

Research Article

# In-silico and In-vitro Evaluation of the Anti-diabetic Potential of p-Propoxybenzoic Acid

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## I N F O

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## A B S T R A C T

**Background:** p-propoxybenzoic acid (p-PBA) is reported as an active chemical constituent of medicinal plants that possess anti-diabetic activity. It is termed a Multiple-Designed Ligand (MDL) having the ability to block more than one enzyme. A molecular docking study justifies the binding ability of p-PBA with acarbose and NaVO<sub>4</sub> which were considered standard compounds having the ability to block target enzymes.  $\alpha$ -amylase inhibition assay was used as an in-vitro screening model to evaluate the activity of p-PBA against diabetes on an initial basis.

**Methods:** For the molecular docking study, a PDB file of p-PBA was prepared and PDB files of  $\alpha$ -amylase (1C8Q),  $\alpha$ -glucosidase (5KZW) and PTP1B (5K9W) were procured. p-PBA was docked against the enzymes using the blind docking method. The binding score of p-PBA and standard with enzymes was obtained and compared. The percentage inhibition of an  $\alpha$ -amylase enzyme by p-PBA was measured by using a DNS-modified  $\alpha$ -amylase inhibition assay and half-maximal inhibitory concentration (IC<sub>50</sub>) was calculated.

**Results:** p-PBA has a significant inhibitory effect against  $\alpha$ -amylase,  $\alpha$ -glucosidase, and PTP1B with docking scores of  $8.43 \pm 0.44$  kcal/mol,  $9.19 \pm 0.49$  kcal/mol, and  $9.40 \pm 0.47$  kcal/mol respectively. IC<sub>50</sub> calculated from the results of  $\alpha$ -amylase inhibition assay p-PBA was 56.59  $\mu$ g/mL.

**Conclusion:** A combination of in-silico and in-vitro methods assessed p-PBA's anti-diabetic potential on an initial basis. A molecular docking study involving p-PBA concluded the affinity of p-PBA to  $\alpha$ -amylase,  $\alpha$ -glucosidase, and PTP1B was significantly correlated with the affinity of acarbose and NaVO<sub>4</sub>. In-vitro  $\alpha$ -amylase assay validated the compound's inhibitory action against the enzyme.

**Keywords:** p-Propoxybenzoic Acid,  $\alpha$ -amylase Assay, Molecular Docking, Anti-diabetic, In-vitro, In-silico

## Background

p-propoxybenzoic acid (p-PBA) is a weak organic acid consisting of propoxy group on the para position of benzoic acid. It was found to be an active chemical constituent of numerous herbal plants reported to give certain medicinal properties including anti-diabetic action. A report suggests the therapeutic activity of plant extract containing p-PBA against microbial growth.<sup>1</sup> p-PBA is also reported to have aphrodisiac effects.<sup>2</sup> Certain evidence from the studies performed on p-PBA suggests the utilisation of this compound against cancer as well.<sup>3</sup>

A study conducted previously emphasised on the beneficial effects of p-PBA against streptozotocin-nicotinamide-induced type-II diabetic condition.<sup>4</sup> p-PBA is also found to have an inhibitory effect on protein tyrosine phosphatases 1B (PTP1B) and  $\alpha$ -glucosidase receptors which are responsible for inducing diabetes.<sup>5</sup> Hence, it can be coined as a multitarget anti-diabetic drug. The half-maximal inhibitory concentration ( $IC_{50}$ ) value of this compound is also significant for blocking both receptors. Based on the in-vitro study performed for the inhibition of PTP1B and  $\alpha$ -glucosidase enzyme,  $IC_{50}$  was found to be  $14.8 \pm 0.9$  ( $\mu$ M) and  $10.5 \pm 0.5$  ( $\mu$ M) for PTP1B and  $\alpha$ -glucosidase respectively.<sup>6</sup>

