***Original Research Article***

# Investigation of Enzymes Binding to "Voglibose- An Antidiabetic Drug" and the choice of Enzyme to be used for Biosensing.


## ABSTRACT

**Aims:** This paper concentrates on the investigation of the enzymes binding to Voglibose and the choice of the enzyme which is suitable for Biosensing. Voglibose is an antidiabetic drug which is reactive and hence used in very low concentrations. The existing methods of analysis of this drug are associated with certain disadvantages. Hence there is a need to establish alternative, simpler method of analysis to overcome the disadvantages of available methods.
**Study design:** This is based on biosensing which requires a bioreceptor for this drug. Thus, enzymes interacting with the drug are studied and the choice of the enzyme that can act as bioreceptor for this drug is confirmed and the same could be used for the next experimentation.
**Place and Duration of Study:** This experiment was carried out as a part of the project under Indian Nanotechnology User Program (INUP) at IITB, Mumbai.
**Methodology:** The approach is based on two steps for the confirmation and choice of the enzyme binding to the drug. The first approach is Molecular Docking which is a simulation done to check the enzymes binding with the drug and the second approach is Chemical Analysis to confirm the same.
**Results:** The experiments conducted shows that Voglibose inhibits both Alpha Amylase and Alpha Glucosidase. The literature mentions that Voglibose is an inhibitor of Alpha Glucosidase. This experiment confirms that Voglibose is an inhibitor of Alpha Amylase also. Thus in future Alpha Amylase can also be chosen as an appropriate target Voglibose.
**Conclusion:** The present *In-silico* and *In-vitro* swot analysis indicates that like Alpha glucosidase even Alpha amylase binds to Voglibose. The interaction outcome suggests that in future one can consider Alpha amylase as a choice of target in applications of Biosensing.


*Keywords: Voglibose, Alpha Amylase, Alpha Glucosidase, Molecular Docking, Chemical Analysis.*

## 1. INTRODUCTION

Diabetes Mellitus is an important concern for the health care sector. Postprandial hyperglycemia(which are normally measured two hours after and before eating in a postprandial glucose test) is one of the earliest abnormalities of glucose homeostasis associated with type 2 diabetes and is markedly exaggerated in diabetic patients with fasting hyperglycemia. It is known that Postprandial hyperglycemia (PPHG) contributes to the increased risk of both micro- and macrovascular complications in patients with diabetes mellitus. It appears in the literature that managing postprandial plasma glucose is more important in order to prevent the complications of type-2 diabetes [1].

Different types of drug are available for lowering PPHG levels. Amongst the oral drug are the Alpha Glucosidase inhibitors like Acarbose, Miglitol and Voglibose. These drugs are comparatively inexpensive and can be orally taken for longer durations [2].

The literature mentions that Miglitol and Voglibose have equal efficacy in reducing PPHG as compared to Acarbose. The clinical benefit of Voglibose was its better safety profile as compared to Miglitol and Acarbose. Looking at the efficacy and safety profile amongst the available Alpha glucosidase inhibitors, Voglibose has a preferential choice in the management of postprandial hyperglycaemia in treatment of type-2 diabetes mellitus [1].

Voglibose [3] is a research product of Takeda Pharma, a Japan based company. Voglibose is a highly reactive drug and is recommended in small dosages of 0.2mg to 0.3mg, which is supposed to be lowest in the pharmaceutical industry. The analysis of this drug is of great importance because of its reactivity and low concentration and also as it is an anti- diabetic drug; which is used for the treatment of type 2 diabetes; one of the prevalent ailments in the society.

