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SOME NEW REACTIONS OF CINNAMOYLMORPHOLINE DERIVATIVES WITH AMINO ACIDS

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Cinnamoylmorpholine-4-sulfonylamino acids (2-13) and some of the corresponding methyl esters (14-18) were successively prepared. Hydrazinolysis of the methyl esters in methanol yielded the corresponding hydrazides (19-23). Coupling of amino acid derivatives (2-13) with amino acid methyl ester hydrochlorides in THF-Et_3N medium using the dicyclohexylcarbodiimide method afforded the desired dipeptide methyl esters (25-30). Some of the synthesized derivatives (2-30) were found to be active against a number of micro-organisms.

Key words: Reactions, Cinnamoylmorpholine derivatives, Amino acids.

Introduction

Several nitrogen, oxygen, and sulfur containing heterocyclic compounds incorporating amino acid residues were found to possess interesting biological properties [1-3].

Recently, we have reported the synthesis and structure activity relationship of a variety of aromatic and heterocyclic derivatives of amino acids and dipeptides [4-9].

Moreover, various aromatic sulfonylamino acid derivatives were found to have hypoglycaemic, antipyretic and other biological properties [10-20].

The reaction of cinnamoylmorpholine-4-sulfonyl chloride(1) [21] with the appropriate amino acid in water – THF – triethylamine medium yielded the desired cinnamoylmorpholine-4-sulfonylamino acids (2-13).

Synthesis of cinnamoylmorpholine-4-sulfonylamino acid methyl esters (14-18) was carried out by treating cinnamoylmorpholine-4-sulfonylamino acids (2-13) with methanol and pure thionyl chloride at -10°. The time required for completion of the reaction of the amino acids to corresponding methyl ester derivatives (14-18) was monitored by TLC. Complete hydrolysis of compound (9 or 17) (6N HCl, 24 hrs 105°) followed by subsequent paper chromatography yielded a positive spot of leucine. Hydrazinolysis of the methyl esters (14-18) in methanol afforded the corresponding hydrazides (19-23) which gave positive benzidine and silver nitrate reactions.

For the preparation of cinnamoylmorpholine-4-sulfonyldipeptide methyl esters (24-30), the appropriate amino acid methyl ester hydrochloride was reacted with cinnamoylmorpholine-4-sulfonylamino acids in THF-Et_3N medium using the dicyclohexylcarbonmide (DCC) method. The dipeptide methyl esters (24-30) were purified by repeated recrystallizations from the appropriate solvent and the products were chromatographically homogeneous.

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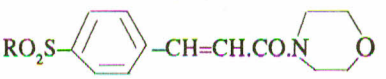
Complete hydrolysis of compound (26) with 6N HCl at 105° for 24 hrs and subsequent chromatography gave positive spots of both alanine and leucine.

The dipeptide methyl esters (24-30) gave a blue coloured 1:1 copper (II) complexes exhibiting λ_{max} at 640-660nm characteristic of normal dipeptide esters [22].

The structure of compounds (2-30) were assigned on the basis of elemental analysis, chromatographic studies, spot tests, IR, and NMR spectra.



Compounds (2-30)

Biological screening results. The antimicrobial activity of the compounds synthesized (2-30) were tested using the hole plate and filter paper disc methods (23-26). All compounds were tested for activity against gram-positive and gram-negative bacteria. A qualitative screening was performed on all compounds, while quantitative assays were carried out on active compounds only. The results were compared with the activity of compound (1) and the data are summarized in Table 1. Cinnamoylmorpholine-4-sulfonyl chloride (1) was antimicrobially inactive.

Studies of the antimicrobial activity of the synthesized compounds (2-30) showed that cinnamoylmorphone-4-sulfonylamino acids, methyl esters, and the corresponding hydrazides were found to be inactive against the tested microorganism while dipeptide derivatives were found to be active towards the tested microorganisms.

Cinnamoylmorpholine-4-sulfonyldipeptide methyl esters containing L-leucine residue such as: L-Ala-L-Leu-OMe (26), L-Lue-L-Leu-OMe (29), and DL-Leu-L-Lue-OMe (30) were found to possess higher antimicrobial activity than the corresponding dipeptides containing DL-Alanine residues

(25, 27, 28). Most of the synthesized dipeptide methyl esters were found to possess various antimicrobial activities toward all the tested microorganisms (Table 1).

