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Synthesis, spectroscopic characterization and computational chemical study of 5-cyano-2-thiouracil derivatives as potential antimicrobial agents

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ABSTRACT

A series of 5-cyano-2-thiouracil derivatives, containing diverse hydrophobic groups in the 2-, 4- and 6-positions, were synthesized through one pot reaction of thiophene 2-carboxaldehyde, ethylcyanoacetate and thiourea using classic reflux-based method as well as microwave-assisted methods. Such prepared compounds were reacted with different electrophilic reagents to synthesize potent anti-microbial agents, e.g. 1,3,4-thiadiazinopyrimidine, hydrazide and triazolopyrimidine derivatives (compounds **4**a-e, **9** and **10**–**12**) respectively. The density functional theory (DFT) was then applied to explore the structural and electronic characteristics of these materials. It is found that compound **12** exhibited the highest antibacterial and antifungal activity against *C. Albicans* showing six-fold increasing biological affinity compared to that of *Colitrimazole* drug with MIC values 7.8 and 49 μg/mL, respectively. All the synthesized compounds have been characterized based on their elemental analyses and spectral data. Such compounds can be submitted to *in vivo* antimicrobial studies in future works.

1. Introduction

Pyrimidines are an important component of nucleic acids and they have been used as building blocks in pharmaceuticals for the synthesis of antiviral [1], antineoplastic [2], antibacterial and antifungal [3] agents. Similarly, the related thiouracil derivatives are potential therapeutic agents with antiviral, anticancer and microbial activities [4–6]. For example, *S*-alkylation and *N*-alkylation products have been recently reported as novel antibacterial, cytotoxic agents [7,8] and unique HIV reverse transcriptase inhibitors [9,10]. Thiadiazine derivatives, particularly 1,3,5-thiadiazine, as well its fused heterocyclic compounds possessed broad spectrum of biological interests [11,12]. However, 1,3,5-thiadiazines derivatives are scarcely reported in the literature. It was found that functionalized thiadiazines have insecticidal [13], antibacterial [14], herbicidal [15], and fungicidal [16] effects. In connection to our previous studies on the biological activities of pyrimidine and fused derivatives [17–27], the present work expands the scope to new pyrimidine derivatives based on a 6-aryl-5-cyano-2-thiouracil derivative. One pot reaction has become significant in combinatorial and green chemistry due to its process simplicity, mild conditions, atomic economy and extension of the scope of substrates [28,29]. So, the authors decided to synthesize the pyrimidine derivatives through microwave assisted organic synthesis that has revealed broad applications as a facile and efficient method to proceed many organic reactions, producing high yields and higher selectivity, lower quantities of side products and, consequently, easier work-up and purification of the products [30,31].

2. Experimental

2.1. Materials and methods

All the chemicals and solvents, procured from Sigma-Aldrich (Egyptian branch, Egyptian International Center for Significance, Cairo, Egypt), were used without further purification. All melting points were measured on a Gallenkamp melting point apparatus (MFB 595-0366). The IR spectra were recorded on a Pye-Unicam SP-3-300 infrared spectrophotometer (KBr Pellets) and expressed in wave number (cm^{-1}). ^1H NMR spectra were run at 300 and 400 MHz, on a Varian Mercury VX-300 and Bruker Avance III NMR

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spectrometer respectively, while ^{13}C NMR spectra were recorded on the Varian Mercury VX-300 spectrometer at 125 MHz. TMS was used as an internal standard in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ) are quoted in ppm. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. All coupling constant (J) values are given in hertz. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. Elemental analyses were performed on CHN analyzer and all compounds were within ±0.1–0.4% of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases. All the newly synthesized compounds gave satisfactory elemental analysis.

2.1.1. General procedure for synthesis of 4-oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1)

a) Conventional method.

A mixture of thiourea (0.01 mol), ethyl cyanoacetate (0.01 mol), 2-thiophene carboxaldehyde (0.01 mol) and potassium carbonate (0.01 mol) in absolute ethanol (50 mL) was refluxed for 8 h. The reaction was monitored by thin-layer chromatography. After completion of the reaction, it was neutralized with concentrated hydrochloric acid solution, and the precipitate was filtered and recrystallized with proper solvent. ethanol.

b) Microwave method.

A mixture of thiourea (0.01 mol), ethyl cyanoacetate (0.01 mol), substituted benzaldehyde (0.01 mol) and potassium carbonate (catalytic amount) was taken in about 30 mL of ethanol (one-pot reaction). The reaction mixture was subjected to microwave pulse for (450 w) for about 20 min. The completion of reaction was monitored by TLC, the product was obtained in the form of potassium salt which was dissolved in warm water and acidified by acetic acid to precipitate pure nucleobase. The crude product was recrystallized from acetic acid.

Yield (88%); White crystal; m.p. 360–362 °C (EtOH); IR (cm^{-1}): 3189 (NH), 3099 (CH aromatic), 2222 (CN), 1675 (CO amide), 1154 (C=S amide); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.31 (m, 1H, Ar-H), 8.06 (d, 1H, Ar-H, $J = 8$ Hz), 8.07 (d, 1H, Ar-H, $J = 8$ Hz), 13.08 (br. s, 2H, 2NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.2 (C_5, pyrid), 118.5 (CN), 127.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 159.8 (C_4, pyrid), 166.3 (CO, pyrid), 172.2 (C=S); Anal. Cal. for $C_9H_5N_3OS_2$ (235.28): % C, 45.95; % H, 2.14; % N, 17.86; Found: % C, 45.81; % H, 2.02; % N, 17.79.

2.1.2. Synthesis of 3,5-dioxo-7-(thiophen-2-yl)-2,3-dihydro-5H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (2)

A mixture of **1** (0.01 mol), monochloroacetic acid (0.01 mol) in glacial acetic acid (20 mL) and acetic anhydride (10 mL) was heated under refluxed for 5–9 h. The precipitate was filtered off, dried and crystallized from acetic acid. Yield (79%); yellow crystal; m.p. 192–194 °C (EtOH); IR (cm^{-1}): 3013 (CH aromatic), 2218 (CN), 1737 (CO), 1655 (CO); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.08 (s, 2H, CH_2), 7.31 (m 1H, Ar-H, 8.07 (d, 1H, Ar-H, $J = 8$ Hz), 8.23 (d, 1H, Ar-H, $J = 8$ Hz); Anal. Cal. for $C_{11}H_5N_3O_2S_2$ (275.30): % C, 47.99; % H, 1.83; % N, 15.26; Found: % C, 47.75; % H, 1.75; % N, 15.19%.

2.1.3. General procedure for the preparation of compounds (3a-c)

Method 1

A mixture of compound **2** (0.01 mol) and different aldehydes namely, 4- methoxybenzaldehyde, thiophenecarboxaldehyde and 4- nitrobenzaldehyde (0.02 mol) in ethanol (30 mL) and few drops of acetic acid was refluxed for 5–8 h. The solid obtained after cooling was filtered off, dried on suction and recrystallized from ethanol afforded compounds **3a-c**.

Method 2

A mixture of **1** (0.01 mol) and different aldehydes namely, 4-methoxybenzaldehyde, thiophenecarboxaldehyde and 4- nitrobenzaldehyde (0.01 mol) in a mixture of glacial acetic acid (30 mL)/acetic anhydride (15 mL) in the presence of anhydrous sodium acetate (2 g) was refluxed for 5–8 h. The solution was cooled, gradually poured onto cold water, and the formed precipitate was washed several times with water, filtered off, and recrystallized from ethanol to give compounds **3a-c**.

2.1.3.1. 2-(4-methoxybenzylidene)-3,5-dioxo-7-(thiophen-2-yl)-2,3-dihydro-5H-thiaz olo[3,2-a]pyrimidine-6-carbonitrile (3a). Yield (78%); yellow crystal; m.p. 212–214 °C (EtOH); IR (cm^{-1}): 3013 (CH aromatic), 2218 (CN), 1735 (CO), 1669 (CO); MS (*m*/*z*) 395/393. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.82 (s, 3H, OCH_3), 7.31–8.24 (m, 7H, Ar-H), 9.80 (s, 1H, CH ethylinic); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.7 (CH_3O), 102.2 (C_5, pyrid), 118.5 (CN), 119.2 (C=, thiaz), 121.1 ($2C_{3,5}$,Ar), 127.2 (C_1, Ar), 127.9 ($2C_{3,4}$, thioph), 129.5, 131.6 ($2C_{2,6}$, Ar), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 148.3 (CH=), 157.8 (C_4–O, Ar), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid), 168.2 (CO, thiaz); Anal. Cal. for $C_{19}H_{11}N_3O_3S_2$ (393.44) % C, 58.00; % H, 2.82; % N, 10.68; Found: % C, 57.78; % H, 2.71; % N, 10.59.

2.1.3.2. 2-(4-nitrobenzylidene)-3,5-dioxo-7-(thiophen-2-yl)-2,3-dihydro-5H-thiazolo [3,2-a]pyrimidine-6-carbonitrile (3b). Yield (81%); White crystal; m.p. 212–213 °C (EtOH); IR (cm^{-1}): 3044 (CH aromatic), 2985, 2926, 2842 (CH aliphatic), 2219 (CN), 1728 (CO ester), 1667 (CO amide); MS (*m*/*z*) 410/408. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.24–7.32 (m, 1H, Ar-H), 7.94–8.23 (m, 6H, Ar-H), 8.24 (s, 1H, CH ethylinic), ^{13}C NMR (125 MHz, DMSO-d_6) δ 101.2 (C_5, pyrid), 115.5 (CN), 120.1 (C=, thiaz), 122.1 ($2C_{3,5}$,Ar), 127.8(C_1, Ar), 128.3 ($2C_{3,4}$, thioph), 129.5, 131.6 ($2C_{2,6}$, Ar), 130.8 (C_5, thioph), 137.3 (C_2, thioph), 148.8 (CH=), 156.9 (C_4–O, Ar), 159.6 (C fused ring), 160.2 (C_4, pyrid), 165.7 (CO, pyrid), 168.2 (CO, thiaz); Anal. Cal. for $C_{18}H_8N_4O_4S_2$ (408.41): % C, 52.94; % H, 1.97; % N, 13.72; Found: % C, 52.79; % H, 1.89; % N, 13.65.

