaliplatin[b]	1.3 ± 0.2	0.3 ± 0.2	23.4 ± 0.2	1.2 ± 0.3

[a] Results shown correspond to mean ± standard deviation of two experiments performed in triplicates. [b] Cisplatin and oxaliplatin are taken as reference compounds.

We next aimed to evaluate whether these compounds could also be effective against metastatic tumours that have acquired multiplatinum resistance (MPR), for prostate PC-3-MPR and colorectal SW620-MPR, along with their age-matched controls, PC-3O or SW620-O (see SI). Strikingly, we obtained that **3a-3b'** exhibited similar antiproliferative effects in controls and resistant cells of both metastatic resistant cell models, with resistance indexes (RI_{resist}) close to 1, denoting a complete absence of cross-resistance. Indeed, other examples of low cross-resistance have been reported in the literature.[5],[22],[7] However, in such cases, the low cross-resistance is related to the axial ligands, bioactive agents *per se* (i.e. HDAC, COX or PDK inhibitors). On the contrary, in our case the absence of cross-resistance must arise from the platinacycle itself, since the axial ligands are halogen groups. In support of this notion, a similar cross-resistance was observed for the Pt^{II} structure **2a**. Encouragingly, we also found that **2a**, **3a** and **3a'** displayed excellent selectivity profiles for cancer cells at low doses (1-10 µM), when assessing their antiproliferative effect on all the cancer cell lines tested compared to normal human foreskin fibroblast cells (BJ) (Figures S13-S14).

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Table 3. Antiproliferative activity (IC_{50}, μM) and resistance index (RI) of the studied compounds, cisplatin and oxaliplatin on the generated colorectal cancer (CRC) model of multiplatinum resistance (SW620-MPR) and its age-matched control (SW620-O).

Compound[a]	SW620-O	SW620-MPR	RI_{aging}[b]	RI_{resist}[c]	RI_{total}[d]
2a	7 ± 3	7.1 ± 0.6	1	**1**	1.3
3a	2.2 ± 0.3	3 ± 2	2.4	**1.2**	2.8
3b	3.6 ± 1.2	4.8 ± 1.4	2	**1.3**	2.7
3a'	1.1 ± 0.7	1.64 ± 0.01	2.8	**1.4**	4
3b'	0.8 ± 0.4	1.4 ± 0.7	1.2	**1.7**	2
Cisplatin	1.1 ± 0.9	21 ± 4	0.8	**19**	15
Oxaliplatin	0.3 ± 0.2	3.2 ± 1.1	1	**10**	10

[a] Results shown correspond to mean ± standard deviation of two experiments performed in triplicates. [b] RI_{aging} corresponds to the ratio of IC50 between SW620-O (age-matched control) and SW620 (parental). [c] RI_{resist} corresponds to the ratio of IC50 between SW620-MPR (resistant) and SW620-O (age-matched control). [d] RI_{total} corresponds to the ratio of IC50 between SW620-MPR (resistant) SW620 (parental).

Table 4. Antiproliferative activity (IC_{50}, μM) and resistance index (RI) of the studied compounds, cisplatin and oxaliplatin on the generated castration-resistant prostate cancer (CRPC) model of multiplatinum resistance (PC-3-MPR) and its age-matched control (PC-3-O).

Compound[a]	PC-3-O	PC-3-MPR	RI_{aging}[b]	RI_{resist}[c]	RI_{total}[d]
2a	0.67 ± 0.11	1.6 ± 0.2	0.6	**2.5**	1.5
3a	1.4 ± 0.8	1.5 ± 0.3	1.5	**1**	1.6
3b	5.3 ± 0.3	3.7 ± 1.3	3.7	**0.7**	2.5
3a'	2 ± 2	2.9 ± 0.4	1.9	**1.3**	2.4
3b'	4 ± 2	3.7 ± 0.4	2.4	**1**	2.5
Cisplatin	2.5 ± 0.9	23 ± 9	1.7	**9**	15
Oxaliplatin	0.69 ± 0.02	51 ± 12	0.6	**74**	42

[a] Results shown correspond to mean ± standard deviation of two experiments performed in triplicates. [b] RI_{aging} corresponds to the ratio of IC_{50} between PC-3-O (age-matched control) and PC-3 (parental). [c] RI_{resist} corresponds to the ratio of IC_{50} between PC-3-MPR (resistant) and PC-3-O (age-matched control). [d] RI_{total} corresponds to the ratio of IC_{50} between PC-3-MPR (resistant) and PC-3 (parental).

