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Research Article

Novel Palladium(II) and Platinum(II) Complexes with a Fluoropiperazinyl Based Ligand Exhibiting High Cytotoxicity and Anticancer Activity *In Vitro*

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cis-Dichloro-palladium(II) and *cis*-dichloro-platinum(II) complexes (**2**, **4**) of the general formula $[M(N\text{-}N)Cl_2]$ (M=Pd(II) and Pt(II), N-N= 1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl) benzene, (DFMPB)) and the dicationic palladium(II) complex $[Pd(N\text{-}N)(CH_3CN)_2](BF_4)_2$ (**3**) have been prepared and characterized by elemental analysis, ^{1}H-NMR-, mass spectroscopy, and IR spectroscopy. The cytotoxic effect of these complexes against MDA-231 and MCF-7 human breast cancer cell lines and K562 human leukemia cell line has been studied. The influence was dose dependent and varies with cell type. The palladium(II) complex (**2**) showed superior cytotoxic effect compared with the corresponding platinum(II) complex and the standard, cisplatin, when tested against all the above cell lines.

1. Introduction

The similarity between the coordination chemistry of palladium(II) and platinum(II) compounds has led to a large research effort towards Pd(II) antitumor drugs that are efficient against Pt(II) resistant therapies and have less side effects [1]. A key factor that might explain why platinum is most useful comes from the ligand-exchange kinetics. The hydrolysis in palladium complexes is too rapid, 10^5 times faster than that of the corresponding platinum analogues [2]. These complexes dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets. Accordingly, compared to cisplatin, the corresponding *cis*-palladium, *cis*-$[Pd(NH_3)_2Cl_2]$, does not show antitumor activity [3, 4]. Therefore, if an antitumor palladium complex is to be designed, it must be stabilized by a chelate or a strongly coordinating nitrogen ligand [5].

Studies of platinum and palladium compounds with biologically active carriers have yielded promising results in the field of anticancer chemistry and there is potential for varying the biological activity of these complexes by changing the structure of the carrier [6, 7]. Significant advances have emerged from this methodology of design [8–10].

Previously, we reported the synthesis and molecular structure of an enantiomerically pure, *trans*-palladium(II) complex, *trans*-$[Pd\{(R)\text{-}(+)\text{-bornyl-amine}\}_2Cl_2]$ that bears the bulky amine ligand R-(+)-bornylamine (*endo*-(1R)-1,7,7-trimethylbicyclo[2-2-1]-heptan-2-amine) [11]. The complex showed similar antitumor activity against HeLa cells when compared with the activity of the standard references, cisplatin, carboplatin, and oxaliplatin. In addition, a palladium complex which contains the bulky nitrogen ligand harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole), *trans*-$[Pd(harmine)(DMSO)Cl_2]$, exhibited a greater cytotoxic activity against P388, L1210, and K562 cell lines than cisplatin [12, 13].

Connors et al. [14] and Meischen et al. [15] have reported different platinum(II) complexes with aromatic amines, such

as *cis*-dichloro(4-chloro-1,2-phenylenediamine)platinum(II) and *cis*-dichloro(1,2-phenylenediamine)platinum(II). Although these complexes were less active than cisplatin, they have showed relevant biological activity against the L1210 leukemic cell lines. de Almeida et al. also reported that platinum(II) complexes with ligands derived from 1,2-phenylenediamine have potential cytotoxicity [16]. The evaluated compounds were less active *in vitro* than cisplatin. Cytotoxic evaluation results suggested that the presence of the strong electron-withdrawing group in the aromatic ring lead to a decrease in the cytotoxicity against human cancer cell lines such as MCF7 and EVSAT (mammary cancers), WiDr (colon cancer), and H226 (lung cancer).

