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Synthesis and *in vitro* Biological Evaluation of a Series of Benzothiazole-Sulfonylurea Hybrids as Novel Class of α-Glucosidase Inhibitors

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A series of benzothiazole-sulfonylurea hybrids **(C1-C9)** were synthesized and the molecular structures were characterized using physical (state, colour, melting point) and spectral (FT-IR, ^{1}H NMR, ^{13}C NMR and ESI-Mass) methods. All the compounds were screened for their bioactive potential as antidiabetic agents through α-glucosidase enzyme inhibitory properties at 100 µM concentration. The results were compared with a standard drug, voglibose. The bioassay results revealed that compound **C2** was found to be the hit molecule that has exhibited a percentage enzyme inhibition of 49.10%, which is relatively better than that of voglibose at 37.75%. The observed activity is primarily due to the synergistic or addition potential of the pharmacophores benzothiazole and sulfonylurea hybridized into one molecule; the structural analogues of these pharmacophores were earlier reported as α-glucosidase inhibitors.

Keywords: Benzothiazole, Sulfonylurea, Benzothiazole-sulfonylurea hybrid, α-Glucosidase inhibitor, Voglibose.

INTRODUCTION

Diabetes mellitus is a chronic and progressive metabolic disorder in which the body's glucose management is impaired. It is mainly caused by the decrease in insulin secretion by pancreatic β-cells, which leads to insulin resistance. Insulin is a pancreatic hormone that helps the body with blood glucose management. An insulin deficient body causes hyperglycemia (elevated blood glucose levels above 120 mg/dL). This condition leads to other health issues, such as ketoacidosis, peripheral neuropathy, retinopathy, *etc*. The global statistics of the International Diabetes Federation (IDF) reported that the prevalence of diabetes is predicted to be 643 million people by 2030 and 783 million people by 2045 [1]. Currently available drugs for diabetes treatment have major issues related to their safety and efficacy. Therefore, the need for new drug discovery is continuously alarming medicinal chemists to design and develop novel chemotypes.

α-Glucosidases are essential enzymes found in the small intestine, responsible for the digestion process and carbohydrate metabolism. They catalyze the chemical hydrolysis of glycosidic bonding with a terminal glucose moiety in the target tissue. They are also involved in the biosynthesis and metabolism of N-linked glycoproteins linked to oligosaccharide chains [2]. α-Glucosidase has sparked interest in drug discovery by chemists due to its ability to delay glucose absorption, preventing spikes in postprandial blood glucose levels. Various α-glucosidase inhibitors, such as miglitol, acarbose and voglibose, are currently in clinical use for treating type 2 diabetes mellitus. However, developing these inhibitors requires time-consuming, multistep processes. Recently, due to their significant selectivity and effectiveness, there has been an increased focus on non-sugar based small or hybrid organic compounds as inhibitors of α-glucosidase.

Benzothiazole is a fused heterocyclic compound where the thiazole ring is fused to the benzene. The functional group substitution at various positions in the ring unfolds its chemical diversity for possible modifications and molecular arrangements in drug discovery [3]. It is one of the privileged scaffolds

