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Antibacterial and antifungal studies on some new acetylcinnolines and cinnolinyl thiazole derivatives

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Synthesis of some new 3-acetylcinnolines **2a-d** has been carried out by the intramolecular cyclisation of phenylhydrazonoacetylacetone **1a-d**. 3-Acetyl-4,8-dimethylcinnoline **2b** has been converted to 3-(bromoacetyl)-4,8-dimethylcinnoline **3b**. The 3-(bromoacetyl)-4,8-dimethylcinnoline **3b** has then been treated with thioacetamide **4a**, thiourea **4b** and substituted thioureas **4c-f** in ethanol to get 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole **5a**, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole **5b** and N- (aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines **5c-f**. The newly synthesized compounds have been characterized by elemental analysis, IR, ^{1}H NMR and mass spectral studies. Compounds have been screened for their antifungal and antibacterial activity. 4,7-dimethyl-3-acetylcinnoline **2c** has emerged as a promising antibacterial agent and N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine **5d** has emerged as a promising antifungal agent.

Keywords: Synthesis, acetylcinnolines, cinnolinyl thiazole, antibacterial activity, antifungal activity

IPC: **Int.Cl.[8] C07D**

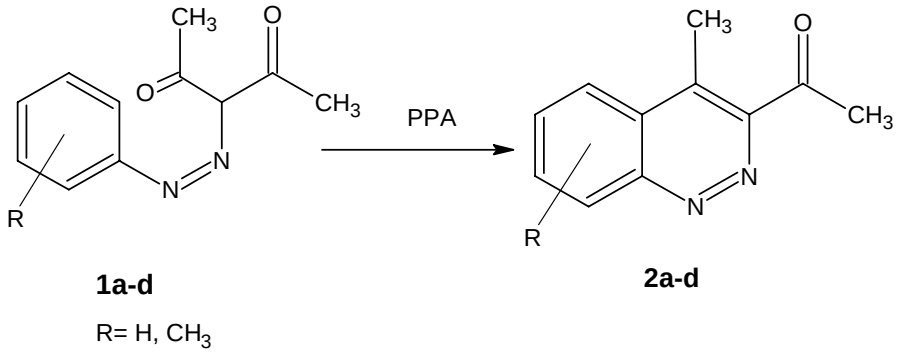
Thiazole and its derivatives are known for their antifungal and antibacterial activities[1-6]. A series of 7-(1-substituted-4-thiazolyl and thiazolidinyl)quinolones were prepared by Zhang *et al.*[5] and their antibacterial activity was tested *in vivo*. Some of these compounds showed good activity against gram-positive bacteria and mycobacteria.

Parent system cinnoline was found to possess antimicrobial action on *Escherichia coli*[7]. Cinnoline carbonitriles have been reported as bactericides[8] and recent studies have shown that cinnoline and their derivatives exhibit biological activity such as anti-hypertensive, antitumour, antisecretory, anti-inflammatory, antipyretic, analgesic and antibacterial in addition to their insecticidal properties[9-16]. Recently the antifungal properties of thiazolyl benzamides[17] have also been reported.

Prompted by the biological activity of cinnoline derivatives and in view of the ongoing search for the most potent antifungal and antibacterial agents, new derivatives of 3-acetylcinnolines **2a-d** and their thiazole derivatives 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole **5a**, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole **5b** and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines **5c-f** have been synthesized and their antifungal and antibacterial activity studied. Interestingly, most of the compounds have shown promising antimicrobial activity.

Synthesis of 3-acetylcinnolines **2a-d** was carried out by the intramolecular cyclization of substituted phenylhydrazonoacetylacetones **1a-d**, (**Scheme I**). Cyclization was done in concentrated sulphuric acid, with anhydrous aluminium chloride in chlorobenzene and in polyphosphoric acid. But the best yields were obtained when reactions were carried out with polyphosphoric acid as the cyclising agent. Cyclization reaction was found to be highly exothermic and external cooling had to be provided initially to avoid charring of the product. The temperature was maintained between 60-65°C.

