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In-Silico Screening of some Pyrimidines as DPP-IV inhibitors for Anti-hyperglycemic Activity.

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ABSTRACT

The objective of the present work is to study the *in silico* DPP4 inhibitory activity of commercially available pyrimidines. Molecular docking helps in studying drug/ligand or receptor/protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand- receptor complex which is evaluated by the energy of interaction for different ligands. We report *in silico* screening to obtain best fit molecules as DPP-IV inhibitors. In silico screening of compound was performed with the help of Vlife MDS 4.4 software as well as ADME studies were performed on PreADMET online software. This has been done for prioritization of molecules for actual synthesis and *in vitro* evaluation. *In silico* screening of 1000 pyrimidnes and derivatives of pyrimidines containing molecules was carried out on DPP-IV enzyme to obtain best fit nitrogenous heterocyclic compound for DPP-IV inhibition. Molecules were prioritized with comparable docking score as compared with Sitagliptin (-40.60) used as standard in docking. ADMET parameters such as HIA, Caco 2 cell permeability; MDCK and PPB were considered for prioritization. Prioritized compounds are DLN1, DLN2, DLN3, DLN4, DLN5, DLN6, DLN7, and DLN8. These can further use for development of anti-hyperglycemic agent.

Keywords: Pyrimidines, Pyrimidinones, *In silico*, ADME, Molecular docking, anti-hyperglycemic.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and associated with impaired fat, carbohydrate, and protein metabolism.DPP-4 inhibitors have been evaluated as monotherapy and used in combinations with other glucose-lowering agents in treatment of type 2 diabetes mellitus. Due to rapid cleavage and inactivation of incretin a therapy with native GLP-1 administered parentally is not feasible for the continuous treatment of type 2 diabetes. DPP-4 inhibitors are orally active in contrast to incretin. Diabetes is major leading cause of mortality. According to WHO serve till 2030 diabetes will be the 7th major factor of death. Inhibition of human Dipeptidyl peptidase (DPP-IV) plays a major role in diabetes therapy.

DPP-IV (Dipeptidyl peptidase):

DPP4 responsible for the degradation of incretins such as GLP-1, degradation of incretins leads to increase in blood glucose level. DPP4 inhibitors block DPP4 enzyme which prevent degradation of incretins and regulate blood glucose levels[1]. It is 766 amino acid proline peptidase which cleaves peptidase after a proline residue [2] . Compound that inhibit DPP4 enzyme exhibit an important role in medicine used as anti-hyperglycemic agent.

Pyrimidines as DPP4 inhibitors:

Research on pyrimidines derivative lead to formation of new anti- hyperglycemic agent. By considering importance of DPP4 enzyme, it was chosed as target and with literature of pyrimidines heterocycle moiety of aloglitin is recognized as a key pharmacophore that contributes to its good pharmacokinetics profile, potency, and selectivity, these were found to be potent DPP4 enzyme inhibitor hence chosen for this research. Using structure based drug design, it was hypothesized that pyrimidine scaffold could effectively display groups know to interact with the active sites residues of DPP-IV enzyme. In silico prioritization of these lead moieties as DPP4 enzyme inhibitor is being done by V life science MDS 4.4 drug design software. This research work reports in silico prioritization performed before actual synthesis and in-vitro evaluation of some pyrimidines for anti-hyperglycemic activity.

MATERIALS AND METHODS

Docking study was performed on V-life MDS 4.4 Drug design software and Marvin bean software was used to draw molecular structures, for conversion of 2D structure to mol files. 2D structure of ligand were prepared in Marvin sketch and convert to 3D by V-life science MDS 4.4 Drug design software. The 3D structure was stabilized by minimizing energy using molecular mechanics followed by Merck molecular force field (MMFF).The PDB of DPP-IV inhibitor receptor was obtained from protein data base with www.rcsb.org. The PDB was subjected for docking study.

