SYNTHESIS, ANTI-INFLAMMATORY AND ANTI-MICROBIAL ACTIVITY OF PYRIMIDINE-5-CARBOXYLATE DERIVATIVES

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Abstract

Ethyl-2-amino-4-chloro-6-(2, 4, 6 trichloro phenyl amino) pyrimidine-5-carboxylate and ethyl-2-amino 4-chloro-1, 3-dimethyl-6-(phenyl amino)-1,2,3,4 tetrahydro pyrimidine-5-carboxylate derivatives were synthesized through Cyclocondensation and Michel type of addition reaction with the aromatic anilines, guanidine and benzamidines. Synthesized novel derivatives were confirmed by elemental analysis viz., IR, 1HNMR and Mass spectroscopy. These novel derivatives have been screened for Antibacterial, Antifungal and Anti-inflammatory activity.

Keywords: Pyrimidine-5-Carboxylate, Aromatic anilines, Guanidines, Benzamidine, Anti-inflammatory and Anti-microbial activity

1. Introduction:

Pyrimidine and their derivatives are considered to be important for drugs and a survey of the pertinent literature has revealed versatile application of pyrimidines-5-carboxylate[1-2] viz., as Antibacterial, Antifungal and Anti-inflammatory[3-4] which improve their solubility and enhance their biological activity, have been reported as promising Antimalerial[5], Anticancer, Anti-HIV[6] and Adenosine kinase inhibitor activity. Pyrimidines-5-carboxylate derivatives have attracted attention to explore some new derivatives. Inflammatory diseases like arthritis, allergy, asthma, multiple sclerosis etc. are quite common and need a considerable attention. Literature survey reveals that vast amount of research is going on in search of safer Anti-inflammatory drugs. Pyrimidine derivatives act as H1-Antihistamines as selective type-IV phosphodiesterase inhibitors and as Anti-inflammatory agents.

2. Materials and Method:

2.1 Materials: Ciprofloxacin, Tetracycline, Indomethacin and

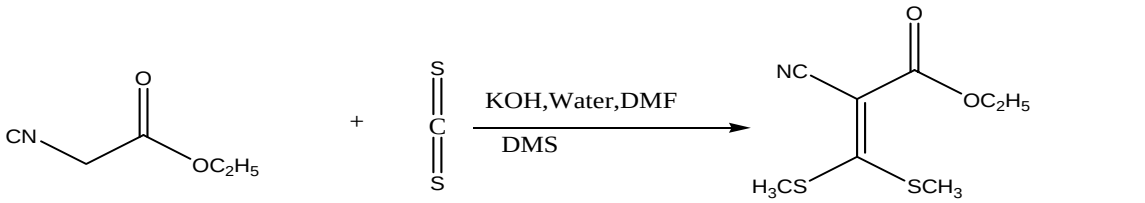
Ketoconozole were gifted by Micro Labs., (India). All the chemicals and reagents were of analytical grade and purchased from Loba Chem. and Hi-Media, Mumbai. The purity of the synthesized compound were ascertained by TLC and melting point were determined by Thiele's tube apparatus and uncorrected. The synthesized compounds were determined by IR, ^{1}HNMR and Mass spectroscopy.

2.2 Method:

Synthesis of Pyrimidine-5-Carboxylate Derivatives

STEP 1: Preparation of Ethyl 3, 3 bis (methylthio)-2- cyanoacrylate.[7]

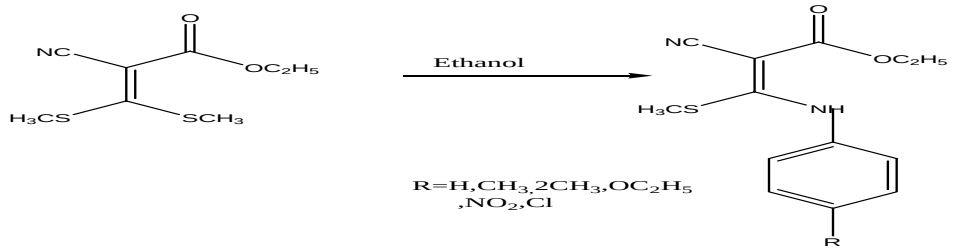
To an ice cold solution of potassium hydroxide (13.2 g, 0.2 mol, 85%) in 10 ml of water and 30 ml of DMF was added, with cooling and stirring, ethyl cyanoacetate (11.3 g, 0.1 mol) followed by carbon disulphide (7.6 g, 0.1 mol). The mixture was stirred for one hour at room temperature, cooled and treated drop wise with DMS (25.2 g, 0.2 mol) maintaining temperature at 20 ^{0}C. The reaction mixture was allowed to stand at room temperature for 12 hours and poured into 500ml of ice water mixture. The solid obtained was filtered, washed with cold water and dried. Recrystallzation from n-hexane yield a crystalline product.



