Research Paper www.ijcps.com

International Journal of
Chemical and Pharmaceutical Sciences
2010, Aug., Vol.1 (1)

ISSN: 0976-9390
IJCPS

Synthesis, Characterization and Pharmacological Activities of Coumarin derivatives

[1]Sandhya B[*], [1]Vinod Mathew, [1]Lohitha P, [2]Ashwini T and [1]Shravani A

[1]Acharya and B. M. Reddy College of Pharmacy, Bangalore, Karnataka, India.

[2]Gokaraju Rangaraju College of Pharmacy, Hyderabad, Andhra Pradesh, India.

***Corresponding Author: Sandhya B.** E-mail- bsandhya05@gmail.com.

ABSTRACT

Coumarins are a class of compounds with benzopyrone ring system. In the present work 7-hydroxy-4-methyl coumarin was prepared by the reaction of resorcinol and ethylacetoacetate mixture in the presence of concentrated sulphuric acid. 7-hydroxy-4-methyl coumarin was acetylated with acetic anhydride in the presence of acetic acid. The product formed was treated with bromine in glacial acetic acid to form brominated derivative which on further treatment with different amines to form 4-methyl-2-oxo-2*H*-chromen-7yl substituted acetates. The structures of the final newly synthesized compounds were confirmed from IR, ^{1}HNMR & Mass Spectra. The newly synthesized compounds were screened for their anti-inflammatory activity using carrageenan induced paw oedema method and for analgesic activity using acetic-acid induced writhing method in mice. Among the synthesized compounds S8 possesses good anti inflammatory and analgesic activity when compared to that of other synthesized compounds.

Keywords: 7-hydroxy-4-methyl coumarin, Anti-inflammatory, Analgesic, Acetic anhydride.

1. INTRODUCTION

Coumarins are both naturally occurring as well as synthetic derivatives, and are having widespread applications as HIV protease inhibitors, anticoagulant, spasmolytic and bacteriostatic agents.[1-2] However, the most widely reported activities for coumarin derivatives are their anti-inflammatory and anti-cancer activities.[3-4] Coumarin derivatives with anticancer activities include aromatase inhibitors, carbonic anhydrase inhibitors, and steroid sulfatase inhibitors. Other coumarin derivatives based anticancer compounds include the naturally occurring GUT-70 from *C. brasiliense*, 7-isopentenyloxycoumarin from *H. lanatum*, 5-oxygenated-6,7 methylenedioxycoumarins from *P. polystachyum* as well as the synthetic coumarin derivatives such as 7-hydroxycoumarin, 6-nitro-7-hydroxycoumarin, coumarin 3-(Naryl) sulfonamides, and 3-bromophenyl 6-acetoxymethyl-2-oxo-2*H*-benzopyran-3-carboxylate.[4-5] Some of the medicinal compounds[6] containing coumarin nucleus are Warfarin (vitamin K antagonists), Ensaculin (antidementia agent), Umbelliferone or 7- hydroxycoumarin (find use in sunscreen creams and lotions), Psoralen (for psoriasis, eczema and vitiligo).

Coumarins scavenge reactive oxygen species and suppress inflammation, oedema and pain. Linkage of various heterocycles at C-4 position in 2-arylamino thiazoles has resulted in good pharmacological activity.[3] Number of 2, 4-disubstituted thiazoles, imidazolyl thiazoles, and pyrazolyl thiazoles have been recognized as potent anti-inflammatory and analgesic agents. Synthesis of many 3-substituted biheterocyclic coumarins with thiazoles and fused thiazoles possessing

antimicrobial and anti-inflammatory agents has been reported.[4,7-8]

It is observed that ester linked with amino group possesses good anti-inflammatory activity and analgesic activity. Coumarin possesses good anti-inflammatory and analgesic activity. So, it was decided to carry out amino group linked by ester linkage in coumarin nucleus. There was no much structural modification at 7th position of coumarin ring. Hence it was decided to modify the structural nucleus coumarin at 7th position by substituting with different amines linked by ester.[9-10]

Thus in the present investigation it was contemplated to synthesize a series of coumarin derivatives by substituting biologically active amino alkanone group with a view to increase their biologically activity.