studied by medicinal chemists by virtue of their chances to transform into clinically useful drugs like ethoxzolamide, riluzole, zopolrestat, *etc*. [4]. The substituted benzothiazole analogues revealed a diverse array of pharmacological agents includes antiviral agents [5], human DNA topoisomerase IIα inhibitors [6], anticonvulsant agents [7], antifungal agents [8], anti-influenza agents [9], non-carboxylic PTP1B inhibitors [10], antitubercular agents [11], β-glucuronidase activity [12], COX-2/5-LOX inhibitors [13], antiproliferative agents [14], tyrosine kinase inhibitors [15], histone deacetylase inhibitors [16], diuretics [17], vasorelaxants and inhibitors of insulin releasing process [18], human estrogen receptor modulators [19], anti-tumor agents [20], selective PI3Kβ inhibitors [21], bacterial type II topoisomerase inhibitors [22], plant growth regulators [23], α-glucosidase inhibitors [24], analgesic agents [25], schistosomicidal agents [26], antichagasic agents [27], Aurora B kinase inhibitors [28], antidiabetic agents [29], β-amyloid imaging agents [30], cyclooxygenase inhibitors [31], anti-cancer agents [32], neuroprotective agents [33], 17β-HSD10 inhibitors [34], chemokine receptor 2 (CXCR2) inhibitors [35], KATP channel openers [36], photosensitizing agents [37], Raf-1 inhibitors [38], anti-inflammatory agents [39], antimicrobial agents [40], human DNA topoisomerase IIα inhibitors [6], PPARα antagonists [41], CK-1δ inhibitors [42], cytotoxic agents [43], falcipain inhibitors [44], antidepressant activity [45], mono-amine oxidase A/B inhibitors [46], DNA gyrase B inhibitors [47], topoisomerase I inhibitors [48], antileishmanial agents [49], antioxidant agents [50], β-amyloid imaging agents [30] and hemostatic agents [51].

Sulfonylureas are the organic compounds with a basic structure consisting of a sulfonyl group covalently linked to the urea moiety. The substitution of either a sulfonyl group or a urea group leads to the chemical diversity of sulfonylureas with greater therapeutic value as antidiabetic agents such as glyburide, gliclazide, glibenclamide, *etc*. On the other hand, sulfonylurea-based compounds attracted medicinal chemists not only because of their synthetic feasibility but also because of their biological significance, which showed a range of therapeutic benefits that include selective β3 adrenergic receptor agonist [52], hypoglycemic [53], peroxisome proliferator activated receptor γ-agonistic [54], antimicrobial [55], herbicidal [56], selective EP4 receptor antagonists [57], selective bombesin receptor subtype-3 (SCS-3) agonist [58], antagonists of the CXCR2 receptor [59], antimalarial [60], Vibrio fischeri quorum sensing regulator [61], selective antagonists TPα and TPβ isoforms human thromboxane A2 receptor [62], reversible inhibitors human steroid sulfatase [63], KATP-channel openers [64], inhibitors of aldehyde dehydrogenase [65], cancer chemotherapeutic [66] and vasodilator [67]. In this study, based on the research question, what would be the structure-activity relationship of benzothiazole-fused tosylurea hybrids as α-glucosidase inhibitors? We designed our study with specific objectives to synthesize and evaluate *in vitro* α-glucosidase inhibitory potential and due to their pharmacophore and approved bioactivity profile. In addition, we also hypothesized that the compounds **C1-C9** synthesized in this investigation will demonstrate inhibitory activities against α-glucosidase.

