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Original Research Paper

AN *IN SILICO* EXPLORATION FOR NOVEL INHIBITORS OF α-AMYLASE AND α-GLUCOSIDASE EXTRACTED FROM *TINOSPORA CORDIFOLIA*

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ABSTRACT

Present study reports the potential of α-amylase and α-glucosidase inhibitory activities of phytoconstituents of the plant, *Tinospora cordifolia* using an *in silico* structure-based molecular docking approach. *In silico* screening of seventeen molecules from *T. cordifolia* was performed and compared with the activity of a known inhibitor acarbose. For this study AutoDock 1.5.6, PyRx and Discovery Studio 4.1 Visualizer softwares were used. Out of the seventeen molecules screened, five: Verbascoside, Hesperetin 7-rhamnoglucoside, 3-(1-Naphthoyl) benzoate, (4-Cinnamoyl-3,5-dihydroxyphenoxy) acetate and 4-(2,4-dimethoxy-3,6-dimethylbenzoyl) oxy-2-hydroxy-3,6-dimethyl benzoate showed lowest binding energies for α-amylase and α-glucosidase. Further, comparative analysis of PDB structures of both enzymes using 3DLigandSite server and Discovery Studio 4.1 Visualizer analysis of docked enzyme structures revealed involvement of almost similar amino acids in ligand binding sites of both the enzymes. This *in silico* investigation may felicitate the development of α-amylase and α-glucosidase inhibitors from *T. cordifolia* for treating diabetes.

Keywords: Diabetes Mellitus, Phytoconstituents, *Tinospora cordifolia, In silico*, Molecular docking, Virtual screening, α-Amylase and α-Glucosidase inhibitors.

INTRODUCTION

Diabetes Mellitus is a common endocrine disorder based on deficiency of insulin which leads to chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism.[1] One of the therapeutic approaches for treating diabetes is to decrease postprandial hyperglycemia which can be mediated by two enzymes, α-amylase and α-glucosidase.[2] This can be done by partially inhibiting or blocking the activity of these enzymes with the help of inhibitors which are also called as starch blockers because they contain substances that prevent absorption of dietary starch in the body.[3] Acarbose, voglibose, and miglitol are clinically used antidiabetic agents which however confer some adverse effects like diarrhoea, abdominal discomfort, flatulence, and hepatotoxicity.[4] Thus it is necessary to screen some inhibitors from natural products which could be used as alternatives for prevention and treatment of type 2 diabetes mellitus without any side effects.[3]

Tinospora cordifolia is one of the well known ayurvedic herbs with tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties.[5,6] Various concentrations of *T. cordifolia* extracts as well as powder forms are frequently prescribed for the diabetic patients. So we initially focused on its phytoconstituents analysis. Our previous studies on phytochemical constituents of *T. cordifolia* have revealed the presence of several compounds. Some of them, like Hesperetin-7-Rhamnoglucoside[7] and Verbascoside[8] are already reported to have antidiabetic activity. While other

compounds like (2S)-2-Pyrrolidinecarboximidate; (4-Cinnamoyl-3,5-dihydroxyphenoxy) acetate; (15Z)-12-Oxophyto-10,15-dienoate; 1-cyclohexenylmethanone; 2-[(3-Amino-4-propoxybenzoyl)-oxy]-N,N-diethylethanaminium; 2-Hydroxy-3-oxoicosanoate; 2-Hydroxy-5,10-dioxo-4-phenyl-3,4,5,10-tetrahydro-2H-benzo[g]chromene-2-carboxylate; 3-(1-Naphthoyl) benzoate; 3,3-Bis-(3,4-dimethoxyphenyl) propanoate; 3,5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate; 3-Carbamoyl-1-(5-O-phosphono-β-D-ribofuranosyl) pyridinium; 6,9,12,15-Octadecatetraenoatato; 4-(2,4-dimethoxy-3,6-dimethyl-benzoyl)-oxy-2-hydroxy-3,6-dimethyl benzoate; Petunidin-3-O-coumaroylrutinoside-5-O-glucoside and Imidazole-Pyrazole are identified by us.[6] These phytochemicals are not reported for anti-diabetic activity. So, in continuation of our research work, we focussed on virtual screening of these molecules from *T. cordifolia* for their potential α-amylase and α-glucosidase inhibitory activity. This was achieved using the molecular docking approach of virtually screening and predicting the binding of the small molecules to known target structures.[9,10]

MATERIALS AND METHODS

Preparation of Macromolecules for Docking

The structure of α-amylase (PDB Code: 3BC9) and α-glucosidase (PDB Code: 2QMJ) macromolecules were obtained from protein data bank (http://www.rcsb.org). 3D structures in PDB format were prepared for molecular docking with the help of AutoDock Tools-1.5.6[11,12] using the steps prescribed in the AutoDock Tutorial.[13] From each of the individual macromolecule water molecules were deleted, polar hydrogen were added and then subjected for GRID preparation which helps to define the binding sites where compounds/ligands are to be docked. Later, the structures were saved in PDBQT file format, the format that is accepted by AutoDock tool which contains a protein structure with hydrogen in all polar residues.

Preparation of Ligands for Docking

The ligand structures (Compounds from our earlier studies on *T. cordifolia)* were obtained from PubChem database (http://pubchem.ncbi.nlm.nih.gov) and ChemSpider[14] in Mol/SD format. The obtained Mol/SD formats were then converted to PDB formats using Open Babel software.[15,16] Ligand molecules in PDB format were further energy minimized by computing gasteiger changes using AutoDock tool and the structures were saved in PDBQT file format.[16,17]

Molecular Docking of Macromolecules and Ligand

Interaction of the enzymes, α-amylase and α-glucosidase with individual phytoconstituents of *T. cordifolia* was performed using AutoDock Vina[11] via PyRx software.[13] This was done to obtain a number of possible conformations and orientations for the ligand at binding site of macromolecule. For this, the macromolecules and ligands were loaded in PyRx software, in a PDBQT file obtained from AutoDock and docking was executed by clicking on Run Vina option in PyRx. After completion of docking, the binding energies (kcal/mol) of each ligand with both the individual enzymes were obtained. The best conformation of each ligand with respective enzyme having lowest binding energy was chosen. In case of more than one pose of ligand and enzyme, the pose with lowest binding energy was selected.[18] These docked structures i.e. enzyme-ligand complexes were saved in PDB format for further analysis.

Molecular Docking Analysis

Acarbose, a standard α-amylase and α-glucosidase inhibitor was also docked with both the enzymes as a reference inhibitor and the binding energies with each of the enzymes were predicted. The binding energy of each ligand with respective enzyme was compared with that of the acarbose and ligands with binding energy less than that of acarbose were marked as best inhibitor. Such ligands with low binding energy than acarbose for both the enzymes simultaneously were preferred.