2.1.3.3. 3,5-Dioxo-7-(thiophen-2-yl)-2-(thiophen-2-ylmethylene)-2,3-dihydro-5H-thiaz olo[3,2-a]pyrimidine-6-carbonitrile (3c). Yield (78%); White crystal; m.p. 212–213 °C (EtOH); IR (cm^{-1}): 3097 (CH aromatic), 2833, 2842 (CH aliphatic), 2223 (CN), 1725 (CO ester), 1689 (CO amide); MS (*m*/*z*) 372/369; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.23–7.34 (m, 1H, Ar-H), 7.84 (d, 1H, Ar-H), 7.94 (s, 1H, CH ethylinic), 8.02–8.24 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.2 (C_5, pyrid), 118.5 (CN), 119.2 (C = , thiaz), 127.1 ($2C_{3,4}$, thioph 2), 127.9 ($2C_{3,4}$, thioph), 129.5, 131.6 (C_5, thioph 2), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 138.8 (C_2, thioph2), 148.3 (CH =), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid), 168.2 (CO, thiaz); Anal. Cal. for $C_{16}H_7N_3O_2S_3$ (369.43): % C, 52.02; % H, 1.91; % N, 11.37; Found: % C, 51.78; % H, 1.80; % N, 11.29.

2.1.4. General procedures for synthesis is of 8-arylpyrimido[2,1-b]-1,3,5-thiadia zine derivatives. 4 (a–e) and 5

A mixture of compound **1** (0.01 mol), primary amines such as 2-aminothiazole, 2-aminopyridine, *p*-aminobenzaldehyde, ethyl 4-aminobenzoate, *p*-toluidine and β-naphthylamine (1.1 mmol) and

formaldehyde (2 mL) was stirred in acetonitrile (20 mL) at room temperature for 2–3 h. The resulting precipitate was collected by filtration, washed with water several times and dried well. The crude product was recrystallized from the proper solvent to give pyrimido[2,1-b]-1,3,5-thiadiazine derivatives **4 (a–e)**. and the derivatives **5**.

2.1.4.1. 6-Oxo-3-(thiazol-2-yl)-8-(thiophen-2-yl)-3,4-dihydro-2h,6h-pyrimido[2,1-b] [1,3,5] thiadiazine-7-carbonitrile (4a). Yield (82%); Pale yellow crystal; m.p. 220–222 °C (EtOH); IR (cm^{-1}): 3055 (CH aromatic), 2994 (CH aliphatic), 2214 (CN) 1645 (CO); MS (*m/z*) 362/359. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.46 (s, 2H, N-CH_2-N), 5.75 ((s, 2H, S-CH_2-N), 7.22–7.33 (m, 3H, Ar-H), 8.04 (d, 1H, Ar-H, J = 8 HZ), 8.09 (d, 1H, Ar-H, J = 8 HZ); ^{13}C NMR (125 MHz, DMSO-d_6), δ 54.7 (CH_2S), 65.8 (CH_2N_2), 102.2 (C_5, pyrid), 117.5 (CN), 112.2(C_5, thiazol), 127.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 137.3 (C_4, thiazol), 156.8 (C_4, thiazol), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Cal. for: $C_{14}H_9N_5OS_3$ (359.44): % C, 46.78; % H, 2.52; % N, 19.48; Found: % C, 46.62; % H, 2.41; % N, 19.39.

2.1.4.2. 6-Oxo-3-(pyridin-2-yl)-8-(thiophen-2-yl)-3,4-dihydro-2h,6h-pyrimido[2,1-b] [1,3,5] thiadiazine-7-carbonitrile (4b). Yield (82%); White crystal; m.p. 228–230 °C (EtOH); IR cm^{-1}): 3095 (CH aromatic), 2973, 2836 (CH aliphatic), 2213 (CN) 1662 (CO) cm^{-1}; MS (*m/z*) 355/353. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.87 (s, 2H, S-CH_2-N), 6.81 (s, 2H, N-CH_2-N), 6.86–8.27 (M, 7H, Ar-H), ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.2 (CH_2S), 69.2 (CH_2N_2), 102.2 (C_5, pyrid), 115.9 (CN), 121.1 ($3C_{3,4,5}$, pyrid), 127.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 148.3 (C_6, pyrid), 157.8 (C_2, pyrid), 159.8 (C_4, pyrid), 160.2 (C fused ring), 166.3 (CO, pyrid); Anal. Calc. for $C_{16}H_{11}N_5OS_2$: 353.42: % C, 54.38; % H, 3.14; % N, 19.82; Found: % C, 54.11; % H, 3.03; % N, 19.71.

2.1.4.3. 3-(4-Formylphenyl)-6-oxo-8-(thiophen-2-yl)-3,4-dihydro-2H,6H-pyrimido [2, 1-b][1, 3,5] thiadiazine-7-carbonitrile (4c). Yield (84%); reddish-brown crystals; m.p. 225–227 °C (EtOH); IR (cm^{-1}): 3101 (CH aromatic), 2955 (CH aliphatic), 2219 (CN), 1736 (CO), 1683 (CO) amide; MS (*m/z*) 382/380. ^{1}H NMR (300 MHz, DMSO-d_6) *δ:* (ppm): 4.32 (s, 2H, S-CH_2), 6.21 (s, 2H, N-CH_2), 6.74–7.99 (m, 7H, Ar-H), 11.2 (s, 1H, N-CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 58.7 (CH_2S), 69.2 (CH_2N_2), 102.2 (C_5, pyrid), 116.5 (CN), 122.4 ($2C_{3,5}$,Ar), 127.6 (C_1, Ar), 128.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 131.6 ($2C_{2,6}$, Ar), 136.8 (C_2, thioph), 157.8 (C_4–N, Ar), 159.8 (C_4, pyrid), 161.2 (C fused ring), 166.3 (CO, pyrid), 188.2 (CO, ald); Anal. Cal. for $C_{18}H_{12}N_4O_2S_2$ (380.44): % C, 56.83; % H, 3.18; % N, 14.73; Found: % C, 56.65; % H, 3.09; % N, 14.65.

2.1.4.4. Ethyl-(4-(7-cyano-6-oxo-8-(thiophen-2-yl)-2h,6h-pyrimido [2,1-b][1,3,5] thiadiazin-3(4H)-yl))benzoate (4d). Yield 85%; colorless crystals; m.p. 248–250 °C (benzene); IR (cm^{-1}): 3059 (CH aromatic), 2925 (CH aliphatic), 2221 (CN), 1754 (CO) ester, 1651 (CO) amide; MS (*m/z*) 427/425. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 1.8 (t, 3H, CH_3), 4.3 (s, 2H, S-CH_2), 5.1 (s, 2H, CH_2OCO), 6.2 (q, 2H, NCH_2N), 6.74–7.99 (m, 7H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6), δ 18.3 (CH_3), 49.6 (SCH_2N), 61.2 (CH_2O), 68.6 (NCH_2N), 102.2 (C_5, pyrim), 116.5 (CN), 122.4 ($2C_{3,5}$,Ar), 127.6 (C_1, Ar), 128.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 131.6 ($2C_{2,6}$, Ar), 136.8 (C_2, thioph), 157.8 (C_4–N, Ar), 159.8 (C_4, pyrim), 161.2(C fusedring), 166.3 (CO, pyrid), 168.2 (COO); Anal. Calcd for $C_{20}H_{16}N_4O_3S_2$ (424.5): % C, 56.59; % H, 3.80; % N, 13.20; Found: % C, 56.37; % H, 3.69; % N, 13.09.

2.1.4.5. 6-Oxo-8-(thiophen-2-yl)-3-(p-tolyl)-3,4-dihydro-2h,6h-pyrimido[2,1-b] [1,3,5]thiadiazine-7-carbonitrile (4e). Yield (70%); Pale yellow crystal; m.p. 218–220 °C (EtOH); IR (cm^{-1}): 3101 (CH aromatic), 2916, 2858 (CH aliphatic), 2218 (CN), 1660 (CO); MS (*m/z*) 369/366. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 4.46 (s, 2H, S-CH_2-N), 5.65 (s, 2H, N-CH_2-N),7.01–7.30 (m, 3H, Ar-H), 8.06 (d, 2H, Ar-H, J = 8 Hz), 8.19 (d, 2H, Ar-H, J = 8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 41.2 (CH_3), 55.7 (CH_2S), 68.8 (CH_2N_2), 102.2 (C_5, pyrid), 118.5 (CN), 122.1 ($2C_{3,5}$,Ar), 127.2(C_1, Ar), 127.9 ($2C_{3,4}$, thioph), 130.6 ($2C_{2,6}$, Ar), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 157.8 (C_4–N, Ar), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Cal. for $C_{18}H_{14}N_4OS_2$ (366.46): % C, 59.00; % H, 3.85; % N, 15.29; Found: % C, 58.76; % H, 3.76; % N, 15.08.

2.1.4.6. 2-(((Naphthalen-2-ylamino)methyl)thio)-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (5). Yield (85%); reddish brown crystals; m.p. 220–222 °C (EtOH); IR (cm^{-1}): 3346 (NH), 3061 (CH aromatic), 2966, 2876 (CH aliphatic), 2209 (CN), 1667 (CO); MS (*m/z*) 393/390. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 4.61 (s, 2H, CH_2), 7.06–8.15 (m, 10H, Ar-H), 8.30 (s, 1H, NH), 9.95 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 54.7 (CH_2S), 102.2 (C_5, pyrid), 116.5 (CN), 122.5 ($4C_{3,4,5,6}$, naph), 127.9 ($2C_{3,4}$, thioph), 129.5, 131.6 ($2C_{2,6}$, Ar), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 148.3 ($C_{7,8}$–O, naph), 157.8($C_{1,10,}$ naph), 159.2 (C fusedring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Cal. for $C_{20}H_{14}N_4OS_2$ (390.48): % C, 61.52; % H, 3.61; % N, 14.35; Found: % C, 61.28; % H, 3.50; % N, 14.18.

