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Synthesis and Evaluation of New Substituted Pyrazoline Derivatives as Biological Agents

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ABSTRACT

Twelve new 3-(4-substituted benzyl)-5-(4-substituted phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole derivatives were synthesized by reacting 1-(4-substituted phenyl)-4-(4 substituted phenyl) but-3-en-2-one and phenyl hydrazine in ethanol. 1-(4-substituted phenyl)-4-(4 substituted phenyl) but-3-en-2-one were synthesized by condensation of substituted phenylacetone and substituted benzaldehyde in NaOH solution. The synthesized compounds were confirmed by spectra data. The compounds were evaluated in-vitro for their antibacterial, antifungal, anti-tubercular activity and in-vivo for their anti-inflammatory activity. Compounds P_{25}, P_{29}, P_{31} and P_{32} were found to exhibit good antimicrobial and anti-inflammatory activity.

Key words: Pyrazoline, Antibacterial, Antifungal, Anti-tubercular, Anti-inflammatory activity.

INTRODUCTION

Infections play an important role in more diseases; among them many communicable diseases have been effectively contained. Infectious diseases associated with pain and inflammation remains a major cause of morbidity and mortality; particularly in developing countries. Especially tuberculosis (TB) is the second leading cause of death. However the increasing prevalence of antimicrobial resistance among pathogenic microbes is increasing. It is currently estimated that within the next 10 years many antimicrobial agents currently employed for treatment of infectious diseases will no longer be effective due to microbial resistance. The emergence of such resistance may in part depend on the acquisition of new mechanism of interference with antimicrobial activity and on the spread of resistant isolates between patients[1-3].

Billions of dollars are being spent by pharmaceutical companies to identify and develop potent therapeutic agent to over come these challenge. According to a literature survey, in present years a significant portion of research work is been carried out in heterocyclic chemistry. Among them considerable attention has been focused on pyrazoline derivatives, as they posses a variety of

significant and diverse pharmacological activities such as antibacterial[4], antifungal[5], antiviral[6], anti-tubercular[7], antiamoebic[8], anti-inflammatory[9], analgesic[10], antidepressant[11], anticancer[12] and anti-diabetic activity[13].

Keeping in view, it was though to design the novel new chemical entities and in continuation of our previous work[14] we report the synthesis of new substituted pyrazoline derivatives and evaluate them for their antibacterial, antifungal, anti-tubercular and anti-inflammatory activity.

MATERIAL AND METHODS

The chemical and solvents were purchased commercial of analytical grade. Melting points were determined in open tube capillary method and were uncorrected. The homogeneity of the synthesized compounds was routinely checked by thin layer chromatography. IR spectra of all compounds were recorded on a Jasco FTIR-460 spectrometer using KBr disc method. ^{1}HNMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using DMSO/ $CDCl_3$ as solvent and TMS as an internal standard.

General procedure for the synthesis of 1-(4-substituted phenyl)-4-(4 substituted phenyl) but-3-en-2-one[4] ($C_{25, 26, 28, 29, 31, 32, 34, 35}$).[15]

An equimolar mixture of 4-substituted phenylacetone and 4-substituted benzaldehyde was added to a rapidly mechanically stirred solution of NaOH (2g in200 ml) at 65^{0}C. After 18 hours the mixture was cooled at room temperature and extracted with five 50 ml portion of CH_2Cl_2. The extract was washed with water and dried over $MgSO_4$ and solvent was evaporated under reduced pressure. The crude product obtained was recrystallized from appropriate solvent.

General procedure for the synthesis of 1-(4-substituted phenyl)-4-(4-nitro-phenyl) but-3-en-2-one[5] ($C_{27, 30, 33, 36}$).

A solution of 10% NaOH (8-10ml) was added drop wise to a well stirred equimolar solution of substituted phenylacetone and 4-nitro-benzaldehyde in 20ml of ethanol and was stirred for 24 hours in ice-bath. The reaction was monitored by TLC. Then the mixture was diluted with ice-water and acidified with conc. HCl. The crude product was filtered and recrystallized from appropriate solvent.

General procedure for the synthesis of 3-(4-substituted benzyl)-5-(4-substituted phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole[6] (P_{25}-P_{36}).[16]

To a magnetically stirred solution of 1-(4-substituted phenyl)-4-(4 substituted phenyl) but-3-en-2-one (C_{25-36}) in ethanol (30 ml) at room temperature equimolar quantity of phenyl hydrazine was added drop wise. The mixture was stirred continuously till the disappearance of starting material (6-10 hours); the reaction was monitored by TLC. Then the solvent was evaporated under reduced pressure to give a crude mass, which was recrystallized from appropriate solvent.

