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Synthesis and Characterization of Thieno[2,3-*d*]pyrimidin-4-ol Tethered with 1,2,3-Triazole Derivatives and Their Antimicrobial Activity

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In effort to develop biologically active compounds, novel heterocyclic compounds of thieno[2,3-*d*]pyrimidine tethered with 1,4-disubstituted-1,2,3-triazoles through methylene-oxy group were synthesized and characterized. The synthesized technique possesses a number of standout characteristics, including few reaction steps, high yield, simple reaction conditions and no need for any further purification processes. The *in vitro* antimicrobial efficacy of the synthesized derivatives was evaluated and several compounds were found to be moderate to excellent inhibition.

Keywords: Thieno[2,3-*d*]pyrimidine, 1,4-Disubstituted-1,2,3-triazole, Antimicrobial activity.

INTRODUCTION

Among the pyrimidine fused compounds with a variety of biological uses, thieno[2,3-*d*]pyrimidines have excellent pharmacophoric activities [1-3]. The synthesis of molecules with fused rings benefits from the usage of pyrimidine analogs, in addition to its relevance in pharmacology [4,5]. It appears that the unique biological effects provided on pyrimidine compounds are significantly affected by the inclusion of a second ring to their core [6]. Thieno[2,3-*d*]pyrimidine ring systems have been synthesized in a variety of ways, but there is still a lot of interest in them. The pharmaceutical characteristics of various fused pyrimidines, such as thienopyrimidines, triazolothieno[2,3-*d*]pyrimidines, pyrazolopyrimidines, quinazolines, purines, furopyrimidines and pyrrolopyrimidines, have been studied during the last 10 years [7].

The 1,2,3-triazoles have also been shown to exhibit a range of biological activities including antiviral [8], antibacterial [9-11], antifungal [12,13], antioxidant [14] and anticancer [15-17] activiies. The 5-phenylthieno[2,3-*d*]pyrimidine moiety of the compound is a heterocyclic ring system that has been reported to possess various biological activities, such as antitumor [18-20], antipyrine [21,22], anti-inflammatory [23,24] and antiproliferative [25] effects. The introduction of triazole moiety through a copper-catalyzed azide-alkyne cycloaddition reaction has been shown to enhance the biological activity of compounds. Additionally, thieno[2,3-*d*]pyrimidine-1*H*-1,2,3-triazol-1-yl)-acetamide compound has a potential pharmacophore feature due to the presence of an acetamide group and its synthesis and potential biological activities are yet to be investigated [26].

In present study, thieno[2,3-*d*]pyrimidine-1*H*-1,2,3-triazol-1-yl)acetamide was used to synthesize of novel *N*-aryl-2-(4-(((5-phenylthieno[2,3-*d*]pyrimidin-4-yl)oxy)methyl) and various substituted alkyl/aryl azide derivatives *via* Cu(I) catalyzed 1,3-dipolar cycloaddition reaction. All the synthesized compounds were characterized using IR, mass, ^{1}H NMR and ^{13}C NMR spectroscopic techniques. The antimicrobial activity of all the synthesized compounds were also evaluated.

EXPERIMENTAL

All the chemicals and reagents were purchased and used without further purification. Reaction progress were monitored by analytical TLC precoated on silica gel-G plates (G60, F_{254}) and visualized the spots were located on iodine vapours and UV light. Melting points were determined using open capillary method melting point apparatus and were uncorrected. FT-IR spectra were obtained on a Shimadzu FT-IR-8400 instrument in cm^{-1}. The NMR spectra were recorded on a Bruker Avance

400 MHz spectrometer ^{1}H NMR (400 MHz) & ^{13}C NMR (101 MHz) spectra were recorded in DMSO-d_6/$CDCl_3$ solvent with respect to TMS as standard. Mass spectra were recorded on Water Acquity Ultra performance LC equipped with SQ detector (ESI), mass range 100-1500 Da, 30 V cone voltage.

