Journal Name

ARTICLE

PtI_2(DACH), the Iodido Analogue of Oxaliplatin as a Candidate for Colorectal Cancer Treatment: Chemical and Biological Features

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Colorectal cancer (CRC) is a global health problem being the fourth most common cause of death for cancer worldwide. Oxaliplatin plays a key role in current CRC treatment but shows serious drawbacks, such as a high systemic toxicity and the frequent insurgence of Pt resistance. In search of novel and more efficacious Pt-based drugs for CRC treatment, we synthesized and characterised PtI_2(DACH), an oxaliplatin analogue. PtI_2(DACH) was obtained through the replacement of bidentate oxalate with two iodides. PtI_2(DACH) turned out to be more lipophilic than Oxaliplatin, a fact that led to an enhancement of its cellular uptake. At difference from oxaliplatin, PtI_2(DACH) showed a scarce reactivity towards model proteins, while keekping affinity for a standard DNA oligo. Notably, PtI_2(DACH) induced cytotoxicities roughly comparable to those of oxaliplatin in three representative CRC cell lines. Moreover, it was able to trigger cell apoptosis, to an extent even better that cisplatin and oxaliplatin. Overall, a rather promising picture emerges for this novel Pt drug that merits, in our opinion, a deeper and more extensive preclinical evaluation.

Introduction

Today, colorectal cancer (CRC) is the fourth most common cause of death for cancer worldwide. Prognosis is highly correlated with TNM staging‡ (describing size of tumour, involvement of nearby lymph nodes, presence of metastasis), with a 5-year survival of 90% for patients in earlier stages but less than 25% for those with metastatic disease.[1] The cornerstone of therapy is represented by "*en bloc*" surgical resection of tumour and regional nodes, although perioperative chemotherapy may provide important advantages in subjects with advanced disease and metastases.[2] Adjuvant chemotherapy for CRC mainly relies on fluoropyrimidine compounds combined with platinum-based drugs, mainly cisplatin and its analogue oxaliplatin.

Oxaliplatin (Eloxatin™) is a third-generation platinum compound approved by United States Food and Drug Administration (FDA) on 2002 for the treatment of advanced colorectal cancer. To date thousands of patients worldwide have been treated with oxaliplatin, especially in combination with other drugs.[3]

Indeed, either the FOLFOX (5-FU, leucovorin, and oxaliplatin) or CapeOx (capecitabine and oxaliplatin) regimens are used most often.[4,5]

Despite its success, important side effects such as acute and chronic neurotoxicity are, still today, limiting factors to its clinical use.[6]

With the aim to overcome the main drawbacks of oxaliplatin treatment *i.e.* side effects as well as frequent intrinsic or acquired pharmacological resistance, in the last decades, many analogues of this clinically established drug have been synthesised and tested. [7-11]. In this frame we have prepared the oxaliplatin analogue {(1R,2R)-cyclohexane-1,2-diamine} diiodidoplatinum(II), (PtI_2(DACH) hereafter) (Figure 1) where the bidentate oxalate ligand of oxaliplatin is replaced by two iodide ligands. Notably, this oxaliplatin derivative had been reported and structurally characterised in 2011 by R. Pažout and coworkers,[12] but no biological studies were then attempted.

Reasons that make PtI_2(DACH) of particular interest for inorganic medicinal chemists are essentially twofold.

i) We believe that replacement of the bidentate oxalate ligand with two iodides may affect importantly the activation process of the Pt centre, both kinetically and thermodynamically, influencing accordingly its overall biological profile.

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Electronic Supplementary Information (ESI) available: NMR characterisation, ESI-MS analysis after incubation with protein and theoretical calculations. See DOI: 10.1039/x0xx00000x

ii) In addition, insertion of two iodide ligands in the place of oxalate is expected to increase considerably the lipophilic character of the resulting drug hopefully enhancing cellular uptake and bioavailability.

