

Synthesis, characterization and cytotoxicity of (3,4-diaminobenzoic acid)-haloplatinum(II)/palladium(II)

N Jain & T S Srivastava*

Department of Chemistry, Indian Institute of Technology, Powai, Bombay 400 076

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The synthesis of platinum compounds of the type $[\text{Pt}(\text{daba})\text{X}_2]$ (where $\text{daba} = 3,4\text{-diaminobenzoic acid}$ and $\text{X} = \text{Cl}, \text{Br}, \text{or I}$) and the analogous chloro and bromo compounds of $\text{Pd}(\text{II})$ has been carried out. These compounds have been characterized by molar conductance measurements, electronic absorption, infrared, ^1H NMR and X-ray photoelectron spectroscopy. The complexes are non-electrolytic in nature and have square planar geometry with two coordination positions occupied by two amino groups of 3,4-diaminobenzoic acid and remaining two positions occupied by halide ions. The sodium salts of the above compounds have been tested against P388 lymphocytic leukemia cells. $[\text{Pt}(\text{daba})\text{Cl}_2]$ and $[\text{Pt}(\text{daba})\text{Br}_2]$ have I.D.₅₀ values less than that of *cisplatin* and $[\text{Pt}(\text{daba})\text{I}_2]$ has I.D.₅₀ values comparable to that of *cisplatin*.

The antitumor activity of *cis*-diamminedichloroplatinum(II) (*cisplatin*) first discovered by B. Rosenberg and coworkers in 1969 has led to its use as a leading drug in cancer chemotherapy^{1,2}. In order to develop more effective platinum compounds which do not have unfavourable toxicity of *cisplatin*, one strategy is to prepare close analogs of *cisplatin* by appropriate modification of carrier ligands and by choosing appropriate leaving ligands³. This approach has revealed that the strongly bound leaving ligands such as iodide, nitrite, and cyanide show no activities⁴. Recently, Carraher, Jr. and coworkers have reported a polymeric diamine platinum iodide complex (where diamine is methotrexate) which has shown good growth inhibition of 3T3 cells⁵. In this paper, we report the synthesis, characterization and cytotoxicity of $[\text{Pt}(\text{daba})\text{X}_2]$ and analogous $\text{Pd}(\text{II})$ compounds (where daba is 3,4-diaminobenzoic acid and X is Cl, Br or I). These compounds have shown the cytotoxicity against P388 lymphocytic leukemia cells. The I.D.₅₀ (50% growth inhibition) value of the iodo compound is comparable to that of *cisplatin*⁶.

Materials and Methods

$[\text{Pt}(\text{daba})\text{Cl}_2]$ (where daba is 3,4-diaminobenzoic acid) was prepared by the literature method⁷, yield 80%. Analysis: [Found: C, 20.03; H, 2.00; N, 6.76. Calc. for $\text{C}_7\text{H}_8\text{N}_2\text{O}_2\text{Cl}_2\text{Pt}$: C, 20.08; H, 1.91; N, 6.69%].

Preparation of complexes

1. $[\text{Pt}(\text{daba})\text{Br}_2]$

K_2PtCl_4 (207.5 mg, 0.5 mmol) was dissolved in 50

ml of distilled water and sodium bromide (1.23 g, 12 mmol) was added to it. The reaction mixture was stirred for 20 min followed by addition of 3,4-diaminobenzoic acid (76.1 mg, 0.5 mmol) in methanol. The reaction mixture was further stirred for 24 h. The green solid obtained was washed with water, methanol and finally with diethyl ether. The compound was recrystallized from dimethyl sulfoxide. The crystals were filtered, washed with methanol and finally with diethyl ether. The compound was air dried and its yield was 78%. Analysis: [Found: C, 17.06; H, 1.65; N, 6.03%. Calc. for $\text{C}_7\text{H}_8\text{N}_2\text{O}_2\text{Br}_2\text{Pt}$: C, 16.56; H, 1.57; N, 5.52%].

