Research Article

Identification of potent Cyanoacetylhydrazone derivatives as antidiabetic activity by *in silico* method

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Abstract

Objective: The high diabetic mellitus rate in India, the identification of novel molecules is important in the development of novel and potent antidiabetic drugs. Herein, novel series of acetohydrazide derivatives were analyzed for antidiabetic activity using combined approach of molecular docking study and ADMET (computational pharmacokinetic elucidation). **Material and methods:** The *in silico* molecular docking study and ADMET calculation were carried out using BIOVIA Discovery Studio (DS) 2017 software. **Results and conclusion:** The molecular interaction analysis revealed that the compounds have good interaction with the active site of 1IR3. The ADMET results show these molecules contain drug likeness properties. So this examination would be an approach to recognize new therapeutics for diabetic patients.

Keywords: Anti-diabetic, Molecular docking, ADMET, insulin receptor

Introduction

Diabetes mellitus (DM), considered by hyperglycemia and carbohydrate, protein and fat metabolism disturbances, is an extensive metabolic disease. It's influence the patient personal satisfaction as far as social, psychological prosperity and physical sick wellbeing (Dewanjee et al., 2009). DM is a chronic metabolic disorder manifested with elevated levels of glucose in the body, which is an impact of debilitated insulin emission, insulin impact, or both. DM is a common scenario in the South and East Asia region mostly in Bangladesh, India, Sri Lanka, Bhutan, Mauritius, and Maldives (Nanditha et al., 2016). According to the latest reports, more than 382 million people are affected with diabetes in 2013 and estimated to reach a total of 592 million by 2035 (International Diabetes Federation, 2013). DM is classified by Two forms. One is Types 1 (T1DM) and another one is Type 2 (T2DM), it is differ in their pathogenesis, but both have hyperglycaemia as a common trademark. T1DM body fails to produce insulin, whereas in T2DM shows resistance to insulin. T2DM is a contribution of many alteration genes and their products.

The general population in Southeast Asia district are being under more serious hazard, and the lion's shares of patients have T2DM. Insulin protection regularly goes before the beginning of T2DM and is ordinarily joined by other cardiovascular hazard factors, for example, dyslipidemia, hypertension, and prothrombotic factors. DM related cardiovascular complications occur due to altered lipoprotein metabolism-mediated atherosclerosis, and DM are two to four times more likely to suffer from stroke (Oranje and Wolffenbuttel, 1999). Even though different classes of effective drugs are available to control T2DM, still it is a challenging task to bring a better molecule which is devoid of undesirable adverse effects than existing drugs. In this study, we have considered acetohydrazide derivatives as an antidiabetic compound and also possible drug candidate for insulin receptor. For which, acetohydrazide derivatives were evaluated by molecular docking studies and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) calculations. This *in silico* analysis may provide possible binding information of interaction between insulin receptor and the acetohydrazide derivatives. And also, ADMET calculations gives details of Aqueous solubility level, Blood Brain Barrier Level (BBB), Cytochrome P4502D6 (CYP2D6), Hepatotoxicity Level, Plasma protein binding logarithmic level (PPB) in human body.

Materials and methods

Chemicals were procured from E. Merck (India), S. D. Fine

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Chemicals (India) and reagent/solvents were used without distillation procedure. Melting points were taken in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (ν in cm–1) and NMR spectra were recorded on a Bruker spectrometer DPX-300MHz (Bruker, Germany) by using $CDCl_3$ as solvent with TMS as an internal standard. All the spectral data are consistent with the assigned structures of the desired product and the progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

The *in silico* molecular docking study and ADMET calculation were carried out using BIOVIA Discovery Studio (DS) 2017 software (Dassault Systèmes BIOVIA, 2017).

Preparation of S1, S2, S3 and S4

3-methyl-2,6-diphenylpiperidin-4-one was prepared by adopting the literature method. Condensation of 2-butanones, benzaldehyde and ammonium acetate in warm ethanol in the ratio of 1:2:1 respectively afforded the formation of 3-methyl-2,6-diphenylpiperidin-4-ones.

Preparation of 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone (Figure 1)

A mixture of 3-methyl-2,6-diphenylpiperidin-4-one (0.1 mol), cyanoacetic hydrazide (0.1 mol) in the presence of few drops of concentrated acetic acid in methanol was refluxed for 2 hours. After the completion of reaction, the reaction mixture was cooled to room temperature. The solid product was separated by filtration and washed with warm water and recrystallized by methanol to afford 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone.