Inferring Amino Acids and Interactions Involved In Molecular Docking

After the docking process, docked structures saved in the PDB format were analyzed for the amino acids involved in the ligand binding sites of the enzyme along with the type of interactions like Vander Waals, hydrogen bonding etc involved in the docking. This was performed using the Discovery Studio 4.1 Visualizer (http://accelrys.com/products/discovery-studio).[19] Docked structures showing the amino acid residues involved in the docking as well as the interactions between the residues and ligand were image captured using image save option in Discovery Studio 4.1 Visualizer.

3DLigandSite Analysis of α-Amylase and α-Glucosidase Enzymes

Enzymes α-amylase and α-glucosidase were subjected independently to the 3DLigandSite server (http://www.sbg.bio.ic.ac.uk/3dligandsite) for interpreting the amino acid residues involved in the ligand binding site of the enzyme.[20] 3DLigandSite is an online server which accepts protein query in FASTA format or PDB structure of the enzyme and predicts the amino acid residues involved in ligand binding site of enzyme through a binding site library comprising of protein-ligand complexes.

RESULTS

Seventeen phytoconstituents of *T. cordifolia*[6] and acarbose, a standard inhibitor, as ligands in PDBQT format as shown in figure 1 were docked with α-amylase and α-glucosidase in PDBQT format shown in figure 2a and 2c. All the seventeen compounds were docked in the active sites of both the enzymes and showed different binding energies in kcal/mol as depicted in table 1. Out of the seventeen, only five compounds showed lower binding energies with both the enzymes as compared to binding energies shown by acarbose (0.8, -8.7 kcal/mol, for α-amylase and α-glucosidase respectively). Verbascoside showed lowest binding energy followed by Hesperetin-7-rhamnoglucoside, 3-(1-Naphthoyl)-benzoate, (4-Cinnamoyl-3,5-dihydroxyphenoxy)-acetate and 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethyl-benzoate with scores of -10.5, -10, -9, -7.5 and -7 kcal/mol for α-amylase and -11.3, -10.4, -10.9, -9.7 and -9.3 kcal/mol for α-glucosidase respectively. All these five compounds and the standard acarbose docked with α-amylase and α-glucosidase have been shown in figure 3 and figure 4 respectively. All the compounds were docked with the enzymes in different poses having varying binding energies. However pose with the lowest binding energy indicate highest affinity to the enzyme, thus was considered significant and selected for further docking analysis.

Further subjecting the docked structures in PDB format to Discovery Studio 4.1 Visualizer software, amino acid residues involved in the ligand binding sites of α-amylase and α-glucosidase were predicted and the predictions for the best five compounds and the standard acarbose has been shown in figure 5 and figure 6 respectively. Our analysis revealed the type of interaction and amino acid residues involved in these interactions between the ligand and both the enzymes. Interactions like Van der Waals, conventional hydrogen bond, carbon hydrogen bond, attractive charges, alkyl, Pi-alkyl, Amide Pi- Stacked, Pi-Sigma, Pi-Pi T-Shaped, Pi-Cation, Pi-Anion, Pi-Donor hydrogen bond, Pi-Pi Stacked, unfavourable acceptor-acceptor, Pi-Lone pair, salt bridge, unfavourable bumps, unfavourable positive-positive, unfavourable negative-negative, unfavourable donor-donor, and halogen (Cl, Br, I) were predicted. Predicted interactions of the best five compounds and the standard acarbose have been shown in figure 5 and figure 6. Out of these interactions, α-amylase and α-glucosidase amino acid residues involved in major interactions like Vander Waals, conventional hydrogen bond, carbon hydrogen bond interactions with all the ligands have been depicted in table 2 and table 3 respectively.

Simultaneously, the PDB structures of α-amylase and α-glucosidase submitted to online 3DLigandSite server predicted the amino acid residues involved in the ligand binding site of the enzymes. The server identified a total of 4 and 18 clusters for α-amylase and α-glucosidase respectively, out of which data for the first 4

clusters for both the enzymes have been shown in table 4. The probable ligand binding pocket for α-amylase and α-glucosidase, identified in cluster 1 of 3DLigandSite analysis has been shown in figure 2b and 2d respectively.

DISCUSSION

Phytochemical profiling and identification of *T. cordifolia* plant extracts from our previous reports revealed a diverse range of bioactive phenolics.[6] Few of them were reported for antidiabetic activities but we found no details of molecular docking analyses. So, in the present study we tried to put forth the *in silico* studies of phytochemicals obtained via *T. cordifolia* plant extracts.

Enzyme and ligand complex models generated after successful docking were obtained based on the parameters such as hydrogen bond interactions, π – π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site.[17] In the present study, Verbascoside, Hesperetin-7-rhamnoglucoside, 3-(1-Naphthoyl)-benzoate, (4-Cinnamoyl-3,5-dihydroxyphenoxy)-acetate and 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethyl-benzoate showed lowest binding energies thus they could be considered as potential inhibitors of α-amylase as well as α-glucosidase. Earlier reports has revealed the antidiabetic properties of Hesperetin-7-rhamnoglucoside[7] and Verbascoside[8] which confirms their potency to consider them for further studies on their involvement in antidiabetic drugs. Our study suggest that 3-(1-Naphthoyl)-benzoate, (4-Cinnamoyl-3,5-dihydroxyphenoxy)-acetate, 4-(2,4-dimethoxy-3,6-dimethyl-benzoyl)-oxy-2-hydroxy-3,6-dimethyl-benzoate could be considered as potential antidiabetic agents. Further, comparing the analysis performed using Discovery Studio 4.1 Visualizer and 3DLigandSite server; it could be revealed that the amino acid residues predicted in the ligand binding site of both the enzymes on 3DLigandSite server were involved in either of the interactions predicted by Discovery Studio 4.1 Visualizer. This was an added advantage of performing two independent analyses of α-amylase and α-glucosidase enzymes using Discovery Studio 4.1 Visualizer and 3DLigandSite online server.

So, our studies put forward this as a first report on potential antidiabetic property of these compounds based on *in silico* studies as well as through TLC Autography and α-amylase inhibition assay.[6] However, the individual molecules need to be tested through wet lab experiments.

