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Index

Recent Trends in Life sciences-I

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dedicated to

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Book Chapter 14

Recent Trends in Life Sciences

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EFFECT OF VANADIUM METAL COMPLEXES ON REDUCING SERUM GLUCOSE LEVELS IN WISTAR RATS

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ABSTRACT

The vanadium metal complexes were synthesized, characterized and their biological activities were evaluated. The purpose of this investigation is, to evaluate the inhibitory properties of these vanadium metal complexes on enzyme PTP-1B and know the ease with which these metal complexes are being transported by transporting proteins like BSA. Evidences from biochemical, genetic, and pharmacological studies strongly suggest that inhibition of Protein Tyrosine Phosphatase-1B (PTP-1B) enzyme could address both diabetes and obesity and thus making PTP-1B as an exciting target for drug development. Although many natural PTP-1B inhibitors showed to be promising clinical and potential agents, there is no clinically used PTP-1B inhibitor due to relatively low activities or lack of selectivity. Search for more potent and selective PTP-1B inhibitors is still necessary. To synchronize these results, experiments were conducted on induced diabetic Wistar Rats. Molecular modeling studies were also performed to support the *in vitro* results. It has been shown by the kinetic studies that vanadium complexes are good inhibitors of PTP-1B enzyme. These complexes upon injecting reduced the serum glucose levels to normal range in induced diabetic Wistar rats within 3 days of experiments. The order of glucose reducing properties of these metal complexes from different experiments is found to be the same.

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INTRODUCTION

Diabetes mellitus (DM) is a group of diseases characterized by high levels of blood sugars and are classified into different types.

Type I: Insulin dependent (IDDM), it affects children and adolescent. There is complete insulin deficiency. They require insulin injection lifelong and shots have to be taken two to three times in a day.

Type II: Non-insulin dependent (NIDDM), caused by ageing, obesity, spiritual stress or other environmental factors. Generally it affects adults. Does not require insulin for control of sugar levels except in advanced stages. Method of treatment is largely medication.

Type III: This is proposed for Alzheimer's disease as there is insulin resistance in the brain.

MODY: Maturity Onset Diabetes of the Young (MODY) is a new form of diabetes due to genetic defect and is inherited. This is not due to obesity and is found in those who are young and lean. It is often compared with Type I. It has 14 genetic variants and a novel gene NKX6-1 is found to cause MODY. There is often confusing between MODY and Type-I as both are found to affect young people or children. Type I is diagnosed in those suffering from MODY, leading to misdiagnosis. MODY can be diagnosed only through genetic testing. There are 14 different forms of MODY and each one has its own unique clinical characters. In Indians it is found that MODY 12, 13 and 14 are very common. MODY do not require insulin injections but can be effectively treated by inexpensive sulphonyl urea tablet.

The discovery by Lyonnet and Martin in 1899 [1] that DM patients excreted less glucose in their urine after treatment with Vanadate indicated that transition metal compounds may play an important role in the treatment of DM. It was shown that $VOSO_4$ (Vanadyl sulphate) does not enhance insulin action in Type I patients but selectively improve insulin action [2] in Type II DM patients.

Later it was established that therapeutic agents are effective in treating Type II DM. Protein tyrosine phosphatases (PTPs) are a large family of enzymes with regulations of innumerable cellular process by dephosphorylating proteins in living organisms. Four human PTPs [3] are PTP- 1B, TCPTP, He PTP and SHP-1. Among these PTPs, the enzyme PTP 1B acts as a key negative regulator of insulin [4, 5] signalling through directly inactivating insulin receptor (IR) by dephosphorylating tyrosine residues in the regulatory domain.

It has been established that Vanadyl compounds have inhibitory action [6, 7, and 8] on Protein Tyrosine Phosphatases (PTPases). There is a perfect tune of balance [9] between action of two proteins, **Protein Tyrosine Kinase** (PTK) and **Protein Tyrosine Phosphatase** (PTP)

(i.e. phosphorylation and dephosphorylation) which is the essence of the mechanism of opening and closing the cell doors for glucose intake.

Overactive PTPases disturb the concerted action between PTK and PTP which is essential for opening & closing of the cell doors, making the cell wall not to respond to insulin.

Inhibitors: Enzyme inhibitors are usually compounds that combine with the enzyme to form in enzyme- inhibitor complex, either by reducing or completely inhibiting the catalytic activity of the enzyme and therefore reducing the rate of reaction.

Binding of an inhibitor to the active site of the enzyme can block the entry of the substrate to the site. Some common examples of inhibitors are listed in table 1.

Over active enzymes are usually attractive targets for development of inhibitor molecule to alleviate disease conditions.

Table-1: Common examples of inhibitors used as pharmaceuticals.

S. No.	Inhibitor	Enzyme	Function
1	Paracetamol	COX	Analgesics
2	Lovastatin	HMG-CoA reductase	Hypercholesterdemic agent
3	Allopurinold	Xanthine oxidase	Gout control / Uric acid control
4	Rifampicin	DNA/ dependent RNA polymerize	Antibiotic
5	Sildanafil Citrate	Phosphodiesterise V	Erectile dysfunction
6	Duxorubicin	Topoisomerase	Anticancer
7	5-fluoro uracil	Thymidylate synthase	Anticancer
8	Gleevec	Tyrosine kinase	Anticancer
9	Captopril	Angiotansin converting enzyme(ACE)	Blood pleasure control

Owing to the importance of vanadium metal complexes in biology, in earlier papers [10], we have reported the synthesis and characterization of few vanadium metal complexes using different substituted acetyl acetones.

The inhibitory action of various vanadium metal complexes on PTP-1B (enzyme kinetics) were studied *in vitro* in our lab using 96 well Micro Plate Reader and found that they have excellent inhibitory properties on PTP-1B [11].

Further, binding studies were also performed on vanadium complexes and PTP-1B using Fluorescence Emission Spectroscopy [12, 13]. These results suggest vanadium binding occurs between inhibitor & PTP-1B in their active site.

To further conform, we relied on molecular modeling studies. The order of inhibition (Kinetics), the order of binding (binding constant) and results of molecular modeling with respect to various vanadium complexes are in consistent with each other.

Finally *in vivo* studies of these metal complexes on Wistar Rats were also conducted to test the glucose reducing effect of these metal complexes under study. Other supporting studies like molecular modeling were also done to support the obtained results.

EXPERIMENTAL

1. Materials and instrumentation

Vanadium metal complexes, which were reported early [10],abbreviated as [7-B], [7-imi], [7-me-imi], [7-et-imi],[6-B], [6-imi], [6-me-imi], [6-et-imi][5-B], [5-imi], [5-me-imi], [5-et-imi]were prepared and characterized in our lab. Enzyme PTP-1B was obtained from Cell Sciences, Newburyport, MA 01950, USA. p-Nitro phenyl phosphate (pNPP) of analytical reagent grade obtained from Sisco Research Laboratories Pvt Ltd. Tris buffer of analytical reagent grade was obtained from Finar and used without further purification. Bovine Serum Albumin (BSA) was purchased from Sigma-Aldrich.