Preparation of Ligands

3D dimensional format of 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone(S1), 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S2), 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3), 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4), and standard Glibenclamide drug were drawn in MarvinSketch software. Further subjected to single step energy minimization with the help of steepest descent method in DS for 200 steps at RMS gradient of 0.01. Energy minimization is an important step in molecular docking studies. It was utilized to compute the equilibrium configuration of the compounds.

Preparation of Proteins

The X-ray crystal structure of insulin receptor 1IR3 for in this anti-diabetes mellitus study was retrieved from RCSB Protein Data Bank (http:// www.rcsb.org/pdb). Subsequently, all the heteroatoms were removed from 1IR3 receptor. Further the protein was subjected to multiple steps energy minimization to remove the bad steric 500 steps at RMS gradient of 0.1. The CHARMM force field was applied to the 1IR3 receptors. The receptor protein is separated into the ligand part and protein part. The protein part was selected as a "Define selected molecule as receptor" under define and edit binding site, where in, the protein is marked as receptor molecule. The ligand part was click and made of "Define sphere from selection" so that the crystal ligand can be used to define the binding site of 1IR3. This 'input receptor molecule' is used as input parameter in the CDOCKER protocol.

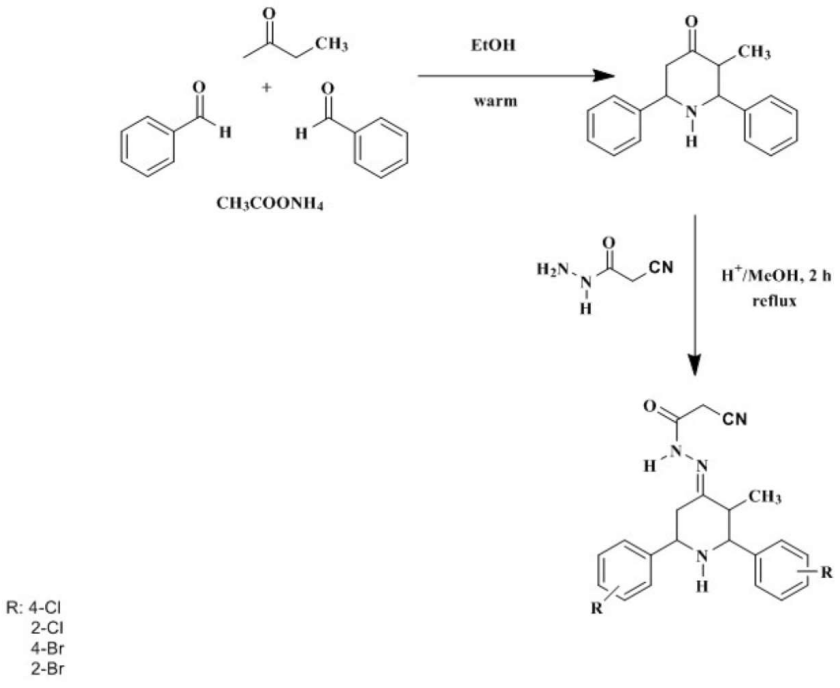


Figure 1. Preparation of 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone

Molecular Docking

Molecular docking was performed by the CDOCKER docking method applied in DS. In this docking method ligands are in fully flexible type and protein is kept as constant. Both minimized ligands and receptor are used as input ligand and input receptor in CDOCKER protocol. The other parameter in this protocol was mentioned in table1.

Table 1. Parameter of CDOCKER protocol

Input Receptor	Input/1ir3.dsv
Input Ligands	/Input/Total_min_ligands.sd
Input Site Sphere	-23.9454, 29.2003, 7.29961
Top Hits	1
Random Conformations	10
Random Conformations Dynamics Steps	1000
Random Conformations Dynamics Target Temperature	1000
Include Electrostatic Interactions	True
Orientations to Refine	10
Maximum Bad Orientations	800
Orientation vdW Energy Threshold	300
Simulated Annealing	True
Heating Steps	2000
Heating Target Temperature	700
Cooling Steps	5000
Cooling Target Temperature	300
Forcefield	CHARMm
Use Full Potential	Yes
Grid Extension	8.0
Ligand Partial Charge Method	CHARMm
Random Number Seed	314159
Final Minimization	Full Potential
Final Minimization Gradient Tolerance	0
Parallel Processing	False
Parallel Processing Batch Size	25
Parallel Processing Server	localhost
Parallel Processing Server Processes	2
Parallel Processing Preserve Order	True
Random Dynamics Time Step	0.002

ADMET Study

In silico ADME studies were performed by using ADMET Descriptors algorithm of DS in which various pharmacokinetic parameters like Aq. Solubility, Human Intestinal, Plasma protein binding (PPB) ,blood-brain-barrier (BBB) penetration cytochrome P450 inhibition and hepatotoxicity levels were estimated for 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone(S1), 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S2), 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3), 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4).

