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SYNTHESIS, MOLECULAR DOCKING AND ANTI DIABETIC EVALUATION OF SOME NEW SULFONAMIDO SUBSTITUTED IMIDAZOLONE DERIVATIVES.

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ABSTRACT:

A new series of *N*-[(4*Z*)-4-(4-arylbenzylidene)-5-oxo-2-phenyl-4, 5-dihydro-1*H*-imidazolyl-benzene sulfonamide derivatives were synthesized and identified by spectral and physico-chemical data. The title compounds were also screened for their Molecular properties and toxicity prediction followed by Molecular docking studies with the help of the Crystal structure of the pancreatic ATP-sensitive K+ channel SUR1/Kir6.2 complexes with ATP taking Glibenclamide (PDB ID: 6PZA) as the standard to predict the binding affinity of the synthetic compounds.Compounds with highest docking scores were selected for further i*nvivo*antidiabetic evaluation.

Key Words: Imidazolones, Hypoglycemic activity, Glibenclamide.

INTRODUCTION:

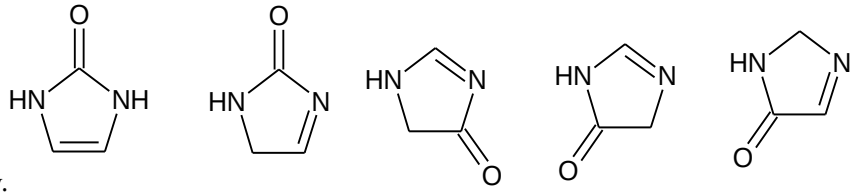
Diabetes mellitus is a major degenerative disease associated with a group of disorders of carbohydrate metabolism which results from the body's failure to produce insulin in type 1 and insulin resistance in type 2 diabetes through altered secretion, decreased insulin activity known as hyperglycemia.[1]A series of sulfonamide substituted imidazolone derivatives were designed and synthesized as hypoglycemic agents. Initially Docking studies were performed using AUTODOCK program to predict the binding affinity and to understand interaction with various residues with the help of the Crystal structure of the pancreatic ATP-sensitive K+ channel SUR1/Kir6.2 complexes with ATP and glibenclamide (PDB ID: 5TWV).Compounds exhibited good binding affinities comparable to glibenclamide, and Compounds with highest docking scores were selected for synthesis and all the synthesize compounds were characterized by melting points, TLC, IR spectroscopy, Mass spectroscopy, 1H-NMR and 13C-NMR. So further these newly synthesized compounds were screened for their in vivo hypoglycemic activity using alloxan induced diabetic rat model.

INTRODUCTION TO IMIDAZOLONES:

Heterocycles are also of considerable interest because of their synthetic utility as synthetic intermediates, protecting groups, chiral auxiliaries, organ catalysts, and metal ligands in asymmetric catalysts inorganic synthesis.

Therefore, substantial attention has been paid to develop efficient new methods to synthesize heterocycles.

Imidazolone is a 5 membered heterocyclic ring containing (N) atom at 1 and 3 positions and carbonyl group at 5 th position, it is a ketodihydroimidazole.Various biologically active synthetic compounds have five-membered nitrogen-containing heterocyclic ring in their structures. The present study incorporates imidazolone nucleus emerging from drug intermediate azalactone which possess



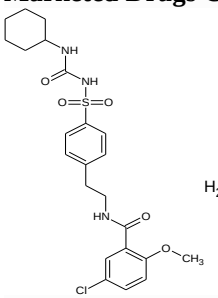
oxazolone moiety.

INTRODOCTION TO SULFONAMIDES:

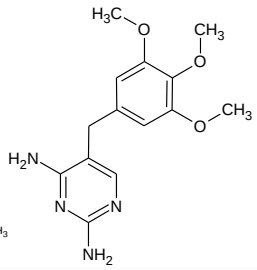
- Sulfonamides are a synthetic substance created from the sulfonamide chemical group. They are used as a medication to treat a variety of illnesses and conditions. Sulfonamide medications are supplied as oral tablets, oral liquids, or even injections.

- Most commonly they are **antibiotic**medications.they are often been classified under carbonic anhydrase inhibitors (CAIs), thiazide diuretics, loop diuretics, cyclooxygenase 2 (COX-2) inhibitors, and sulfonylureas[4].

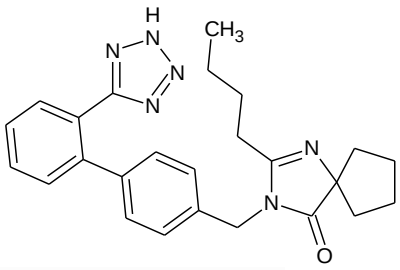
Marketed Drugs Containing Imidazolone and SulfonylUreas Nucleus:



Glibenclamide



Trimethoprim



Irbesartan

MATERIALS AND METHODS

All the chemicals (reagents and solvents) were purchased from commercial suppliers (Merck grade) and they were used further without purification.

MELTING POINT ANALYSIS

Melting points of the synthesized compounds were determined using micro controller based melting point apparatus of CHEMLINE Company CL726 and were uncorrected.

THIN LAYER CHROMATOGRAPHY ANALYSIS

Purity of the compounds was checked by TLC using silica gel G (0.5mm thickness) coated over glass plate (12 x 20 cm). For the determination RF value the dried silica gel G coated over glass plate were used.

PREPARATION OF TLC PLATE: By using distilled water silica gel G slurry is prepared and poured on to a glass plate which is maintained on a level surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plates are dried in an oven at 50ºC or 15 minutes for activating the TLC plate. Chromatogram was developed by ascending technique when solvent front travelled appropriate distance, plates were taken out and dried. The location of spot was detected using iodine chamber.

