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Ditolyldithiophosphates of titanium: Synthesis, characterization and *in vitro* antimicrobial and cytotoxic studies

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ABSTRACT

Ditolyldithiophosphates of titanium corresponding to $[(C_5H_5)_2Ti\{S_2P(OAr)_2\}_nCl_{2-n}]$ (Ar = o-, m-, p-$CH_3C_6H_4$, p-Cl-m-$CH_3C_6H_3$; n = 1 and 2) have been synthesized and characterized by elemental analyses, IR, mass and heteronuclear NMR (^1H, ^{13}C and ^{31}P) spectroscopic analyses. The combined DTA/DTG thermal analysis of $[\{(p\text{-}CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2]$ has yielded final thermolysis product as TiS_2. Cyclic voltammetry probed the redox capabilities of $[\{(m\text{-}CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2]$. Comparison of antimicrobial activity of the ligands and complexes has shown that the complexes are more effective than the ligands. The in vitro cytotoxic investigations against the cultivated human cell lines revealed that the titanium complexes are more active.

Keywords: Ditolyldithiophosphates, Titanium, Thermogravimetric analysis, Antimicrobial, Cytotoxicity.

INTRODUCTION

In recent decades the field of drug designing has seen a surge toward pharmaceuticals that are inorganic in nature [1]. A variety of increasingly effective titanocene derivatives have been developed for use as chemotherapeutic agents since titanocene dichloride was discovered to possess antitumor characteristics [2]. In fact, titanocene is the first non-platinum coordination complex to undergo clinical trials [3]. The transition metal complexes with sulfur based donors are of significant interest as synthetic analogs for the active sites of metalloproteins since these are known to possess affinity toward sulfur ligands [4]. The interest in this research area has grown considerably in the recent years [5].

The biological aspect of dithiophosphates has been well established in the rapidly growing field of phosphorus-sulfur chemistry [6-9]. The dithiophosphates have received much attention for their extensive applications as biocides [10] analytical reagents [11], antiwear and antioxidant additives in motor oils [12]. Cyclic voltammetry has been used to study the electrochemistry of the new compounds. The examination of electrochemistry of bis(cyclopentadienyl)titanium dichloride has demonstrated a single electron redox process [13]. Further, the dependence of standard potential on steric behavior of cyclopentadienyl rings has also been observed [14]. Recently, the complexes of alkylenedithiophosphates with titanocene and zirconocene have been reported [15-17]. However, no attention has yet been given to ditolyldithiophosphate complexes of titanocene. Considering the promising antitumor properties of titanocene dichloride it seemed reasonable to investigate the titanium complexes with ditolyldithiophosphates using titanocene. Moreover, considering the extensive biological features of titanocene and the ligand, the antimicrobial as well as cytotoxic screening studies have been reported herein.

EXPERIMENTAL SECTION

Titanocene dichloride (Himedia) was used as supplied. Solvents (toluene and dichloromethane) were distilled and dried before use. The sodium salts of *O,O'*-(*o*-, *m*-, *p*-, *p*-Cl-*m*-ditolyl)dithiophosphates were synthesized according

to the literature procedure [18]. Moisture was carefully excluded throughout the experimental manipulations by using standard Schlenk's techniques. Infrared spectra were recorded in the range of 4000-200 cm^{-1} on a Perkin Elmer-spectrum RX1 FT-IR spectrophotometer as KBr pellets. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II 400 (400 MHz) using TMS as internal reference for ^{1}H and ^{13}C NMR and H_3PO_4 (85%) as external reference for ^{31}P NMR. The ESI mass spectra were recorded on VG 70-S spectrophotometer. The thermogram was analyzed by using Perkin Elmer, diamond TG/DTA instrument. Recrystallized alumina sample holder was used and the heating rate of 20 ^{o}C per minute. The thermogram was recorded in the temperature range from 30 ^{o}C to 1000 ^{o}C. The experiment was carried out under a flow rate of 50 mL per minute of nitrogen atmosphere. The cyclic voltammogram was recorded on Metrom Autolab. The potential is applied between the reference electrode (Ag/AgCl) and the working electrode (Gold electrode) and the current is measured between the working electrode and the counter electrode (Platinum wire). 0.1 M phosphate buffer solution (pH = 7.0) was used. Titanium was estimated gravimetrically as TiO_2 [18]. Chlorine was estimated by Volhard's method[18]. Elemental analyses (C, H, N, S) were conducted using the Elemental Analyser Vario EL-III.

2.1. Synthesis of *bis*(cyclopentadienyl)(*O,O'-o*-ditolyldithiophosphato)titaniumchloride(IV) [{(*o*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$Cl] (1)

