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Unveiling Cyclic Tetra Amino Acids-based Peptides as Insulin Degrading Enzyme Inhibitors: Insight from *Insilico* Approach

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Abstracts:
Type II diabetes has become one of the ailment that poses a grave universal wellbeing trial and the desire to curtail its dangerous activities has drawn the attention of various researchers globally. Several resistances developed by type II diabetes to series of previous drug-like small molecules has trigger the desire of researchers to attempt the use of tetra-amino acid based peptides to combat this menace. Thus, inhibiting activity of tetra amino acid based-peptides against insulin degrading enzyme (pdb id: 4re9) resulted into series of binding affinities values and (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((4-chlorophenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (compound **F**) with -8.16984558kcal/mol proved to have greatest strength to inhibit the studied target. The calculated binding energy generated from molecular dynamic simulation (MDS) supported the efficiency of compound F than referenced compound (Metformin). The report from ADMET investigations were presented and interpreted accordingly.

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Introduction:

The reports by several researchers on diabetes mellitus type II have shown that it is a metabolic syndrome of manifold etiologies which are branded by continuing hyperglycemia and it was observed to be originated from deficiencies in insulin discharge [1, 2]. Several issues encountered from diabetes mellitus complications have been reported to be a major challenge among the public in many nations [3]. According to Wang *et al.,* 2019, type II diabetes is a lingering ailment which is connected to improper secretion of insulin [4]. Type II diabetic patients are liable to be attacked by various diseases such as depression, heart failure and polyneuropathy etc. [5].

The connectivity between insulin and insulin degrading enzyme has been observed to have strong attraction [6]. The increase in insulin degrading enzyme has the ability to lower the secretion of insulin in human body and this has been reported to result to increase in blood sugar in human system [7]. According to report by various Researchers, decrease in insulin degrading enzyme has great tendency to lower insulin degradation which will thereby sustain usual human blood sugar levels [8-11] and several proteins such as amyloid-β, glucagon, insulin-like growth factor-I and II can be degraded by insulin degrading enzyme. Attempts to regulate the activities of insulin degrading enzyme via various chemical compounds have been reported by several Scientists to be successful, yet it's level of resistance to numerous drug-like compounds has drawn the attention of many Researchers globally.

Cyclo-tetra amino acid based- peptides possess distinctive biotic features and they also have some residues such as N-methylamino acids, β-amino acids etc. that are not proteinogenic in nature [12]. The practical roles of cyclo-peptides in several areas like drug design and discovery as well as imaging have drawn the attention of several scientists globally [13]. According to Dougherty *et al*., 2019, series of properties of cyclo-tetra amino acid based-peptides have been observed to be high binding affinity, high selectivity, low toxicity, and high specificity [14]. Also, several reports on differences between cyclic and linear peptides have shown that cyclic peptides possess high metabolic steadiness and this has enhanced its usefulness in the area of drug design and discovery [15].

More so, computational chemistry comprise of several approaches which are proficient in developing innovative measure for qualifying, predicting and understanding chemical processes and this has made density functional theory method to be exceptional [16]. Therefore, density functional theory helped to recognize the reactivity of any compounds via the descriptors by linking the observed information to calculated molecular descriptors. [17]. Thus, the aim of this study was targeted at predicting the biological features of the lead compound as

well as the role of the derivatives attached to the parent compound of the lead compound.

Methodology

Ligand Minimization and Optimization

Two dimensional structures of the studied cyclic tetra amino acid based peptide (Figure 1) were modeled using ChemDraw 22.2.0.3300 version before transferring to Spartan '14 software for changing to 3-dimensional format. In this study, 6-31+G* was used as basis set for the calculation in vacuum, water and ethanol and the parameters obtained from the calculated compounds were reported appropriately. In this work, the calculation at ground state was executed from current geometry together with neutral charge at zero (0) unpaired electron and the completion of the optimization of the studied ligands was observed to be a function of the atoms present in the compounds. The IUPAC names of studied compounds were: (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(mercaptomethyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**A**), (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-6-methyl-3-((p-tolylthio)methyl)-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**B**), methyl 4-((((2R,8S,11S)-8-((R)-1-(benzyloxy)ethyl)-11-methyl-3,6,9,12-tetraoxo-1,4,7,10-tetraazacyclododecan-2-yl)methyl)thio)benzoate (**C**), (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((4-fluorophenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**D**), (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((3,5-dimethylphenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**E**), (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((4-chlorophenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**F**) and (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((4-bromophenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**G**).

