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# Synthesis, Characterisation, Molecular Docking, Anti-microbial and Anti-diabetic Screening of Substituted 4-indolylphenyl-6-arylpyrimidine-2-imine Derivatives

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**ABSTRACT**

The purpose of the research is to synthesise a novel series of (E)-2-(4-(1H-indol-3-yl)-6-p-substituted phenylpyrimidin-2-yl)dimethylguanidine derivatives since 3-(1H-indol-3-yl)-1-p-substituted phenylprop-2-en-1-one and evaluate their molecular docking studies, antimicrobial, and anti-diabetic activities. Among all the synthesized compounds (**11a-g**), compound **11a** exhibits excellent CDOCKER energy (−11.36 kcal/mol). The entire compounds (**11a-g**) confirm very good antimicrobial activity towards the tested microorganisms. In the in vitro anti-diabetic studies, compounds **(11a, 11c, and 11g)** confirm higher alpha-amylase and alpha-glucosidase inhibition activity. In the in vivo anti-diabetic activities, the synthesized compounds **(11a-g)** (10 mg/kg, p.o.) investigated by the streptozotocin (60 mg/kg, ip) – nicotinamide (120 mg/kg, p.o.) – induced model in adult male albino Wistar rat and these derivatives show considerable fasting blood glucose level when compared to metformin hydrochloride a potent and well-known anti-diabetic drug as a reference.



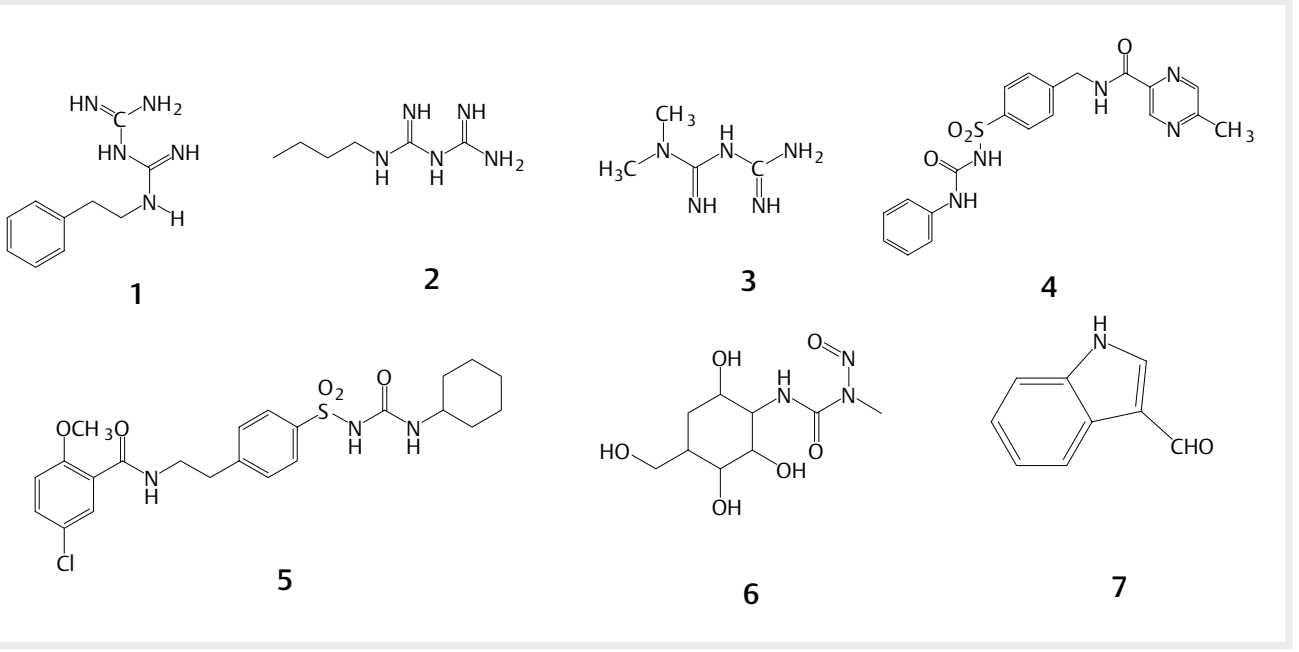
## Abbreviation

| | |
|---|---|
| DM | Diabetes Mellitus |
| MIC | Minimum Inhibitory Concentration |
| GK | Glucokinase |
| DMSO | Dimethylsulfoxide |
| STZ | Streptozotocin |
| p.o | per oram |
| i.p | Intraperitonial |
| NIDDM | Non –Insulin-Dependent Diabetes Mellitus |
| TMS | Tetramethylsilane |

## Introduction

Diabetes mellitus (DM) is a metabolic disorder [1] characterized by chronic hyperglycemia with disturbances in fat, protein, and carbohydrate metabolism, resulting from defects in insulin action, insulin secretion, or both [2]. Diabetes Mellitus is the major effect of final-stage renal disease, non-traumatic lower extremity amputation, adult blindness, neuropathy, kidney failure, strokes, and heart attack. The collective evidence has been used to divide diabetes into four types namely, insulin dependent diabetes mellitus (type 1, IDDM), non-insulin dependent diabetes mellitus (type 2, NIDDM), malnutrition-related diabetes and other types of diabetes [3]. In type 1 diabetes, there is destruction of beta-cells of the pancreas, with consequent insulin deficiency. The signs of IDDM are polydipsia, polyurea, and weight loss. In type-2 diabetes the pancreas produces insulin, but the body does not take the insulin correctly. This may be due to peripheral tissue insulin obstruction where insulin receptors or other intermediates in the insulin signalling pathways within body cells are insensitive to insulin and consequently, glucose does not readily enter the tissues leading to hyperglycemia or elevated blood glucose concentrations [4, 5].

Obesity is a common problem for this type, and most patients with NIDDM are obese [6] and will require multiple antihyperglycemic agents to maintain glycemic control [7]. Four types of oral anti-diabetic agents (▶ **Fig. 1**) are available namely, insulin secre-




▶ **Fig. 1** Chemical structure of some known anti-diabetic agents and some other compounds.