It is worthy reminding that this kind of strategy was recently exploited, in our research group, and applied to cisplatin. Indeed, the iodide analogue of cisplatin was prepared and characterised and shown to possess interesting chemical and biological properties, that are definitely distinct from those of cisplatin.[13]

The obtainment of the desired Pt compound, *i.e.* PtI_2(DACH), was confirmed and supported by NMR and CHN analysis. Afterward, the complex was studied in depth in terms of its solution behaviour and its interactions with model proteins (i.e. lysozyme, RNase) and with a short single strand oligonucleotide bearing the GG motif. Also, cytotoxic and pro-apoptotic effects were investigated in a few representative CRC cell lines and mechanistic hypotheses for the different pharmacological effects proposed. Finally, uptake experiments were carried out to assess whether replacement of the oxalate ligand with two iodide ligands affords indeed an enhanced uptake hopefully improving drug's bioavailability.

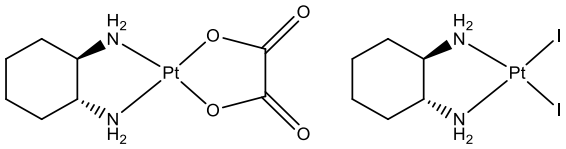

Fig. 1 Schematic drawing of oxaliplatin and its iodide analogue

Experimental

Chemicals and reagents

Chemicals were purchased from Sigma-Aldrich and used without further purification. Dodecameric oligonucleotide ODN2, with sequence CTACGGTTTCAC and proteins were also purchased from Sigma- Aldrich.

Chemistry

Synthesis and characterisation of PtI_2(DACH)

For the synthesis of PtI_2(DACH), 99.3 mg of K_2PtCl_4 (2.4 x 10^{-4} mol), have been solubilised in 1.5 mL di of milli-q water. A water solution (2 mL) of KI (158.7 mg,9.6 x 10^{-4} mol) was added to K_2PtCl_4 and stirred (50° C) in the dark for five minutes until quantitative conversion of K_2PtCl_4 in K_2PtI_4 was obtained. 1,2-Diaminocyclohexane (1R,2R)-(-), DACH (27.3 mg, 2.4 x 10^{-4} mol) was then solubilised in 2 mL of milli-q water and the obtained solution slowly added to K_2PtI_4 water solution. After further four hours of stirring precipitate appeared, and complete precipitation of yellow crystals of PtI_2(DACH) allowed over night at room temperature. The solid was then collected through vacuum filtration and washed with hot water and ice-cooled ethanol and ether. 135.5 mg of PtI_2(DACH) were obtained (yield 81 %). Complex was characterised through ^{1}H-NMR and elemental analysis. ^{1}HNMR (DMF-d^7, 400.13 MHz, 298 K): 5.56 (b, 1H); 4.96 (b, 1H); 2.50 (t, *J* = 8 Hz, 2H); 2.23 (d, *J* = 12 Hz, 2H); 1.56 (m, 4H); 1.15 (m, 2H). Elemental analysis of C, N and H [calculated C: 12.8%, H: 2.32%, N: 4.98%, experimental: C: 12.5%, H: 2.32%, N: 4.86%]; see FIG. S1, S2, S3 in the ESI for ^{1}H NMR, ^{13}C, ^{195}Pt spectra with assignments.

Log P value determination

The octanol-water partition coefficients for PtI_2(DACH) was determined by modification of reported shake-flask method. Water (50 mL, distilled after milli-Q purification) and n-octanol (50 mL) were shaken together for 72 h to allow saturation of both phases. Solution of the complex was prepared in the water phase (3x10^{-3} M) and an equal volume of octanol was added. Biphasic solutions were mixed for ten minutes and then centrifuged for five minutes at 6000 rpm to allow separation. Concentration in both phases was determined by UV-VIS. Reported logP is defined as log $[complex]_{oct}/[complex]_{wat}$. Final values were reported as mean of three determinations.