Synthesis of 2-(1-phenylethylidene)malononitrile (1): A mixture of acetophenone (9 mmol), malononitrile (10 mmol) in toluene (70 mL) was added containing ammonium acetate (9 mmol) and glacial acetic acid (9 mmol) was heated at 110 ºC for 2-4 h. Using a Dean-Stark trap, the condensed water was removed from the reaction. After the completion of reaction as checked by TLC, solvent was evaporated using vacuum and the resulting solution was cooled to room temperature. The separated solid was filtered, washed with distilled water, dried and finally recrystallized from ethanol to afford 2-(1-phenylethylidene)malononitrile (m.p. 94-96 ºC).

Synthesis of 2-amino-4-phenylthiophene-3-carbonitrile (2): 2-(1-Phenylethylidene)malononitrile (**1**) (5 mmol) and elemental sulfur (6.5 mmol) were suspended in 20 mL THF and warmed to an internal temperature of 35 ºC. A solution of sodium bicarbonate (1.6 g in 20 mL H_2O) was added over 1 h. The reaction mixture was stirred at 35 ºC for 35 min before the solution was transferred to a separatory funnel. Then, the organic layers were separated and the water phase was extracted with ethyl acetate by combining the organic phase. After removal of solvent, the residue was recrystallized from ethanol to obtain 2-amino-4-phenylthiophene-3-carbonitrile (**2**) (m.p. 123 ºC).

Synthesis of 5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (3): A mixture of 2-amino-4-phenylthiophene-3-carbonitrile (**2**) (10 mmol) and formic acid (10 mL) was added. After refluxation of for 16-18 h, the reaction mixture was cooled and water (20 mL) was added. The precipitated solid was filtered and washed thoroughly with cold water and hexane to give 5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) (yield: 0.8 g, 85%, m.p. 205-208 ºC).

Synthesis of 4-chloro-5-phenylthieno[2,3-*d*]pyrimidine (4): A reaction of mixture of 5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) (8 g, 1 mmol) and $POCl_3$ (70 mL) was stirred at 80 ºC for 2 h. The reaction monitored by TLC, after completion of reaction mixture slowly poured on crushed ice and neutralized using sodium bicarbonate. The separated solid product was filtered using vacuum and washed with cold water and dried to afford 4-chloro-5-phenylthieno-[2,3-*d*]pyrimidine (**4**) as white colour solid (yield: 8.1 g, 94.23%, m.p. 125-127 ºC).

Synthesis of 5-phenyl-4-(prop-2-yn-1-yloxy)thieno[2,3-*d*]pyrimidine (5): To a solution of propargyl alcohol (2.33 g, 41.73 mmol) in 50 mL dry THF under nitrogen atmosphere and stirred solution under cooling. Then after added portionwise potassium *tert*-butoxide (5.04 g, 45.21 mmol) and the resulting reaction mixture was stirred for 20 min at the same temperature. Then after solution of 4-chloro-5-phenylthieno-[2,3-*d*]pyrimidine (**4**) (8.0 g, 34.78 mmol) in dry THF was added dropwise and after addition the reaction mixture was stirred for 0.5 h at room temperature. The reaction product was extracted using petroleum ether-hexane solvent and the organic layer was removed using anhydrous sodium sulphate under reduced pressure to afford light yellow colour solid product (yield: 7.8 g, 90.13%, m.p. 130-132 ºC. Mass (*m/z*): exact mass 266.32, observed mass: 267.1$(M+H)^+$.