Moreover, the biological action of the synthesized cinnamoylmorpholine derivatives depends on the amino acids constitutions, its configuration and the C-terminal protecting groups in these compounds. Other pharmacological activity studies are currently in progress.

Experimental

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography (R_f) was carried out on silica gel-Gl plastic sheets, using: *n*-butanol - acetic acid-water (4:1:1) as the solvent system and iodine as detection reagent. Optical rotations $[\alpha]_D$ were measured in a Bellingham - stanely polarimeter (C=5 in dimethylformamide DMF) at λ_{max} 589nm using 5cm tube at 20° (Table 2). IR spectra (υ_{max} in cm^{-1}) were taken on Shimadzu 440 instrument and NMR spectra in DMSO-d_6 were recorded on Varian EM-360L, chemical (δ) in ppm using TMS as internal standard.

Cinnamoylmorpholine-4-sulfonyl chloride (1). The title compound was prepared in 60% yield by treatment of cinnamoylmorpholine with chlorosulfonic acid using the procedure described earlier [21].

General procedure for the synthesis of cinnamoylmorpholine-4-sulfonylamino acids (2-13). Triethylamine (5ccm) followed by cinnamoylmorpholine-4-sulfonyl chloride (1) (0.01 mole) were added portionwise during 30 mins. to a solution of the appropriate amino acid (0.1 mole) in water (25ccm) – THF (15 ccm) mixture. The temperature of the reaction mixture during the process of addition was kept at 10° and stirring was continued by concentration under reduced pressure and water (30 ccm) was added. The mixture was cooled to 0° and acidified with 2N HCl until acidic to Congo red (pH 5). The crude product was filtered washed with water and recrystallized from methanol-water. All the products (2-13) were chromatographically homogeneous (detection with iodine or benzidine) and showed negative ninhydrin reaction.

The IR spectra of all cinnamoylmorpholine-4-sulfonylamino acids (2-13) showed bands at: 3300, 3080 (NH, SO_2NH); 1420 (CH=CH): 1725 (>C=O) 1360 (SO_2NH) and other bands characteristic of the cinnamoylmorpholine and amino acid residues. The NMR spectra of (2-13) exhibit the chemical shifts at δ: 12.2 (1H, COOH); (8.7 – 8.2 aromatic and morpholine protons); 4.5 (CH=CH); 7.4 (1H, NH) and other protons assignable to the amino acid residues.

General procedure for the synthesis of cinnamoylmorpholine-4-sulfonylamino acid methyl esters (14-18). A solution of cinnamoylmorpholine-4-sulfonylamino acid (0.01 mole) in absolute methanol (80ccm) was cooled to –10° and pure thionyl chloride (0.011 mole) was added dropwise during 30 mins. The temperature of the mixture was kept below 0° during the addition of thionyl chloride. The reaction mixture was then stirred for additional 3-4 hrs at room temperature, kept overnight and the solvent was removed in vacuo. Methanol was added and re-evaporated several times and the residual solid material was recrystallized from methanol-water. The isolated methyl esters (14-18) were chromatographically homogeneous when developed with iodine or benzidine and hydroxamate reactions, and gave a negative ninhydrin test.

The IR spectra of the methyl esters (14-18) showed characteristic bands at: 1760, 1725 (>C=O); 1750 ($COOCH_3$) and other bands characteristic of the amino acid and cinnamoylmorpholine-4-sulfonyl residues. The NMR spectra show signals at δ: 4(3H, $COOCH_3$); 8.2 (1H, NH) and other signals in support of their assigned structures.

General procedure for the synthesis of cinnamoylmorpholine-4-sulfonylamino acid hydrazides (19-23). A solution of cinnamoylmorpholine-4-sulfonylamino acid methyl esters (0.001 mole) in absolute methanol (25 ccm) and hydrazine hydrate (85%, 0.003 mole) in methanol (25 ccm) was first kept for 24 hrs at 0°. The crystalline product was separated, collected, washed with cold water and recrystallized from ethanol-water. The hydrazides (19-23) were chromatographically homogeneous when detected with iodine or benzidine and gave negative hydroxamate reaction.

The IR spectra of the hydrazides (19-23) exhibited bands at: 3430, 3300 (NH, NH_2); 1550 ($CONHNH_2$), 3300, 1160 (SO_2NH) and other bands assignable to the cinnamoylmorpholine and amino acid moieties.

