SYNTHESIS AND EVALUATION OF SOME NEW PYRAZOLO PHENOXY ACETIC ACID DERIVATIVES FOR THEIR ANTIMICROBIAL ACTIVITIES

Shasikant R.Pattan*[1] ,Ravi L Hullolikar--, Nachiket S Dighe[1] ,Santosh S.Dengale[1]
Jayshri S.Pattan[2] ,Ashok M.Bhosale[2],Pandurang M.Gaikwad[3]
Department of Medicinal Chemistry, Pravara Rural College Of Pharmacy, Pravaranagar
Department of Microbiology, PVPC, Pravaranagar
Department of Pharmacology PDVVP College Of Pharmacy, Ahmednaga

ABSTRACT

The present work is a bonafide and novel for the synthesis of phenoxy acetic acid derivatives. Around 10 new derivatives were synthesized, with the standard chemicals and well established procedures. The synthesized compounds were tested for their preliminary tests, physical constants, TLC, solubility etc.The structures of the final compounds were confirmed by IR, ^{1}H-NMR Spectra and CHN analysis. The proposed compounds were screened for their antibacterial and antifungal activities by disc agar diffusion methods against Ciprofloxacin and Griseofulvin at 100 mcg/ml.

Key-words: Anti bacterial, Anti fungal**,** Pyrazolo phenoxy acetic acid derivatives.

1.INTRODUCTION

The need of new anti-microbial agents is justified because more microorganisms are being resistance to the present drugs available in the market. Word wide researchers are trying to synthesize new drugs with better pharmacokinetic and pharmacodynamic properties with less adverse effects. The literature survey suggests that the Pyrazolo phenoxy acetic acid have proved to be good bioactive molecules. They have shown diverse biological activities like anti-bacterial, anti-fungal, anti-inflammatory, anti-tubercular, anticonvulsant, anti-HIV, cardiac stimulant, diuretic and anticancer etc.Therefore in view of above facts it was thought of interest to synthesize some Pyrazolo phenoxy acetic acid derivatives. The structures of the final compounds were confirmed by IR, ^{1}H-NMR Spectra and CHN analysis. The proposed compounds were screened for their antibacterial and antifungal activities with the standard drugs in the well-equipped microbiology and pharmacology lab by using standard methods.

Address for correspondence*
Dr.Shashikant R Pattan
Principal
Pravara Rural College of Pharmacy, Pravaranagar
A/P Loni Bk,Tal-Rahata, Dist-Ahmednagar 413736
shashipattan@yahoo.com
Telephone-+91-2422-273528, Fax-+91-2422-273528

2.MATERIALS AND METHODS

EXPERIMENTAL:

Synthesis of 4-formyl phenoxy acetic acid (I):

To a mixture of 1.0gm of the p-hydroxy benzaldehyde and 3.5ml of 33% of NaOH solution in a beaker, add 2.5ml of 50% chloroacetic acid solution. Add a little water to dissolve the sodium salt of the phenol and heated gently on boiling water bath for one hour. After cooling dilute with 10 ml of water, acidify to congo red with hydrochloric acid and extract with 30 ml of ether, wash the ethereal extract with 10ml of water and extract the aryloxyacetic acid by shaking with 25ml of 5% sodium carbonate solution. Acidify the sodium carbonate extract with dilute hydrochloric acid, collect the 4-formyl phenoxy acetic acid which separates and recrystallized with ethanol. Melting point was 132°C.

Synthesis of chalcone derivatives from 4-formyl phenoxy acetic acid (II):

A mixture of 4-formyl phenoxy acetic acid (0.01mole) and aryl aldehyde (0.01 moles) was stirred in ethanol (30 ml) and then an aqueous solution of KOH (40%, 15 ml) added to it. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with hydrochloric acid. The solid separated was filtered and crystallized from ethanol. Melting point observed 145-155° C.

Synthesis of 2-(4-(5-substituted phenyl-4*H*-pyrazol-3-yl) phenoxy) acetic acid (III):

A mixture of chalcone derivative (0.01mole) and hydrazine hydrate (0.02mole) in 20 ml of ethanol and 2-3 drops of glacial acetic acid was refluxed for 8 hours, the resulting solution was left overnight in a refrigerator, the product was filtered and recrystallized from ethanol. yield of the product 65-75%.

