Inorg. Chem. **2007**, *46*, 4267−4276

Phosphinic Platinum Complexes with 8-Thiotheophylline Derivatives: Synthesis, Characterization, and Antiproliferative Activity

P. Bergamini,*,† V. Bertolasi,† L. Marvelli,† A. Canella,‡ R. Gavioli,‡ N. Mantovani,† S. Mañas,§ and A. Romerosa*,§

Dipartimento di Chimica dell'Universitá di Ferrara, via L. Borsari 46, 44100 Ferrara, Italy, Dipartimento di Biochimica e Biologia Molecolare dell'Università di Ferrara, via L. Borsari 46, 44100 Ferrara, Italy, and Área de Química Inorgánica, Facultad de Ciencias Experimentales, Universidad de Almería, 04071 Almería, Spain

Received November 10, 2006

The platinum mixed-phosphine complexes (*SP*-4,2)-[PtCl(8-MTT)(PPh_3)(PTA)] (**2**) and *cis*-[Pt(8-MTT)$_2$(PPh_3)(PTA)] (**3**) ($MTTH_2$ = 8-(methylthio)theophylline, PTA = 1,3,5-triaza-7-phosphaadamantane) have been prepared from the precursor *cis*-[$PtCl_2$(PPh_3)(PTA)] (**1**), which has been fully characterized by X-ray diffraction determination. Antiproliferative activity tests indicated that the presence of one lipophilic PPh_3 and one hydrophilic PTA makes **1**–**3** more active than the analogues bearing two PPh_3 or two PTA. The reactivity of *cis*-[$PtCl_2$(PPh_3)$_2$], *cis*-[$PtCl_2$(PTA)$_2$], and *cis*-[$PtCl_2$(PPh_3)(PTA)] with the bis(thiopurines) bis(*S*-8-thiotheophylline)methane ($MBTTH_2$), 1,2-bis(*S*-8-thiotheophylline)ethane ($EBTTH_2$), and 1,3-bis(*S*-8-thiotheophylline)propane ($PBTTH_2$) has also been investigated. New binuclear complexes have been prepared and identified by spectroscopic techniques and their antiproliferative activities on T2 and SKOV3 cell lines evaluated.

Introduction

The design and synthesis of Pt(II) complexes capable of interacting with biomolecules producing a pharmacological action is still a lively topic of research, spanning from the production of new analogues of the classical Pt-containing anticancer drugs[1] to the more recent attempts to develop complexes with an anti-HIV activity.[2]

It is now well-known that the biological activities of platinum complexes originate from their interactions with intranuclear molecules, in particular from the formation of specific DNA−platinum adducts. They are responsible of deformations of the DNA double strand structure which hamper the cell replication mechanism, particularly in fast replicating populations such as cancer cells.[3] While the ideal model structure for reaching the nucleic acids inside the cell nucleus seems to be a cisplatin-resembling pattern, i.e., a neutral square planar Pt(II) complex bearing two neutral and two anionic ligands, the complex distribution and concentration in various body areas are presumably influenced by some properties of the ligands like polarity, hydrophilicity, steric requirements, etc.

In this perspective, we report here a group of new Pt−phosphine complexes bearing 8-thiotheophylline derivatives[4] (Chart 1) as anionic ligands.

Although some Pt−phosphine complexes have been tested unsuccessfully in the past as anticancer compounds,[5] we believe it is worthwhile to reconsider the issue by associating phosphines with other bioactive ligands or by using phosphines with peculiar characteristics like the protonable

* To whom correspondence should be addressed. E-mail: p.bergamini@unife.it (P.B.); romerosa@ual.es (A.R.).

† Dipartimento di Chimica dell'Università di Ferrara.

‡ Dipartimento di Biochimica e Biologia Molecolare dell'Università di Ferrara.

§ Universidad de Almería.

(1) (a) Dyson, P. J.; Sava, G. *Dalton Trans.* **2006**, 1929−1933. (b) Barnes, K. R.; Kutikov, A.; Lippard, S. J. *Chem. Biol.* **2004**, *11*, 557. (c) Ang, W. H.; Khalaila, I.; Allardyce, C. S.; Juillerat-Jeanneret, L.; Dyson, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 1382.

(2) (a) Vzorov, A. N.; Bhattacharyya, D.; Marzilli, L. G.; Compans, R. W. *Antiviral Res.* **2005**, *65* (2), 57−67. (b) Margiotta, N.; Fanizzi, F. P.; Kobe, J.; Natile, G. *Eur. J. Inorg. Chem.* **2001**, *5*, 1303−1310. (c) Cerasino, L.; Intini, F. P.; Kobe, J.; De Clercq, E.; Natile, G. *Inorg. Chim. Acta* **2003**, *344*, 174−182.

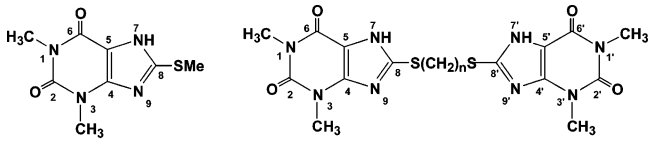
(3) (a) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153−1181. (b) Takahara, P. M.; Rosenzweig, A. C.; Frederick, C. F.; Lippard, S. J. *Nature* **1995**, *377*, 649−652. (c) Reedijk, J. *Chem Comm*, **1996**, 801−806.

(4) Romerosa, A.; López-Magaña, C.; Goeta, A. E.; Mañas, S.; Saoud, M.; Benbdelouahab, F. B.; El Guemmout, F. *Inorg. Chim. Acta* **2003**, *353*, 99−106.

(5) (a) Berners-Price, S. J.; Sadler, P. J. *Struct. Bonding* **1988**, *70*, 27−102. (b) Shi, J. C.; Cheng, L. J.; Huang, X. Y.; Wu, D. X.; Kang, B. S. *J. Organomet. Chem.* **1997**, *535*, 17−23.

10.1021/ic062133c CCC: $37.00
Published on Web 04/20/2007

Chart 1



8-MTTH, a

n = 1, $MBTTH_2$ b
n = 2, $EBTTH_2$ c
n = 3, $PBTTH_2$ d

amino−phosphine PTA (1,3,5-triaza-7-phosphaadamantane), which is at present attracting great interest in the field of metal-based drugs.[6] In a recent paper, we showed that Pt complexes containing PPh_3 and the N7-coordinated anionic ligand 8-MTT^- show on some cancer cell lines a remarkable antiproliferative activity, which decreases when PPh_3 is replaced by the more hydrophilic PTA.[7] On both cisplatin-sensitive T2 and cisplatin-resistant SKOV3 cell lines, the complex *cis*-[PtCl(8-MTT)$(PPh_3)_2$] (8-MTT^- = 8-(methylthio)theophyllinate) showed an activity comparable with cisplatin while the analogue complex *cis*-[PtCl(8-MTT)$(PTA)_2$], containing PTA ligands instead of PPh_3, displayed only a residual activity.

In this paper we expand the series of Pt−phosphine−thiopurine complexes with the aim of finding a favorable combination of ligands enhancing both the antiproliferative activity and solubility in water. Following the hypothesis that complexes with one PPh_3 and one PTA could represent the right balance between lipophilicity and hydrophilicity, we have prepared the 8-MTTH derivatives (*SP*-42)-[PtCl(8-MTT)(PPh_3)(PTA)] (**2**) and *cis*-[Pt(8-MTT)$_2(PPh_3)$(PTA)] (**3**) from *cis*-[$PtCl_2(PPh_3)$(PTA)] (**1**).

The comparative study has also been extended to new binuclear complexes bearing PPh_3, PTA, and the bis-(thiopurines): bis(*S*-8-thiotheophylline)methane ($MBTTH_2$) (Chart 1, b); 1,2-bis(*S*-8-thiotheophylline)ethane ($EBTTH_2$) (Chart 1, c); 1,3-bis(*S*-8-thiotheophylline)propane ($PBTTH_2$) (Chart 1, d). These ligands, containing two purines linked by a dithiopolymethylenic chain of variable length, are able to bind two platinum nuclei via the N7 imidazolic atom of the two linked purines. Such bis(purines) bear two coordination sites at a variable distance depending on the length of the spacing group. The study of the interaction of such ligands with metals could provide new information about the interaction between platinum and oligonucleotides. In fact the coordination of a Pt to a site of the ligand could favor the coordination of the second nucleus on the other one, or on the contrary, the two sites could display an independent reactivity and, therefore, it should be possible to obtain species where a single N7 is bonded to platinum and to synthesize heterobimetallic complexes with purinic sites bonded to different metals. At last, cooperative or synergic effects of the two metal nuclei, likely to amplify the antiproliferative activity, could be observed.

Experimental Section

Ligands 8-(methylthio)theophylline (8-$MTTH_2$), bis(*S*-8-thiotheophylline)methane ($MBTTH_2$), 1,2-bis(*S*-8-thiotheophylline)-ethane ($EBTTH_2$), and 1,3-bis(*S*-8-thiotheophylline)propane ($PBTTH_2$) have been prepared by following the procedure described by literature methods.[4,8] The ligand PTA was prepared as reported[9] as well as Pt complexes *cis*-[$PtCl_2(PPh_3)_2$] and *cis*-[$PtCl_2(PTA)_2$].[10]

All the other chemicals and solvents were used as purchased (reagent grade). Elemental analyses (C, H, N, S) were performed using a Carlo Erba instrument model EA1110. FT-IR spectra were recorded on a Nicolet 510P FT-IR instrument (4000−200 cm^{-1}) using CsI. NMR spectra were recorded on a Bruker AM spectrometer operating at 200 MHz (^1H) and 81.15 MHz (^{31}P) and a Bruker DRX300 spectrometer operating at 300.13 MHz (^1H), 75.47 MHz (^{13}C), and 121.49 MHz (^{31}P). A Varian Gemini 300 spectrometer (121.42 MHz) was also used for variable-temperature studies. Peak positions relative to tetramethylsilane, were calibrated against the residual solvent resonance (^1H) or the deuterated solvent multiplet (^{13}C), and were measured relative to external 85% H_3PO_4 with downfield values taken as positive (^{31}P). All the NMR spectra were acquired at 293 K, unless differently specified.

