Synthesis and antimicrobial activity of 6,6'-Arylidene-bis-[5-hydroxy - 9-methyl -2,3-diaryl thieno[3,2-*g*]thiocoumarins]

P.Vijay Kumar Reddy, P. Naveen Kumar and G. V. P. Chandramouli

Department of Chemistry, National Institute of Technology, Warangal-506004, A.P, India.

Received January 2, 2004

A variety of novel 6,6'-arylidene-bis-[5-hydroxy-9-methyl-2,3-diaryl-thieno[3,2-*g*]thiocoumarins] **3a-d**, **4a-d**, **5a-d**, and **6a-d** were obtained by a reaction between 5-hydroxy-9-methyl-2,3-diarylthieno[3,2-*g*]-thiocoumarins **1a-d** with aromatic aldehydes **2a-d** in isopropyl alcohol. The synthesized compounds were tested for their antimicrobial activity.

J. Heterocyclic Chem., **42**, 283 (2005).

Introduction.

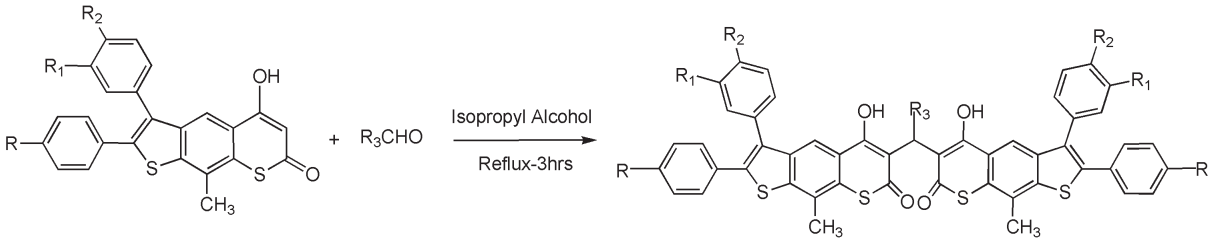
Coumarins show antifungal [1], anticoagulant [2], antimicrobial [3] and insecticidal [4] activities. They are also useful in curing psoriasis [5] and cancer [6]. The marked biological importance and therapeutic activity of 3,3'-methylene-bis-(4-hydroxycoumarin), the causative agent of the hemorrhagic sweet clover disease of cattle [7-10] prompted us to design the synthesis of 6,6'-arylidene-bis-[5-hydroxy-9-methyl-2,3-diarylthieno[3,2-*g*]thiocoumarins] and to investigate their biological activity.

A. K. Shah *et al* [11] and W. R. Sullivan *et al* [12] synthesized 3,3'-arylidene-bis-4-hydroxy coumarin by refluxing two moles of 4-hydroxy coumarin and one mole of aromatic aldehydes in ethanol for 18 hours. However the yields obtained were poor. Here we report the synthesis of 6,6'-arylidene-bis-[5-hydroxy-9-methyl-2,3-diarylthieno[3,2-*g*]thiocoumarins] **3a-d**, **4a-d**, **5a-d**, and **6a-d** by refluxing two moles of 5-hydroxy-9-methyl-2,3-diarylthieno[3,2-*g*]thiocoumarins **1a-d**, with one mole of various aromatic aldehydes **2a-d** in isopropyl alcohol. The time required to complete the reaction is substantially lower and the yields obtained in this method are much higher than previously reported. The structures of all the title compounds were established by analytical and spectral data.

Biological Screening.

