RESEARCH ARTICLE

In silico Design, Synthesis, and Characterization of New Diazole-benzamide Derivatives as Glucokinase Activators

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ABSTRACT

Background: Glucokinase (GK) is an important enzyme expressed in β-cells of pancreas and liver hepatocytes, acting as an insulin sensor and has a key role in glucose homeostasis. GK, therefore could be an attractive target for treating type 2 diabetes (T2DM). Activators of GK are novel drug candidates that activate the enzyme allosterically and exert their anti-hyperglycemic activity.

Methodology: new derivatives with benzamide nucleus were designed carefully, underwent energy minimization, and then docked using the Genetic Optimization of Ligand Docking (GOLD). The *in silico* design and docking process was followed by the chemical syntheses using a well-defined synthetic scheme ended with the syntheses of these compounds, which were purified and then characterized successfully.

Results: The docking analysis of the synthesized compounds (1c and 2c) suggested a complimentary fitting in the allosteric binding site of GK protein. Considering the PLP fitness, the hydrogen bonding, and the hydrophobic interactions, compound 1c was superior to compound 2c. All the compounds were synthesized with a good yield. Characterization and identification of these compounds were done individually using fourier transform infrared (FTIR) spectroscopy, ^{1}H & ^{13}C nuclear magnetic resonance (NMR), and mass spectrometry (MS). The results of these analyses were consistent with the compounds' proposed structures. These new benzamide derivatives might be used as the starting hits for the establishment of safe, potent, and orally bioavailable GKAs for the treatment of T2DM.

Keywords: Glucokinase activators, *In silico* design, Synthesis.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder; characterized by disturbed glucose and fat metabolism. It results from a deficiency of insulin secretion or poor insulin sensitivity.[1] Type 2 diabetes (T2DM) is the most predominant form of the disease, which constitutes about 90-95% of worldwide cases.[2] Although different oral anti-hyperglycemic agents are available, no mono-therapy or combination regimen is useful for achieving long-lasting control of blood glucose levels in most known cases.[3]

Glucokinase (ATP: D- glucose 6- phosphotransferase, EC 2.7.1.2) is a cytoplasmic enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate (G-6-P).[4] Furthermore, the human type has a low affinity for glucose than its Km value; the concentration of the substrate at which half of the maximum enzyme activity is achieved is about 8 mM.[5] Glucokinase (GK) is highly expressed in hepatocytes, and pancreatic β-cells, wherein both the intracellular and extracellular glucose levels are almost the same (below or around the Km value).[6] Therefore, any change in the plasma concentration of glucose leads to a major and direct change in glucokinase activity. By this, GK plays a unique role in the control of the blood glucose level.[7]

GK has an allosteric activator site that mediates an enhanced catalytic rate (Kcat) and decreases Km for glucose. Glucokinase activators (GKAs) exert there effect by binding to this activator site.

GKAs stimulate in vivo both insulin release and glucose uptake by the liver due to their effect on pancreatic islets and liver hexokinase, making them potentially effective therapeutic agents for the treatment of diabetes.[8,9]

The basic roles of GK in hepatocytes and β-cells are primarily different but complementary. Therefore, the

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pharmacological effects of GKAs in these organs are also different.

GK has a regulatory effect in glucose metabolism of pancreatic β-cells (glycolysis and oxidation), providing that even little changes in enzyme activity (by as little as 10–20%) have an obvious effect on glucose metabolism.[10] On the other hand, the enzyme sustains the large capacity process of glucose clearing in the liver from the postprandial portal blood as the first step in glycogen biosynthesis.

In a few words, GK serves as a "glucose sensor" or "glucoreceptor" molecule, while the GK-containing cells serve as "glucose sensor cells" or "glucoreceptor cells" concerned in the "glucose sensing" process.[11]

The discovered GKAs can be classified chemically into the following groups:

1. Carbon-centered model (alkanes, alkenes, and cyclopropyl subtypes).
2. Aromatic ring-centered model (standard H- bond donor/acceptor).
3. Amino acid-based model.
4. Nitrogen-centered model.

Structure-activity relationship (SAR) studies using crystallography for different series of GKAs revealed the importance of the molecular recognition interacting moiety necessary for binding of these ligands to the allosteric activator binding site of the enzyme. These interacting moieties can be simplified as following[12]:

- R1 aryl moiety provides hydrophobic (pi –pi interactions) with Tyr214 and Tyr215 residues.
- R2 provides hydrophobic interactions with the Met235 side chain.
- R3 provides H-bond donor/acceptor interaction with the Arg63 carbonyl and amide NH (Figure 1).

A large number of new derivatives of GKAs have been discovered and patented. Their mechanisms of action at molecular, cellular, and organ levels had been well established and explained in detail. These investigations showed that the biological and medical bases on which GKAs depend are valid and considered a promising new class of antidiabetic therapy to normalize hyperglycemia in patients with T2DM without medically significant side effects except moderate hypoglycemia at high drug doses.

This work aimed to design, synthesize, and characterize new diazole benzamide derivatives as glucokinase activators and assess their biological activity and properties *in silico* using modern docking software supplied by Cambridge Crystallographic Data Center.

MATERIALS AND METHODS

Materials

The materials used in this work are shown in Table 1.

Instruments

The instruments used in this work are shown in Table 2.

