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Synthesis and Antimicrobial Activity of Some New Triazine, 1, 3-Oxazine, Fused Pyridine and Pyrimidine Derivatives

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Abstract: The reaction of 2, 4-dichlorobenzoyl chloride 1 with ammonium thiocyanate afforded 2, 4-dichlorobenzoyl isothiocyanate 2. Treatment of 2 with urea, malononitrile, and 4-phenylaminopent-3-en-2-oneandacetylacetone afforded the corresponding triazine 3, oxazine 4, pyrimidinethion 5 and mercapto acetyl pyridine 6. Mercapto acetyl pyridine 6 was used as a precursor for preparation of pyrido [2, 3-d] pyrimidine 7, pyrazolo [3, 4-b] pyridine 8 and N-phenylpyrazolo [3, 4-b] pyridine 9. Also, pyrazolo [2, 3-d] pyrimidine 10, diphenylhydrazonopyrimidine 11, pyrimido [4, 5-d] pyrimidine 12, dihydro pyrimidine pyridine 13, pyrimidine thion 14a and 14b were obtained by the reaction of pyrimidinethione 5 with urea, hydrazinehydrate, phenylhydrazine, 4-nitrobenzaldehydeor/and benzaldehyde. The antimicrobial activity of these new compounds has been evaluated against 4 microbial strains. Some of the newly synthesized compounds showed a good activity against bacterial and fungal strains.

Key words: Dichlorobenzoylisothiocyanate · Triazine · Pyrimidinethion · Mercaptoacetyl · Pyridine and antimicrobial Activity

INTRODUCTION

Acyl isothiocyanates, well-known as very important synthons in synthetic organic chemistry, particularly in the construction of heterocyclic ring systems such as functionalized pyrimidines, triazines, triazoles, pyridines, pyrazoles, isothiazoles, pyrans andpyridazines [1-3]. In particularly, the synthesis of pyrimidine and pyridine derivatives continues to attract considerable attention because of their great practical usefulness, primarily due to very wide spectrum of biological activities [4-7]. Some literature survey revealed that pyrimidine and pyridine derivatives are widely used for many applications in medicinal science as anti-mycobacterial [8] antitumor [9] antiviral [10] anticancer [11] anti-inflammatory [12] antimicrobial [13] anti-diabetic [14] antioxidant [15] anti-amoebic [16] antimalarial [17] and analgesic activity [18]. Therefore, our research work directed to design and synthesis of biologically important heterocyclic systems from readily available reagents by utilizing 2, 4-dichlorobenzoylisothiocyanate as a synthetic precursor and evaluation of their antimicrobial activity.

MATERIALS AND METHODS

Chemistry: Melting points of studied chemicals were measured using an Electrothermal IA 9100 apparatus with open capillary tube and are uncorrected. All experiments were carried out using drying solvents (type of solvents). Products were purified by recrystallization. The IR spectrum (Kbr disc) was recorded on a PyeUnicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The ^{1}H NMR 400 MHz and ^{13}CNMR 100 MHz spectrum were measured on a JEOL-JNM-LA spectrometer using DMSO as a solvent. All chemical shifts were expressed on the δ (ppm) scale using TMSas an internal standard reference. The coupling constant (*J*) values are given in Hz. Analytical data were obtained from the Microanalysis Center at Cairo.

All bacterial and fungi strains were obtained from the Department of Microbiology, Faculty of Science, Zagazig University (Cairo, Egypt) and were as follows*:* *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19115, *Aspergillusfalvus* ATCC9643 and *Aspergillusniger* ATCC 16404. All the

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newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare final concentration (0.5g/1ml DMSO).

2, 4-Dichlorobenzoylisothiocyanate (2): A mixture of 2, 4-dichlorobenzoylchloride **1** (14ml, 0.1mol) and ammonium thiocyanate (7.91g, 0.1mol) in dry acetone (50ml) was heated under reflux at 90°C for 1hr. The product was isolated to give the 2, 4-dichlorobenzoyl isothiocyanate **2** as yellow solution.

4-(2, 4-Dichlorophenyl)-6-thioxo-5, 6-dihydro-1, 3,5-triazin-2(1*H*)-one (3)

A mixture of 2, 4-dichlorobenzoylisothiocyanate **2** (1mmol) with urea (1mmol) in ethanol (30 mL) was refluxed for 4 hrs then the mixture cooled at 0°C and recrystallized from ethanol to give compound **3** as yellow powder. Yield 60%, m.p. 188-190°C. Elem. Anal. Calcd (%) for $C_9H_5Cl_2N_3OS$ (274.13): C, 39.43; H, 1.84; N, 15.33. Found: C, 39.37; H, 1.89; N, 15.42.IR (KBr, υ, cm^{-1}): 3344 (NH), 1685 (C=O), 1234 (C=S).^{1}H NMR (400MHz, DMSO-d_6) δ (ppm):11.65(s, 1H, NH), 10.41(s, H, NH),7.51 (d, 1H, J = 8.32 Hz, Ar-H), 7.59 (d, 1H, J = 8.32 Hz,Ar-H), 7.74 (s, 1H, Ar-H).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 181.9 (C=S),166.8 (C=O),142.2 (C=N),136.1, 133.9, 131.6, 130.9, 129.5, 127.7(Ar-C) [19].

