SYNTHESIS AND ANTIBACTERIAL EVALUATION OF SOME NEW COUMARIN DERIVATIVES OF PHARMACEUTICAL INTEREST

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ABSTRACT

A series of new coumarin derivatives were prepared by condensation of resorcinol and ethyl acetoacetate followed by sulfonation with chlorosulphonic acid and amination with various water soluble primary amines. The synthesised compounds were characterised by physical properties and spectral studies (IR, $^1$HNMR, $^{13}$CNMR). The compounds were evaluated for antibacterial activity against both gram positive and gram negative organisms with standard benzyl penicillin.

Key words: coumarin, sulfonation, antibacterial activity

INTRODUCTION

Coumarins constitute an important class of heterocyclic compounds. The structure consists of benzene ring fused with oxane ring containing a keto group at 2nd position and unsaturation at 3rd and 4th position. Coumarin functionalities have been widely used but still generate much interest due to their wide range of applications in medicinal chemistry. The compounds containing this heterocyclic moiety are also widely found as additives in food, in cosmetic products, as pharmaceutical agents and as luminescent materials. Among them coumarins with different alkyl, aryl and heterocyclic groups were found to have various biological activities such as antitumor, anti-HIV, anticoagulant, antimicrobial, antioxidant, and anti-inflammatory agents. The antitumor activities of coumarin compounds have been extensively examined. Therefore, the synthesis of some new coumarin derivatives attracts much interest in heterocyclic chemistry.

MATERIALS AND METHODS

Materials and Instruments: Materials and reagents were obtained from commercial suppliers (Merck grade) and were used without further purification. Progress of the reaction and purity of the compounds was checked on thin layer chromatography (TLC) plates (Silica Gel G). Melting points were determined on Gallenkamp (MFB-600) melting point apparatus and were uncorrected. IR spectra were recorded in KBr discs on a Bruker analyzer. $^1$H NMR and $^{13}$CNMR spectra were recorded on a Bruker (400 MHz) and (125 MHz) spectrometer (chemical shifts in ppm) in DMSO using TMS as internal standard.

Typical Procedure for the Synthesis of Coumarin Derivatives (SC1-10):

Step-1(SC-1): 7-hydroxy- 4-methyl coumarin was prepared by condensation of resorcinol (1 mole) and ethyl acetoacetate (2 moles) under acidic conditions performed in a microwave oven at 70volts for 3 min. Reaction mixture was transferred into ice cold water and the precipitate was filtered under vacuum and dried. Recrystallization was done by using methanol and water 1:1 ratio.

Step-2(SC-2): 7-hydroxy- 4-methyl coumarin 6-sulfonyl chloride was prepared by sulfonation of product obtained from step-1 with chlorosulphonic acid.
acid. The product was recrystallized from methanol and confirmed by thin layer chromatography.

Step-3(SC3SC10): Amidation of 7-hydroxy-4-methyl coumarin 6-sulfonyl chloride was carried by using various water soluble primary amines. Amine and water in 1:1 ratio was added to the sulfonated product and heated for 5 to 6 min and the PH was adjusted to obtain precipitated product. The complete preparation described under SCHEME-1

7-hydroxy 4-methyl chroomen-2-one(SC1): Reaction time:10min; %yield:83;Rf:0.508(ethylacetate:hexane1:1); M.P.:167-172; IR(KBr,V max.,

\[C\text{m}^{v}) :3210(\text{OH}),3087(\text{C=O}),2812(\text{CH}),1665(\text{C=O}),3 244(\text{CO}),1210(\text{COC}),1600(\text{C=Caromatic}),1384(\text{asymmetricSO}) , 1132(\text{SymmetricSO}),\text{HNMR}(400MHz, CDCl3):2.4(\text{CH}2(\text{s})),6.24,6.84,7.59(\text{CH3protons}),7.00(\text{OH(s)}) ;\text{13CNMR}(125MHz,CDCl3):11.50(\text{CH3}),15.49,112.20,152.67,112.00,159.56,102.35,161.20,11 2.95,126.35(basicring carbons)

