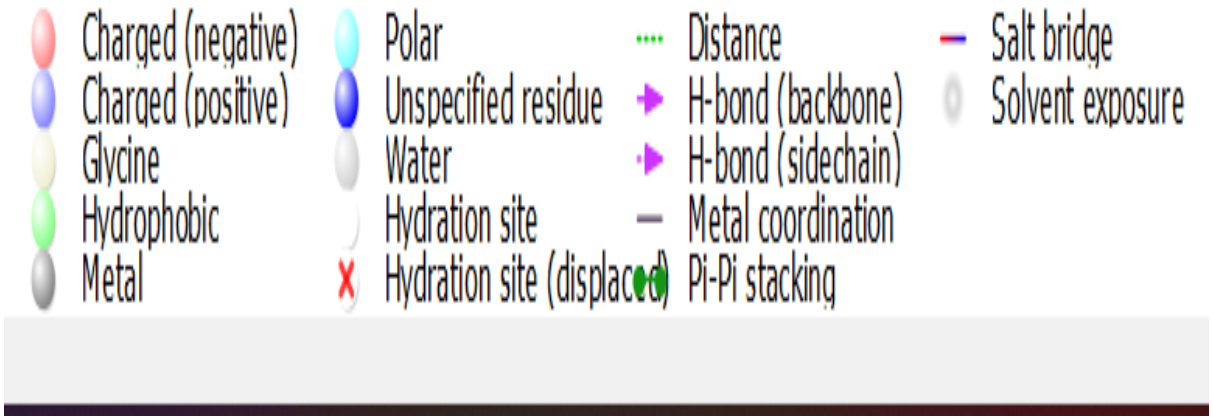
Table S1: Docking score of synthesized compounds within the active site of Human Pancreatic Alpha-glucosidase and Alpha-amylase.

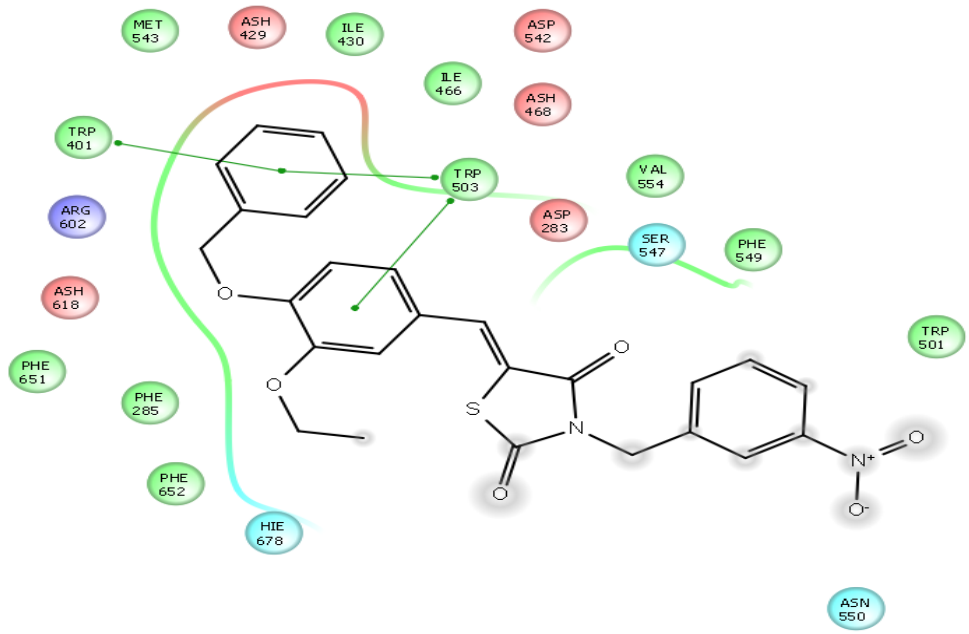
Docking Score		
Compound	Alpha Glucosidase (Uniprot id: P00689)	Alpha-Amylase (PDB id: 1B2Y)
9a	-6.227	-5.365
9b	-7.300	-2.713
9c	-6.224	-3.247
9d	-7.432	-1.202
9e	-7.253	-4.687
9f	-6.235	-2.942
9g	-6.757	-3.096
9h	-6.590	-5.467
9i	-7.236	-4.543
11a	-4.373	-3.792
11b	-6.576	-4.372
11c	-5.710	-4.216
Acarbose	-11.690	-12.270

Colour scheme

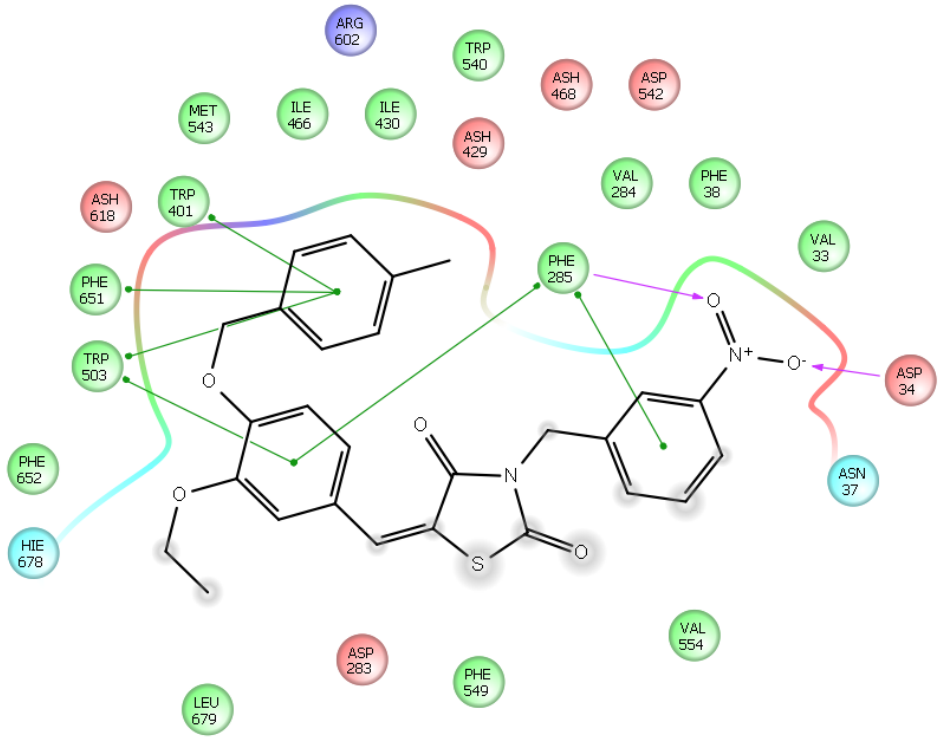


A. 2D-poses of synthesized molecules within the active site of Human Pancreatic Alpha Glucosidase (Uniprot id: P00689)

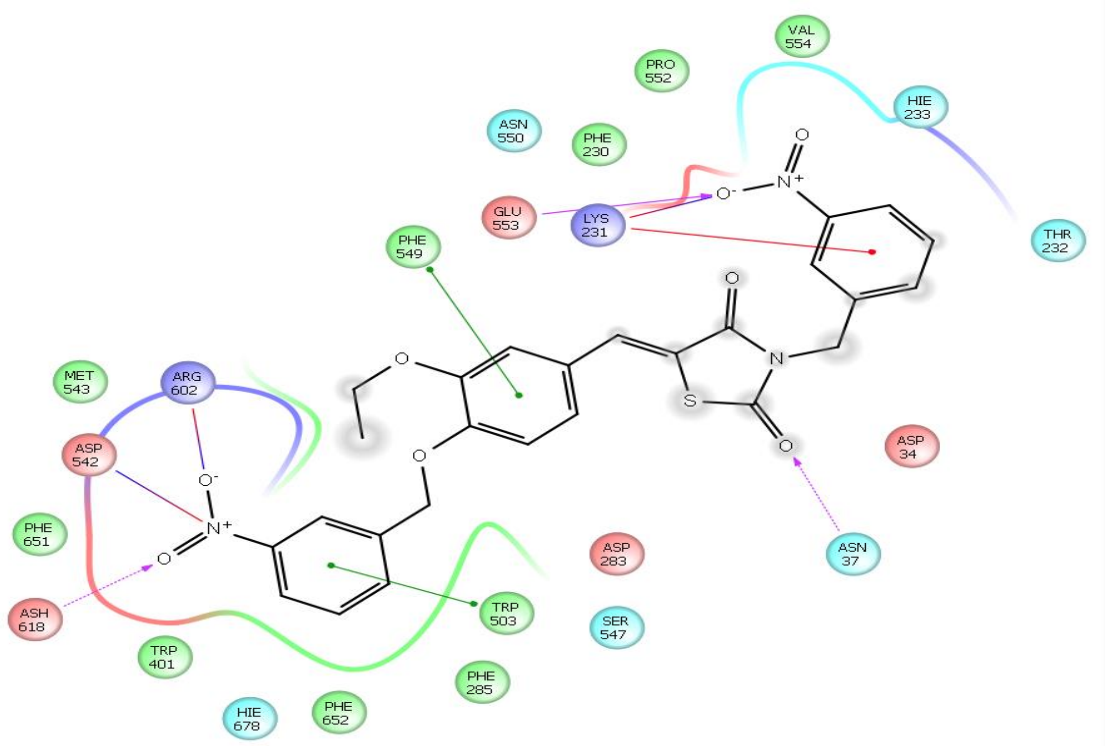
9a



9b



9c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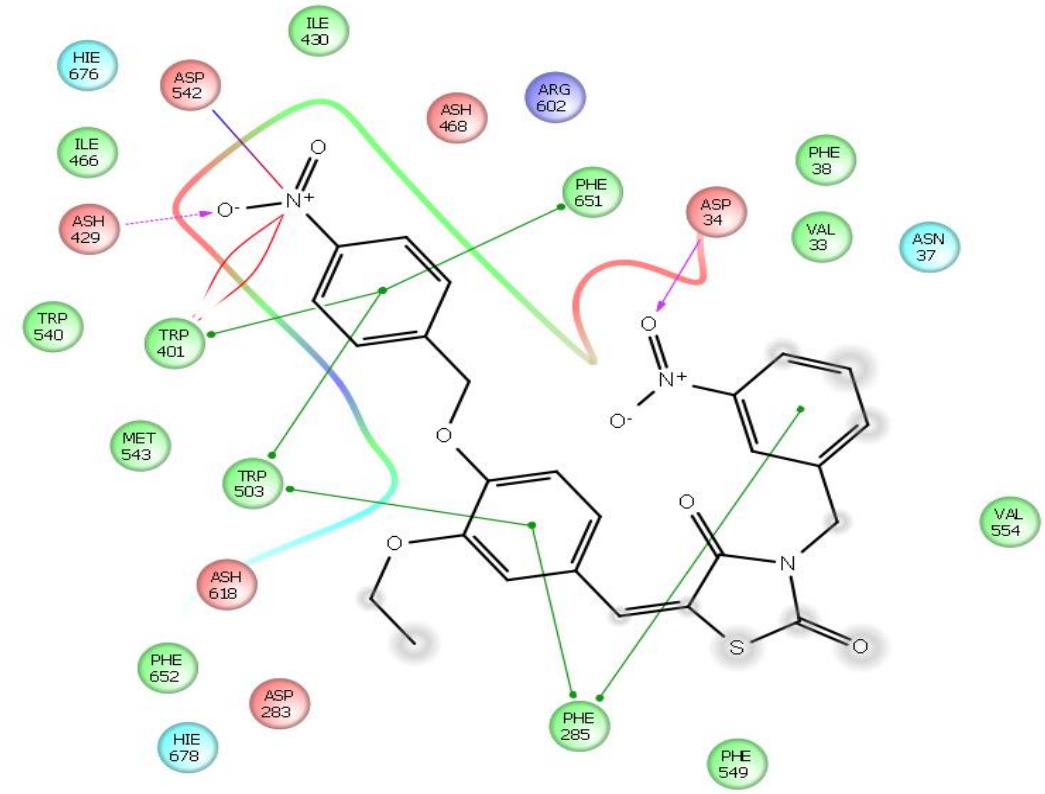
PRO 552
HIE 233
ASN 550
PHE 230
GLU 553
LYS 231
THR 232
PHE 549
MET 543
ARG 602
ASP 542
ASP 34
PHE 651
ASP 283
ASN 37
ASH 618
TRP 503
SER 547
TRP 401
PHE 285
HIE 678
PHE 652

