



Anticancer 4: Anticancer and DNA cleavage studies of some new Schiff base titanium (IV) complexes

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ABSTRACT

Two metal complexes of Ti(IV) were synthesized with newly prepared biologically active ligands. These ligands and their functional groups were carefully designed and selected from well known anticancer drugs. The ligands were prepared by the condensation of a mixture of sulfamethoxazole and the appropriate aldehyde namely, 3,4-dimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde. The structure of the ligands and their complexes were confirmed by spectroscopic data i.e. IR, ¹H NMR, electronic and elemental analysis. All compounds were screened for their *in vitro* anticancer potential using HELA and PC3 cells. All compounds showed limited cytotoxicity and complete cleavage of calf thymus deoxyribonucleic acid (CT-DNA).

1. Introduction

Coordination compounds of biologically active ligands have received much attention [1-3]. Chelation causes drastic change in the biological properties of the ligands and also the metal moiety. A number of Schiff-base complexes [4-7] have been tested for biological activities and showed antibacterial [8-11], antifungal [10-12], anticancer [13-14] and herbicidal [15] activities. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities [16-18]. Following the discovery of *cis*-platin and its anticancer activity, there has been a growing interest in investigations of other platinum-based compounds [19] as well as non-platinum systems, and evaluating their potential value as anticancer reagents [20-23].

Among other metals that have been studied, two families of titanium complexes, titanocene dichloride and derivatives, and budotitane and analogs, showed interesting activities towards a number of tumor cell types, including those resistant to *cis*-platin, and with reduced toxicity [24-30].

A variety of possible Schiff base metal complexes with a wide choice of ligands, and coordination environments, have prompted us to undertake research in this area [31-32]. Metallocene dichlorides (Cp₂MCl₂) with M = Ti, V, Nb and Mo

show remarkable antitumor activity [33-34]. We are interested in studying titanium complexes in more detail, and investigating additional non-Cp-based systems.

In continuation of our previous studies [35] in this work, Schiff base ligands were prepared by condensation of an equimolar mixture of sulfamethoxazole and the aldehyde (3,4,5-trimethoxybenzaldehyde or 3,4-dimethoxybenzaldehyde). The ligands were reacted with titanium(IV) chloride to get Schiff-base complexes. Characterization, electronic properties, DNA cleavage and anticancer effects were reported.