4-Fluoro-5-(4-piperazinyl)-1,2-diaminobenzene has recently been proved to be a valuable intermediate for the synthesis of various benzimidazole derivatives of biological interest, for example, as anticancer agents and bactericides [17]. Furthermore, ferrocenyl-based complexes with 5-fluoro-6-(4-substituted-1-piperazinyl)benzimidazoles have been reported. These complexes were shown to have potency comparable to that of azole-based antifungal agents (miconazole) [18]. It seems that the benzimidazoles with both fluorine and piperazine as substituents lead to a considerable enhancement of the antibacterial potency [19]. The substituted 1-piperazinyl derivative belongs to a group of DNA binding fluorochromes used in chromosome staining and some of them exhibit antihistaminic activity [20, 21].

It was proposed that a combination between Pd(II) or Pt(II) and 4-fluoro-5-(4-piperazinyl)-1,2-diaminobenzene could led to the formation of compounds with potent antitumor activity. To our knowledge, as we are aware, no study of palladium(II) and platinum(II) complexes containing 4-fluoro-5-(4-piperazinyl)-1,2-diaminobenzene was reported.

As an extension of our studies on both the coordination chemistry of heteroatom containing ligands [22] and the biological activity [23–25] of their metal complexes, we describe here the synthesis and characterization of new square-planar platinum(II) and palladium(II) complexes bearing the bidentate chelate, 1,2-diamino-4-fluoro-5-(4-methyl)-1-piperazinyl benzene (DFMPB). For comparison purposes, the biological activity of the corresponding water soluble, dicationic, diacetonitrile palladium(II) complex was also investigated. The aim of our study was to investigate the influence of DFMPB as a biologically active carrier on the cytotoxic properties of the platinum(II) and palladium(II) complexes against MDA-231 and MCF-7 human breast cancer cell lines and K562 leukemia cell line.

2. Experimental

2.1. Materials and Instrumentation. The complex $[Pd(CH_3CN)_4](BF_4)_2$ was purchased from Aldrich. Reagent grade chemicals were used as received unless otherwise stated. 1,2-Diamino-4-fluoro-5-(4-methyl-1-piperazinyl) benzene was prepared as previously described [26]: (M.p.) 97°C; IR (KBr, cm^{-1}): ν = 3382 (mbr), 3224 (m), 2943 (m), 2811 (w), 1634 (m), and 1523 (s); ^{1}H NMR (ppm, DMSO-d_6): δ = 6.27 (m, H arom.2H), 2.78 (br, CH_2CH_2, 4H), 2.41 (br, CH_2CH_2, 4H), and 2.19 (s, CH_3, 3H); MS (EI) (%): 225 (M^+, 100).

Elemental analyses were performed using a EURO EA 3000 instrument. ^{1}H-NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz using DMSO-d_6 as a solvent with TMS as an internal standard. Infrared spectra (KBr discs) were measured on a Nicolet-Magna-IR 560 Spectrophotometer. Mass spectra (EI) were acquired using a Shimadzu-QP5050A. Melting points were measured by a Stuart Scientific melting Apparatus (uncorrected ±0.1°C).

2.2. Synthesis of Complexes

*2.2.1. cis-Dichloro(1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl)benzene)-palladium(II) ([Pd(DFMPB)Cl$_2$], **2**).* A filtered solution of the ligand (**1**) (0.065 g, 0.29 mmol) in acetone (30 mL) was added to a solution of $[Pd(PhCN)_2Cl_2]$ (0.50 g, 1.30 mmol) in acetone (50 mL) with continuous stirring. Upon addition, an orange solid was formed. After 5 h stirring, the precipitate was filtered, washed with acetone (2 × 5 mL) and Et_2O (2 × 10 mL), and dried in vacuum.

