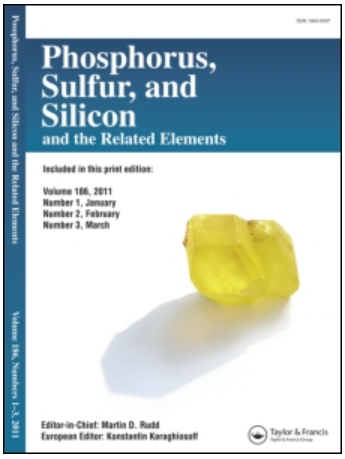

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SYNTHESIS AND STUDIES OF SOME NEW 3-SUBSTITUTED COUMARIN DERIVATIVES

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Communication

SYNTHESIS AND STUDIES OF SOME NEW 3-SUBSTITUTED COUMARIN DERIVATIVES

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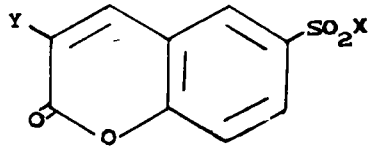
The synthesis of different 3-acetamido-coumarin-6-sulphonylamino acids **2a–e**; corresponding methyl esters **3a–e**; dipeptides **4a–e** and some related 3-amino or 3-hydroxy derivatives **5a–6d** are described. All the 3-amino or 3-hydroxycoumarin-6-sulphonylamino acid derivatives were found to possess remarkable antimicrobial properties towards different microorganisms.

Key words: 3-Substituted coumarins; amino acid derivatives; antimicrobial properties.

INTRODUCTION

In previous communications,[1–4] we reported the synthesis of some coumarins incorporating amino acid and dipeptide moieties. Most of these compounds were found to have interesting antimicrobial properties. However, the effect of variation of functional groups in coumarin derivatives on the antimicrobial and pharmacological properties has not yet been investigated.

We now report the synthesis of a series of 3-substituted coumarin-6-sulphonylamino acid derivatives **2a–6d**, as well as studies on their biological properties (cf. Scheme 1 and Table I).



(2a-6d)

	X	Y
2a-e,	amino acid	$NHCOCH_3$
3a-e,	amino acid-OMe	$NHCOCH_3$
4a-e,	dipeptide-OMe	$NHCOCH_3$
5a-d,	amino acid	NH_2
6a-d,	amino acid	OH

SCHEME 1

TABLE I

Physical data of various 3-substituted coumarin-6-sulphonylamino acid derivatives **2a–6d**

