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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of newer N-benzimidazol-2yl benzamide analogues as allosteric activators of human glucokinase

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Abstract

Allosteric activators of human glucokinase (GK) had revealed significant hypoglycemic effects for therapy of type-2 diabetes (T2D) in animal as well as human models. Some newer N-benzimidazol-2yl substituted benzamide analogues were prepared and assessed for activation of GK accompanied by molecular docking investigations for predicting the bonding interactions of these derivatives with the residues in allosteric site of GK protein. Amongst the derivatives synthesized, compounds **2** and **7** strongly increased catalytic action of GK (GK activation fold >2.0 in comparison to control) in vitro. The results of in-vitro testing were supported by the molecular docking investigations of these analogues with GK protein's allosteric site residues (showed appreciable H-bond interactions with Arg63 residue of GK). Derivatives investigated in present study afforded few lead compounds for the discovery of harmless and strong allosteric GK activating compounds for treating T2D.

Keywords Allosteric · Benzamides · Benzimidazoles · Glucokinase · Docking · GK assay

Introduction

Type-2 diabetes (T2D) is a chronic disease of the food metabolic pathways owing to decreased insulin action resulting in hyperglycemia and prevalent amongst most of the patients suffering from diabetes [1–3]. Although ample types of oral antidiabetic agents are existing to be used for the management of T2D, no individual antidiabetic agent is valuable in attaining persistent homeostasis of plasma sugar within usual physiological range in majority of the persons suffering from T2D. Owing to the above points, these days doctors advise combination of hypoglycemic agents at an initial phase of T2D treatment. Additionally, overdose of hypoglycemic drugs may possibly result in serious hypoglycemia triggering brutal adverse reactions, and patients generally require urgent medical treatment [2, 4, 5]. Now-a-days, medicinal chemistry scientists are aiming at designing newer effective hypoglycemic agents having distinct mechanism of action at molecular level which could be used as single drug with improved safety [6, 7]. Glucokinase (GK) is a cytosolic enzyme that is primarily expressed in pancreatic β-cells and liver hepatocytes; as well as lifts up glucose transformation to glucose-6-phosphate with the aid of adenosinetriphosphate (ATP) [8, 9]. In beta-cells of pancreas gland, GK regulates glucose-instigated discharge of insulin and in liver hepatocytes of liver; it commands the breakdown of sugars. GK acts as an emergent medication focus for treatment and management of T2D due to its key function in controlling sugar breakdown. Small molecule activators of human GK are the unique class of therapeutically useful agents that allosterically activate GK and illustrate their plasma sugar lowering potential [6, 9–12]. Several GK activators had been progressed into clinical trials (phase II) including AZD6370, AZD1656, MK-0941, Piragliatin, and AMG151; even though strong decrease in blood sugar was observed, potential adverse reactions were also reported, such as hypoglycemia and elevated levels of triglycerides. Literature survey revealed that most of the drug discovery and development research associated with allosteric activators of human GK were mainly focused on the substituted benzamide analogues (specially bearing a hetero-aromatic ring connected to the benzamide NH- atom) probably owing towards their corresponding alignment outline and bonding interactions with the residues of allosteric binding site of GK protein [4, 6, 13–27].

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Based on the above facts few newer N-benzimidazol-2-yl benzamide analogues were proposed as potential activators of human GK enzyme.

Materials and methods

Chemicals were acquired from reputed companies such as Spectrochem, SRL, S.D. Fine-Chem, Merck, Fisher Scientific and Sigma-Aldrich etc., and employed without purification. Melting points of the synthesized molecules were estimated by uncorrected Veego VMP-D melting point device. Silica Gel-G TLC was used for monitoring the progress of chemical reaction. Shimadzu FTIR spectrophotometer using potassium bromide pellet process was utilized for recording IR spectra. 'Avance-II (Bruker) 400 MHz NMR spectrophotometer' was employed for taking ^{1}H-NMR and ^{13}C-NMR spectra employing appropriate dutereated solvents. Results were represented in 'parts per million' ('δ', ppm) downfield to 'Si $(CH_3)_4$' (internal reference).

General procedure for preparation of designed molecules

Dry benzoic acid (1 mmol) was transferred to a flat base flask fixed on a magnetic agitator at constant temperature around 10 °C. Excess of $HClSO_3$ (8.0 mL) was added carefully and observed to avoid any escape. When all the acid was liquefied and the exothermic response terminated, the flat bottom flask was heated at 70–80 °C using water bath for 2 h followed by cooling. The materials of flask were poured to crushed ice (150 g) cautiously with stirring and crystals of 3-(chlorosulphonyl)benzoic acid were filtered employing vacuum subsequent to cold water wash followed by air drying. The precipitates prepared above (1 mmol) were then reacted with the corresponding aliphatic and aromatic amines (1 mmol) under reflux using acetone till completion of reaction (observed using silica gel G TLC) following cooling and drying of the precipitates. The different sulphonamides prepared above (1 mmol) were refluxed for 3 h with sulfinyl chloride (1 mmol) and to receive equivalent acid chlorides excess sulfinyl chloride was withdrawn. Acid chlorides prepared above (1 mmol) were refluxed with 2-aminobenzimidazole (1.5 mmol). The end products (compounds 1–10) obtained by the evaporation of solvent were purified using recrystallization from ethanol [28–30].

N-(1H-Benzimidazol-2-yl)-3-(phenylsulfamoyl)benzamide (1)

Pale white solid; Yield: 72%; Mp (°C) 158–161; FTIR (KBr Pellets) ν cm^{-1}: 3867.78 (NH str., CONH), 3737.50 (NH str., Benzamide), 3432.08.46 (NH str., SO_2NH), 2973.38 (CH str., Aromatic), 1642.58 (Carbonyl C=O str., Benzamide), 1558.12 (NH bend, Aromatic amine), 1463.36 (C=N str., Aromatic, Benzimidazol-2-yl), 1417.54 (C=C str., Aromatic), 1296.70 (SO_2 asym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 1076.13 (SO_2 sym. str., SO_2NH, Sulphonamide), 752.08 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 12.68 (s, 1H, NH, CONH), 8.02–8.64 (m, 4H, CH, C_2, C_4, C_5 and C_6 of C_6H_4CO), 7.36–8.46 (m, 4H, CH, Benzimidazol-2-yl), 6.38–7.19 (m, 5H, CH of C_2, C_3, C_4, C_5 and C_6 of C_6H_5), 3.00 (s, 1H, NH, Benzimidazol-2-yl), 2.58 (s, 1H, NH, SO_2NH); ^{13}C-NMR (δ ppm, DMSO-d6): 168.67 (C=N), 164.15 (C=O), 151.78 (C), 136.16 (C), 134.89 (C), 131.78 (C), 130.13 (CH), 129.17 (CH), 128.41 (C), 128.02 (CH), 125.23 (CH), 123.67 (CH), 120.15 (CH), 119.34 (CH), 117.97 (CH), 115.42 (CH).

