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Synthesis, Characterization and *in vitro* Biological Evaluation of Benzothiazole-Phenylsulfonyl Urea Hybrid Analogues as Antibacterial and Antidiabetic Agents

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The global burden of antibacterial resistance, along with the increasing prevalence of diabetes worldwide, has intensified the search for new therapeutic agents with novel chemical scaffolds that possess both antibacterial and antidiabetic potential. In this study, a series of benzothiazole-phenylsulfonyl urea derivatives (**C1-C6**) were synthesized and their molecular structures were confirmed by HRMS-ESI, ATR FT-IR, ^{1}H and ^{13}C NMR spectral data. The *in vitro* antibacterial activity was evaluated against both susceptible and resistant bacterial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter baumannii*, using the broth microdilution method. All compounds exhibited low antibacterial activity (MIC > 128 µM), indicating limited potential as antibacterial agents. In addition, the same derivatives were also tested for *in vitro* α-glucosidase (*Saccharomyces cerevisiae* Type 1) inhibitory activity using an enzyme-kinetics assay. All compounds demonstrated varied degrees of inhibition (IC_{50} = 682.25 ± 2.90 to 801.51 ± 13.46 µM) relative to voglibose (IC_{50} = 711.04 ± 3.88 µM). Compound **C3**, bearing an ethoxy group at position 6 of the benzothiazole ring, showed the highest α-glucosidase inhibitory activity (IC_{50} = 682.25 ± 2.90 µM), while compound **C6**, with a fluoro substitution at position 6, was the most potent within its subgroup (IC_{50} = 744.37 ± 4.86 µM). Structure-activity relationship (SAR) analysis suggested that electron-donating substitutions at position 6 of the benzothiazole moiety enhance inhibitory potency. Overall, while these benzothiazole-phenylsulfonyl urea derivatives demonstrated only weak antibacterial activity, their α-glucosidase inhibition potential is found worthwhile for further structural optimization to develop as antidiabetic agents.

Keywords: Benzothiazole-Phenylsulfonyl urea hybrid, Antibacterial activity, Antidiabetic activity, α-Glucosidase inhibitors, SAR.

INTRODUCTION

Benzothiazole is a heterocyclic compound where thiazole is fused to the benzene ring, the substitution of varied functional groups at different positions in the ring explores the chemical diversity of the benzothiazole for various possible chemical modifications [1]. In addition, benzothiazole derivatives have been experimented by the researchers in the design, discovery and development of various classes of therapeutic agents approved for the clinical use [2]. Medicinal chemists have been continuously embarking on synthesising of novel compounds of this chemotype due to the broad spectrum of medicinal uses associated with the benzothiazole scaffold [3]. The modified analogues of the benzothiazole revealed a significant number of pharmacological properties such as antimicrobial [4], inhibitors of the insulin releasing process [5], α-glucosidase inhibitor [6], β-glucuronidase activity [7], antibacterial [8], anti-infective agents [9], 17β-HSD10 inhibitors [10], antidiabetic [11], K_{ATP} channel openers [12], *etc*.

Based on the structure-activity relationship (SAR) reported in the literature, position 2 in the benzothiazole ring has been identified as a key structural modification carried out and resulted as a potential antimicrobial agent [13], in addition based on earlier studies also exhibit α-glucosidase inhibitory properties. Therefore, we conducted this study with the primary objective of synthesizing a series of 2-substituted benzo-

thiazole-phenylsulfonyl urea hybrids and evaluate their *in vitro* antibacterial and α-glucosidase inhibitory activities as part of our ongoing systematic investigation on the identification of bioactive benzothiazole analogues as antibacterial and antidiabetic agents. Based on earlier studies [14], we have independently identified benzothiazole and sulfonylurea as key pharmacophoric moieties towards observed antibacterial and α-glucosidase inhibitory properties. Hence, in this study, we aim to determine whether benzothiazole-phenylsulfonyl urea hybrids could exhibit both activities and function as dual-targeted therapeutics.