ethyl cyanoacetate **carbon disulphide** **Ethyl 3, 3 bis (methylthio)-2- cyanoacrylate**

STEP 2: Preparation of Ethyl-2cyano-3-(methylthio)-3-phenylamino acrylate.

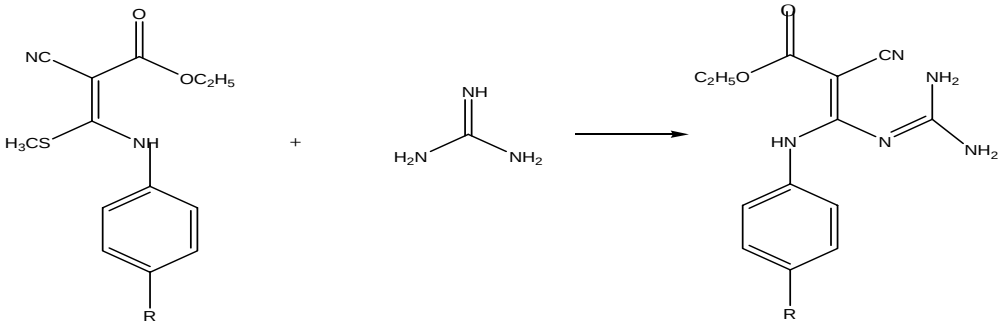
A mixture of step-1 product and freshly distilled aniline (1.86 g, 0.02 mol) in 30 ml of ethanol was refluxed for one hour. After allowing to stand at room temperature for 24 hour. The reaction mixture was filtered, washed with cold ethanol and dried. Recrystallization from hexane yielded the product. In this work different aromatic amines like O-toludine, 2, 4, 6-triachloroaniline, O-dianisidine, N, N, dimethylaniline, m-nitroaniline, 2-chloro aniline were used for the synthesis of pyrimidine derivatives in laboratory condition.



Ethyl-2cyano-3-(methylthio)-3-phenylamino acrylate

STEP 3: Preparation of Ethyl-3-(amino phenyl)methyleneamino-2-cyno-3-(phenylamino) acrylate.[8]

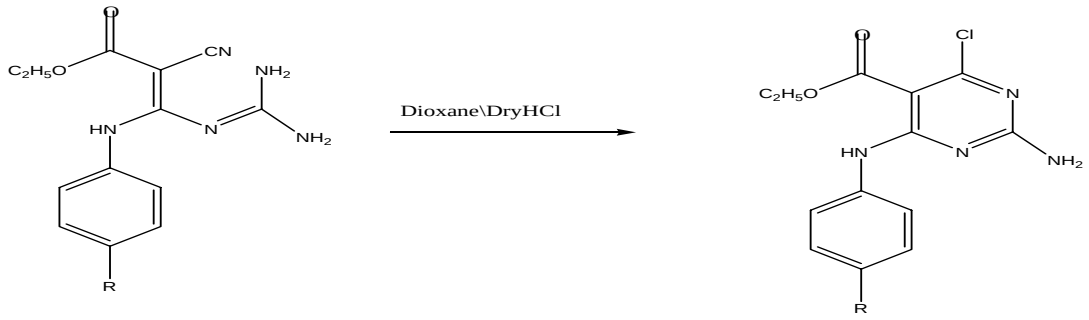
To an ice cold suspension of sodium hydride (0.96 g, 0.02 mol, 50%) in 20 ml of dimethylforamide was added with stiffing guanidine hydrochloride. The mixture was stirred for 30 min and treated drop wise under cooling and stirring with a solution of step-2 product in 15 ml of DMF. The reaction mixture was stirred at 10^0C for 4 hours. After allowing to stand at room temperature for 24 hours. The reaction mixture was poured into 800 ml of ice water mixture. The solid obtained was filtered and dried. Recrystallization from hexane yielded a colorless crystalline compound.