***Existing Methods for the Analysis of Voglibose***

There are different methods of analysis of this particular drug. JP describes the post derivatization methods with Fluorescent detectors for the estimation (assay) of Voglibose in its monographs. This describes about an LC_FD method with post column derivatization for the determination of Voglibose in pharmaceutical tablets. For the post column derivatization, Sodium Periodate and Taurine dissolved in water was used as post column reagent. Voglibose was detected at an excitation wavelength of 350nm and an emission wavelength of 430nm [3]. The paper on “Development and validation of UV-Spectroscopic method for estimation of Voglibose in bulk and tablets” mentions about UV spectroscopic methods for the analysis of Voglibose in pharmaceutical formulation. This also mentions of the post derivatization method. Voglibose was estimated at 282nm. This method was used for the estimation of Voglibose in bulk and tablet dosage forms [4]. The paper on “Development and Validation of RP-HPLC Method for Quantitative Analysis Voglibose in Pure and Pharmaceutical Formulations” mentions of reverse phase HPLC method for the analysis of Voglibose in tablet and bulk forms. In this method Voglibose was derivatized with Sodium Periodate and Taurine as Voglibose cannot be directly detected. Voglibose was detected at 282nm [5].

The paper on “Stability Indicating RP-HPLC Mehtod for Estimation of Voglibose in Bulk and Tablet Dossage forms” mentions about two methods used for the analysis of Voglibose. The first method describes about an LC_FD method with post column derivatization for the determination of Voglibose in pharmaceutical tablets. In this Voglibose was detected at an excitation wavelength of 350nm and an emission wavelength of 430nm [6]. The second method mentions about an LC_MS assay procedure for the analysis of Voglibose which is without derivatization. Voglibose was detected in an ESI mode with single ion recording (SIR,m/z 268.1) [6]. The paper on “Quantitative determination of Voglibose in pharmaceutical tablets using high-performance liquid chromatography-fluorescence detection with post-

column derivatization and mass spectrometric detection" mentions about a methods used for the analysis of Voglibose in bulk and tablet dosage forms using HPLC. Voglibose was detected at 272nm [7].

***Challenges of the Existing Methods***

Assay method of analysis has to be performed for every drug. When the concentrations are less than 10mg per tablet, Uniformity of Content (UOC) is also an important parameter to be tested.

If a standard method is not available for analysis of any drug, then a method has to be established for the analysis.

Voglibose is antidiabetic drug used in very low concentrations and has least absorbability. There are methods established using UV detector but they suffers low response.

In all the above mentioned methods of analysis, Voglibose is detected after performing post derivatization which requires expensive instrumentation and skilled personnel.

Thus in cases of Assay and UOC the method either suffer poor resolution or requires expensive instrumentation and skilled personnel.

There is another parameter called the Dissolution which is a method of evaluation of absorption rate of drug in the body where the drug is diluted to a still lower concentration. Because of its dilution no method of analysis is established for this parameter. Even JP does not mention any method of analysis for dissolution studies because of the dilution to low concentrations.

Thus there is requirement for a new method of analysis to estimate the drug in low concentration using simpler techniques and also to provide a method for dissolution studies [8]

***The Approach:***

This alternative method can be based on the principle of Biosensing [9][10][11] which is based on the Drug Enzyme interaction.

Thus the first step towards this biosensing is to identify the Enzyme binding to the Drug.

This paper concentrates on the investigation of the enzymes interacting to the drug and then the choice of the enzyme for further studies.

For the investigation there are three enzymes considered

1. CYP450[12][13][14] series of enzymes which are involved in the formation (synthesis) and breakdown (metabolism)
2. Alpha Glucosidase as Voglibose is an Alpha Glucosidase Inhibitor AGI [15]
3. Alpha Amylase

Enzymes produced from the Cytochrome P450 [12][13][14] genes are involved in the formation (synthesis) and breakdown (metabolism) of various molecules and chemicals within cells.

The cytochrome P450 enzymes account for the metabolism of approximately 20% of therapeutic drugs including certain oral antidiabetic drugs (OADs). 2C9 is the CYP450 enzyme for metabolizing antidiabetic drug [12]. But the CYP enzymes are associated with their disadvantages of instability and are expensive.

Thus, these drawbacks limit the use of CYP 450 series of enzymes as potential enzymes to be used to bind with Voglibose.