EXPERIMENTAL

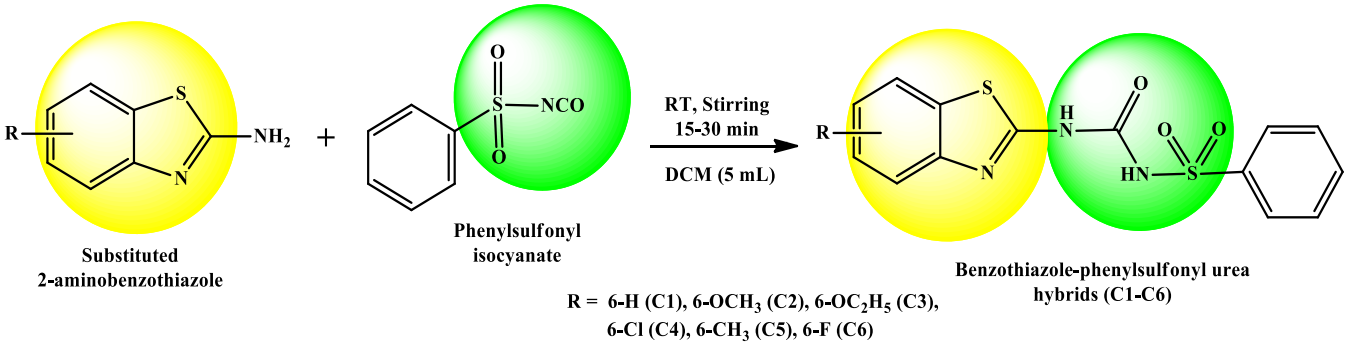
The reaction progress and purity of the compound were checked on pre-coated 60 F_{254} silica gel TLC plates (Merck, 0.25 mm) thickness by means of a gradient solvent system with *n*-hexane and ethyl acetate. FT-IR spectrometer (model: MIRAffinity-1S, Shimadzu, Japan) used to record the spectra. ^{1}H NMR & ^{13}C NMR spectra recorded on a Varian NMR System (Varian, USA, 500 MHz) using TMS as an internal standard, Weighing Balance (model: ML204, Mettler Toledo, USA) was used to weigh the chemicals used in the synthetic protocols. The electrospray ionization mass spectra (ESI-MS) were recorded using high-resolution mass spectrometry (HRMS) (Thermo Scientific, Q Exactive Focus (Orbitrap LC-MS/MS System, USA). Melting point apparatus (SMP1, Stuart Scientific, U.K.) were determined in open capillary tubes and are uncorrected. BioTek Synergy HTX multimode reader (USA), Tecan Infinite Pro microplate reader (Switzerland).

All chemicals and solvents were of analytical or reagent grade and used as received from commercial suppliers unless otherwise specified. Dimethyl sulfoxide (biotech grade), phosphate-buffered saline (PBS, pH 7.3), sodium carbonate, 4-nitrophenyl-α-D-glucopyranoside and crude α-glucosidase from *Saccharomyces cerevisiae* Type I (≥ 10 U/mg protein) were purchased from Sigma-Aldrich (USA). Voglibose (standard α-glucosidase inhibitor) was obtained from a certified supplier. Cation-adjusted Mueller–Hinton broth (CAMHB) and resazurin sodium salt were procured from Merck (Germany). Normal saline (0.9% NaCl) was freshly prepared for each experiment.

The bacterial panel comprised both susceptibility and resistant strains: *Staphylococcus aureus* ATCC 35923, methicillin resistant *S. aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* ATCC BAA-2108, *Escherichia coli* ATCC 25922, *E. coli* ATCC BAA-196, *Acinetobacter baumannii* ATCC 19606 and *A. baumannii* ATCC BAA-1797. Consumables included 1.5 mL microcentrifuge tubes, sterile 96-well clear-bottom plates (enzyme assays), sterile 96-well round-bottom plates (antibacterial assays), sterile micropipette tips and analytical-grade glassware. Weighing was performed using an analytical balance (± 0.1 mg). Enzyme assay absorbance was measured at 405 nm using a BioTek Synergy HTX multimode reader (USA). Bacterial inoculum turbidity was standardized at 625 nm using a Tecan Infinite Pro microplate reader (Switzerland). Incubations were conducted in a temperature controlled incubator at 37 ºC. *Saccharomyces cerevisiae* Type I α-glucosidase enzyme solution, voglibose and *p*-nitrophenyl-α-D-glucopyranoside (pNPG) were purchased from Sigma-Aldrich (USA).

