Turkish Journal of Chemistry
http://journals.tubitak.gov.tr/chem/
Research Article

Turk J Chem
(2013) 37: 936 – 945
© TÜBİTAK
doi:10.3906/kim-1302-36

Syntheses, characterization, and antibacterial study of titanium complexes

Raj KAUSHAL,[1,*] **Nitesh KUMAR,**[1] **Pamita AWASTHI,**[1] **Kiran NEHRA**[2]
[1]Department of Chemistry, National Institute of Technology, Hamirpur, India
[2]Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal (Sonepat), Haryana, India

Received: 19.02.2013 • **Accepted:** 01.06.2013 • **Published Online:** 04.11.2013 • **Printed:** 29.11.2013

Abstract: Titanium(II) complexes of composition $TiCl_2(L)_2$ [where L = 2,2'-bipyridine (bipy), 4,4'-dimethyl-2,2'-bipyridine (bpMe), 4,4'-dimethoxy-2,2'-bipyridine (bpoMe), 6,6'-dimethyl-2,2'-bipyridine (dpMe), adamantylamine (ada)] were prepared by reacting titanium tetrachloride and N-containing bulky ligands in predetermined molar ratios. The complexes synthesized were characterized by different spectroscopic techniques, viz. UV-visible, FTIR, ^{1}H NMR, and mass spectrometry. The stoichiometry of the complexes was established by their elemental analysis (chlorine and titanium estimation). The unit cell parameters calculated by using the powder XRD tool confirm the monoclinic unit system and P lattice type for all complexes. Antibacterial screening of the ligands as well as the complexes was carried out to check their biological potential. It was found that the complex with 4,4'-dimethyl-2,2'-bipyridine ligand was a more potent bactericide than complexes with other ligands.

Key words: Titanium, FTIR, ^{1}H NMR, mass spectra, powder XRD, 2,2'-bipyridine, antibacterial activity

1. Introduction

The role of transition metal complexes in medicinal chemistry has been known since the serendipitous discovery of platinum-based cisplatin by Rosenberg in 1969.[1,2] Transition metal complexes have been widely studied as antibacterial[3,4] and anticancer agents[5,6] for many years. Due to different oxidation states, coordination sphere, and redox potential, coordination complexes show kinetic and thermodynamic properties towards biological receptors. The nonplatinum drugs after cisplatin were budotitane and titanocene dichloride, which are titanium-based anticancer drugs.[7,8] In addition to anticancer properties, titanocene dichloride also exhibits antiviral, antiarithmetic, and anti-inflammatory activities.[9] Since titanium is present in many biomaterials such as food in the form of whitening pigment, it may be incorporated into living systems.[10] The complexes of 2,2'-bipyridine and substituted bipyridine with cobalt, copper, zinc,[11] and gold[12] have been found to show antibacterial and anticancer activity, respectively. Due to bacterial resistance to the currently available antibiotics, there has been growing interest in developing new drugs with better activity. Since metal and ligand interact with various steps of the pathogen life cycle,[13] they can be used to synthesize new drugs. Moreover, it is known that ligands having N, O, and S atoms show pronounced biological activity due to enhancement in coordination behaviour.[14,15] After chelation, metal complexes are assumed to act as antimicrobial agents due to inhibition of enzymes, interaction with intracellular biomolecules, and enhanced lipophilicity.[16] In the present paper, synthesis of

*Correspondence: kaushalraj384@gmail.com

titanium(II) complexes with nitrogen-containing ligands is reported. The structure of the synthesized complexes was confirmed by UV-visible, FTIR, ^{1}H NMR, mass spectrometry, and powder XRD techniques. The average crystallite size of the complexes, calculated by using Scherrer's formula, confirms the nano dimension of the complexes. The antibacterial screening of complexes was performed against different bacterial strains and compared with standard antibiotic ampicillin.

2. Materials and methods

Ligands (2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine, 4,4'-dimethoxy-2,2'-bipyridine, 6,6'-dimethyl-2,2'-bipyridine, and adamantylamine) from Sigma Aldrich were used as such after checking their melting points. Titanium tetrachloride and solvents were obtained from E. Merck. Solvents were purified by the standard procedure. UV-visible spectra of the complexes were recorded on a PerkinElmer Lambda 750 and FTIR spectra were recorded on a PerkinElmer 1600 spectrophotometer by making KBr pellets. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Electrospray mass spectrum (ESIMS) was obtained by using electron spray ionization on a Waters Micromass Q-Tof Micro. Powder XRD was recorded on Philips 1710 X-ray diffractometer and molecular weight was determined by Rast's method.