Synthesis of *cis*-[$PtCl_2$(PTA)(PPh_3)] (1). Solid PTA (0.09 g, 0.632 mmol) was added to a clear solution obtained by dissolving *cis*-[$PtCl_2(PPh_3)_2$] (0.5 g, 0.632 mmol) in 16 mL of CH_2Cl_2. The reaction mixture was kept under stirring for 1 h, and then the solvent was removed under vacuum. The remaining oil was treated with diethyl ether giving a white suspension, which was filtered out and dried over P_2O_5.

Crystals for X-ray determination of **1** were obtained by recrystallization of the crude product from $CHCl_3$ and Et_2O.

Yield: 0.412 g, 95%. Anal. Found: C, 41.94; H, 4.26; N, 5.76. Calcd for $C_{24}H_{27}Cl_2N_3P_2Pt$: C, 42.06; H, 3.97; N, 6.13. IR (CsI, cm^{-1}): 3049 (CH arom), 2944 ν(CH PTA); 1610, 1500, 1436 (ν(CC) Ph), 1010, 975, 950 (PTA), 280 and 258 ν(PtCl). ^1H NMR ($CDCl_3$), δ (ppm): 3.8 NCH_2N, 6H; 4.5 NCH_2P, 6H; 7−8 Ph, 15H. $^{31}P\{^1H\}$ NMR ($CDCl_3$), δ (ppm): 15.1 (d, PPh_3, $^1J_{PtP}$ = 3750 Hz); −63.3 (d, PTA, $^1J_{PtP}$ = 3195 Hz), $^2J_{PP}$ = 18 Hz.

Synthesis of (*SP*-42)-[PtCl(8-MTT)(PPh_3)(PTA)] (2). A solution containing 0.04 g of 8-MTTH (0.20 nmol) in 2 mL of 1 N NaOH was added to a suspension of *cis*-[$PtCl_2(PPh_3)$(PTA)] (0.13 g, 0.20 mmol) in CH_2Cl_2 (14 mL). The biphasic reaction mixture was kept under stirring at room temperature for 1 h, and then the two phases were separated. The water solution was extracted three times with CH_2Cl_2, and the washings were added to the organic

(6) (a) Ang, W. H.; Daldini, E.; Scolaro, C.; Scopelliti, R.; Juillerat-Jeannerat, L.; Dyson, P. J. *Inorg. Chem.* **2006**, *45*, 9006−9013. (b) Dorcier, A.; Ang, W. H.; Bolano, S.; Gonsalvi, L.; Juillerat-Jeannerat, L.; Laurenczy, G.; Peruzzini, M.; Phillips, A. D.; Zanobini, F.; Dyson, P. J. *Organometallics* **2006**, *25*, 4090−4096. (c) Romerosa, A.; Campos-Malpartida, T.; Lidrissi, C.; Saoud, M.; Serrano-Ruiz, M.; Peruzzini, M.; Garrido-Cardenas, J. A.; Garcia-Maroto, F. *Inorg. Chem.* **2006**, *45*, 1289−1298. (d) Scolaro, C.; Geldbach, T. J.; Rochat, S.; Dorcier, A.; Gossens, C.; Bergamo, A.; Cocchietto, M.; Tavernelli, I.; Sava, G.; Rothlisberger, U.; Dyson, P. J. *Organometallics* **2006**, *25*, 756−765. (e) Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T. J.; Sava, G.; Dyson, P. J. *J. Med. Chem.* **2005**, *48*, 4161−4171.

(7) Romerosa, A.; Bergamini, P.; Bertolasi, V.; Canella, A.; Cattabriga, M.; Gavioli, R.; Mañas, S.; Mantovani, N.; Pellicani, L. *Inorg. Chem.* **2004**, *43*, 905−913.

(8) (a) Romerosa, A.; López-Magaña, C.; Saoud, M.; Colacio, E.; Suárez-Varela, J. *Inorg. Chim. Acta* **2000**, *307*, 125−130. (b) Romerosa, A.; López-Magaña, C.; Saoud, M.; Mañas, S. *Eur. J. Inorg. Chem.* **2003**, 348−355.

(9) Daigle, D. J.; Decuir, T. J.; Robertson, J. B.; Darensbourg, D. J. *Inorg. Synth.* **1998**, *32*, 40−43.

(10) (a) Bailar, J. C.; Itatani, H., Jr. *Inorg. Chem.* **1965**, *11*, 1618−1620. (b) Darensbourg, D. J.; Decuir, T. J.; Stafford, N. W.; Robertson, J. B.; Draper, J. D.; Reibenspies, J. H. *Inorg. Chem.* **1997**, *36*, 4218−4226.

phase. The solution was dried over Na_2SO_4, and then the solvent was removed under vacuum. The yellow solid residue was dried over P_2O_5.

Yield: 0.06 g, 54%. Anal. Found: C, 43.82; H, 3.75; N, 10.98; S, 4.20. Calcd for $C_{32}H_{36}ClN_7O_2P_2PtS$: C, 43.88; H, 4.11; N, 11.20; S, 3.66. IR (CsI, cm^{-1}) (selected data): 1650 and 1684 ν(CO), 1527 ν(C═C) + ν(C═N). ^1H NMR (CD_2Cl_2), δ (ppm): 2.5 (SMe), 3.2 (N1Me) and 3.3 ppm (N3Me), 3.7−4.4 (PTA) and 7−8 ppm (PPh_3). $^{31}P\{^1H\}$ NMR ($CDCl_3$), δ (ppm): 11.06 (d, PPh_3, $^1J_{PtP}$ = 3817 Hz), −73.8 (d, PTA, $^1J_{PtP}$ = 2942 Hz), $^2J_{PP}$ = 21.4 Hz.

Synthesis of *cis*-[Pt(8-MTT)$_2$(PTA)(PPh$_3$)] (3). A solution containing 0.129 g of 8-MTTH (0.57 mmol) in 0.1 N NaOH (5.7 mL) was added to a suspension of *cis*-[$PtCl_2(PPh_3)$(PTA)] (0.196 g, 0.29 mmol) in 20 mL of CH_2Cl_2. The biphasic reaction mixture was treated as above-reported for **2**.

Yield: 0.192 g, 62%. Anal. Found: C, 44.7; H, 3.97; N, 13.98; S, 6.80. Calcd for $C_{40}H_{45}N_{11}O_4P_2PtS_2$: C, 45.00; H, 4.23; N, 14.47; S, 6.01. IR (CsI, cm^{-1}) (selected data): 1653 and 1684 ν(CO), 1529 ν(C═C) + ν(C═N). ^1H NMR (CD_2Cl_2), δ (ppm): 2.2 and 2.45 (2 SMe), 3.2 and 3.3 (2 N1Me), 3.45 and 3.5 (2 N3Me), 3.6−4.3 (PTA), 7.3−8.5 (PPh_3). $^{31}P\{^1H\}$ NMR ($CDCl_3$), δ (ppm): 1.6 (d, PPh_3, $^1J_{PtP}$ = 3403 Hz), −76.0 (d, PTA, $^1J_{PtP}$ = 3111 Hz), $^2J_{PP}$ =24 Hz.

Reactivity of Complex 1 with 8-MTTH$_2$. A biphasic reaction similar to the previously described synthesis of **3** was studied by extraction and $^{31}P\{^1H\}$ NMR checking of 0.3 mL aliquots every 10 min using a capillary containing C_6D_6. The fully consumption of **1** was observed after ca. 15 min to give **2** which finally evolved to **3** after 1 h.

Synthesis of *cis*-[{PtCl(PPh$_3$)$_2$}$_2$(μ-*N*,*N*-MBTT)] (4). The ligand $MBTTH_2$ (0.028 g, 0.063 mmol) was dissolved in 1.3 mL of aqueous NaOH (0.1 M) and then added dropwise to a solution of *cis*-[$PtCl_2(PPh_3)_2$] (0.1 g, 0.13 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was kept under stirring at room temperature for 4 h. In this time the organic phase became yellow, and it was then separated and dried over Na_2SO_4. The solvent was then removed under vacuum and the yellow solid residue dried over P_2O_5.

Yield: 0.116 g, 95%. Anal. Found: C, 53.48; H, 3.86; N, 5.50; S, 3.55. Calcd for $C_{87}H_{74}Cl_2N_8O_4P_4Pt_2S_2$: C, 53.69; H, 3.80; N, 5.76; S, 3.30. IR (CsI, cm^{-1}) (selected data): 1688 + 1650 ν(CO); 1526 ν(C═C) + ν(C═N). ^1H NMR ($CDCl_3$), δ (ppm): 3.1 (s, N1CH_3, 3H), 3.4 (s, N3CH_3, 9H), 5.0 (m, SCH_2S, 2H), 7.0−8.0 (m, Ph, 60H). $^{31}P\{^1H\}$ NMR ($CDCl_3$), δ (ppm): P_a + $P_{a'}$ 7.7 and 8.0 (2 d, PPh_3 trans to N7-purine, $^2J_{PP}$ = 20 Hz, $^1J_{PtP}$ = 3259 Hz); P_b + $P_{b'}$ 13.5 and 13.9 (2d, PPh_3 trans to Cl, $^2J_{PP}$ = 20 Hz, $^1J_{PtP}$ = 3811 Hz). $^{31}P\{^1H\}$ NMR (DMSO-d_6), δ (ppm): P_a + $P_{a'}$ 8.01 and 8.17 (2 d, PPh_3 trans to N7-purine, $^2J_{PP}$ = 18 Hz, $^1J_{PtP}$ = 3222 Hz); P_b + $P_{b'}$ 14.08 and 14.26 (2 d, PPh_3 trans to Cl, $^2J_{PP}$ = 18 Hz, $^1J_{PtP}$ = 3808 Hz). $^{31}P\{^1H\}$ NMR in DMSO-d_6 was checked every 10 °C from 25 to 170 °C.

