DOI: 10.1002/ejoc.201500998

Synthesis and Evaluation of the Antiproliferative Properties of a Tethered Tubercidin–Platinum(II) Complex

Stefano D'Errico,[a] Giorgia Oliviero,*[a] Nicola Borbone,[a] Elena Di Gennaro,[b] Andrea Ilaria Zotti,[b] Alfredo Budillon,[b] Vincenzo Cerullo,[c] Fabrizia Nici,[a] Luciano Mayol,[a] Vincenzo Piccialli,[d] and Gennaro Piccialli[a,e]

Keywords: Platinum / Nucleosides / Antitumor agents / Medicinal chemistry

Herein, the synthesis of a nucleoside platinum(II) complex in which a cisplatin-like unit is joined to 7-deazaadenosine through an amino alkyl chain installed at the C6 position of purine was explored. The capability of the new complex to react with DNA purine bases was confirmed by a model reaction with deoxyguanosine monophosphate, whereas its antiproliferative activity against A549 and Cal27 human cancer cell lines was studied by sulforhodamine B assay in comparison with its unplatinated precursor and cisplatin.

Introduction

More than 50 years after its discovery,[1] cisplatin (**1**, Figure 1) is still one of the most effective drugs for the treatment of several types of tumors, including testicular, ovarian, bladder, cervical, and lung cancer and head and neck carcinomas.[2] The mechanism of action of cisplatin involves DNA as the final target. In fact, cisplatin essentially binds the guanine bases at the N7 position and forms stable intrastrand adducts or interstrand crosslinks that cause severe local changes in the secondary structure of DNA.[3] This DNA distortion hampers its binding with crucial cellular proteins and is recognized as DNA damage that eventually leads to cell death by apoptosis.[4] The clinical use of cisplatin is partially limited by intrinsic resistance of many tumors and by the insurgence of acquired resistance during treatments.[2,5] Carboplatin (**2**) and oxaliplatin (**3**), second- and third-generation platinum-based antineoplastic agents, respectively, have dosages and clinical administration protocols that ameliorate the quality of life of patients, even if they do not resolve the problems of drug resistance.[6]

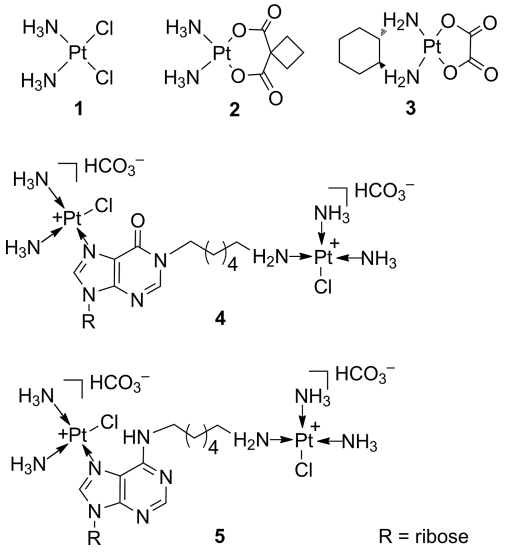


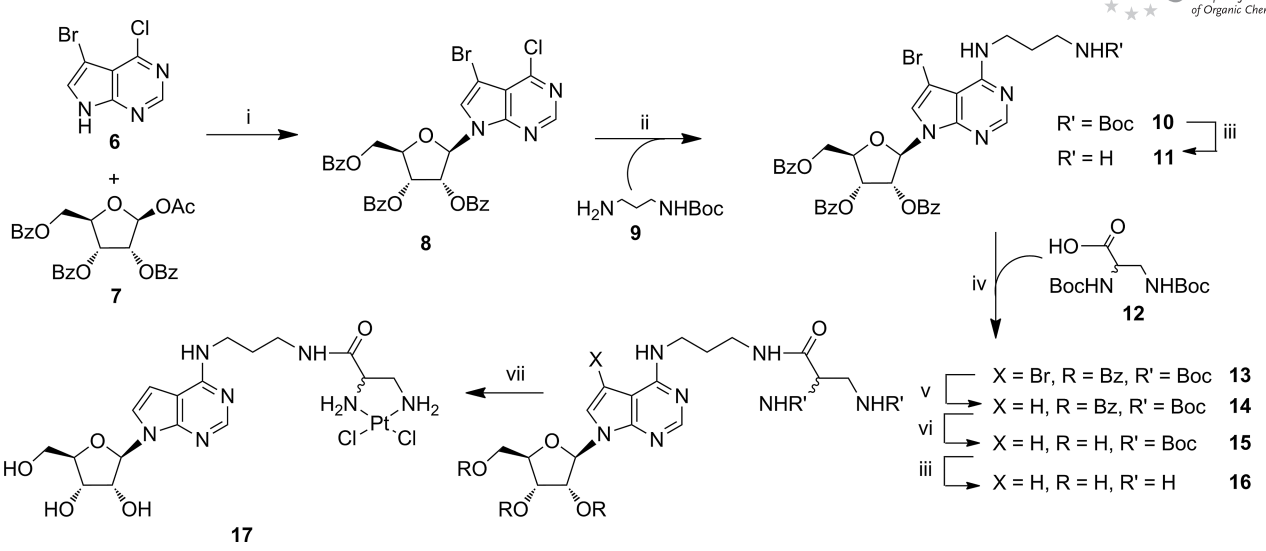
Figure 1. Structures of platinum drugs **1**–**3** and bis-platinated nucleoside complexes **4** and **5** carrying purine nucleoside scaffolds.

For these reasons, research in this field is still very active, proposing multinuclear platinum complexes[7–10] and composite molecules in which the platinum complex is conjugated to biologically important substances, drugs, and carriers.[11–18] Furthermore, thanks to the synergistic effect that may result, tethering between biologically active molecules and platinum units could afford novel complexes with improved drug-targeting and drug-delivery properties.[12]

Nucleosides and nucleotides (NNs) and their analogues play a key role in the regulation of cellular processes and in signal transduction.[19–22] Furthermore, NN analogues are

[a] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II,
Via Domenico Montesano 49, 80131 Napoli, Italy
E-mail: giorgia.oliviero@unina.it
http://www.unina.it
[b] Experimental Pharmacology, Istituto Nazionale Tumori Fondazione G. Pascale – IRCCS,
Via Mariano Semmola 52, 80131 Napoli, Italy
[c] Division of Biopharmaceutics and Pharmacokinetics, Faculty of Pharmacy, University of Helsinki,
Viikinkaari 5 E, 00790 Helsinki, Finland
[d] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II,
Via Cinthia 4, 80126 Napoli, Italy
[e] Institute of Protein Biochemistry, National Research Council of Italy,
Via Pietro Castellino 111, 80131 Napoli, Italy

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201500998.



