Synthesis of 1,2,3-benzotriazin-4(3H)-one derivatives as α-glucosidase inhibitor and their in-silico study

Zunera Khalid1,2 · Syed S. Shafqat3 · Hafiz A. Ahmad2 · Hafiz M. Rehman4 · Munawar A. Munawar2,5 · Matloob Ahmad6 · Abdullah M. Asiri7 · Muhammad Ashraf8

Received: 18 January 2022 / Accepted: 21 March 2022 / Published online: 14 April 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
α-Glucosidase inhibition is considered as an effective strategy for the treatment of diabetes mellitus. Currently, three α-glucosidase inhibitors are being used as drugs; Acarbose, Voglibose and Miglitol. The side effects of these drugs are forcing researchers to search for new and effective molecules. In this research work, novel 1,2,3-benzotriazin-4(3H)-one sulfonamides were synthesized and investigated for their α-glucosidase inhibition activity. 2,4,6-Trichloro-1,3,5-triazine: N, N-dimethylformamide (TCT : DMF) adduct have been utilized for the direct synthesis of targeted sulfonamides. All reactions were performed at room temperature under mild conditions. In-vitro enzyme inhibition studies led us to discover many potent inhibitors demonstrating good to excellent activity. The compound 5c with dimethyl substituent was found to be a more potent inhibitor than acarbose with the IC50 value of 29.75 ± 0.14 μM. Compounds 5a, 5b, 5d, 5e, 5f, and 5m showed good inhibition results with IC50 value 31.97 ± 0.03, 33.24 ± 0.01, 33.76 ± 1.05, 35.98 ± 0.03, 30.87 ± 0.51, and 37.24 ± 0.04 μM respectively. Further structure activity relationship was analyzed by molecular docking studies.

Graphical abstract

Keywords TCT : DMF adduct · 1,2,3-Benzotriazin-4(3H)-one · α-Glucosidase inhibitor · Sulfonamide · Molecular docking

Introduction
Diabetes mellitus is a chronic disorder that is adversely affecting the quality of life of a large population worldwide.

1 Department of Chemistry, Kinnaird College for Women, Lahore, Pakistan
2 School of Chemistry, University of the Punjab, Lahore, Pakistan
3 Department of Chemistry, University of Education, Township, Lahore, Pakistan
4 School of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
5 Department of Chemistry, University of the Central Punjab, Lahore, Pakistan
6 Department of Chemistry, Government College University, Faisalabad, Pakistan
7 Department of Chemistry, King Abdul Aziz University, Jeddah, Saudi Arabia
8 Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
In type 2 diabetes mellitus, the cell secrets insulin but the body becomes irresponsive leading to an uncontrolled blood glucose level. α-Glucosidase inhibitors, Voglibose, Acarbose, and Miglitol are being utilized to treat this health condition. These inhibitors control blood sugar by inhibiting α-glucosidase that is responsible for starch and disaccharides’ conversion to glucose. The available α-glucosidase inhibitors are associated with a number of side effects and thus, there is an urgent need to discover new drugs for the treatment of diabetes [1]. The sulfonamides based on heterocyclic systems are emerging as potent α-glucosidase inhibitors. Various sulfonamide molecules based on indole [2], chalcone [3], Celebrex [4], sulfaguanidine [5], and quinoline [6] have demonstrated effective α-glucosidase inhibition activity as compared to the available drug, acarbose.

In general, sulfa compounds are known for their applications in pharmaceuticals and agrochemicals. The sulfadizine (antibacterial), darunavir (antiviral drug), and celecoxib (anti-inflammatory drug) are among the well-known examples of sulfa-drugs [7]. According to a survey, 15% of the most prescribed drugs in cardiovascular, neurological and infectious diseases belong to the family of sulfonamides [8].

On the other hand, 1,2,3-benzotriazin-4(3H)-one compounds well known for various pharmaceutical applications [9] and have been reported as active antiproliferative agent [10], anti-inflammatory [11, 12], anti-depressant [13], anticancer [14], antidiarrheal [15] as well as anesthetics [16] (Fig. 1). Benzotriazinonephenyllithio-N-hydroxy-propionamides have been found as potent matrix metalloprotease inhibitor. 3-Indolymethyl-1,2,3-benzotriazinones have been reported as excellent chorismate mutase inhibitor [17]. Recently some benzotriazinone (Fig. 1) derivatives have tested as efficient inhibitor of HepG2 liver carcinoma [18]. Structures of some active 1,2,3-benzotriazin-4(3H)-ones are shown in Fig. 1 which prompted us to synthesize their new derivatives. In continuation of our efforts to improve synthesis methodologies along with development of new drugs [19–21] in stated work, we have synthesized a new series of 1,2,3-Benzotriazin-4(3H)-one derivatives with sulfonamide functionality and evaluate their α-glucosidase inhibition potency.