Following instruments were used to perform different experiments. Spectrophotometer (EPOCH-Bio Tek, SN 1701311.), JASCO spectrofluorometer FP-8500, 96 microwell plate readers and Shimadzu IR Prestige-21spectrophotometer.

2. Methods

2.1Molecular Modeling Studies of Vanadium metal complexes with Enzyme PTP-1B and BSA

The interaction of the proteins (Bovine serum albumin (BSA) and Protein tyrosine phosphatase -1B (PTP-1B)) with the different vanadium compounds was studied using molecular docking. The crystal structure of PTP-1B enzyme was retrieved from the Protein Data Bank (PDB) of the Research Collaboration for structural Bioinformatics (RCSB, http://www.rcsb.org).

2D structures of the metal complexes were drawn using "Chem Sketch" software. Molegro Virtual Docker (Molegro 2011) was employed [14] for this purpose to analyze compound binding, at specific cavity for both proteins BSA and PTP (PDB ID: 4F5S and 2HNP) respectively. The accuracy of MVD is higher compared with other stock softwares such as Glide, Sorflex and Flex X [15].The structure of protein loaded on to MVD platform for finding potential active sites or cavities. BSA consists of 583 amino acid residues present in three homologous alpha helical domains (I, II, III). Each domain comprises sub-domain A and sub-domain B [16, 17]. On the other hand, Protein tyrosine phosphatase (PTPs) with 321 amino acids is a monomer with one unique protein chain A consisting of alpha helix, beta strands, and coils [18]. Nearly 20 vanadium derivatives were synthesized and tested for bioinformatics. Five binding cavities obtained from site map were employed in reporting the scoring functions. The lead targets were identified based on the scoring functions like Mol dock score. The accuracy of the data was further analyzed by Plant score functionality. To obtain more potent compounds as inhibitors the optimal binding mode of a molecule (compound) to the active site of macromolecule is interpreted.

2.2 In vivo studies of Vanadium metal complexes on Wistar Rats

2.2.1 Protocols followed for In *vivo* studies of Vanadium metal complexes on Wistar Rats.

"All animal experiments were conducted as per guidelines of the International council for laboratory Animal Science (ICLAS). Efforts also taken in a direction to minimize animal sufferings and only the minimum number of animals necessary to produce reliable scientific data were used. The STZ – diabetes rats were used in the study since they offer a reproducible and reliable test for *in vivo* insulin mimetic behaviours".

Subjects: 3 to 5 months old healthy male Wistar rats (weighting about 150-220 g, specific pathogen free) were purchased from animal breeder and they were housed six per cage at 20 $\pm$ 2°C (12 hours light / dark cycle). Animals in cages were placed in large spacious hygienic place during the course of experimental period (43 days). Animals fed with feed (Pellated) and water.

Induction of Diabetes in subjects: Diabetes was induced in Wistar rats with **Streptozotocin** (STZ – from sigma) by intravenous tail vein injections under alight halothane anaesthesia. The diabetes state was confirmed three days following the STZ injection by a blood glucometer test and rats with a blood glucose level $\geq$ 13 mM shall be diagnosed as diabetes.

Animal Trials: In all trails the rats were grouped into 9 groups of 6 animals each and treated.

The animals were divided in to 9 groups of 6 animals each as labeled below

Group-I - Negative control (NC) (Non diabetic, Normal healthy rats)

Group-II- Positive control (PC) (Untreated, Induced diabetic rats)

Group-III - Control treated (CT) (treated with Metformin, 1mg/rat/day)

Group IV- This group of diabetic rats treated with Vanadium metal complexes

a) Group treated with [7-imi]
b) Group treated with [7-B]
c) Group treated with [6-imi]
d) Group treated with [6-B]
e) Group treated with [5-me-imi]
f) Group treated with [8-B]

Diabetes was induced in all groups except Negative control by a single intraperitoneal (IP) injection of 65mg/ Kg body weight of STZ dissolved in freshly prepared 0.1M Citrate buffer (pH = 4.5). After 72 hours, blood was withdrawn from the tail vein of all the rats to check the onset of diabetes in the rats and the blood glucose levels were estimated. To check the effectiveness of vanadium complexes on the diabetes, 10uL of the complex was administered daily (orally) by gastric intubation to the rats in groups IVa to IVf up to 28 days. The standard anti diabetic drug (Metformin, 1mg / rat) was administered to group-III rats for 28 days. Blood glucose levels were estimated every week. The plasma glucose estimations were done using a digital glucometer (consisting of a digital meter and test strips) using blood samples obtained from the tail vein of the rats. Treatment was stopped after 28th day. Glucose levels were recorded from 28th day to 43rd day even after terminating the treatment with metal complexes.

Statistical analysis:

Results (Blood glucose levels estimated from blood sample) were analyzed by using the software "SPSS version 21" and expressed as MEAN ±SEM .The difference between the groups

were tested by one-way analysis of variance (ANOVA) followed by Tukey's Posthoc test. Results were considered significantly different if $p < 0.05$.

ANOVA of Blood Glucose levels					
Response					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48433.595	10	4843.359	108.183	.000
Within Groups	2462.344	55	44.770		
Total	50895.938	65			

Tukey's Posthoc test: (for blood glucose levels)								
	NC	Met f	[7-imi]	[6-B]	[6-imi]	[8-B]	[5-meimi]	[7-B]
NC		**	-	**	*	**	-	**
Met f			**	**	**	**	**	**
[7-imi]				**	-	**	-	**
[6-B]					**	-	**	-
[6-imi]						**	-	-
[8-B]							**	**
[5-meimi]								**
[7-B]								

- * ----- The mean difference is significant at the 0.05 (5%) level.
- **------ The mean difference is significant at the 0.01 (1%) levels.

Plots of Plasma glucose levels as a function of time for each vanadium compound is presented in Fig 1.

