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Titanium Based Mixed Lig and Complexes: Synthesis, Characterization And In Vitro Antiproliferative Studies

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ABSTRACT

Titanium complexes with composition $TiCl_2(L)(L1)$ [where, L = benzoylacetone (bzac), L1 = 1,2-diaminocyclohexane, 1,10-phenanthroline (phen), 1-adamantylamine (ada)] have been synthesized by reacting titanium tetrachloride with benzoylacetone and nitrogen containing ligands in predetermined molar ratio. The structure of complexes was confirmed by different spectroscopic techniques i.e. FT-IR, ^{1}H NMR and mass spectrometory. Cytotoxic studies were done on HeLa (cervical), C6 (glioma), and CHO (Chinese hamster ovarian) cell lines. It has been found that complex 2 was more potent cytotoxic agent against all the three cell lines tested.

Keywords— Titanium; Benzoylacetone; FT-IR; ^{1}H NMR; Mass spectra; Cytotoxicity.

INTRODUCTION

Transition metal complexes can be cationic, neutral or anionic species in which metal atom is coordinated by the ligands. Transition metals show different coordination sphere and exhibit different oxidation states and can interact with a number of negatively charged bio molecules. Transition metal complexes have shown wide applications as antibacterial [1] and anticancer agents for many years. However, the role of transition metals in medicinal chemistry has been explored after the by chance discovery of cisplatin in 1969. Cisplatin was found much effective against testis, ovary, neck and small cell lung cancers but show side effects such as nausea, vomiting, alopecia, renal impairment, neurotoxicity and ototoxicity [2] etc. Later on many

derivatives of platinum and non platinum [3] based complexes have been synthesized and tested on different cancer cell lines by many researchers. The first non platinum anticancer drug was titanium based budotitane and titanocene dichloride [3] reached the phase I in clinical trial. In the last few decades many derivatives of budotitane and titanocene dichloride have been synthesized and tested on various cancer cell lines; however the mechanism of action of titanium complexes is different than that of platinum and other metal complexes. But, very limited literature is available on studies related to cytotoxicity of titanium complexes, so this can be an emerging area for future research. Researchers all over the world are engaged in developing new metal based compounds with improved cytotoxic activity and lesser side effects in the hope of adding new chemotherapeutic agents in chemotherapy. In the present study, we have found that the synthesized titanium complexes act as potent cytotoxic agents which kill cells through apoptosis.

EXPERIMENTAL

Synthesis of benzoylacetonato-1,2-diamnocyclohexane dichloro titanium(III): $TiCl_2$(bzac)(dach), (1). The solution of benzoylacetone (0.42 g, 2.63 mmol) in 25 mL of dichloromethane was added drop wise in colorless solution of titanium tetrachloride (0.5 g, 2.63 mmol) in 20 mL of dichloromethane, with continuous stirring. The color of solution changed to reddish brown immediately with evolution of HCl gas. The reaction mixture was stirred for 12 h, till the evolution of HCl gas ceased. The color of solution changed to reddish brown. In this reaction mixture 1,2-diaminocyclohexane (221 µL, 1.9 mmol) in 25 mL of dichloromethane was added drop wise with continuous stirring and color of solution changed to orange immediately. The reaction mixture was stirred for 2 h and refluxed for 4 h till the evolution of chlorine gas get ceased and color of solution changed to dark brown. The solvent was removed by vacuum distillation and the compound was dried under vacuum followed by recrystallization in methanol. Yield: 0.5 g (78.5 %). m.p. = 205-210˚C. $TiCl_2C_{16}H_{23}N_2O_2$: Elemental anal. calcd (%): C 48.7, H 6.1, N 7.1; found (%): C 48.4, H 5.8, N 7.5. FT-IR (KBr, cm^{-1}) $\bar{\nu}$: 3404 (NH streching), 2940 and 2866 (CH Streching), 1602 (C=O Streching), 1513 (C=C Streching), 1452 (C=N streching) 1350, 1296 (CH bending), 1106 (C-O streching), 806 (CH out of plane deformation), 562 (Ti-O streching), 440 (Ti-N streching). ^{1}H NMR (DMSO-d^6, 400 MHz): Benzoylacetone δ, ppm C_6H_5 = 7.94, 7.6, 7.5 (H_2 & H_6, H_4, H_3 & H_5), 6.48 (s, CH proton), 2.19 (s, CH_3 protons). 1,2-diaminocyclohexane δ, ppm = 3.58 (2H, CH), 3.2 (4H, NH_2), 2.14 (CH_2, 2H, H^3), 1.89 (CH_2, 2H, H^6), 1.53 (CH_2, 2H, H^4), 1.4 (CH_2 ,2H, H^5).

