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SYNTHESIS AND BIOLOGICAL EVALUATION OF COUMARIN DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

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ABSTRACT: All the newly synthesized Coumarin derivatives have shown considerable anti-inflammatory activity. In the present research work, we synthesized a series of novel Coumarin derivatives. The Coumarin derivatives such as substituted 7-hydroxy-4-methyl-2H-chromen-2-one and substituted 6-chloro-7-hydroxy-4-methyl-2H-chromen-2-one were synthesized from substituted benzoyl chloride. The chemical structures of the synthesized compounds were confirmed by means of IR and ¹H-NMR. These compounds were screened for anti-inflammatory activity by carrageenan induced paw edema method in rats at a dose of 30 mg/kg body weight. All the tested compounds of Coumarin derivatives were found to be more potent when compared to standard drug Diclofenac.

INTRODUCTION: The process of inflammation is accompanied by the activation of the immune system, local vascular system, and cells of the damaged tissue. ¹ It is a complex physiological and pathological process caused by physical trauma, noxious chemicals, microbiologic agents and is associated with various diseases such as cardiovascular diseases, cancers, diabetes, Alzheimer's disease, pulmonary diseases, and autoimmune diseases rheumatoid arthritis (RA), inflammatory bowel disease, psoriasis and multiple sclerosis. ²⁻⁴ Coumarins have been the field of interest in the development of anti-inflammatory agents. According to the extensive literature studies various coumarin derivatives have been found to possess anti-inflammatory activity by varying mechanisms. ⁵

The versatility of coumarin to accompany wide range of substitutions to show anti-inflammatory activity is obtained by the research inputs of K. Upadhyay et al. ⁶ showing the anti-inflammatory activity of 4-styryl Coumarins by TNF- α inhibition and IL-6 inhibition, E.Y. Bissonnette et al. ⁷ described modifications on 3-carboxylate coumarins as moderate inhibitors of TNF- α thus exhibiting the anti-inflammatory activity, S. Kumar et al. ⁸ evaluated oxygenated coumarins and corresponding thiocoumarins for their effects on TNF- α induced expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, Zhao D. et al. ⁹ reported the anti-inflammatory activity of umbelliferone 6-carboxylic acid which containing coumarin as the basic nucleus, Various structural derivatives of 6- hydroxycoumarin and 7- hydroxycoumarin were synthesized and evaluated by Koketsu et al. ¹⁰

Various other contributions made on coumarins also justifies its role in development of molecules possessing anti-inflammatory activity. ^{11, 12}

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Along with this, coumarin nucleus supports multiple varieties of substituent and that to on multiple positions that again make it a versatile nucleus to work on. With the consideration of the potential of the coumarin nucleus towards treatment of inflammation, SAR generated by Cheng et al.¹¹ and comparative SAR reviewed by Kayal et al.⁵, various derivatives have been synthesized and evaluated for anti-inflammatory activity.

MATERIAL AND METHODS:

The chemicals used were procured from Sigma Aldrich, Highmedia, and E. Merck. All the melting points were determined by open capillary method and uncorrected. The reactions were monitored by TLC on silica gel G plates using Petroleum Ether: Ethyl Acetate as developing solvent system in the ratio of 8: 2. Iodine vapor and UV detector (long wavelength) were used as detecting agents. The purification of intermediates and final compounds was carried out through recrystallization. IR spectrum of compounds in was recorded on SHIMADZU spectrophotometer. ¹H-NMR Spectra of compounds was recorded on BRUKER AVANCE II 400 NMR Spectrometer in deuterium-substituted chloroform using TMS as internal Standard (Chemical Shift in δ ppm). Anti-

inflammatory activity of the synthesized compounds was tested using carrageenan induced acute paw edema in albino rat. The dose of the synthesized compounds (30 mg/kg body weight) and Diclofenac (100 mg/kg body weight) were administered orally in 2% acacia.