EXPERIMENTAL

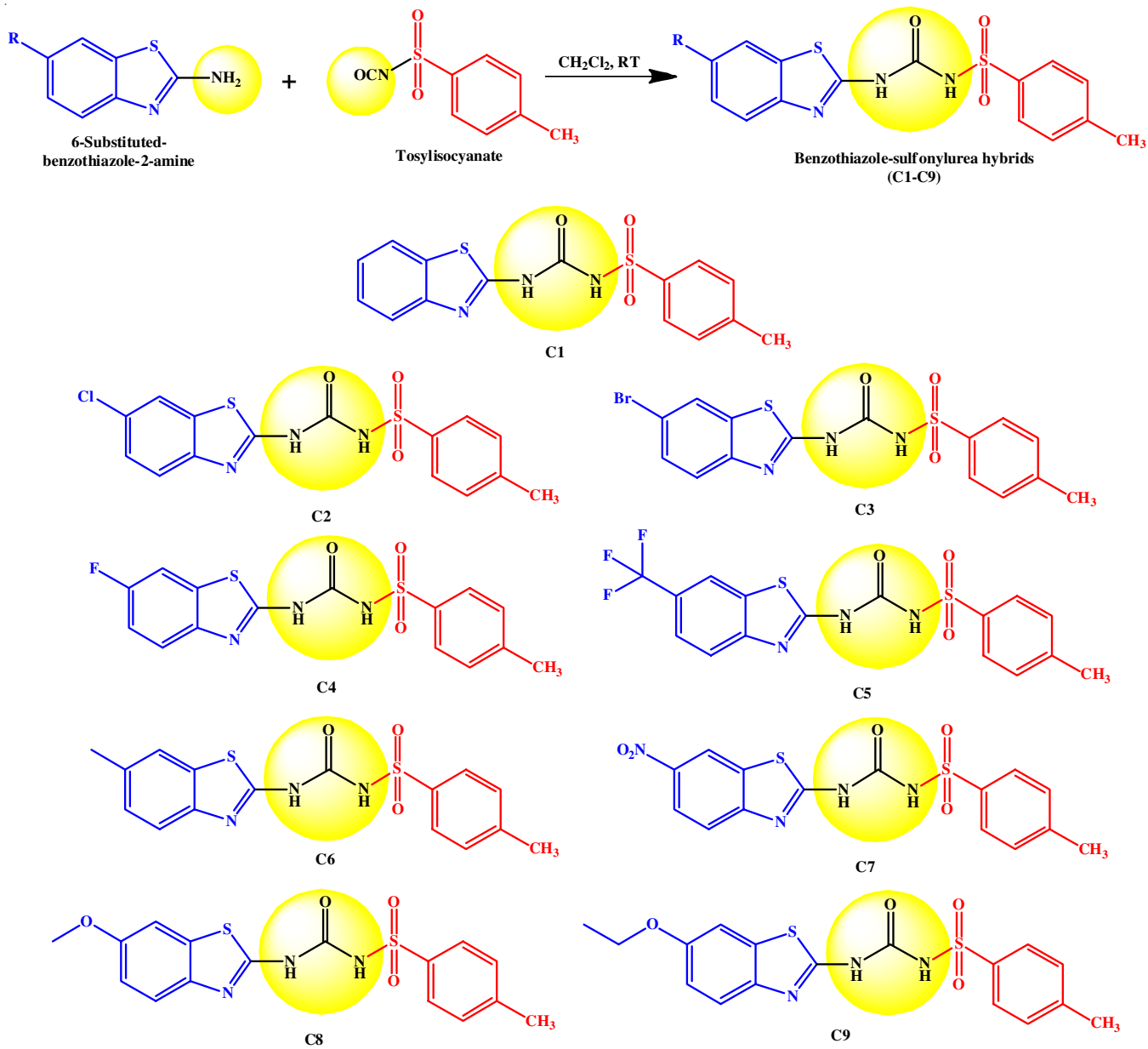
All the reagents and chemical were purchased from Sigma-Aldrich, USA. The compound's purity was checked on pre-coated 60 F_{254} silica gel TLC plates (Merck, 0.25 mm) thickness by means of a gradient solvent system with *n*-hexane and ethyl acetate. Flourier-transform infrared (FT-IR) spectrometer (MIRAffinity-1S, Shimadzu, Japan) used to record the spectra. ^{1}H NMR & ^{13}C NMR spectra recorded on a Varian NMR System (USA, Varian, 500 MHz) using TMS (tetramethylsilane) as an internal standard. The electrospray ionization mass spectra (ESI-MS) were recorded using high-resolution mass spectrometry (HRMS) (Q Exactive Focus (Orbitrap LC-MS/MS System, Thermo-Scientific, USA). The melting point apparatus (Stuart Scientific, Model: SMP1, UK) were determined in open capillary tubes and are uncorrected.

General procedure for the synthesis of benzothiazole-sulfonylurea hybrids (C1-C9): Benzothiazole-sulfonylurea hybrids (**C1-C9**) were synthesized by transferring tosylisocyanate (0.015 M) into a conical flask (100 mL) charged with 6-substituted benzothiazole-2-amines (0.01 M) in 20 mL of methylene chloride. The reaction mixture was gently stirred at room temperature using a glass rod continuously for 10 min. The flask was warmed in a water bath for 15 min and the solution was cooled down to room temperature. A solid precipitate was observed commonly for all compounds **C1-C9**. After washing the products under vacuum filtration with cold methanol were recrystallized with ethanol (**Scheme-I**).