In silico docking

In silico docking study were carried out using Vlife MDS 4.4 software. In silico screening was performed with selection of appropriate protein structure. The protein data bank file 4DSA for Anti-hyperglycemic receptor was selected after comparative analysis from PDB sum and subjected to docking study. The obtained docking score were comparable with standard sitagliptin having score of (-40.60).

EXPERIMENTAL

In silico screening

ADME Prediction

Online PreADMET software gives in silico ADME parameters by following parameters.

Caco2 cell permeability

To predict Caco2 cell permeability in Pre ADMET, molecules were solvated *in silico* at pH 7.4. By using Caco2 cells the apparent permeability values of compounds were determined. The range of Caco 2 cell was found to be 4-70 nm/sec.

MDCK cell permeability

MDCK cell are nothing but the Madin-Darby Canine Kidney cell. These are used to determine the apparent permeability values of compound. The range of MDCK is 25- 500 nm/sec.

Human Intestinal Absorption (HIA)

PreADMET abel to predict percent human intestinal absorption (% HIA). HIA data is the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile and faces. The range of HIA is 20- 70%.

Plasma Protein Binding (PPB)

Only the unbound drug can diffuse or transport across cell membranes and can do interaction with a pharmacological target. As a result a degree of plasma protein binding of drug influences not only the drugs action but also its disposition and efficacy. The range of PPB is about 90%. In silico ADME prediction are shown in Table 1.

Table 1: It shows in silico ADME Prediction data for selected compound.

Compound	HIA@	Caco2 cell permeability++	MDCK+++	PPB$
DLN1	91.88	20.53	0.0512	84.52
DLN2	88.123	20.388	0.165	74.62
DLN3	80.76	17.38	0.055	90.93
DLN4	90.23	21.001	0.0567	88.10
DLN5	81.38	20.71	0.890	83.03
DLN6	84.10	20.97	0.083	89.11
DLN7	76.61	18.56	0.076	95.94

@HIA = Human Intestinal Absorption.
++Caco 2 cell permeability = human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium.
+++MDCK = Madin-Darby canine kidney cell.
$PPB = Plasma Protein Binding.

Docking study

Computer-assisted simulated docking experiments were carried out in Vlife MDS 4.4 software separately. Docking studies involves following steps:

Selection of Protein file from the database (pdb selection)

Protein pdb (4DSA) was selected after a comparative analysis of different pdb protein structures.

Protein Validation

By using Ramchandran plot and Errata plot validation of protein can take place. The Ramchandran plot showed 96.2% residues in favored region; moreover 99.5% in allowed region. Figure 1 shows Ramachandran plot of protein PDB. Further this protein subjected to active site analysis, optimization and

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docking studies on VLife MDS 4.4 tools. Errata report was obtained from the NIH MBI sever for evaluation of protein structures and is shown in Figure 2.

