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ORIGINAL PAPER

A novel method for the synthesis of 1,2,4-triazole-derived heterocyclic compounds: enzyme inhibition and molecular docking studies

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Abstract

Two series of new *N*-aryl/aralkyl derivatives (**9a**–**q**) of 2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide and *N*-aryl/aralkyl derivatives (**10a**–**q**) of 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide were synthesized. The methods included successive conversions of thiophen-2-acetic acid (**a**) into its respective ester, hydrazide and *N*-aryl/aralkyl 1,3,4-triazole. The target compounds (**9a**–**q**; **10a**–**q**) were obtained by the reaction of *N*-aryl/aralkyl 1,3,4-triazole (**5**, **6**) with various electrophiles, (**8a**–**q**), in *N*,*N*-dimethyl formamide (DMF) and sodium hydroxide at room temperature. The characterization of these compounds was done by FTIR, ^{1}H-, ^{13}C-NMR, EI-MS and HR-EI-MS spectral data. All compounds were evaluated for their enzyme inhibitory potentials against electric eel acetylcholinesterase, AChE (**10f**, **10d**; IC_{50} values 32.26 ± 0.12, 45.72 ± 0.11 µM, respectively), equine butyrylcholinesterase, BChE (**9d**, **9l**, **9b**, **10d**, **10h**; IC_{50} values 12.52 ± 0.19, 12.52 ± 0.19, 21.72 ± 0.18, 23.62 ± 0.22, 24.52 ± 0.21 µM, respectively), jack bean urease (**10i**, **10n**, **9e**; IC_{50} values 7.27 ± 0.05, 7.35 ± 0.04, 8.79 ± 0.05 µM, respectively) and yeast α-glucosidase enzymes (**9o**, **10i**; IC_{50} values 62.94 ± 0.19, and 69.46 ± 0.15 µM, respectively). The molecular docking studies supported these findings. This study provides cheaper bioactive triazole amides as promising future lead molecules.

Keywords Triazole amides · Synthesis · Characterization · NMR spectroscopy · Mass spectrometry · Enzyme inhibition · Molecular docking studies

Introduction

During the last few decades, pharmaceutical and agrochemical industries have been focusing on the synthesis of nitrogen-rich heterocyclic compounds. Among them, 1,2,4-triazole-derived synthetic drugs, like etizolam, alprazolam, furacylin, are used for the treatment of inflammation, as muscle relaxants and anxiety disorders, respectively. 1,2,4-Triazoles mostly are synthetic aromatic compounds possessing an extensive chemistry [1, 2]. These compounds with mercapto substitution on ring systems exhibit a diversity of biological activities like antitumor [3], fungicidal [4], antibacterial, antidepressant [5], anticonvulsant [6], anticancer [7], and antioxidant [8], while some are hypotensive [9]. In addition, they are reported to exhibit enzyme inhibition activities against thymidine phosphorylase [10], carbonic anhydrase and cholinesterase [11], glycogen phosphorylase [12], histone deacetylase [13], angiotensin converting enzyme [14], cyclooxygenase [15], lipase and α-glucosidase [16], nitric oxide synthase [17], and CYP19 aromatase [18]. The previous reports revealed that 1,2,4-triazole-derived motifs are strong candidates for future drug discovery and development.

There is a demand to address the new complex biological malfunctions, such as neurodegeneration, dementia, and

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cancer for the betterment of health across the world. Therefore, medicinal chemists are designing chemicals that are able to interact with two or more targets to produce synergistic clinical effects, decreasing the risks and lack of compliance associated with combinations of several drugs. In this regard, enzymes remain prime targets for the drug design because the altering enzyme activity has the immediate and defined effects. Recent multitarget-directed ligand (MTDL) approaches in medicinal chemistry have combined inhibitors of cholinesterases (ChE) to combat the loss of neurotransmitters in Alzheimer's disease (AD) [19].

Structure-based drug design for a single enzyme target has been facilitated with crystal structures enabling the computational searches to identify lead compounds for refinement. With such large-scale computational approaches including analysis of off-target activity, combining suitable pharmacophores for enzyme combinations such as ChE for AD is entirely feasible. However, such compounds still have to be synthesized and tested experimentally, to confirm the predicted inhibitory effects against each of the targets [11].

The present work was designed to explore a new series of biologically active mercapto *N*-aryl/aralkyl acetamide triazoles, which may assist in designing new synthetic drug leads; therefore, *N*-aryl/aralkyl derivatives (**9a**–**q**) of 2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide and *N*-aryl/aralkyl derivatives (**10a**–**q**) of 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide were synthesized in a series of steps and were subsequently screened against electric eel acetylcholinesterase (AChE), equine butyrylcholinesterase (BChE), jack bean urease and yeast α-glucosidase enzymes.

Materials and methods

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker instrument operating at 500 and 125 MHz, respectively, using TMS as an internal standard. IR spectra were recorded on Shimadzu 460 FTIR spectrometer using KBr pellets. Mass spectra were measured on JMSA 500 mass spectrometer and JMS H × 110 spectrometer with data system. Melting points were found on Gallen Kemp electrothermal apparatus. All the chemicals and solvents of analytical grade were purchased from local supplier of Sigma, Aldrich and Alfa Aesar. During synthesis, reaction progress was monitored using TLC.

Synthesis of ethyl 2-(thiophen-2-yl)acetate (1)

Thiophen-2-acetic acid (**a**; 10 g) was dissolved in an excess amount of ethanol in a round-bottom flask. Six ml of H_2SO_4 was added to the flask and the reaction mixture was refluxed for 3–4 h. Excess amount of distilled water was added to the reaction mixture. A reddish brown oily product was generated at basic pH (8–9 maintained by Na_2CO_3) and was extracted with $CHCl_3$ to get compound **1**. The physical and spectroscopic data of **1** are as follows:

Reddish brown oil; yield: 71%; IR (KBr) ν_{max}: 3022, 2928, 1755, 1597 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–O), 3.45 (s, 2H, H-6), 4.43 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–O), 6.53 (d, J = 3.0 Hz, 1H, H-3), 6.83 (dd, J = 3.5, 3.0 Hz, 1H, H-4), 7.10 (d, J = 3.5 Hz, 1H, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.1 (CH_3), 35.3 (C-6), 63.3 (CH_2), 127.3 (C-4), 128.0 (C-3), 131.8 (C-5), 141.4 (C-2), 166.9 (C-7); HR-EI-MS (*m/z*): 170.0410 [M]$^+$ calculated for $C_8H_{10}SO_2$; 170.0402.

Synthesis of 2-(thiophen-2-yl)acetohydrazide (2)

Ethyl thiophene-2-acetate (**2**; 0.05 mol, 7.5 ml) and 0.05 mol (2.4 ml) of hydrazine hydrate (80%) were stirred for 4–5 h in 100-ml round-bottom flask till the completion of the reaction. The light yellow crystalline product was filtered, washed with *n*-hexane and dried. The physical and spectroscopic data of **2** are as follows:

Yellow crystals; yield 76%; M.P.: 108–110 °C; IR (KBr) ν_{max}: 3417, 3320, 3024, 2935, 1692, 1533 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.45 (s, 2H, H-6), 4.02 (s, 1H, –NH), 6.53 (d, J = 3.0 Hz, 1H, H-3), 6.83 (dd, J = 3.5, 3.0 Hz, 1H, H-4), 7.10 (d, J = 3.5 Hz, 1H, H-5), 8.90 (s, 2H, –NH_2); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 35.5 (C-6), 127.3 (C-4), 128.0 (C-3), 129.8 (C-5), 135.4 (C-2), 168.9 (C-7); HR-E-IMS (*m/z*): 156.0357 [M]$^+$ calculated for $C_6H_8SN_2O$; 156.0350.

Synthesis of 4-ethyl-1-(2-(thiophen-2-yl)acetyl) thiosemicarbazide (3)

Carbohydrazide (**2**, 2.5 g, 0.02 mol) was dissolved in 25 ml of ethanol in a 100-ml round-bottom flask. Ethyl isothiocyanate (0.02 mol) was added in the reaction mixture, and contents were refluxed for 3–4 h. Precipitated final product was cooled, filtered, washed and dried. The physical and spectroscopic data of **3** are as follows:

Amorphous solid; yield 79%; M.P.: 121–123 °C; IR (KBr) ν_{max}: 3325, 2938, 1697, 1601 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, J = 6.4 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.41 (s, 2H, H-6), 3.53 (q, J = 6.4 Hz, 2H, CH_3–$\underline{CH_2}$–N), 6.52 (d, J = 3.0 Hz, 1H, H-3), 6.85 (dd, J = 3.5, 3.0 Hz, 1H, H-4), 7.11 (d, J = 3.5 Hz, 1H, H-5); ^{13}C-NMR (125 MHz $CDCl_3$): δ 20.1 (CH_3), 36.3 (C-6), 39.5 (CH_2), 126.3 (C-4), 129.0 (C-3), 131.8 (C-5), 138.4 (C-2), 171.0 (C-7), 180.6 (C=S); HR-EI-MS (*m/z*): 243.0510 [M]$^+$ calculated for $C_9H_{13}S_2N_3O$; 243.0499.

Synthesis of 4-phenyl-1-(2-(thiophen-2-yl)acetyl) thiosemicarbazide (4)

Two grams (0.012 mol) of carbohydrazide (**3**) was dissolved in 25 ml of ethanol in a 100-ml round-bottom flask. Two ml of phenyl isothiocyanate (0.012 mol) was added in the reaction mixture, and the contents were refluxed and stirred for 3–4 h. Precipitated crude product was cooled, filtered, washed and dried. The physical and spectroscopic data for **4** are as follows:

Amorphous powder; yield 68%; M.P.: 119–120 °C; IR (KBr) ν_{max}: 3317, 3031, 2929, 1685, 1601, 1524 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.13 (s, 2H, H-6), 6.48 (d, J = 3.0 Hz, 1H, H-3), 6.69 (dd, J = 3.2, 3.0 Hz, 1H, H-4), 7.10–7.15 (m, 5H, Ar–H), 7.19 (d, J = 3.2 Hz, 1H, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 34.3 (C-6), 126.9 (C-3), 128.0 (C-4), 129.8 (C-5), (C-2) 136.4, Ar–C [125.0 (2 × CH), 127.3 (2 × CH), 128.1 (CH), 128.3 (C)], 168.5 (C-7), 180.6 (C=S); HR-EI-MS (*m/z*): 291.0505 $[M]^+$ calculated for $C_{13}H_{13}S_2N_3O$; 291.0500.

Synthesis of 4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2, 4-triazole-3-thiol (5)

4-Ethyl-1-(2-(thiophen-2-yl)acetyl)thiosemicarbazide (**3**) was dissolved in 10% aqueous NaOH in a 100-ml round-bottom flask and was refluxed for 4 h. The reaction mixture was poured into ice cold water, acidified with conc HCl to pH 4–6 and the precipitated product was cooled, filtered, washed and dried. The physical and spectroscopic data for **5** are as follows:

Amorphous powder; yield 79%; M.P.: 121–123 °C; IR (KBr) ν_{max}: 3032, 2925, 1593, 1543 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.10 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.25 (s, 2H, H-6′), 3.98 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 6.50 (d, J = 3.0 Hz, 1H, H-3′), 6.85 (dd, J = 3.4, 3.0 Hz, 1H, H-4′), 7.10 (d, J = 3.4 Hz, 1H, H-5′), 13.6 (s, SH, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.7 (CH_3), 35.3 (C-6′), 39.5 (CH_2), 126.3 (C-4′), 129.0 (C-3′), 131.8 (C-5′), 138.4 (C-2′), 140.4 (C-5), 149.7 (C-3); HR-EI-MS (*m/z*): 225.0380 $[M]^+$ calculated for $C_9H_{11}S_2N_3$; 225.0394.

Synthesis of 4-phenyl-5-(thiophen-2-ylmethyl)-4H-1, 2,4-triazole-3-thiol (6)

4-Phenyl-1-(2-(thiophen-2-yl)acetyl)thiosemicarbazide (**4**) was dissolved in 10% aqueous NaOH in 100-ml round-bottom flask and refluxed for 4 h. The progress of the reaction was monitored using TLC. The reaction mixture was poured into ice cold water, acidified to pH 4–6 using concentrated HCl, and the precipitated product was cooled, filtered, washed and dried. The physical and the spectroscopic data of compound **6** are as follows:

Amorphous powder; yield 78%; M.P.: 123–125 °C; IR (KBr) ν_{max}: 3033, 2929, 1601, 1524 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.25 (s, 2H, H-6′), 6.57 (d, J = 2.8 Hz, 1H, H-3′), 6.98 (dd, J = 3.2, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.2 Hz, 1H, H-5′), Ar–H, [7.01–7.09 (m, 5H)], 13.6 (s, SH, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 35.3 (C-6′), 126.9 (C-4′), 128.0 (C-3′), 131.0 (C-5′), 136.0 (C-2′), Ar–C [125.8 (2 × CH), 128.1 (2 × CH), 129.1 (CH), 136.4 (C)], 141.2 (C-5), 151.0 (C-3); HR-EI-MS (*m/z*): 273.0381 $[M]^+$ calculated for $C_{13}H_{11}S_2N_3$; 273.0394.

