

Biological Evolution of Titanium(IV) Complex $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ Bearing Bidentate Heteroditopic Schiff Base Ligand: Synthesis, Structure and Biological Studies

Ravi K. Kottalanka^{1,*}, Eswar Pagadala¹, Shiva K. Loke¹, S. Rex Jeya Rajkumar²,
Venkatesan Srinivasadesikan¹ and Balajee Ramchandran²

¹Division of Chemistry, Department of Sciences and Humanities, Vignan's Foundation for Science, Technology and Research, Vadlamudi-522213, India

²Department of Biotechnology, Vignan's Foundation for Science, Technology and Research, Vadlamudi-522213, India

*Corresponding author: E-mail: ravikottalanka@gmail.com

Received: 24 September 2019;

Accepted: 16 November 2019;

Published online: 30 December 2019;

AJC-19740

Titanium(IV)-complex of chemical composition $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ (**2**) bearing bidentate heteroditopic Schiff base $[(\text{C}_5\text{H}_4\text{OH})\text{-N=CH-C}_6\text{H}_3\text{-NH}]$ (**L1**) ligand in titanium coordination sphere was reported and its biological significance was evaluated. The *in vitro* cytotoxicity of **L1** and **2** were evaluated by using MTT assay on cancer cell lines (MCF-7 & A549) and observed significant cytotoxicity. Further, the LDH and NO assay studies on both **L1** and **2** on cancer cell lines revealed that the enhanced cytotoxicity compared to standard anticancer drug *i.e.* cisplatin. The DNA binding studies of tested compounds with Ct-DNA molecule by using UV-visible and fluorescence spectra and molecular docking studies revealed that moderate to good binding interactions with test molecules. Thus, the present work contributes and implies the biological significance of Ti-complex (**2**) and the correlation between the structure and the biological activities of such Ti-complexes supported by Schiff base systems opens up opportunities for further exploitation of similar biologically active titanium systems.

Keywords: Titanium(IV) complex, Bidentate heteroditopic Schiff base, Biological activity, DNA Binding, Docking studies.

INTRODUCTION

Tetravalent titanium coordination complexes were evaluated as first alternative non-platinum anticancer-drugs and had reached the clinical trial stages [1-7]. The enormous benefit of titanium is that, it's low toxicity, high catalytic efficacy [8], therapeutic activity and the hydrolysis of titanium(IV) coordination complexes in biological environment yield inert TiO_2 . After the discovery of platinum based anticancer metallodrugs, two classes of titanium(IV) compounds supported by cyclopentadienide and diketonato ancillary ligands were evaluated as efficient anticancer drugs and they have been showed substantial activity and moderate to good toxicity *in vivo* [9-22]. However, the rapid hydrolysis of these Ti(IV) compounds to form multiple aggregates and low solubility of these complexes under biological environment disadvantaged further advancement in that area [14,23]. However, Salan type diaminobis(phenolato)titanium(IV) coordination complexes $[\text{LTiX}_2]$ type; where L = diaminobis(phenolato) ligand and X = labile group]

were developed three decades later and found that high efficacy both *in vivo* and *in vitro* and markedly improved stability in biological solution [24-35]. Further, structure-activity relationship (SAR) studies on various Salan type substituted diaminobis(phenolato)titanium(IV) complexes revealed that a negative effect of steric bulk and a positive effect of *ortho*-halogenation on hydrolytic stability and cytotoxicity. Further, mechanistic insight of various bis(phenolato)titanium(IV) complexes of the type $[\text{LTiX}_2]$ based on DNA binding, protein binding and enzyme binding studies suggested that labile ligands are not essential for reactivity and being the first to hydrolyze, they reduce the overall hydrolytic stability of the complexes. Thus, design and synthesis of new water stable therapeutic Ti(IV) metal complexes, with no labile ligands, are highly desirable for anticancer applications and clinical trails.

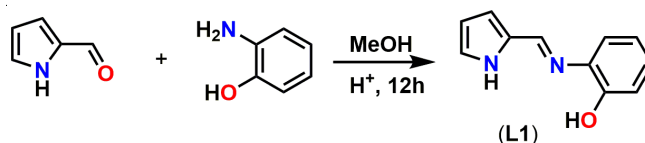
In this work, we report a new water stable titanium(IV) complex of composition $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ (**2**) bearing bidentate heteroditopic Schiff base $[(\text{C}_5\text{H}_4\text{OH})\text{-N=CH-C}_6\text{H}_3\text{-NH}]$ (**L1**) ligand in titanium coordination sphere. The obtained

Ti-complex (**2**) and the ligand (**L1**) were characterized using spectroscopic and analytical techniques to conform the structures and composition of the compounds. The *in vitro* cytotoxicity of ligand (**L1**) and Ti(IV) complex (**2**) were further evaluated by using MTT assay dilution method on cancer cell lines (MCF-7 & A549) and observed significant cytotoxicity. Furthermore, lactose dehydrogenase assay (LDH) and nitric oxide assay (NO) studies on both ligand (**L1**) and titanium(IV) complex (**2**) on cancer cell lines (MCF-7 and A549) revealed that the enhanced cytotoxicity compared to the standard anti-cancer therapeutic drug *i.e.* cisplatin by releasing significant amount of LDH and NO from the cancer cells. The ligand and its Ti(IV) complex were further examined on various Gram-positive and Gram-negative bacterial and fungal strains under *in vitro* condition, exhibited significant antibacterial and anti-fungal activity. The DNA binding studies were carried out to evaluate the binding interactions of these compounds with Ct-DNA helix by using UV-visible and fluorescence spectral measurements and molecular docking studies. DNA binding and molecular docking studies revealed that Ti-complex (**2**) is more active towards protein molecules having multiple binding interactions with more than one type bindings such as inter-chelation and electrostatic binding interactions.

EXPERIMENTAL

All manipulations of air-sensitive materials were performed with the rigorous exclusion of oxygen and moisture in flame dried Schlenk-type glassware on a dual manifold Schlenk line, interfaced to a high vacuum (10^{-4} torr) line. Hydrocarbon solvents (toluene and *n*-hexane) were distilled under nitrogen from LiAlH₄ and stored under N₂ atmosphere. HPLC grade methanol was purchased from Sigma-Aldrich and it was further dried by using 3 Å molecular sieves and followed by distillation. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a VARIAN INOVA 400 spectrometer at Accu Analytical, Hyderabad, India. Perkin-Elmer spectrum IR version 10.6.0 and Agilent Technologies FT-IR was used for FT-IR measurement fitted in the 4000–450 cm⁻¹ range. Elemental analyses were performed on a Truespec Micro CHNS 209-190. Electronic absorption spectra were obtained on a Shimadzu Lambda-1800 UV-Vis spectrophotometer. The starting materials like pyrrole-2-carbaldehyde, 2-aminophenol (99 % pure), Titanium(IV) isopropoxide (97 % pure) were purchased from Sigma-Aldrich and used without further purification.

