Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(8):164-172

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Molecular Docking and Synthesis of Some Substituted Sulphonylurea/Pyrrolidine -Based Derivatives as Hypoglycemic Agents

Ishan I Panchal[1*], Dhrubo Jyoti Sen[2], Ashish Shah[3], Ashish Patel[1] and Vashisth Bhavsar[4]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Parul University, Vadodara, Gujarat, India
[2]Department of Pharmaceutical Chemistry, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India
[3]Department of Pharmacy, Sumandeep Vidhyapeeth, Vadodara, India
[4]Department of Pharmacology, Faculty of Pharmacy, Parul University, Vadodara, Gujarat, India

---

ABSTRACT

A series of 1-(4-substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1 yl)ethyl)phenylsulfonyl)urea/gaunidine(5A-5F) and1-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)urea (5G-5J) derivatives was design and synthesized as hypoglycemic agents. The structures of all the newly synthesized compounds were characterized by their melting point, TLC, IR spectroscopy, 1H-NMR and 13C-NMR. All these newly synthesized compounds were screened for their in vivo hypoglycemic activity using alloxan induced diabetic rat model. Docking studies were performed using iGEMDOCK program to predict the binding affinity and to understand interaction with various residues. Compounds 5B, 5G, and5I exhibited good hypoglycemic activity and binding affinity comparable to glibenclamide. Good binding affinity and in vivo hypoglycemic activity of 5B, 5G, 5I suggest that it can be further explored as hypoglycemic agents.

Keywords: Diabetes mellitus; Sulphonylureas; ADME/T; AKR1C1; QikProp; Alloxan

---

INTRODUCTION

Diabetes mellitus (DM) is a major degenerative disease in the today's world. It is a group of disorders of carbohydrate metabolism resulting from body's failure to produce insulin in Type 1 and insulin resistance in Type 2 diabetes mellitus through altered secretion, decreased insulin activity, or a combination of both factors and characterized by hyperglycaemia [1]. Several epidemiological and clinical studies indicate a direct relationship between hyperglycemia and long-term complications such as retinopathy, nephropathy, neuropathy like micro and macrovascular complications. This disease is associated with reduced life expectancy significant morbidity due to specific diabetes related micro vascular complications that diminish the quality of life. India became the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 [2,3].
Numerous drugs such as sulfonylureas and Biguanides are presently available to reduce hyperglycemia in diabetes mellitus. Inhibitors of DPP-4 have long been sought as tools to elucidate the functional significance of the enzyme [4]. The onset of insulin in the body, which causes an abnormal effect on glucose metabolism, is related not only to the development of Type II diabetes but also to cardiovascular disease. The most restricted item in the diets of both noninsulin-dependent and insulin dependent diabetic individuals is refined carbohydrate [5]. Pyrrolidine heterocycles [6-10] play important role in antidiabetic therapy for many years. Sulphonylureas [11-17] are backbone for antidiabetic therapy. Thus, there remains an urgent need to develop new antidiabetic agents with higher efficacy and lower toxicity for the long term treatment of T2DM. With the help of literature we have decided to design

sulphonylureas and pyrrolidine derivatives as hypoglycemic agents. Abbas Ahmadi et al. has reported Synthesis and Investigating Hypoglycemic and Hypolipidemic Activities ofSome Glibenclamide Analogues in Rats [16]. Xun Ji et al has reported. Design, synthesis and biological evaluation of 4-fluoropyrrolidine-2-carbonitrile and octahydrocyclopenta[b]pyrrole-2-carbonitrile derivatives as dipeptidyl peptidase IV inhibitors [10]. With reference [10,16] of we have decided to design, synthesis and *in vivo* biological activity of novel hypoglycemic agents. Figures 1 and 2 shows the lead compounds and targeted derivatives, design and modification in targeted molecule.

