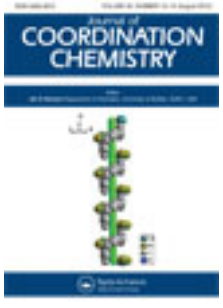

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Anticancer, DNA cleavage, and antimicrobial activity studies of some new Schiff-base titanium(IV) complexes

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Anticancer, DNA cleavage, and antimicrobial activity studies of some new Schiff-base titanium(IV) complexes

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Two Ti(IV) complexes have been synthesized with biologically active ligands. These ligands and their functional groups were carefully designed and selected from well-known anticancer drugs because of substituents on the aromatic ring. The ligands were prepared by condensation of a mixture of phenylenediamine and the appropriate aldehyde, vanillin, and 3,4-dimethoxybenzaldehyde. The structures of ligands and complexes have been confirmed by spectroscopic data, i.e., IR, ^{1}H NMR, ^{13}C NMR, electronic spectra, elemental (C, H, and N) analyses, magnetic and conductance measurements. Anticancer, DNA, and antibacterial activities are reported. Some compounds showed promising activity against Hela and PC3 cells.

Keywords: Schiff-base complexes; Titanium; Antibacterial activity; Anticancer activity, DNA studies

1. Introduction

Schiff-base metal complexes have been of great interest. N and S play a key role in the coordination of metals at the active sites of numerous metallobiomolecules [1]. Schiff-base metal complexes have numerous industrial, antifungal, antibacterial, anticancer, and herbicidal applications [2, 3]. They serve as models for biologically important species and find applications in biomimetic catalytic reactions.

Chelating ligands containing N, S, and O donors show the biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions. Metal ions bonded to biologically active compounds may enhance their activities [4–6]. Following the discovery of Cisplatin and its anticancer activity, there has been growing interest in investigations of other platinum-based compounds [7] as well as non-platinum systems, and evaluating their potential as anticancer reagents [8–11].

Among other metals that have been studied, two families of titanium complexes, titanocene dichloride and derivatives, and budotitane and analogs showed an

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interesting activity towards a number of tumor cell types, including those resistant to Cisplatin, and were characterized by reduced toxicity [12–18].

A variety of Schiff-base metal complexes with a wide choice of ligands, and coordination environments, have prompted us to undertake research in this area [19]. Metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb, and Mo show a remarkable antitumor activity [20, 21]. We are interested in studying titanium complexes in more detail, investigating additional non-Cp-based systems. In this work, Schiff-base ligands were prepared and complexed with titanium(IV) chloride. Characterization, electronic properties, biological activity, DNA, and anticancer effects are reported.

2. Experimental

2.1. *Materials/instrumentation*

Chemicals, synthesized or purchased (Aldrich chemical), were recrystallized before use. Phenylenediamine and vanillin were recrystallized from water. Titanium tetrachloride and 3,4-dimethoxybenzaldehyde were obtained commercially from Aldrich chemicals. Solvents were of reagent grade; dry absolute ethanol, dimethylformamide (DMF), chloroform, DMSO, and methanol were used as received.

Melting points were measured on an electrothermal melting point apparatus and are not corrected. Fourier-transform infrared (FTIR) spectra were recorded using KBr discs on a JASCO 410 FTIR spectrophotometer. Elemental (C, H, and N) analyses were performed using an Exeter CE-440 elemental analyzer. ^{1}H NMR spectra were recorded on GEMINI-200 and 300 MHZ instruments in DMSO-d_6, and TMS was used as an internal reference. ^{13}C NMR spectra of the free ligands (**1**, **2**) in $CDCl_3$ and DMSO were obtained using a Bruker 500 MHZ instrument using TMS as an internal standard. UV-Vis absorption spectra were measured in DMF ($\approx 10^{-5}$ mol L^{-1}) using a Pye–Unicam 8800a UV-Vis automatic scanning spectrophotometer. Solid state magnetic susceptibility measurements were carried out at room temperature using a Bartington MS 2B single sample dual frequency sensor. Molar conductivity was measured on a Systronic conductivity bridge with a dip-type cell using 2.5×10^{-3} mol L^{-1} solutions of complexes in DMF. Microbiological analyses were carried out by the Microanalytical Center, Faculty of Science, Sana'a University. Anticancer activity was evaluated at the International Center for Chemical Sciences and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi, Pakistan.