Compd. No.	X	Y	(*) Yield (%)	M.P. °C	R_f	(**) $[\alpha]_D^{20}$	Molecular formula	Elemental analysis (%) Calculated / Found					
								C	H	N	C	H	N
2a	β-Ala	$NHCOCH_3$	70	246-48	0.50	-----	$C_{14}H_{14}N_2O_7S$	47.45	3.95	7.90	47.6	4.1	8.1
b	L-Pro	$NHCOCH_3$	65	226-28	0.45	-37.2	$C_{16}H_{16}N_2O_7S$	50.52	4.21	7.36	50.7	4.4	7.5
c	L-Leu	$NHCOCH_3$	66	208-10	0.49	+77.9	$C_{17}H_{20}N_2O_7S$	51.51	5.05	7.07	51.7	5.1	7.2
d	L-Met	$NHCOCH_3$	59	120-22	0.47	+85.4	$C_{16}H_{18}N_2O_7S_2$	46.37	4.34	6.76	46.4	4.4	6.8
e	L-Phe	$NHCOCH_3$	61	271-73	0.52	+55.26	$C_{20}H_{18}N_2O_7S$	55.81	4.18	6.51	55.9	4.2	6.7
3a	β-Ala-OMe	$NHCOCH_3$	55	118-20	0.37	-----	$C_{15}H_{16}N_2O_7S$	48.91	4.34	7.60	49.1	4.5	7.7
b	L-Pro-OMe	$NHCOCH_3$	51	137-39	0.49	-95.5	$C_{17}H_{18}N_2O_7S$	51.77	4.56	7.10	51.9	4.8	7.3
c	L-Leu-OMe	$NHCOCH_3$	50	188-90	0.75	+32.6	$C_{18}H_{22}N_2O_7S$	52.68	5.36	6.82	52.8	5.5	6.9
d	L-Met-OMe	$NHCOCH_3$	48	148-50	0.51	-75.4	$C_{17}H_{20}N_2O_7S_2$	47.66	4.67	6.54	47.8	4.7	6.7
e	L-Phe-OMe	$NHCOCH_3$	40	116-18	0.68	+65.2	$C_{21}H_{20}N_2O_7S$	56.75	4.50	6.30	56.8	4.6	6.5
4a	β-Ala-Gly-OMe	$NHCOCH_3$	65	205-7	0.32	-----	$C_{17}H_{19}N_3O_8S$	48.00	4.47	9.88	48.2	4.6	10.1
b	L-Pro-L-Ser-OMe	$NHCOCH_3$	61	168-70	0.45	+10.1	$C_{20}H_{23}N_3O_9S$	49.89	4.78	8.73	50.1	4.9	8.9
c	L-Phe-L-Val-OMe	$NHCOCH_3$	67	156-58	0.57	+60.3	$C_{26}H_{29}N_3O_8S$	57.45	5.34	7.73	57.6	5.5	7.9
d	L-Leu-L-Tyr-OMe	$NHCOCH_3$	59	201-3	0.59	+72.8	$C_{27}H_{31}N_3O_9S$	56.54	5.41	7.32	56.7	5.5	7.4
e	L-Met-L-Phe-OMe	$NHCOCH_3$	55	215-17	0.56	+47.7	$C_{26}H_{29}N_3O_8S_2$	54.26	5.00	7.30	54.4	5.1	7.4
5a	L-Pro	NH_2	40	207-9	0.39	-57.8	$C_{14}H_{14}N_2O_6S$	49.70	4.14	8.28	49.9	4.3	8.4
b	L-Leu	NH_2	52	150-52	0.22	+42.8	$C_{15}H_{18}N_2O_6S$	50.84	5.08	7.90	50.9	5.2	8.1
c	L-Met	NH_2	42	170-72	0.66	-40.2	$C_{14}H_{16}N_2O_6S_2$	45.16	4.30	7.52	45.2	4.4	7.7
d	L-Phe	NH_2	45	226-28	0.37	+27.6	$C_{18}H_{16}N_2O_6S$	55.67	4.12	7.21	55.8	4.3	7.4

6a	β-Ala	OH	41	218-20	0.47	-----	$C_{12}H_{11}NO_7S$	46.00	3.51	4.47	46.2	3.7	4.6
b	L-Pro	OH	46	221-23	0.51	-85.4	$C_{14}H_{13}NO_7S$	49.55	3.83	4.12	49.7	4.1	4.3
c	L-Leu	OH	48	196-98	0.54	+30.1	$C_{15}H_{17}NO_7S$	50.70	4.78	3.94	50.9	4.9	4.1
d	L-Phe	OH	43	143-45	0.65	+17.6	$C_{18}H_{15}NO_7S$	55.52	3.85	3.59	55.7	3.9	3.7

*) Crystallization solvent for compounds 2a-5d = ethanl-water and for compounds 6a-d = abs. ethanol.

**) Optical rotations $[\alpha]_D^{20}$ were measured (C=5) in acetone.

TABLE II

Minimal inhibitory concentration (MIC in μg/ml) of the biologically active compounds

Compd. No.	B. subtilis	B. megaterium	E. coli	S. marscence
1	250	500	500	500
5a	100	100	125	100
b	100	100	150	150
c	100	100	75	100
d	50	50	50	100
6a	20	25	25	25
b	10	10	15	25
c	10	10	15	15
d	5	5	10	10

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography (R_f values) was carried out on silica gel-G (BDH), using benzene-ethyl acetate (3:1) as solvent system and iodine-KI (20%) as detection reagent. Paper chromatography (spot reactions) was carried out using Whatman No. 1 paper and *n*-butanol-pyridine acetic-acid-water (30:20:5:24) as solvent system. Benzidine, ninhydrin and hydroxamate reactions were used for visualizing the spots. IR spectra (λ_{max} in cm^{-1}) were measured by a Shimadzu IR spectrometer (IR 440) in KBr pellets and ^1H NMR data were obtained on a Varian EM-360L spectrophotometer in DMSO-d_6 and shifts are reported in (δ) ppm relative to internal TMS. Optical rotations $[\alpha]_D^{20}$ were taken in a Bellingham-Stanley polarimeter in 1 dm tube, C = 5 in acetone.