7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl chloride(SC2): Reaction time:10min; %yield:70.4%:Rf:0.508(ethylacetate:hexane1:1); M.P.:167-172; IR(KBr,V max.,

\[C\text{m}^{v}) :3232(\text{-OH}),3085(\text{-C=C-}),2810(\text{CH}),1669(\text{C=O}),1238(\text{C=O}),1208(\text{C=O-C}), 1600(\text{C=arylic}),1383(\text{asymmetricSO}),1132(\text{SymmetricSO}),\text{HNMR}(500MHz,CDCl3):2.4(\text{CH}2(\text{s})),6 .27,6.88,8.11(\text{CH3protons}),8.54(\text{OH(s)}) ;\text{13CNMR}(125MHz,CDCl3):19.23(\text{CH3}),11.4,38,154.47,114.38, 160.5,101.15,153.39,123.24,120.41(basicringcarbons).

7-hydroxy-2-oxo-2H-chromene-6sulfonamide(SC3): Reaction time:10min; %yield:62.7%;Rf:0.509(ethylacetate:hexane1:1); M.P.:170-178; IR(KBr,V max.,

\[C\text{m}^{v}) :3440(-NH),3232(-OH),3085(-C=C-),2810(CH),1669(C=O),1238(C=O),1208 (COC),1600(C=Caromatic)1208,\text{HNMR}(500MHz,CDCl3)11 32(\text{SymmetricSO}),\text{HNMR}(500MHz,CDCl3):2.4(C H3(\text{s})),6.27,6.895,8.22(\text{CH3protons}),8.54(\text{OH(s)}),4.4 6(\text{NH}),\text{13CNMR}(125MHz,CDCl3):19.23(CH3(\text{s})),15 5.82,112.46,153.42,112.46,160.50,99.83,155.82,124, 33,124.26(basicringcarbons)

7-hydroxy-4-methyl-2-oxo-N-propyl-2H- chromene-6-sulfonamide(SC4): Reaction time:10min; %yield:57.1%;Rf:0.509(ethylacetate:hexane1:1); M.P.:172-177; IR(KBr,V max.,

\[C\text{m}^{v}) :3439(-NH),3223(-OH),3087(-C=C-),2810(CH),1665(C=O),1206(CO),1336 (COC),1597(C=Caromatic),1385(\text{asymmetricSO}) ,1128(\text{SymmetricSO}),\text{HNMR}(500MHz,CDCl3):2.4(C H3(\text{s})),6.27,6.092,8.23(\text{CH3protons}),8.54(\text{OH(s)}),4.65 (\text{NH(s)}),1.52,3.18(\text{sidechainprotons}),\text{13CNMR}(125MH Z,CDCl3):19.23(CH3(\text{s})),100.83,113.17,112.46,12 3.88,126.51,156.70,157.07,160.50(basicring carbons),46.30,22.14,11.55(\text{NH-side chain carbons})

N-butyl-7-hydroxy-4-methyl-2-oxo-2H- chromene-6-sulfonamide(SC5): Reaction time:10min; %yield:61.0%;Rf:0.59(ethylacetate:hexane1:1); M.P.:172-179; IR(KBr,V max.,

\[C\text{m}^{v}) :3456(-NH),3234(OH),3019(C=C),2809(CH),1673(C=O),1233(CO),1207 (COC),1598(C=Caromatic),1385(\text{asymmetricSO}) ,11 51(\text{SymmetricSO}),\text{HNMR}(500MHz,CDCl3):2.4(C H3(\text{s})),6.24,6.94,8.24(\text{CH2-protons}),8.54(\text{OH(s)}),4 50(\text{NH2(s)}),1.91,1.49,1.12(sidechainprotons) .\text{13CNMR}(125MHz,CDCl3):19.23(CH3(\text{s})),100.83,113.17,112.46, 123.88, 126.51,156.70,157.07,160.50(basicring carbons),13.52,19.67,31.45,43.46(\text{NH-side chain})

