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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 1, 3, 4-TRISUBSTITUTED PYRAZOLYL CHALCONES FOR THEIR ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

Pyrazoles are an important class of heterocyclic compounds due to their synthetic adaptability and effective biological activities.Pyrazoles and their derivatives exhibit a wide variety of biological activities like antifungal, anti-inflammatory, anti-bacterial and anti-convulsant etc. So an attempt has been made towards the synthetic design and development of pyrazoles because of their high demand in academic and pharmaceutical sectors.In view of the biological importance of pyrazoles in the present work ,we have planned to synthesize some novel pyrazoles, followed by characterization using chromatographic and spectroscopy which include TLC, NMR, Mass spectroscopy and IR and to evaluate them for analgesic activity The structures of these synthesized compounds were confirmed by IR, NMR and CHN analysis.The results of this spectral and elemental analysis are found to be in the normal range. Further research on these versatile compounds may lead to potent lead molecules.

INTRODUCTION

Pyrazoles are five numbered ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are known as azoles. Pyrazole chemically known as 1, 2-diazole has become a popular topic due to its manifold use. They are classified as alkaloids, though they are rare in nature. The N-atom at position 2 with two electrons is basic and therefore reacts with electrophilies. The N-atom at position 1 is unreactive, but loses its proton in the presence of base. The combined two N-atoms reduce the charge density at C3 and C5, making C4 available for electrophilic attack. Deprotonation at C3 can occur in the presence of sttrong base, leading to ring opening.

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Protonation of pyrazoles leads to pyrazolium cations that are less likely to undergo electrophilic attack at C4, but attack at C3 is facilitated. The pyrazole anion is much less reactive toward nucleophiles, but the reactivity to electrophiles is increased.

Pyrazole framework plays an essential role in biologically active compounds and therefore represents an interesting template for combinatorial as well as medicinal. The recent success of pyrazole based COX II inhibitors such as Celecoxib and Rimonabant. . Pyrazoles and its derivatives represent one of the most active classes of compounds, which posses wide range of biological activities like anti-bacterial, anti-convulsant, analgesic, anti-microbial, anti-inflammatory, anti-diabetic, sedative, anti-rheumatic, anti-cancer and anti-tubercular, anti-pyretic, anxiolytic, growth inhibitor (insecticides) activites. In view of the above biological activities of pyrazole derivatives, in the present work, it was planned to synthesize some novel pyrazole.

EXPERIMENTAL WORK:

Materials and Methods: All the chemicals and solvents used were of synthetic grade from Finar chemicals Ltd., (Mumbai, India), E. Merck; NR chem. Melting points were determined in open capillary tubes using melting point apparatus and are uncorrected. Purity of the compounds was verified single spot TLC using F_{254}, 0.25mm aluminum plates. The IR spectra were recorded on SCHIMADZU FTIR Spectrophotometer by using 1% potassium bromide discs. Mass spectra of the compounds were recorded on mass spectrometer (Agilent 1100 series; EI/ES-MS) at Indian institute of chemical technology (IICT), Hyderabad and Hetero labs, Hyderabad. All the ^{1}H NMR spectra were recorded on Brucker 300 MHz spectrometer using $CDCl_3$ / DMSO as solvent and tetramethylsilane as an internal standard. Chemical shift values are listed in δ scale.

STEPS:

1) Condensation of substituted acetophenones with differently substituted phenyl hydrazines to yield phenyl hydrazones.
2) Vilsmeier-Hacck reaction of substituted phenyl hydrazones to afford 1, 3-substituted diphenyl-1H-pyrazole-4-carbaldehydes.
3) Condensation of aldehydes containing different substituted acetanilides in the presence of sodium hydroxide to give chalcones.

EXPERIMENTAL:

Step 1: Synthesis of 1-substituted phenyl ethanone 3-substituted phenyl hydrazones*:*

A mixture consisting (0.01 mol) of substituted acetophenone and (0.01 mol) of phenyl hydrazine were taken in a 250 ml beaker and to the above mixture 15-20 ml of methanol and heated on a water bath at 50-60^{0}C for 1 hr and to this catalytic amounts of glacial acetic acid was added and stirred for another 10 min until the reaction mixture was solidified. The lumps were broken into powder in mortar and pestle, the mass was washed with water and ice cold methanol, and the precipitate was filtered on a buchner funnel. It was re-crystallized from methanol. Yield (93%). Their melting points, percentage yields, molecular formula, molecular weights were given in table. 1A. and spectral data was given in table.1B.