General procedure for the synthesis of compounds C1-C6: Target benzothiazole-2-sulfonylurea derivatives (**C1-C6**) were synthesized by reacting the corresponding 2-aminobenzothiazole derivative (1.0 mmol) with phenylsulfonyl isocyanate (0.13 mL, 1.0 mmol) in dichloromethane (DCM, 5 mL). Each 2-aminobenzothiazole derivatives; **C1**: 2-aminobenzothiazole (0.1502 g), **C2**: 2-amino-6-methoxybenzothiazole (0.1802 g), **C3**: 2-amino-6-ethoxybenzothiazole (0.1943 g), **C4**: 2-amino-6-chlorobenzothiazole (0.1847 g), **C5**: 2-amino-6-methylbenzothiazole (0.1642 g) and **C6**: 2-amino-6-fluorobenzothiazole (0.1682 g) was dissolved in DCM and cooled to 0 ºC in an ice bath. Phenylsulfonyl isocyanate was then added dropwise *via* syringe under stirring and the reaction mixture was allowed to warm to ambient temperature and stirred for an additional 15-30 min until precipitation was complete. The crude solids were collected by vacuum filtration, washed thoroughly with cold methanol to remove unreacted starting materials and recrystallized from ethanol (**Scheme-I**). The purified crystals were filtered, dried at 70 ºC for 15 min in a dryer and stored for subsequent physical and spectroscopic characterization.

***N*-(Benzo[*d*]thiazol-2-ylcarbamoyl)benzenesulfonamide (C1):** Yield: 91%; white amorphous powder; m.p.: 210-213 ºC; m.f.: $C_{14}H_{13}N_4O_3S_2$; Relative molecular mass: 333; FT-IR (ATR, ν_{max}, cm^{-1}): 3349.45 (N-H *str.* 2º amide), 3258.79 (N-H *str.* 2º amide), 1688.71 (C=O *str.*), 1576.83 (C=C *str.* aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.23 (s, 1H, Ar-H), 7.37 (d, 2H, *J* = 5 MHz, Ar-H), 7.68-7.54 (m, 5H, Ar-H), 7.82 (s, 1H, Ar-H), 8.00 (d, 1H, *J* = 5 MHz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 144.57, 140.22, 133.88,



Scheme-I: Synthesis scheme of benzothiazole-phenylsulfonyl urea hybrids (**C1-C6**)

132.22, 129.51, 129.36, 128.00, 126.96, 126.01, 123.88, 122.51, 40.43, 40.26, 40.09, 39.93, 39.76, 39.59, 39.42; ESI-HRMS (*m/z*): 332.2241 $[M-H]^-$ (negative-ion mode).

***N*-((6-Methoxybenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C2):** Yield: 59%; white crystalline powder; m.p.: 220-222 ºC; m.f.: $C_{15}H_{13}N_3O_4S_2$; Relative molecular mass: 363; FT-IR (ATR, ν_{max}, cm^{-1}): 3354.27 (N-H *str*. 2º amide), 3257.83 (N-H *str*. 2º amide), 1654.95 (C=O *str*.), 1525.72 (C=C *str*. aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, Ar-OCH_3), 6.98 (d, 1H, *J* = 10 MHz, Ar-H), 7.47 (d, 2H, *J* = 15 MHz, Ar-H), 7.70-7.61 (m, 3H, Ar-H), 7.99 (d, 2H, *J* = 5 MHz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 156.46, 140.21, 133.90, 129.53, 129.36, 127.96, 126.00, 115.28, 105.85, 56.07, 55.34, 40.44, 40.28, 40.11, 39.94, 39.77, 39.61, 39.44; ESI-HRMS (*m/z*): 362.2740 $[M-H]^-$ (negative-ion mode).