RF = Distance travelled by solute / Distance travelled by solvent

SPECTRAL STUDIES CONFIRMATION OF THE STRUCTURE OF SYNTHESIZED COMPOUNDS

INFRARED SPECTRAL ANALYSIS

The IR Spectra of the synthesized compounds were recorded at RbvrrWomens College of pharmacy by SHIMADZU-FT/IR spectrophotometer in KBr disc. The IR value was measured in cm^{-1}.

NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

The NMR Spectra of the synthesized compounds were recorded at IICT Hyderabad (tarnaka) by Bruker 300 MHz FT- NMR using $CDCl_3$(DEUTERIATED CHLOROFORM) as internal standard. The PMR (Proton Magnetic Resonance) spectroscopic values are measured in δ ppm in DMSO-d6.

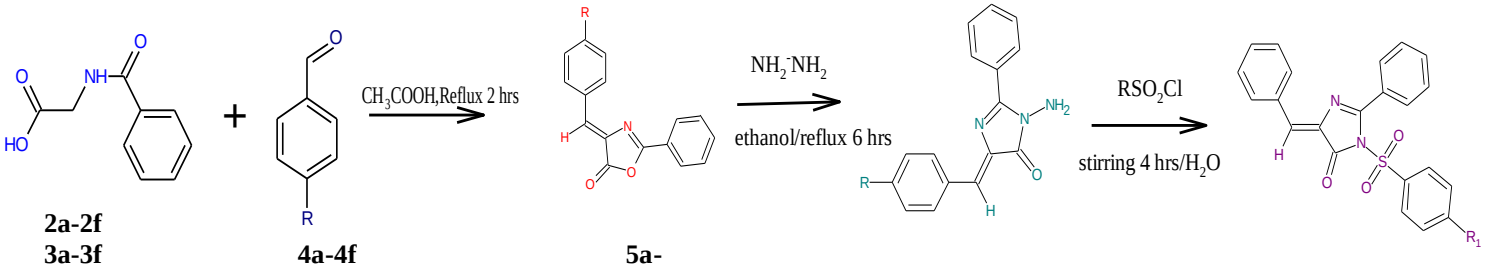
MASS SPECTRAL ANALYSIS

The Mass Spectra of the synthesized compounds were recorded at IICT HYDERABAD in MS (EI) JEOL GC MATE 700 EV.

The name of all compounds given in the experimental section were generated from ACD\chemsketch freeware.

The chemicals and reagents used in the experimentation were of AR and LR grade procured from sigma, Aldrich and SD-fine chem.Ltd

SCHEME OF SYNTHESIS TO ACHIEVE TARGETED COMPOUND



2a-2f
3a-3f **4a-4f** **5a-**
5f

R = OCH_3, -Cl,-OH, -N (CH_3), -$C_5H_4O_2$, -C_8H_7N. **R_1= H, CH3**

EXPERIMENTAL METHODS

Step 1: **General Procedure for the synthesis of (4*Z*)-4-(4-aryl benzylidene)-2-phenyl-1, 3-oxazol-5(4*H*)-ones:**[5]:A mixture of aromatic aldehydes (2a-f) (0.25 moles), Hippuric acid (1a) (44.8 gm., 0.25 mole) / acetyl glycine (29 gm, 0.25 moles), anhydrous sodium acetate (15 gm), and acetic anhydride (59 ml) was heated at 110°C, with constant stirring. The mixture become almost solid, and then as the temperature rises, it gradually liquefies and turns deep yellow in colour. After completion of the reaction monitored by TLC the reaction is allowed to cool and ethanol (100 ml) is added slowly to the contents of the flask. After allowing the reaction mixture is left to stand overnight, the yellow colour product is filtered and washed with ice cold ethanol and finally with boiling water and recrystallized in ethanol.The crystalline products were then dried and characterized for various physio-chemical properties.All the derivatives were soluble in ethanol under hot condition.The yields were in a range of 70-90% with melting points ranging within 165-210˚C.

Step 2:General Procedure for the synthesis of (5*Z*)-3-amino-5-(4-arylbenzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one.

To a solution of compound (oxazolone) (0.0lmole) in 50 ml of absolute ethanol and hydrazine hydrate (0.03 mole), was added and the reaction mixture was refluxed for 6hrs. On cooling, the precipitate formed was filtered off, recrystallization by ethanol.The product obtained is very fine crystalline precipitate which is then dried on air to prevent the exhaustion of product under microwave oven and the yield obtained was 50-80% with the melting points ranging from 205-225˚C and the derivatives were freely soluble in water under normal room temperature.

Step 3:General procedure for the synthesis of *N*-[(4*Z*)-4-(arylbenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-4-methylbenzenesulfonamides &*N*-[(4*Z*)-4-(arylmethylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]benzenesulfonamides:[7-8]:Na_2CO_3 (2.785 g, 26.25 mmol) was added to a solution of imidazolone (12.5 mmol) in H2O (15 ml) at -5˚C to 10°C, followed by addition of p-Toulenesulphonyl chloride (2.86 g, 15 mmol) in three portions over a period of 1h. The slurry was warmed to room temperature and allowed to stir for 4h. Upon completion of the reaction which was monitored with TLC using CHCl3/CH3OH solvent system (9:1). The reaction mixture was acidified with 20% concentrated aqueous HCl solution to pH2, after which crystallization occurred and the product was obtained via suction filtration. And the yield obtained was 80-95% with the melting points ranging from 120-140 ˚C and the derivatives were freely soluble in water under normal room temperature. Compounds are freely soluble in ethanol.

Molecular properties of synthetic derivatives

Lipinski used molecular properties in formulating his “rule of five”.

The rule states that most molecules with good membrane permeability have

- logP ≤5,molecular weight ≤500,number of hydrogen bond acceptors ≤10,Hydrogen bond donor’s ≤5.

Along with the above rules the other molecular descriptors like total polar surface area (TPSA), molecular volume and number of rotatable bonds explain the pharmacodynamics properties

Osiris property explorer

The Osiris property explorer is an integral part of actelion'sinhouse substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid.