Step 2: Synthesis of 1, 3-substituted diphenyl-1H-pyrazole-4-carbaldehydes:

To an ice-cold solution of DMF (0.1 mol), phosphorous oxychloride (0.012 mol), was added drop-wise to maintain the temperature below 10^{0}C, since an exothermic reaction takes place. To the above mixture, ice-cold solution of phenyl hydrazone (0.01mol) was added in lots wise with stirring under ice-cold conditions. After the completion of the addition, the reaction mixture was stirred and refluxed at 60-70^{0}C for 4-5 hr. Solution was cooled, poured into crushed ice with stirring, and neutralized with $NaHCO_3$ solution. The solid obtained was filtered under suction and re-crystallized from methanol. Yield (90%). Their melting points, percentage yields, molecular formula, molecular weights were given in table. 2A. and spectral data was given in table.2B.

Step 3: Synthesis of 1, 3-substituted diphenyl-1H-pyrazole-4 substituted phenylacrylamides:

To a mixture of substituted acetanilides (0.01mol) and substituted pyrazole-1H-carbaldehydes (0.01mol) in ethanol, 2% sodium hydroxide solution (1ml) was added drop wise with constant stirring over a period of 30 min. The reaction mixture was stirred for another 10 hr at room temperature and then refluxed for 6 hr. The reaction mixture was poured into ice-cold water. The solid thus obtained was filtered, dried and re-crystallized from ethanol. Their melting points, percentage yields, molecular formula, molecular weights were given in table. 3A. and spectral data was given in table. 3B.

PHARMACOLOGICAL ACTIVITIES:

Anti-microbial Activity: The anti-bacterial activity of all the compounds was determined by Cup Plate method using 24 – hr old cultures of gram-positive bacteria (*Bacillus subtilis*) and gram-negative bacteria (Escherichia coli). Ciprofloxacin and Ampicillin were used as standard drugs. DMSO was used as a control. Zone of inhibition was measured in mm. The anti-fungal activity of the synthesized compounds was performed by turbidimetric method using the fungi (*Candida albicans*). Ketoconazole was used as a standard drug and DMSO was used as control. All the compounds (3a-f) showed no activity against fungal strains.

Anti-Inflammatory activity: Inflammation is a tissue reaction to infection, irritation or foreign substance. It is a part of host defence mechanism during these tissue reactions the permeablility of the vasculature is increased and leads to extrusion of cells and cellular fluid into the extra vascular areas which results in the formation of oedema. The complete process of inflammation general consists of three phases. Dilation and increased permeability of blood vessels resulting in edema and swelling. migration of leucocytes from venules and capillaries, cellular infiltration and a general mopping of reaction. Proliferation of fibroblasts and synthesis of new connective tissue to repair the injury.

Table 1. A: *Physical data of 1-phenyl ethanone phenyl hydrazone derivatives:*

S.no	Cpd	R	R^1	Molecular formula	Molecular weight	M.P (^{0}C)	Yield (%)
1	1 a	H	H	$C_{14}H_{14}N_2$	210	125-130	93
2	1c	4-Cl	H	$C_{14}H_{13}ClN_2$	244	118-122	91
3	1g	4-OCH_3	H	$C_{15}H_{16}N_2O$	240	119-122	91

Table 2.A: *Physical data of 3-(1, 3-diphenyl)-1H-pyrazole-4-carbaldehydes:*

S.No	Cpd	R	R^1	Molecular formula	Molecular weight	M.P (^{0}C)	Yield (%)
1	2a	H	H	$C_{16}H_{12}N_2O$	248	135-140	92
2	2b	4-Cl	H	$C_{16}H_{11}ClN_2O$	282	138-142	90
3	2c	4-OCH_3	H	$C_{17}H_{14}N_2O_2$	278	139-142	90

Table 3.A: *Physical data of 3-(1,3-diphenyl)-1H-pyrazolyl chalcones :*

S.No	**Cpd**	**R**	**R^1**	**Molecular formula**	**Molecular weight**	**M.P (^{0}C)**	**Yield (%)**
1	3a	H	H	$C_{24}H_{19}N_3O$	365	115-120	92
2	3b	4-Cl	H	$C_{24}H_{18}ClN_3O$	399	118-122	87
3	3c	4-OCH_3	H	$C_{26}H_{21}N_3O_2$	407	119-125	89
4	3d	H	NO_2	$C_{24}H_{18}N_4O_3$	410	117-120	90
5	3e	H	Br	$C_{24}H_{18}N_3OBr$	444	118-124	92
6	3f	4-OCH_3	Br	$C_{25}H_{20}N_3O_3Br$	490	115-123	85

