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# Synthesis of novel pyrimidines thiazolopyrimidines, triazolopyrimidines and pyrimidotriazines as potent antimicrobial agent

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**ABSTRACT**

*A new seriesof 4-aryl-5-cyano thiouracilderivatives* $4_{(a-c)}$ *was synthesized by reacting ethyl cyanoacetate, thiourea or substituted thiourea and aromatic aldehyde in presence of anhydrous potassium carbonate. The main target compound 4-(p-chlorophyll) -2- mercapto -6 -oxo-1,6- dihydropyrimidine-5- carbonitriles (* $\mathbf{4_a}$ *) were reacted with chloroacetyl chloride , chloroacetonitrile or oxalyl chloride to give 2,5-dioxothiazolopyrimidines, 3-amino-5-oxothiazolopyrimidines and 2,3,5-trioxothiazolopyrimidines* ***5,6,7*** *respectively . Derivatives of 2-substituted benzylidne 3, 5-dioxothiazolopyrimidines* $\mathbf{8_{a,\ b}}$ *were prepared via reacting* $\mathbf{4_a}$ *with chloroacetic acid, aromatic aldehyde and anhydrous sodium acetate in presence of a mixture of acetic acid and acetic anhydride. Reacting compound* ***9*** *which was obtained by refluxing compound* $\mathbf{4_a}$ *with hydrazine hydrate , with formic acid or acetic anhydride give the respective triazolopyrimidines* ***10,11*** *.On the other handthe pyrimidotriazines* ***12,13*** *were obtained by the action of compound* ***9*** *with diethyl oxalate and ethyl bromoacetate in sodium ethoxide respectively. Reacting compound* ***9*** *with different aromatic aldehyde in glacial acetic acid gave a series of azomethene compounds* $\mathbf{14_{(a-d)}}$. *All the newly formed compounds are evaluated for their antimicrobial activity .It was found that some of the tested compoundshas variable activity against the selected strain.*

**Keywords:** Pyrimidines, Thiazolopyrimidines, triazolopyrimidines, pyrimidotriazines,

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## INTRODUCTION

Heterocycles are widely incorporated in many of pharmaceutical compound[1, 2].One of the most importantheterocycles that are widely used as a key building unit for preparation of many pharmaceutical compounds is the pyrimidines. It exhibits a wide spectrum of pharmacophore activities, as it can act as bactericidal, fungicidal [3], analgesic [4] andantihypertensive [5].Among the substituted pyrimidines, thiouracils that are well known for anti-inflammatory and virucidal agents [6].The literature survey indicates continuing research in polysubstituted pyrimidine as potential anti-tumor agents [7-9].The biological and synthetic significance places this moiety at a prestigious position in medicinal chemistry research. Thiazoles and their derivatives are also found to be associated with various biological activities such as antibacterial, antifungal and anti-inflammatory [10]. Since the tow heterocyclic moieties thiazole and pyrimidine constitute two active pharmacophores that are highly active against inflammation and pain, combining the two is expected to have a synergistic effect on their analgesic properties. This idea has been utilized to prepare thiazolopyrimidine derivatives [11-13] and their pharmacological activity has been reported[14,15].Thiazolopyrimidine derivatives are the biosteric analogues of purines and are potentially bioactive molecules which show anti-inflammatory activity comparable to that of some standard drugs in vivo, with no or minimal ulcerogenic effects[16]. Triazolopyrimidines, in particular, were tested for their medicinal, bactericidal, and fungicidal activity[17-19]. Wide variety of interesting biological activities were observed for those compounds, such as anticancer[20], antiviral[21], anti-H1V-1 activity[22], anti-inflammatory[23],and antimicrobial activities[24].On the other hand,1,2,4-triazines have been proved to be very useful in the synthetic chemistry, especially in various one-step heterocyclization reactions proceeding by insertion of two carbon atoms bearing

bifunctional group[25,26]. The structural diversity and biological significance of 1, 2, 4-triazines have aroused much attention due to the wide range of applications [27-29].In view of all these facts and the main aim of the present work is the study of the reactivity of polyfunctional pyrimidines with the aim of constructing fused heterobicyclic nitrogen systems containing thiazole, triazole and triazine moiety starting from2-mercaptodihydropyrimidine and 2-hydrazinopyrimidine.

## MATERIALS AND METHODS

### Chemistry

General remarks; Melting point are uncorrected and determined in one end capillary tube using Gallen Kamp melting point apparatus MFP-595-010M ( Gallen Kamp). Microanalysis was carried out at The Regional Center For Mycology and Biotechnology Al-Azhar University , Analysis indicated were within ± 0.5% of the theoretical value. Infrared spectra were recorded on Schimadzue FT-IR 8400S spectrophotometer (Schimadzue), and expressed in wave number ($Cm^{-1}$) using potassium bromide disc. The NMR spectra were recorded on Bruker Hight Performance Digital FT-NMR Spectrophotometer Avance III 400 MHz,$^{1}H$ spectra were run at 400 $MH_Z$ and $^{13}C$ at 100.6 $MH_Z$ in dimethylsulphoxide($DMSO-d_6$). Chemical shift were quoted at$\delta$ and were related to that of the solvents. TLC were carried out using Art. DC-Plastikfolien, Kieselgel 60F254 sheets ( Merck) , the devolping system were benzene acetone(4:1) and the spot were visualized at 366, 254 nm by UV Vilbre Lourmat 77202 (Vilber). Compound **4a**[30, 31] and compound **9**[32] were prepared according to the reported methods.

**1-methyl-4-oxo-6-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile(4b,c).**

A mixture of thiourea (0.05 mole), ethyl cyanoacetate (0.05 mole.), 2-thiophenaldehyde or pyridine-4-carboxaldehyde (0.05 mole) and potassium carbonate (0.05 mole) in absolute ethanol (100 ml) was refluxed overnight, cooled and filtered. The precipitate formed was dissolved in hot water and neutralized with glacial acetic acid. The precipitate was filtered off , washed with water and dried and crystallized from the appropriate solvent.

**1-methyl-4-oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile(4b).**

**Yield** 77% .**mp** :200-202°C(ethanol).**IR** $cm^{-1}$.: 3240(NH),3040 (CH aromatic),2216 (C≡N), 1685 (C=O of pyrimidine ring) and 1591,1556 (C=N and C=C).**$^{1}$H-NMR**(400 MHz, DMSO-*d6*): *δ*: 3.58 (s,3H,N-$CH_3$);*δ* 7.32–7.35( t,1H, *J*=4.0 Hz C4 of thiophene); *δ* 8.06–8.10( d,1H, *J*=3.7 Hz C5 of thiophene); *δ* 8.11–8.15 ( d,1H, *J*=5.0 Hz C3 of thiophene);and *δ*: 8.5(s,1H,NH,exchangeable by $D_2O$) .**$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ**:** 175.1, 170.0, 165.2, 136.5, 134.3, 129.5, 128.0, 117.0,88.2,43.5.**Anal.Calcd**.for $C_{10}H_7N_3OS_2$ (249): C, 48.20; H, 2.83; N, 16.86. Found: C, 48.17; H, 2.64; N, 16.24.

