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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 aceto acetate 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 [1]and as luminescent materials [2]. Among them coumarins with different alkyl, aryl and heterocyclic groups were found to have various biological activities such as antitumor[3,4], anti-HIV[5,6], anticoagulant[7,8], antimicrobial[9, 10], antioxidant [11, 12], and anti-inflammatory [13, 14] agents. The antitumor activities of coumarin compounds have been extensively examined [15,16,17,18]. 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. IRspectra 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 (1mole)and ethyl aceto acetate(2 moles) under acidic conditions performed in a microwave oven at 70volts for 3min. 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. The product was recrystallized from methanol and confirmed by thin layer chromatography.

Step-3(SC3-SC10): 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 chromen-2-one(SC-1): Reactiontime:10min;%yield:83;R_f:0.508(ethylacetate :hexane1:1);M.P(^{0}c):167-172;IR(KBr,V_{max}, Cm^{1}):3210(OH),3087(C=C),2812(CH_3),1665(C=O),1244(CO),1210(COC),1600(C=Caromatic),1384(assymetricSO_2)1132(SymmetricSO_2).;^{1}HNMR(400MHz, $CDCl_3$)2.4($CH_{3,}$(S)),6.24,6.84,7.59(CH_2protons),7.00 (OH(s));^{13}CNMR(125MHZ,$CDCl_3$):18.50($CH_{3,}$(S)),154.95,112.20,152.67,112.00,159.56,102.35,161.20,112.95,126.35(basic ring carbons)

7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl chloride (SC-2):
Reaction time:10min; %yield: 70.4% ;R_f:0.508(ethyl acetate:hexane1:1); M.P:167-172^{0}C.IR (KBr,V_{max},Cm^{-1}):3223(-OH),3085(-C=C-),2810(CH_3),1669(C=O),1238(C-O),1208(C-O-C), 1600(C=CAromatic),1383(assymetric$SO_{2),}$1132(SymmetricSO_2).^{1}HNMR(500MHZ,$CDCl_3$):2.4($CH_{3,}$(S)),6.27,6.88,8.11(CH_2protons),8.54(OH(s)).^{13}CNMR(125MHZ,$CDCl_3$):19.23($CH_{3,}$(S)),11,4.38,154.47,114.38, 160.50,101.15,153.39,123.24,120.41(basic-ringcarbons).

7-hydroxy-2-oxo-2H-chromene-6 sulfonamide(SC-3):
Reaction time:10min; %yield: 62.7%;R_f:0.59(ethyl acetate:hexane1:1); M.P(^{0}c):170-178 ;IR (KBr,V_{max},Cm^{-1}):3440(-NH),3233(-OH),3085(-C=C-),2810(CH_3),1669(C=O),1238(C-O),1208 (COC),1600(C=CAromatic)1208,(assymetric$SO_{2),}$1132(SymmetricSO_2).^{1}HNMR(500MHZ,$CDCl_3$):2.4($CH_{3,}$(S)),6.27,6.893,8.22(CH_2protons),8.54(OH(s)),4.46(NH).^{13}CNMR(125MHZ,$CDCl_3$):19.23($CH_{3,}$(S)),155.82,112.46,153.42,112.46,160.50,99.83,155.82,124.33,124.26(basicringcarbons)

7-hydroxy-4-methyl-2-oxo-N-propyl-2H-chromene-6-sulfonamide (SC-4)
Reaction time:10min; %yield: 57.1% ;R_f:0.59(ethyl acetate:hexane1:1); M.P(^{0}c):172-177;IR (KBr,V_{max},Cm^{-1}):3439(-NH),3223(-OH),3087(-C=C-),2810(CH_3), 1663(C=O), 1206(CO),1336 (COC),1597(C=CAromatic),1385(assymetric$SO_{2),}$1128(SymmetricSO_2).^{1}HNMR(500MHZ,$CDCl_3$):2.4($CH_{3,}$(S)),6.27,6092,8.23(CH_2protons),8.54(OH(s))4.65 (NH(s)),1.52,3.18(sidechainprotons).^{13}CNMR(125MHZ,$CDCl_3$):19.23($CH_{3,}$(S)),100.83,113.17,112.46,123.88,126.51,156.70,157.07,160.50(basic ring carbons),46.30,22.14,11.55 (NH-side chain carbons)

N-butyl-7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonamide (SC-5):
Reaction time:10min; %yield:61.0 ;R_f:0.59 (ethyl acetate:hexane1:1); M.P(^{0}c):172-179;IR (KBr,V_{max},Cm^{-1}):3456(NH),3234(OH), 3019(C=C),2806(CH_3), 1673(C=O),1233(CO),1207 (COC),1598(C=CAromatic),1385(assymetric$SO_{2),}$1151(SymmetricSO_2).^{1}HNMR(500MHZ,$CDCl_3$):2.4($CH_{3,}$(S)),6.24,6.94,8.24 (CH_2-protons),8.54(OH (s)) 4.50 ($NH_{2,}$(s)),1.91,1.49,1.12(side chain protons) .^{13}C NMR (125MHZ,$CDCl_3$): 19.23($CH_{3,}$(S)) ,100.83,113.17,112.46, 123.88, 126.51,156.70,157.07,160.50(basic ring carbons),13.52,19.67,31.45,43.46(NH-side chain)