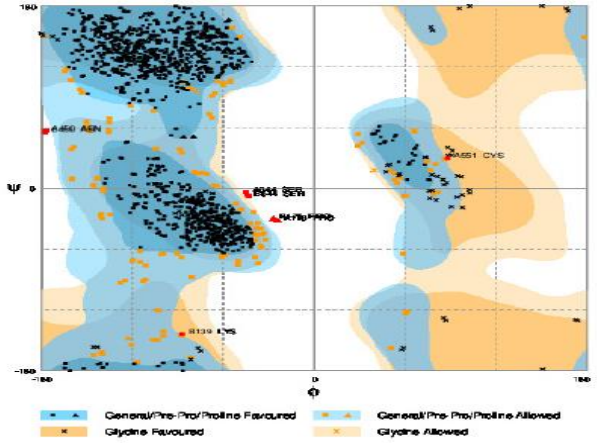


Figure 1: Ramchandran plot of PDB

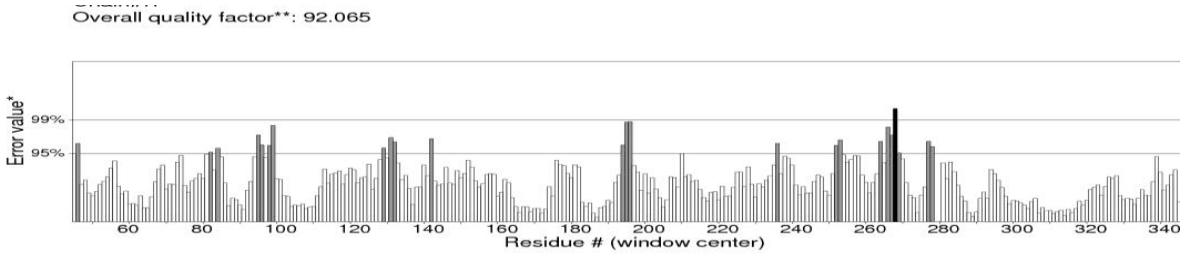


Figure 2: Errata report of PDB

Active site analysis: It shows 3 cavities out of which cavity 1 was selected since it contained co-crystallized ligand. This site abundantly had lipophilic residues and cylindrical in shape. Fig.3.

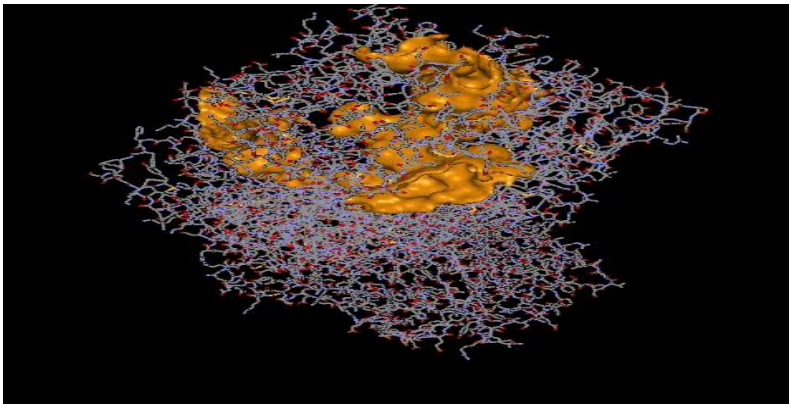


Fig 3: It shows the co-ordinates, shape and active residues involved in 4DSA.

Library design and Ligand preparation

The Chemdraw ultra 8.0 software was used to draw molecular structures of ligands and then converted 2D structure in to 3D mol files. Structures of ligands were designed shown from Series 1 (N-Alkyl-3-[3-amino-4-carbonitrile benzimidazolo pyrimidin5-yl]indoles), Series 2 (5-(5-(substituted phenyl)-2,3-dihydro-6H-thiazolo-pyrimidine-4-one-8 yl)-4,6,7-trimethoxy benzofuran), Series 3 (2-arylidene-4-(4-methoxyphenyl)-5-(3,4-dimethyl phenylamino)carbonyl -6-phenyl-4,7 dihydro thiazolo-pyrimidine-3-ones) and series 4 (2-amino-4-(7-substituted/unsubstituted coumarin-3-yl)6-(chlorosubstitutedphenyl)pyrimidines),series 5(2-(4-

