Inorganic Chemistry

Article

pubs.acs.org/IC

Novel Imino Thioether Complexes of Platinum(II): Synthesis, Structural Investigation, and Biological Activity

Paolo Sgarbossa,[†] Silvia Mazzega Sbovata,[†] Roberta Bertani,[†,∥] Mirto Mozzon,[†] Franco Benetollo,[‡] Cristina Marzano,[§] Valentina Gandin,[§] and Rino A. Michelin*[,†]

[†]Department of Industrial Engineering, University of Padua, Via F. Marzolo, 9, 35131 Padua, Italy

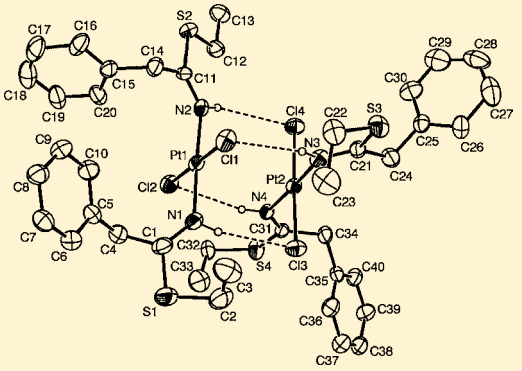
[‡]ICIS-CNR, Corso Stati Uniti, 4-35100 Padua, Italy

[§]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, I-35131 Padova, Italy

[∥]C.N.R.-ISTM, Istituto di Scienze e Tecnologie Molecolari, Via F. Marzolo 1, I- 35131 Padova, Italy

Supporting Information

ABSTRACT: The reactions of the nitrile complexes *cis*- and *trans*-$[PtCl_2(NCR)_2]$ (R = Me, Et, CH_2Ph, Ph) with an excess of ethanethiol, EtSH, in the presence of a catalytic amount of *n*-BuLi in tetrahydrofuran (THF), afforded in good yield the bis-imino thioether derivatives *cis*-$[PtCl_2\{E\text{-}N(H)=C(SEt)R\}_2]$ (R = Me (**1**), Et (**2**), CH_2Ph (**3**), Ph (**4**)) and *trans*-$[PtCl_2\{E\text{-}N(H)=C(SEt)R\}_2]$ (R = Me (**5**), Et (**6**), CH_2Ph (**7**), Ph (**8**)). The imino thioether ligands assumed the *E* configuration corresponding to a *cis* addition of the thiol to the nitrile triple bond. The spectroscopic properties of these complexes have been reported along with the molecular structures of **1**, **2**, and **7** as established by X-ray crystallography which indicated that these compounds exhibit square-planar coordination geometry around the platinum center. Four N−H···Cl intermolecular contacts (N−H···Cl ca. 2.5−2.7 Å) between each chlorine atom and the N−H proton of the imino thioether ligand gave rise to "dimers" $Pt_2Cl_4L_4$ (L = imino thioether) formed by two $PtCl_2L_2$ units. The cytotoxic properties of these new platinum(II) complexes were evaluated against various human cancer cell lines. Among all derivatives, *trans*-$[PtCl_2\{E\text{-}N(H)=C(SEt)CH_2Ph\}_2]$ showed the greatest in vitro cytotoxic activity being able to decrease cancer cell viability roughly 3-fold more effectively than cisplatin.



1. INTRODUCTION

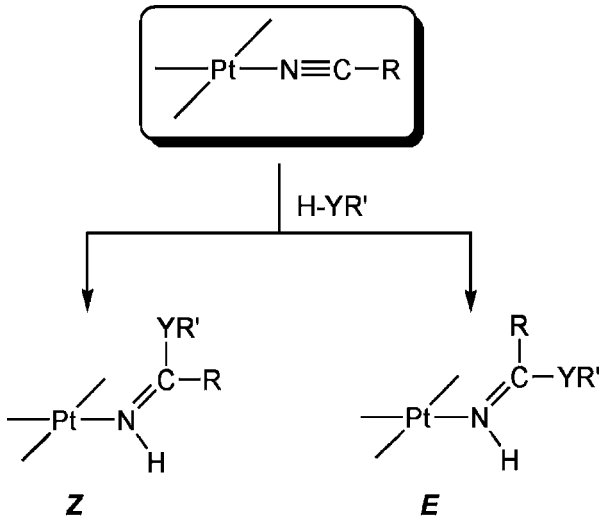
In the past ten years the addition reactions of nucleophiles and electrophiles to organonitrile ligands coordinated to electron-withdrawing transition metal ions have experienced a rapid growth.[1] The RCN metal-promoted and/or catalytic conversion into other organic ligands as a consequence of chemical processes such as insertion, coupling, and nucleophilic or electrophilic attack has been reviewed in a number of articles[2] and is of current interest in view of its importance in synthetic chemistry[3] and catalysis,[2a] as well as for its biological implications. Interestingly, a class of relevant biologically active Pt(II)-based drugs has been prepared from the synthetically useful organonitrile Pt(II) complexes *cis*- and *trans*-$[PtCl_2(NCR)_2]$ (R = Me, Ph) by taking advantage of their ability to undergo nucleophilic addition of alcohols[4] and amines[5] at the C≡N triple bond affording iminoether[6] and amidine[7] derivatives, respectively (Scheme 1). The Pt(II)-mediated alcohol-nitrile[4] and amine-nitrile[7] coupling reactions have been broadly explored and established using nitrile complexes of the type *cis*- and *trans*-$[PtCl_2(NCR)_2]$ (R = alkyl, aryl). The configuration of the reaction products can be either *Z* or *E* corresponding to the *trans* or *cis* addition of the protic nucleophile along the C≡N triple bond, respectively.

Most of the biological studies reported by Coluccia and Natile have been concerned with the *trans*-configured iminoether Pt(II) species.[8] On the other hand, some of us previously reported that also the *cis*-configured benzylimiinoether complex *cis*-$[PtCl_2\{E\text{-}N(H)=C(OMe)CH_2Ph\}_2]$ possesses a significant in vitro and in vivo cytotoxic activity.[6e] Recently, we have also reported that Pt(II) amidine complexes of the type *cis*- and *trans*-$[PtCl_2\{amidine\}_2]$, derived by the addition of primary and secondary amines to the dibenzonitrile complex *cis*- and *trans*-$[PtCl_2(N{\equiv}CPh)_2]$, were endowed with significant antitumor activity.[7e] It is worthwhile noting that, among this type of complexes, the benzamidine Pt(II) species *trans*-$[PtCl_2\{N(H)=C(NMe_2)Ph\}_2]$ appeared as the most effective derivative in the biological assays. It has been proposed that amidine ligands behave as carrier ligands which are retained within the cell, in agreement with the superbasic properties recently stated.[9] In general, *trans* platinum

Received: November 8, 2012
Published: May 6, 2013

   dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

Scheme 1. Configuration of Iminoether and Amidine Complexes



complexes are attracting great attention[10] because some classes of compounds (mononuclear, polynuclear,[11] Pt(IV) derivatives[12]) showed a better cytotoxic activity and ability to overcome cisplatin resistance due to their distinct cellular pharmacological properties with respect to cisplatin based also on different interactions with DNA.[13] It seems that trans platinum compounds form interstrand cross-links stabilizing DNA double helix, whereas cisplatin has an intrastrand cross-link pattern.[14] Detailed studies concerning the speciation of *trans* complexes in water solution, the influence of the spectator ligands, and the role of the leaving group have been carried out by the Navarro-Ranninger's research team in particular for *trans* complexes bearing aliphatic amines[15] and by Farrell for mononuclear *trans* platinum complexes with a heterocyclic planar system in the coordination sphere.[16] Quite recently it was shown that irradiation at 365 nm can be used to activate trans-Pt(IV) diamine complexes improving the selective interaction with biomolecules.[17]

Expanding the investigation of new ligands and/or new complexes having the ability to selectively impact the life cycle of cancerous cells remains an important goal of the bioinorganic chemistry of anticancer metallodrugs. Consequently, it is not surprising that a wide range of ligands and metal complexes have been designed and are currently under investigation.[18]

Platinum is known to have a large affinity for intracellular sulfur nucleophiles[19] and some mechanisms of platinum drug resistance are associated with drug inactivation by sulfur molecules.[20] Thiols and thioethers such as cysteine, glutathione, and methionine decrease DNA platination.[21] Glutathione and metallothionein are overproduced in cell lines resistant to cisplatin.[22] While the fixation of thiols on platinum is irreversible, thioether adducts can be displaced by thiourea and N7 atom of guanine moieties.[23] This reversibility is considered to be involved in cisplatin induced nephrotoxicity,[24] but the formation of adducts with the methionine residues of proteins might act as a reservoir for the drug[21d,25] and/or activate it.[26] Glutathione, thiosulfate, D-methionine, diethyldithiocarbamate (DDTC), aminothiazole,[27] and N,S-chelated ligands[28] have been studied and tested to reduce the nephro- and ototoxicity of cisplatin.[29] It was recently demonstrated that the DNA platination rate of a trans antitumor drug was dramatically enhanced by thioether binding and a detailed study carried out on the interaction between *trans*-$PtCl_2$(*E*-iminoether)$_2$ with thioether indicated that coordination to DNA of platinum-protein adducts was highly feasible for trans-configuration platinum complexes.[30]

In this context, owing to our interest in the investigation of reactivity of Pt(II) coordinated organonitriles and in the design of novel biologically active metal drugs, we have focused our attention on the utilization of S-protic nucleophiles such as thiols in the metal-promoted addition reactions to coordinated nitrile ligands. In the present article, we wish to report preliminary results on the synthesis and characterization of the novel iminothioether Pt(II) complexes some of which display high levels of cytotoxic activity against both cisplatin sensitive and resistant cell lines.

It is noteworthy that, to our knowledge, only one example has been reported of metal-promoted addition reactions of S-protic nucleophiles such as thiols and thiophenols to the coordinated CN group in the cationic cyanobenzyl *cis*-[Pt-(*o*-$CH_2C_6H_4CN$)$(PPh_3)_2]_2(BF_4)_2$ complex to afford the corresponding imino thioether derivatives.[31]

2. EXPERIMENTAL SECTION

2.1. Materials and Methods. Reagents were obtained from commercial sources and used as supplied. All solvents were reagent grade and were distilled prior to use. Deuterated solvents were purchased from Cambridge Isotope Laboratories (CIL) and stored under molecular sieves. The infrared spectra were taken on a Perkin-Elmer Spectrum 100 FT IR Spectrophotometer (CsI films); the frequencies are given in cm^{-1}. ^1H, ^{13}C, and ^{15}N NMR solution spectra were obtained at 298 K (unless otherwise stated) on a Bruker Avance-400 spectrometer (9.4 T field) operating at 400.13, 100.61, and 40.56 MHz, respectively, and using a Bruker AvanceIII-200 spectrometer operating at 200.12 and 50.32 MHz for ^1H and ^{13}C, respectively; δ values (parts per million, ppm) are relative to Me_4Si for ^1H and ^{13}C. Suitable integral values for the proton spectra were obtained with a prescan delay of 10 s. The assignments of the proton resonances were performed by standard chemical shift correlations, as well as by COSY, TOCSY, and NOESY experiments. In the phase-sensitive NOESY measurements the presence of intense cross-peaks, in phase with the diagonal, indicates a chemical exchange between the correlated nuclei (EXSY).[32] The ^{13}C resonances were attributed through 2D-heterocorrelated COSY experiments: heteronuclear multiple quantum correlation (HMQC) with bilinear rotation decoupling[33] and quadrature along F1 achieved using the time proportional phase increment method[34] for the hydrogen-bonded carbon atoms, heteronuclear multiple bond correlation (HMBC)[35] for the quaternary ones. The purity of compounds **1**−**7** was stated to be higher than 98% by elemental analyses which were performed by the Microanalysis Laboratory of the Department of Chemical Sciences, University of Padova. Electrospray ionization mass spectrometry (ESI-MS) analyses were performed using a LCQ-Duo (Thermo-Finnigan, San Jose, CA, U.S.A.) operating in positive ion mode. Instrumental parameters: capillary voltage 10 V, spray voltage 4.5 kV; capillary temperature 200 °C; mass scan range from 150 to 2000 amu; N_2 was used as sheath gas; the He pressure inside the trap was kept constant. The pressure directly read by an ion gauge (in the absence of the N_2 stream) was 1.33×10^{-5} Torr. The collision-induced dissociation experiments were performed by applying a supplementary RF voltage (tickle voltage) to the end-caps of the ion trap in the range 0−80% of its maximum value (5 V peak to peak). Sample solutions were prepared by dissolving the compounds (1 mg) in CH_2Cl_2 (500 μL) and then diluted 1:1000 with CH_3CN (unless otherwise stated). Sample solutions were directly infused into the ESI source by a syringe pump at 8 μL/min flow rate. The formation of ionic species reported in the experimental section 2.2

dx.doi.org/10.1021/ic3024452 | Inorg. Chem. 2013, 52, 5729−5741

and discussed in the text was confirmed by MS/MS experiments and isotope pattern analysis.

2.2. Preparation of the Complexes. *cis-* and *trans-*$[PtCl_2(NCMe)_2]$ were synthesized as described in the literature.[36] *cis*-Complexes **1**−**4** were prepared by a similar procedure which is described for **1**, while *trans*-complexes were prepared according to the procedure described for **5**.