Uv-Vis experiments

Solution behaviour of PtI_2(DACH) was assessed through spectrophotometric experiments performed with a Varian Cary 50 Bio UV-Vis spectrophotometer (1 cm pathlength quartz cell) in buffered solutions without the use of DMSO and NaCl. A solution of the complex (10^{-4} M) was prepared in 50 mM phosphate buffer at pH = 7.4. The absorbance was monitored in the wavelength range between 200 and 800 nm for 72 h at 25° C.

ESI-MS experiments

ESI-MS spectrum of the ODN2-PtI_2(DACH) mixture was recorded by direct injection at 5 µl/min flow rate in an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The working conditions were as follows: negative polarity, spray voltage -2.7 kV, capillary voltage -20 V, capillary temperature 280° C, tube lens voltage -113 V. The sheath and the auxiliary gases were set at 23 and 4 (arbitrary units), respectively. For acquisition, Xcalibur 2.0. software (Thermo) was used and deconvoluted masses were obtained by using the ProMass 2.8 rev. 2 tool for Xcalibur (Novatia).

ESI-MS spectra of the PtI_2(DACH)-protein mixtures were recorded by direct injection at 3 µl/min flow rate in the same instrumentation. The working conditions were as follows: positive polarity, spray voltage 3.1 kV, capillary voltage 45 V, capillary temperature 220° C, tube lens voltage 230 V. The sheath and the auxiliary gases were set at 17 and 1 (arbitrary units), respectively. For acquisition, Xcalibur 2.0. software (Thermo) was used and deconvoluted masses were obtained by using the integrated Xtract tool.

For spectra acquisition a nominal resolution (at m/z 400) of 100,000 was used.

Chemicals and Drugs

For cellular experiments cisplatin, *cis*-$PtI_2(NH_3)_2$, oxaliplatin and PtI_2(DACH) were dissolved in bidistilled water at the concentration of 8.3, 1, 5 and 1 mM, respectively. All stock solutions were stored at -20° C.

Cell culture

Human colorectal cancer cell lines (HCT-116, HCT-8 and HT29) and mouse fibroblast cell line L929 were cultured in RPMI-1640 medium (Euroclone; Milan, Italy), supplemented with 2% L-Glut, 10% fetal

bovine serum (FBS) and 1% penicillin/streptomycin (complete medium). HT29 cells were kindly provided by Dr. R. Falcioni (Regina Elena Cancer Insitute, Roma).

Cell viability assay

To evaluate the IC_{50} of each compound, cell viability was assessed through the Trypan Blue exclusion test (Sigma-Aldrich). Cells were seeded at $1x10^4$/well in 96-well plates (Costar Corning) in complete medium and incubated for 24h before compound addiction. Following the addition, cells were further incubated for 24 hours. Cells were then harvested and counted by the Trypan Blue exclusion test using a hemocytometer. All experiments were performed in triplicate. The IC_{50} values were calculated for each cell line and data fitted using a Hill1-type equation by using Origin Software (Microcal Origin 8.0 software; OriginLab Corporation, Nothampton, MA).

Proliferation experiments.

HCT116 cells were seeded at 1x104/well in 96-well plates (Costar Corning) in complete medium and incubated for 24h before compound addiction. After 24, 48 and 72 h of incubation viable cells (determined by the Trypan Blue exclusion test) were counted in triplicate using a haemocytometer. Each experimental point represents the mean±sem of three separate experiments (see S7 in the ESI for proliferation experiment details and graph).

Cell cycle analysis

Cell cycle distribution was assessed by flow cytometry after staining the cells with propidium iodide (PI). Cells were seeded and treated generally with different compound at their IC_{50} (unless otherwise noted in figure legend) for 24h. At the end of incubation, cells were harvested, washed with PBS and resuspended in 300 µl Propidium Iodide staining solution and incubated in the dark for 20 minutes at room temperature. The DNA content of the cells was measured by BD FACSCanto (Becton Dickinson, Franklin Lakes, NJ, USA) and the percentage of cells in each cell cycle phase was determined using ModFit LT 3.0 analysis software (Verity Software House, Topsham, ME USA).