Synthesis of Schiff based ligand (L1): A bidentate heteroditopic Schiff base ligand [(C₅H₄OH)-N=CH-C₄H₃-NH] (**L1**) was synthesized as per the literature procedure [36]. In brief, a solution of pyrrole-2-aldehyde (2.0 g, 21.0 mmol) was added drop by drop to a boiling solution of 2-aminophenol (2.29 g, 21 mmol) in 50 mL MeOH. The reaction mixture was refluxed for 1 h and then concentrated to 30 mL. After cooling, the solution was mixed with 30 mL of water (**Scheme-I**). The precipitate was recrystallized from MeOH/H₂O (1:2): (2.06 g) yield: 85 %. FT-IR (selected frequencies, KBr, $\nu_{\max}$, cm⁻¹): 3410br (N-H), 3061w (ArC-H), 1618s (C=N). Elemental analysis of C₁₂H₁₀N₂O (m.w. 202.22), calcd. (found) %: C 74.21 (74.21), H 4.95 (4.95), N 10.84 (10.84).



Scheme-I: Synthesis of heteroditopic Schiff base ligand (**L1**)

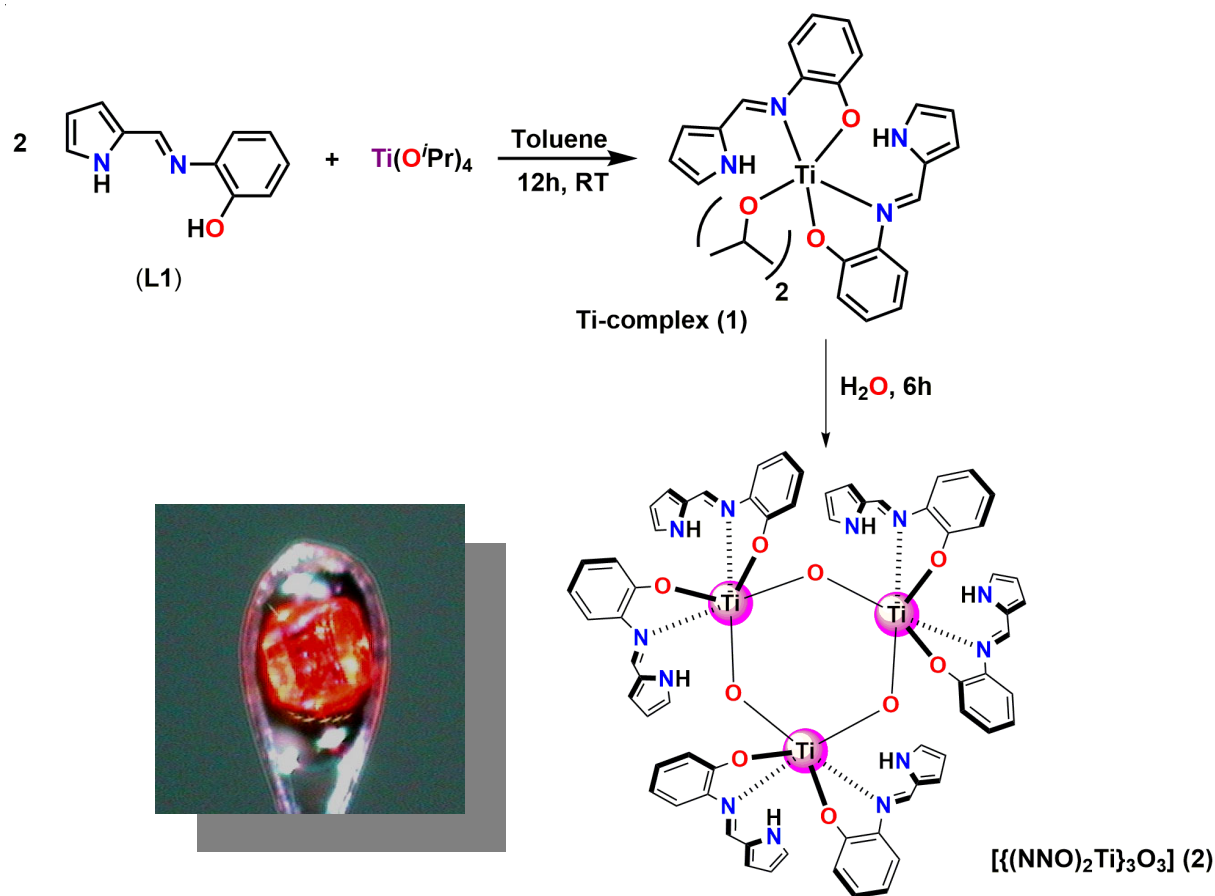
Synthesis of [(NNO)₂Ti]₃O₃] (2**):** To a dried toluene solution (10 mL) of bidentate heteroditopic Schiff base ligand (**L1**) (1.0 g, 5.30 mmol), toluene solution (10 mL) of titanium isopropoxide (0.7g, 2.65 mmol) was added under stirring condition at room temperature. The reaction mixture was then allowed to proceed for another 12 h under inert atmospheric conditions and then all volatile materials and solvent were removed under vacuum to afford titanium(IV) complex of composition [(C₅H₄O)N=CHC₄H₃NH)₂Ti(OiPr)₂] (**1**) as reddish-yellow solid. The obtained titanium(IV) compound (**1**) was further treated with H₂O in 1:10 molar ratio for 12 h under constant stirring at room temperature. Volatile materials and solvents were removed under vacuum to give deep red colour solid and then it was washed with *n*-hexane (**Scheme-II**). The recrystallization of compound **2** from hot toluene gave the required compound as crystalline product in 89 % yield (3.57 g). ¹H NMR (400 MHz, CDCl₃): δ 12.45 (br, 1H, N-H), 7.36 (s, 1H, N=C-H), 6.81 (m, 1H, ArC), 6.69 (m, 1H, ArC), 6.58 (m, 1H, ArC), 6.34 (m, 1H, ArC), 6.27-6.29 (d, 2H pyr), 5.88 (d, 1H, 5-pyr). ¹³C NMR (100 MHz, CDCl₃): δ 159.2 (N=CH), 149.6 (ArC), 140.9 (ArC), 127.2 (ArC), 124.6 (p-ArC), 119.2 (PyC), 115.4 (PyC), 114.3 (PyC), 109.6 (PyC). FT-IR (selected frequencies, KBr, $\nu_{\max}$, cm⁻¹): 3145 (br, N-H), 2860 (w, ArC-H), 1654 (s, C=N), 1245(s, C-O). Elemental analysis of C₆₆H₅₄N₁₂O₉Ti₃ (m.w. 1302.83), calcd. (found): C 60.85 (60.82), H 4.18 (4.21), N 12.90 (12.86).

