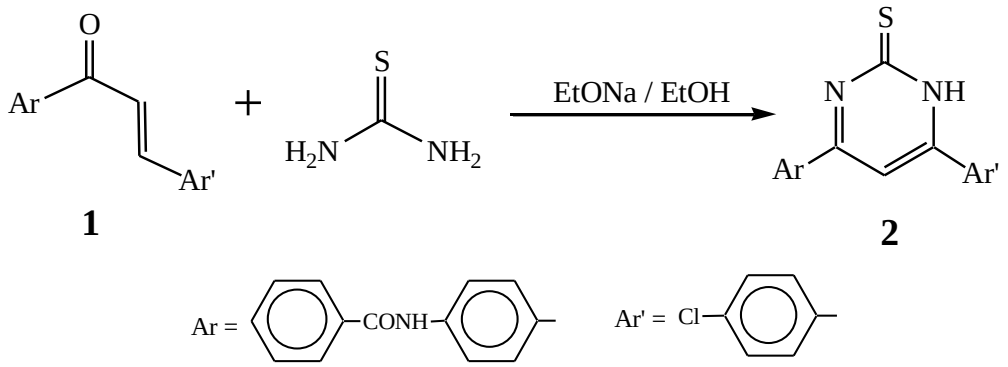
RESULTS AND DISCUSSION

Synthesis and reactions of 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-1*H*-pyrimidine-2-thione

Pyrimidine rings are an integral part of DNA bases and various natural products. They serve as building blocks for numerous pharmaceuticals and occupy a unique place in heterocyclic and medicinal chemistry. In addition, they are pharmacologically active and display anticonvulsant [83], anti-inflammatory [84-86], antibacterial [87], antihistaminic [88], antifungal [89], anti-tumor [90], antitubercular [91] and antileishmanial activities [92]. Condensed pyrimidine derivatives possessing antimicrobial and analgesic activities [93-96] are well-known. Based on these facts, we planned to prepare new pyrimidine derivatives with the aim of increasing potency and improving their pharmacological profile.

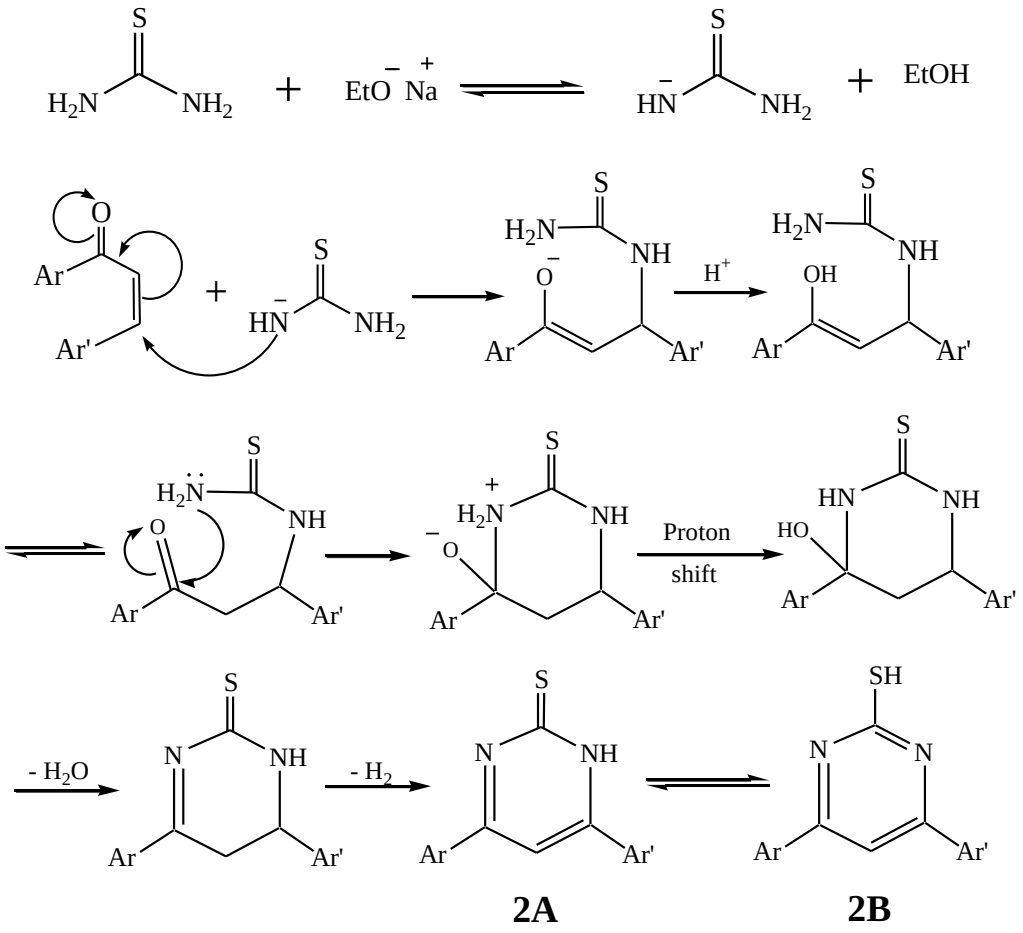
Synthesis of 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-1*H*-pyrimidine-2-thione (2)

When the chalcone **1** was subjected to react with thiourea in ethanolic solution of sodium ethoxide, it afforded 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-1*H*-pyrimidine-2-thione (**2**).



1 **2**

The reaction possibly takes place according to the following mechanism:



2A **2B**

The structure of **2** was confirmed from:

i. Correct analytical data.

ii. IR spectrum, which showed νNH'^{S} at 3393 and 3348, νCH'^{S} aromatic centered at 3056, weak band at 2578 due to νSH, νC=O of amide at about 1670, νC=N at 1610 and νC=S at 1271 cm^{-1}. Such IR data proves that compound **2** exists in thione (**2A**) ⇌ thiol (**2B**) tautomeric equilibrium (*cf.*, *Fig.1*).

iii. ^{1}HNMR spectrum (DMSO-d$_6$), showed signals at δ = 6.65-8.32 (m, 16H, ArH and 2NH). (*cf.*, *Fig.2*).

iv. Mass spectrum showed molecular ion peak (M$^{\cdot+}$) at m/z = 417, (16.4%) and the base peak at m/z= 105, (100%). (*cf.*, *Fig.3*, *chart 1*).