Synthesis of compounds (8a–q)

Calculated amount of each of the aryl/aralkyl amines (**7a**–**q**, 11.0 mmol) separately was added to 10 ml distilled water in an iodine flask. Basic pH (9–10) was adjusted by adding 5% Na_2CO_3 solution. The reaction mixture was stirred for 10 min at 0–5 °C, and at the same temperature, it was supplemented drop-wise with bromoacetyl bromide (1.0 ml, 11.0 mmol). The reaction flask was then shaken vigorously till the formation of precipitates. The precipitates were stirred for further 45 min, and the solid product was filtered, washed with distilled water and dried to yield the respective electrophiles, *N*-aralkyl/aryl-substituted-2-bromoacetamides (**8a**–**q**).

General method for the synthesis of compounds (9a–q)

Compound **5** (0.1 mmol, 0.002 g) was dissolved in 10 ml of DMF in a 50-ml round-bottom flask. The solution was then supplemented with sodium hydride (0.002 g, 0.1 mmol), and the mixture was stirred for 30 min at room temperature, followed by the slow addition of electrophiles (**8a**–**q**, each separately) while stirring for 2–3 h. Distilled water was then added to the reaction mixture and the products were recovered by filtration or solvent extraction according to the product nature.

N-(2,3-dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl) -4H-1,2,4-triazol-3-ylthio)acetamide (9a)

Amorphous powder; yield: 71%; M.P.: 135–137 °C; IR (KBr) ν_{max}: 3020, 2920, 1696, 1597, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.13 (s, CH_3, 3H), 2.28 (s, CH_3, 3H), 3.83 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.01 (s, 2H, H-6′), 4.17 (s, 2H, H-2″), thiophene-H [6.57 (d, J = 2.8 Hz, 1H, H-3′), 6.98 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′], Ar–H [7.10–7.20 (m, CH, 3H)], 7.97 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.4 (CH_3), 14.1 (CH_3), 19.7 (CH_3), 31.9 (C-6′), 32.1 (C-2″), 45.8 (CH_2), 127.3 (C-4′), 128.0 (C-3′), 131.8 (C-5′), 141.4 (C-2′), Ar–C [122.8 (C-4‴),

125.4 (C-5‴), 125.8 (C-6‴), 130.4 (C-2‴), 135.7 (C-3‴), 137.9 (C-1‴)], 151.0 (C-5), 155.0 (C-3), 166.9 (C-1″); HR-EI-MS (*m/z*): 386.1226 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(2,4-dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**9b**)

Amorphous powder; yield: 69%; M.P.: 129–130 °C; IR (KBr) ν_{max}: 3030, 2915, 1696, 1596, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.12 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.11 (s, CH_3, 3H), 2.24 (s, CH_3, 3H), 3.81 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 2H, H-6′), 4.13 (s, 2H, H-2″), thiophene-H [6.57 (d, J = 2.8 Hz, 1H, H-3′), 6.98 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H, [7.23 (br s, 1H, H-3‴), 7.33 (d, J = 8.0 Hz, 1H, H-5‴), 7.38 (d, J = 8.0 Hz, 1H, H-6‴)], 7.98 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 16.2 (CH_3), 20.1 (CH_3), 31.1 (C-6′), 36.5 (C-2″), 44.4 (CH_2), 126.4 (C-4′), 130.1 (C-3′), 132.5 (C-5′), 138.2 (C-2′), Ar–C [126.1 (C-5‴), 127.2 (C-6‴), 127.2 (C-3‴), 128.4 (C-2‴), 129.6 (C-4‴), 136.7 (C-1‴)], 151.6 (C-5), 155.0 (C-3), 166.5 (C-1″); HR-EI-MS (*m/z*): 386.1224 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(2,5-dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**9c**)

Amorphous powder; yield: 69%; M.P.: 126–127 °C; IR (KBr) ν_{max}: 3025, 2910, 1685, 1580, 1540 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.08 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.15 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 3.85 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.01 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 3.0 Hz, 1H, H-3′), 6.80 (dd, J = 3.5, 3.0 Hz, 1H, H-4′), 7.15 (d, J = 3.5 Hz, 1H, H-5′)], Ar–H, [7.01 (br s, 1H, H-6‴), 6.97 (d, J = 7.8 Hz, 1H, H-3‴), 7.37 (d, J = 7.8 Hz, 1H, H-4‴), 7.79 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 16.2 (CH_3), 20.1 (CH_3), 30.1 (C-6′), 35.3 (C-2″), 44.5 (CH_2), 126.6 (C-4′), 130.0 (C-3′), 134.9 (C-5′), 136.1 (C-2′), Ar–C [125.0 (C-3‴), 125.2 (C-4‴), 126.2 (C-6‴), 126.8 (C-2‴), 129.0 (C-5‴), 137.3 (C-1‴)], 150.5 (C-5), 154.6 (C-3), 167.3 (C-1″); HR-EI-MS (*m/z*): 386.1227 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(2,6-dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**9d**)

Amorphous powder; Yield: 62%; M.P.: 151–153 °C; IR (KBr) ν_{max}: 3025, 2915, 1689, 1570, 1540 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.05 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 1.82 (s, 3H, CH_3), 1.93 (s, 3H, CH_3), 3.84 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H [6.86 (d, J = 2.8 Hz, 1H, H-3′), 6.90 (dd, J = 3.0, 2.8 Hz, 1H, H-4″), 7.18 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H [7.23–7.26 (m, 3H, H-3′-5′), 7.80 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 16.2 (CH_3), 20.1 (CH_3), 30.1 (C-6′), 36.3 (C-2″), 42.5 (CH_2), thiophene-C [126.6 (C-4′), 130.0 (C-3′), 134.9 (C-5′), 136.1 (C-2′)], Ar–C [125.0 (C-5‴), 125.2 (C-3‴), 126.2 (C-4‴), 126.8 (C-2‴), 129.0 (C-6‴, 137.3 (C-1‴)], 150.5 (C-5), 154.6 (C-3), 167.3 (C-1″); HR-EI-MS (*m/z*): 386.1224 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(3,4-dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**9e**)

Amorphous powder; yield: 69%; M.P.: 127–129 °C; IR (KBr) ν_{max}: 3026, 2928, 1693, 1591, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.04 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 1.94 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.80 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.02 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H, [6.54 (d, J = 2.8 Hz, 1H, H-3′), 6.91 (dd, J = 2.9, 2.8 Hz, 1H, H-4′), 7.09 (d, J = 2.9 Hz, 1H, H-5′)], Ar–H [7.23 (br s, 1H, H-2‴), 7.33 (d, J = 8.0 Hz, 1H, H-5‴), 7.38 (d, J = 8.0 Hz, 1H, H-6‴)], 7.90 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.6 (CH_3), 19.3 (CH_3), 21.3 (CH_3), 31.4 (C-6′), 35.4 (C-2″), 46.1 (CH_2), thiophene-C [126.4 (C-4′), 130.0 (C-3′), 134.9 (C-5′), 138.7 (C-1′)], Ar–C [125.2 (C-5‴), 126.2 (C-6‴), 127.2 (C-2‴), 128.3 (C-3‴), 128.7 (C-4‴), 137.1 (C-1‴)], 151.2 (C-5), 153.7 (C-3), 168.5 (C-1″); HR-EI-MS (*m/z*): 386.1227 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(3,5-Dimethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**9f**)

Amorphous powder; yield: 67%; M.P.: 127–129 °C; IR (KBr) ν_{max}: 3020, 2925, 1697, 1591, 1530 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.04 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 1.94 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.83 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.01 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H, 6.62 [(d, J = 2.8 Hz, 1H, H-3′), 6.85 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.10 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H [7.23 (br s, 1H, H-2‴), 7.33 (br s, 1H, H-4‴), 7.35 (br s, 1H, H-6‴)], 7.98 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.6 (CH_3), 16.3 (CH_3), 20.3 (CH_3), 31.4 (C-6′), 33.2 (C-2″), 45.1 (CH_2), thiophene-C [126.4 (C-4′), 130.0 (C-3′), 134.9 (C-5′), 138.7 (C-2′)], Ar–C [125.2 (C-2‴), 126.2 (C-4‴), 127.2 (C-6‴), 128.3 (C-3‴), 130.7 (C-5‴), 137.1 (C-1‴)], 151.2 (C-5), 153.7 (C-3), 168.5 (C-1″); HR-EI-MS (*m/z*): 386.1227 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

_N_-phenyl-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9g)

Amorphous powder; yield: 66%; M.P.: 147–150 °C; IR (KBr) ν_{max}: 3020, 2928, 1692, 1597, 1542 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.08 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.78 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 3.99 (s, 2H, H-6′), 4.09 (s, 2H, H-2″), thiophene-H, [6.54 (d, J = 3.0 Hz, 1H, H-3′), 6.83 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.10 (d, J = 2.8 Hz, 1H, H-5′)], Ar–H 7.24–7.27 (m, 5H, H-2‴–6‴), 8.01 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.2 (CH_3), 30.0 (C-6′), 35.0 (C-2″), 45.1 (CH_2), thiophene-C [126.4 (C-4′), 130.2 (C-3′), 132.4 (C-5′), 138.9 (C-2′)], Ar–C [125.2 (C-3‴,5‴), 127.1 (C-2‴,6‴), 128.4 (C-4‴), 129.6 (C-1‴)], 151.6 (C-5), 154.9 (C-3), 168.3 (C-1″); HR-EI-MS (*m/z*): 358.0912 $[M]^+$ calculated for $C_{17}H_{18}S_2N_4O$; 358.0922.

_N_-(2-methylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9h)

Amorphous powder; yield: 73%; M.P.: 123–125 °C; IR (KBr) ν_{max}: 3016, 2924, 1696, 1590, 1532 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.08 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.24 (s, 3H, CH_3), 3.79 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.01 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H, [6.59 (d, J = 2.8 Hz, 1H, H-3′), 6.82 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.15 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H 7.23–7.29 (m, 4H, H-3‴–6‴), 7.81 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.0 (CH_3), 22.2 (CH_3), 30.4 (C-6′), 35.7 (C-2″), 46.1 (CH_2), thiophene-C [126.5 (C-4′), 131.8 (C-3′), 132.3 (C-5′), 136.6 (C-2′)], Ar–C [125.9 (C-4‴), 126.2 (C-5‴), 126.0 (C-3‴), 127.1 (C-6‴), 130.3 (C-2‴), 135.2 (C-1‴)], 151.9 (C-5), 155.0 (C-3), 167.3 (C-1″); HR-EI-MS (*m/z*): 372.1060 $[M]^+$ calculated for $C_{18}H_{20}S_2N_4O$; 372.1078.

_N_-(3-methylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9i)

Amorphous powder; yield: 77%; M.P.: 120–122 °C; IR (KBr) ν_{max}: 3019, 2930, 1699, 1594, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.12 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N,), 2.12 (s, 3H, CH_3), 3.80 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H, [6.62 (d, J = 3.2 Hz, 1H, H-3′), 6.88 (dd, J = 3.2, 2.8 Hz, 1H, H-4′), 7.19 (d, J = 2.8 Hz, 1H, H-5′)], Ar–H [7.23 (s, 1H, H-2‴), 7.33 (m, 3H, H-4‴–6‴), 7.80 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.0 (CH_3), 21.2 (CH_3), 30.4 (C-6′), 35.7 (C-2″), 46.1 (CH_2), thiophene-C [126.7 (C-4′), 137.3 (C-3′), 128.3 (C-5′), 138.0 (C-2′)], Ar–C [116.8 (C-4‴), 120.2 (C-5‴), 124.8 (C-2‴), 125.0 (C-6‴), 126.2 (C-3‴), 138.4 (C-1‴)], 150.3 (C-5), 154.6 (C-3), 166.5 (C-1″); HR-EI-MS (*m/z*): 372.1060 $[M]^+$ calculated for $C_{18}H_{20}S_2N_4O$; 372.1078.