In this research work, the synthesis of N-alkyl/aryl-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamides were achieved through one pot, simple and low cost methodology under mild conditions. The most common method for sulfonamide synthesis is by the reaction of sulfonyl chloride and amine under basic conditions. But this reaction is two step and undesired di-sulfonamides are formed [22]. Only a few sulfonyl chlorides are commercially available due to their instability. To avoid these problems, different reagents have been developed to replace sulfonyl chlorides, such as pentafluorophenyl vinylsulfonate and sulfonylbenzotriazole followed by aminolysis [23]. In-addition, triphenylphosphine/pyridine or triethylamine salt, sodium salt sulfonic acid/ 2,4,6-trichloro-1,3,5-triazine [22] and alkylisocyanides have also been reported for the direct conversion of sulfonic acid to sulfonamides [24].

In continuation of our strategy employed for the direct conversion of sulfonic acid to sulfonamides by using 2,4,6-trichloro-1,3,5-triazine (TCT) and N,N-

![Fig. 1 Different bioactive 1,2,3-benzotriazin-(3H)-ones lead to current synthesis](image-url)
dimethylformamide (DMF). Synthesized products were evaluated for their antidiabetic potential and structure activity relationship was explained by molecular docking studies.

Result and discussion

Chemistry

Isatin (1) was used as basic precursor to prepare isatoic anhydride (2) by the oxidation reaction with hydrogen peroxide in the presence of formic acid. Compound 2 was treated with sulfanilic acid to form 4-(2-aminobenzamido) benzenesulfonic acid (3) that was subjected to cyclization with the help of nitrous acid to 4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonic acid (4). Then triazin-based sulfonic acid (4) was directly converted to series of sulfonamide compounds 5a–m (Scheme 1) by using TCT : DMF adduct.

For direct conversion of sulfonic acid to sulfonamide, cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) was taken in cold DMF and stirred until adduct forms then dried dichloromethane (DCM) and DMF was used in 1:1 ratio. 4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonic acid (4) and p-bromo aniline was added to adduct mixture and 85% product yield was obtained. Reaction was also tried in only DMF and DCM : DMSO mixture but no product was observed. Then all reactions were done in DCM : DMF (1:1) solvent.

The reaction conditions and results of all reactants are explained in Table 1. Aliphatic amine, heteroaryl amine and aniline were utilized in reaction. Anilines with electron donating groups showed better yield rather than anilines with electron withdrawing groups. Among all reactants nitro anilines and amino pyridines did not give any result. All products were confirmed by IR, 1HNMR, and 13CNMR.

α-Glucosidase inhibition studies

The results of α-glucosidase inhibition studies revealed moderate to excellent activity of newly synthesized compounds 5a–5m (Table 2). Among the series 5a–5m, compound 5c with two methyl groups showed excellent activity with 94.53 ± 0.42% inhibition assay (IC50 value 29.75 ± 0.14 µM) that was better than acarbose inhibitory results 92.23 ± 0.16% (IC50 37.38 ± 0.12 µM). Most probably the presence of two methyl groups on compound 5c enabled it to exhibit the more hydrophobic interactions with enzyme than monosubstituted compounds, 5d (m-methyl) and 5f (p-methyl). Furthermore, the position of methyl substituent on the benzene ring of synthesized compounds also has significant effects and showed distinct potential results. p-Methylated compound 5f with 93.01 ± 0.93% (IC50 value 30.87 ± 0.51 µM) was found to be a better inhibitor as compared to m-methylated compound 5d with 92.29 ± 1.43% (IC50 33.76 ± 1.05 µM) value. Compound 5a with one chloro group showed comparable results to di-methylated compound 5d with 92.29 ± 1.43% (IC50 33.76 ± 1.05 µM) value. Compound 5a with one chloro group showed comparable results to di methyl compound with 93.58 ± 0.2% inhibition (IC50 value 31.97 ± 0.03 µM). Because of big size of bromo atom than other substituents, its derivative 5b inhibitory potency was less than 5a compound. Compounds 5a–5f had aryl sulfonamide groups exhibited better results than alkyl sulfonamide compounds 5g, 5h, 5i, 5j, 5k, 5l that just showed moderate activity toward this enzyme.

