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Research Article

Postprandial Blood Glucose Lowering Properties Of Pristriol Isolated From Symphonia Globulifera And Its Binding Interactions With Carbohydrate Hydrolysis Enzymes

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ABSTRACT

The alarming figures for diabetics in the world have accentuated the search for new drugs that can help manage and reverse the incidence for the well-being of all. In this article, we present the effects of pristriol in modulating postprandial glycaemia and its physicochemical parameters in silico. The antihyperglycemic effect was obtained following an overload of starch on the one hand and sucrose on the other hand in normoglycemic rats. Pristriol was tested at a dose of 3 mg/kg.Bw, as was Acarbose. Blood sugar levels were measured at 0, 30, 90 and 150 min. subsequently the glycosylation test were carried out in vitro. Conformational analysis of the site and docking parameters such as binding energy, inhibition constant, interaction profiles with diabetes target residues (α -amylase and invertase) were determined using AutoDock 4.2 and Discovery Studio viewer. Its physicochemical parameters were carried out using the server SwissADME. The results showed that pristriol considerably reduced the glyceamic peak by 62.91% and 81.98% respectively in rats after overloading starch solutions on the one hand and sucrose on the other hand. However, it forms direct bonds with glucose with a binding rate of 87% at a concentration of 500 μ g/mL. Analyzes of molecular interactions have shown that this compound has good affinities with α -amylase (- 9.36 kcal/mol) and invertase (- 8.8 kcal/mol). This interacts with Asp 197 of

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α -amylase through a carbon hydrogen bond. The analysis of the physicochemical parameters shows that it can be valued orally because it has a score of 4/5 ($MW < 500$, $NRB \leq 15$, $HBD \leq 5$ and $HBA \leq 10$) on the Lipinski rules. All its effects of pristirol lead to modulate postprandial glycaemia and may be a starting point for the development of an anti-hyperglycemic compound capable of stabilizing post-meal glycaemia in diabetic patients.

INTRODUCTION

Diabetes is a major global health challenge, affecting over 537 million people worldwide (Ong et al., 2023). Diabetes is associated with several complications include retinopathy causing blindness, nephropathy, neuropathy, and cardiovascular diseases (CVD). Therefore, effective management of diabetes is vital to prevent or delay the onset and progression of diabetes-related health issues (Ansari et al., 2023). One of the main therapeutic strategy is to control postprandial plasma glucose (PPG). PPG is influenced by multiple factors, including the amount and type of dietary carbohydrates consumed, the rate of gastric emptying, the activity of carbohydrate-digesting enzymes like α -amylase and invertase, glucose absorption in the intestine, secretion and action of insulin and glucagon, and glucose clearance (Barber et al., 2022). Strict regulation of PPG levels through modulation of these various factors is important to improve glycemic control and reduce the risk of diabetic complications. However, available antidiabetic medications are still associated with unwanted side effects (DeMarsilis et al., 2022). There is a continuing need to discover and develop safer and more effective antidiabetic agents. For this purpose, plant compounds are ideal candidates because of their scaffolding role (Ambamba Akamba et al., 2023). Among the plants we find *Symphonia globulifera* which is used in traditional medicine to treat various conditions such as malaria, skin diseases, coughs, intestinal worms, pre-hepatic jaundice, fever and diabetes (Téné et al., 2021; Fromentin et al., 2015). Among the

compounds recently isolated from *S. globulifera*, pristirol is a xanthone derivative that has been reported to have potent antioxidant activities (Téné et al., 2021). However, the effect of pristirol on PPG levels and its interaction with other carbohydrate hydrolysis enzymes, such as α -amylase and invertase, have not been investigated. Therefore, the aim of this study was to evaluate the postprandial blood glucose lowering properties of pristirol isolated from *S. globulifera* and its binding interactions with carbohydrate hydrolysis enzymes. We hypothesized that pristirol would reduce PPG levels by inhibiting the activity of α -amylase and invertase, and that this effect would be mediated by specific molecular interactions between pristirol and the active sites of these enzymes. To test this hypothesis, we performed the following experiments:

1. we assessed the antihyperglycemic effect of pristirol in normoglycemic rats after oral administration of starch and sucrose solutions;
2. we assessed the Pristirol's ability to reduce bioavailability of glucose through glucose binding capacity assay;
3. we performed molecular docking simulations to analyze the binding modes and interactions of pristirol with α -amylase and invertase; and
4. we evaluated the physicochemical parameters of pristirol using in silico tools.