**1-methyl-4-oxo-6-(pyridin-4-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5carbonitrile(4c).**

**Yield:** 57%.**mp** :283-285°C(ethanol).**IR** $cm^{-1}$: 3240(NH),3040 (CH aromatic),2216 (C≡N), of 1685 (C=O of pyrimidine ring) and 1591,1556 (C=N and C=C).**$^{1}$H-NMR**(400 MHz, DMSO-*d6*)**::** *δ*: 3.50 (s,3H,N-$CH_3$),*δ:* 7.62–7.83(d,2H, *J*=1.6 Hz C2,6 of pyridine); *δ* 8.1(s, 1H,NH, exchangeable by $D_2O$); and *δ* 8.72–8.83(d,2H, *J*=1.6 Hz C3,5 of pyridine).**$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ: 178,172,159.2,150,143,122,114, ,88,39.5.**Anal.Calcd**.for $C_{11}H_8N_4OS$ (244): C, 54.13; H, 3.21; N, 22.93. Found: C, 53.97; H, 2.95; N, 22.74.

**7-(p-Chlorophenyl)-2,5-dioxo-3,5-dihydro-2H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile(5)**

To a solution of 4a (0.01mole) in dry benzene (15 ml) containingtriethyl amine (0.0003 mole),chloroacetyl chloride (0.016 mole) was added in small portions with continuous cooling and stirring and the mixture was refluxed in a water bath for about 6 hours. The solid obtained was filtered off and crystallized from ethanol to give compound 5 as brown crystals..

**Yield:** 77%.**mp** :192-194 °C(ethanol).**IR** $cm^{-1}$: 3040 (CH aromatic), 2939 and 2900 (CH aliphatic), 2216 (C≡N),1732(C=O of thiazolidinone ring) 1685 (C=O of pyrimidine ring) and1591,1556 (C=N and C=C).**$^{1}$H-NMR**(400 MHz, DMSO-*d6*)*δ*: 4.58 (s.2H ,$COCH_2$); *δ* 7.44–7.63 (d, 2H, *J*=8.6 Hz ,2ArH); and *δ* 7.84–7.94( d, 2 H, *J*=8.6 Hz ,2ArH). **$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ:191.0, 171.5, 163.5, 159.0, 135.3, 132.5, 128.5, 127.5, 116.5, 94.2, 62.1.**Anal. Calcd**.for $C_{13}H_6ClN_3O_2S$ (303.5): C, 51.40.31; H, 1.98; N, 13.83. Found: C, 51.17; H, 1.84; N, 14.04.

**3-Amino-7-(p-Chlorophenyl)- 5-oxo- 5H-[1,3]thiazolo[3,2-a]pyrimidine-6-Carbonitrile (6).**

To a solution of 4a (0.01mole) in ethanol (15 ml) , triethyl amine (15 ml) and chloroacetonitrile (0.01 mole) was added drop wise with continuous stirring during about 30 minutes and the mixture was refluxed for about 3 hours. The solid obtained upon dilution with water and acidification with HCl was filtered off and crystallized from ethanol to give compound 6 as yellow crystals.

**Yield**:57%.**mp**:240-242 °C(ethanol). **IR** $cm^{-1}$:3439,3332($NH_2$) 3050 (CH aromatic), 2950 (CH aliphatic), 2220(C≡N), 1660 (C=O of pyrimidine ring)1620 and1591 (C=N and C=C).**$^{1}$H-NMR**: (400 MHz, DMSO-*d6*)**:**

*δ*:5.83(s,1H,C=CH), *δ*:6 .77(s,2H,$NH_2$,exchangeable by $D_2O$),*δ*: 7.64–7.72( d, , 2H, *J*=8.6 Hz, 2ArH), *δ*: 7.74–7.92( d, , 2H, *J*=8.6 Hz, 2ArH).**$^{13}$C-NMR**(100 MHz, DMSO-*d6*) *δ*: 167.5, 161.0, 156.0, 145.2, 136.5, 135.6,129.1,128.7,116.6,88.21,78.9 .**Anal.Calcd**.For $C_{13}H_7ClN_4OS$ (302.5): C, 51.57; H, 2.31; N, 18.51. Found: C, 51.64; H, 2.52; N, 18.40.

**7-(p-Chlorophenyl)-2,3,5-triioxo-3,5-dihydro-2H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (7).**
To a solution of 4a (0.01mole) in dry benzene (15 ml) containing triethyl amine (1 ml), oxalyl chloride (0.01 mole) in dry benzene was added drop wise with continuous cooling and stirring during about 30 minutes and the mixture was stirred at room temperature for about 6 hours and then left overnight. The solid obtained was filtered off and crystallized from ethanol to give compound 7 as brown crystals.

**Yield**:77% .**mp** : 262-264°C(ethanol)**.IR** $cm^{-1}$: 3040 (CH aromatic), 2950 and 2930 (CH aliphatic), 2219 (C≡N),1752,1728 (2C=O of thiazolidinone ring) 1675 (C=O of pyrimidine ring) and 1585,1558 (C=N and C=C).**$^1$H-NMR**(400 MHz, DMSO-*d6*) *δ*: 7.56–7.82(d,2H, *J*=8.3 Hz, 2ArH),), *δ*: 8.16–7.82(d,2H, *J*=8.3 Hz, 2ArH),). **$^{13}$C-NMR**(100 MHz, DMSO-*d6*) *δ*:185, 171.5, 166.5, 165.5,159.5, ,135.6, 133.5, 129.5, 128.6,116, 95.**Anal.Calcd**.for $C_{13}H_4ClN_3O_3S$ (317.5): C, 49.13; H, 1.25; N, 13.22. Found: C, 49.15; H, 1.02; N, 13.70.

**7-(p-chlorophenyl-2-(Substitutedbenzylidene)-3,5-dioxo-2,3-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile $8_{(a,b)}$**
A mixture of 4a (0.01mole), chloroacetic acid (0.01mole),aromatic aldehyde(0.01mole) and 2g of anhydrous sodium acetate was refluxed in a mixture 30 ml of glacial acetic acid and 15 ml of acetic anhydride for about 7 hours . The reaction mixture was cooled and poured gradually onto crushed ice .The solid obtained was filtered off and crystallized from dioxan-water to give compound $8_{(a,b)}$ .

**2-(p-chlorobenzylidene)-7-(p-chlorophenyl)-3,5-dioxo-3,5-dihydro-2H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (8a).**
Pale brown small needle crystals **.Yield**:88%.**mp** :310-312(dioxan-water)°C**.IR** $cm^{-1}$: 3012 (CH aromatic), 2939 and 2900 (CH aliphatic), 2225 (C≡N),1762(C=O of thiazolidinone ring) 1665 (C=O of pyrimidine ring) and1595,1574 (C=N and C=C) .**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*: 7.48–8.06 (m,8H, 8 ArH), 8.23 (s.1H,C=CH)..**Anal.Calcd**.for $C_{20}H_9Cl_2N_3O_2S$ (426): C, 56.33; H, 2.11; N, 9.85. Found: C, 56.80; H, 2.49; N, 9.67.

