Anti-Cancer and Anti-Microbial Activity Studies of Some Complexes of Trimethoprim

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Abstract: 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine, trimethoprim (TMP), was reacted with Cu, Zn, Fe and Ti. The products were characterized by elemental (CHN) analysis, FTIR, magnetic moment measurements, conductance measurements, electronic spectra, ^{13}C spectra. All complexes were investigated for their anti-bacterial activities against Gram-positive and Gram-negative bacteria and were also screened for their *in vitro* anti-cancer potential using Hela and PC3 cells. All the complexes showed excellent anti-bacterial activity even more than TMP while TMP-Ti displayed good cytotoxic activity *in vitro* against Hela cells and TMP-Cu showed moderate cytotoxicity against PC3 cells.

Keywords: Trimethoprim complexes, anti-bacterial activity, anti-cancer activity.

1. INTRODUCTION

A great deal of interest has been directed to pyrimidines due to their biological importance as components of nucleic acid. Many compounds of therapeutic importance contain the pyrimidine ring system. Substituted 2,4-diaminopyrimidines are widely employed as metabolic inhibitors of pathways leading to the synthesis of proteins and nucleic acids in the chemotherapy of malaria and neoplastic diseases [1]. Many studies have recently stressed the role of metal ions in important biological processes [2-6], whereas the inorganic pharmacology started to be an important field with more than 25 inorganic compounds being used in therapy as anti-bacterial, anti-viral and anti-cancer drugs [7-11].

Trimethoprim has potential binding sites for metal ions. Several authors have studied the interaction of this ligand with biological metal ions and the coordination of trimethoprim *via* NH_2. Nitrogen atom was inferred on the basis of IR and UV-visible measurements [12, 13]. However, other authors have shown by X-ray diffraction methods that the coordination site of trimethoprim molecule is the N_1 of the pyrimidine ring [14-18]. On the other hand, more recently, Sekhon and co-authors [19] prepared and characterized complexes of trimethoprim with Ag, Zn, Cd, Hg and Ni and infrared data showed that the ligand acts as a monodentate through the 4-NH_2 group.

This study aims at complexing the famous anti-microbial drug, trimethoprim, with Cu, Zn, Fe and Ti metals to study their anti-bacterial and anti-cancer properties.

2. EXPERIMENTAL

2.1. Chemicals

Trimethoprim was purchased from Inventa Chemical pvt. Ltd. All reagents used are of analytical grade.

2.2. Instrumentation

The melting points were measured on an electrothermal melting point apparatus and were not corrected. Fourier-transform infrared spectra were recorded using the KBr disc technique on a JASCO 410 FTIR spectrophotometer. Elemental (CHN) analyses were performed on an elemental analyses system GmbH Varioel V2.3 1998 CHNS Mode. ^{13}C spectra were recorded on Bruker 500 MHZ, using TMS as an internal standard in DMSO-d^6 as a solvent. UV-visible absorption spectra were measured in DMF (≈10^{-5}molel^{-1}) using a Pye–Unicam 8800a UV-Visible 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 1×10^{-3} M solution of complexes in DMF. Microbiological analysis was carried out by the microanalytical center, Faculty of Science, Sana'a University. Anti-cancer activity was evaluated at the International Center For Chemical Sciences and Dr. Panjwani Center For Molecular Medicine and Drug Research, University of Karachi.

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2.3. Synthesis of the Complexes

2.3.1. Synthesis

All complexes were prepared according to literature [19, 20].

Trimethoprim (0.58 gm, 2 mmol) was dissolved in 50 ml of ethanol followed by slow addition of 1 mmol $ZnCl_2$, $CuCl_2$, $FeCl_3.6H_20$ or $TiCl_4$ (0.14 gm, 1.34 gm, 0.27 gm, and 0.11 ml, respectively) in 20 ml ethanol. The mixture was refluxed for 24 hrs then filtered, washed and dried. Complexes were recrystallized from methanol.

2.4. Biological Testing

2.4.1. Anti-Microbial Activity

For anti-microbial activity, a filter paper sterilized disk saturated with a measured quantity of the sample is placed on the plate containing solid bacterial medium (nutrient agar broth), which 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 [21, 22]. The tested compounds were dissolved in (DMF) to get a concentration of 1×10^{-3} M.

