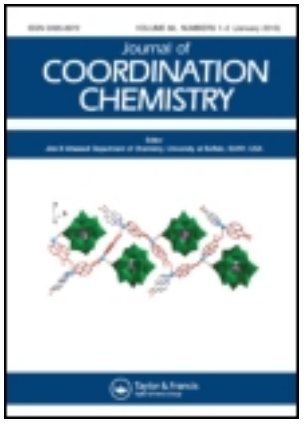

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Platinum(IV) complexes with N-alkylphenothiazines: synthesis, characterization, and antibacterial activity

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Two new platinum(IV) complexes (**1**, trifluoperazinehydrochloride-aquapentachloridoplatinate(IV) and **2**, chlorpromazine-chlorpromazinehydrochloridepentachloridoplatinate(IV)) were synthesized in the reaction of $K_2[PtCl_6]$ with trifluoperazine dihydrochloride (TF·2HCl) or chlorpromazine hydrochloride (CP·HCl). The complexes were characterized by elemental analysis, molar conductivity measurement, and spectral (IR, ^{1}H, ^{13}C, 2D ^{1}H–^{13}C heteronuclear correlation spectra, ^{195}Pt NMR, and MS) methods. Outer-coordination sphere was proposed for **1**; while in **2**, the ligand was coordinated to the metal. The complexes exhibit antibacterial effect on strains of *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pumilus*, and methicillin-resistant *Staphylococci* as Gram-positive bacteria and an *Escherichia coli* as Gram-negative bacteria, as well as the reference strains.

Keywords: Platinum(IV); N-alkylphenothiazines; Antibacterial activity

1. Introduction

Interest in the antitumor properties of platinum(IV) complexes has been growing steadily [1–6] and some have even entered for clinical examination [7–9]. The screening has been extended to include a number of synthesized platinum(II) [10–12] and platinum(IV) [13, 14] complexes usually containing N, S, and O donors with some promising antibacterial activity against different bacterial strains.

Phenothiazines and related compounds have been found to have extensive application in many fields of medicine [15–19]. Among these, antihistaminic and antipsychotic are the ones that are mostly exploited therapeutically. They also possess antimicrobial [20–22] and antifungal [23] effects. Despite extensive studies on complexes with N-alkylphenothiazines [24–27], only some have tested for their biological properties [28–31]. The complexes with these compounds of ruthenium(II) show promising antiproliferative activity against human cancer cells [28, 32]; copper(II) [29] and dinuclear tantalum(V) [30] complexes exhibit greater antifungitoxicity, while palladium(II) complexes demonstrate antibacterial activity [31]. There is, however, tremendous interest to obtain further insight into the structure of

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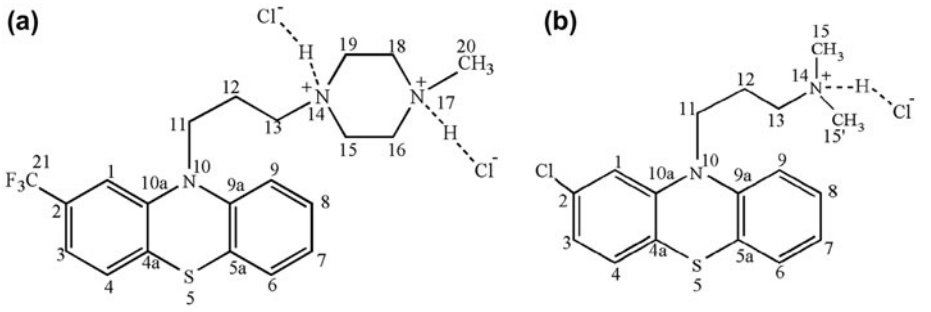


Figure 1. The structures with atom numbering of the ligands: (a) trifluoperazine dihydrochloride (TF·2HCl), 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride; and (b) chlorpromazine hydrochloride (CP·HCl), 2-chloro-10-(3-dimethylaminopropyl) phenothiazine hydrochloride.

these complexes because N-alkylphenothiazines have been found mostly as outer-sphere molecules.