3- benzyl-5-(4-chloro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{25})

Yield-58.09%, m.p-95-98^{0}C, R_f: 0.65 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3068.96 (-ArC-H Str), 2978.42 (C-H Str), 2883.03 (C-H Str sym), 1592.30 (-C=N Str), 1519.03 (Ar-C=C-Str), 750.67 (C-Cl Str).

4-(5 -benzyl-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl)-phenol (P_{26})

Yield-57.62%, m.p-151-153^{0}C, R_f: 0.60 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3273.80 (OH Str), 3028.74 (-ArC-H Str), 2938.08 (C-H Str), 2858.28 (C-H Str sym), 1598.88 (-C=N Str), 1497.03 (Ar-C=C- Str). ^{1}H NMR ($CDCl_3$, δ ppm)**:** 7.96-7.26 (m, 14H, Ar-H), 4.62-4.42 (t, $1H_x$, CH), 4.16 (s, 1H, OH), 3.89 (s, 2H, CH_2), 3.15-3.12 (d, $1H_b$, CH), 2.20-2.17 (d, $1H_a$, CH). GC- MS: m/z-328 (M^+).

3- benzyl-5-(4-nitro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{27})

Yield-55.43%, m.p-123-124^{0}C, R_f: 0.70 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3040.19 (-ArC-H Str), 2921.66 (C-H Str), 2826.11 (C-H Str sym), 1587.29 (-C=N Str), 1501.62 (Ar-C=C-Str), 1338.83 (C-NO_2 Str).

5-(4-chloro-phenyl)-3-(4-fluoro-benzyl)-1-phenyl-4,5-dihydro-1H-pyrazole (P_{28})

Yield-62.08%, m.p-129-131^{0}C, R_f: 0.57 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3066.61 (-ArC-H Str), 2942.11 (C-H Str), 2885.76

(C-H Str sym), 1586.08 (-C=N Str), 1501.31 (Ar-C=C- Str), 1114.44 (C-F Str), 746.73 (C-Cl Str). ^{1}H NMR (DMSO-d_6, δ ppm)**:** 7.70-7.09 (m, 13H, Ar-H), 4.31-4.30 (t, 1H$_x$, CH), 3.64 (s, 2H, CH$_2$), 3.26-3.24 (d, 1H$_b$, CH), 2.18-2.17 (d, 1H$_a$, CH). ES MS: m/z-364.4 (M+1$^+$), 366.4 (M+2$^+$).

4-[5-(4-fluoro-benzyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-phenol(P_{29})

Yield-60.69%, m.p-157-159^{0}C, R_f: 0.63 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3350.08 (OH Str), 3134.25 (-ArC-H Str), 2916.54 (C-H Str), 2855.22 (C-H Str sym), 1596.88 (-C=N Str), 1536.21 (Ar-C=C- Str), 1164.73 (C-F Str). ^{1}H NMR ($CDCl_3$, δ ppm)**:** 7.70-7.16 (m, 13H, Ar-H), 5.19-5.16 (t, 1H$_x$, CH), 4.72 (s, 1H, OH), 3.56 (s, 2H, CH$_2$), 3.15-3.12 (d, 1H$_b$, CH), 2.19-2.17 (d, 1H$_a$, CH).

3-(4-fluoro-benzyl)-5-(4-nitro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{30})

Yield-52.40%, m.p-145-147^{0}C, R_f: 0.65 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3073.12 (-ArC-H Str), 2945.97 (C-H Str), 2854.73 (C-H Str sym), 1596.49 (-C=N Str), 1516.70 (Ar-C=C- Str), 1314.15 (C-NO$_2$ Str), 1115.07 (C-F Str).

3-(4-chloro-benzyl)-5-(4-chloro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{31})

Yield-58.79%, m.p-103-105^{0}C, R_f: 0.63 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3115.50 (-ArC-H Str), 2945.60 (C-H Str), 2871.96 (C-H Str sym), 1591.16 (-C=N Str), 1515.07 (Ar-C=C- Str), 710.47 (C-Cl Str). ^{1}H NMR ($CDCl_3$, δ ppm)**:** 7.85-7.33 (m, 13H, Ar-H), 5.38-5.25 (t, 1H$_x$, CH), 3.69-3.67 (d, 1H$_b$, CH), 2.55 (s, 2H, CH$_2$), 2.17-2.15 (d, 1H$_a$, CH). ES MS: m/z-381.3 (M$^+$).