3. Experimental

3.1. Synthesis of bis(2,2'-bipyridyl) dichloro titanium(II), $TiCl_2(bipy)_2$, (A1)

To a white solution of titanium tetrachloride (1 g, 5.27 mmol) in dichloromethane (15 mL), a colorless solution of 2,2'-bipyridine (1.64 g, 10.54 mmol) in dichloromethane (30 mL) was added dropwise in ice cold conditions with continuous stirring and the color of the solution changed to dark yellow during addition. The reaction mixture was stirred for 20 h and resulted in a light yellow solution. The reaction mixture was refluxed for 5 h to ensure the completion of the reaction until the cessation of evolution of chlorine gas. The complex was extracted from the reaction mixture by vacuum filtration and dried under vacuum. A light yellow complex was obtained. Recrystallization of the complex was carried out in methanol. Yield 87%, mp 220 °C; UV (MeOH) λ_{max} 235, 281 nm; FTIR (KBr) $\bar{\nu}(cm^{-1})$ 3077, 3034 (C-H stretching), 1602 (C=C stretching), 1472, 1441 (C=N stretching), 1278 (C-H bending), 767 (C-H out of plane deformation), 416 (Ti-N stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ, ppm = 8.94 (d, ^{3}J = 5.1 Hz, 4H, H_6), 8.83 (d, ^{3}J = 8.1, 4H, H_3), 8.4 (t, ^{3}J = 7.9, 7.7 Hz, 4H, H_4), 7.88 (t, ^{3}J = 5.7, 6.9 Hz, 4H, H_5).

3.2. Synthesis of bis(4,4'-dimethyl-2,2'-bipyridyl) dichloro titanium(II), $TiCl_2(bpMe)_2$, (B1)

To a white solution of titanium tetrachloride (0.125 g, 0.659 mmol) in dichloromethane (10 mL), a colorless solution of 4,4'-dimethyl-2,2'-bipyridine (0.24 g, 1.31 mmol) in dichloromethane (10 mL) was added dropwise with continuous stirring in ice cold conditions. The color of solution changed to light yellow for a few minutes and then turned colorless. The reaction mixture was stirred for 45 h to ensure the completion of the reaction until the evolution of chlorine gas ceased. The solvent was removed from the reaction mixture by vacuum filtration and complex was dried under vacuum. A white complex was obtained, which was recrystallized from methanol. Yield 85%, mp 200–210 °C; UV (MeOH) λ_{max} 243, 283 nm; FTIR (KBr) $\bar{\nu}(cm^{-1})$ 3026 (C-H stretching), 1619 (C=C stretching), 1504, 1432 (C=N stretching), 1218, 1116 (C-H bending), 834 (C-H deformation), 419 (Ti-N stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ, ppm = 8.75 (d, ^{3}J = 5.5 Hz, 4H, H_6), 8.63 (s, 4H, H_3), 7.72 (d, ^{3}J = 5.4 Hz, 4H, H_5), 2.65 (s, 12H, CH_3).

3.3. Synthesis of bis(4,4'-dimethoxy-2,2'-bipyridyl) dichloro titanium(II) $TiCl_2(bpoMe)_2$, (C1)

To a colorless solution of titanium tetrachloride (0.2 g, 1.05 mmol) in benzene (15 mL), a solution of 4,4'-dimethoxy-2,2'-bipyridine (0.456 g, 2.10 mmol) in benzene (20 mL) was added dropwise in ice cold conditions. The color of the solution changed from colorless to pale yellow after addition. The reaction mixture was stirred for 3 h and then refluxed for 15 h until the evolution of chlorine gas ceased; with the passage of refluxing, a light yellow solid separated out. The solvent was removed by vacuum filtration and the complex was dried under vacuum. A light yellow complex was obtained. Recrystallization of the complex was done in methanol. Yield 95%, mp 180–185 °C (decompose); UV (MeOH) λ_{max} 215, 279 nm; FTIR (KBr) $\bar{\nu}(cm^{-1})$ 3088, 3007 (C-H stretching), 1617 (C=C stretching), 1481, 1452 (C=N stretching), 1292 (C-H bending), 825 (C-H out of plane deformation), 430 (Ti-N stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ, ppm = 8.65 (d, ^{3}J = 6.2 Hz, 4H, H_6), 8.34 (s, 4H, H_3), 7.32 (d, ^{3}J = 3.8 Hz, 4H, H_5), 4.15 (s, 12H, OCH_3).