2.1.5. General procedure for the preparation of compounds 6 and 7

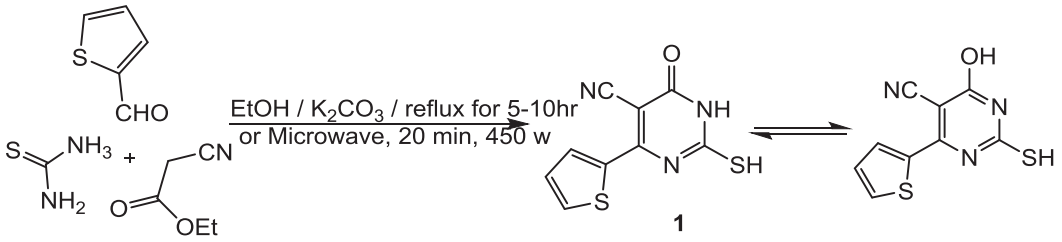
A mixture of compound **1** (0.01 mol), 2,3-dichloroquinoxaline, *p*-toluene sulphonyl chloride (0.01 mol) in the presence of anhydrous potassium carbonate in DMF (20 mL), was added and the reaction mixture was heated under reflux for 3–5 h for compound **6** and stirred at room temperature for compound **7**. The reaction mixture was cold; the precipitate was collected by filtration and recrystallized from absolute ethanol.

2.1.5.1. 4-Oxo-2-((3-oxo-3,4-dihydroquinoxalin-2-yl)thio)-6-(thiophen-2-yl)-1,4-dihy dropyrimidine-5-carbonitrile (6). Yield (89%); brown crystals m.p. 284–286 °C (EtOH); IR (KBr, cm^{-1}): 3190, 3147 (2NH), 3049 (aromatic C-H), 1643 (C=O), 1611 (C=N), 1549, 1504 (C=C); ^{1}H NMR (125 MHz, DMSO-d_6) δ ppm: 7.27–8.30 (m, 7H, Ar-H), 10.56 (s, 1H, NH, D_2O exchangeable), 11.02 (s, 1H, NH-CO, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 102.2 (C_5, pyrid), 116.5 (CN), 122.4 ($2C_{4,5}$,Ar), 127.6 ($2C_{3,6}$, Ar), 128.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 141.6 ($2C_{1,2}$, Ar fused ring), 152.3 (C-S), 159.8 (C_2, pyrid), 164.2 (CO), 166.3 (CO, pyrid), 168.2 (C_4, pyrid); Anal. Calcd. for $C_{17}H_9N_5O_2S_2$ (379.41): % C, 53.82; % H, 2.39; % N, 18.46; Found: % C, 53.60; % H, 2.25; % N, 18.37.

2.1.5.2. 4-Oxo-6-(thiophen-2-yl)-2-thioxo-1,3-ditosyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (7). Yield (75%); reddish brown crystal; m.p. 212–214 °C (EtOH); IR (cm^{-1}): 3098 (CH aromatic), 2218 (CN), 1651 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.71, 2.78 (s. s, 6H, $2CH_3$, 7.25–8.31 (m, 11H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6), δ 33.2 ($2CH_3$), 102.2 (C_5, pyrid), 116.5 (CN), 127.8 ($4C_{3,3',5,5'}$, Ar), 128.6 ($2C_{3,4}$, thioph), 128.9 ($4C_{2,2',6,6'}$ Ar), 130.8 (C_5, thioph), 131.2 ($2C_{4,4'}$, Ar), 136.8 (C_2, thioph), 131.2 ($2C_{1,1'}$, Ar), 159.8 (C_2, pyrid), 166.3 (CO, pyrid), 168.2(C_4, pyrid); 177.4 (C=S), Anal. Calcd. for $C_{23}H_{17}N_3O_5S_4$ (543.65): % C, 50.81; % H, 3.15; % N, 7.73; Found: % C, 50.59; % H, 3.05; % N, 7.61.

2.1.6. Formation of Ethyl 2-((5-cyano-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidin-2-yl) thio)acetate (8)

To a solution of compound **1** (0.01 mol)) and potassium carbonate (20 mmol) in ethanol (20 mL), ethyl chloroacetate (0.02 mol)) was added and the reaction mixture was heated under reflux for 3–5 h. The reaction mixture was left to cool, poured onto ice water. The precipitate was collected by filtration and



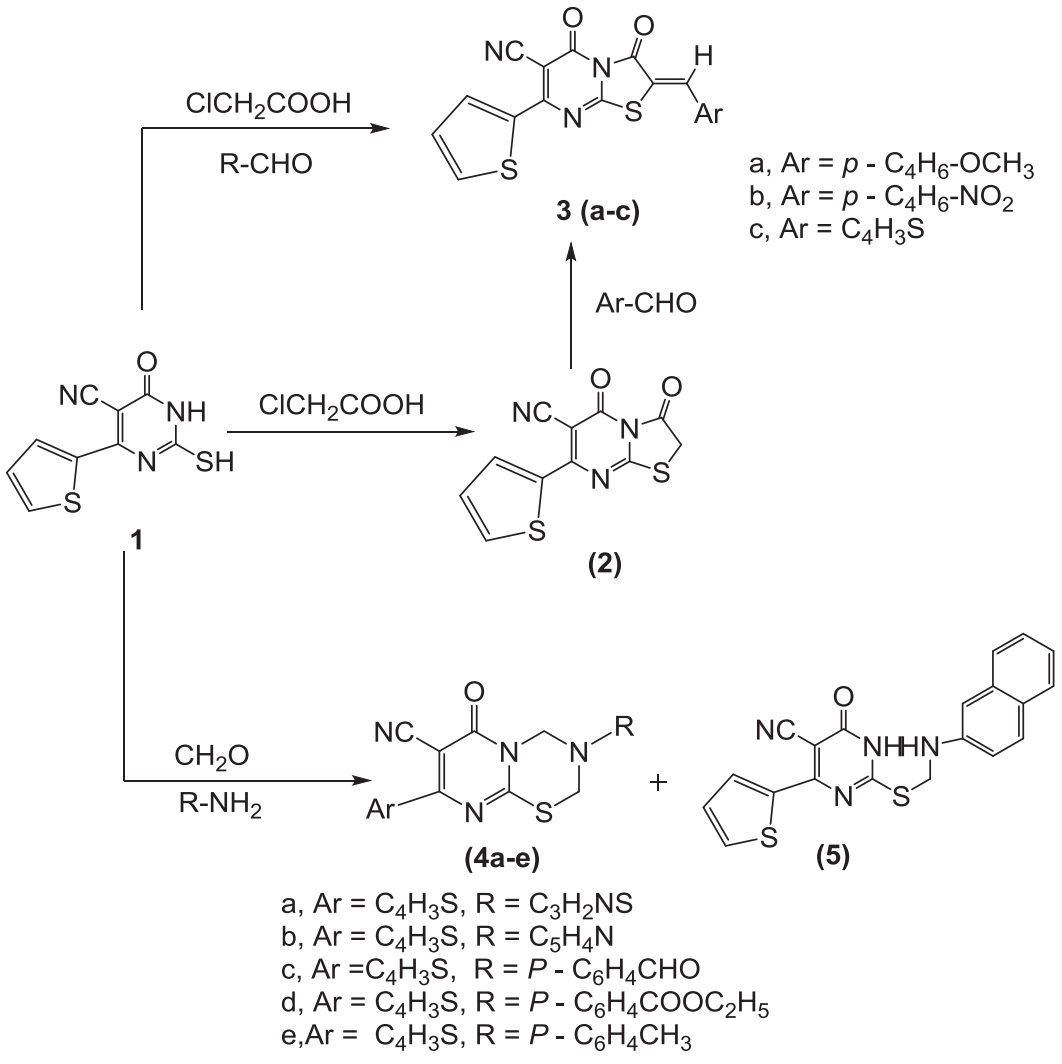
Scheme 1. Synthetic pathway for starting compound **1.**

recrystallized from absolute ethanol.

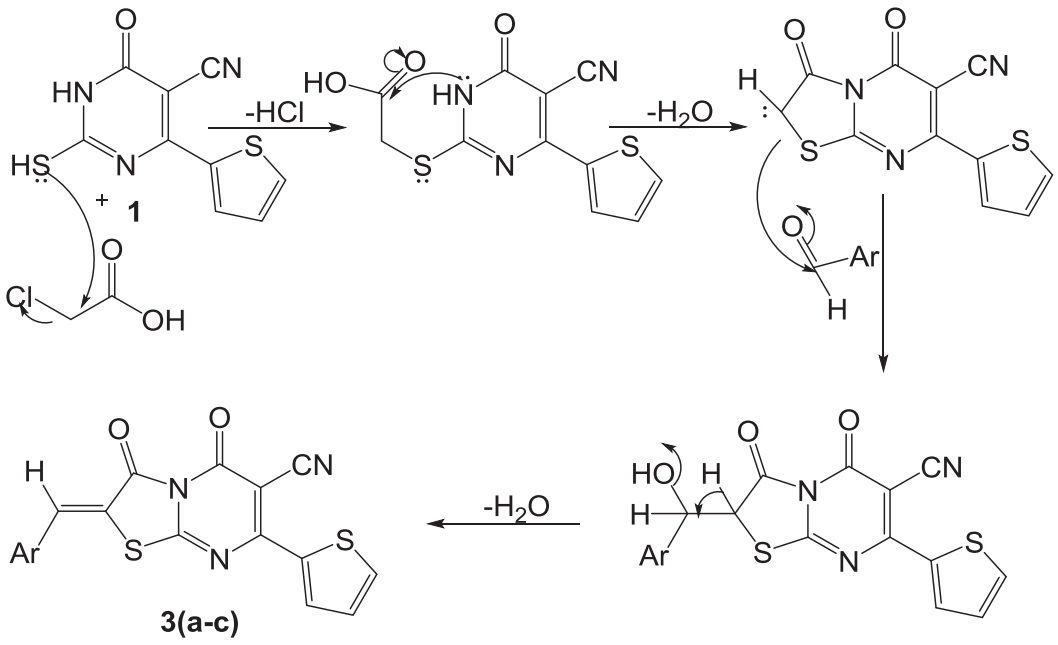
Yield (77%); White crystal; m.p. 212–213 °C (EtOH); IR (cm^{-1}): bro 3222 (NH), 3098 (CH aromatic), 2935, 2871 (CH aliphatic), 2221 (CN), 1746 (CO ester), 1662 (CO amide); ^{1}H NMR (125 MHz, DMSO-d_6) δ (ppm): 1.25 (t, 3H, $2CH_3$, J = 7.2 Hz), 4.12 (q, 2H, 2 CH_2, J = 6 Hz), 4.51 (s, 2H, CH_2), 7.33 (m, 1H, Ar-H), 8.04 (d, 1H, Ar-H, J = 8.4 Hz), 8.12 (d, 1H, Ar-H, J = 8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.3 (CH_3), 28.2 (CH_2), 68.7 (CH_2S), 102.2 (C_5, pyrid), 116.5 (CN), 128.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 159.8 (C_4, pyrid), 161.2, 166.3 (CO, pyrid), 168.2 (COO); Anal. Calcd for $C_{13}H_{11}N_3O_3S_2$ (321.46): % C, 48.59; % H, 3.45; % N, 13.08; Found: % C, 48.28; % H, 3.34; % N, 12.95.