Synthesis of 2-(4-(4-((2-Substituted) methyl)-5-Substituted phenyl-4*H*-pyrazol-3-yl) phenoxy) acetic acid (P_1-P_{10}):

0.01 mole of 2-(4-(5-substituted phenyl-4*H*-pyrazol-3-yl) phenoxy) acetic acid (III) and 0.01mole of secondary amine was dissolved in ethanol, to above mixture 0.01 mole of formaldehyde was added and refluxed for 2 hour, the reaction mixture was cooled and poured over crushed ice and kept in refrigerator for over night, the product was filtered, dried and recrystallized. The melting point and percentage (%) yields were reported in the Table.

BIOLOGICAL EVALUATION

ANTIBACTERIAL ACTIVITY:

The compounds were tested in-vitro for their antibacterial activity against two microorganisms viz. Escherichia coli (NCTC 10418) and Staphylococcus aureus (NCTC 6571) which are pathogenic in human beings.

Method: Disc Agar diffusion method using Mueller-Hinton agar using Escherichia coli and Staphylococcus aureus

ANTIFUNGAL ACTIVITY:

The compounds were tested in-vitro for their antifungal activity against Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16)

Method: Cup-Plate agar diffusion method using Sabouraud-dextrose agar using Candida albicans & Aspergillus niger.

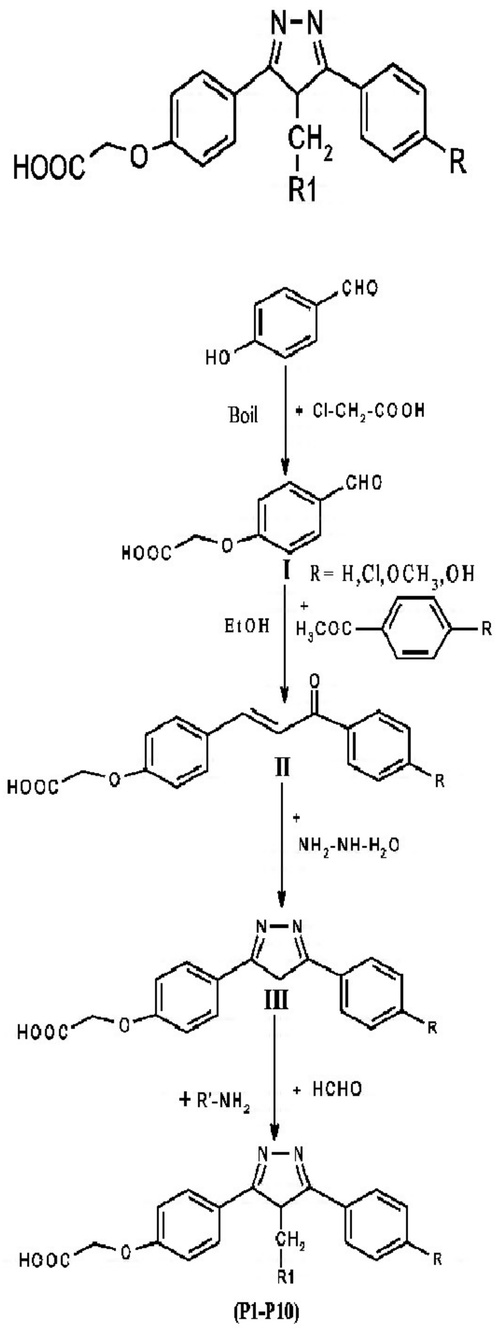
3.RESULTS AND DISCUSSION

p- hydroxy benzaldehyde was treated with chloroacetic acid to get phenoxy aldehyde which were further treated with substituted acetophenones to get their respective chalcones (II) . These chalcones were treated with hydrazine hydrate to get pyrazolo phenoxy acids (III).This intermediate was further treated with various amino compounds through Mannich reaction to get 10 new compounds .The structure of newly synthesized compounds were confirmed by IR, NMR and Elemental analysis. These compounds were evaluated for antibacterial and antifungal activity.

Compounds P_1, P_2, P_6, P_7, P_8 and P_{10} and Compounds P_1, P_2, P_6 and P_8 have shown promising antibacterial and antifungal activities respectively. However with suitable molecular modification these compounds may be expected to get more promising antibacterial and antifungal activities in future.

