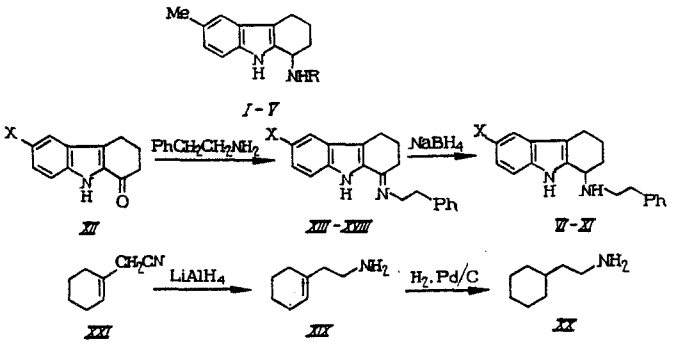
ANTITUBERCULAR, ANTIFUNGAL, AND ANTIBACTERIAL ACTIVITY IN VITRO OF 1-PHENETHYLAMINO-1,2,3,4-TETRAHYDROCARBAZOLES

T. V. Akalaeva, L. Yu. Amel'kin, O. V. Baklanova, A. I. Bokanov, P. Yu. Ivanov, E. N. Padeiskaya, T. P. Radkevich, L. N. Filitis, and V. I. Shvedov

UDC 615.28:[547.759.32].-015.4.07

The biological activity of 1-aminotetrahydrocarbazoles was discovered by in vitro experiments in [1, 6]. Of the compounds studied, the most active was 6-methyl-1-phenethylamino-1,2,3,4-tetrahydrocarbazole (I). In order to find the relationship between the biological activity and the structure of the compounds, we studied the analogs of compound I: its homologs (II, III), the hydrogenated derivatives (IV, V), and also compounds with various substituents in the 6-position (VI-XI).

R=CH_2CH_2Ph (I), CH_2Ph (II), $(CH_2)_3Ph$ (III), 2(cyclohexen-1-yl)ethyl (IV), 2-(cyclohexyl)ethyl (V);
X=H (VI, XIII), F (VII, XIV), Cl (VIII, XV), Br (IX, XVI), cyclohexyl (X, XVII), Ph (XI, XVIII).



Compounds (VI-XI) were synthesized by a known method [6] from phenethylamine and substituted ketocarbazoles (XII) via the intermediate amines (XIII-XVIII).

Aminotetrahydrocarbazoles I-V were obtained in a similar way from ketocarbazole XII (X = Me) and alkylamines. The alkylamines XIX and XX required for the synthesis of compounds IV and V were obtained by stepwise reduction of cyclohexenylacetonitrile (XXI).

The reduction of nitrile XXI to amine was described in [9]; the hydrogenation of amine XIX has not been described in the literature. In [7], amine XX was obtained by hydrogenation of nitrile XXI over Raney Ni (100 atm., 60°C). We carried out the transformation of amine XIX into cyclohexylamine XX under milder conditions (15 atm, 20°C) and with complete conversion (according to GLC data). The refractive index of the amine XX obtained (n_D^{20} 1.4646 was considerably lower than that given in [7]) n_D^{17} 1.4720. It is possible that the authors of [7] dealt with a mixture of amines XIX and XX since the n_D^{20} of pure XIX is 1.4875.

To carry out the biological tests, the aminotetrahydrocarbazoles were converted into their hydrochlorides.

It should be noted that the antifungal activity of aminotetrahydrocarbazoles was studied for the first time.

EXPERIMENTAL (CHEMICAL)

The results of the elemental analysis correspond to the empirical formulas.

2-Cyclohexylethylamine (XX). A solution of 12.4 g (99 mmoles) of amine (XIX) [9] in 50 ml of ethanol and 0.6 g of palladium hydroxide on carbon (content of palladium 17%) were

Central Chemical Laboratory of Synthesis, All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 11, pp. 46-48, November, 1990. Original article submitted December 4, 1989.

