Chapter-6

Co(II) complex of 2-amino-6-methyl-benzothiazole

6. 1. Introduction

Benzothiazoles are bicyclic ring system which has a thiazole ring fused with benzene ring. Thiazole is a five-member ring with one nitrogen and one sulphur atom in the ring. A number of 2-aminobenzothiazoles have been studied as central muscle relaxants and found to interfere with glutamate neurotransmission in biochemical experiments. Benzothiazole derivatives have been studied and found to have various chemical reactivity and biological activity like anti-viral[1], anti-cancer [2-11], anti-bacterial, anti-microbial and fungicidal [12-19] activities. Some of the novel benzothiazolesulphonamides act as potent inhibitors of HIV-1-protease[20]. Benzothiazole derivatives are also reported as anti-leishmanial[21], anti-inflammatory [22, 23], anti-convulsant [24-29], anti-diabetic[30], diuretic[31] and antiproliferative[32] agents. 2-aryl substituted benzothiazoles show antitumor activity while condensed pyrimido-benzothiazoles and benzothiazolo-quinazolines showed anti-viral activity. Substituted 6-nitro and 6-aminobenzothiazoles have been reported for antimicrobial activity[33].

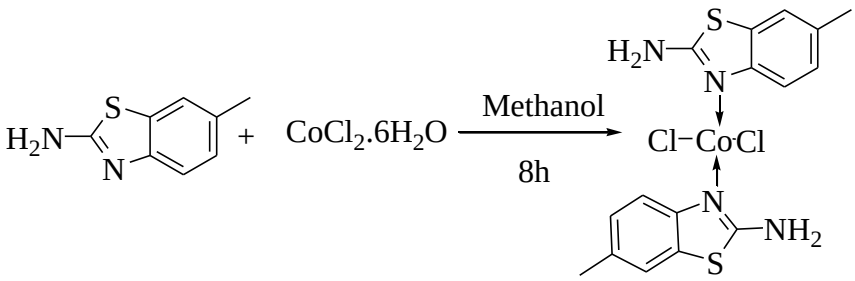
El-Shazly *et al.,* studied the reactions of 2-mercaptobenzothiazole with Cu(II), Ni(II) and Co(II) complexes and the reaction with Co(II) produced a five coordinate polymeric type compound[34]. **M. R. Chaurasia *et al.,*** studied the complexes of the type ML_2X_2 for Co(II) where L=benzothiazole derivative and X=bromide and characterized using analytical and other spectroscopic techniques[35]. **T. M. Bhagat *et al.,*** synthesized the heterocyclic compound, 4-bromo-2-hydrazino-6-methylbenzothiazole and their chelating tendency towards metal ions like Fe^{2+}, Co^{2+}, Ni^{2+} at different pH. The complexes were characterized by analytical, thermal and spectral parameters [36]. **Rendell *et al.,*** synthesized and characterized a series of Co(II), Cu(II) and Zn(II) complexes of 2,2'-o-phenylenebisbenzothiazole. Six coordinate 1:1 derivative was obtained for the Co(II) complexes[37]. **Chohan *et al.,*** synthesized and characterized Co(II) complexes of benzothiazole derived compounds. They were screened for antibacterial properties against various pathogenic species and showed enhanced activity of 80-85%[38]. **Joseph *et al.,*** synthesized and characterized novel copper complexes of 2-aminobenzothiazole derivatives. Antibacterial screening of the ligands and their complexes were done[39].

In view of the biological importance of the benzothiazole nucleus containing compounds, in the present work, it was planned to synthesize Co(II) complex of 2-amino-6-methylbenzothiazole and evaluate its biological activity.

6.2. Experimental

6.2.1. Synthesis of Co(II) complex [CoMBT]

The methanolic solution of 2-amino-6-methyl benzothiazole (2 mmol, 0.328g) was added to the methanolic solution of cobaltouschloride (1 mmol, 0.236g) with stirring. The resulting blue solution was refluxed for 8h. The reaction mixture was then cooled to room temperature, which results in the formation of blue color crystals. It was filtered off and dried. Yield: 74%. m.pt: 238-242°C. Anal. Calcd. for C_{16} H_{16} Cl_2 Co N_4S_2(%): C, 41.93; H, 3.52; N, 12.23; S, 13.99; Co, 12.86. Found (%):C, 41.85; H, 3.42; N, 12.26; S, 13.91, Co, 12.78. IR(KBr, cm^{-1}): 3254, 3171, 1603, 1470, 1336, 801, 694. UV-vis: 265, 310. μ: 4.12 BM.



