New water-soluble platinum(II) phenanthroline complexes tested as cisplatin analogues: first-time comparison of cytotoxic activity between analogous four- and five-coordinate species

Sandra A. De Pascali,[a] Danilo Migoni,[a] Paride Papadia,[a] Antonella Muscella,[a] Santo Marsigliante,[a] Antonella Ciccarese[a] and Francesco P. Fanizzi*[a,b]

Received 31st July 2006, Accepted 11th September 2006
First published as an Advance Article on the web 25th September 2006
DOI: 10.1039/b610945d

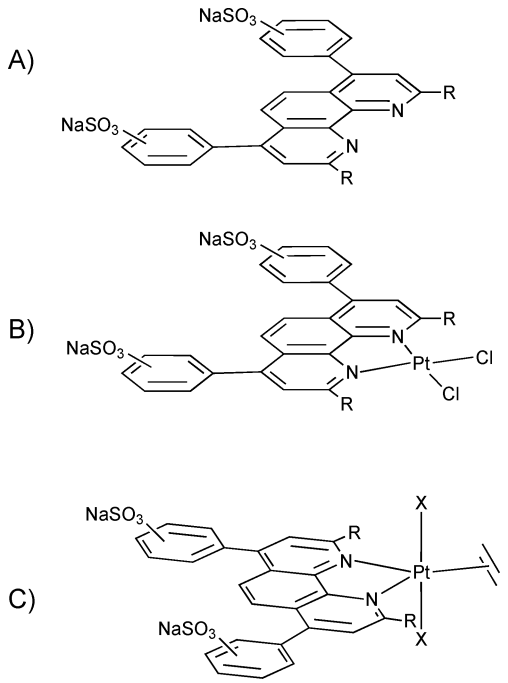
Four- and five-coordinate platinum(II) complexes, *cis*-[$PtCl_2(A_2)$] (**1**) and [$PtCl_2(A_2)(\eta^2$-ethylene)] (**2**) {A_2 = 4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt, BPS (mixture of isomers) (**a**); 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt, BCS (mixture of isomers) (**b**)} have been synthesized and characterized by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy. The stability and high water solubility of complexes **1a**, **1b** and **2b**, due to the presence of the polar SO_3^- groups on the ligands skeleton, allowed to test their *in vitro* cytotoxicity on HeLa tumour cells in a wide range of drug concentration. At low and medium incubation doses (<200 μM) **1a**, **1b** and **2b** all showed similar *in vitro* cytotoxicity, negligible or much lower with respect to cisplatin. At doses higher than 200 μM their activity increased and **1b**, the most active among the new complexes, exhibited a cytotoxicity comparable, although still lower, with respect to cisplatin. GFAAS Platinum analytical data showed that the tested compounds **1a**, **1b** and **2b**, although carrying sulfonate charged groups, may undergo cellular uptake, which, in the case of **1b** and **2b**, is even higher with respect to cisplatin. Furthermore, in the case of **1b** and **2b** it has been possible to compare, for the first time, the cytotoxic activity for square-planar four-coordinate and trigonal-bipyramidal five-coordinate platinum(II) complexes having the same carrier ligand. The tendency of the five-coordinate species **2b** to give at longer incubation time similar cytotoxicity with respect to the square-planar compound **1b** suggests a possible use of the trigonal-bipyramidal five-coordinate complexes as prodrugs.

Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II), is one of the most widely used anticancer drug,[1,2] highly effective in the treatment of testicular and ovarian cancer.[3] Subsequently to the serendipitous discovery of cisplatin, much attention has been turned to platinum complexes as potential anticancer drugs.[4] Furthermore, the significant side effects and the acquired or intrinsic resistance to cisplatin encourage the synthesis of new platinum compounds to afford new drugs with improved pharmacological properties and broader antitumor activity.[5,6] DNA is the main cellular target of cisplatin, the platinum atom forming covalent bonds to the N7 positions of the adjacent purine bases.[7] In the last years a number of diamine[8] or diimine[9] carrier ligands, such as Me_2ppz, phen and Me_2phen (Me_2ppz = chelate *N*,*N*′-dimethylpiperazine; phen = 1,10-phenanthroline; Me_2phen = 2,9-dimethyl-1,10-phenanthroline), have been found able to introduce steric hindrance in the coordination sphere of platinum and therefore to slow down the dynamic motions of the nucleobases about the Pt–N(7) bonds in *cis*-PtA_2G_2 adducts (A_2 = diamine or diimine carrier ligand, G = guanine derivative). Interestingly, in these systems it has been possible to characterize different conformers not only for *cis*-PtA_2G_2 but also in the case of *cis*-PtA_2(GpG) adducts used as retro model of the cisplatin-DNA d(GpG) intrastrand cross link.[10] Unfortunately, the low solubility of the *cis*-PtA_2Cl_2 complexes, when A_2 = phenanthrolinic ligand, prevented *in vitro* and *in vivo* assays to correlate the structure of the *cis*-PtA_2G_2 adducts with the biological activity of the corresponding platinum dichloro species. On the other hand, phenanthrolinic ligands such as 2,9-dimethyl-1,10-phenanthroline (neocuproine) or 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, (bathocuproine), are well known in Pt(II) chemistry for their ability to stabilize five-coordination in [PtX_2(N–N)(η^2-olefin)] complexes (N–N = bidentate N-donor ligand; X = halogen).[11,12] The trigonal-bipyramidal ground-state geometry of these complexes shows, in the metal coordination sphere, the two anionic ligands in axial position and the bidentate N-donor ligand together with the π-bonded alkene in the equatorial plane.[12] The high stability of these five-coordinate species stems from both the π-acceptor ability of the olefin ligand on the trigonal plane (which reduces the electron density on the metal centre) and the steric clashes between the *ortho*-methyl substituents of the phenanthroline and the *cis* halogen ligands, which destabilize the corresponding square-planar [PtX_2(N–N)] species and inhibits olefin release.[13] In principle, five-coordinated platinum(II) species are suitable as potential anticancer compounds beside the most common square-planar Pt(II) or octahedral Pt(IV) complexes and, in some

[a]*Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università di Lecce, Prov.le Monteroni/Lecce, I-73100, Lecce, Italy. E-mail: fp.fanizzi@unile.it; Fax: 0039(0)832-298626; Tel: 0039(0)832-298867*
[b]*Consortium C.A.R.S.O., Cancer Research Center, I-70100, Valenzano-Bari, Italy*

cases, have been actually tested for their biological activity.[14,15] In this context, we report the synthesis, the characterization, the *in vitro* cytotoxic activity on HeLa cells and the total platinum cellular uptake for two new water-soluble square-planar complexes with disulfonate phenanthroline carrier ligands: $[PtCl_2(BPS)]$ (**1a**) and $[PtCl_2(BCS)]$ (**1b**) [BPS = 4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt (mixture of isomers); BCS = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt (mixture of isomers)]. We also report the synthesis and characterization of the five-coordinate species $[PtCl_2(BPS)(\eta^2\text{-ethylene})]$ (**2a**) and $[PtX_2(BCS)(\eta^2\text{-ethylene})]$ (X = Cl, **2b**; X = I, **2c**) together with the *in vitro* cytotoxic activity and the total platinum cellular uptake of **2b** (Scheme 1).



Scheme 1 (A) Ligands (R = H, BPS; R = CH_3, BCS. (B) Square-planar complexes, R = H, **1a**; R = CH_3, **1b**. (C) Five-coordinated complexes, R = H, X = Cl, **2a**; R = CH_3, X = Cl, **2b**; R = CH_3, X = I, **2c**.

In the case of BCS complexes, we obtained stable water-soluble species for both square-planar (**1b**) and five-coordinate (**2b**, **2c**) geometries. Therefore, we were able to test their *in vitro* biological activity with respect to cisplatin and to compare for the first time the cytotoxicity on HeLa cancer cells of analogous four- and five-coordinate Pt(II) complexes. The possible effects of the measured total platinum cellular uptake on cytotoxicity for compounds **1a**, **1b**, **2b** and cisplatin are also discussed.

Results and discussion

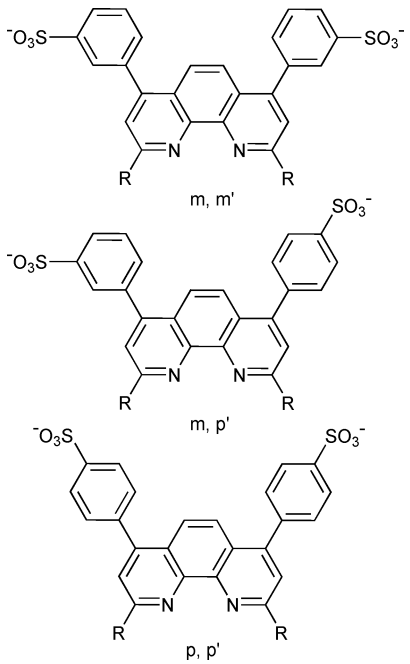
Characterization of BPS and BCS ligands

The nitrogen ligands BPS and BCS are commercially available as a mixture of isomers carrying different sulfonate substitution on the phenyl groups of the 4,7-diphenyl-1,10-phenanthroline or 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline. The *ortho* sulfonation on the phenyl groups in 4 and 7 positions of the phenanthroline is expected to be both electronically and sterically inhibited.