N-(1H-Benzimidazol-2-yl)-3-[(2-chloro-4-nitrophenyl) sulfamoyl]benzamide (2)

Yellowish brown solid; Yield: 78%; Mp (°C) 162–165; FTIR (KBr Pellets) ν cm^{-1}: 3836.20 (NH str., CONH), 3446.91 (NH str., SO_2-NH), 2928.28 (CH str., Aromatic), 1641.34 (C=O str., CONH), 1632.23 (NH bend, Ar–NH), 1551.35 (C=N str., Aromatic), 1464.11 (NO_2 sym. str., NO_2), 1413.94 (NO_2 asym. str., NO_2), 1299.66 (SO_2 asym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 1079.66 (SO_2 sym. str., SO_2NH), 684.36 (C–Cl str., Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 9.28 (s, 1H, NH, CONH), 8.67–8.43 (m, 4H, CH, C_6H_4CO), 7.34–7.91 (m, 4H, CH, Benzimidazol-2-yl), 7.08–7.24 (m, 3H, CH, $C_6H_3ClNO_2$), 4.48 (s, 1H, NH, Benzimidazol-2-yl), 4.05 (s, 1H, NH, SO_2NH); ^{13}C-NMR (δ ppm, DMSO-d6): 169.78 (C=N), 164.58 (C=O), 147.46 (C), 139.06 (C), 137.12 (C), 135.03 (C), 134.45 (C), 132.08 (CH), 129.12 (CH), 128.69 (C), 127.58 (CH), 126.47 (C), 125.07 (CH), 124.17 (CH), 119.58 (CH), 118.14 (CH), 114.36 (CH), 112.94 (CH).

N-(1H-Benzimidazol-2-yl)-3-(benzylsulfamoyl)benzamide (3)

Light brown solid; Yield: 62%; Mp (°C) 160–163; FTIR (KBr Pellets) ν cm^{-1}: 3755.80 (NH str., Benzamide), 3448.08 (NH str., SO_2NH), 2996.40 (CH str., Aromatic), 2912.85 (CH str., Aliphatic), 1659.53 (C=O str., CONH), 1429.38 (NH bend, Ar–NH), 1311.51 (SO_2 asym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 1025.25 (SO_2 sym. str., SO_2NH), 696.02 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 10.93 (s, 1H, NH, CONH), 8.20–8.51 (m, 4H, CH, C_6H_4CO), 7.70–8.14 (m, 4H, CH, Benzimidazol-2-yl), 7.12–7.58 (m, 5H, CH, C_6 of C_6H_5), 6.64 (t, 1H, NH, Sulphonamide), 2.54 (s, 1H, NH, Benzimidazol-2-yl), 2.51 (d, 1H, CH, CH_2); ^{13}C-NMR (δ ppm, DMSO-d6): 175.18 (C=N), 164.76 (C=O), 153.46 (C), 141.24 (C), 140.15 (C),

135.14 (C), 135.03 (C), 132.26 (CH), 131.92 (CH), 128.08 (CH), 124.14 (CH), 122.16 (CH), 120.94 (CH), 119.04 (CH), 116.68 (CH), 45.88 (CH_2).

N-(1H-Benzimidazol-2-yl)-3-(butylsulfamoyl)benzamide (4)

Light brown solid; Yield: 59%; Mp (°C) 156–158; FTIR (KBr Pellets) ν cm^{-1}: 3754.38 (NH str., CONH, Benzamide), 3448.26 (NH str., SO_2NH, Sulphonamide), 2930.77 (CH str., Aromatic), 2962.66 (CH str., Aliphatic), 1643.43 (C=O str., CONH, Benzamide), 1554.13 (NH bend, Ar–NH), 1464.82 (C=C str., Aromatic), 1415.35 (SO_2 asym. str., SO_2NH, Sulphonamide), 1100.00 (C–N str., Benzimidazol-2-yl), 1076.78 (SO_2 sym. str., SO_2NH); ^{1}H-NMR (δ ppm, DMSO-d_6): 8.59 (s, 1H, NH, CONH), 7.34–7.88 (m, 4H, CH, C_6H_4CO), 7.05–7.86 (m, 4H, CH, Benzimidazol-2-yl), 2.54 (t, 1H, NH, Sulphonamide), 2.50 (s, 1H, NH, Benzimidazol-2-yl), 1.31 (m, 9H, Butyl); ^{13}C-NMR (δ ppm, DMSO-d6): 175.18 (C=N), 165.29 (C=O), 151.49 (C), 139.65 (C), 135.26 (C), 135.02 (C), 131.07 (CH), 128.44 (CH), 127.68 (CH), 124.59 (CH), 119.28 (CH), 118.93 (CH), 118.02 (CH), 115.02 (CH), 45.38 (CH_2), 25.58 (CH_2), 21.07 (CH_2), 15.08 (CH_3).

N-(1H-benzimidazol-2-yl)-3-(methylsulfamoyl)benzamide (5)

Dark brown solid; Yield: 52%; Mp (°C) 148–152; FTIR (KBr Pellets) ν cm^{-1}: 3798.48 (NH str., CONH), 3448.44 (NH str., SO_2NH), 3017.57 (CH str., Aromatic), 2966.14 (CH str., Alkyl), 1654.21 (Carbonyl str., CONH), 1598.09 (NH bend, Aromatic amine), 1544.68 (C=N str., Aromatic), 1388.45 (SO_2 asym. str., SO_2NH), 1189.77 (SO_2 sym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 789.65 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 8.60 (s, 1H, NH, CONH), 7.49–7.98 (m, 4H, CH, C_6H_4CO), 7.10–7.58 (m, 4H, CH, C_4, C_5, C_6 and C_7 of Benzimidazol-2-yl), 5.20 (t, 1H, NH, Sulphonamide), 2.54 (s, 1H, NH, Benzimidazol-2-yl), 2.51 (s, 3H, Methyl); ^{13}C-NMR (δ ppm, DMSO-d6): 174.27 (C=N), 165.16 (C=O), 152.86 (C), 141.06 (C), 138.84 (C), 135.38 (CH), 133.08 (C), 132.66 (CH), 127.58 (CH), 126.05 (CH), 122.98 (CH), 120.94 (CH), 118.18 (CH), 117.08 (CH), 31.14 (CH_3).