Spectral data:

T*able 1.B:* *Spectral data of the compounds (1a-c)*

Comp	***R***	***R^1***	***IUPAC NAME***
1a	H	H	1-Phenyl ethanone phenyl hydrazone
1b	4-Cl	H	1-(4-chloro phenyl) ethanone phenyl hydrazone
1c	4-OCH_3	H	1-(4-methoxy phenyl) ethanone phenyl hydrazone

Table 2.B: *Spectral data of the compounds (2a-c)*

Comp	***R***	***R^1***	***IUPAC NAME***	***IR(KBrdisc) stretching frequency in cm^{-1}***	***MS (m/z)***
2a	H	H	1, 3-diphenyl-1H-pyrazole -4-carbaldehyde	C=O(CHO):1672.1 3118.68(Ar C-H str), C=C(aromatic): 1595.02	248:M^+
2b	4-Cl	H	3(4-chlorophenyl-1H-pyrazole-4-carbaldehyde)	C=O(CHO):167O.24 ArC-H:3120.25,C=C (aromatic): 1596.95,C-Cl:752.19	282
2c	4-OCH_3	H	3-(4-methoxyphenyl)-1-phenyl-4-carbaldehyde	C=O(CHO): 1670.24 C=Cl604.68, Ar C-H: 3118.68	_

Table 3 B: *Spectral data of the compounds* ***(3a,d)***

Comp	***R***	***R^1***	***IUPAC NAME***	***IR(KBr disc) stretching frequency in cm^{-1}***	***Mass m/12***
3a	H	H	1, 3-diphenyl-1H-pyrazole-4-phenyl acrylamide	C=O:1733, Aromatic conjugated C=C,1628 NH stretch=3433	----
3d	4-methoxy	H	3(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-car;baldehyde	----	410

Table 4-*A.* *List of microorganisms used for Anti-microbial studies:*

Organism	**Type**
Bacillus subtilis	Gram +ve
Escherichia coli	Gram-ve
Candida albicans	Fungi

Table 4 B*:* *Inhibitory zones of substituted pyrazole derivatives on gram positive and gram negative bacterial strains*

Compound	**Inhibition zones of Bacillus subtilis in mm**	**Inhibition zones of Escherichia coli in mm**
	1000 µg/ml	1000 µg/ml
Solvent (control)	---	---
Ampicillin(50µg/ml) and Ciprofloxacin (25µg/ml)	38	29
3a	13	13
3b	29	18
3c	23	20
3d	21	23
3e	13	11
3f	28	18

