Synthesis, Characterization and Genotoxicity of Platinum(II)Complexes With Subtituted Benzimidazole Ligands

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Five Pt(II) complexes with the ligands 2-ethyl-/ or-benzyl-/ or-phenoxymethyl- and 1-methyl-2-hydroxymethyl- and 1-methyl-2-phenyl-benzimidazoles were synthesized and characterized by elemental analysis, and IR and ^{1}H-NMR spectra and evaluated for their in vitro genotoxic activities by Rec-Assay test. Based on the data obtained in this study, the Pt(II) complexes tested might be taken into consideration as promising antitumor compounds.

Key Words: Benzimidazol, Platinum(II) complexes, In vitro genotoxic activity, Rec-Assay test.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] is one of the most commonly used anticancer drugs in the treatment of testicular, ovarian, bladder and head and neck cancers[1]. Despite the great success in treating certain kinds of cancer there are several side effects, and both intrinsic and acquired resistance limit the organotropic profile of the drug[2].

There is continuing interest in the development of new platinum complexes that are less toxic and have a broader spectrum of activity. Variations in the nature of the amine can have a significant effect on the activity and toxicity of these complexes. Several platinum complexes with N-heterocyclic ligands such as imidazol, thiazole, benzimidazole, benzoxazole and benzothiazole have been reported[3−12]. Some of these platinum complexes showed significant cytotoxicity[3,4,7−12].

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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, and research in this area has predominated[2].

It is now generally accepted that platinum(II) antitumor agents exert their activity by reacting with the nucleobases of cellular DNA and that tumor cells, being deficient in DNA repair capability, are more sensitive than normal cells to cisplatin[13].

Research conducted with bacterial systems has shown that compounds that cause DNA damage and induce the DNA repair system could also be promising as antitumoral agents in mammals, including humans, as repair deficient persons exhibit defects in their ability to repair damaged DNA and thus are prone to developing cancer[14,15].

In a previous paper, we reported the synthesis and characterization of complexes of the structure *cis*-[Pt(L$_2$)Cl$_2$].H$_2$O, where L is 5(6)-non/or- chlorosubstituted-2-hydroxymethyl-benzimidazole, and the determination of their preliminary in vitro cytotoxic effects by Rec-Assay test[8]. The DNA-binding properties of these 2 Pt(II) complexes were also examined and it was determined that the DNA platinated with these compounds was specifically recognized by the high mobility group (HMG) domain protein HMG1[9]. It was concluded that the adducts formed by the compounds distort the DNA in a manner similar to cisplatin diadducts. It was also determined that some of the new 2-substituted benzimidazole Pt(II) complexes we synthesized have in vitro cytotoxic effects on the human RD Rhabdomyosarcoma[16], and MCF-7 breast cancer[17] cell lines.

In this study, 5 Pt(II) complexes with the ligands 2-ethyl/ or-benzyl/ or-phenoxymethyl and 1-methyl-2-hydroxymethyl and 1-methyl-2-phenylbenzimidazole were synthesized. The DNA damaging characteristics and the interaction with the DNA repair system of the Pt(II) complexes were determined on *Escherichia coli* LE 392 (RecA$^+$) and JM 109 (RecA$^-$) strains.

Experimental

Chemistry

Materials

All chemicals and solvents used were of reagent grade (Merck or Aldrich), and were used without further purification. Thin layer chromotographic (TLC) analyses were performed on pre-coated aluminum plates (silicagel 60 F$_{254}$, Merck). TLC spots were visualized with UV light, Dragendorff reagent or iodine vapor.

Melting points were measured on a Electrothermal 9200 melting point apparatus and are uncorrected. Elemental analyses were performed by TÜBİTAK Laboratory (Ankara, Turkey). Infrared (IR) spectra were recorded in KBr pellets and in Nujol mulls on a Mattson 1000 FTIR spectrometer in the range 4000-200 cm^{-1}. For the region 400-200 cm^{-1}, the samples were prepared as Nujol mulls on CsI windows. Proton magnetic resonance (^{1}H-NMR) spectra were recorded in DMSO-d$_6$ (Merck) on a Bruker 400 MHz spectrometer.

