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Synthesis and antimicrobial activity of some new 2,6-*bis*((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene)cyclohexanones

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ABSTRACT

*Seven new 2,6-bis((1-phenyl-3-aryl-1H-pyrazol-4-yl)methylene)cyclohexanones **(3a-g)** were synthesized from cyclohexanone and 4-formylpyrazole aldehyde, via Claisen-Schmidt condensation. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, mass and elemental analyses. All the seven compounds were tested in vitro for their antibacterial activity against two gram positive bacteria namely Staphylococcus aureus and Bacillus subtilis and two gram negative bacteria Escherichia coli and Pseudomonas aeruginosa. The compounds **3b, 3c, 4f, 3g** displayed good antibacterial activity when compared with commercially available antibiotic, ciprofloxacin. These compounds also were screened for their antifungal activity against two ear pathogenic fungi, namely Aspergillus niger and A. flavus. The compounds **3c, 3d, 3f, 3g** exhibited good antifungal activity when compared with commercially available antifungal, fluconazole.*

Keywords: Cyclohexanone, Pyrazole aldehyde, antibacterial activity**,** antifungal activity.

INTRODUCTION

α,β-Unsaturated carbonyl compounds occupy a prominent place among various classes of molecular targets due to their broadspectrum biological and pharmacological activities such as antitubarculosis [1], antimicrobial [2], anti-inflammatory and anticancer [3-7].

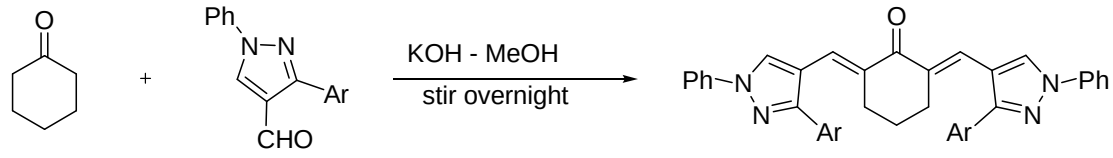
On the other hand, pyrazole and its derivatives, a class of well known nitrogen containing heterocyclic compounds, occupy an important position in the medicinal and pesticide chemistry with having a wide range of bioactivities such as antimicrobial [8], anticancer [9], anti-inflammatory [10], antibacterial [11], antifungal [12] and herbicidal [13,14]. A literature survey revealed that the title compounds 2,6-*bis*((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene) cyclohexanones remain unknown. Led by these observations, the synthesis of some new 2,6-bis((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene)cyclohexanones **(3a-3g)** was undertaken with a view to evaluate their antibacterial and antifungal activities.

MATERIALS AND METHODS

Melting points were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. The IR spectra were recorded with a Buck scientific IR M-500 spectrophotometer. The ^{1}H NMR spectra were scanned on a Bruker (300 MHz) spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. TLC was run on silica gel G plates using chloroform-methanol (9:1) as irrigant. All the new compounds gave satisfactory analytical results.

General procedure for synthesis of 2,6-*bis*((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene)cyclohexanones (3a-g)

To the methanolic solution of KOH (0.008 mol), mixture of THF and pyrazole aldehyde (0.002, **2**) was added and contents were stirred for 20 min in ice cold condition. Then cyclohexanone (0.001, **1**) was added dropwise. The resulting mixture was stirred at room temperature overnight and then poured into ice cold water (100 mL). The mixture was neutralized with dil. hydrochloric acid to give a yellow solid, which was recrystallized from chloroform- methanol. Pyrazole aldehydes **2** used in this reaction were synthesized according to literature method [15].



1 **2** **3**

Ar: Phenyl (a); 4-methylphenyl (b); 4-methoxyphenyl (c); 4-fluorophenyl (d); 4-chlorophenyl (e); 4-bromophenyl (f); 4-nitrophenyl (g)

Scheme 1: Synthetic Scheme for the formation of title compounds

***In-vitro* antibacterial activity**

The antibacterial activity of seven new compounds **3a-3g** was evaluated by agar well diffusion method. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 ×10^8 cfu/ml. Then 20 ml of Mueller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 4.0 mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 ^{0}C for 24 hrs. Antibacterial activity of compounds **3a-3g** was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism [16, 17].

