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Research Article

SYNTHESIS AND EVALUATION OF INVITRO ANTI-INFLAMMATORY ACTIVITY OF COUMARIN DERIVATIVES

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ABSRACT

Reaction of resorcinol with ethylacetoacetate by the addition of cold conc., sulphuric acid, gives 7-hydroxy-4-methyl coumarin **1.** Further**, c**oumarin derivatives **2, 3** & **4** have been synthesized from 7-hydroxy-4-methyl coumarin **1**. The method includes mild conditions, required less time and the yields were satisfactory. The reactions led to the expected products and the products were purified by recrystallization**.** The structure of synthesized coumarin derivatives has been established by elemental analysis, IR and ^{1}H NMR spectral data. All the synthesized compounds have been screened for their invitro anti-inflammatory activity by HRBC Membrane Stabilization. The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by these four synthesized coumarin derivatives to predict the anti-inflammatory activity. The four synthesized compounds at the concentration of 25, 50 and 100 µg/ml were incubated separately with HRBC solution and the % of haemolysis was compared with standard drug Diclofenac sodium at the same concentration. All the tested compounds possess significant invitro anti-inflammatory activity when compared with standard.

Keywords: 7-hydroxy-4-methyl coumarin, anti-inflammatory activity, HRBC Membrane Stabilization Method.

INTRODUCTION

Coumarin, 5, 6-benzo -2-pyrone is an important class of oxygen heterocycles. The name derived from a French word “Coumarou” for the tonka been (Dipteryx odarata). Coumarin and its derivatives represent one of the most active classes of compound possessing a wide spectrum of biological activity. Many of these compounds have proven to be active as antifungal[1], antibacterial[2], antioxidant[3], anticoagulant[4], anti-inflammatory[5], analgesic[6], antitumor[7], anti cancer[8, 9] and anti-HIV[10]. Coumarins are widely used as additives in food, perfumes, and cosmetics and would dispersed fluorescent and laser dyes.

Most of the coumarin derivatives were attempted at 4th&7th positions of basic nucleus and found that the pharmacological and biological properties and therapeutic application of simple coumarin depend upon the pattern of substitutions. Present work is carried out on 7-hydroxy, 4-methyl derivatives for invitro anti-inflammatory activity.

Anti inflammatory activity possess the stability of lysosomal membrane. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. In present invitro anti-inflammatory activity HRBC membrane are similar to lyso somal membrane components. The prevention

of hypotonicity induced HRBC membrane lysis is taken as a measure of anti inflammatory activity of coumarin derivatives.

The preparation of 4-methyl- 7-hydroxy coumarin which was used as a starting material for the synthesis of compounds **2, 3** and **4** is presented in the study. The chemical structure of the synthesized compounds **1-4** were conducted and confirmed.

EXPERIMENTAL

Melting points were determined in open capillaries on a Thiel's tube apparatus and are uncorrected. The purity of compounds was checked by TLC (Merck precoated plates, silica Gel G and ethylacetate: benzene 3:1). IR spectra were recorded on a BRUKER-FTIR spectrophotometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}HNMR spectra were recorded in DMSO-*d6* on AVANCE-300. The synthesized compounds gave satisfactory C, H, O and N analysis. Starting materials were purchased from s d fine.chem.limited or finar chemicals limited and used without further purification. All solvents were of analytical grade and freshly distilled prior to use.

Synthesis of 7-Hydroxy-4-methyl Coumarin (1)

To the mixture of resorcinol (5.5 g, 0.05 mol) and ethylacetoacetate (11ml), 10 ml of cold conc., sulphuric acid was added drop wise in an ice cold condition. The reaction mixture stirred for about 30 min by placing the beaker on ice bath and the precipitated product was filtered off, washed with cold water and purified by recrystalization. IR (KBr, v_{max}, cm^{-1}): 3532 (OH), 848 (C-H, Ar), 1688 (C=O, Lactone), 1508 (C=C). ^{1}H NMR δ (ppm): 2.36 (s, 3H, C_4-CH_3), 10.5 (s, 1H, C_7, OH), 6.7-8.6 (m, 4H, Ar).