Complex (**1**) was prepared by addition of dichloromethane solution (20 mL) of titanocene dichloride, $(C_5H_5)_2TiCl_2$, (0.75 g, 3.01 mmol) to a dichloromethane solution (30 mL) of sodium *O,O'-o*-ditolyldithiophosphate, (*o*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) dropwise with constant stirring which was accompanied with increasing intensity of red color of the solution. The contents were refluxed for 3 h with constant stirring until there was no further change in the bright red color of the reaction mixture. The precipitated sodium chloride was removed by filtration using a funnel fitted with G-4 disk. The removal of volatiles from the filtrate in *vacuo* yielded [{(*o*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$Cl] as red solid. Yield: 1.43 g (91%); M.Pt.: 218-219 ^{o}C; *Anal*. Calcd. for $C_{24}H_{24}O_2PS_2ClTi$: Calculated (%): C, 55.13; H, 4.63; S, 12.27; Cl, 6.78; Ti, 9.15, Found (%): C, 54.83; H, 4.51; S, 12.02; Cl, 6.59; Ti, 8.97; FTIR (cm^{-1}): 1239.4, s [*v*(P)−O−C], 973.4, s [*v*P−O−(C)], 859.2, s [*v*P═S], 681.7, m [*v*P−S], 431.9, w [*v*Ti−S], 363.2, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.2 (s, 6 H, CH_3), 6.6 (d, 2 H^{e}), 6.9 (d, 2 H^{b}), 7.2 (t, 2 H^{d}), 7.3 (t, 2 H^{c}), 5.7 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 91.4; ^{13}C NMR ($CDCl_3$, ppm): 20.8 (CH_3), 121.9 (C^{6}), 124.6 (C^{2}), 129.5 (C^{4}), 130.1 (C^{3}), 138.3 (C^{5}), 147.7 (C^{1}), 121.3 (C_5H_5); ESI MS (*m/z*) (%): [{(*o*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$(Cl)]=522.8(7); [{(*o*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$] = 487.4 (5); [{($C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$] = 457.3 (11); [(O_2PS_2)Ti($C_5H_5)_2$] = 305.2 (8); [($C_5H_5)_2$Ti] = 178.1 (14); [*o*-$CH_3C_6H_4O$] = 107.1 (78).

2.2. Synthesis of *bis*(cyclopentadienyl)(*O,O'-m*-ditolyldithiophosphato)titaniumchloride(IV) [{(*m*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$Cl] (2)

Complex (**2**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'-m*-ditolyldithiophosphate, (*m*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.75 g, 3.01 mmol) were used. Yield: 1.40 g (89%); M.Pt.: 220-221 ^{o}C; *Anal*. Calcd. for $C_{24}H_{24}O_2PS_2ClTi$: Calculated (%): C, 55.13; H, 4.63; S, 12.27; Cl, 6.78; Ti, 9.15, Found (%): C, 54.96; H, 4.42; S, 12.13; Cl, 6.66; Ti, 8.89; FTIR (cm^{-1}): 1243.9, s [*v*(P)−O−C], 967.3, s [*v*P−O−(C)], 846.6, s [*v*P═S], 662.6, m [*v*P−S], 437.8, w [*v*Ti−S], 386.3, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.3 (s, 6 H, CH_3), 6.5 (s, 2 H^{a}), 6.7 (d, 2 H^{e}), 6.8 (d, 2 H^{c}), 7.0 (t, 2 H^{d}), 5.8 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 93.3; ^{13}C NMR ($CDCl_3$, ppm): 19.9 (CH_3), 113.4 (C^{2}), 132.5 (C^{6}), 120.5 (C^{4}), 127.2 (C^{5}), 128.9 (C^{3}), 152.5 (C^{1}), 117.4 (C_5H_5).

2.3. Synthesis of *bis*(cyclopentadienyl)(*O,O'-p*-ditolyldithiophosphato)titaniumchloride(IV) [{(*p*-$CH_3C_6H_4O)_2PS_2$}Ti($C_5H_5)_2$Cl] (3)

Complex (**3**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'-p*-ditolyldithiophosphate, (*p*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.75 g, 3.01 mmol) were used. Yield: 1.41 g (90%); M.Pt.: 224-225 ^{o}C; *Anal*. Calcd. for $C_{24}H_{24}O_2PS_2ClTi$: Calculated (%): C, 55.13; H, 4.63; S, 12.27; Cl, 6.59; Ti, 9.15, Found (%): C, 54.91; H, 4.48; S, 12.09; Cl, 6.61; Ti, 8.93; FTIR (cm^{-1}): 1267.2, s [*v*(P)−O−C], 978.2, s [*v*P−O−(C)], 831.4, s [*v*P═S], 672.5, m [*v*P−S], 429.6, w [*v*Ti−S], 375.8, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.1 (s, 6 H, CH_3), 6.8 (d, 4 $H^{a,e}$), 7.1 (d, 4 $H^{b,d}$) 5.8 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 91.2; ^{13}C NMR ($CDCl_3$, ppm): 20.4 (CH_3), 127.2 ($C^{2,6}$), 129.7 (C^{4}), 132.6 ($C^{3,5}$), 153.3 (C^{1}), 118.4 (C_5H_5).