2.4.2. Anti-Cancer Activity

2.4.2.1. Cytotoxicity

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay [23]. 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 of penicillin and 100 µg/mL of streptomycin in 25 cm^3 flask, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells

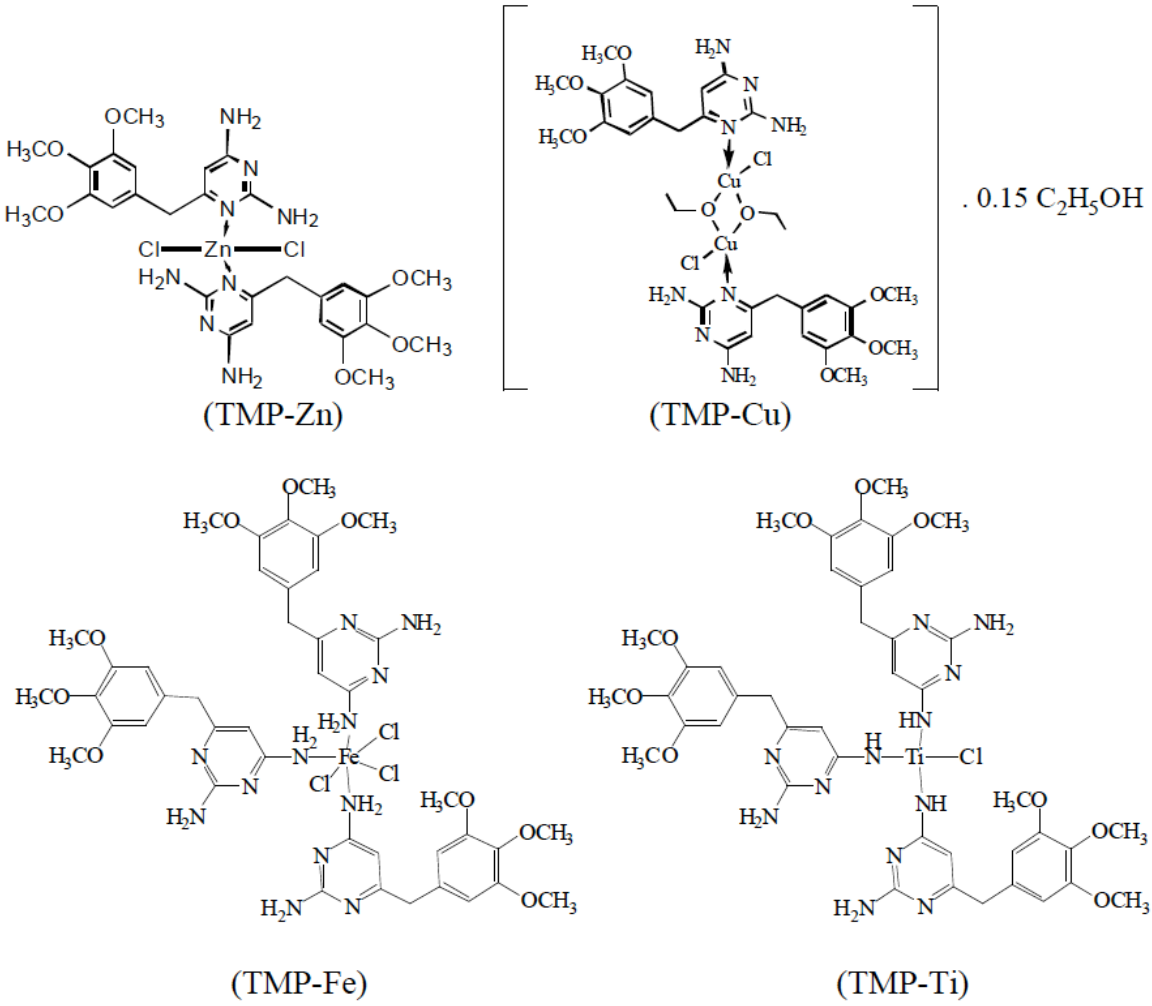

Figure 1: Proposed structures for trimethoprim complexes.