***N*-(Benzo[*d*]thiazol-2-ylcarbamoyl)-4-methylbenzenesulfonamide (C1):** Yield: 64%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{15}H_{13}N_3O_3S_2$; relative molecular mass: 347; FT-IR (ATR, ν_{max}, cm^{-1}): 3167.12 (2º amine N-H *str.*), 3122.75 (2º amine N-H *str.*), 1647.27 (C=O *str.*), 1541.12 (2º amine NH *bend.*), 1307.74 (SO_2, asymmetrical), 1142.22 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, Ar-CH_3), 7.25 (t, 1H, J_1 = 7 MHz, J_2 = 9 MHz, Ar-H), 7.42-7.36 (m, 3H, Ar-H), 7.52 (s, 1H, Ar-H), 7.82 (d, 1H, *J* = 8 MHz, Ar-H), 7.86 (d, 2H, *J* = 8 MHz, Ar-H); ESI-MS (*m/z*): 348 $[M+H]^+$ (positive-ion mode), 346 $[M-H]^-$ (negative-ion mode).

***N*-((6-Chlorobenzo[*d*]thiazol-2-yl)carbamoyl)-4-methylbenzenesulfonamide (C2):** Yield: 96%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{15}H_{12}ClN_3O_3S_2$; relative molecular mass: 381; FT-IR (ATR, ν_{max}, cm^{-1}): 3116.97 (2º amine N-H *str.*), 3072.60 (2º amine N-H *str.*), 1649.14 (C=O *str.*), 1541.12 (2º amine NH *bend.*), 1338.60 (SO_2, asymmetrical), 1151.50 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.36 (s, 3H, Ar-CH_3), 7.39-7.38 (m, 1H, Ar-H), 7.42 (d, 2H, *J* = 7.5 MHz, Ar-H), 7.56 (d, 1H, *J* = 8 MHz, Ar-H), 7.86 (d, 2H, *J* = 8 MHz, Ar-H), 7.98 (s, 1H, Ar-H); ESI-MS (*m/z*): 382 $[M+H]^+$ (positive-ion mode), 380 $[M-H]^-$ (negative-ion mode).

***N*-((6-Bromobenzo[*d*]thiazol-2-yl)carbamoyl)-4-methylbenzenesulfonamide (C3):** Yield: 55%; orange colour powder; m.p.: > 250 ºC; m.f.: $C_{15}H_{12}BrN_3O_3S_2$; relative molecular mass: 426; FT-IR (ATR, ν_{max}, cm^{-1}): 3115.04 (2º amine N-H *str.*), 3024.38 (2º amine N-H *str.*), 1651.07 (C=O *str.*), 1541.12 (2º amine N-H *bend.*), 1338.60 (SO_2, asymmetrical), 1151.50 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37



Scheme-I: Reaction scheme for the synthesis of benzothiazole-sulfonylurea hybrids **(C1-C9)**

(s, 3H, Ar-CH_3), 7.42 (d, 2H, J = 8 MHz, Ar-H), 7.52-7.51 (m, 3H, Ar-H), 7.85 (d, 2H, J = 8 MHz, Ar-H), 8.11 (s, 1H, Ar-H); ESI-MS (*m/z*): 428 $[M+2]^+$ (positive-ion mode), 424 $[M-2]^-$ (negative-ion mode).

