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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY (MIC) OF SOME NEW PYRIMIDINE DERIVATIVES

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ABSTRACT

Synthesis of substituted pyrazolo pyrimidine by the reaction of substituted pyrazole-4-carbaldehyde and 3-methyl 1H-pyrazole -5(4H)-one and 1H-benzo[d]imidazol-2-amine, acetonitrile as solvent and piperidine as a catalyst, was performed. The constitution of all the synthesized compounds has been characterized by using IR, MASS, ^{1}HNMR spectroscopy. All synthesized compounds were screened for their antimicrobial activity.

Keywords: Pyrazole-4-carbaldehyde, 3-methyl 1H-pyrazole -5(4H)-one, 1H-benzo[d]imidazol-2- amine, Piperidine

1. INTRODUCTION

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, alkoxan, barbituric acid and a mixture of anti-malarial and anti-bacterial also contain the pyrimidine ring.

Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological and therapeutic activities. They have been recognized as important heterocyclic compounds due to their diverse biological activities. The pyrimidine ring is also found in vitamins like thiamine vitamin- B1, riboflavin and folic acid [1]. Barbitone, the first barbiturate used as hypnotic sedative and anticonvulsant is a pyrimidine derivative. Some of the therapeutic activities of pyrimidine derivatives are Anti-thyroid [2-3], Anti-tumor [4], Anti-hypertensive [5], Anti-inflammatory [6-8], Anticonvul - sant [9], Anti-neoplastic [10], Anthelmintic [11], Antimicrobial [12], Cardiovascular [13], Anti-viral [14] and Anti-tubercular [15]

Pyrazolopyrimidines have attracted considerable interest because of their biological activity. For instance, this heterocyclic system is found as purine analogues and has useful properties as antimetabolites in purine biochemical reactions [16-17]. Several compounds of this class display interesting antitrypanosomal [18] and antischistosomal activities [19]. They are used as HMG-CoA reductase inhibitors [20]. Some pyrazolo - pyrimidines serve as efficient sedative-hypnotic and anxiolytic drugs like zaleplon (Sonata, hypnotic) [21], indiplon (hypnotic) [22] and ocinaplon (anxiolytic) [23] fasiplon (anxiolytic) [24].

2. EXPERIMENTAL

All the melting points were determined in open capillary tubes and are uncorrected.IR spectral were recorded in solid state using KBr pellet method and recorded on Shimadzu-spectrophotometer and ^{1}H NMR spectral on broker advance 400 MHz spectrometer with DMSO as a solvent and TMS as internal standard. Mass spectra of synthesized compounds were taken on GSMS-GP mass spectrometer. All the reactions were monitored by TLC. The physical data of synthesized compounds are given in table 1.

2.1.Synthesis of of 4-(3-(2-methoxy)-1-phenyl -1h-pyrazol-4-yl)-3-methyl-4,11-dihydro- 1h-benzo [4, 5] imidazo [1, 2-a] pyrazolo [3, 4-d] pyrimidine

A mixture of 3-(2-methoxy phenyl)-1-phenyl-1H pyrazole-4-carbaldehyde (2.62gm,0.01 mole) and 3-methyl 1H-pyrazole -5(4H)-one (0.98 gm,0.01 mole) and 1H-benzo[d] imidazol-2-amine (1.33gm,0.01 mole) 20 ml acetonitrile as solvent and piperidine as a catalyst has been taken in RBF. Reaction mass was refluxed for 6-7 hrs. After completion of reaction, the mixture was cooled and poured in crushed ice. The solid thus obtained was crystallised in methanol. Yield 48%. MP-

182°C. Calculated: C (71.02%), H (4.90%), N (20.71%), Found: C (70.58%), H (4.86%), N (20.65%). ***IR(KBr; cm^{-1}):*** 2924, 2870, 3055.1674, 1435, 1319, 1026, 1597; ***^{1}HNMR (δppm):*** 2.11(s,3H,CH_3), 3.86 (s,3H,OCH_3) 6.92 to 7.89 (m,15H,Ar-H), 10.20 to 11.30 (s,2H,NH) 400 MHz DMSO, ***MS (m/z):*** 473 (M^+),419, 78, 200.