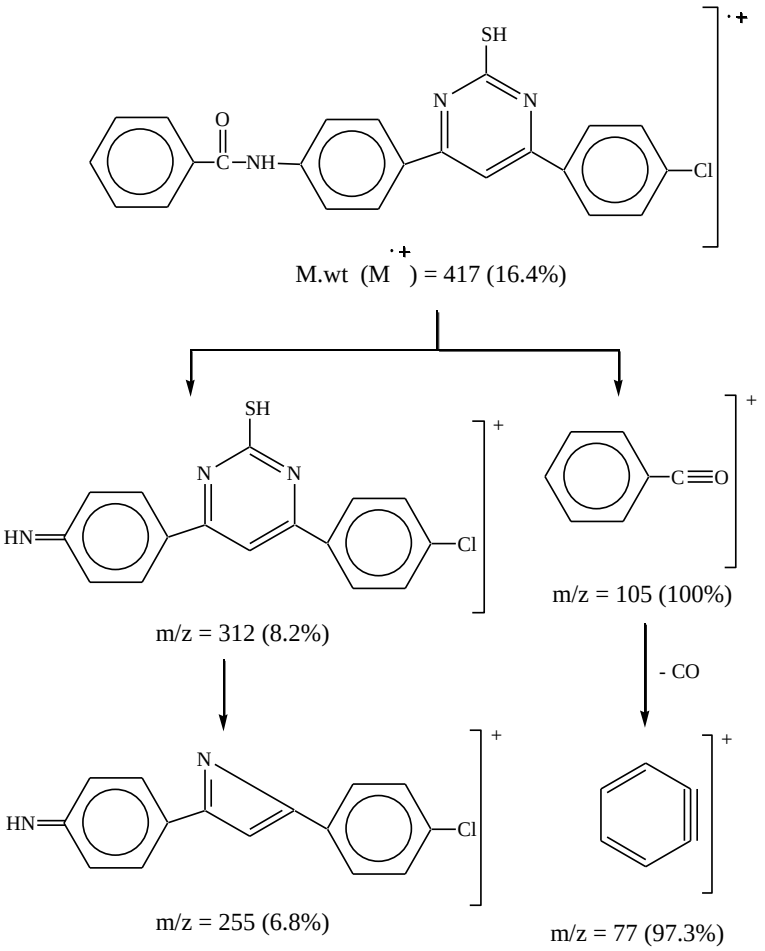


Chart 1

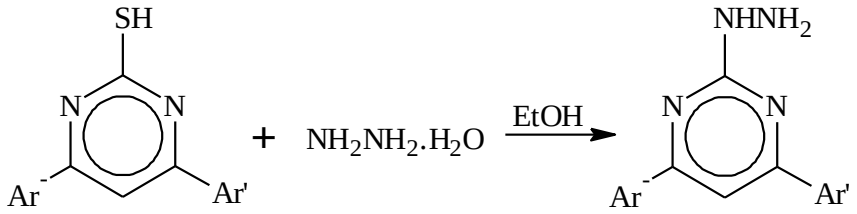
m/z	Abundance %
417	16.4
312	8.2
255	6.8
105	100
77	97.3

Reactions of 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-1*H*-pyrimidine-2-thione (2)

In the present investigation, the behavior of pyrimidine-2-thione **2** towards some nucleophilic and electrophilic reagents was studied with the aim of obtaining newer heterocycles having pyrimidinyl moiety, and also to investigate the phenomena of thione ⇌ thiol tautomerism of this compound.

I) Reaction with hydrazine hydrate:

Treatment of pyrimidine-2-thione derivative **2** with hydrazine hydrate in refluxing ethanol gave 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-2-hydrazinopyrimidine (**3**).



2 **3**

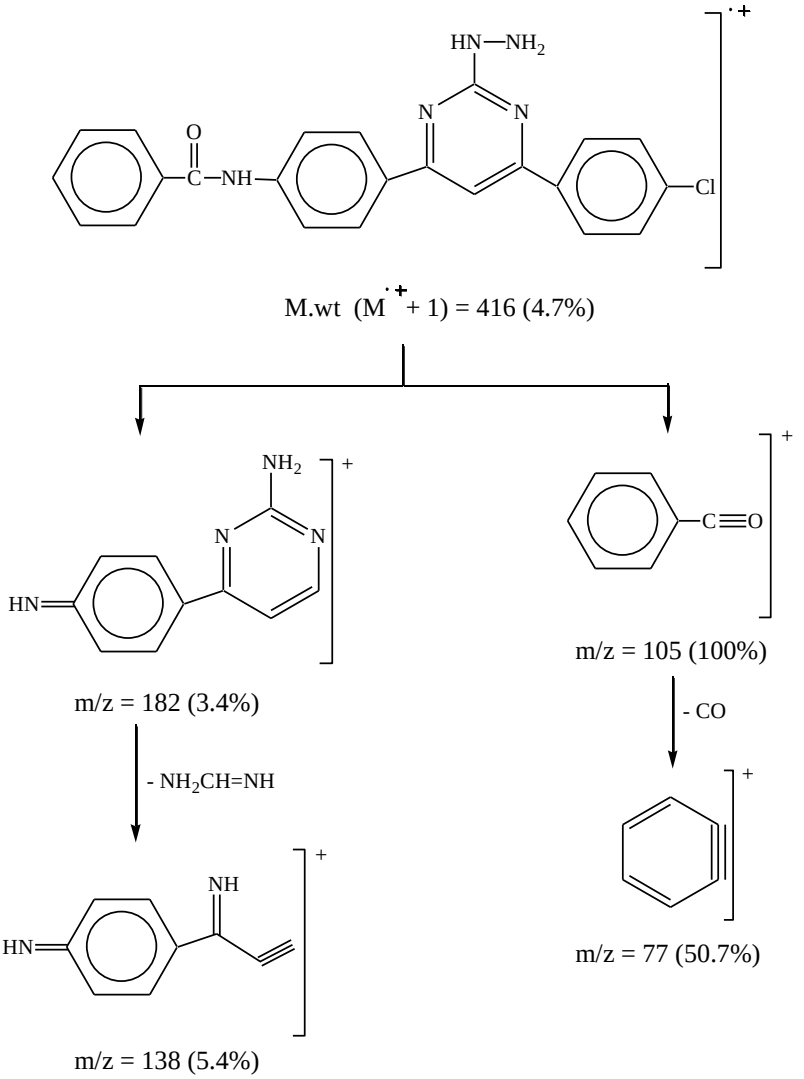
The structure of compound **3** was established via:

i. Correct analytical data.

ii. IR spectrum, which revealed absorption bands at 3334 and 3216 attributable to νNHNH$_2$, νCH'^{S} aromatic centered at 3056, at 1658 due to νC=O of amide, and at 1595 cm^{-1} due to νC=N, devoid any characteristic bands for νC=S or νSH. (*cf.*, *Fig.4*).

iii. ^{1}HNMR spectrum (DMSO-d$_6$), showed signals at δ = 6.54 (s, 2H, NH$_2$) and 6.75-8.32 (m, 16H, ArH and 2NH). (*cf.*, *Fig.5*).

iv. Mass spectrum assigned molecular ion peak (M$^{\cdot+}$ + 1) at m/z = 416, (4.7%) and the base peak at m/z = 105, (100%). (*cf.*, *Fig.6, chart 2*).

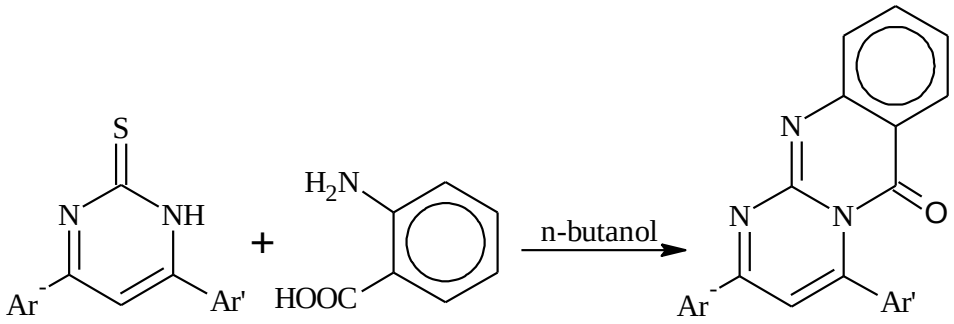


m/z	Abundance %
416	4.7
182	3.4
138	5.4
105	100
77	50.7

Chart 2

II) Reaction with anthranilic acid:

Refluxing of 2-mercaptopyrimidine derivative **2** with anthranilic acid in *n*-butanol furnished 2-(4-benzoylaminophenyl)-4-(4-chlorophenyl)-6*H*-pyrimido[2,1-b]quinazolin-6-one (**4**).



2 **4**

The structure of **4** was proved from:

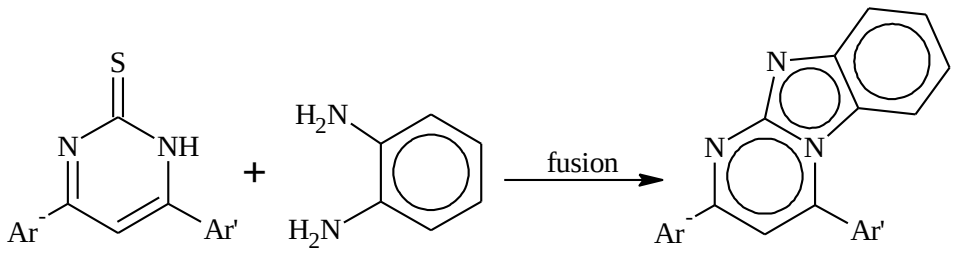
i. Correct analytical data.

ii. IR spectrum, which revealed νNH at 3383, νCH'^{S} aromatic centered at 3058, νC=O of amide at 1665 and νC=N at 1596 cm^{-1}, devoid any characteristic bands for νC=S or νSH.