In the course of research in this field [28, 32], we here report the preparation and properties of two new Pt(IV) complexes (**1** and **2**) with N-alkylphenothiazines (trifluoperazine dihydrochloride (TF·2HCl) or chlorpromazine hydrochloride (CP·HCl), respectively, figure 1). The effect of the ligands on the antibacterial activities of the platinum(IV) complexes was evaluated and the structure–activity relationships were established. Investigated bacterial strains, however, are important for food industry as they are responsible for food spoilage (*Bacillus*) or foodborne diseases (*Staphylococci*, *Escherichia coli*). *Staphylococci* are also significant agents in clinical–medical practice – especially MRSA – as they are causative agents of purulent infections which are hard to treat. The same is observed with *E. coli* (gastrointestinal infections, septicaemia, etc.). Thus, the possible usage of tested complexes as antimicrobial agents was screened.

2. Experimental

2.1. *Materials and methods*

N-Alkylphenothiazines, trifluoperazine dihydrochloride (TF·2HCl), and chlorpromazine hydrochloride (CP·HCl) were purchased from Aldrich (spectral data for them are given in Section 2.4); while for the biological assays, the used chemicals were of reagent grade (Merck) and were used as received. The starting complex $K_2[PtCl_6]$ was prepared as described [33].

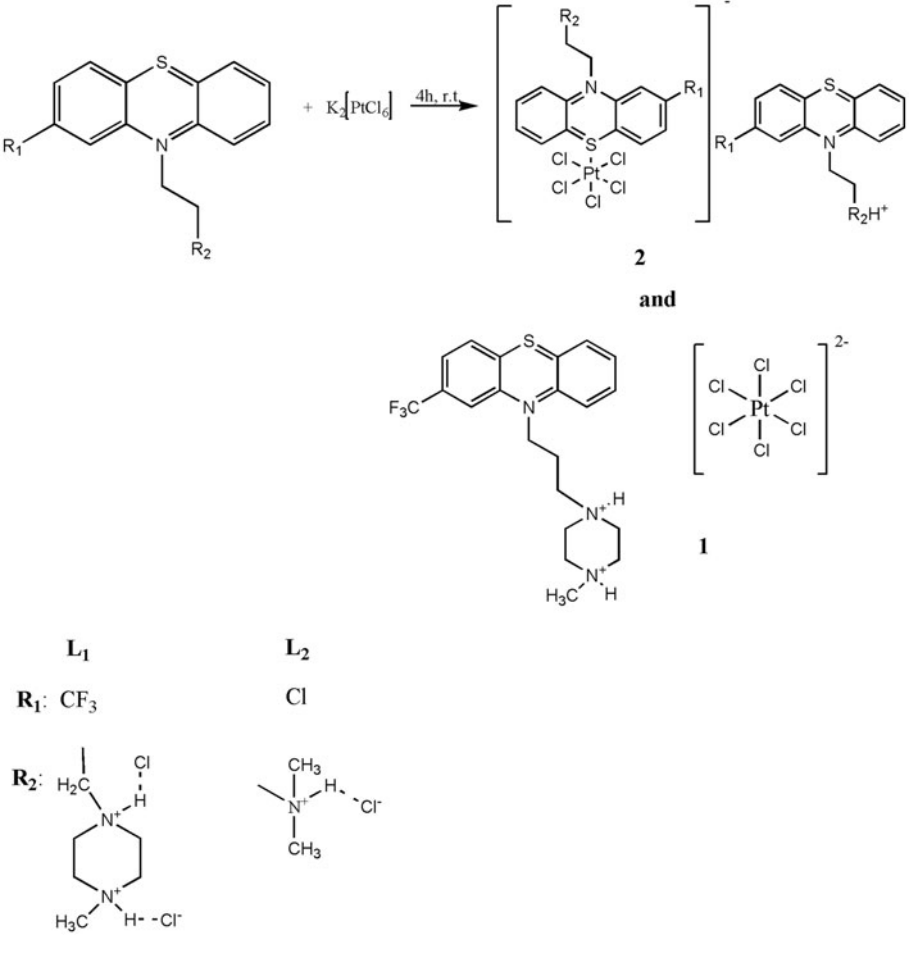
Elemental analyses were carried out on an Elemental Vario EL III microanalyser. Infrared spectra of the ligands and complexes were recorded on a Nicolet 6700 FT-IR spectrometer by ATR technique. ^{1}H NMR, ^{13}C NMR, 2D ^{1}H–^{13}C heteronuclear correlation spectra, and ^{195}Pt NMR spectra were run on Bruker Avance III (500, 50, and 107.54 MHz, respectively) in DMSO-d_6 at room temperature. Mass spectra were carried out on an MS system consisting of a 6210 TOF LC/MS (G1969 A, Agilent Technologies) in DMSO. Molar conductivity measurements were carried out on the Crison Multimeter MM41 in 10^{-3} mol dm^{-3} DMSO solution at room temperature.