**7-(p-Chlorophenyl)-2-(m-flurobenzylidene)-3,5-dioxo-3,5-dihydro-2H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile (8b).**
Yellow crystals,**Yield**: 80% .**mp**: above 350 °C(dioxin-water).**IR** $cm^{-1}$: 3042 (CH aromatic), 2960 and 2900 (CH aliphatic), 2221 (C≡N), 1768(C=O of thiazolidinone ring) 1662 (C=O of pyrimidine ring) and1595,1574 (C=N and C=C).**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*: 7.48–8.20 (m, 8H,8ArH), 8.24 (s.1H,C=CH). **$^{13}$C-NMR** (100 MHz, DMSO-*d6*)*δ***:** 175.7, 172.1, 164.1, 160.66, 159.2, 145.24, 136.69, 135.14, 132.9, 131.32, 129.64, 128.65, 124.65 , 118.25, 115.11, 114.5, 113.2. 95.9 **Anal. Calcd**.for $C_{20}H_9ClFN_3O_2S$ (409.5): C, 58.60; H, 2.19; N, 10.25. Found: C, 58.35; H, 2.22; N, 9.95.

**7-(p-chlorophenyl -5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile(10)**:
A mixture of compound 9 (0.01 mole) and formic acid(35 ml of) was heated under reflux for about 8-10 hours .The solid obtained after cooling and pouring onto ice cold water were filtered off, washed with water and crystallized from acetic acid to give compounds 10.

**Yield**:56% .**mp**.:315-317°C(ethanol). **IR** $cm^{-1}$:3159(NH) 3075 (CH aromatic), 2955 and 2932 (CH aliphatic), 2223 (C≡N), 1691 (C=O of pyrimidine ring)1620 and1591 (C=N and C=C) .**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*:7.3(s,1H,N=CH) ,*δ*: 7.44–7.62( d, , 2H, *J*=8.1 Hz, ArH), *δ*: 7.74–7.92( d, , 2H, *J*=8.1 Hz, ArH) *δ*:),.*δ*8.1(s,1H,NH,exchangeable by $D_2O$). **$^{13}$C-NMR**(100 MHz, DMSO-*d6*)*δ***:** 172.1, 166.4, 153.0, 138.2, 136.0, 134.2, 129.5, 128.0, 116.0, 95.0.**Anal.Calcd**.for $C_{12}H_6ClN_5O$ (271.5): C, 53.03; H, 2.21; N, 25.78 Found: C, 53.49; H, 2.38 N, 26.24.

**7-(p-chlorophenyl)-3-methyl-5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile(11) .**
A mixture of compound 9 (0.01 mole) and acetic anhydride(35 ml of) was heated under reflux for about 8-10 hours .The solid obtained after cooling and pouring onto ice cold water were filtered off, washed with water and crystallized from acetic acid to give compounds 11.

**Yield**:63%.**mp** : 180-182 °C(ethanol).**IR** $cm^{-1}$:3185(NH) 3077 (CH aromatic), 2958 and (CH aliphatic), 2225(C≡N), 1695 (C=O of pyrimidine ring)1580 and1577 (C=N and C=C).**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*:2.43(s,3H,$CH_3$) ,*δ*:7.64-7.72(d,2H, *J*=8.5 Hz, 2ArH),*δ*:7.84-8.12(d,2H, *J*=8.5 Hz ,2ArH), *δ*:8.24 1H,NH exchangeable by $D_2O$,).**$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ**:** 171.0,165.5,156,151.5,135.2, 134.0.2,129.1,

128.0,116,94.5,22.5 .**Anal. Calcd**.For $C_{13}H_8ClN_5O$ (285.5): C, 54.64; H, 2.80; N, 24.51 Found: C, 55.08; H, 2.56; N, 24.32.

**8-(p-Chlorophenyl)-3,4,6-trioxo-2,3,4,6-tetrahydro-1H-pyrimido[2,1-c][1,2,4]triazine-7-carbonitriles (12).**
To a solution of sodium ethoxide (0.23 gm sodium in 25 ml absolute ethanol),diethyl oxalate (0.015 mole) was added followed by compound 9 (0.015 mole). The reaction mixture was refluxed for about 10 hours. The solid obtained upon neutralization with dilute HCl was filtered off and recrystallized from ethanol to give compound 12 .
**Yield** :63% .**mp** ; 260-262 °C(ethanol).**IR** $cm^{-1}$:3327,3032(2 NH ) 3032 (CH aromatic), 2223(C≡N), 1743,1728( 2 C=O of triazine ring) 1654 (C=O of pyrimidine ring),1590 ,1558 (C=N and C=C) .**$^1$H-NMR**(400 MHz, DMSO-*d6*) *δ*:3.32(1H,NH, exchangeable by $D_2O$), *δ*:7.62-7.87(d,2H, *J*=8.5 Hz,2ArH),*δ*:7.91-8.02(d,2H, *J*=8.5 Hz 2ArH); and *δ* 10.74 (1H,CO-NH, exchangeable by $D_2O$). **$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ**:**171, 166, 161, 158, 153, 135.5, 134, 129, 127.5, 116, 94**.Anal.Calcd**.for $C_{13}H_6ClN_5O_3$ (315.5): C, 49.44; H, 3.49; N, 22.19 Found: C, 49.89; H, 3.21; N, 22.48.

**8-(p-Chlorophenyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrimido[2,1-c][1,2,4]triazine-7-carbonitriles (13).**
A solution of compound 9 (0.01 mole) and ethyl bromoacetate (0.01 mole)in ethanolic sodium ethoxide (prepared by dissolving o.o1 mole atom of sodium metal in 50 ml. of ethanol) was heated under reflux for 12 hours .The reaction mixture was then cooled and poured onto ice-cold water. The solid product obtained after acidification with HCl were filtered off, washed with water then crystallized from ethanol to afford compound 13 .

**Yield** :50% .**mp** :265-267°C ; **IR** $cm^{-1}$:3315,3151(2 NH ) 3032 (CH aromatic), 2212(C≡N), 1718(C=O of triazine ring) 1664 (C=O of pyrimidine ring),1590 ,1558 (C=N and C=C) ;**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*:3.16(s,2H,$CH_2$), *δ* 3.34( broad,1H, NH, exchangeable by $D_2O$),δ:3.66 (broad,1H,NH, exchangeable by $D_2O$), *δ*:7.52-7.72(d,2H, *J*=8.5 Hz, 2ArH),*δ*:7.84-8.02(d,2H, *J*=8.5 Hz, 2ArH).**$^{13}$C-NMR**(100 MHz, DMSO-*d6*) δ**:**171, 167, 166, 154, 153.5 133.5, 129,128.2, 116, 95, 56;**Anal. Calcd**.for $C_{13}H_8ClN_5O_2$ (301.5): C, 51.74; H, 2.65; N, 23.21 Found: C, 52.15; H, 2.40; N, 23.17.