Synthesis of 7-hydroxy- 4-methyl coumarin:

A beaker containing 100 ml of concentrated sulphuric acid was kept in an ice bath, to this solution 0.1 mol of resorcinol and ethyl acetoacetate was added, with continuous stirring for two hours, maintaining the temperature below 10⁰C. The reaction mixture was kept at room temperature for 18 hrs and then poured into the mixture of 200g of crushed ice and 300 ml of distilled water, with vigorous stirring. The white precipitate formed was collected by vacuum filtration, washed with 350 ml of cold water, dissolved in 150 ml of 5% w/v sodium hydroxide solution and filtered. The filtrate was added in 55 ml of concentrated sulphuric acid with vigorous stirring until the solution was acidic. The crude product of 7-hydroxy-4-methyl coumarin was collected by filtration, washed with cold water, dried, and purified by recrystallization from ethanol.

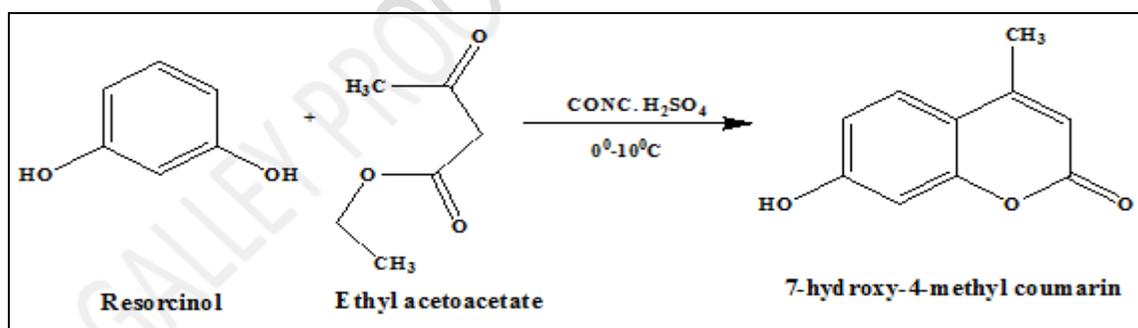


FIG.1: SYNTHESIS OF 7-HYDROXY-4-METHYL COUMARIN

Synthesis of 6-chloro-7-hydroxy- 4-methyl coumarin:

A beaker containing 100 ml of concentrated sulphuric acid was kept in an ice bath, to this solution 0.1 mol of 4-chloro resorcinol and ethyl acetoacetate was added, with continuous stirring for two hours, maintaining the temperature below 10⁰C. The reaction mixture was kept at room temperature for 18 hrs and then poured into the mixture of 200g of crushed ice and 300 ml of distilled water, with vigorous stirring. The white

precipitate formed was collected by vacuum filtration, washed with 350 ml of cold water, dissolved in 150 ml of 5% w/v sodium hydroxide solution and filtered. The filtrate was added in 55 ml of concentrated sulphuric acid with vigorous stirring until the solution was acidic. The crude product of 6-chloro-7-hydroxy-4-methyl coumarin was collected by filtration, washed with cold water, dried, and purified by recrystallization from ethanol.