*2.2.1. cis-[PtCl$_2${E-N(H)=C(SEt)Me}$_2$] (**1**).* A solution of CH_3CH_2SH (8.62 mmol; ρ = 0.839 g/mL) in THF (30 mL) was treated with BuLi^n (0.17 mmol, 1.6 M) at room temperature. Then, complex *cis*-$[PtCl_2(NCMe)_2]$ (0.3 g, 0.86 mmol) was added, and the reaction mixture was stirred for 1 day at room temperature. The solution was then concentrated to a small volume (5 mL) and treated with *n*-hexane to afford a pale yellow solid. The product was collected and dried under vacuum. Yield: 73%. Anal. Calcd for $C_8H_{18}Cl_2N_2S_2Pt$ (**1**, M = 472.36): C, 20.34; H, 3.84; N, 5.93; S, 13.58. Found: C, 20.32; H, 3.90; N, 5.88; S, 13.63. IR (λ_{max}, KBr, cm^{-1}): 3456 ν_{as}(N−H), 3222 ν_s(N−H); 1594ν(C=N); 705 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 333 and 326 ν(Pt−Cl). ^{1}H NMR (200 MHz, $CDCl_3$, t = triplet, q = quadruplet, s = singlet): δ 1.25 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 3.04 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 2.76 (s, $NCCH_3$, 6H); 9.27 (s, N*H*, 2H). ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$, s = singlet): δ 13.08 (s, SCH_2CH_3); 25.51 (s, SCH_2CH_3); 27.75 (s, $NCCH_3$); 178.71 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 495 ([M + Na]$^+$, 27); 511 ([M + K]$^+$, 5); 967 ([2 M + Na]$^+$, 100); 983 ([2 M + K]$^+$, 8); 1439 ([3 M + Na]$^+$, 4).

*2.2.2. cis-[PtCl$_2${E-N(H)=C(SEt)Et}$_2$] (**2**).* Yield 76%. Anal. Calcd for $C_{10}H_{22}Cl_2N_2S_2Pt$ (**2**, M = 500.42): C, 24.00; H, 4.43; N, 5.60; S, 12.82. Found: C, 23.93; H, 4.47; N, 5.58; S, 12.80. IR (λ_{max}, KBr, cm^{-1}): 3434 ν_{as}(N−H); 3228 ν_s(N−H); 1583 ν(C=N); 678 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 337 and 326 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet): δ 1.24 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 1.41 (t, $^3J_{H-H}$ = 7.6 Hz, $NCCH_2CH_3$, 6H); 3.05 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 3.30 (q, $^3J_{H-H}$ = 7.6 Hz, $NCCH_2CH_3$, 4H); 9.15 (s, N*H*, 2H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 13.11 (s, SCH_2CH_3); 12.35 (s, $NCCH_2CH_3$); 25.04 (s, SCH_2CH_3); 35.07 (s, $NCCH_2CH_3$); 184.71 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 523 ([M + Na]$^+$, 5); 1023 ([2 M + Na]$^+$, 55); 1523 ([3 M + Na]$^+$, 15); 506 [M − Cl + CH_3CN]$^+$, 3); 1006 [$Pt_2C_{24}H_{57}N_4S_6Na$]$^+$, 100); 1506 [$Pt_3C_{34}H_{77}N_6S_8Na$]$^+$, 45).

*2.2.3. cis-[PtCl$_2${E-N(H)=C(SEt)CH$_2$Ph}$_2$] (**3**).* Yield 77%. Anal. Calcd for $C_{20}H_{26}Cl_2N_2S_2Pt$ (**3**, M = 624.56): C, 38.46; H, 4.20; N, 4.49; S, 10.27. Found: C, 38.38; H, 4.18; N, 4.42; S, 10.19. IR (λ_{max}, KBr, cm^{-1}): 3436 ν_{as}(N−H); 3239 ν_s(N−H); 1568 ν(C=N); 703 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 326 and 319 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet, m = multiplet): δ 1.24 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 3.03 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 4.45 (s, CH_2Ph, 4H); 9.38 (s, N*H*, 2H), 7.26−7.30 (m, Ph, 10H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 13.02 (s, SCH_2CH_3); 25.83 (s, SCH_2CH_3); 47.11 (s, CH_2Ph,); 127.89 (s, Ph, *o*-C); 128.93 (s, Ph, *m*-C); 130.12 (s, Ph, *p*-C); 134.56 (s, Ph,C_j); 183.02 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 647 ([M + Na]$^+$, 51); 1271([2 M + Na]$^+$, 100); 1895 ([3 M + Na]$^+$, 20); 1208 (2 M + Na − CH_3CH_2SH]$^+$, 20).

*2.2.4. cis-[PtCl$_2${E-N(H)=C(SEt)Ph}$_2$] (**4**).* Yield 77%. Anal. Calcd $C_{18}H_{22}Cl_2N_2S_2Pt$ (**4**, M = 596.50): C, 36.24; H, 3.72; N, 4.70; S, 10.75. Found: C, 36.28; H, 3.75; N, 4.68; S, 10.72. IR (λ_{max}, KBr, cm^{-1}): 3467 ν_{as}(N−H); 3253 ν_s(N−H); 1583 ν(C=N); 714 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 344 and 325 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet, m = multiplet): δ 1.22 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 2.53 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 8.12 (s, N*H*, 2H), 7.45−7.69 (m, Ph, 6H); 7.99−8.04 (m, Ph, 4H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 12.50 (s, SCH_2CH_3); 28.40 (s, SCH_2CH_3); 128.21 (s, Ph, *o*-C); 128.79 (s, Ph, *m*-C); 132.28 (s, Ph, *p*-C); 136.79 (s, Ph,C_j); 178.56 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 619 ([M + Na]$^+$, 100); 635 ([M + K]$^+$, 28); 1215 ([2 M + Na]$^+$, 80); 1231 ([2 M + K]$^+$, 30); 561 ([M − Cl]$^+$, 50); 525 ([M − Cl − HCl]$^+$, 85).

*2.2.5. trans-[PtCl$_2${E-N(H)=C(SEt)Me}$_2$] (**5**).* A solution of CH_3CH_2SH (8.62 mmol; ρ = 0.839 g/mL) in THF (30 mL) was treated with BuLi^n (0.17 mmol, 1.6 M) at −10 °C. Then, complex *trans*-$[PtCl_2(NCMe)_2]$ (0.3 g, 0.86 mmol) was added, and the reaction mixture was stirred for 1 h at low temperature (−10 °C) and then reacted at room temperature for additional 2 h. The solution was then concentrated to a small volume (5 mL) and treated with *n*-hexane to afford a pale yellow solid. The product was collected and dried under vacuum. Yield 70%. Anal. Calcd for $C_8H_{18}Cl_2N_2S_2Pt$ (**5**, M = 472.36): C, 20.34; H, 3.84; N, 5.93; S, 13.58. Found: C, 20.30; H, 3.88; N, 5.90; S, 13.55. IR (λ_{max}, KBr, cm^{-1}): 3399 ν_{as}(N−H), 3196 ν_s(N−H); 1594ν(C=N); 702 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 344 ν(Pt−Cl). ^{1}H NMR (200 MHz, $CDCl_3$, t = triplet, q = quadruplet, s = singlet): δ 1.29 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 3.00 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 2.89 (s, $NCCH_3$, 6H); 9.14 (s, N*H*, 2H). ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$, s = singlet): δ 12.86 (s, SCH_2CH_3); 25.02 (s, SCH_2CH_3); 27.03 (s, $NCCH_3$); 178.03 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 967 ([2 M + Na]$^+$, 20); 1439 ([3 M + Na]$^+$, 25); 950 [$Pt_2C_{20}H_{48}N_4S_6Na$]$^+$, 100); 966 [$Pt_2C_{20}H_{48}N_4S_6K$]$^+$, 25); 401 [$PtC_8H_{19}N_2S_2$]$^+$, 17); 934 [$Pt_2C_{19}H_{44}N_4S_6Na$]$^+$, 40).

*2.2.6. trans-[PtCl$_2${E-N(H)=C(SEt)Et}$_2$] (**6**).* Yield 80%. Anal. Calcd for $C_{10}H_{22}Cl_2N_2S_2Pt$ (**6**, M = 500.42): C, 24.00; H, 4.43; N, 5.60; S, 12.82. Found: C, 23.93; H, 4.47; N, 5.58; S, 12.80. IR (λ_{max}, KBr, cm^{-1}): 3433 ν_{as}(N−H); 3257 ν_s(N−H); 1601 ν(C=N); 702 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 338 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet): δ 1.28 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 1.45 (t, $^3J_{H-H}$ = 7.6 Hz, $NCCH_2CH_3$, 6H); 2.71 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 3.36 (q, $^3J_{H-H}$ = 7.6 Hz, $NCCH_2CH_3$, 4H); 9.02 (s, N*H*, 2H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 12.76 (s, SCH_2CH_3); 11.70 (s, $NCCH_2CH_3$); 23.90 (s, SCH_2CH_3); 33.75 (s, $NCCH_2CH_3$); 182.22 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 1006 [$Pt_2C_{24}H_{57}N_4S_6Na$]$^+$, 100); 1506 [$Pt_3C_{34}H_{77}N_6S_8Na$]$^+$, 45).

*2.2.7. trans-[PtCl$_2${E-N(H)=C(SEt)CH$_2$Ph}$_2$] (**7**).* Yield 76%. Anal. Calcd for $C_{20}H_{26}Cl_2N_2S_2Pt$ (**7**, M = 624.56): C, 38.46; H, 4.20; N, 4.49; S, 10.27. Found: C, 38.48; H, 4.22; N, 4.45; S, 10.20. IR (λ_{max}, KBr, cm^{-1}): 3447 ν_{as}(N−H); 3246 ν_s(N−H); 1589 ν(C=N); 707 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 337 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet, m = multiplet): δ 1.24 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 2.99 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 4.88 (s, CH_2Ph, 4H); 9.48 (s, N*H*, 2H); 7.62 (m, Ph, *o*-H, 4H); 7.32 (m, Ph, *m*-H, 4H); 7.28 (m, Ph, *p*-H, 2H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 12.77 (s, SCH_2CH_3); 24.97 (s, SCH_2CH_3); 46.61 (s, CH_2Ph,); 129.80 (s, Ph, *o*-C); 128.48 (s, Ph, *m*-C); 127.34 (s, Ph, *p*-C); 134.91 (s, Ph,C_j); 181.23 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 647 ([M + Na]$^+$, 20); 589 ([M − Cl]$^+$, 30); 552 ([M − HCl − Cl]$^+$, 35); 630 ([M − Cl + CH_3CN]$^+$, 12); 1255 ([$Pt_2C_{42}H_{54}Cl_2N_6S_4Na$]$^+$, 35); 1271 ([$Pt_2C_{42}H_{54}Cl_2N_6S_4K$]$^+$, 15); 1313 ([$C_{42}H_{54}Cl_3N_6S_4Pt_2Na_2$]$^+$, 10); 1879 ([$Pt_3C_{62}H_{79}Cl_4N_8S_4Na$]$^+$, 70); 1895 ([$Pt_3C_{62}H_{79}Cl_4N_8S_4K$]$^+$, 20). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%), 300 μM in DMF/water physiological solution 1/1): 647 ([M + Na]$^+$, 45); 589 ([M − Cl]$^+$, 50); 552 ([M − HCl − Cl]$^+$, 100); 1319 ([2M − Cl + DMF]$^+$, 30).

*2.2.8. trans-[PtCl$_2${E-N(H)=C(SEt)Ph}$_2$] (**8**).* Yield 30%. Anal. Calcd $C_{18}H_{22}Cl_2N_2S_2Pt$ (**8**, M = 596.50): C, 36.24; H, 3.72; N, 4.70; S, 10.75. Found: C, 36.35; H, 3.79; N, 4.61; S, 10.68. IR (λ_{max}, KBr, cm^{-1}): 3399 ν_{as}(N−H); 1586 ν(C=N); 695 ν(C−S). IR (λ_{max}, PE, cm^{-1}): 317 ν(Pt−Cl). ^{1}H NMR (200 MHz, CD_2Cl_2, t = triplet, q = quadruplet, s = singlet, m = multiplet): δ 1.20 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 6H); 2.97 (q, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3, 4H); 9.67 (s, N*H*, 2H), 7.51−7.73 (m, Ph, 6H); 8.06−8.08 (m, Ph, 4H). ^{13}C {^{1}H} NMR (50 MHz, CD_2Cl_2, s = singlet): δ 12.96 (s, SCH_2CH_3); 28.42 (s, SCH_2CH_3); 128.29 (s, Ph, *o*-C); 128.97 (s, Ph, *m*-C); 129.39 (s, Ph, *p*-C); 135.22 (s, Ph,C_j); 187.38 (s, *C*=N). ESI-MS (fragments were based on ^{195}Pt; m/z (rel.ab.%)): 619 ([M + Na]$^+$, 15); 1215 ([2 M + Na]$^+$, 100); 1811 ([3 M + Na]$^+$, 35); 523 [M − H − 2HCl]$^+$, 65); 561 [M -Cl]$^+$, 25); 1199 [2 M + Na − CH_4]$^+$, 70); 1795 [3 M + Na − CH_4]$^+$, 40); 1773 [3 M − CH_3]$^+$, 15).

