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Synthesis, Type II diabetes inhibitory activity, antimicrobial evaluation, and docking studies of *N*'-arylidene-2-((7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazol-3-yl)thio)acetohydrazides

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ABSTRACT

N'-Arylidene-2-((7-methylbenzo[4, 5]thiazolo[2,3-*c*][1, 2, 4]triazol-3-yl)thio)acetohydrazides (6a-j) were prepared by condensation of 2-((7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4] triazol-3-yl)thio)acetohydrazide with appropriately substituted benzaldehydes in dry methanol and a catalytic amount of glacial acetic acid. The prepared compounds tested for *in vitro* Type II diabetes inhibition and antimicrobial (antibacterial and antifungal) activities employing α-amylase inhibition assay and the serial dilution method, respectively. Type II diabetes inhibitory assay results of all the tested derivatives revealed that precursor 3 (IC_{50} = 0.16 μM) and acetohydrazide 6i (IC_{50} = 0.38 μM) showed comparable activity with standard drug acarbose (IC_{50} = 0.15 μM). The derivatives 6i against *B. subtilis* and *E. coli* with MIC values of 0.0300 μmol/mL, compound 6c against *S. aureus* (MIC = 0.0312 μmol/mL) and compound 6e against *P. aeruginosa* (MIC = 0.0316 μmol/mL) exhibited remarkable antibacterial activity, however, compound 6b was found to be more active against the fungal strain *C. albicans* with MIC value of 0.0135 μmol/mL. All acetohydrazides (6a-j) showed greater potency against all strains tested than their precursors 1-4, which is also supported by the results of molecular docking analysis. Furthermore, no general trend for structure activity relationships was established for Type II diabetes inhibitory activity, nor antimicrobial activities of the tested hydrazones (6a-j).

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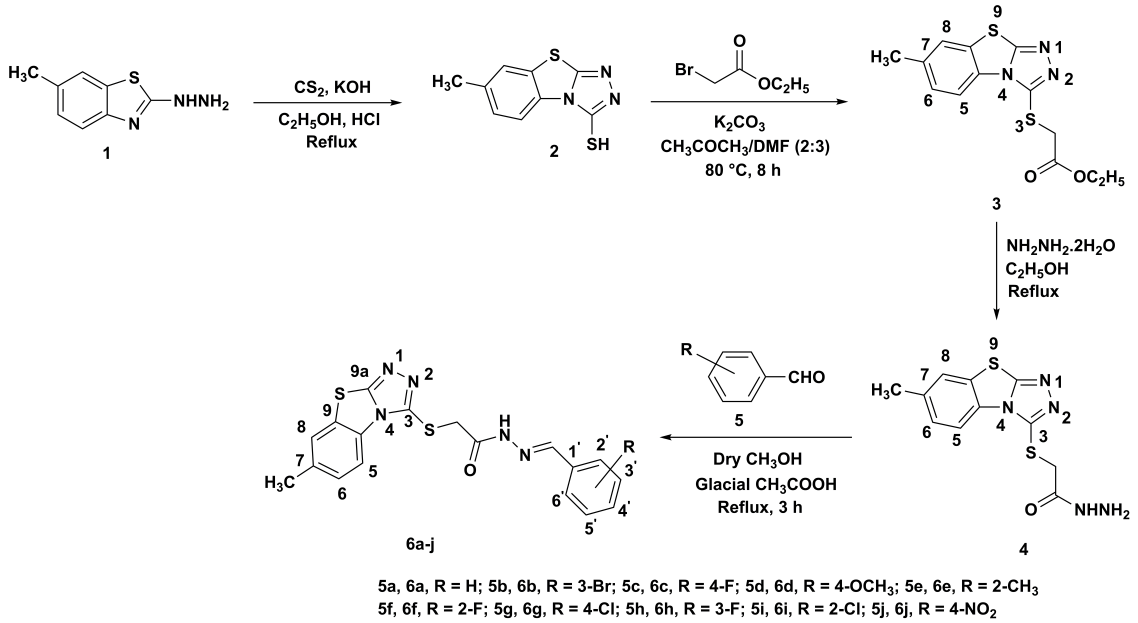
1. Introduction

Diabetes mellitus is a metabolic disorder resulting from insufficient insulin secretion characterized by chronic hyperglycemia due to high-calorie diets rich in carbohydrates, fats, and proteins [1]. Reports on this disease by the International Diabetes Foundation (IDF) have estimated that cases of diabetes may increase to 629 million by 2045 [2]. There are 352 million people at risk of developing Type II diabetes [3]. The emergent factors responsible for the spreading of Type II diabetes include a progressive technological society, food habits, and an inactive lifestyle [4]. Type II diabetes is associated with obesity, hypertension, dyslipidemia, cardiovascular disease, *etc*. It may cause tissue or vascular damage leading to severe diabetic problems such as retinopathy, neuropathy, and nephropathy [5]. α-Glucosidase and α-amylase are the key enzymes that tend to reduce postprandial hyperglycemia seen in Type II diabetes mellitus (DM2) [6]. α-Amylase inhibits the absorption of dietary starch and lowers blood glucose into the body system. Fundamentally, α-amylase inhibitors are classified into two groups: (i) proteinaceous inhibitors and (ii) non-proteinaceous inhibitors [7]. Non-proteinaceous inhibitors include chalcones, flavones, benzothiazoles, *etc*. as potential antidiabetic agents [8]. Similarly, the development of bacterial resistance of pathogenic microorganisms is rapidly becoming one of the most urgent public health challenges in the world [9]. Antibiotic resistance can disturb people at any stage of life, as well as the healthcare, and also has the potential to affect veterinary, and agriculture industries [10]. Therefore, the demand for newer antimicrobial agents is increasing dramatically day by day, and molecular architectures have gained a great deal of interest in the synthesis of safer and new molecules with excellent activity to combat this challenge [11].

The appearance of a benzothiazole nucleus in nature is rare, but is found in complex molecules [12]. Derivatives containing benzothiazole cores are recognized to exhibit a wide spectrum of pharmacological activities, *viz.* antifungal, antibacterial, antiviral, anti-inflammatory, anticancer, anti-diabetic, analgesic, antileishmanial, anticonvulsant, anti-tubercular, antihelmintic, antioxidant, antipsychotic, and many more [13-15]. Some of the important drugs marketed that contain the benzothiazole nucleus are riluzole, sibenadet hydrochloride (Viozan), zopolrestat, and pramipexole [16].



Scheme 1. Protocol for the preparation of *N'*-arylidene-2-((7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazol-3-yl)thio)acetohydrazides (**6a-j**).