The purpose of molecular docking is to anticipate the structure of the ligand-receptor complex using computational approaches. Docking can be accomplished in two interdependent steps: first, by sampling conformations of the ligand in the active site of the protein; and second, by rating these conformations using a scoring function.<sup>7</sup> In an ideal situation, sampling algorithms should be able to duplicate the experimental binding mode, and the scoring function should rank it best among all produced conformations.<sup>8</sup> The objective of the scoring function is to distinguish between proper and wrong postures or active and inactive chemicals in a fair amount of time.<sup>9,10</sup> However, scoring functions include estimation rather than the calculation of the binding affinity between the protein and ligand, as well as the adoption of numerous assumptions and simplifications.<sup>11,13</sup>

$\alpha$ -amylase is a major enzyme involved in the breakdown of carbohydrates and their absorption in the gut.<sup>14</sup> To estimate in-vitro  $\alpha$ -amylase inhibition activity, a modified DNS method was used to quantify the reducing sugar (maltose) liberated during the assay, utilising starch as a substrate and acarbose as a standard reference compound.<sup>15</sup>

This research was structured as an exploratory pilot study to determine the anti-diabetic potential of p-PBA by in-silico molecular docking study and in-vitro  $\alpha$ -amylase inhibition assay. The inhibitory activity of p-PBA against a computational model of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and

PTP1B was measured by obtaining the binding energy of interaction from blind docking as a part of molecular docking study. The purpose of these studies was to minimise unnecessary discomfort to experimental animals.

## Methods

### Materials

5 g of p-PBA (98%) [5438-19-7] and 5 g potassium sodium tartrate tetrahydrate [6381-59-5] were procured from Thermo Fisher Scientific®. 10 g diastase (fungal)  $\alpha$ -amylase [9000-90-2], 50 g soluble potato starch [9005-84-9], 20 g 3,5-dinitrosalicylic acid (98%) [609-99-4] was procured from Sigma Aldrich. Acarbose [56180-94-0] (25 mg) tablets were ordered from Eleganta Pharmaceuticals.

### In-silico Study: Molecular Docking

#### Protein Preparation

The structure of enzyme protein  $\alpha$ -amylase (PDB: 1C8Q),  $\alpha$ -glucosidase (PDB: 5KZW) and PTP1B (5K9W) were downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) in the Protein Data Bank (PDB) format. Swiss-PDB Viewer was utilised to gain better visualisation of the molecules. Extra groups including heteroatoms and water molecules were removed.

#### Ligand Preparation

The structure of the ligand (p-PBA) was identified from ChempSpider, drawn in ChemDraw 20.1.1, and saved as a 2D mol file 2000. The file was converted into PDB format using Open Babel 2.3.2. Similarly, the ligand was prepared for acarbose and  $NaVO_4$ . Acarbose was taken as a standard for  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.  $NaVO_4$  was taken as a standard for PTP1B enzyme.<sup>16</sup>

#### Molecular Docking Analysis

Molecular docking studies were performed by utilising AutoDock Tools. Input PDB file was prepared for p-PBA. The target compound was docked against the  $\alpha$ -amylase enzyme (PDB:1C8Q). Similarly, the target compound was docked against the  $\alpha$ -glucosidase enzyme (PDB: 5KZW). In each docking, the receptor protein was kept rigid and protein polar hydrogens were added.<sup>17</sup> The docking was performed within parallel rectangular boxes of  $126 \times 126 \times 126$  Å dimensions. The centre of the grid was placed at the centre of the mass of the original protein receptor in its apo form in crystal structures. A total of 20 blind docking runs were carried out against each enzyme starting from random positions.<sup>18</sup> The output files were extracted and evaluated for the binding energy of the enzyme-ligand interaction.