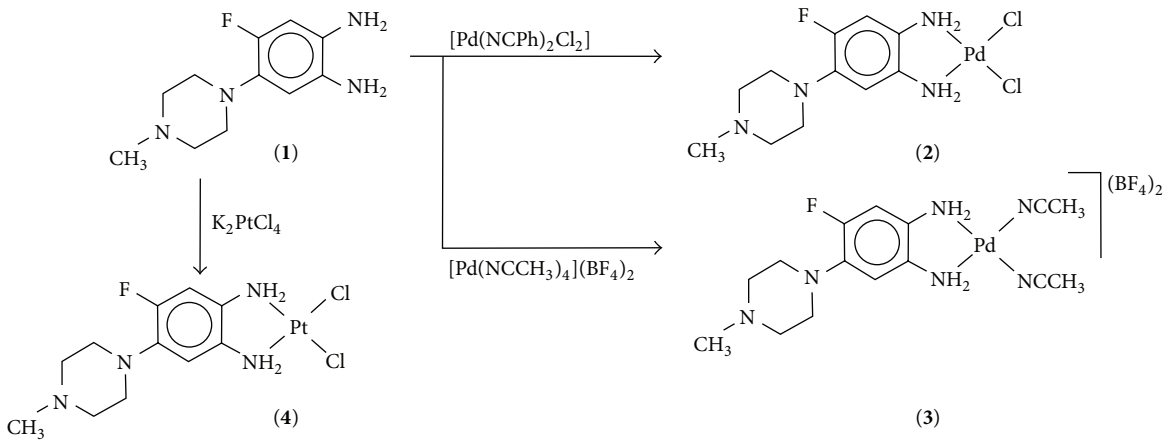
Yield of 0.41 g (78%). M.p. (dec.) 230°C. Found: C, 33.63; H, 4.54; N, 13.75. Anal. Calc. for $C_{11}H_{17}N_4FPdCl_2$: C, 32.89; H, 4.27; N, 13.95. IR (KBr, cm^{-1}): ν = 3389 (mbr), 3181 (m), 3037 (m), 2725 (w), 1620 (w), 1513 (s). MS (EI) (%): 403 (M^+, 10), 225 (M^+-$PdCl_2$, 45).

*2.2.2. cis-Diacetonitrile(1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl) benzene) palladium(II) bis(tetraflouroborate) ([Pd(DFMPB)(CH$_3$CN)$_2$](BF$_4$)$_2$), **3**).* To a solution of $[Pd(CH_3CN)_4](BF_4)_2$ (0.39 g, 0.88 mmol) in acetonitrile (3 mL) was added a filtered solution of the ligand (**1**) (0.88 mmol) in acetonitrile (4 mL) with continuous stirring at room temperature. Upon addition, a dark orange solution was formed. After 5 h stirring, the solvent was evaporated to dryness and the isolated product was washed with Et_2O (2 × 5 mL) and dried in vacuum.

Yield of 0.45 g (87%). M.p. (dec.) 210°C. Found: C, 27.60; H, 3.80; N, 12.24. Anal. Calc. for $C_{15}H_{23}N_6F_9PdB_2{\cdot}4H_2O$: C, 27.36; H, 3.52; N, 12.76. IR (KBr, cm^{-1}): ν = 3391 (mbr), 3189 (m), 3037 (m), 2715 (w), 1656 (m), 1509 (s), 1036 (ssh, BF_4). ^{1}H NMR (ppm, DMSO): δ = 6.50 (m, H arom.2H), 3.51 (br, CH_2CH_2, 4H), 3.20 (br, CH_2CH_2, 4H), 2.89 (s, $NCCH_3$, 6H), 2.00 (s. CH_3, 3H). MS (EI) (%): 411 (M^+-B_2F_8, 12), 370 (M^+-$C_2H_3NB_2F_8$, 14), 225 (M^+-$C_4H_6N_2F_8PdB_2$, 100).

*2.2.3. cis-Dichloro(1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl) benzene)-platinum(II) ([Pt(DFMPB)Cl$_2$], **4**).* To a solution of $K_2[PtCl_4]$ (0.42 g, 1 mmol) in water (4 mL) was added dropwise the ligand (1 mmol) in methanol (5 mL) with continuous stirring. After 24 h in the dark at room temperature, the brown solid formed was filtered, washed with water, and dried.