Synthesis of the ligands

The ligands **L1-L5** were synthesized according to the reported methods[18−21] as shown in the Figure.

L1(Ebim): ^{1}H NMR (DMSO-d_6) δ ppm, 1.22 (3H, t, J=7.6 Hz, CH_3), 2.73 (2H, q, J=7.6 Hz, CH_2), 7.00 (2H, dd, J=9.4 and 3.2 Hz, aromatic H), 7.36 (2H, m, aromatic H), 12.40 (1H, broad s, –NH).

L2(Bbim): ^{1}H NMR (DMSO-d_6) δ ppm, 4.17 (2H, s, CH_2), 7.12 (2H, dd, J=9.4 and 3.1 Hz, aromatic H), 7.21-7.25 (1H, m, aromatic H), 7.29-7.37 (4H, m, aromatic H), 7.45-7.50 (2H, m, aromatic H), 12.50 (1H, broad s, –NH).

L3(Phmbim):^{1}H NMR (DMSO-d_6) δ ppm, 5.31 (2H, s, CH_2), 6.96-6.99 (1H, m, aromatic H), 7.09 (2H, d, J=8.2 Hz, aromatic H), 7.17-7.20 (2H, m, aromatic H), 7.30-7.34 (2H, m, aromatic H), 7.54-7.56 (2H, m, aromatic H), 12.50 (1H, broad s, –NH).

L4(Mphbim):^{1}H NMR (DMSO-d_6) δ ppm, 3.88 (3H, s, N-CH_3), 7.27 (2H, dtd, J=10.4, 7.2 and 1.2 Hz, aromatic H), 7.55-7.62 (4H, m, aromatic H), 7.69 (2H, d, J=7.4 Hz, aromatic H), 7.84-7.87 (2H, m, aromatic H).

L5(Mhmbim):^{1}H NMR (DMSO-d_6) δ ppm, 3.83 (3H, s, N-CH_3), 4.72 (2H, s, CH_2), 5.52 (1H, s, O-H), 7.18 (2H, dtd, J=7.2, 6.8 and 1.1 Hz, aromatic H), 7.52 (1H, d, J=7.8 Hz, aromatic H), 7.58 (1H, d, J=7.8 Hz, aromatic H).

Synthesis of the platinum(II) complexes

cis-[Dichloro-di(2-ethylbenzimidazole)platinum(II)] [Pt(Ebim)$_2$Cl$_2$] (C1).

To a stirred solution of L1(Ebim) (0.292 g, 2 mmol) in ethanol-water mixture (5/20 mL) was added an aqueous solution of K_2PtCl_4 (0.415 g, 1 mmol in 5 ml H_2O) dropwise over 30 min at room temparature. The pH was adjusted to ∼ 8 and kept constant with the addition of 0.1 M KOH. The reaction mixture, protected from light, was heated at 60 ^{o}C for 11 days. The mixture was cooled to 5 ^{o}C. The resulting precipitate was filtered off, washed several times with small portions of water, ethanol, and diethylether and dried in vacuo. Yield: 56% , 0.323 g pure. Anal. Calc. for $C_{18}H_{20}Cl_2N_4Pt$: C, 38.71; H, 3.60; N ,10.03. Found: C, 38.01; H, 3.66; N, 9.40% . IR (KBr): 3193 (N-H), 1620 (C=N), 320 (Pt-Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ ppm, 1.05-1.70 (6H, m, 2x CH_3), 3.48-3.60 (4H, m, 2x CH_2), 6.91-8.40 (8H, m, aromatic H), 12.59 (2H, broad s, 2x –NH).

cis-[Dichloro-di(2-benzylbenzimidazole)platinum(II)] [Pt(Bbim)$_2$Cl$_2$] (C2).

