ORGANOMETALLICS

Article

pubs.acs.org/Organometallics

Organoplatinum(II) Complexes with 2-Acetylthiophene Thiosemicarbazone: Synthesis, Characterization, Crystal Structures, and in Vitro Antitumor Activity

Awadelkareem A. Ali,[†] Hassan Nimir,[†] Cenk Aktas,[‡] Volker Huch,[§] Ulrich Rauch,[⊥] Karl-Herbert Schäfer,[⊥] and Michael Veith*[,‡,§]

[†]Department of Chemistry, University of Khartoum, P.O. Box 321, Khartoum 11115, Sudan

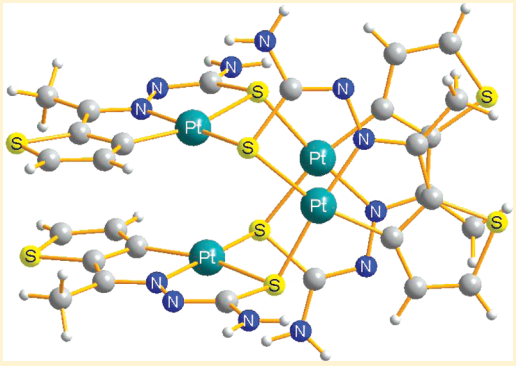
[‡]INM—Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbruecken, Germany

[§]Institute of Inorganic Chemistry, Saarland University, 66041, Saarbruecken, Germany

[⊥]University of Applied Science, Amerikastraße 1, 66482 Zweibruecken, Germany

Supporting Information

ABSTRACT: Novel organoplatinum(II) complexes with 2-acetylthiophene thiosemicarbazone (ATTSC) were synthesized. The reaction of K_2PtCl_4 with ATTSC in 1:1 and 1:2 metal to ligand ratios yielded **1** [Pt_4(ATTSC-2H)$_4$·4DMF] and **2** [Pt(ATTSC-H)(ATTSC)Cl·3CH_3OH)]. The crystal structures of these platinum chelates have been solved by single-crystal X-ray diffraction structure determinations and revealed metalation of the thiophene ring at C2 through platinum atoms. Further characterization of **1** and **2** was performed using electronic, IR, UV/vis, and NMR spectroscopies and elemental analysis. The in vitro antitumor activity of the ligand as well as **1** and **2** was determined against two different human tumor cell lines (HT-29 and HuTu-80). These tests revealed that the platinum(II) complexes are more cytotoxic than their ligand. The tetranuclear complex **1** shows higher antiproliferative activity (IC_{50} = 1.2 and 1.5 μM) when compared to **2** (IC_{50} = 3 and 5.9 μM), while the ligand has IC_{50} values of 8.6 and >10 μM. Compounds **1** and **2** can therefore be considered as agents with potential antitumor activity.



INTRODUCTION

The synthesis of transition metal complexes with thiosemicarbazone ligands has received considerable attention due to the pharmacological properties of both ligands and complexes.[1−3] Thiosemicarbazone derivatives exhibit a great variety of biological activities, which include notably their antitumor,[4] antifungal,[5,6] antibacterial,[6,7] and antiviral[8] properties depending on the parent aldehyde and ketone and, of course, metal ion. The deprotonated thiosemicarbazone ligands usually coordinate to platinum, palladium, copper, ruthenium, and osmium through oxygen, nitrogen, and sulfur donor atoms in their (N, S) bidentate form or (N, N, S or O, N, S) tridentate form to give metallic complexes of different molecular geometry.[9−11] Many complexes of Pd(II) and Pt(II) with thiosemicarbazone derived from different aldehydes and ketones have been synthesized, and their cytotoxic activities were investigated using different cell line cultures: complexes of pyridine carboxyaldehyde thiosemicarbazone were active in vivo against leukemia P388 cells,[12] and Pd(II) complexes of N_4-alkyl-2-acetylpyridine thiosemicarbazones showed in vitro inhibitory activity of DNA syntheses in L1210 and P388 cell cultures.[13] Platinum(II) complexes of N_4-ethyl 2-formyl and 2-acetylpyridine thiosemicarbazones showed cytotoxicity and were found to be able to overcome the cisplatin resistance of A2780/Cp8 cells.[14] Pd(II) and Pt(II) complexes of phenylacetaldehyde thiosemicarbazone[15] and binuclear chloro-bridged palladated and platinated complexes derived from *p*-isopropylbenzaldehyde thiosemicarbazone showed activity against several human and murine cell lines (HL-60, U937, HeLa, and 3T3) that were resistant to the clinically used cisplatin.[16] The great majority of antitumor metal complexes synthesized and characterized are those that are structurally analogous to cisplatin. However, due to severe neurotoxicity, nephrotoxicity,[17] myelosuppression, and cross-resistance against cisplatin-resistant cell lines,[18] there has been a decrease in the number of new compounds of this type,[19] possibly because it has just started to be revealed that substantial advances are unlikely to be reached with such molecules.[20] At the same time there has been an emergence of new structural types of metallic complexes often with promising activity and the ability to circumvent cisplatin resistance. This focus on the design of new coordination compounds with antitumoral properties, increased efficiency, and decreased toxicity along with the establishment

Received: November 24, 2011
Published: March 8, 2012

   dx.doi.org/10.1021/om201178q | *Organometallics* 2012, 31, 2256−2262

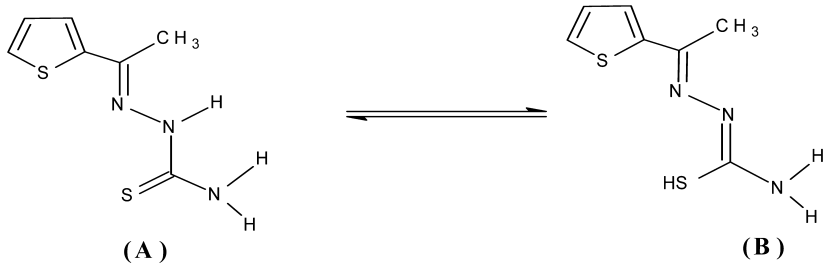


Figure 1. Thione (A) and thiol (B) tautomeric forms of the synthesized Schiff base.

of new biological targets led to the emergence of the bioinorganic chemistry of palladium and platinum complexes. We here present our results with 2-acetylthiophene thiosemicarbazone and some platinum complexes obtained with this ligand.