Compounds **3a**, **3c**, **4b**, **4c**, **5a**, **5d**, **6b**, and **6c** were screened for their antibacterial activity against both gram-positive and gram-negative bacteria as shown in Table-1. Minimum inhibitory concentration (MIC) of these compounds was determined by tube dilution method using Flucanazole (0.5 mg/ml) as standard in 10% DMSO in methanol solvent. Table 1 indicates that compounds **3a** R= H, R_1= H, R_2= H, R_3= C_6H_5 and **3c** R= H, R_1= H, R_2= H, R_3= $C_6H_3Cl_2$ (2,3) show significant antibacterial activity at 10 mg/ml against S. aureus, however they did not show any significant antifungal activity upto 100 mg/ml. Compounds **4b** R= H, R_1= H, R_2= CH_3, R_3= $C_6H_3Cl_2$(3,4) and **4c** R= H, R_1= H, R_2= CH_3, R_3= $C_6H_3Cl_2$(2,3) show significant antibacterial activity at 10 mg/ml against *S. aureus* and *E. coli*. These compounds further exhibit significant antifungal activity at 10 mg/ml against *Candida. albicans*. Compounds **5a** R= H, R_1= H, R_2= Br and R_3= C_6H_5 and **5d** R= H, R_1= H, R_2= Br, R_3= $C_6H_4OH(p)$ show significant antifungal activity at 10 mg/ml against *Aspergillus fumigatus*, *Candida. albicans*, *Candida. krusei*, and *Candida. glabrata*. However **5a** show significant antibacterial activity at 10 mg/ml against *S. aureus* and 50 mg/ml against *E. coli*.

Scheme 1



1 **2a-d** **3a-d**, **4a-d**, **5a-d** and **6a-d**

3a) R= H, R_1=H, R_2=H, R_3= C_6H_5
3c) R= H, R_1=H, R_2=H, R_3= $C_6H_3Cl_2$(2,3)
4a) R= H, R_1=H, R_2=CH_3, R_3= C_6H_5
4c) R= H, R_1=H, R_2=CH_3, R_3= $C_6H_3Cl_2$(2,3)
5a) R= H, R_1=H, R_2=Br, R_3= C_6H_5
5c) R= H, R_1=H, R_2=Br, R_3= $C_6H_3Cl_2$(2,3)
6a) R= H, R_1=Cl, R_2=Cl, R_3= C_6H_5
6c) R= H, R_1=Cl, R_2=Cl, R_3= $C_6H_3Cl_2$(2,3)

3b) R= H, R_1=H, R_2=H, R_3= $C_6H_3Cl_2$(3,4)
3d) R= H, R_1=H, R_2=H, R_3= $C_6H_4OH(p)$
4b) R= H, R_1=H, R_2=CH_3, R_3= $C_6H_3Cl_2$(3,4)
4d) R= H, R_1=H, R_2=CH_3, R_3= $C_6H_4OH(p)$
5b) R= H, R_1=H, R_2=Br, R_3= $C_6H_3Cl_2$(3,4)
5d) R= H, R_1=H, R_2=Br, R_3= $C_6H_4OH(p)$
6b) R= H, R_1=Cl, R_2=Cl, R_3= $C_6H_3Cl_2$(3,4)
6d) R= H, R_1=Cl, R_2=Cl, R_3= $C_6H_4OH(p)$

Table 1
Minimum Inhibitory Concentration

Sr. No	R,R_1,R_2,R_3	S. aureus 209p	E. coli 2231.	*Aspergillus* fumigatus	*Candida* albicans	Candida Krusei	*Candida* Glabrata
3a	R_3=C_6H_5	+++					
3c	R_3=$C_6H_3Cl_2$(3,4)	+++					
4b	R_2=CH_3, R_3=$C_6H_3Cl_2$(3,4)	+++	+++	+	+++	+	+
4c	R_2=CH_3, R_3=$C_6H_3Cl_2$(2,3)	+++	+++	+	+++		
5a	R_2=Br, R_3=C_6H_5	+++	++	+++	+++	+++	+++
5d	R_2=Br, R_3=C_6H_5OH(*p*)	+	+	+++	+++	+++	+++
6b	R_1,R_2=Cl, R_3=$C_6H_3Cl_2$(3,4)	++		+	+	+	+
6c	R_1,R_2=Cl, R_3=$C_6H_3Cl_2$(2,3)	+++		+	+	+	+