Annexin/PI assay

Apoptosis was determined through the Annexin V/propidium iodide test (Annexin-VFLUOS staining kit; Roche Diagnostics, Mennheim, Germany). Cells, treated as above, were harvested after 24h of treatment with the different compounds (at their IC_{50} value, unless otherwise noted in figure legend), washed with PBS, re-suspended in 100 µl of buffer and incubated with FITC-conjugated Annexin V and propidium iodide for 15 min. Flow cytometry was performed using the BD FACSCanto (Becton Dickinson, Franklin Lakes, NJ, USA). Data were analyzed through the BD FACSDiva Software 6.1.3.

Statistical analysis

Data are generally given as mean values ± standard error of the mean (sem), unless otherwise specified in figure legend. The normality of data distribution was checked with the Kolmogorov-Smirnov test. For multiple comparisons, we used one-way ANOVA, with post-hoc Bonferroni test to derive P values.

Uptake measurement

The determination of platinum concentration in the cellular pellets was performed by a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) equipped with a CETAC U5000 AT+ ultrasonic nebulizer, in order to increase the method sensitivity as fully described in Marzo T. et al, 2015.[13]

Results and Discussion

Synthesis and characterization of PtI_2(DACH)

The synthesis of PtI_2(DACH) was performed as described in the experimental section, with a good yield. Synthesis started from K_2PtCl_4, that was quantitatively converted into K_2PtI_4. At variance with previously reported synthetic routes,[5] 1,2-diaminocyclohexane (1R,2R)-(-) was used in the place of DACH tartrate and slowly added to K_2PtI_4 , at room temperature. The purity of the resulting product was checked by ^{1}H-NMR and elemental analysis and found to be > 95%.

A detailed description of all the procedures and of the final chemical characterization is given in the experimental section and ESI (fig. S1, S2, S3). Remarkably, in the ^{1}H-NMR spectrum, the two signal at 4.96 and 5.56 ppm, are assignable to the ammine protons. This attribution was validated by direct comparison with the spectrum of $PtCl_2$(DACH) -previously synthesized- recorded in the same condition. It is interesting to note that at difference of $PtCl_2$(DACH) for which the two signals attributable to the amine protons can be suppressed by adding D_2O to the DMF-d^7 sample solution, for PtI_2(DACH) the exchange of proton with deuterium (and thus amine signals suppression), doesn't occur even after 24 h after the addition of deuterated water, indicating that replacement of chloride with iodide ligands strongly decreases the mobility of amine protons (FIG. S4 and part S4 in the ESI for details on the synthesis of $PtCl_2$(DACH) and NMR spectra).

Log P determination

The octanol-water partition coefficient is an important parameter in drug design with a large impact on the overall ADME‡ profile of the drug itself. Generally speaking, a more lipophilic character commonly facilitates cellular drug uptake and bioavailability. However, the situation is somewhat more complex when dealing with a prodrug requiring chemical activation prior to performing its pharmacological actions as it is the case for anticancer Pt compounds. A log P value of 0.76 was determined for PtI_2(DACH) by modification of classical flask method previously used (see experimental section for further details).[13,14] For comparison purposes log P values for a few parent Pt complexes are also reported in table 1.

Complex	Log P value
Cisplatin	-2.40*
cis-$PtI_2(NH_3)_2$	-0.13
Oxaliplatin	-1.58**
PtI_2(DACH)	0.76

***Value previously determined in our laboratories, Ref. 13**
****The log P here determined for oxaliplatin is close to that reported in literature (-1.76, Ref. 15)**

Table 1 Log P values for Pt-based drugs

As expected, the bis-iodide analogue i.e. PtI_2(DACH), manifests a far larger lipophilic character than oxaliplatin, being the most lipophilic compound in the series.