Ethyl-3-(amino phenyl)methyleneamino-2-cyno-3-(phenylamino) acrylate

STEP 4: Preparation of Ethyl-4-chloro-2-phenyl-6-(phenylamino) pyrimidine-5-carboxylate[8]

A steam of dry HCL gas was passed through an ice cold solution of above product (2.7 g, 0.01 mol) in 25 ml of dioxane for 24 hours. The reaction mixture was allow to stand at room temperature for 24 hours and poured into 500 ml of ice water mixture. The solid obtained was filtered, washed with water and dried. Recrystaliazation from ethanol yielded a colorless compound.



Ethyl-4-chloro-2-phenyl-6-(phenylamino) pyrimidine-5-carboxylate

3. **Biological Studies:**

1. The Antibacterial and Antifungal activities[9] of different sample were done in disc diffusion method against *Escherichia coli, Psueudomonoas aeruginosa (Gram negative), Bacillus cereuves, Staphylococus auereus* (Gram positive) and *Aspergillus Niger, Candida albicans (Fungi)* as directed by Ellen Jo Boron. The test samples used in concentration 1 mg/ml using dimethylsulfoxaide as solvent and 1mg/ml using their respective solvent Gentamicin, Ciprofloxacin and Tetracycline were used as standards for *Staphylococcus auereus* and *Escherichia coli.* Ketoconozole were used as standard against *Aspergillius Niger.*

2. Anti-inflammatory activity[10] was evaluated using Carrageenan induced rat hind paw edema method. Albino rats of either sex weighing between 150-200 g were divided into eight groups of six animals. The first group served as control and received the vehicle (Saline) only. Second group of animals were treated with standards drug Indomethacin (20 mg/kg). The mean difference in initial paw volume and subsequent reading was noted and percentage inhibition of edema was calculated using the formula.

$$\text{Percentage Inhibition} = 100 \times \{1 - Vt/Vc\}$$

Where, Vt = represent edema volume in test

Ve = represent edema volume in control.

4. Result & Discussion:

At present in the scheme the acrylate was synthesized with 65-70% yield, it further converted as substituted Pyrimidine-5-caboxylate derivatives with yield between 70-80%. This research work was oriented towards the finding of newer Pyrimidine-5-caboxylate with Anti-microbial and

Anti-inflammatory activities. The different substituted Pyrimidine-5-carboxilate derivatives were synthesized by Michel type addition reaction using guanidine and different substituted aromatic aniline as followed by cyclization and condensation reaction. The structures of the different substituted Pyrimidine-5-carboxylate were confirmed by using different analytical techniques, Elemental analysis, IR, ^{1}HNMR and Mass spectroscopes. The results of this analysis showed that the expected different substituted Pyrimidine-5-carboxylate were prepared.

5. Conclusion:

The newly synthesized Pyrimidine-5-carboxylate derivatives were evaluated for their Anti-microbial and Anti-inflammatory activities. The synthesized compounds 1b and 1e showed effective Anti-microbial activity, also all the compounds expect compound 1c was found to exhibit good Anti-inflammatory activity. This clearly indicates that new Pyrimidine-5-carboxylate derivatives can be effectively synthesized by the method mentioned in this study and these synthesized compounds exhibited significant Anti-microbial and Anti-inflammatory activities. However further studies can be conducted to synthesize newer Pyrimidine-5-carboxylate derivatives with potent Anti-bacterial and Anti-inflammatory activities.

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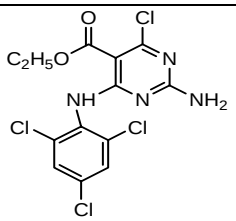
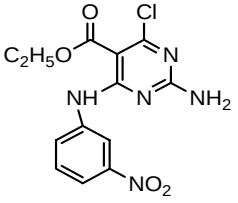
Table I-Different Substitution in Compounds

Sr. No.	**Compound**	**IUPAC Name**	***Mol. Formula***	**Structure**
1	1a	Ethyl -2-amino 4-chloro-6-(2,4,6,trichloro phenyl amino) -pyrimidine-5-carboxylate.	$C_{13}H_{10}Cl_4N_4O_2$	
2	1b	Ethyl -2-amino-4-chloro-6-(3-nitro phenyl amino) -Pyrimidine- 5-carboxylate.	$C_{13}H_{11}ClN_4O_4$	