Detection method: *in vitro* Cytotoxicity activity studies were performed by using MTT assay dilution method and further confirmed by using LDH and NO assay methods [37,38]. The antibacterial and antifungal studies were evaluated by determining the MIC studies on various Gram-positive and Gram-negative bacterial and fungi strains.

Biological studies

Cell cultures: The cancer cell lines used in present study include human lung carcinoma (A549) and human breast carcinoma (MCF-7), whereas the normal cell lines used were human Keratinocytes (HaCaT). All cells were purchased from National Centre for Cell Science (NCCS), Pune, India. A549 were grown in RPMI-1640 (Roswell Park Memorial Institute), while MCF-7 and HaCaT were grown in DMEM (Dulbecco modified eagles medium), respectively. To ensure growth and viability of the cells, the mediums were supplemented with 10 % FBS (HiMedia, India) and incubated in a humidified atmosphere with 5 % CO₂ at 37 °C.

MTT assay: All the three cells (2.5×10^3) were seeded in 96-well plates and allowed to adhere overnight at 37 °C. Briefly, following treatment of cells with cisplatin and all the other compounds for 24 h, MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and incubated for 4 h at 37 °C. Dimethyl sulphoxide was used as a control. Absorbance was recorded at 595 nm and sensitivity



Scheme-II: Synthesis of tri-nuclear Ti-Schiff base complex $[\{(NNO)_2Ti\}_3O_3]$ (2)

to cisplatin and the compounds were calculated based on cell proliferation measurements at 24 h.

Lactate dehydrogenase release: LDH assay was performed according to the literature method as described by Wacker *et al.* [37].

Nitric oxide assay: Nitric oxide assay was performed according to the literature method [38].

in vitro Antibacterial and antifungal activities: The test microorganisms used for screening four bacterial pathogens (Gram-positive and Gram-negative) and 5 fungal pathogens which were procured from Institute of Microbial Technology (IMTECH-CSIR), Chandigarh, India. The pure bacterial stock cultures were maintained on nutrient agar medium, whereas the fungal stock cultures were maintained on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub-culturing regularly on the same medium and stored at 4 °C. The antibacterial and antifungal activities of both bidentate heteroditopic Schiff base (L1) and its Ti-complex (2) were evaluated by using standard agar diffusion method as reported, by taking Gram-positive and Gram-negative pathogens viz. *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, *A. baumannii* and fungal pathogens viz. *C. albicans*, *T. rubrum*, *A. fumigatus*, *A. niger* and *C. tropicalis*. Gentamycin and ketoconazole were used as positive controls to study the antibacterial and antifungal activities, respectively. The antimicrobial activity of the test compounds was checked with various concentrations (25, 50 and 100 µg/mL) against all the test pathogens.

Each experiment was performed in triplicate and the results are represented as an average zone of inhibition and minimum inhibitory concentration of all the test pathogens.

Minimal inhibitory concentration (MIC) determination: The antibacterial and antifungal activity of bidentate heteroditopic Schiff base (L1) and its titanium(IV) complex (2) were determined using sterile 2 mL 96-well plates [39]. The 12 wells of each row were filled with 0.5 mL sterilized Mueller Hinton agar. Sequentially, wells 2-11 received an additional 0.5 mL of a mixture of culture medium and plant extract serially diluted to create a concentration sequence from 0.512 mL to 0.008 mL. Well 1 served as growth control, well 12 as antibiotic control. Gentamycin (10 µg/mL) and ketoconazole (10 µg/mL) were used as controls for the bacteria and fungi, respectively. The respective antibiotics were chosen because they are often employed as first line antibiotics in the respective bacterial and fungal infections. The deep wells were incubated for 24 h at 37 °C. The resulting turbidity was observed and after 24 h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm with a UV-Vis spectrophotometer.

Molecular docking: The Schrodinger molecular modeling suite was employed to carry out the molecular docking protocols. The binding affinity plays a crucial role to determine the biological activity between the receptor and inhibitor. It was influenced by the geometrical positions, steric and the physical properties. The small molecule was synthesized in our laboratory. It was re-drawn using ACD/Chemsketch ver

12.0, it was geometrically optimized and converted into 3D format. the PDBID:3G7B was extracted from Protein Data Bank (www.rcsb.org). Later, the protein molecule was prepared using Protein preparation wizard, Schrodinger. The molecule was optimized, and it was minimized with the OPLS-2005 force field.

Docking methodologies: The molecular docking was performed using GLIDE, Schrodinger with the vdW scaling of 0.8 and partial cut-off of 0.15 to soften the potential for non-polar sites and implemented without the constraints. The docking was performed using GLIDE-SP. An attention paid to obtain the better GLIDE energy and interactions to favour the outcome.

RESULTS AND DISCUSSION

The bidentate heteroditopic Schiff base ligand of chemical composition $[(C_5H_4OH)-N=CH-C_4H_3-NH]$ (**L1**) was successfully reproduced accordingly the literature method [36]. The Schiff base ligand (**L1**) was then treated with $Ti(O^iPr)_4$ in 2:1 molar ratio in dry toluene. Under inert atmospheric conditions, titanium(IV) complex of chemical composition $[(C_5H_4O)N=CHC_4H_3NH)_2Ti(O^iPr)_2]$ (**1**) was first isolated as an intermediate compound (reddish-yellow solid). Without any further purification the obtained titanium(IV) complex (**1**) was immediately treated with H_2O in 1:10 molar ratio at atmospheric conditions. The resultant deep red colour compound so obtained was recrystallized in hot toluene to afford the required Ti-complex of composition $[{(NNO)_2Ti}]_3O_3$ (**2**) in good yield (89 %).

The FT-IR spectrum of Ti-complex (**2**) showed a broad absorption band at 3145 cm^{-1} , which indicate the presence of N-H bond stretching. The enhanced broadness is due to the inter-molecular hydrogen bonding exists between the pyrrole N-H and adjacent phenolic oxygen atom (N-H...O) of the ligand moiety. Further, in FT-IR spectra, the absorption band at 1654 cm^{-1} could be ascribed to the $-C=N-$ (imine) bond stretching and absorption band at 1245 cm^{-1} could be best ascribed to the

$-C-O$ bond stretching. All other pyrrole ring and aromatic ring C-H vibrations were best fitted with the literature reports [40-44]. The 1H NMR spectrum of Ti-complex (**2**), clearly showed the broad resonance signal at δ 12.45 ppm, could be best assigned to the N-H proton and a singlet signal observed at δ 7.36 ppm could be assigned to imine $-N=C-H$ proton, respectively. The resonance signals for pyrrole ring protons were obtained in the region of δ 5.88-6.29 ppm are in good agreement with literature reports [44,45]. The aromatic ring protons as well as ^{13}C NMR spectrum of Ti-complex (**2**) were showed resonance signals at expected regions and fully supported the formation of Ti-complex (**2**) of composition $[{(NNO)_2Ti}]_3O_3$ in good yield.