MATERIAL AND METHODS

In vivo study

Glycaemic modulating effect of pristirol on rats Animals and food

The potential effects of Pristirol against sucrose and starch overload were carried out respectively with twenty (20) male rats of the Wistar strain. They were provided by the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Department of Biochemistry, Faculty of Sciences, University of Yaoundé I. The animals were kept at room temperature ($25 \pm 1^\circ\text{C}$), subjected to a day-night cycle of 12h (7h-19h day and 19h-7h night)



in good conditions of ventilation and natural lighting. They had ad libitum access to running tap water and a conventional diet for breeding (normal diet, ND) rodents. None of these animals was subjected to previous experiments and showed signs of abnormalities. The animals were introduced in standard cages with the respect of the conditions of hygiene and ethics for animals, with the guidelines of the ethics committee of the University of Yaoundé 1 and the Guide for the care and use of laboratory animals (8th edition).

Evaluation of the effect of Pristriol on starch overload in normoglycemic rats

One day before handling, the rats were fasted for a period of 12 h. At the beginning of the experiment, the rats were weighed using an electronic scale. Blood glucose level was assessed using glucose test strips and a glucometer (OneTouch Ultra-Easy) by collecting blood from the caudal tail using a sterile lancet. Subsequently, the rats were divided into 4 groups (five rats each) depending on the average blood glucose level (mg/dL) as follows

Negative control (NC) group:

receiving water

Positive control group (PC):

receiving water + starch solution at dose of 2 g/kg.Bw

Reference group (Ref):

receiving Ascarbose (3 mg/kg.Bw) + starch solution at dose of 2 g/kg.Bw

Assay group:

receiving Taraxerol (3 mg/kg.Bw) + starch solution at dose of 2 g/kg.Bw

At the initial time (t_0), the negative control (NC) and positive control (PC) groups received water by esophageal gavage while the reference and test groups received respectively Ascarbose, and Pristriol solutions. Thirty minutes after administration of these different solutions, the glucose overload solution was administered to all groups except the NC group; the blood glucose

levels were measured at 30, 90 and 150 min respectively and the results recorded.

Evaluation effect of pristriol on sucrose overload in normoglycemic rats

The effect of pristriol on sucrose overload in normoglycemic rats was carried out by evaluating the hydrolysis of the latter in rats. The protocol used above for the effect on starch with slight modifications. These modifications were the replacement of starch with sucrose at a dose of 2 g/kg.Bw. Acarbose at a dose of 3 mg/kg.Bw was used as the reference drug. As in the above protocol, rats received water (NC and PC), acarbose (reference) and test at a dose of 3 mg/kg.Bw. After 30 minutes, gelatinized starch solution (2 g/kg.Bw) was administered to all groups except the NC group. Subsequently (30 min later), blood glucose levels were measured 90, 150 and 240 min using impregnated strips and a OneTouch Ultra brand blood glucometer by pricking the rat's tail and the results were noted.

In vitro study

Evaluation of potential glucose binding effect of pristriol

The ability of pristriol to complex with free glucose was determined using the method described by Ou et al., (2001). Experimentally, 25 μ L of pristriol at different concentrations (100, 200, 300, 400 and 500 μ g/mL) and 25 μ L of glucose solution (25 mM) were introduced respectively into a 96-well microplate (Sigma Aldrich). The mixture was shaken then incubated in a water bath at 37 °C for 1 hour. A control consisting of glucose and distilled water was also prepared. Then, the remaining glucose was measured using the glucose reagent according to the method of Trinder, (1969). The absorbance of the solutions from each well were read at 505 nm and the rates of glucose reductions in solution were calculated as follows:



$$\text{Remaining glucose content} = \left(\frac{[\text{Glucose content}]_{\text{Control}} - [\text{Glucose content}]_{\text{Test}}}{[\text{Glucose content}]_{\text{Control}}} \right) * 100$$

In silico study

Preparation of the target enzyme

The crystal structures of α -amylase and invertase proteins were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Database (<http://www.rcsb.org/pdb/home/home.do>). Once downloaded in pdb format, ions, water molecules, co-crystallized ligands and other unnecessary molecules were removed from these proteins using AutoDock 4.2.7 docking software. The resulting structures in pdb format were submitted to a modeller where a series of python code provided the missing residues and affinity the loops generated in order to minimize the proteins' own energies. Three-dimensional affinity grids (which

ferment the active site amino acids of each protein) sized with a spacing of 0.375 Å were centered on the geometric center of the proteins (Trott and Olson, 2010).