3.4. Synthesis of bis(6,6'-dimethyl-2,2'-bipyridyl) dichloro titanium(II) $TiCl_2(dpMe)_2$, (D1)

To a colorless solution of titanium tetrachloride (0.2 g, 1.05 mmol) in benzene (15 mL), a colorless turbid solution of 6,6'-dimethyl-2,2'-bipyridine (0.388 g, 2.10 mmol) in benzene (20 mL) was added dropwise with continuous stirring. The color of solution changed from colorless to light yellow immediately. The reaction mixture was stirred for 3 h, and then refluxed for 10 h until the evolution of chlorine gas ceased. The solvent was removed through vacuum filtration and the complex was dried under vacuum. Recrystallization of the complex was done in methanol. Yield 85%, mp 170–175 °C (decompose); UV (MeOH) λ_{max} 235, 290 nm; FTIR (KBr) $\bar{\nu}$ (cm^{-1}) 3030 (C-H stretching), 1634 (C=C stretching), 1413 (C=N stretching), 1282 (C-H bending), 794 (C-H out of plane deformation), 447 (Ti-N stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ, ppm = 8.45 (d, ^{3}J = 7.8 Hz, 4H, H_3), 8.25 (t, ^{3}J = 7.9, 7.8 Hz, 4H, H_4), 7.7 (d, ^{3}J = 7.84 Hz, 4H, H_5), 2.87 (s, 12H, CH_3).

3.5. Synthesis of bis(adamantylamine) dichloro titanium(IV), $TiCl_2(ada)_2$, (E1)

To a white solution of titanium tetrachloride (0.08 g, 0.439 mmol) in dichloromethane (10 mL), a solution of adamantylamine (0.132 g, 0.878 mmol) in dichloromethane (15 mL) was added dropwise with continuous stirring in ice cold conditions. The color of the solution changed to yellow for a moment and then to orange immediately. The reaction mixture was stirred for 12 h and color changed to light yellow. Completion of the reaction was indicated by cessation of the evolution of HCl gas. The solvent was decanted out and the complex was dried under vacuum. A light yellow complex was obtained and recrystallized in methanol. Yield = 83.3%, mp 210–215 °C; UV (MeOH) λ_{max} 224 nm; FTIR (KBr) $\bar{\nu}$ (cm^{-1}) 3347 (N-H stretching), 2920, 2854 (C-H stretching), 1595 (C-C stretching), 1360 (C-N stretching), 412 (Ti-N stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ, ppm = 2.12 (s, NH proton), 1.84 (d, ^{3}J = 2 Hz, CH protons), 1.7, 1.6 (d, ^{3}J = 12.5, 12.2 Hz, CH_2 protons).

4. Results and discussion

Complexes of composition $TiCl_2(L)_2$ were prepared by reacting titanium tetrachloride and nitrogen containing ligands in 1:2 molar ratio, which can be rationalized in terms of the following chemical equations:

$$TiCl_4 + 2L \xrightarrow{CH_2CL_2 \text{ or } C_6H_6} TiCl_2(L)_2(II) + Cl_2 \quad \text{where, } L = \text{2,2-bipyridine}$$
$$= \text{4,4'dimethyl-2,2'-bipyridine}$$
$$= \text{4,4'-dimethoxy-2,2'-bipyridine}$$
$$= \text{6,6'-dimethyl-2,2'-bipyridine}$$

$$TiCl_4 + 2 \text{ adamantylamine} \xrightarrow{CH_2Cl_2} TiCl_2(\text{ada})(IV) + 2HCl$$

Elemental analyses, i.e. chlorine and titanium estimations, were performed to check the composition of the complexes (Table 1) by using Volhard's method and through gravimetrical measurements.

4.1. Electronic spectra

The electronic spectrum was recorded in 10^{-7} M solution of respective complex within 200–600 nm range in dry methanol. The values of transition observed in the UV region (Figure 1) were assigned to intraligand $\Pi \rightarrow \Pi^*$ and $n \rightarrow \Pi^*$ charge transfer transitions. The band in the range 280–285 nm due to $n \rightarrow \Pi^*$ transition of 2,2'-bipyridine and substituted bipyridine gets shifted to a lower wavelength and the band due to $\Pi \rightarrow \Pi^*$ transition gets shifted to a slightly higher wavelength in the metal complexes, confirming the coordination of ligand to the metal atom.[17] Since the metal ion has d^0 configuration, there is no possibility of d-d transition; however, the color of complexes may be due to charge transfer transitions from the ligand to metal.

Table 1. Physical and analytical data of titanium complexes.

