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SYNTHESIS AND CHARACTERIZATION OF (4Z)-4-(SUBSTITUTED BENZYLIDENE)-5-METHYL-2, 4-DIHYDRO-3H-PYRAZOL-3-ONE FOR THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

A new series of (4Z)-4-(substituted benzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4a-h) were synthesized by reacting 5-methyl-2, 4-dihydro-3H-pyrazol-3-one (2) with substituted benzaldehydes, anhydrous sodium carbonate under reflux. The title compounds have been characterized by IR, ^{1}H NMR and Mass spectral data. The synthesized compounds were evaluated for their qualitative antibacterial and *in vitro* antioxidant activity by agar diffusion and DPPH$^{\cdot}$ methods, respectively. In general, the title compounds have shown mild to moderate antibacterial and antioxidant activity as well.

Key words: Pyrazole, Characterization, Antibacterial, Antioxidant activity.

INTRODUCTION

The pyrazole ring is a prominent structural found in numerous pharmaceutically active compounds. Pyrazole framework plays an essential role in biologically active compounds and therefore represents an interesting template for combinatorial as well as medicinal chemistry[1-2]. The pyrazole nucleus is a ubiquitous feature of pharmacological interest and has been proven to be a fertile source of medicinal agents such as antibacterial[3], antifungal[4], antiviral[5], antitubercular[6], antiamoebic[7], antiandrogenic[8], etc. Some of these compounds have also exhibited anti-inflammatory[9], antidiabetic[10], anaesthetic[11], analgesic[12] and antiparasitic[13] properties. We have aimed at the synthesis of (4Z)-4-(substituted benzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4a-h) for antibacterial activity against two gram positive and two gram negative organisms using standard substance. The antioxidant activity was performed by DPPH$^{\cdot}$ method using ascorbic acid as standard. The

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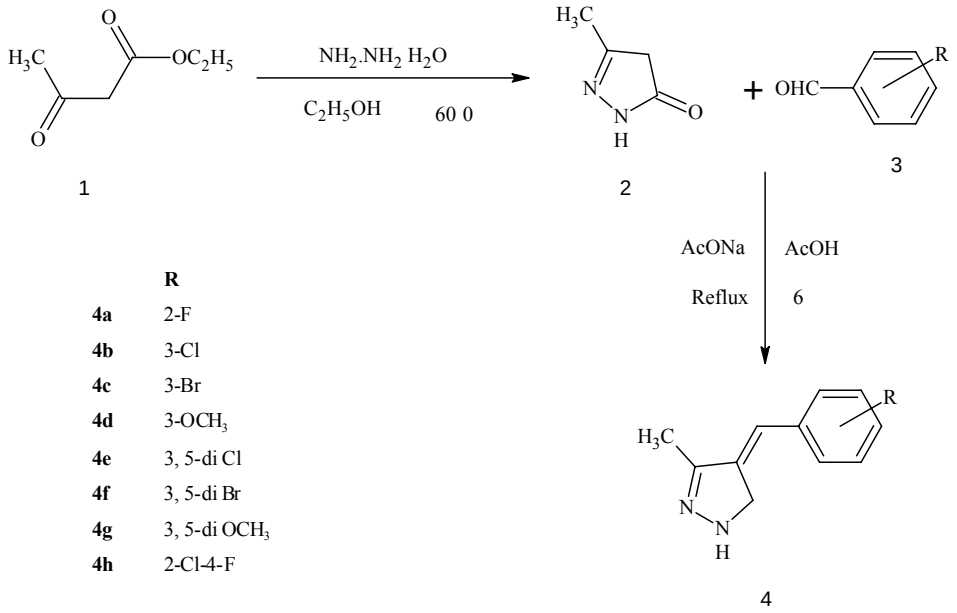
title compounds were synthesized by reacting 5-methyl-2,4-dihydro-3H-pyrazol-3-one (2) with substituted benzaldehydes, anhydrous sodium carbonate (Fig. 1). The yields of the title compounds (4a-h) were found to be in the range of 60.00-80.00%. The title compounds (4a-h) were confirmed by characteristic IR peak at 3200.72-34500.42 cm^{-1} (-NH) for amino function. The title compound showed NMR peaks. Compounds were also characterized by MS in which molecular ion peaks were in good agreement with the molecular weight of the title compounds. Partition coefficient of the title compounds have been determined by using ACD/labs software v 11.0 and found to be in the range of 1.35-2.85.