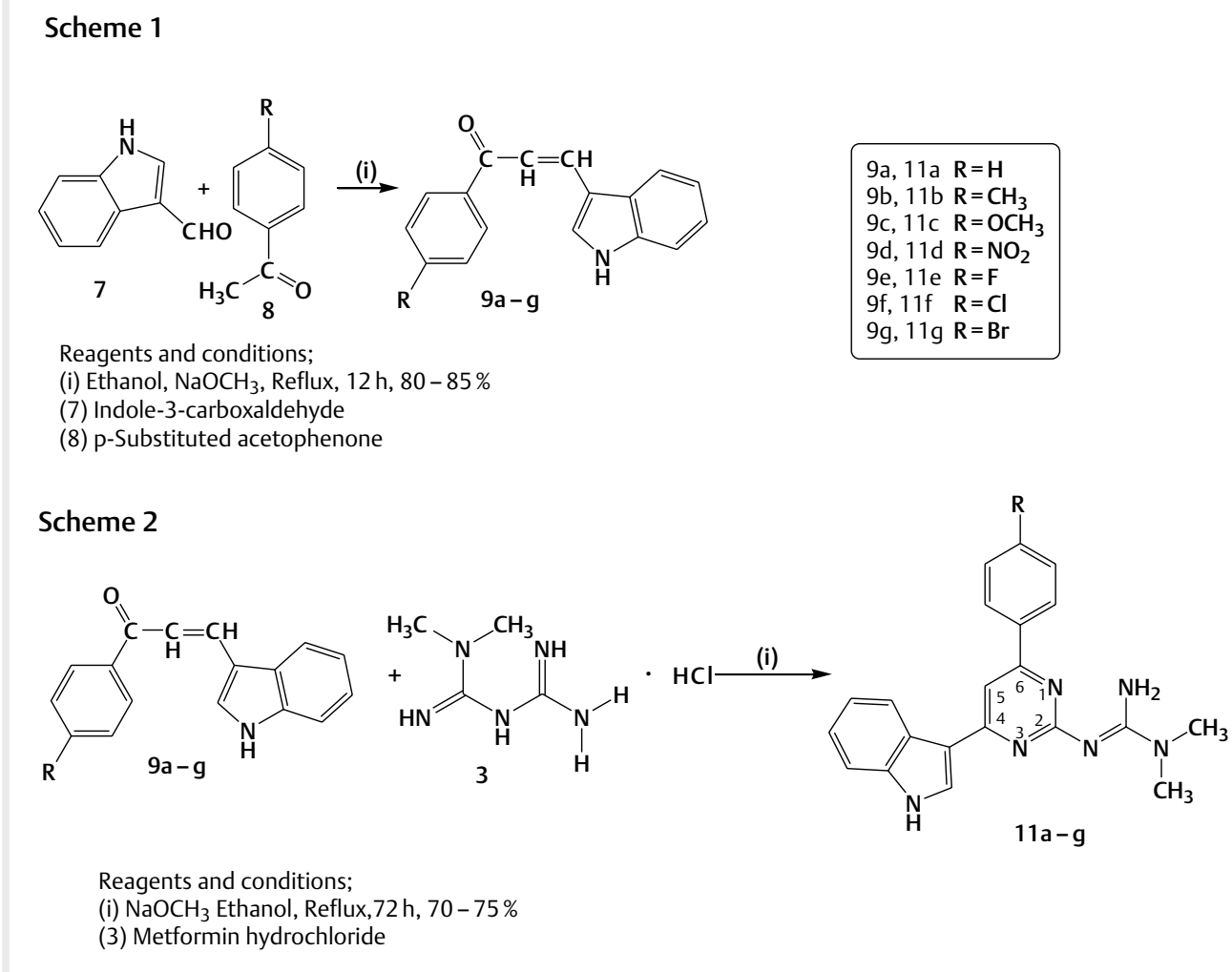
tagogues (sulfonylureas), biguanides (phenformin, buformin, and metformin), thiazolidinediones (pioglitazone) and alpha-glucosidase inhibitors. The main function of sulfonylureas (tolbutamide, glimepiride, and glipizide) is to stimulate pancreatic insulin secretion. Metformin originates from Galega officinalis, reduces the sign of diabetes. The active compound of metformin is gelatine, guanidine derivatives. Metformin is not only an inexpensive drug but also has several other beneficial pharmacological effects which include reduction and stabilization of weight [8], reduces the changes of hypoglycemia [9] and other beneficial vascular effects. Metformin is not metabolized; it's important sites of concentration are the intestinal mucosa and the salivary glands. The mode of action of metformin is not fully understood. It has been postulated that metformin might potentiate the impact over insulin then that would possibly decorate the impact about insulin in the peripheral receptor site. This accelerated sensitivity follows an increase in number of insulin receptors on cell surface membranes.

The hetero cyclic molecules, which possess indole [10] moieties, exhibit an extensive extent of biological activities [11]. Indole alkaloids prove to be medicinally essential natural compounds. From the above discussion, of considering the biological properties of the indole ring, it is planned to synthesize a novel series of 4-indolylphenyl-6-arylpyrimidine-2-imine derivatives having a side chain with different structures; such derivatives could possess very useful and important anti-diabetic activity.

## Result and Discussion

### Chemistry

The novel series of 4-indolylphenyl-6-arylpyrimidine-2-imines **(11a-g)** are synthesized according to the method shown in ▶ **Fig. 2 (Scheme 1 & Scheme 2)**. In the first scheme synthesis of various substituted 3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one (**9a-g**) derivatives are carried out by the condensation of indole-3-carboxaldehyde **7** by p-substituted acetophenones **8** and the products are purified by recrystallising ethyl alcohol. In the second scheme synthesis of 4-indolylphenyl-6-arylpyrimidine-2-imines **(11a-g)** is carried out by the reaction of substituted 3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one (**9a-g**) derivatives with metformin hydrochloride **10** and sodium methoxide in absolute ethanol and the products are purified by recrystallisation from ethyl alcohol as a suitable solvent. The structures of the synthesized products are established by FT-IR, $^{1}H$ & $^{13}C$ NMR, mass spectrometry, and elemental analysis. In FT-IR spectra, the disappearance of the C = O band in the characteristic range and appearance of the C = N band in the range of 1 543–1 606 $cm^{-1}$ are evidence for ring closure of the pyrimidine ring of compounds **(11a-g)**. The synthesized compounds (**11a-g**) also show the presence of -$NH_2$ band in the region of 3 162–3 463 $cm^{-1}$ and the compounds **(11a-g)** reveal characteristic bands at 3 107–3 426 $cm^{-1}$ (NH-indole), 2 342–2 957 $cm^{-1}$ (aromatic C-H), 2 106–2 853 $cm^{-1}$ (aliphatic C-H). The $^{1}H$ NMR spectra of compounds **(11a-g)** reveal the following signals: a singlet equivalent to one proton in the δ 12.17–10.76 ppm range characteristic of an indole -NH proton, a singlet equivalent to two protons at δ 8.75-8.41 ppm characteristic of -$NH_2$ protons, a multiplet at δ 8.58-6.44 ppm characteristic for the aromatic protons, a doublet at δ 8.21–8.65 ppm characteristic of the pyrimidine ring proton which confirmed the cyclization of the chalcone into the pyrimidine ring. The singlet between δ 3.82-2.50 ppm due to -$N(CH_3)_2$ protons, besides the presence of two singlets at δ 3.82 ppm and δ 2.49 ppm corresponding to the methyl protons of –$OCH_3$ and –$CH_3$ respectively. In the $^{13}C$ NMR spectra of compounds the chemical shift values of carbon atoms appear between δ 168.8-158.0 ppm due to pyrimidine ring ipso carbons, δ 158.0-157.4 ppm due to guanidine group ipso carbon, δ 137.6-111.3 ppm due to aromatic




▶ **Fig. 2** Synthetic scheme of 4-indolylphenyl-6-arylpyrimidine-2-imine derivatives **(11a-g).**

carbon atoms, δ 106.7-103.9 ppm due to the one proton attached carbon of the pyrimidine ring, δ 37.3-36.7 ppm due to guanidine group -$N(CH_3)_2$ carbon atoms and δ 27.3 ppm, δ 55.2 ppm due to phenyl ring attached –$CH_3$, -$OCH_3$ respectively. Moreover, the elemental analysis of compounds **(11a-g)** agrees with the proposed structure. The mass spectra of compounds **(11a-g)** are observed as characteristic molecular ion peaks corresponding to their molecular formula.