CONCLUSION

In silico studies can provide valuable insights into target drug interactions. Furthermore, this approach can reduce time and cost of clinical trials. So our focus was to screen inhibitors of α-amylase and α-glucosidase especially the phytoconstituents via *in silico* approach. Owing to the side effects of chemical or synthetic inhibitors already available in the market, screening and selection of phytoconstituents as inhibitors with less or no side effects is the basic purpose of this study. Our results showed the ability to inhibit α-amylase as well as α-glucosidase by all the compounds. When compared with a standard inhibitor, five compounds: Hesperetin-7-rhamnoglucoside (-10 and -10.4 kcal/mol), Verbascoside (-10.5 and -11.3 kcal/mol), 3-(1-Naphthoyl)-benzoate (-9 and -10.9 kcal/mol), (4-Cinnamoyl-3,5-dihydroxyphenoxy)-acetate (-7.5 and -9.7 kcal/mol), and 4-(2,4-dimethoxy-3,6-dimethyl-benzoyl)-oxy-2-hydroxy-3,6-dimethyl-benzoate (-7 and -9.3 kcal/mol) were better than that of the standard drug acarbose (0.8 and -8.7 kcal/mol) with respect to α-amylase and α-glucosidase. This molecular docking analysis could lead to discovery of naturally occurring potent α-amylase and α-glucosidase inhibitors from *T. cordifolia* against Diabetes Mellitus.

Table 1: Binding affinity (kcal/mol) of compounds with α-amylase and α-glucosidase predicted through virtual docking

Sr. No.	Compounds	α-Amylase no. of poses	Binding affinity with α-amylase (kcal/mol)	α-Glucosidase no. of poses	Binding affinity with α-glucosidase (kcal/mol)
1	Acarbose	1	0.8	3	-8.7
2	(2S) 2 Pyrrolidinecarboximidate	9	-4.4	9	-5.7
3	(4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate	3	-7.5	7	-9.7
4	(15Z)-12-Oxophyto-10,15-dienoate	8	-6	9	-7.8
5	1-cyclohexenylmethanone	9	-4.4	9	-5.9
6	2-[(3-Amino-4-propoxybenzoyl)oxy]-N,N-diethylethanaminium	9	-5.6	9	-7.7
7	2-Hydroxy-3-oxoicosanoate	1	-0.7	4	-7.3
8	2-Hydroxy-5,10-dioxo-4-phenyl-3,4,510-tetrahydro-2H-benzo[g]chromene-2-carboxylate	1	-5.8	4	-7.5
9	3-(1-Naphthoyl)benzoate	9	-9	9	-10.9
10	3,3-Bis(3-4-dimethoxyphenyl)propanoate	9	-7.6	6	-8.3
11	3,5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate	9	-7.8	9	-8.3
12	3-Carbamoyl-1-(5-O-phosphono-β-D-ribofuranosyl)pyridinium	3	-6.7	7	-8.5
13	6,9,12,15-Octadecatetraenoatato	9	-4.7	9	-7
14	4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate	7	-7	9	-9.3
15	Petunidin-3-O-coumaroylrutinoside-5-O-glucoside	1	37.6	2	-1
16	Imidazole-Pyrazole	9	-4.6	9	-5.4
17	Hesperetin 7-rhamnoglucoside	10	-10	10	-10.4
18	Verbascoside	9	-10.5	9	-11.3

Table 2: α-Amylase amino acid residues showing different interactions with ligands predicted using Discovery Studio Visualizer 4.1

Sr. No.	Compounds docked with α-amylase	α-Amylase amino acid residues showing different interactions		
		Van der Waal's interactions	Conventional Hydrogen Bond	Carbon Hydrogen Bond
1	Acarbose	PRO 347; THR 351; SER 27; GLY 418; ARG 25; GLN 22; GLN 23; GLY 24	ARG 413; ASP 417	_
2	(2S) 2 Pyrrolidinecarboximidate_Amylase	ARG 436; PRO 347; GLY 418; THR 351; PHE 350	THR 26	_
3	(4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate_Amylase	PHE 350; THR 21; ARG 25; GLY 418; VAL 416	ARG 436	ASP 417; THR 26; PRO 347
4	(15Z)-12-Oxophyto-10,15-dienoate_Amylase	THR 21; PHE 350; ARG 25; THR 26	GLY 418; ARG 436	ASP 417; PRO 347
5	1-cyclohexenylmethanone_Amylase	ARG 267; GLY 349; SER 27	THR 351; THR 26	PHE 350
6	2-[(3-Amino-4-propoxybenzoyl)oxy]-N,N-diethylethanaminium_Amylase	THR26; GLY 418; ARG 436; GLY 349, VAL 416; THR 351; ARG 267; THR 21	_	PRO 347; ASP 417
7	2-Hydroxy-3-oxoicosanoate_Amylase	ALA275; ALA322; PHE 271; ILE 250; ASP 212; THR 279; ALA 325;GLY 324; LEU 252; GLY 323; ILE 327; ASN 316; GLY 321; HIS 320; ASN 313, ASP 315, GLU 248; ARG 210	SER 326	_
8	2-Hydroxy-5,10-dioxo-4-phenyl-3,4,510-tetrahydro-2H-benzo[g]chromene-2-	THR 21; ARG 25; THR 26; ASP 417;GLY 418;ARG 436;PRO 347;TYR 348	SER 304; ARG 267	ASP 305; ASP 417
9	3-(1-Naphthoyl)benzoate_Amylase	THR 351; THR 26; GLY 418; VAL 416; ARG 267; GLY 349; PRO 347	ARG 413; ARG 436	_
10	3,3-Bis(3-4-dimethoxyphenyl)propanoate_Amylase	GLY 349; THR 351; THR 26; VAL 416; ARG 436; GLY 418; PRO 347; ASP 305	ARG 267	SER 304
11	3,5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate_Amylase	GLY 418; SER 304; THR 26; PHE 350; ARG 267	ARG 436	PRO 347
12	3-Carbamoyl-1-(5-O-phosphono-β-D-ribofuranosyl)pyridinium_Amylase	ALA 325; GLY 324; GLY 323; ILE 327; VAL 311; GLY 274; PHE 310; ASN 316; ILE 250	SER 326	_

13	6,9,12,15-Octadecatetraenoatato_Amylase	GLY 418; HIS 346; ASP 417; THR 26; PHE 350; PRO 19; ARG 267	ARG 436	PRO 347
14	4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_Amylase	HIS 346; VAL 416; THR 26; PHE 350; GLY 349; GLY 418; SER 304; ASP 305	ARG 436	PRO 347; ASP 417
15	Petunidin-3-O-coumaroylrutinoside-5-O-glucoside_Amylase	TRP 73; HIS 320; TYR 273; GLY 274; ASP 312, ASP 212; GLU 248; ASN 313; SER 326; GLY 324; GLY 323; ALA 325	ASN 316; ALA 322	PHE 310; GLU 270; GLY 321; ASN 316
16	Imidazole-Pyrazole_Amylase	THR 26;GLY 418; ARG 436; PRO 347; GLY 349	_	_
17	Hesperetin 7-rhamnoglucoside_Amylase	LEU 180; TYR 77; HIS 116; ALA 213; ARG 210; LEU 177; ILE 250; ALA 322; GLN 78	GLU 248; GLY 321	_
18	Verbascoside_Amylase	VAL 249; LYS 215; GLU 164; ASP 162; TYR 166; THR 178; LEU 180; TRP 73	ILE 163; ASP 315; HIS 314; ARG 210; GLU 248	ALA 213

