

SYNTHESIS AND BIOLOGICAL STUDIES OF TRIHYDRO PYRIDO [2, 3-D] PYRIMIDINES 6 –CARBONITRILE

PARTHIV. K. CHAUDHARI

Shri R.R.Lalan College, Chemistry Department, Affiliated with KSKV Kachchh University Bhuj-Kutch-370001, Gujarat, India

ABSTRACT

The synthesis of 5-amino-4-aryl-7-oxo-2-mercato-4,8,4a-trihydro pyrido [2,3-d]pyrimidines-6-carbonitrile (VIIIa-m) have been undertaken by the condensation of 4-amino -5-cyano-6-aryl-2-mercato-5,6-dihydro pyrimidines with ethylcyanoacetate. The constitution of the products has been delineated by elemental analyses IR, PMR and mass spectral data.

Keywords: Trihydro pyrido [2, 3-d] pyrimidines-6-carbonitrile, Multicomponent reaction, cyclocondensation, antibacterial activity, antifungal activity, Therapeutic agents.

INTRODUCTION

Trihydro pyrido [2, 3-d] pyrimidines-6-carbonitrile derivatives form a class of fused heterocyclic compound which have interesting pharmacological and biological activities, particularly the oxo and amino derivatives of pyrido [2, 3-d] pyrimidines stand out for their antitumor^[1] and antiviral^[2] activities. The extensive use of pyrido[2,3-d] pyrimidines in medicine is due to its vast biological activities, antimycobacterial^[3-7], anticancer^[8-9], diuretics^[10-12], anticonvulsant^[13-14], antitumor^[15-16], antiallergic agent^[17], antiphlogistic^[18], CNS depressant^[19], antitussive^[20], coronary vasodilator^[21], antihypertensive^[22], antiarrhythmic agent^[23], immunosuppressing agent^[24], antispasmodic^[25], cardiovascular^[26], antiepileptic^[27], anxiolytic agent^[28], antiasthmatics^[29], antitubercular^[30], anti HIV^[31] activities.

Due to various biodynamic activities of Pyrido [2, 3-d] pyrimidines The products (VIIIa-m) were assayed for their *in vitro* biological assay like antibacterial activity towards positive and Gram negative bacterial strain and antifungal activity towards *Aspergillus Niger* and *Candida albicans* at different concentration for their Minimum

Inhibitory Concentration (MIC) values. The biological activities of the synthesized compounds were compared with standard drugs.

Antimicrobial Activity

Antimicrobial was carried out by using cup-plate method [90]. which has been described as under.

*Antibacterial Activity

Gram positive bacteria were grown in nutrient broth and Gram negative bacteria in Peptone water (PW, 1% bacteriological peptone and 0.5% NaCl) for 24 hours; this gave an optimum growth of the test bacteria. Each purified compound was dissolved in DMF sterilized by filtration by using sintered glass filter and stored at 4°C. Each agent was then added to molten nutrient agar in the following concentration (µg/ml): 0 (control), 25, 50, 100, 200, 500, 800 and poured into sterile Petri dishes. The pH of the media was maintained at 7.2-7.4. The inoculums consisted of an overnight growth broth culture of a bacterium diluted in such a manner that a 2mm (internal diameter) loopful of the culture contain 10⁶ colony-forming units (CFU). These were then spot inoculated on nutrient agar plates containing increasing amount of a compound,

incubated at 37⁰C up to 24 hrs. for determination of the minimum inhibitory concentration (MIC)⁹¹⁻⁹². The antibacterial activity of the compounds (VIII a-n) was compared with known standard reference drugs like Ampicillin, Ciprofloxacin, Chloramphenicol, Griseofulvin, at same concentration. The moderate and comparable antibacterial activities of compound are recorded.

*Antifungal activity

Aspergillus Niger MTCC-282 and *Candida albicans* MTCC-227 were employed for testing fungicidal activity using cup plate method. The culture was maintained on Sabouraud's agar for 72 hrs. This gave on optimum growth of the test fungal spores. Each purified compound was dissolved in DMF sterilized by filtration by using sintered glass filter and stored. Each agent was then added to Sabouraud's agar in the following concentration (μ M/ml) 0(control), 25, 50,100,200,500,800 and poured into sterile Petri dished... The inoculums consisted of an overnight growth broth culture of a bacterium diluted in such a manner that a 2mm (internal diameter) loopful of the culture contain 10⁵ colony-forming units (CFU). These were then spot inoculated on Sabouraud's agar plates containing increasing amount of a compound, incubated at 37⁰C up to 48 hrs. For determination of the minimum inhibitory concentration (MIC)⁹¹⁻⁹². The MIC value of test solutions are recorded in table No 2.