Note: +=100mg/ML, ++ =50mg/ML, +++ =10mg/ML

Compounds **6b** R= H, R_1= Cl, R_2= Cl, R_3= $C_6H_3Cl_2$ and **6c** R= H, R_1= Cl, R_2= Cl, R_3= $C_6H_3Cl_2$(2,3) show antifungal activity at 100 mg/ml against *Aspergillus. fumigatus*, *Candida. albicans*, *Candida. krusei*, *Candida. glabrata*, whereas **6c** shows antibacterial activity at 10 mg/ml against *S. aureus*.

EXPERIMENTAL

General.

All the melting points were determined by an open capillary method and are uncorrected. The IR spectra were recorded on SHIMADZU FTIR model 8010 Spectrophotometer and given in cm-' in KBr. The 'HNMR spectra in $CDCl_3$ were recorded on a C 17-20-ZM-390-200MHZ NMR spectrophotometer using TMS as an internal standard (chemical shifts in δ (ppm)) and mass spectra of compounds described were recorded on JOEL TMS-D 300 at 70 eV. Compounds were obtained in 80%-90% yields. Elementary analysis was carried out using CARLO-ERBA EA-1108-Analyser. The purity of the compound was monitored by TLC using silica gel during reactions.

6,6'-Arylidene-bis-[5-hydroxy-9-methyl-2,3-diaryl-thieno[3,2-g]-thiocoumarin] (**3a-6d**).

Substituted 5-hydroxycoumarinobenzothiophene (0.02 mol) was dissolved in (30 ml) of isopropyl alcohol and heated on a water bath until a clear solution formed. Substituted benzaldehyde (0.01mol) was added to the hot solution and refluxed for 2-hours. The solvent was then removed by distillation and 6,6'-arylidene bis[5-hydroxy-9-methyl-2,3-diarylthieno[3,2-*g*]-thiocoumarin], the separated product, was recrystallized from ethanol.

6,6'-Benzylidene-bis-[5-hydroxy-9-methyl-2,3-diphenylthieno-[3,2-*g*]thiocoumarin] (**3a**).

Compound **3a** was obtained in 80% yield; mp: 280-282 °C; IR (KBr): 1680(C=O), 3450(-OH), 750(C-S); ^{1}H NMR: δ 2.42 (s, 6H, 2 × CH_3), δ 6.21(s, 1H, -CH), δ 6.86-6.98 (m, 10H, Ar-H), δ 7.10-7.18 (m, 10H, Ar-H), δ 7.26-7.32 (s, 2H, Ar-H), δ 7.40-7.53 (m, 5H, Ar-H), δ 12.40 (s, 2H, coumarin-OH); ms: m/z 888 (M^+).

Anal. Calcd. for $C_{55}H_{36}O_4S_4$: C, 74.32; H, 4.05; S, 14.41. Found: C, 74.30; H, 4.03; S, 14.39.

6,6'-(3,4-Dichlorobenzylidene)-bis-[5-hydroxy-9-methyl-2,3-diphenylthieno[3,2-*g*]thiocoumarin] (**3b**).

Compound **3b** was obtained in 85% yield; mp: 270-272 °C; IR (KBr): 1710(C=O), 3456(-OH), 710(C-Cl); ^{1}H NMR: δ 2.48 (s, 6H, 2 × CH_3), δ 6.26 (s, 1H, -CH), δ 6.72-6.78 (m, 10H, Ar-H), δ 6.90-6.96 (m, 10H, Ar-H), δ 7.20-7.28 (s, 2H, Ar-H), δ 7.45-7.62 (m, 3H, Ar-H), δ 12.45 (s, 2H, coumarin-OH); ms: m/z 957 (M^+).

Anal. Calcd. for $C_{55}H_{34}O_4S_4Cl_2$: C, 68.96; H, 3.55; S, 13.37. Found: C, 68.92; H, 3.52; S, 13.33.

