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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2012, Vol. 28, No. (1):
Pg. 507-512

Preparation and Biological Evaluation of 3-amino-4-aryl-4, 5-dihydro-1-N-tolyl pyrazolo [3, 4-d] pyrimidines Derivative

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(Received: January 05, 2012; Accepted: February 02, 2012)

ABSTRACT

The preparation of the 3-amino-4-aryl-4, 5- dihydro-1-N-tolyl pyrazolo [3, 4-d] pyrimidines derivative (V a-m) have been undertaken by the cyclocondensation of aryl aldehyde, urea and 1-N-phenyl-3-amino-5-pyrazolone. The constitution of the product (V a-m) has been characterized by using elemental analyses, IR and PMR spectral data. The products (V a-m) were assayed for their in vitro biological assay like antibacterial activity towards Gram 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. Some of the obtained compounds showed the interesting antimicrobial activity comparable to standard drugs like amplicillin, chloramphenicol, amoxicillin, ciprofloxacin, norfloxacin and griseofluvin...

Keywords: pyrazolo [3, 4-d] pyrimidines, antimicrobial activity and antituberculosis activity antimycobacterial activity

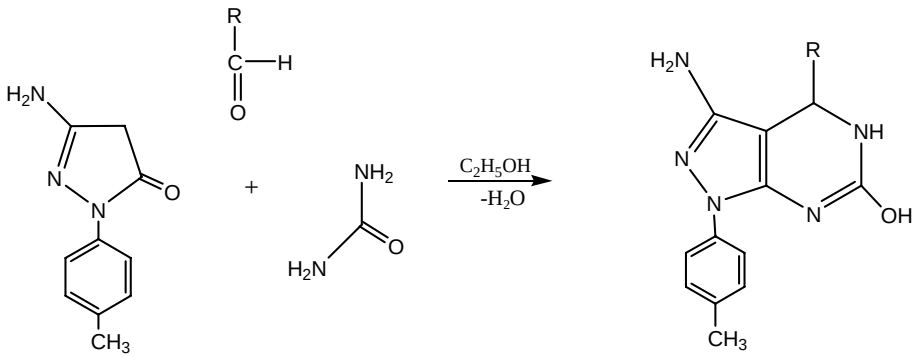
INTRODUCTION

Extensive studies have been made in the field of synthesis of pyrazol [3, 4-d] pyrimidines [1-10] Consideration research has been under taken to extend activity and reduce toxicity of pyrazolo [3, 4-d] pyrimidine. The specific biological activities have been Hyperuricemia[11], Protozoacidal[12], Hypoglycemic[13], Antimalerial[14], Anticancer[15], Analgesic[16], Antipyretic[16] , Aniinflammatory[16], Antimycotic agent[17], Xanthine oxidaseinhibitor[18], Antitumor[19], Anti-HIV[20], Anxiolytic agent[21], Cardiovascular[22], Antileishmanial[23], Tranquilizer[24], Bloodsugar lower agent[25], Anticonvulant[26], Antiviral[27], Antiallergic[28].

The product(Va-m) 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* and *Candida albicans* at different concentration for their MIC values. The biological activities of the synthesized compounds were compared with standard drugs. [Table II]. The physical constant, antimicrobial and antimicobacterial activities of compounds (Va-m) recorded in Table II respectively.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of



(V a-n)
Where R=Aryl

Reaction Scheme

the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

General Method for Synthesis of 3-amino-4-aryl-4, 5- dihydro-1-N-tolyl pyrazolo [3, 4-d] pyrimidines derivative (Va-m)

A mixture of 3-amino-1-p-tolyl-1H-pyrazol-5(4H)-one (0.01 mole), urea (0.01 mole) and aldehyde (0.01mole) in ethanol (30ml) under reflux condition for three hours. The reaction mixture was kept at room temperature for 2 hrs. The product was

Table-1:Physical data
3-amino-4-aryl-4, 5- dihydro-1-N-tolyl pyrazolo [3, 4-d] pyrimidines derivative (Va-m)