Similarly, other compounds (vc1-vc13) were synthesized by above mentioned process from different carbaldehyde. The physical data are recorded in Table no-1.

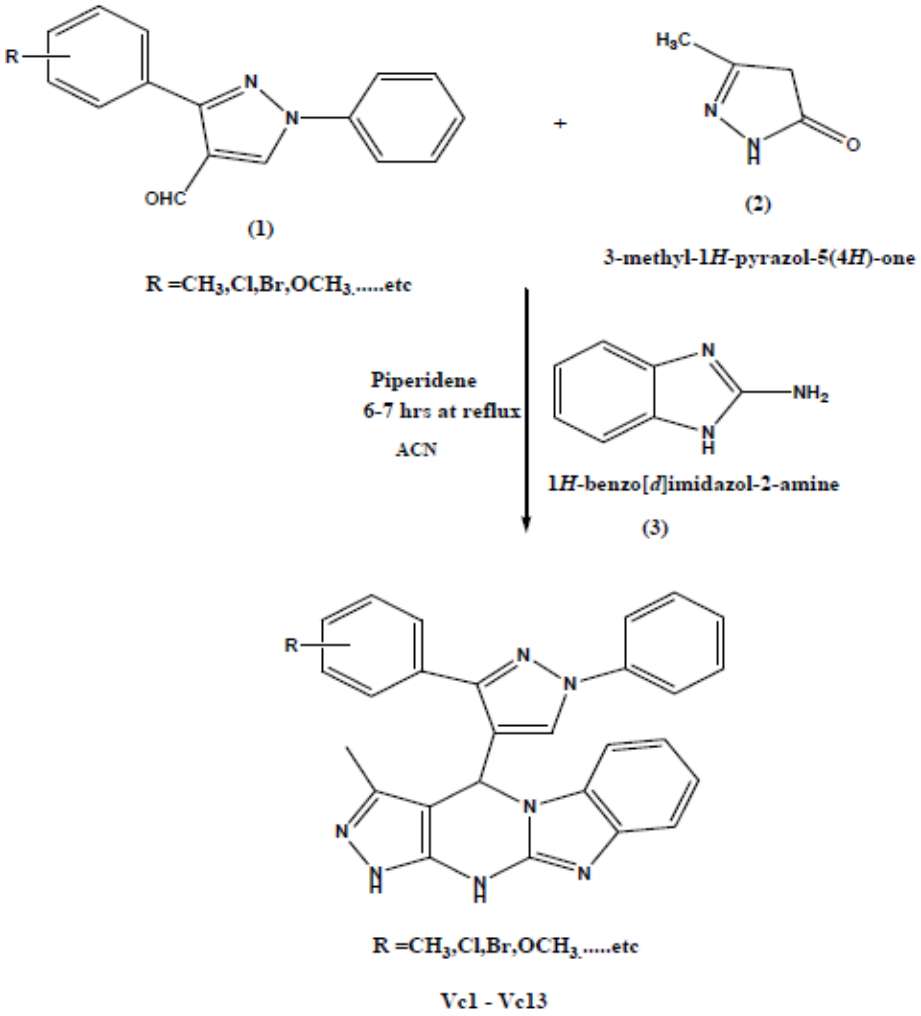

Fig. 1: Reaction Scheme

2.2. Antimicrobial Screening (Minimum Inhibitory Concentration)

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella para typhosa B* and they were also evaluated for antifungal activity against *Candida albicans and Aspergillus niger* at different concentrations: *i.e.* Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 15.26 (μg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (vc1-vc13) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by NCCLs).

