Study of Dipeptidil Peptidase 4 Inhibitors based on Molecular Docking Experiments

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Abstract: The lack of physical activity and poor nutrition triggers various diseases, among them is diabetes. In this context, several researches seek ways that can mitigate these diseases to provide a better quality of life for people. Therefore, the present work aims to analyze the possible inhibitors of the enzyme Dipeptidil Peptidase 4 that hypotheses will be stipulated for the creation of new drugs through molecular docking techniques, that is, a computational simulation of combinations of drugs of the family of gliptins with other antidiabetics (metformin, glyburide and cucurbitacin). Among the results, it was observed that the antidiabetic cucurbitacin combined with the gliptines obtained greater energy during the process.

1 INTRODUCTION

There is great concern about several diseases that have been genetically changing over time, among them is diabetes, one of the most studied in the scientific world. According to (Vignesh and Amalarethinam, 2017), in 2011 there were 347 million diabetics and by 2030 that number will be approximately 552 million people. Being that its main characteristic is the high level of glucose in the blood motivated as a result of defects in the action of the insulin produced by the pancreas. According to the author, insulin aims to allow the entry of glucose into the body's cells, from which it will be used for various cellular activities, however, the lack of insulin or malformation of its function in the human system leads to accumulation of glucose in the body. blood, thus triggering what is called hyperglycemia.

This disease is a worldwide concern, as more and more people, due to their sedentary habits, poor diet and the growing number of obese, end up acquiring it (Nachabe et al., 2017). As a result, there are several studies in the literature that seek to elucidate some solutions to mitigate the damages caused by diabetes.

The pharmaceutical industry perfects efficient methods for creating new medicines. In this context there are molecular docking techniques that perform computational simulations to better predict the adjustment orientation of a ligand to a receptor. The ligand are molecules produced by cells that interact as a puzzle with their receptor as Figure 1. Already the receptor is the target protein in which it is desired to perform the interaction between the parts to verify compatibility information.

With the docking, one can characterize the behavior of small molecules in connection with the target proteins. Therefore, when using this virtual tech-

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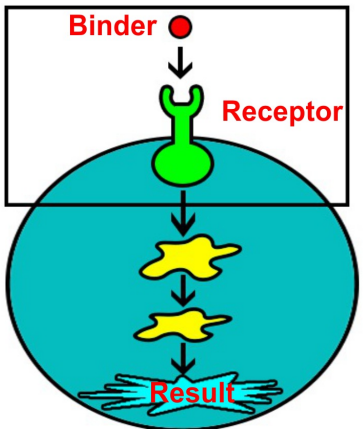

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Figure 1: Illustration of the Binder with its Receptor Molecule.

nique, it will be possible to propose structural hypotheses of how the ligands are connected to their targets.

In this context, the present work aims to use computational simulation in different substances in order to present solutions that may soften the causes of diabetes, that is, the purpose of this research is look for the relation of gliptine families to then create hypotheses of new drugs that will be combined with other antidiabetics. With the study and results presented in this research, hypotheses can be formulated and tested in future lab work.

The target protein of this work is dipeptidyl peptidase 4 (DPP4) whose enzyme is directly bound to type 2 diabetes mellitus because it acts directly on the degradation of incretins (Richter et al., 2008). Incretin works in the body regulating glucose levels, acting on the pancreatic secretion of insulin. According to studies presented throughout this work, the inhibition of DPP4 enzyme may have the function of regulating glucose control in the body.

An approach used for studies on diabetes mellitus is related to dipeptidyl peptidase 4 inhibitors (Richter et al., 2008). In her study, she presented analyzes of the use of two drugs that are already studied in the treatment of type 2 diabetes mellitus, Sitagliptin and Vildagliptin.

Both are drugs that are part of the gliptins family. Gliptins is a class of oral hypoglycemic agents used in the treatment of type 2 diabetes mellitus. Five different types of gliptins (Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin and Alogliptin) have been marketed (Biftu et al., 2014), and in this work four of them will be approached Sitagliptin, Vildagliptin, Saxagliptin and Linagliptin.