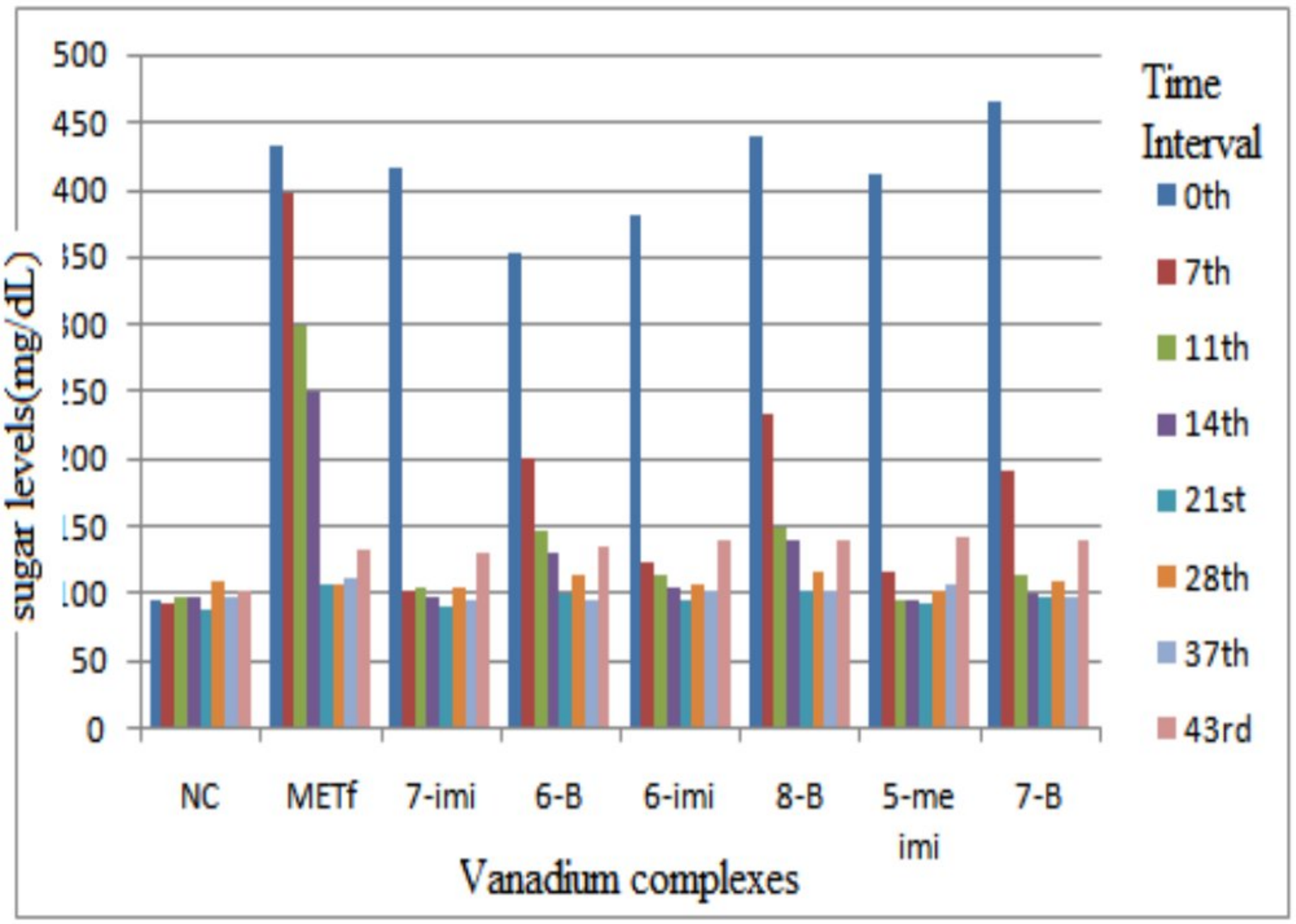


Figure 1: Trend of sugar levels in STZ induced diabetic rats treated with various vanadium metal complexes during the period of 43 days

For all studies, plasma glucose was monitored at zero day (before administration) and at ~~0th~~, 7th, 11th, 14th, 21st, 28th, 37th and 43rd days of post administration of the compound. Plots were constructed taking plasma glucose levels as a function of time for each complex. Blood sugar levels were monitored even after stopping the treatment of vanadium complexes for 15 days.
The results were compared with those of the standard compound (Metformin) to draw the logical conclusion.
Note: Administration of 10uL of 6-B complex to group –5 male rats become lethal, then drug dosage was subsided to 5uL.

Histopathological studies:

At the end of the experiment period all animals were physically anesthetized by chloroform inhalation. A mid line incision was performed at the thoracic region by a veterinary pathologist. The organs were dissected out, weighed and filled in 10% formalin saline for the process of histopathology.
Processing of tissue for histopathology was carried out in 10% buffered formalin were sliced to approximately 1cm thick, and placed into the cassettes. Then, the cassettes are placed in a tissue processor machine, which comprise of dehydration with alchohol, clearing with Xylene and wax, and impregnating process automatically over night. The cassettes were embedded in molten paraffin, which later cooled down to formed blocks of paraffin. Each block was trimmed then sectioned about 5µm by using semi automatic microtome. A good section without wrinkles was placed on the glass slide with due care for microscopic observation.

RESULTS AND DISCUSSION

1. Molecular modeling studies on PTP-1B:

3D structure of protein was downloaded PDB 1D: 2HNP. Its active site was determined and binding of Vanadium metal complexes into its active site was studied. Require parameters were evaluated.

Analysis of dock score for 2HNP reveals that the binding affinity of the molecules is more towards the cavity site-1(Vol= 84.48). The mol dock score and the plant scores of compounds with PTP-1B at site-1 are provided in Table-2. The 2HNP molecule showed three hydrogen bonds with amino acid residues Lys 116, Lys 120 and Arg 221 incase of [7-imi] as shown in Fig-2. As a result, higher binding capacity of 2HNP was reported for [7-imi] compared to remaining vanadium complexes during docking. [7-imi] secondary structures, hydrogen bond interactions and detected cavities images after docking are shown in the Figure 3 to 4.

Table 2: Binding affinity of inhibitors (vanadium metal complexes) to PTP-1B based on Plants, Mol Dock and Re-ranking scores at cavity 1.

S. No.	Complex	Plants Score	Mol Dock Score	Re-rank Score
1	[5-B]	-59.8664	-99.3417	-74.1286
2	[5-Me-IMI]	-71.5439	-143.525	-102.973
3	[6-B]	-60.9324	-122.596	-63.7707
4	[6-IMI]	-73.3549	-146.491	-50.2767
5	[6-Me-IMI]	-77.1689	-156.14	-110.169
6	[7-B]	-76.7183	-115.075	-91.0184
7	[7-Et-IMI]	-74.7142	-143.11	-88.3915
8	[7-Me-IMI]	-78.99	130.782	-94.3242
9	[7-IMI]	-81.6045	-128.711	-79.1859
10	[8-B]	-50.5335	-75.3733	-38.2218

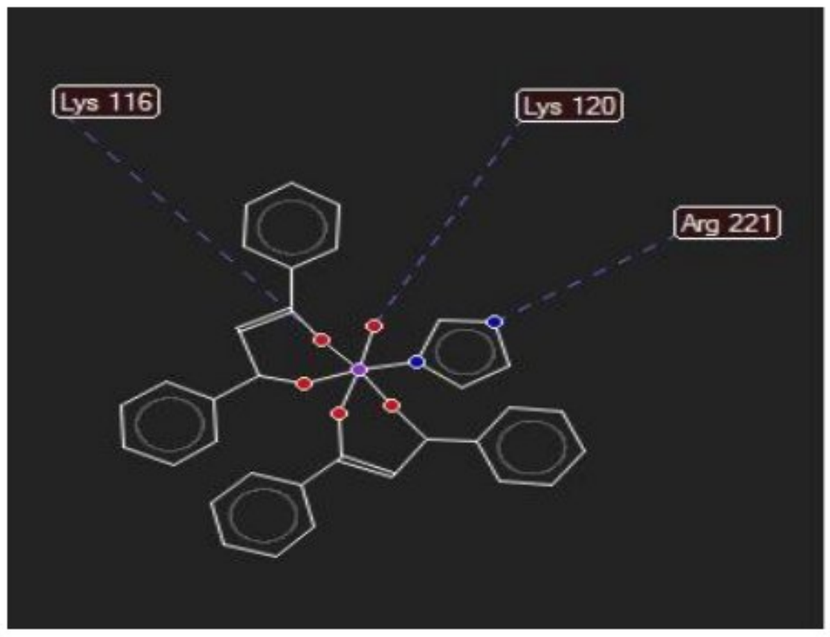


Figure 2: Active site of enzyme interaction picture of PTP-1B with [7-imi]

