Synthesis and Biological Activity of Some New Aminoacylcarbazole Derivatives. Part I.

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The syntheses of different 9-(*N*-phthalyl- or *N*-tosyl- or free aminoacyl)carbazoles and the corresponding derivatives of 3,6-dinitrocarbazoles and some derivatives of 3,6-diamino-9-(*N*-phthalylaminoacyl)carbazoles (II-XXXII) are described. Compounds VIII, XIII-XVII and XXIII-XXVII were found to be active against a number of microorganisms.

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The carbazole group of naturally occurring compounds has aroused in the past twenty years considerable interest on account of various aspects of biological activity (2-7). It therefore seemed desirable to synthesize some aminoacylcarbazole derivatives which may have interesting and improved biological effects. In continuation of our work on the synthesis of heterocyclic amino acid derivatives (8-11), the synthesis of some 9-(*N*-phthalyl- or *N*-tosyl-aminoacyl or free aminoacyl)carbazoles and 3,6-dinitro- or 3,6-diamino-9-(*N*-phthalyl- or *N*-tosylaminoacyl or free aminoacyl)carbazoles (II-XXXII) is described in this paper.

For preparation of 9-(*N*-phthalyl- or *N*-tosylaminoacyl)-carbazole or the corresponding 3,6-dinitrocarbazole derivatives (II-XII and XIX-XXVI), the appropriate *N*-phthalyl- or *N*-tosylamino acid was reacted with 9*H*-carbazole (I) (12-13) or 3,6-dinitro-9*H*-carbazole (XVIII) (14-15) in THF-triethylamine medium (or in the absence of triethylamine (13-14) using the dicyclohexylcarbodiimide (DCC) procedure. All the products (II-XII and XIX-XXVI) were obtained in crystalline form in 55-65% yield and all were chromatographically homogeneous.

Alternatively, coupling of the acid chlorides of *N*-tosyl- or *N*-phthalylamino acids with 9*H*-carbazoles or 3,6-dinitro-9*H*-carbazole (I or XVIII) in dioxane or DMF-triethylamine medium gave the products II-XII and XIX-XXVI with the same melting points, R_f and $[\alpha]_D^{20}$ as those obtained by the DCC procedure. The products obtained by this method needed several recrystallizations (yield 16-28%). In general, the DCC method gave purer products with higher yield and hence was preferred.

Hydrazinolysis of 9-(*N*-phthalylaminoacyl)carbazoles (II-VII) and 3,6-dinitro-9-(*N*-phthalylaminoacyl)carbazole (XXI) with 1*M* hydrazine hydrate in ethanol under mild reflux afforded 9-(aminoacyl)carbazoles (XIII-XVII) and 3,6-dinitro-9-(L-Ala)carbazole (XXVII), respectively. Chromatographic and electrophoretic studies on XIII-XVII and XXVII revealed their homogenity (positive ninhydrin reaction, $E_{(start)}$ = zero, $E_{(XIII-XVII\ and\ XXVII)}$ = 11.5-17 cm, *cf*, Table 1), and their structures were convincingly supported by the ir, uv, and nmr spectral data. The structures were further confirmed by their complete acid hydrolysis (6*N* hydrochloric acid, 24 hours) affording the corresponding amino acids. Treatment of 3,6-dinitro-9-(*N*-phthalylaminoacyl)carbazoles (XX-XXIV) with Tin-hydrochloric acid gave 3,6-diamino-9-(*N*-phthalylaminoacyl)carbazole hydrochlorides which on treatment with sodium hydrogen carbonate affored the desired 3,6-diamino-9-(*N*-phthalylaminoacyl)carbazoles (XXVIII-XXXII) as white crystalline materials (yield 50-55%).

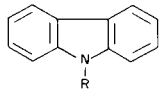
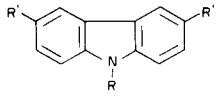
Compounds II-XXXII were prepared and characterized for the first time. All of the compounds which were synthesized (II-XXXII) gave ir, uv and nmr spectra consistent with their assigned structures.

Biological Screening Results.