Section 2 will perform a dosing of DPP4 inhibitors and antidiabetic agents used in this work and section 3 will explain the molecular docking technique. In section 5, we present the molecular docking simulations of the drugs of the family gliptins together with the DPP4 protein, and later the affinity table obtained and the joining of three molecules individually with each result will be displayed. However, we tried to present the best results through the affinity table between the drugs and the molecules selected for the study, then we will point out a hypothesis to be developed in the future by the drugs.

2 DIPEPTIDYL PEPTIDASE 4 INHIBITORS

DPP4 is an enzyme that degrades incretin, a substance responsible for regulating glucose metabolism (Johari et al., 2011). In this section, the relationship between the Gliptines family and the DPP4 enzyme will be elucidated, thus performing a molecular docking, showing the characteristics of each drug and exemplifying its role as a DPP4 inhibitor.

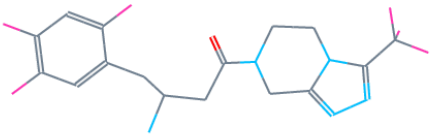
There are studies that show the efficiency of gliptins as the work of (Davidson et al., 2008), who presented new therapeutic treatments to treat type 2 diabetes. Or as in the work of (Nauck et al., 2017) in which it conducts a study on the addition of a DPP4 inhibitor, Sitagliptin, to explore whether the addition of this drug to pre-existing liraglutide therapy alters glycemic levels after a meal. In the study by (Berger et al., 2018) comparisons were made between DPP4 inhibitors for the treatment of type 2 diabetes. Animal experiments aimed at finding a direct relationship between enzyme inhibition in plasma and glucose reduction were done.

2.1 Sitagliptin

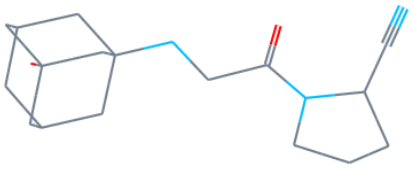
Sitagliptin, as exemplified in Figure 2, is one of the inhibitors of DPP4 because it is blocked by this drug, thus causing the elimination of insulin hormone when blood sugar levels decrease (Rosenstock et al., 2006). Sitagliptin Phosphate and Hydrochloride act as supporters of physical activity and dietary practices in order to control the glycemia of patients with type 2 diabetes mellitus, benefiting people who are sensitive to high insulin levels.

2.2 Vildagliptin

Vidagliptin is a drug that acts on the alpha and beta cells of the pancreas and just like Sitagliptin prevents the proliferation of DPP4. According to (Berger et al.,



Source:PubChem. (PubChem, 2005)
Figure 2: Sitagliptin.

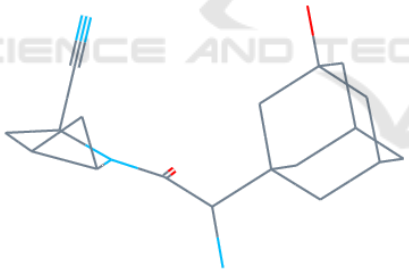


Source:PubChem. (PubChem, 2005)
Figure 3: Vildagliptin.

2018) to Vidaglipline shown in Figure 3, by inhibiting DPP4, incretin gradually increases its efficacy.

2.3 Saxagliptin

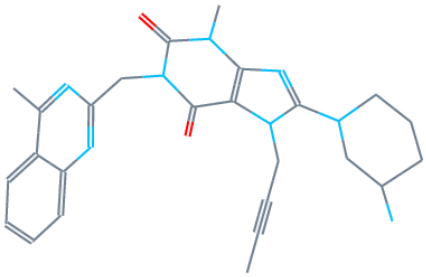
Saxagliptin shown in Figure 4 aims to control blood sugar after meals as well as between meals. This drug also helps increase insulin in the body, thereby attenuating the production of sugar by the liver after meals.