4-[5-(4-chloro-benzyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-phenol (P_{32})

Yield-55.24%, m.p-165-167^{0}C, R_f: 0.72 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3268.28 (OH Str), 3070.32 (-ArC-H Str), 2978.21 (C-H Str), 2895.36 (C-H Str sym), 1587.80 (-C=N Str), 1503.01 (Ar-C=C- Str), 749.59 (C-Cl Str).

3-(4-chloro-benzyl)-5-(4-nitro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{33})

Yield-44.75%, m.p-118-120^{0}C, R_f: 0.51 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3141.76 (-ArC-H Str), 2921.86 (C-H Str), 2852.22 (C-H Str sym), 1595.55 (-C=N Str), 1544.57 (Ar-C=C- Str), 1313.38 (C-NO$_2$ Str), 724.50 (C-Cl Str).

3-(4-bromo-benzyl)-5-(4-chloro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{34})

Yield-74.52%, m.p-110-112^{0}C, R_f: 0.60 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3032.73 (-ArC-H Str), 2960.93 (C-H Str), 2906.44 (C-H Str sym), 1614.76 (-C=N Str), 1516.96 (Ar-C=C- Str), 760.61 (C-Cl Str), 638.21 (C-Br Str). ^{1}H NMR ($CDCl_3$, δ ppm)**:** 7.68-7.21 (m, 13H, Ar-H), 4.25-4.21 (t, 1H$_x$, CH), 3.87-3.86 (d, 1H$_b$, CH), 2.39 (s, 2H, CH$_2$), 2.18-2.17 (d, 1H$_a$, CH).

4-[5-(4-bromo-benzyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-phenol (P_{35})

Yield-70.44%, m.p-171-173^{0}C, R_f: 0.60 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3261.87 (OH Str), 3077.27 (-ArC-H Str), 2959.86 (C-H Str), 2895.85 (C-H Str sym), 1615.87 (-C=N Str), 1516.87 (Ar-C=C- Str), 638.70 (C-Br Str).

3-(4-bromo-benzyl)-5-(4-nitro-phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole (P_{36})

Yield-47.24%, m.p-159-161^{0}C, R_f: 0.70 (methanol: chloroform/1:9), IR (KBr) $_{Vmax}$ cm^{-1}: 3045.63 (-ArC-H Str), 2942.72 (C-H Str), 2872.25 (C-H Str sym), 1593.44 (-C=N Str), 1492.41 (Ar-C=C- Str), 1304.34 (C-NO$_2$ Str), 630.66 (C-Br Str). ^{1}H NMR ($CDCl_3$, δ ppm)**:** 7.85-6.92 (m, 13H, Ar-H), 4.67-4.65 (t, 1H$_x$, CH), 3.86-3.84 (d, 1H$_b$, CH), 2.71 (s, 2H, CH$_2$), 2.17-2.15 (d, 1H$_a$, CH).

Antimicrobial Activity[17-19]

The antibacterial activity of all newly synthesized pyrazoline derivatives was carried out in-vitro by cup plate agar diffusion method, against Staphylococcus aureus, Enterococcus. Faecalis (Gm +ve bacteria), Escherichia coli, Klebsiella. Pneumonia (Gm -ve bacteria) at 75 µg level. Similarly the antifungal activity was carried out against two strains of fungi Aspergillus niger and Candida albicans at 75 µg level. Ciprofloxacin and Fluconazole were used as standard drugs respectively at 75 µg /ml. Dimethyl formamide was Control test which showed no inhibition of the microbial growth. The antibacterial and antifungal activity of synthesized compounds is given in table 1.

Anti-Tubercular Activity [20]

The anti-tubercular screening of all synthesized pyrazoline derivatives was carried out by Middle brook 7H9 broth against *Mycobacterium tuberculosis* of H37Rv strain by Microplate Alamar Blue Assay (MABA) method. This method is non-toxic and shows good correlation with BACTEC radiometric method.