Synthesis of *cis*-[{PtCl(PPh$_3$)$_2$}$_2$(μ-*N*,*N*-EBTT)] (5). The complex **5** was prepared in the same way as **4**, mixing a solution of $EBTTH_2$ (0.029 g, 0.065 mmol) in 1.3 mL of 0.1 M NaOH with a solution of *cis*-[$PtCl_2(PPh_3)_2$] (0.1 g, 0.13 mmol) in 10 mL of CH_2Cl_2.

Yield: 0.117 g, 99%. Anal. Found: C, 54.11; H, 4.10; N, 5.67; S, 3.19. Calcd for $C_{88}H_{76}Cl_2N_8O_4P_4Pt_2S_2$: C, 53.98; H, 3.88; N, 5.72; S, 3.27. IR (CsI, cm^{-1}) (selected data): 1690 + 1654 ν(CO); 1527 ν(C═C) + ν(C═N). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.2, 3.4 (2s, N1CH_3 + N3CH_3, 12H), 3.6 (m, $S_2CH_2CH_2S$, 4H), 7.0−8.0 (m, Ph, 60H). $^{31}P\{^1H\}$ NMR ($CDCl_3$), δ (ppm): P_a + $P_{a'}$ 8.0 and 8.1 (2 d, PPh_3 trans to N7-purine, $^2J_{PP}$ = 20 Hz, $^1J_{PtP}$ = 3221 Hz); P_b + $P_{b'}$ 13.5 and 13.7 (2 d, PPh_3 trans to Cl, $^2J_{PP}$ = 20 Hz, $^1J_{PtP}$ = 3830 Hz). $^{31}P\{^1H\}$ NMR (DMSO-d_6), δ (ppm): P_a + $P_{a'}$ 8.05 and 8.2 (2 d, PPh_3 trans to N7-purine, $^2J_{PP}$ = 19 Hz, $^1J_{PtP}$ = 3218 Hz); P_b + $P_{b'}$ 13.9 and 14.1 (2 d, PPh_3 trans to Cl, $^2J_{PP}$ = 19 Hz, $^1J_{PtP}$ = 3827 Hz). $^{31}P\{^1H\}$ NMR in DMSO-d_6 was checked every 10 °C from 25 to 100 °C.

Synthesis of *cis*-[{PtCl(PPh$_3$)$_2$}$_2$(μ-*N*,*N*-PBTT)] (6). The synthesis of complex **6** was achieved by the same way as **4,** using $PBTTH_2$ (0.029 g, 0.063 mmol) dissolved in 1.3 mL of 0.1 M NaOH and *cis*-[$PtCl_2(PPh_3)_2$] (0.1 g, 0.13 mmol) in 10 mL of CH_2Cl_2.

Yield: 0.122 g, 99%. Anal. Found: C, 54.37; H, 4.03; N, 5.31; S, 3.74. Calcd for $C_{89}H_{78}Cl_2N_8O_4P_4Pt_2S_2$: C, 54.14; H, 3.95; N, 5.68; S, 3.24. IR (CsI, cm^{-1}) (selected data): 1688 + 1649 ν(CO); 1527 ν(C═C) + ν(C═N). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.24 (qt, $SCH_2CH_2CH_2S$, 2H), 3.31, 3.32 (2s, N1-CH_3, 6H), 3.40 (s, N3-CH_3, 6H), 3.41 ($SCH_2CH_2CH_2S$, 4H), 7.0−8.0 (m, Ph, 60H). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.42 MHz), δ (ppm): P_a + $P_{a'}$ 8.35 and 8.38 (d, PPh_3 trans to N7-purine, $^2J_{PP}$ = 19.2 Hz, $^1J_{PtP}$ = 3240 Hz), P_b + $P_{b'}$ 14.01 and 14.03 (d, PPh_3 trans to Cl, $^2J_{PP}$ = 19.2 Hz, $^1J_{PtP}$ = 3831 Hz).

Reactivity in 1:1:1 and 1:2:1 Molar Ratio of *cis*-[PtCl$_2$(PPh$_3$)$_2$], NaOH, and Respectively MBTTH$_2$, EBTTH$_2$, and PBTTH$_2$. In a 1:1:1 ratio experiment, $MBTTH_2$ (0.028 g, 0.063 mmol) was dissolved in 0.65 mL of aqueous 0.1 M NaOH (0.065 mmol) and the resulting solution added to *cis*-[$PtCl_2(PPh_3)_2$] (0.05 g, 0.063 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was treated as above. From the organic phase **4** was recovered (0.060 g, 48% based on the ligand).

Similar experiments were carried out using $EBTTH_2$ and $PBTTH_2$: $EBTTH_2$ gave **5** (42%); $PBTTH_2$ gave **6** (43%).

In the same way three experiments in 1:2:1 molar ratio were performed and gave respectively **4** (48%), **5** (47%), and **6** (49%).

Synthesis of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-MBTT)]Cl (7). The ligand $MBTTH_2$ (0.050 g, 0.114 mmol) was suspended in 10 mL of water, and 2.3 mL of aqueous NaOH (0.1 M) was added leading to a colorless solution. Addition of solid *cis*-[$PtCl_2(PTA)_2$] (0.134 g, 0.23 mmol) gave a pale yellow suspension. After 18 h, a yellow solid was obtained by evaporation of the solvent under reduced pressure. The solid was extracted with CH_2Cl_2 (3 × 3 mL) and the solution filtered and taken to dryness, leaving the product as a yellow solid.

Yield: 0.133 g, 77%. Anal. Found: C, 30.47; H, 4.35; N, 18.37; S, 3.99. Calcd for $C_{39}H_{62}Cl_2N_{20}O_4P_4Pt_2S_2$: C, 30.73; H, 4.07; N, 18.38; S, 4.20. IR (CsI, cm^{-1}) (selected data): 1685 + 1639 ν(CO); 1527 ν(C═C) + ν(C═N). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.32−3.55 (8 s, N1−CH_3 + N3−CH_3, 4 × 3 H); 3.88−4.5 (m, PTA, 48 H); 4.21 (s; SCH_2S, 2 H). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.49 MHz), δ (ppm): P_a −68.5 (m, PTA trans to N7(purine), $^1J_{PtP}$ = 2950 Hz), P_b −57.7 (m, PTA trans to Cl, $^1J_{PtP}$ = 3378 Hz). $^{31}P\{^1H\}$ NMR in $CDCl_3$ was checked every 10 °C from 50 to −60 °C. $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.47 MHz), δ (ppm): 27.99 (s, N1−CH_3); 30.01 (s, N3−CH_3); 35.85 (SCH_2); 52.3 (NCH_2 PTA); 56.05 (PCH_2 PTA, $^1J_{PC}$ = 50.9 Hz); 114.60 (s, C5); 149.093 (s, C4); 150.52 (s, C8); 151.78 (s, C6); 155.85 (s, C2)

Anion Exchange of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-MBTT)]Cl (7) with NaBPh$_4$. Synthesis of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-MBTT)](BPh$_4$) (7BPh$_4$). A solution of **7** (0.05 g, 0.033 mmol) in 2 mL of MeOH was added to a solution (1 mL) of $NaBPh_4$ (0.012 g, 0.035 mmol) in the same solvent. As soon as the solutions were mixed, a white solid began to precipitate. After 1 h the precipitate was filtered out, washed with Et_2O (2 × 1 mL), and air-dried. The ^{31}P NMR in $CDCl_3$ allowed us to identify the product as the BPh_4^- salt of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-MBTT)]$^+$.

Yield: 97%. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.49 MHz), δ (ppm): P_a −67.8 (m, PTA trans to N7(purine), $^1J_{PtP}$ = 2895 Hz), P_b −56.8 (m, PTA trans to Cl, $^1J_{PtP}$ = 3415 Hz).

Synthesis of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-EBTT)]Cl (8). Complex **8** was prepared by reaction of $EDTTH_2$ (0.050 g, 0.110 mmol) dissolved in 2.2 mL of 0.1 M NaOH with *cis*-[$PtCl_2(PTA)_2$] (0.129 g, 0.22 mmol) in 10 mL of water. After 18 h under stirring, the solvent was eliminated by reduced pressure. The yellow solid residue was extracted with CH_2Cl_2 (10 mL), and the obtained yellow solution was then reduced to 1.0 mL. The addition of 5 mL of Et_2O induced the precipitation of a yellow solid, which was filtered out, washed with Et_2O (2 × 2 mL), and air-dried.

Yield: 0.06 g, 36%. Anal. Found: C, 31.17; H, 4.17; N, 18.24; S, 4.01. Calcd for $C_{40}H_{64}Cl_2N_{20}O_4P_4Pt_2S_2$: C, 31.23; H, 4.16; N, 18.22; S, 4.16. IR (CsI, cm^{-1}) (selected data): 1685 + 1644 ν(CO); 1526 ν(C═C) + ν(C═N). ^1H NMR (CD_2Cl_2, 300.13 MHz), δ (ppm): 1.92 (s, SCH_2CH_2S, 4 H), 3.36 (s, N1−CH_3, 6 H); 3.48, 3.54 (2 s, N3−CH_3, 6 H); 3.65−4.58 (m, PTA, 48 H). $^{31}P\{^1H\}$ NMR (CD_2Cl_2, 121.49 MHz), δ (ppm): $P_a + P_{a'}$ −69.1 (m, PTA trans to N7(purine), $^1J_{PtP}$ = 2847 Hz), $P_b + P_{b'}$ −57.9 (m, PTA trans to Cl, $^1J_{PtP}$ = 3460). $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 75.47 MHz), δ (ppm): 27.99 (s, N1−CH_3); 28.04 (s, N3−CH_3); 34.22 (s, SCH_2-CH_2); 35.86 (CH_2CH_2S); 52.3 (NCH_2 PTA); 56.0 (PCH_2 PTA, $^1J_{PC}$ = 50.9 Hz); 114.65 (s, C5); 149.09 (s, C8); 150.53 (s, C6); 151.76 (s, C4); 155.84 (s, C2)

Reactivity of $EBTTH_2$ with NaOH and *cis*-[$PtCl_2(PTA)_2$]. Observation of [{PtCl(PTA)$_2$}$_2$(μ-*N*,*N*-EBTT)] (8a). The reaction of $EBTTH_2$ (0.050 g, 0.110 mmol) with 2.2 mL of 0.1 M NaOH and *cis*-[$PtCl_2(PTA)_2$] (0.129 g, 0.22 mmol) in 10 mL of water produced a yellow solution. After overnight stirring it was taken to dryness leaving a yellow residue. The NMR analysis of the solid dissolved in CD_2Cl_2 showed the presence of two products: **8** (80%) and [{PtCl(PTA)$_2$}$_2$(μ-*N*,*N*-EBTT)] **(8a)** (20%). $^{31}P\{^1H\}$ NMR (CD_2Cl_2, 121.49 MHz), δ (ppm): **8**, see above for data; *cis*-[{PtCl-(PTA)$_2$}$_2$(μ-*N*,*N*-EBTT)], **8a**, $P_a + P_{a'}$ −71.6 (bt, PTA trans to N7-(purine), $^1J_{PtP}$ = 2940 Hz), $P_b + P_{b'}$ −57.8 (bt, PTA trans to Cl, $^1J_{PtP}$ = 3329 Hz). $^{31}P\{^1H\}$ NMR in CD_2Cl_2 was checked every 10 °C from 50 to −60 °C.