MOLECULAR DOCKING:

Sulfonylurea receptor 1 As Anti diabetic target

- Numerous drugs such as sulfonylureas and biguanides are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome this problem.
- Sulfonylureas are the backbone of antidiabetic therapy for many years. Thus, it was thought to develop new antidiabetic agents with higher efficacy and lower toxicity for the long-term treatment of type II diabetes mellitus.
- These drugs work by binding to and inhibiting the ATP-sensitive potassium channels (K_{ATP}) inhibitory regulatory subunit sulfonylurea receptor 1 (SUR1)in pancreatic beta cells This inhibition causes cell membrane depolarization, opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the pancreatic beta cell and subsequent stimulation of insulin release.
- Glibenclamide which is a well-known generic drug belonging to sulfonylureas class is used as a standard drug in the current study on various sulfonamido derivatives.

Molecular Docking procedure using autodockvina

- Molecular docking studies on pancreatic ATP-sensitive K+ channel SUR1Enzyme was carried out in order to access the antihyperglycemic activity of all the synthesized compounds.
- Auto dock vina software was used to perform docking studies, the pancreatic ATP-sensitive K+ channel SUR1structure(pdb id-6PZA)was downloaded from RCSPDB and 3D structure was redefined by removing the inhibitor molecule from the active site, then water molecules were deleted and polar hydrogens and gasteiger charges were added.
- The 3D structures of all the ligands were prepared in ligprep, and energies were minimised.the protein grid was defined which reflects the active site of the Enzyme.

- In autodock,co-ordinates X,Y and Z were set to 51.14,17.63,24.123respectively at 40,40,40 points and lamarcian genetic algorithm (LGA) was used for protein rigid /ligand flexible docking calculations .
- The maximum number of energy evaluations before the termination of LGA run was 2,50,000 and the maximum number of generations was 27,000 for each ligand.
- Total number of GA runs were set to 10 and other parameters were set to the software default values.

Docking analysis was done by considering the low energy confirmation among the top 10 docked confirmations and binding energy was taken as parameter to assess the potency of ligand and results are presented in following table along with number of rotatable bonds of each ligand.

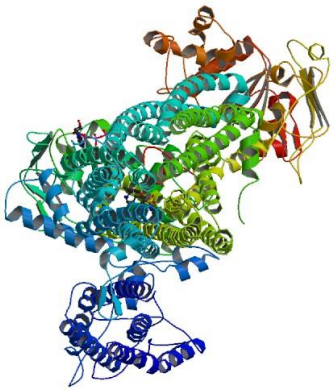


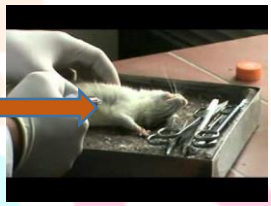
Fig 1-Cryo-EM structure of the pancreatic beta-cell SUR1 bound to ATP and glibenclamide.

INVIVO ANTI DIABETIC EVALUATION

Wister albino rat method:

In the experiment a total of 36 rats (30 diabetic surviving rats +6control group) were used and the study used male Wister White Albino rats (3-4 weeks old) that weighed 150 g-250 g with a mean weight of 170 g. These were bred in the Animal house at the Department of pharmacology, Rbvrr women's college of pharmacy. All the rats were acclimatized for one week to the laboratory conditions before commencing the experiments. The animals were housed at a temperature of 25° C in the polypropylene cages for 12 hrs/12 hrs dark and light cycle and fed on rodent pellets and water ad libitum. . The experimental protocols and procedures used in this study were approved by the Institutional Animal Ethics Committee under the supervision of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi bearing registration number 1328/PO/Re/S/10/CPCSEA.

+ 0.9% saline



The acute study data of all the synthesized compounds were depicted in below tables in relation to the standard drug Glibenclamide. The compounds 5a and 5b have shown significant hypoglycaemic activity. The effect of compounds on insulin levels during day1and 7 in control and alloxan induced diabetic rats are measured.

Induction of hyperglycaemia

Alloxan induced method:

Alloxan is also called as mesozalylurea, mesoxalylcarbamide 2, 4, 5, 6-tetraoxohexa hydro pyrimidine or pyimidinetetrone. It is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. Alloxan generates reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low ant oxidative defence capacity, and ensure state of insulin-dependent alloxan diabetes. Alloxan causes triphasic response in animals Stage I-early hyperglycaemia of short duration (about 1-4 hr) due to a sudden short lasting decrease or cessation of insulin release and direct glycogenolytic effects on the liver. Stage II-hyperglycaemia phase lasting up to 48 hrs and often resulting in convulsion and death. Stage III-chronic diabetic phase consequence of insulin lack histologically only a few ß- cells if any are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restores normal blood glucose levels

Hyperglycaemia was induced experimentally by a single intraperitoneal administration of 120 mg/kg alloxan monohydrate i.p. in normal saline obtained from merck industry(LR-grade) To overcome the early hypoglycaemic phase 5% dextrose solution was given in feeding bottle for a day Forty-eight hours after alloxan administration, blood glucose level was measured using a

glucometer. Rats with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8 hours-12 hours but allowed free access to water until the end of the experiment.

Experimental design

For the oral route of drug administration, the experimental rats were randomly divided into six groups of six animals in each..

- Group 1: normal control rats fed with 0.5 ml of normal saline solution.
- Group 2: Diabetic control (DC) rats; fed with 0.5 ml of normal saline.
- Group 3: Diabetic rats treated with standard drug Glibenclamide 5 mg/kg body wt.
- Group 4: Diabetic rats treated with synthesized drug No 5A in 1% CMC 50 mg/kg of body weight.
- Group 5: Diabetic rats treated with synthesized drug No 5B in 1% CMC 50 mg/kg of body weight
- Group 6: Diabetic rats treated with synthesized drug No 5C in 1% CMC 50 mg/kg of body weight.