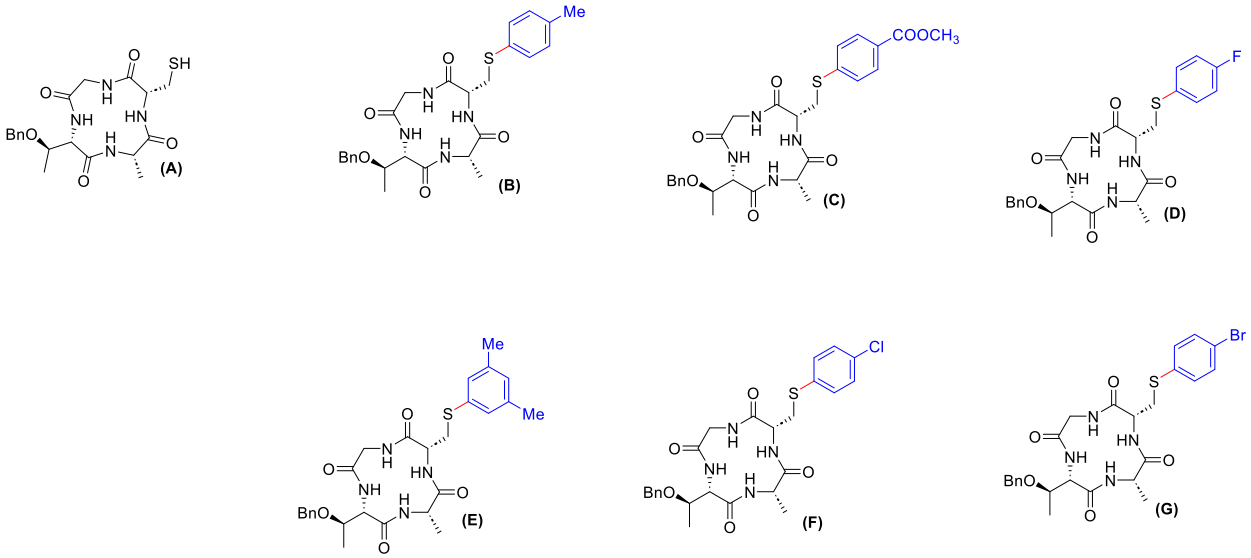

Figure 1: Two dimensional structure of cyclic tetra amino acid based compounds A-G

Preparation and Optimization of Insulin Degrading Enzyme (pdb id: 4re9)

The retrieved receptor (Insulin Degrading Enzyme; pdb id: 4re9) [18] from protein data bank was subjected to quickPrep tool which comprises of appropriate sub tools for receptor preparations and saved in .moe format before docking calculation using molecular operating environment software. The selected receptor was optimized and the any missing amino residue was replaced before further preparations. Also, the binding site was located using site finder and the suitable site was chosen for the docking calculation. The treated and prepared protein structure was saved in .moe format before docking calculation using induced fit method. The obtained results from docked complexes were presented in kcal/mol and the types of interactions involved in the docked complexes were displayed and reported.

Molecular Dynamic Simulation Analysis

Compound 6- Insulin Degrading Enzyme complex and Metformin - Insulin Degrading Enzyme complex were chosen for molecular dynamic simulation study and their choice of selection was due to calculated highest binding affinity for compound 6 and Metformin (reference compound). The chosen compounds were subjected to Swissparam software (https://www.swissparam.ch/) for parameterization. In this work, Charmm36m was employed as force field for the simulation of the parameterized compounds and the target using Gromacs software. Also, appropriate quantity of water molecules was added to the simulating system so as to accomplish solvation. More so, suitable ions were added at constant pressure and temperature. The final simulation was executed via 100 nanoseconds and the obtained results were reported accordingly.

Calculation of Pharmacokinetic Properties of Selected Compounds

The features obtained for Lipinski rule of five and other pharmacokinetic properties for compound 6 and the referenced compound (Metformin) were observed and reported. ADMETSar 1 was employed to execute this analysis and the reported results were presented appropriately.

Results and Discussion

Calculated frontier molecular orbital

Table 1 showed the frontier molecular orbitals obtain from the optimized geometrical structures in three phases (vacuum, water and ethanol). Highest occupied molecular orbital (E_H), lowest unoccupied molecular orbital (E_L) as well as energy gap were the descriptors generated from the optimized geometries. The calculated E_H, E_L and energy gap in eV in the three phases were -6.47, -1.19, 5.28 (vacuum); -6.78, -1.31, 5.47 (water); -6.74, -1.24, 5.50 (ethanol) for **A**; -6.11, -1.16, 4.95 (vacuum); -6.37, -1.05, 5.32 (water); -6.33, -1.00, 5.33 (ethanol) for **B**; -6.41, -1.05, 5.36 (vacuum); -6.39, -1.16, 5.23 (water); -6.36, -1.09, 5.27 (ethanol) for **C**; -6.59, -1.57, 5.02 (vacuum); -6.36, -1.42, 4.94 (water); -6.35,

-1.36, 4.99 (ethanol) for **D**; -6.35, -1.02, 5.33 (vacuum); -6.37, -1.14, 5.23 (water); -6.35, -1.08, 5.27(ethanol) for **E**; -6.47, -1.20, 5.27 (vacuum); -6.50, -1.37, 5.13 (water); -6.47, -1.29, 5.18 (ethanol) for **F**; -6.64, -1.42, 5.22(vacuum); -6.45, -1.41, 5.04 (water); -6.44, -1.34, 5.10 (ethanol) for **G**.

As displayed in table 1, it was observed that compound B optimized in vacuum and ethanol as well as compound D optimized in water has highest strength to donate electron to the neighboring compound since capability of any compound to react well could is a function of its strength to donate electron to nearby compound [19]. It showed that methylbenzene (Toluene) and fluorobenzene played crucial roles in enhancing the electron donating strength of the selected compounds. Thus, as presented in table 1, compound B optimized in vacuum proved to have highest ability to release electron than its optimization in ethanol and compound D in water; this denotes that the solvent used in this work hinder the electron releasing capacity of the compound with ability to donate to the nearby molecules.