N-cyclohexyl-7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonamide (SC-6):
Reaction time:10min; %yield:65.8; R_f:0.58(ethyl acetate:hexane1:1); M.P(^{0}c):176-179 ;IR (KBr,V_{max},Cm^{-1}):3541(-NH),3133(-OH),3083(-C=C-),2811(CH_3),1666(C=O),1241(C-O), 1206 (C-O-C),1600(C=CAromatic),1382(assymetric$SO_{2),}$1128(SymmetricSO_2).^{1}H NMR (500MHZ, $CDCl_3$): 2.4($CH_{3,}$(S)),6.27,6.92,8.27 (CH_2-protons), 4.65 ($NH_{2,}$(s)), 1.34, 1.57 ,1.86 ,2.83 (sidechainprotons),8.54(OH(s)).^{13}CNMR(125MHZ,CDCl$_3$):19.23($CH_{3,}$(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(cyclohexane ring carbons)

7-hydroxy-4-methyl-2-oxo-N-(propan-2-yl)-2H-chromene-6-sulfonamide(SC-7) :
Reaction time:10min; %yield:50.5 ;R_f:0.58 (ethyl acetate:hexane1:1); M.P(^{0}c):176-179;IR (KBr,V_{max}, Cm^{-1}): 3537 (-NH),3140(-OH),3087(-C=C-),2810(CH_3),1663(C=O),1207(C-O),1128(C-O-C),1597(C=C Aromatic) ,1385 (assymetric $SO_{2),}$1154(Symmetric SO_2) .^{1}H NMR(500MHZ,$CDCl_3$): 2.4($CH_{3,}$(S)),6.27,6.91,8.29 (CH_2-protons), 8.54(OH (s)) , 2.75(side chain protons) .^{13}C NMR (125MHZ,$CDCl_3$): 19.23($CH_{3,}$(S)), 100.83,113.17,112.46, 123.88,126.51,156.70, 157.07,160.50(basic ring carbons),37.37(N-CH_3).

(7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl)urea(SC-8):
Reaction time:10min; %yield: 45.8 ;R_f:0.61 (ethyl acetate:hexane1:1); M.P(^{0}c):172-178;IR

(KBr,V_{max},Cm^{-1}):3438(-NH),3210(OH),3017(C=C),2804(CH_3),1672(C=O),1232(CO),1207 (COC),1577(C=CAromatic),1384(assymetric$SO_{2)}$,1154(SymmetricSO_2).;^{1}HNMR(500MHZ,$CDCl_3$):2.4(CH_3,(S)),6.27,6.96,8.21(CH_2protons),8.54(OH(s))6.81(NH_2,(s)),6.81(NH(S));^{13}CNMR(125MHZ,$CDCl_3$):19.23(CH_3,(S)),100.83,113.17,112.46,123.88,126.51,156.70,157.07,160.50(basic ring carbons),155.47(NH-C).

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

Reaction time:10min; %yield:44.2;R_f:0.59(ethyl acetate:hexane1:1); M.P(^{0}c):170-176;IR (KBr,V_{max}, Cm^{-1}):3457(-NH),3235(-OH),3088(-C=C-), 2811 (CH_3), 1667(C=O),1597(C-O),1209 (C-OC),1597 (C=CAromatic), 1386(assymetric$SO_{2)}$,1155 (SymmetricSO_2).^{1}H NMR (500MHZ ,$CDCl_3$): 2.4(CH_3,(S)), 6.27,6.89,8.20 (CH_2-protons), 8.54(OH (s))6.64 (NH_2,(s)),4.26(NH(S)) .^{13}C NMR (125MHZ,$CDCl_3$): 19.23(CH_3,(S)), 100.83,113.17,112.46, 123.88,126.51,156.70, 157.07,160.50 (basic ring carbons),166.70(NH-C).

7-dihydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonamide (SC-10):

Reaction time:10min; %yield:43.5 ;R_f:0.60(ethyl acetate:hexane1:1);M.P(^{0}c):172-178;IR (KBr,V_{max},Cm^{-1}):3436(NH),3235(OH),3090(C=C), 2813(CH_3),1663(C=O),1211(CO),1246(CO C),1597(C=CAromatic),1384(assymetric$SO_{2)}$,1131(SymmetricSO$_2$).^{1}HNMR(500MHZ,$CDCl_3$):2.4(CH_3,(S)),6.27,6.89,8.19(CH_2protons),8.54(OH(s)),3.50(NH OH(S)).^{13}CNMR(125MHZ,$CDCl_3$):19.23(CH_3,(S)),100.83,113.17,112.46,123.88,126.51,156.70,157.07,160.50(basic ring carbons).

ANTIBACTERIAL ACTIVITY:

Cup plate method [20,21]using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of **SC1-10** against Bacillus subtilis, Staphylococcus aureus, Eischerria coli and Pseudomonas aeroginosa. 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 pencilln 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:

The results of antibacterial activity revealed that the compounds **(SC1 -10)** exbhited 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 o f sulphonamide 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.aeroginosa. 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.