A similar procedure was carried out using L2(Bbim) (0.288 g, 1 mmol) and K_2PtCl_4 (0.208 g, 0.5 mmol) at 60 ^{o}C for 10 days. Yield: 36% , 0.180 g pure. Anal. Calc. for $C_{28}H_{24}Cl_2N_4Pt$: C, 49.27; H, 3.54; N, 8.20. Found: C, 49.04; H, 3.61; N, 8.00% . IR (KBr): 3236 (N-H), 1625 (C=N), 320 (Pt-Cl) cm^{-1}.^{1}H NMR (DMSO-d_6) δ ppm, 4.37-4.53 (1H, dd, J=15.7 and 45.47 Hz, and J_{Pt-H}= 45.5 Hz, -C-H), 4.75-4.79 (1H, d, J=15.7 Hz,-C-H), 4.93-5.05 (2H, m, -CH_2), 7.17-8.48 (18H, m, aromatic H), 12.89-13.18 (2H, t, J_{N-H}= 52.02 and 60.29 Hz, 2x NH).

cis-[Dichloro-di(2-phenoxymethylbenzimidazole)platinum(II)][Pt(Phmbim)$_2$Cl$_2$] (C3).

A similar procedure was carried out using L3(Phmbim) (0.196 g, 0.8 mmol) and K_2PtCl_4 (0.166 g, 0.4 mmol) at 60 ^{o}C for 11 days. Yield: 56% , 0.200 g pure. Anal. Calc. for $C_{28}H_{24}Cl_2N_4O_2Pt$: C, 47.06; H, 3.39; N, 7.84. Found: C, 46.60; H, 3.88; N, 7.49% . IR (KBr): 3350 (N-H), 1625 (C=N), 328 (Pt-Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ ppm, 5.31-5.43 (4H, m, 2xCH_2), 6.76-7.32 (18H, m, aromatic H), 12.60 (2H, broad s, 2x NH).

cis-[Dichloro-di(1-methyl-2-phenylbenzimidazole)platinum(II)] [Pt(Mphbim)$_2$Cl$_2$] (C4).

A similar procedure was carried out using L4(Mphbim) (0.324 g, 2 mmol) and K$_2$PtCl$_4$ (0.415 g, 1 mmol) at 60 ^{o}C for 48 h. Yield: 51% , 0.350 g pure. Anal. Calc. for C$_{28}$H$_{24}$Cl$_2$N$_4$Pt: C, 49.27; H, 3.54; N, 8.20. Found: C, 50.88; H, 3.31; N, 8.65% . IR (KBr): 3040 (O-H), 1625 (C=N), 325, 307 (Pt-Cl) cm^{-1}.^{1}H NMR (DMSO-d$_6$) δ ppm, 3.88 and 3.99 (6H, s and s, anti N-CH$_3$, and syn N-CH$_3$), 7.10-8.19 (18H, m, aromatic H).

cis-[Dichloro-(1-methyl-2-hydroxymethylbenzimidazole)platinum(II)] [Pt(Mhmbim)Cl$_2$] (C5).

A similar procedure was carried out using L5(Mhmbim) (0.416 g, 2 mmol) and K$_2$PtCl$_4$ (0.415 g, 1 mmol) at 60 ^{o}C for 12 days. Yield: 64% , 0.226 g pure. Anal. Calc. for C$_9$H$_9$Cl$_2$N$_2$OPt: C, 25.30; H, 2.12; N, 6.55. Found: C, 25.25; H, 2.14; N, 6.52% . IR (KBr): 3650-2300 (O-H, =C-H, -C-H), 1676 (C=N), 325 (Pt-Cl) cm^{-1}.^{1}H NMR (DMSO-d$_6$) δ ppm, 3.10-4.50 (5H, m, N-CH$_3$, -CH$_2$), 5.35 (1H, s, -O-H), 6.60-8.50 (4H, m, aromatic H).

Genotoxicity

DNA-Damaging Activity: Rec-Assay test

E. coli LE 392 (RecA$^+$) and JM 109 (RecA$^-$) strains were obtained from New England Biolabs MA, USA.

Two strains of *E. coli*, RecA$^+$and RecA$^-$, were grown to the exponential phase in LB medium (tryptone 10 g, yeast extract 5 g, NaCl 10 g, agar agar 12 g, L. pH: 7.0 ± 0.2) at 37 ^{o}C overnight with aeration. Ten microliters of the culture was then plated on LB agar. After overnight incubation at 37 ^{o}C with aeration, single colonies of each strain were grown overnight to the stationary phase in LB medium.

