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Original Research Article

Molecular Docking, Synthesis, *In-vitro* Alpha Amylase and Antibacterial Activities of Newer Generation Pyrimidine Derivatives

Sakshi Duklan [1], Supriyo Saha [1,*], Vikash Jakhmola [1], Nidhi Gairola [1], Pallavi Pandey [1], Mahipal Singh [2], Sarkar Mohammad Abe Kawsar [3]

[1] *Department of Pharmaceutical Chemistry, Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Premnagar, Dehradun-248007, Uttarakhand, India*
[2] *School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India*
[3] *Laboratory of Carbohydrate and Nucleoside Chemistry, Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong 4331, Bangladesh*

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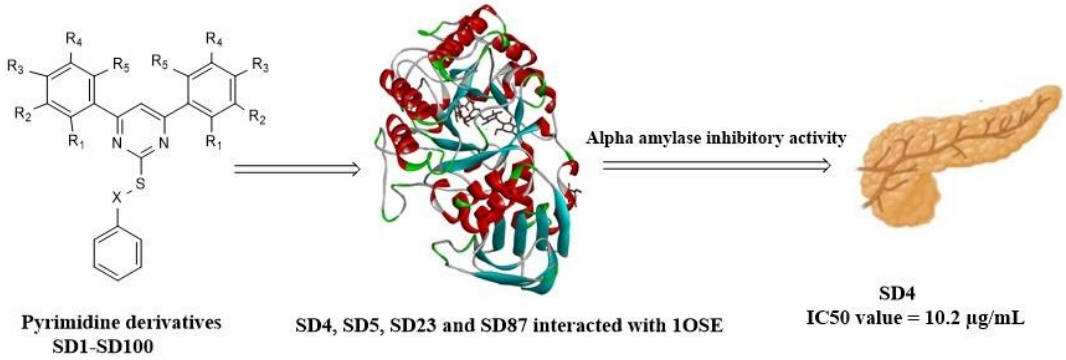
ABSTRACT

A newer generation pyrimidine derivatives were designed, synthesized, and evaluated *in vitro* alpha amylase and bacterial growth inhibitor. The molecules' design fully depended upon the structural features of previously pyrimidine derivatives. Then all the designed molecules (SD1-SD100) were docked with 1OSE pig pancreatic alpha-amylase isoenzyme. S-[4-(2-hydroxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate, S-(4,6-diphenylpyrimidin-2-yl) benzenecarbothioate, S-[4-(4-hydroxy-3-methoxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate, and S-[4,6-bis(4-hydroxyphenyl)pyrimidin-2-yl] benzenecarbothioate showed good docking interaction scores, as compared to acarbose. The interacting residues of the synthesized molecules and 1OSE showed similar amino acid lining as present in the active site. The synthetic procedure of the molecules was divided into two steps such as synthesis of chalcone derivative using aromatic aldehyde and acetophenone, reaction between chalcone and thiourea to form substituted pyrimidine-2-thiol, then finally substituted pyrimidine-2-thiol and benzoyl chloride reacted in presence of glacial acetic acid to obtain the best docked molecules. All the molecules show characteristic peaks in FTIR, ^{1}H-NMR and Mass spectrometric data. Among all the synthesized molecules S-[4-(2-hydroxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate showed best *in vitro* alpha amylase inhibition activity. Also, all synthesized molecules showed moderate to good antibacterial activities.

GRAPHICALABSTRACT



* Corresponding author: Saha, Supriyo
✉ E-mail: supriyo9@gmail.com

Introduction

Diabetes mellitus is a chronic disease in which the blood sugar level or blood glucose reaches too high. Glucose can be made by our body itself and also comes from the food we eat. Insulin is a hormone that is produced by the beta cells of islets of langerhans of the pancreas and helps to regulate or decrease the high blood sugar level. In diabetes, the beta cells of pancreas are unable to produce enough insulin or there may be the resistance create from insulin that the body has no effect from the insulin produced [1]. Also there may be damage to the pancreas, pancreatic cancer, and pancreatitis due to which it does not produce insulin. Diabetes is also known as the hyperglycaemia [2]. The glucose that stay in your blood stream does not reach to the cells causes several health problems and damage delicate internal organs progressively and effectively. Diabetes mellitus has three main types such as insulin dependent type I diabetes, non-insulin type II diabetes and gestational diabetes [3].

Various anti-diabetic agents such as chlorpropamide, tolbutamide as GLUT2 (glucose transport 2) inhibitor, metformin activated the enzyme 5'- AMP- activated protein kinase enzyme which inhibit gluconeogenesis process, rosiglitazone, and pioglitazone showed PPAR (peroxisome proliferator activating receptor) gamma agonistic activity, acarbose as alpha amylase inhibitor, miglitol as alpha glucosidase inhibitor, sitagliptin as dipeptidyl peptidase-4 inhibitor, and dapagliflozin, empagliflozin, and ipragliflozin as SGLT2i (Sodium-Glucose cotransporter Type 2 Inhibitors) [4].

Pyrimidine is a heterocyclic aromatic compound which has 6 members in the ring and somewhat similar to benzene. It has 2 nitrogen atoms at positions 1 and 3. Pyrimidines are also the nitrogenous bases in the nucleic acids DNA and RNA. Namely, Cytosine and Thymine are present in the DNA whereas in RNA Uracil and Cytosine is present. Pyrimidine is a 6-membered heterocyclic compound containing 2 nitrogen and 4 carbon atoms. Its molecular formula is $C_4H_4N_2$ [5].

The Pyrimidine ring has resonance between the atoms therefore it is stable and it provides a partial double bond characteristic. Pyrimidines are weakly basic in nature as they can be considered Lewis bases due to presence of lone pair of electrons on nitrogen atoms. Pyrimidines have planar structure and show UV absorption near 260 nm. Anagliptin and Gosogliptin are established pyrimidine derivatives effective as anti-diabetic agents [6].

Molecular docking is an important method used in drug design for predicting the binding score, binding affinity, and amino acid orientation of ligand molecules upon interaction with receptors. AutoDock Vina and Glide software were used to evaluate the most important molecular docking interactions. AutoDock Vina software used the Monte Carlo sampling technique along with the Broyden-Fletcher-Goldfarb-Shanno (BFGS) local optimization method, which produced good predictability and a shorter docking time. The Glide software used the OPLS-AA force field (Optimized Potentials for Liquid Simulations) for conformation, orientation, and position searches of docked ligands in three-dimensional spaces, and in the final stage, the Monte Carlo sampling technique was used to refine the best conformation.