Preparation of the ligand

The two-dimensional (2D) structures of Pristriol and acarbose were drawn with ChemDraw 2D software (Figure 1). Once the three-dimensional (3D) structures were formed with the help of ChemDraw 3D software, Gasteiger-type partial charges and rotatable bonds were assigned to the ligand by fusion of non-polar hydrogen atoms. Energy minimization was performed using the Molecular Mechanics (MM2) force field and saved in PDB format using the same software (Solis and Wets, 1981).'

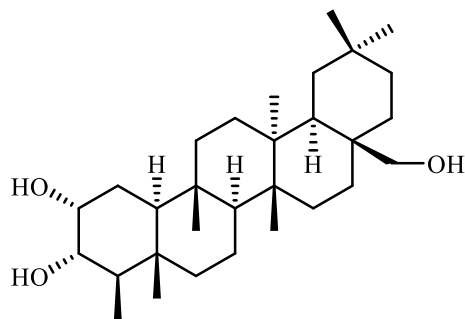


Figure 1: Structure of Pristriol

Docking protein-ligand

The prediction of the binding affinities of α -amylase and invertase towards pristriol was carried out following commonly used molecular docking protocols as described by the parameters of the AutoDock 4.2.7 software (<https://www.ccdc.cam.ac.uk/>). Technically, after recruiting the pdb files in the software's graphical interface, polar hydrogens and Kollman charges were assigned in order to represent the partial distributions of positive or negative charges. These were derived from the corresponding electrostatic potential using ab initio quantum mechanical calculations. The structure of Pristriol and the resulting protein were saved as PDBQT files

containing atom coordinates, partial charges, and atom types. The software exploiting a semi-flexible type docking approach in which the ligand has rotatable bonds a set of coordinates delimited by a grid box were represented by referring to the amino acid residues involved in the catalytic mechanism (see table 1). The Lamarckian genetic algorithm with 1000 runs was used to predict the ligand binding poses. The Python codes Prepare_gp4.py and Prepare_dp4.py were used to generate the grid parameter files (gpf) and anchor parameter files (dpf) (Morris et al., 1998). Finally, after analysis of the orders, the conformation with the lowest binding energy among the 25 best simulations obtained were exploited.

Table 1: Grid centre coordinates

Protein	α-amylase	Invertase
Grid point (X, Y, Z)	40 × 40 × 44	48 × 48 × 40
Grid center dimension (x, y, z)	8.22, 47.41 and 19.66	18.917, -5.028 and 20.750

Physicochemical properties of Pristriol

With the aim of determining the acceptability properties of a drug candidate, Pristriol was subjected to a theoretical in silico using the SwissADME web tool (<http://www.sib.swiss>). To predict the appropriate properties, the 2D structural model of the latter was drawn in SDF format and transferred to a simplified Online Molecular Input System (SMILES) format. The SwissADME server tool was used to measure the physicochemical properties of the compounds such as molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds, and lipophilicity (ALogP). The drug efficacy of Pristriol was examined using Lipinski's rules based on physicochemical properties were also examined (Lipinski et al., 2001).

Statistical analysis

The results of the in vivo and in vitro studies were performed in triplicates and the data were expressed as mean $\pm$ SE. One-way analysis of variance (ANOVA) was performed for statistical analysis of the data using Statistica version 20.0 software. Results with a p value < 0.05 were

considered significant. Using Autodock version 4.2.7 and the BIOVIA Discovery studio viewer, the docking calculations were carried out and the differences obtained were used.

RESULTS

Effect of Pristriol on blood glucose level after starch overload in normoglycemic rats

The evolution of blood sugar after administration of a starch overload is shown in Figure 2. The administration of a starch overload led to a significant ($p < 0.05$) increase in blood glucose levels (glycemic peak) in all groups except the negative control (NC) group from 0 to the 30th minute. However, compared to positive control (PC) group, Pristriol significantly ($p < 0.05$) (62.91%) prevented the establishment of this glycemic peak twice, with lower blood glucose levels at 30 min. Subsequently, a progressive but more pronounced significant decrease of glucose levels with the pristriol-treated group was observed in the groups tested up to 150 minutes, approaching levels close to normal control rats. Acarbose also significantly decrease glucose levels after starch overload, but the reduction was less pronounced than with pristriol.