2.2. *Preparation of starting complex $K_2[PtCl_6]$*

Platinum powder (1 g, 5.13 mmol) was dissolved in a mixture (25 cm^3) of concentrated HNO_3 and HCl in 1:3 volume ratio. The excess HNO_3 and formed nitrogen oxides were removed by adding 15 cm^3 of concentrated HCl and the volume was reduced to 10 cm^3. Solution was then cooled to room temperature and 0.766 g of potassium chloride was added. An yellow precipitate formed immediately was filtered off and washed with a cold mixture of ethanol and water in 1:1 volume ratio and then with cold ethanol, and was left to dry in air for 2–3 days.

2.3. *Preparation of complexes*

To a solution containing $K_2[PtCl_6]$ (0.20 mmol, 100 mg) in water (30 cm^3), the corresponding ligand (i.e. 0.20 mmol, 110 mg for trifluoperazine dihydrochloride and 0.40 mmol, 140 mg for chlorpromazine hydrochloride, respectively) was added in 1 h. Precipitate formed in the next four hours of synthesis. The reaction mixture was stirred and protected from light



Scheme 1. Reaction scheme for synthesis of **1** and **2**.

at room temperature. Precipitates, light brown for **1** and green for **2**, were filtered off and dried *in vacuo*. The complexes (scheme 1) were obtained as analytically pure samples.

2.3.1. Complex 1 ((TFH·HCl)[PtCl$_5$H$_2$O]). Yield: 90.00 mg, 54.46%. Anal. Calcd for $C_{21}Cl_6F_3H_{28}N_3OPtS$ (Mr 835.33) (%): C, 30.19; H, 3.38; N, 5.03; S, 3.84. Found: C, 30.09; H, 3.29; N, 4.98; S, 4.49. IR (ATR): ν(R_3NH^+) 2757.8, 2367.3 cm^{-1}, ν(CH)ar 1605.6, 1570.6 cm^{-1}, δ_{as}(CH), δ_s(CH) 1463.2, 1424.6 cm^{-1}, γ(CH) 879.9 cm^{-1}, π(CH) 824.3 cm^{-1}, ν(CSC) 753.7 cm^{-1}. ^{1}H NMR (ppm) (DMSO-d$_6$, 500 MHz) δ: 2.06 (m, 2H, $CH_2C\boldsymbol{H}_2CH_2$); 2.90 (s, 3H, N**CH$_3$**); 3.16–3.71 (m, 2H, C***H*$_2$**N-piperazine, 8H, piperazine); 4.05 (t, J = 5.8 Hz, 2H, C***H*$_2$**N-phenothiazine); 7.04–7.42 (m, 7H, phenothiazine); 10.04 (s, 2H); ^{13}C NMR (ppm) (DMSO-d$_6$, 50 MHz) δ: 21.59 (C_{12}), 42.52 (C_{20}), 43.79 (C_{11}), 48.88 (C_{13}), 50.32 (C_{16}, C_{18}), 53.75 (C_{15}, C_{19}), 112.09 (C_1), 116.76 (C_3), 119.40 (C_7), 123.25 (C_9), 123.58 (C_8), 125.23 (C_6), 127.45 (C_{21}), 128.40 (C_4), 129.03 (C_{4a}), 129.83 (C_2, C_{4a}, C_{5a}), 143.46 (C_{9a}), 145.45 (C_{10a}). ^{195}Pt NMR (ppm) (DMSO-d$_6$, 107.54 MHz) δ: 406.76; ESI-MS, *m/z*, positive mode: 408.17 ([TF·H]$^+$); negative: 450.83 ([PtCl$_5$ + DMSO]$^-$), 372.81 ([PtCl$_5$–H]$^-$), 335.84 ([PtCl$_4$–H]$^-$); λ_m (1.0×10^{-3} mol dm^{-3}, DMSO, 25 °C): 50.5 Ω^{-1} cm^2 mol^{-1}.

