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Design, synthesis and biological evaluation of some novel pyrazoline derivatives

Sagar A. Jadhav*, Kiran M. Kulkarni, Pramod B. Patil, Vikas R. Dhole and Shitalkumar S. Patil

Department of Pharmaceutical Chemistry, Ashokrao Mane College of Pharmacy, Peth Vadgaon, Kolhapur, Maharashtra, India

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ABSTRACT

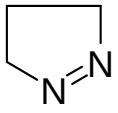
Pyrazoline is dihydropyrazole, a five member heterocyclic compound containing two nitrogen atoms in adjacent positions and possessing only one endocyclic double bond. The compound of different Pyrazoline derivatives were synthesized by cyclisation of substituted chalcone derivatives in the presence of 2, 4 dintro phenyl hydrazine hydrate. All synthesized compounds were characterized by spectral analysis. All synthesized compounds screened for their anticancer activities by MTT assay.

Keywords: Chalcones, 2,4 dinitro phenyl hydrazine hydrate, anticancer activity, MTT assay.

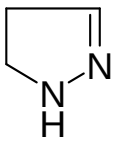
INTRODUCTION

Heterocyclic compounds have gained much importance in medicinal chemistry due to its presence in large number of pharmacologically active moieties. Among the five membered heterocyclic containing two hetero atoms in its ring structure , pyrazole is one of the most important one as large variety of biological activities have been reported for various pyrazole derivatives. Pyrazoline is dihydropyrazole, a five membered heterocyclic compound containing two nitrogen atoms in adjacent positions and possessing only one endocyclic double bond. Depending on the position of the double bond three forms of pyrazoline are possible. These are 1-pyrazoline, 2-pyrazoline and 3 – pyrazoline. Among all the pyrazolines, 2-pyrazoline has gained attraction and is frequently studied one [1-42].

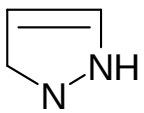
Increasing evidence suggests that pyrazoline derivatives possess a broad spectrum of biological activities such as tranquillizing, muscle relaxant, antidepressant, anticonvulsant, psycoanaleptic, antimycobacterial and antihypotensive activities[1-42].



1-Pyrazoline



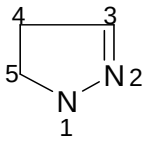
1,2-pyrazoline



3-pyrazoline

The above three represents the tautomeric forms of pyrazoline structures.

Nomenclature:
The application of present heterocyclic nomenclature to pyrazolines requires that nitrogen atoms be numbered one and two in each structure. Substituted 1-pyrazolines are numbered to produce the lower of two possible numbers for substituent group locants, or in the case of complicated structures to produce the simplest name consistent with clarity of meaning. Numbering of the 2-pyrazolines begins with the amino nitrogen and 3-pyrazolines are numbered to obtain for the double bond the lower of the two possible numbers. Thus, here this structure will be referred as[1-42],



1,2-Pyrazoline

CHEMISTRY :
The chalcones were synthesized from an aldol condensation between benzaldehyde and an acetophenone by Aldol-condensation reaction. An aldol condensation is an organic reaction in which an enol or an enolate ion reacts with a carbonyl compound to form a β-hydroxyaldehyde or β-hydroxyketone, followed by a dehydration to give a conjugated enone**.**

The pyrazoline derivatives were synthesized from chalcones by refluxing with 2,4-dinitro phenyl hydrazine in ethanol.

MATERIALS AND METHODS

The chemicals used in the present work were AR grade and LR grade, purchased from Ranbaxy, Merck, S.D. Fine chemicals and Research Lab and used as received. The list of chemicals used were 4-hydroxyacetophenone, 4-methylacetophenone, 2-Acetyl furan,4-methoxy benzaldehyde, 4-fluoro benzaldehyde, sodium hydroxide, 2,4-dintro phenyl hydrazine, ethyl-acetate, HCl, ethanol, conc.HCl, light paraffin oil, pet ether, glacial acetic acid, chloroform. The water used was double distilled deionised water..All the compounds showed satisfactory elemental analysis for C, H & N.

