

Design, Synthesis, Molecular Docking and Biological Evaluation of Dopamine Carbamodithiolates Metal Complexes

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ABSTRACT

Carbamodithiolates are a class of sulfur-based metal-chelating compounds with various applications in medicine. A new series of new transition metal [Cu (II) and Ru (II)] complexes of Carbamodithiolates were synthesized from Dopamine and Carbon disulfide and further characterized. The investigation of these complexes confirmed that the stability of metal–ligands coordination through, S & S, N atoms as bidentate chelates. Docking studies showed that blocking the division of cancer cells and resulting in cell death. It is necessary to understand the binding properties in developing new potential Protein targeting against neurological disorders.

1. Introduction

Insights gained from decades of research have begun to unlock the pathophysiology of these complex diseases and have provided targets for disease-modifying therapies. In the last decade few therapeutic agents designed to modify the underlying disease process have progressed to clinical trials and none have been brought to market. With the focus on disease modification, biomarkers promise to play an increasingly important role in clinical trials. Among the histamine receptor subtypes, H3 receptors play an important regulatory role in the CNS. Activation of H3 auto receptors can inhibit histamine synthesis and release from histaminergic neurons [1, 2] while activation of H3 hetero receptors can inhibit release of other neurotransmitters such acetylcholine, noradrenaline, dopamine and 5-HT from non-histaminergic neurons [3]. Conversely, blockade of H3 receptors with selective antagonists can increase the release of neurotransmitters involved in cognitive processes [4, 5]. Selective H3 receptor antagonists have been shown to improve performance in a diverse range of rodent cognition paradigms [6] and can also increase wakefulness [7]. This has led to the development of H3 receptor antagonists for the potential treatment of several CNS disorders including cognitive dysfunction in Alzheimer's disease (AD) [8].

Protein-ligand binding studies involved in neurological disorders of the metal complexes of new carbamodithiolates is very scarce. Hence the present studies carried out a research program and analyzed the importance of protein binding studies of the new carbamodithiolates with metal complexes like Cu, Ru, Ni, Mn, La, Pd, Y, Co, Zn and Fe [9].

Parkinson's disease (PD) is one of the most common diseases of the central nervous system (CNS). It is frequently heralded by speech disturbances which are one of its first symptoms. Parkinson's disease (PD) is a progressive extra pyramidal motor disorder. Pathologically this disease is characterized by the selective dopaminergic (D-Aergic) neuronal degeneration in the substantia Nigra. Correcting the DA deficiency in PD with levodopa (L-dopa) significantly attenuates the motor symptoms however, its effectiveness often declines and L-dopa-related adverse effects emerge after long-term treatment. Nowadays, DA receptor agonists are useful medication even regarded as first choice to delay the starting of L-dopa therapy. In advanced stage of PD they are also used as adjunct therapy together with L-dopa. DA receptor agonists act by

stimulation of presynaptic and postsynaptic DA receptors. Despite the usefulness they could be causative drugs for valvulopathy and nonmotor complication such as DA dysregulation syndrome (DDS) [10].

Over the past decade the Protein-ligand binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA foot printing and sequence-specific cleaving agents and potential anticancer drugs. The numerous biological experiments performed so far suggest that DNA is the primary intracellular target of anticancer drugs because the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death. It is necessary to understand the binding properties in developing new potential Protein targeting against neurological disorders [11-13].

The present work has been extended to know antibacterial activity, anti-inflammatory activity and antifungal activity of the carbamodithiolates metal complexes.

The main thrust of the study is to prepare as such new carbamodithiolates ligand from an amine having considerable amount of applications on its own with the hope that the resultant carbamodithiolates may possess much more applications and find a place in major fields of interest.

The field of carbamodithiolates metal complexes are vast and fast developing on account of their spectacular applications in various fields, also owing to the varieties of structural forms of the ligands. The ever-increasing applications of transition metal complexes in different fields of sciences are the driving force for the present research. The carbamodithiolates are organosulphur compounds considered to be versatile organic ligands which form complexes with many transition metals like Cu, Fe, Ni, Mn and Zn leading to the stabilization of a wide range of oxidation states.

