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Synthesis and biological evaluation of 4,5-dihydro-4-(4,5-dihydro-1-aryl -3-methyl -1h-pyrazol-4-yl)-1-phenyl-3-methyl-1h-pyrazolo[3,4-d]pyrimidine-6-thiol

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Abstract

The synthesis of 4,5-dihydro-4-(4,5-dihydro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-aryl-3-methyl-1H-pyrazolo[3,4-d]pyrimidine-6-thiol (I a-h) have been undertaken by the condensation of 1-Aryl 3-methyl-1H-pyrazol-5(4H)-one with thiourea and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde. The constitution of the products (I a-h) have been characterized by using elemental analysis IR, 1 NMR and mass spectral data. The products (a-h) were assayed for their in vitro biological assay like antibacterial activity towards gram positive and Gram negative bacterial strain and antifungal activity towards Aspergillus nigor MICC-282 and Candida albicans MTCC-227 at a concentration of 40 µg/ml.

Key words: pyrazolo [3, 4-d] pyrimidines, antibacterial activity, antifungal activity antimicrobial activity and antituberculosis activity antimycobacterial activity.

Introduction

Pyrazolopyrimidinone derivatives have attracted the attention of numerous researchers over many years due to their important biological activities[1–4]. Structural analogs of pyrazolo [3, 4-d]-pyrimidines have displayed good activities as inhibitors of cyclin-dependent kinase[5] and PI3kinase-A[6], anticancer and radioprotective activity[7], antimicrobial[8] and other biology activity[9]. The importance of pyrazolo [3, 4-*d*] pyrimidines had resulted in the development of several synthetic methods for their construction[10-12].

Further more, a large number of pyrimidine derivatives are reported to exhibit antimycobacterial[13], antitumor[14], antiviral[15], anticancer[16], antiinflammatory[17], analgesic[18], antifolate[19], antimicrobial[20], antifungal[21], antiproliferative[22] and antihistaminic[23] activities.

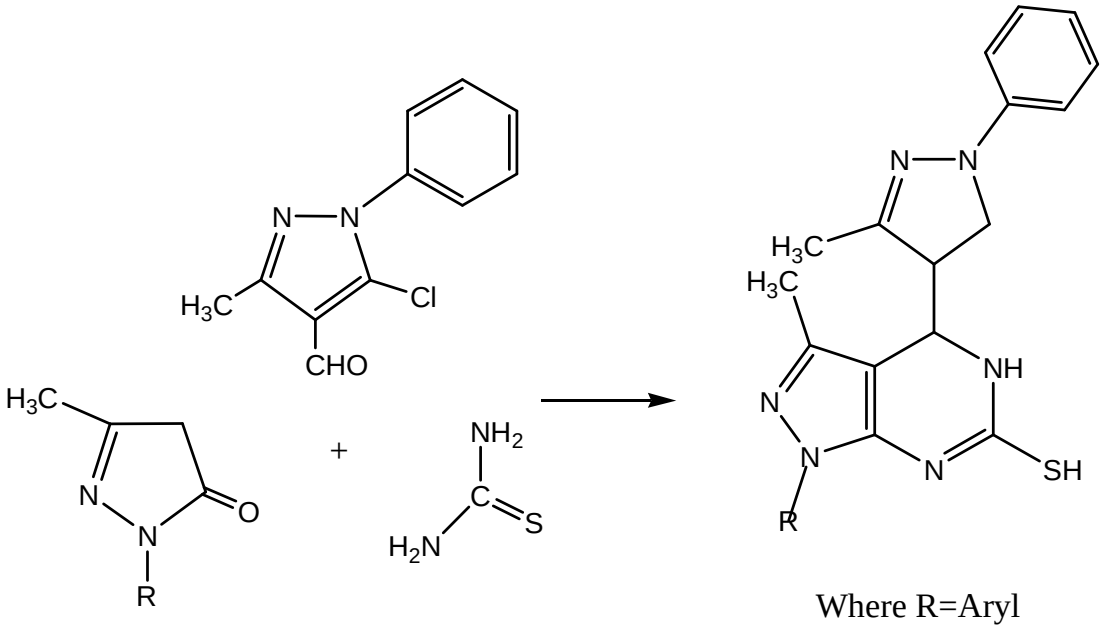
Materials and Methods

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 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 (□ 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 4, 5-dihydro-4-(4, 5-dihydro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-aryl-3-methyl-1H-pyrazolo [3, 4-d] pyrimidine-6-thiol (I a-h):

A mixture of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (0.01M), 1-Aryl-3-methyl-1H-pyrazol-5(4H)-one (0.01M) and thiourea (0.01M) 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 (I a-h).

