Anti-Cancer Drugs 2001, **12,** pp. 65–70

Preclinical report

Platinum(II) and palladium(II) complexes with 2-acetylpyridine thiosemicarbazone: cytogenetic and antineoplastic effects

Z Iakovidou,[1] A Papageorgiou,[3] MA Demertzis,[2] E Mioglou,[1] D Mourelatos,[1] A Kotsis,[1] P Nath Yadav[2] and D Kovala-Demertzi[2]

[1]Laboratory of Medicinal Biology and Genetics, Faculty of Medicine, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece. [2]Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece. [3]Laboratory of Experimental Chemotherapy, Theagenion Anticancer Institute, 54007 Thessaloniki, Greece.

The effect of three novel complexes of Pt(II) and three complexes of Pd(II) with 2-acetylpyridine thiosemicarbazone (HAcTsc) on sister chromatid exchange (SCE) rates and human lymphocyte proliferation kinetics on a molar basis was studied. Also, the effect of Pt(II) and Pd(II) complexes against leukemia P388 was investigated. Among these compounds, the most effective in inducing antitumor and cytogenetic effects were the complexes $[Pt(AcTsc)_2].H_2O$ and $[Pd(AcTsc)_2]$ while the rest, i.e. (HAcTsc), [Pt(AcTsc)Cl], $[Pt(HAcTsc)_2]Cl_2.2H_2O$, [Pd(AcTsc)Cl] and $[Pd(HAcTsc)_2]Cl_2$, displayed marginal cytogenetic and antitumor effects. [© 2001 Lippincott Williams & Wilkins.]

Key words: 2-Acetylpyridine thiosemicarbazone, antileukemic activity, cell kinetics, palladium(II), platinum(II), sister chromatid exchanges.

Introduction

Thiosemicarbazone (Tsc) derivatives are of considerable interest due to their antibacterial, antimalarial, antiviral and antitumor activities.[1] Pyridine-2-carbaldehyde Tsc was the first α-(*N*)-heterocyclic carboxyaldheyde Tsc, HFoTsc, reported to have carcinostatic effects.[2,3] The mechanism of action of HFoTsc, like in other α-(*N*)-Tscs, is due to its ability to inhibit the biosynthesis of DNA, possibly by blocking the enzyme ribonucleotide diphosphate reductase; binding to the nitrogen bases of DNA, hindering or blocking base replication; and creation of lesions in DNA strands by oxidative rupture.[2,3] In some cases the highest activity is associated with a metal atom. For instance, 3-ethoxy-2-oxobutyraldehydebis(thiosemicarbazonato) Cu(II) has proved to be an efficient antitumor agent.[4]

We have initiated an investigation on complexes of Pd(II) with N^4-substituted derivatives of 2-acetylpyridine Tsc (HAcTsc) in an effort to investigate whether Tscs act synergistically in new complexes containing both the parent ligand and Pd(II), and also to correlate structure with biological activity.[5–9] This work is an extension of previously studied Pd(II) complexes of HAcTsc with potentially interesting biological activity in order to obtain information on structure–activity relationships.[5,6]

We have previously synthesized and studied the effect of Pt(II) and Pd(II) complexes with N^4-substituted derivatives of HAcTsc on sister chromatid exchange (SCE) rates and on human lymphocyte proliferation kinetics [proliferation rate index (PRI)]. Also the acute toxicity and antitumor activity were evaluated on leukemia P388-bearing mice.[7–9] SCEs have been proposed as a very sensitive method for detecting genotoxicity, and lately as one of the methods of evaluating chemotherapeutic efficiency *in vitro* and *in vivo*.[10–12] in the present study, the same parameters were used to examine the antitumor and cytogenetic effects of novel Pt(II) and Pd(II) complexes with HAcTsc.