***N*-((6-Ethoxybenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C3):** Yield: 83%; cream amorphous powder; m.p.: 225-227 ºC; m.f.: $C_{16}H_{15}N_3O_4S_2$; Relative molecular mass: 377; FT-IR (ATR, ν_{max}, cm^{-1}): 3354.27 (N-H *str*. 2º amide), 3257.83 (N-H *str*. 2º amide), 1638.56 (C=O *str*.), 1559.47 (C=C *str*. aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.30 (t, 3H, J_1 = 5 MHz and J_2 = 5 MHz, Ar-OCH_2-CH_3), 4.02 (q, 2H, J_1 = 10 MHz, J_2 = 5 MHz and J_3 = 5 MHz, Ar-OCH_2CH_3), 6.97 (d, 1H, *J* = 10 MHz, Ar-H), 7.35 (s, 1H, -NH), 7.46 (t, 1H, J_1 = 10 MHz and J_2 = 10 MHz, Ar-H), 7.69-7.55 (m, 5H, Ar-H), 7.83 (d, 1H, *J* = 5 MHz, Ar-H), 8.00 (d, 1H, *J* = 10 MHz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 155.70, 144.57, 140.17, 133.92, 132.22, 129.54, 129.36, 127.97, 126.01, 115.65, 106.48, 64.09, 40.44, 40.27, 40.11, 39.94, 39.77, 39.61, 39.44, 15.08; ESI-HRMS (*m/z*): 376.2705 $[M-H]^-$ (negative-ion mode).

***N*-((6-Chlorobenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C4):** Yield: 40%; white amorphous powder; m.p.: 233-235 ºC; m.f.: $C_{14}H_{10}ClN_3O_3S_2$; Relative molecular mass: 367; FT-IR (ATR, ν_{max}, cm^{-1}): 3351.37 (N-H *str*. 2º amide), 3257.83 (N-H *str*. 2º amide), 1599.02 (C=O *str*.), 1477.50 (C=C *str*. aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.41 (d, 1H, *J* = 10 MHz, Ar-H), 7.56 (d, 1H, *J* = 10 MHz, Ar-H), 7.64-7.61 (m, 2H, Ar-H), 7.71 (t, 1H, J_1 = 10 MHz and J_2 = 5 MHz, Ar-H), 7.99-7.88 (m, 3H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 140.06, 133.98, 129.54, 128.00, 127.91, 127.09, 122.09, 40.45, 40.28, 40.11, 39.94, 39.78, 39.61, 39.44; ESI-HRMS (*m/z*): 366.2336 $[M-H]^-$ (negative-ion mode).

***N*-((6-Methylbenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C5):** Yield: 25%; white amorphous powder; m.p.: 250-254 ºC; m.f.: $C_{15}H_{13}N_3O_3S_2$; Relative molecular mass: 347; FT-IR (ATR, ν_{max}, cm^{-1}): 3288.63 (N-H *str*. 2º amide), 3258.79 (N-H *str*. 2º amide), 1700.28 (C=O *str*.), 1596.12 (C=C *str*. aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.33 (s, 3H, Ar-CH_3), 7.20 (d, 1H, *J* = 10 MHz, Ar-H), 7.42 (d, 1H, *J* = 10 MHz, Ar-H), 7.63 (t, 3H, J_1 = 5 MHz and J_2 = 10 MHz, Ar-H), 7.70 (t, 1H, J_1 = 10 MHz and J_2 = 5 MHz, Ar-H), 7.99 (d, 2H, *J* = 10 MHz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 140.27, 133.85, 133.36, 129.50, 128.10, 127.98, 122.21, 40.44, 40.28, 40.11, 39.94, 39.78, 39.61, 39.44, 21.26; ESI-HRMS (*m/z*): 346.2831 $[M-H]^-$ (negative-ion mode).