The 4,8-dimethyl-3-acetylcinnoline **2b** was converted to 3-(bromoacetyl)-4,8-dimethyl cinnoline **3b** by a slow bromination in ethylacetate-methanol mixture with bromine and a catalytic amount of HBr with temperatures ranging between 10-15°C (**Scheme II**).

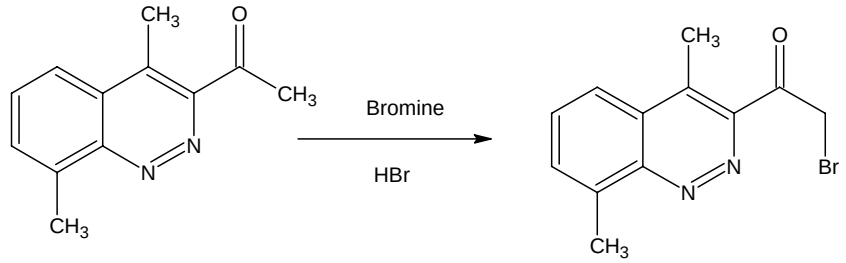


1a-d

R= H, CH_3

2a-d

Scheme I



2b **3b**

Scheme II

The 3-(bromoacetyl)-4,8-dimethylcinnoline **3b** was then treated with thioacetamide **4a**, thiourea **4b** and substituted thiourea **4c-f** in ethanol to get 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole **5a**, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole **5b** and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol -2-amines **5c-f** (**Scheme III**).

Substituted thioureas **4c-f** were synthesized by the reaction of benzoylchloride and ammonium thiocyanate with the appropriate aniline[6].

The formation of 3-acetyldimethylcinnolines **2a-d** and cinnolinylthiazoles **5a-f** were confirmed by elemental analysis, IR, ^{1}H NMR and mass spectra. Characterization data of the compounds phenylhydrazonoacetylacetone **1a**, 3-acetyldimethylcinnolines **2a-d**, 3-(bromoacetyl)-4,8-dimethylcinnoline **3b**, 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole **5a**, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole **5b** and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3thiazol-2-amines **5c-f** are given in **Table I** and **Table II** respectively.

