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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 4-BENZYLIDENE- PYRAZOLIDINE-3, 5-DIONE DERIVATIVES

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ABSTRACT

In the present research work, diethyl malonate and phenyl hydrazine were reacted together to give pyrazolidine-3,5-dione nucleus which was further derivatized at fourth position by reacting with different aromatic aldehydes to give 4-benzylidene-pyrazolidine-3,5-diones, followed by the 4-chloro-butyl and 4-nitrooxy-butyl substitution at nitrogen atom. The synthesized products were characterized by physicochemical and analytical means. Few of the synthesized derivatives showed excellent antibacterial and antifungal activities.

INTRODUCTION: Pyrazole, also known as 1, 2-diazole [1], belonging to one of the most important classes of heterocycles, has been considered to be pharmacologically very important nucleus owing to the potent and broad spectrum activity of the pyrazole scaffold.

It has been the topic of research for thousands of researchers all over the world because of its wide spectrum activities like anti-inflammatory, antipyretic, analgesic [2], antitubercular [3], antiviral [4], anti hypertensive [5], antiglaucoma [6], antioxidant [7], anti depressent, anxiolytic, neuroprotective [8], antimicrobial [9], cytotoxic, antiproliferative [10], antidiabetic [11], anticancer [12] and anti-alzheimer [13].

Several methods have been reported in literature for the synthesis of pyrazoles. Most common method of pyrazole synthesis is the reaction between 1, 3-diketocompounds and hydrazine or hydrazine hydrate or substituted hydrazines [14].

Wang *et al.,* reported the synthesis of 1, 3, 4, 5-tetrasubstituted pyrazoles taking hydrazonyl chlorides and 2-azidoacrylates as reactants using triethylamine as base [15].

Antipyretic action of a pyrazole derivative in man was discovered by Knorr, in 1884 and the compound was named *"antipyrine"*.

Pyrazolones and 1-phenyl-Pyrazolidine-3,5-diones are the most important derivatives of pyrazole and are present as a basic moiety in a number of pharmaceutical compounds like Phenylbutazone, oxyphenbutazone, butazolidine, antipyrine, amino-pyrine and novalgin.

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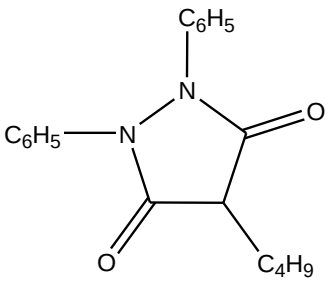

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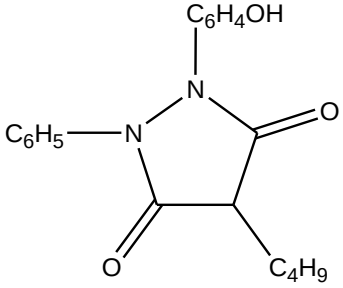

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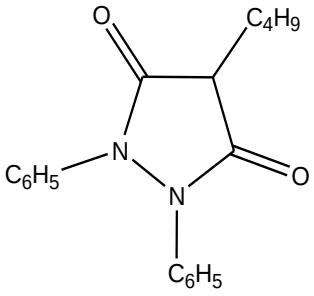

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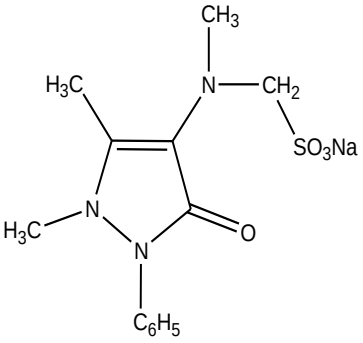

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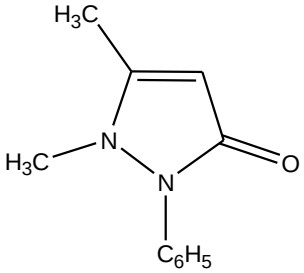

Antipyrine

MATERIALS AND METHODS: The reagent grade chemicals were obtained from commercial sources and were purified by either recrystallization or distillation before use. Melting points were determined by decibel melting point apparatus and were uncorrected. All reactions were monitored by thin layer chromatography (TLC) using silica gel G (Spectrochem Pvt. Ltd., Mumbai).

