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DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ARYL PYRAZOLE-INDANONE HYBRIDS

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ABSTRACT

A series of aryl pyrazole-indanone hybrids were synthesized by the Knoevenagel condensation of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **4** with various 1-indanones **5** at room temperature. The structural investigations were carried out with IR, ^{1}H NMR and mass spectral data. All the newly synthesized compounds were assessed for their anticancer and antimicrobial properties. Among the compounds screened **6d**, **6e** and **6f** displayed moderate anticancer potential against MCF-7 (IC_{50} 42.6-53.9 µM). On the other hand compounds **6a**, **6b**, **6c**, **6d** and **6g** unveiled potent activity against S. aureus and compound **6c** and **6b** displayed moderate activity against E. coli.

Keywords: Aryl Pyrazole, Indanone, Anticancer, Antimicrobial.

INTRODUCTION

Pyrazoles and its derivatives are a class of well-known nitrogen-containing heterocycles with diverse biological properties. Pyrazole analogs have also found to use as versatile building blocks in organic synthesis for designing pharmaceuticals and agrochemicals. They have been known to exhibit antimicrobial,[1] anticancer,[2] anti-inflammatory,[3] and selective enzyme inhibitory activities.[4] Moreover, a series of pyrazole derivatives are also used as insecticides,[5] fungicides,[6] and herbicides.[7]The pyrazole ring is present as the core in a variety of leading drugs such as Celebrex, Sildenafil (Viagra), ionazolac, Rimonabant and Difenamizole, etc. Furthermore, pyrazole compounds, such as pyrazophos, penthiopyrad and pyraclostrobin, have been found to have potential antifungal properties for the control of some plant diseases (Fig.-1).

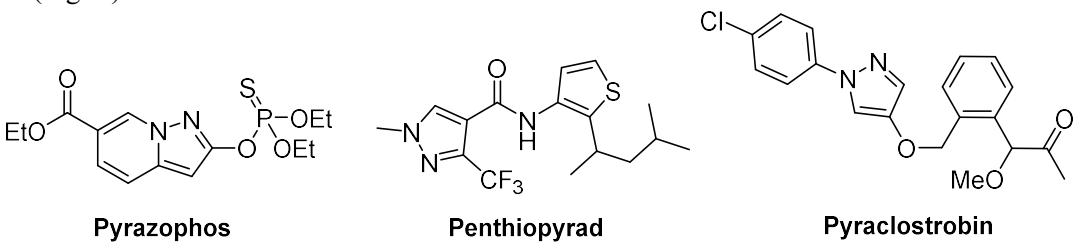

Fig.-1: Pyrazole Nucleus Containing Marketed Drugs

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1-Indanone and its structural analogs have also been played an important role in the field of medicine and agriculture. The presence of active methylene hydrogens adjacent to the carbonyl group of indanone makes it important in condensation reactions or some organic transformations.[8] In recent years indanone derivatives with anticancer,[9] antibacterial,[10] antiviral,[11] anticonvulsant,[12] antimicrobial,[13] antidiabetic,[14] antimalarial,[15] anti-inflammatory[16] activities have been reported. Indanone derivatives also found useful in the treatment of Alzheimer's diseases,[17] as well as insecticidal in the agrochemical field.[18] The structures of some biologically active 1-indanone derivatives[14,15,19] are depicted in Fig.-2.