Similarly, 1,2,4-triazoles are among the important classes of heterocyclics known for their usefulness in the production of insecticides, herbicides, and fungicides [17]. The compounds containing 1,2,4-triazole nuclei are associated with various biological activities such as analgesic and anti-inflammatory, antibacterial, antifungal, antiviral, anticonvulsant, antitubercular, antiparasitic, antihypertensive, antileishmanial, anticancer, *etc.* [18]. Various drugs such as letrozole, fluconazole, estazolam, etizolam, rizatriptan and many others are available in clinical use that contain 1,2,4-triazole as the main nucleus [19-22]. Several commercial plant protection fungicides containing the triazole moiety include metconazole, prothioconazole, tebuconazole, propiconazole, cyproconazole, epoxiconazole, triadimenol, and triadimefon [23]. Similarly, hydrazones containing the azomethine group (-NHN=CH-) have gained interest in drug development [24], which are associated with multiple pharmacological properties such as antidepressant, analgesic, antiinflammatory, antiplatelet, antimalarial, antimicrobial, antimycobacterial, vasodilatory, and antiviral. *etc.* [25]. Furthermore, the hydrazone core is an effective bioactive skeleton that acts as a part of the structures of several marketed drugs, such as dantrolene, nifuroxazide, nitrofurazone, carbazochrome, nitrofurantoin, and azumolene [26]. Moreover, hydrazones are appreciated building blocks in organic synthesis and are used as precursors for the synthesis of a variety of heterocyclics.

Molecular docking has become a significant component of the drug discovery process. It is a computational technique used for the study of molecular recognition, due to its ability to predict the binding mode and binding affinity of a complex formed by two or more constituent molecules with known structures [27]. Nowadays, it is mostly utilized in amalgamation with other computational approaches within integrated workflows [28]. In addition, it has the most important role in virtual screening, bioremediation, and drug discovery. Therefore, this is a useful tool for a researcher to reduce the time and cost of research activity and also gives a better understanding of the study of the ligand and receptor complex [29].

Taking into account the above facts, and in continuation of our program directed for the development of new, simple, safer, and efficient procedures for the synthesis of biologically active heterocyclic compounds utilizing readily accessible starting substrates and intermediates [30-35], here we report synthesis, characterization, Type II diabetes inhibitory activity and antimicrobial evaluation and docking studies of *N'*-arylidene-2-((7-methylbenzo[4, 5]thiazolo[2, 3-*c*][1, 2, 4]triazol-3-yl)thio) acetohydrazides (**6a-j**) and their precursors **1**-**4**.

2. Experimental

2.1. Chemistry

All chemicals used in this study were purchased from commercial suppliers and used without further purification. Melting points (M.p., °C) of the synthesized compounds were determined in open head capillaries with an Electrothermal Melting Point apparatus, LABCO Co, India, and are uncorrected. FT-IR spectra were taken on an IR Affinity-1 FTIR (Shimadzu) spectrophotometer in the region 500-4000 cm^{-1} using KBr, and peaks are reported in cm^{-1}. NMR (^1H and ^{13}C) spectra were recorded on a Bruker AVANCE III NMR spectrometer operating at 400 MHz using tetramethyl silane (TMS) as internal standard (chemical shift in δ, ppm), and the values of the coupling constant (*J*) are presented in Hertz (Hz). HRMS analysis was performed using LC-MS on SCIEX 5600+QTOF operating in a positive full scan mode (120,000 FWMH) in the range of 100-1000 *m/z* using the electrospray ionization (ESI) method.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 2-hydrazinyl-6-methylbenzo[d]thiazole (1)

Hydrazine hydrate (10 g, 0.2 mol) was taken in a 250 mL round bottom flask equipped with a reflux condenser and concentrated HCl (20 mL) was added dropwise to this under stirring on a magnetic stirrer keeping the temperature of the reaction mixture below 10 °C. Subsequently, ethylene glycol (40 mL) was added followed by 6-methylbenzo[*d*]thiazol-2-amine (8.21 g, 0.05 mol) gradually to the reaction mixture above and the resulting mixture was refluxed for 2 h. The solid thus obtained was filtered, dried, and recrystallized from aqueous ethanol to give 2-hydrazinyl-6-methylbenzo[*d*]thiazole (**1**) as colorless crystals, Yield: 59.6%, M.p.: 212-214 °C (Scheme 1) (Lit. M.p.: 218 °C [30]).

2.2.2. General procedure for the synthesis of 7-methylbenzo [4,5]thiazolo[2,3-c][1,2,4]triazole-3-thiol (2)

A suspension of 2-hydrazinyl-6-methylbenzo[*d*]thiazole (**1**, 7.16 g, 0.04 mol) in ethanol (40 mL) was prepared in a 250 mL round bottom flask equipped with a reflux condenser and a solution of potassium hydroxide (2.24 g, 0.04 mol) in ethanol (10 mL) was added to this suspension. Subsequently, carbon disulfide (12 mL, 0.2 mol) was added dropwise to the above reaction mixture and the contents were heated to reflux gently on a water bath for 7 h. Thereafter, the solvents were removed under reduced pressure to give a solid upon cooling. The aqueous solution of the solid thus obtained was acidified with conc. HCl to achieve pH = 5 of the solution to obtain a solid that was separated by filtration, washed with cold water several times and recrystallized from ethanol to give 7-methylbenzo [4,5]thiazolo[2,3-*c*][1,2,4]triazole-3-thiol (**2**) as a yellow solid (Scheme 1), Yield: 60%, M.p.: 308-310 °C (Lit. M.p.: 312 °C [36]).

2.2.3. General procedure for the synthesis of ethyl 2-((7-methylbenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio) acetate (3)

To a solution of 7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4] triazole-3-thiol (**2**, 0.5 g, 0.002 mol) in assortment of acetone (14.0 mL) and DMF (21.0 mL) taken in a 100 mL round bottomed flask equipped with a reflux condenser was added K_2CO_3 (0.345 g, 0.0025 mol) and ethyl bromoacetate (0.24 mL, 0.002 mol). The reaction mixture was heated to reflux while stirring on a magnetic stirrer for 7 h. Subsequently, the contents were poured onto ice while stirring. The precipitates thus separated out were filtered off, washed with cold water, and recrystallized from aqueous ethanol to yield compound **3** as a white solid (Scheme 1).

Ethyl 2-((7-methylbenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)acetate (**3**): Color: White solid. Yield: 72%. M.p.: 115-117 °C. FTIR (KBr, *ν*, cm^{-1}): 804, 1023, 1228, 1741 (C=O stretch), 2910, 2982 (aliphatic C-H stretch), 3092 (aromatic C-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.33 (t, 3H, *J* = 6.96 Hz, -$CO_2CH_2CH_3$), 2.67 (s, 3H, C_7-CH_3), 4.15-4.21 (m, 4H, -$CO_2CH_2CH_3$, -SCH_2-), 7.73 (d, 1H, *J* = 8.56 Hz, 6-H), 8.13 (d, 1H, *J*= 8.52 Hz, 5-H), 8.43 (s, 1H, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm): 20.48 (-$CO_2CH_2CH_3$), 21.38 (C_7-CH_3), 48.09 (-SCH_2-), 57.69 (-$CO_2CH_2CH_3$), 119.17, 127.27, 127.68, 128.80, 129.07, 129.62, 139.56, 151.35, 168.59 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{13}H_{13}N_3O_2S_2$ [M+H]$^+$: 308.0527, Found: 308.0525.

2.2.4. General procedure for the synthesis of 2-((7-methyl benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)aceto-hydrazide (4)

A mixture of ethyl 2-((7-methylbenzo[4,5]thiazolo[2,3-*c*] [1,2,4]triazol-3-yl)thio)acetate (**3**, 0.325 g, 0.0009 mol), hydrazine hydrate (10 mL) and ethanol (5 mL) was taken in a 100 mL round bottomed flask equipped with a reflux condenser and heated to reflux for 2 h on a water bath. The completion of the reaction was ascertained by TLC. After completion of reaction, the contents were cooled upon subsequent usual workup and recrystallization from ethanol gave the desired product 2-((7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazol-3-yl)thio)acetohydrazide (**4**) as a white solid (Scheme 1).