X-ray crystallographic studies: The single crystals suitable for X-ray diffraction analysis were obtained in hot toluene after two days. The X-ray crystallographic studies showed that Ti-complex (**2**) crystallizes in monoclinic I_2/a space group having four molecules in the unit cell. However, we couldn't obtained suitable crystallographic data miserably, due to the decomposition of crystal when it is exposed to X-rays for long period of time. The obtained crystallographic data was used for structure refinement by using (SIR92) [46] and refined on F^2 by full-matrix least-squares methods using SHELXL-97 [47]. The Diamond 3 and Mercury 4.1 programs were used to draw the solid-state structures of Ti-complex (**2**) and those are shown in Fig. 1.

The crystallographic data reveals that each titanium(IV) cationic center present in the Ti-complex $[{(NNO)_2Ti}]_3O_3$ (**2**) is six-coordinated due to the mono- and bidentate chelation from four oxygen atoms (*i.e.* two are from phenolic oxides and two are bridging oxides derived from replacement of isopropoxide groups) and two imine nitrogen atoms of the two ligand moieties. Furthermore, no coordination from N-atoms of the pyrrole rings were also observed. Therefore, at each Ti(IV) cationic center there exists distorted octahedral geometry and a six-membered ring is constructed in a trinuclear form having D_3 symmetry with three Ti-O units. To support this result similar

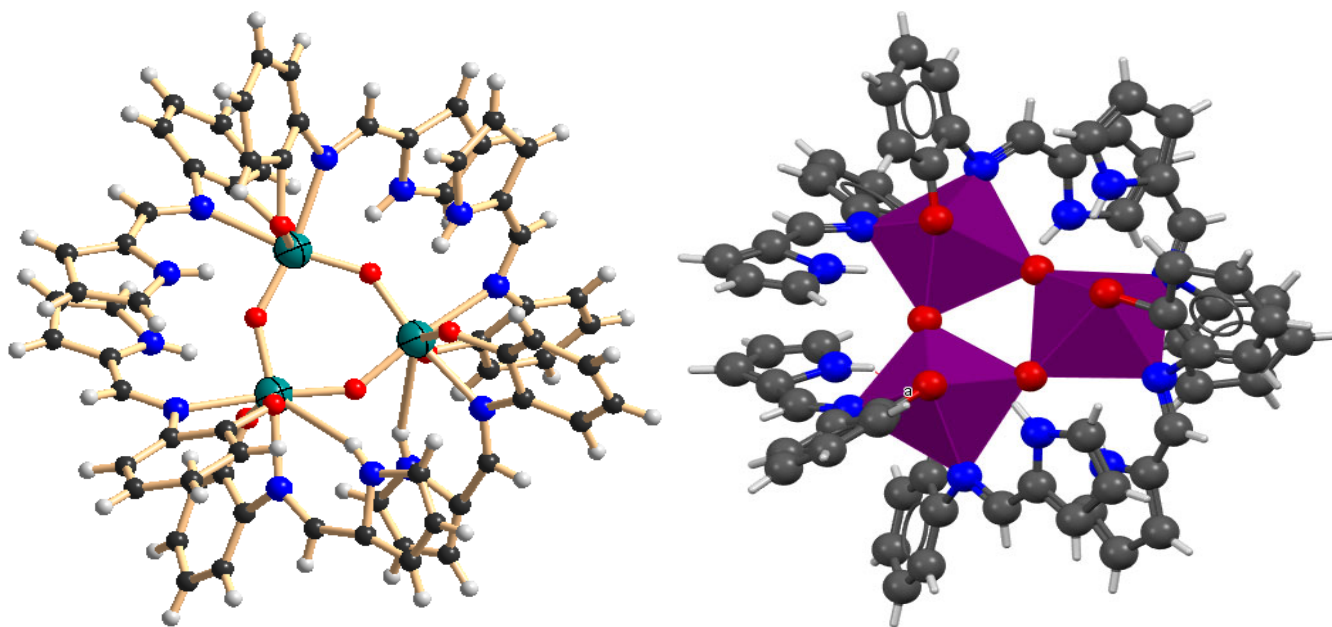


Fig. 1. Solid-state structure of $[{(NNO)_2Ti}]_3O_3$ (**2**) obtained by diamond (left) and mercury (right)

kind of trinuclear six-membered ring was also observed in Ti-complex of chemical composition $[(\text{L}^{\text{Cl-H}})_3\text{Ti}_3\text{O}_3]$ (where $\text{L}^{\text{Cl-H}} = p\text{-Cl-C}_6\text{H}_4\text{-CH=N-N-CH-C}_6\text{H}_4\text{-(}o\text{-O)}$) in the literature [48]. In addition, the crystallographic studies also revealed that each titanium octahedral core is vertex shared with other two titanium octahedral cores through bridging oxide ligands. The obtained structure was best fitted with the NMR data further indicate that trinuclear ring structure was still remains in the solution state as well.

Biological activity studies

in vitro Cytotoxicity activity: Bidentate heteroditopic Schiff base ligand (**L1**) and Ti-complex (**2**) were subjected to MTT assay towards, the normal human Keratinocytes (HaCaT), human lung carcinoma (A549) and human breast carcinoma (MCF-7) cell lines. After 24 h of incubation, ligand and metal complex showed good cytotoxic reduction in the cell viability on comparing with cisplatin. The cytotoxic ability of synthesized ligand and Ti-complex occurred in a dose-dependent manner as shown in Fig. 2 and furthermore, the IC_{50} values were also calculated. The metal complex was found to have potential cytotoxic effect than the ligand against both cancer cell lines tested.

The bidentate chelation of the ligand with Ti(IV) might be responsible for the cytotoxic nature of the metal complex

over ligand. Ligand (**L1**) and Ti-complex (**2**) were screened for their cytotoxic activity on the normal cell lines *viz.* HaCaT and it was found to be non-toxic in nature as shown in Fig. 2. The enrichment of cytotoxic activity of Ti-complex (**2**) can be corroborate with structure-activity relation (SAR) where electron donating N-terminal substitution on the coordinated ligand play major role. Ti-complex (**2**) owing un-coordinated nitrogen as a terminal N-substitution in the coordinated ligand and probably, which is responsible for the higher cytotoxic ability than the titanium complexes bearing only coordinated nitrogen ligands. In addition, anticancer effectiveness of new Ti-complex (**2**) was found to be better than Ti-complexes already reported in the literature [11-14].

LDH assay: The cancer cell lines MCF-7 and A549 were treated with the different amounts of the Ti-complex (**2**), ligand (**L1**) and standard anticancer drug (cisplatin) for a period of 24 h. A significant amount of LDH release was observed in the culture medium (Fig. 3) by Ti-complex (**2**) compared to cisplatin indicates the efficiency of Ti-complex (**2**) by inducing the cell death of membrane integrity is collapsed. Compared to cisplatin, Ti-complex (**2**) showed a good level of leakage of LDH in MCF-7 and A549 cells. From the present findings, it can be determine that Ti-complex (**2**) was found to be more effective than the corresponding Schiff base ligand (**L1**).