9d


ILE 430
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ASP 542
ARG 602
ASH 468
ILE 466
PHE 38
PHE 651
ASP 34
ASH 429
VAL 33
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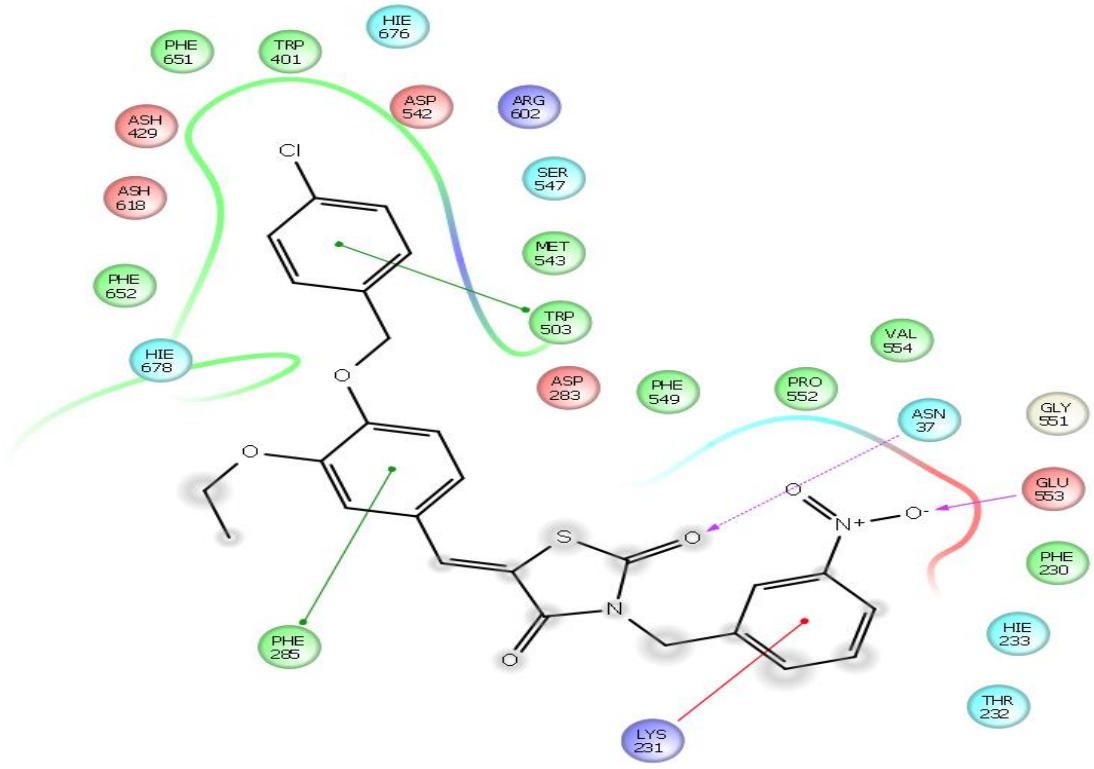
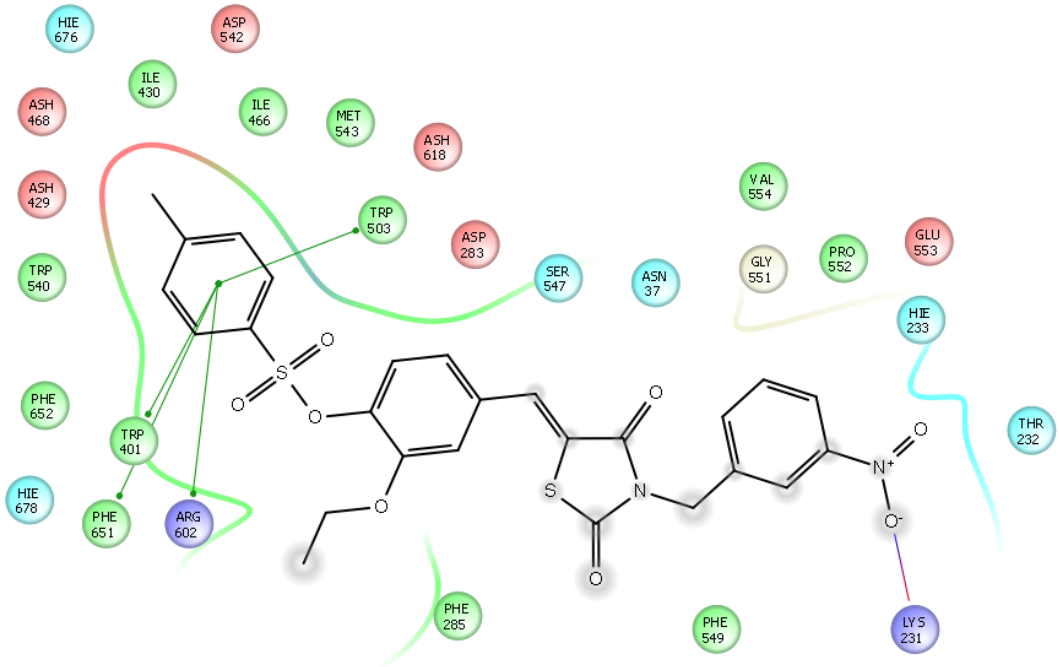
VAL 554
ASH 618
PHE 652
ASP 283
HIE 678
PHE 285
PHE 549

9e


HIE 676
PHE 651
TRP 401
ASP 542
ARG 602
ASH 429
SER 547
ASH 618
MET 543
PHE 652
TRP 503
VAL 554
HIE 678
ASP 283
PHE 549
PRO 552
ASN 37
GLY 551
GLU 553
PHE 230
HIE 233
PHE 285
THR 232
LYS 231

9h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ASP 542
ILE 430
ASH 468
ILE 466
MET 543
ASH 618
ASH 429
TRP 503
ASP 283
VAL 554
GLU 553
PRO 552
TRP 540
SER 547
ASN 37
GLY 551
HIE 233
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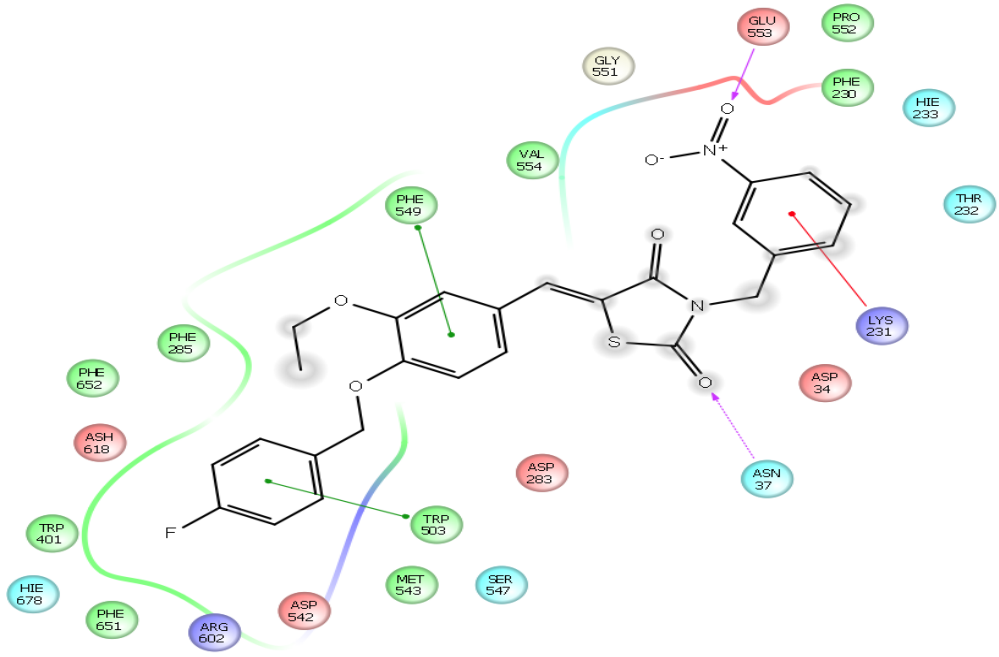
TRP 401
THR 232
HIE 678
PHE 651
ARG 602
PHE 285
PHE 549
LYS 231

9i


GLU 553
PRO 552
GLY 551
PHE 230
HIE 233
VAL 554
THR 232
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LYS 231
PHE 285
ASP 34
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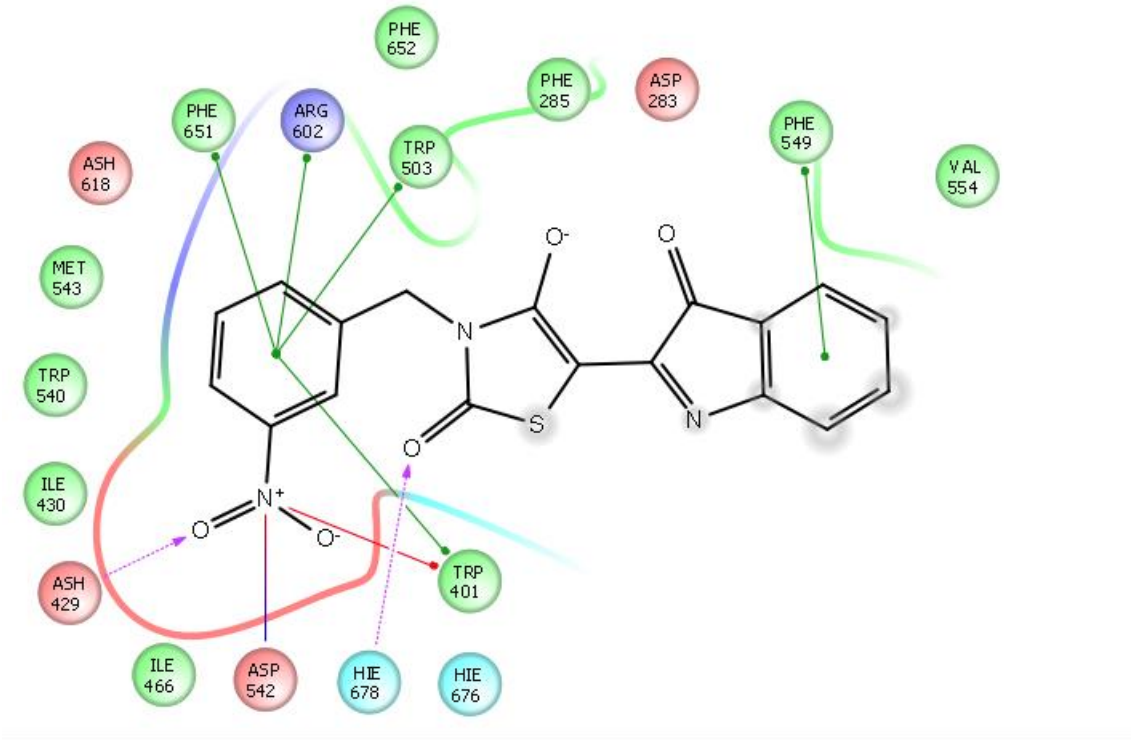
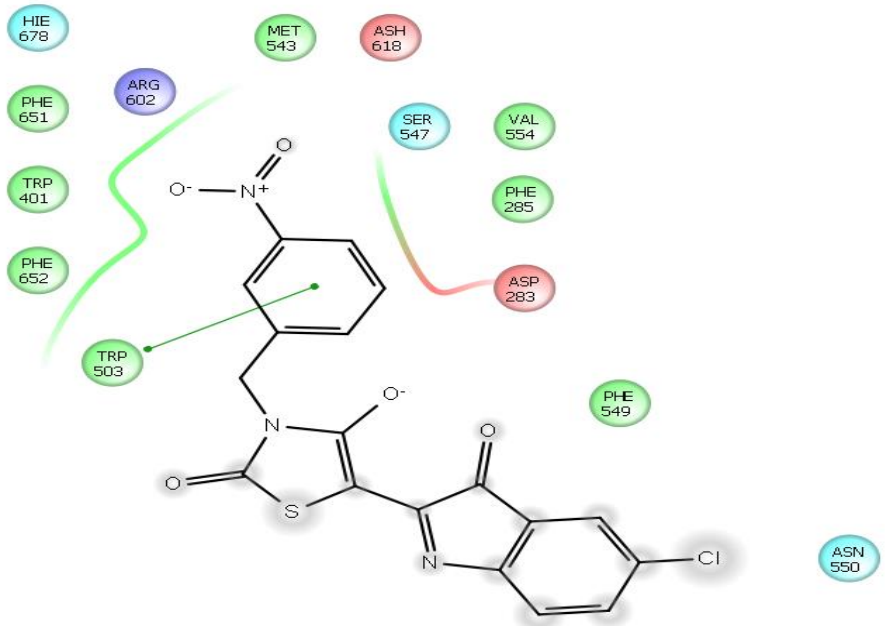
ASP 283
ASN 37
TRP 503
TRP 401
HIE 678
MET 543
SER 547
ASP 542
PHE 651
ARG 602

11a


PHE 652
PHE 285
ASP 283
PHE 651
ARG 602
TRP 503
PHE 549
VAL 554
ASH 618
MET 543
TRP 540
ILE 430
ASH 429
TRP 401
ILE 466
ASP 542
HIE 678
HIE 676
O-
O
N
S
O
N+
O
O-
N

11b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MET 543
ASH 618
ARG 602
PHE 651
SER 547
VAL 554
TRP 401
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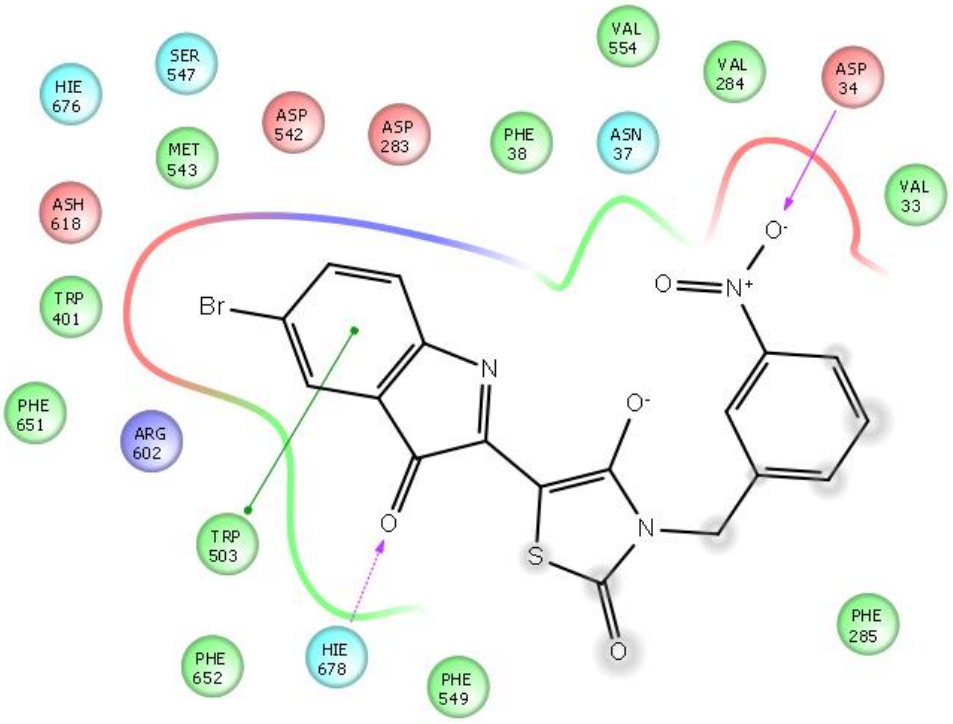
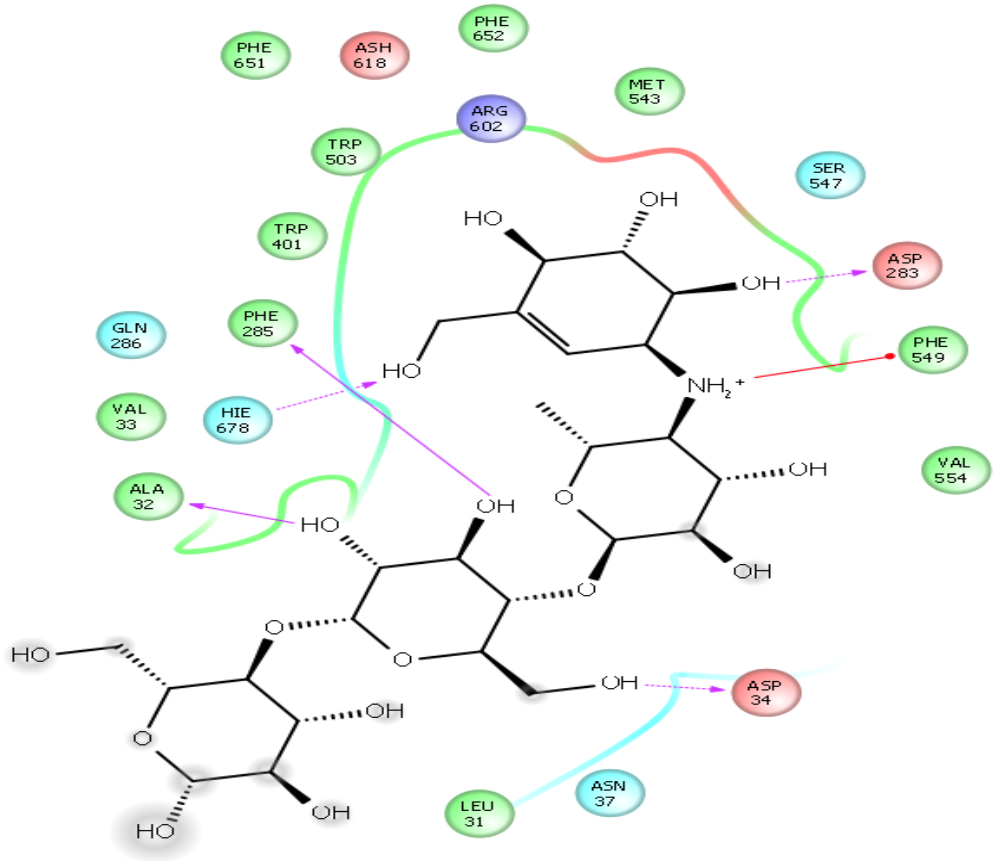
PHE 652
ASP 283
TRP 503
PHE 549
ASN 550
O-
N+
O
N
S
O
Cl