2.4. Synthesis of *bis*(cyclopentadienyl)(*O,O'-p*-chloro-*m*-ditolyldithiophosphato)titaniumchloride(IV) [{(*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2$}Ti($C_5H_5)_2$Cl] (4)

Complex (**4**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'-p*-Cl-*m*-ditolyldithiophosphate, (*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2Na$, (1.00 g, 2.49 mmol) and titanocene dichloride (0.62 g, 2.49 mmol) were used. Yield: 1.35 g (92%); M.Pt.: 221-222 ^{o}C; *Anal*. Calcd. for $C_{24}H_{22}O_2PS_2Cl_3Ti$: Calculated (%): C, 48.71; H, 3.75; S, 10.84; Cl, 17.97; Ti, 8.09, Found (%): C, 48.54; H, 3.59; S, 10.78; Cl, 17.76; Ti, 7.87; FTIR (cm^{-1}): 1226.9, s [*v*(P)−O−C], 956.0, s [*v*P−O−(C)], 877.7, s [*v*P═S], 687.4, m [*v*P−S], 444.8, w [*v*Ti−S], 368.5, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.2 (s, 6 H, CH_3), 6.3 (s, 2 H^{a}), 6.5 (d, 2 H^{e}), 7.1 (d, 4 H^{d}), 5.9 (s, 10 H, C_5H_5);

^{31}P NMR ($CDCl_3$, ppm): 94.2; ^{13}C NMR ($CDCl_3$, ppm): 19.1 (CH_3), 119.4 (C^2), 121.8 (C^6), 128.5 (C^3), 129.6 (C^5), 131.7 (C^4), 151.5 (C^1), 117.1 (C_5H_5).

2.5. Synthesis of *bis*(cyclopentadienyl)*bis*(*O,O'*-*o*-ditolyldithiophosphato)titanium(IV) [{(*o*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] (5)

Complex (**5**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'*-*o*-ditolyldithiophosphate, (*o*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.37 g, 1.49 mmol) were used. Yield: 1.08 g (90%); M.Pt.: 239-240 °C; *Anal*. Calcd. for $C_{38}H_{38}O_4P_2S_4Ti$: Calculated (%): C, 57.28; H, 4.81; S, 16.10; Ti, 6.01, Found (%): C, 57.19; H, 4.63; S, 15.93; Ti, 5.83; FTIR (cm^{-1}): 1253.1, s [*v*(P)−O−C], 982.1, s [*v*P−O−(C)], 807.4, s [*v*P═S], 661.4, m [*v*P−S], 442.5, w [*v*Ti−S], 372.3, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.3 (s, 12 H, CH_3), 6.5 (d, 4 H^e), 6.7 (d, 4 H^b), 7.0 (t, 4 H^d), 7.2 (t, 4 H^c), 5.8 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 92.1; ^{13}C NMR ($CDCl_3$, ppm): 18.1 (CH_3), 117.2 (C^6), 128.8 (C^2), 135.8 (C^4), 134.3 (C^3), 136.2 (C^5), 149.2 (C^1), 120.1 (C_5H_5).

2.6. Synthesis of *bis*(cyclopentadienyl)*bis*(*O,O'*-*m*-ditolyldithiophosphato)titanium(IV) [{(*m*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] (6)

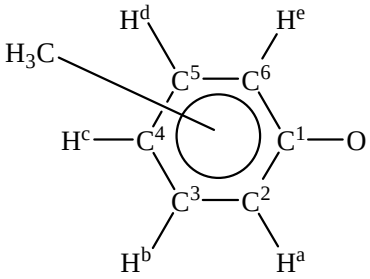
Complex (**6**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'*-*m*-ditolyldithiophosphate, (*m*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.37 g, 1.49 mmol) were used. Yield: 1.09 g (91%); M.Pt.: 241-242 °C; *Anal*. Calcd. for $C_{38}H_{38}O_4P_2S_4Ti$: Calculated (%): C, 57.28; H, 4.81; S, 16.10; Ti, 6.01, Found (%): C, 57.11; H, 4.69; S, 15.89; Ti, 5.77; FTIR (cm^{-1}): 1253.5, s [*v*(P)−O−C], 962.7, s [*v*P−O−(C)], 865.3, s [*v*P═S], 668.1, m [*v*P−S], 428.1, w [*v*Ti−S], 365.9, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.2 (s, 12 H, CH_3), 6.6 (s, 4 H^a), 6.8 (d, 4 H^e), 6.9 (d, 4 H^c), 7.1 (t, 2 H^d), 5.9 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 93.8; ^{13}C NMR ($CDCl_3$, ppm): 19.3 (CH_3), 113.1 (C^2), 136.5 (C^6), 119.7 (C^4), 124.8 (C^5), 128.7 (C^3), 153.0 (C^1), 116.8 (C_5H_5); ESI MS (*m/z*) (%): [{(*m*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] = 796.8 (9); [{($C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] = 736.6 (11); [{($C_6H_4O)_2PS_2$}Ti(C_5H_5)$_2$] = 457.4 (14); [(O_2PS_2)$_2$Ti(C_5H_5)$_2$] = 432.3 (23); [(C_5H_5)$_2$Ti] = 178.1 (15); [*m*-$CH_3C_6H_4O$] = 107.1 (69).

2.7. Synthesis of *bis*(cyclopentadienyl)*bis*(*O,O'*-*p*-ditolyldithiophosphato)titanium(IV) [{(*p*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] (7)

Complex (**7**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'*-*p*-ditolyldithiophosphate, (*p*-$CH_3C_6H_4O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.37 g, 1.49 mmol) were used. Yield: 1.10 g (92%); M.Pt.: 245-246 °C; *Anal*. Calcd. for $C_{38}H_{38}O_4P_2S_4Ti$: Calculated (%): C, 57.28; H, 4.81; S, 16.10; Ti, 6.01, Found (%): C, 57.07; H, 4.59; S, 15.97; Ti, 5.90; FTIR (cm^{-1}): 1287.9, s [*v*(P)−O−C], 997.6, s [*v*P−O−(C)], 799.3, s [*v*P═S], 658.9, m [*v*P−S], 424.9, w [*v*Ti−S], 378.8, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.1 (s, 12 H, CH_3), 6.9 (d, 8 H^{a,e}), 7.1 (d, 8 H^{b,d}), 5.8 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 94.6; ^{13}C NMR ($CDCl_3$, ppm): 17.4 (CH_3), 126.1 ($C^{2,6}$), 127.9 (C^4), 130.3 ($C^{3,5}$), 152.2 (C^1), 118.1 (C_5H_5).