#### In-vitro Study: $\alpha$ -amylase Inhibition

The study was carried out according to Kato-Schwartz et al.'s recommendations.<sup>19</sup> 0.02 M sodium phosphate

buffer was prepared, and pH was adjusted to 7.4. 250 mg of soluble potato starch was added to 50 mL water and incubated for 15 minutes. 100 mL sodium phosphate buffer was added to 0.1 mg of  $\alpha$ -amylase to prepare the enzyme solution. Initially, a maltose calibration curve was obtained by taking maltose as a reactant. Five different concentrations ranging from 20, 40, 60, 80, and 100  $\mu$ g/mL solutions were prepared for acarbose and p-PBA. 1.0 g of 3,5-dinitrosalicylic acid (DNS) was added to 50 mL distilled water followed by dropwise addition of 30.0 g sodium potassium tartrate tetrahydrate, along with 20 mL of 2 N sodium hydroxide, and was diluted up to 100 mL distilled water to prepare DNS reagent. For the conduction of the assay, 1 mL of  $\alpha$ -amylase was added to 1 mL of test/ standard and incubated for 30 minutes. After the incubation period, 1 mL of starch solution was added and furtherly incubated for 3 minutes. The reaction mixture was placed in a closed tube and incubated at 85 °C for 15 minutes. The reaction mixture was cooled, diluted with 9 mL distilled water, and subjected to UV-spectroscopy for the determination of absorbance at 540 nm.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} * 100$$

A graph was plotted for absorbance against concentration ( $\mu$ g/mL).  $IC_{50}$  for p-PBA and acarbose was calculated from the linear equation of the plotted graph.

## Results

**Table I. Results of Molecular Docking Studies**

Enzyme	Ligand	Binding Energy of Individual Docking Runs (kcal/mol)										Docking Score (kcal/mol) <sup>a</sup>
		1	2	3	4	5	6	7	8	9	10	
$\alpha$ -amylase	Acarbose (standard)	7.92	8.47	8.95	8.89	9.04	8.98	8.80	8.76	8.44	8.67	8.69 $\pm$ 0.34
	p-PBA <sup>b</sup>	6.93	7.45	7.32	6.94	7.85	7.98	7.73	8.20	7.92	7.82	8.43 $\pm$ 0.44*
$\alpha$ -gl	Acarbose (standard)	8.90	9.91	9.56	9.45	9.04	9.42	9.93	8.96	9.70	9.31	9.42 $\pm$ 0.37
	p-PBA	7.43	8.34	8.50	7.98	8.33	8.10	8.24	9.15	9.03	8.35	9.19 $\pm$ 0.49 <sup>#</sup>
PTP1B <sup>c</sup>	Standard drug X <sup>d</sup>	9.95	10.25	10.11	10.29	9.70	9.95	9.89	10.25	10.11	10.08	10.05 $\pm$ 0.19
	p-PBA	8.14	8.02	8.24	8.18	9.01	8.66	8.92	8.95	9.21	7.94	9.40 $\pm$ 0.47 <sup>§</sup>

Analysed by one-way ANOVA followed by Tukey's test

<sup>a</sup>Data are represented as mean  $\pm$  SEM of 10 experiments.

\*Indicates the statistical correlation of test drug with Acarbose with p value < 0.0001

<sup>#</sup>Indicates the statistical correlation of test drug with Acarbose with p value < 0.0001

<sup>§</sup>Indicates the statistical correlation of test drug with standard X with p value < 0.0001

<sup>b</sup>p-PBA

<sup>c</sup>PTP1B

<sup>d</sup>Standard drug X: 3-((5-((n-acetyl-3-(4-((carboxy carbonyl)(2-carboxyphenyl) amino)-1-naphthyl)-l-alanyl)amino]pentyl)oxy)-2-naphthoic acid

