RESEARCH ARTICLE

Synthesis, molecular docking and molecular dynamic simulation studies of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives as antidiabetic agents

Samridhi Thakral¹, Rakesh Narang², Manoj Kumar¹ and Vikramjeet Singh¹*²

Abstract
A series of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives (5a–5v) has been synthesized and confirmed by physicochemical (Rf, melting point) and spectral means (IR, ¹H NMR, ¹³C NMR). The results of in vitro antidiabetic study against α-glucosidase indicated that compound 5o bearing 2-CH₃-5-NO₂ substituent on phenyl ring was found to be the most active compound against both enzymes. The electron donating (CH₃) group and electron withdrawing (NO₂) group on a phenyl ring highly favoured the inhibitory activity against these enzymes. The docking simulations study revealed that these synthesized compounds displayed hydrogen bonding, electrostatic and hydrophobic interactions with active site residues. The structure activity relationship studies of these compounds were also corroborated with the help of molecular modeling studies. Molecular dynamic simulations have been done for top most active compound for validating its α-glucosidase and α-amylase inhibitory potential, RMSD analysis of ligand protein complex suggested the stability of top most active compound 5o in binding site of target proteins. In silico ADMET results showed that synthesized compounds were found to have negligible toxicity, good solubility and absorption profile as the synthesized compounds fulfilled Lipinski’s rule of 5 and Veber’s rule.

Keywords: α-Glucosidase, α-Amylase, Molecular docking, Molecular dynamic simulations, ADMET

Introduction
Diabetes mellitus (DM) is a complex metabolic disorder resulting either due to relative or absolute deficiency of pancreatic insulin secretion or insensitivity to insulin action, ensuing in postprandial hyperglycemia and assorted diabetic complications [1, 2]. According to World Health Organization reports, at present around 250 million peoples are living with diabetes and this number is expected to be more than 366 million by 2030 [3] and these statistics are predicted to reach 592 million by 2035 of which 46% may still remain undiagnosed. The reduction of postprandial hyperglycemia by inhibiting carbohydrate hydrolyzing enzymes in gastrointestinal tract is one of the promising approaches for management of diabetes [4, 5]. α-Amylase is involved in hydrolyzing long chain of starch and α-glucosidase release glucose into the small intestine by breaking down oligosaccharides and disaccharides [2, 6]. α-Glucosidase and α-amylase inhibitors reduced postprandial blood glucose level by delaying the hydrolysis of carbohydrate by inhibiting the digestive enzymes [7]. Acarbose, Miglitol

*Correspondence: vikramjeetsinghjudge@gmail.com
1 Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India
Full list of author information is available at the end of the article

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and Voglibose are currently available drugs used as α-glucosidase and α-amylase inhibitors, but due to their deleterious side effects such as abdominal distention, diarrhoea and bloating, flatulence [8–10] there is need to explore and synthesize new drug candidates for the management of type-II diabetes mellitus with no or low risk of side effects.

The sulphonamide moiety (–SO₂NH₂) is an effective pharmacophore revealing the clinical and medicinal importance of sulphonamide drugs in the field of drug discovery [11]. The lead molecules bearing sulphonamide structure exhibited diverse biological properties viz. antibacterial [12, 13], diuretics, carbonic anhydrase (CA) inhibitors [14], antithyroid, anti-diabetic [11, 15, 16], antianxiety [17], antitubercular [18], selective Cox II inhibitors [19], anti-inflammatory [20], aldose reductase inhibitor [21], anti-oxidant [22], and anti-cancer [20] etc. Benzamides are the carbonic acid amide of benzoic acid and have also been described for exhibiting various biological activities i.e. antimicrobial [23, 24], anti-inflammatory [25], anticancer [26, 27], anti-diabetic [28], antidepressant, antitubercular [29], anticonvulsant [30] and analgesic [31] etc. 2,4-Dichlorobenzoic acid derivatives have also been reported for their anti-diabetic potential exhibiting α-glucosidase and α-amylase inhibitory activity, as described in our previous studies [32, 33]. Singh et al., reported the benzamides as glucokinase activators possessing hypoglycaemic activity [34]. Thiazole-2-yland N-pyridin-2-yl benzamides from benzoic acids showed glucokinase activation and possessed good anti-diabetic potential in animal rat model [35, 36]. A series of sulfamoyl benzamide derivatives have also been reported by Grewal et al., having glucokinase activation potential for the treatment of type 2 diabetes [37]. In view of the vital importance of benzamides in management of type 2 diabetes, we have synthesized a series of 2-chloro-5-[[4-chlorophenyl]sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamides and evaluated its antidiabetic potential in the current report.

Results and discussion

Chemistry

The 2-chloro-5-[(chlorosulfonyl)-4-nitrobenzoic acid (2) was prepared from 2-chloro-4-nitro benzoic acid according to our previously reported procedure [32]. The reaction of commercially available para chloro substituted aniline with compound 2 in DMF yielded 2-chloro-5-[[4-chlorophenyl]sulfamoyl]-4-nitrobenzoic acid in appropriate amount. The treatment of compound 3 with excess of thionyl chloride in presence of DMF as a catalyst afforded intermediate 4, which was further refluxed with aromatic/aliphatic/heterocyclic amines in DMF to provide the target compounds 5a–5v (Table 1, Scheme 1).

The structure of 2-chloro-5-[[4-chlorophenyl]sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamides was elucidated by IR, ¹H NMR and ¹³C NMR spectral analysis. The stretching frequency due to NH and carbonyl of amide bond were obtained at 3294–3524 cm⁻¹ and 1614–1692 cm⁻¹ respectively. The bands around 1302–1398 cm⁻¹ and 1127–1183 cm⁻¹ were assigned to asymmetric and symmetric stretching of SO₂ of sulfonamide group respectively. The IR spectrum of synthesized compounds exhibits a band around 1506–1587 cm⁻¹ to 1302–1378 cm⁻¹ assignable to asymmetric and symmetric stretching of NO₂. In the ¹H NMR spectra of compound, singlet for NH protons of SO₂NH and CONH appeared at δ 3.37–4.08 ppm and δ 10.19–10.81 ppm, respectively. The two aromatic protons of 2-chloro-4-nitro benzoic acid appeared around at δ 8.50 ppm and δ 7.50 ppm. The aromatic protons showed the chemical shift values in region of δ 6.58–8.58 ppm based on their chemical structure. In ¹³C NMR, signals for various carbons appeared in the region of δ 17.72 to 168.51 ppm.