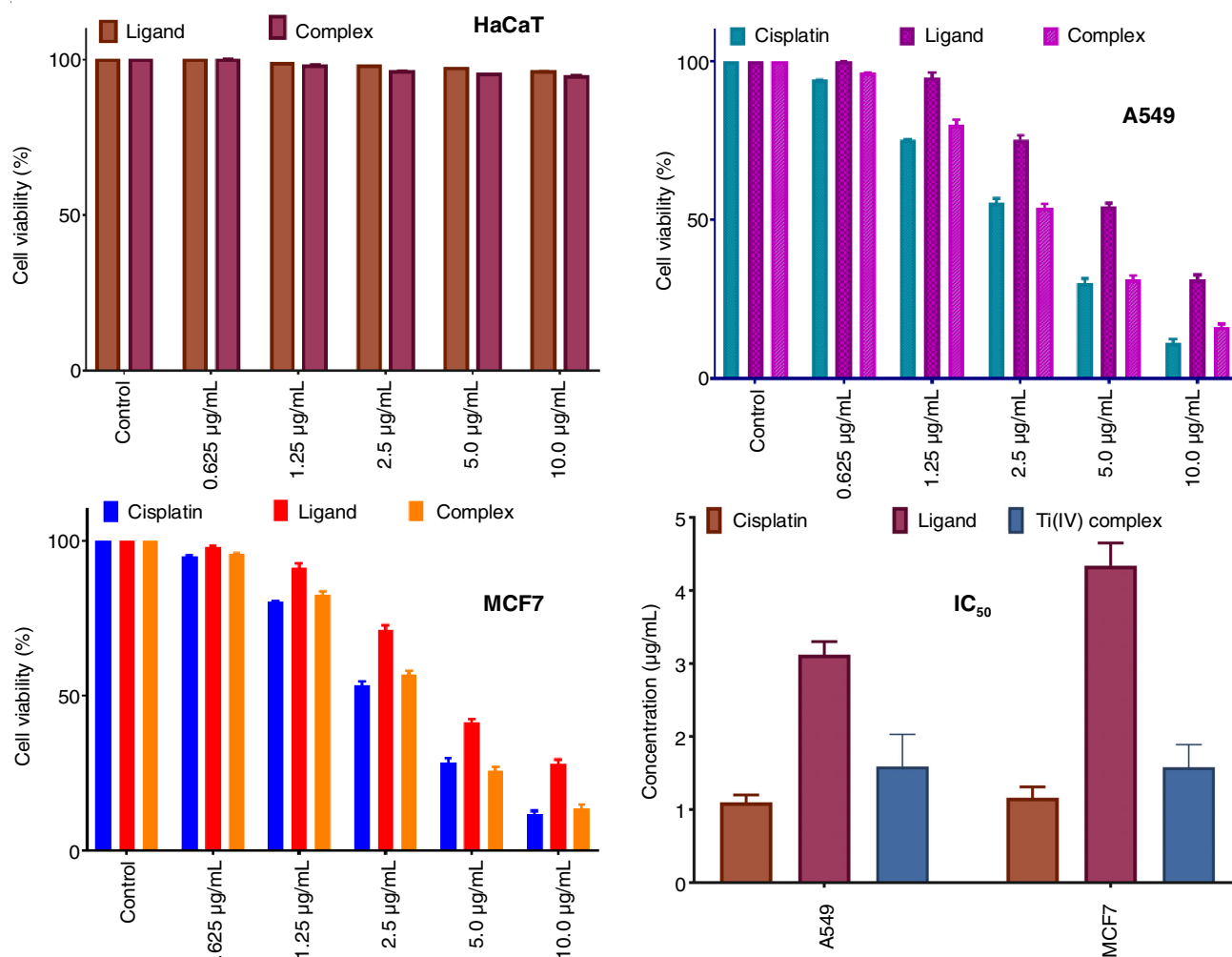


Fig. 2. *in vitro* Cytotoxicity activity of ligand; Ti-complex with standard drug (*i.e.*, cisplatin)

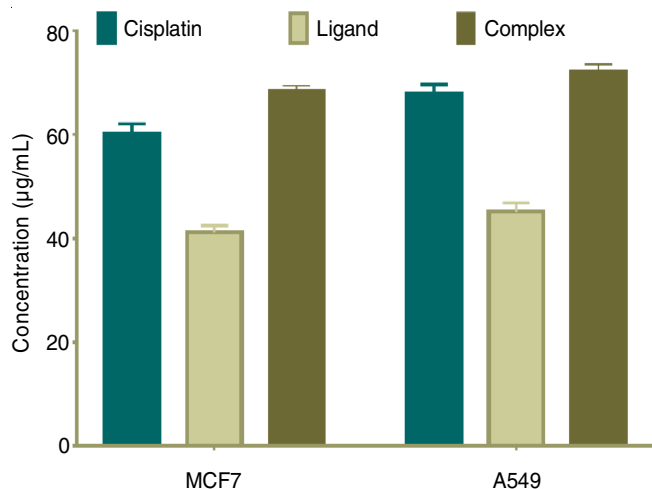


Fig. 3. Amount of LDH released by the MCF-7 and A549 cells after an incubation period of 24 h with the Ti-complex (2)

NO assay: In this study, amount of nitric oxide released by Ti-complex (2), when it is treated with MCF-7 and A549 cancer cells was evaluated. The determination of nitrite released in the cell media by Griess assay [38] is one of the cost effective measurements. The Griess assay showed that Ti-complex (2) exceptionally effective to release high amount of NO in culture medium than standard anticancer drug *i.e.* cisplatin and corresponding ligand (L1) (Fig. 4), hence NO assay study useful to conform the cytotoxicity activity of the studied Ti-complex (2).

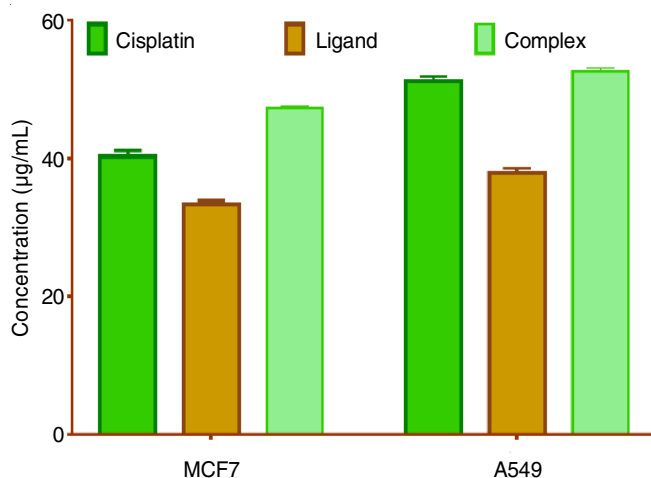


Fig. 4. Nitrite released (n moles) by the MCF7 and A549 after an incubation period of 24 h with the test compounds

in vitro Antibacterial activity: The antibacterial activities of heteroditopic Schiff base ligand (L1) and its Ti-complex (2) were tested with reference to gentamycin (10 µg) on Gram-positive bacteria: *S. aureus* & *S. pneumonie* and Gram-negative bacteria: *P. aeruginosa* & *S. typhi*. The antibacterial activities of the ligand (L1) and its Ti-complex (2) were evaluated by measuring the inhibition zone observed around the tested materials (Fig. 5). The maximum zone of inhibition was observed for Ti-complex (2) when compared to corresponding heteroditopic Schiff base ligand and it can effectively decrease the population of bacterial species. This is mainly because of chelation effect [49], where the donor groups of chelating ligands