2-((7-Methylbenzo[4,5]thiazolo[2, 3-c][1, 2, 4]triazol-3-yl) thio)aceto hydrazide (**4**): Color: White solid. Yield: 52%. M.p.: 280-282 °C. FTIR (KBr, *ν*, cm^{-1}): 822, 1080, 1174, 1508, 1662 (C=O stretch), 2934 (aliphatic C-H stretch), 3061 (aromatic C-H stretch), 3206, 3304 (NH_2, NH stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.39 (s, 3H, -CH_3), 2.50 (s, 2H, NH_2), 4.45 (s, 2H, -SCH_2-), 7.30 (d, 1H, *J* = 8.32 Hz, 6-H), 7.72 (s, 1H, 8-H), 7.76 (s, 1H, NH), 7.93 (d, 1H, *J* = 8.32 Hz, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.21 (-CH_3), 44.36 (-SCH_2-), 115.16, 125.19, 127.67, 127.69, 131.29, 135.78, 151.11, 154.12, 169.29. HRMS (ESI-TOF, *m/z*) calcd. for $C_{11}H_{11}N_5OS_2$[M+H]$^+$: 294.0483, Found: 294.0436.

2.2.5. General procedure for the synthesis of 7-methylbenzo [4,5]thiazolo[2,3-c][1,2,4]triazole based hydrazones (6a-j)

A mixture of compound **4** (0.0002 mol), dry methanol (15 mL), appropriately substituted benzaldehyde (**5**, 0.0002 mol), and a catalytic amount of glacial acetic acid (2-3 drops) was taken in a 100 mL round bottom flask equipped with a reflux condenser. The reaction mixture was heated to reflux on a heating mantle for 3 h. The progress of the reaction was monitored by TLC using hexane:ethylacetate (30:70, *v:v*) on aliquots withdrawn from the reaction mixture at different intervals of time. After completion, the reaction mixture was poured onto crushed ice and the precipitates thus obtained were filtered under suction in a Büchner funnel and washed with water and cold ethanol to obtain the corresponding 7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole based hydrazones (**6a-j**) in high yields (Scheme 1).

N'-Benzylidene-2-((7-methylbenzo[4, 5]thiazolo[2, 3-c][1, 2, 4]triazol-3-yl)thio)acetohydrazide (**6a**): Color: Yellow solid. Yield: 91%. M.p.: 264-266 °C. FTIR (KBr, *ν*, cm^{-1}):755, 1035, 1255, 1355, 1508, 1596 (C=N stretch), 1654 (C=O stretch), 2868 (aliphatic C-H stretch), 3061 (aromatic C-H stretch), 3338 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.39 (s, 3H, C_7-CH_3), 4.11 (s, 2H, -SCH_2-), 7.30 (d, 1H, *J* = 7.24 Hz, 6-H), 7.40 (d, 2H, *J* = 8.00 Hz, 3'-H, 5'-H), 7.62 (d, 1H, *J* = 6.68 Hz, 5-H), 7.71 (s, 1H, H-8), 7.80 (t, 1H, *J* = 7.60 Hz, 4'-H), 7.93 (d, 2H, *J* = 8.12 Hz, 2'-H, 6'-H), 8.22 (s, 1H, -N=C*H*-), 11.10 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):21.22 (-CH_3), 49.07 (-SCH_2-), 115.85, 125.46, 126.73, 127.85, 128.01, 128.88, 129.36, 131.65, 134.88, 136.38, 148.99, 149.58, 152.50, 168.25 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{15}N_5OS_2$ [M+H]$^+$: 382.0796, Found: 382.0741.

N'-(3-Bromobenzylidene)-2-((7-methylbenzo[4, 5]thiazol[2, 3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6b**): Color: Yellow solid. Yield: 85%. M.p.: 267-269 °C. FTIR (KBr, *ν*,c m^{-1}):770, 1079, 1230, 1508, 1585, 1638 (C=N stretch), 1650 (C=O stretch), 2789, 3441 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm):2.42 (s, 3H, C_7-CH_3), 4.10 (s, 2H, -SCH_2-), 7.34 (d, 1H, *J* = 7.88 Hz, 6-H), 7.38 (d, 1H, *J* = 8.48 Hz, 5'-H), 7.51 (d, 1H, *J* = 8.48 Hz, 4'-H), 7.66 (d, 1H, *J* = 7.76 Hz, 5-H), 7.72-7.77 (m, 1H, 6'-H), 7.83 (s, 1H, 8-H), 7.92 (d, 1H, *J* = 8.20 Hz, 2'-H), 8.17 (s, 1H, -N=C*H*-), 11.28 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):21.34 (-CH_3), 48.14 (-SCH_2-), 114.06, 121.83, 122.68, 125.00, 125.68, 126.65, 128.12, 128.98, 129.08, 131.01, 133.59, 135.58, 142.14, 147.13, 155.37, 169.22 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}BrN_5OS_2$ [M+H]$^+$: 459.9901 (^{79}Br), 461.9881 (^{81}Br), Found: 460.0026 (^{79}Br), 461.9847 (^{81}Br).

N'-(4-Fluorobenzylidene)-2-((7-methylbenzo[4, 5]thiazolo[2, 3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6c**): Color: Yellow solid. Yield: 90%. M.p.: 260-262 °C. FTIR (KBr, *ν*, cm^{-1}): 713, 1114, 1233, 1508, 1609 (C=N stretch), 1648 (C=O stretch), 2875 (aliphatic C-H stretch), 3109 (aromatic C-H stretch), 3398 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.35 (s, 3H, C_7-CH_3), 4.13 (s, 2H, -SCH_2-), 7.19 (d, 2H, *J* = 9.04 Hz, 3'-H, 5'-H), 7.26 (d, 1H, *J* = 8.28 Hz, 6-H), 7.63 (d, 1H, *J* = 8.60 Hz, 5-H), 7.75 (s, 1H, 8-H), 7.84 (d, 2H, *J* = 8.44 Hz, 2'-H, 6'-H), 8.17 (s, 1H, -N=C*H*-), 11.04 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):21.22 (-CH_3), 45.80 (-SCH_2-), 115.75, 116.38 (d, *J*= 21.73 Hz), 124.92, 125.46, 127.87, 128.80 (d, *J*= 8.21 Hz), 131.50 (d, *J*= 2.69 Hz), 131.64, 136.36, 142.99, 149.54, 152.51, 160.68 (*J*= 246.96 Hz), 164.38 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}FN_5OS_2$ [M+H]$^+$: 400.0702, Found: 400.0676.

