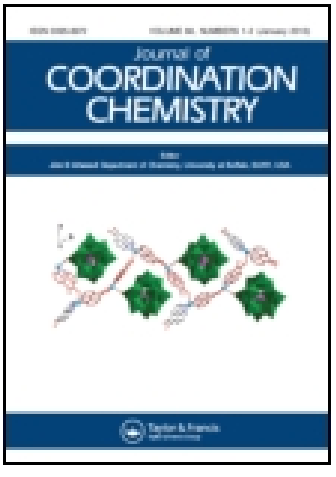

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The role of diaminocyclohexane and diaminobenzene linking bridges on the aqua substitution of chelated dinuclear Pt(II) complexes by nitrogen donor heterocycles. A kinetic and mechanistic study

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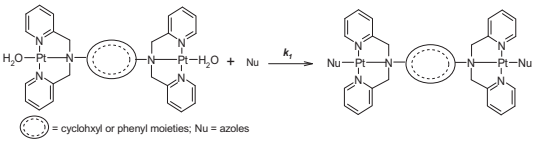

The substitution of the aqua ligands from six Pt(II) complexes, viz., [Pt(H_2O)(*N,N*-bis(2-pyridylmethyl)cyclohexylamine](ClO_4)$_2$ (**Pt1**); [{Pt(H_2O)}$_2$(*N,N,N′,N′*-tetrakis(2-pyridylmethyl)-*trans*-1,4-cyclohexyldiamine)](ClO_4)$_4$ (**Pt2**); [{Pt(H_2O)}$_2$(*N,N,N′,N′*-tetrakis(2-pyridylmethyl)-4,4′-methylenedicyclohexyldiamine)](ClO_4)$_4$ (**Pt3**); [Pt(H_2O)*N,N*-bis(2-pyridylmethyl)phenylamine)](ClO_4)$_2$ (**Pt4**); [{Pt(H_2O)}$_2$(*N,N,N′,N′*-tetrakis(2-pyridylmethyl)-1,4-phenyldiamine](ClO_4)$_4$ (**Pt5**); and [{Pt(H_2O)}$_2$(*N,N,N′,N′*-tetrakis(2-pyridylmethyl)-4,4′-methylenediphenyldiamine)](ClO_4)$_4$ (**Pt6**), by nitrogen heterocyclic ligands{viz., pyrazole (**Pz**); 3-methylpyrazole (**mPz**); 1,2,4-triazole (**Tz**) and pyrazine (**Pzn**)} were studied in an aqueous 0.01 M perchloric acid medium. The substitutions were investigated under pseudo-first-order conditions as a function of the concentration of nucleophiles and reaction temperature using UV–visible spectrophotometry. The substitution of the aqua ligands by all the nitrogen donor heterocycles proceeded via a single step whose rate decreased in the respective orders: **Pt1** > **Pt3** > **Pt2** and **Pt4** > **Pt6** > **Pt5** in the two sets of complexes. Of the nucleophiles used in this study, pyrazine was the most reactive and the complete order of the rate of aqua substitution was **Pzn** >> **Pz** > **Tz** > **mPz**. The large and negative activation entropies and low but positive enthalpies of activation values support a significant contribution from bond making in the transition state of the substitution process.

Keywords: Aqua Pt(II) complexes; Substitution reaction; Heterocyclic ligands; Diaminocyclohexane; Diaminobenzene

Introduction

The serendipitous discovery of the anticancer activity of cis-diaminedichloroplatinum(II) (cisplatin) by Rosenberg [1] and its subsequent clinical approval by the FDA in 1978 was followed by numerous studies on the cytotoxicity [2], reactivity, and mechanistic pathways

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[3] of platinum complexes, all with a common objective of coming up with platinum-based drugs of improved efficacy [4]. Today, cisplatin is used in the first-line treatment for a number of neoplasms including cancers of the testicles, bladder, ovaries, and lungs [5]. It remains the main chemotherapeutic regimen with a remarkable cure rate of above 90% in testicular cancers [6].

However, the therapeutic index of cisplatin is shrouded by its non-selective mode of action [7], which causes severe side effects such as nephrotoxicity, neurotoxicity, and myelosuppression. Its clinical use is further aggravated by intrinsic and acquired resistance in some cancer cells [8]. Apart from this, cisplatin is partially soluble in water and can only be administered by intravenous fusion which makes it a costly drug [9].

Current research in this area is focused on designing new platinum drugs with improved pharmacological properties, broader spectrum of cytotoxic activity, and aqueous solubility for oral administration [10]. Thousands of Pt(II) complexes have been synthesized and screened for their cytotoxicity with only a few analogs of cisplatin entering clinical practice [11]. Studies [12] have shown that the mere structural analogs of cisplatin will not offer substantial clinical advantages over the parent drug in terms of potency and spectra of activity.

The pioneering work of Farrell [13] involving the synthesis of multinuclear Pt(II) complexes, the majority of which were bridged by aliphatic diamines, extended this search to Pt(II) complexes defying the classical "structure–activity relationship" as previously outlined by Cleare and Hoeschele [14]. These multinuclear Pt(II) complexes can form long-range DNA-Pt adducts which are thought to easily circumvent the DNA repair machinery [15] and potentially provide an alternative cytotoxic mechanism which overcomes cisplatin resistance [16]. Pt(II) complexes which are bridged by ligands ranging from alkyldiamines [17], bifunctional thioureas [18], polyamines [19], dipyrazolylmethane [20], azine [21(a)–(c)], and azoles [21(d)–(f)] have been synthesized and some of them have been screened for their cytotoxicity. In general, complexes bridged by polyamines [19] have shown superior activity. A synthetic reward which came from the systematic optimization of structure–activity–reactivity relationships using polyamines is BBR3464, a tri-nuclear Pt(II) complex which has now entered phase II clinical trials [22].

