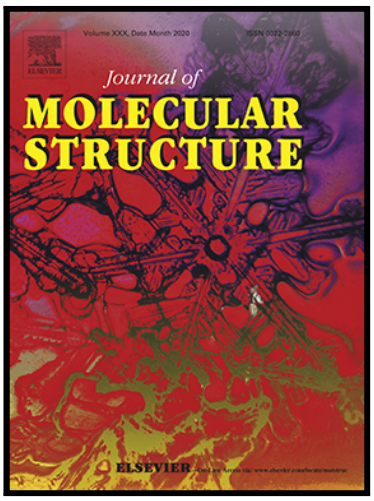


Hetero-substituted sulfonamido-benzamide hybrids as Glucokinase activators: Design, Synthesis, Molecular docking and in-silico ADME evaluation

Saurabh C. Khadse , Nikhil D. Amnerkar , Krushna S. Dighole , Ashish M. Dhote , Vikas R. Patil , Deepak K. Lokwani , Vinod G. Ugale , Nitin B. Charbe , Vivekanand A. Chatpalliwar

PII: S0022-2860(20)31241-2
DOI: https://doi.org/10.1016/j.molstruc.2020.128916
Reference: MOLSTR 128916

To appear in: *Journal of Molecular Structure*

Received date: 16 April 2020
Revised date: 6 July 2020
Accepted date: 15 July 2020

Please cite this article as: Saurabh C. Khadse , Nikhil D. Amnerkar , Krushna S. Dighole , Ashish M. Dhote , Vikas R. Patil , Deepak K. Lokwani , Vinod G. Ugale , Nitin B. Charbe , Vivekanand A. Chatpalliwar , Hetero-substituted sulfonamido-benzamide hybrids as Glucokinase activators: Design, Synthesis, Molecular docking and in-silico ADME evaluation, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.128916

Highlights

- Design and synthesis of a novel hetero-substitutued sulfonamido-benzamides.
- In vivo and in vitro GK activation study by OGTT and protein 1V4S assay.
- Molecular docking study performed on glucokinase 1V4S enzyme.
- In-silico ADME evaluation: No violation of Lipinski's rule.
- Compounds **12** and **15** appeared as most promising GK activators.

Hetero-substituted sulfonamido-benzamide hybrids as Glucokinase activators: Design, Synthesis, Molecular docking and *in-silico* ADME evaluation

Authors: Saurabh C. Khadse*[1], Nikhil D. Amnerkar*[2], Krushna S. Dighole[1], Ashish M. Dhote[1], Vikas R. Patil[1], Deepak K. Lokwani[1], Vinod G. Ugale[1], Nitin B. Charbe[3,4], and Vivekanand A. Chatpalliwar[5]

[1]Department of Pharmaceutical Chemistry, R.C. Patel Institute of Pharmaceutical Education and Research, Karvand Naka Shirpur, Dist. Dhule, Maharashtra 425 405, India

[2]Adv. V. R. Manohar Institute of Diploma in Pharmacy, Wanadongri, Hingna Road, Nagpur 441110, India

[3]Departamento de Química Orgánica, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Av. Vicuña McKenna 4860, Macul, Santiago 7820436, Chile

[4]Sri Adichunchunagiri University, Sri Adichunchunagiri College of Pharmacy, BG Nagar, Karnataka 571418, India

[5]Shri Sureshdada Jain College of Pharmacy, Nemi Nagar, Chandwad, Mumbai-Agra Highway, Dist. Nashik, Maharashtra 423 101, India

*Address correspondence to SCK: E-mail: khadse_s@rediffmail.com, *Address correspondence to NDA: E-mail: nikhilamnerkar@gmail.com

Abstract

A series of hetero-substituted sulphonamido-benzamide derivatives which can activate glucokinase (GK) were synthesized and screened *in-vitro* using Human GK activation assay and *in-vivo* following oral glucose tolerance test (OGTT) assays. All the molecules were docked into the active site of 1V4S receptor grid by XP docking method utilizing Schrodinger software to assess the binding interactions. Compounds **12** (EC_{50} = 495 nM) and **15** (EC_{50} = 522 nM), revealed maximum *in-vitro* GK activation. Selected compounds were subjected for *in-vivo* OGTT assay. The data revealed that same compounds **12** (135 mg/dL) showed maximum reduction in blood glucose level followed by compound **15** (142 mg/dL) at 120 min. The docking results as glide score, binding energy and interactions were reported and compounds with maximum pharmacological activity were studied precisely. *In-silico* ADME parameters, pharmacokinetic properties and toxicity studies were carried out and all compounds were found to have good bioavailability and nontoxic. Overall, the series of hetero-substituted sulphonamido-benzamide hybrids are safe and could be explored further for better therapeutic efficacy as GK activators.

Keywords

Glucokinase; Glucokinase activator; Sulfonamido-benzamides; Docking; ADME

1. Introduction

Diabetes mellitus especially type-2 (known as T2DM) is a metabolic disorder often caused due to inadequate secretion of insulin by pancreas resulting in hyperglycaemia.[1] The market is flooded with the antidiabetic drugs but safe and effective drug for long term therapy still remained a great challenge. Looking at the complications of available anti-diabetic drugs & their significant side effects or drug tolerance, medicinal chemists are still engaged in the development of newer targets and scaffolds to exhibit antihyperglycaemic activity with minimum side effects.[2] The enzyme glucokinase (GK, also called hexokinase IV or hexokinase D) was identified as an outstanding drug receptor for developing anti-diabetic agents. GK facilitates phosphorylation of glucose to glucose-6-phosphate and is expressed in cells of the liver, pancreas, gut, and brain of humans and many other vertebrates.[3–6] Glucokinase activators (GKA) not only lowers blood glucose concentrations by enhancing glucose uptake in the liver but also increases insulin secretion from pancreatic beta cells which makes it a promising molecular target for antidiabetic therapy.[7–11]

Acquisition of the facts of GK enzyme and other parameters, several small molecules were designed and explored targeting GK to activate the allosteric site of protein. Abundant information is available on molecules binding in the allosteric site of GK.[2,12–15] Despite of the several scaffold reported, the main focus is on the benzamide motif owing to its orientation, structural binding, and interactions in the allosteric site. The crystallography study revealed that carboxyl (C=O) group and amino (-NH) group are involved in anchoring the ligand in allosteric site and observed interactions of these groups with Arg63.[9,16,17] Also, the long extensions with wide range of fragments at the ortho/meta positions of benzamide ring has a potential to form a hydrogen bonding with Arg250, Tyr214, Tyr215, Leu451, and Glu98. [8,11] The primary amino (-NH) group in the benzamide ring showed crucial pharmacophoric interactions with the allosteric site of GK, but due to potential risk of mutagenicity it has to be modified for absolute binding with receptor site and better therapeutic potential.[9,16,18] Several benzamide derivatives have been reported to be potent GK activators and showed good binding poses on docking.[8,11,19–28] Many pharmaceutical companies and research groups have successfully designed benzamide analogues including AstraZenecas GKA-22, GKA-50, GKA-60, GKA-71, AZD-1656, AZD-6370; Merck's MK-0941;Pfizer's PF-04937319; YuhanResearch Institute's YH-GKA and few of which are in various phases of clinical trials.[29–33]

These benzamide scaffolds possessing diverse structural attributes discussed above promised to lower the blood sugar level through activation of GK enzyme, but many of them couldn't succeed beyond the trials due to the toxicities associated with them. Keeping this in mind, it was thought to modify primary amino group at second position of benzamide ring by adding substituted-sulfone fragment which imparts oral bioavailability.[11,20,22–25,34] The amido nitrogen of benzamide nucleus was substituted with heterocyclic moieties in such a way that a strong hydrogen bonding and hydrophobic interactions with the amino acid residues in the allosteric site of 1V4S GK protein can be targeted. Heterocyclic ring structures such as thiazoles [9,11,19,20,22–25], indole [35,36], arylthiazole [25,29,37], pyrazole [8,11,20,21,28], 1,3,4-thiadiazole [4,26], pyrazin [21,34,38,39], and 1,2,4-thiadiazole [21,40] were selected based on the maximum GK activation observed during literature review to get good GK activation and favourable pharmacokinetics.

Herein, we report the design and synthesis of *N*-(heterosubstituted)-2-(substituted-sulfonamido)benzamide derivatives as a promising preclinical candidate for the treatment of T2DM.

2. Materials and Methods

2.1. Instrumentation and chemicals

All chemicals and reagents used were of laboratory grade and procured from Sigma-Aldrich (St. Louis, MO, USA), Rankem (Gurugaon, India), and S.D. Fine Chemicals Pvt Ltd. (Mumbai, MH, India). Melting points were recorded using Analab scientific instrument (Thermocal- sr.no.2010-11/1205) open capillary method. Solvents were redistilled and dried as and when required. Precoated silica gel 60 (HF-254, E. Merck, India) plates were used to develop TLC for routine monitoring of reactions. The synthesized compounds were purified by recrystallization and column chromatography. FTIR spectra were recorded using Shimadzu FTIR 8400S spectrophotometer (Kyto, Japan). ^{1}HNMR (DMSO/$CDCl_3$) and ^{13}CNMR spectra of the synthesized compounds were recorded on Bruker Avance-II 400 MHz spectrometer (Billerica, MA, USA), using tetramethylsilane (TMS) as internal standard. Mass spectra were determined using WATERS, Q-TOF MICROMASS (Waters, Milford, MA, USA). Elemental analyses were carried out using FLASH EA 1112 CHN analyzer (Thermo Finnigan, Italy) and found within ±0.4% of theoretical values.

2.2. Synthesis

The reaction of isatin (**1**) with acetic anhydride and acetic acid in presence of urea-hydrogen peroxide complex yielded 3,1-Benzoxazine 2,4(1*H*)-dione [Isatoic anhydride] (**2**).[41] The reaction of isatoic anhydride (**2**) with appropriate amines yielded 2-amino-*N*-(heterosubstituted)benzamides (**3-11**). In this reaction, nitrogen nucleophile of heteroamines readily attacks the reactive 4th position of isatoic anhydride followed by ring opening to generate benzamides. Further, the reaction of Tosyl/Mesyl chloride with 2-amino-*N*-(heterosubstituted)benzamides (**3-11**) in dry pyridine yielded corresponding *N*-(heterosubstituted)-2-(substituted-sulfonamido)benzamide derivatives (**12-29**) as portrayed in **Scheme 1**.

Scheme 1

2.2.1. General procedure for the synthesis of 2-amino-N-(heterosubstituted)benzamides (3-11)

Isatoic anhydride (**2**) (0.01 mol) was suspended in dry toluene (10 ml) contained in a 50 ml round bottom flask. Appropriate amines (0.01 mol) were added to the content of flask and the reaction mixture was refluxed for about 4-6 h. Further, the mixture was allowed to cool overnight to obtain solid precipitate. The resultant products were washed with water, dried and recrystallized from ethanol.

*2.2.1.1. 2-Amino-N-(5-chloro-1,3-thiazol-2-yl)benzamide (**3**)*

Yield: 62%; m. p.: 96-98 °C; IR (KBr) cm^{-1}: 3421 (NH str., Ar-NH), 3280 (NH, CO-NH), 3026 (C-H), 1639 (C=O), 1582 (C=N), 1422 (C=C), 634 (C-S); ^{1}H NMR ($CDCl_3$): δ 5.11 (s, 2H, NH_2), 6.61-7.44 (m, 5H, Ar-H), 11.81 (s, 1H, CO-NH).

