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PYRAZOLO[3,4-a]CARBAZOLES: SYNTHESIS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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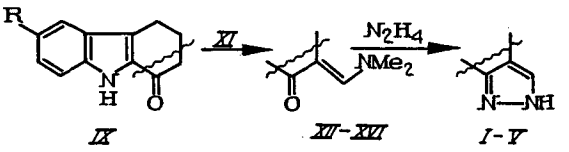
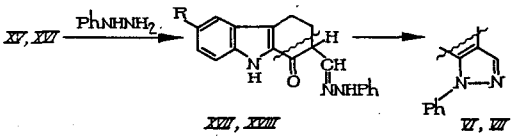
Pyrazolo[3,4-a]carbazoles constitute a new class of heterocyclic compounds. We obtained the first representatives of this class – the 7-R-4,5-dihydropyrazolo[3,4-a]carbazoles (I-VIII) from the corresponding ketocarbazoles (IX, X). Ketocarbazoles IX were condensed with dimethylformamide diethylacetal (XI) and the enamines (XII-XVI) obtained were converted into pyrazolocarbazoles I-V by the action of hydrazine hydrate in boiling ethanol.



R = Cl (I, XII), Br (II, XIII), Me (III, XIV), H (IV, XV), cyclohexyl (V, XVI).

Under the same conditions (boiling in ethanol) the reaction of enamines XV, XVI with phenylhydrazine stopped at the stage of formation of hydrazones (XVII, XVIII), the cyclization of which could be carried out under more rigorous conditions – boiling in anhydrous acetic acid.



R = H (VI, XVII), cyclohexyl (VII, XVIII).

The PMR spectrum of compound XVII (a solution in D_6-DMSO) shows only one set of signals. The hydrazone structure of compound XVII was established by NMR using the double resonance method: the assignment of the δ 3.61 ppm signal (quintet, ^3J 6 Hz) to the H2 carbazole ring proton was confirmed by the interaction of this proton with the CH=N group proton (δ 7.45 ppm, doublet, ^3J 6 Hz).

The product of the reaction of ketocarbazole X with acetal XI could not be obtained in a crystalline form; it was converted without purification into pyrazolocarbazole VIII by the action of hydrazine hydrate.

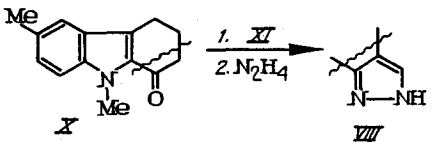


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TABLE 1. Properties of Enamines XII-XVI and Hydrazones XVII, XVIII

Compound	Yield, %	mp, °C,	Empirical formula
XII	82	258—60	$C_{15}H_{15}N_2OCl$
XIII	65	245*	$C_{15}H_{15}N_2OBr$
XIV	70	250*	$C_{16}H_{18}N_2O$
XV	82	220—5	$C_{15}H_{16}N_2O$
XVI	76	235*	$C_{21}H_{26}N_2O$
XVII	75	155*	$C_{19}H_{17}N_3O$
XVIII	54	165*	$C_{25}H_{27}N_3O$

*Melts with decomposition.

TABLE 2. 4,5-Dihydropyrazolo[3,4-a]carbazoles

Compound	Yield, %	mp, °C	Empirical formula
I	59	205—7	$C_{13}H_{10}N_3Cl$
II	65	202—6	$C_{13}H_{10}N_3Br$
III	75	235—40	$C_{14}H_{13}N_3$
IV	66	181—3	$C_{13}H_{11}N_3$
V*	87	176—8	$C_{19}H_{25}N_3 \cdot C_2H_5OH$
VI	83	99—100	$C_{19}H_{15}N_3$
VII	89	176—80	$C_{25}H_{25}N_3$
VIII	23**	177—9	$C_{15}H_{15}N_3$

*A solvate with ethanol.

**The overall yield after two stages, based on ketone X.

EXPERIMENTAL (CHEMICAL)

The electron impact mass spectra were run on a MAT-112 mass-spectrometer (FRG). The PMR spectra were run on an XL-200 spectrometer (Varian, Switzerland), using TMS as an internal standard. The elemental analysis results corresponded to the empirical formulas given.

