Neutral and ionic platinum compounds containing a cyclometallated chiral primary amine: synthesis, antitumor activity, DNA interaction and topoisomerase I–cathepsin B inhibition†

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ABSTRACT:

The synthesis and preliminary biological evaluation of neutral and cationic platinum derivatives of chiral 1-(1-naphthyl)ethylamine are reported, namely cycloplatinated neutral complexes [PtCl{(R or S)-NH2CH(CH3)C10H6}-(L)] [L = SOMe2 (1-R or 1-S), L = PPh3 (2-R or 2-S), L = P(4-FC6H4)3 (3-R), L = P(CH2)3N3(CH2)3 (4-R)], cycloplatinated cationic complexes [Pt{(R)-H2CH(CH3)C10H6}{L}]Cl [L = Ph2PCH2CH2PPh2 (5-R), L = (C6F5)2PCH2CH2P-(C6F5)2 (6-R)] and the Pt(II) coordination compound trans-[PtCl2{(R)-NH2CH(CH3)C10H6}2] (7-R). The X-ray molecular structure of 7-R is reported. The cytotoxic activity against a panel of human adenocarcinoma cell lines (A-549 lung, MDA-MB-231 and MCF-7 breast, and HCT-116 colon), cell cycle arrest and apoptosis, DNA interaction, topoisomerase I and cathepsin B inhibition, and Pt cell uptake of the studied compounds are presented. Remarkable cytotoxicity was observed for most of the synthesized Pt(II) compounds regardless of (i) the absolute configuration R or S, and (ii) the coordinated/cyclometallated (neutral or cationic) nature of the complexes. The most potent compound 2-R (IC50 = 270 nM) showed a 148-fold increase in potency with regard to cisplatin in HCT-116 colon cancer cells. Preliminary biological results point out to different biomolecular targets for the investigated compounds. Neutral cyclometallated complexes 1-R and 2-R, modify the DNA migration as cisplatin, cationic platinacycle 5-R was able to inhibit topoisomerase I-promoted DNA supercoiling, and Pt(II) coordination compound 7-R turned out to be the most potent inhibitor of cathepsin B. Induction of G-1 phase (2-R and 5-R), and S and G-2 phases (6-R) arrests are related to the antiproliferative activity of some representative compounds upon A-549 cells. Induction of apoptosis is also observed for 2-R and 6-R.

INTRODUCTION

It is well known that cisplatin is an efficient anticancer drug through formation of platinum–DNA adducts that are capable of inducing programmed cell death.1 Since double-helical DNA has a chiral structure, the stereochemistry of the adducts formed with complexes containing enantiomeric ligands can influence the antitumor activity.2

Early studies carried out for compounds cis-[PtX2A2] (A = amine ligand and X = anionic ligand), indicated that the activity decreases in the order A = NH3 > RNH2 > R2NH, and, therefore, most investigations concerning chiral monodentate ligands were restricted to platinum complexes with primary amines. The negligible difference generally observed for the biological activities of the enantiomers was related to the free rotation that offset any stereospecificity in the interactions with biological substrates. The degree of rotational freedom can be reduced using chelate ligands, an outstanding example being oxaliplatin, a third generation anti-cancer drug containing trans-1R,2R-diaminocyclohexane. The lower activity of the trans-1S,2S and cis-1R,2S isomers are attributed to the distinct stereochemical structures of the complexes.3–5 Moreover, it is interesting to point out that three out of the six platinum compounds (namely oxaliplatin, lobaplatin and heptaplatin) approved for clinical use have a chiral structure (see Chart 1).1,5

On the other hand, cycloplatinated complexes have raised great interest as anticancer agents,6,7 and compounds containing either bidentate [C,N]8–21 or terdentate [C,N,N′]22–27 ligands have been tested against tumor cells with very promising results. The high stability of these compounds allows them to reach the cell unaltered. Furthermore, the labile ligands favor covalent coordination to DNA as for cisplatin while the aromatic groups in the cyclometallated ligand might favour intercalative binding to DNA through π–π stacking.

In spite of the continuous progress in the field of cyclometallation, the cycloplatination of primary amines remains relatively unexplored.28–31 Since it has been claimed that cycloplatinated amines represent a suitable organometallic scaffold for cytostatic drugs,31 and in view of the small number of cyclometallated platinum compounds containing a primary amine described so far, we decided to prepare new neutral and cationic platinum derivatives of chiral 1-(1-naphthyl)ethylamine and to study their biological activities. These compounds combine several features that make them very suitable candidates for biological studies: a platinum center, a cyclometallated chiral primary amine, the possibility of being involved in hydrogen bonds through the NH2 group, ancillary ligands with different stereo-electronic properties and, for the neutral compounds, a labile chlorido ligand.

RESULTS AND DISCUSSION

Synthesis of the cycloplatinated compounds

The preparation of parent compound [PtCl{(R)-NH2CH(CH3)-C10H6}(SOMe2)] (1-R) from the platinum(II) precursor cis-[PtCl2(SOMe2)2] has been previously reported.30 The success of the synthesis under mild conditions has been related to the presence of both a naphthyl substituent and a methyl group at the α-position of the amine in agreement with the fact that the cyclometallation of amines is promoted by steric encumbrance, as established for palladium analogues.32 In particular, substitution of the carbon at the α position to the amino group plays a pivotal role related to a noticeable decrease in entropic requirements.33 The lability of the dimethylsulfoxide ligands allows for the synthesis of neutral compound [PtCl{(R)-NH2CH(CH3)C10H6}(PPh3)] (2-R) upon reaction with PPh3, while ionic compound [Pt{(R)-NH2CH(CH3)C10H6}-{Ph2PCH2CH2PPh2}]Cl (5-R) containing a chelating diphosphine is obtained upon reaction with 1,2-bis(diphenylphosphine) ethane.30

As shown in Scheme 1, the same synthetic strategies were used in this work to expand the family of 1-(1-naphthyl)ethylamine derivatives including neutral complexes [PtCl{(R)-NH2CH(CH3)C10H6}{P(4-FC6H4)3}] (3-R) and [PtCl{(R)-NH2CH-(CH3)C10H6}{P(CH2)3N3(CH2)3)}] (4-R), as well as the ionic compound [Pt{(R)-NH2CH(CH3)C10H6}{(C6F5)2PCH2CH2P(C6F5)2}]Cl(6-R). Interest in fluorinated derivatives 3-R and 6-R is related to the fact that fluoro substituents have been proposed as an excellent choice to modify the electronic properties and hydrophobicity of related cycloplatinated complexes34 while keeping stereochemical changes to a minimum. In addition, introduction of fluorinated groups would permit the use of 19F NMR spectroscopy in the characterization of the compounds.35 On the other hand, the water-soluble monodentate phosphine 1,3,5-triaza-7-phosphaadamantane (PTA) in compound 4-R is a useful hydrophilic co-ligand, which may impart a pH-dependent behavior in biologically active transition metal compounds.36–40 Compounds 3-R, 4-R and 6-R were prepared from compound [PtCl{(R)-NH2CH(CH3)C10H6}(SOMe2)] (1-R) after stirring an equimolar mixture of this compound and the corresponding mono or diphosphine in acetone at room temperature. Under the reaction conditions, 4-R precipitates as a white solid, very slightly soluble in most common solvents including water, in spite of the ability of PTA to solubilize transition metal complexes in the aqueous phase.37,38 In contrast, precipitation of 3-R and 6-R from the reaction mixture requires the addition of hexane.

The new compounds 3-R, 4-R and 6-R were characterised by elemental analyses and 1H, 19F (3-R and 6-R), 31P and 195Pt NMR spectra. NMR data were collected in CDCl3 except for 4-R that could only be dissolved in d6-dmso. 1H-NMR data are in agreement with the proposed structures; in particular, coupling of the amino protons to platinum was only observed for compound 6-R (2JH–Pt = 75.2 Hz) while for compound 3-R the aromatic proton at the ortho position was coupled to platinum (3JH–Pt = 52.0 Hz). For compounds 3-R and 4-R the JP–Pt values obtained from both 31P and 195Pt NMR spectra indicate that the phosphine ligand is trans to the amino group. For compound 6-R, two resonances are observed in the 31P NMR spectrum with 1JP–Pt values of 1594.0 and 3878.8 Hz. The higher J value is assigned to the phosphorus atom trans to the amino and the lower one to that trans to the metallated carbon in agreement with the expected values.30,41 The 19F NMR spectrum of compound 3-R displays one multiplet corresponding to the three equivalent para-fluoro substituents of the phosphine; in contrast, a much higher complexity is obtained for the spectrum of compound 6-R in which three sets of four signals in the regions −124 to −129 ppm assigned to 8 Fortho, −140 to −143 ppm assigned to 4 Fpara, and −155 to −157 ppm assigned to 8 Fmeta are observed. These values are in good agreement with those previously reported for complexes containing diphosphines bearing pentafluorophenyl groups and suggest the non-equivalence of the four pentafluorophenyl groups.35,42 All 195Pt chemical shift values, collected in Table 1 along with those for previously reported compounds 1-R, 2-R and 5-R, are

in the expected range for square-planar platinum(II) compounds with the corresponding donor atom set [C, N, Cl, P] for compounds 3-R and 4-R and [C, N, P, P] for compound 6-R.43

In order to complete this study, the coordination compound [PtCl2{(R)-NH2CH(CH3)C10H6}2] (7-R), containing two non-cyclometallated amine ligands, was also prepared upon reaction of two equivalents of the amine with K2PtCl4 in water–methanol mixtures at room temperature. Both 1H and 195Pt NMR spectra of the latter compound indicate the presence of a single isomer, and the δ(195Pt) value is in the range expected for a platinum(II) coordinated to two Cl and two N donor atoms. Based on previous results for similar compounds, 29,44 and taking into account the steric bulk of the amine ligands, it is expected that the amine ligands are mutually trans. Suitable crystals of 7-R were grown from dichloromethane–methanol at room temperature and they were analyzed by X-ray diffraction. The obtained molecular structure confirms both the mutual trans arrangement of the amine ligands and the R configuration of both chiral carbons (see Fig. 1). As shown in Fig. S1–S3,† the molecules are associated in dimers, with a Pt⋯Pt short contact of 3.222 Å, and the dimers are arranged in columnar stacks. It is interesting to point out that, although previous structure–activity relationship studies suggest a higher activity for cis-configured compounds, in the last few decades many trans platinum compounds have been shown to be cytotoxic.45 Finally, in order to address the influence of the chirality upon the biological properties of the compounds, the corresponding enantiomers 1-S and 2-S derived from (S)-NH2CH-(CH3)C10H6 were also prepared using identical procedures as those described for 1-R and 2-R 30 and the compounds were characterized by NMR spectra.