N'-(4-Methoxybenzylidene)-2-((7-methylbenzo[4, 5]thiazolo [2,3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6d**): Color: Yellow solid. Yield: 87%. M.p.: 258-260 °C. FTIR (KBr, *ν*, cm^{-1}): 811, 1031, 1244, 1508, 1618 (C=N stretch), 1650 (C=O stretch),

2837 (aliphatic C-H stretch), 3109 (aromatic C-H stretch), 3344 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.35 (s, 3H, C_7-C*H*$_3$), 4.04 (s, 2H, -SC*H*$_2$-), 3.73 (s, 3H, -OC*H*$_3$), 6.93 (d, 2H, *J* = 8.80 Hz, 3'-H, 5'-H), 7.26 (d, 1H, *J* = 8.32 Hz, 6-H), 7.51 (d, 2H, *J* = 8.72 Hz, 2'-H, 6'-H), 7.70 (d, 1H, *J* = 8.52 Hz, 5-H), 7.74 (s, 1H, 8-H), 8.12 (s, 1H, -N=C*H*-), 10.86 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):2 1.23 (-*C*H$_3$), 47.77 (-S*C*H$_2$-), 55.74 (-O*C*H$_3$), 114.86, 125.44, 127.48, 127.82, 128.05, 128.26, 128.74, 131.63, 136.30, 144.16, 148.91, 149.87, 160.80, 167.61 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{19}H_{17}N_5O_2S_2$ [M+H]$^+$: 412.0902, Found: 412.0875.

2-((7-Methylbenzo[4, 5]thiazolo[2, 3-c][1, 2, 4]triazol-3-yl) thio)-N'-(2-methylbenzylidene)acetohydrazide (**6e**): Color: Yellow solid. Yield: 82%. M.p.: 266-268 °C. FTIR (KBr, *ν*, cm^{-1}): 713, 1090, 1258, 1603 (C=N stretch), 1654 (C=O stretch), 2868 (aliphatic C-H stretch), 3091 (aromatic C-H stretch), 3447 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.35 (s, 3H, C_7-C*H*$_3$), 2.38 (s, 3H, C_2-C*H*$_3$), 4.04 (s, 2H, -SC*H*$_2$-), 7.17-7.22 (m, 2H, 3'-H, 4'-H), 7.27 (d, 2H, *J* = 8.52 Hz, 6-H, 5'-H), 7.64 (d, 1H, *J* = 7.56 Hz, 5-H), 7.75 (s, 1H, 8-H), 7.89 (d, 1H, *J* = 8.32 Hz, 6'-H), 8.46 (s, 1H, -N=C*H*-), 10.96 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 19.58 (-*C*H$_3$), 21.23 (-*C*H$_3$), 49.33 (-S*C*H$_2$-), 115.85, 125.51, 126.71, 127.82, 127.95, 128.74, 129.60, 131.38, 131.65, 132.77, 136.37, 136.55, 143.01, 149.61, 152.85, 164.21 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{19}H_{17}N_5OS_2$ [M+H]$^+$: 396.0953, Found: 396.0912.

N'-(2-Fluorobenzylidene)-2-((7-methylbenzo[4, 5]thiazolo[2, 3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6f**): Color: Yellow solid. Yield: 84%. M.p.: 262-264 °C. FTIR (KBr, *ν*, cm^{-1}): 764, 1086, 1234, 1500, 1636 (C=N stretch), 1651 (C=O stretch), 2868 (aliphatic C-H stretch), 3091 (aromatic C-H stretch), 3318 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.43 (s, 3H, C_7-C*H*$_3$), 4.13 (s, 2H, -SC*H*$_2$-), 7.27 (d,1H, *J* = 7.28 Hz, 5'-H), 7.35 (d, 2H, *J* = 8.32 Hz, 6-H, 3'-H), 7.47 (dd, 1H, *J* = 7.60 Hz and 13.20 Hz, 4'-H), 7.76 (s, 1H, 8-H), 7.80 (d, 1H, *J* = 6.96 Hz, 5-H), 7.96 (d, 1H, *J* = 8.28 Hz, 6'-H), 8.46 (s, 1H, -N=C*H*-), 11.33 (s, 1H NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):21.22 (-*C*H$_3$), 47.31 (-S*C*H$_2$-), 114.19, 120.98 (d, *J* = 2.43 Hz), 123.96, 124.93, 126.18, 127.89 (d, *J* = 5.64 Hz), 128.12, 130.59 (d, *J* = 2.21 Hz), 131.66 (d, *J* = 6.14 Hz), 135.54, 136.71 (d, *J* = 4.66 Hz), 142.64, 149.22, 152.58, 160.63 (d, *J* = 247.81 Hz), 166.51 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}FN_5OS_2$ [M+H]$^+$: 400.0702, Found: 400.0684.

N'-(4-Chlorobenzylidene)-2-((7-methylbenzo[4, 5] thiazolo [2,3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6g**): Color: Yellow solid. Yield: 83%. M.p.: 254-258 °C. FTIR (KBr, *ν*, cm^{-1}): 734, 805, 1089, 1257, 1508, 1603 (C=N stretch), 1654 (C=O stretch), 2873 (aliphatic C-H stretch), 3097 (aromatic C-H stretch), 3435 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.40 (s, 3H, C_7-C*H*$_3$), 4.12 (s, 2H, -SC*H*$_2$-), 7.31 (d, 1H, *J* = 7.96 Hz, 6-H), 7.48 (d, 2H, *J* = 8.64 Hz, 3'-H, 5'-H), 7.64 (d, 1H, *J* = 8.40 Hz, 5-H), 7.73 (s, 1H, 8-H), 7.89 (d, 2H, *J* = 8.20 Hz, 2'-H, 6'-H), 8.21 (s, 1H, -N=C*H*-), 11.19 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.23 (-*C*H$_3$), 47.24 (-S*C*H$_2$-), 115.74, 125.49, 127.91, 128.33, 128.78, 128.94, 129.43, 131.65, 133.83, 136.40, 142.68, 147.43, 153.12, 165.62 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}ClN_5OS_2$ [M+H]$^+$: 416.0407 (^{35}Cl), 418.0377 (^{37}Cl), Found: 416.3555 (^{35}Cl), 418.3586 (^{37}Cl).

N'-(3-Fluorobenzylidene)-2-((7-methylbenzo[4,5]thiazolo [2, 3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6h**): Color: Yellow solid. Yield: 83%. M.p.: 254-258 °C. FTIR (KBr, *ν*, cm^{-1}): 771, 814, 1261, 1514, 1594, 1637 (C=N stretch), 1648 (C=O stretch), 2794 (aliphatic C-H stretch), 3094 (aromatic C-H stretch), 3435 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.34 (s, 3H, C_7-C*H*$_3$), 4.01 (s, 2H, -SC*H*$_2$-), 7.12-7.17 (m, 1H, 4'-H), 7.33-7.41 (m, 4H, 6-H, 2'-H, 5'-H, 6'-H), 7.67 (d, 1H, *J* = 7.92 Hz, 5-H), 7.74 (s, 1H, 8-H), 8.15 (s, 1H, -N=C*H*-), 11.18 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm):21.23 (-*C*H$_3$), 47.98 (-S*C*H$_2$-), 109.97, 114.77, 116.43 (d, *J* = 15.32 Hz), 123.89 (d, *J* = 2.79 Hz), 124.98, 125.52, 128.09, 129.08 (d, *J* = 2.23 Hz), 130.81 (d, *J* = 1.47Hz), 135.53 (d, *J* = 2.54 Hz), 136.46, 142.52, 149.25, 153.29,162.82 (d, *J* = 201.42 Hz), 164.19 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}FN_5OS_2$ [M+H]$^+$: 400.0702, Found: 400.0675.