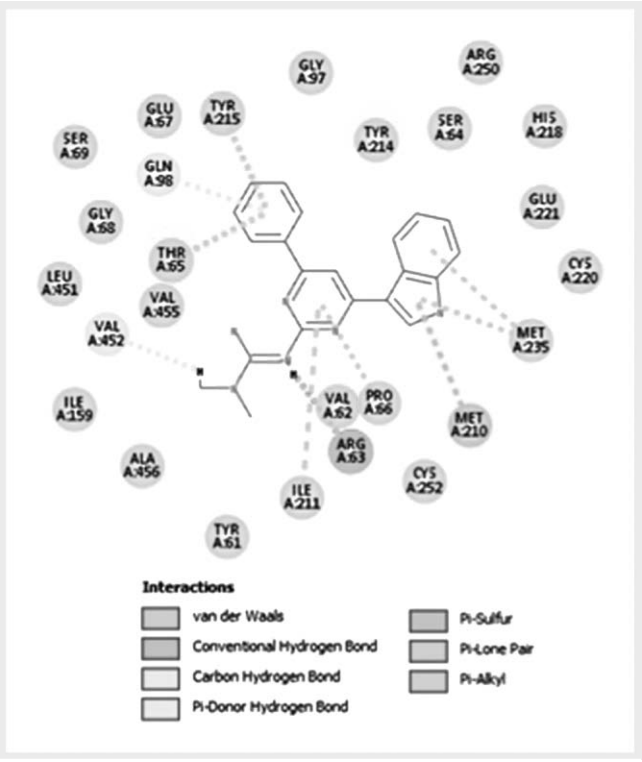
## Molecular docking studies

Molecular docking study conveys out over glucokinase **(1v4s)** as much an anti-diabetic protein and synthesized compounds **(11a-g)** are performed using a CDOCKER protocol over Discovery Studio (v 16.1.0.15350). The results are analyzed for docking interaction [12] of the human glucokinase **(1v4s)** with compounds **(11a-g)**. Glucokinase [13] is found only in pancreatic beta cells and liver performs a key role in the regulation of glucose metabolism. In the beta cells, glucokinase is believed to be in accordance with the remaining portion of the glucose absorbance and to be involved in the regulation of insulin release. The diabetic mice are treated along

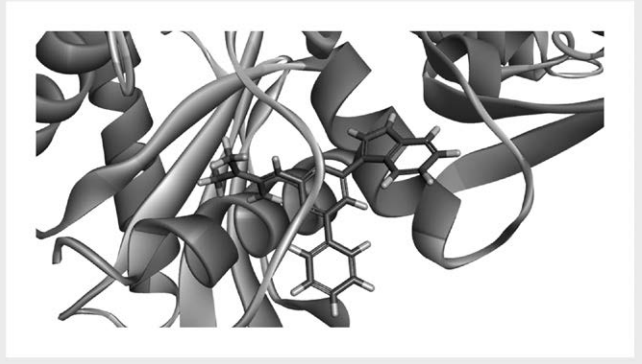
▶ **Table 1** Docking results of the designed compounds **(11a-g)** towards **1v4s.**

| S.NO | ENTRY | −CDOCKER ENERGY kcal/mol | −CDOCKER INTERACTION ENERGY kcal/mol |
|---|---|---|---|
| 1 | 11a | **11.36** | **44.70** |
| 2 | 11b | 8.77 | 42.08 |
| 3 | 11c | 4.87 | 41.17 |
| 4 | 11d | 4.84 | 39.75 |
| 5 | 11e | 5.43 | 36.47 |
| 6 | 11f | 5.93 | 42.53 |
| 7 | 11g | **9.13** | **41.16** |
| 8 | Metformin | 21.60 | 28.75 |

3-HMX active principle beyond plant expanded glucokinase activity [14]. From that report, the compounds (**11a-g**) increased glucokinase activity by binding with the metformin. Therefore, increasing the application of glucose is preferred to reduce blood glucose level. The ligands have been ranked after docking, based

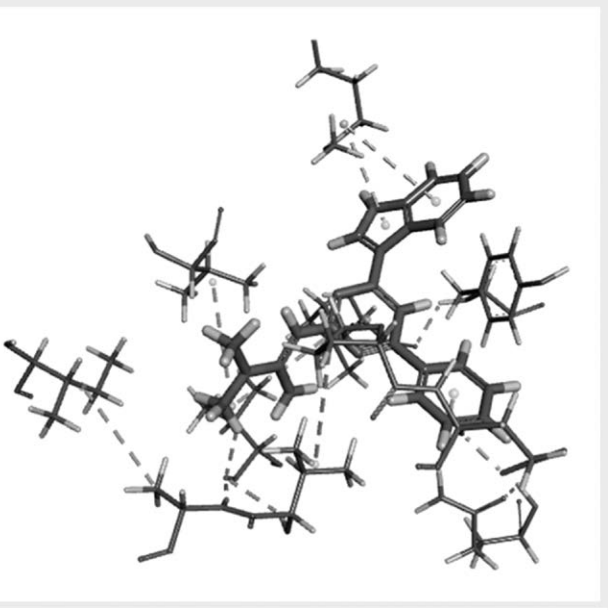



▶ **Fig. 3** Two-dimensional diagram of compound **11a** docked with **1v4s** protein.



▶ **Fig. 4** The structure of glucosidase (1v4s) protein in complex with compound **11a.**

on their binding and CDOCKER energy. The final docked results of selected ligands are given in the ▶ **Table 1**. From that result, the synthesized compound **11a** shows a good cdocker energy (−11.36 kcal/mol) and cdocker interaction energy (−44.70 kcal/mol). The cdocker energy value of compounds **11b** and **11g** are −8.77 kcal/mol and −9.13 kcal/mol respectively. A two-dimensional structure of the synthetic ligand (**11a**) is produced using discovery studio (v 16.1.0.15350) **(**▶ **Fig. 3)**. In the two-dimensional structure, the pale green dotted lines represent the Van der Waals interaction, dark green dotted lines represent the conventional hydrogen bonding, pale blue dotted lines represent the Carbon-Hydrogen bond and pink dotted lines represent the Pi-alkyl interaction. Docking of these optimized compounds against a glucokinase



▶ **Fig. 5** Docking interactions of glucokinase (1v4s) protein with compound **11a.**

**(1v4s)** structure of the active site residues is performed by discovery studio. The structure of **11a** shows interaction with the residues MET A: 235; MET A: 210; PRO A: 66; ARG A: 63; ILF A: 211; VAL A: 452; THR A: 65; GLN A: 98; TYR A: 215. The interaction of the best-docked structure **11a** is shown in the ▶ **Fig. 4, 5**. It is evident from this analysis that the best inhibitors are located in the center of the active site and is stabilized by H-bonding interactions. Out of seven docked complexes, only one best docking compound (**11a**) is got which shows lowest CDOCKER energy with the amino acid residues of the receptor molecule.

## In vitro antimicrobial activity

The review of literature [15] indicates that the metformin is an antihyperglycemic drug having antimicrobial activity, for diabetic patients it would be an additional advantage. It could produce more immunity in the body of the diabetic patient. Hence one should examine the synthesized compounds (**11a-g**) containing a metformin moiety which also been possesses the same microbial activity or not. Hence the antimicrobial activities of the synthesized compounds (**11a-g**) are reported. In vitro antimicrobial activity [16] was evaluated by measuring the minimum inhibitory concentration (MIC in µg/ml) and the diameter zone of inhibition (DZI in mm). ▶ **Table 2**, **3** illustrate the screening test of compounds (**11a-g**) on five fungal strains (Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis and Candida parapsilosis) and two Gram-positive (Staphylococcus aureus, Bacillus subtilis), three Gram-negative bacterial strains (Escherichia coli, Vibrio cholerae and Proteus vulgaries). Compounds **11a, 11c, 11e, 11f,** and **11g** exhibits significantly high antibacterial activity at a diameter of inhibition zones (>21 mm) and minimum inhibition concentration (<6.25) against the bacterial strains as compared to standard drug Ciprofloxacin. Compounds **11b** and **11c** are exhibit moderate antibacterial activity at MIC (12.5 µg/mL) and DIZ (<20 mm) towards Vi-

▶ **Table 2** In vitro anti-microbial activity of compounds (**11a-g**) by MIC method.