Table 1: Physical Properties of Trimethoprim Complexes

Comp.	Unit formula	Unit wt.	M.p. (°C)	Color	Molar conductivity ^M [Smol^{-1}cm2]	[BM]
TMP-Zn	$C_{28}H_{36}O_6N_8ZnCl_2$	716.95	280-282	White	17.7	Dia
TMP-Cu	$C_{32}H_{46}N_8O_8Cu_2Cl_2.2C_2H_5OH$	875.66	167-169	Olive green	46.7	Dia
TMP-Fe	$C_{42}H_{54}O_9N_{12}FeCl_3$	1033.16	244-247	Brown	60.5	Dia
TMP-Ti	$C_{42}H_{51}O_9N_{12}TiCl$	951.25	280-283	White	61.2	Dia

were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 1x10^5 cells/mL was prepared and introduced (100 µL/well) into the 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-100 µM). After 48 hrs, 50 µL MTT (2 mg/mL) was added to each well and incubated further for 4 hrs. 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. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization

All Complexes were prepared as follows: a solution containing 1 mmol metal salt in ethanol (20 ml) was added to a solution of 2 mmol TMP in the same solvent (50 ml).

Table **1** summarizes the physical properties (melting point, color, magnetic moment, Molar conductivity and elemental analysis) of complexes. The proposed structures of the trimethoprim complexes are illustrated in Figure **1**.

3.2. IR Spectra of TMP and its Complexes

The main IR spectra of the compounds are summarized in Table **2**, and shown in Figure **2**. TMP has potential binding sites for metal ions. It has four nitrogen atoms, which can donate electron pairs, two nitrogen atoms of the pyrimidine ring and two nitrogen atoms of the amino groups. IR spectral data of TMP and its complexes showed that the intense absorption bands of TMP shown at 3469 and 3317 cm^{-1} are due to asymmetric while symmetric NH_2 stretching vibrations of the amino groups appeared at 3423, 3337, 3405,

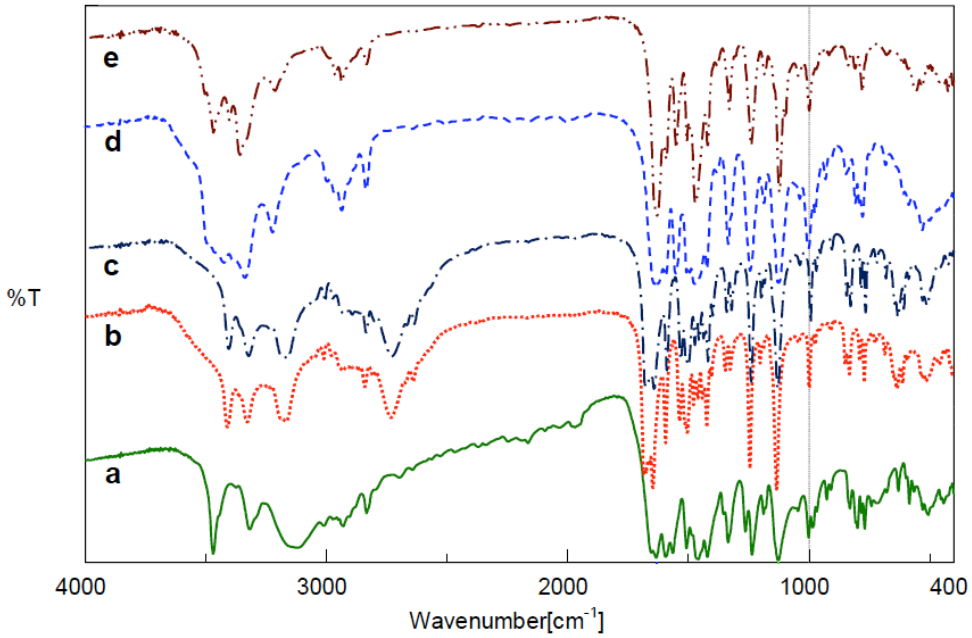

Figure 2: FTIR spectra of (**a**) TMP, (**b**) TMP-Zn, (**c**) TMP-Cu, (**d**) TMP-Fe and (**e**) TMP-Ti.

3323, 3405, 3323 and 3469, 3358 cm^{-1} in Zn(II), Ti(IV), Fe(III) and Cu(II), respectively.