In vitro antidiabetic evaluation

α-Glucosidase inhibitory activity

All the synthesized compounds were tested for their in vitro α-glucosidase inhibitory activity and revealed their varying degree of inhibitory potential with IC₅₀ values of 10.75±0.52 to 130.90±2.42 μM (Table 2) as compared to reference acarbose (IC₅₀=39.48±0.80 μM). The compound 5o (R=2-CH₃-5-NO₂) was found to be most active among this series of synthesized compounds. Most of the compounds exhibited good inhibitory potential with significant IC₅₀ as compared to positive reference.

α-Amylase inhibitory activity

All the compounds were also evaluated for α-amylase inhibitory activity and the inhibition potential with IC₅₀ values were found in range of 0.90±0.31 μM to 55.14±0.71 μM (Table 2). The compound 5o showed excellent inhibitory potential against α-amylase with IC₅₀ value of 0.90±0.31 μM. Compounds 5b, 5m, 5p showed most significant inhibitory potential against α-amylase with IC₅₀ values of 5.30±1.23, 1.52±0.84 and 2.10±0.52 μM, respectively, when compared to acarbose, used as reference compound (IC₅₀=5.60±0.30 μM).

Structure activity relationship

The compound 5o (R=2-CH₃-5-NO₂) was the most active compound (IC₅₀=10.75±0.52 μM; 0.90±0.31 μM) which may be due to the presence of electron withdrawing and electron donating group which generate an uniform electron flow, leading the compound to be more active and potent inhibitor against both enzymes. This fact is supported by the similar results
of Adegboye et al. [38]. In compounds 5m (R=2-CH3-3-NO2) and 5p (R=2-CH3-4-NO2) difference in inhibitory potential was mainly affected by position of NO2 substituent. However the inhibitory activity increased when the phenyl ring was substituted with CH3 at meta position, as observed in compound 5b (IC50 = 24.78 ± 2.69 μM; 5.30 ± 1.23 μM) in comparison to compounds 5a and 5c having CH3 substitution at para and ortho positions. Further a decrease in inhibitory activity was observed for compounds 5d (IC50 = 38.57 ± 0.01 μM; 38.00 ± 0.51 μM) and 5e (IC50 = 41.75 ± 1.08 μM; 50.30 ± 0.21 μM) bearing OCH3 substituted phenyl ring instead of compounds having CH3 substituted phenyl ring. The compounds 5f–5k, 5q and 5r bearing electron withdrawing groups were found to have considerable inhibitory potential. The results illustrated that compounds 5g (R=3-Br), 5i (R=3-Cl), 5r (R=3-NO2), substitution at meta position of phenyl ring was found to be most favored for the α-glucosidase inhibitory activity while compounds 5f and 5q bearing electron withdrawing groups at para position were found to be most favorable for α-amylase inhibitory activity. This fact is supported by Taha et al. [39]. The compounds 5u (IC50 = 89.04 ± 1.76 μM, 38.20 ± 0.34 μM) and 5v (IC50 = 52.37 ± 1.92 μM, 40.40 ± 0.87 μM) substituted with heterocyclic amine displayed reduced inhibitory activities compared to aryl amines. This fact is supported by similar results of Kumar et al. [40] and Charaya et al. [35]. Substituting the compounds with n-propyl amine and butyl amine resulted in diminished activity as in compounds 5s (IC50 = 106.23 ± 0.61 μM, 48.05 ± 0.23 μM) and 5t (IC50 = 130.90 ± 2.42 μM,

| Comp. | R              | Comp. | R              |
|-------|----------------|-------|----------------|
| 5a    | CH3            | 5l    | H3C Cl         |
| 5b    | CH3            | 5m    | H3C NO2        |
| 5c    | H3C            | 5n    | Cl NO2         |
| 5d    | - OCH3         | 5o    | H3C NO2        |
| 5e    | H3CO           | 5p    |                 |
| 5f    | Br             | 5q    |                 |
| 5g    | Br             | 5r    |                 |
| 5h    | Br             | 5s    |                 |
| 5i    | Cl             | 5t    |                 |
| 5j    | Cl             | 5u    |                 |
| 5k    | O2N            | 5v    |                 |

Table 1 List of synthesized 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide compounds
55.14 ± 0.71 μM). This fact is supported by the similar study on benzamide derivatives by Charaya et al. [35].

**Molecular docking**

In silico molecular docking study was performed to investigate binding interactions and to explore binding modes of synthesized compounds with their respective targets. The binding affinities of all the synthesized compounds are reported in Table 2.

**α-Glucosidase enzyme**

The docking results revealed that all the synthesized compounds displayed binding energy ranging from −9.7 to −8.0 kcal/mol and depicted various types of significant binding interactions like hydrogen bonding, electrostatic and hydrophobic interactions with the amino acid residues of active site of enzyme. The binding mode of most active compound 5o and modeled protein is presented in Fig. 1. The oxygen of 2-Cl-4-NO2 established hydrogen bonding interaction with Glu:276 amino acid residue at a distance of 3.35 Å whereas Phe:298 amino acid was found to engage in hydrogen bond interactions with both protonated nitrogen of NO2 of same with bond lengths of 2.49 Å. The nitrogen of 2-CH3-5-NO2 substituted compound displayed charge–charge interaction with Asp:349 amino acid residue (3.78 Å) while the nitrogen of 2-Cl-4-NO2 presented charge–charge interaction with Glu:276 amino acid residue (3.80 Å). The 2-Cl-4-NO2 substituted phenyl ring created pi-anion interaction with residue Glu:276 of modeled protein at a distance of 3.37 Å. It was noticed that Phe:157 residue (5.51 Å) formed pi–pi T shaped interaction with 2-CH3-5-NO2 substituted phenyl ring and para chloro substituted phenyl ring displayed two pi–pi stacked interaction with His:348, Tyr:344 and Phe:298 amino acid residues. In addition 2-Cl-4-NO2 substituted phenyl ring created pi–pi stacked and pi–pi T shaped interaction with His:279 amino acid residues with bond length of 5.77 Å. Pi-alkyl interactions were established by chlorine of 2-Cl-4-NO2 substituted phenyl ring with His:279 residues at a distance of 4.14 Å. The chlorine of para chlorosubstituted phenyl ring was found to engage in forming pi–alkyl interactions with Tyr:344, His:348, Phe:298, Trp:57 amino acid residues of modeled protein. The involvement of 2-CH3-5-NO2 substituted phenyl ring in forming more hydrophobic interactions i.e. pi–pi interactions may be contributing to better activity of compound 5o as compared to compounds 5n (R=2-CH3-3-NO2) and 5p (2-CH3-4-NO2). Comparison of compound 5c (R=2-CH3) with