**4-(p-Chlorophenyl)-2-[2$^-$-(substitutedbenzylidene or methylidene)hydrazinyl]-oxo-1,6-dihydropyrimidine-5-carbonitrile 14$_{(a-d)}$.**
A mixture of compound 9 (0.01 mole) and the appropriate aromatic aldehyde(0.01 mole) in glacial acetic acid (50 ml)was heated under reflux for about from 4-6 hours. The reaction mixture was cooled and poured gradually into crushed ice. The solid obtained was filtered off and crystallized from DMF-$H_2O$ to give 14 $_{(a-d)}$

**2-[2$^-$-(p-chlorobenzylidene )hydrazinyl]-4-(p-chlorophenyl)- 6-oxo-1,6-dihydropyrimidine-5-carbonitrile(14a).**
Pale yellow crystals .**Yield:** 66% **.mp** :350 °C(DMF-$H_2O$).**IR** $cm^{-1}$:3350,3275(2NH) 3082 (CH aromatic), 2955 and (CH aliphatic), 2210 (C≡N), 1666 (C=O of pyrimidine ring)1637 and1584 (C=N and C=C) .**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*:7.49-8.1(m,8H,8 ArH), *δ*:8.71(s.1H.N=CH)and *δ*:12.54, 12.67 (2s,2NH, ,exchangeable by $D_2O$).**Anal.Calcd**.for $C_{18}H_{11}Cl_2N_5O$ (384): C, 56.25; H, 2.86; N, 18.22. Found: C, 56.29; H, 2.89; N, 18.21.

**4-(p-chlorophenyl)-2-[2$^-$-(m-fluorobenzylidene)hydrazinyl]--6-oxo-1,6-dihydropyrimidine-5-carbonitrile(14b).**
Pale yellow crystals.**Yield** :72%..**mp**:340-342°C(DMF-$H_2O$) . **IR** $cm^{-1}$:3300,3220(2NH) 3087 (CH aromatic), 2985 and (CH aliphatic), 2214 (C≡N), 1670 (C=O of pyrimidine ring)1599 and1574(C=N and C=C).**$^1$H-NMR**(400 MHz, DMSO-*d6*)*δ*:7.49-8.4(m,8H,8 ArH),*δ*:8.81(s.1H.N=CH)and *δ*:12.64, 12.50 (2s,2NH, ,exchangeable by $D_2O$). **$^{13}$C-NMR** 172.1, 167.1, 162.1, 154.0, 146.39, 136.5, 135.4, 133.0, 130.5, ,129.5,128.1, 126.9,118, 117.3, 115.5, 92.29 **.Anal.Calcd**.for $C_{18}H_{11}ClFN_5O$ (367.5): C, 58.77; H, 2.99; N, 19.04. Found: C, 58.42; H, 2.67; N, 19.39.

**4-(p-Chlorophenyl)-6-oxo-2-[2$^-$-(thiophene-2-ylmethylene)hydrazinyl]-1,6-dihydropyrimidine-5-carbonitrile(14c).**
Pale yellow crystals .**Yield** : 60%.**mp** : 298-300°C(DMF-$H_2O$) . **IR** $cm^{-1}$:3280,3220(2NH) 3087 (CH aromatic), 2985 and (CH aliphatic), 2212 (C≡N), 1668 (C=O of pyrimidine ring)1602 and1591 (C=N and C=C) . **$^1$H-NMR**(400 MHz, DMSO-*d6*):*δ*:7.29-7.94(m,7H,7 ArH), *δ*:8.85(s.1H.N=CH)and *δ*:11.64, 12.40(2s,2NH, ,exchangeable by $D_2O$).**Anal.Calcd**.for $C_{16}H_{10}ClN_5OS$ (355.5): C, 54.00; H, 2.81; N, 19.69. Found: C, 54.45; H, 2.65; N, 19.43.

**4-(p-Chlorophenyl)-6-oxo-2-[2$^-$-(furan-2-ylmethylene)hydrazinyl]1,6-dihydropyrimidine-5-carbonitrile(14d).**
Dark yellow crystals .**Yield**: 57% .**mp** : 270-272 °C(DMF-$H_2O$) .**IR** $cm^{-1}$:3285,3207(2NH) 3087 (CH aromatic), 2985 and (CH aliphatic), 2220 (C≡N), 1672 (C=O of pyrimidine ring)1612 and1575 (C=N and C=C) .**$^1$H-NMR**(400 MHz, DMSO-*d6*): . *δ*:7.29-7.81(m,7H,7 ArH), *δ*:8.88(s.1H.N=CH)and *δ*:11.80, 12.30(2s,2NH, ,exchangeable by $D_2O$) .**Anal. Calcd**.for $C_{16}H_{10}ClN_5O_2$ (339.5): C, 56.55; H, 2.94; N, 20.60. Found: C, 56.34; H,2.69 ; N,20.44.

**3.2-Antimicrobial activity screening**

The newly synthesized compounds were evaluated for their in vitroantibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans* ATCC 60193 *and Aspergillus niger*ATCC 1718109 using the microbroth dilution method. [33]The Gram-positive antibacterial agent, amoxacillin, the Gram-negative antibacterial agent, gentamycin, and the anti-fungal agent, amphotericin B, were used as controls. In addition to, MICs (minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC50 (the concentration which inhibits 50% of microorganisms) of all compounds were determined according to reported method.[33,34] The invitroantimicrobial properties against a number of Gram-negative and Gram-positive bacteria, and yeasts are presented in Tables 1.

**3.2.1, Determination of the Minimum Inhibitory Concentration (MIC)**

The preliminary MICs were firstly determined by the microbroth dilution method .[33]Briefly, 100 µL of double strength DMSO (Sigma-Aldrich, Germany) were placed in each well of a 96-well microtiter plate. Aliquot of 100 µL of the solutions to be tested were added to the first column. Then two fold dilutions were carried out from one well to the next up to final well in each row for each tested compound.

MICs were then determined using agar streaking technique as per Clinical Laboratory Standard Institute guidelines.[33] A total of 15 mL molten (45 °C) Neutrient agar (Sigma-Aldrich, Germany) were supplemented with the required concentration then were added into sterilized Petri dishes, allowed to solidify. Then 10 µL of each bacterial or fungal suspension (105 CFU mL-1) were streaked onto the surface. Finally all plates were incubated at 37 °C for 24 hours for bacterial strains and 25 °C for 48 hours for fungal strains under aerobic conditions. MIC was determined as the average between the last plate had growth and the first plate with no growth.