Results and discussion

Docking Study

The *in silico* docking study was achieved with the help of CDOCKER protocol which is one of the Receptor-Ligand Interactions protocols in DS. This protocol is a grid-based molecular docking method that employs CHARMm. The docking score in this protocol was reported as the negative value (i.e., -CDOCKER_ENERGY), where a higher value indicates a more favourable ligand-protein binding. This score covers all the energies like docking, Van der Waals, electrostatic, hydrophobic interaction energies etc. The docking score and results of the 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone(S1), 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S2), 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3), 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4), and standard Glibenclamide are presented in (Table 3 and Figure 2 – 6). Each compound of Hydrogen bond, Van der Waals, Pi-Sigma, Alkyl, Pi-Alkyl interactions are clearly depicted in (Figure 2 (B) to 5

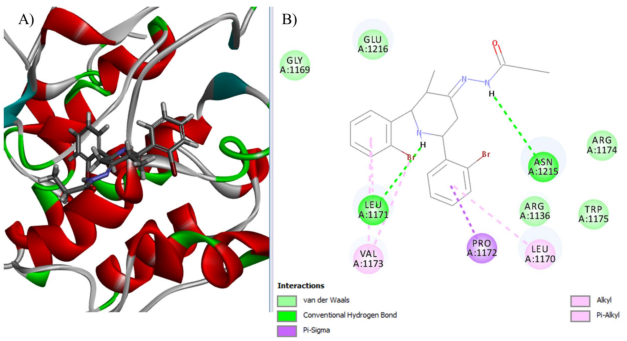


Figure 2. A) 3D and B) 2D Interaction of 3-methyl-2,6 di(bis-o-bromophenyl) piperidin -4-one cyanoacetyl hydrazone, in active site of receptor (1IR3).

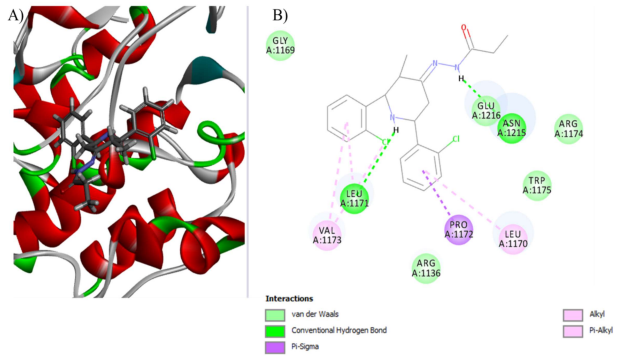
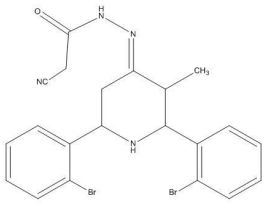
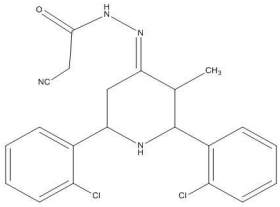
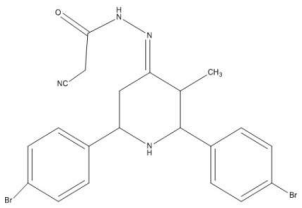
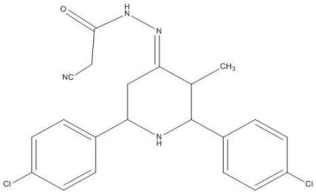


Figure 3. A) 3D and B) 2D Interaction of 3-methyl-2,6 di(bis-o-chlorophenyl) piperidin -4-one cyanoacetyl hydrazone, in active site of receptor (1IR3).

Table 2. The physical data of Synthesized Cyano Acetyl Hydrazone Derivatives

Compounds	Structure	Yield (%)	M. Formula	M.Weight	M. Point
S1		79.65	$C_{21}H_{18}Br_2N_4O$	502	174-176°C
S2		80.69	$C_{21}H_{18}Cl_2N_4O$	412	175-177°C
S3		78.6	$C_{21}H_{18}Br_2N_4O$	502	180-183°C
S4		74.5	$C_{21}H_{18}Cl_2N_4O$	412	178-180°C

Abbreviations: 2BR- 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S1); 2CL- 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S2); 4BR-3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3); 4CL-3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4)