MD simulation analysis was used to predict the atomic-level interaction dynamics upon drug-receptor interaction. MM/PBSA analysis was used to predict free binding energies after completing molecular dynamics simulation analysis by means of van der Waals energy, electrostatic energy, and polar solvation energy. Density functional theory analysis was used to predict the electrical and thermodynamic behavior of chemical structures. Molecular electrostatic potential analysis was used to determine the electrophilic and nucleophilic attack regions and calculate the chemical

potential of the chemical structure. In this study, we designed and synthesized newer generation pyrimidine derivatives with *in vitro* alpha amylase inhibitory and antibacterial activities.

Experimental

Materials and methods

All the chemicals were procured from Sigma Aldrich. The progression of reaction was accessed by precoated Merck TLC plates using n-hexane and ethyl acetate as solvent system with (9:1) ratio. Melting points of the samples were accessed by EI digital melting point apparatus. Infrared spectrum of synthesized molecules was recorded using Perkin Elmer Fourier Transform Infrared spectrophotometer. Proton NMR spectra were recorded using Bruker Avance DRX300 300MHz FTNMR spectrometer using dimethylsulfoxide. The chemical shifts were measured at δ units (reported as ppm) relative to Tetramethylsilane (TMS) and signals are reported as singlet (s), doublet (d), triplet (t), quartet (q), and multiple (m). Mass spectra are also recorded. FTIR, ^{1}H-NMR and Mass spectra was done by Central Instrumentation Facility of DRI, Uttaranchal University and CDRI, Lucknow. UV spectroscopy was done by the help of Shimadzu UV-1900i Spectroscopy. Elemental analysis was performed using microanalytical unit.

Molecular docking study

Preparation of protein

The protein was modelled by MGL Tools 1.5.6 package (Molecular Graphics Laboratory, the Scripps Research Institute, La Jolla, USA). 1OSE is the Crystal structure of pig pancreatic alpha-amylase isoenzyme II, in complex with the carbohydrate inhibitor acarbose [7]. At first, all the hetero atoms (water molecules and the co-crystal occupying the substrate binding site) were removed. The complexed ligand acarbose interacted with TRP 59, GLN 63, HIS 101, ASP 197, HIS 201, GLY 164, VAL 163, SER 105, GLY 106, HIS 299, ASP 300M GLU 233, HIS 305, and GLY 306 by hydrogen bond interactions; GLU 60, GLU 240, and ALA 307 by Van der Waals interactions (Figure 1).

During the process the deviation in coordinates were rectified by energy-minimization using Swiss PDB viewer (SPDBV 4.1.0, Swiss Institute of Bioinformatics). The energy-minimized protein in pdb format was then subjected to Python Molecular viewer. Later, bond orders were assigned, polar and missing hydrogens were merged with the inclusion of partial Gasteiger atomic charges. The missing hydrogens and protonation states were by H++ server. Finally, in order to make it docking software compatible, all the atoms in the protein were made to Autodock4 type (t) and the pdb file of the protein was converted to pdbqt, where q defines the charge and t for Autodock4 type [8].

Preparation of ligand molecules

The programme Avogadro was used to create the structures of the designed molecules (SD1-SD100). MMFF94 force field and steepest descent techniques were used to optimise the molecules. After that, the Open Babel programme was used to add all three-dimensional coordinates to the layout. The molecules were then saved in the PDBQT format after AutoDock Vina was used to add all of the polar hydrogens and gasteiger charges. Gasteiger charges were applied to the receptor and ligand molecules, which were then stored in the PDBQT format. The optimal ligand binding site inside each protein was found using the Lamarckian genetic algorithm [9]. The following is a list of the default grid box dimensions for protein-ligand docking. The output of the ligand-protein docking experiments was set to conformations.