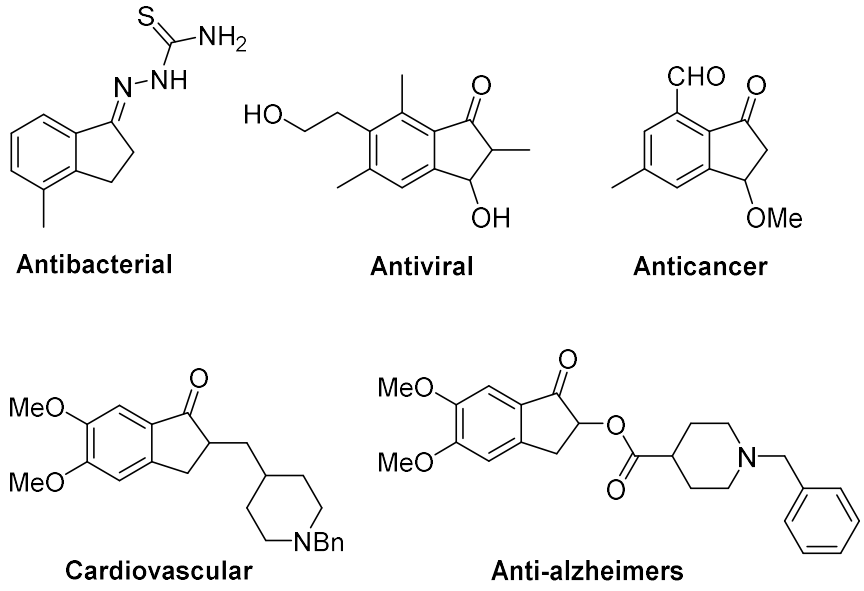


Fig.-2: Biologically Active 1-Indanones and their Derivatives

The combination of active scaffolds may offer a synergistic effect to improve therapeutic potential. Based on these observations and in continuation of our interest[20] in the synthesis of novel bioactive molecules with potential anticancer properties we herein synthesized hybrid scaffolds containing aryl pyrazole framework combined with various substituted 1-indanones and evaluated for their anticancer and antimicrobial properties.

EXPERIMENTAL

Material and Methods

IR spectra were recorded on FT-IR Nicolet iS 10 spectrophotometer and ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ using TMS as an internal standard and chemical shifts are reported in δ units and the coupling constants (J) are reported in Hertz. Mass spectra were obtained with a Shimadzu LCMS-2010EV. TLC was performed on an aluminum-backed silica plate with visualization by UV-light.

General Procedure for the Preparation of (2*E*)-2-[(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-methylidene]-2,3-dihydro-1*H*-inden-1-one (6a-f)

In a round bottom flask, a mixture of 5-chloro-3-methyl-1-phenyl-*1H*-pyrazole-4-carboxaldehyde **4** (0.220 g, 1 mmol) and 1-indanone **5** (0.132 g, 1 mmol) was dissolved in ethanol (15 mL) under stirring. To this solution was added sodium hydroxide (0.12 g, 3 mmol) dissolved in a minimum quantity of water and stirring continued for 1h. The completion of the reaction was monitored by TLC. After completion of the reaction, the solid product obtained was filtered off and washed with little cold ethanol. The crude product was dried and recrystallized from ethanol to obtain the desired product **6** in pure form.

Preparation of 3-Methyl-1-phenyl-2-pyrazolin-5-one (3)

A mixture of ethyl acetoacetate (5.2 g, 5.2 mL, 0.04 mol) and phenylhydrazine (4.3 g, 3.94 mL, 0.04 mol) was taken in a 100 mL round bottom flask and heated at 120 ºC with constant stirring under the solvent-

free condition for 4h on an oil bath. The completion of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled and diethyl ether (20 mL) was added to it. The obtained solid was filtered, washed with diethyl ether and recrystallized from ethanol to obtain the pure product 3-methyl-1-phenyl-2-pyrazolin-5-one **(3)** in excellent yield.

Preparation of 5-Chloro-3-methyl-1-phenyl-*1H*-pyrazole-4-carbaldehyde (4)
A mixture of 3-methyl-1-phenyl-2-pyrazolin-5-one **3** (2.205 g, 0.018 mol) and dimethylformamide (DMF) (10 mL, 0.13 mol) was taken in a three-neck round-bottomed flask equipped with reflux condenser under an inert atmosphere. The reaction mixture was cooled at 0 ºC and treated with $POCl_3$ (4.6 g, 2.8 mL, 0.03 mole), maintaining the temperature between 10-15 ºC. After complete addition, the reaction mixture was heated on a water bath for about 3h, cooled, and poured into ice water with vigorous stirring to obtain the desired compound **4** in good yield. The product obtained was recrystallized from ethanol as yellow needles.

Spectral data of representative compounds:
(E)-2-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-2,3-dihydro-1H-inden-1-one (6a)
^{1}H NMR ($CDCl_3$ 400 MHz): δ 2.44 (s, 3H, -*CH_3*), 3.94 (s, 2H, -*CH_2*-), 7.42-7.47 (m, 2H, -*ArH indanone*), 7.50-7.91 (m, 7H, *6 x –ArH, =CH*), 7.92 (d, J= 7.6 Hz, 1H, -*ArH*); LCMS (ESI): 335.45 (M+1).