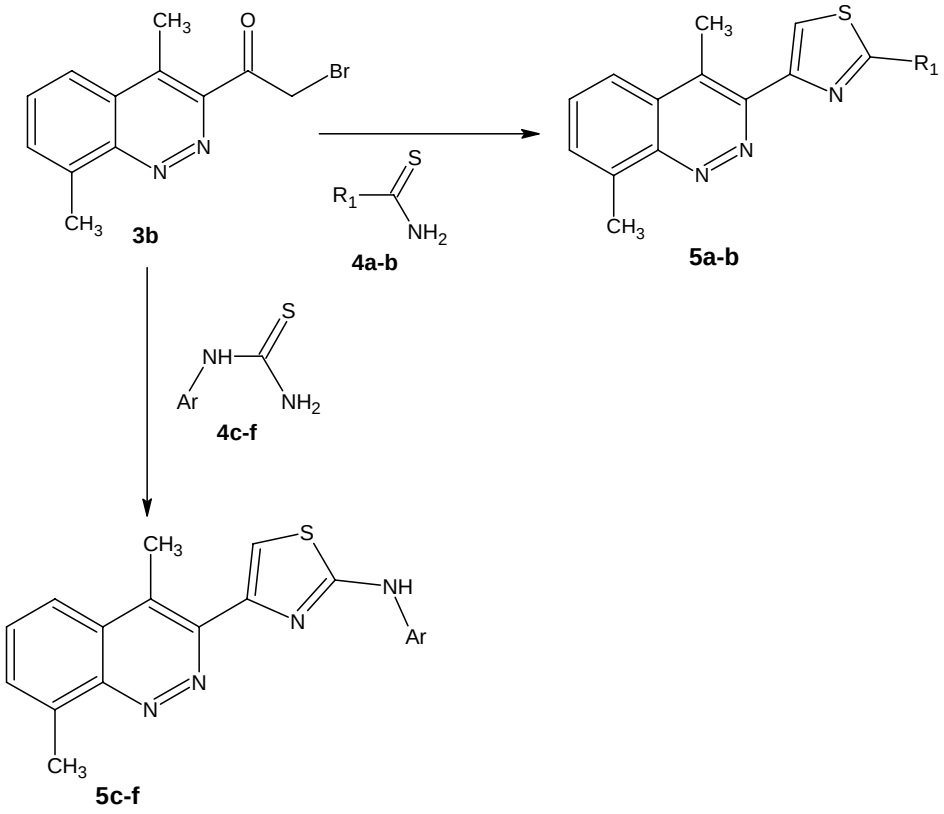
Biological activity

Antibacterial studies

Acetylcinnolines **2a-d** and cinnolinyl thiazoles **5a-f** were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonus aeruginosa* (ATTC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method[18,19]. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Ampicillin was used as standard drug. The results of such studies are given in **Table III**. Among tested acetyl cinnolines, the compound 4,7-dimethyl-3-acetylcinnoline **2c** was found to be the most active against *S. aureus* and *K. pneumoniae* and 4-methyl-3-acetylcinnoline was found to be the least active against tested bacterial strains. Among the tested thiazolyl derivatives **5a-f**, all the compounds showed moderate activity against *K. pneumoniae* (60 μg/mL) and *S. aureus.* But *E. coli* was found to be resistant to all the tested compounds. 4,7-Dimethyl-3-acetylcinnoline **2c** emerged as a promising compound, which requires further evaluation.

Antifungal studies

Acetylcinnolines **2a-d** and cinnolinylthiazoles **5a-f** were screened for their antifungal activity against *Aspergilus flavus* (NCIM No.524), *Aspergilus fumigatus* (NCIM No.902), *Candida albicans* (NCIM No.3100), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[18,19]. Antifungal activity



Scheme III

Table I − Characterization data of compounds **1a-d, 2a-d** and **3b**

Compd	**R**	Yield [a] %	m.p. °C [b]	Mol. formula	%Nitrogen	
					Found	Calcd
1a	H	75	101-03	$C_{11}H_{12}N_2O_2$	13.70	13.72
1b	2-CH_3	72	95-6	$C_{12}H_{14}N_2O_2$	12.80	12.84
1c	3- CH_3	73	100-02	$C_{12}H_{14}N_2O_2$	12.80	12.84
1d	4-CH_3	78	96-8	$C_{12}H_{14}N_2O_2$	12.80	12.84
2a	H	20	110-12	$C_{11}H_{10}N_2O$	15.07	15.04
2b	8-CH_3	25	120-22	$C_{12}H_{12}N_2O$	13.96	13.99
2c	7- CH_3	28	128-30	$C_{12}H_{12}N_2O$	13.96	13.99
2d	6-CH_3	26	160-62	$C_{12}H_{12}N_2O$	13.96	13.99
3b	-	80	120-22	$C_{12}H_{11}BrN_2O$	10.01	10.04

[a] All the yields are on isolated basis.
[b] Recrystallization solvent: Methanol for **1a-d** and **2a-d**, Ethyl acetate/hexane for **3b**.

Table II − Characterization data of compounds **5a-f**

Compd	R_1/Ar	Yield [a] %	m.p. °C [b]	Mol. formula	% Nitrogen	
					Found	Calcd
5a	-CH_3	32	105-07	$C_{14}H_{13}N_3S$	16.42	16.46
5b	-NH_2	36	176-78	$C_{13}H_{12}N_4S$	21.83	21.86
5c	-C_6H_5	35	190-92	$C_{19}H_{16}N_4S$	16.82	16.85
5d	-3Cl C_6H_4	33	170-72	$C_{19}H_{15}ClN_4S$	15.23	15.27
5e	-2$CH_3C_6H_4$	30	178-80	$C_{20}H_{18}N_4S$	16.14	16.17
5f	-4$CH_3C_6H_4$	32	182-84	$C_{20}H_{18}N_4S$	16.14	16.17

[a] All the yields are on isolated basis.
[b] Recrystallization solvent Ethanol/DMF.