2.1.7. Formation of 2-hydrazinyl-6-oxo-4-(thiophen-2-yl)-1,6-dihydropyrimidine-5-carbonitrile (9)

A mixture of **1** (0.01 mol)) and hydrazine hydrate (0.02 mol) was heated for 8 h and then allowed to cool at room temperature. The solid product was collected by filtration and recrystallized from ethanol to give the title compounds. Yield (79%); colorless crystals; m.p. 292–294 °C (EtOH); IR (cm^{-1}): 3315-3236 ($2NH + NH_2$), 3093 (CH aromatic), 2942 (CH aliphatic), 2209 (CN), 1667 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.29 (bs, 2H, $NH\underline{NH_2}$), 7.18 (m, 1H,

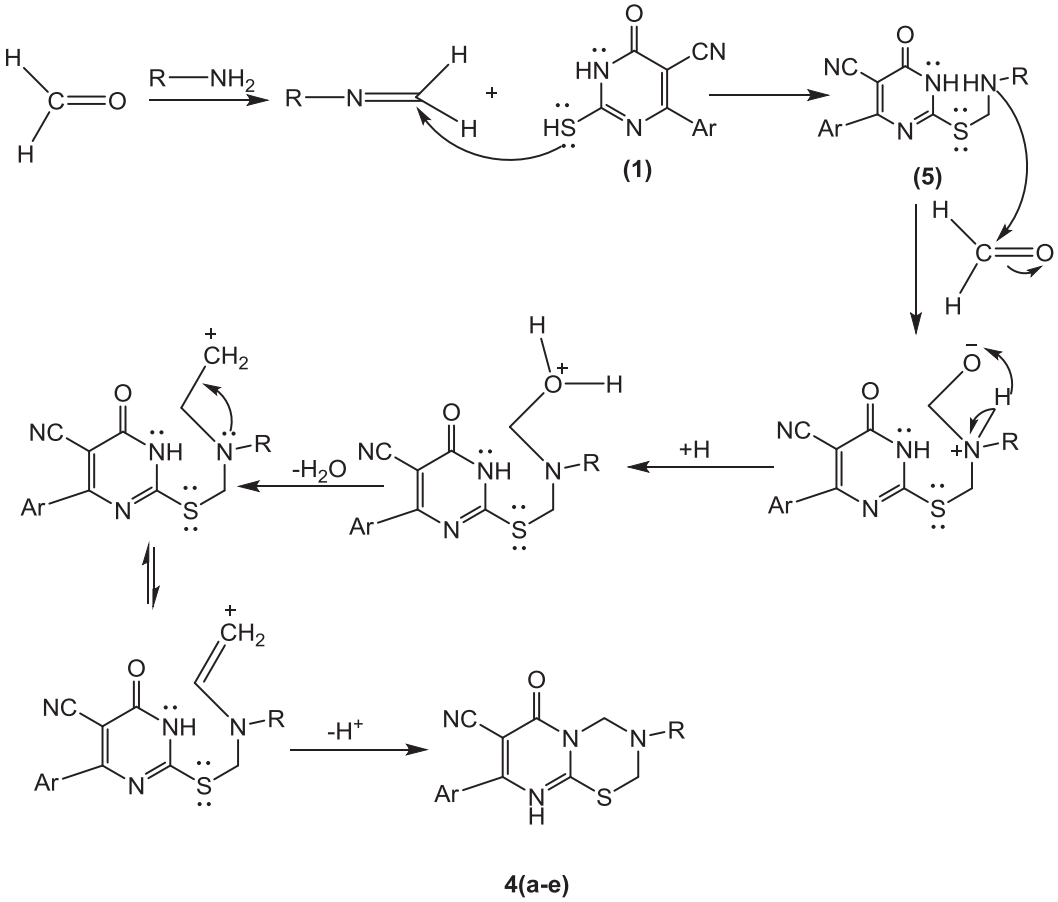


Scheme 2. General procedure for synthesis of target compounds **2**–**5**.



Scheme 3. The proposed mechanism for synthesis of compound **3**.

Ar-H), 7.80 (d, 1H, Ar-H, $J = 8$ Hz), 8.06 (d, 1H, Ar-H, $J = 8$ Hz), 10.20 (bs, 1H, NH), 11.53 (bs, 1H, NH-C=O); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.2 (C_5, pyrid), 116.5 (CN), 128.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 157.8 (C_2, pyrid), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Cal. for $C_9H_7N_5O_S$ (233.04): % C, 46.34; % H, 3.03; % N, 30.03; Found: % C, 46.06; % H, 3.12; % N, 29.92.



Scheme 4. The suggested mechanism for Mannish reaction of thiouracil **1** with 2 mol of formaldehyde and primary amines as follows.

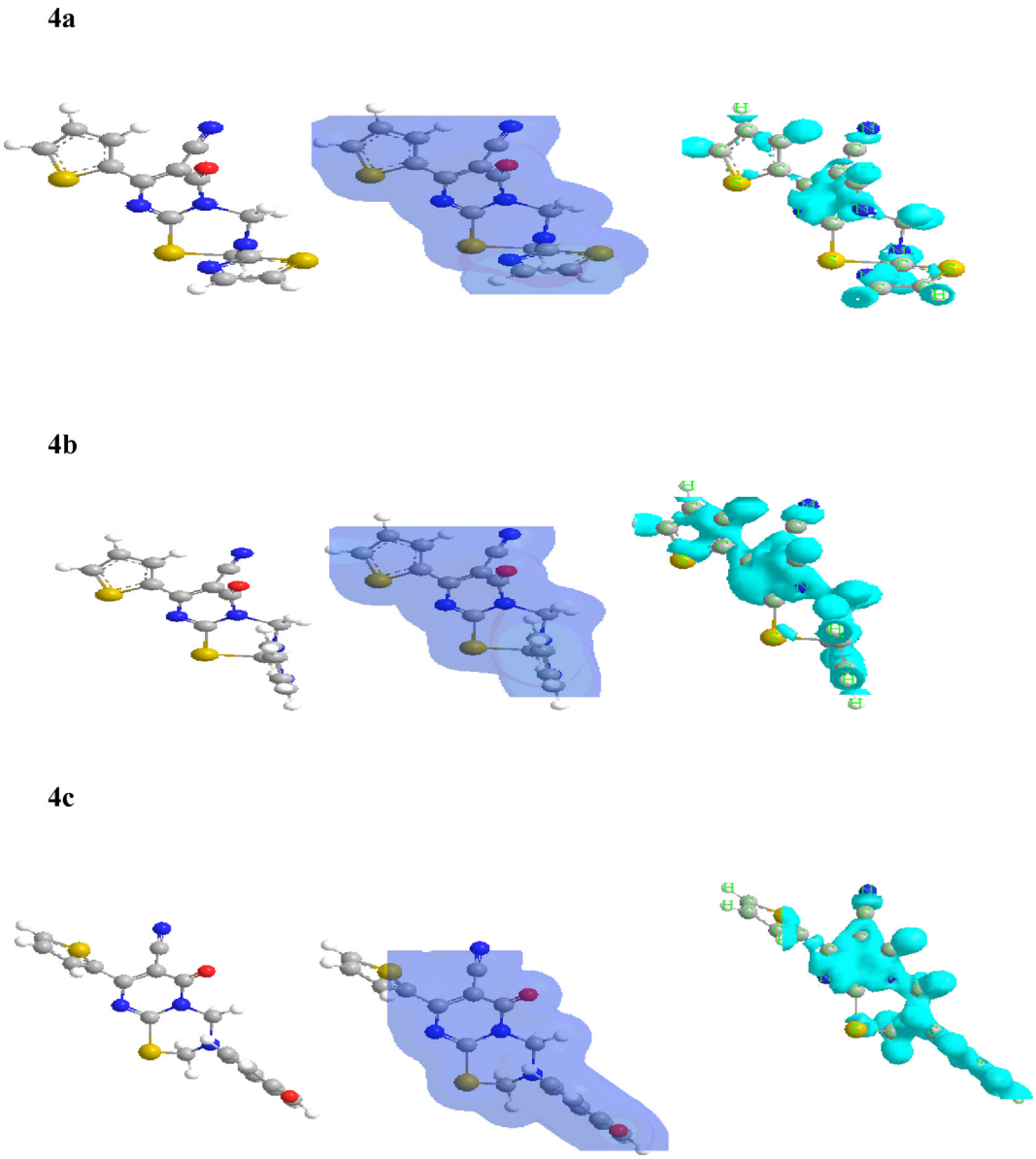


Fig. 1. Optimized structures (left), overall molecular charge distribution overlapped electron density of R with attached amino group (middle) and their solvation with Solvent of reaction (right) obtained for the synthesized compounds **4** and **5**. Color index: White H, Grey C, Blue N, Red O, yellow S and Cyan MeCN solvent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.1.8. General procedure for the preparation of compounds 10, 11 and 12

A mixture of **9** (0.01 mol)) was refluxed with acetyl chloride, benzoyl chloride and phenylisothiocyanate (10 mmol) in (30 mL) ethanol for 5–8 h. The reaction mixture was allowed to cool at room temperature. The solid product was filtered, drying and recrystallized from ethanol to give compounds **10, 11** and **12.**

2.1.8.1. 3-Methyl-5-oxo-7-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo [4,3-a] pyrimidin e-6-carbonitrile (10). Yield (75%); colorless crystals; m.p. 270–272 °C (EtOH); IR (cm^{-1}): 3321 (NH), 3072 (CH aromatic), 2940 (CH aliphatic), 2207 (CN), 1713 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.89 (b. s, 1H, NH), 2.71 (s, 3H, CH_3), 7.95 (m, 1H, Ar-H), 7.96 (d, 1H, Ar-H, J = 8 Hz), 8.23 (d, 1H, Ar-H, J = 8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 44.3 (CH_3), 102.2 (C_5, pyrid), 118.5 (CN), 127.9 ($2C_{3,4}$, thioph), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 152.8 (C, triaz), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Calcd for $C_{11}H_7N_5OS$ (257.30): % C, 51.35; % H, 2.74; % N, 27.22; Found: % C, 51.10; % H, 2.62; % N, 27.11.