Materials and methods

Syntheses

Solvents were purified and dried according to standard procedures. The ligand HAcTsc (**1**) was prepared according to the method described.[5] The complexes of Pd(II) [PdCl(AcTsc)] (**2**), $[Pd(HPyTsc)_2]Cl_2$ (**3**) and

Correspondence to D Mourelatos, Laboratory of Medicinal Biology and Genetics, Faculty of Medicine, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece.
Tel: (+30) 31 999017; Fax: (+30) 31 999019;
E-mail: mourelat@hotmail.com

$[Pd(PAcTsc)_2]$ (**4**) were prepared by some of us, as described in the literature.[6] The complexes of Pt(II) were prepared according to reactions (1) and (2), in methanolic or aqueous solutions in the pH range 1–8.

$$[PtCl_4]^{2-} + HAcTsc \xrightarrow{CH_3OH} [Pt(AcTsc)Cl] + 2Cl^- + HCl \quad (1)$$

$$[PtCl_4]^{2-} + 2HAcTsc \xrightarrow{CH_3OH} [Pt(AcTsc)_2]Cl_2 + 2Cl^- \quad (2)$$

$$[PtCl_4]^{2-} + 2HAcTsc + 2NH_4OH \xrightarrow{CH_3OH//H_2O/pH\ 8} [Pt(AcTsc)_2 + 2Cl^- + 2NH_4Cl + 2H_2O \quad (3)$$

Details concerning synthesis, and spectroscopic and X-ray diffraction studies for the Pt(II) complexes will be reported in a future paper.

In vitro SCE assay

We added 10 drops of whole blood (containing about 3×10^6 white cells) prepared lymphocyte cultures from normal subjects to 5 ml of chromosome medium (Gibco, Middlesex, UK; including phytohemaglutinin). (Metaphases were collected during the last 2 h with colchicine at 0.3 μg/ml.) Cultures were treated with chemicals for 72 h at the beginning of culture. The chemicals were dissolved in dimethylsulfoxide (DMSO) and then further diluted in culture medium. The final concentration of DMSO in culture medium was always less than 0.2%, which does not induce SCEs. For the demonstration of SCEs,[7] 5-bromodeoxyuridine (5-BrdUr) at 5 μg/ml was present in culture for two rounds of replication. During the entire period, all cultures were maintained in the dark to minimize photolysis of BrdUr. Air-dried preparations were made and stained by the fluorescence plus Giemsa procedure.[7] The preparations were scored for cells in their first, second, third and subsequent divisions, and second division cells that were suitably spread were scored blindly for SCEs and lymphocyte proliferation kinetics. A minimum of 30 cells were scored for each culture in order to establish mean SCE values. For PRIs, at least 100 cells were scored. The PRI was calculated according to the formula $PRI=(M_1+2M_2+3M_3)/N$, where M_1, M_2 and M_3 indicate those metaphases corresponding to first, second and third or subsequent divisions, and N is the total number of metaphases scored.[7] For the statistical evaluation of the experimental data, the χ^2-test was used for comparisons of cell kinetics, whereas Student's *t*-test was performed for the SCE frequencies to determine whether any values significantly differed from the controls ($p < 0.05$). We also calculated the correlation between SCEs and PRI values. The formula for the Pearson product moment correlation coefficient r was applied. Then a criterion for testing whether r differed significantly from zero was applied, whose sampling distribution is Student's *t*-test with $n-2$ d.f.

Mice

Male and female DBA/2 and BDF/1 (C57BL × DBA/2) mice that were 8–10 weeks old and weighed 20–25 g were used for toxicity studies and antitumor testing. The mice were obtained from the Experimental Laboratory of Theagenion Anticancer Institute, and kept in sterile cages under conditions of constant temperature and humidity, with water and food. Lymphocyte P388 leukemia was maintained in ascetic form in DBA/2 mice by injection of 10^6 cells into the peritoneal cavity at 7-day intervals.

Compounds

For i.p. treatment, stock solutions of the compounds used in this study were prepared immediately before use. They were suspended in corn oil in the desired concentration following initial dissolution in a small amount of 10% DMSO. This concentration by itself produced no observable toxic effect.

Survival testing

The toxicity of the compounds was tested on BDF1 mice (groups of 10 animals per dose). For each compound, five different doses were chosen. The number of surviving animals was determined after 30 days. The animals were observed for later deaths, but these were not included in the calculations because they were not due to a general pharmacological effect. The i.p. toxicity of the seven compounds for BDF1 mice is shown in Table 2. For chemotherapy testing, the highest dose used for a single treatment was LD_{10}. Therefore the drugs in these experiments were compared at equitoxic doses (see Table 2).