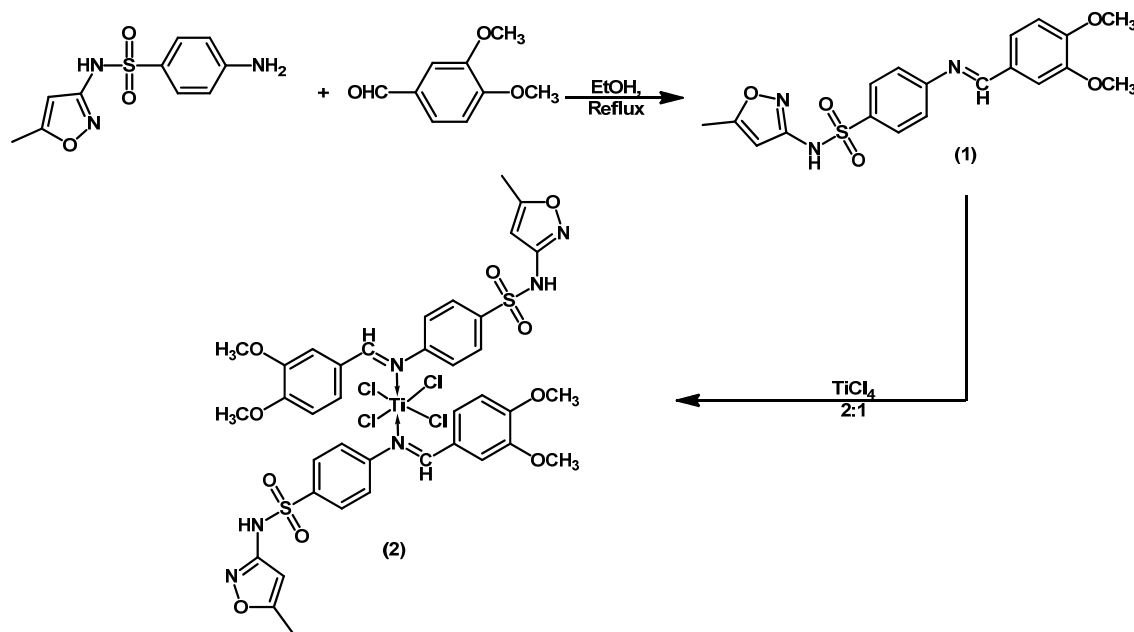
2. Experimental

2.1. Chemicals

Sulfamethoxazole was purchased from Virchow Drugs Ltd., India. Titanium tetrachloride, 3,4,5-trimethoxybenzaldehyde and 3,4-dimethoxybenzaldehyde were obtained commercially from Aldrich Chemicals. The solvents: dry absolute ethanol and diethyl ether were reagent grade and were used as received.

2.2. Instrumentation

The melting points were measured on an electrothermal melting point apparatus and were not corrected.



Scheme 1

Fourier-transform infrared spectra were recorded using the KBr disc technique on a JASCO 410 FTIR spectrophotometer. Elemental (CHN) analysis was performed using an Exeter CE-440 elemental analyzer. UV-visible absorption spectra were measured in DMF using a Pye-Unicam 8800a UV-visible automatic scanning spectrophotometer. Molar conductivity was measured on a systronic conductivity bridge with a dip-type cell, using 1×10^{-3} M solution of complexes in DMF. ^1H NMR spectra of the ligands and their complexes were recorded on a Varian Gemini-200 spectrometer using $\text{DMSO-}d_6$ as solvent and TMS as internal reference. Anticancer activity was evaluated at the International Center for Chemical Sciences and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Pakistan.

2.3. Synthesis of Schiff base ligands

Schiff base compounds were prepared by condensation of an equimolar mixture (0.01 mol) of sulfamethoxazole and the aldehyde (0.01 mol) 3,4,5-trimethoxybenzaldehyde or (0.01 mol) 3,4-dimethoxybenzaldehyde, respectively, in ethanol (25 mL). The mixture was refluxed for 24 hours under nitrogen atmosphere. Schiff base compounds were isolated and purified by diethyl ether after reduction of solvent volume by evaporation in a rotary evaporator (Scheme 1 and 2).

4-(3,4-Dimethoxybenzylideneamino)-N-(5-methylisoxazol-3-yl)benzene sulfonamide (1): Color: Orange. Yield: 92%. M.p.: 204-205 °C. FT-IR (KBr, ν , cm^{-1}): 3432 (NH), 1654 (C=N), 1619 (C=N), 1576 (C=C). ^1H NMR (200 MHz, $\text{DMSO-}d_6$, δ , ppm): 10.99 (s, 1H, D_2O exchangeable NH), 9.79 (s, 1H, CH, azomethine), 6.59-7.47 (m, 7H, Ar-H), 6.06 (s, 1H, isoxazole), 3.72 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3 methyl isoxazole). Anal. calcd. for $\text{C}_{19}\text{H}_{19}\text{O}_5\text{N}_3\text{S}$: C, 56.85; H, 4.77; N, 10.47. Found: C, 56.08; H, 3.90; N, 9.24%. UV/Vis (DMF, λ_{max} , nm): 215, 292, 343.

N-(5-Methylisoxazol-3-yl)-4-(3,4,5-trimethoxybenzylideneamino)benzene sulfonamide (3): Color: Yellow. Yield: 75%. M.p.: 220-223 °C. FT-IR (KBr, ν , cm^{-1}): 3430 (NH), 1656 (C=N), 1623 (C=N ring), 1580 (C=C). ^1H NMR (200 MHz, $\text{DMSO-}d_6$, δ , ppm): 10.95 (s, 1H, D_2O exchangeable NH), 9.88 (s, 1H, CH,

azomethine), 6.60-7.49 (m, 6H, Ar-H), 6.08 (s, 1H, isoxazole), 3.74 (s, 3H, OCH_3), 3.85 (s, 6H, OCH_3), 2.28 (s, 3H, CH_3 methyl isoxazole). Anal. calcd. for $\text{C}_{20}\text{H}_{21}\text{O}_6\text{N}_3\text{S}$: C, 55.67; H, 4.91; N, 9.74. Found: C, 54.20; H, 3.77; N, 9.10%. UV/Vis (DMF, λ_{max} , nm): 213, 292, 344.