Same procedure was followed for the preparation of other two titanium complexes mentioned below.

Synthesis of benzoylacetonato-1,10-phenanthroline dichloro titanium(III), $TiCl_2$(bzac)(phen), (2). Yield: 0.85 g (70.24 %). m.p. = 195-200˚C. $TiCl_2C_{22}H_{17}N_2O_2$: Elemental anal. calcd (%): C 57.3, H 3.9, N 6; found (%): C 57.8, H 3.5, N 6.4. FT-IR (KBr, cm^{-1}) $\bar{\nu}$: 3058 and 2936 (aromatic and aliphatic CH Streching), 1588 (C=O Streching), 1533 (C=C Streching), 1520 (C=N streching) 1330, 1303 (CH bending), 1099 (C-O streching), 834 (CH out of plane deformation), 561 (Ti-O streching), 427 (Ti-N streching). ^{1}H NMR (DMSO-d^6, 400 MHz): Benzoylacetone δ, ppm C_6H_5 = 7.9, 7.6, 7.5 (H_2 & H_6, H_4, H_3 & H_5), 6.48 (s, CH proton), 2.19 (s, CH_3 protons). 1,10-phenanthroline δ, ppm = 9.3 (d, ^{3}J = 4.12 Hz, 2H, H^2&H^9), 9.0 (d, ^{3}J = 7.96 Hz, 2H, H^4&H^7), 8.31 (s, 2H, H^5&H^6), 8.16 (dd, 2H, ^{3}J = 3.24, 4.8 Hz, 2H, H^3&H^8).

Synthesis of adamantylaminobenzoylacetonato dichloro titanium (IV), $TiCl_2$(bzac)(ada), (3). Yield: 0.96 g (84.9%). m.p. = > 210˚C. $TiCl_2C_{20}H_{25}O_2N$: Elemental anal. calcd (%): C 55.8, H 6.2, N 3.2;

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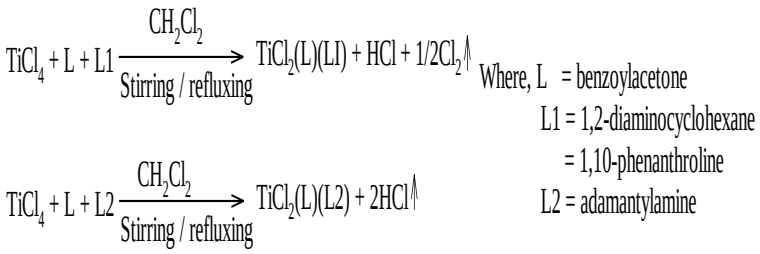
found (%): C 56.2, H 6.6, N 3.8. FT-IR (KBr, cm^{-1}) $\bar{\nu}$: 3415 (NH streching), 2927 and 2852 (CH Streching), 1602 (C=O Streching), 1527 (C=C Streching), 1440 (C=N streching) 1350 (CH bending), 1106 (C-O streching), 963 (CH out of plane deformation), 556 (Ti-O streching), 433 (Ti-N streching). ^{1}H NMR (DMSO-d^6, 400 MHz): Benzoylacetone δ, ppm C_6H_5 = 7.92, 7.60, 7.50 (H_2 & H_6, H_4, H_3 & H_5), 6.49 (s, CH proton), 2.26 (s, CH_3 protons). Adamantylamine δ, ppm = 2.09 (s, NH), 1.84 (d, ^{3}J = 2.16, CH_2 protons), 1.63 (dd, ^{3}J = 17.16, 12.36 Hz, CH protons).

RESULTS AND DISCUSSION

Three new titanium complexes having composition $TiCl_2(L)(L1)$ and $TiCl_2(L)(L2)$, where L = benzoylacetone, L1 = 1,2-diaminocyclohexane, 1,10-phenanthroline and L2 = adamantylamine were synthesized. These complexes (1-3) were synthesized by reacting titanium tetrachloride with benzoylacetone and respective bidentate/monodentate ligand in 1:1:1 molar ratio with the evolution of HCl and chlorine gas [4-6] as shown in Scheme 1. Elemental analysis, i.e. chlorine and titanium estimation was done to check the composition of complexes by Volhard's method and gravimetrically respectively.



