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Synthesis and Biological Activity of Some New Substituted Aminoacyl-Carbazole Derivatives. Part II.

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ABSTRACT. 3-Nitro-9-(N-phthalyl- and N-tosylaminoacyl) carbazoles (II-XI) have been synthesized by the action of 3-nitro-9H- carbazole (I) on N-phthalyl- or N-tosylamino acid in THF-Et_3N medium using DCC method. Treatment of the 3-nitro derivatives (II-XI) with Sn/HCl gave the corresponding 3-amino-9-(N-phthalyl- or N-tosylaminoacyl) carbazoles (XII-XIX). Hydrazinolysis of the N-phthalyl-carbazole derivatives (III-VII) in ethanol gave the corresponding 3-nitro-9-(aminoacyl) carbazoles (XX-XXIII). Compounds (II-XI and XXI, XXII) were found to be active against some microorganisms.

Carbazoles and various substituted carbazole derivatives have been found previously to have different biological activities (Burtner and Lehman 1940, Maisin *et al.* 1927, Knoefel 1933, Sumpter and Miller 1954, Albert 1968, Freudenberg 1952). Recently, the authors have reported the synthesis of some carbazoles incorporating amino acid moieties, (El-Naggar *et al.* 1982), and some of these compounds were found to display antimicrobial properties. However, the effect on the biological and pharmacological activities of variations of the functional groups in both the carbazole and amino acid moieties has not yet been studied.

The present investigation involved the synthesis of some new 3-nitro-9-(N-phthalyl- or N-tosylaminoacyl or free aminoacyl)-carbazoles (II-XI and XX-XXIII) and some of

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their corresponding 3-amino-9-(N-phthalyl- or N-tosylaminoacyl) carbazoles (XII-XIX), and studies on their microbiological activities.

3-Nitro-9-(N-phthalyl- or N-tosylaminoacyl) carbazoles (II-XI) were readily prepared by the reaction of 3-nitro-9H-carbazole (I) (Cadogan and Cameron-wood 1962, Morgan and Mitchell 1931) with the appropriate N-phthalyl- or N-tosylamino acid in THF-triethylamine medium using the dicyclohexylcarbodiimide (DCC) procedure. However, the preparation of (II-XI) could be also accomplished in absence of the base. Compounds (II-XI) were chromatographically homogeneous and did not give a ninhydrin reaction.

Attempts to synthesize II-XI by reaction of N-Pht-amino acid chlorides or N-Tos-amino acid chlorides with 3-nitro-9H-carbazole (I) in DMF, THF, benzene, methylene chloride or dioxane using the acid chloride method were unsuccessful. Moreover, coupling of Tos-amino acid azides *via* the azide method in ethyl acetate and DMF gave very poor yields.

Treatment of 3-nitro-9-(N-phthalyl- or tosylaminoacyl)-carbazoles (II-VII and X-XI) with Sn/HCl gave 3-amino-9-(N-phthalyl- or N-tosylaminoacyl) carbazole hydrochlorides which on treatment with sodium hydrogen carbonate (3%) afforded the desired 3-amino-9-(N-Pht- or N-Tos-aminoacyl) carbazoles as white crystals (yield 50-60%).

Hydrazinolysis of 3-nitro-9-(N-phthalylaminoacyl) carbazoles (III-VII) with 1M hydrazine hydrate in ethanol under mild reflux afforded 3-nitro-9-(aminoacyl) carbazoles (XX-XXIII). Chromatographic and electrophoretic studies on (XX-XXIII) revealed their homogenity (positive ninhydrin reaction, E(III-VII) start = zero, E(XX-XXIII) = 15.5-18 cm, *cf.* Table 1), and their structures were supported by the IR, UV and NMR spectral data. This was further confirmed by their complete acid hydrolysis affording the corresponding amino acids.

Compounds (II-XXIII) were prepared and characterized for the first time. All of the compounds which were synthesized (II-XXIII) 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 (Carlson 1948, Vincent and Vincent 1944, Epstein 1944, Irving 1946, El-Gazzar 1981). The results were compared with the activity of carbazole and 3-nitro-9H-carbazole (I), (*cf.* Table 2).

