Synthesis, Characterization and Antimicrobial Screening on New 1,5-Disubstituted Pyrazoline Derivatives Bearing P-Methoxy-M-Chloro Phenyl Moiety

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Abstract- A new series of 2-(ortho & para substituted phenoxy)-1-(3-(3-chloro-4-methoxyphenyl)-5-(substituted phenyl)-4,5-dihydropyrazol-1-yl)ethanone were synthesized. Ortho and para substituted phenol was refluxed with ethylchloroacetate in dry acetone in presence of anhydrous potassium carbonate to yield ethyl (ortho & para substituted phenoxy) acetate (1). The substituted ester on reaction with hydrazine hydrate yields 2-(ortho & para -substituted phenoxy) acetohydrazide (2). Chalcones (3a-3j) were prepared from the reaction between substituted aromatic aldehydes and 3-chloro-4-methoxy acetophenone in presence of a strong base. (2) On reaction with chalcones afforded the pyrazoline derivatives. The chemical structures of these compounds were confirmed by means of IR, ^{1}H NMR, mass spectral data and elemental analysis. Newly synthesized compounds were screened *in vitro* for their antimicrobial activity against varieties of gram positive and gram negative bacterial strains and fungi strains *Candida albicans* & *Aspergillus nigar* at 100 µg/mL.

Index Terms- 3-Chloro-4-methoxy acetophenone, chalcones, pyrazolines, antimicrobial.

I. INTRODUCTION

Many heterocyclic compounds due to their specific activity are employed in the treatment of many infectious diseases. Their use in the treatment is attributed to their inherent toxicity to various pathogens. Among a wide range of heterocyclic compounds that have been explored for the development of pharmaceutically important molecules. Many compounds possessing pyrazoles and their reduced forms pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities such as anticancer[1], antioxidant[2], antibacterial[3], antifungal[4], antidepressant[5-7], anti-inflammatory[8] , anticonvulsant[9], antitumor[10] , analgesic[11],properties. Literature survey reveals several synthetic protocols for the synthesis of these compounds and the presence of this core in any molecule plays a key role in enhancing the activity. Phenyl ring containing halogen and methoxy groups have shown significant biological activities enhance the biological activities of heterocyclic derivatives drastically[12-14]. Keeping in view of the above interesting pharmacological features, in the present study the synthesis of chalcones of 1-(3-chloro-4-methoxyphenyl)-3-(substituted)prop-2-en-1-ones have been carried out according to claisen-schmidt condensation of aromatic aldehydes with 3-chloro-4-methoxy acetophenone in presence of base and alcohol as solvent medium[15]. Ethyl 2-(substituted phenoxy)acetate 1(a-j) and 2-(substituted phenoxy) aceto hydrazide 2(a-j) were synthesized as reported in the literature[16].

II. EXPERIMENTAL

All the reported melting points were taken in open capillaries and are uncorrected. Infrared spectra were measured on Shimadzu- FTIR-8400S spectrophotometer (cm^{-1}), using KBr disks. ^{1}H-NMR spectra were measured by Bruker amx 400MHz spectrometer, deuterated solvents such as dimethyl-sulphoxide (DMSO-d_6), methanol (CD_3OD) and also chloroform ($CDCl_3$) were used as solvents and the chemical shifts were quoted as δ-value relative to tetramethyl silane (TMS δ=O) as an internal standard. Mass spectra were recorded on LC-MS Schimadzu 2010A using dimethyl sulfoxide as solvent. C, H and N analysis were performed at Sophisticated Test & Instrumentation centre, cochin university, Cochin, Kerala, India. The purity of the compounds was monitored by thin layer chromatography on silica gel plates and iodine was used as a visualizing agent.

General procedure for the synthesis of ethyl 2-(substituted phenoxy)acetate (1): To the substituted phenol (0.05mole) in dry acetone, ethylchloroacetate (0.05mole) was added and refluxed on water bath for 24hours in presence of potassium carbonate as catalyst. The reaction mixture was cooled and filtered, the excess solvent was distilled off to get phenoxy ester.