2.4. Synthesis of Schiff base complexes

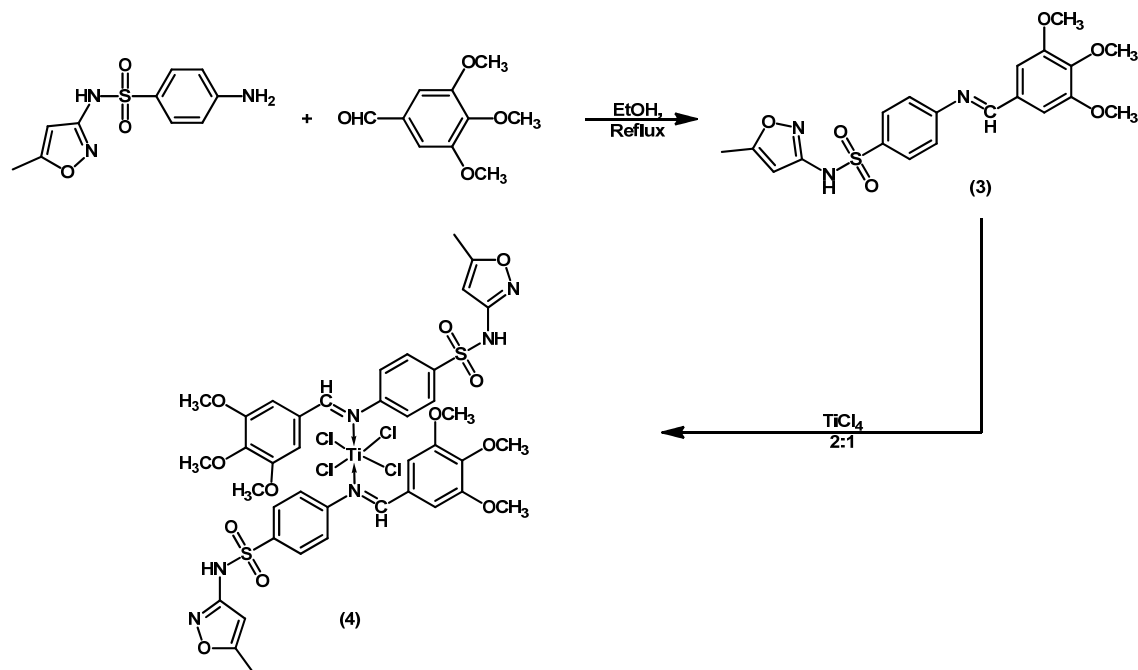
Schiff base compounds (0.02 mol) were reacted as ligands with (0.01 mol) titanium tetrachloride in refluxing ethanol for 24 hrs under nitrogen to give Schiff dibase complexes. The precipitates were crystallized from ethanol (Scheme 1 and 2).

4-(3,4-Dimethoxybenzylideneamino)-N-(5-methylisoxazol-3-yl)benzene sulfonamide titanium complex (2): Color: Reddish brown. Yield: 50%. M.p.: 232-233 °C. FT-IR (KBr, ν , cm^{-1}): 3432 (NH), 1652 (C=N), 1622 (C=N ring), 1594 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, δ , ppm): 10.94 (s, 2H, D_2O exchangeable NH), 9.84 (s, 2H, CH, azomethine), 6.57-7.58 (m, 14H, Ar-H), 6.08 (s, 2H, isoxazole), 3.82 (s, 6H, OCH_3), 3.86 (s, 6H, OCH_3), 2.27 (s, 6H, CH_3 methyl isoxazole). Anal. calcd. for $\text{C}_{38}\text{H}_{38}\text{O}_{10}\text{N}_6\text{S}_2\text{TiCl}_4$: C, 45.98; H, 3.86; N, 8.47. Found: C, 45.20; H, 3.90; N, 8.20%. UV/Vis (DMF, λ_{max} , nm): 215, 281, 363, 414. Λ_m ($\text{S.cm}^2.\text{mol}^{-1}$): 42.