Reaction scheme :



Where R=Aryl

Table 1. Physical and analytical data

Sr. No.	R	Molecular Formula	Molecular Weight	m.p. ^{O}C	Yield (%)	R_f Value	% of Nitrogen	
							Calcd.	Found
I-a	C_6H_5	$C_{22}H_{19}N_6SCL$	434.5	142	67	0.49	19.33	19.29
I-b	2-Cl-C_6H_4	$C_{22}H_{18}N_6SCl_2$	469.0	172	64	0.56	17.91	17.89
I-c	3-Cl-C_6H_4	$C_{22}H_{19}N_6SCl_2$	469.0	160	69	0.54	17.91	17.88
I-d	4-CH_3-C_6H_4	$C_{23}H_{21}N_6SCl$	448.5	144	70	0.58	18.73	18.70
I-e	3-SO_3-C_6H_4	$C_{22}H_{19}N_6O_3S_2Cl$	514.5	198	54	0.51	16.33	16.26
I-f	4- SO_3-C_6H_4	$C_{22}H_{19}N_6O_3S_2Cl$	514.4	>280	63	0.60	16.33	16.27
I-g	2-chloro-5- SO_3-C_6H_4	$C_{22}H_{18}N_6O_3S_2Cl_2$	549.0	202	58	0.46	15.30	15.25
I-h	2,5-Dichloro-5-SO_3-C_6H_4	$C_{22}H_{17}N_6O_3S_2Cl_2$	583.5	248	56	0.51	14.40	14.35

TLC Solvent systems: Acetone: Benzene= 4:6

Synthesis of 4, 5-dihydro-4-(4, 5-dihydro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidine-6-thiol (I-a):

IR: 2950 str. (C-H asym.), 2875 str. (C-H sym.),1465 def (C-H. asym.), 1520(C=C ring skeletal vib. Of pyrimidine),1430(C=N ring skeletal vib. pyrimidine),3044 str. (C-H sym.), 1190 (C-H i.p.def.), 690 (C-H o.o.p.def.),3390 (N-H str. 2^0 amine),1606 (N-H def.),1320 (C-N str.),1560 (C=N str.of pyrazol),1628 (N-N def.of pyrazol),1170(C-N str.of Pyrazol),765 (C-Cl str.),1106(C-S-C str. Of Thiol),2635 (S-H str.of Thiol)

^{1}*H–NMR:*(DMSO+ CDCl3, BRUKER spectrometer (300 MHz, δ ppm):1.845(3H,-CH_3), 2.123 (3H,-CH_3), 4.955(1H, -CH), 6.536-8.5987 (11H, Ar-H+NH+SH).

MASS spectra: The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=434.5 was consistent with molecular formula $C_{22}H_{19}N_6SCl$

Synthesis of 4, 5-dihydro-4-(4, 5-dihydro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-methyl-1-phenyl-1H-pyrazolo [3, 4-d] pyrimidine-6-thiol (I-d) :

IR: 2920 str. (C-H asym.), 2858 str. (C-H sym.),1455 def (C-H. asym.),1365 def (C-H. sym.),1512(C=C ring skeletal vib. Of pyrimidine),1404(C=N ring skeletal vib. pyrimidine), 3040 str. (C-H sym.),1170 (C-H i.p.def.), 692 (C-H o.o.p.def.), 3400 (N-H str. 2^0 amine),1596 (N-H def.),1323 (C-N str.), 1550 (C=N str.of pyrazol),1620 (N-N def.of pyrazol),1130(C-N str.of Pyrazol),761 (C-Cl str.),1101(C-S-C str. Of Thiol),2630 (S-H str.of Thiol)