Solution Chemistry

PtI_2(DACH) may be easily solubilised under physiological-like conditions (*i.e.* in phosphate buffer) upon short sonication, thus avoiding the use of DMSO and the associate chemical interferences. The solution behaviour of PtI_2(DACH) was then assessed through UV-Vis analysis. Similarly to *cis*-$PtI_2(NH_3)$, PtI_2(DACH) shows two main absorption bands located at 280 and 350 nm (Figure 2).[13] With time, the intensity of both bands slowly decreases; the residual band intensity after 72 hours is only ~ 20% of the original intensity according to a roughly monoexponential decay.

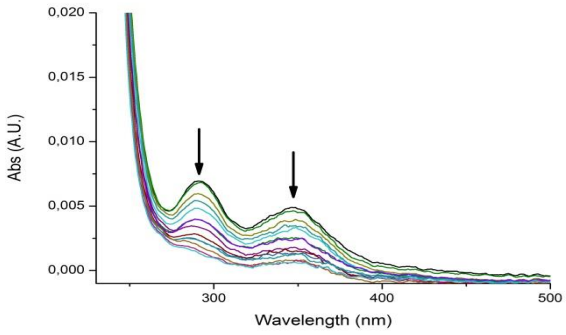

Fig. 2 Time course spectra of PtI_2(DACH), 10^{-4} M in 50 mM phosphate buffer recorded over 72 h, RT.

The observed spectral changes are ascribed to the progressive release of the two iodido ligands[13,14] being the hydrolysis of PtI_2(DACH) significantly slower than cis-$PtI_2(NH_3)_2$ but faster than oxaliplatin (see Table 2).[17]

Complexes	k_{obs} (s^{-1}) [1/t]	$t_{1/2}$ (min) [t*ln(2)]
PtI_2(DACH)	9,75 x 10^{-6}	1184,58
cis-$PtI_2(NH_3)_2$*	2,08 x 10^{-5}	555,58
Oxaliplatin**	6,32 x 10^{-6}	1827,92

*** Value previously determined in our laboratories, Ref. 13**

**** The K_{obs} here determined for oxaliplatin is close to that reported in literature (7,76 x 10^{-6} S^{-1}, Ref. 16)**

Table 2 k_{obs} was calculated fitting the variation of absorbance as function of time with a single exponential function (UV-Vis time course spectra of complexes, 10^{-4} M in 50 mM phosphate buffer recorded over 72 h, RT).

Biomolecular Interactions of PtI_2(DACH)

Next, to study its interactions with model biological targets, cisPtI_2(DACH) was incubated with two model proteins *i.e.* hen egg white lysozyme and RNase (see experimental part for details) and possible adduct formation investigated. These two proteins were chosen since their structures have been often used to characterize the interactions occurring between proteins and metallodrugs; in addition, crystal structures of their adducts with both cisplatin and oxaliplatin are available.[17-20] Notably, these model proteins are well suitable also for ESIMS studies, offering a very important substrate for comparative mechanistic studies of metal-based drugs. In both cases no metallodrug-protein adduct was detected, indicating that PtI_2(DACH), at variance with oxaliplatin, is not able to bind these proteins (see FIG. S5 and S6 in the ESI for ESI-MS spectra).[18-20] An Explanation for this unexpected behaviour is not straightforward. We previously reported that coordination of oxaliplatin to lysozyme and RNase implies a multi-step reaction at the level of aspartate residues. First non-covalent coordination of oxaliplatin to the protein occurs; then detachment of one oxygen atom of oxalate from Pt coordination (a ring-opening step leading to monodentatate oxalate) is observed with concomitant Pt coordination of the carboxylate group from an aspartate residue. Finally the oxalate ligand is fully released.[19,20] We might hypothesize that, this multi-step mechanism is inhibited when the oxalate ligand is replaced by two iodides due to the lack of "recognition" of intact PtI_2(DACH) by these model proteins.

Conversely, and in analogy with its parent drug oxaliplatin, PtI_2(DACH) interacts extensively with a standard oligonucleotide bearing the GG motif (ODN2) through a classical reaction pattern involving preferential release of iodide ligands (Figure 3).[21] These results are fully supported by theoretical calculations of ESI-MS peaks (see FIG. S6 in the ESI).