3-Nitro-9-(N-Pht-Gly) carbazole (II) and the corresponding N-Pht -ß- Ala (III), N-Pht-L-Ala (IV), N-Phe-L-Val (V), N-Pht-DL-Phe (VII), N-Tos-L-Leu (XI) and L-Val (XXI) derivatives were found to be active against *Bacillus subtilis* (ICC-Strain) and *Bacillus cereus* (NRRL-B-569) with MIC values ranging from 25-50 μg/ml and inactive against *Bacillus mycoids* (USSR, NRRL-B-315), *Salmonella typhosa* (NRRL-B-573), *Escherichia coli* (NRRL-B-210) and *Penicillium chrysogenum* (MIC 250-500 μg/ml). 3-

Nitro-9-(N-Pht-L-Leu)-carbazole (VI) and the corresponding N-Tos-Gly (VIII), N-Tos-ß-Ala (IX), N-Tos-L-Val (X) and L-Leu (XII) derivatives showed marked antibacterial activity as compared to (I) against *Bacillus cereus* with MIC 10-25 μg/ml and inactive against the remaining microorganisms (MIC 250-500 μg/ml). None of the 3-amino-9-(N-Pht- or N-Tos-aminoacyl)-carbazole derivatives (XII-XVII) showed any significant activity (MIC 250-500 μg/ml) against any microorganism tested as compared to carbazole or (I).

The present investigation shows that introduction of a nitro group at the 3-position of the carbazole moiety induces remarkable and specific biological properties in 3-nitro-9-(N-phthalyl- or N-tosylaminoacyl or free aminoacyl) carbazole derivatives. However, reduction of 3-nitro group to the corresponding 3-amino derivatives results in biologically inactive compounds. In addition, hydrazinolysis of the N-phthalyl protecting group to the unprotected aminoacyl derivatives leads in some 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. Thin layer chromatography (TLC, 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 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 hr in pyridine-acetate buffer (pH 5.6). IR spectra (KBr, ν_{max} in cm^{-1}) were recorded using a Unicam SP 1200 spectrophotometer, UV spectra (ethanol), λ_{max} in nm, ($\log\epsilon$), in 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 the internal standard. Optical rotations $[\alpha]_D^{20}$ were taken in a Zeiss polarimeter, 1 dm tube in the solvents: (A) = ethanol, (B) = acetone and (C) = acetic acid, (*cf.* Table 1). The analytical results obtained from the new compounds for C, H, and N are in the range of 0.3% of error.

3-Nitro-9H-Carbazole (I)

The title compound was prepared according to the procedure described earlier (Cadogan and Cameron-wood 1962, Morgan 1931).

General Procedure for the Synthesis of 3-Nitro-9-(N-Phthalyl- or N-tosylaminoacyl)-Carbazoles (II-XI)

N-Phthalyl- or N-tosylamino acid (0.001 mole) and 3-nitro-9H-carbazole (I, 0.001 mole) was dissolved in tetrahydrofuran (30 ml). The mixture was cooled to 0°C and dicyclohexylcarbodiimide (0.42 g) was added. The mixture was then stirred for 2 hr at 0°C, left for 24 hr at 0°C and for another 24 hr at room temperature. The

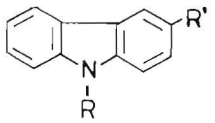
Table 1. Physical Data of Various 3-Nitro- (or 3-Amino)-9-(N-Phtha