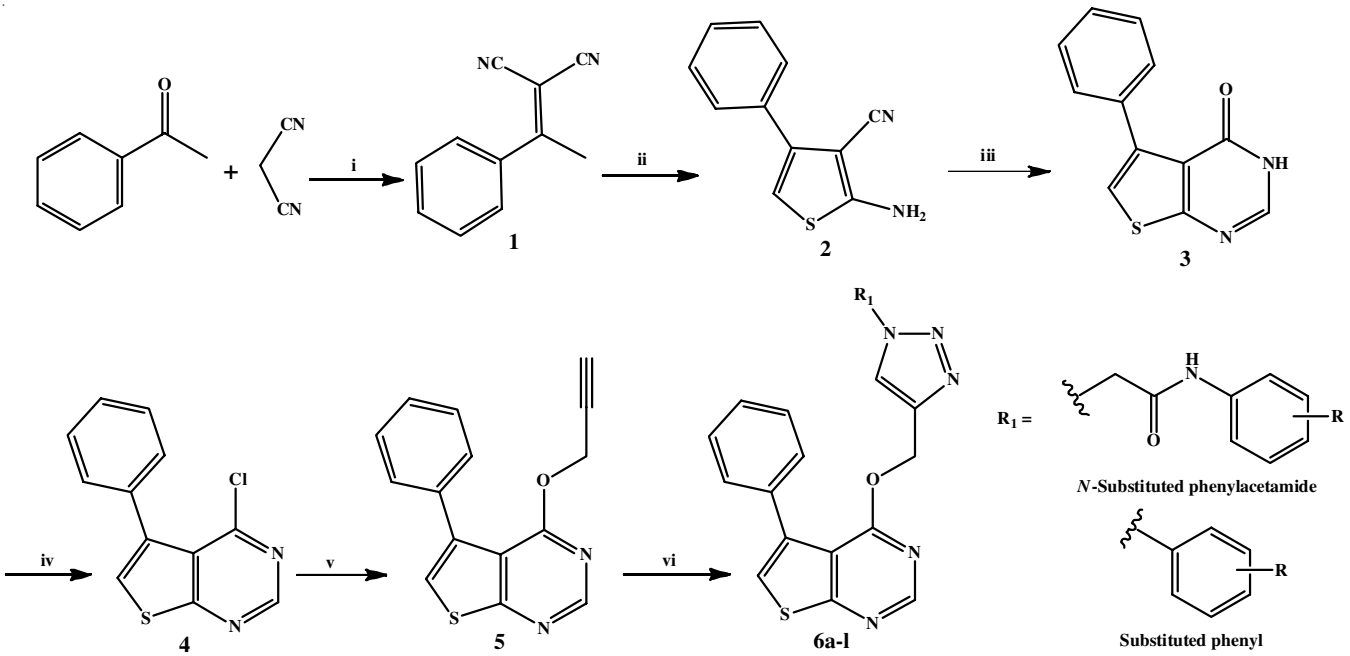
Synthesis of substituted 2-chloro-N-phenylacetamide derivatives: To a solution of various aniline (1.0 equiv.) in acetone were added potassium carbonate (1.1 equiv.) and chloroacetyl chloride (1.1 equiv.) and reaction mixture stirred at room temperature for 4-5 h, reaction was monitored by TLC, after completion of reaction mixture poured in cold water, the separated solid product collected and washed with cold water and dry in vacuum, filter to afford 2-chloro-N-phenylacetamide derivatives.

Synthesis of substituted 2-azido-*N*-phenylacetamide derivatives: To a solution of 2-chloro-N-phenylacetamide derivatives (1.0 equiv.) in DMF were added to sodium azide (3.0 equiv.) and reaction mixture was stirred at room temperature for 9-11 h, reaction was monitored by TLC. After the completion of reaction mixture was poured in ice-cold water, the separated solid collected and washed with cold water and dry in vacuum filter to afford 2-azido-N-phenylacetamide derivatives.

Synthesis of substituted azidobenzene derivatives: To a solution of various amine derivatives (1 equiv.) dissolved in 6 N HCl and stirred for 15 min were added solution of sodium nitrite (1.5 equiv.) at 0-5 ºC and the reaction mixture stirred for 45 min at the same temperature. Then solution of sodium azide (1.5 equiv.) was added under cooling and reaction mixture stirred at room temperature for 4-5 h. The progress of the reaction was monitored by TLC and after the completion of reaction mixture was poured into ice cold water and extracted with ethyl acetate. The organic layer was removed using anhydrous sodium sulphate under reduced pressure to afford azido benzene derivatives.

