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Synthesis and biological evaluation of 4-aryl-3-methyl-1-phenyl-4, 4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-a]pyrimidine-5-ones

A.R.Trivedi, N.R.Ravat, V.H.Shah*

Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Rajkot-360005, (INDIA)

E-mail: shah_v_h@yahoo.com

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ABSTRACT

A novel series of the pyrazolo[3,4-d]pyrimidines fused with thiazolidino neswere prepared by the cyclocondensation of 1-phenyl-3-methyl-4-aryl-6-mercepto-4,5-dihydropyrazolo[3,4-d]pyrimidines with chloraceticacid. Elemental analysis, IR, ^{1}H-NMR, and mass spectral data established identification of the compounds. Products were evaluated for their antimicrobial and antituberculosis activity. Some of the obtained compounds showed the interesting antimicrobial activity comparable to standard drugs like ampicillin, chloramphenicol, amoxycillin, ciprofloxacin, norfloxacin and griseofulvin.

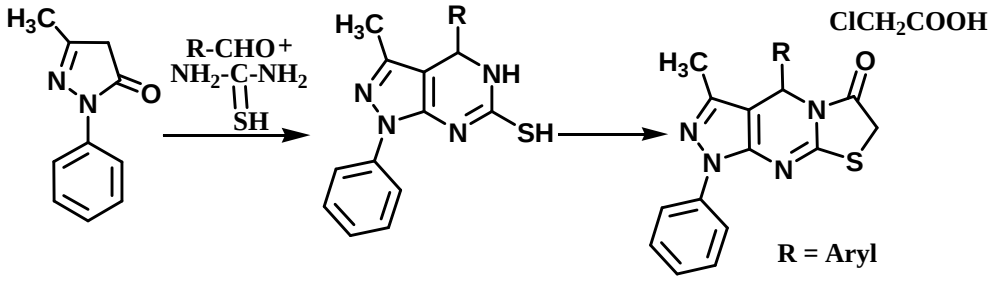
KEYWORDS

Pyrazolo[3,4-d]pyrimidines;
Thiazolidinones;
Antimicrobial activity;
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 A1 adenosine receptors(A_1AR). They may have therapeutical use as cognitive enhancers, antidementia drugs(e.g., for Alzheimer's disease and cerebrovascular dementia), psycostimulants, antidepressant drugs, and ameliorants of cerebral function[9]. Furthermore, a large number of thiazolidinones derivatives are reported to exhibit various pharmacological activities[10-20].

Due to various biodynamic activities of pyrazolo [3,4-d]pyrimidines and thiazolidinones, Synthesis of 4-aryl-3methyl-1-phenyl-4,4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-a]pyrimidine-5-ones**(IVa-m)** have been undertaken by the cyclocondensation of 1-phenyl-3-methyl-4-aryl-6-mercepto-4,5-dihydropyra zolo[3,4-d] pyrimidines with chloracetic acid. For all the compounds a general reaction scheme is outlined in reaction SCHEME 1. The compounds were obtained in excellent yield and were assayed for their *in vitro* biological assay like antibacterial activity towards the gram positive and gram negative bacterial strain and antifungal activity towards *Aspergillus niger* MTCC-282 and *Candida albicans* MTCC-227 at a concentration of 40 μg/ml. The biological activities of the synthesized compounds were compared with reference standard drugs(TABLE 2). The compounds**(IVa-m)** were also evaluated for their *in Vitro* antimycobacterial activity against *Mycobactrium tuberculosis* H_{37}Rv at 6.25 μg/mL concentration. The physical constants, antimicrobial activitiy and antimycobacterial activity of compounds**(IVa-m)** are recorded in TABLES 1, 2 and



SCHEME

TABLE 1 : Physical constant of 4-aryl-3methyl-1-phenyl-4, 4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-]pyrimidine-5-ones (IVa-m)