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

The reaction of *trans*-$[PtCl_2(NCPh)_2]$ with CH_3CH_2SH gave rise to a mixture of *E,E* (**8**), *E,Z* and *Z,Z* atropisomers, as previously observed in the case of the reaction of *trans*-$[PtCl_2(NCPh)_2]$ with $MeNH_2$ and Me_2NH,[5b] together with a small amount (ca.10%) of a cationic species. The NMR spectra of the mixture obtained by the reaction of *trans*-$[PtCl_2(NCPh)_2]$ with CH_3CH_2SH showed the presence of *trans*-$[PtCl_2\{EZ\text{-}N(H)=C(SEt)Ph\}_2]$ (ca. 30%) [*Z system*: ^1H, δ 1.36 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3); 3.50 (q, SCH_2CH_3); 9.57 (s, N*H*, NOE correlation with phenyl protons), 8.12 (*o*-Ph); 7.45 (*m*-Ph); 7.74 (p-Ph); ^{13}C $\{^1H\}$: δ 12.33 (SCH_2CH_3); 28.36 (SCH_2CH_3); 128.87 (*o*-C); 128.01 (*m*-C); 131.02 (*p*-C); 186.70 (*C*=N); *E system*: ^1H, δ 1.34 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3); 3.10 (q, SCH_2CH_3); 9.32 (s, N*H*, NOE correlation with SCH_2 protons); ^{13}C $\{^1H\}$: δ 12.70 (SCH_2CH_3); 26.77 (SCH_2CH_3); 178.90(C=N)] and of *trans*-$[PtCl_2\{ZZ\text{-}N(H)=C(SEt)Ph\}_2]$ (ca. 30%) [^1H, δ 1.41 (t, $^3J_{H-H}$ = 7.4 Hz, SCH_2CH_3); 3.16 (q, SCH_2CH_3); 9.38 (s, N*H*, NOE correlation with SCH_2 protons), 8.38 (*o*-Ph); 7.59 (*m*-Ph); 7.67 (p-Ph); ^{13}C $\{^1H\}$: δ 12.74 (SCH_2CH_3); 26.08 (SCH_2CH_3); 129.01 (*o*-C); 128.00 (*m*-C); 132.00 (*p*-C); 178.70 (C=N)]. A cationic species originated by the substitution of a chlorine atom with CH_3CH_2SH was also observed. The presence of broad signals of the thioethereal SCH_2CH_3 moiety at δ 1.52 (δ ^{13}C 12.55) and 3.50 (δ ^{13}C 28.27) and of the entering CH_3CH_2SH system at δ 1.75 and 2.51 (δ ^{13}C 27.4) indicated in solution the occurrence of a slow exchange (on the NMR time scale) within the coordination sphere of the cationic species $[PtCl_2\{N(H)=C(SEt)Ph\}(HSCH_2CH_3]Cl$ (δ ^{13}C 187.4, C=N).

2.3. X-ray Structure Determinations. White crystals of **1**, **2**, and **7** were obtained by slow diffusion at −4 °C of diethyl ether into solutions of the complexes in CH_2Cl_2. Crystals were lodged in a Lindemann glass capillary and centered on a four circle Philips PW1100 diffractometer using graphite monochromated Mo*Kα* radiation (0.71073 Å), following the standard procedures at room temperature. All intensities were corrected for Lorentz polarization and absorption.[37] The structures were solved by standard direct methods.[38] Refinement was carried out by full-matrix least-squares procedures (based on F_o^2) using anisotropic temperature factors for all non-hydrogen atoms. Hydrogen atoms were placed in calculated position with isotropic thermal parameters (1.2 U_{equiv} of the parent carbon atom). Structure refinement and final geometrical calculations were carried out with the SHELXL-97[39] program, implemented in the WinGX package.[40] Crystallographic data and selected bond lengths and angles have been reported in Table 1 and 2, respectively.

2.4. Biological Assays. Platinum(II) imino thioether derivatives were dissolved in dimethylformamide (DMF) just before the experiment and a calculated amount of drug solution was added to the growth medium containing cells to a final solvent concentration of 0.15% which had no discernible effect on cell killing. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), cisplatin, transplatin, and doxorubicin were obtained from Sigma Chemical Co, St.Louis, U.S.A.

2.4.1. Cell Cultures. Human breast (MCF-7) and lung (A549) carcinoma along with melanoma (A375) cell lines were obtained by ATCC, Rockville, MD. 2008 and its cisplatin resistant variant, C13*, are human ovarian cancer cell lines, and they were kindly provided by Prof. G. Marverti (Dept. of Biomedical Science of Modena University, Italy). A431 and A431/Pt are sensible and resistant human cervical carcinoma cells, respectively, kindly provided by Prof. F. Zunino (Division of Experimental Oncology B, Istituto Nazionale dei Tumori, Milan, Italy). LoVo human colon-carcinoma cell line and its derivative multidrug-resistant subline (LoVo MDR) were kindly provided by Prof. F. Majone (Dept. of Biology of Padova University, Italy). Cell lines were maintained in the logarithmic phase at 37 °C in a 5% carbon dioxide atmosphere using the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units·mL^{-1} penicillin and 50 μg·mL^{-1} streptomycin) and 2 mM L-glutamine: (*i*) RPMI-1640 medium (Euroclone) for MCF-7, 2008, C13*, A431 and A431/Pt; (*ii*) F-12 HAM'S (Sigma Chemical Co) for A549, LoVo and LoVo MDR cells (LoVo MDR culture medium also contained 0.1 μg·mL^{-1} doxorubicin); (*iii*) D-MEM medium (Euroclone) for A375 cells.

2.4.2. Cytotoxicity Assay. The growth inhibitory effect toward tumor cell lines was evaluated by means of MTT (tetrazolium salt

Table 1. Crystallographic Data for 1, 2, and 7

	1	2	7
empirical formula	$C_8H_{18}N_2Cl_2S_2Pt$	$C_{10}H_{22}N_2Cl_2S_2Pt$	$C_{20}H_{26}N_2Cl_2S_2Pt$
fw	472.36	500.41	624. 54
T, K	293(2)	293(2)	293(2)
λ (Å)	0.71073	0.71073	0.71073
crystal-system	triclinic	monoclinic	triclinic
space-group	$P\bar{1}$	$P21/n$	$P\bar{1}$
a (Å)	8.797(2)	12.989(3)	13.337(3)
b (Å)	9.277(2)	14.486(3)	14.836(3)
c (Å)	10.069(2)	18.594(3)	13.275(3)
α (deg)	106.68(2)		94.14(2)
β (deg)	104.19(2)	94.17(3)	109.02(3)
γ (deg)	93.56(2)		104.16(3)
V (Å^3)	755.3(3)	3489(1)	2374.5(9)
Z	2	8	4
ρ_{calc}, g-cm^{-3}	2.077	1.905	1.747
μ(Mo−K*α*), mm^{-1}	9.893	8.572	6.038
$R(F^2)^a$	0.025	0.063	0.056
$Rw(F^2)^b$	0.061	0.063	0.129
goodness of fit	1.202	1.352	1.330
F(000)	448	1920	1216
θ range/deg	3−27	3−25	3−25
no. reflections collected	3729	6695	8719
no. observed [$I \geq 2\sigma(I)$]	3500	5238	7285

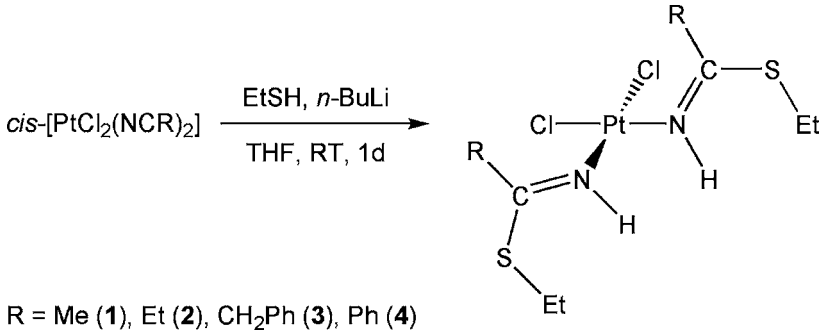
$^aR = \sum||F_o| - |F_c||/\sum|F_o|$. $^bR_w=[\sum\{w(|F_o|^2 - |F_c|^2)^2\}/\sum\{w(|F_o|^2)^2\}]^{1/2}$.

Table 2. Selected Bond Lenghts (Å) and Angles (deg) in the Coordination Metal Sphere for *cis*-$[PtCl_2\{E\text{-}N(H)=C(SEt)Me\}_2]$ (1), *cis*-$[PtCl_2\{E\text{-}N(H)=C(SEt)Et\}_2]$ (2), and *trans*-$[PtCl_2\{E\text{-}N(H)=C(SEt)CH_2Ph\}_2]$ (7)

	1	2	7
Pt(1)−Cl(1)	2.309(2)	2.302(4)	2.304(3)
Pt(1)−Cl(2)	2.307(2)	2.314(5)	2.305(3)
Pt(2)−Cl(3)		2.314(4)	2.301(3)
Pt(2)−Cl(4)		2.307(4)	2.306(3)
Pt(1)−N(1)	2.017(5)	2.000(12)	2.018(10)
Pt(1)−N(2)	2.010(4)	2.020(12)	2.006(10)
Pt(2)−N(3)		2.017(99)	2.025(9)
Pt(2)−N(4)		1.997(12)	2.019(8)
Cl(1)−Pt(1)−Cl(2)	91.3(1)	90.0(2)	179.7(1)
Cl(1)−Pt(1)−N(1)	177.3(1)	177.1(2)	87.0(3)
Cl(1)−Pt(1)−N(2)	90.1(1)	92.4(4)	93.0(3)
N(1)−Pt(1)−Cl(2)	91.1(1)	89.2(4)	93.1(3)
N(1)−Pt(1)−N(2)	87.6(2)	88.4(5)	177.7(4)
N(2)−Pt(1)−Cl(2)	177.9(1)	177.5(4)	86.8(3)
Cl(3)−Pt(2)−Cl(4)		91.5(2)	177.8(1)
Cl(3−Pt(2)−N(3)		89.5(4)	88.9(3)
Cl(3)−Pt(2)−N(4)		177.1(4)	90.8(3)
N(3)−Pt(2)−Cl(4)		178.9(4)	90.5(3)
N(3)−Pt(2)−N(4)		88.6(5)	176.5(4)
N(4)−Pt(2)−Cl(4)		90.4(4)	90.0(3)

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

Scheme 2. Synthesis of the Imino Thioether Complexes *cis*-[$PtCl_2${*E*-N(H)=C(SEt)R}$_2$]



reduction) assay.[41] Briefly, between 3 and 8 × 10^3 cells, dependent upon the growth characteristics of the cell line, were seeded in 96-well microplates in growth medium (100 μL) and then incubated at 37 °C in a 5% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with fresh media containing the compound to be studied at the appropriate concentration. Triplicate cultures were established for each treatment. After 48 h, each well was treated with 10 μL of a 5 mg·mL^{-1} MTT saline solution, and after 5 h of incubation, 100 μL of a sodium dodecylsulfate (SDS) solution in HCl (0.01 M) was added. After overnight incubation in the dark at 37 °C in a 5% carbon dioxide atmosphere, the inhibition of cell growth induced by tested compounds was detected by measuring the absorbance of each well at 570 nm using a Bio-Rad 680 microplate reader (Milan, Italy). Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted vs drug concentration. IC_{50} values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.

2.4.3. Cellular Uptake. 2008 and C13* cells (2 × 10^6) were seeded in 75 cm^2 flasks in growth medium (20 mL). After 24 h, the medium was replaced, and the cells were incubated for different times (6, 12, and 24 h) with IC_{50} doses of the tested complexes. Cell monolayers were washed twice with cold PBS and harvested. Samples were subjected to three freezing/thawing cycles at −80 °C and then vigorously vortexed. Aliquots were removed for the determination of protein content by the BioRad protein assay (BioRad). The samples were treated with 1 mL of highly pure nitric acid ([Pt] ≤ 0.01 ng kg^{-1}, Trace SELECT Ultra, Sigma Chemical Co.) and transferred into a microwave Teflon vessel. Subsequently, samples were submitted to the standard procedure using a speed wave MWS-3 Berghof instrument (Eningen, Germany). After cooling, each mineralized sample was analyzed for platinum by using a Varian AA Duo graphite furnace atomic absorption spectrometer (Varian, Palo Alto, CA) at a wavelength of 324.7 nm. The calibration curve was obtained using known concentrations of standard solutions purchased from Sigma Chemical Co.

2.4.4. Caspase-3 Activity. Caspase-3 activity was detected with the ApoAlert Caspase-3 Fluorescent Assay Kit (Clontech, Mountain View, CA) according to the producer's recommended procedures. 1 × 10^6 2008 cells were collected following 12 or 24 h of incubation of tested compounds (at concentrations corresponding to IC_{50} values) and lysed on ice in 50 μL of lysis buffer for 10 min. Subsequently, cell lysates were then treated with 50 μL of reaction buffer containing dithiothreitol (DTT) and 5 μL of caspase-3 substrate solution (Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl-coumarin [DEVD-AFC], Clontech). Fluorescence was determined on a Perkin-Elmer 550 spectrofluorometer (excitation 440 nm, emission 505 nm). Caspase-3 activity was expressed as the increase in AFC-emitted fluorescence.

2.5. Statistical Analysis. All the values are the means ± SD of not less than five measurements. Multiple comparisons were made by ANOVA followed by Tukey−Kramer multiple comparison test (**P < 0.01; *P < 0.05), using GraphPad Software.