Scheme 6.1. Synthesis of Co(II) complex

6.2.2. Computational details

The structure of the complex were further considered for molecular docking analysis using HEX 6.3, an interactive protein docking and molecular superposition program that is mainly used for feasible docking of various ligands with proteins, enzymes, DNA, and also in protein–protein docking. Docking parameters were set to include complex–DNA interactions and various parameters for non-covalent interactions were used as implemented in the program. The duplex DNA D(*CP*GP*CP*GP*AP*AP*TP*TP*CP*GP*CP*G)-3') dodecamer was taken from the Protein Data Bank (PDB ID: 1BNA) and used in docking studies. All possible docking poses were considered and the docking was performed.

6.3. Results and Discussion

6.3.1. Single crystal XRD studies of Co(II) complex

The single crystal XRD of the Co(II) complex shows that the complex has the empirical formula $C_{16}H_{16}Cl_2CoN_4S_2$ with formula weight 458.28. It crystallizes in the monoclinic system with the space group P121/c1 with unit cell dimensions a=11.549(3) Å, b=13.395Å, c=11.682Å, β=97.414(3)°. The bond lengths of Co(1)-Cl(1), Co(1)-Cl(2) are almost same *i.e*, 2.2677(14), 2.2471(13)Å respectively. The co-ordinate bond Co(1)-N(1) and Co(1)-N(3) are almost similar 2.021(4) and 2.034(4) respectively. The crystal data and the structure refinement parameters are given in Table 6.1. The Co-Cl bond is longer than the Co-N co-ordinate bonds. The chlorine atom forms hydrogen bonding with the amino nitrogen on two sides which is obvious from Table 6.2. The chlorine atoms are

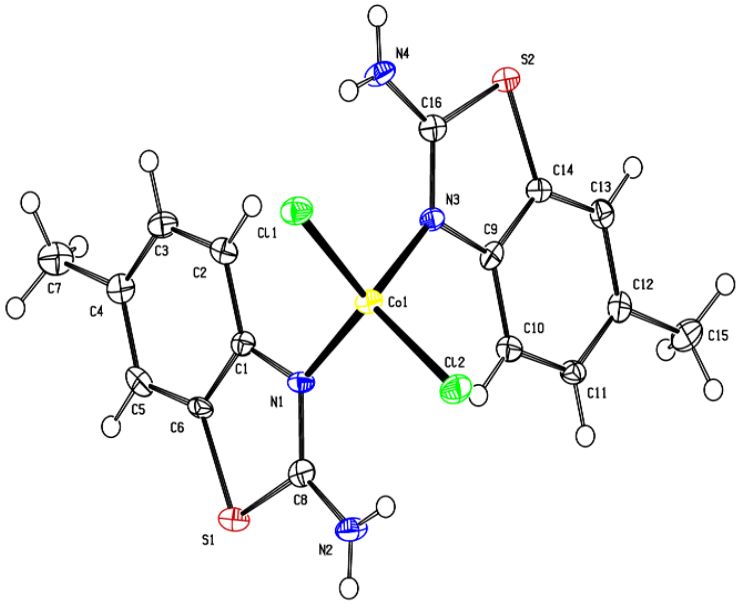


Fig. 6.1. ORTEP diagram of the Co(II) complex

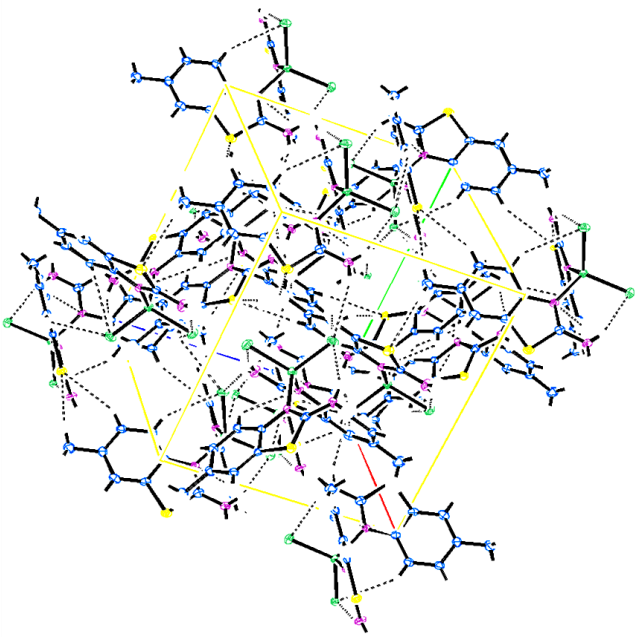


Fig. 6.2. Crystal packing diagram of Co(II) complex showing hydrogen bonds along an axis

in opposite direction which have the bond angles Cl(2)-Co(1)-Cl(2) as 110.73(5). The bond angles of N(3)-Co(1)-Cl(1) and N(3)-Co(1)-Cl(2) are 108.75(10) and 108.23(10) confirming the near the tetrahedral structure. The other important bond angles, bond lengths are shown in Table 6.3. The ORTEP diagram of the title complex is given in Fig. 6.1. The packing diagram of the title complex is given in Fig. 6.2 showing both the intermolecular and the intramolecular interactions. As shown in Fig. 6.2, intermolecular interactions are between the hydrogen of the benzothiazole ring and the chlorine atom attached to the central metal atom. The intramolecular interactions are between the thiazole nitrogen and the hydrogen of benzothiazole ring and between the chlorine atom and the methyl hydrogen. These interactions contribute to the packing stabilization.