Biological studies. Antiproliferative assay

Human lung, breast and colon cancer cell lines (A-549, MDA-MB-231 and MCF-7 and HCT-116, respectively) were used to test the cytotoxic activity of the platinum(II) complexes 1-R–7-R and 1-S–2-S. Cisplatin, as a positive control, was evaluated under identical experimental conditions. The effects of the assayed metallacycles on the growth of the selected cell lines were evaluated after 72 h and the obtained IC50 values are listed in Table 2. For comparison purposes, these results are represented as a bar graph in Fig. 2.

Data presented in Table 2 and Fig. 2 show that the investigated compounds exhibit variable selectivity against the adenocarcinoma cell lines tested. Several complexes showed greater cytotoxicity effectiveness than cisplatin. Taking into account the cisplatin resistant HCT-116 colon cancer cell line, the following trend can be derived for complexes 1-R–7-R, 1-S–2-S and cisplatin:

2-R > 6-R > 5-R > 7-R > 2-S > 3-R > 1-R > 1-S > cisplatin ≫ 4-R

Considering the R configured neutral platinacycles 1-R–4-R, it is noteworthy that complex 2-R with a Pt–P bond (L = PPh3) exhibits lower IC50 values than compound 1-R with a Pt–S bond (L = SOMe2). Actually compound 2-R is 4.5–52 times, depending on the cell line selected, more potent than complex 1-R. Furthermore compound 2-R also showed a lower IC50 value than compounds 3-R [L = P(4-FC6H4)3] and 4-R [L = P(CH2)3N3(CH2)3] both with a Pt–P bond. Interestingly, platinacycle 2-R (IC50 = 270 nM) showed a 148-fold increase in potency as cytotoxic agent with regard to cisplatin in HCT-116 (cisplatin resistant) colon adenocarcinoma cells (Table 2). The platinacycle 2-R was up to 21-fold more potent as a cytotoxic agent than the corresponding cyclometallated palladium(II) complex, previously described in our research group, in A-549 lung human cancer cells.46 This may be due to the greater lability and faster hydrolysis rate of palladium complexes compared to their platinum analogues.47 Surprisingly, no cytotoxic activity was observed for the PTA derivative 4-R at 100 μM concentration, a fact that can be related to the low solubility of this compound, or to the steric

encumbrance of the PTA ligand. The absolute configuration (R/S) of the cyclometallated ligand [1-(1-naphthyl)ethylamine] does not play a role in the target molecular discrimination between the two pairs of enantiomers 1-R vs. 1-S and 2-R vs. 2-S.2 Platinacycles 1-R and 1-S, and 2-R and 2-S, in pairs exhibited comparable IC50 values in the four adenocarcinoma cell lines assayed.

The ionic complexes with a chelating diphosphine 5-R and 6-R showed remarkable potency against the four adenocarcinoma cell lines tested and were up to 22- and 25-fold more potent as cytotoxic agents than cisplatin. Nevertheless, we did not find the expected increase in potency34 in the complex exhibiting the fluorinated chelating diphosphine (6-R) with regard to the complex containing the non-fluorinated one (5-R). Finally, a notable potency was detected for the coordination compound 7-R containing two non-cyclometallated amine ligands. Compound 7-R (IC50 = 4.5 μM) showed approximately a 9-fold increase in potency as a cytotoxic agent with regard to cisplatin in HCT-116 (cisplatin resistant) colon cancer cell lines (Table 2).

Effect of compounds 2-R, 5-R and 6-R on cell cycle distribution

Cell cycle dysregulation is considered to be one of the main hallmarks of cancer cells and those proteins that control the critical events of cell cycle have been proposed as useful antitumor targets.48–50 There are three main phases in the cell cycle distribution: quiescent and gap1 (G0 and G1), synthesis (S), gap2 and mitosis (G2 and M) phases. The effect of the compounds 2-R, 5-R and 6-R were studied over A-549 cells, by incubating them with these compounds at a concentration equal to their IC50 values, for 72 hours and the results were analyzed by FACS (Fluorescence Activated Cell Sorting) using propidium iodide (PI) staining to quantify their DNA content. The results of the analysis (Fig. 3) show that all the three compounds affect the cell cycle distribution at different levels. Compounds 2-R and 5-R show an increase of about 29% and 21%, respectively, of the cell population at the G1 checkpoint with a concomitant decrease at the S and G2 phases. In contrast, compound 6-R shows an increase of the cell populations of about 17% and 33%, respectively at the S and G2 phases.

Effect of compounds 2-R, 5-R and 6-R on apoptosis induction

The antiproliferative effects of these compounds were further tested for their effects in apoptosis induction in A-549 cells. Because cancer is characterized by uncontrolled cellular proliferation, there is a considerable interest in chemotherapeutic-induced apoptosis. By using fluorescein-labeled annexin V (AV–FITC, annexin V-fluorescein isothiocyanate) and PI (propidium iodide), the apoptosis-inducing properties of 2-R, 5-R and 6-R in A-549 cells were examined by performing flow cytometry. In the earlier events of the apoptotic process, plasma membrane asymmetry is lost, accompanied by phosphatidylserine (PS) translocation from the inner to the outer membrane. 51 Thus PS is exposed to the external environment of the cell and can bind to the annexin V-FITC conjugate with a high affinity.51,52 During the late apoptotic and necrotic stages, the cell membranes lose their integrity, allowing PI to access the nucleus and intercalate between DNA bases. The differential distribution between non-apoptotic cells (annexin V− and PI−), early apoptotic (annexin V+ and PI−) and necrotic or later apoptotic (PI+) cells were achieved by FACS analysis using annexin V-FITC staining and PI accumulation.

The results (Fig. 4) from the apoptosis assay do not show a drastic effect of the compounds 2-R, 5-R and 6-R at their IC50 concentrations (9.3, 1.2 and 4.6 μM) for 72 h upon the A-549 cells. However there are some notable effects, such as an increase in early apoptosis induction by all the three compounds, especially 5 and 7 times increase by 2-R and 6-R, respectively, and more than 3 times increase in necrosis induction by 2-R, with respect to the untreated control cells. Therefore compounds 2-R and 6-R at their IC50 concentrations (9.3 and 4.6 μM), generated early apoptosis in 6.9 and 9.0% respectively of

the total cells population, as compared to the 1.3% of the early apoptotic cells in the untreated controls, and 2-R generated late apoptosis/necrosis in 8.9% of the total cell population, as compared to the 2.6% of the late apoptotic/ necrotic cells in the untreated controls.

All these results strongly suggest that the main antiproliferative activities shown by the compounds are by cell cycle arrest, in G1 phase for 2-R and 5-R, and in S and G2 phases for 6-R.

DNA interaction

The interaction of cyclometallated complexes 1-R–6-R and 1-S–2-S and coordination compound 7-R with DNA was studied by their ability to modify the electrophoretic mobility of the supercoiled closed circular (sc) and the open circular (oc) forms of pBluescript SK+ plasmid DNA. The sc form usually moves faster due to its compact structure. To provide a basis for comparison, incubation of DNA with cisplatin and ethidium bromide (EB) was also performed using the same concentrations and conditions. Platinacycles 1-R and 2-R induce significant changes in the mobility of plasmid DNA (Fig. 5). Complex 1-R shows a coalescence point at 100 μM and positive supercoiling at higher concentrations. Platinacycle 2-R greatly alter the mobility of plasmid DNA; at 50 μM concentration the rate of migration of the supercoiled band (sc) decreased to that of the nicked relaxed band (oc) and above this concentration the DNA is no longer visible. Platinacycles 1-S and 2-S were much less efficient in removing the supercoils from DNA than the corresponding enantiomers R (Fig. 5).

It is noteworthy that the complex 2-R with one of the lowest IC50 value of the investigated compounds showed the greatest alteration on plasmid DNA mobility. On the basis of the gel mobility shift assay, it is hypothesized that platinacycles 1-R and 2-R alter the DNA tertiary structure by the same mechanism as the standard reference, cisplatin. In contrast with these findings, compounds 3-R–7-R (Fig. 5) were not effective at all for removing the supercoils of plasmid DNA, pointing to a different mechanism of action or an alternative biomolecular target.