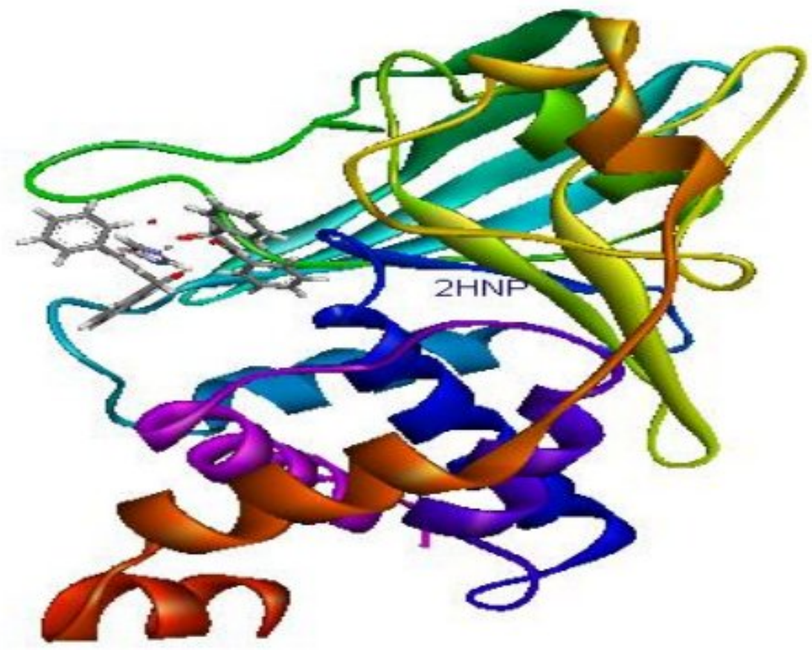


Figure 3: Docking picture of [7-imi] with 2HNP

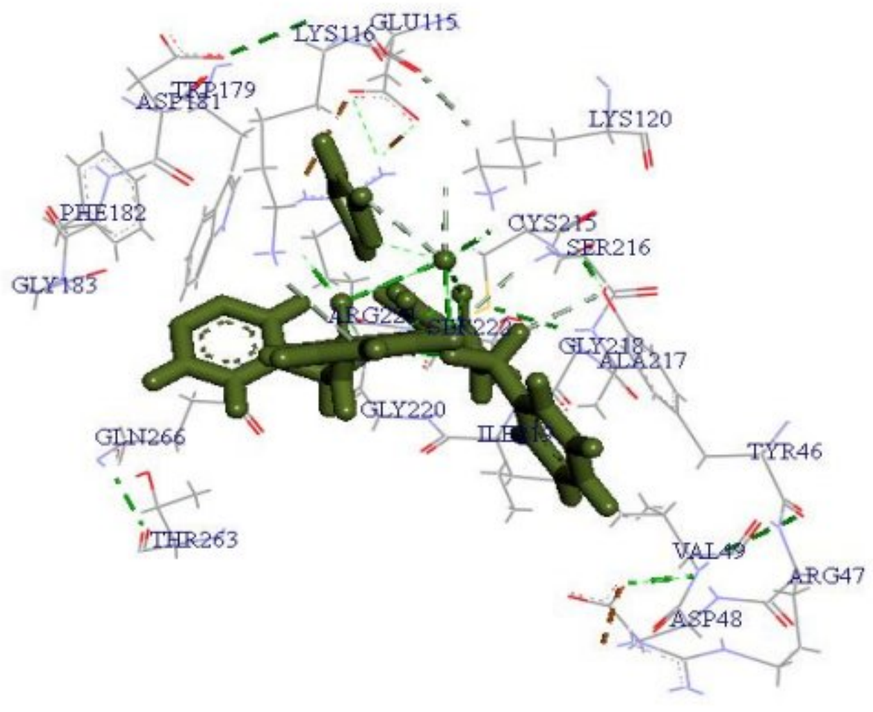


Figure 4: Molecular docking of [7-imi] with Protein tyrosine phosphatase-1B, Showing hydrogen bond interaction of compound [7-imi] with 2HNP residues Arg 221 (2.60 Å), Lys 116 (1.93 Å) and Lys 120 (2.06 Å) along with π - Donor bond.

2: Molecular modeling studies on BSA:

The importance of BSA in transporting a drug is discussed in chapter-3, Section-2. Theoretical studies would be much useful to correlate the experimentally obtained data. Hence molecular modeling on BSA was performed. The 3D structure of protein was downloaded from PDB 1D: 4F5S. Analysis of dock score for BSA reveals that the binding affinity of the complexes is more towards the cavity site-1 (Vol=8539.14) as compared to the other cavities. Site-1 and site-4 correspond to drug binding site I and site II. The mol dock score and the plant scores of compounds with BSA at site-1 are provided in Table 3. The BSA molecule showed only one hydrogen bond interaction with amino acid residues Lys 116 in case of 7-IMI as shown in Fig-5. No specific electrostatic and hydrophobic interactions were seen. Higher binding capacity of BSA was reported for [7-IMI] compared to rest of the metal complexes as a result of docking

simulation. [7-IMI] secondary structures, hydrogen bond interactions and detected cavities images after docking are shown in the Fig 6 to 7.

Table 3: Binding affinity of inhibitors (vanadium metal complexes) to BSA based on Plants, Mol Dock and Reranking scores at cavity 1.

S.No	Compound	Plants Score	MolDock Score	Rerank Score
1	[5-et-imi]	-66.2262	-91.9445	-64.5476
2	[5-me-imi]	-62.8204	-107.667	-72.6972
3	[6-B]	-73.7097	-99.7275	-71.0608
4	[6-et-imi]	-73.3462	-129.19	-87.9003
5	[6-imi]	-71.0031	-118.739	-79.1581
6	[6-me-imi]	-69.984	-122.082	-78.5366
7	[7-B]	-75.9075	-97.2944	-77.0017
8	[7-et-imi]	-80.074	-131.204	-75.2863
9	[7-imi]	-79.4278	-108.386	-74.5589
10	[7-me-imi]	-78.1773	-127.746	-71.2719