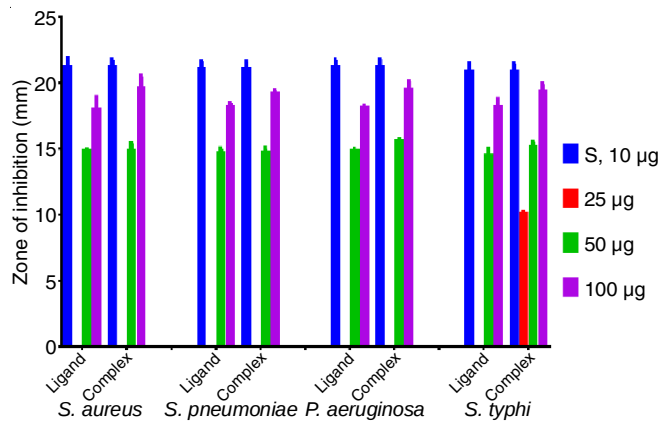


Fig. 5. Antibacterial activity of Schiff base ligand and Ti-complex (2)

considerably reduce the polarity of the central atom by partial sharing of its positive charge with donor groups and possible π -electron delocalization within the whole chelating rings. This eventually increases the lipophilic nature of metal complexes [50].

Therefore, an increase in the lipophilic character of Ti(IV)-complex (2) seems to be responsible for their enhanced antibacterial activity. The antibacterial activity results also displayed all the compounds possess higher inhibitory activity against the bacterial species at 100 µg/mL concentration than at 50 µg/mL concentration.

in vitro Antifungal activity: *in vitro* Antifungal activity of newly synthesized heteroditopic Schiff base ligand (L1) and its Ti-complex (2) were carried out against five different fungi cells such as *Candida albicans*, *Trichophyton rubrum*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida tropicalis* and compared with standard antifungal drug ketoconazole at minimum (10 µg/mL) concentration. Antifungal activity data is shown in Fig. 6, which indicates that Ti-complex (2) was found to be more toxic than heteroditopic Schiff base ligand, whereas at higher (100 µg/mL) concentrations both Schiff base ligands and Ti-complex (2) were found to be more toxic towards fungi cells. The activity is greatly enhanced at the higher concentration.

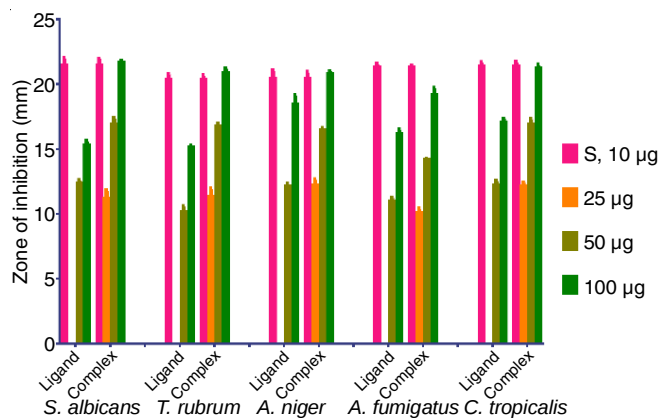


Fig. 6. Antifungal activity of Schiff base ligand and Ti-complex (2)

DNA binding studies: The DNA binding studies were carried out by using UV-absorption and fluorescence emission spectral investigations. The amount of Schiff base ligand (L1)

and Ti-complex (**2**) were kept constant at $[\text{Test sample}] = 2.26 \times 10^{-4} \text{ mol/dm}^3$ and the aliquots of Ct-DNA were added up to a final concentration of $[\text{Ct-DNA}] = 0.121 \times 10^{-4} \text{ mol/dm}^3$. As the addition of Ct-DNA increases, the absorption band at 261 nm enhances (Fig. 7). The band at 261 nm is mainly because of the absorption of light by Ct-DNA helix and hence, this blue shift and the absorbance values were not applied for the calculation of the binding constant in case of ligand and Ti-complex (**2**) with Ct-DNA. However, in addition to absorption band at 261 nm, we observed another less intense absorption band at 314 nm in case of Ti-complex (**2**). The addition of portion of Ct-DNA does not alter this band due to the enrichment of the 261 nm band which defeats the intense changes in the 314 nm band. However, no such kind of less intense absorption band was observed in case of Schiff base ligand (**L1**) when it treated with different aliquots of Ct-DNA.

The binding titrations were performed using fluorescence emission spectroscopy. The fluorescence emission spectra of both Schiff base ligand (**L1**) and Ti-complex (**2**) with different amounts of Ct-DNA are shown in Fig. 8. The ligand fluorescence spectra displayed two emission bands viz. 310 and 355

nm in 300-400 nm region and less intense fluorescence band at 410 to 436 nm was also observed. On addition of Ct-DNA, there is no change in 310 fluorescence band and little down field (towards higher energy) shift was observed in 355 nm fluorescence band with increased intensity. A considerable quenching of fluorescence band at 410 to 436 nm was observed at higher concentration of added Ct-DNA. On the other hand, fluorescence spectra of Ti-complex (**2**) displayed three intense fluorescence bands viz. 309, 355 and 415 to 440 nm. On addition of Ct-DNA, there is no change in 309 fluorescence band. The intensity of 355 nm band was slightly decreased and little down filed shift towards higher energy or shorter wavelength side was observed. However, a very significant quenching of fluorescence band of 415 to 440 nm was observed at higher concentration of added Ct-DNA. These results clearly indicate that Ti-complex (**2**) was more active towards DNA helix compared to the corresponding Schiff base ligand (**L1**) with more than one mode of binding.