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**Scheme 1** General scheme for synthesis of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives
Table 2  α-Glucosidase and α-amylase inhibitory activity (IC<sub>50</sub>) of synthesized derivatives (5a–5v) and their docking affinity with α-glucosidase (modeled protein) and α-amylase (PDB-1qho)

| Comp. | IC<sub>50</sub> α-glucosidase (µM) | Binding score (α-glucosidase: modeled protein) | IC<sub>50</sub> α-amylase (µM) | Binding score (α-amylase: 1qho) |
|-------|---------------------------------|-----------------------------------------------|-------------------------------|---------------------------------|
| 5a    | 31.39 ± 1.66                   | -9.4                                          | 7.40 ± 0.15                   | -8.9                            |
| 5b    | 24.78 ± 2.69                   | -9.7                                          | 5.30 ± 1.23                   | -9.0                            |
| 5c    | 26.77 ± 1.13                   | -9.3                                          | 8.00 ± 0.71                   | -9.8                            |
| 5d    | 38.57 ± 0.01                   | -9.2                                          | 38.00 ± 0.51                  | -9.1                            |
| 5e    | 41.75 ± 1.08                   | -9.3                                          | 50.30 ± 0.21                  | -8.7                            |
| 5f    | 50.24 ± 0.89                   | -9.4                                          | 16.00 ± 0.33                  | -9.7                            |
| 5g    | 35.92 ± 0.60                   | -9.6                                          | 16.70 ± 0.41                  | -9.8                            |
| 5h    | 40.64 ± 1.49                   | -9.6                                          | 19.30 ± 0.63                  | -9.7                            |
| 5i    | 14.02 ± 0.93                   | -9.5                                          | 27.12 ± 0.51                  | -8.5                            |
| 5j    | 15.75 ± 0.90                   | -9.6                                          | 20.90 ± 1.24                  | -9.5                            |
| 5k    | 36.93 ± 1.30                   | -9.7                                          | 12.50 ± 0.91                  | -8.8                            |
| 5l    | 29.01 ± 0.86                   | -9.3                                          | 6.30 ± 0.42                   | -9.3                            |
| 5m    | 24.47 ± 1.23                   | -9.4                                          | 1.52 ± 0.84                   | -9.7                            |
| 5n    | 29.54 ± 1.53                   | -9.4                                          | 35.30 ± 0.45                  | -9.6                            |
| 5o    | 10.75 ± 0.52                   | -9.3                                          | 00.90 ± 0.31                  | -9.2                            |
| 5p    | 19.51 ± 0.43                   | -9.4                                          | 02.10 ± 0.52                  | -9.1                            |
| 5q    | 43.88 ± 1.18                   | -9.4                                          | 11.20 ± 0.67                  | -9.0                            |
| 5r    | 34.36 ± 0.62                   | -9.7                                          | 15.30 ± 1.24                  | -9.4                            |
| 5s    | 106.23 ± 0.61                  | -8.2                                          | 48.05 ± 0.23                  | -7.9                            |
| 5t    | 130.90 ± 2.42                  | -8.6                                          | 55.14 ± 0.71                  | -8.3                            |
| 5u    | 89.04 ± 1.76                   | -8.9                                          | 38.20 ± 0.34                  | -8.6                            |
| 5v    | 52.37 ± 1.92                   | -9.0                                          | 40.40 ± 0.87                  | -8.9                            |
| Acarbose | 39.48 ± 0.88                  | -8.0                                          | 5.60 ± 0.30                   | -8.4                            |

Fig. 1  a 3D Binding confirmation of compound 5o with active site residues of α-glucosidase. b 2D binding confirmation of compound 5o with amino acid residue of nearby active site.
5n (R = 2-CH$_3$-3-NO$_2$), 5o (R = 2-CH$_3$-5-NO$_2$), 5p (R = 2-CH$_3$-4-NO$_2$). 5n, 5o, 5p displayed more hydrophobic interactions with Phe:177, Arg:312, Val:108, His:279, Phe:157, His:348, Tyr:344, Phe:298 amino acid residues of modeled protein which may have resulted in their higher inhibitory potential. The binding interaction between compounds 5c (R = 2-CH$_3$) and residues of modeled protein was nearly same as 5a (R = 4-CH$_3$) and 5b (R = 3-CH$_3$). The difference was that ortho methyl substituted phenyl ring maintained pi–pi stacked, pi–alkyl and pi–pi T interactions (hydrophobic interactions) with Try:344, His:348, Phe:298, Phe:177, Phe:158, Tyr:344 amino acid residue that made 5c more active than 5a and 5b.

The compound 5e (R = 2-OCH$_3$) formed less number of hydrogen bonding, electrostatic and hydrophobic interactions as compared to compound 5d (R = 4-OCH$_3$), resulting in decreased inhibitory potential of compound 5e. The binding of compound 5i (R = 3-Cl) facilitated one more pi–alkyl interaction with other hydrogen bonding, hydrophobic and electrostatic interactions same as that of compound 5j (R = 2-Cl), which may be contributing to better potential of compound 5i. Considering the moderately active compound 5r (R = 3-NO$_2$), additional hydrophobic interaction such as pi–pi interactions with amino acid residues were observed as compared to compounds 5k (R = 2-NO$_2$) and 5q (R = 4-NO$_2$). In comparison to compounds bearing aromatic anilines, a decrease in inhibitory potential was observed in compounds 5s (R = n-propyl), and 5t (R = n-butyl), due to less pi–pi interactions between the inhibitory compounds and amino acid residues. The binding interactions of compound 5u (R = C$_4$H$_3$O–CH$_2$ (2-furfuryl)) with residues of modeled protein were nearly same as that of 5v (R = C$_6$H$_4$N-(pyridine-2-yl)) but the difference was that 2-furfuryl ring exhibited pi–pi T shaped interaction with Trp:177 residue and four hydrogen bond interaction with Asp:329, Arg:376, His:90, Trp:93 residues of α-glucosidase with other interactions while compound 5v formed three hydrogen bond interactions, which made 5u more active than 5v against α-glucosidase enzyme.