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 6.54-8.36 (m, 19H, ArH and NH). (*cf.*, *Fig.7*).

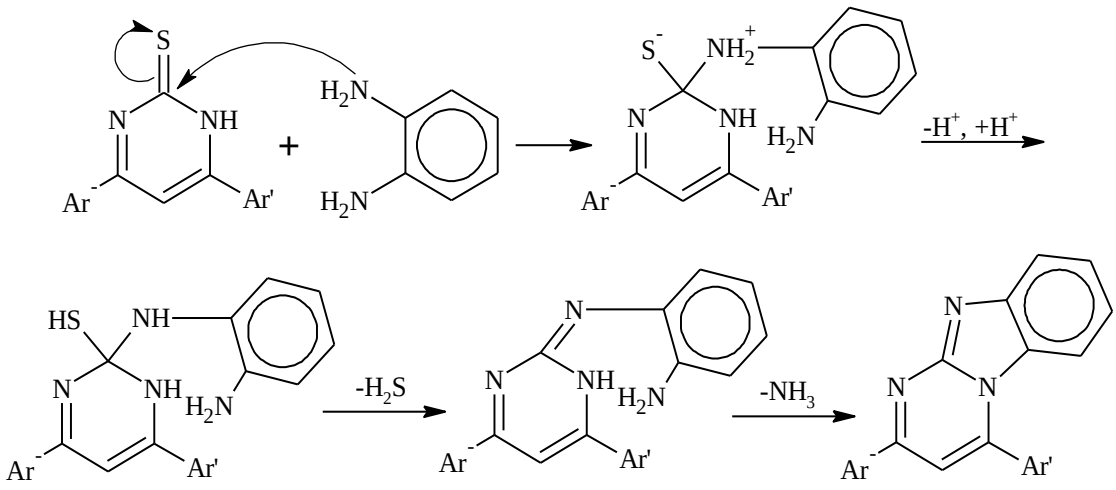
III) Reaction with *o*-phenylenediamine:

Fusion of pyrimidine-2-thione **2** with *o*-phenylenediamine in an oil bath for two hours afforded 2-(4-benzoylaminophenyl)-4-(4-chlorophenyl)-pyrimido[1,2-a]benzimidazole (**5)**.



2 **5**

The possible mechanism for this reaction may be as follows:



The structure of compound **5** was confirmed from:

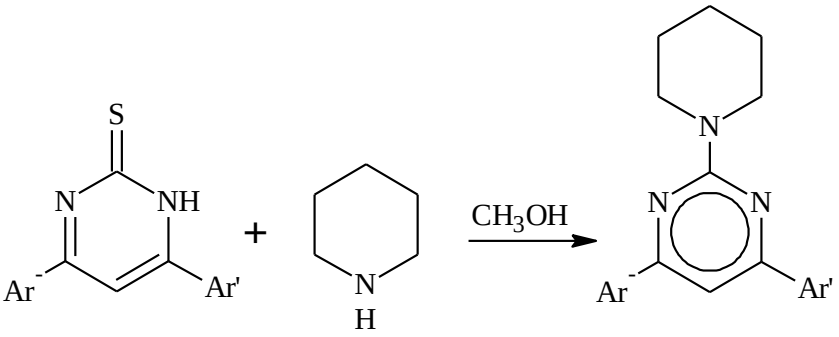
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3335, νCH'^{S} aromatic centered at 3060, νC=O of amide at 1658 and νC=N at 1596 cm^{-1}. (*cf*., *Fig.8*).

iii. Mass spectrum showed molecular ion peak ($M^{\cdot+}$) at m/z = 474, (5.3%) and the base peak at m/z = 105, (100%). (*cf*., *Fig.9*).

IV) Reaction with piperidine:

Reaction of 2-mercaptopyrimidine derivative **2** with piperidine in refluxing methanol provided 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-2-(piperidin-1-yl)pyrimidine (**6**).



2 **6**

The structure of **6** was inferred from:

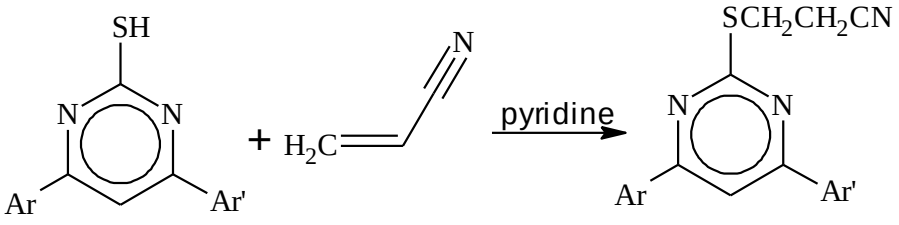
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3352, νCH'^{S} aromatic centered at 3058, νCH'^{S} aliphatic at 2925 and 2853, νC=O of amide at 1665 and νC=N at 1610 cm^{-1}. (*cf.*, *Fig.10*).

iii. ^{1}HNMR spectrum ($CDCl_3$), showed signals at δ = 1.41 (m, 2H, CH_2), 1.61 (m, 4H, $2CH_2$), 2.86 (m, 4H, $2CH_2$), 7.07-7.69 (m, 14H, ArH) and 8.11 (s, 1H, NH exchangeable by D_2O). (*cf.*, *Fig.11*).

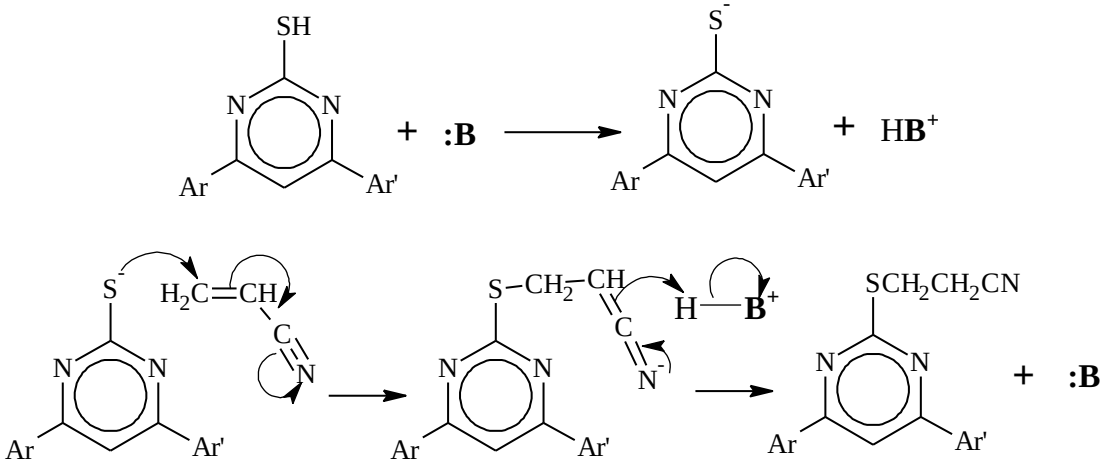
V) Reaction with acrylonitrile:

Pyrimidine-2-thione derivative **2** underwent Michael type addition reaction with acrylonitrile in pyridine to give 2-[β-(cyanoethylthio)]-pyrimidine derivative **7**.



2 **7**

The reaction proceeds via the following mechanism:



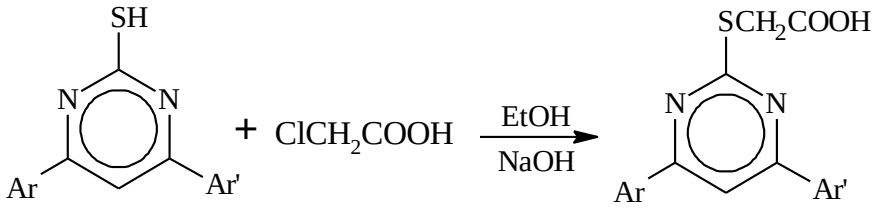
The structure of **7** was elucidated from:

i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3352, νCH'^{S} aromatic centered at 3064, νCH'^{S} aliphatic at 2919 and 2850, νC≡N at 2210, νC=O of amide at 1665 and νC=N at 1594 cm^{-1}. (*cf.*, *Fig.12*).

