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SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRIDO-PYRIMIDINE CARBOXYLATE DERIVATIVES AS POTENTIAL ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
A new series of novel derivatives of Pyrido-Pyrimidine Carboxylate were synthesized. These derivatives were identified on the basis of melting point range, R_f values, IR and ^{1}H NMR spectral analysis. The derivatives were screened for anti-microbial and anti-inflammatory activities. The derivatives exhibited significant to moderate anti-microbial and anti-inflammatory activities.

KEYWORDS: Pyrido-Pyrimidine Carboxylate, anti-microbial and anti-inflammatory activity.

INTRODUCTION
A novel series of Pyrido Pyrimidine Carboxylate derivatives were synthesized by cyanoacetate in ethyl alcohol. It was amazinging that next aromatization took place automatically compared with other synthetic methods. This new method has the advantage of low work, easy reaction condition and good yield.

Pyrido pyrimidine carboxylate derivatives synthesis have great interest in organic chemistry, because they shows significant biological and pharmacological activities, such as antibacterial activity,[1] antifungal activity,[2] analgesic activity,[3] anti-inflammatory activity,[4] anticancer activity,[5] anti HIV,[6] anti-hperlepidemic activity,[7] thermodynamical property,[8] CCR4 antagonist,[9] antidiabetic property,[10] antiherpes activity,[11] antiviral.[12] and calcium channel blocker.[13] etc. There are more number of methods have been derived for the synthesis of pyrido pyrimidine carboxylate derivatives, which usually takes much time, very complex synthetic procedure and expensive catalyst . Research in this area is still unexplored, therefore the present study is directed towards the synthesis of newer derivatives of Pyrido Pyrimidine Carboxylate with good yield and enhance anti-microbial anti-inflammatory.

MATERIALS AND METHODS
All the chemicals procured from Fisher chemical, Himedia, Loba chemicals, CDH etc. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Shimadzu FTIR system in KBr pellets and noted the absorption levels (cm^{-1}) were listed. ^{1}H NMR spectra were run on Bruker DPX 400 FTNMR in DMSO-d_6 as solvent and TMS as an internal standard. The Mass spectra were recorded on JEOL JMS600H mass spectrometer.

STEP 1: **Preparation of Ethyl 3, 3 bis (methyl thio) -2-cyano acrylate**
To an ice cold solution of potassium hydroxide (13.2g., 0.2 mol, 85%) in 10 ml of water and 30 ml of dimethyl formamide will be added, with cooling and stirring, ethyl cyanoacetate (11.3g., 0.1 mol) followed by carbon disulfide (7.6g., 0.1 mol) . The mixture was stirred for one hour at room temperature, cooled and treated dropwise with dimethyl sulphate (25.2 g, 0.2 mol) maintaining temperature at 20°C.The reaction mixture was allowed to stand at room temperature for 12 hours and poured into 500 ml of ice water mixture. The solid obtained was filtered, washed with cold water and dried. Recrystalization by n-hexane.

STEP 2: **Preparation of 2-substituted- 4- (methyl thio) -6-oxo-1,6-dihydro pyrimidine -5-carbo nitrile .**
A mixture of ethyl 2- cyano-3,3-bis (methylthio) acrylates (4.3g, 0.02 mol) and freshly distilled aromatic amidines (1.86g ,0.02 mol) in 30 ml of ethanol will be refluxed for one hour. After allowed stand at room temperature for 24 hours, the reaction mixture was filtered, washed with cold ethanol and dried. Recrystalliozation by benzene-hexane mixture.

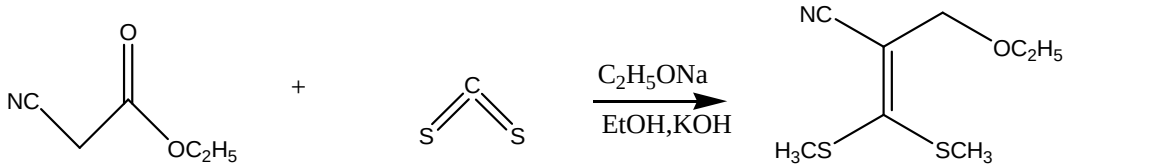
STEP3: Preparation of 4- (4-halo phenyl amino) -2-substituted-6-oxo-1,6-dihydropyrimidine-5-carbonitrile.