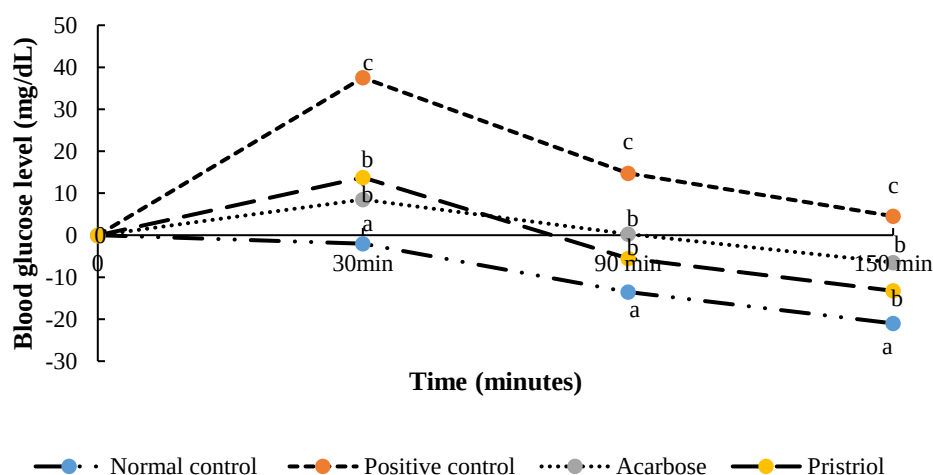


Figure 2: Effect of Pristriol on blood glucose level variation after administration of starch overload in rats

The groups assigned different letters (a,b,c and d) in a t are significantly different at $p < 0.05$. Negative Control (NC): Distilled water, Positive Control (PC): Starch (2 g/kg.Bw) + distilled water, Acarbose: Starch (2 g/kg.Bw) + Acarbose (3 mg/kg.Bw), Pristriol: Starch (2 g/kg.Bw) + Pristriol (3 mg/kg.Bw).

Effect of Pristriol on blood glucose level after sucrose overload in normoglycemic rats

The evolution of blood glucose after administration of a sucrose overload is presented in Figure 3. The administration of a sucrose

overload led to a significant glycemic peak ($p < 0.05$) in all groups except the negative control (NC) group from 0 to the 30th minute. However, compared to the positive control (PC), we found that Pristriol significantly ($p < 0.05$) or 81.98% prevented the establishment of this glycemic peak. After 90 minutes of treatment, blood glucose level in the Pristriol-treated group was normalized until the 150th minute. Acarbose also lowered glucose, but the decline was more uniform, suggesting a different inhibitory mechanism than pristirol.

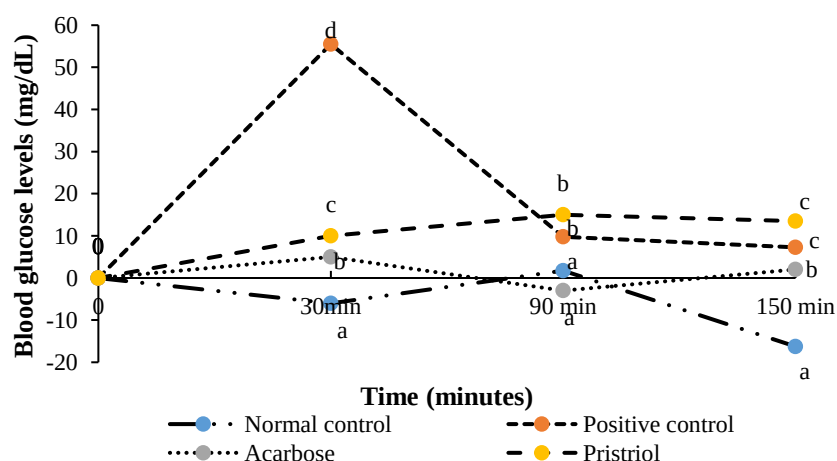


Figure 3: Effect of Pristriol on blood glucose variation after administration of sucrose overload in rats

the groups assigned different letters (a,b,c and d) in a t are significantly different at $p < 0.05$. Negative Control (NC): Distilled water, Positive Control (PC): sucrose (2 g/kg.Bw) + distilled water, Acarbose: sucrose (2 g/kg.Bw) + Acarbose (3

mg/kg.Bw), Pristriol: sucrose (2 g/kg.Bw) + Pristriol (3 mg/kg.Bw).