The search for more effective platinum-based chemotherapeutics remains an active area of research in drug design. The key to underpinning potency of platinum drugs during their design is a sound knowledge of their general reactivity and molecular mechanisms in biological systems. In our laboratory [23] and other groups [24], we have taken a keen interest in understanding the intrinsic role of the diamine bridge on the substitutional reactivity of the dinuclear Pt(II) complexes, using strong thiourea nucleophiles. These reactions model the kinetic aspects underlying the possible deactivation reaction pathways involving thiol biomolecules, which are ubiquitous in the cytoplasm of cells [25].

However, not much is known about the substitutional reactivity of dinuclear platinum complexes with nitrogen-containing biomolecules such as proteins or their mimics. In biological systems, such reactions have potential to affect not only the efficacy of candidate drugs but also the prototype and regulatory functions of the biomolecules by transforming them into toxic metabolites. This can reduce the amount of the platinum drug, destined for reactions with DNA nucleobases [26]. Azoles and azines (nitrogen donor heterocycles) are good nucleophilic mimics for modeling substitution reactions of platinum drugs with proteins. For example, histidinal residues of hemoproteins act as azole donor sites which can readily react with the platinum(II) drugs enrouted for DNA [27]. Such reactions between nitrogen donor heterocycles and platinum compounds have potential to bring about increased toxicity and severe side effects. Thus, the reactivity of the platinum-based

candidate drugs may be detrimental to the homeo functions of proteins and at the same time deactivating the drugs and thus, preventing them from effectively reacting with the targeted DNA nucleotides.

In this study, we report on the substitution of aqua ligands from dinuclear Pt(II) complexes bridged by diamines bearing cyclohexyl and phenyl groups, using nitrogen donor heterocycles in an acidified medium (pH 2). These diamine-linking bridges endow variable structural rigidity and planarity within the complexes. Shown in figure 1 are the chemical structures of the platinum complexes and the geometry optimized structures of the nitrogen donor heterocycles used in this study. Also included in the figure are the abbreviations used to name them.

Experimental

Materials and procedures

All ligands and complexes were synthesized under an inert atmosphere of nitrogen, using the Schlenk system. Organic solvents used in the syntheses were purchased from Merck and used without purification. The diamines were procured from Fluka. 2-Picolylchloride hydrochloride; the azole nucleophiles {viz., pyrazine (**Pzn**); pyrazole (**Pz**); 1,2,4-triazole (**Tz**); and 3-methylpyrazole (**mPz**)}; sodium perchlorate monohydrate ($NaClO_4 \cdot H_2O$); and silver perchlorate ($AgClO_4$, 99%) were purchased from Sigma Aldrich and used without

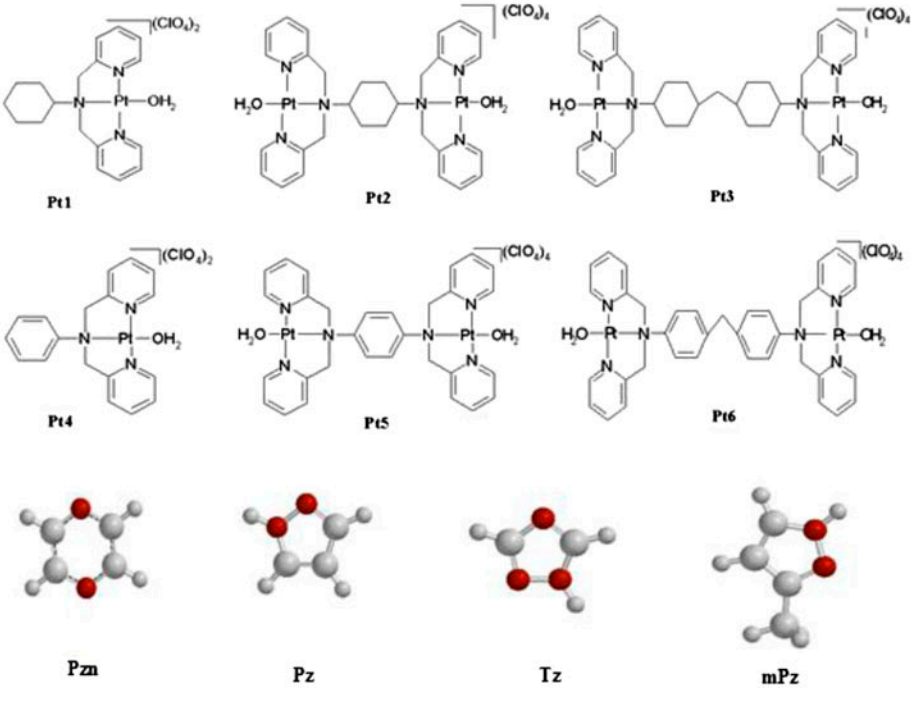


Figure 1. Chemical structures of the Pt(II) complexes and the geometry-optimized structures of the nucleophiles used in the study.

purification. Potassium tetrachloroplatinate (K_2PtCl_4, 99.99%) was purchased from Strem Chemicals. Ultrapure water (ELGA, Pure-lab. ultra-system) was used to make all aqueous solutions.

Synthesis of ligands

The tridentate and hexadentate ligands synthesized for this investigation were *N,N*-bis(2-pyridylmethyl)cyclohexylamine (**L1**), *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-trans-1,4-cyclohexyldiamine (**L2**), *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-4,4′-dicyclohexylmethylenediamine (**L3**), *N,N*-bis(2-pyridylmethyl)phenylamine (**L4**), *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-1,4-phenyldiamine (**L5**), and *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-4,4′-diphenylmethylenediamine (**L6**).