Yield of 0.36 g (73%). M.p. (dec.) 240°C. Found: C, 24.62; H, 3.54; N, 10.92. Anal. Calc. for $C_{11}H_{17}N_4FPtCl_2{\cdot}2H_2O$: C, 25.10; H, 3.26; N, 10.65. IR (KBr, cm^{-1}): ν = 3449 (mbr), 3051 (mbr), 2745 (w), 1618 (m), 1516 (s), 1178 (m). MS (EI) (%): 491 (M^+, 10), 321 (M^+-$C_5H_{11}N_2Cl_2$, 10).



Scheme 1: Synthesis of the complexes used in the present study (**2**–**4**).

2.3. Biology

2.3.1. Cell Culture. Human MDA-231 breast cancer cell line was cultured in high glucose DMEM (Gibco, USA) supplemented with 20% fetal calf serum (FCS) (Euroclone, Italy). Human MCF-7 breast cancer cell line was cultured in RPMI-1640 medium (Euroclone, Italy) supplemented with 10% FCS. Human K562 chronic myelogenous leukemic cells were cultured in RPMI-1640 supplemented with 10% FCS. Trypsin-EDTA (Lonza, Switzerland) was routinely used for subcultures. Cell growth was accomplished at 37°C in a 5% carbon dioxide atmosphere.

2.3.2. In Vitro Cytotoxicity (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT Test. Cytotoxicity of the various complexes on MDA-231, MCF-7, and K562 cells was evaluated by means of MTT (tetrazolium salt reduction) test [27, 28]. Briefly, 5×10^4 viable cells were added to each well of a 96-well tissue culture plate containing growth media supplemented with FCS [29]. Cells were kept in a humified 5% CO_2 incubator at 37°C for 24 h. The complexes (**2**–**4** and cisplatin) were tested and for each complex six concentrations were prepared in growth media: 0.1, 0.5, 2.5, 5, 25, and 50 μg/mL. The complexes were solubilized in 10% DMSO. The next morning, the different concentrations were added, and the cells were incubated for 24 h, 48 h, and 72 h. Freshly prepared MTT salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well to give a final concentration of 0.5 μg/μL. The plates were incubated for 4 h and the formation of formazan crystals was checked using an inverted microscope. Equal volume of 1 : 1 (200 μL) DMSO and isopropanol mixture was added to each well and incubated for 30–45 min. The inhibition of cell growth induced by the various complexes was detected by measuring the absorbance of each well at 570 nm using a Statfax microplate reader. For comparison purposes, the cytotoxicity of cisplatin was evaluated under the same experimental conditions.

2.3.3. Clonogenic Assay. 2×10^5 cells were seeded in tissue culture dishes containing growth media supplemented with FCS. Cells were kept in a humified 5% CO_2 incubator at 37°C for 24 h. Afterwards, the medium was replaced and the cells were incubated for 3 h in the presence of an increasing concentration of tested complexes (0.1, 0.5, 2.5, 5, 25, and 50 μg/mL). Aliquots of 200 cells were seeded on soft agar for MDA-231 and MCF-7 cell lines and on methyl cellulose for K562 cell line and incubated for 12 days. The colonies were then stained and counted, discarding colonies with less than 50 cells. The surviving fraction (SF) was calculated according to Alverdi et al. [30] and Franken et al. [31].

3. Results and Discussion

3.1. Chemistry. The palladium(II) (**2**) and platinum(II) (**4**) complexes were prepared by treating each of the starting materials, $[Pd(PhCN)_2Cl_2]$ and K_2PtCl_4, with one equivalent of the diamine ligand, 1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl) benzene (DFMPB, **1**), at room temperature (Scheme 1). The palladium(II) complex, $[Pd(DFMPB)(CH_3CN)_2]$ (**3**) was prepared by reacting the ligand (**1**) with $[Pd(CH_3CN)_4](BF_4)_2$ following our previously published standard procedure [32].