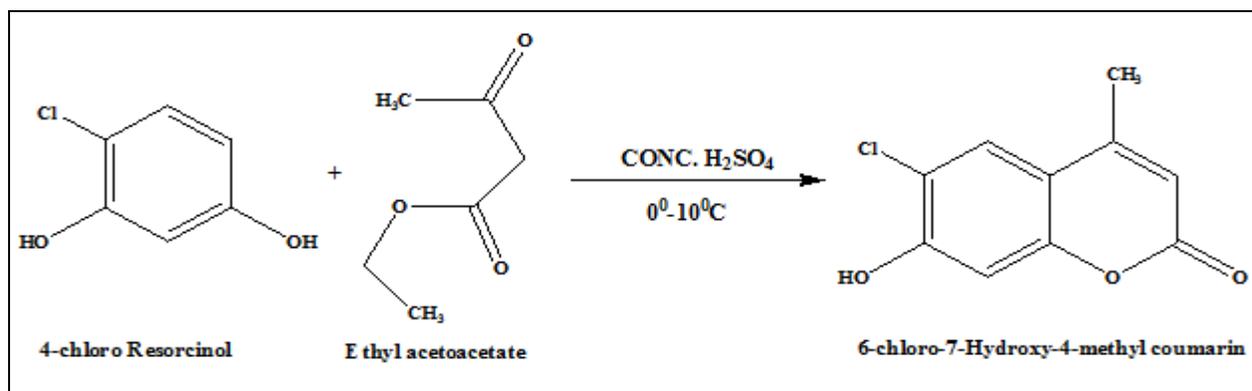


FIG.2: SYNTHESIS OF 6-CHLORO-7-HYDROXY-4-METHYL COUMARIN

Synthesis of 7-hydroxy-4-methyl Coumarin Derivatives (Ia-Ic):

Benzoyl chloride analogs were taken into 20 ml of Dichloromethane in round bottom flask and cooled to 0°C. To this reaction mixture triethylamine (0.005mol) was added slowly with constant stirring. Followed by 7-hydroxy-4-methyl Coumarin (0.005 mol), was added with continuous stirring. The reaction mixture was stirred at 0°C for

another 2 hrs. and stirring continued at room temperature for overnight. Progress of the reaction mixture was monitored through TLC. Then reaction mixture was washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and passed through anhydrous Na₂SO₄. Solvent was removed under vacuum and recrystallized by ethanol.

