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Research Paper :

Synthesis and biological studies of trihydro pyrido [2, 3-d] pyrimidines 6 -carbonitrile

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ABSTRACT

Complexes of the type [ML_2] and [MLL'.H_2O] [where, M = Cu(II), Ni(II), Co(II), Zn(II) and Mn(II); HL = N-(2-thiazole)-2'-carboxylic acid; and L' = oxine] have been synthesized. The complexes are octahedral in nature. The synthesized ligand behaves as tridendate OON donor. The characterization of the complexes has been done on the basis of analytical, molar conductance, magnetic susceptibility, molecular weight, infrared and electronic spectral data. Antibacterial activity of these ligands and their metal complexes has been determined on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria at 37^{0}C and antifungal activity has been determined on common fungi *viz*., *Aspergillus niger*, *Aspergillus nidulense* and *Candida albicans* at 28^{0}C. It has been found that the biocidal activity of these ligands increases on being coordinated with suitable metal ion.

KEY WORDS : Trihydro pyrido [2, 3-d] pyrimidines-6-carbonitrile,Multicomponent reaction, Cyclocondensation, Antibacterial activity, Antifungal activity, Therapeutic agents

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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 standout 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], antiarrythmic agent[23], immunosuppressing agent[24], antispasmodic[25], cardiovascular[26], antiepileptic [27], anxiolytic agent[28], antiaththaminitics[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 per cent bacteriological peptone and 0.5 per cent 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^{0}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 dished. 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 10^0 colony-forming units (CFU). These were then spot inoculated on nutrient agar plates containing increasing amount of a compound, incubated at 37^{0}C 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, Chloramphenical, 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 an 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 (µM/ml) 0(control), 25, 50,100,200,500,800 and poured into sterile Petri dish. 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 10^5 colony-forming units (CFU). These were then spot inoculated on Sabouraud's agar plates containing increasing amount of a compound, incubated at 37^0C up to 48 hrs. For determination of the minimum inhibitory concentration (MIC) [91-92].Th MIC value of test solutions are recorded in Table 2.

EXPERIMENTAL METHODOLOGY

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 (? in cm^{-1}). ^{1}H NMR spectra were recorded in $CDCl_3$ 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 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 per cent , M.P. :138^{0C}, Rf:0.71 (required :C,52.50%; H,3.40%; N2.41%, For $C_{18}H_{14}ON_7SCl$, Found: C,52.49%; H,3.37%, N,20.38%.

Table 1 : Physical and analytical data

Sr. No.	R	M.W.	Molecular formula	M.P. ^{O}C	Yield (%)	R_f value	% of nitrogen	
							Required	Found
VIII a	C_6H_5	297.0	$C_{14}H_{11}ON_5S$	167	60	0.58	23.59	23.54
VIII b	2-Cl-C_6H_4	331.5	$C_{14}H_{10}ON_5S$ Cl	159	66	0.55	21.11	21.05
VIII c	4-Cl-C_6H_4	331.5	$C_{14}H_{10}ON_5SCl$	148	58	0.49	21.11	21.06
VIII d	3-NO_2-C_6H_4	359.0	$C_{14}H_{10}ON_5S$ Br	192	68	0.53	19.49	19.42
VIII e	2-NO_2-C_6H_4	342.0	$C_{14}H_{10}O_3N_6S$	168	63	0.63	24.56	24.50
VIII f	3- C_6H_4-O-C_6H_4	389.0	$C_{20}H_{15}O_2N_5S$	178	55	0.59	17.99	17.93
VIII g	3-OCH_3-C_6H_4	327.0	$C_{15}H_{13}O_2N_5S$	155	61	0.65	21.40	21.35
VIII h	2-OH-C_6H_4	313.0	$C_{14}H_{11}O_2N_5S$	130	69	0.63	22.36	22.30
VIII i	4-OH-C_6H_5	313.0	$C_{14}H_{11}O_2N_5S$	167	52	0.48	22.36	22.29
VIII j	C_6H_4 CH=CH_2	323.0	$C_{16}H_{13}ON_5S$	198	68	0.54	21.67	21.62
VIII k	4-SCH_3-C_6H_4	343.0	$C_{15}H_{13}ON_5S_2$	136	47	0.63	20.40	20.35
VIII l	C_4H_3O	287.0	$C_{12}H_9O_2N_5S$	280	69	0.61	24.39	24.35
VIII m	1-N-C_6H_5-3-CH_3-5-Cl-C_3H_2	411.5	$C_{18}H_{14}ON_7SCl$	138	56	0.71	20.41	20.38