The antimicrobial activity of the compounds which were synthesized were tested using the hole plate and filter paper disc methods (16-19). The results were compared with the activity of the parent compounds (I and XVIII). 9-(*N*-Tos-Gly)carbazole (VIII) showed a maximum activity at (MIC 25 μg/ml) against *Bacillus subtilis* (ICC-Strain) and inactive against *Bacillus mycoids* (USSR), *Bacillus cereus* (NRRL-B-569), *Salmonella typhosa* (NRRL-B-573), *Escherichia coli* (NRRL-B-210) and *Penicillum chrysogenum* (500 υg/ml). In addition, 3,6-dinitro-9-(*N*-Pht-L-Leu)carbazole (XXIII) showed marked antibacterial activity as compared to XVIII against *Bacillus subtilis* with MIC 10 μg/ml and inactive against the remaining microorgainsms. 3,6-Dinitro-9-(*N*-Pht-DL-Phe)carbazole (XXIV) was found to be active (MIC 50 μg/ml) against *Escherichia coli* only. 3,6-Dinitro-9-(*N*-Tos-B-Ala)carbazoles (XXV) and the corresponding *N*-Tos-DL-Phe (XXVI) and L-Ala (XXVII) derivatives were found to be active aganist *Bacillus subtilis* and *Bacillus cereus* with MIC values ranging from 25-50 μg/ml, and inactive against *Bacullus mycoids, Escherichia coli, Salmonella typhosa* and *Pencillum chroysogenum* (MIC 250-500 μg/ml). All 9-(aminoacyl)carbazoles (XIII-XVII) showed activities against *Bacillus subtilis* with MIC values ranging from 125-250 μg/ml and inactive against all the remaining microorganisms (MIC 250-500 μg/ml). None of the 3,6-diamino-9-(*N*-phthalylaminoacyl)carbazole derivatives (XXVIII-XXXII) showed any significant activity (MIC 250-500 μg/ml) against all

Table 1

Physical Data of Various 9-(*N*-Phthalyl or *N*-Tosyl-aminoacyl or Free Aminoacyl)carbazoles (II-XVII), 3,6-Dinitro-9-(N-Phthalyl or *N*-Tosyl-aminoacyl or Free Aminoacyl)carbazoles (XIX-XXVII) and 3,6-Diamino-9-(*N*-Phthalylaminoacyl)carbazoles (XXVIII-XXXII)

Compound No.	R-	Yield (a) (%)	mp °C	R_f	$[\alpha]_D^{20}$ (b)	Molecular formula	Elemental Analysis % Calcd. C	H	N	Found C	H	N
						Compounds II-XVII of the Type (A)						
												
						(Compounds II – XVII) – A						
II	Pht-Gly	60	135-137	0.45	—	$C_{22}H_{14}N_2O_3$	74.58	3.95	7.91	74.69	4.14	7.96
III	Pht-β-Ala	65	165-167	0.48	—	$C_{23}H_{16}N_2O_3$	75.00	4.34	7.61	75.01	4.64	7.75
IV	Pht-L-Ala	61	190-192	0.46	+50.5 (c, 2.5 A)	$C_{23}H_{16}N_2O_3$	75.00	4.34	7.61	75.02	4.42	7.96
V	Pht-L-Val	63	151-153	0.53	+52 (c, 3 A)	$C_{25}H_{20}N_2O_3$	75.76	5.05	7.07	75.92	5.11	7.23
VI	Pht-L-Leu	62	170-172	0.57	+46 (c, 3 A)	$C_{26}H_{22}N_2O_3$	76.10	5.37	6.35	76.19	5.51	6.85
VII	Pht-DL-Phe	64	158-160	0.59	—	$C_{29}H_{20}N_2O_3$	78.38	4.50	6.31	78.41	4.71	6.32
VIII	Tos-Gly	60	186-188	0.54	—	$C_{21}H_{18}N_2O_3S$	66.67	4.76	7.41	67.03	5.07	7.42
IX	Tos-β-Ala	55	189-191	0.57	—	$C_{22}H_{20}N_2O_3S$	67.35	5.10	7.14	67.61	5.15	7.22
X	Tos-L-Val	62	204-206	0.59	+55 (c, 2.5 A)	$C_{24}H_{24}N_2O_3S$	68.57	5.71	6.67	68.49	6.01	6.69
XI	Tos-L-Leu	57	198-200	0.62	+50 (c, 2.5 A)	$C_{25}H_{26}N_2O_3S$	69.12	5.99	6.45	69.15	6.00	6.51
XII	Tos-DL-Phe	55	178-180	0.66	—	$C_{28}H_{24}N_2O_3S$	71.80	5.13	5.98	72.14	5.16	6.10
XIII	β-Ala (c)	60	160-162	0.40	—	$C_{15}H_{14}N_2O$	75.63	5.88	11.76	75.82	5.89	11.81
XIV	L-Ala	58	176-178	0.43	+74 (c, 3 B)	$C_{15}H_{14}N_2O$	75.63	5.88	11.76	75.83	5.91	11.79
XV	L-Val	62	144-146	0.48	+64.5 (c, 3 B)	$C_{17}H_{18}N_2O$	76.69	6.77	10.53	76.97	6.82	10.60
XVI	L-Leu	64	180-182	0.51	+54.2 (c, 3 B)	$C_{18}H_{20}N_2O$	77.14	7.14	10.00	77.16	7.24	9.98
XVII	DL-Phe	55	210-212	0.54	—	$C_{21}H_{18}N_2O$	80.26	5.73	8.92	80.30	5.78	8.98
						Compounds XIX-XXVII (R′ = NO_2) and Compounds XXVIII-XXXII (R′ = NH_2) of the Type (B)						
												