Scheme 1. Reagents and conditions: (i) *N*,*O*-Bis(trimethylsilyl)acetamide (BSA), trimethylsilyl trifluoromethanesulfonate (TMSOTf), CH_3CN, 2 h, 80 °C, 60%; (ii) **9**, EtOH, 5 h, reflux, 80%; (iii) trifluoroacetic acid (TFA)/CH_2Cl_2 (1:1), 1 h, r.t., 99%; (iv) **12**, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt), *N*,*N*-diisopropylethylamine (DIPEA), 16 h, r.t., 83%; (v) MeOH, AcONa, H_2/Pd/C, 2 h, r.t., 99%; (vi) MeONa, MeOH, 1 h, r.t., 95%; (vii) K_2PtCl_4, H_2O/MeOH (1:1), 16 h, r.t., in the dark, 70%. Bz = benzoyl, Boc = *tert*-butoxycarbonyl.

known to inhibit the growth of several cancer lines[23–26] and many of them are clinically used as antiviral drugs.[27–29] Despite this, only a few platinum complexes carrying nucleoside scaffolds have been proposed.[30–33] In principle, a platinum complex conjugated to a biologically active nucleoside could combine the reactivity of the platinum center with the pharmacological properties of the nucleoside analogue (e.g., antimetabolite activity or active transmembrane transport). However, the properties of such a composite molecule are not predictable because each moiety of the complex can strongly influence the chemical and pharmacological behavior of the other. In this context, we recently focused our attention on the development of new nucleoside dinuclear platinum(II) complexes.

In particular, we synthesized bis-platinated nucleoside complexes **4** and **5** (Figure 1)[34,35] carrying two monofunctional platinum(II) centers, the first coordinating the N7 position of the purine system and the second coordinating the terminal amino group of an alkyl chain installed on the purine base. These substances showed moderate (or low) antiproliferative activities upon testing against A278, HeLa, A549, and MCF7 cell lines.

In this paper, we describe the synthesis and the preliminary biological evaluation of a novel nucleoside platinum complex (i.e., **17**, Scheme 1) carrying a neutral cisplatin unit conjugated to the 1,2-diamine moiety of 7-deazaadenosine (7-deaza-A) analogue **16**. 7-Deaza-A (also known as tubercidin) is an interesting antimetabolite endowed with antibiotic and antitumor properties.[36,37] In particular, tubercidin shows significant in vitro cytotoxicity against the murine P388 and the human lung adenocarcinoma A549 cell lines.[38,39] In our context, the absence of the N7 purine atom in 7-deaza-A avoids the issue of the fast reactivity of N7 with platinum during the coordination reaction, which thus allows the construction of a monoplatinated complex involving exclusively the exocyclic 1,2-diamine function of the linker. The capability of **17** to mimic the reactivity of cisplatin with the DNA bases was assessed by monitoring a model reaction of **17** with deoxyguanosine 5′-monophosphate (dGMP) by ^{1}H NMR spectroscopy.[40,41] The preliminary information on the antiproliferative activity of **17** and its unplatinated precursor **16** against Cal27 and A549 tumor cell lines are also reported.

Results and Discussion

Synthesis and Characterization

Our synthetic route started with the construction of bis-halogenated 7-deazapurine-β-D-riboside **8** (Scheme 1). The nucleoside scaffold was prepared according to a procedure by Seela and Ming,[42] which performs the glycosylation step by using 7-bromo-6-chloro-7-deazapurine **6** as the aglycon moiety. As outlined in that paper, the presence of the electron-withdrawing bromine atom at the C7 position of the purine atom makes the formation of the glycosidic bond with suitably protected ribose moiety **7** possible in good yields and with complete β stereoselectivity. Nucleoside **8** was then treated with mono-Boc-protected 1,3-diaminopropane unit **9** to afford derivative **10**. After quantitative removal of the Boc group (TFA in CH_2Cl_2), resulting nucleoside **11** was treated with bis-Boc-protected 2,3-diaminopropanoic acid (racemic mixture, **12**)[43] by using EDC/HOBt as the activating agents of the carboxylic function to give **13** (83% yield) as a mixture of diastereoisomers. Compound **13** was then subjected to hydrogenolysis in a Parr apparatus by using Pd/C as the catalyst to remove the C7 bromine. It was reported that the presence of a base (AcONa) in the reaction mixture is indispensable to neutralize HBr that is formed during the reaction.[44] The

hydrogenolysis proceeded very cleanly to give **14** quantitatively after a standard workup procedure. The benzoate protecting groups were then removed from **14** with a solution of MeONa in MeOH to give **15** after chromatographic purification (95% yield). Finally, the Boc protecting groups in **15** were removed by TFA treatment, and the crude was treated with a concentrated aqueous solution of NH_4OH to affording 7-deaza-A derivative **16**, as the free diamine almost quantitatively (98%). The reaction of **16** with K_2PtCl_4 in a H_2O/MeOH (1:1) solution gave platinum complex **17** as a pale-yellow solid that precipitated from the reaction mixture in 70% yield.

The structures of all intermediates and of complex **17** were supported by spectroscopic analyses. High-resolution mass spectrometry and CHN analysis further confirmed the structure and purity of final complex **17**.

NMR Spectroscopy Study of the Reactivity of 17 towards dGMP

To assess the capability of **17** to react with the guanine bases of DNA as a bifunctional coordinating agent, such as cisplatin, we incubated **17** with a fourfold excess amount of dGMP in a water buffer containing 50 mM KH_2PO_4 and 50 mM KCl in a NMR tube in the dark at 37 °C. It is well known that the chemical shifts of H8 and H1′ of dGMP complexed with platinum(II) species are very sensitive to the geometry and the composition of the complex.[40] Thus, we monitored the reaction by recording the ^{1}H NMR spectrum every 30 min for the first 6 h and then every 6 h until no further changes were noticed. 10% [D_7]DMF was added to the buffer to assure the complete water solubility of **17**.