Sr.no.	R	Molecular formula	Molecular weight	M.P.^{0}C	Yield(%)	R_f value	% of nitrogen required	% of nitrogen found
IVa	Phenyl	$C_{20}H_{16}N_4OS$	363	157	74	0.55	15.56	15.50
IVb	2-Hydrxyphenyl	$C_{20}H_{16}N_4O_2S$	376	186	76	0.60	14.89	14.84
IVc	4-Hydroxyphenyl	$C_{20}H_{16}N_4O_2S$	376	147	71	0.54	14.89	14.85
IVd	2-Chlorophenyl	$C_{20}H_{15}N_4OSCl$	394.5	204	68	0.58	14.20	14.15
IVe	3-Nitrophenyl	$C_{20}H_{15}N_5O_3S$	405	228	78	0.49	17.28	17.22
IVf	3-Phenoxyphenyl	$C_{26}H_{20}N_4O_2S$	452	242	70	0.52	12.39	12.32
IVg	4-Methoxyphenyl	$C_{21}H_{18}N_4O_2S$	390	264	73	0.56	14.36	14.31
IVh	3-methoxy-4-hydroyphenyl	$C_{21}H_{18}N_4O_3S$	406	230	79	0.49	13.79	13.74
IVi	Styryl	$C_{22}H_{18}N_4OS$	386	214	66	0.57	14.51	14.45
IVj	2-Chloro-quinolinyl	$C_{23}H_{16}N_5OSCl$	445.5	222	64	0.54	15.71	15.65
IVk	2,5-dichloro-quinolinyl	$C_{23}H_{15}N_5OSCl_2$	480	194	69	0.50	14.58	14.53
IVl	2-Chloro-8-methyl-quinolinyl	$C_{24}H_{18}N_5OSCl$	459.5	186	74	0.51	15.23	15.20
IVm	2-Chloro-5-methyl- quinolinyl	$C_{24}H_{18}N_5OSCl$	459.5	178	75	0.53	15.23	15.19

TABLE 2 : Antimicrobial activity of of 4-aryl-3methyl-1-phenyl-4, 4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-a] pyrimidine-5-ones (IVa-m)

Sr. no.	R	Antibecterial activity zones of inhibition in mm				Antifungal activity zones of inbition in mm	
		S.pyogens	*S.aureus*	*E.Coli*	*B. subtilis*	*C.albicans*	*A. niger*
IVa	Phenyl	11	13	14	19	20	22
IVb	2-Hydrxyphenyl	10	15	16	16	22	14
IVc	4-Hydroxyphenyl	12	15	17	18	19	16
IVd	2-Chlorophenyl	16	14	21	17	19	18
IVe	3-Nitrophenyl	15	17	23	16	18	19
IVf	3-Phenoxyphenyl	18	16	18	20	17	17
IVg	4-Methoxyphenyl	14	18	19	21	21	23
IVh	3-methoxy-4-hydroyphenyl	18	22	15	18	17	15
IVi	Styryl	12	11	14	15	16	13
IVj	2-Chloro-quinolinyl	14	14	16	15	15	16
IVk	2,5-dichloro-quinolinyl	17	19	22	19	18	18
IVl	2-Chloro-8-methyl-quinolinyl	18	17	19	17	16	17
IVm	2-Chloro-5-methyl- quinolinyl	17	15	20	16	20	20
Antimicrobial activity of known chosen standard drugs							
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	-	-
Griseofluvin		-	-	-	-	25	22

3 respectively.

EXPERIMENTAL

Melting points were determined routinely in open capillary tube and are uncorrected. The completion of reaction was monitored 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 Erba 1108 analyzer and are found within the range of theoretical value. IR spectra were recorded on Shimadzu-8400 FT-IR spectrometer in KBr(λ in cm^{-1}). ^1H NMR spectra were recorded in $CDCl_3$ on a Brucker DRX-300 at 300MHz. 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 43m/z less than the molecular ion peak. No particular fragmentation pattern is observed from the spectra.

