Titanium complexes have been synthesized by the reaction between titanium tetrachloride (TiCl₄), respective bidentate ligand [4,4'-dimethoxy-2,2'-bipyridine (bpome), 6,6'-dimethyl-2,2'-bipyridine (dpme), 1,2-diaminocyclohexane (dach), 1,10-phenanthroline (phen), and benzoylacetone (bzac)], and adamantylamine (ada) in 1:2:2 molar ratios, respectively. The structure of synthesized complexes was confirmed using elemental analysis, FTIR, UV-visible, ¹H NMR, and mass spectrometry techniques. The nanocrystalline nature of complexes was confirmed by powder XRD study. The complexes were evaluated for cytotoxic potential in HeLa (cervical), C6 (glioma), and CHO (Chinese hamster ovarian) cell lines. The complex E was found to be more effective cytotoxic agent against HeLa cell line with an IC₅₀ value of 4.06 μM. Furthermore, the effect of synthesized complexes was studied on different stages of the cell cycle in CHO cells. All complexes exhibited the dose dependent increase in cytotoxicity. The results have shown an increase in sub-G₀ population with increase in concentration which is an indicative measure of apoptosis.

1. Introduction

The discovery of cisplatin, a metal (platinum) based anticancer drug by Rosenberg et al. in 1965, has created interest in the development of metal based anticancer drugs [1–3]. The effect of transition metal complexes, other than platinum such as ruthenium [4–8], palladium [9–13], gold [14, 15], and titanium [16–25] has also been studied on several cancer cell lines. In addition to cisplatin, many other platinum based drugs, namely, carboplatin, oxaliplatin, tetraplatin, and satraplatin [3], and nonplatinum based drugs, namely, budotitane, titanocene dichloride [16], NAMI-A, KP1019 [26–29], and auranoitin [14] have shown remarkable results. Out of these, the first nonplatinum anticancer drugs were budotitane and titanocene dichloride which are titanium based drugs [16]. These titanium complexes offer an alternative to chemotherapy, although these complexes do not follow a mechanism similar to that of other metal complexes. Previous studies have revealed that titanium compounds are effective against those cell lines which are resistant to platinum based drugs and kill the cancer cells through apoptosis. It has also been confirmed that lability of ligand is not a mandatory condition for a compound to show cytotoxicity [30], but other ligand properties have been found to be necessary for this activity [31]. It is well established that ligands having electron donating atom(s) show increased cytotoxicity due to enhanced coordination capacity [32, 33]. Since few efforts have been made towards the synthesis and use of titanium complexes as chemotherapeutic agents, this is an important area of research. In the present work, we report the synthesis, structural characterization, and antiproliferative potential of some of titanium complexes.

2. Experimental Section

2.1. Materials and Methods. Ligands and titanium tetrachloride used were obtained from Sigma Aldrich. All the solvents were of AR grade (Merck) and purified by standard procedure before use and stored over 4 Å molecular sieves. Purity of ligands was checked by checking their melting points. Elemental
analyses were performed by using Perkin-Elmer, Series 2400. The UV-visible spectra of the complexes were recorded on Perkin Elmer Lambda 750 in the range of 200–800 nm and FTIR Spectra were recorded from 4000–200 cm⁻¹ on Perkin Elmer 1600. The mass spectrum was recorded by using the electron spray ionization technique on Waters Micromass Q ToF Micro. ¹H NMR Spectra were recorded on Bruker Avance 400 MHz spectrometer. Crystalline nature of the complexes has been confirmed by powder XRD technique on Philips 1710 X-ray diffractometer.

2.2. Synthetic Procedures (A–E)

2.2.1. Synthesis of Bis(adamantylamino)bis-(4,4'-dimethoxy-2,2'-bipyridyl)titanium(II), Ti(ada₂)bpmoe₂, (A). To a colorless solution of 4,4'-dimethoxy-2,2'-bipyridyl (0.45 g, 2.1 mmol) in 25 mL of toluene, a pale yellow colored solution of titanium tetrachloride (0.2 g, 1.05 mmol) in 25 mL of toluene was added dropwise with continuous stirring under ice cold conditions. The reaction mixture was stirred for 2 h followed by refluxing for 10 h till the evolution of chlorine gas ceased. The evolution of chlorine gas was checked by passing the gas through a potassium iodide solution which results in reddish brown color of potassium iodide due to liberation of iodine. After removing solvent through vacuum distillation-compound was dried under vacuum. A light yellow colored solid compound [TiCl₂(bpmoe)₂] was obtained. Yield: 0.5 g (86.2%).