Nature and position of substituent on the phenyl ring influence the inhibition activity. Aryl sulfonamides make more pi-stacking and pi-cationic interactions with enzyme cavity as compared to alkyl sulfonamide. Compounds 5a and 5b contains chloro and bromo substituent that showed...
| Sr. No. | Reactants | Products | Time | Yield(%) |
|--------|-----------|----------|------|----------|
| 1.     | \(\text{NH}_2\)-Cl | ![Image of 5a] | 24 h | 75       |
| 2.     | \(\text{NH}_2\)-Br | ![Image of 5b] | 24 h | 85       |
| 3.     | \(\text{NH}_2\)-H\(_3\)C | ![Image of 5c] | 24 h | 64       |
| 4.     | \(\text{NH}_2\)-H\(_3\)C | ![Image of 5d] | 24 h | 78       |
| 5.     | \(\text{NH}_2\)-H\(_3\)CO | ![Image of 5e] | 24 h | 79       |
| 6.     | \(\text{NH}_2\)-H\(_3\)C | ![Image of 5f] | 24 h | 72       |
| 7.     | \(\text{NH}_2\)-CH\(_2\)NH\(_2\) | ![Image of 5g] | 24 h | 85       |
| 8.     | \(\text{NH}_2\)-CH\(_2\)NH\(_2\) | ![Image of 5h] | 24 h | 79       |
better result than methoxy 5e because in halogens more pair of electrons are available for interactions than methoxy group. It is noticeable that compounds 5g, 5h, 5i, 5j, 5k and 5l contain alkyl groups and their enzyme inhibition potential increases as number of carbons atoms increase in alkyl chain i.e., 5g with propyl chain showed 79.33 ± 0.97% (102.16 ± 0.41 µM) while 5l with octyl chain had 88.74 ± 0.17% (50.66 ± 0.05 µM) inhibition. This reason of increase in inhibition from 5g to 5l is due to increase in alkyl chains hydrophobic interactions. However, for the inhibitory activity, one generalization can be postulated that sulfonamide with aryl substitution shows better results as compared to compounds with alkyl groups.

**Table 1** (continued)

| Sr. No. | Reactants | Products | Time | Yield(%) |
|---------|-----------|----------|------|----------|
| 9.      | NH₂       | ![Structure](image) | 24 h | 77       |
| 10.     | NH₂       | ![Structure](image) | 24 h | 71       |
| 11.     | NH₂       | ![Structure](image) | 24 h | 73       |
| 12.     | NH₂       | ![Structure](image) | 24 h | 74       |
| 13.     | ![Structure](image) | ![Structure](image) | 24 h | 74       |

**Table 2** Results of α-Glucosidase Inhibition studies

| Sr. NO. | Compounds | Inhibition (%) | IC₅₀(µM) |
|---------|-----------|----------------|----------|
| At 0.5 mm |           |                |          |
| 1.      | 5a        | 93.58 ± 0.20   | 31.97 ± 0.03 |
| 2.      | 5b        | 92.54 ± 0.11   | 33.24 ± 0.01 |
| 3.      | 5c        | 94.53 ± 0.42   | 29.75 ± 0.14 |
| 4.      | 5d        | 92.29 ± 1.43   | 33.76 ± 1.05 |
| 5.      | 5e        | 92.88 ± 0.12   | 35.98 ± 0.03 |
| 6.      | 5f        | 93.01 ± 0.93   | 30.87 ± 0.51 |
| 7.      | 5g        | 79.33 ± 0.97   | 102.16 ± 0.41 |
| 8.      | 5h        | 81.88 ± 1.57   | 95.57 ± 1.03 |
| 9.      | 5i        | 83.97 ± 1.59   | 75.52 ± 1.06 |
| 10.     | 5j        | 85.69 ± 0.15   | 57.51 ± 0.01 |
| 11.     | 5k        | 86.13 ± 0.39   | 54.54 ± 0.09 |
| 12.     | 5l        | 88.74 ± 0.17   | 50.66 ± 0.05 |
| 13.     | 5m        | 91.93 ± 0.66   | 37.24 ± 0.04 |
| 14.     | Acarbose  | 92.23 ± 0.16   | 37.38 ± 0.12 |

**In silico docking studies**

In order to define enzyme inhibition, Auto Dock Vina computational studies were applied to explore possible interaction mechanism of α-glucosidase and ligand. In docking studies,
ligand and enzyme best orientation and confirmation was chosen and α-glucosidase counting function algorithm was used to calculate their binding affinity. All synthesized compounds (5a–5m) interacted with the binding sites of α-glucosidase and their inhibition potency was justified. These novel compounds presented good binding energies against α-glucosidase (Cat No. 5003-1KU Type I) (Table 3). Among all ligands, 5c have highest free binding energy $9.7 \text{ kcal mol}^{-1}$ proves as potent inhibitor of target proteins. Ligands 5a, 5b, 5d, 5f and 5m were also found as good glucosidase inhibitor with binding energy $9.5 \text{ kcal mol}^{-1}$. Remaining analogues, 5e, 5g, 5h, 5i, 5j and 5k presented less interactions as compared to other compounds with binding energy 8.4–9.2 kcal mol$^{-1}$.

For complete docking studies, ligand 5c was selected against α-glucosidase (Cat No. 5003-1KU Type I). 2D and 3D structural interactions were appeared with the several amino acid residues and its picture shown in Fig. 2. These results demonstrated that ligand 5c fits perfectly at the catalytic sites of α-glucosidase with a binding energy of $-9.7 \text{ kcal/mol}$.

The interaction studies displayed that ligand bonded to the active site through five H-bonds. There is one hydrogens bond exists in Phe157 with the distance of 2.39 Å (Table 4a). Asn241 interacted through H-bond have distance of 2.77 Å. Two oxygen atoms of SO$_2$ make H-bonding with the cavity amino acid residues i.e., Arg312 and Asp408 make H-bond with the distance of 2.78 Å and 2.86 Å. His279 form another hydrogen bond with the oxygen atom of benzotriazinone motif with the distance of 3.40 Å.