Antitumor evaluation

Antileukemia experiments were initiated by implanting BDF1 mice with the appropriate number of ascites cells according to the protocol of the National Cancer Institute.[13] Drug in a single dose was given on the first day after tumor transplantation. Each drug-treated group consisted of six mice; 12 mice comprised the leukemic control group which was treated with saline only. The antitumor activity was assessed from the percent increase in median life span of treated animals over the controls (T/C%). Differences in survival time were evaluated by the Wilcoxon test.

Results

Results concerning SCEs and PRIs are presented in Table 1, and chemical formula of the ligand (HAcTsc) appears in Figure 1. The most effective compounds in inducing cytogenetic and antineoplastic effects are the 1:2 (metal to ligand molar ratio) complexes with deprotonated ligands, $[Pt(AcTsc)_2].H_2O$ and $[Pd(AcTsc)_2]$ (Tables 1 and 3). Both induced a statistically significant increase in antitumor activity ($p<0.01$) and in SCE rates ($p<0.01$) at all concentrations tested. The increases were directly related to the concentrations used (5, 10 and 15 μM). $[Pt(AcTsc)_2].H_2O$ and $[Pd(AcTsc)_2]$ induced statistically significant cell division delays at all concentrations studied, while the ligand (HAcTsc) appears as an effective inducer of SCEs, of cell division delays and as achieving the highest acute toxicity (Tables 1 and 2). The remaining compounds, [Pt(AcTsc)Cl], $[Pt(HAcTsc)_2]Cl_2.2H_2O$, [Pd(AcTsc)Cl] and $[Pd(HAcTsc)_2]Cl_2$, induce marginal cytogenetic and antineoplastic effects (Tables 1 and 3). In Table 1, in the group of seven compounds, a correlation was observed between the magnitude of the SCE response and the cell division delay ($p<0.002$).

Discussion

Chemistry

HAcTsc (Figure 1) behaves both as a weak base and a weak acid, and in aqueous solutions there are three independent species. The equilibria between the species are given from the equations:

Table 1. The effect of the ligand (HAcTsc) and of the six complexes on SCE rates and PRIs in cultured human lymphocytes

Compound	Concentration (μM)	SCE/cell $\pm$ SE	PRI
Control	–	9.54 ± 0.53	2.03
HAcTsc	10	19.51 ± 1.03[a]	1.44
	15	–[b]	–
[Pt(AcTsc)Cl]	10	15.20 ± 0.88[a]	1.53[c]
	15	11.97 ± 0.57	1.48[c]
$[Pt(HAcTsc)_2Cl_2].2H_2O$	10	15.46 ± 0.90[a]	1.26[c]
	15	–	–
$[Pt(AcTsc)_2].H_2O$	10	15.77 ± 1.06[a]	1.35[c]
	15	27.02 ± 1.26[a]	1.22[c]
Control	–	7.92 ± 0.93	1.97
HAcTsc	12.5	14.99 ± 1.10[a]	1.22[c]
$[Pt(HAcTsc)_2Cl_2].2H_2O$	12.5	18.89 ± 1.21[a]	1.04[c]
Control	–	8.42 ± 0.35	2.22
HAcTsc	5	12.51 ± 0.54[a]	1.57[c]
	10	13.62 ± 1.66[a]	1.02[c]
	12.5	–	–
	15	–	–
Control	–	9.71 ± 0.61	1.68
[Pd(AcTsc)Cl]	5	10.68 ± 0.62	1.64
	10	12.31 ± 0.70[a]	1.20[c]
	15	–	–
$[Pd(HAcTsc)_2]Cl_2$	5	11.45 ± 0.61	1.53[c]
	10	11.69 ± 0.71	1.29[c]
	15	–	–
$[Pd(AcTsc)_2]$	5	13.25 ± 0.74[a]	1.44[c]
	10	16.92 ± 1.10[a]	1.23[c]
	15	17.89 ± 1.06[a]	1.22[c]

[a]Statistically significant ($p<0.01$) increase over the control (by *t*-test).
[b]Non-dividing cells.
[c]Statiscally significant ($p<0.01$) reduction over the control (by χ^2-test).
SCEs were correlated with corresponding PRI values ($r=-0.65$, $t=3.72$, $p<0.002$). Mean SCE values were established after counting 30 metaphases and PRIs were established after evaluating 100 metaphases for each culture. Details for statisical comparisons and PRI definition are in the text.