Synthesis of 4-methyl-2-oxo-2H-Chromen-7-yl benzoate (2)

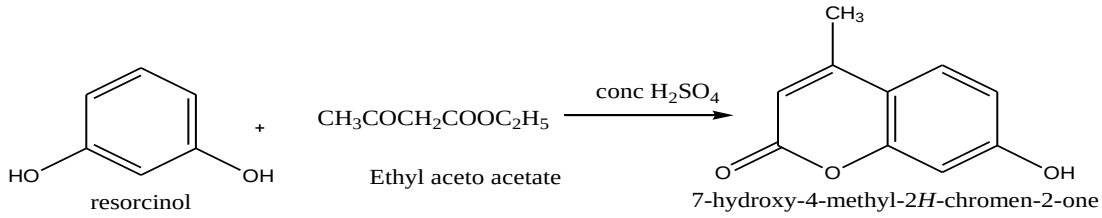
Dissolve compound **1** (1 g, 0.004 mol) in 5 ml of acetone and add 2.5 ml of benzoyl chloride. Add 10 ml of aqueous sodium hydroxide slowly. Stopper the flask and shake profusely till the odour of benzoyl chloride is vanished. The final solution must be alkaline to litmus. Filter off the solid benzoyl derivative, wash first with dilute hydrochloric acid then with cold water and purified by recrystalization. IR (KBr, v_{max}, cm^{-1}): 704 (C-H Ar), 1733 (C=O, Lactone), 1621 (C=C), 1142 (C-O, ester).^{1}H NMR δ (ppm): 1.72 (s, 3H, C_4-CH_3), 6.23-7.11 (m, 4H, Ar-H), 7.41-8.14 (m, 5H, Ar-H).

Synthesis of N, N-bis-(7-hydroxy-4-methyl-Chromen-2-ylidene)-hexa-1, 2, 3, 4, 5-pentaenamide (3)

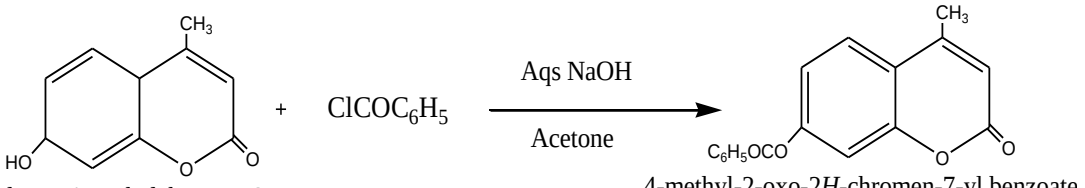
Dissolve compound **1** (1 g, 0.004 mol) and *o*-phenylenediamine (0.54 g, 0.005 mol) in ethanol (10 ml) was refluxed on a water bath for about 3 hours. The precipitated product was filtered off and purified by recrystalization. IR (KBr, v_{max}, cm^{-1}): 3532 (OH), 844(C-H Ar), 1660 (C=O), 1508 (C=C). ^{1}H NMR δ (ppm): 1.71 (s, 6H, C_4-CH_3), 5.01 (s, 2H, C_7-OH), 5.1-6.96 (m, 8H, Ar-H), 7.3 (m, 4H, Ar-H).

Synthesis of (2z)-2-(2, 4-dinitro phenyl) hydrazinyldine-4-methyl-2H-Chromen-4-ol (4)

2,4-Diphenyl hydrazine (1 g, 0.005 mol), 1.5 g of sodium acetate dissolve in 10 ml of water and add to the solution of compound **1** (1g, 0.004 mol) in ethanol reflux on a water bath for about 30 min. Cool and filtered off and purified by recrystalization. IR (KBr, v_{max}, cm^{-1}): 3291 (OH), 3112 (N-H), 847 (C-H Ar), 1616 (C=N), 1510 (C=C), 1332 (C-N). ^{1}H NMR δ (ppm): δ 1.7 (s, 3H, C_4-CH_3), 5.01 (s, H, C_7-OH), 5.1-6.96 (m, 4H, Ar-H), 7.0 (s, 1H, NH), 6.98-8.87 (m, 3H, Ar-H).



