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Synthesis and biological evaluation of oxygen-, nitrogen-, and sulphur-containing non-fused heterocycles and tricyclic fused pyrimidines

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Abstract

A series of non-fused heterocycles containing oxygen, nitrogen, and sulfur (compounds 2, 3, 6, 9, 10, and 12), as well as furo-imidazo and thiazolo-thieno fused pyrimidines (compounds 7 and 13), were synthesized in good yields using a catalyst-free, convenient, general, and facile method. The molecular structures of all newly synthesized compounds were confirmed by spectral data and elemental microanalyses. Furthermore, the biological activities of all synthesized heterocyclic derivatives were evaluated *in vitro* against various common pathogenic microorganisms.

Keywords: furo-imidazo pyrimidines, thiazolo-thieno pyrimidines, biological activity, bioactivity.

Síntese e avaliação biológica de heterociclos não fundidos contendo oxigênio, nitrogênio e enxofre e de pirimidinas tricíclicas fundidas

Resumo

Uma série de heterociclos não fundidos contendo oxigênio, nitrogênio e enxofre (compostos 2, 3, 6, 9, 10 e 12), bem como pirimidinas fundidas furo-imidazo e thiazolo-thieno (compostos 7 e 13) foi sintetizada em bons rendimentos por um método geral, conveniente, simples e livre de catalisador. As estruturas moleculares de todos os compostos recém-sintetizados foram confirmadas por dados espectrais e análises elementares. Além disso, as atividades biológicas de todos os derivados heterocíclicos sintetizados foram avaliadas *in vitro* contra diversos microrganismos patogênicos comuns.

Palavras-chave: pirimidinas furo-imidazo, pirimidinas thiazolo-thieno, atividade biológica, bioatividade

1. Introduction

Since the discovery of DNA intercalating agents (Lerman, 1961; Luzzati et al., 1961), a growing number of organic, inorganic, and metallic compounds have been developed to treat life-threatening microbial infections. Oxygen, nitrogen, sulfur-based non-fused and Fused-heterocycles are amongst the most important groups of compounds that can interact with DNA. The development of physiologically highly potent fused pyrimidines is a challenging task for synthetic organic chemists (Katrizky; Rees, 1984).

Due to a wide range of biological activities exhibited by heterocyclic derivatives, these compounds occupy a unique place in the field of biological and medicinal chemistry. Most of the non-fused and fused heterocycles exhibited antifungal (Azeredo et al., 2017), antibacterial (Jang et al., 2011), antidiabetic (Bassyouni et al., 2021), analgesic (Barot et al., 2014), anti-inflammatory (Tozkoparan et al., 1999), anti-tubercular (Malnuit et al., 2015), anticancer (Pogorelcnik et al., 2015), antioxidant (Quiroga et al., 2016), antiviral (Rashad et al., 2008), anticonvulsant (Raj et al., 2006), antimalarial (Wang et al., 2015), antitumor (Jansen; Mathes, 1955) activities. In view of such wide applications, a few numbers of non-fused and fused pyrimidine heterocycles were synthesized in good yields by a convenient, general, and facile method. Elemental and spectral analyses (such as UV, IR, NMR, and MS) were performed for the new fused pyrimidine and non-fused derivatives, as well as investigated for their anti-microbial activities.

2. Materials and Methods

2.1 Synthesized compounds

Melting points of all synthesized compounds are uncorrected and were taken in open capillaries on a Gallenkamp apparatus. UV, IR, 1H-NMR, and ^{13}C-NMR spectral analysis and elemental analysis were used to confirm the molecular structures of all compounds. The UV spectra were recorded on a Shimadzu UV-*VIS* spectrophotometer, and the IR spectra were recorded on a Shimadzu IR spectrophotometer, using KBr discs. ^{1}H-NMR and ^{13}C-NMR spectra were performed on a Ultra Shield Bruker DPX 400 spectrophotometer, using TMS as internal standard. All chemical shifts are quoted in δ values using the ppm scale. The MS Spectra were recorded on a mass spectrometer at 70 eV. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer. TLC monitored all reactions, and detection of the components was made by short and long UV light. Analytical-grade solvents and reactants were used in the synthesis and were procured from Fulka, Mark (Germany), and Sigma-Aldrich Company Ltd (Dorset, UK).

*2.1 4,5-Dihydro-1H-imidazole-2-thiol, **2***

To a mixture of ethylenediamine **1** (5.0 g, 83.0 mmol), 100 mL rectified spirit, and 100 mL water, carbon disulfide (3.95 mL, 83.33 mmol) was added drop-wise with occasional shaking for 2 h. After that, the reaction mixture was refluxed on a water-bath for 1 h before adding concentrated 15 mL HCl, and then continued for another 9-10 h. The resulting solid was filtered with 80 mL of cold acetone and recrystallized from ethanol to give 4,5-dihydro-1H-imidazole-2-thiol, **2** as yellowish crystals. The yield was 3.98 g (69%); m. p.: 155-156 °C.