3. RESULT AND DISCUSSION

3.1. Spectral Data of 4-(3-(4-methyl)-1-phenyl -1h-pyrazol-4-yl)-3 –methyl-4,11-dihydro-1h-benzo[4,5]imidazo[1,2-a]pyrazolo[3,4-d] pyrimidine

IR (KBr; cm^{-1}): 2960, 2910.3020, 1670, 1437, 1030, 1610, ***^{1}H NMR δ ppm:*** 1.86 (s,3H, CH_3) 2.1

(s,3H,CH_3), 7.26 to 8.34 (m,15H,Ar-H), 10.20 to 11.23 (NH) 400 MHz DMSO, ***MS (m/z):*** 457 (M^+), 77, 91.175, 367.

3.2. Spectral Data of 4-(3-(2-cholro)-1-phenyl -1h-pyrazol-4-yl)-3-methyl-4,11-dihydro-1h-benzo[4,5]imidazo[1,2-a]pyrazolo[3,4-d] pyrimidine

IR (KBr, cm^{-1}): 2907, 2957, 3075, 1645, 1560, 1130, 770, 670, ***^{1}H NMR δ ppm***: 1.96 (s,3H, CH_3) 6.97 to 7.89 (m,15H,Ar-H), 11.01 (NH) 400 MHz DMSO, ***MS (m/z)***: 478 (M^+),77,112.200.

3.3. Spectral Data of 4-(3-(4-cholro)-1-phenyl -1h-pyrazol-4-yl)-3-methyl-4 ,11-dihydro-1h-benzo[4,5]imidazo[1,2-a]pyrazolo[3,4-d] pyrimidine

IR (KBr, cm^{-1}): 2917, 2957, 3079, 1645, 1564, 1130, 770, 670, ***^{1}H NMR δ ppm***:1.94 (s,3H, CH_3) 6.95 to 7.59 (m,15H,Ar-H), 11.01 (NH) 400 MHz DMSO, ***MS (m/z)***: 478 (M^+),77,112.212.

Table 1: Physical Constants of 4-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-3-methyl-4,11-dihydro - 1H-benzo [4,5] imidazo [1,2-a]pyrazolo[3,4-d]pyrimidine derivative

Compound Name	R=	Molecular Formula	MP (C^0)	Molecular Weight	Yield %	% Composition (Calcd.)/found		
Vc_1	4-Cl	$C_{27}H_{20}ClN_7$	178	478	56	(67.85) 67.03	(4.22) 3.98	(20.51) 20.34
Vc_2	4-Br	$C_{27}H_{20}BrN_7$	204	522	61	(62.08) 61.98	(3.86) 3.92	(18.77) 18.68
Vc_3	2-OCH_3	$C_{28}H_{23}N_7O$	182	473	48	(71.02) 70.58	(4.90) 4.86	(20.71) 20.65
Vc_4	4-CH_3	$C_{28}H_{23}N_7$	167	457	52	(73.56) 73.09	(5.07) 5.02	(21.43) 21.26
Vc_5	2-Cl	$C_{27}H_{20}ClN_7$	171	478	58	(67.85) 67.25	(4.22) 4.19	(20.51) 20.21
Vc_6	2-Br	$C_{27}H_{20}BrN_7$	196	522	53	(62.08) 61.88	(3.86) 3.81	(18.77) 18.65
Vc_7	4-NO_2	$C_{27}H_{20}N_8O_2$	182	488	62	(66.38) 66.21	(4.13) 4.02	(22.94) 22.52
Vb_8	4-OCH_3	$C_{25}H_{23}N_7O$	158	437	61	(68.63) 67.85	(5.30) 5.02	(22.41) 22.02
Vc_9	2-NO_2	$C_{27}H_{20}N_8O_2$	179	488	65	(66.38) 66.12	(4.13) 4.08	(22.94) 22.68
Vc_{10}	H	$C_{27}H_{21}N_7$	158	443	58	(73.12) 73.02	(4.77) 4.58	(22.11) 21.86
Vc_{11}	2-CH_3	$C_{28}H_{23}N_7$	168	457	63	(73.56) 73.25	(5.07) 5.02	(21.43) 21.08
Vc_{12}	4-F	$C_{27}H_{20}FN_7$	156	461	57	(70.27) 70.06	(4.37) 4.25	(21.25) 21.16
Vc_{13}	2-F	$C_{27}H_{20}FN_7$	163	461	59	(70.27) 69.58	(4.37) 4.28	(21.25) 21.08

