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Synthesis, characterization and mutagenicity of new *cis*-[Pt(2-substituted-benzimidazole)$_2$Cl$_2$] complexes

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In this study, four new platinum(II) complexes with the structures *cis*-[Pt(Ligand)$_2$Cl$_2$] (ligand=2-(p-methoxy-/or-p-chlorobenzyl or *p*-methoxyphenyl)benzimidazol (**1**, **2**, **4** respectively) and 5(6)-methyl-2-phenoxymethylbenzimidazole (**3**) were synthesized and characterized by their elemental analysis, and IR and ^{1}H NMR spectra. The potentials of the Pt(II) complexes for short-term bacterial mutagenicity were tested in reverse-mutation assays using *Salmonella typhimurium* frame-shift strain T 98 and *S. typhimurium* TA 100 and TA 102 strains, which carry mutations particularly sensitive to reversion by DNA base-pair substitution. The tests were performed in the absence of S9 rat liver fraction. Among the complexes tested **1** had no mutagenic activity. Complex **4** was found to be weakly mutagenic in TA 98 only. The Pt(II) complexes **2** and **3** were found to be mutagenic in TA 98, TA 100 and TA 102.

1. Introduction

In recent years a great deal of effort has been devoted to developing transition metal antitumor agents which have better therapeutic properties than the prototype drug cisplatin [*cis*-diamminedichloroplatinum (II)]. The clinical usefulness of cisplatin has been frequently limited by the following drawbacks: serious toxicities, such as nephrotoxicity, gastrointestinal toxicity, ototoxity, and neurotoxicity, low activity for certain kinds of cancers, development of acquired resistance, and poor solubility in water [1].

Second generation platinum (II) antitumor complexes that carry "non-leaving ligands" other than simple ammonia are of interest for their altered affinity to tissues and their ability to modulate drug metabolism and target binding through steric and electronic effects on the substitution mechanism [2]. We have chosen some benzimidazole derivatives as a "non-leaving ligand" since the benzimidazole nucleus is found in a variety of naturally occuring compounds such as vitamin B_{12} and its derivatives, and it is structurally similar to purine bases. Furthermore, benzimidazoles are known to exhibit a wide variety of pharmacological proporties including antitumor activity [3] and inhibition of nucleic acid synthesis [4]. It was reasoned that by combining certain benzimidazoles with platinum it might be possible to obtain compounds with a superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity and lower side effects than cisplatin.

We previously described the synthesis and characterisation of some complexes of the structure, [Pt(L)$_{1\text{ or }2}$Cl$_2$], where L is 5(6)-non/or-chloro/or-methylsubstituted-2-hydroxymethylbenzimidazole and evaluated the ability of these compounds to damage DNA or interfere with DNA replication by determining their cytotoxic effects on the repair proficient *E. coli* strain AB 1157 (Rec A$^+$) and the repair deficient mutant AB 1157 (Rec A$^-$) [5]. We have also characterized the DNA binding properties of some of the Pt(II) complexes synthesized and the affinity of DNA modified by the complexes to the HMG-domain protein HMG-1. It was concluded that the adducts formed by the complexes having 2-hydroxymethylbenzimidazole ligands as "non-leaving groups" distort the DNA in a manner similar to cisplatin diadducts [6]. And it was also determined that some of the new benzimidazole Pt(II) complexes we synthesized have *in vitro* antitumor activities (unpublished results). These results encouraged us to design the new benzimidazole Pt(II) complexes.

Although there is some evidence to suggest that other biological targets may be important in the cisplatin mechanism, it is generally accepted that DNA is the primary target [7]. The predominant lesions produced in DNA are 1,2 d (GpG) (65%) and 1,2 (ApG) (25%) intrastrand adducts [8]. These are believed to be responsible not only for the cytotoxicity but also for the mutagenicity of cisplatin [9].