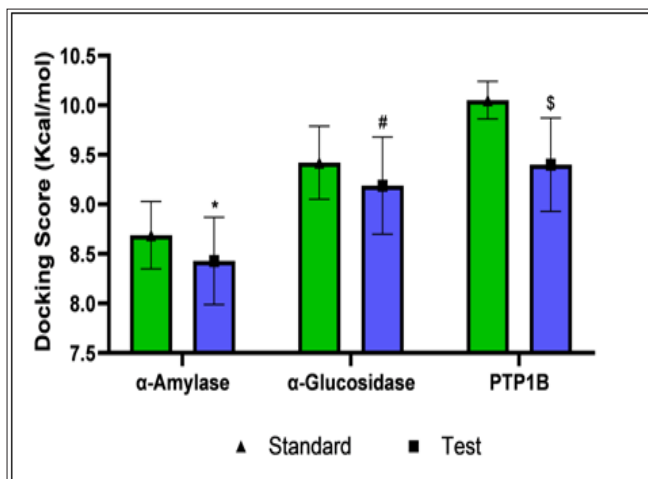
## Molecular Docking

The docking scores of acarbose and p-PBA for binding with  $\alpha$ -amylase were 8.69  $\pm$  0.34 kcal/mol and 8.43  $\pm$  0.44 kcal/mol, respectively. The statistical correlation of p-PBA with acarbose was archived with a p value < 0.001. Similarly, a significant statistical correlation was established between the docking score of p-PBA (9.19  $\pm$  0.49 kcal/mol) and acarbose (9.42  $\pm$  0.37 kcal/mol) for binding with  $\alpha$ -glucosidase enzyme with p value < 0.001. NaVO<sub>4</sub> gave a docking score of 10.05  $\pm$  0.19 kcal/mol compared to the docking score of p-PBA (9.40  $\pm$  0.47 kcal/mol) for the PTP1B enzyme. The statistical correlation was recorded between NaVO<sub>4</sub> and p-PBA with p value < 0.001. Results of docking studies were collected and recorded as provided in Table 1. A graphical representation of the results is described in Figure 1.

## $\alpha$ -amylase Inhibition

### In-vitro Study: $\alpha$ -amylase Inhibition

p-PBA reported a successive increase in percentage inhibition of  $\alpha$ -amylase enzyme with the increase in concentration. The inhibition trend was comparable with the standard drug acarbose. The maximum dose of acarbose reported 87.10% inhibition compared to p-PBA with 70.25% inhibition. The maltose standard curve was plotted and has been represented in Figure 2. The percentage inhibition of  $\alpha$ -amylase by the standard and test drug is provided in Table 2.

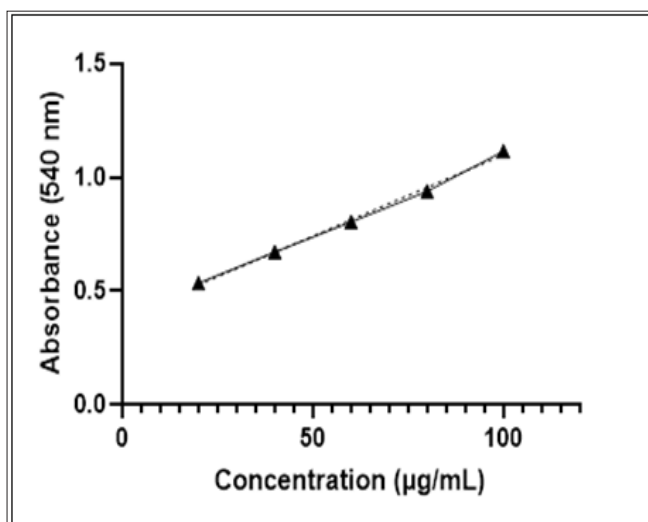


\*, #, and \$ indicate statistically significant correlation ( $p$ -value < 0.001) between respective groups

**Figure 1. Graphical Representation of  $\alpha$ -amylase Inhibition Assay**

**Table 2.  $\alpha$ -amylase Percentage Inhibition**

Concentration ( $\mu\text{g/mL}$ )	Percentage Inhibition (%)	
	p-PBA	Acarbose
20	35.48	44.27
40	42.03	59.95
60	55.38	71.86
80	62.90	78.32
100	70.25	87.10