2.8. Synthesis of *bis*(cyclopentadienyl)*bis*(*O,O'*-*p*-chloro-*m*-ditolyldithiophosphato)titanium(IV) [{(*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] (8)

Complex (**8**) was synthesized as red solid according to the protocol as described for complex (**1**); sodium *O,O'*-*p*-Cl-*m*-ditolyldithiophosphate, (*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2Na$, (1.00 g, 3.01 mmol) and titanocene dichloride (0.37 g, 1.49 mmol) were used. Yield: 1.09 g (91%); M.Pt.: 242-243 °C; *Anal*. Calcd. for $C_{38}H_{34}O_4P_2S_4Cl_4Ti$: Calculated (%): C, 48.84; H, 3.67; S, 13.72; Cl, 15.17; Ti, 5.12, Found (%): C, 48.71; H, 3.55; S, 13.61; Cl, 15.03; Ti, 5.06; FTIR (cm^{-1}): 1273.4, s [*v*(P)−O−C], 971.4, s [*v*P−O−(C)], 828.1, s [*v*P═S], 662.9, m [*v*P−S], 439.6, w [*v*Ti−S], 369.7, w [*v*Ti−Cl]; ^{1}H NMR ($CDCl_3$, ppm): 2.1 (s, 12 H, CH_3), 6.5 (s, 4 H^a), 6.9 (d, 4 H^e), 7.3 (d, 2 H^d), 5.9 (s, 10 H, C_5H_5); ^{31}P NMR ($CDCl_3$, ppm): 92.7; ^{13}C NMR ($CDCl_3$, ppm): 16.5 (CH_3), 119.3 (C^2), 122.8 (C^6), 125.8 (C^3), 129.7 (C^5), 130.6 (C^4), 150.5 (C^1), 116.5 (C_5H_5); ESI MS (*m/z*) (%): [{(*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] = 934.6 (9); [{(*m*-$CH_3C_6H_3O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] = 792.8 (38); [(O_2PS_2)$_2$Ti(C_5H_5)$_2$] = 432.3 (26); [(C_5H_5)$_2$Ti] = 178.1 (8); [*p*-Cl-*m*-$CH_3C_6H_3O$] = 141.6 (11); [*m*-$CH_3C_6H_3O$] = 106.1 (54).



Scheme 1: Ring labelling for NMR spectroscopic assignments of complexes 1-8 [H^a = CH_3 (1, 5), H^b = CH_3 (2, 4, 6, 8), H^c = CH_3 (3, 7), H^d = Cl (4, 8)]

2.9. Biological Studies

2.9.1. Antibacterial activity

The antibacterial screening was tested against two pathogenic bacteria, *i.e. E. coli* and *E. faciolus* by agar well diffusion technique [19]. Test samples were prepared in different concentrations (100, 200, 400 and 800 ppm) in DMSO. Agar medium (20 mL) was poured into each Petri plate and plates were swabbed with broth cultures of the respective micro-organisms and kept for 15 minutes for adsorption to take place. Using a punch ≈ 6 mm diameter wells were bored in the seeded agar plates and 100 µL of the DMSO solution of each test compound was added into the wells. An additional control well without any sample but with an equivalent amount of solvent (DMSO) was used as reference in the assay. After holding the plates at room temperature for 2 h to allow diffusion of the compounds into the agar the plates were incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameter of the inhibition zone. The entire tests were made in triplicates and the mean of the diameter of zone of inhibition was calculated.

2.9.2. Antifungal activity

The antifungal activity of ligands and a few representative metal complexes was evaluated by the poisoned food technique against pathogenic strain of fungus *F. oxysporum* [19]. Potato dextrose medium (PDA) was prepared in a flask and sterilized. 100 µL of each test sample (as prepared for antibacterial screening) was added to the PDA medium and poured into each sterilized Petri plate. Mycelial disks taken from the standard culture of fungus, were grown on PDA medium for 7 days. These cultures were used for aseptic inoculation in the sterilized Petri dish. Standard cultures, inoculated at 28 ± 1 °C, were used as the control. The efficacy of each sample was determined by measuring the radial fungal growth. The radial growth of the colony was measured in two directions at right angle to each other, and the average of two replicates was recorded in each case. Data were expressed as percent inhibition over the control from the size of the colonies. The percent inhibition was calculated using the formulae: % Inhibition = ((C–T)/C)×100, where, C is the diameter of the fungus colony in the control plate after 96 h incubation and T is the diameter of the fungus colony in the tested plate after the same incubation period.