The plates were developed by exposing to iodine chamber. Infrared spectra were recorded by FTIR-ATR Thermo Scientific NICOLET Is10 spectrophotometer using KBr disks. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on Bruker Avance Π 400 NMR Spectrophotometer using DMSO as solvent from Central laboratory, Punjab University, Chandigarh. Chemical shifts are expressed as δ values (ppm).

Synthetic Procedure: The general scheme for the synthesis of 4-Benzylidene-Pyrazolidine-3,5-dione derivatives has been represented in **Figure 1.**

Step 1. Synthesis of Parent Compound (1-Phenyl-pyrazolidine-3, 5-diones): Heated a mixture of 0.01 M (1.60 mL) Diethyl malonate and 0.01 M (1.08 mL) Phenyl hydrazine at 120^{o}C on an oil bath for 1 hour with constant stirring followed by cooling the resulting red oil and stirring with 20 mL ether until solidification occurs, the product was filtered and washed with 20 mL ether. The resultant compound was recrystallized using 50% aqueous ethanol. Melting point measurement was done. The formation of the parent compound was then confirmed by performing TLC using Chloroform:Methanol (10:10) as mobile phase. Spots were identified by placing the plates in iodine chamber and R_f value was calculated.

Step 2. Synthesis of 4-Benzylidene-1-Phenyl-pyrazolidine-3, 5-diones: To a mixture of 0.01 M 1-Phenyl-pyrazolidine-3, 5-diones, was added 0.01 M of substituted Benzaldehyde using 0.01 M weak base. The mixture was refluxed for 2 hours. The solution thus obtained was cooled by keeping over ice or cold water. After cooling colored solid mass was separated and recrystallized using ethanol. Crystals so obtained were dried and melting point was determined followed by the TLC using Chloroform:Methanol (10:10) as mobile phase.

Step 3. Synthesis of 4-Benzylidene-1-(4-chloro-butyl)-2-Phenyl-pyrazolidine-3, 5-diones: A mixture of 0.01 M of 4-Benzylidene-1-Phenyl-pyrazolidine-3, 5-diones with 0.008% v/v 1, 4-dichlorobutane in dried toluene was refluxed using 0.01 M triethylamine as base. The assembly was protected by anhyd.$CaCl_2$ dry tube for about 8-10 Hours, after refluxing excess solvent was distilled off and remaining solution was filtered; residue was kept overnight and dried by keeping in a desiccator.

Step 4. Synthesis of 4-Benzylidene-1-(4-nitrooxy-butyl)-2-Phenyl-pyrazolidine-3, 5- diones: Final product was synthesized by dissolving 0.01 M 4-Benzylidene-1-(4-chloro-butyl)-2-Phenyl-pyrazolidine-3, 5-diones in 0.015 % w/v Silver nitrate in dried benzene. The resultant solution was heated for 2 hours. After refluxing, excess solvent was distilled off.

Remaining solution was cooled; crystals were filtered, kept overnight and dried by keeping in a desiccator.

Table 1 and Table 2 respectively represent the list of synthesized 4-Benzylidene-Pyrazolidine-3, 5-dione derivatives and their physicochemical characterization. The antimicrobial activity (MIC values) of 4-Benzylidene-Pyrazolidine-3, 5-dione derivatives for various bacterial and fungal strains has been enlisted in **Table 3**.