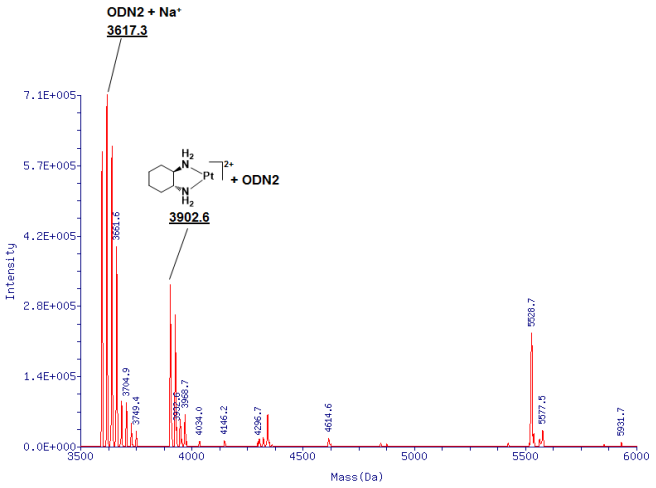

Fig. 3 Deconvoluted ESI-MS spectrum of $PtI_2(DACH)$ 10^{-4} M incubated with ODN2 in LC-water 72 h, 37° C, metal to oligonucleotide ratio 1:1

Cellular effects

We then measured the cytotoxic effects of $PtI_2(DACH)$ in a small panel of CRC cell lines (HT-29, HCT-116, HCT-8) in comparison with cisplatin, oxaliplatin and cis-$PtI_2(NH_3)_2$. A normal cell line (L929 fibroblast cells) was also included. *cis*-$PtI_2(NH_3)_2$ compound was also evaluated since we previously showed that it is fairly active on a cisplatin resistant CRC cell line, and can hence be considered a potential drug candidate to overcome, at least partially, Pt resistance.[13]

Cells were exposed to increasing concentrations of the drugs, given in the 0-300 µM range, for 24 hours, and the respective IC_{50} concentrations were determined (Table 3).

Cell line	cisplatin	*cis*-PtI2(NH3)2	oxaliplatin	PtI2(DACH)
HT-29	15.2±1.3	5.8±0.3	19.7±1.2	25.7±1.6
HCT-116	22.9±1.0	9.1±0.2	49.2±0.9	34.5±1.2
HCT-8	9.3±0.6	23.6±1.0	33.1±0.2	51.1±2.0
L929	70.2±1.5	72.1±2.3	72.1±1.1	82.4±2.4

Table 3 IC_{50} (µM) of cisplatin, *cis*-$PtI_2(NH_3)_2$, Oxaliplatin and $PtI_2(DACH)$ in HT29, HCT116 and HCT8 CRC cell lines, as well as in a normal fibroblastic cell line (L929). Cell vitality was measured after 24 hours of treatment with the four different compounds, by the Trypan Blue exclusion test. IC_{50} values (means ± sem of four independent experiments) were calculated using the Origin Software (Microcal Origin 8.0 software; OriginLab Corporation, Northampton, MA) fitting experimental data with Hill1 type equation.

In line with our previous results[13], *cis*-$PtI_2(NH_3)_2$ turned out to be more active than cisplatin, and even more compared to oxaliplatin, especially in the case of partially cisplatin-resistant cell lines HT29 and HCT116. Though less cytotoxic than cisplatin and slightly less cytotoxic than oxaliplatin, $PtI_2(DACH)$ was more effective than oxaliplatin in HCT116 cells, that are poorly sensitive to either compounds. These data point out that $PtI_2(DACH)$ maintains appreciable cytotoxic properties, not different from, and in some case even better than, oxaliplatin. This implies that replacement of oxalate with two iodide does not impair the cellular effects of this drug that may be attributed mainly to the $[Pt(DACH)]_2$ chemical moiety. Additionally, when evaluating the effects of all compounds on fibroblast cell line L929, we can state that the cytotoxicity is on the whole similar and rather low, being the iodide analogue of oxaliplatin slightly less cytotoxic.