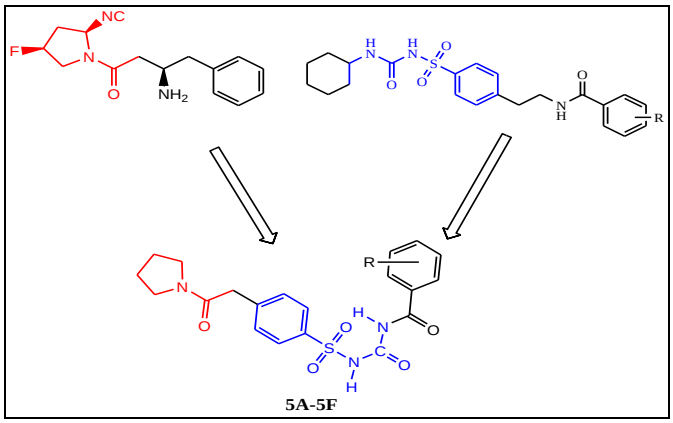


Figure 1: Design and modification strategies of novel 5A-5E compound

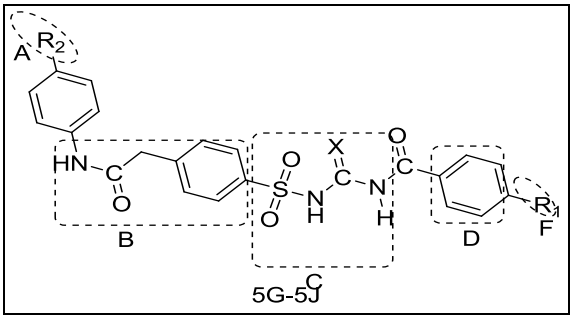


Figure 2: Influences of structural components in determining the structure–activity relationship: A) methoxy group larger groups increasing the binding affinity and insulin secretion; B) acid amide moiety enhancing binding affinity by the factor 1000 in comparison to tolbutamide; C) acidic sulfonylurea group essential for binding; [17] D) benzene moiety increasing lipophilicity; F) electron withdrawing group like nitro, fluoro, chloro at the para position in compound to produce significant blood glucose lowering activity [18]

MATERIALS AND METHODS

Materials

All the chemicals and reagents collected were of LR grade from Sigma Alderich & Loba Chemie. The reactions were monitored by thin layer chromatography on TLC silica gel 60 F254 plates for completion of the reaction; mobile phase solvents were selected as n-hexane: ethyl acetate (7:3). Melting points of all the synthesized compounds were checked in capillary tubes by using a melting point apparatus (VEEGO melting point apparatus). All the compounds were characterized by FT-IR spectrometer (Bruker); 1H NMR, 13C NMR spectra were obtained from 400 MHz NMR Spectrometer (Brüker Biospin, Switzerland) CoE Rajkot, Gujarat. Molecular weight of synthesized compounds was performed in Mass spectrophotometer (O2h discovery, Ahmedabad and Synzeal Research Pvt. Ltd, Gandhinagar).

Experimental Section

Molecular Docking Study: The Crystal structure of the pancreatic ATP-sensitive K+ channel SUR1/Kir6.2 complexed with ATP and glibenclamide (PDB ID: 5TWV) was imported [19]. Docking, screening and post-analysis of the designed compounds were done using iGEMDOCK program with the protein target 5TWZ. The binding sites of the targets were prepared and the energy minimized compound was imported. During docking, at first the molecules were prepared and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands. From the docking, wizard ligands were selected and the scoring function used was

iGEMDOCK score. If hydrogen bonding is possible, the hydrogen bond energy contribution to the docking score is assigned a penalty based on the deviations from the ideal bonding angle. This option can significantly reduce the number of unlikely hydrogen bonds and also internal electrostatic interaction; internal hydrogen bond sp2-sp2 torsions are calculated from the pose by enabling the ligand evaluation terms. The search algorithm is taken as iGEMDOCK and numbers of runs taken are 70 and max interactions were 2000 with population size 200 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lowest energy is chosen. If the energy is positive (i.e., because of a clash or an unfavourable electrostatic interaction), then additional 'max' positions will be tested. If the pose being docked is closer to one of the ligands in the list than specified by the Root Mean Square Deviation (RMSD) threshold, an extra penalty term (the energy penalty) is added to the scoring function. This ensures a greater diversity of the returned solutions since the docking engine will focus its search on poses different from earlier poses found. The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation standard docking were set. Docking was conducted between protein and inhibitor which results in binding affinities in kcal/mol. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was 8Å. The empirical scoring function of iGEMDOCK was estimated as:

$$\text{Fitness} = \text{vdW} + \text{Hbond} + \text{Elec.}$$

Here, the vdW term is vander Waal energy. H-bond and Elect terms are hydrogen bonding energy and electro-static energy, respectively.

General Procedure for Synthesis of 1-(4-substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea/gaunidine (5A-5F)

They were prepared by following the literature method [20]. Fridalcraft alkylation of 1-(4-substitutedbenzoyl)-3-(phenylsulfonyl)urea/gaunidine (0.1 mmol) and 2-chloro-1-(pyrrolidin-1-yl)ethanone (1 mmol) was done for 7 hrs in the presence of anhydrous $AlCl_3$ and dry DCM (50 ml) as solvent. A reaction mixture was cooled and washes with ice cold water. Solid product was recrystalize with rectified spirit.