	R
4a	2-F
4b	3-Cl
4c	3-Br
4d	3-OCH_3
4e	3, 5-di Cl
4f	3, 5-di Br
4g	3, 5-di OCH_3
4h	2-Cl-4-F

Fig. 1: Scheme of the reaction

EXPERIMENTAL

Melting points of the synthesized compounds were determined using Thiele's melting point apparatus and were found uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellets in the range 4000-400 cm^{-1} on a Fourier Transform IR Spectrophotometer (Model Shimadzu 8700, at Strides Arcolab Limited, Bangalore) and frequencies were recorded in wave numbers (cm^{-1}). The ^{1}H NMR spectra were recorded on Amx-400 liquid state PMR spectrometer (Indian Institute of Science, Bangalore). Chemical shifts (δ) are reported in parts per million (ppm) downfield from internal reference tetramethylsilane (TMS). Mass spectrum was recorded by LC-MS (model-Shimadzu, Quest, Bangalore). Purity of the compounds was checked by thin layer chromatography. The physical constants of the title compounds are reported in Table 1.

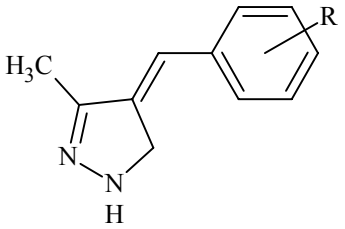
General procedure for the preparation of 5-methyl-2,4-dihydro-3H-pyrazol-3-one (2)[14]

Ethyl acetoacetate (0.1 mole) was taken in conical flask and hydrazine hydrate (0.2 mole) in ethanol (20 mL) was added drop wise to it with stirring. The temperature raised during this addition and it was maintained at 60^{o}C when a crystalline solid separated. The reaction-mixture was further stirred for 1 hr at room temperature then cooled in an ice bath to complete the crystallization. Separated solid was washed with ice cold ethanol.

General procedure for the preparation of 4-(Substituted benzylidene)-5-Methyl-2,4-dihydro-3H-pyrazol-3-one (4a-h)

5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one (0.01 mole), substituted benzaldehyde (3a-h) (0.01 mole) and anhydrous sodium acetate (0.02 mole) were dissolved in acetic acid and refluxed for 6 hr. The reaction-mixture was filtered and the filtrate was poured on crushed ice. The solid obtained was recrystallized from ethanol.

Table 1: Physical properties of 4-(substituted benzylidene)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one (4a-h).



Comp.	R	MF	MW	Yield (%)	M.P. (^{o}C)	R_f[a]	*C*log*P*[b]
4a	2-F	$C_{11}H_9FN_2O$	204	72.6	193	0.79	1.97
4b	3-Cl	$C_{11}H_9ClN_2O$	220	79.2	188	0.73	1.75
4c	3-Br	$C_{11}H_9BrN_2O$	263	64.1	201	0.75	2.02
4d	3-OCH_3	$C_{12}H_{12}N_2O_2$	216	68.3	216	0.61	1.72
4e	3,5-di Cl	$_{11}H_8Cl_2N_2O$	254	77.6	176	0.73	3.25
4f	3,5-di Br	$C_{11}H_8Br_2N_2O$	344	64.8	171	0.80	3.55
4g	3,5-di OCH_3	$C_{13}H_{14}N_2O_3$	246	66.2	191	0.86	1.88
4h	2-Cl, 4-F	$_{11}H_8ClFN_2O$	238	75.7	163	0.81	2.68

[a] n-hexane: ethyl acetate (8 : 2)

[b] Clog P was calculated using ACD/labs software v11.0

(4Z)-4-(2-fluorobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4a)

IR (KBr) (υ, cm^{-1}): 3310 (N-H str.), 3014 (C-H str., Ar-H), 2858 (C-H str., CH_3), 1642(C = O str.), 1580 (C = N str.), 527 (C-F str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.92 (s, 3H, -CH_3), 6.91 (s, 1H, = CH-Ar), 7.42-8.08 (m, 4H, Ar-H), 8.71 (s, 1H, -NH); LCMS m/z: 204 (M^+). Elemental analysis Calcd: C (64.70%) H (4.44%) N (13.72%) Found: C (65.32%) H (4.46%) N (13.31%).