EXPERIMENTAL SECTION

General Procedures and Measurements. The experimental work was carried out under atmospheric pressure. Reagents were used as obtained from commercial sources (Alfa Aesar and Aldrich) without further purification. Solvents were purified and dried according to standard procedures. Melting points were determined using an EZ-Melt automated melting point apparatus. Elemental analyses were determined on an Leco CHN-932 analyzer. The infrared (IR) spectra were recorded in the solid state on a Varian 2000 FTIR spectrophotometer in the 4000−400 cm^{-1} range. The ^1H and ^{13}C NMR spectra were recorded on a Bruker 300, using DMSO-d_6 as a solvent. The chemical shifts (δ) in ppm were measured relative to tetramethylsilane (TMS). The electronic spectra were recorded using a Varian 5000 spectrophotometer.

Synthesis of the Ligand. The ligand 2-acetylthiophene thiosemicarbazone (ATTSC) was prepared similarly to the literature procedure.[21]

To a hot solution of thiosemicarbazide (1.08 g, 11.88 mmol) in 70 mL of methanol was added dropwise a solution of 2-acetylthiophene (11.88 mmol) in 30 mL of methanol. A few drops of glacial acetic acid were also added to the mixture. The mixture was stirred and refluxed for 10 h (70−80 °C) and then filtered. The volume of the filtrate was reduced to half through solvent removal at 10^{-2} atm and was kept in the refrigerator. After several hours, small rectangular pale yellow crystals were obtained. These crystals were collected by filtration, washed with cold ethanol, and dried in vacuo.

Yield: 84.91%, mp 135−136 °C. Anal. Calcd for $C_7H_9N_3S_2$ (199.30 g/mol): C, 42.19; H, 4.55; N, 21.08. Found: C, 42.31; H, 4.61; N, 20.93. IR (cm^{-1}): $\nu(NH_2)$ 3403.95, 3228.17; ν(NH) 3142.02; ν(C═N) 1581.39; ν(C═S) 950.65; ν(N−N)1042.68. ^1H NMR (DMSO-d_6): δ 1.62 (s, 3H, CH_3), 6.19−6.69 (m, C_4H_3S ring); 7.44, 6.71 (d, 2H, NH_2); 9.49 (s,1H, =N−NH). ^{13}C NMR (DMSO-d_6): δ 15.17(CH_3); 128.16−143.27 (C_4H_3S ring); 145.32 (HC═N); 178.95 (C═S). Electronic spectra (λ_{max} nm): 292.

Synthesis of Complexes. *Synthesis of 1, Pt_4(ATTSC-2H)$_4$·4DMF.* A solution of K_2PtCl_4 (0.208 g, 0.5 mmol) in methanol was added dropwise to a stirred solution of ATTSC (0.5 mmol) in 20 mL of methanol. The solution was refluxed for 2 h and stirred for 24 h at room temperature. The yellowish-brown solid precipitate was collected by filtration, washed with ethanol and ether, dried at 10^{-2} atm, and recrystallized from DMF. These crystals were suitable for structural analysis by X-ray diffraction.

Yield: 73.60%. Mp dec > 335 °C. Anal. Calcd for $C_{28}H_{28}N_{12}Pt_4S_8$ (1569.41 g/mol): C, 21.43; H, 1.80; N, 10.71. Found: C, 20.52; H, 2.14; N, 10.28. IR (solid state, cm^{-1}): $\nu(NH_2)$ 3307.73; ν(C═N) 1597.83; ν(C═S) 875.26; ν(N−N) 1072.56. ^1H NMR (DMSO-d_6): δ 1.08 (s, 3H, CH_3), 7.35, 7.36 (d, 2H ring); 7.44 (b, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 13.98(CH_3); 128.99−159.28 (C_4H_3S ring); 163.87 (HC═N); 172.56 (C═S). Electronic spectra (λ_{max} nm): 277, 355, 509. Crystals of **1** are subject to loss of DMF. This explains the lower C value compared to the calculated one.

Synthesis of 2, Pt(ATTSC)(ATTSC-H)Cl·3CH_3OH. A solution of K_2PtCl_4 (0.208 g, 0.5 mmol) in methanol was added dropwise to a stirred solution of ATTSC (1.0 mmol) in 30 mL of methanol. The solution was refluxed for 2 h and stirred for 24 h at room temperature. The colored solution was condensed to half of its volume under reduced pressure (10^{-2} atm) and kept in a refrigerator overnight. Small, dark red rectangular crystals were obtained. These crystals were collected by filtration, washed with cold ethanol and diethyl ether, and dried at 10^{-2} atm. These crystals were suitable for structure analysis by X-ray diffraction.

Yield: 86.82%, mp 209−210 °C. Anal. Calcd for $C_{17}H_{29}ClN_6O_3PtS_4$ (724.23 g/mol): C, 28.18; H, 4.04; N, 11.60. Found: C, 20.54; H, 2.83; N, 10.75. IR (solid state, cm^{-1}): $\nu(NH_2)$ 3390.68, 3244.62; ν(C═N) 1592.59; ν(C═S) 853.89; ν(N−N)1089.22. ^1H NMR (DMSO-d_6): δ 1.06(s, 3H), 7.46, 7.48 (d, 2H ring); 7.52, 7.54 (b, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 14.72(CH_3); 128.16−159.05 (C_4H_3S ring); 163.77 (HC═N); 172.36 (C═S). Electronic spectra (λ_{max} nm): 277, 303, 349, 521. Compound **2** contains three methanol molecules per platinum atom: As the crystals decompose slowly at room temperature with loss of methanol (NMR proof), especially the carbon content shows a discrepancy.

In Vitro Growth Inhibition Assay. *Cell Culture.* The antitumor assays were performed employing the following cell lines: HT-29 (human colon adenocarcinoma); HuTu-80 (human duodenal adenocarcinoma). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 50 μg/mL gentamycin and grown at 37 °C in a 5% CO_2 humidified environment.

