Synthesis and *In Vitro* Antimicrobial, Anthelmintic and Insecticidal Activities Study of 4(4'-Bromophenyl)-6-substituted-aryl-1-acetyl pyrimidine-2-thiols

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Abstract: A new series of 4(4'-bromophenyl)-6-substituted aryl-1-acetyl pyrimidine-2-thiol derivatives were synthesized by heating chalcones with thiourea, in the presence of ethanolic potassium hydroxide, followed by treatment with acetyl chloride. The structure of the compounds was characterized by IR and ^1H NMR spectral study and elemental analysis. The compounds were screened for their antimicrobial, anthelmintic and insecticidal activities. All the compounds exhibited significant to moderate biological activities.

Keywords: Pyrimidine-2-thiols, Antimicrobial, Anthelmintic, Insecticidal activity.

Introduction

Heterocyclic system containing pyrimidine ring, constitute an important class of potentially bio-active compounds. The earlier workers reported that, pyrimidine derivatives are associated with a wide spectrum of biological activities viz. antimicrobial^{1,4}, anthelmintic⁵⁻⁷, anticancer^{8,9}, anti-tumor¹⁰, anti-inflammatory¹¹⁻¹², anti-HIV agents¹³, calcium-channel-blocker¹⁴ and insecticidal agents¹⁵ and this significance led us to synthesize the titled compounds.

In the present communication, we have reported, the synthesis of a new series of 4(4'-bromophenyl)-6-substituted aryl-1-acetyl pyrimidine-2-thiols (Scheme 1) and evaluated for their antimicrobial, anthelmintic and insecticidal activities.

Where - R¹ = Bromo, R² = 4-O-CH₃, 4-OH-3-OCH₃, H, 4-Cl, 2-Cl, 4-N(CH₃)₂, 2-NO₂, 3-NO₂, Furyl, 2-OH.

Scheme 1

Experimental

The melting points were recorded by open capillary method and are uncorrected. IR spectra ($\nu_{\max}$ in cm⁻¹) were recorded on a Shimadzu FTIR 8300 spectrophotometer, using KBr pellets. The ¹H NMR spectra were recorded on a DRX-300 (300 MHz) instrument using CDCl₃ as solvent (Chemical Shift in δ ppm), using TMS as internal standard. The completion of reactions was monitored by TLC.

General procedure

Synthesis of 4-bromochalcones (1a-j)

4-Bromoacetophenone (0.01 mol) and anisaldehyde (0.01 mol) were stirred in ethanol (20 mL) and to it 10 mL of 40% NaOH solution was added. The mixture was kept overnight at room temperature. The contents were then poured on crushed ice and acidified with dil. HCl. The solid was filtered, dried and recrystallised from ethanol. The other chalcones were prepared with the same procedure using different aromatic aldehydes.

4(4'-Bromophenyl)-6-substituted phenyl-1, 5, 5, 6-tetrahydropyrimidine-2-thiones (2a-j)

The chalcones (0.01 mol), thiourea (0.01 mol) and KOH (1.0 g) in ethanol (20 mL) was refluxed on water bath for 6 h and completion of reaction was monitored by TLC. After keeping in ice-cold condition, the solid was collected and recrystallised from ethanol.

Yield 71%, m.p. 206 °C, m.wt.374.9, (% of C : found 54.38 - calculated 54.41), (% of H: found 03.98, calculated 4.00), (% of N: found 7.40, calculated 7.46), IR (KBr) in cm⁻¹ : 536-600 (Ar-Br str), 1595-1560 (C=N str), 1220-1200 (C=S str.), 820-740 (Ar-H def. pyrimidine ring breath), 3351-3300 (N-H Str). The other derivatives were prepared with different chalcones, applying the same procedure.

4(4'-Bromophenyl)-6-substitutedphenyl-1-acetyl-5, 6-dihydropyrimidine-2-thiols (3a-j)

The different thiones (2a-j), (0.005 mol) and acetyl chloride (16 mL) were heated at 30-40 °C, under reflux on water bath for 2 h, completion of reaction was monitored by TLC. Excess of acetyl chloride was evaporated and the oily product obtained, was treated with pet ether. The solid obtained was filtered off, dried and recrystallised from ethanol. The physical and spectral data of all the synthesized compounds described as follows:

Table 1. Physical data of compounds **3a-j**.