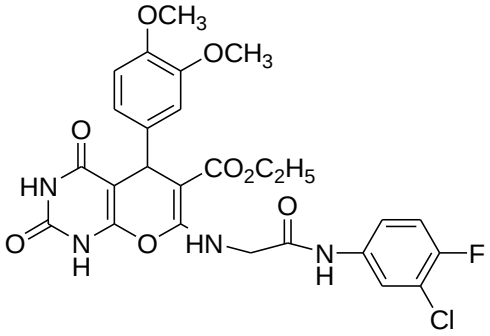
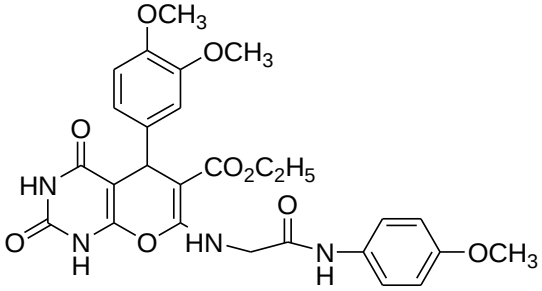
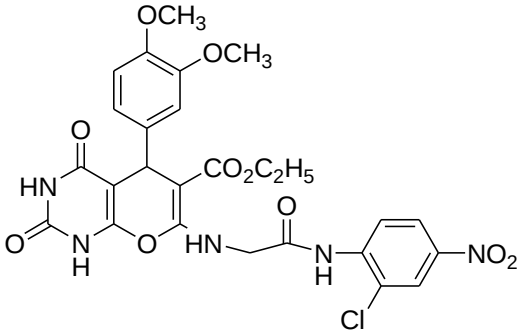
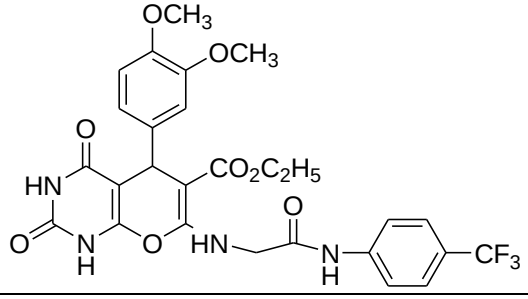
sulphonamoyl)hydrazono-3,5-heptane dione),series 6 (2-amino-4,6-diethyl-5-(4-sulphonamoyl)azopyrimidine) . Library of (1000) compounds was developed.

Molecules were drawn using 2D draw in Vlife MDS 4.4 tool. These ligand were further converted in to 3D. General structures of these ligands are shown in following Tables.

Docking of ligands

V-Life MDS 4.4: Docking studies were performed using Biopredicta modules of VLife MDS 4.4 software. Docking was done by grip based docking. For performance of grip based docking, it requires a set of ligand with its conformers to be docked in receptor cavity. Grip docking consist of the pre-computation of grid, its gives the best possible interaction of ligand and receptor and minimizing the steric unfavorable and repulsive interaction. Result of docking score, are shown in following table no. 2-13.

Table no 2:

Compound no.	Structure	Docking score
DLN1		-50.84
DLN2		-53.78
DLN3		-50.10
DLN4		-53.59

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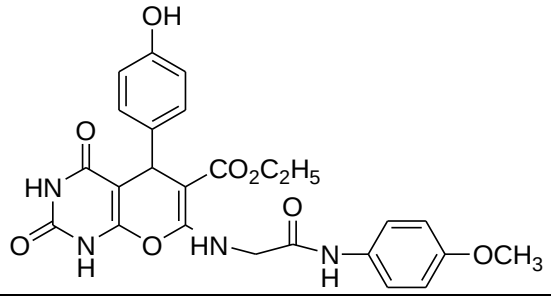
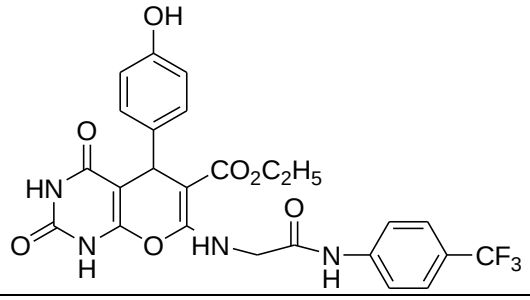
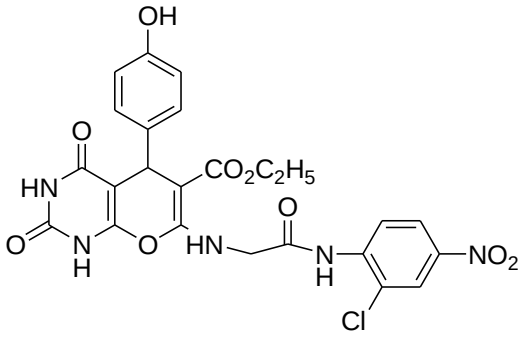
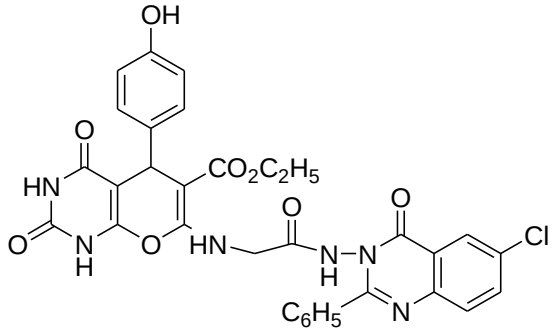
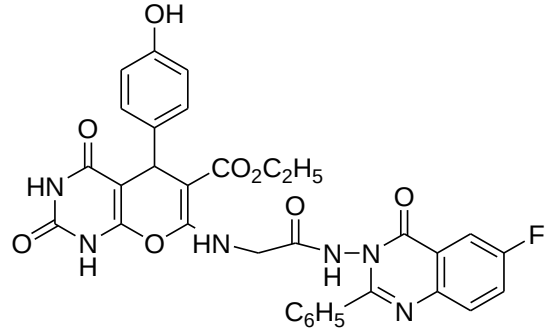
DLN5		-50.78
DLN6		-51.39
DLN7		-52.17
DLN8		-53.43
DLN9		-55.87
Sitagliptin	-	-40.60