Assessment of Cytotoxicity. Cells were inoculated into 24-well tissue culture plates at a density of 10 000 cells per well and incubated at 37 °C with their corresponding growth medium for 24 h to allow cells to attach. A plate containing each of these cells was fixed in situ with formaldehyde in order to obtain the number of cells at zero time before adding the test compounds. The rest of the plates containing the different cell lines received serial dilutions (0.1−10 μM) of the ligand and platinum complexes in aqueous DMSO and were incubated at 37 °C for 48 h. The control cultures were supplemented with the same amount of solvent containing DMSO. The assay was terminated by the addition of cold formaldehyde. The number of cells in each well was determined using the 4,6-diamidino-2-phenylindole (DAPI) assay. Formaldehyde-treated plates were incubated at room temperature for 30 min, and then the cells were washed with phosphate-buffered saline (PBS). The cells were stained for 20 min with a solution of 1.0% DAPI in PBS. At the end of the staining period, unbound dye was removed by washing with PBS. The pictures of the bound dye were taken using a fluorescent microscope, and the number of living cells was calculated using imaging software. The IC_{50} value was defined as the concentration of test sample resulting in a 50% reduction of the number of cells as compared with untreated controls that received a serial dilution of the solvent in which the test samples were dissolved.

RESULTS AND DISCUSSION

Spectroscopy. Compounds containing the thioamide functional group −NH−C(S) are capable of exhibiting

dx.doi.org/10.1021/om201178q | *Organometallics* 2012, 31, 2256−2262

thione−thiol tautomerism, and therefore, the Schiff base, in principle, can also exist as either the thione (A) or the thiol (B) tautomeric form or as a mixture of both tautomers[22] (Figure 1). However, on the basis of IR data, it is easy to distinguish between them in the solid state; no peak in the range 2150−2600 cm^{-1} attributed to the −SH vibration is observed, indicating that the Schiff base exists in the thione form in the solid state. ^1H NMR spectra of the Schiff base in DMSO-d_6 exhibit the secondary −NH− proton signal at 9.49 ppm. No signal at 4.00 ppm (attributed to the −SH proton) is observed in the ^1H NMR spectra of the Schiff base, indicating that it remains solely as the thione form, even in a polar solvent such as DMSO. In addition to the possibility of thione and thiol tautomers, the Schiff base is also capable of existing as *E* and *Z* isomeric forms (Figure 2) or as a mixture of both isomers. In

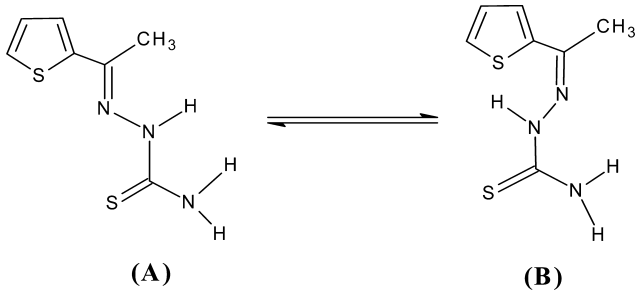


Figure 2. *E* and *Z* isomeric forms of the synthesized Schiff base: (A) *E*-form; (B) *Z*-form.

solution, the related N heterocyclic NNS thiosemicarbazones have been shown to exist as a mixture of *E* and *Z* isomers,[23] and it was possible to assign the correct configuration to the different products using ^1H NMR spectroscopy.[24] The most sensitive signal that has been used to distinguish between the *E* and *Z* isomers is that belonging to the NH group. Compounds possessing the *Z* isomer generally exhibit the NH signal in the 14−15 ppm range, whereas those having an *E* form exhibit the signal in the 9−12 ppm range. The presence of a signal at 9.49 ppm due to the NH proton and the absence of any signal at 14−15 ppm in the ^1H NMR spectra of the present Schiff base is strong evidence for only the *E* isomer in DMSO.

Although the Schiff base remains in the thione forms both in the solid state and in solution, it, nonetheless, undergoes a rapid conversion to the thiol forms in the presence of Pt(II) salt. This conversion is concomitant with the formation of its Pt(II) complexes, which have the empirical formulas ML_2 and M_nL_n (L = deprotonated forms of the Schiff base). The related tridentate NNS thiosemicarbazones derived from heterocyclic carbonyls have been shown to coordinate to metal ions in both the protonated thione and deprotonated thiolate[25] forms. There are also interesting examples of metal−thiosemicarbazone complexes in which both the protonated thione and deprotonated thiolate forms of the ligand are present in the same complex.[26]

The IR spectra of the synthesized complexes **1** and **2** do not contain a sharp μ NH band of the free ligand, indicating that the proton on the imine nitrogen atom of the ligand is lost during coordination with the metal ion or is involved in hydrogen bridging, giving a broad undissolved signal. The IR spectral data containing the relevant vibrational bands of the ligand and its platinum(II) complexes are given in Table 1. The data support coordination of the ligands via the azomethine

Table 1. IR and Electronic Spectral Data for the Synthesized Schiff Base (ATTSC) and Its Pt(II) Complexes

	IR bands (cm^{-1})				electronic spectra
compound	−NH	−C=N	−C=S	N−N	λ_{max} (nm)
ATTSC	3142.02	1581.39	950.65	1042.68	292
Pt_4(ATTSC-2H)$_4$·4DMF (**1**)		1597.83	875.26	1072.56	277, 355, 509
Pt(ATTSC)(ATTSC-1H)Cl·3CH_3OH (**2**)		1592.59	853.89	1089.22	277, 303, 349, 521

nitrogen atom (shift of the hydrazinic μ(N−N) band of the ligand to higher value in the spectra of the complexes) and via the sulfur atom (shifting of the CS band of the free ligand to higher value in the spectra of the complexes).