						(Compounds XIX-XXXII) – B						
XIX (d)	Pht-Gly	54	301-303	0.62	—	$C_{22}H_{12}N_4O_7$	59.46	2.70	12.61	59.63	2.97	12.70
XX	Pht-β-Ala	56	183-185	0.65	—	$C_{23}H_{14}N_4O_7$	60.26	3.06	12.23	60.30	3.36	12.39
XXI	Pht-L-Ala	58	216-218	0.66	+37.7 (c, 2.5 A)	$C_{23}H_{14}N_4O_7$	60.26	3.06	12.23	60.32	3.21	12.30
XXII	Pht-L-Val	59	257-259	0.69	+43 (c, 2.5 A)	$C_{25}H_{18}N_4O_7$	61.73	3.70	11.52	61.92	4.02	11.58
XXIII	Pht-L-Leu	60	250-252	0.73	+47 (c, 2.5 A)	$C_{26}H_{20}N_4O_7$	62.40	4.00	11.20	62.51	4.21	11.31
XXIV	Pht-DL-Phe	57	292-294	0.74		$C_{29}H_{18}N_4O_7$	65.17	3.71	10.49	65.19	3.91	10.70
XXV	Tos-β-Ala	60	247-249	0.67	—	$C_{22}H_{16}N_4O_7S$	54.77	3.73	11.62	54.99	3.99	11.81
XXVI	Tos-DL-Phe	55	238-240	0.75	—	$C_{28}H_{22}N_4O_7S$	60.22	3.94	10.04	60.33	4.15	10.12
XXVII	L-Ala	60	263-265	0.62	+50.5 (c, 2.5 C)	$C_{15}H_{12}N_4O_5$	54.88	3.66	17.07	54.91	3.65	17.10
XXVIII	Pht-B-Ala	52	250-252	0.69	—	$C_{23}H_{18}N_4O_3$	69.35	4.52	14.07	69.43	4.63	14.26
XXIX	Pht-L-Ala	54	265-267	0.68	+43.5 (c, 3 C)	$C_{23}H_{18}N_4O_3$	69.35	4.52	14.07	69.44	4.54	14.09
XXX	Pht-L-Val	55	286-288	0.72	+51.7 (c, 3 C)	$C_{25}H_{22}N_4O_3$	70.42	5.16	13.15	70.58	5.24	13.21
XXXI	Pht-L-Leu	53	281-283	0.76	+55.6 (c, 3 C)	$C_{26}H_{24}N_4O_3$	70.91	5.45	12.73	71.01	5.52	12.77
XXXII	Pht-DL-Phe	55	300-302	0.79		$C_{29}H_{22}N_4O_3$	73.42	4.64	11.81	73.85	4.80	12.01

(a) Crystallization solvent for compounds II-XII and XIX-XXVI = ethanol-water (1:1) mixture and for compounds XIII-XVII and XXVII-XXXII = ethanol. (b) Optical rotations $[\alpha]_D^{20}$ were measured in the solvents: (A) = ethanol, (B) = acetone, and (C) = acetic acid. (c) Electrophoretic mobilities (E) for compounds (XIII) = 11.5 cm, (XIV) = 12.5 cm, (XV) = 13.8 cm, (XVI) = 14.5 cm, (XVII) = 15.4 cm and (XXVII) = 17.7 cm, and for the remaining compounds (E) = zero. (d) Compounds XIX-XXVII, (R′ = NO_2) and for compounds XXVIII-XXXII, (R′ = NH_2).

microorganisms tested as compared to compound (XVIII).