6,6'-(2,3-Dichlorobenzylidene)-bis-[5-hydroxy-9-methyl-2,3-diphenylthieno[3,2-*g*]thiocoumarin] (**3c**).

Compound **3c** was obtained in 80% yield; mp: 293-295 °C; IR (KBr): 1712(C=O), 3480(-OH), 710

(C-Cl); ^{1}H NMR: δ 2.45 (s, 6H, 2 × CH_3), δ 6.20(s, 1H, -CH), δ 6.70-6.76 (m, 10H, Ar-H), δ 6.93-6.98 (m, 10H, Ar-H), δ 7.24-7.32 (s, 2H, Ar-H), δ 7.42-7.56 (m, 3H, Ar-H), δ 12.55 (s, 2H, coumarin-OH); ms: m/z 957 (M^+).

Anal. Calcd. for $C_{55}H_{34}O_4S_4Cl_2$: C, 68.96; H, 3.55; S, 13.37. Found: C, 68.94; H, 3.53; S, 13.35.

6,6'-*p*-Hydroxybenzylidene-bis-[5-hydroxy-9-methyl-2,3-diphenylthieno[3,2-*g*]thiocoumarin] (**3d**).

Compound **3d** was obtained in 87% yield; mp: 263-265 °C; IR (KBr): 1680(C=O), 3450(-OH), 1345(br-OH); ^{1}H NMR: δ 2.51 (s, 6H, 2 × CH_3), δ 5.42 (s, 1H, Ar-OH), δ 6.24(s, 1H, -CH), δ 6.77-6.80 (m, 10H, Ar-H), δ 6.99-7.08 (m, 10H, Ar-H), δ 7.20-7.28 (s, 2H, Ar-H), δ 7.46-7.54 (m, 4H, Ar-H), δ 12.46 (s, 2H, coumarin-OH); ms: m/z 904 (M^+).

Anal. Calcd. for $C_{55}H_{36}O_5S_4$: C, 73.01; H, 3.98; S, 14.16. Found: C, 72.98; H, 3.94; S, 14.13.

6,6'-Benzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-methyl)-phenylthieno[3,2-*g*]thiocoumarin] (**4a**).

Compound **4a** was obtained in 85% yield; mp: 323-325 °C; IR (KBr): 1720(C=O), 3500(-OH); ^{1}H NMR: δ 2.39 (s, 12H, 4 × CH_3), δ 6.20(s, 1H, -CH), δ 6.75-6.78 (m, 8H, Ar-H), δ 6.96-7.04 (m, 10H, Ar-H), δ 7.18-7.26 (s, 2H, Ar-H), δ 7.36-7.45 (m, 5H, Ar-H), δ 12.40 (s, 2H, coumarin-OH); ms: m/z 916 (M^+).

Anal. Calcd. for $C_{57}H_{40}O_4S_4$: C, 74.67; H, 4.37; S, 13.97. Found: C, 74.63; H, 4.35; S, 13.93.

6,6'-(3,4-Dichlorobenzylidene)-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-methyl)phenylthieno[3,2-*g*]thiocoumarin] (**4b**).

Compound **4b** was obtained in 89% yield; mp: 295-297 °C; IR (KBr): 1710(C=O), 3500(-OH); ^{1}H NMR: δ 2.46 (s, 12H, 4 × CH_3), δ 6.24 (s, 1H, -CH), δ 6.70-6.75 (m, 8H, Ar-H), δ 7.02-7.08 (m, 10H, Ar-H), δ 7.20-7.28 (s, 2H, Ar-H), δ 7.48-7.72 (m, 3H, Ar-H), δ 12.47 (s, 2H, coumarin-OH); ms: m/z 985 (M^+).

Anal. Calcd. for $C_{57}H_{38}O_4S_4Cl_2$: C, 69.47; H, 3.86; S, 12.99. Found: C, 69.46; H, 3.84; S, 12.95.