2-(2,4-Dichlorophenyl)-6-imino-4-thioxo-5,6-dihydro-4H-1,3-oxazine-5-carboxamide (4): A mixture of 2, 4-dichlorobenzoyl isothiocyanate **2**(1mmol) with malononitrile (1mmol) in ethanol (30 mL) was refluxed for 4 hr. the precipitate was obtained after filtration and recrystallization from ethanol to give compound **4** as pale yellow powder. Yield 65 %, m.p.300-312°C. Elem.Anal.Calcd (%) $C_{11}H_7Cl_2N_3O_2S$ (316.16): C, 41.79; H, 2.23; N, 13.29. Found: C, 41.88; H, 2.17; N, 13.36.IR (KBr,υ, cm^{-1}): 3344 (NH), 3325(NH_2), 1680 (C=O), 1233 (C=S). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 11.65 (s, H, NH).9.59 (s, 2H, NH_2),7.74 (s, 1H,Ar-H),7.59 (d, 1H, J = 8.28 Hz, Ar-H),7.51 (d, 1H, J = 8.28 Hz, Ar-H),2.99 (s, 1H, CH of oxazine ring).^{13}CNMR(100MHz,DMSO-d_6) δ (ppm): 181.9(C=S),166.8 (C=O), 158.6 (C=N) ,142.3 (C=N), 136.1, 133.9, 131.6,131.0, 129.5,127.7(Ar-C), 48.00 (Oxazine-C) [20].

1-(2-(2, 4-Dichlorophenyl)-6-methyl-1-phenyl-4-thioxo-1,4-dihydro pyrimi din-5-yl) ethanone (5): A mixture2, 4-dichlorobenzoyl isothiocyanate **2** (1mmol) and 4-phenylamino pent-3-en-2-one (1mmol) in dioxane (50ml) was refluxed for 1 hr. then the mixture was cooled and recrystallized from ethanol to give white powder of compound **5**. Yield73%, m.p. 320-322°C. Elem. Anal. Calcd. (%) $C_{19}H_{14}Cl_2N_2OS$ (389.30): C, 58.62; H, 3.62; N, 7.20. Found: C, 58.71; H, 3.58; N, 7.16.IR (KBr, υ, cm^{-1}): 1701 (C=O), 1242 (C=S). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm):7.39-7.68 (m, 8H, Ar-H), 2.50 (s, 3H, CH_3CO), 1.81 (s, 3H, CH_3). ^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 201.1(C=S), 193.5(C=O), 152.3 (C=N),144.1, 137.0,136.5, 135.5,132.5,132.4,132.2,130.9,130.0,129.0,127.5,126.9(Ar-C), 17.83(CH_3), 30.85(CH_3CO) [21].

1-(6-(2,4-Dichlorophenyl)-4-hydroxy-2-mercaptopyridin-3-yl)ethanone (6): A mixture of 2, 4-dichlorobenzoylisothiocyanate **2** (1mmol) with acetyl acetone (1mmol) in ethanol (30 mL) was refluxed for 6 hrs, then cooled and the obtained precipitate was recrystallized from acetic acid. Yield 70 %, m.p. 210-212 °C. Elem. Anal. Calcd. (%) $C_{13}H_9Cl_2NO_2S$ (314.19): C, 49.70; H, 2.89; N, 4.46 Found: C, 49.61; H, 2.85; N, 4.50.IR (KBr, υ, cm^{-1}): 3375(OH),2650 (SH) 1697 (C=O). ^{1}HNMR (400MHz, DMSO-d_6) δ (ppm):13.47 (s, 1H, SH),7.92 (s, 1H, OH),7.74 (s, 1H,Ar-H),7.67 (s, 1H, pyridine-H),7.59 (d, 1H, J = 8.28 Hz, Ar-H),7.51 (d, 1H, J = 8.28 Hz, Ar-H), 2.90 (s, 3H,CH_3CO).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 172.3 (C=O), 137.0 (C=N), 127.6, 127.7, 127.9, 129.5, 129.7, 130.5, 132.8, 133.5, 134.7, 136.3(Ar-C),28.50 (CH_3-C).