N-(1H-Benzimidazol-2-yl)-3-[(2-methylphenyl)sulfamoyl] benzamide (6)

Light brown solid; Yield: 60%; Mp (°C) 162–165; FTIR (KBr Pellets) ν cm^{-1}: 3791.96 (NH str., CONH, Benzamide), 3456.56 (NH str., SO_2NH, Sulphonamide), 3013.67 (CH str., Aromatic), 2912.67 (CH str., Aliphatic), 1667.25 (NH str., SO_2NH), 1604.66 (NH bend, Aromatic amine), 1578.56 (C=N str., Aromatic), 1345.34 (SO_2 asym. str., SO_2NH, Sulphonamide), 1103.78 (SO_2 sym. str., SO_2NH, Sulphonamide), 1100.00 (C–N str., Benzimidazol-2-yl), 850.55 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 10.27 (s, 1H, NH, CONH), 8.01–8.46 (m, 4H, CH, C_6H_4CO), 7.34–8.02 (m, 4H, CH, Benzimidazol-2-yl), 7.04–7.39 (m, 4H, CH, $C_6H_4CH_3$), 2.57 (s, 1H, NH, Benzimidazol-2-yl), 2.18 (s, 1H, NH, SO_2NH), 2.14 (s, 3H, Methyl); ^{13}C-NMR (δ ppm, DMSO-d6): 175.16 (C=N), 165.26 (C=O), 151.42 (C), 139.55 (C), 138.06 (C), 136.32 (C), 134.87 (CH), 133.34 (C), 129.67 (C), 129.12 (CH), 126.55 (CH), 125.12 (CH), 123.56 (CH), 121.68 (CH), 117.36 (CH), 17.32 (CH_3).

N-(1H-Benzimidazol-2-yl)-3-[(4-bromophenyl)sulfamoyl] benzamide (7)

Grayish black solid; Yield: 74%; Mp (°C) 158–160; FTIR (KBr Pellets) ν cm^{-1}: 3837.14 (NH str., Benzamide), 3732.98 (NH str., Benzamide), 3441.64 (NH str., SO_2NH, Sulphonamide), 2974.87 (CH str., Aromatic carbon), 1641.67 (Carbonyl str., Benzamide), 1553.91 (NH bend, Aromatic amine), 1464.33 (C=N str., Aromatic), 1415.88 (SO_2 asym. str., SO_2NH), 1296.76 (SO_2 sym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 809.70 (CH bend, Aromatic), 753.82 (C–Br str., Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 9.49 (s, 1H, NH, CONH), 7.78–8.66 (m, 4H, CH, C_6H_4CO), 7.14–7.92 (m, 4H, CH, Benzimidazol-2-yl), 6.20–7.44 (m, 4H, CH, C_6H_4Br), 4.48 (s, 1H, NH, Benzimidazol-2-yl), 4.02 (s, 1H, NH, SO_2NH); ^{13}C-NMR (δ ppm, DMSO-d6): 175.28 (C=N), 165.82 (C=O), 152.47 (C), 142.05 (C), 139.10 (C), 136.97 (C), 136.26 (C), 132.98 (C), 132.02 (CH), 130.36 (CH), 130.01 (CH), 129.67 (CH), 127.12 (CH), 124.84 (CH), 122.04 (CH), 120.18 (CH), 116.45 (CH).

N-(1H-Benzimidazol-2-yl)-3-[(4-methylphenyl)sulfamoyl] benzamide (8)

Light brown solid; Yield: 63%; Mp (°C) 160–163; FTIR (KBr Pellets) ν cm^{-1}: 3870.59 (NH str., Benzamide), 3755.40 (NH str., CONH), 3452.66 (NH str., SO_2NH), 2997.49 (CH str., Aromatic), 1708.27 (C=O str., Amide), 1429.03 (NO_2 sym. str., Aromatic nitro group), 1362.55 (NO_2 asym. str., Aromatic nitro group), 1323.98 (SO_2 asym. str., SO_2NH), 1223.02 (SO_2 sym. str., SO_2NH), 1100.00 (C–N str., Benzimidazol-2-yl), 696.02 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 7.95 (s, 1H, NH, CONH), 7.16–7.90 (m, 4H, CH, C_6H_4CO), 7.26–7.84 (m, 4H, CH, Benzimidazol-2-yl), 7.16–7.88 (m, 4H, CH, $C_6H_4NO_2$), 5.00 (s, 1H, NH, Benzimidazol-2-yl), 2.50 (s, 1H, NH, Sulphonamide); ^{13}C-NMR (δ ppm, DMSO-d6): 174.63 (C=N), 164.94 (C=O), 151.68 (C), 142.21 (C), 139.04 (C), 138.14 (C), 135.28 (C), 132.76 (C), 132.12 (CH), 128.69 (CH), 125.36 (CH), 122.09 (CH), 120.01