The tendency of any compound to accept electron from other molecules with ability to donate electron has great connection with lowest E_L value. As presented in table 1, compound D proved to possess the strength to accept electron from the studied target as observed from all studied solvents. Also, the order of the effect of the studied solvent on compound D with respect to lowest unoccupied molecular orbital is $D_V > D_W > D_E$. This proved that the ability of compound D to receive electron in gas phase was greater than the compound in water and ethanol. More so, ethanol hindered it ability to accept electron from the other neighboring compound than water and this denote that compound D in water has higher strength accept electron than c compound D in ethanol.

According to report by Morakinyo *et al*., 2022 [20], the lower the energy gap value, the better the interaction between two molecules; thus, compound B which was optimized in vacuum and compound D which was optimized in water and ethanol proved to have ability to interact well in vacuum, water and ethanol media.

Table 1: Calculated features for the studied compounds in vacuum, water and ethanol

	Vacuum			Water			Ethanol		
	E_H (eV)	E_L (eV)	Energy Gap (eV)	E_H (eV)	E_L (eV)	Energy Gap (eV)	E_H (eV)	E_L (eV)	Energy Gap (eV)
A	-6.47	-1.19	5.28	-6.78	-1.31	5.47	-6.74	-1.24	5.50
B	-6.11	-1.16	4.95	-6.37	-1.05	5.32	-6.33	-1.00	5.33
C	-6.41	-1.05	5.36	-6.39	-1.16	5.23	-6.36	-1.09	5.27
D	-6.59	-1.57	5.02	-6.36	-1.42	4.94	-6.35	-1.36	4.99

E	-6.35	-1.02	5.33	-6.37	-1.14	5.23	-6.35	-1.08	5.27
F	-6.47	-1.20	5.27	-6.50	-1.37	5.13	-6.47	-1.29	5.18
G	-6.64	-1.42	5.22	-6.45	-1.41	5.04	-6.44	-1.34	5.10

Predicted induced fit docking study

The optimized compound docked against insulin degrading enzyme (pdb id: 4re9) resulted into various binding affinity for each docked complex. The effect of various derivatives attached to the parent compounds was investigated via its inhibiting capability against the studied target and the outcome for the inhibiting strength of each was reported in table 3. The calculated binding affinity in kcal/mol for compound A –G was -6.21969795, -7.21045113, -7.29078817, -7.92481852, -7.98367596, -8.16984558, -7.27570343 respectively. The report by Latona *et al* 2022 [21] revealed that the lower the binding affinity value, the stronger the molecule to inhibit than other ligands under study; thus, compound **F** proved to possess the adequate capacity to inhibit insulin degrading enzyme than other studied tetra amino acid based peptides as well as the referenced compound. More so, it was observed that the ability of Chlorine to reduce the electron density of the ring thereby have a great effect on compound **F** and enhanced its ability to inhibit the studied target than other compounds studied in this work (Figure 2-8). As shown in figure 7, compound F showed no type of interaction with any amino acid residue and this is an indication that other studied compounds with nonbonding interaction were weak. Also, it showed that possible presence of nonbonding interaction in compound F will alter it biological capability.

Table 3: The calculated values for docking analysis

	Scoring (kcal/mol)	Ligand	Receptor	Interaction	Distance
A	-6.21969795	S25	ASP 197	H-acceptor	4.34
		S25	LYS 308	H-acceptor	3.29
B	-7.21045113	O7	ASN 376	H-acceptor	2.97
		O27	LYS 364	H-acceptor	3.11
		6-ring	PHE 202	pi-pi	3.94
C	-7.29078817	O27	TYR 594	H-acceptor	2.69
		6-ring	ARG 668	pi-cation	4.19
D	-7.92481852	O27	LYS 364	H-acceptor	2.91
		N22	TYR 314	H-pi	4.63
		6-ring	TYR 302	pi-pi	3.80

E	-7.98367596	S25 O7 O21 O27	GLY 366 LYS 364 GLY 366 LYS 364	H-donor H-acceptor H-acceptor H-acceptor	4.09 3.13 3.38 2.88
F	-8.16984558	-	-	-	-
G	-7.27570343	S25 N28 O2 O16	THR 358 ASN 312 TYR 314 ASN 312	H-donor H-donor H-acceptor H-acceptor	3.64 3.24 3.02 3.01
Ref	-5.4				