Synthesis of 4-(4'-methoxyphenyl)-3-methyl-6-mercapto-4,5-dihydro-1-phenylpyra-zolo[3,4-d]pyrimidines

A mixture of 1-aryl-3-methyl-5-pyrazolones (0.01M), 4-methoxy benzaldehyde(0.01M) and thiourea (0.01M) in ethanol(30mL) was heated under reflux condition for 9hours. The reaction mixture was kept at room temperature for 3 hr. The product was filtered, dried and recrystallized from ethanol. Yield: 72%, m.p. 118^0C, R_f: 0.33, Found: C,65.00%, H,4.90%, N,15.60%. Calculated for $C_{19}H_{18}N_4OS$: C,65.12%, H,5.18%, N,15.99%. The constitution has been delineated by IR, PMR and Mass spectra as followed. I.R.(KBr)(ν_{max} cm^{-1}): 3045(C-H str.Aromatic1, 4-disubstituted), 2920(C-H str. asym. Alkane-CH_3), 2858 (C-H str. sym. Alkane-CH_3), 1515(C=C ring skeletal vib.), 1404(C=N ring skeletal vib.), 3400(N-H str.), 1596(N-H def.), 1323(C-N str.), 1550(C=N Str.Of pyrazole), 1620(N-N def. of pyrazole), 1265(C-O-C str. of ehter), 1101(C-S-C str.), 2630(S-H str.). ^1H-NMR(TMS)(δppm): 1.832(s,3H,-CH_3), 3.108(s,3H,-OCH_3), 4.949(s,1H,-CH), 6.526-8.595(m,11H,Ar-H,-NH,-SH).

Mass spectra

The mass spectrum fragmentation shows molecular ion(M^+) peak at m/z=350 was consistent with the molecular formula $C_{19}H_{18}N_4OS$. Similarly other pyrazolo[3,4-d]pyrimidines were prepared and utilized for further reaction.

Synthesis of 4-(4'-methoxyphenyl)-3methyl-1-phenyl-4,4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-a]pyrimidine-5-ones(IVg)

A mixture of 4-(4'-methoxyphenyl)-3-methyl-6-mercapto-4,5-dihydro-1-phenylpyrazolo[3,4-d] pyrimidines(0.01M), chloracetic acid(0.01M) and anhydrous sodium acetate(3gm) fused in gl.acetic acid(30ml) and acetic anhydride(10ml) was refluxed for 3hours. Then the reaction mixture was allowed to cool and poured gradually with stirring in to cold water. The solid formed was filtered off, washed with water and recrystallized from ethanol. Yield: 73%, m.p. 264°C, $R_{f:}$ 0.56, Found : C,64.62%, H, 4.58%, N, 14.31%. Calculated for $C_{21}H_{18}N_4O_2S$: C, 64.62%, H, 4.62%, N, 14.36%. The constitution of **(IVg)** has been delineated by IR, PMR and Mass spectra as followed. I.R.(KBr)**(IVg)**(ν_{max} cm^{-1}): 3050(C-H str. Aromatic1,4-disubstituted), 2928(C-H str. asym. Alkane-CH_3), 2939(C-H str. sym. Alkane-CH_3), 1558(C=C ring skeletal vib.), 1431(C=N ring skeletal vib.), 3421(N-H str.), 1590(N-H def.), 1118(C-N str.), 1639(C=N Str.Of pyrazole), 1590(N-N def. Of pyrazole), 1709(C=O str. of thiazolidinone), 667(C-S-C str. of thiazolidinone), 1265(C-O-C str. of ether). P.M.R (TMS)(δppm)**(IVg)**: 2.549(s,3H,CH_3), 3.516(s,3H,-OCH_3), 4.683(s,2H,-CH_2), 5.808(s,1H,-CH), 7.164-7.970(m,8H,-Ar-H). Mass spectra **(IVg)**: The Mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=391 was consistent with the molecular formula $C_{21}H_{18}N_4O_2S$. Similarly other compounds (IVa-m) were also prepared and their physical constants are shown in TABLE 1.