*2.2.1.2. 2-Amino-N-(4-(2-ethoxy-2-oxyethyl)-1,3-thiazol-2-yl)benzamide (**4**)*

Yield: 47%; m. p.: 114-116 °C; IR (KBr) cm^{-1}: 3382 (NH str., Ar-NH), 3240 (NH, CO-NH), 3020 (C-H), 1632 (C=O), 1565 (C=N), 1469 (C=C), 612 (C-S); ^{1}H NMR ($CDCl_3$): δ 1.31 (t, *J* = 5.6 Hz, 3H, CH_3), 3.42 (s, 2H, CH_2-CO), 4.09 (q, *J* = 6.8 Hz, 2H, OCH_2), 4.91 (s, 2H, NH_2), 6.84-7.53 (m, 4H, Ar-H), 10.27 (s, 1H, CO-NH).

*2.2.1.3. 2-Amino-N-(1H-indol-5-yl)benzamide (**5**)*

Yield: 63%; m. p.: 185-187 °C; IR (KBr) cm^{-1}: 3375 (NH str., Ar-NH), 3253 (NH, CO-NH), 3012 (C-H), 1648 (C=O), 1457 (C=C); ^1H NMR ($CDCl_3$): δ 4.68 (s, 2H, NH_2), 6.48-7.78 (m, 9H, Ar-H), 9.53 (s, 1H, CO-NH), 10.11 (s, 1H, NH).

*2.2.1.4. 2-Amino-N-(6-methyl-1,3-benzothiazol-2-yl)benzamide (**6**)*

Yield: 57%; m. p.: 212-214 °C; IR (KBr) cm^{-1}: 3402 (NH str., Ar-NH), 3247 (NH, CO-NH), 2990 (C-H), 1675 (C=O), 1546 (C=N), 1451 (C=C), 598 (C-S); ^1H NMR ($CDCl_3$): δ 2.24 (s, 3H, CH_3), 4.16 (s, 2H, NH_2), 6.52-7.92 (m, 7H, Ar-H), 9.86 (s, 1H, CO-NH).

*2.2.1.5. 2-Amino-N-(1-methyl-1H-pyrazol-3-yl)benzamide (**7**)*

Yield: 65%; m. p.: 166-168 °C; IR (KBr) cm^{-1}: 3387 (NH str., Ar-NH), 3213 (NH, CO-NH), 3033 (C-H), 1655 (C=O), 1585 (C=N), 1455 (C=C), 1095 (C-N); ^1H NMR ($CDCl_3$): δ 3.16 (s, 3H, CH_3), 4.51 (s, 2H, NH_2), 6.64-7.76 (m, 6H, Ar-H), 10.08 (s, 1H, CO-NH).

*2.2.1.6. 2-Amino-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide (**8**)*

Yield: 59%; m. p.: 192-194 °C; IR (KBr) cm^{-1}: 3455 (NH str., Ar-NH), 3232 (NH, CO-NH), 3012 (C-H), 1666 (C=O), 1553 (C=N), 1464 (C=C), 621 (C-S); ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 4.21 (s, 2H, NH_2), 6.66-7.74 (m, 4H, Ar-H), 10.32 (s, 1H, CO-NH).

*2.2.1.7. 2-Amino-N-(5-ethoxycarbonyl-1,3,4-thiadiazol-2-yl)benzamide (**9**)*

Yield: 71%; m. p.: 201-203 °C; IR (KBr) cm^{-1}: 3387 (NH str., Ar-NH), 3234 (NH, CO-NH), 2997 (C-H), 1645 (C=O), 1534 (C=N), 1443 (C=C), 616 (C-S); ^1H NMR ($CDCl_3$): δ 1.51 (t, J = 5.8 Hz, 3H, CH_3), 3.26 (q, J = 7.2 Hz, 2H, CH_2), 4.12 (s, 2H, NH_2), 6.71-7.87 (m, 4H, Ar-H), 10.17 (s, 1H, CO-NH).

*2.2.1.8. 2-Amino-N-(pyrazin-2-yl)benzamide (**10**)*

Yield: 60%; m. p.: 186-188 °C; IR (KBr) cm^{-1}: 3416 (NH str., Ar-NH), 3264 (NH, CO-NH), 3052 (C-H), 1652 (C=O), 1512 (C=N), 1436 (C=C); ^1H NMR ($CDCl_3$): δ 4.21 (s, 2H, NH_2), 6.81-7.60 (m, 7H, Ar-H), 9.87 (s, 1H, CO-NH).

*2.2.1.9. 2-Amino-N-(3-methyl-1,2,4-thiadiazol-5-yl)benzamide (**11**)*

Yield: 74%; m. p.: 176-178 °C; IR (KBr) cm^{-1}: 3422 (NH str., Ar-NH), 3228 (NH, CO-NH), 3036 (C-H), 1595 (C=O), 1521 (C=N), 1458 (C=C), 609 (C-S); ^1H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 4.68 (s, 2H, NH_2), 6.54-7.61 (m, 4H, Ar-H), 9.83 (s, 1H, CO-NH).

2.2.2. General procedure for the synthesis of N-(heterosubstituted)-2-(substituted-sulfonamido)benzamides (12-29)

Benzamides (**3-11**) (0.01 mol) were suspended in dry pyridine (10 ml) contained in a 50 ml round bottom flask. Tosyl/Mesyl chloride (0.02 mol) was added to the content of flask and reaction mixture was refluxed for about 6 h on oil bath. Then the mixture was allowed to cool and poured into cold water (20 mL), to obtain precipitate. The product was filtered, washed with HCl (0.1N) and water, dried and recrystallized from ethanol.

*2.2.2.1. N-(5-chloro-1,3-thiazol-2-yl)-2-(methylsulfonamido)benzamide (**12**)*

Yield: 55%; m. p.: 156-158 °C; IR (KBr) cm^{-1}: 3373 (N-H), 2935 (C-H), 1645 (C=O), 1564 (C=N), 1448 (C=C), 1305, 1174 (SO_2, asym., sym. str.), 643 (C-S); ^{1}H NMR ($CDCl_3$): δ 2.23 (s, 3H, CH_3), 4.31 (s, 1H, SO_2-NH) ,6.61-7.92 (m, 5H, Ar-H), 10.81 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 164.6, 161.8 144.2, 135.7, 132.2, 129.5, 119.4, 118.3, 115.1, 111.1, 42.2; HRMS (ESI) *m/z* (M+1) 331; Anal. Calcd. for $C_{11}H_{10}ClN_3O_3S_2$: C, 39.82; H, 3.04; N, 12.66; found: C, 39.78; H, 3.06; N, 12.75.

*2.2.2.2. N-(4-(2-ethoxy-2-oxyethyl)-1,3-thiazol-2-yl)-2-(methylsulfonamido)benzamide (**13**)*

Yield: 63%; m. p.: 181-183 °C; IR (KBr) cm^{-1}: 3434 (N-H), 3017 (C-H), 1706 (C=O), 1555 (C=N), 1421 (C=C), 1321, 1110 (SO_2, asym., sym. str.), 642 (C-S); ^{1}H NMR ($CDCl_3$): δ 1.33 (t, *J* = 6.5 Hz, 3H, CH_2-CH_3), 2.56 (s, 3H, SO_2-CH_3), 3.53 (s, 2H, CH_2-CO), 4.17 (q, *J* = 7.8 Hz, 2H, OCH_2), 4.42 (s, 1H, SO_2-NH), 6.06-7.68 (m, 5H, Ar-H), 10.45 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 170.2, 165.1, 164.1 152.1, 145.9, 134.5, 126.8, 118.2, 117.7, 117.5, 105.6, 60.4, 40.5, 39.2, 14.4; HRMS (ESI) *m/z* (M+1) 384; Anal. Calcd. for $C_{15}H_{17}N_3O_5S_2$: C, 46.99; H, 4.47; N, 10.96; found: C, 46.85; H, 4.24; N, 10.81.

*2.2.2.3. N-(1H-indol-5-yl)-2-(methylsulfonamido)benzamide (**14**)*

Yield: 43%; m. p.: 202-204 °C; IR (KBr) cm^{-1}: 3382 (N-H), 2953 (C-H), 1672 (C=O), 1458 (C=C), 1365, 1159 (SO_2, asym., sym. str.); ^{1}H NMR ($CDCl_3$): δ 2.92 (s, 3H, CH_3), 4.76 (s, 1H, SO_2-NH), 6.32-7.98 (m, 9H, Ar-H), 9.15 (s, 1H, CO-NH), 10.92 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 164.2, 144.3, 133.8, 132.4, 130.2, 129.3, 127.6, 124.5, 119.5, 119.1, 116.5, 113.8, 112.4, 111.8, 101.8, 42.6; HRMS (ESI) *m/z* (M+1) 330; Anal. Calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76; found: C, 58.67; H, 4.43; N, 12.91.

*2.2.2.4. N-(6-methyl-1,3-benzothiazol-2-yl)-2-(methylsulfonamido)benzamide (**15**)*

Yield: 57%; m. p.: 172-174 °C; IR (KBr) cm^{-1}: 3298 (N-H), 3053 (C-H), 1612 (C=O), 1543 (C=N), 1448 (C=C), 1365, 1165 (SO_2, asym., sym. str.), 629 (C-S); ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, Ar-CH_3), 2.87 (s, 3H, SO_2-CH_3), 4.14 (s, 1H, SO_2-NH), 6.67-8.09 (m, 7H, Ar-H), 9.56 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 174.8, 164.1, 147.2, 144.4, 13.8, 132.2, 127.5, 125.8, 124.7, 121.4, 120.6, 119.1, 118.2, 115.9, 41.9, 22.8; HRMS (ESI) *m/z* (M+1) 362; Anal. Calcd. for $C_{16}H_{15}N_3O_3S_2$: C, 53.17; H, 4.18; N, 11.63; found: C, 53.46; H, 4.51; N, 11.87.

*2.2.2.5. N-(1-methyl-1H-pyrazol-3-yl)-2-(methylsulfonamido)benzamide (**16**)*

Yield: 67%; m. p.: 234-236 °C; IR (KBr) cm^{-1}: 3341 (N-H), 3098 (C-H), 1654 (C=O), 1523 (C=N), 1412 (C=C), 1342, 1161 (SO_2, asym., sym. str.); ^1H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 3.78 (s, 3H, Ar-CH_3), 4.63 (s, 1H, SO_2-NH), 6.67-8.09 (m, 6H, Ar-H), 9.12 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 165.3, 146.8, 144.2, 133.1, 129.5, 127.9, 118.2, 117.3, 116.8, 92.1, 43.2, 41.1; HRMS (ESI) *m/z* (M+1) 295; Anal. Calcd. for $C_{12}H_{14}N_4O_3S$: C, 48.97; H, 4.79; N, 19.04; found: C, 48.21; H, 4.84; N, 19.51.

*2.2.2.6. 2-(Methylsulfonamido)-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide (**17**)*

Yield: 48%; m. p.: 173-175 °C; IR (KBr) cm^{-1}: 3318 (N-H), 3068 (C-H), 1626 (C=O), 1512 (C=N), 1446 (C=C), 1313, 1127 (SO_2, asym., sym. str.), 588 (C-S); ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 3.15 (s, 3H, SO_2-CH_3), 4.48 (s, 1H, SO_2-NH), 6.92-7.67 (m, 4H, Ar-H), 9.72 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 166.1, 152.6, 145.8, 135.2, 127.1, 119.5, 119.2, 117.4, 110.2, 41.9, 16.8; HRMS (ESI) *m/z* (M+1) 313; Anal. Calcd. for $C_{11}H_{12}N_4O_3S_2$: C, 42.30; H, 3.87; N, 17.94; found: C, 42.87; H, 3.13; N, 17.27.