α-Amylase enzyme

The docking results revealed that all the synthesized compounds displayed binding energy ranging from −9.8 to −7.9 kcal/mol. The binding mode of most active compound 5o and 1qho is presented in Fig. 2. The oxygen of 2-CH$_3$-5-NO$_2$ established hydrogen bonding interaction with His:90 amino acid residue at a distance of 3.01 Å whereas His:232 amino acid was found to engage in hydrogen bond interactions with both oxygen of NO$_2$ of 2-Cl-4-NO$_2$ substituted phenyl ring with bond lengths of 2.04 Å and 1.86 Å. The nitrogen of 2-CH$_3$-5-NO$_2$ displayed charge–charge interaction with Asp:372 amino acid residue (4.82 Å) while the protonated nitrogen of 2-CH$_3$-5-NO$_2$ presented salt bridge charge–charge interaction with Asp:190 amino acid residue (3.14 Å). The charge–charge interaction was also found between the nitrogen of 2-Cl-4-NO$_2$ substituted phenyl ring and Glu:256 amino acid residue with bond length of 5.09 Å. The 2-CH$_3$-5-NO$_2$ substituted phenyl ring created pi-anion interaction with residue Asp:372 of α-amylase.
while nitrogen of 2-CH$_3$-5-NO$_2$ substituted phenyl ring formed pi-cation interaction with His:90 residue. It was shown that His:90 residue (4.88 Å) formed pi–pi T shaped interaction with 2-CH$_3$-5-NO$_2$ substituted phenyl ring and para chloro substituted phenyl ring displayed pi–pi stacked interaction with Trp:177 residue (4.60 Å). In addition 2-Cl-4-NO$_2$ substituted phenyl ring created pi–pi stacked interaction with Tyr:258, Phe:188 amino acid residues with bond lengths of 5.3 Å and 5.07 Å, respectively. The pi–alkyl interactions were established by cholorine of 2-Cl-4-NO$_2$ substituted phenyl ring and methyl of 2-CH$_3$-5-NO$_2$ substituted phenyl ring with Phe:188 and His:328 residues at a distance of 4.58 Å and 4.50 Å, respectively. The CH$_3$ of 2-CH$_3$-5-NO$_2$ substituted phenyl ring was found to engage in forming pi-sigma interaction with Tyr:92 residue (3.56 Å) while oxygen of NO$_2$ created pi–pi stacked and pi–alkyl methyl substituted phenyl ring interaction capacity (Additional file 1: Table S2). Bioactivity and ADMET properties prediction

In silico ADMET properties prediction

Lipinski’s rule of five, topological polar surface area, aqueous solubility and number of rotatable bonds, these calculated parameters are presented in Additional file 1: Table S1. The human intestinal absorption values were found in range of 93.10 to 95.93% which established the moderate to good absorption capacity of synthesized compounds and supported their interaction with target cell.

The in vitro Caco-2 cell permeable property in the range of 0.36–0.55 nm/s, in vitro MDCK cell permeability in range of 0.01–0.97 nm/s designated low permeability of target compounds with the concerned cell line. The synthesized compounds displayed values in range of 95.75–100% confirmed their strong binding capacity with proteins. The in vivo blood brain barrier penetration ranges from 0.01 to 0.32 supported their low to moderate distribution in vivo with medium to good penetration capacity (Additional file 1: Table S2). Bioactivity and...
toxicity risk values of synthesized compounds are illustrated in Additional file 1: Table S3.

**Conclusion**

A series of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives (5a-5v) has been synthesized and all the compounds were found to possess potent to moderate inhibitory potential against α-glucosidase and α-amylase. Compound 5o (2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(2-methyl-5-nitrophenyl)-4-nitrobenzamide) was found to be highly active having fourfold inhibitory potential against α-glucosidase and around six times inhibitory activity against α-amylase in comparison to standard drug.
Molecular docking results of antidiabetic study showed reasonable dock score and binding interactions of synthesized molecules with their respective targets. Analysis of RMSD of ligand protein complex during molecular dynamic simulations suggested stability of the most active compounds in binding site of respective target proteins i.e. α-glucosidase and α-amylase enzymes. Prediction of computational drug like properties showed that most of synthesized compounds are safe with acceptable ADMET and druggable properties.

Materials and methods

Chemicals

The analytical grade chemicals and reagents were used as such in experiments without any purification. Decibel melting point apparatus was used for checking the melting point of the synthesized compounds and are reported as uncorrected. The silica gel-precoated aluminum sheets for thin-layer chromatography (TLC) were employed to keep a vigil of the reaction progress. FT-IR (Diffuse Reflectance Method (DRS) -8000A, Shimadzu, Japan) spectrophotometer was utilized for recording infrared spectra and the Bruker Avance III, 400 MHz NMR spectrometer was employed for nuclear magnetic resonance spectra (1H NMR, 13C NMR; Chemical shift δ values-ppm). α-Glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20, Sigma Aldrich) and α-amylase from malt (232-588-1, HiMedia) have been used for in vitro studies.

General procedure for synthesis of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide (5a–5v)

**Synthesis of 2-chloro-5-(chlorosulfonyl)-4-nitro benzoic acid (2)**

Compound 2 was synthesized from 2-chloro-4-nitro benzoic acid (1) as previously reported method in literature [32].

**Synthesis of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzoic acid (3)**

2-Chloro-5-(chlorosulfonyl)-4-nitrobenzoic acid (1 g, 0.003 mol) was refluxed with p-nitro aniline (0.003 mol) using dimethyl formamide as solvent, till the completion of reaction [34]. The reaction progress was monitored by TLC. The reaction mixture was cooled and yielded precipitates were washed and recrystallized.

**Synthesis of 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(alkyl/aryl)-4-nitrobenzamide (5a–5o)**

Compound 3 (0.5 g, 0.0012 mol) was further treated with excess of thionyl chloride in presence of catalytic amount of DMF with calcium chloride (CaCl₂) guard tube to get 2-chloro-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzoyl chloride (4). Compound 4 was dissolved in DMF and refluxed with anilines/amines/heterocyclic amines to get the desired products in appropriate yield [41]. After refluxing, mixture was cooled and poured on crushed ice, separated product was filtered and washed with dilute HCl and dried.

Physicochemical and spectral characterization

**2-Chloro-5-[(4-chlorophenyl)sulfamoyl]-N-(4-methylphenyl)-4-nitrobenzamide (5a)**

% Yield: 37.70; m.p.: 90–92 °C; Rf 0.81 (Chloroform); FTIR (KBr): ν max (cm⁻¹): 3502.79 (N–H str.), 3171.70 (C–H str., Ar), 2977.30, 2889.01 (C–H str., Aliphatic), 1641.45 (C=O), 1600.41 (C–H bend), 1586.48 (asym. NO₂ str.), 1349.25 (sym. NO₂ str.), 1315.47 (asym. SO₂ str.), 1157.31 (sym. SO₂ str.), 1157.31 (sym. SO₂ str.), 733.44 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 2.15 (s, 3H, CH₃), 4.01 (s, 1H, NH),
2-Chloro-5-(4-chlorophenyl) sulfamoyl-N-(3-methylphenyl)-4-nitrobenzamide (5b)