To evaluate the ability of the investigated platinum(II) complexes to intercalate into DNA, a topoisomerase-based gel assay was performed with complexes 3-R–7-R. Supercoiled pBluescript plasmid DNA was incubated in the presence of topoisomerase I at increasing concentrations of compounds 3-R–7-R. The results are given in Fig. 6 and they show that 5-R prevent unwinding of DNA by the action of topoisomerase I, indicating that this compound is either intercalator or topoisomerase I inhibitor. Ethidium bromide (EB), used as an intercalator control, prevents the shift of supercoiled DNA into a relaxed state. Complexes 3-R, 4-R, 6-R, and 7-R do not prevent unwinding of DNA by the action of topoisomerase I, indicating that these compounds are neither intercalators nor topoisomerase I inhibitors.53

To dilucidate whether platinacycle 5-R is a DNA-intercalator or a topoisomerase I inhibitor, relaxed pBluescript plasmid DNA was incubated in the presence of topoisomerase I at increasing concentrations of compound 5-R. It is well known that compounds that are topoisomerase I inhibitors will prevent topoisomerase I from changing the state of the relaxed DNA into a supercoiled state, whereas in the presence of an intercalator such as ethidium bromide, topoisomerase I will convert the relaxed DNA into a supercoiled state.54 The results are given in Fig. 7 and show that 5-R prevents unwinding of DNA by the action of topoisomerase I, indicating that this compound is the inhibitor of topoisomerase I. In higher eukaryotes, DNA topoisomerases I are essential enzymes whose main role is to relieve DNA supercoiling (torsional tension) ahead of replication and transcription complexes.55,56 Nowadays topoisomerase I is considered an important molecular target for anticancer drug development. The anticancer activity of trinuclear (TriplatinNC, TriplatinNC-A)57 and naphthoquinone58 Pt(II) complexes, and luminescent cyclometallated Pt(II)59 compounds have being associated with their ability to inhibit topoisomerase I. Interestingly, in topoisomerase-based gel assay, complex 5-R acts as topoisomerase I inhibitor like camptothecin, a well-known topoisomerase I inhibitor.58

Cathepsin B inhibition

Cathepsin B is a cysteine metalloprotease highly upregulated in a wide variety of cancers by mechanisms ranging from gene amplification to post-transcriptional modification. The exact role of cathepsin B in solid tumors has yet to be defined, but it has been proposed to participate in metastasis, angiogenesis, and tumor progression. Recently, compounds based on palladium, platinum, ruthenium, rhenium, gold and tellurium were shown to be effective inhibitors of cathepsin B.60 In addition, an excellent correlation between cathepsin B inhibition and cytotoxicity for some dinuclear biphosphine palladacycles61 and mononuclear platinacycles containing a fluorinated phosphine34 has been reported. In spite of these later results, we have recently reported53,62 that a series of cyclopalladated and cycloplatinated benzophenone imines were not efficient inhibitors of cathepsin B, although showing in vitro high cytotoxicity.

We have determined the cathepsin B inhibition activity for compounds 1-R–7-R and 1-S–2-S and the results are given in Table 3. All the compounds with the exception of compound 4-R, which was not cytotoxic in the antiproliferative assay, inhibited cathepsin B in a dose dependent manner. Inhibition of cathepsin B with IC50 values below 50 μM was achieved for compounds 1-R, 1-S, 6-R and 7-R. The non-cyclometallated trans-Pt(II) compound 7-R turned out to be the most potent (IC50 = 17 μM) of the studied compounds inhibiting cathepsin B in vitro and showed also a quite high cytotoxic activity. For instance, in MDA-MB-231 breast adenocarcinoma cell line complex 7-R showed an IC50 of 1.9 μM in the viability assay. This is noteworthy taking into account that not always the cathepsin B inhibition properties of metallodrugs correlate with their antiproliferative activity.63 On the other hand, the trans-platinum(II) compound 7-R did not modify the DNA tertiary structure and therefore may act on alternative biological targets in addition to cathepsin B.64 It have been reported that trans-platinum compounds show a biological behavior rather different from that of cisplatin (ability to form structurally different DNA adducts, different reactivity in front of cellular components such as glutathione, HMG proteins, DNA repair proteins, etc.) and hence these compounds may be the key to overcoming intrinsic or acquired resistance to cisplatin.64,65

Cell accumulation

Cellular uptake is considered an important issue concerning the cytotoxicity of platinum compounds. It is believed to be dependent of both passive diffusion and active transport.66 The inhibition of drug accumulation and increase of efflux have been directly related to mechanisms of acquired resistance to cisplatin.67 A high cellular uptake of structurally different palladium68,69 and platinum58,70,71 compounds by several human cancer cell lines has been reported. Furthermore, within a series of complexes the highest cellular accumulation is in line with the highest cytotoxic activity.58,69–71

The cellular accumulation of platinum was used as a measure of the cellular accumulation of compounds 1-R–7-R and 1-S–2-S (mole of Pt per cell × 10−16) and cisplatin was used as the reference compound. Table 4 illustrates cellular accumulation of the nine tested compounds and cisplatin over 4 h, as applied to the breast cancer cell line MDA-MB-231.

Accumulation of the studied platinum complexes in MDA-MB-231 breast cancer cells was considerably greater than that of cisplatin for all the compounds tested and follows the order:

7-R > 1-R > 6-R > 5-R > 2-S > 3-R > 4-R > 1-S > 2-R> cisplatin

The coordination compound 7-R was associated with the highest cellular accumulation and this data was in line with the notable potency assessed for this compound (IC50 = 1.9 μM). The cationic cyclometallated compounds 5-R and 6-R (IC50 = 1.59 and 0.82 μM, respectively) exhibiting a high cytotoxic activity displayed a remarkable amount of platinum accumulated per cell. These results are in agreement with the elevated platinum cellular uptake described for cationic mononuclear58 and polynuclear71 platinum complexes with regard to cisplatin. The accumulation of complexes 5-R, 6-R and 7-R was about 10, 15 and 28 times higher than that of cisplatin, respectively.

Concerning the neutral cycloplatinated complexes 1-R–4-R and 1-S–2-S, no correlation was observed between the antiproliferative efficacy in MDA-MB-231 breast cancer cells and the Pt cellular uptake. These data are in agreement with previous studies reporting that cell uptake per se, may not necessarily give an indication of the level of the antitumor activity.20,72 The relative contribution of cellular accumulation to overall cytotoxicity merits further biological studies which are in progress.

CONCLUSIONS

Eight cyclometallated platinum(II) compounds (1-R–6-R and 1-S–2-S) containing a chiral primary amine ligand [(R or S)-1- (1-naphthyl)ethylamine] and the coordination compound 7-R, containing two non-cyclometallated chiral (R) amines, were tested as new antitumor drugs. Complexes 3-R, 4-R, 6-R and 7-R were synthesized in this work and characterized by spectral and elemental analysis. The molecular structure of compound 7-R was solved by X-ray analysis. Cytotoxicity studies revealed the high effectiveness of either neutral cycloplatinated compounds (2-R and 2-S), cationic cycloplatinated compounds (5-R–6-R) or coordination compound (7-R) against human lung, breast and colon adenocarcinoma cell lines (A-549, MDA-MB-231, MCF-7 and HCT-116 respectively). In the cisplatin resistant HCT-116 colon cancer cells, neutral platinacycle 2-R (IC50 = 270 nM), and the ionic compounds containing the chelating diphosphine 5-R (IC50 = 1.8 μM), and 6-R (IC50 = 1.6 μM) were up to 148-, 22- and 25-fold more potent than when cisplatin is used as a positive control. Based on IC50 data, the absolute configuration (R or S) of the cyclometallated ligand [1-(1-naphthyl)ethylamine] does not produce relevant differences in the biological properties of the two pairs of enantiomers 1-R vs. 1-S and 2-R vs. 2-S.

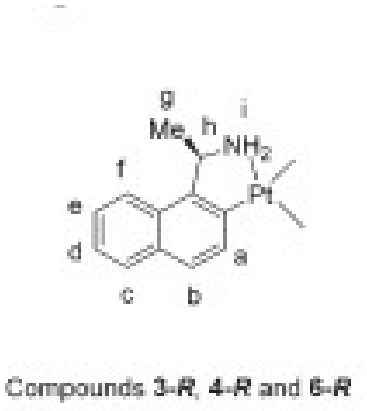
Considering plausible target biomolecules for the studied compounds, the electrophoretic mobility of complexes of 2-R and 2-S indicates alteration of the DNA tertiary structure in a similar way as cisplatin, although at higher concentrations. Compounds 3-R–7-R were not efficient at all for removing the plasmid DNA supercoils. Among them, cationic platinacycle 5-R turned out to be a topoisomerase I inhibitor in a topoisomerase- based gel assay. All the compounds with the exception of neutral platinacycle 4-R, which was not cytotoxic in the antiproliferative assay, inhibited cathepsin B in a dose dependent manner. The coordination Pt(II) complex 7-R turned out to be the most potent (IC50 = 17 μM) of the studied compounds inhibiting cathepsin B. As representative complexes of the series, platinacycles 2-R and 5-R (G-1 phase) and 6-R (S and G-2) were found to suppress A-549 lung cancer cell growth mainly through cell cycle arrest. Induction of apoptosis is also observed for compounds 2-R and 6-R. Further studies are in progress upon the studied complexes for both understanding structure–activity relationship and gaining further insight into their biological behavior.