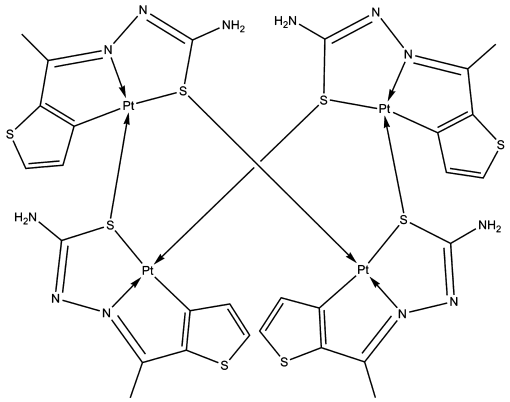
The electronic spectra of the complexes showed the intraligand bands in the 277−292 and 348−402 nm ranges, corresponding to the n → π^* and π → π^* transitions, respectively. These spectra also exhibit a strong absorption band at 509−521 nm. These absorption bands can be assigned to S → M charge-transfer bands. The presence of an S → M (LMCT) band in the electronic spectra of these complexes further supports bonding of the ligand to the Pt(II) ion via a sulfur atom. As shown by crystal structure analyses, the complex **2** is monomeric and has a square-planar structure around platinum, while **1** is tetrameric. Two-dimensional chemical formulas of the complexes **1** and **2**, as confirmed by X-ray structure analysis, are shown in Scheme 1. The platinum(II) ion having a $4d^8$ electronic configuration is expected to exhibit three bands in a square-planar environment, corresponding to the transitions $^1A_{1g}$ → $^1A_{2g}$,$^1A_{1g}$ → $^1B_{1g}$, and $^1A_{1g}$ → ^1E_g.[27] However, the electronic spectra of Pt(II) complexes are complicated due to the presence of S and N donor atoms in the ligands since the tails of the strong S → M (LMCT) bands may extend up to the visible portion of the spectrum, thereby masking the expected d−d bands.

Therefore, the electronic spectra of the present complexes are of little help in assigning a definitive structure to these complexes. However, based on IR and ^1H and ^{13}C NMR results, a square-planar structure may be tentatively assigned to these complexes.

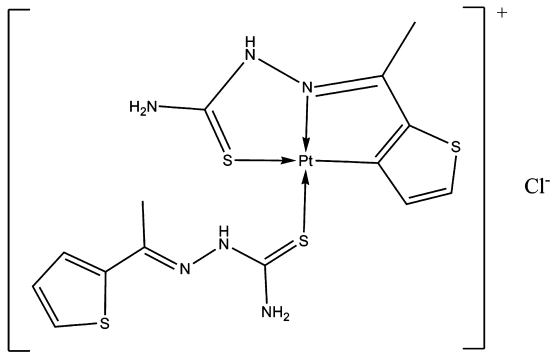
The ^1H and ^{13}C NMR spectral data of the Schiff base, together with assignments of relevant signals, are compiled in Tables 2 and 3. The assignments are based on those made previously for structurally related compounds.[25] An examination of the NMR data shows that the spectra of the complexes **1** and **2** do not contain the NH proton signals of the free ligand, lending further support to the IR results that the Schiff base might be coordinated to the metal ion in its deprotonated mercaptide form. As may be seen from the X-ray diffraction results below, this is clearly the case for **1**, whereas in compound **2**, at least in the solid state, the N−H bond is still present being engaged in a hydrogen bridge. A comparison of the ^1H NMR spectral data for the platinum(II) complexes with those of the free ligand (Table 2) reveals the following points: (1) The peaks at 9.49 ppm due to the imino proton in the free ligand disappeared after complexation, indicating that the ligand in **1** is deprotonated and coordinated with the metal ion through the mercaptide sulfur ion. For the bonding in **2**, the solution spectrum does not correspond to the solid state, which

dx.doi.org/10.1021/om201178q | *Organometallics* 2012, 31, 2256−2262

Scheme 1



Complexe 1



Complexe 2

Table 2. ^{1}H NMR Spectra for the Schiff Base (ATTSC) and Its Pt(II) Complexes

compound	δ of (NH_2) ppm	δ of NH ppm	δ of CH_3–C ppm	δ of ring 3H ppm
ATTSC	7.44, 6.71	9.49	1.62	6.19, 6.69
Pt_4(ATTSC-2H)$_4$·4DMF (1)	7.44		1.08	7.35, 7.36
Pt(ATTSC)(ATTSC-1H) Cl·3CH_3OH (2)	7.52, 7.54		2.33, 1.07	7.46, 7.48

Table 3. ^{13}C NMR Spectra for the Schiff Base (ATTSC) and Its Pt(II) Complexes 1 and 2

compound	δ of CH_3C	δ of ring (C_4H_3S ring) 3C	δ of C=N	δ of C=S
ATTSC	15.17	128.16–143.27	145.32	178.95
Pt_4(ATTSC-2H)$_4$·4DMF (1)	13.98	128. 99–159.28	163.87	172.56
Pt(ATTSC)(ATTSC-1H) Cl·3CH_3OH (2)	14.72	128.16–159.05	163.77	172.36/1.72 (broad)

might indicate that the structures are different. (2) The signal due to the azomethine CH_3 proton in the spectra of the free ligand undergoes a downfield shift in the spectra of the complexes, indicating that coordination occurs via the azomethine nitrogen atom.

Because of the conversion of the ligand from the thione to the thiol form and consequent deprotonation during coordination in **1**, shielding of the CS carbon atom causes an upfield shifting of the signal due to the CS carbon atom in the ^{13}C NMR spectra of the complexes relative to those in the free ligand. This observation is commensurate with coordination of the ligands via one of the sulfur atoms to the Pt(II) ion. The spectral data for **2** are similar as well as different, the most remarkable feature being the two signals for the CH_3 group in the ^{1}H and ^{13}C NMR. As found by X-ray diffraction, the splitting of the CH_3 signal in **2** is explained by two different ligands, one being charged −1 and tridentate, while the other is neutral and monodentate. The signal due to the carbon atoms of the azomethine (C=N) also shows downfield shifts, lending further support to the IR and ^{1}H NMR evidence presented before. This coordination of the ligand to the platinum ion in **1** and partly in **2** occurs via the azomethine nitrogen atom, the thiolate sulfur atom, and a carbene species created by abstraction of a hydrogen atom of the β-carbon atom, in the thiophene part of the ligand. It should be mentioned here that ATTSC ligands may be metalated at the β-C carbon atom if the aromatic ring leads to a chelate coordination via C, N, and S. As may be seen from the X-ray structural analyses (see below), this is indeed the case here in compound **1** and partly in compound **2**, as the two ligands are either singly negatively charged or neutral.