3-Acetamidocoumarin-6-sulphonyl chloride 1: Compound **1** was prepared by literature method.[5]

General procedure for the synthesis of 3-acetamidocoumarin-6-sulphonylamino acids **2a–e**: The amino acid (0.1 mole) was dissolved in a mixture of THF (15 ml), water (25 ml), then triethylamine (5 ml) was added. 3-Acetamidocoumarin-6-sulphonyl chloride **1** was added portionwise to the reaction mixture during 30 min at 10°C and stirring continued for 3–4 hrs at room temperature. Tetrahydrofuran was removed from the reaction mixture under reduced pressure. The mixture was cooled and acidified with 2M-HCl until acidic to congo red (pH = 5). The crude product was filtered, washed with cold water several times and recrystallized from ethanol-water. The compounds **2a–e** were chromatographically homogeneous when detected with iodine solution, benzidine and showed negative ninhydrin test.

General procedure for the synthesis of 3-acetamidocoumarin-6-sulphonylamino acid methyl esters **3a–e**: A suspension of 3-acetamidocoumarin-6-sulphonylamino acid (0.02 mole) in abs. methanol (20 ml) was cooled to −5°C and pure thionyl chloride (0.022 mole) was added dropwise during 1 hr. The temperature of the reaction mixture was kept below 0°C during the addition of thionyl chloride. The reaction mixture was stirred for an additional 4 hrs at room temperature, kept over night at room temperature and the solvent removed in vacuo. Methanol was added and reevaporated several times and the residual solid was recrystallized from ethanol-water. The isolated methyl esters **3a–e** were chromatographically homogeneous when developed with iodine solution and showed ninhydrin negative test, but hydroxamate positive reaction.

General procedure for the synthesis of 3-acetamidocoumarin-6-sulphonyldipeptide methyl esters **4a–e**: To a solution of amino acid methyl ester hydrochloride (0.02 mole) in THF (20 ml), triethylamine (3 ml) was added. The reaction mixture was stirred at 20°C for 30 min. and cooled to 0°C. The precipitated triethylamine hydrochloride was filtered off. To the filtrate, at −5°C were added 3-acetamidocoumarin-6-sulphonylamino acid (0.021 mole) and dicyclohexylcarbodiimide (DDC, 0.022 mole) successively. The reaction mixture was stirred for 4 hrs at 0°C and for another 4 hrs at room temperature. Dicyclohexylurea was filtered off and the filtrate evaporated in vacuo. The residual solid was recrystallized from ethanol-water. The compounds **4a–e** were chromatographically homogeneous when developed with iodine solution and gave ninhydrin negative test and hydroxamate positive reaction.

General procedure for the synthesis of 3-aminocoumarin-6-sulphonylamino acids **5a–d**: The appropriate 3-acetamidocoumarin-6-sulphonylamino acid (0.05 mole) was suspended in a mixture of acetic acid (25 ml) and sulphric acid (25 ml). The reaction mixture was heated at 50–60°C for 30–45 min. The clear solution was poured in an equal volume of cold water and neutralized with sodium carbonate. The crude product was filtered and recrystallized from ethanol-water. The compounds **5a–d** were chromatographically homogeneous when developed with benzidine, iodine solution and showed ninhydrin negative test.

General procedure for the synthesis of 3-hydroxycoumarin-6-sulphonylamino acids **6a–d**: The appropriate 3-acetamidocoumarin-6-sulphonylamino acid (0.05 mole) was dissolved in a minimum quantity of ethanol and refluxed with 3N-HCl (30 ml) for 3–4 hrs. On cooling the crude product was filtered, washed with cooled water several times and recrystallized from abs. ethanol. The compounds **6a–d** were chromatographically homogeneous when detected with benzidine and iodine solution and showed ninhydrin negative test.

RESULTS AND DISCUSSION

A. Chemistry

3-Acetamidocoumarin-6-sulphonylamino acids **2a–e** were easily prepared by the reaction of 3-acetamidocoumarin-6-sulphonyl chloride **1**[5] with the appropriate amino

acid in THF-H_2O-Et_3N medium at 10°C. The time required for completion of the reaction (3–4 hrs) was monitored by TLC.