Table 1 Isomer ratio (%) ± error for the commercial ligands, as evaluated by ^{1}H NMR spectroscopy

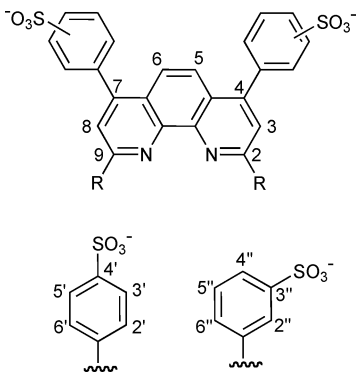
Ligand	*m*–*m*′ (%)	*m*–*p*′ (%)	*p*–*p*′ (%)
BPS (Fluka)	19.4 ± 1.2	63.8 ± 3.9	16.8 ± 1.0
BPS (Carlo Erba)	47.5 ± 2.9	43.2 ± 2.6	9.3 ± 0.6
BCS (Avocado)	61.7 ± 3.7	29.0 ± 1.4	9.3 ± 0.9

Moreover, the first aromatic ring sulfonation definitely deactivate the substrate towards a second attack on the same ring. Therefore in the commercial mixtures of BPS or BCS only two substitution position (either *m* or *p*), and consequently a mixture of three isomers, two symmetric (*m*–*m*′ and *p*–*p*′) and one asymmetric (*m*–*p*′), were found (Table 1, Scheme 2).



Scheme 2 The three sulfonation isomers in the commercial mixtures.

Relevant NMR data for ligands and complexes according to the numbering given in Scheme 3 are reported in Table 2 (^{1}H) and Table 3 (^{13}C).



Scheme 3 Atom numbering scheme for the phenanthroline skeleton and the two types of phenyl substitution.

Table 2 ^{1}H NMR data (δ) for complexes in CD_3OD. Values of J(H–H) (in parenthesis) and values of J(H–Pt) [in square brackets] are given when assignable

	2/9	3/8	5/6	2′/6′	3′/5′	2″	4″	5″	6″	C_2H_4
BPS	9.176 (d) *m*–*m*′ 9.174 (d), 9.166 (d) *m*–*p*′ 9.164 (d) *p*–*p*′ (4.6)	7.768 (d) *m*–*m*′ 7.765 (d), 7.749 (d) *m*–*p*′ 7.747 (d) *p*–*p*′ (4.6)	7.945 (s) *m*–*m*″ 7.944 (m) *m*–*p*′ 7.942 (s) *p*–*p*	7.669 (d) (8.1; 0.6)	8.044 (dd) (8.1; 0.6)	8.044 (m)	8.008 (m)	7.658 (m)	7.665 (m)	
BCS	2.950 (s) *m*–*m*′ 2.948 (s), 2.944 (s) *m*–*p*′ 2.943 (s) *p*–*p*′	7.626 (s)[a] 7.605 (s)[a]	7.808 (s) *m*–*m*″ 7.801 (m) *m*–*p*′ 7.795 (s) *p*–*p*	7.634 (dd) (8.7; 0.7)	8.024 (dd) (8.7; 0.7)	8.016 (m)	7.988 (m)	7.634 (m)	7.642 (m)	
1a	9.834 (d) *m*–*m*′ 9.812 (d), 9.800 (d) *m*–*p*′ 9.779 (d) *p*–*p*′ (5.7)	8.067 (d) *m*–*m*′ 8.057 (d) *m*–*p*′ 8.047 (d) *p*–*p*′ (5.7)	8.174 (s)[a]	7.837 (dd) (7.8; 0.9)	8.107 (dd) (7.8; 0.9)	8.162 (d) (0.7)	8.096 (dd) (7.7; 2.9)	7.740 (dd) (7.7; 0.7)	7.847 (dd) (7.7; 2.9)	
1b	3.237 (s)[a]	7.813 (m)[a] 7.796 (m)[a]	7.948 (m)[a]	7.756 (dd) (8.0; 0.6)	8.062 (dd) (8.0; 0.6)	8.104 (d) (0.6)	8.050 (dd) (7.7; 3.0)	7.695 (dd) (7.7; 0.6)	7.773 (dd) (7.7; 3.0)	
2b	3.528 (s) *m*–*m*′ 3.525 (s), 3.520 (s) *m*–*p*′ 3.518 (s) *p*–*p*′	7.948 (s) *m*–*m*′ 7.812 (s) *m*–*p*′ 7.798 (s) *p*–*p*′	7.994 (s) *m*–*m*″ 8.012 (m) *m*–*p*′ 8.023 (s) *p*–*p*	7.736 (dd) (8.9)	8.070 (dd) (8.9)	8.100 (d) (1.4)	8.043 (dd) (7.7; 2.9)	7.692 (dd) (7.7; 1.4)	7.748 (dd) (7.7; 2.9)	3.567 (s) [60][a]
2c	3.495 (s)[a]	7.995 (m)[a]	7.999 (m)[a]	8.071 (m)	8.071 (m)	8.071 (m)	7.745 (m)	7.698 (m)	7.745 (m)	3.939 (s) [66]

[a] Signals overlap for the three isomers.

Table 3 ^{13}C NMR data (δ) for complexes in CD_3OD. *m* and *p* indicate the *meta*- and the *para*-substituted rings, respectively

	2,9-Me	C_2H_4	2/9	3/8	5/6	4/7	11/13	12/14	2′/6′	3′/5′	2″	4″	5″	6″	1′/1″	4′/3″
BPS			150.8	125.4	125.6	150.0 *m* 149.9 *p*	147.5	127.8	130.9	127.7	128.3	127.5	130.1	132.7	140.9 *p* 139.2 *m*	147.1 *m* 147.4 *p*
BCS	24.8		160.1	125.5	124.1	149.8	146.7	125.8	130.1	127.7	127.4	127.0	130.4	133.1	139.5	147.2 *m* 147.5 *p*
1a			150.1	127.2	127.2	152.3	150.1	130.4	131.0	127.8	128.1	128.4	130.4	132.6	137.2 *m* 138.7 *p*	147.7 *m* 147.9 *p*
1b	27.8		167.0	128.4	125.7	150.7 *m* 150.8 *p*	151.3	127.8	130.6	127.9	127.9	125.5	130.4	132.2	136.9 *m* 138.5 *p*	147.6 *m* 147.7 *p*
2b	28.2	35.8	162.2	123.2 125.8 128.4	125.3	151.8	146.2	128.0	130.5	127.9	128.1	127.9	130.2	132.7	137.9 *m* 138.1 *p*	147.5
2c	29.7	26.9	162.9	127.6	127.9	151.5	146.6	127.9	131.0	127.8	127.8	127.8	130.7	131.0	138.0 *m* 139.5 *p*	147.4

The isomer distribution could be obtained from the pattern and integrals of the NMR signals for the H2/9 protons and of the 2/9 methyl groups for the BPS and BCS ligands, respectively. Four signals are expected for the 2/9 substituents (H or CH_3 for BPS or BCS, respectively): one each for the two symmetric (*m*–*m*′ and *p*–*p*′) and two for the asymmetric (*m*–*p*′) species. In the case of the BPS ligand, where H2/9, due to the coupling with H3/8 protons, give overlapping doublets for the three species, ^{1}H 2D *J*-resolved spectroscopy allowed to clearly identify the four signals of the three isomers. Assignment of the signals to the different isomers was straightforward on the basis of both the prevalence of the *meta* over *para* sulfonation and the need for the asymmetric species (*m*–*p*′) to give two resonances having the same integrals. ^{1}H NMR spectra acquired for several batches of the commercial BPS ligands used, Carlo Erba and Fluka, showed different concentrations of the species with a prevalence of the asymmetric isomer *m*–*p*′ in the Fluka and the symmetric isomer *m*–*m*′ in the Carlo Erba product, respectively. Moreover, in the Fluka ligand the two symmetric isomers *m*–*m*′ and *p*–*p*′ showed approximately a similar concentration, while in the Carlo Erba product the symmetric isomer *m*–*m*′ was dominant with respect to the *p*–*p*′.

By using a combination of multinuclear, multidimensional NMR techniques it was possible to assign the key resonances to the different species. The distribution of the three isomers in the batch commercial products was evaluated by deconvolution of selected resonances of the ^{1}H NMR spectra (see Experimental section).

