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Synthesis and Biological Studies of Pyrazolo [3, 4-d] Pyrimidines

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ABSTRACT

An novel series of the pyrazolo [3,4-d] pyrimidines (IIIa-n) were prepared via one pot reaction of 4-amino-5-cyano-6-aryl-2- mercapto-5,6-dihydro pyrimidines(I) with hydrazine hydrate(II) in ethanol. Elemental analysis, IR, 1NMRand mass spectral data established identification of the compounds (IIIa-n) was evaluated for their antimicrobial activity. Some of the obtained compounds showed the interesting antimicrobial activity comparable to standard drugs like amplicillin, chloramphenicol, amoxicillin, ciprofloxacin, norfloxacin and griseofluvin.

Key words: pyrazolo [3, 4-d] pyrimidines, Antimicrobial activity and Antituberculosis activity, Antimycobacterial activity.

INTRODUCTION

pyrazolo [3,4-d] pyrimidines are of considerable chemical and pharmaceutical importance as purines analogs[1-3] of naturally occurring fused uracils that possess diverse biological activities[4]. These derivatives[5-8] were found to be selective ligands with antagonist activity for A_1 adenosine receptors (A_1AR). They may have therapeutically use as cognitive enhancers , antidementia drugs (e.g. for Alzheimer's disease and cerebrovascular dementia),psycostimulants, antidepressant drugs and ameliorants of cerebral fuction[9], Further more, a large number of pyrimidine derivatives are reported to exhibit antimycobacterial[10], antitumor[11], antiviral[12], anticancer[13], antiinflammatory[14], analgesic[15], antifolate[16], antimicrobial[17,] antifungal[18], antiproliferative[19] and antihistaminic[20] activities.

Due to various biodynamic activities of pyrazolo [3,4-d] pyrimidines ,one pot synthesis of 3-Amino-4-Aryl-6-Mercapto-3a,4-Dihydro-1H-Pyrazolo[3,4-d] Pyrimidines[IIIa-n] have been undertaken by the condensation of 4-amino-5-cyano-6-aryl-2- mercapto-5,6-dihydro pyrimidines with hydrazine hydrate in ethanol. The product (IIIa-n) were assayed for their in vitro biological assay like antibacterial activity towards Gram positive and Gram negative bacterial strain and antifungal activity towards *Aspergillus niger* and *Candida albicans* at different concentration for their MIC values. The biological activities of the synthesized compounds were compared with standard drugs [Table 2]. The physical constant, antimicrobial and antimicobacterial activities of compounds (IIIa-n) recorded in Table 1, 2 and 3 respectively.

MATERIAL 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 by 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 (Δ in cm^{-1}). ^1H 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 3-Amino-4-Aryl-6-Mercapto-3a, 4-Dihydro-1H-Pyrazolo [3, 4-d] Pyrimidines (III a-n)

Amixture of 4-amino-5-cyano-6-aryl-2-mercapto-5, 6-dihydro pyrimidines (0.01mole) with hydrazine hydrate (0.01mole) in ethanol (30ml) under reflux for a specific period.The reaction mixture was kept at room temperature for 3 hrs. The product was isolated and crystallized from a suitable solvent to give the desire product (IIIa-n).

3-Amino-4-(1'-N-Phenyl-3'-Methyl-5'-Chloro-Pyrazol-4'-YL)-6-Mercapto-3a,4-Dihydro-1H-Pyrazolo[3,4-d] Pyrimidines(IIIn)

IR: 3042(C-H) str.Aromatic), 1506 (C=C ring skeletal vib. Of pyrimidine) ,1454 (C=N ring skeletal vib. pyrimidine), 2929(C-H str.asym.), 2839 (C-H sym.), 1409(C-H sym.), 1454(C-H def.asym.), 3417(N-H str.), 3375 (N-H str.), 1598 (N-H def.) ,1290 (C-N str.), 1598 (C=N str.of pyrazol), 1572 (N-N def.of pyrazol), 1109(C-N str.of Pyrazol), 813 (C-Cl str.)