^{1}*H–NMR*: (DMSO+CDCl3, BRUKER spectrometer (300 MHz, δ ppm):1.832(3H,-CH_3),2.032(3H,-CH_3),2.275(3H,-CH_3), 4.949(1H, -CH),6.526-8.595(11H, Ar-H+ NH+SH)

MASS spectra: The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=448.5was consistent with molecular formula $C_{23}H_{21}N_6SCl$

Antimicrobial activity of 4, 5-dihydro-4-(4, 5-dihydro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-aryl-3-methyl-1H-pyrazolo [3, 4-d] pyrimidine-6-thiol (I a-h) :

Antimicrobial activity was carried out by using the cup-plate method, which has been described as under.

*****Antibacterial activity:*

The purified products were screened for their antibecterial activity. The nutrient agar bath prepared by the usual method was inoculated specially with 0.5 ml for 24 hours. Old subculture os ***Streptococcus pyogens MTCC-442 ,Staphylococcus aureus supsp. Auresus MTCC-96, Bacillus subtillis MTCC-441, Escherichia coli MTCC-443*** in separate conical flask at 40^0-50^0C and mix well by gentle shaking. About 25 ml of the contents of the flask were poured and evently spread in a petridish (13 mm in diameter) and allowed to set for two hours. The cups (10mm in diameter) were formed by the help of a sample in DMF. The plates were incubated at 37^0C for24 hrs and the control was also maintained with 0.04 ml of DMF in similar manner anf the zones of inhibition of the bacterial growth are mesuredinmm and are compared with known choosen standard drugs at the same concentration.The name of the drugs used are Ampicillin, Chloramphenicol, Amoxycillin, Ciprofloxacin, Nofloxacin, Grieseofulvin. The moderate and comparable antibacterial activity of compounds (Ia-h) are reported in Table 2 and table 3.

*****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 slants. Purified compounds were used for their antifungal activity. Sterilized Sabouraud's agar medium was inoculated with 72 hours old, 0.5 ml suspension of fungal spores, in a separate flask. About 25 ml of the inoculated medium was evenly spreader in a sterilized petridish and allow setting for two hours. The cups (10mm in diameter) were punched in Petridis and loaded with 0.04 ml (40µg/ml) of the solution of a DMF. The plates were incubated at room temperature (30^0C) for78 hours. After the completion of inoculate period, the zones of inhibition of growth of compounds (Ia-h) in the form of diameter in mm was measured. Along the test solution in each petridish, one cup was filled up with solvent which acts as control. The antifungal activity of compounds (I a-h) is compared with known standard drugs. The name of the drugs Grisefulvin. The moderate and comparable antifungal activity is reported in Table 2 and Table 3.

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.alibicans MTCC-227	A.niger MTCC-282
I-A	C_6H_5	12	11	17	14	23	16
I-b	2-Cl-C_6H_4	17	16	19	16	17	18
I-c	3-Cl-C_6H_4	16	19	18	17	19	17
I-d	4-CH_3-C_6H_4	15	15	22	24	17	15
I-e	3-SO_3-C_6H_4	14	14	20	18	20	14
I-f	4- SO_3-C_6H_4	13	19	18	22	18	13
I-g	2-chloro-5-SO_3-C_6H_4	18	16	24	16	19	21
I-h	2,5-Dichloro-5-SO_3-C_6H_4	18	18	19	18	20	18

Table 3. Comparable Activity of compounds (I a-h) with known chosen standard drugs

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
	I b(17)	I c(19)	I d(22)	I d(24)	-	-
	I c(16)	I f(19)	I g(24)	I f(22)	-	-
	I g (18)	I h(18)				
	I h (18)					
Ampicillin	16	17	23	19	-	-
Chloramphenicol	19	22	23	25	-	-
Amoxycillin	17	20	21	25	-	-
Ciprofloxacin	21	22	28	22	-	-
Norfloxacin	20	25	26	23	-	-
Griseofulvin	-	-	-	-	25	22

N.B. :(-): No activity

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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