Table 2: Main IR Absorption Bands NH_2 of TMP and its Complexes

Comp.	NH_2 asymmetric	NH_2 symmetric
TMP	3469	3317
TMP-Zn	3423	3337
TMP-Cu	3469	3358
TMP-Fe	3405	3323
TMP-Ti	3405	3323

These frequency shifts and intensity changes of the NH_2 group on complexation suggest that the NH_2 group is involved in coordination in case of Zn, Ti and Fe complexes.

In TMP-Cu, the band of NH_2, due to asymmetric vibrations, is present in the same region 3469. This band didn't shift with respect to the ligand. This assignment suggests that the coordination of ligand probably occurred through the pyrimidine nitrogen atom.

3.3. Electronic Spectra

The main electronic spectral absorptions are summarized in Table **3** and illustrated in Figure **3**. UV-Vis spectra of TMP and its 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 the complexes generally showed the characteristic band of TMP with some changes, both in wavelengths (λ_{max}) and intensity, together with appearance of new bands at longer wavelengths. The spectra of TMP and its complexes exhibit bands in the regions 210-215 and

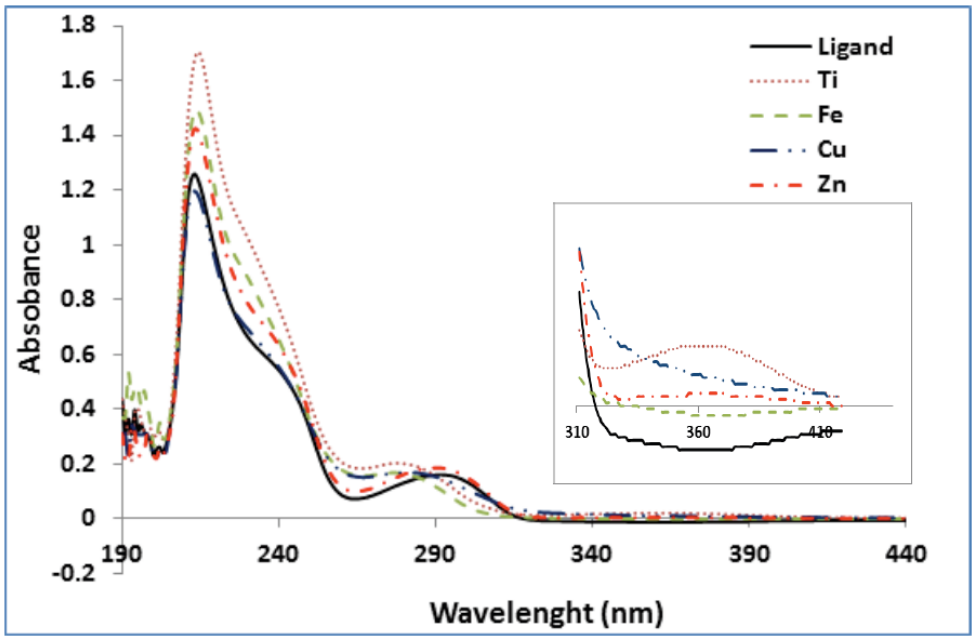

Figure 3: UV-visible spectra of the trimethoprim complexes. The insert is the UV-Vis. in the range 310-410 nm.

Table 3: Main Electronic Absorptions of TMP and its Complexes

Comp.	n- π* nm, (cm^{-1})	π-π* nm, (cm^{-1})
TMP	293 (34130)	215 (46511)
TMP-Zn	289 (34602)	210 (47619)
TMP-Cu	279 (35842)	213 (46948)
TMP-Fe	275 (36363)	214 (46729)
TMP-Ti	278(35971) 361(27701)	215(46511)