The isolated compounds are microcrystalline or powder-like and stable at atmospheric conditions. The new compounds have been characterized using variety of techniques including elemental analysis, IR-, ^{1}H-NMR-spectroscopy, and mass- (EI-) spectroscopy.

Elemental analyses of the complexes (**2**–**4**) showed that the metal to the fluoropiperazinyl ligand ratio in the dichloro complexes is 1 : 1. The presence of the ligands in the complexes was also confirmed by IR-analyses (Table 1). The peaks due to the stretching vibration of the amine (N-H) showed slight shift to higher frequency. This slight stiffness of vibration refers to complexation of the ligands with the metal (Table 1). Although some of the complexes were microcrystalline, attempts to obtain crystals of suitable quality for an X-ray

Table 1: Analysis of the compounds[a].

Entry	Compound	Compound number	Color	m.p (dec) ˚C	IR[a] cm^{-1}
1	DFMPB	**1**	Colorless	97	3382, 1634, 1523
2	$[(DFMPB)PdCl_2]$	**2**	Orange	230	3389, 1620, 1513
3	$[Pd(DFMPB)(CH_3CN)_2](BF_4)_2$	**3**	Red	210	3391, 1656, 1509
4	[Pt(DFMPB) Cl_2]	**4**	Brown	240	3449, 1618, 1516

[a]N-H stretching absorption, C=C absorption, and in-plane NH_2 scissoring absorption, respectively.

Table 2: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) results, the values shown are IC_{50}[a].

Entry	Compound	Compound number	MDA-231	MCF-7	K562
1	DFMPB	**1**	(After 24 h)[+] 30.7 μg/mL 136.27 μM	(After 24 h)[+] 29.2 μg/mL 129.61 μM	(After 48 h)[+] 25.1 μg/mL 110.9 μM
2	$[Pd(DFMPB)Cl_2]$	**2**	(After 24 h)[+] 31.1 μg/mL 63.83 μM	(After 24 h)[+] 28.8 μg/mL 71.53 μM	(After 48 h)[+] 23.9 μg/mL 59.36 μM
3	$[Pd(DFMPB)(CH_3CN)_2](BF_4)_2$	**3**	No effect[b]	(After 72 h)[+] 50.1 μg/mL 85.29 μM	(After 48 h)[+] 43.6 μg/mL 74.22 μM
4	$[Pt(DFMPB)Cl_2]$	**4**	(After 48 h)[+] 42.8 μg/mL 87.12 μM	(After 48 h)[+] 44.6 μg/mL 90.79 μM	(After 48 h)[+] 35.4 μg/mL 72.1 μM
5	Cisplatin		(After 48 h)[+] 43.0 μg/mL 143.31 μM	(After 24 h)[+] 40.1 μg/mL 133.65 μM	(After 48 h)[+] 25.9 μg/mL 86.32 μM

[a]The results are shown in terms of IC_{50} values (the concentration needed to inhibit 50% of the cellular proliferation). For comparison purposes, the cytotoxicity of cisplatin, a standard antitumor drug was tested under the same conditions.
[+]The time it took for the drug to affect cell viability.
[b]After 72 h of drug addition, the drug killed at most 15% of the cells at 50 μg/mL.

structure determination were unsuccessful and could be due to the marginal solubility of the dichloride complexes. Therefore, only the solution behavior of complex **3** was determined by NMR spectroscopy in DMSO-d_6 at room temperature. The ^{1}H-NMR experiments showed that the metal to ligand ratio is 1 : 1. It has been indicated that slight shift to higher delta values of the ligand signals is observed upon coordination with palladium.