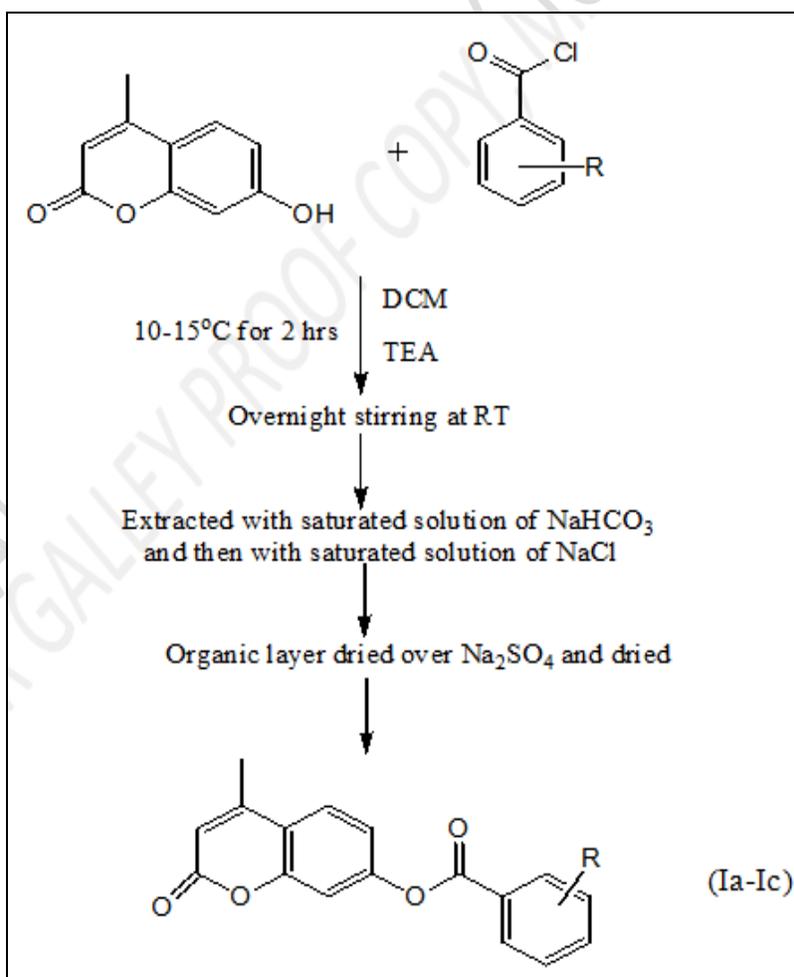


FIG.3: SYNTHESIS OF 7-HYDROXY-4-METHYL COUMARIN DERIVATIVES BY ESTERIFICATION

TABLE 1: STRUCTURE OF SYNTHESIZED COMPOUNDS (Ia-Ic)

Comp. Name	Structure of Proposed Comp.	IUPAC NAME
I-a		4-methyl-2-oxo-2H-chromen-7-yl benzoate
I-b		4-methyl-2-oxo-2H-chromen-7-yl 4-methoxybenzoate
I-c		4-methyl-2-oxo-2H-chromen-7-yl 2-chlorobenzoate

Synthesis of 6-chloro-7-hydroxy-4-methyl Coumarin Derivatives (Id-Ie):

Benzoyl chloride analogs were taken into 20 ml of Dichloromethane in round bottom flask and cooled to 0°C. To this reaction mixture triethylamine (0.005 mole) was added slowly with constant stirring. Followed by 6-chloro-7-hydroxy-4-methyl Coumarin (0.005 mole), was added with continuous stirring. The reaction mixture was stirred at 0°C for

another 2 hrs. and stirring continued at room temperature for overnight. Progress of the reaction mixture was monitored through TLC. Then reaction mixture was washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and passed through anhydrous Na₂SO₄. Solvent was removed under vacuum and recrystallized by ethanol.

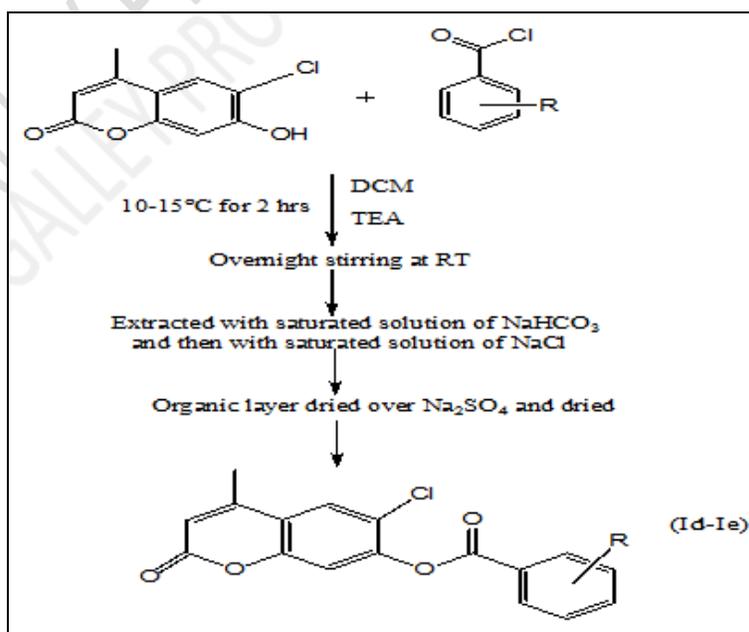
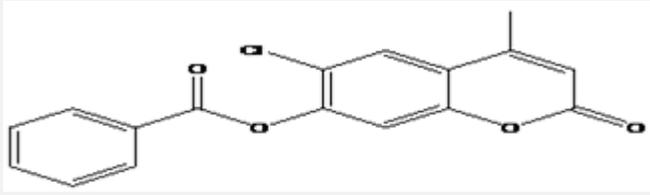
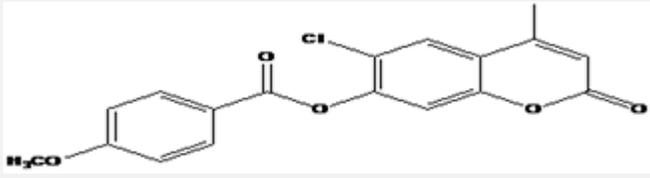