Table III — Antibacterial screening results of compounds **2a-d** and **5a-f**

Compd	Zone of inhibition in mm			
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonus aeruginosa*	*Klebsiella pneumoniae*
2a	6	-	-	13
2b	6	6	14	13
2c	6	18	12	20
2d	7	13	6	13
5a	6	6	5	8
5b	-	-	-	12
5c	6	9	5	13
5d	-	12	5	12
5e	-	12	-	12
5f	-	12	-	13
Ampicillin	20	28	-	-

Table IV − Antifungal screening results of compounds **2a-d** and **5a-f**

Compd	Zone of inhibition in mm				
	Aspergilus flavus	*Aspergilus fumigatus*	*Penicillium marneffei*	*Candida albicans*	*Trichophyton mentagrophytes*
2b	6	6	-	18	6
2c	20	-	-	6	7
2d	5	7	5	12	18
5a	-	-	-	-	-
5b	6	-	19	5	5
5c	-	18	20	-	-
5d	6	20	12	8	19
Itraconozole[20]	-	-	-	26	-

was determined by measuring the diameter of the inhibition zone. The results of these studies are given in **Table IV**. Activity of each compound was compared with Itraconozole as standard drug. From the studies it was found that N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine **5d** was found to be the most active against *A. fumigatus, P. marneffei* and *T. mentagrophytes* and 4-(4,8-dimethylcinnolin-3-yl)-N-phenyl-1, 3-thiazol-2-amine **5c** emerged as the most active against *A. fumigatus* and *P. marneffei.* The compound, 4-(4,8-dimethyl-cinnolin-3-yl)-2-methyl-1,3-thiazole **5a** was found to be the least active. Hence N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine **5d** emerged as a promising compound, which requires further evaluation.

In conclusion, 4,7-dimethyl-3-acetylcinnoline **2c** emerged as a promising antibacterial agent and N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thia-zol-2-amine **5d** emerged as a promising antifungal agent.

Experimental Section

TLC was run on Merck silica gel coated aluminium plates and melting points were taken in open capillary tubes and are uncorrected. IR spectra in KBr pellets were recorded on Shimadzu FT-IR Infrared spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian (300 MHz) spectrometer using TMS as internal standard and mass spectra were recorded on a VG-s-70 Micro Mass, mass spectrometer operating at 70eV.

Synthesis of phenylhydrazonoacetylacetones 1a-d. Benzenediazonium chloride was prepared by dissolving sodium nitrite (7.4 g, 0.1072 mole) in 26 mL of water and adding it dropwise to a solution of aniline (10 g, 0.1025 mole) in 1N HCl (200 mL) at 0°C while stirring. The temperature was maintained at 0°C for another 10 min under stirring. Benzenediazonium chloride solution obtained was then added to a well stirred solution of ethanol (30 mL), water (500 mL) and acetylacetone (12.9 g, 0.129 mole) at 0°C with stirring. Sodium acetate was then added to keep the mixture alkaline to litmus. After 3 hr stirring at 0°C, the crude product was filtered, washed with water and air-dried. Recrystallisation from ethanol afforded yellow needles of purified phenylhydrazonoacetylacetone **1a** with a yield of 16.3 g (75%) IR (KBr): 3500 (broad, enolic -OH),

1691 (C=O), 1614 (N=N) 1140 cm^{-1} (-C-N=N-); ^1H NMR ($CDCl_3$): δ 14.7 (s, 1H, enolic OH), 7.1-7.4 (m, Ar-H), 2.4 (s, 3H, $-CH_3$), 2.6 (s, 3H, $-CH_3$), 1.6 (s, 1H, -CH); MS: m/z 204.8 (M^+, I=45%), 203 (M-1, I=100%), 187 (M-H_2O, I=22%).

Other substituted phenylhydrazozonoacetylacetones **1b-d** were also prepared in a similar way starting from *o*-toluidine, *m*-toluidine and *p*-toluidine.