Alkyl and pi-alkyl bonds found to have hydrophobic interactions at six sides of enzyme cavity (Table 4b) and they observed in Phe177X, Phe157, Phe158, Glu304, Pro309, Arg312 with the distance of 3.35, 3.31, 3.83, 3.90, 3.55, 3.61 Å.

In ligand there are number of non-polar and aromatic residues that build π-stacking interactions with Phe177 (Table 4c). Pi-cation interactions were also formed because of phenyl rings and these interactions were located at two points with residue His279 (Table 4d).

The analogue structures 5a, 5b, 5d, 5f and 5m demonstrated the second level inhibitory results with α-glucosidase binding energy 9.5 kcal/mol. These compounds form one or two hydrogen bonds with enzyme cavity while 5c showed strong interactions because of five H-bonds. These compounds 5a, 5b, 5d, 5f and 5m make same type of hydrophobic interactions with Phe158, Phe300, and Phe177 at two sides of enzyme cavity while Thr215, Glu304 have these interactions at one side Figs. 3–7. 5a, 5b, 5d and 5f form interactions with the active site Glu276 through the hydrogen bond with the distance of 2.34–2.36 Å but 5m make its three H-bonds with Glu276 (2.74 Å), His279 (3.20 Å), Arg312 (3.16 Å). His279 in all these compounds bonded to vicinity residues through Pi-cation interaction.

These interactions stabilize the ligand protein complex and forms its strong inhibition effect. From docking studied

**Table 3** Docking binding energies (kcal mol$^{-1}$) of the docked compounds 5a–m into the active site of α-glucosidase

| Compound | Free binding energy (kcal mol$^{-1}$) |
|----------|--------------------------------------|
| 5a       | $-9.5$                               |
| 5b       | $-9.5$                               |
| 5c       | $-9.7$                               |
| 5d       | $-9.5$                               |
| 5e       | $-9.2$                               |
| 5f       | $-9.5$                               |
| 5g       | $-8.5$                               |
| 5h       | $-8.4$                               |
| 5i       | $-8.5$                               |
| 5j       | $-8.7$                               |
| 5k       | $-8.5$                               |
| 5l       | $-8.5$                               |
| 5m       | $-9.5$                               |

**Fig. 2** 2D and 3D interactions between amino acid residues of α-glucosidase and ligand 5c
result elucidated that 5c have strong binding within enzyme protein. 5a, 5b, 5d, 5f, 5m have low binding energy than 5c because of less hydrogen bonds. 5g, 5h, 5i, 5j, 5k and 5l are alkyl sulfonamides and have less hydrophobic and pi stacking interactions to amino acid residues that reduce its binding energy as compared to 5c.

| Index | Residue | AA | Distance H-A | Distance D-A | Donar Angle | Donar Atom | Acceptor Atom |
|-------|---------|----|-----------|-----------|-------------|------------|---------------|
| 1.    | 157X    | PHE | 2.39      | 3.30      | 146.32      | 9435[Npl]  | 2528[O2]     |
| 2.    | 241X    | ASN | 2.77      | 3.65      | 145.77      | 3858[Nam]  | 9433[Nar]    |
| 3.    | 279X    | HIS | 3.40      | 4.04      | 122.64      | 4486[Npl]  | 9457[O2]     |
| 4.    | 312X    | ARG | 2.78      | 3.56      | 134.24      | 4975[Ng+]  | 9459[O2]     |
| 5.    | 408X    | ASP | 2.86      | 3.59      | 133.55      | 6548[O-]   | 9458[O2]     |

**Conclusion**

TCT: DMF Adduct has been used successfully for the synthesis of N-alkyl or aryl 4-(4-oxobenzo[1,2,3]triazin-3 (4H)-yl)benzenesulfonamides. All reactions were done at the same time under same conditions. This method is simple.
Fig. 4 2D and 3D structure represents 5b ligand and \( \alpha \)-glucosidase interactions

Fig. 5 2D and 3D structures represent 5d ligand and \( \alpha \)-glucosidase interactions

Fig. 6 2D and 3D structures represent 5f ligand and \( \alpha \)-glucosidase interactions

Fig. 7 2D and 3D structure represents 5m ligand and \( \alpha \)-glucosidase interactions
one step conversion that provides rapid and good yield product under mild conditions. So, it is efficient method to convert 1,2,3-benzotriazin-4(3H)-one sulfonic acid to their corresponding sulfonamides. In comparison to acarbose all synthesized compounds were screened for their potency to inhibit α-glucosidase. Compound 5c found potent inhibitor (IC$_{50}$ = 29.75 ± 0.14 μM) than acarbose (IC$_{50}$ = 37.38 ± 0.12) and most of the elaborated benzotriazinone derivatives showed inhibitory activity close to acarbose. Molecular docking studies clearly demonstrate that structures 5a–m bind to the active sites of α-glucosidase and possesses strong inhibitory potential.