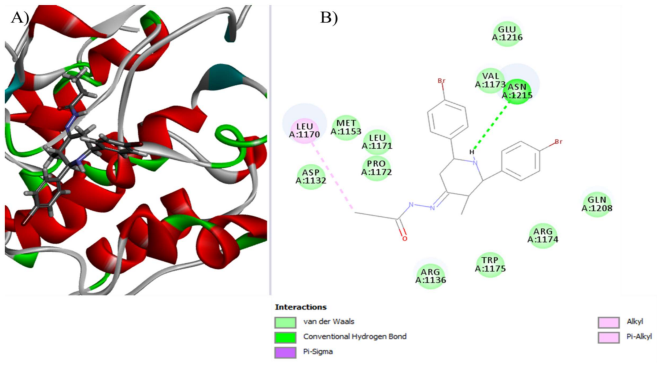


Figure 4. A) 3D and B) 2D Interaction of 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone in active site of receptor (1IR3)

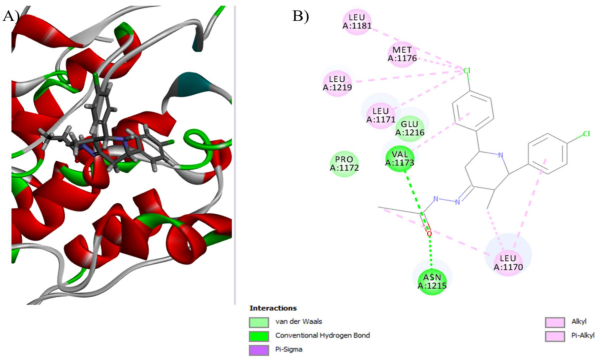


Figure 5. A) 3D and B) 2D Interaction of 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone, in active site of receptor (1IR3).

(B)). It was found that the docking results of those four compounds has higher binding affinity comparing the result of Glibenclamide (Table 3 and Figure 6). The high docking score (more negative value) of the ligands reflects a strong interaction in the cavity site of 1IR3 receptor. These score also suggested a strong binding between the target protein and the compounds. The -CDOCKER score of the compounds are not only due to hydrogen bond but also due to Hydrogen bond, Van der Waals, Pi-Sigma, Alkyl, Pi-Alkyl interactions that take place between these compounds and active site of residues of 1IR3 receptor.

ADMET Study

The ADMET result of 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone(S1), 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one

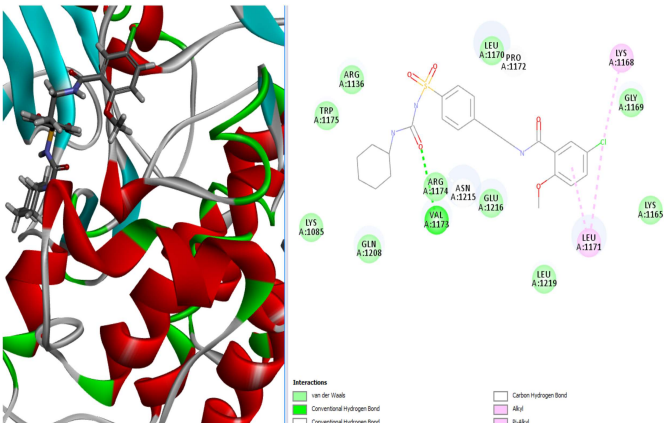


Figure 6. A) 3D and B) 2D Interaction of Glibenclamide in active site of receptor (1IR3).

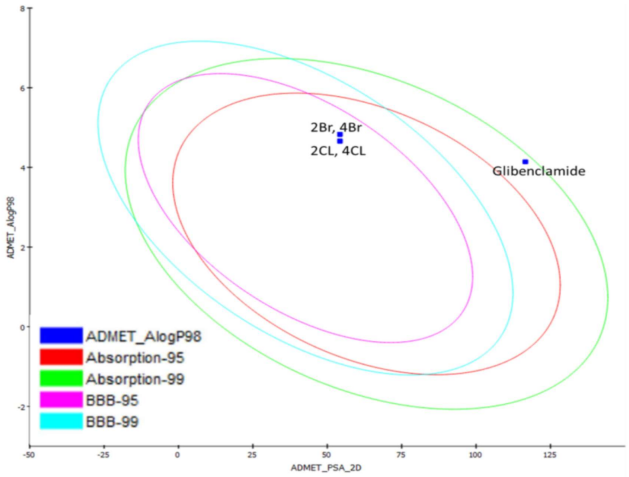


Figure 7. Plot of polar surface area (PSA) versus ALogP for capsazepine and its derivatives showing the 95% and 99% confidence limit ellipses corresponding to the blood brain barrier (BBB) and intestinal absorption.