2.9.3. Cytotoxicity analysis

The cytotoxicity was measured in vitro using the cultivated human cell lines: lung adeno carcinoma cell line A549, leukemia cell line THP-1, prostate cancer cell line PC3 and colorectal cancer cell line HCT116. The inhibition capacity was assessed using the sulforhodamine B (SRB) protein staining assay by 96-well technique described previously by Skehan *et al* [20]. The seeded 96-well plates are incubated for 48 h after addition of test samples. Then the cells were fixed in 30% TCA (trichloroacetic acid) and placed for 1 h at 4 °C followed by washing with distilled water. After air-drying, the fixed cells were stained with 0.4% SRB (prepared in 1% acetic acid), left at room temperature for 30 minutes, washed with 1% acetic acid and dried. Solubilization is carried out with 10 mM Tris buffer followed by recording the optical density (OD) with ELISA reader at 540 nm wavelength.

RESULTS AND DISCUSSION

The reaction of titanocene dichloride, Cp_2TiCl_2, with sodium salts of *O,O'*-(*o*-, *m*-, *p*- and *p*-Cl-*m*-ditolyl)dithiophosphoric acids, $\{(ArO)_2PS_2\}Na$, in 1:1 and 1:2 molar ratio yielded the ditolyldithiophosphates of titanocene corresponding to $[(C_5H_5)_2Ti\{S_2P(OAr)_2\}_nCl_{2-n}]$ (Ar = *o*-, *m*-, *p*-$CH_3C_6H_4$, *p*-Cl-*m*-$CH_3C_6H_3$; n = 1 and 2) as red solid in 89-92% yield.

$$(C_5H_5)_2TiCl_2 + n\,(ArO)_2PS_2Na \xrightarrow[-n\ NaCl]{CH_2Cl_2} [(C_5H_5)_2Ti\{S_2P(OAr)_2\}_nCl_{2-n}]$$

Ar = *o*-$CH_3C_6H_4$ (**1**, **5**), *m*-$CH_3C_6H_4$(**2**, **6**) , *p*-$CH_3C_6H_4$ (**3**, **7**) or *p*-Cl-*m*-$CH_3C_6H_3$ (**4**, **8**);
n = 1 (**1-4**) or 2 (**5-8**)

Scheme 2: Synthesis of ditolyldithiophosphate derivatives of titanium

3.1. Spectral studies

IR spectra of these complexes have been interpreted on the basis of relevant literature reports [15,21-22]. The comparison of IR spectra of these complexes with starting materials has provided seminal information. Two strong intensity bands were observed in the region 1287.9-1226.9 cm^{-1} and 997.6-956.0 cm^{-1} for *ν*(P)−O−C and *ν*P−O−(C) vibrations of the ditolyldithiophosphate moiety, respectively. The bands for *ν*P=S and *ν*P−S of the ditolyldithiophosphate moiety were observed in the region 877.7-799.3 cm^{-1} and 687.4-658.9 cm^{-1}, showing significant shifting of the bands toward lower frequency. The presence of a new band for *ν*Ti−S in the region 444.8-424.9 cm^{-1} is indicative of the formation of titanium-sulfur bond. The weak band in the region 386.3-363.2 cm^{-1} is attributed to *ν*Ti−Cl in the complexes **1-4**.

The ^{1}H NMR spectra exhibited the chemical shifts for the protons of the cyclopentadienyl and tolyl moiety in their characteristic region without much appreciable shift. The protons of the cyclopentadienyl resonate in the region 5.7-5.9 ppm as a singlet. The chemical shift for the methyl (−CH_3) protons of the tolyl ring was observed in the region 2.1-2.3 ppm as singlet. The phenyl ring protons resonated in the region 6.3-7.3 ppm. The *o*- and *m*-tolyl ring protons exhibited four chemical shifts, *p*-tolyl ring protons exhibited two chemical shifts and *p*-Cl-*m*-tolyl ring exhibited three chemical shifts. The splitting patterns of the peaks in the spectra of all the complexes were found to be consistent with the predicted structures.

The phosphorus atom of the ditolyldithiophosphate moiety shows one signal in the region 91.2-94.6 ppm, depicting a ~15 ppm upfield shift compared to parent ligand. The singlet signifies the equivalent and symmetric nature of the phosphorus atom. This range observed for ^{31}P nucleus is consistent with bidentate behavior of dithiophosphate moiety according to Glidewell [15,21,23].

The ^{13}C NMR resonances for the cyclopentadienyl and tolyl moieties were found retained with a marginal shift in their values compared to the parent compounds. The carbon nuclei of the cyclopentadienyl ring resonate in the region 116.5-121.3 ppm. The chemical shift for the methyl (–CH_3) carbon was found in the region 16.5-20.8 ppm. The carbon nuclei of the phenyl ring have displayed their resonance in the region 113.1-153.3 ppm. The chemical shifts for C−O carbon of *meta* and *para* derivatives were found in the region 150.5-153.3 ppm. However, the chemical shift for C−O carbon of *ortho* derivatives was observed in the region of 147.7-149.2 ppm.