(4Z)-4-(3-chlorobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4b)

IR (KBr) (υ, cm^{-1}): 3472 (N-H str.), 3120 (C-H str., Ar-H), 3112 (C-H str., CH_3), 1571(C = O str.), 1610 (C = N str.), 784 (C-Cl str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.74 (s, 3H, -CH_3), 6.75 (s, 1H, = CH-Ar), 7.57-7.98 (m,4H, Ar-H), 8.47 (s, 1H, -NH); LCMS m/z: 220 (M^+). Elemental analysis Calcd: C (59.88%) H (4.11%) N (12.70%) Found: C (60.01%) H (4.14%) N (13.01%).

(4Z)-4-(3-bromobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4c)

IR (KBr) (υ, cm^{-1}): 3417 (N-H str.), 3124 (C-H str., Ar-H), 3017 (C-H str., CH_3), 1547(C = O str.), 1628 (C = N str.), 587 (C-Br str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.57 (s, 3H, -CH_3), 6.75 (s, 1H, = CH-Ar), 7.57-7.86 (m,4H, Ar-H), 8.47(s, 1H, -NH); LCMS m/z: 263 (M^+). Elemental analysis Calcd: C (49.84%) H (3.42%) N (10.57%) Found: C (48.97%) H (3.84%) N (11.02%).

(4Z)-4-(3-methoxybenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4d)

IR (KBr) (υ, cm^{-1}): 3397 (N-H str.), 3027 (C-H str., Ar-H), 3241 (C-H str., CH_3), 1617(C = O str.), 1684 (C = N str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.64 (s, 3H, -CH_3), 2.62 (s, 3H, OCH_3), 6.68 (s, 1H, = CH-Ar), 7.28-7.46 (m,4H, Ar-H), 8.29(s, 1H, -NH); LCMS m/z: 216 (M^+). Elemental analysis Calcd: C (66.65%) H (5.59%) N (12.96%) Found: C (66.82%) H (5.14%) N (13.02%).

(4Z)-4-(3,5-dichlorobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4e)

IR (KBr) (υ, cm^{-1}): 3375 (N-H str.), 3221 (C-H str., Ar-H), 2982 (C-H str., CH_3), 1641(C = O str.), 1684 (C = N str.), 749 (C-Cl str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.62 (s, 3H, -CH_3), 6.87 (s, 1H, = CH-Ar),

7.45-7.92 (m,3H, Ar-H), 8.84(s, 1H, -NH); LCMS m/z: 254 (M^+). Elemental analysis Calcd: C (51.79%) H (3.16%) N (10.98%) Found: C (52.07%) H (3.43%) N (11.14%).

(4Z)-4-(3,5-dibromobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4f)

IR (KBr) (υ, cm^{-1}): 3415 (N-H str.), 3011 (C-H str., Ar-H), 3212 (C-H str., CH_3), 1587(C = O str.), 1642 (C = N str.), 549 (C-Br str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.75 (s, 3H, $-CH_3$), 6.41 (s, 1H, = CH-Ar), 7.36-7.73 (m,3H, Ar-H), 8.57 (s, 1H, -NH); LCMS m/z: 344 (M^+). Elemental analysis Calcd: C (38.41%) H (2.34%) N (8.14%) Found: C (38.92%) H (2.43%) N (8.72%).

(4Z)-4-(3,5-dimethoxybenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4g)

IR (KBr) (υ, cm^{-1}): 3287 (N-H str.), 3118 (C-H str., Ar-H), 3012 (C-H str., CH_3), 1684(C = O str.), 1586 (C = N str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.31 (s, 3H, $-CH_3$), 2.47 (s, 6H, OCH_3), 6.84 (s, 1H, = CH-Ar), 7.48-7.76 (m,3H, Ar-H), 8.74(s, 1H, -NH); LCMS m/z: 246 (M^+). Elemental analysis Calcd: C (63.40%) H (5.73%) N (11.38%) Found: C (64.02%) H (5.54%) N (11.84%).