6,6'-(2,3-Dichlorobenzylidene)-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-methyl)phenylthieno[3,2-*g*]thiocoumarin] (**4c**).

Compound **4c** was obtained in 82% yield; mp: 285-287 °C; IR (KBr): 1680(C=O), 3500(-OH); ^{1}H NMR: δ 2.50 (s, 12H, 4 × CH_3), δ 6.25(s, 1H, -CH), δ 6.73-6.78 (m, 8H, Ar-H), δ 6.93-6.98 (m, 10H, Ar-H), δ 7.24-7.30 (s, 2H, Ar-H), δ 7.46-7.68 (m, 3H, Ar-H), δ12.55 (s, 2H, coumarin-OH); ms: m/z 985 (M^+).

Anal. Calcd. for $C_{57}H_{38}O_4S_4Cl_2$: C, 69.47; H, 3.86; S, 12.99. Found: C, 69.40; H, 3.90; S, 12.92.

6,6'-*p*-Hydroxybenzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-methyl)phenylthieno[3,2-*g*]thiocoumarin] (**4d**).

Compound **4d** was obtained in 89% yield; mp: 212-215 °C; IR (KBr): 1720(C=O), 3450(-OH), 1340(Br-OH); ^{1}H NMR: δ 2.38 (s, 12H, 4 × CH_3), δ 5.42 (s, 1H, Ar-OH), δ 6.27 (s, 1H, Ar-H), δ 6.75-6.82 (m, 8H, Ar-H), δ 7.00-7.06 (m, 10H, Ar-H), δ 7.16-7.24 (s, 2H, Ar-H), δ 7.40-7.52 (m, 4H, Ar-H), δ 12.35 (s, 2H, coumarin-OH); ms: m/z 932 (M^+).

Anal. Calcd. for $C_{57}H_{40}O_5S_4$: C, 73.39; H, 4.29; S, 13.73. Found: C, 73.35.45; H, 4.26; S, 13.70.

6,6'-Benzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-bromo)phenylthieno[3,2-*g*]thiocoumarin] (**5a**).

Compound **5a** was obtained in 82% yield; mp: 275-277 °C; IR (KBr): 1710(C=O), 3450(-OH); ^{1}H NMR: δ 2.42 (s, 6H, 2 × CH_3), δ 6.21 (s, 1H, -CH), δ 6.86-6.90 (m, 8H, Ar-H), δ 6.98-7.08 (m, 10H, Ar-H), δ 7.28-7.32 (s, 2H, Ar-H), δ 7.46 (m, 5H, Ar-H), δ 12.55 (s, 2H, coumarin-OH); ms: m/z 1046 (M^+).

Anal. Calcd. for $C_{55}H_{34}O_4S_4Br_2$: C, 63.09; H, 3.25; S, 12.24. Found: C, 63.11; H, 3.20; S, 12.31.

6,6'-(3,4-Dicholrobenzylidene)-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-bromo)phenylthieno[3,2-*g*]thiocoumarin] (**5b**).

Compound **5b** was obtained in 86% yield; mp: 253-257 °C; IR (KBr): 1720(C=O), 3500(-OH); ^{1}H NMR: δ 2.38 (s, 6H, 2 × CH_3), δ 6.27(s, 1H, -CH), δ 6.80-6.86 (m, 8H, Ar-H), δ 7.02-7.06 (m, 10H, Ar-H), δ 7.24-7.26 (s, 2H, Ar-H), δ 7.42-7.66 (m, 3H, Ar-H), δ 12.35 (s, 2H, coumarin-OH); ms: m/z 1115 (m^+).

Anal. Calcd. for $C_{55}H_{32}O_4S_4Br_2Cl_2$: C, 59.19; H, 2.87; S, 11.48. Found: C, 59.16; H, 2.84; S, 11.46.

6,6'-(2,3-Dichlorobenzylidene)-bis[5-hydroxy-9-methyl-2-phenyl-3-(*p*-bromo)phenylthieno[3,2-*g*]thiocoumarin] (**5c**).