2.2.2. Synthesis of Bis(adamantylamino)bis-(6,6'-dimethoxy-2,2'-bipyridyl)titanium(II), Ti(ada₂)dpmoe₂, (B). The complex was synthesized in accordance to the procedure used for complex A. Yield: 0.4 g (84.38%). TiC₄H₅N₆O₄: elemental anal. Calcd (%): C 67.67, H 7.17, N 10.41; found (%): C 67.77, H 10.23, N 14.72. FTIR (KBr, cm⁻¹) ¯: 3379 (NH stretching), 2925, 2900 (CH stretching), 1595 (C–N stretching), 1522 (CH bending), 1084, 1020 (CH out of plane deformation), 444 (Ti–N stretching). ¹H NMR (D₂O, 400 MHz): adamantylamine δ, ppm = 2.09 (s, NH), 1.7, (3J = 4 (d, CH₂ protons), 1.63, 1.55, (dd, 3J = 12, 36.76 CH protons). 1,2-Diaminocyclohexane δ, ppm = 3.63 (t, 3J = 4, 4.64 CH), 3.3(s, CH₂), 2.01, 1.76, 1.68, 1.3 (H₃, H₅, H₆, H₂).

2.2.3. Synthesis of Bis(adamantylamino)bis-(1,2-diaminocyclohexane)titanium(II), Ti(ada₂)dach₂, (C). The complex was synthesized similarly to complex A. Yield = 0.48 g (87.2%). TiC₃₂H₅₆N₆: elemental anal. Calcd (%): C 66.63, H 10.41, N 14.57; found (%): C 65.77, H 10.23, N 14.72. FTIR (KBr, cm⁻¹) ¯: 3379 (NH stretching), 2925, 2900 (CH stretching), 1595 (C–N stretching), 1522 (NH bending), 1360, 1311 (CH stretching), 2927 (CH stretching), 1612 (CH out of plane deformation), 444 (Ti–N stretching). ¹H NMR (D₂O, 400 MHz): adamantylamine δ, ppm = 2.09 (s, NH), 1.7, (3J = 4 (d, CH₂ protons), 1.63, 1.55, (dd, 3J = 12, 36.76 CH protons). L-Diaminocyclohexane δ, ppm = 3.63 (t, 3J = 4, 4.64 CH), 3.3(m, CH₂), 2.01, 1.76, 1.68, 1.3 (H₃, H₅, H₆, H₂).

2.2.4. Synthesis of Bis(adamantylamino)bis-(1,10-phenanthroline)titanium(II), Ti(ada₂)(phen)₂, (D). The procedure described above for complex A was followed for the synthesis of complex D. Yield: 0.42 g (87.5%). TiC₄₄H₆₆N₆: elemental anal. Calcd (%): C 74.55, H 6.77, N 11.85; found (%): C 74.46, H 6.42, N 11.52. FTIR (KBr, cm⁻¹) ¯: 3412 (NH stretching), 3039 (aromatic CH stretching), 2927 (CH stretching), 1612 (C=C stretching), 1514 (N–H bending), 1449 (C=N stretching), 1368, 1319 (CH bending), 1084 (CH out of plane deformation), 411 (Ti–N stretching). ¹H NMR (D₂O, 400 MHz): adamantylamine δ, ppm = 2.04 (s, NH), 1.75 (d, 3J = 2.52, CH₂ protons), 1.61, 1.55, (dd, 3J = 12, 24.8 CH protons). 10-Phenanthroline δ, ppm = 8.56 (d, 3J = 7.43, 4H, H₄ H₆), 7.7 (s, 4H, H₂ H₄), 7.4 (d, 3J = 9.28, 4H, H₄ H₆), 6.8 (dd, 3J = 28, 40, 4H, H₄ H₃).