(4Z)-4-(2-chloro-4-fluorobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (4h)

IR (KBr) (υ, cm^{-1}): 3247 (N-H str.), 3132 (C-H str., Ar-H), 3022 (C-H str., CH_3), 1672(C = O str.), 1627 (C = N str.), 674 (C-Cl str.), 549 (C-F str.).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.42 (s, 3H, $-CH_3$), 6.76 (s, 1H, = CH-Ar), 7.54-7.87 (m,3H, Ar-H), 8.34 (s, 1H, -NH); LCMS m/z: 238 (M^+). Elemental analysis Calcd: C (55.36%) H (3.38%) N (11.74%) Found: C (56.02%) H (3.45%) N (11.27%).

Antibacterial activity[15]

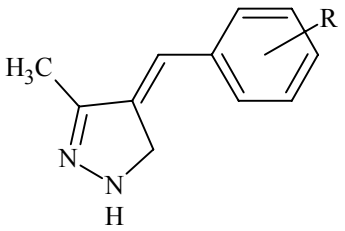
The synthesized compounds were screened for their antibacterial activity against two gram positive organisms such as *Staphylococcus aureus, Bacillus stearothermophilus,* and two gram negative organisms such as *Escherichia coli* and *Salmonella typhi*. The technique used was agar diffusion method using 100 µg/100 mL of Penicillin and Streptomycin as standard for gram positive and gram negative, respectively.

Antioxidant Activity[16]

The antioxidant potential of all the compounds were screened by *in-vitro* free radical scavenging activity using $DPPH^{\cdot}$ (2, 2-diphenyl-1picryl hydrazyl) reduction method.

Ascorbic acid was taken as the standard. Significant effect is not to be seen as most of the compounds had IC_{50} value near 10.

Table 2: Antibacterial study of 4-(substituted benzylidene)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one (4a-h)



Compd. Code	R	Antibacterial Activity (Zone of Inhibition in mm)			
		S. aureus	***E. coli***	***B. Stearothermophilus***	***S. typhi***
4a	2-F	13	12	14	14
4b	3-Cl	16	11	14	17
4c	3-Br	18	16	11	16
4d	3-OCH_3	15	16	18	16
4e	3,5-di Cl	14	14	12	14
4f	3,5-di Br	16	15	11	12
4g	3,5-di OCH_3	19	16	18	16
4h	2-Cl,4-F	21	12	18	16
Standard	Penicillin	-	22	-	24
	Streptomycin	22	-	23	-

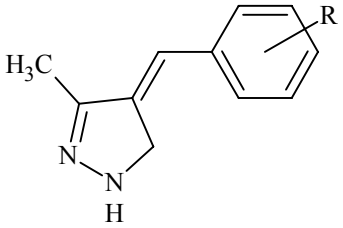
RESULTS AND DISCUSSION

Antibacterial activity of the synthesized compounds was expressed as zone of inhibition in mm (Table 2). Antibacterial activity of the synthesized compounds (4a-h) revealed that moderate activity is shown by compounds 4a-h against *S. aureus*. Similarly in the case of *Bacillus stearothermophilus* almost all compounds showed mild antibacterial activity. Medium activity is shown by almost all compounds within the series against *E. coli*

and low antibacterial activity was shown by all the compounds against *S. typhi*. However, none of the compounds showed activity more than the standard.

However, compared to other compounds 4e and 4f showed good activity with IC_{50} value of 10 µg/mL compared to the standard (Table 3).

Table 3: Antioxidant activity of 4-(substituted benzylidene)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one (4a-h)



Compound code	R	Antioxidant activity (IC_{50} in µg/mL)
4a	2-F	51
4b	3-Cl	47
4c	3-Br	39
4d	3-OCH_3	41
4e	3,5-di Cl	28
4f	3,5-di Br	26
4g	3,5-di OCH_3	42
4h	2-Cl, 4-F	39
Standard	Ascorbic acid	10.72

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