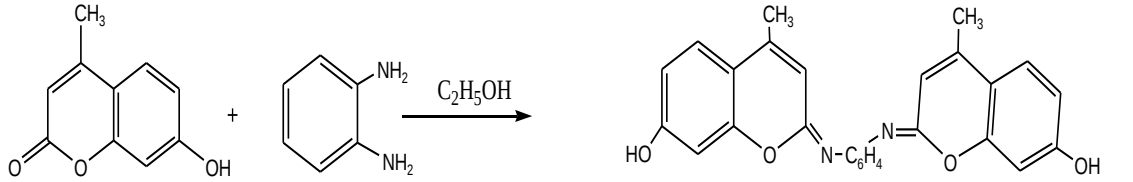
Scheme I



7-hydroxy-4-methylchroman-2-one

4-methyl-2-oxo-2*H*-chromen-7-yl benzoate

Scheme II

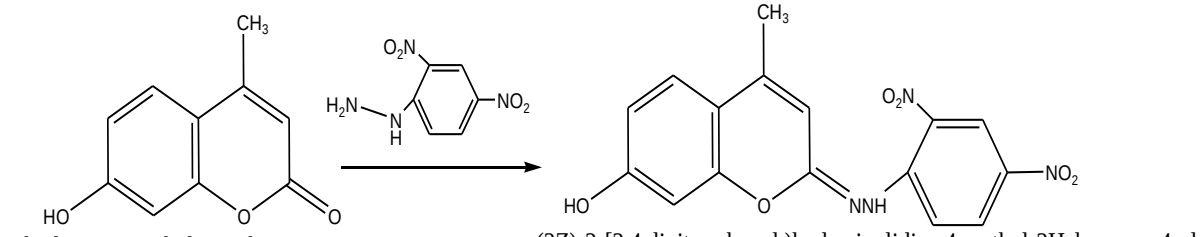


7-hydroxy-4-methyl-2H-chromen-2-one

o-Phenyl diamine

N,N-bis-(7-hydroxy-4-methyl-chromen-2-ylidene)-hexa-1,2,3,4,5-pentaenamide

Scheme III



7-hydroxy-4-methyl-2*H*-chromen-2-one

(2Z)-2-[2,4 dinitro phenyl)hydrazinylidine 4-methyl-2Hchromen -4-ol

Scheme IV

RESULTS AND DISCUSSION

7-hydroxy4-methyl coumarin **1** was prepared in good yield by condensation of resorcinol with ethylacetoacetate in acidic medium (**Scheme I**). Compound **1** was the starting material for the synthesis of compound **2, 3 & 4** (**Scheme II, III & IV**). Further, the synthesized compounds were purified by recrystallisation and the physical data of coumarin derivatives were mentioned in Table **1**.

The molecular formulae of compounds were confirmed by elemental analysis and their structures were determined from IR and ^{1}H NMR & m.p., which were consistent with the literature data. The ^{1}H NMR spectra of the synthesized compounds gave characterized peaks in the expected region.

The title compounds were also screened for their biological activity i.e., anti-inflammatory by using HRBC membrane stabilization method. Since, HRBC membrane is similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity. The title compounds showed highly significant activity when compared to that of standard Diclofenac sodium. The results of anti-

inflammatory activity were shown in **Table 2** and **Figure 1**

***In-vitro* anti inflammatory activity**

HRBC Membrane Stabilization Method[11]

The principle concerned in this method is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. Blood was collected (2ml) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3000 rpm .The packed cells were washed with isosaline solution and a 10% v/v suspension was prepared with normal saline. Different concentrations of synthesized coumarin derivatives (25µg/ml, 50µg/ml, 100µg/ml) Diclofenac sodium (25µg/ml, 50µg/ml, 100µg/ml) as standard and control (distilled water instead of hyposaline to produce 100% haemolysis) were separately mixed with 1ml of phosphate buffer, 2ml hyposaline solution and 0.5ml of 10% HRBC suspension was added to prepared reaction mixture. All the assay mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the formula.