The reactivity of the *cis*-dichloroplatinum moiety of **17** towards dGMP was confirmed by the appearance of downfield-shifted aromatic signals (δ = 8.4–9.0 ppm), attributable to H8 of dGMP complexed with **17**, already in the first hour of incubation (Figure 2). Because of the asymmetry of the diaminoalkyl moiety coordinated with the platinum(II) center, up to six aromatic signals belonging to H8 of dGMP complexed with **17** were expected [two signals for each of the two complexes 7-deaza-A-Pt(dGMP)(Cl) and 7-deaza-A-Pt(dGMP)(H_2O); two signals for 7-deaza-A-Pt-(dGMP)$_2$]. This spectral complexity was observed in the NMR spectra recorded at an incubation time ≥3 h. Although the detailed assignment of all the dGMP H8 signals was not accomplished, because it is beyond the scope of this paper, the analysis of the literature data and the relative intensity of the NMR signals recorded at increasing incubation times (Figure 2) allowed us to tentatively assign (1) the signals at δ = 8.55 and 8.62 ppm of the dGMP H8 proton of the complexes 7-deaza-A-Pt(dGMP)(Cl) and 7-deaza-A-Pt(Cl)(dGMP) (**+** in Figure 2); (2) the signal at δ = 8.99 ppm (visible at incubation times ≥ 6 h) of the dGMP H8 proton of one of the two aqua complexes 7-deaza-A-Pt(dGMP)(H_2O) or 7-deaza-A-Pt(H_2O)(dGMP) (●); (3) the signals at δ = 8.52 and 8.65 ppm of the H8 protons of the two dGMP in the 7-deaza-A–Pt(dGMP)$_2$ complex (♦). The formation of platinum–d(GMP) complexes during the incubation time was also confirmed by the appearance of new upfield-shifted signals for the H2, H7, H8, and H1′ protons of **17** in the NMR spectra. In particular, the relative intensity of the NMR signals of H7 and H8 before and after coordination with d(GMP) disclosed that more than 50% of **17** reacted with one or two molecules of d(GMP) at incubation times within 12 and 36 h (Figure 2).

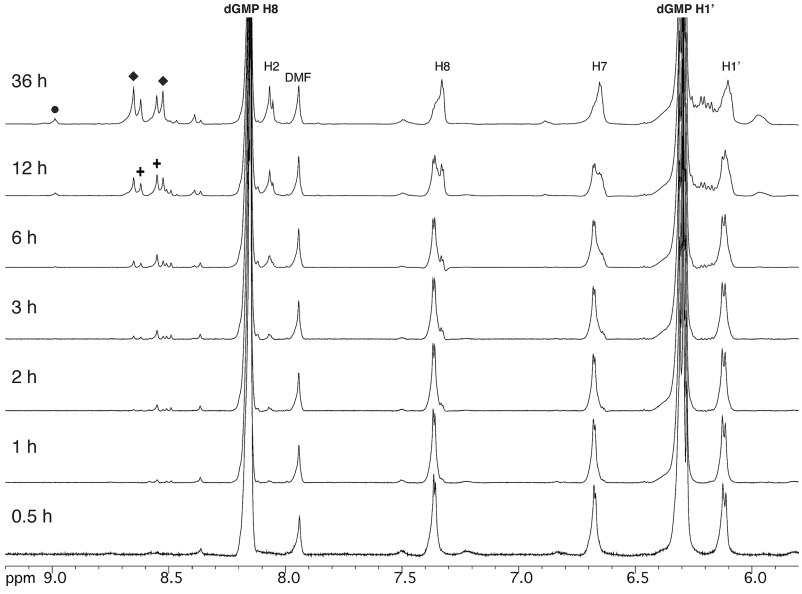


Figure 2. Aromatic region of the ^{1}H NMR spectra recorded at different time points monitoring the reaction between **17** and a fourfold excess amount of dGMP (50 mM KH_2PO_4, 50 mM KCl, pH 7.0, 37 °C in H_2O/D_2O = 9:1 containing 10% [D_7]DMF). The signals of **17** and dGMP are labeled by using regular and bold-type fonts, respectively. The H8 NMR signals of dGMP complexed with **17** are labeled with ●, ♦, and **+** (see also the Results and Discussion section).

Antiproliferative Studies on the A549 and Cal27 Cell Lines

Compound **17**, its unplatinated precursor **16**, and cisplatin were examined by sulforhodamine B (SRB) assay[45] for their capability to inhibit growth of the A549 and Cal27 cell lines. To this end, cisplatin was dissolved in phosphate-buffered saline (PBS), whereas compound **16** and complex **17** were dissolved in 10% DMSO/PBS. All compounds were diluted immediately to the final concentrations by using the cell growth medium. The results showed that, although less active than cisplatin, both compounds induced dose-dependent growth inhibition in both cell lines after 96 h of treatment (Figure 3). Unexpectedly, for the tested cell lines, we observed that the antiproliferative activity of unplatinated precursor **16** was 1.5-fold higher than that of platinated complex **17** (Table 1). Further studies are needed to ascertain whether the lower activity of **17** is due to the residual "tubercidin-like" activity found for **16** or to the target "cisplatin-like alkylation" of purine bases by the dichloroplatinum moiety.

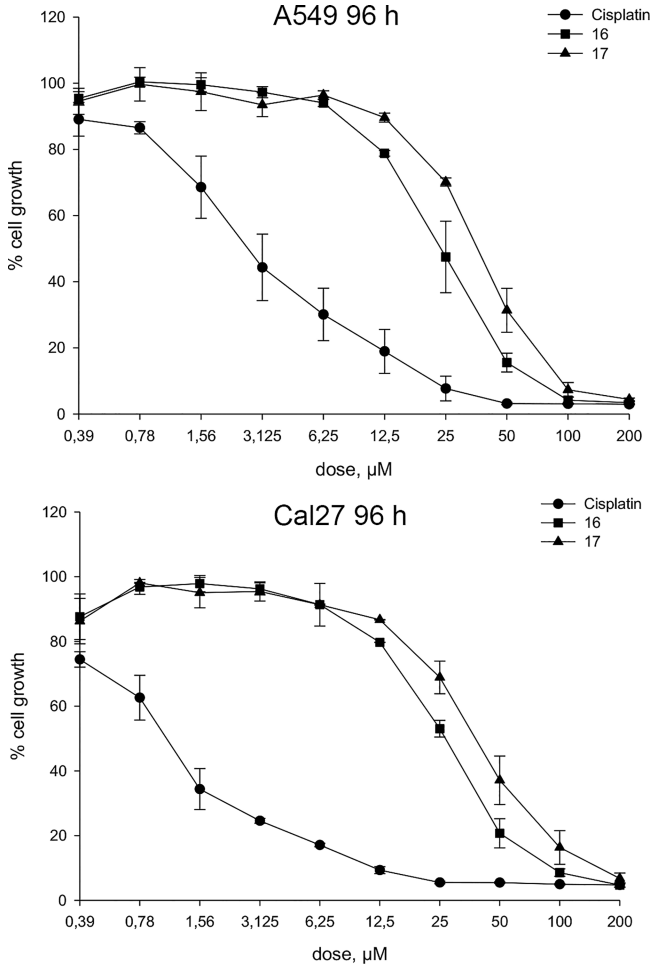


Figure 3. Antiproliferative effect of cisplatin, **16**, and **17** in the A549 (top) and Cal27 (bottom) cell lines. Cell growth assessment was performed by sulforhodamine B colorimetric assay (see the Experimental Section) and is expressed as the percentage of control for each time point. Values are the mean ± SD from at least three independent experiments performed in quadruplicate.