The mass spectra of the complexes **1**, **6** and **8** represented molecular ion peak [M^+] at 522.8, 796.8 and 934.6, respectively. In addition to molecular ion peak several other peaks of different fragments were also observed, which were formed after consecutive dismissal of different groups. The occurrence of molecular ion peak in the complexes is supporting the monomeric nature of the complexes. Furthermore, the presence of chlorine atoms in these complexes (**1**) and (**8**) resulted in the appearance of isotopic peaks at intervals of M, M+2 and M+4 in the mass spectra. The masses of the fragmented ions are calculated using one chlorine atom mass equal to 35 amu as it is the most abundant isotope of chlorine atom.

3.3. Thermal Analysis

The thermogravimetric analysis of the complex (**7**), [{(*p*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] displayed a thermolysis step that covers a temperature range from 150 to 900 °C. The thermogram (Figure 1) exhibited the characteristic decline curve for dithiophosphate complexes. The diagnostic weight loss of initial weight occurs in the steeply descending segment of the TGA curve. This weight loss *i.e.* 49.82% at 461.6 °C is due to the formation of the dithiometaphosphate fragment corresponding to [{(OPS_2}$_2$Ti(C_5H_5)$_2$], (the calculated weight loss is 49.77%) as an intermediate product, which agrees with thermogravimetric data for dithiophosphates. The weight loss at 886.6 °C leading to the formation of the final residue (the calculated weight loss is 85.94%) is 88.01% of the initial weight of the sample (2.07% is attributed to contamination with other thermolysis products) corresponds to TiS_2.

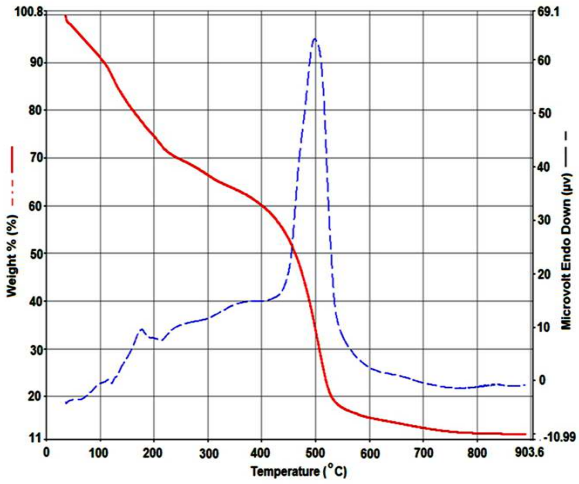


Figure 1. Graph showing TGA curve of [{(*p*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$]

3.4. Cyclic Voltametric Analysis

Cyclic voltammogramm (Figure 2) of the complex (**6**), [{(*m*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] at scan rate 100 Vs^{-1} exhibited a one-electron reversible reduction wave. It is well established that the reduction process is a reversible one-electron reduction taking place on titanium. The cyclic voltagramm depicts a cathodic peak at -0.78 V (E_{pc}) and the anodic peak at 0.56 V (E_{pa}). The cathodic peak current, i_c, and the anodic peak current, i_a, were found to be -5.8 x 10^{-6} A and 5.6 x 10^{-6} A, respectively. The one-electron reduction is reversible as is indicated by the value for i_c/i_a which is close to unity i.e. 1.04.

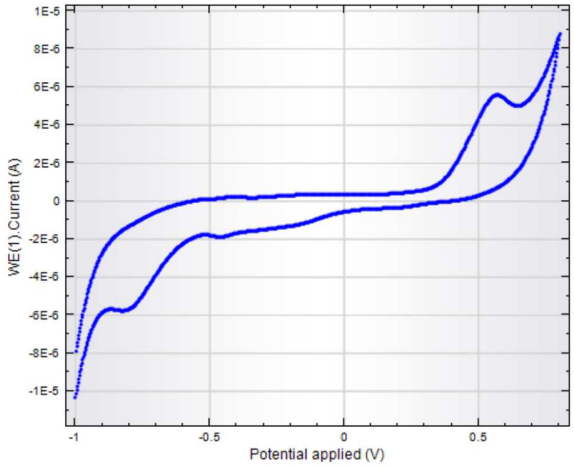


Figure 2. Graph showing cyclic voltammetric curve of [{(*m*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$]

3.5. Biological Studies

3.5.1. Antibacterial activity

The ligands as well as their few representative metal complexes were screened for their antibacterial activity against two bacterial species *Eischerichia coli* (*E. coli*) and *Enterococcus faciolus* (*E. faciolus*). The results revealed insignificant activity towards *E. faciolus* species in comparison to *E. coli*. Furthermore, antibacterial activity of the ligands ((*o*-$CH_3C_6H_4O)_2PS_2Na$ and (*p*-Cl-*m*-$CH_3C_6H_3O)_2PS_2Na$) was studied and compared with the results obtained for antibacterial activity of the titanium complexes. A comparative study of the ligands and their complexes indicates that complexes exhibit higher antimicrobial activity than the free ligands. From the zone of inhibition values, complexes are found to be more potent than the ligands. Increased activity on metal chelation can be explained on the basis of chelation theory [24]. While chelation is not the only criterion for antimicrobial activity, it is an intricate blend of several contributions such as the nature of the metal ion and the ligand, the geometry of the complex, the lipophilicity, the steric, and the pharmacokinetic factors. It has also been proposed that concentration plays a vital role in increasing the degree of inhibition because the activity increases with the increase of concentration. The comparative results achieved by these studies have been enlisted in the Table 1.