L1 was prepared using a reported procedure by Karlin and co-workers [28]. To a 5 mL of 5.3 M NaOH was added a chilled solution of 2-picolylchloride hydrochloride while stirring, until a pink emulsion was observed. Doubly distilled cyclohexylamine, dissolved in 10 mL of dichloromethane was added dropwise at room temperature. An additional 40 mL of 5.3 M NaOH was added dropwise over a period of two days, while maintaining the color of the reaction mixture pale orange. Further additions were resumed whenever the color of the mixture turned pale green. The product was extracted using five aliquots of 10 mL of dichloromethane. The crude extract was then passed through a short column packed with silica and a small amount of anhydrous $MgSO_4$ (drying agent). Fifty percent chloroform in dichloromethane was used as the eluting solvent and the solution was concentrated by rotor evaporation to get an orange oil. A few drops of ethanol/diethyl ether were layered onto the oil and left to slowly evaporate for two days, yielding plate-like crystals. **L4** was prepared in a similar manner to **L1**, incorporating additional steps as described in the method of Buchen *et al.* [29]. Slow evaporation of its ethanolic solutions afforded off-white powders.

L2 and **L3** were prepared using a procedure by Sato *et al.* [30], while **L5** and **L6** were prepared following a procedure by Schindler *et al.* [31]. The crude extracts of **L2**, **L3**, and **L4** were further purified by eluting through a chromatographic column packed with 2 g of activated charcoal, 5 g of neutral alumina and sodium sulfate with chloroform as the eluting solvent resulting in pale yellow solutions [32]. Slow evaporation of the ethanol solutions of **L5** and **L6** gave off-white powders. The ligands were fully characterized by NMR, TOF, MS, ESI^+, and microanalysis. Details for the characterization of ligands are given in the supplementary information together with some representative spectra.

Synthesis of the Pt(II) complexes

The salts of the chloro derivatives of two mononuclear (**Pt1**; **Pt4**) and dinuclear Pt(II) complexes (**Pt2**, **Pt3**, **Pt5,** and **Pt6**) were synthesized using **L1**–**L6**, following a literature procedure by Hofmann [33].

To a solution of 200 mg (0.48 mM) K_2PtCl_4 in 50 mL of 0.01 M HCl, a solution of 0.24 mM of the corresponding bridging ligand was added. The mixture was refluxed for 24 h, filtered, and the product was precipitated by adding 1.5 mL of saturated $NaClO_4$ solution. The resulting powder was filtered off; washed with H_2O, ethanol, and diethyl ether; and dried under vacuum. The resulting product was further purified by recrystallizing from water. The complexes were characterized by NMR, IR, and microanalysis. Representative NMR spectral data for **Pt1** is provided in figures SI 3–5 (Supplemental data, see online at http://dx.doi.org/10.1080/00958972.2014.930138). The characterization data (listed below) confirmed the structures and purity of the complexes.

Pt1. $PtC_{18}H_{23}N_3Cl_2O_4$. Yield: 38 mg (13%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.92(d, 2H), 8.39(t, 2H), 8.05(d, 2H), 7.79(t, 2H), 5.40(d, 2H), 5.30(d, 2H), 3.05(m, 1H), 2.34(m, 2H), 1.69(m, 2H), 1.29(m, 6H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 25.1, 25.4, 30.1, 66.0, 73.0, 123.3, 126.0, 142.0, 149.2, 167.3. ^{195}Pt NMR (85 MHz, DMF-d_7) δ/ppm: −2334. Anal. Calcd for $PtC_{18}H_{23}N_3Cl_2O_4$: C, 38.56; H, 4.19; N, 7.56%. Found: C, 38.89; H, 4.16; N, 7.26%.

Pt2. $Pt_2C_{30}H_{34}N_6Cl_4O_8$. Yield: 122 mg (44.7%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.81(d, 4H), 8.30(t, 4H), 7.74(t, 4H), 5.31(d, 4H), 5.10(d, 4H), 3.45(s, 4H), 2.33(m, 2H), 2.10(m, 4H), 1.61(m, 4H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 27.8, 66.1, 70.3, 123.5, 125.6, 141.7, 167.0, 179.1. IR (4000–600 cm^{-1}) ^{195}Pt NMR (85 MHz, DMF-d_7) δ/ppm: −2331. Anal. Calcd for $Pt_2C_{30}H_{34}N_6Cl_4O_8$: C, 32.64; H, 3.07; N, 7.43%. Found: C, 32.69; H, 3.10; N, 7.40%.

Pt3. $Pt_2C_{37}H_{46}N_6Cl_4O_8$. Yield: 46 mg (16%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.90 (d, 4H), 8.36(t, 4H), 7.90(d, 4H), 7.75(t, 4H), 5.39(d, 4H), 5.26(d, 4H), 3.05(m, 2H), 2.30 (m, 4H), 1.61(m, 4H), 1.47(m, 4H), 1.40(m, 2H), 0.90(m, 6H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 31.4, 33.0, 66.0, 72.3, 123.0, 126.0, 142.0, 149.0, 167.0. IR (4000–600 cm^{-1}) ^{195}Pt NMR (85 MHz, DMF-d_7) δ/ppm: −2322 Anal. Calcd for $Pt_2C_{37}H_{46}N_6Cl_4O_8$: C, 36.89; H, 3.88; N, 6.74 %. Found: C, 36.85; H, 3.91; N, 6.75%.