2.3.2. Complex 2 ((CP·H)[Pt(CP·HCl)Cl$_5$]·H$_2$O). Yield: 140.00 mg, 65.69%. Anal. Calcd for $C_{34}Cl_8H_{40}N_4PtS_2{\cdot}H_2O$ (Mr 1065.56) (%): C, 38.32; H, 3.97; N, 5.26; S, 6.02. Found: C, 38.33; H, 3.90; N, 5.29; S, 5.93. IR (ATR): ν(R_3NH^+) 2731.4 cm^{-1}, ν(CH)ar 1567.3 cm^{-1}, δ_{as}(CH), δ_s(CH) 1456.9, 1408.2 cm^{-1}, γ(CH) 931.0 cm^{-1}, π(CH) 804.0 cm^{-1}, ν(CSC) 754.7 cm^{-1}. ^{1}H NMR (ppm) (DMSO-d$_6$, 500 MHz) δ: 2.04–2.17 (m, 4H, $CH_2C\boldsymbol{H}_2CH_2N(CH_3)_2$); 2.67–2.70 (m, 12H, $N(C\boldsymbol{H}_3)_2$); 3.14 (m, 4H, $CH_2CH_2C\boldsymbol{H}_2N(CH_3)_2$); 3.98 and 4.47 (m, 4H, $C\boldsymbol{H}_2CH_2CH_2N(CH_3)_2$); 7.02–8.02 (m, 14H, phenothiazine); 10.40 (s, 2H, $^+HN(CH_3)_2$). ^{13}C NMR (ppm) (DMSO-d$_6$, 50 MHz) δ: 21.55 (C_{12}), 42.14 (C_{15}), 43.90 (C_{11}), 54.31 (C_{13}), 115.94 (C_1), 116.50 (C_3), 122.49 (C_9), 123.33 (C_7), 127.94 (C_6, C_8), 132.68 (C_4), 137.43 (C_2), 137.88 (C_{5a}), 139.14 (C_{4a}), 143.74 (C_{9a}), 146.18 (C_{10a}). ^{195}Pt NMR (ppm) (DMSO-d$_6$, 107.54 MHz) δ: –824.65; ESI-MS, *m/z*: positive: 319.10 ([CP·H]$^+$); negative: 690.91 ([Pt(CP·HCl)Cl$_4$–2H]$^-$), 372.81 ([PtCl$_5$–H]$^-$), 335.84 ([PtCl$_4$–H]$^-$); λ_m (1.0×10^{-3} mol dm^{-3}, DMSO, 25 °C): 54.3 Ω^{-1} cm^2 mol^{-1}.

2.4. *Spectral data for ligands*

2.4.1. Trifluoperazine dihydrochloride (TF·2HCl). Anal. Calcd for $C_{21}H_{26}F_3N_3SCl_2$ (Mr 480.42) (%): C, 52.50; H, 5.45; N, 8.75; S, 6.67. Found: C, 52.25; H, 5.37; N, 8.56; S, 6.65. IR (ATR): ν(R_3NH^+) 2340.2 cm^{-1}, ν(CH)ar 1599.9 cm^{-1}, δ_{as}(CH), δ_s(CH) 1467.7, 1423.6 cm^{-1}, γ(CH) 939.8 cm^{-1}, π(CH) 823.5 cm^{-1}, ν(CSC) 754.4 cm^{-1}. ^{1}H NMR (ppm) (DMSO-d$_6$, 500 MHz) δ: 2.13 (m, 2H, $CH_2C\boldsymbol{H}_2CH_2$); 2.80 (s, 3H, N***H*$_3$**); 3.26–3.62 (m, 2H, C***H*$_2$**N-piperazine, 8H, piperazine); 4.06 (t, J = 5.8 Hz, 2H, C***H*$_2$**N-phenothiazine); 7.03–7.40 (m, 7H, phenothiazine); 11.90 (s, 2H). ^{13}C NMR (ppm) (DMSO-d$_6$, 50 MHz) δ: 21.24 (C_{12}), 41.95 (C_{20}), 43.98 (C_{11}), 48.26 (C_{13}), 49.64 (C_{16}, C_{18}), 53.43 (C_{15}, C_{19}), 112.15 (C_1), 116.68 (C_3), 119.36 (C_7), 123.12 (C_9), 123.55 (C_8), 125.27 (C_6), 127.50 (C_{21}), 127.95 (C_4), 128.67 (C_{4a}), 129.63 (C_2, C_{4a}, C_{5a}), 143.50 (C_{9a}), 145.52 (C_{10a}); ESI-MS, *m/z*, positive mode: 408.17 ([TF·H]$^+$).