% Yield: 50.81; m.p.: 102–104 °C; Rf: 0.82 (H:–8:2); FTIR (KBr): v max (cm⁻¹): 3502.97 (N–H str.), 3117.98 (C–H str., CH₃), 2982.94, 2882.00 (C–H str., Aliphatic), 1621.03 (C=O), 1602.20 (N–H bend), 1544.11 (asym. NO₂ str.), 1370.45 (asym. SO₂ str.), 1340.55 (sym. NO₂ str.), 1170.07 (sym. SO₂ str.), 766.16 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 2.35 (3H, CH₃), 3.92 (1H, NH), 5.48 (1H, CH of C₃–CONH–C₆H₅CH₃), 6.88 (1H, CH of C₆ of ClC₆H₅CONH–), 7.10–7.12 (2H, CH of C₂ and C₆ of ClC₆H₅NH), 7.19 (1H, CH of C₃ of ClC₆H₅CONH–), 7.23–7.25 (1H, CH of C₅ of ClC₆H₅CONH–), 7.35–7.40 (m, 2H, CH of C₄, C₅ of ClC₆H₅CONH–), 7.51–7.53 (2H, CH of C₂ and C₆ of ClC₆H₅NH), 10.31 (1H, NH); ¹³C NMR (300 MHz, DMSO-d₆), δ ppm: 168.51 (C=O), 151.75 (C–S), 143.40 (C–NO₂), 138.92 (C–NH), 138.59 (C–Cl), 132.07, 130.56, 130.11, 129.23, 128.68, 124.09, 120.89, 119.21, 115.21, 21.41.

2-Chloro-5-(4-chlorophenyl) sulfamoyl-N-(2-methoxyphenyl)-4-nitrobenzamide (5e)

% Yield: 85.30; m.p.: 164–166 °C; Rf: 0.53 (B:EA–7:3); FTIR (KBr): v max (cm⁻¹): 3447.82 (N–H str.), 3012.86 (C–H str., Ar), 1682.45 (C=O), 1597.64 (N–H bend), 1530.54 (asym. NO₂ str.), 1378.16 (sym. NO₂ str.), 1307.76 (asym. SO₂ str.), 1252.68 (C=O–C str.), 1174.67 (sym. SO₂ str.), 752.65 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 3.38 (3H, OCH₃), 3.98 (1H, NH), 7.07–7.09 (d, 1H, CH of C₃–CONH–C₆H₅OCH₃), 7.27–7.33 (m, 3H, CH of C₅, C₆ of –CONH–C₆H₅OCH₃), 7.52 (1H, CH of C₆ of ClC₆H₅CONH–), 7.81–7.82 (d, 2H, CH of C₂ and C₆ of ClC₆H₅NH), 8.28–8.30 (d, 2H, CH of C₃ and C₅ of ClC₆H₅NH), 8.49 (1H, CH of C₃ of ClC₆H₅CONH–), 10.48 (1H, NH); ¹³C NMR (300 MHz, DMSO-d₆), δ ppm: 164.21 (C=O), 157.32 (C–S), 145.07 (C–NO₂), 139.07 (C–NH), 136.16 (C–Cl), 131.34, 129.45, 128.24, 127.14, 125.18, 121.68, 121.51, 120.55, 35.60.

N-(4-Bromophenyl)-2-chloro-5-(4-chlorophenyl) sulfamoyl-4-nitrobenzamide (5f)

% Yield: 91.30; m.p.: 200–202 °C; Rf: 0.56 (Chloroform); FTIR (KBr): v max (cm⁻¹): 3392.85 (N–H str.), 3012.55 (C–H str., Ar), 1614.45 (C=O), 1591.36 (N–H bend), 1562.37 (asym. NO₂ str.), 1353.50 (sym. NO₂ str.), 1308.72 (asym. SO₂ str.), 1145.63 (sym. SO₂ str.), 732.07 (C–Cl), 691.49 (C–Br); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 3.60 (1H, NH), 7.46 (1H, CH of C₆ of ClC₆H₅CONH–), 7.54–7.55 (d, 2H, CH of C₂ and C₆ of ClC₆H₅NH), 8.00–8.02 (d, 2H, CH of...
C₂, C₆ of –CONH–C₆H₄Br–), 8.24–8.26 (d, 2H, CH of C₃ and C₅ of C₆H₂(NO₂)C₆H₂CONH–), 8.36–8.37 (d, 2H, CH of C₃, C₅ –CONH–C₆H₄Br–), 8.51 (s, 1H, CH of C₃ of ClNO₂C₆H₂CONH–), 10.55 (s, 1H, NH); ¹³CNMR (300 MHz, DMSO-d₆), δ ppm: 166.34 (C=O), 158.33 (C=S), 144.35 (C–NO₂), 140.21 (C–NH), 135.49 (C–Cl), 132.90, 130.58, 129.89, 129.21, 128.24, 127.08, 120.18.

N-(3-Bromophenyl)-2-chloro-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzamide (5g)
% Yield: 44.92; m.p.: 181–183 °C; Rf: 0.73 (Chloroform); FTIR (KBr): v max (cm⁻¹): 3502.79 (N–H str.), 3058.19 (C=H str., Ar), 1614.45 (C=O), 1588.41 (N–H bend), 1566.61 (asym. NO₂ str.), 1372.38 (asym. SO₂ str.), 1302.95 (sym. NO₂ str.), 1175.63 (sym. SO₂ str.), 778.76 (C=Cl), 675.80 (C=Br); ¹¹HNMR (300 MHz, DMSO-d₆), δ ppm: 3.71 (s, 1H, NH), 7.37 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.51 (s, 1H, CH of –CONH–C₆H₄Br–), 7.71–7.80 (d, 2H, CH of C₂ and C₆ of ClNO₂C₆H₂CONH–), 7.93–7.98 (m, 3H, CH of C₄, C₅ and C₆ of –CONH–C₆H₄Br–), 8.21–8.23 (d, 2H, CH of C₃ and C₅ of ClNO₂C₆H₂CONH–), 8.84 (s, 1H, CH of C₃ of ClNO₂C₆H₂CONH–), 10.40 (s, 1H, NH); ¹³CNMR (300 MHz, DMSO-d₆), δ ppm: 165.33 (C=O), 157.35 (C=S), 143.24 (C=NO₂), 139.51 (C=NH), 136.54 (C=Cl), 133.05, 130.77, 129.10, 128.39, 128.17, 127.63, 126.71, 126.12, 121.49.