The complexing ability of carbamodithiolates is well established and this is due to two sulphur atoms, which are capable to donate a lone pair of electrons to the central metal atom to form the stable metal complexes. These are known to stabilize unusual oxidation states of metal complexes, because of the delocalization of positive charge from the metal to nitrogen. Besides carbamodithiolates ligand and its metal complexes exhibit their striking and diversified applications in many fields like Medicine, Agriculture Industry, Polymer technology, Inorganic and Environmental trace analysis.

Carbamodithiolate ligands are soft bases. They had been found to act almost as uni negative bi dentate ligands coordinating through sulphur atoms and also both tetra and hexa co-ordinate complex of many transitional metal ions have been isolated. They exhibit various

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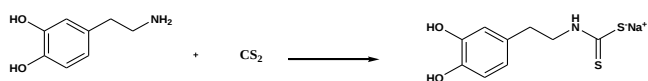
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applications in different fields especially in the production of petroleum derivatives, lubricants as accelerators for vulcanization in Polymer Industry as antioxidants and anti-humidity agents. They are also having fungicidal, bactericidal, insecticidal, anticancer and photochemical activities. Carbamodithiolate ligands itself acts as a good antibacterial and antifungal activity and are used as biocides (vapam, nabam) [14–15].

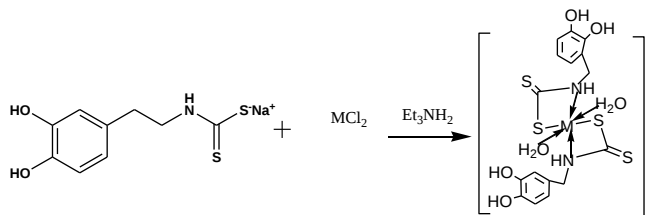
2. Experimental Methods

2.1 Design Aspects

Copper chloride anhydrous was obtained from Fluka, Dopamine and carbon di sulfide were purchased from Aldrich. Other chemicals used were of analytical reagent or higher purity grade. Solvents used were of reagent grade and purified before use by the standard methods. Conductivity measurement was carried out by a Systronics Conductivity Bridge 305, using a conductivity cell of cell constant 1.0 double distilled water was used as solvent. Electronic absorption spectra on JASCO UV/VIS-7850 recording spectrophotometer. Infrared spectra was recorded on a JASCO-460 plus FT-IR spectrophotometer in the range of 4000 - 400 cm^{-1} in KBr pellets. Micro chemical analysis of carbon, hydrogen and nitrogen for the complexes were carried out on a Heraeus CHNO-Rapid elemental analyzer. ^1H NMR spectra were recorded on a Bruker DRX-500 Advance spectrometer at 500 MHz in DMSO- d_6 discussing tetra methyl silane as internal reference standard. Melting points were measured on a unimelt capillary melting point apparatus and reported uncorrected.



Scheme 1 Synthesis of pramepexole carbamodithiolate ligand



Scheme 2 Synthesis of pramepexole carbamodithiolate metal complex

2.1 Preparation of Sodium Salt of Carbamodithiolates Ligands

A 0.05 M of amine was dissolved in 30 mL of absolute alcohol in a clean beaker which was placed in ice bath. To this cold solution add 5 mL of sodium hydroxide (10 N) solution and then add pure carbon disulphide (0.05 mL) in drop wise with constant stirring. The contents were stirred mechanically for about 30 min sodium salt of carbamodithiolates precipitated out. It was dried over and recrystallized from ethanol.

2.2 Preparation of Cu (II) and Ru (II) Complexes

2.2.1 Synthesis of $[\text{Cu}(\text{DCDT})_2]\text{Cl}_2$

The aqueous solution of 0.05 M of Copper Chloride was added with constant stirring to an aqueous solution of 0.01 M of Sodium salt of Dopamine carbamodithiolates ligand. The reaction mixture was stirred at room temperature for 2 h. The colored (yellow) precipitates were obtained. The precipitates were filtered and washed with water and then with methanol and dried over calcium chloride in desiccator's yield 78% and decomposes at 110 $^{\circ}\text{C}$.