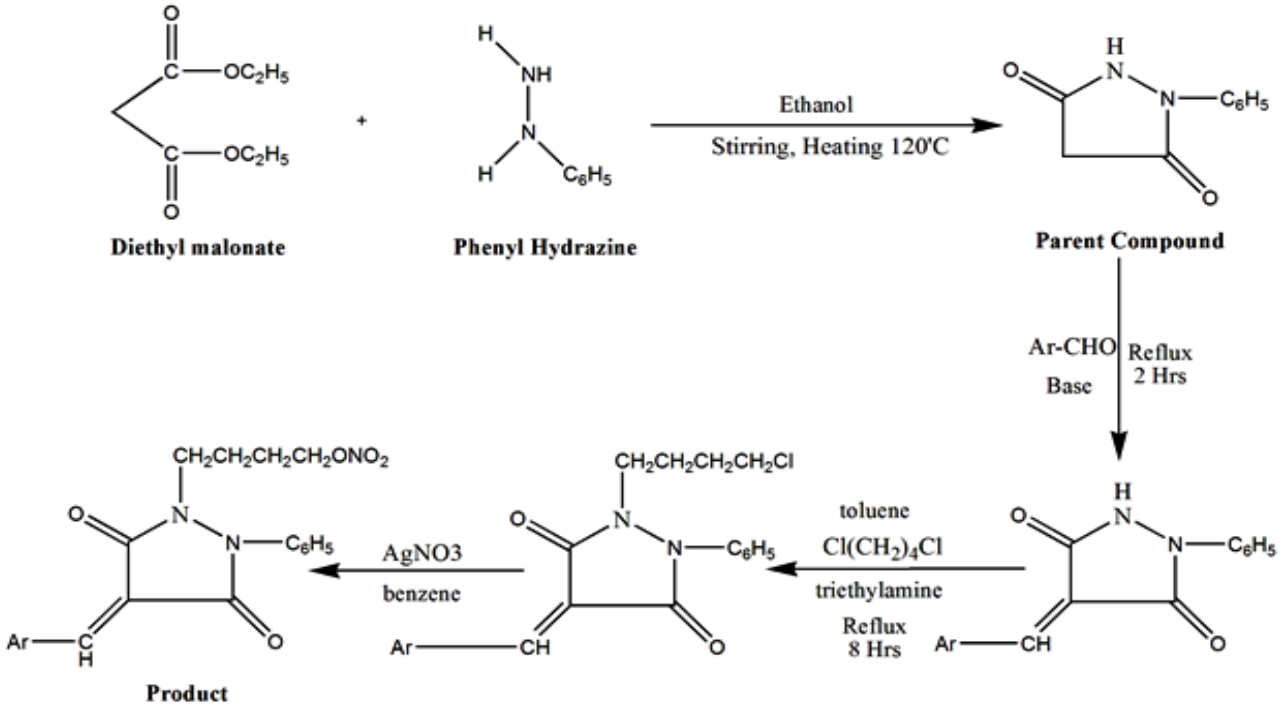

FIGURE 1: GENERAL SCHEME FOR THE SYNTHESIS OF 4-BENZYLIDENE-PYRAZOLIDINE-3,5-DIONE DERIVATIVES

TABLE 1: LIST OF 4-BENZYLIDENE-PYRAZOLIDINE-3,5-DIONE DERIVATIVES SYNTHESIZED

Compound	Ar	Compound	Ar
CN- 1	C_6H_5	**CN- 12**	4-OCH_3-Ph
CN- 2	4-F-Ph	**CN- 13**	4-CH_3-Ph
CN- 3	4-OH-3,5-$(OCH_3)_2$-Ph	**CN- 14**	3-Cl-Ph
CN- 4	3-Br-Ph	**CN- 15**	4-NO_2-Ph
CN-5	2-Cl-Ph	**CN- 16**	H
CN- 6	PDMA-Ph	**CN- 17**	2-OH-Ph
CN- 7	4-Cl-Ph	**CN - 18**	2,4 - $(OH)_2$-Ph
CN- 8	2,3-$(OCH_3)_2$-Ph	**CN - 19**	3-OCH_3-Ph
CN- 9	2-NO_2-Ph	**CN- 20**	2,6-$(OCH_3)_2$-Ph
CN- 10	4-OH-Ph	**CN- 21**	4-Br-Ph
CN- 11	3-OH-Ph		