2.2.5. Synthesis of Bis(adamantylamino)bis(benzoylacetonato) titanium(IV), Ti(ada₂)bzac₂, (E). The procedure for the synthesis of complex A was followed for the preparation of complex E. However, there was evolution of HCl gas in both the steps. Yield: 0.43 g (86%). TiC₄₈H₆₄N₆O₄: elemental anal. Calcd (%): C 71.96, H 7.45, N 4.17; found (%): C 72.10, H 7.27, N 4.10. FTIR (KBr, cm⁻¹) ¯: 3379 (NH stretching), 2933, 2866 (CH stretching), 1612 (C=O stretching), 1449 (NH bending), 1109, 1004 (C–H bending), 557 (Ti–O stretching), 427 (Ti–N stretching). ¹H NMR (D₂O, 400 MHz): adamantylamine δ, ppm = 2 (s, NH), 1.71 (d, 3J = 2.56, CH₂ protons), 1.58, 1.49, (dd, 3J = 12.56, 12.08 CH protons). Benzoylacetone δ, ppm C₅H₄: δ = 8.08 (d, 3J = 7.52, 4H, H₄ and H₆), 7.96 (t, 3J = 7.16, 4.28, 4H, H₄ and H₆), 7.6 (t, 3J = 7.64, 7.28, 2H, H₂), 3.92 (s, CH protons), 2.5 (s, CH₃ protons).
2.3. Cytotoxicity Studies

2.3.1. Cell Lines and Culture. The cytotoxic studies of synthesized complexes were performed on HeLa (cervical cancer cell line), C6 (glioma), and CHO (Chinese hamster ovarian) cell line. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing fetal calf serum (FCS) (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37°C with 90% humidity and 5% CO2. The complexes were dissolved in dimethyl sulphoxide (DMSO) to prepare the solutions of different concentrations. The selected cell lines were treated with these solutions to calculate the IC50 values by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay while control cells received only DMSO.

2.3.2. MTT Assay. The growth inhibitory effect of newly synthesized titanium complexes on HeLa, C6, and CHO cells was determined by MTT assay [34]. For this, cells were supplemented in complete growth medium to get 1×10⁵ cells/mL and 100 μL of cell suspension per well was seeded in tissue culture plate. The assay was carried out in 96 well plates in triplicate in which cells were treated with three different concentrations of complexes and incubated for 12 h in CO2 incubator. Thereafter, 20 μL of freshly prepared MTT solution, 5 mgmL⁻¹ in PBS (phosphate buffered saline) after sterile filtering, was added to each well. Now, culture plates were stirred at 150 rpm for 5 min to thoroughly mix MTT into the media. The plates were further incubated for 4 h at 37°C to allow metabolization of MTT. MTT formazan crystals were resuspended in 100 μL of DMSO and plates were stirred for 20 min in order to dissolve formazan crystals and optical density was measured at 570 nm. The phase contrast imaging was done by using Nikon Eclipse TS100 inverted microscope.

2.3.3. Cell Cycle Analysis. 1 × 10⁶ cells/dish well was plated in 24 well plates which were allowed for adhesion for 6 h and then treated with complexes at three different concentrations. After 24 h of treatment, cells were harvested from the plate. The cell suspension having 1 × 10⁶ cells was centrifuged and the resultant cell pellet was resuspended in phosphate buffered saline (1 mL) solution. The cells were fixed in ice cold 70% ethanol and stained with propidium iodide followed by analysis on the FL-2 channel by BD Accuri C6 flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA). DNA content histograms and cell cycle phase distributions were modeled from at least 15,000 single events.

3. Results and Discussion

Synthesis of titanium complexes was carried out in two steps. In the first step, titanium tetrachloride reacted with respective bidentate ligand, that is, 4,4’-dimethoxy-2,2’-bipyridine, 6,6’-dimethyl-2,2’-bipyridine, 1,2-diaminocyclohexane, 1,10-phenanthroline, and benzoylaceton which are ligands in 1:2 molar ratio under continuous stirring and refluxing by using toluene as a solvent. There was evolution of chlorine gas during the course of reaction. In the next step, respective titanium complexes reacted with main ligand (adamantylamine) in 1:2 molar ratio in the same solvent, which results in evolution of HCl gas [35] as shown in Scheme 1. Elemental analysis, that is, Titanium and chlorine estimation, was performed to check the composition by gravimetric and Volhard’s method, respectively, and molecular weight was determined by Rast’s camphor method (Table 1). The analytical data and spectroscopic characterization of complexes confirm the proposed structure of complexes. The proposed structure of complexes and their corresponding ligands has been shown in Table 2.

