Synthesis and Biological Activity of Some New 3- and 6-Substituted Coumarin Amino Acid Derivatives. Part I

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The synthesis of 6-nitrocouarmin-3-CO-amino acids and their corresponding methyl esters (II-XVII) and some dipeptide methyl esters (XVIII-XXVI) are described. 6-(*N*-Tosyl- or *N*-phthalylaminoacyl)aminocoumarin-3-carboxylic acid methyl esters (XXXIV-XL) and 3-(*N*-phthalyl- or *N*-tosylaminoacyl)aminocoumarins (XLV-LVI) have been prepared *via* the carbodiimide and acid chloride methods. Hydrazinolysis of 3- or 6-(*N*-phthalylaminoacyl)aminocoumarin derivatives in tetraline gave the corresponding 3- and 6-(aminoacyl)aminocoumarins and the carboxylic acid hydrazides (XLI-LVIII), respectively. 3-(*N*-Tosyl-L-Val-L-Leu-)aminocoumarin (LIX) was synthesized *via* the azide method. Twenty four of various substituted 3- and 6-aminoacylcoumarin derivatives were found to possess specific antimicrobial activities towards different microorganisms.

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A number of coumarin derivatives are known to be physiologically active and some of them possess antibacterial and antifungal properties (2). Also, 3-pyridyl and 3-aminocoumarins have been reported to act as nervous system depressants (3) and antibacterial agents (4) respectively.

In view of the above observations and in continuation of our previous studies in the same field (5-7), we synthesized a new class of 6-nitrocoumarin-3-CO-amino acids and some of their corresponding methyl esters and dipeptide methyl esters (II-XXVI), 6-(*N*-tosyl- or *N*-phthalylaminoacyl)-aminocoumarin-3-carboxylic acid methyl esters (XXXIX-XL), 3-(*N*-phthalyl- or *N*-tosylaminacyl)aminocoumarins (XLV-LVI) and some of their corresponding aminoacyl and hydrazide derivatives (XLI-LVIII) and the dipeptide (LIX). These compounds were tested for biological activity.

The reaction of 6-nitrocoumarin-3-acid chloride (I) (8) with different amino acids and their methyl esters was conducted in dioxane containing triethylamine. The reaction mixture was stirred at room temperature followed by a brief refluxing till completion as monitored by tlc. 6-Nitrocoumarin-3-CO-amino acids (II-X) and their corresponding methyl esters (XI-XVII) were chromatographically homogeneous. Complete acid hydrolysis of II for 24 hours afforded glycine.

The ir spectrum of the methyl of 6-nitrocoumarin-3-CO-Gly (XI) in potassium bromide showed the characteristic bands at 3430, 3320 and 3080 (NH and CONH), 1740, 1650 and 1560 (α-pyrone), 1650, 1530 and 1280 (amide I, II and III), 1445, 1380 (-$COOCH_3$), 1690 ($>C=O$), 1640, 1490 and 1380 cm^{-1} (NO_2), thereby supporting the structure of XI. The nmr spectrum of XI in deuteriochloroform showed: δ 3.60 (s, 3H, OCH_3), 3.49 (s, 2H, CH_2), 5.56 (s, 1H, NH), 7.6-8.4 (s, 8H, aromatic protons). The uv spectrum of XI in ethanol showed: λ max (log ϵ) at 213 nm (4.65) and 296 nm (4.48) characteristic of the coumarin residue. The ir, uv and nmr spectra of the other compounds (II-XVII) had analogous peaks confirming their structures.

6-Nitrocoumarin-3-CO-dipeptide methyl esters (XVIII-XXVI) were prepared by the carbodiimide method. Coupling of 6-nitrocoumarin-3-CO-amino acids (II-X) with amino acid methyl ester hydrochlorides in dioxane or DMF containing triethylamine and using the dicyclohexylcarbodiimide (DCC) technique afforded dipeptides (XVIII-XXVI).