7-(2,4-Dichlorophenyl)-5-hydroxy-4-methylpyrido[2,3-d]pyrimidin-2(1H)-one (7): A mixture of compound **6** (1mmol) and urea (1mmol) in sodium ethoxide (0.015mol in 30mL ethanol) was refluxed for 4 hr, then the content of the flask, acidified by dilute HCL (20 mL, 1:10) to give yellow powder of compound **10**and recrystallized from of acetic acid. Yield 68 %,m.p.164-166°C.Elem.Anal.Calcd. (%) $C_{14}H_9Cl_2N_3O_2$ (322.15): C, 52.20; H, 2.82; N, 13.04. Found: C, 52.28; H, 2.85; N, 12.98.IR (KBr, υ, cm^{-1}): 3444 (OH), 3325 (NH), 1693 (C=O), ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 12.57 (s, 1H, NH),8.12 (s, 1H, OH),7.74 (d, 1H,J = 8.40Hz,Ar-H),7.65-7.58(d_2,1H,Ar-H),7.43(d,1H,J=8.40Hz, Ar-H), 2.58(s, 3H, CH_3).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 148.8(C=N), 166.2(C=O), 125.6,126.0, 126.5, 127.0,127.9, 130.5, 130.6, 132.8, 133.5, 137.0, 142.0(Ar-C),32.12 (CH_3-C) [22].

6-(2,4-Dichlorophenyl)-3-methyl-1H-pyrazolo[3,4-b] pyridin-4-ol (8): A mixture of compound **6** (1mmol) with hydrazine hydrate (1mmol) in ethanol (30 mL) was refluxed for 6 hr then, cooled and recrystallized from acetic acid to

give pale yellow powder of compound **8**.Yield 75 %,m.p.282-284°C. Elem.Anal.Calcd. (%) $C_{13}H_9Cl_2N_3O$ (294.14): C, 53.08; H, 3.08; N, 14.29. Found: C, 53.00; H, 3.05; N, 14.34.IR (KBr, υ, cm^{-1}): 3450 (OH), 3152 (NH), 1680(C=O)1526 (C=N). ^{1}HNMR (400MHz, DMSO-d_6) δ (ppm): 12.97 (s, 1H, OH, D_2O exchangeable),8.16 (s, 1H, NH, D_2O exchangeable),7.74 (d, 1H,J = 8.40Hz,Ar-H), 7.65-7.58(d_2,1H,Ar-H), 7.43 (d,1H,J=8.40Hz,Ar-H),2.58 (s, 3H, CH_3).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 133.0 (C=N), 126.8, 127.5, 127.9, 128.1, 129.8, 130.4,131.1,131.9,132.1,132.7,132.8 (Ar-C), 30.28 (CH_3-C)[23].

6-(2,4-Dichlorophenyl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-4-ol (9): A mixture of compound **6** (1mmole) with phenyl hydrazine (1mmole) in ethanol (30 mL) was refluxed for 6 hr, then cooled and recrystallized from acetic acid. Yield 62 %,m.p.168-170°C.Elem.Anal.Calcd. (%) $C_{19}H_{13}Cl_2N_3O$ (370.23): C, 61.64; H, 3.54; N, 11.35. Found: C, 61.71; H, 3.52; N, 11.39.IR (KBr, υ, cm^{-1}): 3344 (OH). 1586(C=N)^{1}HNMR (400MHz, DMSO-d_6/D_2O) δ (ppm):10.59 (s, 1H, OH, D_2O exchangeable), 7.27-7.74 (m, 8H, Ar-H), 2.57 (s, 3H, CH_3) [23].

6-(2,4-Dichlorophenyl)-3,4-dimethyl-5-phenyl-5H-pyrazolo[3,4-d]pyrimidine (10): A mixture of compound **5** (1mmol) with hydrazine hydrate (1mmol) in ethanol (15mL) was refluxed for 3 hrs, then the precipitate cooled, collected and recrystallized from ethanol to give yellow crystals from **10**.Yield 70 %,m.p. 292-294°C.Elem.Anal.Cald. (%) $C_{19}H_{14}Cl_2N_4$ (369.25): C, 61.80; H, 3.82; N, 15.17. Found: C, 61.89; H, 3.79; N, 15.22.IR (KBr, υ, cm^{-1}): 1620 (C=N). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 7.27-7.90 (m, 8H, Ar-H),3.20 (s, 3H, CH_3-C=N),2.34 (s, 3H, CH_3).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm):160.1, 158.0, 152.3, 144.0, 137.0, 136.6, 135.6,132.5, 132.4, 132.2, 130.9,130.1, 129.0, 127.4, 126.9 (Ar-C), 30.85 ($\underline{CH_3}$-C=N), 29.06 ($\underline{CH_3}$-C) [24].