A mixture of 2-subsutitued-4- (methylthio) -6-oxo-1,6dihydropyrimidine-5-carbonitrile (4.3g, 0.02 mol) and freshly distilled aromatic halo anilines (1.12g, 0.01mol; 1.275g, 0.01mol &1.55g, 0.01mol) in 30ml of ethanol was refluxed for one hour. After allowed stand at room temperature for 24 hours, the reaction mixture was filtered, washed with cold ethanol and dried . Recrystallization by hexane. In this work different anilines like p-fluroaniline, p- choloaniline, p-bromoaniline will be used.

STEP 4:Preparation of Ethyl-5-amino-8- (4-halo phenyl) -2-substituted-4, 7-dioxo- 3,4,5,6,7,8-hexa hydro pyrido (2,3-d) pyrimidine -6-carboxylate.

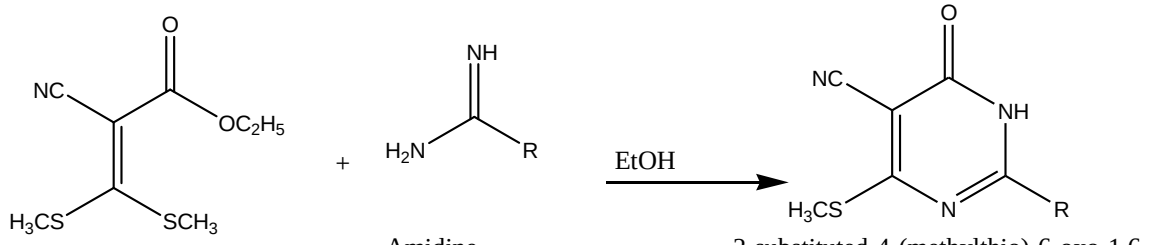
A mixture of step 3 product (2.45g, 0.01mol; 2.62g, 0.01mol & 2.89g,0.01mol for Benzamidine related derivatives and 2.46g, 0.01mol; 2.63g, 0.01mol & 2.91g, 0.01mol for Guanidine related derivatives) and malonic acid (2.04g, 0.02mol) and 30ml of ethanol was added and refluxed for one hour . After allowed stand at room temperature for 24 hours, the reaction was filtered, washed with cold ethanol and dried. Recrystallization by benzene-hexane mixture.

STEP: 1



ethyl 2-cyanoacetate carbon di sulphide ethyl 2-cyano-3,3-bis(methylthio)acrylate

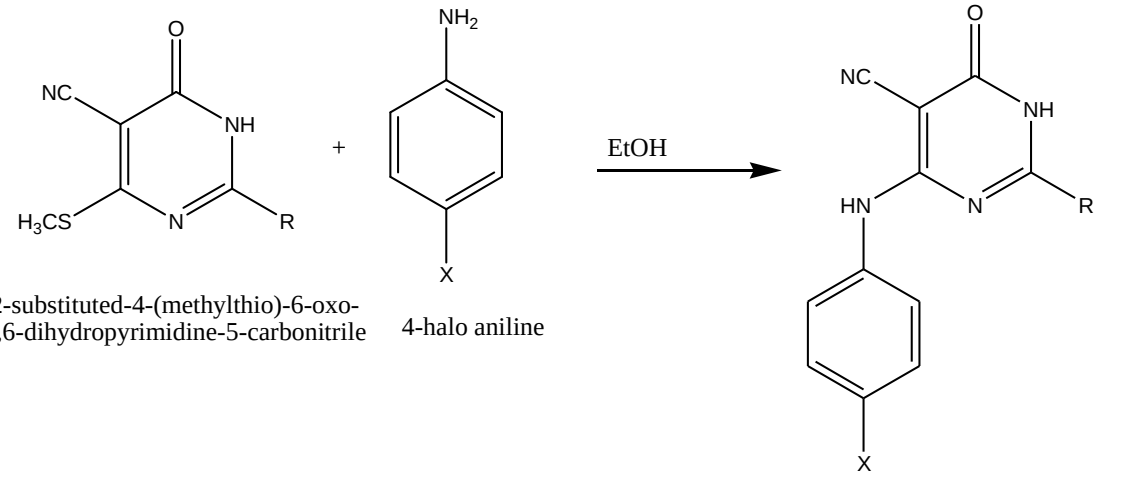
STEP: 2



ethyl 2-cyano-3,3-bis(methylthio)acrylate Amidine 2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile

R =C_6H_5, Benzamidine,
R = $COOCH_3$, Formamidine acetate

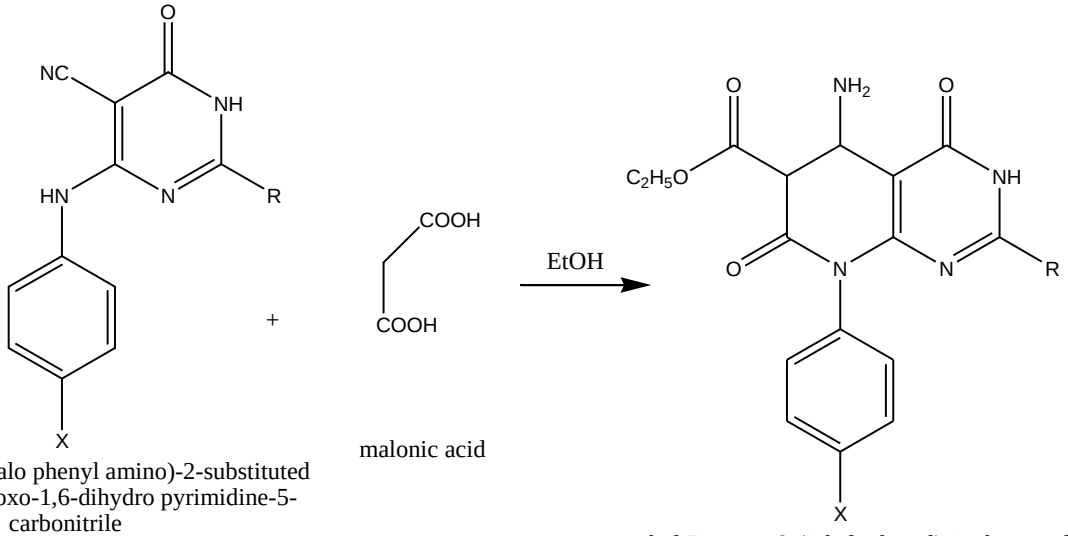
STEP: 3



2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile 4-halo aniline 4-(4-halo phenyl amino)-2-substituted -6-oxo-1,6-dihydro pyrimidine-5-carbonitrile

Where, X = F, Cl, Br etc

STEP: 4



4-(4-halo phenyl amino)-2-substituted -6-oxo-1,6-dihydro pyrimidine-5-carbonitrile

malonic acid

ethyl 5-amino-8-(4-halophenyl)-2substituted-4,7-dioxo-3,4,5,6,7,8-hexahydropyrido [2,3-*d*]pyrimidine-6-carboxylate

Antimicrobial screening[14]

Cup-plate method

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37^0C before inoculation. The organisms were inoculated in the plates prepared earlier, by dipping sterile swab in the previously standardized inoculums, removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times, rotating the plates through an angle of 60^0 after each application. Finally press the swab round the edge of the agar surface. It is allowed to dry at room temperature, with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of the Mueller Hinton agar plate and it was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug. Plates were prepared in triplicate and they were then incubated for 18-24 hrs. Observations were made for zone of inhibition around the drugs and compared with that of standard. All the compounds synthesized were tested for antibacterial activity.