2.4.2. Chlorpromazine hydrochloride (CP·HCl). Anal. Calcd for $C_{17}H_{20}N_2SCl_2$ (Mr 355.32) (%): C, 57.46; H, 5.67; N, 7.88; S, 9.02. Found: C, 57.25; H, 5.52; N, 7.68; S, 8.86. IR (ATR): $\nu(R_3NH^+)$ 2398.4 cm^{-1}, ν(CH)ar 1562.1 cm^{-1}, δ_{as}(CH), δ_s(CH) 1455.6 cm^{-1}, γ(CH) 928.0 cm^{-1}, π(CH) 802.3 cm^{-1}, ν(CSC) 749.4 cm^{-1}. ^{1}H NMR (ppm) (DMSO-d_6, 500 MHz) δ: 2.04–2.10 (m, 2H, $CH_2C\boldsymbol{H}_2CH_2N(CH_3)_2$); 2.67 (m, 6H, $N(C\boldsymbol{H}_3)_2$); 3.12 (m, 2H, $CH_2CH_2C\boldsymbol{H}_2N(CH_3)_2$); 3.98 (m, 2H,$C\boldsymbol{H}_2CH_2CH_2$ $N(CH_3)_2$); 6.99–7.26 (m, 7H, phenothiazine); 10.84 (s, 1H, $^+HN(CH_3)_2$). ^{13}C NMR (ppm) (DMSO-d_6, 50 MHz) δ: 21.44 (C_{12}), 41.94 (C_{15}), 43.93 (C_{11}), 54.15 (C_{13}), 115.90 (C_1), 116.45 (C_3), 122.43 (C_9), 123.97 (C_7), 127.88 (C_6, C_8), 128.21 (C_4), 132.00 (C_2, C_{5a}, C_{4a}), 143.70 (C_{9a}), 146.16 (C_{10a}); ESI-MS, *m/z*: positive: 319.10 ($[CP\cdot H]^+$).

2.5. *Antimicrobial screening*

Investigation of antibacterial activity of the N-alkylphenothiazines and their platinum(IV) complexes has been performed with bacterial strains isolated from clinical specimens as with referential strains from the ATCC collection: MRSA ATCC 43360, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633BB and *E. coli* ATCC 10536. Investigated *B. cereus* and *Bacillus pumilus* were isolated from fresh milk and cheese specimens. *E. coli* strains were isolated from fecal specimens originating from domestic poultry and pigs. Investigated methicillin-resistant *Staphylococci* were isolated from ear swabs taken from dogs and cats. The isolation was made from a clinical material delivered to the Department of Microbiology of the Faculty of Veterinary Medicine, University of Belgrade.

For isolation and identification of Gram-positive bacteria and *E. coli*, conventional microbiological methods were applied using commercial kits for species identification – BBL Crystal Gram-positive ID kit and BBL Crystal Enteric/Nonfermenter ID kit (Becton Dickinson). For the detection of methicilin-resistant *Staphylococcus aureus* (MRSA) strains, cefoxitin disks were used (Becton Dickinson) in routine disk diffusion investigations, and confirmation was done by detecting *mec*A gen using PCR according to the previously described protocols [34]. For antibacterial susceptibility testing, broth microdilution was applied for determining the minimal inhibitory concentration (MIC) values in accord with the CLSI recommendations (Clinical and Laboratory Standards Institute, 2006) prescribed references [35] for antimicrobial susceptibility testing. Cation-adjusted Mueller Hinton II broth was used (CAMHB, Becton Dickinson) and the antibacterial activity of N-alkylphenothiazines and its platinum(IV) complexes were investigated in concentrations of 1280; 640; 320; 160; 80; 40; 20; 10; 5; 2.5; 1.25; and 0.625 μg mL^{-1}. The compounds were previously dissolved in concentration of 1280 μg mL^{-1} in DMSO, and then the two fold serial dilutions of extracts down to the lowest tested concentration were prepared. The desired inoculum density of 5×10^5 CFU mL^{-1} was achieved by preparing suspension of bacteria of approximately 1–2 $\times$ 10^8 CFU mL^{-1}, which was the density equal to McFarland standard, 0.5 (Becton Dickinson). The prepared suspension was diluted 10 times to obtain the final inoculum density of approximately 1–2 $\times$ 10^7 CFU mL^{-1} and 50 μL of this suspension was applied to CAMHB, after which the number of bacteria in the media was approximately 5×10^5 mL^{-1}. The media were incubated at the temperature of 37 °C for 18 h. For MIC values, the broth with lowest concentration of investigation compounds, with no visible bacterial growth, was used.