11c

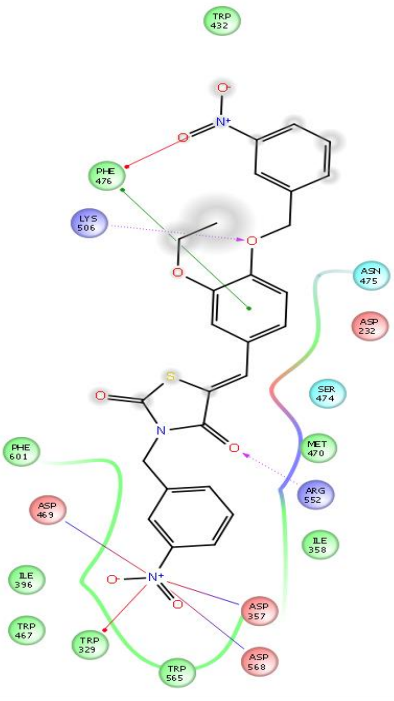


Acarbose

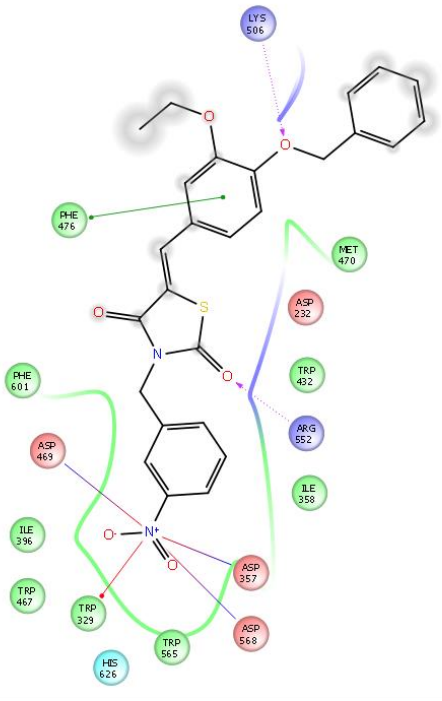


b. Docking Against 3W37

Using the Schrodinger Maestro interface, all synthesized molecules were docked at the active site α-glucosidase (PDB id:3W37). The ligands fit well within the protein's active site and display prominent binding interaction at the active site of the α-glucosidase. The obtained 2-D poses are summarized below. 3-NO_2 (9a-c & 9e-i) attached to the phenyl ring at the 3-position of the TZD core exhibited salt bridge formation between the nitrogen atom and ASP357, ASP568, and ASP568. The 4-NO_2 group in compound 9d displayed similar interaction within the active site of the molecule. π-cation interaction between the N of NO_2 groups and TRP329 was also observed. Hydrogen bond formation between oxygen atoms of -**O**CH_2Ph and Lys506 was observed in the 9A, 9F, and 9G. Another hydrogen bond between Arg552 and the TZD core's oxygen of C-2 (9A, 9C, 9I, 11A & 11C) and C-4 (9F) carbonyl groups was also observed. The SO_2 and 3-NO_2 groups also form H-bonds with Arg552 in compounds 9H and 11B, respectively. π-π stacking interaction between PHE476 and the phenyl ring of vanillin and 3-NO_2 groups was also observed. Compound 9B exhibited four π-π stacking interactions, i.e., PHE376 & 3-NO_2Ph, TRP432 & 3-NO_2Ph, Vanillin and TRP329 & 4-MePh. The compound 9E displayed the weakest interactions, which justified its poor *in vitro* activity against α-glucosidase.

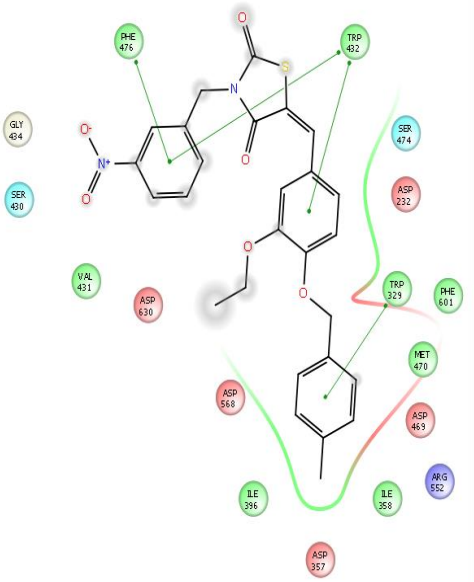


B. 2-D pose of



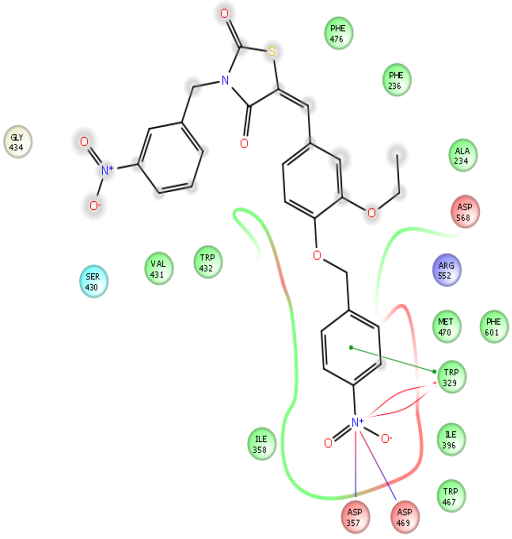
synthesized compounds against α-Glucosidase (PDB id: 3W37)

9a

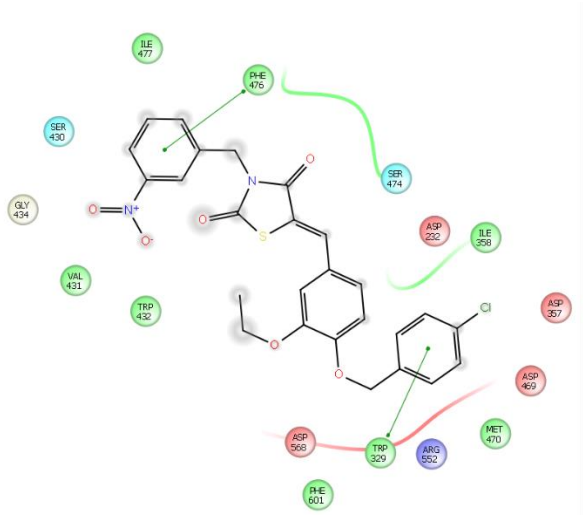


9b

9c



9d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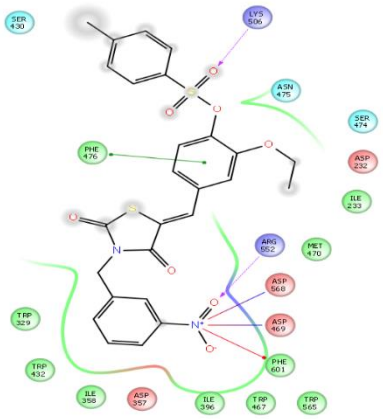
SER 430
SER 474
GLY 434
ASP 232
ILE 358
VAL 431
TRP 432
ASP 357
ASP 469
MET 470
ASP 568
TRP 329
ARG 552
PHE 601

9e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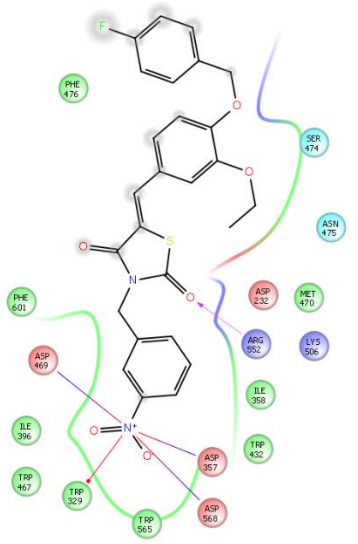
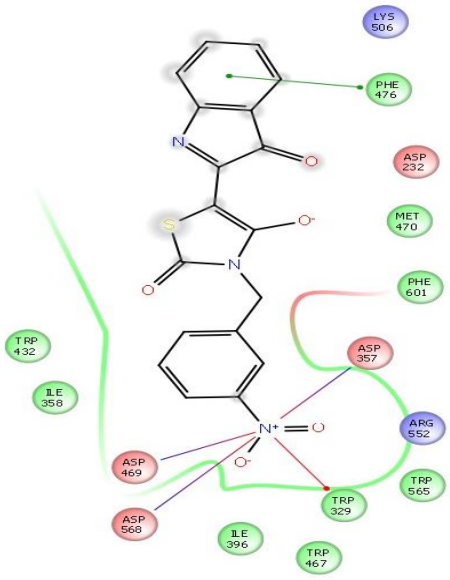
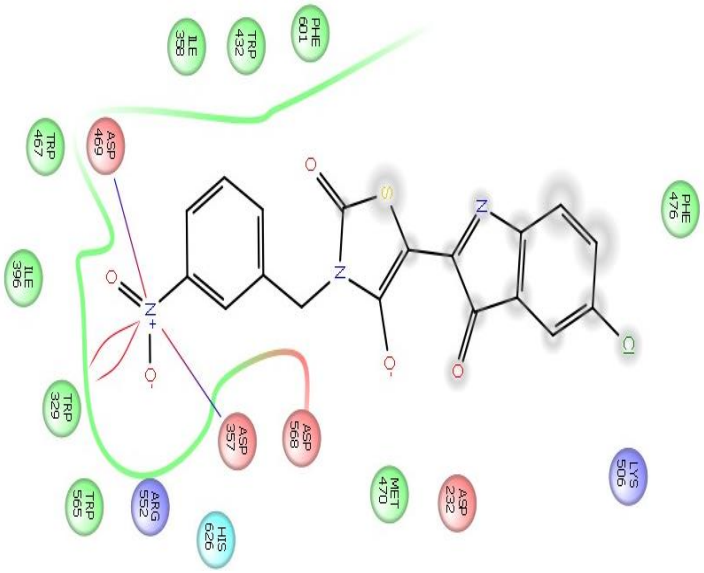
LYS 506
ASN 475
SER 474
PHE 476
ASP 232
ILE 233
ARG 552
MET 470
ASP 568
ASP 469
TRP 329
PHE 601
TRP 432
ILE 358
ASP 357
ILE 396
TRP 467
TRP 565

9h

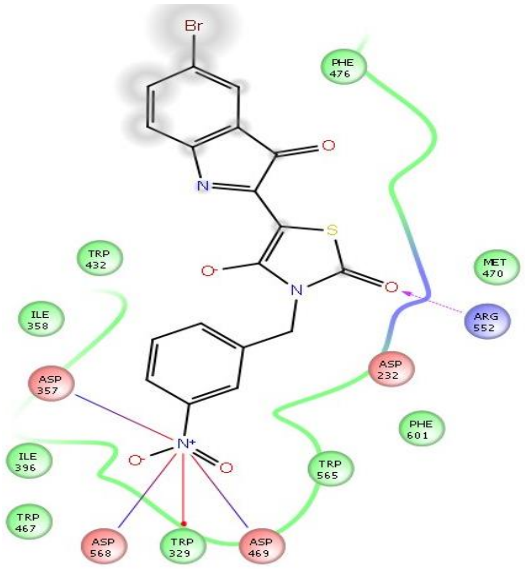

F
PHE 476
SER 474
ASN 475
PHE 601
ASP 232
MET 470
ARG 552
LYS 506
ASP 469
ILE 358
ILE 396
TRP 432
ASP 357
TRP 467
TRP 329
TRP 565
ASP 568


LYS 506
PHE 476
ASP 232
MET 470
PHE 601
TRP 432
ILE 358
ASP 357
ARG 552
ASP 469
TRP 565
TRP 329
ASP 568
ILE 396
TRP 467

9i

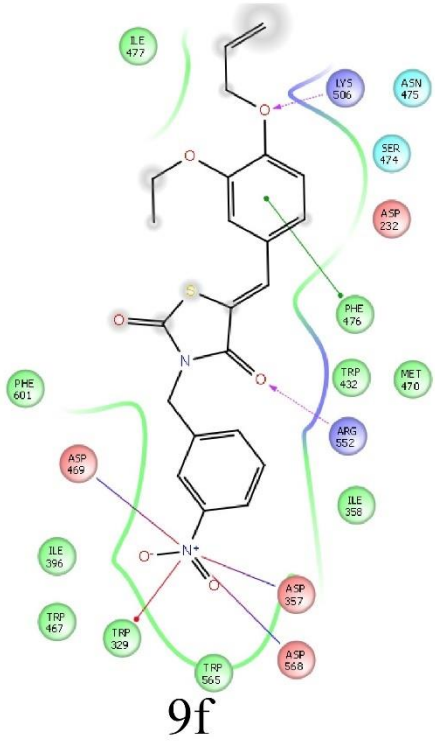

ILE 358
TRP 432
PHE 601
TRP 467
ASP 469
PHE 476
ILE 396
TRP 329
ASP 357
ASP 568
LYS 506
MET 470
ASP 232
TRP 565
ARG 552
HIS 526

11a



11b

11c



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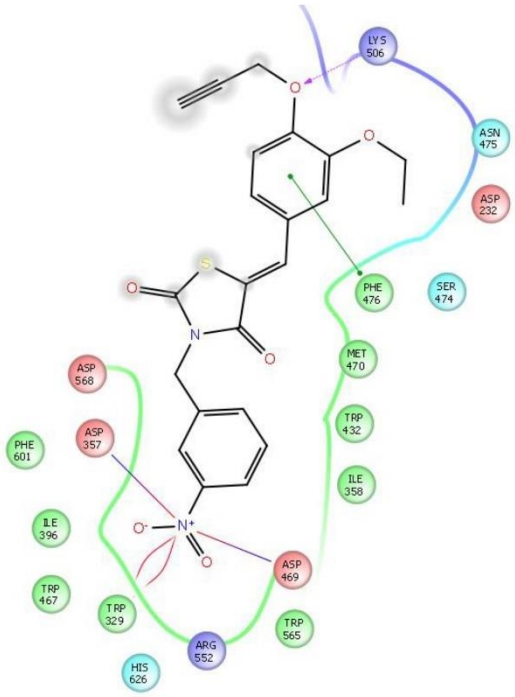


9g

C. MD simulation within the active site of alpha-glucosidase (PDB id: 3W37)

The molecule was simulated for 100 ns, and RMSD, RMSF, and radius of gyration were calculated. The results showed that the ligand and the protein formed a stable complex within the protein's active site after 40 sec, which remained stable for 100 ns. The RMSD

variation was in the range of 0.8 -1.8 Å for the 9G within the protein's active site. The radius of gyration was found in the range of 5.1-6.0 Å. Further, no intramolecular hydrogen bonding was observed. The RMSF value for the different atoms present in the ligand was in the range of 2-6 Å. Furthermore, various interactions within the active site of the protein, such as H-bonds with Ser430, Lys506, Asn569, Arg624, and Arg629, Hydrophobic interaction with Trp329, Trp432, Phe476, Phe601, and Ionic interaction with residues like Asp469, Lys506, and Asn568 were observed. Furthermore, water bridges were also seen, further enhancing ligand stability within the active site of 3W37.