2.2. *Synthesis of Schiff bases*

General procedure: Schiff bases **1** and **2** were prepared by condensation of a mixture (0.01 mole) of phenylenediamine and the aldehyde (0.02 mole), vanillin, and (0.02 mole) 3,4-di-methoxybenzaldehyde, respectively, in ethanol (25 mL). The mixture was refluxed for 24 h under nitrogen. Schiff bases were filtered, washed, and recrystallized to yield 87% and 89% of **1** from chloroform and **2** from methanol : chloroform (1 : 1), respectively.

2.3. *Synthesis of Schiff-base complexes*

General procedure: Schiff bases **1** and **2** were reacted with (0.01 mole) titanium tetrachloride in refluxing DMF and ethanol, respectively, for 24 h under nitrogen to give Schiff-dibase complexes. Complex **3** was purified by methanol and few drops of DMF, and washed several times with methanol in 65% yield. Complex **4** was recrystallized from ethanol in 72% yield.

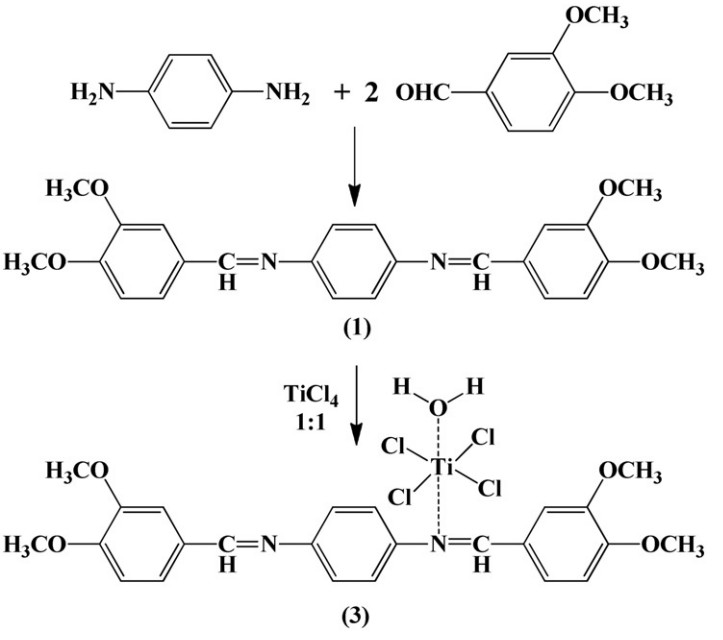
2.4. *Biological testing*

2.4.1. Gel electrophoresis. DNA cleavage experiments were conducted using CT-DNA by gel electrophoresis with the ligand or metal complex in the absence and presence of H_2O_2 as an oxidant. The reaction mixture was incubated before electrophoresis experiments at 37°C for 2 h as follows: CT-DNA 30 μmol L^{-1}, 50 μmol L^{-1}, each complex, and 500 μmol L^{-1} H_2O_2 in 50 m mol L^{-1} Tris-HCl buffer (7.1). Samples were subjected to electrophoresis experiments for 2 h at 50 V on 1% agarose gel using tris-acetic acid–EDTA buffer at pH = 8.3. After electrophoresis, the gel was stained using 3 μL ethidium bromide (EB) and photographed under UV light using a digital camera.

2.4.2. Antimicrobial activity. For antimicrobial activity, a filter paper sterilized disk saturated with a measured quantity of the sample was placed on the plate containing a solid bacterial medium (nutrient agar broth) or a fungal medium (Doxs medium) that has been heavily seeded with spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [22–24].