2-(2,4-Dichlorophenyl)-6-methyl-1-phenyl-4-(2-phenylhydrazono)-5-(1-(2-phenylhydrazono) ethyl)-1,4-dihydropyrimidine (11): A mixture of compound **5** (1mmol) with phenyl hydrazine (1mmol) in ethanol (15 mL) was refluxed for 3 hrs, then the precipitate left at room temperature, collected and recrystallized from ethanol to give yellow crystals of compound **11**.Yield 52 %, m.p. 168-170 °C. Elem. Anal. Calcd. (%) $C_{31}H_{26}Cl_2N_6$ (553.48): C, 67.27; H, 4.73; N, 15.18 Found: C, 67.35; H, 4.70; N, 15.23IR (KBr, υ, cm^{-1}): 3321(NH), 3132 (NH), 1608 (C=N). ^{1}HNMR (400MHz, DMSO-d_6/D_2O)ä(ppm):11.20(s, 1H, NH, D_2O exchangeable), 11.61 (s, 1H, 1NH, D_2O exchangeable), 7.27-7.74 (m, 18H, Ar-H),2.50 (s, 3H, CH_3-C=N), 1.50 (s, 3H, CH_3) [25].

7-(2,4-Dichlorophenyl)-4,5-dimethyl-6-phenylpyrimido[4,5-*d*]pyrimidin-2(6*H*)-one(12): A mixture of compound **5** (1 mmol) and urea (1 mmole) was refluxed for 3 hrs. The obtained solid cooled and recrystallized from ethanol to give pale yellow of compound **12**.Yield 63 %, m.p. 180-182 ?. Elem. Anal. Calcd. (%) $C_{20}H_{14}Cl_2N_4O$ (397.26): C, 60.47; H, 3.55; N, 14.10. Found: C, 60.56; H, 3.52; N, 14.14.IR (KBr, υ, cm^{-1}): 1701 (C=O), 1604 (C=N). ^{1}HNMR (400MHz, DMSO-d_6/D_2O) ä (ppm): 7.39-7.68 (m, 8H, Ar-H), 2.50 (s, 3H, CH_3-C=N), 1.81 (s, 3H, CH_3).^{13}C NMR (100MHz, DMSO-d_6) ä (ppm): 169.2(C=O), 158.0 (C=N), 152.4, 144.1,136.9, 136.5,135.5, 132.4, 132.2, 130.9, 130.0, 129.06, 129.01, 127.5, 126.9 (Ar-C), 30.83 ($H_3\underline{C}$-C=N), 17.81 (CH_3-C) [25].

6-(2-(2,4-dichlorophenyl)-6-methyl-1-phenyl-4-thioxo-1,4-dihydropyrimidin-5-yl)-4-(3-nitrophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (13): A mixture of compound **5** (1mmole),4-nitrobenzaldehyde (1mmole), ethylcyanoacetate (1mmol) and ammonium acetate (1mmol) in acetic acid was refluxed for 6 hrs, then the obtained solid collected by filtration and recrystallized from acetic acid. Yield 60 %, m.p. 300-302°C. Elem. Anal. Calcd. (%) $C_{29}H_{17}Cl_2N_5O_3S$ (586.45): C, 59.39; H, 2.92; N, 11.94. Found: C, 59.28; H, 2.88; N, 11.89.IR (KBr, υ, cm^{-1}): 2218 (C=N), 1701 (C=O), 1242 (C=S), (NO_2). ^{1}H NMR (400MHz, DMSO-d_6/D_2O) δ (ppm): 12.32 (s, 1H, NH),7.39-7.68 (m, 12H, Ar-H),6.98 (s, 1H, pyridine-H),2.50 (s, 3H, CH_3).^{13}CNMR (100MHz, DMSO-d_6) δ (ppm): 169.8 (C=O)160.3(C=S),158.1(C=N),157.1,154.0,152.4,148.2,144.1, 137.0, 136.5, 135.5, 132.5, 132.4, 132.2,130.9,130.0,129.0,128.9,128.2,127.8,127.5,126.9,126.6 ,126.0, 125.5 (Ar-C , pyrimidine-C and pyridine-C),114.5(C=N), 30.83, (CH_3-C) [26].

1-(2-(2,4-Dichlorophenyl)-6-methyl-1-phenyl-4-thioxo-1,4-dihydropyrimi din-5-yl)-3-phenylprop-2-en-1-one (14a): A mixture of compound **5** (1mmol), benzaldehyde (1mmol) and sodium hydroxide (1mmol) was refluxed for 3 hrs, the obtained solid was collected by filtration and recrystallized from ethanol. Yield 71 %, m.p. 340-342°C. Elem. Ana. Calcd. (%)$C_{26}H_{18}Cl_2N_2OS$ (477.40): C, 65.41; H, 3.80; N, 5.87. Found: C, 65.33; H, 3.77; N, 5.91. IR (υ, cm^{-1}):

1701 (C=O), 1604 (C=N), 1242 (C=S). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm):7.68 -6.80 (m, 15H, Ar-H and CH=CH), 2.50 (s, 3H, CH_3).^{13}C NMR (100MHz, DMSO-d_6) δ (ppm):182.4(C=S),169.0 (C=O), 158.0 (C=N),158.0, 154.2,152.4, 144.1, 136.9, 136.5, 135.5, 132.5, 132.4, 132.2,130.9,130.0, 129.0, 127.5, 126.9,126.6, 126.0, 125.5, 112.5, 90.8(Ar-C and ethene–C), 30.83 (CH_3-C)[27].