Scheme 1. Synthetic route for titanium complexes (1-3).

The determination of molecular weight was done by Rast's camphor method. The proposed structure of complexes has been shown in Figure 1.

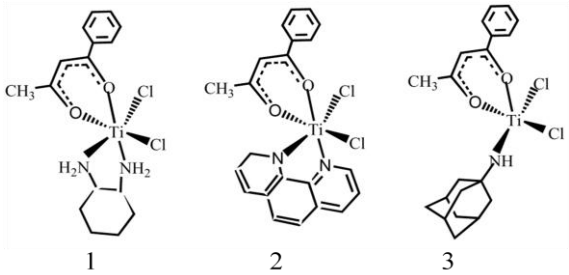


Figure 1. Proposed structure of titanium complexes.

CHARACTERIZATION:

FT-IR, ^{1}HNMR AND MASS SPECTRA:

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The disappearance of broad band observed in the region 3100-2700 cm^{-1} due to diketone suggests that bonding takes place through both the oxygen of (C=O) carbonyl groups in the complexes, however $\bar{\nu}$(C=O) band shows marginal change even after coordination [7]. The appearance of new bands at 440, 427, and 433 cm^{-1} in complexes 1, 2, and 3 confirm the coordination of titanium with the nitrogen atom of second ligand [8]. In ^{1}H NMR Spectra, it has been observed that there is a downfield shift in 1,2-diaminocyclohexane and 1,10-phenanthroline protons after complexation in metal complexes In mass spectra, appearance of expected base peaks and fragment ion peaks confirm the formation of complexes.

MTT assay

The IC_{50} values were determined by using linear regression model and on comparing with negative control i.e. only media. From the calculated IC_{50} values it has been found that complex 2, showed good activity i.e. 12.27, 19.24 and 13.55 µM against HeLa, C6 and CHO cell lines respectively. The change in morphological features (formation of small apoptotic bodies, plasma membrane blebbing, shrinkage of cells) was observed at different concentration of complexes which indicate that change in morphology was dose dependent [9]

CONCLUSIONS

In the present studies, we have reported the synthesis of mixed ligand titanium complexes with labile groups. The structure of synthesized titanium complexes was proposed by using FT-IR, UV-visible, ^{1}H NMR and mass spectrometry techniques. Complex 2, was proved to be more potent cytotoxic agent against all the three cell lines tested. In present attempt we have established the *in vitro* study of synthesized titanium complexes however efficacy of these complexes must also be studied *in vivo* to understand the detailed mechanism of action of these titanium complexes for clinical implications.

References

1. P. Kamala kannan, D. Venkappayya, “Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents,” J. Inorg. Biochem. vol. 90, pp 22-37, 2002.
2. V. Cepeda, M.A. Fuertes, J Castilla, C. Alonso, C. Quevedo, J. M Perez, “Biochemical mechanisms of cisplatin cytotoxicity”. Anti-Cancer Agents Med. Chem., vol 7, pp 3-18, 2007.
3. E. Menendez, “Titanium complexes in cancer treatment”. Crit. Rev. Oncol. Hemat. vol 42, pp 309-315, 2002.
4. R. Kaushal, N. Kumar, P. Awasthi, K. Nehra, “Syntheses, characterization and antibacterial study of titanium complexes”. Turk. J Chem, vol 37, pp 936-945, 2013.
5. R. Kaushal, N. Kumar, A. Chaudhary, S. Arora, P. Awasthi, “Synthesis, Spectral Characterization, and Antiproliferative Studies of Mixed Ligand Titanium Complexes of Adamantylamine”. Bioinorganic chemistry and applications, vol 2014, pp1-12, 2014,

6. N. Kumar, R. Kaushal, A. Chaudhary, S. Arora, P. Awasthi, "Synthesis, structural elucidation, and *in vitro* antiproliferative activities of mixed-ligand titanium complexes". Med. Chem. Res. vol 23 pp 3897-3906, 2014.
7. L. Ravagnan, T. Roumier, G. Kroemer, "Mitochondria, the killer organelles and their weapons". J Cell Physiol., vol 192, pp 131-137, 2002.
8. C.V Christodoulou, A.G Eliopoulos, L.S.Young, L Hodgkins, D.R Ferry, D.J. Kerr, "Antiproliferative activity and mechanism of action of titanocene dichloride". Brit J Cancer, vol 77, pp 2088-2097, 1988.
9. T. Mosmann, "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays". J Immunol Methods, vol 65, pp 55-63, 1983.