Potential glucose binding effect of pristirol

The glucose binding capacity of Pristriol was evaluated by glucose adsorption capacity and is

shown in Figure (4). The compound bound glucose efficiently and the glucose binding capacity was directly proportional to the concentration of the

compound. Glucose binding percentages are between 9 and 87%.

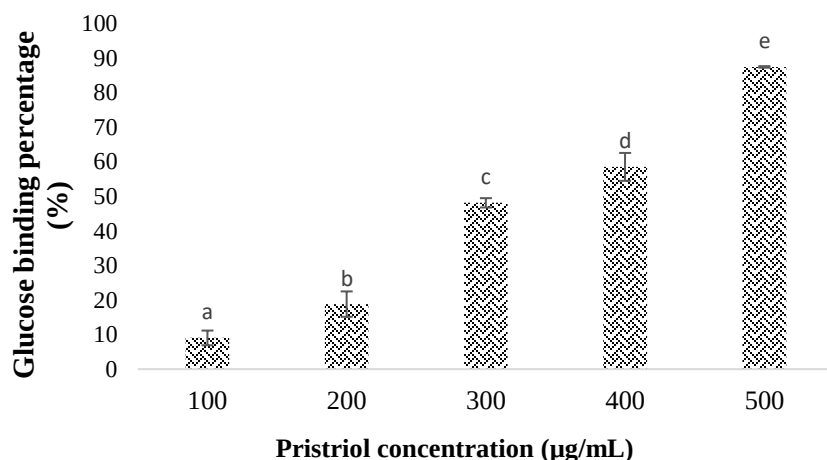


Figure 4: Glucose binding capacity of Pristriol

Numbers assigned different letters (a, b, c, d and e) are significantly different at $p < 0.05$

Lipinski rules parameters

The predicted physicochemical parameters of Pristriol are presented in Table 2. Lipinski's rule of five represents a set of parameters that an oral drug candidate must meet. According to Lipinski's rule

of five, a drug candidate is likely to be produced as a potential oral drug if the applicant violates none or less one of these five conditions. Pristriol violated only one of its parameters (octanol-water partition coefficient (Clog P) and therefore should be used orally.

Table 2: Predicted physicochemical parameters of Pristriol

Composés	Règle de Lipinski					Score
	MW < 500	NRB ≤ 15	HBD ≤ 5	HBA ≤ 10	LogP ≤ 5	
Pristriol	460.39	1	3	3	5.18	4

MW: Molecular Weight; NRB: Number of Rotatable Bound; HBD: Hydrogen Bound Donor; HBA: Hydrogen Bound Acceptor; Log P: Solubility

3.3. Molecular docking result of Pristriol against interest enzyme

3.3.1. Docking score of Pristriol against α -amylase and invertase

The docking scores of Pristriol and acarbose are shown in Table 3. According to Autodock's parameterization elements, the binding energy

(ΔG) between a ligand and its target varies inversely with the affinity set. Pristriol presented docking scores of -9.36 and -8.8 kcal/mol respectively with α -amylase and invertase, which in both cases had better affinities than that of acarbose.

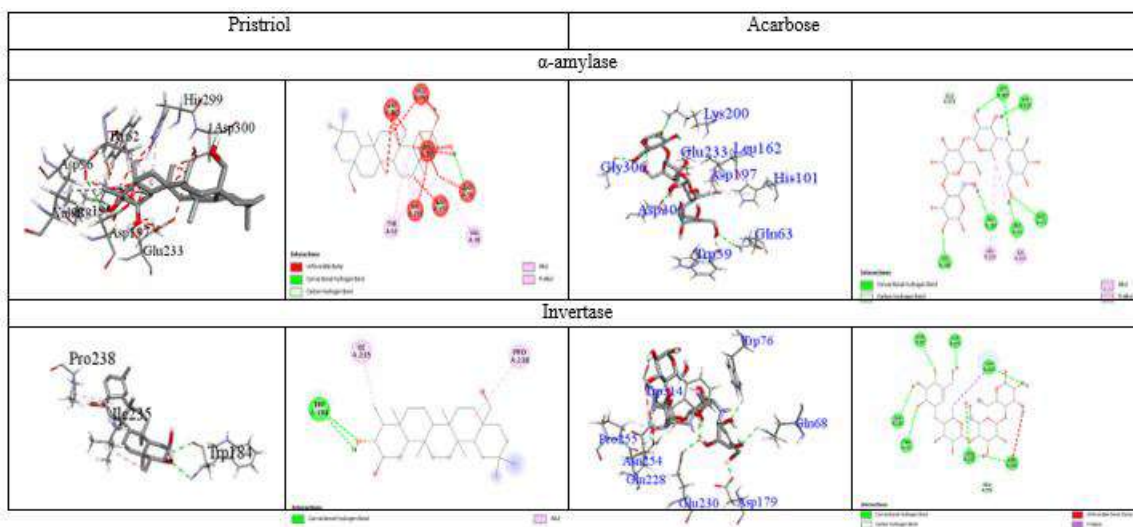
Table 3: Molecular docking scores of Pristriol against α -amylase and invertase