**3.2.2, Determination of the MBC and IC50**

MBC and IC50 were determined in 96 well microtiter plate where a 100 µL of trypcase soya broth (Oxoid, USA) for bacterial isolates or sabaroud's dextrose broth for fungal strains were placed in each well. A proper amount of the stock solution of the tested compounds was added to reach the desired concentration. All columns were then inoculated with 20 µL of bacterial suspension (106 CFU mL-1) and incubated for 5-6 hours. An aliquot of 100 µL from each well was transferred into another pre-supplemented with 100 µL f Dey-engly broth medium (Fluka, USA) and allowed to stand for 10-20 minutes to neutralized any antimicrobial activities. Then these neutralized solutions were subjected to proper dilutions and streaked onto trypcase soya agar or sabaroud's dextrose agar plates to determine the viable count. [34] Controls were done for sterility and growth and subjected to the same regimen of treatment. MBC was determined as the lowest concentration which decreased the number of viable bacteria by 3 log units. IC50 was determined as the lowest concentration reduced the viable count by about 50%.

## RESULTS AND DISCUSSION

Chemistry

***The*** *synthesis of the designed compounds is outlined in* ***scheme 1****and* ***scheme*** **2**.The main precursor for the synthesis of our target compounds 4-(p-chlorophenyl)-2- mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitriles( **$4_a$)** was prepared according to previously published synthetic methods [30,31]. The newly formed 6-(4-p-chlorophenyl)-4-oxo-1-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles(**$4_{(b,c)}$**)were prepared adopting the same method used for the preparation of compound **$4_a$** by the condensation cyclization ofN-methyl thiourea, ethyl cyanoacetate and the respective aromatic aldehyde in presence of anhydrous potassium carbonate. This newly formed compound was confirmed by, IR, $^1$H-NMR, $^{13}$C-NMR and microanalysis.

IR spectra of this compound showed the characteristic C≡ N stretching absorption atυ 2200 $Cm^{-1}$in addition to one NH absorption at υ 3400 $Cm^{-1}$.The $^1$H-NMR spectra revealed an exchangeable proton, NH at $\delta$ 8.5 ppm and singlet signal at $\delta$ 3.58 of N-$CH_3$. Attempts to S-alkylate such compounds by methyl iodide in presence of catalytic amount of potassium hydroxide in alcohol or even stronger base like sodium ethoxide was unsuccessful which is attributed to the absence of tatumerism as shown in( **Figure 1)**

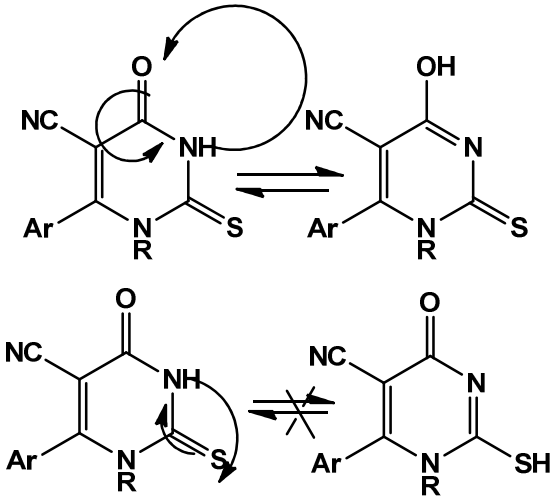
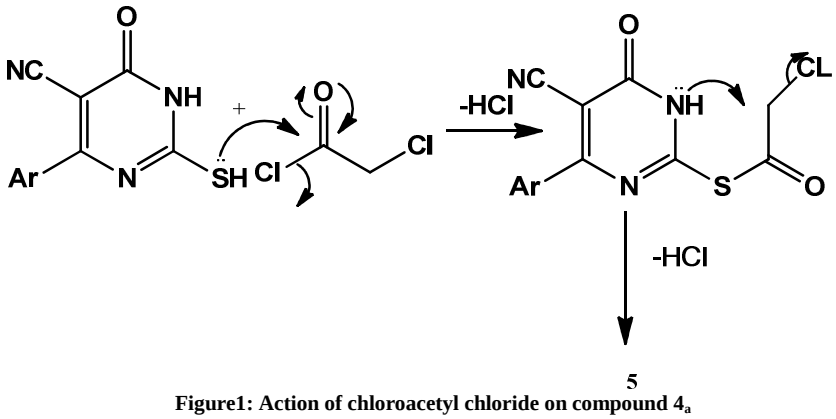


**Figure 1:NO tautomerism where N atom at position 1 was substituted**

Also preparation of 2-hydrazino-1-substituted pyrimidine was failed duo to the same reason. On the other hand the synthesis of the novel compound 7-(p-Chlorophenyl)-2,5-dioxo-2,3-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile(**5**) was achieved by reacting compound **4a** with chloroacetyl chloride in dry benzene and in the presence of triethylamine as in ( **Figure 2).**



**5**

**Figure1: Action of chloroacetyl chloride on compound $4_a$**

Compound **5** was confirmed by the complete absence of the NH band and appearance of new band at 1730 $cm^{-1}$ of the C=O group of the thiazolidinone ring in the IR and also by $^1$H NMR, which revealed disappearance of the two singlet exchangeable signals corresponding to 2 NH of the precursor **4a** and appearance of the singlet signal atδ 4.58 ($COCH_2$) which is more deshielded because of the high –I effect of both N atom and C=O.

Moreover 3-Amino-7-(p-Chlorophenyl)-5-oxo- 5H-[1,3]thiazolo[3,2-a] pyrimidine-6-carbonitril(**6)** was prepared by refluxing compound **4a** with chloroacetonitrile in ethanol containing few drops of triethyl amine via a mechanism discussed in **( Figure 3).** Such compound was confirmed in the IR by the appearance of new band at υ 2950$Cm^{-1}$ which is corresponding to aliphatic CH .Also presence of singlet signal at *δ*:5.83 (s,1H,C=CH) in $^1$H NMR is an indication about the structure of the compound.

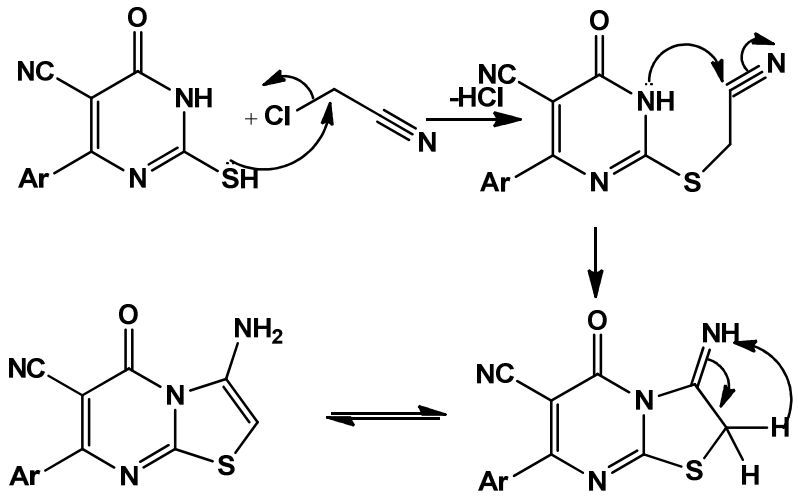


**Figure2: Action of chloroacetonitriles on compound 4a**

Treatment of compound **4**$_a$ with oxalyl chloride in dry benzene afforded the 7-(p-Chlorophenyl)-2,3,5-triioxo-2,3-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carbonitrile **(7)** via two successive nucleophilic attacks upon the carbonyl carbon atom of the oxalyl chloride as shown in **(Figure 4).** Compound **7** was confirmed by the absence of NH band and appearance of two bands at1752,1728 cm$^{-1}$ of the two C=O of the thiazolidinone ring in IR and also by $^1$H NMR, which revealed disappearance of the two singlet $D_2O$ exchangeable signals corresponding to 2 NH .