Table 4-C. *Anti-Inflammatory Activity- Mean paw Oedema volume*

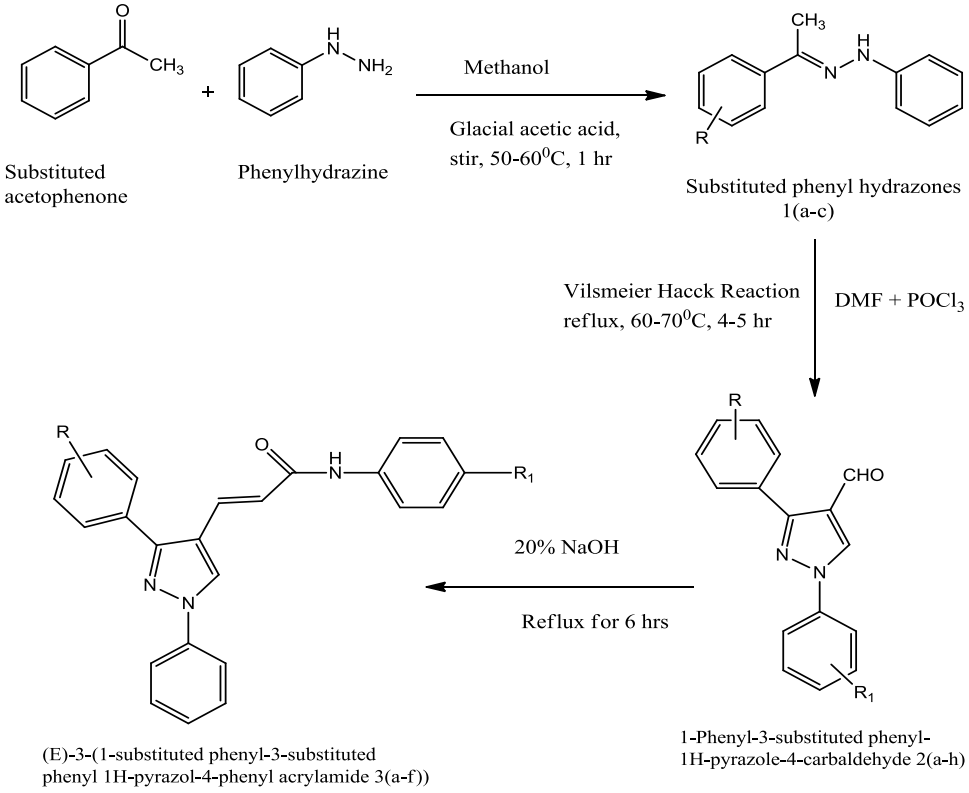
Treatment	**Dose (mg/kg)**	**MEAN EDEMA VOLUME (ml)**				
		30 min	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Control	1% carragenon	0.21+0.04	0.34+-0.11	0.50+0.08	0.69+-0.09	0.53+_0.08
Ibuprofen (standard)	50 mg/kg	0.04+0.06	0.22+-0.03	0.23+0.02 *	0.31+_0.06 ***	0.22+_0.03 **
3b	100	0.17+0.04	0.23+-0.02	0.91+0.03 **	0.31+_0.02 **	0.25+_0.01 **
3c	100	0.18+0.03	0.28+-0.05	0.31+0.07	0.41+_0.16 **	0.33+_0.07 *
3d	100	0.14+0.04	0.26+0.06	0.35+0.06	0.41+_0.04 **	0.31+_0.23 *
3e	100	0.19+0.04	0.20+0.01	0.35+0.04	0.40+_0.04 **	0.34+_0.04

Table 4-D.*Anti- Inflammatory Activity-Percentage Protection inhibition edema formation*

Treatment	Dose Mg/kg	% Protection				
		30 min	1 hr	2 hr	3 hr	4 hr
Ibuprofen (standard)	15 mg/kg	32.94	34.56	54.17	55.60	57.82
3b		20	33.09	57.29	50.18	53.08
3c	100	15.29	16.91	35.18	41.54	37.91
3d	100	34.19	23.53	29.65	40.79	41.23
3e	100	9.41	40.44	30.65	42.60	35.55

N=4, Results are expressed as MEAN+SEM. The statistical analysis was performed on absolute data *pc 0.05

**pc 0.01



S.No	*CPD*	*R*	*R^1*
1	3a	H	H
2	3b	4-Cl	H
3	3c	4-OCH_3	H
4	3d	H	NO_2
5	3e	H	Br
6	3f	4-OCH_3	Br

Inflammation may be acute or chronic.

Acute inflammation is usually characterized by fundamental symptoms like redness, swelling, pain and loss of function at the injured area depending upon site and extent of injury.

Chronic inflammation usually occurs when acute inflammation remains and resolved. It is associated with many inflammatory diseases e.g. Rheumatoid arthritis, hepatitis etc.

CONCLUSION:

Six derivatives have been synthesized in high quality and in good yields by the condensation of different substituted pyrazole aldehydes with substituted acetanilides. The chemical structures of synthesized compounds were confirmed based on physical, IR and mass spectral data. The compounds were screened for anti bacterial activity against gram-positive bacteria (Bacillus subtilis) and two gram negative bacteria (Escherichia coli) by cup-plate and anti inflammatory activity was carried using carrageenan induced rat paw edema method. The compounds 3(b), 3(c), 3(d) and 3(e) exhibited significant protection against the edema formation at a concentration of 100mg/kg and the results are comparable with the standard drug ibuprofen (100 mg/kg). The activity may be attributed to the presence of chloro, methyl, methoxy, and bromo substitutions on the phenyl ring. (3b, 3c, 3d and 3f) has shown minimal anti-bacterial activity at a concentration of 1000 µg/ml against all the two organisms. In anti-fungal activity, none of the compounds exhibited activity against *candida albicans*.

Conflict of interest:

The author declares no conflict of interest.

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