| Compounds | Minimum Inhibitory Concentration (MIC) in µg/mL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Antibacterial activity | | | | | Antifungal activity | | | | |
| | Gram ( + ve) | | Gram( − ve) | | | | | | | |
| | Bacillus subtilis | Staphylococcus aureus | Vibrio cholerae | Escherichia coli | Proteus vulgaries | Candida albicans | Candida glabrata | Candidakrusei | Candida tropicalis | Candida parapsilosis |
| 11a | **6.25** | **6.25** | **3.125** | 12.5 | 12.5 | **3.125** | **6.25** | 25 | **12.5** | **3.125** |
| 11b | 25 | 12.5 | 50 | 12.5 | 50 | 25 | 12.5 | **6.25** | 100 | 12.5 |
| 11c | 50 | 50 | 12.5 | **6.25** | 25 | 100 | 25 | **12.5** | **12.5** | 50 |
| 11d | 25 | 50 | 25 | 25 | 50 | 50 | 25 | 100 | 50 | 50 |
| 11e | **6.25** | **3.125** | 50 | 12.5 | **6.25** | **6.25** | 12.5 | **12.5** | 100 | 50 |
| 11f | 25 | 25 | **6.25** | **6.25** | 25 | **6.25** | **3.125** | 25 | **12.5** | 25 |
| 11g | 25 | **3.125** | **6.25** | 12.5 | **3.125** | 25 | 12.5 | **6.25** | 25 | **3.125** |
| Ciprofloxacin | 6.25 | 3.125 | 3.125 | 6.25 | 3.125 | – | – | – | – | – |
| Amphotericin B | – | – | – | – | – | 6.25 | 6.25 | 12.5 | 12.5 | 6.25 |

▶ **Table 3** In vitro anti-microbial activity of compounds (**11a-g**) by disc diffusion method.

| Compounds | Disc Diffusion Method Zone of inhibition (in mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Antibacterial activity | | | | | Antifungal activity | | | | |
| | Gram ( + ve) | | Gram( − ve) | | | | | | | |
| | Bacillus subtilis | Staphylococcus aureus | Vibrio cholerae | Escherichia coli | Proteus vulgaries | Candida albicans | Candida glabrata | Candida krusei | Candida tropicalis | Candida parapsilosis |
| 11a | **23** | **23** | 17 | **22** | **22** | **16** | 10 | **15** | **14** | **16** |
| 11b | 14 | **20** | **19** | 15 | 11 | 12 | 11 | 11 | 11 | 9 |
| 11c | 11 | **19** | **20** | **18** | 9 | 8 | 9 | 12 | 14 | 10 |
| 11d | 15 | 12 | 16 | 13 | 11 | 7 | 9 | 10 | 10 | 12 |
| 11e | **21** | 19 | 19 | **23** | **21** | **15** | 10 | **18** | 13 | 11 |
| 11f | 17 | **23** | **22** | **22** | **20** | 12 | **17** | 9 | 10 | **15** |
| 11g | **21** | **23** | **23** | **24** | 18 | 13 | **15** | **17** | **16** | 12 |
| Ciprofloxacin | 22 | 23 | 21 | 23 | 21 | – | – | – | – | – |
| Amphotericin B | – | – | – | – | – | 16 | 14 | 15 | 15 | 15 |

▶ **Table 4** In vitro anti-diabetic activity of compounds (**11a-g**).

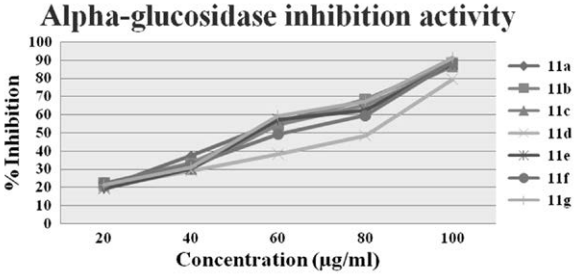
| Compounds | α-glucosidase inhibitory activity Concentrations (µg/ml) | | | | | $IC_{50}$ (µg/ml) | α-amylase inhibitory activity Concentrations (µg/ml) | | | | | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 | | 20 | 40 | 60 | 80 | 100 | |
| 11a | 20.01 | 37.21 | 56.21 | 68.32 | 89.21 | **55.98** | 30.33 | 56.34 | 63.32 | 78.31 | 83.23 | **49.50** |
| 11b | 22.12 | 33.33 | 54.43 | 68.32 | 87.65 | 56.94 | 31.22 | 54.32 | 67.43 | 71.21 | 92.22 | 51.02 |
| 11c | 21.23 | 30.23 | 54.32 | 65.32 | 86.67 | 58.25 | 31.21 | 55.45 | 65.66 | 74.44 | 93.43 | 50.09 |
| 11d | 20.32 | 29.34 | 38.43 | 48.44 | 79.43 | 66.01 | 30.21 | 56.32 | 61.98 | 75.33 | 90.21 | 53.53 |
| 11e | 19.33 | 30.32 | 57.32 | 62.27 | 88.12 | 57.46 | 32.56 | 50.59 | 67.77 | 77.56 | 91.56 | **49.94** |
| 11f | 22.21 | 32.13 | 49.21 | 59.54 | 89.02 | 60.73 | 32.45 | 58.32 | 60.33 | 74.78 | 90.78 | 52.61 |
| 11g | 21.32 | 31.21 | 59.32 | 67.43 | 91.21 | **56.27** | 31.76 | 52.58 | 68.56 | 75.44 | 91.12 | **49.90** |

Values are expressed as mean ± SD (n = 3)

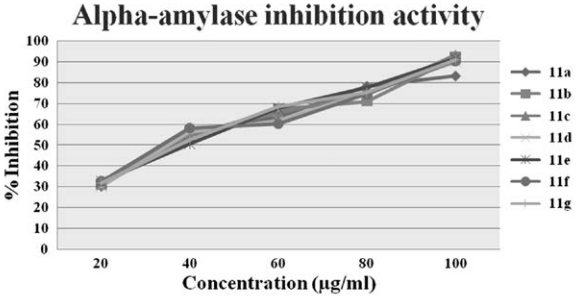
brio cholerae, Staphylococcus aureus, and Escherichia coli. Similarly compounds **11a, 11e** and **11g** exhibit better antifungal activity against Candida glabrata, Candida krusei, Candida tropicalis and Candida parapsilosis at MIC ( < 12.5 µg/mL) and DIZ ( > 15 mm) as compared to the standard drug Amphotericin B. The screening consequences revealed as the newly designed compounds (**11a-g**) containing halogen groups have shown significant antibacterial [17] and antifungal activity compared to corresponding standard drugs. It reveals that the metformin residue and the indole site attached to the compounds may increase the antimicrobial activities [18–23] of the entire compounds (**11a-g**).

## In vitro enzyme inhibition activity assay

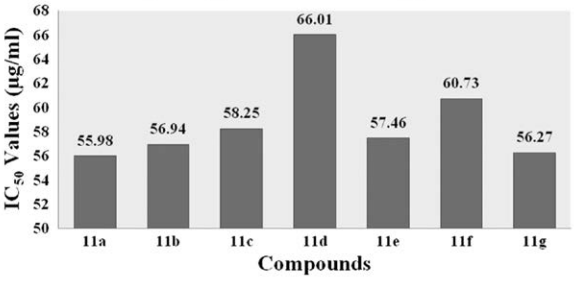
The novel series of 4-indolylphenyl-6-arylpyrimidine-2-imines (**11a-g**) hold definite promises in the remedy regarding diabetes mellitus (DM). Two carbohydrates hydrolysing enzymes [24] (alpha-amylase and alpha-glucosidase) are responsible for postprandial hyperglycemia. Alpha-glucosidase catalyzes the disaccharides to a monosaccharide, leading to postprandial hyperglycemia and alpha-amylase begins the process of carbohydrate metabolism by hydrolysis of 1, 4-glycosidic linkage of polysaccharides (starch, glycogen) to disaccharides [25, 26]. Hence, inhibitors of alpha-amylase [27] and alpha-glucosidase are beneficial within the control of hyperglycemia as they delay carbohydrate metabolism, which consequently reduces the postprandial blood glucose level. In the current study, compounds **(11a-g)** have been evaluated because of their inhibitory effect on alpha-amylase and alpha-glucosidase [28] enzymes by in vitro method. The inhibitory activity of the designed compounds against alpha-amylase and alpha-glucosidase are proved within ▶ **Table 4,** ▶ **Fig. 6, 7**. Compounds of different concentrations are used to assess their inhibitory potential against alpha-glucosidase. Hence the compounds **11a** and **11g** show alpha-glucosidase inhibitory activity with an $IC_{50}$ value of 55.98 µg/ml and 56.27 µg/ml respectively (▶ **Fig. 8**). Among the seven synthesized compounds, compound **11a, 11e,** and **11g** exhibit the highest inhibition of alpha-amylase with an $IC_{50}$ value of 49.50 µg/ml, 49.94 µg/ml, and 49.90 µg/ml respectively (▶ **Fig. 9**). Overall, our result suggests that compounds of 4-indolylphenyl-6-arylpyrimidine-2-imines (**11a-g**) show maximum potent as natural alpha-amylase and alpha-glucosidase inhibitors decrease the metabolism of dietary starch.