General procedure for the synthesis of acetohydrazide (2): To a solution of compound **1** (0.05mole) in ethanol (60ml), hydrazine hydrate (0.07 mole) was refluxed for 12 hours. The excess of solvent is distilled off and the solid thus seperated was recrystallized from ethanol

General procedure for the synthesis of chalcones (3a-j): Appropriate aromatic aldehydes (0.01 mol) were stirred with 3-Chloro-4-methoxy acetophenone in ethanol in presence of 40% sodium hydroxide for 24 hours. The resultant reaction mixture was poured on to crushed ice and acidified with dilute HCl. The seperated solid was filtered , dried and recrystallized from ethanol.

1-(3-chloro-4-methoxyphenyl)-3-phenylprop-2-en-1-one (3a)

IR (KBr, γ_{max}, cm^{-1}): 2924 (Ar-CH), 1691 (C=O), 1563 (C=C), 830 (Ar-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 7.99-7.01 (8H,

m, Ar-H), 7.04 (1H, d, CH), 7.41 (1H, d, CH), 3.73 (3H, s, OCH_3). MS (m/z): 273.4 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (3b)

IR (KBr, γ_{max}, cm^{-1}): 3024 (Ar-CH), 1698 (C=O),1567 (C=C), 836 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.75-7.10 (8H, m, Ar-H), 7.08 (1H, d, CH), 7.45 (1H, d, CH), 3.70 (3H, s, OCH_3). MS (m/z): 342.34 (M^+).

3-(5-bromo-2-hydroxyphenyl)-1-(3-chloro-4-methoxyphenyl)prop-2-en-1-one (3c)

IR (KBr, γ_{max}, cm^{-1}): 3028 (Ar-CH), 1712 (C=O),1544 (C=C), 840 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.78-7.03 (8H, m, Ar-H), 7.00 (1H, d, CH), 7.41 (1H, d, CH), 3.78 (3H, s, OCH_3). MS (m/z): 368.60 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (3d)

IR (KBr, γ_{max}, cm^{-1}): 2978 (Ar-CH), 1699 (C=O),1566 (C=C), 832 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.99-6.78 (8H, m, Ar-H), 7.01 (1H, d, CH), 7.41 (1H, d, CH), 3.81 (3H, s, OCH_3). MS (m/z): 303.57 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one (3e)

IR (KBr, γ_{max}, cm^{-1}): 2900 (Ar-CH), 1708 (C=O), 1563 (C=C), 830 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.99-7.04 (8H, m, Ar-H), 7.07 (1H, d, CH), 7.48 (1H, d, CH), 3.78 (3H, s, OCH_3). MS (m/z): 289.52 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (3f)

IR (KBr, γ_{max}, cm^{-1}): 3007 (Ar-CH), 1715 (C=O), 1560 (C=C), 831 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.78-7.11 (8H, m, Ar-H), 7.05 (1H, d, CH), 7.44 (1H, d, CH), 3.78 (3H, s, OCH_3). MS (m/z): 318.37 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(2-methoxynaphthalen-1-yl)prop-2-en-1-one (3g)

IR (KBr, γ_{max}, cm^{-1}): 2935 (Ar-CH), 1707 (C=O),1567 (C=C) 834 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.86-7.05 (8H, m, Ar-H), 7.08 (1H, d, CH), 7.47 (1H, d, CH), 3.77 (3H, s, OCH_3). MS (m/z): 353.77 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(4-chlorophenyl)prop-2-en-1-one (3h)

IR (KBr, γ_{max}, cm^{-1}): 3001 (Ar-CH), 1710 (C=O), 1568 (C=C) 835 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.55-7.01 (8H, m, Ar-H), 7.45 (1H, d, CH), 6.95 (1H, d, CH), 3.75 (3H, s, OCH_3). MS (m/z): 308.07 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (3i)

IR (KBr, γ_{max}, cm^{-1}): 2995 (Ar-CH), 1697 (C=O), 1566 (C=C) 838 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.68-7.09 (8H, m, Ar-H), 7.95 (1H, d, CH), 6.95 (1H, d, CH), 3.77 (3H, s, OCH_3). MS (m/z): 289.62 (M^+).