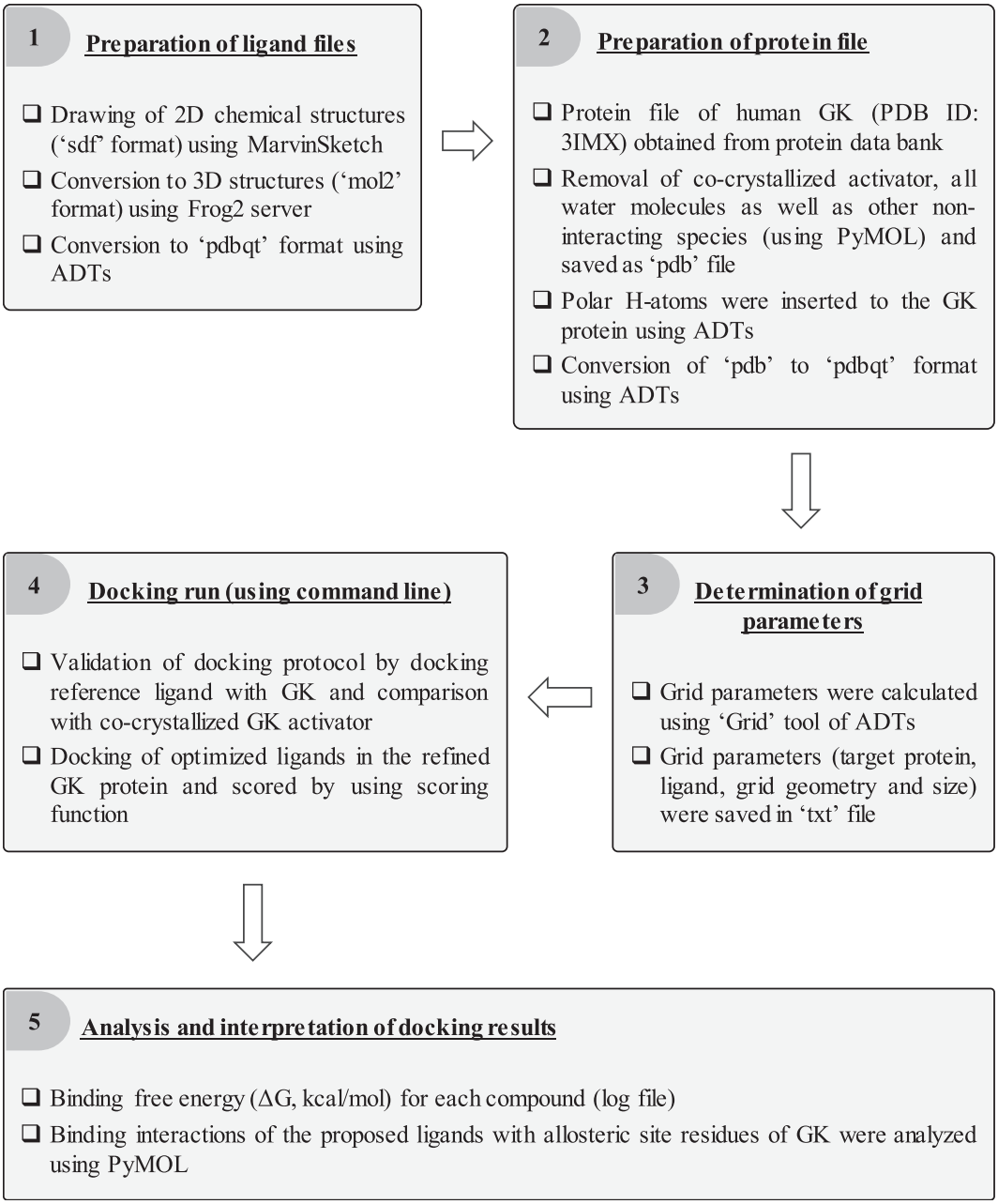
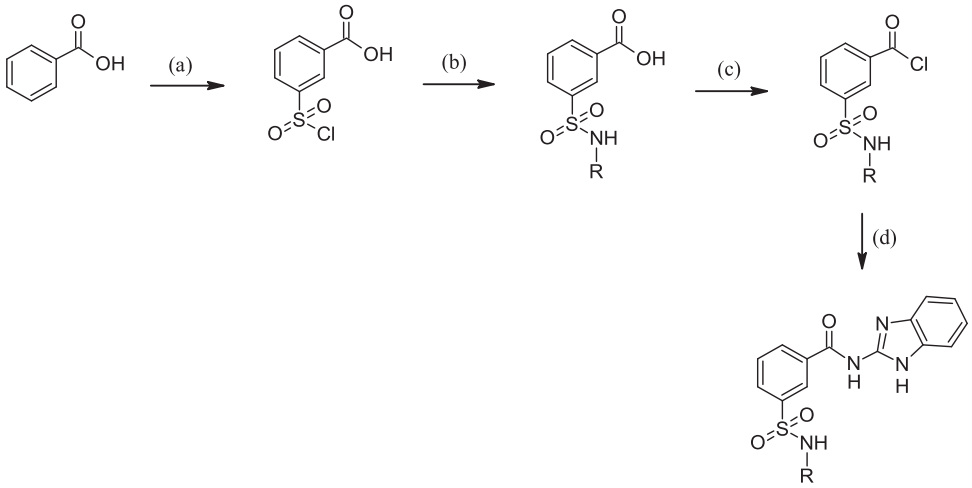


Fig. 1 Procedure used for docking of designed analogues in the allosteric site of GK protein

(CH), 119.25 (CH), 118.42 (CH), 117.68 (CH), 115.18 (CH), 24.14 (CH_3).

N-(1H-Benzimidazol-2-yl)-3-[(4-nitrophenyl)sulfamoyl] benzamide (9)

Pale yellow solid; Yield: 74%; Mp (°C) 165–168; FTIR (KBr Pellets) ν cm^{-1}: 3868.16 (NH str., CONH), 3754.28 (NH str., CONH), 3448.36 (NH str., SO_2NH), 2930.77 (CH str., Aromatic), 1643.31 (C=O str., CONH), 1553.03 (NH bend), 1524.42 (NO_2 sym. str.), 1464.83 (C=N str., Aromatic), 1441.52 (NO_2 asym. str.), 1415.35 (CH bend, Aliphatic), 1300.62 (SO_2 asym. str., SO_2NH), 1100.00 (C–N str., Aromatic), 1076.79 (SO_2 sym. str., SO_2NH), 717.52 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 10.47 (s, 1H, NH, CONH), 7.89–8.32 (m, 4H, CH, C_6H_4CO), 7.39–7.56 (m, 4H, CH, Benzimidazol-2-yl), 7.20–7.50 (m, 4H, CH, $C_6H_4CH_3$), 2.50 (s, 1H, NH, Benzimidazol-2-yl); ^{13}C-NMR (δ ppm, DMSO-d6): 175.56 (C=N), 165.08 (C=O), 153.84 (C), 143.80 (C), 139.46 (C), 138.86 (C), 137.47 (CH), 135.31 (C), 133.94 (C), 131.08 (CH), 129.95 (CH), 129.18 (CH), 126.02 (CH), 122.91



Scheme 1 General scheme employed for synthesis of N-benzimidazol-2-yl benzamide analogues. Reagents and conditions: **a** '$HClSO_3$'; **b** 'NH_2-R', reflux; **c** 'SO_2Cl', reflux; **d** 2-Amino benzimidazole, reflux

(CH), 121.27 (CH), 120.67 (CH), 119.37 (CH), 116.62 (CH).

