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Anti-inflammatory and Anti-cancer studies of newly synthesized derivatives of Para-bromo benzoic acid

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Abstract
Synthetic derivatives of pyrazole have widespread biological actions like antibacterial, anti-mycobacterial, antifungal, anti-HIV, anti-malarial, herbicidal, analgesic, anti-inflammatory, anticonvulsant etc. Pyrazole is a π-excessive heterocycle and contain 2 nitrogen atoms adjacent to each other. In the current work newly synthesized derivatives of P-bromo benzoic acid were tested for their anti-cancer as well as anti-inflammatory activity. The results of the in-vitro assays show that the derivative 1-(4-bromobenzoyl)-3-(4-bromo phenyl)-1H-pyrazole-4-carbaldehyde has the highest anti-inflammatory and anti-cancer activity.

Keywords: anti-inflammatory, anti-cancer, pyrazole derivatives

1. Introduction
Since ages Pyrazole derivatives [1, 2] have been known to have Anti-cancer and anti-inflammatory activity. So the newly synthesized 1-(4-bromobenzoyl)-3-(4-substituted phenyl)-1H-pyrazole-4-carbaldehyde[47] were selected for carrying out the *in-vitro* studies.

2. Materials and Methods
The Dalton's Lymphoma Ascites cell lines (DLA) were obtained from Amala Cancer Research Center, Thrissur. The Trypan blue exclusion method was used to determine the extent of cytotoxicity.

2.1 Methodology
2.1.1 *In-vitro* anti-inflammatory study
The anti-inflammatory activity studies of the synthesized compounds were studied at department of pharmacology, SJCP, Cherthala. The test compounds were studied for *invitro* anti-inflammatory activity studies by means of Human Red Blood Corpuscles(HRBC) membrane stabilizing method. The test compounds AV1[1-(4-bromobenzoyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carbal dehyde],AV2[1-(4-bromobenzoyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde], AV3[1-(4-bromobenzoyl)-3-(4-bromophenyl)-1H-pyrazole-4-carbaldehyde], AV4[1-(4-bromobenzoyl)-3-(4-flourophenyl)-1H-pyrazole-4-carbaldehyde],andAV5[1-(4-bromobenzoyl)-3-(4-Chlorophenyl)-1H-pyrazole-4-carbaldehyde] at the concentration (500µg/ml,100µg/ml) exhibited varying degree of anti inflammatory activities when compared to that of the standard drug Diclofenac(500µg/ml).

2.1.1.1 Human Red Blood Corpuscle (HRBC)Membrane stabilizing method [3, 4]
2.1.1.1.1 Preparation of Alsever solution
Alsever solution was prepared by dissolving 2% Dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42 %sodium chloride in 100 ml distilled water and sterilized.

2.1.1.1.2 Procedure
The blood was collected from an healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution. Then these mixture was transferred into centrifuge tubes which is then centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10 % suspension in normal saline was made. The reaction mixture (4-5 ml)consisted 2 ml hypotonic saline (0.25%w/v NaCl), 1ml of 0.15 M phosphate buffer (pH 7.4), 1ml of test solution (500µg/ml) in normal saline and 0.5 ml of 10 % HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 37^0C for 30 min and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The haemoglobin content of the solution was estimated by using colorimetry at 560nm. The control represents 100 % lysis. The percentage membrane stabilization was calculated using the formula.

$$\% \text{ inhibition of haemolysis} = 100 \times \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of Control}}$$

2.1.2 Anti-cancer activity study [5, 6]
2.1.2.1 Procedure for Trypan blue exclusion method
The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice using normal saline or Phosphate Buffered Saline (PBS). Viability of the cells was determined by trypan blue exclusion method. Viable cell suspension was taken from the peritoneal cavity of tumour bearing mice. Viable cell suspension ($1x10^6$ cells in 1.0 ml) was added to the tubes containing various

concentrations like 200, 100, 50, 20, 10µg of the test compounds and the volume was made up to 1ml using Phosphate Buffered saline (PBS). Control tube contained only the cell suspension. These tubes were incubated for 3hrs at 37^0C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes. Then it was loaded on a haemocytometer. On observation it was found that the dead cells take up the blue colour of Trypan blue and while the live cells do not take up the dye. The number of stained and unstained cells was separately counted. The anti-cancer activities of the compounds were represented as percentage cell death.

3. Results & Discussion

3.1 *In-vitro* anti-inflammatory study

The results of anti-inflammatory studies using Human Red Blood Corpuscle (HRBC) Membrane stabilizing method on the newly synthesized pyrazole derivatives showed that the compound AV3 has the maximum anti-inflammatory activity.

Table 1: *In vitro* anti-inflammatory activity screening data

Compound Code	Absorbance at 560nm		Percentage Haemolysis	
	500µg/ml	100 µg/ml	500 µg/ml	100 µg/ml
AV1	0.18	0.20	70%	66.67%
AV2	0.16	0.19	73.33%	68.33%
AV3	0.14	0.16	76.67%	73.33%
AV4	0.20	0.21	66.67%	65%
AV5	0.17	0.22	71.67%	63.33%
Diclofenac(500µg/ml)	0.010		98.33%	

Absorbance of Control = 0.60

3.2 Anti-cancer activity Study

All the synthesized derivatives were tested for their short term *in-vitro* cytotoxicity using Dalton's Lymphoma Ascites cells (DLA) by means of Trypan blue exclusion method. The anti-cancer activities of the compounds were represented as percentage cell death. The percentage cell death of the DLA cells corresponding to the various concentrations (200µg/ml, 100µg/ml, 50µg/ml) of the compounds was reported and summarized in TableNo:2

Table 2: Anti-cancer activity results

Compound Code	Sample Concentration(µg/ml)	Percentage Cell Death (DLA)
AV1	200	56
	100	30
	50	18
AV2	200	54
	100	32
	50	18
AV3	200	**57**
	100	34
	50	20
AV4	200	52
	100	32
	50	19
AV5	200	54
	100	32
	50	19
+ve Control (5-Fluro Uracil)	200	100
	100	100
	50	80
Control (DMSO)	200	00
	100	00
	50	00

From the table it was clearly seen than the newly synthesized compound AV3 has profound anti-cancer activity when compared with the other derivatives.

4. Conclusions

1-(4-bromobenzoyl)-3-(4-substitutedphenyl)-1H-pyrazole-4-carbaldehyde derivatives were prepared and screened for anti-inflammatory activity by HRBC method and anti-cancer activity by Trypan Blue Exclusion method. The compound AV3 exhibited the best anti-inflammatory as well as anti-cancer activity among the derivatives.

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6. References

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