**Material and methods**

**Experimental**

**Synthesis of Isatoic anhydride (2)**

In conical flask, 14.7 g Isatin (0.1 mol) was taken in formic acid (80 mL). Hydrogen peroxide (30%, 20 mL) was added dropwise with slight cooling. The reaction contents were stirred for 1 h at 25 °C and precipitates were filtered. Product was washed with methanol and dried at 70 °C. Light yellow precipitates, m.p.: 240 °C (Lit. m.p.: 240 °C). (C13H12N2O4S) [M+H] NMR: (DMSO, 400 MHz): δ: 7.60 (2H, d, J = 8.4 Hz, Ar-H), 7.78 (2H, t, J = 8.0 Hz, Ar-H), 7.98 (1H, t, J = 7.4 Hz, Ar-H), 8.13 (1H, t, J = 7.6 Hz, Ar-H), 8.27 (1H, d, J = 8.0 Hz, Ar-H), 8.33 (1H, d, J = 7.6 Hz, Ar-H). 13C NMR: (DMSO, 126 MHz): δ: 155.31, 150.49 (2C), 148.82, 143.71, 139.40, 136.24, 133.90, 128.78, 126.70 (2C), 125.55, 120.49. EI-MS (m/z): (C13H9N3O4S) 339 (0.4%), 269 (7), 195 (53), 167 (41), 139 (17), 119 (7), 93 (83), 64 (100).

**Synthesis of 4-(2-Aminobenzamido)benzenesulfonic acid (3)**

Sulfanilic acid (3.46 g, 20 mmol) was dissolved in pyridine (70 mL). Hydrogen peroxide (30%, 20 mL) was added slowly to mixture and the temperature was maintained below 25 °C. After 30 min stirring, white solid precipitates was formed that were filtered and recrystallized with methanol to have pure 4-(2-Aminobenzamido)benzenesulfonic acid (3). (C13H9N3O4S) [M+H] NMR: (DMSO, 400 MHz): δ: 7.60 (2H, d, J = 8.0 Hz, Ar-H), 7.58 (2H, d, J = 8.5 Hz, Ar-H), 7.96 (1H, s, NH2), 8.11 (1H, t, J = 7.5 Hz, Ar-H), 8.26 (1H, d, J = 8.0, 1H, Ar-H), 8.31 (d, J = 8.0, 1H, Ar-H), 11.15 (s, NH, 1H). 13C NMR: (DMSO, 126 MHz): δ: 155.26 (C δ 1), 149.15, 143.71, 139.40, 136.24, 133.90, 128.78, 126.70 (2C), 125.55, 120.49. EI-MS (m/z): (C13H9N3O4S) 339 (0.4%), 269 (7), 195 (53), 167 (41), 139 (17), 119 (7), 93 (83), 64 (100).

**4-(4-Oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonic acid (4)**

4-(Aminobenzamido)benzene sulfonic acid (3 g, 10.26 mmol) was suspended in ice cold water (30 mL) and 30% HCl solution (20 mL) was added. Sodium nitrite (5.1 g, 52 mmol) solution in distilled water (10 mL) was added slowly to mixture and the temperature was maintained at 0–5 °C. The it was stirred for 1 h, precipitates were filtered and recrystallized with methanol. Off white precipitate, m.p.: >300 °C, Yield: 2.80 g (90%), FT-IR (v-cm$^{-1}$): 3383 (OH), 1697 (CO), 1140 (SO$_4$), 1030 (SO$_4$) [M+H] NMR: (DMSO, 400 MHz): δ: 7.60 (2H, d, J = 8.4 Hz, Ar-H), 7.78 (2H, t, J = 8.0 Hz, Ar-H), 7.98 (1H, t, J = 7.4 Hz, Ar-H), 8.13 (1H, t, J = 7.6 Hz, Ar-H), 8.27 (1H, d, J = 8.0 Hz, Ar-H), 8.33 (1H, d, J = 7.6 Hz, Ar-H). 13C NMR: (DMSO, 126 MHz): δ: 155.31, 150.49 (2C), 148.82, 143.71, 139.40, 136.24, 133.90, 128.78, 126.70 (2C), 125.55, 120.49. EI-MS (m/z): (C13H9N3O4S) 393 (83), 371 (9), 359 (9), 339 (83), 269 (7), 195 (53), 167 (41), 139 (17), 119 (7), 93 (83), 64 (100).

**General preparation for the preparation of N-alkyl or aryl-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamides (5a–5k)**

2,4,6-Trichloro-1,3,5-triazine (0.182 g, 0.99 mmol) was added to cold N,N-DMF (0.39 mL, 0.99 mmol) and temperature maintained below 25 °C. After 30 min stirring, white solid was formed. Then adduct was dissolved in DCM (10 mL). 4-(4-Oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonic acid (4) (300 mg, 0.99 mmol) was dissolved in N,N-DMF (10 mL) and poured to adduct solution followed by the amine (0.99 mmol) addition. After reaction completion precipitates formed that were filtered and washed with 1 N HCl followed by recrystallization with methanol to have pure N-alkyl or aryl sulfonamides. But in case of N-alkyl sulfonamides, product was purified by chloroform: n-hexane mixture.