All dipeptide methyl esters (XVIII-XXVI) were highly purified through repeated recrystallizations and chromatographically homogeneous materials were obtained in 51-76% yields. E = zero for all dipeptides indicating high purity of the products. Ir, uv and nmr confirmed the identity of the dipeptide derivatives (XVIII-XXVI). The dipeptide methyl esters (XVIII-XXVI) gave deep blue 1:1 complexes with copper(II), λ max 650-670 nm.

The synthesis of 6-(*N*-phthalyl- or *N*-tosyl aminoacyl)-aminocoumarin-3-carboxylic acid methyl esters (XXIX-XL) was achieved through treatment of 6-aminocoumarin-3-carboxylic acid methyl ester (XXVII) (8) with the appropriate *N*-phthalylamino acid chloride or *N*-tosylamino acid chloride (9-11) in dioxane containing triethylamine. For the preparation of 3-(*N*-phthalyl- or *N*-tosylaminoacyl)-aminocoumarins (XLV-LVI), the appropriate *N*-phthalyl- or *N*-tosylamino acid was reacted with 3-aminocoumarin (XXVIII) (8) in dioxane-DMF-triethylamine medium using the DCC procedure. All the products (XXIX-XL and XLV-LVI) were obtained in crystalline form in 46-91% yield and gave chromatographically homogeneous spots.

The ir spectrum of 3-(*N*-phthalyl-Gly)aminocoumarin (XLV) in potassium bromide showed the characteristic bands at 3360, 3320 and 3140 (NH and CONH), 1690 ($>C=O$), 1740, 1650, 1560 (α pyrone), 2960, 2840 (-CH_2), 1650, 1540, 1280 (amide I, II and III) and other

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characteristic bands due to the coumarin and phthalylamino acid moieties, thereby supporting the structure of XLV. The uv spectrum of XLV in ethanol showed; λ max (log ε) at 225 nm (4.27) and 292 nm (4.86), characteristic of the coumarin residue. The nmr spectrum of XLV in DMSO-d_6 showed: δ 7.4-8.4 (s, 9H, aromatic protons), 3.49 (s, 2H, CH_2), 5.6 (s, 1H, NH). Ir, uv and nmr studies on the derivatives (XXIX-XL and XLV-LVI) confirmed their structures.

Treatment of the 6-(*N*-phthalylaminoacyl)aminocoumarin-3-carboxylic acid methyl esters (XXIX-XXXIV) with a 0.5*M* (or 1*M*) solution of hydrazine hydrate in ethanol at 20° (or even at 0°) for 1-2 hours lead to the destruction of the α-pyrone ring as indicated by the uv data. However, when compounds XXIX-XXXIV were refluxed at 45° with a 0.5*M* hydrazine hydrate in tetraline, the desired hydrazides (XLI-XLIV) were obtained in 60-85% yield, and no side reactions were observed. The stability of the α-pyrone ring was established by the uv, ir and nmr spectra, elemental analysis and chromatographic studies.

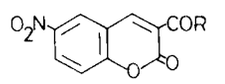
Similarly, treatment of 3-(*N*-phthalylaminoacyl)aminocoumarins (XLV-L) with 0.5*M* or 1*M* solutions of hydrazine hydrate in ethanol for 2-3 hours at 30° led to rupture of the α-pyrone ring (uv spectrum). However, hydrazinolysis could be achieved by conducting the reaction with 0.5*M* hydrazine hydrate in tetraline or a dioxane-ethanol (3:1) mixture under mild reflux affording 3-(aminoacyl)aminocoumarins (LVII-LVIII). Chromatographic and electrophoretic studies on LVII-LVIII revealed their homogenity and their structures were supported by the uv and ir spectral data.

The uv spectra of compounds XLI-L in ethanol showed: λ max (log ε) at 218-220 nm (4.84-4.87) and 293-295 nm (4.93-4.97), characteristic of the coumarin residue.