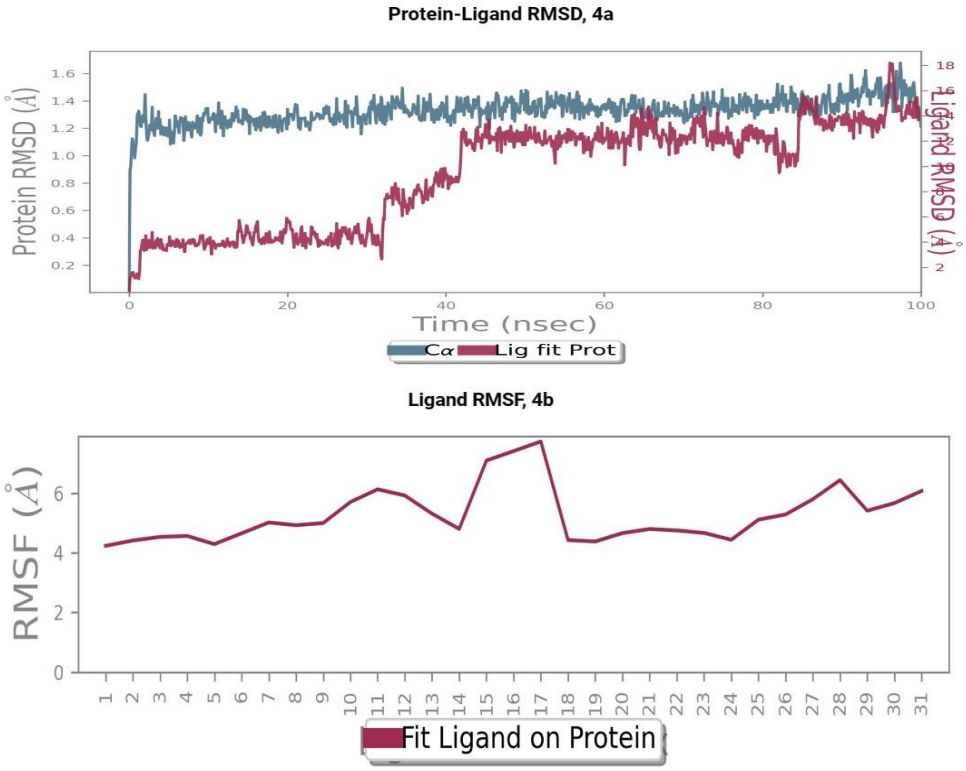
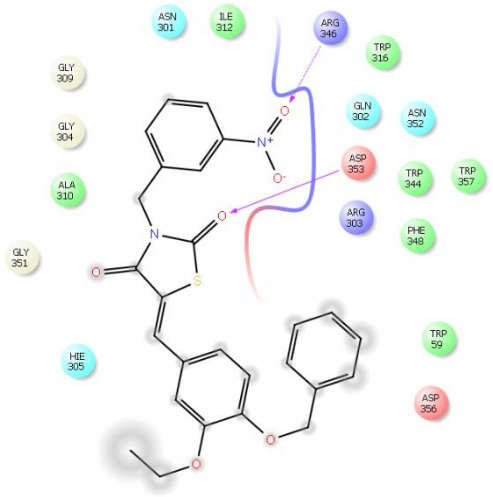


Figure S1: **Simulation of 9G within the active site of alpha-glucosidase (PDB id: 3W37).**

D. 2-D pose of synthesized compounds against human pancreatic α-amylase (PDB id: 1B2Y)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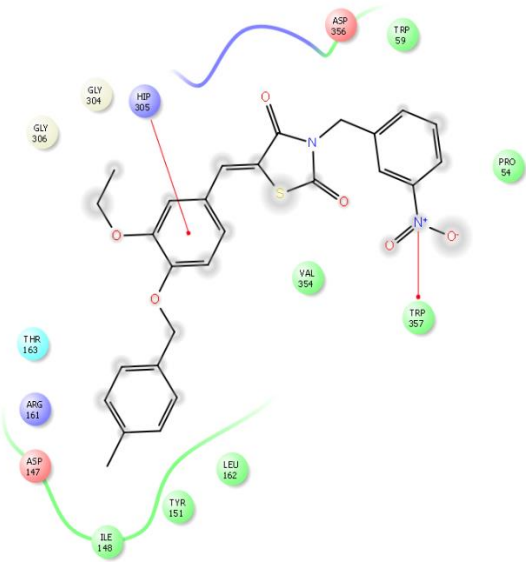
ARG 346
TRP 316
GLY 309
GLN 302
ASN 352
GLY 304
ASP 353
TRP 344
TRP 357
ALA 310
ARG 303
PHE 348
GLY 351
TRP 59
HIE 305
ASP 356

9a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TRP 59
GLY 304
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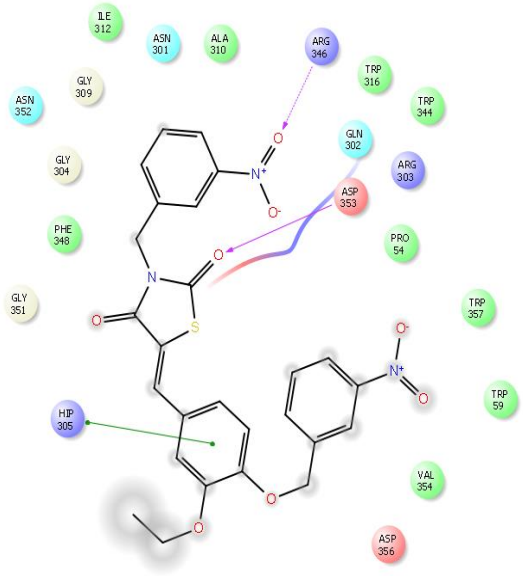
GLY 306
PRO 54
VAL 354
TRP 357
THR 163
ARG 161
ASP 147
LEU 162
TYR 151
ILE 148

9b


ILE 312
ASN 301
ALA 310
ARG 346
TRP 316
GLY 309
ASN 352
TRP 344
GLN 302
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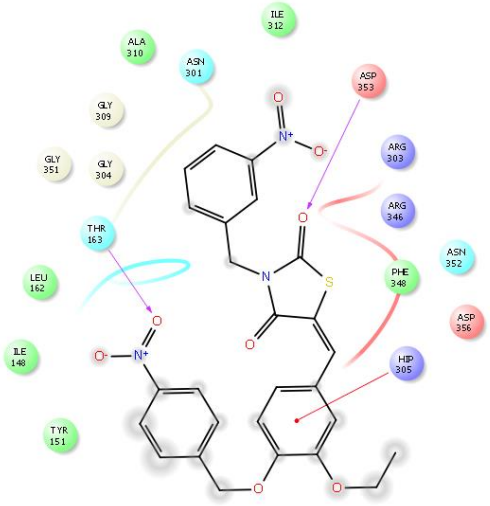
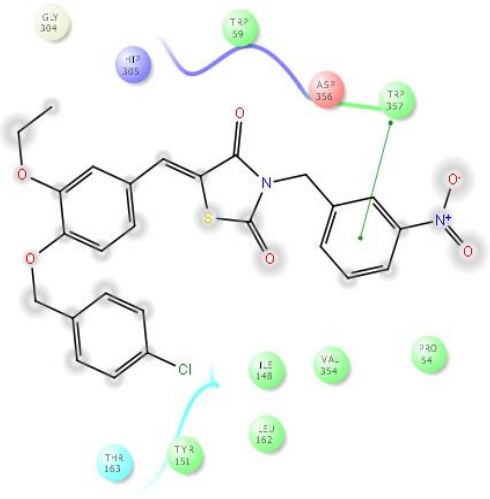
ARG 303
ASP 353
PHE 348
PRO 54
GLY 351
TRP 357
HIP 305
TRP 59
VAL 354
ASP 356

9c


ILE 312
ALA 310
ASN 301
ASP 353
GLY 309
GLY 351
GLY 304
ARG 303
ARG 346
THR 163
LEU 162
ASN 352
PHE 348
ASP 356
ILE 148
HIP 305
TYR 151

9d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TRP 59
HIP 305
ASP 356
TRP 357
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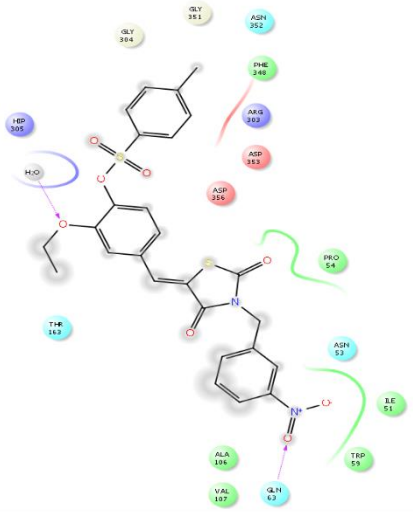
O
N
S
O
O
O
N+
O
Cl
ILE 148
VAL 354
PRO 54
LEU 162
THR 163
TYR 151

9e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GLY 304
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ARG 303
ASP 353
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ASP 356
O
S
O
O
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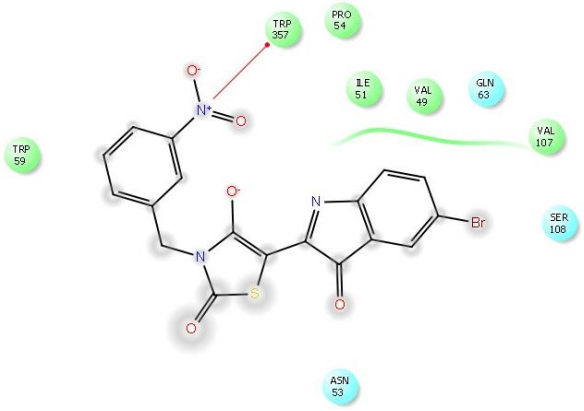
O-
O
ALA 106
TRP 59
VAL 107
GLN 63

9h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PRO 54
O-
N+
O
ILE 51
VAL 49
GLN 63
TRP 59
VAL 107
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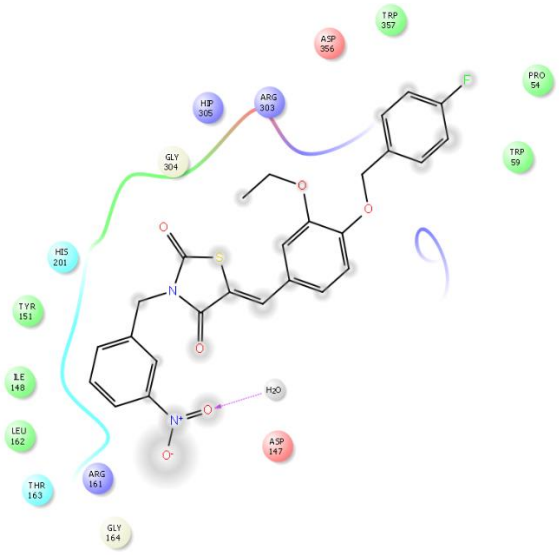
Br
SER 108
N
S
O
O
ASN 53


TRP 357
ASP 356
PRO 54
HIP 305
ARG 303
F
GLY 304
TRP 59
O
O
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HIS 201
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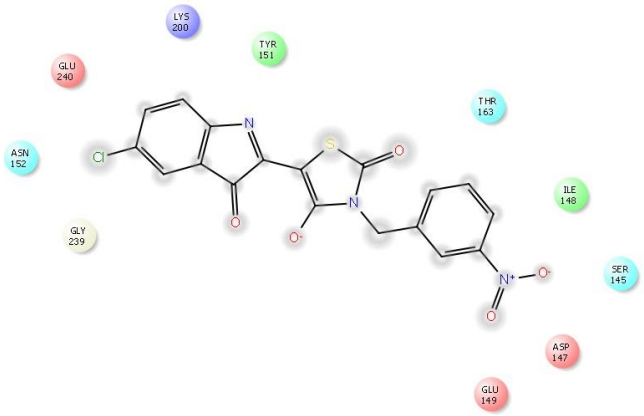
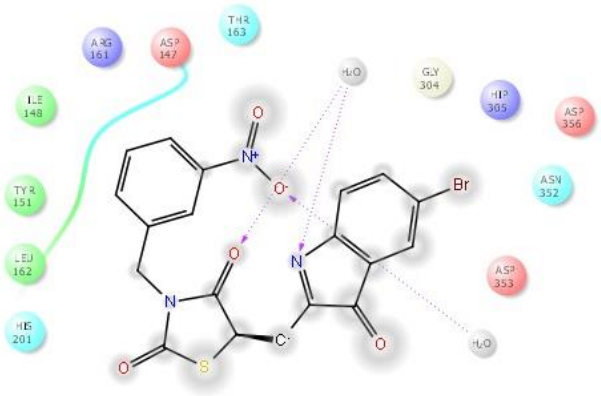
O
TYR 151
ILE 148
N+
O
H2O
O-
LEU 162
ASP 147
ARG 161
THR 163
GLY 164