(E)-2-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-6-methoxy-2,3-dihydro-1H-inden-1-one (6b)
^{1}H NMR ($CDCl_3$ 400 MHz): δ 2.41 (s, 3H, *Pyr-*CH_3), 3.86 (s, 2H, -*CH_2*-), 3.88 (s, 3H, -*OCH_3*), 7.22 (dd, J = 2.8 Hz, 8.4 Hz, 1H, -*ArH indanone*), 7.37 (d, J = 2.4 Hz, 1H, *ArH indanone*), 7.42 (d, J = 8.8 Hz, 1H, -*ArH indanone*), 7.46 (dd, J = 1.6 Hz, 7.2Hz, 1H, -*ArH*,), 7.50-7.54 (m, 3H, *2 x -ArH, =CH*), 7.57-7.59 (m, 2H, -*ArH*); LCMS (ESI): 365.30 (M+1).

(E)-2-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (6c)
^{1}H NMR ($CDCl_3$ 400 MHz): δ 2.42 (s, 3H, *Pyr-*CH_3), 3.84 (s, 2H, -*CH_2*-), 3.95 (s, 3H, -*OCH_3*), 3.99 (s, 3H, -*OCH_3*), 6.95 (s, 1H, -*ArH indanone*), 7.34 (s, 1H, -*ArH indanone*), 7.44 (m, 2H, -*ArH*), 7.49-7.52 (m, 2H, -*ArH*), 7.56-7.58 (m, 2H, -*ArH, =CH*); LCMS (ESI): 395.10 (M+1).

(Z)-3-((5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)indolin-2-one (6f)
^{1}H NMR ($CDCl_3$ 400 MHz): δ 2.32 (s, 3H, -*CH_3 Pyr*), 6.91(d, J = 8Hz, 1H, -*ArH indanone*), 6.98 (d, J = 8Hz, 1H, -*ArH indanone*), 7.20 (d, J = 7.6 Hz, 1H, -*ArH indanone*), 7.24 (d, J = 7.6 Hz, 1H, -*ArH indanone*), 7.64 (dd, J = 1.2 Hz, J = 8Hz, 2H, -*ArH*), 7.46 (d, J = 7.2 Hz, 1H, -*ArH*), 7.50-7.56 (m, 3H, *2 x –ArH, 1 =CH*), 8.23 (bs, 1H, -*NH*-); LCMS (ESI): 336.05 (M+1).

MTT Assay For Anticancer Screening
The cells were seeded at a density of approximately 5×10^3 cells/well in a 96-well flat-bottom microplate and maintained at 37 ºC in 95% humidity and 5% CO_2 overnight. Different concentration (500, 400, 300, 200, 100, 50 µg/ml) of samples was treated. The cells were incubated for another 48 hours. The cells in well were washed twice with phosphate buffer solution, and 20 µL of the MTT staining solution (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37 °C. After 4h, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using a microplate reader (1, 2).

Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control ×100

Using graph Pad Prism Version 5.1, we calculated the IC_{50} values of compounds.
Note: DMSO Concentration is less 1.5% in experiments. Concentrations are in duplicates

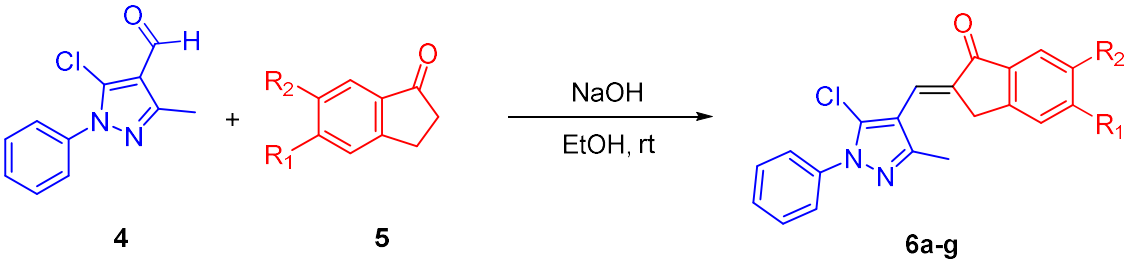
Protocol for Antimicrobial Evaluation: MIC Test (Aerobic)