The most deshielded signal observed in the ^{1}H 2D *J*-resolved spectra of BPS (one doublet at δ 9.176) was assigned to the phenanthroline H2/9 of the *m*–*m*′ isomer, the two doublets of equal intensity at δ 9.174 and 9.166 were attributed to the *m*–*p*′ isomer and the doublet at δ 9.164 was assigned to the less abundant *p*–*p*′ species.[12] Accordingly, the 2/9-Me region of the ^{1}H NMR spectra of the BCS ligand shows four singlets (resolution enhanced spectra): the most deshielded signal at δ 2.950 was related to the *m*–*m*′ isomer, the two equal intensity singlets at δ 2.948 and 2.944 were assigned to the *m*–*p*′ isomer, and the singlet at δ 2.943 was attributed to the *p*–*p*′ species. COSY cross-peaks with H2/9 and both COSY and NOESY cross peaks with 2/9-Me allowed assignment of H3/8 signals of the BPS and BCS ligand, respectively. Furthermore, by ^{1}H 2D *J*-resolved spectra it was possible to distinguish the phenanthroline H5/6 signals for the three isomers in both BPS and BCS ligands. On the other hand, at the instrument fields used, the sulfonate phenyl protons give overlapping resonances which only allowed differentiation between the two types of substituted rings (see Scheme 3), not between the three isomers. In particular the *para* substituted rings were easily identified by the typical AA′XX′ pattern for the spin system characterized by the strong COSY cross peaks between the H2′/6′ and H3′/5′ phenyl signals. NOESY cross peaks selectively correlating the phenyl H2′/6′ with the phenanthroline H5/6 and H3/8 protons allowed to identify the hydrogens in *ortho* position with respect to the sulfonate groups as the more deshielded in the AA′XX′ proton systems. Both COSY and NOESY data allowed to assign all the proton resonances also for the *meta* substituted phenyl rings where, again, the hydrogens in *ortho* (H2″/4″) and *para* (H6″) to the sulfonate substituent resulted more deshielded with respect to those in *meta* (H5″) positions. ^{1}H–^{13}C HETCOR and ^{1}H–^{13}C long-range HETCOR two dimensional experiments allowed the assignment of relevant ^{13}C resonances.

Synthesis and characterization of square-planar complexes [$PtCl_2$-(BPS)] (1a) and [$PtCl_2$(BCS)] (1b)

The synthesis of square-planar dichloro phenanthroline platinum complexes such as [$PtCl_2$(phen)] and [$PtCl_2$(4,7-Ph_2-Phen)] or [$PtCl_2$(2,9-Me_2-phen)] and [$PtCl_2$(2,9-Me_2-4,7-Ph_2-phen)] by reaction of *cis*-[$PtCl_2(DMSO)_2$] and the appropriate ligand in alcoholic solution has been already reported.[12,16] Methanol is normally used as solvent, or ethanol in the case of hindered phen ligands bearing 2/9-Me substituents, where the reaction requires higher temperatures.[12] Unfortunately, the established procedure which takes advantage of the extremely low solubility, in the reaction medium, of the neutral target phenanthroline platinum complexes, could not straightforward be used in the present case. The presence of sulfonate substituents on the 4/7-phenyls of BPS and BCS ligands, used in this work, considerably increases the solubility of the square-planar dichloro complexes [$PtCl_2$(BPS)] (**1a**) and [$PtCl_2$(BCS)] (**1b**) in polar solvents and introduces the need of diethyl ether in order to quantitatively precipitate the product at the end of the reaction. Moreover, due to the steric clashes between the 2/9-methyl substituents of the phenanthroline and the *cis* halogen ligands also **1b**, as already reported for [$PtCl_2$(2,9-Me_2-phen)] and [$PtCl_2$(2,9-Me_2-4,7-Ph_2-phen)],[12] easily undergoes addition reaction of an extra ligand L with formation of [$PtCl_2$(BCS)(L)] *via* dissociation of one end of the chelate. Therefore, when *cis*-[$PtCl_2(DMSO)_2$] is the starting platinum compound, both the presence of excess DMSO released during the reaction and the increased solubility in alcohols of the target product result in reduced reaction yield for [$PtCl_2$(BCS)] with respect to those reported for [$PtCl_2$(2,9-Me_2-phen)] and [$PtCl_2$(2,9-Me_2-4,7-Ph_2-phen)] complexes. In order to reduce the presence of excess DMSO in the reaction medium, K[$PtCl_3$(DMSO)] was used as starting material for the synthesis of the **1b** complex and a specific working up procedure was used to remove the KCl formed (see Experimental section). It should be also noted that, due to the presence of 2/9-Me substituents, the BCS ligand is expected to be unable to form bis-chelates. In the case of BPS ligand, in order to avoid the formation of bis-chelate $[Pt(BPS)_2]^{2+}$ complexes, a slight excess of the starting platinum species *cis*-[$PtCl_2(DMSO)_2$] was used. The latter was easily removed from the final product **1a** taking advantage of its much lower solubility in water.

The ^{1}H NMR spectra in CD_3OD of the complexes **1a** and **1b** show a high frequency shift for all signals with respect to the free ligands. This shift is more remarkable for the phenanthroline with respect to the phenyl protons. Moreover, in the platinum complexes the signals of the phenyl sulfonate rings for the different substitution systems are much less overlapped with respect to the free ligand and therefore much easier to assign. The higher frequency signals in the ^{1}H NMR spectra of **1a** were attributed, as in the case of the free ligand, to the H2/9 hydrogens. Due to metal coordination through the two N donor atoms, a deshielding of ~0.66 ppm with respect to the free BPS was observed for these protons. The relative intensities of the four separate doublets which constitute this group of signals, allowed a straightforward assignment of the chemical shifts for each one of the three isomers, confirmed by the 2D ^{1}H *J*-resolved spectrum (Fig. 1). Analogously,

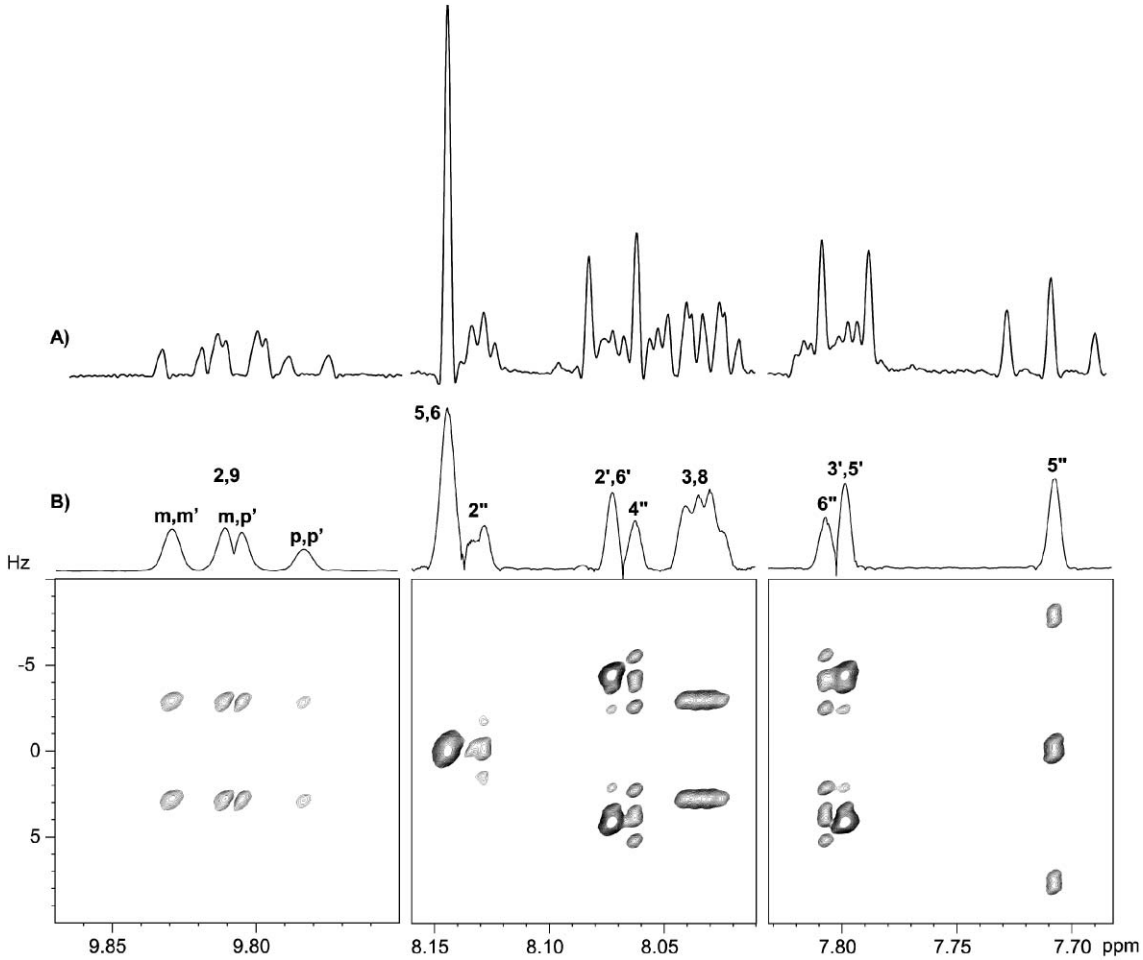


Fig. 1 Expansion of the 2D ^{1}H *J*-resolved spectra of [$PtCl_2$(BPS)] (**1a**), showing the aromatic protons. (A) expansion of the resolution enhanced 1D ^{1}H NMR spectrum; (B) projection along the ^{1}H (chemical shift axis) of the 2D ^{1}H *J*-resolved spectra with atom labeling. All spectra were acquired with a ^{1}H frequency of 400.13 MHz in CD_3OD.

the 2/9-Me signals of **1b** show a high frequency shift of ~0.3 ppm with respect to the free BCS ligand. As in the case of the free ligands, COSY cross-peaks with H2/9 and both COSY and NOESY cross peaks with 2/9-Me allowed assignment of H3/8 signals of **1a** and **1b**, respectively. Interestingly, the phenanthroline H5/6 hydrogens for the different isomers of both **1a** and **1b** are much more overlapped in the complexes with respect to the free ligands. Moreover, coordination to the metal causes a high-frequency shift for the phenanthroline H5/6 protons, considerably higher in the case of **1a** with respect to **1b**. This may be due to the different combined electronic and steric changes that coordination to the metal causes in phen ligands bearing 2/9-Me substituents such as **1b** with respect to phen based complexes like **1a**.[13,16]

Also in the case of BPS and BCS square-planar complexes, 2D ^{1}H *J*-resolved (Fig. 1), COSY and NOESY spectra allowed the complete NMR characterization of the two types of phenyl substitution systems (*meta* and *para*) but, again, at the instrument fields used, it was not possible to assign the phenyl resonances for each one of the three isomers.