***N*-((6-Fluorobenzo[*d*]thiazol-2-yl)carbamoyl)-4-methylbenzenesulfonamide (C4):** Yield: 98%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{15}H_{12}FN_3O_3S_2$; relative molecular mass: 365; FT-IR (ATR, ν_{max}, cm^{-1}): 3128.54 (2º amine N-H *str.*), 3070.68 (2º amine N-H *str.*), 1645.28 (C=O *str.*), 1546.91 (2º amine NH *bend.*), 1327.03 (SO_2, asymmetrical), 1147.65 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, Ar-CH_3), 7.25 (t, 1H, J_1 = 9 MHz, J_2 = 11.5 MHz, Ar-H), 7.42 (d, 2H, J = 8 MHz, Ar-H), 7.58 (s, 1H, Ar-H), 7.78 (d, 1H, J = 10.5 MHz, Ar-H), 7.86 (d, 2H, J = 8.5 MHz, Ar-H); ESI-MS (*m/z*): 366 $[M+H]^+$ (positive-ion mode), 364 $[M-H]^-$ (negative-ion mode).

4-Methyl-*N*-((6-(trifluoromethyl)benzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C5): Yield: 51%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{16}H_{12}F_3N_3O_3S_2$; relative molecular mass: 415; FT-IR (ATR, ν_{max}, cm^{-1}): 3253.91 (2º amine N-H *str.*), 3207.62 (2º amine N-H *str.*), 1747.51 (C=O *str.*), 1552.70 (2º amine NH *bend.*), 1323.17 (SO_2, asymmetrical), 1114.86 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, Ar-CH_3), 7.43 (d, 2H, J = 8.5 MHz, Ar-H), 7.70-7.67 (m, 3H, Ar-H), 7.86 (d, 2H, J = 8.5 MHz, Ar-H); ESI-MS (*m/z*): 416 $[M+H]^+$ (positive-ion mode), 414 $[M-H]^-$ (negative-ion mode).

4-Methyl-*N*-((6-methylbenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C6): Yield: 93%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{16}H_{15}N_3O_3S_2$; relative molecular mass: 361; FT-IR (ATR, ν_{max}, cm^{-1}): 3161.33 (2º amine N-H *str.*), 3118.90 (2º amine N-H *str.*), 1647.21 (C=O *str.*), 1541.12

(2º amine NH *bend.*), 1307.74 (SO_2, asymmetrical), 1153.43 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H, Ar-CH_3), 2.36 (s, 3H, Ar-CH_3), 7.19 (d, 2H, J = 8.5 MHz, Ar-H), 7.41 (d, 2H, J = 8 MHz, Ar-H), 7.60 (s, 1H, Ar-H), 7.86 (d, 2H, J = 8 MHz, Ar-H); ESI-MS (*m/z*): 362 $[M+H]^+$ (positive-ion mode), 360 $[M-H]^-$ (negative-ion mode).

4-Methyl-*N*-((6-nitrobenzo[*d*]thiazol-2-yl)carbamoyl)-benzenesulfonamide (C7): Yield: 62%; light yellow colour powder; m.p.: > 250 ºC; m.f.: $C_{15}H_{12}N_4O_5S_2$; relative molecular mass: 392; FT-IR (ATR, ν_{max}, cm^{-1}): 3128.54 (2º amine N-H *str.*), 3080.52 (2º amine N-H *str.*), 1660.71 (C=O *str.*), 1546.91 (2º amine NH *bend.*), 1519.91 (NO_2, asymmetrical), 1339.10 (NO_2, symmetrical), 1307.74 (SO_2, asymmetrical), 1153.43 (SO_2, symmetrical); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, Ar-CH_3), 7.42 (d, 2H, J = 8 MHz, Ar-H), 7.72 (d, 1H, J = 8.5 MHz, Ar-H), 7.86 (d, 2H, J = 8 MHz, Ar-H), 8.23 (d, 2H, J = 9 MHz, Ar-H), 8.85 (s, 1H, Ar-H); ESI-MS (*m/z*): 393 $[M+H]^+$ (positive-ion mode), 391 $[M-H]^-$ (negative-ion mode).