3.2. Biological Investigations. The cytotoxic activities of the ligand (**1**) and the corresponding complexes (**2**–**4**) were evaluated against human MDA-231 breast cancer cell line, human MCF-7 breast cancer cell line, and human K562 leukemia cell line. The results are shown in Table 2 in terms of IC_{50} values (the concentration needed to inhibit 50% of the cellular proliferation). For comparison purposes, the cytotoxicity of cisplatin, a standard antitumor drug, was tested under the same conditions.

The obtained data (Table 2) indicate a superior activity for the palladium(II) complex, $[Pd(DFMPB)Cl_2]$ (**2**), against all cell lines: MDA-231 and MCF-7 breast cancer cell lines and K562 leukemia cell lines (IC_{50} = 63.83, 71.53, and 59.36 μM) under similar condition. It showed the highest activity among the compounds investigated in the present study. In addition, the organic carrier (ligand, DFMPB, **1**) showed substantial activity compared with the standard antitumor drug, cisplatin, and the platinum(II) complex (**4**) against MDA-231 and MCF-7 human breast cancer cell lines. The noticeable activity of the organic ligand could be due to the presence of the piperazinyl moiety. Previous investigation showed that the presence of a strong electron-withdrawing group in the aromatic ring leads to a decrease in the cytotoxic activity of platinum(II) complexes [16].

However, the platinum(II) complex, $[Pt(DFMPB)Cl_2]$ (**4**) is more active than cisplatin against only MDA-231 breast cancer and human K562 leukemia cell lines. The corresponding dicationic complex (**3**) showed higher cytotoxic activity compared with cisplatin and the ligand only against K562 leukemia cell line.

According to Table 2, coordination of the organic biological carrier with palladium significantly increases its biological activity towards all cancer cell lines. However, the corresponding platinum(II) complex showed lower activity than compounds **1** and **2**. This behavior could be due to the relatively lower rate of hydrolysis of the palladium(II) complex caused by the coordination with the ridged aromatic