The tested compounds were dissolved in dimethylsulphoxide (DMSO) (Merck) at the concentration examined. *E. coli* strains were diluted with compound-free LB medium. The cells were incubated for a further 2 h at 37 ^{o}C.

The percentage survival of the cells was determined with respect to the cells not treated with compounds. For counting, 90 μL of LB medium was placed into each well of microplates, and then 10 μL of each solution was added to the first rows. Tenfold dilutions (10^{-1},10^{-2}, 10^{-3}, 10^{-4}) were made by dispensing the solution into the remaining wells. The cells not treated with the compounds underwent the same applications. Twenty microliters of each dilution from the last rows of the microplates was inoculated on the dry surface of LB agar. The plates were incubated at 37 ^{o}C for 24 h. At the end of this period the numbers of surviving colonies were counted. The values of the RecA$^+$ strain were compared with those of the RecA$^-$ strain. Cell survival at different doses of the compounds was expressed as a percentage with respect to culture not treated. The tests were prepared in triplicate, and cisplatin used as a standard.

Results and Discussion

Synthesis and characterization of the complexes

Five mono- or disubstituted benzimidazole ligands (**L1-L5**) used as "non-leaving ligands" in the structure of the Pt(II) complexes (**C1-C5**) were synthesized as shown in the Figure, and their melting points were in accordance with the literature[18−21].

The 5 new Pt(II) complexes (**C1-C5**) were synthesized by the reaction of the ligands with K_2PtCl_4 in ethanol-water solution as shown in the Figure. The melting points of all complexes were above 400 ^oC.
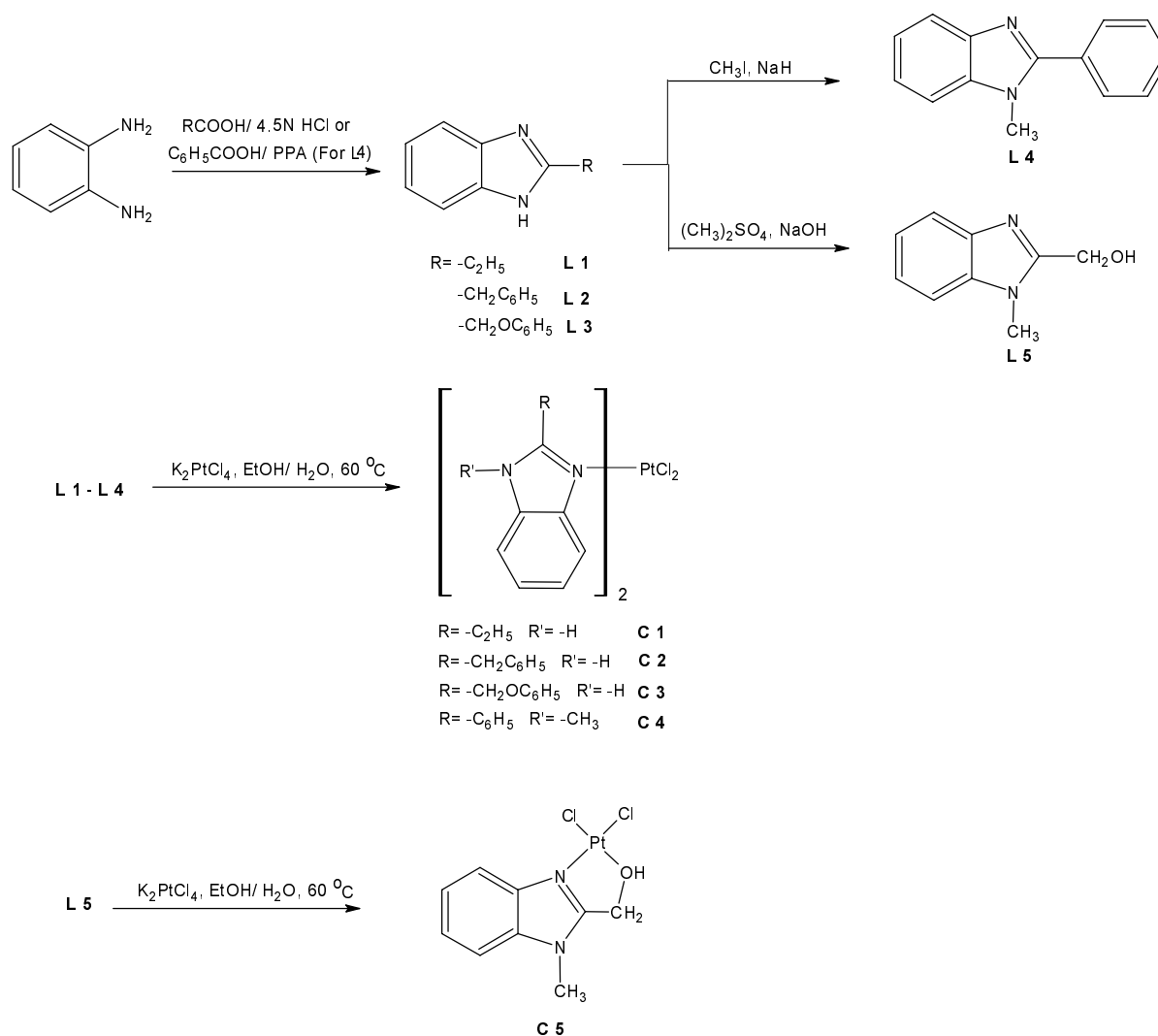
Figure. Synthesis of the ligands and their Pt(II) complexes.