1-(4-Methoxybenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5A):

Yield: 65%, m.p 150-152°C; Rf=0.75 (ethyl acetate: hexane 2:8 v/v) IR (KBr) (ƛmax in cm^{-1}): 3408(N-H Str), 2850(C-H Str), 1695(C=O str). MS (ESI) m/z: 445[M+]1H NMR (DMSO): δ (ppm) 3.76(s,3H,OCH3), 7.87(d,4H,ArH), 7.21(d,4H,ArH), 10.18(s,1H,NH), 2.34(t,4H,Pyrrolidine), 3.39(t,4H,Pyrrolidine)

1-(4-Fluorobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5B):

Yield: 75%; gray crystalline powder; m.p=124-126ºC; Rf=0.75 (ethyl acetate: hexane 2:8 v/v) IR (KBr) (ƛmax in cm^{-1}): 3263(N-H Str), 2973(C-H Str), 1672(C=O str), 1378(S=O), 1150(C-F Str); MS(ESI); 435(M+2); 1HNMR(DMSO): δ(ppm), 7.87(d, 4H, ArH), 7.25(d, 4H, ArH), 10.03(s, 1H, NH), 2.14(t,4H,Pyrrolidine), 3.48(t,4H,Pyrrolidine)

1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5C):

Yield: 75%; yellow crystalline powder; m.p=160-164ºC; Rf=0.60 (ethyl acetate: hexane 2:8 v/v) IR (KBr) (ƛmax in cm^{-1}): 3449(N-H Str), 2989(C-H Str), 1680(C=O str), 1187(S=O), 1549 and 1318(N-O str), 1HNMR(DMSO): δ(ppm), 7.67 (d, 4H, ArH), 7.20 (d, 4H, ArH), 10.23 (s, 1H, NH), 2.17(t,4H,Pyrrolidine), 3.28(t,4H, Pyrrolidine)MS: m/z: 460 (M+)

1-Benzoyl-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5D):

Yield: 65%; m.p: 116-118°C. Rf=0.60 (ethyl acetate: hexane 3:7 v/v) IR (KBr) (ƛmax in cm^{-1}): 3274(N-H Str), 2993(C-H Str), 1724(C=O str), 1102(S=O); MS:(m/z): 415[M+]; 1H NMR(δppm): 7.25(d, 4H, ArH), 7.49(s,1H,ArH),7.89(d, 4H, ArH), 2.17(t,4H,Pyrrolidine), 3.28(t,4H, Pyrrolidine)

1-(4-Chlorobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5E)

Yield: 70%; m.p=122-126°C. Rf value: 0.5 (ethyl acetate: hexane: 0.7:0.3). IR (KBr) (ƛmax in cm^{-1}): 3363(N-H Str), 2975(C-H Str), 1682(C=O str), 1378(S=O), 850(C-Cl Str); MS (m/z): 452[M+2]; 1HNMR (δ ppm), 7.87(d, 4H, ArH), 7.25(d, 4H, ArH), 10.03(s, 1H, NH), 2.14(t,4H,Pyrrolidine), 3.48(t,4H,Pyrrolidine)

1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)guanidine(5F):

Yield: 70%; yellow crystalline powder; m.p=160-164ºC; Rf=0.60 (ethyl acetate: hexane 2:8 v/v) IR (KBr) (ƛmax in cm^{-1}): 3449(N-H Str), 2989(C-H Str), 1680(C=O str)1187(S=O), 1575 and 1336(N-O str), 1HNMR(DMSO):

δ(ppm), 7.67(d, 4H, ArH), 7.20(d, 4H, ArH), 10.23(s, 1H, NH), 2.17(t,4H,Pyrrolidine), 3.28(t,4H,Pyrrolidine). MS: m/z: 459.2 (M+)

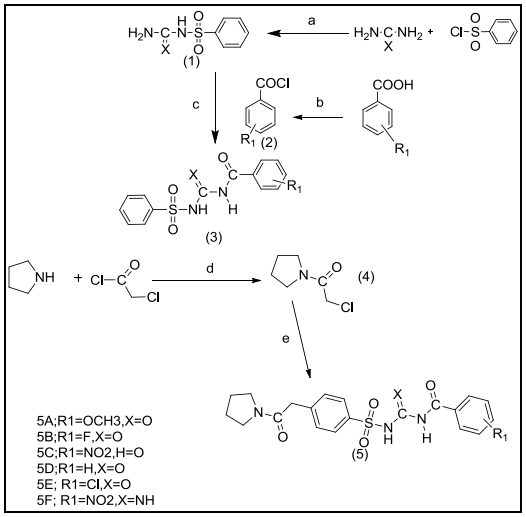
General Procedure for Synthesis of 1-(4-substitutedbenzoyl)-3-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)urea (5G-5J) were prepared by following the literature method [20]

1-(4-(2-(4-Methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)gaunidine (5G):
Yield: 60%; m.p=144-146°C. Rf value: 0.5 (mobile phase: ethyl acetate: hexane: 0.3:0.7); IR (KBr) (ƛmax in cm^{-1}); 3449(N-H Str), 2989(C-H Str), 1684(C=O str)1187(S=O), 1554 and 1320(N-O str); MS(ESI)(m/z): 510.8[M-1]; 1HNMR (δ ppm): 7.67.21(d,4H,ArH), 8.18-8.206(d,4H,ArH), 10.24(s,1H,NH), 3.76(s,3H,OCH3), 3.062(s,2H,CH2)