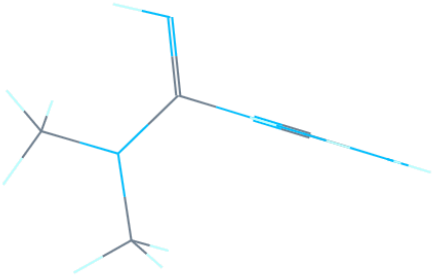
Source:PubChem. (PubChem, 2005)
Figure 4: Saxagliptin.

2.4 Linagliptin

Linagliptin also inhibits the enzyme DPP4, since it prevents the breakdown of incretins, GLP-1 and GIP, hormones that help the pancreas to produce insulin when a high level of glucose in the blood is detected, and Linagliptin also reduces another hormone called Glicacon, this hormone produced by the pancreas that increases blood glucose. When DPP4 inhibits the drug Linagliptin, GLP-1, responsible for releasing insulin according to the needs of the body, acts longer. Figure 5 shows how this drug is structured.



Source:PubChem. (PubChem, 2005)
Figure 5: Linagliptin.



Source:PubChem. (PubChem, 2005)
Figure 6: Metformin.

3 ANTIDIABETIC AGENTS

The drugs of the gliptins families are strong candidates for DPP4 inhibitors, thus leading to improvements in patients with diabetes. Therefore, when making combinations with these medicines can obtain possible positive results for treatments. One of these drugs, in which there are already studies of combinations, is Metformin, in which it is already used in several studies and in this research will play the role of parameter for the tests performed that will be compared with the following substances: glyburide and cucurbitacin.

The glyburide substance is very viable to make combinations with the gliptins. In the studies of (Marbury et al., 1999) the efficacy of this drug in the fight against diabetes is already evident.

Finally, cucurbitacin, a substance extracted from plants of the Cucurbitacese family, in which it has several oxidants, was used. This substance has high hyperglycemic power as evidence the work of (Alarcon-Aguilar et al., 2002). Their study explored the hyperglycemic effect of cucurbitacease.

3.1 Metformin

Metformin is a suitable substance to act as an initial therapy in patients with type 2 diabetes mellitus. This

drug helps improve glycemic control by enabling insulin sensitivity and decreasing intestinal absorption of glucose. This drug is structured according to Figure 6.

3.1.1 Glyburide

It acts as a strong antihyperglycemic agent that can be used in the treatment of non-insulin dependent diabetes mellitus. This substance when used in conjunction with adequate diets and physical exercises can help to lower blood sugar levels, thus avoiding various types of problems caused by increased glucose.

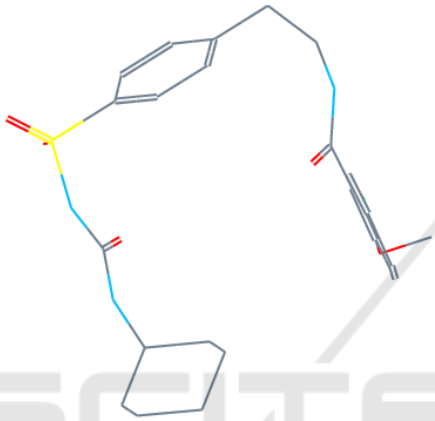


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Figure 7: Glyburide.

3.2 Cucurbitacin

Cucurbitacin is a highly oxygenated triterpene substance found free or glycosylated, extracted from plants of the family Cucurbitaceae such as pumpkins, cucumbers and gourds. This substance has aroused the interest of several researchers to present high levels of toxicity acting as an antitumor, antiinflammatory, antifertilizer, phage repellent among others.

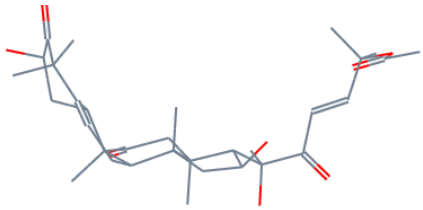

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Figure 8: Cucurbitacin.