Compd. No.	R—	—R′	Yield* %	m.p. °C	R_f (TLC)	$[\alpha]_D^{20}$ **
II	Pht-Gly	NO_2	60	159-161	0.59	—
III	Pht-ß-Ala	NO_2	70	144-146	0.60	—
IV	Pht-L-Ala	NO_2	65	175-177	0.62	+56.5 (c, 3 A)
V	Pht-L-Val	NO_2	62	172-174	0.64	+62.5 (c, 2.5 A)
VI	Pht-L-Leu	NO_2	68	184-186	0.67	+70 (c, 2.5 A)
VII	Pht-DL-Phe	NO_2	60	169-171	0.70	—
VIII	Tos-Gly	NO_2	65	148-150	0.61	—
IX	Tos-ß-Ala	NO_2	64	182-184	0.64	—
X	Tos-L-Val	NO_2	60	178-180	0.67	+68.5 (c, 2.5 A)
XI	Tos-L-Leu	NO_2	65	164-166	0.73	+75.5 (c, 2.5 A)
XII	Pht-Gly	NH_2	60	284-286	0.62	—
XIII	Pht-ß-Ala	NH_2	55	270-272	0.64	—
XIV	Pht-L-Ala	NH_2	61	279-281	0.65	+61.5 (c, 2.5 B)
XV	Pht-L-Val	NH_2	52	274-276	0.69	+67 (c, 2.5 B)
XVI	Pht-L-Leu	NH_2	55	225-227	0.71	+79.5 (c, 3 B)
XVII	Pht-DL-Phe	NH_2	50	261-263	0.72	—
XVIII	Tos-L-Val	NH_2	54	268-270	0.70	+72 (c, 3 B)
XIX	Tos-L-Leu	NH_2	55	258-260	0.75	+76.5 (c, 3 B)
XX	ß-Ala***	NO_2	55	178-180	0.55	—
XXI	L-Val	NO_2	59	164-166	0.59	+80 (c, 3 C)
XXII	L-Leu	NO_2	61	136-138	0.61	+92.5 (c, 3 C)
XXIII	DL-Phe	NO_2	56	130-132	0.64	—

* Crystallization solvent for compounds II-XI = ethanol-water (1:1) mixture and for compounds XII-

** Optical rotations $[\alpha]_D^{20}$ were measured in the solvents: (A) = ethanol, (B) = acetone and (C) = acetic

*** Electrophoretic mobilities (E) for compounds (XX) = 15.5 cm, (XXI) = 16.8 cm, (XXII) = 17.4 cm,

lyl or N-Tosylaminoacyl or Free Aminoacyl) Carbazole Derivatives (II-XXIII) 

Molecular formula	Elemental analysis, %					
	Calc.			Found		
	C	H	N	C	H	N
$C_{22}H_{13}N_3O_5$	66.17	3.26	10.53	66.19	3.29	10.71
$C_{23}H_{15}N_3O_5$	66.83	3.63	10.17	66.89	3.65	10.19
$C_{23}H_{15}N_3O_5$	66.83	3.63	10.17	67.11	3.65	10.40
$C_{25}H_{19}N_3O_5$	68.03	4.31	9.52	68.27	4.32	9.90
$C_{26}H_{21}N_3O_5$	68.57	4.62	9.23	68.60	4.83	9.53
$C_{29}H_{19}N_3O_5$	71.17	3.89	8.59	71.22	4.13	8.64
$C_{21}H_{17}N_3O_5S$	59.58	4.02	9.93	59.70	4.11	9.91
$C_{22}H_{19}N_3O_5S$	60.41	4.35	9.61	60.69	4.56	9.63
$C_{24}H_{23}N_3O_5S$	61.94	4.95	9.03	61.99	4.89	9.21
$C_{25}H_{25}N_3O_5S$	62.63	5.22	8.77	62.67	5.47	8.82
$C_{22}H_{15}N_3O_3$	71.55	4.06	11.38	71.81	4.36	11.67
$C_{23}H_{17}N_3O_3$	72.06	4.44	10.97	72.08	4.53	10.98
$C_{23}H_{17}N_3O_3$	72.06	4.44	10.97	72.10	4.60	10.88
$C_{25}H_{21}N_3O_3$	72.99	5.11	10.22	73.01	5.34	10.23
$C_{26}H_{23}N_3O_3$	73.41	5.41	9.88	73.50	5.58	9.89
$C_{29}H_{21}N_3O_3$	75.82	4.58	9.15	75.91	4.63	9.22
$C_{24}H_{25}N_3O_3S$	66.21	5.75	9.66	66.31	5.86	9.72
$C_{25}H_{27}N_3O_3S$	66.82	6.01	9.35	66.89	6.34	9.51
$C_{15}H_{13}N_3O_3$	63.60	4.59	14.84	63.70	4.81	14.91
$C_{17}H_{17}N_3O_3$	65.60	5.57	13.50	65.91	5.59	13.66
$C_{18}H_{19}N_3O_3$	66.46	5.85	12.92	66.79	5.91	13.05
$C_{21}H_{17}N_3O_3$	70.20	4.74	11.70	70.21	4.91	11.81

XXIII = ethanol.

acid.