Synthesis of *N*-substituted aryl-2-(4-((1-(substituted aryl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-phenylthieno[2,3-*d*]-pyrimidine (6a-l): To an equimolar mixture of 5-phenyl-4-(prop-2-yn-1-yloxy)thieno[2,3-*d*]pyrimidine (**5**) and substituted azidoaryl in round bottom flask were added DMF:water:*t*-BuOH (1:1:1) followed by the further addition of the catalytic amount of sodium ascorbate and aqueous copper sulphate solution. The resulting reaction mixture was stirred at room temperature for 4-6 h and progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture poured in cold water, collected the separated solid and washed with ammonium chloride solution to remove copper and then washed again thoroughly with distilled water and small amount of methanol and finally dried over vacuum to afford titled compounds (**6a-l**) (**Scheme-I**).

***N*-Phenyl-2-(4-(((5-phenylthieno[2,3-*d*]pyrimidin-4-yl)-oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (6a):** Yield: 86%, tan solid, m.p.: 134-136 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3610.86, 3271.38, 3140.22, 3047.63, 2800.73, 1890.30, 1689.70, 1603.83, 1543.10, 1450.52, 1319.35, 1249.91, 1126.47, 1033.88, 956.72, 840.99, 756.12, 702.11, 648.10, 563.23, 493.79; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.50 (s, 1H, CONH), 8.79 (s, 1H, thiophene), 8.02 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.60-7.58 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.49-7.47 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.36-7.27 (m, 4H, Ar-H), 7.11-7.07 (t, *J* = 7.4 Hz, 1H,



6a: R = H; **6b:** R = 4-F; **6c:** R = 4-Cl; **6d:** R = 4-Me; **6e:** R = 4-Ome; **6f:** R = 2-Me;
6g: R = 2,4,6-triCl; **6h:** R = 4-Me; **6i:** R = 4-Cl; **6j:** R = 2-Cl,4-NO_2; **6k:** R = 4-NO_2; **6l:** R = 4-OMe

(i) Ammonium acetate, acetic acid, reflux, toluene, 1-2 h; (ii) S_8, $NaHCO_3$, THF, 35 °C, 30 min; (iii) HCOOH, reflux, 18 h; (iv) $POCl_3$, 90 °C, 2-4 h; (v) propargyl alcohol, *t*-BuOK, THF, 35 min; (vi) various azide, Na-ascorbate, $CuSO_4$, DMF:water:*t*-BuOH, 4-6 h

Scheme-I: Synthetic route for desire compounds **6a-l**

Ar-H), 5.58 (s, 2H, OCH_2), 5.32 (s, 2H, $COCH_2$); MS (*m/z*): exact mass 442.12, observed mass: 443.2 $(M+H)^+$.

***N*-(4-Fluorophenyl)-2-(4-(((5-phenylthieno[2,3-*d*]-pyrimidin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (6b):** Yield: 90%, off-white solid, m.p.: 168-170 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3271.38, 3063.06, 2955.64, 1890.30, 1681.98, 1550.82, 1450.52, 1357.93, 1311.64, 1219.05, 1118.75, 1018.45, 956.72, 833.28, 756.12, 694.40, 570.95, 509.22; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.56 (s, 1H, CONH), 8.78 (s, 1H, thiophene), 8.03 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.61-7.47 (m, 4H, Ar-H), 7.30-7.19 (m, 4H, Ar-H), 5.58 (s, 2H, OCH_2), 5.32 (s, 2H, $COCH_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 169.36, 164.46, 163.45, 157.49, 153.43, 135.51, 135.26, 129.90, 128.09, 127.96, 124.10, 121.46, 116.14, 115.91, 60.15, 52.61; MS (*m/z*): Exact mass 460.49, observed mass: 461.3 $(M+H)^+$.