*2.2.2.7. N-(5-ethoxycarbonyl-1,3,4-thiadiazol-2-yl)-2-(methylsulfonamido)benzamide (**18**)*

Yield: 52%; m. p.: 165-167 °C; IR (KBr) cm^{-1}: 3324 (N-H), 2926 (C-H), 1645 (C=O), 1524 (C=N), 1423 (C=C), 1329, 1146 (SO_2, asym., sym. str.), 612 (C-S); ^1H NMR ($CDCl_3$): δ 1.46 (t, J = 6.1 Hz, 3H, CH_2-$\underline{CH_3}$), 2.83 (s, 3H, SO_2-CH_3), 4.51 (q, J = 7.1 Hz, 2H, CH_2), 4.76 (s, 1H, SO_2-NH), 6.52-7.84 (m, 4H, Ar-H), 9.87 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 171.1, 165.4, 159.6, 151.2, 146.2, 134.2, 129.6, 119.9, 119.2, 118.1, 61.4, 42.7, 14.7; HRMS (ESI) *m/z* (M+1) 371; Anal. Calcd. for $C_{13}H_{14}N_4O_5S_2$: C, 42.15; H, 3.81; N, 15.13; found: C, 42.23; H, 3.44; N, 15.54.

*2.2.2.8. 2-(Methylsulfonamido)-N-(pyrazin-2-yl)benzamide (**19**)*

Yield: 67%; m. p.: 213-215 °C; IR (KBr) cm^{-1}: 3285 (N-H), 3082 (C-H), 1745 (C=O), 1556 (C=N), 1434 (C=C), 1311, 1125 (SO_2, asym., sym. str.); ^1H NMR ($CDCl_3$): δ 2.65 (s, 3H, SO_2-CH_3), 4.26 (s, 1H, SO_2-NH), 6.43-8.14 (m, 7H, Ar-H), 10.24 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 165.6, 148.3, 146.7, 144.8, 135.5, 135.1, 133.2, 129.2, 119.7, 119.2, 118.3, 42.7; HRMS (ESI) *m/z* (M+1) 293; Anal. Calcd. for $C_{12}H_{12}N_4O_3S$: C, 49.31; H, 4.14; N, 19.17; found: C, 49.86; H, 3.83; N, 19.45.

*2.2.2.9. 2-(Methylsulfonamido)-N-(3-methyl-1,2,4-thiadiazol-5-yl)benzamide (**20**)*

Yield: 52%; m. p.: 174-177 °C; IR (KBr) cm^{-1}: 3326 (N-H), 3034 (C-H), 1732 (C=O), 1534 (C=N), 1411 (C=C), 1324, 1142 (SO_2, asym., sym. str.); ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 2.94 (s, 3H, SO_2-CH_3), 4.87 (s, 1H, SO_2-NH), 6.36-7.85 (m, 4H, Ar-H), 9.58 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 178.3, 172.8, 165.5, 145.2, 132.6, 129.5, 119.7, 119.1, 117.3, 42.7, 18.4; HRMS (ESI) *m/z* (M+1) 313; Anal. Calcd. for $C_{11}H_{12}N_4O_3S_2$: C, 42.30; H, 3.87; N, 17.94; found: C, 42.47; H, 3.35; N, 17.63.

*2.2.2.10. N-(5-chloro-1,3-thiazol-2-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**21**)*

Yield: 71%; m. p.: 186-188 °C; IR (KBr) cm^{-1}: 3408 (N-H), 3062 (C-H), 1676 (C=O), 1526 (C=N), 1435 (C=C), 1354, 1126 (SO_2, asym., sym. str.), 583 (C-S); ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, Ar-CH_3), 4.72 (s, 1H, SO_2-NH), 6.53-7.87 (m, 5H, Ar-H), 7.35 (d, J = 9.7 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.67 (d, J =8.1 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.75 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 165.8, 161.3, 146.2, 140.8, 137.2, 134.6, 134.2, 129.7, 129.3, 127.5, 120.4, 119.2, 118.5, 111.1, 23.6; HRMS (ESI) *m/z* (M+1) 408; Anal. Calcd. for $C_{17}H_{14}ClN_3O_3S_2$: C, 50.06; H, 3.46; N, 10.30; found: C, 50.56; H, 3.68; N, 10.61.

*2.2.2.11. N-(4-(2-ethoxy-2-oxyethyl)thiazol-2-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**22**)*

Yield: 68%; m. p.: 165-167 °C; IR (KBr) cm^{-1}: 3352 (N-H), 2976 (C-H), 1739 (C=O), 1516 (C=N), 1447 (C=C), 1353, 1148 (SO_2, asym., sym. str.), 597 (C-S); ^1H NMR ($CDCl_3$): δ 1.46 (t, *J* = 6.2 Hz, 3H, CH_2-$\underline{CH_3}$), 2.42 (s, 3H, Ar-CH_3), 3.65 (s, 2H, CH_2-CO), 4.27 (q, *J* = 7.1 Hz, 2H, OCH_2), 4.68 (s, 1H, SO_2-NH), 6.11-7.87 (m, 9H, Ar-H), 7.36 (d, J = 8.9 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.71 (d, J = 9.2 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.37 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 168.4, 165.2, 164.6, 151.4, 146.2, 140.5, 138.3, 134.2, 129.8, 129.3, 128.2,

119.8, 119.3, 118.6, 105.6, 61.8, 39.2, 25.7, 14.9; HRMS (ESI) *m/z* (M+1) 460; Anal. Calcd. for $C_{21}H_{21}N_3O_5S_2$: C, 54.89; H, 4.61; N, 9.14; found: C, 54.55; H, 4.47; N, 9.23.

*2.2.2.12. N-(1H-indol-5-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**23**)*

Yield: 64%; m. p.: 177-179 °C; IR (KBr) cm^{-1}: 3367 (N-H), 3026 (C-H), 1712 (C=O), 1432 (C=C), 1335, 1123 (SO_2, asym., sym. str.); ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H, Ar-CH_3), 4.81 (s, 1H, SO_2-NH), 6.52-7.87 (m, 9H, Ar-H), 7.24 (d, J = 9.4 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.87 (d, J =8.5 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.21 (s, 1H, CO-NH), 10.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 165.6, 146.4, 140.4, 137.2, 134.2, 132.5, 130.4, 129.8, 129.7, 129.2, 128.7, 128.3, 123.1, 119.6, 118.8, 113.5, 110.2, 109.3, 101.4, 24.7; HRMS (ESI) *m/z* (M+1) 406; Anal. Calcd. for $C_{22}H_{19}N_3O_3S$: C, 65.17; H, 4.72; N, 10.36; found: C, 65.42; H, 4.53; N, 10.41.

*2.2.2.13. N-(6-methyl-1,3-benzothiazol-2-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**24**)*

Yield: 68%; m. p.: 181-183 °C; IR (KBr) cm^{-1}: 3345 (N-H), 3053 (C-H), 1693 (C=O), 1532 (C=N), 1458 (C=C), 1312, 1131 (SO_2, asym., sym. str.), 603 (C-S); ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3H, Het-CH_3), 2.65 (s, 3H, Ar-CH_3), 4.35 (s, 1H, SO_2-NH), 6.61-8.23 (m, 7H, Ar-H), 7.43 (d, J = 8.6 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.84 (d, J =9.7 Hz, 2H, C_3, C_5 of Ar-CH_3), 10.13 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 172.1, 164.1, 147.2, 145.2, 137.2, 135.2, 134.3, 130.5, 129.5, 128.2, 125.9, 125.3, 122.3, 122.1, 119.4, 118.2, 117.8, 25.4, 24.5; HRMS (ESI) *m/z* (M+1) 438; Anal. Calcd. for $C_{22}H_{19}N_3O_3S_2$: C, 60.39; H, 4.38; N, 9.60; found: C, 60.14; H, 4.47; N, 9.51.

*2.2.2.14. N-(1-methyl-1H-pyrazol-3-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**25**)*

Yield: 74%; m. p.: 222-224 °C; IR (KBr) cm^{-1}: 3357 (N-H), 2967 (C-H), 1752 (C=O), 1544 (C=N), 1428 (C=C), 1349, 1104 (SO_2, asym., sym. str.); ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 3H, Ar-CH_3), 3.74 (s, 3H, Het-CH_3), 4.72 (s, 1H, SO_2-NH), 6.51-7.48 (m, 6H, Ar-H), 7.58 (d, J = 5.8 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.96 (d, J = 11.2 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.63 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 164.3, 146.7, 145.8, 142.5, 137.2, 134.8, 130.2, 129.2, 128.7, 119.9, 119.2, 117.4, 91.7, 41.4, 26.4; HRMS (ESI) *m/z* (M+1) 371; Anal. Calcd. for $C_{18}H_{18}N_4O_3S$: C, 58.36; H, 4.90; N, 15.12; found: C, 59.21; H, 4.88; N, 16.16.

*2.2.2.15. 2-[(4-Methyphenyl)sulfonamido]-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide (**26**)*

Yield: 69%; m. p.: 218-220 °C; IR (KBr) cm^{-1}: 3304 (N-H), 3057 (C-H), 1721 (C=O), 1536 (C=N), 1478 (C=C), 1298, 1153 (SO_2, asym., sym. str.), 634 (C-S); ^1H NMR ($CDCl_3$): δ 2.78 (s, 3H, Ar-CH_3), 3.17 (s, 3H, Het-CH_3), 4.26 (s, 1H, SO_2-NH), 6.67-7.93 (m, 4H, Ar-H), 7.44 (d, J = 6.4 Hz, 2H, C_2, C_6 of Ar-CH_3), 8.09 (d, J = 7.6 Hz, 2H, C_3, C_5 of Ar-CH_3), 10.35 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 163.8, 152.8, 146.7, 140.8, 138.2, 135.2, 129.4, 129.2, 119.5, 119.2, 117.4, 114.2, 25.4, 16.7; HRMS (ESI) *m/z* (M+1) 389; Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2$: C, 52.56; H, 4.15; N, 14.42; found: C, 52.98; H, 4.22; N, 14.04.

*2.2.2.16. N-(5-ethoxycarbonyl-1,3,4-thiadiazol-2-yl)-2-[(4-methyphenyl)sulfonamido]benzamide (**27**)*

Yield: 78%; m. p.: 168-170 °C; IR (KBr) cm^{-1}: 3286 (N-H), 2965 (C-H), 1655 (C=O), 1552 (C=N), 1467 (C=C), 1346, 1134 (SO_2, asym., sym. str.), 631 (C-S); ^1H NMR ($CDCl_3$): δ 1.32 (t, *J* = 5.8 Hz, 3H, CH_2-$\underline{CH_3}$), 2.56 (s, 3H, Ar-CH_3), 4.14 (q, *J* = 8.4 Hz, 2H, CH_2), 4.76 (s, 1H, SO_2-NH), 6.45-7.46 (m, 4H, Ar-H), 7.48 (d, J = 8.3 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.86 (d, J = 6.8 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.56 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 171.5, 165.6, 159.6, 152.5, 144.3, 140.6, 138.6, 134.2, 131.7, 129.2, 128.4, 119.6, 119.3, 117.4, 61.3, 25.7, 15.6; HRMS (ESI) *m/z* (M+1) 447; Anal. Calcd. for $C_{19}H_{18}N_4O_5S_2$: C, 51.11; H, 4.06; N, 12.55; found: C, 51.76; H, 4.14; N, 12.36.