VI) Reaction with chloroacetic acid:

The reaction of 2-mercaptopyrimidine derivative **2** with chloroacetic acid in ethanol in the presence of sodium hydroxide yielded (pyrimidin-2-yl)-thioacetic acid derivative **8**.



2 **8**

The structure of **6** was inferred from:

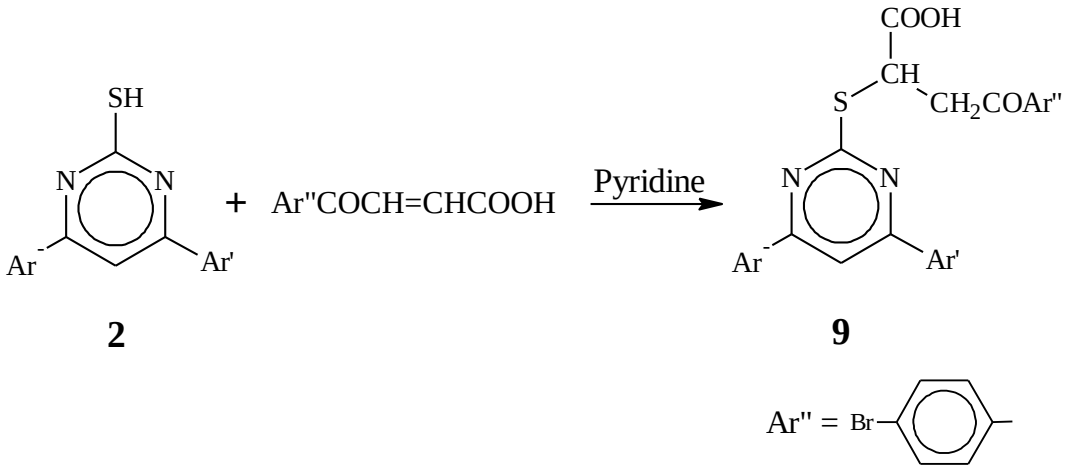
i. Correct analytical data.

ii. IR spectrum, which showed broad band of νOH and νNH at 3500-2500, νCH'^{S} aromatic centered at 3061, νCH'^{S} aliphatic at 2924 and 2851, νC=O of acid at 1695 and νC=O of amide at 1658 cm^{-1}. (*cf.*, *Fig.13*).

iii. ^{1}HNMR spectrum (DMSO-d$_6$), showed signals at δ = 3.37 (s, 2H, CH$_2$), 7.32-8.36 (m, 15H, ArH and NH) and 10.5 (s, 1H, OH). (*cf.*, *Fig.14*).

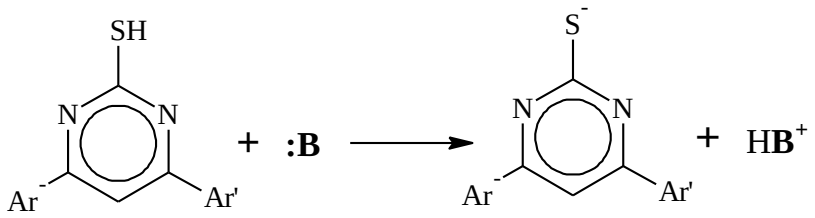
VII) Reaction with β-aroylacrylic acid:

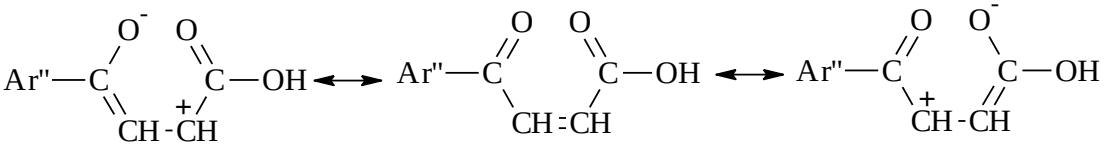
The interaction of pyrimidine-2-thione derivative **2** with β-(*p*-bromo-benzoyl)acrylic acid in refluxing pyridine gave 3-aroyl-2-(pyrimidin-2-yl)thiopropanoic acid (**9**).



2 **9**

The reaction may proceed via the following pathway:

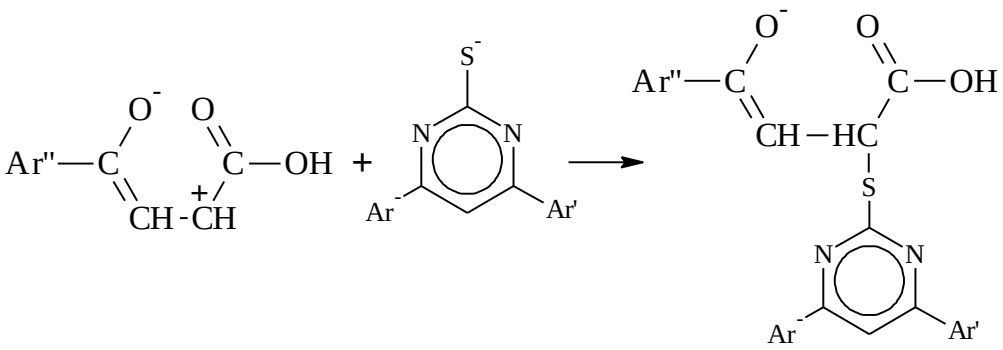


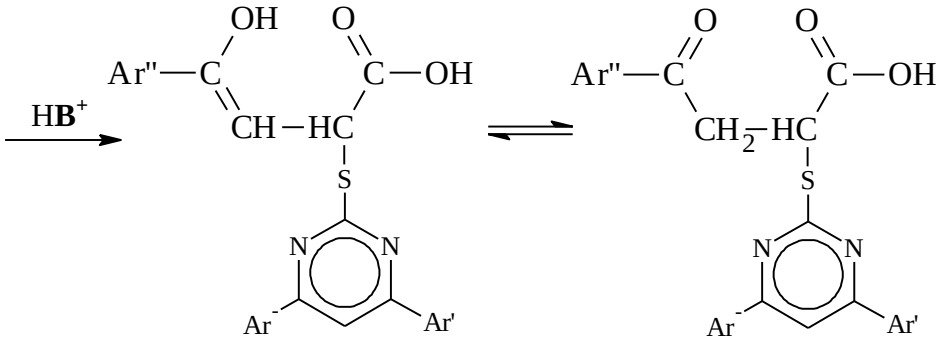


(a) **(b)**

less electrostatic repulsion **more electrostatic repulsion**

Structure **(a)** is thermodynamically favored and can accept nucleophiles easily than **(b)**.





The structure of compound **9** was confirmed from:

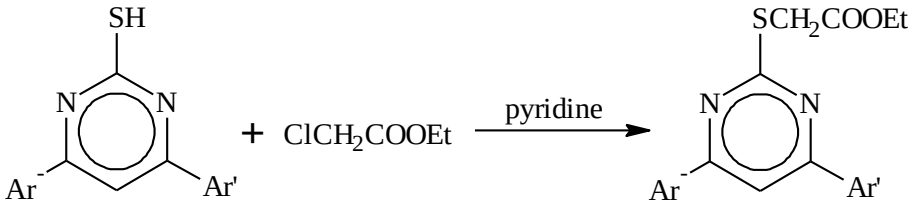
i. Correct analytical data.

ii. IR spectrum, which showed broad band due to νOH and νNH at 3500-2500, band of νCH'^{S} aliphatic at 2948 and 2842, νC=O of carboxyl group at 1690 cm^{-1}. (*cf.*, *Fig.15)*

iii. Mass spectrum showed molecular ion peak ($M^{\cdot+}$ + 3) at m/z = 675, (3.3%) and the base peak at m/z = 105, (100%). (*cf.*, *Fig.16, chart 3*).

VIII) Reaction with ethyl chloroacetate:

Alkylation of 2-mercaptopyrimidine derivative **2** with ethyl chloro-acetate in dry pyridine gave ethyl {[4-(4-benzoylaminophenyl)-6-(4-chloro-phenyl)pyrimidin-2-yl]thio}acetate (**10**).