Minimum inhibitory concentration (MIC) of the various compounds **3a-3g** against bacterial strains was tested through a macrodilution tube method as recommended by NCCLS. In this method, various test concentrations of synthesized compounds **3a-3g** were made from 128 to 0.25µg/ml in sterile tubes No.1 to10. 100 µl sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 µl test compound in tube 1. Two fold serial dilutions were carried out from the tube 1 to the tube 10 and excess broth (100 µl) was discarded from the last tube No.10. To each tube, 100 µl of standard inoculum (1.5 ×10^8 cfu/ml) was

added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 ^{0}C for 24 hrs [18].

***In-vitro* antifungal activity**

The antifungal activity of the synthesized compounds **3a-3g** was evaluated by poison food technique .The moulds were grown on Sabouraud dextrose agar (SDA) at 25 ^{0}C for 7 days and used as inocula. The 15 ml of molten SDA (45 ^{0}C) was poisoned by the addition of 100 µl volume of each compound having concentration of 4.0mg/ml reconstituted in the DMSO and poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8mm diameter) obtained from the actively fungus growing on margins of the SDA plates and incubated at 25 ^{0}C for 7 days. DMSO was used as the negative control whereas Fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition by applying the formula [19].

$$\text{Percentage inhibition of myelial growth} = (dc-dt) / dc \times 100$$

dc = average diameter of fungal colony in negative control sets
dt = average diameter fungal colony in experimental sets

Table 1: Characterization data of compounds 3a-3g

Compound	M.P. (^{o}C)	Yield (%)	Mol. Formula	Elemental analysis Calcd/(found)%		
				C	H	N
3a	208	79	$C_{38}H_{30}N_4O$ (558.67)	81.70 (81.68)	5.41 (5.43)	10.03 (10.00)
3b	222	82	$C_{40}H_{34}N_4O$ (586.72)	81.88 (81.82)	5.84 (5.80)	9.55 (9.50)
3c	210	81	$C_{40}H_{34}N_4O_3$ (618.72)	77.65 (77.63)	5.54 (5.58)	9.06 (9.02)
3d	186	74	$C_{38}H_{28}N_4OF_2$ (594.65)	76.75 (76.78)	4.75 (4.72)	9.42 (9.44)
3e	130	78	$C_{38}H_{28}N_4OCl_2$ (627.56)	72.73 (72.70)	4.50 (4.52)	8.93 (8.93)
3f	142	81	$C_{38}H_{28}N_4OBr_2$ (716.46)	63.70 (63.72)	3.94 (3.96)	7.82 (7.79)
3g	240	69	$C_{38}H_{28}N_6O_5$ (648.66)	70.36 (70.40)	4.35 (4.30)	12.96 (12.97)

Table 2: *In vitro* antibacterial activity of synthesized compounds 3a-3g

Compounds	Diameter of growth of inhibition zone (mm)[a]			
	Staphylococcus aureus	*Bacillus Subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	20.3	20.2	15.6	18.3
3b	25.0	21.6	18.3	18.6
3c	24.6	23.6	16.6	15.0
3d	21.0	21.2	19.3	18.6
3e	18.6	20.3	18.6	17.0
3f	25.6	22.3	18.6	18.6
3g	24.0	20.3	18.6	17.2
Ciprofloxacin	26.0	24.0	25.0	22.0

[a] *Values, including diameter of the well (8mm), are means of three replicates*

Table 3: Minimum Inhibitory Concentration (µg/ml) of snthesized compounds 3a-3g

Compounds	*Staphylococcus aureus*	*Bacillus Subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	128	128	>128	64
3b	16	64	128	64
3c	32	32	128	128
3d	64	64	64	64
3e	128	128	>128	>128
3f	16	64	64	128
3g	32	64	64	64
Ciprofloxacin	5	5	5	5