9i



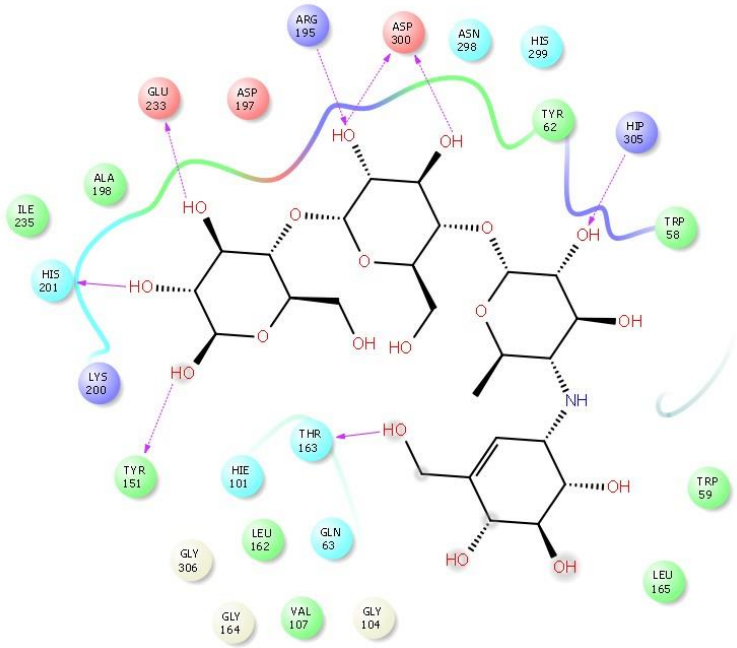
11a



11b

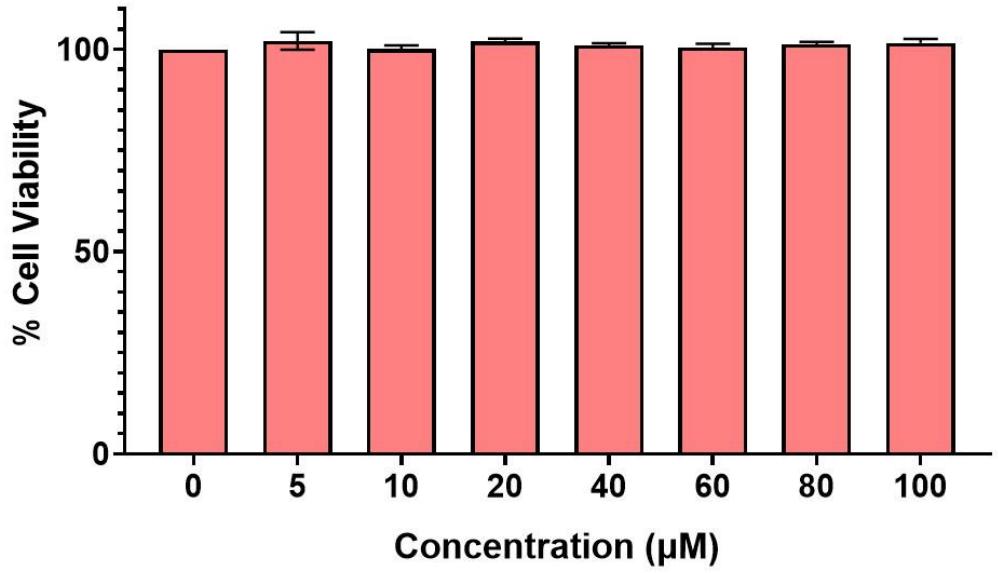
11c

Acarbose

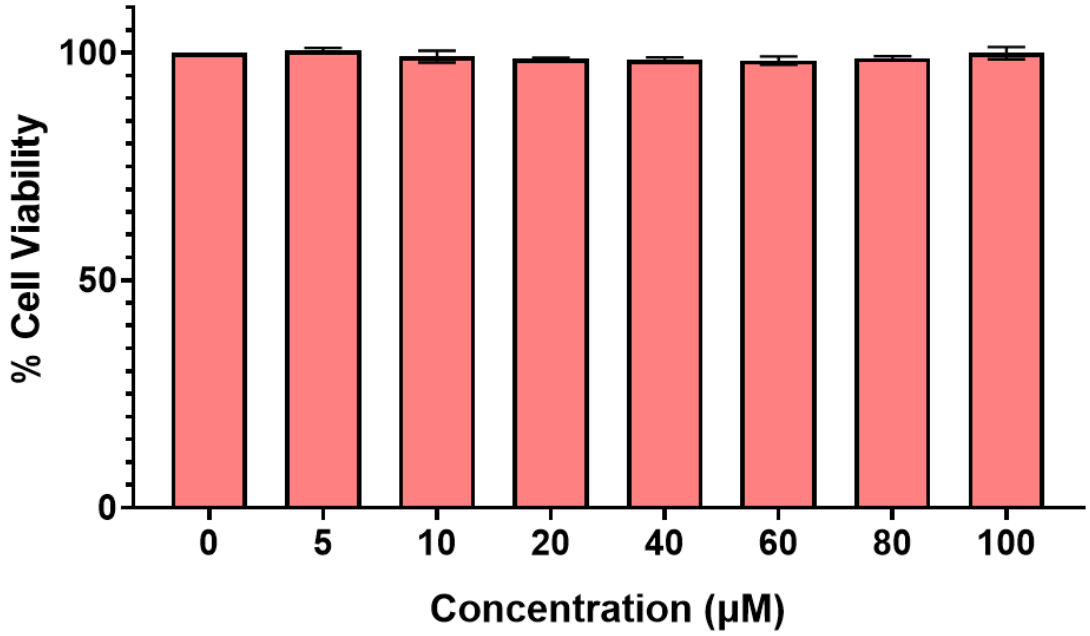


E. Cytotoxicity Data (a. PANC-1)

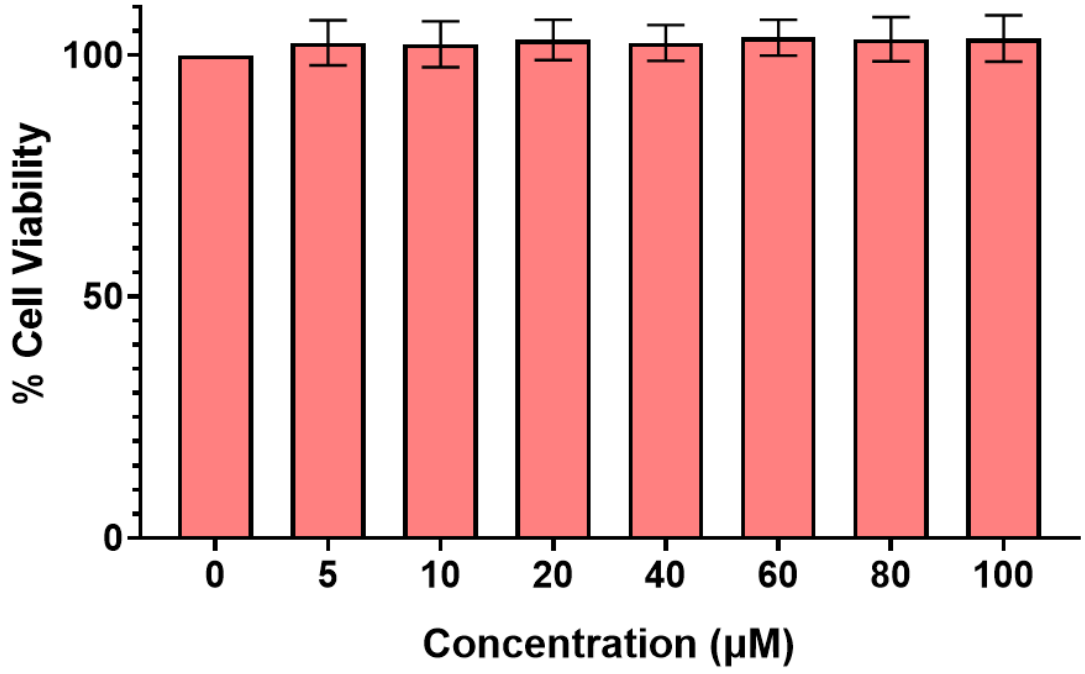
9a



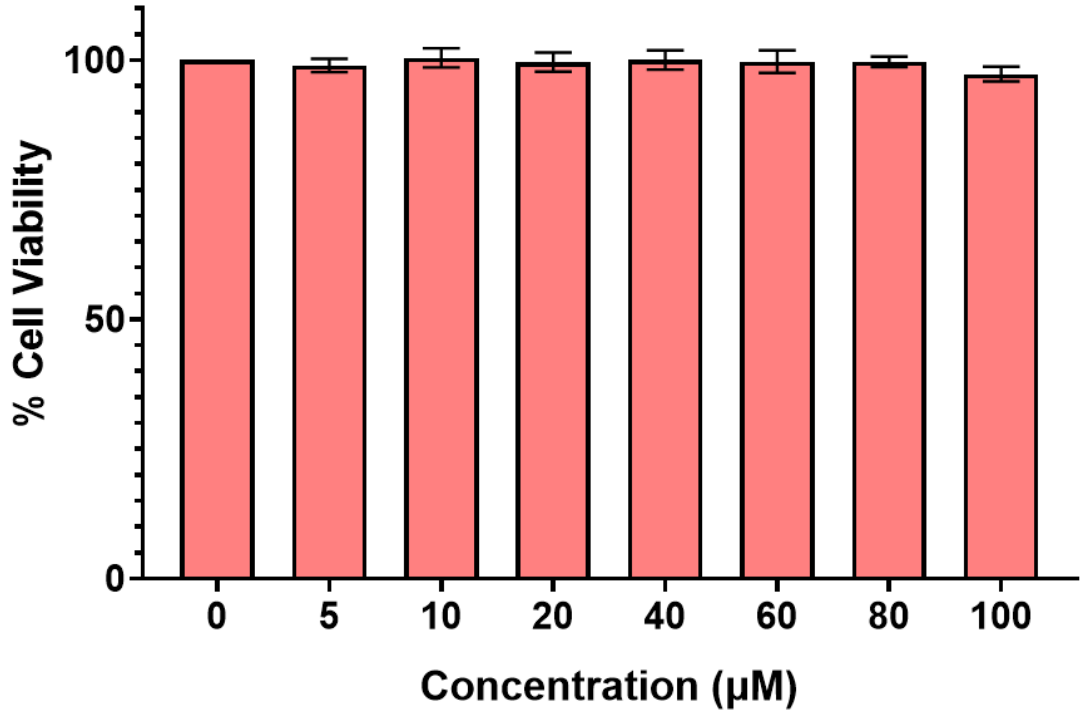
9b



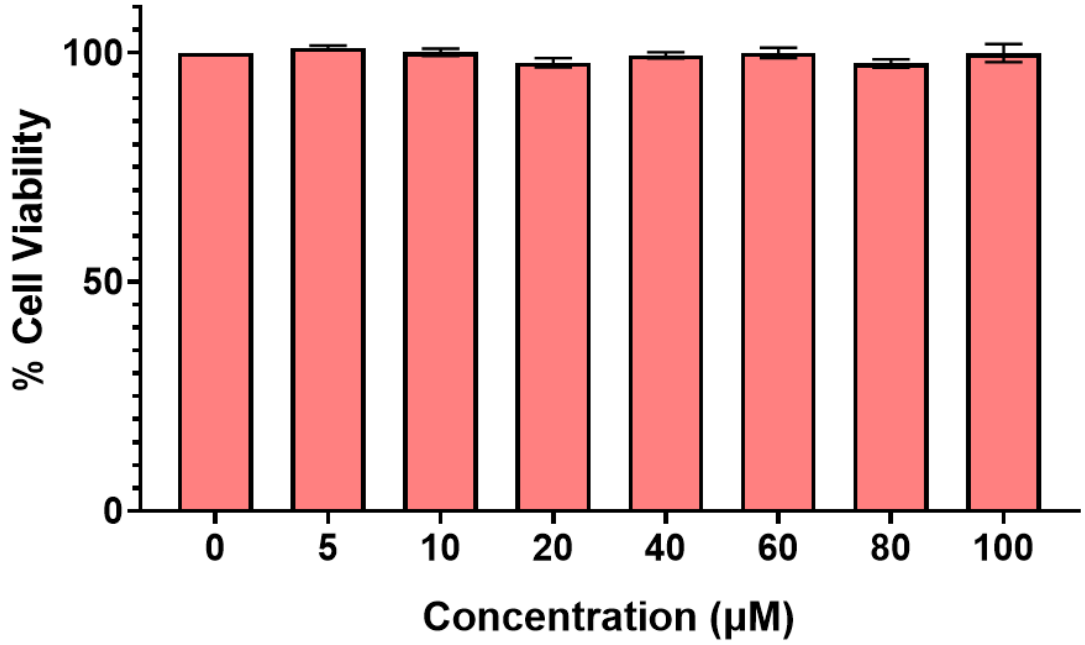
9c


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

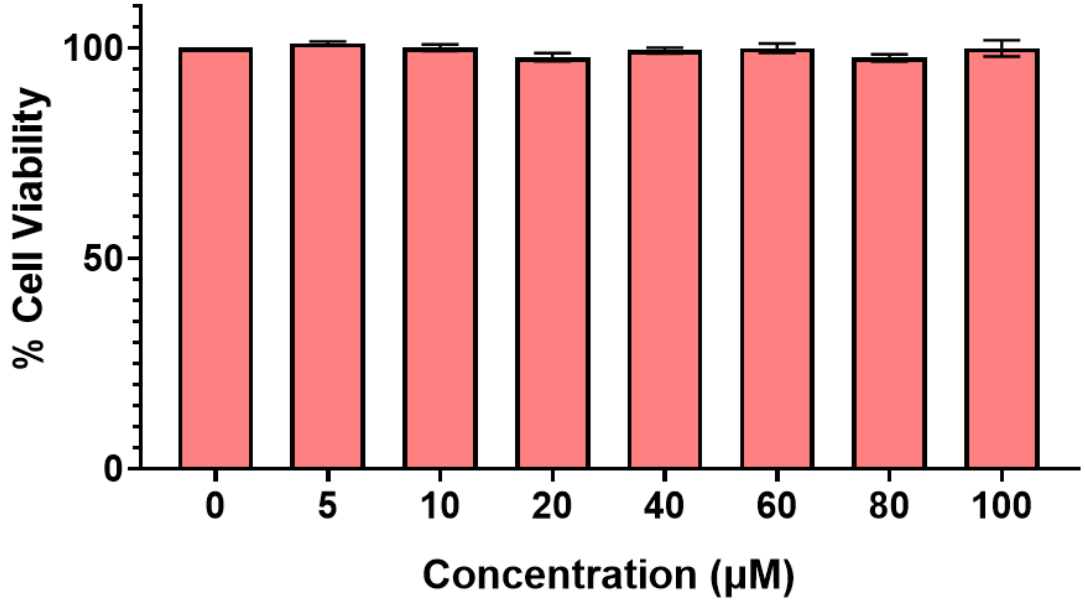
9d


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

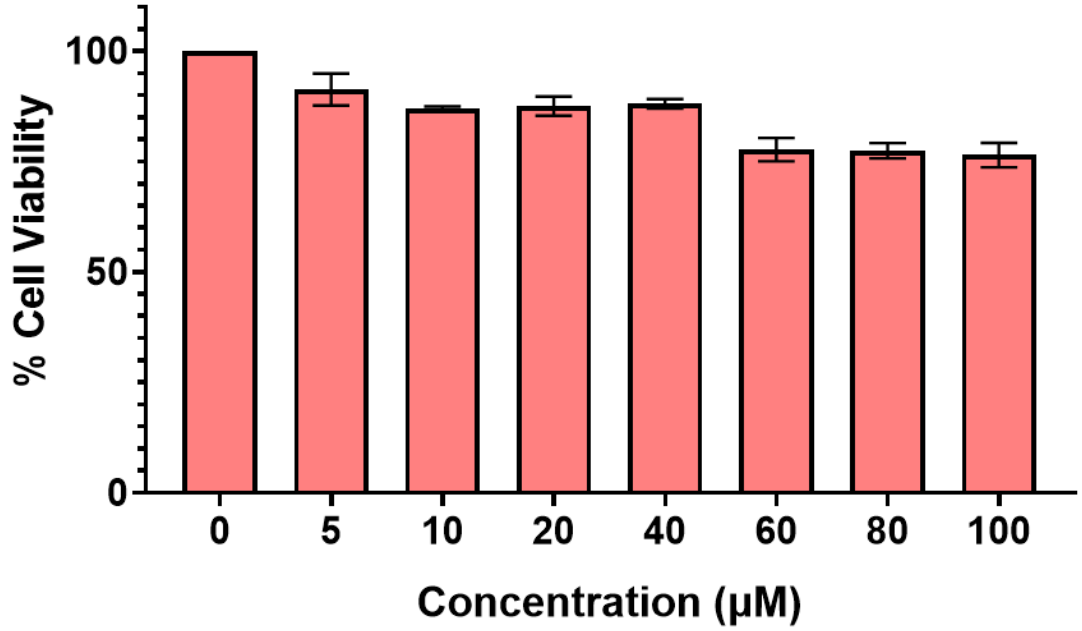
9e


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