**Ref denotes Metformin*

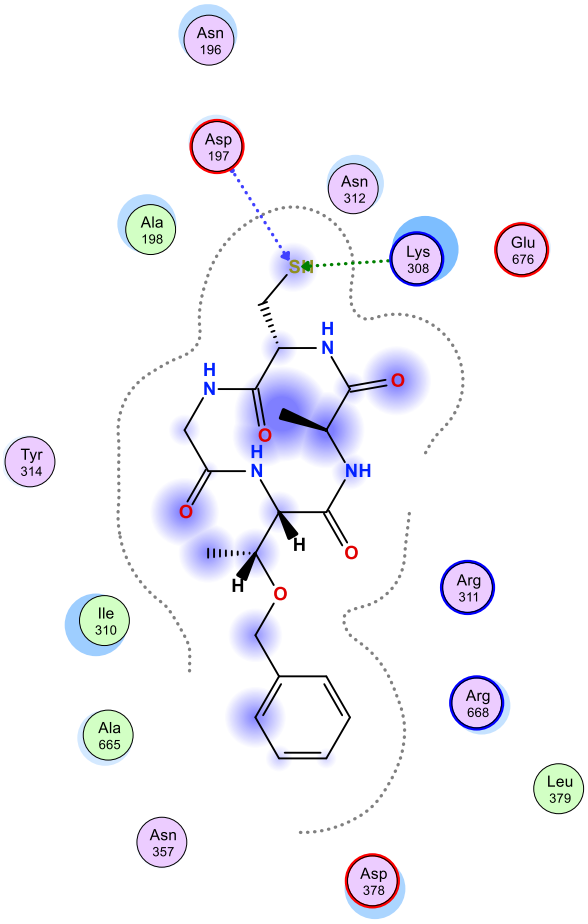

Figure 2: compound A surrounded with amino acid residues in active site of the studied target

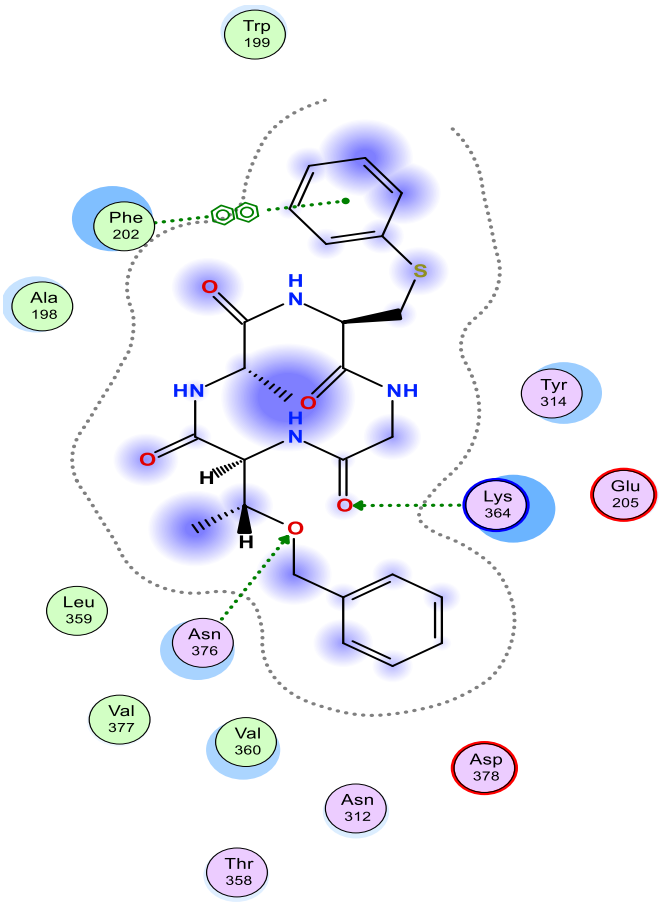

Figure 3: compound B surrounded with amino acid residues in active site of the studied target

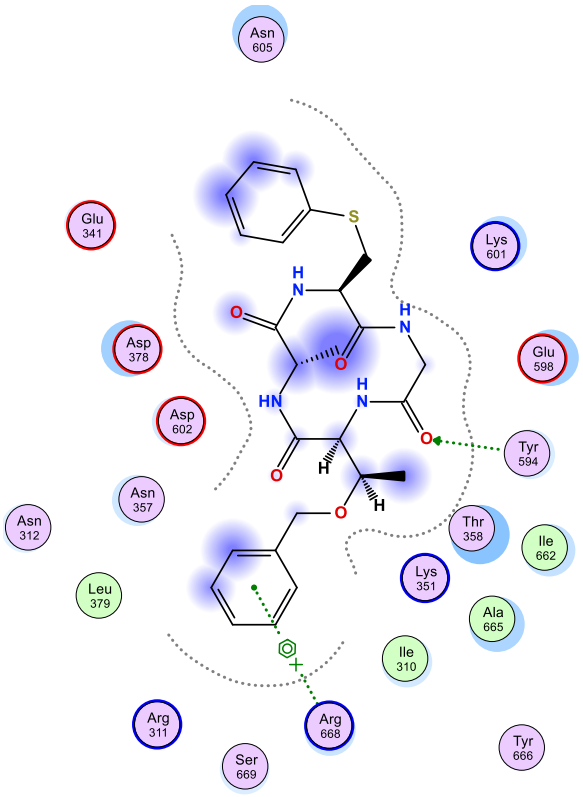

Figure 4: compound C surrounded with amino acid residues in active site of the studied target