Comp. No.	R ²	M.F.	M.wt.	M.P. °C	Yield, %	Elemental Analysis %			
							C	H	N
3a	4-O-CH ₃	C ₁₉ H ₁₇ O ₂ N ₂ SBr	416.9	215	85	found	54.62	4.05	6.69
						calc.	54.68	4.07	6.71
3b	4-OH-3-OCH ₃	C ₁₉ H ₁₇ O ₃ N ₂ SBr	432.9	185	86	found	52.65	3.90	6.42
						calc.	52.66	3.92	6.46
3c	H	C ₁₈ H ₁₅ N ₂ OSBr	386.9	218	65	found	55.80	3.82	7.20
						calc.	55.82	3.87	7.23
3d	4-Cl	C ₁₈ H ₁₄ N ₂ OSClBr	421.4	194	75	found	51.21	3.30	6.61
						calc.	51.25	3.32	6.64
3e	2-Cl	C ₁₈ H ₁₄ N ₂ OSClBr	421.4	220	70	found	51.20	3.30	6.60
						calc.	51.25	3.32	6.64
3f	4-N(CH ₃) ₂	C ₂₀ H ₂₀ N ₃ OSBr	429.9	200	72	found	55.80	4.61	9.72
						calc.	55.82	4.65	9.76
3g	2-NO ₂	C ₁₈ H ₁₄ N ₃ O ₃ SBr	431.9	190	72	found	49.99	3.21	9.70
						calc.	50.01	3.24	9.72
3h	3-NO ₂	C ₁₈ H ₁₄ N ₃ O ₃ SBr	431.9	180	84	found	50.00	3.20	9.69
						calc.	50.01	3.24	9.72
3i	6-Furyl	C ₁₆ H ₁₃ N ₂ O ₂ SBr	376.9	176	85	found	50.90	3.40	7.40
						calc.	50.94	3.44	7.42
3j	2-OH	C ₁₈ H ₁₅ N ₂ O ₂ SBr	402.9	205	60	found	53.58	3.70	6.90
						calc.	53.61	3.72	6.94

Spectral data of compounds (3a-j)

4(4'-Bromophenyl)-6-(4"-methoxyphenyl)-1-acetyl- 5, 6-dihydropyrimidine-2-thiol (3a)

IR (KBr) in cm⁻¹: 2550.11(S-H str), 1681.98 (N-C=O str), 1606.76 (C=C str), 1581.68(C=N str), 582.52 (Ar-Br str), 1114.89 (C-O-C str.); H¹NMR (CDCl₃) δ (ppm) : 1.50 (δ, 1H, S-H), 2.03 (δ, 3H, N-CO-CH₃), 5.51 (d, 1H, H-C₆-pyrimidine), 6.73(d, 1H, H-C₅-pyrimidine), 7.17-7.66 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-(4"-hydroxy-3-methoxy-phenyl)-1-acetyl-5,6-dihydropyrimidine-2-thiol (3b)

IR (KBr) in cm⁻¹: 2540.28 (S-H str), 3450.25 (O-H str), 1712.85 (N-C=O str), 1589.40 (C=N str), 1599.04 (C=C str), 594.10 (Ar-Br str), 1074.39 (C-O-C str), H¹ NMR (CDCl₃) δ (ppm); 1.52(δ, 1H, S-H), 2.03 (δ, 3H, NCOCH₃), 2.11 (δ, 3H, C-O-CH₃), 4.90 (δ, 1H, Ar-OH), 5.52 (d, 1H, H-C₆ pyrimidine), 6.74 (d, 1H, H-C₅ pyrimidine), 7.12-7.66 (m, 7H, Ar-H)

4(4'-Bromophenyl)-6-phenyl-1-acetyl,5,6-dihydro-pyrimidine-2-thiol (3c)

IR (KBr) in cm⁻¹ : 2565.44 (S-H str), 1689.97 (N-C=O str), 1606.76 (C=C str), 1585.64 (C=N str), 590.24 (Ar-Br); H¹ NMR (CDCl₃) δ (ppm) : 1.50 (δ, 1H, S-H), 2.04 (δ, 3H, NCOCH₃), 5.50 (d, 1H, H-C₆ pyrimidine), 6.72 (d, 1H, H-C₅ pyrimidine), 7.11-7.50 (m, 9H, Ar-H).