*2.2.2.17. 2-[(4-Methyphenyl)sulfonamido]-N-(pyrazin-2-yl)benzamide (**28**)*

Yield: 62%; m. p.: 187-189 °C; IR (KBr) cm^{-1}: 3335 (N-H), 3065 (C-H), 1710 (C=O), 1521 (C=N), 1443 (C=C), 1345, 1126 (SO_2, asym., sym. str.); ^1H NMR ($CDCl_3$): δ 2.72 (s, 3H, Ar-CH_3), 4.37 (s, 1H, SO_2-NH), 6.64-8.32 (m, 7H, Ar-H), 7.52 (d, J = 5.4 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.75 (d, J = 7.5 Hz, 2H, C_3, C_5 of Ar-CH_3), 10.22 (s, 1H, CO-NH); ^{13}C NMR (DMSO-d_6): δ 165.7, 151.4, 146.7, 144.2, 140.6, 137.7, 136.3, 134.7, 134.8, 134.2, 13.0.2, 129.3, 127.5, 119.4, 118.5, 117.3, 24.2; HRMS (ESI) *m/z* (M+1) 369; Anal. Calcd. for $C_{18}H_{16}N_4O_3S$: C, 58.68; H, 4.38; N, 15.21; found: C, 58.34; H, 4.45; N, 15.43.

*2.2.2.18. 2-[(4-Methyphenyl)sulfonamido]-N-(3-methyl-1,2,4-thiadiazol-5-yl)benzamide (**29**)*

Yield: 63%; m. p.: 165-167 °C; IR (KBr) cm^{-1}: 3358 (N-H), 3032 (C-H), 1630 (C=O), 1587 (C=N), 1412 (C=C), 1365, 1132 (SO_2, asym., sym. str.), 578 (C-S); ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, Ar-CH_3), 3.43 (s, 3H, Het-CH_3), 4.82 (s, 1H, SO_2-NH), 6.65-7.80 (m, 4H, Ar-H), 7.24 (d, J = 4.8 Hz, 2H, C_2, C_6 of Ar-CH_3), 7.94 (d, J = 6.4 Hz, 2H, C_3, C_5 of Ar-CH_3), 9.56 (s, 1H,

CO-NH); ^{13}C NMR (DMSO-d_6): δ 178.4, 170.3, 165.7, 145.8, 140.5, 138.6, 132.4, 130.5, 128.8, 12.3, 119.2, 118.9, 117.2, 25.1, 16.4; HRMS (ESI) *m/z* (M+1) 389; Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2$: C, 52.56; H, 4.15; N, 14.42; found: C, 52.98; H, 4.22; N, 14.04.

2.3. Antidiabetic study

The *in vitro* glucose-phosphorylating activity of GK enzyme was measured by an enzyme-coupled spectrophotometric assay, as described. [16] The proteins and the reagents used in the assay were procured from Sigma-Aldrich Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd. GK catalyses glucose phosphorylation to give glucose-6-phosphate dehydrogenase (G6PDH) reducing NADPH which was monitored at 340 nm. All the test compounds were prepared in dimethyl sulfoxide (DMSO). The assay was performed in 96 well plates consisting of 25 mM/L 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethane sulfonic acid (HEPES, pH 7.1), 1 mM/L adenosine triphosphate (ATP), 5 mM/L beta-mercaptoethanol, 2.5 mM/L $MgCl_2$, 1 mM/L NADP, 25 mM/L KCl, 2 U/mL glucose-6-phosphate dehydrogenase, 60 nM/L GK, and 10mM glucose. The EC_{50} values were calculated as a mean of three readings for each test compound and standard. [42,43]

OGTT assay was performed *in vivo* using male wistar rats weighing 150-200g were procured from Central Animal House facility of R. C. Patel Institute of Pharmaceutical Education & Research (Registered under CPCSEA, India, 651/PO/RcBi/S/CPCSEA). The rats were made ready for the experimental protocol as per the ethical guidelines and approved by Institutional Animal Ethical Committee. The test compounds (20 mg/kg) or vehicle (0.5% carboxymethyl cellulose) alone was administered orally to fasted animals (n=5) 30 min before glucose loading (2 g/kg). The blood glucose levels were measured using portable glucometer (Accu-Check Active GN03770116) at different time intervals (t=0, 30, 60, 90 and 120 min).[38,42,43]

2.4. Molecular docking protocol

The docking simulations were carried out using GLIDE, version 7.4, Schrodinger, LLC, New York, USA, 2017. The crystal structure of GK (PDB ID 1V4S) was retrieved from the RCSB Protein Data Bank (PDB) [44] and prepared for docking with the Protein Preparation Wizard in Maestro v11.1. The protein 1V4S is a crystal structure of human glucokinase and revealed allosteric site through which small molecules may modulate the kinetic properties of the

enzyme that provides the basis for the activation of glucokinase for treating type 2 diabetes mellitus. Further, the optimization of hydrogen bonds was carried out, followed by energy minimization of protein structure using OPLS3 force field.[45] The ligands were prepared using LigPrep tool that generates accurate and energy minimized 3D molecular structures. Low energy conformers were generated for the 3D structures of designed ligands. The receptor grid was generated by applying van der Waals radii and the size of the grid box was fixed so that ligand with the size of </= 20 Å can be docked.[46] Docking was carried out by applying the generated low energy conformers of ligands into the active site of 1V4S receptor grid by extra precision (XP) mode. The docking glide score, binding free energy (using Prime MM-GBSA method), hydrogen bonding and π-π interactions with the surrounding amino acids were calculated and best-suited conformations of ligands with maximum dock score were studied precisely.

2.5. *In-Silico* ADMET prediction

In-silico ADMET studies was performed using SwissADME[47] and PreADMET[48] web tools. Various pharmacokinetic parameters related to Absorption, Distribution, Metabolism, and Excretion were studied and discussed.

3. Results and Discussion

3.1 Chemistry

The general route for the synthesis of the target compounds *N*-(heterosubstituted)-2-(substituted-sulfonamido)benzamide derivatives (**12-29**) is outlined in **Scheme 1**. The compounds were synthesized in a good yield (43-78%). All the newly synthesized compounds were purified using column chromatography and confirmed from the single spot TLC reports. The IR spectrum of the compounds (**12-29**) showed SO_2 asymmetric/ symmetric stretching in the range 1365-1298 cm^{-1}/1174-1110 cm^{-1} in addition to C=O stretching and N-H stretching in the range of 1752-1612 cm^{-1} and 3434-3285 cm^{-1}, respectively shown by compounds (**3-11**). Thus, it is supporting the fact that sulphonamide and amide functional groups are present in the structure of target synthesized *N*-(heterosubstituted)-2-(substituted-sulfonamido)benzamide derivatives. ^{1}HNMR spectra of the compounds (**12-29**) showed the singlet signals for -CH_3 in the range of δ 2.23-2.92 and for the NH proton of SO_2-NH group around δ 4.14-4.87. The doublet signals in the range of δ

7.24-7.58 for the proton at C_2 and C_6 and around δ 7.67-8.09 belonging to proton at C_3 and C_5 of the phenyl ring in the compounds (**21-29**) confirmed that the sulphonamide bond and methyl bond (Ar-CH_3) were placed at para- position to each other. The presence of singlet signal for the amide NH proton of CONH group was observed in the range of δ 9.12-10.81. However, all the other protons were resolved in appropriate regions confirming the assigned structures. In the ^{13}C NMR spectra, characteristic singlet signal of the carbonyl (C=O) carbon was observed at around δ 165 indicating the presence of amide linkage. The presence of the singlet signal at around δ 25 and δ 45 corresponds to the presence of methyl CH_3 and Ar-CH_3 group. A singlet signal at around δ 145 corresponding to the C_2 of the benzamide phenyl ring depicted the sulphonamide group attached to the phenyl ring. Mass spectrum as M+1 shows the molecular ion peaks of the synthesized benzamides. Elemental analysis data were found in the range of ±0.4% for the theoretical values of the analyzed elements (C, H, N).

3.2. Pharmacological evaluation

Acute toxicity studies of the selected compounds (**12**, **15, 17, 21** and **24**) were carried out in rats administering dose of 2000 mg/kg. The compounds (**12, 15, 17, 21** and **24**) were selected based on the results of *in vitro* GK assay with good EC_{50} values. The effects were observed for a period of 14 days. None of the animals showed any toxic effect and all the animals were survived in each group (n=5).

The GK activation by the newly synthesized compounds was evaluated *in-vitro* using Human GK activation assay and *in-vivo* following oral glucose tolerance test (OGTT) assays. The enzymatic activity on recombinant human GK was first studied and reported as EC_{50} in **Table 1**. Standards RO-28-1675, Dorzagliatin and Piragliatin was considered for comparison with test compounds and revealed that the test compounds exhibited less ability to activate GK. However, EC_{50} values of the compounds **12, 15, 17, 21** and **24** are encouraging. To our delight, compound **12** having 5-chloro-thiazole as heterocyclic moiety and methyl- group as alkyl residue in benzamide ring structure displayed an EC_{50} = 495 nM exhibiting maximum potency. The 6-methylbenzothiazole- and methylsulfonamido- substituted benzamide derivative (compound **15**) showed GK activation of EC_{50} = 522 nM. Thus, it states that thiazolyl or arylthiazolyl compounds showed promising GK activation. However, in compound **21**, replacing the methylsulfonamido group with 4-methyl-phenylsulfonamido- moiety exhibits lower potency (EC_{50}= 657 nM) which states that replacing methyl group with 4-methylphenyl fragment alters and lowers the GK activation. The compound **17** with 5-

methyl-1,3,4-thiadiazole- and methylsulfonamido- fragment and compound **24** bearing 6-methylbenzothiazole- and methyl-phenylsulfonamido- residues in benzamide ring structure showed good GK activation potency (EC_{50}= 958 nM, and EC_{50}= 836 nM, respectively). The remaining tested compounds showed EC_{50} > 1000 nM. Overall, *in vitro* GK activation study revealed that replacing thiazolyl heterocycles with pyrazolyl/pyrazin/1,2,4-thiadiazolyl (EC_{50} = 1063 nM/ EC_{50} = 1052 nM/ EC_{50} = 2327 nM) fragments remarkably reduced the GK activation.

Table 1

On the basis of the *in vitro* GK activation results and structure activity relationships (SAR) studies described above, compounds **12, 15, 17, 21** and **24** were exploited further for OGTT assay in animal models. The results of the OGTT assay were reported as mg/dL at different time intervals (0, 30, 60, 90 and 120 min). The tested compounds showed significant reduction in the blood glucose levels on comparison with control in 0-120 min at a dose of 20 mg/kg (**Fig. 1**). The compound **12** showed maximum reduction in blood glucose level (135 mg/dL) followed by compound **15** (142 mg/dL) at 120 min. The compound **21** and **24** showed moderate reduction in blood glucose level (168 and 162mg/dL, respectively) compared with control. On the other hand, compound **17** (172 mg/dL) showed similar pattern of blood glucose reduction as that of control. The data were statistically analysed by one-way ANOVA and expressed as mean ± SEM (n=6) ($p < 0.05$) and revealed that both the results from *in-vitro* and *in-vivo* assay were in agreement with each other. It indicated that compounds with thiazolyl or arylthiazolyl moieties led to increase in glucose reduction compared to other heterocyclic substitutions on benzamide ring. However, with same heterocyclic moiety and replacing methylsulfonamido group with methylphenylsufonamido moiety led to decrease in activity in both *in vitro* and *in vivo* assays.

Fig. 1

3.3. Molecular docking studies

The designed GK activators were docked in the allosteric site of 1V4S GK to understand the hydrogen bond interactions and binding mode of ligands with the receptor site. The glide score, binding energy, hydrogen bonding and $\pi - \pi$ interaction of all the newly synthesized

compounds (**12-29**) are reported in **Table 1** along with the *in vitro* human GK activation assay results for comparative analysis. The glide score of the compounds were found to be in the range of to -5.673 to -9.185. The most active compounds **12** and **15** in the series in terms of pharmacological actions are considered and discussed precisely.