The AA′XX′ systems of the *para* phenyl substituted rings were clearly identified by 2D ^{1}H *J*-resolved and COSY spectra. The *meta* substituted rings, on the other hand, show the typical spin system where again the hydrogens in *ortho* (H2″/4″) and *para* (H6″) to the sulfonate substituent result more deshielded with respect to those in *meta* (H5″) positions. ^{1}H–^{13}C HETCOR and ^{1}H–^{13}C long-range HETCOR two dimensional experiments allowed the assignment of relevant ^{13}C resonances for **1a** and **1b** (Table 3). Single ^{195}Pt resonances were observed for **1a** and **1b** (Table 4) indicating that the differences in the ^{195}Pt chemical shift for the *m*–*m*′, *m*–*p*′ and *p*–*p*′ isomers are within the line width of the observed broad signal.

Table 4 ^{195}Pt NMR data (δ) for complexes in CD_3OD

Complex	δ
1a	−2336
1b	−1892
2b	−2186
2c	−4070

Synthesis and characterization of five-coordinate [$PtCl_2$(BPS)(η^2-ethylene)] (2a) and [$PtCl_2$(BCS)(η^2-ethylene)] (2b)

Five-coordinate compounds with BPS and BCS, [$PtCl_2$(BPS)(η^2-ethylene)] (**2a**) and [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**), were obtained by reaction of the Zeise salt K[$PtCl_3$(η^2-ethylene)] with

the corresponding phenanthroline ligand in CH_3OH. The BPS complex **2a** rapidly loses olefin with formation of the square-planar analogue, therefore it could be only spectroscopically detected by ^{1}H NMR, monitoring the reaction of K[$PtCl_3(\eta^2$-ethylene)] with BPS in CD_3OD (Fig. 2). On the other hand, as already observed for other five-coordinate platinum(II) olefin complexes with phenanthrolines carrying 2/9-Me substituents, such as [$PtCl_2$(2,9-Me_2-phen)(η^2-ethylene)] and [$PtCl_2$(2,9-Me_2-4,7-Ph_2-phen)(η^2-ethylene)],[12] compound **2b** resulted particularly stable. Again, as for the square-planar complexes with BPS and BCS, the presence of sulfonate substituents on the phenyl rings renders **2b** soluble in methanol and introduces the need of diethyl ether in order to quantitatively precipitate the product from the filtered solution at the end of the reaction. The stability of five-coordinate complexes stems from the π-acceptor ability of the olefin ligand on the trigonal plane, which reduces the electron density on the metal centre. In the particular cases of phenanthrolines carrying 2/9-Me substituents, the stability is considerably increased due to the steric clashes between the 2/9-methyl of the chelate and the *cis* halogen ligands. This interaction destabilize the corresponding square-planar [PtX_2(N–N)] species and inhibits olefin release.[13] Five-coordinate ethylene complexes are generally obtained by reaction of the Zeise salt K[$PtCl_3(\eta^2$-ethylene)][17] or dimer [$PtCl_2(\eta^2$-ethylene)]$_2$[18] with the corresponding N–N ligand but, in the case of neocuproine (N–N = 2,9-Me_2-phen) or bathocuproine (N–N = 2,9-Me_2-4,7-Ph_2-phen) complexes the synthesis of the five-coordinate species [PtX_2(2,9-Me_2-phen)(η^2-olefin)] and [PtX_2(2,9-Me_2-4,7-Ph_2-phen)(η^2-olefin)] (X = Cl, Br, I) could also be performed by direct olefin uptake starting from the corresponding square-planar [PtX_2(2,9-Me_2-phen)] and [PtX_2(2,9-Me_2-4,7-Ph_2-phen)] analogues.[12] The formation constants for the direct olefin uptake strongly depend on both the steric and the electronic characteristics of the olefin (K_f for the formation of [$PtCl_2$(2,9-Me_2-phen)(η^2-olefin)] are 8.3×10^2, 3.3×10^2 and 1.1×10 for η^2-olefin = ethylene, propene and *cis*-but-2-ene, respectively)[16] and the bulk of the halogen ligand (K_f for the formation of [PtX_2(2,9-Me_2-phen)(η^2-*trans*-but-2-ene)] are 1.7, 1.5×10 and 4.6×10^2 for X = Cl, Br and I, respectively).[12] In the present work, being interested in the dichloro ethylene species **2b**, we used the synthetic procedure discussed above, starting from the Zeise salt and the BCS ligand. Nevertheless, we found that direct uptake of olefin also takes place in the case of [PtX_2(BCS)] complexes, as demonstrated by the reaction with ethylene of [PtI_2(BCS)], synthesized from [$PtCl_2$(BCS)] by halogen metathesis (see experimental section).

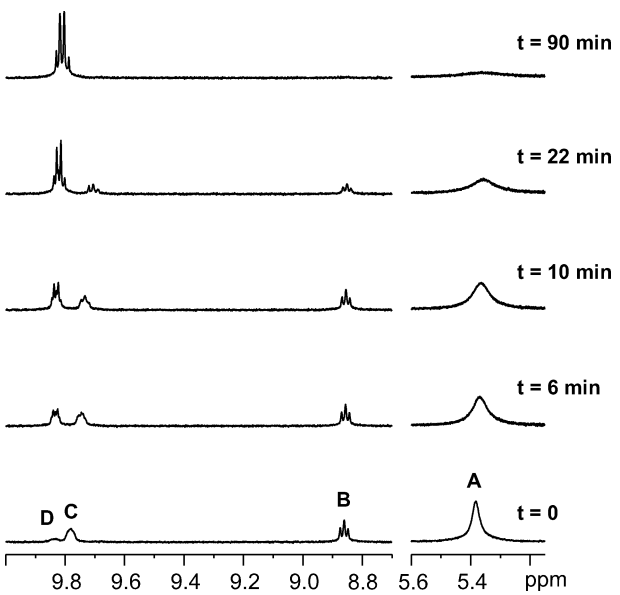


Fig. 2 ^{1}H NMR spectra monitoring the reaction of Zeise salt with BPS, acquired with a ^{1}H frequency of 400.13 MHz in CD_3OD. (A) = Free ethylene; (B) = free BPS ligand; (C) = five-coordinated complex [$PtCl_2$(BPS)(η^2-ethylene)] (**2a**); (D) = square-planar complex [$PtCl_2$(BPS)] (**1a**).

^{1}H NMR monitoring of the reaction of K[$PtCl_3(\eta^2$-ethylene)] with BPS in CD_3OD allowed the identification of two new species (two separate groups of overlapping phenanthroline H2/H9 doublets at 9.72 and 9.82 ppm) together with the free ligand and free ethylene (Fig. 2). Comparison of the spectra recorded during the reaction with the spectrum of **1a** led to the conclusion that the BPS ligand reacts with the Zeise salt to form the five-coordinate complex **2a**, which rapidly loses olefin to give the four-coordinate species **1a**. The ^{1}H NMR spectrum of **2b** in CD_3OD shows a group of ^{195}Pt coupled convoluted signals approximating a singlet at 3.567 ppm, which were readily assigned to the olefin hydrogens. As expected the ^{1}H NMR signals of the η^2-ethylene protons exhibit a different shielding in the five-coordinate with respect to the square-planar complexes.[16] The 2/9-methyl groups are deshielded in the five-coordinate species with respect to both the corresponding square-planar complex and the free ligand and could be easily assigned to the three isomers on the basis of their relative intensities. Also for the complex **2b** the NMR characterization in CD_3OD of 4/7-phenyls allowed differentiation of the two sulfonate substitution systems but not between isomers. The ^{1}H NMR spectrum of **2b** in D_2O remained almost unchanged for 24 h. Interestingly, in D_2O the chemical shift differences for the isomers are considerably enhanced as clearly observed from the four singlets of the methyl groups. This is probably due to the greater solvation, in water solution, of the functional groups which lie on the equatorial coordination plane of the metal. Also in the case of stable five-coordinate complexes [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) and [PtI_2(BCS)(η^2-ethylene)] (**2c**) ^{1}H–^{13}C HETCOR and ^{1}H–^{13}C long-range HETCOR two dimensional experiments allowed the assignment of relevant ^{13}C resonances (Table 3). Single ^{195}Pt resonances were observed also for the five-coordinate species **2b** and **2c** (Table 4) indicating that the differences in the ^{195}Pt chemical shift for the *m*–*m*′, *m*–*p*′ and *p*–*p*′ isomers are within the line width of the broad observed signal.