FIG.4: SYNTHESIS OF 6-CHLORO-7-HYDROXY-4-METHYL COUMARIN DERIVATIVES (Id-Ie)

TABLE 2: STRUCTURE OF SYNTHESIZED COMPOUNDS (Id-Ie)

Comp. Name	Structure of Proposed Comp.	IUPAC NAME
I-d		6-chloro-4-methyl -2-oxo-2H-chromen -7-yl benzoate
I-e		6-chloro-4-methyl -2-oxo-2H-chromen-7-yl 4-methoxybenzoate

In-vivo Biological Evaluation:

Induction of Inflammation: The acclimatized animals were kept under controlled standard conditions ($23 \pm 1^\circ\text{C}$, $55 \pm 10\%$ humidity and a 12-h light/dark cycle). Food and water was provided *ad libitum*. The test compounds were administered orally to the rats suspended in 2% Acacia. The control animals received 2% Acacia. Thirty minutes after drug administration, 0.1 ml of 1% carrageenan in normal saline solution was injected into sub plantar region of left hind paws. Anti-inflammatory activity of the synthesized compounds was carried out in the group of 6 rats.

Group 1: Inflammation control (Carrageenan induced)

Group 2: Reference standard (Diclofenac 100 mg/kg body weight)

Group 3: Ia compound (30 mg/kg body weight for acute study)

Group 4: Ib compound (30 mg/kg body weight for acute study)

Group 5: Ic compound (30 mg/kg body weight for acute study)

Group 6: Id compound (30 mg/kg body weight for acute study)

Group 7: Ie compound (30 mg/kg body weight for acute study)

Anti-inflammatory activity of the synthesized compounds was tested using carrageenan induced acute paw edema in albino rat. The dose of the synthesized compounds (30 mg/kg body weight) and Diclofenac (100 mg/kg body weight) were administered orally in 2% acacia. The diameter of sub plantar region of left hind paw was monitored at different times 0.5, 1, 2, 3, 5 and 6h respectively. The decrease in paw diameter against each compound was measured (Table 3).

RESULT AND DISCUSSION:**Spectral Data of the synthesized compounds:**

4-methyl-2-oxo-2H-chromen-7-yl benzoate (I-a): $^1\text{H NMR}$ (CDCl_3) δ 8.20-8.22 (m, 2H, arom. CH), 7.65-7.69 (t, 2H, arom. CH), 7.52-7.56 (m, 2H, arom. CH), 7.20-7.26 (m, 2H, arom. CH), 6.29-6.30 (d, 1H, ethylene CH), 2.46-2.51 (m, 3H, methyl CH). M.P. $148-150^\circ\text{C}$, % Yield: 63.13%

4-methyl-2-oxo-2H-chromen-7-yl - 4 - methoxy benzoate (I-b): $^1\text{H NMR}$ (CDCl_3) δ 8.08-8.17 (m, 2H, arom. CH), 7.64-7.66 (d, 1H, arom. CH), 7.18-7.26 (m, 2H, arom. CH), 6.97-7.02 (m, 2H, arom. CH), 6.28-6.29 (d, 1H, ethylene CH), 3.87-3.91 (t, 3H, methoxy CH), 2.45-2.46 (d, 3H, methyl CH). M.P. $156-158^\circ\text{C}$, % Yield: 42.05%

4-methyl-2-oxo-2H-chromen-7-yl - 2 - chloro benzoate (I-c): $^1\text{H NMR}$ (CDCl_3) δ 8.05-8.08 (m, 1H, arom. CH), 7.66-7.68 (d, 1H, arom. CH), 7.51-7.56 (m, 2H, arom. CH), 7.40-7.44 (m, 1H, arom. CH), 7.23-7.27 (m, 2H, arom. CH), 6.300-6.303 (d, 1H, methylene CH), 2.464-2.467 (d, 3H, methyl CH). M.P. $158-160^\circ\text{C}$, % Yield: 67.48%