Structural Data. *Structure of Pt_4(ATTSC-2H)$_4$·4DMF (1).*[28] As result of the X-ray structure determination on single crystals of **1**,[28] the molecular part of **1** (omitting DMF) is shown in Figure 3. The ATTSC had reacted with K_2PtCl_4 in

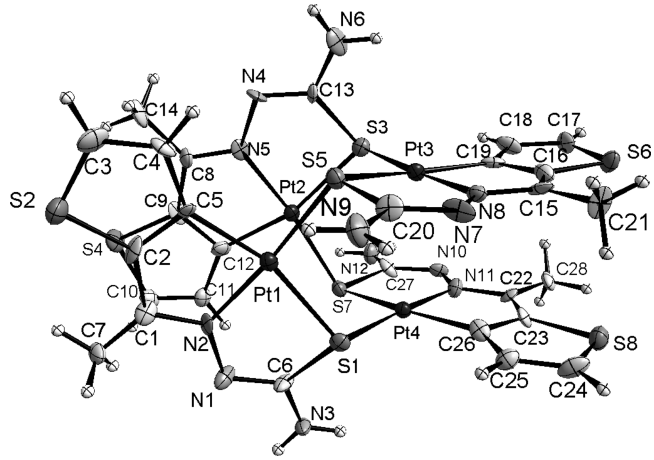


Figure 3. Stereographic representation of part of the structure of Pt_4(ATTSC-2H)$_4$ (**1**). The ellipsoids of atoms are represented at the 50% level. The coordinating diethylformanide (DMF) molecules are omitted for clarity.

1:1 ratio, eliminating 2KCl and 2HCl. The molecule is tetrameric with four platinum atoms, each metal atom displaying almost square-planar geometry and being coordinated by the dianion of ATTSC (ATTSC-2H) through sulfur, nitrogen, and a carbene (thiophene ring). The fourth coordination site at each platinum atom is occupied by a sulfur atom of a neighboring ligand. As each of the ATTSC-2H ligands has two negative charges, the oxidation number of the platinum atoms is +2. The sulfur interaction by an adjacent ligand leads to an eight-membered puckered central ring of

dx.doi.org/10.1021/om201178q | Organometallics 2012, 31, 2256–2262

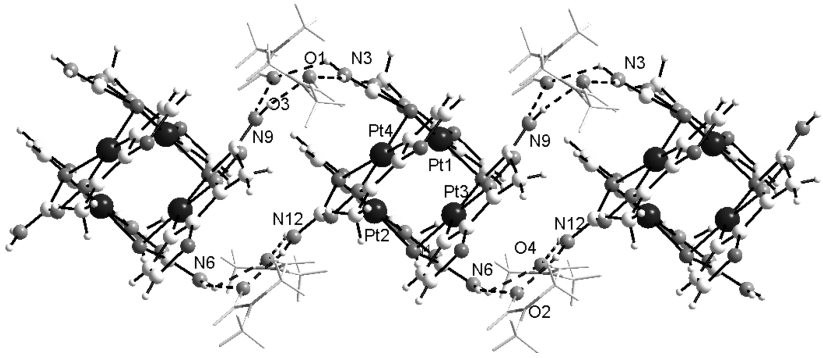


Figure 4. Representation of the hydrogen-bridged one-dimensional alignment of cluster molecules **1** in the crystal. For better viewing, the DMF molecules are represented as wire models.

alternating Pt and S atoms. The molecule in the crystal has no higher symmetry, but, in an approximation, this molecular cluster does not deviate much from S_4-symmetry. Furthermore, in the crystal, two chiral and enantiomeric forms are present, which are related by an inversion center of the space group ($P\bar{1}$).

The crystal structure of **1** is dominated by the interaction of $Pt_4(ATTSC\text{-}2H)_4$ with four dimethylformamide (DMF) molecules, resulting in a hydrogen-bridged superstructure. In the form of pairs always two of the four DMF molecules through their oxygen atoms are participating in double N–H···O bridges (N···O ranging from 2.912(9) to 3.004(9) Å), thus creating a one-dimensional hydrogen-bridged aggregate. A view of this superstructure is shown in Figure 4.

As stated above, the platinum atoms are in a distorted square-planar environment (d^8 configuration) with mean Pt–C and Pt–N distances of 2.006(6) and 2.010(4) Å (Table 4). The

Table 4. Selected Bond Lengths of "Heavy Atoms" within $[Pt_4(ATTSC\text{-}2H)_4]$ (1)

bond	length [Å]	bond	length [Å]
Pt(1)–C(5)	2.006(9)	Pt(4)–S(1)	2.289(4)
Pt(1)–N(2)	2.014(9)	Pt(4)–S(7)	2.334(4)
Pt(1)–S(5)	2.302(4)	S(1)–C(6)	1.81(2)
Pt(1)–S(1)	2.353(4)	S(2)–C(3)	1.70(2)
Pt(2)–C(12)	2.010(9)	S(2)–C(2)	1.72(2)
Pt(2)–N(5)	2.016(9)	S(3)–C(13)	1.79(2)
Pt(2)–S(7)	2.307(4)	S(4)–C(10)	1.73(2)
Pt(2)–S(3)	2.351(4)	S(4)–C(9)	1.75(2)
Pt(3)–N(8)	1.997(9)	S(5)–C(20)	1.77(2)
Pt(3)–C(19)	2.013(9)	S(6)–C(17)	1.70(2)
Pt(3)–S(3)	2.306(4)	S(6)–C(16)	1.74(2)
Pt(3)–S(5)	2.346(4)	S(7)–C(27)	1.80(2)
Pt(4)–C(26)	1.995(9)	S(8)–C(24)	1.70(2)
Pt(4)–N(11)	2.014(9)	S(8)–C(23)	1.75(2)