Synthesis of *cis*-[{Pt(PTA)$_2$}$_2$(μ-Cl)(μ-*N*,*N*-PBTT)]Cl (9). Complex **9** was prepared as above-described for **7**, using $PDTTH_2$ (0.040 g, 0.086 mmol) dissolved in 1.72 mL of aqueous NaOH (0.1 M) and *cis*-[$PtCl_2(PTA)_2$] (0.100 g, 0.172 mmol) in 10 mL of water.

Yield: 0.165 g, 0.106 mmol, 62%. Anal. Found: C, 31.80; H, 4.33; N, 18.21; S, 4.52. Calcd for $C_{41}H_{66}Cl_2N_{20}O_4P_4Pt_2S_2$: C, 31.75; H, 4.25; N, 18.05; S, 4.13. IR (CsI, cm^{-1}) (selected data): 1685 + 1644 ν(CO); 1526 ν(C═C) + ν(C═N). ^1H NMR ($CDCl_3$, 300.13 MHz), δ (ppm): 2.034 (bs, SCH_2CH_2, 2H); 3.39 (bm, SCH_2CH_2, 4H), 3.32 (s, N1−CH_3, 6 H); 3.54 (s, N3−CH_3, 6 H); 3.87−4.56 (m, PTA, 48 H). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.49 MHz), δ (ppm): $P_a + P_{a'}$ −68.72 ($^1J_{PtP}$ = 3016 Hz) and −71.4 ($^1J_{PtP}$ = 2990 Hz) (2m, PTA trans to N7(purine), $P_b + P_{b'}$ −57.5 ($^1J_{PtP}$ = 3387 Hz) and −58.1 ($^1J_{PtP}$ = 3396 Hz) (2m, PTA trans to Cl). $^{31}P\{^1H\}$ NMR in $CDCl_3$ was checked every 10 °C from 50 to −60 °C. $^{13}C\{^1H\}$ NMR (CD_2Cl_2, 75.47 MHz), δ (ppm): 28.06 (bs, N1−CH_3); 30.10 (bs, N3−CH_3); 32.00 (bs, SCH_2CH_2−); 32.20 (bs, SCH_2CH_2−); 52.70 (m, NCH_2 PTA); 56.0 (d, PCH_2 PTA, $^1J_{PC}$ = 50.6 Hz); 112.20 (bs, C5); 150.78 (bs, C4); 151.86 (bs, C8); 152.03 (bs, C6); 155.90 (bs, C2).

Reactivity of *cis*-[$PtCl_2(PPh_3)$(PTA)] with NaOH and Respectively MBTTH$_2$, EBTTH$_2$, and PBTTH$_2$. In three parallel experiments, $MBTTH_2$ (0.050 g, 0.114 mmol), $EBTTH_2$ (0.050 g, 0.110 mmol), and $PBTTH_2$ (0.050 g, 0.110 mmol) dissolved in 2.3 mL of 0.1 M NaOH (0.23 mmol) were mixed with a solution of *cis*-[$PtCl_2(PPh_3)$(PTA)] (0.16 g, 0.228 mmol) in 10 mL of CH_2Cl_2. After 18 h, the $^{31}P\{^1H\}$ NMR inspection of aliquots of the organic phase of each reaction showed the formation of a mixture of isomeric platinum complexes.

$^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.49 MHz), δ (ppm) data for detectable species:

(i) Reaction with $MBTTH_2$: 10a, [{Pt(PPh_3)(PTA)Cl}$_2$(μ-*N*,*N*-MBTT)] (66%) $P_a + P_{a'}$ −73.6 and −73.9 (2d, PTA trans N7-(purine), $^1J_{PtP}$ = 2975 Hz), $P_b + P_{b'}$ 10.7 and 10.9 (2d, PPh_3 trans Cl, $^1J_{PtP}$ = 3790 Hz), $^2J_{PP}$ = 20 Hz; **10b**, [{Pt(PPh_3)(PTA)Cl}-[{Pt(PTA)(PPh_3)Cl}(μ-*N*,*N*-MBTT)] (ca. 20%) P_a −64.7 (2d, PTA trans to Cl, $^1J_{PtP}$ = 3424 Hz) and $P_{a'}$ −76.0 (2d, PTA trans to N, $^1J_{PtP}$ = 2900 Hz), P_b 7.0 (2d, PPh_3 trans to N, $^1J_{PtP}$ = 3314 Hz), $^2J_{PP}$ = 20 Hz.

(ii) Reaction with $EBTTH_2$: 11a, [{Pt(PPh_3)(PTA)Cl}$_2$(μ-*N*,*N*-EBTT)] (64%) $P_a + P_{a'}$ −73.5 and −74.0 (2d, PTA trans to N7-(purine), $^1J_{PtP}$ ca. 2950 Hz), $P_b + P_{b'}$ 10.7 and 10.9 (2d, PPh_3 trans to Cl, $^1J_{PtP}$ = ca. 3810 Hz), $^2J_{PP}$ = 22 Hz; **11b**, [{Pt(PPh_3)(PTA)-Cl}[{Pt(PTA)(PPh_3)Cl}(μ-*N*,*N*-EBTT)] (16%) P_a −64.7 (2d, PTA trans to Cl, $^1J_{PtP}$ = 3408 Hz) and $P_{a'}$ −76.7 (2d, PTA trans to N, $^1J_{PtP}$ = 2933 Hz), P_b 7.0 (2d, PPh_3 trans to N, $^1J_{PtP}$ = 3244 Hz), $^2J_{PP}$ = 20 Hz.

(iii) Reaction with $PBTTH_2$: 12a, [{Pt(PPh_3)(PTA)Cl}$_2$(μ-*N*,*N*-PBTT)] (ca. 70%) $P_a + P_{a'}$ −73.7 (m, PTA trans to N7, $^1J_{PtP}$ = 2961 Hz) −76.7 (m, PTA trans to N7, $^1J_{PtP}$ = 2924 Hz), $P_b + P_{b'}$ 10.8 (m, PPh_3 trans to Cl, $^1J_{PtP}$ = 3798 Hz), together with other unidentified species.

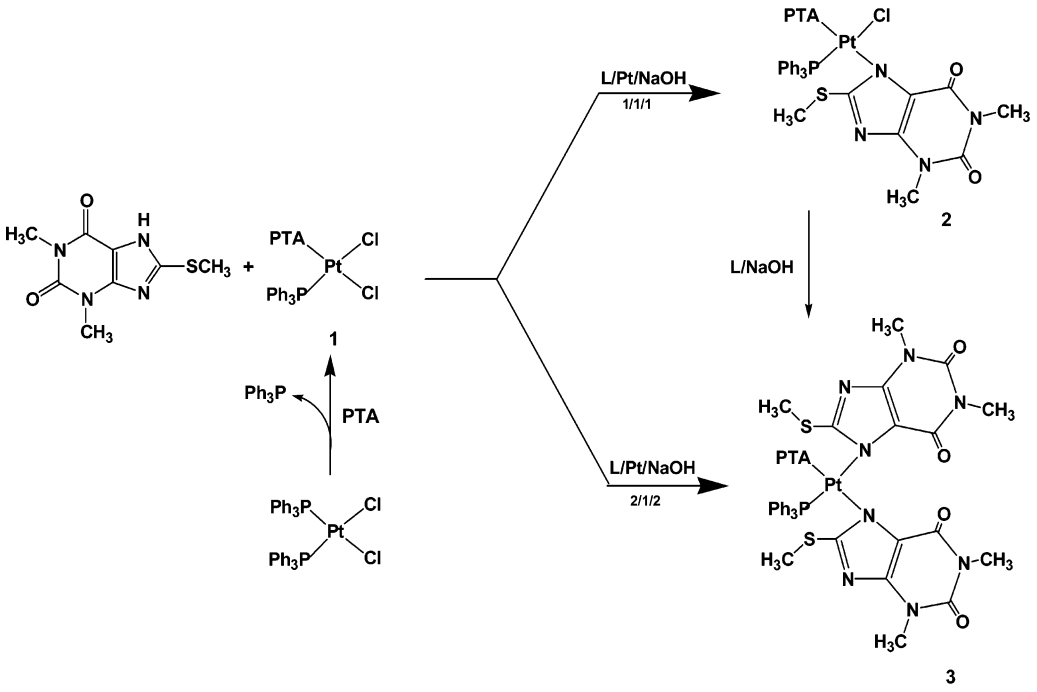
Table 1. Crystallographic Data for **1**

formula	$C_{24}H_{27}Cl_2N_3P_2Pt$
M_r	685.42
space group	$P\bar{1}$
cryst system	triclinic
a/Å	9.8553(1)
b/Å	10.5419(2)
c/Å	13.0706(3)
α/deg	84.685(1)
β/deg	72.933(1)
γ/deg	70.871(1)
V/Å^3	1226.46(4)
Z	2
D_c/g cm^{-3}	1.856
F(000)	668
μ(Mo Kα)/cm^{-1}	60.87
measd reflcns	19 814
unique reflcns	7031
R_{int}	0.068
obsd reflcns [$I \geq 2\sigma(I)$]	6355
$\theta_{min}-\theta_{max}$/deg	3.2−30.0
hkl ranges	−13, 13; −13, 14; −17, 18
R(F^2) (obsd reflcns)	0.0330
wR(F^2) (all reflcns)	0.0825
no. of variables	290
goodness of fit	1.051
ρ_{min}, ρ_{max}/e Å^{-3}	−1.70; 1.57

X-ray Crystal Structure Determination. X-ray diffraction data for compound **1** (Table 1) were collected on a Nonius Kappa CCD diffractometer, at room temperature (T = 295 K), with graphite-monochromated Mo Kα radiation (λ = 0.7107 Å) and corrected for Lorentz, polarization, and absorption (SORTAV)[11] effects. The structure was solved by direct methods (SIR97)[12] and refined (SHELXL-97)[13] by full-matrix least squares with anisotropic non-H and hydrogen atoms on calculated positions, riding on their carrier atoms.