3. Results and discussion

3.1. *Synthesis*

Complex **1** was synthesized from $K_2[PtCl_6]$ and trifluoperazine dihydrochloride in 1:1 molar ratio (ratio of 1:2 gave the same product), while **2** was prepared from the starting complex and chlorpromazine hydrochloride in 1:2 molar ratio. The analytical data were in good agreement with the proposed stoichiometry of the complexes. Both complexes were stable under atmospheric conditions and insoluble in water and common organic solvents, but soluble in DMSO.

3.2. *Spectroscopic characterization*

3.2.1. IR spectra. Infrared spectra of uncoordinated N-alkylphenothiazine hydrochloride exhibit a strong broad band at 2500–2300 cm^{-1}, characteristic for interaction of the tertiary amine R_3NH^+ with chloride [27, 28]. In the spectra of the obtained complexes, these bands shift to higher wave numbers, indicating weakening of hydrogen bonds between nitrogen and chloride. The sharp band at 750 cm^{-1} in the spectrum of free trifluoperazine dihydrochloride assigned to the heterocyclic ν(CSC) remains relatively unchanged in the spectrum of the corresponding **1**, suggesting non-coordination of the heterocyclic sulfur. This band has reduced intensity in the spectrum of **2**, suggesting coordinated N-alkylphenothiazine via sulfur.

3.2.2. NMR spectra. Comparison of the signals in the ^1H NMR spectra of free ligands with those of the corresponding complexes exhibits some changes in the resonance of signals due to complexation. The aromatic heterocyclic ring protons from phenothiazine in free ligands are observed as multiplets around 6.99–7.40 ppm. In the spectrum of **2,** these protons shift to 7.02–8.02 ppm and the peaks were divided into two groups of signals, as multiplets. 2-D ^1H–^{13}C heteronuclear correlation spectrum of **2** showed that the shifts for protons that were directly under the influence of coordination for platinum ion (protons on C_4 and C_6) were moved to 8 ppm. The aromatic protons in the spectrum of **1** demonstrate almost the same chemical shifts as the corresponding N-alkylphenothiazine, indicating their outer-sphere position [14]. Broad singlet at 10.84–11.90 ppm in spectra of free ligands attributed to R_3NH^+ protons exhibited downfield shifts in complexes, indicating a change in this proton environment [27]. Carbons of **1** showed the same shifts as free TF·2HCl, also reflecting non-coordination. Carbons near sulfur (C_{5a}, C_{4a}) had the same chemical shifts (132.00 ppm) in the spectrum of chlorpromazine hydrochloride; while in **2,** these signals for carbons were separated and slightly shifted downfield (137.88 (C_{5a}) and 139.14 (C_{4a})), indicating coordination via sulfur.

Presence of ligand to metal coordination in **2** was confirmed by ^{195}Pt NMR spectrum. The sharp singlet at −824.65 ppm indicates coordination of ligand to platinum via sulfur. In the spectrum of **1**, the peak in the ^{195}Pt NMR spectrum at 406.76 ppm corresponds to $[PtCl_5H_2O]^-$ [36]. This was in agreement with supposed structure using other spectral data.

