Synthesis, Characterization, Theoretical Calculations and Anti-Tumor Study of 2-Aminopyridine trifluorotitanium (IV) complex

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Summary: A new titanium compound that is 2-Aminopyridine trifluorotitanium (IV) complex was synthesized and identified. This Complex was prepared through the reaction of 2-Amino pyridine and titanium tetrafluoride salt of in acetonitrile. Identification and Characterization of complex was performed by elemental analysis and spectroscopic methods. Structural and spectroscopic study of the mentioned complex was carried out at DFT- B3LYP/6-311G level of theory. This new compound was used to biological studies; and its anticancer properties against the two types of cancer cells such as k562 (human chronic myeloid leukemia) and Jurkat (human T lymphocyte carcinoma) was revealed.

Keywords: Ti (IV) complex; 2-Aminopyridine trifluorotitanium (IV), theoretical study, Anti-tumor, K562, Jurkat.

Introduction

It has long been known that metal ions involve in biological processes of life and have been subject of interest. The modes of action of metal ions are often complex but are believed to involve bonding to the heteroatoms of the heterocyclic residues of biological molecules, *i.e.*, proteins, enzymes, nucleic acids, etc. In ligand type compound, substituted amine compounds take special place in particular processes. The mechanism of their interaction with surrounding molecules in condensed medium in most cases is determined by hydrogen bonds, providing flexibility, speed and variety of biochemical reactions [1, 2]. 2-Aminopyridine (2Apy)-tagged oligosaccharides have been widely used for sensitive qualitative and quantitative analysis by high performance liquid chromatography with fluorescence detection. Moreover, 2Apy is used in the preparation of cytidine analogs. In addition to these applications, 2Apy is also immensely used as a reagent in analytical chemistry. Therefore, it was found interesting to investigate vibrational frequencies of 2Apy in a series of transition metal complexes and to compare the vibrational results of isostructural complexes depending on the transition metal strength [3]. On the other hand the use of titanium fluorides as catalysts for organic reactions had been limited because of their low solubility in common organic solvents and lack of well established synthetic routes to substituted titanium fluorides in contrast with widely used titanium chlorides. Nonetheless, titanium fluoride complexes have been attracting considerable attention recently due to their strong Lewis acidity, resulting from the high electro negativity of fluorine. Also, a strong Ti-F is useful because it prevents ligand exchange reactions between substrates, which are often observed in titanium triflate mediated reactions [4]. On this basis, studying different kinds of complexes of transition metals from among the ligands with biological properties and activities seems interesting. In this paper the synthesis, characterization and anti-tumor properties of a transition metal complex has been studied.

The structure of combination was optimized using the density functional theory (DFT) at B3LYP/6-311G levels method [5-7]. The geometries of this compound were optimized using standard gradient techniques with default parameters set in Gaussian 03 program. The comparison between theoretical and experimental results has been shown good agreements.

Experimental

Materials and methods

2-Amino pyridine and Titanium (IV) tetrafluoride were Merck chemicals and were used without further purification. All organic solvents were reagent grade. Infrared (IR) spectra were recorded as KBr pellets by using the Bruker Tensor

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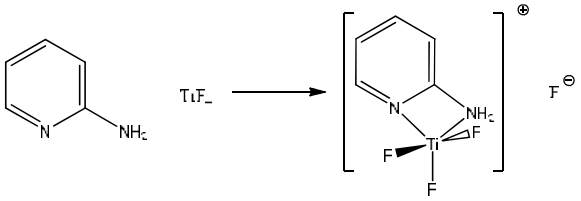
27-Model 420 IR spectrofotometer. The electronic spectra of all combinations have been recorded by model 350 UV/Visible machines of Camspec and Wpa bio Wave S2 100. Elemental analyses were performed by Heraeus CHNO-Rapid elemental analyzer. ^{1}H-NMR and ^{19}F-NMR spectra were recorded on the Bruker-AVANCE DRX 500 machine; All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H NMR and ^{19}F- NMR spectra were referenced to external $SiMe_4$ and $CFCl_3$ respectively, and finally for the purpose of obtaining the melting point Electro-thermal 9200 machine was used.