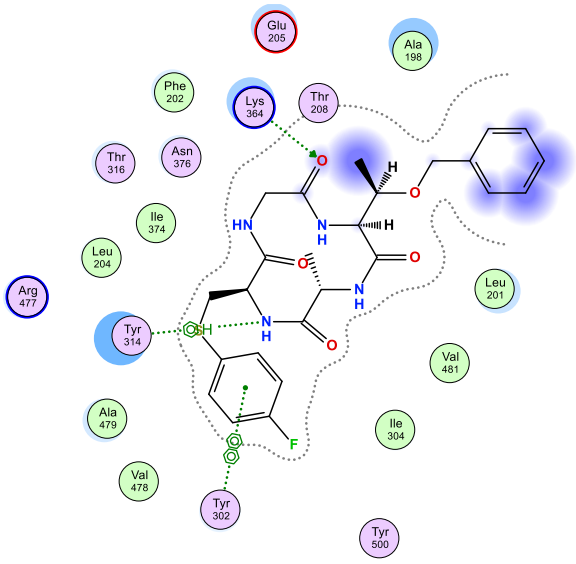

Figure 5: compound D surrounded with amino acid residues in active site of the studied target

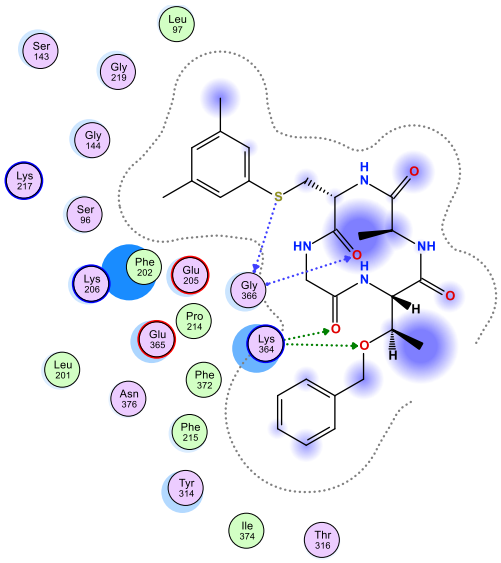

Figure 6: compound E surrounded with amino acid residues in active site of the studied target

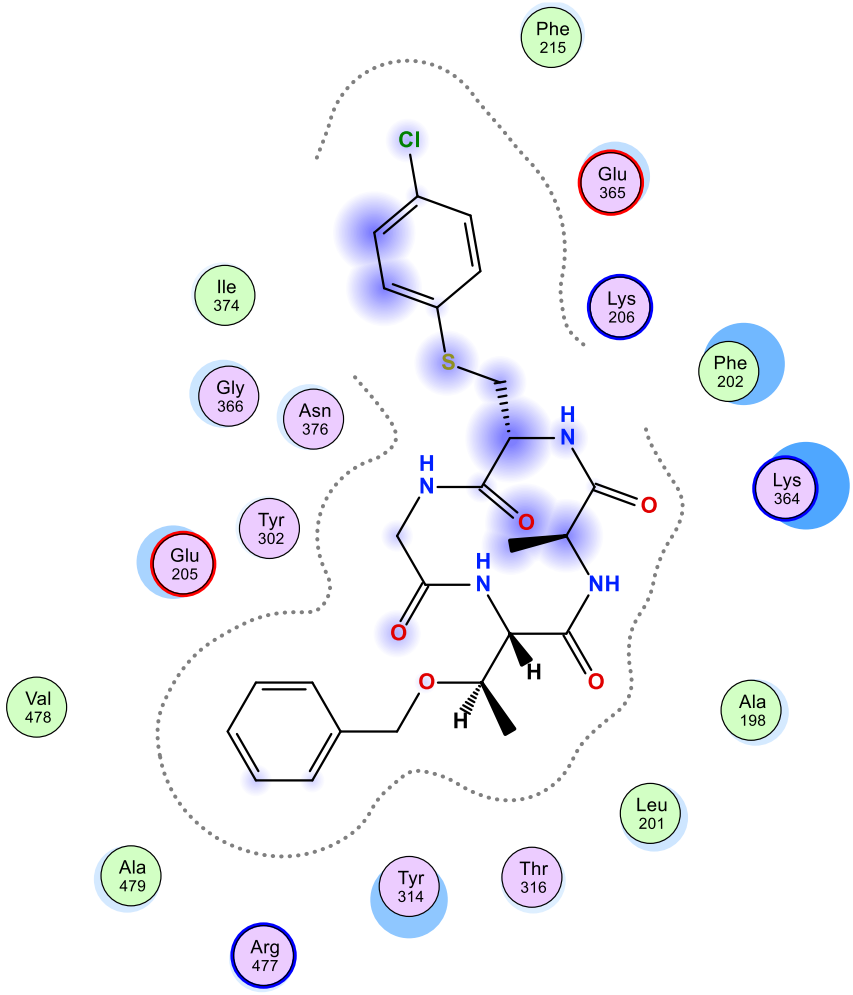

Figure 7: compound F surrounded with amino acid residues in active site of the studied target

