Bioorganic & Medicinal Chemistry 21 (2013) 2379–2386

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Cytotoxic effect of (1-methyl-1*H*-imidazol-2-yl)-methanamine and its derivatives in Pt^{II} complexes on human carcinoma cell lines: A comparative study with cisplatin

Nicola Ferri [a], Stefano Cazzaniga [a], Luca Mazzarella [b], Giuseppe Curigliano [b], Giorgio Lucchini [c], Daniele Zerla [d], Raffaella Gandolfi [d], Giorgio Facchetti [d], Michela Pellizzoni [d], Isabella Rimoldi [d,*]

[a] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, 20133 Milano, Italy
[b] Division of Medical Oncology, Department of Medicine, Istituto Europeo di Oncologia, Milano, Italy
[c] Dipartimento di Scienze Agrarie e Ambientali-Produzione, Territorio, Agroenergia, Università degli Studi di Milano,Via Celoria 2, 20133 Milano, Italy
[d] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Venezia 21, 20133 Milano, Italy

ARTICLE INFO

Article history:
Received 19 October 2012
Revised 18 January 2013
Accepted 24 January 2013
Available online 5 February 2013

Keywords:
Cytotoxicity
Cisplatin
Cancer cell line
Platinum(II) complex
Imidazole

ABSTRACT

The synthesis and pharmacological characterisation of (1-methyl-1*H*-imidazol-2-yl)-methanamine and its derivatives in Pt^{II} complexes are described. Six out of eleven new Pt^{II} complexes showed a significant cytotoxic effect on NCI-H460 lung cancer cell line with EC_{50} values between 1.1 and 0.115 mM, determined by MTT assay. Compound **Pt-4a** showed a particularly more potent cytotoxic effect than the previously described Pt^{II} complex with 2,2′-bipyridine, [Pt(bpy)Cl_2], with an EC_{50} value equal to 172.7 μM versus 726.5 μM respectively, and similar potency of cisplatin (EC_{50} = 78.3 μM) in NCI-H460 cell line. The determination of the intracellular and DNA-bound concentrations of ^{195}Pt, as marker of the presence of the complexes, showed that the cytotoxic compound **Pt-4a** readily diffused into the cells to a similar extent of cisplatin and directly interacted with the nuclear DNA. **Pt-4a** induced both p53 and $p21^{Waf}$ expression in NCI-H460 cells similar to cisplatin. A direct comparison of the cytotoxic effect between compound **Pt-4a** and cisplatin on 12 different cancer cell lines demonstrated that compound **Pt-4a** was in general less potent than cisplatin, but it had a comparable cytotoxic effect on non-small-cell lung cancer NCI-H460 cells, and the colorectal cancer cells HCT-15 and HCT-116. Altogether, these results suggested that the Pt^{II} complex with 1-methyl-1*H*-imidazol-2-yl)-methanamine (compound **Pt-4a**), displayed a significant cytotoxic activity in cancer cells. Similarly to cisplatin this compound interacts with nuclear DNA and induces both p53 and $p21^{waf}$, and thus it represents an interesting starting point for future optimisation of new Pt^{II} complexes forming DNA adducts.

1. Introduction

The great impact of cisplatin, discovered in 1969 by Rosenberg et al., for the treatment of cancer opened the era of platinum-based anticancer drugs.[1,2] Cisplatin, carboplatin and oxaliplatin are highly polar molecules that do not readily diffuse across lipid membranes. Although a variety of pumps and transporters can influence the cellular uptake of the platinum-containing drugs; the copper transporter 1 (CTR1) plays a major role.[3] Once inside the cells, cisplatin becomes activated by the *acquation* of one of the two chloride leaving groups, it covalently binds to DNA, forming DNA adducts and realising its cytotoxic effect.[4,5]

This effect triggers a variety of signal-transduction pathways involved in DNA-damage recognition and repair, cell-cycle arrest, and programmed cell death/apoptosis. Tumor suppressor genes also influence cisplatin-induced apoptosis.

Protein p53 is considered a 'guardian of the genome' and facilitates DNA repair before DNA replication. Cisplatin DNA damage leads to expression of p53 protein that subsequently induces both expression of downstream $p21^{waf}$ protein and G1 phase cell cycle arrest.[6] Cisplatin and its derivatives are still used for more than 50% of cancer diseases and a request for an alternative is highly needed especially when their toxicity and inefficiency against platinum-resistant tumors are considered.[7–10] The resistance to cisplatin and carboplatin could be mediated through three broad mechanisms: first, a failure of a sufficient amount of platinum to reach the target DNA; second, a failure to achieve cell death after platinum-DNA adduct formation; and third the cellular efflux of Pt^{II} complexes through the ATP7A and ATP7B channels.[11]

Chemical modifications of platinum containing drugs that could lead to better cell-membrane permeability and facilitate the *acquation* of the chloride leaving group, may generate a new class of

* Corresponding author. Tel./fax: +39 0250314609.
E-mail address: isabella.rimoldi@unimi.it (I. Rimoldi).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.01.063

chemotherapic agents suitable also as antiproliferative agents. Regarding these aspects organometallic compounds based on complexes of different transition metals have been proven to be a valid alternative,[12–14] even if platinum still furnishes the best results.[15]

In order to improve the selectivity, one possible strategy could be the introduction of changes to the ligands' properties.[16–23] In the last decade bipyridines, histamines and pyrrolidines have been used as bidentate ligands in Pt^{II} complexes and their antineoplastic activity has been known for years.[24–27]

Based on these premises, in the present study the Pt^{II} complexes with bidentate diamines derived from imidazol moiety are described. The choice of different substituents, as proposed easily functionalised methylaminoimidazolic ligands, is carried out with the aim to modulate the properties of the considered complexes affecting intracellular distribution.

2. Results and discussion

Imidazole belongs to the π-electronrich heteroaromatic family, with 6 electrons arranged on 5 atoms, even if they are mainly centred on N-atoms. Various substitute imidazoles form complexes with many metal ions in which the donation is realised by the pyridinic N-atom. The presence in variously substituted 2-methylaminoimidazoles of another aminic group, that can coordinate to the metal centre, leads to the formation of bidentate ligands.

The starting point of the present report was the preparation of these types of ligands derived from variously substituted 2-methylaminoimidazoles, because of imidazolic moiety's chemical features: functionalised easily with hydrophobic or hydrophilic groups of various nature and length influencing the complex properties. The second important target was the modification of *trans* effect that arises from the *trans*-activation mechanism in the cell, where the active complex is the corresponding aqua species (activated form of the complex of Pt^{II}).

For a metal complex, the so-called '*trans* effect' is intended as the ability of a ligand coordinated to a metal (imidazole ligand), to facilitate the departure of a second ligand *trans* to the first (chloride) and its replacement or substitution by an external ligand (water).[28] By increasing this effect the intracellular concentration of the activated aqua ion might be raised, making the drug much more available for binding to DNA.