2. MATERIALS AND METHODS

- All FT-IR spectra were recorded in Tensor 27 spectrophotometer, Bruker Optik (Germany) using ATR method.
- All ^{1}H NMR spectra were recorded in Bruker spectrophotometer AMX- 400 (400 MHz), Bruker Optik (Germany) in CD3OD using TMS as an internal standard.
- All Mass spectra were recorded using a Jeol-D-300 Mass spectrophotometer (70ev), SHIMADZU (Japan) by LCMS-2010A.
- All melting points were determined in open capillaries using Thermonik precision apparatus (Model-C-PMP-2, Mumbai, India) and are uncorrected.
- TLC was performed on precoated TLC plates (Silica Gel 60; F254: Merck, Germany) and visualized under UV light.

2.1. METHODOLOGY

2.1.1. Synthesis of 7-hydroxy-4-methyl Coumarin (A)

Concentrated sulphuric acid (50 ml) was taken in a two necked round bottomed flask (250 ml) and cooled to 10°C. Powdered resorcinol (5 g, 0.04 M) dissolved in freshly distilled ethylacetoacetate (6.8 ml, 0.05M) was taken in a dropping funnel. The mixture of resorcinol and ethylaetoacetate was added drop wise to cold conc sulphuric acid solution with constant stirring. The temperature was maintained always below 10°C. After adding all the portions of the resorcinol and ethylacetoacetate mixture, the solution was kept aside for 12 hrs, without further cooling. Then the reaction mixture was poured into crushed ice (150 g). The precipitate obtained was filtered and dried. The dried product was dissolved in sodium hydroxide solution (10%) then acidified with dil. sulphuric acid till the solution was acidic to litmus. This solution was kept aside for 10 min. The formed precipitate was washed with water and recrystallized from the ethanol.

Yeild-50.6%, m.p-178-180^{o}C, FT-IR-1664cm^{-1}(C=O), 3490 cm^{-1}(OH), 1267 cm^{-1}(C=O), 2950 cm^{-1}(Aliphatic C-H), 1HNMR (DMSO) δ 2.3(s, CH_3), δ 9.8(s, OH), δ 6.3-7(m, aromatic).Molecular formula - $C_{10}H_8O_3$.

2.1.2. Preparation 4- methyl-2-oxo-2*H*-chromen-7yl acetate (B)

7- hydroxy-4-methyl coumarin (1 g, 0.005 M) was dissolved in a mixture of acetic anhydride (1.5 ml, 0.014 M) and glacial acetic acid (1 ml, 0.016 M). The mixture was heated on a water bath for 20-30 min, with occasional shaking. The reaction mixture was poured into crushed ice, drop wise with continuous stirring. The precipitate was collected, washed with water, dried and recrystallized from the ethanol.

Yield-78.90%, m.p-145-148^{o}C, 1715 cm^{-1}(C=O), 1621 cm^{-1}(C=C), 1379 cm^{-1}(aliphatic C-H), 1320 cm^{-1}(C-O), 1HNMR (DMSO) δ2.2(s, CH_3), δ2.4(s, CH_3), 7-8(m, Aromatic). Molecular formula-$C_{12}H_9O_4$.

2.1.3. Preparation of 4- methyl-2-oxo-2*H*-chromen-7yl bromo acetate (C)

4-methyl-2-oxo-2*H*-chromen-7yl acetate (1.09 g, 0.01 M) was dissolved in 10

ml of absolute ethanol, to this bromine (2 ml, 0.01 M) in glacial acetic acid mixture was added drop wise with constant stirring. After adding all the portions of the solution the reaction mixture was stirred at 40°C, for 4 h and cooled. After cooling the reaction mixture was poured into crushed ice. Filter the precipitate and washed it with water several times. Dried the precipitate and recrystallized from ethanol.