EXPERIMENTAL

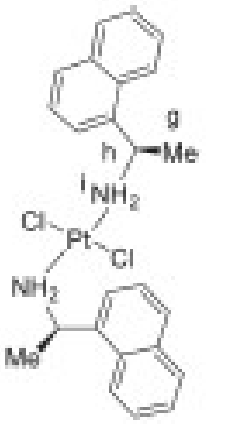
General

Elemental analyses of C, H and N were performed with an Eager 1108 microanalyzer. NMR spectra were recorded in $CDCl_3$ at 298 K with Mercury 400 (1H, 19F) and Bruker 400 Avance III HD (31P, 195Pt) spectrometers. Chemical shifts are given in δ values (ppm) relative to $SiMe_4$ (1H), $CFCl_3$ (19F), 85% H_3PO_4 ($^{31}P\{^1H\}$) and H_2PtCl_6 in D_2O (195Pt), and coupling constants are given in Hz. Multiplicity is expressed as: s (singlet), d (doublet), t (triplet), and q (quadruplet). Compounds 1-R, 2-R and 5-R have been previously reported.30

NMR labelling



Compounds 3-*R*, 4-*R* and 6-*R*



Compound 7-*R*

Preparation of the compounds

Compound [PtCl{(R)-NH2CH(CH3)C10H6}{P(4-FC6H4)3}] (3-R) was obtained after stirring at room temperature for 2 hours a solution containing 50 mg (0.104 mmol) of compound 1-R and 33 mg (0.104 mmol) of P(4-FC6H4)3 in 20 mL of acetone. The solvent was evaporated and the residue was treated with hexane. The white solid was filtered and dried under vacuum. Yield: 40 mg (53%). 1H NMR (400 MHz, $CDCl_3$), δ = 7.72–7.67 (m, 7H, Ho,PR3 + Hf or Hc), 7.61 (d, 3JH–H = 8.0, 1H, Hf or Hc), 7.40 (t, 3JH–H = 8.0, 1H, Hd or He), 7.33 (t, 3JH–H = 8.0, 1H, Hd or He), 7.07–7.02 (m, 6H, Hm,PR3), 6.94 (d, 3JH–H = 8.0, 1H, Hb), 6.62 (dd, 3JH–H = 8.0, 4JH–P = 2.8, 3JH–Pt = 52.0, 1H, Ha), 5.47 (q, 3JH–H = 6.0, 3JH–Pt = 58.8, 1H, Hh), 4.62 (m, 1H, Hi), 4.15 (m, 1H, Hi), 1.91 (d, 3JH–H = 6.0, 3H, Hg).19F NMR (376.45 MHz, $CDCl_3$), δ = −108.2 (m). 31P NMR (161.98 MHz, $CDCl_3$), δ = 20.99 (s, 1JP–Pt = 4344.3). 195Pt NMR (85.68 MHz, $CDCl_3$), δ = −3973.8 (d, 1JP–Pt = 4352.5). EA (calc. for $C_{30}H_{24}ClF_3NPPt \cdot H_2O$): C: 48.79% (49.02%); H: 3.73% (3.57%); N: 1.74% (1.91%).

Compound [PtCl{(R)-NH2CH(CH3)C10H6}{PTA}] (4-R) was obtained after stirring at room temperature for 2 hours a solution containing 63 mg (0.131 mmol) of compound 1-R and 21 mg (0.134 mmol) of P(CH2)3N3(CH2)3 (PTA) in 20 mL of acetone. Precipitation of a white solid was observed; the solid was filtered, washed with ether and dried under vacuum. Yield: 63 mg (86%). 1H NMR (400 MHz, d6-dmso), δ = 7.73 (d, 3JH–H = 8.0, 1H), 7.69 (d, 3JH–H = 8.0, 1H), 7.49 (dd, 3JH–H = 8.0, 4JH–H = 2.8, 1H, Hc or Hf), 7.42 (d, 3JH–H = 8.0, 1H), 7.35 (t, 3JH–H = 7.2, 1H, Hd or He), 7.35 (t,

3JH–H = 7.2, 1H, Hd or He), 6.00 (m, 1H, Hh), 5.09 (m, 1H, Hi), 4.97 (dd, 2JH–H = 10.8, 3JH–H = 2.8, 1H, Hi), {4.52 (d, 2JH–H = 12.8, 3H), 4.41 (d, 2JH–H = 12.8, 3H), AB system, PCH2N}, {4.34 (d, 2JH–H = 15.0, 3H), 4.27 (d, 2JH–H = 15.0, 3H), AB system, NCH2N}, 1.55 (d, 3JH–H = 6.0, 3H, Hg). 31P NMR (161.98 MHz, d6-dmso), δ = −67.03 (s, 1JP–Pt = 3699.6). 195Pt NMR (85.68 MHz, d6-dmso), δ = −3911.6 (d, 1JP–Pt = 3721.9). EA (calc. for C18H24ClN4PPt·H2O): C: 37.70% (37.54%); H: 4.72% (4.55%); N: 9.36% (9.73%).

Compound [Pt{(R)-NH2CH(CH3)C10H6}{(C6F5)2PCH2CH2P- (C6F5)2}]Cl (6-R) was obtained after stirring at room temperature for 2 hours a solution containing 55 mg (0.115 mmol) of compound 1-R and 87 mg (0.115 mmol) of (C6F5)2PCH2CH2P- (C6F5)2 in 20 mL of acetone. The solvent was evaporated and the residue was treated with hexane. The white solid was filtered and dried under vacuum. Yield: 104 mg (78%). 1H NMR (400 MHz, CDCl3), δ = 7.72 (d, 3JH–H = 8.0, 1H, Hf or Hc), 7.67 (dd, 3JH–H = 7.6, 4JH–H = 1.6, 1H, Hf or Hc), 7.43 (td, 3JH–H = 6.8, 4JH–H = 1.6, 1H, Hd or He), 7.41 (m, 1H, Hb), 7.39 (td, 3JH–H = 6.8, 4JH–H = 1.0, 1H, Hd or He), 7.30 (m, 1H, Ha), 5.99 (m, br, 3JH–Pt = 56.0, 1H, Hh), 5.80 (m, br, 2JH–Pt = 75.2, 2H, Hi), 3.28–2.96 (m, 2H); 2.70–2.68 (m, 1H); 2.49–2.47 (m, br, 1H), 1.73 (d, 3JH–H = 6.4, 3H, Hg).19F NMR (376.45 MHz, CDCl3), δ = −124.20 (m, 2F, Fo), −126.70 (m, 2F, Fo), −122.73 (d, 2JF–F = 18.9, 2F, Fo), −129.17 (d, 2F, 2JF–F = 15.0, Fo), −140.04 (t, 2JF–F = 20.7, 1F, Fp), −142.06 (t, 2JF–F = 18.9, 1F, Fp), −142.23 (t, 2JF–F = 20.7, 1F, Fp), −142.72 (t, 2JF–F = 20.7, 1F, Fp), −155.73 (t, 2JF–F = 18.9, 2F, Fm), −156.31 (t, 2JF–F = 18.9, 2F, Fm), −156.35 (t, 2JF–F = 18.9, 2F, Fm), −156.62 (t, 2JF–F = 18.9, 2F, Fm). 31P NMR (161.98 MHz, CDCl3), δ = 13.01 (d, 1JP–Pt = 1594.0, 2JPA–PB = 17.0, PA), 5.49 (d, 1JP–Pt = 3878.8, 2JPA–PB = 17.0, PB). 195Pt NMR (85.68 MHz, CDCl3), δ = −4327.8 (dd, 1JPA–Pt = 3926.9, 1JPB–Pt = 1601.8). EA (calc. for C38H16ClF20NP2Pt·2H2O): C: 37.74% (38.19%); H: 1.82% (1.69%); N: 1.20% (1.17%).

Compound [PtCl2{(R)-NH2CH(CH3)C10H6}2] (7-R) was obtained after stirring at room temperature for 4 days a mixture of 200 mg (0.48 mmol) of K2PtCl4 in 10 mL of water and 165 mg of (R)-NH2CH(CH3)C10H6 (0.96 mmol) in 10 mL of methanol. The obtained solid was filtered, washed with water and extracted with dichloromethane (10 mL). The obtained solution was dried over MgSO4, and methanol was added. After cooling to 10 °C, a yellowish solid was obtained. Yield: 55 mg (46%). 1H NMR (400 MHz, CDCl3), δ = 8.34 (d, 3JH–H = 8.0, 1H), 7.86 (d, 3JH–H = 8.0, 1H), 7.80 (d, 3JH–H = 8.0, 1H), 7.61 (td, 3JH–H = 8.0, 4JH–H = 1.6, 1H), 7.51 (td, 3JH–H = 8.0, 4JH–H = 1.6, 1H), 7.48 (d, 3JH–H = 8.0, 1H), 7.46 (t, 3JH–H = 8.0, 1H), 5.23 (m, 1H, Hh), 3.95 (d, 2JH–H = 12.0, 1H, Hi), 3.68 (t, 2JH–H = 12.0, 1H, Hi), 2.00 (d, 3JH–H = 6.8, 3H, Hg). 195Pt NMR (85.68 MHz, CDCl3), δ = −2179.0 (s). EA (calc. for C24H26Cl2N2Pt·H2O): C: 45.67% (46.01%); H: 4.43% (4.50%); N: 4.37% (4.47%).

Crystal data and structure refinement for 7-R. A yellow prism-like specimen of 7-R, grown in dichloromethane-methanol at room temperature, was used for the X-ray crystallographic analysis. X-ray intensity data were collected on a D8 VENTURE system equipped with a multilayer monochromator and a Mo microfocus (λ = 0.71073 Å) at 100 K and the structure was solved and refined using the Bruker SHELXTL software package.73 Further information is given in Table 5.

Cell culture. Lung (A-549), colon (HCT-116) and breast (MCF-7 and MBA-MD-231) cancer cells were grown as a monolayer culture in minimum essential medium (DMEM with L-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum (FCS), 10 mM D-glucose and 0.1% streptomycin/penicillin, under standard culture conditions (humidified air with 5% CO2 at 37 °C).

Cell viability assay. For A-549 cell viability assays, compounds were suspended in high purity DMSO at 20 mM as stock solution. To obtain the final assay concentrations, they were diluted in DMEM (Dulbecco's Modified Eagle Medium) (final concentration of DMSO was the same for all conditions, and was always lower than 1%). The assay was performed by a variation of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay described by Mosmann et al.74 and Matito and coworkers75 which is based on the ability of live cells to cleave the tetrazolium ring of the

MTT thus producing formazan, which absorbs at 550 nm. In brief, 2.5 × 103 A-549 cells per well were cultured in 96 well plates for 24 hours prior to the addition of different compounds at different concentrations, in triplicate. After incubation for 72 h more, the medium was aspirated and 100 μL of filtered MTT (0.5 mg mL−1) were added to each well. Following 1 h of incubation with the MTT, the supernatant was removed and the precipitated formazan was dissolved in 100 μL DMSO. Relative cell viability, compared to the viability of untreated cells, was measured by absorbance at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Salzburg, Austria). Concentrations that inhibited cell growth by 50% (IC50) after 72 h of treatment were subsequently calculated.

