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Original Article

Synthesis, Characterization and Molecular Level Interaction Study of Insulin Receptor with Some Cyanoacetyl Hydrazone Derivatives as a Potent Antidiabetic Study

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ABSTRACT

The present investigations describe about the synthesis, characterization and biological studies of novel cyanoacetyl hydrazone derivatives. However their derivatives have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities. The structures of all the synthesized compounds were elucidated by using spectral data. In vitro anti-diabetic activity of cyanoacetyl hydrazones were evaluated in insulin receptor. The docking results showed higher binding activity of this novel compounds (32.5054, 33.2148 and 30.917 Kcal/mole-1 respectively) compare with standard drug Glibenclamide -29.6323 Kcal/mole-1 in insulin receptor. For the reason that novel compounds have interaction with most of the residues in cavity site insulin protein. Overall studies indicate that compound S2 is a promising compound leading to the development of selective inhibition insulin receptor.

Key words: Cyanoacetyl hydrazone, CDOCKER, Insulin receptor, Gilbenclamide.

1. INTRODUCTION

Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. However their derivatives having N-C linkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities. Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries [1]. This is a complex chronic illness associated with a state of high blood glucose level or

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hyperglycemia, occurring from deficiencies in insulin secretion, action or both. It is estimated that 25% of the world population is affected by this disease. The World Health Organization (WHO) estimates that about 200 million people all over the globe are suffering from diabetes and this figure is likely to be doubled by 2030 [2]. WHO says that about 80% of the deaths occur every year due to diabetes in middle-income countries. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. The recently published Indian Council for Medical Research-India diabetes (ICMR-INDIAB) national study reported that there are 62.4 million people with type II diabetes (T2DM) and 77 million people with prediabetes in India. This will be increased to 100 million by 2030 [3]. Diabetes is a metabolic disorder where in human body does not produce or properly us insulin, a hormone that is required to convert sugar, starches and other food into energy. Diabetes mellitus is characterized by constant high levels of blood glucose. It involves multiple disorders like hyperglycemia, glycosuria, and abnormal metabolism of lipids, carbohydrates and proteins. T2DM is a genetically heterogeneous, polygenic disease with a complex inheritance pattern and is caused by genetic predisposition and environmental factors and is associated with hypertension and dyslipidemia. Type 1 Diabetes leads to inability to release insulin results in low rates of glucose uptake into adipose tissue. Human body has to maintain the blood glucose levels at a very narrow range which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood or the production of energy.

Different classes of anti-diabetic drugs available in market that includes insulin secretagogues known as sulfonylureas and meglitinides. The basic mechanism of antidiabetic medications is stimulating insulin production from the pancreas or increasing the sensitivity of the body cells to insulin and is commonly used along with insulin. Insulin sensitizers are biguanides, thiazolidinediones and metformin important inhibitors are α-glucosidase inhibitors include acarbose and miglitol etc. The side-effects of these medications include extreme hypoglycemia, liver cell injury, lactic acidosis, digestive discomfort, permanent neurological deficit, headache, dizziness and even death. The basic challenge in curing diabetes is to maintain blood glucose level close to normal levels. These therapies are used as monotherapy or in combination for optimal control of glycemia. As mentioned before that these drugs are normally expensive and come with side effects.

Protein-ligand interaction (docking) is comparable to the lock-and key principle, in which the lock encodes the protein and the key is grouped with the ligand. The major driving force for binding appears to be hydrophobic interaction. *In silico* techniques helps identifying drug target via bioinformatics. This study has been carried out in order to identify the binding affinity of molecule 1, 2 and 3 as efficient antidiabetic compound and its analogues. ChemDraw, Accelry Discovery Studio 3.5 [6] were used for studying molecular docking and ligand–protein interactions, respectively.

2. MATERIALS AND METHODS

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

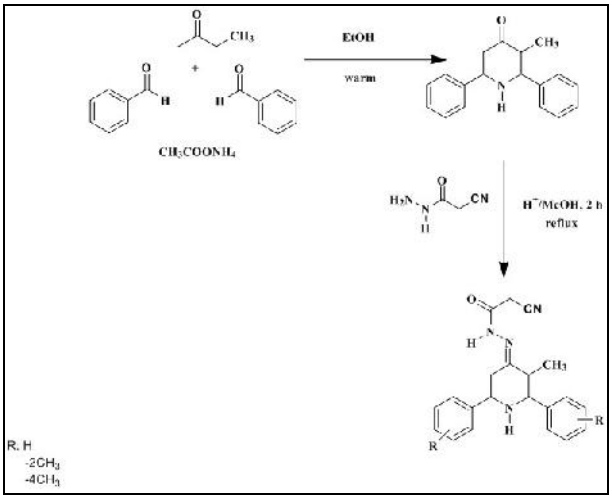
In our present study, *in silico* molecular docking studies were carried out using BIOVIA Discovery Studio (DS) 2017 software.