1-(3-chloro-4-methoxyphenyl)-3-p-tolylprop-2-en-1-one (3j)

IR (KBr, γ_{max}, cm^{-1}): 2970 (Ar-CH), 1696 (C=O),1568 (C=C) 832 (Ar-H). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.90-6.90 (8H, m, Ar-H), 7.91 (1H, d, CH), 7.11 (1H, d, CH), 3.70 (3H, s, OCH_3). MS (m/z): 287.75 (M^+).

General procedure for the synthesis of 2-(ortho & para substituted phenoxy)-1-(3-(3-chloro-4-methoxyphenyl)-5-(substituted phenyl)-4,5-dihydropyrazol-1-yl)ethanone (4a-j):

To a solution of chalcone (0.01 mol) in ethanol, acetohydrazide (0.01 mol) was added . The mixture was refluxed for 8-10 hours in presence of catalytic amount of glacial acetic acid and left overnight. The reaction mixture was poured onto crushed ice and the solid mass that seperated out was filtered, washed with ethanol, dried and recrystallized from DMF

1-(3-(3-chloro-4-methoxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)-2-phenoxyethanone (4a)

IR (KBr, γ_{max}, cm^{-1}): 3040 (Ar-CH), 1675 (C=O), 1590 (C=C), 1510(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 8.27-7.67 (13H,m, Ar), 5.35-5.26 (1H,dd, Hx of pyrazoline), 4.70 (2H,s,OCH2), 3.75(3H,s,OCH_3), 3.53-3.48 (1H,dd,Hb of CH2 of pyrazoline), 3.25-3.18 (1H,Ha of CH2 of pyrazoline). MS (m/z): 433.29 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(2,4-dichlorophenyl)-4,5-dihydropyrazol-1-yl)-2-(2-chlorophenoxy)ethanone (4b)

IR (KBr, γ_{max}, cm^{-1}): 3028 (Ar-CH), 1688 (C=O), 1546 (C=C), 1520(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 8.27-7.67 (10H,m, Ar), 5.40-5.29 (1H,dd, Hx of pyrazoline), 4.50 (2H,s,OCH2), 3.72(3H,s,OCH_3), 3.55-3.50 (1H,dd,Hb of CH2 of pyrazoline), 3.20-3.13 (1H,Ha of CH2 of pyrazoline). MS (m/z): 537.13 (M^+).

1-(5-(5-bromo-2-hydroxyphenyl)-3-(3-chloro-4-methoxyphenyl)-4,5-dihydropyrazol-1-yl)-2-(2,4,6-trichlorophenoxy)ethanone (4c)

IR (KBr, γ_{max}, cm^{-1}): 3497(OH), 3030 (Ar-CH), 1690 (C=O), 1548 (C=C), 1518 (N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 10.20 (1H,s,OH), 8.30-7.67 (8H,m, Ar), 5.38-5.29 (1H,dd, Hx of pyrazoline), 4.59 (2H,s,OCH2), 3.72(3H,s,OCH_3), 3.51-3.46 (1H,dd,Hb of CH2 of pyrazoline), 3.23-3.16 (1H,Ha of CH2 of pyrazoline). MS (m/z): 632.07 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl)-2-(4-hydroxyphenoxy)ethanone (4d)

IR (KBr, γ_{max}, cm^{-1}): 3500 (OH), 3021 (Ar-CH), 1686 (C=O), 1588 (C=C), 1515 (N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 9.83 (1H,s,OH) , 8.27-7.67 (11H,m, Ar), 5.35-5.26 (1H,dd, Hx of pyrazoline), 4.56 (2H,s,OCH2), 3.73(3H,s,OCH_3), 3.72(3H,s,OCH_3) 3.53-3.48 (1H,dd,Hb of CH2 of pyrazoline), 3.25-3.18 (1H,Ha of CH2 of pyrazoline). MS (m/z): 479.52 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(2-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-2-(4-nitrophenoxy)ethanone (4e)