Yield-69.05%, m.p-95-96^{o}C, FT-IR 3103 cm^{-1}(Aromatic C-H), 1715 cm^{-1}(C=O), 1205 cm^{-1}(C-O). Molecular formula- $C_{12}H_9O_4Br$.

2.1.4. General procedure for synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl substituted acetate (S1 –S10)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.09 g, 0.01 M) and different aromatic heterocyclic amines (0.01 M) were dissolved in acetone (40 ml, 0.01 M). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

2.1.5. Synthesis of 4- methyl-2-oxo -2*H*-chromen-7yl (1, 5-dihydro-4*H*-1, 2, 4-triazol-4-yl amino) acetate (S1)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and 4-amino- 1, 2, 4-triazole (0.85 g, 0.01 M) were dissolved in acetone (40 ml, 0.01 M). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-77.9%, m.p-198^{o}C, FT-IR 3488 cm^{-1}(NH), 1664 cm^{-1}(C=O), 1266 cm^{-1}(C-O), 1599 cm^{-1} (C-N),2850 cm^{-1}(Aliphatic C-H).Molecular formula- $C_{14}H_{14}O_4N_4$.

2.1.6. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7-yl [(4methoxyphenyl) amino] acetate (S2)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and *p*-anisidine (1 g, 0.01 M) were dissolved in acetone (40 ml, 0.01 M). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield 60.7%, M.P-221^{o}C, 3488 cm^{-1}(NH), 1742 cm^{-1}(C=O), 1234 cm^{-1}(C-O), 1505cm^{-1}(C-N), 2842cm^{-1}(Aliphatic C-H). Molecular formula - $C_{19}H_{17}O_5N$.

2.1.7. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7-yl (1, 3-benzothiazol-2-ylamino) acetate (S3)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and 2-amino benzothiazole (0.58 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-44.6%, m.p-198^{o}C, FT-IR-3333 cm^{-1}(NH), 1746 cm^{-1}(C=O), 1232 cm^{-1}(C-O), 1451 cm^{-1} (C-N), 2832 cm^{-1}(Aliphatic C-H). Molecular formula- $C_{18}H_{13}O_4N_2S$.

2.1.8. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl (1, 3-thiazol-2-yl amino) acetate (S4)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and 2-amino thiazole (0.53 g, 0.01 M) were dissolved in acetone (40 ml, 0.01 M). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield - 60.3%. M.P 234-236^{o}C, FR-IR-3306 cm^{-1}(N-H), 1746 cm^{-1}(C=O), 1231 cm^{-1}(C-N), 1610 cm^{-1}(C-N), 2862 cm^{-1}(Aliphatic C-H). Molecular formula- $C_{15}H_{11}O_4N_2S$.

2.1.9. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl [(4-nitrophenyl) amino] acetate (S5)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and *p*-nitro aniline (0.54 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-86.44%, M.P-213^{o}C, 3366 cm^{-1}(N-H), 1628 cm^{-1}(C=O), 1266 cm^{-1}(C-O), 1588 cm^{-1}(C-N), 2707 cm^{-1}(Aliphatic C-H). Molecular formula-$C_{18}H_{13}O_6N_2$.

2.1.10. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl [(2-nitrophenyl) amino] acetate (S6)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and *o*- nitro aniline (0.54 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-83.47%, M.P-203-205^{o}C, 3366 cm^{-1}(N-H), 2920 cm^{-1}(Aliphatic C-H),1729 cm^{-1}(C=O), 1250 cm^{-1}(C-O), 1575 cm^{-1}(C-N).Molecular formula-$C_{18}H_{13}O_6N_2$.

2.1.11. Synthesis of 4-methyl-2oxo-2*H*-chromen-7yl [(4-chlorophenyl) amino] acetate (S7)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and *o*- nitro aniline (0.63 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-66.34%, M.P-185-187^{o}C, FT-IR-3387 cm^{-1}(N-H), 1718 cm^{-1}(C=O), 1595 cm^{-1}(C-O), 1372 cm^{-1}(CH), 1595 cm^{-1}(C-N), 2854 cm^{-1}(Aliphatic C-H). Molecular formula-$C_{18}H_{13}O_4NCl$.