***N*-((6-Fluorobenzo[*d*]thiazol-2-yl)carbamoyl)benzenesulfonamide (C6):** Yield: 78%; white solid crystalline; m.p.: 240-244 ºC; m.f.: $C_{14}H_{10}FN_3O_3S_2$; Relative molecular mass: 351; FT-IR (ATR, ν_{max}, cm^{-1}): 3349.45 (N-H *str*. 2º amide), 3257.83 (N-H *str*. 2º amide), 1653.02 (C=O *str*.), 1598.05 (C=C *str*. aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.33 (s, 3H, Ar-CH_3), 7.20 (d, 1H, *J* = 10 MHz, Ar-H), 7.42 (d, 1H, *J* = 10 MHz, Ar-H), 7.63 (t, 3H, J_1 = 5 MHz and J_2 = 10 MHz, Ar-H), 7.70 (t, 1H, J_1 = 10 MHz and J_2 = 5 MHz, Ar-H), 7.99 (d, 2H, *J* = 10 MHz, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): δ 159.90, 157.99, 140.01, 134.01, 129.56, 128.00, 114.84, 114.65, 109.12, 108.90, 40.43, 40.26, 40.09, 39.93, 39.76, 39.59, 39.42; ESI-HRMS (*m/z*): 350.2554 $[M-H]^-$ (negative-ion mode).

General procedure for *in vitro* antibacterial evaluation

Preparation of bacterial inoculum: Bacterial suspensions were prepared by transferring colonies into 1 mL of normal saline under sterile conditions. The turbidity of each suspension was adjusted to match a 0.5 McFarland standard (OD 0.08-0.10 at 625 nm) using a Tecan Pro Microplate reader. For final inoculum preparation, 0.1 mL of the OD-adjusted bacterial suspension was diluted into 9.9 mL of normal saline to yield ~5×10^5 CFU/mL.

Broth microdilution assay: Sterile 96-well round-bottom plates were used for susceptibility and resistant strain testing. In each well, 100 μL of the compound solution was combined with 100 μL of bacterial suspension. CAMHB containing 100 μL of bacterial suspension served as the growth control. Plates were incubated at 37 ºC for 18 h. Following incubation, 10 μL of resazurin dye was added to each well and plates were incubated for a further 2 h.

Minimum inhibitory concentration (MIC) determination: MIC values were determined using a broth microdilution assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines, with a maximum test concentration of 128 μM. For each compound, 3-5 mg was weighed into labelled microtubes and dissolved in DMSO to prepare a 0.01024 M stock solution. A working solution of 256 μM was prepared by diluting 25 μL of stock with 975 μL of cation-adjusted Mueller-Hinton broth (CAMHB), corresponding to 128 μM in 100 μL.

Data interpretation: MIC was defined as the lowest compound concentration at which the well colour remained blue, indicating inhibition of bacterial growth. A pink or purple colour indicated bacterial growth. The MIC range tested was 0.125-128 μM. All tested compounds **C1-C6** showed colour change at concentrations >128 μM for all bacterial strains, indicating no significant antibacterial activity within the tested range.

General procedure for *in vitro* α-glucosidase inhibition assay

Sample preparation: For each test compound **C1-C6** and the standard voglibose, the exact masses (1-5 mg) were weighed using an analytical balance and transferred into labelled 1.5 mL Eppendorf tubes. Stock solutions (10 mM) were prepared in biotech grade DMSO solvent. Working concentrations (1.0-0.125 mM) were prepared by dilution with phosphate-buffered saline (PBS). Enzyme solutions were

prepared from a 10 U/mL stock to yield a working concentration of 0.1 U/mL in freshly prepared 0.1 M potassium phosphate buffer (pH 7.3). Further dilutions (0.8-0.0125 U/mL) were prepared for calibration. The pNPG substrate (0.1 mM) was similarly prepared in buffer and diluted to concentrations between 0.8 and 0.0125 mM.