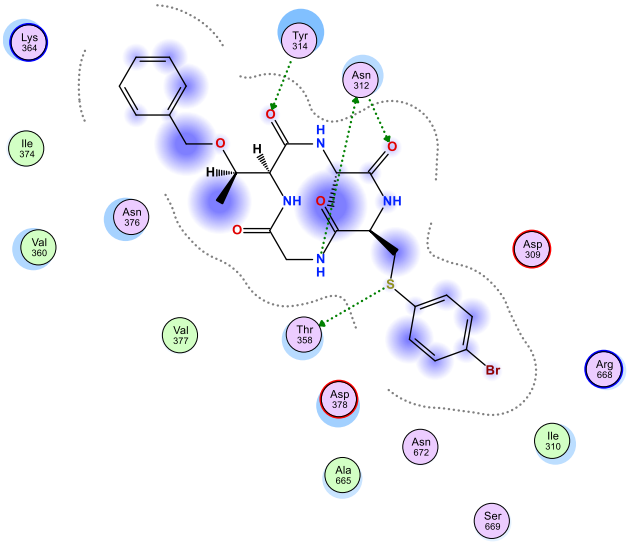

Figure 8: compound G surrounded with amino acid residues in active site of the studied target

Molecular dynamic Simulation Evaluation

In this study, the compound with highest binding affinity towards the studied target and the referenced compound were investigated using molecular dynamic simulation study. According to Oyebamiji *et al*., 2020, calculated binding affinity obtained from the docking of ligand and receptor is not enough to judge the inhibiting capacity of any compound; thus there is need for further investigations [20]. According to the report shown in table 3, (3R,6S,9S)-9-((R)-1-(benzyloxy)ethyl)-3-(((4-chlorophenyl)thio)methyl)-6-methyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone (**F**) has highest tendency to inhibit insulin degrading enzyme but the report shown in table 4 proved otherwise. Reviewing the docking result, it was observed that the difference between calculated binding affinity for compound F and reference compound was 2.76984558, but the calculated binding energy for compound F and the reference compound was 0.57± 0.00 kcal/mol and 0.48 ±0.19 kcal/mol respectively (Table 4). This showed that the ability of compound F to inhibit the target as claimed using docking result differs in the result presented for molecular dynamic simulation. Also, the lower binding affinity revealed for referenced compound was not validated using molecular dynamic simulation study. Also, the predicted RMSD for compound F-target complex (denoted by black) (Figure 9) was observed to be stable in the last 30ns which was contrary to the predicted RMSD for the reference-target complex and this was also supported by the predicted RMSF as shown in Figure 10.

Table 4 : Binding Energy Components

Complexes	Binding Energy Components (kcal/mol)			
	ΔE_{ele}	ΔG_{gas}	ΔG_{sol}	ΔG_{bind}
F-4re9	-0.87± 0.13	-0.87± 0.13	1.45± 0.14	0.57± 0.00
REF-4re9	0.12 ± 0.11	0.12 ± 0.11	0.37 ± 0.24	0.48 ±0.19

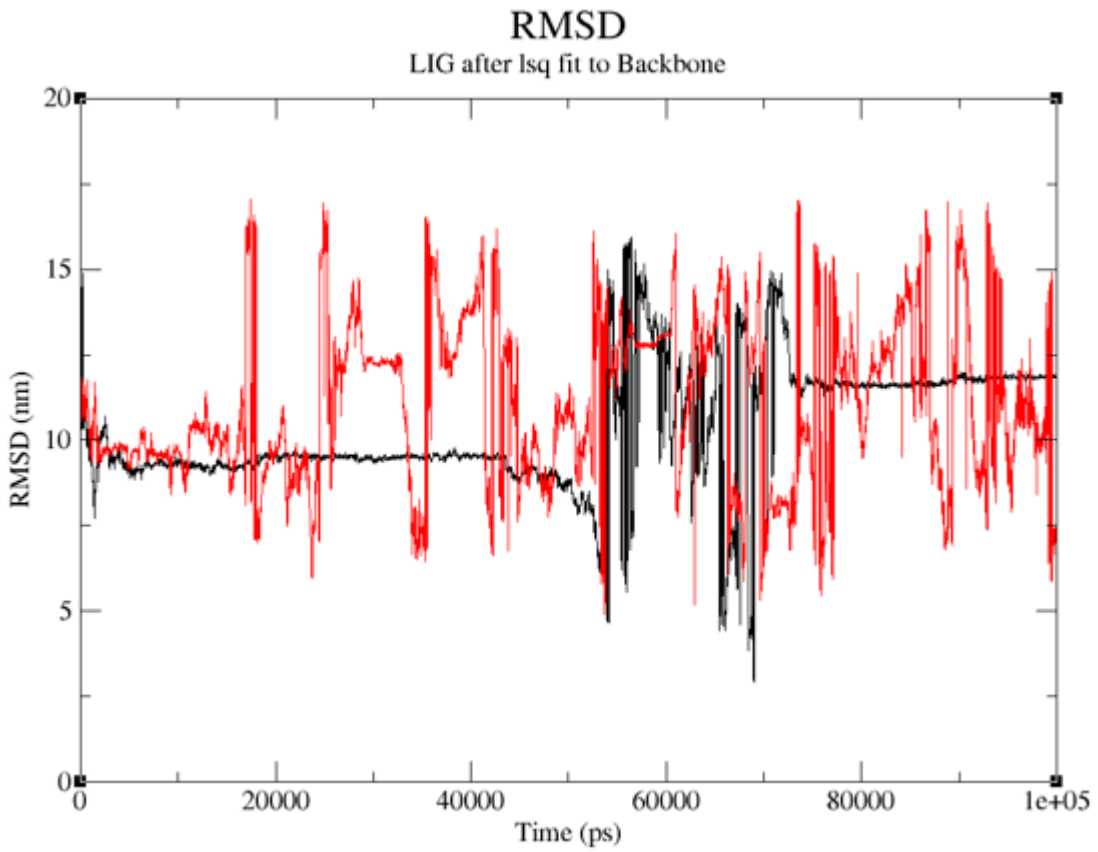

Figure 9: Predicted RMSD for compound F-Target complex (black) and reference-target complex (Red)