***N*-(4-Chlorophenyl)-2-(4-(((5-phenylthieno[2,3-*d*]-pyrimidin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (6c):** Yield: 85%, light grey solid, m.p.: 174-176 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3626.29, 3271.38, 3117.07, 3055.35, 2955.04, 1890.30, 1689.70, 1550.82, 1450.52, 1303.92, 1249.91, 1103.32, 1026.16, 949.01, 833.28, 756.12, 678.97, 563.23, 501.51; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.64 (s, 1H, CONH), 8.78 (s, 1H, Ar-H), 8.02 (s, 1H, thiophene), 7.77 (s, 1H, Ar-H), 7.63-7.61 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.49-7.47 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.42-7.40 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.33-7.27 (p, *J* = 7.0 Hz, 3H, Ar-H), 5.58 (s, 2H, OCH_2), 5.33 (s, 2H, $COCH_2$); MS (*m/z*): Exact mass 476.94, observed mass: 477.2 $(M+H)^+$.

2-(4-(((5-Phenylthieno[2,3-*d*]pyrimidin-4-yl)oxy)-methyl)-1*H*-1,2,3-triazol-1-yl)-N-(*p*-tolyl)acetamide (6d): Yield: 88%, brown solid, m.p.: 124-126 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3618.58, 3255.95, 3047.63, 2862.46, 1890.30, 1689.70, 1543.10, 1450.52, 1311.64, 1249.91, 1118.75, 1026.16, 949.01, 825.56, 756.12, 694.40, 563.23, 501.51; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.41 (s, 1H, CONH), 8.78 (s, 1H, Ar-H), 8.01 (s, 1H), 7.77 (s, 1H, Ar-H), 7.49-7.47 (m, 4H, Ar-H), 7.33-7.27 (m, 3H, Ar-H), 7.15-7.13 (d, *J* = 8.2 Hz, 2H, Ar-H), 5.58 (s, 2H, OCH_2), 5.30 (s, 2H, $COCH_2$), 2.26 (s, 3H, CH_3); MS (*m/z*): Exact mass 456.52, observed mass: 457.2 $(M+H)^+$.

***N*-(4-Methoxyphenyl)-2-(4-(((5-phenylthieno[2,3-*d*]-pyrimidin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (6e):** Yield: 94%, grey solid, m.p.: 160-162 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3294.53, 3063.06, 2955.04, 2839.31, 1882.59, 1681.98, 1543.10, 1450.52, 1357.93, 1311.64, 1242.20, 1118.75, 1026.16, 956.72, 825.56, 763.84, 694.40, 524.66; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.36 (s, 1H, CONH), 8.79 (s, 1H, Ar-H), 8.01 (s, 1H, thiophene), 7.77 (s, 1H, Ar-H), 7.50 (dd, *J* = 12.9, 7.9 Hz, 3H, Ar-H), 7.35-7.25 (m, 4H, Ar-H), 6.92 (d, *J* = 8.6 Hz, 2H, Ar-H), 5.58 (s, 2H, OCH_2), 5.28 (s, 2H, $COCH_2$), 3.72 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm: 163.97, 155.98, 135.52, 131.99, 129.91, 128.04 (d, *J* = 13.1 Hz), 124.09, 121.16, 114.48, 55.60.