**N-(4-Chlorophenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5a)**

Beige brown precipitates, m.p.: >300 °C, Yield: 0.30 g (75%), FT-IR (v-cm$^{-1}$): 2865 (NH), 1684 (CO), 1331 (SO$_4$), 1160 (SO$_4$), [M+H] NMR: (DMSO, 400 MHz): δ: 7.17 (d, J = 8.5 Hz, 2H, Ar-H), 7.42 (d, J = 9.0 Hz, 2H, Ar-H), 7.58 (d, J = 8.5 Hz, 2H, Ar-H), 7.75 (d, J = 8.5 Hz, 2H, Ar-H), 7.96 (t, J = 7.8, 1H, Ar-H), 8.11 (t, J = 7.5, 1H, Ar-H), 8.26 (d, J = 8.0, 1H, Ar-H), 8.31 (d, J = 8.0, 1H, Ar-H), 11.15 (s, NH, 1H). 13C NMR: (DMSO, 126 MHz): δ: 155.26 (C δ 1), 149.15, 143.71, 139.40, 136.24, 133.89, 130.10 (2C), 128.84, 126.65 (3C), 126.64 (3C), 125.56, 123.84, 120.65.

**N-(4-Bromophenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5b)**

Apricot pink precipitates, m.p.: >300 °C, Yield: 0.38 g (85%), FT-IR (v-cm$^{-1}$): 2866 (NH), 1685 (CO), 1328 (SO$_4$), 1150
benzenesulfonamide (5d)

Off white precipitates, m.p.: >300 °C, Yield: 0.32 g

N-(3,4-Dimethylphenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5c)

Pale pink precipitates, m.p.: >300 °C, Yield: 0.3 g

N-(3-Methylphenyl)- 4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5d)

Persian orange precipitates, m.p.: >300 °C, Yield: 0.32 g

N-(4-Methylphenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5e)

N-(4-Methylphenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5f)

N-(4-Methylphenyl)-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5g)

N-Propyl-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5h)

N-Butyl-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl)benzenesulfonamide (5i)
(S=O), 1H NMR: (500 MHz, DMSO): δ 8.11 (m, 3H, Ar-H), 8.27 (d, 2H, Ar-H), 8.12 (td, J = 8.4 Hz, 2H, Ar-H), 7.73 (d, J = 8.4 Hz, 2H, Ar-H), 7.61 (d, J = 8.1 Hz, 2H, Ar-H), 8.27 (d, J = 8.1 Hz, 1H, Ar-H), 8.32 (d, J = 7.8 Hz, 1H, Ar-H), 8.46 (s, NH, 1H). 13C NMR: (126 MHz, DMSO): δ 155.32 (C=O), 150.49, 148.85, 143.73, 139.38, 136.24, 135.21, 133.93, 128.79, 126.71 (2C), 126.68 (2C), 125.55 (2C), 120.44, 34.74, 01, 31.21, 27.40, 25.98, 24.32, 14.36.

N-Heptyl-4-(4-oxobenzo[1,2,3]triazin-3(4H)-yl) benzenesulfonamide (5j)

Off white precipitates, m.p.; >300 °C, Yield: 0.30 g (74%), FT-IR (v=cm−1): 2855 (NH), 1689 (C=O), 1299 (S=O), 1041 (S=O), 1H NMR: (300 MHz, DMSO): δ 1.49 (d, J = 6.9 Hz, CH2), 4.37 (q, J = 6.0 Hz, NH), 7.39 (m, 3H, Ar-H), 7.49 (d, J = 7.2 Hz, 2H, Ar-H), 7.61 (d, J = 8.1 Hz, 2H, Ar-H), 7.73 (d, J = 8.4 Hz, 2H, Ar-H), 7.98 (t, J = 7.5 Hz, 1H, Ar-H), 8.13 (t, J = 7.5 Hz, 1H, Ar-H), 8.27 (d, J = 8.1 Hz, 1H, Ar-H), 8.32 (d, J = 7.8 Hz, 1H, Ar-H), 8.46 (s, NH, 1H). 13C NMR: (126 MHz, DMSO): δ 155.32 (C=O), 150.49 (5C), 148.85, 143.73, 139.38, 136.24, 135.21, 133.93, 128.79, 126.71 (2C), 126.68 (2C), 125.56, 120.52, 34.67.

Procedure for α-glucosidase inhibition studies

α-Glucosidase (Cat No. 5003-1KU Type I) belongs to Saccharomyces cerevisiae was used for the enzyme inhibition studies because its structure and function is like yeast/ mammalian enzymes. Pierre et al. method was adopted with some changes.