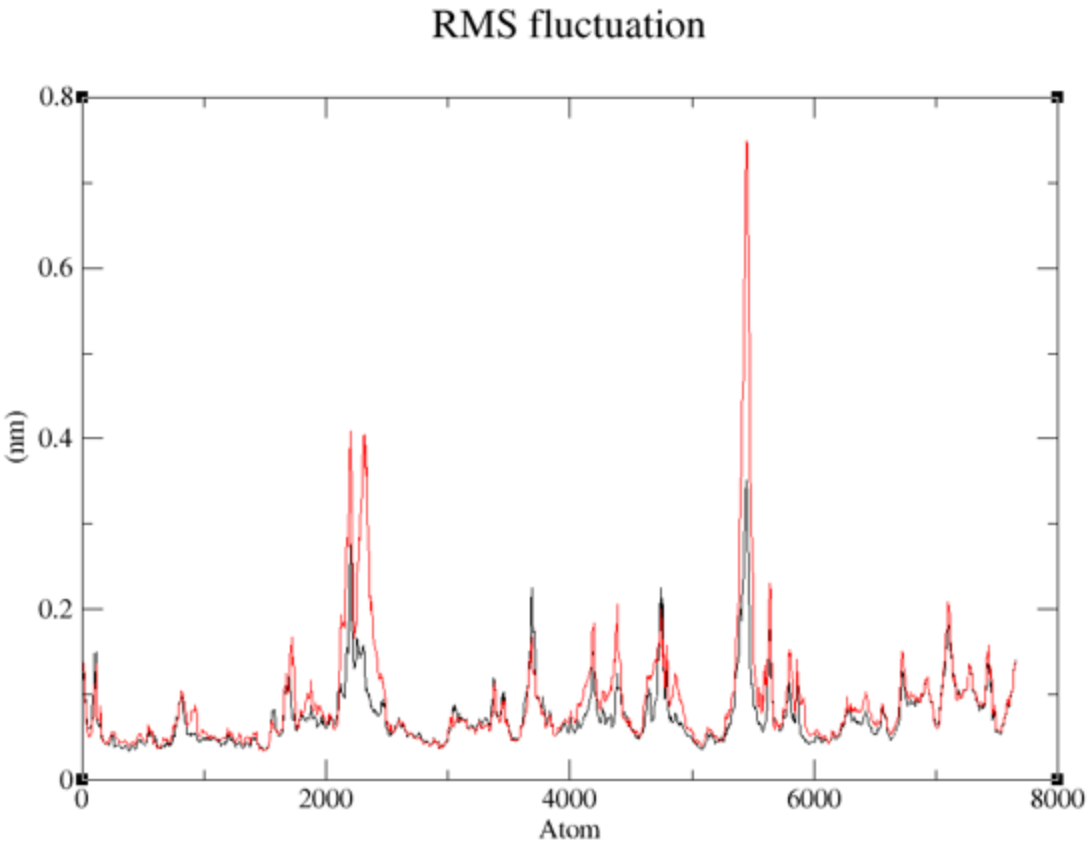

Figure 10: Predicted RMSF for compound F-Target complex (black) and reference-target complex (Red)

Pharmacokinetics Evaluation

The compounds with lowest binding affinity value and the referenced compound were selected for the purpose of investigating their absorption, distribution, metabolism, excretion and toxicity level. As shown in table 5 and 6, it was observed that the ADMET report for compound F and metformin were similar and this denotes that compound F has greater attribute to be a save drug-like agent.

Table 5: ADMET Features for compound **F**

Physicochemical Property	
Molecular Weight (MW)	532.150
Volume	510.580
Density	1.042
nHA	9
nHD	4
nRot	7

nRing	3
MaxRing	12
nHet	11
fChar	0
nRig	28
Flexibility	0.250
Stereo Centers	4
TPSA	125.630
logS	-3.856
logP	3.280
logD	3.147
Medicinal Chemistry	
QED	0.402
SAscore	4.375
Fsp^3	0.360
MCE-18	64.706
NPscore	0.484
Lipinski Rule	Accepted
Pfizer Rule	Accepted
GSK Rule	Rejected
Golden Triangle	Rejected
PAINS	0 alert(s)
ALARM NMR Rule	2 alert(s)
BMS Rule	0 alert(s)
Chelator Rule	0 alert(s)
Absorption	
Caco-2 Permeability	-5.717
MDCK Permeability	2.6e-05
Pgp-inhibitor	--
Pgp-substrate	++
HIA	---
$F_{20\%}$	---
$F_{30\%}$	---
Distribution	