2.1.12. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7-yl *N*-(4'-amino-3,3'-dimethoxybiphenyl-4-yl)glycinate (S8)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and *o*-anisidine (1.2 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

Yield-81.16%, M.p-235-258^{o}C, FT-IR-3373 cm^{-1}(N-H), 2905 cm^{-1}(Aliphatic C-H), 1717 cm^{-1} (C=O), 1173 cm^{-1}(C-H), 1306 cm^{-1}(C-N), ^{1}HNMR(DMSO), δ3.2(s,CH_3), δ6.8-7(m, aromatic), δ5.9(s, NH), M-411M^{+}. Molecular formula-$C_{26}H_{23}O_6N_2$.

2.1.13. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl [acetyl (phenyl) amino] acetate (S9)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and acetanilide (0.75 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.Yield-64.4%, M.P-210-212^{o}C. Molecular formula-$C_{20}H_{17}O_5N$.

2.1.14. Synthesis of 4-methyl-2-oxo-2*H*-chromen-7yl 1*H*-imidazol-1-ylacetate (S10)

4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate (1.53 g, 0.01 M) and 1*H*imidazole (0.25 g, 0.01 M) were dissolved in acetone (40 ml). The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.
Yield-47.3%. M.P-189-191^{o}C.Molecular formula-$C_{15}H_{12}O_4N_2$.

3.PHARMACOLOGICAL SCREENING

3.1 Anti-inflammatory activity study [11-13]

Carrageenan-induced paw oedema model

The animals were weighed, numbered and divided into twelve groups of six each & mark was made on the ankle joint of each rat, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume.10 ml/kg body weight of normal saline (0.9% NaCl) was injected to the control group and 15 mg/kg of Indomethacin was injected to the standard group. The test compounds (100 mg/kg11) were injected to the remaining groups (Group I-X). After one hour 0.1 ml of 1% (w/v) carrageenan in normal saline was injected into the plantar region of the left paw of control group animals, standard group and test compound treated groups i.e. Group I-X. Paw volume up to the ankle joint was measured in drug treated and untreated groups after carrageenan challenge at 30, 60, 90 and 120 min using a plethysmograph filled with mercury. The edema was expressed as an increase in the volume of paw, and the percentage of inhibition for each rat and each group was obtained as follows and the reading were specified in table I and II

Percentage of inhibition = Vc – Vt / Vc * 100

Vc = the mean increase in paw thickness in the control group of rats.

Vt = the mean increase in paw thickness in rats treated with test compounds.

3.2. Analgesic activity study[11]

Acetic-acid induced writhing in mice

The animals were weighed, numbered, and divided into twelve groups of six each. 10ml/kg body weight of normal saline (0.9% NaCl) was administered intraperitonealy to the control group and 15 mg/kg body weight of Diclofenac Sodium was administered to the standard group. The test compounds (100 mg/kg) were injected intraperitonealy to the remaining groups (Group I-X). Fifteen minutes later, 10 ml/kg of acetic acid (0.6%) was administered to all the groups. The onset and severity of writhing response was noted. The mean writhing scores in control, standard and compound treated groups were calculated and specified in table: III

4. RESULTS AND DISCUSSION

In step 1; 7- hydroxy coumarin was synthesized from resorcinol and ethyl acetoacetate in the presence of conc. sulphuric acid. The mixture was stirred for 2 h at below 10°C. The reaction was well monitored through thin layer chromatography technique. The synthesized compound was dried completely and recrystallised from ethanol. The M.P. of the synthesized compound was found to be 178-180°C & the yield was 50.6%.