In tubes, 10 μL test compound (0.5 mM), 70 μL saline phosphate buffer (50 mM at pH 6.8) and 10 μL α-glucosidase enzyme (0.0234 units) was added. Then tubes were incubated for ten minutes at 37 °C and absorbance was observed at 400 nm. In test tube, 10 μL p-nitrophenyl-α-D-glucopyranoside (0.5 mM, ‘substrate’, code No. N1377 from Sigma) was added to start the reaction and tubes were placed for 30 min. Then to observe free substrate change, absorbance of all tubes was measured at 400 nm. The percentage inhibition was calculated by the formula given below and IC50 values were calculated at EZ-Fit enzyme kinetics software version 5.03 (Perrella Scientific Inc. Amherst, USA).

\[
\%\text{Inhibition} = \frac{Abs\text{ of test} - Abs\text{ of Control}}{Abs\text{ of Control}} \times 100
\]

Molecular modeling studies

The crystal structure of eukaryotic yeast (Saccharomyces cerevisiae) was not found on Protein Data Bank, only some bacterial glucosidase structures were available. The sequence of saccharomyces cerevisiae’s α-glucosidase is based on sequence of 584 amino acid residues (uniprot ID: P53341). NCBI’s BLAST algorithm was used to have suitable template for homology modelling of target protein.
For homology modeling, highest sequence similarity was observed in oligo-1,6-glucosidase (P53051) and selected to be used as a basic pattern. Sequence alignment was conducted by using Needleman-Wunsch Global Alignment Algorithm via Chimera. Structure modelling was processed on Modeller. Quality of the new structure was checked by using Ramachandran plot, which showed 97.3% residues were in the favored region, and 99.7% residues were in the allowed region. To review the quality of created homology model, molecular dynamics simulation was carried out on NAMD. Visualization of molecular dynamics trajectories was done on VMD. Protein molecule was solvated and equilibrated in a water box and modeled at physiological temperature of 310 K for 10 ps. Finally optimized structure was applied for further docking studies on BioSolveIT’s LeadIT. Discovery Studio visualizer was used for presentation of docked conformations.

Acknowledgements
The authors acknowledge the School of Chemistry, University of the Punjab Lahore, Pakistan for providing us chemical and instrumental facilities.