3. RESULTS AND DISCUSSION

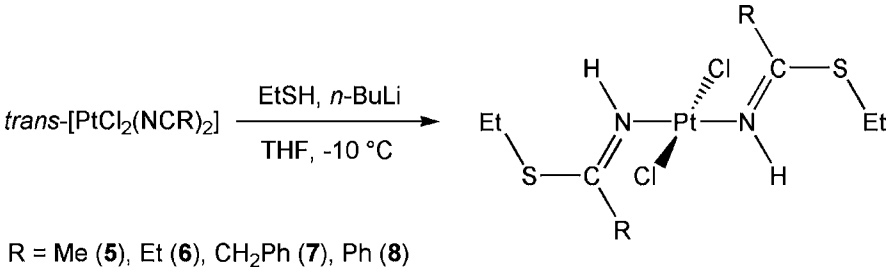
3.1. Synthesis and Characterization. By reaction of the dinitrile complexes *cis*-[$PtCl_2(NCR)_2$] (R = Me, Et, CH_2Ph, Ph) in THF with a 10-fold excess of EtSH in the presence of of $LiBu^n$ at room temperature for 24 h, the imino thioether derivatives *cis*-[$PtCl_2${*E*-N(H)=C(SEt)R}$_2$] (R = Me (**1**), Et (**2**), CH_2Ph (**3**), Ph (**4**)) have been prepared in good yields, where both the imino thioether ligands had the *E* configuration (Scheme 2) as can be readily determined on the basis of NMR data and also observed in the X-ray structures carried out for **1** and **2** (see below).

In fact, the ^{1}H NMR spectra showed a downfield shift of the chemical shifts of the protons of the R groups being closer to platinum, while this did not occur when they are far from the metal center as in the *Z* configuration. This feature had been already reported in the study of complexes of similar structure such as iminoether *cis*- and *trans*-[$PtCl_2${*E*-N(H)=C(OMe)-R}$_2$][7d] (R = Me, Ph, CH_2Ph) and amidine *cis*- and *trans*-[$PtCl_2${*E*-N(H)=C(NMe_2)R}$_2$][7c] (R = Me, Ph, CH_2Ph) derivatives. Thus, the chemical shifts of the methyl and methylene R protons of complexes **1**−**3** were observed at δ 2.76, 3.30, and 4.45, respectively. On the other hand, the $-SCH_2$ protons of the thiolate group were found in the range 3.03−2.53 ppm, the lowest value being observed for **4**. From a synthetic point of view, there was no evidence of formation of *cis* derivatives with a *Z* arrangement of the imino thioether ligand, even carrying out the reactions at room temperature in shorter reaction times (30 min), which, as proposed for the corresponding reactions of alcohols and amines, should favor the formation of this type of configuration.[4b] Complexes **1**−**4** were characterized by microanalysis, IR, ^{1}H and ^{13}C NMR spectroscopies. The IR spectra showed the N−H stretching bands in the range 3222−3467 cm^{-1}, while the C=N stretching appeared in the range 1568−1594 cm^{-1} at lower values compared to those found for amidine (1609−1628 cm^{-1}) and iminoether (1630−1660 cm^{-1}) complexes.[6e,7b] Two bands typical of the *cis* geometry were observed for the Pt−Cl absorptions in the expected range of 319−337 cm^{-1}.[36] The ^{1}H NMR spectra exhibited the N−H signal as a broad singlet in the range δ 8.12−9.38. NOESY experiments confirmed the *E* conformation of the complexes showing correlations between the N*H* and SCH_2CH_3 signals. In the ^{13}C NMR spectra, the resonances of the *C*=N carbons fell in the range δ 178−184 as expected for Pt(II) imino derivatives and downfield shifted with respect to iminoether (170−176 ppm)[4,6] and amidine (165−169 ppm)[6e] ligands. The resonances of the $-SCH_2$ methylene carbons were observed in the range δ 25−28.

The imino thioether derivatives *trans*-[$PtCl_2${*E*-N(H)=C(SEt)R}$_2$] (R = Me (**5**), Et (**6**), CH_2Ph (**7**), Ph (**8**)) were

dx.doi.org/10.1021/ic3024452 | Inorg. Chem. 2013, 52, 5729−5741

Scheme 3. Synthesis of the Imino Thioether Complexes *trans*-[$PtCl_2${*E*-N(H)=C(SEt)R}$_2$]



prepared by reaction of the corresponding complexes *trans*-[$PtCl_2(NCR)_2$] in THF with a 10-fold excess of EtSH in the presence of LiBu^n at low temperature for 1 h and then reacted at room temperature for additional 2 h (see Scheme 3). Complexes **5**–**8** contained both imino thioether moieties in *E* configuration.

The chemical shifts of the methyl and methylene R protons of complexes **5**–**7** were observed at δ 2.89, 3.36, and 4.88, respectively. On the other hand, the $-SCH_2$ protons of the thiolate group were found in the range 2.71–3.00 ppm. Complexes **5**–**8** were characterized by microanalysis, IR, ^{1}H and ^{13}C NMR spectroscopies. The IR spectra showed the N–H stretching bands in the range 3196–3447 cm^{-1}, while the C=N stretching appeared in the range 1586–1601 cm^{-1} at lower values compared to those found for amidine and iminoether complexes.[6e,7b] One band typical of the *trans* geometry was observed for the Pt–Cl absorptions in the expected range of 317–344 cm^{-1}. The ^{1}H NMR spectra exhibited the N–H signal as a broad singlet in the range δ 9.02–9.67. NOESY experiments confirmed the *E* conformation of the complexes showing correlations between the N*H* and the SCH_2CH_3 signals. In Figure 1 the ^{1}H-^{1}H NOESY spectrum of **7** has been reported. In the ^{13}C NMR spectra, the resonances of the C=N carbons fell in the range δ 178–187 as expected for

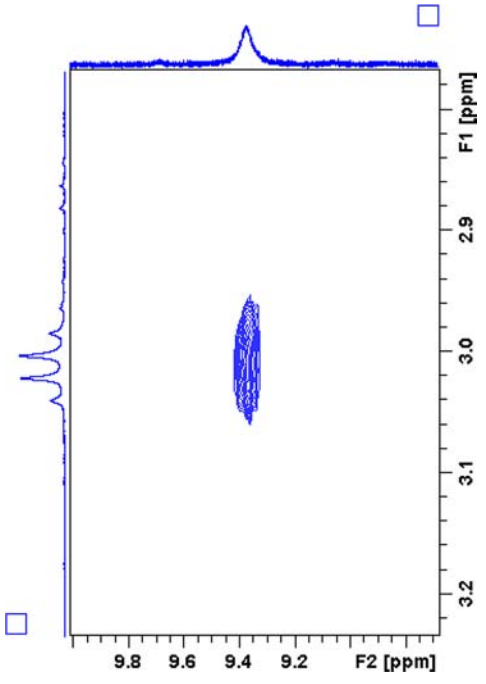


Figure 1. Portion of 400 MHz NOESY Spectrum of **7** showing the correlation peak between Pt–N*H* and $-SCH_2$- protons.

Pt(II) imino derivatives. The resonances of the $-SCH_2$ methylene carbons were observed in the range δ 24–28.

The characterization of compounds **1**–**8** included also the ESI-MS spectra. The ESI spectra of the *cis*-complexes **1**–**4** were characterized by the presence of the molecular ionic species cationized with Na^+ and K^+ together with the dimeric species $[2M+Na]^+$ which represented the base peak for **1** and **3**. Similar behavior has been previously observed for a series of platinum amidine complexes.[42] Retrosynthetic processes with loss of CH_3CH_2SH and subsequent addition in multinuclear species has been observed for **1**–**3**. In the case of **4** the loss of chlorine atoms might be favored by the formation of an orthometalated species because of the presence of the phenyl ring close to the metal center in the *E* arrangement. The most relevant feature in the ESI-MS spectra of **5**, **7**, and **8** was the presence of abundant dinuclear and trinuclear species retaining intact the imino thioether ligands. A striking feature of the iminoether complexes in the solid state, as shown by the X-ray determination reported below for **1**, **2**, and **7**, and previously observed for iminoether and amidine complexes in either *cis*- or *trans*-configuration, is that individual molecules are connected to each other through intermolecular hydrogen bonds forming dimeric or oligomeric species that could play a role in the concentration dependence of their hydrolysis rate in the biological tests.[43] This aspect has been previously investigated as for the stability of infusion solutions of carboplatin and oxaliplatin.[44] It is noteworthy that in the ESI-MS spectra of **5** dissolved in DMF (300 μM) and then diluted 1/1 with CH_3CN, H_2O, or physiological water solution, respectively (Figure 2), the base peak was at *m*/*z* 967 corresponding to the $[2M+Na]^+$ ionic species together with the $[M+Na]^+$ ionic species with increased abundance in the presence of Cl^- (15% in DMF/CH_3CN, 25% in DMF/water and 35% in DMF/physiological water solution). The presence in the ESI MS spectrum of **7** (300 μM), in DMF/physiological water solution (1/1) of the ionic species at *m*/*z* 589 corresponding to chlorine loss with retaining of the N donor ligands, was in agreement with the proposed mechanism explaining the antitumor activity of *trans*-PtL_2Cl_2 complexes and their interaction with biological substrates.[45] The ^{1}H NMR spectra, recorded in water free CD_2Cl_2 (Figure 3) confirmed the dependence on association processes from concentration showing a highfield shift of the PtNH proton (involved in N–H···Cl intermolecular bonds) from 8.93 ppm for a 18.0 mM solution to 8.08 ppm for a 0.35 mM solution. The solution association equilibria and the speciation in water solution of *trans*-iminothioether compounds to understand the nature of the platinum species present in culture media are under investigation. It is noteworthy that also interaction between DNA and dinuclear platinum species has been demonstrated.[46]

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729–5741

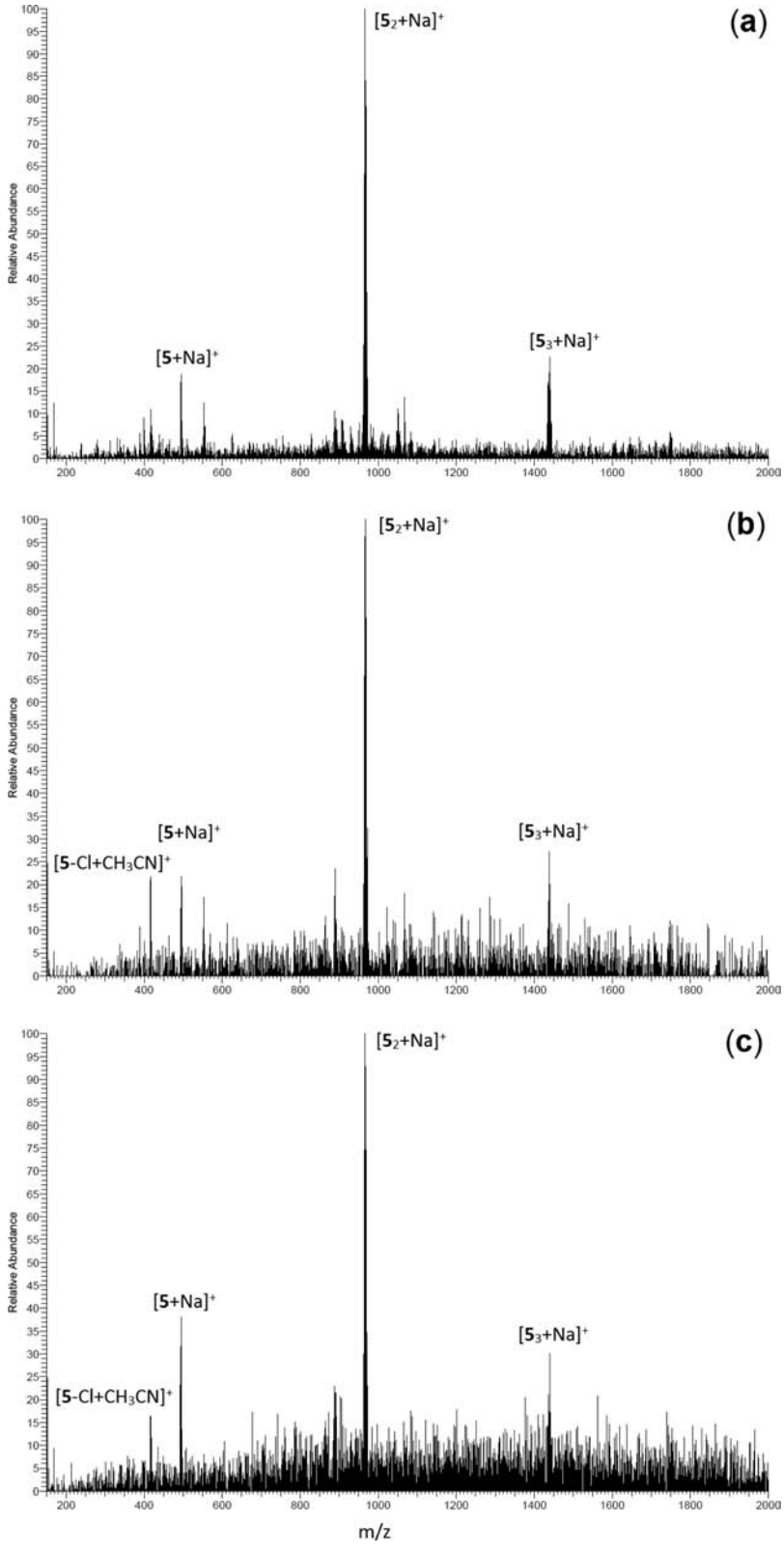


Figure 2. ESI mass spectra of **5** dissolved in DMF (300 μM) and then diluted 1/1 with CH_3CN (**a**), H_2O (**b**), or physiological water solution (**c**).