For HCT-116, MDA-MB-231 and MCF-7 cell viability assays, a stock solution (50 mM) of each compound was prepared in high purity DMSO. Then, serial dilutions were made with DMSO/DMEM (1 : 1) and finally a 1 : 500 dilution on culture medium was prepared. The final assay concentration of DMSO was the same in all experiments and was 0.2%. The assay was performed as described by Givens et al.76 HCT-116, MDA-MB-231 and MCF-7 cells were plated at 5000 cells per well, respectively, in 100 μL media in tissue culture 96-well plates (Cultek). After 24 h, the media were replaced by a 100 μL per well of drug serial dilutions. Control wells did not contain the compounds under study. Each point concentration was run in triplicate. Reagent blanks, containing media and colorimetric reagent without cells were run on each plate. Blank values were subtracted from the test values and were routinely 5–10% of the control values. Plates were incubated 72 h. Hexosaminidase activity was measured according to the following protocol. The media were removed and cells were washed once with PBS (phosphate-buffered saline). 60 μL of substrate solution (p-nitrophenol-N-acetyl-β-D-glucosamide 7.5 mM, sodium citrate 0.1 M at pH 5.0, and 0.25% Triton X-100) was added to each well and incubated at 37 °C for 1–2 hours. After this incubation time, a bright yellow colour appeared. Then, the plates were developed by adding 90 μL of the developer solution (glycine 50 mM, pH 10.4; EDTA 5 mM) and the absorbance was recorded at 410 nm.

Cell cycle analysis. Cell cycle was assessed by flow cytometry using a fluorescence activated cell sorter (FACS). For this assay, 4 × 104 A-549 cells were seeded in 6 well plates with 2 mL of medium. After 24 h of incubation, 2-R, 5-R, or 6-R was added at their IC50 values (9.3, 1.2 and 4.6 μM, respectively). Following 72 h of incubation, cells were harvested by mild tripsinization, collected by centrifugation and resuspended in Tris buffered saline (TBS) containing 50 mg mL−1 PI, 10 mg mL−1 DNase-free RNase and 0.1% Igepal CA-630. The cell suspension was incubated for 1 h at room temperature to allow for the staining of the cells with the PI, and afterwards FACS analysis was carried out at 488 nm in an Epics XL flow cytometer (Coulter Corporation, Hialeah, FL). Data from 1 × 104 cells were collected and analyzed using the Multicycle program (Phoenix Flow Systems, San Diego, CA).

Apoptosis assay. Apoptosis was assessed evaluating the annexin-V binding to phosphatidylserine (PS), which is externalized early in the apoptotic process. 4 × 104 A-549 cells per well were seeded in 6 well plates with 2 mL of medium and treated as described above for the cell cycle analysis assay. After cell collection and centrifugation, cells were resuspended in 95 μL binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2). 3 μL of an Annexin-V FITC conjugate (1 mg mL−1) were then added and the suspension was incubated in the dark for 30 min, at room temperature. Just before FACS analysis, the cell suspension was added to a vial containing 500 μL of binding buffer, and then stained with 20 μL of 1 mg mL−1 PI solution. Data from 1 × 104 cells were collected and analyzed.

Data analysis. For each compound, a minimum of three independent experiments with triplicate values to measure cell viability and a minimum of two independent experiments for cell cycle analysis and assessment of apoptosis were conducted. Data are given as the mean ± standard deviation (SD).

DNA migration studies. A stock solution (10 mM) of each compound was prepared in high purity DMSO. Then, serial dilutions were made in MilliQ water (1 : 1). Plasmid pBluescript SK+ (Stratagene) was obtained using a QIAGEN plasmid midi kit as described by the manufacturer. Interaction of drugs with pBluescript SK+ plasmid DNA was analyzed by agarose gel electrophoresis following a

493 modification of the method described by Abdullah et al.77 Plasmid DNA aliquots (40 μg mL−1) were
494 incubated in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) with different concentrations of
495 compounds 1-R–7-R and 1-S–2-S ranging from 0 μM to 200 μM at 37 °C for 24 h. The final DMSO
496 concentration in the reactions was always lower than 1%. For comparison, cisplatin and ethidium
497 bromide (EB) were used as reference controls. Aliquots of 20 μL of the incubated solutions containing
498 0.8 μg of DNA were subjected to 1% agarose gel electrophoresis in TAE buffer (40 mM tris-acetate, 2
499 mM EDTA, pH 8.0). The gel was stained in TAE buffer containing ethidium bromide (0.5 mg mL−1)
500 and visualized and photographed under UV light.

501 Topoisomerase I-based experiments were performed as described previously.78 Supercoiled pBluescript
502 DNA, obtained as described above, was treated with topoisomerase I in the absence or presence of
503 increasing concentrations of compounds 3-R–7-R. Assay mixtures contained supercoiled pBluescript
504 DNA (0.8 μg), calf thymus topoisomerase I (3 units) and complexes 3-R–7-R (0–100 μM) in 20 μL of
505 relaxation buffer Tris-HCl buffer (pH 7.5) containing 175 mM KCl, 5 mM MgCl2 and 0.1 mM EDTA.
506 Ethidium bromide (EB, 10 μM) was used as a control of intercalating agents and etoposide (E, 100 μM)
507 as a control of the non-intercalating agent. Reactions were incubated for 30 min at 37 °C and stopped by
508 the addition of 2 μL of agarose gel loading buffer. Samples were then subjected to electrophoresis and
509 DNA bands stained with ethidium bromide as described above.

510 To distinguish whether compounds act as topoisomerase inhibitors or DNA intercalators the conversion
511 of relaxed DNA to a supercoiled state caused by the compounds was analysed in the presence of
512 topoisomerase I. Relaxed DNA was obtained by incubation of supercoiled DNA with topoisomerase I as
513 described above. Assay mixtures (20 μL) contained: relaxed DNA, topoisomerase I (3 units) and
514 compound (50 μM or 100 μM). Reactions were incubated 20 min at 37 °C and stopped as described
515 above. Ethidium bromide (10 μM) was used as a control of intercalative drug.

516 **Cathepsin B inhibition assay.** The colorimetric cathepsin B assay was performed as described by
517 Casini et al.79 with few modifications. Briefly, the reaction mixture contained 100 mM sodium
518 phosphate (pH 6.0), 1 mM EDTA and 200 μM sodium N-carbobenzoxy-L-lysine p-nitrophenyl ester as
519 the substrate. To have the enzyme catalytically active before each experiment the cysteine in the active
520 site was reduced by treatment with dithiothreitol (DTT). For this purpose, 5 mM DTT was added to the
521 cathepsin B sample, before dilution, and incubated 1 h at 30 °C. To test the inhibitory effect of the
522 platinum compounds on cathepsin B, activity measurements were performed in triplicate using fixed
523 concentrations of enzyme (500 nM) and substrate (200 μM). The platinum compounds were used at
524 concentrations ranging from 5 to 100 μM. Previous to the addition of substrate, cathepsin B was
525 incubated with the different compounds at 25 °C for 2 h. The cysteine proteinase inhibitor E-64 was
526 used as a positive control of cathepsin B inhibition. Complete inhibition was achieved at 10 μM
527 concentration of E-64. Activity was measured over 90 s at 326 nm on a UV-spectrophotometer.

528 **Cell accumulation.** Cell accumulation of platinum compounds was measured in MDA-MB-231. A total
529 of 1 × 106 cells were seeded in 6 mm tissue culture dishes for 16 h in DMEMhigh glucose plus 10%
530 FCS (fetal calf serum). Then, compounds were added at 50 μM for 4 h at 37 °C, 5% CO2. Cisplatin was
531 used as a positive control at the same concentration, and DMSO as a vehicle control. After treatment, the
532 cells were washed twice with PBS, tripsinized and harvested in PBS. The cell suspension was
533 centrifuged and pellets were digested with 12 M HCl and diluted to 1.2 M HCl. Each treatment was
534 done in duplicate. The samples were analyzed by ICPMS using an ICP-MS Perkin Elmer (Elan 6000).
535 Results are expressed as mol Pt per cell.

536

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686 **Legends to figures**

687

688 **Chart 1** Chemical structures of platinum-based anticancer drugs in clinical use. * Indicates a chiral
689 center.

690

691 **Scheme 1** Synthesis of the studied compounds.

692

693 **Figure 1**. Molecular structure of compound 7-R. Selected bond lengths (Å) and angles (°) with
694 estimated standard deviations: Pt(01)–N(1): 2.065(3); Pt(01)–N(2): 2.072(3); Pt(01)–Cl(2): 2.2977(9);
695 Pt(01)–Cl(1): 2.3106(8); N(1)–Pt(01)–N(2): 179.55(15); Cl(2)–Pt(01)–Cl(1): 178.28(3); N(1)–Pt(01)–
696 Cl(2): 87.90(9); N(2)–Pt(01)–Cl(2): 91.67(11); N(1)–Pt(01)–Cl(1): 91.22(10); N(2)–Pt(01)–Cl(1):
697 89.22(9)..

698

699 **Figure 2.** Comparison of the IC50 (μM) values obtained for the platinum(II) complexes 1-R–7-R, 1-S–
700 2-S and cisplatin in the A-549 (lung), MDA-MB-231 and MCF-7 (breast) and HCT-116 (colon) cancer
701 cell lines. Compound 4-R (IC50 value >100) is not shown.

702

703 **Figure 3** The percentages of cell cycle distributions in A-549 cells are depicted in the histogram and
704 represented as the cell number vs. the DNA content plot. The conditions include untreated cells (control)
705 and those treated with compounds 2-R, 5-R and 6-R at concentrations equal to their IC50 values (9.6
706 μM, 1.2 μM and 4.6 μM, respectively) for 72 h. The harvested cells were stained with PI (propidium
707 iodide) and their DNA content analyzed by flow cytometry.