Biological activity

The high water solubility of the new four- and five-coordinate platinum complexes with BPS and BCS allowed us to perform *in vitro* cytotoxicity assays in order to analyse the biological activity of the compounds with respect to cisplatin and to compare for the first time the cytotoxic effects of two homologous species [$PtCl_2$(BCS)] (**1b**) and [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) with square-planar and trigonal-bipyramidal geometry, respectively. Experiments were performed on human cervix carcinoma cell line HeLa using the colorimetric MTT test,[19] as reported in the Experimental section. Cells were treated with various concentrations of **1a**, **1b**,

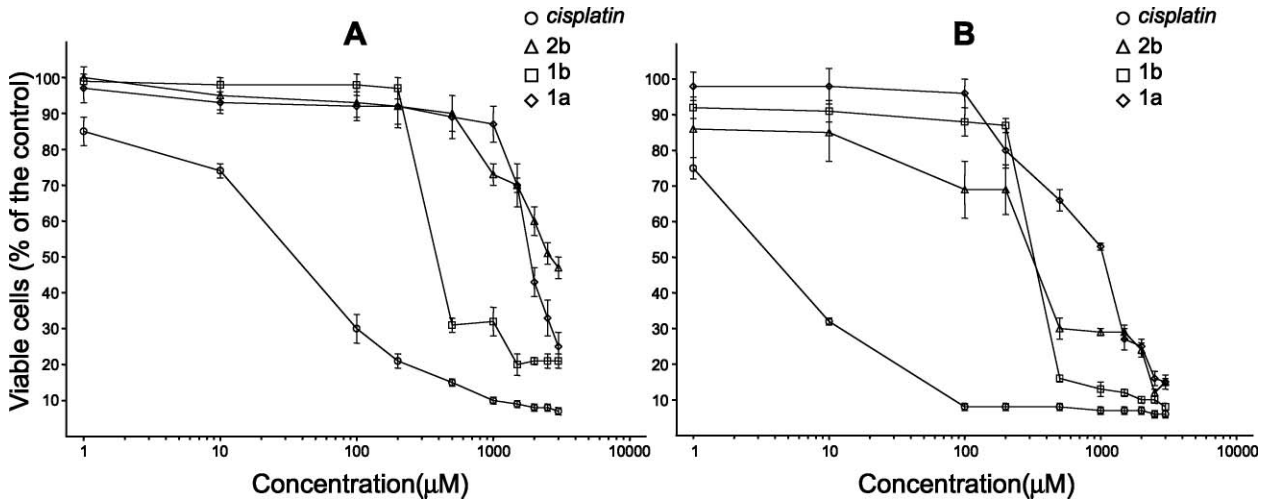


Fig. 3 Cytotoxic effect on HeLa cells after 24 (A) and 48 h (B) of cisplatin, [$PtCl_2$(BPS)] (**1a**), [$PtCl_2$(BCS)] (**1b**) and [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) at growing concentrations (from 1 μM up to 3 mM).

2b and cisplatin. The viable cell number was determined 24, 48 and 72 h later. The presence of the SO_3^- groups and the consequent high water solubility allowed to test the novel compounds also at very high concentrations. The effects of **1a**, **1b** and **2b** were therefore monitored in a concentration range from 1 μM up to the complete destruction of cultured cells (*ca.* 3 mM). As shown in Fig. 3(A), after 24 h of treatment, the cytotoxicity profiles for **1a**, **1b** and **2b** complexes were clearly different with respect to cisplatin. The new platinum complexes all exhibited a negligible cytotoxicity in the concentration range 1–200 μM whereas 100 μM cisplatin already resulted in a 70% depletion of viable cells (O.D. ~30% of the control in the MTT test at 100 μM). At higher doses, **1b** was more cytotoxic than **1a** and **2b** but still less effective than cisplatin. After 48 h of treatment (Fig. 3(B)) again cisplatin cytotoxicity was much higher with respect to **1a**, **1b** and **2b** in the drug concentration range 1–200 μM and reached the maximum value at 100 μM. Among the three new platinum complexes tested, only the five-coordinate species [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) showed after 48 h significant cytotoxicity at relatively low doses (O.D. ~70% of the control in the MTT test at 100 μM).

At higher doses, **1b** appeared the most cytotoxic showing the greatest effect at 500 μM, whereas ~2.5 mM was needed to gain the same effects in the case of **1a** and **2b**. On the other hand, it may well be that the steric bulk, introduced by the two methyl groups of the BCS carrier ligand, which distorts the square-planar coordination geometry of the metallic centre, renders **1b** more reactive than **1a** and therefore more cytotoxic.

It should be noted that compound **1b** is expected to be also more reactive than **2b** due to both the higher saturation of the coordination sphere and the release of the 2,9-methyl groups steric constraints observed in the five-coordinate with respect to the square-planar geometry.[20] Consistently the square-planar complex **1b** results also more cytotoxic than the corresponding five-coordinate species **2b** at high incubation doses. On the other hand **2b** shows considerably enhanced cytotoxicity in the longer term (48 h) with respect to the short term (24 h) treatment. This effect is very peculiar at low doses where after 48 h **2b** appears to be even more cytotoxic than **1b**. The cytotoxicity profile exhibited by **2b** may be rationalized on the basis of two factors. The higher saturation of the coordination sphere and the lower reactivity of **2b** with respect to **1b** may be responsible of an increased cellular uptake. On the other hand, the five-coordinate complex, in the long term treatment, may lose the olefin to convert into the square-planar species **1b**. Time-course of the cytotoxic effect at 500 μM incubation doses for **1b** and **2b** (Fig. 4) clearly shows the tendency of both complexes to exhibit similar cytotoxicity at longer incubation time. The general low toxicity exhibited by **1a**, **1b** and **2b**, at low doses, with respect to cisplatin, could be, in principle, related to a reduced cellular uptake. It is well known that, in the case of cisplatin, the neutral charge of the drug does not interfere with passive diffusion through the plasma membrane, which is considered the most important process for platinum cellular uptake,[21,22] although active transports have been also reported.[23] The new platinum complexes, **1a**, **1b** and **2b** are

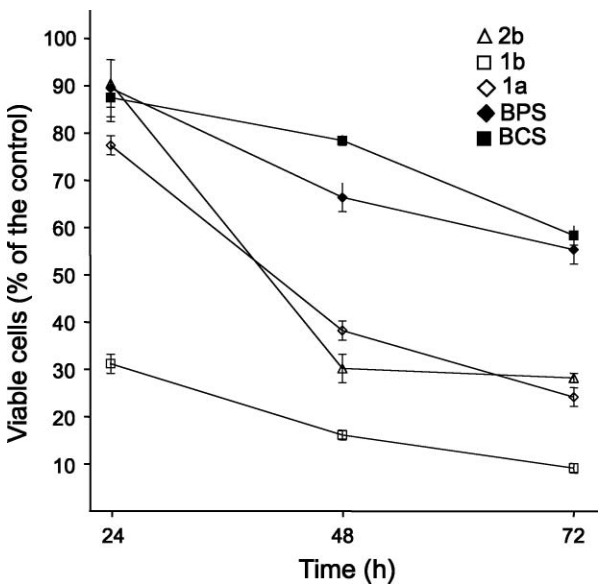


Fig. 4 Time-course of cytotoxic effect on HeLa cells of the free ligands BPS, BCS and the complexes [$PtCl_2$(BPS)] (**1a**), [$PtCl_2$(BCS)] (**1b**) and [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) at equimolar concentrations (500 μM).

all characterised by two negative charges, making them scarcely liposoluble and probably limiting cellular uptake through the membrane.

In order to better understand cytotoxicity behaviour of the tested compounds we have measured by standard literature[24] procedures the total amount of cellular platinum uptake on HeLa cell cultures treated with 1, 10 and 100 μM of cisplatin, **1a**, **1b** and **2b**. According to the standard protocol, the concentration range used was chosen considering the minimal doses exhibiting the highest cytotoxicity difference between cisplatin and new compounds. Moreover, Pt cellular uptake was determined after a short-term exposure (3 h) in order to avoid bias due to apoptosis induction and consequent severe membrane permeability alterations. Total Pt cellular uptake obtained by GFAAS measurement is reported as ng of metal per mg of cell proteins for the tested complexes in the selected concentration range in Fig. 5.

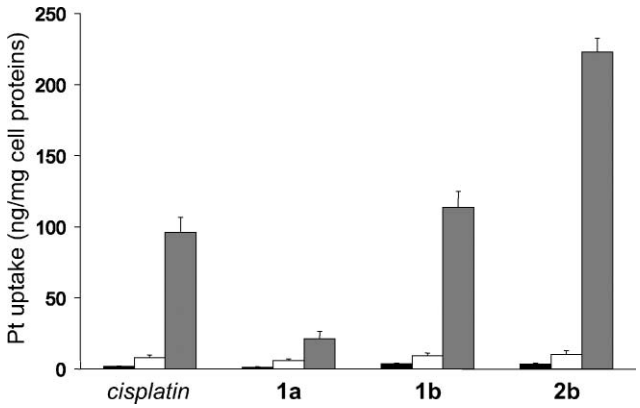


Fig. 5 Platinum intracellular accumulation in HeLa cells after 3 h continuous exposure to cisplatin, **1a**, **1b** and **2b**, administered at three different concentrations (1, 10 and 100 μM). The data were the results of three independent experiments presented as means ± S.D.