Anti- inflammatory activity[15]

Carrageenan induced rat paw oedema method

The anti-inflammatory activity of the standard drug Acetaminophen and synthesized derivatives was determined against carrageenan induced paw oedema in albino rats (weighing 150-175g). The albino rats were divided into 4 groups containing 1 animal each. The animals were fasted for 12 hrs prior to the experiment. The 1% w/v solution of carrageenan for injection is prepared in normal saline and 0.1 ml is injected under.

subplanter region. The standard drug (200mg/Kg) and synthesized derivative (PZ3) (100mg/Kg, 200mg/Kg) was administered in animals by oral route. Volume of the injected paw after 3hr was measured with a plethysmometer. The differences in the paw volumes (i.e. oedema volumes) of each animal were calculated and compared with the changes in the oedema volumes of control and the drug treated animals. The results were expressed as percentage reduction in oedema volume, which can be calculated by using the formula:

Percent Reduction = (Cvt – Tvt)/ Cvt × 100

Where,

Cvt = oedema volume of control animals at time 't'

Tvt = oedema volume of drug treated animals at time 't'

RESULTS AND DISCUSSION

The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The structures of the synthesized compounds were supported by physical data (Table-1) and following spectral analysis.

Table1: Physical data of the derivatives.

Sl. No.	Compound code	Molecular Formula	Mol. Wt.	M.P. (^{0}C)	Rf. value	Solvent system
1	A	$C_{17}H_{17}N_4O_4F$	360.34	More than 353 ^{0}C	0. 71	Butanol: Hexane (3 : 2)
2	B	$C_{17}H_{17}N_4O_4Cl$	376.79	More than 353 ^{0}C	0. 90	Butanol: Hexane (3 : 2)
3	C	$C_{17}H_{17}N_4O_4Br$	421.25	More than 353 ^{0}C	0 .73	Butanol: Hexane

						(3 : 2)
4	1	$C_{22}H_{19}N_4O_4F$	422.40	More than 353 ^{0}C	0. 80	Butanol: Hexane (3 : 2)
5	2	$C_{22}H_{19}N_4O_4Cl$	438.863	More than 353 ^{0}C	0.70	Butanol: Hexane (3 : 2)
6	3	$C_{22}H_{19}N_4O_4Br$	483.31	More than 353 ^{0}C	0. 60	Butanol: Hexane (3 : 2)

The IR spectra were recorded on Shimadzu FTIR system in KBr pellets and noted the absorption levels (cm^{-1}) were listed. The results of IR spectra were given in spectral detail heading which showed absorption bands for different groups.

^{1}H NMR spectra were run on Bruker DPX 400 FTNMR in DMSO-d_6 as solvent and TMS as an internal standard. The results of the ^{1}HNMR spectra given under spectral detail heading showed that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds.

The Mass spectra were recorded on JEOL JMS600H mass spectrometer. The results presented in the spectral heading showed that the molecular mass of the synthesized compounds was nearer to the molecular mass of the expected compounds.

The spectral details of the synthesized compounds

Ethyl-5-amino-8- (4-fluorophenyl) -2-acetyl-4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate (A)

IR (KBr) ν_{max} :1550(Ar,C=C),3132(Ar,C-H), 2938(C-H,s), 1378(C-H,b), 900(C-C,ring), 1310(C-N,s), 1661(C=N,s), 670(C-F,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.2(Ar-H,m), 2.2(CH_3,m), 2.4(CH_2,m), 4.2(NH,d), 11.4(OH or COOH,s) ; LC-MS:*m/z* 360.54(M^+)

Ethyl-5-amino-8- (4-chlorophenyl) -2- acetyl -4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate (B)

IR (KBr) ν_{max} :1553(Ar,C=C),2923(Ar,C-H), 2940(C-H,s), 1308(C-H,b), 814(C-C,ring), 1413(C-N,s), 1610(C=N,s), 693(C-Cl,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.2(Ar-H,m), 2.2(CH_3,m), 2.4(CH_2,m), 4.2(NH,d), 11.4(OH or COOH,s), 6.5(OCH_3,s) ; LC-MS:*m/z* 378.65(M^+)

Ethyl-5-amino-8- (4-bromophenyl) -2- acetyl -4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate (C)

IR (KBr) ν_{max} :1558(Ar,C=C),3170(Ar,C-H), 2992(C-H,s), 1378(C-H,b), 956(C-C,ring), 1315(C-N,s), 1604(C=N,s), 660(C-Br,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.2(Ar-H,m), 2.2(CH_3,m), 4.2(NH,d); LC-MS:*m/z* 421.39(M^+)

Ethyl-2,5-diamino-8- (4-fluorophenyl) 2-benzyl- 4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate (1)