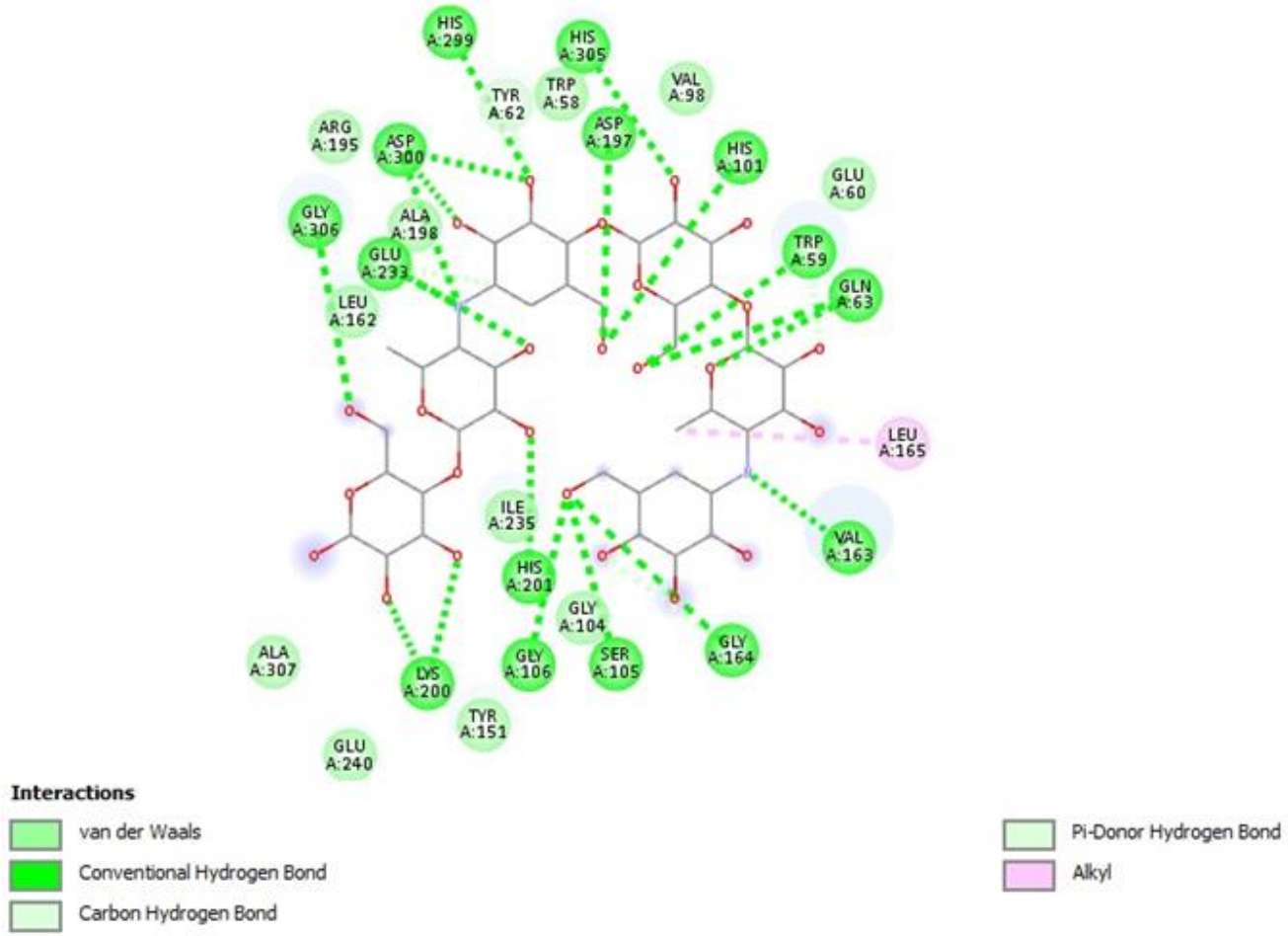


Figure 1. Interacting amino acid residues of Acarbose within 1OSE receptor.

Lastly, BIOVIA Discovery Studio Visualizer 4.5 was used to visualise and analyse the docking results. Grid size dimensions of of 1OSE: center_x = 28.087, center_y = 37.451, center_z = 3.997. size_x = 24, size_y = 24, size_z = 24, and exhaustiveness = 8.

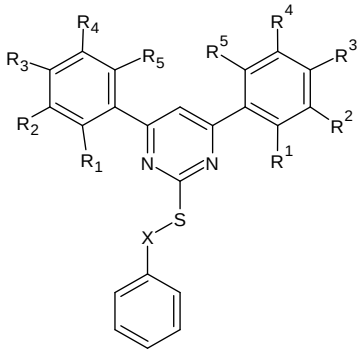
Validation of molecular docking

Validation is an important step to ensure the reliability and reproducibility of docking process. It is done through re-docking the ligand preoccupying the active site. The active site was emptied by removing the complexed ligand from the structure of the protein (PDB: 1OSE) and thereafter re-docked within the active site of the protein. The root means square deviation (RMSD) between the re-docked conformation and the unprocessed crystallographic conformation of compound was found to be <1.5 Å. This ensures the reliability of the docking method in regenerating the experimentally observed binding mode for the receptor [10].

Synthesis

Based on the docking score of the designed molecules (SD1-SD100), we synthesized top 4 molecules SD4, SD5, SD23 and SD87. Table 1 indicates all the structures of the designed pyrimidine derivatives [11].

Table 1. Designed structures of newer generation pyrimidine derivatives



SN	SAMPLE CODE	R^1	R^2	R^3	R^4	R^5	R_1	R_2	R_3	R_4	R_5	X
1	SD1	H	H	OH	H	H	H	H	H	H	H	C=O
2	SD2	OH	H	H	H	H	H	H	H	H	H	C=O
3	SD3	H	Br	H	H	H	H	H	H	OH	H	C=O
4	SD4	H	H	H	H	H	OH	H	H	H	H	C=O
5	SD5	H	H	H	H	H	H	H	H	H	H	C=O
6	SD6	H	H	H	OH	H	H	H	H	H	H	C=O
7	SD7	H	H	H	H	OH	H	H	H	H	H	C=O
8	SD8	H	H	CH_3	H	H	CH_3	H	H	H	H	C=O
9	SD9	H	H	$N(CH_3)_2$	H	H	OH	H	H	OH	H	C=O
10	SD10	H	H	Cl	H	H	H	H	H	H	H	C=O
11	SD11	H	H	H	H	H	H	H	Cl	H	H	C=O
12	SD12	H	H	H	Cl	H	H	H	H	H	H	C=O
13	SD13	Cl	H	H	H	H	Cl	H	H	H	H	C=O
14	SD14	F	H	H	H	H	H	H	H	H	H	C=O
15	SD15	H	H	NH_2	H	H	H	H	H	H	H	C=O
16	SD16	OH	H	H	H	H	H	H	OH	H	H	C=O
17	SD17	NH_2	H	H	H	H	H	H	H	H	H	C=O
18	SD18	H	H	NH_2	H	H	H	H	NH_2	H	H	C=O
19	SD19	NO_2	H	H	H	H	H	H	H	H	H	C=O
20	SD20	H	H	NO_2	H	H	H	H	NO_2	H	H	C=O
21	SD21	H	Br	H	H	H	H	H	H	H	H	C=O
22	SD22	H	Br	H	H	H	H	Br	H	H	H	C=O
23	SD23	H	H	H	H	H	H	OCH_3	OH	H	H	C=O
24	SD24	H	Cl	H	H	H	H	H	H	H	H	C=O
25	SD25	H	Cl	H	H	H	H	Cl	H	H	H	C=O
26	SD26	H	Cl	H	H	H	H	Br	H	H	H	C=O
27	SD27	H	NO_2	H	H	H	NH_2	H	H	H	H	C=O
28	SD28	H	F	H	H	H	H	Cl	H	H	H	C=O
29	SD29	H	H	F	H	H	H	Cl	H	H	H	C=O
30	SD30	H	H	H	OH	H	H	H	H	OH	H	C=O
31	SD31	H	H	H	Cl	H	H	H	H	OH	H	C=O
32	SD32	H	H	OCH_3	H	H	H	H	H	OH	H	C=O
33	SD33	H	H	OCH_3	H	H	H	H	H	H	H	C=O
34	SD34	H	H	H	H	H	H	H	H	OCH_3	H	C=O
35	SD35	H	H	OCH_3	OH	H	H	H	H	H	H	C=O
36	SD36	H	H	OCH_3	OH	H	H	H	OCH_3	OH	H	C=O