In step 2; 4- methyl-2-oxo-2*H*-chromen-7yl acetate was synthesized from 7- hydroxy4-methyl coumarin in the presence of acetic anhydride and acetic acid. The mixture was heated for 30 min with stirring at room temperature. The reaction was well monitored through thin layer chromatography technique. The synthesized compound was dried completely and recrystallized from ethanol. The M.P. of the synthesized compound was found to be 145-148°C & the yield was 78.9%.

In step 3; 4- methyl-2-oxo-2*H*-chromen-7yl bromo acetate was synthesized from 4-methyl-2-oxo-2*H*-chromen-7yl acetate and bromine in the presence of acetic acid. The mixture was heated for 30 min with stirring at room temperature. The reaction was well monitored through thin layer chromatography technique. The synthesized compound was dried completely and recrystallized from ethanol. The M.P. of the synthesized compound was found to be 95-96°C & the yield was 69.09%.

In step 4; 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate was refluxed for 6 h with different amines in presence of

acetone to form the final product. The product is recrystallized from ethanol.

S1: 4-methyl-2-oxo-2*H*-chromen-7yl (1, 5-dihydro-4*H*-1,2,4-triazol-4-ylamino) acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with 4-amino1,2,4-triazole in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 77.9%, The M.P was found to be 198°C.

S2: 4-methyl-2-oxo-2*H*-chromen-7-yl [(4-methoxyphenyl) amino] acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with *p*-anisidine in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 60.7%, The M.P was found to be 221°C.

S3: 4-methyl-2-oxo-2*H*-chromen-7-yl (1,3-benzothiazol-2-ylamino)acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with 2-amino benzothiazol in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 44.6%, The M.P was found to be 198°C.

S4: 4-methyl-2-oxo-2*H*-chromen-7yl (1,3-thiazol-2-yl amino) acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with 2-aminothiazole in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 60.3%. The M.P was found to be 234-236°C.

S5: 4-methyl-2-oxo-2*H*-chromen-7yl [(4-nitrophenyl) amino] acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with *p*-nitro aniline in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 86.44%. The M.P was found to be 213°C.

S6: 4-methyl-2-oxo-2*H*-chromen-7yl [(2-nitrophenyl) amino] acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with *o*-nitro aniline in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 83.47%. The M.P was found to be 205°C.

S7: 4-methyl-2oxo-2*H*-chromen-7yl [(4-chlorophenyl) amino] acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with 4-chloroaniline in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 63.34%. The M.P was found to be 185-187°C.

S8: 4-methyl-2-oxo-2*H*-chromen-7-yl *N*-(4'-amino-3,3'-dimethoxybiphenyl-4-yl)glycinate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with *o*-dianisidine in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 81.16%. The M.P was found to be 235-238°C.

S9: 4-methyl-2-oxo-2*H*-chromen-7yl [acetyl (phenyl) amino] acetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with acetanilide in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 64.4%. The M.P was found to be 210-212°C.

S10: 4-methyl-2-oxo-2*H*-chromen-7yl 1*H*-imidazol-1-ylacetate was synthesized by refluxing 4-methyl-2-oxo-2*H*-chromen-7yl bromo acetate with imidazole in presence of acetone. The reaction was well monitored with thin layer chromatography and the synthesized compound was recrystallized from ethanol. The percentage yield was found to be 47.3%. The M.P was found to be 189-191°C.

Table No-I
Anti-inflammatory activity data for newly synthesized compounds

Treatment	Mean increase in paw volume (mean ± SEM) Time in minutes			
	30	**60**	**90**	**120**
Control	0.056±0.004	0.078±0.05	0.094±0.003	0.118±0.005
Standard	0.026±0.003**	0.025±0.001**	0.023±0.002**	0.022±0.006**
Group-I (S1)	0.045±0.005*	0.059±0.004*	0.089±0.006*	0.108±0.004*
Group-II (S2)	0.060±0.003	0.068±0.003	0.086±0.001	0.108±0.004*
Group-III (S3)	0.062±0.001	0.079±0.002	0.088±0.003	0.110±0.001
Group-IV (S4)	0.066±0.007	0.080±0.001	0.099±0.002	0.128±0.005
Group-V (S5)	0.052±0.004*	0.064±0.001*	0.083±0.005*	0.117±0.007
Group-VI (S6)	0.044±0.001**	0.065±0.004**	0.079±0.001**	0.103±0.007**
Group-VII (S7)	0.050±0.001*	0.069±0.001*	0.093±0.007*	0.110±0.006*
Group-VIII (S8)	0.051±0.003*	0.076±0.004*	0.099±0.001*	0.127±0.006
Group-IX (S9)	0.059±0.001	0.066±0.001	0.083±0.006	0.120±0.004
Group-X (S10)	0.052±0.008	0.072±0.001	0.095±0.001	0.121±0.006