2-(4-(((5-Phenylthieno[2,3-*d*]pyrimidin-4-yl)oxy)-methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(*o*-tolyl)acetamide (6f): Yield: 86%, white solid, m.p.: 196-198 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3610.86, 3279.10, 3140.22, 2962.76, 2908.75, 2353.23, 1674.27, 1543.10, 1458.23, 1357.93, 1303.92, 1249.91, 1118.75, 1018.45, 956.72, 794.70, 756.12, 655.82, 570.95, 447.50; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.80 (s, 1H, CONH), 8.77 (s, 1H, Ar-H), 8.01 (s, 1H, thiophene), 7.76 (s, 1H, Ar-H), 7.47

(s, 4H, Ar-H), 7.29 (s, 4H, Ar-H), 7.17-7.11 (s, 1H, Ar-H), 5.57 (s, 2H, OCH_2), 5.37 (s, 2H, $COCH_2$), 2.49-2.23 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 169.39, 164.74, 163.50, 153.45, 142.05, 135.98, 135.54, 130.95, 129.93, 128.05 (d, *J* = 14.3 Hz), 126.88, 126.58, 126.05, 125.15, 124.09, 60.16, 52.37, 18.29.

2-(4-(((5-Phenylthieno[2,3-*d*]pyrimidin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-(2,4,6-trichlorophenyl)acetamide (6g): Yield: 91%, white solid, m.p.: 186-188 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.32 (s, 1H, CONH), 8.79 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 8.04 (s, 1H, thiophene), 7.98 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.50 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.33-7.31 (m, 3H, Ar-H), 5.59 (s, 2H, OCH_2), 5.48 (s, 2H, $COCH_2$); MS (*m/z*): Exact mass 545.82, observed mass: 545.3 $(M+H)^+$.

5-Phenyl-4-((1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-thieno[2,3-*d*]pyrimidine (6h): Yield: 87%, grey solid, m.p.: 162-164 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3938.77, 3850.04, 3144.07, 3101.64, 3043.77, 2951.19, 2866.32, 2731.29, 2600.13, 2515.26, 2457.39, 2364.81, 2291.51, 2106.34, 1979.03, 1909.59, 1832.44, 1670.41, 1523.82, 1442.80, 1357.93, 1315.50, 1219.05, 1118.75, 1037.74, 1006.88, 918.15, 817.85, 756.12, 702.11, 655.82, 551.66, 516.94; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (s, 1H, Ar-H), 8.53 (s, 1H, thiophene), 7.79 (s, 1H, Ar-H), 7.74 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.55-7.48 (m, 3H, Ar-H), 7.44 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.27 (d, *J* = 6.5 Hz, 2H, Ar-H), 5.62 (s, 2H, OCH_2), 2.41 (s, 3H, CH_3).

4-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-phenylthieno[2,3-*d*]pyrimidine (6i): Yield: 92%, offwhite solid, m.p.: 197-199 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.70 (s, 1H, Ar-H), 8.35 (s, 1H, thiophene), 7.71-7.63 (m, 2H, Ar-H), 7.45-7.38 (m, 2H, Ar-H), 7.21-7.15 (m, 2H, Ar-H), 7.08 (d, *J* = 8.5 Hz, 2H, Ar-H), 5.52 (s, 2H, OCH_2), 3.76 (s, 2H); MS (*m/z*): Exact mass 419.89, observed mass: 420.4 $(M+H)^+$.

4-((1-(2-Chloro-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)-methoxy)-5-phenylthieno[2,3-*d*]pyrimidine (6j): Yield: 96%, m.p.: 149-151 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3352.39, 3155.65, 3113.21, 2951.19, 2870.17, 2673.43, 2607.85, 2468.97, 2360.95, 2272.22, 1921.16, 1678.13, 1527.67, 1438.94, 1350.22, 1319.35, 1261.49, 1122.61, 1022.31, 887.28, 837.13, 759.98, 702.11, 659.68, 509.22; MS (*m/z*): Exact mass 464.88, observed mass: 465.36 $(M+H)^+$.