Table 3: α-glucosidase amino acid residues showing different interactions with ligands predicted using Discovery Studio Visualizer 4.1

Sr. No.	Compounds docked with α-glucosidase	α-Glucosidase amino acid residues showing different interactions		
		Van der Waal's interactions	Conventional Hydrogen Bond	Carbon Hydrogen Bond
1	Acarbose	HIS 688; GLN 355; PHE 821; ARG 565; PRO 566; LEU 556; ILE 557	THR 354	LYS 579
2	(2S) 2 Pyrrolidinecarboximidate_glucosidase	PHE 567; VAL 568; TYR 580; VAL 583; PHE 612; ILE 610; SER 611; GLN 578; GLN 355	_	_
3	(4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate_glucosidase	TYR 580; PHE 821; PRO 359; MET 357; PHE 567; PRO 358; GLN 355; PHE 684; GLY 609; ILE 610	VAL 568; PHE 612; SER 611	ALA 584; GLY 581; GLN 578
4	(15Z)-12-Oxophyto-10,15-dienoate_glucosidase	GLY 581; SER 611; ILE 610; GLN 355; GLN 578; PHE 821; ALA 553; LEU556; ARG 565; VAL 568; PHE 567; SER 362	PRO 566	_
5	1-cyclohexenylmethanone_glucosidase	PRO 359; PHE 567; VAL 568; TYR 580; GLN 355; PHE 612	GLN 578	GLY 581
6	2-[(3-Amino-4-propoxybenzoyl)oxy]-N,N-diethylethanaminium_glucosidase	ALA 553; LEU556; PRO 566; PHE 567; SER 362; SER 611; GLN 355; PRO 359; PHE 821; ARG 565; MET 357	VAL 568	TYR 580; GLN 578
7	2-Hydroxy-3-oxoicosanoate_glucosidase	HIS 350; GLY 353; THR 354; HIS 688; PHE 821; ALA 553; LEU 556; PRO 359; PHE567; GLY 581; ALA 582; TYR 580; ALA 356; MET 357; LYS 579	ARG 565; PRO 566; VAL 568	PRO 358
8	2-Hydroxy-5,10-dioxo-4-phenyl-3,4,510-tetrahydro-2H-benzo[g]chromene-2-carboxylate_glucosidase	PRO 566; ARG 565; THR 354; HIS 688; LYS 579; MET 357; GLN 355	_	_

9	3-(1-Naphthoyl)benzoate_glucosidase	SER 362; PHE 612; GLN 578; TYR 580; LEU 556; PHE 821; LYS 579; PRO 566; MET 357; PHE 567	VAL 568	_
10	3,3-Bis(3-4-dimethoxyphenyl)propanoate_glucosidase	ILE 557; PRO 566; PHE 567; PRO 358; VAL 568; PRO 359; MET 357; LYS 579; TYR 580; PHE 821	_	_
11	3,5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate_glucosidase	ALA 553; LEU556; TYR 580; PRO 566; GLY 581; PHE 567; PRO 358;PHE 821; ARG 565; ALA 356	VAL 568	PRO 359; MET 357
12	3-Carbamoyl-1-(5-O-phosphono-β-D-ribofuranosyl)pyridinium_glucosidase	PRO 358; PHE567; VAL 568; ALA 582; GLY 581; PHE 612; TYR 580; PRO 566; LEU 556	LYS 579; MET 357	PRO 359; ARG 565
13	6,9,12,15-Octadecatetraenoatato_glucosidase	GLY 581; PRO 358; PHE 567; PRO 359; LYS 579; VAL 568; ARG 565; PRO 566; ILE 610; GLY 609; GLN 578; GLN 355; ALA 356	SER 611; PHE 612	ALA 582
14	4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_glucosidase	TYR 580; PRO 566; GLY 581; SER 362; MET 357; PHE 821	VAL 568	GLN 578
15	Petunidin-3-O-coumaroylrutinoside-5-O-glucoside_glucosidase	LEU 102; PHE 821; MET 357; PRO 566; PRO 358; GLY 581; VAL 568; PHE 567; GLN 355	THR 354; ALA 582	GLY 353; THR 354; HIS 688; ALA 356; LYS 579
16	Imidazole-Pyrazole_glucosidase	TYR 580; VAL 568; PHE 567; PHE 612	_	_
17	Hesperetin 7-rhamnoglucoside_glucosidase	PRO 566; LEU 556; ARG 565; ALA 553; ALA 356; GLN 822	GLU 564; TYR 580; LYS 579	LYS 579; TYR 580
18	Verbascoside_glucosidase	GLY 520; VAL 517; PRO 515; SER 516; TRP 472; TRP 584; TRP 509; ASP 398; ILE 435	GLU 522; PHE 518; ARG 571; ASP 587; ASP 511	PHE 518; ASP 252; TYR 254

Table 4: Amino acid residues involved in the ligand binding sites of α-amylase and α-glucosidase predicted using 3DLigandSite server

Clusters identified	Ligands bound	Potential amino acid residues and their positions associated with the ligand binding site															
α-Amylase																	
Cluster 1	24	ASN	ARG	HIS													
		115	173	216													
Cluster 2	15	ASN	GLY	ASN													
		474	475	476													
Cluster 3	6	TRP	TRP	TYR	GLN	LEU	ASP	HIS									
		73	74	77	78	180	315	320									
Cluster 4	3	TYR	LYS	ILE	GLU	GLY	ALA										
		166	215	250	255	321	322										
α-Glucosidase																	
Cluster 1	27	ASP	TRP	ILE	TRP	ASP	MET	PHE	ARG	ASP							
		252	370	399	472	511	512	518	571	587							
Cluster 2	26	LEU	ILE	SER	GLY	LYS	GLU	ARG	PRO	HIS	SER	PHE	GLN	TYR	LEU	HIS	GLN
		556	557	560	562	563	564	565	566	819	820	821	822	823	824	825	826
Cluster 3	10	ASP	VAL	LEU	THR	PHE	LEU	PHE	TYR	LEU	THR	GLY	ALA	GLY			
		100	101	102	103	283	285	334	348	351	352	353	575	576			
Cluster 4	10	HIS	PHE	VAL	LYS	GLU	ASP	PHE	GLU	GLY	SER	SER	TYR	LEU	HIS	TYR	
		439	458	459	460	465	466	467	468	469	476	477	478	479	531	544	