Pt4. $PtC_{18}H_{17}N_3Cl_2O_4$. Yield: 132 mg (45.5%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.99 (d, 2H), 8.35(t, d merged, 4H), 7.80(d, 2H), 7.70(m, 2H), 7.46(m, 2H), 7.30(m, 1H), 6.0(d, 2H), 5.69(d, 2H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 72.0, 125.0, 125.5, 127.2, 130.6, 131.6, 143.0, 150.7, 151.0, 166.7. IR (4000–600 cm^{-1}) ^{195}Pt NMR (85 MHz, DMF-d_7) δ/ppm: −2334. Anal. Calcd for $PtC_{18}H_{17}N_3Cl_2O_4$: C, 35.72; H, 2.83; N, 6.94%. Found: C, 35.76; H, 2.85; N, 6.91%.

Pt5. $Pt_2C_{30}H_{28}N_6Cl_4O_8$. Yield: 136 mg (33%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.95 (d, 4H), 8.60(m, 8H), 8.35(m, 4H), 7.91(m, 4H), 6.0(d, 4H), 5.60(d, 4H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 59.0, 113.5, 122.0, 123.5, 136.0, 140.5, 151.0, 162.5.IR (4000–600 cm^{-1}) ^{195}Pt NMR (85 MHz, DMF-d_7) δ/ppm: −2331. Anal. Calcd for $Pt_2C_{30}H_{28}N_6Cl_4O_8$: C, 31.71; H, 2.49; N, 7.42%. Found: C, 31.70; H, 2.51; N, 7.41%.

Pt6. $Pt_2C_{37}H_{34}N_6Cl_4O_8$. Yield: 146.5 mg (32%). ^{1}H NMR (400 MHz, DMF-d_7) δ/ppm: 8.80(d, 4H), 8.60(t, 4H), 7.81(t, 4H), 7.43(m, 4H), 7.29(m, 4H), 6.95(d, 4H), 6.62(d, 4H), 4.4(s, 2H). ^{13}C NMR (100 MHz, DMF-d_7) δ/ppm: 41.1, 59.0, 113.7, 113.9, 122.6, 123.8, 130.5, 138.3, 150.6, 160. ^{195}Pt NMR (85 MHz, DMF-d_7)δ/ppm: −2321. Anal. Calcd for $Pt_2C_{37}H_{34}N_6Cl_4O_8$: C, 36.34; H, 2.80; N, 6.87%. Found: C, 36.30; H, 2.81; N, 6.90%.

Preparation of solutions for kinetics

The chloro complexes were converted into their aqua solutions following a procedure described by Bugarčić *et al.* [34]. A known amount of the complex was dissolved in 10 mL

of 0.01 M $HClO_4$ and stirred under an inert atmosphere of nitrogen. An equivalent of anhydrous $AgClO_4$ was dissolved in a little amount of solvent and added dropwise to an agitated solution of the complex. The temperature was raised to 40–50 °C and the mixture was left to stir for a day in the dark. The silver precipitate formed was removed by filtering through a 0.45 μm pore membrane filter. Care was taken to ensure that the resulting solution was free from Ag^+ and that the chloro complex had been converted completely into the aqua species. The kinetics of the substitution reactions were studied in a perchlorate media of pH 2 because, the perchlorate ion does not coordinates with Pt(II) in aqueous solution [35]. The ionic strength of the final solution was maintained at 0.1 M, using a solution comprising of 0.01 M $HClO_4$ and 0.09 M $NaClO_4$. Under these conditions, the studied Pt(II) complexes exist in their aqua forms and their hydrolysis products are negligible as deduced from the pK_a data of the same complexes reported [23] with values varying from 4.1 to 5.7 as shown in table SI 1 (Supplemental data).

Preparation of nucleophiles for kinetic analysis

Stock solutions of **Pzn**, **Pz**, **Tz**, and **mPz** were prepared by dissolving appropriate amounts of each nucleophile in 0.1 M ionic strength solution of pH 2. The nucleophile concentration was prepared in at least 20-fold excess over the concentration of the dinuclear Pt(II) complexes and 10-fold excess of concentration in cases of the mononuclear complexes. This guaranteed pseudo-first-order conditions for the substitution reactions. This also forced reactions to go to completion.

Physical measurements and instrumentation

A Thermo Scientific Flash 2000 Elemental Analyzer and a Bruker Avance DPX 400 spectrometer were used for elementary analysis and NMR measurements of the complexes, respectively. IR spectra of all the complexes were recorded from 4000 to 600 cm^{-1} on a Perkin Elmer Spectrum 100 FT-IR spectrometer. The mass spectral data of both ligands and complexes were obtained on a micro-mass LCT Premier coupled to an ESI^+-TOF mass spectrometer (see figures SI 2–17). All kinetic measurements were performed on a Cary 100 UV–visible spectrophotometer (Varian) equipped with a Varian Peltier thermostated cell holder. The temperature of the instrument was controlled within ±0.1 °C, throughout all kinetic measurements for both reference and sample compartments. All data were analyzed using Origin 7.5® [36] graphical analysis software.

Computational modeling

In an effort to gain further insight into the role of the diamine linker on tuning the reactivity of the complexes, the ground-state electronic structures of **Pz**-coordinated derivatives of the complexes (product of the substitution reactions with **Pz**) were optimized as cations of +2 charge for mononuclear and +4 for dinuclear complexes, and selected metric data were compared with literature values of the unsubstituted aqua complexes [23]. The calculations were performed in the gaseous phase at the density functional theoretical (DFT) level run on Spartan 08' for windows® program. The B3LYP [37] method, utilizing the LACVP** [38] pseudo potentials basis set, was used for optimization. A summary of the energy-minimized

structures and electrostatic potential surfaces are presented in table SI 6 (Supplemental data), while the key selected data is summarized in table 2.

Results and discussion

Kinetics measurements

The rate of substitution of the coordinated aqua ligands of the Pt(II) complexes by nitrogen donor heterocycles was monitored spectrophotometrically, by measuring the changes in the absorbance of their mixtures as a function of time at suitable wavelengths. A list of wavelengths, at which kinetic measurements for the substitution of the coordinated aqua ligands from the complexes, is summarized in table SI 2 (Supplemental data).

