Proc. Indian natn. Sci. Acad., **60**, A, No. 2, 1994, pp. 433-439.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 7-METHOXY-4-METHYLCOUMARIN-6-SULPHONYL-AMINO ACID DERIVATIVES

T M IBRAHIM, F S M AHMED AND S.A SHEDID
Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

(*Received* 14 *June* 1993; *Revised* 1 *September* 1993; *Accepted* 28 *October* 1993)

Synthesis of 7-methoxy-4-methylcoumarin-6-sulphonylamino acids **2a-h**, corresponding methyl esters **3a-h**, hydrazides **4a-h**, dipeptides **5a-6b**, tripeptide derivative **7**, dipeptide hydrazides **8a-c** are described. Twenty-four of various 7-methoxy-4-methylcoumarin-6-sulphonylamino acid derivatives were found to possess specific antimicrobial activity against a number of microorganisms.

Key Words: Coupling Reactions; 7-Methoxy-4-methylcoumarin-6-sulphonyl Chloride; Biological Properties

Introduction

Recently, we reported that some substituted coumarin derivatives exhibited various antimicrobial properties[1,2]. In continuation of our studies in this field, we now report the synthesis of a series of 7-methoxy-4-methylcoumarin-6-sulphonylamino acid derivatives **2a-8c**. These compounds were also tested for antimicrobial activity against different microorganisms.

Results and Discussion

A. Chemistry

7-Methoxy-4-methylcoumarin-6-sulphonylamino acid **2a-h** and 7-methoxy-4-methylcoumarin-6-sulphonyl-Gly-Gly **6a** were readily prepared by the reaction of 7-methoxy-4-methylcoumarin-6-sulphonyl chloride 1[3] with the appropriate amino acid (or Gly-Gly) in water-THF-Et_3N medium. The time required for completion of the reaction (4 hrs) was monitored by TLC. Synthesis of Ser, Tyr-derivatives did not require prior protection of the side chain and no side reactions were observed. Compounds **2a-h** and **6a** were chromatographically homogeneous and gave negative results with ninhydrin (cf. Scheme 1 and Table I).

The methyl esters **3a-h** and **6b** were prepared by treating the amino acid derivatives **2a-h** or Gly-Gly derivative **6a** with methanol and pure thionyl chloride at $-5°C$. All the methyl esters **3a-h** and **6b** (cf. Scheme 1 and Table I) were chromatographically homogeneous. Complete acid hydrolysis of compounds **2a** and **6b** (6M-HCl, 24 hrs, 100°C) followed by paper chromatography afforded alanine and glycine spots.

Hydrazinolysis of the methyl esters **3a-h** in ethanol gave the corresponding hydrazides **4a-h** (cf. Scheme 1 and Table I), as crystalline solids which gave positive benzidine and silver nitrate reactions.

Table I

Physical data of various 7-*methoxy*-4-*methyl-coumarin*-6-*sulphonylamino acid derivatives* (**2a-8c**)

