SYNTHESIS AND BIOLOGICAL ACTIVITIES OF (4Z)-2-(1H-BENZIMIDAZOL-2-YLMETHYL)-4-ARYLIDENE-5-METHYL-2,4-DIHYDRO-3H-PYRAZOL-3-ONE COMPOUNDS

ABSTRACT

Pyrazoles are reported to be ~~well known~~well-known pharmacophores. This has motivated ~~to~~ the synthesis of some of the pyrazole derivatives by using hydrazine hydrate as well as adding benzimidazole in pyrazoles. A series of (4Z)-2-(1H-benzimidazol-2-ylmethyl)-4-arylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-ones (IIIa3 to IIIe3) was synthesized by the conventional method by refluxing compounds (IIIa2-IIIe2) with O-Phenylene diamine in absolute ethanol. A series of compounds (IIIa2-IIIe2) was prepared by reacting compounds (IIIa1-IIIe1) with chloroacetic acid. A series of (4E)-4-arylidene-5-methyl-2,4–dihydro-3H-pyrazol-3-ones (IIIa1-IIIe1) was prepared by the reaction between 5-methyl-2,4-dihydro- 3H-pyrazol-3-one (II) ~~and~~ and different aldehydes in presence of Sodium acetate. All the compounds were synthesized with good yield (58-80%) and characterized by IR, ^{1}H NMR spectral data, and C, H, N elemental analysis. All the synthesized compounds exhibited antibacterial and antifungal activities at various MIC levels as well as exhibited analgesic and anti-inflammatory activities. The synthesized compounds are believed to exert various other activities such as anticonvulsant, CNS depressant, ulcerogenic and anthelmintic.

KEYWORDS: Pyrazole, Benzimidazole, Antibacterial, Antifungal, Analgesic, Anti-inflammatory

INTRODUCTION:

Pyrazoles and Benzimidazoles are reported with the wide range of biological activities. Many attempts were made by researchers to find out an able potent pyrazole and benzimidazole derivatives and also combining both heterocyclic ring to enhance the biological activity. Antiviral, antimicrobial properties possessing compounds is the need today as we are facing Covid-19 pandemic. Pyrazole and Benzimidazole compounds possess biological activities as antimicrobial, antitumor anti-inflammatory, analgesic, antiviral, anti-Alzheimer's, antiulcer, antidiabetic. "Pyrazole-Benzimidazole combined compounds are reported to possess antimicrobial properties"- is the conclusion from the recent literature.

Comment [D1]: Where the refrence, please add referace

MATERIALS AND METHODS

Well dried apparatus was used to conduct the reactions requiring anhydrous conditions. laboratory reagent grade solvents and reagents were and were purified by distillation and

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crystallization wherever necessary. Catalyst's scientific microwave synthesizer (CATA-R, 32 litre, 850 W, 2450 MHz) was used for synthesis. Open capillary method was used for determining melting points of newly synthesized compounds. The final products were purified by recrystalization and purity was checked by micro TLC. The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pressed pellet. ^{1}H NMR spectra were recorded in a BRUKER DPX-200MHz spectrometer using TMS as internal standard. Perkin Elmer 2400 elemental analyzer was used for analysis of C, H and N which were found within ± 0.4 % of the theoretical values. The prior permission of Institutional Animal Ethics Committee [IAEC Registration No. 1153/PO/Re/S/08/CPCSEA (present), 1153/PO/ac/08/CPCSEA (previous)] was taken before conducting activity on animals.

SYNTHETIC SCHEME:

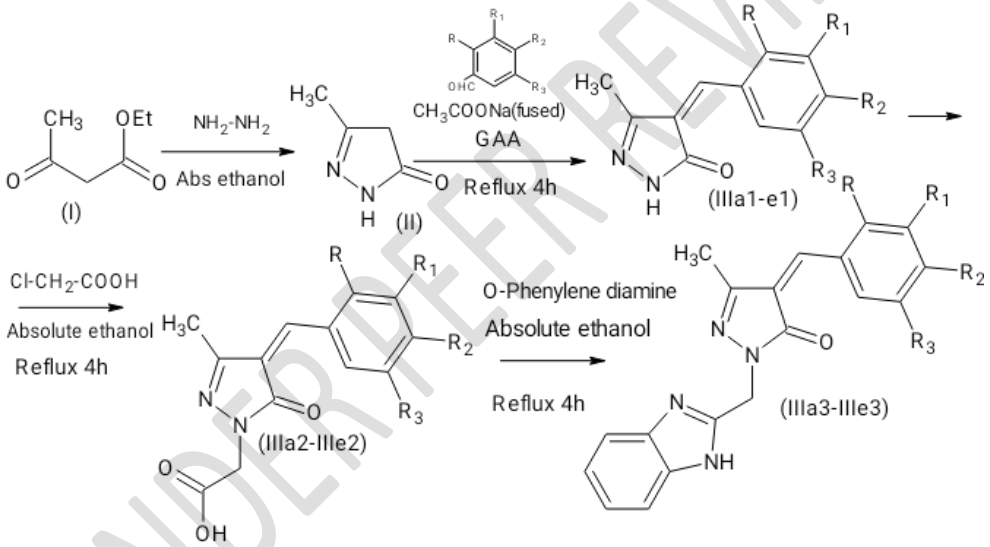

Fig. 1: Synthesis Scheme

A series of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-arylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-ones (IIIa3 to IIIe3) was prepared by the reaction between [(4*Z*)-4-arylidene-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl]acetic acids (IIIa2 to IIIe2) and o-phenylene diamine by refluxing it in presence of absolute ethanol. (IIIa2 to IIIe2) were prepared by reaction of (IIIa1 to IIIe1) with chloroacetic acid by refluxing with absolute ethanol. (IIIa1 to IIIe1) were obtained by reacting compound II (5-methyl-2,4-dihydro- 3H-pyrazol-3-one) with glacial acetic acid in presence of fused sodium acetate. Compound II was obtained by cyclization of ethyl acetoacetateate (I) with hydrazine hydrate by stirring in absolute ethanol.

PROCEDURES FOR SYNTHESIS

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Conventional synthesis method was preferred for the study. The purpose was to synthesize non-toxic/ less toxic derivatives with good activity, high yield and purity, less solvent requirement, less reaction time and novelty.

Conventional method for the synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II)

Ethyl acetoacetate (1.3g, 0.01mol) was placed in a conical flask and stirred magnetically during the slow drop wise addition of solution of hydrazine hydrate (98%,0.5 ml, 0.01 mol) in absolute ethanol (1ml) and temperature of about 60^0 C was maintained, a crystalline deposit was separated. After stirring for 1 h at room temp, the reaction mixture was cooled in an ice bath to complete ~~recrystalisation~~recrystallizations, filtered, washed with ice-cold ethanol, dried, m.p.222^0 C. Yield 0.88g,90%. [1]

General procedure for the synthesis of (4E)-4-arylidene-5-methyl-2,4–dihydro-3*H*-pyrazol-3-ones (IIIa1-e1)

A mixture of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (0.98g,0.01mol), appropriate aldehyde (0.01 mol), anhydrous sodium acetate (0.82g,0.01mol) and glacial acetic acid (40ml), was heated under reflux on heating mantle for 4 hours, cooled to room temperature and poured in an ice cold water, filtered, washed with water and ~~recrystalised~~recrystallized from methanol/ glacial acetic acid. The yield and m. p. were reported. [2, 3]

General procedure for the Synthesis of compounds (IIIa2-e2)

Compounds (IIIa1-e1) (0.01 mol) were added in methanol (20 ml) and stirred well to dissolve. To this chloroacetic acid (0.01mol) was added drop wise with continuous stirring to get clear solution and then refluxed for 2 hours on water bath to get solid residue of compounds (IIIa2-e2). The yield and m. p. were reported. [4]

General procedure for the Synthesis of compounds (IIIa3-b3)

Compounds (IIIa2-b2) (0.01 mol) were refluxed with O-Phenylene diamine (0.01 mol) for 4 hours in absolute ethanol (20 ml). The solvent was reduced to one third of its volume and then acidified with 10% HCl to yield final products (IIIa3-b3). The yield and m. p. were reported. [5]

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Table 1: Physical constants of compounds (IIIa3-e3)

Compound	Recrystalization Solvent	% yield	m.p. (^{0}c)	Molecular formula	Molecular weight	*Rf