Significant differences in the Pt cellular uptake of the compounds were observed only at the highest dose 100 μM. At this drug concentration Pt cellular content was the lowest for **1a** (21.15 ng mg^{-1} cell proteins), the highest for **2b** (222.81 ng mg^{-1} cell proteins), while cisplatin and **1b** gave similar intermediate values (96.06 and 113.60 ng mg^{-1} cell proteins, respectively). These results show that the different *in vitro* cytotoxic effects of cisplatin and of the three new compounds could not be explained in terms of different platinum uptake. Although the new synthesized Pt(II) complexes were all less cytotoxic *in vitro* with respect to cisplatin, only in the case of **1a** 100 μM administration to the cell cultures resulted in a total Pt uptake lower than cisplatin. Moreover, despite the lower short term cytotoxicity exhibited by **2b** with respect to **1b** (Fig. 3(A)), 100 μM administration of **2b** resulted in total Pt cellular uptake nearly double with respect to **1b**. The platinum cellular accumulation data strongly suggest that cisplatin cytotoxicity is based on a different pharmacodynamic with respect to the other tested compounds (**1a**, **1b** and **2b**). On the other hand, the higher platinum cellular uptake shown by **2b** with respect to **1b** supports the idea that the five-coordinate species (**2b**) may act as prodrug for the square-planar species (**1b**). In fact, the tendency of **2b** to give even higher cytotoxicity with respect to **1b** at low doses and longer incubation time could be related to the differences in cellular platinum uptake shown by the two complexes (higher for **2b** with respect to **1b**). At doses higher than 200 μM, the square-planar compound **1b** is more toxic than **2b** for every incubation time (24, 48 and 72 h) suggesting that the higher platinum coordination sphere saturation in **2b** makes this compound less reactive than its square-planar homologue **1b** toward cellular targets. Interestingly, the tendency of the five-coordinate species **2b** to give at longer incubation time and high doses similar cytotoxicity (or even higher cytotoxicity at low doses) with respect to the square-planar compound **1b** suggests a possible general use of the trigonal-bipyramidal complexes as prodrugs. Finally, reduced cellular uptake and chemical reactivity of **1a** with respect to both **1b** and **2b** may account for its lower cytotoxicity.

Other results were reported in the literature about trigonal-bipyramidal platinum complexes *in vitro* toxicity[14,15] but this is the first time that a comparison of the behaviour of a square-planar compound and an analogous five-coordinate species could be obtained.

It should be noted that in order to use $[PtCl_2(N–N)(\eta^2\text{-olefin})]$ complexes as possible prodrugs for the generation of $[PtCl_2(N–N)]$, the olefin release from the former species must be kept under control. In this case, the presence of 2/9 methyl groups on the carrier ligand BCS, responsible for the higher stability of **2b** with respect to **2a**, allows to keep under control the olefin release.[11] In principle, other features related to the stability of five-coordinate species toward olefin release, such as the π-acidity of the alkene ligand,[11] may be also used for this purpose. Comparison of the cytotoxicity profiles for **1a** and the free BPS ligand (Fig. 6(A) and (B)) or **1b**, **2b** and the free BCS ligand (Fig. 6(C) and (D)) is also reported. After 24 h treatment the cytotoxicity exhibited by BPS is very similar to that of **1a** in the whole concentration range used, whereas after 48 h the free ligand seems to be constantly and significantly even more cytotoxic than its platinum complex.

In the case of BCS both after 24 and 48 h treatment the free ligand exhibited, in the concentration range 1–200 μM, the similar negligible cytotoxicity of **1b** and **2b**. On the other hand, **1b** (after 24 h) or both **1b** and **2b** (after 48 h) resulted, at incubation doses higher than 200 μM, much more cytotoxic than BCS. The toxicity profiles of the free ligands with respect to their platinum complexes are easily explained taking into account the well known ability of phenanthroline based chelates such as BPS[25,26] to bind essential metals together with the reduced and more selective binding activity exhibited by 2,9-Me_2-phenanthroline (neocuproine) based systems, such as BCS.[27,28]

Conclusion

New water-soluble square-planar and five-coordinate complexes with disulfonate phenanthroline carrier ligands BPS and BCS (BPS = 4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt (mixture of isomers); BCS = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt (mixture of isomers)) have been synthesized and characterized. In the case of BPS ligand only the square-planar complex $[PtCl_2(BPS)]$ (**1a**) could be isolated as stable product, whereas in the case of BCS, both square-planar $[PtCl_2(BCS)]$ (**1b**) and five-coordinate $[PtX_2(BCS)(\eta^2\text{-ethylene})]$ (X = Cl, **2b**; X = I, **2c**) stable complexes could be obtained. The five-coordinate complex $[PtCl_2(BPS)(\eta^2\text{-ethylene})]$ (**2a**) could be only characterized as a transient species due to fast olefin release with formation of $[PtCl_2(BPS)]$ (**1a**).

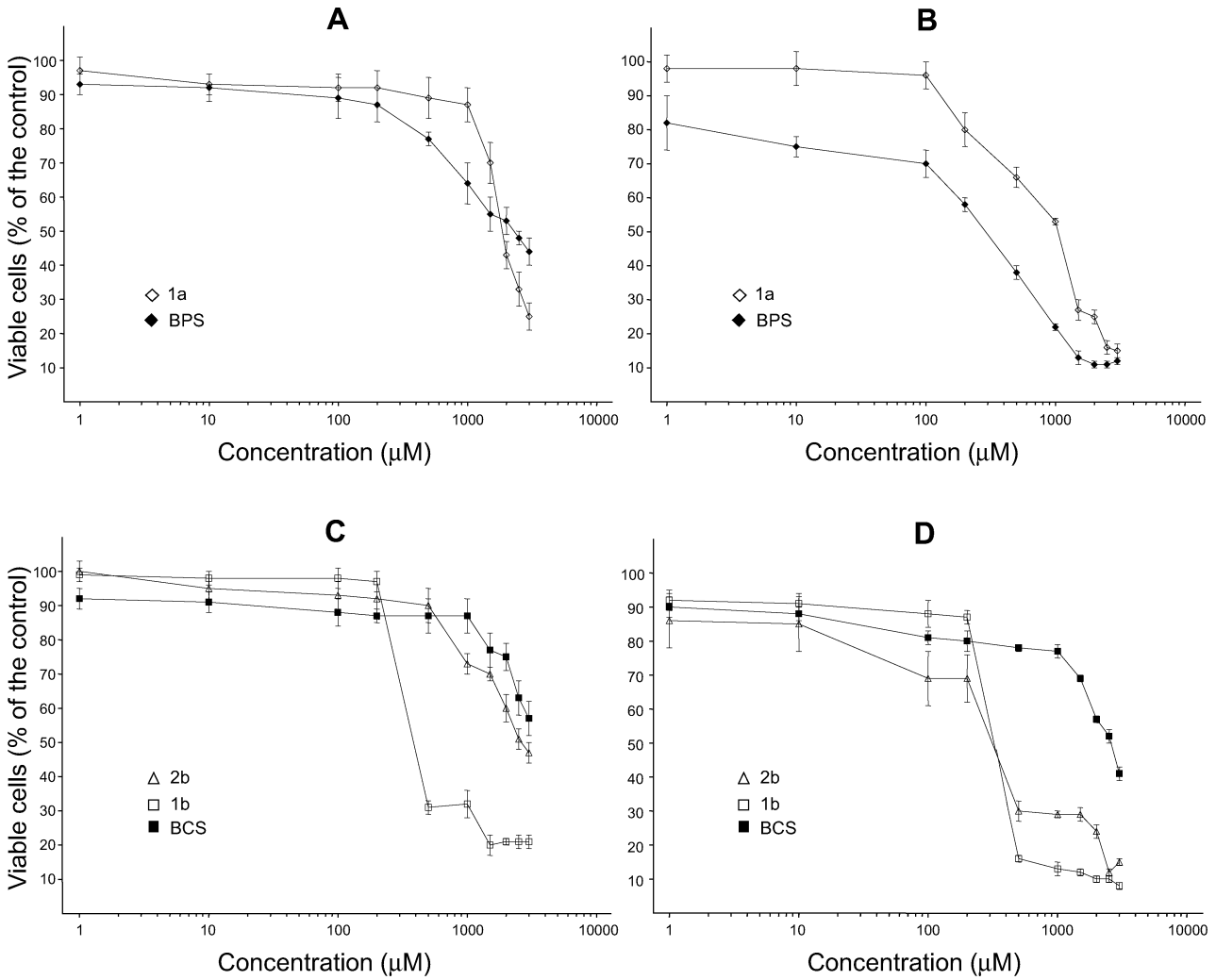


Fig. 6 Comparison of the cytotoxic effect at growing concentrations (from 1 μM up to 3 mM) on HeLa cells: the free ligand BPS with respect to the square-planar complex [$PtCl_2$(BPS)] (**1a**) after 24 (A) and 48 h (B); the free ligand BCS with respect to the square-planar complex [$PtCl_2$(BCS)] (**1b**) and the five-coordinated complex [$PtCl_2$(BCS)(η^2-ethylene)] (**2b**) after 24 (C) and 48 h (D).