Preparation of S1, S2 and S3

3-methyl-2,6-diphenylpiperidin-4-one was prepared by adopting the literature method. Condensation of 2-butanone,substituted aldehydes 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

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.



Scheme 1

Preparation of protein

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). Hydrogen added to the protein 1IR3 by applied the forcefield algorithm subsequently the energy of protein was minimized using CHARM force field in DS.

Ligand preparation

The standard Glibenclamide drug, S1, S2 and S3 were drawn in chemdraw software, subsequently energy minimized and saved in SDF file format for docking studies.

Docking Studies

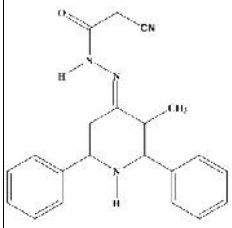
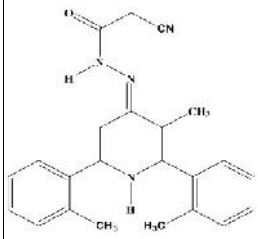
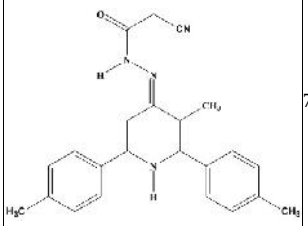
Molecular docking study was performed, with the aim of evaluating the most preferred geometry of protein-ligand complex. Computational docking study was used to analyze structural complexes of the 1IR3 with Glibenclamide drug, S1, S2 and S3 in order to understand the structural basis of this target proteins. Possible binding modes between the ligands and this target proteins were studied by CDOCKER (CHARMm-based DOCKER) protocol incorporated within DS. The parameter to run the CDOCKER was tabularized in Table 1. The algorithm offers full ligand flexibly and employs CHARMm force fields. Ligand binding affinity was calculated using CDOCKER energy, CDOCKER interaction energy, Hydrogen bonds, binding energies, protein energy and ligand protein complex energy. The CDOCKER energy mentioned in negative values. More negative value energy indicated as higher binding affinity of the ligands with target protein [7].

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, 9
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

3. RESULTS AND DISCUSSION

Table 2: The physical data of Synthesized Cyanoacetyl Hydrazone Derivatives

Compound	Structure	Yield(%)	M.Formula	M.Weight	m.pt
S1		79.65	$C_{21}H_{20}N_4O$	344	137-140°C
S2		82.6	$C_{23}H_{26}N_4O$	374	141-144 °C.
S3		78.69	$C_{23}H_{26}N_4O$	374	140-142°C.

3-methyl-2,6-diphenylpiperidin-4-one cyanoacetylhydrazone (S1) : Yield. 79.65%. mp. 137-140°C. **FT-IR (KBr) ν_{max} (cm^{-1}):** 3036-2834 (C-H Aliphatic & Aromatic stretching), 1702 (C=O), 1604 (C=N), 2263 (C≡N), 3212-3115 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm** :142.66(C-2 ipso carbon), 142.18 (C-6 ipso carbon), 126.56-128.79 (Aromatic carbons), 164.74 (C=O), 157.54 (C=N), 114.05 (C≡N), 24.59 (CH_2 carbon of cyanoacetohydrazone moiety) , 69.20 (C-2), 60.80 (C-6), 45.36 (C-3), 36.04 (C-5), 12.06 (3-CH_3). **^{1}H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.50-7.26(m, 10H, Aromatic Protons), 9.24 (b s, 1H, N-H Hydrazone Moiety), 2.08 (b s, 1H, N-H Piperidin moiety), 3.78 (q, 2H, CH_2 – Protons in hydrazone moiety), 0.89 (d, J = 6.3Hz, 3H, 3-CH_3), 3.90 (dd, $J^3_{a,e}$ = 2.4Hz, $J^3_{a,a}$ = 11.4Hz, 1H, H-6a), 3.53 (d, $J^3_{a,a}$ = 9.9Hz, 1H, H-2a), 2.23 (dd, $J^3_{a,e}$ = 13.8Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 2.90 (dd, $J^3_{a,e}$ = 2.7 Hz, $J^2_{a,e}$ = 13.8Hz, 1H, H-5e), 2.58 (m, 1H, H-3a Proton).