IIIa3	Ethanol	58	226-227	$C_{19}H_{16}N_4O$	316.356	0.61
IIIb3	Glacial acetic acid + Ethanol(1:1)	70	215-216	$C_{19}H_{15}ClN_4O$	277.709	0.39
IIIc3	Glacial acetic acid	65	206-208	$C_{19}H_{16}N_4O_2$	259.263	0.58
IIId3	Glacial acetic acid	82	223-224	$C_{19}H_{14}Cl_2N_4O$	312.154	0.61
IIIe3	Ethanol	80	244-246	$C_{21}H_{21}N_5O$	286.332	0.43

Characterization of (4*Z*)-4-benzylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIa1)

The compound **IIIa1** with melting point 197-199^0 C was analysed for $C_{11}H_{10}N_2O$. It exhibits intense bands at 3416 cm^{-1}(aromatic N-H str), 3095 cm^{-1} (aromatic C-H str), 2903 cm^{-1} (C-H str in CH_3), 1680 cm^{-1} (C=O) 1613 cm^{-1}, 1585 cm^{-1} (C=C and C=N), 1134 cm^{-1}, 1052 cm$^{-1,}$ 787 cm^{-1} (monosubstituted benzene ring).

Characterization of (4Z)-4-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIb1)

The compound **IIIb1** with melting point 175-177^0 C was analysed for $C_{11}H_9ClN_2O$. It exhibits intense bands at 3456 cm^{-1} (aromatic N-H str), 3000 cm^{-1} (aromatic C-H str), 2921 cm^{-1} (C-H str in CH_3), 1687 cm^{-1} (C=O) 1613 cm^{-1}, 1556 cm^{-1} (C=C and C=N), 1120 cm^{-1}, 1051 cm$^{-1,}$ 757 cm^{-1} (1, 2-disubstituted benzene ring)

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-benzylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIa3)

The compound **IIIa3** with melting point 226-227^0 C was analyzed for $C_{19}H_{16}N_4O$. It exhibits intense bands at 3416 cm^{-1}(aromatic N-H str), 3095 cm^{-1} (aromatic C-H str), 2903 cm^{-1} (C-H str in CH_3), 1680 cm^{-1} (C=O), 1513 cm^{-1} and 1585 cm^{-1} (C=C and C=N), 1052 cm^{-1} , 787 cm^{-1}. (monosubstituted benzene ring).The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.20. It shows peaks at δ: 7.25 (d, 1H, =C<u>H</u>- on C4), 4.22 (s, 2H, -C<u>H</u>$_2$), 7.53- 7.99 (m, 9H, Ar-<u>H</u>), 12.1832 (bs, 1H, benzimidazole N-<u>H</u>) and 2.348 (s, 3H,-C<u>H</u>$_3$). Elemental analysis for composition of C, H and N is given as calculated: C(72.13%) H(5.10%) N(17.71%) found: C(72.10%) H (5.14%) N(17.72%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIb3)

The compound **IIIb3** with melting point 215-216^0 C was analyzed for $C_{19}H_{15}ClN_4O$. It exhibits intense bands at 3423 cm^{-1}(aromatic N-H str), 3056 cm^{-1} (aromatic C-H str), 2856

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cm^{-1} (C-H str in CH_3), 1716 cm^{-1} (C=O)1581 cm^{-1} and 1466 cm^{-1} (C=C and C=N), 1265 cm^{-1} (C-N str),1052 cm^{-1}, 787 cm^{-1} (1,2-disubstituted benzene ring). The ^1H NMR spectrum in $CDCl_3$ is given in figure 5.22. It shows peaks at δ: 7.91 (d, 1H, =CH- on C4), 4.22 (s, 2H, -CH_2), 7.12- 7.58 (m, 8H, Ar-H), 12.1833 (bs, 1H, benzimidazole N-H) and 2.342 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(65.05%) H(4.31%) N(15.97%) found: C(65.08%) H(4.29%) N(15.94%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-[(1*H*-benzimidazol-2-yl) methyl]-4-[(2-hydroxyphenyl) methylidene]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIc3)