6.3.2. Spectral characterization of Co(II) complex

The complex is non-hygroscopic and air stable in solution and in the solid state at room temperature. It is insoluble in water but soluble in common organic solvents like ethanol, acetonitrile, DMSO and DMF. The molar conductance of the complex in 10^{-3} M solutions is negligible indicating their non-electrolytic behavior. In the literature, Co(II) complexes having tetrahedral symmetry should have the following absorptions, 640, 590nm (υ_3) attributed to $^4A_2 \rightarrow {}^4T_1(F)$, $^4T_1(P)$ transitions. In the present complex, the transitions observed are 263, 310, 420 and 730nm. The characteristic band around 625nm for the tetrahedral complexes is not observed in the

complex. This may be due to the reason that the solvent DMSO has been co-ordinated to the central Co(II) metal ion making it distorted octahedral. The electronic spectrum of the Co(II) complex is compared with $CoCl_2$ in DMSO and shown in Fig. 6.3. To study the thermal behavior of the title complex, thermogravimetric analysis were carried out from 35 to 670°C at a heating rate of 10°C/min. The TGA curve shows it is stable upto 254.79°C. When the temperature is higher than 254.79°C, the coordination sphere has been disturbed eliminating the organic ligands in two decomposition steps at 254.79°C and 468.04°C leaving a stable metal oxide. The thermogram of the Co(II) complex is given in Fig.6.5.

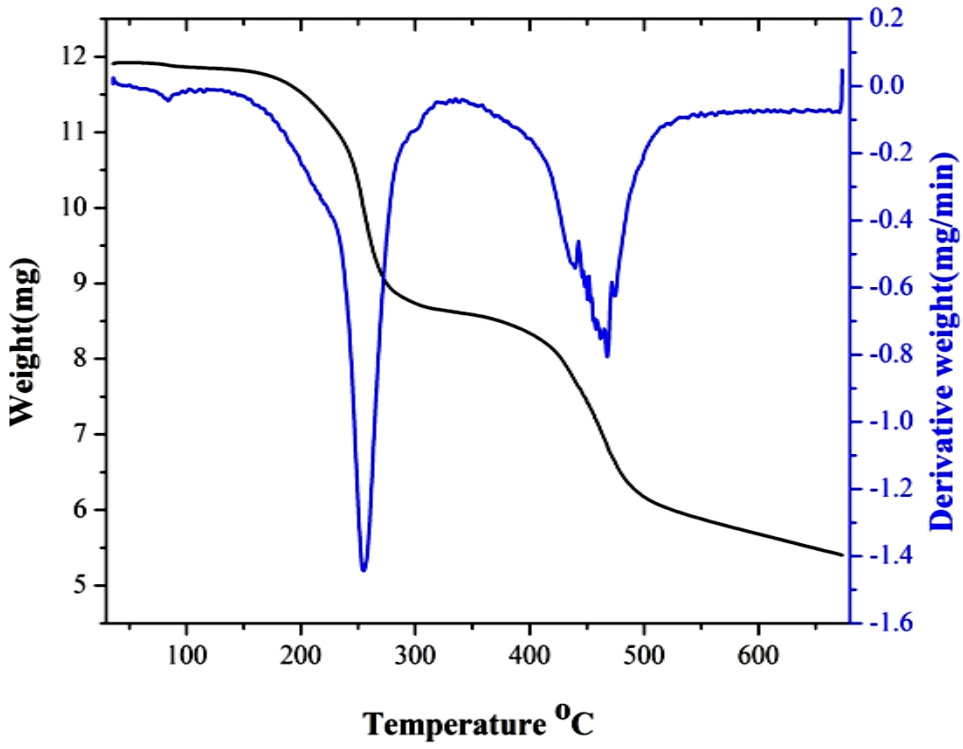


Fig. 6.5. Thermogram of the title complex

6.3.3. Magnetic properties

The observed magnetic moment value of the Co(II) complex is 4.12BM. This is the expected value of 4.0 to 4.88 BM for the tetrahedral Co(II) complexes which is the sum of contributions due to the spin only moments and spin orbit coupling. Thus the title complex shows the tetrahedral geometry[40]. The magnetic properties of the synthesized crystals were analyzed using a Magnetometer (VSM) at room temperature. Figure 6.6 show the M-H curves of the prepared Co(II) complex. The saturation magnetization (Ms) and coercivity (Hc) values have been directly extracted

from these curves. The plots how the hysteresis loop for complexes at room temperature and give a saturation magnetization of 0.00754 emu/g. The coercivities of the Co(II) complexes were 410.35G. The low saturation magnetization and the presence of coercivities for the samples indicate that these complexes are weakly ferromagnetic.