MATERIALS AND METHODS

Melting points were determined routinely in open capillary tube and are uncorrected. The completion of reaction was routinely checked by TLC on silica gel-G plates of 0.5mm thickness and spots were located iodine. Elemental analyses of the newly synthesized compounds was carried out on Carlo Reba 1108 analyzer and are found within the range of theoretical value. IR spectra were recorded on Shimadzu-8400 FT-IR spectrometer in Ker ($\square$ in cm⁻¹). ¹H NMR spectra were recorded in CDCl₃ on a Bruckner DRX-300 at 300 MHz. EI-MS spectra were recorded on Shimadzu GC-MS QP-2010 by Electron Impact method. In all the compounds, the molecular weights were found to be 43 m/z less than the molecular ion peak. No particular fragmentation pattern is observed from the spectra.

General Method for Synthesis of 5-amino-4-aryl-7-oxo-2-mercato-4, 8, 4a-trihydro pyrido [2, 3-d] pyrimidines-6-carbonitrile (VIIIa-m)

A mixture of 4-amino-5-cyano-6-aryl-2-mercato-5, 6-dihydro pyrimidines (0.01M) and ethylcyanoacetate (0.01M) in ethanol and Sodium ethoxide (5 ml) was heated on waterbath for 8 hours. The product was isolated and recrystallized from ethanol: Dioxane (2:1) or suitable solvent. The physical data of compounds (VIIa-m) are recorded in Table No.1

Synthesis of 5-amino-4-(1'-N-phenyl-3'-methyl -5'-chloro-pyrazol-4'-yl)-7-oxo-2-mercapto-4,8-4a-trihydro pyrido [2, 3-d] pyrimidin-6-carbonitrile (VIIIm)

A mixture of 4-amino-5-cyano-6- (5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidine (3.44 gm,0.01M) and ethylcyanoacetate(1.31ml) in ethanol and Sodium ethoxide (5 ml) was heated on waterbath for 8 hours. The product was isolated and recrystallized from ethanol:Dioxane (2:1).Yield:65%, M.P.:138⁰C,Rf:0.71(required :C,52.50%; H,3.40%;N2.41%, For C₁₈H₁₄ON₇SCl ,Found: C,52.49%; H,3.37%,; N,20.38%.

5-amino-4-(1'-N-phenyl-3'-methyl -5'-chloro-pyrazol-4'-yl)-7-oxo-2-mercapto-4,8-4a-trihydro pyrido [2, 3-d] pyrimidin-6-carbonitrile (VIIIm)

IR: 1500 (C=C ring skeletal vib. Of pyrimidine),1400 (C=N ring skeletal vib. pyrimidine), 3036(C-H str.asym.), 1101 (C-H i.p.),2925(C-H asym.), 1450(C-H def.asym.), 2843 (C-H str. sym.) 3400(N-H str.), 1595 (N-H def.),1315 (C-N str.), 1622 (C=N str.of pyrazol), 1595 (N-N def.of pyrazol), 1101(C-N str.of Pyrazol), 2206(C=N str.of Pyridine), 1672 (C=O str.),1610 (Pyridine str.)

¹ H -NMR (DMSO+ CDCl₃, BRUKER spectrometer (300 MHz, δ ppm): 2.41(3H,-CH₃), 5.73 (1H, -CH), 7.31-7.83(8H, Ar-H+NH₂+SH), 8.32 (1H, -NH)

MASS spectra: The mass spectrum fragmentation shows molecular ion (M⁺) peak at m/z=523.5 was consistent with molecular formula C₁₈H₁₄ON₇SCl

5-amino-4-(4 - Chlorophenyl)-7-oxo-2-mercapto-4, 8-4a-trihydro pyrido [2, 3-d] pyrimidin-6-carbonitrile (VIIIc)

IR: 1506 (C=C ring skeletal vib. Of pyrimidine), 1454 (C=N ring skeletal vib. pyrimidine), 2929(C-H str.asym.), 2839 (C-H sym.-CH₃), 1409(C-H sym.), 1154(C-H i.p.def.), 3417(N-H str.), 3375 (N-H str.), 1598 (N-H def.), 1290 (C-N str.), 1610(C=C str.of Pyridine) 761 (C-Cl str.)