4-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-phenylthieno[2,3-*d*]pyrimidine (6k): Yield: 93%, white solid, m.p.: 191-193 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3730.45, 3637.87, 3607.01, 3144.07, 3097.78, 2943.47, 2854.74, 2627.13, 2511.40, 2453.54, 2353.23, 2322.37, 1928.88, 1728.28, 1674.27, 1600.97, 1527.67, 1442.80, 1350.22, 1315.50, 1249.91, 1114.89, 1014.59, 922.00, 852.56, 756.12, 702.11, 547.80, 509.22; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.71 (s, 2H, Ar-H), 8.43 (s, 2H, Ar-H), 8.14-8.07 (s, 1H, thiophene), 7.70 (s, 2H, Ar-H), 7.43 (s, 2H, Ar-H), 7.19 (s, 3H, Ar-H), 5.57 (s, 2H, OCH_2); MS (*m/z*): Exact mass 430.44, observed mass: 431.40 $(M+H)^+$.

4-((1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-methoxy)-5-phenylthieno[2,3-*d*]pyrimidine (6l): Yield: 89%, white solid, m.p.: 153-155 ºC; FT-IR (KBr, ν_{max}, cm^{-1}): 3749.74, 3448.84, 3136.36, 3101.64, 2951.19, 2912.61, 2835.45, 2654.14, 2553.84, 2503.69, 2368.66, 2326.23, 2052.33, 1979.03, 1925.02, 1720.56, 1608.69, 1523.82, 1442.80, 1354.07, 1311.64, 1253.77, 1192.05, 1114.89, 1037.74, 929.72, 829.42, 794.70, 759.98, 702.11, 659.68, 613.38, 540.09; MS (*m/z*): Exact mass 415.47, observed mass: 416.14 $(M+H)^+$.

Antimicrobial activities: The *in vitro* antibacterial assessment of the synthesized compounds was investigated. *Escherichia coli*, *Pseudomonas aeruginosa* (Gram +ve) and *Staphylococcus aureus*, *Bacillus subtilis* (Gram -ve) were selected for antibacterial activity whereas *Aspergillus niger* and *Aspergillus flavus* were selected for antifungal activity using microbroth dilution technique. Mueller-Hinton Broth technique was utilized to grow bacteria as a neutriant media, while Sabouraud Dextrose broth was used to cultivate fungi. After examining the turbidity, the inoculant concentration for the test strain had to be adjusted to 10^8 CFU/mL. For the primary and secondary screenings, the serial dilutions were prepared. The research compounds and standard drugs were combined twice to prepare the stock solution (2000 µg/mL). At concentrations of 1000, 500, 250 and 125 µg/mL, respectively, the synthesized compounds were evaluated. More study was conducted on the substances that survived this first analysis and proved to be effective. Concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/mL were utilized in secondary screening. The injection wells were incubated at 37 ºC in a humid environment for 24 h [27,28].

RESULTS AND DISCUSSION

Starting from the synthesis of 2-(1-phenylethylidene)-malononitrile (**1**) using knoevenagel condensation [29,30] followed by Gewald reaction with sulphur, sodium bicarbonate in THF:water to obtain 2-amino-4-phenylthiophene-3-carbonitrile (**2**) [31,32], which underwent intermolecular condensation reaction through refluxing in formic acid to afford 5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) [33], which was heated in $POCl_3$ to afford 4-chloro-5-phenylthieno[2,3-*d*]-pyrimidine (**4**) [34]. Compound **4** was then reacted with propargyl alcohol in the presence of potassium *tert*-butoxide to afforded 5-phenyl-4-(prop-2-yn-1-yloxy)thieno[2,3-*d*]pyrimidine (**5**) [35]. The copper(I) catalyzed azide-alkyne cycloaddition (Cu AAC) reaction of terminal alkyne [36] reacted with various alkyl or aryl azide in the presence of Cu(I) to afford desired triazole compounds (**6a-l**) [37]. The synthesized targeted compounds carried out is outlined in **Scheme-I**.