Anal. Calcd. For C, 38.59; H, 4.97; N, 6.16; Cu, 12.70; O, 18.67; S, 24.61; Found: C, 37.59; H, 4.27; N, 5.16; Cu, 11.70; O, 17.67, S, 23.61.

2.2.2 Synthesis of $[\text{Ru}(\text{DCDT})_2]\text{Cl}_2$

The aqueous solution of 0.05 M of Manganese Chloride was added with constant stirring to an aqueous solution of 0.01 M of Sodium salt of dopamine carbamodithiolates ligand in the presence of small quantity of triethylamine. The reaction mixture was stirred at room temperature for 2 h. The colored (gray) precipitates were obtained. The precipitates were filtered and washed with water and then with methanol and dried over calcium chloride in a desiccator Yield 80% and decomposes at 110 $^{\circ}\text{C}$. Anal. Calcd. For C, 26.43; H, 4.44; N, 6.17; Cl, 15.60; Mn, 12.09 Found: C, 35.16; H, 3.9; N, 4.82; O, 16.53; Ru, 17.40; S, 22.09.

3. Result and Discussion

Solid reflectance spectra data for the Cu and Ru of dopamine metal complexes. The complexes $[\text{Cu}(\text{DCDT})_2]\text{Cl}_2$ and $[\text{Ru}(\text{DCDT})_2]\text{Cl}_2$ complexes exhibit magnetic property and has an electronic spectrum which can be assigned to low spin Cu (II) and Ru (II) in an octahedral environment. Intra ligand electronic transition in then C...S...S and S...C...S chromophores of the carbamodithiolates moiety. Thus the peak at 646 nm and the shoulder at 499 nm arise from $^1\text{A}_{1g} \rightarrow ^1\text{T}_{1g}$ and $^1\text{A}_{1g} \rightarrow ^1\text{T}_{2g}$ transitions, respectively. The other lower peaks are probably charge-transfer in origin.

3.1 Infrared Spectrum

Two regions of the IR spectrum of the $[\text{Cu}(\text{DCDT})_2]\text{Cl}_2$ and $[\text{Ru}(\text{DCDT})_2]\text{Cl}_2$ complex have proven valuable in arguments concerning the electronic and structural characteristics of this compound. The presence of the thiouride band between 1545 - 1430 cm^{-1} suggest a considerable double bond character in the C...N bond vibration of the $\text{S}_2\text{C}-\text{NR}_2$ group. The band present in the 967 cm^{-1} range is attributed to the prevailing contribution of (C...S) vibrations in these ranges have been used defectively in differentiating between monodentate, bidentate carbamodithiolates ligands. The presence of only one strong band supports bidentate coordination of the dithioligands, where as a doublet is expected in the case of monodentate coordination. (C...S) and (C...N) Stretching frequencies fall in the 1035 cm^{-1} (1001 cm^{-1} for the free ligand) and 1478 cm^{-1} respectively. The methyl group in the complex as medium strong bands in the 2960 cm^{-1} range can be related to the asymmetric CH_3 stretching vibration.

3.2 ^1H -NMR Spectra

The NMR spectrum of the $[\text{Cu}(\text{DCDT})_2]\text{Cl}_2$ and $[\text{Ru}(\text{DCDT})_2]\text{Cl}_2$ complexes showed at 2.3 - 2.4 ppm. Which may be assigned to the hydroxyl protons. The peak at 7.9 - 7.98 attributed to NH protons of thiouramide nitrogens in both complexes. In other signals is also appeared in the region 0.98, 1.5 and 3.8 ppm.

3.3 Docking Studies

Docking techniques, designed to find the correct conformation of a ligand and its receptor, have now been used for decades. The process of binding a small molecule to its protein target is not simple several entropic and enthalpic factors influence the interactions between them. The mobility of both ligand and receptor the effect of the protein environment on the charge distribution over the ligand and their interactions with the surrounding water molecules, further complicate the quantitative description of the process. The idea behind this technique is to generate a comprehensive set of conformations of the receptor complex and then to rank them according to their stability. The most popular docking programs include DOCK, Auto Dock, FlexX, GOLD, and GLIDE among others.