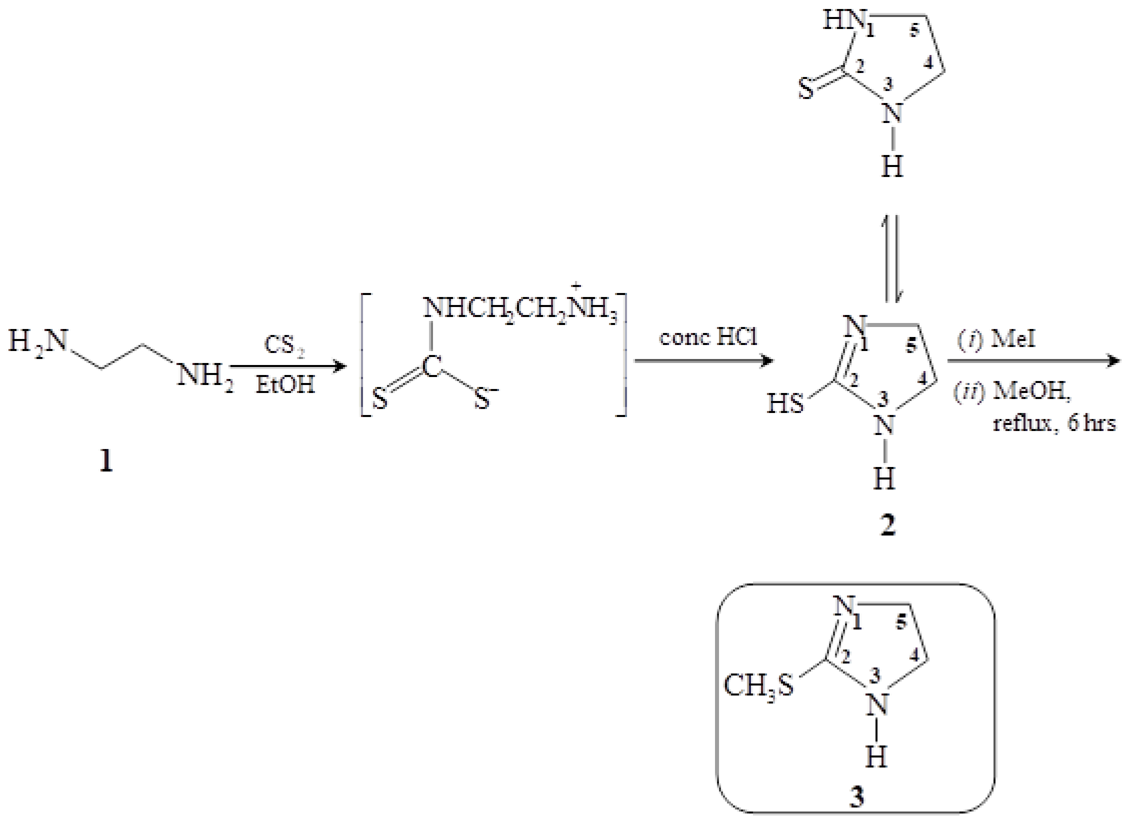
UV(EtOH), λmax (nm): 245.6; **IR (KBr)**, **υmax (cm^{-1}):** 3244 (NH str.), 2880 (CH str.), 2571 (SH str.) as well as 1275 (C=S str.); **^{1}H-NMR (400 MHz, $CDCl_3$):** δ 8.15 (s, 1H, NH), 2.0 (s, 1H, SH), 3.98 (m, 4H, 2xCH_2) which give the three signals for three kinds of protons using TMS as an internal standard; **^{13}C-NMR (100 MHz, $CDCl_3$):** δ 163.3 (C=N, C2), 44.8 (CH_2, C-4) and 44.8 (CH_2, C-5) which showed three signals for three nonequivalent carbon atoms; **MS (m/z):** 103.02 (M^+) consistent with molecular formula $C_3H_6N_2S$ was confirmed by the elemental analysis (% C, H, N, S).

Found (%): C, 35.25; H, 6.00; N, 27.32; S, 31.35

Calcd. C3H6N2S (%): C, 35.27; H, 5.92; N, 27.42; S, 31.39

2.3 2-Methylthioimidazoline, 3

Annulated reagent 2-methylthioimidazoline, **3**, was obtained by the reaction of 4,5-dihydro-1H-imidazole-2-thiol, **2**, with methyl iodide in the presence of methanol. Methylation of 2-imidazolinethione (3 g, 29.41 mmol) was carried out by adding methyl iodide (1.85 mL, 29.0 mmol) to a solution of compound **2** in 18 mL of absolute methanol. After that, the reaction mixture was heated to reflux on a water bath for 2 h with stirring. Methanol was then removed under reduced pressure, and the solid white hydroiodide salt was neutralized with 50% NaOH (2.4 mL). Then it was stirred for about 2 h, extracted with $CHCl_3$ (30 mLx4), dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness under reduced pressure in a vacuum evaporator to give annulated reagent 2-methylthioimidazoline, **3** in 72% (1.5 g) yield as white crystals m. p. 119-121 °C shown in (Scheme 1). The materials, equipment, and methodologies used to develop the study must be systematically described. These aspects must be presented in such a way that other researchers who consult the article can reproduce them based solely on what was described in the article.



Yield : 72%
State : yellowish crystals
m.p. : 119-121°C

Scheme 1. Synthesis of annulated reagents 4,5-dihydro-1H-imidazole-2-thiol, **2** and 2-methylthioimidazoline, **3**. Source: Authors, 2025.