IR (KBr) ν_{max} :1561(Ar,C=C),3434(Ar,C-H), 2983(C-H,s), 1323(C-H,b), 980(C-C,ring), 1380(C-N,s), 1656(C=N,s), 619(C-F,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.0(Ar-H,m), 2.08(CH_3,m), 2.9(CH_2,m), 6.6(OCH_3,s) ; LC-MS:*m/z* 422.27(M^+)

Ethyl-2,5-diamino-8- (4-chlorophenyl) 2-benzyl -4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate(2)

IR (KBr) ν_{max} :1561(Ar,C=C),3785(Ar,C-H), 2956(C-H,s), 1390(C-H,b), 899(C-C,ring), 1311(C-N,s), 1685(C=N,s), 685(C-Cl,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.2(Ar-H,m), 2.9(CH_2,m), 4.2(NH,d), 6.5(OCH_3,s) ; LC-MS:*m/z* 438.29(M^+)

Ethyl-2,5-diamino-8- (4-bromophenyl) 2-benzyl -4,7-dioxo-3,4,5,6,7,8 hexahydropyrido (2,3-d) pyrimidine-6-carboxylate (3)

IR (KBr) ν_{max} :1572(Ar,C=C),3322(Ar,C-H), 2990(C-H,s), 1358(C-H,b), 960(C-C,ring), 1325(C-N,s), 1660(C=N,s), 657(C-Br,s) ; ^{1}HNMR (DMSO-d_6)δ: 7.2(Ar-H,m), 2.9(CH_2,m), 6.6(OCH_3,s) ; LC-MS:*m/z* 483.95(M^+)

The Antimicrobial activities of all synthesized compounds were screened by cup plate method. For all six compounds minimum inhibitory concentration was determined using standard Ciprofloxacin. All the compounds showed significant inhibitory activity against the microbes with the 100µg/ml which produces 100% inhibition against the microorganism. The results were tabulated in table 2 were given as zone of inhibition and MIC. Results showed that the compounds were having a very good antimicrobial activity.

Table 2: Antibacterial activity.

COMPOUND	CONCENTRATION (µg/ml)	Gram Negative	
		E. coli	*S. typhi*
A	100	7	9
	200	12	13
	300	14	15
B	100	11	12
	200	12	7
	300	16	13
C	100	10	11

	200	12	14
	300	17	16
1	100	10	11
	200	12	11
	300	13	14
2	100	17	13
	200	18	15
	300	10	18
3	100	10	11
	200	13	16
	300	18	17
Standard (Ciprofloxacin)	100	25	23
Control (DMSO)	-	-	-

The synthesized compounds were screened for their anti-inflammatory activity. The results were tabulated in table 3. All the compounds showed near about good anti-inflammatory activity. Out of all the synthesized compounds B, 2 and 3 showed good anti-inflammatory activity. The SEM values were calculated by one way ANOVA method followed by Dunnet multiple comparison tests using a computer program.

Table3: Anti-inflammatory activity.

Compound No.	Inhibition (%)a		
	3 Hours	**4 Hours**	**Potency**
A	26.29 ± 2.64	27.29 ± 2.70b	0.38
B	59.78 ± 2.58	60.73 ± 2.26b	0.81
C	54.27 ± 2.33	56.59 ± 2.74b	0.69
1	29.27 ± 2.83	30.54 ± 2.89b	0.40
2	66.01 ± 2.92	64.16 ± 2.62b	0.70
3	68.72 ± 1.98	67.60 ± 2.92	0.85
Ibuprofen	79.00 ± 2.54	80.53 ± 2.35	1.00
Control	-	-	-

Dose: 35 mg kg–1 b.m. of the tested compound and standard drug.
a Mean ± SEM (n = 6).
b Significant difference relative to ibuprofen: $p < 0.01$.

CONCLUSION

The research work was oriented towards the finding of novel derivatives of Pyrido-Pyrimidine Carboxylate with enhance anti-microbial and anti-inflammatory activities. The different derivatives were synthesized. The synthesized derivatives showed very good anti-microbial and anti-inflammatory activities against previously reported derivatives of Pyrido-Pyrimidine Carboxylate**.**

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