TABLE 2: PHYSICOCHEMICAL CHARACTERIZATION OF SYNTHESIZED 4-BENZYLIDENE-PYRAZOLIDINE-3,5-DIONES DERIVATIVES

Serial No.	Molecular formula	Molecular weight	Melting point (^oC)	R_f Value	% Yield
CN- 1	$C_{20}H_{19}N_3O_5$	381.38	137-139	0.63	51.19
CN- 2	$C_{20}H_{18}FN_3O_5$	399.96	119-122	0.47	49.20
CN- 3	$C_{22}H_{23}N_3O_8$	457.43	121-125	0.51	55.00
CN- 4	$C_{20}H_{18}BrN_3O_5$	460.00	127-131	0.60	60.57
CN- 5	$C_{20}H_{18}ClN_3O_5$	415.83	130-134	0.69	51.87
CN- 6	$C_{22}H_{24}N_4O_5$	424.17	135-138	0.47	57.10
CN- 7	$C_{20}H_{18}ClN_3O_5$	415.83	137-139	0.61	53.66
CN- 8	$C_{22}H_{23}N_3O_7$	441.25	125-126	0.54	58.12
CN-9	$C_{20}H_{18}N_4O_7$	426.17	129-133	0.69	49.54
CN-10	$C_{20}H_{19}N_3O_6$	397.41	124-126	0.54	69.54

CN- 11	$C_{20}H_{19}N_3O_6$	397.14	124-128	0.44	54.86
CN- 12	$C_{24}H_{31}N_3O_7$	473.00	131-139	0.65	56.19
CN- 13	$C_{24}H_{31}N_3O_6$	457.12	131-136	0.54	45.18
CN- 14	$C_{20}H_{18}ClN_3O_5$	415.83	134-135	0.62	41.57
CN- 15	$C_{20}H_{18}N_4O_7$	426.17	121-124	0.64	49.19
CN- 16	$C_{14}H_{15}N_3O_5$	305.10	130-135	0.41	59.41
CN- 17	$C_{20}H_{19}N_3O_6$	397.14	134-136	0.63	60.66
CN- 18	$C_{20}H_{19}N_3O_7$	413.12	121-124	0.54	64.12
CN-19	$C_{21}H_{21}N_3O_6$	473.00	123-126	0.71	50.02
CN-20	$C_{22}H_{23}N_3O_7$	441.25	128-134	0.59	61.58
CN-21	$C_{20}H_{18}BrN_3O_5$	460.00	134-141	0.49	58.10

RESULTS AND DISCUSSION:

Evaluation of Antimicrobial Activity:

1. **Antibacterial activity:** Synthesized 4-benzylidene pyrazolidine-3, 5-dione derivatives (CN-1 to CN-21) were tested *in vitro* for their antibacterial profile using Tube Dilution Method [16, 17] against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* MIC values were calculated for all the synthesized compounds using Ciprofloxacin as the standard. The tested solutions were serially diluted to give concentrations of 5, 2.5, 0.125, 0.625, 0.312 mg/mL respectively. All the microbial strains were procured as lyophilized form from Himedia Labs Pvt. Ltd, Mumbai.

2. **Antifungal activity:** Antifungal activity evaluation of synthesized compounds against *Candida albicans* were performed similar to antibacterial activity by use of Sabouraud's glucose broth as media for assay taking Fluconazole as the standard drug. MIC was determined by the lowest concentration of sample that prevented the development of turbidity.