Table 1. Sensitivity of the A549 and Cal27 cell lines to cisplatin, **16**, and **17**.

Compound	IC_{50}[a] at 96 h [μM] ± SD	
	A549	Cal27
Cisplatin	2.69 ± 0.80	1.04 ± 0.20
16	23.62 ± 4.83	26.44 ± 1.64
17	36.20 ± 2.72	37.75 ± 5.58

[a] The half-maximal inhibitory concentration (IC_{50}) values were computed after 96 h of treatment (mean ± SD from at least three separate experiments performed in quadruplicate). SD: standard deviation.

Conclusions

In this paper, we described the synthesis of a new nucleoside platinum(II) complex (i.e., **17**) in which a cisplatin-like unit is joined to 7-deazaadenosine through an amino alkyl chain installed at the C6 position of purine. The cisplatin-like capability of **17** to react with the purine bases of DNA was confirmed by monitoring a model reaction with dGMP, whereas preliminary antiproliferative data on **17** and on unplatinated precursor **16** demonstrated that both compounds, although less potent than cisplatin, were able to inhibit the proliferation of the A549 and Cal27 human cell lines in a dose-dependent manner. Interestingly, the antiproliferative activity of free diamine **16** was 1.5-fold higher than that of platinated complex **17** on both cell lines. Studies are ongoing in our laboratories to assess the suitability of compound **16** to chelate other metals involved in cancer research and to construct novel unprecedented tubercidin derivatives with polyaminoalkyl chains at the C6 position of purine.

Experimental Section

General Methods: All reagents and solvents for the chemical syntheses were obtained from commercial sources and were used without further purification. All cell culture media, serum, antibiotics, and glutamine were purchased from LONZA (Basel, Switzerland). Sulforhodamine B (SRB) was from ICN Biomedicals (Irvine, CA, USA). ^1H NMR and ^{13}C NMR spectra were acquired with Varian Mercury Plus 400 MHz and Varian UNITY Inova 500 MHz instruments by using CD_3OD, $(CD_3)_2SO$, or C_6D_6 as solvents. A total of 450 μL of a 50 mM KH_2PO_4, 50 mM KCl, pH 7 buffer in H_2O/D_2O (9:1) was used to record the ^1H NMR spectra required to monitor the reaction of **17** (dissolved in 50 μL of [D_7]DMF) with dGMP. A double pulsed gradient spin echo (DPFGSE) module was included in the NMR pulse sequences to suppress the water signal.[46,47] Chemical shifts are reported in parts per million (δ) relative to the residual solvent signal [^1H: CD_2HOD δ = 3.31 ppm, $(CD_3)(CD_2H)SO$ δ = 2.54 ppm, C_6D_5H δ = 7.15 ppm; ^{13}C: CD_3OD δ = 49.0 ppm, $(CD_3)_2SO$ δ = 40.4 ppm, C_6D_6 δ = 128.6 ppm] and were assigned by 2D NMR experiments. UV spectra were recorded with a Jasco V-530 UV spectrophotometer. IR spectra were recorded with a Jasco FTIR 430 spectrophotometer. Optical rotations were determined with a Jasco polarimeter by using a 1 dm cell at 25 °C; concentrations are expressed in g 100 mL^{-1}. High-resolution MS spectra were recorded with a Thermo Orbitrap XL mass spectrometer by using the electrospray ionization (ESI) tech-

nique in positive mode. Elemental analyses were performed with a Thermo Finnigan Flash EA 1112 CHN analyzer.

Cell Culture and Proliferation Assay: Human lung-cancer-derived A549 cells and human tongue squamous-carcinoma-derived Cal27 cells were grown in adhesion in Dulbecco's modified Eagle's medium. The medium was supplemented with 10% heat-inactivated fetal bovine serum, penicillin (50 U mL^{-1}), streptomycin (500 μg mL^{-1}), and glutamine (4 mmol L^{-1}) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cell proliferation of A549 and Cal27 was evaluated in the presence and absence of increasing concentrations of the compounds by colorimetric assay with sulforhodamine B (SRB, ICN Biomedicals, Irvine, CA, USA). In detail, 1000 cells well^{-1} were seeded in 96-multiwell plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) in quadruplicate. After 24 h, the cells were treated with the indicated compounds for 96 h and then the SRB assay was performed as described before.[45] The growth was evaluated as percentage compared to untreated cells.

Compound 10: A mixture of compound **8** (300 mg, 0.44 mmol), *tert*-butyl (3-aminopropyl)carbamate (**9**; 383 mg, 2.2 mmol), and Et_3N (61 μL, 0.44 mmol) was heated at reflux in EtOH (12 mL). During the reaction, a colorless solid, identified as compound **10**, precipitated. After 5 h (TLC monitoring: CH_2Cl_2/MeOH, 98:2), the system was cooled to room temperature, and the solid was then filtered, washed with cold EtOH, and dried. Colorless amorphous solid (287 mg, 80%). $[a]_D$ = –122.1 (c = 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, C_6D_6): δ = 8.46 (s, 1 H, 2-H), 8.31–8.24 (m, 2 H, arom.), 8.02–7.92 (complex signal, 4 H, arom.), 7.20–6.81 (complex signal partially covered by residual solvent signal, 10 H, 8-H and arom.), 6.75 (d, J = 5.6 Hz, 1 H, 1′-H), 6.37–6.31 (m, 1 H, 2′-H), 6.25–6.16 (complex signal, 2 H, 3′-H and NH), 4.74–4.65 (m, 1 H, 4′-H), 4.64–4.56 (br. t, J = 6.1 Hz, 1 H, NH), 4.40–4.32 (m, 2 H, 5′-$H_{a,b}$), 3.41–3.31 (m, 2 H, CH_2NH), 2.96–2.87 (m, 2 H, CH_2NHBoc), 1.44 (s, 9 H, Boc), 1.37–1.26 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, C_6D_6, 40 °C): δ = 166.0, 165.3, 165.2, 156.9, 156.4, 153.7, 150.4, 133.3, 133.2, 130.4, 130.1, 129.8, 129.5, 128.8, 128.6, 128.5, 120.6, 103.0, 89.1, 86.9, 80.6, 78.6, 75.0, 72.3, 64.1, 37.5, 30.7, 28.6 ppm. IR (KBr pellet): $\tilde{\nu}$ = 3411, 3382, 2980, 2936, 1728, 1687, 1602, 1562, 1513, 1451, 1270, 1175, 1127, 1027, 711 cm^{-1}. UV (CH_2Cl_2): λ_{max} = 235, 280 nm. HRMS (ESI): m/z = 836.1911 [M + Na]$^+$ ($C_{40}H_{40}BrN_5O_9Na$ requires 836.1907).