N-(1H-benzimidazol-2-yl)-3-(propylsulfamoyl)benzamide (10)

Light brown solid; Yield: 48%; Mp (°C) 155–158; FTIR (KBr Pellets) ν cm^{-1}: 3450.06 (NH str., CONH), 2996.68 (NH str., SO_2NH), 2912.98 (CH str.), 1689.51 (C=O str., CONH), 1428.92 (NH bend), 1311.73 (C=N str., Aromatic), 1100.00 (C–N str., Benzimidazol-2-yl), 1023.65 (SO_2 asym. str., SO_2NH), 950.47 (SO_2 sym. str., SO_2NH), 696.27 (CH bend, Aromatic); ^{1}H-NMR (δ ppm, DMSO-d_6): 10.25 (s, 1H, NH, CONH), 8.37–8.71 (s, 3H, CH, C_6H_3CO), 7.56–7.94 (m, 4H, CH, Benzimidazol-2-yl), 2.61 (s, 1H, NH, Benzimidazol-2-yl), 2.58 (s, 1H, NH, SO_2NH), 2.44 (m, 2H, Methylene), 2.05 (m, 2H, Methylene), 1.27 (t, 3H, methyl); ^{13}C-NMR (δ ppm, DMSO-d6): 174.24 (C=N), 165.02 (C=O), 151.33 (C), 139.00 (C), 136.88 (C), 134.65 (C), 130.34 (CH), 129.17 (CH), 125.37 (CH), 124.92 (CH), 120.49 (CH), 118.78 (CH), 118.02 (CH), 117.33 (CH), 46.04 (CH_2), 32.48 (CH_2), 12.44 (CH_3).

In silico estimation of pharmacokinetic properties

The designed molecules were analyzed for the prediction of pharmacokinetics using FAF-Drugs4 server; as well as accessed for their "drug likeness" potential utilizing Lipinski's rule [27, 31, 32].

In vitro GK assay

GK activation potential of all the derivatives was assessed employing a combined response with glucose-6-phosphate dehydrogenase (G6PDH) using spectrometry. All the samples were made using DMSO and the in-vitro GK test was done in an ultimate quantity of '2000 µL' comprising of 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4) (0.25 mM), 10 mM dextrose (10 mM), KCl (25 mM), $MgCl_2$ (1 mM), 1,4-dithio-D-threitol (1 mM), 1 mM nicotinamide adenine dinucleotide, 1 mM ATP, G6PDH (2.5 U/mL), 0.5 µg GK, and derivatives to be tested (10 µM). Readings were taken at 340 nm following nurture time of 3 min and GK activation was computed in comparison to DMSO (activation of GK by DMSO alone was treated as 100%). All the results were represented as mean ($n = 3$) ± standard deviation (S.D.). The in vitro GK assay data for test groups was statistically analyzed by one-way ANOVA for comparison and significance from control group (value of $p < 0.05$) using GraphPad Prism (GraphPad Software Inc.) [22, 24, 33, 34].

Molecular docking investigations

AutoDock Vina and AutoDock Tools were used for executing molecular docking of all the analogues in the allosteric location of the GK (PDB ID: '3IMX') [35, 36]. The protocol followed for docking of designed analogues with GK is presented in Fig. 1 as previously reported [22, 24, 26, 30, 37, 38].

In silico prediction of toxicity

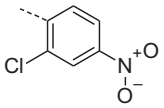
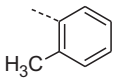
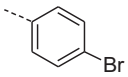
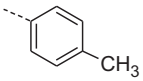
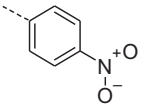
All samples were screened in silico to estimate the potential toxicity of such substances using online computer software pkCSM [39–41].

Results and discussion

Chemistry

3-(Chlorosulphonyl)-benzoic acid acquired through chlorosulphonation of benzoic acid was reacted with different amine derivatives to obtain various sulphonamide analogues

Table 1 Physicochemical characteristics of the prepared N-benzimidazol-2-yl benzamide analogues

Compound	R	Mol. formula	M. Pt. (°C)	R_f*	% Yield
1	-C_6H_5	$C_{20}H_{16}N_4O_3S$	158-161	0.61	72
2		$C_{20}H_{14}ClN_5O_5S$	162-165	0.53	78
3	-$CH_2C_6H_4$	$C_{21}H_{18}N_4O_3S$	160-163	0.62	62
4	-C_4H_9	$C_{18}H_{20}N_4O_3S$	156-158	0.73	59
5	-CH_3	$C_{15}H_{24}N_4O_3S$	148-152	0.57	52
6		$C_{21}H_{18}N_4O_3S$	162-165	0.78	60
7		$C_{20}H_{15}BrN_4O_3S$	158-160	0.47	74
8		$C_{21}H_{18}N_4O_3S$	160-163	0.72	63
9		$C_{20}H_{15}N_5O_5S$	165-168	0.68	74
10	-C_3H_7	$C_{17}H_{18}N_4O_3S$	155-158	0.67	48

[a]TLC; mobile phase: Toluene: Ethyl acetate (7:3)

which were then reacted with SO_2Cl followed by reaction with 2-aminobenzimidazole to synthesize the desired compounds (Scheme 1) in good yield (Table 1).