IR (KBr, γ_{max}, cm^{-1}): 3310 (OH), 3098 (Ar-CH), 1694 (C=O), 1535 (C=C), 1517(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 10.01 (1H,s,OH) , 8.00-7.67 (11H,m, Ar), 5.65-5.56 (1H,dd, Hx of pyrazoline), 4.50 (2H,s,OCH2), 3.67 (3H,s,OCH_3), 3.55-3.50 (1H,dd,Hb of CH2 of pyrazoline), 3.20-3.13 (1H,Ha of CH2 of pyrazoline). MS (m/z): 494.19 (M^+).

2-(4-bromophenoxy)-1-(3-(3-chloro-4-methoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydropyrazol-1-yl)ethanone (4f)

IR (KBr, γ_{max}, cm^{-1}): 3008 (Ar-CH), 1699 (C=O), 1583 (C=C), 1515 (N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 7.57-6.67 (11H,m, Ar), 5.42-5.33 (1H,dd, Hx of pyrazoline), 4.36 (2H,s,OCH2), 3.70(3H,s,OCH_3), 3.50-3.45 (1H,dd,Hb of CH2 of pyrazoline), 3.19-3.12 (1H,Ha of CH2 of pyrazoline). MS (m/z): 557.39 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(2-methoxynaphthalen-1-yl)-4,5-dihydro pyrazol-1-yl)-2-(4-methoxyphenoxy)ethanone (4g)

IR (KBr, γ_{max}, cm^{-1}): 3038 (Ar-CH), 1679 (C=O), 1590 (C=C), 1515(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 8.27-6.83 (13H,m, Ar), 5.37-5.20 (1H,dd, Hx of pyrazoline), 4.50 (2H,s,OCH2), 3.73(6H,s,OCH_3), 3.72(3H,s,OCH_3), 3.58-3.48 (1H,dd,Hb of CH2 of pyrazoline), 3.20-3.14 (1H,Ha of CH2 of pyrazoline). MS (m/z): 529.08 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)-2-(2,4-dichlorophenoxy)ethanone (4h)

IR (KBr, γ_{max}, cm^{-1}): 3001 (Ar-CH), 1700 (C=O), 1590 (C=C), 1513(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 8.11-7.13 (10H,m, Ar), 4.99-4.90 (1H,dd, Hx of pyrazoline), 4.83 (2H,s,OCH2), 3.73(3H,s,OCH_3), 3.54-3.47 (1H,dd,Hb of CH2 of pyrazoline), 3.25-3.18 (1H,Ha of CH2 of pyrazoline). MS (m/z): 537.13 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-2-(2-hydroxyphenoxy)ethanone (4i)

IR (KBr, γ_{max}, cm^{-1}): 3498 (OH), 3040 (Ar-CH), 1682 (C=O), 1596 (C=C), 1513(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 10.20 (2H,s,OH) , 8.27-7.67 (11H,m, Ar), 5.35-5.26 (1H,dd, Hx of pyrazoline), 4.56 (2H,s,OCH2), 3.73(3H,s,OCH_3), 3.53-3.48 (1H,dd,Hb of CH2 of pyrazoline), 3.25-3.18 (1H,Ha of CH2 of pyrazoline). MS (m/z): 465.17 (M^+).