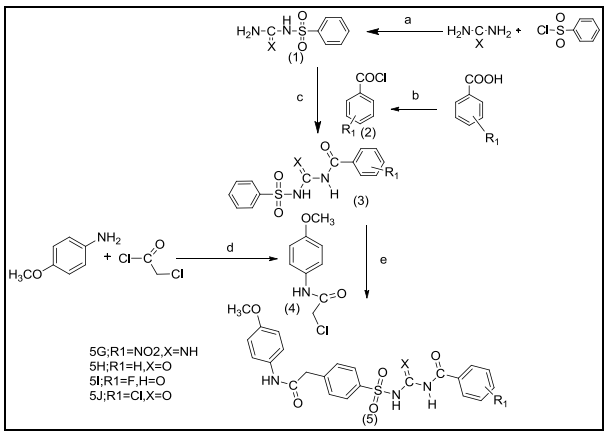
1-Benzoyl-3-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)urea(5H):
Yield: 42%; m.p=112-114°C. Rf value: 0.5 (mobile phase: ethyl acetate: hexane: 0.3:0.7); IR (KBr) (ƛmax in cm^{-1}): 3449(N-H Str), 2989(C-H Str),1688(C=O str)1187(S=O); MS(ESI) (m/z): 468(M+1); 1HNMR (δ ppm): 7.69-7.21(d,4H,ArH), 8.18-8.206(d,4H,ArH), 10.24(s,1H,NH), 3.76(s,3H,OCH3), 3.062(s,2H,CH2)

1-(4-((4-Methoxyphenyl)carbamoyl)phenylsulfonyl)-3-(4-fluorobenzoyl)urea(5I):
Yield: 42%; m.p=112-114°C. Rf value: 0.55(mobile phase: ethyl acetate: hexane: 0.3:0.7); IR (KBr) (ƛmax in cm^{-1}): 3439(N-H Str), 2989(C-H Str), 1670(C=O str)1187(S=O); Mass (m/z): 471(M+1); 1H NMR (δ ppm): 7.69-7.21(d,4H,ArH), 8.18-8.206(d,4H,ArH), 10.24(s,1H,NH), 3.76(s,3H,OCH3), 3.062(s,2H,CH2)

1-(4-Chlorobenzoyl)-3-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)urea(5J):
Yield: 42%; m.p=112-114°C. Rf value: 0.55(mobile phase: ethyl acetate: hexane: 0.3:0.7); IR (KBr) (ƛmax in cm^{-1}): 3352(N-H Str), 2949(C-H Str), 1690(C=O str), 850(C-Cl), 1167(S=O); MS(ESI) (m/z):503[M+1];1H NMR (δ ppm) :7.69-7.21(d,4H,ArH), 8.18-8.206(d,4H,ArH), 10.24(s,1H,NH), 3.76(s,3H,OCH3), 3.062(s,2H,CH2)



Scheme 1: Synthetic route for the preparation of the sulphonylureas/gaunidine derivatives Reagents and conditions: (a) Pyridine, CH_3CH_2OH, Reflux (yield >75%); (b) $SOCl_2$, reflux, 3 h, (yield >60%); (c) Pyridine, CH_3CH_2OH, Reflux (yield >70%); (d) DRY THF, 00C TO RT, Stirring, 4 h, RT (yield >85%). (e) Dry DCM, $AlCl_3$, reflux, 6 hrs, (yield>60%)



Scheme 2: Synthetic route for the preparation of the sulphonylureas/gaunidine derivatives Reagents and conditions: (a) Pyridine, CH_3CH_2OH, Reflux (yield >75%); (b) $SOCl_2$, reflux, 3 h, (yield >60%); (c) Pyridine, CH_3CH_2OH, Reflux (yield >70%); (d) DRY THF, 0 TO RT, Stirring, 4 h, RT (yield >85%). (e) Dry DCM, $AlCl_3$, reflux, 6 hrs, (yield>60%)

In vivo Biological Evaluation

Rats of Wistar strain were procured form the Animal House, Department of Pharmacology, Parul institute of pharmacy, Parul University, Vadodara were used in this study. Experiments were carried out in male rats weighing between 150 g and 200 g. They were housed (six per cage) in plastic cages (47 cm × 34 cm × 18 cm) lined with husk renewed every 24 h. The rats were fed on a pellet diet (Hindustan Lever, India). Drinking water was allowed ad libitum. Diabetes was induced in the rats by a single intraperitonial injection of alloxan (150 mg kg^{-1} body weight). Since alloxan is capable of producing fatal hypoglycaemia as a result of the massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneal after 6 h. The rats were then kept for the next 24 h on 5% glucose solution to prevent hypoglycemia. [21] After 1 week, rats with moderate hyperglycaemia with blood glucose range of 200-400 mg dl^{-1} were used for the study. Blood was collected from the tail vein. All of the target molecules were given to the diabetic rats orally in the form of suspension in carboxy methyl cellulose.