2.4.3. Anticancer activity

Cytotoxicity: Cytotoxic activity was evaluated in 96-well flat-bottomed micro plates using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay [25]. For this purpose, PC-3 cells (Prostate Cancer) and Hela cells were cultured in Dulbecco's modified Eagle's medium and minimal essential medium (MEM), respectively, supplemented with 5% of fetal bovine serum (FBS), 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin in 25 cm^3 flask, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with a haemocytometer, and diluted with a particular medium. Cell culture with a concentration of 1×10^5 cells mL^{-1} was prepared and introduced (100 μL per well) into 96-well plates. After overnight incubation, the medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1–100 μmol L^{-1}). After 48 h, 50 μL of MTT (2 mg mL^{-1}) was added to each well and incubated further for 4 h. Subsequently, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm using a microplate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as a concentration causing 50% growth inhibition (IC_{50}).



Scheme 1. Synthesis of ligand **1** and its complex **3**.

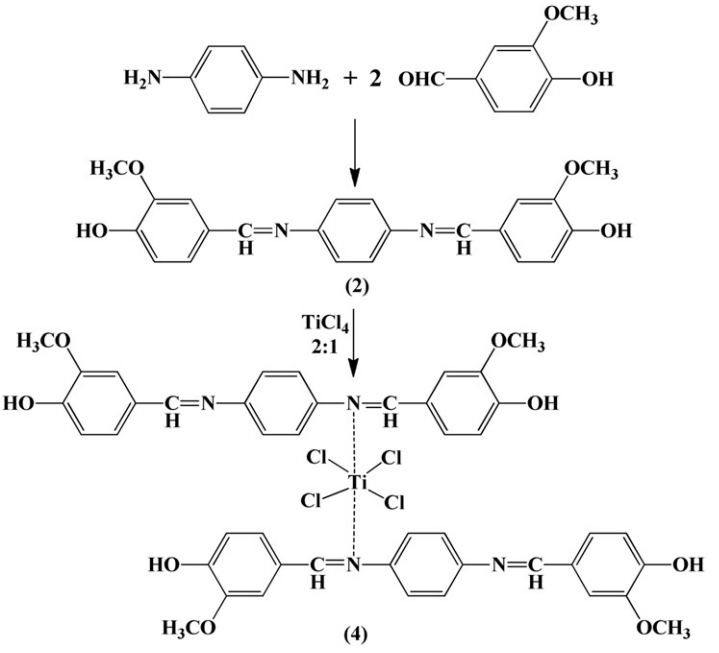
3. Results and discussion

3.1. *Synthesis and characterization*

Schiff bases were prepared by the reaction of phenylenediamine with 3,4-dimethoxybenzaldehyde and vanillin in a refluxing solvent. Schiff base of vanillin (**1**) (0.02 mole) was reacted with $TiCl_4$ (0.01 mole) to give a Schiff-dibase complex, (**3**) $[TiCl_4L(H_2O)]$, as confirmed by elemental and other analyses. Schiff base of 3,4-dimethoxybenzaldehyde (**2**) (0.02 mole) was reacted with $TiCl_4$ (0.01 mole) to give **4** as $[TiCl_4L_2]$ (schemes 1 and 2). All attempts at recrystallization yielded powder products. Hence, X-ray crystallography could not be performed. Table 1 summarizes the physical properties (melting point, color, percentage yield, magnetic moment and elemental analysis) of Schiff bases and Schiff-base complexes. Results of molar conductivity are given in table 2.

3.1.1. Conductance measurements. Ti complexes (**3** and **4**) were electrolytes, as shown by their molar conductivity (Λ_M) measurements in DMF, which are 31 and 64.2 $S\,mol^{-1}cm^{-1}$, respectively.

3.1.2. IR spectra of ligands. IR spectra of ligands are summarized in table 2. The absence of the aldehydic carbonyl stretch and the appearance of the characteristic azomethine $\nu_{C=N}$ at 1615 and 1618 cm^{-1} confirmed the formation of the Schiff bases. Medium intensity bands at 1213–1210 cm^{-1} were attributed to ν_{OCH3}. Single ν_{OH} of



Scheme 2. Synthesis of ligand **2** and its complex **4**.