4 MOLECULAR DOCKING

According to (Ferreira et al., 2015), molecular docking is a versatile computational technique for the study of biological macromolecules, this technique studies the production of drugs based on molecular structures where they are simulated through numerical interactions by algorithms. The main objective of this technique is the matching of these molecules for identification and characterization of the binding sites in the target proteins, generating a table of evaluation of the interaction potential as shown in Figure 9.

```
mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -9.3      0.000      0.000
   2         -9.1    120.847    124.985
   3         -9.0    121.206    124.330
   4         -9.0      2.154      3.997
   5         -8.9     76.201     80.717
   6         -8.9     53.326     56.975
   7         -8.9     52.867     56.820
   8         -8.7      0.821      2.025
   9         -8.7     53.310     56.939
Writing output ... done.
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Figure 9: Example of Affinity Result between Binder and Receptor.

According to (Ferreira et al., 2015), The software associates two main components: search algorithm and score function, in which the algorithm is responsible for the search for possible combinations in the connections and the score demonstrates the best binding results obtained during the procedure. The algorithms allow the exploration of several angles, both rotational and translational and conformational of the ligand in the target protein.

In Figure 9, a result table after the molecular dock in the autodock vina software is exemplified. This table shows the nine best binding results from the linker to the receiver, where the first column shows the sequence of the numbered results and the second column shows the binding affinity in kcal / mol, representing the highest energy. In the next columns, two variants of RMSD metrics are provided: rmsd / lb (lower limit of RMSD) and rmsd / ub (upper limit of RMSD). The rmsd / ub combines each atom in a conformation with itself in the other conformation, ignoring any symmetry, since rmsd / lb is defined as follows: rmsd / lb (c1, c2) = max (rmsd '(c1, c2) , rmsd '(c2, c1)).

5 METHODOLOGY OF WORK