2.1.8.2. 5-Oxo-3-phenyl-7-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo[4,3-a] pyrimidin e-6-carbonitrile (11). Yield (78%); colorless crystals; m.p. 256–258 °C (EtOH); IR (cm^{-1}): 3224 (NH), 3081 (CH aromatic), 2919 (CH aliphatic), 2215 (CN), 1700 (CO); MS (m/z) 320/319; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.95 (m, 1H, Ar-H), 7.96 (d, 1H, Ar-H, J = 8 Hz), 7.99–8.20 (m, 5H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8 Hz), 10.49 (b. s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 101.93 (C_5, pyrid), 107.88 (CN), 122.41 ($2C_{3,5}$,Ar), 123.86 (C_1, Ar), 126.27 ($2C_{3,4}$, thioph), 131.6 ($2C_{2,6}$, Ar), 130.8 (C_5, thioph), 136.8 (C_2, thioph), 147.8 (C_4, Ar), 150.8 (C, triaz), 154.2 (C fused ring), 159.8 (C_4, pyrid), 166.67 (CO, pyrid); Anal. Cal. for $C_{16}H_9N_5OS$ (319.34): % C, 60.18; % H, 2.84; % N, 21.93; Found: % C, 59.88; % H, 2.71; % N, 21.85.

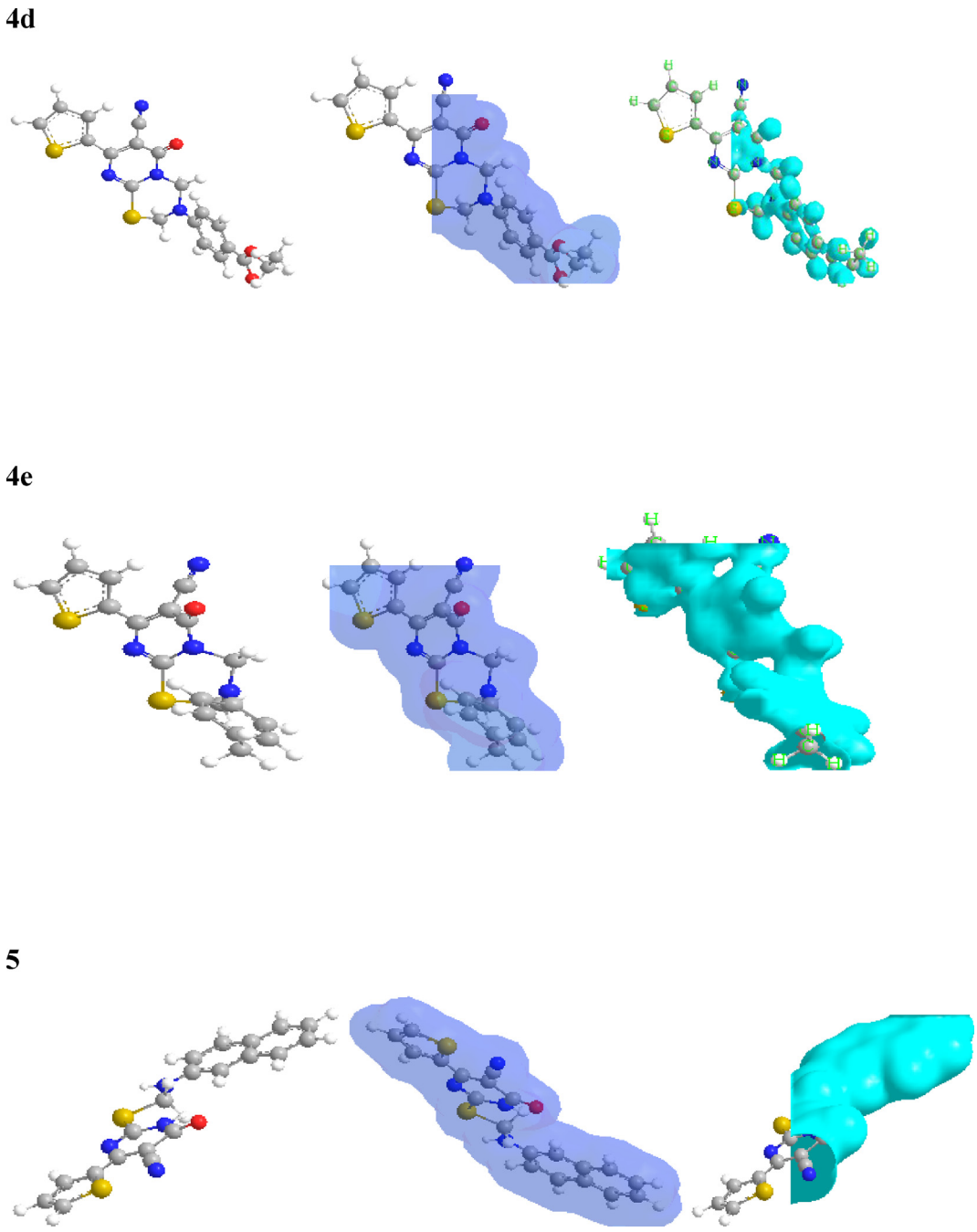


Fig. 1. (*continued*).

2.1.8.3. 5-Oxo-3-phenyl-7-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo[4,3-a] pyrimidin e-6-carbonitrile. (12). Yield (79%); colorless crystals; m.p. 298–300 °C (EtOH); IR (cm^{-1}): 3228-3126 (4NH), 3028 (CH aromatic), 2975 (CH aliphatic), 2215 (CN), 1700 (CO); MS (*m*/*z*) 336/334. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.96 (b. s, 1H, NH), 6.98–7.32 (m, 5H, Ar-H), 7.51 (m, 1H, Ar-H), 7.77 (d, 1H, Ar-H, J = 8 Hz), 8.07 (d, 1H, Ar-H, J = 8 Hz), 9.77 (b.s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.2 (C_5, pyrid), 118.5 (CN), 121.1 ($2C_{3,5}$,Ar), 127.8 (C_1, Ar), 28.9 ($2C_{3,4}$, thioph), 131.6 ($2C_{2,6}$, Ar), 131.8 (C_5, thioph), 137.8 (C_2, thioph), 141.3 (C_4, Ar), 152.8 (C, triaz), 159.2 (C fused ring), 159.8 (C_4, pyrid), 166.3 (CO, pyrid); Anal. Cal. for $C_{16}H_{10}N_6OS$ (334.4): % C, 57.48; % H, 3.01; % N, 25.14; Found: % C, 57.22; % H, 2.92; % N, 25.04.

2.2. Antimicrobial activity

Chemical compounds were individually tested against a panel of two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) using Muller Hinton agar medium (Oxoid). The anti-fungal activities of the compounds were tested against two fungi (*Candida albicans*and *Aspergillus flavus*) using Sabouraud dextrose agar medium (Oxoid). 1 mg/mL solutions of each compound were prepared in DMSO and added Whatman filter paper disc of standard size (5 cm). The disks were sterilized in autoclave [32]. The treated paper discs were soaked in petri dishes containing Muller Hinton agar medium seeded with *Staphylococcus*

aureus, Bacillus subtilis, E. coli, Pseudomonas aeuroginosa and Sabouraud dextrose agar medium seeded with *Candida albicans*, *Aspergillus flavus*. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin and antifungal Clotrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under:

$$\%\,Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)} \times 100$$

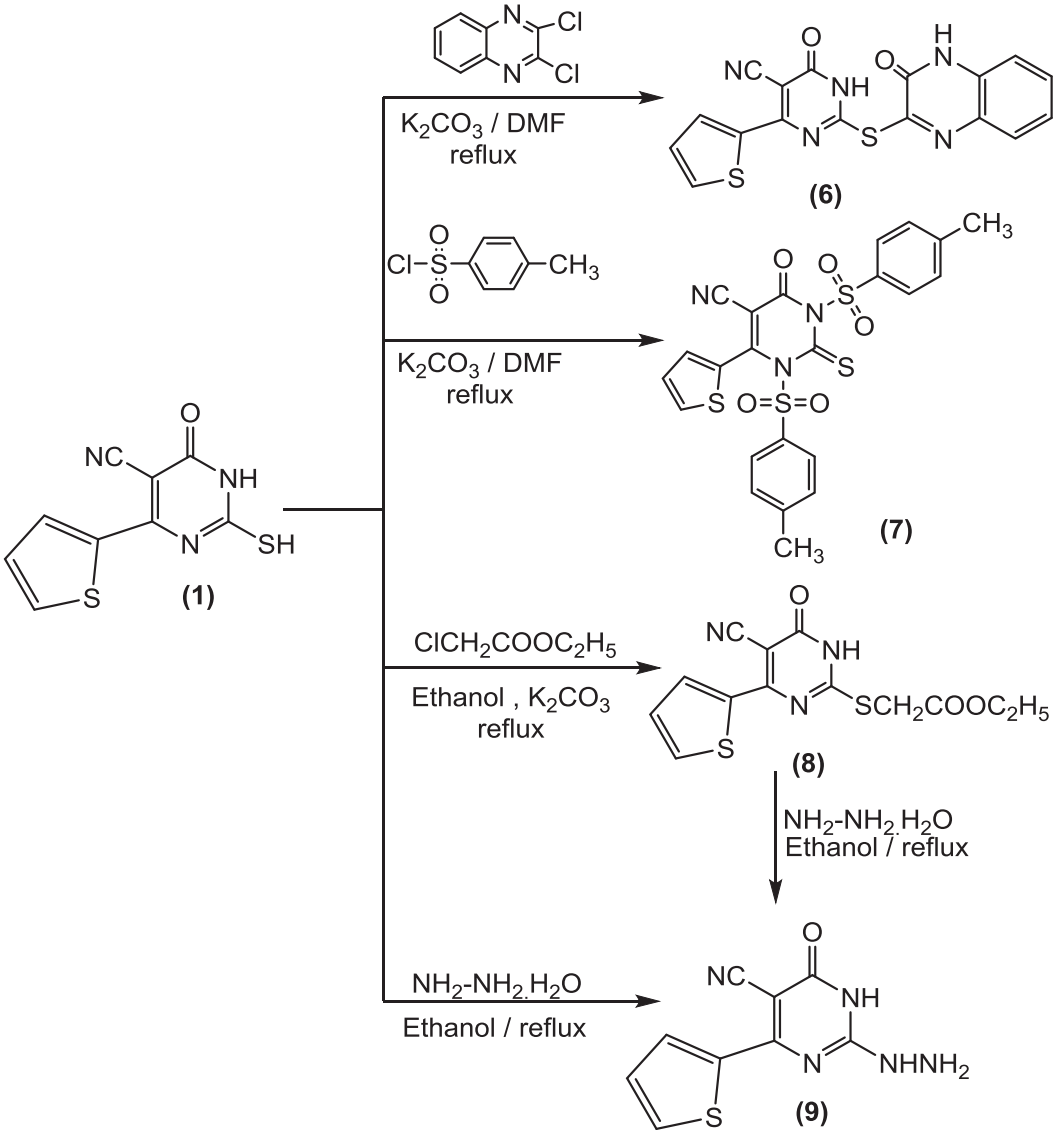
2.3. Computational methods

DFT studies were carried out for the Mannich compounds (4 and 5) using Materials Studio 6.0 (MS 6.0) software from Accelrys, Inc. DMol3 module was used to perform the DFT calculations using Perdew and Wang LDA exchange-correlation functional and DND basis set. The calculated parameters involved the electron density, dipole moment and Frontier molecular orbitals and the molecular surface area. Frontier molecular orbitals include the highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMOs) [33].

3. Result and discussion

3.1. Chemistry

The synthetic pathway adopted to obtain the starting compound **1** was depicted in (Scheme 1). The structure of the synthesized compound was established based on their elemental analyses and spectral data. One-pot reaction condensation of aromatic aldehydes with ethylcyanoacetate and thiourea afforded 1,6-dihydro-2-mercapto-6-oxo-4-arylpyrimidine-5-carbonitrile according to the reported procedure [34,35]. The IR spectrum of starting compound was characterized by the presence of NH stretching bands at 3410 cm^{-1}, C≡N bands at 2214 cm^{-1} along with C=O bands at



Scheme 5. General procedure for synthesis of target compounds **6**–**9**.

1652 cm^{-1} and C=S bands at 1425 cm^{-1}.

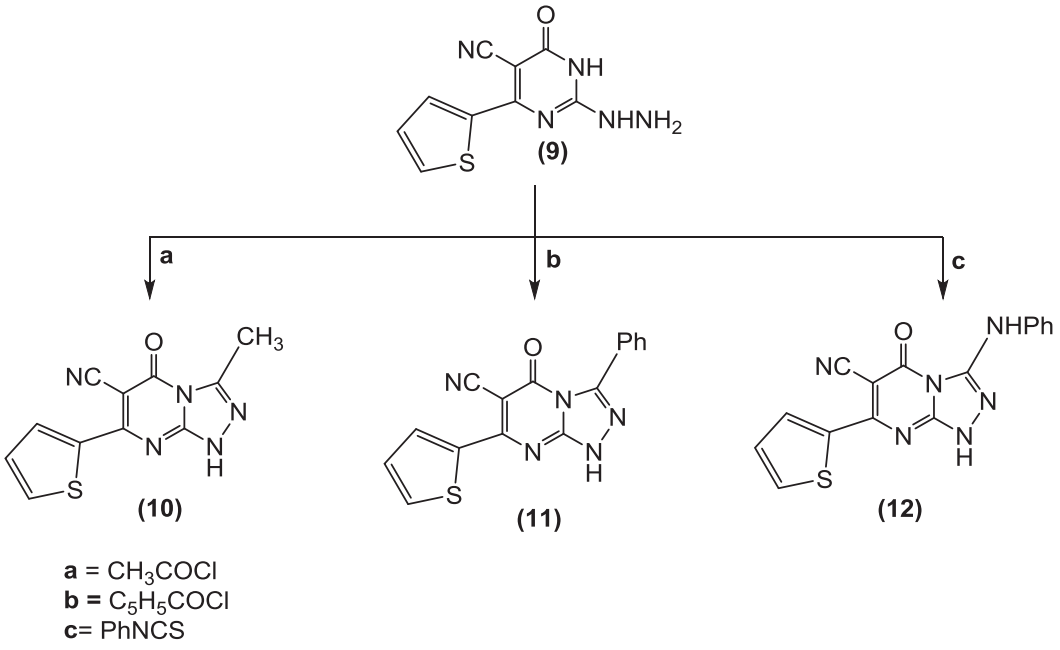
Multicomponent reactions (MCRs) of thioxopyrimidine **1** and chloroacetic acid linked with diverse aromatic aldehyde e.g. *p*-methoxy-benzaldehyde, *p*-nitro-benzaldehyde and thiophene-2-carboxaldehyde, in mixed solvent afforded various thiazolopyrimidine derivatives **3a-c.** Such compounds were previously conventionally prepared *via* stepwise reaction progressions [36,37].

The structures of compounds **3a-c** were confirmed by their analytical and spectral data. The IR spectra of **3a-c** showed disappearance of NH band with presence of characteristic absorption bands at 1728 cm^{-1} and 1635 cm^{-1}. These bands pointed to the vibrational coupling of the carbonyl groups revealing existence of successful cyclization route. The course of cyclization may be due to the steric hindrance of arylidine group [38]. ^{1}H NMR spectra for this group exhibited singlet band corresponding to the ethylinic protons at the range 7.65–9.8.46 ppm. Further evidence was gained from their mass spectra that showed the correct molecular ion peaks beside some of abundant peaks (cf. experimental). The mechanistic pathway for the transformation of compound **1** to compound **3** was represented in (Scheme 3) [39].

In this study, we focused on the susceptibility and selectivity of the cyclization 4-oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **1** towards the double Mannich reaction. The synthesis of N-Mannich bases of the type 1, based on the reaction of compound 1 with formaldehyde and primary amines (2-aminothiazole, 2-aminopyridine, 4- amino-benzaldehyde, ethyl-4-amino benzoate, 4-toluidine or 2-naphthylamine) gave the compounds of 3-aryl-pyrimido[2,1-b][1,3,5]thiadiazine-7-carbonitrile (4a-e) and 2-Naphthalen-2-yl aminomethylthio-1,6-dihydropyrimidine-5-carbonitrile (5), respectively (Scheme 2), with good yields. The reactions were carried out in acetonitrile (MeCN) at room temperature afforded the products (**4a-e)** *via* S- and N-cyclo-alkylation by addition of 2 mol of formaldehyde and compound (**5)** *via* S-alkylation with simultaneous addition of 1 mol according to the mechanism (Scheme 4) [40]. The yields of the Mannich condensation seem to be directly correlated to the promoted charge density of the substituted precursors in the position 3 of the pyrimidine moiety(Ar). The high yield of compounds **4a-d** are depended on the charge density of the aryl group (Fig. 1). The attack of the thiol and amino groups to the carbonyl group of the formaldehyde and the stability of products follow the order: 4d > 4c > 4a > 4e [41]. The ^{1}H NMR spectra of heterocycles **4a-e** in the region of 4.3–6.8 ppm had two singlet signals of methylene protons located between the heteroatoms, which is typical for [1,3,5] thiadiazine system [42]. In the IR spectrum of product **5** exhibited the absorption band of carbonyl at 1667 cm^{-1} and 1701 cm^{-1}. The absorption band of carbonyl group remained and the bands of stretching vibrations of NH groups disappeared compared with the IR spectra of the starting thiouracils **1**. However, the ^{1}H NMR spectrum of compound **5** revealed two singlet bands at 7.70 and 9.95 ppm for 2NH protons. Solvation of the product **5** and the lower electron density of the naphthylamino group restricted its interaction with another formaldehyde molecule (Fig. 1).

The alkylation of thiopyrimidone **1** with different carbon electrophiles e.g. 2,3-dichloroquinoxaline, *p*-toluene sulphonyl chloride and ethylchloroacetate gave S-, N- alkylated derivatives **6**, **7** and **8** (Scheme 5).

The IR spectra of compounds 6 and 7 showed absorption bands at (2218, 2221) and (1643, 1651) cm^{-1} corresponding to C≡N and C=O groups, respectively. The IR spectrum of compound **8** showed two strong absorption bands at 1746 and 1662 cm^{-1} assigned to two C=O groups and broad band at 3220 cm^{-1} corresponding to NH. ^{1}H NMR spectrum of such compound showed two singlet bands at δ = 10.56 and 11.02 ppm corresponding to the two NH. Hydrazinolysis of compound **1** displayed aminopyrimidine derivative **9**. IR spectrum of compound **9** showed absorption bands at 3315-3236 cm^{-1} corresponding to NH and NH_2, in addition to two bands at 2209 cm^{-1} and 1667 cm^{-1} corresponding to C≡N and C=O groups, respectively. Its ^{1}H NMR spectrum showed three singlets at 3.29 and 11.15 ppm for NH_2 and NH, respectively. Acylation and addition reaction of compound **9** with acetyl chloride, benzoyl chloride and phenylisothiocyanate afforded triazolopyrimidine derivatives **10**, **11** and **12** respectively (Scheme 6). The structures were elucidated by their IR and ^{1}H NMR spectra. IR spectra of compounds 10–12 showed absorption bands at (2207-2015) and (1700–1713) cm^{-1} corresponding to C≡N and C=O groups, respectively. Whereas ^{1}H NMR spectrum of compounds **10** and **11** showed a broad singlet at 10.49 ppm corresponding to NH.