Identification and characterization of synthesized products:
The synthesized compounds were scaled for yield and purified by recrystallization with suitable solvent system. The purified compounds were assigned for physical constant determination and further subjected for spectral analysis like thin layer chromatography, Infrared spectroscopy, Nuclear magnetic resonance spectroscopy.

1.Melting point determination:
The melting point of the synthesized compounds were determined using Veego VMP-I melting point apparatus and recorded in degree Celsius.

2.Thin layer chromatography:
Thin layer chromatography was performed on percolated silica gel plates with suitable solvent system. The R_F values were recorded accordingly.

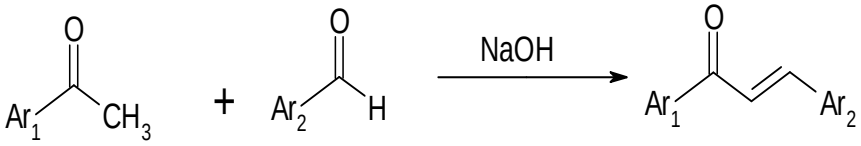
3.Infrared spectroscopy:
The infrared spectra for the synthesized compounds were recorded using JASCO-FTIR 8400 spectrophotometer using potassium bromide pellet technique.

4.Nuclear magnetic resonance spectroscopy:
^{1}HNMR and ^{13}CNMR spectra of the synthesized compounds were taken using tetramethyl silane as an internal standard. ^{1}HNMR spectra were recorded with DMSO and $CDCl_3$ as a solvent and the chemical shift data were expressed as δ values relative to TMS. ^{13}CNMR spectra were recorded with DMSO and $CDCl_3$ as a solvent and the chemical shift data were expressed as δ values relative to TMS. (Samples were sent to Chemistry department, Shivaji University, Kolhapur. For NMR analysis.)

Step-1. Synthesis of chalcones:-
Equimolar quantity of substituted acetophenone (0.01 mol) and substituted aldehyde (0.01 mol) was dissolved in ethanol (10ml) under stirring & aq. NaOH (30 %) was added dropwise. The reaction mixture stirred at room temperature using magnetic stirrer & kept for few hour. The reaction mixture was diluted with water & acidified with HCl . The separated solid was filtered and recrystallised from ethanol.

Step-2. Synthesis of pyrazoline derivatives:-
A mixture of chalcones (0.01 mol) , 2,4-dinitrophenyl hydrazine reagent dissolved in ethanol. The resulting mixture was refluxed for 2 or 3 hours. The resulting mixture was poured into ice water. The separated solid were filtered and recrystallised from ethanol.

EXPERIMENTAL DESIGN :
Step 1



Step 2

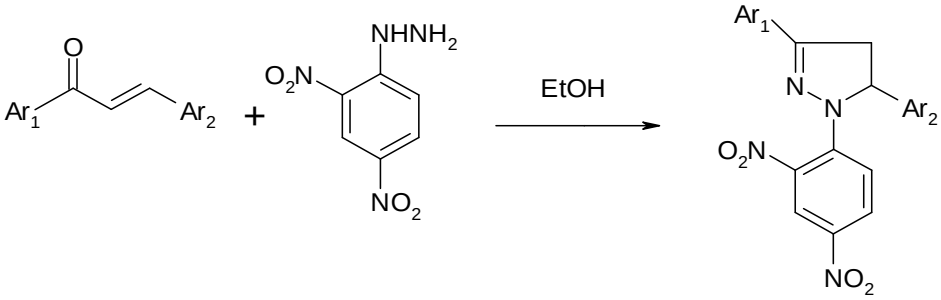


Table 1 : Newly Synthesized pyrazoline containing functional group

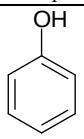
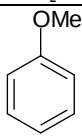
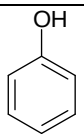
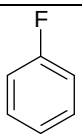
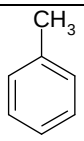
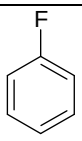
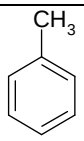
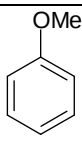
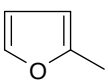
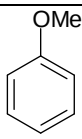
Compound	Ar_1	Ar_2
A		
B		
C		
D		
E		