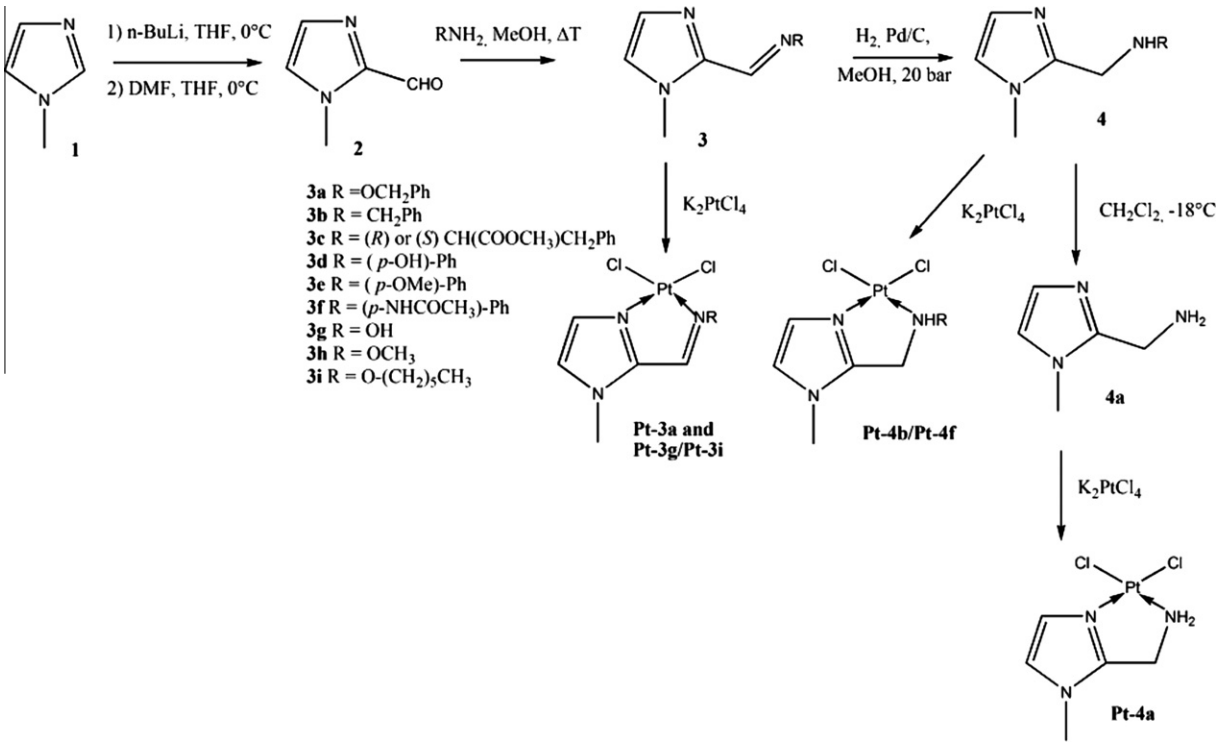
The aim being to study the influence of substitution, initially the change only occurred on NH_2 moiety maintaining fixed *N*-methyl-1*H*-imidazole scaffold. The synthesis of ligands and their corresponding Pt^{II} complexes are recorded in Scheme 1.

At first, *N*-Methyl-1*H*-imidazole-2-carbaldehyde **2** was synthesised,[29] then a condensation with selected amines occurred obtaining the corresponding Schiff bases **3a**–**l**.[30] For imines **3a**–**f** a reduction was realised by hydrogenation with Pd/C. The diamines **4a**–**f** thus obtained were purified by recrystallisation; finally the Pt^{II} complexes **Pt-4a**/**Pt-4f** and **Pt-3g**/**Pt-3i** were formed by reacting with $PtCl_4K_2$[31] (Scheme 1).

The cytotoxic effect of all the Pt^{II} complexes was then studied in cultured non-small lung carcinoma (NSLC) NCI-H460 cell line in a concentration range from 0.25 to 1 mM. After 48 h incubation with tested compounds, the MTT assay was performed and the cell viability was compared to the control condition. A significant cytotoxic effect was observed for all the compounds tested at 1 mM concentration, except for compound **Pt-3g** and **Pt-4f**. Being the more efficient compound **Pt-4a**, the free ligand **4a** was tested and did not demonstrate a significant cytotoxic effect.

These results indicate that these platinum complexes are cytotoxic and that the coordination with Pt^{II} is required for such effect. Compounds **Pt-4b**, **Pt-3a**, **Pt-3i** and **Pt-4a** showed a significant effect starting from 0.25 mM concentration (Fig. 1). Beyond cisplatin, a second reference compound utilised was the Pt^{II} complexes of the 2,2′-bipyridine, $[Pt(bpy)Cl_2]$ with known cytotoxic effect.[24] Under our experimental conditions, this compound showed a lower cytotoxic potency compared to compounds **Pt-4b**, **Pt-3a**, **Pt-3i**, and **Pt-4a** (Fig. 1).

In order to study a possible correlation between the cytotoxic effect and the cellular uptake of the new Pt^{II} complexes, we



Scheme 1. Synthesis of ligands and their corresponding Pt^{II} complexes.

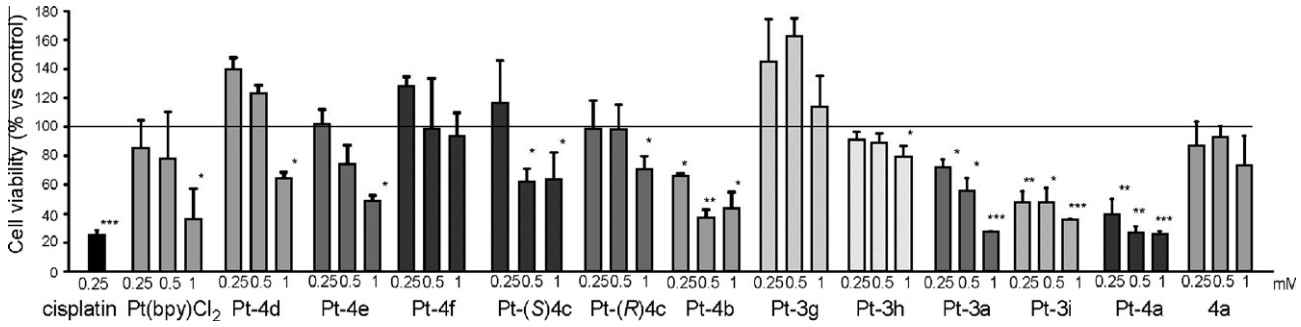


Figure 1. Cytotoxic effects of Pt^{II} complexes on NCI-H460 cell line. Cells were seeded (35,000/well of 48 well tray) and incubated with DMEM supplemented with 10% FCS; 48 h later the medium was changed with one containing 0.4% FCS to stop cell growth and the cultures were incubated for 48 h. At this time the medium was replaced with one containing 10% FCS and the reported concentrations of compounds and cisplatin and the incubation was continued for a further 48 h at 37 °C. At the end of this incubation period the cell viability was determined by MTT assay. Each bar represents the mean ± SD of three independent experiments. Inhibitors versus control: $^{*}P$ <0.05; $^{**}P$ <0.001; $^{***}P$ <0.0001.

determined the ^{195}Pt concentrations from total cell homogenates. This analysis was conducted after 3 h incubation of NCI-H460 cell lines with 1 mM concentration of compounds using ICP-MS spectroscopy (Table 1).

Cells were seeded (250,000/35 mm petri dish) and incubated with RPMI 1640 supplemented with 10% FCS; 24 h later the medium was changed with one containing 0.4% FCS to stop cell growth and the cultures were incubated for 48 h. At this point, the medium was replaced with one containing 10% FCS and 1 mM of compounds or cisplatin and the incubation continued for 3 h at 37 °C. At the end of this incubation period the total cell lysates were prepared and ^{195}Pt concentrations determined by ICP-MS. The cytotoxic effect was determined under the same experimental conditions described in Fig. 1 and the EC_{50} values calculated by nonlinear regression curve.

The concentrations of ^{195}Pt from total cell lysates after incubation with the two reference drugs, cisplatin and compound [Pt(bpy)Cl_2], were 1.55 and 3.80 μM, respectively. Considering the compounds that inhibited the cell viability by more than 50%, similar ^{195}Pt levels were observed for compounds **Pt-3i** (2.98 μM) and **Pt-4a** (1.98 μM). Compound **Pt-3a** showed a lower intracellular concentration (0.7 μM) possibly due to the presence of oxyme moiety[32] that probably decreases the diffusion across the cellular membranes. Thus, the lower cytotoxic effect of **Pt-3a** compared to **Pt-4a** may be due to an unfavourable pharmacokinetic property, since ligand **3a** contains an oxymic group hydrolysed to corresponding **4a** that is the real molecule interacting with DNA. For complex **Pt-3i**, in which the ligand is a long alkylic chain substituted oxyme, the intracellular concentration of platinum is very high thanks to the presence of a hydrophobic group.

The low concentrations of ^{195}Pt observed after incubation with compounds **Pt-4d** (0.11 μM), **Pt-4e** (0.45 μM) and **Pt-4f** (0.42 μM) may explain their lower cytotoxic effect on NCI-H460 cell line (Fig. 1). Indeed, a significant linear correlation between the intracellular levels of ^{195}Pt and EC_{50} values was found ($R^2 = 0.648$) (Fig. 2). From this analysis it is interesting to note that **Pt-4a** is the complex that more closely mimics the cisplatin's properties (Fig. 2), recently showing a very potent cytotoxic activity without a particularly high intracellular accumulation (1.55 μM).

Finally, compounds **Pt-(*S*)4c** and **Pt-(*R*)4c**, although accumulated efficiently into the cells (4.11 and 2.20 μM, respectively), were less cytotoxic than other compounds, suggesting an impaired formation of active species (*aquation*) and thus interaction with DNA. Considering the above, compound **Pt-4a** was chosen as the leading compound for further investigations.