Table 1. Comparative antibacterial screening results of complexes

Compound	Concentration (ppm)	Zone of Inhibition (in cm)	
		E. coli.	*E. fac.*
$(C_5H_5)_2TiCl_2$	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	800	0.8	0.0
$(o\text{-}CH_3C_6H_4O)_2PS_2Na$	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	800	0.0	0.0
$(p\text{-Cl-}m\text{-}CH_3C_6H_3O)_2PS_2Na$	100	0.0	0.0
	200	0.8	0.0
	400	1.1	0.0
	800	1.4	0.4
$[(m\text{-}CH_3C_6H_4O)_2PS_2\}Ti(C_5H_5)_2Cl]$	100	0.6	0.0
	200	0.7	0.0
	400	0.8	0.0
	800	0.9	0.0
$[(o\text{-}CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2]$	100	0.0	0.0
	200	0.0	0.0
	400	0.0	0.0
	800	0.6	0.0
$[(p\text{-Cl-}m\text{-}CH_3C_6H_3O)_2PS_2\}_2Ti(C_5H_5)_2]$	100	0.0	0.0
	200	1.0	0.0
	400	1.4	0.8
	800	2.1	0.9

3.5.2. Antifungal activity

The antifungal activity of the ligands and few representative complexes was also studied against the *Fusarium oxysorum* (*F. oxysorum*) fungal species. The complexes exhibited pronounced antifungal behavior. The fungicidal screening data (Table 2) established a linear relationship between concentration and percent inhibition. The increase in antifungal activity is due to faster diffusion of complexes as a whole through the cell membrane, or due to combined activity effect of the metal and the ligand. Moreover, the complexes may also indulge in the formation of bridge between the coordinated chloride anion with the active centre of cell constituents. The factors capable of increasing lipophilicity are expected to enhance the antifungal activity. The order of antifungal activity was found in the order of $[\{(p\text{-Cl-}m\text{-}CH_3C_6H_3O)_2PS_2\}_2Ti(C_5H_5)_2]$ > $[\{(m\text{-}CH_3C_6H_4O)_2PS_2\}Ti(C_5H_5)_2Cl]$ > $[\{(o\text{-}CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2]$. This trend is attributed to the presence of chlorine atoms. The results of fungitoxicity analysis have been illustrated as a bar graph in Figure 3.

Table 2. Comparative antifungal screening results of complexes

Compound	Conc. (ppm)	Mean colony diameter (in cm)	% Inhibition = ((C–T)/C)×100 (C = 3.2 cm)
$(o\text{-}CH_3C_6H_4O)_2PS_2Na$	100	2.4	25.0
	200	1.9	40.6
	400	1.5	53.1
	800	1.4	56.2
$(p\text{-Cl-}m\text{-}CH_3C_6H_3O)_2PS_2Na$	100	2.2	31.3
	200	1.9	40.6
	400	1.3	59.4
	800	0.9	71.9
$[(m\text{-}CH_3C_6H_4O)_2PS_2\}Ti(C_5H_5)_2Cl]$	100	2.2	31.3
	200	1.8	43.8
	400	1.2	62.5
	800	1.0	68.8
$[(o\text{-}CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2]$	100	2.4	25.0
	200	2.0	37.5
	400	1.4	56.3
	800	1.2	62.5
$[(p\text{-Cl-}m\text{-}CH_3C_6H_3O)_2PS_2\}_2Ti(C_5H_5)_2]$	100	1.9	40.6
	200	1.5	53.1
	400	1.0	68.8
	800	0.8	75.0

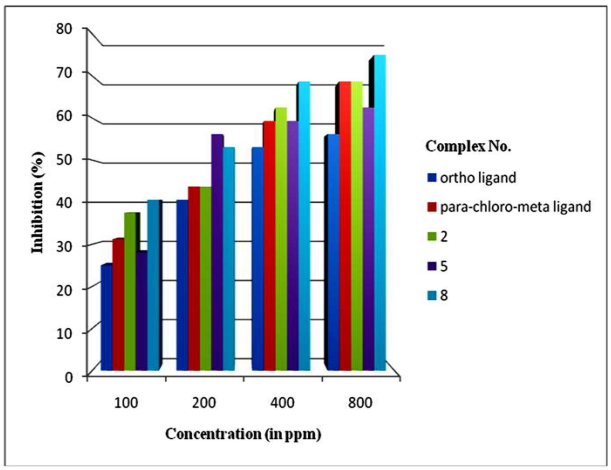


Figure 3. Comparative results of antifungal screening data of complexes

3.5.3. Cytotoxicity analysis

Cytotoxic study of the complex [{(*o*-$CH_3C_6H_4O)_2PS_2$}$_2$Ti(C_5H_5)$_2$] (**5**) and ligand (*o*-$CH_3C_6H_4O)_2PS_2Na$ against the cultivated human lung adeno carcinoma cell line A549, leukemia cell line THP-1, prostate cancer cell line PC3 and colorectal cancer cell line HCT116 revealed the maximum potential against the prostate cancer cell line PC3 while minimum activity was observed against colon cancer cell line HCT 116 for complex **5**. The ligand showed moderate cytotoxic activity against all cell lines. The cytotoxicity of the ligand is attributed to the presence of sulfur donor atoms that bind effectively with DNA and modify the cancer cell processes. Higher potential for complex can be explained by the fact that titanium protects the ligand ensuring its arrival intact at the active site. It is reported that cyclopentadienyl moiety of the stable titanocene dichloride is labile under physiological conditions and this property results in binding of $(C_5H_5)_2TiCl_2$ to the iron-transport protein transferrin and also induces a conformational change to iron(III) binding [2,3,25]. The comparative cytotoxicity data is well illustrated in the form of bar graphs in Figure 4 and Figure 5.