The animals were housed under standard laboratory conditions maintained at 25 ± 10°C and under 12/12 hour light /dark cycle. The experimental protocol was approved by the institutional animal ethics committee (Protocol No: PIPH 03/16) and by the animal regulatory body of the Indian Government (Registration No: 921/PO/EReBi/S/05/CPCSEA/PIPH03).

Experimental Design

In the experiment, a total of 66 rats (60 diabetic surviving rats + 6 normal rats) was used. Diabetes was induced in rats, 1 week before starting the treatment. The rats were divided into eleven groups as follows, after the induction of alloxan diabetes and each containing six rats Animals with blood glucose levels between 200-400 mg/dl were divided into the following groups.

Group I: Normal -Normal controlled rats fed with 0.5 ml of normal saline.
Group II: Diabetic control (DC) rats; fed with 0.5 ml of normal saline.
Group III: Diabetic rats treated with standard drug Glibenclamide 5 mg/kg body wt.
Group IV: Diabetic rats; treated with synthesized drug No 5A in 1% CMC 50 mg/kg of body weight.
Group V: Diabetic rats; treated with synthesized drug No 5B in 1% CMC 50 mg/kg of body weight.
Group VI: Diabetic rats; treated with synthesized drug No 5C in 1% CMC 50mg/kg of body weight.
Group VII: Diabetic rats; treated with synthesized drug No 5D in 1% CMC 50 mg/kg of body weight.
Group VIII: Diabetic rats; treated with synthesized drug No 5E in 1% CMC 50 mg/kg of body weight.
Group IX: Diabetic rats; treated with synthesized drug No 5F in 1% CMC 50 mg/kg of body weight.
Group X: Diabetic rats; treated with synthesized drug No 5G in 1% CMC 50 mg/kg of body weight.
Group XI: Diabetic rats; treated with synthesized drug No 5H in 1% CMC 50 mg/kg of body.
Group XII: Diabetic rats; treated with synthesized drug No 5I in 1% CMC 50 mg/kg of body.
Group XIII: Diabetic rats; treated with synthesized drug No 5J in 1% CMC 50mg/kg of body.

The dose for the newly synthesized compounds was decided on the basis of literature survey [22]. Glibenclamide was taken as the standard. The blood glucose level was determined at 0 and 3 hours after administration of test

compound using glucometer (Johnson & Johnson Pvt. Ltd.) Percentage reduction in plasma glucose level was calculated for each animal.

Statistical Analysis

Measurement data were tabulated as means ± S.E.M. Comparisons were carried out using one way analysis of variances (ANOVA) followed by post-hoc Tukey test and p-value<0.05 as the level of significance. Data was analyzed using the Graph Pad Prism 5.3, San Diego, CA.

RESULTS AND DISCUSSION

Molecular Docking Studies

The results were obtained as docking score, i.e. binding energy which is mentioned in Table 1. The entire designed compound has shown good binding affinity in comparison with standard glibenclimide, out of this Compund 5B, 5G, 5I showed more binding affinity in comparison with glibenclamide. Binding cavity and interaction with the various amino acid residues with the compound 5G and 5I were shown in Figure 3.

***In silico* Toxicity Studies**

In silico toxicity profile of designed compounds was performed using SWISS ADME programme. The Lipnski rule of five was applied. The acceptability of analogues based on Lipinski's rule of five which was essential to ensure drug like properties. The results of *in silico* toxicity studies mentioned in Table 2.