N'-(2-Chlorobenzylidene)-2-((7-methylbenzo[4, 5]thiazolo[2, 3-c][1,2,4]triazol-3-yl)thio)acetohydrazide (**6i**): Color: Yellow solid. Yield: 88%. M.p.: 268-270 °C. FTIR (KBr, *ν*, cm^{-1}): 734, 809, 1241, 1514, 1635 (C=N stretch), 1651 (C=O stretch), 2864 (aliphatic C-H stretch), 3084 (aromatic C-H stretch), 3346 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.46 (s, 3H, C_7-C*H*$_3$), 4.09 (s, 2H, -SC*H*$_2$-), 7.37-7.41 (m, 1H, 6-H), 7.45-7.48 (m, 1H, 4'-H), 7.53-7.58 (m, 2H, 3'-H, 5'-H), 7.63 (dd, 1H, *J* = 8.08 Hz and *J* = 1.16 Hz, 5-H), 7.69-7.73 (m, 1H, 8-H), 7.88 (dd,1H, *J* = 7.68 Hz and *J* = 1.76 Hz, 6'-H), 8.46 (s, 1H, -N=C*H*-), 10.36 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.44 (-*C*H$_3$), 53.99 (-S*C*H$_2$-), 100.96, 127.34, 128.36, 128.51, 129.93, 130.15, 130.67, 131.20, 132.52, 132.62, 134.70, 135.62, 136.73, 151.52, 155.38, 167.23 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}ClN_5OS_2$ [M+H]$^+$: 416.0407 (^{35}Cl), 418.0377 (^{37}Cl), Found: 416.0387 (^{35}Cl), 418.0345 (^{37}Cl).

2-((7-Methylbenzo[4, 5]thiazolo[2, 3-c][1, 2, 4]triazol-3-yl) thio)-N'-(4-nitrobenzylidene)acetohydrazide (**6j**): Color: Yellow solid. Yield: 83%. M.p.: 271-272 °C. FTIR (KBr, *ν*, cm^{-1}):749, 827, 1014, 1044, 1105, 1207, 1252, 1341 (symmetrical N-O stretch), 1508, 1548 (asymmetrical N-O stretch), 1590 (C=N stretch), 1652 (C=O stretch), 2928 (aliphatic C-H stretch), 3091 (aromatic C-H stretch), 3447 (N-H stretch). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.47 (s, 3H, C_7-C*H*$_3$), 4.09 (s, 2H, -SC*H*$_2$-), 7.46-7.48 (m, 2H, 5-H, 6-H), 7.91 (s, 1H, 8-H), 8.17 (d, 2H, *J* = 8.84 Hz, 2'-H, 6'-H), 8.38 (d, 2H, *J* = 8.88 Hz, 3'-H, 5'-H), 8.87 (s, 1H,-N=C*H*-), 10.18 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.47 (-*C*H$_3$), 50.04 (-S*C*H$_2$-), 115.09, 119.97, 124.66, 126.49, 130.05, 131.37, 135.51, 136.88, 139.35, 140.77, 145.40, 148.14, 152.71, 164.84 (C=O). HRMS (ESI-TOF, *m/z*) calcd. for $C_{18}H_{14}N_6O_3S_2$ [M+H]$^+$: 426.0569, Found: 426.0538.

2.3. Biological evaluation

2.3.1. In vitro α-amylase inhibition

All synthesized compounds **1**-**4** and **6a**-**j** were tested for their *in vitro* α-amylase inhibitory activity against α-amylase enzyme using the protocol reported by Xiao *et al.* and Yoshikawa *et al.* with slight modifications [37,38]. The results of *in vitro* α-amylase inhibitory activity of the compounds **1**-**4** and **6a**-**j** reported in terms of % inhibition and IC_{50} values are shown in Table 1.

2.3.2. Antimicrobial evaluation

All synthesized compounds 1-4 and *N*'-arylidene-2-((7-methylbenzo[4,5]thiazolo[2 ,3-*c*][1, 2, 4]triazol-3-yl)thio)aceto hydrazides (**6a**-**j**) were evaluated for their antimicrobial (antibacterial and antifungal) activities *in vitro* using a serial dilution method according to the literature procedure [39]. The *in vitro* inhibitory activity results of the synthesized compounds **1**-**4** and **6a**-**j** were expressed in terms of minimum inhibition concentration (MIC, µmol/mL) and are shown in Table 2.

2.4. Molecular docking study

Molecular docking analysis of compounds **1**-**4** and **6a**-**j** was performed to find the plausible mechanism of action of their antifungal activity against *C. albicans* (MTCC 227). The protocol utilized for molecular docking is reported as follows: The protein-ligand crystal structure has been optimized to its lower energy conformation using a protein preparation wizard where all water molecules were removed during pre-process and missing side chains of residues have been added using prime.

Table 1. *In vitro* α-amylase inhibitory activity of compounds **1**-**4** and **6a**-**j**.

Compounds	Percentage inhibition±SD				IC_{50} (µM)
	12.5 (µg/mL)	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	
1	70.10±0.00	77.95±0.30	79.61±0.75	85.35±0.15	3.96
2	75.09±0.30	80.67±0.75	87.77±0.15	93.81±0.45	3.71
3	75.69±0.75	80.97±0.15	82.03±0.45	85.96±0.30	0.16
4	88.22±0.45	88.97±0.15	67.84±0.30	96.07±0.00	11.31
6a	73.58±0.30	77.80±0.45	84.75±0.30	90.64±0.15	2.17
6b	68.29±0.60	79.01±0.30	80.97±0.15	91.54±0.60	4.99
6c	74.18±0.15	82.78±0.00	84.75±0.45	87.77±0.30	0.80
6d	60.44±0.75	65.27±0.30	68.90±0.15	85.96±0.45	18.98
6e	58.33±0.00	81.88±0.45	83.99±0.60	91.54±0.15	17.14
6f	61.20±0.60	63.76±0.15	66.33±0.30	81.73±0.75	17.97
6g	73.73±0.75	77.80±0.45	82.78±0.00	88.82±0.30	1.23
6h	75.69±0.45	81.58±0.30	87.01±0.15	92.45±0.45	1.25
6i	79.46±0.15	82.48±0.75	85.05±0.45	92.60±0.00	0.38
6j	53.04±0.00	67.54±0.45	77.50±0.15	89.13±0.30	23.19
Acarbose	80.52±0.00	87.46±0.30	90.64±0.45	93.96±0.15	0.15

Table 2. *In vitro* antimicrobial activity of compounds **1**-**4** and **6a**-**j**.