The present investigation reveals that the introduction of nitrogroups at the 3- and 6-positions of the carbazole moiety induces high and specific biological properties in 3,6-dinitro-9-(*N*-phthalyl- or *N*-tosylaminoacyl or free aminoacyl)carbazole derivatives. However, reduction of the 3,6-dinitro groups to the corresponding 3,6-diamino derivatives results in biologically inactive compounds. In addition, introduction of *N*-phthalyl- or *N*-tosylaminoacyl or free aminoacyl moieties to the carbazole nucleus leads in many cases to a remarkable increase in their antimicrobial activity. Other pharmacological studies are in progress.

EXPERIMENTAL

Melting points were recorded on Gallenkamp melting point apparatus and are uncorrected. The ir spectra (potassium bromide, ν max in cm^{-1}) were recorded on a Varian or a Unicam SP 1200 spectrophotometer, uv spectra (ethanol, λ max in nm (log ϵ) on a Unicam SP 8000 spectrophotometer, and nmr spectra in DMSO-d_6 were run on a Varian T-60 A instrument (chemical shift in (δ), ppm) using TMS as internal standard. Thin layer chromatography (R_f values) was carried out on Silica Gel G (BDH), using benzene-ethyl acetate (1:1) as solvent system and an iodine-potassium iodide (20%) or chlorosulphonic acid-acetic acid (1:3) mixture as a detection reagent. Benzidine, ninhydrin, silver nitrate and hydroxamate reactions were used for detection of amino acid derivatives on Whatman No. 1 paper chromatograms (spot reactions). The electrophoretic mobilities (E) were measured on Whatman No. 1 paper using vertical high voltage electrophoresis, with 1000 V, 2 hours, in pyridine-acetate buffer (*p*H 5.6). Optical rotations $[\alpha]_D^{20}$ were taken with a Zeiss polarimeter, 1 dm tube in the solvents: (A) = ethanol, (B) = acetone and (C) = acetic acid (*cf.* Table 1).

9*H*-Carbazole (I) and 3,6-Dinitro-9*H*-carbazole (XVIII).

The title compounds were prepared according to the procedures described earlier (12-15).

General Procedure for the Synthesis of 9-(*N*-Phthalyl- or *N*-Tosyl-aminoacyl)carbazoles (II-XII) and 3,6-Dinitro-9-(*N*-phthalyl- or *N*-Tosylaminoacyl)carbazoles (XIX-XXVI).

N-Phthalyl- or *N*-tosylamino acid (0.003 mole) and 9*H*-carbazole or 3,6-dinitro-9*H*-carbazole (I or XVIII, 0.003 mole) were dissolved in tetrahydrofuran (50 ml). The mixture was cooled to 0° and dicyclohexylcarbodiimide (0.63 g) was added. The mixture was then stirred for 2 hours at 0°, allowed to stand for 24 hours at 0° and for another 24 hours at room temperature. The dicyclohexylurea was filtered off and the filtrate was evaporated *in vacuo*. The residual solid was recrystallized from ethanol or ethanol-water (1:1) mixture. The products II-XII and XIX-XXVI were soluble in DMSO, alcohols, DMF, dioxane, THF and nitromethane and insoluble in water, ether and petroleum ether. The materials were chromatographically homogeneous when developed with benzidine or chlorosulphonic acid-acetic acid (1:3) mixture and showed negative ninhydrin reactions. Complete acid hydrolysis of II and XIX (6*N* hydrochloric acid, 24 hours) afforded glycine.

9-(*N*-Phthalyl-L-alanyl)carbazole (IV).

This compound had ir 1760 (>C=O), 3200, 3020 (CON, N), 2950, 2860 (CH_3), 1270, 1120, 1050, 790, 785, 775 (carbazole and phthalyl moieties); uv: 213 (4.70), 233 (4.63), 247 (4.48), 255 (4.47), 281 (4.49), 285 (4.12), 310 (4.27) and 380 (4.29) characteristic of the carbazole nucleus; nmr: 3.20 (s, 1H, >CH-), 2.36 (s, 3H, C-CH_3), 6.94, 7.35, 7.89 (s, 12H, aromatic and ring protons).

3,6-Dinitro-9-(*N*-Phthalyl-L-Valyl)carbazole (XXII).