PPB	91.815%
VD	0.887
BBB Penetration	--
Fu	3.172%
Metabolism	
CYP1A2 inhibitor	---
CYP1A2 substrate	---
CYP2C19 inhibitor	-
CYP2C19 substrate	---
CYP2C9 inhibitor	-
CYP2C9 substrate	---
CYP2D6 inhibitor	---
CYP2D6 substrate	--
CYP3A4 inhibitor	++
CYP3A4 substrate	--
Excretion	
CL	2.855
$T_{1/2}$	0.313
Toxicity	
hERG Blockers	--
H-HT	-
DILI	--
AMES Toxicity	---
Rat Oral Acute Toxicity	---
FDAMDD	---
Skin Sensitization	--
Carcinogencity	---
Eye Corrosion	---
Eye Irritation	---
Respiratory Toxicity	--
Environmental Toxicity	
Bioconcentration Factors	0.718

IGC_{50}	3.749
$LC_{50}FM$	4.911
$LC_{50}DM$	5.987
Tox21 Pathway	
NR-AR	-
NR-AR-LBD	---
NR-AhR	---
NR-Aromatase	---
NR-ER	-
NR-ER-LBD	--
NR-PPAR-gamma	++
SR-ARE	-
SR-ATAD5	---
SR-HSE	---
SR-MMP	--
SR-p53	---
Toxicophore Rules	
Acute Toxicity Rule	0 alert(s)
Genotoxic Carcinogenicity Rule	0 alert(s)
NonGenotoxic Carcinogenicity Rule	1 alert(s)
Skin Sensitization Rule	1 alert(s)
Aquatic Toxicity Rule	1 alert(s)
NonBiodegradable Rule	2 alert(s)
SureChEMBL Rule	0 alert(s)
FAF-Drugs4 Rule	2 alert(s)

Table 6: ADMET Features for compound **F**

Physicochemical Property	
Molecular Weight (MW)	129.100
Volume	127.451
Density	1.013
nHA	5

nHD	5
nRot	2
nRing	0
MaxRing	0
nHet	5
fChar	0
nRig	2
Flexibility	1.000
Stereo Centers	0
TPSA	91.490
logS	-1.163
logP	-1.584
logD	-1.471
Medicinal Chemistry	
QED	0.282
SAscore	3.206
Fsp^3	0.500
MCE-18	0.000
NPscore	-0.278
Lipinski Rule	Accepted
Pfizer Rule	Accepted
GSK Rule	Accepted
Golden Triangle	Rejected
PAINS	0 alert(s)
ALARM NMR Rule	0 alert(s)
BMS Rule	0 alert(s)
Chelator Rule	0 alert(s)
Absorption	
Caco-2 Permeability	-5.745
MDCK Permeability	0.0025
Pgp-inhibitor	---

Pgp-substrate	+++
HIA	---
$F_{20\%}$	---
$F_{30\%}$	---
Distribution	
PPB	5.598%
VD	1.083
BBB Penetration	--
Fu	76.538%
Metabolism	
CYP1A2 inhibitor	---
CYP1A2 substrate	---
CYP2C19 inhibitor	---
CYP2C19 substrate	---
CYP2C9 inhibitor	---
CYP2C9 substrate	---
CYP2D6 inhibitor	---
CYP2D6 substrate	++
CYP3A4 inhibitor	---
CYP3A4 substrate	---
Excretion	
CL	3.531
$T_{1/2}$	0.369
Toxicity	
hERG Blockers	--
H-HT	++
DILI	--
AMES Toxicity	---
Rat Oral Acute Toxicity	-
FDAMDD	---
Skin Sensitization	++

Carcinogencity	++
Eye Corrosion	---
Eye Irritation	---
Respiratory Toxicity	++
Environmental Toxicity	
Bioconcentration Factors	-0.247
IGC_{50}	1.341
$LC_{50}FM$	2.019
$LC_{50}DM$	2.923
Tox21 Pathway	
NR-AR	---
NR-AR-LBD	---
NR-AhR	---
NR-Aromatase	---
NR-ER	--
NR-ER-LBD	---
NR-PPAR-gamma	---
SR-ARE	--
SR-ATAD5	---
SR-HSE	---
SR-MMP	---
SR-p53	---
Toxicophore Rules	
Acute Toxicity Rule	0 alert(s)
Genotoxic Carcinogenicity Rule	0 alert(s)
NonGenotoxic Carcinogenicity Rule	0 alert(s)
Skin Sensitization Rule	0 alert(s)
Aquatic Toxicity Rule	1 alert(s)
NonBiodegradable Rule	1 alert(s)
SureChEMBL Rule	0 alert(s)
FAF-Drugs4 Rule	1 alert(s)

4.0. Conclusion

The inhibiting activities of tetra amino acid based peptides were evaluated using various computational tools such as Spartan '14, molecular operating environment software (MOE), Gromacs and swissparam. The activity of the studied compound in various media (vacuum, water, and ethanol) were executed and reported. Also, compound F with -8.16984558kcal/mol proved to have highest inhibiting than other studied compound and this is an indication that it can act as a potential drug like agent. The report from molecular dynamic simulation revealed that compound F has ability to inhibit the target and the calculated RMSD and RMSF supported this claim and this was compared with the calculated reference compound. Also, ADMET evaluation revealed the potential drug-like activities of compound F compared to the referenced compound. Thus, the studied compounds have greater prospect to be potential Insulin Degrading Enzyme inhibitors.

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