708

709 **Figure 4**. The histogram shows the percentage variations of the quadrants depicting alive (Q4), early
710 apoptotic (Q3) and late apoptotic/necrotic (Q2/Q1) phases in A-549 cells. The same is represented by
711 the adjoining figure. The conditions include untreated cells (control) and those treated with compounds
712 2-R, 5-R and 6-R at concentrations equal to their IC50 values (9.6 μM, 1.2 μM and 4.6 μM,
713 respectively) for 72 h.

714

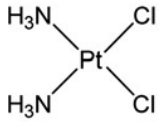
715 **Figure 5** Interaction of pBluescript SK+ plasmid DNA (0.8 μg) with increasing concentrations of
716 compounds under study, cisplatin and ethidium bromide (EB). Lane 1: DNA only. Lane 2: 2.5 μM. Lane
717 3: 5 μM. Lane 4: 10 μM. Lane 5: 25 μM. Lane 6: 50 μM. Lane 7: 100 μM. Lane 8: 200 μM; sc =
718 supercoiled closed circular DNA; oc = open circular DNA.

719

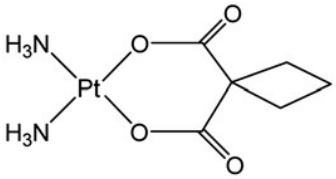
720 **Figure 6** Analysis of 3-R–7-R as putative DNA intercalators or topoisomerase I inhibitors. Conversion
721 of supercoiled pBluescript plasmid DNA (0.8 μg) to relaxed DNA by the action of topoisomerase I (3

722 units) in the absence or in the presence of increasing amounts of compounds 3-R–7-R was analyzed by
723 agarose gel. Ethidium bromide (EB) was used as a control of intercalating agent and etoposide (E) as a
724 control of nonintercalating agent. Lane 1: (–) scDNA only. Lane 2: 0 μM drug. Lane 3: 10 μM drug.
725 Lane 4: 25 μM drug. Lane 5: 100 μM drug. Except for lane 1, all lanes included topoisomerase I; sc =
726 supercoiled closed circular DNA; oc = open circular DNA.
727
728 **Figure 7** Effect of compound 5-R on the activity of topoisomerase I. Lane 1, (–) scDNA as a control.
729 Lane 2, relaxed DNA as a control. Relaxed pBluescript plasmid DNA was incubated with topoisomerase
730 I (3 units) in the presence of 50 μM (lane 3) or 100 μM (lane 4) of compound 5-R and 10 μM (lane 5) of
731 ethidium bromide (EB). The conversion of relaxed DNA to supercoiled DNA was analysed after a 20
732 min incubation. Reaction containing EB is included as an example of an intercalative drug. sc =
733 supercoiled closed circular DNA; oc = open circular DNA.
734
735
736
737
738
739

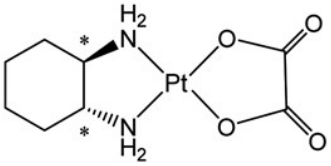
CHART 1



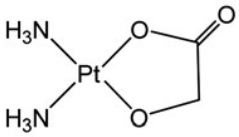
cisplatin



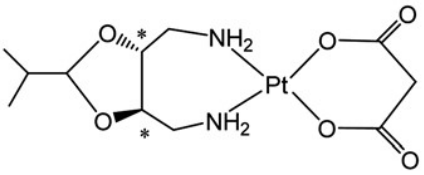
carboplatin



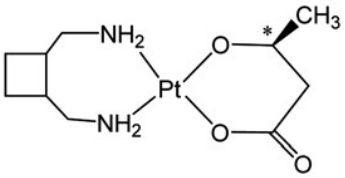
oxaliplatin



nedaplatin



heptaplatin



lobaplatin

745 **SCHEME 1**

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747

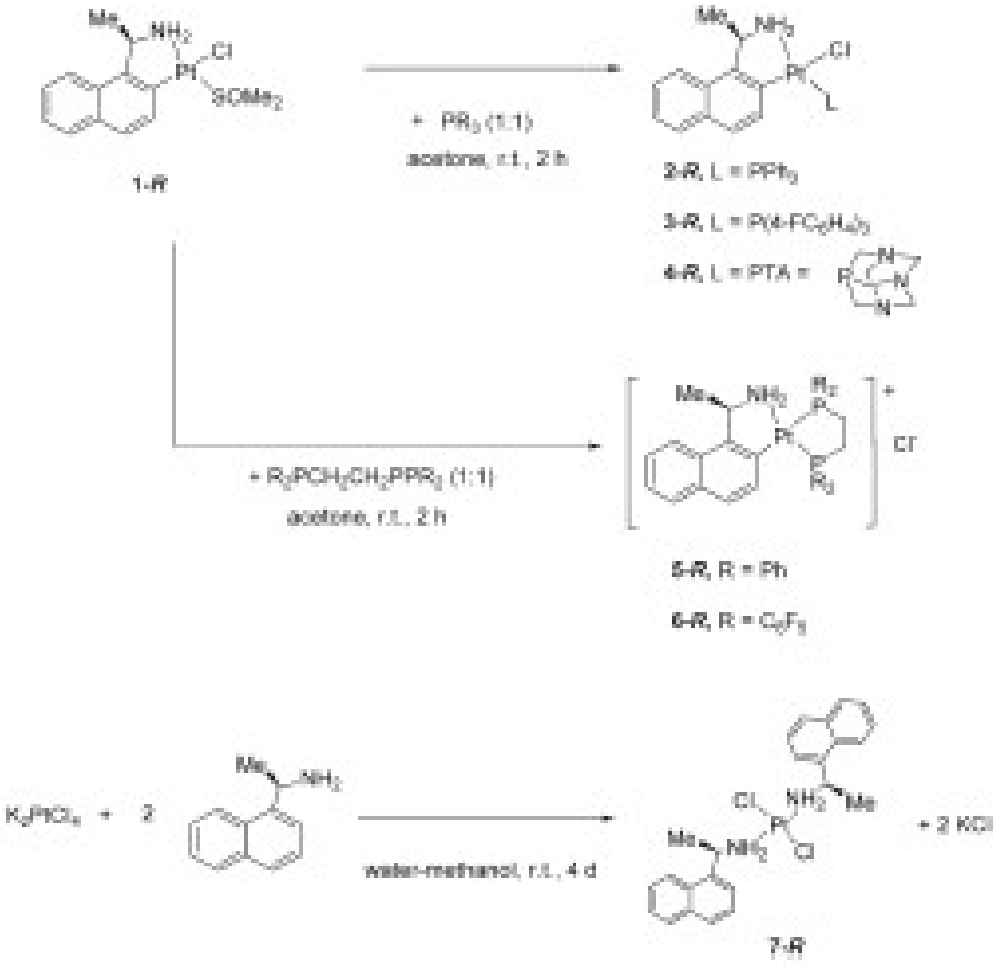

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FIGURE 1.

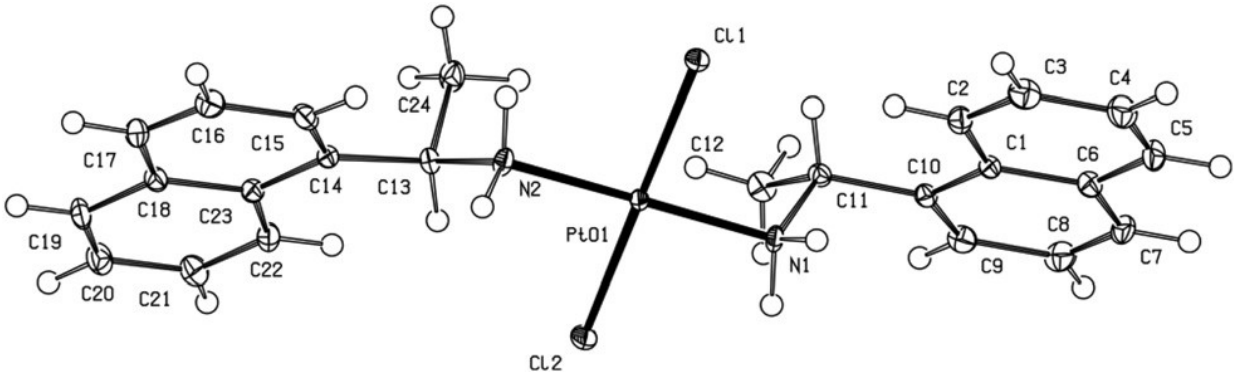
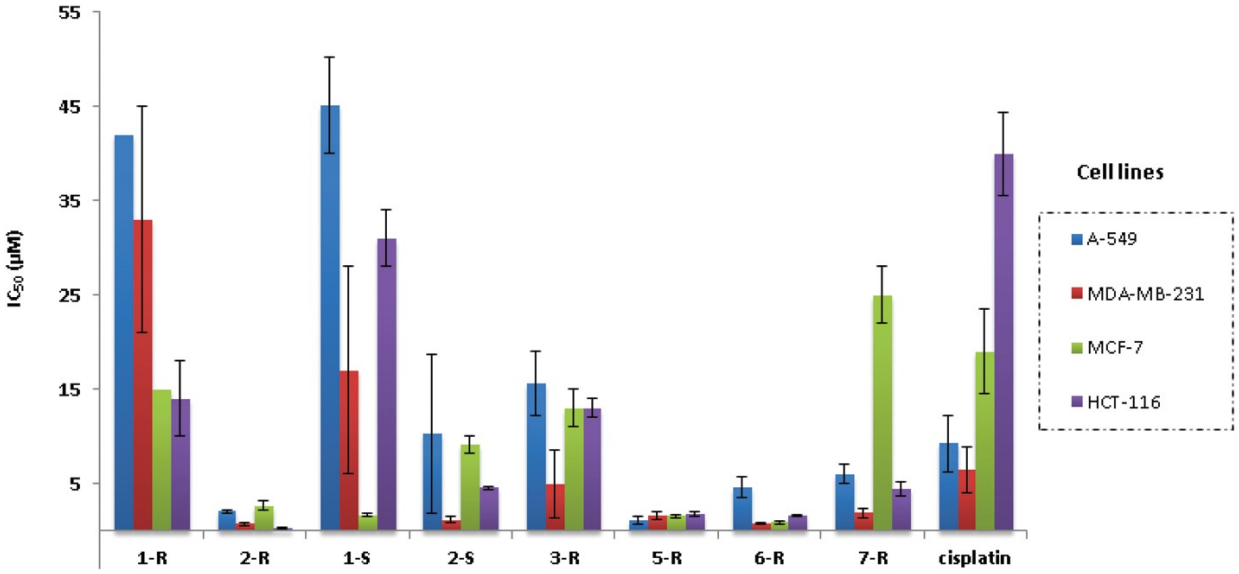