Statistical analysis was done by ANOVA followed by Dunnet's test.
All the values are expressed as mean ± SEM
*P<0.05, **P<0.01. When compared to control

Table No-II
Anti-inflammatory activity (Percentage inhibition of paw volume)

Treatment	(Percentage Inhibition of Paw edema) Time in minutes			
	30	**60**	**90**	**120**
Standard	50%	65%	76%	80%
Group-I (S1)	19.6%	24.3%	14.8%	0.084%
Group-II (S2)	----	---	---	---
Group-III (S3)	---	---	---	---
Group-IV (S4)	---	---	---	---
Group-V (S5)	7.14%	4.79%	1.179%	---
Group-VI (S6)	21.4%	16.6%	15.9%	12.7%
Group-VII (S7)	10.7%	5.39%	1.06%	1.01%
Group-VIII (S8)	8.92%	2.56%	1.03%	---
Group-IX (S9)	----	----	---	---
Group-X (S10)	---	---	---	---

Table No:III
Analgesic activity data for newly synthesized compounds

Treatment	No. of writhings
Control	51±0.3651**
Diclofenac sodium	25±0.577**
Group-I	50±0.3651
Group-II	49.6±0.3333
Group-III	47±0.3651*
Group-IV	48.33±0.4216
Group-V	47±0.7303*
Group-VI	41±1.856**
Group-VII	40.83±1.014**
Group-VIII	36.16±1.352**
Group-IX	47.5±0.7638
Group-X	49±0.5774

Statistical analysis was done by ANOVA followed Dunnet's test.

All the values are expressed as mean±SEM.

*P<0.05, **P<0.01 when compared to control.

Several aromatic and heterocyclic 4-methyl-2-oxo-2*H*-chromen-7yl substituted acetates were synthesized from 7-hydroxy-4-methyl coumarin and the progress of the reaction was monitored using precoated TLC plates. The absence of TLC spots for starting materials and appearance of new TLC spot at different R*f* value were ensured to declare completion of reaction. The TLC plates were visualized either by Iodine vapors or by viewing in UV-visible chamber. The reaction products of all the reactions were purified initially by different workup processes to remove unreacted starting materials if any and then by recrystallization using suitable solvents. Most of the steps were optimized in order to achieve quantitative yields. The FTIR spectra of final derivatives showed the expected bands for the characteristic groups which are present in the compounds such as C=O at 1660 cm-1. The N-H stretching bands at 3343 -3185 show the presence of –NH group. In NMR spectra of some derivatives, band was observed at δ 6.3-7

which showed the presence of aromatic ring and bands at δ2.4 showed the presence of CH3. The mass spectra of compound (A) was taken and found to have 177 M+, and Mass spectra of compound (S8) found to be 411 M+. The synthesized derivatives were screened for anti-inflammatory and analgesic activity. Among the synthesized compounds S8 possesses good anti-inflammatory and analgesic activity when compared to that of other synthesized compounds. And S1, S5, S7 showed moderate anti-inflammatory activity. S3, S5, S6 showed moderate analgesic activity.