2-Dimethylaminomethylene-1-oxo-6-chloro-1,2,3,4-tetrahydrocarbazole (XII). A 15 g portion (68 mmoles) of ketone IX (R = Cl) in 100 ml of acetal XI was gradually heated (acetal, bp 130-135°C, n_D^{20} 1.4035, contains according to PMR data 15 wt. % of DMFA, 15% of mixed methylethylacetal and 70% of diethylacetal). The heating was carried out with simultaneous distillation of the alcohol formed. At 110°C the product began to precipitate from solution. At 125°C the downward condenser was replaced by a reflux condenser and the suspension formed was boiled with stirring for further 3 h. The mass was cooled and the product was filtered. Yield 15.5 g. Mass spectrum (I_{rel}): 274 (100%), 261(8), 259(25), 257(30), 233(13), 231(30).

In a similar way, enamines XIII-XVI were obtained from ketones IX (R = Br, Me, H, C_6H_{11}), respectively (Table 1). All the enamines obtained were purified by crystallization from DMFA.

7-Chloro-4,5-dihydropyrazolo[3,4-a]carbazole (I). A mixture of 15.5 g (56 mmoles) of enamine XII, 5.65 g (113 mmoles) of hydrazine hydrate and 90 ml of absolute ethanol was boiled for 4 h. The solution obtained was evaporated *in vacuo* and the residue was crystallized from ethanol. Yield, 8.15 g of pyrazolocarbazole I. PMR spectrum in $CDCl_3$ (δ, ppm): 2.98 (m, CH_2CH_2), 7.12 (dd, ^3J 8.57 Hz, ^4J 2 Hz, H-8), 7.29 (d, ^3J 8.57 Hz, H-9), 7.38 (s, H-3), 7.51 (d ^4J 2 Hz, H-6), 9.13 (NH). Mass spectrum (I_{rel}: 243(100), 207(50).

In a similar way, pyrazolocarbazoles II-V were synthesized from enamines XIII-XVI (Table 2).

Phenylhydrazone of 1-oxo-1,2,3,4-tetrahydrocarbazole-2-carboxaldehyde (XVII). A mixture of 13.2 g (55 mmoles) of enamine XV, 11.9 g (110 mmoles) of phenylhydrazine and 80 ml of absolute ethanol was boiled for 5 h, and then cooled. The hydrazone XVII (13.5 g) that separated out was filtered and crystallized from acetonitrile. PMR spectrum in D_6-DMSO (δ, ppm): 2.42 (m, H-3), 3.11 (m, H-4), 3.61 (m, H-2), 7.45 (d, ^3J 6 Hz, CH=N), 7.72-6.60 (m, CH_{ar}). Mass spectrum (I_{rel}): 303(100), 285(70), 183(30). The relative intensity of the peaks is dependent on the admission temperature: at 230°C the peak with m/z 303 (M^+) is the main peak, while at 260°C it is the peak with m/z 285 $(M-H_2O)^+$

TABLE 3. *In vitro* Activity of Pyrazolocarbazole Derivatives (MIC, μg/ml)

Compound	St. aureus 6538-P ATCC	Bact. subtilis 6633 ATCC	E. coli 25922 ATCC	Prot. vulgaris 6896 ATCC	Ps. aeruginosa 27853 ATCC	Microsporum canis 3/83	C. albicans 885-653 ATCC	Tr. gypseum 5/85
I	3,9	3,9	>250	150	>250	3,9	31,2	7,8
II	1,0	2,0	250	250	>250	2,0	3,9	2,9
III	7,8	31,2	>250	>250	>250	31,2	62,5	31,2
IV	31,2	31,2	>250	>250	>250	7,8	125	15,6
V	1,0	2,0	>250	>250	>250	3,9	>250	3,9
VI	>250	>250	>250	>250	>250	31,2	>250	62,5
VII	>250	>250	>250	>250	>250	>250	>250	>250
VIII	3,9	3,9	3,9	>250	>250	31,2	>250	3,9

In a similar way, phenylhydrazone XVIII was obtained from enamine XVI, and was crystallized from dioxane (see Table 1).

1-Phenyl-4,5-dihydropyrazolo[3,4-a]carbazole (VI). A solution of 34 g (0.112 mole) of hydrazone XVII in 120 ml of glacial acetic acid was boiled for 6 h and then cooled. The precipitate that separated out was filtered off and dissolved in benzene. The solution obtained was washed from the AcOH impurity, first with a 5% solution of sodium carbonate, and then with water; it was then dried over sodium sulfate and evaporated under vacuum. The residue was ground with hexane and filtered to yield 26.5 g of compound VI. Mass spectrum (I_{rel}): 285(100), 257(20), 256(15).

In a similar way, pyrazolocarbazole VII was obtained from hydrazone XVIII. Compound VI was crystallized from cyclohexane and VII from ethanol.