Table 1. Continued…

SN	SAMPLE CODE	R^1	R^2	R^3	R^4	R^5	R_1	R_2	R_3	R_4	R_5	X
37	SD37	H	H	H	OCH_3	H	H	H	OCH_3	H	H	C=O
38	SD38	H	H	OCH_3	OH	H	H	H	H	Cl	H	C=O
39	SD39	H	H	H	NO_2	H	H	H	OCH_3	OH	H	C=O
40	SD40	H	H	H	NH_2	H	H	H	OCH_3	OH	H	C=O
41	SD41	H	H	OCH_3	OH	H	H	H	NO_2	H	H	C=O
42	SD42	H	H	OCH_3	OH	H	H	H	H	NO_2	H	C=O
43	SD43	H	H	H	OCH_3	OH	H	H	H	NO_2	H	C=O
44	SD44	H	OCH_3	OH	H	H	H	H	OCH_3	OH	H	C=O
45	SD45	H	OCH_3	OH	H	H	H	H	H	OCH_3	OH	C=O
46	SD46	H	OCH_3	OH	H	H	H	OCH_3	OH	H	H	C=O
47	SD47	H	OCH_3	OH	H	H	H	H	H	H	NO_2	C=O
48	SD48	OCH_3	OH	H	H	H	H	H	H	H	H	C=O
49	SD49	OCH_3	OH	H	H	H	OCH_3	OH	H	H	H	C=O
50	SD50	OCH_3	OH	H	H	H	F	H	H	H	H	C=O
51	SD51	OCH_3	OH	H	H	H	Br	H	H	H	H	C=O
52	SD52	OCH_3	OH	H	H	H	Cl	H	H	H	H	C=O
53	SD53	OCH_3	OH	H	H	H	NH_2	H	H	H	H	C=O
54	SD54	OCH_3	OH	H	H	H	H	F	H	H	H	C=O
55	SD55	OCH_3	OH	H	H	H	H	Cl	H	H	H	C=O
56	SD56	OCH_3	OH	H	H	H	H	Br	H	H	H	C=O
57	SD57	OCH_3	OH	H	H	H	H	NH_2	H	H	H	C=O
58	SD58	OCH_3	OH	H	H	H	H	H	F	H	H	C=O
59	SD59	OCH_3	OH	H	H	H	H	H	Cl	H	H	C=O
60	SD60	OCH_3	OH	H	H	H	H	H	Br	H	H	C=O
61	SD61	OCH_3	OH	H	H	H	H	H	NH_2	H	H	C=O
62	SD62	OCH_3	OH	H	H	H	H	H	H	F	H	C=O
63	SD63	OCH_3	OH	H	H	H	H	H	H	Br	H	C=O
64	SD64	OCH_3	OH	H	H	H	H	H	H	Cl	H	C=O
65	SD65	OCH_3	OH	H	H	H	H	H	H	NH_2	H	C=O
66	SD66	OCH_3	OH	H	H	H	H	H	H	H	F	C=O
67	SD67	OCH_3	OH	H	H	H	H	H	H	H	Cl	C=O
68	SD68	OCH_3	OH	H	H	H	H	H	H	H	Br	C=O
69	SD69	OCH_3	OH	H	H	H	H	H	H	H	NH_2	C=O
70	SD70	H	CH_3	OH	H	H	H	H	H	H	H	C=O
71	SD71	H	CH_3	OH	H	H	H	H	F	H	H	C=O
72	SD72	H	CH_3	OH	H	H	H	H	Cl	H	H	C=O
73	SD73	H	CH_3	OH	H	H	H	H	Br	H	H	C=O
74	SD74	H	CH_3	OH	H	H	H	H	NH_2	H	H	C=O
75	SD75	H	CH_3	OH	H	H	H	H	H	F	H	C=O
76	SD76	H	CH_3	OH	H	H	H	H	H	Cl	H	C=O
77	SD77	H	CH_3	OH	H	H	H	H	H	Br	H	C=O
78	SD78	H	CH_3	OH	H	H	H	H	H	NH_2	H	C=O
79	SD79	H	CH_3	OH	H	H	H	H	H	H	F	C=O
80	SD80	H	CH_3	OH	H	H	H	H	H	H	Cl	C=O
81	SD81	H	CH_3	OH	H	H	H	H	H	H	Br	C=O
82	SD82	H	CH_3	OH	H	H	H	H	H	H	NH_2	C=O

Table 1. Continued…

SN	SAMPLE CODE	R^1	R^2	R^3	R^4	R^5	R_1	R_2	R_3	R_4	R_5	X
83	SD83	H	H	CH_3	OH	H	H	H	H	H	F	C=O
84	SD84	H	H	CH_3	OH	H	H	H	H	H	Cl	C=O
85	SD85	H	H	CH_3	OH	H	H	H	H	H	Br	C=O
86	SD86	H	H	OH	H	H	OH	H	H	H	H	C=O
87	SD87	H	H	OH	H	H	H	H	OH	H	H	C=O
88	SD88	H	H	CH_3	OH	H	H	H	H	Cl	H	C=O
89	SD89	H	H	CH_3	OH	H	H	H	H	Br	H	C=O
90	SD90	H	H	CH_3	OH	H	H	H	H	NH_2	H	C=O
91	SD91	H	H	H	CH_3	OH	H	F	H	H	H	C=O
92	SD92	H	H	H	CH_3	OH	H	Cl	H	H	H	C=O
93	SD93	H	H	H	CH_3	OH	H	Br	H	H	H	C=O
94	SD94	H	H	H	CH_3	OH	H	NH_2	H	H	H	C=O
95	SD95	H	H	H	CF_3	OH	H	H	H	H	H	C=O
96	SD96	H	H	H	CF_3	OH	H	F	H	H	H	C=O
97	SD97	H	H	H	CF_3	OH	H	Cl	H	H	H	C=O
98	SD98	H	H	H	CF_3	OH	H	Br	H	H	H	C=O
99	SD99	H	H	H	CF_3	OH	H	NH_2	H	H	H	C=O
100	SD100	H	Cl	H	H	CF_3	H	H	NH_2	H	H	C=O

Synthesis of S-[4-(2-hydroxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate (SD4)

In the first step, 2-hydroxybenzaldehyde and acetophenone reacted in presence of methanol to produce (2*E*)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one followed by reaction with thiourea to obtain 2-(6-phenyl-2-sulfanylpyrimidin-4-yl)phenol. Finally, 2-(6-phenyl-2-sulfanylpyrimidin-4-yl)phenol and benzoyl chloride reacted in presence of glacial acetic to get final 2S-[4-(2-hydroxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate.

SD4: Yield= 42.51%, Melting point= 128˚C. FT-IR (KBr) v/cm^{-1}: 3431 (OH str), 1667 (C=O str), 2925 (C=S str), and 1812-1994 (Aromatic ring str). ^{1}H-NMR DMSO (300 MHz) δ ppm: 2.50 (-CH_2- str), 9.31 (OH str), 7.13 (=CH- str), (7.29-8.77) Ar-H str. $C_{23}H_{16}N_2O_2S$: original C: 71.84%, H: 4.16%, N: 7.29%, and O: 8.33%. $C_{23}H_{16}N_2O_2S$: calculated C: 70.92%, H: 4.12%, N: 7.14%, and O: 8.21%. (M+1): 384.45 (EIMS).