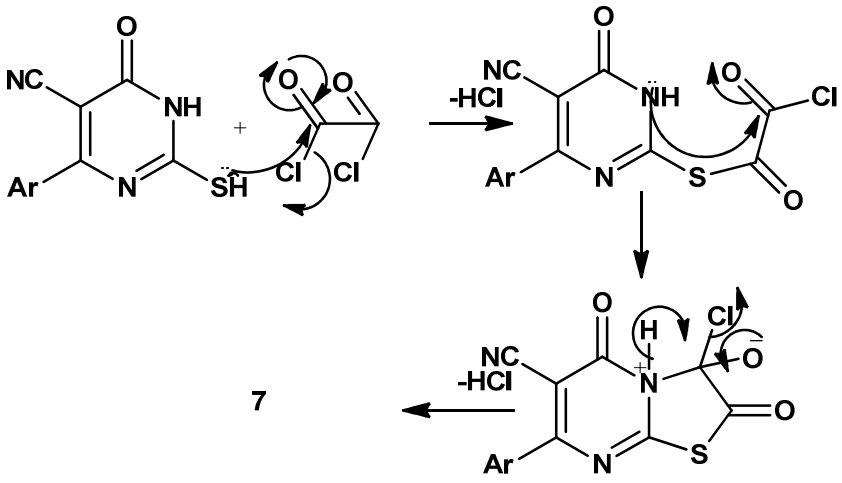


**Figure4:Action of oxallyl chloride on compound 4**$_a$

On the other hand the synthesis of 7-(p-chlorophenyl -2-(Substituted benzylidene) -3,5-dioxo-2,3-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile **8a,b** was outlined in scheme 1 in one pot reaction by refluxing compound **4a** with chloroacetic acid, appropriate aldehyde and sodium acetate in a mixture of acetic acid and acetic anhydride according to the mechanism in **(Figure 5).**

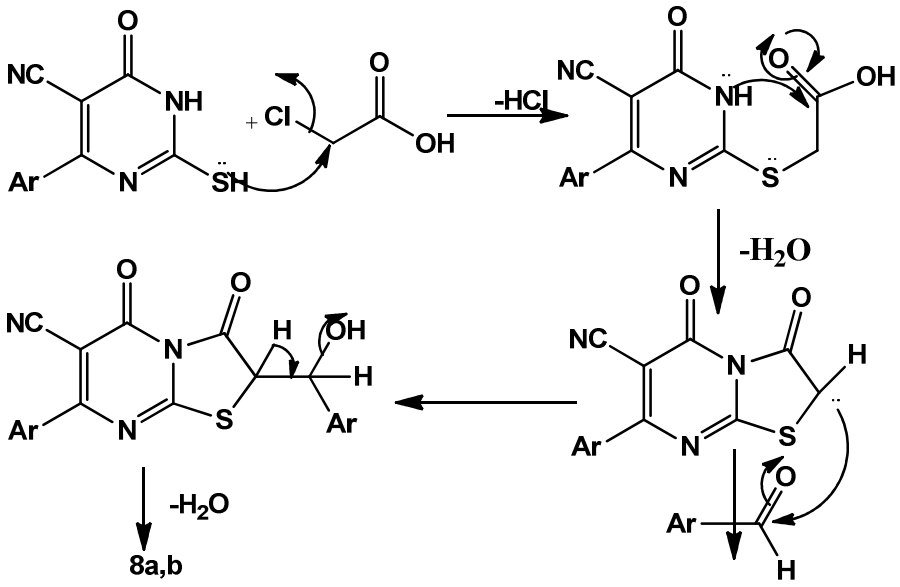



**Figure 5:action of chloroacetic acid and aromatic aldehyde on compound 4$_a$**

The IR spectra of compounds **8$_{a,b}$** showing the complete absence of NH absorption band which is characteristic for the precursor 4a and the appearance of another band at υ 1762Cm$^{-1}$ which is due to the C=O of the thiazolidinone ring. 1H NMR spectra of the same compound showing the presence of signal at $\delta$ 8.81 of (s.1H,C=CH).

The synthesis of 7-(p-chlorophenyl -5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile and 7-(p-chlorophenyl)-3-methyl-5-oxo-1,5-dihydro-[1,2,4] triazolo[4,3-a] pyrimidine-6-carbonitrile **(10)** and **(11)** was achieved via the reaction of the previously prepared 2- hydrazinopyrimidine [32,] with formic acid or acetic acid respectively for a long time as shown in **(Figure 6).**The structure of compound 10 and 11 were approved and confirmed by the complete absence of a second C=O group band in the IR(of the opened NHNHCHO or NHNHCOCH$_3$) and the presence of a singlet signal of one NH exchangeable by D$_2$O , $\delta$ 7.3(s,1H,N=CH) in compound 10 and $\delta$ 2.43(s,3H,CH$_3$) in compound 11 in $^1$H-NMR.