1-(2-(2,4-dichlorophenyl)-6-methyl-1-phenyl-4-thioxo-1,4-dihydropyrim idin-5-yl)-3-(3-nitro phenyl) prop-2-en-1-one (14b): A mixture of pyrimidine thione**5**(0.02 mole), 4-nitrobenzaldehyde (0.02 mole) and sodium hydroxide (0.02 mole) was refluxed for 3 hours, the obtained solid was collected by filtration and recrystallized from ethanol. Yield 70%,m.p. 350-352°C. Elem. Anal.Cald. (%) $C_{26}H_{17}Cl_2N_3O_3S$ (522.40): C, 59.78; H, 3.28; N, 8.04. Found: C, 59.88; H, 3.32; N, 8.09.IR (υ, cm^{-1}): 1701 (C=O), 1704 (C=N), 1242 (C=S)(NO_2). ^{1}HNMR (400MHz, DMSO-d_6) δ (ppm):7.96-6.89 (m, 14H, Ar-Hand CH=CH), 2.50 (s, 3H, CH_3).^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 182.5(C=S),169.0(C=O),158.0(C=N),158.0,154.2,152.4,144.1, 137.9, 136.5, 135.5,132.5,132.4,132.2, 130.9,130.0, 129.0, 127.5, 126.9,126.6, 126.0, 125.5, 101.0, 116.5 (Ar-C and ethene–C), 30.85 (CH_3-C) [27].

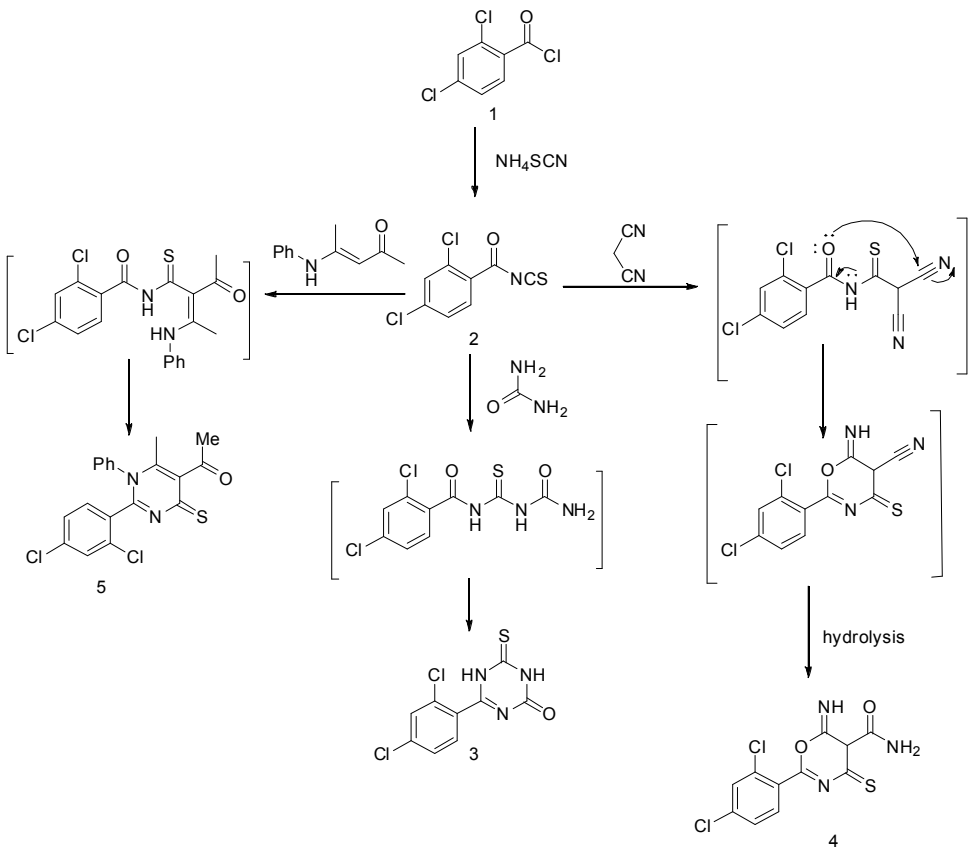
Antimicrobial Activity: The agar diffusion disc method was followed for antibacterial and antifungal susceptibility test [28].Two pathogenic fungi (*Aspergillus flavus* and *Aspergillus niger*) and two multi-drug resistant bacteria (*Enterococcus faecalis* and *Listeria monocytogens*) were used in biological activity. Petri plates were prepared by pouring 10 ml of nutrient Agar for bacteria and czapexDox Agar for fungi and allowed to solidify. Plates were dried and 1 ml of standardized bacterial and fungal inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the inoculum was allowed to dry for 5 min. four sterile paper discs (4 mm in diameter) were placed on the surface of each agar plate and were impregnated with 100 µL of the diluted tested samples (0.5g/1ml DMSO). The plates were incubated at 37 °C for 24 h (Bacteria) and 28 °C for 72–96 h (mycelial fungi). The inhibition zone was measured (as 4 mm) from the edge of the disc to the inner margin of the surrounding fungal and bacterial pathogens. Each assay in this experiment was repeated triplicate.