Synthesis of 4-methyl-3-acetylcinnoline 2a and 3-acetyldimethylcinnolines 2b-d. Phenylhydrazonoacetylacetone **1a** (10 g, 0.0537 mole) was added to polyphosphoric acid (16 g) in small lots over 2 hr while maintaining the temperature between 60-65°C. Once the addition of **1a** was over, the same temperature was maintained for an additional 4 hr. Reaction was monitored by TLC. After completion of the reaction, chilled water (200 mL) was added carefully to decompose the black residue at 0-5°C. The product was then extracted with ethyl acetate. Ethyl acetate layer was then treated with charcoal and concentrated to get the crude product as a brownish-black residue. After purification by recrystallisation from methanol, **2a** was yielded as light yellow crystals with a yield of 0.9 g (20%). IR (KBr): 2976.5($-CH_3$), 1682 (C=O), 1522 (N=N) 1248 (-C-N=N) and 1128 cm^{-1} (-N=N-C-); ^1H NMR ($CDCl_3$): δ 2.9 (s, 3H, $-CH_3$), 3.1 (s, 3H, $-CH_3$), 8.6 (d, 1H,-Ar-H, *J*=7.8), 8.2 (d, 1H, -Ar-H, *J*= 7.8), 7.8 (t, 1H, -Ar-H), 7.8 (t, 1H, -Ar-H); MS: m/z 187(M+1, I= 100%).

In the same way 3-acetyl-4,8-dimethylcinnoline **2b**, 3-acetyl-4,7-dimethylcinnoline **2c** and 3-acetyl-4,6-dimethylcinnoline **2d** were prepared starting from the corresponding phenylhydrazonoacetylacetones **1b**, **1c** and **1d** respectively.

2b. IR (KBr): 2900 ($-CH_3$) 1698 (C=O) 1354 cm^{-1} (-N=N-); ^1H NMR ($CDCl_3$): δ2.9(s, 3H, $-CH_3$),3.1(s, 3H, $-CH_3$),3.2 (s, 3H, $-CH_3$),7.7 (m, 2H, -Ar-H), 8.1 (t,1H,-Ar-H);MS: m/z 201(M+1, I = 100%).

2c. ^1H NMR ($CDCl_3$): δ 2.67 (s, 3H, $-CH_3$), 2.94 (s, 3H, $-CH_3$), 3.01 (s, 3H, $-CH_3$), 7.65-7.61 (dd, 2H, -Ar-H, *J*=10), 8.1-8.0 (d, 1H, -Ar-H, *J*=10), 8.32 (s, 1H, -Ar-H); MS: m/z 200(M^+, I=100%), 199(M-1, I=40%), 184 (M-CH_3, I=20%), 172 (M-N_2, I=30%), 157(M-$COCH_3$, I=42%).

2d. ^1H NMR ($CDCl_3$): δ 2.65 (s, 3H, $-CH_3$), 2.92 (s, 3H, $-CH_3$), 3.01 (s, 3H, $-CH_3$), 7.06-7.14 (dd, 2H, -Ar-H, *J*=10.35), 8.46-8.43 (d, 1H, -Ar-H, *J*=8.7), 7.92 (s, 1H, -Ar-H); MS: m/z 200(M^+, I=100%), 199(M-1, I=40%), 184 (M-CH_3, I=20%), 172 (M-N_2, I=30%), 157(M-$COCH_3$, I=42%).

Synthesis of (3-bromoacetyl)-4,8-dimethyl cinnoline 3b. Ethyl acetate (30 mL) and HBr (0.1 mL) were taken in a flask. Reaction mixture was then cooled to 5-10°C. Bromine (3.07 g, 0.0192 mole) was then slowly added with stirring at 5-10°C over a period of 1 hr. In another flask, 3-acetyl-4,8-dimethylcinnoline **2b** (3 g, 0.015 mole) in 15 mL of ethyl acetate was taken and 0.1 mL of HBr was added. The reaction mixture was cooled to 5-10°C and the above prepared bromine solution was slowly added over a period of 6 hr. The reaction mixture was stirred for an additional 12 hr. The precipitated solid was filtered and purified by recrystallisation from ethyl acetate/hexane mixture. The product was obtained as yellow crystals with a yield of 2.77 g (80%).

IR (KBr): 3058 and 2912.3 ($-CH_2-$), 1701 (C=O), 1149 and 771 cm^{-1} (CH_2-Br); ^1H NMR ($CDCl_3$): δ 2.79(s, 3H, $-CH_3$), 3.03 (s, 3H, $-CH_3$), 5.44 (s, 2H, -CH_2), 6.2 (d, 1H, -Ar-H, *J*=8.3), 6.20 (s, 1H,Ar-H), 9.4 (d, 1H, -Ar-H, *J*=8.4); MS: m/z 280(M+1, I=20%), 199 (M-80, I=100%).