Table 1. Physical properties of Schiff bases and their complexes.

No.	Unit formula	CHN, Calcd/found	F. wt.	m.p.	Yield	Color
1	$C_{24}H_{24}O_4N_2$	71.2/71.02, 5.9/5.47, 6.9/6.66	404	197–198	87%	Yellow
2	$C_{22}H_{20}O_4N_2$	70.2/69.83, 5.31/4.97, 7.4/7.16	376	200–201	89%	Pale yellow
3	$C_{24}H_{24}O_4N_2TiCl_4 \cdot H_2O$	47.13/47.75, 4.2/4.00, 4.5/4.8	611.8	>300	65%	Brown
4	$C_{44}H_{40}O_8N_4TiCl_4$	56.1/55.6, 4.24/4.6, 5.9/6.3	941.8	>300	72%	Reddish/brown

Table 2. IR absorptions and molar conductivity of **1**–**4**.

Comp. No.	OCH_3	OH	CH bending	CH aliphatic stretch	C–H aromatic stretch	C=N	C=C aromatic ring stretch	Molar conductivity, Λ_M ($S\,mol^{-1}\,cm^{-1}$)
1	1184		1374	2960	3065	1615	1598, 1466	–
2	1210	3407	1364	2962	3083	1618	1594, 1462	–
3	1184		1375	2960	3080	1649	1597, 1467	31
4	1216	3410	1367	2965	3055	1646	1594, 1464	64.2

vanillin was at 3407 [26]. The presence of additional bands between 1598 and 1462 cm^{-1} are attributed to $\nu_{C=C}$ of aromatic rings.

3.1.3. IR spectra of Ti(IV) complexes. Comparison of infrared spectral data of complexes (table 2) and their corresponding ligands confirmed complexation as significant shifts in $\nu_{C=N}$ were observed. The strong band at 1615–1618 cm^{-1} assigned

Table 3. Electronic absorptions of **1**–**4**.

λ_{max} (nm)		
Comp. No.	p–p*	n–p*
1	289 (34602)	365 (27397)
2	259 (38610)	367 (27247)
3	259 (38610)	356 (28089)
4	311 (32154)	358 (27932)

to $\nu_{C=N}$ in the free ligand shifted to higher wavenumber in complexes, indicating participation of azomethine nitrogen in coordination. Complex **3** showed a broad band of water hydroxyls at 3448 cm^{-1}. This was confirmed by CHN analysis.

3.1.4. NMR. The ^{1}H NMR spectrum of N_1,N_4-bis(3,4-dimethoxybenzylidene) benzene-1,4-diamine (**1**) showed the presence of azomthine proton at δ 8.47 ppm (m, 2H), while the multiplets at δ 6.89–7.52 ppm (m, 10H) were ascribed to aromatic protons, the OCH_3 were observed at δ 3.82 ppm (s, 6H), and δ 3.95 ppm (s, 6H). The ^{1}H NMR spectrum of 4,4′−(1,4−phenylenebis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(2-methoxyphenol) (**2**) showed the presence of azomethine proton at δ 8.51 ppm (s, 2H), with multiplets around δ 6.91–7.54 ppm (m, 10H) ascribed to aromatic protons, OCH_3 were noted at δ 3.85 ppm (s, 6H), and the OH at δ 9.79 ppm (s, 2H).

For the ^{1}H NMR spectra of **3** and **4**, the electron density shift was observed from ligand to the metal. Signals of azomethine protons appeared at δ 8.38, δ 7.85 ppm and δ 8.74, δ 8.57 ppm in the Ti complexes, respectively, as compared to δ 8.47 ppm and δ 8.51 ppm in the Schiff base, confirming coordination through the azomethine nitrogen.