Complex	Color	Yield (%)	Melting point (°C)	Found (calculated) %		
				Cl	Ti	MW
$TiCl_2(bipy)_2$ (A1)	Light yellow	87	220	16 (16.4)	11.9 (11.2)	430 (431)
$TiCl_2(bpMe)_2$ (B1)	White	85	200–210	14.42 (14.56)	10 (9.8)	490 (487)
$TiCl_2(bpoMe)_2$ (C1)	Light yellow	95	180–185	12.42 (12.88)	8.99 (8.69)	550 (551)
$Ti(Cl)_2(dpMe)_2$ (D1)	Light yellow	85	170–175	14.2 (14.57)	9 (9.8)	490 (487)
$TiCl_2(ada)_2$ (E1)	Light yellow	83	210–215	17.7 (17)	10.6 (11)	420 (419)

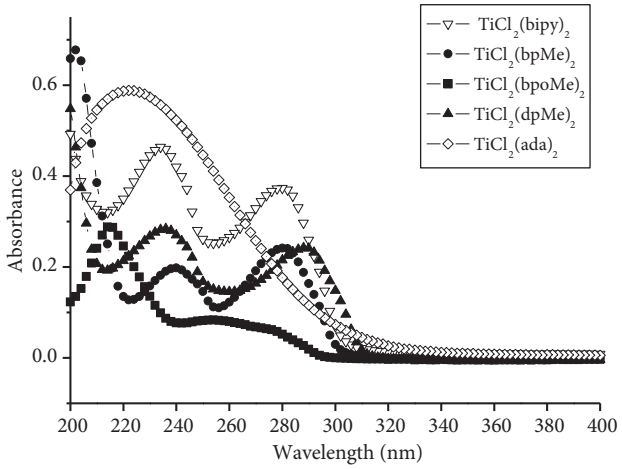

Figure 1. Electronic spectra of complexes.

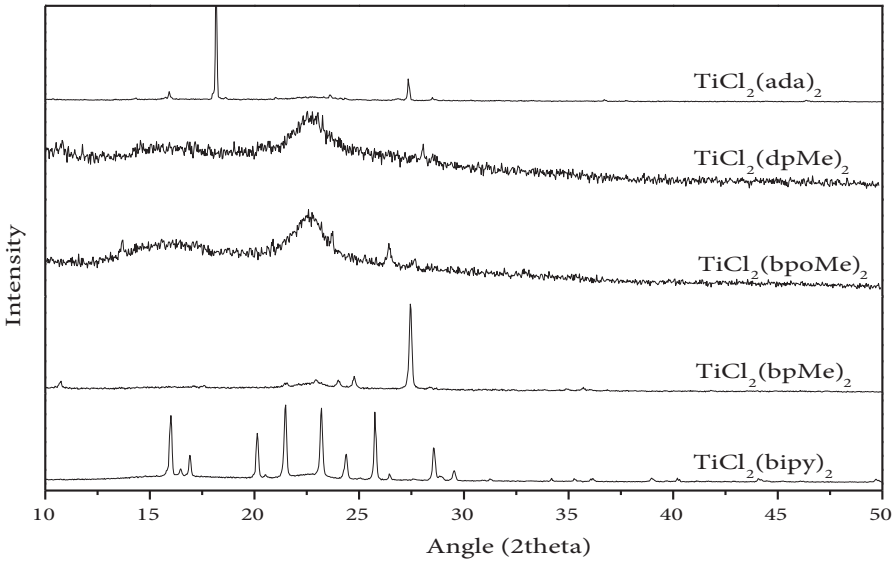

Figure 2. Powder XRD pattern of complexes.

4.2. FTIR study

Generally, the $\bar{\nu}_{C=C}$ and $\bar{\nu}_{C=N}$ stretching vibrations of 2,2'-bipyridine and substituted bipyridine are observed around 1580–1590 cm^{-1}, which get shifted by 20–25 cm^{-1} to higher wave numbers on coordination.[18] The absorption bands due to $\bar{\nu}_{C=C}$ in these ligands at around 1589 cm^{-1} get shifted to 1602, 1619, 1617, and 1634 cm^{-1} for complexes A1, B1, C1, and D1, respectively. This upward shift of about 20–25 cm^{-1} due to $\bar{\nu}_{C=C}$ stretching shows increased conjugation due to complexation with metal. This shift may be due to a reduction in electron density after complexation with metal, and spatial effects (field effect, steric effect, and ring strain) may also be responsible for the frequency change in the vibrational spectrum. Bands around 3000–2900 cm^{-1} were due to $\bar{\nu}_{C-H}$ stretching of the ring, which remained unaltered even after the formation of the complex. The absorption bands of complexes A1, B1, C1, and D1 at 1026, 1061, 1104, 1156, 1236, 1316 cm^{-1}; 1026, 1116, 1218, 1292, 1363 cm^{-1}; 1076, 1117, 1233, 1292, 1317 cm^{-1}; and 1010, 1047, 1089, 1184, 1282, 1336 cm^{-1}, respectively, were assigned to in-plane $\bar{\nu}_{C-H}$ bending vibrations of respective ligands. The bands at 1082 and 1305 cm^{-1} were attributed to $\bar{\nu}_{C-N}$ stretching of adamantylamine in E1 complex. Moreover, the absorption at 1600 cm^{-1} in complex E1 represents $\bar{\nu}_{N-H}$ bending and formation of the single peak at 3347 cm^{-1} in complex E1 indicates deprotonation of primary amine, i.e. adamantylamine. Appearance of new absorption bands at 416, 419, 430, 447, and 412 cm^{-1} can be attributed to $\bar{\nu}_{Ti-N}$ stretching in complexes A1, B1, C1, D1, and E1, respectively, indicating the coordination of nitrogen atom to titanium.[19]