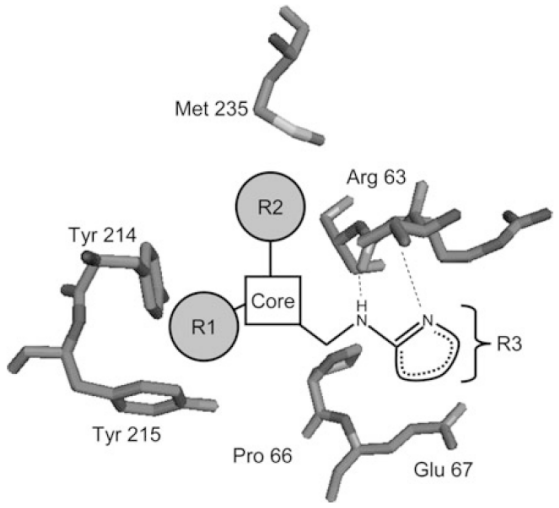


Figure 1: Shows molecular recognition interactions between GKA pharmacophores and corresponding allosteric activator binding sites.[13]

Table 1: The materials utilized in this study, their manufacturers, and country of origin.

No.	*Material*	*Manufacturer*	*Country*	*Purity %*
1	5-amino-1H-imidazole-4-carbonitrile	Hangzhou Hyper Chem	China	95.0
2	Chloroform	Merk	Germany	99.5
3	2-Chloroaniline	Sigma-Aldrich	USA	98.0
4	3-(Chlorosulfonyl) benzoic acid	Sigma-Aldrich	USA	95.0
5	Ethanol	BDH	England	99.0
6	2-Nitroaniline	Sigma-Aldrich	USA	98.0
7	Thionyl chloride (SOCl2)	alpha chemical	India	97.0

Table 2: The instruments utilized in this study, their manufacturers, and country of origin.

No.	*Instrument*	*Manufacturer*	*Country*
1	Chiller Julabo VC (F30)	GMBH	Germany
2	Electronic melting point apparatus	Electrothermal 9300	USA
3	FT-IR spectrophotometer	Schimadzu	Japan
4	^{1}H, ^{13}C-NMR spectrophotometer	Bruker	Germany
5	Mass spectrometer	Schimadzu	Japan

Methods

In silico Docking Studies

Energy Minimization

Both protein and ligand energies were minimized using Chem3D and Mercury (version 4.1.0), then the energy-minimized protein was saved as PDB format while the energy-minimized ligand was saved as mol2 format.

Preparation of Ligand and Protein Receptor

The crystal structure of GK enzyme (PDB ID: 5V4X) was downloaded from the protein data bank (Figure 2). This protein removed all water molecules and metals, and hydrogen atoms were added to obtain amino acid residues' correct ionization and tautomerization.[14]

Docking Procedure

Molecular docking was performed using the full licensed version of GOLD (*v.* 5.7.1). Receptors were set up for the docking process using the Hermes visualizer software and the GOLD suite. The binding sites used for the docking process were identified by detecting all the protein residues within a 10 Å distance away from the reference ligand that existed originally with the downloaded protein structures complex.[15]

Chem piecewise linear potential (PLP) was used as a scoring function. According to Chem PLP, the conformation of the docked molecule with the highest GOLD score (fitness) was selected, and the mode of binding was analyzed consecutively. After GOLD running was finished, the solutions were saved to study and evaluate the predicted interaction and forces between the amino acid residues of GK protein (binding sites) and the functional groups of the candidate ligands.[16]

Depending on the docking results, two compounds were selected to be synthesized; these compounds were listed in Table 3 below with their structures and IUPAC names.

Chemical Synthetic Methods

The synthesis of the diazole benzamide derivatives was achieved following the procedures listed below, and steps were summarized in (Scheme 1).[17,18]

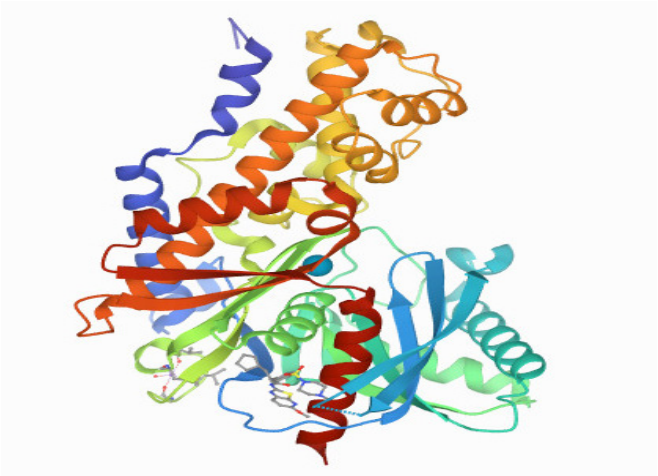


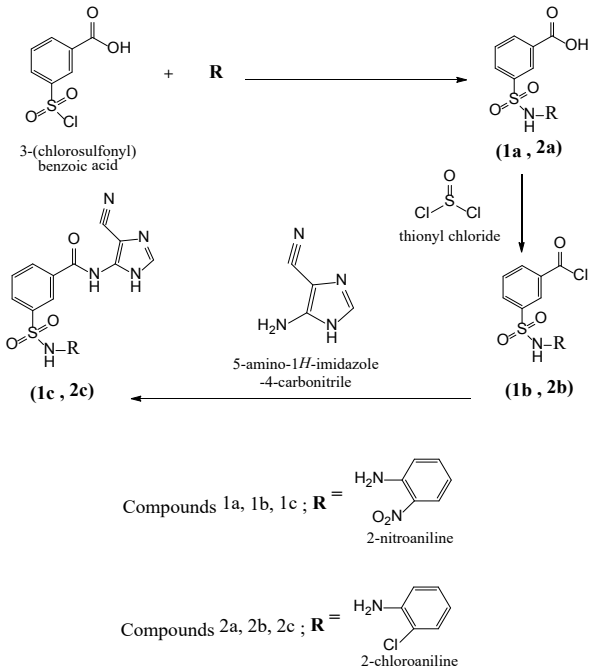
Figure 2: Crystal Structure of human glucokinase in complex with a synthetic activator

Synthesis of compound (1a): 3-(N-(2-nitrophenyl) sulfamoyl) benzoic acid.