3.2. X-ray Crystal Structures. *3.2.1. X-ray Structure of Compound (1).* Compound **1** exhibits square-planar coordination geometry around the platinum center, with the chlorine ligands in *cis* positions. Four N−H···Cl intermolecular contacts (average N−H···Cl 2.665(2) Å, Table 3) between each chlorine atom and the N−H proton of the imino thioether ligand (L) shows that the complex consisted of one dimer $Pt_2Cl_4L_4$ formed by two centrosymmetrically related $PtCl_2L_2$ units (Figure 4). The Pt···Pt contact distance of 3.438(1) Å was not indicative of a metal−metal interaction (as already observed in other Pt(II) complexes[5b,7b,31] such as *cis*-[$PtCl_2${*Z*-N(H)=C(OCH_3)$CH_2C_6H_5$}$_2$],[6e] *trans*-[$PtCl_2${*E*-N(H)=C(OCH_3)-

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

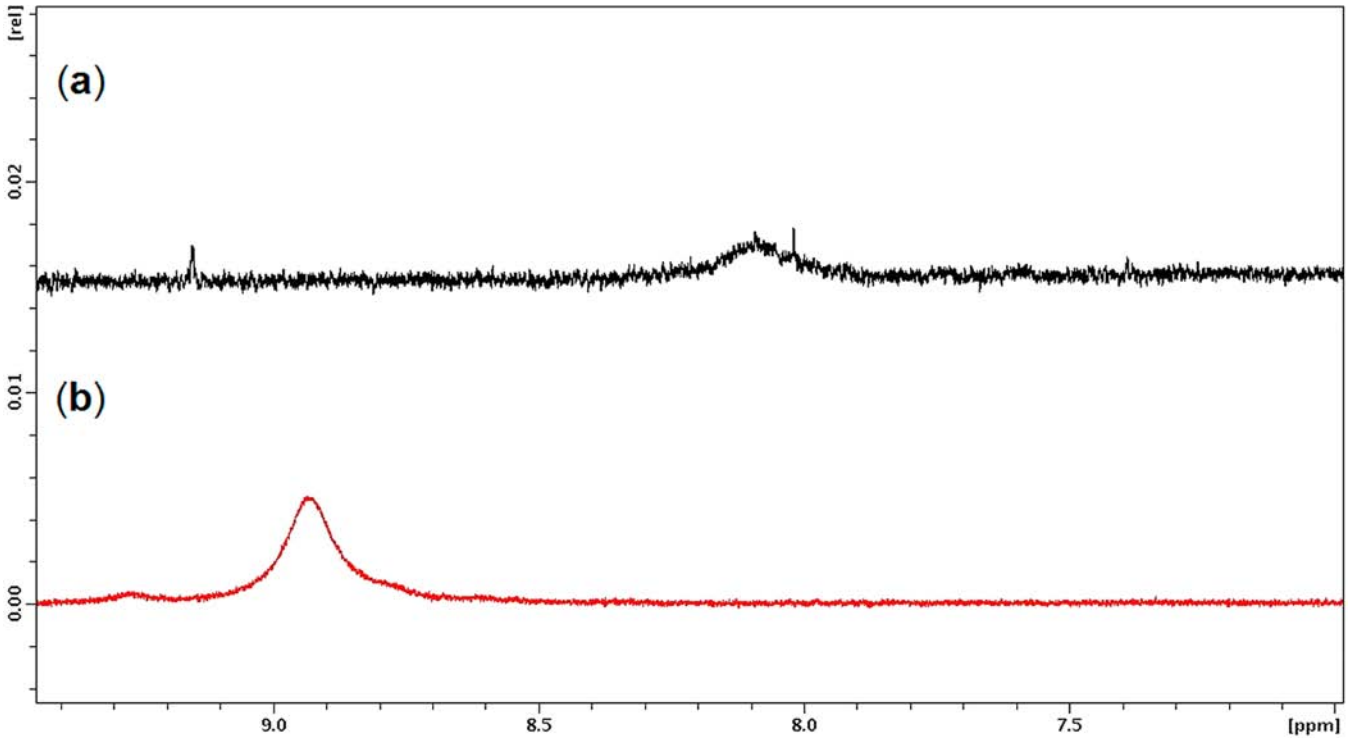


Figure 3. Portion of 400 MHz ^{1}H NMR Spectrum of **7** in CD_2Cl_2 at 0.35 mM (**a**) and 18.0 mM (**b**) concentration showing the Pt−N*H* peak.

Table 3. Intermolecular Hydrogen Bond Interactions (Å) and Angles (deg)

1 (*cis*)	**2** (*cis*)	**7** (*trans*)
Cl(1)···H−N(1)′ 2.595(2) 157.2(3)	Cl(2)···H−N(4) 2.720(2) 139.8(8)	Cl(2)···H−N(4) 2.6682) 157.8(7)
Cl(2)···H−N(2)′ 2.736(2) 154.3(3)	Cl(3)···H−N(2) 2.619(2) 155.1(8)	Cl(3)···H−N(1) 2.707(2) 147.4(8)
′ at 1−*x*, −*y*, 1−*z*	Cl(1)···H−N(3) 2.681(2) 143.5(9)	Cl(1)···H−N(3) 2.5262) 150.2(8)
	Cl(4)···H−N(1) 2.592(2) 148.8(8)	Cl(4)···H−N(2) 2.750(2) 137.9(7)

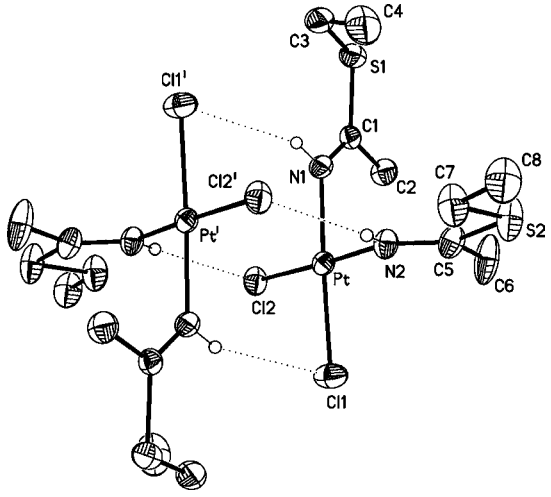


Figure 4. Perspective ORTEP drawing of a dimer of **1** with the atom labeling scheme. H atoms involved in the N−H···Cl bridges were set in calculated positions (′ at 1−*x*, −*y*, 1−*z*); (with the exception of the Pt−N*H*, the H atoms have been omitted for clarity).

$CH_2-C_6H_4$-*p*-F$\}_2$],[6f] and *cis*-[$PtCl_2\{E$-N(H)=C(N$(CH_3)_2$)-$CH_3\}_2$]).[47] The Pt−Cl (average 2.308(2) Å) and Pt−N (average 2.013(5) Å) bond distances as well as the Cl(1)−Pt−Cl(2) (91.3(1)°), N(1)−Pt−N(2) (87.6(2)°), N(1)−Pt-Cl(2) (90.1(1)°) and N(2)−Pt-Cl(1) (91.1(1)°) angles have normal values for Pt(II) square planar complexes.[48] The two iminothioether ligands had *E* configuration and comparable geometric parameters. The N(1)−C(1) [1.279(6) Å] and N(2)−C(5) [1.280(6) Å] bond distances were in agreement with a double-bond character and a sp^2 hybridization of the N atom.[4b,49] The C(sp^2)−S and C(sp^3)−S bond distances were 1.736(6) Å and 1.803(7) Å, respectively (averaged values). The difference (less than 0.1 Å) could be explained on the basis of a partial double-bond character of C−S [typical values: C(sp^3)−S, 1.82; C(sp^2)=S, 1.60 Å][50] as indicated also by the values of the N(1)−C(1)−S(1), N(1)−C(1)−C(2), and S(1)−C(1)−C(2) angles of 125.3(4)°, 121.8(5)°, and 112.9(4)°, respectively. The bond distance average of 1.736(6) Å found for C(1)−S(1) and C(5)−S(2) fell halfway between those of single and double carbon−sulfur bonds. This could indicate that whenever a sulfur atom is bound to a carbon atom engaged in a double bond, a lone pair of the sulfur participates in the π system. It is worthwhile to note that the N=C bond distances in the imino thioether ligands (N(1)−C(1) 1.279(7) Å and N(2)−C(5) 1.280(6) Å) were comparable to those observed in the iminoether derivatives such as *cis*-[$PtCl_2\{Z$-N(H)=C-$(OCH_3)CH_2C_6H_5\}_2$][6e] (1.281(6) Å) while they were shorter compared with N=C distance in amidine derivatives such as *cis*-[$PtCl_2\{E$-N(H)=C(N$(CH_3)_2)CH_3\}_2$][48a] (1.304(4) Å). The two NCS planes of the imino thioethereal ligands in each unit of the dimer formed with the coordination plane dihedral angles of 70° and 80°, and of 85° each other, while within the dimeric system the corresponding NCS planes were exactly parallel.

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

*3.2.2. X-ray Structure of Compound (**2**).* Compound **2** which differs from **1** only in an ethyl substituent to C(6) (instead of methyl) had the same square planar coordination geometry around the metal ion with the same *cis* position of the imino thioether ligands (Figure 5). Also in this case the

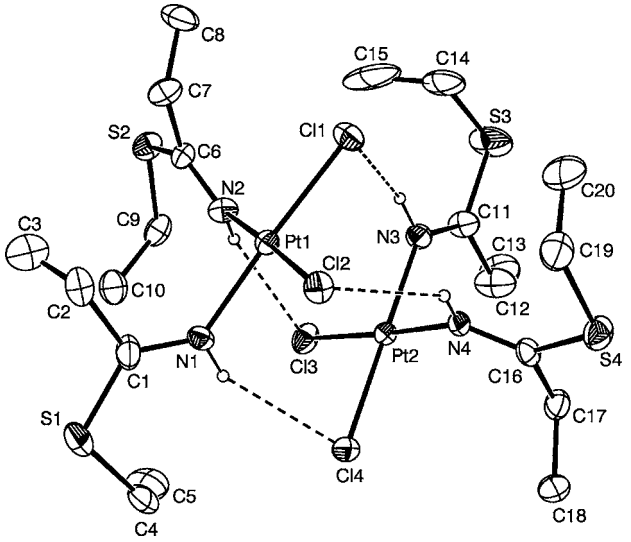


Figure 5. ORTEP drawing of a dimer of compound **2** (with the exception of the Pt−N*H*, the H atoms have been omitted for clarity).

structure consisted of dimers $Pt_2Cl_4L_4$ (L = N(H)=C(SEt)-CH_2CH_3) formed by two $PtCl_2L_2$ units intermolecularly held together by four N−H···Cl hydrogen bonds involving the chlorines and the imino thioether NH protons of two adjacent molecules. The Pt···Pt contact distance of 3.258(1) Å indicated a degree of metal−metal interaction. The Pt−Cl and Pt−N bond distances (Table 2) were comparable to those of **1** as well the N−H···Cl intermolecular contacts (Table 3). The imino thioether moieties had *E* configuration and comparable geometrical parameters. Again the C(sp^2)−S bond distance average of 1.73(2) Å could be explained on the basis of a partial double-bond character of C−S as indicated also by the average values of the N−C−S, N−C−C, and S−C−C angles of 126(2)°, 120(2)°, and 114(2)°, respectively. The N=C bond distances in the imino thioether ligands (average 1.30(2) Å) were comparable to those observed in **1**. The two NCS planes of the imino thioethereal ligands in each unit of the dimer formed with the coordination plane dihedral angles of 60° and 90°, and were almost perpendicular each other, whereas the corresponding NCS planes were not parallel in the dimer forming dihedral angles of 70° and 30°, respectively.

*3.2.3. X-ray Structure of Compound (**7**).* Compound **7** presented a square planar coordination geometry around the metal center with the ligands in *trans* positions. The unit was formed by two independent $PtCl_2L_2$ (L = N(H)=C(SEt)-CH_2Ph) moieties, where the two Pt-square planes were parallel but rotated each other of 95° (Figure 6). The Pt−Cl and Pt−N bond distances were again comparable to those of compound **1** and **2** and was evidenced by the presence of a dimer due to the occurrence of four intermolecular Cl···H−N hydrogen bonds with lengths comparable to those of **1** and **2** (see Table 3). The Pt···Pt contact distance of 3.428(1) Å indicated the absence of metal−metal interaction. The imino thioether moieties had *E* configuration and comparable geometrical parameters. Some relevant bond distances in the iminothioether ligands (average Pt−N 2.017(9) Å, C=N 1.28(2) Å and =C−CH_2 1.49(2) Å) resembled the values observed in the iminoethereal ligands of *cis*-[$PtCl_2${*Z*-N(H)=C(OCH_3)$CH_2C_6H_5$}$_2$][6e] (average Pt−N 2.006(4) Å, C=N 1.281(6) Å, =C−CH_2 1.499(7) Å). Again the C(sp^2)−S bond distance average of 1.75(2) Å could be explained on the basis of a partial double-bond character of C−S as indicated also by the average values of the N−C−S, N−C−C, and S−C−C angles of 123(1)°, 123(1)°, and 112(1)°, respectively. The N=C bond distances in the imino thioether ligands (average 1.28(2) Å) were comparable to those observed in **1** and **2**. Both NCS planes of the imino thioether ligands in the Pt(1) unit formed with the coordination plane dihedral angles of 60° and of 80° in the Pt(2) unit, the corresponding NCS planes in the dimer being almost perpendicular. Within each unit the phenyl ring planes were almost perpendicular with respect the corresponding NCS planes, and despite the presence of several aromatic moieties, the inspection of the

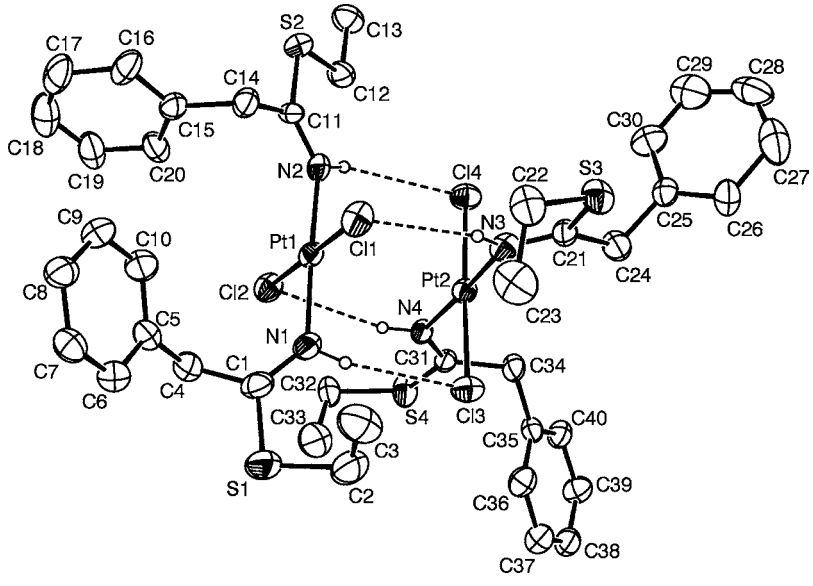


Figure 6. ORTEP drawing of a dimer of compound **7** (with the exception of the Pt−N*H*, the H atoms have been omitted for clarity).