(XXIII) = 18 cm, and for the remaining compounds (E) = zero.

Table 2. Antimicrobial activity (A)* and minimal inhibitory concentration (MIC in μg/ml) of the biologically active compounds.

Compd. No.	*B. subtilis*		*B. mycoids*		*B. cereus*		*E. coli*		*Salm. typhosa*		*Pen. chrysogenum*	
	MIC	A	MIC	A	MIC	A	MIC	A	MIC	A	MIC	A
I	250	+	—	—	500	+	—	—	—	—	—	—
II	25	+++	—	—	50	+++	—	—	—	—	—	—
III	50	+++	—	—	25	+++	—	—	—	—	—	—
IV	25	+++	—	—	25	+++	—	—	—	—	—	—
V	25	+++	—	—	25	+++	—	—	—	—	—	—
VI	—	—	—	—	10	+++	—	—	—	—	—	—
VII	50	++	—	—	25	+++	—	—	—	—	—	—
VIII	—	—	—	—	25	+++	—	—	—	—	—	—
IX	—	—	—	—	10	+++	—	—	—	—	—	—
X	—	—	—	—	10	+++	—	—	—	—	—	—
XI	50	++	—	—	25	+++	—	—	—	—	—	—
XII	—	—	—	—	25	+++	—	—	—	—	—	—
XXI	25	+++	—	—	25	+++	—	—	—	—	—	—
carbazole	—	—	—	—	500	+	—	—	—	—	—	—

* Antimicrobial activity (A) +++ = highly active, ++ = moderately active, + = slightly active, — = inactive; and MIC = minimal inhibitory concentration in μg/ml.

dicyclohexylurea was filtered off and the filtrate was evaporated *in vacuo*. The residual solid was recrystallized from ethanol-water (1:1) mixture. The products (II-XI) were soluble in DMSO, alcohols, DMF, dioxane, THF and insoluble in chloroform, 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 (VI and XI), (6 N hydrochloric acid, 24 hr, 105°C) followed by subsequent paper chromatography afforded leucine (positive ninhydrin spot).

3-Nitro-9-(N-Pht-Gly)Carbazole (II)

This compound had IR spectra: 3130, 3080 (N, CON), 1760, 1720 ($\geq$C=O), 1720, 1500, 1380, 1220 (NO_2), 1680, 1540, 1320, 1120, 1060, 790 and 760 cm^{-1} (aminoacyl and carbazole moieties); UV: 215 nm (4.64), 233 (4.65), 247 (4.48), 255 (4.49), 278 (3.39), 287 (3.88), 303 (4.12), 330 (4.31) characteristic of the carbazole nucleus; NMR: 2.36 ppm (s=chemical shift, 2H, CH_2), 6.94, 7.35, 7.89 (s, 11H aromatic and ring protons).

General Procedure for the Synthesis of 3-Amino-9-(N-Phthalyl- or N-Tosylaminoacyl) Carbazoles (XII-XIX)

A mixture of 3-nitro-9-(Nphthalyl- or N-tosylaminoacyl) carbazole (0.003 mole), tin (2.9 g), stannous chloride (4.26 g) and ethanol (60 ml) was cooled to 5°C and conc. HCl (15 ml) added. The reaction mixture was stirred for 24 hr at room temperature, filtered and the solution concentrated to half its volume in vacuum. The product was separated, collected, filtered and washed several times with water, $NaHCO_3$ (3%) and water and dried over Na_2SO_4. The products were further purified by repeated recrystallizations from ethanol. All the compounds (XII-XIX) were chromatographically homogeneous when developed with benzidine or chlorosulphonic acid-acetic acid (1:3) mixture and gave negative reaction with ninhydrin or hydroxamate test.

Complete acid hydrolysis of (XIV) (6 N hydrochloric acid, 24 hr, 105°C), followed by subsequent paper chromatography afforded alanine (positive spot with ninhydrin).