_N_-(4-methylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9j)

Amorphous powder; yield: 62%; M.P.: 122–124 °C; IR (KBr disk,) ν_{max}: 3020, 2920, 1696, 1597, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.04 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.76 (s, 3H, CH_3), 3.81 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.01 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H, [6.55 (d, J = 2.6 Hz, 1H, H-3′), 6.92 (dd, J = 3.0, 2.6 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′), Ar–H [7.23 (d, J = 8.4 Hz, 2H, H-2‴,3‴), 7.33 (d, J = 8.4 Hz, 2H, H-5‴,6‴), 7.90 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 22.1 (CH_3), 31.9 (C-6′), 35.7 (C-2″), 46.1 (CH_2), thiophene-C [126.3 (C-4′), 128.1 (C-3′), 128.4 (C-5), 137.3 (C-2′)], Ar–C [116.8 (C-3‴,5‴), 125.2 (C-2‴,6‴), 126.3 (C-4‴) 138.8 (C-1‴)], 150.3 (C-5), 154.5 (C-3), 168.4 (C-1″); HR-EI-MS (*m/z*): 372.1060 $[M]^+$ calculated for $C_{18}H_{20}S_2N_4O$; 372.1078.

Methyl-(2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamido)-1-benzoate (9k)

Amorphous powder; yield: 71%; M.P.: 173–175 °C; IR (KBr) ν_{max}: 3020, 2920, 1696, 1597, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.05 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.80 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 3.98 (s, 3H, –OCH_3), 4.02 (s, 2H, H-6′), 4.11 (s, 2H, H-2″), thiophene-H, [6.49 (d, J = 2.6 Hz, 1H, H-3′), 6.85 (dd, J = 3.4, 2.6 Hz, 1H, H-4′), 7.11 (d, J = 3.4 Hz, 1H, H-5′)], Ar–H [7.23 (m, 4H, H-3‴–6‴), 7.90 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.3 (CH_3), 31.2 (C-6′), 34.5 (C-2″), 45.1 (CH_2), 55.3 (OCH_3), thiophene-C [124.5 (C-3′), 128.7 (C-4′), 128.4 (C-5′), 136.2 (C-2′)], Ar–C [115.3 (C-4‴), 125.0 (C-3‴), 126.0 (C-5‴), 127.9 (C-6‴), 128.3 (C-2‴) 139.4 (C-1‴)], 148.3 (C-5), 152.0 (C-3), 169.4 (C-1″); HR-EI-MS (*m/z*): 416.0965 $[M]^+$ calculated for $C_{19}H_{20}S_2N_4O_3$; 416.0976.

_N_-(2-ethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9l)

Amorphous powder; yield: 62%; M.P.: 133–135 °C; IR (KBr) ν_{max}: 3025, 2929, 1689, 1593, 1539 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.00 (t, J = 6.6 Hz, 3H, $\underline{CH_3}$–CH_2), 1.05 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.60 (q, J = 6.6 Hz, 2H, CH_3–$\underline{CH_2}$), 3.83 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 3.99 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H, [6.53 (d, J = 2.6 Hz, 1H, H-3′), 6.87 (dd, J = 3.5, 2.6 Hz, 1H, H-4′), 7.11 (d, J = 3.5 Hz, 1H, H-5′)], Ar–H 7.23 (m, 4H, H-3‴–6‴), 7.91 (s, 1H, NH); ^{13}C-NMR (125 MHz,

$CDCl_3$): δ 13.6 (CH_3), 18.1 (CH_3), 22.0 (CH_2), 31.4 (C-6′), 34.7 (C-2″), 44.1 (CH_2), thiophene-C [126.5 (C-4′), 130.2 (C-3′), 132.2 (C-5′), 136.7 (C-2′)], Ar–C [124.7 (C-4‴), 125.9 (C-3‴), 126.2 (C-5‴), 127.1 (C-6‴), 134.5 (C-2‴), 138.4 (C-1‴)], 151.9 (C-5), 155.1 (C-3), 167.7 (C-1″); HR-EI-MS (*m/z*): 386.1226 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(4-ethylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazo-3-ylthio)acetamide (9m)

Amorphous powder; yield: 62%; M.P.: 129–131 °C; IR (KBr) ν_{max}: 3035, 2934, 1682, 1581, 1550 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.01 (t, *J* = 6.6 Hz, 3H, $\underline{CH_3}$–CH_2), 1.16 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.25 (q, *J* = 6.6 Hz, 2H, CH_3–$\underline{CH_2}$), 3.86 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H [6.53 (d, *J* = 2.6 Hz, 1H, H-3′), 6.89 (dd, *J* = 3.5, 2.6 Hz, 1H, H-4′), 7.11 (d, *J* = 3.5 Hz, 1H, H-5′)], Ar–H [7.30 (d, *J* = 8.2 Hz, 2H, H-2‴,3‴), 7.45 (d, *J* = 8.2 Hz, 2H, H-5‴,6‴)], 7.88 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.7 (CH_3), 14.8 (CH_3), 21.2 (CH_2), 31.5 (C-6′), 39.3 (C-2″), 45.9 (CH_2), thiophene-C [126.2 (C-3′), 126.7 (C-4′), 135.7 (C-5′), 137.3 (C-2′)], Ar–C [119.8 (C-3‴,5‴), 126.7 (C-2‴,6‴), 135.7 (C-4‴), 140.4 (C-1‴)], 150.3 (C-5), 154.5 (C-3), 166.4 (C-1″); HR-EI-MS (*m/z*): 386.1226 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O$; 386.1235.

N-(4-ethoxyphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (9n)

Amorphous powder; yield: 65%; M.P.: 135–137 °C; IR (KBr) ν_{max}: 3035, 2934, 1682, 1581, 1550 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.03 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 1.10 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–O), 3.83 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 3.98 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–O), 404 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H, [6.51 (d, *J* = 2.6 Hz, 1H, H-3′), 6.78 (dd, *J* = 3.2, 2.6 Hz, 1H, H-4′), 7.10 (d, *J* = 3.2 Hz, 1H, H-5′)], Ar–H [7.35 (d, *J* = 8.2 Hz, 2H, H-5‴,6‴), 7.38 (d, *J* = 8.2 Hz, 2H, H-2‴,3‴)], 7.91 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 14.1 (CH_3), 14.2 (CH_3), 31.5 (C-6′), 36.3 (C-2″), 44.5 (CH_2), 65.4 (CH_2), thiophene-C [126.2 (C-4′), 126.3 (C-3′), 126.9 (C-5′), 130.9 (C-2′)], Ar–C [114.4 (C-3‴,5‴), 125.1 (C-2‴,6‴), 136.9 (C-4‴), 150.5 (C-1‴)], 154.4 (C-5), 155.9 (C-3), 166.2 (C-1″); HR-EI-MS (*m/z*): 402.1170 $[M]^+$ calculated for $C_{19}H_{22}S_2N_4O_2$; 402.1184.

N-(2-ethyl-6-methylphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (9o)

Amorphous powder; yield: 65%; M.P.: 121–122 °C; IR (KBr) ν_{max}: 3020, 2922, 1687, 1587, 1545 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.01 (t, *J* = 6.6 Hz, 3H, $\underline{CH_3}$–CH_2), 1.05 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 2.16 (q, *J* = 6.6 Hz, 2H, CH_3–$\underline{CH_2}$), 2.23 (s, 3H, CH_3), 3.81 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.58 (d, *J* = 3.2 Hz, 1H, H-3′), 6.80 (dd, *J* = 3.4, 3.2, Hz, 1H, H-4′), 7.15 (d, *J* = 3.4 Hz, 1H, H-5′)], Ar–H 7.30 (m, 3H, H-3‴–5‴), 7.89 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 13.9 (CH_3), 17.1 (CH_3), 22.4 (CH_2), 31.8 (C-6′), 37.4 (C-2″), 45.0 (CH_2), thiophene-C [126.5 (C-3′), 133.0 (C-4′), 135.8 (C-5′), 141.4 (C-2′)], Ar–C [125.2 (C-4‴), 126.1 (C-3‴), 126.9 (C-5‴), 127.5 (C-2‴), 127.6 (C-6‴) 136.9 (C-1‴)], 150.8 (C-5), 154.5 (C-3), 167.5 (C-1″); HR-EI-MS (*m/z*): 400.1380 $[M]^+$ calculated for $C_{20}H_{24}S_2N_4O$; 400.1391.

N-(2-methoxyphenyl)-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (9p)

Amorphous powder; yield: 65%; M.P.: 141–143 °C; IR (KBr) ν_{max}: 3016, 2929, 1691, 1590, 1533 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.09 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.81 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 4.00 (s, 3H, O$\underline{CH_3}$), 4.10 (s, 2H, H-6′), 4.15 (s, 2H, H-2″), thiophene-H, [6.60 (d, *J* = 3.5 Hz, 1H, H-3′), 6.84 (dd, *J* = 3.5, 3.2 Hz, 1H, H-4′), 7.12 (d, *J* = 3.2 Hz, 1H, H-5′), Ar–H [7.30 (m, 4H, H-3‴–6‴), 7.84 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.4 (CH_3), 30.6 (C-6′), 36.4 (C-2″), 45.6 (CH_2), 57.6 (OCH_3), thiophene-C [126.4 (C-4″), 129.9 (C-3″), 132.1 (C-5″), 136.4 (C-2″)], Ar–C [114.5 (C-3‴), 121.5 (C-5‴), 126.7 (C-4‴), 127.9 (C-6‴), 136.4 (C-2‴), 152.2 (C-1‴)], 154.8 (C-5), 155.8 (C-3), 166.1 (C-1″); HR-EI-MS (*m/z*): 388.1019 $[M]^+$ calculated for $C_{18}H_{20}S_2N_4O_2$; 388.1027.

N-benzyl-2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (9q)

Amorphous powder; yield: 76%; M.P.: 118–120 °C; IR (KBr) ν_{max}: 3035, 2910, 1670, 1584, 1527 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.05 (t, *J* = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–N), 3.84 (q, *J* = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–N), 3.98 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H, [6.58 (d, *J* = 3.0 Hz, 1H, H-3′), 6.85 (dd, *J* = 3.2, 3.0 Hz, 1H, H-4′), 7.15 (d, *J* = 3.2 Hz, 1H, H-5′)], Ar–H 7.23–7.34 (m, 5H, H-2‴–6‴), 7.99 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.6 (CH_3), 32.2 (C-6′), 35.1 (C-2″), 45.2 (CH_2), 46.3 (CH_2), thiophene-C [126.3 (C-4′), 128.3 (C-3′), 128.2 (C-5′), 138.0 (C-2′)], Ar–C [114.5 (C-2‴, 6‴), 119.0 (C-4‴), 128.1 (C-3‴, 5‴), 137.3 (C-1‴)], 150.2 (C-5), 154.5 (C-3), 168.3 (C-1″); HR-EI-MS (*m/z*): 372.1061 $[M]^+$ calculated for $C_{18}H_{20}S_2N_4O$; 372.1078.

Synthesis of compounds (10a–q)

In a 50-ml round-bottom flask, 4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol (**6**; 0.002 g, 0.1 mmol) was dissolved in 10 ml of DMF followed by the addition of sodium hydride (0.002 g, 0.1 mmol). The mixture was stirred for 30 min at 30 °C, and then the electrophiles (**8a**–**q**, each separately) were added slowly to the mixture with further stirring for 2–3 h. Distilled water was added to the flask, and the products were recovered by filtration or solvent extraction according to the nature of the product.