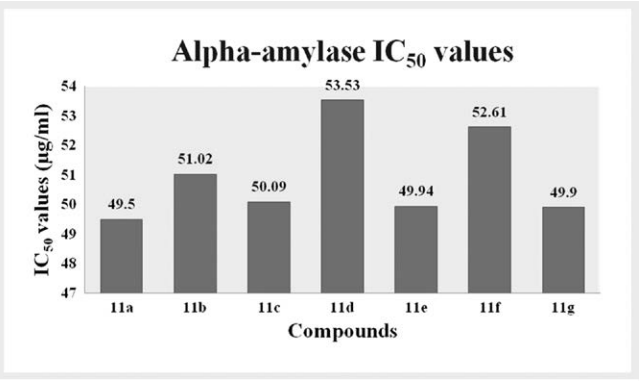

▶ **Fig. 6** Alpha-glucosidase inhibition activity of compounds (**11a-g**).




▶ **Fig. 7** Alpha-amylase inhibition activity of compounds (**11a-g**).




▶ **Fig. 8** Alpha-glucosidase $IC_{50}$ values of compounds (**11a-g**).




▶ **Fig. 9** Alpha-amylase $IC_{50}$ values of compounds (**11a-g**).

## In vivo anti-diabetic activity

Administration over streptozotocin [29] causes diabetes by the rapid destroy of pancreatic beta cells, thereby brings about a reduction concerning insulin release and induction of insulin resistance, both of which are associated with type 2 diabetes[30]. In the present study, an increase in blood glucose level among diabetic animals confirms the installation of diabetes mellitus. The anti-diabetic effect about more than a few doses of compounds **(11a-g)** in normal (Group 1) and streptozotocin (60 mg/kg, ip) -nicotinamide (120 mg/kg p.o.) -induced (Group 2) diabetic rats are assessed at different time periods. The oral administration of a single dose of designing compounds brought about a massive reduction of serum glucose in diabetic rats.

### Blood glucose level analysis

The anti-diabetic recruited on synthesized compounds **(11a-g)** is investigated in streptozotocin (60 mg/kg, ip) - nicotinamide (120 mg/kg, p.o.) - induced diabetic adult male albino Wistar rats [31]. The change of blood glucose level from the initial to 28th day fasting glycemia is shown in the ▶**Table 5**. ▶**Fig. 10** indicates the change in blood glucose level in control and experimental normal rats received (**11a-g)** (10 mg/kg, p.o.) and standard drug metformin (10 mg/kg, p.o.) [32]. The highest blood glucose level is noted on the 28th day of the test for the experimental positive and negative control rats. Hence, the compound (**11a**, 10 mg/kg, p.o. 152.23 mg/dl) and the positive control (metformin, 10 mg/kg, p.o. 154.23 mg/dl) retained [33] the blood glucose level to the fasting glycemia after the 15th day. However for compounds **11b, 11c,** and **11d** the blood glucose level on the final day is 182.5 ± 11 mg/dl, 180.232 ± 12 mg/dl, and 181.32 ± 12 mg/dl respectively are less potent and take a longer period during the experiments. The moderate blood glucose levels are observed in the compounds **11e** (170.21 mg/dl), **11f** (167.45 mg/dl) and **11g** (173.44 mg/dl) on the last day of experiments. The compounds **(11a-g)** steadily exert an anti-diabetic effect at the tested doses when compared to the negative control (diabetes mice). The improved diabetic control between oral glucose tolerance tests with the aid of the 4-indolylphenyl-6-arylpyrimidine-2-imine derivatives suggests that the compounds lower the blood glucose levels also in normal rats. The effect of decreasing blood glucose level in normal healthier rats is due to the increased efficiency of the peripheral tissues for the uptake of glucose from the blood.

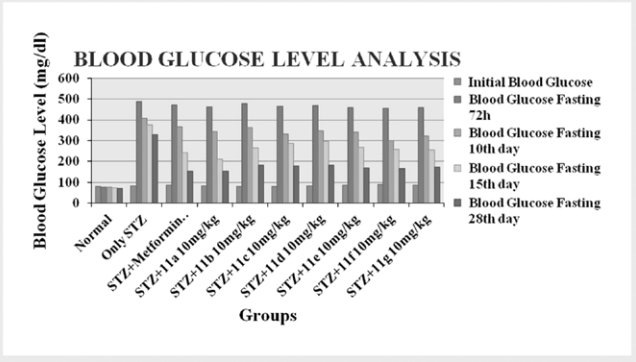
▲ **Table 5** Effect of compounds (**11a-g**) on blood glucose level in diabetic rats.

| Groups | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Initial Blood Sugar | Blood Sugar Fasting 72h | Blood Sugar Fasting 10th day | Blood Sugar Fasting 15th day | Blood Sugar Fasting 28th day |
| Normal | 79.2112 ± 2.31245 | 76.5521 ± 4.213 | 75.21 ± 2.132 | 73.723 ± 4.2718 | 70.2738 ± 1.317 |
| Only STZ | 83 ± 2.2901 | 487.2039 ± 40.231*** | 410.212 ± 23.123*** | 377.290 ± 12.328*** | 330.219 ± 21.29938*** |
| STZ + Metformin 10mg/kg | 87.112 ± 32.12 | 472.2839 ± 42.321*** | 368.321 ± 21.234** | 241.21 ± 13.238*** | 154.23 ± 13.213*** |
| STZ + **11a** 10mg/kg | 82.34 ± 23.321*** | 464 ± 38.098** | 344.321 ± 43.212*** | 212.21 ± 32.345 | 152.34 ± 12.342*** |
| STZ + **11b** 10mg/kg | 80 ± 3.87298 | 478.33 ± 54.185*** | 365 ± 38.449*** | 266.66 ± 26.7914*** | 182.5 ± 11.529*** |
| STZ + **11c** 10mg/kg | 81.231 ± 32.342*** | 465.435 ± 21.390 | 332.231 ± 43.22*** | 287.32 ± 23.454*** | 180.232 ± 12.324** |
| STZ + **11d** 10mg/kg | 84.343 ± 24.33** | 470.32 ± 32.435*** | 349.897 ± 35.655 | 298.321 ± 30.435*** | 181.323 ± 12.980 |
| STZ + **11e** 10mg/kg | 85.434 ± 32.43 | 460.434 ± 34.121*** | 340.231 ± 26.435 | 269.987 ± 43.232*** | 170.213 ± 31.234*** |
| STZ + **11f** 10mg/kg | 88.453 ± 23.434 | 455.342 ± 33.78*** | 300.342 ± 40.432*** | 260.564 ± 24.876*** | 167.453 ± 28.675*** |
| STZ + **11g** 10mg/kg | 86.564 ± 27.7654 | 459.545 ± 44.342*** | 321.543 ± 36.432*** | 255.654 ± 37.654 | 173.44 ± 36.876 |