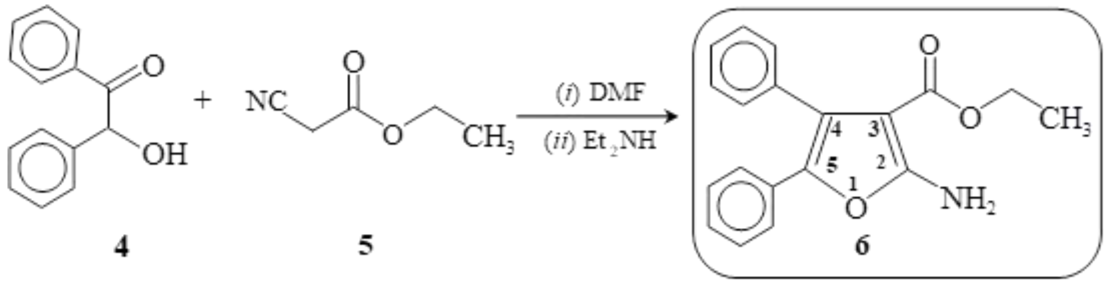
UV(EtOH), λmax (nm): 251.6; **IR (KBr)**, **υmax (cm^{-1}):** 3393 (NH str.), 2999 (CH str. in CH_3), 2865 (CH str in CH_2) as well as 1604 (CN str.); **^{1}H-NMR (400 MHz, $CDCl_3$):** δ 7.00 (s, 1H, NH), 3.65 (m, 4H, 2xCH_2) and 2.50 (s, 3H, SCH_3) which give the three signals for three kinds of protons in the moleceule; **^{13}C-NMR (100 MHz, $CDCl_3$):** δ 165.5 (C=N), 47.9 (CH_2), 47.9 (CH_2) and 12.7 (-SCH_3) which showed four signals for four carbon atom; **MS (m/z):** 117.04 (M^+) consistent with molecular formula $C_3H_8N_2S$ was confirmed by the elemental analysis (% C, H, N, S).

Found (%): C, 40.12; H, 7.02; N, 25.00; S, 28.00

Calcd. C3H8N2S (%): C, 41.35; H, 6.94; N, 24.11; S, 27. 60

*2.4 Ethyl-2-amino-4,5-diphenylfuran-3-carboxylate, **6***

A solution of benzoin, **5** (5.25 g, 25 mmol) and ethylcyanoacetate, **4** (2.125 g, 33.2 mmol) in 7.5 mL N, N-dimethylformamide (DMF) was treated with 4 mL diethylamine. After stirring for 12 h at room temperature, the mixture was poured into 60 mL of water, the separated solid was collected by filtration, and recrystallized from ethanol to give ethyl 2-amino-4,5-diphenylfuran-3-carboxylate **6** as shown in (Scheme 2). The yield was 3.52 g, 68% as white needle-shaped crystals; m.p.: 163-165 °C.



Yield : 68%
State : white needle shaped crystals
m.p. : 163-165°C

Scheme 2. Synthesis of annulated substrate Ethyl-2-amino-4,5-diphenylfuran-3-carboxylate, 6. Source: Authors, 2025.

UV (EtOH), λmax (nm): 253; **IR (KBr)**, **υmax (cm^{-1}):** 3383, 3262 (N-H str.), 1677 (C=O str.), 1595 (C=C str.), 1207 and 1100 (C-O str.) as well as 977, 929 and 755 (ArC-H bending); **^{1}H-NMR (400 MHz, $CDCl_3$):** δ 7.21 (s, 1H, NH2), 7.88 (m, 4H, Ar-H), 7.49 (t, 2H, Ar-H, J = 8.1 Hz), 7.26 (t, 2H, Ar-H, J = 8.0 Hz), 7.28 (t, 2H, Ar-H, J= 7.6 Hz), 4.27 (q, 2H, CH2, J= 7.2 Hz), 1.07 (t, 2H, CH2, ,7=7.2 Hz). which give seven signals for seven kinds of protons in the molecule; **^{13}C-NMR (100 MHz, $CDCl_3$):** d 164.87 (C=O), 156.47 (C5), 150.0 (C2), 137.88 (ArC), 136.05 (C4), 132.89 (ArC), 130.77 (ArC), 130.77 (ArC), 130.77 (ArC), 130.77 (Ar-C), 129.1 (ArC), 129.1 (ArC), 128.76 (ArC), 128.76 (ArC), 128.97 (ArC), 128.97 (ArC), 112.0 (C3), 61.95 (CH2), 13.0 (CH_3) which indicated the presence of nineteen nonequivalent carbons; **MS (m/z):** 307.12 (M^+) consistent with molecular formula $C_{19}H_7NO_3$ which was confirmed by the elemental analysis (% C, H, N, O).