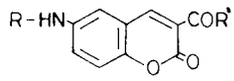
Synthesis of the dipeptide (LIX) was achieved starting from the hydrazide tosyl-L-Val-L-Leu-N_2H_3, which was converted into the corresponding azide. The azide on coupling with 3-aminocoumarin (XXVIII) furnished the dipeptide (LIX) which was isolated and purified in the usual manner (12). Elemental analysis, chromatographic studies, ir, uv and nmr spectra confirmed the structure of LIX.

Table I

Physical Data of Various 3- and 6-Substituted Coumarin-Amino Acid Derivatives (II-LIX) (a)



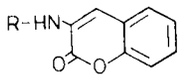
(Compounds Type A)



(Compounds Type B)

Compound No.	R	Yield %	M.p. °C	R_f	$[\alpha]_D^{26}$	Molecular Formula	Elemental analysis, % Calcd. C	H	N	Found C	H	N
					Compounds (II-XXVI) Type (A)							
II	-Gly	49	183-185	0.80	----	$C_{12}H_8N_2O_7$	49.32	2.73	9.58	49.45	2.81	9.84
III	-L-Ser	59	155-157	0.45	+20.0 (c, 6.3)	$C_{13}H_{10}N_2O_8$	48.44	3.10	8.69	48.50	3.00	8.71
IV	-L-Thr	54	65-67	0.66	+16.5 (c, 6.5)	$C_{14}H_{12}N_2O_8$	50.00	3.57	8.33	50.09	3.55	8.37
V	-L-Val	69	88-90	0.63	−38.0 (c, 6.5)	$C_{15}H_{14}N_2O_7$	53.89	4.19	8.38	53.80	4.25	8.41
VI	-L-Leu	59	195-197	0.91	+22.5 (c, 6)	$C_{16}H_{16}N_2O_7$	55.17	4.59	8.04	55.20	4.58	8.09
VII	-DL-nor-Leu	49	190-192	0.92	----	$C_{16}H_{16}N_2O_7$	55.17	4.59	8.04	55.16	4.61	8.09
VIII	-L-Phe	46	180-182	0.86	+130.5 (c, 6.5)	$C_{19}H_{14}N_2O_7$	59.68	3.66	7.32	60.01	3.65	7.35
IX	-L-Tyr	50	165-167	0.52	+39.0 (c, 6.8)	$C_{19}H_{14}N_2O_8$	57.28	3.51	7.03	57.16	3.77	7.03
X	-L-Try	50	165-167	0.52	+39.0 (c, 6.8)	$C_{21}H_{15}N_3O_7$	59.85	3.56	9.97	59.88	3.60	9.95
XI	-Gly-OMe	59	65-67	0.70	----	$C_{13}H_{10}N_2O_7$	50.98	3.23	9.15	50.99	3.34	9.20
XII	-L-Ser-OMe	62	127-129	0.86	−32.5 (c, 6)	$C_{14}H_{12}N_2O_8$	50.00	3.57	8.33	50.02	3.50	8.35
XIII	-β-Ala-OMe	72	140-142	0.77	----	$C_{14}H_{12}N_2O_7$	52.50	3.75	8.75	52.53	3.77	8.76
XIV	-DL-nor-Leu-OMe	59	136-138	0.78	----	$C_{17}H_{18}N_2O_7$	56.35	4.97	7.73	56.35	4.95	7.88
XV	-L-Phe-OMe	58	155-157	0.75	+16.5 (c, 6.5)	$C_{20}H_{16}N_2O_7$	60.60	4.04	7.07	60.59	4.08	7.08
XVI	-L-Tyr-OMe	63	120-122	0.90	+20.0 (c, 6)	$C_{20}H_{16}N_2O_8$	58.25	3.88	6.79	58.27	3.90	6.80
XVII	-L-Try-OMe	43	115-117	0.67	+80.5 (c, 6.7)	$C_{22}H_{17}N_3O_7$	60.68	3.90	9.65	60.95	3.89	9.71
XVIII	-Gly-DL-Ser-OMe	54	255-257	0.85	----	$C_{16}H_{15}N_3O_9$	48.85	3.82	10.68	48.80	3.90	10.69
XIX	-L-Val-DL-Ser-OMe	53	190-192	0.73	+144.0 (c, 6)	$C_{19}H_{21}N_3O_9$	52.41	4.82	9.65	52.45	4.86	9.70
XX	-Gly-L-Phe-OMe	60	168-170	0.94	+28.5 (c, 6.5)	$C_{22}H_{19}N_3O_8$	58.27	4.19	9.27	58.25	4.20	9.30
XXI	-Gly-L-Tyr-OMe	68	180-182	0.64	+37.5 (c, 6)	$C_{22}H_{19}N_3O_9$	56.28	4.05	8.95	56.30	4.06	8.96
XXII	-L-Tyr-DL-Ser-OMe	76	148-150	0.43	+89.0 (c, 6.8)	$C_{23}H_{21}N_3O_{10}$	55.31	4.21	8.42	55.34	4.22	8.47
XXIII	-L-Val-L-Phe-OMe	62	178-180	0.72	−17.5 (c, 6)	$C_{23}H_{23}N_3O_8$	60.60	5.05	8.48	60.62	5.06	8.47
XXIV	-L-Val-L-Tyr-OMe	59	201-203	0.54	+22.3 (c, 6.5)	$C_{23}H_{23}N_3O_9$	58.70	4.89	8.21	58.71	4.87	8.20
XXV	-L-Tyr-L-Phe-OMe	59	190-192	0.70	−18.7 (c, 6.6)	$C_{29}H_{25}N_3O_9$	62.25	4.47	7.51	62.26	4.50	7.50
XXVI	-L-Tyr-L-Tyr-OMe	54	120-122	0.62	+25.5 (c, 6)	$C_{29}H_{25}N_3O_{10}$	60.52	4.34	7.30	60.55	4.37	7.35