Using a reported procedure, the chemical reaction using the conventional approach in the presence of Cu(I) catalyzed in different solvents or mixtures of solvents was executed. The ideal reaction parameters for the synthesis of specified molecules using the conventional method and DMF:water:*t*-BuOH (1:1:1) as solvent (Table-1, entries 8 to 10) were determined. This allowed to obtain the excellent yields and reduced reaction times without any purification.

Biological evaluation: The synthesized titled compounds exhibited moderate to excellent inhibition inhibition based on the MIC values (Table-2). Compounds **6a**, **6b**, **6c**, **6d**, **6e**, **6g**, **6i** and **6k** showed the strong antibacterial activity. According to the MIC values, compounds **6a** and **6d** were shows good antifungal activity. Therefore, compounds **6a**, **6b**, **6d**, **6e**, **6g** and **6k** are considered as potent drug against the studied Gram-

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS FOR THE SYNTHESIS OF TITLED COMPOUNDS

Entry	Solvent	Catalyst	Time (h)	Yield (%)
1	Methanol	$CuSO_4$, Na-ascorbate	14	21
2	Water	$CuSO_4$, Na-ascorbate	14	15
3	*t*-BuOH	$CuSO_4$, Na-ascorbate	10	37
4	DMF	$CuSO_4$, Na-ascorbate	10	51
5	DMF:*t*-BuOH (1:1)	$CuSO_4$, Na-ascorbate	8	70
6	Water:*t*-BuOH (1:1)	$CuSO_4$, Na-ascorbate	8	65
7	DMF:water (1:1)	$CuSO_4$, Na-ascorbate	8	67
8	DMF:water:*t*-BuOH (1:1:1)	$CuSO_4$, Na-ascorbate	6	96
9	DMF:water:*t*-BuOH (1:1:1)	CuBr	6	69
10	DMF:water:*t*-BuOH (1:1:1)	CuI	6	78

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF N-SUBSTITUTED ARYL-2-(4-((1-(SUBSTITUTED ARYL)-1*H*-1,2,3-TRIAZOL-4-YL)METHOXY)-5-PHENYLTHIENO[2,3-*d*]PYRIMIDINE DERIVATIVES (**6a-l**)

Compound	Antibacterial MIC (µg/mL)				Antifungal MIC (µg/mL)	
	B. megaterium (MTCC 2444)	*S. aureus* (MTCC 737)	*E. aerogenes* (MTCC 2823)	*P. aeruginosa* (MTCC 3541)	*A. flavus* (MTCC 418)	*A. niger* (MTCC 282)
Streptomycin	–	–	50	50	–	–
Ampicillin	100	100	–	–	–	–
Nystatin	–	–	–	–	100	100
6a	250	250	125	125	250	250
6b	250	250	125	125	500	500
6c	125	125	125	125	1000	1000
6d	125	125	125	125	125	125
6e	250	250	125	125	500	500
6f	250	250	1000	1000	1000	1000
6g	125	125	125	125	500	500
6h	250	125	500	500	500	500
6i	250	250	125	125	1000	1000
6j	500	500	250	250	1000	1000
6k	250	250	125	125	250	250
6l	500	250	500	250	1000	1000

positive, Gram-negative bacteria and fungi. Compound **6f** showed antagonistic activity against the Gram-positive bacteria, whereas compounds **6a**, **6d** and **6k** exhibited excellent antifungal activity.

Conclusion

In conclusion, we designed, synthesized and characterized thieno[2,3-*d*]pyrimidine derivatives tethered with 1,2,3-triazole moiety by employing click chemistry. This was accomplished by utilizing a traditional approach and a Cu(I)-catalyzed azide-alkyne cycloaddition procedure. By using spectroscopic techniques, the synthesized molecules was identified and confirmed. All the synthesized compounds had moderate to excellent antibacterial and antifungal abilities evaluated compared to common drugs. Some of the compounds were shown to be the most potent against evaluated antibacterial and antifungal activities during the *in vitro* antimicrobial testing.

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CONFLICT OF INTEREST

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

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