4.3. ^{1}H NMR spectra

^{1}H NMR spectra of the complexes were recorded in DMSO-d_6 solution using TMS as internal standard and the data are given in Table 2. There is a considerable downfield shift in 2,2'-bipyridine and substituted bipyridine protons on complexation in complexes A1, B1, C1, and D1. The downfield shift in delta values of protons indicates the coordination of ligand to metal. These shifts may be assigned to the deshielding of protons due to transfer of electron density from aromatic protons to the metal atom (N$\rightarrow$M). Similar observations have been reported for gold(III) complexes with modified bipyridine and bipyridyl amine ligands.[12] The

appearance of signals in the upfield region (2.12–1.62 ppm) in the spectra of complex E1 confirms the presence of adamantylamine protons. Integration of signals also supports the formation of complexes.

Table 2. ^{1}H NMR data of complexes (δ, ppm).

Ligand/Complex	Bipyridine				CH_3	OCH_3	Adamantylamine		
	H^6	H^3	H^4	H^5	-		NH	CH	CH_2
2,2'-bipyridine	8.68	8.4	7.82	7.31					
4,4'-dimethyl-2,2'-bipyridine	8.54	8.26	-	7.15	2.45				
4,4'-dimethoxy-2,2'-bipyridine	8.47	7.99	-	6.85		3.95			
6,6'-dimethyl-2,2'-bipyridine	-	7.15	7.68	8.18	2.63				
adamantylamine	-	-	-	-	-		2.04	1.64	1.6, 1.29
$TiCl_2(bipy)_2$ (A1)	8.94	8.83	8.4	7.88			-	-	-
$TiCl_2(bpMe)_2$ (B1)	8.75	8.63	-	7.72	2.65		-	-	-
$TiCl_2(bpoMe)_2$ (C1)	8.65	8.34	-	7.32	-	4.15			
$TiCl_2(dpMe)_2$ (D1)	-	8.45	8.25	7.70	2.87				
$TiCl_2(ada)_2$ (E1)							2.12	1.84	1.69, 1.62

4.4. Electrospray mass spectral data of titanium complexes

The formation of complexes was further established by recording the mass spectrum of each of the complexes. The mass spectrum of $TiCl_2(bipy)_2$ showed a base peak due to the $C_{10}H_8N_2$ fragment that appeared at m/z = 157. Complexes $TiCl_2(bpMe)_2$ and $TiCl_2(bpoMe)_2$ showed their respective base peaks at m/z = 325 and 185 due to formation of $C_{18}H_{18}N_3Ti$ and $C_{12}H_{12}N_2$ fragment ions, respectively. Complex $TiCl_2(bpoMe)_2$ also shows peaks at m/z = 202 and 239 due to $TiC_{10}H_6N_2$ and $TiClC_{10}H_6N_2$ fragments. The mass spectrum of complex $TiCl_2(dpMe)_2$ shows a base peak at m/z = 185 due to 6,6'-dimethyl-2,2'-bipyridine ligand with 100% intensity. A detailed description of the results of mass spectrometry of all complexes is given in Table 3. Complex $TiCl_2(ada)_2$ shows a base peak due to fragment ion $TiCl_2C_{12}H_{17}N_2$ at m/z = 306. The presence of different fragment peaks in these complexes may be considered to support their stoichiometric formulation.

Table 3. Electrospray mass spectral data.