(11) Blessing, R. H. *Acta Crystallogr., Sect. A* **1995**, *51*, 33.
(12) 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.

Chart 2



Growth Inhibition Assays. Cell growth inhibition assays were carried out using the cisplatin-sensitive T2 human cell line and the cisplatin-resistant SKOV3 cell line. T2 is a cell hybrid obtained by the fusion of the human lynphoblastoid line 174 (B lynphocyte transformed by the Epstein−Barr virus) with the CEM human cancer line (leukaemia T) while SKOV3 is derived from a human ovarian tumor. The cells were seeded in triplicate in 96-well trays at a density of 50×10^3 in 50 μL of AIM-V medium for T2 and 25×10^3 in 50 μL of AIM-V medium for SKOV3. Stock solutions (10 mM) of the Pt(II) complexes were made in DMSO and diluted in AIM-V medium to give final concentration of 2, 10, and 50 μM. Cisplatin was employed as a control for the cisplatin-sensitive T2 cell line and for the cisplatin-resistant SKOV3. Untreated cells were placed in every plate as a negative control. The cells were exposed to the compounds for 72 h, and then 25 μL of a (4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide solution (12 mM) was added. After 2 h of incubation, 100 μL of lysing buffer (50% DMF + 20% SDS, pH 4.7) was added to convert (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into a brown formazane. After an additional 18 h the solution absorbance, proportional to the number of live cells, was measured by spectrophotometer and converted in % of growth inhibition.[14]

Results and Discussion

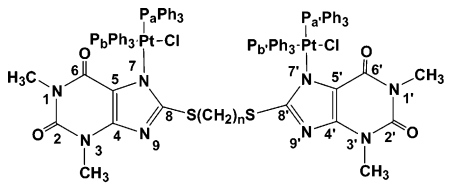
Complexes Containing 8-MTTH. We have recently reported[7] the lipophilic thiopurinic complexes *cis*-[PtCl(8-MTT)$(PPh_3)_2$] (**I**) and *cis*-[Pt(8-MTT)$_2(PPh_3)_2$] (**II**), which display antiproliferative activity either on cisplatin-sensitive T2 or on cisplatin-resistant SKOV3 cell lines, while the more hydrophilic analogues *cis*-[PtCl(8-MTT)$(PTA)_2$] (**III**) and *cis*-[Pt(8-MTT)$_2(PTA)_2$] (**IV**) are less active on the same cell lines.

Trying to find a convenient compromise between the lipophilicity which seems to be associated with a higher activity and the hydrophilicity generally welcomed for a convenient drug formulation and administration,[15] we reasoned that the mixed analogues with one PPh_3 and one PTA could represent the right balance between the two types and therefore we report here the preparation of (*SP*-42)-[PtCl(8-MTT)(PPh_3)(PTA)] (**2**) and *cis*-[Pt(8-MTT)$_2(PPh_3)$(PTA)] (**3**) from their precursor, the new complex *cis*-[$PtCl_2(PPh_3)$(PTA)] (**1**) (Chart 2).

We found that the most convenient way to obtain the new precursor **1** is the treatment of *cis*-[$PtCl_2(PPh_3)_2$] with 1 equiv of PTA in dichloromethane. The more nucleophilic PTA replaced one PPh_3 molecule giving **1** that was isolated by quantitative precipitation with ether and was characterized spectroscopically and through the determination of its X-ray crystal structure. The $^{31}P\{^1H\}$ NMR spectrum of **1** in $CDCl_3$ shows two coupled doublets ($^2J_{PP} = 18$ Hz), the first due to coordinated PPh_3 at 15.1 ppm ($^1J_{PtP} = 3750$ Hz) and the second to PTA at −63.3 ($^1J_{PtP} = 3195$ Hz). It is interesting to point out that the $^1J_{PtP}$ for coordinated PTA is ca. 500 Hz smaller than that for PPh_3 although both phosphines are trans to chloride. This significant difference could be the consequence of the smaller size of PTA cone angle (102°) with respect to PPh_3 (145°).[16] The ^1H NMR spectrum of **1** displays few broad signals for PTA aliphatic protons at 3.8 ppm (NCH_2N) and 4.5 ppm (NCH_2P) and a 5:4 relative integrals ratio for aromatic versus aliphatic protons. The IR shows

(13) Sheldrick, G. M. *SHELXL-97, Program for Crystal Structures Refinement*; University of Göttingen: Göttingen, Germany, 1997.
(14) Hansen, M. B.; Nielsen, S. E.; Berg, K. *J. Immunol. Methods* **1989**, *119*, 203.
(15) Henderson, W.; McCaffrey, L. J.; Nicholson, B. K. *J. Chem. Soc., Dalton Trans.* **2000**, 2753−2760.
(16) Assefa, Z.; McBurnett, B. G.; Staples, R. J.; Fackler, J. P.; Assmann, B., Jr.; Angermaier, K.; Schmidbaur, H. *Inorg. Chem.* **1995**, *34*, 75−83.

Chart 3



two characteristic PtCl stretching signals at 280 and 258 cm^{-1}, which are consistent with a cis geometry.[17]

The thiopurinic complexes **2** and **3** were prepared from **1** by reaction respectively with 1 and 2 equiv of 8-MTTH and NaOH in a double phase mixture of dichloromethane and water (Chart 2). In their ^{1}H NMR spectrum, the signals due to coordinated 8-MTT$^-$ are present: for **2** three singlets at 2.5 (SMe), 3.2 (N1Me), and 3.3 (N3Me) ppm are observed, while for **3** two close sets of signals are visible belonging to the two inequivalent thiopurines (trans to PPh_3 and trans to PTA): there are two singlets at 2.2 and 2.45 ppm (SMe), two singlets at 3.2 and 3.3 ppm (N1Me), and two singlets at 3.45 and 3.5 ppm (N3Me).

The synthesis of **3** by reaction of **1** with 2 equiv of 8-MTTH was followed by ^{31}P{^{1}H} NMR. A two step process was observed: the first one, complete in about 15 min, led to **2** through the substitution of the chloride trans to PTA by MTT$^-$, as indicated by the 10 ppm upfield shift of the PTA signal (from −63.3 ppm in **1** to −73.8 ppm in **2**). The second step provided **3** in a further 60 min by substitution of the residual chloride by the remaining equiv of deprotonated ligand MTT$^-$.

Reactivity of Bis(thiopurines) MBTTH$_2$, EBTTH$_2$, and PBTTH$_2$ with Pt Phosphine Complexes. (a) Reactions with *cis*-[PtCl$_2$(PPh$_3$)$_2$]: Synthesis of 4−6. The reactions of MBTTH$_2$, EBTTH$_2$, or PBTTH$_2$ in CH_2Cl_2/H_2O with 2 equiv of NaOH and 2 equiv of *cis*-[PtCl$_2$(PPh$_3$)$_2$] gave the binuclear complexes **4**−**6**, respectively (Chart 3).

The ^{31}P{^{1}H} NMR for complex **4** displays two partially overlapped sets of signals, each made up by two doublets with satellites at 7.7, 8.0 (PPh_3 trans to N7-purine, $^2J_{PP}$ = 20.0, $^1J_{PtP}$ = 3259 Hz) and 13.5, 13.9 ppm (PPh_3 trans to Cl, $^2J_{PP}$ = 20.0 Hz, $^1J_{PtP}$ = 3811 Hz).

The assignment of signals to P trans to Cl (downfield shift, larger $^1J_{PtP}$ coupling constant) and P trans to N (upfield shift, smaller $^1J_{PtP}$ coupling constant) is based on the comparison with the data of the previously reported mononuclear complexes *cis*-[PtCl$_2$(PPh$_3$)$_2$], *cis*-[PtCl(8-MTT)(PPh$_3$)$_2$], and *cis*-[Pt(8-MTT)$_2$(PPh$_3$)$_2$].[7] The two sets of signals show very close chemical shifts and undistinguishable values for coupling constants. We believe this feature is due to the inequivalence of the corresponding phosphorus nuclei bonded to different platinum centers ($P_a \neq P_{a'}$, $P_b \neq P_{b'}$).

It is known[4] that, in the ligand MBTTH$_2$ itself, the two thiotheophyllinic moieties are magnetically inequivalent at room temperature. Variable-temperature NMR experiments showed that the inequivalence survives in solution up to 110 °C in DMSO-d_6 due to a high conformational barrier. Finally, the X-ray crystal structure for MBTTH$_2$ gave evidence for a stacking interaction between the two thiotheophyllines. To confirm the hypothesis of a rotational origin also for the inequivalence of the two sides observed for **4**, a variable-temperature ^{31}P{^{1}H} NMR experiment was carried out for a solution of **4** in DMSO-d_6 by running the spectrum every 10 °C from 25 up to 170 °C. The complete coalescence of the two sets of signals was not obtained, but it was only partial at 170 °C.