platinum–sulfur distances vary with their bonding: those that belong to the five-membered Pt–N–N–S–C cycle have mean values of 2.346(8) Å, whereas those that are created by the interaction of a second ligand are shorter, with mean values of 2.301(5) Å. This difference may be attributed to the acute bite angles of the chelates with N–Pt–S mean values of 82.8(4)°, which is due to the fact that each platinum atom is a member of two fused five-membered rings (Pt–C–C–C–N and Pt–N–N–C–S). As two of the four chelates are oriented in parallel, the six intramolecular Pt–Pt distances can be divided into short ones (Pt(1)–Pt(2) 3.444(1) and Pt(3)–Pt(4) 3.364(1) Å) and four longer ones, with a mean value of 3.78(6) Å. Apparently, the parallel orientation of chelates with Pt(1) and Pt(2) and also Pt(3) and Pt(4) could give some platinum–platinum electronic interaction. Within the chelate rings one observes a lengthening of the C–S bonds in proximity to platinum (mean value: 1.793(7) Å) and a shortening of the neighboring N–C bond (mean value: 1.314(9) Å), compared to the free ligand, in accordance with the fact that in the chelate C–S is almost a single bond and C–N has almost a double-bond character.[21]

Structure of Pt(ATTSC)(ATTSC-H)Cl·3CH$_3$OH (2).[29] Using two molar ratios of thiosemicarbazone (ATTSC) with respect to K_2PtCl_4 results in the formation of complex **2**, which has two ATTSC molecules coordinated to platinum. Interestingly, only one of the chlorides is replaced by the monoanion (ATTSC-H) and the second ATTSC ligand coordinates in its neutral form. The compound can be described as the ion pair $[Pt(ATTSC)(ATTSC\text{-}H)]^+$ with Cl^- as counterion and with three methanol molecules serving as links between the chloride anion and the platinum formal cationic moiety (Figure 5). The ATTSC-H

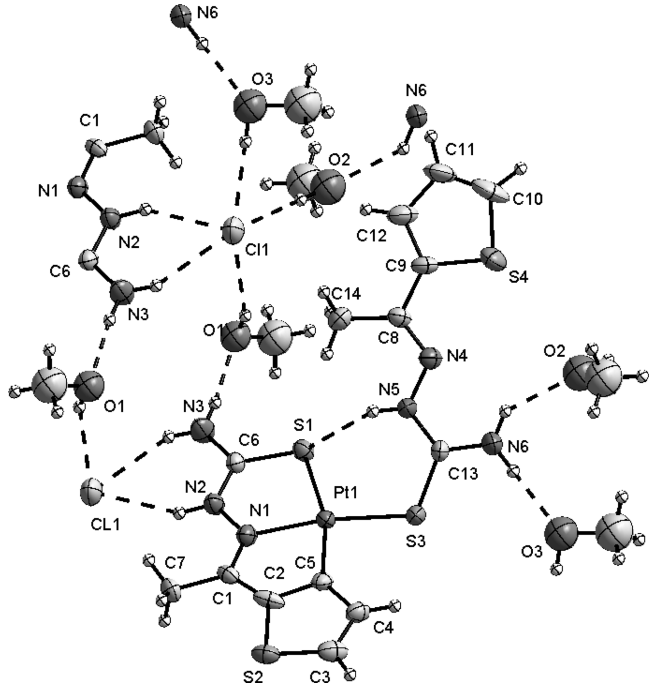


Figure 5. Part of the crystal structure of Pt(ATTSC-1H)(ATTSC)-Cl·3CH$_3$OH (**2**).

dx.doi.org/10.1021/om201178q | *Organometallics* 2012, 31, 2256–2262

ligand is singly negatively charged, has a hydrogen atom bonded to one of the hydrazine atoms (N(2)), and bonds to platinum very similarly to the ATTSC-2H ligands in compound **1** in a chelate fashion through the ring carbene, nitrogen, and sulfur. The fourth coordination place at the distorted square-planar platinum atom is occupied by sulfur of a neutral ATTSC ligand. The chloride anion in the crystal structure is bonded through hydrogen bridges to the three methanol molecules and to two nitrogen atoms of the N(2)−C(6)−N(3) part of the chelating ATTSC-H ligand. All hydrogen atoms at the oxygen atoms of the methanol molecules and at the nitrogen atoms of ATTSC and ATTSC-H are involved in hydrogen bridges (mean values: Cl···O[Cl···H−O] 3.19(2), Cl···N[Cl···H−N] 3.20(2), O···N[O···H−N] 2.83(1), N(5)···S(1) [N···H−S] 3.313(9) Å); by simple geometrical calculations, the positions of the hydrogen atoms can thus be calculated, without relying on the diffraction data, with good precision.

The most remarkable differences between the platinum bonding in **1** and **2** are the following: because of the lesser steric constraint in **2**, the S(3)−Pt(1) bond (2.264(2) Å) is 0.037 Å shorter than the comparable bond in **1**. The longer Pt(1)−S(1) bond in **2** (2.366(2) versus the mean Pt−S distance in the chelate 2.346(8) Å in **1**) seems to relate to the different nature of sulfur, which is sulfidic in **1**, while it is engaged in a C═S double bond in **2** (C(6)−S(1) = 1.712(9) Å). Interestingly, the C−Pt and N−Pt bonds in **1** and **2** are almost the same, which is in line with the similar chemical bonding (in **1**: Pt−C = 2.006(6), Pt−N 2.010(4) Å, in **2**: Pt(1)−C(5) 2.015(8), Pt(1)−N(1) = 2.016(7) Å).