Compounds of the Type B: For Compounds XXIX-XL R' = OCH_3 and for Compounds XLI-XLIV, R' = N_2H_3



(Compounds Type C)

XXIX	Pht-Gly-	67	135-137	0.53	---	$C_{21}H_{14}N_2O_7$	62.06	3.45	6.89	62.09	3.50	6.93
XXX	Pht-β-Ala-	62	99-101	0.72	---	$C_{22}H_{16}N_2O_7$	62.85	3.81	6.66	62.86	3.83	6.69
XXXI	Pht-L-Ala-	91	94-96	0.75	+22.5 (c, 6.8)	$C_{22}H_{16}N_2O_7$	62.85	3.81	6.66	62.84	3.97	6.67
XXXII	Pht-L-Val-	75	80-82	0.63	+33.6 (c, 6)	$C_{24}H_{20}N_2O_7$	64.28	4.46	6.25	64.29	4.48	6.30
XXXIII	Pht-L-Leu-	51	75-77	0.34	+19.8 (c, 6)	$C_{25}H_{22}N_2O_7$	64.93	4.76	6.06	64.99	4.75	6.07
XXXIV	Pht-L-Phe-	55	160-162	0.74	−23.5 (c, 6.5)	$C_{28}H_{20}N_2O_7$	67.74	4.03	5.64	67.75	4.04	5.69
XXXV	Tos-Gly-	52	120-122	0.54	---	$C_{20}H_{18}N_2O_7S$	55.81	4.18	6.51	55.93	4.34	6.58
XXXVI	Tos-β-Ala-	71	165-167	0.80	---	$C_{21}H_{20}N_2O_7S$	56.75	4.50	6.31	56.80	4.51	6.30
XXXVII	Tos-L-Ala-	60	151-153	0.79	+19.5 (c, 6)	$C_{21}H_{20}N_2O_7S$	56.75	4.50	6.31	56.77	4.49	6.32
XXXVIII	Tos-L-Val-	57	80-82	0.84	+35.5 (c, 6.8)	$C_{23}H_{24}N_2O_7S$	58.47	5.08	5.93	58.54	5.19	6.09
XXXIX	Tos-L-Leu-	45	175-177	0.67	−16.0 (c, 6)	$C_{24}H_{26}N_2O_7S$	59.25	5.35	5.76	59.30	5.39	5.79
XL	Tos-L-Phe-	54	105-107	0.59	+20.5 (c, 6.5)	$C_{27}H_{24}N_2O_7S$	62.30	4.62	5.38	62.31	4.66	5.47
XLI	L-Ala (HCl)-	83	250-252	0.29	+119 (c, 2.3 CH_3OH)	$C_{13}H_{15}ClN_4O_4$	47.77	4.59	17.15	48.01	4.63	17.41
XLII	L-Val (HCl)-	81	270-272	0.31	+216 (c, 2.2 CH_3OH)	$C_{15}H_{19}ClN_4O_4$	50.77	5.35	15.79	50.85	5.41	16.01
XLIII	L-Leu (HCl)-	85	299-301	0.29	+200 (c, 2.3 CH_3OH)	$C_{16}H_{21}ClN_4O_4$	52.10	5.69	15.19	52.31	5.71	15.21
XLIV	L-Phe (HCl)-	79	220-222	0.38	−69 (c, 3.5 CH_3OH)	$C_{19}H_{19}ClN_4O_4$	56.64	4.72	13.91	56.68	4.79	13.99