Dry 3-(chlorosulphonyl) benzoic acid (2.2 g, 0.01 mol) in (25 mL) chloroform was placed in a round-bottomed flask 100 mL in size, fitted with a stirring bar and condenser, stirred, and refluxed to (70°C). After 15 minutes, (1.38 g, 0.01 mol) of 2-nitroaniline (aromatic amine) was added, and the mixture was refluxed for 7 hours (until the reaction was completed as checked by TLC. The contents of the flask were cooled under room temperature, then filtered, washed, and dried to obtain the precipitate of benzoic acid sulphonamide (compound 1a).

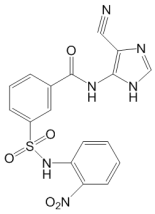
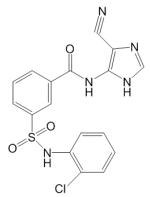
Synthesis of Compound (1b): 3-(N-(2-nitrophenyl) sulfamoyl) benzoyl chloride

Compound (1a) (3.22 g, 0.01 mol) was dissolved in 25 mL chloroform, stirred for 15 minutes, then (0.73 mL, 0.01 mol)



Scheme 1: General synthetic pathway of compounds (1c, 2c)

Table 3: Structures of the designed compounds

Compound	*Structure*	*IUPAC name*
1c		N-(4-cyano-1H-imidazol-5-yl)-3-(N-(2-nitrophenyl) sulfamoyl) benzamide
2c		3-(N-(2-chlorophenyl) sulfamoyl)-N-(4-cyano-1H-imidazol-5-yl)benzamide

of thionyl chloride was added to the solution and refluxed for 4 hours. After completion of reflux, the solvent was evaporated by rotary evaporator, and the product was filtered, then recrystallized by ethanol, dried, and collected as compound (1b).

Synthesis of compound (1c): N-(4-cyano-1H-imidazol-5-yl)-3-(N-(2-nitrophenyl) sulfamoyl) benzamide

The benzoyl chloride (compound 1b) (3.4 g, 0.01 mol) was refluxed with the diazole amine (5-amino-1H-imidazole-4-carbonitrile) (1.08 g, 0.01 mol) in 30 mL chloroform for 7 hours, and the final product compound (1c) was received after the evaporation of chloroform and recrystallization from ethanol to get the final pure product.

Synthesis of compound (2a): 3-(N-(2-chlorophenyl) sulfamoyl) benzoic acid

Dry 3-(chlorosulphonyl) benzoic acid (2.2 g, 0.01 mol) in (25 mL) chloroform was placed in a round-bottomed flask 100 mL in size, fitted with a stirring bar and condenser, stirred, and refluxed to (70°C). After 15 minutes, (1.28 g, 0.01 mol) of 2-chloroaniline (aromatic amine) was added, and the mixture was refluxed for 7 hours (until the reaction was completed as checked by TLC. The contents of the flask were cooled at room temperature, then filtered, washed, and dried to obtain the precipitate of benzoic acid sulphonamide (compound 2a).

Synthesis of compound (2b): 3-(N-(2-chlorophenyl) sulfamoyl) benzoyl chloride

Compound (2a) (3.12 g, 0.01 mol) was dissolved in 25 mL chloroform, stirred for 15 minutes, then (0.73 mL, 0.01 mol) of thionyl chloride was added to the solution and refluxed for 4 hours. After completion of reflux, the solvent was evaporated by a rotary evaporator, and the product was filtered, then recrystallized by ethanol, dried, and collected as compound (2b).

Synthesis of compound (2c): 3-(N-(2-chlorophenyl) sulfamoyl)-N-(4-cyano-1H-imidazol-5-yl) benzamide.

The benzoyl chloride (compound 2b) (3.3 g, 0.01 mol) was refluxed with the diazole amine (5-amino-1H-imidazole-4-carbonitrile) (1.08 g, 0.01 mol) in 30 mL chloroform for 7 hours, and the final product compound (2c) was received after the evaporation of chloroform and recrystallization from ethanol to get the final pure product.

Methods of Characterization and Identification

Thin-layer Chromatography (TLC)

Kieslgel GF_{254} (60) aluminum plates, E. Merck (Germany), were used to run the ascending Thin Layer Chromatography to check the purity of the synthesized compounds and observe the progression of the progression the reactions. Compounds were viewed by the exposure to UV_{254} light.[19]

The following solvent system was used to elute the chromatogram: Benzene: Ethyl acetate (70:30).[20]

Melting Points

The melting points of the synthesized compounds and their precursors were accomplished using the electronic melting point apparatus (digital Stuart scientific SMP30) and are uncorrected.

Infrared Spectra

Infrared spectra were determined and recorded using Shimadzu FTIR-8400 (with resolution; 4[1/cm] and no. of scans; 15) at the chemistry department, College of Science, Mustansiriyah University.

^{1}H and ^{13}C-Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR)

The ^{1}H and ^{13}C-NMR spectra were recorded for the final synthesized compounds; (1c and 2c) using Bruker DMX-500 NMR spectrophotometer (300 MHZ, solvent DMSO-d_6) with TMS (tetramethylsilane) as a standard internal reference at Sharif University of Technology, Iran.

Mass Spectroscopy

Mass spectroscopy was done for the final products; compounds (1c and 2c) using a mass spectrometer (Varian 3900, 5793 Network Mass Selective Detector), Tehran University, Iran.