In the simulations developed, the following configurations were used: core i7 fourth generation, 12 GB ram, nvidia p6000 video card, HD Sdd 240 gb.

An important step for the accomplishment of the experiment is the obtaining of the biological macromolecules in the format supported by the software that will be used in the work. There are several databases that provide the chemical compounds, these same compounds are converted into tri-dimensional molecular structures and made available in databases such as PubChem, ChemSPider, Zinc, RCSB.org among others.

The molecules presented as ligands in this work were extracted from Pub-Chem, a database with a large diversity of molecules maintained by the National Center for Biotechnology Information. The DPP4 protein was obtained from the work of (Hiramatsu et al., 2003) in which it is made available in the RCSB database PDB.

In order to perform the process and visualization of the molecular docking results, we used the autodock vina software (to perform the docking experiments and the simulation of the best fit between the binder and the protein), Mgltools (conversion of molecules formats) and oPyMol (for visualization of results).

6 RESULTS

The first part of the experiment consists of carrying out molecular docking simulations using first the gliptins (Sitagliptin, Vildagliptin, Saxagliptin and Linagliptin) molecules presented in section 2 together with the dipeptidyl peptidase 4 target protein. the combination with other antidiabetics and then observe the behavior of the other molecules.

All the connection models obtained are divided into files for multimodal visualization in three-dimensional format. For the analysis of the experiments we need a function to predict the binding affinity, there are several computational ways to obtain them with different sofwares, each based on their calculations.

For the experiments we used the autodock vina where from the obtained results we will explore the affinity (kcal / mol). In this software the results that released more energy (represented by the results of smaller value) are the best binder fittings in the receiver. (Shityakov and Förster, 2014) explains that the lower the value presented in the autodock vina simulations, the more significant it will present to the connection found, where the values for the affinity in the molecular docking process are favorable only when they are represented negatively, that is, the more negative the value obtained , the better the interaction.