5. CONCLUSION

The objective of the present study was to synthesize and characterize some novel coumarin derivatives and to carry out the analgesic and anti-inflammatory activities. The synthesis of the coumarin derivatives were carried out in the following steps

- Synthesis of 7-hydroxy coumarin by the reaction of resorcinol and ethylacetoacetate in the presence of conc. sulphuric acid.
- Above prepared coumarin treated with acetic anhydride in the presence of glacial aceticacid.
- And that acetylated coumarin treated with bromine in the presence of acetic acid.
- And finally treated with different aromatic amines.

Synthesis of all the coumarin derivatives by the above described method results in products with good yields. IR, ^{1}H NMR and Mass spectroscopic analysis was done to confirm the structures of the newly synthesized compounds. Research program for the discovery of new anti-inflammatory and analgesic drug for improving the evaluation criteria are under way of many laboratories. Small and simple heterocyclic structures often have surprising complex biological properties. Coumarins play a vital role in the field of medicinal chemistry. The literature study reveals that coumarin moiety exhibits outstanding biological activities.

It was decided to carry out substitution at 7th position using various primary and secondary amines which were confirmed by spectral data. The synthesized derivatives were screened for anti-inflammatory and analgesic activity. Among the synthesized compounds S8 possesses good anti-inflammatory and analgesic activity when compared to that of other synthesized compounds and S1, S5, S7 showed moderate antiinflammatory activity. S3, S5, S6 showed moderate analgesic activity.

6. REFERENCES

1. **Chemistry of coumarins**. http://pubs.acs.org/doi/abs/10.1021/cr60113a001. 2009.
2. Joule JA, Mills K & Smith GF. **Heterocyclic chemistry**. 3rd ed. Stanley thornes publishers Ltd. 1999: 177-8.
3. Venugopala KN and Jayashree BS. Synthesis of carboxamides of 2-amino-4-(6- bromo-3-scoumarinyl) thiazole as analgesic and anti-inflammatory agents. **Indian Journal of Heterocyclic Chemistry** 2003; 12: 307-10.
4. Hoda El-Diwani I and Hend El-Sahrawi. Synthesis and anticoagulant, antipyretic and analgesic activities of some 4*H*-1-benzopyran-4-one derivatives. **Indian Journal of Chemistry**. Sec B 1995; 34B: 27-31.
5. Vijaya Kumar P and Rajeswar Rao V. Synthesis and biological activity of some 3- imidazo[1,2-*a*]pyridin-2-yl-chromen-2-one and 3-indolizin-2-yl-chromen-2-one. **Indian Journal of Chemistry.** Sec B 2005; 44B: 2120-5.
6. Coumarins.http://en.wikipedia.org/wiki/Coumarin**.** 2009.
7. Rang HP and Dale MM. **Text book of Pharmacology**. 5th ed. Churchill Livingstone publishers; 2005; 244-52.

8. Tripathi KD. **Essentials of Medicinal Pharmacology**. 5th ed. Jaypee brother's biomedical publishers, New Delhi. 2005; 561-8.
9. Halnor VB, Dalvi NR, Joshi NS, Gill CH and Karale BK. Condensation reactions of various nucleophiles with 3-formylchromone. **Indian Journal of Chemistry.** Sec B 2006; 45B: 288-91.
10. Freddy Havaldar H and Sanjay Bhise S. Synthesis and biological activity of 7-methyl-3-phenyl-4*H*-furo [3,2-*c*] chromen-4-one and 2,7-dimethyl-4*H*-furo [3,2- *c*] chromen-4-one and their derivatives. **Indian Journal of Heterocyclic Chemistry.** Sec B 2003; 13: 15-8.
11. Kulkarni SK. **Hand book of Experimental Pharmacology.** 3rd ed. Vallabh prakashan publishers. Delhi. 2003; 127-31.
12. Anti-inflammatory activity. http://en.wikipedia.org/wiki/Anti-inflammatory. 2009.
13. Rajeshwar Rao GV & Madan Mohan Reddy. A facile step synthesis of 3-(2-hydroxy benzalhydrazino-4-thiazolyl) coumarins under solvent free conditions and their biological activity. **Indian Journal of Chemistry.** Sec B 2003; 45B: 69-72.