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

Table 4. Cytotoxic Activity of the Platinum(II) Imino Thioether Complexes in Human Cancer Lines Derived from Solid Tumors

	IC_{50}[a] (μM) ± SD[b]											
compound	MCF-7	A549	A375	A431	A431/Pt	R.F.	2008	C13*	R.F.	LoVo	LoVo MDR	R.F.
5	97.0 ± 1.2	86 ± 2	88 ± 3	93.2 ± 1.9	92 ± 2	1.0	97.2 ± 1.6	88 ± 2	0.9	91 ± 2	98.8 ± 1.6	1.1
6	43.0 ± 1.6	39 ± 2	34 ± 2	43.0 ± 0.8	46.3 ± 1.1	1.1	47.2 ± 1.6	43 ± 2	0.9	44.1 ± 1.0	51.8 ± 1.4	1.3
7	7.0 ± 0.5	5.1 ± 1.1	6.5 ± 1.0	7.1 ± 1.4	6 ± 2	0.9	7 ± 2	8.1 ± 1.1	1.1	6.9 ± 1.1	5.8 ± 0.7	0.8
transplatin	>100	>100	>100	>100	>100		>100	>100		>100	>100	
cisplatin	23 ± 2	27.2 ± 1.5	21 ± 2	17.2 ± 1.1	41.3 ± 1.2	2.6	12.7 ± 1.7	89.2 ± 1.5	7.0	16.5 ± 1.9		

[a] IC_{50} values were calculated by PL model ($P < 0.05$). Cells ($3\text{-}8 \times 10^4$ ml^{-1}) were treated for 48 h with increasing concentrations of tested compounds dissolved in DMF. Cytotoxicity was assessed by MTT test. The RF is defined as IC_{50} resistant/parent line. [b] S.D. = standard deviation.

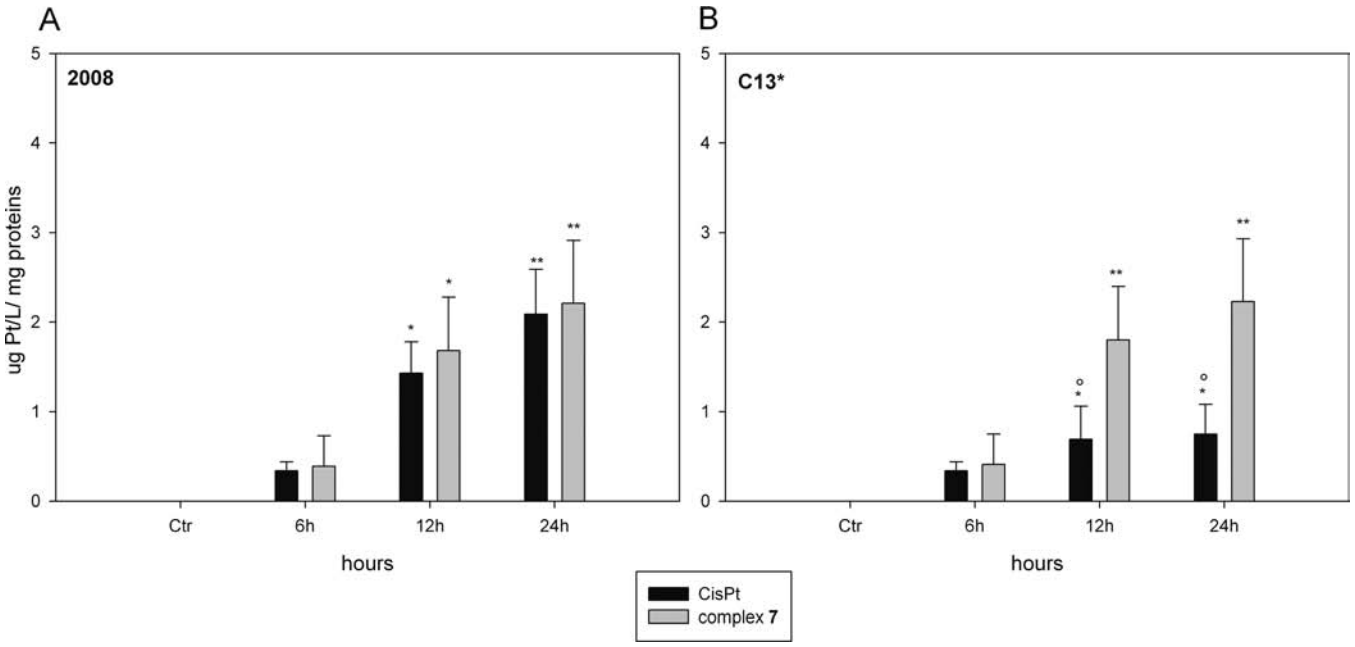


Figure 7. Intracellular accumulation of platinum complexes. 2008 (A) and C13* (B) cells were incubated with IC_{50} doses of complex **7** and cisplatin for 6, 12, and 24 h. Intracellular platinum accumulation was detected by GF-AAS analysis. Error bars indicate the standard deviation. Key: *, $p < 0.05$; **, $p < 0.01$ compared to the control, °, $p < 0.05$ compared to cisplatin-sensitive cells.

packing diagram did not reveal the presence of π staking interactions.

3.3. Biological Assays. *3.3.1. Cytotoxicity Assays.* The newly synthesized platinum(II) imino thioether derivatives have been evaluated for their cytotoxic activity toward a panel of nine human tumor cell lines derived from solid tumors and including examples of ovarian (2008 and C13*), cervical (A431 and A431/Pt), lung (A549), colon (LoVo and loVo MDR), and breast (MCF-7) cancers and melanoma (A375). Cytotoxicity has been evaluated by means of MTT tests after 48 h of treatment with increasing concentrations of the tested compounds. For comparison purposes, the cytotoxicity of cisplatin, the most widely used anticancer metallodrug, and of transplatin were evaluated in the same experimental conditions. IC_{50} values, calculated from dose-survival curves, are shown in Table 4. The *cis* complexes **1**–**4** were not tested for their biological activity, because of their very low solubility in the experimental conditions. All the *trans*-imino thiother complexes appeared to be more effective than transplatin, which, as well documented,[6a] was found to be scarcely effective. Among Pt(II) *trans*-derivatives, the complex *trans*-[$PtCl_2${*E*-N(H)=C(SEt)-Me}$_2$] (**5**) was the least active agent, showing an antiproliferative activity roughly 5-fold lower than that of cisplatin, the mean IC_{50} (μM) values being 93.2 and 20.0 (excluding C13* and A431/Pt IC_{50} values) for **5** and cisplatin, respectively. Similarly, the complex *trans*-[$PtCl_2${*E*-N(H)=C(SEt)Et}$_2$] (**6**) showed across the various cancer cell lines a cytotoxic activity lower than that of cisplatin, the mean IC_{50} (μM) values being respectively 43.2 and 20.0 (excluding C13* and A431/Pt IC_{50} values) for **6** and cisplatin, respectively. Among all derivatives the complex *trans*-[$PtCl_2${*E*-N(H)=C(SEt)CH_2Ph}$_2$] (**7**) showed the greatest in vitro antitumor activity being able to decrease cell viability roughly 3-fold more effectively than cisplatin (mean IC_{50} (μM) value 6.5).

Cytotoxic activity of imino thioethers was also evaluated onto additional human cancer cell lines, including two cisplatin-resistant cell lines (C13* ovarian adenocarcinoma and A431/Pt cervical squamous carcinoma cells) and a multidrug resistant (MDR) phenotype (LoVo MDR colon adenocarcinoma cells). Cross-resistance profiles were evaluated by means of the resistance factor (R.F.) which is defined as the ratio between IC_{50} values calculated for the resistant cells and those obtained with the sensitive ones. Although cisplatin resistance is multifactorial, the main molecular mechanisms involved in C13* and A431/Pt cell resistance have almost been defined. In particular, in human ovarian adenocarcinoma C13* cells, cisplatin resistance has been correlated to reduced cell drug uptake, high cellular thioredoxin reductase and glutathione

dx.doi.org/10.1021/ic3024452 | Inorg. Chem. 2013, 52, 5729–5741

levels, and enhanced repair of DNA damage.[51] In human squamous cervical carcinoma A431/Pt cells, resistance is due to defect in drug uptake and to decreased levels of proteins involved in DNA mismatch repair (MSH2), causing an increased tolerance to cisplatin-induced DNA damage. As shown in Table 4, all derivatives exhibited a different cross-resistance profile than that of cisplatin, possessing a quite similar cytotoxic potency both on cisplatin sensitive and resistant cell lines. The R.F. for all derivatives, calculated on the A431-A431/Pt and 2008-C13* cell pairs, were about 2 and 7 times lower than those of cisplatin, respectively, clearly revealing no cross-resistance phenomena and thus supporting the hypothesis that these Pt(II) imino thioether complexes have different cytotoxic mechanisms compared to cisplatin. Tested on LoVo MDR colon cancer cells, suitably selected for their resistance to doxorubicin and thus retaining the MDR phenotype, all derivatives yielded R.F. values roughly 28 times lower than that obtained with doxorubicin (R.F.: 30.7). Given that in LoVo MDR cells doxorubicin-resistance is mainly associated with an overexpression of the multispecific drug transporters, such as the 170 kDa P-glycoprotein,[52] the ability of Pt(II) imino thioether complexes to overcome multidrug resistance phenomena in this cell line may suggest that these agents are not potential MDR substrates.

3.3.2. Cellular Uptake. It is well-known that cellular uptake is an important factor influencing drug efficacy. Moreover, since one of the main mechanisms controlling cisplatin resistance is cellular uptake, uptake experiments were performed in human ovarian adenocarcinoma cells, sensitive (2008) and resistant (C13*) to cisplatin. Cancer cells were treated for 6, 12, and 24 h with IC_{50} doses of complex **7**, the most promising Pt(II) imino thioether derivative, and cisplatin. The intracellular platinum accumulation was quantified by means of GF-AAS analysis, and the results, expressed as μg of metal mg^{-1} of cellular proteins, are summarized in Figure 7. Cellular uptake of complex **7** was time dependent, both in sensitive as well as in cisplatin resistant cells. There was, however, a marked difference between sensitive and resistant cells as far as the discrimination between cisplatin and complex **7** is concerned. In 2008 sensitive cells the amount of internalized Pt amount was very similar for both cisplatin and Pt(II) imino thioether derivative **7** (Figure 7A). In resistant C13* cells (Figure 7B) the cellular uptake was remarkably decreased for cisplatin but not for compound **7**, which was internalized with the same efficacy in 2008 and C13* cells. These data can well justify the lack of cross-resistance between compound **7** and cisplatin in ovarian C13* cells.

3.3.3. Caspase-3 Activation. To investigate if the decrease in cell viability induced by *trans*-[$PtCl_2${*E*-N(H)=C(SEt)-CH_2Ph}$_2$] could be due to apoptosis, caspase-3 activity was assayed. Caspase-3, a downstream caspase, playing a pivotal role in the terminal execution phase of apoptosis induced by diverse stimuli, including anticancer metallodrugs.[53] The cleavage of various substrates contributes to the typical morphological and biochemical features observed in apoptosis. Because of the diversity of its substrates, caspase-3 is thought to be a general mediator of physiological and stress-induced apoptosis. Caspase-3 activity was assayed in 2008 human ovarian carcinoma cells treated for 24 h with IC_{50} of compound **7**, or cisplatin, the first line chemotherapeutic drug against ovarian cancer. Figure 8 shows that *trans*-[$PtCl_2${E-N(H)=C(SEt)-CH_2Ph}$_2$] markedly stimulated caspase-3 activity, similarly to cisplatin. In particular, in *trans*-[$PtCl_2${E-N(H)=C(SEt)-CH_2Ph}$_2$] treated cells, protease activity was four times enhanced with respect to control cells.