The complexes obtained were characterized by their elemental analyses and IR and ^{1}H-NMR spectra. Although the ligands synthesized in this study were reported previously by other researchers and the melting points of the ligands (**L1-L5**) agree with the literature for the characterization of the complexes in comparison with the data of the ligands, IR and ^{1}H-NMR data of the ligands were also obtained. ^{1}H-NMR data of the ligands were presented in the Experimental section.

Elemental analyses suggested a 1:2 (metal:ligand) stoichiometry for Pt(II) complexes **C1-C4** and 1:1 stoichiometry for **C5**.

When the IR spectra of the free ligands and the related complexes are compared, some characteristic changes are seen. The ligands, except **L4** and **L5**, show broad bands in the region 3200-2200 due to the intermolecular hydrogen bonded imidazole N-H. The complexes that have free imidazole N-H exhibit N-H stretching bands ranging from 3350 to 3193 cm^{-1}, which become sharper than those of the ligands due to the breaking of tautomerism, indicating that imidazoles N-H were not involved in the coordination[22,23].

The strong bands at around 1616 cm^{-1} in the free ligands assigned to C=N stretches shifted (2-26 cm^{-1}) towards higher frequencies in the spectra of complexes, suggesting that the tertiary nitrogen of the benzimidazole was coordinated to the metal ion[24−26].

According to the kinetic trans effect[27], the synthesis method used is expected to yield complexes with cis geometry. In the far-IR region of the spectra of all the complexes appeared a new broad band assigned to v(Pt-Cl) centered around 330-307 cm^{-1} characteristic for *cis*-configurated dichloro-Pt(II) complexes[28]. It is well known that *cis*-dichloro complexes should show 2 bands of medium intensity, because the vibrations are additive, but in many cases the second band is only a shoulder. In some cases, *cis*-dichloro complexes show only one band due to low resolutions independent of *cis* or *trans* configurations[29]. Although no shoulder was observable at the Pt-Cl stretching band of the complexes **C1-C3**, and **C5** the broad nature of the band suggested the presence of bands overlapped in this domain. In addition, the position of the (Pt-Cl) band of the complexes was at 328-320 cm^{-1} and the geometry can be assigned as *cis*.

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 insolubility of the complexes in the other organic solvents made it necessary to record ^{1}H-NMR spectra in dimethylsulfoxide-d$_6$ (DMSO-d$_6$). All ^{1}H-NMR measurements were recorded immediately in order to avoid ligand exchange reactions between the Pt(II) complexes and DMSO-d$_6$. The ^{1}H-NMR spectrum of the Pt(II) complexes in DMSO-d$_6$ is indicative of complex formation. The spectra of the complexes compared to those of the free ligands showed considerable differences. The large downfield shift in the imidazole N-H signal in the spectra of all complexes with respect to their ligands is a result of an increase in the N-H acid character after platinum binding[30]. The chemical shift variation upon coordination ($\Delta\delta = \delta$ complex $- \delta$ free ligand) observed shows that most of the values are positive, indicating a decrease in electronic density on the 2-substituted benzimidazole ligands with coordination to platinum[30].