Biological Activity. The preliminary investigation for the anticancer activity of ATTSC using two different human tumor cell lines (HT-29 and HuTu-80) shows that the ligand ATTSC alone has a 50% inhibitory concentration (IC_{50}) > 10.0 μM against these human tumor cell lines (Table 6). The synthesized complexes **1** and **2** exhibit stronger cytotoxicity in comparison to the ligand. These results reveal that the cytotoxic activity increases considerably when ligands are coordinated to the metal ion.[30] The tetranuclear Pt(II) complex with formula $Pt_4(L)_4$ shows the highest cytotoxicity among the three compounds against the two tested human tumor cell lines. These results indicate that the cytotoxic activity is enhanced when four ligands are coordinated to four platinum atoms. Probably, the high cytotoxicity of the $Pt_4(L)_4$ complex may be related to the intercalation of the metal complex between nitrogen bases of the DNA tumor cells, causing greater conformational changes in the double helix of DNA and then producing cell death.[28] On the other hand, these results also show that the synthesized ligand and complexes have more effectiveness against HT-29 cancerous cell line in comparison to the HuTu-80 cell lines in the concentration range used (0.1−10 μM) and are thus more effective in comparison to cisplatin. Here the IC_{50} has been shown to be 11.9 μM for the HT29 cells.[31]

Table 5. Selected Bond Lengths within [Pt(ATTSC)(ATTSC-H)Cl] (2)

bond	length [Å]	bond	length [Å]
Pt(1)−C(5)	2.015(8)	C(4)−C(5)	1.44(1)
Pt(1)−N(1)	2.016(7)	S(3)−C(13)	1.720(9)
Pt(1)−S(3)	2.264(2)	S(4)−C(10)	1.709(9)
Pt(1)−S(1)	2.366(2)	S(4)−C(9)	1.735(9)
S(1)−C(6)	1.712(9)	N(4)−C(8)	1.29(1)
S(2)−C(3)	1.702(9)	N(4)−N(5)	1.38(1)
S(2)−C(2)	1.726(9)	N(5)−C(13)	1.34(1)
N(1)−C(1)	1.30(1)	N(6)−C(13)	1.32(1)
N(2)−C(6)	1.34(1)	C(8)−C(9)	1.45(1)
N(3)−C(6)	1.34(1)	C(8)−C(14)	1.49(1)
C(1)−C(2)	1.40(1)	C(9)−C(12)	1.38(1)
C(1)−C(7)	1.49(1)	C(10)−C(11)	1.33(1)
C(2)−C(5)	1.42(1)	C(11)−C(12)	1.41(1)
C(3)−C(4)	1.34(1)	C(4)−C(5)	1.44(1)

Table 6. IC_{50} (μM) Values for the Tested Ligand and Complexes against Two Different Human Cancerous Cell Lines

	$IC_{50\%}$ (μM)	
compound	HT-29	HuTu-80
HATTSC	8.6	>10.0
$Pt(ATTSC)_2$	3.0	5.9
$Pt_4(ATTSC)_4$	1.5	1.2

CONCLUSIONS

New potential anticancer Pt(II) complexes were synthesized through the reaction of a heterocyclic thiosemicarbazone ligand with Pt(II) ion in 1:1 and 1:2 molar ratios.

The structures of the synthesized compounds were elucidated on the basis of spectroscopic and diffraction data (IR, ^{1}H and ^{13}C NMR, UV−vis, and XRD).

As the experimental results show, the used Schiff base reacts with Pt(II) ions in different modes of bonding. In one case, the ligand is twice deprotonated at the β-C carbon atom of the thiophene ring and at the sulfur atom, forming a dinegative tridentate chelate around platinum. By intermolecular coordination, a planar C, N, S_2-configuration around platinum atoms is obtained, resulting in a tetranuclear cluster. In the other case, a mononuclear complex of platinum is obtained with two different Schiff base ligands (one is neutral bonding through sulfur, the other is single negative bonding through carbon, nitrogen, and sulfur).

All ligands and complexes tested show a concentration-dependent reduction of cell proliferation. The test results show that the change of the ligand metal ratio has significant effects on the antiproliferative activities of the platinum(II) complexes. In general, it was found that complexes were more active than the corresponding metal-free ligand. The complex with the general formula Pt_4L_4 was found to be slightly more active than the complexes with formula PtL_2 against HT-29 and HuTu cancer cell line.

ASSOCIATED CONTENT

Supporting Information

CIF files giving crystallographic data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Michael.veith@inm-gmbh.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support for this work was provided by DAAD. The experimental part of this work was performed at INM−Leibniz Institute for New Materials and at the Institute of Inorganic Chemistry, Saarbruecken/Germany. A.A.A. would like to thank Prof. Yosif Sulfab, Hameed Ullah Wazir, and Siddieg Elsiddieg for kind discussions.