The dose for the newly synthesized compounds was decided on the basis of literature survey. Glibenclamide was taken as the standard. The blood glucose level was determined at 0 and 3 h after administration of test compound using glucometer (Johnson and Johnson Pvt. Ltd.) % reduction in plasma glucose level was calculated for each animals.

Statistical analysis:

Measurement data were tabulated as means±SEM Data were analysed using One-Way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison post hoc tests using the Graph Pad Prism 5.3, San Diego, CA and **P≤0.01 as the level of significance.

Blood sampling:

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and repeated whenever required. The blood glucose levels were determined with Accu Sure Blood Glucose Monitoring System (Dr. Gene Health & Wellness).

Determination of body weight:

The body weight of each rat was assessed after every 24 hrs during the dosing period up to and including the 7th day of experiment .and the day.

Feed and water intake:

Anti-diabetic activity evaluation

In vivo acute toxicity studies: The synthesized compounds showed no serious toxicity up to dose level 100 mg/kg body weight in experimental rats. Blood glucose level before starting the treatments, blood glucose level of all the animals was within normal range. After 72 hours of alloxan treatment, the blood glucose level was significantly changed more than 240 mg/dL.

S.no	Group name	Doses mg/kg body wt.
1.	Normal Control group	0.9% saline
2.	Diabetic control	0.9% saline
3.	Diabetic+glibenclamide	5%

4. Diabetic rats + compound 5a 50%
5. Diabetic rats + compound 5b 50%
6. Diabetic rats + compound 5c 50%

RESULTS AND DISCUSSION:

Table 1-Physical data of (4*Z*)-4-(4-aryl benzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one derivatives.

Compound S.no	Name of aryl Aldehyde used	Name of oxazolone derivative obtained	Physical State	Melting point ˚C	Yield %	Rf value Hexane:ethyl- -acetate(7:3)
2a	p-chlorobenzaldehyde	(4*Z*)-4-(4-chlorobenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one	Pale yellow	189-190	75.4	0.46
2b	p-hydroxybenzaldehyde	(4*Z*)-4-(4-hydroxybenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one	Crystal white	174-175	79.02	0.66
2c	Di methyl amino benzaldehyde	(4*Z*)-4-[4-(dimethylamino)benzylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	White	207-208	67.2	0.54
2d	Anisolaldehyde	(4*Z*)-4-[2-(4-methoxyphenyl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	White fine crystalline	199-201	78.8	0.75
2e	Indole -3 carboxaldehyde	(4*Z*)-4-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	Brick red crystalline powder	203-205	95	0.72
2f	furfuraldehyde	(4*Z*)-4-[2-(furan-2-yl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	Black crystalline powder	167-169	57.2	0.56

Table 2-Physical data of (5*Z*)-3-amino-5-(4-arylbenzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one derivatives

Compound S.no	Name of oxazolone used	Name of imidazolone derivative obtained	Physical State	Melting point ˚C	Yield %	Rf value
3a	(4*Z*)-4-(4-chlorobenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-(4-chlorobenzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**White ppt**	**201-204**	**78.4**	0.76
3b	(4*Z*)-4-(4-hydroxybenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-(4-hydroxy benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**White crystalline ppt**	**206-207**	**69.1**	0.71
3c	(4*Z*)-4-[4-(dimethylamino)benzylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-(4-(dimethyl amino)benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**Pale yellow crystalline ppt**	**214-217**	**52.6**	0.68
3d	(4*Z*)-4-[2-(4-methoxyphenyl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-(4-methoxyl phenyl ethylidine)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**White ppt**	**207-210**	**75.8**	
3e	(4*Z*)-4-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**White ppt**	**224-225**	**72.6**	0.56

3f	(4*Z*)-4-[2-(furan-2-yl)ethylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one	(5*Z*)-3-amino-5-[2-(furan-2-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	**White ppt**	**198-201**	**81.7**	0.57

Ppt = Precipitate ;Rf Value Calculated Using Hexane:Ethyl Acetate (8:2) Ratio.

Characterization of imidazolone formation by using irspectroscopy:Example : The Synthesis (5*Z*)-3-Amino-5-[2-(Furan-2-Yl)Ethylidene]-2-Phenyl-3,5-Dihydro-4*H*-Imidazol-4-One Is Identified By 1°NH (S)-2PEAKS=3296.35 Cm^{-2} ,C=N(S)= 1636cm^{-2} ,C-N(S) =1080.14 Cm^{-2} C=O (Lactam)= 1645 Cm^{-2}.

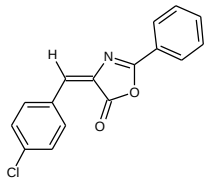
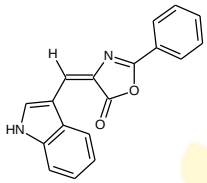
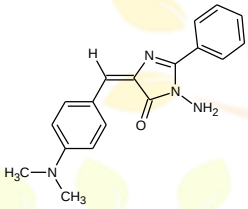
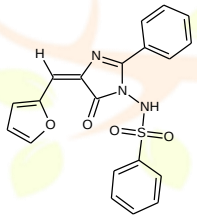
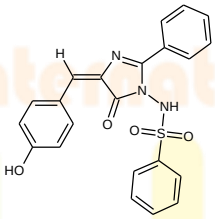
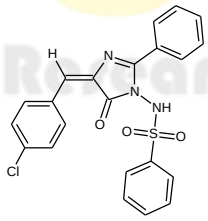
Table 3-Physical data of synthesized N-[(4Z)-4-(arylbenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-yl) -methylbenzenesulfonamide derivatives.