***N*-((6-Methoxybenzo[*d*]thiazol-2-yl)carbamoyl)-4-methylbenzenesulfonamide (C8):** Yield: 99%; white colour powder; m.p.: > 250 ºC; m.f.: $C_{16}H_{15}N_3O_4S_2$; relative molecular mass: 377; FT-IR (ATR, ν_{max}, cm^{-1}): 3201.83 (2º amine N-H *str.*), 3130.47 (2º amine N-H *str.*), 1645.28 (C=O *str.*), 1541.12 (2º amine NH *bend.*), 1307.74 (SO_2, asymmetrical), 1159.22 (SO_2, symmetrical), 1087.85 (C=O *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H, Ar-CH_3), 3.75 (s, 3H, Ar-OCH_3), 6.98 (d, 2H, J = 9 MHz, Ar-H), 7.42 (d, 2H, J = 8 MHz, Ar-H), 7.44 (s, 2H, Ar-H), 7.85 (d, 2H, J = 8 MHz, Ar-H); ESI-MS (*m/z*): 378 $[M+H]^+$ (positive-ion mode), 376 $[M-H]^-$ (negative-ion mode).

***N*-((6-Ethoxybenzo[*d*]thiazol-2-yl)carbamoyl)-4-methyl-benzenesulfonamide (C9):** Yield: 73%; cream colour powder; m.p.: > 250 ºC; m.f.: $C_{17}H_{17}N_3O_4S_2$; relative molecular mass: 391; FT-IR (ATR, ν_{max}, cm^{-1}): 3163.26 (2º amine N-H *str.*), 3126.61 (2º amine N-H *str.*), 1645.28 (C=O *str.*), 1543.05 (2º amine NH *bend.*), 1307.74 (SO_2, asymmetrical), 1159.22 (SO_2, symmetrical), 1087.85 (C=O *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.31 (t, 3H, J_1 = 7 MHz, J_2 = 7 MHz, Ar-OCH_2CH_3), 2.36 (s, 3H, Ar-CH_3), 4.02-3.98 (q, 2H, J_1 = 7 MHz, J_2 = 7 MHz, J_3 = 7 MHz, Ar-OCH_2CH_3), 6.96 (d, 2H, J = 9 MHz, Ar-H), 7.42 (d, 2H, J = 8 MHz, Ar-H), 7.45 (s, 2H, Ar-H), 7.85 (d, 2H, J = 8 MHz, Ar-H); ESI-MS (*m/z*): 392 $[M+H]^+$ (positive-ion mode), 390 $[M-H]^-$ (negative-ion mode).

General procedure for *in vitro* α-glucosidase inhibitor screening: The α-glucosidase inhibitory activity of compounds **(C1-C9)** were evaluated using *in vitro* α-glucosidase enzymatic kinetics. Initially, 100 mL of phosphate buffer solution (PBS) has been prepared using pre-adjusted buffer tablet dissolved using distilled water. The enzyme concentrations (0.8 to 0.0125 U/mL) were prepared in PBS, alongside 4-nitrophenyl-D-glucopyranoside (pNPG) prepared in PBS (0.8 to 0.0125 mM), in addition test compounds and the standard were also prepared 100 µM concentration in DMSO. A calibration graph was plotted for the reaction mixture concentrations enzyme (0.1 U/mL) against substrate (0.8 to 0.0125 mM) at UV 405 nm. The screening was performed by measuring the absorbances of liberated *p*-nitrophenol (yellow) in sample/blank reaction mixtures at 405 nm. The total microplate well volume of 130 µL that includes control (enzyme: 120 µL, phosphate buffer: 5 µL, phosphate buffer + substrate: 5 µL), Reaction control–blank (enzyme: 120 µL, phosphate buffer: 10 µL), reaction test (enzyme: 120 µL, DMSO + test compound: 5 µL, phosphate buffer + substrate: 5 µL), reaction solvent blank (enzyme: 120 µL, DMSO: 5 µL, phosphate buffer + substrate-5 µL), reaction standard (enzyme: 120 µL, phosphate buffer + substrate: 5 µL, DMSO + voglibose: 5 µL (100 µM to 0.5 µM). All the solutions were subjected to enzyme kinetics for 20 min to measure the absorbance. The percentage (%) enzyme inhibition calculated using the formula:

$$\text{Enzyme inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

The statistical analysis was carried out using Microsoft Excel.