Found (%): C, 76.70; H, 2.32; N, 4.75; O, 16.10

Calcd. $C_{19}H_7NO_3$ (%): C, 76.77; H, 2.37; N, 4.71; O, 16.15

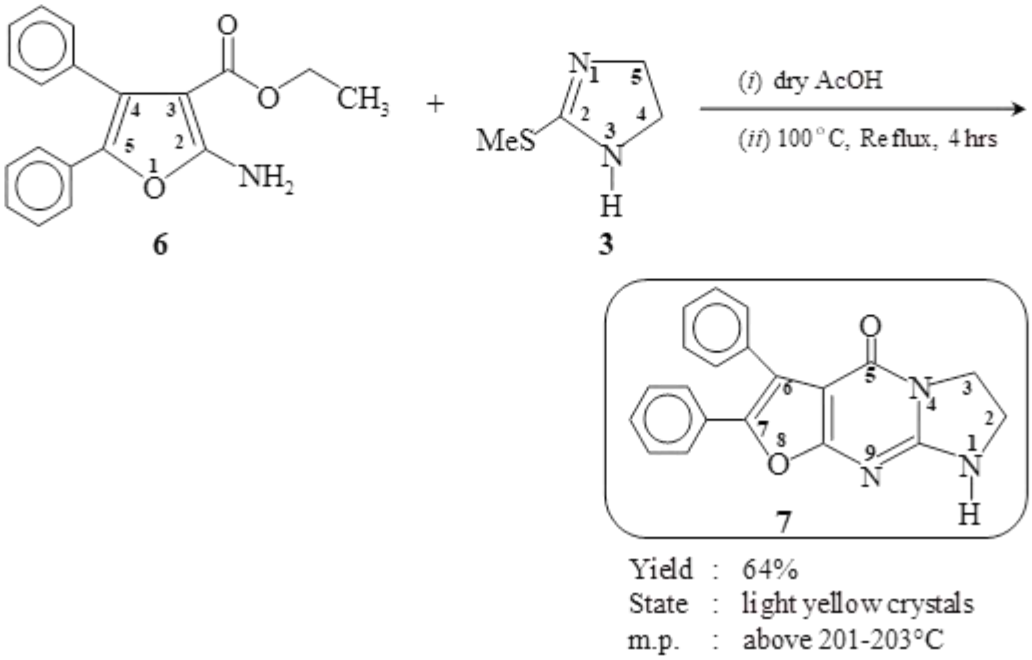
2.5 6,7-Diphenyl-2,3-dihydrofuro[2,3-d]imidazo[1,2-a] pyrimidin-5(1H)-one, **7**

A solution of 2-amino-4,5-diphenylfuran-3-carboxylate, **6** (0.78 g, 3 mmol), and 2-methylthio-imidazoline, 16 (0.522 g, 4.5 mmol), in 6 mL dry acetic acid was refluxed for 6 h. After cooling the reaction mixture to room temperature, crushed ice (30 g) was added, and the mixture was stirred for an hour. The precipitate was collected and crystallized from methanol to give a new fused pyrimidine compound, **7**, as light-yellow crystals in 64% yield (0.50 g), m.p. 201-203 °C, shown in (Scheme 3).

UV(EtOH), λmax (nm): 288; **IR (KBr)**, **υmax (cm^{-1}):** 3392 (NH str.), 2995 (CH str. in CH2), 1661 (C=O str.), 1580 (C=C) as well as 988, 923 and 772 (C-H bending in aromatic ring); **^{1}H-NMR (400 MHz, $CDCl_3$):** δ 8.76 (s,1H, NH), 7.88 (m, 4H, Ar-H), 7.49 (t, 2H,Ar-H, J = 8.1), 7.26 (t, 2H, Ar-H, J = 8.0), 7.11 (t, 2H, Ar-H, J= 7.6), 3.77 (q, 2H, CH2, J = 7.8), 2.21 (t, 2H, CH2, J = 7.8) which give the seven signals for seven kinds of protons in the molecule; **^{13}C-NMR (100 MHz, $CDCl_3$):** δ 162.47 (C5, C=O), 158.38 (C7), 146.74, 145.88, 137.50 (ArC), 132.00 (C6), 131.5 (ArC), 130.77 (ArC), 130.77 (ArC), 130.77 (ArC), 130.77 (ArC), 129.1 (ArC), 129.1 (ArC), 128.97 (ArC), 128.97, (ArC) 126.76 (ArC), 126.76 (ArC), 122.8. 46.2 (CH_2), 37.1 (CH_2), which indicated the presence of twenty nonequivalent carbon atoms; **MS (m/z):** 330 (M^+), consistent with molecular formula $C_{20}H_{15}N_3O_2$, as confirmed by the elemental analysis (% C, H, N, O).

Found (%): C, 72.52; H, 5.00; N, 12.00; O, 9.43

Calcd. $C_{20}H_{15}N_3O_2$ (%): C, 72.94; H, 4.59; N, 12.76; O, 9.72



Scheme 3. Synthesis of target fused pyrimidine 6,7-diphenyl-2,3-dihydrofuro[2,3-d]imidazo[1,2-a] pyrimidin-5(1H)-one, **7**. Source: Authors, 2025.

*2.6 4,5-Dihydrothiazole-2-thiol, **9***

A mixture of ethanolamine, **8** (3.66 g), and sodium hydroxide (9.61 g) in water (26 mL) was cooled to 30 °C. Then, carbon disulfide (12 g) was added to the reaction mixture with constant stirring under cooling conditions. The reaction mixture was warmed to keep it at 45 °C. After refluxing for seven hours, the reaction mixture was heated more strongly at 100 °C for three hours. Upon cooling to room temperature, the 4,5-dihydrothiazole-2-thiol, **9**, was deposited as a solid, the remainder being precipitated by the addition of 200 mL of concentrated hydrochloric acid. The product was filtered off, washed with water, and dried to afford **9** as light-yellow crystals in 84% yield, m.p. 102-104 °C shown in (Scheme 4).