Compd. No.	X	(*) Yield (%)	M.P. °C	R_f	(**) $[\alpha]_D^{20}$	Molecular formula (****)	Minimal inhibitory concentration (MIC in μg/ml) of the biologically active compounds†			
							B. subtilis	*B. megaterium*	*E. coli*	*S. marscena*
1	2	3	4	5	6	7	8	9	10	
2a	β-Ala	80	191-93	0.35	—	$C_{14}H_{15}NO_7S$	200	—	200	200
b	L-Ser	61	201-3	0.87	−140.7	$C_{14}H_{15}NO_8S$	50	250	250	50
c	L-Pro	57	210-12	0.27	−180.9	$C_{16}H_{17}NO_7S$	200	200	200	100
d	L-Leu	72	214-16	0.47	+5.1	$C_{17}H_{21}NO_7S$	150	200	250	150
e	P-Aba***	50	250-52	0.56	—	$C_{18}H_{15}NO_7S$	100	200	250	100
f	L-Met	48	182-84	0.28	−115.6	$C_{18}H_{19}NO_7S_2$	50	200	50	50
g	L-Phe	73	217-19	0.41	−10	$C_{20}H_{19}NO_8S$	100	—	100	100
g	L-Tyr	65	194-98	0.79	−45.1	$C_{20}H_{19}NO_8S$	50	250	100	50
3a	β-Ala-OMe	74	165-67	0.28	—	$C_{15}H_{17}NO_7S$	200	—	100	200
b	L-Ser-OMe	51	158-60	0.68	−16.6	$C_{15}H_{17}NO_8S$	50	50	100	100
c	L-Pro-OMe	62	154-56	0.39	−85.4	$C_{17}H_{19}NO_7S$	200	100	100	100
d	L-Leu-OMe	71	176-78	0.94	+200	$C_{18}H_{23}NO_7S$	100	100	100	100
e	P-Aba-OMe	55	236-38	0.96	—	$C_{17}H_{19}NO_7S$	50	100	100	100
f	L-Met-OMe	54	147-49	0.37	−220.3	$C_{17}H_{21}NO_7S_2$	100	100	100	100
g	L-Phe-OMe	73	135-37	0.89	−55.3	$C_{21}H_{21}NO_7S$	100	—	100	100
h	L-Tyr-OMe	66	181-83	0.24	+22.6	$C_{21}H_{21}NO_8S$	50	100	50	25
4a	β-Ala-N_2H_3	63	204-6	0.71	—	$C_{14}H_{17}N_3O_6S$	50	—(*****)	50	50
b	L-Ser-N_2H_3	50	199-201	0.90	−130.6	$C_{14}H_{17}N_3O_7S$	10	100	50	25
c	L-Pro-N_2H_3	55	183-85	0.83	−70.3	$C_{16}H_{19}N_3O_6S$	100	50	50	100
d	L-Leu-N_2H_3	58	179-82	0.59	+7.3	$C_{17}H_{23}N_3O_6S$	25	50	50	50

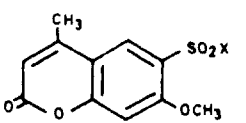
Note: Please see footnote of this table on next page

Table I C'ontd.

Contn. of Table I

1	2	3	4	5	6	7	8	9	10	11
e	P-Aba-N_2H_3	51	271-73	0.68	—	$C_{18}H_{17}N_3O_6S$	25	50	50	50
f	L-Met-N_2H_3	52	165-67	0.50	−281.3	$C_{16}H_{21}N_3O_6S_2$	25	250	50	50
g	L-Phe-N_2H_3	61	202-4	0.37	+95.4	$C_{20}H_{21}N_3O_6S$	25	100	50	50
h	L-Tyr-N_2H_3	57	235-37	0.53	−37.7	$C_{20}H_{21}N_3O_7S$	10	50	25	10
5a	L-Pro-L-Ser-OMe	60	175-77	0.52	−95.5	$C_{20}H_{24}N_2O_9S$	—	—	—	—
b	L-Met-Gly-OMe	51	228-30	0.60	−7.5	$C_{19}H_{24}N_2O_8S_2$	—	—	—	—
c	L-Phe-L-Leu-OMe	55	156-58	0.51	−21.1	$C_{27}H_{32}N_2O_8S$	—	—	—	—
d	L-Leu-L-Tyr-OMe	58	197-99	0.47	−10	$C_{27}H_{32}N_2O_9S$	—	—	—	—
6a	Gly-Gly	80	200-2	0.43	—	$C_{14}H_{16}N_2O_8S$	—	—	—	—
b	Gly-Gly-OMe	71	188-90	0.51	—	$C_{16}H_{18}N_2O_8S$	—	—	—	—
7	Gly-Gly-β-Ala	49	195dec.	0.63	—	$C_{18}H_{21}N_3O_9S$	—	—	—	—
8a	L-Met-Gly-N_2H_3	45	204-6	0.58	−24.1	$C_{18}H_{24}N_4O_7S_2$	—	—	—	—
b	L-Leu-L-Tyr-N_2H_3	42	300-2	0.54	−5.1	$C_{26}H_{32}N_4O_8S$	—	—	—	—
c	Gly-Gly-N_2H_3	63	232-34	0.32		$C_{15}H_{18}N_4O_7S$	—	—	—	—