The stability and high water solubility of **1a**, **1b** and **2b** allowed to perform a range of *in vitro* assays on HeLa cancer cells, at increasing drug concentrations and incubation times, up to the complete destruction of the cell cultures. All new tested compounds showed similar *in vitro* cytotoxicity negligible or much lower with respect to cisplatin at low and medium incubation doses (<200 μM) for every incubation time. At doses higher than 200 μM **1a**, **1b** and **2b** showed an increasing cytotoxicity and **1b** resulted the most cytotoxic among the new complexes for every incubation time. GFAAS Platinum analytical data showed that the tested compounds **1a**, **1b** and **2b**, although carrying sulfonate charged groups, may undergo cellular uptake which, in the case of **1b** and **2b**, is even higher with respect to cisplatin. Therefore, cytotoxicity of cisplatin and the three new compounds could not be explained only in terms of different drug cellular uptake. Platinum uptake and cytotoxic activity data strongly suggest that cisplatin cytotoxicity is based on a different pharmacodynamics with respect to the other tested compounds (**1a**, **1b** and **2b**). Higher cytotoxicity generally shown by **1b** with respect to **1a** and **2b** appears to be related to its higher chemical reactivity toward cellular targets, induced by the steric constraints of 2/9-methyl groups in the coordination sphere. The five-coordinate species **2b**, is less cytotoxic with respect to **1b** at short incubation time but in the long term shows similar cytotoxicity (or even higher at low doses) with respect to its square-planar analogue. This behaviour may be connected to both the lower chemical reactivity of the five-coordinate species **2b** with respect to the square-planar **1b** and the possibility for the former to act as prodrug undergoing slow olefin release to give the latter. Consistently, total platinum cellular uptake for **2b** was nearly double with respect to **1b**. Reduced cellular uptake and chemical reactivity account for the lower cytotoxicity of **1a** with respect to both **1b** and **2b**. Noteworthy, for the first time the cytotoxicity on HeLa cancer cells of analogous four- and five-coordinate Pt(II) complexes has been reported. Although both unresolved optical[29,30] isomers and geometrical[31] isomeric mixtures are currently used as drugs in the treatment of specific diseases, hypothetical pharmacological applications of the new tested compounds (which consist of sulfonation isomers) appear a minor issue to be considered at this stage. Nevertheless, the tendency of the five-coordinate species **2b** to give at longer

incubation time similar or even higher cytotoxicity (at high and low doses, respectively) with respect to the square-planar compound **1b** suggests a possible general use of the trigonal-bipyramidal five-coordinate complexes as prodrugs.

Experimental

Starting materials

Commercial reagent grade chemicals purchased either from Fluka or Carlo Erba, 4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt hydrate (mixture of isomers), 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid disodium salt hydrate (mixture of isomers) from Avocado, and solvents were used without further purification. *cis*-$[PtCl_2(DMSO)_2]$,[32] $K[PtCl_3(DMSO)]$,[33] $K[PtCl_3(\eta^2\text{-ethylene})]$[34,35] (Zeise salt) were prepared by previously reported procedures.

Physical measurements

^1H NMR and ^{195}Pt NMR spectra, ^1H–^1H COSY, ^1H–^1H NOESY, ^1H *J*-resolved, ^1H–^{13}C HETCOR and ^1H–^{13}C long-range HETCOR two-dimensional experiments were recorded on a Bruker Avance DPX 400 and on a Bruker Avance DRX 500 (CARSO) using CD_3OD and D_2O as solvents. ^1H and ^{13}C chemical shifts were referred to TMS for CD_3OD and TSP for D_2O. ^{195}Pt chemical shifts were referenced to Na_2PtCl_6 ($\delta(Pt) = 0$ ppm) in D_2O as an external reference.[36] ESI mass spectra were recorded on a LC-MSD-Trap-VL_01002 spectrometer by directly injecting the complexes previously dissolved in methanol.

Spectral deconvolution

In order to evaluate the isomeric ratio of the sulfonation mixture of the commercial reagent grade BPS purchased either from Fluka or Carlo Erba, or BCS purchased from Avocado, spectral deconvolution was carried out. In the case of the BPS ligand, the deconvolution was carried out on the 3,8-phenanthroline proton signals, while in the case of the BCS ligand, on the 2,9-methyl proton signals. The spectra, acquired on the DRX500 spectrometer, were exported to standard xy coordinate pairs text format and fitted with the *Fityk* 0.7.4 curve fitting software,[37] using Lorentzian functions and the Nelder–Mead simplex until convergence was reached. The fitted area were used to evaluate the mixture composition.

Biological assays

Cell lines. HeLa cells from human tumoral endometrium were grown in DMEM (Euroclone). Culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Euroclone), 0.2 mg mL^{-1} streptomycin, 200 IU mL^{-1} penicillin. Cells were cultured routinely at 37 °C and 5% CO_2 in a humidified incubator.

MTT assay. The MTT assay, already described by Mosmann,[19] is a cytotoxicity test based on the metabolic reduction of a soluble tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma) by a mitochondrial enzyme of cultured cells into an insoluble colored formazan product. After harvesting, the cells were counted and diluted appropriately with culture medium; 100 μL containing 3000 cells were seeded in each well of a 96-well microtiter plate (Corning). After 24 h of incubation cisplatin and its analogues were administrated to each well in appropriated concentrations (from 1 μM to 3 mM). The toxicity of these compounds was tested for 24, 48 and 72 h. At the end of every incubation, MTT was added at the final concentration of 500 μg mL^{-1}. After 4 h of incubation, the amount of formazan was spectrophotometrically measured at 550 nm wavelength in isopropanol. Optical density was used to calculate cell growth inhibition, as percentage with respect to the control.

For the statistical analysis of data, the Bonferroni–Dunn test was used and a p value <0.05 was considered significant. All the results are the mean of three, separately performed, experiments. Cytotoxicity differences obtained from different batches of Fluka or Carlo Erba ligands were not statistically significant.

Total cellular platinum uptake determination. After 3 h incubation at three different concentrations (1, 10 and 100 μM), HeLa cells treated with cisplatin, **1a**, **1b** and **2b** were washed twice with PBS (Phosphate Buffered Saline) and harvested with a lysis buffer (SDS 0.1%, Na deossicolate 0.1%, Nonidet P40 10 μL mL^{-1}, Leupeptine 10 μg μL^{-1}, Pepstatine 10 μg μL^{-1}, PMSF 0.5 mM, pH 7.5); after which the pellets were digested in 65% nitric acid at room temperature in a closed vial.[24] The total amount of cellular platinum uptake was performed at central facility of CIRCMSB (Consorzio Interuniversitario di Chimica dei Metalli nei Sistemi Biologici, Bari, Italy) by a Varian SpectrAA-880 Zeeman graphite furnace atomic absorption spectrometer (GFAAS). A five-point calibration curve was prepared for all measurements. The data were expressed as nanogram Pt per milligram of cell proteins (means ± S.D.) and were the results of three independent experiments. Protein amounts of the treated cells were determined with the Bio-Rad protein assay kit 1, using lyophilised bovine serum albumin as a standard.

Synthesis of compounds

$[PtCl_2(BPS)]$, 1a. To a suspension of *cis*-$[PtCl_2(DMSO)_2]$ (0.115 g, 0.272 mmol) in methanol (50 mL), 0.140 g (0.237 mmol) of bathophenanthroline disulfonate (BPS) were added, and the reaction mixture left under stirring at 40 °C for 24 h. The resulting yellow solution was cooled, filtered and concentrated to 15 mL then Et_2O was added and the solution kept at 5 °C overnight. The yellow precipitate formed was separated by filtration, washed with $CHCl_3$ and Et_2O, and dried under vacuum. The precipitate was extracted with water (10 mL) and the yellow solution obtained was filtered and taken to dryness by evaporation of the solvent under vacuum to give $[PtCl_2(BPS)]$ as a yellow powder (yield 216 mg, 84%). Elemental analysis: calc. for $C_{24}H_{14}Cl_2N_2Na_2O_6PtS_2 \cdot 6H_2O$ (910.56): C, 31.66; H, 2.88; N, 3.08. Found: C, 31.31; H, 3.04; N, 2.72%. MS (ESI) (*m*/*z*): 778 (loss of Na^+), 513 (loss of Na^+ and $PtCl_2$).

$[PtCl_2(BCS)]$, 1b. To a solution of $K[PtCl_3(DMSO)]$ (0.101 g, 0.241 mmol) in ethanol (80 mL), 0.136 g (0.241 mmol) of bathocuproine disulfonates (BCS) were added and the reaction mixture was left under stirring at 80 °C. The colour of reaction mixture slowly changed from yellow to red–brown. After four days the stirring was stopped, and the solution was concentrated to 20 mL, then Et_2O was added. The yellow–brown precipitate of $[PtCl_2(BCS)]$ which formed was separated by filtration, washed

with $CHCl_3$ and Et_2O, and dried under vacuum. Then, the powder was dissolved in a solution of MeOH–$CHCl_3$ (1 : 3 ratio) and kept at 5 °C. After 2 h the mixture was filtered and the solvent was removed under vacuum. The yellow residue was finally washed with $CHCl_3$, dried and collected (yield 0.157 g, 63%). Elemental analysis: calc. for $C_{26}H_{18}Cl_2N_2Na_2O_6PtS_2 \cdot 11H_2O$ (1028.693): C, 30.36; H, 3.92; N, 2.72. Found: C, 30.37; H, 3.35; N, 2.83%. MS (ESI) (*m*/*z*): 806 (loss of Na^+), 770 (loss of Na^+ and Cl^-), 541 (loss of Na^+ and $PtCl_2$).