N-(2,3-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (10a)

Amorphous powder; yield: 74%; M.P.: 131–135 °C; IR (KBr) ν_{max}: 3020, 2914, 1696, 1596, 1542 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.90 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 3.87 (s, 2H, H-6′), 4.15 (s, 2H, H-2″), thiophene-H [6.54 (d, J = 2.8 Hz, 1H, H-3′), 6.88 (dd, J = 3.0, 2.8, Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H 7.10–7.20 (m, 3H, H-4‴–6‴), Ph–N 7.43–7.47 (m, 5H), 7.89 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.7 (CH_3), 22.3 (CH_3), 30.4 (C-6′), 35.0 (C-2′), thiophene-C [126.2 (C-4′), 130.2 (C-3′), 132.3 (C-5′), 136.6 (C-2′)], 120.2 (C-4‴), 120.7 (C-5‴), 124.6 (C-6‴), 126.2 (C-2‴), 126.8 (C-3‴), 132.3 (C-1‴), Ph–N [124.8 (CH), 126.6 (2×CH), 129.6 (2×CH), 149.4 (C)], 151.2 (C-5), 155.0 (C-3), 169.9 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(2,4-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (10b)

Amorphous powder; yield: 81%; M.P.: 116–117 °C; IR (KBr) ν_{max}: 3023, 2929, 1688, 1592, 1534 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.90 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 4.00 (s, 2H, H-6′), 4.15 (s, 2H, H-2″), thiophene-H [6.58 (d, J = 2.6 Hz, 1H, H-3′), 6.98 (dd, J = 2.8, 2.6 Hz, 1H, H-4′), 7.18 (d, J = 2.8 Hz, 1H, H-5′)], Ar–H [7.23 (br s, 1H, H-3‴), 7.33 (d, J = 8.2 Hz, 1H, H-6‴), 7.38 (d, J = 8.2 Hz, 1H, H-5‴)], Ph–N 7.43–7.47 (m, 5H), 7.99 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.7 (CH_3), 22.4 (CH_3), 31.2 (C-6′), 36.5 (C-2″), thiophene-C [127.2 (C-4′), 130.1 (C-3′), 132.3 (C-5′), 132.5 (C-2′)], 119.6 (C-3‴), 124.7 (C-5‴), 126.1 (C-2‴), 126.4 (C-4‴), 128.4 (C-6‴), 136.7 (C-1‴), Ph–N [124.0 (2×CH), 128.4 (CH), 129.6 (2×CH), 138.6 (C)], 151.6 (C-5), 155.0 (C-3), 166.5 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(2,5-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (10c)

Amorphous powder; yield: 76%; M.P.: 119–121 °C; IR (KBr) ν_{max}: 3020, 2920, 1696, 1587, 1530 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.10 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 4.02 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.62 (d, J = 3.5 Hz, 1H, H-3′), 6.92 (dd, J = 3.5, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 2.8 Hz, 1H, H-5′)], Ar–H 7.23 (br s, 1H, H-6‴), 7.33 (d, J = 8.6 Hz, 1H, H-4‴), 7.38 (d, J = 8.6 Hz, 1H, H-3‴), Ph–N 7.49–7.52 (m, CH, 5H), 7.93 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 17.4 (CH_3), 20.3 (CH_3), 30.6 (C-6′), 35.7 (C-2″), thiophene-C [126.4 (C-2′), 130.5 (C-3′), 131.0 (C-5′), 136.5 (C-2′)], 124.2 (C-3‴), 125.0 (C-6‴), 126.1 (C-2‴), 127.1 (C-4‴), 132.1 (C-5‴), 135.6 (C-1‴), Ph–N [126.4 (2×CH), 127.1 (CH), 129.9 (2×CH), 152.4 (C)], 153.7 (C-5), 155.0 (C-3), 167.2 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(2,6-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (10d)

Amorphous powder; yield: 70%; M.P.: 120–121 °C; IR (KBr) ν_{max}: 3031, 2934, 1697, 1581, 1522 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 4.01 (s, 2H, H-6′), 4.16 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 2.8 Hz, 1H, H-3′), 6.88 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′), Ar–H 7.10–7.20 (m, 3H, H-3‴–5‴), Ph–N 7.44–7.47 (m, CH, 5H), 7.88 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.9 (CH_3), 22.6 (CH_3), 31.4 (C-6′), 35.6 (C-2″), thiophene-C [126.4 (C-4′), 130.4 (C-3′), 132.7 (C-5′), 136.3 (C-2′)], 123.6 (C-3‴), 125.4 (C-4‴), 129.8 (C-5‴), 126.7 (C-2‴), 132.6 (C-6‴), 134.9 (C-1‴), Ph–N [124.5 (2×CH), 127.1 (CH), 129.5 (2×CH), 138.6 (C)], 150.5 (C-5), 155.1 (C-3), 170.2 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(3,4-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (10e)

Amorphous powder; yield: 71%; M.P.: 118–120 °C; IR (KBr) ν_{max}: 3032, 2931, 1698, 1580, 1522 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.91 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 4.05 (s, 2H, H-6′), 4.15 (s, 2H, H-2″), thiophene-H [6.59 (d, J = 3.2 Hz, 1H, H-3′), 6.82 (dd, J = 3.2, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 2.8 Hz, 1H, H-5′)], Ar–H 7.25 (br s, 1H, H-2‴), 7.37 (d, J = 8.6 Hz, 1H, H-6‴), 7.42 (d, J = 8.6 Hz, 1H, H-5‴), Ph–N 7.52–7.55 (m, 5H), 7.90 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.7 (CH_3), 22.6 (CH_3), 31.8 (C-6′), 35.6 (C-2″), thiophene-C [126.4 (C-2′), 131.4 (C-3′), 132.7 (C-5′), 136.3 (C-2′)], 123.6 (C-2‴), 124.9 (C-6‴), 125.4 (C-6‴), 126.7

(C-4‴), 133.6 (C-3‴), 134.9 (C-1‴), Ph–N [125.5 (2×CH), 127.1 (CH), 129.5 (2×CH), 138.6 (C)], 150.5 (C-5), 155.1 (C-3), 170.2 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(3,5-dimethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10f**)

Amorphous powder; yield: 78%; M.P.: 117–119 °C; IR (KBr) ν_{max}: 3025, 2905, 1702, 1575, 1510 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3), 2.12 (s, 3H, CH_3,), 4.02 (s, 2H, H-6′), 4.16 (s, 2H, H-2″), thiophene-H [6.55 (d, J = 2.6 Hz, 1H, H-3′), 6.80 (dd, J = 3.2, 2.6 Hz, 1H, H-4′), 7.15 (d, J = 3.2 Hz, 1H, H-5′)], Ar–H 7.23 (br s, 1H, H-2‴), 7.33 (s, 1H, H-4‴), 7.35 (s, 1H, H-6‴), Ph–N 7.11–7.15 (m, CH, 5H), 7.86 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.9 (CH_3), 22.6 (CH_3), 31.4 (C-6′), 35.6 (C-2″), thiophene-C [126.4 (C-4′), 130.4 (C-3′), 132.7 (C-5′), 136.3 (C-2′)], 123.6 (C-2‴), 124.5 (C-4‴), 124.9 (C-6‴), 126.7 (C-5‴), 132.6 (C-3‴), 134.9 (C-1‴), Ph–N [125.4 (2×CH), 127.9 (CH), 129.8 (2×CH), 138.6 (C)], 150.5 (C-5), 155.1 (C-3), 170.2 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-phenyl-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10g**)

Amorphous powder; yield: 81%; M.P.: 151–153 °C; IR (KBr) ν_{max}: 3021, 2910, 1701, 1577, 1514 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.01 (s, 2H, H-6′), 4.15 (s, 2H, H-2″), thiophene-H [6.55 (d, J = 2.6 Hz, 1H, H-3′), 6.80 (dd, J = 3.2, 2.6 Hz, 1H, H-4′), 7.15 (d, J = 3.2 Hz, 1H, H-5′)], Ar–H 7.23–7.25 (m, 5H, H-2‴–6‴), Ph–N 7.45–7.47 (m, 5H), 9.72 (s, NH, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 26.0 (C-6′), 35.7 (C-2″), thiophene-C [126.4 (C-4′), 131.1 (C-3′), 133.6 (C-5′), 136.9 (C-2′)], 123.6 (C-4‴), 125.0 (C-2‴,6‴), 130.9 (C-3‴,5‴), 134.4 (C-1‴), Ph–N [126.9 (2×CH), 127.9 (CH), 130.0 (2×CH), 136.9 (C)], 153.0 (C-5), 154.8 (C-3), 166.9 (C-1″); HR-EI-MS (*m/z*): 406.0909 $[M]^+$ calculated for $C_{21}H_{18}S_2N_4O$; 406.0922.

N-(2-methylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10h**)

Amorphous powder; yield: 81%; M.P.: 157–160 °C; IR (KBr) ν_{max}: 3025, 2929, 1695, 1597, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.93 (s, 3H, CH_3), 4.02 (s, 2H, H-6′), 4.14 (s, 2H, H-2″), thiophene-H [6.58 (d, J = 2.8 Hz, 1H, H-3′), 6.80 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.10 (d, J = 3.0 Hz, 1H, H-5′)], 7.23–7.25 (m, 4H, H-3‴–6‴), Ph–N 7.42–7.48 (m, CH, 5H), 7.69 (s, NH, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 19.9 (CH_3), 31.0 (C-6′), 34.7 (C-2″), thiophene-C [126.3 (C-4′), 130.4 (C-3′), 131.5 (C-5′), 135.2 (C-2′)], 124.3 (C-4‴), 124.3 (C-3‴), 124.9 (C-5‴), 125.8 (C-6‴), 130.4 (C-2‴), 132.3 (C-1‴), Ph–N [126.6 (2×CH), 129.8 (CH), 130.3 (2×CH), 136.5 (C)], 152.1 (C-5), 155.0 (C-3), 167.2 (C-1″); HR-EI-MS (*m/z*): 420.1060 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O$; 420.1078.

N-(3-Methylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10i**)

Amorphous powder; yield: 81%; M.P.: 143–145 °C; IR (KBr) ν_{max}: 3019, 2921, 1696, 1590, 1532 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.33 (s, 3H, CH_3), 4.01 (s, 2H, H-6′), 4.17 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 2.8 Hz, 1H, H-3′), 6.89 (dd, J = 3.2, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.2 Hz, 1H, H-5′)], Ph–N 7.20–7.29 (m, 5H), 7.45 (*s*, 1H, H-2‴), 7.49 (d, J = 8.0 Hz, 1H, H-4‴), 7.52 (dd, J = 8.5, 8.0 Hz, 1H, H-5‴), 7.54 (d, J = 8.5 Hz, 1H, H-6‴), 7.69 (s, NH, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.1 (CH_3), 31.2 (C-6′), 35.5 (C-2″), thiophene-C [126.4 (C-4′), 130.1 (C-3′), 132.5 (C-5′), 136.7 (C-2′)], 120.2 (C-4‴), 124.7 (C-5‴), 126.7 (C-3‴), 127.2 (C-2‴), 128.3 (C-6‴), 138.0 (C-1‴), Ph–N [124.8 (2×CH), 126.1 (CH), 129.3 (2×CH), 138.4 (C)], 151.6 (C-5), 155.0 (C-3), 166.5 (C-1″); HR-EI-MS (*m/z*): 420.1060 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O$; 420.1078.

N-(4-methylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10j**)

Amorphous powder; yield: 81%; M.P.: 124–126 °C; IR (KBr) ν_{max}: 3024, 2916, 1685, 1590, 1530 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.07 (s, 3H, CH_3), 3.83 (s, 2H, H-6′), 4.17 (s, 2H, H-2″), thiophene-H [6.58 (d, J = 2.8 Hz, 1H, H-3′), 6.82 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.11 (d, J = 3.0 Hz, 1H, H-5′), Ph–N 7.22–7.29 (m, 5H), 7.53 (d, J = 8.2 Hz, 2H, H-2‴, 6‴), 7.57 (d, 2H, J = 8.2 Hz, H-3‴, 5‴), 7.99 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.2 (CH_3), 30.8 (C-6′), 35.0 (C-2″), thiophene-C [126.5 (C-4′), 130.2 (C-3′), 129.6 (C-5′), 132.4 (C-2′)], 124.7 (C-3‴, 5‴), 126.5 (C-2‴, -6‴), 130.2 (C-4‴), 136.7 (C-1‴), Ph–N [125.9 (2×CH), 128.4 (CH), 129.3 (2×CH), 138.9 (C)], 151.6 (C-5), 154.9 (C-3), 168.3 (C-1′); HR-EI-MS (*m/z*): 420.1060 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O$; 420.1078.

Methyl-(2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamido) benzoate (**10k**)

Amorphous powder; yield: 81%; M.P.: 170–173 °C; IR (KBr) ν_{max}: 3025, 2928, 1696, 1597, 1531 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.57 (s, 3H, OCH_3), 3.83 (s, 2H, H-6′), 4.14 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 2.8 Hz, 1H, H-3′), 6.83 (dd, J = 3.2, 2.8 Hz, 1H, H-4′), 7.11 (d, J = 3.2 Hz, 1H, H-5′), Ar–H 7.23 (m, 4H, H-3‴-6‴), Ph–N

7.43–7.49 (m, 5H), 7.88 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 31.8 (C-6′), 35.0 (C-2″), 55.2 (OCH_3), thiophene-C [126.5 (C-4′), 130.2 (C-3′), 129.6 (C-5′), 132.4 (C-2′)], 124.7 (C-4‴), 124.7 (C-3‴), 125.9 (CH), 126.5 (C-5‴), 127.1 (C-6‴), 130.2 (C-2‴), 136.7 (C-1‴), Ph–N [128.1 (2×CH), 129.3 (CH), 129.6 (2×CH), 138.9 (C)], 151.6 (C-5), 154.9 (C-3), 168.3 (C-1′); HR-EI-MS (*m/z*): 464.0964 $[M]^+$ calculated for $C_{23}H_{20}S_2N_4O_3$; 464.0976.