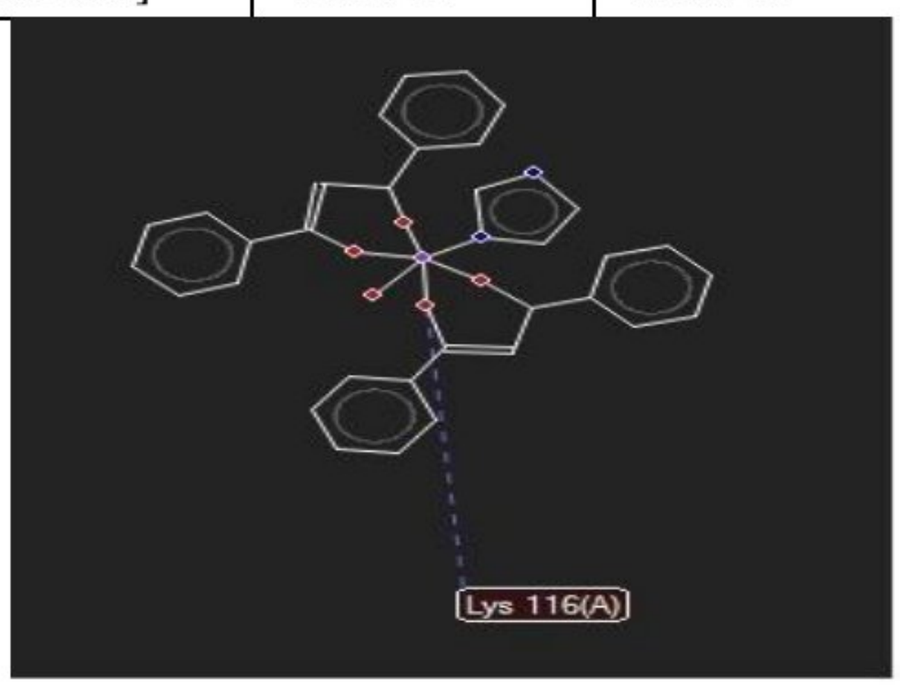


Figure 5: Active site of enzyme interaction picture of BSA with [7-imi].

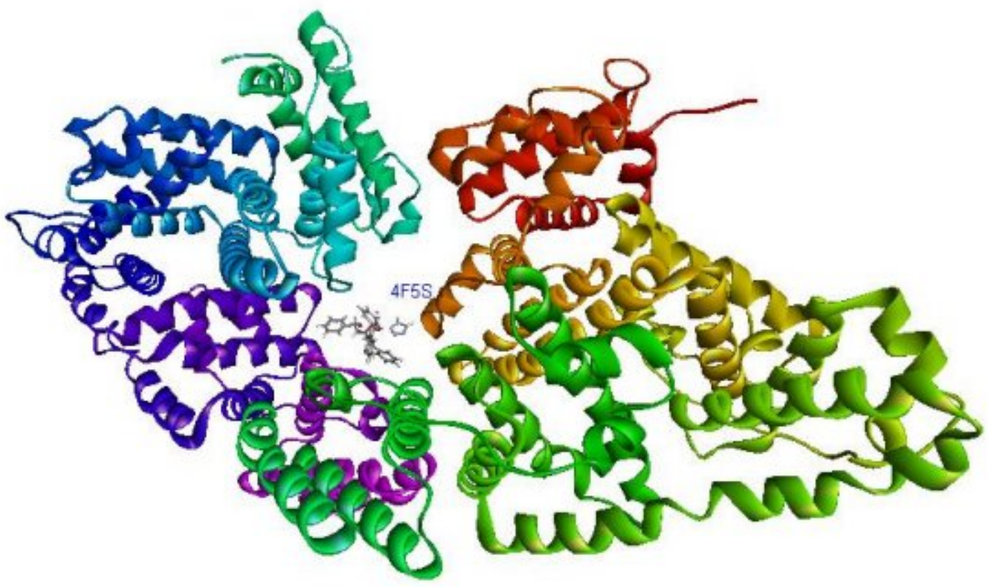


Figure 6: Docking picture of [7- imi] with BSA

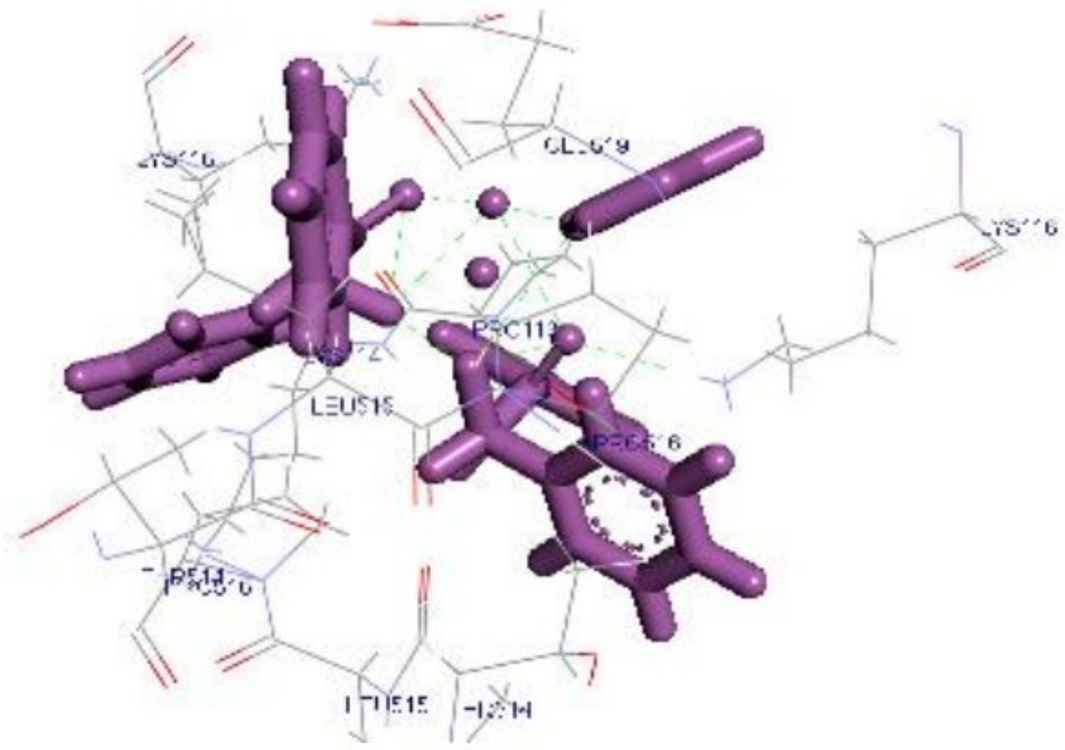


Figure 7: Molecular docking of [7-imi] with Bovine Serum Albumin, Showing hydrogen bond interaction of compound [7-imi] with BSA residues Lys 116.

In docking with BSA the top found vanadium complex ([7-et imi]) had binding affinity of -80.074 based on PLANTS score which is equal to -131.204 Mol dock score.

In docking with PTP-1B,t he top found vanadium complex ([7-imi]) had binding affinity of -81.604 based on PLANTS score which is equal to -128.711 Mol dock score, basing on this model of potential inhibition, this study suggest that the [7-imi] complex which was found in this research can be used for subsequent laboratory studies.

3. In vivo studies of Vanadium metal complexes on Wistar Rats

3.1 Effect of Vanadium metal complexes on reducing serum glucose levels in induced diabetes Wistar rats

3.1.1 Animals:

In present study, 54 Wistar rats (aged 8 to 9 weeks, weighting 150-220 g) were selected and tested; Rats were divided into 9 experimental groups of 6 animals each.