4(4'-Bromophenyl)-6-(4''-chlorophenyl)-1-acetyl 5,6- dihydropyrimidine-2-thiol (3d)

IR (KBr) in cm^{-1} : 2542.38 (S-H str), 1703.20 (N-C=O str), 1587.47 (C=C str), 1576.89 (C=N str), 619.17 (Ar-Br str), 750.01 (Ar-Cl str); ^1H NMR (CDCl_3) δ (ppm) : 1.54 (δ , 1H, S-H), 2.06 (δ , 3H, N-CO-CH₃), 5.53 (d, 1H, H-C₆ pyrimidine), 6.72 (d, 1H, H-C₅ pyrimidine), 7.15 - 7.60 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-(2'-chlorophenyl)-1-acetyl, 5,6-dihydropyrimidine-2-thiol (3e)

IR (KBr) in cm^{-1} : 2596.27 (S-H str), 1681.98 (N-C=O str), 1583.61 (C=C str), 1568.18 (C=N str), 513.08 (Ar-Br str), 702.11 (Ar-Cl str). ^1H NMR (CDCl_3) δ (ppm) : 1.56 (δ , 1H, S-H), 2.08 (δ , 3H, N-CO-CH₃), 5.55 (d, 1H, H-C₆ pyrimidine), 6.73 (d, 1H, H-C₅ pyrimidine), 7.16 - 7.62 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-(4''-dimethylaminophenyl)-1-acetyl-5,6-dihydropyrimidine-2-thiol (3f)

IR (KBr) in cm^{-1} : 2548.05 (S-H str), 1680.99 (N-C=O str), 1583.61 (C=C str), 1508.38 (C=N str). ^1H NMR (CDCl_3) δ (ppm) : 1.57 (δ , 1H, S-H), 2.08 (δ , 3H, N-COCH₃), 3.03 (δ , 6H, N(CH₃)₂), 5.54 (d, 1H, H-C₆ pyrimidine), 6.74 (d, 1H, H-C₅ pyrimidine), 7.19-7.68 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-(2'-nitrophenyl)-1-acetyl-5, 6-dihydropyrimidine-2-thiol (3g)

IR (KBr) in cm^{-1} : 2580.77 (S-H str), 1680.98 (N-C=O str), 1605.09 (C=C str), 1560.08 (C=N str), 560.02 (Ar-Br str), 1590.25 (Ar-NO₂ Asym str), 1340.22 (Ar-NO₂ sym str). ^1H NMR (CDCl_3) δ (ppm) : 1.60 (δ , 1H, S-H), 2.10 (δ , 3H, N-COCH₃), 5.60 (d, 1H, H-C₆ pyrimidine), 6.78 (d, 1H, H-C₅ pyrimidine), 7.20-8.10 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-(3'-nitrophenyl)-1-acetyl- 5,6 - dihydropyrimidine-2- thiol (3h)

IR (KBr) in cm^{-1} : 2580.77 (S-H str), 1680.90 (N-C=O str), 1605.08 (C=C str), 1560.04 (C=N str), 560.00 (Ar-Br str), 1540.00 (Ar-NO₂ Asym. str), 1320.99 (Ar-NO₂ sym. str). ^1H NMR (CDCl_3) δ (ppm) : 1.52 (δ , 1H, S-H), 2.05 (δ , 3H, N-COCH₃), 5.58 (d, 1H, H-C₆ pyrimidine), 6.74 (d, 1H, H-C₅ pyrimidine), 7.27-8.12 (m, 8H, Ar-H).

4(4'-Bromophenyl)-6-furyl-1-acetyl-5,6-dihydro- pyrimidine- 2-thiol (3i)

IR (KBr) in cm^{-1} : 2569.27 (S-H str), 1683.91 (N-C=O str), 1585.54 (C=C str), 1489.01 (C=N str), 569.02 (Ar-Br str), 1072.46 (Furyl ring C-O-C str), 2980.04 (C-H str), ^1H NMR (CDCl_3) δ (ppm) : 1.61 (δ , 1H, S-H), 2.10 (δ , 3H, N-COCH₃), 5.59 (d, 1H, H-C₆ pyrimidine), 6.76 (d, 1H, H-C₅ pyrimidine), 7.20-7.78 (m, 7H, Ar-H).

4(4'-Bromophenyl)-6-(2'-hydroxyphenyl)-1-acetyl- 5,6-dihydropyrimidine-2-thiol (3j)

IR(KBr) in cm^{-1} : 2600.01 (S-H str), 1680.88 (N-C=O str), 1585.55 (C=C str), 1568.18 (C=N str), 599.88 (Ar-Br str), 3400.01 (Ar-OH), ^1H NMR (CDCl_3) δ (ppm) : 1.61 (δ , 1H, S-H), 2.09 (δ , 3H, N-COCH₃), 4.99 (δ , 1H, Ar-OH), 5.61 (d, 1H, H-C₆ pyrimidine), 6.75 (d, 1H, H-C₅ pyrimidine), 7.18-7.78 (m, 8H, Ar-H).