N-(5-Nethylisoxazol-3-yl)-4-(3,4,5-trimethoxybenzylideneamino)benzene sulfonamide titanium complex (4): Color: Dark yellow. Yield: 32%. M.p.: 169-171 °C. FT-IR (KBr, ν , cm^{-1}): 3438 (NH), 1650 (C=N), 1623 (C=N ring), 1583 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, δ , ppm): 11.05 (s, 2H, D_2O exchangeable NH), 9.83 (s, 2H, CH, azomethine), 6.80-7.59 (m, 12H, Ar-H), 6.09 (s, 2H, isoxazole), 3.82 (s, 6H, OCH_3), 3.87 (s, 12H, OCH_3), 2.27 (s, 6H, CH_3 methyl isoxazole). Anal. calcd. for $\text{C}_{40}\text{H}_{42}\text{O}_{12}\text{N}_6\text{S}_2\text{TiCl}_4$: C, 45.64; H, 4.02; N, 7.98. Found: C, 45.86; H, 3.40; N, 7.78%. UV/Vis (DMF, λ_{max} , nm): 214, 268, 400. Λ_m ($\text{S.cm}^2.\text{mol}^{-1}$): 93.6.

2.5. Gel electrophoresis

The DNA cleavage experiment was conducted using CT-DNA by gel electrophoresis with the ligand and metal complex in the absence and presence of H_2O_2 as an oxidant.



Scheme 2

The reaction mixture was incubated before electrophoresis experiment at 37 °C for 2 h as follows: Calf thymus DNA, CT-DNA 30 μM, 50 μM, each complex and 500 μM H₂O₂ in 50 mM Tris-HCl buffer (pH = 7.1). The samples were electrophoresed 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.

2.6. Cytotoxicity

The cytotoxic activity of the compounds was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay [36]. 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), supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 μg/mL of streptomycin in 25 cm³ flask, and kept in 5% CO₂ incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 1×10⁵ cells/mL was prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1, 10 and 100 μM). After 48 h, 50 μL MTT (2 mg/mL) was added to each well and incubated further for 4 hours. 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 micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC₅₀).

3. Result and discussion

3.1. Synthesis and characterization

Schiff base ligand was prepared by reaction of sulfamethoxazole with 3,4-dimethoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde in refluxing solvent. Schiff base ligand (0.02 mole) was reacted with TiCl₄ (0.01 mole) to give Schiff dibase complexes (Scheme 1 and 2).

3.2. Conductance measurements

All Ti complexes (2 and 4) are electrolytes as shown by their molar conductivity (Λ_m) measurements in DMF, which are 42 and 93.6 S.cm².mol⁻¹, respectively.

3.3. IR spectra

The assigned absorption bands are consistent with the suggested structures. The aromatic C=C vibrations were observed in the range 1576-1596 cm⁻¹; the azomethane band is seen at 1650-1656 cm⁻¹; NH single bond of sulfamethoxazole at 3430-3438 cm⁻¹ [37] and C=N oxazole ring at 1619-1623 cm⁻¹ [38].

3.4. ¹H NMR study of Schiff base

¹H NMR spectrum of the Schiff base (1), 4-(3,4-dimethoxybenzylideneamino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide, showed NH proton of sulfonamide at 10.99 ppm (s, 1H, D₂O exchangeable), CH₃ of methyl isoxazole at 2.29 ppm (s, 3H), azomethine proton noted at 9.79 ppm (m, 1H). The multiple signals around 6.59-7.47 ppm (m, 7H) are ascribed to aromatic protons. The signal observed at 6.06 ppm (s, 1H) is due to isoxazole proton. OCH₃ protons showed up at 3.72 ppm (s, 3H) and at 3.84 ppm (s, 3H).

¹H NMR spectrum of the Schiff base (3), N-(5-methylisoxazol-3-yl)-4-(3,4,5-trimethoxybenzylideneamino)benzenesulfonamide, showed NH proton of sulfonamide at 10.95 ppm (s, 1H, D₂O exchangeable), CH₃ of methyl isoxazole at 2.28 ppm (s, 3H) and azomethine proton noted at 9.88 ppm (s, 1H). The multiple signals around 6.60-7.49 ppm (m, 6H) are ascribed to aromatic protons. Signal observed at 6.08 ppm (s, 1H) is due to

isoxazole proton. OCH₃ protons were noted at 3.74 ppm (s, 3H) and at 3.85 ppm (s, 6H).

3.5. ¹H NMR study of the complexes

In the ¹H NMR spectra of the complexes (2 and 4) an electron density shift from the ligand to the metal complex was observed. The signals of azomethine protons appeared at 9.84 and 9.83 ppm in the compound 2 and 4, respectively, as compared to 9.79 ppm and 9.88 ppm in the Schiff base, confirming coordination through the azomethine nitrogen atom of the ligand [39].