To determine whether compound **Pt-4a** is capable as efficiently diffusing into the cells and reaching the nuclear DNA, the presence of ^{195}Pt was measured from DNA extracts of NCI-H460 cell line incubated with increasing concentrations of compound **Pt-4a** or cisplatin for 3 h (Fig. 3).

Cisplatin and compound **Pt-4a** appears to have a similar capacity to accumulate into NCI-H460 cell line and form DNA adducts. The accumulation of platinum complex was linearly dependent on the concentration of the compound in the cultured media, suggesting a diffusion process across the membrane as a major route of cellular uptake. In this regard it is worth mentioning that the cellular uptake of cisplatin is partially regulated by the copper transporter CTR1,[33] while the effect of this cell membrane channel on compound **Pt-4a** is still unknown. In agreement with previous evidence, the amount of platinum observed in the DNA extract

Table 1
Intracellular concentration of ^{195}Pt and cytotoxic potency of newly synthesised complexes in NCI-H460 cell line

Compound	[Pt] μM	EC_{50} (μM)
Cisplatin	1.55	78.3
[Pt(bpy)Cl_2]	3.80	736.5
Pt-4d	0.11	1110.1
Pt-4e	0.45	540.3
Pt-4f	0.42	N/A
Pt-(*S*)4c	4.11	N/A
Pt-(*R*)4c	2.20	N/A
Pt-4b	3.13	250.3
Pt-3g	0.70	N/A
Pt-3h	0.13	N/A
Pt-3a	0.70	437.4
Pt-3i	2.98	115.2
Pt-4a	1.98	172.7

N/A: Not applicable.

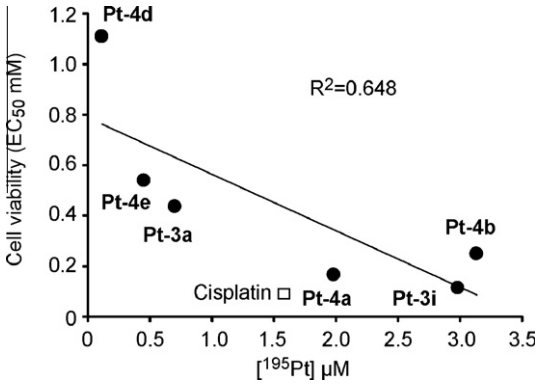


Figure 2. Correlation between ^{195}Pt concentrations and EC_{50} values of cytotoxic effect.

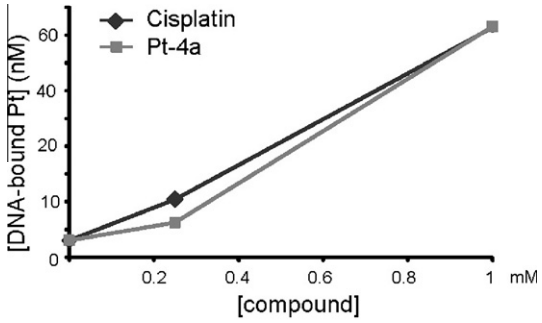


Figure 3. DNA-bound concentration of ^{195}Pt after incubation of NCI-H460 cell line with compound **Pt-4a** and cisplatin. Cells were seeded (250,000/35 mm petri dish) and incubated with DMEM supplemented with 10% FCS; 24 h later the medium was changed with one containing 0.4% FCS to stop cell growth and the cultures were incubated for 48 h. At this time, the medium was replaced with one containing 10% FCS and compound **Pt-4a** and cisplatin (0.25 and 1 mM) and the incubation was continued for a further 3 h at 37 °C. At the end of this incubation period the nuclear DNA was extracted and ^{195}Pt concentration determined by ICP-MS.

was approximately 3–4% of that measured in the cells from a total cell homogenate (0.06 μM vs 2 μM after incubation with 1 mM of compound **Pt-4a** or cisplatin).[6,34]

Thus, compound **Pt-4a** showed a significant cytotoxic effect because it is able to reach the nuclear DNA, the intracellular target to cisplatin. To investigate whether this compound was capable of activating the same cellular responses as cisplatin, the intracellular levels of p53 and p21^{waf} were determined by Western blot analysis from total protein extracts.

As shown in Fig. 4, 24 h incubation of NCI-H460 cell line with 0.1 mM cisplatin determined a significant induction of both p53 and p21^{waf} compared to control cells. Compound **Pt-4a** had a similar effect to cisplatin on p21^{waf} at 0.25 and 0.5 mM, while a less pronounced effect was observed on p53 levels. These data showed that compound **Pt-4a** had a cytotoxic effect on tumor cell line NCI-H460 with a similar mechanism of action to cisplatin, although a more detailed investigation will be needed to determine the formation of the **Pt-4a-**DNA adducts.

Finally, the cytotoxicity of compound **Pt-4a** was compared to that of cisplatin in 12 different cancer cell lines with a range of concentration between 0.01 and 0.5 mM.

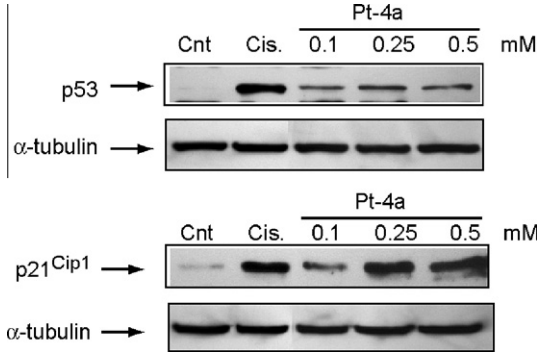


Figure 4. Effect of compound **Pt-4a** on p53 and p21^{Waf} in NCI-H460 cell line. Cells were seeded (250,000/35 mm petri dish) and incubated with DMEM supplemented with 10% FCS; 24 h later the medium was changed with one containing 0.4% FCS to stop cell growth and the cultures were incubated for 48 h. At this time, the medium was replaced with one containing 10% FCS and the reported concentrations of compound **Pt-4a**, and cisplatin at 0.1 mM, and the incubation was continued for a further 24 h at 37 °C. At the end of this incubation period the total cell lysates were prepared and protein expression evaluated by Western blotting analysis with a specific antibody anti p53 or p21^{waf}. The same membrane was then probed with anti-α-tubulin antibody (Sigma–Aldrich) as a loading control. Cis. stands for cisplatin and Cnt for control.

Table 2
Cytotoxic effect of compound **Pt-4a** and cisplatin on different tumor cell lines. Cells were seeded (35,000/well of 48 well tray) and incubated with appropriate medium supplemented with 10% FCS; 24 h later the medium was changed with one containing 0.4% FCS to stop cell growth and the cultures were incubated for 48 h. At this time, the medium was replaced with one containing 10% FCS and compounds or cisplatin (from 0.01 mM to 0.5 mM concentrations) and the incubation was continued for a further 48 h at 37 °C. At the end of this incubation period the cell viability was determined by MTT assay. The EC_{50} values were then calculated by nonlinear regression curve.

Cell line	EC_{50} cisplatin (μM)	EC_{50} **Pt-4a** (μM)	EC_{50} cisp/EC_{50} Pt-4a
H460	78.3 ± 1.8	172.7 ± 20.5	0.45
HCT-15	88.5 ± 36.1	145.5 ± 9.2	0.61
A2780	31.5 ± 4.9	265.5 ± 2.1	0.12
IGROV-1	107.8 ± 27.2	NE	—
HEY	197.0 ± 161.2	NE	—
A549	238.0 ± 62.2	NE	—
HCT-116	170.5 ± 27.6	201.3 ± 37.8	0.85
A431	107.5 ± 2.8	NE	—
KYSE-150	203.0 ± 15.6	NE	—
MCF-7	NE	NE	—
MDA-MB-231	NE	NE	—
DLD-1	NE	NE	—

NE: Not effective.

The cytotoxic effect of compound **Pt-4a** was statistically significant in four cell lines tested, the non-small lung carcinoma NCI-H460, the epithelial ovarian cancer A2780, the colorectal cancer cells HCT-15 and HCT-116, with EC_{50} values between 145.5 μM and 265.5 μM (Table 2). In all these cell lines cisplatin was more potent than compound **Pt-4a**, although comparable EC_{50} values were observed for the NCI-H460. HCT-116 and HCT-15, with EC_{50} ratio of cisplatin and compound **Pt-4a** equal to 0.45, 0.85 and 0.61, respectively. Thus compound **Pt-4a** was not active on five cancer cell lines that were effect by cisplatin: the epithelial ovarian cancer IGROV-1, the ovarian cancer HEY, the lung adenocarcinoma A549, the cutaneous squamous carcinoma A431 and the esophageal squamous cell carcinoma KYSE-150. The breast cancer cell lines MCF-7, MDA-MB-231 and colorectal cancer DLD-1 were shown to be resistant to both chemical entities.