To initiate reactions, equal amounts of the nucleophile and complex were mixed in a tandem cuvette and the reaction was followed spectrophotometrically. Representative spectra for the reactions are shown in figure 2(a) and (b) for the reactions of **Pt3** with **mPz** and **Pt2** with **Pzn**, respectively. All kinetic traces taken at specific wavelengths gave perfect fits to a single exponential function, shown as insets in figure 2(a) and (b).

The single-step reaction pathway was true for mononuclear and dinuclear Pt(II) complexes, alike even when the reactions were left for over 16 h (see figure SI 1). This is unlike the spectral changes observed when the same Pt(II) complexes were reacted with thioureas [23], where two reaction steps were observed. The first step was considered to be the simultaneous substitution of the coordinated aqua ligands followed by a subsequent step in which one of the pyridylmethyl units in each pycolyl chelate framework was dechelated. The results obtained from this study suggest that the substitution of the coordinated aqua ligands from the Pt(II) dinuclear complexes occurs simultaneously. The indication is that the nitrogen donor heterocycles are too weak to de-coordinate the pyridyl units as observed for the reactions, involving stronger thiourea nucleophiles [23].

The pseudo-first-order rate constant, k_{obs}, calculated by fitting data to a single exponential function, was plotted against the concentration of the azole nucleophiles. Representative

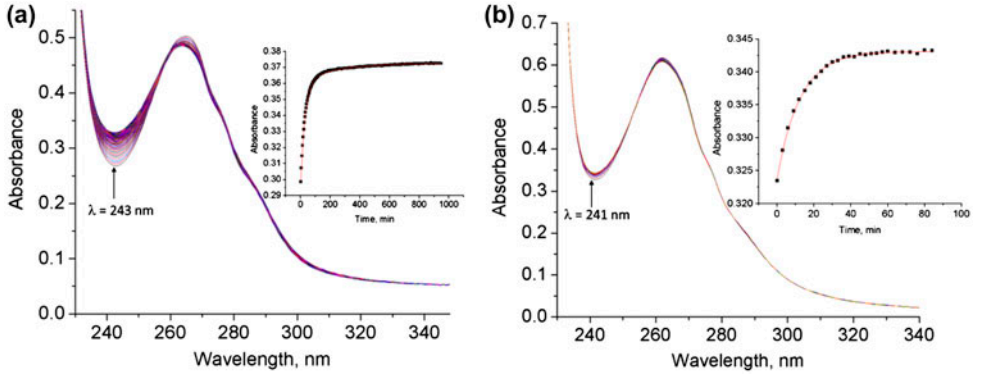


Figure 2. UV–visible spectra evolving from the simultaneous substitution of the aqua ligands of (a) **Pt3** (0.1 mM) with **mPz** (4 mM) and of (b) **Pt2** (0.1 mM) with **Pz** (5.3 mM), respectively. Insets are kinetic traces taken at 243 and 241 nm, respectively.

plots for the reactions between the Pt(II) complexes (**Pt2** and **Pt5**) and the nitrogen donor heterocycles for the substitution of the aqua ligands are shown in figure 3(a) and (b), respectively (additional Supplemental data is given in figure SI 19). Linear plots with no meaningful intercepts were observed in all cases. This indicated that neither the parallel solvent pathway nor the reverse reaction occurred significantly.

Thus, the dependence of the pseudo-first-order rate constants on the concentration of nucleophiles is described by equation (1):

$$k_{obs} = k_2[\mathrm{Nu}] \tag{1}$$

(Nu = **Pz**, **mPz**, **Tz**, and **Pzn**)

The slopes of the plots were used to calculate the values of the second-order rate constant, k_2, a measure of the rate of direct substitution of the aqua ligands from the Pt(II) complexes by the nitrogen donor heterocycles. Their values at 298 K are presented in table 1.

Activation parameters

The temperature dependence of the second-order rate constants was determined from 15 to 40 °C. The thermal activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) for the simultaneous substitution of aqua ligands by the nitrogen donor heterocycles were calculated from the slopes and intercepts of the Eyring plots, respectively, according to the equation: $\ln(k_2/T) = -\Delta H^{\neq}/RT + (23.8 + \Delta S^{\neq}/R)$ where the parameters represent the usual terms. Representative plots are shown in figure 4(a) and (b) and the values of the activation parameters calculated therefrom are summarized in table 1. The values of $\Delta S^{\neq}$ are all negative (ranging from −45 to $-146\ \mathrm{J\,K^{-1}\,M^{-1}}$) while $\Delta H^{\neq}$ values are positive and relatively small (within the range 42–58 $\mathrm{kJ\,M^{-1}}$) for the reactions of the complexes with all nucleophiles. This is typical of an associatively activated mode of substitution known to proceed via a trigonal bipyramidal transition state.