					Compounds XLV-LIX of the Type C							
XLV	Pht-Gly-	60	250-252	0.83	---	$C_{19}H_{12}N_2O_5$	65.51	3.44	8.04	65.83	3.62	8.45
XLVI	Pht-L-Ala-	46	170-172	0.70	−18.9 (c, 7.1)	$C_{20}H_{14}N_2O_5$	66.29	3.86	7.73	66.30	3.89	7.86
XLVII	Pht-β-Ala-	55	180-182	0.79	---	$C_{20}H_{14}N_2O_5$	66.29	3.86	7.73	66.45	3.88	7.79
XLVIII	Pht-L-Val-	45	177-179	0.90	+22.5 (c, 5)	$C_{22}H_{18}N_2O_5$	67.69	4.61	7.17	67.82	4.70	7.19
XLIX	Pht-L-Leu-	54	200-202	0.93	+14.5 (c, 4.3)	$C_{23}H_{20}N_2O_5$	68.31	4.95	6.93	68.52	4.98	6.99
L	Pht-L-Phe-	48	182-184	0.85	−20.5 (c, 5.1)	$C_{26}H_{18}N_2O_5$	71.23	4.10	6.39	71.42	4.23	6.38
LI	Tos-Gly-	43	162-164	0.80	---	$C_{18}H_{16}N_2O_5S$	58.06	4.30	7.52	58.15	4.38	7.56
LII	Tos-L-Ala-	50	215-217	0.66	−16.5 (c, 9.1)	$C_{19}H_{18}N_2O_5S$	59.07	4.66	7.25	59.21	4.68	7.32
LIII	Tos-DL-Ala-	64	187-189	0.59	---	$C_{19}H_{18}N_2O_5S$	59.07	4.66	7.25	59.09	4.78	7.35
LIV	Tos-β-Ala-	53	178-180	0.89	---	$C_{19}H_{18}N_2O_5S$	59.06	4.66	7.25	59.31	4.69	7.28
LV	Tos-DL-Val-	48	200-202	0.70	---	$C_{21}H_{22}N_2O_5S$	60.86	5.31	6.76	60.89	5.40	6.68
LVI	Tos-DL-Ser-	50	174-176	0.74	---	$C_{19}H_{18}N_2O_6S$	56.71	4.47	6.96	56.80	4.49	6.99
LVII	β-Ala-	65	160-162	0.93	---	$C_{12}H_{12}N_2O_5$	62.06	5.17	12.06	62.23	5.27	12.35
LVIII	L-Val-	77	140-142	0.95	+14.5 (c, 4.9)	$C_{14}H_{16}N_2O_5$	64.61	6.15	10.76	64.73	6.23	10.82
LIX	Tos-L-Val-L-Leu-	62	165-167	0.73	+17.8 (c, 5.6)	$C_{26}H_{33}N_3O_6S$	64.59	6.83	8.69	64.72	6.92	8.78

(a) Electrophoretic mobilities (E) for compounds: (II) = 9.5 cm, (III) = 7 cm, (IV) = 13.5 cm, (V) = 6.5 cm, (VI) = 11 cm, (VII) = 10.5 cm, (VIII) = 12.5 cm, (IX = 8.5 cm, (X) = 14.5 cm, (XLI-XLIV & LVII & LVIII) = 7.5-8 cm, E = zero for the remaining compounds.