Complex	Major ESMS fragment ions (m/z, %)
$TiCl_2(bipy)_2$	$C_{10}H_8N_2$ (157, 100%); $TiCl_2C_{15}H_{12}N_3$+Na^+ (377, 17%); $TiCl_2C_{20}H_{16}N_4$+Na^+ (453, 7%).
$TiCl_2(bpMe)_2$	$C_{12}H_{12}N_2$ (185, 98%); $C_{12}H_{12}N_2TiCl_2$ (304, 25%); $C_{18}H_{18}N_3Ti$ (325, 100%), $C_{21}H_{15}N_4TiCl$ (406, 4%); $C_{20}H_{12}N_4Cl_2Ti$ (427, 3%), $C_{24}H_{24}N_4TiCl$ (453, 17%).
$TiCl_2(bpoMe)_2$	$C_{12}H_{12}N_2O_2$ (217, 100%); $TiC_{10}H_6N_2$ (202, 5%); $TiClC_{10}H_6N_2$ (239, 100%).
$TiCl_2(dpMe)_2$	$C_{12}H_{12}N_2$ (185, 100%); $TiClC_{11}H_9N_2$ (249, 35%); $TiClC_{12}H_{12}N_2$ (265, 25%); $TiC_{15}H_9N_3$ (276, 100%); $TiCl_2C_{21}H_{15}N_4$ (443, 5%); $TiClC_{24}H_{24}N_4$ (453, 10%)
$TiCl_2(ada)_2$	$C_9H_{14}NTi$ (185, 99%); $C_9H_{14}NTiCl_2$ (249, 7%); $C_{11}H_{17}N_2TiCl_2$ (299, 3%); $C_{12}H_{17}N_2TiCl_2$ (306, 100%); $C_{16}H_{25}N_2TiCl_2$ (360, 6%); $C_{19}H_{30}N_2TiCl_2$ (408, 95%); $C_{20}H_{32}N_2TiCl_2$ (419, 15%).

4.5. Powder XRD study

The XRD study was done on a Philips 1710 X-ray diffractometer with CuKα radiation (λ = 1.5405 Å). Scherrer's equation D = ($\lambda\times$ 0.9)/($\beta\times$ Cosθ),[20] [where D is the crystallite size of (h k l) plane, β is full width half maximum (FWHM) in radians, and λ is the wavelength of incident radiation] was used to calculate the

crystallite size (d_{XRD}) of complexes. The calculated crystallite size was found to be 77.5, 71.2, 4.6, 3.9, and 206 nm for complexes A1, B1, C1, D1, and E1 respectively, which confirm their nanocrystalline nature. The unit cell parameters were calculated with the help of powder X software[21] and are summarized in Table 4. Figure 2 shows that peaks for complexes $TiCl_2(bpoMe)_2$ and $TiCl_2(dpMe)_2$ become broader as the grain size decreases. On the basis of XRD and other spectroscopic techniques, an octahedral geometry may be proposed for A1, B1, C1, and D1 complexes and a tetrahedral geometry for E1 complex.[22]

Table 4. XRD data of complexes.

Empirical formula	$TiCl_2C_{20}H_{16}N_4$	$TiCl_2C_{24}H_{24}N_4$	$TiCl_2C_{24}H_{24}N_4O_4$	$TiCl_2C_{24}H_{24}N_4$	$TiCl_2C_{20}H_{32}N_2$
Formula weight	431	487	551	487	419
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Lattice type	P	P	P	P	P
a (Å)	14	14	16.5	13.5	14.5
b (Å)	13	12	16	15	17.5
c (Å)	17	17	17	16	20
α (°)	90	90	90	90	90
β (°)	98	113	94	105	115
γ (°)	90	90	90	90	90
Crystallite size (nm)	77.5	71.2	4.6	3.9	206
V (Å)^3	3094	2856	4488	3240	5075
2θ start	10	10	10	10	10
2θ end	50	50	50	50	50
Radiation	Cu	Cu	Cu	Cu	Cu
Wavelength	1.54	1.54	1.54	1.5	1.54

4.6. Antibacterial activity

Antimicrobial activity of ligands and titanium complexes was determined by using agar well diffusion[23] against 10 pathogenic bacterial strains. The activity was determined at a concentration of 1 mg/mL in dimethylformamide (DMF) against 4 gram-positive, viz. *Bacillus cereus* MTCC 6728, *Micrococcus luteus* MTCC 1809, *Staphylococcus aureus* MTCC 3160, and *Staphylococcus epidermidis* MTCC 3086, and 6 gram-negative, viz. *Aeromonas hydrophila* MTCC 1739, *Aclaligenes faecalis* MTCC 126, *Shigella sonnei* MTCC 2957, *Klebsiella pneumoniae* MTCC 3384, *Pseudomonas aeruginosa* MTCC 1035, and *Salmonella typhimurium* MTCC 1253, bacterial strains. The diameter of zone of inhibition produced by complexes was measured in millimeters and compared with the standard antibiotic ampicillin (250 μg/mL). From the results, it was established that in synthesized complexes the $TiCl_2(bpMe)_2$ complex was more potent than the standard antibiotic ampicillin (Table 5). It is also evident that $TiCl_2(bpMe)_2$ complex possesses more activity against all the bacterial strains than other complexes. The complex $TiCl_2(bpMe)_2$ appears to be more effective than 4,4'-dimethyl-2,2'-bipyridine ligand, but complexes $TiCl_2(dpMe)_2$ and $TiCl_2(ada)_2$ show antibacterial activity similar to their respective ligands. It was observed that the position of substituent (electron releasing/withdrawing group) on the bipyridine ring plays a significant role in biological efficacy. The synthesized complexes showed different activity towards various pathogenic strains, which may be attributed to their unique biocidal mechanism, hydrophilicity, and inability to penetrate inside the cell membrane. It is well established that beside chelation other factors such as lability of ligand, nature of metal ion, nature of ligand, coordination sites, geometry of the complex, concentration, conductivity, dipole moment, and cell permeability (influenced by the presence of metal ion) may