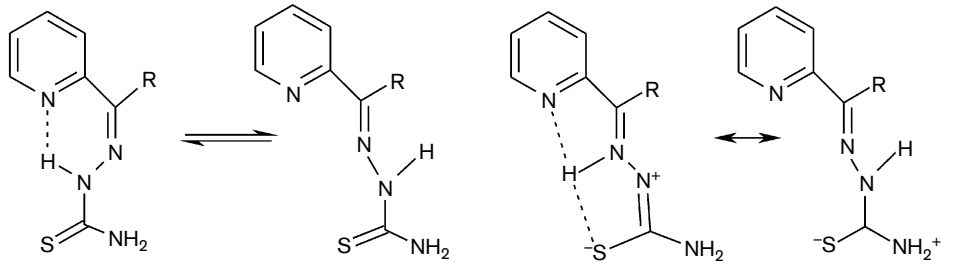

Figure 1. The tautomeric forms of HAcTsc (R is $-CH_3$) and HFoTsc (R is $-H$).

Table 2. Acute toxicity of the ligand (HAcTsc) and its complexes in BDF1 mice

Compound	LD_{50}[a] (μg/g body weight)	LD_{10}[a] (μg/g body weight)
Control	saline	saline
HAcTsc	25	10
[Pt(AcTsc)Cl]	105	60
$[Pt(HAcTsc)_2Cl_2.2H_2O]$	50	35
$[Pt(AcTsc)_2].H_2O$	75	50
[Pd(AcTsc)Cl]	80	50
$[Pd(HAcTsc)_2]Cl_2$	68	45
$[Pt(HAcTsc)_2]$	65	40

[a]LD_{50} and LD_{10} values were estimated graphically: percent deaths due to the toxicity of each dose are shown on the ordinate, while the administered doses are indicated on the abscissa on semilogarithmic paper. LD_{50} and LD_{10} = lethal doses for 50 and 10% respectively, of the mice used (10 animals per dose).

$$AcTsc^- + H_3O^+ \overset{K_{a1}}{\rightleftharpoons} HAcTsc + H_2O \quad (1)$$

$$HAcTsc^- + H_3O^+ \overset{K_{a2}}{\rightleftharpoons} H_2AcTsc^+ + H_2O \quad (2)$$

The protonation constants of the ligand, K_{a1} and K_{a2}, were determined by spectrophotometry and the logarithms of their values were found to be equal to 11.43 ± 0.02 and 3.98 ± 0.02, respectively.[6] The crystal structure of [Pd(AcTsc)Br] has been solved.[6]

A polymeric structure of an extended network of intermolecular hydrogen bonding contacts of the type N–H–N and Br–H–C is formed (Figure 2). The most plausible structure, based on spectroscopic results, for [M(AcTsc)X] and $[M(AcTsc)_2]$ is square planar. Anions [loss of N^3 hydrogen] of HAcTsc coordinate in a planar conformation to a central Pd(II) or Pt(II) through the pyridyl N, azomethine N and thiolato S atoms. The fourth coordination site is occupied by either a chloro or a second ligand bonding via only its thiolato S atom. The $[M(AcTsc)_2]$ complexes seem to be similar to $[Pd(Ac4DM)_2]$, where Ac4DM is the anion of 2-acetylpyridine N^4-dimethylthiosemicarbazone. The crystal structure determination showed that this complex has one NNS tridentate and a S monodentate ligand (Figure 2).[19] For $[M(HAcTsc)_2]X_2$ it is suggested that the neutral ligand, in the zwitterion form, exhibits bidentate (azomethine N and thiolato S)-chelated coordinating behavior. The counter (halogen) ions seem to be hydrogen bonded by the protonated pyridine ring $\geqslant N\text{–}H^+\text{—}Cl^-$. It was found that in solution these complexes are converted to 1:1 complex and protonated ligand, according to the reaction.[14]