Synthesis of N-substituted thioureas, 4c-f[6]. Benzoyl chloride (0.01 mole) was added over 5 min to a freshly prepared solution of ammonium thiocyante (0.012 mole) in dry acetone and the mixture was heated under reflux for about 15 min. Heating was stopped and appropriate quantity of aniline in acetone was added over a period of 15 min. The mixture was heated under reflux for 30 min and then poured into crushed ice. The resulting solid was collected, washed with water, followed by a cold mixture of water and methanol (1:1). Suitably substituted benzoyl thioureas were added to pre-heated solution of aqueous sodium hydroxide (5%) and stirred. The mixture was then poured into crushed ice containing hydrochloric acid (5%). The benzoic acid which separated was removed by treating the reaction mixture with sodium carbonate. The product was collected, washed with water, dried and then directly taken for the next reaction.

Synthesis of 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethyl cinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines 5c-f. (3-Bromoacetyl)-4,8-dimethylcinnoline **3b** (0.01 mole) and appropriate thioacetamide/thiourea/substituted thiourea **4a-f** (0.01 mole) in ethanol was refluxed for 6 hr and allowed to stand undisturbed overnight. The solid which separated on cooling was filtered and purified by recrystallisation from a mixture of ethanol

and DMF (5-10% of DMF in ethanol). All the compounds were isolated in 32-35% yield.

5a. IR (KBr): 2923.9 (-CH_3), 1691.5(-C=N), 1359(C-N), 1259 and 1028 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6): δ 2.96 (s,3H,CH_3), 3.049 (s,3H,-CH_3), 3.07 (s,3H,-CH_3), 7.26 (s,3H,-ArH), 7.74 (dd., 2H,-Ar-H), 8.07 (t, 1H,Ar-H); MS: m/z 256 (M+, I=12%), m/z 214 (M- CH_3CN, I=100%).

5b. IR (KBr): 3251(-NH_2), 1635.5 (C=N), 1460(C-N), 1251 and 1051cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6): δ 1.95 (s,3H,CH_3), 1.89 (s,3H,-CH_3), 2.94-3.57 (dd,2H,-NH_2), 6.49-6.91 (m, 3H,Ar-H), 7.3 (s,1H,-thiazole-H), 8.076 (t, 1H,-Ar-H); MS: m/z 256 (M^+, I= 15%), m/z 241 (M-NH_2, I= 40%).

5c. IR (KBr): 3311.6 (-NH-), 1687 and 1604 (-C=N), 1490 (-C-N), 1245.9 and 1026.1cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6): δ 2.4(s,3H,CH_3), 3.02 (s,3H,-CH_3), 7.86(s,1H,-$NH_)$, 7.63 (m, 3H,Ar-H), 7.62 (m, 5H,Ar-H), 7.2(s,1H,-thiazole-H); MS: m/z 300 (M-2, 10%), m/z 214 (M-$NCNHC_6H_5$).

5d. ^{1}H NMR (DMSO-d_6): δ 2.34(s, 3H, CH_3), 2.40 (s, 3H, -CH_3),10.43 (s, 1H, -NH), 7.62 (s, 1H,Ar-H), 7.0 (m, 3H,Ar-H), 7.33 (d,1H, Ar-H, *J*=7.74), 7.42 (d, 1H, Ar-H, *J*=7.63), 7.7 (s, 1H,-thiazole-H); MS: m/z 368 (M+1, I=10%), m/z 256 (M-C_6H_5ClNH, I=12%), m/z157 (M-thiazole ring, I= 20%).

5e. MS: m/z 346 (M^+, I= 8%), 256(M-$C_6H_5CH_3NH$, I=12%), 157(M-thiazole ring, I= 20%)

5f. IR (KBr): 3312 (-NH-), 1687 and 1604 (-C=N), 1490 (-C-N), 1245 and 1026 cm^{-1} (-C-S).

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