Table 4: *In vitro* antifungal activity of synthesized compounds 3a-3g

Compounds	Mycelial growth inhibition (%)	
	Aspergillus niger	*Aspergillus flavus*
3a	44.4	62.2
3b	48.7	55.9
3c	57.2	67.6
3d	59.8	70.6
3e	33.3	64.1
3f	58.0	67.6
3g	53.6	60.1
Fluconazole	81.1	77.7

Spectral data:

2,6-*Bis*((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3a)
: IR ν max (KBr) Cm^{-1}1659 (C=O). ^{1}**H NMR (300 MHz, $CDCl_3$):** δ 1.979- 2.018 (quintet, 2H, CH_2, J= 5.7 Hz), 2.904- 2.942 (t, 4H, CH_2, J= 5.7 Hz), 7.341- 7.391 (m, 2H), 7.407- 7.455 (m, 2H), 7.476- 7.566(m, 6H), 7.723- 7.746 (m, 6H), 7.815- 7.842 (m, 4H), 7.887 (s, 2H, =CH), 8.174 (s, 2H). **MS (m/z):** 559.11.

2,6-*Bis*((1-phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3b)
: IR ν max (KBr) Cm^{-1}1659 (C=O). ^{1}**H NMR (300 MHz, $CDCl_3$):** δ 1.877- 1.985 (quintet, 2H, CH_2, J= 4.2 Hz), 2.431 (s, 6H, CH_3), 2.999- 3.038 (t, 4H, CH_2, J= 4.2 Hz), 7.282- 7.315 (m, 4H), 7.352- 7.377 (m, 2H), 7.483- 7.534(m, 4H), 7.605- 7.631 (m, 4H), 7.801- 7.827 (m, 4H), 7.870 (s, 2H, =CH), 8.146 (s, 2H). **MS (m/z):** 587.11.

2,6-*Bis*((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4yl)methylene)cyclohexanone (3c)
: IR ν max (KBr) Cm^{-1}1674 (C=O). ^{1}**H NMR (300 MHz, $CDCl_3$):** δ 1.971- 1.992 (quintet, 2H, CH_2, J= 4.1 Hz), 2.815- 2.914 (t, 4H, CH_2, J= 4.1 Hz), 3.884 (s, 6H, OCH_3), 7.017- 7.045 (m, 4H), 7.328- 7.378 (m, 2H), 7.485- 7.537(m, 4H), 7.655- 7.683 (m, 4H), 7.801- 7.828 (m, 4H), 7.875 (s, 2H, =CH), 8.148 (s, 2H). **MS (m/z):** 619.17.

2,6-*Bis*((3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3d)
: IR ν max (KBr) Cm^{-1} 1666(C=O). ^{1}**H NMR (300 MHz, $CDCl_3$):** δ 1.988- 2.008 (quintet, 2H, CH_2, J= 3.3 Hz), 2.889- 2.906 (t, 4H, CH_2, J= 3.3 Hz), 7.162- 7.218 (m, 4H), 7.347- 7.395 (m, 2H), 7.495- 7.568(m, 4H), 7.683- 7.728 (m, 4H), 7.794- 7.822 (m, 6H), 8.154 (s, 2H). **MS (m/z):** 595.02.

2,6-*Bis*((3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3e) : IR ν max (KBr) Cm^{-1}1659 (C=O). ^{1} **H NMR (300 MHz, $CDCl_3$):** δ 1.874 (quintet, 2H, CH_2, J= 4.2 Hz), 2.898 (t, 4H, CH_2, J= 4.2 Hz), 7.350- 7.399 (m, 2H), 7.459- 7.547 (m, 8H), 7.664- 7.692 (m, 4H), 7.791- 7.820 (m, 6H), 8.149 (s, 2H). **MS (m/z):** 626.11, 628.01.