% Membrane Stabilization= {(Absorbance of control-Absorbance of test)/Absorbance of control} × 100

CONCLUSION

In this present study, the four derived compounds have been successively synthesized and characterized by using various spectroscopic methods. All these compounds showed anti-inflammatory activity with potent HRBC membrane stabilization using three different concentration levels (25µg/ml, 50µg/ml, and 100µg/ml) tested. The results obtained revealed that the four coumarin derivatives showed significant invitro anti-inflammatory activity. Basing on the results further work may be carried on using animal models.

Table 1: Physical data of coumarin derivatives

Compound	Physical state	Mol. Formlula	Mol. Wt.	M. P. (°C)	Yield (%)
1	white color solid	$C_{10}H_{12}O_3$	180	115	72
2	white color solid	$C_{17}H_{12}O_4$	280	110	56
3	pale brown solid	$C_{28}H_{28}N_2O_2$	356	172	70
4	yellow color solid	$C_{16}H_{12}N_4O_6$	424	100-104	75

Recrystillization solvent : Ethanol

Table 2: Results of anti-inflammatory activity

Conc., (µg/ml)	Compd 1	Compd 2	Compd 3	Compd4	Standard
25	0.078± 0	0.064± 0.001	0.301 ±0.029	0.204 ±0.0005	0.163 ±0.0005
50	0.061± 0.0005	0.039± 0.001	0.126± 0.123	0.108± 0.019	0.054± 0.012
100	0.031± 0	0.022± 0	0.054 ±0.00006	0.017± 0.00006	0.013± 0.0005

Each value represents the mean ± SEM. N=3, Experimental group were compared with control p<0.01, considered extremely significant.

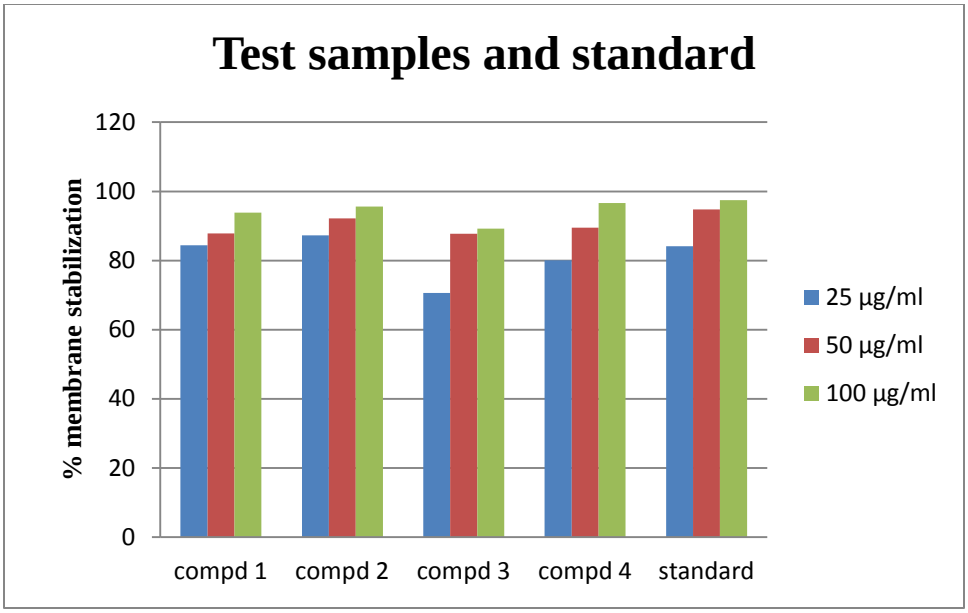


Fig. 1: Effect of coumarin derivatives on HRBC Membrane Stabilization

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