3. Conclusions

This study describes Pt^{II} complexes, where the ligands are variously substituted bidentate diamines derived from imidazol moiety and revealed that same complexes showed a significant cytotoxic effect on the non-small-cell lung cancer NCI-H460 cell line. The Pt^{II} complex with (1-methyl-1*H*-imidazol-2-yl)-methanamine **Pt-4a** showed the most potent cytotoxic effect with comparable activity to cisplatin on three cancer cell lines: the non-small-cell lung cancer cells NCI-H460 and the colorectal cancer cells HCT-15 and HCT-116. This compound was shown to interact with the nuclear DNA and to activate similar intracellular response to cisplatin inducing the expression of p53 and p21^{waf}. In order to improve both pharmacokinetic and pharmacodynamic properties of the lead compound **Pt-4a** additional substitutions on N^1 of the imidazole ring will be performed.

4. Experimental section

4.1. Chemistry

4.1.1. General

All manipulations involving air sensitive materials were carried out in an inert atmosphere glove box or using standard Schlenk line techniques under an atmosphere of nitrogen or argon in oven-dried glassware. All solvents were used anhydrous. ^{1}H

NMR, ^{13}C NMR and ^{195}Pt NMR spectra were recorded on a Bruker DRX Avance 300 MHz equipped with a non-reverse probe and also on a Bruker DRX Avance 400 MHz. MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system MS spectrometer with an electronspray ionisation source and an 'Ion Trap' mass analyser. The MS spectra were obtained by direct infusion of a sample solution in MeOH under ionisation, ESI positive. FT-IR spectra were collected by using a Perkin–Elmer (MA, USA) FT-IR Spectrometer 'Spectrum One' in a spectral region between 4000 and 450 cm^{-1} and analysed by transmittance technique with 32 scansions and 4 cm^{-1} resolution. Elemental analyses were performed using a Perkin–Elmer SeriesII/CHNS/O 2400 analyzer. ICP-MS data were recorded with BRUKER aurora M90 ICP-MS (MA, USA)

4.1.2. Preparation of ligands

4.1.2.1. *N*-Methyl-1*H*-imidazole-2-carbaldehyde (2). The synthesis of this compound was realised according with literature procedure.[29] Yield = 0.400 g (73%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 9.80 (1H, s, HC=O), 7.25 (1H, s, H_5 imz), 7.09 (1H, s, H_4, imz), 4.00 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 182.5 (C=O), 144.0 (imz), 131.6 (imz), 129.1 (imz), 35.1 (imz). Elemental analysis for $C_5H_6N_2O$: calcd C, 54.54; H, 5.49; N, 25.44; found C, 55.08; H, 5.97; N, 24.23.

4.1.2.2. (1-Methyl-1*H*-imidazol-2-yl)methanimines (3). To a solution of *N*-Methyl-1*H*-imidazole-2-carbaldehyde (**2**) (0.400 g, 3.47 mmol) in anhydrous CH_3OH (40 mL), K_2CO_3 (0.483 g, 3.5 mmol) was added at 0 °C. A solution of amine (3.5 mmol) was added dropwise. The temperature was maintained at 0 °C for 20 min, then wormed to room temperature and stirred overnight. The mixture was filtered and the product was obtained as yellow oil.

*4.1.2.2.1. 1-Methyl-1H-imidazole-2-carbaldehyde O-benzyloxime (**3a**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.19 (1H, s, N=CH), 7.26–7.42 (5H, m, aromatic), 7.07 (1H, s, imz), 6.88 (1H, s, imz), 5.23 (2H, d, CH_2), 3.79 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 142.5 (N=CH), 139.7 (imz), 138.0 (aromatic), 129.7 (aromatic), 129.1 (imz), 129.0 (imz), 128.5–128.8 (aromatic), 76.4 (CH_2), 35.3 (CH_3). Elemental analysis for $C_{12}H_{13}N_3O$: calcd C, 66.96; H, 6.09; N, 19.52; found C, 68.80; H, 5.56; N, 18.98. MS (ESI) *m*/*z* 216.1 $[M+H]^+$, 238.2 $[M+Na]^+$. IR *v* cm^{-1} 3248w, 2875w, 1617w, 1519w, 1443w, 1365w, 1288w, 1015w, 943w, 813w, 748w, 698w. Yield = 0.715 g (95%).

*4.1.2.2.2. N-((1-Methyl-1H-imidazol-2-yl) methylene)(phenyl)methanamine (**3b**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.42 (1H, s, N=CH), 7.32–7.34 (5H, m, aromatic), 7.11 (1H, s, imz), 6.92 (1H, s, imz), 4.78 (2H, d, CH_2), 4.00 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 145.9 (imz), 145.5 (CH=N), 139.6 (aromatic), 132.4 (imz), 126.1–129.0 (aromatic), 120.0 (imz), 64.3 (CH_2), 34.5 (CH_3). Elemental analysis for $C_{12}H_{13}N_3$: calcd C, 72.33; H, 6.58; N, 21.09; found C, 71.58; H, 6.12; N, 20.78. MS (ESI) *m*/*z* 200.1 $[M+H]^+$, 223.3 $[M+Na]^+$. IR *v* cm^{-1} 3109w, 2932w, 2876w, 1608w, 1520w, 1496w, 1444w, 1365w, 1288w, 1016w, 943w, 815w, 748w, 700w. Yield = 0.663 g (83%).

*4.1.2.2.3. (R)- or (S)-Methyl 2-((1-methyl-1H-imidazol-2-yl)methyleneamino)-3-phenylpropanoate (**3c**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.04 (1H, s, N=CH), 7.14–7.26 (5H, m, aromatic), 7.08 (1H, s, imz), 6.92 (1H, s, imz), 4.18 (1H, t, CH), 3.98 (3H, s, OCH_3), 3.73 (3H, s, CH_3), 3.71 (1H, m, CH*H*), 3.33 (1H, m, C*H*H). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 168.5 (C=O), 148.8 (CH=N), 145.3 (imz), 135.4 (aromatic), 131.5 (imz), 126.3–129.6 (aromatic), 119.7 (imz), 70.6 (CH), 52.2 (OCH_3), 37.4 (CH_2), 35.7 (CH_3). Elemental analysis for $C_{15}H_{17}N_3O_2$: calcd C, 66.40; H, 6.32; N, 15.49; found C, 65.86; H, 5.98; N, 15.01. MS (ESI) *m*/*z* 272.1 $[M+H]^+$, 294.2 $[M+Na]^+$. IR *v* cm^{-1} 3014w, 2957w, 2673w, 1622w, 1581w, 1529w, 1510w, 1479w, 1438w, 1270w, 1248w, 1140w, 814w, 760w. Yield = 0.288 g (71%).

*4.1.2.2.4. 4-((1-Methyl-1H-imidazol-2-yl) methyleneamino)phenol (**3d**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.47 (1H, s, N=CH), 7.21–7.26 (2H, s, aromatic), 7.11 (1H, d, imz), 7.07 (2H, m, aromatic), 6.89 (1H, d, imz), 4.13 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 154.3 (aromatic), 147.5 (CH=N), 145.3 (aromatic), 141.6 (imz), 131.6 (imz), 122.8 (aromatic), 120.0 (imz), 116.3 (aromatic), 35.3 (CH_3). Elemental analysis for $C_{11}H_{11}N_3O$: calcd C, 65.66; H, 5.51; N, 20.88; found C, 65.43; H, 5.60; N, 20.12. MS (ESI) *m*/*z* 202.1 $[M+H]^+$, 224.3 $[M+Na]^+$. IR *v* cm^{-1} 3431w, 3013w, 2671w, 2613w, 1622w, 1581w, 1458w, 1440w, 1271w, 1246w, 1143w, 837w, 756w. Yield = 0.359 g (40%).