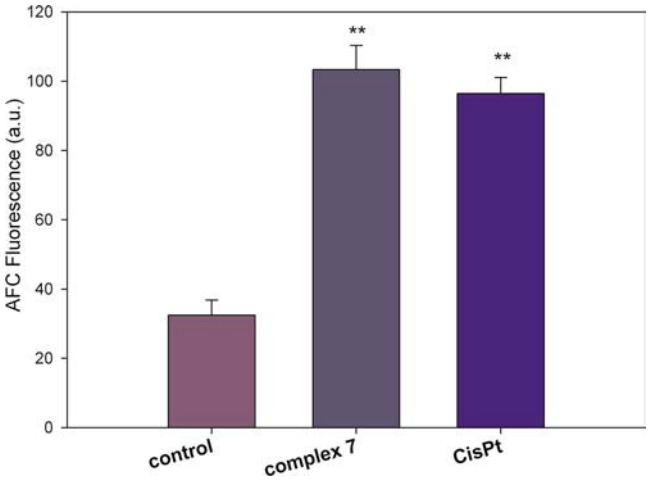


Figure 8. Induction of caspase-3 activity. 2008 cells were incubated for 24 h with complex **7** or cisplatin and then processed for caspase-3 activity detection as described in the Experimental Section. Data are the mean values of at least three independent experiments. Error bars indicate standard deviation.

4. CONCLUSIONS

In this paper we reported the high yield synthesis of novel biologically active imino thioether complexes of the type *cis*- and *trans*-[$PtCl_2${*E*-N(H)=C(SEt)R}$_2$] by addition of CH_3CH_2SH to coordinated nitrile ligands in *cis*- and *trans*-[$PtCl_2(NCR)_2$] (R = Me, Et, CH_2Ph, Ph). These complexes complete a series of structurally similar Pt(II)-imino derivatives bearing amidine or iminoether ligands of the type *cis*- and *trans*-[$PtCl_2${*E*-N(H)=C(NR′R″)R}$_2$] and *cis*- and *trans*-[$PtCl_2${*E*-N(H)=C(OR′)R}$_2$], respectively, which have been also found to exhibit cytotoxic activity higher than cisplatin.[6a−d,7e] It is worthwhile noting that the nature of the heteroatom affects the biological activity of these imino-Pt(II) complexes. Generally, the S- and O-derivatives show higher activity compared to the N-analogues. The cytotoxicity of the new imino thioether products has been studied in different cell lines derived from solid tumors and endowed with different sensitivity to cisplatin. The compound *trans*-[$PtCl_2${*E*-N(H)=C(SEt)CH_2Ph}$_2$] (**7**) proved to be the most active cytotoxic agent and exhibited a biological activity similar to that shown by *trans*-[$PtCl_2${*E*-N(H)=C(OMe)Me}$_2$] and *trans*-[$PtCl_2(NH_3)${*E*-N(H)=C-(OMe)Me}] previously reported by Natile et al.[6a] We also noticed that the presence of a benzyl group increases the cytoxicity of this type of complexes. In fact, **7** shows the highest biological activity within the series of imino thioethers prepared. Moreover, the cellular uptake of compound **7** is similar into cisplatin-sensitive and -resistant cells. This indicates that, somehow, **7** can easily escape the resistance mechanisms that allow for a decreased uptake and/or increased export of platinum drugs in resistant cells. In conclusion, it appears that the imino thioether platinum complexes are good candidates for further investigation in the field of cytotoxic drugs and therefore a wider series of imino thioether derivatives will be developed. In particular, a synthetic strategy for the preparation of complexes bearing cyclic imino thioethers, which are of relevant interest for biomedical applications,[54] will be investigated.

dx.doi.org/10.1021/ic3024452 | *Inorg. Chem.* 2013, 52, 5729−5741

■ ASSOCIATED CONTENT

Supporting Information

Cystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +39 049 8275525. Phone: +39 049 8275522. E-mail: rino.michelin@unipd.it.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the University of Padova (PRAT Project 2011, code CPDA114144, SA 31/10/2012, and CPDA121121973/12) and MIUR (PRIN 2010 project, code 2010L9SH3K) for financial support and the Inter-University Consortium for Research on the Chemistry of Metal Ions in Biological Systems (CIRCMSB, Bari, Italy).

■ REFERENCES

(1) (a) Kukushkin, V. Y.; Pombeiro, A. J. L. *Chem. Rev.* **2002**, *102*, 1771−1882. (b) Pombeiro, A. J. L.; Kukushkin, V. Yu. Reactions of Coordinated Nitriles. In *Comprehensive Coordination Chemistry II*; McCleverty, J. A., Meter, T. J., Eds.; Elsevier: Amsterdam, The Netherlands, 2004; Vol. *1*, Chapter 1.34, pp 639−660; (c) Michelin, R. A.; Mozzon, M.; Bertani, R. *Coord. Chem. Rev.* **1996**, *147*, 299−338.

(2) (a) Kukushkin, V. Yu.; Pombeiro, A. J. L. *Inorg. Chim. Acta* **2005**, *358*, 1−21. (b) Bokach, N. A.; Konovalova, N. P.; Wang, Y.; Moskalenko, Y. E.; Gribanov, A. V.; Kukushkin, V. Y. *Dalton Trans.* **2010**, 4619−4623. (c) Eglin, J. *Comments Inorg. Chem* **2002**, *23*, 23−43. (d) Murahashi, S. I.; Takaya, H. *Acc. Chem. Res.* **2000**, *33*, 225−233. (e) Kukushkin, V. Y.; Pakhomova, T. B.; Kukushkin, Y. N.; Herrmann, R.; Wagner, G.; Pombeiro, A. J. L. *Inorg. Chem.* **1998**, *37*, 6511−6517. (f) Makarycheva-Mikhailova, A. V.; Kukushkin, V. Y.; Nazarov, A. A.; Garnovskii, D. A.; Pombeiro, A. J. L.; Haukka, M.; Keppler., B. K.; Galanski, M. *Inorg. Chem.* **2003**, *42*, 2805−2813.

(3) (a) Stepanenko, I. N.; Cebrián-Losantos, B.; Arion, V. B.; Krokhin, A. A.; Nazarov, A. A.; Keppler, B. K. *Eur. J. Inorg. Chem.* **2007**, 400−411. (b) Hopmann, K. H.; Guo, J.-D.; Himo, F. *Inorg. Chem.* **2007**, *46*, 4850−4856.

(4) (a) Fanizzi, F. P.; Intini, F. P.; Natile, G. *J. Chem. Soc., Dalton Trans.* **1989**, 947−951. (b) Cini, R.; Caputo, P. A.; Intini, F. P.; Natile, G. *Inorg. Chem.* **1995**, *34*, 1130−1137.

(5) (a) Bertani, R.; Catanese, D.; Michelin, R. A.; Mozzon, M.; Bandoli, G.; Dolmella, A. *Inorg. Chem. Commun.* **2000**, *3*, 16−18. (b) Belluco, U.; Benetollo, F.; Bertani, R.; Bombieri, G.; Michelin, R. A.; Mozzon, M.; Tonon, O.; Pombeiro, A. J. L.; Guedes da Silva, F. C. *Inorg. Chim. Acta* **2002**, *334*, 437−447.

(6) (a) Coluccia, M.; Natile, G. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 111−123. (b) Natile, G.; Coluccia, M. In *Metal Complexes in Tumor Diagnosis and as Anticancer Agents*; Sigel, A., Sigel, H., Eds.; Metal Ions in Biological Systems, Vol. *42*; Marcel Dekker: New York, 2004; pp 209−250; (c) Natile, G.; Coluccia, M. *Coord. Chem. Rev.* **2001**, *216−217*, 383−410. (d) Liu, Y.; Sivo, M. F.; Natile, G.; Sletten, E. *Met.-Based Drugs* **2000**, *7*, 169−176. (e) Mazzega Sbovata, S.; Bettio, F.; Mozzon, M.; Bertani, R.; Venzo, A.; Benetollo, F.; Michelin, R. A.; Gandin, V.; Marzano, C. *J. Med. Chem.* **2007**, *50*, 4775−4784. (f) Mazzega Sbovata, S.; Bettio, F.; Marzano, C.; Tassan, A.; Mozzon, M.; Bertani, R.; Benetollo, F.; Michelin, R. A. *J. Inorg. Biochem.* **2008**, *102*, 882−891.

(7) (a) Michelin, R. A.; Bertani, R.; Mozzon, M.; Sassi, A.; Benetollo, F.; Bombieri, G.; Pombeiro, A. J. L. *Inorg. Chem. Commun.* **2001**, *4*, 275−280. (b) Belluco, U.; Benetollo, F.; Bertani, R.; Bombieri, G.; Michelin, R. A.; Mozzon, M.; Pombeiro, A. J. L.; Guedes da Silva, F. C. *Inorg. Chim. Acta* **2002**, *330*, 229−239. (c) Marzano, C.; Mazzega Sbovata, S.; Bettio, F.; Michelin, R. A.; Seraglia, R.; Kiss, T.; Venzo, A.; Bertani, R. *J. Biol. Inorg. Chem.* **2007**, *12*, 477−493. (d) Mazzega Sbovata, S.; Bettio, F.; Marzano, C.; Mozzon, M.; Bertani, R.; Benetollo, F.; Michelin, R. A. *Inorg. Chim. Acta* **2008**, *361*, 3109−3116. (e) Michelin, R. A.; Sgarbossa, P.; Sbovata Mazzega, S.; Gandin, V.; Marzano, C.; Bertani, R. *ChemMedChem* **2011**, *6*, 1172−1183.

(8) (a) Janovska, E.; Novakova, O.; Natile, G.; Brabec, V. *J. Inorg. Biochem.* **2002**, *90*, 155−158. (b) Coluccia, M.; Nassi, A.; Loseto, F.; Boccarelli, A.; Mariggiò, M. A.; Giordano, D.; Intini, F. P.; Caputo, P. A.; Natile, G. *J. Med. Chem.* **1993**, *36*, 510−512. (c) Liu, Y.; Vinje, J.; Pacifico, C.; Natile, G.; Sletten, E. *J. Am. Chem. Soc.* **2002**, *124*, 12854−12862.

(9) Perera, T.; Fronczek, F. R.; Marzilli, P. A.; Marzilli, L. G. *Inorg. Chem.* **2010**, *49*, 7035−7045.

(10) (a) Kalinowska-Lis, U.; Ochocki, J.; Matlawska-Wasowska, K. *Coord. Chem. Rev.* **2008**, *252*, 1328−1345. (b) Novakova, O.; Kasparkova, J.; Malina, J.; Natile, G.; Brabec, V. *Nucleic Acids Res.* **2003**, *31*, 6450−6460. (c) Zorbas-Seifried, S.; Jakupec, M. A.; Kukushkin, N. V.; Groessl, M.; Hartinger, C. G.; Semenova, O.; Zorbas, H.; Kukushkin, V. Y.; Keppler, B. K. *Mol. Pharmacol.* **2007**, *71*, 357−365. (d) Kelland, L. R.; Barnard, C. F. J.; Mellish, K. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Bryant, A.; Murrer, B. A.; Harrap, K. R. *Cancer Res.* **1994**, *54*, 5618−5622. (e) Kelland, L. R.; Barnard, C. F. J.; Evans, I. G.; Murrer, B. A.; Theobald, B. R. C.; Wyer, S. B.; Goddard, P. M.; Jones, M.; Valenti, M.; Bryant, A.; Rogers, P. M.; Harrap, K. R. *J. Med. Chem.* **1995**, *38*, 3016−3024.

(11) Farrell, N. Polynuclear charged platinum compounds as a new class of anticancer agents.. In *Platinum Based drugs in Cancer Therapy*; Kelland, L.R., Farrell, N.P., Eds.; Humana Press: Totowa, NJ, 2000; pp 321−338.

(12) (a) Gonzalez-Vadillo, A. M.; Alvarez-Valdes, A.; Moneo, V.; Blanco, F.; Diaz, R. G.; Carnero, A.; Navarro-Ranninger, C. *J. Inorg. Biochem.* **2007**, *101*, 551−558. (b) Kelland, L. R.; Abel, G.; McKeage, M. L.; Jones, M.; Goddard, P. M.; Valenti, M.; Murrer, B. A. *Cancer Res.* **1993**, *53*, 2581−2586. (c) Lee, Y. A.; Won Kang, S.; Yul Park, Y.; Jung, O. S. *J. Mol. Struct.* **2003**, *659*, 129−133. (d) Lemma, K.; Shi, T.; Elding, L. I. *Inorg. Chem.* **2000**, *39*, 1728−1734. (e) Tai, H. C.; Brodbeck, R.; Kasparkova, J.; Farrer, N. J.; Brabec, V.; Sadler, J.; Deeth, R. J. *Inorg. Chem.* **2012**, *51*, 6830−6841.

(13) (a) Aris, S. M.; Gewirtz, D. A.; Ryan, J. J.; Knott, K. M.; Farrell, N. P. *Biochem. Pharmacol.* **2007**, *73*, 1749−1757. (b) Leng, M.; Schwartz, A.; Giraud Panis, M. J. Transplatin-modified oligonucleotides as potential antitumor drugs. In *Platinum Based drugs in Cancer Therapy*; Kelland, L.R., Farrell, N.P., Eds.; Humana Press: Totowa, NJ, 2000; pp 63−85; (c) Muller, J.; Drumm, M.; Boudvillain, M.; Leng, M.; Sletten, E.; Lippert, B. *J. Biol. Inorg. Chem.* **2000**, *5*, 603−611. (d) Nayajreh, Y.; Prilutski, D.; Ardeli-Tzaraf, Y.; Perez, J. M.; Khozanov, E.; Barenholz, Y.; Kasparkova, J.; Brabec, V.; Gibson, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 2885−2887. (e) Griffith, D. M.; Duff, B.; Suponitsky, K. Y.; Kavanagh, K.; Morgan, M. P.; Egan, D.; Marmion, C. J. *J. Inorg. Biochem.* **2011**, *105*, 793−799. (f) Mori, H.; Hirayama, N.; Komeiji, Y.; Mochizuki, Y. *Comput. Theor. Chem.* **2012**, *986*, 30−34. (g) Bartel, C.; Bytzek, A. K.; Scaffidi-Domianello, Y. Y.; Grabmann, G.; Jakupec, M. A.; Hartinger, C. G.; Galanski, M.; Keppler, B. K. *J. Inorg. Inorg. Chem.* **2012**, *12*, 465−474.