Compound **5c** was obtained in 90% yield; mp: 212-213 °C; IR (KBr): 1720(C=O), 3460(-OH); ^{1}H NMR: δ 2.49 (s, 6H, 2 × CH_3), δ 6.21(s, 1H, -CH), δ 6.82-6.88 (m, 8H, Ar-H), δ 7.05-7.10 (m, 10H, Ar-H), δ 7.26-7.30 (s, 2H, Ar-H), δ 7.42 –7.58(m, 3H, Ar-H), δ 12.42 (s, 2H, coumarin-OH); ms: m/z 1115 (M^+).

Anal. Calcd. for $C_{55}H_{32}O_4S_4Br_2Cl_2$: C, 59.19; H, 2.87; S, 11.48. Found: C, 59.15; H, 2.85; S, 11.46.

6,6'-*p*-Hydroxybenzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(*p*-bromo)phenylthieno[3,2-*g*]thiocoumarin] (**5d**).

Compound **5d** was obtained in 86% yield; mp: 222-225 °C; IR (KBr): 1680(C=O), 3500(-OH), 1350(Br-OH); ^{1}H NMR: δ 2.40 (s, 6H, 2 × CH_3), δ 5.42 (s, 1H, Ar-OH), δ 6.24 (s, 1H, -CH), δ 6.76-6.84 (m, 8H, Ar-H), δ 6.98-7.08 (m, 10H, Ar-H), δ 7.22-7.26 (s, 2H, Ar-H), δ 7.38-7.52 (m, 4H, Ar-H), δ 12.52 (s, 2H, coumarin-OH); ms: m/z 1062 (M^+).

Anal. Calcd. for $C_{55}H_{34}O_5S_4Br_2$: C, 62.15; H, 3.20; S, 12.04. Found: C, 62.13; H, 3.16; S, 12.00.

6,6'-Benzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(3,4-dichloro)phenylthieno[3,2-*g*]thiocoumarin] (**6a**).

Compound **6a** was obtained in 87% yield; mp: 255-257 °C; IR (KBr): 1710(C=O), 3450(-OH); ^{1}H NMR: δ 2.51 (s, 6H, 2 × CH_3), δ 6.25(s, 1H, -CH), δ 6.80-6.98 (m, 10H, Ar-H), δ 7.18-7.24 (m, 6H, Ar-H), δ 7.26-7.32 (s, 2H, Ar-H), δ 7.35-7.40 (m, 5H, Ar-H), δ 12.40 (s, 2H, coumarin-OH); ms: m/z 1026 (M^+).

Anal. Calcd. for $C_{55}H_{32}O_4S_4Cl_4$: C, 64.33; H, 3.12; S, 12.48. Found: C, 64.30; H, 3.09; S, 12.46.

6,6'-(3,4-Dichlorobenzylidene)-bis-[5-hydroxy-9-methyl-2-phenyl-3-(3,4-dichloro)phenylthieno[3,2-*g*]thiocoumarin] (**6b**).

Compound **6b** was obtained in 82% yield; mp: 281-283 °C; IR (KBr): 1680(C=O), 3450(-OH); ^{1}H NMR: δ 2.47 (s, 6H, 2 × CH_3), δ 6.26 (s, 1H, -CH), δ 6.98-7.06 (m, 10H, Ar-H), δ 7.16-7.24 (m, 6H, Ar-H), δ 7.26-7.28 (s, 2H, Ar-H), δ 7.36-7.62 (m, 3H, Ar-H), δ 12.32 (s, 2H, coumarin-OH); ms: m/z 1095 (M^+).

Anal. Calcd. for $C_{55}H_{30}O_4S_4Cl_6$: C, 60.27; H, 2.75; S, 11.69. Found: C, 60.25; H, 2.71; S, 11.66.