The ^{13}C NMR spectra of the Schiff base had characteristic signals at δ 159.17 and δ 164 ppm of the azomethine carbon, C=N. Spectra were further characterized by the absence of the aldehydic signal at δ 190 ppm from the corresponding aromatic aldehydes (starting materials). ^{13}C NMR (ppm) in $CDCl_3$ of **1**: δ = 56.04, 108.86, 110.50, 121.81, 122.23, 124.43, 129.65, 149.49, 149.89, 152.02, 159.17. ^{13}C NMR (ppm) in DMSO of **2**: δ = 60.75, 115.48, 119.42, 120.57, 127.03, 127.27, 129.30, 133.23, 133.90, 153.37, 154.46, 155.36, 164.68.

3.1.5. Electronic spectra. Electronic absorptions are summarized in table 3 with one p–p* (K-band) λ_{max} at 259–311 nm and another n–p* (R-band) at λ_{max} 358–367 nm. The K band of **2** is 259 and **4** is 311. The K band of **1** is 289 nm and **3** is 259 nm. The R band of **2** is 367 nm while **4** is 358 nm. The R band of **1** is 365 nm and **3** is 356 nm [27].

3.1.6. Antimicrobial activity. The investigated compounds were tested against the bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, and fungi candidates. Values indicate that most complexes have higher antimicrobial activity than the free ligand. Table 4 shows the results of the bioassay. Compounds **1** and **4** show fair

Table 4. Antimicrobial activity of the Schiff base and their complexes.

	Microorganisms			
	Gram negative		Gram positive	
Compound	Fungus candidate	*E. coli*	*S. aureus*	*B. subtilis*
1	20 (+2)	18 (+2)	–	–
2	–	25 (+3)	18 (+2)	20 (+2)
3	–	–	16 (+2)	25 (+3)
4	20 (+2)	–	19 (+2)	23 (+3)

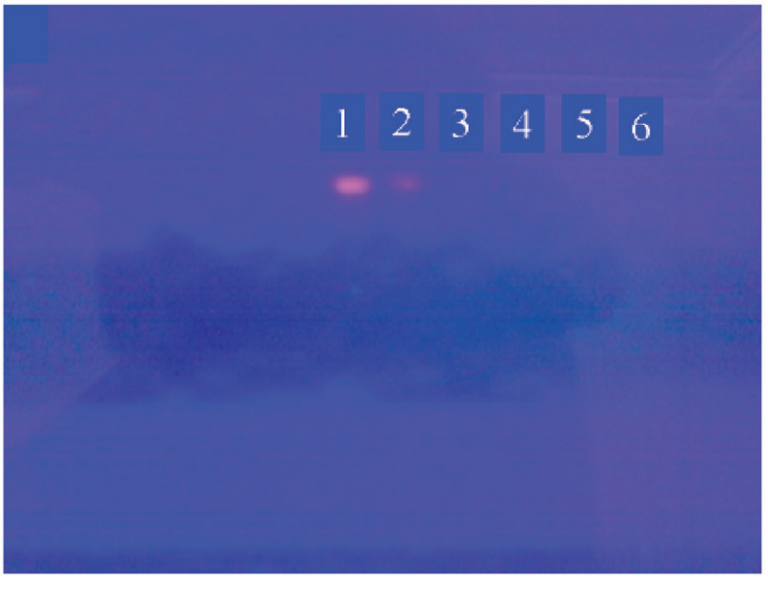


Figure 1. DNA with hydrogen peroxide and **1**–**4**: 1 – DNA alone; 2 – DNA + H_2O_2; 3 – DNA + H_2O_2 + **1**; 4 – DNA + H_2O_2 + **2**; 5 – DNA + H_2O_2 + **3**; 6 – DNA + H_2O_2 + **4**.

sensitivity to fungus candidate, **1** and **2** show high to fair sensitivity to *E. coli*, while **2**–**4** showed high to fair sensitivity to *S. aureus* and *B. subtilis*.