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex Lengauer T, Rarey M. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes (Kitchen DB et al., 2004). During the course of the docking process the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustment resulting in the overall binding is referred to as "induced-fit". Molecular docking research focusses on computationally simulating the molecular recognition process. It aims to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design most drugs are small organic molecules and docking may be applied to hit identification-docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest (see virtual screening). Lead optimization-docking can be used to predict in where and in which relative orientation a ligand binds to a protein (also referred to as the binding mode or pose). This information may in turn be used to design more

potent and selective analogs. Bioremediation-Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.

The present work all the calculations were performed on a workplace by AMD 64 bits dual processing hi end server machines. Molecular docking calculations were performed with Auto Dock 4.0. If not otherwise stated, default settings were used during all calculations.

Keeping the aim of constructing novel ligand complexes for H3, a library of 10 molecules was synthesized. The Auto Dock 4.0/ADT (Laskowski RA et al., 2005) program was used to investigate ligand binding to structurally refined H3 model using a grid spacing of 0.375 Å and the grid points in X, Y and Z axis were set to 60 × 60 × 60. The search was based on the Lamarckian genetic algorithm [13] and the results were analyzed using binding energy. For each ligand a docking experiment consisting of 100 stimulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values. Substrate docking with synthesized substrates was also performed on to H3 model with same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compounds to the H3 model.

Binding energy for each docking was calculated using a semi-empirical free energy force field. Out of these 5 docked ligands and its complexes molecules with receptor top two molecules were filtered out on the basis of binding energy. The binding modes and geometrical orientation of all compounds were almost identical, suggesting that all the inhibitors occupied a common cavity in the receptor. The binding energy of top three inhibitor molecules with an active site of receptor protein is given in Table 1.

Table 1 Summary of docking results high ranked ligands and complex molecules with H3 receptor

S.No	Compound name	Receptor name	Cluster Rank	RMSD	Lowest binding energy (Kcal/mole)
1	Dopamine (DCDT) Ligand	H3 Receptor	1	0.00	-4.41
2	[Cu(DCDT)]	H3 Receptor	1	1.05	-7.42
3	[Ru(DCDT)]	H3 Receptor	2	0.23	-6.53

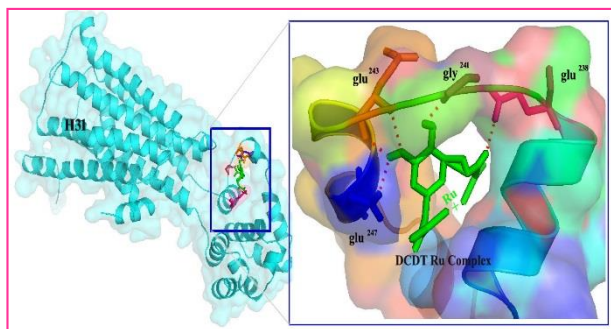


Fig. 1 The cartoon and electrostatic surface representation of the binding site of (e1, e2) H3 receptor model in sky blue, ligand complexes (Ru) with sticks in green and amino acids same in e1 and e2 that are represented Glu 238 in pink, Glu 241 in limon, Glu 243 in orange and Glu 247 in blue colour

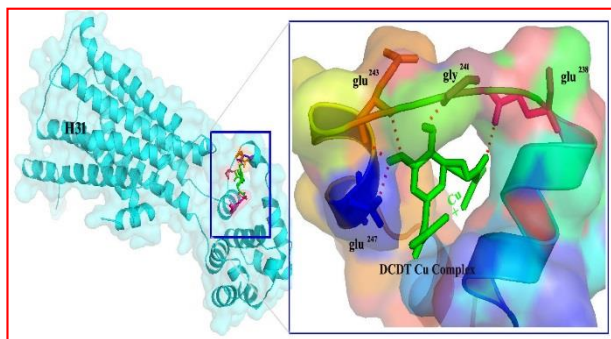


Fig. 2 The cartoon and electrostatic surface representation of the binding site of (e1, e2) H3 receptor model in sky blue, ligand complexes (Cu) with sticks in green and amino acids same in e1 and e2 that are represented Glu 238 in pink, Glu 241 in limon, Glu 243 in orange and Glu 247 in blue colour