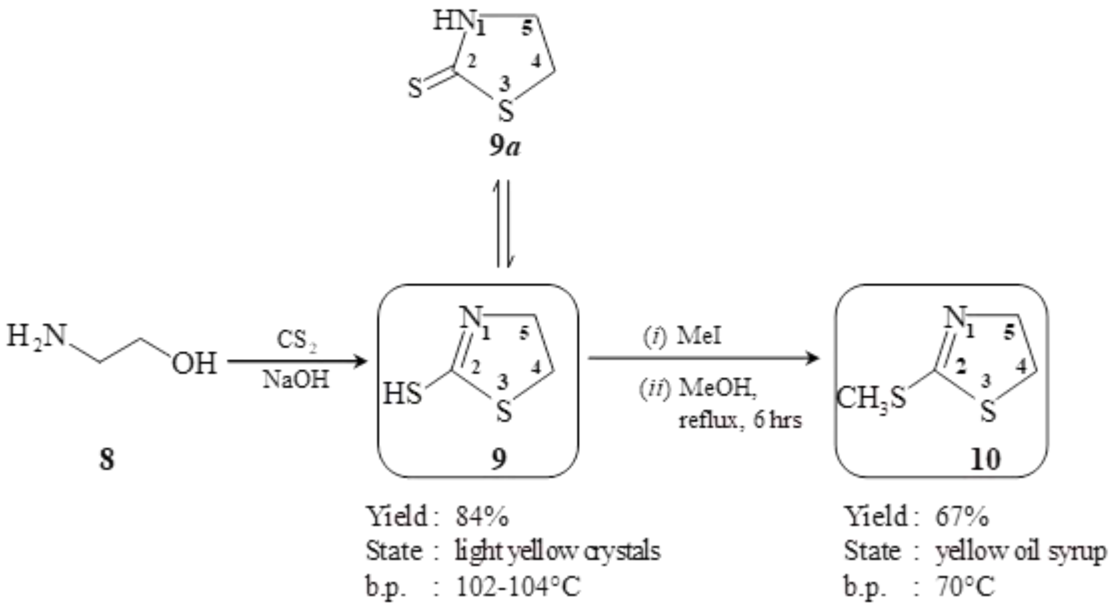
UV (EtOH), λmax (nm): 240; **IR (KBr)**, **υmax (cm^{-1}):** 3133 (NH str.), 2853 (CH str), 2570 (SH str) as well as 1296 (C=S str.); **^{1}H-NMR (400 MHz, CDCl$_3$):** δ 4.20 (t, 2H, CH_2, J = 7.9 Hz), 3.39 (t, 2H, CH2 J = 7.9 Hz) and 2.00 (s, 1H, SH) which give three signals for three kinds of protons in the molecule; **^{13}C-NMR (100 MHz, CDCl$_3$):** δ 165.47 (C2, C=N), 63.74 (C5, CH2), 35.26 (C5, CH2) which indicated three signals for three carbons; **MS (m/z):** 119.99 (M^+) consistent with molecular formula $C_3H_5NS_2$ was confirmed by the elemental analysis (% C, H, N, S).

Found (%): C, 30.20; H, 4.37; N, 11.80; S, 53.72

Calcd. $C_3H_5NS_2$ (%): C, 30.23; H, 4.23; N, 11.75; S, 53.79

*2.7 2-Methylthio-2-thiazoline, **10***

The solution of 4,5-dihydrothiazole-2-thiol, **9** (6 g, 50.4 mmol) and methyl iodide (7.15 g, 50.4 mmol) in 30.25 mL absolute methanol was heated under reflux for 1.5 h. The solvent was removed to give the product hydroiodide as a white crystalline mass. The solid white hydroiodide salt was dissolved in 35 mL of water and neutralized with 4.10 mL of 15% NaOH. After that, the reaction mixture was stirred for one hour, extracted with chloroform (30 mL x 4). The combined organic layer was dried over anhydrous sodium sulfate, and chloroform was evaporated to dryness under reduced pressure in a vacuum evaporator to give a syrup, which was distilled to give yellow 2-methylthio-2-thiazoline, **10** as a syrup. The yield was 6.1 g (67%) as yellow oil syrup. b. p. 70-71 °C shown in (Scheme 4).



Scheme 4. Synthesis of annulated reagents 4,5-dihydrothiazole-2-thiol, **9**, and 2-methylthio-2-thiazoline, **10**. Source: Authors, 2025.

UV (EtOH), λmax (nm): 253; **IR (KBr)**, **υmax (cm^{-1}):** 2932 (CH str in CH_3), 2853 (CH str in CH_2) as well as 1564 (C=N str.); **^{1}H-NMR (400 MHz, $CDCl_3$):** δ 4.20 (t, 2H, CH2, J = 7.9 Hz), 3.39 (t, 2H, CH2 J = 7.9 Hz) and 2.50 (s, 3H, CH_3) which give three signals for three kinds of protons in the molecule. **^{13}C-NMR (100 MHz, $CDCl_3$):** δ 164.0 (C2, C=N), 65.5 (C5, CH2), 37.1 (C4, CH_2) and 12.12 (-SCH_3) which indicated three signals for three carbons; **MS (m/z):** 134.00 (M^+) consistent with molecular formula $C_3H_7NS_2$ was confirmed by the elemental analysis (% C, H, N, S).

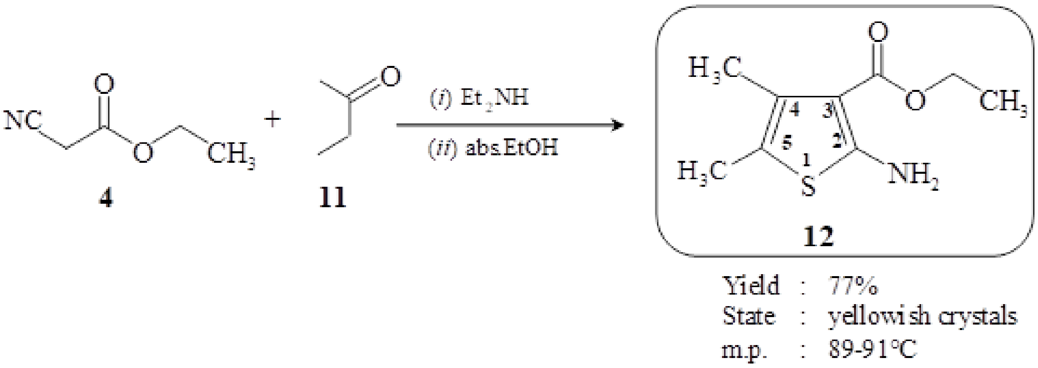
Found (%): C, 36.10; H, 5.20; N, 10.55; S, 48.18