$$[M(HAcTsc)_2]Cl_2 \rightleftharpoons [M(AcTsc)Cl] + H_2AcTscCl \quad (3)$$

Antitumor activity

Most antitumor agents have been identified to be either clastogenic, carcinogenic, mutagenic or teratogenic.[15] Antitumor treatments provide models of controlled mutagen exposure, which facilitate the comparison of the sensitivity and specificity of various mutagen assay systems.[16] Chemically induced cytotoxicity, in that it delays cell turnover times, is clearly manifested as a change in the relative proportions of cells in their first, second and subsequent divisions.[17] Studies in search of a relationship between SCE induction and other expressions of genotoxicity have shown a positive relationship between SCEs and reduced cell survival and alteration in cell cycle kinetics.[18] There are findings indicating that the effectiveness in SCE induction by potential antitumor agents in cancer cells *in vitro*[10] and *in vivo*[11] are positively correlated with *in vivo* tumor response to these agents, and suggest that the SCE assay could be used to predict both the sensitivity of human tumor cells to chemotherapeutics and the heterogeneity of drug sensitivity of individual tumors. The SCE assay has predictive value as a clinical assay for drugs for which a strong correlation between cell killing and induction of SCEs has been established.[12] A correlation between potency for SCE induction, antitumor activity and

Table 3. Antitumor activity of the ligand, HAcTsc, and its complexes on leukemia P388-bearing BDF1 mice

Compound	Dosage[a] (μg/g body weight)	MST[a] (days $\pm$ SE)	T/C%[a]
Control[a]	saline	8.5 $\pm$ 0.15	100
HAcTsc[a]	10	9.0 $\pm$ 0.19	106
[Pt(AcTsc)Cl][a]	60	9.2 $\pm$ 0.21	108
$[Pt(HAcTsc)_2]Cl_2.2H_2O$[a]	35	9.8 $\pm$ 0.22	115
$[Pt(AcTsc)_2].H_2O$[a]	50	10.4 $\pm$ 0.24	122[c]
[Pd(AcTsc)Cl][a]	50	9.4 $\pm$ 0.22	111
$[Pd(HAcTsc)_2]Cl_2$[a]	45	9.4 $\pm$ 0.24	111
$[Pd(AcTsc)_2]$[a]	40	10.8 $\pm$ 0.25	127[c]
Control[b]	saline	8.70 $\pm$ 0.18	100
HFoTsc[b]	20	11.25 $\pm$ 0.21	129
[Pt(FoTsc)Cl][b]	100	10.00 $\pm$ 0.14	115
$[Pt(HFoTsc)_2]Cl_2$[b]	70	12.20 $\pm$ 0.22	138
$[Pt(FoTsc)_2]$[b]	120	12.25 $\pm$ 0.21	141
[Pd(FoTsc)Cl][b]	38	11.50 $\pm$ 0.19	132
$[Pd(HFoTsc)_2]Cl_2$[b]	40	12.20 $\pm$ 0.22	138
$[Pd(FoTsc)_2]$[b]	150	13.00 $\pm$ 0.25	149

[a]This work; LD_{10} given as a single dose on the first day after tumor transplantation. Twelve mice were used in the control group, while each drug-treated group consisted of six mice; in each group equal numbers of male and female mice were used. MST, mean survival time; T/C%, median survival time of the drug-treated animals versus corn oil-treated controls (C).
[b]Where HFoTsc is the related pyridine-2-carbaldehyde Tsc.[8]
[c]Statistically significant increase ($P < 0.01$) over the control (by the Wilcoxon test).

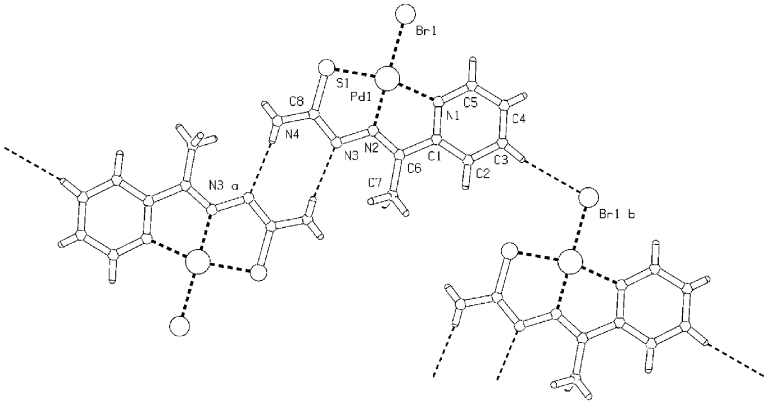

Figure 2. Arrangement of intermolecular hydrogen bonds in [Pd(AcTsc)Br].[6,19]