Calibration: To evaluate the linearity of the enzyme substrate reaction, two standard calibration curves were plotted: (i) seven substrate concentrations (0.8-0.0125 mM) against a fixed enzyme concentration (0.1 U/mL) and (ii) seven enzyme concentrations (0.8-0.0125 U/mL) against a fixed substrate concentration (0.1 mM). Linearity (R^2) values between 0.7 and 1.0 indicated a strong positive relationship between enzyme and substrate concentrations.

Assay procedure: Assays were performed in sterile 96-well clear-bottom microplates with a final well volume of 90 μL. For test wells, 10 μL of test compound was mixed with 40 μL of α-glucosidase (0.1 U/mL) and incubated for 15 min at 37 ºC, followed by the addition of 40 μL pNPG (0.1 mM) and further incubation for 45 min at 37 ºC. Blank wells contained enzyme (40 μL), substrate (40 μL) and phosphate buffer (10 μL) without the test compound. Voglibose was included as the positive control. Reactions were terminated by adding 100 μL of 0.1 M Na_2CO_3. The release of *p*-nitrophenol (yellow colour) was quantified at 405 nm using a microplate reader. All tests were performed in triplicate.

Statistical analysis: The percentage inhibition was calculated using the formula:

$$\text{Inhibition } (\%) = \left(1 - \frac{\text{Abs}_{\text{test}}}{\text{Abs}_{\text{blank}}}\right) \times 100$$

IC_{50} values (concentration required to inhibit 50% of enzyme activity) were determined from the curve obtained by plotting percentage inhibition against test concentration and expressed as mean ± standard error of the mean (SEM). Statistical significance between test compounds **C1-C6** and voglibose was determined using a *t*-test, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

The conventional reaction between benzenesulfonyl isocyanate and substituted benzothiazoles affords a series of benzothiazole-sulfonylurea derivatives (**C1-C6**). This transformation establishes a characteristic covalent –NH–CO–NH–SO_2– linkage, forming the benzothiazole–sulfonylurea scaffold. Based on this standard synthetic route, the theoretical structures of the resulting molecules were rationalized and their molecular formulas were confirmed by HRMS in ESI negative ionization mode. The calculated exact masses ranged from *m/z* 333 to 377, corresponding to molecular formulas depending on the specific substituents such as methoxy, ethoxy, methyl, chloro or fluoro groups.

The FT-IR spectra of all compounds exhibited strong absorption bands in the regions of 3340–3250 cm^{-1}, characteristic of N–H stretching vibrations of secondary amide groups and 1700–1630 cm^{-1}, typical of C=O stretching vibrations of urea or amide carbonyl groups. Additional intense bands observed between 1600–1500 cm^{-1} were attributed to aromatic C=C stretching, confirming the presence of aromatic rings within the benzothiazole and benzenesulfonyl moieties.

The ^{1}H NMR spectra of compounds **C1-C6** showed well-resolved aromatic proton resonances within the δ 6.8–8.2 ppm region, consistent with substituted benzothiazole and phenyl sulfonyl environments. Characteristic aliphatic or substituent signals such as methoxy (δ ~3.7 ppm), ethoxy (δ 1.3–4.0 ppm) or methyl (δ ~2.3 ppm) confirmed the presence of various substituents on the aromatic rings. The observed splitting patterns and coupling constants (J = 5–15 Hz) agreed with those expected for aromatic substitution patterns.

The ^{13}C NMR spectra displayed chemical shifts typically spanning δ 155–20 ppm, with downfield signals (δ > 150 ppm) corresponding to carbonyl and aromatic quaternary carbons, and upfield signals to methyl or methylene carbons of alkoxy substituents. The observed chemical shift ranges and multiplicities are consistent with the proposed sulfonylurea framework. Collectively, the combined HRMS, FT-IR, ^{1}H NMR, and ^{13}C NMR data confirm the successful synthesis and structural integrity of the benzothiazole–sulfonylurea derivatives (**C1-C6**). The spectral features are in good agreement with literature values for related sulfonylurea compounds, thereby validating the presence of the characteristic benzothiazole–sulfonylurea scaffold in all synthesized molecules.