Preparation of 2-Amino pyridine titanium (IV) fluoride complex (APTF)

In order to prepare the above complex which is briefly called APTF, ligand of 2-amino pyridine (0.210g, 2.23 mmol) was poured into a waterless 50 mL Beaker containing a magnetic stirrer and 20 mL acetonitrile. After dissolving, ligand solution became pale yellow. Then mixture of TiF_4 (0.281g, 2.256 mmol) which was into the 50 ml Beaker containing distilled water and magnetic stirrer of colorless and clear solution was added. Total compound was mixed at room temperature; and in order to ensure the completeness of the reaction stirring was continued for one hour. Together with the increase in sediment the mixture became milky in color at the time of reaction; and finally milky color precipitation of the formed complex was rinsed using Hexane and diethyl ether and dried in vacuum desiccators over anhydrous $CaCl_2$ for the purpose of subsequent reviews.



Scheme-1: Schematic representation of the reaction of complex synthesis.

Theoretical Details

Hybrid-DFT calculations were carried out using the B3LYP method with the 6-311G basis set with the Gaussian 03 package of programs [6, 8] for optimizing the initial-estimated structural geometries and spectroscopic behavior of complex. Hybrid-DFT methods combines the exact Hartree-Fock exchange with Becke's and uses the Lee-Yang-Parr correlation function in order to include the most important correlation effects. Representative structural parameters for the mentioned complex as calculated at the B3LYP/6-311G level of theory, are shown in Table-1.

The optimized structural parameters were used in the NMR spectroscopy and vibrational frequency calculations at the HF and DFT levels to characterize all stationary points as minima [9, 10]. Harmonic vibrational frequencies (n) in cm^{-1} and infrared intensities in km/mole of compound were performed at the same level on the respective fully optimized geometries. This complex and its data are in agreement with recent works on the formation of five coordinate intermediates.

Results and Discussion

Analytical data of Ti $[C_5H_5N_2]$ F_3

^{19}FNMR (ppm DMSO, 470.54 MHz): 81.01. ^{1}HNMR (ppm DMSO, 300MHz): 6.67-7.9 [2q, 2d, 4H, pyridine), 4.26 [s, 1H, NH]. IR absorptions (cm^{-1} KBr): 3161.5 (N-H), 1669.9 (C=N), 1473 (C=C), 767.3 (Ti-N), 722.9 (Ti-F), 636-656 v(C-H). Elemntal Analysis data for Ti $[C_5H_5N_2]$ F_3: Calculated (%): C, 31.31; H, 2.07; N, 14.14; found: C, 31.01; H, 1.86; N, 14.31. UV- vis (MeCN): λ_{max} 302 nm (300), 412nm (10).

Complex Characterization

Table-1: Geometrical parameters optimized of complex, bond length (A˚) and angle (°).

Bond lengths(A°)	complex	ligand	Bond angles(°)	Complex	ligand
Ti14-F15	1.788180	-	F15-Ti14-F16	115.9158	-
Ti14-F16	1.788261	-	F15-Ti14-F17	103.1353	-
Ti14-F17	1.794088	-	F16-Ti14-F17	103.1465	-
Ti14-N6	2.255493	-	N6-Ti14-F15	90.6165	-
Ti14-N11	1.971565	-	N6-Ti14-F16	90.5491	-
C1-N6	1.371091	1.349838	N6-Ti14-F17	153.528	-
C1-N11	1.371154	3.551593	N11-Ti14-F15	118.2695	-
C5-N6	1.342487	1.357371	N11-Ti14-F16	118.3884	-
C5-H10	1.079788	2.170413	N11-Ti14-F17	91.1912	-
N11-H12	1.007291	1.001688	N6-C1-N11	106.7766	115.826

The reaction of TiF_4 salt with 2-Aminopyridine as ligand produced a compound with [ML] stoichiometry in which M= Ti (IV). A completely fixed and stable complex was formed that can be kept without any considerable changes and used for tests on identification of structure and investigation of spectrometry. APTF complex was characterized by several techniques using elemental analysis (C, H, N), ^{1}H NMR, $^{19}FNMR$, FT-IR, electronic spectra and molar conductance measurements. The elemental analysis data suggest the stoichiometry to be 1:1 [M:L] ratio formation. The molar conductance measurements reveal the presence of 1:1 electrolytic nature complex.