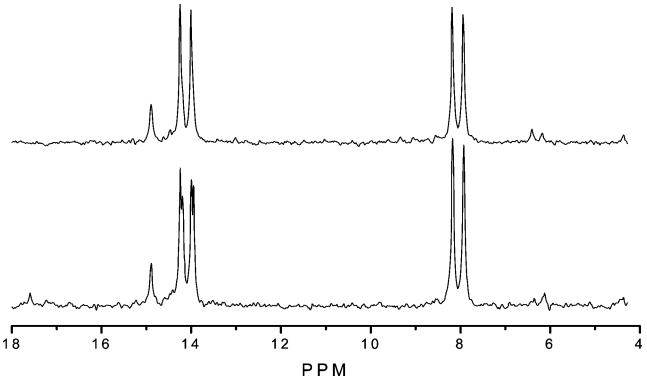


Figure 1. ^{31}P{^{1}H} NMR of **5** in DMSO-d_6 at 25 °C (lower trace) and 80 °C (upper trace).

The identity of **4** is supported also by the IR spectrum that shows absorptions for the thiopurine and PPh_3 but no bands for ν(NH). The ^{1}H NMR displays signals for MBTTH$_2$ and phosphines with an integral ratio in agreement with the proposed molecular composition. The SCH_2S group signal arises at 5.00 ppm, very close to the corresponding signal of the free ligand (5.12 ppm).[4]

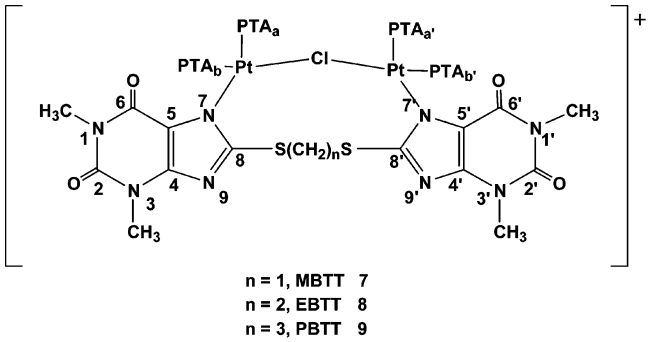
The longer central chain in EBTTH$_2$ and PBTTH$_2$ allows a major conformational freedom, and therefore, for the free ligands the inequivalence of the two extremities of the bis-(purine) molecule was not observed by ^{1}H NMR at room temperature.[4] On the contrary, both complexes **5** and **6** show in ^{31}P{^{1}H} NMR a double pattern of signals resembling the one just mentioned for **4**, as a consequence of the loss of conformational freedom upon the coordination of two hindered [PtCl(PPh$_3$)$_2$]$^+$ units. The two sets of signals in the ^{31}P{^{1}H} NMR ($CDCl_3$) of **5** are closer in chemical shift than the corresponding ones in **4** (the differences ($\Delta(\delta)$) in ppm between the same signal in the two sets are the following: for **5**, $\Delta(\delta)PPh_3-Pt-Cl$ = 0.1 and $\Delta(\delta)PPh_3-Pt-N7$ = 0.2; for **4**, $\Delta(\delta)PPh_3-Pt-Cl$ = 0.3 and $\Delta(\delta)PPh_3-Pt-N7$ = 0.4). For **5** the coalescence of the two sets was observed in DMSO-d_6 at about 80 °C (Figure 1).

Similarly, complex **6** shows a single ^{31}P{^{1}H} NMR pattern at 81.15 MHz, but at 121.42 MHz the signals show some splitting indicating the presence of a second almost coincident pattern (**6**: $\Delta(\delta)PPh_3-Pt-Cl$ = 0.02 and $\Delta(\delta)PPh_3-Pt-N7$ = 0.03).

We have performed a series of experiments trying to obtain mononuclear complexes to be subsequently used as starting materials for heterobimetallic complexes. The choice of

(17) (a) Adams, D. M.; Chatt, J.; Gerrat, J.; Westland, A. D. *J. Chem. Soc.* **1964**, 734−739. (b) Gillard, R. D.; Pilbrow, M. F. *J. Chem. Soc., Dalton Trans.* **1974**, 2320−2325.

Chart 4



n = 1, MBTT 7
n = 2, EBTT 8
n = 3, PBTT 9

$MBTTH_2$ is particularly convenient because the mono- and bimetallic species should be easily distinguishable by $^{31}P\{^1H\}$ NMR. In fact, while the above-described binuclear complex **4** shows two close but clearly separated sets of signals, the expected mononuclear complex should be characterized by a single pattern. When we tried to obtain the mononuclear 8-membered chelate, the stoichiometric ratio of the reaction among $MBTTH_2$, *cis*-$[PtCl_2(PPh_3)_2]$, and NaOH was at first set at 1:1:2, but also under these conditions, the above-described binuclear complex **4** was obtained as the only Pt-containing product in less than 50% yield. Analogue experiments with $EBTTH_2$ and $PBTTH_2$ gave the respective products in less than 50% yields.

A further confirmation of the tendency of these ligands to give binuclear complexes was provided by the reaction of L/*cis*-$[PtCl_2(PPh_3)_2]$/NaOH in 1:1:1 ratio (L = $MBTTH_2$, $EBTTH_2$, or $PBTTH_2$), which gave for each ligand 0.5 equiv of the respective binuclear platinum complex. Therefore, it is possible to conclude that the two imidazolic N7 atoms of the two sides in $MBTTH_2$, $EBTTH_2$, and $PBTTH_2$ do not have independent reactivity with *cis*-$[PtCl_2(PPh_3)_2]$, but the coordination of the first Pt seems to favor the entrance of the second, the two steps occurring nearly simultaneously. As a consequence, it was not possible to obtain monoplatinum or heterobimetallic complexes in this way. It is worth noticing that we have previously reported[4] that the reaction of $MBTTH_2$ with *cis*-$[PdCl_2(PPh_3)_2]$ and NaOH under similar reaction conditions produced exclusively the dipalladium complex *trans*-$[\{PdCl(PPh_3)_2\}_2(\mu\text{-}N,N\text{-MBTT})]$. This sort of "cooperative effect" between two metal nuclei may be involved in multiple-platinum coordination by oligonucleotides or nucleic acids interacting with platinum-based drugs.

(b) Reactions with *cis*-$[PtCl_2(PTA)_2]$: Synthesis of 7−9. Reactions of $MBTTH_2$, $EBTTH_2$, and $PBTTH_2$ in H_2O with 2 equiv of NaOH and *cis*-$[PtCl_2(PTA)_2]$ allowed us to prepare the cationic platinum/PTA complexes **7**−**9**, in which two metal nuclei are bridged by a chloride atom and a bis(thiopurinic) dianion through its imidazolic N7 atoms (Chart 4).

Compound **7**, obtained from $MBTTH_2$, displays a $^{31}P\{^1H\}$ NMR characterized by two groups of very close signals, one at −57.7 ppm ($^1J_{PtP}$ = 3378 Hz), assigned to PTA trans to chloride, and the second one at −68.5 ppm ($^1J_{PtP}$ = 2950 Hz), attributed to PTA trans to N7, on the basis of a comparison with chemical shifts in **2** and **3** (PTA trans to chloride downfield with respect to PTA trans to N7). Variable-temperature NMR experiments from 40 to −60 °C showed further splitting of the signals indicating the existence of an electronic relation between the two $\{(PTA)_2Pt\}$ moieties. This hypothesis was confirmed by a $^{31}P-^{31}P\{^1H\}$ COSY experiment which showed a clear coupling ($^4J_{Pa/Pa'}$ = ca. 8 Hz) between phosphorus nuclei bonded to different Pt centers.

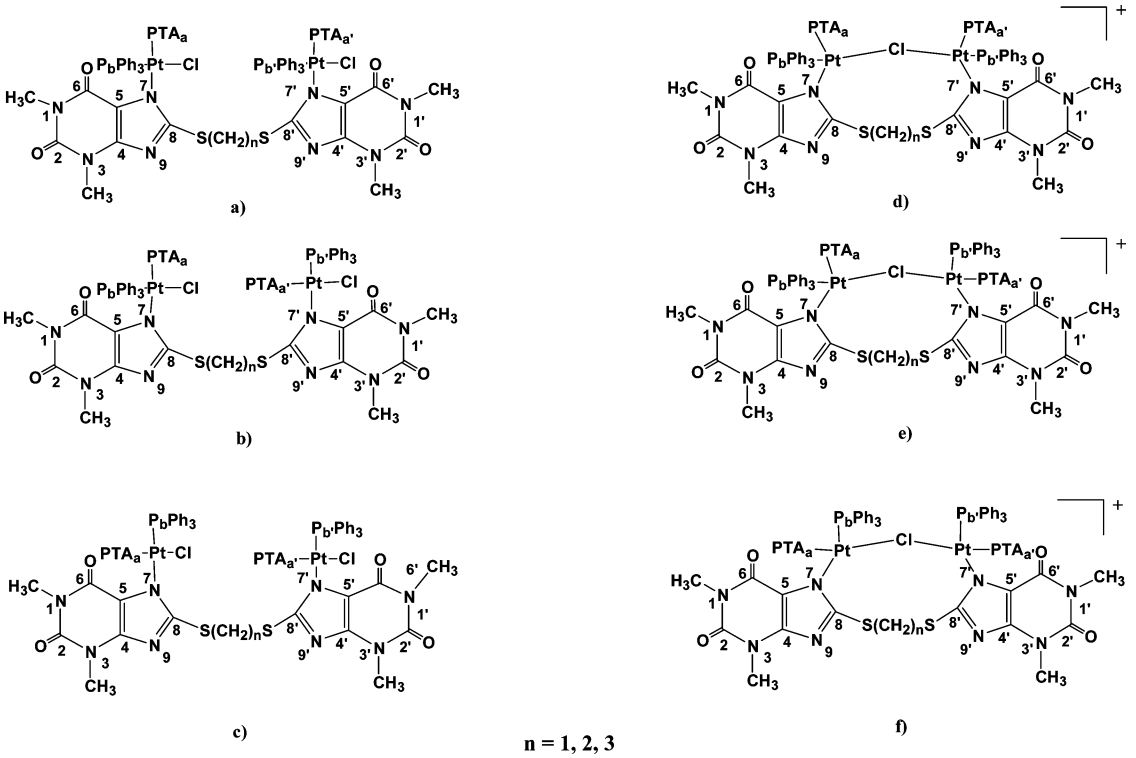
The cationic structure *cis*-$[\{Pt(PTA)_2\}_2(\mu\text{-Cl})(\mu\text{-}N,N\text{-MBTT})]^+$ (**7** in Chart 4), in which two platinum moieties are bridged by a chloride that electronically connects the two metal nuclei, is consistent with the above-reported NMR observations, allowing coupling through a four-bonds distance. The cationic nature of the complex was confirmed also by mixing a solution of the chloride of **7** with $NaBPh_4$ in MeOH, producing the precipitation of the insoluble complex *cis*-$[\{Pt(PTA)_2\}_2(\mu\text{-Cl})(\mu\text{-}N,N\text{-MBTT})](BPh_4)$ (**7BPh$_4$**).