The dipeptide (LIX) gave a deep blue 1:1 complex with copper(II), λ max 670 nm.

Biological Screening Results.

The antimicrobial activity of the synthesized compounds were determined according to the procedure described earlier (13-16), and the results compared with the activity of the starting amino- and nitrocoumarin (I, XXVII and XXVIII) derivatives.

6-Nitrocoumarin-3-CO-Gly (II) and the corresponding L-Ser (III) derivative were found to possess high antimicrobial activities against *Bacillus subtilis, Bacillus cereus, Bacillus mycoids* and *Penicillum chrysogenum* and inactive against *Escherichia coli* and *Salmonella typhosa.* 6-(*N*-tosyl-L-Phe)aminocoumarin-3-carboxylic acid methyl ester (XL) produced antimicrobial effects and gave promising results against *Bacillus subtilis, Bacillus cereus, Bacillus mycoids* and *Escherichia coli.* 3-(L-Val)aminocoumarin (LVIII) had a marked growth inhibitory effect against *Bacillus subtilis, Bacillus mycoids, Escherichia coli* and *Salmonella typhosa.* 3-(*N*-Tosyl-L-Ala)aminocoumarin (LII) and the corresponding DL-Ala (LIII) derivative and 6-(*N*-phthalyl-L-Ala)aminocoumarin-3-carboxylic acid methyl ester (XXXI) and the corresponding *N*-Phthalyl-L-Val (XXXII) derivatives were found to possess high antimicrobial activity against *Bacillus subtilis, Bacillus mycoids* and *Bacillus cereus.* 6-Nitrocoumarin-3-CO-L-Val (V) and 3-(*N*-tosyl-Gly)aminocoumarin (LI) were found to have a marked growth inhibitory effect against *Bacillus subtilis* and *Bacillus cereus* only. 3-(*N*-Phthalyl-L-Ala)aminocoumarin (XLVI) and the corresponding *N*-tosyl-DL-Ser- (LVI) derivatives produced high antimicrobial effects against *Bacillus subtilis* and *Bacillus mycoids.* 6-Nitrocoumarin-3-CO-L-Tyr (IX) and the corresponding -β-Ala-OMe (XIII) derivatives were found to possess high antimicrobial activities against *Bacillus subtilis* and *Penicillum chrysogeneum.*

6-(*N*-Phthalyl)-L-Phe)aminocoumarin-3-carboxylic acid

methyl ester (XXXIV) was found to be active against *Bacillus cereus* and *Bacillus mycoids*. 3-(*N*-Phthalyl-Gly)-aminocoumarin (XLV) and the corresponding *N*-phthalyl-β-Ala (XLVII), *N*-Phthalyl-L-Leu (XLIX) and *N*-Tosyl-DL-Val (LV) derivatives and 6-nitrocoumarin -3-CO-L-Leu (VI) and 6-(*N*-phthalyl-L-Leu)-aminocoumarin-3-carboxylic acid methyl ester (XXXIII) and the corresponding *N*-phthalyl-β-Ala- (XXX) derivatives were found to possess high antimicrobial activities against *Bacillus subtilis* and inactive towards the remaining microoorganisms. 3-(*N*-Phthalyl-L-Val)aminocoumarin (XLVIII) and 6-nitrocoumarin-3-CO-Gly-L-Phe-OMe (XX) were found to be active against *Bacillus mycoids* only. The remaining amino acid and peptide derivatives were inactive towards all the tested microorganisms.