Cisplatin is also a carcinogen in both rats and mice [10], and humans treated with cisplatin in combination with other chemotherapeutic agents show an elevated frequency of micronuclei in the peripheral blood several years after treatment indicating that this drug causes long-lasting genetic damage [11]. Inactivation of the mismatch repair system also augments the intrinsic mutagenicity of cisplatin [12]. Mutagenicity may represent a serious limitation to the therapeutic use of potential drugs. This adverse property should be eliminated by rational drug design if possible.

In this study, as an extension of the planned investigation into the antitumor activity of Pt(II) complexes of the benz-

imidazoles, the mutagenic potentials of some new Pt(II) complexes of 2-substituted-benzimidazoles were tested for short-term bacterial mutagenicity in reverse-mutation assays using *Salmonella typhimurium* frame-shift strain TA 98 and *S. typhimurium* TA 100 and TA 102 strains which carry mutations particularly sensitive to reversion by DNA base-pair substitution.

2. Investigations, results and discussion

2.1. Syntheses and characterization of the complexes

The ligands L^1-L^4, used as 'non-leaving ligands' in the structure of the Pt(II) complexes, were prepared as shown in the Scheme. The ligands had been reported in the literature previously and their melting points were in accordance with the literature [13–15].

The platinum(II) complexes **1**, **4** and **2**, and **3** were synthesized by the reaction of the corresponding ligands and potasiumtetrachloroplatinate in the media of dimethylformamide or 0.5 N hydrochloric acid respectively as described in the Experimental section. Heating and stirring for longer periods of time did not improve the yields for these reactions. Purification of [Pt(benzimidazole-ligand)$_2$Cl$_2$] proved to be problematic with some impurities appearing in all cases. The crude product precipitated was a mixture of desirable product and a small amout of unreacted starting compounds and some unidentified water-soluble (presumably solvolysis products) and insoluble (presumably polymeric products) materials and it was purified by repeated washing with water, ethanol and diethylether.

Purification processes to avoid the undesirable components caused some loss in yields, but the Pt(II) complexes **1**–**4** obtained were pure. The melting points of all the complexes were above 300 °C. All the complexes synthesized were stable under at normal conditions, and the reactions were reproducible.

The complexes were characterized by their elemental analyses and IR and ^{1}H NMR spectra compared with those of the ligands. It was not possible to obtain suitable crystals for structural determinations. However, from the results of the different techniques employed it is possible to propose structures. Elemental analysis suggested a 1 : 2 (metal : ligand) stoichiometry for all the complexes as expected. For complexes **2** and **3** molecules of water were included as justified by the analytical results. This was also observed clearly in the IR spectra of the complexes. It is expected that complex **3** bearing L^3 is probably a mixture of constitutional isomers since two [Pt(5/and 6-methyl-2-phenoxymethylbenzimidazole)$_2$ Cl$_2$] isomers could be formed from 5(6)-methyl-2-phenoxymethylbenzimidazole upon coordination to platinum.

The IR spectra of the complexes showed some characteristic changes when compared to free ligands. The ligands showed broad bands in the region 3200–2300 cm^{-1} due to the intermolecular hydrogen bounded imidazole N–H. All the complexes exhibited N–H stretching bands ranging from 3550 cm^{-1} to 2500 cm^{-1}, sharper than those of the ligands due to breaking of tautomerism, indicating that the N–H group was not involved in the coordination [16, 17].

Pt–N and Pt–Cl vibrations are considered to be characteristic for dichloro-diamine platinum complexes. But in all cases the metal-nitrogen stretching bands could not be distinguished from other ring skeleton vibrations present in the spectra. According to the kinetic trans effect [18] the synthesis method used would be expected to yield