Table 1 shows the best binding affinity results obtained in the docking process. It was observed that the affinity of Linagliptin with DPP4 generated the highest energy during the process, releasing a value of -9.3 kcal / mol, higher than the others, which obtained values between -7.6 to -7.9, both being drugs used as inhibitors of DPP4.

Table 1: Gliptine affinity table with DPP4.

	Ligante	Receptor	Aff
(a)	Sitagliptin	DPP4	-7.6
(b)	Vildagliptin	DPP4	-7.3
(c)	Saxagliptin	DPP4	-7.9
(d)	Linagliptin	DPP4	-9.3

Aff = Affinity(kcal/mol).

In Figure 10, we find the simulation results in the three-dimensional view, in which the DPP4 enzyme is represented in green color and the others (drugs) are highlighted in a red circle. This image represents the best allocation of gliptins in the molecule.

6.1 Mixing Gliptines with Metformin

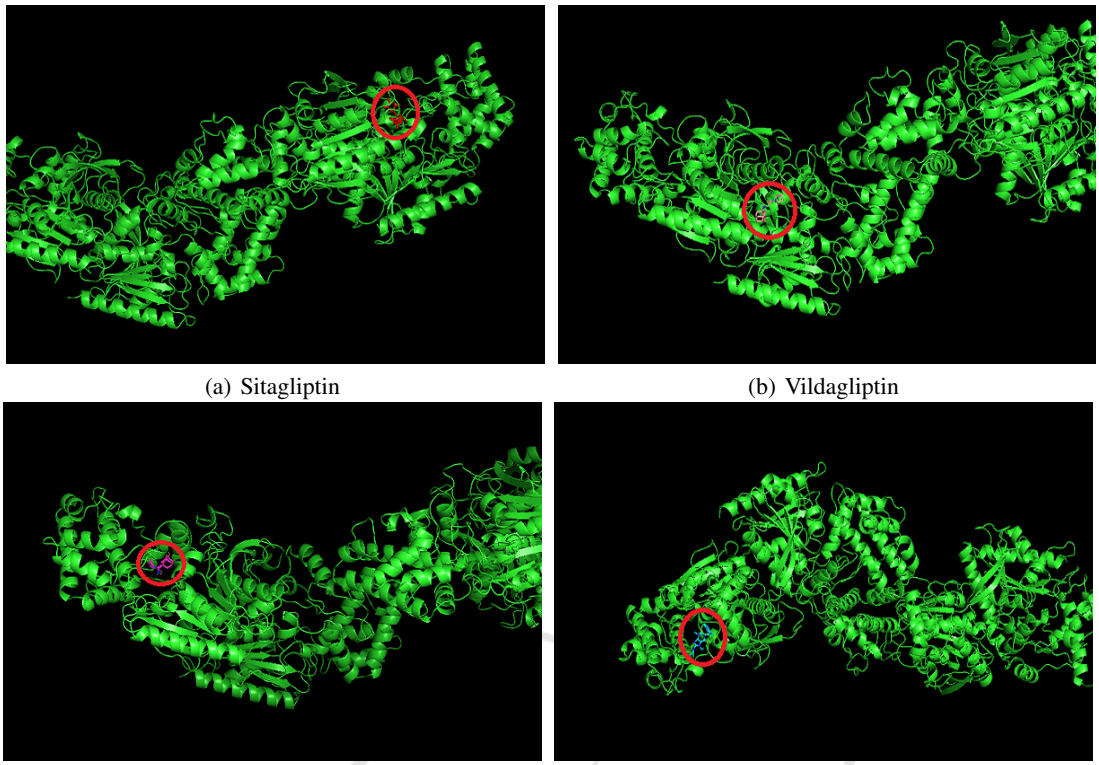
The following sections aim to explore the results obtained from the combinations of the gliptins presented in table 1 with the subtances previously presented, aiming at the behavior (affinity in kcal / mol) of antidiabetic agents in docking with DPP4. The first is Metformin, which according to the study by (Davidson et al., 2008) is a good candidate to effectively inhibit DPP4. In table 2 it was observed that all combinations obtained the same affinity values with DPP4.

Table 2: Gliptines affinity table with Metformin.

	Ligantes	Receptor	Aff)
(a)	Sitagliptin + Metformin	DPP4	-5.4
(b)	Vildagliptin + Metformin	DPP4	-5.4
(c)	Saxagliltina + Metformin	DPP4	-5.4
(d)	Linagliptin + Metformin	DPP4	-5.4

Aff = Affinity(kcal/mol).

In the three-dimensional simulations presented in Figure 11, it was observed that the ideal fit of Metformin is very close to the gliptins presented previously, however, they generate less energy in this process, but due to their increasing research advances this combination can be very useful for treatments of diabetes.



(a) Sitagliptin (b) Vildagliptin

(c) Saxagliltina (d) Linagliptin