^1H –NMR (DMSO+ $CDCl_3$, δ ppm): 2.41(3H,-CH_3), 5.73 (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=360 was consistent with molecular formula $C_{15}H_{14}N_7SCl$

3-amino-3a, 4-dihydro-4-phenyl-1H-pyrazolo [3, 4-d] pyrimidine-6-thiol(III-a)

IR: 3004(C-H) str.Aromatic), 1504 (C=C

Table 1: Physical and analytical data

S. No.	R	X	Molecular Formula	m.p. °C	Yield (%)	R_f Value	% of Nitrogen Calcd.	% of Nitrogen Found
III a	C_6H_5	S	$C_{11}H_{11}N_5S$	140	61	0.62	28.57	28.52
III b	2-Cl-C_6H_4	S	$C_{11}H_{10}N_5SCl$	144	63	0.58	25.04	25.00
III c	4-Cl-C_6H_4	S	$C_{11}H_{10}N_5SCl$	158	48	0.49	25.04	24.99
III d	3-Br-C_6H_4	S	$C_{11}H_{10}N_5SBr$	165	51	0.51	21.67	21.61
III e	3-NO_2-C_6H_4	S	$C_{11}H_{10}N_6O_2S$	175	46	0.53	27.63	27.57
III f	3-C_6H_5-O-C_6H_4	S	$C_{17}H_{15}N_5OS$	138	54	0.50	20.77	20.71
III g	4-OCH_3-C_6H_4	S	$C_{12}H_{13}N_5OS$	147	50	0.63	25.45	25.40
III h	2-OH-C_6H_4	S	$C_{11}H_{11}N_5OS$	172	67	0.64	26.81	26.76
III i	4-OH-C_6H_5	S	$C_{11}H_{11}N_5SO$	230	52	0.52	26.81	26.8
III j	C_6H_4-CH=CH	S	$C_{13}H_{13}N_5S$	151	56	0.55	25.83	25.80
III k	4-CH_3S-C_6H_4	S	$C_{12}H_{13}N_5S_2$	168	48	0.57	24.05	23.97
III l	á- C_4H_3O	S	$C_9H_9N_5OS$	250	68	0.58	29.78	29.73
III m	4-N-$(CH_3)_2$-C_6H_4	S	$C_{13}H_{16}N_6S$	180	74	0.61	29.16	29.11
III n	1-N-C_6H_5-3-CH_3-5Cl-C_3N_4	S	$C_{15}H_{14}N_7SCl$	128	68	0.62	27.26	27.20

TLC Solvent systems: Acetone: Benzene= 1:9

ring skeletal vib. Of pyrimidine) ,1456(C=N ring skeletal vib. pyrimidine), 2925(C-H str.asym.), 2837 (C-H sym.), 1365(C-H sym.), 3471(N-H str.), 3135 (N-H str.), 1581 (N-H def.) ,1355 (C-N str.), 1651 (C=N str.of pyrazol), 1620 (N-N def.of pyrazol), 1165(C-N str.of Pyrazol).

^{1}H –NMR (DMSO+ $CDCl_3$, δ ppm): 5.62 (1H, -CH), 6.74-7.93(9H, Ar-H+CH+NH_2+SH).

MASS spectra: The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=245.0 was consistent with molecular formula $C_{11}H_{11}N_5S$

Table 2: Antimicrobial Activity of 3-Amino-4-Aryl-6-Mercapto-3a, 4-Dihydro-1H-Pyrazolo [3, 4-d] Pyrimidines (III a-n)