Table 1: ^{1}H NMR data of the ligands and their Pt(II) complexes

Compd.	CH_2	OCH_3	CH_3	NH	Ar–H
L^1	4.14 (s, 2 H)	3.77 (s, 3 H)	–	12.16 (s, 1 H)	7.54–7.36 (m, 2 H) 7.24 (d, J = 8.6 Hz, 2 H) 7.12–7.09 (m, 2 H) 6.89–6.86 (m, 2 H)
1	4.98 (s, 2 H) 4.86 (s, 2 H)	3.77 (s, 3 H) 3.70 (s, 3 H)	–	13.10 (s, 2 H)	8.46 (d, J = 8.6 Hz, 1 H) 8.20 (d, J = 7.5 Hz, 1 H) 7.71–7.07 (m, 10 H) 7.07–6.76 (m, 4 H)
L^2	4.12 (s, 2 H)	–	–	12.27 (brs, 1 H)	7.47–7.39 (m, 2 H) 7.34–7.22 (m, 4 H) 7.06 (dd, J = 3.1 and 6.7 Hz, 2 H)
2	4.52 (s, 4 H)	–	–	14.70 (brs, 2 H)	7.73 (dd, J = 3.1 and 6.0 Hz, 4 H) 7.49–7.41 (m, 12 H)
L^3	5.27 (s, 2 H)	–	2.40 (s, 3 H)	12.53 (s, 1 H)	7.33–7.29 (m, 4 H) 7.09–7.07 (m, 2 H) 7.01–6.95 (m, 2 H)
3	5.76 (s, 2 H) 5.53 (s, 2 H)	–	a	13.68 (s, 2 H)	7.65–7.63 (m, 2 H) 7.54–7.50 (m, 2 H) 7.45–7.11 (m, 10 H) 7.06–7.02 (m, 2 H)
L^4	–	3.90 (s, 3 H)	–	14.62 (brs, 1 H)	8.26 (d, J = 8.8 Hz, 2 H) 7.74 (dd, J = 3.1 and 6.0 Hz, 2 H) 7.44 (dd, J = 3.1 and 6.0 Hz, 2 H) 7.25 (d, J = 8.7 Hz, 2 H)
4	–	3.91 (s, 6 H)	–	14.94 (brs, 2 H)	8.22 (d, J = 8.5 Hz, 4 H) 7.79 (dd, J = 3.1 and 6.0 Hz, 4 H) 7.52–7.50 (m, 4 H) 7.29 (d, J = 8.7 Hz, 4 H)

[a] Obscured by solvent signals

complexes with *cis* geometry. In the far IR region of the complexes' spectra a new band appeared assigned to ν (Pt−Cl), centered at 320 cm^{-1} and characteristic for *cis*-configurated dichloro-Pt(II) complexes [19]. The broad band centered at 3480 cm^{-1} and 3550 cm^{-1} in the spectra of complexes **2** and **3** respectively was assigned to ν (O−H) of hydration water.

The other bands in the spectrum of each complex were similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

The ^{1}H NMR spectral data for the ligands and their Pt(II) complexes are presented in Table 1. The ^{1}H NMR spectrum of the Pt(II) complexes in dimethylsulfoxide-d_6 (DMSO-d_6) is indicative of complex formation. The spectra of the complexes showed considerable differences compared to those of the free ligands. The large downfield shifts in the imidazole N−H signal in the spectra of all complexes compared to those of their ligands are a result of an increase in the N−H acid character after platinum binding [20]. The chemical shift variation observed upon coordination ($\Delta\delta = \delta$ complex-δ free ligand) shows that most of the values are positive, indicating a decrease in electronic density on the 2-substituted-benzimidazole ligands with coordination to the platinum.

2.2. Mutagenicity

Four Pt(II) complexes having 2-substituted-benzimidazole ligands, which were synthesized as potential antitumor compounds on the basis of previous findings on some Pt(II) complexes of benzimidazole derivatives [5, 6], were investigated for their mutagenicity in *Salmonella* strains. It was the aim of the present study to eliminate the complexes which govern the mutagenic potency.