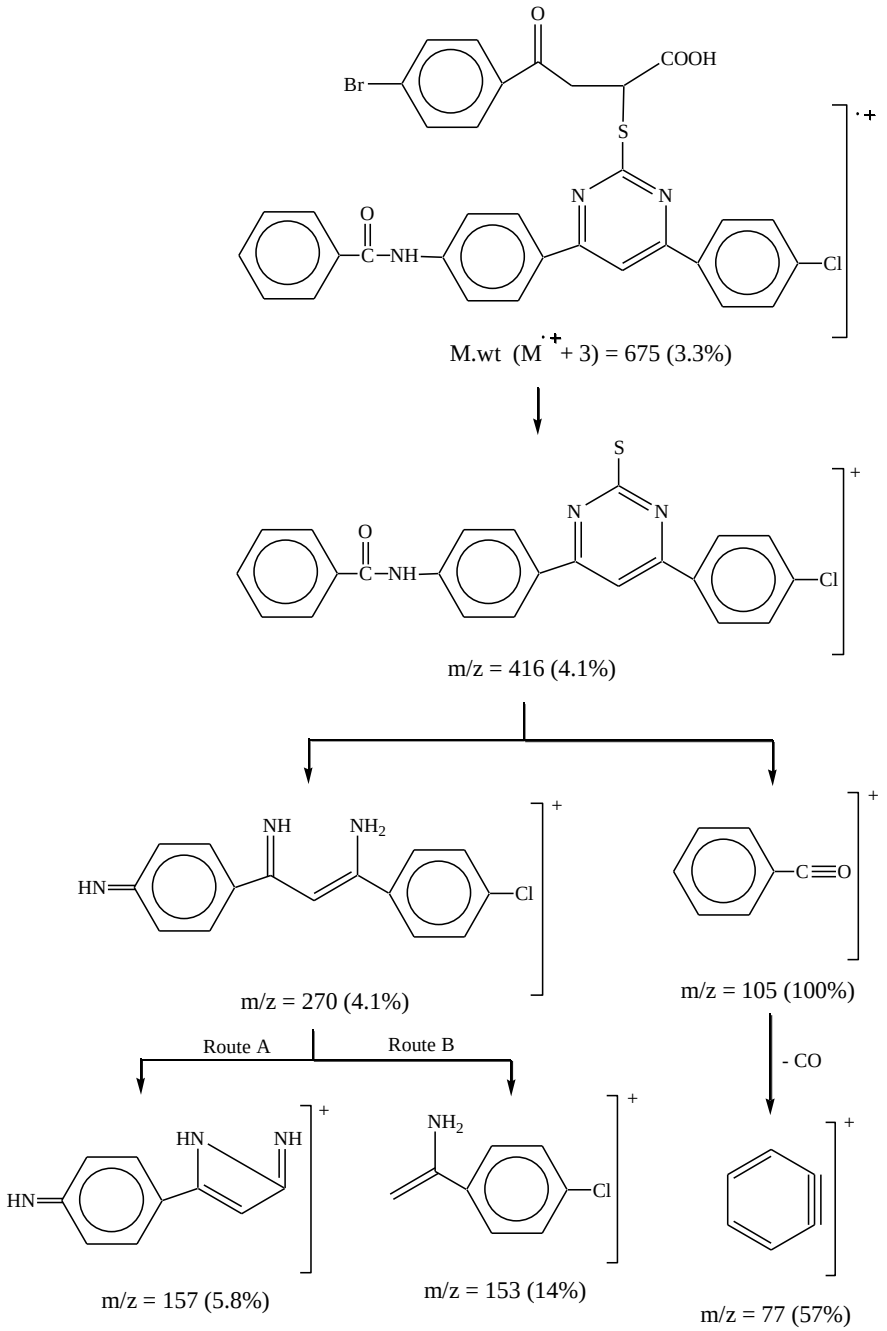


2 **10**

The structure of compound **10** was elucidated from:

i. Correct analytical data.

ii. IR spectrum, which assigned band of νNH at 3360, νCH'^{S} aliphatic at 2977 and 2926, νC=O of ester at 1730cm^{-1}. (*cf.*, *Fig.17*).

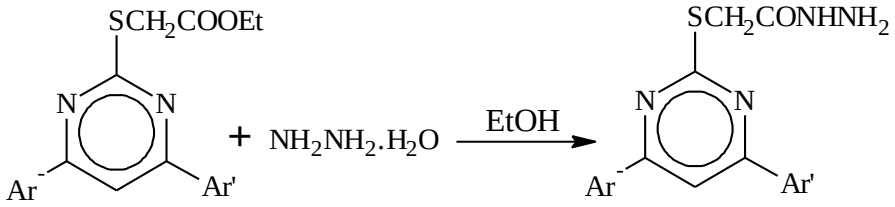


m/z	Abundance %
675	3.3
416	4.1
270	4.1
157	5.8
153	14
105	100
77	57

Chart 3

Behavior of ethyl (pyrimidin-2-yl)thioacetate derivative 10 towards hydrazine hydrate:

Ethyl (pyrimidin-2-yl)thioacetate derivative **10** was treated under reflux with hydrazine hydrate in absolute ethanol to give 2-{[4-(4-benzoyl-aminophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl]thio}acetohydrazide (**11**).



10 **11**

The structure of **11** was established via:

i. Correct analytical data.

ii. IR spectrum, which showed bands at 3388 and 3205 attributable to νNH and $\nu NHNH_2$, band of $\nu CH'^{S}$ aromatic centered at 3057, $\nu CH'^{S}$ aliphatic at 2976 and 2925, νC=O of amide at 1659 cm^{-1}. (*cf.*, *Fig.18*).

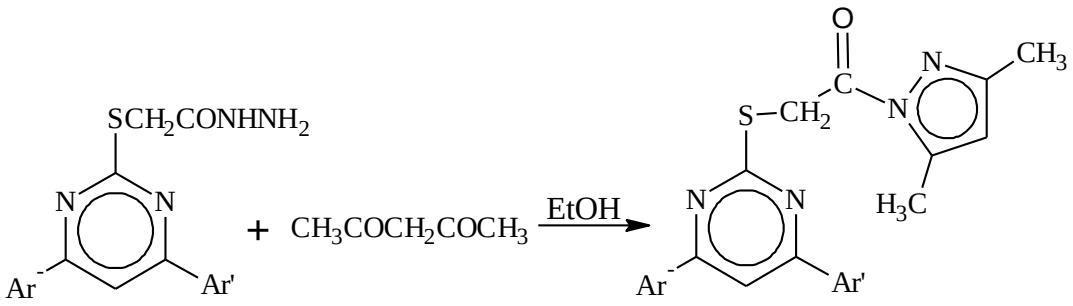
iii. Mass spectrum showed molecular ion peak ($M^{\cdot+}$ + 3) at m/z = 493, (57.1%) and the base peak at m/z = 105, (100%). (*cf.*, *Fig.19*).

Reactions of 2-{[4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-pyrimidin-2-yl]thio}acetohydrazide (11)

In the present work, we have studied the reactivity of hydrazide **11** towards some active methylene compounds with the aim of synthesizing pharmaceutically interest pyrazole [97,98] derivatives.

a) Reaction with acetylacetone:

When the hydrazide derivative **11** was reacted with acetylacetone in refluxing ethanol it gave pyrazolylpyrimidine derivative **12**.