All outer perimeter wells of sterile 96 wells plate were added 200µl of sterile deionzed water to minimized evaporation of medium in the test wells during incubation. 100 µl of the Middlebrook 7H9 broth was added in 96 wells plate and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 0.01 to 20.0 µl/ml. 100 µl of mycobacterium tuberculosis inoculum was added to the 96 wells plate yielding a final volume of 200 µl per well. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. Isoniazid was used as standard drug. (Table 1)

Acute oral toxicity

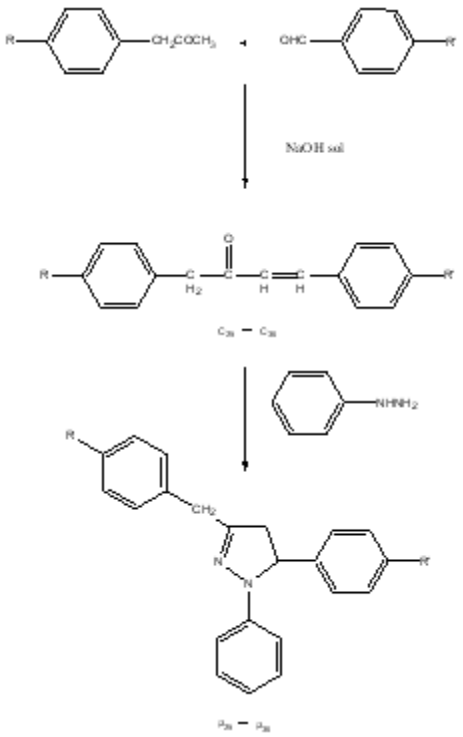
The acute oral toxicity of newly synthesized pyrazoline derivatives was determined by using Swiss albino mice (22-30 g) as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA. Animal ethical committee clearance was obtained for carrying out the experiment (IAEC/MMCP/2011-12/06).

Anti-inflammatory Activity[21]

All the synthesized compounds were screened for in-vivo anti-inflammatory activity by carrageenan induced rat hind paw method. Rats were assigned into 14 groups of 6 animals each weighing 150-200 g. The first group was administered 0.5% w/v of sodium CMC, which served as control. Group II was administered 100 mg/kg body weight of Diclofenac sodium as standard drug. Group III to XIV received 200 mg/kg body weight of synthesized compounds. All the drugs were administered orally 30 mins before the carrageenan injection. Acute inflammation was induced in each group by injecting sub-cuteanous 0.1ml of freshly prepared 1% carrageenan (0.1 ml of 1% suspension in 0.9% saline) into the sub-plantar region of left hind paw The initial reading was taken at 0hr., i.e., immediately after injecting carrageenan and the procedure was repeated at 1, 2, 3, and 4 hours after carrageenan injection with the help of Plethysmometer by water displacement. The mean paw volume at different times was calculated and compared with the control. (Table 2)

RESULTS AND DISCUSSION

In this study twelve new 3-(4-substituted benzyl)-5-(4-substituted phenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole derivatives (P_{25}-P_{36}) were prepared by the action of phenyl hydrazine on 1-(4-substituted phenyl)-4-(4 substituted phenyl) but-3-



Comp. code	R	R'	Comp. code	R	R'
P_{25}	H	Cl	P_{31}	Cl	Cl
P_{26}	H	OH	P_{32}	Cl	OH
P_{27}	H	NO_2	P_{33}	Cl	NO_2
P_{28}	F	Cl	P_{34}	Br	Cl
P_{29}	F	OH	P_{35}	Br	OH
P_{30}	F	NO_2	P_{36}	Br	NO_2

Scheme 1:

en-2-one (C_{25}-C_{36}), in turn generated by hydroxide catalyzed condensation of phenylacetone and substituted phenylacetone with substituted benzaldehyde except 4-nitro-benzaldehyde, which was proceeded by piperidine catalyzed condensation in NaOH solution.

Formation of intermediate and final derivatives was confirmed on the basis of their spectral data. The intermediate derivatives (C_{25}-C_{36}) showed a characteristic IR absorption peak in range of 1623-1725 cm^{-1} indicating the presence of a

Table 1: Antibacterial, Antifungal and Anti-tubercular activity of synthesized compounds. (P_{25}-P_{36})