N-(2-Bromophenyl)-2-chloro-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzamide (5h)
% Yield: 55.07; m.p.: 180–182 °C; Rf: 0.70 (Chloroform); FTIR (KBr): v max (cm⁻¹): 3392.85 (N–H str.), 3028.29 (C=H str., Ar), 1614.62 (C=O), 1594.19 (N–H bend), 1565.96 (asym. NO₂ str.), 1349.23 (sym. NO₂ str.), 1377.20 (asym. SO₂ str.), 1152.15 (sym. SO₂ str.), 754.34 (C=Cl), 661.15 (C=Br); ¹¹HNMR (300 MHz, DMSO-d₆), δ ppm: 3.83 (s, 1H, NH), 6.57–6.61 (t, 1H, CH of C₅ of –CONH–C₆H₄Br–), 6.90–6.92 (d, 1H, CH of C₆ –CONH–C₆H₄Br–), 7.12–7.14 (t, 1H, CH of C₄ of –CONH–C₆H₄Br–), 7.32–7.40 (d, 2H, CH of C₂ and C₆ of ClNO₂C₆H₂CONH–), 7.51–7.52 (d, 1H, CH of C₃ of –CONH–C₆H₄Br–), 7.82 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 8.16–8.18 (d, 2H, CH of C₃ and C₅ of ClNO₂C₆H₂CONH–), 8.50 (s, 1H, CH of C₅ of ClNO₂C₆H₂CONH–), 10.39 (s, 1H, NH); ¹³CNMR (300 MHz, DMSO-d₆), δ ppm: 167.04 (C=O), 156.56 (C=S), 143.06 (C=NO₂), 138.20 (C=NH), 135.47 (C=Cl), 135.07, 130.23, 129.04, 128.93, 126.13, 125.34, 122.21.

2-Chloro-N-(3-chlorophenyl)-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzamide (5i)
% Yield: 93.25; m.p.: 180–182 °C; Rf: 0.73 (B:EA–7:3); FTIR (KBr): v max (cm⁻¹): 3337.42 (N–H str.), 3060.18 (C=H str., Ar), 1655.65 (C=O), 1592.43 (N–H bend), 1533.43 (asym. NO₂ str.), 1377.20 (sym. NO₂ str.), 1173.70 (sym. SO₂ str.), 755.22 (C=Cl); ¹¹HNMR (300 MHz, DMSO-d₆), δ ppm: 3.89 (s, 1H, NH), 7.44 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.85–7.90 (m, 3H, CH of C₃ and C₅ of –CONH–C₆H₂CONH–), 7.98–8.00 (d, 2H, CH of C₂ and C₆ of ClNO₂C₆H₂CONH–), 8.21–8.22 (d, 2H, CH of C₃ and C₅ of ClNO₂C₆H₂CONH–), 8.31–8.33 (d, 1H, CH of C₃ of –CONH–C₆H₂CONH–), 8.52 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 10.19 (s, 1H, NH); ¹³CNMR (300 MHz, DMSO-d₆), δ ppm: 166.34 (C=O), 157.02 (C=Cl), 146.93 (C=NO₂), 139.24 (C=NH), 135.56 (C=Cl), 131.57, 131.25, 129.53, 128.55, 127.21, 125.24, 121.86.

2-Chloro-N-(2-chlorophenyl)-5-[(4-chlorophenyl)sulfamoyl]-4-nitrobenzamide (5j)
% Yield: 87.71; m.p.: 176–178 °C; Rf: 0.5 (B:EA–7:3); FTIR (KBr): v max (cm⁻¹): 3337.42 (N–H str.), 3060.18 (C=H str., Ar), 1655.65 (C=O), 1592.43 (N–H bend), 1533.43 (asym. NO₂ str.), 1377.20 (sym. NO₂ str.), 1392.63 (asym. SO₂ str.), 755.22 (C=Cl); ¹¹HNMR (300 MHz, DMSO-d₆), δ ppm: 3.89 (s, 1H, NH), 7.44 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.85–7.90 (m, 3H, CH of C₃ and C₅ of –CONH–C₆H₂CONH–), 7.98–8.00 (d, 2H, CH of C₂ and C₆ of ClNO₂C₆H₂CONH–), 8.21–8.22 (d, 2H, CH of C₃ and C₅ of ClNO₂C₆H₂CONH–), 8.31–8.33 (d, 1H, CH of C₃ of –CONH–C₆H₂CONH–), 8.52 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 10.19 (s, 1H, NH); ¹³CNMR (300 MHz, DMSO-d₆), δ ppm: 166.34 (C=O), 157.02 (C=Cl), 146.93 (C=NO₂), 139.24 (C=NH), 135.56 (C=Cl), 131.57, 131.25, 129.53, 128.55, 127.21, 125.24, 121.86.
2-Chloro-N-(3-chloro-2-methylphenyl)-5-[(4-chlorophenyl) sulfonyl]4-nitrobenzamide (5i)

% Yield: 57.62; m.p.: 212–214 °C; Rf: 0.65 (B:EA– 7:3); FTIR (KBr): v_max (cm⁻¹): 3447.82 (N–H str.), 3096.77 (C–H str., Ar), 2947.05, 2885.31 (C–H str., Aliphatic), 1692.29 (C=O), 1592.79 (N–H bend), 1531.51 (asym. NO₂ str.), 1380.09 (asym. SO₂ str.), 1306.80 (sym. NO₂ str.), 1174.67 (sym. SO₂ str.), 772.81 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 2.26 (s, H, CH₃), 3.45 (s, 1H, NH), 7.24–7.30 (m, 2H, CH C₅ and C₆ of –CONH–C₆H₂CH₃Cl–), 7.63 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.84–7.87 (d, 2H, CH of C₂ and C₆ of ClC₆H₄NH–), 8.28–8.30 (d, 2H, CH of C₃ and C₅ of ClC₆H₄NH–), 10.59 (s, 1H, NH); ¹³C NMR (300 MHz, DMSO-d₆), δ ppm: 165.37 (C=O), 158.36 (C–S), 147.85 (C–NO₂), 140.10 (C–NH), 138.55 (C–Cl), 137.21, 135.46, 130.80, 129.61, 129.04, 125.15, 121.47, 118.28, 23.57.

2-Chloro-5-[(4-chlorophenyl)sulfonyl]-N-(2-methyl-3-nitrophenyl)-4-nitrobenzamide (5m)

% Yield: 38.29; m.p.: 170–172 °C; Rf: 0.61 (C₁₇– 9:1); FTIR (KBr): v_max (cm⁻¹): 3469.45 (N–H str.), 3095.80 (C–H str., Ar), 2882.55 (C–H str., Aliphatic), 1692.32 (C=O), 1598.62 (N–H bend), 1530.06 (asym. NO₂ str.), 1351.59 (sym. NO₂ str.), 1302.76 (asym. SO₂ str.), 1177.56 (sym. SO₂ str.), 734.45 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 2.14 (s, H, CH₃ of –CONH–C₆H₂CH₃NO₂–), 3.37 (s, 1H, NH), 7.45 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.94–7.95 (d, 2H, CH of C₂ and C₆ of ClC₆H₄NH–), 8.09–8.13 (m, 2H, CH C₅ and C₆ of –CONH–C₆H₂CH₃NO₂–), 8.46–8.47 (d, 2H, CH of C₃ and C₅ of ClC₆H₄NH–), 8.72 (s, 1H, CH of C₉ of ClNO₂C₆H₂CONH–), 10.50 (s, 1H, NH); ¹³C NMR (300 MHz, DMSO-d₆), δ ppm: 166.48 (C=O), 158.10 (C–S), 146.02 (C–NO₂), 138.52 (C–NH), 137.46 (C–Cl), 136.78, 136.15, 131.20, 129.89, 129.25, 128.19, 126.71, 126.26, 117.19, 22.76.