11 **12**

The structure of **12** was inferred from:

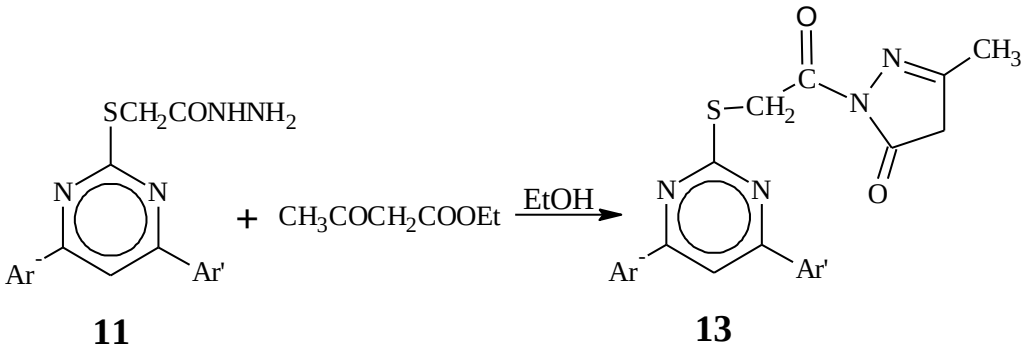
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3365, νCH'^{S} aromatic centered at 3049, νCH'^{S} aliphatic at 2919 and νC=O of amide at 1660 cm^{-1}. (*cf.*, *Fig.20*).

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 2.50 (s, 6H, $2CH_3$), 4.19 (s, 2H, CH_2) and 6.72-8.38 (m, 16H, ArH and NH). (*cf.*, *Fig.21*).

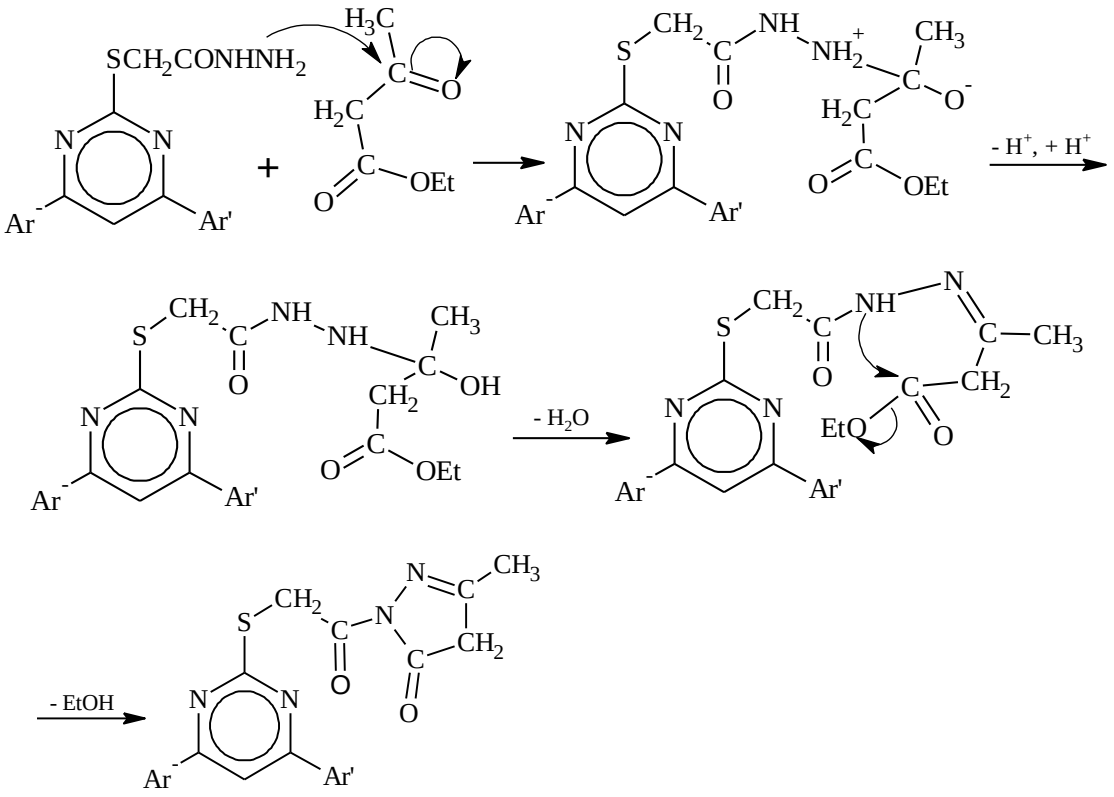
b) Reaction with ethyl acetoacetate:

The hydrazide **11** was subjected to react with ethyl acetoacetate in boiling ethanol and furnished the non-condensed pyrimidine derivative **13**.



11 **13**

The reaction possibly proceeds via the following mechanism:



The structure of **13** was confirmed from:

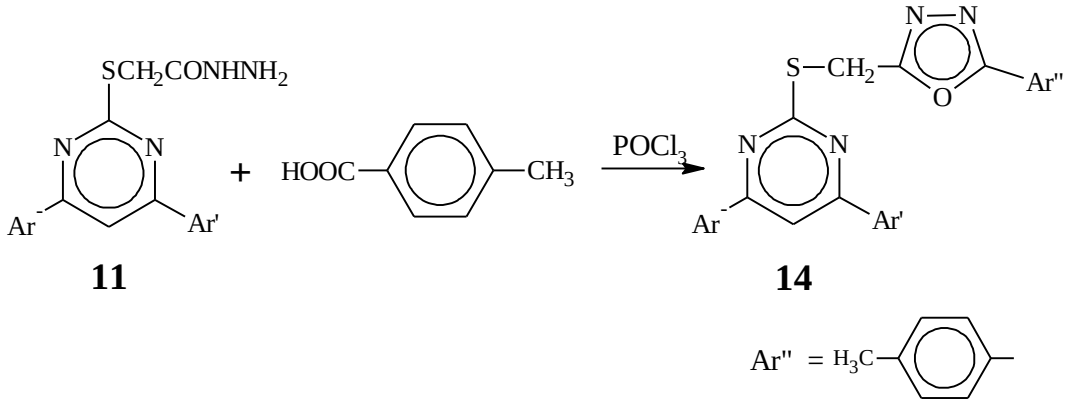
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3395, νCH'^{S} aromatic centered at 3059, νCH'^{S} aliphatic at 2926 and νC=O of amide at 1664 cm^{-1}.

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 1.19 (s, 3H, CH_3), 2.50 (s, 2H, CH_2 of pyrazolone ring), 4.18 (s, 2H, CH_2) and 7.29-8.38 (m, 15H, ArH and NH). (*cf.*, *Fig.22*).

c) Reaction with *p*-toluic acid:

Another point of interest in this investigation is the synthesis of oxadiazole derivative which associated with different biological activities [99,100]. Thus, the reaction of equimolar amounts of hydrazide **11** and *p*-toluic acid under reflux in the presence of $POCl_3$ afforded 4-(4-benzoyl-aminophenyl)-6-(4-chlorophenyl)-2-({[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]methyl}sulfanyl)pyrimidine (**14**).



11 **14**

The structure of **14** was established via:

i. Correct analytical data.

ii. IR spectrum, which showed ν*N*H at 3388, ν*CH*'^S aromatic at 3059, ν*CH*'^S aliphatic at 2923 and 2857 , ν*C*=O of amide at 1660 and ν*C*-O at 1095 cm^{-1}. (*cf*., *Fig.23*).

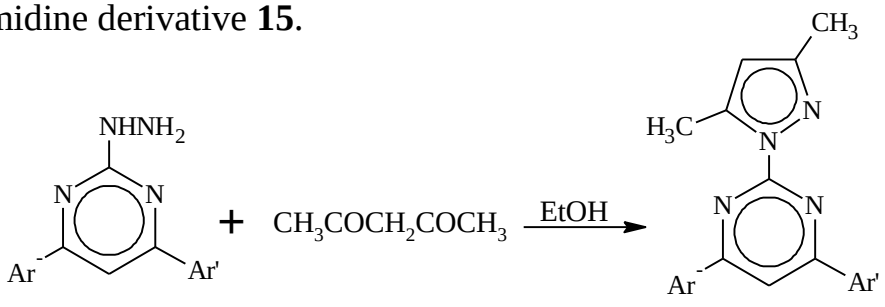
iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 2.35 (s, 3H, CH_3), 4.12 (s, 2H, CH_2) and 6.82-8.35 (m, 15H, ArH and NH). (*cf*., *Fig.24*).

Reactions of 4-(4-benzoylaminophenyl)-6-(4-chlorophenyl)-2-hydrazinopyrimidine (3)

2-Hydrazinopyrimidine derivative **3** was used as starting material for the synthesis of some condensed and non-condensed pyrimidines.