TABLE 3: ANTIMICROBIAL ACTIVITY (MIC VALUES) OF 4-BENZYLIDENE-PYRAZOLIDINE-3, 5-DIONE DERIVATIVES

Compound	MIC *(Bacillus subtilis)*	MIC *(Staphylococcus aureus)*	MIC *(Escherichia coli)*	MIC *(Pseudomonas aeruginosa)*	MIC *(Candida albicans)*
Parent 1	2.5	2.5	1.25	1.25	1.25
CN-1	2.5	2.5	2.5	1.25	1.25
CN-2	1.25	1.25	1.25	2.5	1.25
CN-3	0.625	0.625	1.25	1.25	1.25
CN-4	1.25	2.5	2.5	1.25	1.25
CN-5	1.25	1.25	2.5	2.5	0.625
CN-6	1.25	1.25	1.25	2.5	2.5
CN-7	2.5	2.5	2.5	1.25	0.625
CN-8	1.25	1.25	2.5	1.25	0.625
CN-9	2.5	2.5	2.5	2.5	1.25
CN-10	0.625	0.625	1.25	2.5	2.5
CN-11	1.25	1.25	0.625	1.25	2.5
CN-12	0.625	0.625	2.5	2.5	1.25
CN-13	1.25	1.25	1.25	2.5	1.25
CN-14	2.5	2.5	2.5	2.5	1.25
CN-15	1.25	1.25	2.5	2.5	1.25
CN-16	1.25	2.5	2.5	2.5	1.25
CN-17	2.5	2.5	0.625	0.625	2.5
CN-18	1.25	2.5	0.625	0.625	1.25
CN-19	2.5	2.5	0.625	1.25	1.25
CN-20	2.5	2.5	1.25	1.25	0.625
CN-21	1.25	1.25	2.5	2.5	1.25
Ciprofloxcin	0.625	0.625	0.625	0.625	-
Fluconazole	-	-	-	-	0.625

CONCLUSION: A novel class of 4-benzylidene-pyrazolidine-3, 5-diones were synthesized and characterized for their structure activity relationship. Antibacterial and Antifungal studies of these compounds indicated that the compounds CN-3, CN-10 and CN-12 were found to be the most active antibacterial compounds. Compounds CN-3, CN-10 and CN-12 showed potent antibacterial effect against gram positive bacteria. Compounds CN-11, CN-17, CN-18 and CN-19 showed potent antibacterial effect against gram negative bacteria. Compounds CN-5, CN-7, CN-8, CN-20 were found to possess better antifungal activity than antibacterial.

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REFERENCES:

1. Yet L: 4.01-Pyrazoles. In Comprehensive Heterocyclic Chemistry III. Elsevier: Oxford, 2008: 1-141.
2. Williams M, Kowaluk EA and Arneric SP: Emerging molecular approaches to pain therapy. Journal of Medicinal Chemistry 1999;42:1481-1500.
3. Pattan SR, Rabara PA, Pattan JS, Bukitagar AA, Wakale VS and Musmade DS: Synthesis and evaluation of some novel substituted 1,3,4-oxadiazole and pyrazole derivatives for antitubercular activity. Indian Journal of Chemistry 2009; 48B:1453-1456.
4. Shih SR, Chu TY, Reddy G, Tseng SN, Chen HL, Tang WF, Wu MS, Yeh JY, Chao YS, Hsu J, Hsieh HP and Horng JT: Pyrazole compound BPR1P0034 with potent and selective anti-influenza virus activity. Journal of Biomedical Science 2010;17(1):13.
5. Lo HY, Man CC, Fleck RW, Farrow NA, Ingraham RH, Kukulka A, Proudfoot JR, Betageri R, Kirrane T, Patel U, Sharma R, Hoermann MA, Kabcenell A and Lombaert SD: Substituted pyrazoles as novel sEH antagonist: Investigation of key binding interactions within the catalytic domain. Bioorganic & Medicinal Chemistry Letters 2010;20(22):6379-6383.
6. Kasımogullari R, Bulbul M, Arslan BS and Gokçe, B: Synthesis, characterization and antiglaucoma activity of some novel pyrazole derivatives of 5-amino-1,3,4-thiadiazole-2-sulfonamide. European Journal of Medicinal Chemistry 2010;45(11):4769-4773.
7. Padmaja A, Rajasekhar C, Muralikrishna A and Padmavathi V: Synthesis and antioxidant activity of oxazolyl/thiazolylsulfonyl methyl pyrazoles and isoxazoles. European Journal of Medicinal Chemistry 2011;46(10):5034-5038.
8. Gokhan-Kelekci N, Koyunoglu S, Yabanoglu S, Yelekci K, Ozgen O, Ucar G, Erol K, Kendi E and Yesilada A: New pyrazoline bearing 4(3H)-quinazolinone inhibitors of monoamine oxidase-Synthesis, biological evaluation, and structural determinants of MAO-A and MAO-B selectivity. Bioorganic & Medicinal Chemistry 2009;17(2):675-689.
9. Tanitame A, Oyamada Y, Ofuji K, Terauchi H, Kawasaki M, Wachi M and Yamagishi JI: Synthesis and antibacterial activity of a novel series of DNA gyrase inhibitors: 5-[(E)-2-arylvinyl]pyrazoles. Bioorganic & Medicinal Chemistry Letters 2005;15(19):4299-4303.
10. Tandon VK, Yadav DB, Chaturvedi AK and Shukla PK: Synthesis of (1,4)-naphthoquinono-[3,2-c]-1H-pyrazoles and their (1,4)-naphthohydroquinone derivatives as antifungal, antibacterial, and anticancer agents. Bioorganic & Medicinal Chemistry Letters 2005;15(13):3288-3291.
11. Shen DM, Brady EJ, Candelore MR, DallasYang Q, Ding VDH, Feeney WP, Jiang G, McCann ME, Mock S, Qureshi SA, Saperstein R, Shen X, Tong X, Tota LM, Wright MJ, Yang X, Zheng S, Chapman KT, Zhang BB, Tata JR and Parmee ER: Discovery of novel, potent, selective and orally active human glucagon receptor antagonists containing a pyrazole core. Bioorganic & Medicinal Chemistry Letters 2011;21(1):76-81.
12. Zheng LW, Zhu J, Zhao BX, Huang YH, Ding J and Miao JY: Synthesis, crystal structure and biological evaluation of novel 2-(5-(hydroxymethyl)-3-phenyl-1H-pyrazol-1-yl)-1-phenylethanol derivatives. European Journal of Medicinal Chemistry 2010;45(12):5792-5799.
13. Chioua M, Samadi A, Soriano E, Lozach O, Meijer L and Marco-Contelles J: Synthesis and biological evaluation of 3,6-diamino-1H-pyrazolo[3,4-b]pyridine derivatives as protein kinase inhibitors. Bioorganic & Medicinal Chemistry Letters 2009;19(16): 4566-4569.
14. Litvinov YM, Shestopalov AA, Rodinovskaya LA and Shestopalov AM: New Convenient Four-Component Synthesis of 6-amino-2,4-dihydropyrano[2,3-c]pyrazol-5-carbonitriles and one pot synthesis of 6′-aminospiro[(3H)-indol-3,4′-pyrano[2,3-c]pyrazol]-(1H)-2-on-5′-carbonitriles. Journal of Combinatorial Chemistry 2009; 11(5):914-919.
15. Li Y, Hong D, Lu P and Wang Y: Synthesis of pyrazoles from 2-azidoacrylates and hydrazonyl chlorides. Tetrahedron Letters 2011;52(32):4161-4163.
16. Ragavan RV, Vijayakumar V and Kumari NS: Synthesis and antimicrobial activities of novel 1,5-diaryl pyrazoles. European Journal of Medicinal Chemistry 2010;45(3):1173-1180.
17. National Committee for clinical Laboratory standard, Reference method for broth dilution antifungal susceptibility testing of yeasts approved standard M27A, NCCLS, Wayne, PA, 1997.

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