¹ **H –NMR** (DMSO+ CDCl₃, BRUKER spectrometer (300 MHz, δ ppm): 5.13 (1H, -CH), 7.16-7.63 (6H, Ar-H+CH+NH+SH), 7.96-8.02(2H,-NH₂).

MASS spectra: The mass spectrum fragmentation shows molecular ion (M⁺) peak at m/z=331.5 was consistent with molecular formula C₁₄H₁₀ON₅SCl

5-amino-4-(4-methoxyphenyl)-7-oxo-2-mercapto-4, 8-4a-trihydro pyrido [2, 3-d] pyrimidin-6-carbonitrile (VIIIg)

IR: 1500 (C=C ring skeletal vib. Of pyrimidine), 1390 (C=N ring skeletal vib. pyrimidine), 3030(C-H str.), 1112 (C-H i.p.def.), 850(C-

H o.o.p.def.), 2925(C-H asym. alkane), 2840(C-H str.,sym.,-CH₃),1413 (C-H asm.),3415(N-H str.), 1591 (N-H def.) ,1168 (C-N str. Of 1618(C=C str.of Pyridine), 1660(C=O str.), 761 (C-Cl str.)

¹ **H –NMR** (DMSO+ CDCl₃, δ ppm): 5.73 (1H, -CH), 3.88 (3H, -OCH₃), 6.73-8.92(7H, Ar-H+CH+NH+SH).

MASS spectra: The mass spectrum fragmentation shows molecular ion (M⁺) peak at m/z=327.0 was consistent with molecular formula C₁₅H₁₃O₂N₅S

RESULT AND DISCUSSION

It was interesting to note that the reaction occurred immediately. This work demonstrates a very simple and efficient method for the synthesis of a well functionalized pyrido [2, 3-d] pyrimidines of biological importance in excellent yields.

Table – I: Physical and analytical data

Sr. No.	R	M.W.	Molecular Formula	M.P. °C	Yield (%)	R _f Value	% of Nitrogen	
							Required	Found
VIII a	C ₆ H ₅	297.0	C ₁₄ H ₁₁ ON ₅ S	167	60	0.58	23.59	23.54
VIII b	2-Cl-C ₆ H ₄	331.5	C ₁₄ H ₁₀ ON ₅ S Cl	159	66	0.55	21.11	21.05
VIII c	4-Cl-C ₆ H ₄	331.5	C ₁₄ H ₁₀ ON ₅ SCl	148	58	0.49	21.11	21.06
VIII d	3-NO ₂ -C ₆ H ₄	359.0	C ₁₄ H ₁₀ ON ₅ S Br	192	68	0.53	19.49	19.42
VIII e	2-NO ₂ -C ₆ H ₄	342.0	C ₁₄ H ₁₀ O ₃ N ₆ S	168	63	0.63	24.56	24.50
VIII f	3-C ₆ H ₄ -O-C ₆ H ₄	389.0	C ₂₀ H ₁₅ O ₂ N ₅ S	178	55	0.59	17.99	17.93
VIII g	3-OCH ₃ -C ₆ H ₄	327.0	C ₁₅ H ₁₃ O ₂ N ₅ S	155	61	0.65	21.40	21.35
VIII h	2-OH-C ₆ H ₄	313.0	C ₁₄ H ₁₁ O ₂ N ₅ S	130	69	0.63	22.36	22.30
VIII i	4-OH-C ₆ H ₅	313.0	C ₁₄ H ₁₁ O ₂ N ₅ S	167	52	0.48	22.36	22.29
VIII j	C ₆ H ₄ CH=CH ₂	323.0	C ₁₆ H ₁₃ ON ₅ S	198	68	0.54	21.67	21.62
VIII k	4-SCH ₃ -C ₆ H ₄	343.0	C ₁₅ H ₁₃ ON ₅ S ₂	136	47	0.63	20.40	20.35
VIII l	C ₄ H ₃ O	287.0	C ₁₂ H ₉ O ₂ N ₅ S	280	69	0.61	24.39	24.35
VIII m	1-N-C ₆ H ₅ -3-CH ₃ -5-Cl-C ₃ H ₂	411.5	C ₁₈ H ₁₄ ON ₇ SCl	138	56	0.71	20.41	20.38