be responsible for increased activity.[24,25] From our studies we find that position of substituent, aromaticity, lipophilicity, and lability of ligand are important factors in determining the antibacterial activity of a complex.

Table 5. Antibacterial activity results of the ligands and their titanium complexes.

Ligand/Complex	Diameter of inhibition in mm after 24 h									
	Gram-positive				Gram-negative					
	B. cereus	*M. luteus*	*S. aureus*	*S. epidermidis*	*A. hydrophila*	*A. faecalis*	*S. sonnei*	*P. pneumoniae*	*P. aeruginosa*	*S. typhimurium*
bipy	12	10	9	7	9	7	9	7	9	8
bpMe	13	11	11	9	10	9	11	10	13	12
bpoMe	14	10	11	13	9	9	10	-	11	12
dpMe	5	11	7	-	7	6	6	-	6	6
ada	6	5	5	7	5	8	-	4	-	6
$TiCl_2(bipy)_2$	6	5	6	6	-	7	-	-	-	6
$TiCl_2(bpMe)_2$	15	13	12	9	13	12	12	7	5	11
$TiCl_2(bpoMe)_2$	9	6	11	6	8	7	8	6	6	8
$TiCl_2(dpMe)_2$	6	6	6	-	8	6	8	6	6	6
$TiCl_2(ada)_2$	6	7	8	5	7	6	6	6	-	6
Ampicillin	7	8	11	9	10	9	5	7	10	9

4.7. MIC measurements

MIC is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after 24 h incubation at 37 °C. Ten bacterial species, 4 gram-positive (*B. cereus*, *M. luteus*, *S. aureus*, and *S. epidermidis*), and 6 gram-negative (*A. hydrophila*, *A. faecalis*, *S. sonnei*, *K. pneumoniae*, *P. aeruginosa*, and *S. typhimurium*), were used. The calculation of MICs involves a semiquantitative test procedure. It gives an approximate value of minimum concentration of an antibacterial agent needed to prevent bacterial growth. The serial dilution method was used for the determination of MICs of these complexes. The method involves the addition of 10 μL of microbes grown in nutrient broth and 10 μL of solutions of varying concentrations of each complex dissolved in DMF to small tubes containing 300 μL of nutrient broth. The end result of the test is the minimum concentration of the complex that gives a clear solution, i.e. no visible growth after 24 h in a BOD incubator at 37 °C. Amongst the various bacterial strains tested, the lowest MICs were obtained against *S. sonnei* and *A. hydrophila*, showing that these bacteria were most sensitive to the complexes. The MICs of all complexes against each bacterial strain are given in Table 6. The lowest MICs were observed for the complex $TiCl_2(bpMe)_2$ against most of the bacterial strains, which indicates the high effectiveness of this complex.

We have described the synthesis of titanium complexes with bipyridine, substituted bipyridine, and adamantylamine ligands. The synthesized complexes were characterized by using FTIR, UV-visible, ^{1}H NMR, and mass spectrometry techniques. The downfield shift in protons and formation of $\bar{\nu}_{Ti-N}$ bond in the far IR region (400–450 cm^{-1}) confirms the formation of complexes. Scherrer's equation was used to calculate the crystallite size (d_{XRD}) of complexes, and the calculations showed that the complexes were nanocrystalline. The study of antibacterial screening showed variation in activity across different complexes, and $TiCl_2(bpMe)_2$ complex was found to be the most potent complex.

Table 6. Minimum inhibitory concentrations (MICs) of titanium complexes (μg/mL).