The mutagenic potential of the Pt(II) complexes synthesized was investigated by short-term bacterial mutagenicity in a reverse-mutation assay using *Salmonella typhimurium* frameshift strain TA 98 and S.t. TA 100 and TA 102, which carry mutations particularly sensitive to reversion by DNA base-pair substitution. The tests were performed in the absence of S9 rat liver fraction. Cisplatin was tested for comparison.

The direct-acting mutagenicity results of the compounds tested are summarized in Table 2.

Complex **2** bearing 2-(*p*-chlorobenzyl)benzimidazoles as "non-leaving ligands" caused a two or three fold increase in the number of revertants at 50 or 100 μg/plate respectively in TA 98. Complex **3** bearing 5(6)-methyl-2-phenoxymethylbenzimidazole ligands had the same degree of increase in the number of revertants at 10 or 25 μg/plate in TA 98. These complexes were also found to be mutagenic in TA 100 and TA 102. The TA 102 strain carries the his G 428 ochre (TTA) mutation [21] and detects as mutagens a variety of oxidants and other tester strains. The his G 428 mutation has A:T base pairs at the mutated site, while the other standard tester strains have G:C base pairs at the critical site. In particular, complex **3,** which caused a four fold increase in the number of revertants at 25 μg/plate, was found to be highly mutagenic in TA 102. This may be due to its high binding selectivity for AT sequences. The presence of a methoxy substituent on the *para* position of the benzyl group (complex **1**), turned out to be completely inactive in all test strains in the range of doses tested.

Complex **4** having *p*-methoxyphenylbenzimidazole ligands was found to be weakly mutagenic in TA 98, while no activity was evaluated in the TA 100 and TA 102 strains.

Cisplatin, by comparison, showed mutagenic response at extremely low doses (1 μg/plate) in TA 100 and a very low level of frame shift reversion in TA 98.

It was observed that the nature of the side groups on the benzimidazole ligands greatly influenced the mutagenic activity of the Pt(II) complexes. This is in agreement with reports in the literature on the mutagenicity of some benzimidazole derivatives [3, 22, 23], and may be explained by their different physicochemical properties.

Strain specificity indicates that the molecular mechanisms of mutagenicity of the complexes **2** and **3** involve both a frameshift and base-pair substitution process.

Although relationships between patterns of DNA alkylation and mutagenic potential are complex, DNA binding reactions of platinium complexes are usually viewed as kinetically controlled processes [24]. Steric hindrance of the "non-leaving ligands" can slow reaction kinetics [25]. The presence of bulky, planar amine ligands in [Pt(aninon)$_2$] complexes and their orientation with respect to the coordination plane, as well as their substituents, can reduce the rates of DNA binding compaired to aliphatic ammine and amine complexes [26]. The ligands used in this study are not flat due to the benzyl or phenoxymethyl moiety. This may result in severe steric constraints around the Pt atom, making the complexes less reactive than cisplatin. For the same reason Pt−Cl hydrolysis of the platinum complexes **1**–**4** may be relatively slow compared to cisplatin. A detailed study to determine the complex binding and hydrolysis rate constants of the complexes will be necessary to investigate these hypotheses.

Table 2: Mutagenic activities of the platinum complexes in *Salmonella typhimurium* TA 98, TA 100 and TA 102