Compounds	Zone of inhibition at 75 µg/ml						MIC
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumonia*	*C. albicans*	*A. biger*	µg/ml
P_{25}	22	20	18	21	18	15	3.125
P_{26}	18	17	15	18	15	17	6.25
P_{27}	12	13	14	15	12	14	25
P_{28}	14	12	10	14	11	15	25
P_{29}	21	21	20	22	17	15	3.125
P_{30}	10	15	16	17	14	12	50
P_{31}	20	19	18	22	23	20	3.125
P_{32}	21	20	21	20	24	22	3.125
P_{33}	11	14	16	15	13	15	25
P_{34}	13	15	11	12	10	10	25
P_{35}	17	13	14	19	16	14	6.25
P_{36}	15	16	17	13	11	17	25
Ciprofloxacin	28	27	26	29	-	-	-
Fluconazole	-	-	-	-	30	31	-
Isoniazid	-	-	-	-	-	-	0.2

Table 2: Anti-inflammatory activity of synthesized compounds (P_{25}-P_{36})

Comp	Mean paw oedema volume ± SEM				
	0 hour	1 hour	2 hour	3 hour	4 hour
Ct.	1.002±0.038	1.348±0.017	1.593±0.024	1.743±0.034	1.773±0.026
Std.	1.037±0.043	1.2320.026	1.418±0.017**	1.338±0.020***	1.215±0.021***
P_{25}	1.070±0.041	1.303±0.030	1.508±0.033	1.498±0.036***	1.427±0.029***
P_{26}	1.042±0.048	1.253±0.044	1.488±0.042	1.475±0.037***	1.423±0.038***
P_{27}	1.098±0.032	1.310±0.042	1.515±0.038	1.497±0.036***	1.433±0.027***
P_{28}	1.070±0.044	1.300±0.034	1.510±0.028	1.463±0.016***	1.420±0.013***
P_{29}	1.102±0.051	1.332±0.048	1.557±0.045	1.558±0.052**	1.480±0.047***
P_{30}	1.160±0.040	1.380±0.041	1.558±0.034	1.552±0.024***	1.483±0.019***
P_{31}	1.087±0.052	1.3330.044	1.535±0.031	1.468±0.013***	1.353±0.018***
P_{32}	1.072±0.057	1.337±0.045	1.522±0.043	1.455±0.035***	1.327±0.026***
P_{33}	1.118±0.031	1.395±0.034	1.573±0.032	1.538±0.031***	1.497±0.026***
P_{34}	1.113±0.038	1.342±0.053	1.545±0.047	1.510±0.048***	1.427±0.042***
P_{35}	1.085±0.027	1.342±0.027	1.547±0.032	1.497±0.034***	1.435±0.030***
P_{36}	1.100±0.044	1.308±0.033	1.503±0.032	1.468±0.035***	1.395±0.030***

All values are expressed as mean±SEMs, ***p<0.001, **p<0.05, *p<0.01, One way ANOYA followed by Dunnett's't' test

conjugated carbonyl group (>C=O) which was absence in the spectra of final derivatives (P_{25}-P_{36})

The compounds were tested in-vitro for their antimicrobial activity. Almost all the compounds have shown some antimicrobial activity. The compound P_{25}, P_{29} and P_{32} showed promising activity against Staphylococcus aureus where as other compounds showed moderate to poor activity. Compound P_{25} and P_{32} showed significant activity against E. Faecalis and other have shown low activity. Compound P_{29} and P_{32} have shown significant activity against Escherichia coli and compound P_{25}, P_{29} and P_{31} have shown significant activity against K. pneumonia and rest compounds have shown moderate and low activity. The compound P_{31} and P_{32} have shown excellent activity against Albican Candida and Aspergillus Niger where as rest of compounds have shown moderate to low activity against these fungi. The synthesized compounds were screened in-vitro for anti-tubercular activity and compound P_{25}, $P_{29,}$ $P_{31}$and P_{32} have exhibited good activity (MIC-3.125 µg/ml) against M. tuberculosis and rest of compounds have have shown moderste to low activity compared with standard Isoniazid (MIC-0.2 µg/ml).

The synthesized compounds were also screened for their in-vivo anti-inflammatory activity. All the compounds have shown varying degree of anti-inflammatory activity by inhibition of rat paw edema volume. Compound P_{31} and P_{32} have shown good activity as compared with standard Diclofenac sodium and rest of compounds has shown poor activity.

From the study it can be reported that the presence of P-Chloro-phenyl and P-Hydroxy-phenyl at position 5th along with P-Chloro-phenyl/ P-Fluoro-phenyl/ phenyl at position 3rd of the pyrazoline nucleus have exhibited better all the activities as compared with the other synthesized pyrazoline derivatives. Further optimization of these compounds may result into a more potent and effective agents.

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