The literature strongly mentions in the Mechanism of Voglibose [15] that Voglibose is a competitive inhibitor of Alpha Glucosibase thus it is clear that Voglibose binds to Alpha Glucosidase. This point is as mentioned in the Drug Bank of Voglibose. There are references that Acarbose another anti diabetic drug inhibits Alpha Amylase so an attempt was done to test if Voglibose also inhibits Alpha Amylase [16]. The binding of Alpha Amylase and Voglibose was tested using Molecular Docking process. The same was confirmed by chemical analysis [17]. As the literature of binding of Alpha Glucosidase with Voglibose is already in the Drug Bank of Voglibose, this paper more concentrates to confirm the binding of Voglibose and Alpha Amylase. Molecular Docking is the first step to confirm the binding of the Alpha Amylase and Voglibose which is followed by the chemical analysis.

## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

### 2.1 In-Silico Methodology.

**2.1.1. Receptor Protein:**

The 3D crystallized complex structure of Alpha glucosidase with Acarbose Pdb-id:2QMJ [18] and crystal complex structure of alpha-amylase with Acarviostatin amylase Pdb-id: 3OLD [19] were downloaded from the RCSB Protein Data Bank

**2.1.2. Active site:**

Identification of an active site or binding site on a protein is a principal analysis in a structure based drug discovery. In present work the default binding site for Acarbose and Acarviostatin was considered for the Voglibose too.

**2.1.3. Ligand preparation:**

The 3D structure of Voglibose in sketched using Chemsketch 12.0 [20] and optimized the structure using UFF [21] in Argus lab [22] and converted to .Pdb file format.

**2.1.4 Molecular docking:** Molecular interactions play a key role in all biological reactions. Chemical structure are either mimicking or mitigating the effect of natural ligands binding on to the receptor by exerting the pharmacological reactions. Computational methods are used to understand this mode of binding of ligands to their receptors is known as Molecular Docking [23]. Molecular docking study is carried out in AutodockVina [24].

Gasteiger charges are added to the ligand and maximum numbers of calculated rotatable bonds were considered torsions using AutoDock4.0 [25] tool. Kollman charges and the solvation term were then added to the protein structure using the same. Considering the default active site, grid box is adjusted with the number of points in X, Y, Z-axis so that the entire active site of protein is covered. Due to large volume of active site is acquired by Acarbose and Acarviostatin we have increased the spacing value to

1.0 Å from default value of 0.375 Å between grid points so to investigate the large volume space. Considering with default parameters the molecular docking process is successfully carried out.

## 2.2 Chemical Analysis to verify the Binding of Alpha Amylase with Voglibose.

The chemical analysis was done to confirm the following

1. The activity of Alpha Amylase
2. Inhibition of Alpha Amylase by Voglibose

### 2.2.1 Materials used

1. Starch Solution
2. Buffer solution
3. Alpha Amylase Solution
4. Iodine Indicator

*2.2.1.1 Preparation of Starch Solution:*

The starch used was potato starch and 50mg was dissolved in 100ml water with continuous stirring at 100 degree C.

*2.2.1.2 Preparation of buffer*

The Alpha Amylase used is Fungal Diastase which is active in Acetate buffer of pH=5-6. This was obtained as a complimentary sample from Anthem Solutions.

357ml of 0.1M Acetic Acid was mixed with 643ml of 0.1M Sodium Acetate to get Acetate buffer of pH=5.

*2.2.1.3 Preparation of Alpha Amylase Solution*

The Alpha Amylase used is fungal diastase with strength of 1:800

100units of Alpha Amylase corresponds to 125mg of the Fungal Diastase used. This was dilute 1000ml of the buffer. Then 10ml of this solution was diluted in 100ml Acetate Buffer.1ml of this test solution should be capable of digesting about 10mg of starch.

*2.2.1.4 Iodine Indicator*

The Indicator used is Iodine Indicator of 2mM. This was prepared mixing 0.2% Iodine and 2% Potassium Iodide.

## 2.3 Procedure for testing the activity of Alpha Amylase used in the experiment (Fungal Diastase)

Starch Iodine Test is used to confirm the activity of the Alpha Amylase.

Weigh accurately a quantity containing 100units of Alpha Amylase. Make a solution in 200ml of acetate buffer solution of pH=5. 10ml of Alpha Amylase is diluted to 100ml with acetate buffer. Different volumes like 3.5ml, 4.5ml, 5ml were added with 5ml of starch solution. The test tubes were kept in water bath at 40 degree C for 1 hour. The absorbance value was noted after the addition of 0.5ml Iodine Indicator.