This reaction does not have any other productions. It expresses the possibility of the usage of ether and hexane solvents for removing impurities. When the complex was investigated in terms of physical characteristics, it was observed that although melting temperature of ligand 2- amino-pyridine and TiF_4 salt were 54-58 and 284°C, it was melted at temperatures 220-222 ˚C .The complex is soluble in solvents such as DMSO and DMF and also in Acetonitrile and Chloroform, Dichloromethane and Hexane and in no-polar solvents such as Benzene Toluene and diethyl ether the complex is insoluble. Another reason for the formation of the mentioned complex [Ti ($C_5H_6N_2$] F_4 is elemental analysis [11, 12]. As it can be observed experimental percentages are in good agreement with calculated percentages. Thus, the closed formula [Ti ($C_5H_6N_2$)] F_4 can be proposed for synthesized material.

Computational Data of Ti [$C_5H_5N_2$] F_3

The density function theory and ab initio calculation have been performed with the Gaussian program and the basis sets implemented therein [6, 13-15]. DFT (B3LYP/6-311G) calculations are performed for Ti [$C_5H_5N_2$] F_3 complex. The structure of complex is shown in Fig. 1. The suggested numbers for 2-Aminopyridine and Ti [$C_5H_5N_2$] F_3 complex are shown in Fig. 2. We have calculated charges using the natural bond orbital (NBO) and Mulliken method at the level of DFT- B3LYP/6-311G. For 2-Aminopyridine the NBO and Mulliken charges on each nitrogen is -0.509 and -0.403, and for 2-Aminopyridine trifluorotitanium (IV) complex (APTF) the NBO and Mulliken charges on each nitrogen is -0.549 and -0.761, the chargs on each of the other atoms as shown in Fig. 3. It has been improved that according to geometry optimization calculation, the symmetry for APTF complex is C_1. Selected bond lengths and bond angles are reported in Table-1.

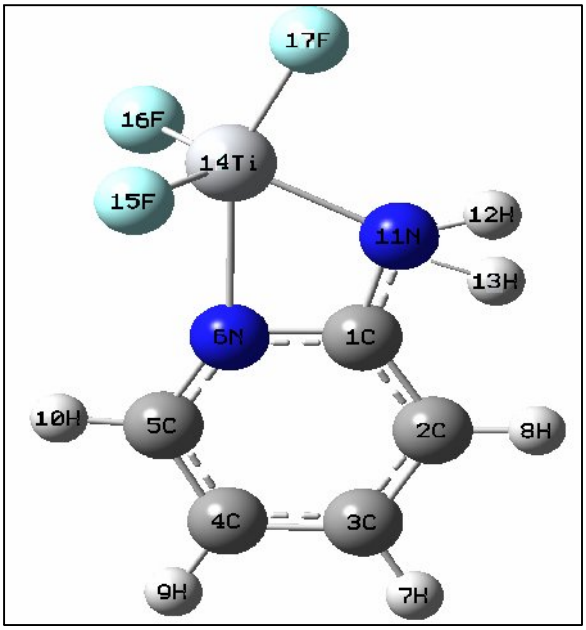


Fig. 1: Optimized geometries of complex at B3LYP/6-311G level of theory.

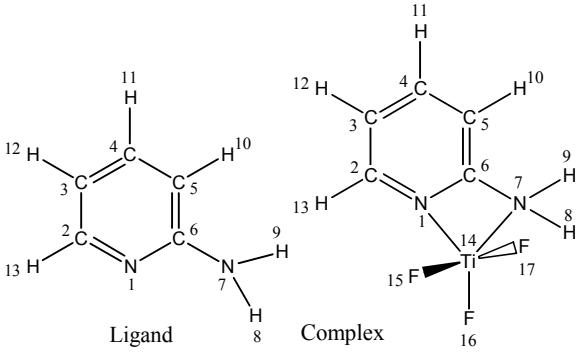


Fig.2. Suggested numbers for 2-Amino pyridine and APTF.

The calculated IR spectrum of APTF complex is used for the assignment of IR frequencies that are observed in the experimental IR spectrum. The calculated infrared spectra of 2-Amino pyridine titanium (IV) fluoride complex are presented in Fig. 4. The vibrational frequencies and intensities of the stationary points of complex at the B3LYP/6-311G level of theory and related experimental data, were presented in Table-2.

In this study, Calculations of absolute chemical shielding were carried out at the B3LYP/6-311G (2d, p) GIAO level, assuming the solvation (DMSO, PCM model).