Compound 11: A solution of compound **10** (250 mg, 0.29 mmol) in CH_2Cl_2 (1.0 mL) was cooled to 0 °C. TFA (1.0 mL) was added in one portion. The mixture was then warmed to room temperature and stirred for 1 h (TLC monitoring: CH_2Cl_2/MeOH, 8:2). Solvents were evaporated under reduced pressure, and the crude material containing compound **11** as its TFA salt was used for the next step without purification. Oil (238 mg, 99%). $[a]_D$ = –76.8 (c = 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.18 (s, 1 H, 2-H), 8.10–8.05 (m, 2 H, arom.), 8.01–7.96 (m, 2 H, arom.), 7.85–7.89 (m, 2 H, arom.), 7.65–7.30 (complex signal, 10 H, 8-H and arom.), 6.61 (d, J = 5.3 Hz, 1 H, 1′-H), 6.26–6.19 (m, 1 H, 2′-H), 6.16–6.11 (m, 1 H, 3′-H), 4.95–4.80 (complex signal partially covered by residual solvent signal, 2 H, 4′-H and 5′-H_a), 4.67 (dd, J = 11.7, 3.5 Hz, 1 H, 5′-H_b), 3.71 (t, J = 6.7 Hz, 2 H, CH_2NH), 3.01 (t, J = 7.2 Hz, 2 H, CH_2NH$_3^+$), 2.08–1.97 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 167.5, 166.7, 166.4, 161.2 (q, J = 37.7 Hz), 156.6, 151.5, 149.8, 134.9, 134.6, 130.9, 130.8, 130.7, 130.2, 129.8, 129.6, 123.9, 115.8, 103.4, 89.8, 88.1, 81.5, 75.4, 72.8, 64.8, 38.5, 37.9, 28.7 ppm. IR (neat): $\tilde{\nu}$ = 3060, 2884, 1722, 1682, 1599, 1448, 1269, 1204, 1127, 711 cm^{-1}. UV (MeOH): λ_{max} = 277 nm. HRMS (ESI): m/z = 736.1390 [M + Na]$^+$ ($C_{35}H_{32}BrN_5NaO_7$ requires 736.1383).

Compound 13: Racemic acid **12** (177 mg, 0.58 mmol) was dissolved in dry DMF (4 mL) and then DIPEA (0.4 mL, 2.3 mmol), EDC (222 mg, 1.2 mmol), and HOBt (157 mg, 1.2 mmol) were added. The mixture was stirred for 30 min at room temperature. Compound **11** (200 mg, 0.24 mmol), dissolved in dry DMF (2 mL), was added to the previous solution, and the mixture was stirred for 16 h at room temperature (TLC monitoring: *n*-hexane/AcOEt, 4:6). The solvents were removed under reduced pressure, and the crude material was diluted with AcOEt (20 mL) and extracted with brine (3 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material containing compound **13** was purified by chromatography (silica gel, EtOAc in *n*-hexane, gradient up to 70%). The fractions containing the product were collected and concentrated to afford pure **13** as a mixture of diastereomers. Amorphous white solid (200 mg, 83%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.19 (s, 2 H, 2 × 2-H), 8.12–8.07 (m, 4 H, arom.), 8.02–7.97 (m, 4 H, arom.), 7.91–7.86 (m, 4 H, arom.), 7.66–7.33 (complex signal, 20 H, 2 × 8-H and arom.), 6.61 (d, J = 5.5 Hz, 2 H, 2 × 1′-H), 6.25–6.19 (m, 2 H, 2 × 2′-H), 6.18–6.12 (m, 2 H, 2 × 3′-H), 4.93–4.78 (complex signal partially covered by residual solvent signal, 4 H, 2 × 4′-H and 2 × 5′-H_a), 4.67 (dd, J = 3.6, 12.2 Hz, 2 H, 2 × 5′-H_b), 4.15–4.08 (m, 2 H, 2 × CH), 3.71–3.54 (m, 4 H, 2 × CH_2NH), 3.41 (dd, J = 4.7, 14.1 Hz, 2 H, 2 × CH_aNHBoc), 3.36–3.26 (2 H, 2 × CH_bNHBoc, covered by residual solvent signal), 3.24–3-14 (m, 4 H, 2 × CH_2NHCO), 1.83–1.72 (m, 4 H, 2 × CH_2), 1.41 (s, 9 H, Boc), 1.39 (s, 9 H, Boc), 1.37 (s, 9 H, Boc), 1.36 (s, 9 H, Boc) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 173.2, 167.5, 166.7, 166.4, 158.9, 157.9, 157.7, 153.9, 150.4, 134.8, 134.7, 134.5, 130.9, 130.8, 130.7, 130.3, 129.9, 129.7, 129.6, 129.5, 122.5, 103.4, 89.7, 87.7, 81.4, 80.9, 80.5, 75.4, 72.9, 64.9, 57.4, 43.1, 38.4, 37.0, 30.7, 28.7 ppm. IR (KBr pellet): $\tilde{\nu}$ = 3330, 2923, 1723, 1597, 1262, 1160, 1117, 708 cm^{-1}. UV (MeOH): λ_{max} = 275 nm. HRMS (ESI): m/z = 1000.3085 [M + H]$^+$ ($C_{48}H_{55}BrN_7O_{12}$ requires 1000.3092).