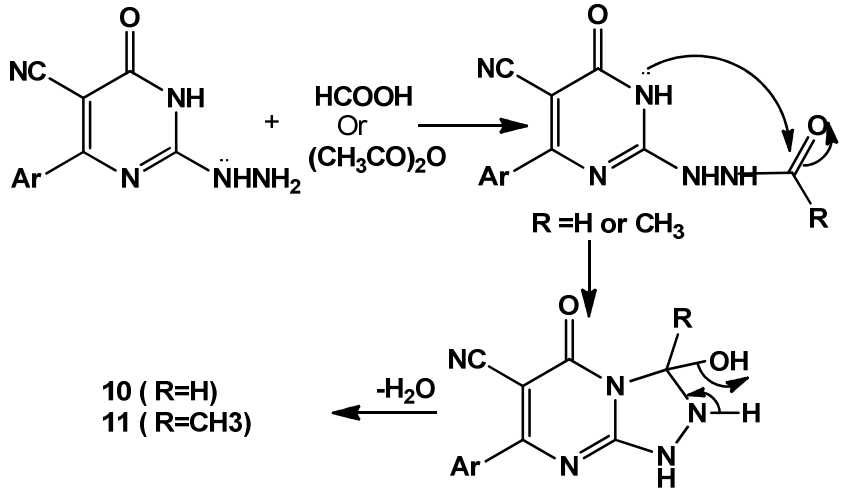



**Figure 6: Action of acetic or formic acid on compound 9**

The synthesis of 8-(p-Chlorophenyl)-3,4,6-trioxo-2,3,4,6-tetrahydro-1H-pyrimido[2,1-c][1,2,4]triazine-7-carbonitriles**(12)** and 8-(p-Chlorophenyl)-4,6-dioxo-2,3,4,6-tetrahydro-1H-pyrimido[2,1-c][1,2,4]triazine-7-carbonitriles (**13**) was adopted by reaction of compound **9** with diethyl oxalate or ethyl bromoacetate respectively in strongly basic media like sodium ethoxide, as shown in( **Figure 7)**.Compound **12** was confirmed by the presence of an additional absorption bands at 1743,1728( 2 C=O of triazine ring) in IR. While compound **13** was approved by the appearance of another absorption band at1718(C=O of triazine ring) in the IR and presence of singlet signal at $\delta$ 3.36 (2H,CH$_2$) in the $^1$H-NMR.

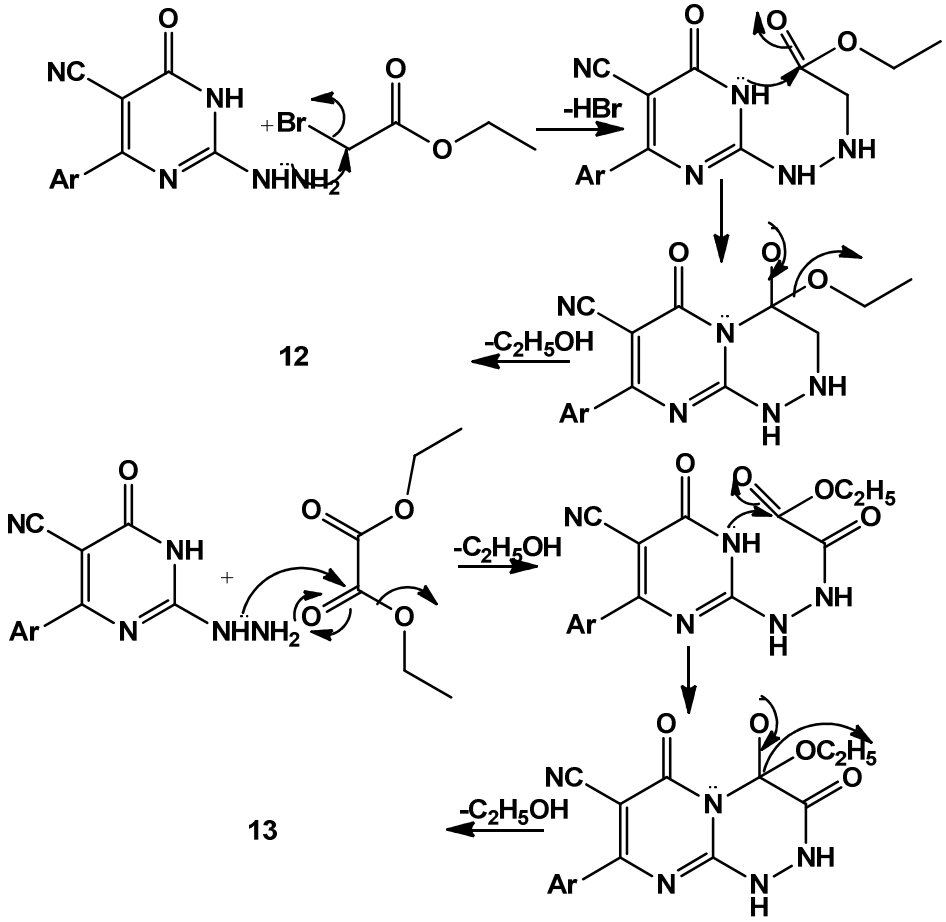
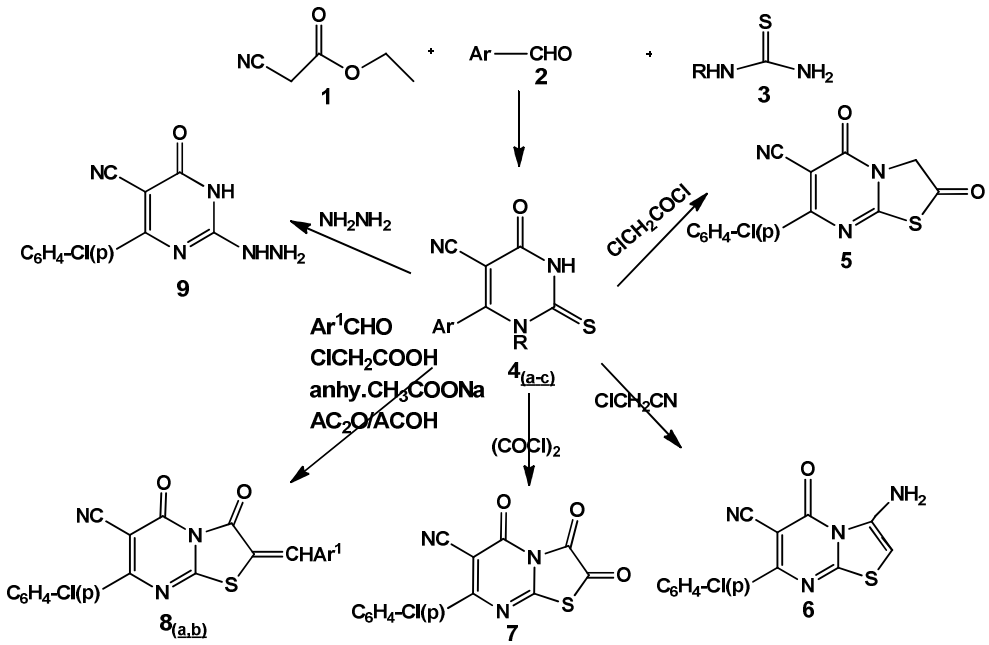
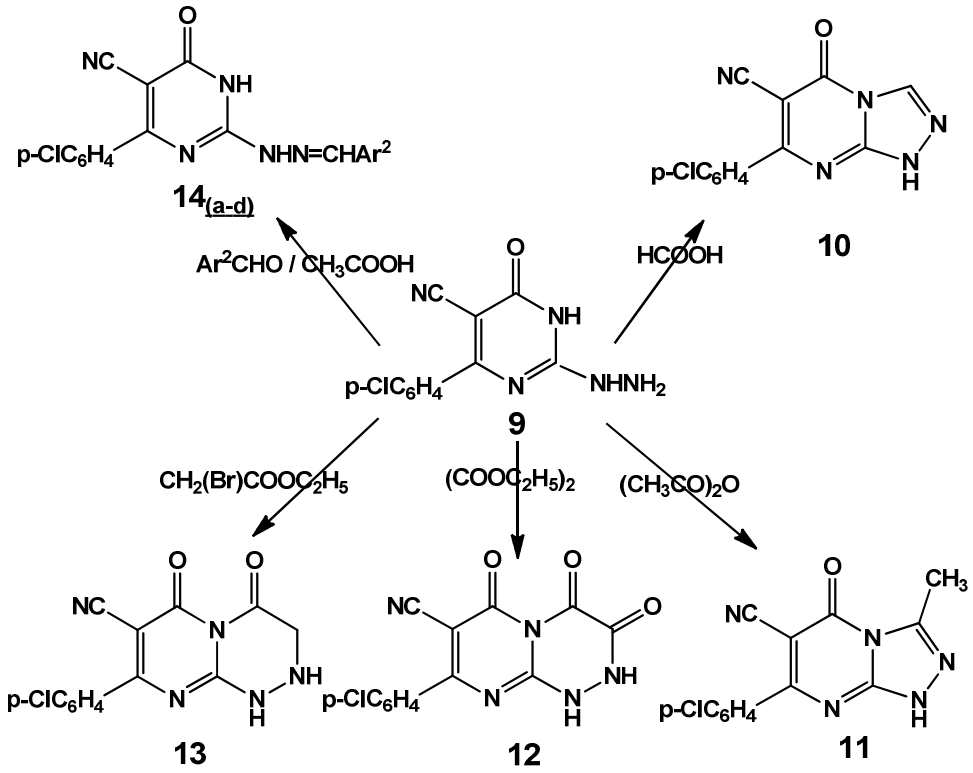