The present investigation revealed that introduction of nitro- or amino- substitutents in the 3- and 6-positions in the coumarin residue in combination with *N*-phthalyl- or *N*-tosyl-aminoacyl and aminoacyl moieties gave coumarin amino acid derivatives of highly specific biological properties. The L-Val, L-Ala, L-Phe, L-Ser, and L-Leu derivatives were found to possess high antimicrobial properties when compared with the corresponding Gly- and β-Ala-derivatives or with their racemic modifications. The phthalyl- and tosyl- protecting groups did not affect the biological activity of these compounds, since hydrazinolysis of the phthalyl group did not enhance or verify the biological properties of most unprotected aminoacyl derivatives (*cf.*, compounds XLI-LVII).

Other pharmacological studies are currently in progress.

EXPERIMENTAL

All melting points are uncorrected. All thin-layer chromatography was done on Silica Gel-G plates using benzene-ethyl acetate (1:1) as a solvent system and iodine-potassium iodide (20%) as a detection reagent. Benzidine, ninhydrin and silver nitrate reactions were used for detection of the amino acid derivatives on paper chromatograms (spot reactions) (12). The electrophoretic mobilities (E) were measured with high voltage electrophoresis 1000 V, 2 hours in pyridine-acetate buffer (*p*H 5.6) (12). The uv spectra were taken in ethanol with a Unicam SP 8000 and the ir spectra were taken with a Unicam SP 1200 in potassium bromide. The nmr data were measured on a Varian T-60A spectrometer and shifts are reported in ppm (δ) relative to internal TMS. $[\alpha]_D^{20}$ were taken in Zeiss polarimeter, 1 dm tube in DMF.

6-Nitrocoumarin-3-carboxyl Chloride (I).

This compound was prepared from 6-nitrocoumarin-3-carboxylic acid using the procedure described in the literature (8).

General Procedure for the Synthesis of 6-Nitrocoumarin-3-CO-amino Acids or Amino Acid Methyl Esters (II-XVII).

6-Nitrocoumarin-3-carboxyl chloride (I, 0.9 g, 0.0035 mole) was dissolved in dioxane (20 ml) and added to a solution of the appropriate amino acid or its methyl ester hydrochloride (0.0039 mole) in dioxane (25 ml) containing triethylamine (3 ml). The reaction mixture was stirred at room temperature followed by refluxing (1-2 hours) until completion of the reaction as checked by tlc. After cooling the reaction mixture, triethylammonium chloride was filtered and a benzene-ether mixture (250 ml) (1:1) was added. The reaction mixture was washed with water, 10% sodium bicarbonate, water and dried over sodium sulphate. Evaporation of the solvent *in vacuo* gave solid products which were recrystallized from ethanol, methanol, water, dioxane or their mixtures. Compounds II-XVII were chromatographically homogeneous when developed with benzidine-iodine solution and all gave negative test with ninhydrin.

General Procedure for the Synthesis of 6-Nitrocoumarin-3-CO-dipeptide Methyl Esters (XVIII-XXVI).

The 6-nitrocoumarin-3-CO-amino acid (0.014 mole) and the appropriate amino acid methyl ester hydrochloride (0.016 mole) were dissolved in a mixture of dioxane (60 ml) and DMF (10 ml) containing triethylamine (2.5 ml). The mixture was cooled to 0-5°, dicyclohexylcarbodiimide (2.7 g, 0.014 mole) was added and the mixture was stirred for 3-4 hours at 0° and then left for 24 hours at room temperature. Dicyclohexylurea was filtered and the filtrate was evaporated *in vacuo*. The residual solid was recrystallized from methanol, ethanol, acetone, DMF or their mixtures. The dipeptides (XVIII-XXVI) were easily soluble in alcohols, DMF, nitromethane and dioxane and insoluble in water, ether and petroleum ether. The dipeptides were chromatographically homogeneous when detected with benzidine-iodine solution and hydroxamate reactions.

6-Aminocoumarin-3-carboxylic Acid Methyl Ester (XXVII) and 3-Aminocoumarin (XXVIII).

These compounds were synthesized using the procedure described in the literature (8,17).