Calcd. $C_3H_7NS_2$ (%): C, 36.06; H, 5.30; N, 10.51; S, 48.13

*2.8 Ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate, **12***

A suspension of sulfur (3.20 g, 100 mmol), methyl ethyl ketone i.e., MEK (8.9 mL, 100 mmol), ethylcyanoacetate (11.30 g, 100 mmol) in diethylamine (4 mL) and 30 mL 95% ethanol was stirred at 60 °C, the internal temperature being maintained below 60 ºC by means of an ice-bath. After the mixture was stirred for 2 h at room temperature. Then the mixture was poured into ice water.

The precipitate was filtered, washed with water, and re-crystallized from ethanol. to give an ortho-aminoester ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate, **12** as shown in (Scheme 5). The yield was 15.2 g, 77% as yellowish crystals, m.p. 89-91 °C.



Scheme 5. Synthesis of annulated substrate ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate, **12**. Source: Authors, 2025.

UV (EtOH), λmax (nm): 240; **IR (KBr)**, **υmax (cm^{-1}):** 3433 and 3285 (N-H str.), 3096 and 3197 (CH str.), 1648 (C=O str), 1562 (C=C str) as well as 1261 and 1026 (C=O str); **^{1}H-NMR (400 MHz, CDCl$_3$):** δ 5.98 (s, 2H, NH2), 3.00 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.29 (q, 2H, CH2, J = 7.1Hz) and 1.39 (s, 3H, CH3, J = 7.2Hz) which give five signals for five kinds of protons in the molecule.

^{13}C-NMR (100 MHz, CDCl$_3$): d 164.31 (C2), 150.86 (C=O), 128.96 (C4), 126.65 (C5), 106.76 (C3), 59.29 (CH2), 13.87 (CH_3), 13.00 (CH_3), 11.72 (CH_3) which indicated the presence of nine nonequivalent carbons; **MS (m/z):** 200.07 (M^+) consistent with molecular formula $C_9H_{13}NO_2S$ was confirmed by the elemental analysis (% C, H, N, O).

Found (%): C, 54.00; H, 6.60; N, 7.00; O, 16.12; S, 16.00

Calcd. $C_9H_{13}NO_2S$ (%): C, 54.25; H, 6.58; N, 7.03; O, 16.06; S, 16.09

*2.9 6,7-Dimethyl-2.3-dihydro-5H-thiazolo [3,2-a]thieno[2,3-d] pyrimidine-5-one, **13***

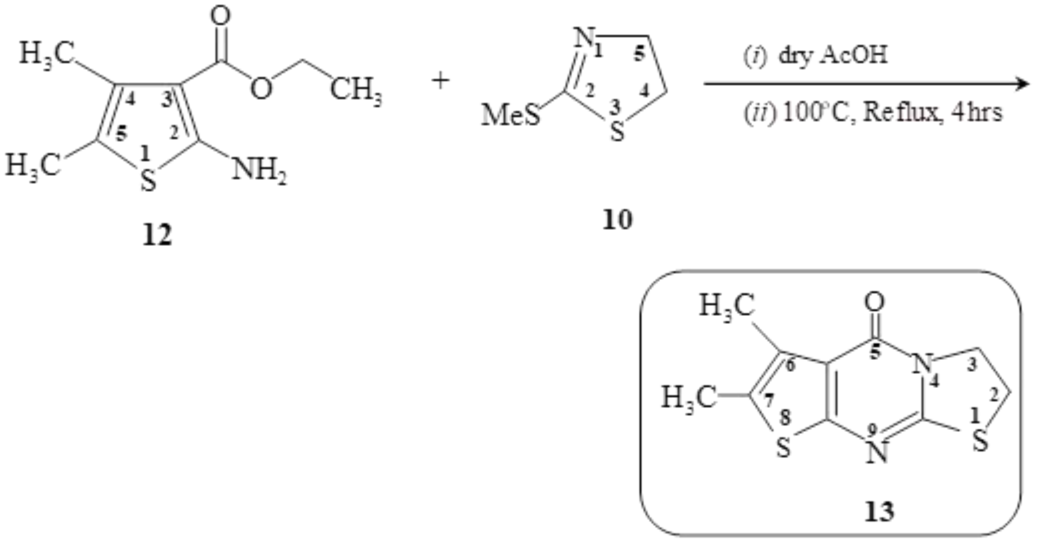
The solution of ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate, **12** (1.00 g, 5.025 mmol) and 2-methylthio-2-thiazoline, **10** (0.668 g, 5.025 mmol) in 6 mL dry acetic acid was heated under reflux for 8 h. The progress of the reaction was monitored by TLC (*n*-hexane: ethylacetate, 9:1), which showed complete disappearance of the starting material with the formation of the product. After that, the mixture was poured into ice water. The resulting precipitate was filtered off and washed with water.