3.4. Antimicrobial Screening

All the compounds were found active against all the bacteria and fungi but out of which 9 compounds are more active. so we kept that compounds for tertiary screening. Vc_3, Vc_4, Vc_5, Vc_7, Vc_{12} are active against gram Positives, Gram Negative bacteria and *A.niger, C.albicans* at 125 ug/ml conc. Out of which **Vc_5** is most active compound it shows activity at 62.5 ug/ml concentration. The structure of **Vc_5** is as under which can be a potent antimicrobial agent after further extensive investigations.

Table 2: Biological evaluation of 4-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-3-methyl-4,11-dihydro-1H-benzo [4,5] imidazo [1,2-a]pyrazolo[3,4-d]pyrimidine

Comp No	Antibacterial Activity												Antifungal Activity					
	Gram Positives						Gram Negative											
	S.aureus (μg/ml)			*B.Subtilis* (μg/ml)			*E.Coli* (μg/ml)			*S.peratyphi B* (μg/ml)			*A.niger* (μg/ml)			*C.albicans* (ug/ml)		
	1000	*500*	*250*	*1000*	*500*	*250*	*1000*	*500*	*250*	*1000*	*500*	*250*	*1000*	*500*	*250*	*1000*	*500*	*250*
Vc_1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vb_8	+	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+
Vc_9	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Vc_{10}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_{11}	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+
Vc_{12}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vc_{13}	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
CPFN	1.9			7.8			1.4			0.4			*			*		
FCNZ	*			*			*			*			0.7			0.4		

CPFN: Ciprofloxacin; FCNZ: Fluconazole

Tertiary screening for minimum inhibition Concentration
(Extensive investigation for the powerful antimicrobials of the series)

Comp No.	Antibacterial Activity												Antifungal Activity					
	Gram Positives						Gram Negative											
	S.aureus (μg/ml)			*B.Subtilis* (μg/ml)			*E.Coli* (μg/ml)			*S.peratyphi B* (μg/ml)			*A.niger* (μg/ml)			*C.albicans* (ug/ml)		
	125	**62.5**	**31.25**	**125**	**62.5**	**31.25**	**125**	**62.5**	**31.25**	**125**	**62.5**	**31.25**	**125**	**62.5**	**31.25**	**125**	**62.5**	**31.25**
Vc_1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vc_2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
Vc_3	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-
Vc_4	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-
Vc_5	+	+	-	+	+	-	+	-	-	+	+	-	+	+	-	+	+	-
Vc_6	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
Vc_7	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-
Vc_{10}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vc_{12}	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-
CPFN	1.9			7.8			1.4			0.4			*			*		
FCNZ	*			*			*			*			0.7			0.4		

CPFN: Ciprofloxacin; FCNZ: Fluconazole

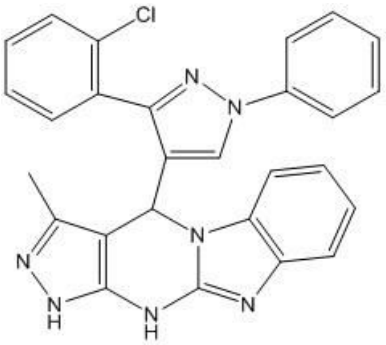

Fig. 2: Structure of Vc_5

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