The binding poses and the interactions of the compounds **12**, **15** and co-crystallized ligand (MRK) are illustrated in **Fig. 2(a-f)**. The docking poses of co-crystallized ligand (MRK) will help to distinguish the essential interaction in the crystal structure 1V4S with the most active compounds **12** and **15** in the series. The compound **12** showed glide score of -9.185 and binding energy of -53.76 kcal/mol, which is greater than the glide scores of standards Dorzagliatin (glide score -7.949), Piragliatin (glide score -7.891) and RO-28-1675 (glide score -6.394). It binds with the active site of 1V4S receptor forming hydrogen bonds between nitrogen NH of sulfonamide and Arg63at a distance of 1.91 Å, and carbonyl oxygen C=O of amide group and Arg63 at a distance of 2.06 Å. One additional hydrogen bond was observed between the aromatic hydrogen and Tyr215 at a distance of 2.54 Å. Moreover, the compound showed hydrophobic interactions with Tyr61, Val62, Cys252, Met235, Pro66, Met210, Ile211, Tyr214, Tyr215, Leu451, Val452, Val455, Ala456 and Ile159 (**Fig. 2a** and **2b**).

Fig. 2(a-f)

Compound **15**, the second most potent compound in the series, showed glide score of -6.482 and binding energy of -51.79. It showed four hydrogen bond interactions, viz.; one between NH of the sulfonamido group and Arg63; second between sulfone group S=O and Thr65 at a distance of 2.55 Å; third between carbonyl oxygen C=O and Arg63 at a distance of 2.29 Å and forth between thiazolyl ring nitrogen N and Arg63 at a distance of 2.46 Å. Additionally, one hydrogen bond is also observed in 3D docking pose between hydrogen of benzamide aromatic ring and Tyr215 at a distance of 2.59 Å and π–π stacking appeared between phenyl ring of benzothiazole moiety and Lys459. Furthermore, compound **15** forms hydrophobic cloud with Tyr61, Val62, Pro66, Met235, Cys220, Met210, Ile211, Tyr214, Cys252, Tyr215, Leu451, Val452, Val455, Ala456 and Ile159 (**Fig. 2c** and **2d**). Hydrogen bonds with Arg63 was shown by compounds **12** and **15**, similar to the standard drugs piragliatin and RO-28-1675. The compound **15** showed additional hydrogen bond with Thr65 like the standard RO-28-1675.

Thus, the molecular docking of the designed benzamide derivatives may serve to understand mechanism of GK activation and could be optimised to get improved and better GK activators.

3.4. ADMET evaluation

All the newly synthesized compounds (**12-29**) were subjected for ADMET predictions using SwissADME and PreADMET softwares. Various parameters like hydrogen bond acceptor, hydrogen bond donor, number of rotatable bonds, topological polar surface area, partition coefficient, aqueous solubility, blood-brain barrier permeability, cytochrome-P2D6 inhibitor (CYP2D6), Lipinski-no. of violations, hERG inhibition, carcinogenicity, and human intestinal absorption were studied and illustrated in **Table 2**.

Table 2

All the synthesized compounds obeyed the criteria for number of hydrogen bond acceptor, hydrogen bond donor and number of rotatable bonds and remained within the limits. The transport of drug molecules across the biological barriers is determined by Topological polar surface area (TPSA) and observed in the range of 99.44-163.97 $Å^2$ and the partition coefficient (Log P) values were less than 5 indicating good permeability across cell membrane. Thus, the compounds followed Lipinski's rule of five[49] with few exceptions and oral bioavailability could be accounted. Solubility (Log *S*) is one of the criteria that influences absorption and here we find all the compounds displayed in the scale of soluble to moderately soluble (log scale -2.32 to -5.95) in water. The data revealed that none of the compound crosses blood brain barrier. Inhibition of cytochrome (CYP2D6) led to drug-drug interaction[50] and it is observed that except few compounds (compounds **14, 23, 25** and **28**) all others do not inhibit this crucial isoenzyme. All the compounds were observed to be non-carcinogenic and found to be at lower to medium risk of hERG inhibition except compound **23** and **25**. It was also noticed that all the synthesized compounds displayed % human intestinal absorption ranging from 77.01 to 97.65% denoting good bioavailability. On studying all these *in-silico* parameters, it can be predicted that the potent compounds in the series could be potentially promising leads as GK activators for the treatment of diabetes.

4. Conclusion

GK activators are an important tool that helps to regulate glucose level and provides effective therapeutic approach in the treatment of diabetes. The newer GK activators were designed by understanding the binding patterns of the co-crystallized GK proteins from the PDB database. Considering the crucial pharmacophoric features (carboxyl (C=O) and amino (-NH) group) required for the binding to activate GK enzyme, benzamide nucleus was chosen and a series of hetero-substituted sulphonamido-benzamide hybrids (**12-29**) were synthesised and subjected to SAR analysis. Molecular docking study was performed to understand the binding modes of ligands along with the crucial hydrogen bonding and π–π interactions with the amino acid residues in the allosteric site of protein 1V4S. Compounds **12** and **15** showed the expected hydrogen bonding with ARG63 amino acid residue which is a crucial interaction required for optimum binding. All the compounds were evaluated *in-vitro* following human GK activation assay and results reported as EC_{50} values. Selected compounds with good EC_{50} values and SAR outcomes were subjected for *in-vivo* OGTT assay. It is noteworthy that compound **12** and compound **15** possessing thiazolyl or arylthiazolyl ring and methylsulfonamido group in benzamide scaffold exhibited maximum potency in both *in-vitro* and *in-vivo* assay. So, it would be interesting to further modify thiazolyl and arylthiazolyl residue to get improved activity. *In-silico* ADMET prediction for all the compounds showed good oral bioavailability with no toxicity and followed Lipinski's rule of five to exhibit drug-like property. Our study may contribute significantly in designing valuable lead molecules with potential to activate GK for the treatment of diabetic disorder.

Declaration of competing interest

None

CRediT author statement

Saurabh C. Khadse: Conceptualization, Methodology, Funding acquisition, Investigation, Writing- Original draft preparation, Writing - Review & Editing; **Nikhil D. Amnerkar:** Conceptualization, Methodology, Writing- Original draft preparation, Writing - Review & Editing, Validation; **Krushna S. Dighole:** Investigation; **Ashish M. Dhote:** Visualization, Data Curation; **Vikas R. Patil:** Visualization, Data Curation; **Deepak K. Lokwani:** Formal

analysis, Software-docking; **Vinod G. Ugale:** Validation-Pharmacological assays, Resources; **Nitin B. Charbe:** Validation-Pharmacological assays, Resources; **Vivekanand A. Chatpalliwar:** Project administration, Supervision.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgements

We acknowledge the partial support form the University Grant Commission (Minor Research Project No. 47-977/14 (WRO), India). We are also thankful to Dr. S. J. Surana, Principal, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing research facilities.

References

[1] Q. Min, X. Cai, W. Sun, F. Gao, Z. Li, Q. Zhang, L.-S. Wan, H. Li, J. Chen, Identification of mangiferin as a potential Glucokinase activator by structure-based virtual ligand screening, Sci. Rep. 7 (2017) 44681. https://doi.org/10.1038/srep44681.

[2] A.S. Grewal, R. Kharb, J.S. Dua, V. Lather, Molecular docking assessment of N-heteroaryl substituted benzamide derivatives as glucokinase activators, Asian J. Pharm. Pharmacol. 5 (2018) 129–136. https://doi.org/10.31024/ajpp.2019.5.1.18.

[3] M. Puig-Domingo, S. Pellitero, Nuevos agentes terapéuticos para la diabetes tipo 2, Med. Clin. (Barc). 144 (2015) 560–565. https://doi.org/10.1016/j.medcli.2014.03.018.

[4] M. Pal, Recent advances in glucokinase activators for the treatment of type 2 diabetes, Drug Discov. Today. 14 (2009) 784–792. https://doi.org/10.1016/j.drudis.2009.05.013.

[5] L. Agius, Lessons from glucokinase activators: The problem of declining efficacy, Expert Opin. Ther. Pat. 24 (2014) 1155–1159. https://doi.org/10.1517/13543776.2014.965680.

[6] T.J. Kohn, X. Du, S. Lai, Y. Xiong, R. Komorowski, M. Veniant, Z. Fu, X. Jiao, V. Pattaropong, D. Chow, M. Cardozo, L. Jin, M. Conn, W.E. DeWolf, C.F. Kraser, R.J. Hinklin, M.L. Boys, J.C. Medina, J. Houze, P. Dransfield, P. Coward, 5-Alkyl-2-urea-Substituted Pyridines: Identification of Efficacious Glucokinase Activators with Improved Properties, ACS Med. Chem. Lett. 7 (2016) 666–670. https://doi.org/10.1021/acsmedchemlett.6b00145.

[7] D. McKerrecher, J. V. Allen, S.S. Bowker, S. Boyd, P.W.R. Caulkett, G.S. Currie, C.D. Davies, M.L. Fenwick, H. Gaskin, E. Grange, R.B. Hargreaves, B.R. Hayter, R. James, K.M. Johnson, C. Johnstone, C.D. Jones, S. Lackie, J.W. Rayner, R.P. Walker, Discovery, synthesis and biological evaluation of novel glucokinase activators, Bioorg. Med. Chem. Lett. 15 (2005) 2103–2106. https://doi.org/10.1016/j.bmcl.2005.01.087.

[8] K. Park, B.M. Lee, K.H. Hyun, T. Han, D.H. Lee, H.H. Choi, Design and synthesis of acetylenyl benzamide derivatives as novel glucokinase activators for the treatment of t2dm, ACS Med. Chem. Lett. 6 (2015) 296–301. https://doi.org/10.1021/ml5004712.

[9] T. Iino, D. Tsukahara, K. Kamata, K. Sasaki, S. Ohyama, H. Hosaka, T. Hasegawa, M. Chiba, Y. Nagata, J. ichi Eiki, T. Nishimura, Discovery of potent and orally active 3-alkoxy-5-phenoxy-N-thiazolyl benzamides as novel allosteric glucokinase activators, Bioorganic Med. Chem. 17 (2009) 2733–2743. https://doi.org/10.1016/j.bmc.2009.02.038.

[10] B.D. Stevens, J. Litchfield, J.A. Pfefferkorn, K. Atkinson, C. Perreault, P. Amor, K. Bahnck, M.A. Berliner, J. Calloway, A. Carlo, D.R. Derksen, K.J. Filipski, M. Gumkowski, C. Jassal, M. MacDougall, B. Murphy, P. Nkansah, J. Pettersen, C. Rotter, Y. Zhang, Discovery of an intravenous hepatoselective glucokinase activator for the treatment of inpatient hyperglycemia, Bioorg. Med. Chem. Lett. 23 (2013) 6588–6592. https://doi.org/10.1016/j.bmcl.2013.10.057.

[11] K. Park, B.M. Lee, K.H. Hyun, D.H. Lee, H.H. Choi, H. Kim, W. Chong, K.B. Kim, S.Y. Nam, Discovery of 3-(4-methanesulfonylphenoxy)-N-[1-(2-methoxy-ethoxymethyl)-1H-pyrazol-3-yl]-5-(3-methylpyridin-2-yl)-benzamide as a novel glucokinase activator (GKA) for the treatment of type 2 diabetes mellitus, Bioorg. Med. Chem. 22 (2014) 2280–2293. https://doi.org/10.1016/j.bmc.2014.02.009.

[12] E. Ermakova, Structural insight into the glucokinase-ligands interactions. Molecular docking study, Comput. Biol. Chem. 64 (2016) 281–296. https://doi.org/10.1016/j.compbiolchem.2016.08.001.

[13] M.O. Taha, M. Habash, M.M. Hatmal, A.H. Abdelazeem, A. Qandil, Ligand-based modeling followed by in vitro bioassay yielded new potent glucokinase activators, J. Mol. Graph. Model. 56 (2015) 91–102. https://doi.org/10.1016/j.jmgm.2014.12.003.