Based on these results we studied in detail the effects that Pt compounds produce on HCT-116 cells, determining both the distribution of the cells in the various phases of the cell cycle and the percentage of cells in the early apoptotic phase. These effects were measured in the absence or in the presence of the four compounds tested at their respective IC50s. None of the tested Pt compounds caused relevant variation of cell cycle distribution of HCT 116 cells (Table 4 and Figure 4).

	G0/G1 (%)	S (%)	G2/M (%)
control	36.3±0.6	41.5±2.6	22.2±2.8
cisplatin	41.3±1.3	36.3±3.5	22.4±1.3
***cis*-$PtI_2(NH_3)_2$**	45.5±2.8	35.5±5.3	19.1±2.5
oxaliplatin	37.9±3.6	37.0±4.4	25.1±1.9
$PtI_2(DACH)$	40.6±2.3	36.4±3.4	24.1±3.7

Table 4 Cell cycle distribution of HCT 116 cells after 24 hours of treatment with the four different compounds at the IC_{50} values shown in Table 3. Data are means±sem of three independent experiments, and are expressed as percentage of cells in each phase of the cell cycle, determined by flow cytometry after staining the cells with propidium iodide (PI).

On the contrary, both cisplatin and oxaliplatin increased the percentage of early apoptotic cells, as expected (Figure 5A). Furthermore, the two iodide analogues showed an even more evident pro-apoptotic effect, higher than cisplatin and oxaliplatin. Interestingly, the analysis of dot plots (Figure 5B) showed a shifting of the Annexin V/PI labelled population to higher fluorescence in HCT116 cells treated with $PtI_2(DACH)$ compared to oxaliplatin. This suggests that the iodide compound exerts a stronger, possibly earlier, pro-apoptotic effect in HCT 116 cells, which poorly respond to both cisplatin and oxaliplatin.

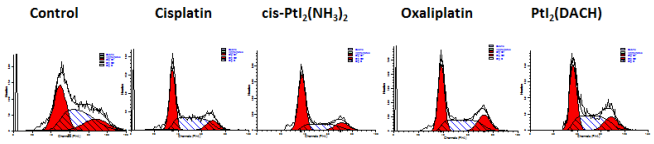

Fig. 4 Representative histograms of data reported in Table 4

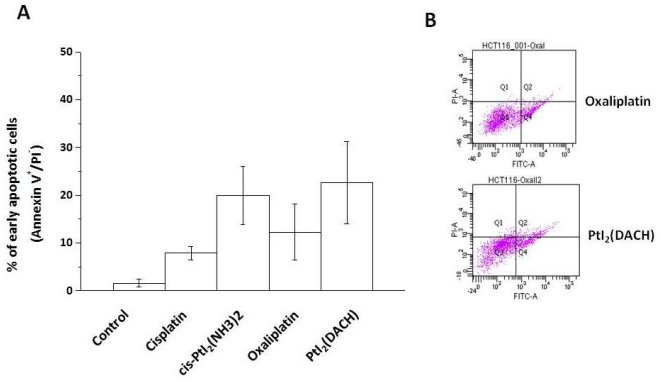

Fig. 5 Percentage of early apoptotic HCT 116 cells (determined as Annexin V+/PI-cells) after 24 hours treatment with the four different compounds at the respective IC_{50} values. Histograms (panel A) are means±sem of three independent experiments. B: representative dot plots of the Annexin V/PI assay relative to treatments with oxaliplatin and PtI2(DACH).

Pt uptake studies

Afterward, to confirm whether the enhanced lipophilic character of PtI_2(DACH) was related to an increase in drug internalisation, comparative uptake experiments were carried out on the HCT-116 cell line. Results reveal that upon replacement of oxalate with iodide, a dramatic enhancement of cellular uptake (~7 fold increase) takes place (Table 6) most likely in relation to the strong increment in the lipophilic character.