1) Reaction with acetylacetone:

Reaction of 2-hydrazinopyrimidine derivative **3** with acetylacetone in refluxing ethanol in the presence of few drops of piperidine gave 2-(pyrazol-1-yl)pyrimidine derivative **15**.



3 **15**

The structure of **15** was confirmed from:

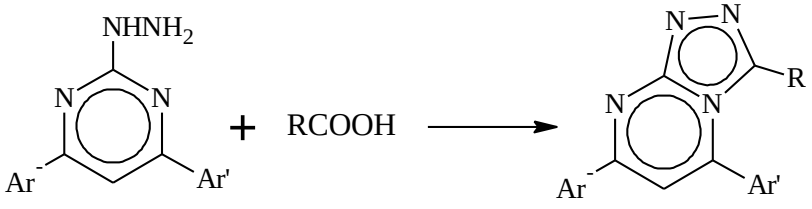
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3310, νCH'^{S} aromatic centered at 3056, νCH'^{S} aliphatic at 2923 and 2854, νC=O of amide at 1660 and νC=N at 1597 cm^{-1}.

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 2.51 (s, 6H, $2CH_3$) and 7.21-8.39 (m, 16H, ArH and NH). (*cf.*, *Fig.25*).

2) Reaction with carboxylic acids:

Refluxing 2-hydrazinopyrimidine derivative **3** with carboxylic acids namely, formic acid and acetic acid afforded triazolo[4,3-a]pyrimidine derivatives **16a** and **16b** respectively.



3 **16a,b** **16a;** R = H **16b;** R = CH_3

The structure of **16a** was elucidated from:

i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3253, νCH'^{S} aromatic centered at 3058, νCH'^{S} aliphatic at 2924 and 2871, νC=O of amide at 1669 and νC=N at 1597 cm^{-1}. (*cf.*, *Fig.26*).

While the structure of **16b** was established via:

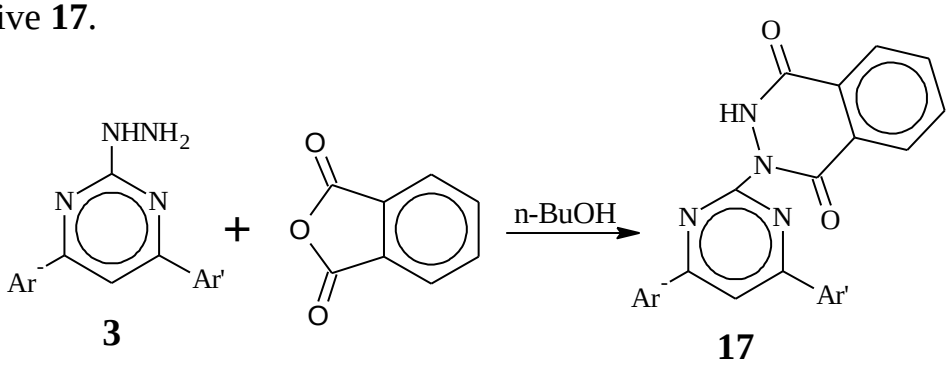
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3319, νCH'^{S} aromatic centered at 3059, νCH'^{S} aliphatic at 2925, νC=O at 1662 and νC=N at 1594 cm^{-1}.

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 2.51 (s, 3H, CH_3) and 7.39-8.32 (m, 15H, ArH and NH). (*cf.*, *Fig.27*).

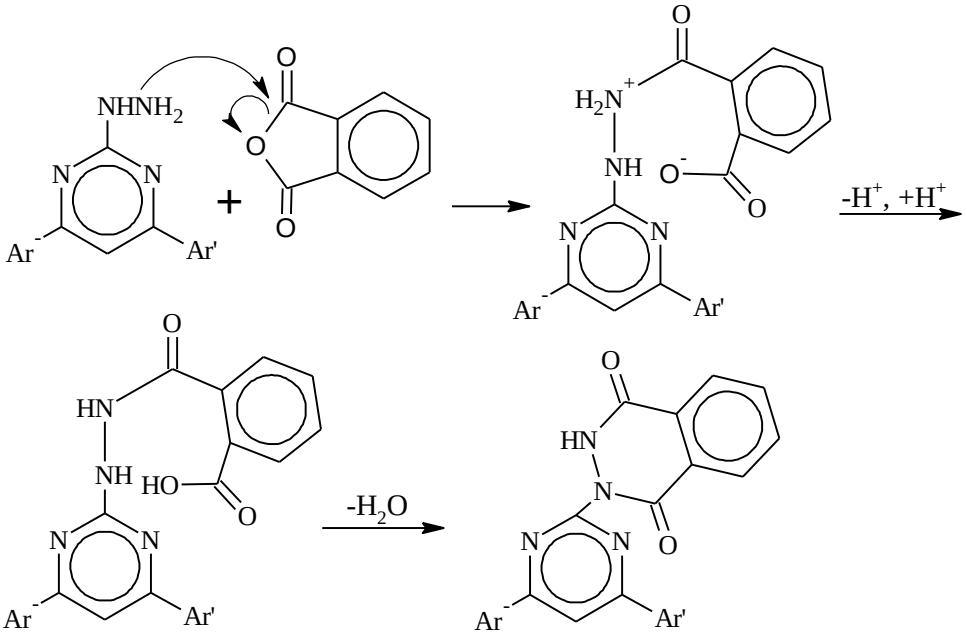
3) Reaction with phthalic anhydride:

When 2-hydrazinopyrimidine derivative **3** was reacted with phthalic anhydride in n-butanol, it gave (pyrimidin-2-yl)phthalazine-1,4-dione derivative **17**.



3 **17**

The possible mechanism for this reaction may be as follows:

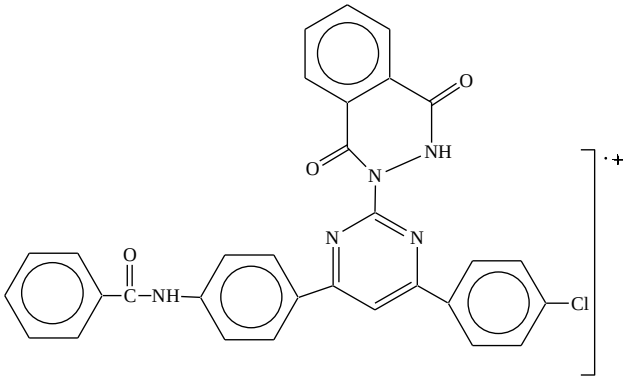


The structure of compound **17** was inferred from:

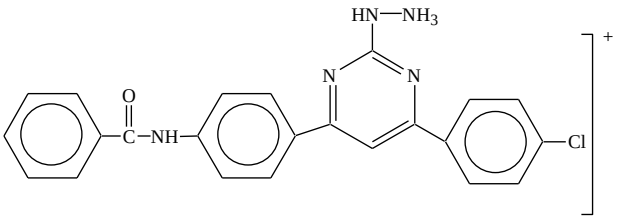
i. Correct analytical data.

ii. IR spectrum, which showed νNH at 3334, νCH'^{S} aromatic centered at 3058, νC=O of phthalazinone ring at 1695 and νC=N at 1595 cm^{-1}.

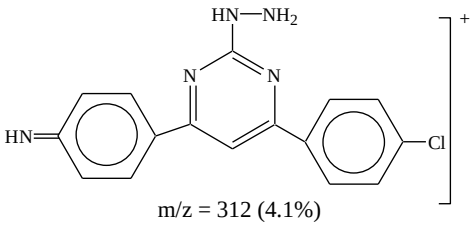
iii. Mass spectrum showed molecular ion peak ($M^{\cdot+}$) at m/z = 545, (1.8%) and the base peak at m/z = 105, (100%). (*cf.*, *Fig.28, chart 4*).