*4.1.2.2.5. 4-Methoxy-N-((1-methyl-1H-imidazol-2-yl)methylene) benzenamine (**3e**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.50 (1H, s, N=CH), 7.20–7.23 (2H, m, aromatic), 7.14 (1H, s, imz), 6.98 (1H, s, imz), 6.89–6.92 (2H, m, aromatic), 4.10 (3H, s, OCH_3), 3.80 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 154.9 (aromatic), 147.0 (CH=N), 146.3 (aromatic), 141.6 (imz), 133.6 (imz), 120.8 (aromatic), 119.5 (imz), 117.3 (aromatic), 61.4 (OCH_3), 34.3 (CH_3). Elemental analysis for $C_{11}H_{13}N_3O$: calcd C, 65.01; H, 6.45; N, 20.68; found C, 64.92; H, 6.02; N, 20.54. MS (ESI) *m*/*z* 194.1 $[M+H]^+$, 216.2 $[M+Na]^+$. IR *v* cm^{-1} 3026w, 2711w, 2608w, 1632w, 1586w, 1464w, 1440w, 1270w, 1248w, 1138w, 827w, 766w. Yield = 0.468 g (32%).

*4.1.2.2.6. N-(4-((1-Methyl-1H-imidazol-2-yl)methyleneamino) phenyl)acetamide (**3f**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.49 (1H, s, N=CH), 7.63 (1H, s, imz), 7.52–7.55 (2H, m, aromatic), 7.17–7.26 (2H, m, aromatic), 7.02 (1H, s, imz), 4.13 (3H, s, OCH_3), 2.19 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 169.3 (C=O), 147.0 (CH=N), 141.6 (imz), 138.3 (aromatic), 133.6 (imz), 126.5 (aromatic), 118.9 (imz), 116.3 (aromatic), 36.3 (CH_3), 25.7 (CH_3). Elemental analysis for $C_{13}H_{16}N_4O$: calcd C, 63.91; H, 6.60; N, 22.93; found C, 64.46; H, 6.14; N, 22.48. MS (ESI) *m*/*z* 245.1 $[M+H]^+$, 267.2 $[M+Na]^+$. IR *v* cm^{-1} 3427w, 3105w, 1680w, 1626w, 1541w, 1437w, 1309w, 1265w, 1107w, 858w, 790w. Yield = 0.500 g (55%).

*4.1.2.2.7. 1-Methyl-1H-imidazole-2-carbaldehydeoxime (**3g**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.27 (1H, s, N=CH), 7.11 (1H, s, imz), 6.92 (1H, s, imz), 3.88 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 144.5 (N=CH), 139.7 (imz), 131.1 (imz), 119.4 (imz), 35.3 (CH_3). Elemental analysis for $C_5H_7N_3O$: calcd C, 47.99; H, 5.64; N, 33.58; found C, 47.78; H, 5.56; N, 33.08. MS (ESI) *m*/*z* 148.2 $[M+Na]^+$. IR *v* cm^{-1} 3429w, 3106w, 2939w, 1677w, 1510w, 1475w, 1445w, 1270w, 1131w, 1036w, 913w, 763w, 720w. Yield = 1.716 g (74%).

*4.1.2.2.8. 1-Methyl-1H-imidazole-2-carbaldehyde O-methyloxime (**3h**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.12 (1H, s, N=CH), 7.00 (1H, s, imz), 6.90 (1H, s, imz), 3.95 (3H, s, OCH_3), 3.86 (3H, s, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 143.7 (N=CH), 141.1 (imz), 131.4 (imz), 119.8 (imz), 56.8 (OCH_3), 33.3 (CH_3). Elemental analysis for $C_6H_9N_3O$: calcd C, 51.79; H, 6.52; N, 30.20; found C, 50.99; H, 6.11; N, 29.60. MS (ESI) *m*/*z* 162.2 $[M+Na]^+$. IR *v* cm^{-1} 3111w, 2941w, 1674w, 1520w, 1469w, 1445w, 1288w, 1147w, 1057w, 922w, 758w, 711w. Yield = 0.718 g (65%).

*4.1.2.2.9. 1-Methyl-1H-imidazole-2-carbaldehyde O-hexyloxime (**3i**).* ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.14 (1H, s, N=CH), 7.09 (1H, s, imz), 6.91 (1H, s, imz), 4.15 (2H, t, OCH_2), 3.87 (3H, s, CH_3), 1.70 (2H, m, CH_2), 1.30 (6H, m, $(CH_2)_3$), 1.00 (3H, t, CH_3). ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 144.7 (N=CH), 143.1 (imz), 131.4 (imz), 120.1 (imz), 69.5 (OCH_2), 32.3 (CH_3), 31.6 (CH_2), 29.1 (CH_2), 26.8 (CH_2), 24.5 (CH_2), 14.6 (CH_3). Elemental analysis for $C_{11}H_{19}N_3O$: calcd C, 63.13; H, 9.15; N, 20.08; found C, 62.98; H, 8.99; N, 19.83. MS (ESI) *m*/*z* 209.3 $[M+Na]^+$. IR *v* cm^{-1} 2932w, 2870w, 1714w, 1467w, 1444w, 1288w, 1059w, 943w, 748w, 711w. Yield = 0.543 g (51%).

4.1.2.3. (1-Methyl-1*H*-imidazol-2-yl)methanamines (4). In a stainless steel autoclave (20 mL), equipped with temperature control and magnetic stirrer, was purged 5 times with hydrogen,

a solution of imine (**3**) (3.0 mmol) in CH_3OH with 5% of Pd/C was transferred, the autoclave was pressurised at 20 atm and maintaining at room temperature. After 24 h, the mixture was filtered on celite and the solvent was evaporated with vacuum.

*4.1.2.3.1. (1-Methyl-1H-imidazol-2-yl) methanamine (**4a**).* The product was purified by crystallization in CH_2Cl_2 at −18 °C. ^{1}H NMR (300 MHz, DMSO) δ_H 6.93 (1H, s, H_5 imz), 6.81 (1H, s, H_4, imz), 3.87 (2H, s, CH_2), 3.63 (3H, s, CH_3). ^{13}C NMR (300 MHz, DMSO) δ_c 145.2 (imz), 129.7 (imz), 125.0 (imz), 40.2 (CH_3), 36.6 (CH_2). Elemental analysis for $C_5H_9N_3$: calcd C, 54.03; H, 8.16; N, 37.81; found C, 53.98; H, 8.01; N, 37.56. MS (ESI) *m*/*z* 111.08 $[M+H]^+$, 95.2 $[M-NH_2]$. IR *v* cm^{-1} 3154w, 2033w, 1632w, 1607w, 1529w, 1466w, 1404w, 1119w, 1182w, 863w, 828w, 768w, 744w. Yield = 0.410 g (98%).

*4.1.2.3.2. N-((1-Methyl-1H-imidazol-2-yl)methyl)(phenyl)methanamine·2HCl (**4b**).* ^{1}H NMR (300 MHz, DMSO) δ_H 7.30–7.32 (5H, m, aromatic), 6.91 (1H, s, imz), 6.79 (1H, s, imz), 4.55 (2H, s, CH_2imz), 4.33 (2H, d, CH_2Ph), 3.93 (3H, s, CH_3). ^{13}C NMR (300 MHz, DMSO) δ_c 148.2 (imz), 146.5 (aromatic), 130.0 (aromatic), 126.8 (imz), 122.3 (imz), 118.5 (aromatic), 113.5 (aromatic), 53.8 (CH_2), 39.0 (CH_2), 35.0 (CH_3). Elemental analysis for $C_{12}H_{15}N_3$·2HCl: calcd C, 52.57; H, 6.25; N, 15.33; found C, 52.54; H, 6.15; N, 15.20. MS (ESI) *m*/*z* 202.1 $[M+H]^+$, 224.3 $[M+Na^+]$. IR *v* cm^{-1} 3337w, 3111w, 2677w, 2376w, 1601w, 1560w, 1498w, 1433w, 1035w, 752w, 688w. Yield = 0.670 g (97%).