[14] Z.S. Cheruvallath, S.L. Gwaltney, M. Sabat, M. Tang, J. Feng, H. Wang, J. Miura, P. Guntupalli, A. Jennings, D. Hosfield, B. Lee, Y. Wu, Design, synthesis and SAR of novel glucokinase activators, Bioorganic Med. Chem. Lett. 23 (2013) 2166–2171. https://doi.org/10.1016/j.bmcl.2013.01.093.

[15] N.K. Yellapu, R.B. Kilaru, N. Chamarthi, S. PVGK, B. Matcha, Structure based design, synthesis and biological evaluation of amino phosphonate derivatives as human glucokinase activators, Comput. Biol. Chem. 68 (2017) 118–130. https://doi.org/10.1016/j.compbiolchem.2017.02.011.

[16] S.C. Khadse, N.D. Amnerkar, M.U. Dave, D.K. Lokwani, R.R. Patil, V.G. Ugale, N.B. Charbe, V.A. Chatpalliwar, Quinazolin-4-one derivatives lacking toxicity-producing attributes as glucokinase activators: design, synthesis, molecular docking, and in-silico ADMET prediction, Futur. J. Pharm. Sci. 5 (2019) 11. https://doi.org/10.1186/s43094-019-0012-y.

[17] Y.Q. Li, Y.L. Zhang, S.Q. Hu, Y.L. Wang, H.R. Song, Z.Q. Feng, L. Lei, Q. Liu, Z.F. Shen, Design, synthesis and biological evaluation of novel glucokinase activators, Chinese Chem. Lett. 22 (2011) 73–76. https://doi.org/10.1016/j.cclet.2010.07.023.

[18] E.R. Muñoz, B.M. Barnett, Evaluation of the genotoxicity of aniline·HCl in Drosophila melanogaster, Mutat. Res. Toxicol. Environ. Mutagen. 413 (1998) 15–22. https://doi.org/10.1016/S1383-5718(98)00007-2.

[19] T. Iino, N. Hashimoto, T. Hasegawa, M. Chiba, J. Eiki, T. Nishimura, Metabolic activation of N-thiazol-2-yl benzamide as glucokinase activators: Impacts of glutathione trapping on covalent binding, Bioorg. Med. Chem. Lett. 20 (2010) 1619–1622. https://doi.org/10.1016/j.bmcl.2010.01.041.

[20] W. Mao, M. Ning, Z. Liu, Q. Zhu, Y. Leng, A. Zhang, Design, synthesis, and pharmacological evaluation of benzamide derivatives as glucokinase activators, Bioorg. Med. Chem. 20 (2012) 2982–2991. https://doi.org/10.1016/j.bmc.2012.03.008.

[21] T. Iino, Y. Sasaki, M. Bamba, M. Mitsuya, A. Ohno, K. Kamata, H. Hosaka, H. Maruki, M. Futamura, R. Yoshimoto, S. Ohyama, K. Sasaki, M. Chiba, N. Ohtake, Y. Nagata, J. ichi Eiki, T. Nishimura, Discovery and structure-activity relationships of a novel class of quinazoline glucokinase activators, Bioorganic Med. Chem. Lett. 19 (2009) 5531–5538. https://doi.org/10.1016/j.bmcl.2009.08.064.

[22] T. Iino, N. Hashimoto, K. Sasaki, S. Ohyama, R. Yoshimoto, H. Hosaka, T. Hasegawa, M. Chiba, Y. Nagata, J. ichi Eiki, T. Nishimura, Structure-activity relationships of 3,5-disubstituted benzamides as glucokinase activators with potent in vivo efficacy, Bioorganic Med. Chem. 17 (2009) 3800–3809. https://doi.org/10.1016/j.bmc.2009.04.040.

[23] N. Charaya, D. Pandita, A.S. Grewal, V. Lather, Design, synthesis and biological evaluation of novel thiazol-2-yl benzamide derivatives as glucokinase activators, Comput. Biol. Chem. 73 (2018) 221–229.

https://doi.org/10.1016/j.compbiolchem.2018.02.018.

[24] A.S. Grewal, R. Kharb, D.N. Prasad, J.S. Dua, V. Lather, Design, synthesis and evaluation of novel 3,5-disubstituted benzamide derivatives as allosteric glucokinase activators, BMC Chem. 13 (2019) 1–14. https://doi.org/10.1186/s13065-019-0532-8.

[25] Z. Wang, X. Shi, H. Zhang, L. Yu, Y. Cheng, H. Zhang, H. Zhang, J. Zhou, J. Chen, X. Shen, W. Duan, Discovery of cycloalkyl-fused N-thiazol-2-yl-benzamides as tissue non-specific glucokinase activators: Design, synthesis, and biological evaluation, Eur. J. Med. Chem. 139 (2017) 128–152. https://doi.org/10.1016/j.ejmech.2017.07.051.

[26] L. Zhang, H. Li, Q. Zhu, J. Liu, L. Chen, Y. Leng, H. Jiang, H. Liu, Benzamide derivatives as dual-action hypoglycemic agents that inhibit glycogen phosphorylase and activate glucokinase, Bioorganic Med. Chem. 17 (2009) 7301–7312. https://doi.org/10.1016/j.bmc.2009.08.045.

[27] A.S. Grewal, S. Singh, D. Pandita, V. Lather, Design , Synthesis and Antidiabetic Activity of Novel Sulfamoyl Benzamide Journal of Pharmaceutical Tehnology Research and Management, 6 (2018) 115–124. https://doi.org/10.15415/jptrm.2018.62008.

[28] K. Park, B.M. Lee, Y.H. Kim, T. Han, W. Yi, D.H. Lee, H.H. Choi, W. Chong, C.H. Lee, Discovery of a novel phenylethyl benzamide glucokinase activator for the treatment of type 2 diabetes mellitus, Bioorg. Med. Chem. Lett. 23 (2013) 537–542. https://doi.org/10.1016/j.bmcl.2012.11.018.

[29] H. Fujieda, M. Kogami, M. Sakairi, N. Kato, M. Makino, N. Takahashi, T. Miyazawa, S. Harada, T. Yamashita, Discovery of a potent glucokinase activator with a favorable liver and pancreas distribution pattern for the treatment of type 2 diabetes mellitus, Eur. J. Med. Chem. 156 (2018) 269–294. https://doi.org/10.1016/j.ejmech.2018.06.060.

[30] A.M. Deshpande, D. Bhuniya, S. De, B. Dave, V.P. Vyavahare, S.H. Kurhade, S.R. Kandalkar, K.P. Naik, B.S. Kobal, R.D. Kaduskar, S. Basu, V. Jain, P. Patil, S. Chaturvedi Joshi, G. Bhat, A.A. Raje, S. Reddy, J. Gundu, V. Madgula, S. Tambe, P. Shitole, D. Umrani, A. Chugh, V.P. Palle, K.A. Mookhtiar, Discovery of liver-directed glucokinase activator having anti-hyperglycemic effect without hypoglycemia, Eur. J. Med. Chem. 133 (2017) 268–286. https://doi.org/10.1016/j.ejmech.2017.03.042.

[31] F. Li, Q. Zhu, Y. Zhang, Y. Feng, Y. Leng, A. Zhang, Design, synthesis, and pharmacological evaluation of N-(4-mono and 4,5-disubstituted thiazol-2-yl)-2-aryl-3-(tetrahydro-2H-pyran-4-yl)propanamides as glucokinase activators, Bioorganic Med. Chem. 18 (2010) 3875–3884. https://doi.org/10.1016/j.bmc.2010.04.038.

[32] K.G. Pike, J. V. Allen, P.W.R. Caulkett, D.S. Clarke, C.S. Donald, M.L. Fenwick, K.M. Johnson, C. Johnstone, D. McKerrecher, J.W. Rayner, R.P. Walker, I. Wilson, Design of a potent, soluble glucokinase activator with increased pharmacokinetic half-life, Bioorganic Med. Chem. Lett. 21 (2011) 3467–3470. https://doi.org/10.1016/j.bmcl.2011.03.093.

[33] D. McKerrecher, J. V. Allen, P.W.R. Caulkett, C.S. Donald, M.L. Fenwick, E. Grange, K.M. Johnson, C. Johnstone, C.D. Jones, K.G. Pike, J.W. Rayner, R.P. Walker, Design of a potent, soluble glucokinase activator with excellent in vivo efficacy, Bioorg. Med. Chem. Lett. 16 (2006) 2705–2709. https://doi.org/10.1016/j.bmcl.2006.02.022.

[34] K.J. Filipski, A. Guzman-Perez, J. Bian, C. Perreault, G.E. Aspnes, M.T. Didiuk, R.L.

Dow, R.F. Hank, C.S. Jones, R.J. Maguire, M. Tu, D. Zeng, S. Liu, J.D. Knafels, J. Litchfield, K. Atkinson, D.R. Derksen, F. Bourbonais, K.S. Gajiwala, M. Hickey, T.O. Johnson, P.S. Humphries, J.A. Pfefferkorn, Pyrimidone-based series of glucokinase activators with alternative donor–acceptor motif, Bioorg. Med. Chem. Lett. 23 (2013) 4571–4578. https://doi.org/10.1016/j.bmcl.2013.06.036.

[35] J. Xu, S. Lin, R.W. Myers, G. Addona, J.P. Berger, B. Campbell, H. Chen, Z. Chen, G.J. Eiermann, N.H. Elowe, B.T. Farrer, W. Feng, Q. Fu, R. Kats-Kagan, M. Kavana, S. Malkani, D.R. McMasters, K. Mitra, M.J. Pachanski, X. Tong, M.E. Trujillo, L. Xu, B. Zhang, F. Zhang, R. Zhang, E.R. Parmee, Novel, highly potent systemic glucokinase activators for the treatment of Type 2 Diabetes Mellitus, Bioorg. Med. Chem. Lett. 27 (2017) 2069–2073. https://doi.org/10.1016/j.bmcl.2016.10.085.

[36] L. Zhang, X. Chen, J. Liu, Q. Zhu, Y. Leng, X. Luo, H. Jiang, H. Liu, Discovery of novel dual-action antidiabetic agents that inhibit glycogen phosphorylase and activate glucokinase, Eur. J. Med. Chem. 58 (2012) 624–639. https://doi.org/10.1016/j.ejmech.2012.06.020.

[37] Y. Li, K. Tian, A. Qin, L. Zhang, L. Huo, L. Lei, Z. Shen, H. Song, Z. Feng, Discovery of novel urea derivatives as dual-target hypoglycemic agents that activate glucokinase and PPARγ, Eur. J. Med. Chem. 76 (2014) 182–192. https://doi.org/10.1016/j.ejmech.2014.02.024.

[38] J.A. Pfefferkorn, A. Guzman-Perez, P.J. Oates, J. Litchfield, G. Aspnes, A. Basak, J. Benbow, M.A. Berliner, J. Bian, C. Choi, K. Freeman-Cook, J.W. Corbett, M. Didiuk, J.R. Dunetz, K.J. Filipski, W.M. Hungerford, C.S. Jones, K. Karki, A. Ling, J.C. Li, L. Patel, C. Perreault, H. Risley, J. Saenz, W. Song, M. Tu, R. Aiello, K. Atkinson, N. Barucci, D. Beebe, P. Bourassa, F. Bourbounais, A.M. Brodeur, R. Burbey, J. Chen, T. D'Aquila, D.R. Derksen, N. Haddish-Berhane, C. Huang, J. Landro, A. Lee Lapworth, M. MacDougall, D. Perregaux, J. Pettersen, A. Robertson, B. Tan, J.L. Treadway, S. Liu, X. Qiu, J. Knafels, M. Ammirati, X. Song, P. Dasilva-Jardine, S. Liras, L. Sweet, T.P. Rolph, Designing glucokinase activators with reduced hypoglycemia risk: Discovery of N,N-dimethyl-5-(2-methyl-6-((5-methylpyrazin-2-yl)-carbamoyl)benzofuran-4- yloxy)pyrimidine-2-carboxamide as a clinical candidate for the treatment of type 2 diabetes mellitus, Medchemcomm. 2 (2011) 828–839. https://doi.org/10.1039/c1md00116g.