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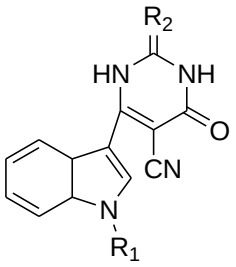

Table no. 3

Compound no.	R1	R2	Docking score
DLN10	H	O	-32.58
DLN11	CH2CH3	O	-31.90
DLN12	CH2C6H5	O	-43.24
DLN13	COC6H5	O	-42.30
DLN14	COC6H4Cl(Para)	O	-44.06
DLN15	COC6H4Cl(Ortho)	O	-42.91
DLN16	SO2C6H5	O	-39.74
DLN17	SO2C6H4Br(para)	O	-38.02
DLN18	H	S	-33.82
DLN19	CH2CH3	S	-33.76
DLN20	CH2C6H5	S	-40.59
DLN21	COC6H5	S	-48.19
DLN22	COC6H4Cl(Ortho)	S	-43.95
DLN23	COC6H4Cl(Para)	S	-42.95
DLN24	SO2C6H5	S	-45.05
DLN26	SO2C6H4Br(para)	S	-42.96
Sitagliptin	-	-	-40.60

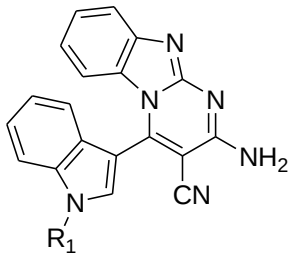

Table no 4:

Compound no.	R1	Docking score
DLN25	H	-38.82
DLN26	CH2CH3	-37.43
DLN27	CH2C6H5	-47.06
DLN29	COC6H4Cl(Ortho)	-54.17
DLN30	COC6H4Cl(Para)	-51.49
Sitagliptin	-	-40.60

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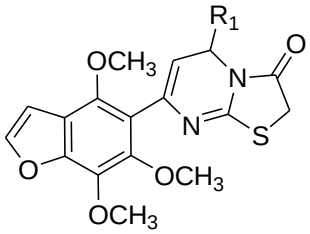

Table no 5:

Compound no.	R1	Docking score
DLN33	C6H5	-41.93
DLN34	C6H4OCH3(Para)	-37.70
DLN35	C6H4NO2(Para)	-39.15
Sitagliptin	-	-40.60

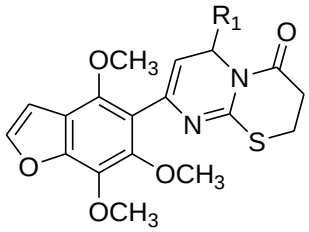

Table no 6:

Compound no.	R1	Docking score
DLN36	C6H5	-37.87
DLN37	C6H4OCH3	-39.63
DLN38	C6H4NO2	-42.37
Sitagliptin	-	-40.60

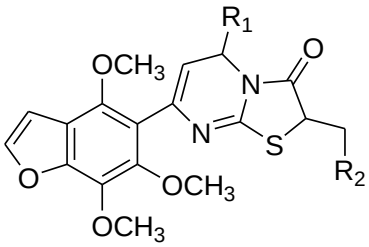

Table no 7

Compound no.	R1	R2	Docking score
DLN39	C6H5	C6H5	-43.49
DLN40	C6H4OCH3 (Para)	C6H4OCH3 (Para)	-42.00
DLN41	C6H4NO2 (Para)	C6H4NO2 (Para)	-47.84
Sitagliptin	-	-	-40.60

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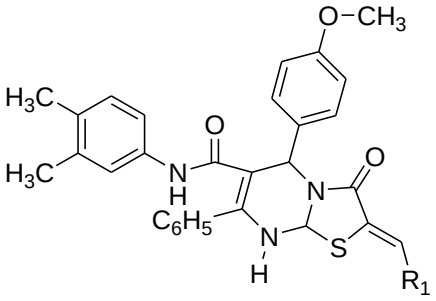
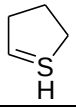


Table no 8:

Compound no	R1	Docking score
DLN42	C6H4OCH3(Para)	-45.27
DLN43	C6H5	-40.60
DLN44	C6H4-O-C6H4	-49.52
DLN45	3,4 dimethoxy benzene	-39.08
DLN46	4-hydroxy,3-methoxy benzene	-45.00
DLN47	3-nitrobenzene	-44.46
DLN48		-38.80
DLN49	p-hydroxy benzene	-46.22
DLN50	4-N(CH3)2C6H4	-40.29
DLN51	4-ClC6H4	-39.86
Sitagliptin	-	-40.60

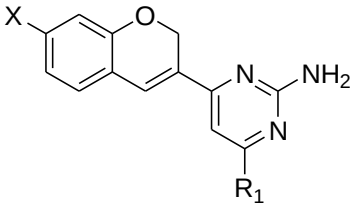


Table no 9:

Compound no	X	R1	Docking score
DLN52	H	2-Chorophenyl	-42.59
DLN53	H	4-Chlorophenyl	-38.87
DLN54	H	2,6-dichlorophenyl	-41.32
DLN55	H	2,4-dichlorophenyl	-39.87
DLN56	H	2,5-dichlorophenyl	-45.56
DLN57	H	3,5-dichlorophenyl	-45.92
DLN58	H	3,4-dichlorophenyl	-44.27
DLN59	Cl	2-chlorophenyl	-43.11
DLN60	Cl	4-Chlorophenyl	-40.43
DLN61	Cl	2,6-dichlorophenyl	-45.17
DLN62	Cl	2,4-dichlorophenyl	-40.86
DLN63	Cl	2,5-dichlorophenyl	-47.18
DLN64	Cl	3,5-dichlrophenyl	-51.12
DLN65	Cl	3,4-dichlorophenyl	-41.75
DLN66	Br	2-chlorophenyl	-43.67
DLN67	Br	4-chlorophenyl	-41.36
DLN68	Br	2,6-dichlorophenyl	-44.67

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DLN69	Br	2,4-dichlorophenyl	-46.52
DLN70	Br	2,5-dichlorophenyl	-49.46
DLN71	Br	3,5-dichlorophenyl	-45.62
DLN72	Br	3,4-dichlorophenyl	-44.29
Sitagliptin	-	-	-40.60

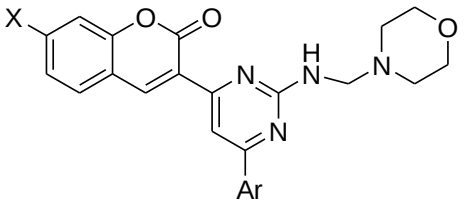

Table no 10:

Compound no	X	Ar	Docking score
DLN73	H	2-chlorophenyl	-49.08
DLN74	H	4-chlorophenyl	-47.24
DLN75	H	2,6-dichlorophenyl	-51.74
DLN76	H	2,4-dichlorophenyl	-41.89
DLN77	H	2,5-dichlorophenyl	-47.76
DLN78	H	3,5-dichlorophenyl	-46.30
DLN79	H	3,4-dichlorophenyl	-50.07
DLN80	Cl	2chlorophenyl	-47.92
DLN81	Cl	4-chlorophenyl	-47.82
DLN82	Cl	2,6-dichlorophenyl	-48.19
DLN83	Cl	2,4-dichlorophenyl	-50.58
DLN84	Cl	2,5-dichlorophenyl	-45.88
DLN85	Cl	3,5-dichlorophenyl	-49.32
DLN86	Cl	3,4-dichlorophenyl	-43.95
DLN87	Br	2-chlorophenyl	-46.28
DLN88	Br	4-chlorophenyl	-43.87
DLN89	Br	2,6-dichlorophenyl	-46.10
DLN90	Br	2,4-dichorophenyl	-47.58
DLN91	Br	2,5-dichlorophenyl	-45.11
DLN92	Br	3,5-dichlorophenyl	-46.37
DLN93	Br	3,4-dichlorophenyl	-49.74
Sitagliptin	-	-	-40.60

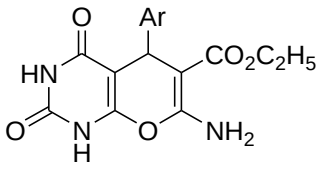

Table no 11:

Compound no	Ar	Docking score
DLN94	4-MeC6H4	-38.33
DLN95	C6H5	-34.22
DLN96	4-MeoC6H4	-42.60
DLN109	3,4-MeoC6H4	-43.23
DLN110	3-OH-C6H4	-38.06
DLN111	4-OH- C6H4	-41.84
DLN112	4-Cl- C6H4	-43.23

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DLN113	3-Br- C6H4	-38.52
DLN114	3-NO2- C6H4	-41.40
DLN115	4-NO2- C6H4	-40.28
Sitagliptin	-	-40.60

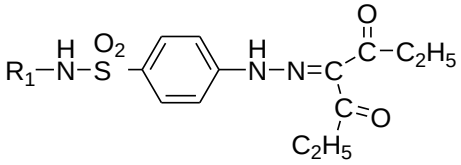
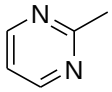
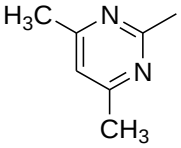


Table no 12:

Compound no	R	Docking score
DLN116		-40.25
DLN117		-37.79
Sitagliptin	-	-40.60

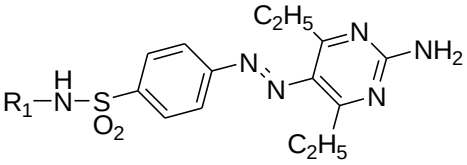
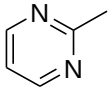
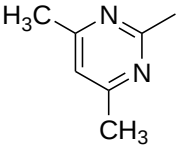


Table no 13:

Compound no	R1	Docking score
DLN118		-38.78
DL119		-39.15
Sitagliptin	-	-40.60

RESULT AND DISCUSSION

Form figure 1 and 2 the protein structure was validated. Around 1000 molecules was docked and screened best score prior to actual synthesis. Changes were made in substituent's position carried out. Alkyl/phenyl substitution on pyrimidine ring results in increased score and activity and it was found that it may results in increased anti-hyperglycemic activity.

All the molecules were found to be fitting the actual binding pocket of molecule Sitagliptin. Most of the good scoring ligands from the synthetic series 1, 2, 3,4,5,6 were obtained. From above work prioritized compounds are shown in fig. 4.