In light of these results, we also investigated if the ability to overcome resistance of the reported compounds involved an alteration of their cytotoxic and cytostatic activities inside the cell compared to cisplatin. For that, we studied the effect of **3a'** on the cell cycle phase distribution (Figure 3) and apoptosis (Figure 4) of SW620-O and SW620-MPR cells.

We found that, compared to cisplatin, **3a'** had only a slight effect on the cell cycle phase distribution of SW620-O cells (Figure 3A). Moreover, whereas cisplatin appeared to induce a significant G2/M arrest, consistent with previous observations [23], **3a'** only occasioned a minor reorganization between the S and G2/M subpopulations, with no effect on the percentage of cells at G0/G1. Indeed, while cisplatin caused a 40% reduction in the G0/G1 subpopulation in SW620-O, it only caused a G0/G1 drop of around 15% in SW620-MPR cells (Figure 3B), with a subsequent G2/M arrest. Interestingly, **3a'** had no significant effect on the cell cycle of SW620-MPR cells. On the contrary, **3a'** exhibited a much greater effect on the induction of cell death (PI^+ cells) than cisplatin in both SW620-O and SW620-MPR (Figure 4). Preapoptotic cells (PI^-/Annexin V^+ cells) were only observed in SW620-O at 72 h, and both compounds had a significantly enhanced effect on SW620-O than in SW620-MPR cells, potentially denoting that the generated resistant cell lines enhanced their potential to evade apoptosis. In this regard, apoptosis levels did not correlate with ROS accumulation in the cells treated with either cisplatin or **3a'** (See Figure S15).

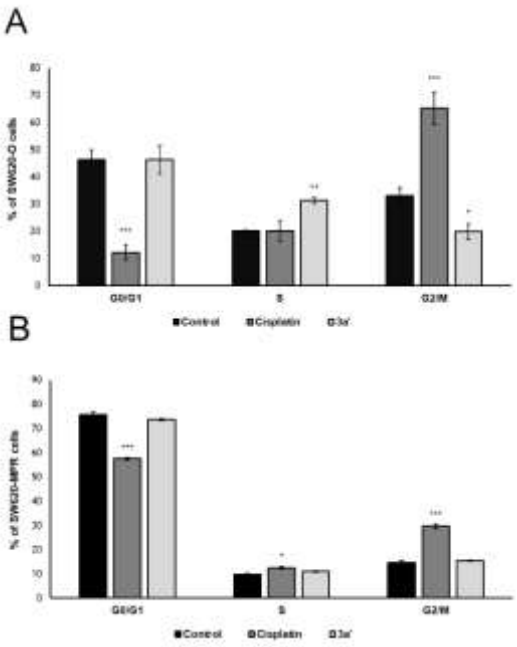

Figure 3. Cell cycle phase distribution at 72 h incubation with cisplatin or **3a'** at their IC_{50} concentration in (A) SW620-O and (B) SW620-MPR.

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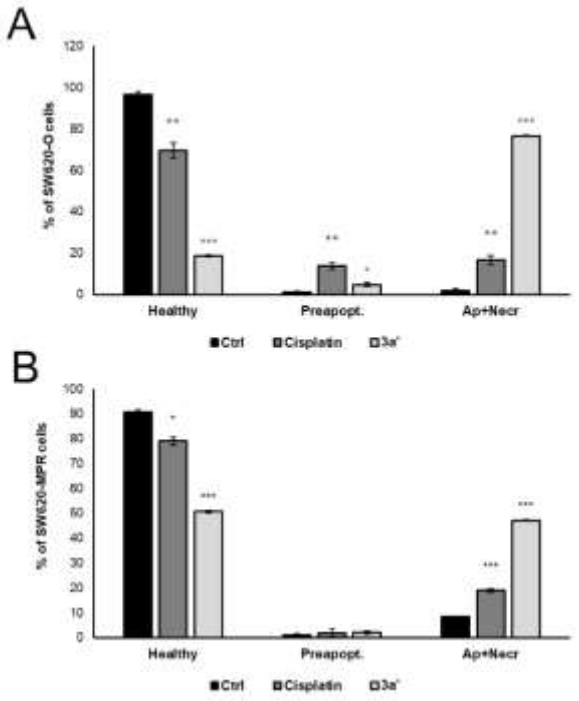