*) Crystallization solvent for compounds **2a-h, 4a-6a** and **7-8c** = ethanol-water and for compounds **3a-h** and **6b** = abs. ethanol.
**) Optical rotations $[\alpha]_D^{20}$ were measured (C = 5) in acetone.
***) p-Aba = p-aminobenzoic acid residue.
****) All the synthesized compounds **2a-8c** gave satisfactory C, H and N analysis.
†) Biologically inactive compound (MIC > 500 μg/ml).



Scheme 1

2a-h; X = amino acid
3a-h; X = amino acid-OMe
4a-h; X = amino acid-N_2H_3
5a-h; X = dipeptide-OMe
6a; X = Gly-Gly
6b; X = Gly-Gly-OMe
7; X = Gly-Gly-β-Ala-OMe
8a; X = dipeptide-N_2H_3

2a-8c

Coupling of 7-methoxy-4-methylcoumarin-6-sulphonylamino acids **2c,d,f,g** and Gly-Gly-derivative **6a** with the appropriate amino acid methyl ester hydrochloride in THF-Et_3N medium and using (DCC) technique[4] afforded the dipeptides **5a-d** and the tripeptide derivative **7** (cf. Scheme 1 and Table I). All the dipeptides **5a-h** and the tripeptide derivative 7 were chromatographically homogeneous and gave positive hydroxamate reaction. Complete acid hydrolysis of **5c** (6M – HCl, 24 hrs, 100°C) afforded phenylalanine and leucine spots.

Hydrazinolysis of the dipeptides **5b,d** and **6b** in ethanol gave the corresponding hydrazides **8a-c** (cf. Scheme 1 and Table I). All hydrazides **8a-c** were chromatographically homogeneous and gave positive silver nitrate reaction.

B. Biology

The antimicrobial activities of synthesized compounds were tested using the hole plate and filter paper disc methods[5-9]. All compounds were tested against gram-positive and gram-negative bacteria: *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Serrati marscena* and selected fungi: *Candida utilis*. The results were compared with the activity of the parent compound 1, which was found to possess a low antimicrobial activity against B. subtilis (250 μg/ml) and E. coli (500 μg/ml), the data for the minimal inhibitory concentrations (MICs in μg/ml) of the active compounds are summarized in Table I.

Results and Conclusion

7-Methoxy-4-methylcoumarin-6-sulphonyl-β-Ala, **2a** and corresponding L-Ser **2b**, L-Pro **2c**, L-Leu **2d**, p-Aba **2e**, L-Met **2f**, L-Phe **2g** and L-Tyr **2h** were found to possess various antimicrobial activities at a minimal inhibitory concentration (MIC) of 50-250 μg/ml against most of tested microorganisms.

All the synthesized methyl ester derivatives **3a-h** and corresponding hydrazides **4a-h** were biologically active and gave promising results against B. subtilis, B. megaterium, E. coli and S. marscena ranging from 10-200 μg/ml.

All the synthesized dipeptides **5a-6b**, tripeptide derivative **7** and dipeptide hydrazides **8a-c** were biologically inactive against all tested microorganisms.

All the synthesized compounds **2a-8c** were found to possess a very low antimicrobial activity against Cand. utilis (500 μg/ml).

The present investigation indicated that the presence of 7-methoxy-4-methylcoumarin-6-sulphonyl residue in combination with amino acids give biologically active compounds **2a-h**. Moreover, esterification and hydrazinolysis of the C-terminal of amino acids improve the biological properties of 7-methoxy-4-methylcoumarin-6-sulphonylamino acid methyl esters **3a-h** and corresponding hydrazides **4a-h**. However, the elongation of the peptide chain gave biologically inactive compounds **5a-8c**.