2. $[\text{Pt}(\text{daba})\text{I}_2]$

This compound was prepared by following the method adopted for (1) except that potassium iodide (1.93 g, 12 mmol) was added in place of sodium bromide. The yield was 90%. Analysis: [Found: C, 14.63; H, 1.39; N, 4.69%. Calc. for $\text{C}_7\text{H}_8\text{N}_2\text{O}_2\text{I}_2\text{Pt}$: C, 13.97; H, 1.33; N, 4.65%].

3. $[\text{Pd}(\text{daba})\text{Cl}_2]$

PdCl_2 (443.7 mg, 2.5 mmol) and sodium chloride (350.7 mg, 6 mmol) were suspended in 50 ml of distilled water. The reaction mixture was stirred for 3 h to get a clear solution. 3,4-Diaminobenzoic acid (380.3 mg, 2.5 mmol) in methanol was added to the above solution and the reaction mixture stirred for 24 h. The brown red compound obtained was filtered, washed with water, methanol and finally with diethyl ether. The compound was air dried, yield 40%. Analysis: [Found: C, 25.68; H, 2.53; N, 8.57%].

Table 1—Electronic absorption spectra of [M(daba)X₂] in DMF

Compound	Band-1	Band-2	Band-3	Band-4	Band-5	Band-6	Band-7
[Pt(daba)Cl ₂]	721 ^a (14100) ^b	589 (2000)	440 (3300)	400 (3400)	329 (7800)	279 (13300)	264 (13400)
[Pt(daba)Br ₂]	720 (6300)	588 (2200)	441 (3800)	402 (3800)	334 (9500)	278 (12100)	265 (12200)
[Pt(daba)I ₂]	721 (2100)	599 (2300)	444 (4400)	418 (4300)	341 (11100)	279 (13000)	264 (11900)
[Pd(daba)Cl ₂]				400(sh) (1200)	326 (5600)	275 (7100)	268 (7700)
[Pd(daba)Br ₂]				400(sh) (1400)	320 (5900)		267 (8900)

^a $\lambda_{\max}$ in nm, ^b $\epsilon_{\max}$ in l.mol⁻¹ cm⁻¹ is given in parentheses, ^csh is shoulder in parentheses.

Table 2—¹H NMR spectral data of [M(daba)X₂] in DMSO-*d*₆

Compound	3,4-Diaminobenzoic Acid Protons		
	-NH ₂	H-6	H-2,5
[Pt(daba)Cl ₂]	8.40 ^a (br) ^b (-2.28) ^c	7.20(m) (-0.05)	7.77(m)
[Pt(daba)Br ₂]	8.54(br) (-2.42)	7.52(m) (-0.37)	7.84(m)
[Pt(daba)I ₂]	8.54(br) (-2.42)	7.54(m) (-0.39)	7.80(m)
[Pd(daba)Cl ₂]	7.30(br) (-1.18)	7.30(m) (-0.15)	7.80(m)
[Pd(daba)Br ₂]	7.20(br) (-1.08)	7.20(m) (-0.15)	7.80(m)

^aChemical shifts are given in δ , ppm.

^bbr is broad and m is multiplet in the parenthesis.

^cChemical shift difference is given in parenthesis and they are relative to free 3,4-diaminobenzoic acid. Negative sign denotes downfield shift.

The molar conductance values in DMF obtained for [Pt(daba)X₂] and [Pd(daba)X₂] are in the range of 3 to 18 cm² ohm⁻¹ mol⁻¹ which are suggestive of their non-electrolytic nature.

The infrared spectra (600-4000 cm⁻¹) of the above compounds in nujol mull and KBr pellets have been recorded. The N-H bending vibration in these compounds is located in the region 1540-1560 cm⁻¹ which is at lower position as compared to that in the free 3,4-diaminobenzoic acid. In addition, the C=O stretching vibration of carboxylic acid group in the metal chelates has been observed in the region 1720-1730 cm⁻¹ which indicates the presence of uncoordinated carboxylic acid group^{11,12}.