RESULTS AND DISCUSSION

Structures Docking

In silico computational docking studies were carried out using GOLD (Genetic Optimization of Ligand Docking), which was provided by the Cambridge Crystallographic Data Center (CCDC). Glucokinase enzyme (PDB ID: 5V4X) is the target protein. Compounds 1c and 2c were docked at the allosteric binding site of the enzyme with different GOLD scoring (based on their PLP fitness involved in the complex formation at the active site) and specifications as listed in Table 4.

The docking of chemical structures, including; binding site amino acid residues, hydrogen bonding, hydrophobic interaction, and lengths of bonds along with poses of these compounds at the allosteric activation site of the enzyme, was shown in Figures 3 to 6.

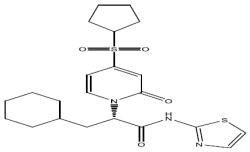
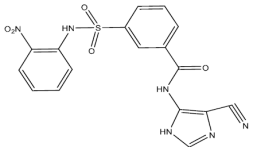
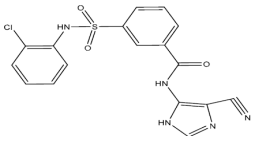
After reviewing several entries, the best ligand-bound complex (PDB ID: 5V4X) was chosen by evaluating the three-dimensional structures with the highest resolution. This enzyme contains a glucose molecule in its catalytic site and an activator molecule (8wj) in its allosteric site.

Depending on the analyses of GK activators reported in the previous studies in the literature binding to the allosteric site of GK, the molecules derived from diazole-benzamide moiety were designed and synthesized. Lead optimization was done by studying the drug-likeness properties of these compounds (molecular weight, log P, hydrogen bond donors (HBD), and hydrogen bond acceptors (HBA) as shown in Tables 5 and 6. According to Lipinski's rule of five, the selected compounds for *in silico* studies had drug-like properties.

The allosteric site of GK is composed primarily of residues from the large and small domains and two loops that connect them, and it is well known that all known GKAs bind at this site.[21]

This allosteric site comprises Arg 63, Tyr 215, Met 210, Tyr 214, Val 452, and Val 455 residues.20 At this site the synthesized compounds were docked. Docking scores which

Table 4: *In silico* design, no. of H-bonding, amino acids included in H-bonding, amino acids included in hydrophobic interactions, and docking score (PLP fitness)

Comp	*Structure*	*No. of H-bonding*	*Amino acids included in H-bonding*	*Amino acids included in hydrophobic interactions*	*Docking score (PLP fitness)*
REF. Ligand (8 wj)		3	TRP 99, TYR 214, ARG 63	TYR 214, TYR 215, TRP 99, ARG 63	121.75
1c		2	TYR 214, TRP 99	TYR 214, TRP 99, ILE 211, CYS 220, GLU 221, MET 210, PRO 66	77.95
2c		1	TRP 99	LEU 451, PRO 66, TRP 99, ILE 211	70.23

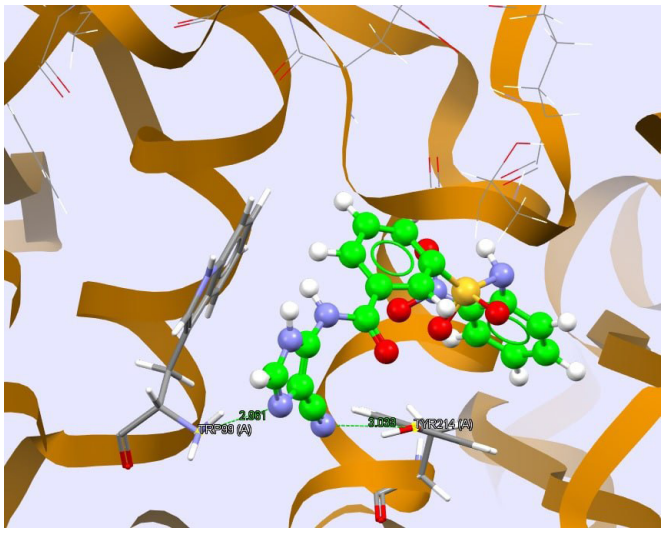


Figure 3: Crystal structure of compound 1c with GK (PDB entry: 5V4X). Amino acid residues included in H-bonding: TYR 214, TRP 99. [1c: Ball and stick style, amino acid residues in a capped stick style].

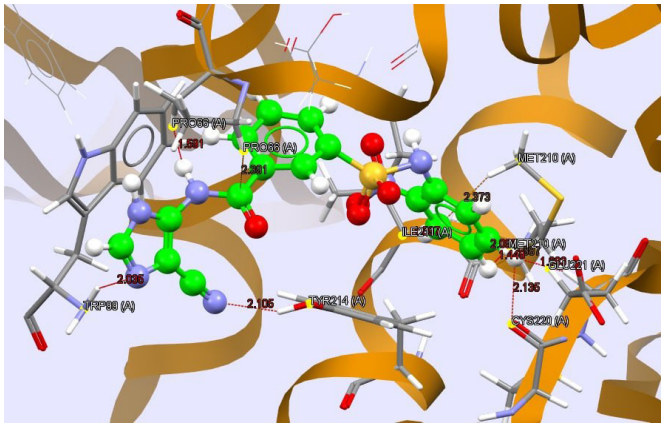


Figure 4: Crystal structure of compound 1c with GK (PDB entry: 5V4X). Amino acid included in hydrophobic interactions: TYR 214, TRP 99, ILE 211, CYS 220, GLU 221, MET 210, PRO 66.