*4.1.2.3.3. (R)- or (S)-Methyl 2-((1-methyl-1H-imidazol-2-yl)methylamino)-3-phenylpropanoate (**4c**).* ^{1}H NMR (300 MHz, DMSO) δ_H 7.11–7.26 (5H, m, aromatic), 6.88 (1H, d, imz), 6.75 (1H, d, imz), 3.89 (1H, s, CH*H*), 3.88 (1H, s, C*H*H), 3.84 (1H, m, CH), 3.68 (3H, s, OCH_3), 3.42 (3H, s, CH_3), 2.98 (1H, m, CH*H*), 2.76 (1H, m, C*H*H). ^{13}C NMR (300 MHz, DMSO) δ_c 168.3 (C=O), 143.2 (imz), 137.1 (aromatic), 129.0–129.3 (aromatic), 127.1 (imz), 115.5 (imz), 59.3 (CH), 53.8 (OCH_3), 48.1 (CH_2), 39.2 (CH_2), 35.0 (CH_3). Elemental analysis for $C_{15}H_{19}N_3O_2$: calcd C, 65.91; H, 7.01; N, 15.37; found C, 65.73; H, 6.98; N, 15.22. MS (ESI) *m*/*z* 274.1 $[M+H]^+$, 296.3 $[M+Na^+]$. IR *v* cm^{-1} 3343w, 3122w, 2689w, 2356w, 1608w, 1610w, 1510w, 1489w, 1423w, 1070w, 762w, 696w. Yield = 0.290 g (97%).

*4.1.2.3.4. 4-((1-Methyl-1H-imidazol-2-yl)methyl amino)phenol (**4d**).* ^{1}H NMR (300 MHz, DMSO) δ_H 6.97 (1H, s, imz), 6.80 (1H, s, imz), 6.63–6.70 (4H, m, aromatic), 4.26 (2H, d, CH_2), 3.70 (3H, s, CH_3). ^{13}C NMR (300 MHz, DMSO) δ_c 151.7 (aromatic, HO–C), 146.2 (imz), 143.3 (aromatic), 126.7 (imz), 122.2 (imz), 114.2–115.2 (aromatic), 39.4 (CH_2), 32.9 (CH_3). Elemental analysis for $C_{11}H_{13}N_3O$: calcd C, 65.01; H, 6.45; N, 20.68; found C, 64.51; H, 6.39; N, 20.22. IR *v* cm^{-1} 3416w, 2934w, 2592w, 1516w, 1439w, 1249w, 1109w, 821w, 738w. Yield = 0.344 g (95%).

*4.1.2.3.5. 4-Methoxy-N-((1-methyl-1H-imidazol-2-yl)methyl)benzenamine (**4e**).* ^{1}H NMR (300 MHz, DMSO) δ_H 6.98 (1H, s, imz), 6.97 (1H, s, imz), 6.79–6.86 (2H, m, aromatic), 6.68–6.71 (2H, m, aromatic), 4.28 (2H, d, CH_2), 3.75 (3H, s, OCH_3), 3.66 (3H, s, CH_3). ^{13}C NMR (300 MHz, DMSO) δ_c 151.8 (aromatic), 146.0 (imz), 143.7 (aromatic), 126.9 (imz), 122.5 (imz), 114.1–115.6 (aromatic), 55.93 (OCH_3), 39.6 (CH_2), 31.9 (CH_3). Elemental analysis for $C_{12}H_{15}N_3O$: calcd C, 66.34; H, 6.96; N, 19.34; found C, 66.47; H, 7.21; N, 19.5. MS (ESI) *m*/*z* 218.1 $[M+H]^+$, 240.3 $[M+Na^+]$. IR *v* cm^{-1} 3246w, 3119w, 2993w, 1861w, 1510w, 1466w, 1286w, 1236w, 1033w, 981w, 823w, 748w. Yield = 0.449 g (96%).

*4.1.2.3.6. N-(4-((1-Methyl-1H-imidazol-2-yl)methylamino)phenyl) acetamide (**4f**).* ^{1}H NMR (300 MHz, DMSO) δ_H 7.26–7.43 (2H, m, aromatic), 6.97 (1H, s, imz), 6.86 (1H, s, imz), 6.63–6.67 (2H, m, aromatic), 4.28 (2H, d, CH_2), 3.65 (3H, s, OCH_3), 2.17 (3H, s, CH_3). ^{13}C NMR (300 MHz, DMSO) δ_c 168.3 (C=O), 146.4 (imz), 145.7 (aromatic), 130.1 (aromatic), 127.0 (imz), 122.7 (imz), 113.3–121.8 (aromatic), 39.9 (CH_2), 31.6 (CH_3), 24.7 (CH_3). Elemental analysis for $C_{13}H_{16}N_4O$: calcd C, 63.91; H, 6.60; N, 22.93; found C, 64.07; H, 6.89; N, 23.6. MS (ESI) *m*/*z* 245.1 $[M+H]^+$, 267.3 $[M+Na^+]$. IR *v* cm^{-1} 3483w, 3383w, 3138w, 3036w, 1664w, 1608w, 1564w, 1520w,1496w, 1313w, 1263w, 1078w, 829w, 750w. Yield = 0.488 g (97%).

4.1.3. General procedure for preparation of Pt^{II}(diamine) dichloride complexes

To a solution of K_2PtCl_4 (0.208 g, 0.500 mmol) in 16 mL of H_2O/HCl 4 M (ratio 15/1), the diamine (0.500 mmol) was added. The mixture was stirred for 6 h and heated under reflux, then it was warmed to room temperature and stirred overnight. The complex was filtered off and washed with water, methanol and diethyl ether. The solid was dried under vacuum at room temperature.

4.1.3.1. (1-Methyl-1*H*-imidazol-2-yl)methanamine dichloroplatinum(II) (Pt-4a). Light grey solid. Yield = 0.164 g (87%). ^{1}H NMR (300 MHz, DMSO) δ_H 7.33 (1H, s, H_5 imz), 7.07 (1H, s, H_4, imz), 6.09 (2H, br, NH_2), 3.90 (2H, dd, CH_2), 3.66 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2079. Elemental analysis for $C_5H_9Cl_2N_3Pt$: calcd C, 15.92; H, 2.41; N, 11.14; found C, 15.32; H, 2.73; N, 10.54. MS (ESI) *m*/*z* 400.3 $[M+Na]^+$.

4.1.3.2. *N*-((1-Methyl-1*H*-imidazol-2-yl)methyl) (phenyl)methanaminedichloroplatinum(II) (Pt-4b). Pale yellow solid. Yield = 0.191 g (82%). ^{1}H NMR (300 MHz, DMSO) δ_H 7.65 (1H, d, NH), 7.32–7.40 (5H, m, aromatic), 7.25 (1H, d, imz), 6.90 (1H, d, imz), 4.33 (2H, dd, CH_2imz), 4.03 (2H, d, CH_2Ph), 3.57 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2098. Elemental analysis for $C_{12}H_{15}Cl_2N_3Pt$: calcd C, 30.85; H, 3.24; N, 8.99; found C, 31.01; H, 3.76; N, 8.83. MS (ESI) *m*/*z* 490.3 $[M+Na]^+$.

4.1.3.3. (*S*)-Methyl 2-((1-methyl-1*H*-imidazol-2-yl)methylamino)-3-phenylpropanoatedichloro platinum(II) (Pt-(*S*)4c). Green solid. Yield = 0.223 g (88%). ^{1}H NMR (300 MHz, DMSO) δ_H 7.03–7.24 (5H, m, aromatic), 6.98 (1H, d, imz), 6.89 (1H, d, imz), 3.79 (1H, s, CH*H*), 3.71 (1H, s, C*H*H), 3.64 (1H, m, CH), 3.61 (3H, s, OCH_3), 3.43 (3H, s, CH_3), 3.26 (1H, m, CH*H*), 3.17 (1H, m, C*H*H). ^{195}Pt NMR (300 MHz, DMSO) δ −2122. Elemental analysis for $C_{15}H_{19}Cl_2N_3O_2Pt$: calcd C, 33.41; H, 3.55; N, 7.79; found C, 33.08; H, 3.77; N, 7.84. MS (ESI) *m*/*z* 562.2 $[M+Na]^+$.

4.1.3.4. (*R*)-Methyl 2-((1-methyl-1*H*-imidazol-2-yl)methylamino)-3-phenylpropanoatedichloro platinum(II) (Pt-(*R*)4c). Moss-green solid. Yield = 0.230 g (85%). ^{1}H NMR (300 MHz, DMSO) δ_H 7.03–7.24 (5H, m, aromatic), 6.98 (1H, d, imz), 6.89 (1H, d, imz), 3.79 (1H, s, CH*H*), 3.71 (1H, s, C*H*H), 3.64 (1H, m, CH), 3.61 (3H, s, OCH_3), 3.43 (3H, s, CH_3), 3.26 (1H, m, CH*H*), 3.17 (1H, m, C*H*H). ^{195}Pt NMR (300 MHz, DMSO) δ −2124. Elemental analysis for $C_{15}H_{19}Cl_2N_3O_2Pt$: calcd C, 33.41; H, 3.55; N, 7.79; found C, 32.73; H, 3.82; N, 7.86. MS (ESI) *m*/*z* 562.3 $[M+Na]^+$.