Table 3. The CDOCKER energy of the compounds

Name	4BR	4CL	2BR	2CL	Glibenclamide
Initial Potential Energy	215.041	212.623	345.721	333.557	235.487
Initial RMS Gradient	106.959	104.87	169.323	146.267	125.358
CHARMm Energy	-14.3582	-14.2869	1.17712	11.9797	-9.8256
Electrostatic Energy	-24.9625	-31.5152	-13.6388	-3.41499	-50.0285
Potential Energy	-14.3582	-14.2869	1.17712	11.9797	10.2468
Van der Waals Energy	-1.56697	-2.12287	-0.75883	-0.71427	-2.42203
RMS Gradient	0.00967	0.00937	0.00879	0.00978	0.00854
-CDOCKER Energy	27.8926	25.5312	20.9243	18.4523	16.5337
-CDOCKER interaction Energy	32.8766	32.6569	31.0585	34.0659	28.4016

Table 4. ADMET properties of the molecule.

Name	Absorption level	Solubility level	BBB level	PPB level	Hepatotoxic level	CYP 2D6	PSA 2D	AlogP98
2BR	0	4	4	0	0	0	54.244	4.828
2CL	0	4	4	0	0	0	54.244	4.66
4BR	0	4	4	0	0	0	54.244	4.828
4CL	0	4	4	0	0	0	54.244	4.66
Glibenclamide	1	4	4	1	0	0	116.563	4.14

cyanoacetyl hydrazone (S2), 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3), 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4), and Glibenclamide are declared in table 3. The obtained results were cross checked with the standard levels listed in table 4 and 5. The plot of polar surface area (2D PSA) and AlogP for these compounds are represented in figure 7. The intestinal absorption and blood brain barrier penetration were predicted by 2D PSA and AlogP that include 95% and 99% confidence ellipses in ADMET study (Egan et al., 2000). The region of ellipses defines, where the compounds are expected as well-absorbed. The absorption level (human intestinal absorption-HIA) of all the molecules shows good absorption (value 0 as good absorption). The absorption levels of HIA model are defined by 95% and 99% confidence ellipses in the ADMET.

Similarly, aqueous solubility level is 4, it means all the compounds has good solubility nature in aqueous media. Further, all ligands are satisfactory with respect to CYP2D6 liver, suggesting that PA are non-inhibitors of CYP2D6. The model orders either as "toxic" or "nontoxic" and gives a certainty level pointer of the probability of the models

Table 5. Standard levels of ADMET descriptors

Aqueous Solubility		BBB		CYP450		Hepatotoxicity		Intestinal absorption	
Level	**Intensity**	**Level**	**Intensity**	**Level**	**Value**	**Level**	**Value**	**Level**	**Value**
0	Extremely low	0	Very High	0	Non inhibitor	0	Non toxic	0	Good
1	No, Very Low	1	High	1	Inhibitor	1	toxic	1	Moderate
2	Yes, Low	2	Medium	PPB				2	Low
3	Yes, good	3	Low	Level	% of Binding			3	Very Low
4	Yes, Optimal	4	Very Low	0	<90%				
5	No, Too soluble			1	>90%				
6	Unknown			2	>95%				

prescient exactness (Table 2). Our results indicate that all compounds are nontoxic to liver (level 0), and thus they experience significant first-pass effect. According to the model for the all compounds to have an optimum cell permeability should follow the criteria (PSA < 140 $Å^2$ and AlogP98 < 5) [7]. All the compounds showed polar surface area (PSA) < 140 $Å^2$. Since the AlogP98 criteria, all the compounds had AlogP98 value <5. From the result of ADMET, we found that the molecules have drug likeness properties and also it will be useful as a potent new drug for diabetes mellitus.

Conclusion

In conclusion, the potential anti-diabetic effect of 3-methyl-2,6 di(bis-o-bromophenyl)piperidin -4-one cyanoacetyl hydrazone(S1), 3-methyl-2,6 di(bis-o-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S2), 3-methyl-2,6 di(bis-p-bromophenyl)piperidin -4-one cyanoacetyl hydrazone (S3), 3-methyl-2,6 di(bis-p-chlorophenyl)piperidin -4-one cyanoacetyl hydrazone (S4) were well analyzed in molecular docking and ADMET study. These studies suggested the same binding orientation inside the 1IR3 binding pockets and have better profiles when compare with Glibenclamide. Further wet lab assessment of these drugs has to be performed to confirm their insulin mimicking activity for anti-diabetic.

Conflicts of interest: Not declared

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