Compliance with ethical standards

Conflict of interest
The authors declare no competing interests.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Taha M, Alrashedy AS, Almandil NB, Iqbal N, Nawaz M, Uddin N, et al. Synthesis of indole derivatives as diabetics II inhibitors and enzymatic kinetics study of α-glucosidase and α-amylase along with their in-silico study. Int J Biol Macromol. 2021;190:301–18. https://doi.org/10.1016/j.ijbiomac.2021.08.207
2. Alomari M, Taha M, Rahim F, Selvaraj M, Iqbal N, Chigurupati S, et al. Synthesis of indole-based-thiadiazole derivatives as a potent inhibitor of α-glucosidase enzyme along with in silico study. Bioorg Chem. 2021;108:104638 https://doi.org/10.1016/j.bioorg.2021.104638
3. Seo WD, Kim JH, Kang JE, Ryu HW, Curtis-Long MJ, Lee HS, et al. Sulfonamide chalcone as a new class of α-glucosidase inhibitors. Bioorg Med Chem Lett. 2005;15:5514–6. https://doi.org/10.1016/j.bmcl.2005.08.087
4. Kausar N, Ullah S, Khan MA, Zafar H, Choudhary MI, Youusf S. Celebrex derivatives: synthesis, α-glucosidase inhibition, crystal structures and molecular docking studies. Bioorg Chem. 2021;106:104499 https://doi.org/10.1016/j.bioorg.2020.104499
5. Akocak S, Taslimi P, Lokal N, Isık M, Durgun M, Budak Y, et al. Synthesis, Characterization, and Inhibition Study of Novel Substituted Phenylureido Sulfaguanidine Derivatives as α-Glycosidase and Cholinesterase Inhibitors. Chem Biodivers. 2021;18: e2000098 https://doi.org/10.1002/cbdv.202000098
6. Devaraj S, Yip YM, Panda P, Ong LL, Wong PWK, Zhang D, et al. Cinnamoyl Sucrose Esters as Alpha Glucosidase Inhibitors for the Treatment of Diabetes. Molecules 2021;26:469 https://doi.org/10.3390/molecules26020469
7. Elgemeie GH, Azizn RA, Elsayed RE. Sulfur drug analogs: new classes of N-sulfonyl aminoazides and their biological and preclinical importance in medicinal chemistry (2000–2018). Med Chem Res. 2019;28:1099–131. https://doi.org/10.1007/s00440-019-02378-6
8. Mukherjee P, Woroch CP, Cleary L, Rusznak M, Franzese RW, Reese MR, et al. Sulfonamide synthesis via calcium trilimide activation of sulfonyl fluorides. Org Lett. 2018;20:3943–7. https://doi.org/10.1021/acs.orglett.8b01520
9. Khalid Z, Ahmad HA, Munawar MA, Khan M-U-A, Gul S, 1, 2, 3-Benzotriazin-4-(3H)-ones: synthesis, reactions and applications. Heterocycles 2017;94:3–54. https://doi.org/10.3987/REV-16-846
10. Fiorino F, Magli E, Perissuti E, Severino B, Frencetese F, Esposito A, et al. Synthesis of 1-naphthylpyrazine derivatives as sertoneronergic ligands and their evaluation as antiproliferative agents. Eur J Med Chem. 2011;46:2206–14. https://doi.org/10.1016/j.ejmech.2011.03.001
11. Ibrahim TS, Rashad AA, Abdel-Samii ZK, El-Feky SA, Abdel-Hamid MK, Barakat W. Synthesis, molecular modeling and anti-inflammatory screening of new 1, 2, 3-benzotriazinone derivatives. Med Chem Res. 2012;21:4369–80. https://doi.org/10.1007/s00044-012-9975-3
12. Raffa D, Migliara O, Maggio B, Plescia F, Cascoferro S, Cusimano MG, et al. Pyrazolobenzotriazinone Derivatives as COX Inhibitors: Synthesis, Biological Activity, and Molecular-Modeling Studies. Arch Pharm. 2010;343:631–8. https://doi.org/10.1002/ardp.200900317
13. Fiorino F, Severino B, De Angelis F, Perissuti E, Frencetese F, Massarelli P, et al. Synthesis and In-vitro Pharmacological Evaluation of New 5-HT1A Receptor Ligands Containing a Benzotriazinone Nucleus. Arch Pharm. 2008;341:20–7. https://doi.org/10.1002/ardp.200700151
14. Raffa D, Daidone G, Maggio B, Schillaci D, Plescia F. Synthesis and Antiproliferative Activity of Novel 3-[(3Indazol-3-yl)-quinazolin-4-(3H)-one and 3-[(3Indazol-3-yl)-benzotriazin-4-(3H)-one Derivatives. Arch Pharm. 1999;332:317–20. https://doi.org/10.1002/s2.1521-4184(19999)332:9%3C317::AID-ARDP317%3E3.0.CO;2-R
15. Fiorino F, Caliendo G, Perissuti E, Severino B, Frencetese F, Preziosi B, et al. Synthesis by microwave irradiation and anti-diarrhoeal activity of benzotriazinone and saccharine derivatives. J Org Chem. 2005;338:548–55. https://doi.org/10.1002/ardp.200500134
16. Caliendo G, Fiorino F, Grieco P, Perissuti E, Santagada V, Meli R, et al. Preparation and local anaesthetic activity of benzotriazinone and benzoyltriazole derivatives. Eur J Med Chem. 1999;34:1043–51. https://doi.org/10.1016/S0223-5234(99)00126-9
17. Reddy GS, Snehalatha AV, Edwin RK, Hossain KA, Giliyaru VB, Hariharapura RC, et al. Synthesis of 3-indolymethyl substituted (pyrazolo/benzo) triazinone derivatives under Pd/Cu-catalysis: Identification of potent inhibitors of chorismate mutase (CM). Bioorg Chem. 2019;91:103155 https://doi.org/10.1016/j.bioorg.2019.103155
18. El Rayes S, Ali I, Fathalla W, Mahmoud M. Synthesis and Biological Activities of Some New Benzotriazinone Derivatives Based on Molecular Docking; Promising HepG2 Liver Carcinoma Inhibitors. ACS Omega. 2020;5:6781–91. https://doi.org/10.1021/acs.omega.0c00116
19. Ahmad HA, Gilliani SS, Babar R, Munawar MA, Gulb S A, Rapid and Efficient Protocol for the Synthesis of Cinnamates. INEOS OPEN. 2020;3:20–4. https://doi.org/10.32931/ino20020a
20. Chaudhry F, Shahid W, al-Rashida M, Ashraf M, Ali Munawar M, Ain Khan M. Synthesis of imidazolyl-pyrazole conjugates bearing aryl spacer and exploring their enzyme inhibition potentials. Bioorg Chem. 2021;108:104686 https://doi.org/10.1016/j.bioorg.2021.104686
21. Ahmad HA, Aslam M, Gul S, Mehmood T, Munawar MA. In vivo Anti Inflammation Studies of Novel 1, 2, 5 Oxadiazole Sulfonamide Hybrids. Pak J Zool. 2021. https://doi.org/10.17582/journal.pjz/20200601040658

22. De Luca L, Giacomelli G. An easy microwave-assisted synthesis of sulfonamides directly from sulfonic acids. J Org Chem. 2008;73:3967–9. https://doi.org/10.1021/jo800424g

23. Caddick S, Wilden J, Bush H, Wadman S, Judd D. A new route to sulfonamides via intermolecular radical addition to pentafluorophenyl vinylsulfonate and subsequent aminolysis. Org Lett. 2002;4:2549–51. https://doi.org/10.1021/ol026181m

24. Shaabani A, Soleimani E, Rezayan AH. A novel approach for the synthesis of alkyl and aryl sulfonamides. Tetrahedron Lett. 2007;48:2185–8. https://doi.org/10.1016/j.tetlet.2007.01.091

25. Clark R, Wagner E. Isatoic anhydride. I. Reactions with primary and secondary amines and with some amides1. J Org Chem. 1944;9:55–67. https://doi.org/10.1021/jo01183a007