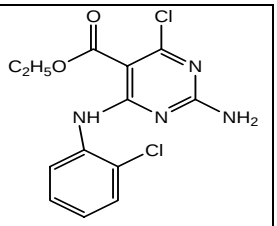
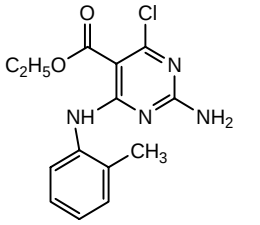
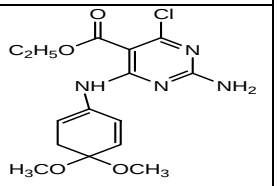
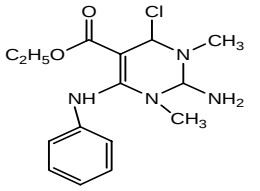
3	1c	Ethyl-2-amino-4-chloro-6-(2-chloro phenyl amino)-Pyrimidine -5-carboxylate.	$C_{13}H_{12}Cl_2N_4O_2$	
4	1d	Ethyl -2-amino- 4-chloro-6-(o-toluidino phenyl amino) - Pyrimidine- 5-carboxylate.	$C_{14}H_{15}ClN_4O_2$	
5	1e	Ethyl -2-amino-4-chloro-6-(4 4, dimethoxy cyclohexa-1, 5-dienyl amino) -Pyrimidine-5-carboxylate.	$C_{15}H_{19}ClN_4O_4$	
6	1f	Ethyl-2-amino- 4-chloro-1, 3 dimethyl-6-(phenyl amino)-1, 2, 3, 4 –tetrahydro- Pyrimidine-5-carboxylate.	$C_{15}H_{21}ClN_4O_2$	

Table II –Elemental Analysis Data of Compounds

Sr. No.	Compound	Mol. Formula	m.p.^{0}C	Molecular weight	Yield in percentage (avg.)	Elemental analysis of compounds (%) found		
						C	H	N
1	1a	$C_{13}H_{10}Cl_4N_4O_2$	290- 292^{o}C	396.60	80	39.40	2.54	4.15
2	1b	$C_{13}H_{11}ClN_4O_4$	189- 193^{o}C	322.70	82	48.38	3.44	17.36
3	1c	$C_{13}H_{12}Cl_2N_4O_2$	294- 297^{o}C	327.17	78	47.72	3.70	17.12
4	1d	$C_{14}H_{15}ClN_4O_2$	282- 286^{o}C	306.75	75	54.82	4.93	18.26

5	1e	$C_{15}H_{19}ClN_4O_4$	289- 291°C	354.78	67	50.70	5.40	15.79
6	1f	$C_{15}H_{21}ClN_4O_2$	296- 298°C	324.14	70	55.47	6.52	17.25

Table III– IR Spectral Data of Compounds

Sr. No.	Compound	Mol. Formula	IR spectral data in CM^{-1}
1	1a	$C_{13}H_{10}Cl_4N_4O_2$	3467, 38, 53082 , 2853, 2610, 1793, 1684, 1466, 1295, 1071, 934, 883 cm^{-1}
2	1b	$C_{13}H_{11}ClN_4O_4$	3441, 2925, 2440, 1951, 1749, 1524, 1223, 930, 861, 667 cm^{-1}
3	1c	$C_{13}H_{12}Cl_2N_4O_2$	3447, 3322, 2924, 2853, 2513, 1616, 1457, 1016, 873, 752, 566 cm^{-1}
4	1d	$C_{14}H_{15}ClN_4O_2$	3469, 3337, 3015, 2854, 1791, 1624, 1456, 1276, 984, 825, 608 cm^{-1}
5	1e	$C_{15}H_{19}ClN_4O_4$	3432, 2956, 2854, 1672, 1408, 1219,1092, 872, 770, 583, 547 cm^{-1}
6	1f	$C_{15}H_{21}ClN_4O_2$	3441,3384, 2923, 2854, 2513, 1795, 1678, 1419, 1016, 873, 752, 566 cm^{-1}

Table IV- ^1H NMR Spectral Data of Compounds.