Sr. No.	R	Molecular Formula	M.W.	M.P. °C	Yield (%)	R_f Value	% of Nitrogen	
							Calcd.	Found
V a	C_6H_5	$C_{18}H_{17}N_5O$	319.36	240	68	0.68	21.80	21.75
V b	2-Cl-C_6H_4	$C_{18}H_{16}ClN_5O$	353.80	252	72	0.63	19.69	19.63
V c	4-Cl-C_6H_4	$C_{18}H_{16}ClN_5O$	353.80	260	67	0.59	19.69	19.65
V d	3-NO_2—C_6H_4	$C_{18}H_{16}N_6O_3$	364.35	271	72	0.53	22.95	22.90
V e	2-NO_2-C_6H_4	$C_{18}H_{16}N_6O_3$	364.35	245	78	0.58	22.95	22.90
V f	3-C_6H_5-O-C_6H_4	$C_{24}H_{21}N_5O_2$	411.45	249	64	0.70	16.94	16.90
V g	2-OCH_3-C_6H_4	$C_{19}H_{19}N_5O_2$	349.38	259	58	0.55	19.94	19.91
V h	4-OCH_3-C_6H_4	$C_{19}H_{19}N_5O_2$	349.38	250	65	0.53	19.94	20.89
V i	2-OH-C_6H_5	$C_{18}H_{17}N_5O_2$	335.35	240	72	0.51	18.56	20.51
V j	4-OH-C_6H_5	$C_{18}H_{17}N_5O_2$	335.35	255	75	0.49	18.56	20.50
V k	C_6H_4-CH=CH	$C_{26}H_{23}N_5O$	421.49	265	62	0.62	20.17	19.11
V l	4-CH_3S-C_6H_4	$C_{19}H_{19}ON_5OS$	365.45	219	71	0.63	19.07	22.02
V m	α- C_4H_3O	$C_{24}H_{21}N_5O_2$	385.45	290	77	0.61	22.50	16.45

TLC Solvent systems: Acetone: Benzene= 1:9

isolated and crystallized from a suitable solvent to give the desire product. The Physical data were recorded in Table-1

Preparation of 3-amino-4, 5-dihydro-4-(4-(methylthio) phenyl)-1-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidin-6-ol (V-l)

A mixture of 3-amino-1-p-tolyl-1H-pyrazol-5(4H)-one (1.88 gm, 0.01M), urea(0.60gm,0.01M) and 4-(methylthio)benzaldehyde (1.52 ml, 0.01mole) in ethanol (30ml) under reflux condition for three hours. The reaction mixture was kept at room temperature for 2 hrs. The product was collected and recrystallized from an ethanol: dioxan (2:1).

Table 2: Antimicrobial Activity of 3-amino-4-aryl-4, 5-dihydro-1-N-tolyl pyrazolo [3, 4-d] pyrimidines derivative (Va-m)

Com pound	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
V a	C_6H_5	100	200	500	500	200	200
V b	2-Cl-C_6H_4	100	50	25	100	25	50
V c	4-Cl-C_6H_4	200	-	-	50	-	100
V d	3-NO_2—C_6H_4	25	-	-	50	-	-
V e	2-NO_2-C_6H_4	500	500	-	800	500	-
V f	3-C_6H_5-O-C_6H_4	50	-	500	500	25	800
V g	2-OCH_3-C_6H_4	-	50	500	-	-	500
V h	4-OCH_3-C_6H_4	50	100	200	500	500	-
V i	2-OH-C_6H_5	100	200	-	500	-	800
V j	4-OH-C_6H_5	500	500	800	500	-	-
V k	C_6H_4-CH=CH	-	-	500	500	-	500
V l	4-CH_3S-C_6H_4	-	500	500	500	500	-
V m	α- C_4H_3O	-	800	500	200	200	200