1-(3-(3-chloro-4-methoxyphenyl)-5-p-tolyl-4,5-dihydropyrazol-1-yl)-2-(4-chlorophenoxy)ethanone (4j)

IR (KBr, γ_{max}, cm^{-1}): 3066 (Ar-CH), 1682 (C=O), 1596 (C=C), 1513(N-N). ^1H NMR (400 MHz, $CDCl_3$, δ ppm); 8.21-7.00 (11H,m, Ar), 5.45-5.36 (1H,dd, Hx of pyrazoline), 4.66 (2H,s,OCH2), 3.77(3H,s,OCH_3), 3.53-3.48 (1H,dd,Hb of CH2 of pyrazoline), 3.25-3.18 (1H,Ha of CH2 of pyrazoline), 2.19 (3H,s,CH_3) MS (m/z): 482.17 (M^+).

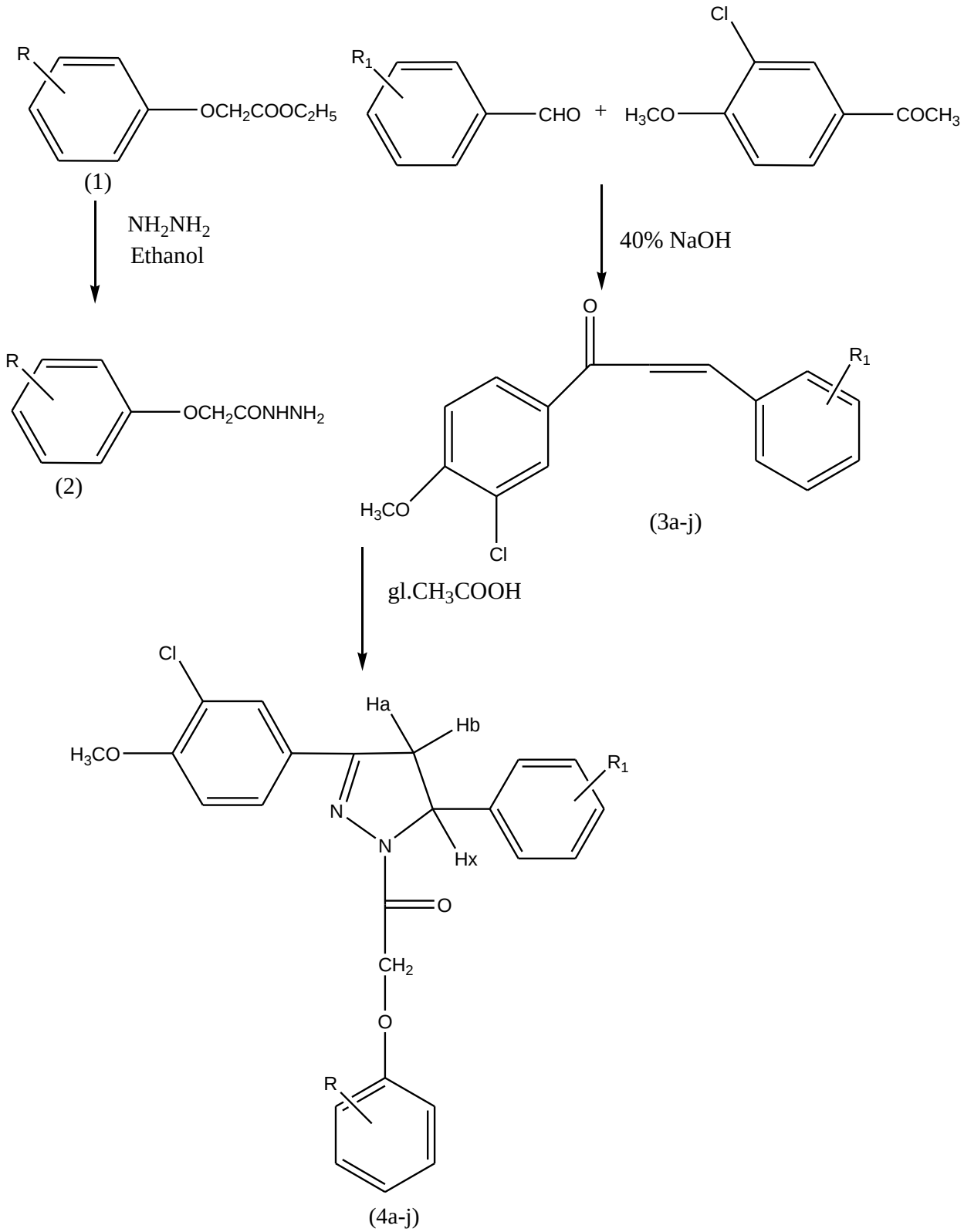

Table I: Physicochemical data of chalcone derivatives (3a-j).