A singlet signal comparable to one CONH proton at around δ 10 ppm was observed in the proton NMR spectra of the synthesized molecules verifying the existence of 'benzamide link' in synthesized compounds. The detection of singlet signal for one 'NH proton' of SO_2NH functional moiety was recorded ~2.5 ppm, demonstrating the development of sulphonamide analogues by an interaction of sulphonyl chloride compounds with the respective amines. The presence of a singlet, two doublets and a triplet signal about δ 8 ppm referring to ^{1}H-atoms at C_2, C_4, C_5 and C_6; correspondingly of the benzoic acid-derived phenyl moiety verified that the 'benzamide link' and the 'sulphonamide linkage' were meta to one another i.e., alienated by C_2. Two doublets were recorded in the proton-NMR spectra of prepared compounds in 7–8 ppm range, apart from 2 'triplet signals' pointing to four ^{1}H-atoms (corresponding to aromatic CH), demonstrating that 2-aminobenzimidazole was combined with benzoyl chloride for development of benzamide derivatives. Occurrence of singlet signal at δ 5 ppm corresponding to NH of benzimidazol-2-yl further confirmed presence of benzimidazol-2-yl scaffold in synthesized compounds. In ^{13}C-NMR pattern of the prepared molecules signals around δ 170 ppm indicated the existence of C=N bond in these derivatives and signals about δ 165 ppm illustrated occurrence of amide C=O bond therefore supporting development of benzamide linkage in produced derivatives. The FTIR spectrum of manufactured N-benzimidazol-2-yl substituted benzamide analogues demonstrated the occurrence of characteristics 'stretching frequencies' at 3300–3200, above 3000, 1400-1300/1200-1100 (asymmetric) and 3400–3100 cm^{-1} which correspond to amide NH, aromatic CH, SO_2, sulphonamide NH functional groups respectively, therefore supporting the linkage of amide (CONH) and sulphonamide (SO2–NH) groups in developed molecules. Furthermore, the presence of carbonyl C=O stretching (1700–1600 cm^{-1}) and NH-bending (1600 cm^{-1}) vibrations in spectrum of molecules signified the occurrence of amide carbonyl and aromatic NH-functional group (Fig. 2).

Prediction of ADME properties

ADME parameters such as 'molecular weight' (MW), 'partition coefficient' (log *P*), distribution coefficient (log *D*),

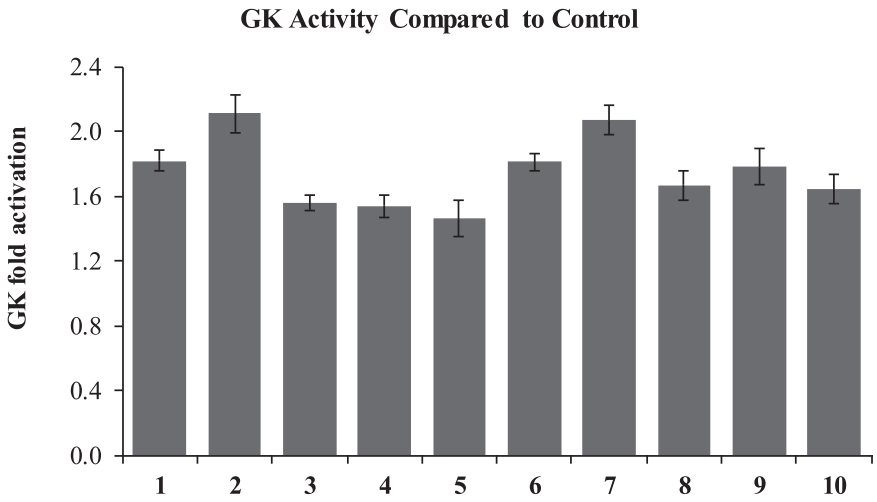


Fig. 2 GK activity (GK fold activation) of the synthesized derivatives (at 10 µM concentration). Values were presented as mean ± S.D. ($n = 3$) and data was significantly dissimilar compared to control ($p < 0.05$)

Table 2 Predicted ADME properties of the designed compounds

Compound	MW	log *P*	log *D*	log S_w	tPSA	HBA	HBD	Solubility	NRB
1	392.43	3.30	3.38	−4.44	112.33	4	3	4623.29	4
2	471.87	3.64	3.55	−5.07	161.72	6	3	2974.48	5
3	406.46	3.12	3.59	−4.33	112.33	4	3	5372.02	5
4	372.44	2.99	3.19	−3.88	112.33	4	3	7705.53	6
5	330.36	1.74	1.86	−3.09	112.33	4	3	15085.68	3
6	406.46	3.54	3.90	−4.66	112.33	4	3	3859.76	4
7	471.33	3.87	4.14	−5.29	112.33	4	3	2377.48	5
8	406.46	3.54	3.92	−4.66	112.33	4	3	3859.76	4
9	437.43	3.01	3.21	−4.46	158.15	6	3	5077.05	5
10	358.41	2.63	2.74	−3.65	112.33	4	3	9341.62	5

water solubility (log S_w), 'topological polar surface area' (tPSA), 'H-bond acceptors' (HBA), 'H-bond donors' (HBD), solubility (mg/L) and 'number of rotatable bonds' (NRB) were predicted for all the designed analogues. All of the designed analogues showed good pharmacokinetic parameters for oral bioavailability (Table 2) and drug-like properties as described using "Lipinski's rule of five" (i.e., MW < 500 Da; log $P < 5$; HBA ≤ 10 and HBD: ≤ 5). All the designed molecules showed MW (330–472 Da), log *P* value (1.70–3.87), HBA (4–6) and HBD (3) within the range of ideal orally bioavailable drug candidate.

In vitro GK assay

The GK catalytic effect of the newly prepared compounds was assessed spectrometrically by calculating the absorbance around 340 nm through coupled interaction with G6PDH. Amongst the tested derivatives, two molecules (compounds **2** and **7**) demonstrated peak GK activation (fold activation exceeding 2.0 in contrasts to control). Other compounds demonstrated modest activation (1.4 to 1.8-fold compared to control) of GK enzyme (Fig. 2).

Amongst the synthesized analogues, compounds bearing N-(2-chloro-4-nitrophenyl) sulphonamide moiety (**2**) and N-(4-bromophenyl) sulphonamide moiety (**7**) exhibited highest GK activity (GK activation fold of 2.11 and 2.07, respectively compared to control). Analogues having N-phenyl, N-2-methylphenyl and N-2- nitrophenyl sulphonamide moiety (**1**, **6** and **9**) demonstrated 1.82, 1.81 and 1.78-fold GK activation, correspondingly. Synthesized compounds having N-4-methylphenyl and N-methyl sulphonamide moiety (**8** and **10**, respectively) exhibited moderate GK activity (1.67 and 1.64-fold GK activation, respectively). Analogues bearing N-benzyl and N-butyl sulphonamide moiety (3 and 4, respectively) exhibited ~1.5 times activation in comparison to control. Derivative having N-methyl sulphonamide moiety (**5**) showed lowest GK fold activation. Outcomes of the GK test demonstrated that replacement of substituted phenyl moiety substituted to SO_2NH resulted in improved GK activity compared to those having alkyl group as can be observed from the GK activation potential of compounds 2, and 7. Substitution of aromatic moiety attached to SO_2NH with alkyl chains have decreased the capacity for GK activation compared to