The compound **IIIc3** with melting point 206-208^0 C was analyzed for $C_{19}H_{16}N_4O_2$. It exhibits intense bands at 3510 cm^{-1} (O-H str), 3315 cm^{-1} (aromatic N-H str), 3140 cm^{-1} (aromatic C-H str), 2894 cm^{-1} (C-H str in CH_3), 1723 cm^{-1} (C=O), 1579 cm^{-1} and 1653 cm^{-1} (C=C and C=N), 1338 cm^{-1} (C-N str), 1047 cm^{-1}, 1097 cm^{-1}, 780 cm^{-1} (1,2- disubstituted benzene ring). The ^1H NMR spectrum in $CDCl_3$ is given in figure 5.24. It shows peaks at δ: 11.85 (s, 1H, OH), 7.75 (d, 1H, =CH- on C4) , 4.22 (s, 2H, -CH_2), 6.65- 7.56 (m, 8H, Ar-H), 12.1833 (bs, 1H, benzimidazole N-H) and 2.497 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(68.66%) H(4.85%) N(16.86%) found: C(68.69%) H(4.82%) N(16.89%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-(2,4-dichlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIId3)

The compound IIId3 with melting point 223-2240 C was analyzed for C19H14Cl2N4O. It exhibits intense bands at 3442 cm-1(aromatic N-H str), 3083 cm-1 (aromatic C-H str), 2922 cm-1 (C-H str in CH3), 1733 cm-1 (C=O), 1579 cm-1 and 1653 cm-1 (C=C and C=N), 1315 cm-1 (C-N str), 1097 cm-1, 780 cm-1 (1,2,4-trisubstituted benzene ring). The 1H NMR spectrum in CDCl3 is given in figure 5.26. It shows peaks at δ: 7.72 (d, 1H, =CH- on C4), 4.22 (s, 2H, -CH2), 7.04- 7.61 (m, 7H, Ar-H), 12.1856 (bs, 1H, benzimidazole N-H) and 2.496 (s, 3H,-CH3). Elemental analysis for composition of C, H and N is given as calculated: C(59.24%) H(3.66%) N(14.54%) found: C(59.26%) H(3.64%) N(14.51%). The data confirms the structure of the compound.

Characterization of (4*Z*)-2-(1*H*-benzimidazol-2-ylmethyl)-4-[4(dimethylamino) benzylidene]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (IIIe3)

The compound IIIe3 with melting point 244-2460 C was analyzed for $C_{21}H_{21}N_5O$. It exhibits intense bands at 3428 cm-1(aromatic N-H str), 3092 cm-1 (aromatic C-H str), 2923 cm-1 (C-H str in CH3), 1702 cm-1 (C=O), 1482 cm-1 and 1682 cm-1 (C=C and C=N), 1323

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cm-1 (C-N str), 1108 cm-1, 781 cm-1(1,4-disubstituted benzene ring). The 1H NMR spectrum in CDCl3 is given in figure 5.28. It shows peaks at δ: 7.75 (d, 1H, =CH- on C4), 4.22 (s, 2H, -CH2), 6.91- 8.25 (m, 8H, Ar-H), 12.1823 (bs, 1H, benzimidazole N-H), 3.065 (s, 6H,-N (CH3)2) and 2.345 (s, 3H,-CH3). Elemental analysis for composition of C, H and N is given as calculated: C(70.17%) H(5.89%) N(19.48%) found: C(70.14%) H(5.87%) N(19.53%). The data confirms the structure of the compound.

BIOLOGICAL ACTIVITY

The LD_{50} values of synthesized compounds (IIIa3-e3) have been determined by the Karber's method. [6] Analgesic activity of synthesized compounds was studied by acetic acid induced writhings method [7, 8]. Anti-inflammatory activity of synthesized compounds was studied by carrageenan-induced rat paw oedema method [7, 8]. The antimicrobial activities of synthesized compounds were evaluated by the disc diffusion method [8]. This method is based on the diffusion of an antibiotic from a filter paper disc through the solidified culture media of a Petri dish used for the study. Growth of inoculated microorganism is inhibited entirely in a circular area "zone" around the filter paper disc containing a solution of the antibiotic and the test compounds.

Antimicrobial assay

The paper disc (No- 2 Whatmann) was cut down into a small disc (6 mm in diameter) and sterilized autoclave and then impregnated with the test solutions and standard solution. The dried discs were placed on the surface of the medium. After placing discs, Petri plates were left standing for 30 minutes at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri plates were incubated for 24h at the required temperatures, i.e. 370C for bacteria and 250C for fungi. After incubation, the diameters of the circular inhibition zones were measured.