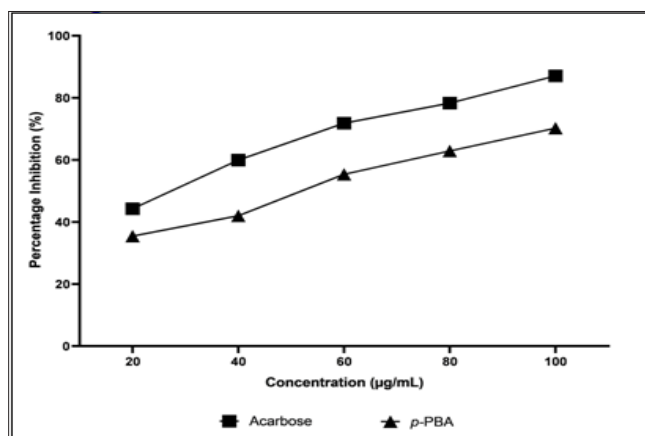
**Figure 2. Maltose Calibration Curve**

A graphical comparison between the inhibitory activity of acarbose and p-PBA was performed as given in Figure 3.

Half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) for p-PBA (24.82  $\mu\text{g/mL}$ ) and acarbose (56.59  $\mu\text{g/mL}$ ) was calculated from the linear equations obtained from the graphs and was recorded as given in Table 3.

**Table 3. Half-maximal Inhibitory Concentration ( $\text{IC}_{50}$ ) of Acarbose and p-PBA**

Concentration ( $\mu\text{g/mL}$ )	$\text{IC}_{50}$ Concentration ( $\mu\text{g/mL}$ )
Acarbose	24.82
p-PBA	56.59



**Figure 3. Graphical Comparison between the Inhibitory Activity of Acarbose and p-PBA**

## Discussion

Single-target oral hypoglycaemic agents target one specific enzyme responsible for glucose elevation and inhibit the same to produce hypoglycaemia.<sup>20</sup> Inhibition of a single target may result in less efficacy over time and may fail to decrease glucose levels. Hence, the requirement for alternatives is mandatory. Multi-target oral hypoglycaemic agents can rectify this limitation and provide a potent anti-diabetic effect. From the results of the study, p-PBA can be claimed as the first multi-target oral hypoglycaemic agent inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase. p-PBA was procured from a highly authentic source ensuring purity, and screening for anti-diabetic activity was conducted at in-silico and in-vitro levels.

Blind docking was performed using Autodock Tools 1.5.6. p-PBA has a significant binding affinity with  $\alpha$ -amylase,  $\alpha$ -glucosidase, and PTP1B compared to acarbose and NaVO<sub>4</sub>. Hence, the anti-diabetic activity of p-PBA can be justified in the quantum chemistry computational domain.

The calculated  $\text{IC}_{50}$  value of p-PBA from  $\alpha$ -amylase assay was 56.59  $\mu\text{g/mL}$  which was comparable with the  $\text{IC}_{50}$  value of the standard drug acarbose (24.82  $\mu\text{g/mL}$ ). Therefore, it can provide a preliminary justification for the in-vitro anti-diabetic activity of p-PBA.

This research was an exploratory study conducted to predict the anti-diabetic potential of p-PBA. The study provides the base for the in-vivo screening of p-PBA against type-II diabetes and can be considered as a rationale for the

same. The positive outcome of the research justifies the mechanism of action by which p-PBA can be coined as an anti-diabetic agent.

## Conclusions

The anti-diabetic potential of p-PBA acid was evaluated by in-silico and in-vitro approaches. p-PBA reported a significant correlation in binding affinity towards  $\alpha$ -amylase,  $\alpha$ -glucosidase, and PTP1B in an in-silico molecular docking study. Inhibitory activity against  $\alpha$ -amylase was further confirmed by in-vitro  $\alpha$ -amylase assay. Hence, p-PBA has shown potential anti-diabetic activity on a laboratory scale. The next step of this research can be to evaluate the potential of p-PBA against anti-diabetic potential through in-vivo studies on experimental animals. p-PBA can also be examined for the possible beneficial effect against the diseases having  $\alpha$ -amylase,  $\alpha$ -glucosidase and PTP1B in their progression.

## Abbreviations

DL: decilitre, DNS: 3,5-dinitrosalicylic acid, g: gram,  $IC_{50}$ : Submaximal inhibitory concentration,  $\mu$ g: microgram, p-PBA: p-propoxybenzoic acid.

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**Conflict of Interest:** None

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