Group-I - Negative control (NC) (Non diabetic, Normal healthy rats)

Group-II- Positive control (PC) (Untreated, Induced diabetic rats)

Group-III - Control treated (CT) (treated with Metformin, 1mg/rat/day)

Group IV- This group of diabetic rats treated with Vanadium metal complexes

a) Group treated with [7-imi]
b) Group treated with [7-B]
c) Group treated with [6-imi]
d) Group treated with [6-B]
e) Group treated with [5-me-imi]
f) Group treated with [8-B]

3.1.2 Induction of diabetes:

Streptozotocin (STZ) (Fig-8) has been widely used for induction of diabetes into the rats. STZ has toxic effects [19] on pancreatic β-cells which result in stoppage of insulin production. Results of numerous experiments revealed that this model of diabetes is useful in studies of different aspects of diabetes [20].

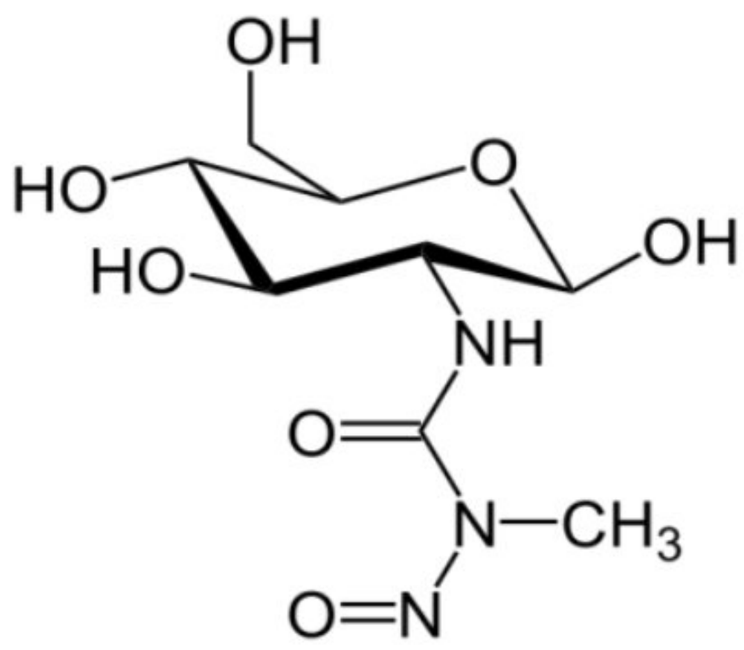


Figure 8: Structure of STZ

3.1.3 Sample collection & testing glucose levels:

One day after STZ injection, diabetes was confirmed by measuring serum glucose levels. Blood samples were collected from the tail vein. One touch gluco meter was used to measure the glucose levels. Rats with blood glucose levels above 140mg/dl were considered as diabetes. Sugar levels were monitored on 7^{th}, 11^{th}, 14^{th}, 21^{st}, and 28^{th} days after treatment. After this, treatment was withdrawn from 28^{th} day to 43^{rd} day. But sugar levels were estimated, monitored and recorded.

3.1.4 Statistical analysis:

Recorded sugar levels in each group and between groups were analyzed using ANOVA followed by Tukey's Posthoc test. P values less than 0.05 was taken as significant values. The obtained data is presented table-4 which is represented in graphical form in fig-9

Anaysis of data (table-4) or graph (fig-9) reveals that vanadium metal complexes proved to be much better in reducing serum glucose levels when compared with the drug (Metformin) (fig-10) available in market. Interesting finding is that no rat was found hypoglycemic. The order of sugar reducing property of these six vanadium complexes is as follows

[7-Imi] > [5-me imi] > [6-imi] > [7-B] > [6-B] > [8-B] > Metformin

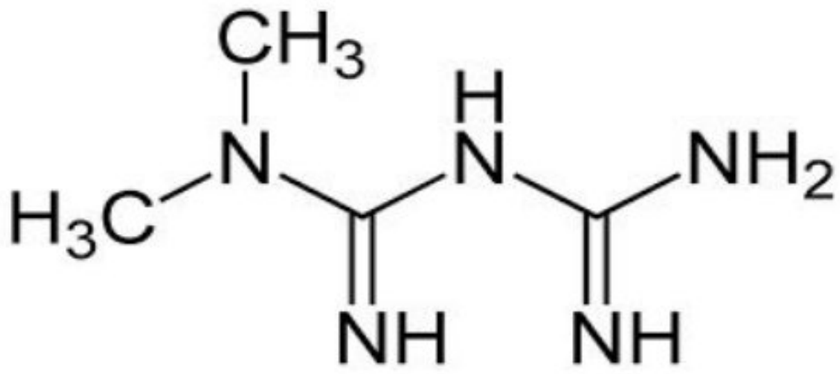


Figure 10 : Structure of Metformin

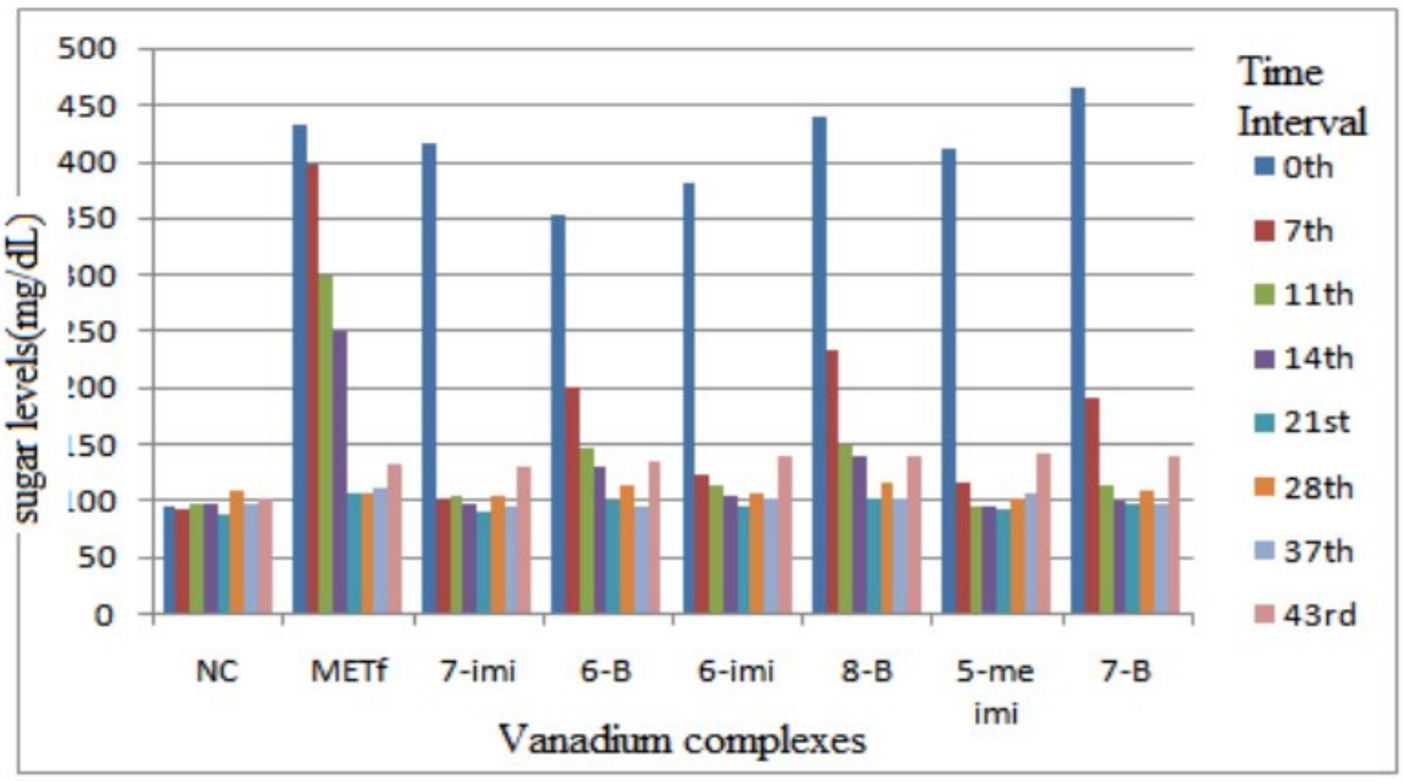


Figure 9: Trend of sugar levels in STZ induced diabetic rats treated with various vanadium metal complexes during the period of 43 days.