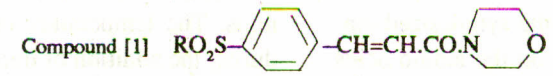
General procedure for the synthesis of cinnamoylmorpholine-4-sulfonyldipeptide methyl ester (24-30). Triethylamine (2 ccm) was added to a solution of amino acid methyl ester hydrochloride (0.0011 mole) in THF (50 ccm). The

TABLE 1. ANTIMICROBIAL ACTIVITY (A*) AND MINIMAL INHIBITORY CONCENTRATION (MIC) IN μg/ml OF THE BIOLOGICALLY ACTIVE COMPOUNDS.

Comp.	*Bac. cereus*		*Cand. utilis*		*Sar. lutea*		*Bac.spharicus*		*Pseud. seruginosa*	
	A	MIC	A	MIC	A	MIC	A	MIC	A	MIC
24	+++	220	++	340	++	440	++	415	+	475
25	++	325	++	410	–	–	–	–	–	–
26	+++	210	+++	210	–	–	+	495	–	–
27	+++	230	+++	345	+	500	+	500	+	470
28	–	–	++	410	+	500	–	–	–	–
29	++	360	++	400	+++	200	+	465	+++	270
30	+++	240	+++	360	+++	270	++	350	+++	210

(A)* +++ = highly active, ++ = moderately active, + = slightly, – = inactive.

TABLE 2. PHYSICAL DATA OF CINNAMOYLMORPHOLINE-4-SULFONYLAMINO ACIDS, METHYLESTERS, HYDRAZIDES, AND DIPEPTIDES DERIVATIVES (2-30).

Compound [1] 