Drug	Platinum level (µg)
oxaliplatin	1.96×10^{-8}
PtI_2(DACH)	1.44×10^{-7}

Table 6. Platinum level (per cell) measured after exposure (24 hours) of cell lines to 25 µM of oxaliplatin and cisPtI_2(DACH).

Conclusions

Despite the success of novel targeted anticancer therapies, cytotoxic platinum drugs still remain a cornerstone in the treatment of CRC being largely used in chemotherapeutic regimens combined with other cytotoxic molecules. Yet, clinically established Pt drugs, *e.g.* cisplatin and oxaliplatin, manifest important drawbacks often leading to treatment failure. Accordingly, new Pt based drugs are still urgently needed. These arguments led us to design and investigate some structural analogues of established anticancer Pt drugs that have been surprisingly overlooked; specifically, we have prepared a few analogues of established Pt drugs through incorporation of non-conventional halido ligands such as iodide and bromide.

We report here on PtI_2(DACH), an analogue of oxaliplatin that is obtained through replacement of the oxalate ligand with two iodide ligands. The kinetics of activation is significantly influenced by oxalate replacement; the biomolecular reactivity is also influenced. At variance with oxaliplatin, PtI_2(DACH) is poorly reactive toward model proteins while retaining a significant affinity for a representative DNA molecule.

The effects of PtI_2(DACH) on a small panel of CRC cells have been explored as well; PtI_2(DACH) similarly to *cis*-$PtI_2(NH_3)_2$ demonstrated an increased capability of inducing apoptosis in the HCT-116 cell line, being this cell line the lest sensitive to cisplatin.

On the other hand, the cytotoxic properties of PtI_2(DACH) versus CRC cell lines were roughly comparable to those of oxaliplatin. This implies that the presence of the oxalate ligand is not crucial for the anticancer activity of oxaliplatin and that the latter may be replaced by other ligands such as iodide with nearly full retention of the cytotoxic properties. Furthermore, analysis of proliferation (up to 72 h) of HCT116 cell line treated with oxaliplatin and PtI_2(DACH) reveal as, even for long incubation time of incubation, differences in the cytotoxicity of these two drugs are comparable. This implies that there isn't any "kinetic" of the cytotoxic activity.

Remarkably, PtI_2(DACH) manifests a larger lipophilicity than oxaliplatin and cisplatin. Increased lipophilicity leads to a strong enhancement of cellular uptake of this drug in HCT-116 cells; however, this enhanced uptake does not result in a net enhancement of cytotoxicity possibly in relation to lower reactivity. The lower reactivity towards portiens might be associated to a better toxicological profile.

Overall, we believe that this novel analogue of oxaliplatin manifests a number of peculiar chemical and biological features that render it particularly attractive and worthy of further preclinical testing in appropriate in vivo models of CRC.

Notes and references

‡ Abbreviations:

TNM Staging: widely used cancer staging system. **T** refers to the size and extent of the main tumor (primary tumor); **N** refers to the number of nearby lymph nodes that have cancer (regional lymph nodes); **M** refers to whether the cancer has metastasized (distant metastasis). Numbers after each letter are used to give more details on the stage of a tumor.

ADME profile: Absorption, Distribution, Metabolism, and Excretion.

# These two authors contributed equally

Acknowledgements

We gratefully acknowledge Beneficentia Stiftung, Ente Cassa Risparmio Firenze (ECR), COST Action CM1105 for financial support, CISM (University of Florence) for recording ESI-MS spectra. T.M. thanks AIRC-FIRC (Fondazione Italiana per la Ricerca sul Cancro, 3-years Fellowship for Italy - Project Code: 18044). CIRCMSB is also acknowledged.
We thank Dr. M. Severi and Prof. R. Udisti for ICP-AES measurements.

Notes and references

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