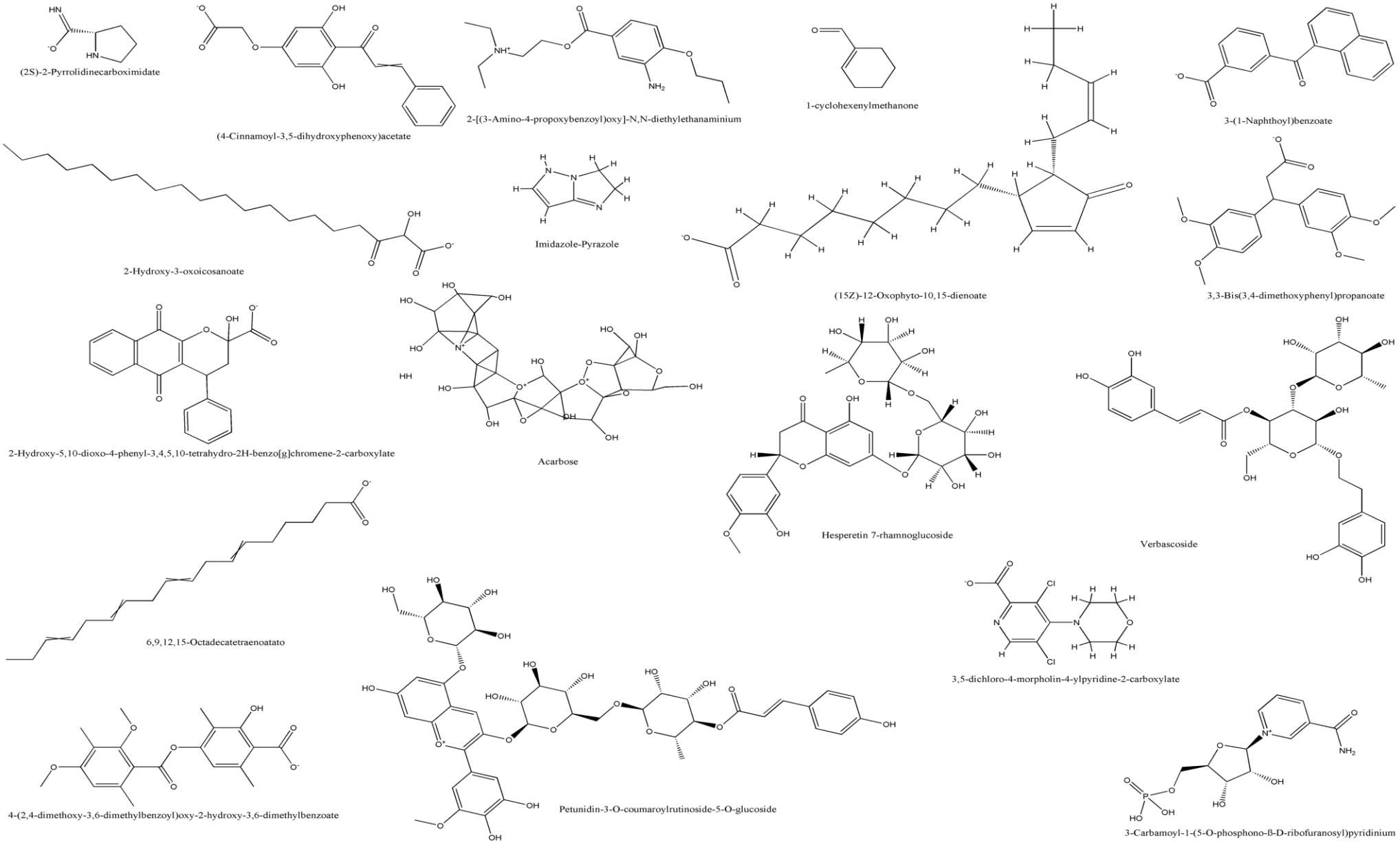

Figure 1: Seventeen phytoconstituents extracted from *Tinospora cordifolia* and standard inhibitor acarbose used as ligands for *in silico* docking with α-amylase and α-glucosidase