[39] M. Jahan, P. Johnström, S. Nag, A. Takano, O. Korsgren, L. Johansson, C. Halldin, O. Eriksson, Synthesis and biological evaluation of [11C]AZ12504948; a novel tracer for imaging of glucokinase in pancreas and liver, Nucl. Med. Biol. 42 (2015) 387–394. https://doi.org/10.1016/j.nucmedbio.2014.12.003.

[40] J. Xu, S. Lin, R.W. Myers, M.E. Trujillo, M.J. Pachanski, S. Malkani, H. Chen, Z. Chen, B. Campbell, G.J. Eiermann, N. Elowe, B.T. Farrer, W. Feng, Q. Fu, R. Kats-Kagan, M. Kavana, D.R. McMasters, K. Mitra, X. Tong, L. Xu, F. Zhang, R. Zhang, G.H. Addona, J.P. Berger, B. Zhang, E.R. Parmee, Discovery of orally active hepatoselective glucokinase activators for treatment of Type II Diabetes Mellitus, Bioorg. Med. Chem. Lett. 27 (2017) 2063–2068. https://doi.org/10.1016/j.bmcl.2016.10.088.

[41] T. Deligeorgiev, A. Vasilev, J.J. Vaquero, J. Alvarez-Builla, A green synthesis of isatoic anhydrides from isatins with urea–hydrogen peroxide complex and ultrasound, Ultrason. Sonochem. 14 (2007) 497–501. https://doi.org/10.1016/j.ultsonch.2006.12.003.

[42] T. Nishimura, T. Iino, M. Mitsuya, M. Bamba, H. Watanabe, D. Tsukahara, K. Kamata, K. Sasaki, S. Ohyama, H. Hosaka, M. Futamura, Y. Nagata, J. ichi Eiki, Identification of novel and potent 2-amino benzamide derivatives as allosteric glucokinase activators, Bioorganic Med. Chem. Lett. 19 (2009) 1357–1360. https://doi.org/10.1016/j.bmcl.2009.01.053.

[43] M. Mitsuya, K. Kamata, M. Bamba, H. Watanabe, Y. Sasaki, K. Sasaki, S. Ohyama, H. Hosaka, Y. Nagata, J. ichi Eiki, T. Nishimura, Discovery of novel 3,6-disubstituted 2-pyridinecarboxamide derivatives as GK activators, Bioorganic Med. Chem. Lett. 19 (2009) 2718–2721. https://doi.org/10.1016/j.bmcl.2009.03.137.

[44] K. Kamata, M. Mitsuya, T. Nishimura, J.I. Eiki, Y. Nagata, Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase, Structure. 12 (2004) 429–438. https://doi.org/10.1016/j.str.2004.02.005.

[45] E. Harder, W. Damm, J. Maple, C. Wu, M. Reboul, J.Y. Xiang, L. Wang, D. Lupyan, M.K. Dahlgren, J.L. Knight, J.W. Kaus, D.S. Cerutti, G. Krilov, W.L. Jorgensen, R. Abel, R.A. Friesner, OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins., J. Chem. Theory Comput. 12 (2016) 281–96. https://doi.org/10.1021/acs.jctc.5b00864.

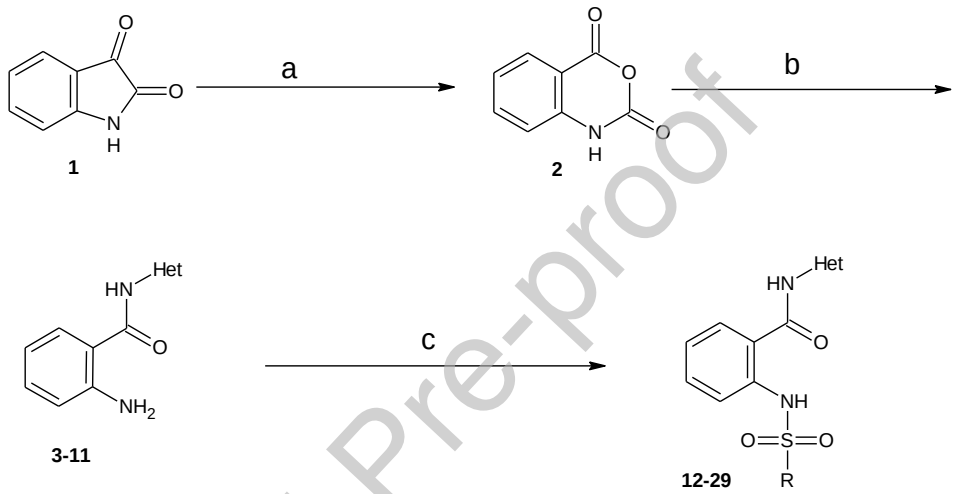
[46] M.O. Taha, M. Habash, M.A. Khanfar, The use of docking-based comparative intermolecular contacts analysis to identify optimal docking conditions within glucokinase and to discover of new GK activators, J. Comput. Aided. Mol. Des. 28 (2014) 509–547. https://doi.org/10.1007/s10822-014-9740-4.

[47] A. Daina, O. Michielin, V. Zoete, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 42717. https://doi.org/10.1038/srep42717.

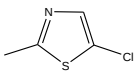
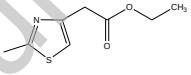
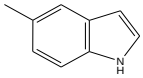
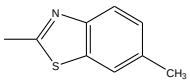
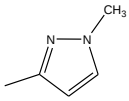
[48] S.K. Lee, Y. Kang, G.S. Chang, I.H. Lee, S.H. Park, J. Park, K.Y. Sung, H.J. Kim, J.E. Chung, Bioinformatics and Molecular Design Research Center, Seoul, South Korea, Yonsei Univ. (2017). https://preadmet.bmdrc.kr.

[49] C.A. Lipinski, Lead- and drug-like compounds: The rule-of-five revolution, Drug Discov. Today Technol. 1 (2004) 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007.

[50] P. Rydberg, L. Olsen, Predicting Drug Metabolism by Cytochrome P450 2C9: Comparison with the 2D6 and 3A4 Isoforms, ChemMedChem. 7 (2012) 1202–1209. https://doi.org/10.1002/cmdc.201200160.



Scheme 1: General scheme for the synthesis of compounds **12-29**. Reagents and conditions: (**a**) $(CH_3CO)_2$, CH_3COOH, H_2SO_4, $NH_2CONH_2.H_2O_2$, stirring at r.t. for 24 h (**b**) Dry toluene, substituted amine, reflux, 4-6 h (**c**) Dry pyridine, TsCl/MsCl, reflux, 6h.

Compd	**3**	**12**	**21**	**4**	**13**	**22**	**5**	**14**	**23**	**6**	**15**	**24**	**7**	**16**	**25**
Het															
R	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-

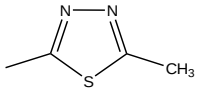
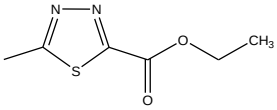
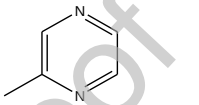
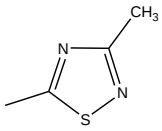
Compd	**8**	**17**	**26**	**9**	**18**	**27**	**10**	**19**	**28**	**11**	**20**	**29**
Het												
R	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-	---	-CH_3	4-CH_3-C_6H_4-

Table 1: *In vitro* Glucokinase assay and molecular docking results

Compd	EC_{50} (nM)	Glide Score	Binding free energy (kcal/ mol)	Hydrogen bonding			$\pi - \pi$ interaction	
				Atom of ligand	Amino acids	Distance (Å)	Ring of ligand	Amino acid
12	495	-9.185	-53.76	N	ARG 63	1.91	NI[b]	NI
				O	ARG 63	2.06		
				H (*Arom.*)	TYR 215	2.54		
13	1586	-5.673	-58.88	N	ARG 63	1.87	NI	NI
				O	ARG 63	2.12		
				H (*Arom.*)	TYR 215	2.52		
14	1113	-7.029	-48.12	N	ARG 63	2.01	Phenyl	TYR 214
				O	ARG 63	1.84	Pyrrole	ARG 250
				O	THR 65	1.72		
				H (*Arom.*)	ARG 63	2.62		
				H (*Arom.*)	TYR 215	2.66		
				H (*Arom.*)	GLU 221	--		
15	522	-6.482	-51.79	N	ARG 63	2.48	Benzothiazole	LYS 459
				O	THR 65	2.55		
				N	ARG 63	2.46		
				O	ARG 63	2.29		
				H (*Arom.*)	TYR 215	2.59		
16	1063	-7.532	-49.12	N	ARG 63	1.93	NI	NI
				O	ARG 63	2.43		
				H (*Arom.*)	TYR 215	2.50		
17	958	-7.706	-53.81	N	ARG 63	1.91	NI	NI
				O	ARG 63	2.11		
				H (*Arom.*)	TYR 215	2.51		
18	1224	-7.524	-57.88	N	ARG 63	1.99	Thiadiazole	TYR 214
				O	ARG 63	1.92		
				O	THR 65	1.74		
				H (*Arom.*)	LEU 451	2.46		
				H (*Arom.*)	TYR 215	2.39		
19	1052	-6.151	-52.83	N	ARG 63	2.07	Phenyl	TYR 214
				O	ARG 63	2.05		
				O	THR 65	1.76		
				H (*Arom.*)	LEU 451			
20	3312	-6.334	-51.6	N	ARG 63	2.09	Phenyl	TYR 214

				O	ARG 63	1.97		
				O	THR 65	1.93		
				N (Thiazole)	ARG 250	2.40		
21	657	-7.624	-60.07	N	ARG 63	1.87	NI	NI
				O	ARG 63	1.97		
				H (*Arom.*)	CYS 220	2.46		
				H (*Arom.*)	TYR 215	2.49		
22	1874	-6.338	-65.44	N	ARG 63	2.25	Phenyl	TYR 214
				O	ARG 63	1.92		
				H (*Arom.*)	CYS 220	2.45		
				H (*Arom.*)	TYR 215	2.48		
23	1648	-7.448	-56.96	N	ARG 63	2.04	Pyrrole	LYS 459
				H (*Arom.*)	CYS 220	2.47		
				H (*Arom.*)	VAL 452	2.80		
				H (*Arom.*)	TYR 215	2.52		
24	836	-7.526	-69.45	N	ARG 63	2.12	Phenyl	TYR 214
				O	ARG 63	2.24	Phenyl	LYS 459
				N (*Arom.*)	ARG 63	2.44		
				H (*Arom.*)	CYS 220	2.65		
				H (*Arom.*)	ARG 63	2.63		
25	2853	-6.28	-53.77	N	ARG 63	1.88	NI	NI
				O	ARG 63	2.28		
				H (*Arom.*)	CYS 220	2.48		
				H (*Arom.*)	TYR 215	2.47		
26	1321	-7.471	-62.48	N	ARG 63	1.90	NI	NI
				O	ARG 63	2.43		
				H (*Arom.*)	TYR 215	--		
27	1625	-6.436	-59.42	N	ARG 63	1.88	NI	NI
				O	ARG 63	2.17		
				H (*Arom.*)	TYR 215	2.51		
				H (*Arom.*)	CYS 220	2.47		
				N (*Arom.*)	ARG 63	2.41		
28	1454	-6.543	-64.59	N	ARG 63	2.09	Phenyl	TYR 214
				O	ARG 63	2.48		
				H (*Arom.*)	CYS 220	2.71		
				H (*Arom.*)	ARG 63	2.58		
				H (*Arom.*)	TYR 215	2.44		
				H (*Arom.*)	TYR 61	2.71		
				N (*Arom.*)	ARG 63	2.48		