Figure 4. Percentage variations of alive, early apoptotic and late apoptotic/necrotic cell populations at 72 h incubation with cisplatin or **3a'** at their IC_{50} concentrations in (A) SW620-O and (B) SW620-MPR cells.

Previous evidence gathered by our group on different related sets of cyclometallated Pt^{II} and Pt^{IV} complexes extensively revealed that cellular accumulation for all platinum cyclometallated compounds was significantly higher than for cisplatin[24],[25] However, no correlation was observed between the cytotoxicity in cancer cells and Pt accumulation within the series of compounds, as previously reported for other metal complexes.[26],[27] Therefore, more information than just intracellular drug concentration, such as reduction potential or differential DNA binding affinity is necessary to explain the displayed enhanced cytotoxicity.

In consequence, the ability of cyclometallated Pt^{II} (**2a**,**2b**,**2a'**,**2b'**) and Pt^{IV} (**3a**,**3b**,**3a'**,**3b'**) compounds to modify the mobility of the supercoiled closed (*sc*) and the open circular (*oc*) forms of pBluescript SK+ plasmid DNA was studied in an agarose gel electrophoresis (Figure 5). For the Pt^{II} complexes significant changes in the mobility of plasmid DNA were observed at 10 μM and even at 5 μM concentration for compounds **2a'** and **2b'**. With regard to Pt^{IV} compounds only **3b'** showed an interaction with plasmid DNA at concentrations as high as 100 μM, while compounds **3a**, **3b** and, **3a'** were not efficient at all in removing the supercoils from DNA.

Since it is generally accepted that Pt^{IV} compounds are rapidly reduced under physiological conditions by cellular reducing agents, the reactions of **3a** with ascorbic acid, glutathione and cysteine were also monitored by ^1H NMR spectroscopy under analogous conditions (Figures S16-S18). The reaction with cysteine (Figure S18) was the most conclusive since it produced from the early stages a compound that is stable in solution up to one week and that can be assigned to Pt^{II} compound **2a-cys** (see Scheme S1). This result is supported by recent studies suggesting that cysteine has the highest reactivity toward reduction of Pt^{IV} anticancer prodrugs under physiological conditions and its reactivity is highly sensitive to pH. [28],[29] Additionally, this result is also in agreement with the kinetics studies previously carried out for **3a** and analogous $Pt^{IV}Cl_3$ compounds[30] which revealed that the reaction of these cyclometallated Pt^{IV} compounds with thiols consists of two consecutive reaction steps: a Pt^{IV} to Pt^{II} reduction (step 1 in Scheme S1) followed by substitution of the remaining chlorido ligand by cysteine (step 2).

In light of this, we hypothesized if the studied Pt compounds could be inhibitors of cysteine metalloprotease Cathepsin B, as recently reported for other metal compounds, [24],[25],[31] but none of the compounds in this study presented significant inhibitory activity.

To evaluate the ability of the Pt^{II} and Pt^{IV} compounds under study to intercalate with DNA and block the action of topoisomerases, topoisomerase I- and topoisomerase-IIα-based gel assays were also performed. [32] We found that none of the compounds are intercalators or topoisomerase I inhibitors (see Figure S19). On the contrary, at 50 μM concentration, topoisomerase-IIα inhibition was detected for compounds **2a'**, **2b'**, **3a'**, **3b** and **3b'** (See Figure S20).