### 2.4 Procedure for testing inhibition activity of Alpha Amylase by Voglibose.

A known concentration of Alpha Amylase was prepared. Voglibose of 0.3mg/ml, 0.4mg/ml, 0.5mg/ml were prepared in acetate buffer. Then 1ml of Voglibose (of the above mentioned concentrations) and 1 ml of Alpha Amylase were maintained at 40 degree C for 60 minutes. Later 1ml of Starch was added and maintained at 40 degree C for 60 minutes. Iodine indicator was added and the spectroscopy was obtained.

### 2.5 Procedure for testing Inhibition activity of Alpha Amylase by Voglibose with the concentration of 0.3mg/ml which is the concentration available in tablets.

A known concentrations of Alpha Amylase were prepared. Voglibose of 0.3mg/ml was prepared in acetate buffer. Then 1ml of Voglibose and 1 ml of Alpha Amylase (of various concentrations) were maintained at 40 degree C for 60 minutes. Later 1ml of Starch was added and maintained at 40 degree C for 60 minutes. Iodine indicator was added and the spectroscopy was obtained.

The inhibition of Alpha Amylase by 0.3mg/ml Voglibose was obtained by the formula: A(control)-A(test)/A(control)*100

## 3. RESULTS AND DISCUSSION

### 3.1 Results of Molecular docking

The 3D optimized structure of Voglibose exhibited a minimum energy of18.3637 kcal/molfrom210.3295 kcal/mol. Molecular docking studies predicts the interaction between selected ligand molecule and element of an amino acid involved in an active site of a receptor protein. Therefore the optimum binding energy, types of bonding, interaction and as well as pharmacophoric points are equally important in justification and validation of results. The binding energy of Voglibose to Gluco-amylase is -6.1kcal/mol and Voglibose to Alpha-amylase is -6.4kcal/mol. Both the generated complexes exhibited a hydrogen bond, vander waal interaction and covalent features. In case of Pdb id 2QMJ Gluco amylase, Voglibose appropriately placed inside the binding site cavity forming a typical hydrogen, Vander Waal and covalent bond interaction to most of the pharmacophoric amino acid residues. Where ASP 203, ASP 443, ARG 526 are core amino acid for binding the Acarbose within the Gluco amylase by hydrogen bond formation, similar binding activity is exhibited with Voglibose too, such as hydrogen bond: ASP 203, ASP 443, ARG 526. Vander Waal interaction: TYR 299, ILE 364, TRP 441, TRP 539, PHE 575 and Covalent bond with ASP 327, TRP 406, MET 444, ASP 542. Whereas in a crystallized structure Pdb id 3OLD Alpha amylase exhibited a binding affinity with ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306 and etc. Here similar binding can be seen with Voglibose too, where ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306 formed a hydrogen bond between Voglibose and Alpha amylase, Vander Waal interaction with TRP 58, GLU 60, VAL 98, HIS 101, LEU 162, THR 163, LEU 165and Covalent bond with TYR 62, GLN 63, ARG 195, HIS 299, HIS 305 given in Table (1), Figure (1) and (2).

**Table 1: Molecular Docking Interaction Analysis**

| Complex | Hydrogen bond | Vander Waal interaction | Covalent bond | Binding energy Kcal/mol |
|---|---|---|---|---|
| Voglibose to 2QMJ | ASP 203, ASP 443, ARG 526 | TYR 299, ILE 364, TRP 441, TRP 539, PHE 575. | ASP 327, TRP 406, MET 444, ASP 542 | -6.1 |
| Voglibose to 3OLD | ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306. | TRP 58, GLU 60, VAL 98, HIS 101, LEU 162, THR 163, LEU 165. | TYR 62, GLN 63, ARG 195, HIS 299, HIS 305. | -6.6 |