Code	R_1	Molecular Formula	M.W	Melting Point(°C)	Rf	% yield
3a	-H(Phenyl)	$C_{16}H_{13}ClO_2$	272.72	152	0.87	84

3b	-2,4-Cl	$C_{16}H_{11}Cl_3O_2$	341.61	174	0.65	80
3c	-5-Br,2- OH	$C_{16}H_{12}BrClO_3$	367.62	186	0.72	90
3d	-4-OCH_3	$C_{17}H_{15}ClO_3$	302.75	140	0.81	78
3e	-2-OH	$C_{16}H_{13}ClO_3$	288.72	176	0.56	86
3f	4-NO_2	$C_{16}H_{12}ClNO_4$	317.72	90	0.60	80
3g	-2-OCH_3-H(Naphthyl)	$C_{21}H_{17}ClO_3$	352.81	191	0.59	88
3h	-4-Cl	$C_{16}H_{12}Cl_2O_2$	307.17	158	0.74	68
3i	-4-OH	$C_{16}H_{13}ClO_3$	288.72	138	0.68	79
3j	-4-CH_3	$C_{17}H_{15}ClO_2$	286.75	124	0.90	76

Table II: physicochemical data of pyrazoline derivatives (4a-j).

Code	R	R_1	Molecular Formula	M.W	M.P (°C)	Rf	% yield	Element % cal (found)		
								C	H	N
4a	-H	-H(Phenyl)	$C_{25}H_{21}ClN_2O_3$	432.89	192	0.57	72	69.30	4.85	6.46
								(69.27)	(4.87)	(6.43)
4b	-2-Cl	-2,4-Cl	$C_{25}H_{18}Cl_4N_2O_3$	536.23	198	0.86	84	55.94	3.35	5.22
								(55.93)	(3.31)	(5.21)
4c	-2,4,6-Cl	-5-Br,2- OH	$C_{25}H_{17}BrCl_4N_2O_4$	631.12	213	0.91	80	47.53	2.69	4.43
								(47.50)	(2.67)	(4.41)
4d	-4-OH	-4-OCH_3	$C_{26}H_{23}ClN_2O_5$	478.92	175	0.59	71	65.14	4.80	5.84
								(65.11)	(4.79)	(5.84)
4e	-4-NO_2	-2-OH	$C_{25}H_{20}ClN_3O_6$	493.89	206	0.68	74	60.74	4.04	8.50
								(60.73)	(4.02)	(8.49)
4f	-4-Br	4-NO_2	$C_{25}H_{19}BrClN_3O_5$	556.79	207	0.70	78	53.88	3.41	7.54
								(53.87)	(3.40)	(7.52)
4g	-4-OCH_3	-2-OCH_3-H (Naphthyl)	$C_{31}H_{29}ClN_2O_5$	528.98	218	0.81	87	70.32	5.48	5.29
								(70.30)	(5.46)	(5.27)
4h	-2,4-Cl	-4-Cl	$C_{25}H_{18}Cl_4N_2O_3$	536.23	188	0.97	83	55.94	3.35	5.29
								(55.91)	(3.33)	(5.27)
4i	-2-OH	-4-OH	$C_{25}H_{21}ClN_2O_5$	464.89	166	0.61	75	64.53	4.51	6.02
								(64.51)	(4.50)	(6.00)
4j	-4-Cl	-4-CH_3	$C_{26}H_{22}Cl_2N_2O_3$	481.37	144	0.78	73	64.81	4.57	5.81
								(64.78)	(4.56)	(5.80)

III. ANTIMICROBIAL ACTIVITY

In vitro antibacterial screening

All the newly synthesized compounds (4a-j) were screened in vitro for their antibacterial activity against *Bacillus subtilis*, and *Pseudomonas aeruginosa* by disc diffusion method[19] was performed using Mueller.Hinton agar (Hi-Media) medium. Each compound was tested at a concentration at 100µg/mL in DMSO. The diameter of zone of inhibition was measured in mm after 24hours incubation at 37°C. The known compound ciprofloxacin was used as standard drug for comparison study. The antibacterial screening data are recorded in Table III.