SCHEME- 1:

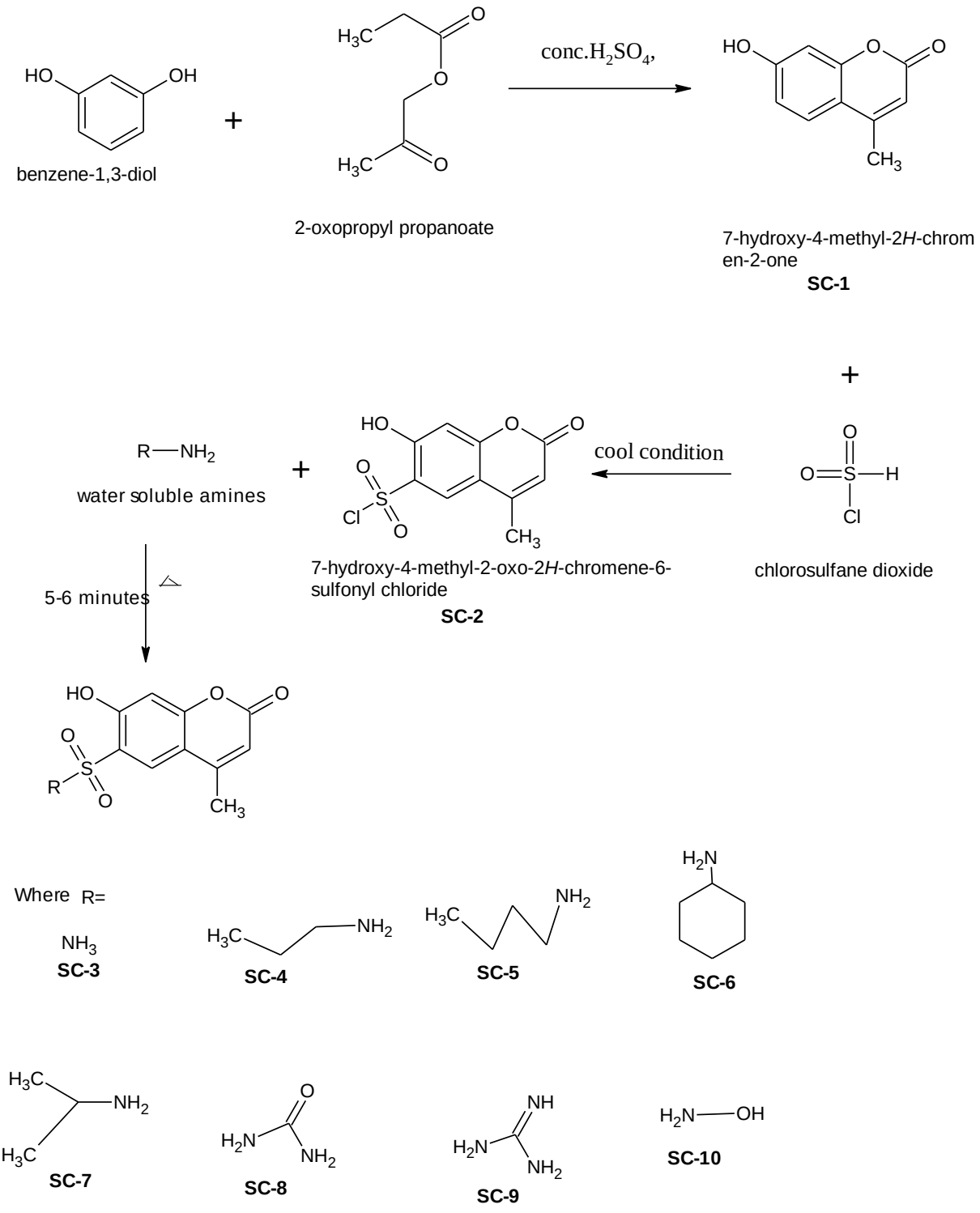
benzene-1,3-diol
2-oxopropyl propanoate
conc.H2SO4,
7-hydroxy-4-methyl-2H-chromen-2-one
SC-1
chlorosulfane dioxide
cool condition
water soluble amines
7-hydroxy-4-methyl-2-oxo-2H-chromene-6-sulfonyl chloride
SC-2
5-6 minutes
Where R=
NH3
SC-3
SC-4
SC-5
SC-6
SC-7
SC-8
SC-9
SC-10

TABLE1: Antibacterial Activity of Compounds **SC1-10**

	Zones of inhibition in mm			
Compound	**Bacillus subtilis**	**Staphylococcus aureus**	**E.coli**	**Pseudomonas aeroginosa**
SC-1	18	18	19	21
SC-2	16	15	13	21
SC-3	12	15	17	19
SC-4	19	17	19	21
SC-5	17	19	21	19
SC-6	11	22	23	17
SC-7	12	19	28	17
SC-8	29	19	12	13
SC-9	23	16	16	18
SC-10	19	12	14	18
STANDARD DRUG	30	24	32	26

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