4.1.3.5. 4-((1-Methyl-1*H*-imidazol-2-yl)methyl amino)phenoldichloroplatinum(II) (Pt-4d). Light green solid. Yield = 0.180 g (76%). ^{1}H NMR (300 MHz, DMSO) δ_H 9.50 (1H, s, OH), 9.04 (1H, d, NH), 7.40 (1H, s, imz), 6.97 (1H, s, imz), 6.96 (2H, d, aromatic), 6.68 (2H, d, aromatic), 4.49–4.47 (1H, dd, C*H*H), 4.26 (1H, d, CH*H*), 3.71 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2052. Elemental analysis for $C_{11}H_{13}Cl_2N_3OPt$: calcd C, 28.16; H, 2.79; N, 8.96; found C, 27.42; H, 2.96; N, 8.59. MS (ESI) *m*/*z* 492.2 $[M+Na]^+$.

4.1.3.6. 4-Methoxy-*N*-((1-methyl-1*H*-imidazol-2-yl)methyl) benzenaminedichloroplatinum(II) (Pt-4e). Light yellow solid. Yield = 0.197 g (83%). ^{1}H NMR (300 MHz, DMSO) δ_H 9.15 (1H, d, NH), 7.42 (1H, s, imz), 6.90 (1H, s, imz), 7.05 (2H, d, aromatic), 6.88 (2H, d, aromatic), 4.55 (1H, dd, CH*H*), 4.28 (1H, d, C*H*H), 3.73 (3H, s, OCH_3), 3.72 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2058. Elemental analysis for $C_{12}H_{15}Cl_2N_3OPt$: calcd C, 29.82; H,

3.13; N, 8.69; found C, 29.87; H, 3.28; N, 8.49. MS (ESI) *m*/*z* 506.2 $[M+Na]^+$.

4.1.3.7. *N*-(4-((1-Methyl-1*H*-imidazol-2-yl)methylamino)phenyl) acetamidedichloro platinum(II) (Pt-4f). Pale yellow solid. Yield = 0.206 g (81%). ^1H NMR (300 MHz, DMSO) δ_H 9.06 (1H, d, NH), 7.26 (1H, d, imz), 7.06–6.93 (4H, m, aromatic), 6.84 (1H, d, imz), 4.63 (1H, dd, CH*H*), 4.36 (1H, d, C*H*H), 3.75 (3H, s, CH_3), 2.67 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2063. Elemental analysis for $C_{13}H_{16}Cl_2N_4OPt$: calcd C, 30.60; H, 3.16; N, 10.98; found C, 29.97; H, 3.22; N, 10.86. MS (ESI) *m*/*z* 533.2 $[M+Na]^+$.

4.1.3.8. 1-Methyl-1*H*-imidazole-2-carbaldehyde *O*-benzyloxi-medichloroplatinum(II) (Pt-3a). Deep yellow solid. Yield = 0.208 g (87%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.84 (1H, d, N=CH), 7.50–7.38 (7H, m, aromatic+ imz), 5.30 (2H, d, CH_2), 3.87 (3H, s, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2943. Elemental analysis for $C_{12}H_{13}Cl_2N_3OPt$: calcd C, 29.95; H, 2.72; N, 8.73; found C, 29.67; H, 2.98; N, 8.62. MS (ESI) *m*/*z* 504.1 $[M+Na]^+$.

4.1.3.9. 1-Methyl-1*H*-imidazole-2-carbaldehydeoximedichloro-platinum(II) (Pt-3g). Orange solid. Yield = 0.150 g (77%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.97 (1H, s, OH), 8.73 (1H, d, N=CH), 7.77 (1H, d, imz), 7.69 (1H, d, imz), 3.91 (3H, d, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2904. Elemental analysis for $C_5H_7Cl_2N_3OPt$: calcd C, 15.35; H, 1.80; N, 10.74; found C, 15.73; H, 2.03; N, 10.74. MS (ESI) *m*/*z* 393.3 $[M+H]^+$, 804.8 $[2M+Na]^+$.

4.1.3.10. 1-Methyl-1*H*-imidazole-2-carbaldehyde *O*-methyloxi-medichloroplatinum(II) (Pt-3h). Pale orange solid. Yield = 0.159 g (79%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.80 (1H, d, N=CH), 7.57 (1H, d, imz), 7.50 (1H, d, imz), 4.03 (3H, d, OCH_3) 3.91 (3H, d, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2910. Elemental analysis for $C_6H_9Cl_2N_3OPt$: calcd C, 17.79; H, 2.24; N, 10.37; found C, 17.80; H, 2.28; N, 10.32. MS (ESI) *m*/*z* 428.1 $[M+Na]^+$.

4.1.3.11. 1-Methyl-1*H*-imidazole-2-carbaldehyde *O*-hexyloxi-medichloroplatinum(II) (Pt-3i). Yellow solid. Yield = 0.194 g (82%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.79 (1H, d, N=CH), 7.55 (1H, d, imz), 7.49 (1H, d, imz), 4.25 (2H, t, OCH_2), 3.82 (3H, d, CH_3), 1.73 (2H, m, CH_2), 1.36 (6H, m, $(CH_2)_3$), 1.00 (3H, t, CH_3). ^{195}Pt NMR (300 MHz, DMSO) δ −2960. Elemental analysis for $C_{11}H_{19}Cl_2N_3OPt$: calcd C, 27.80; H, 4.03; N, 8.84; found C, 28.33; H, 4.13; N, 8.99 . MS (ESI) *m*/*z* 497.3 $[M+Na]^+$, 973.0 $[2\ M+Na]^+$.

4.2. Biological evaluation procedures

4.2.1. Reagents and antibodies

DMEM, trypsin-EDTA, penicillin, streptomycin, non-essential amino acid solution, fetal calf serum (FCS), disposable culture flasks and petri dishes were purchased from Euroclone S.p.A. (Pero, Milan, Italy). For western blot analysis, the following antibodies were used: mouse monoclonal anti-p53 and $p21^{Waf}$ (Santa-Cruz Biotechnology Inc., Santa Cruz, CA, USA), mouse monoclonal anti-α-tubulin (Sigma–Aldrich, Milan, Italy), anti-mouse peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Lab; Cambridgeshire, UK).

4.2.2. Cell culture

The following human cancer cell types have been utilised in the study: NCI-H460 (non-small cell lung cancer) A2780 and IGROV-1 (epithelial ovarian cancer), HEY (ovarian cancer), A431 (cutaneous squamous carcinoma), KYSE510 (esophageal squamous cell carcinoma) HCT15, KM12, SW620, HCT116, DLD1, and COLO205 (colorectal cancer), MCF-7 and MDA-MB-231 (breast cancer), and A549 (lung adenocarcinoma). All cultured media were supplemented with penicillin (10,000 U/mL), streptomycin (10 mg/mL), nonessential amino acid and 10% Fetal Calf Serum (FCS) unless otherwise indicated. Dulbecco's modified eagle's medium (DMEM) with glutamine was utilised for HEY, A2780, A549, MDA-MB-231, MCF-7, A431, DLD-1 cell lines; RPMI 1640 for NCI-H460, IGROV-1 e HCT-15; McCoy's for HCT-116; 49% Ham's plus 49% RPMI with 2% FCS for KYSE-150. Cells were incubated with newly synthesized compounds dissolved in DMSO. The same volume of solvent were added to control conditions and did not exceed 0.5% v/v.

4.2.3. MTT-assay

The determination of the conversion of MTT (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) to formazan was determined by using a commercially available kit (Millipore, Billerica, MA, USA), according to the manufacturer's instructions.[35]

4.2.4. Determination of intracellular and DNA-bound Pt concentration

For the determination of total intracellular ^{195}Pt concentrations, cells were washed twice with PBS and lysed by incubation with 1% Triton X100/0.1% SDS for 5 min at room temperature. Cell lysates were then cleared by centrifugation at 14,000*g* for 10 min, and the ^{195}Pt concentrations determined by ICP-MS. The data were normalised with the protein concentrations determined using the BCA protein assay (Thermo scientific, Rockford, IL USA). For the DNA-bound ^{195}Pt concentrations, the nuclear DNA was extracted by incubating cell monolayers with digest buffer (50 mM Tris–HCl 1 M, 100 mM NaCl, 100 mM EDTA, 1% SDS) then transferred to 1.5 mL microcentrifuge tubes and saturated NaCl solution added. The samples were then clear by centrifugation for 15 min at 13,000 rpm and the supernatant transferred to new microcentrifuge tubes and DNA precipitated by isopropanol. DNA was then washed by centrifugation two times with 70% ethanol and resuspended in TE buffer (10 mM Tris pH 8.0; 0.1 mM EDTA). The ^{195}Pt concentrations were then determined by ICP-MS.