This compound had ir: 3170, 3020, 22960 (CON, N, CH_3), 1740, 1540, 1400, 1260 (NO_2), 1760, 1740 (>C=O), 1260, 1120, 1070, 790, 785, 770 (carbazole and phthalyl moieties); uv: 227 (4.50), 233 (4.59), 251 (4.38), 289 (4.18), 319 (3.45), 329 (3.69), 342 (3.49) (carbazole nucleus); nmr: 3.25 (s, 1H, >CH-), 1.49 (s, gem-dimethyl, 6H), 6.95, 7.42, 7.89, 8.01 (s, 10H, aromatic and ring protons).

General Procedure for the Synthesis of 9-(Aminoacyl)carbazoles (XIII-XVII) and 3,6-Dinitro-9(L-alanyl)carbazole (XXVII).

9-(*N*-Phthalylaminoacyl)carbazole or 3,6-dinitro-9-(*N*-phthalyl-L-alanyl)carbazole (0.003 mole) was dissolved in ethanol (30 ml) and treated with 1*M* hydrazine hydrate in ethanol (10 ml). The reaction mixture was refluxed for 3 hours. The residue obtained after evaporation of the solvent was dissolved in water (20 ml) and acidified with acetic acid (*p*H 6). The reaction mixture was refluxed for one hour, cooled and water (30 ml) added and the insoluble phthalyl hydrazide was filtered. The filtrate was evaporated in vacuum and the residual material was recrystallized from ethanol. The products XIII-XVII and XXVII were tlc pure when developed with benzidine, ninhydrin and chlorosulphonic acid-acetic acid (1:3) mixture.

9-(*N*-Valyl)carbazole (XV).

This compound had 3220, 3140, 3060 (NH_2, N, CON), 1760 (>C=O), 2980, 2790 (CH_3), $CH(CH_3)_2$), 1260, 1120, 1050, 790, 780, 770 (carbazole nucleus); uv: 215 (4.47), 232 (4.69), 249 (4.53), 255 (4.49), 280 (4.51), 285 (4.22), 312 (3.98), 329 (4.46) (carbazole nucleus); nmr: 3.23 (s, 1H, >CH-), 1.49 (s, gemdimethyl, 6H), 1.36 (s, 3H, C-CH_3), 7.98 (s, 2H, NH_2), 6.94, 7.35, 8.02 (s, 8H, ring protons).

3,6-Dinitro-9-(L-alanyl)carbazole (XXVII).

This compound had ir: 3320, 3160, 3080 (NH_2, N, CON), 1760 (>C=O), 2960, 2890 (CH_3), 1740, 1540, 1400, 1230 (NO_2), 1250, 1130, 1080, 790, 780, 760 (carbazole moiety). uv: 225 (4.55), 231 (4.56), 253 (4.33), 282 (4.27), 318 (3.46), 329 (3.68), 345 (3.48); nmr: 3.27 (s, 1H, >CH-), 3.22 (s, 3H, C-CH_3), 7.96 (s, 2H, NH_2), 6.98, 7.85, 8.05 (s, 6H, aromatic ring protons).

General Procedure for the Synthesis of 3,6-Diamino-9-(*N*-phthalyl-aminoacyl)carbazoles (XXVIII-XXXII).

A mixture of 3,6-dinitro-9-(*N*-phthalylaminoacyl)carbazole (0.001 mole), tin (1.2 g), stannous chloride (1.42 g) and ethanol (50 ml) was cooled to 5°, and concentrated hydrochloric acid (7.5 ml) added. The reaction mixtrure was stirred for 24 hours at room temperature, filtered and the solution concentrated in vacuum. The solid product was collected filtered and washed several times with water, sodium bicarbonate (3%) and water and dried (sodium sulfate). The products were further purified by repeated recrystallizations from ethanol. Compounds (XXVIII-XXXII) were chromatographically homogeneous when developed with benzidine, chlorosulphonic acid-acetic acid (1:3) mixture and gave negative reaction with ninhydrin and hydroxamate test.

3,6-Diamino-9-(*N*-phthalyl-L-valyl)carbazole (XXX).

This compound had ir: 3400, 3300, 3080 (Ar-NH_2, N, CON), 2960, 2870 (CH_3), 1760, 1725 (>C=O), 1250, 1130, 1080, 790, 780, 760 (carbazole moiety); uv: 228 (4.57), 233 (4.60), 251 (4.30), 260 (4.01), 288 (4.11), 298 (4.33), 3.19 (3.43), 330 (3.65), 341 (3.47); nmr: 3.21 (s, 1H, >CH-), 1.38 (s, 3H, CH_3), 7.96, 8.03 (s, 4H, 2 NH_2), 6.98, 7.35, 7.89 (s, 10H, aromatic ring protons).

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