Synthesis of S-(4,6-diphenylpyrimidin-2-yl) benzenecarbothioate (SD5)

In the first step, benzaldehyde and acetophenone reacted in presence of methanol to produce (2E)-1,3-diphenylprop-2-en-1-one followed by reaction with thiourea to obtain 4,6-diphenylpyrimidine-2-thiol. Finally 4,6-diphenylpyrimidine-2-thiol and benzoyl chloride reacted in presence of glacial acetic to get final 2S-[4-(2-hydroxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate.

SD5: Yield= 38.48%, Melting point = 122˚C. FT-IR (KBr) v/cm^{-1}: 3403 (OH str), 1685 (C=O str), 2914 (C=S str), and 1883-2032 (Aromatic ring str). ^{1}H-NMR DMSO (300 MHz) δ ppm: 2.50 (-CH_2- str), 8.14 (OH str), 7.43 (=CH- str), (7.52-8.09) Ar-H str. $C_{23}H_{16}N_2OS$: original C: 75.0%, H: 4.34%, N: 7.68%, and O: 4.34 %. $C_{23}H_{16}N_2OS$: calculated C: 74.82%, H: 4.21%, N: 7.45%, and O: 4.14%. (M+1): 368.45 (EIMS).

Synthesis of S-[4-(4-hydroxy-3-methoxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate (SD23)

In the first step, 4-hydroxy-3-methoxybenzaldehyde and acetophenone

reacted in presence of methanol to produce (2E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one followed by reaction with thiourea to obtain 4,6-diphenylpyrimidine-2-thiol. Finally, (2E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one and benzoyl chloride reacted in presence of glacial acetic to get final S-[4-(4-hydroxy-3-methoxyphenyl)-6-phenylpyrimidin-2-yl] benzenecarbothioate.

SD23: Yield= 34.54%, Melting point = 132˚C. FT-IR (KBr) v/cm^{-1}: 3343 (OH str), 1787 (C=O str), 2932 (C=S str), and 1822-2043 (Aromatic ring str). ^{1}H-NMR DMSO (300 MHz) δ ppm: 2.50 (-CH_2- str), 8.92 (OH str), 7.14 (=CH- str), (7.47-8.90) Ar-H str. $C_{24}H_{18}N_2O_3S$: original C: 69.56 %, H: 4.34%, N: 6.76 %, and O: 11.59%. $C_{24}H_{18}N_2O_3S$: calculated C: 69.16%, H: 4.16%, N: 6.51%, and O: 11.42%. (M+1): 414.47 (EIMS).

Synthesis of S-[4,6-bis(4-hydroxyphenyl)pyrimidin-2-yl] benzenecarbothioate (SD87)

In the first step, 4-hydroxybenzaldehyde and 4-hydroxyacetophenone reacted in presence of methanol to produce (2E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one followed by reaction with thiourea to obtain (2E)-1,3-bis(4-hydroxyphenyl)prop-2-en-1-one. Finally, (2E)-1,3-bis(4-hydroxyphenyl)prop-2-en-1-one and benzoyl chloride reacted in presence of glacial acetic to get final S-[4,6-bis(4-hydroxyphenyl)pyrimidin-2-yl] benzenecarbothioate.

SD87: Yield= 37.74%, Melting point = 121˚C. FT-IR (KBr) v/cm^{-1}: 3329 (OH str), 1720 (C=O str), 2976 (C=S str), and 1821-2040 (Aromatic ring str). ^{1}H-NMR DMSO (300 MHz) δ ppm: 2.50 (-CH_2- str), 8.87 (OH str), and 7.11 (=CH- str), (6.98-8.48) Ar-H str. $C_{23}H_{16}N_2O_3S$: original C: 69.00%, H: 4.00%, N: 7.00%, and O: 12.00%. $C_{23}H_{16}N_2O_3S$: calculated C: 57.942%, H: 3.88%, N: 6.88%, and O: 11.88%. (M+1): 400.041 (EIMS).

In vitro alpha amylase inhibition assay

Stock solutions of synthesized molecules were prepared in two phases, in the first step 100 mg of drug sample dissolved in 1 mL DMSO followed by dilute upto 100 mL in methanol, and then prepared 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 60 µg/mL solutions using methanol as diluents. Standard acarbose solution was prepared in water followed by its consecutive dilutions in the same solvent. In 96-well plate reaction mixture 50 µl and 100 mM, pH = 6.8 phosphate buffer, 10 µl 2 Unit/mL α–amylase, and 20 µl of synthesized molecules or standard acarbose were incubated at 37 °C for 20 minute, and then 20 µl of 1% soluble starch (prepared in 100 mM phosphate buffer pH 6.8) was added and incubated at 37 °C for 30 minutes. Further 100 µl of the Dinitrosalicylic acid was added and boiled for 10 minutes. The absorbance of the resulting mixture was taken at 540 nm using UV-Visible spectroscopy (UV-1900i). Acarbose at various concentrations was considered as standard. Control was considered as reagent without sample solution and every experiment was performed in triplicate manner [12].

Antibacterial efficacies of synthesized compounds

10 mg of synthesized molecules (SD4, SD5, SD23, and SD87) was dissolved separately in mg of drug sample dissolved in 1 mL DMSO followed by dilute up to 100 mL in methanol, and then prepared 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, and 6 µg/mL solutions using same diluent. A series of Gram +ve and Gram –ve bacterial strains such as *B. subtilis (+ve), B. cereus* (+ve), *S. aureus* (+ve), *E. coli* (-ve), *P. aeruginosa* (-ve), and *S. typhi* (-ve) were considered for antibacterial assessment using disc diffusion method [13]. The cultures were maintained on nutrient agar slants and incubated at 37 °C for 18-24 h. The nutrient agar medium was prepared by solubilizing peptone, beef extract, sodium chloride, and agar in distilled water followed by pH adjustment and sterilization at 121 °C 15 psi

pressure for 15-30 min. In the same manner nutrient broth was prepared using peptone, beef extract, and sodium chloride. 25 mL of pre autoclaved nutrient agar medium as poured into 90 mm diameter pre sterilized petriplates under aseptic conditions and solidified at room temperature. Fresh microorganism cultures were developed and diluted with sterile physiological solution. 100 µl suspension of test microorganism containing 1.5 × 10^8 CFU/mL of bacteria was inoculated on the surface of agar plates. Three sterile discs of 6 mm diameter were placed on each microorganisms containing agar plate, and then 30 µl of drug solutions were dropped into disc under sterile conditions and incubated at 37±0.1 °C for 24 h [14]. After incubation, the zone of inhibition was measured in mm on all plates. Experiments were performed in triplicate manner. Tetracycline (5 µg/mL) was used as standard. A disc of pure methanol was used as negative control.