3.1. FTIR Spectra. The bands of FTIR were assigned by comparing the spectra of complexes with those of free ligands and were shown in Table 3. From the spectra of complexes, we have found that wave number of \( \nu \) \(_{C=H} \) band appearing around 2900–3000 cm\(^{-1} \) does not change much although the intensity of the band changes and gets weaker upon complexation with titanium metal. The absorption band due to \( \nu \) \(_{C=C} \) stretching at 1595, 1578, and 1603 cm\(^{-1} \) in the bidentate ligand of complexes A, B, and D gets shifted to 1629, 1644, and 1612 cm\(^{-1} \) (Table 3). The shift may be due to reduction in electron density after an increase in conjugation caused by complexation with titanium metal [36]. In previous studies, it has been observed that three factors, namely, field effect, steric effect, and ring strain, can cause shift in vibrational frequencies of complexes. Due to field effect [37], the value of force constant gets changed and there is change in vibration frequencies, due to steric effect [36], the conjugation in the complex is not completed which results in a shift in
absorption frequencies to higher wave number, and due to ring strain in the molecule, more energy is required for vibration of bonds which results in shift of band towards higher wave number. The ring breathing vibration (around 800–900 cm\(^{-1}\)) having more intensity gets shifted to higher wave number in complexes (around 1000 cm\(^{-1}\)). All these changes can be assigned to the coordinated nature of bidentate ligand through nitrogen atoms [12, 13]. The band formed around 3350–3400 cm\(^{-1}\) due to N–H stretching of adamantylamine ring, while the occurrence of a strong band in the region 1600–1580 cm\(^{-1}\) in complex E may be assigned to stretching modes of \(\gamma_{C=O}\) in benzoylaceton ligand. In complex E carbonyl groups are involved in bonding with the metal ion which is further supported by the appearance of an intense band at ~557 cm\(^{-1}\) assignable to \(\gamma_{M-O}\) vibration. Appearance of new bands at 452, 404, 444, 411, and 427 cm\(^{-1}\) in complexes A, B, C, D, and E shows that ligands are coordinated to the metal atom through nitrogen [35, 38] and the absence of bands in the region 385–340 cm\(^{-1}\) due to \(\gamma_{Ti-Cl}\) bond in all complexes indicates the complete removal of chloride ions [39].

### 3.2. UV-Visible Spectra

The UV-visible spectra of the complexes (Figure 1) and ligands were recorded from a solid sample by using diffuse reflectance technique. The transitions observed in the UV-visible spectrum of complexes were due to intraligand charge transfer. The transition around 320–325 nm can be attributed to \(\pi\rightarrow\pi^*\) transition in complexes A, B, D, and E get shifted to lower wavelength after coordination. However bands due to \(\pi\rightarrow\pi^*\) around 240–245 nm remain almost at the same position even after coordination. Since in complex C both the ligands are of cyclic nature, so there is no possibility of these transitions.

![Figure 1: Electronic spectra of titanium complexes.](image)

### 3.3. \(^1\)H NMR Study

The \(^1\)H NMR spectra of the complexes are consistent with the structures proposed in the reaction scheme. We find that bidentate ligands of synthesized complexes show a considerable downfield shift of protons after complexation with titanium. This shift may be due to transfer of electron density from ligand protons to the metal atom [35, 40]. However, protons of adamantylamine in all complexes appearing around 1.2–2.12 ppm show a marginal chemical shift. The cyclic aliphatic nature of both the ligands in complex C creates complications in the spectrum as the peaks corresponding to these falls almost in the same region.

However, the integration of signals in all spectra supports the formation of proposed complexes.