Table 2 : List of compounds synthesized

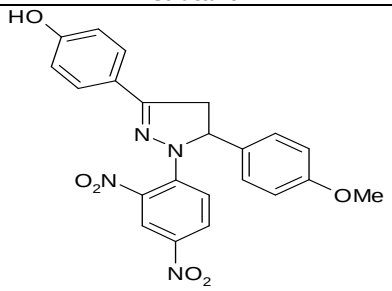
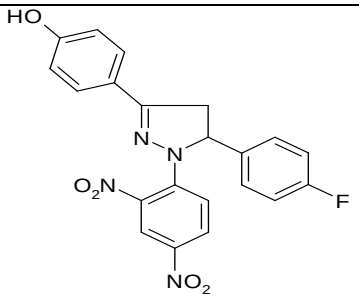
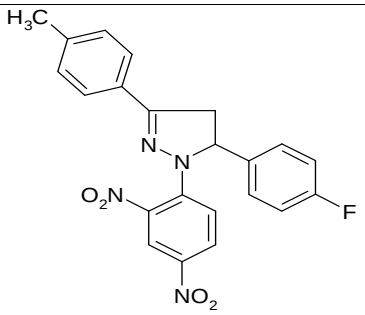
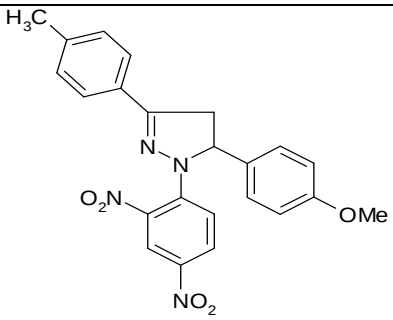
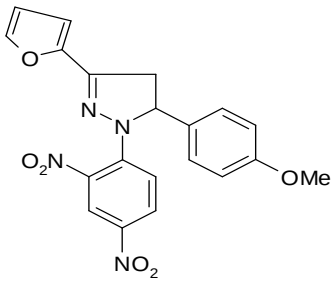
Compound	Chemical name	Structure
A	1(2,4-dintrophenyl)3phenol-5(4-methoxyphenyl)pyrazoline	
B	1(2,4-dintrophenyl)3phenol-5(4-fluorophenyl)pyrazoline	
C	1(2,4-dintrophenyl)3(4-methylphenyl)-5(4-fluorophenyl)pyrazoline	
D	1(2,4-dintrophenyl)3(4-methylphenyl)-5(4-methoxyphenyl)pyrazoline	
E	1(2,4-dintrophenyl)3(2-furyl)-5(4-methoxyphenyl)pyrazoline	

Table 3: data for the Physicochemical compounds (1a-1e) (Chalcones)

Comp. No.	Molecular Formula	Molecular Weight	M.P. range (^{0}C)	Mobile Phase	$^*R_F$ value
1a	$C_{16}H_{14}O_3$	254	120-122	Pet-ether : ethylacetate(5:5)	0.73
1b	$C_{15}H_{11}O_2F$	241.99	100-102	Pet –ether : ethylacetate(5:5)	0.84
1c	$C_{16}H_{13}OF$	239.99	90-92	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.79
1d	$C_{17}H_{16}O_2$	252	80-82	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.65
1e	$C_{14}H_{12}O_3$	228	85-87	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.84

Table .3 : Physicochemical data for the synthesized compounds (Pyrazolines)

Comp. No.	Molecular Formula	Mol. Weight	M.P. range (^{0}C)	Mobile Phase	$^*R_F$ value	React^n time, Hrs.
2a	$C_{22}H_{16}N_4O_6$	432	158-160	Pet-ether : ethylacetate(5:5)	0.58	1.5
2b	$C_{21}H_{13}N_4O_5F$	419.99	140-142	Pet –ether : ethylacetate(5:5)	0.74	2
2c	$C_{22}H_{15}N_4O_4$	417.99	130-132	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.62	1.5
2d	$C_{23}H_{18}N_4O_5$	448.99	110-112	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.70	2
2e	$C_{20}H_{14}N_4O_6$	424.99	100-102	Ethanol:ethylacetate:acetic-acid(5:4:1)	0.90	2