Compd.	Dose (μg/plate)	Revertant colony numbers		
		TA 98 mean ± SD	TA100 mean ± SD	TA102 mean ± SD
Control	0	25.6 ± 6	97.7 ± 10.5	208.6 ± 52.9
1	5	16.4 ± 3.9*	82 ± 14.1	124.57 ± 23.1*
	10	15.6 ± 3.7*	71.6 ± 15.5*	79.14 ± 29.1*
	20	21.6 ± 5.4	100.1 ± 37.3	112 ± 49.3*
	40	18.1 ± 8.5*	80 ± 10.8	139.9 ± 25.3*
Control	0	115.7 ± 9.8	112.7 ± 9.2	223.4 ± 27.8
2	10	117.2 ± 18.2	109 ± 13.8	304.2 ± 45.4^
	25	167.6 ± 14.3*	148.2 ± 17.1^	302.4 ± 74.4
	50	220.6 ± 44.2*	136.8 ± 44	364 ± 122.8
	100	345.3 ± 29.5*	349 ± 114.1^	488.2 ± 162.2
Control	0	73.2 ± 15.5	57.8 ± 6.5	106.4 ± 38.7
3	2.5	77.4 ± 12	77.5 ± 11.5^	74.1 ± 47.6
	5	85 ± 12.4	87.4 ± 8.4^	107.2 ± 77.1
	10	138.9 ± 19.3^	161.3 ± 42^	379 ± 81*
	25	248.6 ± 64.8^	225.2 ± 16.6^	489.4 ± 125.6*
Control	0	37.2 ± 7.2	88.6 ± 8.6	234.5 ± 30.9
4	2.5	34.1 ± 5.7	92.7 ± 9.1	233.4 ± 29.3
	5	33.6 ± 8.6	91.7 ± 10.6	226.9 ± 15.5
	25	51.6 ± 6.9*	108.6 ± 12.5^	278.7 ± 47.8*
	50	64.2 ± 5.2*	115 ± 28.5	318.8 ± 33.4*
Cisplatin	0	20.2 ± 1.6	81 ± 11	
	1	31.6 ± 5.2*	195.2 ± 40*	
	2.5	33.2 ± 8.3*	269 ± 54.1*	
	5	39.5 ± 7.9*	222.5 ± 44.8*	
	10	30.1 ± 7.3	98.4 ± 65.3	

* $p < 0.05$ for Dunnett's t test
^ $p < 0.05$ for Dunnett's C test

The insolubility of the Pt(II) complexes in water compared to cisplatin, may also be related to their lower mutagenicity.

To determine whether the hydrophobic properties of the Pt(II) complexes influence their mutagenicity, theoretical calculations of lipophilicity of the ligands as logP were performed using the software program ACD logP. The LogP values of the ligands L^1–L^4 are 3.23 ± 0.25, 3.91 ± 0.23, 3.58 ± 0.25, 3.87 ± 0.3 respectively. If the complexes, in order of decreasing mutagenicity (**3** > **2** > **4** > **1**), are compared with the order of the logP values of the corresponding ligands ($L^2 > L^4 > L^3 > L^1$), except for complex **3** which has the lipophilic "non-leaving ligands" among the complexes tested and was found to be nonmutagenic, there seems to be no relationship between the mutagenicity and the hydrophobicity of the complexes. This result agrees with the conclusions of a study on the importance of the hydrophobic interaction in the mutagenicity of organic compounds, including some platinum complexes reported by Debnath et al. [27].

In conclusion, the lack of mutagenicity shown by Pt(II) complexes of 2-(*p*-methoxybenzyl) benzimidazole makes this Pt(II) complex worthy of antitumor activity studies. However, the requirement to confirm the nonmutagenicity of this compound with additional testing must also be taken into consideration.

3. Experimental

3.1. Equipment

Melting points were measured on a Electrothermal 9200 melting point apparatus and are uncorrected. Elemental analysis were performed by TÜBİTAK Laboratory (Ankara, Türkiye). All the results were in an acceptable range. IR spectra were recorded in KBr pellets on a Mattson 1000 FTIR spectrometer in the range of 4000–200 cm^{-1}. ^1H NMR spectra were recorded in DMSO-d_6 (Merck) on a Bruker 400-MHz spectromer. The chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, and spin multiplicity are given as s (singlet), brs (broad singlet), d (doublet), dd (doublet and doublet), or m (multiplet). All chemicals and solvents used were of reagent grade (Merck, Aldrich, Sigma) and were used without further purification. TLC was performed on precoated aluminum plates (Silicagel 60 F_{254}, Merck). Plates were visualized by UV light, Dragendorff reagent, and iodine vapor.