All blood glucose levels were recorded in mg/dl. Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dennett's *** P<0.001, ** P<0.01, * P<0.05 calculated by comparing treated group with control group

### Body weight analysis

After Streptozotocin (60 mg/kg, ip) - nicotinamide (120 mg/kg, p.o.) administration, the body weight of rats [34, 35] is observed after compounds (**11a-g**) (10 mg/kg. p.o.) and in standard drug metformin (10 mg/kg, p.o.) administration when compared to diabetic control rats. Normal body weight gain is an indication of efficient glucose homeostasis; but among hyperglycemic, glucose is not available; consequently the cells make use of alternative proteins for energy; Due to excessive breakdown of tissue protein, a loss in body weight occurs. Results on the effect of compounds (**11a-g**) on body weight regarding streptozotocin (60 mg/kg, ip) - nicotinamide (120 mg/kg, p.o.) induce diabetic among the animals after 4 weeks of treatment are summarized in ▶ **Table 6**. At the end of 4th week, diabetic rats gain less body weight with significant decrease in the normal healthy control animals. The novel series of 4-indolylphenyl-6-arylpyrimidine-2-imines (**11a-g**) improve the body weight of diabetic rats with a significant increase compared to the diabetic control rats (▶ **Fig. 11**). This investigation thus indicates that the compounds **11a** (153.89g) and **11g** (155.76g) significantly increase the body weight [36, 37] of nicotinamide (120 mg/kg, p.o.) – streptozotocin (60 mg/kg, ip) - induced diabetic rats almost near to the normal healthier rats (154.12g) compared to all other synthesized compounds.




▶ **Fig. 10** Blood glucose level on compounds (**11a-g**) in diabetic rats

## Conclusions

In conclusion, the docking studies of the seven ligands (**11a-g**) with target protein **1v4s** show that compound **11a** and **11g** are good molecules which docks well with target correlated to diabetes mellitus. Based on a biological evaluation resulting data, the entire compounds (**11a-g**) show good results to moderate antimicrobial activity against tested microorganism by both MIC and disc diffusion method. The compounds **(11a-g)** exhibit significant in vitro anti-diabetic activity using alpha-amylase and alpha-glucosidase enzymes as well as in vivo anti-diabetic activity using streptozotocin-nicotinamide-induced animal model. This study reveals that compounds (**11a** and **11g**) an in vivo anti-diabetic examination, bringing back the blood glucose and body weight normal in diabetic rats. With the results, it is observed that the presence of indole ring and the halogen (Br) substitution of an aryl ring attached to the pyrimidine system enhances the anti-diabetic activity. Thus the synthesised compounds (**11a-g**) can be considered for developing a potent anti-diabetic drug.

## Experimental

### General

All the melting points are uncorrected and determined by an open capillary tube. IR absorption spectra are recorded within the 4000-400 $cm^{-1}$ range of an Agilent Cary 650 FT-IR spectrometer using KBr pellets. $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectra are recorded within DMSO-$d_6$ with a Bruker Avance III spectrometer using TMS as an internal standard. The splitting patterns are precise as follows; s-singlet, d-doublet, t-triplet, m-multiplet. Mass spectra have been recorded on an API 3000 series mass spectrometer. Elemental analysis has been performed on a Vario Micro V2.2.0 C H N analyzer.

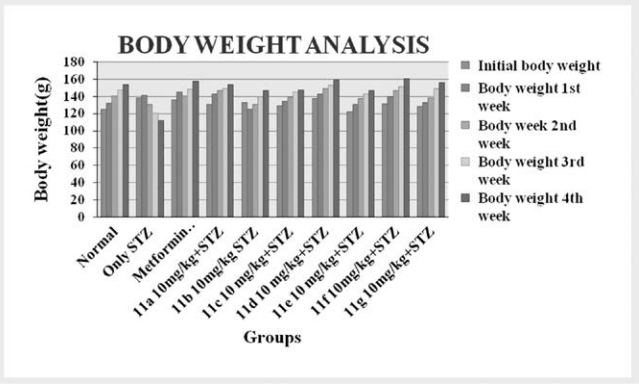
### General procedure for synthesis of 4-indolylphenyl-6-arylpyrimidine-2-imine derivatives (11a-g)

A solution of substituted 3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one (0.01 mole) and metformin hydrochloride (0.01 mole) within

▶ **Table 6** Effect of compounds (**11a-g**) on body weight in diabetic rats.

| Groups | Body Weight (g) | | | | |
|---|---|---|---|---|---|
| | Initial Body Weight | Body Weight 1st week | Body Weight 2nd week | Body Weight 3rd week | Body Weight 4th week |
| Normal | 125.322 ± 1.341 | 132.21 ± 1.823 | 140.23 ± 1.244 | 147.22 ± 1.321 | 154.12 ± 2.13 |
| Only STZ | 138.21 ± 1.09 | 141.21 ± 0.251 * * | 130.32 ± 0.983 * * * | 120.21 ± 1.212 * * | 112.32 ± 1.312 * |
| STZ + Metformin 10 mg/kg | 136.29 ± 2.0113 * * * | 145.32 ± 3.21 * * | 140.32 ± 0.213 * * * | 148.213 ± 3.564 * | 157.67 ± 1.231 |
| STZ + **11a** 10 mg/kg | 130.34 ± 2.312 * | 142.87 ± 1.002 * * | 146.85 ± 1.221 * * * | 149.21 ± 2.11 | 153.89 ± 0.213 * * |
| STZ + **11b** 10 mg/kg | 133 ± 1.095 * * * | 125.33 ± 1.520 * | 130.5 ± 1.147 * * | 140.16 ± 0.980 | 146.83 ± 1.077 * * |
| STZ + **11c** 10 mg/kg | 129.23 ± 1.323 | 134.21 ± 0.321 * | 139.21 ± 3.22 | 144.85 ± 1.322 * * * | 147.21 ± 1.22 * * |
| STZ + **11d** 10 mg/kg | 137.32 ± 1.22 * * | 143.22 ± 1.22 * | 149.23 ± 1.22 * * | 153.23 ± 2.11 * * * | 159.32 ± 1.44 * * * |
| STZ + **11e** 10 mg/kg | 122.32 ± 1.42 | 130.44 ± 0.34 * * * | 137.21 ± 1.22 * * * | 143.21 ± 1.33 * | 146.54 ± 0.88 |
| STZ + **11f** 10 mg/kg | 131.43 ± 1.99 * * | 139.54 ± 1.07 * * | 146.54 ± 1.77 * * | 151.66 ± 1.66 * | 160.34 ± 2.08 * * |
| STZ + **11g** 10 mg/kg | 127.86 ± 0.88 * * * | 132.70 ± 0.44 * * * | 138.55 ± 1.54 | 148.99 ± 0.77 | 155.76 ± 0.33 * * |

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dennett's * * * P < 0.001, * * P < 0.01, * P < 0.05 calculated by comparing treated group with control group




▶ **Fig. 11** Body weight analysis of compounds (**11a-g**) in diabetic rats

25 ml ethyl alcohol is added 0.5 g of anhydrous sodium methoxide. The reaction mixture is refluxed, thin layer chromatography (TLC) confirmed the reaction is completed after 72 h at a solvent ratio of pet-ether: ethyl acetate (2:1) ratio. After finishing about the reaction, the reaction mixture is poured into beaten ice; precipitate filtered, washed with water until the basic catalyst is removed and recrystallized from ethanol to give 4-indolylphenyl-6-arylpyrimidine-2-imine derivatives **(11a-g)** obtained in moderate yields (70–75 %).