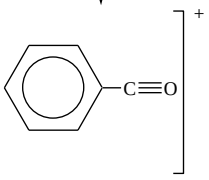
M.wt ($M^{\cdot+}$) = 545 (1.8%)



m/z = 417 (6.7%)

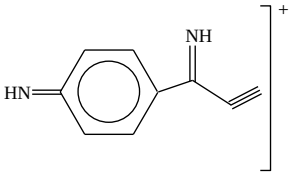


m/z = 312 (4.1%)

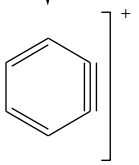


m/z = 105 (100%)

- CO



m/z = 138 (4.9%)



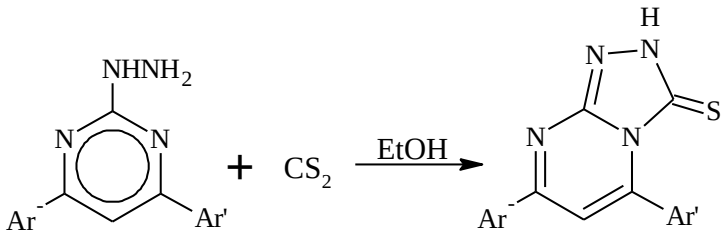
m/z = 77(48%)

m/z	Abundance %
545	1.8
417	6.7
312	4.1
138	4.9
105	100
77	48

Chart 4

4) Reaction with carbon disulphide:

2-Hydrazinopyrimidine derivative **3** was heated under reflux with carbon disulphide in absolute ethanol to give triazolo[4,3-a]pyrimidine-3-thione derivative **18**.



3 **18**

The structure of compound **18** was confirmed from:

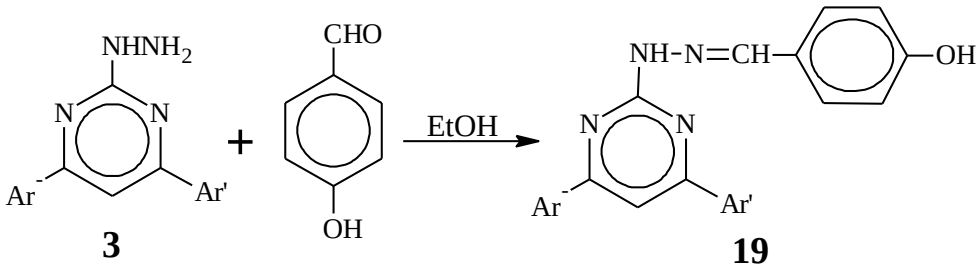
i. Correct analytical data.

ii. IR spectrum, which showed νNH'^{S} at 3338 and 3212, νCH'^{S} aromatic centered at 3058, weak band due to νC=S at 2597, νC=O of amide at 1665 and νC=N at 1594 cm^{-1}. (*cf.*, *Fig.29*).

iii. Mass spectrum showed molecular ion peak ($M^{\cdot+}$ + 2) at m/z = 460, (2.1%) and the base peak at m/z = 105, (100%). (*cf.*, *Fig.30*).

5) Reaction with *p*-hydroxybenzaldehyde:

Condensation of 2-hydrazinopyrimidine derivative **3** with *p*-hydroxy-benzaldehyde in absolute ethanol gave the Schiff's base (pyrimidin-2-yl)-hydrazone derivative **19**.



3 **19**

The structure of **19** was established via:

i. Correct analytical data.

ii. IR spectrum, which showed νNH and νOH at 3344-3200, $\nu CH'^{S}$ aromatic centered at 3060, $\nu CH'^{S}$ aliphatic at 2924 and νC=O at 1660 cm^{-1}. (*cf.*, *Fig.31*)

iii. ^{1}HNMR spectrum (DMSO-d_6), showed signals at δ = 7.20-8.39 (m, 17H, ArH, 2NH and CH) and 9.14 (s, 1H, OH). (*cf.*, *Fig.32*).

Biological activity of the synthesized compounds.

It was reported that pyrimidine derivatives have a potential at the pharmacological level, so some new pyrimidine derivatives were synthesized and quantified. Their effectiveness against a number of micro-organisms was tested with the aim of obtaining specific derivatives that could be potent in medicine, chemistry or agriculture. Some of the synthesized compounds were screened for their antibacterial activity against **[Staphylococcus aureus** (gram-positive) and **Escherichia coli** (gram-negative)**]**, antifungal activity against **(Aspergillus flavus** and **Candida albicans)** and the results are given in table **1**.

Table 1: Antimicrobial activity of some synthesized compounds.

Compd.	Bacteria				Fungi			
	E. coli (G^-)		S. aureus (G^+)		A. flavus		C. albicans	
	A	MIC	A	MIC	A	MIC	A	MIC
2	++	250	++	125	+	250	++	125
3	++	125	+++	250	-	500	-	500
4	++	125	+++	125	++	125	++	125
6	++	250	+++	125	+	250	+	125
9	++	250	++	125	+++	250	++	250
12	+++	125	++	250	++	250	+	250
14	++	125	+++	250	++	125	+++	250

16b	+++	125	++	125	+	250	++	500
17	+	250	++	125	+	500	-	500
18	+++	125	++	250	+	250	+	250
19	-	500	+	500	+	250	++	500

A = Antimicrobial activity of tested compounds; MIC = Minimum inhibitory concentration -, not active; + > 10 mm, slightly active; ++ > ٢٠ mm, moderately active; +++ > 30 mm, highly active.

It is apparent from the data listed in table **1**, that most of the tested compounds have moderate to high activity against micro-organisms. However, concerning the activity against gram-positive bacteria (*Staphylococcus aureus*), compounds **3**, **4**, **6** and **14** showed excellent activities, compounds **2**, **9**, **16b** and **17** exhibited good activities, whereas compounds **12** and **18** showed moderate activities. On the other hand, the activity against gram-negative bacteria (*Escherichia coli*), compounds **12**, **16b** and **18** showed excellent activities, while compounds **3**, **4** and **14** exhibited very good activities.

Concerning the data of antifungal activity, compounds **9** and **14** only showed very strong inhibition against *Aspergillus flavus* and *Candida albicans*. Also, compounds **2**, **4**, **12**, **16b** and **19** exhibited moderate antifungal activities. Compounds **3**, **17** and **18** showed poor antifungal activities against both *Aspergillus flavus* and *Candida albicans*.

Thus it is clear that these compounds are effective and inhibit the growth of most tested micro-organisms.

Structure-activity relationships (SAR) of pyrimidine derivatives

In SAR study, we focused on the effect of substitution of a group in the pyrimidine ring and the effect of construction of a new ring either condensed or non-condensed with pyrimidine ring on the activity against certain micro-organisms.

2-Mercaptopyrimidine derivative **2** showed good inhibitory activity against both bacteria and fungi. Substitution of the mercapto group by a hydrazino group in the pyrimidine ring (in compound **3**) enhanced activity against bacteria, but resulted in drastic loss of activity against fungi. However, replacement of the mercapto group by piperidine moiety (in compound **6**) led to slight improvement of antibacterial activity and slight loss of antifungal activity.

Construction of quinazolinone ring on pyrimidine (in compound **4**) resulted in high improvement of activity against both bacteria and fungi. Also, addition of 2-mercaptopyrimidine derivative **2** to the carbon-carbon double bond of 3-(4-bromobenzoyl)acrylic acid (in compound **9**) improved the antifungal activity to high extent while the antibacterial activity was not changed greatly.

Introduction of pyrazole ring and/or oxadiazole ring in a non-condensed way with pyrimidine (in **12** compounds and **14**) demonstrated potent activity against both types of micro-organisms (bacteria & fungi). Moreover, [1,2,4]triazolo[4,3-a]pyrimidine derivatives **16b** and **18** showed quite enhancement of antibacterial and antifungal activity if compared to 2-hydrazinopyrimidine derivative **3** due to the presence of triazole ring that constructed on pyrimidine.

2-(Pyrimidin-2-yl)phthalazine-1,4-dione derivative **17** was tolerated to maintain the potent activity of 2-hydrazinopyrimidine derivative **3** against bacteria and fungi.

Finally, condensation of 2-hydrazinopyrimidine derivative **3** with aromatic aldehyde [p-hydroxybenzaldehyde] (in compound **19**) led to quite improvement of antifungal and loss of activity against bacteria.