General Procedure for the Synthesis of 6-(*N*-Phthalyl- or *N*-Tosylaminoacyl)aminocoumarin-3-carboxylic Acid Methyl Esters (XXIX-XL).

6-Aminocoumarin-3-carboxylic acid methyl ester (XXVII, 0.01 mole) was dissolved in dioxane (150 ml) containing triethylamine (10 ml). The mixture was then treated with the *N*-phthalylamino acid chloride or the *N*-tosylamino acid chloride (0.012 mole) in dioxane (200 ml). The reaction mixture was stirred at room temperature followed by reflux (2-3 hours) till completion as monitored by tlc. The reaction mixture was worked up as described for synthesis of II-XVII. Compounds XXIX-XL were recrystallized from methanol, acetone, ethanol or their mixtures with water. All the products were chromatically homogeneous when developed with benzidine-iodine solution and hydroxamate reactions.

General Procedure for Synthesis of 3-(*N*-Phthalyl- or *N*-Tosylaminoacyl)-aminocoumarins (XLV-LVI).

To a stirred suspension of 3-aminocoumarin (XXVIII, 0.029 mole) in a mixture of dioxane (45 ml) and dimethylformamide (15 ml) containing triethylamine (4.5 ml) at −5° was added *N*-tosyl- or *N*-phthalylamino acid (0.029 mole) in dioxane (25 ml) followed by DCC (5.4 g). The reaction mixture was stirred for 2-3 hours at 0° and left for 24 hours at room temperature. The reaction mixture was worked up as described for the preparation of XVIII-XXVI. Compounds XLV-LVI were recrystallized from ethanol, acetone, DMF or their mixtures. The products (XLV-LVI) were found to be homogeneous (PC detection with benzidine) and showed negative ninhydrin and silver nitrate reactions.

Reaction Procedure for the Synthesis of 6-(Aminoacyl)aminocoumarin-3-carboxylic Acid Hydrazides (XLI-XLIV) and 3-(Aminoacyl)aminocoumarins (LVII-LVIII).

The appropriate 6-(*N*-phthalylaminoacyl)aminocoumarin-3-carboxylic acid methyl ester (XXIX-XXXIV, 0.003 mole) or 3-(*N*-phthalylaminoacyl)aminocoumarin (XLV-L, 0.003 mole) was dissolved in tetraline (25 ml) and the mixture was then treated with 0.5*M* hydrazine hydrate in ethanol (9 ml). The reaction mixture was refluxed for 15 minutes-1 hour and the rate of the reaction was checked by tlc. The residue obtained after evaporation of the solvent was treated with 2*N* hydrochloric acid (30 ml) for 10 minutes at 50°. The reaction mixture was cooled and the insoluble phthalyl-hydrazide was filtered. The filtrate was evaporated *in vacuo* and the residual material was dissolved in ethyl acetate (100 ml) and treated with triethylamine (10 ml) for 2 hours. The reaction mixture

was washed with water, sodium bicarbonate (3%), water and dried over sodium sulfate. The solvent was evaporated *in vacuo* and the residual material was recrystallized several times from ethanol or methanol. The products (XLI-XLIV and LVII-LVIII) gave a positive ninhydrin reaction.

3-(*N*-Tosyl-L-Val-L-Leu)aminocoumarin (LIX).

N-Tosyl-L-Val-L-Leu-N_2H_3 (4.77 g, 0.012 mole) was dissolved in a mixture of acetic acid (20 ml), concentrated hydrochloric acid (30 ml) and water (100 ml). The mixture was cooled to $-5°$, and a solution of sodium nitrite (2.7 g) in water (15 ml) was added to it. The dipeptide azide was extracted with 120 ml of ethyl acetate, and washed and dried as described earlier (12). The azide was coupled with 3-aminocoumarin (XXVIII, 0.013 mole) in ethyl acetate (120 ml) and the reaction mixture worked up as described earlier (12). The dipeptide was recrystallized from ethanol. The product (LIX) was found to be homogeneous and showed negative ninhydrin and silver nitrate reactions.

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