Recrystallization from ethanol produced new fused pyrimidine, **13** name of 6,7-dimethyl-2,3-dihydro-5H-thiazolo[3,2-a]thieno[2,3-d]pyrimidine-5-one as yellowish crystals with 67% yield (0.66 g), m.p. 171-172 °C, shown in (Scheme 6).

UV(EtOH), λmax (nm): 240; **IR (KBr)**, **υmax (cm^{-1}):** 3096 and 3019 (CH str. in CH_3), 2895 (CH str. in CH^2), 1675 (C=O str.) as well as 1534 (C=C str.). **^{1}H-NMR (400 MHz, CDCl$_3$):** δ 2.53 (s, 6H, 2x3CH_3), 4.22 (t, 2H, CH_2, J=7.5Hz), 3.44 (t, 2H, CH_2, J = 7.5Hz) which give the three signals for three kinds of protons in the molecule; **^{13}C-NMR (100 MHz, CDCl$_3$):** δ 164.1 (C4, C=O), 162.48, 154.95, 130.06 (C7), 128.7 (C6), 115.2, 52.91 (C3, CH2), 25.75 (C2, CH_2), 11.39 (CH_3), 10.60 (CH_3) which indicated the presence of ten non equivalent carbons; **MS (m/z):** 239.03 (M^+) consistent with molecular formula $C_{10}H_{10}N_2OS_2$. which was confirmed by the elemental analysis (% C, H, N, O, S).

Found (%): C, 50.48; H, 4.25; N, 11.70; O, 6.52; S, 27.00

Calcd. $C_{10}H_{10}N_2OS_2$ (%): C, 50.40; H, 4.23; N, 11.75; O, 6.71; S, 26.91



Yield : 67%
State : yellowish crystals
m.p. : 170-171°C

Scheme 6. Synthesis of target fused pyrimidiene compound 6,7-dimethyl-2.3-dihydro-5H-thiazolo [3,2-a]thieno[2,3-d] pyrimidine-5-one, **13**. Source: Authors, 2025.

3. Results

3.1 Synthesis compounds

At the first stage, a simple diamine compound such as ethane-1,2-diamine, **1** reacted with carbon disulfide in presence of ethanol and concentrated HC1 to give 4,5-dihydro-1H-imidazole-2-thiol, **2** as well as the annulated reagent 2-methylthioimidazoline **3** obtained by the reaction of **2** with methyl iodide in presence of methanol by the Jensen & Hofmann method (Gewald, 1966; Gewald et al., 1966) in 82% yield as shown in (Scheme 1). Letter On, an annulated substrate ethyl 2-amino-4,5-diphenylfuran-3-carboxylate, **6** was synthesized by the reaction of ethylcyanoacetate and benzoin in the presence of DMF and diethylamine according to by Gewald method [18,19] in good yields as shown in (Scheme 2). After that, compound **6** reacted with a 2-methylthio-imidazoline, **3** by one-step reaction in dry acetic acid medium under reflux for four hours to afford 6,7-diphenyl-2,3-dihydrofuro[2,3-d]imidazo[1,2-a]pyrimidin-5(1H)-one, **7** as shown in (Scheme 3).

On the other hand, 4,5-dihydrothiazole-2-thiol, **2** as annulated reagent was synthesized from an amino alcohol namely ethanolamine, **1** and sodium hydroxide in presence of carbon disulfide by the Jensen & Hofmann method [16, 17] in 84% yield as light yellow crystals as well as in the next step annulated reagent 2-methylthio-2-thiazoline, **3** was synthesized from 4,5-dihydrothiazole-2-thiol, **2** by the methylation with methyl iodide in presence of methanol to give, **3** as shown in (Scheme 1). Further, another annulated substrate, ethyl-2-amino-4,5-dimethylthiophene-3-carboxylate, **12**, was synthesized from butanone and ethylcyanoacetate by using the Gewald procedure (Gewald, 1966; Gewald et al., 1966) as shown in (Scheme 5). Finally, annulated substrate, **12**, reacted with 2-methylthio-2-thiazoline, **10**, synthesized by one-step reaction in dry acetic acid medium under reflux for four hours to give a new fused pyrimidine compound 6,7-dimethyl-2.3-dihydro-5H-thiazolo-[3,2-a]-thieno-[2,3-d]pyrimi-dine-5-one, **13**, as shown in (Scheme 6). Structures of the synthesized compounds were established based on IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectra well as elemental analysis. After that, eight compounds were tested for their antimicrobial activities against pathogenic bacterial strains and fungal strains.

3.2 Biological investigation

All the synthesized heterocycles (**2**, **3**, **6**, **7**, **9**, **10**, **12**, and **13**) were screened for in vitro antimicrobial activities. Antibacterial screening of all synthesized heterocyclic derivatives was carried out by the disc diffusion method

(EUCAST, 2021; Brown; Kothari, 1975) against four Gram (+) human pathogenic bacteria, as *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus* & *Bacillus subtilis*, and two Gram (-) negative human pathogenic bacteria, *Escherichia coli* & *Shigella dysenteriae*. On the other hand, antifungal screening of synthesized heterocyclic derivatives was carried out by the mentioned previous method against four human pathogenic fungal strains as *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae* & *Saccharomyces cerevisiae*. The Zone of inhibition (in mm) was measured and compared with the reference Kanamycin. The results of the diameter of the zone of inhibition (ZOI, mm) and % inhibition of mycelial growth due to the effect of synthesized compounds are presented in (Table 1).