For complex **C5** with 1-methyl-2-hydroxymethylbenzimidazole ligand, syn- and anti-rotamers were observed in ^{1}H-NMR in the ratio 1:1.

Genotoxicity

Synthesized platinum complexes were investigated for their potential in vitro genotoxicity by evaluating DNA - damage in an E. coli Rec - Assay test.

General genetic recombination is a process, common to all forms of life, by which new combinations of genetic material or nucleic acid sequences are generated by RecA. RecA is a specific cofactor in the cleavage of the repressor related to SOS induction in E. coli and plays a central role in several other homologous processes in bacteria[31−33].

Cisplatin, which is widely used as a cancer chemotherapeutic drug, causes DNA damage and induces

Table. In vitro genotoxic activity of the complexes by Rec-Assay test.

Complex Number		E. coli Rec⁻				E. coli Rec⁺				DNA-damaging activity Rec⁺⁰%/Rec⁻% (at conc. 100 mM)
		Concentration (mM)				Concentration (mM)				
		200	100	50	25	200	100	50	25	
C1	Live m.[a] number	21±4	118±13	189±15	300±15	460±29	500±16	864±94	1200±78	
	Live m. %	1	7	15	34	27	32	56	96	4.57
C2	Live m. number	53±2	110±4	320±2	530±11	860±65	910±70	981±184	1149±128	
	Live m. %	3	7	25	60	53	59	65	80	8.42
C3	Live m. number	17±2	45±4	73±18	730±3	218±13	293±48	487±86	1285±4	
	Live m. %	1	3	6	83	14	19	32	89	6.33
C4	Live m. number	20±3	124±1	241±28	342±42	165±25	270±5	640±6	1264±28	
	Live m. %	1	8	19	39	10	18	43	88	2.25
C5	Live m. number	35±4	50±4	208±55	261±252	477±76	637±90	650±9	750±37	
	Live m. %	2	3	17	30	28	41	42	60	13.6
		Concentration (μM)				Concentration (μM)				Rec⁺⁰%/Rec⁻% (at conc. 400 μM)
		400	200	100	50	400	200	100	50	
Cisplatin	Live m. number	10±2	140±6	180±5	257±3	50±4	570±4	610±6	670±19	
	Live m. %	2	45	63	93	4	49	67	95	2

[a]m.: microorganism

the RecA gene dependent repair system in bacteria[34,35].

The Rec - Assay is a simple and efficient procedure for screening environmental DNA - damaging chemicals[36].

In this study, complexes **C1-C5** were tested for each concentration and cells in duplicate and the entire experiments were repeated to confirm the results on a second occasion using the same testing conditions in all steps of the experiment prepared on another day.

The data were analyzed by Kruskal-Wallis test on a computer. The interaction with the DNA and the ability to induce the DNA repair system of the 5 platinum(II) complexes **C1-C5** were determinated on *E. coli* RecA$^+$ and RecA$^-$ strains as a preliminary indication of their antitumor acitivity.

The survival of *E. coli* cells treated with cisplatin, which was used as a control agent, was lower in the strains with mutations in their recA locus (RecA$^-$) (strains with deficent DNA repair systems) than in non-mutant strains (Rec A$^+$) (strains with efficient DNA repair systems) (Table).

The survival of cells treated with complexes C1-C5 was low in repair deficient *E. coli* cells (Rec A$^-$) when compared to *E. coli* (RecA$^+$) cells. In vitro test results suggested that the repair of DNA damage produced by the complexes depends on the RecA protein. Complexes C2, C3 and C5 induced the RecA dependent the DNA repair system potentially more than the other complexes. Although the platinum(II) complexes tested were not as active as cisplatin in low doses they might be considered promising antitumor agents.

Conclusion

In general, the platinum(II) complexes with substituted benzimidazole ligands, which were found to be less genotoxic than cisplatin, exhibited moderate genotoxicity. In conclusion, complexes **C1-C5** should be considered for further antitumor activity studies.

Acknowledgments

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