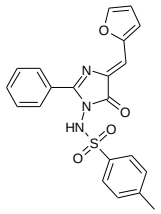
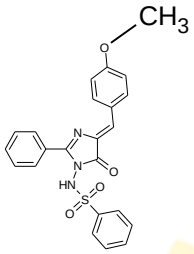
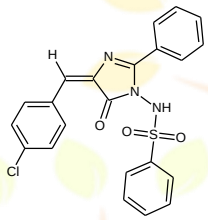
Compound S.no	**Name of imidazolone used**	**Name of sulfonamide derivative obtained**	**Physical State**	**Melting point ˚C**	**Yield %**
5a	(5*Z*)-3-amino-5-(4-chlorobenzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-chlorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-4-methylbenzenesulfonamide	**Pale yellow powder**	**122-124**	78.2
5b	(5*Z*)-3-amino-5-(4-hydroxy benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-hydroxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-4-methylbenzenesulfonamide	**Yellow fine powder**	**130-134**	86.1
5c	(5*Z*)-3-amino-5-(4-(dimethyl amino)benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	(5*Z*)-3-amino-5-(4-(dimethyl amino)benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-1-yl)4-methylbenzene sulphonamide	**Orange yellow powder**	**128-137**	73.9
5d	(5*Z*)-3-amino-5-(4-methoxylphenyl ethylidine)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]4-methyl benzenesulfonamide	**Pale white powder**	**125-130**	89.4
5e	(5*Z*)-3-amino-5-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	(5*Z*)-3-amino-5-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-3,5-dihydro-1*H*-imidazol-1-yl)4-methylbenzene sulphonamide	**Yellow powder**	**130-137**	80.35
5f	(5*Z*)-3-amino-5-[2-(furan-2-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(furan-2-ylmethylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-4-methylbenzenesulfonamide	**Light black crystalline powder**	**120-124**	90.54

Table 5: Physical data characterisation of *N*-[(4*Z*)-4-(arylbenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]benzenesulfonamide derivatives

Compound S.no	Name of imidazolone used	Name of sulfonamide derivative obtained	Physical State	Melting point ˚C	Yield %
6a	(5*Z*)-3-amino-5-(4-chlorobenzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-chlorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]-benzenesulfonamide	Pale yellow powder	124-125	80.45
6b	(5*Z*)-3-amino-5-(4-hydroxy benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-hydroxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]benzenesulfonamide	Yellow powder	132-134	63.89
6c	(5*Z*)-3-amino-5-(4-(dimethyl amino)benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	(5*Z*)-3-amino-5-(4-(dimethyl amino)benzylidene)-2-phenyl-3,5-dihydro-4*H*-imidazol-1-yl)benzene sulphonamide	Pale orange powder	126-127	88.47
6d	(5*Z*)-3-amino-5-(4-methoxyl phenyl ethylidine)-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	*N*-[(4*Z*)-4-(4-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl]benzenesulfonamide	White powder	124-125	93.11
6e	(5*Z*)-3-amino-5-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	(5*Z*)-3-amino-5-[2-(2,3-dihydro-1*H*-indol-3-yl)ethylidene]-2-phenyl-3,5-dihydro-1*H*-imidazol-1-yl)benzene sulphonamide	Yellow powder	132-134	86.74
6f	(5*Z*)-3-amino-5-[2-(furan-2-yl)ethylidene]-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one	5*Z*)-3-amino-5-[2-(-(furan-2-yl)ethylidene]-2-phenyl-3,5-dihydro-1*H*-imidazol-1-yl)benzene sulphonamide	Black powder	127-130	81.98

Table 6 -SPECTRAL DATA OF SYNTHESIZED COMPOUNDS:

Compound code	Molecular structure	(IR) υmax(KBr/cm-1)
2a		C-H Stretching at 2917.98cm-1 C-Cl Stretching at 691cm-1 C-O Stretching at 1793 cm^{-1}(lactone)
2e		2°NH (s)-PEAK at 3340.71 cm^{-1} C-H(S) at 2819.93cm^{-1} C=O (Lactone) at 1759.08 cm^{-1}
3c		1°NH (s)2PEAKS at 3319.49 cm^{-1} N-H(S) at 1521cm^{-1} C-N(S) at 1300-1319.31cm^{-1} C=O (Lactam) at 1637.56 cm^{-1}
6f		1°NH (s)-2PEAKS at 3296.35 cm-1 C=N(S) at 1636cm-1 C-N(S) at 1080.14 cm -1 C=O (Lactam) at 1645 cm-1
6b		2°NH (s) peak at 3342 cm-1 S=O (Asymmetric stretching) at 1415cm-1 and Symmetric stretching at 1029 cm-1 C-N(S) at 1078.21 cm-1 C=O og lactam ring at 1759.08cm-1
6a		2°NH (s) peak at 3342 cm-1 S=O characteristic Asymmetric stretching at 1415 cm-1 and Symmetric stretching at 1029 cm-1 C-N(S) at 1078.21 cm-1 C=O (Lactam) at 1759.08cm-1

5f		Compound Molecular weight is 447gms M+1 peak was observed at 448 Characteristic loss of H+1 from CH3 at 446 Base peak at 356 with the loss of characteristic tropyliumcation (m/z=91)
6d		Mol.wt of the compound was 433 M+1 peak was observed at 434 Base peak at 293with the characteristic loss of -NH-SO2-C6 H5
Compound code 6a	Structure 	1HNMR (δ- ppm) Ar-CH3 hydrogens within the range of 2.61-2.03 Aromatic hydrogens at 7.89-7.03 NH-hydrogen attached to amide group have shown a characteristic peak at 7.5Hz Ar-O-CH3 peak at 3.8

Molecular properties of synthetic derivatives

Table 7-Molecular property calculation and toxicity prediction of the title compounds

Mol.formula	**Mol.wt**	**clog P**	**logS**	**DL**	**MUT**	**REP**	**Rotatable bonds**
$C_{22}H_{16}ClN_3O_3S$	437.89	2.32	-2.31	3.71	None	None	4
$C_{22}H_{17}N_3O_4S$	419.45	1.84	-3.20	1.48	None	None	5
$C_{24}H_{22}N_4O_3S$	446.52	2.09	-3.96	1.73	None	None	5
$C_{20}H_{15}N_3O_4S$	393.41	1.45	-3.47	1.59	None	None	3
$C_{24}H_{18}N_4O_3S$	442.48	1.28	-1.25	2.74	None	None	5
$C_{23}H_{19}N_3O_4S$	433.47	2.63	-3.46	-0.77	None	None	5
$C_{23}H_{18}ClN_3O_3S$	451.92	2.8	-2.24	3.14	None	None	4