RESULTS AND DISCUSSION

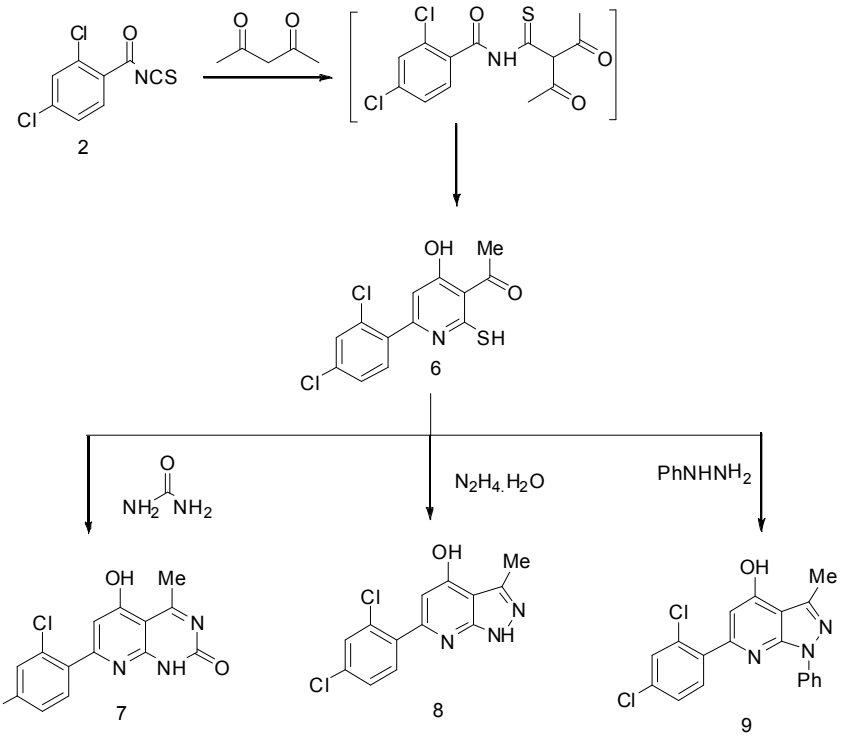
2, 4-Dichlorobenzoyl isothiocyanate (**2**) (Prepared through reaction of 2, 4-dichlorobenzoyl chloride with ammonium thiocyanate in dry acetone) [29]. The triazine **3** was obtained from the reaction of 2, 4-dichlorobenzoyl isothiocyanate (**2**) with urea as shown in **Scheme 1**. ^{1}HNMR was in agreement with the triazine structure which showed two NH signals at11.65 ppm and 10.41 ppm (Controlled by changing D_2O)in addition to aromatic protons were observed as a multiplet signal at 7.74-7.51 ppm. IR spectrum revealed the presence of bands for NH, C=O and C=S absorption bands at 3251, 1685 and 1234 cm^{-1}, respectively. In ^{13}C NMR of compound **3** the C=O, C=S and C=N SP^2 carbon showed signals at 181 ppm, 166 ppm and 142 ppm. Malononitrile and of 2,4-dichlorobenzoyl derivative (**2**) underwent [4+2] cycloaddition to produce oxazine derivative **(4)** presumably *via* acyclic form that underwent addition of enolic OH to cyano function followed by partial hydrolysis of cyano group as depicted in **Scheme 1**. ^{1}HNMR spectrum displayed down field signals at 11.65, 9.63 and 9.95 ppm for NH and NH_2(D_2O-exchangeable) The IR spectrum of compound **4** displayed signals for NH, C=O, C=N and C=S at 3344, 1650, 1608 and 1234 cm^{-1}, respectively. ^{13}C NMR of compound **4** showed signals at 181.9,166.8 and 158 ppm of C=S,C=O and C=Nsp^2 carbons.

Enamine carbon of enaminone derivative underwent nucleophilic attack to heteroallene**2** to furnish pyrimidine thione derivative **5**. The reaction started with the formation of a non-isolable acyclic intermediate that loss a molecule of H_2O. The ^{1}H NMR spectrum showed a multiplet signal at 7.68-7.39 ppm for aromatic protons and singlet signal for CH_3 at 2.50 ppm.IR spectrum of compound **5** displayed signals for C=O and C=S at 1701and 1242 cm^{-1}. Moreover,(C=S), (C=O) and (C=N) groups resonated at 201, 193 and 152 ppm.