Antimicrobial activity

The synthesized compounds (**3a-j**) were screened for their *in vitro* antimicrobial activity against, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and antifungal activity against, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*, *Candida albicans* by measuring the zone of inhibition in mm. The antimicrobial activity was performed by standard filter paper disc diffusion method¹⁶⁻¹⁷ and zone of inhibition is reported in the Table 3. Streptomycin and nystatin were used as standard drug for antibacterial and antifungal activities respectively. Nutrient agar was employed as culture medium and DMSO, was used as solvent.

Table 2. Antimicrobial data of compounds **3a-j**.

S. No.	Compounds	Antibacterial				Antifungal			
		<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>T. viride</i>	<i>C. albicans</i>
1.	3a	++	+++	++	+++	++	++	+++	++
2.	3b	++	++	++	++++	++	-	+	++
3.	3c	++	++	++	+++	++	+	++	-
4.	3d	++++	-	++	++	+	+	++	+
5.	3e	+	+	++	+	++	+	++	-
6.	3f	+++	+++	++	++++	++	++	++	+
7.	3g	++	+	-	+	+++	+++	+++	+++
8.	3h	+++	++	++	+++	+++	++	++	+++
9.	3i	+++	+++	++	+++	++	+++	+++	+++
10.	3j	+++	++	+	+	+	++	+++	++
Std. drug	Streptomycin	++++	+++	+++	++++	-	-	-	-
	Nystatin	-	-	-	-	++++	+++	++++	+++

Zone of inhibition was measured in mm.++++ : Strong activity, +++ : good activity, ++ : moderate activity, + : poor activity, - : inactive.

In vitro anthelmintic activity¹⁸

In vitro anthelmintic screening studies of compounds **3a-j** were performed by the Watkins technique, against common Indian earthworm '*P. posthuma*'. For this purpose 4% and 2% solutions of the synthesized pyrimidine derivatives and standard drug piperazine hydrochloride in ethylene glycol were used for experiment. The experiments were performed in duplicate and average values of paralytic time and lethal time in minutes have given in the Table 3.

Table 3. Anthelmintic activity of compounds **3a-j** (in minutes).

S. No.	Compounds	Concentration, %			
		4%		2%	
		Paralytic time	Lethal time	Paralytic time	Lethal time
1.	3a	7	15	9	18
2.	3b	8	17	8	20
3.	3c	10	18	10	21
4.	3d	9	17	9	17
5.	3e	6	14	7	17
6.	3f	8	16	7	16
7.	3g	9	15	10	20
8.	3h	9	17	9	18
9.	3i	8	15	9	17
10.	3j	7	14	10	18
11.	Std. drug	6	13	7	15

*Insecticidal activity*¹⁹⁻²⁰

Adult cockroaches (*P. americana*) were selected for the testing of, *in vitro* insecticidal activity. 4% solutions of synthesized pyrimidine derivatives and standard drug cypermethrin (w/v), in acetone were used for experiment. The time of death of cockroaches was noted, on an average and denoted as KD (Knock Down) value in minutes. For each sample three replication were performed and same experiments were performed with standard drug.

Table 4. Insecticidal activity of compounds **3a-j** (in minutes).

S. No.	Compounds	R ²	K.D. Value, min
1.	3a	4-O-CH ₃	9
2.	3b	4-OH-3-OCH ₃	9
3.	3c	H	10
4.	3d	4-Cl	12
5.	3e	2-Cl	8
6.	3f	4-N(CH ₃) ₂	8
7.	3g	2-NO ₂	7
8.	3h	3-NO ₂	9
9.	3i	6-Furyl	7
10.	3j	2-OH	9
11.	Std. drug	Cypermethrin	8

Conclusion

All the synthesized pyrimidine-2-thiols **3(a-j)** have given appreciable yield with satisfactory elemental analysis. It is inferred from the Table 2, 3 & 4, that synthesized compounds **3a-j**, have shown significant antibacterial activity, good to moderate antifungal activity and significant anthelmintic and insecticidal activities, due to the presence of thio group, which enhance the respective activities with varied substituent groups, against selected pathogens and organisms.

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