(14) (a) Hambley, T. W. *Coord. Chem. Rev.* **1997**, *166*, 181−223. (b) Zou, Y.; Van Houten, B.; Farrell, N. *Biochemistry* **1993**, *32*, 9632−9638.

(15) (a) Quiroga, A. G. *J. Inorg. Biochem.* **2012**, *114*, 106−112. (b) Montero, E. I.; Diaz, S.; Gonzàlez-Vadillo, A. M.; Pérez, J. M.; Alonso, C.; Navarro-Nanninger, C. *J. Med. Chem.* **1999**, *42*, 4264−4268. (c) Pantoja, E.; Gallipoli, A.; van Zutphen, S.; Komeda, S.; Reddy, D.; Jaganyi, D.; Lutz, M.; Tooke, D. M.; Spek, A. L.; Navarro-Ranninger, C.; Reedijk, J. *J. Inorg. Biochem.* **2006**, *100*, 1955−1964. (d) Cubo, L.; Hambley, T. W.; Miguel, P. J. S.; Carnero, A.; Navarro-Ranninger, C.; Quiroga, A. G. *Dalton Trans.* **2011**, *40*, 344−347. (e) Ramos-Lima, F. J.; Moneo, V.; Quiroga, A. G.; Carnero, A.; Navarro-Ranninger, C. *Eur. J. Med. Chem.* **2010**, *45*, 134−141. (f) Cepero, V.; Garcia-Serrelde, B.; Moneo, V.; Blanco, F.; Gonzalez-

Vadillo, A. M.; Alvarez-Valdes, A.; Navarro-Ranninger, C.; Carnero, A. *Clin. Transl. Oncol.* **2007**, *9*, 521−530. (g) Gonzalez-Vadillo, A. M.; Alvarez-Valdes, A.; Moneo, V.; Blanco, F.; Diaz, R. G.; Carnero, A.; Navarro-Ranninger, C. *J. Inorg. Biochem.* **2007**, *101*, 551−558.
(16) Musetti, C.; Nazarov, A. A.; Farrell, N. P.; Sissi, C. *ChemMedChem* **2011**, *6*, 1283−1290.
(17) (a) Berner-Price, S. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 804−805. (b) Cubo, L.; Pizarro, A. M.; Quiroga, A. G.; Salassa, L.; Navarro-Ranninger, C.; Sadler, P. J. *J. Inorg. Biochem.* **2010**, *104*, 909−918. (c) Heringova, P.; Woods, J.; Mackay, F. S.; Kasparkova, J.; Sadler, P. J.; Brabec, V. *J. Med. Chem.* **2006**, *49*, 7792−7798.
(18) (a) Komeda, S.; Casini, A. *Curr. Topics Med. Chem.* **2012**, *12*, 219−235. (b) Frezza, M.; Hindo, S.; Chen, D.; Davenport, A.; Schmitt, S.; Tomco, D.; Ping Dou, Q. *Curr. Pharm. Design* **2010**, *16*, 1813−1825. (c) Kaluderovic, G. N.; Paschke, R. *Curr. Med. Chem.* **2011**, *18*, 4738−475. (d) Gasser, G.; Metzler-Nolte, N. *Curr. Op. Chem. Biol.* **2012**, *16*, 84−91. (e) Ronconi, L.; Sadler, P. J. *Coord. Chem. Rev.* **2007**, *251*, 1633−1648. (f) Fricker, S. P. *Dalton Trans.* **2007**, 4903−4917.
(19) (a) Reedjik, J. *Chem. Rev.* **1999**, *99*, 2499−2510. (b) Chattaraj, P. K. *J. Phys. Chem. A* **2001**, *105*, 511−513. (c) Ma, Z.; Rao, L.; Bierbach, U. *J. Med. Chem.* **2009**, *52*, 3424−3427. (d) Hahn, M.; Kleine, M.; Sheldrick, W. S. *J. Biol. Inorg. Chem.* **2001**, *6*, 556−566.
(20) Fuertes, M. A.; Alonso, C.; Perez, J. M. *Chem. Rev.* **2003**, *103*, 645−662.
(21) (a) Fokkema, E.; Groen, H. J. M.; Helder, M. N.; deVries, E. G. E.; Mejer, C. *Biochem. Pharmacol.* **2002**, *63*, 1989−1996. (b) Bugarcic, Z. D.; Rosic, J.; Petrovic, B.; Summa, N.; Puchta, R.; van Eldik, R. *J. Biol. Inorg. Chem.* **2007**, *12*, 1141−1150. (c) Vrana, O.; Brabec, V. *Biochemistry* **2002**, *41*, 10994−10999. (d) Li, C.; Li, Z.; Sletten, E.; Arnesano, F.; Losacco, M.; Natile, G.; Liu, Y. *Angew. Chem., Int. Ed.* **2009**, *48*, 8497−8500.
(22) Jansen, B. A. J.; Brouwer, J.; Reedjik, J. *J. Inorg. Biochem.* **2002**, *89*, 197−202.
(23) Marchand, V.; Moreno, V.; Pedroso, E.; Grandas, A. *Chem.—Eur. J.* **2001**, *7*, 808−815.
(24) Lemoers, E. L. M.; Reedjik, J. *Inorg. Chem.* **1990**, *29*, 217−222.
(25) Brabec, V.; Kasparkova, J. *Drug Resist. Updates* **2002**, *5*, 147−161.
(26) Gabbiani, C.; Casini, A.; Mastrobuoni, G.; Kirshenbaum, N.; Moshel, O.; Pieraccini, G.; Moneti, G.; Messori, L.; Gibson, D. *J. Biol. Inorg. Chem.* **2008**, *13*, 755−764.
(27) (a) Gao, E. J.; Liu, L.; Zhu, M. C.; Huang, Y.; Guan, F.; Gao, X. N.; Zhang, M.; Wang, L.; Zhang, W. Z.; Sun, Y. G. *Inorg. Chem.* **2011**, *50*, 4732−4741. (b) Gallardo-Godoy, A.; Gever, J.; Fife, K. L.; Silber, B. M.; Prusiner, S. B.; Renslo, A. R. *J. Med. Chem.* **2011**, *54*, 1010−1021.
(28) (a) Casas, J. S.; Castellano, E. E.; Ellena, J.; Garcia-Tasende, M. S.; Perez-Paralli, M. L.; Sanchez, A.; Sanchez-Gonzalez, A.; Sordo, J.; Touceda, A. *J. Inorg. Biochem.* **2008**, *102*, 33−45. (b) Quiroga, A. G.; Cubo, L.; Sanz Miguel, P. J.; Moneo, V.; Carnero, A.; Navarro-Ranninger, C. *Eur. J. Inorg. Chem.* **2008**, 1183−1187.
(29) (a) Campbell, K. C. M.; Meech, R. P.; Klemens, J. J.; Gerberi, M. T.; Dyrstad, S. S.; Larsen, D. L.; Mitchell, D. L.; El-Azizi, M.; Verhulst, S. J.; Hughes, L. F. *Hear Res.* **2007**, *226*, 92−103. (b) Suchankova, T.; Vojtiskova, S.; Reedijk, J.; Brabec, V.; Kasparkova, J. *J. Biol. Inorg. Chem.* **2009**, *14*, 75−87. (c) Tesei, A.; Brigliadori, G.; Carloni, S.; Fabbri, F.; Ulivi, P.; Arienti, C.; Sparatore, A.; Del Soldato, P.; Pasini, A.; Amadori, D.; Silvestrini, R.; Zoli, W. *J. Cell. Physiol.* **2012**, *227*, 3389−3396.
(30) (a) Li, C.; Huang, R.; Ding, Y.; Sletten, E.; Arnesano, F.; Losacco, M.; Natile, G.; Liu, Y. *Inorg. Chem.* **2011**, *50*, 8168−8176. (b) Chen, S.; Xu, d:; Jiang, H.; Xi, Z.; Zhu, P.; Liu, Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 12258−12261. (c) Ma, G.; Min, Y.; Huang, F. M.; Jiang, T.; Liu, Y. *Chem. Commun.* **2010**, *46*, 6938−6940.
(31) Ros, R.; Michelin, R. A.; Boschi, T.; Roulet, R. *Inorg. Chim. Acta* **1979**, *35*, 43−48.
(32) (a) Perrin, C. L.; Dwyer, T. J. *Chem. Rev.* **1990**, *90*, 935−967. (b) Venzo, A.; Bisello, A.; Ceccon, A.; Manoli, F.; Santi, S. *Inorg. Chem. Commun.* **2000**, *3*, 1−4.
(33) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565−569.
(34) Drobny, G.; Pines, A.; Sinton, S.; Weitekamp, D. P.; Wemmer, D. *Faraday Symp. Chem. Soc.* **1978**, *13*, 49−55.
(35) (a) Otting, G.; Wüthrich, K. *J. Magn. Reson.* **1988**, *76*, 569−574. (b) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(36) (a) Fracarollo, D.; Bertani, R.; Mozzon, M.; Belluco, U.; Michelin, R. A. *Inorg. Chim. Acta* **1992**, *201*, 15−22. (b) Kukushkin, V. Y.; Tkachuk, V. M. *Z. Anorg. Allg. Chem.* **1992**, *61*, 123−126.
(37) North, A. T. C.; Philips, D. C.; Mathews, F. S. *Acta Crystallogr.* **1968**, *A24*, 351−359.
(38) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(39) (a) Sheldrick, G. M. *SHELXL-97, Program for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997; (b) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *64*, 112−122.
(40) Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837−838.
(41) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589−601.
(42) Bertani, R.; Seraglia, R.; Favretto, D.; Michelin, R. A.; Mozzon, M.; Mazzega Sbovata, S.; Sassi, A. *Inorg. Chim. Acta* **2003**, *356*, 357−364.
(43) (a) Neidle, S.; Ismailand, M.; Sadler, P. J. *J. Inorg. Biochem.* **1980**, *133*, 205−212. (b) Abu-Surrah, A. S.; Al-Allaf, T. A. K.; Klinga, M.; Aklgren, M. *Polyhedron* **2003**, *22*, 1529−1535.
(44) Di Pasqua, A. J.; Kerwood, D.; Shi, Y.; Goodisman, J.; Dabrowiak, J. C. *Dalton Trans.* **2011**, *40*, 4821−4825.
(45) (a) Tisato, F.; Refosco, F.; Porchia, M.; Teloni, M.; Gandin, V.; Marzano, C.; Pellei, M.; Papini, G.; Lucato, L.; Seraglia, R.; Traldi, P. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1610−1616. (b) Knipp, M. *Curr. Med. Chem.* **2009**, *16*, 522−537.
(46) (a) Marini, V.; Kasparkova, J.; Novakova, O.; Monsù Scolaro, L.; Romeo, R.; Brabec, V. *J. Biol. Inorg. Chem.* **2002**, *7*, 725−734. (b) Cox, J. W.; Berners-Price, S. J.; Davies, M. S.; Qu, Y.; Farrell, N. *J. Am. Chem. Soc.* **2001**, *123*, 1316−1326.
(47) Michelin, R. A.; Mozzon, M.; Bertani, R.; Benetollo, F.; Bombieri, G.; Angelici, R. J. *Inorg. Chim. Acta* **1994**, *222*, 327−337.
(48) (a) Michelin, R. A.; Bertani, R.; Mozzon, M.; Sassi, A.; Benetollo, F.; Bombieri, G.; Pombeiro, A. J. L. *Inorg. Chem. Commun.* **2001**, *4*, 275−280. (b) Fanizzi, F. P.; Natile, G.; Maresca, L.; Manotti-Lanfredi, A.; Tiripicchio, A. *J. Chem. Soc., Dalton Trans.* **1984**, *7*, 1467−1470.
(49) Natile, G.; Fanizzi, F. P.; Maresca, L.; Manotti-Lanfredi, A.; Tiripicchio, A. *J. Chem. Soc., Dalton Trans.* **1985**, *5*, 1057−1059.
(50) (a) Parker, S. F.; Refson, K.; Bennett, R. D.; Best, J.; Mel'nikov, M. Y.; Weinstein, J. A. *Inorg. Chem.* **2012**, *51* (18), 9748−9756. (b) Casas, J. S.; Castellano, E. E.; Ellena, J.; Garcia-Tasende, M. S.; Sanchez, A.; Sordo, J.; Toucesa, A. *Polyhedron* **2009**, *28*, 1029−1039. (c) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G. *J. Chem. Soc., Perkin Trans 2* **1987**, S1−S19.
(51) Marzano, C.; Gandin, V.; Folda, A.; Scutari, G.; Bindoli, A.; Rigobello, M. P. *Free Radical Biol. Med.* **2007**, *42*, 872−881.
(52) Wersinger, C.; Rebel, G.; Lelong-Rebel, I. H. *Amino Acids* **2000**, *19*, 0667−0685.
(53) Siddik, Z. H. *Oncogene* **2003**, *22*, 7265−7279.
(54) Moraski, G. C.; Markley, L. D.; Chang, M.; Cho, S.; Franzblau, S. G.; Hwang, C. H.; Boshoff, H.; Miller, M. J. *Bioorg. Med. Chem.* **2012**, *20*, 2214−2220.