Results and Discussion

Molecular docking study

The molecular docking studies of the designed pyrimidine derivatives (SD1-SD100) with 1OSE receptor showed docking scores ranges from -5.2 kcal/mol to -9.4 kcal/mol whereas standard acarbose showed docking score of -6.9 kcal/mol. All the synthesized molecules effectively bound within the receptor active site and shared similar amino acid residues as complexed ligand. SD4, SD5, SD23, and SD87 showed good docking interaction scores of -9.4 kcal/mol, -9.2 kcal/mol, -9.1 kcal/mol, and -9.0 kcal/mol, respectively. SD4 interacted with VAL163 by pi-sigma interaction, LEU162 and ILE235 by pi-alkyl interaction, TYR62 by pi-pi interaction, and HIS201 by hydrogen bonding [15].

SD5 interacted with GLY306, GLU240, LYS200, and ASP300 by hydrogen bond interactions. SD23 interacted with TYR62, LEU162, LYS200, ILE325 by pi-pi interactions; HIS201 by pi-cation interaction; and GLY304 by hydrogen bond interaction. SD85 interacted with VAL163, LEU162, and ILE235 by pi-alkyl interactions; HIS101 and LYS200 by hydrogen bond interactions; and also TYR151 and TYR62 by pi-pi interactions. These below mentioned amino acid residues are similar between synthesized molecules and complexed ligand: SD4: VAL163; SD5: GLY306, GLU240, ASP300; SD23: HIS201; SD85: VAL163, and HIS101 (Figure 2 and Table 2) [16,17].

Table 2. Molecular docking interaction data between of SD1-SD100 and standard Acarbose

SN	Code of the Molecules	Receptor used	Dock Score (kcal/mol)	Interacting residues
1	SD1	1OSE	-7.1	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e,
2	SD2	1OSE	-7.8	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
3	SD3	1OSE	-6.8	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
4	SD4	1OSE	-9.4	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
5	SD5	1OSE	-9.2	GLY306e, GLU240e, LYS200e, ASP300e
6	SD6	1OSE	-6.4	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
7	SD7	1OSE	-7.1	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
8	SD8	1OSE	-7.0	TYR62b, LEU162a, LYS200a ILE325a, GLY304e
9	SD9	1OSE	-6.7	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e

Table 2. Continued….

SN	Code of the Molecules	Receptor used	Dock Score (kcal/mol)	Interacting residues
10	SD10	1OSE	-6.8	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
11	SD11	1OSE	-6.6	LEU162a, TRP59a, VAL163a, ALA198a, TYR151b, HIS201, ILE235e
12	SD12	1OSE	-6.9	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
13	SD13	1OSE	-6.4	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
14	SD14	1OSE	-6.2	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
15	SD15	1OSE	-6.9	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
16	SD16	1OSE	-7.3	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
17	SD17	1OSE	-7.0	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
18	SD18	1OSE	-7.0	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
19	SD19	1OSE	-7.1	VAL163, LEU162a, ILE235a, TYR62b, HIS201e
20	SD20	1OSE	-6.6	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
21	SD21	1OSE	-6.4	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b
22	SD22	1OSE	-6.3	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
23	SD23	1OSE	-9.1	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
24	SD24	1OSE	-6.8	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
25	SD25	1OSE	-5.9	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
26	SD26	1OSE	-6.0	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b
27	SD27	1OSE	-6.2	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
28	SD28	1OSE	-6.1	TYR62b, LEU162a, LYS200a ILE325a, HIS201c
29	SD29	1OSE	-6.2	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
30	SD30	1OSE	-6.3	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
31	SD31	1OSE	-6.5	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e
32	SD32	1OSE	-6.2	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
33	SD33	1OSE	-6.4	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
34	SD34	1OSE	-6.6	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
35	SD35	1OSE	-6.1	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g

Table 2. Continued….

SN	Code of the Molecules	Receptor used	Dock Score (kcal/mol)	Interacting residues
36	SD36	1OSE	-5.9	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e
37	SD37	1OSE	-6.2	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
38	SD38	1OSE	-6.4	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
39	SD39	1OSE	-6.8	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
40	SD40	1OSE	-7.6	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
41	SD41	1OSE	-5.9	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e
42	SD42	1OSE	-6.0	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
43	SD43	1OSE	-6.1	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
44	SD44	1OSE	-6.6	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
45	SD45	1OSE	-6.7	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
46	SD46	1OSE	-6.9	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
47	SD47	1OSE	-7.2	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
48	SD48	1OSE	-6.8	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
49	SD49	1OSE	-6.9	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
50	SD50	1OSE	-6.2	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
51	SD51	1OSE	-6.2	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
52	SD52	1OSE	-6.3	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
53	SD53	1OSE	-6.5	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
54	SD54	1OSE	-6.8	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
55	SD55	1OSE	-6.5	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
56	SD56	1OSE	-6.7	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e,
57	SD57	1OSE	-6.9	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
58	SD58	1OSE	-6.4	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
59	SD59	1OSE	-6.6	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
60	SD60	1OSE	-6.7	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g

Table 2. Continued….

SN	Code of the Molecules	Receptor used	Dock Score (kcal/mol)	Interacting residues
61	SD61	1OSE	-6.8	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b,
62	SD62	1OSE	-6.8	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
63	SD63	1OSE	-5.5	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
64	SD64	1OSE	-5.9	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
65	SD65	1OSE	-5.8	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
66	SD66	1OSE	-5.4	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
67	SD67	1OSE	-5.2	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
68	SD68	1OSE	-5.7	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
69	SD69	1OSE	-5.9	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
70	SD70	1OSE	-6.1	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
71	SD71	1OSE	-6.2	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
72	SD72	1OSE	-6.4	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
73	SD73	1OSE	-6.7	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
74	SD74	1OSE	-6.5	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
75	SD75	1OSE	-6.6	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
76	SD76	1OSE	-6.7	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b
77	SD77	1OSE	-6.5	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
78	SD78	1OSE	-6.6	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
79	SD79	1OSE	-6.7	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
80	SD80	1OSE	-6.4	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
81	SD81	1OSE	-5.6	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200h
82	SD82	1OSE	-5.9	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
83	SD83	1OSE	-6.4	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
84	SD84	1OSE	-6.2	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
85	SD85	1OSE	-6.2	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g

Table 2. Continued….