3-Amino-9-(N-Pht-DL-Phe) Carbazole (XVII)

This compound had IR spectra: 3420, 3320, 3080 (NH_2, N, CON), 1760, 1720 ($>C=O$), 1650, 1450, 1240, 1120, 1080, 790 and 760 cm^{-1} (carbazole and aminoacyl moieties); UV: 229 nm (4.57), 233 (4.60), 253 (4.43), 262 (4.11), 289 (4.13), 298 (4.48), 320 (3.42), 342 (3.48); NMR: 3.21 ppm (s = chemical shift, 1H, $>CH-$) 3.42 (s, 2H, $-CH_2-$), 7.96 (s, 2H, Ar—NH_2), 6.98, 7.35, 7.89 (s, 16H, aromatic and ring protons).

General Procedure for the Synthesis of 3-Nitro-9-(aminoacyl)-Carbazoles (XX-XXIII)

3-Nitro-9-(N-phthalylaminoacyl) carbazole (0.002 mole), was dissolved in ethanol (40 ml) and treated with 1M hydrazine hydrate in ethanol (8 ml). The reaction mixture was refluxed for three hours. The residue obtained after evaporation of the solvent was dissolved in water (30 ml) and acidified with acetic acid (pH 6). The reaction mixture was refluxed for one hour, cooled, water was added (30 ml) and the insoluble phthalyl hydrazide was filtered off. The filtrate was evaporated *in vacuo* and the residual material was recrystallized from ethanol. The products (XX-XXIII) were soluble in alcohols, DMF, THF, chloroform and dioxane, and insoluble in water and ether. All compounds (XX-XXIII) were pure on TLC plates when developed with ninhydrin, benzidine and chlorosulphonic acid-acetic acid (1:3) mixture.

Complete acid hydrolysis of (XXI) (6 N hydrochloric acid, 24 hr, (105°C), followed by subsequent paper chromatography afforded valine (positive spot with ninhydrin).

3-Nitro-9-(L-Val) carbazole (XXI)

This compound had IR spectra: 3320, 3140, 3060 (NH_2, CON, N), 1760, 1720 ($>C=O$), 2980, 2780 (CH_3, $CH(CH_3)_2$), 1260, 1120, 1080, 789, and 770 cm^{-1} (carbazole and aminoacyl moieties). UV: 215 nm (4.46), 233 (4.68), 249 (4.56), 258 (4.46), 280 (4.36), 285 (4.39), 312 (4.31), 330 (4.46) characteristic of the carbazole nucleus. NMR: 3.24 ppm (s=chemical shift, 1H, $>CH-$), 1.49 (s, 6H, gemdimethyl),

1.36 (s, 3H, CH_3), 7.98 (s, 2H, NH_2), 6.95, 7.35, 8.03 (s, 7H, aromatic and ring protons).

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التخليق والنشاط البيولـوجي لبعض المشتقات الجديدة لمركبات أمينو آسيل - كربازول. الجزء الثاني.

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تم تخليق مجموعة من مركبات ٣ - نيترو - ٩ - (ن - فثاليل - و ن - توزيل - أمينو آسيل) كربازول (٢ - ١١) وذلك بتأثير ٣ - نيترو - ٩ يد كربازول (١) على ن - فثاليل - أو ن - توزيل حمض أميني في وسط رباعي هيدروفيوران وثلاثي إثيل أمين باستخدام طريقة الكربوثنائي الإميد.

وبمعالجة مشتقات ٣ - نيترو (٢ - ١١) بواسطة القصدير في وجود حمض الهيدروكلوريك، تم فصل مشتقات ٣ - أمينو - ٩ - (ن - فثاليل - أو ن - توزيل أمينو آسيل) كربازول (١٢ - ١٩).

وبتفـاعل مشتقـات ن - فثاليـل - كربـازول (٣ - ٧) بالهيدرازين هيدرات في وجود الإيثانول نتجت مشتقات ٣ - نيترو - ٩ - (أمينو آسيل) كربازول (٢٠ - ٢٣) المناظرة.

وبدراسة النشاط البيولـوجي للمركبـات التي تم تخليقها اتضـح أن مـركبـات (٢ - ١١، ٢١، ٢٢) ذات نشـاط بيولوجي مميز تجاه بعض الكائنات الدقيقة.

* العنوان الحالي: قسم الكيمياء - كلية العلوم - جامعة قطر الدوحة - ص . ب: ٢٧١٣ - دولة قطر.