■ REFERENCES

(1) Kovala-Demertzi, D.; Boccarelli, A.; Demertzis, M. A.; Coluccia, M. *Chemotherapy* **2007**, *53*, 148.
(2) Kovala-Demertzi, D.; Varadinova, T.; Genova, P.; Souza, P.; Demertzis, M. A. *Bioinorg. Chem. Appl.* **2007**, 56165.
(3) Scovill, J. P.; Klayman, D. L.; Franchino, C. F. *J. Med. Chem.* **1982**, *25*, 1261.
(4) Quiroga, A. G.; Pérez, J. M.; López-Solera, I.; Masaguer, J. R.; Luque, A.; Román, P.; Edwards, A.; Alonso, C.; Navarro-Ranninger, C. *J. Med. Chem.* **1998**, *41*, 1399.
(5) Costa, R. F. F.; Rebolledo, A. P.; Matencio, T.; Calado, H. D. R.; Ardisson, J. D.; Cortés, M. E.; Rodrigues, B. L.; Beralso, H. *J. Coord. Chem.* **2005**, *58*, 1307.
(6) Agarwal, R. K.; Singh, L.; Sharma, D. K. *Bioinorg. Chem. Appl.* **2006**, 59509.
(7) Pandey, O. P.; Sengupta, S. K.; Mishra, M. K.; Tripathi, C. M. *Bioinorg. Chem. Appl.* **2003**, *1*, 35.
(8) Shipman, C. Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Antiviral Res.* **1986**, *6*, 197.
(9) Basuli, I.; Pal, F.; Bhattacharya, S. *Proc. Indian Acad. Sci.: Chem. Sci.* **2002**, *114*, 255.
(10) Bakkar, M. S.; Yamin, S. M.; Monshi, M. S. *Synth. React. Inorg., Met.-Org., Nano-Met. Chem.* **2003**, *33*, 1157.
(11) El-Shazly, R. M.; Al-Hazmi, G. A. A.; Ghazy, S. E.; El- Shahawi, M. S.; El-Asmy, A. A. *J. Coord. Chem.* **2006**, *59*, 845.
(12) Kovala, D.; Miller, J. R.; Koukoumelis, N.; Hadjikakou, S. K.; Demetzis, M. A. *Polyhedron* **1999**, *18*, 1005.
(13) Kovala, D.; Domopoulou, A.; Demetzis, M. A.; Papageorgiou, A.; West, D. X. *Polyhedron* **1997**, *16*, 3625.
(14) Kovala, D.; Yadav, P. N.; Demetzis, M. A.; Coluccia, M. J. *Inorg. Biochem.* **2000**, *78*, 347.
(15) Quiroga, A. G.; Perez, J. M.; Montero, E. I.; Masaguer, J. R.; Alonso, C.; Navarro, C. J. *Inorg. Biochem.* **1998**, *70*, 117.
(16) Quiroga, A. G.; Perez, J. M.; Lopez, S. I.; Montero, E. I.; Masaguer, J. R.; Alonso, C.; Navarro, C. J. *Inorg. Biochem.* **1998**, *69*, 275.
(17) Fillastre, J. P.; Ranguenez, V. *Toxicol. Lett.* **1989**, *46*, 163.
(18) Noji, M.; Kizu, R.; Takeda, Y.; Akiyama, N.; Yoshikazi, I.; Eriguchi, M.; Kidani, Y. *Biomed. Pharmacother.* **2005**, *59*, 224.
(19) Matesanz, A.; Pérez, J. M.; Navarro, P.; Moreno, J. M.; Colacio, E.; Souza, P. *J. Inorg. Biochem.* **1999**, *76*, 29.
(20) Kelland, L. R. *Crit. Rev. Oncol. Hematol.* **1993**, *15*, 191.
(21) De Lima, G. M.; Neto, J. L.; Beraldo, H.; Seibald, H. G. L; Duncalf, D. J. *J. Mol. Struct.* **2001**, *604*, 287.
(22) Mohammad, A.; Mahbub, S. M.; Ray, J.; Jerry, P.; John, M. *Polyhedron* **1997**, *16*, 2749.
(23) West, D. X.; Scovill, J. P.; Silverton, J.; Bareso, A. *Transition Met. Chem.* **1986**, *11*, 123.
(24) Koch, K. R. *J. Coord. Chem.* **1991**, *22*, 289.
(25) Tarlok, S. L.; Rekha, S.; Gagandeep, B.; Sonia, K. *Coord. Chem. Rev.* **2009**, *253*, 977.
(26) Souza, P.; Matesanz, A. I.; Fernandez, V. *J. Chem. Soc., Dalton Trans.* **1996**, 3011.
(27) Lever, A. B. P. *Inorganic Electronic Spectroscopy*; Elsevier: NewYork, 1984.
(28) Crystal data for $[Pt_4(ATTSC\text{-}2H)_4\cdot 4DMF]$, **1**: Empirical formula: $C_{40}H_{56}N_{16}O_4Pt_4S_8$; formula weight: 1861.85; temperature: 134(2) K; wavelength: 0.71073 Å; crystal system and space group: triclinic ($P\bar{1}$); unit cell dimensions: a = 9.785(1) Å, b = 11.775(2) Å, c = 24.179(4) Å, α = 96.994(6)°, β = 94.403(7)°, γ = 94.925(6)°; V: 2748.1(7) Å^3; Z: 2; density (calcd): 2.250 Mg/m^3; absorption coefficient: 10.509 mm^{-1}; F(000): 1760; crystal size: 0.13 × 0.04 × 0.03 mm^3; theta range for data collection: 1.70 to 21.67°; index ranges: $-10 \le h \le 9$, $-12 \le k \le 12$, $-25 \le l \le 24$; reflections collected: 25874; independent reflections: 6299 [R(int) = 0.1068]; completeness to theta = 21.67°: 97.4%; absorption correction: multiscan; max. and min. transmission: 0.7791 and 0.3487; refinement method: full-matrix least-squares on F^2; data/restraints/parameters: 6299/0/661; goodness-of-fit on F^2: 1.034; final R indices [$I > 2\sigma(I)$]: R1 = 0.0433, wR2 = 0.0947; R indices (all data): R1 = 0.0687, wR2 = 0.1055; largest diff peak and hole: 1.504 and −1.151 e·Å^{-3}.
(29) Crystal data for $Pt(ATTSC)(ATTSC\text{-}H)Cl\cdot 3CH_3OH$], **2**: Empirical formula: $C_{17}H_{29}Cl_7N_6O_3PtS_4$; formula weight: 715.17; temperature: 222(2) K; wavelength: 0.71073 Å; crystal system and space group: triclinic ($P\bar{1}$); unit cell dimensions: a = 7.701(1) Å, b = 12.887(2) Å, c = 15.119(3) Å, α = 70.480(8)°, β = 75.639(9)°, γ = 75.383(9)°; V: 1346.3(4) Å^3; Z: 2; density (calcd): 1.764 Mg/m^3; absorption coefficient: 5.649 mm^{-1}; F(000): 694; crystal size: 0.863 × 0.39 × 0.07 mm^3; theta range for data collection: 1.45 to 31.68°; index ranges: $-11 \le h \le 11$, $-18 \le k \le 17$, $-22 \le l \le 22$; reflections collected: 28 946; independent reflections: 8866 [R(int) = 0.0775]; completeness to theta = 21.67°: 97.4%; absorption correction: multiscan; refinement method: full-matrix least-squares on F^2; data/restraints/parameters: 8866/0/264; goodness-of-fit on F^2: 1.732; final R indices [$I > 2\sigma(I)$]: R1 = 0.0748, wR2 = 0.1976; R indices (all data): R1 = 0.0839, wR2 = 0.2017; largest diff peak and hole: 7.181 and −4.070 e·Å^{-3}.
(30) Wilfredo, H.; Paz, J.; Vaisberg, A.; Sodine, E.; Richter, R.; Beyer, L. *Bioinorg. Chem. Appl.* **2008**, Article ID 690952, DOI: 10.1155/2008/690952.
(31) Lovejoy, K. S.; Serova, M.; Bieche, I.; Emami, S.; D'Incalci, M.; Broggini, M.; Erba, E.; Gespach, C.; Cvitkovic, E.; Faivre, S.; Raymond, E.; Lippard, S. J. *Mol. Cancer Ther.* **2011**, *10*, 1709.