It is known that the fluorescence phenomenon is being sensitive to the medium, nature of fluorophore and polarity change results in shift of the spectrum [51]. The mechanism

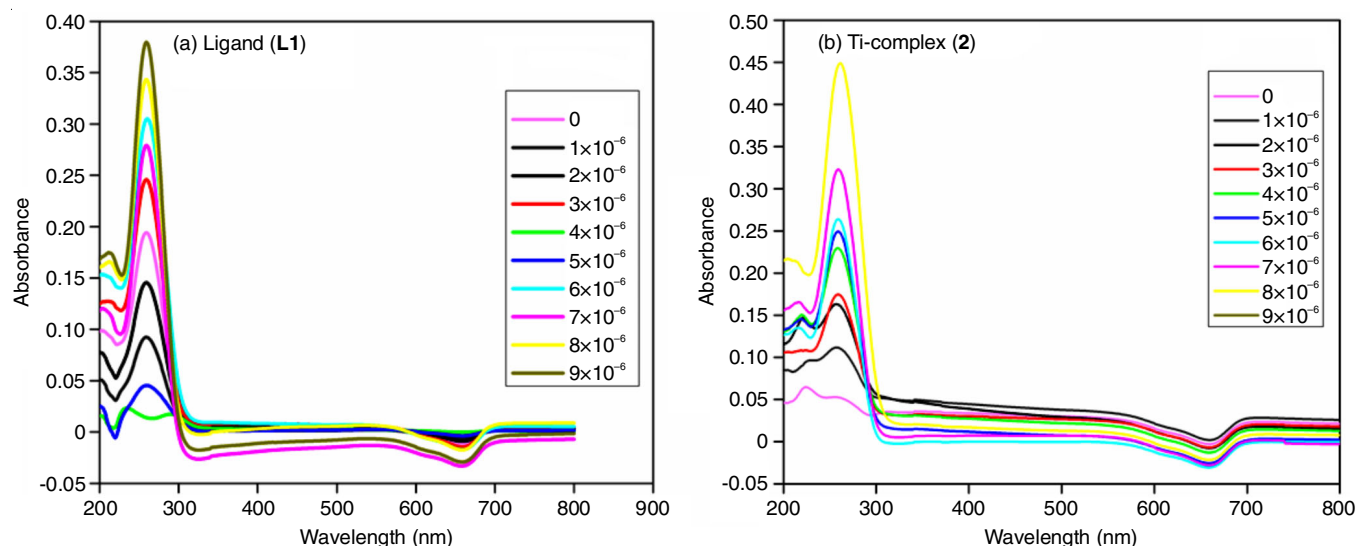


Fig. 7. (a) & (b). Absorption spectra of Schiff ligand (**L1**) and Ti-complex (**2**) with different concentrations of added Ct-DNA

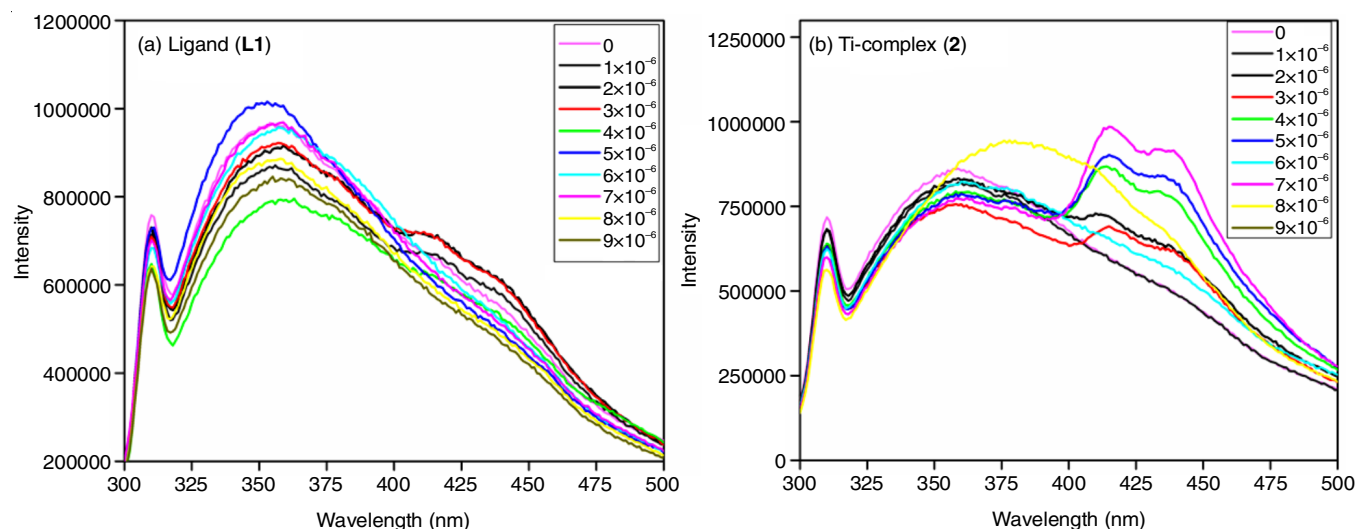


Fig. 8. (a) & (b). Fluorescence spectra of Schiff ligand (**L1**) and Ti-complex (**2**) with different concentrations of added Ct-DNA

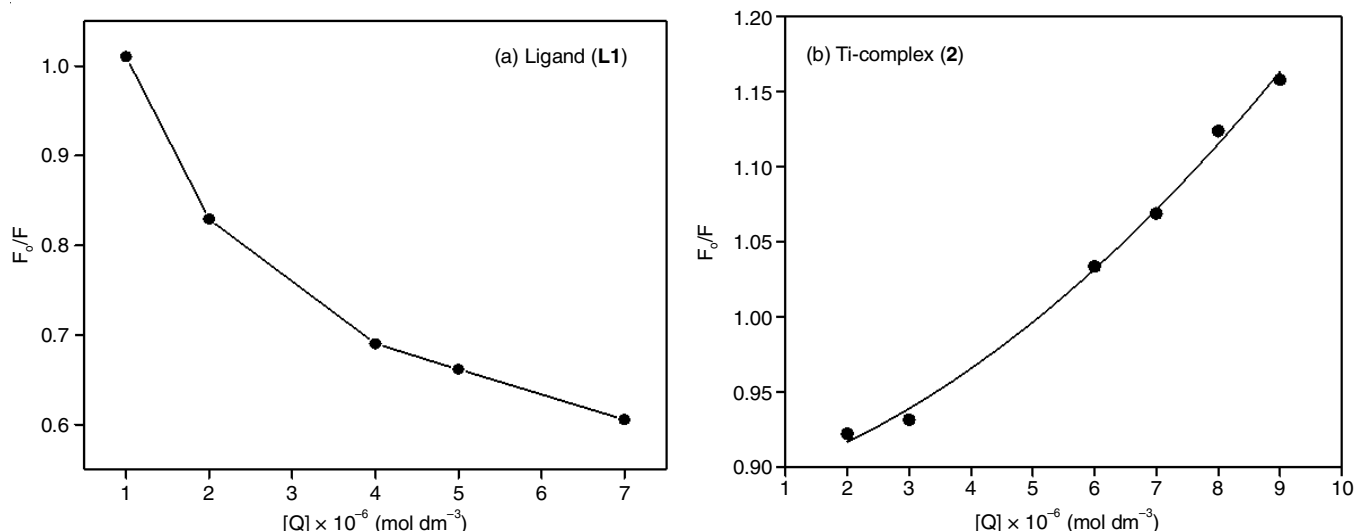


Fig. 9. Stern-Volmer plots of Schiff base (**L1**) and Ti-complex (**2**) with concentration of added Ct-DNA