Most docked inhibitors interacted by the same mode of the inhibitors, histamine H3 receptor binding site. The different surface pocket for residue seems to be an important factor in determining the binding mode of histamine ligand of Glu 241 and Leu 231 amino acid residues (Fig. 1a and Fig. 2). Synthesised ligand metal complexes are showing same interaction and binding pose with high energy values in detailed Table 1.

among all complex molecules dopamine copper complex, dopamine ruthenium complexes gave best scores.

3.4 Antimicrobial Activity

Antimicrobial test was performed on four bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and two fungi (*Candida albicans* and *Candida tropicalis*). The media used were prepared by dissolving separately 2 g of nutrient broth powder and 38 g of the Mueller-Hinton agar powder in 250 mL and 1 L of deionized water, respectively. Two media were sterilized in an autoclave at 121 °C for 15 min and then stored overnight in a refrigerator after cooling. Cultures of the microorganisms were prepared in sterile nutrient broth and incubated for 24 h at 37 °C for the bacteria and 27 °C for the fungi. 0.1 mL of each of the overnight cultures in sterile test tubes with caps were made up to 10 mL with 9.9 mL of sterile deionized water (Table 2).

Table 2 Antimicrobial Activity

Bacteria	Growth inhibition zone in millimeter (mm)					
	Gram + ve				Fungus	
	S.aur	B.subt	E.coli	P.aerug	C.alb	C.trop
Cu(PCDT)	15	15	16	15	15	16
Ru(PCDT)	14	16	15	15.5	17	15
FLU			-	-	16	16
DMSO	13	15	14	16	-	-

To give 1:100 or 10⁻² dilution of the microorganisms. The technique used for the study was agar-well diffusion. Solutions of concentration 10 mg/mL of the compounds were made in dimethyl sulphoxide (DMSO). DMSO was also used as the negative control. Positive controls for bacteria and fungi were discs of commercial antibiotics manufactured by Abtek Biological Limited and Fluconazole dissolved in DMSO. The discs were carefully placed on the inoculated media with the aid of sterile forceps. Plates inoculated with bacteria were incubated at 37 °C for 24 h and those inoculated with fungi were incubated at 27 °C for 72 h. Afterwards, the zones of inhibition of microbial growth that appeared around the wells of the compounds were examined and the diameters measured and recorded in millimeters (mm). Antimicrobial activity of the Cu (II) and Ru (II) complexes was evaluated in vitro against Gram positive bacteria - *Staphylococcus aureus* and *Bacillus subtilis*, Gram negative bacteria - *Escherichia coli*, *Pseudomonas aeruginosa* and fungi, *Candida albicans*, *Candida tropicalis*. The results for the complexes and commercial antibiotics used as positive controls are listed in (Table 1).

4. Conclusion

Cu (II) and Ru (II) complexes of dopamine carbamodithiolates ligand with have been synthesized and characterized. The ligand moiety exhibit a bidentate coordination mode in the Cu (II) and Ru (II) complexes. Solid reflectance spectra and magnetic data indicate that the complexes are paramagnetic and octahedral. The complexes show selective activity towards some of the test microorganisms. In this Study, we have docking studies of H3 receptor model with carbamodithiolates ligand and metal complexes having more favourable rank score, docking score and hydrogen bonding energy and the binding pocket of the H3 receptor. Activation of H3 hetero receptors can inhibit release of other neurotransmitters such acetylcholine, noradrenaline, dopamine, conversely blockade of H3 receptors with our synthesized selective antagonists can increase the release of neurotransmitters involved in cognitive processes. Docking studies of carbamodithiolates ligand and metal complexes with H3 receptor and detailed analyses of metal inhibitors, H3 receptor interactions were done and the residues in binding responsible for binding to the inhibitors of metal substrates with high binding affinity were identified. Hence we conclude that these carbamodithiolates ligands and metal complexes could be a potential anti Neurological disorders lead molecules for modulating the expression of H3 receptor in Parkinson's disease (PD) and Alzheimer's disease (AD) supports for experimental testing.