Experimental

Melting points were determined using an electrothermal melting points apparatus and are uncorrected. Thin layer chromatography (TLC, R_f values) was performed on silica gel-G using benzene-ethyl acetate (2:1) as the solvent system and iodine-KI (20%) as detection reagent. Benzidine, ninhydrin, silver nitrate

and hydroxamate reactions were used for detection of the amino acid derivatives on chromatograms (spot reactions). IR spectra were recorded on Shimadzu IR spectrometer (IR 440) using KBr discs. ^{1}H NMR spectra were recorded on a Varian EM-360L spectrophotometer using DMSO-d_6 as solvent and TMS as internal standard. Optical activities $[\alpha]_D^{20}$ were taken with Bellingham-Stanley polarimeter in 1 dm tube, C = 5 in acetone.

7-Methoxy-4-methylcoumarin-6-sulphonyl chloride **1**

The titled compound **1** was prepared by literature method[3].

General procedure for the synthesis of 7-methoxy-4-methylcoumarin-6-sulphonylamino acids **2a-h** *and 7-methoxy-4-methylcoumarin-6-sulphonylglyceylglycine* **6a**

To a solution of the appropriate amino acid or Gly-Gly (0.1 mole) in THF (15 ml)-water (25 ml) mixture was added triethylamine (5 ml) followed by 7-methoxy-4-methylcoumarin-6-sulphonyl chloride 1 (0.11 mole) portionwise during 30 min. The temperature of the reaction mixture during the addition was kept at 10°C and stirring continued for 4 hrs at room temperature. Tetrahydrofuran was removed by concentration reaction mixture under reduced pressure. The mixture was cooled to 0°C and acidified with 2M-HCl until acidic to congo red (pH = 5). The crude product was filtered, washed with cold water and redcrystallized from ethanol-water. The compounds **2a-h** and **6a** were chromatographically homogeneous when developed with benzidine, iodine solution and gave ninhydrin reaction.

General procedure for the synthesis of 7-methoxy-4-methyl-coumarin-6-sulphonylamino acid methyl esters **3a-h** *and 7-methoxy-4-methylcoumarin-6-sulphonylglyceylglycine methyl ester* **6b**.

A suspension of 7-methoxy-4-methylcoumarin-6-sulphonylamino acid or 7-methoxy-4-methylcoumarin-6-sulphonyl-Gly-Gly (0.01 mole) in absolute methanol (30 ml) was cooled to −5°C and pure thionyl chloride (1.2 ml) was added dropwise during 1 hr. The temperature of the mixture was kept below 0°C during the addition of thionyl chloride. The reaction mixture was then stirred for additional 3 hrs at room temperature, kept over night at room temperature and the solvent was removed in vacuo. Methanol was added and reevaporated several times and the residual solid material was recrystallized from abs. ethanol. The compounds **3a-h** and **6b** were chromatographically homogeneous when developed with benzidine, iodine solution and gave positive hydroxamate reaction and ninhydrin negative test.

General procedure for the synthesis of 7-methoxy-4-methylcoumarin-6-sulphonylamino acid hydrazides **4a-h**

A solution of 7-methoxy-4-methylcoumarin-6-sulphonylamino acid methyl ester **3a-h** (0.01 mole) in ethanol (35 ml) and hydrazine hydrate (85%, 0.05 mole) in ethanol (20 ml) was first kept for 24 hrs at 0°C and then for another 24 hrs at room temperature. The crystalline product which separated from the mixture was collected, washed with cold water, and methanol and recrystal-

lized from ethanol-water. The hydrazides **4a-h** were chromatographically homogeneous (detection with iodine solution and benzidine), and showed positive silver nitrate and negative ninhydrin reactions.