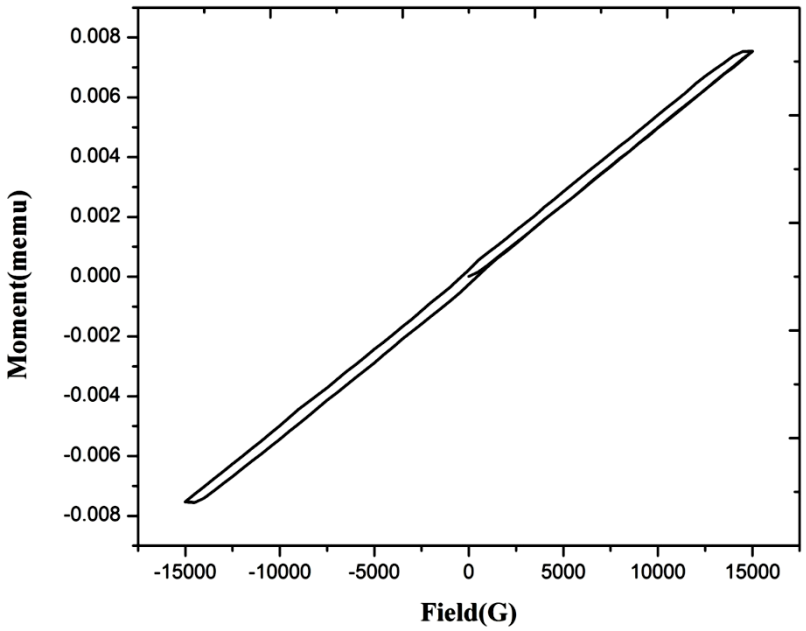


Fig. 6.6.Magnetization *versus* applied field plot for Co(II) complex

6.3.4. Luminescence spectra

The photoluminescence properties of the Co(II) complex (CoMBT) and the ligand, 2-amino-6-methylbenzothiazole (MBT) were studied at room temperature for 10^{-4}M solution in DMSO. Photoluminescence spectra were studied for solutions of the ligand excited at 280 nm. The most striking feature was that the ligand gave an intense emission upon irradiation by UV light. The photoluminescence spectrum of the ligand in DMSO is shown in Figure 6.7. The emission of the ligand, being inhibited in this case, is dependent on the presence of a metal ion, and the ligand photoluminescence in DMSO is regarded as being dependent on the d-block metals that are effective in DMSO solutions. Quenching of fluorescence of the ligand by transition metal ions during complexation is a rather common phenomenon, which can be explained by processes including magnetic perturbation, redox-activity, and electronic energy transfer. Enhancement of fluorescence through complexation is, however, of much interest, as it opens up the opportunity for photochemical

applications of these complexes. It can be seen in Figure 6.7 that upon complexation of the ligand, the photoluminescence intensities of the metal complexes decreases which is a common phenomenon[41].