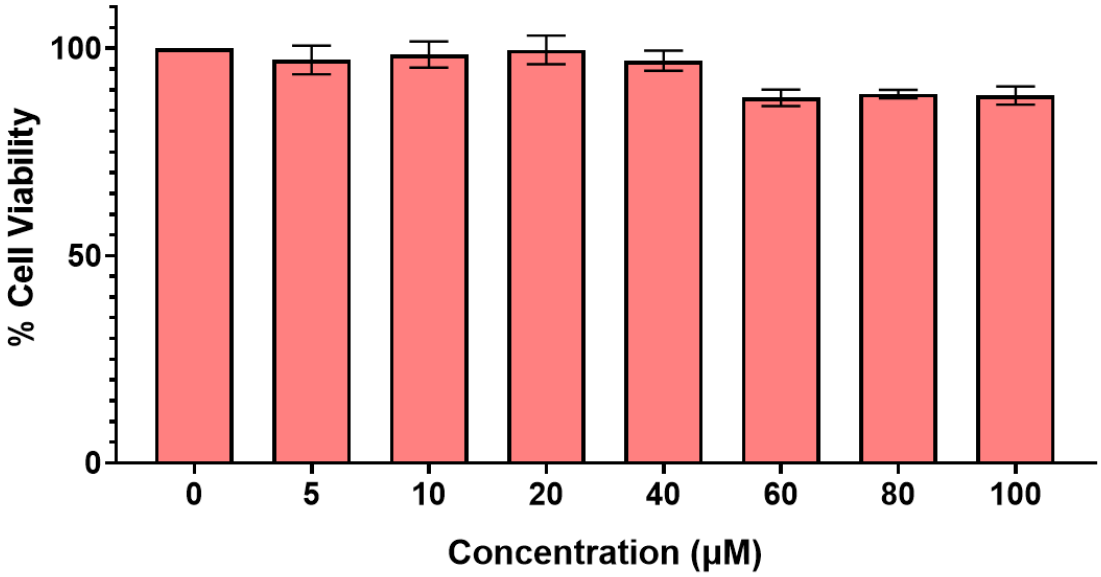
9h


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

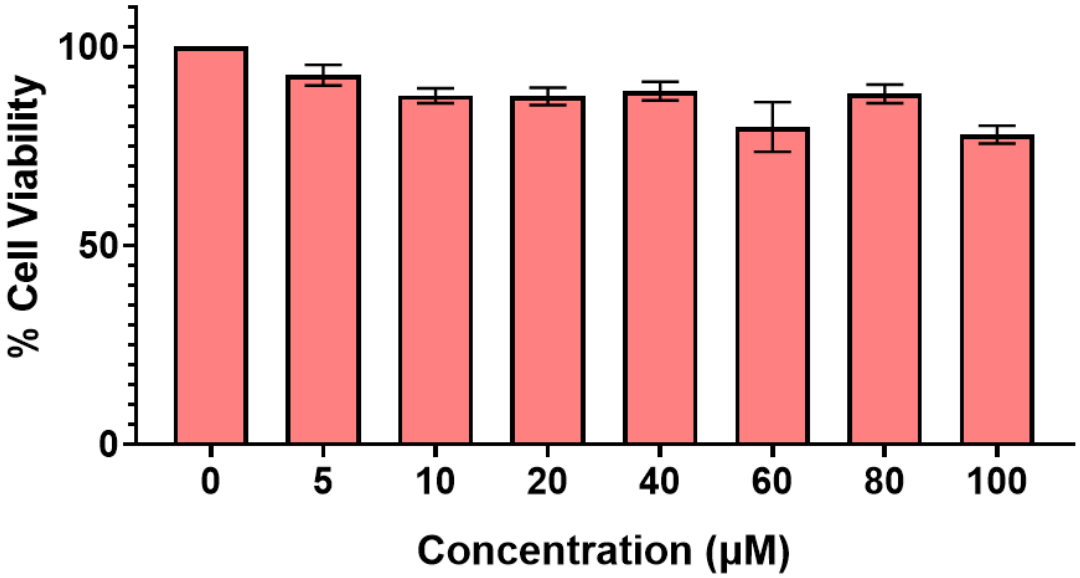
9i


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

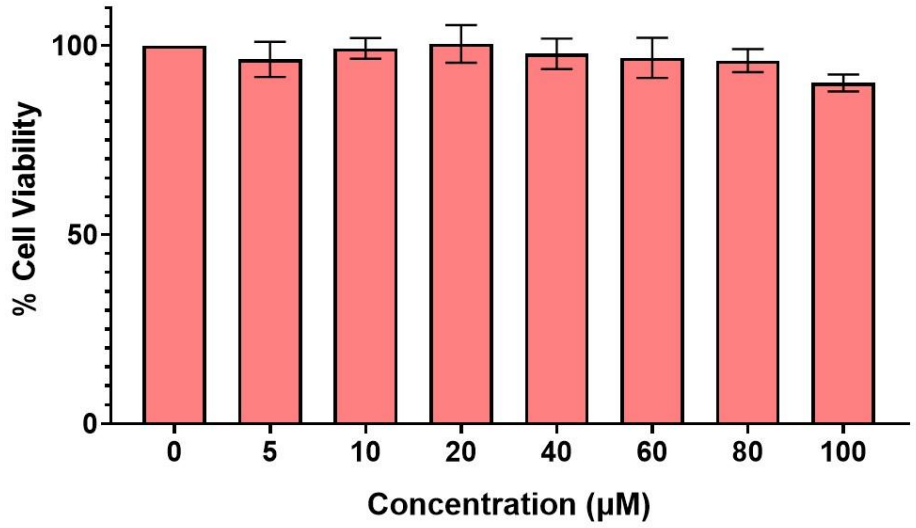
11a


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Concentration (μM)

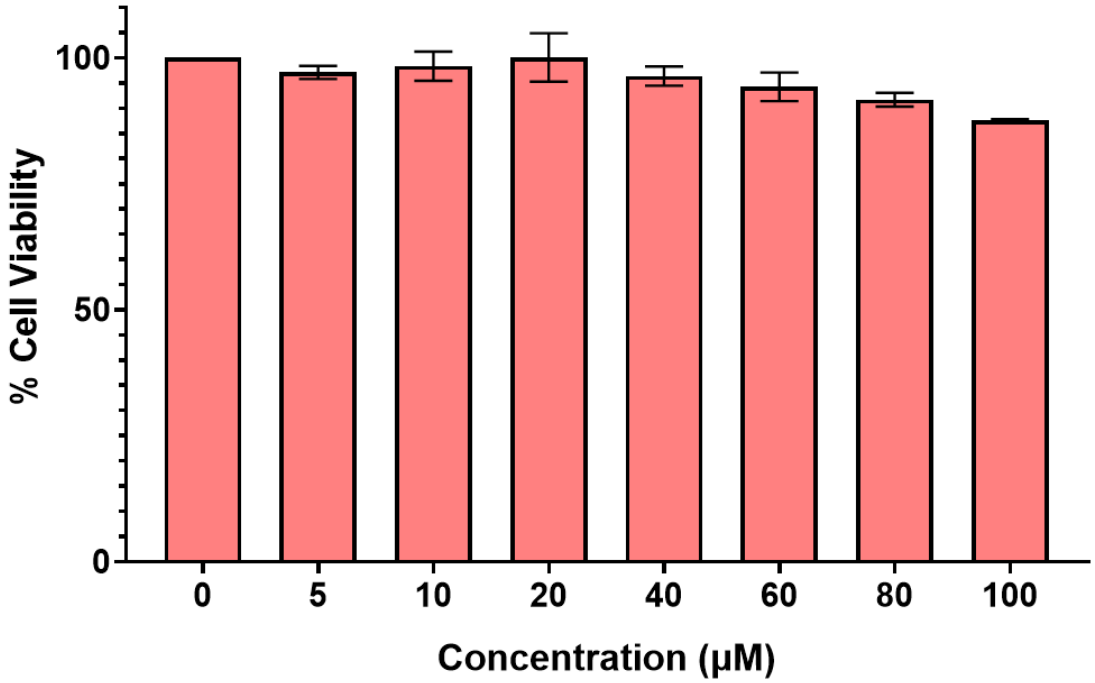
11b



11c

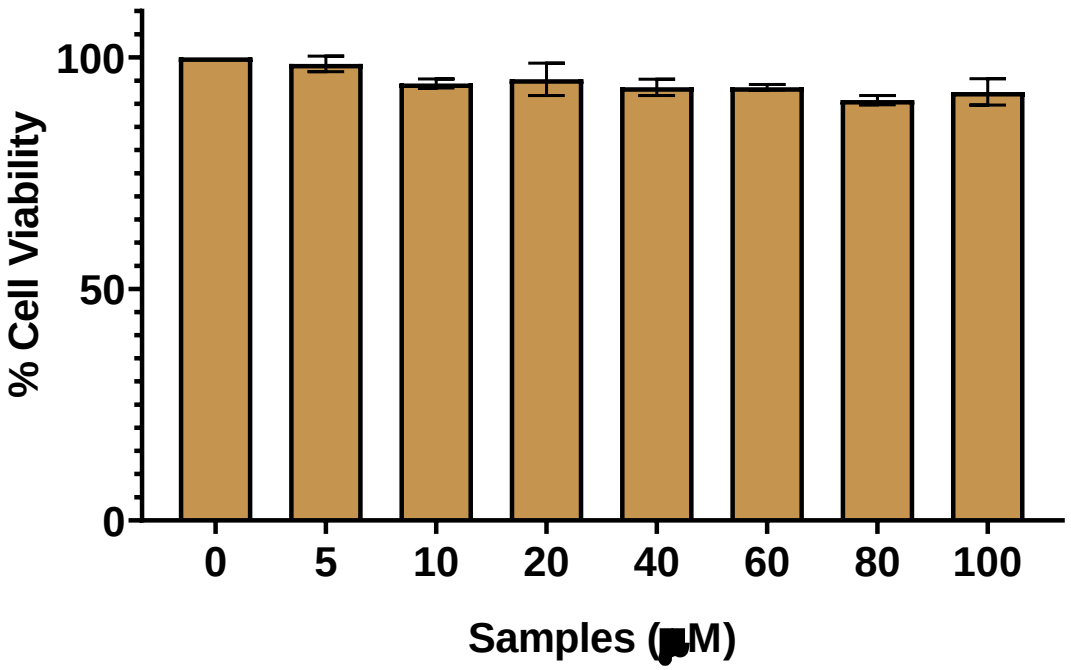


Acarbose

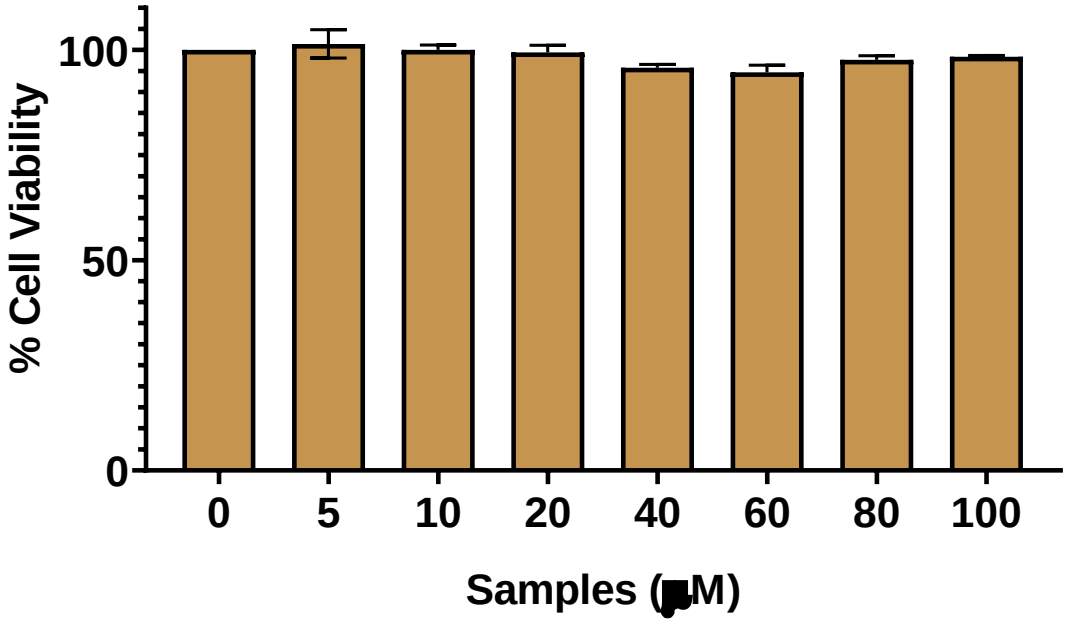


F. Cytotoxicity data (INS1 cell line)

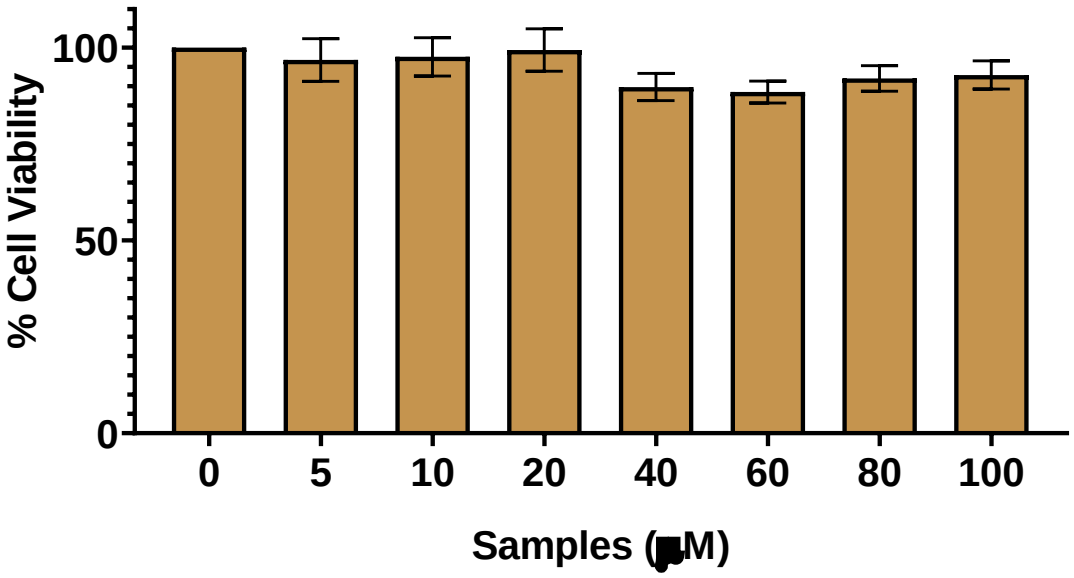
9A


100
50
0
% Cell Viability
0
5
10
20
40
60
80
100
Samples (µM)