4. ACKNOWLEDGEMENTS

Authors wish to thank Honourable Shri.Radhakrishna Vikhe Patil, Minister for Education, Law and Justice, Govt. of Maharashtra for his constant encouragement and support. Our sincere thanks to Maj. Gen. U. M. Maindarkar, Director General Pravara Rural Education Society, Loni for providing all the facilities necessary for research work



(P1-P10)

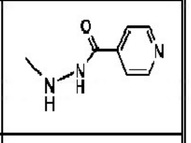
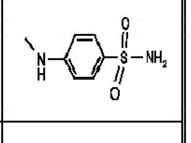
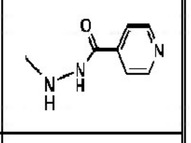
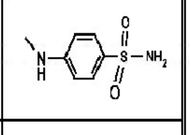
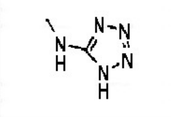
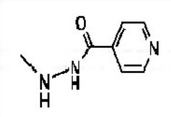
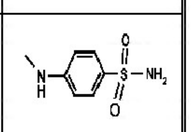
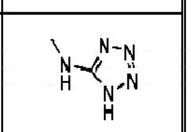
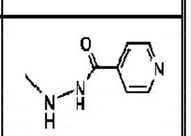
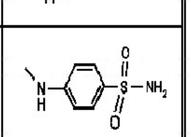
Compounds	R	R_1
P_1	H	
P_2	H	
P_3	Cl	
P_4	Cl	
P_5	Cl	
P_6	OCH_3	
P_7	OCH_3	
P_8	OCH_3	
P_9	OH	
P_{10}	OH	

Table No-1: Infra Red / ^{1}H NMR spectral data of Pyrazolo Phenoxy acetic acid derivatives (P_1-P_{10})

Compd. Code	IR Bands (cm^{-1})	Types of Vibrations	δ Values in ppm	No. Of Protons
P_1	2854,2972 3045,1513 2927,1605 763,838 3214	O-H Str.-C-H Ar. Str.-C=N Str. -C-H def.-C=O str.C-H def.-N-H str.	11.82,1.25 9.78,2.49 3.47-3.56 2.58-2.49 8.7-7.59	1H of –COOH 1H of Pyrazole 1H of 2^O NH 1H of Amine 2H of $-CH_2$ 2H of CH_2 sub.to pyrazole 12H of Ar & Pyridine
P_2	2858,2967 2952,3249 1510,1594	-O-H Str.-C-H Ar.Str.-N-H Str. -C=N Str.-C=O Str.		
P_3	2852,3073 1513,3219 1591,2923 832	-O-H Str.-C-H Ar.Str.-C=N Str. -N-H Str.-C=O str. -C-H Alk.str.-C-Cl str.		
P_4	2853,2924 1512,1596 829,1151 3247	-O-H Str.-C-H Ar.Str.-C=N Str. -C–O Str.-C-Cl str.-S=O str.-N-H str.	10.92,7.39 8.8,7.12-8.10 1.7,3.23,3.49	1H of –COOH 2H of $-SO_2NH_2$ 1H of Ar.-C-NH 12H of Ar.CH 1H of Pyrazole 2H of $-CH_2$sub. Pyrazole 2H of $-CH_2$
P_5	2852,2922 1513,1590 831	-O-H Str.-C-H Ar.Str.C=N Str. -C=O Str.-C-Cl str.		
P_6	2851,2922 1513,1176 3067,1605 836	-O-H Str.-C-H Ar.Str.-C=N Str. -C-O- Str.-N-H str.-C=O str.		
P7	2849,3068 3264,1599 1513,832 2926,1152	-O-H Str.-C-H Ar. Str.-N-H Str. -C=O Str.-C=N Str.-C-H def. -C-H str.-S=O str.	7.08-8.10 12.34,9.7 1.7.3.22 3.49.3.83	12H of Ar. CH 1H of -COOH 1H of Ar-C-NH 1H of Pyrazole 2H of CH2 2H of CH2 sub. to -COOH 1H of –OCH3
P8	2851,2922 3068.1604 1511	O-H Str. -C-H Ar.Str.-N-H Str.-C=O Str.-C=N Str.		
P9	3225.2924 3045.1585 1516.2860	-Ar-O-H Str.-C-H Ar.Str.-N-H Str. -C=O Str.C=N Str. -O-H str.		
P10	3225.2924 3045.1599 1513.2860	-Ar-O-H Str.-C-H Ar.Str.-N-H Str. -C=O Str.-C=N Str.-O-H str.		