The methyl esters **3a–e** were prepared by treating the amino acid derivatives **2a–e** with methanol and pure thionyl chloride at −5°C. Complete acid hydrolysis of compound **3a** [6M-HCl, 24 hrs, 100°C] followed by paper chromatography afforded an alanine spot.

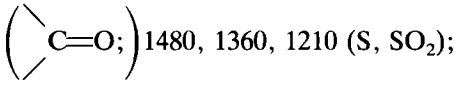
The dipeptides **4a–e** were readily prepared by coupling of 3-acetamidocoumarin-6-sulphonylamino acid **2a–e** with the appropriate amino acid methyl ester hydrochloride in THF-Et_3N medium and using (DCC) method.[6] Complete acid hydrolysis of **4d** [6M-HCl, 24 hrs, 100°C] followed by paper chromatography afforded leucine and tyrosine spots.

3-Aminocoumarin-6-sulphonylamino acids **5a–d** were prepared by heating the appropriate 3-acetamidocoumarin-6-sulphonylamino acid **2b–e** in a mixture of acetic acid and sulphuric acid and using literature technique.[7]

Hydrolysis of the appropriate 3-acetomidocoumarin-6-sulphonylamino acid **2a–c, e** by using literature method[8] in HCl/C_2H_5OH medium gave the related 3-hydroxy derivatives **6a–d**.

Complete acid hydrolysis of **5b** or **6c** [6M-HCl, 24 hrs, 100°C] followed by paper chromatography gave a leucine spot.

The IR spectra of compounds **2a–6d** in KBr showed characteristic bands at cm^{-1}: 3320, 3130 (NH, CONH), 1760, 1720



1480, 1360, 1210 (S, SO_2);

1670, 1550, 1340 (amide I, II and III) and other bands due to amino acid residues.

The ^1H NMR spectra of all compounds **2a–6d** in DMSO-d_6 exhibited the chemical shifts (δ) at: 5.8 (s, 1H, amide-NH), 7.8–8.5 (m, aromatic protons); for compounds **2a–6** and **5a–6d** 10.9 (s, 1H, COOH), for compounds **5a–d** 4.6 (s, 2H, NH_2); for compounds **6a–d** 9.6 (s, 1H, OH) and other signals in support of the proposed structures.

B. Biology

The antimicrobial activities of the synthesized compounds **2a–6d** were tested using the hole plate and filter paper disc method.[9–12] The substances were added to the plates according to the literature methods.[9–12] The substances were dissolved in ethanol (10%) which did not exhibit any effect as recorded by control experiment. All compounds were tested against gram-positive and gram-negative bacteria: Bacillus subtilis; Bacillus megaterium; Escherichia coli and Serratia marscence. The results were compared with the activity of the parent compound **1** [cf. Table II]. The data for the minimal inhibitory concentrations (MIC in μg/ml) of the active compounds are summarized in Table II.

All the synthesized 3-acetamidocoumarin-6-sulphonylamino acids **2a–e**, corresponding methyl esters **3a–e** and dipeptides **4a–e** were biologically inactive towards all tested bacteria.

All 3-aminocoumarin derivatives **5a–d** were found to possess various antimicrobial activities at a minimal inhibitory concentration (MIC) of 50–150 μg/ml against all tested bacteria.

Also, all 3-hydroxy derivatives **6a–d** were biologically active and gave interesting results towards all tested bacteria (MIC 5–25 μg/ml).

The achieved results showed that introduction of an acetamido group in 3-position of the coumarin moiety in combination with sulphonylamino acids, corresponding methyl esters and dipeptide residues gave biologically inactive compounds **2a–4e**. However, the introduction of an amino group in 3-position of the coumarin moiety with sulphonylamino acid residues gave biologically active compounds **5a–d**. Moreover, the introduction of a hydroxy group in the 3-position in coumarin improve the biological properties of the synthesized compounds **6a–d**.

Since the biologically active substances (**5a–6d**) have sulfonamide character, a trial of the reversal inhibition by the addition of *p*-aminobenzoic acid to the plates has been carried out. This resulted in having a clear cut growth of the bacterial test strain which confirms sulfonamide nature of the substances under investigation.

Also, it is well known that work on the action of the present sulfonamides with regard to the binding to plasma proteins will add a strong evidence concerning the nature of the synthesized compounds. This type of mode of action is now under investigation.

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