Cl1
C24
C3
C4
C2
C5
C16
C15
C17
C12
C1
C6
C10
C18
C14
C13
N2
C11
C23
C8
Pt01
C19
N1
C22
C9
C7
C20
C21
Cl2

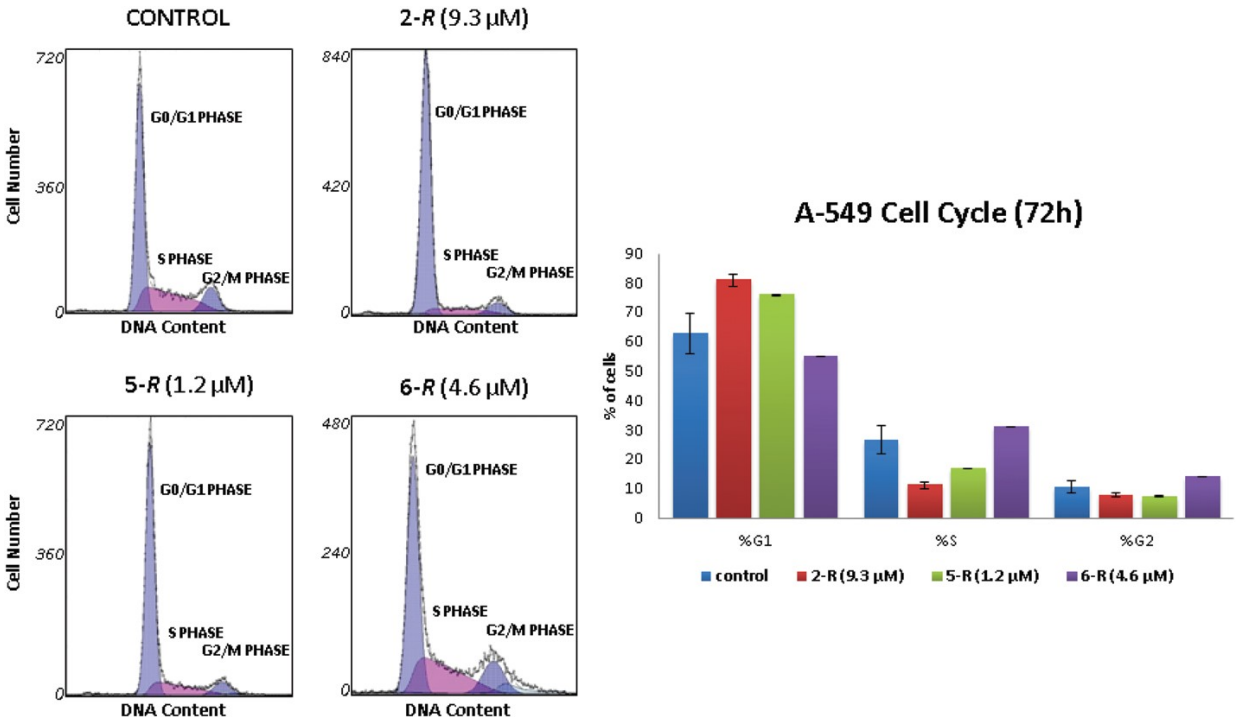
FIGURE 2.


55
45
35
25
15
5
IC50 (µM)
1-R
2-R
1-S
2-S
3-R
5-R
6-R
7-R
cisplatin
Cell lines
A-549
MDA-MB-231
MCF-7
HCT-116

762 **FIGURE 3.**

763

764



765

766

FIGURE 4.

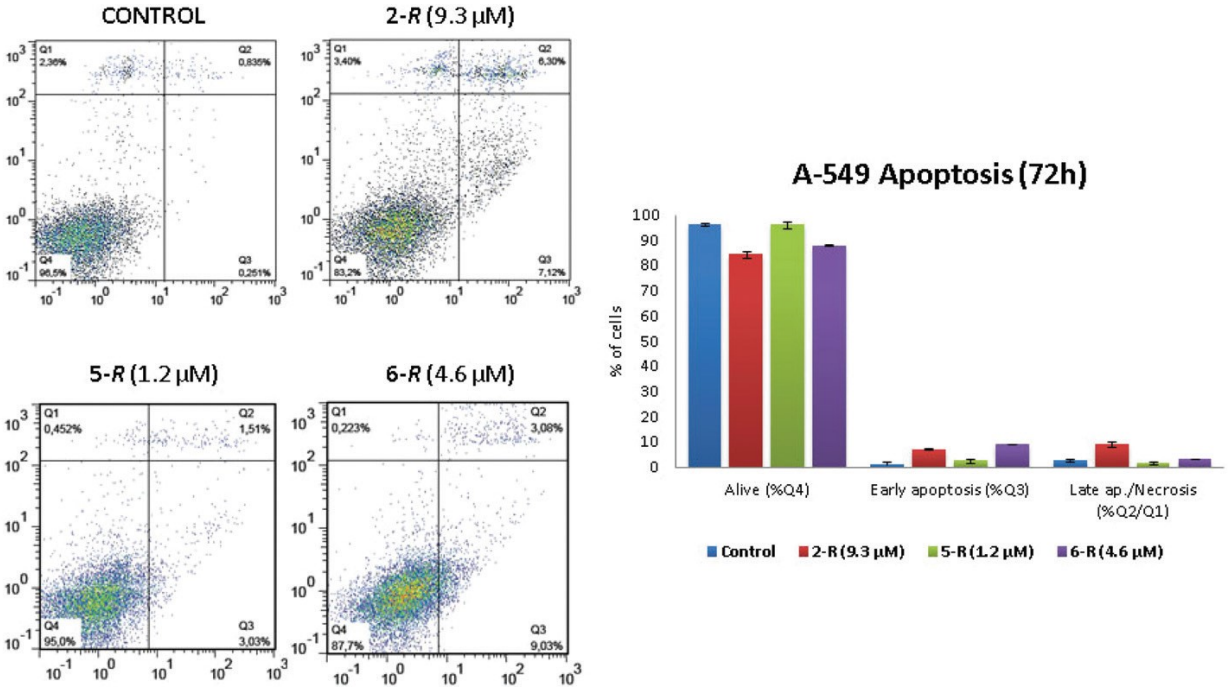

CONTROL
2-R (9.3 µM)
5-R (1.2 µM)
6-R (4.6 µM)
A-549 Apoptosis (72h)
% of cells
Alive (%Q4)
Early apoptosis (%Q3)
Late ap./Necrosis (%Q2/Q1)
Control
2-R (9.3 µM)
5-R (1.2 µM)
6-R (4.6 µM)

FIGURE 5.

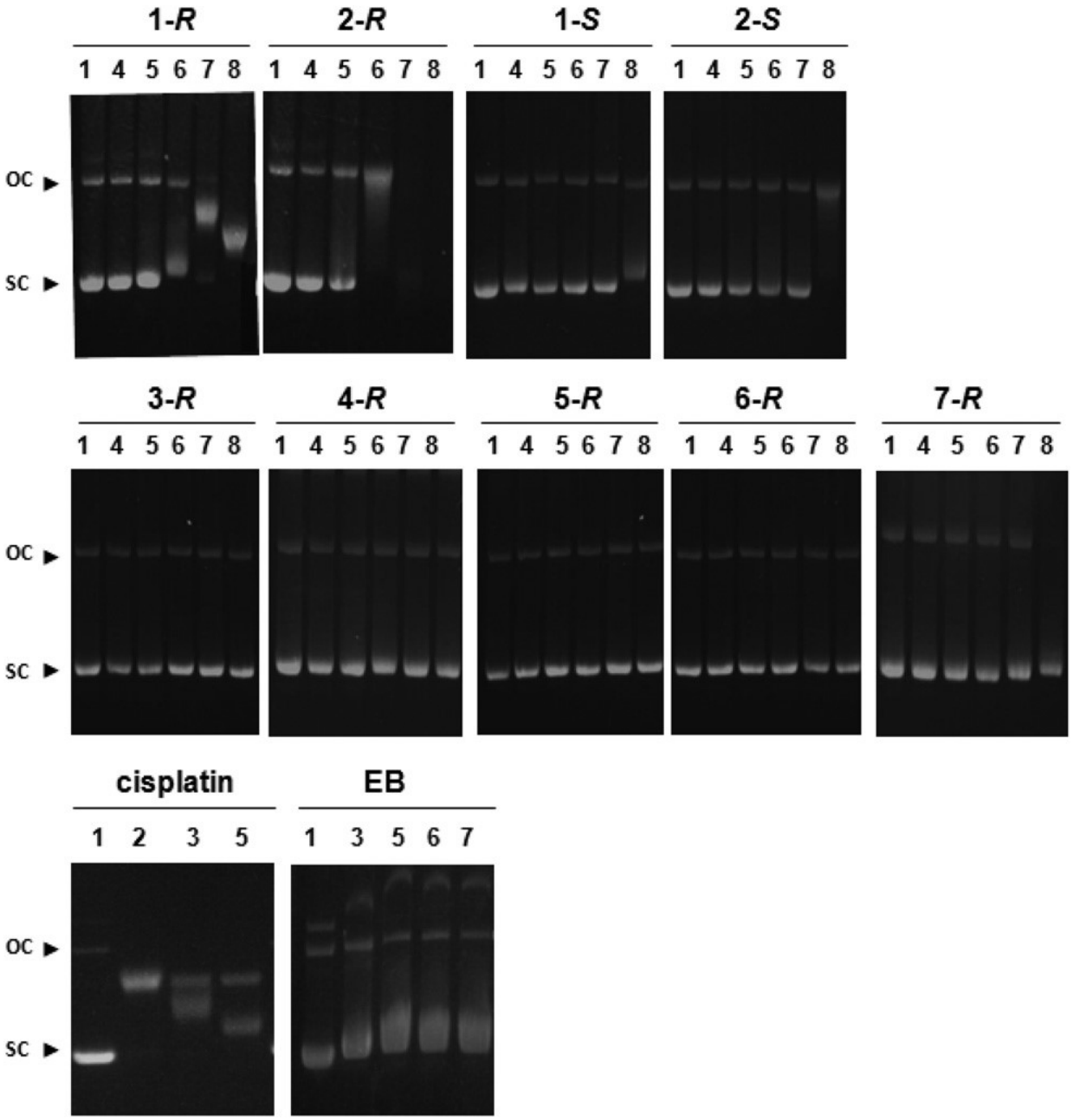


FIGURE 6.