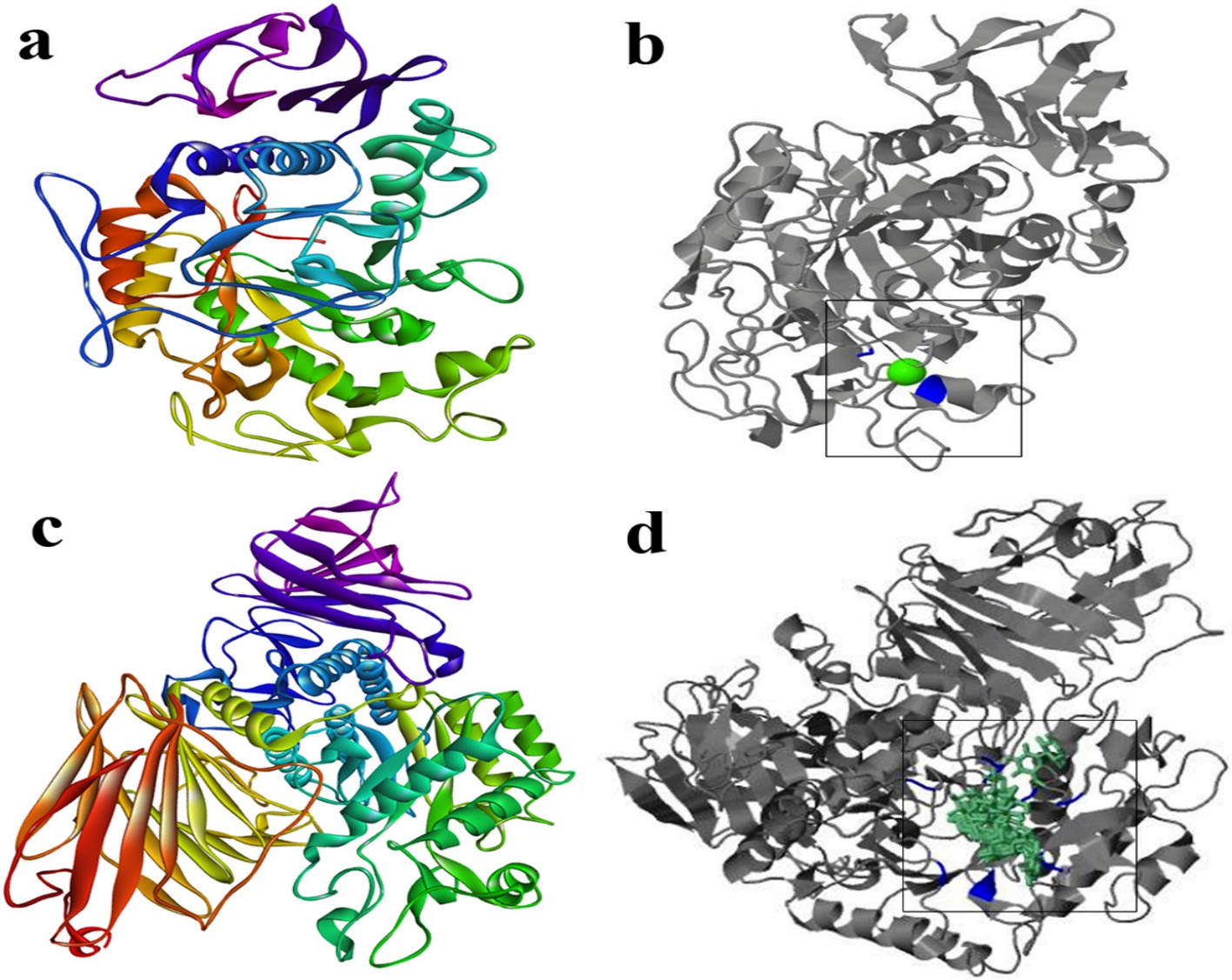

Figure 2: 3D Structures of α-amylase and α-glucosidase; **a, c** PDBQT structures of α-amylase and α-glucosidase respectively. **b, d** α-amylase and α-glucosidase with bound ligands showing ligand binding pockets predicted from 3DLigandSite server respectively

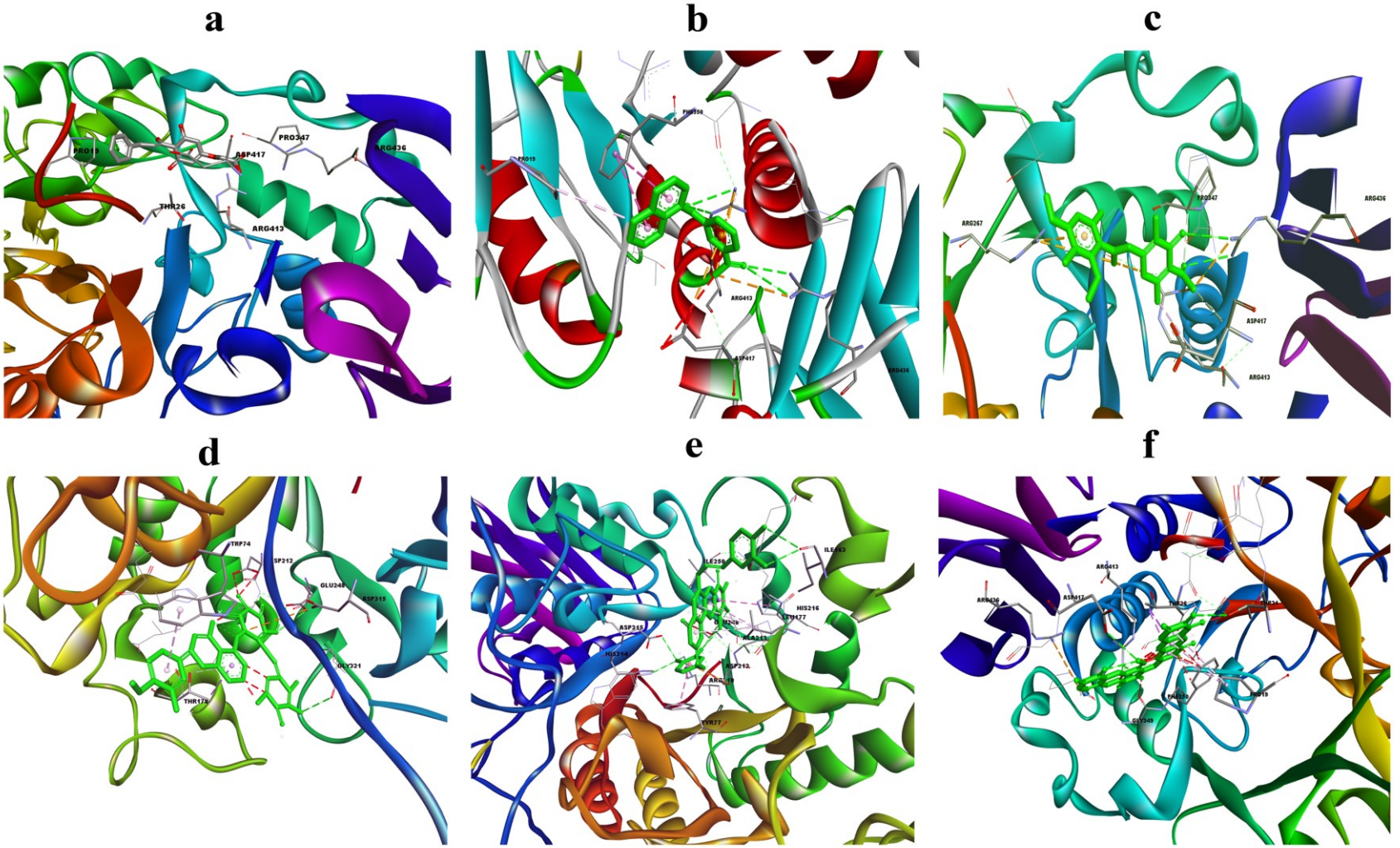

Figure 3: 3D Docking images of α-amylase-ligand complex generated using Discovery Studio 4.1 Visualizer. **a** (4_Cinnamoyl_3_5_dihydroxyphenoxy)acetate_α-amylase complex. **b** 3-(1-Naphthoyl)benzoate_ α-amylase complex. **c** 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_ α-amylase complex. **d** Hesperetin 7_rhamnoglucoside_ α-amylase complex. **e** Verbascoside_ α-amylase complex. **f** Acarbose_ α-amylase complex