Table 4: Effect of vanadium metal complexes on reducing glucose levels in diabetic rats.

	Group-I	Group-III (CT)	Group-IV					
	NC	Metformin	7-imi	6-B	6-imi	8-B	5-me imi	7-B
0^{th}	96.66	432	415.66	353.16	382	438.5	411.5	464
7^{th}	94.166	397.66	102.33	200.83	123.5	233.6	117.16	191.5
11^{th}	97.8333	299.33	104.33	147.33	113.33	149.5	96.3333	114
14^{th}	98.8333	249	99	130.16	105.66	140.5	94.833	100.83
21^{st}	89.5	108	91.3333	100.33	96.66	103.5	92.666	97
28^{th}	109.16	106.83	104.5	115	108.33	116.83	103.5	109
37^{th}	98.8333	112	94.6667	95.1667	102.66	101.66	107.83	98.5
43^{rd}	103.666	134.333	132	135.333	139.666	139.166	142.166	139.5

Note: Treatment is given up to 28th day. Glucose levels from 29th day to 43rd day of project without treatment.

3.2.Effect of Vanadium metal complexes on Kidney, Liver and Pancreas of treated diabetic rats:

Kidney:

Histopathology of Kidney, Liver and Pancreas was studied in normal, diabetic and treated groups. The normal rat Kidney section shows the well arranged cells and central vein, also no inflammation and degeneration was noticed. In the diabetic group, Haemorrhages and inflammatory cells noticed. In the [5-me-imi] treated group, no inflammation was noticed, and histipathological changes are restored near to normal in the Kidney treated rats, as shown in fig 11.

Liver:

The normal rat Liver section shows the well arranged cells, also hepatocytes appeared normal. No inflammation, degeneration and haemorrhage noticed. In the diabetic group, moderate to severe inflammation of Liver noticed. In the [5-me-imi] treated group, Hepatocytes appeared normal, histipathological changes are restored near to normal in the Liver of [5-me-imi] treated rats, as shown in fig 12.

Pancreas:

The normal rat Pancreas section shows no degeneration, inflammation and necrosis. In the diabetic group, moderate to severe necrosis of Pancreas noticed. In the [5-me-imi] treated group, pancreas appeared normal, histipathological changes are restored near to normal in the pancreas of [5-me-imi] treated rats, as shown in fig 13.

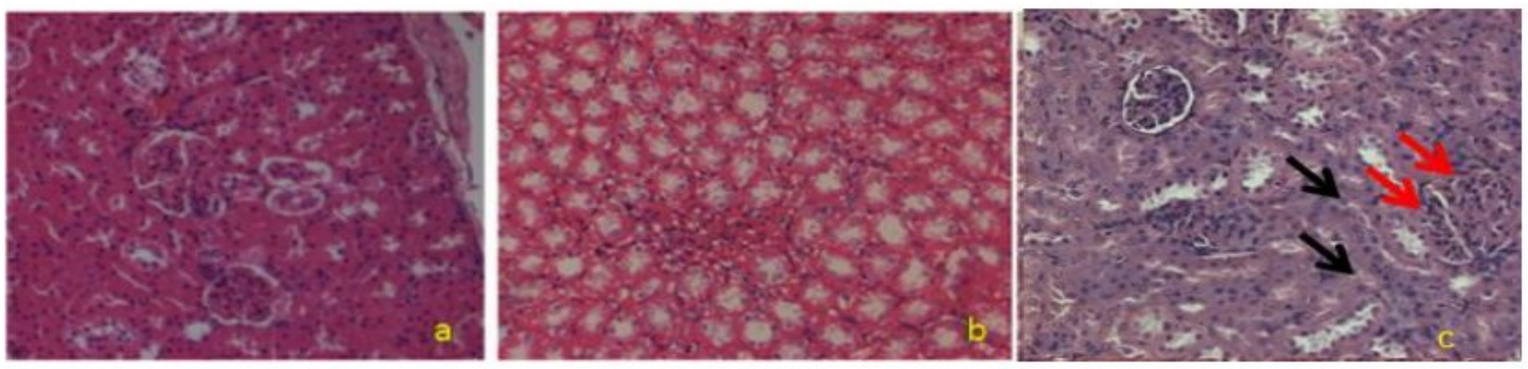


Figure 11: Histopathological changes in Kidney of [5-me-imi] treated diabetic rats

a. No inflammation, no degeneration noticed in Normal rat kidney

b. Haemorrhages and inflammatory cells noticed in diabetic rat kidney

c. No inflammation noticed, histipathological changes are restored near to normal in the Kidney of [5-me-imi] treated rats.

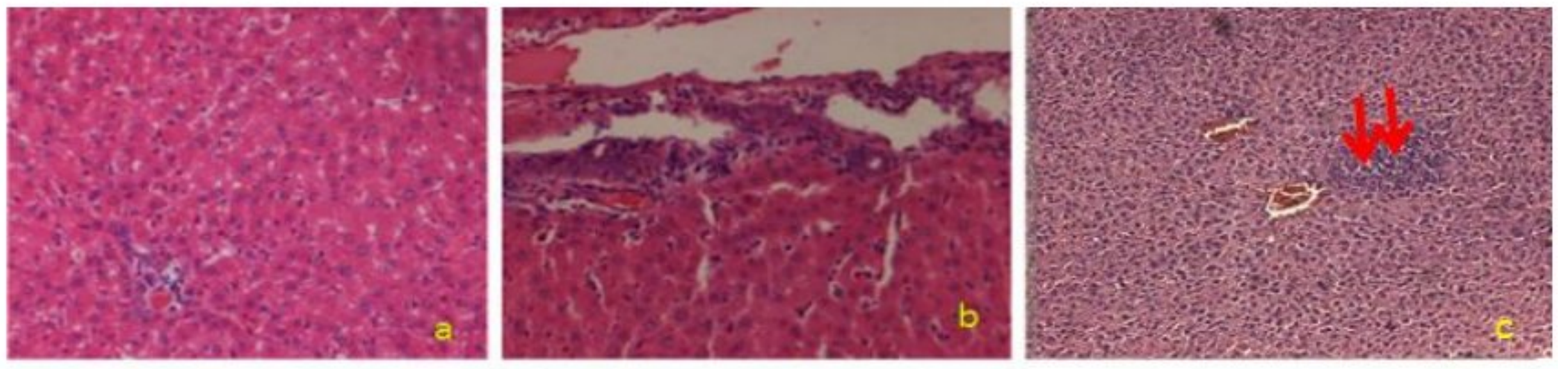


Figure 12: Histopathological changes in Liver of [5-me-imi] treated diabetic rats

a. Hepatocytes appeared normal. No inflammation, degeneration and haemorrhage noticed in Normal rat Liver

b. Moderate to severe inflammation noticed in diabetic rat Liver.