ANTIMICROBIAL ACTIVITY

Products **(IVa-m)** were evaluated for their antimicrobial activity against *Sreptococcus pyogens* MTCC-442, *Staphylococcus aureus* supsp. Aureus MTCC-96, *Bacillus subtilius* MTCC-441, *Escherichia coli* MTCC-443 and antifungal activity against *Aspergillus niger* MTCC-282 and *Candida albicans* MTCC-227 using DMF as a solvent at 40μg/ml concentration by

TABLE 3 : Antimycobacterial Activity of 4-aryl-3methyl-1-phenyl-4,4a-dihydro-6H-pyrazolo[3,4-d]-1,3-thiazolidino-[3,2-a]pyrimidine-5-ones(IVa-m)

Sr. no.	R	Molecular formula	Assay	MIC (μg/mL)	% Inh	Activity
IVa	Phenyl	$C_{20}H_{16}N_4OS$	Alamar	>6.25	0	-
IVb	2-Hydrxyphenyl	$C_{20}H_{16}N_4O_2S$	Alamar	>6.25	0	-
IVc	4-Hydroxyphenyl	$C_{20}H_{16}N_4O_2S$	Alamar	>6.25	7	-
IVd	2-Chlorophenyl	$C_{20}H_{15}N_4OSCl$	Alamar	>6.25	42	-
IVe	3-Nitrophenyl	$C_{20}H_{15}N_5O_3S$	Alamar	>6.25	0	-
IVf	3-Phenoxyphenyl	$C_{26}H_{20}N_4O_2S$	Alamar	>6.25	17	-
IVg	4-Methoxyphenyl	$C_{21}H_{18}N_4O_2S$	Alamar	>6.25	5	-
IVh	3-methoxy-4-hydroyphenyl	$C_{21}H_{18}N_4O_3S$	Alamar	>6.25	6	-
IVi	Styryl	$C_{22}H_{18}N_4OS$	Alamar	>6.25	22	
IVj	2-Chloro-quinolinyl	$C_{23}H_{16}N_5OSCl$	Alamar	>6.25	11	
IVk	2,5-dichloro-quinolinyl	$C_{23}H_{15}N_5OSCl_2$	Alamar	>6.25	0	
IVl	2-Chloro-8-methyl-quinolinyl	$C_{24}H_{18}N_5OSCl$	Alamar	>6.25	1	
IVm	2-Chloro-5-methyl- quinolinyl	$C_{24}H_{18}N_5OSCl$	Alamar	>6.25	10	

using cup-plate method[21]. After 24hours of incubation at 37^0C, the zones of inhibition were measured in mm. The activity was compared with some known antibiotics like ampicillin, chloramphenicol, amoxycillin, ciprofloxacin, norfloxacin and griseofulvin, at same concentration, which are recorded in TABLE 2.

Antituberculosis activity

Primary screening of the compounds**(IVa-m)** was conducted at 6.25 μg/mL against *Mycobacterium tuberculosis* Strain H_{37}Rv(ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay(MABA)[22]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. All the compounds effected <90% inhibition in primary screening (i.e.,MIC>6.25 μg/mL). The compounds**(IVa-m)** were not subjected to the further evaluation due to lake of inhibition in primary screening. The results are depicted in TABLE 3.

RESULT AND DISCUSSION

Infrared spectroscopic investigation was carried out by the use of KBr method, showed a sharp band around 1708cms^{-1} due to ketonic stretching band(C=O str.) of thiazolidinone. Which confirms the desired cyclisation. The ^{1}H NMR spectra of compound**(IVg)** showed a singlet at 4.683 δppm which suggests presence of a -CH_2 next to a ketonic functional group , which confirmed the formation of desired product **(IVg)**.

The newly synthesized compounds **(IVa-m)** were evaluated for their antimicrobial activity Compounds **(IVe)**, **(IVf)**, **(IVg)**, **(IVi)**, **(IVe)** and **(IVg)** demonstrated excellent antimicrobial activity compared to the standard drugs like Ampicillin, Chloramphenical, Ciprofloxacin, Griseofluvin. None of the compounds showed any specific inhibition towards mycobacterium tuberculosis strain H_{37}Rv in primary screening. Antimycobacterial activity is designated in TABLE 3.

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