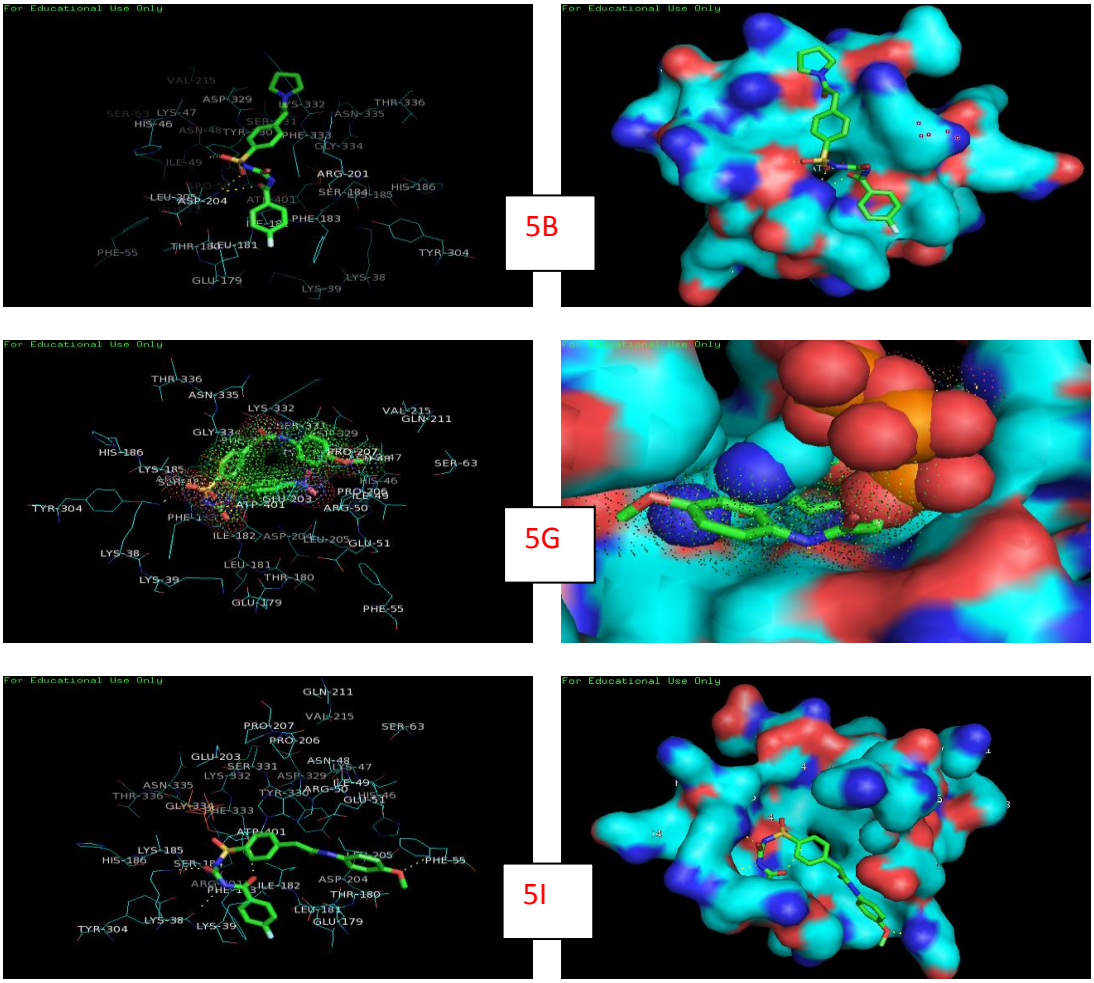


Figure 3: Showing binding pocket and interection with various residues of compound 5B, 5G, 5I

Table 1: Docking results of the designed compounds

Compound code	Docking score	H- bond energy	Vander Waal energy
5A	-104.667	-95.0936	-19.5733
5B	-108.3982	-86.3291	-13.0691
5C	-104.018	-71.8192	-33.386
5D	-99.6336	-90.4049	-9.22871
5E	-102.399	-91.801	-10.5978
5F	-107.77	-89.3005	-19.6134
5G	-114.205	-82.6018	-23.6027
5H	-112.783	-94.8273	-17.9556
5I	-118.063	-105.333	-12.7301
5J	-104.372	-98.9745	-5.39719
Glibenclamide	-108.996	-91.48	-17.5158

Table 2: *In silico* toxicity studies

Compound code	Mol. Wt(g/mol)	HBd	HBa	C Log P
5A	445.49	2	6	1.7
5B	433.45	2	6	1.91
5C	461.47	3	7	0.33
5D	415.46	2	5	1.68
5E	449.91	2	5	2.11
5F	460.48	7	10	0.18
5G	513.5	4	8	0.48
5H	467.49	3	6	2.32
5I	485.48	3	7	2.65
5J	501.94	3	6	2.85

Chemistry

Chemistry (Schemes 1 and 2) general procedure for Synthesis of 1-(4-substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea/gaunidine(5A-5F) were prepared using literature method. Fridal craft alkylation of 1-(4-substitutedbenzoyl)-3-(phenylsulfonyl)urea/gaunidine(0.1 mmol) and 2-chloro-1-(pyrrolidin-1-yl)ethanone (1 mmol) was done in 7 hrs in the presence of anhydrous AlCl3 and dry DCM (50 ml) as solvent. A reaction mixture was cooled and washed with ice cold water. Solid product was Recrystalize with rectified spirit. The synthesis compound 1 was done by reacting benzene sulphonyl chloride with excess amount of urea/guanidine in the presence of pyridine (0.2 ml) under reflux condition for 5 hrs. Reaction mixture was monitored by thin layer chromatography. Different derivatives (NO_2, OCH_3, Cl and F) of benzene carboxylic acid were converted into benzene carbonyl chloride 2 with the help of $SOCl_2$ under reflux condition for 3 hrs. Compound 1 was dissolved in absolute alcohol and reacted with compound 2 under reflux condition for 2 hrs. Pyridine (0.2 ml) was taken was the catalyst. Reaction mixture was poured into ice cold water; solid product was isolated and recrystallizes with rectified spirit. pyrrolidine was stirred with chloro acetyl chloride under cooling condition for 4 hrs in fuming hood. After addition of ice cold water in a reaction mixture solid crystals of 2-chloro-1-(pyrrolidin-1-yl)ethanone 4 were isolated.