 0091-150X/90/2411-0826$12.50

TABLE 1. Characteristics of the Compounds Synthesized for the First Time*

Compound	Yield, %	Mp, °C	Empirical formula
III·HCl	80**	151—1,5	$C_{22}H_{26}N_2$·HCl
IV·HCl	49**	162—3,5	$C_{21}H_{28}N_2$·HCl
V·HCl	50**	165 (dec.)	$C_{21}H_{30}N_2$·HCl
VIII·HCl	92	169—70	$C_{20}H_{21}ClN_2$·HCl
IX·HCl	80	169—9,5	$C_{20}H_{21}BrN_2$·HCl
X·HCl	96	190 (dec.)	$C_{26}H_{32}N_2$·HCl
XI	87	127—9	$C_{26}H_{26}N_2$
XI·HCl	98	180 (dec.)	$C_{26}H_{26}N_2$·HCl
XV·HCl	96	225 (dec.)	$C_{20}H_{19}ClN_2$·HCl
XVI	96	128—129	$C_{20}H_{19}BrN_2$
XVII	92	104—6	$C_{26}H_{30}N_2$
XVIII	82	139—40	$C_{26}H_{24}N_2$

*The characteristics of compounds I, II, VI, VII, XIII, XIV are given in [1, 6].
**Overall yield for the imination and reduction stages.

placed in a 150-ml autoclave. The hydrogenation was carried out for 10 h at room temperature and at a pressure of 15-10 at. The catalyst was filtered off and the filtrate was fractionated. Yield 8.8 g (70%) of amine XX; bp 95-96°C (40 mm), n_D^{20} 1.4646 (180-183°C at 760 mm, n_D^{20} 1.4649 [8]).

6-Methyl-1-(2-cyclohexylethylamino)-1,2,3,4-tetrahydrocarbazole Hydrochloride (V·HCl). A mixture of 25 ml of xylene, 10 g (150 mmoles) of ketocarbazole XII (X = Me) and 9.3 g (73 mmoles) of amine XX was boiled for 3 h in a nitrogen atmosphere in an apparatus equipped with a Dean—Stark adapter. Xylene was evaporated in vacuo, and 25 ml of absolute ethanol was added to the residue. After 24 h, the iminocarbazole that separated out was filtered off (13.3 g) and dissolved in 110 ml of hot ethanol; a 1.6-g portion (43 mmoles) of $NaBH_4$ was gradually added to the solution at 37-40°C. The suspension that formed was stirred for another 2 h at room temperature, and then 200 ml of water was added, and aminocarbazole V was filtered off (13.4 g, retains ethanol). The base V obtained was dissolved in 80 ml of ethanol, and ethanolic HCl was added to the solution. The precipitated hydrochloride was crystallized from MeOH. Yield 8.8 g of V·HCl, yield 50%.

Compounds III·HCl and IV·HCl were obtained in a similar way using 3-phenylpropylamine or cyclohexylethylamine XIX instead of amine XX. Compound III·HCl was crystallized from a mixture of ethanol with ether, and IV·HCl – from MeOH.

1-Phenethylimino-6-phenyl-1,2,3,4-tetrahydrocarbazole (XVIII). A mixture of 50 ml of xylene, 26.13 g (0.1 mole) of ketocarbazole XII (X = Ph), and 18.2 g (0.15 mole) of phenethylamine was boiled for 4 h until no further water separated out. The mixture was cooled and the iminocarbazole XVIII that separated out was filtered off. Yield 30.1 g (82%).

Iminocarbazoles XV-XVII were obtained in a similar way. Compounds XV·HCl and XVII were crystallized from ethanol, XVI and XVIII from MeOH (see Table 1).

1-Phenethylamino-6-phenyl-1,2,3,4-tetrahydrocarbazole (XI). A 2.9-g portion (77 mmoles) of $NaBH_4$ was added at 20-25°C to a suspension of 28 g (77 mmoles) of imine XVIII in 300 ml of absolute ethanol. The mixture was stirred at this temperature for 5 h, the solution formed was evaporated under vacuum, and 200 ml of benzene and 200 ml of water were added to the residue. The benzene layer was washed with water, dried over $MgSO_4$, benzene was evaporated under vacuum, and the residue was crystallized from ethanol. Yield 24.6 g (87%) of aminocarbazole XI. The solution of base XI in hot ethanol was treated with an alcoholic solution of HCl to yield XI·HCl.