### (E)-2-(4-(1H-indol-3-yl)-6-phenylpyrimidin-2-yl)dimethylguanidine (**11a**)

Yield: 0.79 g (75 %); Mp.: 96–98 °C; Color: pale yellow; FT-IR (KBr, $cm^{-1}$); 3316(-$NH_2$), 3201(-NH -indole), 2342(Ar, -CH str), 2106(Al, -CH str), 1594(-C = N str), 1493(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 12.17(s, 1H), 8.64(s, 2H), 8.54(d, 1H), 8.53-7.22 (m, 8H), 3.18(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 163.8, 163.3, 162.9, 157.4, 137.1, 136.6, 130.8, 128.8, 126.9, 125.1, 122.3, 121.9, 120.9, 113.3, 112.2, 105.6, 37.3. MS (m/z): 357(m + H). Elemental analysis for $C_{21}H_{20}N_6$ (%): calculated: C, 70.79; H, 5.62; N, 23.19; found: C, 70.83; H, 5.67; N, 23.21.

### (E)-2-(4-(1H-indol-3-yl)-6-p-tolylpyrimidin-2-yl)dimethylguanidine (**11b**)

Yield: 0.64 g (71 %); Mp.: 72–74 °C; Color: pale brown; FT-IR (KBr, $cm^{-1}$); 3414(-$NH_2$), 3154(-NH -indole), 2850(Ar, -CH str), 2316(Al, -CH str), 1606(-C = N str), 1571(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 11.77(s, 1H), 8.43(s, 2H), 8.41(d, 1H), 8.40-7.18(m, 7H), 3.08(s, 3H), 2.49(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 164.2, 163.8, 163.1, 157.8, 137.6, 135.3, 131.5, 129.2, 129.2, 128.2, 126.6, 124.3, 122.5, 121.2, 114.6, 112.9, 104.9, 36.9, 27.3. MS (m/z): 371(m + H). Elemental analysis for $C_{22}H_{22}N_6$ (%): calculated: C, 71.35; H, 5.94; N, 22.70; found: C, 71.40; H, 5.98; N, 22.72.

### (E)-2-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)dimethylguanidine (**11c**)

Yield: 0.64 g (73 %); Mp: 74–76 °C; Color: pale yellow; FT-IR (KBr, $cm^{-1}$); 3380(-$NH_2$), 3211(-NH -indole), 2928(Ar, -CH str), 2314(Al, -CH str), 1571(-C = N str), 1512(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 11.95(s, 1H), 8.75(s, 2H), 8.65(d, 1H), 8.58-6.44(m, 7H), 3.82 (s, 3H), 2.50(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 163.6, 163.1, 162.4, 157.9, 137.0, 132.3, 130.3, 128.2, 127.7, 125.13, 122.6, 120.4, 118.7, 114.1, 113.8, 111.3, 105.0, 55.2, 36.8. MS (m/z): 387(m + H). Elemental analysis for $C_{22}H_{22}N_6O$ (%): calculated: C, 68.39; H, 5.70; N, 21.76; found: C, 68.42; H, 5.74; N, 21.80.

### (E)-2-(4-(1H-indol-3-yl)-6-(4-nitrophenyl)pyrimidin-2-yl) dimethylguanidine (**11d**)

Yield: 0.79 g (75 %); Mp.: 96–100 °C; Color: pale yellow; FT-IR (KBr, $cm^{-1}$); 3162(-$NH_2$), 3107(-NH -indole), 2918(Ar, -CH str), 2343(Al, -CH str), 1543(-C = N str), 1516(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 11.63(s, 1H), 8.54(s, 2H), 8.52(s, 1H), 8.46-6.56(m, 7H), 3.10(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 168.8, 166.6, 165.4, 157.8, 137.1, 136.9, 129.2, 128.9, 127.6, 125.1, 123.0, 122.0, 121.3, 120.8, 114.2, 112.1, 103.9, 36.9. MS (m/z): 402(m + H). Elemental analysis for $C_{21}H_{19}N_7O_2$ (%): calculated: C, 62.84; H, 4.74; N, 24.44; found: C, 62.88; H, 4.77; N, 24.47.

### (E)-2-(4-(4-fluorophenyl)-6-(1H-indol-3-yl)pyrimidin-2-yl) dimethylguanidine (**11e**)

Yield: 0.78 g (72 %); Mp.: 74–76 °C; Color: pale brown; FT-IR (KBr, $cm^{-1}$); 3311(-$NH_2$), 3053(-NH -indole), 2915(Ar, -CH str), 2847(Al, -CH str), 1594(-C = N str), 1452(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 10.76(s, 1H), 8.46(s, 2H), 8.45(d, 1H), 8.40-6.99(m, 7H), 3.82(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 166.1, 165.8, 164.8, 157.9, 137.1, 136.2, 133.8, 130.8, 128.2, 126.1, 123.7, 122.1, 120.5, 117.1, 112.0, 111.4, 106.6, 36.8. MS (m/z): 375(m + H). Elemental analysis for $C_{21}H_{19}N_6F$ (%): calculated: C, 67.38; H, 5.08; N, 22.46; found: C, 67.40; H, 5.12; N, 22.48.

### (E)-2-(4-(4-chlorophenyl)-6-(1H-indol-3-yl)pyrimidin-2-yl) dimethylguanidine (**11f**)

Yield: 0.80 g (75 %); Mp.: 68–70 °C; Color: pale yellow; FT-IR (KBr, $cm^{-1}$); 3449(-$NH_2$), 3426(-NH -indole), 2957(Ar, -CH str), 2853(Al, -CH str), 1550(-C = N str), 1491(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm) 11.82(s, 1H), 8.41(s, 2H), 8.21(d, 1H), 8.20-7.18(m, 7H), 3.08(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 161.8, 159.3, 158.0, 157.9, 136.2, 135.1, 129.8, 128.8, 128.7, 128.5, 126.2, 123.8, 121.1, 116.3, 114.5, 111.3, 106.7, 36.7. MS (m/z): 391(m + H). Elemental analysis for $C_{21}H_{19}N_6Cl$ (%): calculated: C, 64.61; H, 4.87; N, 21.54; found: C, 64.65; H, 4.92; N, 21.57.

### (E)-2-(4-(4-bromophenyl)-6-(1H-indol-3-yl)pyrimidin-2-yl) dimethylguanidine (**11g**)

Yield: 0.74 g (74 %); Mp.: 78–80 °C; Color: pale brown; FT-IR (KBr, $cm^{-1}$); 3463(-$NH_2$), 3299(-NH -indole), 2921(Ar, -CH str), 2313(Al, -CH str), 1548(-C = N str), 1486(Ar, -C = C); $^1$H NMR (400 MHz, $CDCl_3$): (δ, ppm), 10.77(s, 1H), 8.48(s, 2H), 8.46(d, 1H), 8.33-6.72(m, 7H), 3.02(s, 6H); $^{13}$C NMR(100 MHz, $CDCl_3$); (δ, ppm), 163.7, 162.4, 161.9, 158.0, 136.5, 135.7, 131.7, 130.2, 129.9, 128.8, 127.1, 125.1, 124.0, 122.1, 120.8, 111.3, 105.6, 36.8. MS (m/z): 437(m + H). Elemental analysis for $C_{21}H_{19}N_6Br$ (%): calculated: C, 57.80; H, 4.36; N, 19.27; found: C, 57.81; H, 4.38; N, 19.31.

## Molecular docking

Molecular docking study of target protein **1v4s** is carried out using a CDOCKER docking protocol of Discovery studio (CHARMm-based

DOCKER) is a molecular dynamics based docking algorithm. It uses the CHARMm family about the force field and offers full flexibility to ligand including dihedral, angles, and bonds. Docking helps to predict best binding compounds based on a variety of scoring functions. For the active site, ligands are docked to get the best interaction poses. The top poses along with CDOCKER energy and interaction energy are calculated. The best-docked poses are selected as the ones with the lowest CDOCKER energy the more negative the cdocker energy, the more favourable the binding.