Compound	Ar	Binding scores
5a	*p*-chlorobenzylidine	**-8.8**
5b	***p*-hydroxybenzylidine**	**-9.1**
5c	Di methyl amino benzylidine	**-8.5**

$C_{23}H_{19}N_3O_4S$	433.47	1.81	-3.20	1.28	None	None	5
$C_{25}H_{20}N_4O_3S$	456.51	2.1	-3.54	1.21	None	None	5
$C_{24}H_{21}N_3O_4S$	447.50	2.24	-3.52	2.24	None	None	3
$C_{25}H_{24}N_4O_3S$	460.54	1.47	-3.62	-0.78	None	None	5
$C_{21}H_{17}N_3O_4S$	407.44	2.21	-3.10	1.61	None	None	5

- In this screening all the synthesized compounds complied with the Lipinski rule and none of the compounds amongst synthesized violated the rules and were predicted as potential therapeutic compounds.
- The log S solubility parameter corresponds to good absorption (logS>_4), All the compounds demonstrated logS values ranging from – 3.96 to -1.25 which predicts its extent of absorption.
- The logP values are ranged from 1.28 to 2.8 while the logS values were between – 3.96 to -1.25 both the set of values are within the accepted ranges for drug like molecules as described.
- Moreover, all the synthesized compounds have rotatable bonds 3-5.
- Compounds exhibited positive drug likeness ranging from -0.78 to 3.751 which represent that the molecule consists of fragments that are commonly found in marketed drugs.
- On the basis of drug likeness, compounds were predicted to be promising druggable candidates. Further, the result of molecular docking and *in vitro*antidiabetic studies supports the accuracy of prediction.
- The toxicity of the compounds was also predicted using Osiris, most of the compounds amongst the synthesized ones showed non tumorigenic and non-reproductive effects, which further supports the drug like features in the molecules. This toxicity prediction would be useful for the selection of compounds to test in animal models.
- The acute study data of all the synthesized compounds were depicted in Table ( ) in relation to the standard drug Glibenclamide. The compounds 5a and 5b have shown significant hypoglycaemic activity. The effect of compounds on insulin levels during day1and 7 in control and alloxan induced diabetic rats are measured.

Molecular Docking Studies: The docking studies of the synthesized compounds proved that they showed almost similar as Glibenclamide (reference standard) in binding with the target. The results are presented in the following table.

Table no.8 Binding scores of the synthesized compounds(5a-5f)with SUR1 receptors:

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5d	Anisalbenzylidine	**-8.8**
5e	Indolylidine	**-8.5**
5f	Furfurylidine	**-8.2**
Glibenclamide		**-10.66**

Table no.9 Binding scores of the synthesized compounds (6a-6f)with SUR1 receptors:

compound	Ar	Docking score
6a	***p*-chlorobenzylidine**	**-10.1**
6b	*p*-hydroxybenzylidine	-8.9
6c	Di methyl amino benzylidine	-8.6
6d	Anisalbenzylidine	-8.8
6e	**Indolylidine**	**-8.9**
6f	Furfurylidine	-8.6
Glibenclamide	-	-10.6

Table no.10 Binding interactions of the synthesized derivatives in SUR1 receptors (PDB ID: 6PZA)

Compound	Binding Interactions	Hydrophobic Bonds	H-Bonds
Glibenclamide	**SER-1238,ARG-388,ILE-381,**	**PHE-433**	**ARG-1246 (2), PRO- 589**
6b	TRP-430, GLU-1249	PHE-433,TRP-377	ARG-1246(1) ,ARG-308
6d	ILE-381, GLU-1249, LEU-381	PHE-433, TRP-377	ARG-1300,ARG-306
6a	**ILE-381, ALA-380, LYS-856**	**TRP-377**	**ARG-1246(2),**
5b	**ILE-381, ARG-1300**	**TRP-377**	**ARG-1246(2)**
6e	ASN-1437, PRO-438, LYS-486	PHE-433, TRP-377	-

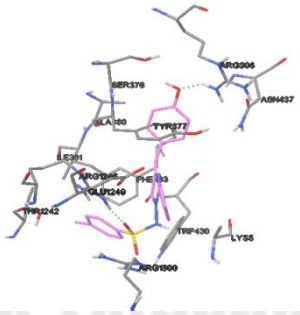


Figure 1: Three dimensional representation of binding mode of compounds 6b derivative (pink)in SUR-1 receptor(PDBID 6PZA) binding site and interacting amino acid residues

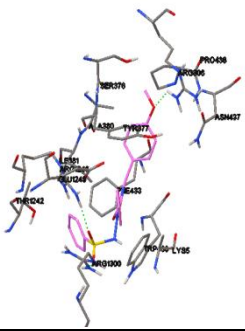


Figure 2: Three dimensional representation of binding mode of compounds 6d derivative (pink) in SUR-1 receptor(PDBID 6PZA) binding site and interacting amino acid residues

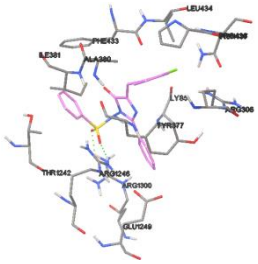


Figure 3: Three dimensional representation of binding mode of 6a derivative (pink) in SUR-1 receptor(PDBID 6PZA) binding site and interacting amino acid residues

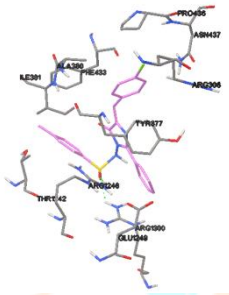


Figure4: Three dimensional representation of binding mode of 5b derivative (pink) in SUR-1 receptor(PDBID 6PZA) binding site and interacting amino acid residue

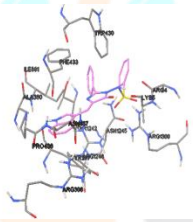


Figure 5: Three dimensional representation of binding mode of 6ederivative (pink) in SUR-1 receptor(PDBID 6PZA) binding site and interacting amino acid residues.