Complex	Gram-positive				Gram-negative					
	B. cereus	*M. luteus*	*S. aureus*	*S. epidermidis*	*A. hydrophila*	*A. faecalis*	*S. sonnei*	*P. pneumoniae*	*P. aeruginosa*	*S. typhimurium*
$TiCl_2(bipy)_2$	62.5	1000	62.5	1000	500	500	125	–	–	500
$TiCl_2(bpMe)_2$	31.25	125	62.5	250	250	125	62.5	1000	250	125
$TiCl_2(bpoMe)_2$	1000	1000	500	1000	500	250	500	250	1000	500
$TiCl_2(dpMe)_2$	500	500	1000	-	250	1000	250	31.25	1000	1000
$TiCl_2(ada)_2$	1000	500	31.25	1000	500	1000	125	250	500	1000

References

1. Cepeda, V.; Fuertes, M. A.; Castilla, J.; Alonso, C.; Quevedo, C.; Perez, J. M. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 3–18.
2. Reedijk, J. *Chem. Rev.* **1999**, *99*, 2499–2510.
3. Kamalakannan, P.; Venkappayya, D. *J. Inorg. Biochem.* **2002**, *90*, 22–37.
4. Islam, M. S.; Farooque, M. A.; Bodruddoza, M. A. K.; Mosaddik, M. A.; Alam, M. S. *Online J. Biol. Sci.* **2002**, *2*, 797–799.
5. Marzano, C.; Pellei, M.; Colavito, D.; Alidori, S.; Lobbia, G. G.; Gandin V.; Tisato, F.; Santini, C. *J. Med. Chem.* **2006**, *49*, 7317–7324.
6. Immel, T. A.; Groth, U.; Huhn, T.; Ohlschlager, P. *PLoS One.* **2011**, *6*, e17869.
7. Melendez, E. *Crit. Rev. Oncol. Hemat.* **2002**, *42*, 309–315.
8. Dubler, E.; Buschmann, R.; Schmalle, H. W. *J. Inorg. Biochem.* **2003**, *95*, 97–104.
9. Fairlie, D. P.; Whitehouse, M. W.; Broomhead, J. A. *Chem.-Biol. Interact.* **1987**, *61*, 277–291.
10. Tshuva, E. Y.; Peri, D. *Coord. Chem. Rev.* **2009**, *253*, 2098–2115.
11. Agwara, M. O.; Ndifon, P. T.; Ndosiri, N. B.; Paboudam, A. G.; Yufanyi, D. M.; Mohamadou, A. *B. Chem. Soc. Ethiopia.* **2010**, *24*, 383–389.
12. Casini, A.; Diawara, M. C.; Scopelliti, R.; Zakeeruddin, S. M.; Gratzel, M.; Dyson, P. J. *Dalton Trans.* **2010**, *39*, 2239–2245.
13. Travis, J.; Potempa, J. *Biochim. Biophys. Acta.* **2000**, *1477*, 35–50.
14. Halder, S.; Peng, S.-M.; Lee, G.-H.; Chatterjee, T.; Mukherjee, A.; Dutta, S.; Sanyal, U.; Bhattacharya, S. *New J. Chem.* **2008**, *32*, 105–114.
15. Kovala-Demertzi, D.; Dermertzis, M A.; Miller, J R.; Papadopoulou, C.; Dodorou, C.; Filousis, G. *J. Inorg. Biochem.* **2001**, *86*, 555–563.
16. Dharmaraj, N.; Viswanathamurthi, P.; Natarajan, K. *Transit. Metal Chem.* **2001**, *26*, 105–109.
17. Poonia, K.; Swami, M.; Chaudhary, A.; Singh, R. V. *Indian J. Chem.* **2008**, *47A*, 996–1003.
18. Shi, X.-M; Wang, H.-Y.; Li, Y.-B.; Yang, J.-G.; Chen, L.; Hui, G.; Xu, W.-Q.; Zhao, B. *Chem. Res. Chinese Univ.* **2010**, *26*, 1011–1015.
19. Gülcan, M.; Sönmez, M.; Berber, İ. *Turk. J. Chem.* **2012**, *36*, 189–200.

20. Dhanaraj, C. J.; Nair, M. S. *Eur. Polym. J.* **2009**, *45*, 565–572.

21. Ade, S. B.; Deshpande, M. N.; Kolhatkar, D. G. *Int. J. Chem. Tech. Res.* **2012**, *4*, 474–478.

22. Priya, N. P.; Arunachalam, S. V.; Sathya, N.; Chinnusamy, V.; Jayabalakrishnan, C. *Transit. Metal Chem.* **2009**, *34*, 437–445.

23. Sathisha, M. P.; Revankar, V. K.; Pai, K. S. R. *Met.-Based Drugs.* **2008**, *2008*, 1–11.

24. Murukan, B.; Mohanan, K. *Transit. Metal Chem.* **2006**, *31*, 441–446.

25. Supuran, C. T.; Scozzafava, A.; Saramet, I.; Banciu, M. D. *J. Enzyme Inhib. Med. Chem.* **1998**, *13*, 177–194.