N-(2-ethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10l**)

Amorphous powder; yield: 71%; M.P.: 129–130 °C; IR (KBr) ν_{max}: 3033, 2935, 1697, 1582, 1522 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2), 2.63 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$), 4.01 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.50 (d, J = 3.0 Hz, 1H, H-3′), 6.87 (dd, J = 3.2, 3.0 Hz, 1H, H-4′), 7.10 (d, J = 3.2 Hz, 1H, H-5′)], Ar–H 7.26 (m, 4H, H-3‴–6‴), Ph–N 7.52–7.59 (m, 5H), 7.79 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.5 (CH_3), 24.0 (CH_2), 31.2 (C-6′), 35.4 (C-2″), thiophene-C [126.3 (C-4′), 130.2 (C-3′), 132.4 (C-5′), 134.5 (C-2′)], 124.7 (C-4‴), 125.6 (C-3‴), 126.2 (C-5‴), 126.5 (C-6‴), 130.5 (C-2‴), 136.7 (C-1‴), Ph–N [125.9 (2×CH), 127.1 (CH), 129.7 (2×CH), 138.4 (C)], 151.1 (C-5), 151.9 (C-3), 167.7 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(4-ethylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10m**)

Amorphous powder; yield: 68%; M.P.: 121–125 °C; IR (KBr) ν_{max}: 3041, 2915, 1701, 1601, 1530 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2), 2.43 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$), 4.01 (s, 2H, H-6′), 4.10 (s, 2H, H-2″), thiophene-H [6.53 (d, J = 2.6 Hz, 1H, H-3′), 6.82 (dd, J = 3.2, 2.6 Hz, 1H, H-4′), 7.17 (d, J = 3.2 Hz, 1H, H-5′)], Ph–N 7.23–7.29 (m, 5H), Ar–H 7.57 (d, J = 8.2 Hz, 2H, H-2‴,6‴), 7.59 (d, J = 8.2 Hz, 2H, H-3‴, 5‴), 7.90 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.5 (CH_3), 24.0 (CH_2), 31.2 (C-6′), 35.4 (C-2″), thiophene-C [126.3 (C-4′), 130.2 (C-3′), 132.4 (C-5′), 134.5 (C-2′)], 124.7 (C-3‴, 5‴), 126.2 (C-2‴, 6‴), 128.5 (C-4‴), 136.7 (C-1‴), Ph–N [125.6 (2×CH), 127.1 (CH), 129.7 (2×CH), 138.4 (C)], 151.1 (C-5), 151.9 (C-3), 167.7 (C-1″); HR-EI-MS (*m/z*): 434.1224 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O$; 434.1235.

N-(4-ethoxyphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10n**)

Amorphous powder; yield: 68%; M.P.: 129–131 °C; IR (KBr) ν_{max}: 3019, 2923, 1696, 1597, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.38 (t, J = 6.5 Hz, 3H, $\underline{CH_3}$–CH_2–O), 3.92 (q, J = 6.5 Hz, 2H, CH_3–$\underline{CH_2}$–O), 3.97 (s, 2H, H-6′), 4.11 (s, 2H, H-2″), thiophene-H [6.58 (d, J = 2.8 Hz, 1H, H-3′), 6.82 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.11 (d, J = 3.0 Hz, 1H, H-5′), Ph–N 7.22–7.29 (m, 5H), Ar–H 7.53 (d, J = 8.0 Hz, 2H, H-2‴, 6‴), 7.57 (d, J = 8.0 Hz, 2H, H-3‴, 5‴), 7.99 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.4 (CH_3), 30.7 (C-6′), 34.3 (C-2″), 63.6 (CH_2), thiophene-C [126.4 (C-4′), 130.9 (C-3′), 132.0 (C-5′), 136.4 (C-2′)], 114.6 (C-3‴, 5‴), 126.8 (C-2‴, 6‴), 128.5 (C-4‴), 130.5 (C-1‴), Ph–N [125.0 (2×CH), 129.4 (CH), 130.4 (2×CH), 152.3 (C)], 154.8 (C-5), 155.7 (C-3), 166.1 (C-1″); HR-EI-MS (*m/z*): 450.1171 $[M]^+$ calculated for $C_{23}H_{22}S_2N_4O_2$; 450.1184.

N-(2-ethyl-6-methylphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10o**)

Amorphous powder; yield: 68%; M.P.: 121–123 °C; IR (KBr) ν_{max}: 3020, 2925, 1694, 1591, 1535 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.16 (t, J = 6.4 Hz, 3H, $\underline{CH_3}$–CH_2), 2.17 (s, 3H, CH_3), 2.63 (q, J = 6.4 Hz, 2H, CH_3–$\underline{CH_2}$), 4.01 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 2.8 Hz, 1H, H-3′), 6.88 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H 7.10–7.20 (m, 3H, H-3‴–5‴), Ph–N 7.44–7.49 (m, 5H), 7.88 (s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 13.5 (CH_3), 17.6 (CH_3), 24.0 (CH_2), 31.2 (C-6′), 36.3 (C-2″), thiophene-C [126.4 (C-4′), 129.7 (C-3′), 130.2 (C-5′), 136.7 (C-2′)], 124.7 (C-3‴), 125.6 (C-4‴), 125.9 (C-5‴), 130.7 (C-6‴), 131.5 (C-2‴), 134.5 (C-1‴), Ph–N [126.2 (2×CH), 127.1 (CH), 130.5 (2×CH), 138.4 (C)], 151.9 (C-5), 155.1 (C-3), 167.7 (C-1″); HR-EI-MS (*m/z*): 436.1018 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O_2$; 436.1027.

N-(2-methoxyphenyl)-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10p**)

Amorphous powder; yield: 63%; M.P.: 140–142 °C; IR (KBr) ν_{max}: 3016, 2929, 1691, 1590, 1533 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.65 (s, 3H, OCH_3), 4.00 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), thiophene-H [6.52 (d, J = 2.8 Hz, 1H, H-3′), 6.88 (dd, J = 3.0, 2.8 Hz, 1H, H-4′), 7.18 (d, J = 3.0 Hz, 1H, H-5′)], Ar–H 7.12–7.18 (m, 4H, H-3‴–6‴), Ph–N 7.44–7.47 (m, 5H), 7.49 (*s*, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 31.6 (C-6′), 36.4 (C-2″), 58.6 (OCH_3), thiophene-C [126.6 (C-4′), 129.8 (C-3′), 132.3 (C-5′), 136.6 (C-2′)], 116.5 (C-3‴), 122.5 (C-4‴), 126.7 (C-5‴), 127.8 (C-6‴), 128.9 (C-2‴), 136.4 (C-1‴), Ph–N [125.2 (2×CH), 127.2 (CH), 129.1 (2×CH), 152.4 (C)], 154.7 (C-5), 155.8 (C-3), 166.6 (C-1″); HR-EI-MS (*m/z*): 436.1018 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O_2$; 436.1027.

N-benzyl-2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide (**10q**)

Amorphous powder; yield: 61%; M.P.: 133–134 °C; IR (KBr) ν_{max}: 3035, 2910, 1690, 1584, 1527 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.02 (s, 2H, H-6′), 4.12 (s, 2H, H-2″), 4.37 (s, 2H, CH_2), thiophene-H [6.54 (d, J = 3.0 Hz, 1H, H-3′), 6.80 (dd, J = 3.2, 3.0 Hz, 1H, H-4′), 7.10 (d, J = 3.2 Hz, 1H, H-5′), Ar–H 7.34–7.39 (m, 5H, H-2‴–6‴), Ph–N 7.45–7.49 (m, 5H), 7.89 9 s, 1H, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.2 (CH_2), 31.2 (C-6′), 33.1 (C-2″), thiophene-C [124.3 (C-4′), 125.3 (C-3′), 127.2 (C-5′), 136.0 (C-2′)], 116.5 (C-2‴, 6‴), 126.9 (C-4‴), 128.2 (C-3‴, 5‴), 137.3 (C-1‴), Ph–N [125.0 (2 × CH), 127.8 (CH), 129.0 (2 × CH), 129.0 (CH), 139.0 (C)], 150.2 (C-5), 154.5 (C-3), 168.3 (C-1″); HR-EI-MS (*m/z*): 420.1068 $[M]^+$ calculated for $C_{22}H_{20}S_2N_4O$; 420.1078.

Enzyme inhibition assays

Cholinesterase assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assays were performed using Ellman method [20] with slight modifications. Reaction mixture of 100 µl containing 60 µl 50 mM Na_2HPO_4 buffer, pH 7.7 and 10 µl (0.5 mM) test compound and 10 µl enzyme (0.005 unit AChE and 0.0005 units BChE) was added per well. The content was mixed and preread at 405 nm. After preincubation at 37 °C for 10 min, the reaction was initiated by the addition of 10 µl of 0.5 mM substrate (acetylthiocholine iodide or butyrylcholine bromide) per well, followed by the addition of 10 µl DTNB (0.5 mM per well). Incubation was continued for further 30 min. Absorbance was measured at 405 nm on Synergy HTX (BioTek, USA) 96-well plate reader. Eserine was used as a positive control. The percent inhibition was calculated as below. IC_{50} values of active compounds were determined by assaying the enzyme with suitable dilutions of the active compounds and computing the data with EZ-Fit software (Perrella Int. USA).

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Urease assay

Phenyl hypochlorite method was used to determine the antiurease activities of the compounds (**9a–q** and **10a–q**) with slight modifications [21]. The reaction mixture contained 60 µl 200 mM phosphate buffer pH 8.0, 20 µl jack bean urease enzyme and 5 µl test compound. Contents were pre-incubated at 25 °C for 10 min, and then 15 µl of 20 mM urea was added and incubation continued at 25 °C for further 10 min. It was followed by the addition of 100 µl freshly prepared alcohol–phenol reagent. The contents were incubated for further 15 min, and absorbance was recorded at 630 nm. The percentage inhibition and IC_{50} values of active compounds were determined as mentioned above for AChE, BChE.

α-Glucosidase assay

The yeast α-glucosidase inhibition assay was performed according to the reported method [22] with some modifications. Total volume of the reaction mixture of 100 µl contained 70 µl 50 mM phosphate buffer, pH 6.8, 10 µl of 0.5 mM test compound and 10 µl (0.057 units) enzyme per well. The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 405 nm. The reaction was initiated by the addition of 10 µl of 0.5 mM substrate *p*-nitrophenylglucopyranoside. After 30 min of incubation, absorbance was measured. All experiments were carried out in triplicate. Acarbose was used as a positive control. The percentage inhibition and IC_{50} values of active compounds were determined as mentioned above for AChE, BChE.

Results and discussion

Chemistry

Ethyl 2-thiopheneacetate (**1**) was initially synthesized by refluxing thiophen-2-acetic acid with ethanol for 3–4 h. The oily product was then extracted with $CHCl_3$ from the reaction mixture at basic pH (8–9 with Na_2CO_3). The next step was the synthesis of 2-(thiophen-2-yl)acetohydrazide (**2**) by stirring **1** with hydrazine hydrate for 4–5 h at room temperature. The resulting light yellow crystalline product was filtered and washed with *n*-hexane. The ethanolic solution of hydrazide (**2**) was refluxed (3–4 h) with ethyl isothiocyanate and phenylisothiocyanate separately to get 4-ethyl-1-{2-(thiophen-2-yl)acetyl}thiosemicarbazide (**3**) and 4-phenyl-1-{2-(thiophen-2-yl)acetyl}thiosemicarbazide (**4**), respectively, on cooling. Intramolecular cyclization was achieved by refluxing **3** and **4** under alkaline (10% NaOH) conditions yielded 4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol (**5**) and 4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol (**6**), respectively, which were precipitated by pouring the reaction mixtures into ice cold water and acidified to pH 4–6 with concentrated HCl [23, 24]. The *N*-aryl/aralkyl-2-bromoacetamides (**8a–q**), the other precursors of target compounds **9a–q** and **10a–q**, were synthesized by stirring aryl/alkyl amines (**7a–q**) with 2-bromoacetyl bromide in a basic medium (pH 9–10). Final step was the coupling of compounds **5** and **6** separately with the precursors **8a–q** to get target compounds **9a–q** and **10a–q**. This task was

accomplished by stirring the solutions of precursors **5** and **6** in *N,N*-dimethyl formamide (DMF, 10 mL) and adding sodium hydride (0.002 g, 0.1 mmol). After 30 min stirring, the reaction mixture was followed by the slow addition of the electrophiles, **8a–q**, respectively, and was further stirred at room temperature for 2–3 h. The reaction mixtures were then supplemented with distilled water, and the end products **9a–q** and **10a–q** were separated by filtration or solvent extraction according to the nature of the products. The structures of all the synthesized compounds (**1–6**) and *N*-substituted derivatives (**9a–q**; **10a–q**) were confirmed by the analysis of spectral data as presented in the experimental section.