Compound	R	Antibacterial activity				Antifungal activity	
		S.pyogens **MTCC-** 442	***S.aureus*** **MTCC-96**	***E.coli*** **MTCC-** 443	***B.subtillis*** **MTCC-** 441	***C.albicans*** **MTCC-** 227	***A.niger*** **MTCC-** 282
III a	C_6H_5	200	200	500	800	-	200
III b	2-Cl-C_6H_4	25	100	200	500	500	800
III c	4-Cl-C_6H_4	-	50	800	-	100	50
III d	3-Br-C_6H_4	800	100	25	200	200	500
III e	3-NO_2-C_6H_4	50	50	100	-	50	-
III f	3-C_6H_5-O-C_6H_4	200	200	800	100	800	200
III g	4-OCH_3-C_6H_4	25	25	-	25	-	100
III h	2-OH-C_6H_4	-	500	50	50	200	800
III i	4-OH-C_6H_5	800	100	200	200	200	-
III j	C_6H_4-CH=CH	100	-	-	800	800	500
III k	4-CH_3S-C_6H_4	500	500	100	200	-	-
III l	á- C_4H_3O	100	200	200	500	500	800
III m	4-N-$(CH_3)_2$-C_6H_4	200	200	100	200	800	-
III n	1-N-C_6H_5-3-CH_3-5Cl-C_3N_4	100	200	25	500	500	-

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

Standard Drug	Antibacterial activity				Antifungal activity	
	S.pyogens **MTCC-442** **IIIb(25), IIIe(50) IIIg(25)**	***S.aureus*** **MTCC-96** **IIIc(50), IIIe(50) IIIg(25)**	***E.coli*** **MTCC-443** **IIId(25) IIIh(50)**	***B.subtillis*** **MTCC-441** **IIIg(25) IIIh(50)**	***C.albicans*** **MTCC-227** **IIIe(50)** **-**	***A.niger*** **MTCC-282** **IIIc(50)** **-**
Ampicillin	30	20	30	30	-	-
Amoxycillin	20	20	20	20	-	-
Cifalexin	20	30	35	20	-	-
Erythromycin	30	35	20	20	-	-
Chlotrimazole	-	-	-	-	20	20
Griseofulvin	-	-	-	-	30	20

N.B. :(-): No activity

3-amino-3a,4-dihydro-4-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine-6-thiol(III-j)

IR: 3030(C-H) str.Aromatic), 1512 (C=C ring skeletal vib. Of pyrimidine) ,1456(C=N ring skeletal vib. pyrimidine), 2920(C-H str.asym.), 2852 (C-H sym.), 1383(C-H sym.), 3435(N-H str.), 3375 (N-H str.), 1311 (N-H def.) ,1178 (C-N str.), 1662 (C=N str.of pyrazol), 1583(N-N def.of pyrazol), 1178(C-N str.of Pyrazol).

^{1}H-NMR (DMSO+ $CDCl_3$, δ ppm): 2.47(3H, SCH_3), 5.95 (1H, -CH), 6.71-8.70(9H, Ar-H+CH+NH_2+SH)

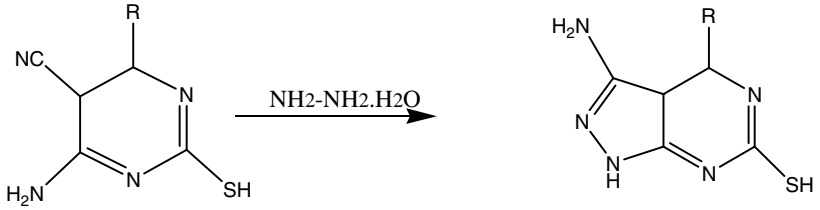
MASS spectra: The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=271.0 was consistent with molecular formula $C_{13}H_{13}N_5S$.

Antimicrobial Activity

Antimicrobial was carried out by using cup-plate method 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



Where R=Aryl

Scheme

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), 25,50,100,200,500,800and 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 unit (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 (III 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 cultures were maintained on Sabouraud's agar for 72 hours 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 at 4^{0}C. Each agent was then added to Sabouraud's agar in the following concentration(µg/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 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^{0}C 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.

CONCLUSION

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 pyrazolo [3, 4-d] pyrimidines of biological importance in excellent yields.

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