1(2,4-dintrophenyl)3phenol-5(4-methoxyphenyl)pyrazoline (A)
IR (KBr,cm^{-1}): 3354 (OH str phenolic), 1601 (C-N), 1514, 1449,1338 (Aromatic C=C str), 1293, 1006 (C-O str),1514(N-O str).
^{1}H-NMR (δ ppm): 6.764-7.736 (, Ar-H), 3.294 (OCH_3), 2.410(OH)
^{13}C-NMR (δ ppm): 128.35**(**C=N), 130.41(C in Aromatic ring), 29.71(C-N), 95.41(C-O), 55.34(C-N), 11.34(CH_3)

1(2,4-dintrophenyl)3phenol-5(4-fluorophenyl)pyrazoline (B)
IR (KBr,cm^{-1}): 3348 (OH str phenolic), 2923 (-CH str), 1607, 1512, 1442 (Aromatic C=C str), 1344 (NO_2),1202(C-N),1344(C-F), 1282(C-O).
^{1}H-NMR (δ ppm): 7.281-7.741(Aromatic ring), 2.410(OH), 7.751(Ar-F), 3.882(Ar-OH)
^{13}C-NMR (δ ppm): C=N(127.41), 128.83(C in Aromatic ring),29.71(C-N),21.46(C-C), 56.27(C-F),11.34(CH_3)

1(2,4-dintrophenyl)3(4-methylphenyl)-5(4-fluorophenyl)pyrazoline (C)
IR (KBr,cm^{-1}): 1042(C-F),1285(C-N),1536(Niro gp),1650(C=C),2309(C-H).
^{1}H-NMR (δ ppm):7.910-8.225(Aromatic ring), 1.239(CH_3)
^{13}C-NMR (δ ppm):): 127.32**(**C=N), 130.41(C in Aromatic ring),29.71(C-N),21.46(C-C), 56.37(C-F), 11.44(CH_3)

1(2,4-dintrophenyl)3(4-methylphenyl)-5(4-methoxyphenyl)pyrazoline(D)
IR (KBr,cm^{-1}): 1035(C-C),1644(C=C),1358(C-N),989(C-O).
^{1}H-NMR (δ ppm): 6.764-7.751(Aromatic ring), 3.291(OCH_3), 1.279(CH_3),
^{13}C-NMR (δ ppm):): 123.11**(**C=N), 128.33(C in Aromatic ring),30.11(C-N),21.44(C-C), 11.23(CH_3)

1(2,4-dintrophenyl)3(2-furyl)-5(4-methoxyphenyl)pyrazoline(E)
IR (KBr,cm^{-1}):1464(Nitro gp),1271(C-O-C), 1332(C-N), 1593(C=C), 1367 (C-H), 992(C-H) .
^{1}H-NMR (δ ppm): 6.742-7.742(Aromatic ring), 2.440(OCH_3)
^{13}C-NMR (δ ppm): 123.11**(**C=N), 128.33(C in Aromatic ring),29.11(C-N),11.82(C-C), 22.16(CH_3), C-O(97.31)123.31(C=C)

BIOLOGICAL ACTIVITY :
Anticancer activity :
Cell line used :
MCF 7 (human cervical carcinoma)

All cell lines were grown and maintained in suitable (MEM -media and were grown and subcultured in medium supplemented with 10% fetal bovine serum,1% L-Glutamine.1%p penicillin streptomycin –streptomycin-amphotericine-B antibiotic solution. All cells were trypsinated using trypsin-EDTA solution and seeded in96 well plates[43-47].

Procedure

The MCF cell line was maintained in MEM medium supplemented with 10 % fetal bovine serum. The cells were plated at a density of 1×10^5 cells per well in a 96-well plate, and cultured for 24 h at 37 °C. The cells were subsequently exposed to 10 µM. The plates were incubated for 48 h, and cell proliferation was measured by adding 10 µL of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg ml^{-1} in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37 °C in a humidified chamber containing 5% CO_2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 200 µl DMSO, and absorbance was read at 490 nm. The results were compared with the standard drug inhibitors 5 flurouracil. (10µM) [43-47].

RESULTS AND DISCUSSION

The main objective of the study was to synthesize pyrazoline from aldehyde and ketone using as a starting material. Total five derivatives were synthesized in fairly good yield and yield of all derivatives were lies in the ranging from 68% to 82 %.

Thin layer chromatography was used to checking the completion of reaction as well as purity of all final products.