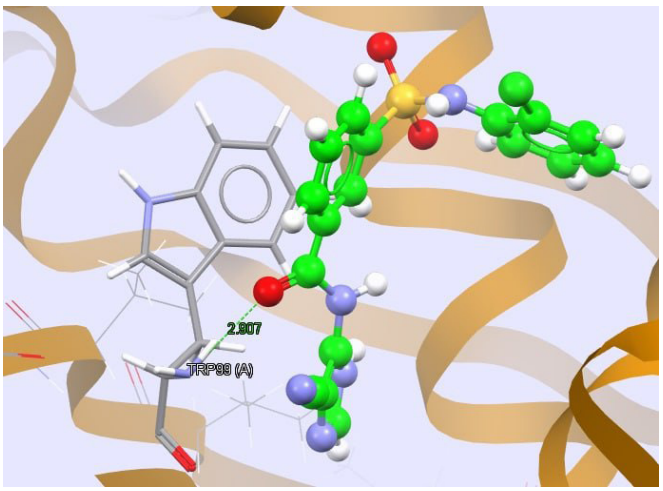


Figure 5: Crystal structure of compound 2c with GK (PDB entry: 5V4X). Amino acid residues included in H-bonding: TRP 99. [1c: Ball and stick style, amino acid residues in a capped stick style].

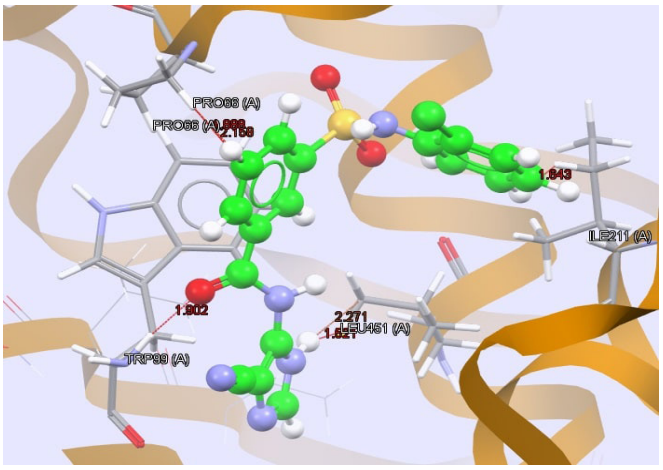


Figure 6: Crystal structure of compound 2c with GK (PDB entry: 5V4X). Amino acid included in hydrophobic interactions: LEU 451, PRO 66, TRP 99, ILE 211.

Table 5: Physical properties of the synthesized compounds:

Comp	*Physical appearance*	*Melting point (°C)*	*Log P*	*tPSA**	*R_f value*	*Water solubility*
1c	Orange-brown precipitate	146-147	1.38	181.94 Å²	0.60	9.80e-04 mg/mL (Moderately soluble)
2c	Yellowish-brown precipitate	144-46	2.25	136.12 Å²	0.78	2.12e-02 mg/mL (Moderately soluble)

*tPSA = topological polar surface area: the surface sum over all polar atoms of the molecules, principally oxygen and nitrogen, together with their attached hydrogen atoms.

Table 6: Chemical properties of the synthesized compounds

Compound	*Chemical formula*	*Molecular weight (g/mol)*	*Yield %*	*H-Bond Donor*	*H-Bond Acceptor*	*No. of rotatable bonds*	*No. of heavy atoms*	*No. of aromatic heavy atoms*
1c	$C_{17}H_{12}N_6O_5S$	412.38	65	3	8	7	29	17
2c	$C_{17}H_{12}ClN_5O_3S$	401.83	87	3	6	6	27	17

include; PLP fitness, H-bonding, hydrophobic interactions, and the amino acids concerned with these interactions, were listed in Table 4.

Compounds (1c and 2c) were analyzed in detail depending on their higher PLP scores and docking interactions in the binding site (regarding number of H-bonding, hydrophobic interactions, and lengths of these bonds).

Analyses of H-bond Interactions

It can be seen (Figure 3) that compound 1c creates two H-bonds with GK: one is between the 'N' of diazole ring of compound 1c and 'H' of the primary amine of Trp 99 on GK protein with a distance of 2.961 A°, and the other is between the 'N' of cyano group of compound 1c and the 'H' of the phenolic hydroxyl group of Tyr 214 on GK protein with a distance of 3.038 A°.

On the other hand, the docked pose showed the H-bond interaction between the 'O' of the carbonyl group of compound 2c and 'H' of the primary amine of Trp 99 on GK protein with a distance of 2.907 A° (Figure 5).

Analyses of Hydrophobic Interactions

In addition to the H-bond forces, compounds (1c and 2c) were bonded in the allosteric site by hydrophobic interactions made with hydrophobic pocket amino acid residues, which determine the pose of each compound in the binding site.

Compound 1c has hydrophobic interactions with pocket comprising residues; Tyr 214, Trp 99, Ile 211, Cys 220, Glu 221, Met 210, and Pro 66. At the same time, compound 2c has hydrophobic interactions with Leu 451, Pro 66, Trp 99, and Ile 211 (Figures 4 and 6).

Compound 1c was observed to have reasonable binding in the allosteric site as specified by studying the H-bond and hydrophobic interactions of the selected best-docked pose where this compound has a similar binding orientation in the allosteric site of the enzyme as that of the co-crystallized ligand (8wj).

This was suggested by the pattern of H-bond and hydrophobic interactions with pocket comprising residues in addition to the higher value of PLP compared with compound 2c.