For compound **8**, a $^{31}P\{^1H\}$ NMR pattern similar to that of **7** was observed and therefore a similar structure was proposed (Chart 4). Nevertheless the NMR observation of the crude reaction mixture of $EBTTH_2$ with NaOH and *cis*-$[PtCl_2(PTA)_2]$ showed the formation of an additional species (**8a**) together with **8**. The NMR features of **8a** are similar to those found for above-described bis-$\{(PPh_3)_2Pt\}$ complexes (**4**−**6**) indicating a noncyclic neutral structure with nonequivalent extremities: the $^{31}P\{^1H\}$ NMR of **8a** is constituted by two pseudotriplets in the chemical shift range characteristic for coordinated PTA trans to Cl (ca. −57.8, $^1J_{PtP}$ = 3329 Hz) and trans to N7 (ca. −71.6, $^1J_{PtP}$ = 2940 Hz). The signals become two doublets of doublets at −40 °C. Considering that **8** and **8a** are formed in a constant ratio, which does not change with the temperature, and the solution of pure **8** does not show any sign of formation of **8a** in 10 days, we can conclude that **8** and **8a** are not in equilibrium but originate from different formation pathways.

Similarly, complex **9** was isolated from the reaction of $PBTTH_2$ with NaOH and *cis*-$[PtCl_2(PTA)_2]$. It displays a $^{31}P\{^1H\}$ NMR constituted by two multiplets of PTA trans to N7 at −68.7 ($^1J_{PtP}$ = 3016) and −71.4 ($^1J_{PtP}$ = 2990) ($P_a + P_{a'}$) and two multiplets of PTA trans to Cl at −57.5 ($^1J_{PtP}$ = 3387 Hz) and −58.1 ($^1J_{PtP}$ = 3396 Hz) ($P_b + P_{b'}$). This feature is due to two partially overlapped sets of signals with satellites. Variable-temperature NMR experiments in $CDCl_3$ did not show signals of coalescence over a temperature range from +50 to −60 °C. A 2D-$^{31}P\{^1H\}$ COSY experiment in $CDCl_3$ showed also in this case cross-peaks between the signals at −68.72 and −71.4 and between the signals at −57.5 and −58.1 ppm, showing a through-bond electronic connexion between the two coordination spheres. This result allows us to confirm the cationic chloride-bridged structure also for **9**.

(c) Reactions with *cis*-$[PtCl_2(PPh_3)PTA]$. In principle, the geometrical isomers obtainable from the reaction of bis(thiopurine) with *cis*-$[PtCl_2(PPh_3)PTA]$ (**1**) are three for the open form (*a*−*c*) and three for the cationic cyclic form (*d*−*f*) (*a* and *d*, both Ph_3P trans to chloride; *b* and *e*, one PPh_3

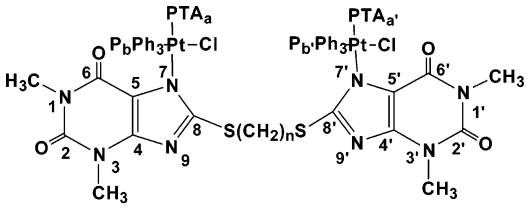
Chart 5



trans to chloride and the other one trans to nitrogen; *c* and *f*, both PPh_3 trans to N) (Chart 5).

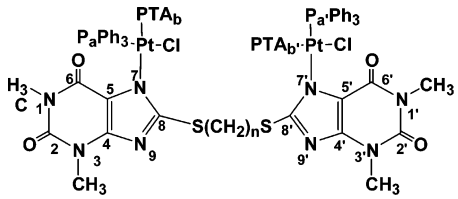
The reaction of $MBTT^{2-}$ with 2 equiv of *cis*-[$PtCl_2(PPh_3)$-PTA] (**1**) gave rise to a mixture of isomeric forms, and we did not succeed in separating them. The major species amounted to ca. 66% of the mixture, on the basis of the integrated signals in $^{31}P\{^1H\}$ NMR. Although it was not possible to separate and purify this complex, its structure could be assigned from its NMR features. A characteristic two partially overlapped doublets at 10.7 and 10.9 ppm was observed in its $^{31}P\{^1H\}$ NMR, both due to Pt coordinated PPh_3 with $^1J_{PtP}$ of ca. 3790 Hz, indicating that this phosphine is trans to chloride, and two other very close doublets at −73.6 and −73.9 ppm ($^1J_{PtP}$ of ca. 2975 Hz), for the coordinated PTA trans to nitrogen. It is reasonable to suppose that all the signals belong to the open form **10a** (Chart 6), and also in this case the double pattern is due to the two inequivalent Pt coordination spheres at the two sides of the complex.

Chart 6



A small amount of a minor species (ca. 20% by ^{13}P NMR) is also observed in the spectrum, giving rise to three doublets of doublets with satellites. Two of these signals (same intensity) are in the range of coordinated PTA (−76 and −64.7 ppm) with $^1J_{PtP}$ indicating that the first one is trans to nitrogen (2900 Hz) while the second one is trans to chloride (3424 Hz). The third signal is located in the PPh_3 zone (7.0 ppm, $^1J_{PtP}$ = 3314 Hz, trans to nitrogen). A further signal (PPh_3 trans to Cl) could be coincident with the corresponding of the main species. These data support the hypothesis that the minor species is the open isomer **10b** (Chart 7).

Chart 7



The same distribution of products can be observed for the mixture obtained from the reaction of **1** with $EBTT^{2-}$, containing **11a** as the main species (yield 64% based on its $^{31}P\{^1H\}$ NMR: $P_a + P_{a'}$ −73.5 and −74.0, 2d, PTA trans to N7(purine), $^1J_{PtP}$ = ca. 2950 Hz; $P_b + P_{b'}$ 10.7 and 10.9, 2d, PPh_3 trans to Cl, $^1J_{PtP}$ = ca. 3810 Hz) and **11b** as minor species (P_a −64.7, 2d, PTA trans to Cl, $^1J_{PtP}$ = 3408 Hz; $P_{a'}$ −76.7, 2d, PTA trans to N, $^1J_{PtP}$ = 2933 Hz; P_b 7.0, 2d, PPh_3 trans to N, $^1J_{PtP}$ = 3244, $P_{b'}$ trans to Cl could be coincident with the corresponding of **11a**).

Finally the reaction of $PBTT^{2-}$ with 2 equiv of *cis*-[$PtCl_2$-(PPh_3)PTA] (**1**) gave a main pattern that tentatively we

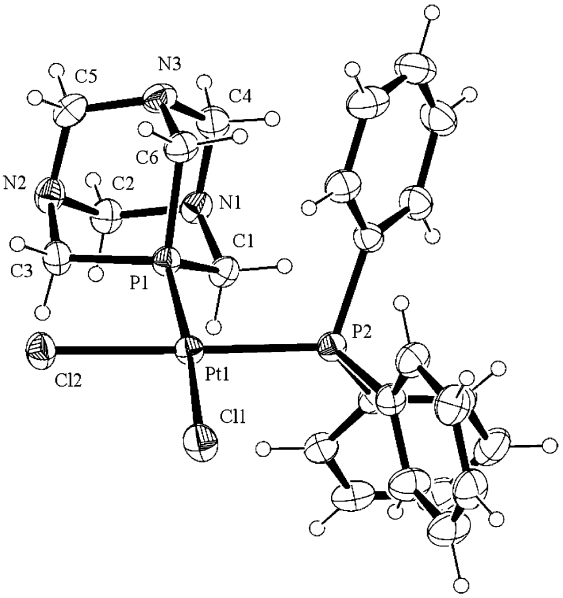


Figure 2. Crystal structure of **1**.

Table 2. Selected Bond Distances (Å) and Angles (deg)

Pt1−Cl1	2.337(1)	Pt1−P1	2.250(1)
Pt1−Cl2	2.369(1)	Pt1−P2	2.255(1)
Cl1−Pt1−Cl2	86.76(4)	Cl2−Pt1−P1	88.33(4)
Cl1−Pt1−P1	175.09(4)	Cl2−Pt1−P2	175.76(4)
Cl1−Pt−P2	90.75(4)	P1−Pt1−P2	94.16(4)

attribute to isomer **12a** (estimated ca. 90% on its $^{31}P\{^1H\}$ NMR), characterized by two multiplets one for two PTA both trans to N7(purine) at −73.7 ($^1J_{PtP}$ = 2961 Hz) and −76.7 ($^1J_{PtP}$ = 2924 Hz) and the other multiplet (or two overlapped multiplets) at 10.8 ppm ($^1J_{PtP}$ = 3798 Hz) due to PPh_3 trans to Cl. Also in this case a group of minor signals is present due to PTA trans to Cl and an unresolved multiplet due to PPh_3 probably trans to nitrogen.