Compound 14: Compound **13** (150 mg, 0.15 mmol) was dissolved in MeOH (10 mL) in a Parr reactor; NaOAc (13 mg, 0.16 mmol) and Pd/C (10% w/w, 42 mg, 0.04 mmol) were added and air was removed by insufflating H_2. The apparatus was then charged with H_2 (1.0 MPa), and the system was stirred for 2 h (TLC monitoring: *n*-hexane/AcOEt, 3:7) at room temperature. H_2 was removed, and the mixture was filtered through a Celite 545 pad that was then washed with MeOH (3 × 10 mL). The solvent was evaporated under reduced pressure, and the crude was diluted with AcOEt (20 mL) and extracted with brine (3 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material containing compound **14** was used for the next reaction step without purification. Oil (137 mg, 99%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (s, 2 H, 2 × 2-H), 8.16–8.08 (m, 4 H, arom.), 8.04–7.97 (m, 4 H, arom.), 7.92–7.83 (m, 4 H, arom.), 7.66–7.34 (complex signal, 18 H, arom.), 7.23 (d, J = 3.7 Hz, 2 H, 2 × 8-H), 6.67 (d, J = 5.9 Hz, 2 H, 2 × 1′-H), 6.58 (d, J = 3.7 Hz, 2 H, 2 × 7-H), 6.28–6.20 (m, 2 H, 2 × 2′-H), 6.19–6.12 (m, 2 H, 2 × 3′-H), 4.93–4.77 (complex signal partially covered by residual solvent signal, 4 H, 2 × 4′-H and 2 × 5′-H_a), 4.71–4.64 (m, 2 H, 2 × 5′-H_b), 4.15–4.08 (m, 2 H, 2 × CH), 3.70–3.45 (m, 4 H, 2 × CH_2NH), 3.44–3.36 (m, 2 H, 2 × CH_aNHBoc), 3.36–3.26 (2 H, 2 × CH_bNHBoc, covered by residual solvent signal), 3.22–3.11 (m, 4 H, 2 × CH_2NHCO), 1.83–1.73 (m, 4 H, 2 × CH_2), 1.40 (s, 9 H, Boc), 1.38 (s, 9 H, Boc), 1.36 (s, 9 H, Boc), 1.35 (s, 9 H, Boc) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 167.5, 166.7, 166.5, 158.9, 158.3, 157.7, 153.1, 150.8, 134.8, 134.7, 134.5, 130.9, 130.8, 130.7, 130.3, 129.9, 129.7, 129.6, 129.5, 122.5, 105.2, 101.6, 87.2, 81.2, 80.9, 80.5, 75.3, 73.0, 65.2, 57.5, 43.0, 38.5, 37.2, 30.6,

28.7 ppm. IR (neat): $\tilde{\nu}$ = 3341, 2972, 1723, 1602, 1503, 1262, 1160, 1119, 708 cm^{-1}. UV (MeOH): λ_{max} = 276 nm. HRMS (ESI) *m/z* = 922.3989 $[M + H]^+$ ($C_{48}H_{55}N_7O_{12}$ requires 922.3987).

Compound 15: Compound **14** (115 mg, 0.12 mmol) was dissolved in MeOH (1.5 mL) and then MeONa (32 mg, 0.60 mmol) was added. The solution was stirred for 2 h at room temperature (TLC monitoring: AcOEt/MeOH, 9:1). The solution was neutralized with a few drops of glacial acetic acid; the solvents were removed under reduced pressure, and the crude was diluted with AcOEt (20 mL) and extracted with brine (3 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel, MeOH in AcOEt, gradient up to 10%). The fractions containing the product were collected and concentrated to afford pure **15**. Amorphous solid (67 mg, 95%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.12 (s, 2 H, 2 × 2-H), 7.25 (d, *J* = 3.6 Hz, 2 H, 2 × 8-H), 6.57 (d, *J* = 3.6 Hz, 2 H, 2 × 7-H), 5.96 (d, *J* = 6.6 Hz, 2 H, 2 × 1′-H), 4.71–4.65 (m, 2 H, 2 × 2′-H), 4.30–4.26 (m, 2 H, 2 × 3′-H), 4.18–4.08 (complex signal, 4 H, 2 × 4′-H and 2 × CH), 3.84 (dd, *J* = 2.1, 12.4 Hz, 2 H, 2 × 5′-H_a), 3.71 (dd, *J* = 2.3, 12.4 Hz, 2 H, 2 × 5′-H_b), 3.68–3.48 (m, 4 H, 2 × CH_2NH), 3.46–3.39 (m, 2 H, 2 × CH_aNHBoc), 3.36–3.26 (2 H, 2 × CH_bNHBoc, covered by residual solvent signal), 3.24–3.11 (m, 4 H, 2 × CH_2NHCO), 1.86–1.75 (m, 4 H, 2 × CH_2), 1.42 (s, 18 H, 2 × Boc), 1.38 (s, 18 H, 2 × Boc) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 158.8, 158.3, 157.7, 152.2, 149.5, 124.7, 105.9, 100.0, 91.6, 87.3, 80.9, 80.5, 75.3, 72.7, 63.7, 57.4, 43.0, 38.4, 37.2, 30.6, 28.7 ppm. IR (KBr pellet): $\tilde{\nu}$ = 3307, 2917, 1695, 1607, 1503, 1366, 1256, 1160, 1048 cm^{-1}. UV (MeOH): λ_{max} = 277 nm. HRMS (ESI): *m/z* =610.3184 $[M + H]^+$ ($C_{27}H_{44}N_7O_9$ requires 610.3201).

Compound 16: A solution of compound **15** (50 mg, 0.082 mmol) in CH_2Cl_2 (0.5 mL) was cooled to 0 °C. TFA (0.5 mL) was added in one portion. The mixture was then warmed to room temperature and stirred for 1 h (TLC monitoring: CH_2Cl_2/MeOH, 8:2). The solvents were evaporated under reduced pressure. The crude was treated with conc. NH_4OH solution (1 mL), evaporated under reduced pressure, dissolved in water (1 mL), and eventually lyophilized. The crude material containing compound **16** was used for the next reaction step without purification. Oil (33 mg, 99%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.12 (s, 2 H, 2 × 2-H), 7.27 (d, *J* = 3.7 Hz, 2 H, 2 × 8-H), 6.58 (d, *J* = 3.7 Hz, 2 H, 2 × 7-H), 5.95 (d, *J* = 6.6 Hz, 2 H, 2 × 1′-H), 4.69–4.63 (m, 2 H, 2 × 2′-H), 4.29–4.24 (m, 2 H, 2 × 3′-H), 4.13–4.08 (m, 2 H, 2 × 4′-H), 3.84 (dd, *J* = 2.6, 12.4 Hz, 2 H, 2 × 5′-H_a), 3.72 (dd, *J* = 2.8, 12.4 Hz, 2 H, 2 × 5′-H_b), 3.58 (t, *J* = 6.7 Hz, 4 H, 2 × CH_2NH), 3.33–3.26 (6 H, covered by residual solvent signal, 2 × CH and 2 × CH_2NHCO), 2.85 (dd, *J* = 5.0, 13.1 Hz, 2 H, 2 × C$H_a$$NH_2$), 2.85 (dd, *J* = 6.7, 13.1 Hz, 2 H, 2 × C$H_b$$NH_2$), 1.91–1.80 (m, 4 H, 2 × CH_2) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 176.1, 158.1, 152.0, 149.7, 124.6, 105.9, 100.1, 91.5, 87.3, 75.3, 72.7, 63.7, 57.7, 46.7, 38.9, 37.7, 30.4 ppm. IR (neat): $\tilde{\nu}$ = 3269, 1610, 1551, 1365, 1314, 1248, 1079, 725 cm^{-1}. UV (MeOH): λ_{max} = 274 nm. HRMS (ESI): *m/z* = 410.2147 $[M + H]^+$ ($C_{17}H_{28}N_7O_5$ requires 410.2152).