Compounds	Minimum inhibitory concentration (MIC in µmol/mL)					
	Gram-positive bacteria *		Gram-negative bacteria *		Fungi *	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1	0.1396	0.1396	0.1396	0.1396	0.0349	0.0698
2	0.1129	0.1129	0.1129	0.1129	0.0282	0.0564
3	0.0813	0.0813	0.0813	0.0813	0.0203	0.0406
4	0.0852	0.0852	0.0852	0.0852	0.0213	0.0426
6a	0.0655	0.0655	0.0655	0.0655	0.0163	0.0327
6b	0.0543	0.0543	0.0543	0.0543	0.0135	0.0271
6c	0.0312	0.0312	0.0312	0.0625	0.0625	0.0625
6d	0.0607	0.0607	0.0607	0.0607	0.0607	0.0303
6e	0.0632	0.0632	0.0316	0.0316	0.0632	0.0316
6f	0.0625	0.0625	0.0625	0.0625	0.1251	0.0312
6g	0.0601	0.0601	0.0601	0.0601	0.0601	0.0300
6h	0.0625	0.0625	0.0625	0.0625	0.0625	0.0312
6i	0.0300	0.0601	0.0300	0.0601	0.0150	0.0300
6j	0.0586	0.0586	0.0586	0.0586	0.0146	0.0293
Ciprofloxacin	0.0047	0.0047	0.0047	0.0047	-	-
Fluconazole	-	-	-	-	0.0102	0.0102

* *Bacillus subtilis* (MTCC 441); *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 1652); *Pseudomonas aeruginosa* (MTCC 424), *Candida albicans* (MTCC 227); *Aspergillus niger* (MTCC 8189).

Keeping in mind the appropriate ionization states for the acidic as well as basic amino acids, hydrogen atoms were added to the protein structure corresponding to the physiological pH = 7.0. Finally, energy minimization with a root mean square deviation (RMSD) value of 0.30 Å was carried out using optimized potentials for liquid simulations (OPLS-2005) force field after assigning charge and protonation state. Ligand preparation was done using the Schrodinger LigPrep utility (Schrodinger, LLC, USA), which generates a low energy 3D structure. The active site of the protein was defined by a bounding box (grid) that was centered on the native ligand in the crystal complex. Extra-precision glide docking (Glide XP), which docks ligands flexibly, was used to rank the docking poses and to gauze the binding affinity of these ligands towards the protein.

3. Results and discussion

3.1. Chemistry

The procedure for the preparation of *N*′-arylidene-2-((7-methylbenzo[4,5]thiazolo[2, 3-*c*][1, 2, 4]triazol-3-yl)thio)aceto hydrazides (**6a-j**) is described in Scheme 1. Firstly, we prepared 2-hydrazinyl-6-methylbenzo[*d*]thiazole (**1**) from *p*-toluidine in accord with the literature procedure [40,41]. Thiazole (**1**) upon reaction with carbon disulfide in the presence of potassium hydroxide/ethanol under reflux conditions followed by acidification with concentrated hydrochloric acid gave 7-methyl benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole-3-thiol (**2**) with good yield [42]. The synthesis of key intermediate 2-((7-methyl benzo[4,5]thiazolo[2, 3-*c*][1, 2, 4]triazol-3-yl)thio)acetohydrazide (**4**) was achieved in two steps: (i) the thiol **2** on alkylation with ethyl bromoacetate in presence of potassium carbonate using acetone/DMF as solvent under stirring at 80 °C yielded 7-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole based ester (**3**), and subsequently (ii) the ester (**3**) was treated with hydrazine hydrate in presence of ethanol under reflux to give acid hydrazide (**4**) [43]. Finally, acid hydrazide (**4**) was condensed with differently substituted benzaldehydes **5** using a catalytic amount of acetic acid (glacial) in methanol under reflux conditions to provide the target *N*′-Arylidene-2-((7-methyl-benzo[4, 5]thiazolo[2, 3-*c*][1, 2, 4]triazol-3-yl)thio)acetohydrazides (**6a-j**) in high yields (82-91%).

The structures of ester **3** and acid hydrazide **4** were well confirmed from FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS spectral data. FTIR spectra of compounds **3** and **4**, in each case, exhibited strong absorption bands at 1741 and 1662 cm^{-1} due to >C=O stretchings of the -$CO_2CH_2CH_3$ and -$CONHNH_2$ groups, respectively [42]. The ^{1}H NMR spectrum of compound **3** showed a triplet integration for three protons resonated at δ 1.33 ppm (^3J = 6.96 Hz) due to the methyl protons of the ester group (-$CO_2CH_2CH_3$). A signal obtained as a three-proton singlet at δ 2.67 ppm was safely assigned to C_7-CH_3. A multiplet that appeared in the region at δ 4.15-4.21 ppm integrated for four protons was attributed to the two protons each of the -SCH_2- and methylene protons of the ester group (-$CO_2CH_2CH_3$). The remaining aromatic protons demonstrated signals in the expected regions. In its ^{13}C NMR spectrum, the signals due to the methyl carbons of -$CO_2CH_2CH_3$ and C_7-CH_3 appeared at δ 20.48 and 21.38 ppm, respectively, while the carbon atom of -SCH_2- and the methylene carbon of the -$CO_2CH_2CH_3$ group displayed signals at δ 48.09 and δ 57.69 ppm, respectively. In the downfield region of the spectrum, the characteristic signal due to the carbonyl carbon of the ester group in compound **3** was obtained at δ 168.59 ppm.

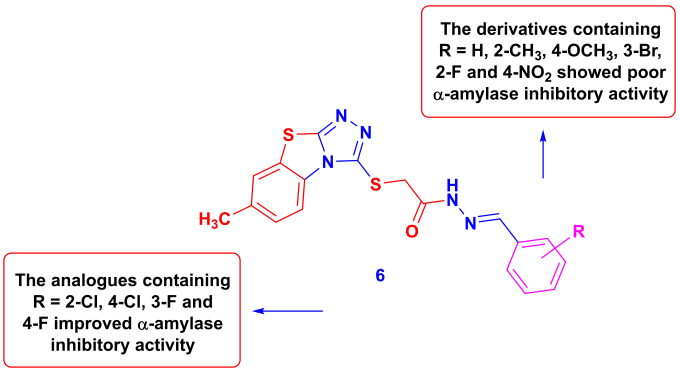


Figure 1. Structure Activity Relationship (SAR) of compounds **6a-j** for α-amylase inhibitory activity.

The remaining aromatic carbon atoms exhibited signals in the expected regions. Similarly, the ^{1}H NMR spectrum of compound **4** showed a singlet of three protons in the most up-field region at δ 2.39 due to C_7-CH_3. The characteristic signals due to -NH_2 and NH were appeared at δ 2.50 and 7.76 ppm integrating for two protons and one proton, respectively. The remaining aromatic protons appeared in the expected regions. In ^{13}C NMR spectrum of compound **4**, the signals resonated at δ 21.21 and 44.36 ppm could easily be assigned to the carbons of C_7-CH_3 and -SCH_2-group, respectively. The most significant feature of ^{13}C NMR spectrum of compound **4** is the resonance exhibited in the most downfield region at δ 169.29 ppm due to the carbonyl carbon of -$CONHNH_2$ group. Furthermore, the results of the HRMS analysis of compounds **3** and **4** were found to agree well with their molecular formulae (vide experimental).