One step Fridal crafts alkylation was performed to prepare1-(4-substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea/gaunidine(5A-5F) as illustrated in scheme 1, an appropriate1-(4-Substitutedbenzoyl) -3-(phenylsulfonyl)urea/guanidine and 2-chloro-1-(pyrrolidin-1-yl)ethanone. Dry dichloromethane was used as solvent and anhydrous $AlCl_3$ act as catalyst. Reflux was done for 6 hrs and the mixture was poured in ice cold water to get final product 5. The synthesis of piperazine derivatives 5G-5J was done as illustrated in scheme 2, by reflux between 1-(4-Substitutedbenzoyl)-3-(phenylsulfonyl) urea/gaunidine and 2-chloro-N-(4-methoxyphenyl)acetamide for 7 hrs in the presence of anhydrous AlCl3 and dry DCM (50 ml) as solvent. The target compounds 5A-5J were purified by recrystallization. Reaction of all targeted molecules was monitored by thin layer chromatography. Ethyl acetate and hexane (3:7) was used as mobile phase. Some impurities were very close to most of our compounds, which leads to obtain a less percentage yield of pure target compounds.

Spectral Characterization

It was done with Infra-red spectroscopy, mass spectroscopy, and 1H and 13C nuclear magnetic resonance for all target compounds. In mass spectroscopy, we got peak in M (5A), (5C), (5D), (5F), (5H) M+1(5I), M-1(5G), and M+2(5B), (5E) for different target compounds. M+2 peak obtained because of halogen atom. In1H NMR doublet

was obtained for aromatic hydrogen between 7 to 8.5 delta values. In the same way singlet of NH of urea/guanidine derivatives is obtained between 7.98 to 10.41. The absorption band at 1670 cm^{-1} to1724 cm^{-1}(5D) corresponds to C=O is stretching of carbonyl and amide for targeted compounds (5A-5I). Also N-O stretching was observed between 1320 cm^{-1} and 1554 cm^{-1} (5G), 1336 cm^{-1} and 1575 cm^{-1} (5F), 1318 cm^{-1} and 1549 cm^{-1} (5C). Halogen stretching was present at 1150 cm^{-1} (C-F, 5B), 850 cm^{-1} (C-Cl, 5E).

Biological Evaluation

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia [23]. The ability of target molecules to bind with sulphonylureas receptor was determined by testing them at an albino wistar rate for measurement of reduction of blood sugar level. Blood Data analysis was done by graph pad prism one way ANOVA followed by turkey test. In our study, we have found that administration of compounds 5A-5J to diabetic rats reversed their blood glucose. The possible mechanism by which they brings about them hypoglycemic action may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form. However, the Compound 5B of pyrrolidine derivatives and 5G, 5I methoxy derivatives of sulfonylureas shows better % reduction of blood glucose level (Table 3 and Figure 4) compare to other derivatives. 5B contain electronegative atom (F) in the para position of benzene ring which has significant effects on blood sugar reduction. In another side, 5G, 5I contain NO2and F functional groups respectively in the para position of sulfonylureas derivatives, which shows better blood sugar reduction compare to other derivatives (Figure 5). Table 3 gives drug and % reduction in Plasma Glucose level (Mean ± SEM). Figure 4 shows the biological activity of synthesized compounds.

Table 3: *In vivo* hypoglycemic activity of compounds 5A-5J

Group	% Reduction in Plasma Glucose level (Mean ± SEM)
Diabetic control	0.29 ± 1.05
Glibenclamide	55.97 ± 3.19
5C	45.88 ± 3.17
5G	51.49 ± 7.73
5H	46.40 ± 6.1
5A	39.24 ± 2.08
5B	49.25 ± 4.11
5E	44.18 ± 3.22
5F	39.07 ± 1.88
5D	41.26 ± 2.78
5I	48.33+ 3.2
5J	41.88 ± 3.17

Note: Values are given as mean ± S.D. for six rats in each group. Experimental groups are compared with Glibenclamide. Values are statistically significant at ∗∗P <0.01 as compared with diabetic control