Compound 17: Compound **16** (20 mg, 0.031 mmol) was dissolved in H_2O/MeOH (1:1, 1 mL). K_2PtCl_4 (13 mg, 0.031 mmol) was added, and the solution was stirred in the dark at room temperature for 16 h. The pale-yellow precipitate (15 mg, 70%) was collected by filtration, washed sequentially with H_2O and MeOH, and then dried. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.21 (br. t, 2 H, 2 × N*H*CO), 8.12 (s, 2 H, 2 × 2-H), 7.55–7.50 (m, 2 H, 2 × N*H*CH_2), 7.33 (br. d, *J* = 3.6 Hz, 2 H, 2 × 8-H), 6.60 (br. d, *J* = 3.6 Hz, 2 H, 2 × 7-H), 5.98 (br. d, *J* = 6.3 Hz, 2 H, 2 × 1′-H), 5.50–5.41 (br. s, 8 H, 2 × PtN$H_2$$CH_2$ and 2 × PtNH_2CH), 5.29 (br. t, 2 H, 2 × 5′-OH), 5.23 (br. d, 2 H, 2 × 2′-OH), 5.09 (br. d, 2 H, 2 × 3′-OH), 4.38–4.45 (m, 2 H, 2 × 2′-H), 4.11–4.04 (m, 2 H, 2 × 3′-H), 3.93–3.85 (m, 2 H, 2 × 4′-H), 3.66–3.40 (complex signal partially covered by residual solvent signal, 14 H, 2 × 5′-$H_{a,b}$, 2 × CH_2NH, 2 × PtNH_2CH_2, 2 × PtNHC*H*), 3.19–3.11 (m, 4 H, CH_2NHCO), 1.80–1.66 (m, 4 H, 2 × CH_2) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 167.0, 157.0, 152.3, 150.1, 123.1, 104.3, 100.1, 88.4, 86.0, 74.6, 71.6, 62.7, 61.9, 38.4, 37.7, 29.7 ppm. IR (KBr pellet): $\tilde{\nu}$ = 3247, 1665, 1610, 1561, 1363, 1311, 1245, 1089, 721 cm^{-1}. UV (H_2O): λ_{max} = 274 nm. HRMS (ESI): *m/z* = 717.1531 $[M – Cl + DMSO]^+$ ($C_{19}H_{33}ClN_7O_6PtS$ requires 717.1549). $C_{17}H_{27}Cl_2N_7O_5Pt$ (675.44): calcd. C 30.23, H 4.03, N 14.52; found C 30.27, H 4.08, N 14.58.

Reaction of 17 with dGMP: Complex **17** (2 mg, 3.0×10^{-3} mmol) was dissolved in [D_7]DMF (50 μL) and then added to 450 μL of a solution of dGMP (4 equiv.) in H_2O/D_2O (9:1) containing 50 mM KH_2PO_4 and 50 mM KCl, pH 7. The mixture was transferred to a NMR tube and was incubated at 37 °C. Water-suppressed ^{1}H NMR spectra (500 MHz, 37 °C) were then recorded as described in the general methods section and in the text.

Acknowledgments

This work was supported by POR Campania FESR 2007-2013 – Rete delle Biotecnolgie in Campania (project "FARMABIONET").

[1] B. Rosenberg, L. VanCamp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385–386.
[2] A.-M. Florea, D. Büsselberg, *Cancers* **2011**, *3*, 1351–1371.
[3] A. Sigel, H. Sigel, *Metal Ions in Biological Systems: Metal Complexes in Tumor Diagnosis and as Anticancer Agents*, CRC Press, London, UK, **2004**.
[4] E. Wexselblatt, E. Yavin, D. Gibson, *Inorg. Chim. Acta* **2012**, *393*, 75–83.
[5] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573–584.
[6] B. W. Harper, A. M. Krause-Heuer, M. P. Grant, M. Manohar, K. B. Garbutcheon-Singh, J. R. Aldrich-Wright, *Chem. Eur. J.* **2010**, *16*, 7064–7077.
[7] L. R. Kelland, N. P. Farrell (Eds.), *Platinum-Based Drugs in Cancer Therapy*, Humana Press, Totowa, NJ, USA, **2000**.
[8] F. Arnesano, G. Natile, *Coord. Chem. Rev.* **2009**, *253*, 2070–2081.
[9] J. Zhang, L. Wang, Z. Xing, D. Liu, J. Sun, *Curr. Med. Chem. Anti-Cancer Agents* **2010**, *10*, 272–282.
[10] K. Wang, E. Gao, *Curr. Med. Chem. Anti-Cancer Agents* **2014**, *14*, 147–169.
[11] P. Sengupta, S. Basu, S. Soni, A. Pandey, B. Roy, M. S. Oh, K. T. Chin, A. S. Paraskar, S. Sarangi, Y. Connor, V. S. Sabbisetti, J. Kopparam, A. Kulkarni, K. Muto, C. Amarasiriwardena, I. Jayawardene, N. Lupoli, D. M. Dinulescu, J. V. Bonventre, R. A. Mashelkar, S. Sengupta, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11294–11299.
[12] R. Cincinelli, L. Musso, S. Dallavalle, R. Artali, S. Tinelli, D. Colangelo, F. Zunino, M. De Cesare, G. L. Beretta, N. Zaffaroni, *Eur. J. Med. Chem.* **2013**, *63*, 387–400.
[13] J. S. Butler, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2013**, *17*, 175–188.
[14] A. Arouri, A. H. Hansen, T. E. Rasmussen, O. G. Mouritsen, *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 419–431.
[15] C. Tomuleasa, O. Soritau, A. Orza, M. Dudea, B. Petrushev, O. Mosteanu, S. Susman, A. Florea, E. Pall, M. Aldea, G. Kacso, V. Cristea, I. Berindan-Neagoe, A. Irimie, *J. Gastrointestin. Liver. Dis.* **2012**, *21*, 187–196.