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RESULTS

Table 2: ED_{50} values of synthesized compounds IIIa3-e3)

Sr. No.	Compound	ED_{50} (mg/kg)
1	IIIa3	140
2	IIIb3	130

3	IIIc3	145
4	IIId3	135
5	IIIe3	120

Table 3: Analgesic activity of compounds (IIIa3-e3)

S. No.	Design of treatment (Groups)	Dose (mg/kg, p.o.)	Number of writhings in 5 minutes	% Inhibition
1	Control(CMC, 0.25%, 1ml)	-	180.00 ±0.607	-
2	Aspirin	100	41.83 ±0.477**	76.76
3	Compound IIIa3	140	82.33±0.731**	54.26
4	Compound IIIb3	130	67.33±0.433**	**62.59**
5	Compound IIIc3	145	74.00±0.472**	58.88
6	Compound IIId3	135	57.00±0.608**	**68.33**
7	Compound IIIe3	120	69.33±0.463**	**61.48**

Comment [D2]: Round SE to nearest .01

Values are expressed as mean ± SEM, N=6, When compared with control, *= P< 0.05, **= P< 0.01, ***= P< 0.001 (One way ANOVA followed by Dunnett's multiple comparison test)

Table 4: Anti-inflammatory activity of compounds (IIIa3-e3)

S. No.	Design of treatment (Groups)	Dose (mg/kg, p.o.)	Change in paw edema at the end of 3h (mm)	% Inhibition
1	Control (CMC, 0.25%,1ml)	-	0.85±0.0067	-
2	Indomethacin	10	0.22±0.0060**	74.11
3	Compound IIIa3	143	0.53±0.0043**	37.64
4	Compound IIIb3	127	0.38±0.0055**	**55.29**
5	Compound IIIc3	148	0.50±0.0055**	41.17
6	Compound IIId3	131	0.32±0.0043**	**62.35**
7	Compound IIIe3	117	0.43±0.0040**	49.41

Values are expressed as mean ± SEM, N=6, When compared with control,*= P< 0.05, **= P< 0.01, ***= P< 0.001 (One-Way ANOVA followed by Dennett's multiple comparison test)

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Table 5: Antimicrobial (Antibacterial and Antifungal) activity of compounds (IIIa3-e3)

S. No.	Design of treatment (1mg/ml)	Diameter of zone of inhibition (mm)					
		Escherichia coli	***Staphylococcus aureus***	***Shigella dysenteriae***	***Streptococcus mutans***	***Candida albicans***	***Rhizopus oryzae***
1	Standard*	20	14	24	16	26	22
2	Compound IIIa3	**13**	**8**	**17**	**14**	10	7
3	Compound IIIb3	11	NA	11	**14**	12	11
4	Compound IIIc3	6	4	NA	4	6	12
5	Compound IIId3	10	4	2	5	4	NA
6	Compound IIIe3	4	6	14	**13**	**18**	**16**

NA: No activity at this amount of test compound or standard

*Standard drugs: Amoxicillin-clavulanic acid (for Gram Positive Bacteria), Cefixime (for Gram Negative Bacteria), Ketoconazole (for Fungi)

Discussion

Synthesis of two new chemical entities incorporating the two active pharmacophores namely pyrazoline and heteronucleus (another Pyrazole and benzimidazole) in a single molecular framework was successfully carried out. Conventional synthesis of new series of pyrazole-benzimidazoles, characterization of synthesized compounds by spectral methods viz. Infra Red, Nuclear Magnetic Resonance spectroscopy and elemental analysis and screening for the analgesic, anti-inflammatory and antimicrobial activity are the major highlights of the research work.

Pyrazoles with benzimidazole derivatives can be synthesized by conventional method. The yield is almost quantitative.

All the synthesized compounds exhibited analgesic, anti-inflammatory antibacterial and antifungal activities. Compounds IIIb3, IIId3 & IIIe3- showed good analgesic activity. Compounds IIIb3 & IIId3- showed good anti-inflammatory activity. Compound IIIa3 showed good activity against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* &

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Streptococcus mutans. IIIb3- showed good activity against *Streptococcus mutans*. IIIe3- showed good activity against *Streptococcus mutans*, *Candida albicans* & *Rhizopus oryzae.*

Comment [D3]: Please add the referance

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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