The FTIR spectra of all synthesized acetohydrazides (**6a-j**), in each case, exhibited an absorption band in the region at 1590-1638 cm^{-1} due to the C=N stretching while the strong absorption bands obtained in the range of 1648-1654 and 3318-3447 cm^{-1} were safely assigned to >C=O and N-H stretchings of the -CONHN= group, respectively. In the ^{1}H NMR spectra of compounds **6a-j**, in their aliphatic regions, in each case, the singlet integrated for three protons centered in the region at δ 2.34-2.47 ppm could undoubtedly be ascribed to the protons of C_7-CH_3. The characteristic signal due to the protons of -SCH_2- as a two-protons singlet, in each case, appeared in the range of δ 4.01-4.13 ppm while a singlet of one proton obtained in the region at δ 8.12-8.87 ppm could easily be assigned to the proton of the -N=C*H*- group. In the most down-field region of the spectra, in each case, a broad singlet (exchangeable with D_2O) integrated for one proton appeared in the region at δ 10.18-11.33 ppm, which was undoubtedly assigned to the NH of hydrazono (-CONHN=) [38]. The resonances due to the remaining protons were appeared in the expected regions. The ^{13}C NMR spectra of compounds **6a-j**, in their aliphatic region, in each case, exhibited a signal in the range of δ 21.22-21.47 ppm which could be securely assigned to the carbon of C_7-CH_3, while the carbon atom of the -SCH_2- group, in each case, resonated in the range of δ 45.80-53.99 ppm. The most significant feature of ^{13}C NMR spectra of compounds **6a-j**, in each case, was the resonance due to carbon atom of carbonyl group that exhibited signal in the most downfield region at δ 164.19-169.22 ppm [42,44]. The signals from the remaining carbons were observed in the expected regions. Furthermore, the structures of hydrazones (**6a-j**) were also supported by their HRMS analysis results.

3.2. Pharmacological assay

3.2.1. Antidiabetic evaluation

All synthesized compounds **1-4** and **6a-j** were evaluated for preliminary *in vitro* α-amylase inhibitory activity (α-amylase isolated from Malt) at various concentrations, that is, 12.5, 25, 50 and 100 µg/mL following the procedure developed by Xiao *et al.* [37] and Yoshikawa *et al.* [38] with slight modifications. The reagent solution without test samples was used as a control, and acarbose was taken as a standard for comparison. The absorbance was recorded on ELISA microplate reader at wavelength (λ) = 650 nm. The α-amylase inhibitory activity reported as a percentage (%) inhibition was calculated using Equation (1) as shown below.

$$\text{Enzyme inhibitory activity (\% Inhibition)} = \left\{1 - \left(\frac{Abs_2 - Abs_1}{Abs_4 - Abs_3}\right)\right\} \times 100 \quad (1)$$

where Abs_1 = Absorbance of incubated solution containing test sample, starch, and α-amylase, Abs_2 = Absorbance of incubated solution containing test sample and starch, Abs_3 = Absorbance of incubated solution containing starch and α-amylase, Abs_4 = Absorbance of incubated solution containing starch.

Each experiment was performed in triplicate and mean % inhibition±SD at each concentration of all the compounds were determined. The IC_{50} values of all compounds **1-4** and **6a-j** were calculated by linear regression and the results obtained thus are depicted in Table 1.

The results summarized in Table 1 inferred that all compounds **1-4** and **6a-j** tested exhibited poor to excellent α-amylase inhibitory activity with IC_{50} values in the range of 0.16-23.19 µM. Among all the tested derivatives (**4**, **6d**, **6e**, **6f** and **6j**) were found to demonstrate poor inhibitory activity whereas compounds **1**, **2**, **6a**, **6b**, **6g** and **6h** were found moderately active and the derivative **6c** (IC_{50} = 0.80 µM) was found to display good inhibitory activity. Compounds **3** (IC_{50} = 0.16 µM) and **6i** (IC_{50} = 0.38 µM) showed comparable activity with standard acarbose with IC_{50} values of 0.15 µM. From the *α*-amylase inhibitory evaluation results of compounds **6a-j**, the following Structure Activity Relationship (SAR) may be deduced: (i) The compound **6i** (R= 2-Cl) demonstrated to increase the α-amylase inhibitory activity followed by compounds **6c** (R= 4-F), **6g** (R = 4-Cl) and **6h** (R = 3-F). (ii) It was found that the derivatives **6a** (R = H), **6b** (R = 3-Br), **6d** (R = 4-OCH_3), **6e** (R = 2-CH_3), **6f** (R = 2-F) and **6j** (R = 4-NO_2) showed poor inhibition against α-amylase.

In general, it is inferred that there are different structural requirements for a compound to be active against *α*-amylase and no general trend for SAR was established for compounds **6a-j** against antidiabetic activity. The findings mentioned above are summarized in Figure 1.

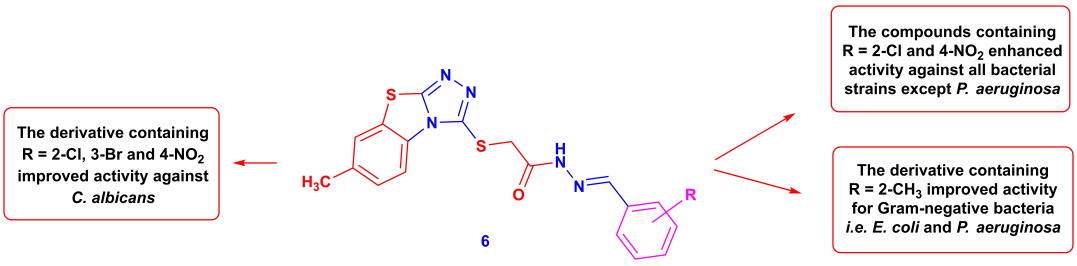


Figure 2. Structure Activity Relationships (SAR) of compounds **6a-j** for antimicrobial activity.

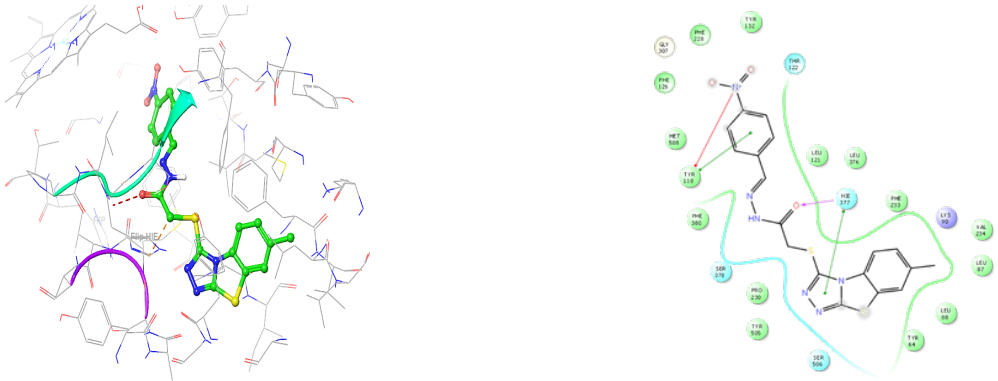


Figure 3. (a) 3D and (b) 2D docked pose of compound **6j** with 5TZ1.

3.2.2. Antimicrobial evaluation

All synthesized compounds **1-4** and acetohydrazides **6a-j** were evaluated for their *in vitro* antibacterial activity against two Gram-positive bacteria, *viz. Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 7443), two Gram-negative bacteria, *viz. Escherichia coli* (MTCC 1652) and *P. aeruginosa* (MTCC 424) and two fungi *viz. Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 8189) using the serial dilution method [39]. Ciprofloxacin and fluconazole were used as standard drugs for preliminary bioassay of bacteria and fungi, respectively, and the results of Minimum Inhibitory Concentrations (MIC) were reported in terms of µmol/mL as depicted in Table 2.