of interaction of Ct-DNA with the binder involves the insertion of the binder into the active base pairs of DNA helix. The freely rotating small (binder) molecule probably deactivates the excited states and hence there will be fluorescence enhancement [52]. Further, narrow-cut binding agents which experience electrostatic interactions with Ct-DNA get close to the backbone of Ct-DNA *viz.* sugar phosphates groups and this leads to the significant quenching of fluorescence activity. In the current fluorescence spectral studies, it is noticed that quenching and the enhancement of fluorescence bands of Ti-complex (**2**), which indicates that when Ti-complex (**2**) treated with Ct-DNA helix probably two type of interactions namely electrostatic and intercalative binding were involved. The Stern-Volmer equation was utilized to find the quenching constant for the interaction of binders such as Schiff base ligand (**L1**) and Ti-complex (**2**) with Ct-DNA.

$$\frac{F_0}{F} = 1 + k_{sv}[Q]$$

where, F_0 = Initial intensity of fluorescence of binder without Ct-DNA; F = intensity of fluorescence of binder with Ct-DNA.

In the presence of quenching agent (ligand/Ti-complex), k_{sv} is the quenching constant and $[Q]$ is the concentration of quenching agent (ligand/Ti-complex). The Stern-Volmer quenching studies were illustrated in Fig. 9. The Stern-Volmer plot of ligand (**L1**) with varying concentration of Ct-DNA represents the non-linear downward curve due to the lesser binding capability of ligand (**L1**), whereas the corresponding Ti-complex (**2**) plot represents the upward straight line which is indicative of static fluorescence quenching process due to the formation of stable complex with binder (Ti-complex) molecules. The quenching constant k_{sv} value obtained as a slope from the plot of $[Q]$ *vs.* F_0/F was found to be $2.9 \times 10^4 \text{ M}^{-1}$. These results further support the strong binding ability of Ti-complex (**2**) over the Schiff base ligand (**L1**) towards the Ct-DNA helix.

Molecular docking analysis: The study aimed to identify the significance of the synthesized small molecule against anti-bacterial target [53,54]. The molecular docking has initiated with reproducing the crystal structure of 3G7B. The hydrogen bonding interactions have been observed from the in-sights of

the conformation. The complex results the docking score of -3.224 kcal/mol with the hydrogen bonding between the Asp81 with the distance of 1.85 Å as shown in Fig. 10.

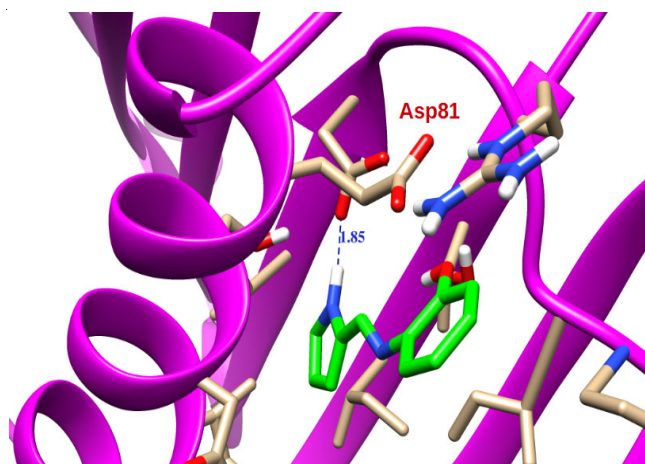


Fig. 10. Binding interactions of heteroditopic Schiff base ligand (**L1**) with protein molecule having non-covalent interactions with Asp81

Therefore, residues are considered as an active site to proceed further. The docking was performed with the ciprofloxacin which shows as an interaction with Asp81 and Ser129 respectively, with the distance ~ 2.0 Å. The test molecule was docked with the reference of the existing molecules. The test molecule revealed that it has an interaction with Asp81 with 1.85 Å, by showing -3.224 kcal/mol. The test molecule clearly shows that it contains strong hydrogen bonding network with N-H...O type interaction. However, corresponding trinuclear Ti-complex $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ (**2**) is having three Ti(IV) cationic centers and each cation is surrounded by two such type of small molecule having uncoordinated N-H groups. Therefore, Ti-complex (**2**) perhaps having multiple interactions with protein used for docking studies and may have approximately -10-18 kcal/mol energy as docking score. It clearly shows that Ti-complex (**2**) is more active towards protein molecules and therefore, it can be considered as active antibacterial/fungal and anticancer agents.

Conclusion

In this study, synthesis of tri-nuclear Ti(IV)-complex of type $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ (**2**) was well demonstrated. To evaluate biological significance of synthesized Ti-complex (**2**) along with corresponding bidentate heteroditopic Schiff base ligand $[(\text{C}_5\text{H}_4\text{OH})\text{-N=CH-C}_4\text{H}_3\text{-NH}]$ (**L1**), we studied systematically *in vitro* cytotoxicity activity by using MTT assay on cancer cell lines *viz.* human breast carcinoma (MCF-7) and human lung carcinoma (A549) and normal cell lines such as human keratinocytes (HaCaT). The results demonstrated that Ti(IV)-complex (**2**) displayed significant cytotoxicity compared to standard anticancer drug (*i.e.* cisplatin). The enhanced cytotoxicity of Ti-complex (**2**) was further supported by the LDH and nitric oxide assay by releasing significant amount LDH and NO. The *in vitro* antibacterial and antifungal activity studies revealed that Ti-complex (**2**) having enhanced antibacterial activity due to its increased lipophilic character and it was found to be more toxic than Schiff base ligand (**L1**). At higher concentration (100 $\mu\text{g/mL}$) both Schiff base ligand (**L1**) and Ti-complex (**2**) were found to be more toxic towards fungi cells. Furthermore, DNA binding studies were performed by using UV-visible and fluorescence investigations to evaluate the binding capacity of the tested compounds with Ct-DNA helix revealed that Ti-complex (**2**) is having significant interactions than the heteroditopic Schiff base ligand (**L1**). The molecular docking studies also conformed that significant hydrogen bonding interactions (N-H---O type) with Asp81 were observed when heteroditopic Schiff base ligand (**L1**) was subjected to docking studies with protein molecule. From all these studies, it is concluded that the synthesized tri-nuclear Ti-complex $[(\text{NNO})_2\text{Ti}]_3\text{O}_3$ (**2**) was found to be active antibacterial/fungal and anticancer agent.

ACKNOWLEDGEMENTS

This work is supported by the VFSTR (Deemed to be University), Vadlamudi, India under the scheme of seed grant for research faculty. Two of the authors, Eswar and Shiva thanks to VFSTR for providing facilities and fellowship.

CONFLICT OF INTEREST

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

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