RESULTS AND DISCUSSION

The starting point for identifying the molecular structures of hybrids **C1-C9** emerged by applying the theoretical understanding of the conventional method of organic synthesis involving isocyanate and amine as reactants, resulting in the formation of a urea bridge as a linkage between benzothiazole-2-amine and tosylisocyanate. The resultant product is a benzothiazole-sulfonylurea hybrid scaffold. The electrospray ionization mass spectrometry (ESI-MS) technique was used to record the mass spectra of compounds **C1-C9** in both positive and negative ion modes using methanol as solvent. The spectral data for synthesized compounds **C1-C9** revealed that pseudo-molecular ions were observed as M+H or M+2 (for bromine-substituted compounds) in positive modes and M-H or M-2 (for bromine-substituted compounds) in negative modes, respectively. The molecular ion signals were detected as base peaks, which correspond to the corresponding relative molecular masses of **C1-C9**. The synthesized compounds **C1-C9** were further analyzed with FT-IR spectroscopy. The vibrational bands observed for compounds **C1-C9** were consistent with the characteristic range of vibrational frequencies that include two secondary amino stretches (3200-3000 cm^{-1}), carbonyl stretch (1720-1650 cm^{-1}), secondary amide bend (1560-1500 cm^{-1}), sulfonyl asymmetrical stretches (1400-1300 cm^{-1}) and sulfonyl symmetrical stretches (1200-1100 cm^{-1}), respectively. Subsequently, the chemical shifts of the ^{1}H NMR spectra of compounds **C1-C9** exhibited characteristic peaks that included three equivalent aromatic methyl protons of the toluene moiety that appeared in the range of 2.33 to 2.37 δ ppm as a singlet and two doublets of equivalent protons of the phenyl ring appeared between the ranges. The characteristic position 2 carbon of the benzothiazole ring is identified in the downfield scale at 203.55 δ ppm. In adding up, all aromatic protons were observed within the range of aromatic protons. Two doublets, each integrated for 2 protons with a coupling constant of J = 8 MHz, show the presence of equivalent phenyl ring protons. In compounds **C1-C9**, the proton integration matched the expected number of protons in the compounds, except for the 2 protons of the secondary amino group (-NH-), which are exchangeable and do not contri-

bute to resonance due to hydrogen-deuterium exchange. In addition, the chemical shifts of the ^{13}C NMR spectra of the representative compound **C1** exhibited a characteristic peak that indicates aromatic methyl carbon of the toluene methyl group, which appeared at 21.51 δ ppm. Another characteristic carbon of the carbonyl group was found to exist at 143.95 δ ppm. In addition, the de-shielded center carbon at position in between the sulfur at position-1 and nitrogen at position-3 appeared at 203.55 δ ppm, respectively. In addition, all the aromatic carbons were observed within the range of aromatic carbons.

***In vitro* screening:** Compound **C1** which is unsubstituted at position-6 on benzothiazole ring exhibited activity close to the standard but less potency than the standard, it is very interesting to see that the substituents at position 6 of benzothiazole shows a variability in potencies based on the type of functional group substituted and the potency order is **C2** (6-chloro) > **C3** (6-bromo) > **C7** (6-nitro) > **C1** > **C8** (6-methoxyl) > **C6** (6-methyl) > **C9** (6-ethoxy) > **C5** (6-trifluromethyl) > **C4** (6-fluoro), respectively as shown in Table-1. This clearly shows that there is no substituent at position-6, compound **C1** possess inhibitory potency, this indicates hybridization of benzothiazole-sulfonylurea moieties is significant for the α-glucosidase inhibitory potency, however the substitution on position-6 of benzothiazole ring led to the variations either increase or decrease in the potency.