The electronic absorption spectra ($\lambda_{\max}$, $\epsilon_{\max}$ values) of the above compounds in DMF are listed in Table 1. The band 1 is assigned to charge-transfer transition from platinum *d* orbitals to π^* orbital of 3,4-diaminobenzoic acid. The band shift of 15 to 20 nm towards blue region is observed on increasing the polarity of solvents from dimethyl sulphoxide to methanol¹³. The bands 2,3,4 and 5 are tentatively assigned to *d-d* + π - π^* transitions. The bands 6 and 7 are due to π - π^* and *n*- π^* transitions of the benzene ring and -COOH group of 3,4-diaminobenzoic acid respectively. These bands show red and blue shifts respectively, with increasing polarity of solvents from dimethyl sulphoxide to methanol.

The ¹H NMR spectra of 3,4-diaminobenzoic acid and their Pt(II) and Pd(II) complexes in (DMSO-*d*₆) were measured on a 100 MHz FT NMR spectrometer (Table 2). The ¹H NMR spectrum of 3,4-diaminobenzoic acid shows a broad peak at δ 6.22 ppm due to protons as indicated by its disappearance after D₂O exchange. In the free ligand spectrum, a doublet at 6.56 ppm is assigned to H-5 proton, and a doublet of a doublet at 7.15 ppm is assigned to

Calc. for C₇H₈N₂O₂Cl₂Pd:C, 25.48, H, 2.42, N, 8.49%].

4. [Pd(daba)Br₂]

This compound was prepared by following the method adopted for (3) except that sodium bromide (617.5 mg, 6 mmol) was used in place of sodium chloride. The yield of the compound was 30%. Analysis: [Found: C, 20.28, H, 1.99; N, 7.30%. Calc. for C₇H₈N₂O₂Br₂Pd:C, 20.07; H, 1.91; N, 6.69%].

The methods used for the physical measurements and cytotoxic studies have been described elsewhere^{6,8,9}.

Results and Discussion

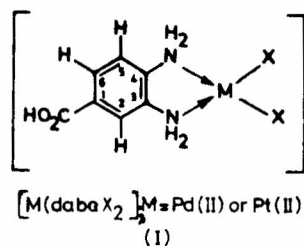
Three platinum(II) compounds of the general formula [Pt(daba)X₂] and two analogous chloro and bromo compounds of Pd(II) have been prepared.

Table 3—X-ray photoelectron spectral data of $[M(\text{daba})X_2]$

Compound	Pt($4d_{5/2}$)	N($1s_{1/2}$)	Cl($2p_{3/2}$)	Br($2p_{1/2,3/2}$)	I($3d_{5/2}$)	Pd($3d_{5/2}$)
[Pt(daba)Cl ₂]	310.6 ^a	400.1	198.3			
[Pt(daba)Br ₂]	314.1	403.7		183.3		
[Pt(daba)I ₂]	321.7	405.7			623.2 636.6	
[Pd(daba)Cl ₂]		401.0	200.6			344.3 338.9
[Pd(daba)Br ₂]		399.6		181.6 188.2		342.7 337.4

^aBinding energies are given in eV.

H-6 proton. The ¹H NMR spectra of Pt(II) and Pd(II) complexes show the downfield shifts of NH₂ protons and H-2, H-5 and H-6 protons as compared to the free ligand¹⁴ values. This suggests that bonding of 3,4-diaminobenzoic acid to metal is through both the nitrogen atoms of the amino groups (Structure 1). The carboxylic acid group of 3,4-diaminobenzoic acid in the above compounds is uncoordinated because the C—O stretching vibration of carboxylic acid is present between 1720 and 1730 cm⁻¹ in the metal chelates¹¹.