Table No-2: Analytical & Physicochemical data of Pyrazolo Phenoxy acetic acid derivatives

Comp.	Mol. Formula	Mol. Wt.	m.p. °C	Yield %	Elemental analysis Calcd. (Found)			LogP	CLogP	CMR
					C	H	N			
P_1	$C_{24}H_{21}N_5O_4$	443.45	124-28	61	65.95 (65.00)	4.98 (4.77)	15.92 (15.79)	1.6	2.39	12.67
P_2	$C_{24}H_{22}N_4O_5S$	478.52	137-42	68	60.25	4.63	11.71	2.78	2.86	13.25
P_3	$C_{24}H_{20}ClN_5O_4$	477.90	114-18	67	60.32	4.22	14.65	2.22	3.10	13.16
P_4	$C_{24}H_{21}ClN_4O_5S$	512.97	171-175	73	56.40 (56.19)	4.25 (4.13)	11.10 (10.92)	3.34	3.57	13.75
P_5	$C_{19}H_{16}ClN_7O_3$	425.83	154-59	55	53.85	3.95	23.20	3.04	2.42	11.30
P_6	$C_{25}H_{23}N_5O_5$	473.48	120-124	65	63.42	4.90	14.79	1.73	2.31	13.29
P_7	$C_{25}H_{24}N_4O_6S$	508.55	144-48	78	59.25 (59.04)	4.85 (4.76)	11.20 (11.02)	2.65	2.77	13.87
P_8	$C_{26}H_{19}N_7O_4$	421.41	105-08	80	57.00	4.54	23.27	2.35	1.63	11.43
P_9	$C_{24}H_{21}N_5O_5$	459.45	164-167	83	63.12 (62.74)	4.82 (4.61)	15.46 (15.24)	1.21	1.75	12.82
P_{10}	$C_{24}H_{22}N_4O_6S$	494.52	88-92	53	58.30	4.48	11.35	2.29	1.91	13.75

The combustion analysis of compounds synthesized is within the limits of permissible errors.

Table No. 3

Antibacterial and Antifungal activity of Pyrazolo Phenoxy acetic acid derivatives (P_1-P_{10})

SL. No.	Compd.	Zone of inhibition at 100 mcg/mL (in mm.)			
		E.coli	*S. aureus*	*A. niger*	*C. albicans*
1	P_1	**24**	**25**	**26**	**27**
2	P_2	**24**	**25**	**27**	**28**
3	P_3	15	16	16	19
4	P_4	16	18	18	20
5	P_5	15	16	19	21
6	P_6	**24**	**23**	**25**	**27**
7	P_7	**23**	**25**	19	21
8	P_8	**24**	**25**	**26**	**27**
9	P_9	16	18	18	19
10	P_{10}	**24**	**23**	24	25
Standard	Ciprofloxacin	**20**	**22**	--	--
Standard	Griseofulvin	–	–	**24**	**24**

Compounds P_1, P_2, P_6, P_7, P_8 and P_{10} have shown promising antibacterial activity. Ciprofloxacin was used as standard drug. Compounds P_1, P_2, P_6 and P_8 have shown promising antifungal activity. Griseofulvin was used as standard drug.

REFERENCES:

A.L. Barry, The antimicrobial susceptibility test: principle & practices, edited by IIIus Le& Febiger (Philadelphia, Pa. USA), 180; Biol. Abstr., 64 (1976), 25783.

A. R, Paniker J. Text book of microbiology. 5th Edition, Madras: orient Longman, 1997; 36-44.

Bhat A.R,Chetan B.P and Srinivas M.T., Indian Journal of Heterocyclic Chemistry;13;2004;225-228

Furniss BS, Hannaford AJ, Smith PWG, Patchel AR. Vogel's Textbook of Practical Organic Chemistry. 5th edition, Singapore, Published by Pearson education (Singapore) Pvt. Ltd. 1996, 1034,1050-53

Indian pharmacopoeia. New Delhi: Govt. of India, 1996; 2: A: 104-08.

Kobayashi Gs, Medofff Gm. Antifungal agents: recent developments. Ann.Rev. Microbiology 1977; 31: 291-08.

Koneman EW, Allen SD, Janda MW, Washington CW. Text book of Diagnostic Microbiology. 5th Edition, Philadelphia: J B Lippincott company (P) Ltd; 1997; 935-939

Salvemini D, Manning P.J, Zweifel B.S, Seibert K, Conner J, Carrie M.G, et al. Dual inhibition of nitric oxide and prostaglandin production contributes to the anti-inflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 1995; 96:301-8.