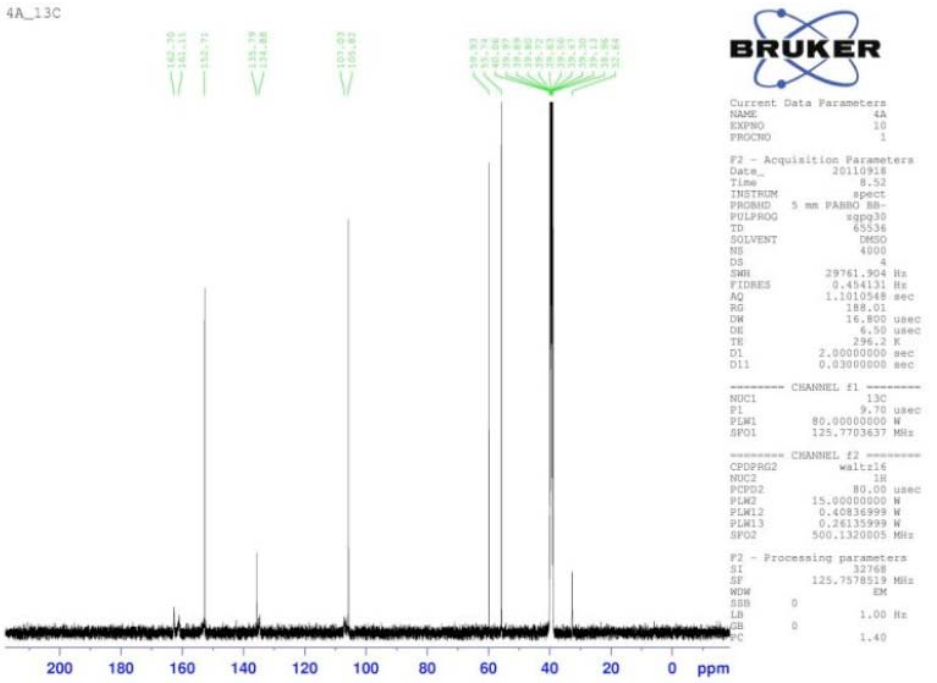

Figure 4: ^{13}C NMR of trimethoprim–Zn complex.

275-361 nm, which may be attributed to π-π∗, n-π∗ transitions, respectively.

3.4. ^{13}C NMR

The ^{13}C NMR signal of C_6 (at 155.91 ppm in TMP) [24] is shifted to high field values in both compounds (at 152.71 ppm for TMP-Zn (Figure **4**), 152.85 ppm for TMP-Fe (Figure **5**) and TMP-Ti (Figure **6**). Likewise, the coordination of TMP-M ion caused deshielding of C_5 in the pyrimidine ring (at 105.96 ppm in TMP and 107.03 ppm for TMP-Zn, 106.22 ppm for TMP-Fe and TMP-Ti).

3.5. Antimicrobial Activity

For *in vitro* anti-microbial activity, the investigated compounds were tested against the bacteria: M. L., B.

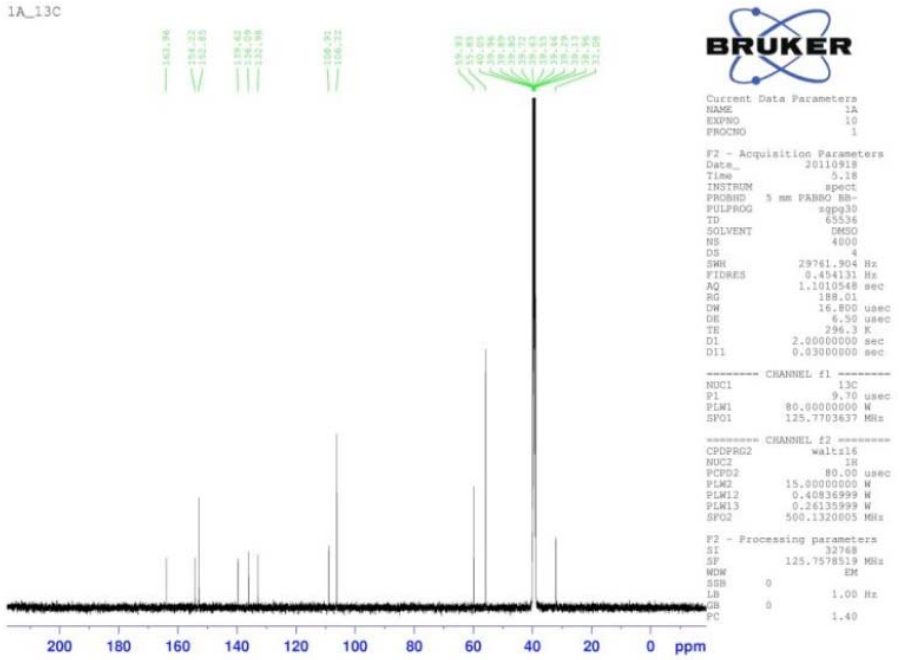

Figure 5: ^{13}C NMR of the TMP–Fe complex.