[16] Y. H. Quan, B. Kim, J.-H. Park, Y. Choi, Y. H. Choi, H. K. Kim, *Exp. Lung. Res.* **2014**, *40*, 475–484.
[17] Y. Min, C.-Q. Mao, S. Chen, G. Ma, J. Wang, Y. Liu, *Angew. Chem. Int. Ed.* **2012**, *51*, 6742–6747; *Angew. Chem.* **2012**, *124*, 6846–6851.
[18] S. Wang, W. Chu, Y. Wang, S. Liu, J. Zhang, S. Li, H. Wei, G. Zhou, X. Qin, *Appl. Organomet. Chem.* **2013**, *27*, 373–379.
[19] Dubyak, G. R, *Am. J. Physiol. Cell Physiol.* **2013**, *304*, C403–C405.
[20] A. H. Guse, *Curr. Mol. Med.* **2002**, *2*, 273–282.
[21] H. C. Lee, *J. Biol. Chem.* **2012**, *287*, 31633–31640.
[22] G. Oliviero, S. D'Errico, N. Borbone, J. Amato, V. Piccialli, M. Varra, G. Piccialli, L. Mayol, *Tetrahedron* **2010**, *66*, 1931–1936.
[23] R. S. Hosmane, *Curr. Top. Med. Chem.* **2002**, *2*, 1093–1109.
[24] S. D'Errico, G. Oliviero, J. Amato, N. Borbone, V. Cerullo, A. Hemminki, V. Piccialli, S. Zaccaria, L. Mayol, G. Piccialli, *Chem. Commun.* **2012**, *48*, 9310–9312.
[25] R. M. Huang, Y. N. Chen, Z. Zeng, C. H. Gao, X. Su, Y. Peng, *Mar. Drugs* **2014**; *12*, 5817–5838.
[26] S. Meike, T. Yamamori, H. Yasui, M. Eitaki, A. Matsuda, M. Morimatsu, M. Fukushima, Y. Yamasaki, O. Inanami, *Mol. Cancer* **2011**, *10*, 92.
[27] C. K. Chu, *Antiviral Nucleosides: Chiral Synthesis and Chemotherapy*, Elsevier, Amsterdam, The Netherlands, **2003**.
[28] L. P. Jordheim, D. Durantel, F. Zoulim, C. Dumontet, *Nat. Rev. Drug Discovery* **2013**, *12*, 447–464.
[29] M. F. Caso, D. D'Alonzo, S. D'Errico, G. Palumbo, A. Guaragna, *Org. Lett.* **2015**, *17*, 2626–2629.
[30] D. Montagner, V. Gandin, C. Marzano, B. Longato, *J. Inorg. Biochem.* **2011**, *105*, 919–926.
[31] P. Starha, I. Popa, Z. Trávníček, J. Vančo, *Molecules* **2013**, *18*, 6990–7003.
[32] M. Coluccia, A. Boccarelli, C. Cermelli, M. Portolani, G. Natile, *Met. Based Drugs* **1995**, *2*, 249–256.
[33] C. Nervi, M. A. Vigna, G. Cavigiolio, M. Ravera, D. Osella, *Inorg. Chim. Acta* **2005**, *358*, 2799–2803.
[34] S. D'Errico, G. Oliviero, V. Piccialli, J. Amato, N. Borbone, V. D'Atri, F. D'Alessio, R. Di Noto, F. Ruffo, F. Salvatore, G. Piccialli, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5835–5838.
[35] S. D'Errico, G. Oliviero, N. Borbone, V. Piccialli, B. Pinto, F. De Falco, M. C. Maiuri, R. Carnuccio, V. Costantino, F. Nici, G. Piccialli, *Molecules* **2014**, *19*, 9339–9353.
[36] L. V. Frolova, I. V. Magedov, A. E. Romero, M. Karki, I. Otero, K. Hayden, N. M. Evdokimov, L. M. Y. Banuls, S. K. Rastogi, W. R. Smith, S.-L. Lu, R. Kiss, C. B. Shuster, E. Hamel, T. Betancourt, S. Rogelj, A. Kornienko, *J. Med. Chem.* **2013**, *56*, 6886–6900.
[37] G. Acs, E. Reich, M. Mori, *Proc. Natl. Acad. Sci. USA* **1964**, *52*, 493–501.
[38] M. F. Biabani, S. P. Gunasekera, *Pharm. Biol.* **2002**, *40*, 302–303.
[39] B. K. Hwang, B. S. Kim, *Pestic. Sci.* **1995**, *44*, 255–260.
[40] A. Marcelis, C. G. Van Kralingen, J. Reedijk, *J. Inorg. Biochem.* **1980**, *13*, 213–222.
[41] E. Pantoja, A. Gallipoli, S. van Zutphen, S. Komeda, D. Reddy, D. Jaganyi, M. Lutz, D. M. Tooke, A. L. Spek, C. Navarro-Ranninger, J. Reedijk, *J. Inorg. Biochem.* **2006**, *100*, 1955–1964.
[42] F. Seela, X. Ming, *Tetrahedron* **2007**, *63*, 9850–9861.
[43] S. Moradell, J. Lorenzo, A. Rovira, S. van Zutphen, F. X. Avilés, V. Moreno, R. de Llorens, M. A. Martinez, J. Reedijk, A. Llobet, *J. Inorg. Biochem.* **2004**, *98*, 1933–1946.
[44] E. A. Véliz, P. A. Beal, *J. Org. Chem.* **2001**, *66*, 8592–8598.
[45] E. Di Gennaro, F. Bruzzese, S. Pepe, A. Leone, P. Delrio, P. R. Subbarayan, A. Avallone, A. Budillon, *Cancer Biol. Ther.* **2009**, *8*, 782–791.
[46] T. L. Hwang, A. J. Shaka, *J. Magn. Reson., Ser. A* **1995**, *112*, 275–279.
[47] C. Dalvit, *J. Biomol. NMR* **1998**, *11*, 437–444.

Received: July 29, 2015
Published Online: November 3, 2015