4.2.5. Western blot analysis

Cells were washed twice with PBS and lysed by incubation with a solution of 50 mM Tris pH 7.5, 150 mM NaCl, 0.5% Nonidet-P40, containing a protease and phosphatase inhibitor cocktails (Sigma–Aldrich, Milan, Italy) for 30 min on ice. Cell lysates were then cleared by centrifugation at 14,000 g for 10 min, and protein concentrations were determined using the BCA protein assay (Thermo scientific, Rockford, IL USA). Equal amount of total protein per sample were separated by SDS-PAGE under reducing conditions, transferred to Immobilon PVDF (GE Healthcare Little Chalfont, Buckinghamshire, UK) and subsequently immunoblotted with primary antibody following appropriate secondary antibody, prior to visualization by enhanced chemiluminescence (LiteAblot Extend Long Lasting Chemiluminescent Substrate, EuroClone).

Quantitative densitometric analyses were performed using Gel Doc acquisition system and Quantity One software (BIO-RAD Laboratories, Hercules, CA, USA).[36]

4.2.6. Statistical analysis

All data shown are representative of at least three replicate experiments. Data are expressed as mean ± SD. Statistical analyses were performed using the unpaired Student's *t* test. *P* values <0.05 were considered significant. The concentration of compounds required to reduce by 50% the cell viability (EC_{50}) was calculated by nonlinear regression curve (SigmaPlot software; Systat Software, Inc., Point Richmond, CA).

References and notes

1. Alderden, R. A.; Hall, M. D.; Hambley, T. W. *J. Chem. Educ.* **2006**, *83*, 728.
2. Rosenberg, B.; Vancamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385.
3. Burger, H.; Loos, W. J.; Eechoute, K.; Verweij, J.; Mathijssen, R. H.; Wiemer, E. A. *Drug Resist. Updat.* **2011**, *14*, 22.
4. Bancroft, D. P.; Lepre, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 6860.
5. Siddik, Z. H. *Oncogene* **2003**, *22*, 7265.
6. Gonzalez, V. M.; Fuertes, M. A.; Alonso, C.; Perez, J. M. *Mol. Pharmacol.* **2001**, *59*, 657.
7. Fuertes, M. A.; Alonso, C.; Pérez, J. M. *Chem. Rev.* **2003**, *103*, 645.
8. Monti, E.; Gariboldi, M.; Maiocchi, A.; Marengo, E.; Cassino, C.; Gabano, E.; Osella, D. *J. Med. Chem.* **2005**, *48*, 857.
9. Todd, R. C.; Lippard, S. J. *Metallomics* **2009**, *1*, 280.
10. Raymond, E.; Faivre, S.; Chaney, S.; Woynarowski, J.; Cvitkovic, E. *J. Mol Cancer Ther.* **2002**, *1*(3), 227.
11. Rabik, C. A.; Dolan, M. E. *Cancer Treat. Rev.* **2007**, *33*, 9.
12. Gasser, G.; Ott, I.; Metzler-Nolte, N. *J. Med. Chem.* **2012**, *54*, 3.
13. Hanif, M.; Nazarov, A. A.; Legin, A.; Groessl, M.; Arion, V. B.; Jakupec, M. A.; Tsybin, Y. O.; Dyson, P. J.; Keppler, B. K.; Hartinger, C. G. *Chem. Commun.* **2012**, *48*, 1475.
14. Messori, L.; Marcon, G.; Orioli, P.; Fontani, M.; Zanello, P.; Bergamo, A.; Sava, G.; Mura, P. *J. Inorg. Biochem.* **2003**, *95*, 37.
15. Klein, A. V.; Hambley, T. W. *Chem. Rev.* **2009**, *109*, 4911.
16. Huq, F.; Daghriri, H.; Yu, J. Q.; Beale, P.; Fisher, K. *Eur. J. Med. Chem.* **2004**, *39*, 691.
17. Munk, V. P.; Diakos, C. I.; Ellis, L. T.; Fenton, R. R.; Messerle, B. A.; Hambley, T. W. *Inorg. Chem.* **2003**, *42*, 3582.
18. Ranaldo, R.; Margiotta, N.; Intini, F. P.; Pacifico, C.; Natile, G. *Inorg. Chem.* **2008**, *47*, 2820.
19. De Pascali, S. A.; Migoni, D.; Papadia, P.; Muscella, A.; Marsigliante, S.; Ciccarese, A.; Fanizzi, F. P. *Dalton Trans.* **2006**, 5077.
20. Hall, M. D.; Mellor, H. R.; Callaghan, R.; Hambley, T. W. *J. Med. Chem.* **2007**, *50*, 3403.
21. Abramkin, S. A.; Jungwirth, U.; Valiahdi, S. M.; Dworak, C.; Habala, L.; Meelich, K.; Berger, W.; Jakupec, M. A.; Hartinger, C. G.; Nazarov, A. A.; Galanski, M.; Keppler, B. K. *J. Med. Chem.* **2012**, *53*, 7356.
22. Annie Bligh, S. W.; Bashall, A.; Garrud, C.; McPartlin, M.; Wardle, N.; White, K.; Padhye, S.; Barve, V.; Kundu, G. *Dalton Trans.* **2003**, 184.
23. Oziminski, W. P.; Garnuszek, P.; Bednarek, E.; Dobrowolski, J. C. *Inorg. Chim. Acta* **2007**, *360*, 1902.
24. Mansuri-Torshizi, H.; Ghadimy, S.; Akbarzadeh, N. *Chem. Pharm. Bull. (Tokyo)* **2001**, *49*, 1517.
25. Diakos, C. I.; Zhang, M.; Beale, P. J.; Fenton, R. R.; Hambley, T. W. *Eur. J. Med. Chem.* **2009**, *44*, 2807.
26. Garnuszek, P.; Karczmarczyk, U.; Maurin, M. *Nucl. Med. Biol.* **2008**, *35*, 605.
27. Summa, N.; Schiessl, W.; Puchta, R.; Van, E. H. N.; Van, E. R. *Inorg. Chem.* **2006**, *45*, 2948.
28. Crabtree, R. H. *The Organometallic Chemistry of Transition Metals*; John Wiley & Sons: Hoboken, NJ, 2005.
29. Howson, S. E.; Allan, L. E. N.; Chmel, N. P.; Clarkson, G. J.; Deeth, R. J.; Faulkner, A. D.; Simpson, D. H.; Scott, P. *Dalton Trans.* **2011**, *40*, 10416.
30. Hansson, G. K. *N. Engl. J. Med.* **2005**, *352*, 1685.
31. Egan, T. J.; Koch, K. R.; Swan, P. L.; Clarkson, C.; Van Schalkwyk, D. A.; Smith, P. J. *J. Med. Chem.* **2004**, *47*, 2926.
32. Scaffidi-Domianello, Y. Y.; Legin, A. A.; Jakupec, M. A.; Roller, A.; Kukushkin, V. Y.; Galanski, M.; Keppler, B. K. *Inorg. Chem.* **2012**, *51*, 7153.
33. Appella, E.; Anderson, C. W. *Eur. J. Biochem.* **2001**, *268*, 2764.
34. Yu, F.; Megyesi, J.; Price, P. M. *Am. J. Physiol. Renal Physiol.* **2008**, *295*, 44.
35. Kwon, J.-Y.; Bai, S.-W.; Kwon, Y.-G.; Kim, S.-H.; Kim, C. H.; Kang, M. H.; Linton, J. A.; Park, Y.-W. *Acta Obstet. Gynecol. Scand.* **2010**, *89*, 565.
36. Ferri, N.; Tibolla, G.; Pirillo, A.; Cipollone, F.; Mezzetti, A.; Pacia, S.; Corsini, A.; Catapano, A. L. *Atherosclerosis* **2012**, *220*, 381.