Name of the Compound		α -amylase	Invertase	
	Binding Energy (Kcal/mol)	Inhibition Constant (kI) (μ M)	Binding Energy (Kcal/mol)	Inhibition Constant (kI) (μ M)
Pristriol	-9.36	0.138	-8.8	0.412
Acarbose	-3.64	2160	-1.67	61280

Profiles of interactions established between pristriol and protein targets (α -amylase and invertase)

Analysis of 2D and 3D diagrams of the interactions established between pristriol and the targets (α -amylase and invertase) showed that Pristriol is capable of penetrating the active site of α -amylase and establishing numerous bonds

hydrogen, the most important. Where the hydrogen bonds established with the Asp 197 residues of the active site of the enzyme. However, with invertase, although having shown a fairly pronounced affinity, we noted the establishment of numerous interactions but no favorable interaction with the amino acid residues of the active site of invertase was observed (table 4 below).

Table 4: 3D and 2D views of molecular interactions of Pristriol and Ascarbose on α -amylase and invertase


DISCUSSION

This study was to evaluate the postprandial blood glucose lowering properties of pristriol isolated from *S. globulifera* and its binding interactions with carbohydrate hydrolysis enzymes. Postprandial glycaemia has a strong impact on fasting glycaemia, so modulating this allows fasting glycaemia to be controlled, which is why it is an important pharmacological target for managing type 2 diabetes (T2DM). Starch is the major complex sugar in the human diet and is

digested primarily by salivary and pancreatic α -amylase. Besides this, sucrose is generally found in the human breakfast and is digested in the intestine by invertase. Inhibition of α -amylase and invertase blocks carbohydrate hydrolysis in the digestive tract, thereby reducing glucose absorption and permeability in the intestine (Gong et al., 2020; Santos et al., 2022). Pristriol significantly prevented the establishment of this blood sugar peak after starch overload, and it was associated with the decline in blood sugar levels

over time. This effect can be attributed to the inhibition of α -amylase, as confirmed by molecular docking studies showing high affinity binding to this target enzyme. Indeed, in silico molecular interaction studies show that the compound has a good affinity with the active site of α -amylase (-9.36 kcal/mol) and interacts with the amino acids of the catalytic triad (Asp 197, Glu 233 and Asp 300); in the same way as acarbose (reference drug), this was of the carbon hydrogen bond type with Asp 197 and could be a competitive inhibitor. This result is similar to those of Kepawou et al (2024) which showed the anti-hyperglycemic effect of two terpenoids isolated from *Coula edulis* on normoglycemic rats having received a starch overload. Pristiol also prevented the establishment of this glycemic peak after sucrose overload. This result is explained at the molecular level by the strong binding affinity of the compound with invertase (-8.8 kcal/mol) reflecting inhibition. Pristiol does not interact with the amino acids of invertase catalytic site and could be a non-competitive inhibitor. This result is similar to those of Franke et al. (2018) which showed that Vitamin C normalized blood sugar levels after overloading with sucrose. The positive effects obtained on blood glucose levels after starch and sucrose overload could also be due to Pristiol's ability to bind remaining glucose after the action of digestive enzymes. Indeed, the compound has the capacity to glycosylate glucose in a concentration-dependent manner with maximum binding to 87%. This results can be explained by the formation of complexes between the hydroxyl groups of glucose and Pristiol (Takuissu et al., 2020). Physicochemical analyses determined pristirol complies with Lipinski's rules for oral drug candidates ($MW < 500$, $NRB \leq 15$, $HBD \leq 5$ and $HBA \leq 10$). This, along with its favorable pharmacokinetic interactions observed in silico, indicate pristirol merits further investigation as an antidiabetic agent.

CONCLUSION

This study shows that pristirol modulates postprandial glycaemia through multiple complementary mechanisms of action. This one can therefore be valued as a hit to lead for the research and development of an antidiabetic compound, which modulates postprandial blood sugar level.

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