6,6'-(2,3-Dichlorobenzylidene-bis-[5-hydroxy-9-methyl-2-phenyl-3-(3,4-dichloro)phenylthieno[3,2-*g*]thiocoumarin] (**6c**).

Compound **6c** was obtained in 88% yield; mp: 290-293 °C; IR (KBr): 1710(C=O), 3450(-OH); ^{1}H NMR: δ 2.54 (s, 6H, 2 × CH_3), δ 6.24 (s, 1H, -CH), δ 7.02-7.08 (m, 10H, Ar-H), δ 7.16-7.20 (m, 6H, Ar-H), δ 7.26-7.28 (s, 2H, Ar-H), δ 7.40-7.60 (m, 3H, Ar-H), δ 12.32 (s, 2H, coumarin-OH); ms: m/z 1095 (M^+).

Anal. Calcd. for $C_{55}H_{30}O_4S_4Cl_6$: C, 60.27; H, 2.75; S, 11.69. Found: C, 60.24; H, 2.71; S, 11.67.

6,6'-*p*-Hydroxybenzylidene-bis[5-hydroxy-9-methyl-2-phenyl-3-(3,4-dichloro)phenylthieno[3,2-*g*]thiocoumarin] (**6d**).

Compound **6d** was obtained in 90% yield; mp: 241-243 °C; IR (KBr): 1680(C=O), 3460(-OH), 1340(Br-OH); ^{1}H NMR: δ 2.43 (s, 6H, 2 × CH_3), δ 5.42 (s, 1H, Ar-OH), δ 6.26(s, 1H, -CH), δ 6.98-7.06 (m, 10H, Ar-H), δ 7.12-7.16 (m, 6H, Ar-H), δ 7.30-7.32 (s, 2H, Ar-H), δ 7.38-7.56 (m, 4H, Ar-H), δ 12.47 (s, 2H, coumarin-OH); ms: m/z 1042 (M^+).

Anal. Calcd. for $C_{55}H_{32}O_5S_4Cl_4$: C, 63.34; H, 3.07; S, 12.28. Found: C, 63.30; H, 3.04; S, 12.24.

REFERENCES AND NOTES

[1] N. K. Sangwan, B. S. Verma, O. P. Malik and S. K. Dhindsa, *Indian. J. Chem*., **29B**, 294 (1990).

[2] A. M. Stahmann, F. C. Huebner and K. P. Link, *J. Biol. Chem*., **138**, 513 (1941).

[3] S. S. Hanmantgad, M. V. Kulkarni and V. D. Patil, *Indian. J. Chem*., **24B**, 459 (1985).

[4] D. J. Hepworth, Comprehensive Heterocyclic Chemistry, Vol **3**, J. A. Boultonand and A. Mikillap, ed, Pergamon Press, Oxford, 1984, pp 737.

[5] A. J. Parrish, B. T. Fitzpatrick, L. Tanenbaurn and M. A. Pathak, *New. Engl. J. Med*., **291**, 206 (1974).

[6] P. F. Top. Schuda, *Org. Chem*., **91**, 75 (1980).

[7] Campbell and Link, *J. Biol. Chem*., **138**, 21 (1941).

[8] F. C. Huebner and Link, *J. Biol. Chem*., **138**, 529 (1941).

[9] Overman, Stahmann, Sullivan, Huebner, Campbell and Link, *J. Biol. Chem.*, **142**, 941 (1942).

[10] For the possible therapeutic use of this drug in rendering bloodless coagulable *in vivo* to control processes of embolism and thrombosis in man see the symposium in *J. Am. Med. Assoc*., **120**, 1009-1226 (1942).

[11] A. K. Shah, S. N. Bhatt, V. R. Raval and M. V. Thakor, *Sci and Culture*., **52(7**), 2289 (1986).

[12] W. R. Sullivan, F. C. Huenbner, M. A. Stahmann and K. P. Link , *J. Am. Chem. Soc*., **65**, 2288 (1943).