29	2327	-6.410	-69.81	N	ARG 63	2.03	NI	NI
				O	ARG 63	2.06		
				H (*Arom.*)	CYS 220	2.59		
				H (*Arom.*)	ARG 63	2.56		
				H (*Arom.*)	TYR 215	2.41		
				N (*Arom.*)	ARG 63	2.69		
Dorzagliatin	--	-7.949	-54.49	OH	TYR 61	1.91	Phenyl	TYR 214
				OH	TYR 61	1.75		
				H (*Arom.*)	CYS 220	--		
Piragliatin	365[a]	-7.891	-63.44	N	ARG 63	2.04		
				N (*Arom.*)	ARG 632	2.2		
				O	GLN 98	2.05		
RO-28-1675	353[a]	-6.394	-48.58	O	THR 65	1.82	Thiazole	TYR 214
				H (*Arom.*)	ARG 63	2.39		
				H (*Arom.*)	ARG 63	2.58		
				H (*Arom.*)	SER 453	2.73		

[a]Data from ref. 16
[b] NI: No interaction

Table 2: *In silico* ADMET predictions using SwissADME and PreADMET software

Compd no.	HBA	HBD	n-rotb	TPSA Å²	Log $P_{o/w}$	Log *S*	BBB	CYP2D6 inhibitor	Lipinski-violations	hERG_ Inhibition	Carcino test	HIA (%)
12	4	2	5	124.78	1.85	-3.61	No	No	0	+	negative	97.65
13	6	2	9	151.08	1.58	-3.1	No	No	0	+	negative	87.96
14	3	3	5	99.44	1.93	-3.55	No	Yes	0	++	negative	88.57
15	4	2	5	124.78	2.77	-4.37	No	No	0	+	negative	95.18
16	4	2	5	101.47	0.56	-2.32	No	No	0	++	negative	92.46
17	5	2	5	137.67	1.05	-2.77	No	No	0	+	negative	87.15
18	7	2	8	163.97	1.15	-2.96	No	No	0	+	negative	77.01
19	5	2	5	109.43	0.53	-2.06	No	No	0	+	negative	91.57
20	5	2	5	137.67	1.06	-3.01	No	No	0	+	negative	87.14
21	5	2	5	137.67	1.06	-3.01	No	No	0	++	negative	95.14
22	6	2	10	151.08	3.22	-4.8	No	No	0	++	negative	95.58
23	3	3	6	99.44	3.46	-5.22	No	Yes	0	+++	negative	91.84
24	4	2	6	124.78	4.3	-5.95	No	No	0	++	negative	95.45
25	4	2	6	101.47	2.24	-4.01	No	Yes	0	+++	negative	94.27
26	5	2	6	137.67	2.7	-4.46	No	No	0	++	negative	95.17
27	7	2	9	163.97	2.8	-4.66	No	No	0	++	negative	92.46
28	5	2	6	109.43	2.06	-3.75	No	Yes	0	++	negative	94.46
29	5	2	6	137.67	2.59	-4.71	No	No	0	++	negative	95.17

HBA (hydrogen bond acceptor): ≤ 10; HBD (hydrogen bond donor): ≤ 5; n-rotb (No. of rotatable bonds): ≤ 10; TPSA (Topological Polar Surface Area): ≤ 130 Å²; Log Po/w (octanol/water partition coefficient): -0.7 to +5.0; Log S (aqueous solubility) Scale: Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very Soluble < 0 < Highly soluble; GI absorption (Oral absorption); BBB (blood brain barrier permeability, central nervous system toxicity); CYP2D6 inhibitor (Hepatotoxicity); Lipinski (number of violations of Lipinski's rule of five): maximum is 4; hERG_inhibition: High risk: +++, medium risk: ++, low risk: +; carcino test (carcinogenicity): Positive or negative; HIA: human intestinal absorption.

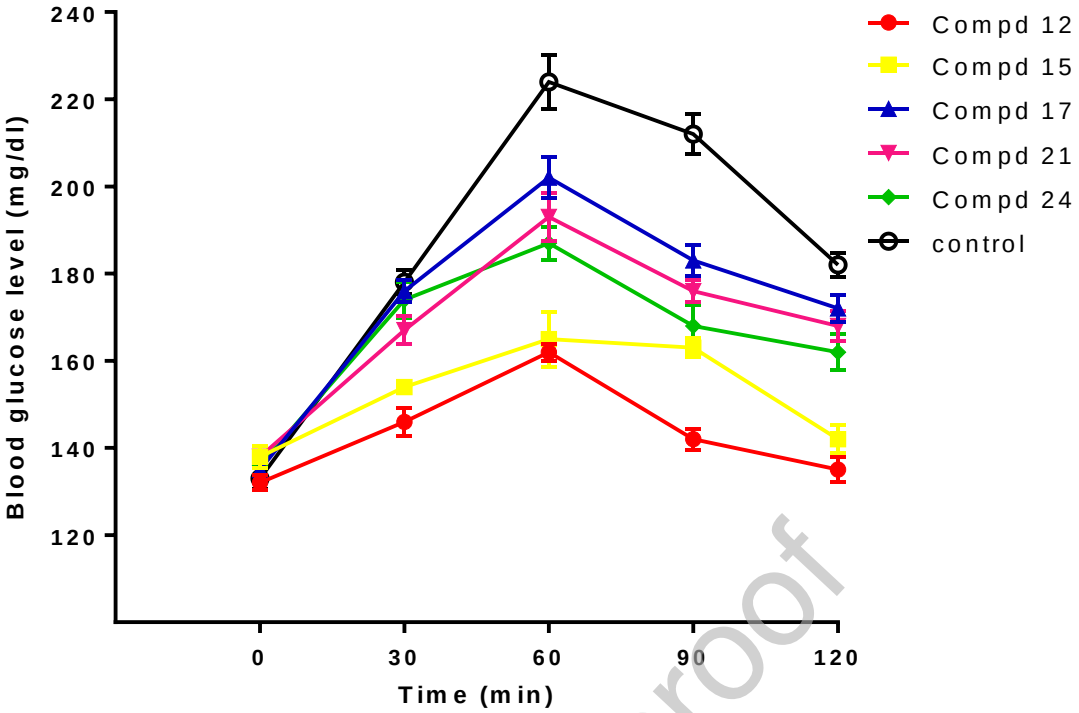


Fig. 1: Reduction in the blood glucose levels at different time intervals (0-120 min). Data expressed as Mean ± SEM (n=6), $p<0.05$

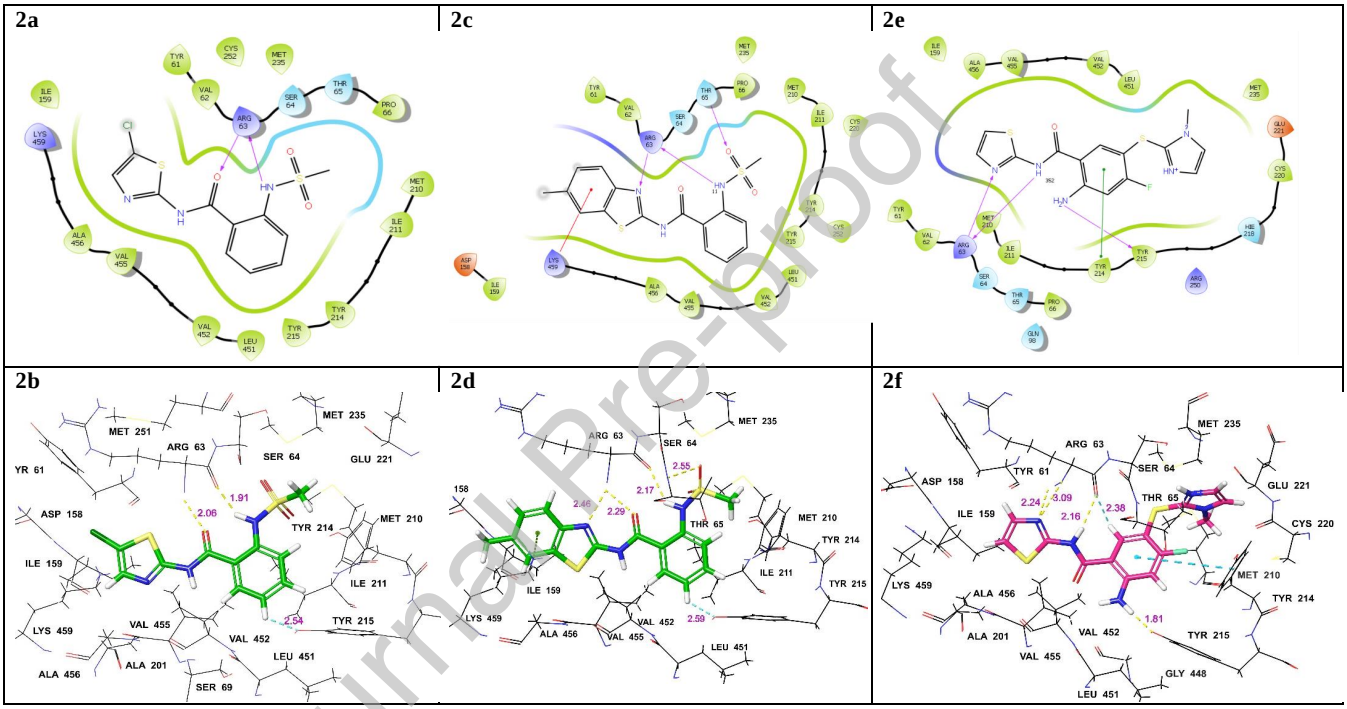
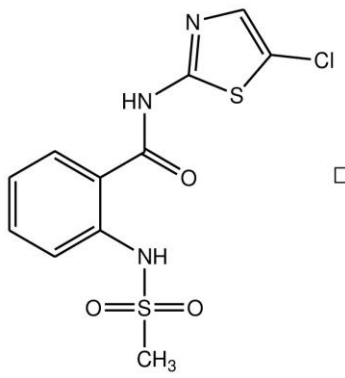
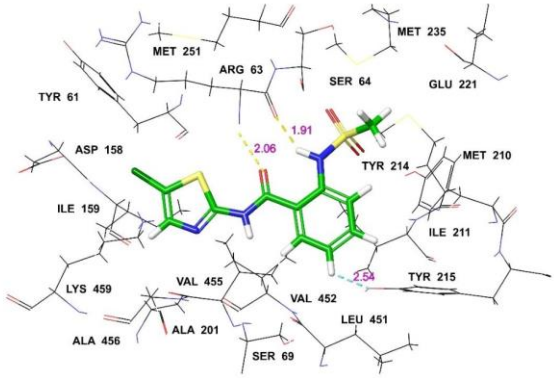


Fig. 2 (a-f): 2D and 3D docking poses of ligands in the allosteric binding site of GK protein (PDB ID: 1V4S). In the 2D poses, hydrogen bonds are represented by pink sticks, and the arrow points to the hydrogen bond acceptor, π-π interactions by a solid green stick and hydrophobic interactions by green amino acid residues; whereas in the 3D poses, hydrogen bonds are depicted as yellow/blue dotted sticks. (**2a**) 2D pose of compound **12**; (**2b**) 3D pose of compound **12**; (**2c**) 2D pose of compound **15**; (**2d**) 3D pose of compound **15**; (**2e**) 2D pose of co-crystallized ligand (MRK); (**2f**) 3D pose of co-crystallized ligand (MRK).



Compound **12**, EC_{50} = 495 nM



3D docking pose of compound **12** in the binding site of 1V4S enzyme

Graphical-Abstract