Figure 10: Affinity Result of Gliptines with DPP4.

6.2 Gliptines Mixture with Glyburide

The next antidiabetic combined with glipins was glyburide. It is observed in Table 3 that the combination Vildagliptin + glyburide was the one that generated the highest energy, standing out from the others, thus showing, that this combination has a greater affinity with DPP4 than the others.

Table 3: Gliptines affinity table with Glyburide.

	Ligantes	Receptor	Aff)
(a)	Sitagliptin + *Glyburide*	DPP4	-6.6
(b)	Vildagliptin + *Glyburide*	DPP4	-8.6
(c)	Saxagliltina + *Glyburide*	DPP4	-6.1
(d)	Linagliptin + *Glyburide*	DPP4	-6.8

Aff = Affinity(kcal/mol).

6.3 Mixture of Gliptin with Cucurbitacin

The last experiment was carried out with Cucurbitacin, a substance extracted from pumpkin plants, gourds and cucumbers, its vegetables are usually consumed in the day to day, which does not diminish its importance in this study. The combinations of cucurbitacin with the selected glipitins generated affinity values between -8.0 and -8.7 as shown in table 4. Among the antidiabetics selected for the research, this was the one that provided the highest gerander energy in combination with the glipins at the DPP4 receptor, showing high affinity in this combination.

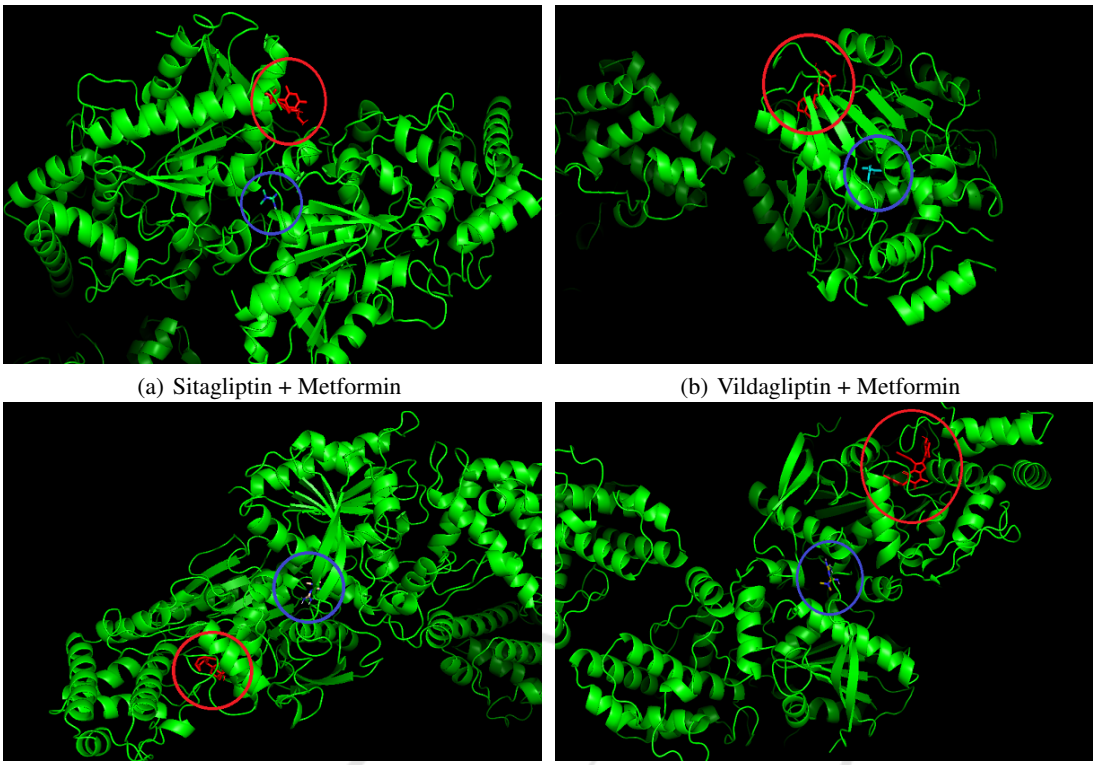
Table 4: Affinity Table of Glucines with Cucurbitacin.

	Ligantes	Receptor	Aff
(a)	Sitagliptin + *Cucurbitacin*	DPP4	-8.0
(b)	Vildagliptin + *Cucurbitacin*	DPP4	-8.3
(c)	Saxagliltina +*Cucurbitacin*	DPP4	-8.1
(d)	Linagliptin + *Cucurbitacin*	DPP4	-8.7

Aff = Affinity(kcal/mol).

7 CONCLUSIONS

With the obtained results, it could be observed that all the co-creations used had affinity with the DPP4 enzyme, thus resulting in options that can be used in clinical laboratory studies to create an effective antidiabetic. However, some combinations stood out more than others regarding the energy release during the



(a) Sitagliptin + Metformin (b) Vildagliptin + Metformin

(c) Saxagliltina + Metformin (d) Linagliptin + Metformin

Figure 11: Affinity Result of Gliptines with Metformin.

docking process, thus creating better hypotheses for future laboratory tests.

In table 5, the three best results were highlighted based on the combinations that released more energy. It may be noted that among the most significant results two are combinations made with cucurbitacin.