3.1.7. DNA cleavage studies. DNA cleavage results in cell death and can be used as an indication of an anticancer agent. Cleavage efficiency of complexes compared to that of the control is due to their DNA-binding ability. The metal complexes were able to convert supercoiled DNA into open circular DNA. The proposed general oxidative mechanisms of DNA cleavage by hydroxyl radicals occurs *via* abstraction of a hydrogen atom. The release of specific residues arising from transformed sugars depends on the position from which the hydrogen is removed [28]. Free radical scavengers inhibit cleavage. This implies that hydroxyl radical or peroxy derivatives mediate the cleavage. The reaction is modulated by bound hydroxyl radical or a peroxo species generated from the co-reactant H_2O_2.

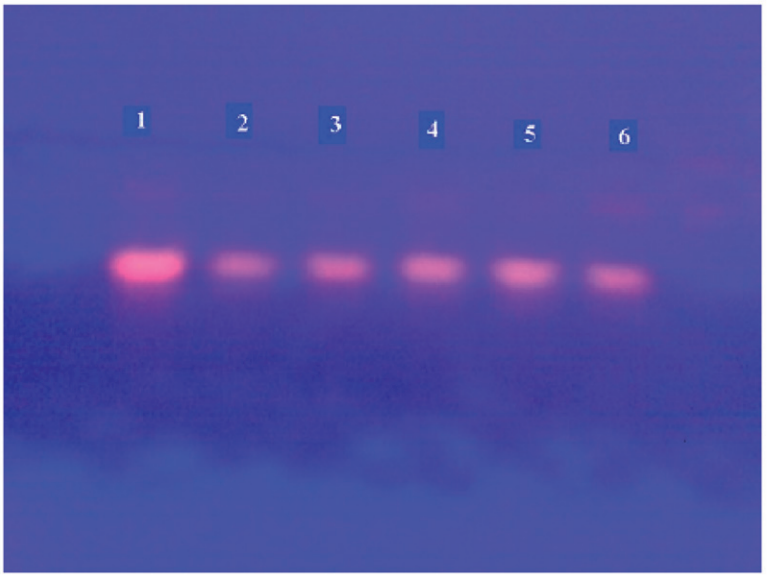


Figure 2. DNA without hydrogen peroxide: 1 – DNA alone; 2 – DNA + DMSO; 3 – DNA + **1**; 4 – DNA + **2**; 5 – DNA + **3**; 6 – DNA + **4**.

Table 5. Cytotoxic activity against Hela cells and PC3 (at 1, 10, and 100 μmol L^{-1}).

	PC3	Hela
Comp. No.	$IC_{50} \pm SD$ (μmol L^{-1})	$IC_{50} \pm SD$ (μmol L^{-1})
1	>100	>100
2	>100	>100
3	18.71 ± 1.2	11.82 ± 1.0
4	5.81 ± 0.6	3.53 ± 0.2
Doxorubicin (as control)	0.912 μM	3.10 ± 0.2

The CT-DNA gel electrophoresis experiment was conducted at 35°C using **1**–**4** in the presence of H_2O_2 as an oxidant. At a very low concentration, all the ligands and their complexes exhibited nuclease activity in the presence of H_2O_2 (figures 1 and 2). Control experiment using DNA alone (line 1) does not show significant cleavage of CT-DNA even on a long exposure time. Control using DNA + H_2O_2 (line 2) does not show significant cleavage of CT-DNA. Ligands (lines 3 and 4) and their complexes (lines 5 and 6) show complete cleavage of CT-DNA.

3.1.8. Anticancer activity. *In vitro* anticancer activity of **4** showed strong cytotoxic effect for PC3 and Hela cells, while moderate activity was observed for **3**. Table 5 presents the cytotoxic activity of the tested compounds.

4. Conclusion

This work describes the synthesis and *in vitro* antimicrobial and anticancer evaluation of two Schiff bases and their complexes. Most of the tested compounds exhibit antibacterial activity. Compound **4** displayed antitumor activity against PC3 and Hela cells.