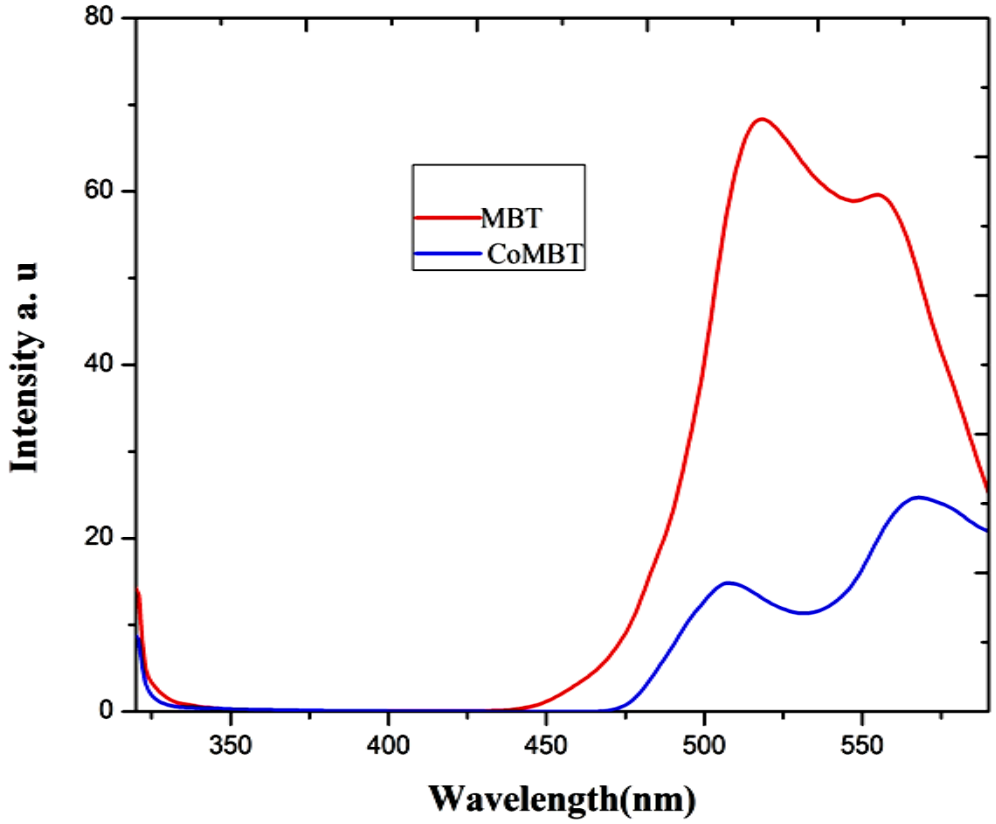


Fig. 6.7. Emission spectra of the ligand and the Co(II) complex

6.4. Pharmacology

6.4.1. In-vitro anti-microbial activity

The *in-vitro* antimicrobial activity of the Co(II) complex was tested against various bacteria *Aeromonas hydrophila MTCC 646, Serratia marcescens MTCC 4822, Thiobacillus thidurance, Pseudomonas aeruginosa* and *Acinetobacter baumanii KCMC 076,* and fungi *Aspergillus niger* and *Candida tropicalis.* The standard error for the experiment is ±0.001cm and the experiment was repeated three times under similar conditions. DMSO was used as the negative control and amikacin was used as the standard for antibacterial studies. Nystatin was used as the reference for antifungal studies.

Table 6.3. Antimicrobial activity of the ligand and the Co(II) complex

		Zone of inhibition(mm)									
	Name of organism	**2-amino-6-methyl benzothiazole**					**Co(II) complex**				
		C	**20%**	**40%**	**60%**	**80%**	**C**	**20%**	**40%**	**60%**	**80%**
1	*Pseudomonas aeruginosa*	-	-	06	08	10	-	-	12	14	16
2	*Aeromonas hydrophila*	-	-	-	09	12	-	-	-	10	14
3	*Thiobacillus thidurance*	-	-	-	11	14	-	-	-	15	18
4	*Serratia marcescens*	-	-	-	15	18	-	-	-	16	22
5	*Acinetobater baumanii*	-	-	06	07	10	-	-	12	14	16
6	*Aspergillus niger*	-	-	08	10	12	-	16	17	20	24
7	*Candida tropicalis*	-	-	-	-	10	-	-	-	-	18

The zone of inhibition for various species is given in Table 6.3. The cobalt complex showed a very good activity against *Aspergillus niger, Thiobacillus thidurance* and *Serratia marcescens*. In all the cases, the antimicrobial activity of the

Co(II) complex is greater than that of the ligand. It is evident from the data that this activity significantly increased on coordination. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups. The activity of the complexes can be related to the strength of the metal–ligand bond, besides other factors such as size of the cation, receptor sites, diffusion and a combined effect of the metal and the ligands for inactivation of the biomolecules. Most of the Co(II) complexes in the literature has a very good activity or equal to the standard value against the test organisms.[42,43].

6.4.2. In-vitro anti-cancer activity

Cisplatin is the anti-cancer drug used but it has a lot of side effects and the search for newer anti-cancer drugs is still going on. Many attempts are being made to replace these drugs with suitable alternatives and in this direction, a number of non platinum complexes have been synthesized and screened for their potential anticancer activity. In the field of non platinum compounds exhibiting anticancer properties, cobalt complexes find very promising alternative to platinum, showing activity on tumors which developed resistance to cisplatin.

In this regard, the ligand 2-amino-6-methyl-benzothiazole and its cobalt(II) complex were evaluated for their cytotoxicity against human breast cancer cell line MCF-7 by means of MTT assay method that measures mitochondrial dehydrogenase activity as a indication of cell viability. The results were analyzed by means of cell viability curves and expressed with IC_{50} values in the studied concentration range from 0.1 to 100μM. The results of MTT assays revealed that the complex showed promising cytotoxic effect than the ligand. The presence of cobalt metal ion brought a higher cytoxicity than the ligand. The IC_{50} values of ligand and the complex are 80.19μM and 14.12μM respectively. The data obtained for our compounds showed cytotoxicity with short incubation period (48h) and hence the data are highly significant. The reported value for cisplatin is 12.75, and thus the title complex shows good *in-vitro* cytotoxic activity equivalent to cisplatin [54-56]. The Co(II) complex of 2-((6-methoxybenzo[d]thiazol-2-ylimino) methyl)-6-ethylphenol of 14.85μM with respect to HeLa cell lines. Novel benzothiazole derivatives synthesized had IC_{50} values ranging from 9.39 to 10.25 $\mu molL^{-1}$; it exhibited 3.1 to 3.4 fold higher anticancer activity than the standard reference drug Doxorubicin with an IC_{50} value of 32.00 $\mu molL^{-1}$ [44,45].