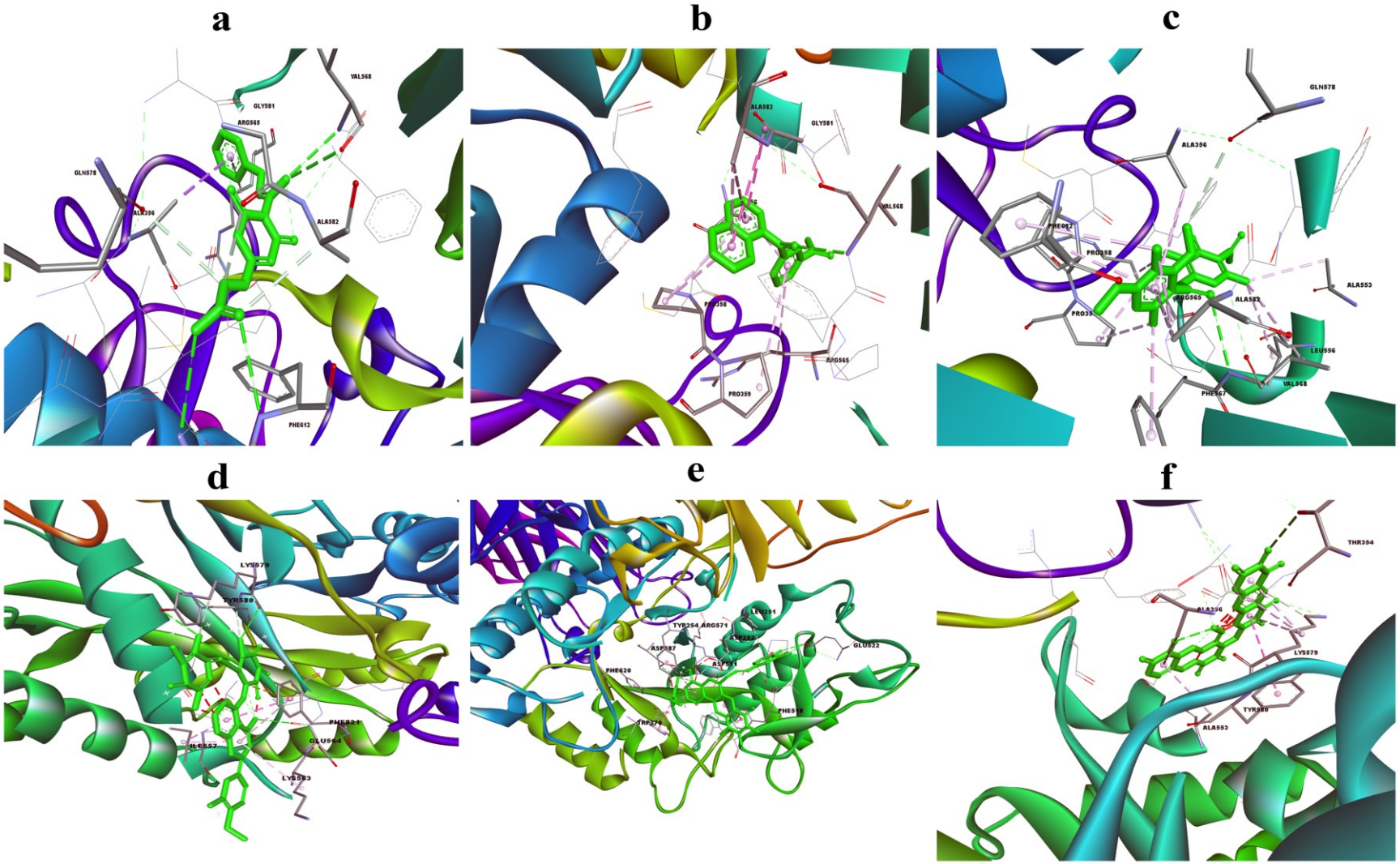

Figure 4: 3D Docking images of α-glucosidase-ligand complex generated using Discovery Studio 4.1 Visualizer. **a** (4_Cinnamoyl_3_5_dihydroxyphenoxy)acetate_ α-glucosidase complex. **b** 3-(1-Naphthoyl)benzoate_ α-glucosidase complex. **c** 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_ α-glucosidase complex. **d** Hesperetin 7_rhamnoglucoside_ α-glucosidase complex. **e** Verbascoside_ α-glucosidase complex. **f** Acarbose_ α- glucosidase complex