References

[1] K. Singh, M.S. Barwa, P. Tagi. *Eur. J. Med. Chem.*, **42**, 394 (2007).
[2] P.G. Cozzi. *Chem. Soc. Rev.*, **33**, 410 (2004).
[3] S. Chandra, J. Sangeetika. *J. Indian Chem. Soc.*, **81**, 203 (2004).
[4] M.B. Ferrari, S. Capacchi, G. Pelosi, G. Reffo, P. Tarasconi, R. Albertini, S. Pinelli, P. Lunghi. *Inorg. Chim. Acta*, **286**, 134 (1999).
[5] E. Canpolat, M. Kaya. *J. Coord. Chem.*, **57**, 1217 (2004).
[6] M. Yildiz, B. Dulger, S.Y. Koyuncu, B.M. Yapici. *J. Indian Chem. Soc.*, **81**, 7 (2004).
[7] E. Wong, C.M. Giandomenico. *Chem. Rev.*, **99**, 2451 (1999).
[8] M.J. Clarke, F. Zhu, D.R. Frasca. *Chem. Rev.*, **99**, 2511 (1999).
[9] F. Kratz, M.T. Schutte. *Cancer J.*, **11**, 176 (1998).
[10] P. Kopf-Maier. *Eur. J. Clin. Pharmacol.*, **47**, 1 (1994).
[11] P. Kopf-Maier, H. Kopf. *Chem. Rev.*, **87**, 1137 (1987).
[12] E. Melendez. *Crit. Rev. Oncol. Hemat.*, **42**, 309 (2002).
[13] F. Caruso, M. Rossi, C. Pettinari. *Expert. Opin. Ther. Pat.*, **11**, 969 (2001).
[14] P. Kopf-Maier, H. Kopf. *Struct. Bond.*, **70**, 103 (1988).
[15] G. Kelter, N.J. Sweeney, K. Strohfeldt, H.H. Fiebig, M. Tacke. *Anticancer Drugs*, **16**, 1091 (2005).
[16] C.V. Christodoulou, A.G. Elipoulos, L.S. Young, L. Hodgkins, D.R. Ferry, D.J. Kerr. *Br. J. Cancer*, **77**, 2088 (1998).
[17] B.K. Keppler, C. Friesen, H.G. Moritz, H. Vongerichten, E. Vogel. *Struct. Bond.*, **78**, 97 (1991).
[18] F. Caruso, M. Rossi, J. Tanski, R. Sartori, R. Sariego, S. Moya, S. Diez, E. Navarrete, A. Cingolani, F. Marchetti, C. Pettinari. *J. Med. Chem.*, **43**, 3665 (2000).
[19] A. Majumder, G.M. Rosair, A. Mallick, N. Chattopadhyay, S. Mitra. *Polyhedron*, **25**, 1753 (2006).
[20] A. Korfel, M.E. Scheulen, H.J. Schmoll, O. Grundel, A. Harstrick, M. Knoche, M. Fels, M. Skorzec, F. Bach, J. Baumgart, G. Sab, S. Seeber, E. Thiel, W.E. Berdel. *Clin. Cancer Res.*, **4**, 2701 (1998).
[21] G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubben. *Cancer Chemother. Pharmacol.*, **42**, 415 (1998).
[22] R.J. Grayer, J.B. Harbone. *Phytochemistry*, **37**, 19 (1994).
[23] D.N. Muanza, B.W. Kim, K.L. Euler, L. Williams. *Int. J. Pharmacogn.*, **32**, 337 (1994).
[24] O.N. Irob, M. Moo-Young, W.A. Anderson. *Intl. J. Pharmacogn.*, **34**, 87 (1996).
[25] T.J. Mosmann. *Immunol. Methods*, **65**, 55 (1983).
[26] M. Silverstein, G. Bassler, C. Morril. *Spectrometric Identification of Organic Compounds*, 4th Edn, John Wiley & Sons, New York (1981).
[27] M. Nath, C.L. Sharma, N. Sharma. *Synth. React. Inorg. Met.-Org. Chem.*, **21**, 807 (1991).
[28] G. Prativel, M. Pitie, J. Bernadou, B. Meunier. *Angew. Chem. Int. Ed. Eng.*, **30**, 702 (1991).