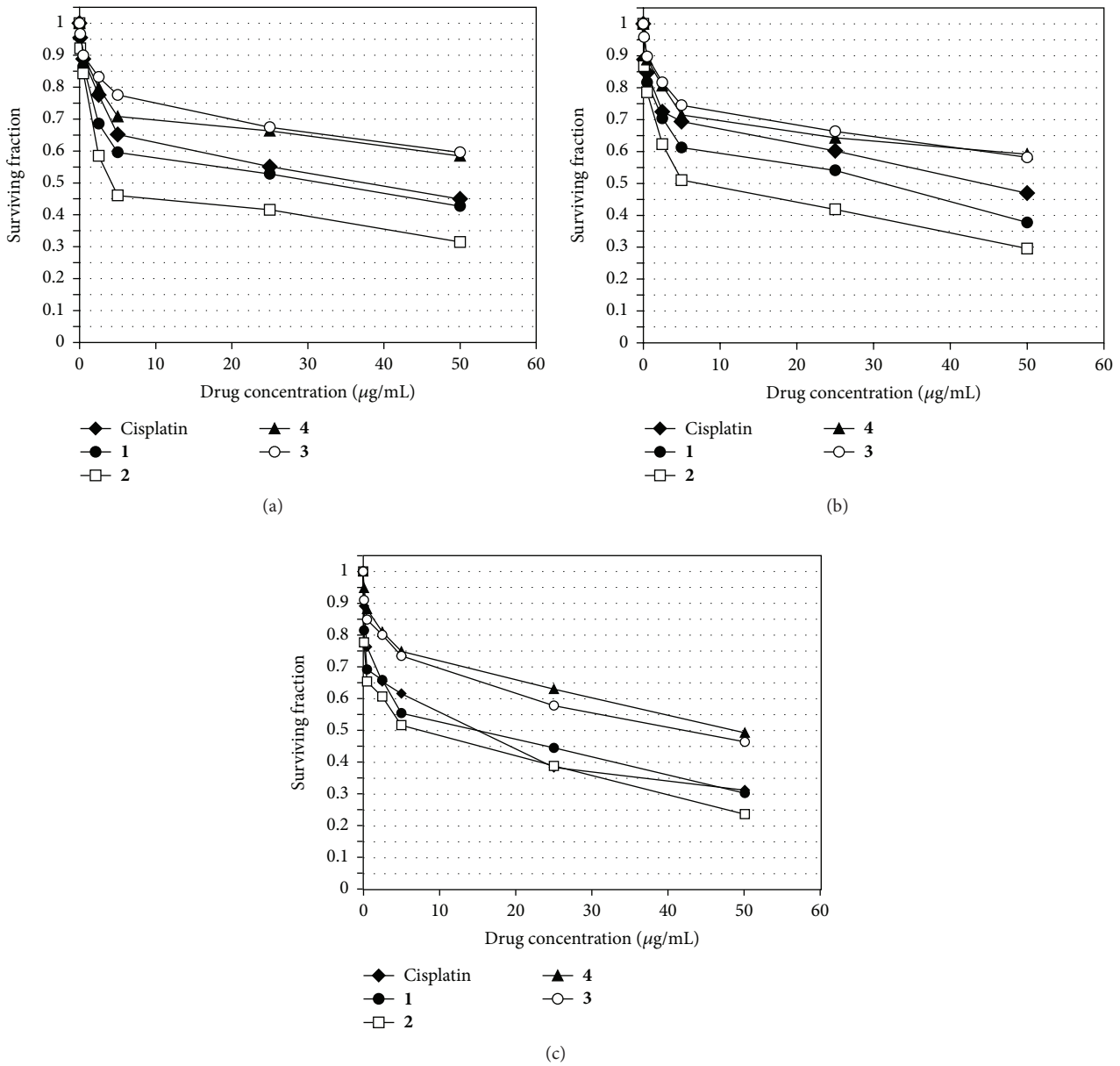


Figure 1: The antiproliferative activity of drugs (**2**–**4** and the standard antitumor drug, cisplatin) was evaluated using the clonogenic assay against (a) human MDA-231 breast cancer cell line, (b) human MCF-7 breast cancer cell line, and (c) human K562 leukemia cell line. The surviving fraction was calculated as number of colonies after treatment/[number of seeded cells (200 cells) × plating efficiency (PE)], where PE = number of colonies/[number of seeded cells (200 cells) × 100%].

diamine ligand (**1**). However, due to the relatively lower stability of the dicationic complex (**3**), the activity towards human MCF-7 breast cancer cell declined.

Based on the cytotoxic activity results above, it seems that both electronic properties and bulkiness of the ligand have a noticeable influence not only on the activity of the palladium(II) but also on its stability. The antiproliferative activity of the new complexes was also evaluated by studying the effect on clonal growth capacity of cells (Figures 1(a)–1(c)). The obtained data suggest that the palladium derivatives show a significant antiproliferative activity similar to that of the reference drug (cisplatin).

4. Conclusions

Several strategies have been utilized in order to design a better antitumor drug. Studies of platinum and palladium compounds with biologically active carriers have yielded promising results in the field of anticancer chemistry. In the present study, we showed that the biologically active compound, 1,2-diamino-4-fluoro-5-(4-methyl-1-piperazinyl)benzene, could generate palladium(II) and platinum(II) complexes with high cytotoxic activity against MDA-231 and MCF-7 breast cancer cell lines and K562 leukemia cell lines. Our experiments illustrate that the activity against the above cell lines for the

palladium(II) complex (**2**) (IC_{50} = 63.83, 71.53, and 59.36 μM, resp.) is much better than that for the corresponding platinum(II) complex (**4**) (IC_{50} = 87.12, 90.79, and 72.1 μM, resp.) and cisplatin (IC_{50} = 143.31, 133.65, and 86.32 μM, resp.) under similar conditions.

Disclosure

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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