7,10-Dimethyl-4,5-dihydropyrazolo[3,4-a]carbazole (VIII). A mixture of 10.66 g (0.05 mole) of ketocarbazole X [2], 8.7 g (0.10 mole) of morpholine and 40 ml of acetal XI was gradually heated in a flask with a downward condenser. The volatile products were distilled off until the temperature in the reaction mixture reached 125°C. The downward condenser was replaced by a reflux condenser and the mixture was boiled for further 3 h. The reaction mixture was evaporated under vacuum, the oily residue was dissolved in 75 ml of absolute ethanol, 5 g (0.10 mole) of hydrazine hydrate was added to the solution obtained, and the mixture was boiled for 3 h. The alcohol was distilled off and the residue was treated with benzene and water. The benzene layer was separated, dried over sodium sulfate and evaporated. The residue was ground in 10 ml of chloroform up to solidification to yield 2.76 g of VIII, which was crystallized from isopropanol (see Table 2). PMR spectrum (in $CD_3)_2CO$ (δ, ppm): 2.404 (s, 7-CH_3), 2.86-2.94 (m, CH_2CH_2), 4.097 (s, NCH_3), 6.97 (dd, ^3J 8.4 Hz, ^4J 1.6 Hz, H-8), 7.25 (d, ^3J 8.4, Hz, H-9), 7.28 (s, H-3), 7.54 (br. s, H-6), 11.8 (NH).

EXPERIMENTAL (BIOLOGICAL)

The antibacterial activity of the pyrazolocarbazole derivatives was studied in *in vitro* and *in vivo* experiments. *In vitro* the activity was studied by the method of multiple serial dilution on a liquid culture medium. In experiments with bacteria Hottinger bullion was used, and in experiments with fungi – Saburo medium was used. The activity with respect to bacteria was studied on strains of *S. aureus* 6538-P ATCC, *B. subtilis* 6633 ATCC, *E. coli* 2592 ATCC, *P. vulgaris* 6896 ATCC and *P. aeruginosa* 27853 ATCC. In experiments with fungi *Microsporum canis* 3/83, *Trichophiton gypseum* 5/85 and *Candida albicans* 885-653 ATCC were used. The microbial charge in experiments with bacteria was $1 \cdot 10^5$ CFU/ml (colony forming units in 1 ml of the solution) and in experiments with fungi – $1 \cdot 10^6$ CFU/ml). The bacteria were incubated at 37°C for 18-20 h; the fungi – for 24 h in experiments with *Candida* and for 5 days with fungi-dermatophytes. The activity of the compounds was studied in concentrations of 250 μg/ml and lower.

The *in vitro* experimental results obtained showed a pronounced activity of compounds I-III, V and VIII with respect to Gram-positive bacteria *S. aureus* and *B. subtilis*; MIC (minimal inhibiting concentration) 1.0-7.8 μg/ml. Compounds I, II, IV and V in the same concentrations inhibited the growth of fungi-dermatophytes: *M. canis* and *T. gipseum*. All the compounds studied had no activity with respect to Gram-negative bacteria (Table 3).

The *in vivo* experiments were carried out with four compounds (I-III and V) on 270 white nonpedigree mice, each weighing 14-15 g. The chemotherapeutic activity of the compounds was studied on three experimental models of murine generalized infection (septicemia) induced by *S. typhy* 4446, *P. aeruginosa* 165, and *S. aureus* 178. The mice were infected intraperitoneally, the culture was introduced in the form of a suspension in 1 ml of a physiological solution in a mixture with

a 0.25% cold agar. The size of the infecting doses causing the death of 100% of control untreated animals amounted to $1 \cdot 10^7$ CFU for the typhoid bacillus, $1 \cdot 10^6$ CFU for the *Bacillus pyocyaneus* and $8 \cdot 10^8$ CFU for *Staphylococcus*.

The treatment was started 30-40 min after infection. The compounds were administered in a volume of 0.5 ml of the physiological solution in a single dose: orally – on a model of a pyocyaneus infection; subcutaneously – on models of septicemia induced by the typhoid *Bacillus* and *Staphylococcus*. The activity of the compounds was evaluated from the survival index of the compounds on the 10-th day after infection.

The *in vivo* experimental results showed the absence of a chemotherapeutic activity in compounds I-III and V on all three models of murine generalized infection, irrespective of the method of administration of the compounds (orally or subcutaneously).

Thus, pyrazolocarbazoles I-V displayed a narrow spectrum of antibacterial activity *in vitro* and did not show chemotherapeutic activity *in vivo*.

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