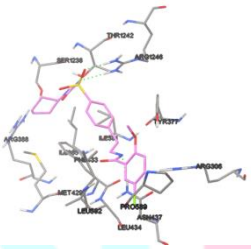


Figure 6: Three dimensional representation of binding mode of Glibenclamide (pink) in SUR-1 receptor (PDBID 6PZA) binding site and interacting amino acid residues.

All the compounds 5a-5f and 6a-6f demonstrated significant binding scores ranging from -8.2 to -10.1. The title compounds which demonstrated highest docking scores reported the hydrogen bonding interactions with the target protein amino acid residueARG-1246which was similar to that of Glibenclamide (reference standard drug) protein interaction as shown in the fig: 6 .The title compounds demonstrated interactions with the hydrophobic pocket of the target protein amino acid residues PHE-433, TRP-377 which was similar to that of Glibenclamide protein interaction.

All the title compounds represented in fig: 1-6. Among all the tested compounds, 6b and 6dreported significantly two H-bonds with the catalytic site of the receptors in correlation to the standard drug.

It could be understood that from analyzing the interaction of compounds with protein, the polar substitution of sulphonamido –NH and S=O groups in the structure accounts for good interaction with receptor through hydrogen bonding. Apart from it, in compounds 5b, 6b, 5d substituted derivatives where there are –OH,-OCH3 groups participated in forming hydrogen bonding with amino acid residues ARG-308,ARG-306 respectively. It can be summarized that the synthesized compounds form the complex with the target at lowest energy and showed better affinity towards SUR-1which suggested that these compounds could be further subjected to *in vivo* studies to assess their hypoglycaemic activity.

***In vivo*Hypoglycemic Activity:**

All the compounds were screened for their oral in vivo hypoglycaemic activity using Alloxan -induced diabetic model in rats. All the tested compounds are having remarkable hypoglycaemic property, however with a degree of variation.

Alloxan causes diabetes by the rapid depletion of β-cells and thereby brings about a reduction of insulin release. In our study, an increase in blood glucose level in diabetic rats confirmed the induction of diabetes mellitus the oral administration of a single dose of synthesized compounds caused a significant reduction in blood glucose in diabetic rats. These results revealed that sulphanamido substituted Imidazolone derivatives may be effective in the treatment of insulin-independent diabetes mellitus. Since the derivatives are having structural resemblance with sulphonylureas and also thiazolidinone with minor modification, it can be expected that the mode of action of these derivatives may result in synergistic hypoglycaemic effect. However, it is expected that the synthesized compounds can act directly/ indirectly by stimulating the release of insulin into the bloodstream.

Blood glucose changes, average food and water intake in treatment of diabetic rats with synthesized sulphonamido substituted imidazolone derivatives were presented in the following tables:

Table 11: Average blood glucose levels of normal and diabetic rats before and after the treatment.

Values are expressed in Mean± SEM. (n=6) *p< 0.01, ** p< 0.001 compared to Diabetic control.

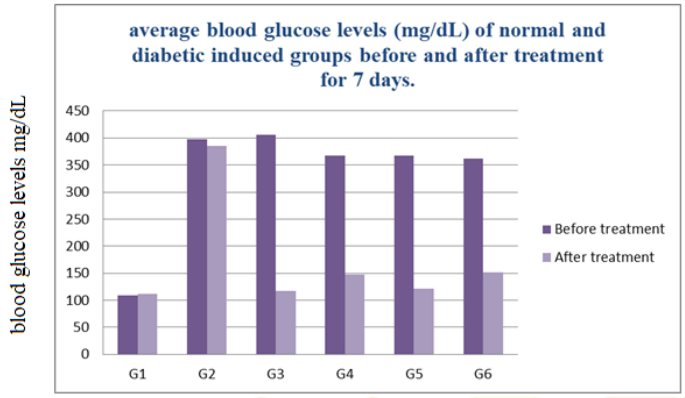


Figure: Average blood glucose levels of normal and diabetic rats before and after the treatment.

Table 12 : Average food and water intake of normal and diabetic rats during 7 days of treatment.

GROUP	Average Food Intake (gms)	Average water intake (ml)
G1(normal control)	10.98±0.52*	17.1±0.44*
G2(diabetic control)	14.9±0.83	25.7±0.89
G3(Standard)	10.8±0.48*	13.6±2.18*
G4(5b)	12.6±0.46	1+9.43±2.23
G5(6a)	9.94±0.54*	14.3±2.9*
G6(6e)	12.48±0.45	19.53±2.7*

Values are expressed in Mean± SEM. (n=6) *p< 0.01, ** p< 0.001 compared to control group
#p< 0.01 ## p<0.001 compared to Diabetic control.Values are expressed in Mean± SEM. (n=6) *p< 0.01, ** p< 0.001 compared to Diabetic control.