Table 3: Comparable Activity of compounds (V a-m) 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
	Vd (25) Vf (50)	Vb(50) Vg(50)	Vb(50)	Vc (50)	Vb (25)	-	Vb (50) -
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

3-amino-4, 5-dihydro-4-(4-(methylthio) phenyl)-1-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidin-6-ol (V-l)

IR: 3030(C-H) str. Aromatic), 1512 (C=C ring skeletal vib. Of pyrimidine) ,1456(C=N ring skeletal vib. pyrimidine), 3030(C-H str.),1178((-C-H i.p.def.), 2920(C-H asym.), 2852(C-H sym.), 1382(C-H def. sym.), 3435(N-H str.), 3373 (N-H str.), 1588 (N-H def.) ,1311 (C-N str.), 1662 (C=N str. of pyrazol), 1583 (N-N def. of pyrazol), 1178(C-N str. of Pyrazol),3325(-OH Str.)

^{1}H –NMR ($DMSOd_6$+ $CDCl_3$, ä ppm): 2.56(3H,-CH_3), 2.47(3H,-SCH_3), 5.95(1H, -CH), 6.71-8.70(16H, Ar-H+NH+NH_2+OH)

MASS spectra

The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=365.45was consistent with molecular formula $C_{19}H_{19}ON_5$ OS

3-amino-4, 5-dihydro-4-(4-methoxyphenyl)-1-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidin-6-ol (V-h)

IR: 3114(C-H) str. Aromatic), 1514 (C=C ring skeletal vibe. Of pyrimidine), 1459(C=N ring skeletal vib. pyrimidine), 2935(C-H str. asym.), 2845(C-H sym.), 1370(C-H sym.), 1460(C-H def. asym.), 3473(N-H str.), 3133 (N-H str.), 1587N-H def.) , 1370 (C-N str.), 1648 (C=N str. of pyrazol), 1625 (N-N def. of pyrazol), 1165(C-N str.of Pyrazol).

^{1}H –NMR ($DMSOd_6$+ $CDCl_3$, ä ppm): 2.45(3H,-CH_3), 3.73(3H, 3-OCH_3), 5.19 (1H, -CH), 6.80-7.02(12 H, Ar-H+NH+NH_2+OH),

MASS spectra

The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z= 349.38was consistent with molecular formula $C_{19}H_{19}N_5O_2$

3-amino-4, 5-dihydro-4-(4-hydroxyphenyl)-1-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidin-6-ol (V-j)

IR: 3004(C-H) str. Aromatic), 1504 (C=C 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.), 1454(C-H def. asym.), 3471(N-H str.), 3135 (N-H str.), 1581 (N-H def.) ,1365 (C-N str.), 1651 (C=N str. of pyrazol), 1620 (N-N def. of pyrazol), 1166(C-N str.of Pyrazol).

^{1}H –NMR ($DMSOd_6$+ $CDCl_3$, ä ppm): 2.35(3H,-CH_3), 5.0(1H, -OH) 5.62 (1H, -CH), 6.61-7.13(12H, Ar-H+NH+NH_2+OH),

MASS spectra

The mass spectrum fragmentation shows molecular ion (M^+) peak at m/z=335.5 was consistent with molecular formula $C_{18}H_{17}N_5$ O_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.

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 growth of the test bacteria. Each purified compound was dissolved in DMF sterilized by filtration by using sintered glass filter and stored at 4^0C.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 units (CFU). These were then spot inoculated on nutrient agar plates containing increasing amount of a compound, incubated at 37^0C up to 24 hrs. for determination of the minimum inhibitory concentration (MIC) .The antibacterial activity of the compounds (Va-m) 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 for72 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 4^0C.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 37^0C up to 48 hrs. For determination of the minimum inhibitory concentration (MIC) .The MIC value of test solutions are recorded in Table No-2 and Table No-3

ACKNOWLEDGEMENTS

The author's wish to thanks to Principal, Shree R.R. Lalan College, Bhuj for providing research facilities.

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