effectiveness in cell cycle delay by potential chemotherapeutics has been recently identified *in vivo*.[16] In the present study our results show a high correlation between the potency for SCE induction, effectiveness in cell division delay ($p < 0.002$) in normal human lymphocytes *in vitro* and *in vivo* established antitumor activity in P388 leukemia-bearing mice (Tables 1 and 3). SCEs have been frequently used as highly sensitive indicators of DNA damage and/or subsequent repair. The ability to excise and repair various types of damage to DNA is probably a general property of living cells.[15] Non-repaired damage expressed as SCEs, in normal cells caused by certain chemicals, may indicate inability for repair of damage induced by the same chemicals in cancer cells. This is of considerable interest to the problem of cancer because it provides a mechanism for the modification of the rate of the potential genetic damage caused by chemotherapeutics. Human cells, like some rodent cells, are proficient in DNA repair. Therefore it is expected that the DNA repair mechanisms in both cell types would be disturbed in a similar manner by the same potential chemotherapeutics.[15]

The ligand (HAcTsc) causes the highest acute toxicity and cell division delay, while the complexes of Pt(I) and Pd(II) with two deprotonated molecules, AcTsc $[Pt(HAcTsc)_2].2H_2O$ and $[Pt(AcTsc)_2]$, show significant reduction of the toxicity, enhancement of

cytogenetic damage and significant increase of survival time of the drug-treated leukemia-bearing mice (Tables 1–3). The acute toxicity and the antitumor activities of these complexes were correlated with the related complexes of HFoTsc.[8,9] The *in vivo* antitumor activities of Pd(II) and Pt(II) are related to the ease of reduction; the lower the potential, the higher the biological activity, probably due to an easier reductive attack on these compounds by intracellular thiols. The compounds $[Pt(FoTsc)_2]$ and $[Pd(FoTsc)_2]$ were found to exhibit the higher *in vivo* antitumor activity and the lower cytotoxicity. The related complexes of HFoTsc as well as the parent ligand exhibit lower values of acute toxicity, and higher values of mean survival time and median survival time.[8,9] The replacement of an acetyl group (HAcTsc) by a formyl group (HFoTsc) in the 2-pyridyl position causes marked differences on the biological results. The compounds $[M(FoTsc)_2]$ and $[M(AcTsc)_2]$, where M=Pd(II) or Pt(II), with *cis*-N_2 and *cis*-S_2 configuration, were found more effective against leukemia.[8,9]

These substances might interact better with DNA and/or proteins than the other compounds. The present experiments showed that the activity of Pt(II) and Pd(II) complexes is different from the activity of the ligand alone and may represent independent cytotoxic entities. All the complexes were less cytotoxic and almost all were found more effective in antitumor activity than the corresponding parent ligand, acting synergistically. The observed correlation between SCE induction, PRI depression and antineoplastic activity encourages this kind of investigation in order to select and synthesize more effective chemotherapeutics.