Scheme 6. Synthesis of heterocycles **10**–**12**.

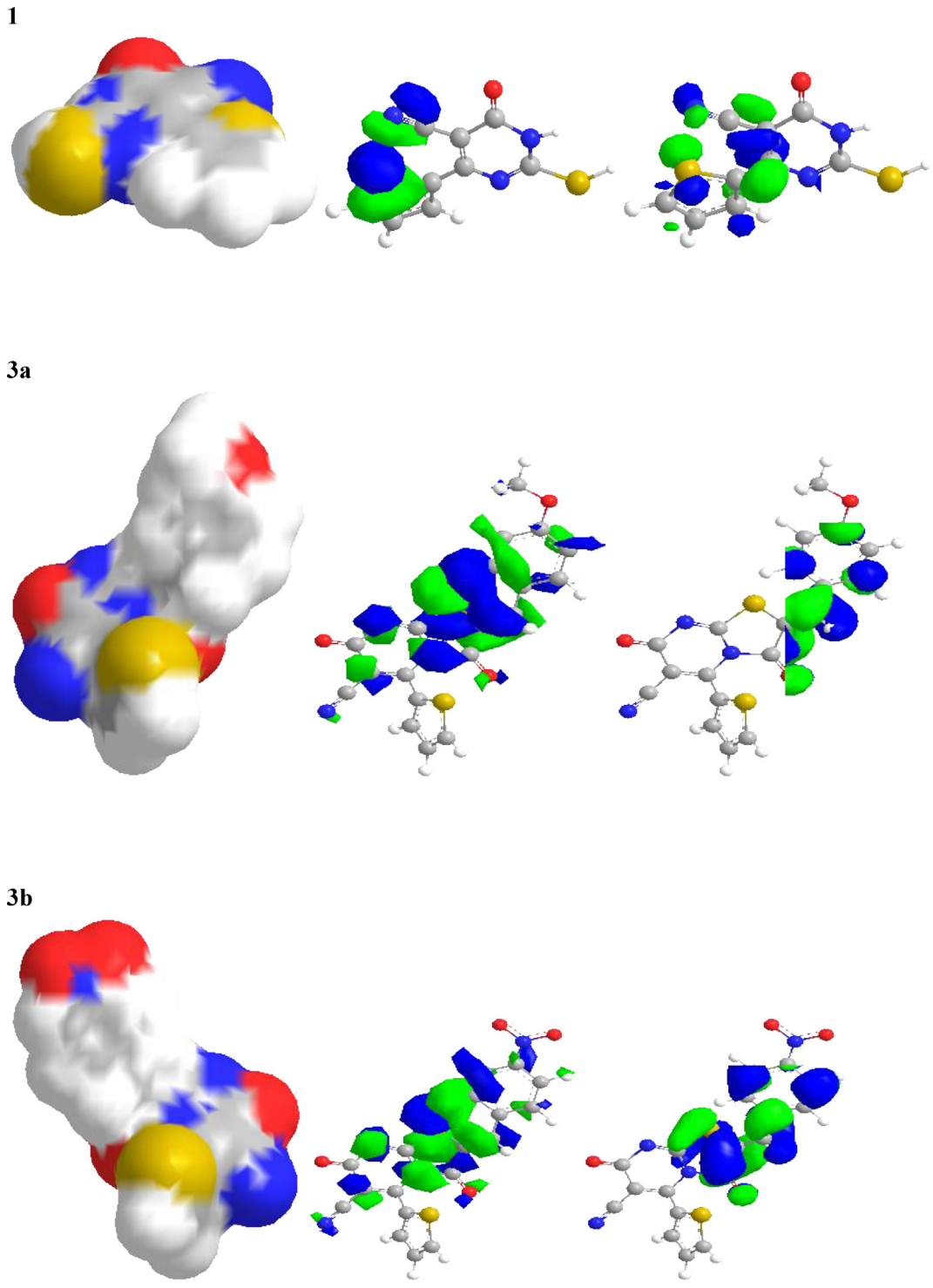


Fig. 2. Surface area (left), HOMO (middle) and LUMO (right) for most potent antimicrobial compounds. Color index: white and grey surface (lipophilicity position), blue, red and yellow surface (hydrophilicity position). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, ^{1}H NMR spectrum of compounds **12** showed two singlets at 3.24 and 10.49 ppm corresponding to 2NH.

3.2. *DFT "studies"*

The optimized geometries of 1,3,4-thiadiazine derivatives in addition to their solvation with acetonitrile (MeCN) displayed wholly distributed over every molecular structure (Fig. 1). Frontier molecular orbitals possess the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) beside their surface area (Fig. 2). The regions of highest electron density (HOMO) represents the electrophilic-attacking sites, whereas the LUMO reflects the nucleophilic-attacked sites [43,44]. However, the electron donating amino-groups (HOMO) in the tolyl and naphthyl moieties didn't attack the electrophilic site e.g. carbonyl group of the formaldehyde due to their high solvating

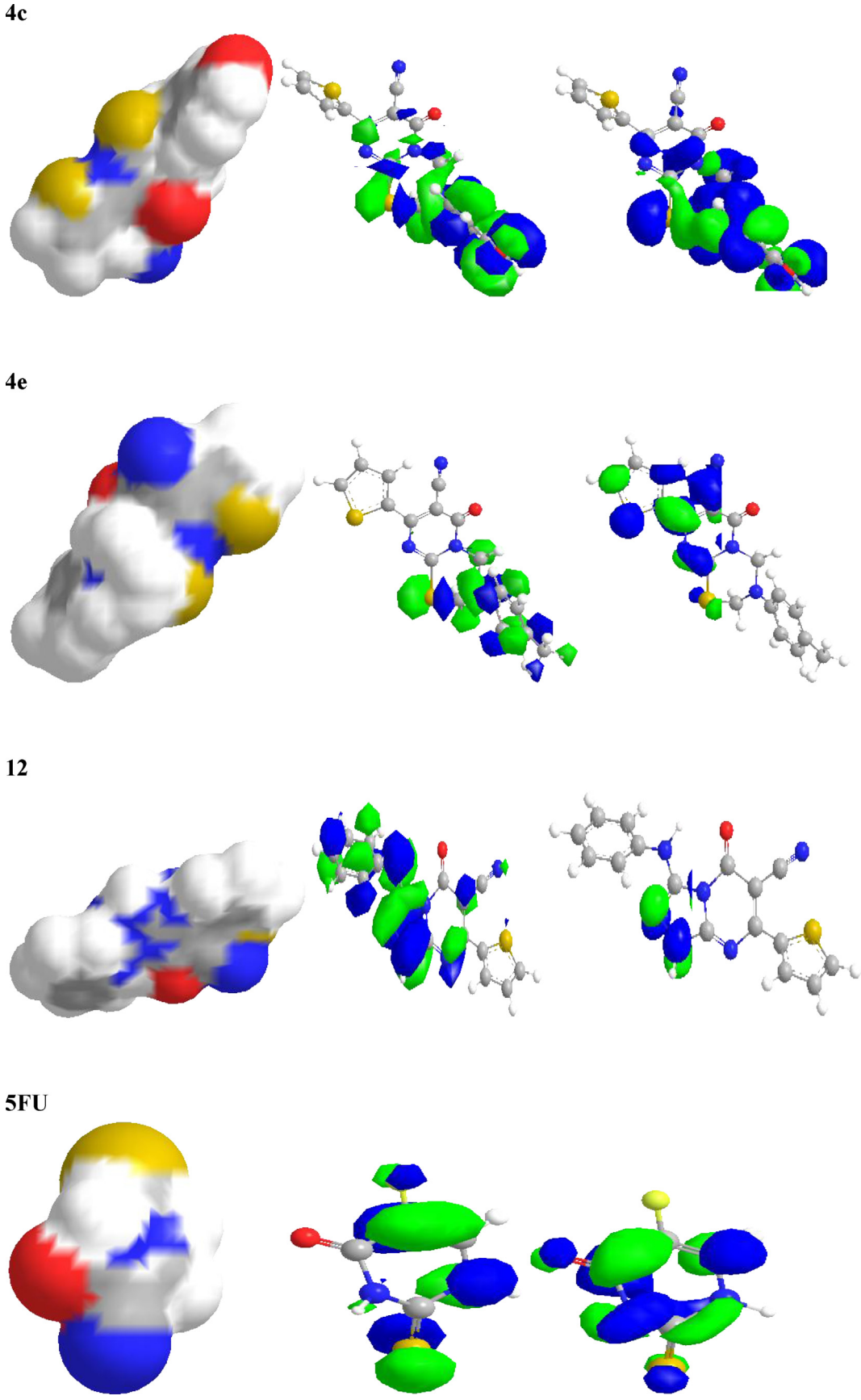


Fig. 2. (*continued*).

Table 1
DFT parameters calculated for the synthesized compounds.

Compd number	E_{HOMO} (eV)	E_{LUMO} (eV)	$\Delta E_{(LUMO-HOMO)}$	Dipole moment μ (Debye)	Lipophilicity coeff. Log P	Polarizability pol (A3)	Hydration E (k cal mol^{-1}	Surface area, A,(nm^2)	Nucleophilicity, ω (eV)
3b	−5.88	3.23	9.03	1.65	0.64	23.37	−19.23	1183.34	4.12
4b	−5.87	3.21	9.08	1.11	0.25	21.87	−19.13	909.23	4.34
4c	−5.74	2.23	7.97	1.33	0.63	24.13	−18.25	1122.25	5.24
4d	−5.43	2.18	7.61	0.98	0.43	25.87	−16.23	1034.24	6.38
4e	−5.64	1.82	7.46	1.87	0.72	34.71	−27.34	1187.32	3.27
5	−4.07	−1.76	2.31	2.65	0.79	23.37	−35.23	1336.34	2.11
9	−3.95	−1.75	2.20	3.11	0.45	31.87	−37.13	1013.83	2.32
10	−3.18	−1.68	1.50	3.98	0.43	78.87	−28.23	1036.24	6.33
11	−2.68	−1.72	0.96	4.11	0.45	87.51	−30.14	1038.34	4.24
12	−10.9	−4.82	6.09	3.87	0.55	64.71	−27.34	1338.32	7.22

Table 2
Antimicrobial activity and diameter inhibition zone (mm) of the synthesized compounds (NA → No Activity).