The hydrochlorides VIII·HCl-X·HCl were obtained in a similar way from iminocarbazoles XV-XVII (see Table 1). Compounds VIII·HCl and IX·HCl were crystallized from MeOH, X·HCl from a mixture of ethanol with DMFA, and XI·HCl from a mixture of MeOH with HCl.

TABLE 2. Antitubercular Activity in Vitro of Aminotetrahydrocarbazole Hydrochlorides (MIC, μg/ml)

Compound	H37RV	Academia	Bovis 8	M. kansasii	M. avium	M. intracellulare	M. fortuitum	M. phlei	M. aquae SN 632	ATCC 607
I·HCl	23	23	0,55	0,55	<0,08	3,5	23	153	23	23
II·HCl	0,55	4,0	0,55	3,5	23	3,5	23	23	23	3,5
III·HCl	0,55	0,55	<0,08	<0,08	3,5	0,55	0,55	0,55	3,5	3,5
IV·HCl	3,5	<0,08	0,55	<0,08	0,55	0,55	3,5	23	23	3,5
V·HCl	<0,08	<0,08	0,55	<0,08	<0,08	0,55	3,5	0,55	<0,08	3,5
VI·HCl	3,5	0,55	3,5	0,55	153	3,5	0,55	153	0,55	23
VII·HCl	3,5	<0,08	3,5	<0,08	3,5	3,5	0,55	23	0,55	3,5
VIII·HCl	<0,08	<0,08	3,5	<0,08	3,5	—	23	0,55	23	3,5
IX·HCl	0,55	<0,08	<0,08	<0,08	<0,08	0,55	0,55	0,55	<0,08	3,5
X·HCl	0,55	3,5	<0,08	<0,08	3,5	0,55	3,5	23	<0,08	23
XI·HCl	0,55	<0,08	<0,08	<0,08	—	—	—	<0,08	—	—

TABLE 3. In Vitro Antifungal and Antibacterial Activity of Aminotetrahydrocarbazole Hydrochlorides (MIC, μg/ml)

Compound	C. albicans 1755	T. gypseum 5/85	M. canis 3/84	S. aureus 209-p	B. subtilis ATCC 3366	E. coli ATCC 25922
I·HCl	31,2	15,6	15,6	15,6	31,2	125
II·HCl	62,5	31,2	31,2	3,9	15,6	>250
III·HCl	125	62,5	62,5	3,9 (15,6)	2,0	125
IV·HCl	62,5	62,5	31,2	2,0 (3,9)	31,2	125
V·HCl	62,5	31,2	31,2	2,0 (3,9)	31,2	125
VI·HCl	125	62,5	62,5	15,6	15,6	125
VII·HCl	62,5	62,5	62,5	7,8 (>31,2)	7,8	62,5
VIII·HCl	31,2	31,2	15,6	3,9 (15,6)	3,9	>250
IX·HCl	62,5	31,2	31,2	2,0 (>15,6)	31,2	125
X·HCl	3,9	15,6	2,0	3,9 (>15,6)	3,9	>250
XI·HCl	15,6	7,8	3,9	2,0 (>31,2)	1,0	>250

Note. The values of minimal bactericidal concentrations are given in brackets.

EXPERIMENTAL (BIOLOGICAL)

The activity of compounds I·HCl-XI·HCl was studied in experiments in vitro with respect to mycobacteria (10 strains), pathogenic fungi (three strains), Gram-positive and Gram-negative bacteria (three strains). The generally accepted method of serial dilutions on liquid culture media [5, 6] was used. The minimal inhibiting concentration (MIC, μg/ml) was determined. The names of the strains used are given in Tables 2 and 3.