Table 3 Binding interactions and docking score (ΔG) of the docked designed derivatives

Ligand	H-bond interactions		Residues involved in hydrophobic interactions	ΔG
	Residues	Distance (Å)		
1	Arg63	3.0, 3.0	Pro66, Pro99, Ile159, Ile211, Tyr214, Ala454, Val455	−10.4
2	Arg63	3.1, 2.9	Pro66, Pro99, Ile159, Met 210, Ile211, Tyr214, Ala454, Val455	−10.9
3	Arg63	4.2, 3.8	Pro66, Ile159, Ile211, Tyr214, Ala454, Val455	−9.1
4	Arg63	3.1, 3.0	Pro66, Ile159, Ile211, Tyr214, Ala454, Val455	−9.0
5	Arg63	3.2, 3.0	Pro66, Ile159, Met 210, Ile211, Tyr214, Ala454, Val455	−8.9
6	Arg63	3.0, 3.0	Pro66, Pro99, Ile159, Ile211, Tyr214, Ala454, Val455	−0.0
7	Arg63	2.9, 2.9	Pro66, Pro99, Ile159, Ile211, Tyr214, Ala454	−10.8
8	Arg63	3.1, 3.0	Pro66, Pro99, Ile159, Met 210, Ile211, Tyr214, Ala454	−9.9
9	Arg63	3.0, 3.0	Pro66, Pro99, Ile159, Met 210, Ile211, Tyr214, Ala454	−10.0
10	Ser69	3.1, 3.0	Pro66, Pro99, Ile159, Met 210, Ile211, Tyr214, Ala454	−9.5

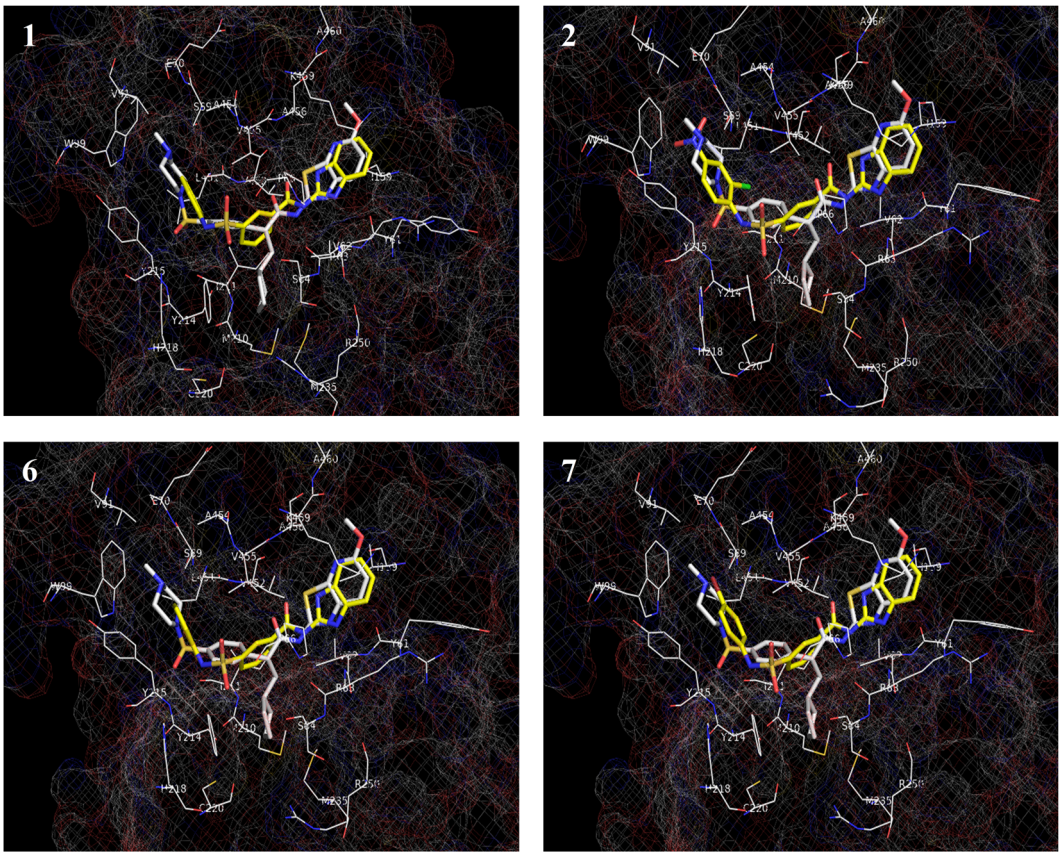


Fig. 3 Superposition of the docked poses of compounds **1, 2, 6** and **7** (yellow) on docked pose of the PDB ligand of 3IMX (gray) in the allosteric binding site of GK protein

compounds 4, 5 and 10 with aromatic moiety as shown by GK activity.

In silico docking investigations

In silico molecular docking investigations were performed to discover the connection and linking behaviors of developed molecules using AutoDock Vina in the allosteric location of GK (PDB ID: 3IMX). The reference GK activator (PDB entry: 3IMX) developed a similar linking sequence and transposed on bonding fashion of co-crystallized GK activator with ΔG of −9.0 kcal/mol validating accuracy of the docking methodology used. Most of the docked ligands showed appreciable linking in allosteric location of GK as established by examining their linkage interactions and ΔG of the paramount docked facades (Table 3). These compounds displayed