Results of Chemical Syntheses

The GKA derivatives were successfully synthesized and the selected chemical and physical parameters of these compounds were summarized in Tables 5 and 6. The final products were obtained in a relatively good yield. The melting points were observed and recorded for all the synthesized compounds. The estimated log P (*n*-octanol / water partition coefficient) parameters, the H-bond donors, and the H-bond acceptors exhibit a very good acceptance for the Lipinski rule of five.[22,23]

However, the total no. of the rotatable bonds within the molecule is another important feature of the drug molecule for oral bioavailability. To be orally absorbable, the drug molecule should not have more than 10 rotatable bonds.[24] All the synthesized derivatives did not exceed this no. and obeyed this rule very well.

Suggested Mechanism of Synthesis for Compounds (1c and 2c)

Step 1: Formation of Sulphonamides

Concisely, a nucleophilic attack of the arylamine [the unshared pair of electrons on the nitrogen] to the corresponding electrophile [the partially positively charged sulfur of 3-(chlorosulfonyl) benzoic acid] to obtain the proposed sulphonamides as shown in Scheme 2.

Step 2: Formation of Benzoyl Chlorides

This step includes the production of benzyl chlorides obtained by refluxing the above products with thionyl chloride. It is also started with a nucleophilic attack of the carboxylic oxygen of the sulphonamide to their corresponding electrophilic sulfur of thionyl chloride and preceded in suggested mechanism illustrated in Scheme 3.

Step 3: Formation of Diazole-benzamide Derivatives

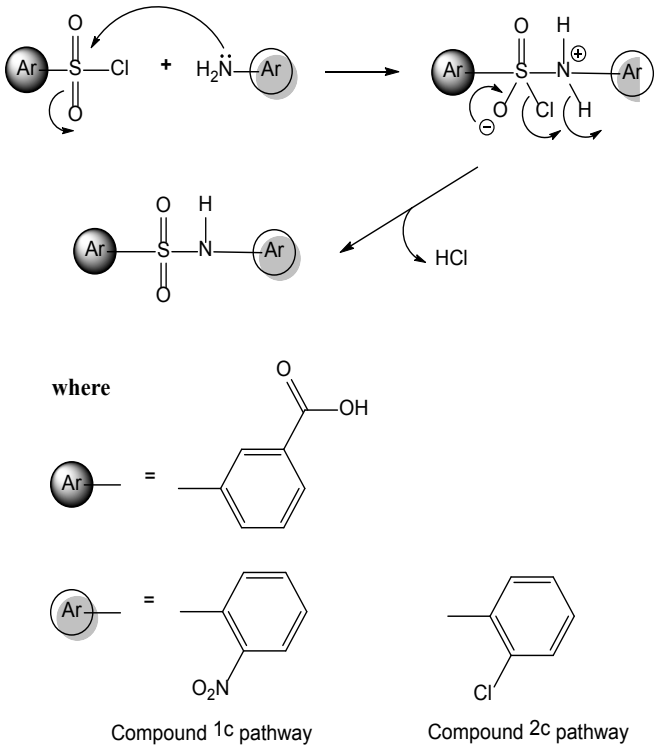
It can be seen from Scheme 4 below that this step's suggested mechanism started with the nucleophilic attack of the diazole amine to the corresponding electrophiles represented by the benzoyl chloride to synthesize the designed diazole-benzamide derivatives.

Results of characterization and identification of the synthesized compounds

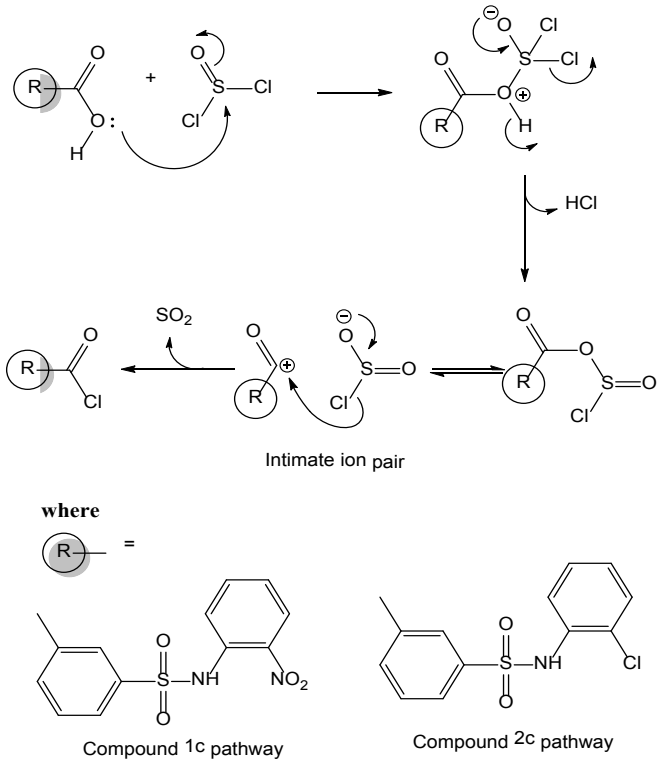
FTIR Characterization: This mainly depends on the appearance or disappearance of specific bands through the IR spectra that correlate to the functional groups of the reactants or the products, respectively.

FTIR Characterization of Compound (1c): 3344 cm^{-1} and 3277 cm^{-1} (N-H stretching vibration of sulfonamide and amide overlap), 2210 cm^{-1} (C≡N stretching vibration), 1693 cm^{-1} (C=O of amide), 1606 cm^{-1} and 1498 cm^{-1} (C=C aromatic stretching vibration), 1548 cm^{-1} and 1338 cm^{-1} (NO2 asymmetric and symmetric stretching vibration), 1377 cm^{-1} and 1170 cm^{-1} (SO2 asymmetric & symmetric stretching vibration).