Sr. No.	Compound	Mol. Formula	^1H NMR Spectral data in δ values
1	1a	$C_{13}H_{10}Cl_4N_4O_2$	1.30(S,3H), 2.10(S,3H), 3.50(S,H), 4.10(S,H), 4.65(d,2H), 6.35(S,H), 6.75(S,H), 7.10(S,H)
2	1b	$C_{13}H_{11}ClN_4O_4$	1.45(d,3H), 3.85(S,H), 4.25(d,2H),5.75(S,H), 6.75(d,H), 7.30(S,H), 7.40(S,H), 7.65(S,H)
3	1c	$C_{13}H_{12}Cl_2N_4O_2$	1.35(S,3H), 3.70(S,H), 4.60(S,2H), 6.60(S,H), 6.75(d,H), 7.40(S,H), 7,65(S,H)
4	1d	$C_{14}H_{15}ClN_4O_2$	1.15(M,3H), 2.85(S,3H), 4.50(S,H), 4.85(d,H), 6.50(d,H), 6.65(M,H), 7.25(S,H), 7.35(d,H)

5	1e	$C_{15}H_{19}ClN_4O_4$	0.95(M,3H), 2.15(d,2H), 3.35(S,6H), 4.40(S,H), 4.85(S,2H), 5.40(S,H), 5.60(S,H), 6.10(S,H), 6.30(d,H)
6	1f	$C_{15}H_{21}ClN_4O_2$	1.35(S,3H), 3.70(S,H), 4.60(S,2H), 6.60(S,H), 6.75(d,H), 7.40(S,H), 7.65(S,H)

Table V – Mass Spectral Data of Compounds

Sr. No.	Compound	Mol. Formula	M/Z Values
1	1a	$C_{13}H_{10}Cl_4N_4O_2$	396 (Mol. ion peak) 372, 346, 294, 268, 256, 238, 211, 178, 149, 139 (Base peak) 116, 72, 56.
2	1b	$C_{13}H_{11}ClN_4O_4$	322 (Mol. ion peak) 312, 304, 293, 273, 262, 240, 226, 213, 199, 172, 162, 139 (Base peak) 133, 123, 112, 96, 82, 68, 56.
3	1c	$C_{13}H_{12}Cl_2N_4O_2$	327 (Mol. ion peak) 302, 290, 270, 259, 236, 223, 209, 197, 183, 172, 160, 139 (Base peak) 131, 121, 112, 98, 71, 57.
4	1d	$C_{14}H_{15}ClN_4O_2$	306 (Mol. ion peak) 293, 268, 256, 235, 228, 212, 189, 174, 166, 151, 139 (Base peak) 131, 120, 99, 85, 69, 57.
5	1e	$C_{15}H_{19}ClN_4O_4$	354 (Mol. ion peak) 345, 331, 308, 291, 272, 260, 239, 224, 210, 193, 184, 170, 160, 139 (Base peak) 132, 121, 110, 92, 80, 67, 55.
6	1f	$C_{15}H_{21}ClN_4O_2$	324 (Mol. ion peak) 294, 280, 268, 256, 236, 224, 203, 193, 175, 164, 139 (Base peak) 122, 109, 92, 83, 66, 55.

Table VI (a) Anti-microbial Activity by Disc Diffusion Method for Pyrimidine-5-Carboxylate Derivatives

Sr. No.	Compound	Anti-bacterial activity zone of inhibition (mm)				Anti-fungal activity zone of inhibition (mm)	
		B. cereus	*S. auereus*	*E.coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1	1a	20	19	17	19	18	15
2	1b	23	22	20	21	21	18
3	1c	22	21	20	22	21	18

4	1d	18	16	15	14	16	13
5	1e	25	23	22	24	24	20
6	1f	19	17	16	18	18	14
7	Ciprofloxacin	30	30	28	28	--	--
8	Ketoconazole	--	--	--	--	31	30

Table VI (b) Anti-inflammatory Activity by Carrageenan Induced Paw Edema Method

Sr. No.	**Groups**	**Mean ± SEM**	**% inhibiton at 4 hour**	**% Activity in relation to Indomethacin**
1	Control	0.83 ± 0.21	0.00	0.00
2	Indomethacin	0.35 ± 0.34	57.0	100
3	Ia	0.61 ± 0.30	26.0	45.0
4	Ib	0.55 ± 0.42	33.0	57.8
5	Ic	0.68 ± 0.40	18.0	31.5
6	Id	0.73 ± 0.44	12.0	21.0
7	Ie	0.38 ±0.28	54.0	94.0
8	If	0.61 ±0.41	26.0	45.0