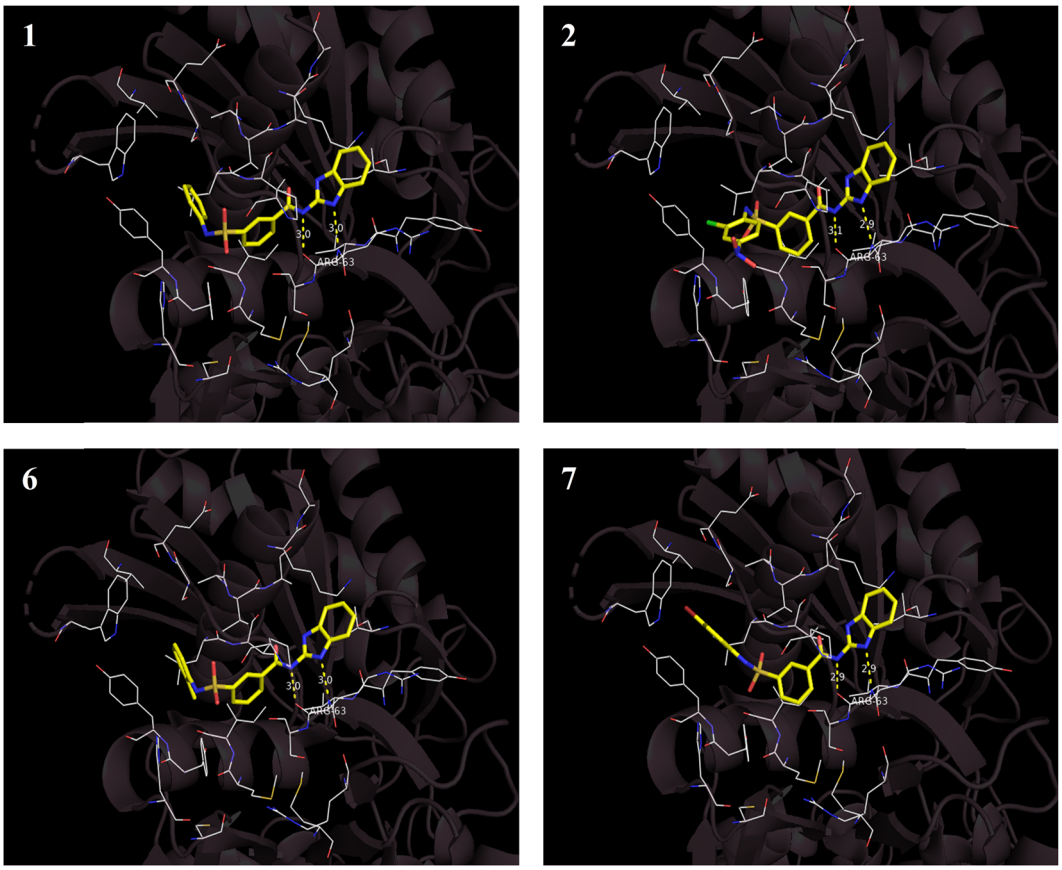


Fig. 4 Best docked poses displaying binding interactions of the analogues **1, 2, 6** and **7** with allosteric site residues of GK protein

strong H-bond interactions between '–NH' of benzamide and 'amide carbonyl' of Arg63; and 'N' of the benzimidazol-2-yl ring and 'amide –NH' of Arg63 in GK protein's allosteric position.

Super-positioning of the docking conformations of analogues **1, 2, 6** and **7**, on that of reference ligand in the allosteric site of GK demonstrated that these molecules had the analogous binding and orientation arrangement in the allosteric site of GK enzyme as that of the x-ray crystallized effector (PDB entry: 3IMX) supporting the outcomes of in vitro GK test for these compounds (Fig. 3).

The docked conformations of analogues **1, 2, 6** and **7** demonstrated strong hydrogen bond interactions between 'N' of benzimidazol-2-yl group and amide –NH of Arg63 residues; and 'benzamide –NH' group and 'backbone carbonyl' of Arg63 in the allosteric location of GK with bond length in the range 2.9–3.1 Å; and 2.9–3.0 Å, respectively. Overall, the benzimidazol-2-yl moiety bonded to the benzamide 'NH' of these compounds protruded in the hydrophobic cavity displaying connections with the Val455 and Lys459 amino acid residues, along with Pro66 and Ile159 amino acid residues, aromatic moiety parceled in the cavity composed of Met210, Tyr214 and Val455 residues (Fig. 4) (Table 3).

In silico prediction of toxicity

The possible toxicity (mutagenic, cardiotoxicity, acute toxicity, hepatotoxicity, skin irritation, and chronic toxicity) for the optimized compounds was accessed using pkCSM online platform. Conferring to the results represented in Table 4; some of the compounds showed little toxicity probability. Mutagenicity was predicted for all the synthesized compounds. Cardiotoxicity (inhibition of hERG-I and hERG-II) was predicted for compounds 1 and 3. Hepatotoxicity was predicted for compounds 1, 3 and 8. In this perspective, the initial evaluation performed in silico using pkCMS online platform, can supplement forthcoming studies related to the safety of these compounds.

Conclusions

In summary, a series of novel N-benzimidazol-2-yl benzamide analogues were designed and prepared using structure-based drug design approach. Among these newly identified derivatives, analogues **2** and **7** unveiled maximum GK activation potential (>2.0-fold increase in GK catalytic

Table 4 Toxicity prediction for the optimized compounds obtained using pkCSM

Compound	Mutagenicity[a]	Cardio-toxicity[b]	Acute toxicity[c]	Chronic toxicity[d]	Hepato-toxicity	Skin irritation	Max. tolerated dose[e]
1	Yes	Yes	2.491	2.516	Yes	No	0.331
2	Yes	No	2.461	3.181	No	No	0.391
3	Yes	Yes	2.494	2.575	Yes	No	0.315
4	Yes	No	2.137	2.592	No	No	0.326
5	Yes	No	2.038	2.613	No	No	0.348
6	Yes	No	2.447	2.865	No	No	0.399
7	Yes	No	2.410	2.774	No	No	0.373
8	Yes	No	2.483	3.224	Yes	No	0.350
9	Yes	No	2.409	2.824	No	No	0.374
10	Yes	No	2.085	2.586	No	No	0.335

[a]Mutagenicity was accessed using "AMES" test

[b]Cardiotoxicity was accessed using hERG-I and hERG-II inhibition

[c]Acute Toxicity: "Oral rat acute toxicity (LD_{50} in mol/kg)"

[d]Chronic Toxicity: "Oral rat chronic toxicity (log mg/kg_bw/day)"

[e]Max. Tolerated Dose (Human): "log mg/kg/day"

activity in vitro). Outcomes of the in-vitro test were found to be analogous to the in-silico docking investigations with the GK enzyme. These analogues followed the 'Lipinski's rule of five' for the "drug-like" characteristics. These newly developed compounds might assist in finding the lead analogues for discovery of strong and harmless activators of GK for T2D handling and management.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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