2-Chloro-N-(2-chloro-4-nitrophenyl)-5-[(4-chlorophenyl)sulfonyl]4-nitrobenzamide (5n)

% Yield: 57.62; m.p.: 212–214 °C; Rf: 0.65 (B:EA– 7:3); FTIR (KBr): v_max (cm⁻¹): 3447.82 (N–H str.), 3096.77 (C–H str., Ar), 2947.05, 2885.31 (C–H str., Aliphatic), 1692.29 (C=O), 1592.79 (N–H bend), 1531.51 (asym. NO₂ str.), 1380.09 (asym. SO₂ str.), 1306.80 (sym. NO₂ str.), 1174.67 (sym. SO₂ str.), 772.81 (C–Cl); ¹H NMR (300 MHz, DMSO-d₆), δ ppm: 2.26 (s, H, CH₃), 3.45 (s, 1H, NH), 7.24–7.30 (m, 2H, CH C₅ and C₆ of –CONH–C₆H₂CH₃Cl–), 7.63 (s, 1H, CH of C₆ of ClNO₂C₆H₂CONH–), 7.84–7.87 (d, 2H, CH of C₂ and C₆ of ClC₆H₄NH–), 8.28–8.30 (d, 2H, CH of C₃ and C₅ of ClC₆H₄NH–), 10.59 (s, 1H, NH); ¹³C NMR (300 MHz, DMSO-d₆), δ ppm: 165.37 (C=O), 158.36 (C–S), 147.85 (C–NO₂), 140.10 (C–NH), 138.55 (C–Cl), 137.21, 135.46, 130.80, 129.61, 129.04, 125.15, 121.47, 118.28, 23.57.
2-Chloro-N-(4-chlorophenyl)-5-[(4-chlorophenyl) sulfamoyl]-4-nitrobenzamide (5q)

% Yield: 95.24; m.p.: 177–179 °C; Rf: 0.65 (B:EA–7:3); FTIR (KBr): \( v_{\text{max}} \) (cm\(^{-1}\)): 3483.02 (N–H str.), 3108.34 (C–H str., Ar), 1633.74 (C=O), 1599.82 (N–H bend), 1530.54 (asym. NO\(_2\) str.), 1396.49 (asym. SO\(_2\) str.), 1353.09 (sym. NO\(_2\) str.), 1183.49 (sym. SO\(_2\) str.), 754.34 (C–Cl); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 3.67 (s, 1H, NH), 7.44–7.46 (d, 2H, CH of C\(_2\) and C\(_5\) of –C\(_6\)H\(_4\)NH–), 7.60 (s, 1H, CH of C\(_6\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 7.95–7.96 (d, 2H, CH of C\(_2\) and C\(_6\) of –CONHCH\(_2\)Cl–), 8.00–8.02 (d, 2H, CH of C\(_3\) and C\(_5\) of –CONH–C\(_6\)H\(_4\)NO\(_2\)–), 8.24–8.26 (d, 2H, CH of C\(_3\) and C\(_5\) of –ClC\(_6\)H\(_4\)NH–), 8.50 (s, 1H, CH of C\(_3\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 10.63 (s, 1H, NH); \(^{13}\)CNMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 166.17 (C=O), 156.21 (C=S), 149.28 (C=NO\(_2\)H), 138.40 (C–NO\(_2\)), 138.06 (C–Cl), 131.16, 131.94, 132.52, 129.89, 126.84, 125.71, 123.20, 112.90.

N-Butyl-2-chloro-5-[(4-chlorophenyl) sulfamoyl]-4-nitrobenzamide (5t)

% Yield: 84.54; m.p.: 111–113 °C; Rf: 0.54 (Chloro-form); FTIR (KBr): \( v_{\text{max}} \) (cm\(^{-1}\)): 3446.85 (N–H str.), 3106.46 (C–H str., Ar), 2959.53, 2871.74 (C–H str., Aliphatic), 1658.64 (C=O), 1597.82 (N–H bend), 1531.51 (asym. NO\(_2\) str.), 1372.38 (asym. SO\(_2\) str.), 1309.80 (sym. NO\(_2\) str.), 1174.67 (sym. SO\(_2\) str.), 750.37 (C–Cl); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 2.08–2.18 (m, 9H, –CONH–C\(_2\)H\(_5\)), 3.71 (s, 1H, NH), 7.46 (s, 1H, CH of C\(_6\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 7.79–7.80 (d, 2H, CH of C\(_2\) and C\(_6\) of CI\(_6\)CH\(_2\)NH), 8.22–8.24 (d, 2H, CH of C\(_2\) and C\(_6\) of NO\(_2\)C\(_6\)H\(_2\)NH), 8.50 (s, 1H, CH of C\(_3\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 10.51 (s, 1H, NH); \(^{13}\)CNMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 160.69 (C=O), 155.52 (C=S), 144.08 (C=NO\(_2\)), 138.84 (C–NH), 136.60 (C–Cl), 133.53, 129.57, 128.48, 127.57, 32.15, 27.34, 18.99.

2-Chloro-5-[(4-chlorophenyl)sulfamoyl]-N-[(furan-2-yl)methyl]-4-nitrobenzamide (5u)

% Yield: 67.48; m.p.: 191–193 °C; Rf: 0.40 (B:EA–7:3); FTIR (KBr): \( v_{\text{max}} \) (cm\(^{-1}\)): 3503.75 (N–H str.), 3506.03 (C–H str., Ar), 2981.34 (C–H str., Aliphatic), 1665.16 (C=O), 1596.15 (N–H bend), 1506.43 (asym. NO\(_2\) str.), 1396.80 (asym. SO\(_2\) str.), 1376.23 (sym. NO\(_2\) str.), 1149.59 (sym. SO\(_2\) str.), 743.60 (C–Cl); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 2.59 (s, 2H, CH of CONH–C\(_2\)H\(_5\)O), 3.71 (s, 1H, NH), 7.61 (s, 1H, CH of C\(_6\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 7.79–7.87 (m, 3H, CH of C\(_2\), C\(_6\) and C4 of CONH–C\(_2\)H\(_5\)O), 7.92–7.93 (d, 2H, CH of C\(_2\) and C\(_6\) of CI\(_6\)H\(_2\)NH), 8.22–8.24 (d, 2H, CH of C\(_6\) of CI\(_6\)H\(_2\)NH), 8.50 (s, 1H, CH of C\(_3\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 10.58 (s, 1H, NH); \(^{13}\)CNMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 165.97 (C=O), 157.03 (C=S), 145.82 (C=NO\(_2\)), 142.54 (C–NH), 136.72 (C–Cl), 130.46, 129.18, 125.49, 121.85, 120.80, 113.64, 32.59.