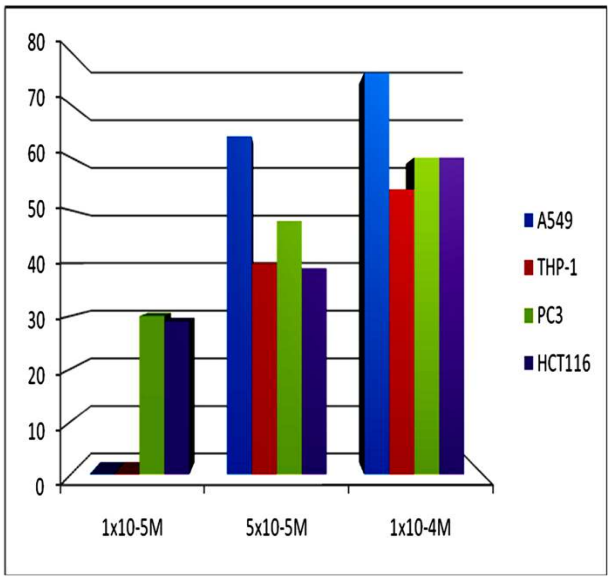


Figure 4. Comparative cytotoxic screening data for the ligand (*o*-$CH_3C_6H_4O)_2PS_2Na$

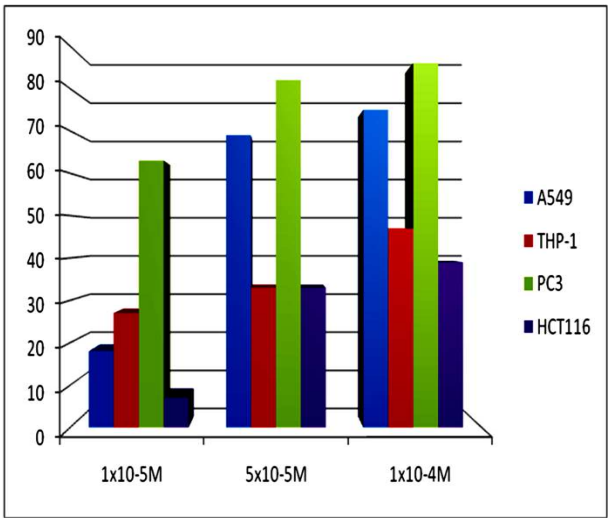
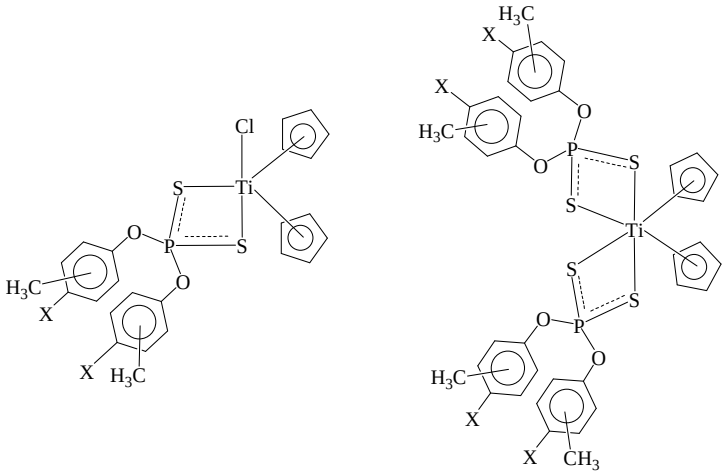


Figure 5. Comparative cytotoxic screening data for (o-$CH_3C_6H_4O)_2PS_2\}_2Ti(C_5H_5)_2$

3.5.4. Structural Features

The suggested structures in these complexes are based on the literature reports [15-17,21] and observations of elemental analysis, IR, NMR (^1H, ^{13}C and ^{31}P), mass spectral studies and thermogravimetric analysis. Pentacoordinate and hexacoordinate geometries around the titanium(IV) atom (Figure 6) are proposed for the complexes **1**-**4** and **5**-**8** respectively.



X = H (**1**, **2**, **3**, **5**, **6**, **7**) and X = Cl (**4**, **8**)

Figure 6. Proposed geometries of the ditolyldithiophosphate complexes of titanium

CONCLUSION

A series of eight new *O,O'*-(*o*-, *m*-, *p*-, *p*-Cl-*m*-ditolyl)dithiophosphate derivatives of cyclopentadienyltitanium has been isolated. The cyclic voltammetric analysis predicted the one electron reduction of the titanium center. A five and six coordinated titanium atom has been proposed in these complexes. The thermogravimetric analysis produced TiS_2 as the final decomposition product. The antimicrobial screening results revealed appreciable inhibitory action. Moreover, cytotoxic potential of a complex has been demonstrated. However, further investigations are essential to explore the exact mechanism of their cytotoxic properties.

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