5-membered heterocycles **3** and **10** showed mild *in vitro* antimicrobial activity against *Staphylococcus aureus* (ZOI 7.9 and 7.4), *C. ablicans* (ZOI 9.4 and 12.0), and *S. cerevisiae* (ZOI 7.8 only for **3**). This may be due to the available SCH_3 group present in **3** and **10**; in the case of **2** and **9**, they were found to be completely resistant against all the tested microorganisms, which may be due to the absence of the SCH_3 group. Again, Oxygen based 5-memberd compound **6** and sulphur based 5-membered compound, **12** showed mild *in vitro* antibacterial activity against to the Gram positive *B. cereus* (ZOI 8.1 mm **12**), *B. megaterium* (ZOI 7.5 mm **12**), *B. subtilis* (ZOI 6.5 mm **6**) and antifungal activity to the fungal strains as *A. niger* (ZOI 9.5 mm **12**), *C. ablicans* (ZOI 9.5 **6**) and *S. cerevisiae* (ZOI 7.8 mm **12**) as well as were found to be completely resistant against the tested other bacterial strains and all the tested fungal strains at a dose level of 200 µg/disc.

On the other hand, the targeted fused pyrimidine, **7** showed mild *in vitro* antimicrobial activity especially against the bacterial strains *B. cereus*, *B. megaterium*, *S. aureus*, *B. subtilis*, *E. coli*, *S. dysenteriae* with the zone of inhibition equal to 8.5, 9.8, 7.0, 8.6, 7.9, 8.2 nm respectively and fungal strains *A. niger*, *C. albicans*, *S.cerevisiae* with the zone of inhibition equal to 9.8, 12, 8.1 mm respectively and were found to be completely resistant against the tested rest bacterial strains and fungal strains whereas 13 showed mild in vitro antimicrobial activity especially against the *B. cereus*, *B. megaterium*, *S. aureus*, *E. coli*, *S. dysenteriae* with the zone of inhibition equal to 7.7, 10.1, 8.6, 6.5, 9.1 nm respectively and fungal strain *A. niger*, *C. albicans* with the zone of inhibition equal to 8.1, 10.5 nm respectively and were found to be completely resistant against the tested rest bacterial strain and fungal strain at a dose level of 200 µg/disc.

Table 1. The *in vitro* antibacterial and antifungal activity of newly synthesized heterocyclic compounds as **2**, **3**, **6**, **7**, **9**, **10**, **12**, and **13**.

Diameter of Zone of Inhibition *i.e.*, ZOI in mm										
Compounds	*Bacillus cereus*	*Bacillus megaterium*	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Shigella dysenteriae*	*Aspergillus niger*		*Rhizopus oryzae*	
Std 30 Kan.	30	30	30	30	30	30	30	30	30	30
Two annulated reagents										
2	–	–	–	–	–	–	–	–	–	–
3	–	–	7.9	–	–	–	–	–	9.4	7.8
Two annulated substrates										
6	–	–	–	6.5	–	–	–	–	–	–
12	8.1	7.5	–	–	8.2	–	9.5	–	–	7.8

Another two annulated reagents										
9	–	–	6.9	–	–	–	–	–	10.0	–
10	–	–	–	7.4	–	–	–	12.0	–	–
Two fused pyrimidines										
7	8.5	9.8	7.0	–	7.9	8.2	9.8	12.0	–	8.1
13	7.7	10.1	8.6	–	6.5	9.1	8.1	10.5	–	–

Source: Authors, 2025.

4. Discussion

The synthetic strategy employed was efficient, producing the target heterocycles in moderate to high yields without the use of catalysts. The introduction of methylthio (-SCH_3) groups in compounds **3** and **10** appears to enhance antimicrobial activity compared to their unmethylated counterparts (**2** and **9**), which showed no activity. This suggests that lipophilic substituents may facilitate better cell membrane penetration.

Comparative activity analysis revealed that oxygen-containing heterocycles (compound **6**) and sulfur-containing heterocycles (compound **12**) showed only mild antimicrobial effects, possibly due to limited interaction with microbial targets. In contrast, the fused pyrimidine systems (compounds **7** and **13**) demonstrated improved antibacterial and antifungal activity. This improvement may be attributed to the rigid fused-ring framework, which could enhance binding affinity to microbial enzymes or nucleic acids.

Overall, the data indicate that ring fusion and the presence of lipophilic groups significantly influence the biological activity of these heterocyclic systems. Among the tested compounds, **7** and **13** emerged as the most promising candidates for further optimization as antimicrobial agents.

5. Conclusions

Overall, we have reported the synthesis of two annulated reagents having Nitrogen, two annulated substrates having oxygen and sulphur, another two annulated reagents having nitrogen and sulphur and two tergated fused heterocyclic compounds containing furo [2,3-d] imidazo [1,2-a] pyrimidine and thiazolo [3,2-a] thieno [2,3-d] pyrimidine by a convenient, general, rapid, facile and catalyst free methods. The structures of all heterocyclic compounds were confirmed by spectroscopic and elemental analyses successfully. Finally, all synthesized and characterized compounds were tested and screened against a few human pathogenic microorganisms. Among the tested compounds, only **7** and **13** (the targeted fused pyrimidines) showed better antimicrobial activity compared with other synthesized compounds, which may be due to the fusion of the ring in these molecules.

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7. Authors' Contributions

Mohammad Mizanur Rahman: writing-original draft preparation. *Rabiul Islam*: reviewing and editing. *Faqir Rafiqul Alam*: reviewing and editing. *Masud Reza*: reviewing and editing.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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