FTIR Characterization of Compound (2c): 3371 cm^{-1} (NH stretching vibration of sulfonamide), 3279 cm^{-1} (N-H stretching vibration of imidazole), 3174 cm^{-1} (NH stretching vibration of amide), 3072 cm^{-1} (C-H aromatic stretching vibration), 2222 cm^{-1} (C≡N stretching vibration), 1653 cm^{-1} (C=O of amide), 1556 cm^{-1} and 1523 cm^{-1} (C=C aromatic stretching vibration), 1381 cm^{-1} and 1176 cm^{-1} (SO2 asymmetric and symmetric stretching vibration), 1033 cm^{-1} (C-Cl aromatic stretching vibration).



Scheme 2: Suggested mechanism of the formation of sulphonamides



Scheme 3: Suggested mechanism of the formation of benzyl chlorides

^{1}H-NMR Characterization

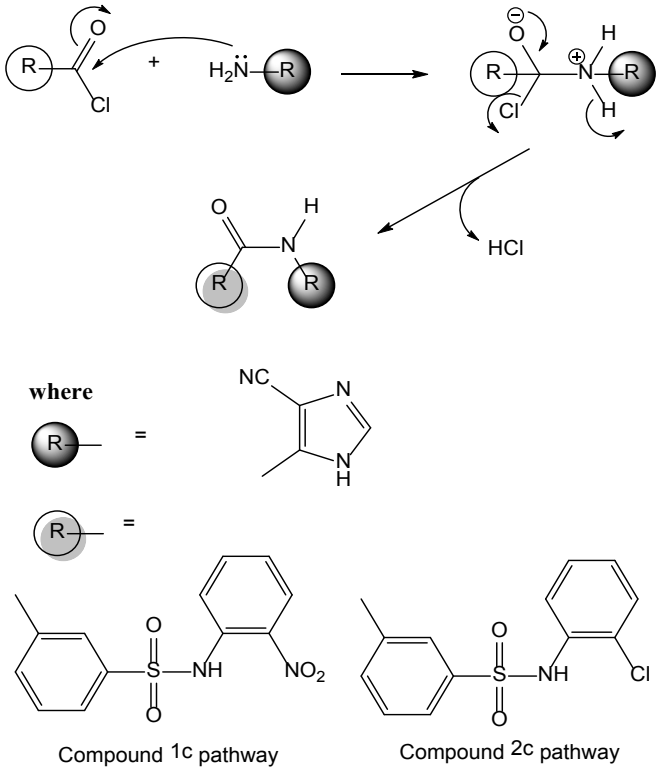
^{1}H-NMR characterization and interpretation of compounds 1c and 2c are tabulated in Tables 7 and 8, respectively. The spectra were recorded in DMSO-*d6* (deuterated dimethyl sulfoxide) solvent.

^{13}C NMR Characterization

^{13}C-NMR characterization and interpretation of compounds 1c and 2c are tabulated in Tables 9 and 10, respectively.

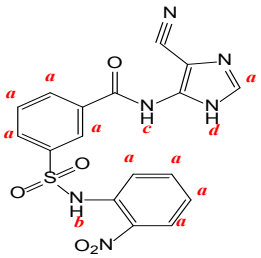
Mass Spectrometric Characterization

Mass spectrometric methods were employed to detect and fully characterize the chemical compounds synthesized in this work. The mass spectra were recorded, the parent ions (m /z $^{+}$) were shown corresponding to the expected molecular mass of the compounds. Tables 11 and 12 summarized the fragmentation with the abundance and formula for each fragment.



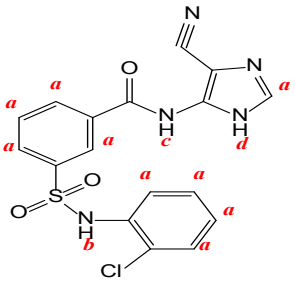
Scheme 4: A suggested mechanism for the formation of diazole-benzamide derivatives

Table 7: ^{1}H NMR data and the interpretations of compound 1c



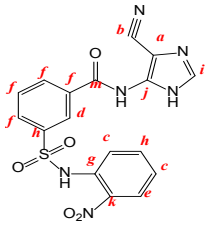
Signal	*Chemical shift (ppm)*	*No. of protons*	*Multiplicity*	*Interpretation*
a	6.99–8.64	9H	Multiplet	Aromatic protons
b	10.55	1H	Singlet	The proton of secondary amine affected by neighboring SO_2 group and nitrobenzene
c	10.90	1H	Singlet	The proton of secondary amine affected by neighboring carbonyl group and imidazole ring
d	11.96	1H	Singlet	The proton of secondary amine of the imidazole ring

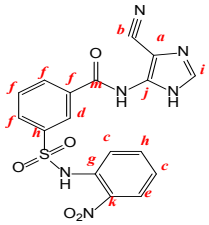
Table 8: ^{1}H NMR data and the interpretations of compound 2c:



Signal	*Chemical shift (ppm)*	*No. of protons*	*Multiplicity*	*Interpretation*
a	6.75–8.61	9H	Multiplet	Aromatic protons
b	10.27	1H	Singlet	The proton of secondary amine affected by neighboring SO_2 group and chlorobenzene ring
c	11.14	1H	Singlet	The proton of secondary amine affected by neighboring carbonyl group and imidazole ring
d	12.05	1H	Singlet	The proton of a secondary amine of the imidazole ring

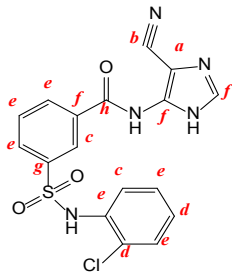
Table 9: ^{13} C NMR data and the interpretations of compound 1c:





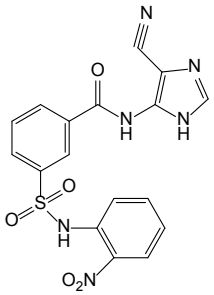
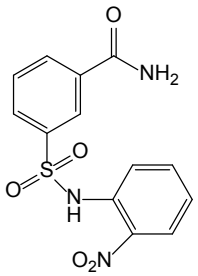
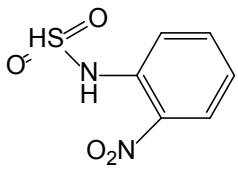
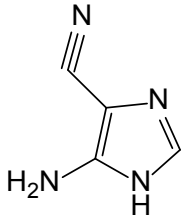
Signal	*Chemical shift (ppm)*	*No. of carbon atoms*	*Interpretation*
a	111.36	1	(C—N) of imidazole ring
b	115.57	1	(C≡N) of cyanide group
c	119.28	2	Carbon of nitrobenzene ring
d	124.98	1	Carbon of benzene ring
e	126.55	1	Carbon of nitrobenzene ring
f	(128.35-129.96)	4	Carbon of benzene ring
g	131.53	1	Carbon of nitrobenzene ring adjacent to amine group
h	133.99	1	Carbon of nitrobenzene ring
i	135.81	1	(C═N) of imidazole ring
j	140.02	1	(C—NH) of imidazole ring
k	143.23	1	Carbon of nitrobenzene ring adjacent to nitro group
l	148.56	1	Carbon of benzene ring adjacent to SO_2 group
m	165.19	1	Carbon of carbonyl group

Table 10: ^{13}C NMR data and the interpretations of compound 2c:



Signal	*Chemical shift (ppm)*	*No. of carbon atoms*	*Interpretation*
a	112.43	1	(C─N) of imidazole ring
b	118.25	1	(C≡N) of cyanide group
c	120.50	2	Carbon of aromatic ring
d	140.01	2	Carbon of chlorobenzene ring
e	(126.47–130.35)	6	Carbon of aromatic ring
f	132.05	3	Carbon of imidazole and phenyl rings
g	148.34	1	Carbon of benzene ring adjacent to SO_2 group
h	166.99	1	Carbon of carbonyl group

Table 11: Mass spectrometric data for compound 1c:

No.	*Molecular mass*	*Chemical formula*	*Abundance*	*Structure*
1	412	$C_{17}H_{12}N_6O_5S$	270000 Molecular ion	
2	321	$C_{13}H_{11}N_3O_5S$	270000	
3	202	$C_6H_6N_2O_4S$	1150000	
4	108	$C_4H_4N_4$	6250000 Base peak	

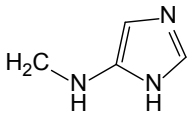
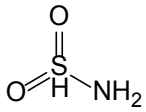
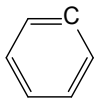
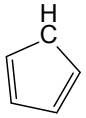
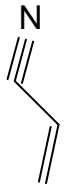
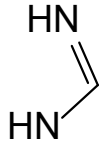
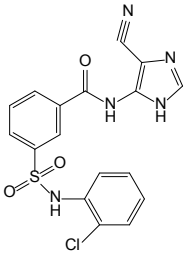
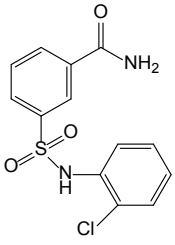
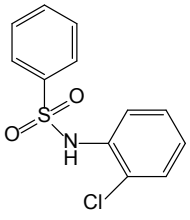
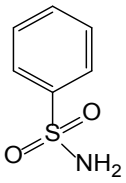
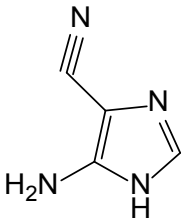
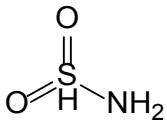
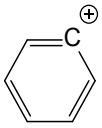
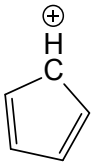
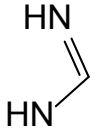
No.	*Molecular mass*	*Chemical formula*	*Abundance*	*Structure*
5	96	$C_4H_6N_3$	520000	
6	81	H_3NO_2S	1700000	
7	77	C_6H_5	900000	
8	65	C_5H_5	370000	
9	53	C_3H_3N	2800000	
10	43	CH_3N_2	450000	

Table 12: Mass spectrometric data for compound 2c:

No.	*Molecular mass*	*Chemical formula*	*Abundance*	*Structure*
1	401	$C_{17}H_{12}ClN_5O_3S$	285000 Molecular ion	
2	310	$C_{13}H_{11}ClN_2O_3S$	285000	
3	267	$C_{12}H_{10}ClNO_2S$	270000	

No.	*Molecular mass*	*Chemical formula*	*Abundance*	*Structure*
4	157	$C_6H_7NO_2S$	270000	
5	108	$C_4H_4N_4$	6500000 Base peak	
6	81	H_3NO_2S	2230000	
7	77	C_6H_5	1100000	
8	65	C_5H_5	360000	
9	53	C_3H_3N	4120000	
10	43	CH_3N_2	550000	

CONCLUSION

In this work, the authors conclude that new diazole- benzamide derivatives as glucokinase activators were successfully designed, synthesized, and characterized. *In silico* docking studies showed that the designed compounds could form characteristic H-bonds with GK proteins in addition to the hydrophobic interaction. The biological evaluation is recommended for these compounds to support GK as a target to normalize hyperglycemia, and the GKAs could be used to treat T2DM.

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