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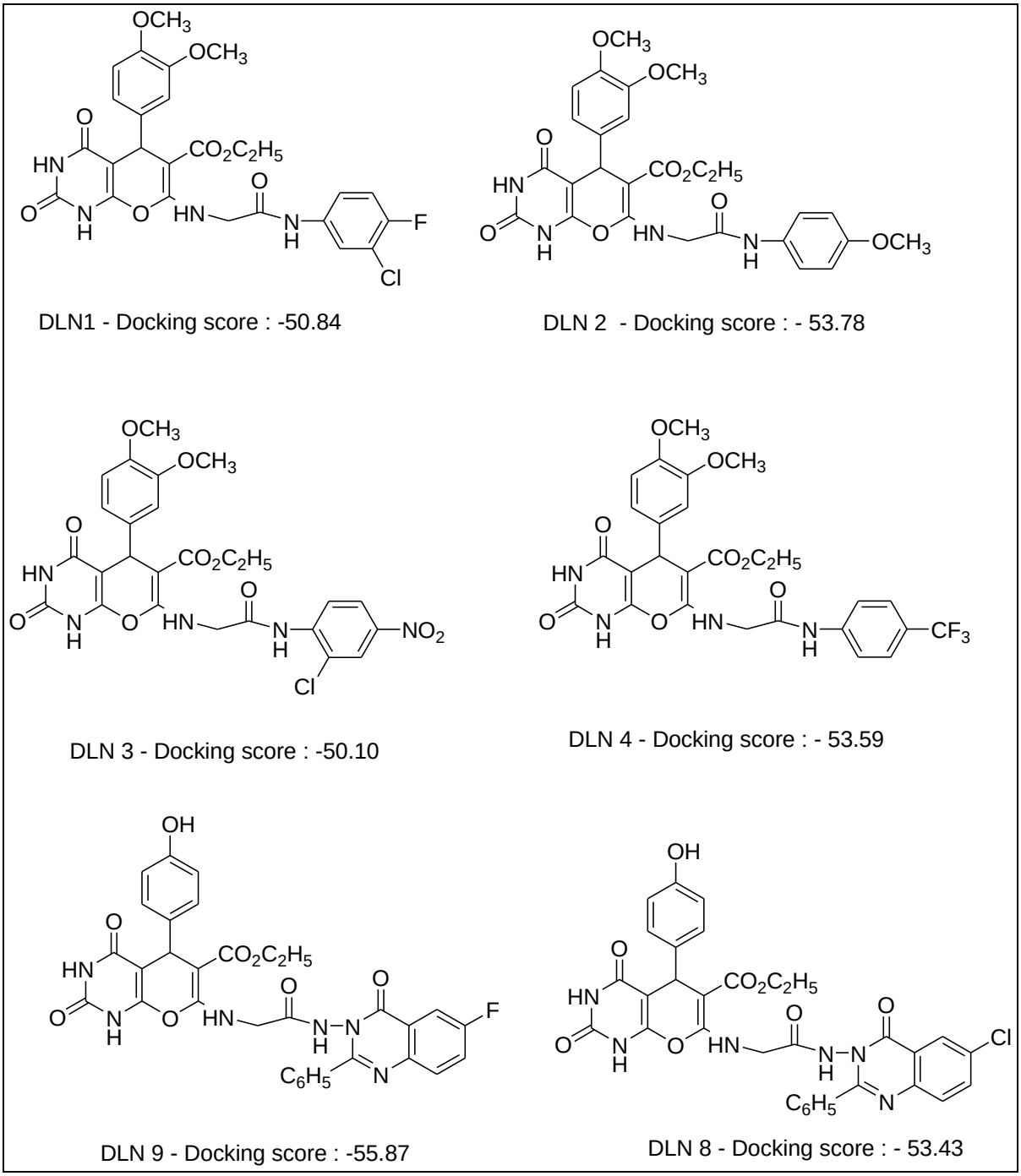
Fig no 4: Prioritized compounds



CONCLUSION

Most of ligands were found to be interacting with the amino acid residues of the active sites. The present work leads to the development of pyrimidine derivatives as anti- hyperglycemic leads by in silico design.

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