SN	Code of the Molecules	Receptor used	Dock Score (kcal/mol)	Interacting residues
86	SD86	1OSE	-6.6	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, HIS201, ILE235e,
87	SD87	1OSE	-9.0	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
88	SD88	1OSE	-7.6	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
89	SD89	1OSE	-6.7	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
90	SD90	1OSE	-6.8	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
91	SD91	1OSE	-6.7	LEU162a, TRP59a, VAL163a, ALA198a, TYR151b, HIS201, ILE235e, GLU233e,
92	SD92	1OSE	-6.1	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
93	SD93	1OSE	-6.8	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
94	SD94	1OSE	-6.4	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
95	SD95	1OSE	-6.2	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
96	SD96	1OSE	-6.4	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a,
97	SD97	1OSE	-6.7	VAL163a, LEU162a, ILE235a, HIS101e, TYR151b, TYR62b, LYS200e
98	SD98	1OSE	-6.6	TYR62b, LEU162a, LYS200a ILE325a, HIS201c, GLY304e
99	SD99	1OSE	-6.7	VAL163g, LEU162a, ILE235a, TYR62b, HIS201e
100	SD100	1OSE	-6.6	GLY306e, GLU240e, LYS200e, ASP300e, HIS201e, HIS201g
101	Acarbose	1OSE	-6.9	LEU162a, TRP59a, VAL163a, ALA198a, ILE235a, LYS200a, TYR151b, HIS201, ILE235e, GLU233e, LYS200g

a: pi-alkyl interactions; b: pi-pi interactions; c: pi-cation/anion interaction; d: pi-sulfur interaction; e: hydrogen bond; f: van der waals interactions; g: pi-sigma interactions

Synthesis

All the synthesized molecules were characterized by melting point, FTIR, ^{1}H-NMR, Mass Spectrometry, and elemental analysis. The synthesized molecules showed single peak during chromatographic assessments. All the spectroscopic characterization data confirmed the synthesis of the designed molecules [18].

In vitro alpha amylase inhibition assay of the synthesized molecules

The IC_{50} values of all the synthesized molecules (SD4, SD5, SD23, and SD87) as per *in vitro* alpha amylase inhibition assay were 10.2 µg/mL, 10.41 µg/mL, 16.88 µg/mL, and 20.0 µg/mL, respectively whereas the IC_{50} value of standard acarbose was 66.65 µg/mL [19]. These data were obtained after three consecutive experiments to minimize the error. Among all the synthesized molecules SD4 showed best activity. The activity profile of the molecule was SD4>SD5> SD23> SD87 (Table 3) [20].

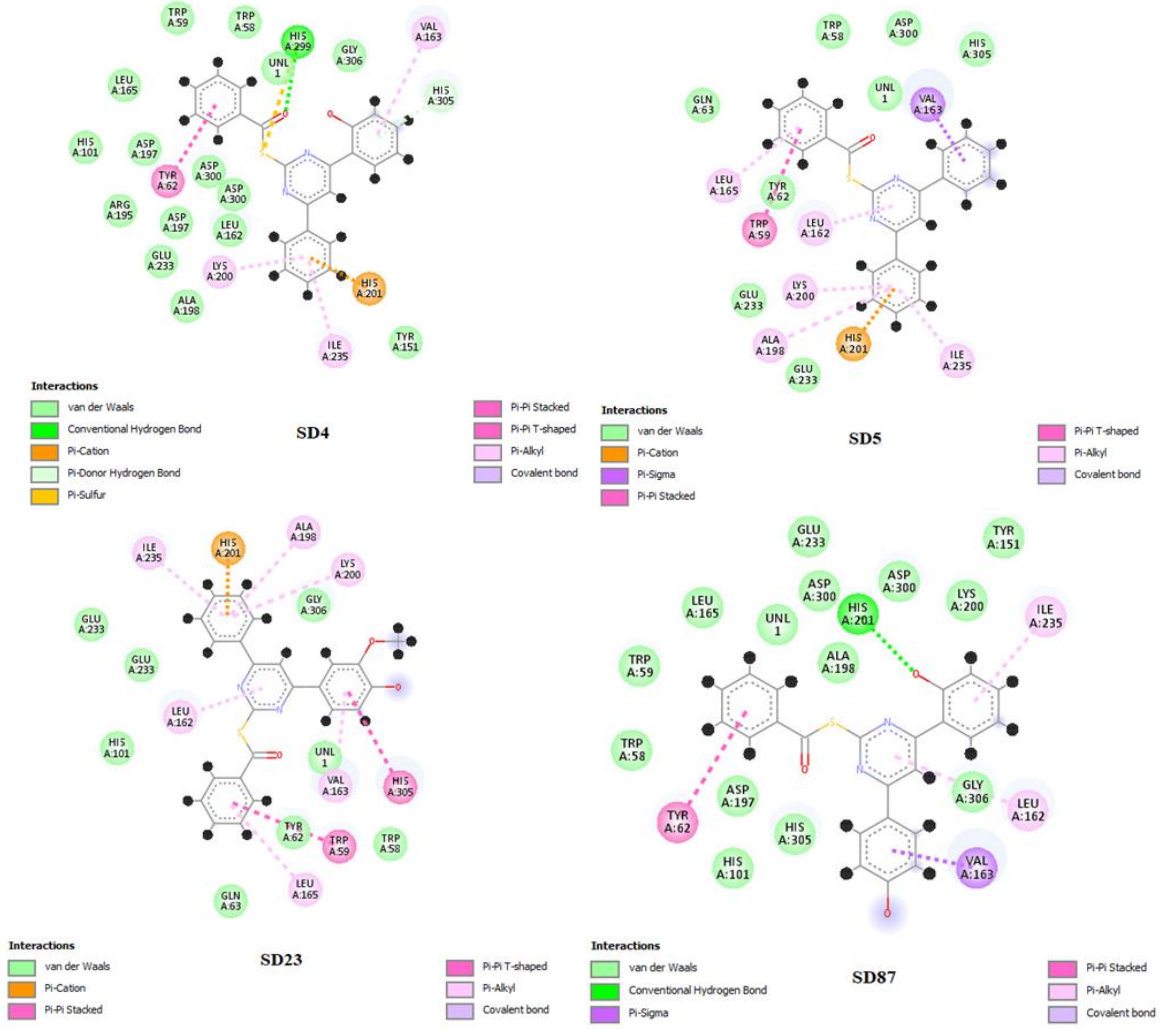


Figure 2. Molecular docking interaction between Synthesized Molecules (SD4, SD5, SD23, and SD85) and 1OSE receptor.