The chemotherapeutic effectiveness of the compounds was studied for microsporia of guinea pigs and Candida-induced encephalomeningitis in mice and on a model of a generalized Staphylococcus infection in mice. The microsporia in guinea pigs was induced by depositing and rubbing M. canis 3/84 on an epilated and scarified skin surface on the backs of guinea pigs. The treatment began 9 days after infection and was carried out for 3 weeks. The results were recorded 24 h and 7 days after the last day of treatment. Compounds I, X, and XI were studied on this model, with local application in the form of a 1% (I and X) or 3% (XI) ointment. The Candida encephalomeningitis of mice was induced by infection of the brain with C. albicans 1755 in a dose of $4 \cdot 10^7$ CFU (colony forming units) [2]. Compound X, which is most active in vitro, was studied on this model with abdominal administration in doses of 62.5-31.2 mg/kg for 5 days. Compounds III-V, VII, VIII, and X were studied on a model of a Staphylococcus septicemia with intraperitoneal infection using the generally accepted methods described in [5].

Most of the compounds displayed high activity in experiments in vitro against mycobacteria (compounds III-V and VII-XI) and considerable activity with respect to pathogenic fungi (compounds X and XI) and Gram-positive bacteria (Tables 2 and 3).

In experiments in vivo on a microsporia model of guinea pigs, compounds I, X, and XI did not have chemotherapeutic action. Compound X in a dose of 62.5 mg/kg had a weak therapeutic effect on a model of Candida encephalomeningitis in mice. The overall lifetime of the animals with respect to the maximally possible in the 10-day period of the experiment was 60%, while in control it was 48%. In treatment with a dose of 31.2 mg/kg, the overall lifetime of mice was 63%. At the end of the experiment (on the 30th day), the overall lifetime in both treated groups differed also inappreciably, but reliably from the corresponding parameter for the control group (28 and 16%). Compounds III-V, VII, VIII, and X did not have a chemotherapeutic activity in the Staphylococcus infection in mice.

The coincidence of the high activity of the compounds against mycobacteria, fungi, S. aureus, and B. subtilis, organisms remotely distributed in the series (and hence not having similar biology [3, 4]) indicates that the biological activity of aminotetrahydrocarbazoles is nonspecific in character. This is also indicated by the fact that for some compounds of this series the activity level does not change over the whole spectrum of the mycobacteria. The compounds can possibly be related to the group of antiseptics. This supposition is also supported by the fact that according to the data obtained in the Laboratory of Chemotherapy of Infectious Diseases (L. D. Shipilova), the compounds were inactive in experiments on models of generalized bacterial infections.

Compounds X-XI have a similar structure; this can possibly explain the fact that in one and the same test culture their activity does not differ by more than two orders of magnitude. Nevertheless, it can be seen that the introduction of bulky substituents into the 6-position (compounds X and XI) increases both the antitubercular and antifungal activity. It should also be noted that on transition from compound I to its hydrogen analogs IV and V, the biological activity does not decrease and, with respect to certain strains of mycobacteria, it even increases. Hence, the activity of aminotetrahydrocarbazoles containing the RCH_2CH_2NH group (where R = cyclohexenyl, cyclohexyl, or phenyl) is determined not by the electronic, but by the steric effect of the substituent R.

LITERATURE CITED

1. T. V. Akalaeva, A. I. Bokanov, P. Yu. Ivanov, et al., Khim.-farm. Zh., No. 3, 299-302 (1989).
2. O. V. Baklanova, E. N. Padeiskaya, and S. N. Kutchak, Antibiotiki, No. 1, 48-52 (1987).
3. A. A. Fedorov (ed.), Plant Life [in Russian], Vol. 1, pp. 289-290; Vol. 2, p. 99, Moscow (1974).
4. L. I. Kursanov and N. A. Kamarnitskii, Course on Lower Plants [in Russian], Moscow (1945), pp. 235-239.
5. G. N. Pershin (ed.), Methods of Experimental Chemotherapy [in Russian], Moscow (1971), pp. 100-187.
6. L. N. Filitis, T. V. Akalaeva, O. Yu. Amel'kin, et al., Khim.-farm. Zh., No. 10, 1217-1222 (1988).
7. R. J. Andersen, P. M. Frearson, and E. S. Stern, J. Chem. Soc., No. 11, 4088-4091 (1956).
8. R. A. Benkeser, C. Arnold, R. F. Lambert, and O. H. Thomas, J. Am. Chem. Soc., 77, 6042-6045 (1955).
9. O. Schnider and J. Hellerbach, Helv. Chim. Acta, 33, 1437-1448 (1950).