6-chloro-4-methyl-2-oxo-2H - chromen – 7 - yl benzoate (I-d): ¹H NMR (CDCl₃) δ 8.23-8.25 (m, 2H, arom. CH), 8.15-8.17 (m, 2H, arom. CH), 8.10-8.13 (m, 1H, arom. CH), 7.66-7.71 (m, 2H, arom. CH), 6.33-6.34 (d, 1H, ethylene CH), 2.44-2.46 (m, 3H, methyl CH). M.P. 158-160°C, % Yield: 27.58%

6-chloro-4-methyl-2-oxo-2H-chromen-7 - yl - 4-methoxy benzoate (I-e): ¹H NMR (CDCl₃) δ 8.18-8.21 (m, 2H, arom. CH), 7.98-8.00 (m, 1H, arom. CH), 6.69-7.03 (m, 3H, arom. CH), 6.323-6.326 (d, 1H, ethylene CH), 3.91 (m, 3H, methoxy CH), 2.443-2.446 (d, 3H, methyl CH). M.P. 168-170°C, % Yield: 22.10%.

TABLE 3: THE ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compound	Dose(mg/kg)	Mean Diameter of left hind paw (mm)						
		0h	0.5h	1h	2h	3h	5h	6h
Control	-	2.57±	2.73±	3.46±	4.51±	4.92±	5.03±	5.13±
		0.043	0.034	0.044	0.041	0.054	0.021	0.073
Standard	100	2.69±	2.8±	3.11±	3.45±	3.39±	3.34±	3.26±
		0.044	0.045	0.041	0.067	0.047	0.034	0.036
Ia	30	2.7±	2.85±	3.35±	3.95±	4.05±	3.51±	3.48±
		0.051	0.046	0.056	0.061	0.042	0.078	0.067
Ib	30	3.1±	3.24±	3.8±	4.12±	4.29±	3.89±	3.87±
		0.052	0.027	0.067	0.023	0.034	0.046	0.035
Ic	30	2.13±	2.27±	2.79±	3.48±	3.3±	3.08±	3.02±
		0.047	0.035	0.056	0.056	0.078	0.041	0.082
Id	30	2.73±	2.86±	3.3±	3.89±	3.87±	3.59±	3.45±
		0.048	0.067	0.023	0.078	0.041	0.073	0.078
Ie	30	1.69±	1.82±	2.23±	2.79±	2.75±	2.51±	2.34±
		0.061	0.028	0.034	0.071	0.058	0.087	0.067

TABLE 4: IN-VIVO ANTI-INFLAMMATORY CONCENTRATION DATA OF SYNTHESIZED COMPOUNDS

Compound	% Inhibition
Standard (Diclofenac)	77.73
Ia	69.53
Ib	69.92
Ic	65.23
Id	71.85
Ie	74.60