The structure-activity relationship (SAR) of the synthesized compounds **C1-C9** based on their percentage inhibition of α-glucosidase enzyme activity in comparison to the standard voglibose was investigated. The structural features analyzed include the heterocyclic ring and the type of substituent at position 6 and position 2. Compounds **C1-C9**, share a common heterocyclic ring, benzothiazole, which contributes to the stability of the molecular structure as a basic skeleton. Likewise, the tosylurea group which is also common to **C1-C9**, it plays a role in modulating α-glucosidase inhibition through exploring the influence of various substituents at position 6 on the benzothiazole, such as chloro, bromo, fluoro, tri-fluoro methyl, methyl, nitro, methoxyl and ethoxyl respectively. The percentage inhibition of α-glucosidase activity at 100 μM concentration varies among the compounds screened, ranging from 19.41% to 49.10%. Among all, compound **C2**, with a chloro substituent on the benzothiazole, displayed the highest inhibition at 49.10%, indicating that the presence of a chlorine atom enhances the activity. Compounds **C2**, **C3** and **C7** exhibited relatively better potency than voglibose. In opposition, compound **C4**, with a fluoro substituent, displays the lowest inhibition at 19.41%. This result suggests that the lightest halogen atom fluorine may not contribute as effectively to the binding interactions, highlighting the importance of relative molecular mass in influencing potency. The substituents at position-6 of benzothiazole ring varies among the studied compounds as observed in case of compound **C3**, with a bromo substituent, exhibits a percentage inhibition of 40.40%, indicating a halogen other than fluorine has showed improvement in the activity. This suggests that the introduction of a bromine atom enhances the interaction with the enzyme, it clearly indicated that the atomic size of the halogen atom has relationship with the activity earlier proven in case of compound **C2** with chloro substituent. In contrast, compound **C6**, with a methyl substituent, shows the percentage inhibition of 26.10%, suggesting a weaker inhibitory effect compared to compound **C3**. The smaller size of the methyl group may reduce steric hindrance, affecting the binding affinity of the compound for the α-glucosidase active site. A close interpretation of chemical nature of functional groups, the presence of electron withdrawing groups, such as chloro, bromo and nitro, at position 6 positively influenced the inhibitory activity. Compounds with electron-donating groups, such as methyl, methoxyl and ethoxyl, exhibited relatively less potency, respectively.

Conclusion

In summary, this study provides an insight into the structure activity relationship (SAR) of benzothiazole-sulfonylurea hybrids as a novel class of α-glucosidase inhibitors. The inhibitory potencies exhibited by the compounds are primarily influenced by the nature of the functional group substituent at position-6 on the benzothiazole ring. Compounds with electron withdrawing groups typically exhibit greater potency; however, this observation has a limitation since there are other substituent groups that were not studied, such as iodine. The findings of this study revealed the valuable insights for designing new hybrids

TABLE-1
PERCENTAGE INHIBITION OF THE α-GLUCOSIDASE ENZYME ACTIVITY DATA OF COMPOUNDS **C1-C9**

Compound (100 μM)	% Inhibition of the α-glucosidase enzyme activity	Structural features			
		Heterocyclic ring	Substituent at the position 6 of the BZT	Type of position 6 substituent	Substituent at the position 2 of the BZT
C1	36.47	BZT	–	–	Tosylurea
C2	49.10	BZT	Chloro	EWG	Tosylurea
C3	40.40	BZT	Bromo	EWG	Tosylurea
C4	19.41	BZT	Fluoro	EWG	Tosylurea
C5	21.13	BZT	Tri-fluoro methyl	EWG	Tosylurea
C6	26.10	BZT	Methyl	EDG	Tosylurea
C7	37.80	BZT	Nitro	EWG	Tosylurea
C8	28.61	BZT	Methoxyl	EDG	Tosylurea
C9	21.55	BZT	Ethoxyl	EDG	Tosylurea
Voglibose	37.75	–	–	–	–

BZT: Benzothiazole, EWG: Electron withdrawing group, EDG: Electron donating group

as α-glucosidase inhibitors consisting of benzothiazole-sulfonylurea moieties. Further research on derivatizing the hybrids with a more functional group will demonstrate a detailed SAR that contributes to the discovery of novel α-glucosidase inhibitors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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