References

1. Liberta AE, West DX. Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. *BioMetals* 1992; **5**: 121–6.
2. French FA, Blanz Jr E. Carcinostatic activity of thiosemicarbazones of formyl heterocyclic compounds. 6. 1-Formylisoquinoline derivatives bearing additional ring substituents with notrs on mechanism of action. *J Med Chem* 1970; **13**: 1117–23.
3. Sartorelli AC, Agrawal KC, Tsiftsoglou AS, Moore AC. Characterization of biochemical mechanism of action of alpha (*N*)-heterocyclic carboxaldehyde thiosemicarbazones. *Adv Enz Reg* 1977; **15**: 117–39.
4. Kraker A, Krezoski S, Schneider J, Mingel D, Petering DH. Reaction of 3-ethyoxy–oxobutyraldehyde bis(thiosemicarbozonato) Cu(II) with Ehrlich cells. Binding of copper metalloprotein and its relationship to zinc metabolism and cell proliferation. *J Biol Chem* 1985; **260**: 13710–8.
5. Kovala-Demertzi D, Domopoulou A, Demetrzis MA, Raptopoulou CP, Terzis A. Coordinating properties of 2-acetylpyridinethiosemicarbazone. Palladium(II) complexes with neutral and deprotonated ligand. X-ray structure of bromo(2-acetylpyridinethiosemicarbazonato)-palladium(II). *Polyhedron* 1994; **13**: 1917–25 (and references therein).
6. Kovala-Demertzi D, Domopoulou A, Demertzis MA, *et al*. Structures and spectral properties of palladium(II) complexes of 2-acetylpyridine *N*(4)-dimethylthiosemicarbazone. *Polyhedron* 1996; **15**: 2587–96.
7. Papageorgiou A, Iakovidou Z, Mourelatos D, *et al*. Antineoplastic and cytogenetic effects of complexes of Pd(II) with 4*N*-substituted derivatives of 2-acetyl-pyridine-thiosemicarbazone. *Anticancer Res* 1997: **17**: 247–52.
8. Kovala-Demertzi D, Demertzis MA, Varagi V, *et al*. Antineoplastic and cytogenetic effects on platinum and palladium complexes with pyridine-2-carboxaldehyde thiosemicarbazone. *Chemotherapy* 1998; **44**: 421–6.
9. Kovala-Demertzi D, Miller JR, Kourkoumelis N, Hadjikakou SK, Demertzis MA. Palladium(II) and platinum(II) complexes of pyridine-2-carbaldehyde thiosemicarbazone with potential biological activity. Synthesis, structure and spectral properties. Extended network via hydrogen bond linkages of [Pd(FoTsc)Cl]. *Polyhedron* 1999; **18**: 1005–13.
10. Tofilon PJ, Basic I, Millas L. Prediction of *in vivo* tumor response to the chemotherapeutic agents by the *in vitro* sister chromatid exchange essays. *Cancer Res* 1985; **45**: 2025–30.
11. Mourelatos D, Dozi-Vasiliadis J, Kotsis A, Gourtsas C. Enhancement of cytogenetic damage and of antineoplastic effect by caffeine in erlich ascites tumor cell chemosensitivity using the sister chromatid exchange essay. *Cancer Res* 1988; **48**: 1129–31.
12. Deen DF, Kendall LE, Marton LJ, Tofilon PJ. Prediction of tumor cell chemosensitivity using the sister chromatid exchange essay. *Cancer Res* 1986; **46**: 1599–602.
13. Goldin A, Sofina Z, Syrkin A. Experimental evaluation of antitumor drugs in the USA and USSR and clinical correlations. *Cancer Inst Mongr* 1980: **55**.
14. Domopoulou A, Michaelidis A, Skoulika S, Kovala-Demertzi D. Crystallization of an unstable molecular compound using a tailor-made additive. *J Crystal Growth* 1998; **191**: 166–8.
15. Mourelatos D. Chromosomes study as predictor of chemoresponse of tumours. *Cancer J* 1996; **9**: 136–41.
16. Eliopoulos P, Mourelatos D, Dozi J. Comparative study on Salmonella mutagenicity and on cytogenetic and antineoplastic effects induced by Cyclophosphamide and 3-aminobenzamide in cells of three transplantable tumours *in vivo*. *Mutat Res* 1995; **342**: 141–6.
17. Morimoto K. Induction of SCEs and cell division delays in human lymphocytes by microsomal activation of benzene. *Cancer Res* 1983; **43**: 1330–4.
18. Morris SM, Heflich RH. A comparison of the toxic and SCE-inducing effects of inhibitors of ADP-rybosyltrasferase in CHO-cells. *Mutat Res* 1984; **126**: 63–71.
19. Spek AL. *PLATON. A program for the automated generation of a variety of geometrical entities*. The Netherlands: University of Utrecht 1997.

(Received 22 June 2000; revised form accepted 26 September 2000)