The structural characterization of synthesized compounds were done by interpretation of IR ,^{1}HNMR ,^{13}CNMR & GCMS All the compound exhibited satisfactory IR , NMR data.

The test compounds were screened for anticancer activity by MTT assay.

6.1 ANTICANCER ACTIVITY

compound were screened for anticancer activity against mcf cancer cell lines.

Percent Cytotoxicity = Reading of control - Reading of treated cells / Reading of control X 100

Fig. 1 : Drug concentration vs percent inhibitory

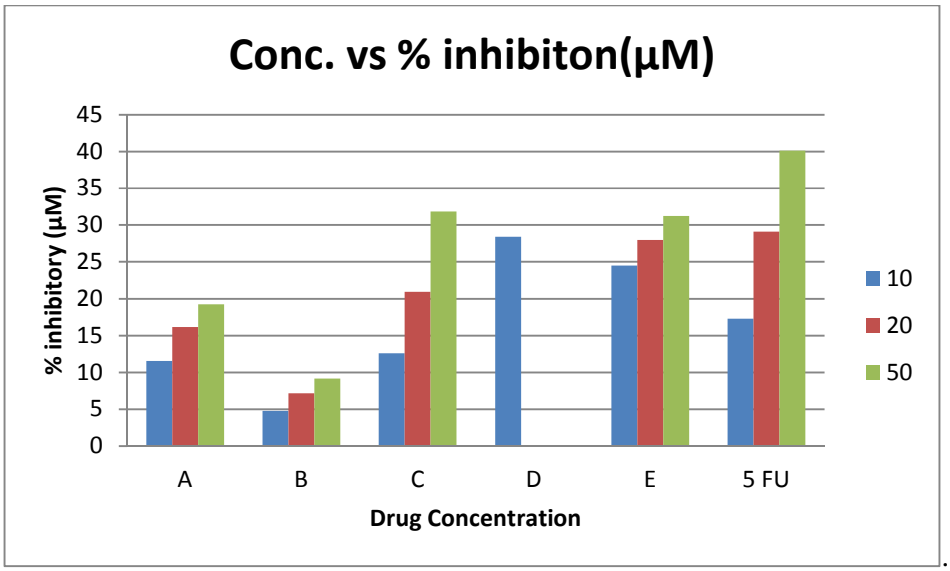


Table 3 : anticancer activity of synthesized compound against MCF cell lines

Compound	% inhibitory (µM)		
	10	20	50
A	11.55	16.17	19.25
B	4.78	7.15	9.14
C	12.58	20.95	31.85
D	28.40	00	00
E	24.51	28.00	31.25
5 FU	17.27	29.11	40.12

5FU : 5 Fluorouracil

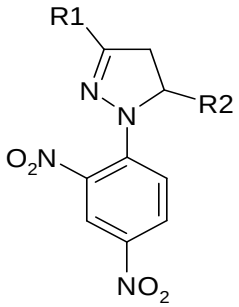
Generally, as shown in table, the prepared synthetic compound (A-E) displayed moderate to good inhibition activities against breast human cancer cell lines. Notably, the compounds C,D,E exhibited significant inhibitory activities against MCF-7 cell lines with 12.58,28.40,24.51% growth inhibition at 10µM /mL concentration compared to the positive control 5-FU (17.27%). The activity is due to the substituted nitro group on C -2 & C-4 position on aromatic group.

CONCLUSION

The synthetic scheme reported in this study design is novel example in heterocyclic synthesis. The synthesis of pyrazoline derivatives was carried out in two steps. These are as

1 Formation of Chalcone
2 Cyclisation to form pyrazolines

In firstly , when substituted aldehyde treated with substituted ketone to form chalcone. Further treated with suitable 2,4- dinitro phenyl hydrazine hydrate to form corresponding pyrazolines. The yield of all derivatives were lies in the ranging from 68% to 82 %. All synthesized compounds were meeting the expected spectral data. General structure confirmed from the collected spectral data is as follows,



All synthesized compounds characterized by spectral analysis.

All synthesized compounds are screened for anticancer activity and compared with standard drug. From the results it can be concluded that the modified pyrazoline shows remarkable anticancer activity.

Acknowledgements
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