3.2.3. Mass spectra. Mass spectra of synthesized complexes were recorded in positive and negative ion mode. In positive ion mode, there are signals for cation (**2**) that correspond to positively charged ligands (i.e. *m/z* 319.10 $[CP{\cdot}H]^+$) and outer sphere of **1** (*m/z* 408.17 for

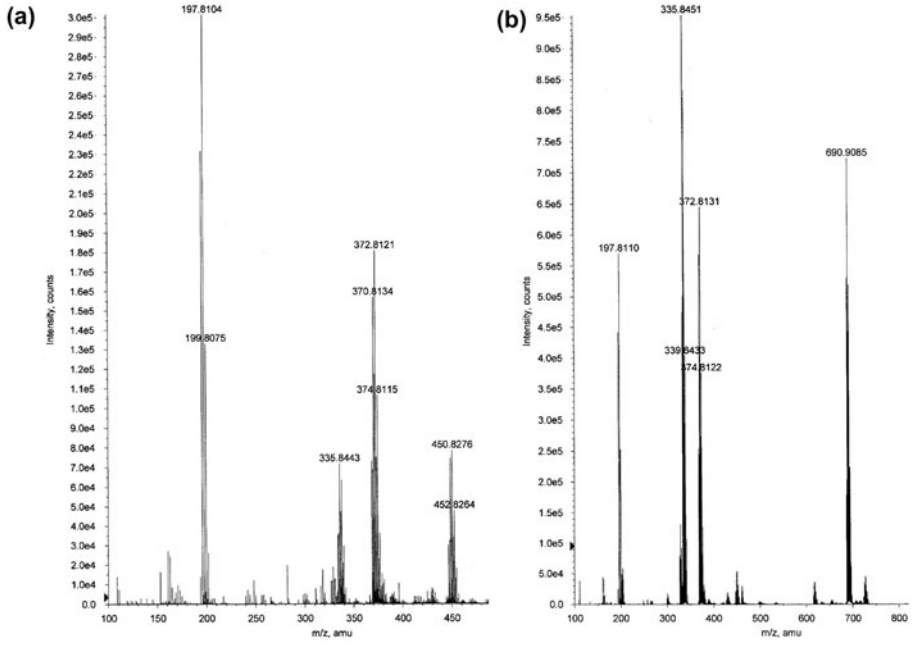


Figure 2. Mass spectra of complexes recorded in negative mode: (a) **1** and (b) **2**.

$[TF{\cdot}H]^+$). In negative mode, several fragment ions confirmed the proposed molecular structure (figure 2). Identical molecular peaks at *m/z* 335.84 for $[PtCl_4–H]^-$ and at *m/z* 372.81 for $[PtCl_5–H]^-$ confirmed the same inner sphere for both complexes. Strong signal for $[Pt(L)Cl_4–H]^-$ (690.91 for $[Pt(CP{\cdot}HCl)Cl_4–2H]^-$) indicates coordination of CP·HCl to platinum (**2**). This is also in agreement with NMR spectra and showed peaks corresponding to complete inner sphere of **2** or their fragments. Signal of $[PtCl_5 + DMSO]^-$ for **1**, however, was in agreement with ^{1}H NMR spectra and indicate outer-sphere position of alkylphenothiazine.

Significantly, different structure of **1** in relation to **2** was probably due to the differences in the ligand structure. TF·2HCl is electrophilic because of the +2 charge and the presence of electron-withdrawing $–CF_3$ [37]. The higher electrophilic nature of Pt(IV) results in strong interaction with chloride, hence substitution by electrophilic TF·2HCl is unfavorable. CP·HCl has an electron donor –Cl, hence it is nucleophilic and easily substituted the chloride coordinated to Pt(IV) in $K_2[PtCl_6]$.

3.2.4. Molar conductivity measurements. Molar conductivity measurements confirmed structures of **1** and **2**. The values for conductivity of these complexes were 50–70 Ω^{-1} cm^2 mol^{-1}, which corespond to 1:1 electrolytes in DMSO [38].

3.3. *Antibacterial activity*

The obtained results with the MIC values of complexes, free ligands, and antibiotic Ceftriaxone are summarized in table 1.

Table 1. *In vitro* antibacterial activity of the tested ligands and corresponding platinum(IV) complexes, MIC (μg mL^{-1}).