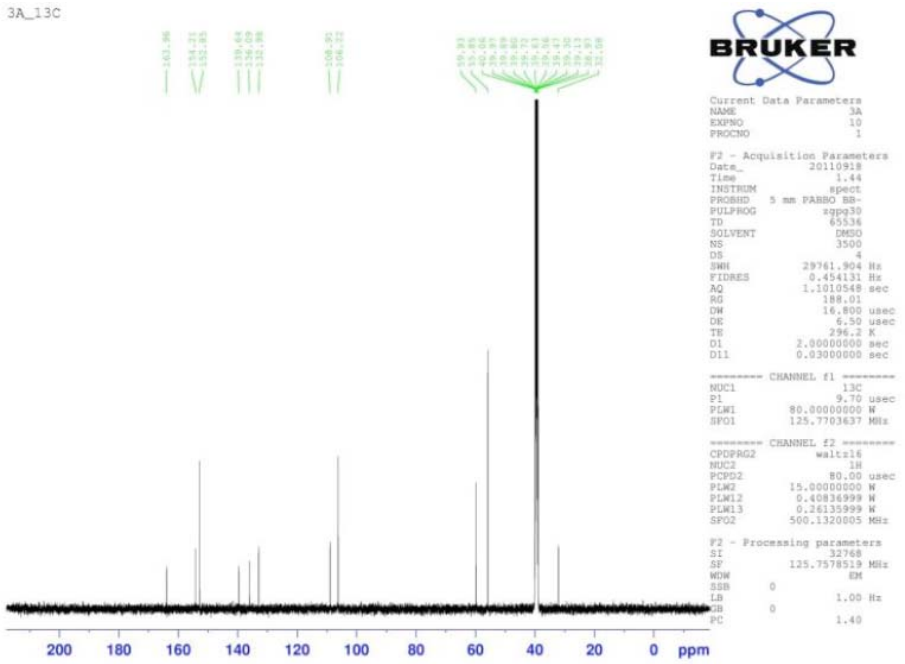

Figure 6: ^{13}C NMR of TMP–Ti complex.

Table 4: Anti-Microbial Activity of TMP and its Complexes. The Inhibition Zones (IZ) are in mm Diameter

Comp.	Gram negative	Gram positive	
	Escherichia coli	Micrococcus luteus	Bacillus subtilis
TMP-Zn	34 (+3)	34 (+3)	31 (+3)
TMP-Cu	32 (+3)	32 (+3)	28 (+3)
TMP-Fe	20 (+2)	32 (+3)	30 (+3)
TMP-Ti	31 (+3)	33 (+3)	28 (+3)
Trimethoprim	20	31	34

(+3) Highly sensitive above (21mm), (+2) Fairly sensitive (17-20 mm), (+1) slightly sensitive (16-12 mm), less than 12 (-) no effect.

subtilis and E. coli. Values indicate that all complexes displayed higher anti-microbial activity than TMP. Table **4** shows the results of the bioassay.

3.6. Anti-Cancer Activity

In vitro anti-cancer activity using Hela cells showed a good cytotoxic effect by TMP-Ti and moderate activity was observed in case of TMP-Cu with PC3 cells. Table **5** represents the cytotoxic activity of the tested compounds.

4. CONCLUSIONS

The present work describes the synthesis, characterization and *in vitro* anti-microbial and anti-

Table 5: Cytotoxic Activity of TMP-M Against Hela Cells and PC3

Comp.	PC3	Hela
	IC50 ± SD(µM)	IC50 ± SD(µM)
TMP-Zn	> 50	> 50
TMP-Cu	31.95± 0.37	45.74±0.9
TMP-Fe	47.48± 0.603	48.21± 0.87
TMP-Ti	41.45± 0.13	29.46± 0.58
Doxorubicin (as control)	0.912	3.10± 0.2

cancer evaluation of TMP and its four complexes with Zn, Cu, Fe and Ti (compounds1-4). All tested compounds exhibited an excellent anti-bacterial activity, even better than TMP. TMP-Ti displayed good anti-cancer activity *in vitro* against Hela cells while TMP-Cu showed moderate activity against PC3.

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