Compound no	Diameter of inhibition zone (mm), % Activity index					
	E. coli	*S. aureus*	*Bacillus subtilis*	*C. Albicans*	*C. Albicans*	*A. flavus)*
1	18 (72)	21 (91.3)	20 (86.9)	19 (82.6)	22 (84.6)	22 (88)
2	17 (68)	21 (91.3)	10 (43.5)	9(39.1)	11 (42.3)	9 (36)
3a	12 (46)	13(54.3)	13 (56.8)	13(56)	15 (55.8)	18 (66)
3b	11(44)	12 (52.2)	13(56.5)	12(52.2)	16(61.5)	17(68)
4a	6 (24)	6 (26.1)	15 (65.2)	17(73.9)	17 (65.4)	18 (72)
4b	NA	2 (8.7)	8 (34.8)	7 (30.4)	13 (50)	12 (48)
4c	15 (60)	16 (69.6)	18 (78.3)	19 (82.6)	20 (76.9)	5 (20)
4d	NA	NA	NA	NA	2 (7.7)	18 (72)
4e	6 (24)	6 (26.1)	15 (65.2)	17 (73.9)	17 (65.4)	18 (72)
5	13 (52)	12 (52.2)	12 (52.2)	10 (43.5)	14 (53.8)	15 (60)
6	5 (20)	4 (17.4)	4 (17.4)	5 (21.7)	5 (19.2)	6 (24)
7	NA	2 (8.7)	NA	NA	NA	NA
8	8 (32)	8 (34.8)	6 (26.1)	6 (26.1)	7 (26.9)	7 (28)
9	9 (36)	10(43.5)	7 (30.4)	6 (26.1)	9 (34.6)	7 (28)
10	NA	NA	NA	NA	2 (7.7)	4 (16)
11	6 (24)	7 (30.4)	2 (9)	3 (13.0)	3 (11.5)	5(20)
12	19 (76)	22 (95.6)	22 (95.6)	21 (91.3)	25 (96.1)	23 (92)
Ampicillin	25 (100)	23 (100)	23(100)	23 (100)	NA	NA
Colitrimazole	NA	NA	NA	NA	26 (100)	25(100)

behavior to afford the products **4e** and **5,** respectively (Fig. 1). As the dipole moment (μ) is a promising measurable parameter for the molecular polarity, it is clearly evident from Table 1 that compounds **4** (a, c and d) exhibit low polarities. The lower polarity (less solvated) of such compounds contributed to attack the carbonyl group of the formaldehyde resulting in production of 1,3,5-thiadiazine compounds (Fig. 1). For more confirmation, the nucleophilicity index (ω) as well as the hydration energy, follow the order: **4**d > **4**c > **4**a > **4**e > **5**, are in conformity to the yield % of Mannich products. Accordingly, the DFT data run in harmony with the previous obtained results (cf. Table 1).

3.3. Antimicrobial evaluation

The anti-bacterial activity of the all synthesized compounds was tested against a panel of two Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli, Pseudomonas aeuroginosa*). The anti-fungal activities of the compounds were tested against two fungi (*Candida albicans, Aspergillus flavus*) using conventional Broth dilution method [45]. *Ampicillin* and *Clotrimazole* were used as reference. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimal inhibitory concentrations (MICs) for compounds that showed significant growth inhibition zones (>10 mm) were determined using two-fold serial dilution method [46].

The inhibition zone diameters and MIC (μg/mL) values are recorded in Tables 2 and 3. It was observed that compounds **1**, **3a**, **3b**, **4c**, **4e** and **12** exhibited the highest activity against *S. aureus* and *E. coli*. Replacing of thiol group in compound **1** by arylidene moieties (compounds **3** and **4**) led to an increased antimicrobial activity. This finding most probably resulted from the high hydrophobicity of such compounds (Table 1). In addition, the presence of an aniline moiety directly attached to the triazole ring as in compound **12** led to an increased activity at against *S. aureus* (MIC = 62.5 <mu>g/mL), *E. coli* (MIC = 93.7 <mu>g/mL) and C. Albicans (MIC = 7.8 <mu>g/mL). Moreover, compounds **3a** and **3b** which contain electron withdrawing groups or heterocyclic rings showed good activity (MIC = 125 μg/mL) against *S. aureus* bacteria. On the other hand, compounds **3a**, **3b**, **4c** and **12** exhibited

Table 3
[a]Minimum Inhibitory Concentration; [b]Calculated values used to generate QSAR models.

Comp. No	[a]MIC (μg/mL)	[b]ADME Weight	[b]HOMO	[b]K appa2 index
1	12–94	320.3	−7.515	8.762
3a	62–250	325.2	−4.071	7.505
3b	31–250	393.1	−8.409	9.718
4c	15–187	314.3	−5.740	7.415
4e	31–250	325.3	−5.642	7.321
10	187–750	319.7	−5.936	
11	187–500	311.7	−6.112	8.563
12	7.8–93	475.3	−10.918	7.914
5CTU	100–200	277.29	−9.509	7.513

Table 4
Minimum inhibitory concentration (MIC, μg/mL) of the newly synthesized compounds.

Compound	*E. coli*	*Pseudomonas aeruginosa*	*S. aureus*	*B. subtilis*	*C. Albicans*	*A. flavus*
1	94	94	47	93	12	16
3a	125	125	187	250	62	93
3b	250	250	125	125	31	46
3c	NA	NA	NA	NA	NA	NA
4a	500	375	93	125	23	46
4b	750	250	250	250	46	93
4c	125	187	62	93	15	31
4d	500	500	NA	NA	250	178
4e	187	250	125	187	31	62
5	500	250	375	500	125	250
6	500	NA	750	NA	750	125
7	375	375	375	500	94	125
8	375	250	250	375	94	187
9	750	NA	750	NA	250	500
10	750	500	500	750	187	375
11	500	375	500	250	187	300
12	62	93	46	62	7.8	11.7
Ampicillin	125	187.5	93.7	187.5	–	–
Colitrimazole	–	–	–	–	7.8	5.8

equipotent to Ampicillin in inhibiting the growth of *E. coli* (MIC = 125 μg/mL) and compound **4e** was equipotent to *Ampicillin* in inhibition the growth of *S. aureus* (MIC = 187.5 μg/mL).

3.4. Structure-activity relationships (SARs)

From the evaluation of the antimicrobial activities of the newly synthesized compounds **1**–**12** demonstrated that the presence of electron withdrawing groups the observed activities in vitro. The most potent thiouracil compounds **3a**, **3b**, **4c**, **4e** and **12** contain heterocyclic rings and electron withdrawing moieties. Moreover, antifungal evaluation displayed conflicting results, considering that compounds having electron-withdrawing substituents had weak inhibitory activities.

The data represented in Tables 2–4 showed that compounds **1**–**12** possess a pronounced antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* compared to the reference drug penicillin. As far as antifungal activity is concerned, compounds **12** exhibited promising activity, which equal to reference drug *Colitrimazole* against *Candida albicans* and compounds **3a, 4c** and **4e** against *Aspergillus flavus.* Compounds **4a**, **4b** and **4d** showed moderate activity against the fungus *Candida albicans* and compounds **4a** and **5** displayed moderate activity against the fungus *Aspergillus flavus*. Compounds **4a**, **4d**, **9**, **10**, **11**, were either inactive or moderately active against the tested bacteria. Triazolo-pyrimidinone **10** and **11** have lower antimicrobial activity, although presenting chemical structures similar to compound **12** (highest antimicrobial activity). DFT studies indicate that compounds **10** and **11** have higher HOMO energies, which can be correlated to their lower antimicrobial activities [39,47]. Table 3 shows that the HOMO energies for such compounds follow the order: **10** > **11** > **12**. Compounds **3a**, **4c**, **4e** and **12** showed the highest antibacterial activity whereas compound 3b exhibited excellent results against *Candida albicans* and *Aspergillus flavus.* On the other hand, incorporation of quinoxaline ring, *p*-toluene sulphonyl moiety or ester group to the thiouracil derivatives as in **6**, **7** and **8** diminished antimicrobial activities. The structure activity relationship suggested that thiouracils containing amide or hydrazide moiety showed higher antibacterial and antifungal activities than other derivatives [48]. The present study revealed that conversion of thiol group at 5′-position to hydrazide **9** caused a pronounced inhibition effect against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria. Compounds **3a**, **3b**, **4c**, **4e** and **12** exhibited the highest lipophilicity, charge density and surface area among the newly synthesized thiouracil derivatives, as revealed by DFT. These hydrophobic compounds were most potent against *E. coli*, *S. aureus* and Candida albicans.

DFT-based QSAR e.g. high molecular weight (ADME), electron withdrawing groups (low HOMO values) and shape indexes in structure-property modeling (high kappa index) [49] of such compounds supported the high antimicrobial activity (c.f. Tables 3 and 4).

4. Conclusion

Here we reported a green synthetic route for important derivatives of 5-cyano-2-thiouracils that contain thiazole, Schiff bases, hydrazides and triazolo moieties. The antimicrobial studies have revealed that the most promising compounds are the newly synthesized thiouracil derivatives **3a**, **3b**, **4c**, **4e** and **12**. DFT-based QSAR of such compounds that have hydrophobic groups support the high antimicrobial activities of the thiouracil containing amide, hydrazide, benzylidene and triazole precursors. Based on the above studies, the promising compounds can be submitted to *in vivo* antimicrobial studies as a future perspective.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molstruc.2017.11.066.

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