Computational calculations

The DFT calculated data in table 2 reveal that **Pz**-substituted complexes have similar ground state conformational metrics to that of the optimized structures of their aqua analogs

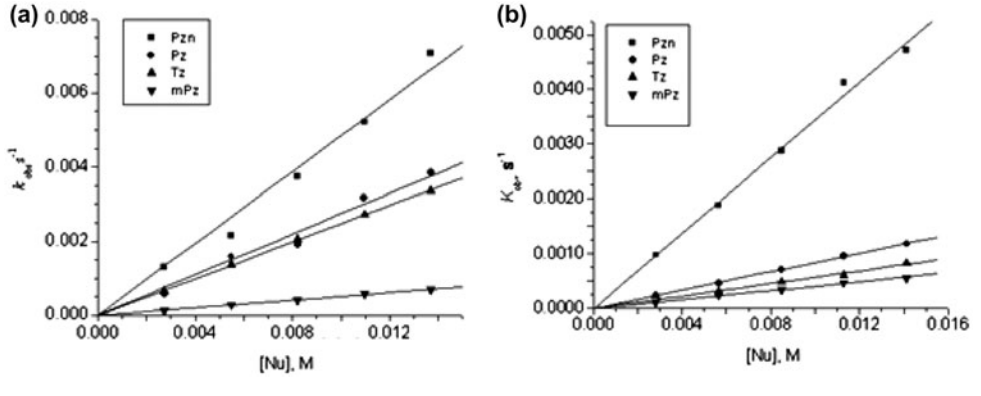
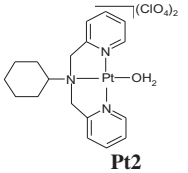
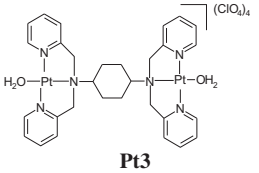
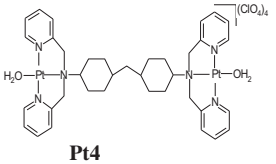
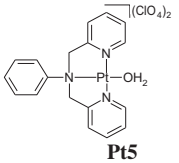
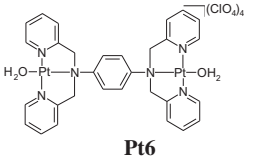
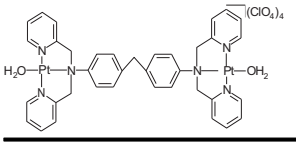


Figure 3. Plots of k_{obs} as a function of concentration of nitrogen donor heterocycles at 298 K for the substitution reactions of (a) **Pt2** and (b) **Pt5**.

Table 1. Second-order rate constants, k_2 and activation parameters for the substitution reactions of Pt(II) dinuclear complexes and their mononuclear analogs with nitrogen donor heterocycles at a pH of 2, and ionic strength of 0.1 M.

Complex	Nucleophile	k_2(M^{-1} s^{-1})	$\Delta H^{\neq}$(kJ M^{-1})	$\Delta S^{\neq}$(J K^{-1} M^{-1})
Pt1 	**Pzn**	4.01 ± 0.10	46 ± 3	−45 ± 10
	Pz	0.91 ± 0.01	49 ± 3	−74 ± 9
	Tz	0.82 ± 0.02	51 ± 3	−82 ± 9
	mPz	0.44 ± 0.04	51 ± 2	−83 ± 6
Pt2 	**Pzn**	0.49 ± 0.04	42 ± 4	−111 ± 10
	Pz	0.28 ± 0.01	46 ± 2	−90 ± 7
	Tz	0.25 ± 0.01	44 ± 3	−109 ± 9
	mPz	0.05 ± 0.01	50 ± 2	−102 ± 7
Pt3 	**Pzn**	1.10 ± 0.01	41 ± 2	−72 ± 5
	Pz	0.30 ± 0.01	47 ± 2	−98 ± 6
	Tz	0.27 ± 0.01	49 ± 1	−90 ± 3
	mPz	0.16 ± 0.01	50 ± 4	−91 ± 10
Pt4 	**Pzn**	0.81 ± 0.01	49 ± 2	−81 ± 7
	Pz	0.48 ± 0.01	55 ± 4	−55 ± 10
	Tz	0.15 ± 0.01	53 ± 2	−92 ± 7
	mPz	0.12 ± 0.01	58 ± 4	−69 ± 12
Pt5 	**Pzn**	0.34 ± 0.01	39 ± 2	−85 ± 6
	Pz	0.08 ± 0.01	42 ± 3	−123 ± 10
	Tz	0.06 ± 0.01	43 ± 4	−125 ± 13
	mPz	0.04 ± 0.01	50 ± 3	−140 ± 9
Pt6 	**Pzn**	0.62 ± 0.01	45 ± 2	−80 ± 6
	Pz	0.16 ± 0.01	47 ± 4	−103 ± 12
	Tz	0.13 ± 0.01	50 ± 2	−94 ± 6
	mPz	0.08 ± 0.01	54 ± 3	107 ± 9

[23](c). As previously reported, the number of carbons, n, between nitrogens of the diamine bridge determines the conformation of the dinuclear Pt(II) complexes. When n is odd, the optimized structure of the **Pz**-substituted product is bowl shaped with a C_{2v} symmetry, while when n is even, the complex takes a slip-up structure with C_{2h} symmetry. This conformational symmetry plays a significant role on the substitutional reactivity of the Pt(II) complexes as discussed later. A similar effect on the reactivity due to the conformational symmetry is observed for the cyclohexyldiamine and phenyldiamine-bridged complexes alike. A summary of key computed data is presented in table 2 while the energy-minimized and EPS structures are found in the Supplemental data (table SI 6).

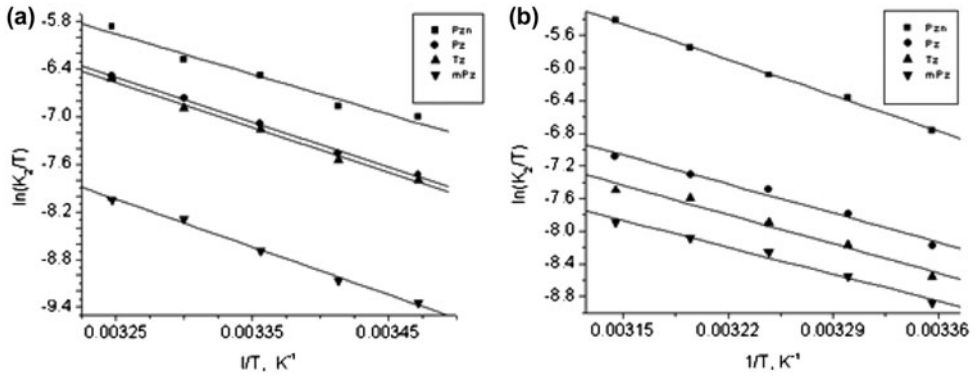


Figure 4. Temperature dependence of k_2, $M^{-1}s^{-1}$, for the simultaneous substitution of the aqua ligands from (a) **Pt2** and (b) **Pt5** by nitrogen donor heterocycles at pH 2.0, $I = 0.1$ M.