**Fig. 7.:action of ethyl bromoacetate or diethyl oxalate on compound 9**

A series of 4-(p-Chlorophenyl)-2- [2-(substituted benzylidene or methylidene)hydrazinyl] - 6-oxo-1,6-dihydropyrimidine-5-carbonitrile **14**$_{(a-d)}$ were prepared by reacting the key compound **(9)** with different aromatic aldehyde in glacial acetic acid. The presence of a singlet signal at $\delta$ 8.88 (1H.N=CH) in the $^1$H-NMR is an indication about the structure.




**Scheme1 . 4aAr = p-ClC$_6$H$_4$ , R = H , 4bAr =2- thienyl, R =CH$_3$, 4cAr =4- pyridyl , R =CH$_3$8a Ar$^1$= m-FC$_6$H$_4$ , 8b Ar$^1$ = p-ClC$_6$H$_4$**




**Scheme1. :14a $Ar^2$= p-$ClC_6H_4$,14b $Ar^2$ = m-$FC_6H_4$,14c $Ar^2$ = thiophen-2-yl, 14d $Ar^2$ = furan-2-yl**

**Antimicrobial activity**

The newly synthesized compounds were subjected for evaluation of their antimicrobial activities using micro broth dilution method [34]. The data presented in table 1 which revealed that compounds **4b** showed broad spectrum antibacterial and antifungal activities, while compounds **8a,8b,10 and 13** showed antibacterial activity against Gram positive bacteria. Compounds **4c, 5,14a and 14b** showed antifungal activity. The remaining compounds had no significant activity against any of the tested strains at concentration up to 50 µg/mL.

**Table .Antimicrobial activity of the synthesized compounds expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and concentration that inhibit 50% of microorganisms (IC50) in µg /mL against the pathologicalstrains based on two fold serial dilution technique**

| *Compound* | | *Gram positive bacteria* | | *Gram negative bacteria* | | *Fungi* | |
|---|---|---|---|---|---|---|---|
| | | *S. aureus* | *B. subtlis* | *E. coli* | *P.aeruginosa* | *C.albicans* | *A. niger* |
| 4b | MIC | 18.75 | 18.75 | 18.75 | 18.75 | 18.75 | 37.5 |
| | MBC | 18.75 | 18.75 | 18.75 | 18.75 | 18.75 | 37.5 |
| | IC50 | 12.5 | 12.5 | 9.3 | 12.5 | 12.5 | 12.5 |
| 4c | MIC | >50 | >50 | >50 | >50 | 16.5 | 16.5 |
| | MBC | >50 | >50 | >50 | >50 | 16.5 | 16.5 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | 12 | 12 |
| 5 | MIC | >50 | >50 | >50 | >50 | 18.75 | 9.38 |
| | MBC | >50 | >50 | >50 | >50 | 18.75 | 12.5 |
| | IC50 | >50 | >50 | >50 | >50 | 12.5 | 9.3 |
| 6 | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | >50 | >50 |
| 7 | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | >50 | >50 |
| 8a | MIC | 37.75 | 37.75 | >50 | >50 | >50 | >50 |
| | MBC | 37.75 | 37.75 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | 25 | 25 | >50 | >50 | >50 | >50 |
| 8b | MIC | 18.75 | 18.75 | >50 | >50 | >50 | >50 |
| | MBC | 18.75 | 18.75 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | 12.5 | 125 | >50 | >50 | >50 | >50 |
| 10 | MIC | 37.5 | 37.5 | >50 | >50 | >50 | >50 |
| | MBC | 37.5 | 37.5 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | 25 | 25 | >50 | >50 | >50 | >50 |
| 11 | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | IC50 | >50 | >50 | >50 | >50 | >50 | >50 |
| 12 | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | >50 | >50 |
| 13 | MIC | 4.7 | 4.7 | >50 | >50 | >50 | >50 |
| | MBC | 4.7 | 4.7 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | 2.3 | 2.3 | >50 | >50 | >50 | >50 |
| 14a | MIC | >50 | >50 | >50 | >50 | 18.75 | 18.75 |
| | MBC | >50 | >50 | >50 | >50 | 18.75 | 18.75 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | 12 | 9.3 |
| 14c | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | >50 | >50 |
| 14d. | MIC | >50 | >50 | >50 | >50 | >50 | >50 |
| | MBC | >50 | >50 | >50 | >50 | >50 | >50 |
| | $IC_{50}$ | >50 | >50 | >50 | >50 | >50 | >50 |
| Amoxicillin | MIC | 10 | 100 | NO Action | NO Action | NO Action | NO Action |
| Gentamycin | MIC | NO Action | NO Action | 10 | 25 | NO Action | NO Action |
| Amphotricin B | MIC | NO Action | NO Action | NO Action | NO Action | 10 | 15 |

## CONCLUSION

Present study describes the synthesis of derivative of pyrimidines, triazolopyrimidines ,thiazolopyrimidines and pyrimidotriazines. Thecompounds were characterized by spectral techniques such as IR and $^{1}$H- NMR,$^{13}$C-NMR spectra and elemental analysis. All the title compoundswere screened for their antibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans*ATCC 60193 *and Aspergillusniger*ATCC 1718109.In addition their MICs (minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC50 (the concentration which inhibits 50% of microorganisms) were determined. The results of antibacterial activity showed that unsubstituted pyrimidines more active than substituted one.

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