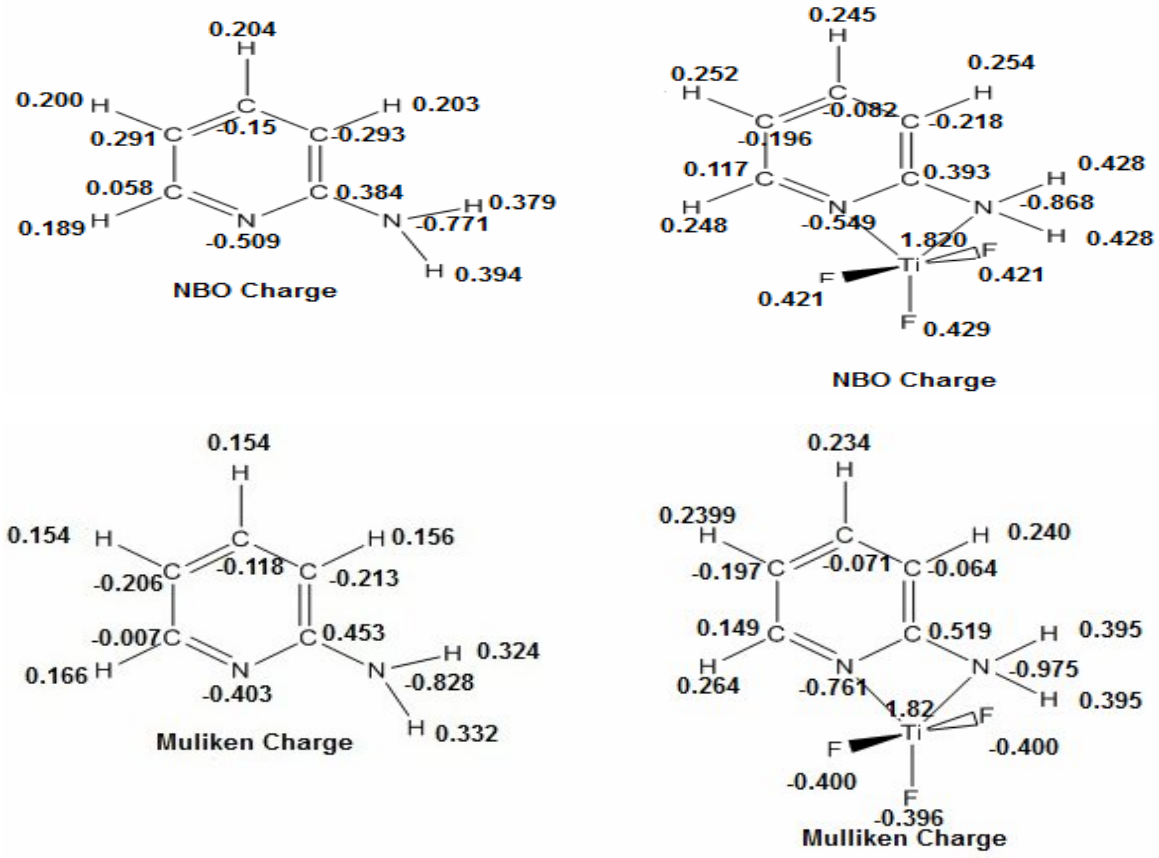


Fig. 3: NBO (a) and Mulliken(b) charges of 2-Amino pyridine and APTF.