Kinetics analysis

As already stated, the substitution reactions of the Pt(II) complexes with the nitrogen donor heterocycles proceeded via a single step which fits perfectly to a single exponential function. The step is taken to be the simultaneous substitution of the coordinated aqua ligands from the dinuclear complexes, by the nitrogen donor heterocycles. No dechelation was observed even when the reactants were left to react for more than 48 h. A plausible explanation is that heterocyclic nucleophiles coordinate with Pt(II) through a nitrogen donor, making them intrinsically weaker nucleophiles when compared with the stronger thiourea reported in literature [23(c)]. Furthermore, the rate of aqua substitution by nitrogen donor heterocycles was found to be slower by a factor of about 10^3 when compared with those reported [23(c)] for thiourea.

The k_2 values in table 1 show a similar reactivity trend in the two groups of complexes, viz., Pt(II) with cyclohexyl groups and those with phenyl groups. In general, the rate of aqua substitution from the complexes decreases in the order: mononuclear Pt(II) with cyclohexylamine/phenylamine pendants > dinuclear Pt(II) with dicyclohexyl/diphenyl methylenediamine bridges > dinuclear Pt(II) with cyclohexyldiamine/phenyldiamine bridges. This is the trend which was observed using thioureas as substituting nucleophiles [23(c)]. The complete order of the rate of substitution of water in each set of complexes decreases in the

Table 2. Summary of DFT computed data for the **Pz**-substituted dinuclear Pt(II) complexes and their mononuclear analogs for comparison with their unsubstituted aqua complexes (*data taken from Ref. [23]).

Property	**Pt1**	**Pt2**	**Pt3**	**Pt4**	**Pt5**	**Pt6**
Bond lengths (Å)						
$Pt–N_{pyrazole}$	2.197	2.165	2.183	2.175	2.155	2.161
$Pt–OH_2$*	2.161	2.144	2.156	2.153	2.140	2.143
Separation distances (Å)						
$N_{1trans}–N_{2trans}$	–	5.980	11.253	–	5.804	11.010
$Pt_1–Pt_2$	–	8.908	13.081	–	8.675	13.752
$Pt_1–Pt_2$*	–	8.770	14.200	–	8.590	10.630
NBO charges						
Natural charges for Pt_1 and Pt_2	1.200	1.221	1.1220	1.095	1.225	1.223
NBO charges*	1.203	1.220	1.215	1.215	1.226	1.221

order **Pt1** > **Pt3** > **Pt2** in the case of complexes bearing cyclohexyl groups and **Pt4** > **Pt6** > **Pt5** for the complexes with phenyl groups.

A comparison of the rate constants in table 1 reveals that the substitutions of the aqua ligands occur much slower for dinuclear Pt(II) complexes when compared with their mononuclear analogs. Taking **Pzn** as a reference nucleophile, the rate of aqua substitution in **Pt1** is about eight times than that of **Pt2**. A similar trend is observed in the complexes bearing a phenyl pendant group. This occurs despite the lower effective charge at the Pt(II) centers of the mononuclear analogs. The observed reactivity differences are attributed to the presence of steric influence on the Pt(II) chelates due to the C_{2h} conformations of the two dinuclear complexes. The short linkers (1,4-diaminocyclohexane or 1,4-diaminobenzene) project similar conformations with overlap geometries that slows the approach of the incoming nitrogen donor heterocycles. Such conformational influence is totally absent on the mononuclear analogs.

As the chain increases, however, from **Pt2** to **Pt3**, the rate of aqua substitution increases noticeably, doubling for **Pzn**. Two reinforcing factors are predominant. First, the incorporated methylene spacer within the diamine bridges in both **Pt3** and **Pt6** results in an increase in the Pt–Pt separation distances. The diamine linker in **Pt3** (13.1 Å), for instance, is about 4 Å longer than **Pt2** (8.9 Å) while the diamine linker in **Pt6** (13.8 Å) is about 5 Å longer than **Pt5** (8.7 Å). This increase reduces the steric effects at each Pt(II) center. Secondly, the switch in conformational symmetry from C_{2h} in **Pt2** to C_{2v} conformations in **Pt3** also accelerates the substitution reactions of the methylene-spaced complexes relative to the former. This is because the nucleophile is entrapped, resulting in increased effective interactions with the complex forming products. To a lesser extent, the same trend is observed in the phenylamine/diamine series of complexes with **Pt6** having a distorted C_{2v} conformation to accommodate the constraints due to the rigidity and planarity of the phenyl groups within its diamine linker. The trend in bond distances and changes in NBO charges, (table 2) both reaffirm the general reactivity trend observed between the respective groups of complexes. The complexes bearing cyclohexyl pendant groups show a higher rate of aqua substitution when compared with their phenyl pendant group-bearing counterparts. This reactivity difference is due to the slightly better σ-inductive donation of electron density from the cyclohexyl groups towards the Pt(II) metal center as opposed to the phenyl groups, which delocalize the electron density within the aromatic rings leading to slightly lower rates of aqua substitution at its Pt(II) metal center.