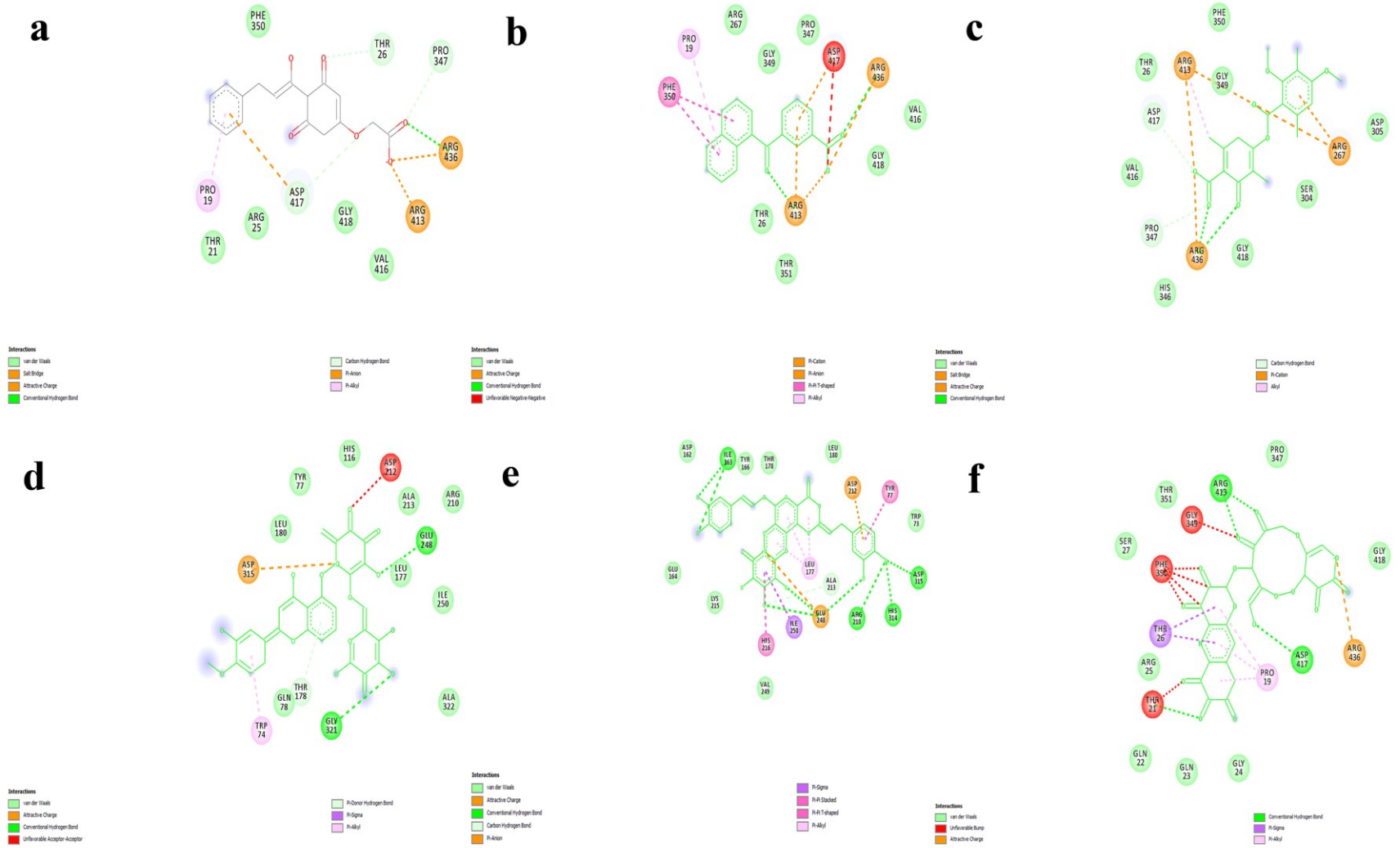

Figure 5: 2D docking images generated using Discovery Studio 4.1 Visualizer showing amino acid residues involved in interactions between α-amylase and ligands. **a** (4_Cinnamoyl_3_5_dihydroxyphenoxy)acetate_α-amylase complex. **b** 3-(1-Naphthoyl)benzoate_ α-amylase complex. **c** 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_ α-amylase complex. **d** Hesperetin 7_rhamnoglucoside_ α-amylase complex. **e** Verbascoside_ α-amylase complex. **f** Acarbose_ α-amylase complex

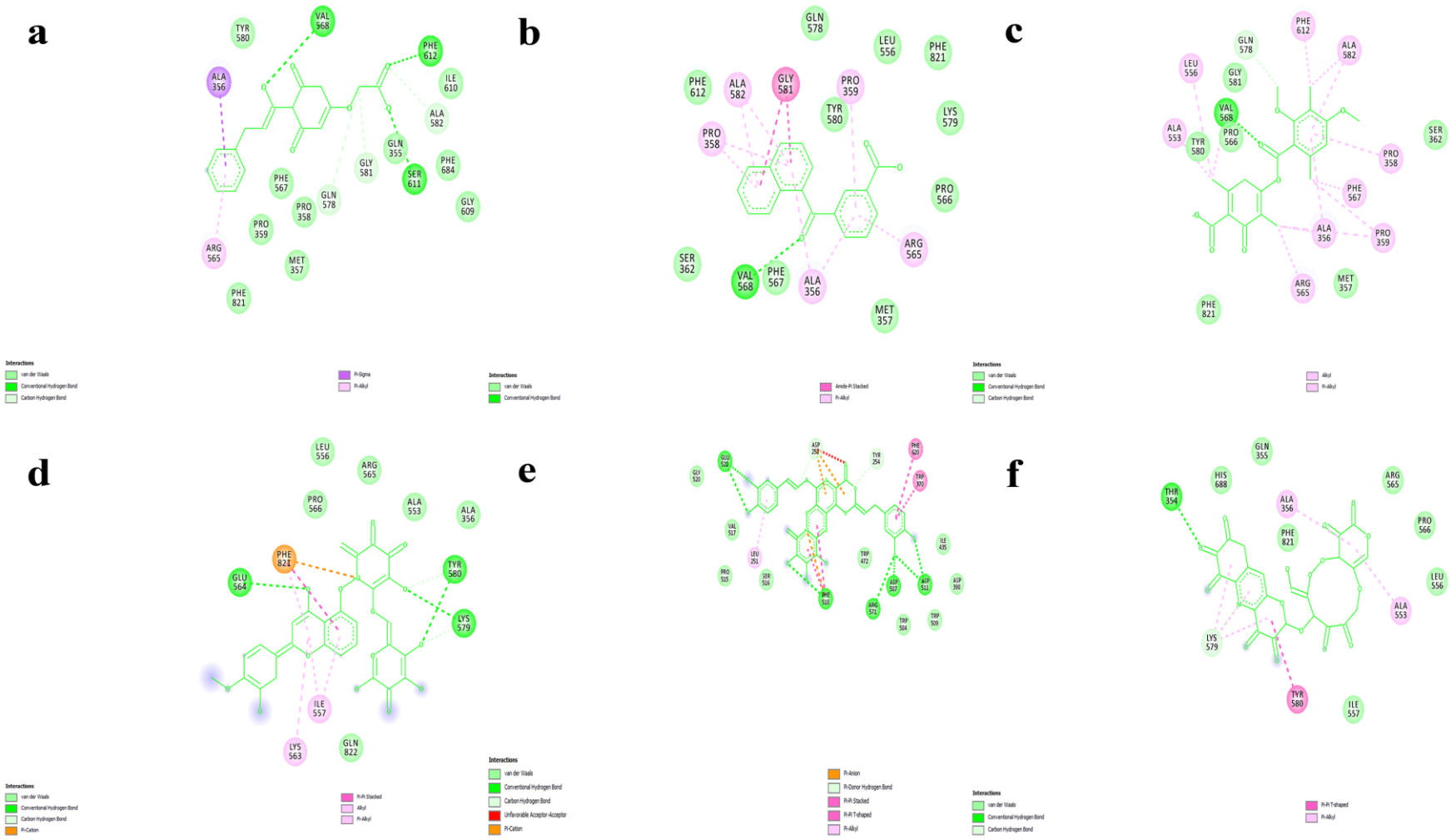

Figure 6: 2D docking images generated using Discovery Studio 4.1 Visualizer showing amino acid residues involved in interactions between α- glucosidase and ligands. **a** (4_Cinnamoyl_3_5_dihydroxyphenoxy)acetate_ α-glucosidase complex. **b** 3-(1-Naphthoyl)benzoate_ α-glucosidase complex. **c** 4-(2,4-dimethoxy-3,6-dimethylbenzoyl)oxy-2-hydroxy-3,6-dimethylbenzoate_ α-glucosidase complex. **d** Hesperetin 7_rhamnoglucoside_ α-glucosidase complex. **e** Verbascoside_ α-glucosidase complex. **f** Acarbose_ α- glucosidase complex.

Abbreviation: *T. cordifolia*_ *Tinospora cordifolia*; RMSD_ Root-Mean-Square-Deviation; PDB_ Protein Data Bank; PDBQT_ PDB File indicating partial charges and atom type.

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