General procedure for the synthesis of 7-methoxy-4-methylcoumarin-6-sulphonyldipeptide methyl esters **5a-d**

The amino acid methyl ester hydrochloride (0.03 mole) was suspended in THF (20 ml) and then triethylamine (3 ml) was added. The mixture was stirred for 30 min at 20°C and cooled to 0°C and the triethylamine hydrochloride filtered off. The filtrate was added to a solution of the appropriate 7-methoxy-4-methylcoumarin-6-sulphonylamino acid (0.03 mole) in THF (20 ml). The mixture was cooled (0-5°C), and dicyclohexylcarbodiimide (0.034 mole) was added and the mixture stirred for 3 hrs at 0°C, then left 24 hrs at room temperature. The dicyclohexylurea was filtered off, and the filtrate evaporated in vacuo. The residual solid was recrystallized from ethanol-water. The compounds **5a-d** were chromatographically homogeneous when developed with iodine solution and benzidine and gave ninhydrin negative test.

7-Methoxy-4-methylcoumarin-6-sulphonylglyceylglyceyl-β-alanine 7

The title compound was prepared starting from β-alanine methyl ester hydrochloride (0.01 mole) and 7-methoxy-4-methylcoumarin-6-sulphonylglyceylglycine **6a** (0.01 mole) using the procedure which described for the synthesis of compounds **5a-h**. Compound **7** was recrystallized from ethanol-water and was chromatographically homogeneous when developed with benzidine, iodine solution.

General procedure for the synthesis of 7-methoxy-4-methylcoumarin-6-sulphonyldipeptide hydrazides **8a-c**

7-Methoxy-4-methylcoumarin-6-sulphonyldipeptide methyl ester (0.01 mole) was dissolved in ethanol (50 ml) containing hydrazine hydrate (3.3 ml). The remaining procedure was as described for the preparation of compounds **4a-h**. The compounds **8a-c** were recrystallized from ethanol-water and were chromatographically homogeneous on TLC, responded positively to benzidine, iodine solution, silver nitrate reactions and gave ninhydrin negative test.

The IR spectra of compounds **2a-8c** in KBr showed characteristic bands at cm^{-1}: 3320, 3210 (NH, CONH); 1780, 1720 (>C=O); 1660, 1535, 1350 (amide I, II and III); 1630, 1480, 1380, 1220, 1050 (S, SO_2, COOH and $COOCH_3$) and other bands characteristic of the amino acids and 7-methoxy-4-methylcoumarin residues.

The ^1H NMR spectra of all compounds **2a-8c** in DMSO-d_6 exhibited the chemical shifts (δ) at: 5.7 (s, 1H, amide-NH), 7.9-8.7 (m, aromatic protons); for compounds **3a-h, 5a-d, 6b** and **7 2.84** (s, 3H, $-COOCH_3$ methyl ester protons); for compounds **4a-h** and **8a-c** 4.7-4.95 (s, 3H, N_2H_3 hydrazide protons) and other protons assignable to each individual amino acid moiety, thus confirming the proposed structures.

References

1 A M El-Naggar, A M Abd El-Salam and T M Ibrahim *Afinidad* **44** (1987) 431
2 T M Ibrahim, M A El-Gazzar, A M El-Naggar and S A Shedid *Indian natn Sci Acad* **59A** No. 2 (1993) 189-195
3 J R Merchant and R C Shah *J Indian Chem Soc* **34** (1957) 35
4 J C Sheehan and G P Hess *J Am Chem Soc* **77** (1955) 1067
5 H J Carlson, H G Douglas and H D Bissel *J Bact* **55** (1948) 607
6 G W Irving, T D Fontaine and S P Dolittle *J Bact* **52** (1946) 10
7 J G Vincent and H W Vincent *Proc Soc Exptl Biol Med* **55** (1944) 162
8 J A Epstein, E J Foley, I Perrine and S W Lee *J Lab Clin Med* **29** (1944) 319
9 A M El-Naggar, F S M Ahmed, M F Badie and K M Kamel *Int J Peptide and Protein Res* **22** (1983) 251