S.No	GROUPS	Before treatment	After treatment	Percentage reduction (%)
1	G1(normal control)	109.5±4.16	111.6±4.8	-
2	G2(diabetic control)	398±11**	385.5±7.05	-
3	G3(Std)	406.6±12.11**	117.5±3.18##	71.18
4	G4(5b)	367.5 ± 14.7**	148.1±3.86#	59.67
5	G5(6a)	368.8±11.24**	120.8±2.89##	67.2
6	G6(6e)	362.8±12.9**	151.9±3.5#	58.12

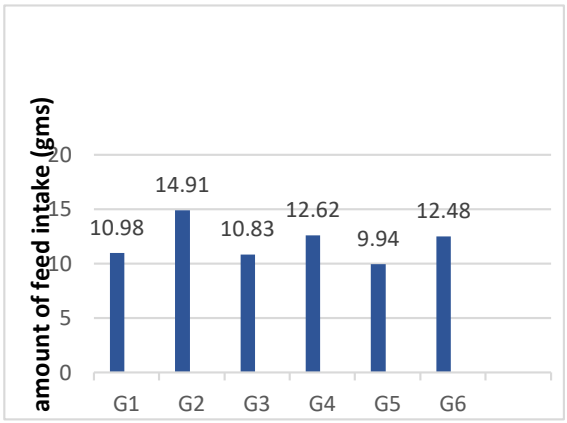
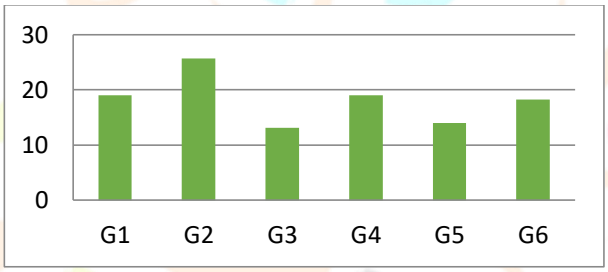


Figure7: Average food intake of normal and diabetic rats during 7 days of treatment.

There is a Significant reduction in food intake in group 3 and group 5 which were treated with glibenclamide and compound 6a



Values are expressed in Mean± SEM. (n=6) *p< 0.01, ** p< 0.001 compared to Diabetic control. **Figure 8:** Average water intake of animals during 7 days of treatment.

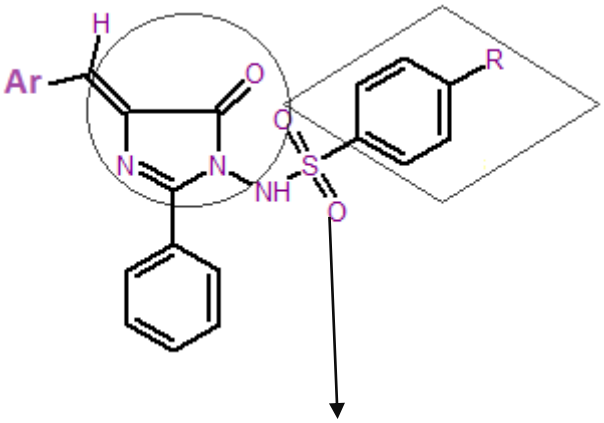
There is also a significant reduction in water intake of groups that were treated with test compounds and standard.

From the observed results it is concluded that almost all the tested compounds reduced glucose level in diabetic rats. However, the effect of **6a**is more pronounced in Alloxan diabetic rats. Further toxicity studies and studies on mode of action of the hypoglycaemic activity can provide an effective drug candidate for diabetes mellitus.

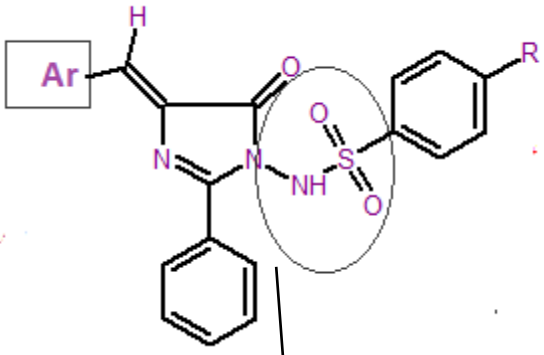
In the meanwhile from the data obtained through docking studies and animal experimentation we have predicted the possible structural activity relationship of various synthetic derivatives. It revealed that the presence of imidazolone nucleus which is isosteric to thiazolidinedione, sulphonamide moiety, and Substituted aryl aldehydes with electron withdrawing groups at Para position enhances the hydrogen bond type of interactions and aromatic phenyl attached to sulphonamide enhances hydrophobic interactions with the active site.

DERIVATION OF SAR OF SULFONAMIDO SUBSTITUTED IMIDAZOLONE DERIVATIVES FROM THE CONCLUSIONS DERIVED THROUGH THE ABOVE EXPERIMENT.

Isosteric Replacement of
Thiol with Amino group resulted in analogues with desired Antidiabetic property and enhanced physico-chemical properties.



R=CH_3 Increases the activity by enhancing interactions with hydrophobic pockets of protein and are more prone to bind with biomolecules.



Substitution of the Ar-phenyl ring at 4th position by an electron withdrawinggroup(-Cl,-OH) showed maximum hypoglycaemic activity because of their high binding affinities and selectivity

Presence of Sulphonamide core structure be replacing R_1 with R-C_6H_5 ,R_2 with H and R_3 with substituted imidazolones resulted in increasing efficacy of lead molecule as Antidiabetic agent

Conclusion:Values are expressed in Mean± SEM. (n=6) *p< 0.01, ** p< 0.001 compared to Diabetic control.

In the present investigation 12 new sulfonamido substituted imidazolone derivatives were synthesized. All the compounds were characterized by physical and spectral analysis. These were screened for molecular properties and toxicity prediction to access the druglikeliness of the synthesized compounds. Followed by molecular docking studies on sulfonylureas receptor having PDB ID-6PZA to identify potential molecules. From this results we have selected the compounds 6a,6e and5b that have shown better binding affinities towards receptor for invivo evaluation. The compounds were then screened for their invivoantidiabetic activity using Wister albino rats considering glibenclamide as standard.It was observed all the compounds used for treatment were selective towards SUR-1 receptor and have shown significant decrease in blood glucose levels especially derivatives possessing electronegative group (compound 6a) are having high potency.

Thus, further research could be carried out in metabolic and toxicity areas to to convert this lead molecule into potent anti-diabetic agent.

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