### 3.4. Mass Spectra

The structure of complexes was further confirmed by recording electron spray mass spectrum. The complex A showed base peak at \(m/z = 152\) due to \(\text{C}_{10}\text{H}_{16}\text{N}\) fragment ion and \(4,4'\)-dimethoxy-2,2'-bipyridine ligand in the complex showed peak at \(m/z = 217\) with relative intensity of 25%. We find that this complex also shows a peak at \(m/z = 478\) due to \(\text{TiC}_{24}\text{H}_{24}\text{N}_{6}\text{O}_{6}\) fragment ion. In case of complex B, one peak at \(m/z = 185\) due to \(\text{C}_{12}\text{H}_{12}\text{N}_{2}\) fragment ion and another peak at \(m/z = 152\) due to \(\text{C}_{10}\text{H}_{17}\text{N}\) fragment ion were found with a relative intensity of 18%. In complex C, peaks were formed at \(m/z = 98, 115, 230\) due to \(\text{C}_{9}\text{H}_{12}\text{N}, \text{C}_{9}\text{H}_{14}\text{N}_{2}\), and \(\text{TiC}_{10}\text{H}_{19}\text{N}_{2}\) fragment ions. The complex D shows peaks at \(m/z = 304\) and 335 due to \(\text{TiC}_{16}\text{H}_{22}\text{N}_{3}\) and \(\text{TiC}_{16}\text{H}_{22}\text{N}_{3}\) fragment ions. In addition to these peaks, complex D shows a molecular ion peak at \(m/z = 708\) with very low intensity. In complexes C, D, and E, formation of the base peak takes place due to \(\text{C}_{10}\text{H}_{17}\text{N}\) fragment ion at \(m/z = 152\). The complex E, in addition to base peak, also shows fragment ion peaks, in which one peak is formed at \(m/z = 401\) due to \(\text{TiC}_{22}\text{H}_{32}\text{N}_{2}\text{O}_{2}\) fragment ion. The complexes A, B, C, and E show their molecular ion peaks at \(m/z = 780, 716, 577,\) and 670.
Table 2: Structure of ligands and proposed complexes.

| Ligands                              | Proposed complexes |
|--------------------------------------|--------------------|
| Adamantylamine                       | Ti(ada)$_2$(bpome)$_2$ |
| H$_3$N                               |                    |
| $\text{H}_3\text{CO}$-$\text{N}$-$\text{N}$-$\text{N}$-$\text{OCH}_3$ | Ti(ada)$_2$(dpme)$_2$ |
| $4,4'$-Dimethoxy-2,2'-bipyridine      | Ti(ada)$_2$(dach)$_2$ |
| H$_3$C-$\text{N}$-$\text{N}$-$\text{CH}_3$ |                    |
| $6,6'$-Dimethyl-2,2'-bipyridine       |                    |
| H$_2$N$_2$                            |                    |
| 1,2-Diaminocyclohexane               |                    |
Table 2: Continued.

| Ligands Proposed complexes |
|-----------------------------|
| N N Ti NH NH N N(phen)2 Ti(ada)2(bpome)2 |
| 1,10-Phenanthroline |
| Benzoylacetone |

Table 3: Selected FTIR bands for titanium complexes and their corresponding ligands $\tilde{\nu}$ (cm$^{-1}$).

| Ligand/Complex | $\tilde{\nu}_{\text{Ti-O}}$ | $\tilde{\nu}_{\text{Ti-N}}$ | $\tilde{\nu}_{\text{C-H bend}}$ | $\tilde{\nu}_{\text{C=O-stretch}}$ | $\tilde{\nu}_{\text{N-H bend}}$ | $\tilde{\nu}_{\text{C-H stretch}}$ | $\tilde{\nu}_{\text{N-H stretch}}$ | $\tilde{\nu}_{\text{C-O stretch}}$ |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Ada            | —             | —             | 1132,1108     | 1457           | 1589           | 2912           | 3345,3372      |                 |
| bpome          | —             | —             | 1303,1230     | 1595           | —              | 3071,2974      |               |                 |
| Ti(ada)$_2$(bpome)$_2$ | 452         | 1313,1229     | 1629           | 1449           | 3015,2927      | 3388           |                 |                 |
| dpme           | —             | —             | 1247,1158     | 1578           | —              | 3063,2917      |               |                 |
| Ti(ada)$_2$(dpme)$_2$ | 404         | 1271,1117     | 1644           | 1441           | 3071,2925      | 3336           |                 |                 |
| dach           | —             | —             | 1373,1072     | 1433           | 1578           | 2924           | 3357,3285      |                 |
| Ti(ada)$_2$(dach)$_2$ | 444         | 1360,1311     | 1470           | 1522           | 2925,2900      | 3379           |                 |                 |
| phen           | —             | —             | 1344,1093     | 1603           | —              | 3055           |               |                 |
| Ti(ada)$_2$(phen)$_2$ | 411         | 1368,1319     | 1612           | 1514           | 3039,2927      | 3412           |                 |                 |
| bzac           | 1255          | 1409          | 1522           | 1449           | 2933,2866      | 3379           | 1603           |                 |
| Ti(ada)$_2$(bzac)$_2$ | 557          | 427           | 1109,1004     | 1152           | 1449           | 2933,2866      | 3379           | 1612           |

indicating the formation of complexes. The existence of these different fragment ion peaks, base peaks, and molecular ion peaks supports the stoichiometric formulation of synthesized complexes [35].