3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S2) : Yield. 82.6%. mp. 141-144 ^{o}C. **FT-IR(KBr) ν_{max} (cm^{-1}):** 3025-2852 (C-H Aliphatic & Aromatic stretching), 1674 (C=O), 1568 (C=N), 2267 (C≡N), 3440-3184 (N-H). **^{13}C NMR(300 MHz, CDCl$_3$) δ ppm:**139.98 (C-2 ipso carbon), 140.49(C-6 ipso carbon), 126.49-129.77 (Aromatic carbons), 164.48 (C=O), 158.05 (C=N), 114.35 (C≡N), 24.16 (CH_2 carbon of cyanoacetohydrazone moiety), 76.57 (C-2), 56.12 (C-6), 44.89 (C-3), 34.56 (C-5), 11.15 (3-CH_3) 19.20 (*o*-CH_3). **^{1}H NMR(300 MHz, CDCl$_3$), δ ppm :** δ ppm 7.32-7.13 (m, 8H, Aromatic Protons),10.09(b s, 1H, N-H, Hydrazone Moiety), 2.09 (b s, 1H, N-H Piperidin moiety), 3.50 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.92 (d, J = 6Hz, 3H, 3-CH_3), 3.89 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.11(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.39 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.07(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.57 (m, 1H, H-3a Proton), 2.33 (s, 3H, *o*-CH_3 protons).

3-methyl-2,6 di(bis-*p*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S3) : Yield. 78.69%. mp. 140-142 ^{o}C. **FT-IR(KBr) ν_{max} (cm^{-1})**: 3026-2963(C-H Aliphatic & Aromatic stretching), 1701 (C=O), 1638 (C=N), 2266 (C≡N), 3195-3097 (N-H). **^{13}C NMR(300 MHz, CDCl$_3$) δ ppm**: 139.33 (C-2 ipso carbon), 139.83 (C-6 ipso carbon), 126.42-129.38 (Aromatic carbons), 164.87 (C=O), 158.07 (C=N), 114.13 (C≡N), 24.56 (CH_2 carbon of cyanoacetohydrazone moiety), 68.92 (C-2), 60.46 (C-6), 45.34 (C-5), 36.16 (C-3), 12.10 (3-CH_3), 21.13(*p*-CH_3) . **^{1}H NMR(300 MHz, CDCl$_3$), δ ppm :** 7.14-7.36 (m, 8H, Aromatic Protons), 9.01(bs, 1H, N-H, Hydrazone Moiety), 2.06 (b s, 1H, N-H Piperidin moiety), 3.73 (q, 2H, CH_2 – Protons in hydrazone moiety), 0.89 (d, J = 6Hz, 3H, 3-CH_3), 3.87 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 11.4Hz, 1H, H-6a), 3.49 (d, $J^3_{a,a}$=9Hz, 1H, H-2a), 2.24 (dd, $J^3_{a,e}$=12Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 2.83 (dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 13.5Hz, 1H, H-5e), 2.57 ((m, 1H, H-3a Proton), 2.06 (s, 3H, p-CH_3 protons).

MOLECULAR DOCKING STUDIES

The X-ray crystallography of insulin receptor 1IR3 protein from homo sapiens with 1.9 (Å) and it contain 306 amino acids. The secondary structure of target protein with active site sphere (Radius 9) was depicted in Figure 1. The crystal structures were refined by removing water molecules and repeating coordinates. Hydrogen atoms were added and charges were assigned to the protein atoms.

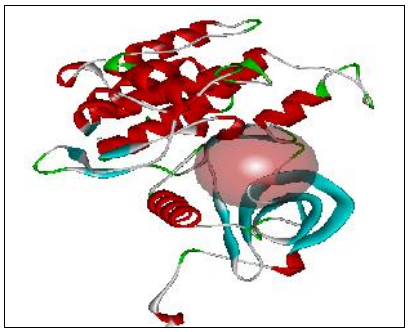


Fig 1: The secondary structure of the target insulin receptor 1IR3 with active site sphere

In this docking study the three molecules such as S1, S2 and S3 with standard molecule Glibenclamide were form good interaction with cavity site of the 1IR3 receptor (Figure 2).

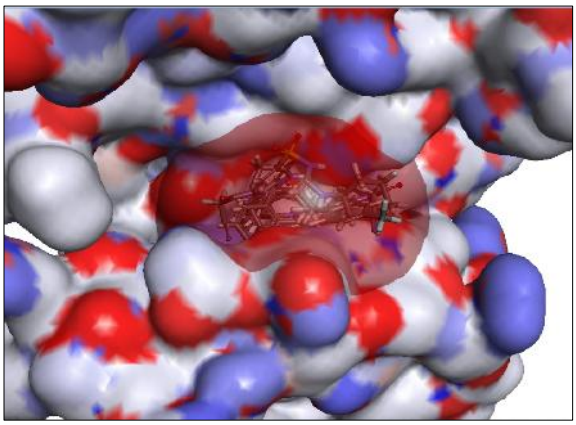


Fig 2: Surface area of receptor with ligands

The S1 molecule formed hydrogen bond with Asp 1083 amino acid (CDOCKER energy is -32.5054 Kcal/mol^{-1}) through NH of the ligand (Figure 3). Similarly S2 molecule also formed hydrogen bond with Asp 1083 amino acid (CDOCKER energy is -33.2148 -Kcal/mol^{-1}) through NH of the ligand (Figure 4). The other electrostatics and vander Waals of this molecule was depicted in Figure 3. The CDOCKER energy of this molecule is -32.5054 Kcal/mol^{-1}. The CDOCKER energy calculates the all the properties of binding interactions (Table 3).