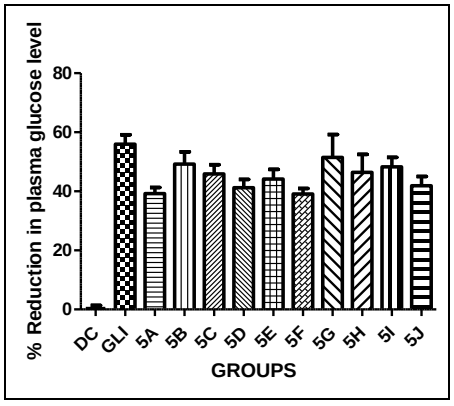


Figure 4: *In vivo* biological evaluation

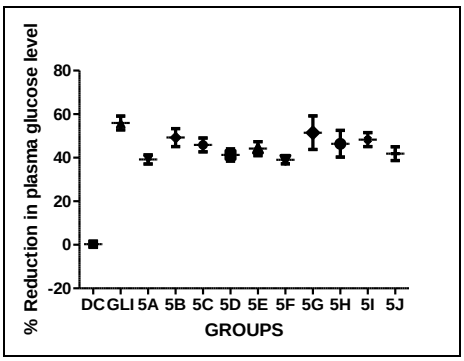


Figure 5: Reduction in plasma glucose level

CONCLUSION

The 4th position of derivatives substituted with F and NO2, which was shown better result compare to unsubstituted or substituted with other derivatives. Subsequently, *in vivo* biological evaluation compound 5B (F), 5G (NO2)and 5I (F) containing electron withdrawing groups which give more % reduction of blood sugar level with compare to glibenclamide. Finally, it was concluded that results obtained from *in vitro* docking analysis and *in vivo* biological activity on rat are significantly same and the compound 5B, 5G and 5I can be used as lead molecule for further development of more potent sulphonylureas /guanidine based derivatives as oral hypoglycemic agents.

REFERENCES

[1] A Salah; AMostafa; A IhabTalat. *Bull Pharm Sci.* **2011**, 34, 149-158.
[2] G Mariappana; B Saha; D Sriparna; K Deepak; P Haldar. *J Chem Sci.* **2011**, 123 (3), 335-341.
[3] G Sridhar. *Curr Sci*. **2000**, 83, 791.
[4] II Panchal; DJ Sen; B Prajapati; SK Shah. *World J Pharm Sci.* **2013**, 1(4), 168-175.
[5] II Panchal; DJ Sen; SK Shah. *Int J Pharma Res Bio Sci.* **2014**, 3(2), 770-784.
[6] BV Edwin; AB John; BN Goli; FB Bryan; E Beth. *J Med Chem.* **2003**, 46 (13), 2774-2789.
[7] BB Lohray; V Bhushan; AS Reddy; PB Rao; NJ Reddy; P Harikishore; N Haritha. *J Med Chem*. **1999**, 42, 2569-2581.
[8] L Tatyana; FG Pavlovskaya; VV Yaremenko. V Lipson; V Svetlana; OV Shishkin; VI Musatov; SK Alexander. *Beilstein J Org Chem*. **2014**, 10, 117-126.
[9] S Ebtehal; Al-Abdullah; M Hanaa; Al Tuwaijri; M Hanan; A Monirah; Al Alshaikh. *Molecules.* **2015**, 20, 8125-8143.
[10] Ji Xun; Xi Chunmei; J Wang; S Mingbo; Lei Zhang; T Dong; Li Zeng. *Eur J Med Chem*. **2014**, 6, 242-256.
[11] P Ishan; P Bibhuranjan; M Kamal; CN Patel. *Der Pharma Chemica.* **2011**, 3(2), 383-391.
[12] Yi Huan; Li Yongqiang; L Zhang; Z Shen; Feng Z. *Chem Med Chem*. **2014**, 9(5), 922-927.
[13] Z Hui; Z Yaan; WG Zhong; Z Jinpei; H Wenlong; H Xiao. *Bioorg Med Chem Lett.* **2009**, 19, 1740-1744.
[14] LH Woo; KB Young; JB Ahn; KS Kwon; L Jung; SJ Soo. *Euro J Med Chem.* **2005**, 40, 862-874.
[15] M Hassan; A Khalid; M Abdullah. *J Flu Chem.* **2011**, 132, 870-877.
[16] A Ahmadi; M Khalili; K Khatamia; M Farsadrooha; B Nahri. *Mini Rev Med Chem.* **2014**, 14, 208-213.
[17] S Schneider; S Ueberberg; A Korobeynikov; W Schechinger; C Schwanstecher; M Schwanstecher; HH Klein; E Schirrmacher. *Regulatory Peptides*. **2007**, 139(1-3), 122-127.
[18] ST Alok; D Ravitas; KJ Arvind; PK Sudhir. *Sau Pharma J.* **2015**, 23, 475-482.
[19] GM Martin; C Yoshioka; EA Rex; JF Fay; Q Xie; MR Whorton; JZ Chen. *eLife*. **2017**, 1-21.
[20] P Whalley. *Plymouth Student Sci*. **2016**, 9(1), 252-296.
[21] M Stanely; N Kamalakkannan; P Venugopal. *J Ethnopharmacol*. **2004**, 91, 209-213.
[22] S Prakash; D Maji; S Samanta; R Sinha. *Med Chem.* **2014**, 4, 345-350.
[23] S Chattopadhyay; M Ramanathan; J Das; SK Bhattacharya. *Ind J Exper Bio.* **1997**, 35, 1141-1145.