3.6. Electronic spectra

The main electronic absorptions are shown in Figure 1. UV-Vis spectra of the ligands and their complexes were measured in the range 200-800 nm. The lower wavelength in the range 200-400 nm is specific for the electronic intra-ligand transitions. The spectra of complexes generally show the characteristic band of the free ligands with some changes both in wavelengths (λ_{max}) and intensity, together with appearances of new bands at longer wavelengths. The spectra of the ligands and their complexes exhibit bands in the regions of 213-215, 268-292 and 343-414 nm, which may be attributed to the transitions of $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, respectively.

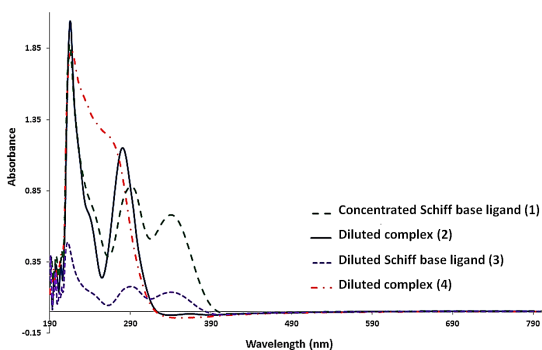


Figure 1. UV-Vis spectra of the two ligands and their metal complexes.

3.7. DNA cleavage studies

The cleavage efficiency of the complexes compared to that of control is due to their efficient DNA-binding ability. The metal complexes were able to convert super coiled DNA into open circular DNA. The proposed general oxidative mechanisms and account of DNA cleavage is by hydroxyl radicals via abstraction of a hydrogen atom from sugar units that predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [40]. Free radical scavengers inhibit cleavage. This implies that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by metal complexes bound hydroxyl radical or a peroxy species generated from the co-reactant H₂O₂.

In the present study, CT-DNA gel electrophoresis experiment was conducted at 35 °C using our synthesized complexes in the presence of H₂O₂ as an oxidant. As can be seen from the results illustrated in Figure 2 and 3, at very low concentrations, all ligands and their complexes exhibited nuclease activity in the presence of H₂O₂. Control-1 experiment, using DNA alone (line 1) did not show any significant cleavage of CT-DNA even on a longer exposure time. Control-2, using DNA+H₂O₂ (line 2) did not show any significant cleavage of CT-DNA. From the observed results, it can be concluded that all

ligands (lines 3 and 5) and their complexes (lines 4 and 6) showed complete cleavage of CT-DNA.



Figure 2. DNA with hydrogen peroxide and chemical compounds: 1-DNA alone; 2-DNA + H₂O₂; 3-DNA + H₂O₂ + Compound 1; 4-DNA + H₂O₂ + Compound 2; 5-DNA + H₂O₂ + Compound 3; 6-DNA + H₂O₂ + Compound 4.

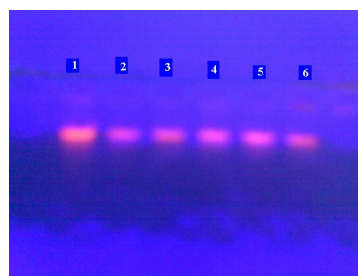


Figure 3. DNA without hydrogen peroxide: 1-DNA alone; 2-DNA + solvent (DMSO); 3-DNA + Compound 1; 4-DNA + Compound 2; 5-DNA + Compound 3; 6-DNA + Compound 4.

3.8. Anti-cancer activity

The results of the *in vitro* anticancer activity of the tested compounds 1-4 were evaluated for cytotoxicity against PC3 cells (Prostate cancer) and Hela cells of humans in comparison with doxorubicin as a positive control. All the tested compounds showed a limited cytotoxic activity. Table 1 represents the cytotoxic activity of the tested compounds.

Table 1. Cytotoxic activity against Hela cells and PC3 cells.

Compound	PC3	Hela
	IC ₅₀ ± SD (μM)	IC ₅₀ ± SD (μM)
1	35.67±1.7	37.66±0.2
2	32.6±0.5	36.75±1.0
3	50.76±0.2	54.98±1.1
4	>100	>100
Doxorubicin (as control)	0.912±0.120	3.10±0.2

4. Conclusion

The present work describes the synthesis, characterization and *in vitro* anticancer evaluation of two sulfonamide derivatives and their complexes. With regard to anticancer activity we expected the titanium complexes to give excellent cytotoxic activity, however all tested compounds showed limited cytotoxic activity and complete cleavage of CT-DNA.

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