3.5. Powder XRD Study. The powder X-ray diffraction study was performed to understand the lattice structure of the complexes. Figure 2 shows XRD pattern obtained for all the complexes with well-defined peaks in these patterns which
indicate the crystalline nature of complexes. Scherrer’s equation \( D = \left( \frac{\lambda \times 0.9}{\beta \times \cos \theta} \right) \) [35, 41], with \( D \) as the crystallite size of (h k l) planes, \( \lambda \) as the wavelength of incident radiation (CuKα, 1.54 Å), and \( \beta \) as full width half maximum (FWHM), was used to calculate the crystallite size of complexes. The calculated crystallite size for complexes A, B, C, D, and E was 69, 26.5, 19.1, 115, and 76.6 nm, respectively, which falls in nanorange. Unit cell parameter of the complexes has been calculated by using Powder X software [42] and the results are summarized in Table 4. We have also observed that as the crystallite size decreases, peaks become broader as seen in Figure 2. On the basis of these different spectroscopic techniques, that is, UV-visible, FTIR, \(^1\)H NMR, and mass spectrometry, an octahedral geometry may be proposed for the synthesized titanium complexes [43].

3.6. MTT Assay. The IC\(_{50}\) values were calculated by using best fit regression model and results have been tabulated in Table 5. The change in morphological features was observed at different concentrations of complexes, which indicates that such change in morphology is dose dependent as shown in Figure 3. The phase contrast imaging was done with a Nikon microscope at 40x after harvesting stage which clearly shows the formation of small apoptotic bodies, rounding of cells, shrinkage of cells, and plasma membrane blebbing. From the calculated IC\(_{50}\) values, it has been observed that complex E with benzoylacetone ligand shows (4.06 \( \mu \)M) better activity than other complexes against the HeLa cell line, which is even better than known anticancer drug camptothecin as seen in Table 5. But ligands were found not much effective against the tested cancer cell lines.

![Figure 2: Powder XRD pattern of titanium complexes.](image-url)
Table 4: XRD data of titanium complexes.

| Empirical formula | TiC_{44}H_{54}N_{6}O_{4} (A) | TiC_{44}H_{54}N_{6} (B) | TiC_{32}H_{58}N_{6} (C) | TiC_{44}H_{46}N_{6} (D) | TiC_{40}H_{48}N_{2}O_{2} (E) |
|-------------------|-----------------------------|------------------------|------------------------|------------------------|-----------------------------|
| Formula weight    | 780                         | 716                    | 576                    | 708                    | 670                         |
| Crystal system    | Monoclinic                  | Monoclinic             | Monoclinic             | Monoclinic             | Monoclinic                  |
| Lattice type      | P                           | P                      | P                      | P                      | P                           |
| 𝑎 (Å)             | 17                          | 13                     | 16                     | 11                     | 17                          |
| 𝑏 (Å)             | 13                          | 11                     | 12                     | 13                     | 11                          |
| 𝑐 (Å)             | 14                          | 20                     | 15                     | 14                     | 16                          |
| 𝛼 (°)             | 90                          | 90                     | 90                     | 90                     | 90                          |
| 𝛽 (°)             | 91                          | 106                    | 113                    | 106                    | 85                          |
| 𝛾 (°)             | 90                          | 90                     | 90                     | 90                     | 90                          |
| Crystallite size (nm) | 69                      | 26.5                   | 19.1                   | 115                    | 76.6                        |
| 𝑉 (Å³)           | 3094                        | 2860                   | 2880                   | 2002                   | 2992                        |
| 2θ start         | 10                          | 10                     | 10                     | 10                     | 10                          |
| 2θ end           | 60                          | 60                     | 60                     | 60                     | 60                          |
| Radiation        | Cu                          | Cu                     | Cu                     | Cu                     | Cu                          |
| Wavelength       | 1.54                        | 1.54                   | 1.54                   | 1.54                   | 1.54                        |

Table 5: Cytotoxic studies of titanium complexes on HeLa, C6 and CHO cancer cell lines as determined by MTT assay.