Antibacterial activity of the synthesized molecules

All the synthesized molecules (SD4, SD5, SD23, and SD87) were evaluated against three Gram +ve and three Gram –ve bacterial strains such as *B. subtilis (+ve), B. cereus* (+ve), *S. aureus* (+ve), *E. coli* (-ve), *P. aeruginosa* (-ve), and *S. typhi* (-ve) by considering tetracycline as reference standard. In case of Gram +ve bacteria: the growth of *B. subtilis*, *B. cereus*, and *S. aureus* were maximum inhibited by SD5, SD87, and SD87, respectively. In case of Gram -ve bacteria: the growth of *E. coli*, *P. aeruginosa*, and *S. typhi* were maximum inhibited by SD4, SD4 and SD23, respectively. The reference tetracycline showed higher zone of inhibitions than synthesized molecules against *B. subtilis (+ve), B. cereus* (+ve), *S. aureus* (+ve), *E. coli* (-ve), *P. aeruginosa* (-ve), and *S. typhi* (-ve) with 28 mm, 29 mm, 29 mm, 31 mm, 28 mm, and 27 mm, respectively. The MIC value (Minimum Inhibitory Concentration) of SD4 against *B. subtilis, B. cereus, S. aureus, E. coli, P. aeruginosa,* and *S. typhi* was 8 µg/mL. MIC value of SD5 against *B. subtilis, B. cereus, E. coli,* and *P. aeruginosa, S. typhi* was 8 µg/mL and against *S. aureus* was 4 µg/mL. MIC value of SD23 against *B. subtilis, B. cereus, S. aureus, E. coli, and P. aeruginosa* was 8 µg/mL and against *S. typhi* was

4 µg/mL. MIC value of SD87 against *B. subtilis, S. aureus, E. coli, P. aeruginosa, and S. typhi* was 8 µg/ml and against *B. cereus* was 4 µg/mL [21]. In overall synthesized molecules showed good to moderate antibacterial activities as compare to standard tetracycline (Table 4).

Conclusion

The development of newer generation pyrimidine derivatives was successfully designed, synthesized, chemically characterized, and biologically evaluated as *in vitro* alpha amylase inhibitor and antibacterial activities. Molecular docking studies of the designed (SD1-SD100) molecules against alpha amylase receptor, which showed that SD4, SD5, SD23, and SD87 observed with good docking scores as compare to standard acarbose. Synthesis of the molecules was carried using simple synthetic procedures and characterized by different spectroscopic methods. Among all the synthesized molecules SD4 showed best activity. The activity profile of the molecule was SD4>SD5> SD23> SD87. Also, all synthesized molecules showed moderate to good antibacterial activities.

Table 3. *In vitro* alpha amylase inhibition assay of synthesized molecules SD4, SD5, SD23, SD87 and Acarbose

SN	Code of the Molecules	Concentration (µg/mL)	% Alpha Amylase Inhibition	IC50
1	SD4	10	49.01	10.2
		20	53.92	
		30	60.29	
		40	70.09	
		60	71.07	
2	SD5	10	48.03	10.41
		20	53.43	
		30	61.76	
		40	71.56	
		60	79.41	
3	SD23	10	43.62	16.88
		20	53.43	
		30	56.86	
		40	72.54	
		60	79.9	
4	SD87	10	36.76	20
		20	50	
		30	58.82	
		40	69.6	
		50	81.37	
		60	43.62	
5	Acarbose	10	5.88	66.65
		20	9.8	
		30	17.64	
		40	30.39	
		60	40.68	
		50	45.09	
		60	52.45	

Table 4. Antibacterial activities of synthesized molecules SD4, SD5, SD23, SD87 and tetracycline

SN	Sample Code	Concentration (µg/mL)	Diameter of Inhibition Zone (mm)					
			B. subtilis (+ve)	B. cereus (+ve)	S. aureus (+ve)	E. coli (-ve)	P. aeruginosa (-ve)	S. typhi (-ve)
1	S4	4	NI	NI	NI	NI	NI	NI
		8	3±0.08	2±0.07	2±0.10	4±0.11	2±0.11	4±0.12
		16	5±0.12	7±0.09	5±0.15	9±0.12	6±0.05	9±0.18
		32	12±0.04	11±0.15	13±0.12	19±0.14	15±0.08	13±0.2
		64	19±0.14	18±0.06	21±0.3	27±0.21	21±0.08	18±0.17
2	S5	4	NI	NI	2 ±0.04	NI	NI	NI
		8	4±0.07	6±0.12	7±0.07	7±0.12	6±0.02	6±0.16
		16	9±0.16	7±0.19	9±0.02	8±0.13	8±0.02	6±0.
		32	16±0.14	9±0.13	13±0.02	11±0.18	11±0.13	9±0.14
		64	23±0.19	16±0.21	19±0.4	16±0.22	15±0.02	14±0.18
3	S23	4	NI	NI	NI	NI	NI	4±0.14
		8	6±0.17	7±0.15	5±0.13	6±0.21	5±0.11	9±0.16
		16	9±0.14	9±0.06	7±0.02	7±0.22	8±0.08	12±0.02
		32	12±0.12	11±0.09	9±0.02	10±0.04	11±0.08	16±0.24
		64	17±0.20	15±0.11	13±0.02	13±0.02	18±0.02	20±0.02
4	S87	4	NI	3±0.17	NI	NI	NI	NI
		8	5±0.02	6±0.16	6±0.02	5±0.04	6±0.16	7±0.18
		16	9±0.02	9±0.02	10±0.02	8±0.07	8±0.13	9±0.16
		32	12±0.02	11±0.02	15±0.02	11±0.02	12±0.18	13±0.14
		64	16±0.14	20±0.23	22±0.16	16±0.11	17±0.12	19±0.18
5.	Tetracycline	5	28±0.06	29±0.05	29±0.006	31±0.06	28±0.05	27±0.09

NI: No Inhibition

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Orcid

Supriyo Saha: 0000-0003-1365-4698
Vikash Jakhmola: 0000-0002-8108-006X
Sarkar M. A. Kawsar: 0000-0001-7964-9117

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