Reaction of [$PtCl_3(\eta^2$-ethylene)] with BPS or BCS in NMR tube. 0.002 g of K[$PtCl_3(\eta^2$-ethylene)] (0.005 mmol), synthesized by a previously reported procedure,[34,35] were dissolved in CD_3OD (0.5 mL) and the resulting solution placed in a NMR tube. The reaction with added stoichiometric BPS (0.0016 g, 0.003 mmol) or BCS (0.0017 g, 0.003 mmol) was time-monitored recording ^1H NMR spectra.

[$PtCl_2$(BCS)(η^2-ethylene)], 2b. A stoichiometric amount of BCS (0.153 g, 0.271 mmol), suspended in methanol (10 mL), was slowly added to a solution of Zeise salt K[$PtCl_3(\eta^2$-ethylene)] (0.10 g, 0.271 mmol) dissolved in methanol (10 mL) and kept under stirring at room temperature. After 12 h, the stirring was stopped and the solution was filtered and Et_2O was added up to the precipitation of [$PtCl_2$(BCS)(η^2-ethylene)] as a yellow powder. The product was filtered, washed with Et_2O and dried under vacuum (yield 0.073 g, 47%). Elemental analysis: calc. for $C_{28}H_{22}Cl_2N_2Na_2O_6PtS_2 \cdot 9H_2O$ (1020.715): C, 32.75; H, 3.93; N, 2.73. Found: C, 32.80; H, 3.52; N, 2.93%. MS (ESI) (*m*/*z*): 834 (loss of Na^+), 806 (loss of Na^+ and C_2H_4), 770 (loss of Na^+, C_2H_4 and Cl^-), 512 (loss of Na^+ and C_2H_4), 770 (loss of Na^+, C_2H_4 and $PtCl_2$).

[PtI_2(BCS)(η^2-ethylene)], 2c. A large excess of KI (0.1 g, 0.602 mmol) was added to a methanol solution (30 mL) of [$PtCl_2$(BCS)] (0.05 g, 0.0602 mmol). After 12 h the red-brown suspension obtained was added of $CHCl_3$ (10 mL) and filtered to remove KI and KCl. The yellow-orange solution obtained was reacted with ethylene for 12 h under stirring. Then, the mixture was concentrated and Et_2O was added up to the precipitation of a red–brown powder of [PtI_2(BCS)(η^2-ethylene)]. The solid was isolated through filtration, washed with Et_2O and dried under vacuum (yield 0.035 g, 46%). Elemental analysis: calc. for $C_{28}H_{22}I_2N_2K_2O_6PtS_2 \cdot 10H_2O$ (1253.85): C, 26.82; H, 3.38; N, 2.23. Found: C, 27.10; H, 3.72; N, 2.39%. MS (ESI) (*m*/*z*): 1034 (loss of K^+), 1006 (loss of K^+ and C_2H_4), 879 (loss of K^+, C_2H_4 and I^-).

Acknowledgements

This work was supported by the University of Lecce and the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR), Cofin. 2004 (No. 2004050714_002).

References

1 B. Rosenberg, L. Van Camp and T. Krigas, *Nature*, 1965, **205**, 698–699.
2 B. Rosenberg, L. VanCamp, J. E. Trosko and V. H. Mansour, *Nature*, 1969, **222**, 385–386.
3 G. Giaccone, *Drugs*, 2000, **59**, 9–17.
4 E. R. Jamieson and S. J. Lippard, *Chem. Rev.*, 1999, **99**, 2467–2498.
5 E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451–2466.
6 C. X. Zhang and S. J. Lippard, *Curr. Opin. Chem. Biol.*, 2003, **7**, 481–489.
7 D. Wang, R. Hara, G. Singh, A. Sancar and S. Lippard, *Biochemistry*, 2003, **42**, 6747–6753.
8 S. T. Sullivan, A. Ciccarese, F. P. Fanizzi and L. G. Marzilli, *J. Am. Chem. Soc.*, 2001, **123**, 9345–9355.
9 N. Margiotta, P. Papadia, F. P. Fanizzi and G. Natile, *Eur. J. Inorg. Chem.*, 2003, 1136–1144.
10 S. T. Sullivan, A. Ciccarese, F. P. Fanizzi and L. G. Marzilli, *Inorg. Chem.*, 2001, **40**, 455–462.
11 (*a*) V. G. Albano, G. Natile and A. Panunzi, *Coord. Chem. Rev.*, 1994, **133**, 67–114; (*b*) G. Natile and L. G. Marzilli, *Coord. Chem. Rev.*, 2006, **250**, 1315–1331.
12 F. P. Fanizzi, G. Natile, M. Lanfranchi, A. Tiripicchio, F. Laschi and P. Zanello, *Inorg. Chem.*, 1996, **35**, 3173–3182.
13 F. P. Fanizzi, L. Maresca, G. Natile, M. Lanfranchi, A. Tiripicchio and G. Pacchioni, *J. Chem. Soc., Chem. Commun.*, 1992, 333–335.
14 S. Bartolucci, M. Rossi, M. Estenoz, A. Panunzi and A. Vitagliano, *Inorg. Chim. Acta*, 1987, **137**, 53–55.
15 M. Bigioni, P. Genis, A. Panunzi, F. Ruffo, C. Salvatore and A. Vito, *Eur. J. Inorg. Chem.*, 2000, **8**, 1717–1721.
16 F. P. Fanizzi, F. P. Intini, L. Maresca, G. Natile, M. Lanfranchi and A. Tiripicchio, *J. Chem. Soc., Dalton Trans.*, 1991, 1007–1015.
17 L. Maresca, G. Natile and L. Cattalini, *Inorg. Chim. Acta*, 1975, **14**, 79–82.
18 H. C. Clark and L. E. Manzer, *J. Am. Chem. Soc.*, 1973, **95**, 3812–3813.
19 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.
20 F. P. Fanizzi, L. Maresca, C. Pacifico, G. Natile, M. Lanfranchi and A. Tiripicchio, *Eur. J. Inorg. Chem.*, 1999, **8**, 1351–1358.
21 G. R. Gale, C. R. Morris, L. M. Atkins and A. B. Smith, *Cancer Res.*, 1973, **33**, 813–818.
22 S. P. Binks and M. Dobrota, *Biochem. Pharmacol.*, 1990, **40**, 1329–1336.
23 D. Wang and S. J. Lippard, *Nat. Rev. Drug Discovery*, 2005, **4**, 307–320.
24 E. Teixeira Cesar, M. Vieira de Almeida, A. P. Soares Fontes, E. C. Pereira Maia, A. Garnier-Suillerot, M. R. Costa Couri and E. de Castro Antunes Felýcio, *J. Inorg. Biochem.*, 2003, **95**, 297–305.
25 W. Calabri, I. Candiani and A. Bedeschi, *J. Org. Chem.*, 1993, **58**, 7423–7426.
26 L. Fallace and D. P. Rillema, *Inorg. Chem.*, 1993, **32**, 3836–3843.
27 E. Lorenzo, J. Fernández, F. Pariente, K. Trible, B. Pendley and H. Abruña, *J. Electroanal. Chem.*, 1993, **356**, 43–58.
28 M. Matsushita, T. Irino, T. Comoda and Y. Sakagishi, *Clin. Chim. Acta*, 1993, **216**, 103–111.
29 O. Michihiro, S. Ryozo and K. Tyuzi, *Biosci., Biotechnol., Biochem.*, 1995, **59**, 1002–1006.
30 T. C. Majerus, J. F. Dasta, J. L. Barman, L. H. Danziger and R. R. Ruffolo, Jr., *Pharmacotherapy*, 1989, **9**, 245–259.
31 W. C. Shyu, U. A. Shukla, V. R. Shah, E. A. Papp and R. H. Barbhaiya, *Pharm. Res.*, 1981, **8**, 992–996.
32 Yu. N. Kukushkin, Yu. E. Vyaz'menskii, L. I. Zorina and Yu. L. Pazukhina, *Russ. J. Inorg. Chem. (Engl. Transl.)*., 1968, **13**, 835–838.
33 Yu. N. Kukushkin, Yu. E. Vyaz'menskii and L. I. Zorina, *Russ. J. Inorg. Chem. (Engl. Transl.)*., 1968, **13**, 1573–1576.
34 R. D. Cramer, E. L. Jenner, R. V. Lindsey and B. G. Stolberg, *J. Am. Chem. Soc.*, 1963, **85**, 1691–1692.
35 J. Chatt and M. L. Searle, *Inorg. Synth.*, 1957, **5**, 210–215.
36 R. K. Harris, E. D. Becker, S. M. Cabral De Menezes, R. Goodfellow and P. Granger, *Pure Appl. Chem.*, 2001, **73**, 1795–1818.
37 http://www.unipress.waw.pl/fityk/.