N-cyclohexyl-7-hydroxy-4-methyl-2-oxo-2H- chromene-6-sulfonamide(SC6): Reaction time:10min; %yield:65.8%; Rf:0.58(ethylacetate:hexane1:1); M.P.:176-179; IR(KBr,V max.,

\[C\text{m}^{v}) :3541(-NH),3133(-OH),3083(-C=C-),2811(CH),1666(C=O),1241(C-O), 1206(C-O-C),1600(C=Caromatic),1382(\text{asymmetricSO}) ,1128(\text{SymmetricSO}),\text{HNMR}(500MHz,CDCl3):2.4(\text{CH3(s) }),6.27,6.92,8.27(\text{CH2-protons}), 4.65 \text{(NH2(s))},1.34,1.57,1.86,2.83 (sidechainprotons),8.54(\text{OH(s)}),\text{13CNMR}(125MHz,CD Cl3):19.23(CH3(\text{s})),100.83,113.17,112. 46,123.88,126.51,156.70,157.07,160.50(basicringcarbons),25.20,25.94,32.37,50.47(cyclohexanecarbons)

7-hydroxy-4-methyl-2-oxo-N-(propan-2-yl)-2H- chromene-6-sulfonamide(SC7): Reaction time:10min; %yield:50.5;Rf:0.58(ethylacetate:hexane1:1); M.P.:176-179; IR(KBr,V max.,

\[C\text{m}^{v}) :3537(-NH),3140(-OH),3087(-C=C-),2810(CH),1663(C=O),1207(C-O),1128(C-O-C),1597(C=Caromatic),1385 (\text{asymmetricSO}),1154(\text{SymmetricSO}) ,\text{HNMR}(500MHz,CDCl3):2.4(CH3(\text{s})),6.27,6.91,8.29(\text{CH2-protons}),8.54(\text{OH(s)}),2.75(sidechainprotons).\text{13CNMR}(125MHz,CDCl3):19.23(CH3(\text{s})),100.83,113.17,112.46, 123.88,126.51,156.70,157.07,160.50(basicring carbons),37.37(\text{N-CH3})

(7-hydroxy-4-methyl-2-oxo-2H-chromene-6 sulfonyl)urea(SC8): Reaction time:10min; %yield:45.8;Rf:0.61(ethylacetate:hexane1:1); M.P.:172-178; IR

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1-[(7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl) guanidine (SC-9):

Result and Discussion:

The results of antibacterial activity revealed that the compounds (SC1 -10) exhibited moderate to considerable activity when compared to reference standard benzyl penicillin. In addition it was found that SC-8 showed maximum activity against gram positive organism B.subtilis and this may be due to the presence of sulphamid group at C-6 of coumarin ring. Moreover it was also observed that the compounds SC-6 and SC-9 carrying cyclohexyl and guanidine substituents showed remarkable activity against gram positive organisms. Compound SC-7 with N-propyl moiety showed maximum activity against E-coli, compounds SC-1, SC-2 and SC-4 showed moderate activity against P.aeruginosa. The results clearly revealed the contribution of electron releasing groups and electron withdrawing groups on the 6-sulfonyl coumarin ring in enhancing the antibacterial activity.

ANTIBACTERIAL ACTIVITY:

Cup plate method using Mueller-Hilton agar medium was employed to study the preliminary antibacterial activity of SC1-10 against Bacillus subtilis, Staphylococcus aureus, Eisherria coli and Pseudomonas aeruginosa. The agar media was purchased from HI-media laboratories limited, Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure each test compound (5mg) was dissolved in 5ml of dimethyl sulfoxide. benzyl penicillin was employed as reference standard (1000µg/ml) to compare the results. All the compounds were tested at a concentration of 0.15ml (150µg) level and DMSO as control did not show any inhibition. The medium was inoculated at one percent level using 18hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for about 30 minutes. The test and standard solutions were added into cups, left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37 0 c, the plates were examined for inhibition zones. The results were represented in Table1.

RESULTS AND DISCUSSION:

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SCHEME- 1:

benzene-1,3-diol + 2-oxopropyl propanoate → 7-hydroxy-4-methyl-2H-chromen-2-one

R−NH₂ (water soluble amines) + 7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl chloride → chlorosulfane dioxide

Where R=

- NH₃ (SC-3)
- SC-4
- SC-5
- SC-6
- SC-7
- SC-8
- SC-9
- SC-10
### TABLE 1: Antibacterial Activity of Compounds SC1-10

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<tr>
<th>Compound</th>
<th>Bacillus subtilis</th>
<th>Staphylococcus aureus</th>
<th>E.coli</th>
<th>Pseudomonas aeruginosa</th>
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### REFERENCES