Crystal Structure of 1. An ORTEP[18] drawing of **1** is shown in Figure 2. Selected bond distances and angles are given in Table 2. The coordination geometry is distorted square planar with angles ranging from 86.76(4) to 94.16(4)° around the Pt1 atom. The Cl−Pt−P angle observed for PTA (Cl2−Pt1−P1 = 88.33(4)°) and PPh_3 (Cl1−Pt−P2 = 90.75(4)°) is in agreement with their respective ligand cone angles (PTA = 102°; PPh_3 = 145°);[16] the biggest volume of the PPh_3 leads to a reduced Cl−Pt−PTA angle. The largest deviations from the least-squares plane through Pt1, Cl1, Cl2, P1, and P2 are 0.076(1) and 0.060(1) Å for Cl2 and P2, respectively.

Both Pt−Cl distances of 2.337(1) and 2.369(1) Å, with Cl atoms in trans positions to tertiary phosphines, are significantly longer than the average Pt−Cl distance of 2.29(1) Å calculated for square-planar Pt complexes having Cl atoms in mutual trans positions.[19] These data reflect the strong trans influence exerted by phosphine groups.[20]

In the crystal packing the molecules of complex are linked by a centrosymmetric dimers by means of C1−H1B···Cl2 and C2−H2A···Cl1 short interactions with H1B···Cl2 and H2A···Cl1 distances of 2.84 and 2.94 Å and C1−H1B···Cl2 and C2−H2A···Cl1 angles of 159 and 140°, respectively.

(18) Burnett, M. N.; Johnson, C. K. *ORTEPIII*; Report ORNL-6895; Oak Ridge National Laboratory: Oak Ridge, TN, 1996.

Table 3. Estimated IC50 (μM) on Cisplatin-Sensitive T2 Cell Line and on Cisplatin-Resistant SKOV3

complex	T2	SKOV 3
1, *cis*-$[PtCl_2(PTA)(PPh_3)]$	ca. 10	10−50
2, *cis*-$[PtCl(8\text{-}MTT)(PPh_3)(PTA)]$	2−10	10−50
3, *cis*-$[Pt(8\text{-}MTT)_2(PTA)(PPh_3)]$	2−10	10
I, *cis*-$[PtCl(8\text{-}MTT)(PPh_3)_2]$	ca. 2	ca. 50
II, *cis*-$[Pt(8\text{-}MTT)_2(PPh_3)_2]$	10−50	>50
III, *cis*-$[PtCl(8\text{-}MTT)(PTA)_2]$	>50	>50
IV, *cis*-$[Pt(8\text{-}MTT)_2(PTA)_2]$	>50	>50
cis-$[PtCl_2(PTA)_2]$	>50	>50
cis-$[PtCl_2(PPh_3)_2]$	ca. 50	>50
Na(8-MTT)	>50	>50
cis-$[PtCl_2(NH_3)_2]$	<2	>50

Cell Growth Inhibition of Complexes 1−3. In Table 3 are reported the estimated concentrations reducing to 50% the cell growth (IC50) of T2 (cisplatin-sensitive) and SKOV3 (cisplatin-resistant) cell lines for the complexes **1**−**3**, in comparison with the previously reported data[7] for *cis*-$[PtCl(8\text{-}MTT)(PPh_3)_2]$ (**I**), *cis*-$[Pt(8\text{-}MTT)_2(PPh_3)_2]$ (**II**), *cis*-$[PtCl(8\text{-}MTT)(PTA)_2]$ (**III**), *cis*-$[Pt(8\text{-}MTT)_2(PTA)_2]$ (**IV**), and their dichloride precursors. The data obtained for cisplatin in the same set of experiments are also given.

The comparison points out that on the T2 cell line the compounds **2** and **3** are about as active as the triphenylphosphine analogues **I** and **II** and more active than PTA complexes **III** and **IV**, while on SKOV3 (cisplatin-resistant) only the mixed phosphine complexes **1**−**3** show appreciable activity.

It is reasonable to suppose that the $\{Pt(PTA)(PPh_3)\}$ group could confer to **1**−**3** the ability either to dissolve in water-based biological fluids and to cross the lipophilic membranes of the cell and of the nucleus, reaching their target, the nucleic acids.

The activities of the dichloride precursors **1**, *cis*-$[PtCl_2(PPh_3)_2]$, and *cis*-$[PtCl_2(PTA)_2]$ and of the salt Na(8-MTT) are reported for comparison in Table 3. The activity of the dichlorides on both cell lines is lower than that found for the corresponding thiopurinic derivatives but shows the same trend: **1** is the most active dichloride, followed by the PPh_3 complex, and then by the nearly inactive *cis*-$[PtCl_2(PTA)_2]$.

The free ligand salt Na(8-MTT) does not show appreciable activity on its own, but the presence of $8\text{-}MTT^-$ as ligand in the platinum complexes in most cases improves their antiproliferative activity as it is evident from the comparison of the Pt dichloride precursors with the corresponding Pt−MTT complexes.

Cancer Cell Growth Inhibition of Binuclear Complexes. The results of the cell growth inhibition obtained with the binuclear complexes **4**−**9** (together with their dichloride precursors and the ligands salts) for cisplatin-

(19) Palenik, G. J.; Giordano, T. J. *J. Chem. Soc., Dalton Trans*. **1987**, 1175−1179.

(20) (a) Oberhauser, W.; Bachman, C.; Stampfl, T.; Haid, R.; Langes C.; Rieder, A.; Brüggeller, P. *Inorg. Chim. Acta* **1998**, *274*, 143−154. (b) Johansson, M. H.; Malmström, T.; Wendt, O. F. *Inorg. Chim. Acta* **2001**, *316*, 149−152. (c) Song, H. B.; Zhang, Z. Z.; Mak, T. C. W. *Polyhedron* **2002**, *21*, 1043−1050.

Table 4. Estimated IC50 (μM) on Cisplatin-Sensitive T2 Cell Line and on Cisplatin-Resistant SKOV3 for Binuclear Complexes

complex	T2	SKOV 3
4	10−50	>50
5	10−50	>50
6	10−50	ca. 50
7	2−10	10−50
8	2−10	10−50
9	2−10	10−50
cis-[$PtCl_2(PPh_3)_2$]	ca. 50	>50
cis-[$PtCl_2(PTA)_2$]	>50	>50
Na_2(MBTT)	>50	>50
Na_2(EBTT)	>50	>50
Na_2(PBTT)	>50	>50
cis-[$PtCl_2(NH_3)_2$]	<2	>50

sensitive T2 cell line and for cisplatin-resistant SKOV3 cell line are reported in Table 4.

It is necessary to underline that, for all the binuclear platinum complexes, the platinum concentration is effectively double that of the complex concentration. In spite of this, on both cancer lines the triphenylphosphine derivatives **4**−**6** present an activity lower than that observed for corresponding mononuclear analogues **I** and **II**, showing the lack of synergic effect between the two platinum centers. On the contrary, the bimetallic PTA complexes **7**−**9** are much more active than the mononuclear complexes **III** and **IV**. This result could be again related to the hydrophilicity/lipophilicity balance: in fact, among all tested PTA complexes activity was found only in binuclear complexes where lipophilic bis-(thiopurinic) ligands are introduced beside the hydrophilic {$(PTA)_2$Pt} group.

In a comparison of the activity of the binuclear complexes **4**−**9** with their dichloride precursors *cis*-[$PtCl_2(PPh_3)_2$] and *cis*-[$PtCl_2(PTA)_2$], a clear conclusion is pulled: a thiopurinic ligand on platinum enhances the antiproliferative activity of the complex which seems to be independent of the length of the bridging group in binuclear complexes. We also tested the disodium salts of $MBTTH_2$, $EBTTH_2$, and $PBTTH_2$ as comparison, and we found that they do not show any appreciable activity on their own.

Unfortunately, the reactions of $MBTTH_2$, $EBTTH_2$, and $PBTTH_2$ with the mixed phosphine precursor **1** invariably gave only mixtures of several isomeric platinum complexes which we did not succeeded in separating in pure form. For this reason it was not possible to draw any definitive conclusion from the tests of the antiproliferative activity of the group of binuclear products bearing the {$Pt(PTA)(PPh_3)$} moiety (see Supporting Information).

Conclusion

The new complex *cis*-[$PtCl_2(PPh_3)$(PTA)] (**1**), containing two different phosphines, one of them being water soluble (PTA), has been prepared and structurally characterized. It has been shown that its derivatives with 8-MTT^- (**2**, **3**) in most cases display an antiproliferative activity on model tumoral cell lines (T2 and SKOV3) better than their analogues (**I**−**IV**) containing a single phosphine type. This improvement is likely to be related with a more favorable balance between lipophilicity and hydrophilicity due to the presence of two different phosphines bonded to platinum.

The reactions of *cis*-[$PtCl_2(PPh_3)_2$], *cis*-[$PtCl_2(PTA)_2$], and *cis*-[$PtCl_2(PPh_3)$(PTA)] with bis(*S*-8-thiotheophylline)methane ($MBTTH_2$), 1,2-bis(*S*-8-thiotheophylline)ethane ($EBTTH_2$) and 1,3-bis(*S*-8-thiotheophylline)propane ($PBTTH_2$) in various stoichiometric ratios gave invariably binuclear products, suggesting a sort of cooperative effect between the two sites of the ligand with regard to coordination.

The antiproliferative activity of triphenylphosphine binuclear complexes was found lower than those for corresponding mononuclear complexes, indicating that biological activity of the two platinum centers is not addictive, while among all the examined PTA complexes only the binuclear **7**−**9** showed some activity, probably because a more convenient hydrophilicity/lipophilicity balance is reached in this case.

Acknowledgment. We thank the PAI (Junta de Andalucía, FQM-317; Project AM56/04), the MCYT (Spain) for the Projects PPQ2003-01339 and CTQ2006-06552/BQU, and Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici. This work was supported by the EC through the MCRTN program AQUACHEM (MRTN-CT-2003-503864) and the COST Actions D17 and D29.

Supporting Information Available: X-ray crystallographic files in CIF format for the structure determination of compound **1** and antiproliferative activity tables including **10**−**12** as isomeric mixtures. This material is available free of charge via the Internet at http://pubs.acs.org. Complete crystallographic data (excluding structural factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 643227. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax +44(0)-1223-336033; e-mail deposit@ccdc.cam.ac.uk or via www.ccdc.cam.ac.uk/conts/retrieving.html].

IC062133C