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References

- [1] P. Blandina, M. Giorgetti, L. Bartolini, M. Cecchi, H. Timmerman, R. Leurs, et al, Inhibition of cortical acetylcholine release and cognitive performance by histamine H3 receptor activation in rats, *Br. J. Pharmacol.* 119 (1996) 1656-1664.
- [2] R.E. Brown, D.R. Stevens, H.L. Haas, The physiology of brain histamine, *Prog. Neurobiol.* 63 (2001) 637-672.
- [3] G.B. Fox, T.A. Esbenshade, J.B. Pan, R.J. Radek, K.M. Krueger, B.B. Yao, et al, Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl} benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist, *J. Pharmacol. Exp. Ther.* 313 (2005) 176-190.
- [4] A.D. Medhurst, A.R. Atkins, I.J. Beresford, K. Brackenborough, M.A. Briggs, A.R. Calver, et al, GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models 19, *J. Pharmacol. Exp. Ther.* 321 (2007) 1032-1045.
- [5] A.J. Barbier, C. Berridge, C. Dugovic, A.D. Laposky, S.J. Wilson, J. Boggs, et al, Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H3 antagonist, *Br. J. Pharmacol.* 143 (2004) 649-661.
- [6] M.B. Passani, J.S. Lin, A. Hancock, S. Crochet, P. Blandina, The histamine H3 receptor as a novel therapeutic target for cognitive and sleep disorders, *Trends Pharmacol. Sci.* 25 (2004) 618-625.
- [7] T.A. Esbenshade, K.E. Browman, R.S. Bitner, M. Strakhova, M.D. Cowart, J.D. Brioni, The histamine H3 receptor: an attractive target for the treatment of cognitive disorders, *Br. J. Pharmacol.* 154 (2008) 1166-1181.
- [8] T. Lengauer, M. Rarey, Computational methods for biomolecular docking, *Curr. Opin. Struct. Biol.* 6(3) (1996) 402-406.
- [9] D.B. Kitchen, H. Decornez, J.R. Furr, J. Bajorath, Docking and scoring in virtual screening for drug discovery: methods and applications, *Nat. Reviews. Drug Discovery* 3(11) (2004) 935-949.
- [10] B.Q. Wei, L.H. Weaver, A.M. Ferrari, B.W. Matthews, B.K. Shoichet, Testing a flexible-receptor docking algorithm in a model binding site, *J. Mol. Biol.* 337(5) (2004) 1161-82.
- [11] B.D. Bursulaya, M. Totrov, R. Abagyan, C.L. Brooks, Comparative study of several algorithms for flexible ligand docking, *J. Comput. Aided Mol. Des.* 17 (11) (2003), 755-63.
- [12] R.A. Laskowski, J.D. Watson, J.M. Thornton, ProFunc: a server for predicting protein function from 3D structure, *Nucl. Acids. Res.* 33 (2005) 89-93.
- [13] T.I. Oprea, A.M. Davis, S.J. Teague, Leeson is there a difference between leads and drugs? A historical perspective, *J. Chem. Inf. Comput. Sci.* 41 (2001) 1308-1315.
- [14] M. Musthak Ahmed, R. Mallikarjuna Rao, M. Md. Rafi, G. Jaffer Mohiddin, J. Sreeramulu, Dithiocarbamate metal complexes, *Scholars Res. Library* 4(2) (2012) 858-862.
- [15] A. Jayaraju, M. Musthak Ahmed, R. Mallikarjuna Rao, J. Sreeramulu, Novel dithiocarbamate metal complexes, *Der. Pharma. Chemica.* 4(3) (2012) 1191-1194.