| Complex                | Cell line (Source) | IC_{50} (µM) |
|------------------------|--------------------|--------------|
| Ti(ada)_{2}(bpome)_{2} | Hela (cervical)    | 13           |
| Ti(ada)_{2}(dpme)_{2}  | Hela (cervical)    | 74           |
| Ti(ada)_{2}(dach)_{2}  | Hela (cervical)    | 20.4         |
| Ti(ada)_{2}(phen)_{2}  | Hela (cervical)    | 11.1         |
| Ti(ada)_{2}(bzac)_{2}  | Hela (cervical)    | 4.06         |
| Adamantylamine         | Hela (cervical)    | 104.5        |
| Camptothecin           | Hela (cervical)    | 6.2          |
| Ti(ada)_{2}(bpome)_{2} | C6 (Rat glioma)    | 17.8         |
| Ti(ada)_{2}(dpme)_{2}  | C6 (Rat glioma)    | 69.8         |
| Ti(ada)_{2}(dach)_{2}  | C6 (Rat glioma)    | 21           |
| Ti(ada)_{2}(phen)_{2}  | C6 (Rat glioma)    | 22.1         |
| Ti(ada)_{2}(bzac)_{2}  | C6 (Rat glioma)    | 21.8         |
| Adamantylamine         | C6 (Rat glioma)    | 148          |
| Camptothecin           | C6 (Rat glioma)    | 6.4          |
| Ti(ada)_{2}(bpome)_{2} | CHO (Ovary)        | 19.9         |
| Ti(ada)_{2}(dpme)_{2}  | CHO (Ovary)        | 16.1         |
| Ti(ada)_{2}(dach)_{2}  | CHO (Ovary)        | 16.6         |
| Ti(ada)_{2}(phen)_{2}  | CHO (Ovary)        | 21.5         |
| Ti(ada)_{2}(bzac)_{2}  | CHO (Ovary)        | 46.1         |
| Adamantylamine         | CHO (Ovary)        | 123          |
| Camptothecin           | CHO (Ovary)        | 6.4          |

The IC_{50} values of main ligand, that is, adamantylamine along with its complexes, has been shown in Table 5. Complexes A, C, and D with 4,4'-dimethoxy-2,2'-bipyridine, 1,2-diaminocyclohexane, and 1,10-phenanthroline ligand shows good activity against all the tested cell lines which may be due to the presence of electron withdrawing nature of methoxy group, cyclic nature of 1,2-diaminocyclohexane, and aromatic nature of 1,10-phenanthroline ligand. However, complex B with 6,6'-dimethyl-2,2'-bipyridine was not found much effective against HeLa and C6 cell lines, which may be due to the presence of electron donating methyl groups in the ligand. So, it could be summarized that electron withdrawing group present in ligand as well as cyclic and aromatic nature of ligand are responsible for the cytotoxicity of titanium complexes.

3.7. Cell Cycle Analysis Using Propidium Iodide. For cell cycle analysis, CHO cells were treated with the complexes at three concentrations almost near to their IC_{50} values which caused the decrease in the number of cells with an increase in dose due to induction of apoptosis. It has been observed that all complexes increases cells in hypo-diploid cells of cell cycle and also increased the cell death with increase in concentration. Among all the complexes, complex E having benzoylaceton ligand showed 44.3% cell death at 80 µM, which is the maximum for all the complexes. However, known anticancer drug Camptothecin showed 45.5% cell death at 6 µM. Abundant evidences suggest that mitochondria plays a key role in the initiation of apoptosis by releasing Cytochrome C [44, 45]. In addition to Cytochrome C, other factors such as apoptosis signaling molecules and apoptosis inducing factor (AIF) can be important triggers of apoptosis [18]. It has been confirmed from cell cycle analysis (Figure 4) that cell death occurred through increase in hypo-diploid cells (Sub-G1 population) which indicates apoptosis. Previous studies showed that titanium affects polymerase proteins and transcription factors which inhibits protein synthesis and causes cytotoxicity [46].

4. Conclusions
We have reported the synthesis of mixed ligand titanium complexes having nitrogen containing ligands. The structure of the complexes has been confirmed by elemental analysis,
Figure 3: Morphology of CHO cells at different concentrations of titanium complexes.
Figure 4: Continued.
FTIR, UV-visible, $^1$H NMR, and mass spectrometry techniques. Cytotoxic studies were done on different cell lines and it has been found that complex E with benzoylacetonate ligand was a more potent cytotoxic agent. The morphological analysis on CHO cells indicates characteristic features of apoptosis and cell cycle analysis indicate increase in hypo-diploid cells. The mechanism of action has been certainly established in vitro; however, the efficacy of these complexes with their action mechanisms should also be demonstrated in vivo.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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