***In vitro* antibacterial activity:** The synthesised compounds **C1-C6** were subjected for the antibacterial activity screening using selected bacterial strains to study the preliminary antibacterial properties of the compounds. There were earlier reports indicated that benzothiazole derivatives exhibits potential antibacterial properties but based on the current investigation, we have observed poor activity of the compounds with respect to their antibacterial properties against the selected strains (Tables 1 and 2). This study clearly provided an insight that the modification of the position 2 of benzothiazole ring system with sulfonylurea leads to the devoid of the antibacterial potency. Therefore, none of the compounds were active at 100 μM concentration, which is our benchmark standard to identify the hit molecules at IMU research laboratories. The compounds **C1-C6** were found to be active above the concentration > 128 μM concentration. It is noteworthy that the compounds screened were found to be inactive in terms of antibacterial potency. However, the SAR derived from this small chemically diversified library of benzothiazole-sulfonylurea derivatives (**C1-C6**) would provide direction to design derivatives that differs from sulfonylurea analogues. The hypothesis generated based on the antibacterial screening results will be tested in our continued exploration of the focused library synthesis of the benzothiazole derivatives. The negative results obtained in this study will enable us to modify basis skeleton synthesized in this study and explore the possible antibacterial potency associated with the new derivatives related to benzothiazole scaffold.

***In vitro* α-glucosidase inhibitory activity:** All compounds (**C1-C6**) demonstrated measurable α-glucosidase inhibitory activity, with IC_{50} values spanning 682.25 ± 2.90 to 909.02 ± 6.44 μM relative to the standard voglibose (IC_{50} = 711.04 ± 3.88 μM) (Table-3). As the IC_{50} value inversely reflects inhibitory potency, lower values indicate stronger

TABLE-1
MIC VALVES OF THE TESTED COMPOUNDS **C1-C6** AGAINST FOUR SUSCEPTIBILITY BACTERIAL STRAINS

Compounds	R	MIC (μM) against susceptibility bacterial strains			
		S. aureus ATCC 35923	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*A. baumannii* ATCC 19606
C1	H	>128	>128	>128	>128
C2	OCH_3	>128	>128	>128	>128
C3	OCH_2CH_3	>128	>128	>128	>128
C4	Cl	>128	>128	>128	>128
C5	CH_3	>128	>128	>128	>128
C6	F	>128	>128	>128	>128

TABLE-2
MIC VALUES OF THE TESTED COMPOUNDS **C1-C6** AGAINST FOUR RESISTANT BACTERIAL STRAINS

Compounds	R	MIC (μM) against resistant bacterial strains			
		MRSA ATCC 43300	*P. aeruginosa* ATCC BAA-2108	*E. coli* ATCC BAA-196	*A. baumannii* ATCC BAA-1797
C1	H	>128	>128	>128	>128
C2	OCH_3	>128	>128	>128	>128
C3	OCH_2CH_3	>128	>128	>128	>128
C4	Cl	>128	>128	>128	>128
C5	CH_3	>128	>128	>128	>128
C6	F	>128	>128	>128	>128

TABLE-3
α-GLUCOSIDASE INHIBITOR ACTIVITY
IC_{50} (μM) OF THE TESTED COMPOUNDS **C1-C6**

Compound	R	IC_{50} (μM)
C1	H	909.02 ± 6.44
C2	OCH_3	865.87 ± 4.28
C3	OCH_2CH_3	682.25 ± 2.90
C4	Cl	777.43 ± 11.26
C5	CH_3	801.51 ± 13.46
C6	F	744.37 ± 4.86
Voglibose	–	711.04 ± 3.88