The goal of the present study was: primarily, to synthesize new thiophene-1,2,4-triazoles (**9a–q**; **10a–q**) containing a variety of substituents and secondarily, to investigate the synthesized compounds for their enzyme inhibitory potential against some enzymes of therapeutical importance like AChE, BChE, urease, and α-glucosidase. Syntheses of the intermediates and target compounds were carried out according to the protocol as shown in Fig. 1.

The starting compound ethyl 2-thiopheneacetate (**1**) was synthesized according to the literature [25], and its structure was confirmed by ^{1}H- and ^{13}C-NMR spectra which exhibited additional peaks for $–COOCH_2CH_3$ compared to the NMR data of the starting material. The treatment of **1** with hydrazine hydrate ($NH_2–NH_2·H_2O$) resulted in the formation of hydrazide derivative **2** in good yield (81%), which was identified as a key intermediate for the synthesis of target compounds. The ^{1}H-NMR spectrum of **2** did not show any signals corresponding to ethoxy ($–OCH_2CH_3$) group; instead, new singlets appeared at δ 4.02 (1H, –NH) and 8.90 (2H, $–NH_2$) attributed to $–NHNH_2$ moiety. The ^{1}H NMR spectrum of compound **3** displayed signals for N-ethyl group at δ 1.15 (*t*, 3H, $N–CH_2–\underline{CH_3}$, $J = 6.4$ Hz) and 3.53 (*q*, 2H, $N–\underline{CH_2}–CH_3$, $J = 6.4$ Hz), while the spectrum of **4** substantiated the presence of phenyl moiety due to a multiplet at δ 7.10–7.15 (*m*, 5H). The C=S carbon nucleus resonated at δ 180.6 in the ^{13}C-NMR spectrum of both the compounds **3** and **4**. The resonance of thiol proton at δ 13.6 in the ^{1}H-NMR spectra of the compounds **5** and **6**, and the absence of –NH and carbonyl carbon signals in their NMR spectra

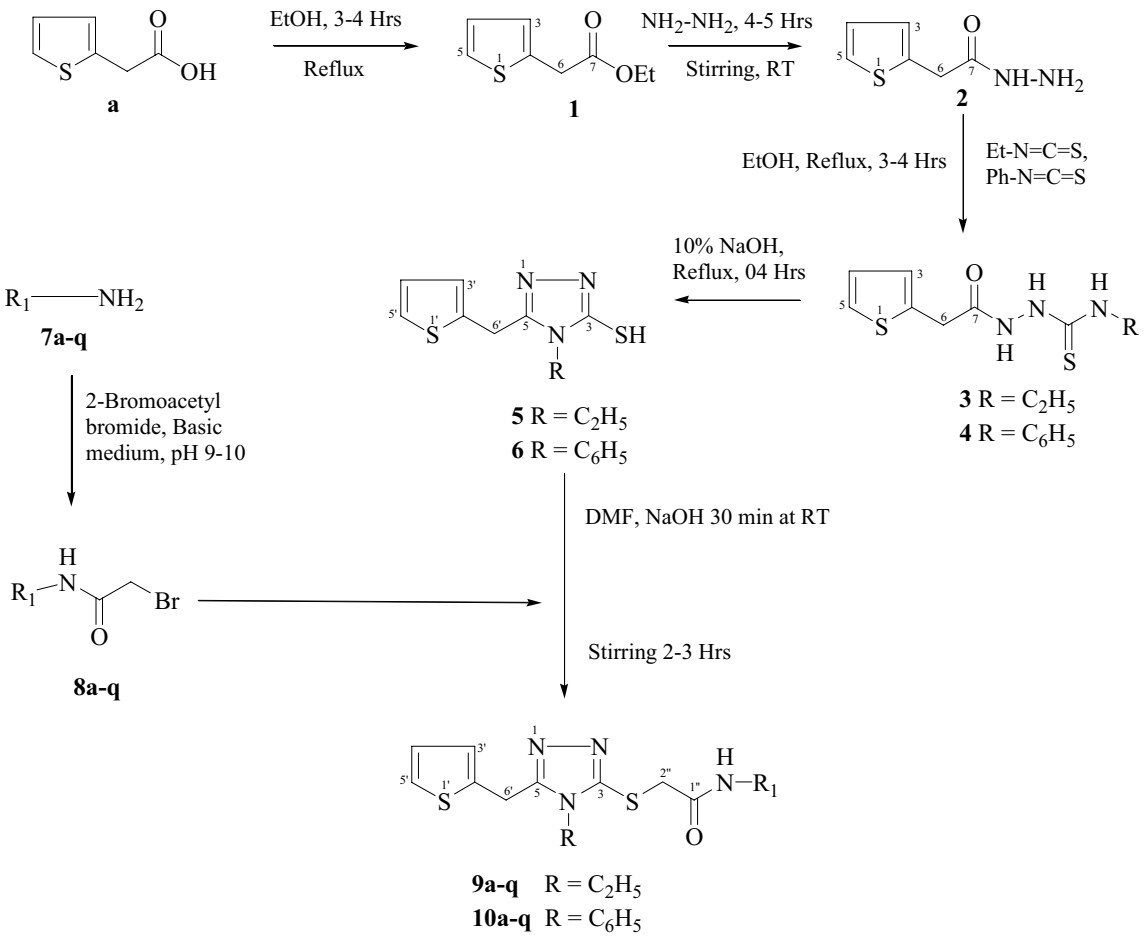
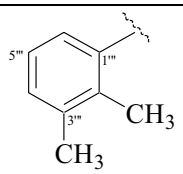
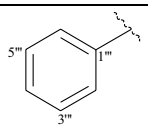
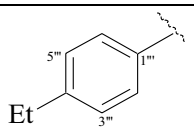
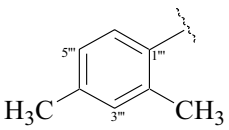
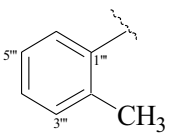
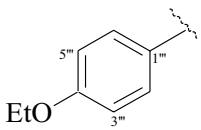
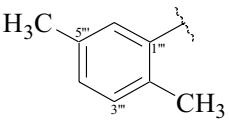
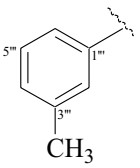
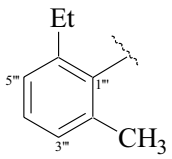
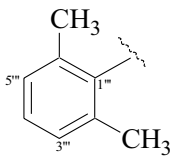
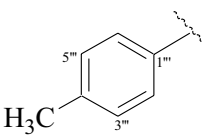
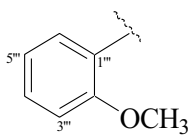
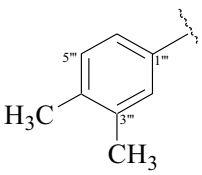
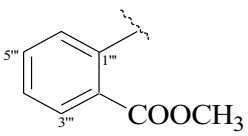
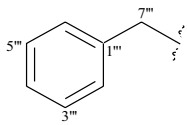
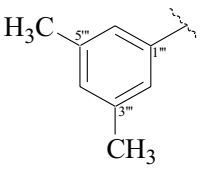
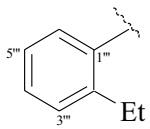


Fig. 1 Protocol for the synthesis of triazole amides from thiophene-2-acetic acid

substantiated the conversion of **3** and **4** into 1,2,4-triazol-3-thiols in compounds **5** and **6**. The syntheses of different aryl/aralkyl substituted bromoacetamides (**8a**–**q**) were carried out by the reaction of different aryl/aralkyl amines (**7a**–**q**; Table 1) with bromoacetyl bromide. Owing to aromatic ring in aryl/aralkyl amines, the ^{1}H-, and ^{13}C-NMR spectra displayed additional signals for these groups. Synthones **5** and **6** were coupled with aryl/aralkyl amines (**8a**–**q**) to get target molecules **9a**–**q** and **10a**–**q** which were characterized and confirmed by spectroscopic analyses, and the data are presented in the experimental section. Compound **9a** was synthesized as a dull white amorphous solid (yield of 71%), which melted at 135–137 °C. The molecular formula $C_{19}H_{22}S_2N_4O$ was determined through HR-EI-MS due to molecular ion $[M]^+$ peak at *m/z* 386.1226. The IR spectrum displayed absorption bands at 3020, 1696, 1597, 1535 cm^{-1}, attested for C-H (aromatic), C=O, C=N, and C=C functionalities. In the ^{1}H NMR spectrum, signals at δ 1.15 (*t*, 3H, $\underline{CH_3}$–CH_2–N, $J = 6.5$ Hz) and 3.83 (*q*, 2H, CH_3–$\underline{CH_2}$–N, $J = 6.5$ Hz) were assigned to *N*-ethyl group in **9a**. Two singlet methylene protons resonating at δ 4.01 and 4.17 were attributed to thiophene-CH_2, and -S-CH_2, respectively. In addition to the aromatic proton resonances at δ 7.10–7.20 (*m*, 3H, CH), the spectrum displayed two singlet methyl signals at δ 2.13 (*s*, 3H, CH_3) and 2.28 (*s*, 3H, CH_3) that confirmed the presence of 2,3-dimethyl aniline residue in **9a**. The resonance of nineteen carbons in the ^{13}C-NMR spectrum of **9a** was in agreement with the molecular formula. The multiplicity of these signals was established as three methyl, three methylene, six methine and seven quaternary carbons through DEPT experiment. Similarly, other compounds (**9b**–**q**; **10a**–**q**) were also characterized by IR,

Table 1 Aryl/aralkyl substituted amines

Compd	R	Compd	R	Compd	R
a		**g**		**m**	
b		**h**		**n**	
c		**i**		**o**	
d		**j**		**p**	
e		**k**		**q**	
f		**l**		–	–

^{1}H-NMR, ^{13}C-NMR, and mass spectral data as described in experimental section.

Biology (biological studies)

Acetylcholinesterase activity

Compounds **9a–q**; **10a–q** were evaluated for their inhibitory activities against AChE (Table 2). Results showed that the compounds **9i**, **9n**, **10a, 10d, 10f**, **10j** and **10m** were found to be moderate inhibitors of AChE activity with IC_{50} values of 73.54 ± 0.16, 69.26 ± 0.12, 165.26 ± 0.17, 45.72 ± 0.11, 32.26 ± 0.12, 117.43 ± 0.12, 72.53 ± 0.13 µM, respectively. The positive control, serine exhibited IC_{50} 0.19 ± 0.05 µM in this assay. The activity of these compounds could be attributed to the presence of dimethylphenyl group attached to the nitrogen atom at *meta* position of acetamide moiety. The phenyl group attached to nitrogen atom at position 4 of 1,2,4-triazole nucleus also enhanced the *ortho* and *para* alkyl group attached to the nitrogen of acetamide moiety. Overall activity of the synthesized compounds was moderately good.

Butyrylcholinesterase activity

The BChE activity of compounds **9a–q**; **10a–q** showed that the compounds **9b**, **9d**, **9h**, **9l**, **10a**, **10d**, **10e**, **10h**, **10j** and **10o** were significantly promising inhibitors with IC_{50} values 21.72 ± 0.18, 12.52 ± 0.19, 19.75 ± 0.22, 12.52 ± 0.19, 27.24 ± 0.19, 23.62 ± 0.22, 27.43 ± 0.16, 24.52 ± 0.21, 28.35 ± 0.18 and 27.24 ± 0.19 µM, respectively, with respect to the reference standard eserine (Table 3). These compounds exhibited activity due to the presence of dimethylphenyl group attached to the nitrogen atom at *ortho* and *para* position of acetamide moiety in these molecules. The ethyl group attached to nitrogen atom at position 4 of 1,2,4-triazole nucleus also enhanced the *ortho* and *para* alkyl group attached to the nitrogen of acetamide moiety. Overall activity of the evaluated compounds was found significant.

Urease activity

Compounds **9a–q**; **10a–q** were also evaluated for their antiurease potential (Table 4). Compounds **9e**, **10i** and **10n** were found to be potent inhibitors with IC_{50} values 8.79 ± 0.057, 7.27 ± 0.05, 7.35 ± 0.04 µM, respectively; thiourea as standard drug displayed IC_{50} value of 21.25 ± 0.15. The SAR study revealed that the activity of these compounds could be attributed to *ortho*, *meta* methyl substitution at the phenyl group attached to the nitrogen atom of acetamide moiety.