### Protein structure preparation

The protein choosing for the present investigation is one of the most crucial aspects. **1v4s** proteins are selected as target receptor due to its significant role in human diabetes. The three-dimensional structure of **1v4s** is retrieved from the Protein Data Bank (PDB) (http://www.pdb.org/). The choice protein is prepared formerly in imitation of the docking studies by using correcting the chemistry of the missing hydrogens and the unfilled valence atoms. Thereafter, the protein is once subjected, according to energy minimization by making use of the CHARMm force field until the best gradient tolerance is obtained.

### Ligand preparation

A quantity of seven synthesized compounds is chosen in conformity with a challenge against the **1v4s** protein receptor molecule. These compounds have been drawn in Chem Draw and their corresponding 3D structures are generated on the Discovery Studio (v 16.1.0.15350). The ligand preparation consists of a series of steps so much operate conversions, request corrections after the structures, eliminate unwanted structures, and optimize the structures. Many of the steps are optional and are managed by selecting choices from the Ligand Preparation panel. The process of converting the structure format, pick out the structures, assemble hydrogen atoms, remove undesirable molecules, neutralize charged groups, grow ionization states, generate low energy ring conformations to get the output file.

## In vitro antimicrobial activity assays

### Disc diffusion method

The in vitro antimicrobial (antibacterial and antifungal) activities of synthesized seven compounds (**11a-g**) are screened by the filter paper disc diffusion method. The various bacterial species (Gram +ve and Gram −ve) and fungal species are first incubated at 48 °C for 24 h. The sterile filter paper discs (6 mm) have been impregnated along a variety of test compounds and standard antibacterial and antifungal agents Ciprofloxacin and Amphotericin B are dried at 48 °C. The discs have been positioned below the nutrient agar Petri plates previously seeded with a suspension of each bacterial and fungal strain. The diameters of zone of inhibition are measured at 35 °C after 24 h. The results are given in ▶ **Table 3**.

### Minimal inhibitory concentration method

The in vitro antifungal and antibacterial activity of all tested compounds are measured by means of the minimal inhibitory concentration (MIC) using the serial dilution method with 96-well Micro test plates. The lowest concentration at which there is no visible growth is taken as the MIC when compared with the growth of the control. The compounds under tests are dissolved in DMSO serially diluted in growth medium, inoculated and incubated at 37 °C. Growth MIC is determined at 48 h for the fungal and bacterial strains.

## Anti-diabetic activity assay

### In vitro anti-diabetic activity assay

**In vitro alpha-amylase inhibition assay** This assay is carried out using alpha-amylase (0.5 mg/mL) premixed along the sample at different concentrations (20–100 µg/ml) and 0.5 % starch as a starch solution is added to start the reaction. The process is carried out at 37 °C for 10 min. The reaction is continued by adding 2 mL of DNS (3, 5-dinitrosalicylic acid) reagent and further incubated in boiling water bath for 10 min and cooled to room temperature. The content of each test tube is diluted with 10 mL of distilled water in an ice bath and the absorbance is measured at 540 nm from the spectrometer. The alpha-amylase inhibitory activity is calculated as in eq. (1)

$$\%\text{Inhibition} = \left\{\frac{\left[\text{Abs}_{540}(\text{control}) - \text{Abs}_{540}(\text{test compound})\right]}{\text{Abs}_{540}(\text{control})}\right\}100 \quad (1)$$

The concentration of the test compounds to inhibit 50 % of alpha-amylase enzyme activity ($IC_{50}$) is determined graphically using Microsoft Excel 2007.

**In vitro alpha-glucosidase inhibition assay** The effect of the compound on alpha-glucosidase activity is determined by premixing alpha-glucosidase (0.07 Units) with the compound at various concentrations 20–100 µg/ml. The substrate solution, p-nitrophenyl glucopyranoside 3 mM is added. The reaction mixture is pre-incubated at 37 °C for 10 min and 2 mL of sodium carbonate is added to terminate the reaction. By measuring the release of p-nitrophenyl from p-nitrophenyl glucopyranoside at 400 nm, the activity of alpha-glucosidase is determined. The results (% Inhibition) are expressed as percentage of control as in eq. (2)

$$\%\,\text{Inhibition} = \left\{\frac{\left[\text{Abs}_{540}(\text{control}) - \text{Abs}_{540}(\text{test compound})\right]}{\text{Abs}_{540}(\text{control})}\right\}100 \quad (2)$$

The concentration of the test compounds to inhibit 50 % of alpha- glucosidase enzyme activity ($IC_{50}$) is determined graphically using Microsoft Excel 2007.

### In vivo anti-diabetic assay

**Experimental animals** Adult healthy albino male Wistar rats of aged 6 weeks and weighing between 150 to 200 g (Sri Venkateshwara Enterprises, Pvt. Ltd, Bangalore) with no prior drug treatment is used in the present investigation. The mice are housed within pure polypropylene cages and a well-ventilated temperature controlled along a constant 12 h dark and 12 h light schedule and fed on rodent pellets and water ad libitum. The experimental animals and procedures used in this investigation are approved by the ethical committee and in agreement with the recommendation for the proper use of laboratory animals.

**Ethical statement** All the experimental protocols and procedures used in this investigation have been approved by the Ethics Committee KMCH College of Pharmacy, Department of Pharmacology, Coimbatore (registration number KMCRET/PhD/01/2016-17).

**Induction of diabetes mellitus** The male albino Wistar rats are randomly divided into 10 groups of six animals each. Diabetes is induced by a single intraperitoneal injection of nicotinamide 120 mg/kg streptozotocin 60 mg/kg body weight. Nicotinamide is dissolved in normal saline and streptozotocin is dissolved in a 0.1 mol/L citrate buffer ($P_H$ 4.5) at a dosage of 35 mg/kg body weight. Diabetes is supported by the elevated level of blood sugar were measured at 72 h.

**Experimental design**

**Group 1:** Animals receive only normal saline and serve as a control group.

**Group 2**: Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin 60 mg/kg/b.w (ip) serve as diabetic control group.

**Group 3:** Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with Metformin 10 mg/kg. (po) serve as standard group.

**Group 4**: Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11a** 10 mg/kg. (po).

**Group 5:** Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11b** 10 mg/kg. (po).

**Group 6**: Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11c** 10 mg/kg. (po).

**Group 7:** Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11d** 10 mg/kg. (po).

**Group 8:** Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11e** 10 mg/kg. (po).

**Group 9:** Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11f** 10 mg/kg. (po).

**Group 10**: Animals receive Nicotinamide 120 mg/kg (po) and Streptozotocin (60 mg/kg) with compound **11g** 10 mg/kg. (po).

This treatment was continued for 28 days. During the period of study, test compounds and metformin were freshly dispersed in regular saline and distilled water before to the administration. The fasting animal serum sugar level was estimated on 1$^{st}$, 10$^{th}$, 15$^{th}$ and the 28$^{th}$ day from the tip of rat tail vein. The animals with blood glucose concentration more than 250 mg/dl will be used for the study. Body weight also determined and recorded while studying period.

**Statistical analysis** The $IC_{50}$ values are determined from the plots of percentage inhibition versus log inhibitor concentration and have been calculated by non-linear regression analysis from the mean inhibitory values. Tests have been carried out in triplicates. All the tests of fasting blood glucose and body weight estimations are carried out in triplicates. The values are expressed as the Mean ± SD (n = 3) analyzed by one-way analysis of variance (ANOVA) and post hoc Dunnett's-test. Differences between groups were considered to be significant if $p < 0.05$.

## Conflict of interest

The authors declare that there is no conflict of interests.

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