No.	R	Yield (%)	m.p. (°C)	R_f TLC	Cryst.* solvent	Mol. formula	$[\alpha]_D^{20**}$	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
2	Gly	55	253-255	0.94	a	$C_{15}H_{18}N_2O_6S$	--	50.85	5.08	7.91	50.61	5.01	7.69
3	L-Ala	54	230-232	0.56	a	$C_{16}H_{20}N_2O_6S$	+13.1	52.17	5.43	7.61	52.11	5.22	7.44
4	DL-Ala	51	242-244	0.79	a	$C_{16}H_{20}N_2O_6S$	--	52.17	5.43	7.61	52.19	5.19	7.55
5	B-Ala	49	235-237	0.78	a	$C_{16}H_{20}N_2O_6S$	--	52.17	5.43	7.61	52.16	5.24	7.60
6	L-Val	44	215-217	0.70	a	$C_{18}H_{24}N_2O_6S$	+16.6	54.55	6.06	7.07	54.51	6.03	7.04
7	DL-Val	47	248-250	0.60	a	$C_{18}H_{24}N_2O_6S$	--	54.55	6.06	7.07	54.46	6.04	7.06
8	L-Ser	64	198-200	0.68	a	$C_{16}H_{20}N_2O_7S$	+17.1	50.00	5.21	7.29	50.00	5.18	7.19
9	L-Leu	53	232-234	0.73	a	$C_{19}H_{26}N_2O_6S$	+14.3	55.61	6.34	6.83	55.49	6.24	6.73
10	DL-Leu	62	235-237	0.75	a	$C_{19}H_{26}N_2O_6S$	--	55.61	6.34	6.83	55.55	6.21	6.75
11	L-Phe	66	244-246	0.71	a	$C_{22}H_{24}N_2O_6S$	+22.1	59.46	5.41	6.31	59.39	5.39	6.29
12	L-Tyr	59	208-210	0.57	a	$C_{22}H_{24}N_2O_7S$	+24.6	57.39	5.22	6.09	57.35	5.19	6.06
13	L-Pro	60	214-216	0.62	a	$C_{18}H_{22}N_2O_6S$	+25.6	54.96	5.60	7.12	54.69	5.44	7.13
14	Gly-OMe	63	143-145	0.64	a	$C_{16}H_{20}N_2O_6S$	--	52.17	5.43	7.61	52.29	5.41	7.60
15	DL-Ala-OMe	57	132-134	0.82	a	$C_{17}H_{22}N_2O_6S$	--	53.40	5.76	7.33	53.33	5.54	7.22
16	L-Val-OMe	66	115-117	0.86	a	$C_{19}H_{26}N_2O_6S$	+19.6	55.61	6.34	6.83	55.54	6.29	6.71
17	L-Leu-OMe	65	98-100	0.65	a	$C_{20}H_{28}N_2O_6S$	+8.0	56.60	6.60	6.60	56.55	6.49	6.59
18	DL-Leu-OMe	53	105-107	0.69	a	$C_{20}H_{28}N_2O_6S$	--	56.60	6.60	6.60	56.54	6.55	6.55
19	Gly-N_2H_3	46	175-177	0.71	b	$C_{15}H_{20}N_4O_5S$	--	48.91	5.43	15.22	48.79	5.39	15.16
20	DL-Ala-N_2H_3	51	185-187	0.48	b	$C_{16}H_{22}N_4O_5S$	--	50.56	5.76	14.66	50.49	5.69	14.56
21	L-Val-N_2H_3	44	70-72	0.44	b	$C_{18}H_{26}N_4O_5S$	--	52.68	6.34	13.66	52.58	6.29	13.58
22	L-Leu-N_2H_3	68	175-177	0.69	b	$C_{19}H_{28}N_4O_5S$	+8.5	53.77	6.60	13.21	53.71	6.60	13.16
23	DL-Leu-N_2H_3	74	192-194	0.53	b	$C_{19}H_{28}N_4O_5S$	--	53.77	6.60	13.21	53.70	6.58	13.20
24	Gly-L-Leu-OMe	75	166-168	0.81	a	$C_{27}H_{31}N_3O_7S$	+14.1	59.89	5.73	7.76	59.76	5.61	7.69
25	DL-Ala-DL-Ala-OMe	78	160-162	0.90	a	$C_{20}H_{27}N_3O_7S$	--	52.98	5.96	9.27	52.89	5.88	9.16
26	L-Ala-L-Leu-OMe	59	148-150	0.69	a	$C_{23}H_{33}N_3O_7S$	+12.5	55.76	6.67	8.48	55.67	6.66	8.41
27	L-Val-DL-Ala-OMe	53	155-157	0.61	a	$C_{22}H_{31}N_3O_7S$	+11.6	54.89	6.44	8.73	54.79	6.44	8.70
28	DL-Val-DL-Ala-OMe	56	140-142	0.37	a	$C_{22}H_{31}N_3O_7S$	--	54.89	6.44	8.73	54.82	6.43	8.71
29	L-Leu-L-Leu-OMe	72	160-162	0.54	a	$C_{26}H_{39}N_3O_7S$	+9.5	58.10	7.24	7.79	58.00	7.21	7.70
30	DL-Leu-L-Leu-OMe	77	179-181	0.60	a	$C_{26}H_{39}N_3O_7S$	+7.5	58.10	7.24	7.79	58.09	7.22	7.70

* Crystallization Solvent: a = Methanol-water, b = Ethanol-water. **optical rotation $[\alpha]_D^{20}$ (C = 5, in DMF at λ_{max} . 589 at 20°C)

solution was stirred at 20° for 30 min. and cooled to 0°. Cinnamoylmorpholine-4-sulfonyl amino acid (0.001 mole) in THF (20 ccm) and dicyclohexylcarbodiimide (DCC) (1.59g) were added to the above mixture. The reaction mixture was stirred for 2 hr. at 0°C and for another 2 hr. at room temperature. Dicyclohexylurea was filtered off, acetic acid (1 ccm) was added and the solution refiltered, the filtrate was evaporated in vacue. The residual solid was recrystallized from methanol-water. The products (24-30) were easily soluble in alcohols, DMF, and dioxane but insoluble in water and ether. The dipeptide were chromatographically homogeneous when detected with iodine or benzidine and gave negative ninhydrin reaction.

IR spectra of the dipeptide methyl esters (24-30) showed bands at: 3300, 3100 (NH, CONH, SO_2NH); 1750 (C=O); 1320 ($COOCH_3$) and other characteristic bands due to cinnamoylmorpholine and dipeptide moieties. The NMR spectra show signals at δ: 3.3 (3H, $COOCH_3$), 8 (1H, NH); 8.2-8.7 (aromatic and cinnamoylmorpholine residue) and other bands supporting its structures.

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