6.4.3. Anti-tuberculosis activity

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, most commonly affecting the lungs. According to world health organization, 8.8 million incident cases of TB were estimated globally in 2010. Treatment of drug resistant TB requires extensive chemotherapy with second line anti-tb drugs, which are costlier than first line drugs and produce severe adverse drug reactions. The emergence of multidrug resistant strains highlighted the need for new drugs for the treatment of tuberculosis. The ligand and Co(II) complex were checked for its anti-tuberculosis activity since much of the reports were not reported in the literature and the results are given in Table 6.4.

The strain used for this study was *M. tuberculosis* (H37 RV strain) and the standards (MIC values) used was Pyrazinamide(3.125µg/ml), Streptomycin(6.25µg/ml), Ciprofloxacin(3.125µg/ml). It is obvious from Table 6.4 that ligand shows a moderate activity but complex has a MIC value of 6.2µg/ml which is equal to the tested standard Streptomycin[46,47].

Table 6.4. Anti-tb results of ligand and Co(II) complex

Sl. No	**Compound**	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**6.25 µg/ml**	**3.12 µg/ml**	**1.6 µg/ml**	**0.8 µg/ml**
1	Ligand	S	S	S	S	R	R	R	R
2	Complex	S	S	S	S	S	R	R	R

6.4.4. Anti-oxidant activity

A survey of literature reveals that the DNA interaction experiments conducted so far revealed that the thiazole compounds exhibit good DNA binding affinity. It is considered worthwhile to study the antioxidant activity of these compounds. 2,2'-diphenyl-2-picryl-hydrazyl (DPPH) assay is widely used for assessing the ability of radical scavenging activity and it is measured in terms of IC_{50} values. Because of the presence of odd electron, DPPH shows a strong absorption band at 517nm in the visible spectrum. As this electron becomes paired off in the presence of a free radical scavenger, this absorption vanishes and the resulting decolourisation is stoichiometric with respect to the number of electrons taken up. The DPPH assay of the tested compounds is shown in Fig. 6.8. It is seen from the

results that the Co(II) complex exhibited low activity compared to the standard ascorbic acid[48].

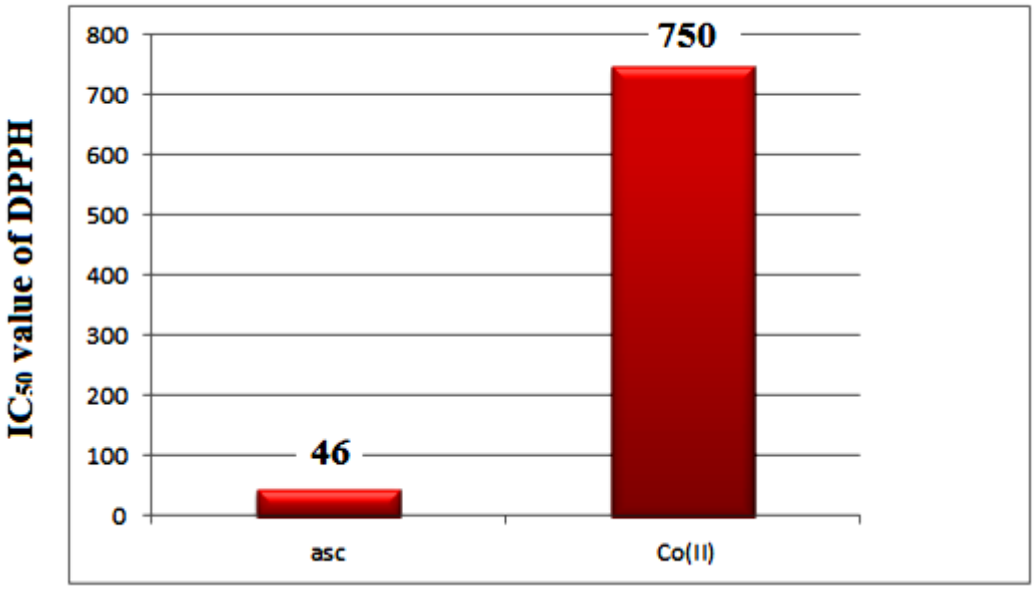


Fig. 6.8. Antioxidant activity of the complex

6.4.5. UV–visible absorption spectral titrations

UV–visible spectral studies provide preliminary information regarding the binding behavior between DNA and small molecules. The concentration of the complex was kept constant and DNA was added to that solution in increasing amounts. Generally, when metal complexes bind with DNA, if hypochromism with red shift in the absorption spectrum of the complex is observed, it indicates an intercalative mode involving a strong stacking interaction between the complex and the base pairs of DNA, whereas a non-intercalative mode of interaction shows hyperchromism with blue shift. The binding constant (K_b) for the complexes have been determined from the following equation.