Bacterial strain	MIC, μg mL^{-1}					
	$K_2[PtCl_6]$	TF·2HCl	Complex **1**	CP·HCl	Complex **2**	Cefriaxone
Bacillus subtilis ATCC 6633BB	1024	16	32	64	64	8
Bacillus cereus ATCC 11778	1024	16	64	128	64	8
B. cereus	1024	16	64	64	32	8
B. pumilus	1024	32	64	64	64	16
MRSA* ATCC 43360	1024	32	128	128	64	>64
MRS 23 *S. pseudintermedius*	1024	32	128	64	32	>64
MRS15 *S. haemolyticus*	1024	32	128	64	64	>64
MRS 2 *S. haemolyticus*	1024	32	128	128	64	>64
MRS 20 *S. haemolyticus*	1024	32	128	512	64	>64
Escherichia coli ATCC 10536	1024	512	1024	512	256	4
E. coli 103	1024	1024	1024	512	512	8
E. coli 107	1024	1024	1024	1024	512	4
E. coli 108	1024	1024	1024	1024	512	4
E. coli 110	1024	256	1024	256	256	2

*MRSA (methicillin resistant *S. aureus*).

All compounds showed strong or moderately strong antibacterial activities against investigated Gram-positive bacteria, except $K_2[PtCl_6]$ which showed no activity against this group of micro-organisms. The free ligands exhibited varying degrees of inhibitory effects on growth of the tested bacteria (MICs 16–1024 μg mL^{-1}) as previously reported [39]. This is particularly in the case of TF·2HCl which showed strong activity against all Gram-positives with just two obtained MIC values – one is 16 μg mL^{-1} against *B. cereus* and *B. subtilis* strains, and the other is 32 μg mL^{-1} against *B. pumilus* and all MRSA strains. The obtained MIC value of 32 μg mL^{-1} against MRSA strains may be interpreted as strong antibacterial activity when compared to that of Ceftriaxone, which was >64 μg mL^{-1} and those strains were categorized as resistant to this antibiotic. Complex **1** showed strong activity against *B. subtilis* ATCC 6633BB (MIC 32 μg mL^{-1}) and moderately strong activity against other Gram-positives (MICs 64–128 μg mL^{-1}). Weaker activity of **1** compared to free TF·2HCl could be attributed to the outer-sphere position which reduces activity of the complex itself. Strong activity against all tested Gram-positive strains is evident for **2** with little more variation in the obtained MIC values (32–64 μg mL^{-1}) as their corresponding ligand CP·HCl. Complex **2** and its ligand CP·HCl showed strong-to-moderate antibacterial activity (MIC = 32–512 μg mL^{-1}) with activity of the complex higher than that of the corresponding ligand. Complex **2** is the only complex that showed some activity against Gram-negative micro-organisms with MIC values of 256–512 μg mL^{-1}, while the other complex showed weak antibacterial activity against Gram-negative micro-organisms with an MIC value of 1024 μg mL^{-1} for most of the investigated strains. The exceptions are TF·2HCl and CP·HCl as well as **2**, which showed moderately strong antibacterial activity against *E. coli* 110, with an obtained MIC of 256 μg mL^{-1}.

These results indicate that the antibacterial activity of the complexes differs from that of free ligands as described for other platinum(IV) complexes [13, 40]. Higher antibacterial effect of **2** could be interpreted in the light of coordination of N-alkylphenothiazines. Presence of a strong bond formed between platinum ion and ligand in the coordination sphere seems to be related to the electronic properties of the inner sphere as a whole, thus producing the corresponding reduced polarity, which favors permeability of this complex through the lipid membrane of the bacteria cell.

4. Conclusion

The present study demonstrates a simple synthetic route to two new Pt(IV) complexes with N-alkylphenothiazine ligands. On the basis of analytical and spectral methods, it is assumed that N-alkylphenothiazines formed complexes with Pt(IV) where one chlorpromazine hydrochloride is directly coordinated to Pt(IV) through sulfur and another ligand is out of the coordination sphere (**2**) while in **1,** only outer-sphere coordination was proposed. Inner sphere coordination of **2** resulted in greater antibacterial activity compared to the outer sphere **1**. Complex **2** exhibited moderately strong or weak activity against all tested Gram-negative micro-organisms, but this complex showed lower activity against *E. coli* than some platinum(IV) complexes. The MICs of **1** and **2** against *B. subtilis* exhibited better inhibition in comparison with those for the previously described platinum(II) complexes. Difference in a way of coordination mode of N-alkylphenothiazines to platinum ion could be a good approximation to the structure–activity relationship.

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