The X-ray photoelectron spectral data of the above compounds are summarized in Table 3. The measured Pt($4d_{5/2}$) binding energies of the $[Pt(\text{daba})X_2]$ increase in the order Cl → Br → I. The increase in binding energy of Pt($4d_{5/2}$) in the $[Pt(\text{daba})X_2]$ may be correlated in terms of electronegativity difference between the platinum and halogen atom and in terms of platinum to ligand π -bonding¹⁵⁻¹⁷. In the platinum complexes, the latter mechanism seems to be of significance. This is expected to increase the overall positive charge on platinum due to increase in platinum-halogen π -bonding in the order Cl → Br → I^{18,19}. On the other hand, the measured Pd($4d_{5/2}$) binding energies of $[Pd(\text{daba})X_2]$ decrease as Cl → Br, which are in accord with the decreasing electronegativity of halogens (Cl > Br > I)¹⁵⁻¹⁷. The N($1s_{1/2}$) binding energy of $[M(\text{daba})X_2]$ shifts towards the higher energy as the anion changes from Cl → Br → I. This observation is

the result of increase in metal halogen π -bonding in the order Cl → Br → I. The lower energies of Cl($2p_{3/2}$) Br($3p_{1/2,3/2}$) and I($3d_{5/2}$) in the above compounds (see Table 2) as compared to the corresponding value of free halide ions [Cl($2p_{3/2}$) (200 eV), Br($3p_{1/2,3/2}$) (189.0 eV) and I($3d_{5/2}$) (620.0 eV)] suggest that there is electron drift towards halogens in the platinum compounds¹⁹.

Solutions of $[M(\text{daba})X_2]$ in 1% sodium bicarbonate solution were initially tested against P388 lymphocytic leukemia cells using concentrations of 1, 20 and 100 $\mu\text{g}/\text{ml}$ ^{6,9}. Those compounds showing I.D.₅₀ (50% inhibitory dose) values less than 20 $\mu\text{g}/\text{mol}$ were further tested at varying concentrations of 1 to 20 $\mu\text{g}/\text{ml}$. The I.D.₅₀ values obtained for $[Pt(\text{daba})Cl_2]$, $[Pt(\text{daba})Br_2]$ and $[Pt(\text{daba})I_2]$ are 9.5, 9.4 and 18.2 $\mu\text{mol}/\text{litre}$ respectively. The I.D.₅₀ values obtained for $[Pt(\text{daba})Cl_2]$, $[Pt(\text{daba})Br_2]$ are less than that of cisplatin (I.D.₅₀ = 17 $\mu\text{mol}/\text{litre}$) whereas I.D.₅₀ value of $[Pt(\text{daba})I_2]$ is comparable to that of cisplatin. The chloride and bromide ions as leaving ligands in the $[Pt(\text{daba})Cl_2]$ and $[Pt(\text{daba})Br_2]$ complexes seem to be lost inside the cancer cells with the formation of $[Pt(\text{daba})(\text{H}_2\text{O})(\text{OH})]^+$ because the leaving ligands in the above complexes have intermediate liability of Pt—Cl and Pt—Br bonds. The cytotoxicity of cancer cells is probably due to attack of $[Pt(\text{daba})(\text{H}_2\text{O})(\text{OH})]^+$ on guanine and cytosine region of DNA, producing damage which is responsible for selective death of cancer cells. A similar mechanism operates for cytotoxicity of cisplatin²⁰. However, the Pt—I bonds in $[Pt(\text{daba})I_2]$ give rise to a kinetically inert complex which is not able to give cytotoxic species like $[Pt(\text{daba})(\text{H}_2\text{O})(\text{OH})]^+$ inside the cancer cells. Therefore, a different mechanism is operative for cytotoxicity of $[Pt(\text{daba})I_2]$ than $[Pt(\text{daba})Cl_2]$ and $[Pt(\text{daba})Br_2]$ complexes. Thus, the iodo compounds may be looked upon as a new class of futuristic antitumor agents.

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