In vitro antifungal screening

The compounds (4a-j) were evaluated for their in vitro antifungal activity against *Candida albicans* and *Aspergillus nigar* using disc diffusion method[20] with sabouraud's dextrose agar (Hi-Media). Each compound was tested at a concentration of 100µg/mL in DMSO. The zone of inhibition (mm) was measured. The known compound Amphotericin B was used as

standard drug for comparison study. The antifungal screening data are recorded in Table III.

Table III: Antimicrobial activity data of pyrazoline derivatives 4(a-j).

Code.	Antibacterial activity Zone of inhibition (in mm)		Antifungal activity Zone of inhibition (in mm)	
	Bacillus subtilis	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillus nigar***
4a	10	8	12	13
4b	13	12	23	10
4c	27	25	23	24
4d	12	11	13	15
4e	25	17	15	12
4f	26	19	16	16
4g	15	28	14	18
4h	11	12	23	15
4i	10	10	7	8
4j	8	7	9	10
Ciprofloxacin	37	38	-ve	-ve
Amphotericin B	-ve	-ve	35	36

IV. Results and Discussion

All the reactions were carried out under prescribed laboratory conditions. The solvents and reagents used in synthetic work were of laboratory grade and were purified by distillation. The title compounds 2-(ortho & para substituted phenoxy)-1-(3-(3-chloro-4-methoxyphenyl)-5-(substituted phenyl)-4,5-dihydro pyrazol -1-yl)ethanone 4(a-j) were obtained by the cyclisation rection shown by the mixture of 2-(ortho and para substituted phenoxy)acetohydrazide and 1-(3-chloro-4-methoxyphenyl)-3-(substituted phenyl)prop-2-en-1-one resulted in good yields (71-87%). All the synthesized pyrazoline derivatives were screened for antibacterial activity showing moderate to significant activity against bacterial strains i.e., *Bacillus subtilis* (Gram positive), *Pseudomonas aeruginosa* (Gram negative) and fungal strains i.e., *Candida albicans* and *Aspergillus nigar* using cup-plate method. Compound (4g) has shown significant activity against *Pseudomonas aeruginosa* due to the presence of substituted naphthyl group. From the above observations it is evident that the compounds that contain chloro, bromo and nitro groups have shown maximum activity against tested microorganisms. The ^{1}HNMR spectra of pyrazolines (4a-j) displayed three characteristic signals due to the diastereotopic proton [17,18.] (Ha,Hb and Hx). The Ha proton, was cis to Hx resonated upfield in the range δ 3.13-3.25 as doublet of doublet (dd, J=～18.07 and 4.60Hz), while the Hb proton was trans to Hx resonated downfield in the range of δ 3.45-3.58 (dd,J=17.89 and 12.21Hz). The Hx proton which was vicinal to two methylene protons (Ha & Hb) was also observed as doublet of doublet at δ values ranging from 5.20-5.65 (dd, J=11.74 and 4.66Hz).

V. Conclusion

The present study is aimed to synthesize, characterize and also to evaluate the antimicrobial activity of some new chalcones and 1,5-disubstituted pyrazoline derivatives bearing p-methoxy-m-chloro phenyl moiety. All the synthesized pyrazoline derivatives have shown promising antimicrobial results and some are showing zone of inhibition (mm) very similar to the standard drug used. Investigations are in progress to explore the possible mechanism of action of the synthesized compounds.

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