activity. Within the **C1**-**C3** subset, **C3** (IC_{50} = 682.25 ± 2.90 μM) surpassed voglibose in potency, whereas **C1** and **C2** (IC_{50} = 909.02 ± 6.44 μM and 866.67 ± 4.28 μM, respectively) were weaker inhibitors. Statistical analysis (t-test, $p < 0.05$) confirmed that all three significantly inhibited α-glucosidase. SAR evaluation revealed that differences in activity were primarily due to substituent effects at position 6 of the benzothiazole moiety. Compound **C1**, lacking substitution, was least potent, while compounds **C2** and **C3**, bearing electron-donating groups (methoxy and ethoxy), displayed higher inhibition. EDGs enhance electron density on the aromatic ring, strengthening interactions with the active site of enzyme. The ethoxy group in **C3** further improved potency *via* its positive mesomeric effect and π–π interactions with catalytic residues, producing an activity order of ethoxy > methoxy > hydrogen. In compounds **C4**–**C6** subset, activity was slightly lower overall (IC_{50} = 744.37 ± 4.86 to 801.51 ± 13.46 μM) compared to voglibose. Compound **C6** (IC_{50} = 744.37 ± 4.86 μM), containing a fluoro group at carbon-6, exhibited the highest potency in this group, followed by compound **C4** (IC_{50} = 777.43 ± 11.26 μM) and compound **C5** (IC_{50} = 801.51 ± 13.46 μM). Incorporation of electron-withdrawing groups (fluoride, chlorine) improved activity by reducing aromatic electron density, thereby increasing electrophilicity and enabling π–π stacking interactions with the enzyme. Fluoride substitution outperformed chlorine due to higher electronegativity, while methyl substitution slightly reduced activity. Overall, the potency ranking across all compounds was **C3** > **C6** > **C4** > **C2** > **C5** > **C1**. These results indicate that both electron-donating and electron-withdrawing substituents at the benzothiazole's 6-position can enhance α-glucosidase inhibitory activity, with EDGs (notably ethoxy) and highly electronegative EWGs (notably fluoride) being most effective. This highlights the importance of fine-tuning electronic effects and substituent properties to optimize the inhibitory potency in benzothiazole–sulfonylurea derivatives.

Conclusion

In summary, we have been successfully prepared a series of organic small molecules **C1-C6** consisting of a substituted benzothiazole scaffold derivatized with a phenylsulfonyl urea moiety and tested for their preliminary antibacterial activity properties using selected Gram-positive and Gram-negative strains. Compounds **C1-C6** were designed by selecting a variety of chemical substituents such as electron-withdrawing (Cl, F) and electron-releasing (methyl, methoxy, ethoxy) groups to understand the effect of functional group derivatization on the substituted benzothiazole–phenylsulfonyl urea moiety. The unsubstituted benzothiazole–phenylsulfonyl urea derivative served as reference molecule to compare among the various substituted derivatives. Although benzothiazole has been well established as one of the potential pharmacophores associated with antibacterial properties, the SAR studies revealed that benzenesulfonylurea derivatization on the benzothiazole ring system at position 6 was unfavourable for antibacterial properties. This observation was proven with specific positional substitution on the benzothiazole ring, where the amino group was modified to phenylsulfonyl urea. The phenylsul-

fonyl urea derivatization does not produce molecules with potential antibacterial properties, unlike azomethine, primary, *sec*. or *tert.*-amides, sulfonamide, alkyl, aryl, urea or thiourea derivatives, which were earlier reported as having potential antibacterial properties. This study offers insights for medicinal chemists in designing benzothiazole derivatives with antibacterial potential. Modification of 2-amino group through sulfonylurea derivatization yielded compounds (**C1-C6**) with poor antibacterial potency, underscoring the critical role of the substituent at this position. Beyond antibacterial testing, the same series was evaluated for α-glucosidase inhibition, where C3 and C6 emerged as promising inhibitors, comparable to voglibose. SAR analysis revealed that introducing electron-donating or -withdrawing groups at position 6 of the benzothiazole core significantly affects activity. Overall, these results highlight the limited antibacterial value of phenylsulfonyl urea derivatization but point to opportunities for optimizing potent α-glucosidase inhibitors for antidiabetic therapy.

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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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