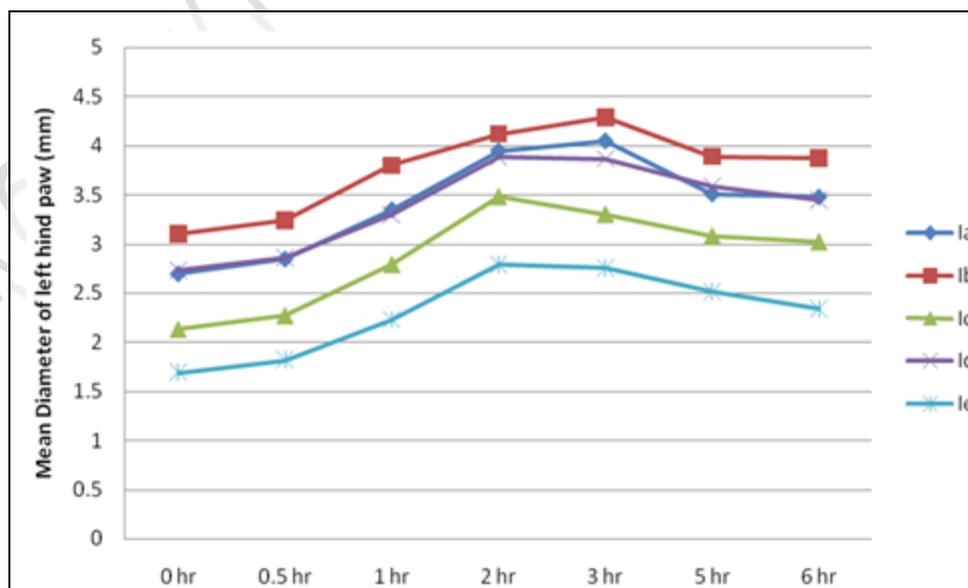


FIG.5: COMPARATIVE ANTI-INFLAMMATORY ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS

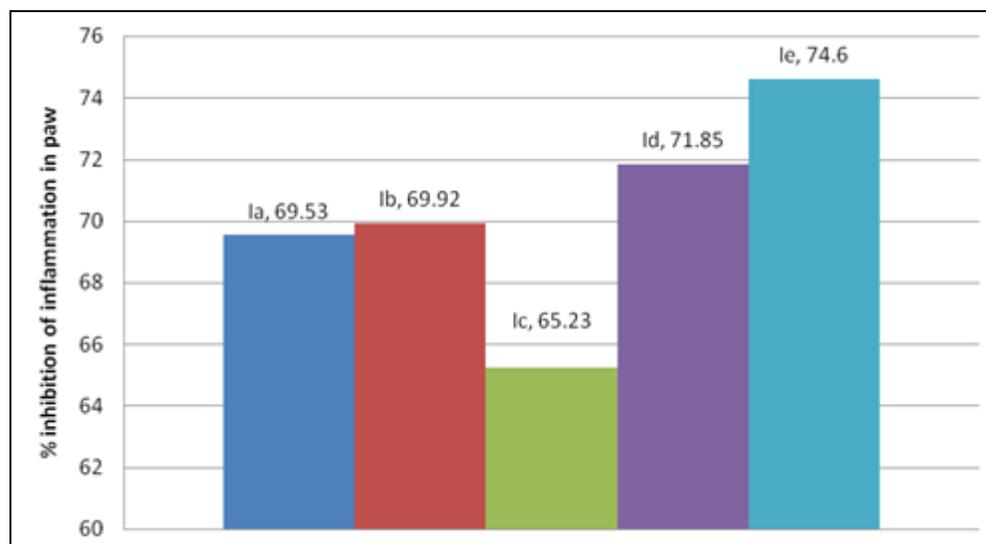


FIG.6: % INHIBITION OF INFLAMMATION BY SYNTHESIZED COMPOUNDS

Coumarin analogs with various substitutions on Coumarin moiety were studied. Synthesized compound shows anti-inflammatory activity in the range of 65.23-74.60% (Table 4). The compound with unsubstituted coumarin moiety (I-a) shows minimum inhibition as compared to substituted compounds. In particular, I-e derivative with 6-Cl and 7-(4-methoxy benzoate) proved to be the most active among these substituted compounds.

The present study has resulted in the identification of 4-methyl-2-oxo-2H-chromen-7-yl benzoate analogs as anti-inflammatory agents. This class of compounds might address the complications of inflammatory disorders with safety.

The field is further open for study of these compounds with respect to toxicity studies, pharmacokinetic studies and mechanism approach so as to establish such coumarin analogs as better and safer agents for the managements of inflammatory complications.

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