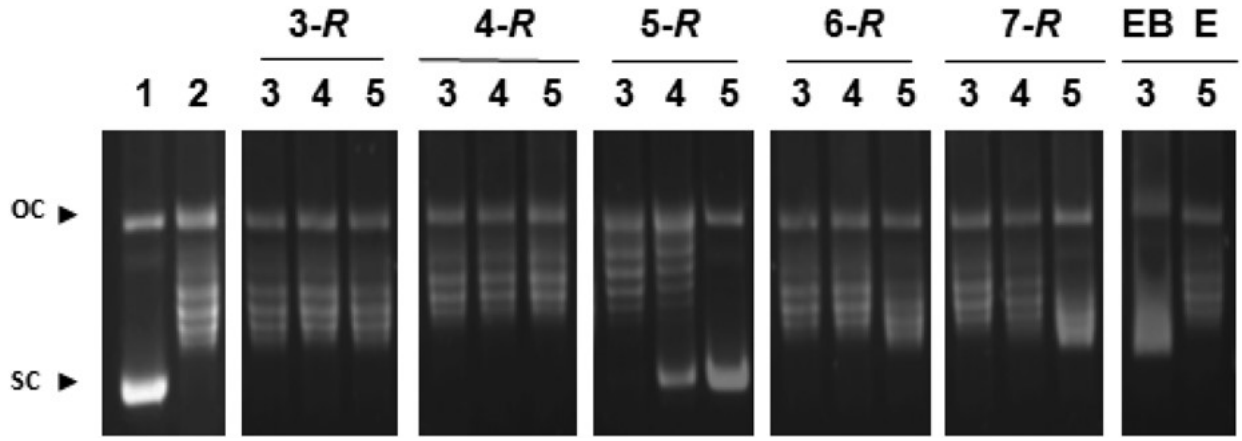


FIGURE 7.

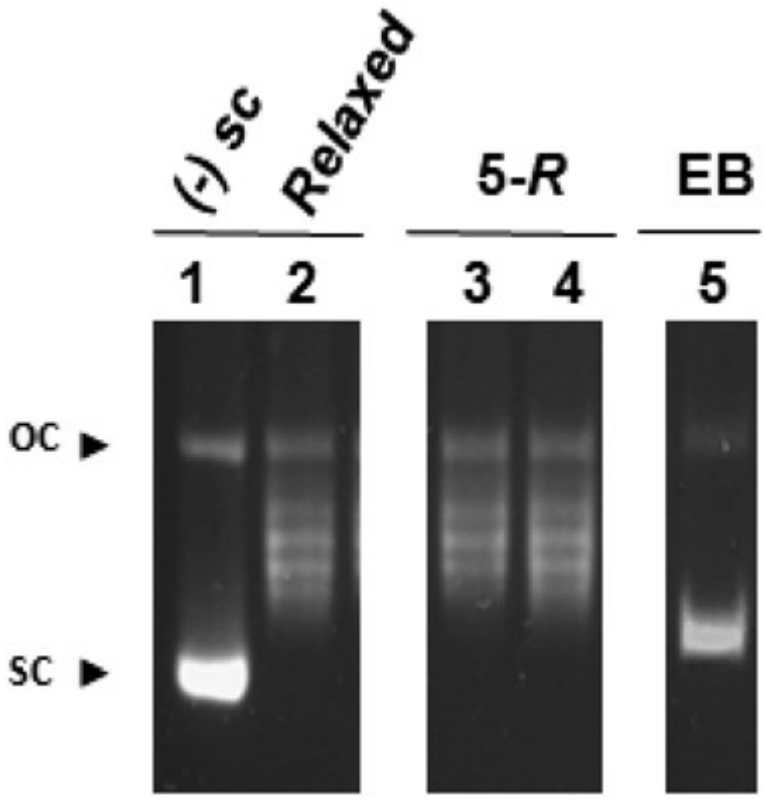


Table 1. 1 195Pt NMR dataa

Compound	$\delta(^{195}Pt)$	$^{1}J_{P\text{-}Pt}$
1-*R*	−3617.1	—
2-*R*	−3970.4	4335.1
3-*R*	−3973.8	4352.5
4-*R*[b]	−3911.6	3721.9
5-*R*	−4560.7	3713.1; 1689.3
6-*R*	−4327.8	3926.9; 1601.8
7-*R*	−2179.0	—

[a] δ in ppm, J in Hz, solvent $CDCl_3$ unless otherwise stated. [b] Solvent d^6-dmso.

797 **Table 2** IC50 (μM) valuesa for compounds under study

798

IC_{50} (μM)				
Compound	A-549	MDA-MB-231	MCF-7	HCT-116
1-*R*	42 ± nd	33 ± 12	15 ± nd	14 ± 4
2-*R*	9.3 ± 2.3	0.76 ± 0.16	2.7 ± 0.5	0.27 ± 0.1
1-*S*	45.2 ± 5.1	17 ± 11	1.7 ± 0.2	31 ± 3
2-*S*	10.3 ± 8.4	1.2 ± 0.3	9.2 ± 0.9	4.6 ± 0.2
3-*R*	15.7 ± 3.4	5.0 ± 3.6	13.0 ± 2.0	13 ± 1
4-*R*	>100	>100	>100	>100
5-*R*	1.2 ± 0.4	1.59 ± 0.4	1.6 ± 0.2	1.8 ± 0.2
6-*R*	4.6 ± 1.1	0.82 ± 0.08	0.9 ± 0.1	1.6 ± 0.05
7-*R*	6 ± 1	1.9 ± 0.5	25 ± 3	4.5 ± 0.8
Cisplatin[b]	9.3 ± 3	6.5 ± 2.4	19 ± 4.5	40 ± 4.4

[a] Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviations. [b] Cisplatin is taken as the reference compound.

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802 **Table 3** IC50 (μM) values and % of residual activity of cathepsin B at 100 μM for compounds 1-R–7-R,
803 and 1-S–2-Sa
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Compound	IC_{50} (μM) *vs.* cathepsin B	% Of residual activity at 100 μM
1-*R*	37 ± 4	1.2 ± 1.0
2-*R*	>50	46 ± 1.7
1-*S*	35 ± 3	5 ± 1.1
2-*S*	>50	40 ± 1.5
3-*R*	>50	20 ± 1.9
4-*R*	>100	100 ± 0.7
5-*R*	>50	18 ± 2.1
6-*R*	30 ± 5	4.5 ± 0.9
7-*R*	17 ± 2	<0.10

[a] The enzyme was preincubated for 2 h with each compound (from 5 to 100 μM). The residual activity is given as a percentage of the enzyme activity determined in the absence of the test compound. Data are shown as the mean values of the experiment performed in triplicate with the corresponding standard deviation.

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809 **Table 4** Platinum accumulation in the MDA-MB-231 cell line

810

Compound	Cell accumulation[a]
1-*R*	9.65 ± 0.10
1-*S*	1.45 ± 0.04
2-*R*	0.96 ± 0.03
2-*S*	4.41 ± 0.41
3-*R*	3.51 ± 0.05
4-*R*	2.86 ± 0.10
5-*R*	4.67 ± 0.21
6-*R*	6.69 ± 0.62
7-*R*	12.49 ± 0.33
Cisplatin	0.45 ± 0.03

[a] Cell accumulation for compounds 1-*R*–7-*R* and 1-*S*–2-*S* and for cisplatin is given as (mol of Pt per cell ± SD) × 10^{-16}, and was measured by ICP-MS after 4 h of treatment at 50 μM with the indicated compounds. Experiments were performed in duplicate. SD = standard deviation.

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816 **Table 5** Crystallographic and refinement data for compound 7-R

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Formula	$C_{24}H_{20}Cl_2N_2Pt$
Fw	608.46
Temp (K)	100(2)
λ (Å)	0.71073
Crystal system	Monoclinic
Space group	*C*2
a (Å)	35.2163(17)
b (Å)	6.7270(3)
c (Å)	9.5287(5)
α (°)	90
β (°)	102.512(2)
γ (°)	90
V (Å^3); Z	2203.74(19); 4
D (calcd), (Mg m^{-3})	1.834
Abs coeff. (mm^{-1})	6.623
F(000)	1184
Rflns coll./independent	24 896/6539 [R(int) = 0.0251]
Data/restraint/parameters	6539/1/264
GOF on F^2	1.049
Final R index ($I > 2\sigma(I)$)	$R_1 = 0.0151$, w$R_2 = 0.0346$
R index (all data)	$R_1 = 0.0158$, w$R_2 = 0.0348$
Peak and hole (e Å^{-3})	1.137 and −1.065
CCDC number	1062343

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