3.2. General procedure for the synthesis of the ligands

Four benzimidazole derivatives (L^1–L^4) used as "non-leaving ligands" in the structure of the Pt(II) complexes were synthesized by condensation of the corresponding carboxylic acid with the 1,2-phenylenediamine derivatives in the presence of 5 N hydrochloric acid (for L^1–L^3) or reaction of the sodium bisulfite adduct of p-anisaldehyde with 1,2-phenylenediamine in dimethylformamide (for L^4) as shown in the Scheme.

3.3. Synthesis of the platinum(II) complexes

3.3.1. Cis-[Pt(2-(p-methoxybenzyl)benzimidazole)$_2$Cl$_2$] (cis-[PtL$^1{}_2$Cl$_2$]) (**1**)

0.21 g (0.90 mmol) L^1 and 0.18 g (0.45 mmol) K_2PtCl_4 were dissolved in 5 mL DMF. The reaction mixture, protected from light, was heated at 50 °C for 12 days. Precipitated KCl was removed by filtration. A solution of 5% aqueous KCl (3 mL) was then added to the filtered solution and the mixture was stirred for 2 h. The resulting crude precipitate (approximately 0.217 g, 65% yield) was filtered off and purified by repeated washing with small portions of water, ethanol and diethylether and dried in vacuo. The yield of purified *cis*-[PtL$^1{}_2$Cl$_2$] was 9%, 0.030 g pure. IR (KBr) ν 317 (Pt–Cl) cm^{-1}.

$C_{30}H_{28}Cl_2N_4O_2Pt$

3.3.2. Cis-[Pt(2-(p-chlorobenzyl)benzimidazole)$_2$Cl$_2$] · 4 H$_2$O, (cis-[PtL$^2{}_2$ Cl$_2$] · 4 H$_2$O) (**2**)

To a stirred solution of L^2 (0.20 g, 0.80 mmol) in 0.5 N HCl (5 mL) was added a solution of K_2PtCl_4 (0.17 g, 0.40 mmol) in 0.5 N HCl (3 mL) dropwise over 30 min at room temperature. The reaction mixture, protected from light, was heated at 60 °C for 24 h, after which time the mixture was kept in the refrigerator at 4 °C for 12 h. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol and diethylether and dried in vacuo. The yield of purified *cis*-[PtL$^2{}_2$Cl$_2$] · 4 H_2O was 17%, 0.050 g pure. IR (KBr) ν 320 (Pt–Cl) cm^{-1}.

$C_{28}H_{22}Cl_4N_4Pt \cdot 4\ H_2O$

3.3.3. Cis-[Pt(5(6)-methyl-2-(phenoxymethyl)benzimidazole)$_2$Cl$_2$] · 2 H$_2$O (cis-[PtL$^3{}_2$ Cl$_2$] · 2 H$_2$O) (**3**)

A similar procedure was carried out as described for complex **2** using L^3 (0.05 g, 0.20 mmol) and K_2PtCl_4 (0.05 g, 0.20 mmol) at 60 °C for 2 days. Yield: 48%, 0.040 g pure. IR (KBr) ν 321 (Pt–Cl) cm^{-1}.

$C_{30}H_{28}Cl_2N_4O_2Pt \cdot 2\ H_2O$

3.3.4. Cis-[Pt(2-(p-methoxyphenyl)benzimidazole)$_2$Cl$_2$] (cis-[PtL$^4{}_2$Cl$_2$]) (**4**)

A similar procedure was carried out as described for complex **1** using L^4 (0.35 g, 1.60 mmol) and K_2PtCl_4 (0.32 g, 0.80 mmol) at 60 °C for 2 days. Yield: 11%, 0.02 g pure. IR (KBr) ν 327, 312 (Pt–Cl) cm^{-1}.