2-Chloro-5-[(4-chlorophenyl)sulfamoyl]-4-nitro-N-(pyridin-4-yl)benzamide (5v)

% Yield: 71.32; m.p.: 197–199 °C; Rf: 0.31 (B:EA–7:3); FTIR (KBr): \( v_{\text{max}} \) (cm\(^{-1}\)): 3503.75 (N–H str.), 3113.16 (C–H str., Ar), 1665.56 (C=O), 1598.05 (N–H bend), 1548.50 (asym. NO\(_2\) str.), 1371.07 (sym. SO\(_2\) str.), 1316.44 (sym. NO\(_2\) str.), 1170.81 (sym. SO\(_2\) str.), 755.14 (C–Cl); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)), \( \delta \) ppm: 3.71 (s, 1H, NH), 7.57 (s, 1H, CH of C\(_6\) of CINO\(_2\)C\(_6\)H\(_2\)CONH–), 7.80–7.83 (d, 2H, CH of C\(_2\) and C\(_6\) of CI\(_6\)H\(_2\)NH–), 7.93–7.96 (d, 2H, CH of C\(_2\) and C\(_6\) of –CONH–C\(_6\)H\(_2\)NH–), 8.24–8.26 (d, 2H,
CH of C₃ and C₅ of CIC₆H₄NH–), 8.36–8.39 (d, 2H, C₃ and C₅ CH of –CONH–C₆H₄NH–), 8.50 (s, 1H, CH of C₃ of CINO₂C₆H₅CONH–), 10.56 (s, 1H, NH); 13CNMR (300 MHz, DMSO–d₆), δ ppm: 164.70 (C=O), 156.22 (C=S), 148.17 (C–NO₂), 144.89 (C–NH), 138.96 (C–Cl), 130.34, 128.50, 125.55, 120.17.

In vitro antidiabetic studies

α-Glucosidase inhibitory assay
The method adopted for performing α-glucosidase inhibitory assay was similar to our previous study, Thakral and Singh [32]. Graph Pad Prism program, version 5 was employed for calculation of the 50% inhibitory concentration (IC₅₀) of all compound [32, 42, 43].

α-Anylase inhibitory assay
Xiao et al., and Yoshikawa et al., illustrated a method, with little modification this method has been adopted for measuring the activity [32, 44].

Homology modeling
The 3D model for α-glucosidase is developed by comparative homology modeling technique using SWISS-MODEL web server (https://swissmodel.expasy.org/) [45] and then the quality of modeled structure was validated by Ramachandran plot (RAMPAGE) (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). The details are available in our previous report [32].

Molecular docking
Ligand molecules were prepared as per reported method [32] using MarvinSketch and AutoDock tools. The crystal structures of α-amylase, 1qho [32, 46] from Bacillus sterothermophilus, maltose/acyarbore complex downloaded from the protein data bank (http://www.rcsb.org) and α-glucosidase modeled structure [32] was used for docking in antidiabetic evaluation. Docking studies were carried out as reported in our previous study and literature using AutoDock Vina program [32, 47].

Molecular dynamic simulations
The respective structures placed in the center of the cubic box, the remaining volume of the box was filled by SPCe [48] water molecules. The whole box is then neutralized by adding the respective number of positive and negative ions using GROMACS 5.4 [49] by replacing the equal number of water molecules. Further energy minimization followed by 10 ns equilibration performed by using OPLS [50] force fields integrated into GROMACS 5.4 package to represent the potential energy of the system.

Computation of drug like parameters and ADMET profiling
Molinspiration (http://www.molinspiration.com/) online tool kit and OSIRIS property explorer was used for computing drug like characteristics from 2D chemical structures of aforementioned compounds [51–54]. Pre-ADMET online server (https://preadmet.bmdrc.kr/) was used for calculating pharmacokinetic parameters like adsorption, distribution, metabolism and excretion and some of the computed properties are human intestinal absorption (HIA %), Caco-2 cell permeability (nm/s), MDCK (Medin-Darbey Canine Kidney Epithelial Cells) cell permeability (nm/s), plasma protein binding (%), blood brain barrier penetration (C. brain/C. blood) and Pgp inhibition [55]. Bioactivity of synthesized compounds was predicted by Molinspiration (http://www.molinspiration.com/) online tool kit [56] and toxicity parameters like mutagenicity, tumorigenicity irritating effects and reproductive effects were computed by OSIRIS property explorer [57].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13065-020-00703-4.

Additional file 1: Table S1. Topological polar surface area, aqueous solubility, number of rotatable bonds, and calculated Lipinski’s rule of five for the synthesized 2-chloro-5-[(4-chlorophenyl)sulamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives; Table S2. ADME property values of synthesized 2-chloro-5-[(4-chlorophenyl)sulamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives using Pre-ADMET online server; Table S3. Bioactivity and toxicity risk of synthesized 2-chloro-5-[(4-chlorophenyl)sulamoyl]-N-(alkyl/aryl)-4-nitrobenzamide derivatives

Acknowledgements
The authors are thankful to Chairman, Department of Pharmaceutical Sciences, G. J. U. S. and T., Hisar for providing necessary facilities to carry out this research work and Prof. Neeraj Dilbaghi, Dept of Bio &Nano Technology, G. J. U. S. and T., Hisar for providing lab facility for in vitro studies. The authors are also thankful to Amit Singh, Discipline of Chemistry, Indian Institute of Technology, Gandhinagar for facilitation in computational studies.

Authors’ contributions
The authors (ST, RN, MK and VS) have done synthetic work, in vitro and in silico evaluation. All authors have read and approved the manuscript.

Funding
The author (VS) gratefully acknowledges the financial support (CIL/2017/356) as minor project for purchase of chemicals and Junior Research Fellow (JRF) award to Ms. Samridhi Thakral by Dr. A. P. J. Abdul Kalam Central Instrumentation laboratory, G. J. U. S. and T., Hisar under DST-PURSE program.

Availability of data and materials
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India. 2 Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136118, Haryana, India.
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