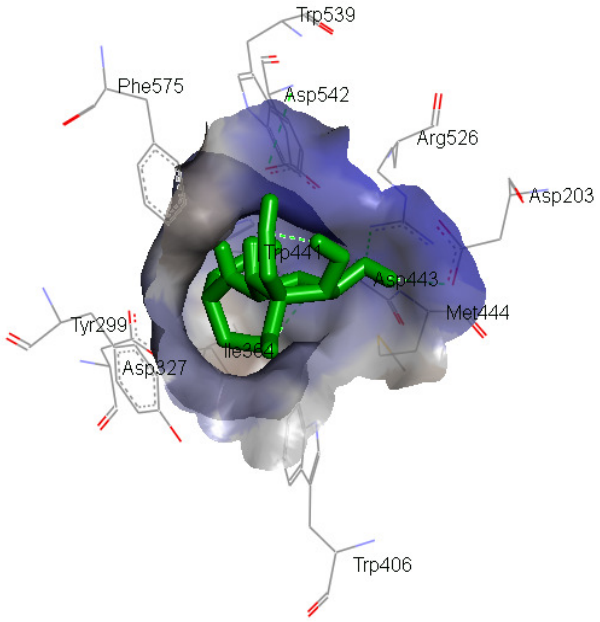


**Fig 1: Interaction of Alpha Glucosidase with Voglibose**

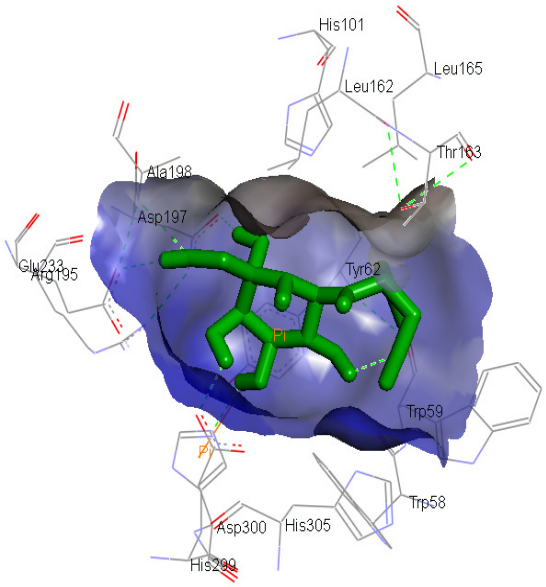


**Fig 2: Interaction of Alpha Amylase with Voglibose**

## 3.2 Results of Chemical Analysis

Alpha amylase is active and as the concentration of alpha amylase increases the absorption of starch increases which is shown in Table (2) and Table (3)

**Table 2: Testing the activity of Alpha Amylase (Fungal Diastase) used for the experiment, the absorbance was observed at wavelength of 576nm**

| Alpha amylase | Starch | Iodine | Absorbance value |
|---|---|---|---|
| 3.5ml | 5ml | 0.5ml | 0.18 |
| 4ml | 5ml | 0.5ml | 0.15 |
| 4.5ml | 5ml | 0.5ml | 0.148 |
| 5ml | 5ml | 0.5ml | 0.11 |

**Table 3: Testing Inhibition activity of Alpha Amylase by Voglibose, the absorbance was observed at wavelength of 576nm.**

| Alpha amylases | Voglibose | Starch | Absorbance value |
|---|---|---|---|
| - | - | 1ml | 0.565 |
| 1ml | - | 1ml | 0.029 |
| 1ml | 0.3mg | 1ml | 0.12 |
| 1ml | 0.4mg | 1ml | 0.16 |
| 1ml | 0.5mg | 1ml | 0.18 |

**3.2.1 Tabulation for testing percentage inhibition of Alpha Amylase by 0.3mg/ml of Voglibose.**

**Experiment 1:** Testing the inhibition of 25ml Alpha Amylase in 25ml buffer by 0.3mg/ml Voglibose which is shown in Table (4).