Table 5: Best Results Based on Energy Release.

Ligantes	Receptor	Aff
Vildagliptin + *Glyburide*	DPP4	-8.6
Vildagliptin +*Cucurbitacin*	DPP4	-8.3
Linagliptin + *Cucurbitacin*	DPP4	-8.7

Aff = Affinity(kcal/mol).

The combinations of gliptines with cucurtbitacin were those that obtained less oscillations and, consequently, better results, being in a scale of energy between 8.0 to 8.7. Already the combinations with Glyburide obtained scale of -6.1 to -8.6, where its combination with Vildagliptin had the best result. Finally, Metformin was the one that achieved the worst energy release indices, in which all combinations were -5.4.

In this work, new hypotheses of efficient combination for the creation of drugs that act as antidiabetics, that is, substances that can inhibit the effects of type 2 diabetes, which affects a large part of the world population, have been presented through the molecular docking technique. But for studies like these to meet the needs of the population it is crucial that these researches go hand in hand with laboratory tests to thus prove the effectiveness of the simulations.

In this work, we hypothesized new drugs for the creation of efficient inhibitors of the DPP4 protein, thus being able to carry out these experiments in laboratories and to verify efficacy. Also look for controversial effects that can negatively affect these mixtures in humans.

Search for other combinations of other antidiabetic agents and perform tests for future comparisons with these drugs.

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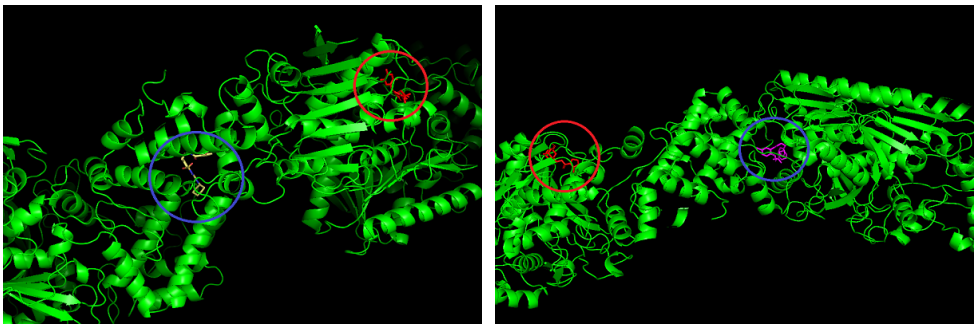
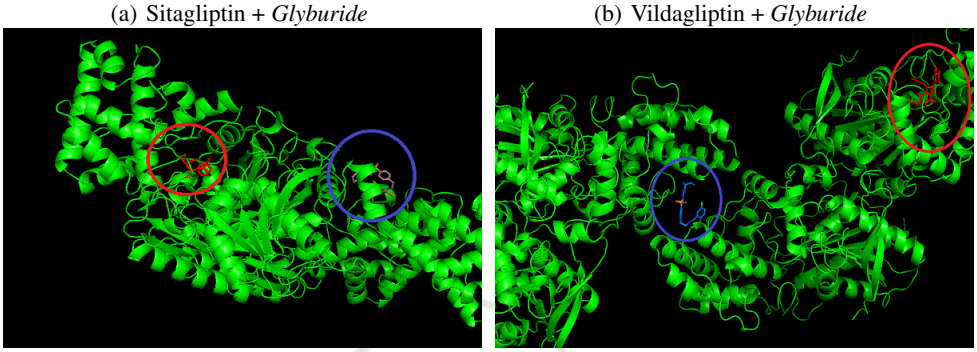
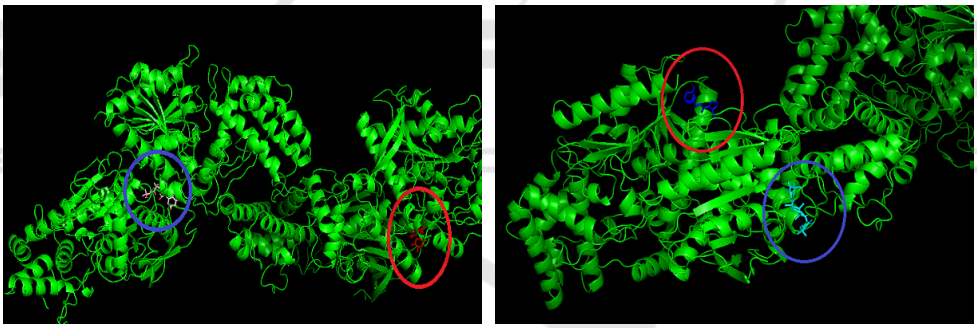


(a) Sitagliptin + *Glyburide* (b) Vildagliptin + *Glyburide*

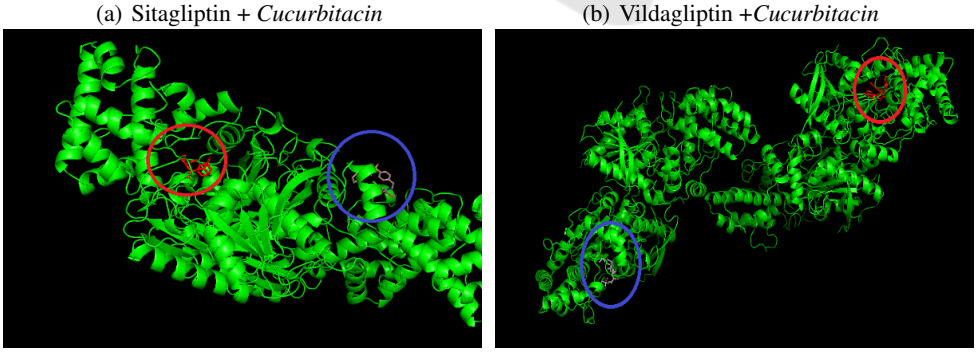


(c) Saxagliltina + *Glyburide* (d) Linagliptin + *Glyburide*

Figure 12: Affinity Result of Gliptines with Glyburide.



(a) Sitagliptin + *Cucurbitacin* (b) Vildagliptin +*Cucurbitacin*



(c) Saxagliltin + *Cucurbitacin* (d) Linagliptin + *Cucurbitacin*

Figure 13: Affinity Result Glucines with Cucurbitacin.

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