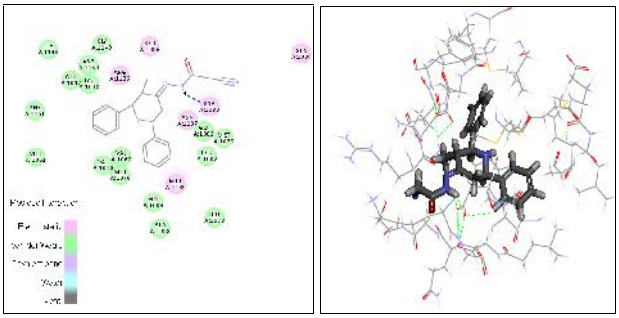


Fig 3: a) 2D and b) 3D view of interaction of S1 in receptor 1IR3.

Table 3: The involved energies of the docking study of 1IR3 protein

Name	CHARMm Energy	Electrostatic Energy	Van de Waals Energy	-CDOCKER Energy	-CDOCKER Interaction Energy
S1	3.38908	-13.0757	-0.38889	-32.5054	44.9646
S2	-7.54258	-21.8682	-2.01422	-30.917	38.5335
S3	12.274	-8.5235	-2.35888	-33.2148	46.2458
Glibenclamide	-9.8256	-50.0285	-2.42203	-29.6323	42.9886

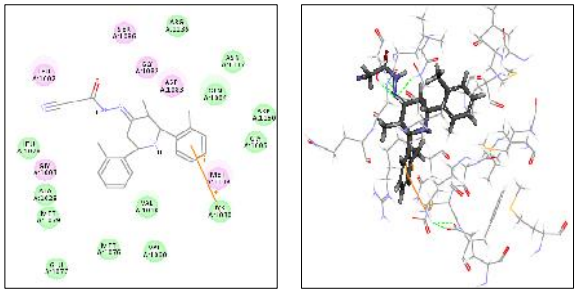


Fig 4: a) 2D and b) 3D view of interaction S2 in receptor 1IR3.

However, the molecule S3 formed pi-pi interaction with Lys 1030 amino acid through phenyl ring of the ligand (Figure 5).

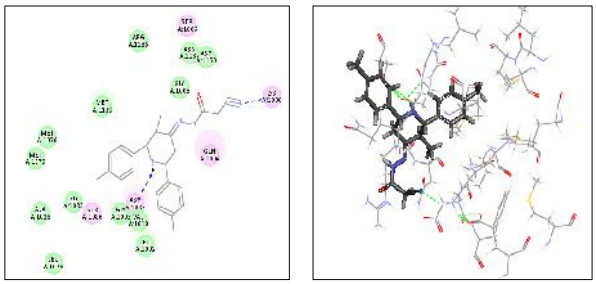


Fig 5: a) 2D and b) 3D view of interaction S3 in receptor 1IR3.

In these studies, we compare the activity of the these three compounds by the help of Glibenclamide drug. This drug shows lowest binding affinity (-29.6323Kcal/mol^{-1}) in the 1IR3 receptor (Figure 6). These results revealed that the compounds such as S1, S2 and S3 shows improved anti-diabetic activity than Glibenclamide drug..

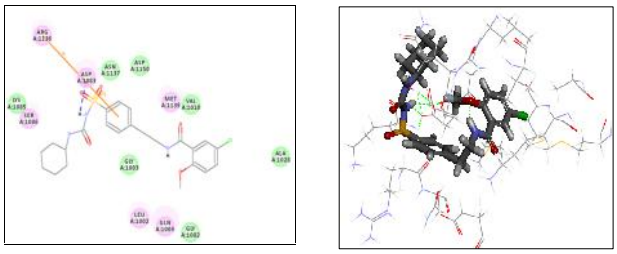


Fig 6: a) 2D and b) 3D view of interaction of Glibenclamide in receptor 1IR3

4. CONCLUSION

The result of present study was to determine the molecules such as S1, S2 and S3 the insilico docking studies revealed that the compounds as insulin mimetics that can mimic the action of insulin and activate insulin receptor. Hence this study suggests that the compounds have a potent anti-diabetic instead of effect which could be used for the management of diabetes effectively instead of Glibenclamide drug. Overall studies indicate that compound S2 is a promising compound leading to the development of selective inhibition insulin receptor

Abbreviation:

S1-3-methyl-2,6-diphenylpiperidin-4-one cyanoacetylhydrazone

S2- 3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone

S3- 3-methyl-2,6 di(bis-*p*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone

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