TLC Solvent systems: Acetone: Benzene= 1:9

Table 2 : 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_6H_5	100	100	200	200	50	100
VIII b	2-Cl-C_6H_4	100	500	25	25	25	200
VIII c	4-Cl-C_6H_4	25	25	500	200	25	100
VIII d	3-NO_2-C_6H_4	500	-	25	-	500	500
VIII e	2-NO_2-C_6H_4	25	50	500	100	200	200
VIII f	3- C_6H_4-O-C_6H_4	-	-	100	500	800	25
VIII g	3-OCH_3-C_6H_4	100	25	100	25	-	-
VIII h	2-OH-C_6H_4	25	100	500	150	-	-
VIII i	4-OH-C_6H_5	100	-	800	800	-	-
VIII j	C_6H_4 CH=CH_2	-	-	800	500	500	500
VIII k	4-SCH_3-C_6H_4	500	500	800	-	500	800
VIII l	C_4H_3O	500	-	-	-	-	100
VIII m	1-N-C_6H_5-3-CH_3-5-Cl-C_3H_2	50	50	-	800	500	-
Comparative activity of (III a-n) with known choosen 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),VIIIm(50)	VIIIg (25),VIIIm(50)	VIIId (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

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,-CH_3), 5.73 (1H, -CH), 7.31-7.83(8H, Ar-H+NH_2+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 $C_{18}H_{14}ON_7SCl$

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.-CH_3), 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,-NH_2).

MASS spectra: The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=331.5 was consistent with molecular formula $C_{14}H_{10}ON_5SCl$

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) ,

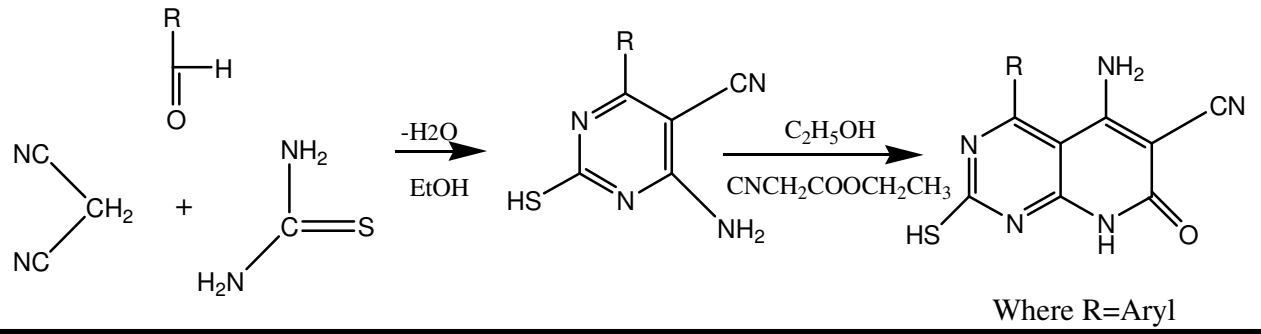

Fig. 1 : Reaction scheme

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.,-CH_3),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-H), 3.88 (3H, -OCH_3), 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 $C_{15}H_{13}O_2N_5S$

EXPERIMENTAL FINDINGS AND ANALYSIS

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.

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