$$\frac{[DNA]}{(\epsilon_A - \epsilon_F)} = \frac{[DNA]}{(\epsilon_B - \epsilon_F)} + \frac{1}{K_b(\epsilon_B - \epsilon_F)}$$

where ε_A, ε_B and ε_F correspond to the apparent, bound and free metal complex extinction coefficients respectively. A plot of [DNA] /(ε_A-ε_F) versus [DNA] gave a slope of 1/(ε_B-ε_F) and a Y intercept equal to 1/K_b(ε_B-ε_F), where K_b is the ratio of slope to the intercept yielding the binding constants, K = 4.07 × 10^3. On the other hand, the observation of hyperchromism is indicative of the breakage of the secondary structure of DNA.

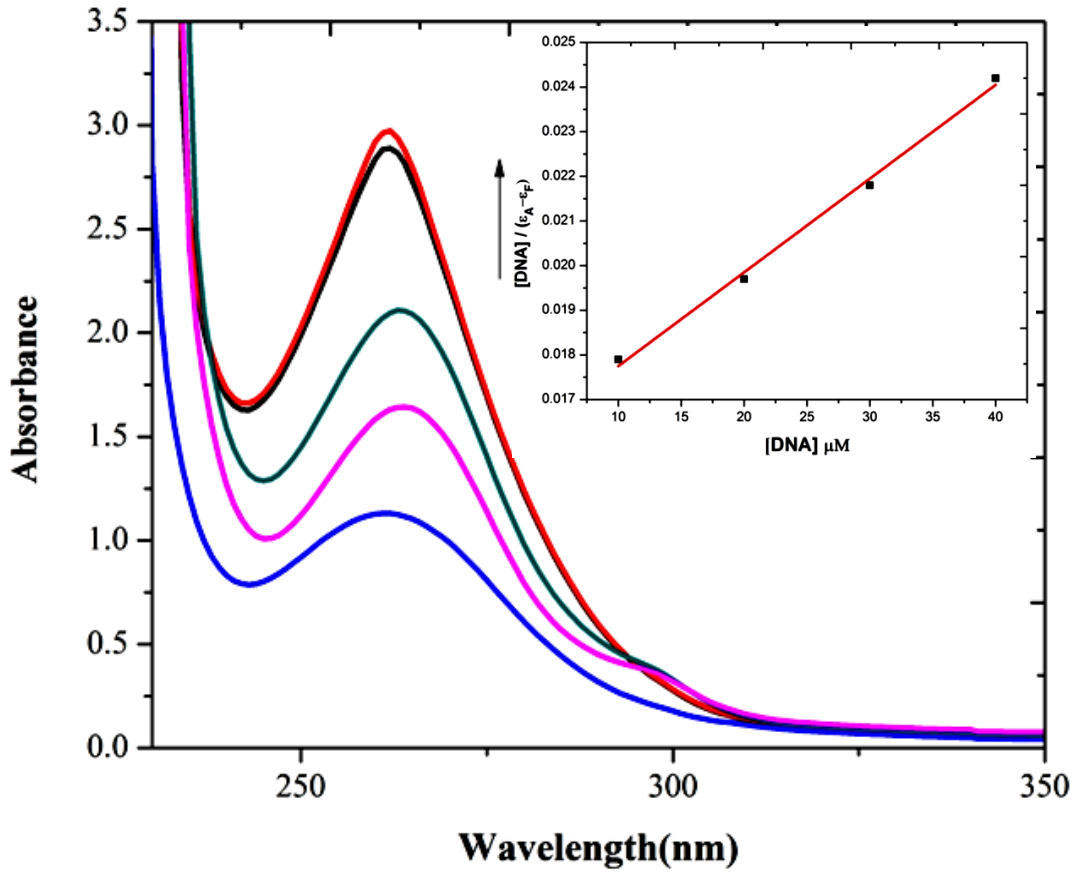


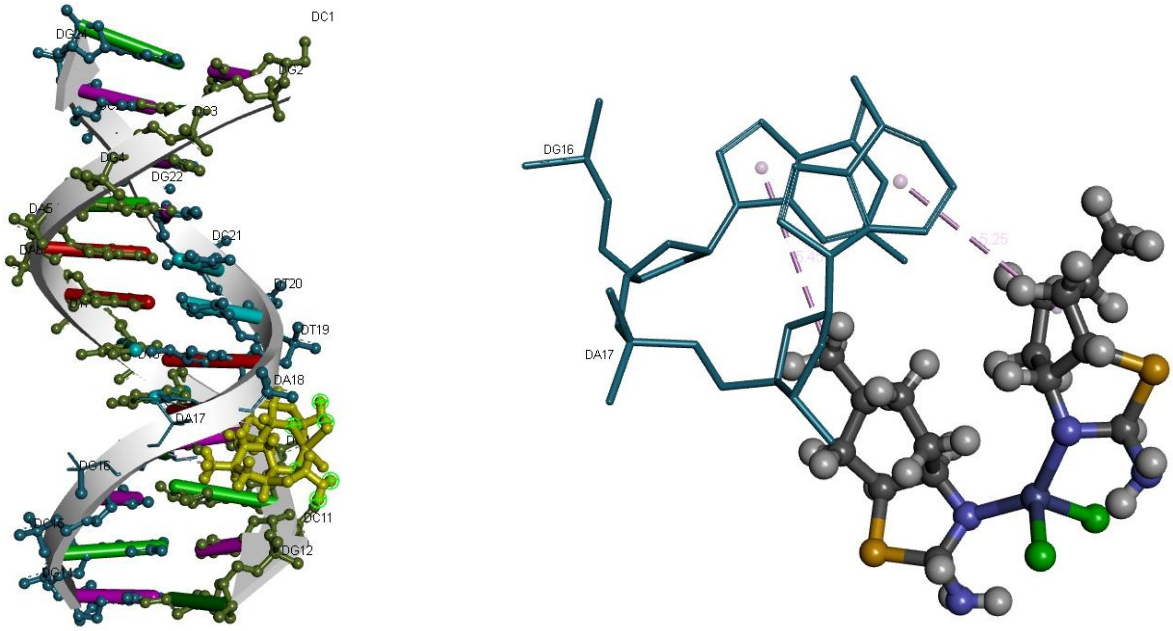
Fig. 6.9. Absorption spectrum of Co(II) complex in the presence and absence of CT-DNA. Conditions: [M] = 10μM, [DNA] = 0-40μM. Arrow (↑) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant, K_b.

Hence the observation of hyperchromism with slight red shift for our complexes showed that the new complex interact with the secondary structure of CT-DNA by breaking its double helix structure. The observed binding constant value for the title complex is 1.24×10^4 M^{-1}. This value is lesser than that of the classical intercalators in the literature and hence it is a minor groove binder[48-51]. Figure 6.9 shows the absorption spectrum of the complex on the addition of the CT-DNA.

6.4.6. Docking with DNA

Possible binding modes of ligands with DNA can be identified using docking analysis. Therefore molecular docking studies were carried out with HEX 6.3 package and the energetically most probable poses are given in Fig. 6.10. The binding energy

of the complex is -6.0Kcal/mol with a pIC_{50} value 39.02 micromolar concentrations. Therefore there is a possibility of intercalation of the benzothiazole containing Co(II) complex between the benzothiazole nucleus and the DNA bases. The Co(II) complex with benzothiazole derivative binds with the DNA in minor groove fashion with the help of hydrogen bonds[52-54].