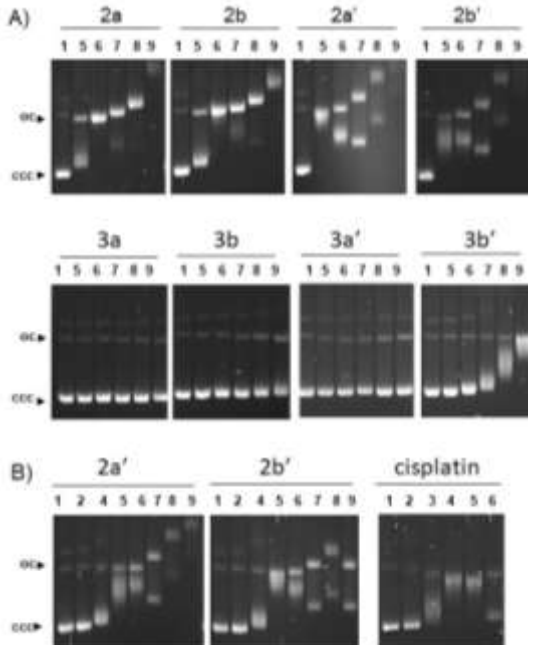

Figure 5. Interaction of pBluescript SK+ plasmid DNA (0.3 μg) with increasing concentrations of compounds under study. A) All compounds were analysed from 10 to 200 μM concentration Lane 1: DNA only. Lane 5: 10 μM. Lane 6: 25 μM. Lane 7: 50 μM. Lane 8: 100 μM. Lane 9: 200 μM. B) Compound **2a'** and **2b'** were also analysed at lower concentrations and compared with cisplatin as a standard reference. Lane 1: 0 μM. Lane 2: 1 μM. Lane 3: 2.5 μM. Lane 4: 5 μM.

Lane 5: 10 µM. Lane 6: 25 µM. Lane 7: 50 µM. Lane 8: 100 µM. Lane 9: 200 µM; *sc* = supercoiled closed circular DNA; *oc* = open circular DNA.

In summary, we have presented a new family of luminescent Pt^{II} and Pt^{IV} (C,N,N')-cycloplatinated compounds with high efficacy and selectivity against a broad cancer cell panel. The studied Pt^{IV} compounds presented enhanced efficacy, capacity to be reduced in solution and, importantly, a complete absence of platinum cross-resistance against metastatic CRC and CRPC multiplatinum-resistant cell models. Indeed, we also proved that the absence of cross-resistance is an intrinsic property of the platinacycle. Therefore, further modulating the nature of the axial ligands could allow to remarkably improve the multi-target action of the compounds, leading to unparalleled levels of efficacy and to ultimately overcome platinum resistance in the clinics.

Acknowledgements ((optional))

This work was supported by the Ministerio de Economia y Competitividad (Projects CTQ-2015-65040-P, CTQ2015-65707C2-1/FEDER, AEI/FEDER CTQ2016-76120-P, CTQ2017-90802-REDT, SAF2017-89673-R and SAF2015-70270-REDT), Instituto de Salud Carlos III and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD CB17/04/00023), Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) – Generalitat de Catalunya (2017SGR-1033). M.Cascante acknowledges the support received through the prize "ICREA Academia" for excellence in research, funded by ICREA foundation – Generalitat de Catalunya.

Keywords: Platinum • Luminescence • Anticancer drugs • Biological Activity • Chemotherapeutic resistance

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10.1002/chem.201905325

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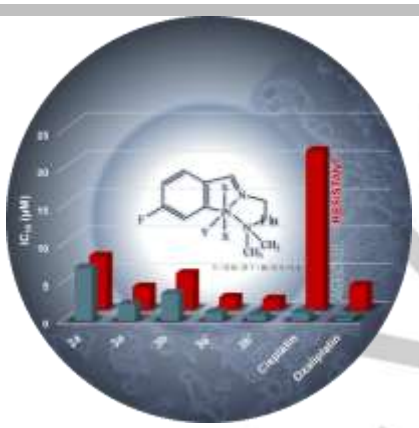

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Layout 2:

COMMUNICATION