TLC Solvent systems: Acetone: Benzene= 1:9

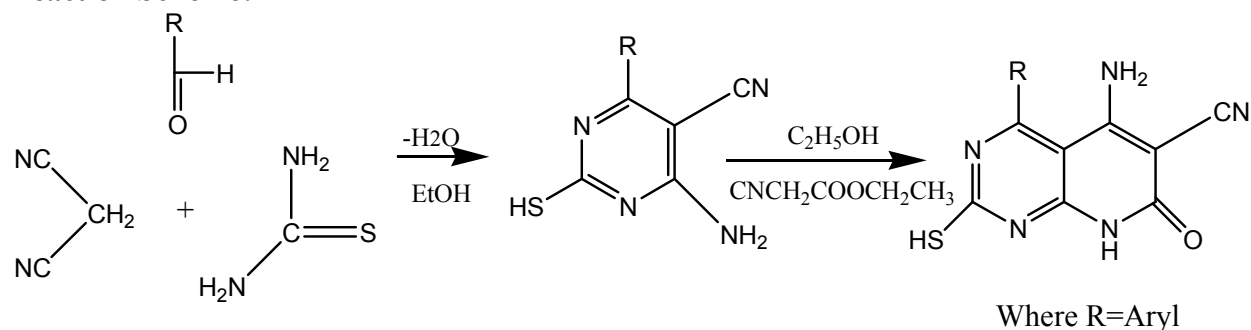
Table-II: 5-amino-4-aryl-7-oxo-2-mercato-4, 8,4a-trihydro pyrido [2, 3-d] pyrimidines-6-carbonitrile (VIIIa-m)

Compound	R	Antibacterial activity				Antifungal activity	
		S.pyogens MTCC- 442	S.aureus MTCC- 96	E.Coli MTCC- 443	B.subtillis MTCC- 441	C.alibicans MTCC- 227	A.niger MTCC- 282
VIII a	C ₆ H ₅	100	100	200	200	50	100
VIII b	2-Cl-C ₆ H ₄	100	500	25	25	25	200
VIII c	4-Cl-C ₆ H ₄	25	25	500	200	25	100
VIII d	3-NO ₂ -C ₆ H ₄	500	-	25	-	500	500
VIII e	2-NO ₂ -C ₆ H ₄	25	50	500	100	200	200
VIII f	3-C ₆ H ₄ -O-C ₆ H ₄	-	-	100	500	800	25
VIII g	3-OCH ₃ -C ₆ H ₄	100	25	100	25	-	-
VIII h	2-OH-C ₆ H ₄	25	100	500	150	-	-
VIII i	4-OH-C ₆ H ₅	100	-	800	800	-	-
VIII j	C ₆ H ₄ CH=CH ₂	-	-	800	500	500	500
VIII k	4-SCH ₃ -C ₆ H ₄	500	500	800	-	500	800
VIII l	C ₄ H ₃ O	500	-	-	-	-	100
VIII m	1-N-C ₆ H ₅ -3-CH ₃ -5-Cl-C ₃ H ₂	50	50	-	800	500	-

Comparative activity of (III a-n) with known chosen standard drugs

Standard Drug	Antibacterial activity				Antifungal activity	
	S.pyogens MTCC-442	S.aureus MTCC-96	E.Coli MTCC-443	B.subtillis MTCC-441	C.alibicans MTCC-227	A.niger MTCC- 282
	VIIIc (25), VIIIe(25)	VIIIc (25), VIIIe(50)	VIIIb (50)	VIIIb (50)	VIIIa (50)	VIIIf (25)
	VIIIh (25), VIIIIm(50)	VIIIg (25), VIIIIm (50)	VIIIId (25)	VIIIg (25)	VIIIc(25)	
Ampicillin	30	20	30	30	-	-
Amoxycillin	20	20	20	20	-	-
Cifalexin	20	30	30	20	-	-
Erythromycin	30	30	20	20	-	-
Chlotrimazole	-	-	-	-	20	20
Griseofulvin	-	-	-	-	30	20

N.B. :(-): No activity

Reaction Scheme:-**ACKNOWLEDGEMENT**

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