(a) **(b)**

Fig. 6.10: The docking poses of the Co(II) complex with the DNA

6.5. Conclusions

Co(II) complex bearing amino benzothiazole ligand is synthesized and characterized. The structure of the complex is confirmed by single crystal XRD. The magnetic property of the complex has been studied and found to be paramagnetic. The synthesized complex has been examined for the biological properties like DNA binding, anti-oxidant and cytotoxicity under *in-vitro* experimental conditions. The DNA binding ability of the ligands and complexes has been assessed by absorption spectra and fluorescence measurements which inferred a minor groove binding with binding constant 1.24×10^4 M^{-1}. The results are validated from docking studies. The anti-oxidant activity showed that it has a low activity against DPPH radical. The *in-vitro* anti-microbial activity against various bacteria and fungi were studied and found that the Co(II) complex showed a better activity than the free ligand. The *in-vitro* cytotoxicity of the Co(II) complex possess pronounced activity against MCF-7 cell line and it is compared with cisplatin and other reported Co(II) complexes. In addition, the anti-tuberculosis activity of the Co(II) complex showed a good activity

equal to the test standard *streptomycin*. At this juncture, it is notable to mention that the major chemical and biological findings of this study throw light on the potential of the synthesized complex in a reasonable range of concentrations under *in-vitro* conditions. In our opinion the significant outcome of the study lies in the search of some biological importance in the synthesized complex and in case of positive and non-toxic promising results it can be later developed into a drug useful to the society.

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