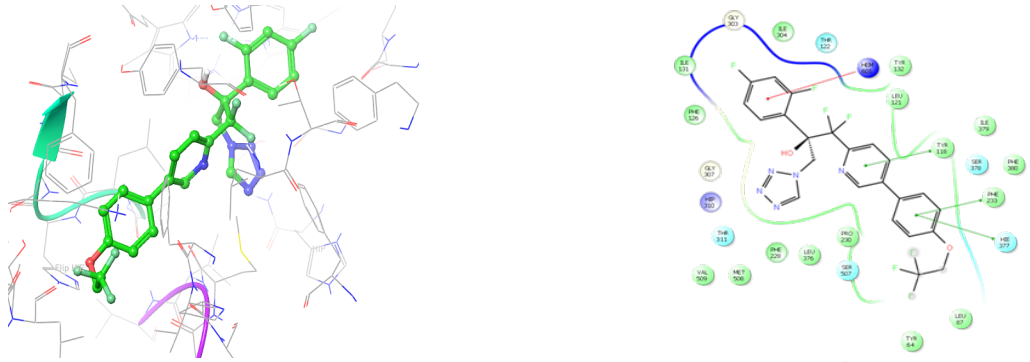
The antimicrobial evaluation data summarized in Table 2 revealed that compounds **6c** and **6i** against *B. subtilis*; compound **6c** against *S. aureus*; compounds **6c**, **6e** and **6i** against *E. coli*, and compound **6e** against *P. aeruginosa* demonstrated noticeable antibacterial activity compared to the reference drug ciprofloxacin. The derivatives **6a**, **6i**, and **6j** against *C. albicans* exhibited substantial antifungal activity compared to the reference drug fluconazole. Interestingly, compound **6b** (MIC, 0.0135 µmol/mL) against *C. albicans* showed activity comparable to the standard drug fluconazole (MIC, 0.0102 µmol/mL). However, the remaining tested derivatives were found to demonstrate moderate to poor inhibitory activity against all bacterial and fungal strains under investigation. Moreover, all synthesized compounds **6a-j** showed more potency against all tested strains compared to compounds **1-4**.

From the results of the *in vitro* antimicrobial assay of compounds **1-4** and **6a-j**, the following structure activity relationship (SAR) can be inferred: (i) The presence of 2-Cl on the benzene ring increased the antibacterial efficiency for *B. subtilis* and *E. coli* followed by 4-F, which increased the antibacterial potency against *B. subtilis*, *S. aureus*, and *E. coli*, (ii) The presence of 2-CH_3 on the benzene ring improved the antimicrobial activity against *E. coli* and *P. aeruginosa*, and (iii) The derivatives bearing the 3-Br, 2-Cl and 4-NO_2 group on the benzene ring augmented inhibition for the fungus *C. albicans.*

Therefore, these results showed that there are different structural requirements for a compound to be active against different strains and a general trend for Structure Activity Relationships (SAR) could not be established for the antimicrobial (antibacterial and antifungal) activities of the tested acetohydrazides **6a-j**. The findings mentioned above are shown in Figure 2.

3.3. Molecular docking

Molecular docking analysis of the synthesized compounds **1-4** and **6a-j** was performed to identify the probable mechanism of action for their antifungal activity against *C. albicans*. The primary target of azoles is the sterol 14α-demethylase/heme protein which co-catalyses cytochrome P-450-dependent 14α-demethylation of sterol [45]. Therefore, in an attempt to find out possible mechanism of binding action of the synthesized compounds, molecular docking analysis was performed with sterol 14α-demethylase of *C. albicans*. Hargrove *et al.* [46] reported the latest crystal structure of sterol 14α-demethylase of *C. albicans* for molecular docking analysis employing PDB code 5TZ1. Initially, the validation of the docking protocol was performed by re-docking the native ligand to the active site of *C. albicans* sterol 14α-demethylase (PDB ID: 5TZ1) with a root mean square deviation (RMSD) of < 1 Å. Among the entire series, compound **6b** and **6j** demonstrated the highest *in vitro* antifungal activity against *C. albicans*; therefore, an analysis of all synthesized compounds was performed to determine the interactions that could be responsible for the inhibition of sterol 14α-demethylase. The derivative **6j** with a docking score of -7.79 showed two aromatic interactions, one each with TYR118 and HIE377 as shown in Figure 3.



(a) (b)

Figure 4. (a) 3D and (b) 2D docked poses of fluconazole with 5TZ1.

On the other hand, fluconazole with a docking score of -10.04 exhibited four aromatic interactions with residues HEM601, TYR118, PHE233, and HIE377 as shown in Figure 4. Comparison of the docked pose of compound **6j** and fluconazole showed that there are two common interacting residues, *i.e.,* TYR118 and HIE377. These common interactions exhibited in the synthesized compounds are responsible for the inhibition of sterol 14α-demethylase and thus antifungal activity.

The docking simulations described above reveal that compounds **6a-j** are more active than their precursors **1-4**, which is in agreement with the experimental *in vitro* antifungal evaluation results.

4. Conclusions

N'-Arylidene-2-((7-methylbenzo[4, 5]thiazolo[2,3-*c*][1,2,4] triazol-3-yl)thio)acetohydrazides (**6a-j**) reported in this paper were easy to prepare from their precursors **1-4**. They were preliminary examined for their *in vitro* Type-II antidiabetic and antimicrobial activities. Among all synthesized compounds **3** (IC_{50} = 0.16 µM) and **6i** (IC_{50} = 0.38 µM) against α-amylase were found to show comparable activity with the standard acarbose with an IC_{50} value of 0.15 µM of both the derivatives. Analogues **6c** and **6i** against *B. subtilis*; compound **6c** against *S. aureus*; compounds **6c**, **6e**, and **6i** against *E. coli*, and compound **6e** against *P. aeruginosa* showed noticeable antibacterial activity compared to the reference drug ciprofloxacin. The derivatives **6a**, **6i**, and **6j** against *C. albicans* demonstrated substantial antifungal activity. Interestingly, compound **6b** (MIC, 0.0135 µmol/mL) against *C. albicans* showed activity comparable to the standard drug fluconazole with a MIC value of 0.0102 µmol/mL. In addition, the antifungal activity of the synthesized compounds **6a-j** was also supported by the docking simulation. From the *α*-amylase inhibitory and antifungal activities, it is inferred that derivatives **3**, **6i** and **6b** can be considered as potential antidiabetic and antifungal agents, respectively, for further drug development.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
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CRediT authorship contribution statement

Conceptualization: Satbir Mor, Suchita Sindhu; Methodology: Suchita Sindhu; Validation: Suchita Sindhu; Formal Analysis: Suchita Sindhu; Investigation: Satbir Mor, Suchita Sindhu; Resources: Satbir Mor, Suchita Sindhu; Data Curation: Suchita Sindhu; Writing - Original Draft: Suchita Sindhu; Writing - Review and Editing: Mohini Khatri, Ravinder Punia, Komal Jakhar; Visualization: Satbir Mor; Funding acquisition: Suchita Sindhu; Supervision: Satbir Mor; Project Administration: Satbir Mor.

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