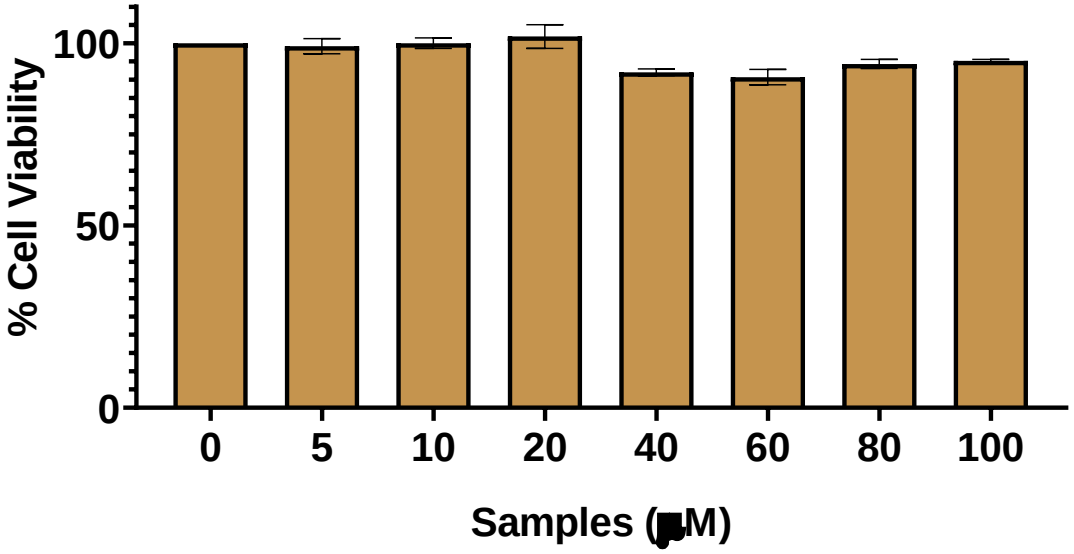
9B

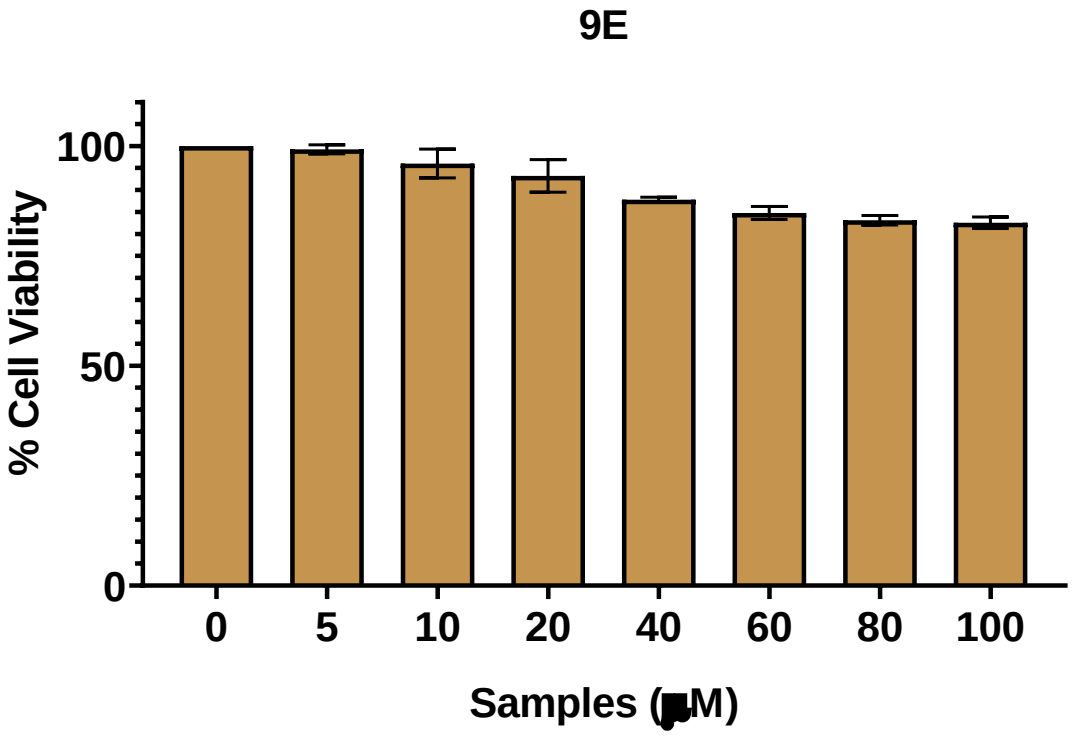

100
50
0
% Cell Viability
0
5
10
20
40
60
80
100
Samples (µM)

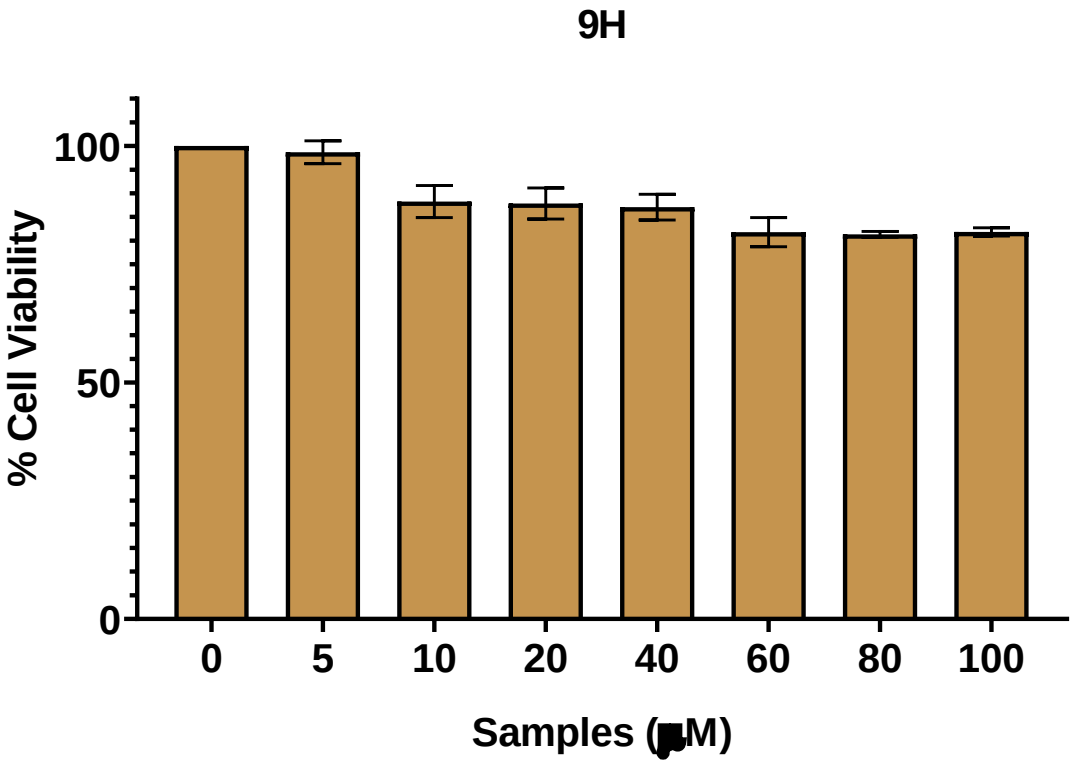
9C

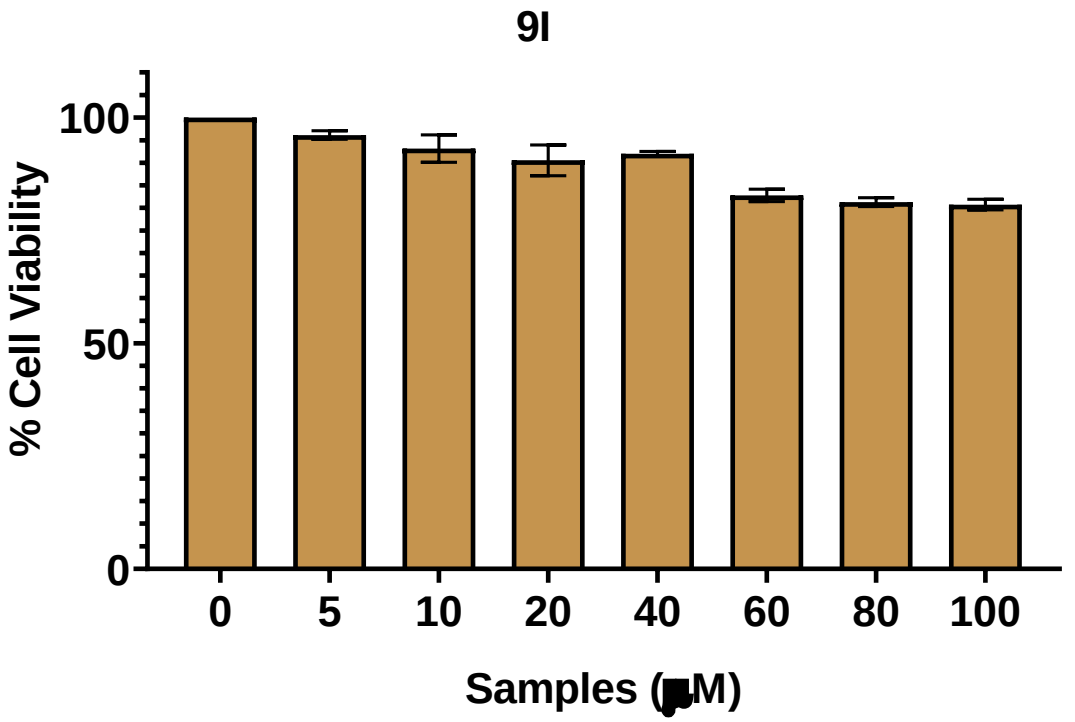

% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)

9D


% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)


9E
% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)


9H
% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)