The 9 dilutions of each drug have to be done with BHI for MIC. In the initial tube, 20 microliters of the drug were added into the 380 microliters of BHI broth. For dilutions, 200 microliters of BHI broth were added into the next 9 tubes separately. Then from the initial tube, 200 microliters were transferred to the first tube containing 200 microliters of BHI broth. This was considered as 10-1 dilution. From 10-1 diluted tube, 200 microliters were transferred to the second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each drug. From the maintained stock cultures of required organisms, 5 microliters were taken and added into 2ml of BHI (brain heart infusion) broth. In each serially, diluted tube 200 microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity.[21]

RESULTS AND DISCUSSION

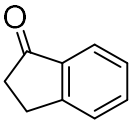
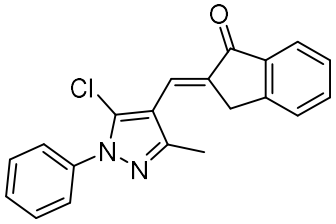
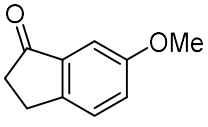
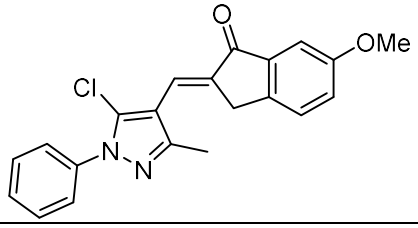
Chemistry

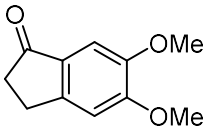
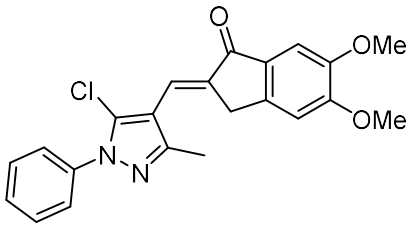
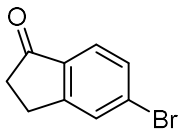
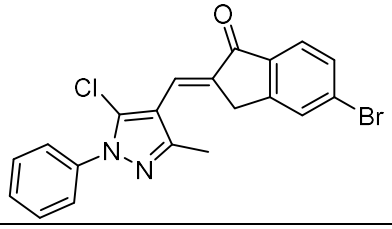
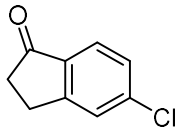
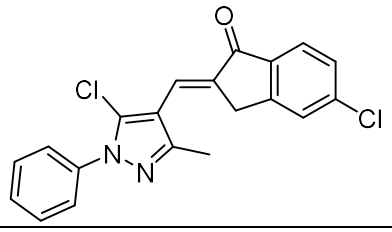
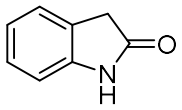
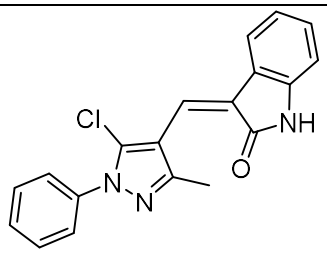
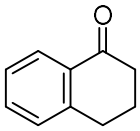
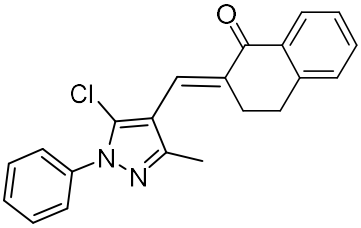
The synthesis of target molecules (**6a-g**) was achieved by the Knoevenagel condensation of 5-chloro-3-methyl-1-phenyl-*1H*-pyrazole-4-carbaldehyde (**4**) with 1-indanone (**5**) in at room temperature in the presence of sodium hydroxide in ethanol in good to excellent yield (Scheme-1). The precursor 5-chloro-3-methyl-1-phenyl-*1H*-pyrazole-4-carbaldehyde (**4**) was synthesized by Vilsmeyer-Hack formylation of 3-methyl-1-phenyl-2-pyrazolin-5-one (**3**). The synthesis of starting compound 3-methyl-1-phenyl-2-pyrazolin-5-one (**3**) was accomplished under solvent-free condition by the condensation of ethyl acetoacetate (**1**) and phenyl hydrazine (**2**) at 120 ºC (Scheme-2). The structural investigation of the synthesized compounds was carried out using IR, ^{1}H NMR and mass spectral data. The structures of synthesized compounds are presented in Table-1.