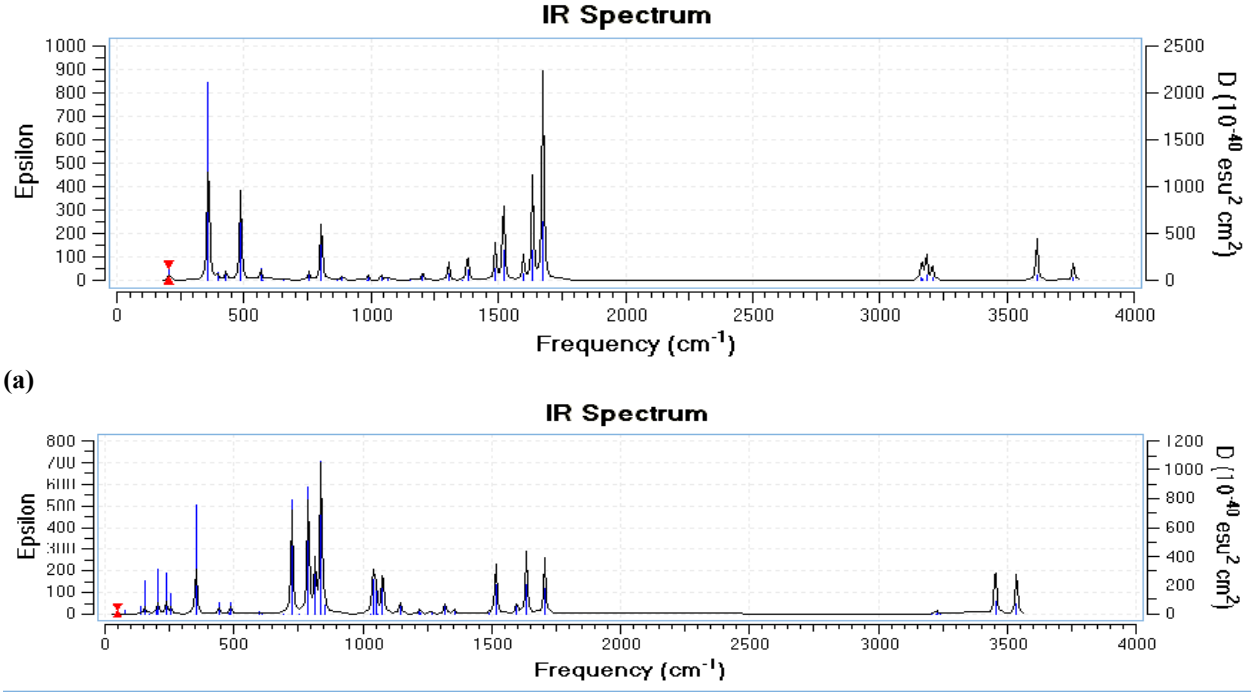


Fig. 4: Calculated infrared spectra of (a) 2-Amino pyridine (b) APTF.

Table-2: Calculated and experimental frequencies of Complex & Ligand.

	Complex			Ligand		
	Cal. freq	Corr. Cal. freq	Exp.	Cal. freq	Corr. Cal. freq	Exp.
N-H	3454.48	3316.30	3161.5	3617.97	3473.25	3307.07
C=N	1705.91	1637.68	1669.9	1675.66	1608.64	1661.76
C=C	1516.30	1455.65	1473	1634.80	1569.48	1443
Ti-N	837.92	804.407	767.3	-	-	-
Ti-F	788.37	756.83	722.9	-	-	-
C-H	725.06	696.62	636.65	804.22	772.05	774.5

Scaling factor of vibrational frequencies that obtained at B3LYP/6-311G level of theory is 0.96

Chemical shift calculations have been performed in order to test how the calculated data reproduced experimental findings and to estimate ^{1}H, ^{13}C and ^{15}N chemical shifts of individual structures of ligand and complex. Additionally, the linear correlation of calculated and experimental chemical shifts was expected to prove the signal assignments.

The absolute shielding scale was recalculated to the chemical shift scale using reference shielding of 182.47 ppm for ^{13}C NMR (TMS reference shielding included in Gaussian 03 program) or −165.31 ppm for ^{15}N NMR (neat CH_3NO_2, calculated at B3LYP/6-311+G(2d,p) level, assuming CH_3NO_2 as a solvent, PCM solvation model). The results have been showed in Tables 3-5. Calculated Ti-NMR data in DMSO as solvent is -144 ppm.

Table-3: Calculated 1H-NMR data in DMSO as solvent.

H-number	H8	H9	H10	H11	H12	H13
ligand	3	3	5.5	6.5	5.6	7.4
complex	3.5	3.5	7.1	8.1	7.2	7.8

Table-4: Calculated 13C-NMR data in DMSO as solvent.

C-Number	C2	C3	C4	C5	C6
Ligand	155	103	135	111	148
Complex	151	125	149	127	145

Table-5: Calculated 15N-NMR data in DMSO as solvent.

N-Number	N1	N7
ligand	300.1	47.2
complex	306.6	76.1

Cytotoxicity Studies

In this research, two cell lines concerning the blood cancer named with K562 (human chronic myeloid leukemia) and Jurkat (human T lymphocyte carcinoma) were selected. After culturing the cells and reaching to the desired value, complex and ligand were prepared in six different concentrations from 0.1-300μM for 2-Aminopyridine, 0.1-500μM for [Ti ($C_5H_6N_2$) F_3] in DMSO; and after filtering by filter (0.2 plastic) 1λ was added to the micro plates of 96 parts which are present in each one of the wells containing 5× 10^4cells. Every 24 hours micro plates were monitored.