Relative to their respective mononuclear complexes, the Pt(II) centers of the two isosteric dinuclear complexes, viz., **Pt5** and **Pt2** experience a reduced σ-inductive effect, since the diamine bridge is shared among two platinum centers. However, in **Pt3** and **Pt6**, the methyl spacers increase the number of functional groups that can donate electron density to their metal centers. Thus, the metal centers in **Pt3** and **Pt6** are expected to experience slightly higher σ-inductive effects than **Pt2** and **Pt5**, respectively. This corroborates well with the trend of reactivity in table 1 as well as the Pt–OH bond lengths recorded in table 2. The Pt–OH_2 bond lengths for **Pt3** (2.143 Å) and **Pt6** (2.156 Å) are longer than those of **Pt2** (2.140 Å) and **Pt5** (2.144 Å), respectively, leading to faster substitution of the aqua ligands of **Pt3** and **Pt6**.

Reactivity of nucleophiles

The nucleophiles used in this study were carefully chosen to explore both electronic and steric effects. **Pzn** was selected to assess the nucleophilicity of a six-atomed ring (azine)

nitrogen donor. Included in the set were three azoles viz., **Pz**, **Tz**, and **mPz**. **Pz** and **Tz** differ only in the number and positions of nitrogens within their ring systems. **mPz**, an azole with a methyl substituent on the ortho position, was chosen to quantify the role of the methyl substituent on the nucleophilicity of the pyrazole ring.

By looking at the data in table 1, the general order of reactivity of the heterocyclic nucleophiles is: **Pzn** > **Pz** > **Tz** > **mPz**. It appears that the trend of reactivity of the nucleophiles depends linearly on their pK_a values, which is a measure of their basicity [39] as reported from other studies [40, 41]. When a nucleophile has a superior basicity index, its rate of substitution at the metal center is expected to be high. The pK_a values of **Pzn**, **Pz**, **Tz**, and **mPz** are reported as 0.37, 2.52, 2.19, and 2.10, respectively [42]. While the pK_a value of **Pzn** may seem odd and lower than the rest of the azoles, **Pzn** is still more reactive than its azole counterparts. This may be because the valence atomic orbitals at its nitrogen donor can overlap more effectively with those at the Pt(II) metal center due to the reduced ring strain and electron repulsion. A large angle (~120°) is subtended at each atom on the **Pzn** ring. Moreover, the symmetrical and *para* positions of the two nitrogen donors in **Pzn** make both of them equally susceptible to coordination at the metal center. Both factors increase its reactivity by factors not less than two when compared with that of **Pz** and other azole nucleophiles, for all complexes. A similar anomalous order of reactivity was also reported by Bugarčić *et al.* [43]. Pyrazine is certainly a better incoming nucleophile when compared with **Pz** with all Pt(II) complexes.

Pz and **Tz** are two isosteric nitrogen (N) donor nucleophiles, only differing in the number of nitrogens in the azole ring [44]. As the number of nitrogens in an aromatic heterocycle increases, so does the acidity. Thus, the three nitrogens on the ring of **Tz** increase its acidity, leading to a lower pK_a value than **Pz**. This makes it a poor substituting nucleophile, and less reactive, as the data in table 1 show.

When comparing the reactivity of **mPz** and **Pz** for all complexes using data in table 1, it is clear that the ortho-methyl substituent slows the approach of the nucleophiles at the Pt(II) metal center. Thus, the steric hindrance of the methyl group dominates over its expected inductive donation of electron density in the aromatic ring which would accelerate the rate of aqua substitution. This is in line with the general trend observed on the values of the enthalpy of activation (refer to table 1). The highest values of the enthalpy of activation are recorded for the reactions of **mPz** which is the weakest incoming nucleophile. The complete order of activation is **mPz** > **Tz** > and **Pz** > **Pzn**. Data in table 1 are characterized by significantly negative values of entropies of activation and the small and positive values of the enthalpies of activation. This indicates that the reactions are associatively activated as commonly occurring at a square-planar Pt(II) center [45].

Conclusion

The results of this study indicated that the substitution of the aqua ligands by nitrogen donor nucleophiles from dinuclear Pt(II) complexes occurs simultaneously in a single step. The aqua substitution is predominantly controlled by steric effects due to the conformations of Pt(II) dinuclear complexes. A weaker electronic effect due to the inductive effects from the groups within the diamine bridge also contributes to the higher reactivity of the mononuclear Pt(II) complexes in comparison with the analogs of dinuclear Pt(II) complexes. Increasing the chain and incorporation of a methyl spacer afford some flexibility in the

complex which results in a change in configuration from C_{2h} to C_{2v}. This increases the reactivity of complexes with bridging methylene spacers due to the entrapment of the nucleophile. The reactivities of the complexes bearing cyclohexyl groups in their backbone were slightly higher than for complexes bearing aromatic groups, showing that cyclohexyl groups have better σ-inductive donation towards the Pt(II) centers when compared with that of an aromatic group. This is supported by DFT calculations. The rate of aqua substitution by nitrogen donor heterocycles in the two sets of complexes decreases in the order: **Pt1** > **Pt3** > **Pt2** and **Pt4** > **Pt6** > **Pt5**. The nucleophilic strength is: **Pzn** >> **Pz** > **Tz** > **mPz**. This reactivity trend correlates linearly with their pK_a values. An exception is **Pzn**, being an azine, reacts faster than all azoles used in this study. Aqua substitution of the Pt(II) complexes with nitrogen-containing heterocycles occurs at relatively slower rates than with sulfur donor nucleophiles and proceeds without de-coordinating the carrier ligands. The slow reactivity with nitrogen donor heterocycles favors low or reduced toxicity of the complexes when they interact with plasma proteins before they react with nucleic acids.

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