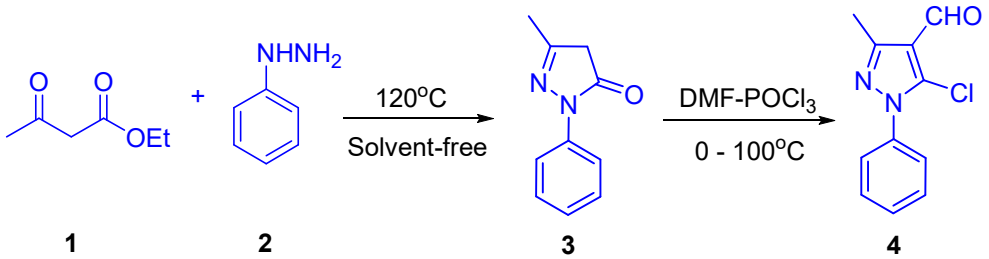


Scheme-1: Synthesis of Aryl Pyrazole-indanone Hybrids

Table-1: Structures of the Aryl Pyrazole-1-indanone Hybrids

Entry	Active methylene Compound	Product	Yield %	M.P. ºC
6a			93	116-118
6b			78	140-142

6c			81	182-186
6d			87	170-172
6e			88	175-176
6f			83	172-175
6g			82	162-164



Scheme-2: Preparation of Precursor Aryl Pyrazole Aldehyde, **4**

Biological Evaluation

Anticancer Activity

All the synthesized compounds were screened for their anticancer potential against breast carcinoma (MCF-7) using MTT assay method using paclitaxel as a reference standard drug. The results are summarized in Table-2. The IC_{50} values revels that compound **6d**, **6e** and **6f** have shown moderate anticancer activities (IC_{50} 42.6-53.9 µM) and all other compounds displayed poor anticancer activities

against MCF-7. Structure-activity relationship study reveals that the compounds bearing electron-withdrawing groups like chloro, bromo on 1-indanone motif enhances anticancer activity.

Antimicrobial Activity

All the synthesized compounds also screened for their antimicrobial potential against gram-positive and gram-negative bacteris *viz*. *S. aureus* and E. coli respectively using ciprofloxacin as a reference standard The MIC values are presented below in Table-2. The results obtained reveal that most of the synthesized compounds possess significant antimicrobial activities against both the bacterial strains. Among the compounds screened compound **6a**, **6b**, **6c**, **6d** and **6g** exhibited excellent activity against *S. aureus* and compound **6c** and **6b** displayed moderate activity against E. coli. However, remaining compounds showed moderate activity against both *S. aureus* and *E. coli*.

Table-2: Anticancer and Antimicrobial Screening of the Aryl pyrazole-1-indanone Hybrids

Entry	Anticancer Activity MCF-7	Antimicrobial Activity MIC (µg/ml)	
	IC_{50} (µM)	*S. aureus*	*E. coli*
6a	383.7	0.4	25
6b	298.5	0.7	8.50
6c	318.1	0.8	6.25
6d	47.0	0.8	25
6e	42.6	1.25	21
6f	53.9	6.25	12.5
6g	260.2	0.9	25
Paclitaxel	0.35	--	--
Ciprofloxacin	--	2.0	2.0

CONCLUSION

In conclusion, we have synthesized hybrid molecules by combining aryl pyrazole with various 1-indanones under basic conditions. The results of the anticancer study reveal that compounds **6d**, **6e** and **6f** showed moderate anticancer potential against MCF-7 with IC_{50} values 42.6 to 53.9 µM. the results of antimicrobial study disclose that compound **6a**, **6b**, **6c**, **6d** and **6g** possess significant inhibition of *S. aureus* (MIC: 0.4 – 0.9 µg/ml) and compound **6c** and **6b** possess moderate inhibition of *E. coli* (MIC: 6.25 – 8.50 mM).

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