**Table 4: Inhibition of 25ml Alpha Amylase in 25ml buffer by 0.3mg/ml Voglibose**

| Starch | Alpha amylase | Voglibose | Wavelength | Absorbance value | Percent |
|---|---|---|---|---|---|
| 1ml | - | - | 578 | 0.983 | 100 |
| 1ml | 1ml | - | 566 | 0.229 | 76.7 |
| 1ml | 1ml | 0.3mg | 574 | 0.39 | 60.32 |

This shows 16.4% inhibition of Alpha Amylase by 0.3 mg/ml Voglibose.

***Experiment 2:*** Testing the inhibition of 30ml Alpha Amylase in 20ml buffer by 0.3mg/ml Voglibose which is shown in Table (5).

**Table 5: Testing the inhibition of 30ml Alpha Amylase in 20ml buffer by 0.3mg/ml Voglibose**

| Starch | Alpha amylase | Voglibose | Wavelength | Absorbance value | Percent |
|---|---|---|---|---|---|
| 1ml | - | - | 578 | 0.983 | 100 |
| 1ml | 1ml | - | 573 | 0.34 | 65.41 |
| 1ml | 1ml | 0.3mg | 577 | 0.487 | 50.45 |

This shows 15% inhibition of Alpha Amylase by 0.3mg/ml Voglibose

***Experiment 3:*** Testing the inhibition of 40ml Alpha Amylase in 10ml buffer by 0.3mg/ml Voglibose which is shown in Table (6)

**Table 6: Testing the inhibition of 40ml Alpha Amylase in 10ml buffer by 0.3mg/ml Voglibose**

| Starch | Alpha amylase | Voglibose | Wavelength | Absorbance value | Percent |
|---|---|---|---|---|---|
| 1ml | - | - | 578 | 0.983 | 100 |
| 1ml | 1ml | - | 570 | 0.409 | 58.39 |
| 1ml | 1ml | 0.3mg | 581 | 0.557 | 43.33 |

This shows 15.06% inhibition of Alpha Amylase by 0.3mg/ml Voglibose.

**Discussion:**

The experiments conducted shows that Voglibose inhibits both Alpha Amylase and Alpha Glucosidase. The literature mentions that Voglibose is an inhibitor of Alpha Glucosidase [14]. This experiment confirms that Voglibose is an inhibitor of Alpha Amylase also. Thus in future Alpha Amylase can also be chosen as an appropriate target Voglibose.

## 4. CONCLUSION

The present *In-silico* and *In-vitro* swot analysis indicates that like Alpha glucosidase even Alpha amylase binds to Voglibose. The interaction outcome suggests that in future one can consider Alpha amylase as a choice of target in applications of Biosensing.

**Reference to a journal**:

1.Ismail E S, deshmukh S A. Comparative study of effects of alpha Glucosidase Inhibitors-Miglitol, Acarbose and Voglibose on Postprandial Hyperglycemia and Glycosylated hemoglobin in type-2 Diabetes Mellitus. Int J Pharm Bio Sci 2012; 3(3): 337 – 343.

2. Lee M Y, Choi D S, Lee M K, Lee H W, Park T S, Kim DM, Chung CH, Kim DK, Kim IJ, Jang HC, Park YS, Kwon HS, Lee SH, Shin HK. Comparison of acarbose and voglibose in diabetes patients who are inadequately controlled with basal insulin treatment: randomized, parallel, open-label, active-controlled study. J Korean Med Sci 2014 ; 29(1) : 90-7.

3. JP XVI OFFICIAL MONOGRAPHS PAGE NUMBER 1567-1569.

4.Rao M, Bagyalakshmi J, Ravi T K. Development and validation of UV- Spectroscopic method for estimation of Voglibose in bulk and tablets. J. Chem. Pharm. Res 2010; 2(2) :350-356

5. Sai kishore V, Jitendrakumar P, Chakravarthy T K, Naik S V, Reddy M H, Babu E N. Development and validation of rp-hplc method for quantitative analysis voglibose in pure and pharmaceutical formulations. IJPCBS 2013; 3(2): 336-341

6. Daswadkar S C, Walode S G, Mahendra Kumar C B. Stability indicating rp-hplc method for estimation of voglibose in bulk and tablet dosage form. Pharmacophore 2013; 4(5): 158-165

7. Woo J S, Ryu J K. Quantitative determination of voglibose in pharmaceutical tablets using high-performance liquid chromatography-fluorescence detection with post-column derivatization and mass spectrometric detection. J Pharm Biomed Anal 2006; 42(3) : 328-33.