The under the hood laminar was filtered by 0.2 plastic filter. This takes place to remove the possible contaminations from bacteria and mushroom. The number of cells into the flask containing K562 and Jurkat cells was calculated using Neobar Lam. Given that the number of cells in each one of the plate vials should be 96 parts of 5× 10^4,185/18μL of cell line of K562 was transferred to each one of the vials of the plate by sampler from the fresh environment. This was repeated for six times in 6 rows of the plate for the purpose of reducing the errors. In each row in order to control the effects, complex of the two vials was used as control. These two vials do not contain any concentration of the complex or ligand. The mentioned plate was transferred to a 37°C and 5% CO_2 incubator. Checking the status of the cells was performed every 24 hours. After 72 hours, the number of cells in each row and any concentration was counted by Neobar Lam. For this purpose at first the number of control vials and then the numbers of vials beginning from the most dilute to the most concentrated ones were counted. The IC_{50} cytotoxicity values of the complex were compared to those found for the starting organic bases as well as for some of the anticancer agents used nowadays, that are cis platin and oxaplatin compounds [16]. The corresponding 50% and 90% inhibitory doses (IC_{50} and IC_{90}) values are shown in Table-6.

Table-6: 72 hour IC50 and IC90 values obtained for two compounds (μM).

Compound	IC50 for Cell line		IC90 for Cell line	
	K562	Jurkat	K562	Jurkat
2-Aminopyridine	>45	>40	-	-
APTF	>15	>15	>100	>95

Cell Culture Assay

The human chronic myeloid leukemia: K562 cell line and the human T lymphocyte carcinoma: Jurkat cell line, used for treatment with the compounds, was provided. K562 and Jurkat cells were grown at 37 °C in an atmosphere containing 5% CO_2, with RPMI-1640 MEDIUM HEPES Modification with L-glutamine and 25mM HEPES

(SIGMA-ALDRICH CHEMIE GmbH) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2.7% sodium bicarbonate and 500 mg/L ampicillin.

In Vitro Activities

The two cell lines were provided by the Pastour Instutiute (Iranian Type Culture Collection (NCBI Code; C122 & C121). The procedure for cytotoxicity studies was similar to that reported earlier [17]. Briefly, K562 and Jurkat cell lines in (SIGMA) RPMI culture medium containing 10% FBS, 2 mmol L-Glotamine, 2gr/liter Sodium Bicarbonate, 100 Iu/ml penicillin and 100 micrograms Streptomycin was cultivated into the flask with 50 ml capacity. After the cell culture, when the number of cells reached to an appropriate level, transferring the cell into the plate was done with 96 parts special for the Nunc culture. So that to each vial of the culture plate, a volume of K562 cell suspension and another volume of Jurkat cell suspension containing 5×10^4 cells in milliliter were added. Then desired ligand and complex with specified dilutions are added to it; and using the Neobar Lam and Trypan blue method cellular fatality and cytotoxicity rate of materials and percentage of dead cells are investigated. The final concentration of DMSO in the growth medium was 2%(v/v) or lower, concentrations without effect on cell replication.[18, 19] After incubation periods 72 h for all cell lines, the cell concentrations were determined both in control and in compond-treated cultures. All experiments were carried out in six times and series.

Conclusion

The elemental and spectroscopic data show that a new complex is synthesized. Comparison of theoretical and practical results revealed an appropriate conformity. The structures optimized at the B3LYP/6-31G is reliable and reasonable. After synthesis and identification of inorganic complex, in accordance with the needs of today's society to the wide inorganic compounds containing macro-cycle ligands for being used in chemotherapy, this compound was investigated for its inhibitory effects concerning the growth of cancer cell and their fatality rate. The present research is completely a new research and it has received popular welcome by scientific community. The results were very interesting and promising; and in the following we deal with this part of the study. It is clear from the above discussion that 2-Amino pyridine titanium (IV) fluoride complex offer a new outlook for chemotherapy. It should be noted that the results suggest that complex showed greater effects than ligand. These results are concerned with the effect of the metal on complex; and this, causes increase in its Cytotoxic and Inhibitory effects. The mechanism by which these complexes act as antitumor agents is apoptosis. It has been proposed that there is close relationship between concentration and inhabitation, so that concentration plays a vital role in increasing the degree of inhabitation. [20-30]. Although apoptosis may be a natural physiological occurrence, excessive apoptosis results in tissue damage. Alternatively, apoptosis may be viewed as the response to hyper proliferation in an attempt to reduce tissue growth.

Acknowledgments

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