c. Hepatocytes appeared normal, histipathological changes are restored near to normal in the Liver of [5-me-imi] treated rats

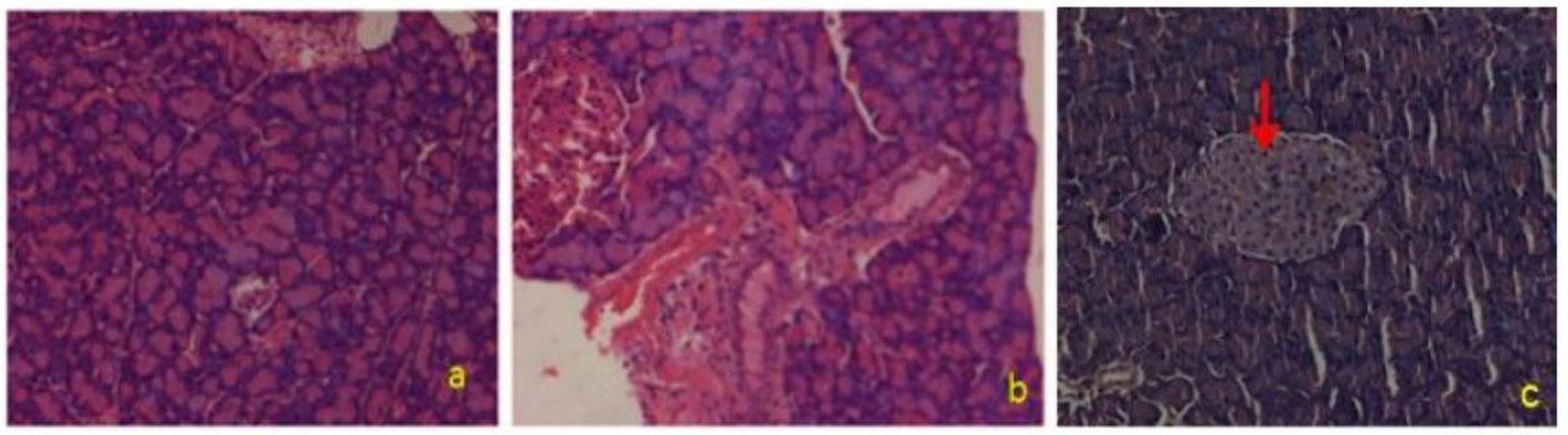


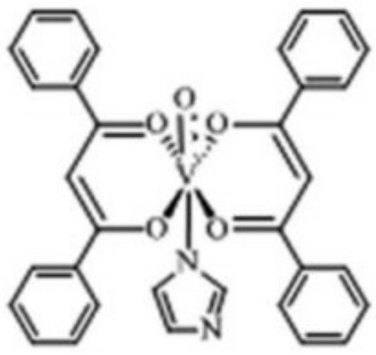
Figure 13: Histopathological changes in Pancreas of [5-me-imi] treated diabetic rats

a. No degeneration, inflammation and necrosis noticed in entire pancreas of Norma rat.

b. Moderate to severe necrosis noticed in diabetic rat Pancreas.

c. Pancreas appeared normal, histipathological changes are restored near to normal in the pancreas of [5-me-imi] treated rats.

3.2.3. Conclusion: In vivo efficiency of Vanadium metal complexes to normalize the serum blood sugar levels of STZ diabetic Wistar rats was tested. All tested complexes reduced plasma glucose levels in rats. All these complexes have showed major advantage over standard drug "Metformin". Among all these compounds [7-imi] metal complex (ternary complex) showed better efficiency than other vanadium complexes tested.



[7-imi]

The order of efficiency is as follows: [7-imi] > [5-me imi] > [6-imi] > [7-B] > [6-B] > [8-B] > Metformin

CONCLUSION

Enzyme inhibitors are usually compounds that combine with the enzyme to form in enzyme-inhibitor complex, either by reducing or completely inhibiting the catalytic activity of the enzyme and therefore reducing the rate of reaction. The binding of an inhibitor to the active site of the enzyme can block the entry of the substrate to the site. Overactive enzymes are usually attractive targets for the development of inhibitor molecules to alleviate disease conditions.

With this background, we have synthesized and characterized the inhibitors - Vanadium metal complexes of different di-ketones ligands exclusive for PTP-1B. The inhibitory action of vanadium metal complexes on PTP-1B (enzyme kinetics) was studied *in-vitro* in our lab using 96 well Micro Plate Reader and found that they have excellent inhibitory properties on PTP-1B. In our present work, we have also studied the interaction of vanadium metal complexes with BSA, since it is known as a carrier of a drug ~~in *in-vitro* studies~~. These results suggest vanadium binding occurs between inhibitor & PTP-1B in their active site. To further confirm, we relied on molecular modeling studies. The order of inhibition (Kinetics), the order of binding (binding constant) and results of molecular modeling concerning various vanadium complexes are in consistent with each other.

Excited with these results, we conducted animal studies after completing all the required formalities. Diabetes-induced STZ Wistar rats were chosen as targets. These rats were treated with four different Vanadyl metal complexes and various tests were conducted (as per protocols) which include blood sugar. The drug "Metformin" was chosen as the standard. Synthesized vanadium metal complexes are shown to reducing blood glucose level. An interesting finding is that no rat was found to be hypoglycemic. The order of sugar reducing properties of four vanadium complexes follows the same trends as observed in different studies i.e. Kinetics, Binding Studies, Molecular Modeling and *In-vivo* studies.

The experimental results from various sources (binding studies, enzyme kinetics, Molecular modeling and animal studies) are given below before making concluding remarks.

BSA binding order: 7-imi > 7-Et imi > 7-Me imi > 7-B > 5-me imi > 6-me imi > 6-imi >6-B > 5-B > V-acac.

*Enzyme Kinetics studies:*7-Imi > 7-Et-imi >7-Me-imi > 7-B > 5-Me-imi > V-Pyridyl > 5-B > V-acac

*Molecular Modeling studies:*7-imi > 7-me imi > 6-me imi > 7-B > 7-Et imi > 6-imi > 5-me imi > 6-B > V-acac

*In Vivo studies:*7-Imi >5-Me imi > 6-imi > 7-B > 6-B > V-acac > Metformin

Based on the above experimental observations, it is concluded that vanadium metal complexes help in reducing serum glucose levels in animals. They behave as inhibitors to suppress the over expression of PTP-1B enzyme. The inhibition is competitive. Among these complexes [7-imi]

is found to be the best for further studies. However, further studies are required to establish its effect on human beings.

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