8.Shreedevi K, Andhe P. A review Paper on the Available Methods for the Analysis of an Anti-Diabetic Drug- “VOGLIBOSE”. Bull. Env. Pharmacol. Life Sci. 2016; 5 (2) January 2016: 89-92

9.IUPAC. Recommended Definitions and Classifications. Pure Applied Chem 71: 2333-2348.

10. Jose I. Reyes De C, Ralph P C. BIOSENSORS. Encyclopedia of Agricultural, Food, and Biological Engineering.2010; 2 2114

11. Verma M S, Chen P Z, Jones L, Gu F X. Controlling “chemical nose” biosensor characteristics by modulating gold nanoparticle shape and concentration. Sensing and Bio-Sensing Research 2015; 5: 13–18.

12. Holstein A, Beil W, Kovacs P. CYP2C metabolism of oral antidiabetic drugs--impact on pharmacokinetics, drug interactions and pharmacogenetic aspects. Informa healthcare. 2012 ; 8(12): 1549-63.

(13) Preissner S, Kuzman D, Pischon N. Drug Interactions Involving the Cytochrome P450 Enzymes: Analysis of Common Combinations of Antibiotics and Pain Relieving Drugs. J Drug Metab Toxicol 2012; 3:5.

(14) Martínez C, Albet C, J A Agúndez , Herrero E, J A Carrillo , Márquez M, Benítez J, Ortiz J A. Comparative in vitro and in vivo inhibition of cytochrome P450 CYP1A2, CYP2D6, and CYP3A by H2-receptor antagonists. Clinical Pharmacology and Therapeutics 1999 ; 65(4) :369-76.

15. DrugBank: Voglibose (DB04878) http://www.drugbank.ca/drugs/DB04878

16. Prasanth Kumar S, Kapopara R G, Patel S K, Patni M I, Jasrai Y T, Pandya H A, Rawal R M. Molecular Descriptor Enhancement of a Common Structure Towards the Development of α- Glucosidase and α-Amylase Inhibitors for Post-Prandial Hyperglycemia (PPHG). Asian Journal of Biomedical and Pharmaceutical Sciences 2011; 2: 01-12.

17. Xiao Z, Storms R, Tsang A. A quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. Analytical Biochemistry 2006; 351(1): 146–148.

18. Sim L, Quezada-Calvillo R, Sterchi EE, Nichols BL, Rose DR. Human intestinal maltase-glucoamylase: crystal structure of the N-terminal catalytic subunit and basis of inhibition and substrate specificity. J Mol Biol. 2008; 375(3):782-92.

19. Qin X, Ren L, Yang X, Bai F, Wang L, Geng P, Bai G, Shen Y. Structures of human pancreatic α-amylase in complex with acarviostatins: Implications for drug design against type II diabetes. J Struct Biol. 2011; 174(1):196-202.

20. ACD/ Chemsketch, version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada,www.acdlabs.com, 2014.

21. Casewit C J, Colwell K S, Rappe A K. J. Am. Chem. Soc., 1992; 114 : 10035–10046

22.Thompson M A, Zerner M C. A theoretical examination of the electronic structure and spectroscopy of the photosynthetic reaction center from Rhodopseudomonasviridis. J. Am. Chem. Soc. 1991; 113(22): 8210–8215.

23.Gupta P P, Sahu B. Identification of Natural Compound Inhibitors against PeptideDeformylase Using Virtual Screening and Molecular DockingTechniques. Bull. Env. Pharmacol. Life Sci., 2015; 4(9): 70-80.

24. Trott O, Olson A J. AutodockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry 2010; 31(2) :455-61

25. Morris G M, Goodsell D S, Halliday R S, Huey R, Hart W E, Belew R K, Olson A. Automated Docking Using a Lamarckian Genetic Algorithm and and Empirical Binding Free Energy Function. J.Computational Chemistry 1998; 19: 1639-1662