α-Glucosidase activity

The compounds **9a–q**; **10a–q** were also evaluated for their inhibitory studies against *α*-glucosidase, and the results are shown in Table 5. Compounds **9o** and **10i** were found to be good inhibitors with IC_{50} values of 62.94 ± 0.19, and

Table 2 AChE inhibitory profiles of compounds **9a–q**; **10a–q**

Comp	AChE		Comp	AChE	
	Inhibition (%) at 0.5 mM	IC_{50} (µM)		Inhibition (%) at 0.5 mM	IC_{50} (µM)
9a	45.32 ± 0.15	–	**10a**	85.29 ± 0.21	165.26 ± 0.17
9b	21.49 ± 0.12	–	**10b**	31.24 ± 0.13	–
9c	42.15 ± 0.16	–	**10c**	18.46 ± 0.12	–
9d	45.56 ± 0.17	–	**10d**	89.52 ± 0.18	45.72 ± 0.11
9e	76.92 ± 0.19	113.62 ± 0.15	**10e**	24.51 ± 0.14	–
9f	32.59 ± 0.12	–	**10f**	87.64 ± 0.19	32.26 ± 0.12
9g	37.54 ± 0.12	–	**10g**	25.75 ± 0.15	–
9h	37.36 ± 0.14	–	**10h**	32.38 ± 0.17	–
9i	81.53 ± 0.19	73.54 ± 0.16	**10i**	43.51 ± 0.14	–
9j	74.12 ± 0.17	157.32 ± 0.13	**10j**	86.64 ± 0.19	117.43 ± 0.12
9k	72.35 ± 0.23	216.72 ± 0.18	**10k**	53.21 ± 0.16	452.42 ± 0.15
9l	71.24 ± 0.24	283.53 ± 0.19	**10l**	43.58 ± 0.12	–
9m	74.53 ± 0.19	145.84 ± 0.15	**10m**	87.31 ± 0.18	72.53 ± 0.13
9n	82.24 ± 0.17	69.26 ± 0.12	**10n**	32.48 ± 0.12	–
9o	27.62 ± 0.14	–	**10o**	38.36 ± 0.14	–
9p	74.58 ± 0.21	165.42 ± 0.18	**10p**	44.15 ± 0.14	–
9q	48.75 ± 0.15	–	**10q**	25.3 ± 0.15	–
Eserine	91.46 ± 1.25	0.19 ± 0.05	**Eserine**	91.46 ± 1.25	0.19 ± 0.05

69.46 ± 0.15 µM, respectively. Other compounds showed moderate to poor activities against the said enzyme.

Molecular docking studies

AChE docking studies

The crystal structure of AChE (PDB id: 4M0E) was downloaded from the PDB. Docking studies were carried out using BioSolveIT's LeadIT software. Discovery Studio Visualizer [26] was used to visualize protein–ligand interactions. The thiophenyl ring of compound **10f** was making a π-anion interaction with Glu292 (Fig. 2). One of the methyl groups was making π-alkyl interaction with Phe297 and Phe338, while the other methyl group was making π-alkyl interaction withTyr341 and Tyr337. The phenyl ring was making a π–π T-shaped interaction with Tyr124. The N-substituted phenyl ring was also making a π–π T-shaped interaction with Trp286. One of the triazole ring nitrogen atoms was making a hydrogen bond with Ser293. The carbonyl oxygen was making a hydrogen bond with Phe295.

Similar interactions were observed for compound **10d** (Fig. 3) with AChE, π-anion interaction was observed between the thiophenyl ring and Glu292, while π-alkyl interactions were observed between one of the methyl groups and amino acids Phe338 and Tyr341, the other methyl group was also making π-alkyl interactions with Tyr341, Tyr72 and Trp286. The phenyl ring was making π–π stacked interaction with Tyr341. Both triazole and *N*-phenyl group were making π–π stacked interaction with Trp286. One of the triazole ring nitrogen atoms was making a hydrogen bond with Ser293.

BChE docking studies

For docking against BChE, crystal structure of human BChE enzyme (PDB id: 4BDS, 2.1 Å) was downloaded from the PDB. To validate the docking protocol, the ligand that had co-crystallized with the enzyme was extracted and re-docked into the same enzyme. The docking protocol was able to reproduce the experimentally observed bound conformation with rmsd of less than 2. The three most active BChE inhibitors **9d**, **9h** and **9l** were docked against BChE. For compound **9d**, the NH of amide group was found to act as a hydrogen bond donor toward Asp70. One of the ring nitrogen atoms was acting as a hydrogen bond acceptor toward Gln71 and Ser72. Several other non-covalent interactions were also observed. Both methyl groups on the phenyl ring were found to make π-alky interactions with Phe329 and Trp82. One of the methyl group was additionally making a π-alkyl interaction with Tyr332. The triazole ring was making a π-alkyl interaction with Asp70. The phenyl ring was making a π–π stacked interaction with Tyr332 (Fig. 4).

For compound **9l** similar interactions as in **9d** were found as can be seen from Fig. 5. One of the nitrogen atoms of the triazole ring was making a hydrogen bond with Ser72, whereas the triazole ring itself was making a π-anion interaction with Asp70. The NH group of amide group was making

Table 3 BChE inhibitory activities of compounds **9a–q**; **10a–q**

Comp	BChE		Comp	BChE	
	Inhibition (%) at 0.5 mM	IC_{50} (µM)		Inhibition (%) at 0.5 mM	IC_{50} (µM)
9a	65.26 ± 0.29	165.43 ± 0.25	**10a**	86.47 ± 0.25	27.24 ± 0.19
9b	83.42 ± 0.25	21.72 ± 0.18	**10b**	29.58 ± 0.32	–
9c	65.87 ± 0.28	166.43 ± 0.23	**10c**	71.35 ± 0.27	48.53 ± 0.23
9d	85.23 ± 0.29	12.52 ± 0.19	**10d**	73.43 ± 0.29	23.62 ± 0.22
9e	67.84 ± 0.27	154.82 ± 0.23	**10e**	87.28 ± 0.23	27.43 ± 0.16
9f	16.52 ± 0.24	–	**10f**	84.25 ± 0.28	84.01 ± 0.23
9g	79.23 ± 0.26	32.24 ± 0.21	**10g**	79.42 ± 0.36	58.52 ± 0.28
9h	87.74 ± 0.28	19.75 ± 0.22	**10h**	73.64 ± 0.29	24.52 ± 0.21
9i	71.25 ± 0.29	112.82 ± 0.23	**10i**	78.32 ± 0.27	54.36 ± 0.22
9j	43.76 ± 0.24	–	**10j**	75.36 ± 0.26	28.35 ± 0.18
9k	62.58 ± 0.26	212.96 ± 0.21	**10k**	79.45 ± 0.37	61.45 ± 0.24
9l	87.63 ± 0.28	12.52 ± 0.19	**10l**	26.32 ± 0.23	–
9m	42.52 ± 0.35	–	**10m**	76.27 ± 0.26	55.63 ± 0.19
9n	35.34 ± 0.27	–	**10n**	78.79 ± 0.28	68.96 ± 0.21
9o	56.23 ± 0.28	326.92 ± 0.24	**10o**	86.47 ± 0.25	27.24 ± 0.19
9p	61.76 ± 0.29	275.24 ± 0.25	**10p**	29.58 ± 0.32	–
9q	82.27 ± 0.26	37.56 ± 0.21	**10q**	71.35 ± 0.27	48.53 ± 0.23
Eserine	83.75 ± 1.16	0.62 ± 0.08	**Eserine**	83.75 ± 1.16	0.62 ± 0.08

Table 4 Urease inhibitory activity of compounds **9a–q**; **10a–q**

Comp	Urease		Comp	Urease	
	Inhibition (%) at 0.25 mM	IC_{50} (μM)		Inhibition (%) at 0.25 mM	IC_{50} (μM)
9a	15.3 ± 0.14	–	**10a**	26.48 ± 0.13	–
9b	11.6 ± 0.13	–	**10b**	9.75 ± 0.12	–
9c	15.2 ± 0.15	–	**10c**	28.56 ± 0.15	–
9d	8.4 ± 0.15	–	**10d**	12.27 ± 0.11	–
9e	98.45 ± 0.12	8.79 ± 0.05	**10e**	19.36 ± 0.14	–
9f	14.2 ± 0.13		**10f**	21.25 ± 0.12	–
9g	7.4 ± 0.12	–	**10g**	6.43 ± 0.13	–
9h	9.3 ± 0.14	–	**10h**	21.25 ± 0.12	–
9i	12.6 ± 0.15	–	**10i**	98.56 ± 0.11	7.27 ± 0.05
9j	38.5 ± 0.17	–	**10j**	16.57 ± 0.13	–
9k	24.7 ± 0.16	–	**10k**	18.36 ± 0.14	–
9l	6.3 ± 0.12	–	**10l**	21.41 ± 0.12	–
9m	7.5 ± 0.11	–	**10m**	13.62 ± 0.15	–
9n	31.2 ± 0.14	–	**10n**	98.75 ± 0.12	7.35 ± 0.04
9o	34.8 ± 0.15	–	**10o**	27.41 ± 0.12	–
9p	15.7 ± 0.13	–	**10p**	31.41 ± 0.12	–
9q	11.5 ± 0.16	–	**10q**	12.5 ± 0.19	–
Thiourea	98.21 ± 0.18	21.25 ± 0.15	**Thiourea**	98.21 ± 0.18	21.25 ± 0.15

Table 5 α-Glucosidase inhibitory activities of compounds **9a–q**; **10a–q**

Comp	**α-Glucosidase**		**Comp**	**α-Glucosidase**	
	Inhibition (%) at 0.5 mM	IC_{50} (μM)		Inhibition (%) at 0.5 mM	IC_{50} (μM)
9a	71.46 ± 0.28	274.52 ± 0.19	**10a**	46.53 ± 0.27	–
9b	65.75 ± 0.29	392.75 ± 0.21	**10b**	35.24 ± 0.28	–
9c	37.14 ± 0.19	–	**10c**	68.46 ± 0.23	319.28 ± 0.16
9d	64.39 ± 0.32	328.26 ± 0.22	**10d**	78.34 ± 0.26	174.57 ± 0.19
9e	27.82 ± 0.25	–	**10e**	18.32 ± 0.25	–
9f	47.65 ± 0.27	–	**10f**	46.45 ± 0.28	–
9g	43.59 ± 0.21	–	**10g**	43.36 ± 0.23	–
9h	65.78 ± 0.28	374.25 ± 0.21	**10h**	32.54 ± 0.27	–
9i	43.65 ± 0.16	–	**10i**	97.68 ± 0.27	69.46 ± 0.15
9j	42.37 ± 0.19	–	**10j**	28.26 ± 0.23	–
9k	76.52 ± 0.34	172.18 ± 0.23	**10k**	12.39 ± 0.21	–
9l	72.34 ± 0.29	289.52 ± 0.24	**10l**	22.54 ± 0.25	–
9m	63.25 ± 0.36	365.46 ± 0.27	**10m**	63.35 ± 0.26	385.14 ± 0.18
9n	48.76 ± 0.24	–	**10n**	78.52 ± 0.27	195.16 ± 0.19
9o	89.43 ± 0.28	62.94 ± 0.19	**10o**	17.5 ± 0.21	–
9p	65.27 ± 0.36	325.83 ± 0.23	**10p**	31.5 ± 0.27	–
9q	47.5 ± 0.31	–	**10q**	17.5 ± 0.11	–
Acarbose	65.73 ± 1.93	375.82 ± 1.76	**Acarbose**	65.73 ± 1.93	375.82 ± 1.76

a hydrogen bond with Asp70. The ethyl group substituted on the phenyl ring was making a π-alky interaction with TRp82, whereas the alkyl part of Ala328 was also making a π-alkyl interaction with the phenyl ring. The phenyl ring was also making π–π stacked interactions with Trp82.