2,6-*Bis*((3-(4-bromorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3f) : IR ν max (KBr) Cm^{-1}:1674 (C=O). ^{1} **H NMR (300 MHz, $CDCl_3$):** δ 1.993- 2.012 (quintet, 2H, CH_2, J= 4.5 Hz), 2.915 (t, 4H, CH_2, J= 4.5 Hz), 7.340- 7.408 (m, 2H), 7.456- 7.541 (m, 8H), 7.720- 7.744 (m, 4H), 7.810- 7.836 (m, 4H), 7.801- 7.885 (s, 2H, =CH), 8.166(s, 2H). **MS (m/z):** 715.12, 717.04.

2,6-*Bis*((3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclohexanone (3g) : IR ν max (KBr) Cm^{-1}: 1682 (C=O), 1335, 1504 (NO_2). ^{1} **H NMR (300 MHz, $CDCl_3$):** δ 1.980- 2.021 (quintet, 2H, CH_2, J= 4.1 Hz), 2.728- 2.970 (t, 4H, CH_2 J= 4.2 Hz), 7.372- 7.455 (m, 2H), 7.562- 7.607 (m, 6H), 7.916- 7.945(d, 4H, J= 8.7 Hz), 8.028- 8.054 (m, 4H),8.390- 8.418 (d, 4H, J= 8.7 Hz), 8.902 (s, 2H). **MS (m/z):** 642.12.

RESULT AND DISCUSSION

All the compounds **3a-3g** were synthesized from cyclohexanone **1** and 4-formylpyrazole aldehyde **2**, via Claisen-Schmidt condensation (Scheme **-1)** in good to excellent yield **(Table -1)**. The physical and analytical data of the compounds are presented in Table –1. The structures of all the newly synthesized 2,6-*bis*((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene)cyclohexanones **(3a- 3g)** were confirmed by their spectral (IR, ^{1}HNMR and mass) data. The appearance of absorption band at ~1659 cm^{-1} in the IR spectra of compounds **3a-3g** showed the presence of *bis*-α, β-unsaturated carbonyl group, thereby suggesting the cross conjugated system. The ^{1}H NMR spectra of compounds **3a-3g** showed one quintet at δ 1.874-1.993 (due to CH_2), one triplet at δ 2.896-2.999 (due to CH_2) of cylcohexanone ring and one singlet at δ ~7.870 (due to =CH) . Other protons appeared as multiplet in the aromatic regions. The C (5)–H of pyrazole ring appeared as a singlet at δ ~ 8.54.

The antibacterial activity of seven new compounds **3a-3g** was evaluated by agar well diffusion method against four bacteria, *Staphylococcus aureus*, *Bacillus subtilis*

(Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative).
The zone of inhibition and MIC values were determined by comparison with the standard drug ciprofloxacin. The outcome of this study is presented in table 2 and 3.

The compounds **3b, 3c, 3f, 3g** have displayed good antibacterial activity when compared with commercially available antibiotic, ciprofloxacin.

The antifungal activity was evaluated against two pathogenic strains (*A.niger* and *A.flavus).*The zone of inhibition was determined by comparison with the standard drug fluconazol. The outcome of this study is presented in table-4. The compounds **3c, 3d, 3f, 3g** exhibited comparable antifungal activity when compared with commercially available antifungal, fluconazole **(Table 4)**.

CONCLUSION

In conclusion, seven new 2,6-*bis*((1-phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene)cyclohexanones **(3a-3g)** were synthesized from cyclohexanone and 4-formylpyrazole aldehydes, via Claisen-Schmidt condensation. All the newly synthesized compounds were evaluated for their antibacterial and antifungal activities. The compounds **3b, 3c, 3f, 3g** have displayed good antibacterial activity when compared with commercially available antibiotic, ciprofloxacin **(Table 2 and 3)**. The compounds **3c, 3d, 3f, 3g** exhibited good antifungal activity when compared with commercially available antifungal, fluconazole **(Table 4)**.

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