$C_{28}H_{24}Cl_2N_4O_2Pt$

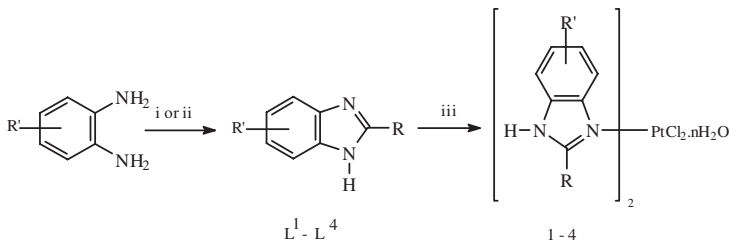
3.4. Bacterial mutagenicity assay

3.4.1. Material and Methods

Chemicals used were obtained from the following sources: sodium azide and D-biotin (Sigma Chemical Co., St. Louis, USA), daunomicine (Deva Holding A.Ş), L-histidin-HCl monohydrate (BDH), Bacto agar and oxoid nutrient broth No: 2 (Oxoid).

Tester strains: *S. typhimurium* TA 98 (his D 3052, rfa, Δuvr B, pKM 101), TA 100 (his G 46, Δuvr B, pKM 101) and TA 102 (PA Q1, his G 428, Δhis, rfa, pKM 101) were kindly provided by Dr. Bruce Ames (University of California, Berkeley Ca., U.S.A.). All strains were stored at −80 °C and were routinely checked to ensure the presence of appropriate genetic markers and spontaneous reversion patterns. The positive control used was sodium azide for *S. typhimurium* TA 100 and daunomicine for *S. typhimurium* TA 98 and TA 102. Overnight growth was initiated with inoculation from the master plate into Oxoid-nutrient broth No: 2. Following overnight growth, all tester strains were diluted into the same culture

Scheme



L^1 - L^4 1 - 4

Ligand	Complex	R′	R	n
L^1	1	H	$CH_2C_6H_4$-p-OCH_3	0
L^2	2	H	$CH_2C_6H_4$-p-Cl	4
L^3	3	CH_3	$CH_2OC_6H_5$	2
L^4	4	H	C_6H_4-p-OCH_3	0

Reagants: (i) RCOOH, 5 N HCl, reflux (for L^1–L^3) ; (ii) R–CHO, $NaHSO_3$, DMF, reflux (for L^4); (iii) K_2PtCl_4, 0.5 N HCl or DMF

and grown with shaking at 37 °C (approximately 5 h). When cultures reached a density of 0.300 OD at 650 nm $1-2.10^9$ cells/mL, they were used in the mutagenicity experiments [28].

3.4.2. Cytotoxicity assay

The amouts of test compouds to be used in the mutation assays were selected by a cytotoxicity assay. 0.1 mL of a suitable dilution of an overnight bacterial culture was added to 2 mL top agar together with a different concentration of the compounds in dimethylsulfoxide. The top agar was poured onto nutrient agar plates and asssesment of cytotoxicity was made after 24 h incubation at 37 °C [29].

3.4.3. Mutagenicity assay

The method used was basically as described by Maron and Ames [28]. Briefly 0.1 mL of bacterial tester strain and the sample to be tested (in freshly prepared dimethylsulfoxide solution) were added to 2 mL of molten top agar. The contents were mixed and poured on agar plates. After 2–3 days of incubation, revertant colonies were counted. At least ten plates were used for each dose. The strains were checked routinuely for ampicillin resistance, ultraviolet-light sensitivity, crystal violet sensitivity, histidine requirement and spontaneous reversion rate. They were stored at −80 °C. In the *Salmonella*/microsome test system, data are interpreted on the basis of a consistent doubling of the spontaneous reversion frequency confirmed by a dose-response relationship. Where the number of induced revertants is less than twice the spontaneous rate but a reproducible dose-related increase in revertants is detected, this is also interpreted as a positive response [29]. The concentrations reported were designed to include the highest non-toxic dose. All Pt(II) complexes were tested in three independent experiments.

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