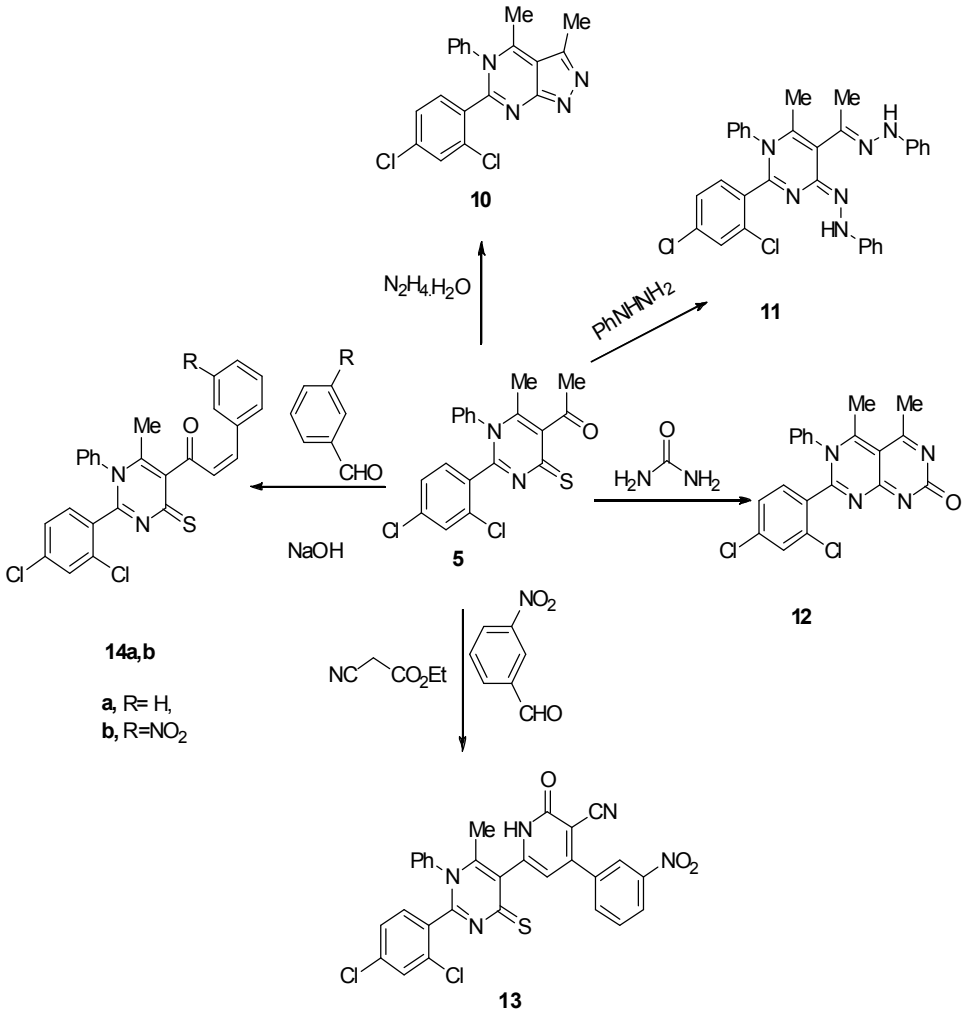
The reaction of acylisothiocyanate with acetyl acetone underwent cyclo condensation of type [3+3] in the presence of triethylamine to afford mercapto pyridine derivative **6** as demonstrated in **Scheme 2**. The IR spectrum of mercapto pyridine derivative **6** showed absorption peaks at 3375, 2560, 1651 and 1234 cm^{-1} for NH, SH, C=O and C=S, respectively. ^{1}H NMR spectrum of compound 6 showed deshielded signals at 13.69 and 7.92 ppm for SH and OH (Controlled by changing D_2O).^{13}C NMR of compound **6** showed signals at 172 and 137 ppm of C=O and C=N sp^2 carbons. Upon reaction of mercaptopyridine **6** with urea the pyridopyrimidine **7** was afforded as a result of attack of urea to carbonyl and thiocarbonyl functions as shown in **Scheme 2**. ^{1}H NMR spectrum of compound **7** showed deshielded signals for NH and OH at 12.57and 8.12 ppm (Controlled by changing D_2O).IR spectrum revealed the presence of bands for OH, NH, C=O absorption bands at 3444, 3325 and 1693cm^{-1}, respectively.



Scheme 1:



Scheme 2:



Scheme 3:

3-Acetylpyridine **6** condensed with hydrazine hydrate to form pyrazolopyridine derivative **8.** ^{1}H NMR data of compound **8** showed two downfield signals at 12.99 ppm for NH proton. ^{1}H NMR spectrum of compound **8** showed two deshielded signal for OH and NH at 12.97 and 8.16ppm (Controlled by changing D_2O). IR spectrum of **8** showed C=O band at 1680 $cm^{-1}$and C=N at 1526 cm^{-1}.Cyclocondensation of mercaptopyridine **6** with phenylhydrazine afforded1-phenylpyrazolo [3, 4-b] pyridine **9**. ^{1}HNMR of **9** showed a downfield signal at 10.59 ppm for OH. Also, the C=N absorption stretching was observed in IR spectrum at 1586 cm^{-1}.