9I
% Cell Viability
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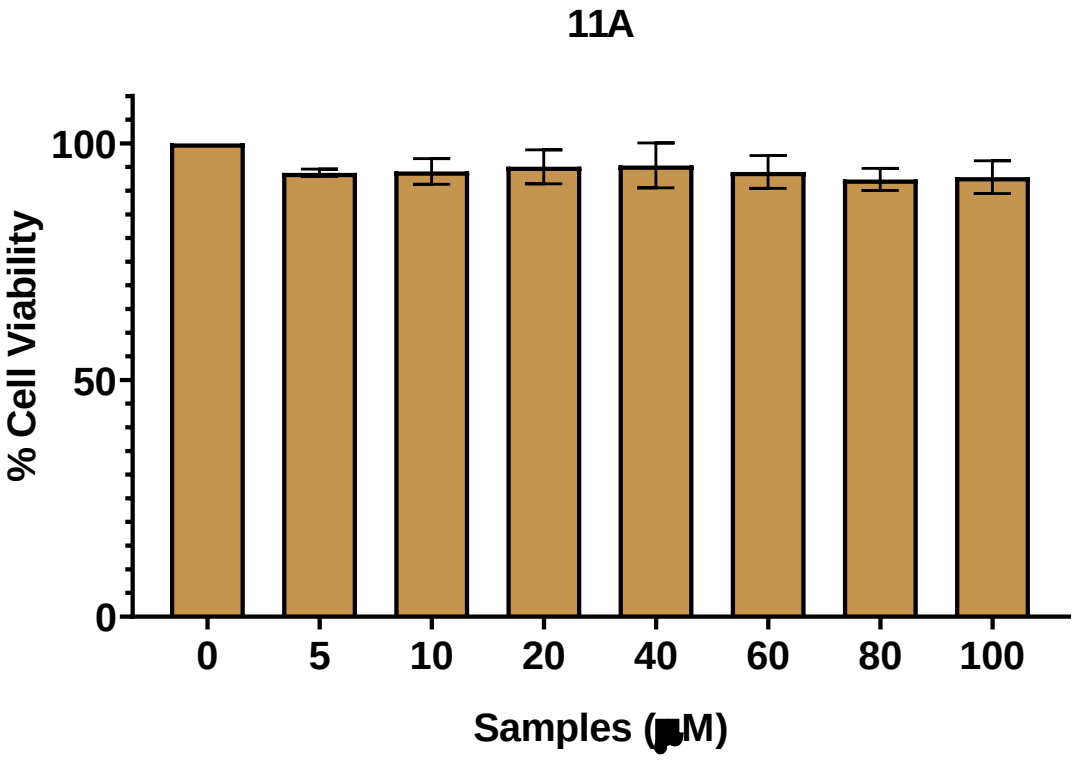
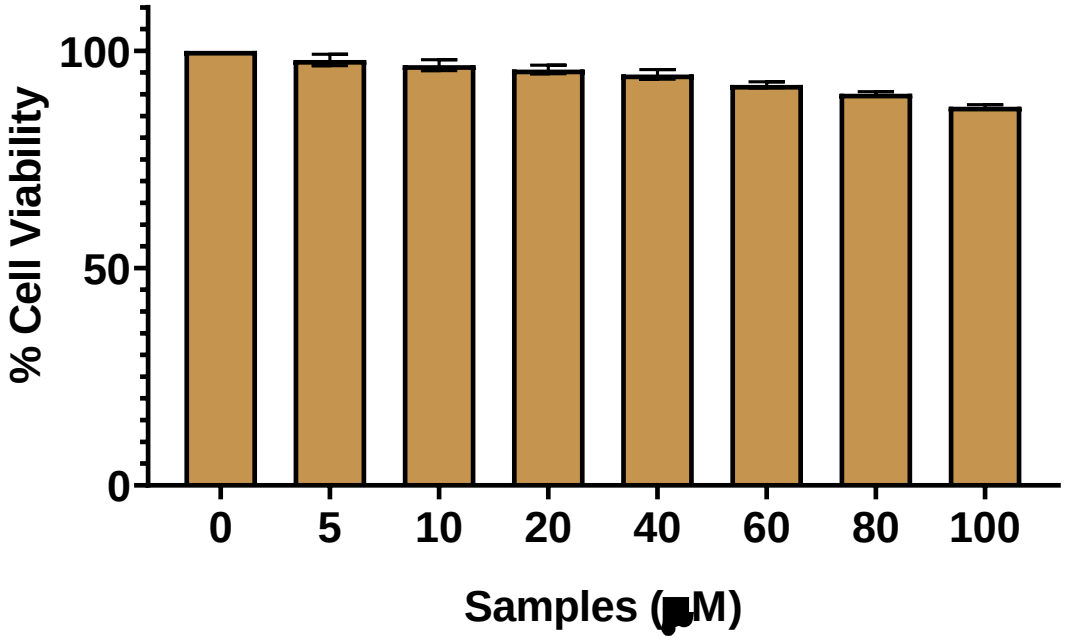
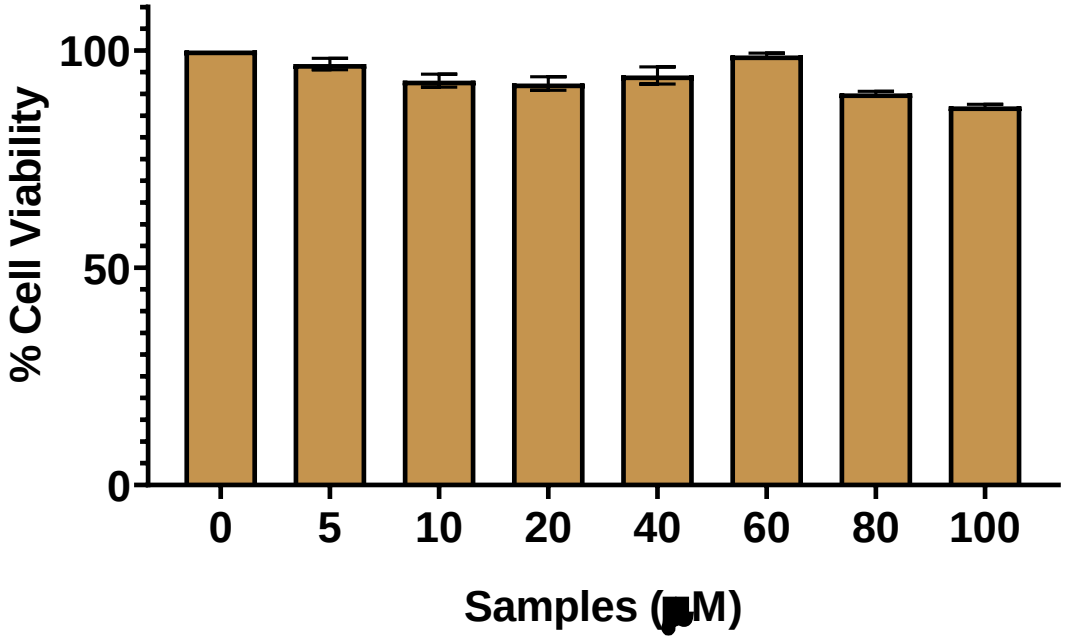
0
0
5
10
20
40
60
80
100
Samples (µM)

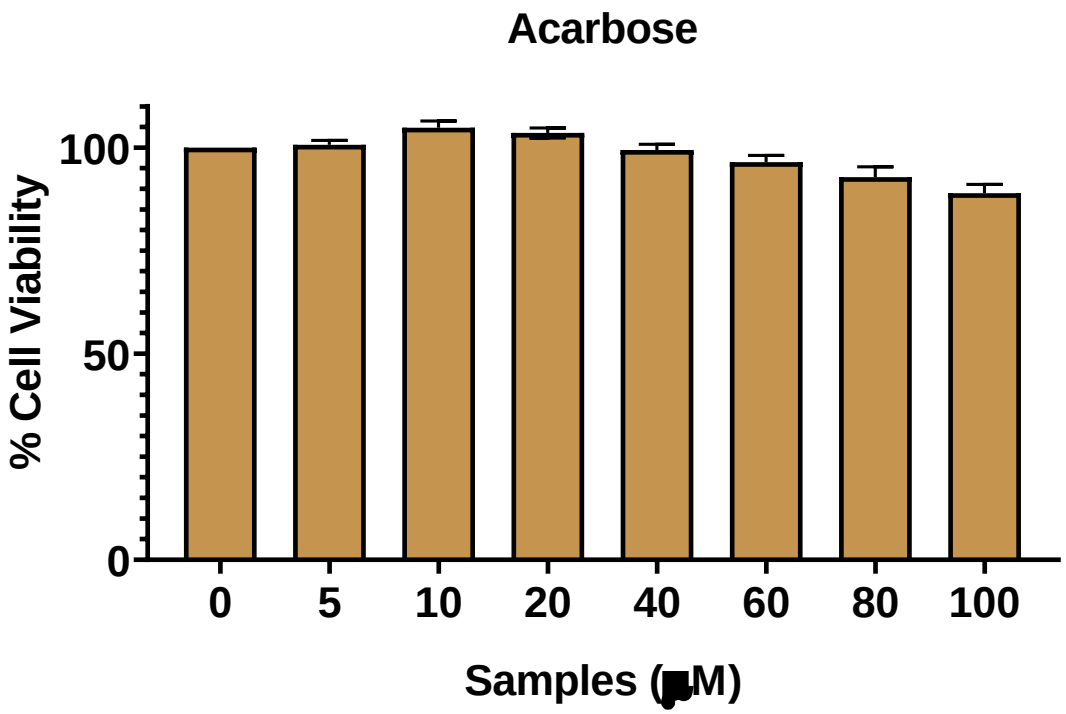

11A
% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)

11B


100
50
0
% Cell Viability
0
5
10
20
40
60
80
100
Samples (µM)

11C


100
50
0
% Cell Viability
0
5
10
20
40
60
80
100
Samples (µM)


Acarbose
% Cell Viability
100
50
0
0
5
10
20
40
60
80
100
Samples (µM)

G. Simulation studies

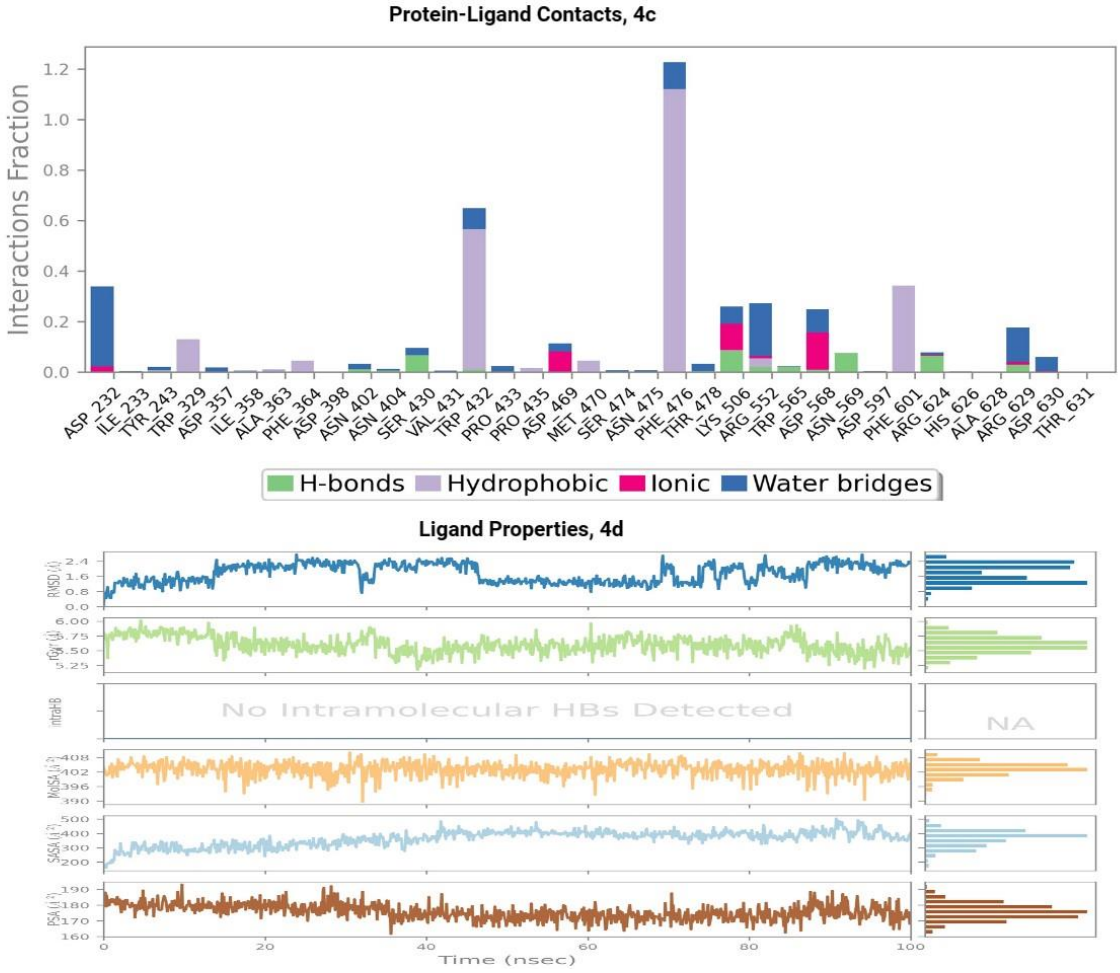


Figure S2: **Simulation of 9G within the active site of alpha-glucosidase (PDB id: 3W37)**

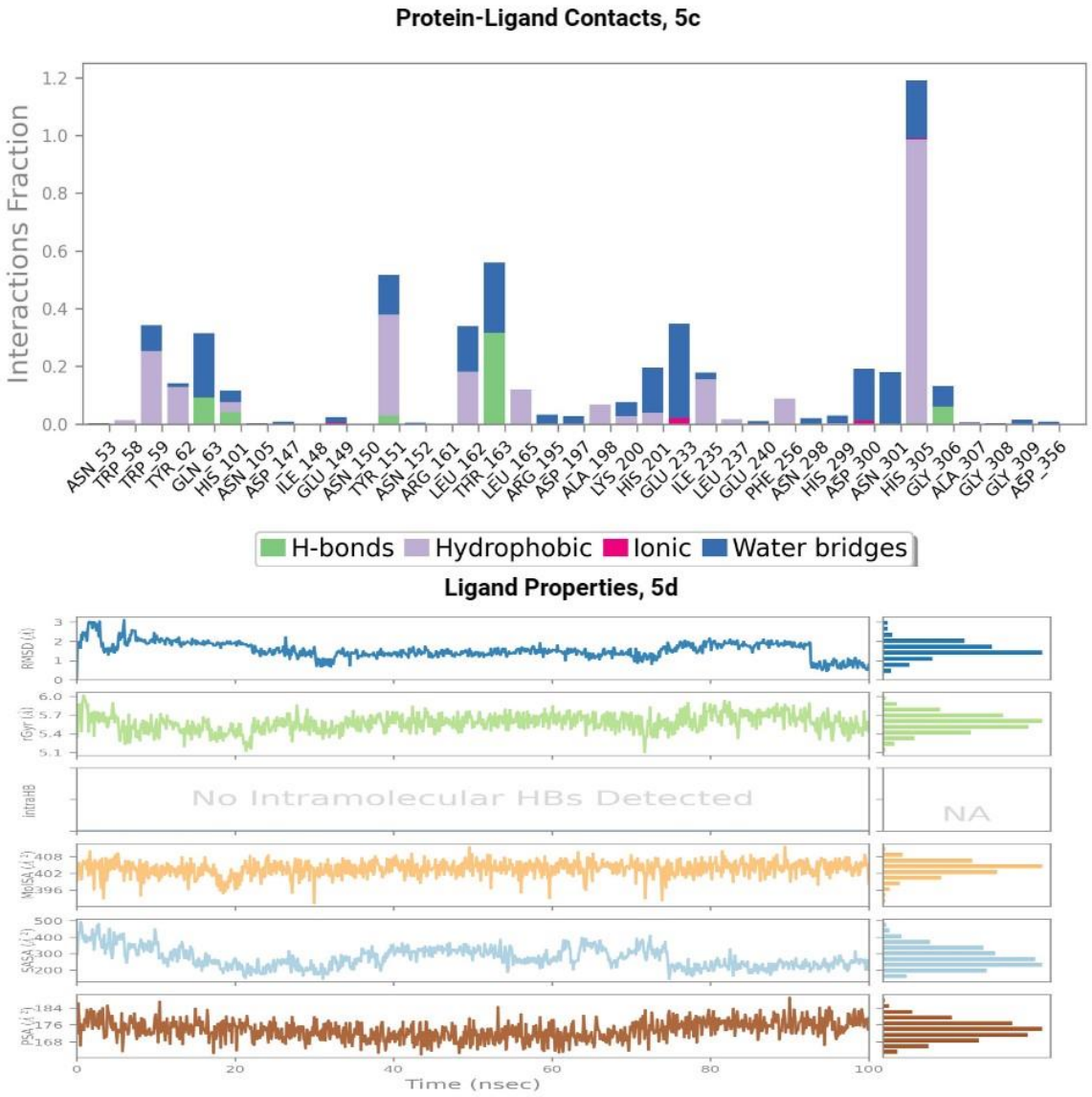


Figure S3: **Simulation of 9G within the active site of alpha-amylase (PDB id: 1B2Y)**

Ethical Approval

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

ISF COLLEGE OF PHARMACY,

GT Road, Ghal Kalan, MOGA-142001 (Punjab) India

Mob.: 087250-55460, 08146562883, 09779980588 Email: director@isfcp.org

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Certificate

This is to certify that the project proposal no. 23 entitled "Effect of N-substituted-5-arylidene derivatives of thiazolidinedione in streptozotocin-induced experimental model of diabetes in rats" submitted by Mr. Gurpreet Singh has been approved/recommended by the IAEC of ISF College of Pharmacy, Moga in its meeting held on 26/11/2022 and 42 Wistar Rats have been sanctioned under this proposal for a duration of next 6 months.

Authorized by	Name	Signature	Date
Chairman:	Dr. G.D. Gupta	.....	26/11/2022
Member Secretary:	Dr. Shamsher Singh	.....	26/11/2022
Main Nominee of CPCSEA:	Dr. Rahul Deshmukh	.....	.....

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)