For compound **9h**, the thiophenyl ring was found to be making π-anion and π-sigma interaction with Asp70; π–π stacked interactions were observed between the triazole ring and Trp82. A π-alkyl interaction was observed between *N*-ethyl side chain and Trp82. The carbonyl oxygen was

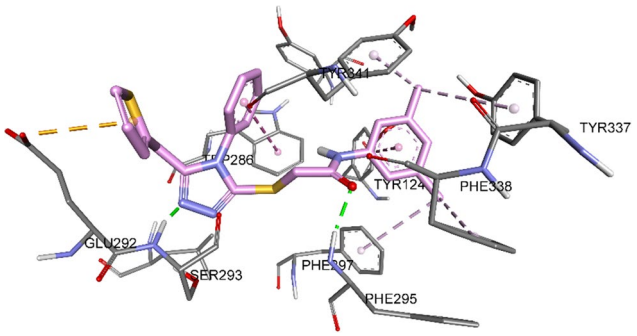


Fig. 2 Docked conformation of **10f** showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π stacked (purple) interactions with AChE

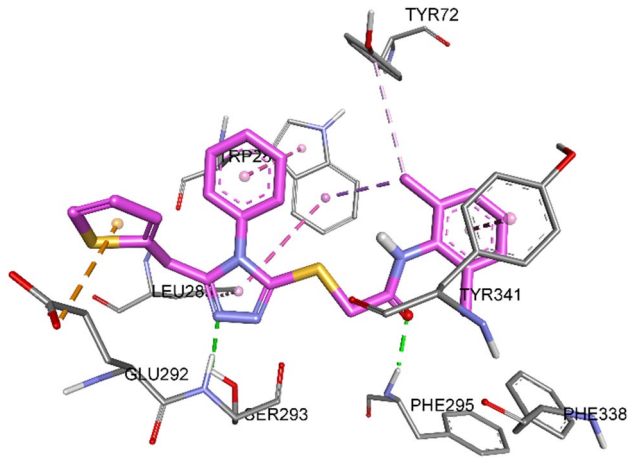


Fig. 3 Docked conformation of **10d** with AChE showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π stacked (purple) interactions

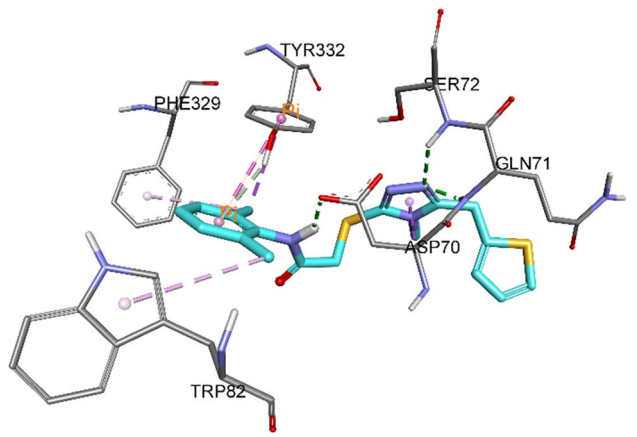


Fig. 4 Docked conformation of **9d** with BChE showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π stacked (purple) interactions

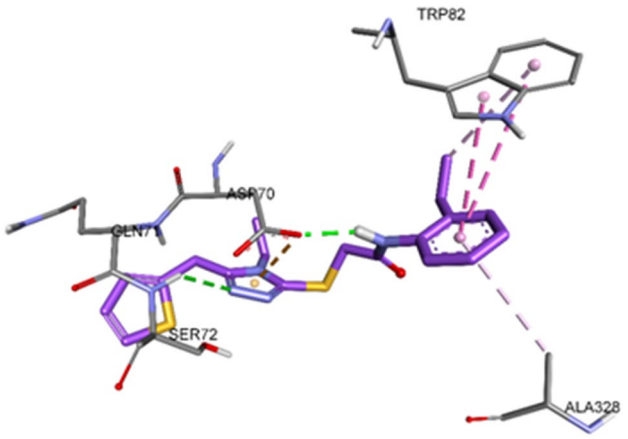


Fig. 5 Docked conformation of **9 l** with BChE showing hydrogen bonds (green), π-alkyl (light purple), and π–π stacked (purple) interactions

within hydrogen bonding distance of amino acids Gly116, Gly117 and Ala199. The phenyl ring was found to be making π–π T-shaped interactions with Phe329 and Trp231 (Fig. 6).

Urease docking studies

The crystal structure of urease (PDB id: 4GY7) was downloaded from the PDB. For compound **10i,** hydrogen bond was observed between the NH of amide group and the amino acid Ala436. The amino acid Arg439 was found to be involved in making hydrogen bonds with the ring nitrogen atoms of the triazole ring and the ring sulfur of thiophene group. Several non-bonded interactions were also observed. The phenyl ring was found to be making a π-anion interaction with Asp494 and π–π T-shaped interaction with aromatic rings of Trp495 (Fig. 7).

For compound **10n** the ethoxide side chain was found to be making a π-alkyl interaction with Ile411. The phenyl and triazole rings were making π-alkyl interactions with Ala440. The triazole ring was additionally making a π-anion interaction with Asp494, and a π–π T-shaped interaction with His593. The NH group of amide group was making a hydrogen bond with carboxymethylated amino acid Cme592 (Fig. 8).

α-Glucosidase docking studies

Since crystal structure of α-glucosidase from *Saccharomyces cerevisiae* is not yet available from the PDB, its homology model was constructed and validated using crystal structure of oligo-1,6-glucosidase as a template, according to our previously reported procedure [27–29]. The most active inhibitors **9o** and **10i** were docked using LeadIT software. Similar binding orientations were found for both compounds. For

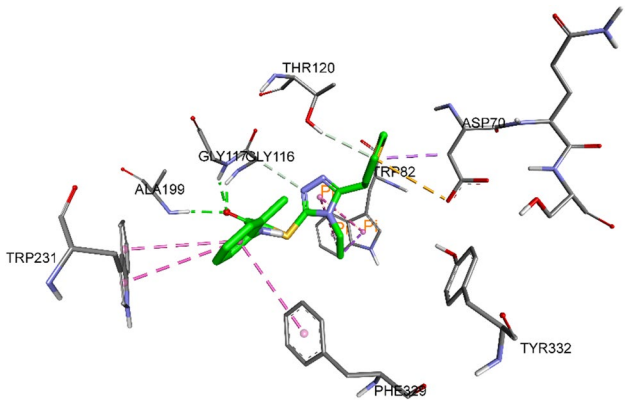


Fig. 6 Docked conformation of **9 h** with BChE showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π T-shaped and π–π stacked (purple) interactions

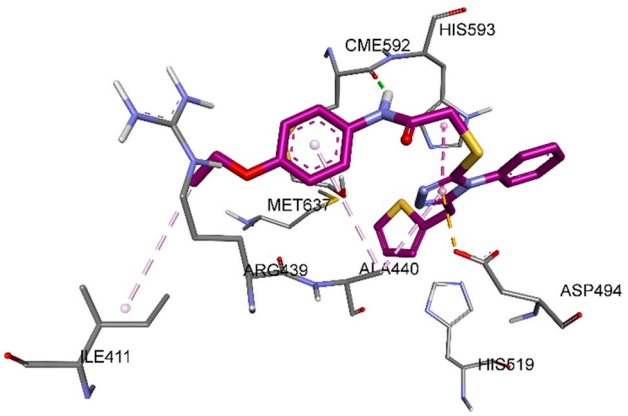


Fig. 8 Docked conformation of **10n** with urease enzyme showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π T-shaped (purple) interactions

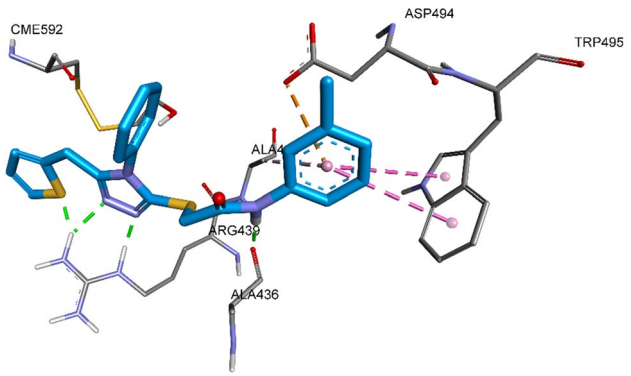


Fig. 7 Docked conformation of **10i** with urease showing hydrogen bonds (green), π-anion (orange), and π–π T-shaped (purple) interactions

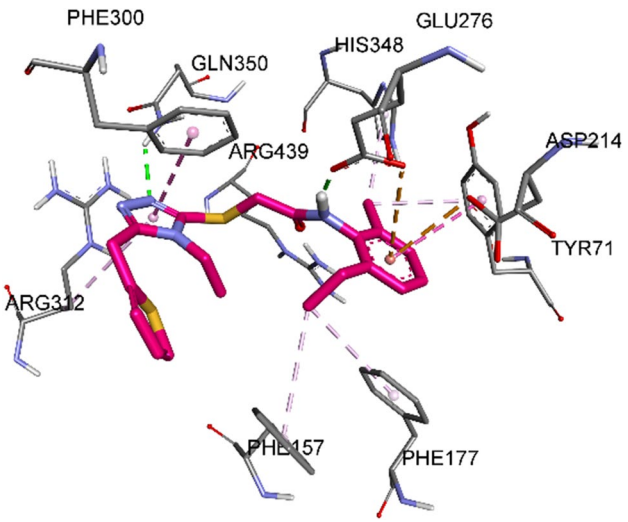


Fig. 9 Docked conformation of **9o** with yeast α-glucosidase showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π T-shaped (purple) interactions

compound **9o**, π-alkyl interactions were observed between ethyl side chain and amino acids Phe177 and Phe157. The methyl group was making π-alkyl interaction with Tyr71 and His348. The phenyl ring was making π-anion interactions with Asp214 and Glu276. The triazole ring was making π-alkyl and π–π T-shaped interactions with Arg312 and Phe300, respectively. One of the triazole nitrogen atoms was making a hydrogen bond with Gln350. Another hydrogen bond was observed between the NH of amide group and amino acid Glu276 (Fig. 9).

For compound **10i**, the methyl group was found to be making a π-alkyl interaction with Tyr71 and His111. The phenyl ring was making π-anion and π–π T-shaped interactions with amino acids Asp214 and Tyr71, respectively. The thiophenyl ring and triazole rings were making π-alkyl interactions with Ala278 and Arg312, respectively; both rings were making π–π T-shaped interactions with Phe300. Additionally, the thiophenyl ring was making a π-anion interaction with His279. The thiophenyl ring and the *N*-substituted phenyl ring were found to be in contact via π–π stacked interaction. Hydrogen bonds were observed between one of the triazole ring nitrogen atoms and Gln350, between NH of amide group and Glu276, and between carbonyl group of amide and Arg439 (Fig. 10).

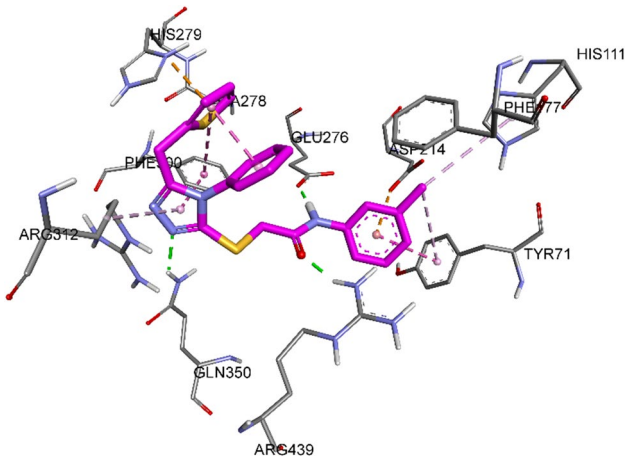


Fig. 10 Docked conformation of **10i** with yeast α-glucosidase showing hydrogen bonds (green), π-alkyl (light purple), π-anion (orange), and π–π T-shaped and π–π stacked (purple) interactions

Conclusions

The targeted *N*-aryl/aralkyl derivatives (**9a**–**q**) of 2-(4-ethyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetamide and *N*-aryl/aralkyl derivatives (**10a**–**q**) of 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio) acetamide were synthesized in good yields, and these molecules possessed various enzyme inhibitory activities moderate to good and potent against AChE (**9i**, **9n**, **10d**, **10f**, **10m** with IC_{50} values 73.54 ± 0.16, 69.26 ± 0.12, 45.72 ± 0.11, 32.26 ± 0.12, 72.53 ± 0.13 µM, respectively), BChE (**9b**, **9d**, **9l**, **10a**, **10d**, **10e**, **10h**, **10j**, **10o**, IC_{50} values 21.72 ± 0.18, 12.52 ± 0.19, 12.52 ± 0.19, 27.24 ± 0.19, 23.62 ± 0.22, 27.43 ± 0.16, 24.52 ± 0.21, 24.52 ± 0.21, 28.35 ± 0.18, and 27.24 ± 0.19 µM, respectively), urease (**9e**, **10i**, **10n** IC_{50} values 8.79 ± 0.05, 7.27 ± 0.05, 7.35 ± 0.04 µM, respectively) and α-glucosidase enzymes (**9o**, **10i** with IC_{50} values of 62.94 ± 0.19, and 69.46 ± 0.15 µM, respectively). Therefore, these studies led to the conclusion that the newly synthesized molecules might serve as promising drug molecules for further structural optimizations and drug designing studies.

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