Pyrazolopyrimidine **10** was obtained by the reaction of pyrimidine thion **5** with hydrazine hydrate as illustrated in **Scheme 3**. The target compound **10** conatined absorption peak at 1620 cm^+ for C=N in addition to multiplet signal in region 7.90-7.27 ppm. The reaction of pyrimidinethione (**5**) with two moles of phenyl hydrazine afforded hydrazone derivative (**11**) as shown in **Scheme 3**. The IR spectrum of compound **(11**) leads to NH and C=O at υ 3321 and 1666 cm^{-1}, respectively. ^{1}H NMR contained two NH's signals at 9.64 and 9.60 ppm. Compound **5** was allowed to react with urea to furnish 7-(2, 4-Dichlorophenyl)-4, 5-dimethyl-6-phenylpyrimido [4, 5-*d*] pyrimidin-2(6*H*)-one (**12**). ^{1}HNMRwas in agreement with the pyrimidopyrimidine structure **12** which revealed aromatic protons at7.39-7.68ppm.IR spectrum showed (C=O) and (C=N) stretching frequency at 1701and 1604 cm^{-1}.

One pot four component reaction of pyrimidinethion **(5**) with 3-nitrobenzaldehyde, ethylcyanoacetate and ammoniumacetate in acetic acid afforded 6-(2-(2, 4-dichlorophenyl)-6-methyl-1-phenyl-4-thioxo-1,4-dihydro pyrimidin-5-yl)-4-(3-nitrophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13**). IR showed absorption bands at 2218, 1701and 1242cm^{-1} for C=N, C=O and C=S,

Table 1: Screening for antimicrobial activity of synthesized compounds

	Mean inhibition zone (M. IZ mm)			
	Bacteria strains			
	Gram +ve		Fungi strains	
Compound number	*E. faecalis*	*L.monocytogens*	*A. falvus*	*A .niger*
3	0.8	0.6	0.5	1.9
4	1.99	2.1	2.16	–
5	0.7	0.8		2.96
6	1.32	1.1	2.36	
7	–	2.56	–	–
8	0.6	–	2.73	1.56
9	–	1.98	–	–
10	1.4	1.03	2.96	–
11	0.88	0.9	2.85	1.77
12	1.24	–	–	1.87
13	2.8	1.58	2.12	–
14a	1.56	1.24	2.79	–
14b	0.56	0.5	2.83	1.93
DMSO(control)	–ve	–ve	-ve	-ve

(-) = No activity. *E. faecalis=Enterococcus faecalis*, *L.monocytogens=Listeria monocytogens, A. falvus=Aspergillus falvus, A.niger= Aspergillus nige*r,

respectively. The ^{1}H NMR showed signals at 12.32 ppm for NH. ^{13}C NMR of compound **13** showed signals at 168.8, 160.3and 158.1 of C=O, C=S and C=N sp^2 carbon. While, (C=N) sp carbon showed at 114 ppm. Acetyl pyrimidinethion **5** underwent aldol condensation with benzaldehydeor 3-nitrobenzaldehydeto form cinnamoylpyrimidine derivative **14a** and **14b.** ^{1}HNMR spectrum of **14a** and **14b** showed multiplet signal at 7.68-6.80 ppm for aromatic protons. Also, the (C=O), (C=S) and (C=N) absorption stretching were observed in IR spectrum for compound **14a** and **14b** at 1701, 1604 and 1242 cm^{-1}. ^{13}CNMR spectra of compound **14a** and **14b** are given in the experimental section.

Antimicrobial Activity: The antimicrobial activity results are summarized in **Table 1**. The synthesized compounds showed a good anti-microbial activity. The anti-fungal and anti- bacterial property of these samples mentioned can be referred to that this sample affected the enzymatic system responsible for the fungal or bacterial growth and reproduction as well as physiological processes.

CONCLUSIONS

Our study reports the synthesis of some heterocyclic compounds containing triazine **3**, oxazine **4**, pyrimidinethion **5** and mercaptopyridine **6**, pyrido [2,3-d] pyrimidine **7**, pyrazolo [3,4-b] pyridine **8** and N-phenylpyrazolo [3,4-b] pyridine **9**, pyrazolo [2, 3-d] pyrimidine **10**, diphenyl hydrazono pyrimidine **11**, pyrimido [4,5-d] pyrimidine **12,** dihydropyrimidinepyridine **13**, pyrimidine thion **14a** and **14b**. Spectral and analytical data of the newly synthesized compounds were in good agreement with proposed chemical structure. The antimicrobial activity study revealed that compounds **3,4,5,6, 7,8 ,9, 10, 11,12,13,14a** and **14b** showed a good activity for microbial strains which studied.

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